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Toward Efficient Zn(II)-Based Artificial Nucleases

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Abstract: A series of *cis-cis*-triaminocyclohexane Zn(II) complex−anthraquinone intercalator conjugates, designed in such a way to allow their easy synthesis and modification, have been investigated as hydrolytic cleaving agents for plasmid DNA. The ligand structure comprises a triaminocyclohexane platform linked by means of alkyl spacers of different length (from C_4 to C_8) to the anthraquinone group which may intercalate the DNA. At a concentration of 5 μM, the complex of the derivative with a C_8 alkyl spacer induces the hydrolytic stand scission of supercoiled DNA with a rate of 4.6×10^{-6} s^{-1} at pH 7 and 37 °C. The conjugation of the metal complex with the anthraquinone group leads to a 15-fold increase of the cleavage efficiency when compared with the anthraquinone lacking Zn-triaminocyclohexane complex. The straightforward synthetic procedure employed, allowing a systematic change of the spacer length, made possible to gain more insight on the role of the intercalating group in determining the reactivity of the systems. Comparison of the reactivity of the different complexes shows a remarkable increase of the DNA cleaving efficiency with the length of the spacer. In the case of too-short spacers, the advantages due to the increased DNA affinity are canceled due to the incorrect positioning of the reactive group, thus leading to cleavage inhibition.

Introduction

The phosphodiester bonds of DNA are exceptionally resistant to hydrolysis under uncatalyzed physiological conditions. At neutral pH and 25 °C, half-life times ranging from hundreds of thousands to billions of years have been estimated for the hydrolysis of phosphodiester bonds of DNA.[1] Nucleases are able to accelerate this reaction up to 10^{16}-fold, thus making possible the DNA manipulations that are essential to life.[2] The enormous effect of these enzymes most often depends on the presence in their active sites of metal ions, particularly Zn(II), Mg(II), and Fe(III), and on their ability to act as Lewis acids.[3] Although many of these enzymes are currently used in the laboratory practice, there is an increasing interest in the realization of small and robust artificial nucleases for their potential applications.[4] As a matter of fact, several examples of metal complexes that promote the hydrolysis of DNA have been reported in the past few years.[4,5] Hovewer, almost all these systems employ metal ions different from those used by the natural catalysts and, in particular, are often based on lanthanide ions[5d−f,6] or Cu(II).[5g−l]

Among the physiologically relevant metal ions, Zn(II) is probably the best suited metal ion for the development of artificial metallonucleases. In fact, being a strong Lewis acid and exchanging ligands very rapidly, it is an ideal candidate to play the role of hydrolytic catalysts. Furthermore, it is characterized by a well-defined coordination chemistry, which allows detailed studies of the reactivity of its complexes, and by the absence of a relevant redox chemistry that, unlike Cu(II) based systems, rules out competing oxidative cleavage pathways.

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However, its reactivity is somewhat lower than that of the other commonly employed transition-metal ions,[4] and this is probably why the examples of Zn(II)-based artificial nucleases reported to date are scarce.[7−9] Among them, the most relevant are two peptide-based systems: (i) a family of mononuclear Zn(II)-binding peptides tethered to a rhodium complex as intercalator, proposed by Barton and co-workers, which promotes plasmid DNA cleavage at the rate of 2.5×10^{-5} s^{-1} at pH 6 and in the presence of 5 μM complex concentration[8] and (ii) a binuclear Zn(II)-binding peptide conjugate with an acridine intercalator, described by Scrimin and co-workers, which cleaves plasmid DNA with a first-order rate constant of 1.0×10^{-5} s^{-1} at pH 7.0 and 3.6 μM complex concentration.[9] These two peptide-based catalysts, quite different in structure, share as a common element the presence of the DNA-intercalating moiety that, as in the case of some oxidative DNA cleaving agents,[10] may be at the source of the high reactivity observed. However, the effect of conjugating hydrolytic metal complexes to intercalating groups is not clearly established. On one hand, in the case of Barton's peptide system, the presence of the rhodium complex intercalator seems to be fundamental for the activity of the system, but its substitution with other intercalators leads to unreactive systems.[8b] On the other hand, in Scrimin's peptide the removal of the acridine group does not affect substantially the DNA cleavage efficiency of the system.[9]

Besides the two systems just discussed, examples of metal complexes appended to intercalating groups as hydrolytic agents are surprisingly rare. Schneider and co-workers appended two naphthalene groups to an azacrown ligand via C_6 alkyl spacers and used it as a cofactor for Eu(III)-promoted DNA hydrolysis.[5a] Again the reported results are puzzling because the conjugation with the intercalating units led to an increase of the intrinsic reactivity but to a decrease of the DNA affinity, resulting in an overall decrease of the DNA cleavage rate at low concentration of metal complex. Finally, an earlier report of Nakamura and Hashimoto,[11] who investigated the reactivity of a hydroxamic acid linked to a phenanthridine intercalator in the presence of different lanthanide ions, points attention to the length of the spacer which tethers the intercalating unit to the catalytic group as a key element for the cleavage activity. However, such mechanistic investigations require systematic variations of the catalyst structure, and therefore, a readily synthetically accessible system is highly desired.

Other strategies than conjunction to intercalating groups have been explored to increase the DNA affinity of hydrolytic agents. Again Schneider and co-workers, using Co(III) cyclen complexes featuring alkylammonium chains of different length, found an increase of the DNA affinity and, as a result, of the overall reactivity as the spacer length was increased.[5b] Finally, evidence of DNA binding was obtained also by the groups of Fujii[5m] and Cowan[5l] with Cu(II) complexes of triaminocyclohexane and the aminoglycoside kanamycin A, respectively. Very high DNA cleavage rate was obtained (plasmid DNA cleavage rate constants of 4.8×10^{-5} s^{-1} for the Fujii complex and 3×10^{-4} s^{-1} for the Cowan system at pH 8.1 and 7.3),[12] and the efficiency of the systems was attributed to specific ligand−DNA interactions presumably via hydrogen bonds.

