Synthesis of a Functionalized Salen−Copper Complex and Its Interaction with DNA

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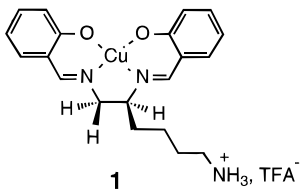
An original procedure for efficient synthesis of a functionalized salen·copper complex is reported. The mode of binding to DNA of the salen·Cu^{II} complex was investigated by viscometry as well as by absorption, circular, and linear dichroism spectroscopy. The complex can induce DNA strand breakage in the presence of a reducing agent as revealed by a plasmid cleavage assay. The spectroscopic and biochemical data indicate that the salen·Cu^{II} complex induces single-stranded breaks via an interaction within one of the grooves of the double helix.

Introduction

There is continuing interest in DNA-binding metal complexes. Cu·phenanthroline, Fe·EDTA, Mn·porphyrin, and Ru·polypyridyl complexes are typical examples of metal chelates capable of triggering single- and/or double-stranded DNA cleavages after activation with appropriate reducing agents.[1] Studies on their DNA binding/cleaving properties are of great importance for the design of sequence-specific or stereospecific artificial nucleases. Metal complexes of salen [bis(salicylidene)ethylenediamine][2] can also induce DNA cleavage[3] but, so far as we are aware, their mechanism of binding to nucleic acids has received little attention.[4] In this paper, we report an original procedure for efficient synthesis of a functionalized salen·Cu complex (**1**). Preliminary studies on the DNA-binding and DNA-cleaving properties of this copper complex using viscometry, measurements of absorption, circular and electric linear dichroism, and gel electrophoresis are described.



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Results and Discussion

Synthesis (Scheme 1). The key intermediate for the synthesis of the functionalized salen·copper complex **1** is the asymmetric α,*β*-diamine **5** which was synthesized following a sequential method.[5] The amide **3** was obtained from the commercially available N^{α}-*Z*-N^{ϵ}-BOC-L-lysine **2** after treatment with ethyl chloroformate in ammonia-saturated THF. Dehydration of the carboxamide **3** with trifluoroacetic anhydride in the presence of triethylamine furnished the nitrile **4** which was then subjected to hydrogenation (over Raney-Ni) under high pressure. The benzyloxycarbonyl protecting group was cleaved during this reaction (hydrogenolysis). The α,*β*-diamine **5** was then condensed with salicylaldehyde in the presence of cuprous acetate monohydrate to give the BOC-protected compound **6**. Finally, deprotection under acid conditions and purification afforded the salen·Cu complex **1** in 61% yield. The ESR parameters ($g_{||} = 2.23$ and $A_{||} = 191$ G) for the Cu^{II}·salen complex **1** are typical of a square planar complex. It is noteworthy that the chemical procedure outlined in Scheme 1 is generally applicable for the synthesis of other metal−salen−amino acid complexes. Recently, the synthesis of Ni and Mn·-salen complexes has been successfully completed using the same route.

Binding to DNA. We set out to define the mode of interaction between the copper·salen complex **1** and calf thymus DNA using a combination of spectroscopic and hydrodynamic measurements.

Absorption. The electronic absorption spectrum of compound **1** is significantly perturbed on binding to DNA. Relative to the absorption spectrum of the DNA-free compound, the absorption band centered at 346 nm is red-shifted by 3 nm and displays 13% hypochromism upon interaction of the ligand with DNA (not shown). Attempts to determine binding isotherms were made by titrating measured quantities of a stock solution of compound **1** into a known volume of calf thymus DNA solution and monitoring the resulting changes in the absorption spectrum of the ligand. The bathochromic shift proved too weak to permit a clear distinction between free and bound molecules and consequently

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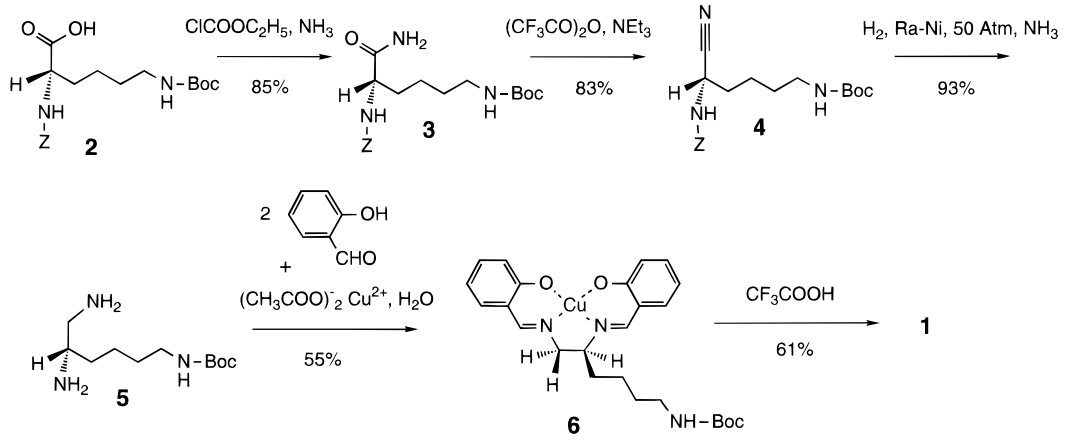
Scheme 1



