Bioorganic & Medicinal Chemistry 20 (2012) 7112–7118

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and DNA interaction of ethylenediamine platinum(II) complexes linked to DNA intercalants

Katerina Duskova, Sara Sierra, María-José Fernández, Lourdes Gude, Antonio Lorente *

Departamento de Química Orgánica, Universidad de Alcalá, 28871 Alcalá de Henares, Madrid, Spain

ARTICLE INFO

Article history:
Received 10 August 2012
Revised 26 September 2012
Accepted 27 September 2012
Available online 17 October 2012

Keywords:
Platinum(II) complexes
DNA intercalants
DNA interactions

ABSTRACT

A series of ethylenediamine platinum(II) complexes connected through semi-rigid chains of 1,2-bis(4-pyridyl)ethane to DNA intercalating subunits (naphthalene, anthracene or phenazine) has been synthesized, and their interactions with calf thymus (CT) DNA have been evaluated by viscometric titrations and equilibrium dialysis experiments. The parent ligands that contain anthracene or phenazine chromophores showed a monointercalative mode of DNA interaction (especially the anthracene derivative), with apparent association constants in the order of $10^4\ M^{-1}$. The corresponding platinum(II) complexes bind CT DNA through bisintercalation, as established by the significant increase of DNA contour length inferred from viscosity measurements, and the association constants are in the order of $10^5\ M^{-1}$. The naphthalene derivatives, however, exhibit a mixed mode of interaction, which suggests a partial contribution of both intercalation and groove binding for the ligand, and monointercalation in the case of the platinum(II) complex. Competition dialysis experiments carried out on the intercalative compounds have revealed a moderate selectivity towards GC DNA sequences for the derivatives containing the anthracene chromophore.

1. Introduction

Small molecules can interact with nucleic acids in different ways: groove binding, intercalation between the DNA or the RNA base pairs, covalent binding, electrostatic interactions, etc.[1] As a result of these interactions, both the structure and function of nucleic acids can be modified and, subsequently, many compounds that bind DNA have found relevant applications in therapy, e.g. as antitumor drugs.[2] Typical DNA intercalation does not affect Watson–Crick base pairing, but notably distorts its regular helical conformation, unwinding the helix at the binding site and thus leading to an interference with the action of DNA binding enzymes, such as DNA topoisomerases and DNA polymerases. DNA topoisomerase interference significantly affects the degree of DNA unwinding, whereas interference with the polymerases may lead to inhibition of DNA extension, proofreading and repair.

Among the family of compounds that can recognize and bind DNA by intercalation, DNA bisintercalants are interesting compounds, because they usually present higher affinity and selectivity for DNA and much slower dissociation kinetics than their monointercalant counterparts.[3] However, many DNA bisintercalants containing flexible linkers between the intercalative subunits hardly represent an improvement in affinity or sequence selectivity, probably due to the existence of an auto-stacking process between the intercalating moieties.[4,5] On the contrary, some natural antibiotics, such as echinomycin, display the two DNA intercalating moieties connected through rigid linkers, which makes them high-affinity DNA binders that resemble molecular staples for duplex DNA.[6,7]

In this paper, we describe the synthesis and DNA interactions of a series of potential DNA bisintercalating compounds containing naphthalene, anthracene or phenazine subunits as chromophores, connected through semi-rigid chains of 1,2-bis(4-pyridyl)ethane, and assembled through an ethylenediamineplatinum(II) moiety. Ethylenediamine platinum(II) complexes and other platinum(II) complexes bound to DNA intercalants have received much attention over the years, mainly due to their interesting DNA binding properties and, in some cases, relevant biological activities.[8] Intercalants with positively charged groups attached typically bind to DNA with the positive charges facing the minor groove. In the case of the compounds reported in this paper, the connecting chains tethered to the two planar chromophores have been designed taking into account that pyridinium groups can favor DNA binding through interactions between the cationic nitrogens and the α hydrogens with the phosphate backbone,[9,10] besides the favorable interactions with adenine N-3 or with thymine C(2)=O. On the other hand, a platinum moiety bound to an intercalant may lead to disposition of the platinum moiety in the minor groove.[11] Thus, additional interactions between amino groups in the ethylenediamine platinum(II) subunit and the phosphate backbone might also favor DNA binding.[12] Furthermore, the chosen spacer provides sufficient solubility in aqueous solutions to study

* Corresponding author. Fax: + 34 91 8854686.
E-mail address: antonio.lorente@uah.es (A. Lorente).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.09.055

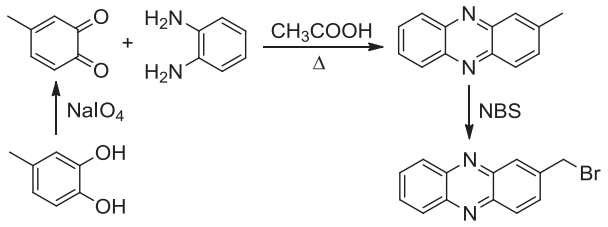
DNA–ligand interactions. Finally, as the distance between chromophores, as measured by CPK models, is approximately 16 Å, a DNA bisintercalation phenomenon for these platinum(II) complexes following the nearest neighbor-exclusion principle would be expected.

2. Results and discussion

2.1. Synthesis

The general synthetic route for the preparation of compounds **1**–**3** (Scheme 1) involved the reaction of 1,2-bis(4-pyridyl)ethane with an appropriate halogen derivative of 2-methylnaphthalene, 9-methylanthracene or 2-methylphenazine, followed by reaction with ethylenediaminedichloroplatinum(II) to afford the metal complexes **4**–**6** in 22 to 61% yield. For compounds containing phenazine (**3** and **6**), the precursor 2-methylphenazine was previously prepared following a reported procedure.[13] This route consisted of a condensation reaction between a diketone, in this case a methyl orthoquinone, and a 1,2-diamino compound, such as *o*-phenylenediamine (Scheme 2). First, the *o*-quinone was prepared by oxidation of 4-methylcatechol with $NaIO_4$, immediately followed by reaction with *o*-phenylenediamine, giving rise to 2-methylphenazine, which was then transformed into 2-bromomethylphenazine using standard NBS bromination conditions (Section 4).