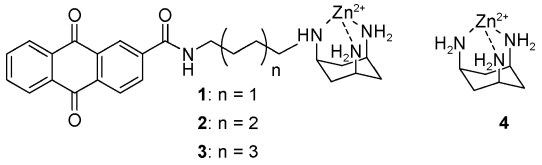

Figure 1. Structures of metal complex−anthraquinone conjugates (**1**−**3**) and of reference compound **4**.

We report here the realization of a series of Zn(II) complex−intercalator conjugates, whose structure features a *cis-cis*-triaminocyclohexane chelating subunit linked to an anthraquinone moiety through alkyl spacers of different length (Figure 1). The hydrolytic subunit was chosen on the basis of our previous studies which have shown that it is a rather efficient catalyst of model phosphate esters cleavage,[13] while the anthraquinone intercalator was selected for the straightforward chemistry and for its well-known ability to intercalate DNA base pairs.[14] The complexes have been designed to test their efficacy in the hydrolytic cleavage of plasmid DNA and to get insight on the role played by the intercalating group in determining the reactivity of the systems. The latter point could be investigated by systematically changing the length of the alkyl spacer using a rather straightforward synthetic procedure.

Results and Discussion

The general synthetic route for the synthesis of the anthraquinone conjugates **5**−**7** is shown in Scheme 1. The di-BOC-protected derivative of the triaminocyclohexane was reacted with the proper Z-protected ω-bromoamine. Removal of the Z protecting group, coupling with the chloride of the anthraquinone-4-carboxylic acid, and final deprotection of the BOC groups led to the desired compounds.

Zn(II) complexes **1**−**3** bind to dsDNA as highlighted by a decrease of the anthraquinone absorbance at 332 nm, diagnostic of the formation of a stable binary complex (see Supporting Information). In the case of compound **2**, we could evaluate a binding constant of about 1×10^4 M^{-1}, a value in the range reported for similar intercalating units.[14]

Incubation of pBR 322 plasmid DNA with the complexes **1**−**4** at pH 7.0 for 24 h at 37 °C results in a different extent of DNA cleavage depending on the nature and concentration of the complex (Figure 2). DNA is converted from the supercoiled form I to the nicked form II and eventually to the linearized form III. On the other hand, control experiments have shown that ligands **5**−**7**, in the absence of Zn(II), are totally unreactive (see Supporting Information).

Complex **4**, lacking the intercalating subunit, has been used as reference compound and shows a much lesser hydrolytic

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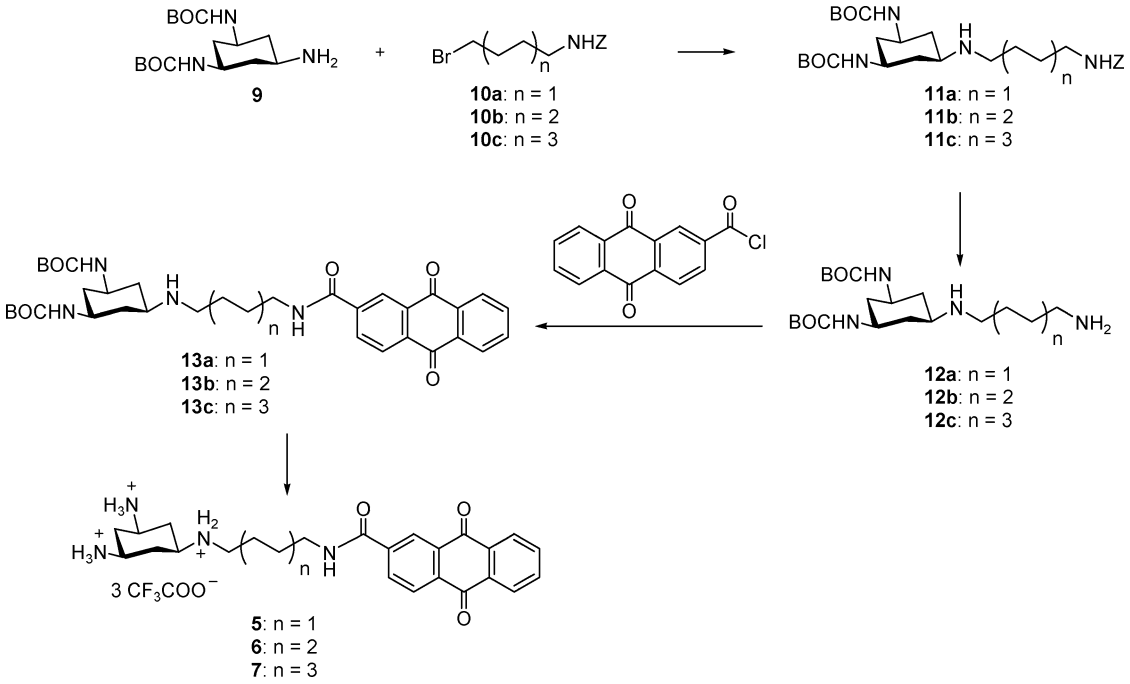
Scheme 1



activity: after incubation of DNA in the presence of 180 μM of **4**, only about 50% of form I has been nicked. The same extension of conversion from form I to II is obtained at concentrations 75 and 36 μM of complexes **2** and **3**, respectively. This indicates a much more pronounced DNA cleavage efficiency for the anthraquinone coniugates. However, in the concentration range investigated, no such reactivity has been reached using complex **1**. Thus, the presence of the anthraquinone group leads to a significant modulation of the reactivity depending upon the length of the spacers.

The use of Zn(II) complexes allows us to discard the possibility of metal ion-driven oxidative cleavage processes, while the absence of cleavage observed with free ligands **5**−**7** and with complex **1** indicates that the possibility of an anthraquinone-driven oxidative process can also be ruled out. The results of DNA cleavage experiments performed in anaerobic conditions with complex **3** (see Supporting Information), which show that the reactivity of the complex does not change in the absence of oxygen, further support such conclusions.