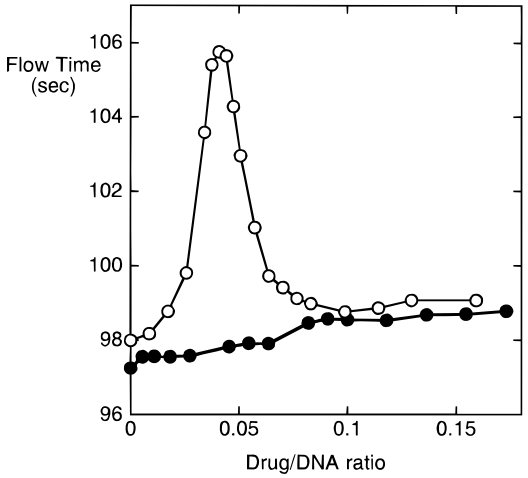


Figure 1. Viscometric titrations of pUC12 closed circular duplex DNA with compound **1** (J) and ethidium bromide (E). The flow time is plotted as a function of the molar ratio of drug added per DNA nucleotide.

attempts to estimate the binding constant from conventional Scatchard analysis failed. As an alternative procedure to examine the strength of the drug−DNA interaction, we employed fluorescence spectroscopy to investigate competition between the test drug and the intercalating drug ethidium bromide for available binding sites. The concentrations of compound **1** required to reduce by 50% the fluorescence of the DNA−ethidium complex under standard conditions (Q_{50}) were 12 μM with calf thymus DNA, 21 μM with poly(dA-dT)·poly(dA-dT) and >50 μM with poly(dG-dC)·poly(dG-dC). The finding that the drug diminishes the fluorescence of the ethidium−poly(dA-dT)·poly(dA-dT) complex more efficiently than the ethidium−poly(dG-dC)·poly(dG-dC) complex is consistent with the CD data (see below) suggesting that the drug interacts preferentially with AT rather than GC sequences. It speaks for an affinity of the drug toward the AT polynucleotide which is at least 2-fold higher than that for the GC polymer.

Viscometry. Compound **1** causes practically no change in the viscosity of a supercoiled DNA solution whereas a classical rise and fall in viscosity is observed with ethidium bromide (Figure 1). Apparently, binding of the salen·Cu complex to DNA does not induce any significant change in the winding of the helix.

Circular Dichroism (CD). The CD spectrum of the Cu·salen chelate **1** in solution reflects the asymmetric structure of the salen moiety. Addition of calf thymus DNA induces significant changes in its CD spectrum (Figure 2a). The band at 330 nm decreases as the DNA concentration is raised; meanwhile the CD in the 350−420 nm region increases significantly. The two isodichroic points at 318 and 354 nm and the constant spectral proportions indicate the existence of a single binding mode. Thus the binding appears geometrically homogeneous.

To evaluate the binding selectivity of compound **1** vis-à-vis GC or AT base pairs, further CD titration experiments were performed using synthetic polynucleotides having defined base compositions. As observed with calf thymus DNA, addition of the alternating copolymer poly(dA-dT)·poly(dA-dT) induces significant changes in the CD spectrum of the salen·Cu complex (Figure 2b). However, the variation in CD intensity is less pronounced than seen with calf thymus DNA which contains a nearly equal proportion of AT and GC base pairs. By contrast, the CD spectrum of the ligand is little affected by addition of poly(dG-dC)·poly(dG-dC) (Figure 2c), therefore suggesting that compound **1** binds preferentially to AT sequences rather than to GC sequences.[6] A preference for AT sequences has also been reported for Mn^{III}·salen complexes.[3a] Substitution of inosine for guanosine does not markedly promote binding of **1** to the polymer (Figure 2d). The CD spectra of complexes between coumpound **1** and either poly(dG-dC)·poly(dG-dC) or poly(dI-dC)·poly(dI-dC) at a polynucleotide/ligand ratio of 20 are almost identical. In neither case does a positive CD band appear at 390 nm, suggesting that the ligand has minimal interaction with the GC- and IC-containing polynucleotides. In other words, unlike what we found recently with AT-specific (e.g. netropsin, Hoechst 33258, berenil, DAPI) and GC-specific (e.g. mithramycin, chromomycin) minor groove binders and with intercalating drugs (e.g. actinomycin, echinomycin),[7] removal of the 2-amino group of guanine (which is exposed in the minor groove of DNA) has practically no influence on the binding of the salen·Cu complex **1** to DNA.