Ligands **1**–**3** were prepared by reacting the corresponding halogen derivative with threefold excess of 1,2-bis(4-pyridyl)ethane for 24 h in acetone at room temperature, with yields ranging from 74% to 80%. Afterward, complexes **4**–**6** were obtained by reaction of the corresponding ligand with a solution of ethylenediaminedichloroplatinum(II) in refluxing water for 6 h. After purification by column chromatography, a subsequent treatment of the complexes in water with ammonium hexafluorophosphate (NH_4PF_6) was required to precipitate the complexes, and to remove the NH_4Cl salt employed in the purification step. A second ion-exchange step was carried out to afford the final, water-soluble products, as the bromide salts to be used in DNA interaction studies.

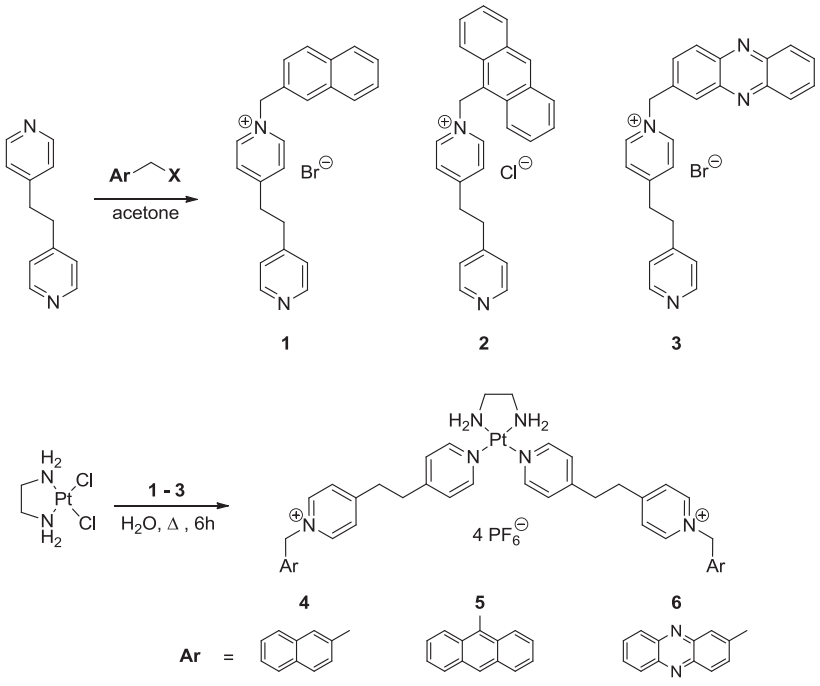


Scheme 2. Preparation of 2-bromomethylphenazine. Reagents and conditions: $NaIO_4$, H_2O, rt; CH_3COOH, reflux, 4 h, 24% (2 steps); NBS, benzoyl peroxide, CCl_4, reflux, 72 h, 53%.

2.2. DNA interaction

2.2.1. Viscometric measurements

After the synthesis of compounds **1–6,** our next goal was to obtain direct evidence of DNA interaction and, specifically, we wanted to discern between DNA intercalation and groove binding modes. It is well established that viscosity measurements provide a simple way for determining the binding modes of DNA ligands.[14] When a DNA intercalant inserts itself between adjacent base pairs within duplex DNA, the helix must unwind to create a gap to accommodate the incoming ligand. This unwinding process increases the overall contour length of the helix and, as a result, the DNA solution becomes more viscous. Because groove binding compounds do not require insertion between the base pairs, DNA lengthening does not occur, and in these circumstances viscosity is not significantly altered. Thus, viscometric experiments can be readily employed to distinguish between different non-covalent DNA binding modes. According to the theory of Cohen and Eisenberg,[15] from gradual titration of DNA solutions with the compounds of interest, linear plots of the cubed root of the relative DNA viscosity $(\eta/\eta_o)^{1/3}$ versus the molar ratio of bound ligand to DNA nucleotide (r) can be easily obtained. The slopes values in these plots correlate well with the DNA-ligand binding modes. Thus, groove binding compounds usually display a slope close to 0.0, whereas classical monointercalants result in a slope close to



Scheme 1. Target compounds (1–6). Reagents and conditions: 1,2-bis(4-pyridyl)ethane, $ArCH_2X$, rt, 24 h, acetone, 74–78%; $PtCl_2(en)_2$, water, reflux, 6 h, 22–61%.

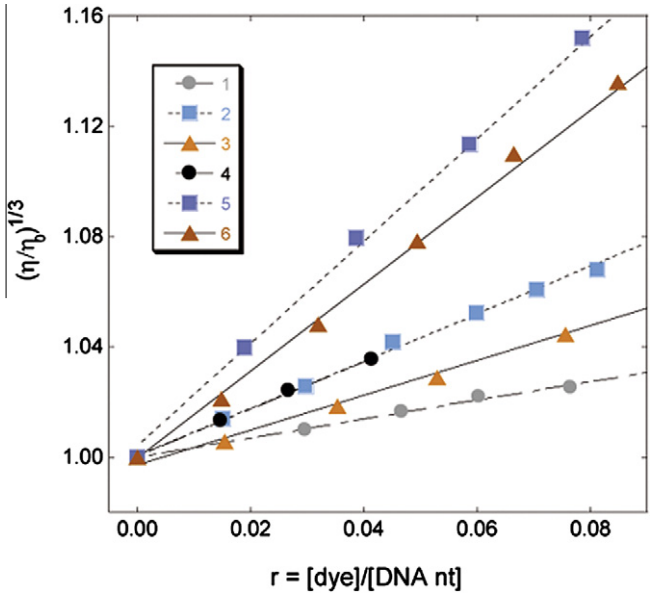


Figure 1. Viscometric measurement experiments of calf thymus DNA and compounds **1**–**6** at 25 °C (10 mM sodium phosphate buffer, pH 7.2). Assays were performed at least in duplicate. The averaged slopes are: **(1)** 0.31 ± 0.04, **(2)** 0.84 ± 0.03, **(3)** 0.65 ± 0.02, **(4)** 0.88 ± 0.06, **(5)** 1.85 ± 0.11 and **(6)** 1.59 ± 0.02.