To better define the behavior of the system, we carried out kinetic measurements of the DNA cleavage reaction using different concentrations of **3**. The kinetic profiles obtained by densitometric quantification of the agarose gels were (Figure 3, left) analyzed using two simplified kinetic models. The first one assumes the occurrence of two consecutive, independent first-order processes in which form II is first produced from form I (single-strand cleavage, k_1) and then is degraded to form III (random double-strand cleavage, k_2). The second one implies three independent first-order processes in which form III can be produced directly either from form I (nonrandom double-strand cleavage, k_3) or from form II (random double-strand cleavage, k_2), which in turn is the product of the cleavage of form I (single-strand cleavage, k_1). The fitting of the kinetic data gave satisfactory results only with the first model, indicating that any relevant nonrandom double-strand clavage, leading to the direct conversion of form I to form III, can be ruled out. At a 45 μM concentration of complex, form I cleavage rates are 3.2×10^{-5} s^{-1} and 2.0×10^{-6} s^{-1} in the presence of **3** and **4**, respectively. Therefore, a 15-fold acceleration in the reaction rate is generated by the introduction of the intercalating subunit.

The rate of conversion of form I to form II increases with the concentration of the complex showing (Figure 3, right) a dose-dependent saturation behavior. This is admittedly not so well-defined since the relatively low solubility of the complexes did not allow us to explore a wider concentration range. At any rate, fitting of the available data with the Michaelis−Menten equation gives a formation constant of approximately 1×10^4 M^{-1}, in agreement with the results of the UV−vis binding experiments. The effective intercalation of the anthraquinone

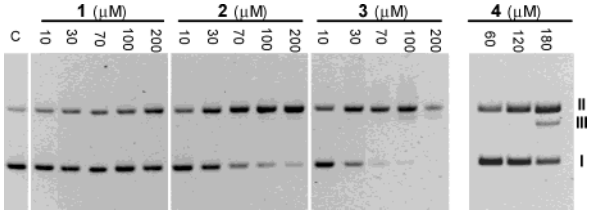


Figure 2. Agarose gel electrophoresis of pBR 322 DNA (12 μM b.p.) after 24 h incubation with the indicated concentration of complexes **1**−**4** in 20 mM HEPES, pH 7.0, 37 °C. Lane C is DNA incubated in the absence of complexes.

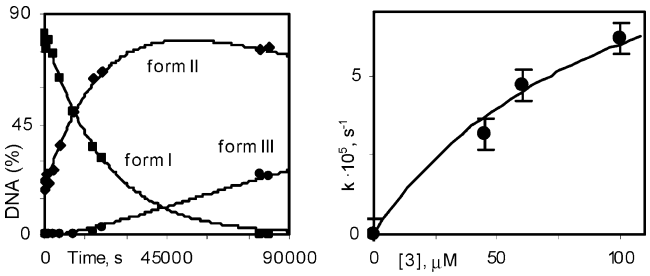


Figure 3. Left: kinetic profiles for plasmid DNA cleavage in the presence of 60 μM **3** (HEPES 20 mM, pH 7, 37 °C). The curves were generated by fitting the data with the kinetic model described in the text. Right: cleavage rate of pBR 322 DNA (forms I to II) as a function of the concentration of **3**. The curve shows the fitting with the Michaelis−Menten equation.

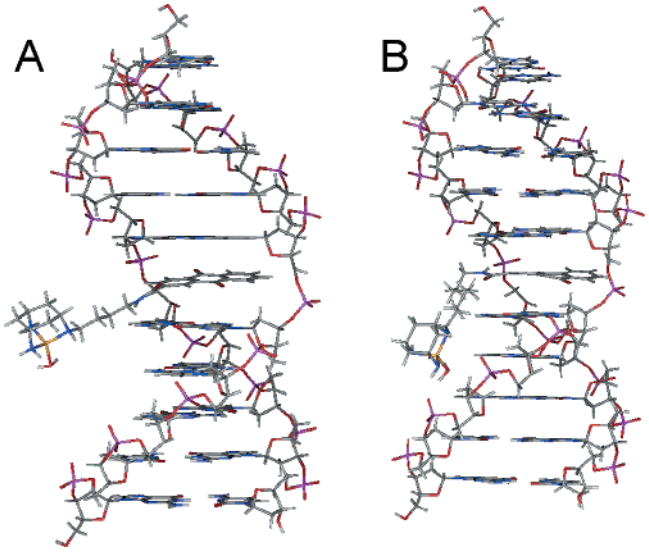


Figure 4. Calculated structures (MMFF94) of the intercalation complexes between complexes **1** (A) and **3** (B) and DNA.

conjugates **1**−**3** in plasmid DNA is further confirmed by agarose gel electrophoresis performed in the absence of SDS in the medium (see Supporting Information). The considerable smearing and decrease of the plasmid mobility observed clearly indicates an increase of the hydrodynamic volume of the negative supercoiled plasmid, as a result of the formation of the intercalation complexes.

The reactivity trend of complexes **1**−**3** (Figure 2) highlights the remarkable effect due to the length of the alkyl spacer. While complex **1**, with a C_4 spacer, is virtually ineffective at every concentration, the reactivity progressively increases using complexes **2** and **3** with C_6 and C_8 spacers. Such behavior indicates that the length and flexibility of the spacer play a fundamental role in the interaction between the catalytic subunit and the phosphate backbone. After intercalation of the anthraquinone moiety, the spacer must be free to fold in such a way as to position the metal complex close to a phosphate group, and a too-short spacer may prevent the correct folding. This is confirmed by molecular mechanics calculations: as shown in Figure 4, only in the intercalation complex between **3** and DNA can the metal complex reach the phosphate backbone and promote its cleavage. In the case of complex **1**, the spacer is too short and does not allow this interaction to occur, so that the intercalation process leads to inhibition rather than enhancement of reactivity of the metal complex, because the metal complex is hampered in interacting with the phosphate groups.

The present results may help to explain the apparently contradictory results obtained by Scrimin,[8] Barton,[9] and Schneider[5a] by appending intercalators to hydrolytic metal complexes. The proper distance between the two subunits is a main factor, and the catalyst efficacy results from a subtle balance between increased DNA affinity and decreased mobility of the metal complex.