(6) Footprinting experiments using DNAase I as cleaving agent have failed to confirm the apparent preference for binding to AT sequences inferred from the CD titration experiments. The lack of detectable footprints may be due to the low affinity of the salen·Cu complex for DNA and possibly from the mechanism of recognition of DNA by DNAase I. The nuclease binds to the minor groove of DNA whereas compound **1** is suspected to interact within the major groove.

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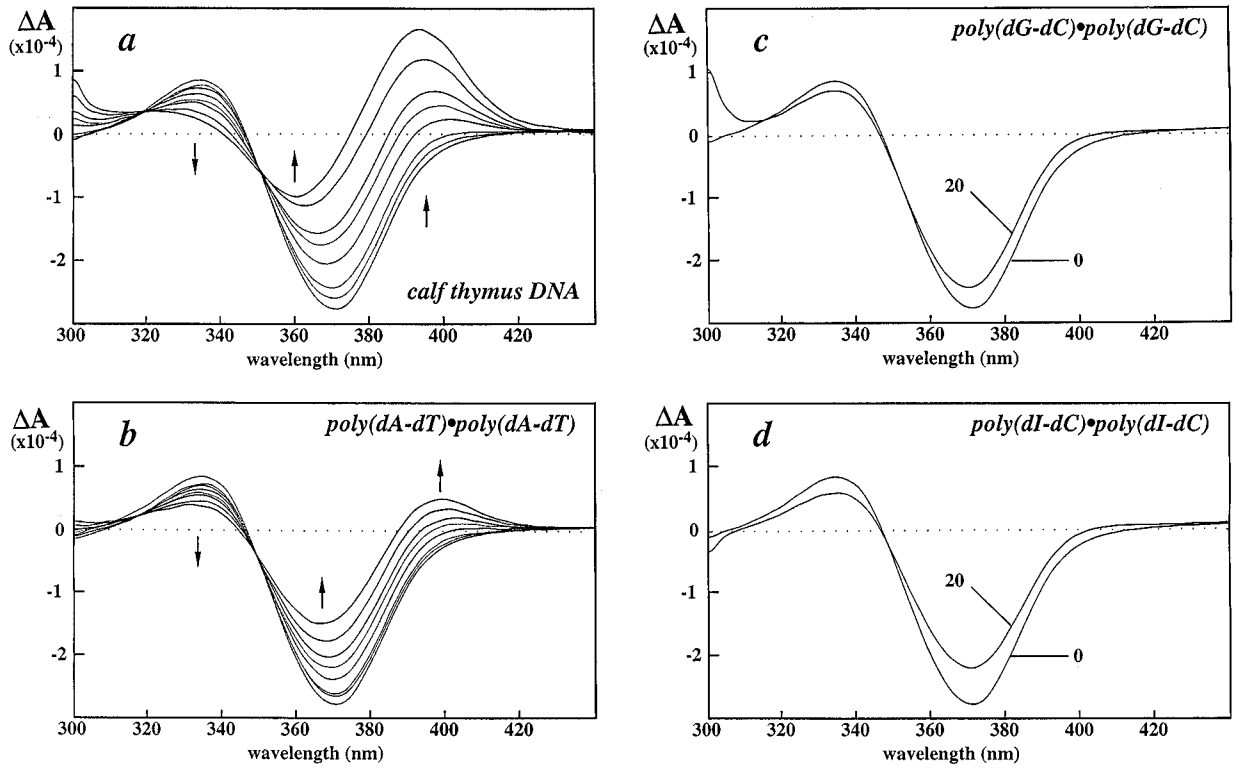

Figure 2. Circular dichroism. Titration of compound **1** with (a) calf thymus DNA, (b) poly(dA-dT)·poly(dA-dT). Panels c and d show the CD spectra of compound **1** in the absence or presence of (c) poly(dG-dC)·poly(dG-dC) and (d) poly(dI-dC)·poly(dI-dC). In panels a and b, the DNA-phosphate/drug ratio (P/D) increased as follows (bottom to top curves at 370 nm): 0, 1, 2.5, 5, 7.5, 10, 15, 20. In panels c and d, the P/D ratio is indicated. Solutions of drug with or without nucleic acids were scanned in 1 cm quartz cuvettes using a Jobin-Yvon CD6 dichrograph. Measurements were made by progressive addition of DNA to a ligand solution of constant concentration in order to obtain the desired drug/DNA ratios.

