Synthesis, Crystal Structure, and DNA-Binding Study on the Trinitrate{2,2′-[2,3-naphthylenebis(oxy)]-bis(*N*,*N*-diisopropyl(acetamide))} Gadolinium(III) Complex

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A 2,2′-[2,3-naphthylenebis(oxy)]-bis(*N*,*N*-diisopropyl(acetamide)) ligand (L) and its Gd(III) complex have been prepared and characterized. The crystal and molecular structure of the complex was determined by single-crystal X-ray diffraction. The interactions of complex with calf thymus DNA were investigated by UV–vis, fluorescence and viscosity measurements. Experimental results indicated that the complex can bind to DNA by intercalation modes. Its intrinsic binding constant is 1.03×10^6 M^{-1}.

Key words complex; structure; UV–vis; fluorescence; viscosity; intrinsic constant

Numerous biological experiments have demonstrated that DNA is the primary intracellular target of anticancer drugs due to the interaction between small molecules and DNA, which cause DNA damage in cancer cells, blocking the division of cancer cells and resulting in cell death.[1—3] Of these studies, the interaction of transition metal complexes containing multidentate aromatic ligands, with DNA has gained much attention. This is due to their possible application as new therapeutic agents and their photochemical properties which make them potential probes of DNA structure and conformation.[4—9]

The design of small complexes that bind and react at specific sequences of DNA becomes important. A more complete understanding of how to target DNA sites with specificity will lead not only to novel chemotherapeutics but also to a greatly expand ability for chemists to probe DNA and to develop highly sensitive diagnostic agents.[4]

Transition–metal complexes are being used at the forefront of many of these efforts. Stable, inert complexes containing spectroscopically active metal centers are extremely valuable as probes of biological systems. As both spectroscopic tags and functional models for the active centers of proteins, metal complexes have helped elucidate the mechanisms by which metalloproteins function.[4]

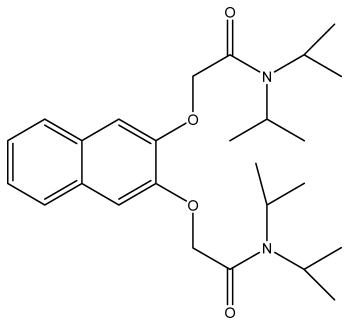
In order to develop new antitumor drugs which specifically target DNA, it is necessary to understand the different binding modes a complex is capable of undertaking. Basically, metal complexes interact with the double helix DNA in either a non-covalent or a covalent way. The former way includes three binding modes: intercalation, groove binding and external static electronic effects. Among these interactions, intercalation is one of the most important DNA binding modes as it invariably leads to cellular degradation. It was reported that the intercalating ability increases with the planarity of ligands.[10,11] Additionally, the coordination geometry and ligand donor atom type also play key roles in determining the binding extent of complexes to DNA.[12,13] The metal ion type and its valence, which are responsible for the geometry of complexes, also affect the intercalating ability of metal complexes to DNA.[14,15]

Amide-based open-chain crown ethers offer many advantages in extraction and analysis of the rare earth ions[16,17] because of their ring-like coordination structure and terminal group effects.[17,18] A series of multi-functional ligands having selective ability to coordinate lanthanides ions have been designed. People reported the novel luminescence properties of these lanthanide complexes. However, up to now, the interactions with DNA of the lanthanide complexes are not reported by any references. This aroused our interest in the synthesis a ligand, 2,2′-[2,3-naphthylenebis(oxy)]-bis(*N*,*N*-diisopropyl-(acetamide)) (L) (Chart 1) and its gadolinium(III) nitrate complex with a view to evaluating the binding behaviors of it with CT-DNA.

Experimental

Instrumentation Carbon, hydrogen and nitrogen were performed using a Vario EL elemental analyzer. Infrared spectra (4000—400 cm^{-1}) were obtained with KBr discs on a Therrno Mattson FTIR spectrometer. The ultraviolet spectra were recorded on a Varian Cary 100 Conc spectrophotometer. ^{1}H-NMR spectra were measured on a Varian Mercury Plus 200 BB, using TMS as an internal standard in $CDCl_3$. Fluorescence measurements were made on a Hitachic RF-4500 spectrofluorophotometer equipped with quartz cuvettes of 1 cm path length at room temperature.