1.0.[15] In the case of bisintercalation, a slope of approximately twice that the one observed for a monointercalant is usually expected.[3] In practice, slope values reported in the literature typically lie in the range 0.8–1.5 for DNA monointercalants[16] and 1.3–2.3 for DNA bisintercalants.[16c,17]

Viscometric measurements were carried out at 25 ± 0.01 °C by adding aliquots of compounds **1**–**6** to the DNA solutions of CT DNA, in 10 mM sodium phosphate buffer (pH 7.2). Flow times were recorded in the presence (η) and in the absence (η_o) of dye. Concentrations of DNA were kept constant in experiments with compounds **1**–**3,** and slightly variable (with the percentage of dilution kept below 5%) in the case of compounds **4**–**6**, because the platinum(II) complexes were found to precipitate at relatively high concentrations, if previously mixed with DNA. Parallel experiments performed with ligands **1**–**3** under slightly variable DNA concentration did not show significant differences in the slope values obtained, in comparison to experiments at constant DNA concentration. The viscosity data for compounds **1**–**6** were plotted as $(\eta\eta_o)^{1/3}$ versus r, as shown in Figure 1.

The tested compounds demonstrated a linear $(\eta\eta_o)^{1/3}$ versus r correlation in the typical *r* range used in these experiments, with the exception of compound **4**, the naphthalene platinum(II) complex, for which no linearity was observed at *r* values $\geqslant$0.05. In the case of the ligands **1**–**3**, the slopes obtained from viscosity plots were 0.31 ± 0.04, 0.84 ± 0.03, and 0.65 ± 0.02, respectively. Clearly, compounds **1** and **3** both interact with CT DNA with a mixed binding mode groove binding and intercalation, with a more significant contribution of intercalation for compound **3**. In the case of compound **2**, containing the anthracene chromophore, the results point towards a monointercalative DNA binding mode.

For platinum(II) complexes **4**–**6**, the slopes obtained from viscosity plots were 0.88 ± 0.06, 1.85 ± 0.11, and 1.59 ± 0.02, respectively. In this case, it is evident that compounds **5** and **6** both interact with CT DNA as bifunctional intercalants, unlike compound **4**, which behavior suggests that it binds DNA through monointercalation.

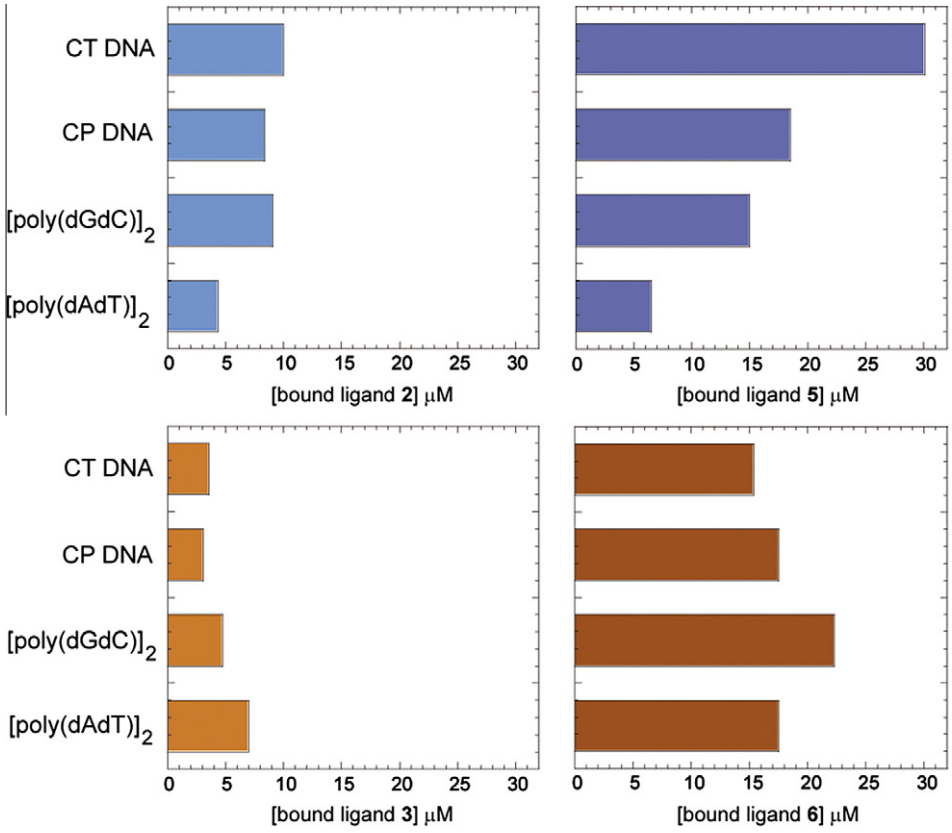


Figure 2. The bar graphs indicate the concentrations of bound ligands **2** (light blue), **3** (orange), **5** (dark blue) and **6** (brown) detected after a 24 h equilibration in competition binding dialysis studies of four DNA sequences. The abbreviations CT DNA, and CP DNA correspond to *Calf thymus*, and *Clostridium perfringens* DNA, respectively.

2.2.2. Competition dialysis

After the establishment of the DNA binding mode for the synthesized compounds, we were interested in investigating whether the studied compounds bind DNA with sequence selectivity. To this end, we employed competition equilibrium dialysis, a technique developed by the Chaires group[18–20] that allows a relative fast screening of multiple DNA structures binding to small-molecule ligands. The method also allows the determination of apparent binding constants. It is based on the fundamental thermodynamic principle of equilibrium dialysis[21] and, in particular, it represents an extension of the Crothers competition dialysis method.[22]

The results obtained for compounds **2**, **3**, **5** and **6** (naphthalene derivatives were not included in the experiments, due to overlap of their UV absorption spectra at DNA absorption maximum) are depicted in Figure 2. It represents a bar graph showing the ligand concentrations bound to *Calf thymus* (CT) DNA and to *Clostridium perfringens* (CP) DNA and to the synthetic, double-helical polynucleotides $[poly(dGdC)]_2$ and $[poly(dAdT)]_2$. The results were obtained following the protocol described by Chaires, with some minor modifications: 2.5 μM solutions of ligands **2**, **3**, **5** and **6** (in the dialysate solution) were equilibrated with 75 μM bp of nucleic acid (in each sample dialysis unit) for 24 h. At the end of the equilibration period, sodium dodecyl sulfate (SDS) was added to the dialysis solutions, and UV–visible spectra were recorded in order to determine the concentrations of free and DNA-bound ligands. The amount of the DNA-bound ligand was averaged over three trials. The competition dialysis data were then used to calculate the apparent association constants of ligands **2**, **3**, **5** and **6**, given by the equation $K_{app} = C_b/(C_f)(S_{total}-C_b)$,[20] where C_b is the amount of ligand bound, C_f is the free ligand concentration and S_{total} = 75 μM bp (Table 1).