The kinetic data obtained allow us to estimate, for a 5 μM concentration of **3**, a first-order rate constant of 4.6×10^{-6} s^{-1} for the cleavage of plasmid form I. This value compares well with those reported for the others Zn(II)-based system, although obtained under somewhat different experimental conditions, and also with the Schneider lanthanide and Co(III)-based agents (see infra). The slightly lower reactivity of the present systems can be substantially attributed to a still low DNA affinity, which could be increased by selecting more efficient intercalating groups. The plainness of the synthetic procedure involved and the modular design of the system will allow the easy modification and optimization of the systems to obtain more efficient and selective catalysts.

Conclusion

In conclusion, we have realized a simple Zn(II)-based artificial nuclease with a remarkable DNA cleavage activity. The source of the enhanced reactivity is the conjugation of the metal complex with a DNA targeting unit, which leads to a 15-fold increase of the cleavage efficiency with respect to the metal complex alone. In particular, the importance of a careful design of the system and the choice of the appropriate spacer has been highlighted. In fact, as the comparison of the reactivity of complexes **1**−**4** clearly shows, a too-short spacer may decrease or even cancel the advantages due to the increased DNA affinity. The availability of a system that is easy to synthesize and, hence, to modify is of paramount importance to reach the optimum efficiency.

Experimental Section

General. Solvents were purified by standard methods. All commercially available reagents and substrates were used as received. TLC analyses were performed using Merck 60 F_{254} precoated silica gel glass plates. Column chromatography was carried out on Macherey-Nagel silica gel 60 (70−230 mesh). NMR spectra were recorded using a Bruker AC250F operating at 250 MHz for ^{1}H and 62.9 MHz for ^{13}C. Chemical shifts are reported relative to internal Me_4Si. Multiplicity is given as follow: s = singlet, d = doublet, t = triplet, q = quartet, qt = quintet, m = multiplet, br = broad peak. UV−vis absorption measurements were performed on a Perkin Helmer Lambda 16 spectrophotometer equipped with a thermostated cell holder. $Zn(NO_3)_2$ was an analytical grade product. Metal ion stock solutions were titrated against EDTA following standard procedures. The buffer 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma) was used as supplied by the manufacturers. *cis,cis*-1,3,5-Triaminocyclohexane (**8**),[15] 8-amino-1-octanol,[16] and 9,10-dioxo-9,10-dihydro-anthracene-2-carbonyl chloride[17] were prepared as reported.

Procedure for DNA Cleavage Experiments. Stock solutions of the anthraquinone conjugates **5**−**7** and model ligand **8** (0.5−1 mmol) were prepared in H_2O/CH_3OH, 4:1 (ligands **5**,**6**, and **8**) and H_2O/CH_3OH, 2:3 (ligand **7**). The ligand was dissolved in the proper solvent mixture, 1 equiv of $Zn(NO_3)_2$ in water was added, and the pH was corrected to 7 by addition of NaOH. In the case of ligand **7**, the solubility at neutral pH in the absence of the metal ion was very low, and stable stock solutions of the free ligand could not be obtained. The methanol content in the final reaction mixtures was always lower than 4%; control experiments confirmed that the presence of such an amount of methanol has no effect on the reaction rates.

DNA cleavage experiments were performed using pBR 322 (Gibco BRL) in 20 mM HEPES, pH 7.0. Reactions were performed incubating DNA (12 μM base pairs) at 37 °C in the presence and absence of increasing amounts of metal complex for the indicated time. Reaction products were resolved on a 1% agarose gel in TAE buffer (40 mM TRIS base, 20 mM, acetic acid, 1 mM EDTA) containing 1% SDS to dissociate the ligands from DNA. The resolved bands were visualized by ethidium bromide staining and photographed. The relative amounts

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of different plasmid structures were quantified using a BioRad Gel Doc 1000 apparatus interfaced to a PC workstation.

The DNA degradation experiments in anaerobic conditions were performed following a modification of the protocol reported by Burstyn and co-workers.[5i] Deoxygenated 10 mM HEPES buffer at pH 7.0 was prepared by five freeze−pump−thaw cycles; before each cycle, the solution was equilibrated with argon under sonication to favor the deoxygenation process. Deaerated methanol was prepared by sonication under argon. Deoxygenated buffer and methanol were used immediately after preparation. All anaerobic stock solutions were prepared in a nitrogen-filled glovebag. Reaction mixtures were immediately prepared by addition of the appropriate amounts of stock solutions to the reactions tubes and incubated at 37 °C for 24 h in the same nitrogen-filled glovebag. All other conditions were the same as reported in the previous paragraph.

Molecular Mechanics Calculations. All calculations were run on SGI Octane R12000 workstation. *cis*,*cis*-1,3,5-Triaminocyclohexane·Zn^{2+} (**4**) structure was optimized using Hartree−Fock calculations with the 6-311++G(d,p) basis set. A water molecule coordinated to the apical position of the Zn^{2+} ion was used. This water molecule was used to saturate, at least partially, the coordination sphere of the zinc ion. Quantum chemistry calculations were carried out with Gaussian 98. Harmonic vibrational frequencies were obtained from RHF/6-311++G(d,p) calculations and used to characterize local energy minima (all frequency real). Atomic charges were calculated by fitting to electrostatic potential maps (CHELPG method).[18]

Structures of metal complex−anthraquinone conjugates (**1**−**3**) were built using the "Builder" module of Molecular Operation Environment (MOE, version 2002.03).[19] Conjugate complexes were minimized using MMFF94 force field. All bond distances involving Zn^{2+} metal ion were frozen during the optimization step. Charges for the *cis*,*cis*-1,3,5-triaminocyclohexane Zn(II) structure were imported from the Gaussian output files.