Materials and Methods All material and solvents employed in this study were analytical reagents. The rare earth(III) nitrates were derived from their oxide (99.9%) acquired from Nong Hua (PR China). EDTA-Fe(II) and KH_2PO_4–K_2HPO_4 buffers were prepared by demonized water. All the experiments involving the interaction of the complex with DNA were carried out in demonized water buffer with tris(hydroxymethyl) aminomethane (Tris, 5 mM) and sodium chloride (50 mM) and adjusted to pH 7.1 with hydrochloric acid. Solution of ctDNA gave ratios of UV absorbance at 260 and 280 nm of about 1.8—1.9, indicating that the DNA was sufficiently free of protein. The DNA concentration per nucleotide was determined spectrophotometri-



2,2′-[2,3-Naphtylenebis(oxy)]-bis(*N*,*N*-diisopropyl(acetamide)) (L).

Chart 1

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Table 1. Elemental Analytical and Molar Conductance Data for the Complex

Complex	C (%)	H (%)	N (%)	Eu (%)	Λ ($S\,cm^2\,mol^{-1}$)
$Gd(NO_3)_3L$	40.01 (39.74)	4.82 (4.87)	8.99 (8.91)	19.52 (19.47)	65

cally by employing an extinction coefficient of $6600\,M^{-1}\,cm^{-1}$ at 260 nm.[19] The complex was dissolved in a mixture solvent of 10% MeCN and 90% Tris–HCl buffer (5 mM Tris–HCl, 50 mM NaCl, pH 7.1) at concentration 1.0×10^{-5} M. Absorption titration experiment was performed by maintaining constant concentration (10 μM) of compounds and varying the concentration of nucleic acid. While measuring the absorption spectra, equal amount of DNA was added to both compound solution and the reference solution to eliminate the absorbance of DNA itself. The intrinsic binding constants K_b of the complex was obtained by the luminescence titration method.[20] Fixed amounts of the compound was titrated with increasing amounts of DNA, over a range of DNA concentrations from 2.5 to 15 μM. An excitation wavelength of 321 nm was used, and the total emission intensity was monitored at 450 nm. The concentration of the bound compounds was calculated using Eq. 1:

$$C_b=C_t[(F-F^0)/(F^{max}-F^0)] \quad (1)$$

where C_t is the total compound concentration, F is the observed fluorescence emission intensity at given DNA concentration, F^0 is the intensity in the absence of DNA, and F^{max} is the fluorescence of the totally bound compound. The concentration of the free compound C_f is equal to C_t-C_b. Binding data were cast into the form of a Scathchard plot[21] of r/C_f *vs.* r, where r isbinding ratio C_b/[DNA]t. All experiments were conducted at 20 °C in a buffer containing 5 mM Tris–HCl (pH 7.1) and 50 mM NaCl concentrations. Further support for the complex binding to DNA by intercalation mode is given through the emission quenching experiment. EB is a highly sensitive fluorescent probe for the structure and quantitative determination of DNA.[22,23] Under appropriate conditions, EB causes a significant increase in the fluorescence of DNA due to the increase in separation of base pairs at intercalation sites. After adding the compound, which can react with DNA, the fluorescence of DNA-EB system cause more pronounced changes, and *vice versa*.[24] So EB is a common fluorescent probe for DNA structure and has been employed in examinations of the mode and process of compound binding to DNA.[25] The complex (10—60 mM) were added dropwise to 2-ml solution composed of DNA (10 μM) and EB (0.33 μM) (at saturating binding levels[26]), respectively.

According to the classical Stern–Volmer equation[27]:

$$F_0/F=K_q[Q]+1$$

Where F_0 is the emission intensity in the absence of quencher, F the emission intensity in the presence of quencher, K_q the quenching constant, and [Q] the quencher concentration. The shape of Stern–Volmer plots can be used to characterize the quenching as being predominantly dynamic or static. Plots of F_0/F *versus* [Q] appear to be linear and K_q depends on temperature.