Figure 2 and Table 1 show that, for all DNA sequences studied, compounds **2** and **3** have less affinity for DNA than the corresponding platinum(II) complexes and, in the case of the phenazine derivatives (compounds **3** and **6**) this difference is more pronounced (about a fivefold increase in DNA affinity for the metal complex). However, the increase in affinity between the parent ligands and the platinum(II) complexes is modest, and it does not correspond to the expected one considering the difference between monointercalative and bisintercalative mechanisms, and the existence of certain cooperativity in DNA binding. This seems to indicate that the presence of the ethylenediamine platinum(II) moiety may have a significant effect, such as conditioning the binding geometry of the semi-rigid 1,2-bis(4-pyridyl)ethane linkers along the DNA grooves.

Regarding selectivity, nor the ligand or the platinum(II) complex of the phenazine derivatives exhibited a clear preference to bind the GC- or AT-rich DNA sequences, whereas the anthracene compound **2** did demonstrate a slight preference to bind GC DNA sequences, which was notably accentuated in the case of the platinum(II) complex, compound **5**. This compound clearly shows a preference for GC base pairs, as exemplified by an increase in levels of binding to CT DNA (42% GC, $K_{app} = 2.68 \times 10^5\ M^{-1}$) relative to CP DNA (31% GC; $K_{app} = 1.31 \times 10^5\ M^{-1}$) and to $[poly(dGdC)]_2$ ($K_{app} = 1.00 \times 10^5\ M^{-1}$) relative to $[poly(dAdT)]_2$ ($K_{app} = 0.38 \times 10^5\ M^{-1}$).

3. Conclusions

In summary, we have reported the synthesis of a family of compounds based on ethylenediamine platinum(II) complexes connected through semi-rigid chains of 1,2-bis(4-pyridyl)ethane to DNA intercalating subunits (naphthalene, anthracene or phenazine). The synthetic procedures described herein allow an easy and efficient preparation of these multifunctional compounds in acceptable yields. Moreover, the interactions of the parent ligands and their platinum(II) complexes with DNA have been studied by viscometric titrations and competition equilibrium dialysis assays. Anthracene and phenazine derivatives have proven, in general, to be more efficacious than the naphthalene compounds as DNA intercalating agents, which was expected based on their increased planar aromatic surface. However, a significant difference in DNA binding between the phenazine and anthracene metal complexes has been observed where, despite the subtle structural difference among these compounds (replacement of the nitrogens atoms at 9,10 positions of the aromatic ring for carbon atoms), there is a considerable increase in DNA sequence selectivity in the anthracene-containing compound. As a general trend, the parent ligands show monointercalative modes of DNA interaction, especially in the case of the anthracene derivative, whereas the platinum(II) complexes recognize DNA via bisintercalative interactions.

Regarding the potential applications of these molecules, the platinum(II) complexes described in this article may serve as useful probes for the study of ligand–DNA interactions, and they may constitute starting points for the development of novel compounds with potential therapeutic use, incorporating other aromatic systems or labile ligands coordinated to the platinum(II) center. These compounds could also be studied, for example, as feasible DNA photocleaving agents, in particular the anthracene platinum(II) complex, compound **5**. Indeed, we have previously reported that certain platinum(II) complexes linked to anthracene derivatives can cleave plasmid DNA very efficiently under physiological conditions at nanomolar concentrations.[23] In addition, the compounds could be used as scaffold structures to create second-generation compounds that can recognize other DNA secondary structures of biological relevance besides duplex DNA. These may include, for example, triplex and quadruplex DNA structures, which are extremely interesting in cancer chemotherapy, e.g. antisense technology, oncogene expression modulating agents, telomerase inhibitors, etc.

4. Experimental

4.1. General

Melting points were determined using an Electrothermal Digital IA9100 apparatus. ^1H and ^{13}C NMR spectra were recorded on either Varian Unity or Varian Mercury-VX (300 MHz) or Varian Inova (500 MHz) spectrometers. Carbon and proton assignments were based on HSQC and HMBC experiments. Infrared spectra were acquired with an FT-IR Perkin Elmer Spectrum 2000 spectrophotometer. Elemental analyses were done on a Leco CHNS-932 automatic analyzer. ESI mass spectra were recorded on a Thermo Scientific

Table 1
Apparent association constants obtained by competition dialysis

DNA	Compound 2	Compound 3	Compound 5	Compound 6
	$K_{app} \times 10^{-5}\ M^{-1}$	$K_{app} \times 10^{-5}\ M^{-1}$	$K_{app} \times 10^{-5}\ M^{-1}$	$K_{app} \times 10^{-5}\ M^{-1}$
CT DNA (42% GC)	0.61 ± 0.08	0.20 ± 0.02	2.68 ± 0.14	1.03 ± 0.07
CP DNA (31% GC)	0.50 ± 0.03	0.17 ± 0.01	1.31 ± 0.07	1.22 ± 0.07
$[poly(dGdC)]_2$ (100% GC)	0.55 ± 0.05	0.27 ± 0.08	1.00 ± 0.02	1.69 ± 0.04
$[poly(dAdT)]_2$ (0% GC)	0.24 ± 0.02	0.41 ± 0.06	0.38 ± 0.05	1.22 ± 0.12

TSQ Quantum LC/MS or an Agilent 6210 LC/MS/TOF instruments. FAB mass spectra were obtained on a V.G. AutoSpec spectrometer with 3-nitrobenzyl alcohol as matrix. UV–visible spectra were recorded on a Lambda 18 Perkin-Elmer spectrometer. Merck silica gel 60 (230–400 ASTM mesh) was employed for flash column chromatography. TLC was performed on precoated aluminum silica gel plates (Merck or Macherey-Nagel 60 F_{254} 0.25 mm). Distilled, deionized water was utilized in the preparation of all buffers and all aqueous reactions. Chemicals were of the highest available purity and were used without further purification. Sodium phosphate dibasic, and sodium phosphate monobasic were obtained from the Aldrich Chemical Company. The concentration of CT DNA solutions was determined by UV–visible spectrophotometry using the extinction coefficient ε_{260} = 12,824 M^{-1} (bp) cm^{-1}.