The present study involved the use of consensus dinucleotide intercalation geometries d(ApT) and d(GpC) initially obtained using Nucleic Acid MOdeling Tool (NAMOT2) software.[20] d(ApT) and d(GpC) intercalation sites were contained in the center of a decanucleotide duplex of sequences d(5′-ATATA-3′)$_2$ and d(5′-GCGCG-3′)$_2$, respectively. Decamers in B-form were built using the "DNA Builder" module of MOE. Decanucleotides were minimized using Amber94 all-atom force field,[21] implemented by MOE modeling package, until the rms value of the truncated Newton method (TN) was <0.001 kcal mol^{-1} Å^{-1}. The dielectric constant was assumed to be distance-independent with a magnitude of 4. The metal complex−anthraquinone conjugates (**1**−**3**) were docked into both intercalation sites using flexible MOE-Dock methodology. The purpose of MOE-Dock was to search for favorable binding configurations between a small, flexible ligand and a rigid macromolecular target. Searching was conducted within a user-specified 3D docking box, using "tabù search" protocol[22] and MMFF94 force field.[23] MOE-Dock performs a user-specified number of independent docking runs (55 in our specific case) and writes the resulting conformations and their energies to a molecular database file. The resulting DNA−anthraquinone intercalated complexes were subjected to MMFF94 all-atom energy minimization until the rms of conjugate gradient was <0.1 kcal mol^{-1} Å^{-1}. Also in these steps, all bond distances involving Zn^{2+} metal ion were frozen during the energy optimization. To model the effects of solvent more directly, a set of electrostatic interaction corrections was used. MOE suite implements a modified version of GB/SA contact function described by Still and coauthors.[24] These terms model the electrostatic contribution to the free energy of solvation in a continuum solvent model. The interaction energy values were calculated as the energy of the complex minus the energy of the ligand, minus the energy of DNA: $\Delta E_{inter} = E_{(complex)} - (E_{(L)} + E_{(rDNA)})$.

***all-cis*-1,3-*N*,*N*-(Di-*tert*-butyloxycarbonyl)-1,3,5-triaminocyclohexane (9).** *all-cis*-1,3,5-Triaminocyclohexane (1.04 g, 8.0 mmol) was dissolved in 400 mL of dry CH_3OH, and triethylamnine (TEA, 1.9 mL, 13.6 mmol) was added. To the resulting solution, being stirred at room temperature, was added dropwise a solution of di-*tert*-butyl dicarbonate (BOC_2O, 3.0 g, 13.6 mmol) in 70 mL of dry CH_3OH during 7 h. After the addition was completed, the reaction mixture was stirred at room for 2 days, protected from moisture with a $CaCl_2$ drying tube, following the reaction by TLC (silica gel, eluant $CHCl_3/CH_3OH/NH_3$, 9:1:0.1, R_f (**9**) = 0.25). The solvent was then evaporated, and the crude product was dissolved in CH_2Cl_2 (500 mL) and washed first with a 5% aqueous solution of $NaHCO_3$ (3 × 100 mL) and then with water (2 × 100 mL). The evaporation of the dried solvent (Na_2SO_4) afforded a crude product which was purified by column chromatography (silica gel, eluant $CHCl_3/CH_3OH/NH_3$, 9:1:0.1), yielding 1.05 g (40%) of pure **9** as a white solid. mp 214−215 °C.

^{1}H NMR ($CDCl_3$) δ: 0.90 (m, 3H), 1.43 (s, 18H), 2.18 (m, 3H), 2.85 (brt, 1H), 3.54 (m, 2H), 4.43 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 28.4, 39.3, 42.5, 46.7, 47.2, 79.3, 154.9.

***N*-Benzyloxycarbonyl-ω-amino-1-alcohol.** The ω-amino-1-alcohol (17 mmol) was dissolved in 10 mL of CH_3OH, and TEA (5.45 mL, 39.2 mmol) was added. To this solution, stirred at room temperature, was added dropwise a solution of benzyloxycarbonyl chloride (ZCl, 3.2 g, 18.77 mmol) in 5 mL of CH_3OH. After the addition was completed, the reaction mixture was stirred at room temperature for 10 h and protected from moisture with a $CaCl_2$ drying tube. The solvent was then evaporated, and the crude product was dissolved in CH_2Cl_2 (150 mL) and washed first with a 5% aqueous solution of $KHSO_4$ (2 × 50 mL) and then with water (2 × 50 mL). The evaporation of the dried solvent (Na_2SO_4) afforded the product as a white solid pure enough to be used in the following steps.

***N*-Benzyloxycarbonyl-4-amino-1-butanol.**[25] Yield 91%, mp 82−83 °C, R_f: 0.7 (silica gel, $CHCl_3$/MeOH, 9:1). ^{1}H NMR ($CDCl_3$) δ: 1.56 (m, 4H), 2.36 (br s, 1H), 3.20 (m, 2H), 3.62 (m, 2H), 4.9 (br s, 1H), 5.08 (s, 2H), 7.32 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 26.4, 29.5, 40.7, 62.1, 66.5, 128.0, 128.4, 136.5, 156.6. ESI-MS (*m*/*z*): 223.9[$M^+ + H^+$], 245.9[$M^+ + Na^+$], 262.0[$M^+ + K^+$].

***N*-Benzyloxycarbonyl-6-amino-1-hexanol.**[26] Yield 82%, mp 81−82 °C, R_f: 0.6 (silica gel, $CHCl_3$/MeOH, 9:1). ^{1}H NMR ($CDCl_3$) δ: 1.24−1.56 (m, 8H), 3.18 (m, 2H), 3.62 (t, J = 6.6 Hz, 2H), 4.69 (br, 1H), 5.09 (s, 2H), 7.35 (m, 5H).