Viscosity experiments were conducted on an Ubbdlodhe viscometer, immersed in a thermostatic water-bath maintained to 25.0 °C. Titrations were performed for the Gd(III) complex and the ligand (0.5—3 μM), and each compound was introduced into a DNA solution (5 μM) present in the viscometer. Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* the ratio of the concentration of the compound and DNA, where η is the viscosity of DNA in the presence of compound and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA containing solution corrected from the flow time of buffer alone (t_0), $\eta=t-t_0$.[28,29]

Preparations of the Ligand Anhydrous K_2CO_3 (5.66 g, 41 mmol) was added slowly to the DMF solution of 2,3-dihydroxy-naphthalene (3.1 g, 20 mmol) at 100 °C. An hour later, a solution of *N*,*N*-diisopropyl chloroacetamide (7.14 g, 40 mmol) in 10 ml DMF was added dropwise and slowly to the mixture. The reaction mixture was stirred for 10 h. Fifty milliliter water was poured and extracted by $CHCl_3$ (3×40 ml). Organic phase combined was evaporated in vacuum. The crude product was chromatographed on silica gel to afford ligand L as a white solid (4.8 g), yield: 80%. ^{1}H-NMR: (200 MHz $CDCl_3$) δ: 7.29—7.69 (m, 6H); 4.75 (s, 4H; 2O–CH_2–C(O)); 3.42 (q, 2H; 2N–CH(R)$_2$, J=9.6 Hz); 1.40 (d, 12H; 2R–(CH_2)$_2$, J=9.9 Hz) IR: ν 1661 (C=O), 1174 (Ar–O–C), Analytical data, Calcd for L: C, 70.85; H, 8.45; N, 6.38. Found: C, 70.56; H, 8.65; N, 6.33%.

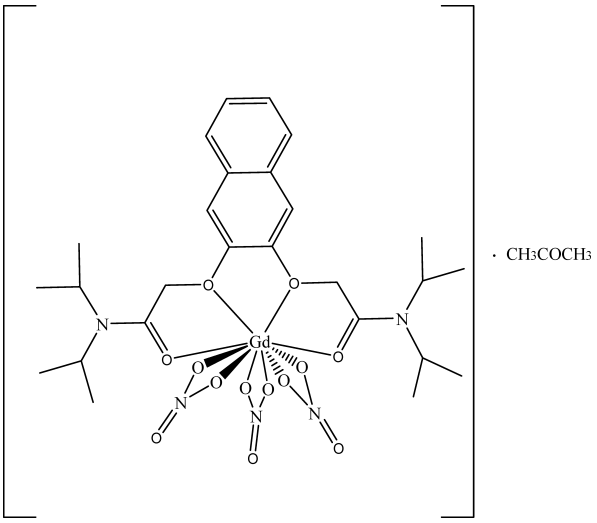

Fig. 1. Chemical Structure

Synthesis of the Complex An ethyl acetate solution of $Gd(NO_3)_3\cdot6H_2O$ (0.1 mmol) was added dropwise to a solution of the ligand (0.1 mmol) in the ethyl acetate (20 ml). The mixture was stirred for 4 h and white precipitate formed. The precipitate was collected and washed three times with ethyl acetate. Further drying in vacuum afforded a pale white powder, yield: 75%.

The crystals of the complex were recrystallized from acetone 1 d later.

Crystallography The X-ray data were collected on a diffractometer equipped with graphite-monochromated Mo$K\alpha$ radiation (λ=0.71073 Å) at 298(2) K. The structure was solved by direct methods and refined by full-matrix least-squares techniques on F^2 with the program SHELXTL-97. All of the non-hydrogen atoms were refined anisotropically. The hydrogen atoms were assigned with common isotropic displacement factors and included in the final refinement by using geometrical restrains. The final agreement factor value was R=0.0483.

Result and Discussion

Analytical data for the complex (Table 1) conform to a 1 : 3 : 1 metal-to-nitrate-to-L stoichiometry (Fig. 1). The complex is soluble in DMSO, DMF, MeCN, methanol and acetone, and slightly soluble in ethanol, ethyl acetate, chloroform. The molar conductance values of the complex in methanol (Table 1) indicate the presence of a non-electrolyte.[31]

IR Spectrum The IR spectrum of free L shows bands at 1660 cm^{-1} and 1174 cm^{-1} which may be assigned to O(C=O), O(C–O–C) respectively. In the IR spectra of the lanthanide complex, these bands shift by 43 cm^{-1}, and 16 cm^{-1} toward lower wavenumbers, thus indicating that the C=O, ether O atoms take part in coordination to the metal ion.

The absorption bands assigned to the coordinated nitrates were observed at 1483, 1308 and 817 cm^{-1} for the complex. It indicates that coordinated nitrate groups in the complex are bidentate in agreement with the result of the conductivity experiments.