4.2. Synthesis

4.2.1. Synthesis of 2-methylphenazine

4-Methylcatechol (3.72 g, 30 mmol) was dissolved in water (300 mL) at room temperature and sodium periodate (6.37 g, 31.4 mmol) was added. The mixture was stirred vigorously for 1 min before extraction with methylene chloride (2 × 70 mL). The organic extract was immediately added to a solution of *o*-phenylenediamine (3.24 g, 30 mmol) in methylene chloride (40 mL) with stirring. Then glacial acetic acid (20 mL) was added dropwise over a period of 10 min and the solution was left to stir for 30 min at room temperature before being heated to reflux for 4 h. The solution was cooled and washed successively with water (100 mL), aqueous sodium hydrogen carbonate (2 × 100 mL) and finally brine (100 mL). The organic layer was dried and evaporated to dryness giving a brown solid. Filtration through a plug of alumina using methylene chloride afforded an orange solid, which was purified by flash chromatography through silica gel (5:1 hexane/ethyl acetate, R_f = 0.3) affording the *title compound* (1.4 g, 24%) as a yellow solid, mp 117–119 °C (lit.[13] 117 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.66 (3H, s, CH_3), 7.70 (1H, dd, *J* 9.2, 1.8 Hz, H-3), 7.80–7.91 (2H, m, H-7, H-8), 8.06 (1H, br s, H-1), 8.17 (1H, d, *J* 9.2 Hz, H-4), 8.25–8.32 (2H, m, H-6, H-9). HRMS (ESI-TOF): *m/z* found 195.0925 $(M+H)^+$, $C_{13}H_{11}N_2$ requires 195.0922.

4.2.2. Synthesis of 2-bromomethylphenazine

2-Methylphenazine (200 mg, 1 mmol) was dissolved in carbon tetrachloride (10 mL) at room temperature. Freshly recrystallized N-bromosuccinimide (NBS) (196 mg) was added, followed by benzoyl peroxide (8 mg) and the reaction mixture was gentle refluxed for 71 h. Then it was cooled at room temperature, filtered, and the filtrate washed successively with water (10 mL) and brine (10 mL). The organic extract was dried, evaporated and dried overnight in a desiccator to give the crude product, which was purified by flash chromatography (5:1 hexane/ethyl acetate, R_f = 0.23) to give the *title compound* as a yellow product (149 mg, 53%), mp 157–159 °C (lit.[13] 156–162 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 4.74 (2H, s, CH_2Br), 7.84–7.88 (3H, m, ArH), 8.21–8.26 (4H, m, ArH); HRMS (ESI-TOF): *m/z* found 273.0035 $(M+H)^+$, $C_{13}H_{10}BrN_2$ requires 273.0027.

4.2.3. 1-(Naphthalen-2-ylmethyl)-4-(2-(pyridin-4-yl)ethyl) pyridinium bromide (1)

To a solution of 1,2-bis(4-pyridyl)ethane (200 mg, 1.08 mmol) in dry acetone (2 mL) a solution of 2-bromomethylnaphtalene (83 mg, 0.36 mmol) in dry acetone (2 mL) was added dropwise. The reaction mixture was stirred under argon at room temperature for 24 h and then cooled at 0 °C. The solid was filtered, washed with cold acetone and dried affording pure product **1** (108 mg, 74%); mp 189–191 °C; [Found: C, 68.18; H, 5.39; N, 7.15. $C_{23}H_{21}BrN_2$ requires C, 68.15; H, 5.22; N, 6.91%]; IR (KBr): *v* 3017, 2980, 1639, 1603, 1518, 1510, 1472, 1413, 1151 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.05 (2H, t, *J* 8.1 Hz, CH_2CH_2), 3.2 (2H, t, *J* 8.1 Hz, CH_2CH_2), 5.95 (2H, s, Py^+CH_2Naph), 7.27 (2H, d, *J* 5.9 Hz, H_βPy), 7.55–7.59 (3H, m, H-3, H-6, H-7 Naph), 7.91–7.95 (2H, m, H-5, H-8 Naph), 7.98 (1H, d, *J* 8.5 Hz, H-4 Naph), 8.04 (1H, br s, H-1 Naph), 8.07 (2H, d, *J* 6.5 Hz, $H_\beta Py^+$), 8.44 (2H, d, *J* 5.9 Hz, H_αPy), 9.13 (2H, d, *J* 6.5 Hz, $H_\alpha Py^+$); ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 33.7, 34.9, 62.9, 124.1, 125.8, 127.1, 127.3, 127.9, 128.2, 128.3, 128.5, 129.2, 132.1, 132.8, 133.1, 144.4, 149.1, 149.8, 161.8.

4.2.4. 1-(Anthracen-9-ylmethyl)-4-(2-(pyridin-4-yl)ethyl) pyridinium chloride (2)

To a solution of 1,2-bis(4-pyridyl)ethane (200 mg, 1.08 mmol) in dry acetone (2 mL) a solution of 9-chloromethylanthracene (78 mg, 0.36 mmol) in dry acetone (2 mL) was added dropwise. The reaction mixture was stirred at room temperature for 24 h. The solid was filtered, washed with cold acetone and dried at 50 °C affording pure product **2** (114 mg, 77%); mp 224–226 °C; [Found: C, 79.15; H, 5.72; N, 6.50. $C_{27}H_{23}ClN_2$ requires C, 78.91; H, 5.64; N, 6.82%]; IR (KBr): *v* 3015, 1633, 1602, 1515, 1467, 1419, 1143 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.97 (2H, t, *J* 8.1 Hz, CH_2CH_2), 3.20 (2H, t, *J* 8.1 Hz, CH_2CH_2), 6.89 (s, 2H, Py^+CH_2Anthr), 7.23 (2H, d, *J* 6.3 Hz, H_βPy), 7.60–7.70 (4H, m, H-2, H-3, H-6, H-7 Anthr), 7.93 (2H, d, *J* 6.9 Hz, $H_\beta Py^+$), 8.25 (2H, d, *J* 8.2 Hz, H-4, H-5 Anthr), 8.34 (2H, d, *J* 8.9 Hz, H-1, H-8 Anthr), 8.42 (2H, d, *J* 6.3 Hz, H_αPy), 8.75 (2H, d, *J* 6.9 Hz, $H_\alpha Py^+$), 8.91 (1H, s, H-10 Anthr). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 33.6, 34.7, 55.3, 122.4, 123.4, 124.1, 125.9, 128.2, 128.4, 129.7, 131.2, 131.3, 131.5, 143.5, 149.1, 149.6, 161.5.