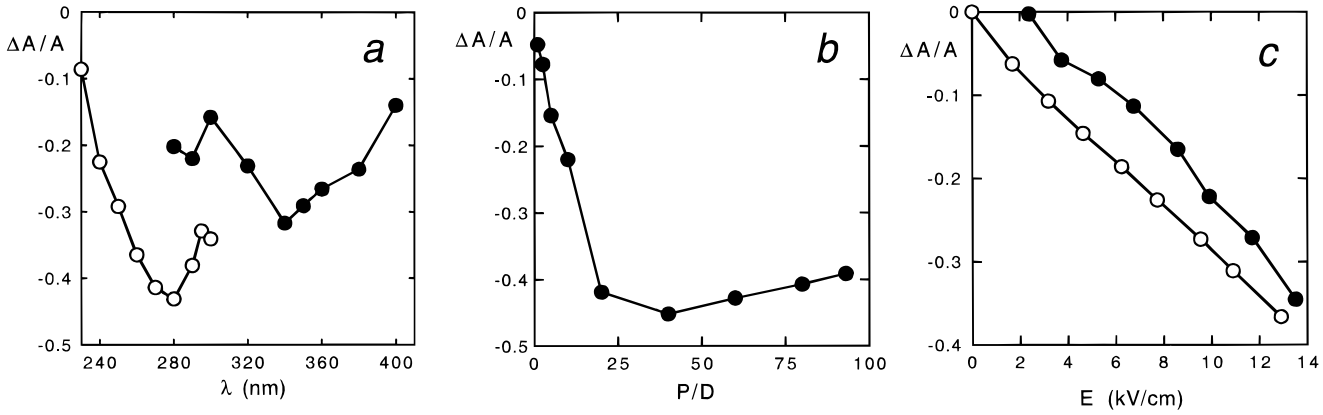

Figure 3. Dependence of the reduced dichroism $\Delta A/A$ on (a) wavelength, (b) the DNA-phosphate to drug ratio (P/D), and (c) electric field strength for compound **1**−DNA complex (J) and for DNA alone (E). Conditions: (a) P/D = 20, 12.5 kV/cm, (b) 340 nm, 12.5 kV/cm, (c) 340 nm for **1**, 260 nm for DNA, P/D = 20 in 1 mM sodium cacodylate buffer, pH 6.5.

Electric linear dichroism (ELD) represents a sensitive method for investigating drug binding to DNA.[8] Figure 3 illustrates a typical experimental data set showing the dependence of the reduced dichroism on the wavelength, the drug/DNA ratio and the electric field. The reduced dichroism $\Delta A/A$ is always negative in sign even in the 300−420 nm absorption band where there is no contribution from the DNA molecules. When compound **1** is fully bound to DNA (at $P/D \geq 20$) the DNA bases and the salen·copper complex display comparable reduced dichroism values. The fact that $\Delta A/A$ depends similarly upon field strength at 260 nm for the DNA bases and at 340 nm for compound **1** indicates that the planar copper complex is tilted close to the plane of the DNA bases.

The spectroscopic and hydrodynamic data reported above provide key information about the binding geometry of the Cu·salen complex **1** when associated with DNA. The copper chelate almost certainly binds to DNA with a single prefered geometry as judged from the CD spectra. The ELD data indicate that the Cu·salen complex is oriented roughly parallel to the plane of the base pairs (within 10°). At first sight, such an orientation would be consistent with an intercalative mode of binding as recently proposed for structurally related Cu·salen derivatives.[4] But the viscometric results are hard to