Crystallography The crystal data and experimental parameters are given in Table 2. The selected bond lengths and

Table 2. Crystal and Experimental Data

CCDC No: 285250
Empirical formula: $C_{29}H_{44}GdN_5O_{14}$
Formula weight=843.94
Wavelength=0.71073 Å
Crystal system: monoclinic
Space group: $P2_1/c$
a=15.083(3) Å
b=17.890(4) Å
c=14.457(3) Å
β=99.29(3)°
Volume=3849.6(14) Å^3
Z=4
Dx=1.456 g/cm^3
Absorption coefficient: 1.789 mm^{-1}
F(000)=1716
Crystal size: 0.48×0.39×0.13 mm
Theta range for data collection: 1.78 to 25.01°
Reflections collected/unique: 19740/6710 [R(int.)=0.0713]
$2\theta_{max}$=50.02° with Mo$K\alpha$
Absorption correction: semi-empirical from equivalents
Max. and min. tranGdission: 0.8008 and 0.4806
Goodness-of-fit on F^2: 1.010
Final R indices [$I>2\sigma(I)$]: $R1$=0.0483, $wR2$=0.1094
$(\Delta\rho)_{min}$=−0.768 e Å^{-3}
$(\Delta\rho)_{max}$=1.271 e Å^{-3}
$(\Delta/\sigma)_{max}$=0.002
No. of reflections used=4231
Measurement: Bruker SMART CCD
Program system: SHELXL-97
Structure determination: direct method
Refinement: full matrix least-squares on F^2

Table 3. Selected Bond Distance and Angle (Å,°)

Gd(1)–O(3)	2.337(5)	Gd(1)–O(1)	2.323(5)
Gd(1)–O(2)	2.606(4)	Gd(1)–O(4)	2.546(5)
O(3)–Gd(1)–O(1)	149.9(2)	O(3)–Gd(1)–O(2)	118.09(16)
O(1)–Gd(1)–O(2)	61.41(16)	O(3)–Gd(1)–O(4)	62.76(15)
O(1)–Gd(1)–O(4)	120.62(16)	O(2)–Gd(1)–O(4)	59.93(14)

bond angles are given in Table 3. An ORTEP drawing of the complex is illustrated in Fig. 2.

The Gd(III) atom is ten-coordinated by four O atoms from the tetradentate 2,2′-[2,3-naphthylenebis(oxy)]-bis(*N*,*N*-diisopropyl(acetamide)) ligand and three nitrate anions in a distorted bicapped dodecahedron. One acetone molecule is in the outerspace. The bond length are within normal ranges. Four O-atoms of L are not quite coplanar. Their deviation from the least-squares plane of O1/O2/O3/O4 is in the range of 0.0652(18)—0.1154(32) Å. The Gd atom lies out of this plane by 0.5763(37) Å. The average distance between the Gd(III) ion and the coordination oxygen atom is 2.453(5) Å. The Gd–O(C=O) distance [mean 2.330(5) Å] are significantly shorter than the Gd–O(Ar–O–C) distance [mean 2.576(5) Å], which suggests that the Gd–O(C=O) bond is stronger than the Gd–O(Ar–O–C) bond.

Electronic Absorption Titration It is a general observation that a red shift and hypochromism in the absorption spectra accompany the binding of intercalative molecules to DNA. The extent of spectral change is related to the strength of binding and the spectra for intercalators are more perturbed than those for groove binders.[32] The absorption spectra of the complex in the absence or presence of CT-DNA (at a constant concentration of complex) are given in Fig. 3. Addition of increasing amounts of CT-DNA results in slight red shifts of about 2 nm and notable hypochromicities were observed. The complex at 230 nm exhibited hypochromism of about 83.1%. These results suggest an intimate association of the compound with DNA and it is also likely that compound bind to the helix by intercalation.

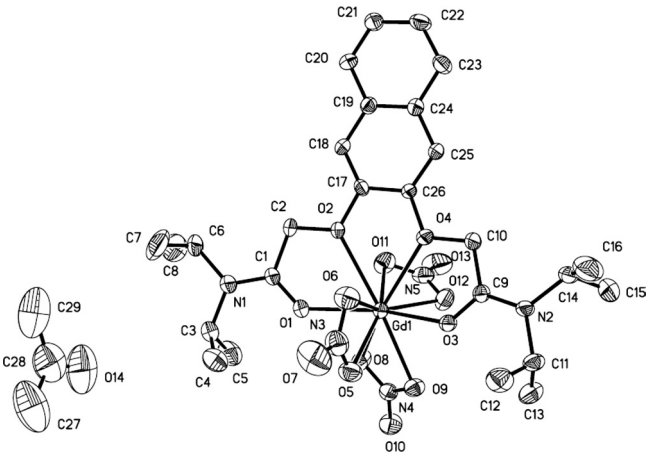

Fig. 2. Molecular Structure of the Gd(III) Complex Showing 30% Probability Displacement Ellipsoids and the Atom-Numbering Scheme

H atoms attached to C atoms have been omitted for clarity.