4.2.5. 1-(Phenazin-2-ylmethyl)-4-(2-(pyridin-4-yl)ethyl) pyridinium bromide (3)

To a solution of 1,2-bis(4-pyridyl)ethane (200 mg, 1.08 mmol) in dry acetone (2 mL) a solution of 2-bromethylphenazine (100 mg, 0.36 mmol) in dry acetone (10 mL) was added dropwise. The reaction mixture was stirred at room temperature for 28 h and then the solid was filtered, washed with cold acetone and dried at 50 °C affording pure product **3** (128 mg, 78%); mp 273–275 °C; [Found: C, 65.52; H, 4.71; N, 12.43. $C_{25}H_{21}BrN_4$ requires C, 65.65; H, 4.63; N, 12.25%]; IR (KBr): *v* 3052, 1645, 1605, 1513, 1475, 1176, 1153 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.05 (2H, t, *J* 7.8 Hz, CH_2CH_2), 3.27 (2H, t, *J* 7.8 Hz, CH_2CH_2), 6.13 (2H, s, Py^+CH_2Phenazine), 7.28 (2H, d, *J* 6.0 Hz, H_βPy), 7.98–8.03 (3H, m, H-3, H-7, H-8 Phenazine), 8.12 (2H, d, *J* 6.7 Hz, $H_\beta Py^+$), 8.25−8.29 (3H, m, H-1, H-6, H-9 Phenazine), 8.33 (1H, d, *J* 9.1 Hz, H-4 Phenazine), 8.46 (2H, d, *J* 6.0 Hz, H_αPy), 9.21 (2H, d, *J* 6.7 Hz, $H_\alpha Py^+$). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 33.7, 35.0, 62.5, 124.2, 128.5, 129.3, 129.5, 129.6, 130.6, 130.7, 131.9, 137.4, 142.6, 142.7, 143.4, 143.5, 144.8, 149.4, 149.6, 162.2.

4.2.9. Bis-[4-(2-(1-(naphthalen-2-ylmethyl)pyridinium-4-yl)ethyl)pyridinium-1-yl](1,2-ethylendiamine)platinum(II) (tetrakis)hexafluorophosphate (4)

A suspension of $PtCl_2(en)_2$ (40 mg, 0.12 mmol) in water (10 mL) was heated at reflux until to obtain a clear solution. Then a solution of **1** (100 mg, 0.24 mmol) in methanol-water (1:10) (3 mL) was added. The reaction mixture was heated at reflux for 6 h. The solid was filtered and the filtrate concentrated under reduced pressure affording a colorless oil. The crude product was purified by column chromatography on silica gel and methanol-374 mM aqueous ammonium chloride solution (2:1) as eluent. The combined fractions were concentrated at reduced pressure and the residue thus obtained was dissolved in water and treated with ammonium hexafluorophosphate. The precipitate was filtered, washed with water and dried in a oven at 50 °C yielding 40 mg (22%) of pure

product **4**; mp 176–178 °C (d). [Found: C, 38.91; H, 3.42; N, 5.26. $C_{48}H_{50}F_{24}N_6P_4Pt$ requires C, 38.80; H, 3.39; N, 5.66%]; IR (KBr): ν 3419, 1639, 1622, 1438, 1157 cm^{-1}. ^1H NMR (D_2O, 300 MHz) δ (ppm): 2.55 (4H, s, NCH_2CH_2N), 2.74 (4H, t, J 7.6 Hz, CH_2CH_2), 2.93(4H, t, J 7.6 Hz, CH_2CH_2), 5.67 (4H, s, Py^+CH_2Naph), 6.96 (4H, d, J 6.5 Hz, $H_\beta Py$), 7.14 (2H, dd, J 8.5, 1.8 Hz, H-3 Naph), 7.30–7.38 (4H, m, H-6, H-7 Naph), 7.51 (4H, d, J 6.7 Hz, $H_\beta Py^+$), 7.60–7.71 (8H, m, H-1, H-4, H-5, H-8 Naph), 8.11 (4H, d, J 6.5 Hz, $H_\alpha Py$), 8.51 (4H, d, J 6.7 Hz, $H_\alpha Py^+$); ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm) 33.4, 34.3, 63.1, 125.7, 126.9, 127.1, 127.3, 127.8, 128.1, 128.4, 129.2, 131.9, 132.8, 133.0, 144.5, 152.1, 154.5, 161.2; LRMS (ESI) m/z found 1340 ($M^{+-}PF_6$), $C_{48}H_{50}F_{18}N_6P_3Pt$ requires 1340.26.

4.2.10. Bis-[4-(2-(1-(anthracen-9-ylmethyl)pyridinium-4-yl)ethyl)pyridinium-1-yl](1,2-ethylendiamine)platinum(II) (tetrakis)hexafluorophosphate (5)