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reconcile with any binding model based on intercalation, even partial, of compound **1** into the DNA double helix. The manifest lack of effect of the Cu·salen derivative **1** on the viscosity of supercoiled DNA is frankly incompatible with an intercalative binding mode, given that all intercalating drugs provoke significant unwinding of the double helix.[9] The intense and positive CD signals obtained with calf thymus DNA and poly(dA-dT)·poly(dA-dT) are also in conflict with intercalation.[10] External binding of the ligand at the surface of the double helix is also unlikely since such a process would be expected to be completely nonspecific as regards orientation whereas the CD spectra attest that the binding is geometrically homogeneous. The possibility that the chelate fits into one of the grooves of DNA has been examined and appears most likely at first sight, though insertion of a ligand into the minor groove of DNA gives rise to large positive ELD signals[8] whereas those observed for the complex between compound **1** and calf thymus DNA are always negative. We considered the possibility of a binding site within the major groove of DNA. This would probably not require unwinding of the helix, and the major groove is certainly large enough to accommodate the Cu·salen chelate oriented parallel to the base pairs. Insertion between the major groove edges of the bases has been proposed for certain metal·trisphenanthroline complexes.[11] Moreover, the binding data obtained with compound **1** are reminiscent of those reported for planar alkaloids[12] and the phenothiazinium dye methylene blue which is believed to bind in the major groove of poly(dA-dT)·poly(dA-dT).[13] Our finding that the substitution of inosine for guanosine residues has no effect on the binding of compound **1** to DNA would be compatible with major groove binding, since all drugs which interact with DNA via the minor groove have been found to be sensitive to the presence or absence of the guanine 2-amino group, even if they do not interact with it directly.[7] The only drugs we have found so far whose binding to DNA is not dependent on the placement of the 2-amino group of guanine are bis-naphthalimide derivatives which are believed to interact in the major groove of the double helix.[14] However, the case for major groove binding of **1** is far from proved, and the hypothesis must at present be considered speculative, though binding of some sort into either or both of the helical grooves seems most probable.

DNA cleavage was analyzed by monitoring the conversion of supercoiled plasmid DNA (form I) to the nicked circular molecules (form II) and linear DNA (form III). The tests were performed under aerobic conditions in the presence of 2-mercaptopropionic acid (MPA) as a reducing agent. As shown in Figure 4, compound **1** is able to catalyze oxidative cleavage of DNA. Incubation of the plasmid at 37 °C for 1 h with 50 μM compound **1** causes the complete conversion of form I to the nicked form II. We therefore conclude that the activation of the copper complex leads mainly to single strand cleavage of duplex

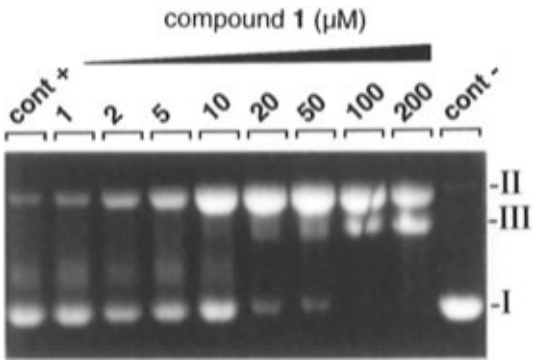

Figure 4. Cleavage of closed circular pUC12 DNA (form I) by compound **1** in the presence of 2-mercaptopropionic acid (MPA) as reducing agent. Forms II and III refer to the nicked and linear DNA forms, respectively. The drug concentration (μM) is indicated at the top of each gel lane. Control lanes marked cont + and cont − refer to the plasmid DNA incubated without drug in the presence and absence of MPA, respectively.

DNA. As the salen concentration increases the probability of double-strand scissions is enhanced once the DNA has undergone a single-strand break. This is manifested in the gel by the appearance of linearized DNA molecules (form III). The reactive species (presumably oxygen-based radicals)[15] have free access to the site of first cleavage. High resolution affinity cleavage studies, to be reported in detail elsewhere (manuscript submitted for publication), reveal that the reaction is essentially non-sequence-specific. The Cu·salen derivative **1** complements the tool box of reagents which can be utilized to produce single-strand cleavage of DNA.

In conclusion, the results suggest that the functionalized salen·Cu^{II} complex **1** studied here induces DNA cleavage via an interaction within one of the grooves of the double helix. Both the peculiar mode of binding to DNA and the DNA-cleaving properties entreat further exploration into the use of metal·salen complexes as tools for investigating DNA structure and for the active interest in developing metal-containing artificial nucleases. The newly introduced butylamino side chain may ultimately allow tailoring of the chelate to facilitate cellular transport and DNA recognition. In particular, it would be interesting to tether the functionalized salen derivative **1** to an oligonucleotide for sequence-specific recognition of DNA via triple-helix formation along the lines already attempted with Mn·porphyrin and Cu·phenanthroline conjugates.[16] Such efforts have now been initiated.