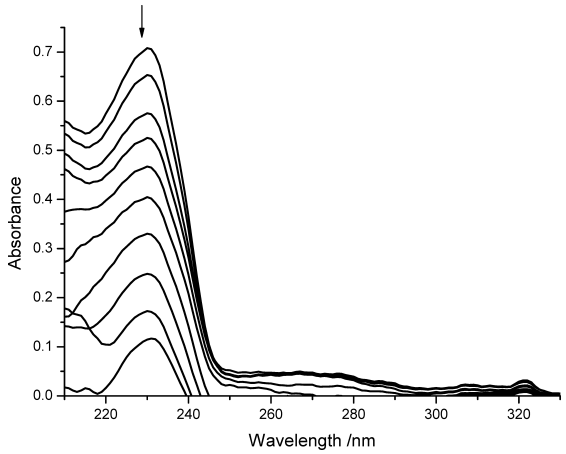

Fig. 3. Electronic Spectra of Gd(III) Complex (10 μM) in the Presence of Increasing Amounts of CT-DNA

[DNA]=0—90 μM. Arrow shows the absorbance changes upon increasing DNA concentration.

Viscosity Measurements To further clarify the interactions between the study compounds and DNA, viscosity measurements were carried out. Hydrodynamic measurements that are sensitive to length change (*i.e.* viscosity and sedimentation) are regarded as the least ambiguous and the most critical tests of binding in solution in the absence of crystallographic structural data.[33] A classical intercalation model results in lengthening the DNA helix as base pairs were separated to accommodate the binding ligand, leading to the increase of DNA viscosity. In contrast, a partial and/or nonclassical intercalation of ligand could bend (or kink) the DNA helix, reducing its effective length and concomitantly its viscosity.[33,34] The effects of the ligand and complex on the viscosity of ct-DNA at 25.0 °C are shown in Fig. 4. Viscosity experimental results clearly show that both the compounds can intercalate between adjacent DNA base pairs,

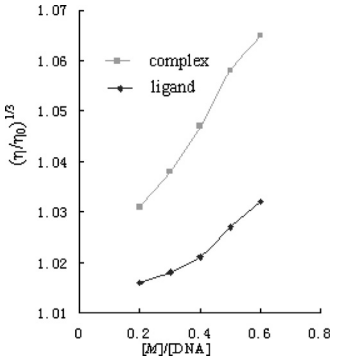

Fig. 4. Effect of Increasing Amounts of Gd(III) Complex and Ligand on the Relative Viscosity of Caltf Thymus DNA at 25.0 °C

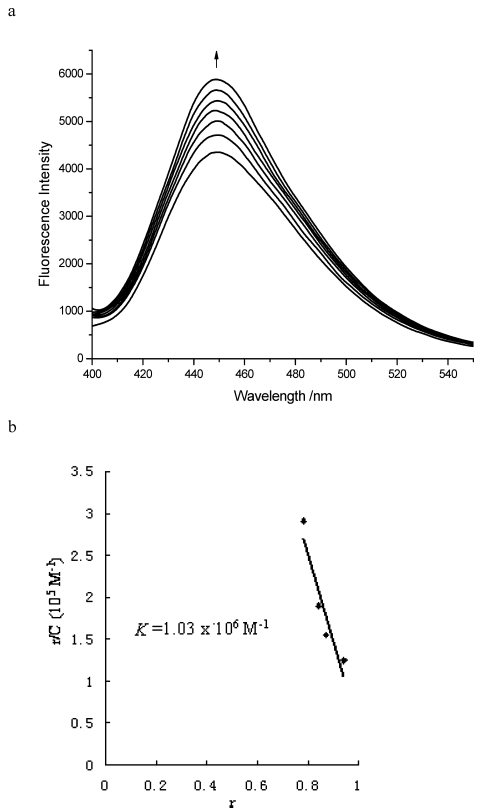

Fig. 5. The Emission Enhancement Spectra of Gd(III) Complex (10 μM) in the Presnce of 0, 2.5, 5, 7.5, 10, 12.5, 15 μM CT-DNA

Arrow shows the emission intensities upon increasing DNA concentration. Inset: Scatchard plot of the flourescence titration data of Gd(III) complex, $K=1.03\times10^6$ M^{-1}.

causing an extension in the helix, and thus increase the viscosity of DNA; and that the Gd(III) complex can intercalate more strongly and deeply than the free ligand, leading to the greater increase in viscosity of the DNA with an increasing concentration of complex.