A suspension of $PtCl_2(en)_2$ (40 mg, 0.12 mmol) in water (10 mL) was heated at reflux until to obtain a clear solution. Then a solution of **2** (100 mg, 0.24 mmol) in water (2 mL) was added. The reaction mixture was heated at reflux for 6 h. The solid was filtered and the filtrate evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel and methanol-374 mM aqueous ammonium chloride solution (2:1) as eluent. The combined fractions were concentrated at reduced pressure and the residue dissolved in water and treated with ammonium hexafluorophosphate. The yellow precipitate was filtered, washed with water and dried in an oven at 80 °C affording 46 mg (24%) of pure product **5**; mp 245-248 °C (d); [Found: C, 42.51; H, 3.39; N, 5.17. $C_{56}H_{54}F_{24}N_6P_4Pt$ requires C, 42.41; H, 3.43; N, 5.30]; IR (KBr): ν 3424, 1640, 1624, 1467, 1450, 1438, 1142 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.55 (4H, s, NCH_2CH_2N), 2.90 (8H, m, CH_2CH_2), 6.30 (4H, br s, NH_2), 6.89 (4H, s, Py^+CH_2Anthr), 7.52 (4H, d, J 6.4 Hz, $H_\beta Py$), 7.59–7.71 (8H, m, H-2, H-3, H-6, H-7 Anthr), 7.93 (4H, d, J 6.4 Hz, $H_\beta Py^+$), 8.25 (4H, d, J 8.0 Hz, H-4, H-5 Anthr), 8.37 (4H, d, J 8.9 Hz, H-1, H-8 Anthr), 8.61 (4H, d, J 6.4 Hz, $H_\alpha Py$), 8.77 (4H, d, J 6.4 Hz, $H_\alpha Py^+$), 8.92 (2H, s, H-10 Anthr); ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 33.4, 34.3, 47.6, 55.5, 119.6, 122.2, 123.3, 126.0, 127.0, 128.1, 128.5, 129.8, 131.3, 131.5, 143.6, 152.1, 154.4, 161.1; FAB-MS (m-NBA) m/z found 1440.3 ($M^{+-}PF_6$), $C_{56}H_{54}F_{18}N_6P_3Pt$ requires 1440.29.

4.2.14. Bis-[4-(2-(1-(phenazin-2-ylmethyl)pyridinium-4-yl)ethyl)pyridinium-1-yl](1,2-ethylendiamine)platinum(II) (tetrakis)hexafluorophosphate (6)

A suspension of $PtCl_2(en)_2$ (28 mg, 0.086 mmol) in water (7 mL) was heated at reflux until to obtain a clear solution. Then a solution of **3** (80 mg, 0.17 mmol) in water (2 mL) was added. The reaction mixture was heated at reflux for 6 h. The solid was filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel and methanol-2.8 M aqueous ammonium chloride solution (2:1) as eluent. The combined fractions were concentrated at reduced pressure and the residue dissolved in water and treated with ammonium hexafluorophosphate. The yellow precipitate was filtered, washed with water and dried in a oven at 80 °C affording 85 mg (61%) of pure product **6**; mp 213–215 °C (d); [Found: C, 39.44; H, 3.06; N, 9.04. $C_{52}H_{50}F_{24}N_{10}P_4Pt$ requires C, 39.28; H, 3.17; N, 8.81%]; IR (KBr): ν 3430, 1644, 1624, 1516, 1473, 1438 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.56 (4H, s, NCH_2CH_2N), 3.0–3.27 (8H, m, CH_2CH_2), 6.14 (4H, s, $Py^+CH_2Phenazine$), 6.20 (4H, br s, NH_2) 7.58 (d, 4H, J 6.0 Hz, $H_\beta Py$), 7.98–8.04 (m, 6H, H-3, H-7, H-8 Phenazine), 8.14 (d, 4H, J 6.4 Hz, $H_\beta Py^+$), 8.24–8.29 (6H, m, H-1, H-6, H-9 Phenazine), 8.34 (2H, d, J 8.9 Hz, H-4 Phenazine), 8.65 (4H, d, J 6.0 Hz, $H_\alpha Py$), 9.24 (4H, d, J 6.4 Hz, $H_\alpha Py^+$); ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 33.5, 34.5, 47.7, 62.6, 127.0, 128.4, 129.2, 129.5, 129.6, 130.7, 130.8, 131.9, 137.4, 142.5, 142.7, 143.4, 143.5, 144.9, 152.2, 154.6, 161.7. FAB-MS (m-NBA) m/z found 1444.3 ($M^{+-}PF_6$). HRMS (ESI-TOF): m/z found 1444.2781 (M^+-PF_6), $C_{52}H_{50}F_{18}N_{10}P_3Pt$ requires 1444.2793.

4.2.15. Anion exchange from hexafluorophosphate to bromide

The hexafluorophosphate salt was dissolved in nitromethane. By addition of a nitromethane solution of tetraethylammonium bromide, the bromide salt precipitates. The filtered precipitate was washed with nitromethane and then with cold acetone.

4.3. Viscometric titrations

The viscometric measurements were performed in a Visco System AVS 470 at 25 ± 0.01 °C, using a microUbbelohde (K = 0.01) capillary viscometer. Solutions of DNA (*Calf thymus*, CT), ligands and platinum complexes were prepared in sodium phosphate buffer (10 mM, pH 7.2). DNA solutions (0.35–0.40 mM, in nucleotides) were equilibrated for 20 min at 25.00 °C and then 20 flow times were registered. Small aliquots (30–60 μL) of solutions of compounds **1**–**3** (0.4–1.5 mM) were added next maintaining the DNA concentration constant. For platinum complexes, compounds **4**–**6**, small aliquots of solutions (0.6–1.6 mM) were added without maintaining constant the DNA concentration, with a maximum dilution of 5%. Before each flow time registration, the solutions were equilibrated for at least 20 min at 25 ± 0.01 °C and then 20 flow times were measured. With the averaged flow times and the viscometer constant, the viscosities (μ) for each point were calculated, with μ_0 representing the DNA solution viscosity in the absence of compound. The viscosity results were then plotted as $(\mu\mu_0)^{1/3}$ versus the molar ratio of bound ligand to DNA nt (r).