Experimental Section

Synthesis. The purity of all compounds was assessed by TLC, ^{1}H- and ^{13}C-NMR, and by mass spectrometry. Kieselgel 60 (004−0063 mesh) was used for flash chromatography columns. TLC was carried out using silica gel 60F-254 (0.25

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(15) The formation of free-radical species generated from oxygenated solutions of compound **1** in the presence of MPA was monitored by spin-trapping experiments with 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO). ESR signals characteristic of DMPO-OH ($A_N = A_H = 14.9$G) were recorded suggesting the production of superoxide anions $O_2^{\bullet-}$. The postulated mechanism of DNA cleavage by the salen·Cu complex involves reduction of **1**·Cu^{II} to **1**·Cu^{I} which reacts with O_2 to give $O_2^{\bullet-}$ followed by the formation of hydrogen peroxide. H_2O_2 would decompose upon reaction with DNA-bound **1**·Cu^{I} to yield OH$^\bullet$ radicals capable of reacting with the deoxyribose residues in DNA.
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mm thick) precoated UV sensitive plates. Spots were visualized by inspection under visible light or UV at 254 nm. Melting points were determined in a hot plate microscope and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker AM 300 WB. Chemical shifts were reported from tetramethylsilane as an internal reference and are given in δ (ppm) units. IR spectra were obtained using KBr pellets and only the principal sharp peaks are given. ESR spectra were recorded at 77 K, with a maximum modulation amplitude of 8 G in a dual cavity operating in the TE_{104} mode. Samples were frozen in liquid nitrogen into 4 mm diameter cylindrical quartz tubes. The g factor measurements were related to the "strong pitch", $g = 2.0028$. FAB mass spectra were determined on a mass spectrometer arranged in EBE geometry. Samples were bombarded using a beam of xenon with a kinetic energy of 7 keV. The mass spectrometer was operated at 8 kV accelerating voltage with a mass resolution of 3000. IE mass spectra were determined on a quadrupolar spectrometer with a kinetic energy of 70 eV.

N$^\alpha$-*Z*-N$^\epsilon$-BOC-L-lysinamide (3). A solution of N$^\alpha$-*Z*-N$^\epsilon$-BOC-L-lysine (**2**) (5.26 mmol, 2 g), NEt_3 (5.31 mmol, 740 mL), and ethyl choroformate (5.75 mmol, 550 mL) in dry THF (30 mL) was stirred under argon at −10°C for 45 min, 30 mL of ammonia-saturated THF were added, and the mixture was stirred for 1 h at −10 °C and then overnight at room temperature. The solvent was removed by distillation under reduced pressure, and the residue was dissolved in ethyl acetate (150 mL). The organic layer was washed with 1 N Na_2CO_3 (100 mL) and water (100 mL). After drying over Na_2SO_4 and filtration, the solvent was removed by distillation under reduced pressure. Compound **3** was obtained as a white solid (1.7 g; 85%), mp 141−142 °C; $[\alpha]^{25}_D$ −1.75 (c 4 × 10^{-2}, MeOH); IR (KBr, cm^{-1}) ν 3370−3310, 3200, 2965, 1680, 1655, 1535; EIMS 380 $(M + 1)^+$; R_f (MeOH/$CHCl_3$ 20:80) 0.74; ^{1}H-NMR ($CDCl_3$) δ 1.4 (s, 9H), 1.47 (m, 4H), 1.62 (q, 2H), 3.06 (m, 2H), 4.15 (m, 1H), 5.10 (s, 2H), 5.75 (m, 2H), 6.40 (m, 2H); ^{13}C-NMR ($CDCl_3$) δ 22.6 (CH_2), 28.40 (CH_3), 29.43 (CH_2), 40.02 (CH_2), 44.38 (CH_2), 64.64 (CH_2), 77.32 (CH), 78.96 (C_q), 126.88 (CH), 127.23 (CH), 141.41(C_q), 156.33 (C_q). Anal. Calcd for $C_{19}H_{29}N_3O_5$: C, 60.16; H, 7.65; N, 11.08. Found: C, 60.1; H, 7.5; N, 10.9.