Fluorescence Spectra The enhancements in the emission intensity of complex with increasing DNA concentration are shown in Fig. 5. In the absence of DNA, complex emit weak luminescence in Tris buffer at ambient temperature, with a maximum appearing at 450 nm. The emission intensity of complex increases in the presence of ct-DNA. These phenomenon are related to the extent to which the complex get into a hydrophobic environment inside the DNA and avoid the quenching effect of solvent water molecules. The

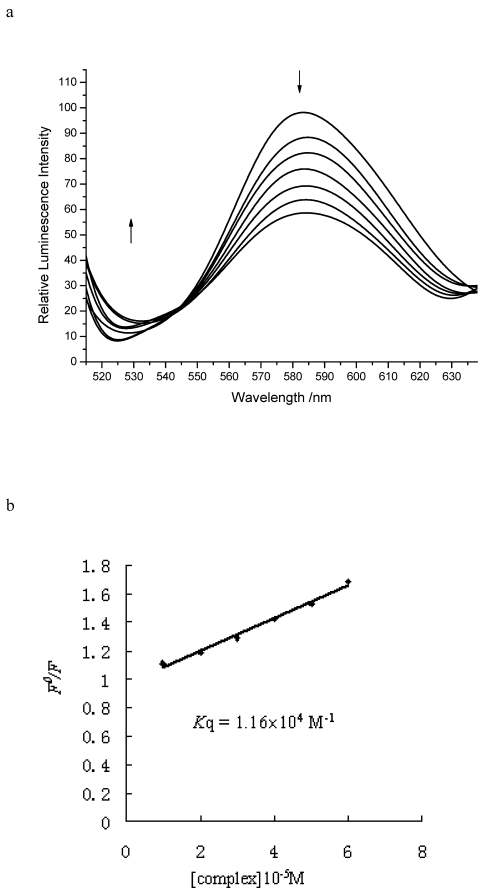

Fig. 6. The Emission Spectra of DNA–EB System (10 μM and 0.32 μM EB), λ_{ex}=500 nm, λ_{em}=515.0—650.0 nm, in the Presence of 0, 10, 20, 30, 40, 50, 60 μM Gd(III) Complex

Arrow shows the emission intensities changes upon increasing complex concentration. Inset: Stern–Volumer plot of the flourescence titration data of complex, $K_q=1.16\times10^4$ M^{-1}.

binding of complex to DNA leading to a marked increased in emission intensity also agrees with those observed for other intercalators.[19] According to the Scathchard equation, a plot of r/C_f *vs.* r gave the binding constant 1.03×10^6 M^{-1} from the fluorescence data for complex. The fluorescence quenching curves of EB bound to DNA by the complex are shown in Fig. 6. The quenching plots illustrate that the quenching of EB bound to DNA by complex are in good agreement with the linear Stern–Volmer equation, which also proves that they bind to DNA. In the plots of F^0/F *versus* [Q], K_q is given by the ratio of the slope to intercep. The K_q values for the complex is 1.16×10^4 M^{-1}. There is a moderate interaction.[35] Since these changes indicate only one kind of quenching process, it may be concluded that the Gd(III) complex bind DNA may be intercalation mode.

Conclusion

Taken together, a novel ligand (L) and its Gd(III) complex have been prepared and full characterized. Single crystal has been examined by X-ray crystal diffraction. The interaction of complex with ct-DNA was investigated by absorption, fluorescence and viscosity measurements. The intrinsic binding of complex with ct-DNA is 1.03×10^6 M^{-1}. Three methods indicate that the complex may intercalate into DNA.

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Supplementary Material Crystallographic data for the structure analysis have been deposited with the Cambridge Crystallographic Data Center, CCDC No. 285250. Copies of this information may be obtained free of charge from the director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (E-mail: linstead@ccdc.cam.ac.uk; deposit@ccdc.cam.ac.uk; Direct Line: +44-1223-762910; Tel: 44-1223-336408; Fax: +44-1223-336033).

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