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***N*-Benzyloxycarbonyl-8-amino-1-octanol.**[27] Yield 42%, mp 87−88 °C, R_f: 0.7 (silica gel, $CHCl_3$/MeOH, 9:1). ^{1}H NMR ($CDCl_3$) δ: 1.49 (m, 12H), 3.17 (t, J = 6.6 Hz, 2H), 3.63 (t, J = 6.6 Hz, 2H), 4.75 (br s, 1H), 5.10 (s, 2H), 7.34 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 25.6, 26.6, 29.1, 29.2, 29.9, 32.7, 54.8, 62.9, 69.6, 128.3, 128.5, 128.6, 136.6, 157.0. ESI-MS (*m*/*z*): 280.2 [$M^+ + H^+$], 297.2 [$M^+ + NH_4^+$], 302.1 [$M^+ + Na^+$], 318.1 [$M^+ + K^+$].

***N*-Benzyloxycarbonyl-ω-bromo-1-alkylamine 10a−c.** The above protected amino alcohol (2 mmol) was dissolved in 15 mL of CH_2Cl_2, and CBr_4 (0.990 g, 2.98 mmol) and Ph_3P (0.783 g, 2.98 mmol) were added to the stirred solution at 0 °C. The solution was warmed at room temperature, and the reaction was followed by TLC (petroleum ether/EtOAc, 7:3). After 90 min at room temperature, water was added in the reaction flask. The solution was diluted with 110 mL of CH_2Cl_2, and the organic phase was washed twice with water and brine and dried over Na_2SO_4. The solvent was evaporated to dryness under vacuum. The crude material was purified by flash chromatography (SiO_2, petroleum ether/AcOEt, 90:10, gradient 2%) to give the bromide as a transparent oil.

***N*-Benzyloxycarbonyl-4-bromo-1-butylamine (10a).**[28] Yield 78%, R_f: 0.6 (silica gel, petroleum ether/AcOEt, 7:3). ^{1}H NMR ($CDCl_3$) δ: 1.65 (qt, J = 6.7 Hz, 2H), 1.86 (qt, J = 6.7 Hz), 3.21 (q, J = 6.7 Hz, 2H), 3.41 (t, J = 6.7 Hz, 2H), 4.83 (br s, 1H), 5.09 (s, 2H), 7.33 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 28.6, 29.8, 33.1, 40.1, 66.6, 128.0, 128.1, 128.5, 136.5, 156.4. ESI-MS (*m*/*z*): 286.1, 288.2 [$M^+ + H^+$], 308.0, 310.0 [$M^+ + Na^+$].

***N*-Benzyloxycarbonyl-6-bromo-1-hexylamine (10b).**[29] Yield 84%, R_f: 0.6 (silica gel, petroleum ether/AcOEt, 7:3). ^{1}H NMR ($CDCl_3$) δ: 1.5 (m, 6H), 1.85 (qt; J = 6.8 Hz, 2H), 3.19 (q, J = 6.8 Hz, 2H), 3.39 (t, J = 6.8 Hz, 2H), 4.76 (br s, 1H), 5.1 (s, 2H), 7.33 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 25.8, 27.7, 29.7, 32.5, 33.7, 40.8, 66.5, 128.0, 128.1, 128.4, 136.6, 156.3. ESI-MS (*m*/*z*): 314.1, 316.2 [$M^+ + H^+$], 331.1, 333.2 [$M^+ + NH_4^+$], 335.8, 337.8 [$M^+ + Na^+$], 351.9, 353.9 [$M^+ + K^+$].

***N*-Benzyloxycarbonyl-8-bromo-1-octylamine (10c).** Yield 88%, R_f: 0.7 (silica gel, petroleum ether/AcOEt, 7:3). ^{1}H NMR ($CDCl_3$) δ: 1.53 (m, 10H), 1.84 (qt, J = 6.9 Hz, 2H), 3.18 (q, J = 6.9 Hz, 2H), 3.39 (t, J = 6.9 Hz, 2H), 4.74 (br s, 1H), 5.09 (s, 2H), 7.33 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 26.6, 28.0, 28.6, 29.0, 29.8, 29.9, 33.9, 41.0, 66.6, 128.0, 128.1, 128.5, 136.6, 155.7. ESI-MS (*m*/*z*): 342.1, 344.2 [$M^+ + H^+$], 364.1, 366.1 [$M^+ + Na^+$], 380.1, 382.1 [$M^+ + K^+$].

***all-cis*-3,5-*N*,*N*-Di-*tert*-butyloxycarbonyl-1-*N*-[ω-*N*-(benzyloxycarbony)aminoalkyl]-1,3,5-triaminocyclohexane (11a−c).** The bromide **10** (1 mmol) was dissolved in 10 mL of dry CH_3CN, and a solution of **9** (0.25 g, 0.76 mmol) in 20 mL of dry CH_3CN was added dropwise under stirring. Diisopropylethylamine (DIPEA, 0.13 mL, 0.76 mmol) was then added to maintain the pH around 8. The mixture was heated at reflux for 8 days following the reaction by TLC ($CHCl_3$/MeOH/NH_3, 9.5:0.5:0.1). The solvent was evaporated under reduced pressure, and the residue was dissolved in 40 mL of CH_2Cl_2 and washed with $NaHCO_3$ 5% (3 × 20 mL) and water (2 × 20 mL). The organic layer was dried over Na_2SO_4, and the solvent was removed under vacuum. The crude product was purified by flash chromatography (SiO_2, CH_2Cl_2 100%, NH_3 1%, gradient 0.5% MeOH), giving the product **11**.

***all-cis*-3,5-*N*,*N*-Di-*tert*-butyloxycarbonyl-1-*N*-[4-*N*-(benzyloxycarbony)aminobutyl]-1,3,5-triaminocyclohexane (11a).** Yield 61%. Pale yellow oil. R_f = 0.6 (silica gel, $CHCl_3$/MeOH/NH_3, 9.5:0.5:0.1). ^{1}H NMR ($CDCl_3$) δ: 0.86 (dd, J = 11.6 Hz, 3H), 1.40 (m, 22H), 2.15 (dd, J = 11.6 Hz, 3H), 2.59 (m, 3H), 3.17 (q, J = 6 Hz, 2H), 3.51 (br, 2H), 4.36 (br, 2H), 5.08 (s, 2H), 5.74 (br, 1H), 7.33 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 27.9, 28.4, 39.5, 40.9, 46.6, 46.7, 53.7, 66.5, 79.4, 128.1, 128.2, 128.4, 128.5, 136.7, 154.9, 156.4. ESI-MS (*m*/*z*): 536.2 [$M^+ + H^+$].