(*S*)-1-Cyano-N^1-(benzyloxycarbonyl)-N^5-(*tert*-butyloxycarbonyl)-1,5-diaminopentane (4). A solution of **3** (4.21 mmol, 1.6 g) and NEt_3 (10.04 mmol, 1.4 mL) in THF (20 mL) was stirred under argon at 0 °C. Trifluoroacetic anhydride (4.95 mmol, 0.7 mL) was added dropwise, and the mixture was stirred for 1 h at 0 °C and then overnight at room temperature. The solvent was removed by distillation under reduced pressure, and the residue was dissolved in Et_2O (150 mL). The organic layer was washed in turn with 0.1 N HCl (100 mL), 0.1 N NaOH (100 mL), and water (100 mL). After drying over Na_2SO_4, the solvent was removed by distillation under reduced pressure. Compound **4** was obtained as a white solid (1.26 g; 83%): mp 81−82 °C, $[\alpha]^{25}_D$ −27 (c 4 × 10^{-2}, MeOH), IR (KBr, cm^{-1}) ν 3370, 2965, 1715, 1690, 1540; EIMS 361 $(M + 1)^+$; R_f (MeOH/$CHCl_3$ 20:80) 0.85; ^{1}H-NMR ($CDCl_3$) δ 1.38 (m, 11H), 1.47 (m, 4H), 3.10 (m, 2H), 4.53 (m, 1H), 5.14 (s, 2H), 5.76 (m, 2H), 7.34 (m, 5H); ^{13}C-NMR ($CDCl_3$) δ 21.27 (CH_2), 28.37 (CH_3), 29.38 (CH_2), 32.11 (CH_2), 39.42 (CH_2), 42.65 (CH), 67.63 (CH_2), 79.49 (C_q), 118.61 (C_q), 128.35 (CH), 128.59 (CH), 135.66 (C_q), 156.38 (C_q), 155.39 (C_q). Anal. Calcd for $C_{19}H_{27}N_3O_4$: C, 63.16; H, 7.48; N, 11.63. Found: C, 63.1; H, 7.5; N, 11.5.

(*S*)-6-(*tert*-Butyloxycarbonyl)-1,2,6-triaminohexane (5). A solution of **4** (1.38 mmol, 500 mg) containing Raney Ni (1 g) in MeOH (20 mL) saturated with ammonia was stirred under 50 atm hydrogen at 50 °C for 24 h. The reaction mixture was filtered, and the solvent was removed under reduced pressure to give compound **5** as a pink solid (93%, 0.3 g): mp 46−48 °C; IR (KBr, cm^{-1}) ν 3200−3600, 2800−2900, 1690, 1540, EISM 232 $(M + 1)^+$; R_f (MeOH/$CHCl_3$ 40:60) 0.15; ^{1}H-NMR ($CDCl_3$) δ 1.3−1.6 (m, 15H) 1.88 (m, 2H) 2.7−3.1 (m, 3H) 5.23 (m, 4H) 7.32 (m, 1H); ^{13}C-NMR ($CDCl_3$) δ 22.28 (CH_2), 23.74 (CH_2), 28.43 (CH_3), 29.43 (CH_2), 32.66 (CH_2), 39.78 (CH_2), 50.50 (CH), 79.04 (C_q), 156.25 (C_q). Anal. Calcd for $C_{11}H_{25}N_3O_2$: C, 57.14; H, 10.82; N, 18.18. Found: C, 57.1; H, 10.9; N, 18.2.

(*S*)-N^1,N^2-Bis(salicylidene)-6-(*tert*-butyloxycarbonyl)-1,2,6-triaminohexane Copper Complex (6). A solution of **5** (1.3 mmol, 300 mg) and salicylaldehyde (6.10 mmol, 650 mL) in dry EtOH (25 mL) and cuprous acetate monohydrate (3.05 mmol, 610 mg in 5 mL of water) was refluxed under argon for 4 h. The solvent was distilled off under reduced pressure, and the residue was triturated with CH_2Cl_2. After filtration, the solvent was removed by distillation under reduced pressure. Compound **6** was recrystallized from ethanol/water (1:1) (55%, 0.36 g): mp 135−136 °C; $[\alpha]^{25}_D$ −25 (c 4 × 10^{-4}, MeOH); IR (KBr, cm^{-1}) ν 3400, 3370, 2950, 1715, 1615; MS (FAB+) 501-$(M + 1)^+$, 1002 $(2 \times (M + 1))^+$; R_f (MeOH) 0.00; ESR $A_{||}$ 192.5 G, $g_{||}$ 2.22.

(*S*)-N^1,N^2-Bis(salicylidene)-1,2,6-triaminohexane, Trifluoroacetate, Copper Complex (1). Trifluoroacetic acid (11.41 mmol, 1.01 mL) was added dropwise at room temperature to a solution of **6** (0.19 mmol, 100 mg) in 15 mL of CH_2Cl_2 containing anisole (4.6 μmol, 5 μL). The resulting mixture was stirred for 30 min and then evaporated to dryness. The crude residue was dissolved in ethanol, and the solvent was removed by distillation under reduced pressure. The final compound was recrystallized from EtOH as green solid (61%, 60 mg): mp 158−160 °C; IR (KBr, cm^{-1}) ν 3200, 1620; MS (FAB+) 401(M)$^+$, 801 $(2 \times (M))^+$; R_f (MeOH) 0.00; ESR $A_{||}$ 191.25 G, $g_{||}$ 2.23.

General. Ethidium bromide was purchased from Boehringer (Mannheim, Germany); stock solutions were prepared in water. A 100 mM stock solution of compound **1** was prepared in DMSO since it is poorly soluble in water. This solution was stored at −20°C in the dark and diluted to a working concentration with water. All other chemicals were analytical grade reagents, and all solutions were prepared using doubly deionized, Millipore-filtered water. DNA from calf thymus and the double-stranded polymers poly(dA-dT)·poly(dA-dT), poly(dG-dC)·poly(dG-dC), and poly(dI-dC)·poly(dI-dC) were from Sigma Chemical Co. (La Verpillière, France). Calf thymus DNA was deproteinized with sodium dodecyl sulfate (protein content <0.2%) and all nucleic acids were dialyzed against 1 mM sodium cacodylate buffer pH 6.5.

Viscosity measurements were carried out in a capillary viscometer submerged in a 45 L water bath which was maintained at 25 ± 0.1 °C. Flow times were measured at least in triplicate to an accuracy of ±0.1 s with a stopwatch, and the average time was calculated. The pUC12 plasmid DNA was isolated by a standard sodium dodecyl sulfate−sodium hydroxide lysis procedure and purified by banding twice in CsCl−ethidium bromide gradients. This procedure yields pure covalently closed circular supercoiled DNA suitable for viscosity measurements. Aliquots (1−5 μL) of the test drug solution (1−2 mM) were titrated directly into the viscometer containing 2 mL of a 250 μM solution of the plasmid. After each addition the solutions were carefully mixed with a small flow of air through the dilution bulb of the viscometer and the flow times measured. Experiments were conducted in buffer containing 10 mM Tris-HCl (pH 7.0) and 10 mM NaCl. The system was calibrated using ethidium bromide as a control.[17]

Absorption Spectroscopy. Absorption spectra were recorded on a Perkin-Elmer Lambda 5 spectrophotometer using a 10 mm optical pathlength. Titrations of the drugs with DNA, covering a large range of drug/DNA-phosphate ratios (D/P), were performed by adding aliquots of a concentrated DNA solution to a drug solution at constant ligand concentration (10 μM).

Fluorescence measurements were made using a 10 mm lightpath cuvette in a 0.01 M ionic strength buffer (9.3 mM NaCl, 2 mM Na acetate, 0.1 mM EDTA) using 20 μM DNA or polynucleotide and 2 μM ethidium bromide. The DNA−ethidium complex was excited at 546 nm and the fluorescence measured at 595 nm.[18]

Circular dichroism (CD) measurements were recorded on a Jobin-Yvon CD6 dichrograph interfaced to a microcomputer.

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Solutions of drugs and/or nucleic acids were scanned in 1 cm quartz cuvettes. Measurements were made by progressive addition of DNA or polynucleotide to a pure ligand solution to obtain the desired drug/nucleic acid ratios.

Electric Linear Dichroism (ELD). The theory and practice for measurements of electric linear dichroism have already been the subject of detailed reports.[19] The optical set-up for a high sensitivity T-jump instrument equipped with a Glan polarizer was used under the following conditions: bandwidth 3 nm, sensitivity limit 0.001 in $\Delta A/A$, response time 3 μs. All experiments were conducted at 20 °C with a 10 mm path length Kerr cell having 1.5 mm electrode separation, in 1 mM sodium cacodylate buffer, pH 6.5. The conductivity of the solutions, measured with a Metrohm conductimeter Model E527, ranged from 0.8 to 1.2 mS. The DNA samples were oriented by rectangular electric pulses at 13 kV/cm, and the drug under test was present at 10 μM together with the DNA or polynucleotide at 100 μM, unless otherwise stated. Linear dichroism ΔA is defined as the difference between the absorbance for light polarized parallel ($A_{\parallel}$) and perpendicular ($A_{\perp}$) to the applied field at a given wavelength. The reduced dichroism is $\Delta A/A = (A_{\parallel} - A_{\perp})/A$, where A is the isotropic absorbance of the sample measured in the absence of electric field at the same wavelength and using the same pathlength.

DNA Cleaving Activity. Each reaction mixture contained 4 μL of supercoiled pUC12 DNA (3 μg), 5 μL of compound **1** (2 to 500 μM), and 1 μL of MPA (25 mM) to initiate the reaction. After 1 h incubation at 37 °C, 1 μL of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol in H_2O) was added to each tube and the solution was loaded on to a 0.9% agarose gel. The electrophoresis was carried out for about 2 h at 100 V in TBE buffer (89 mM Tris-borate pH 8.3, 1 mM EDTA). Gels were stained with ethidium bromide (1 μg/μL) and then destained for 30 min in water prior to being photographed under UV light.

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