***all-cis*-3,5-*N*,*N*-Di-*tert*-butyloxycarbonyl-1-*N*-[6-*N*-(benzyloxycarbony)aminohexyl]-1,3,5-triaminocyclohexane (11b).** Yield 38%. White solid, mp 172−173 °C. R_f = 0.6 (silica gel $CHCl_3$/MeOH/NH_3, 9.5:0.5:0.1). ^{1}H NMR ($CDCl_3$) δ: 0.87 (dd, J = 11.6 Hz, 3H), 1.37 (m, 26H), 2.16 (dd, J = 11.6 Hz, 3H), 2.58 (m, 3H), 3.17 (q, J = 6.3 Hz), 3.54 (br, 2H), 4.42 (br, 2H), 4.78 (br, 1H), 5.09 (s, 2H), 7.33 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 26.5, 26.8, 28.4, 29.8, 30.1, 39.7, 40.9, 46.6, 47.1, 53.6, 66.5, 79.3, 128.0, 128.1, 128.4, 136.6, 154.9, 156.4. ESI-MS (*m*/*z*): 563.2 [$M^+ + H^+$], 585.3 [$M^+ + Na^+$], 601.2 [$M^+ + K^+$].

***all-cis*-3,5-*N*,*N*-Di-*tert*-butyloxycarbonyl-1-*N*-[8-*N*-(benzyloxycarbony)aminooctyl]-1,3,5-triaminocyclohexane (11c).** Yield 36%. White solid, mp 164−165 °C. R_f = 0.6 (silica gel $CHCl_3$/MeOH/NH_3, 9.5:0.5:0.1). R_f = 0.6 ($CHCl_3$/MeOH/NH_3, 9.5:0.5:0.1). ^{1}H NMR ($CDCl_3$) δ: 0.88 (dd, J = 11.5 Hz, 3H), 1.42 (m, 30H), 2.20 (dd, J = 11.5 Hz, 3H), 2.58 (m, 3H), 3.18 (q, J = 6.6 Hz, 2H), 3.55 (m, 2H), 4.48 (br, 2H), 4.82 (br, 1H), 5.09 (s, 2H), 7.32 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 26.6, 27.2, 28.4, 29.1, 29.3, 29.9, 30.2, 39.7, 41.0, 46.6, 47.3, 53.6, 66.5, 79.3, 128.0, 128.1, 128.4, 136.6, 154.9, 156.3. ESI-MS (*m*/*z*): 591.4 [$M^+ + H^+$].

***all-cis*-3,5-*N*,*N*-Di-*tert*-butyloxycarbonyl-1-*N*-(ω-aminoalkyl)-1,3,5-triaminocyclohexane (12a−c).** Compound **11** (0.4 mmol) was dissolved in MeOH, and 25 mg of Pd/C 5% was added to the stirred solution. The mixture was kept under H_2 atmosphere for 5 h, and the removal of the CBz group was followed by TLC (silica gel, $CHCl_3$/MeOH/NH_3, 9:1:0.1). The catalyst was filtered off, the solution was evaporated to dryness, and the residue was coevaporated twice with CH_3CN, giving compound **12** which was used without further purification.

***all-cis*-3,5-*N*,*N*-Di-*tert*-butyloxycarbonyl-1-*N*-(4-aminobutyl)-1,3,5-triaminocyclohexane (12a).** Yield 77%. White solid. R_f = 0.1 (silica gel $CHCl_3$/MeOH/NH_3, 9:1:0.1). ^{1}H NMR (CD_3OD) δ: 1.01 (dd, J = 11.8 Hz, 3H), 1.42 (s, 18H), 1.58 (m, 4H), 2.06 (dd, J = 11.8 Hz, 3H), 2.63 (m, 3H), 2.83 (m, 2H), 3.41 (br, 2H). ESI-MS (*m*/*z*): 302.3 [M^+ − BOC], 401.3 [$M^+ + H^+$].

***all-cis*-3,5-*N*,*N*-Di-*tert*-butyloxycarbonyl-1-*N*-(6-aminohexyl)-1,3,5-triaminocyclohexane (12b).** Yield 78%. White solid. R_f = 0.1 (silica gel $CHCl_3$/MeOH/NH_3, 9:1:0.1). ^{1}H NMR ($CDCl_3$) δ: 1.06 (m, 2H), 1.4 (m, 27H), 2.2 (m, 3H), 2.76 (m, 5H), 3.49 (br, 2H), 4.72 (br, 2H). ESI-MS (*m*/*z*): 229.2 [M^+ − 2BOC], 329.3 [M^+ − BOC], 429.4 [$M^+ + H^+$], 451.3 [$M^+ + Na^+$].

***all-cis*-3,5-*N*,*N*-Di-*tert*-butyloxycarbonyl-1-*N*-(8-aminooctyl)-1,3,5-triaminocyclohexane (12c).** Yield 88%. White solid. R_f = 0.1 (silica gel $CHCl_3$/MeOH/NH_3, 9:1:0.1). ^{1}H NMR (CD_3OD) δ: 0.95 (dd, J = 11.7 Hz, 3H), 1.34 (m, 30H), 2.04 (dd, J = 11.7 Hz, 3H), 2.56 (m, 3H), 2.71 (m, 2H), 3.35 (br, 2H). ESI-MS (*m*/*z*): 457.4 [$M^+ + H^+$].

9,10-Dioxo-9,10-dihydro-anthracene-2-carboxylic Acid [ω-(*cis*-3,5-(*N*,*N*-Di-*tert*-butyloxycarbonyl)diamino-cyclohexylamino)alkyl]-amide (13). To a solution of the above amine **12** (0.16 mmol) in dry THF (5 mL) were added first TEA (0.025 mL, 0.16 mmol) and then 9,10-dioxo-9,10-dihydro-anthracene-2-carbonyl chloride (53 mg, 0.019 mmol). The reaction mixture was stirred at room temperature protected from moisture for 28 h following the reaction by TLC ($CHCl_3$/CH_3CH_2OH, 10:0.5). The solvent was evaporated, and the crude material was purified by two subsequent column chromatography using as eluant first $CHCl_3$/CH_3CH_2OH, 10:0.5 and then $CHCl_3$/CH_3OH/NH_3, 10:1:0.1.

9,10-Dioxo-9,10-dihydro-anthracene-2-carboxylic Acid [4-(*cis*-3,5-(*N*,*N*-Di-*tert*-butyloxycarbonyl)diamino-cyclohexylamino)butyl]-amide (13a). Yield 32%. R_f = 0.3 (silica gel $CHCl_3$/CH_3CH_2OH, 10:0.5). ^{1}H NMR ($CDCl_3$) δ: 0.78−0.99 (m, 3H), 1.41 (s, 18H), 1.61−1.77 (m, 4H), 2.17−2.20 (m, 3H), 2.61−2.75 (m, 3H), 3.49−3.53 (m, 4H), 4.52 (br s, 2H), 7.85−8.53 (m, 7H).

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9,10-Dioxo-9,10-dihydro-anthracene-2-carboxylic Acid [6-(*cis*-3,5-(*N*,*N*-Di-*tert*-butyloxycarbonyl)diamino-cyclohexylamino)hexyl]-amide (13b). Yield 65%. R_f = 0.3 (silica gel $CHCl_3/CH_3CH_2OH$, 10:0.5). ^{1}H NMR ($CDCl_3$) δ: 0.78−0.99 (m, 3H), 1.41 (s, 18H), 1.68−1.80 (m, 8H), 2.19−2.24 (m, 3H), 2.88−2.96 (m, 3H), 3.49−3.51 (m, 2H), 4.49 (br, 2H), 7.77−8.63 (m, 7H).

9,10-Dioxo-9,10-dihydro-anthracene-2-carboxylic Acid [8-(*cis*-3,5-(*N*,*N*-Di-*tert*-butyloxycarbonyl)diamino-cyclohexylamino)octyl]-amide (13c). Yield 24%. R_f = 0.3 (silica gel $CHCl_3/CH_3CH_2OH$, 10:0.5). ^{1}H NMR ($CDCl_3$) δ: 1.07−1.67 (m, 33H), 1.85−2.35 (m, 3H), 2.90−3.90 (m, 7H), 4.62 (m, 2H), 7.77−8.54 (m, 7H).

9,10-Dioxo-9,10-dihydro-anthracene-2-carboxylic Acid [ω-(*cis*-3,5-(*N*,*N*-Diamino-cyclohexylamino)alkyl)]amide (5−7). The above protected compound **13** (0.07 mmol) was dissolved in dry CH_2Cl_2 (2 mL), and trifluoroacetic acid (2 mL) was added. The resulting solution was stirred at room temperature and protected from moisture, following the reaction by TLC (silica gel, CH_2Cl_2/CH_3CH_2OH, 15:1). Evaporation of the solvent gave in quantitative yield the title compound as trifluoroacetate salt.

9,10-Dioxo-9,10-dihydro-anthracene-2-carboxylic Acid [4-(*cis*-3,5-(*N*,*N*-Diamino-cyclohexylamino)butyl)]amide (5). Yield 78%. R_f = 0 (silica gel, CH_2Cl_2/CH_3OH, 10:2). ^{1}H NMR (D_2O) δ: 1.45−1.60 (m, 7), 2.33−2.47 (m, 3H), 3.06 (t, 1H), 3.28 (t, 2H), 3.37−3.41 (m, 4H), 7.44−7.67 (m, 7H). ^{13}C NMR (D_2O) δ: 26.7, 29.1, 34.6, 36.1, 42.7, 48.3, 48.8, 55.5, 128.6, 130.4, 130.8, 135.4, 135.6, 136.0, 137.4, 138.6, 141.5, 170.0, 186.1, 186.3. ESI-MS (*m*/*z*): 435 [$M^+ + H^+$].

9,10-Dioxo-9,10-dihydro-anthracene-2-carboxylic Acid [6-(*cis*-3,5-(*N*,*N*-Diamino-cyclohexylamino)hexyl)]amide (6). Yield 96%. R_f = 0 (silica gel, CH_2Cl_2/CH_3OH, 10:2). ^{1}H NMR (CD_3OD) δ: 1.17 (m, 3H), 1.36−1.55 (m, 8H), 2.39 (m, 3H), 2.97 (t, 2H), 3.32 (m, 5H), 7.76−8.55 (m, 7H). ^{13}C NMR (D_2O) δ: 26.7, 27.0, 29.6, 32.5, 33.8, 34.0, 41.5, 46.5, 46.7, 53.2, 127.8, 128.4, 128.5, 129.0, 134.0, 134.3, 136.4, 140.6, 177.3, 183.3, 183.4. ESI-MS (*m*/*z*): 463 [$M^+ + H^+$].

9,10-Dioxo-9,10-dihydro-anthracene-2-carboxylic Acid [8-(*cis*-3,5-(*N*,*N*-Diamino-cyclohexylamino)octyl)]amide (7). Yield 92%. R_f=0 (silica gel, CH_2Cl_2/CH_3OH, 10:2). ^{1}H NMR (CD_3OD) δ: 1.09−1.57 (m, 15H), 2.21−2.33 (m, 3H), 3.36 (m, 5H), 7.78−8.50 (m, 7H). ^{13}C NMR (D_2O) δ: 26.5, 27.1, 29.6, 30.1, 32.4, 33.5, 34.1, 41.3, 46.4, 46.9, 53.1, 127.3, 128.6, 128.8, 129.2, 134.1, 134.5, 136.1, 140.1, 177.0, 183.2, 183.3. ESI-MS (*m*/*z*): 491 [$M^+ + H^+$].

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Supporting Information Available: DNA cleavage experiments by compounds **1**−**4** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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