4.4. Equilibrium dialysis

4.5.1. Competition dialysis assay

Calf thymus (CT), *Clostridium perfringens* (CP) DNAs and the synthetic polynucleotides $[poly(dGdC)]_2$ and $[poly(dAdT)]_2$ were purchased from Sigma and were used without further purification. A 10 mM sodium phosphate buffer (pH 7.2) was utilized in the preparation of all nucleic acid stock solutions. The concentrations of the nucleic acid solutions were determined by UV–visible spectrophotometry using the λ_{max} values and extinction coefficients listed in Table S1 (Supplementary data). Extinction coefficients for compounds **2**, **3**, **5** and **6** were determined in 10 mM sodium phosphate buffer pH 7.0 in the absence and in the presence of 1% SDS (w/v) or excess of CT DNA (Table S2,Supplementary data). Competition dialysis experiments were performed following a similar protocol as the one described by Chaires.[18–20] For each dialysis assay, a 0.5 mL volume of DNA (75 μM bp DNA in buffer; Table S1) was pipetted into one of 4 individual Spectra/Por DispoDialyzer units (S135062, Spectrum Laboratories, Inc.). The 4 dialysis units were then placed in a beaker containing 225 mL of a 2.5 μM solution of **2**, **3** and **5**, **6** in buffer. The beaker was covered with Parafilm and wrapped in foil, and its contents were allowed to equilibrate with continuous stirring for 24 h at room temperature (20–22 °C). At the end of the equilibration period, the DNA solutions inside the dialysis units were carefully transferred to microcentrifuge tubes and a 10.0% (w/v) stock solution of sodium dodecyl sulfate (SDS) was added to give a final concentration of 1.0% (w/v). The DNA–SDS solutions were allowed to equilibrate for 2 h, after which the total concentration of the corresponding ligand **2**, **3** and **5**, **6** (C_t) was determined by UV–visible absorbance measurements using the extinction coefficient for free ligands in the presence of 1.0% SDS. An appropriate correction for the slight dilution of the sample resulting from the addition of SDS stock solution was made. The concentration of free ligands **2**, **3** and free complexes **5**, **6** (C_f) was also determined spectrophotometrically

using an aliquot of their dialysate solution. The amount of DNA-bound **2**, **3**, **5** and **6** (C_b) was then calculated by difference ($C_b = C_t - C_f$).

Acknowledgements

The research leading to these results has received funding from projects CTQ2011-26822 (A.L.), CCG10-UAH/PPQ-5961 (M.-J.F.) and from the *European Community's Seventh Framework Programme* ([FP7/2007-2013, under grant agreement 248638 (L.G.)]). The authors thank Universidad de Alcalá (UAH) for a postgraduate fellowship to K.D.

Supplementary data

Supplementary data (Table S1 and Table S2) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.09.055.

References and notes

1. (a) Tumir, L.-M.; Piantanida, I. *Mini-Rev. Med. Chem.* **2010**, *10*, 299; (b) Xie, Y.; Tam, V.; Tor, Y. *Chem. Biol. Nucleic Acids* **2010**, 115; (c) Prabal, G.; Suresh, K. G. *Curr. Med. Chem.* **2009**, *16*, 965; (d) Nelson, S. M.; Ferguson, L. R.; Denny, W. A. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* **2007**, *623*, 24; (e) Hendry, L. B.; Mahesh, V. B.; Bransome, E. D.; Ewing, D. E. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* **2007**, *623*, 53; (f) Hannon, M. J. *Chem. Soc. Rev.* **2007**, *36*, 280; (g) Strekowski, L.; Wilson, B. *Mutat. Res.* **2007**, *623*, 3; (h)*Nucleic Acids in Chemistry and Biology*; Blackburn, G. M., Gait, M. J., Loakes, D., Williams, D. M., Eds.; RSC Publishing: Cambridge, 2006; (i)*Sequence-specific DNA Binding Agents*; Waring, M. J., Ed.; RSC Publishing: Cambridge, 2006; (j)*DNA and RNA Binders*; Demeunynck, M., Bailly, C., Wilson, W. D., Eds.; Wiley-VCH: Weinheim, 2003. Vol. 2; (k) Wilson, W. D. In *Comprehensive Natural Products Chemistry*; Kool, E. T., Ed.; Elsevier: Amsterdam, 1999; Vol. 7, pp 427–476; (l)*Bioorganic Chemistry: Nucleic Acids*; Hecht, S. M., Ed.; Oxford University Press: Oxford, 1996; (m)*Nucleic Acid Targeted Drug Design*; Propst, C. L., Perun, T. J., Eds.; Marcel Dekker, 1992.
2. Waring, M. J. *Annu. Rev. Biochem.* **1981**, *50*, 159.
3. Wakelin, L. P. G. *Med. Res. Rev.* **1986**, *6*, 275.
4. Barbet, J.; Roques, B. P.; Combrisson, S.; Le Pecq, J.-B. *Biochemistry* **1976**, *15*, 2642.
5. Capelle, N.; Barbet, J.; Dessen, P.; Blanquet, S.; Roques, B. P.; Le Pecq, J.-B. *Biochemistry* **1979**, *18*, 3354.
6. Cory, M.; McKee, D. D.; Kagan, J.; Henry, D. W.; Miller, J. A. *J. Am. Chem. Soc.* **1985**, *107*, 2528.
7. Jaycox, G. D.; Gribble, G. W.; Hacker, M. P. *J. Heterocycl. Chem.* **1987**, *24*, 1405.
8. Holmes, R. J.; McKeage, M. J.; Murray, V.; Denny, W. A.; McFadyen, W. D. *J. Inorg. Biochem.* **2001**, *85*, 209; (b) Collins, J. G.; Rixon, J. G.; Aldrich-Wright, J. R. *Inorg. Chem.* **2000**, *39*, 4377; (c) Cusumano, M.; Giannetto, A. *J. Inorg. Biochem.* **1997**, *65*, 137; (d) Bowler, B. E.; Hollis, J. S.; Lippard, S. J. *J. Am. Chem. Soc.* **1984**, *106*, 6102; (e) Norden, B. *Inorg. Chim. Acta* **1978**, *30*, 83; (f) Howe-Grant, M.; Wu, K. C.; Bauer, W. R.; Lippard, S. J. *Biochemistry* **1976**, *15*, 4339; (g) Lippard, S. J.; Bond, P. J.; Wu, K. C.; Bauer, W. R. *Science* **1976**, *194*, 726.
9. Raymo, F. M.; Bartberger, M. D.; Houk, K. N.; Stoddart, J. F. *J. Am. Chem. Soc.* **2001**, *123*, 9264–9267.
10. Chae, Y.-H.; Jin, B.; Kim, J.-K.; Han, S. W.; Kim, S. K.; Lee, H. M. *Bull. Korean Chem. Soc.* **2007**, *28*, 2203.
11. Whan, R. M.; Messerle, B. A.; Hambley, T. W. *Dalton Trans.* **2009**, 932.
12. Kieltyka, R.; Fakhoury, J.; Moitessier, N.; Sleiman, H. F. *Chem. Eur. J.* **2008**, *14*, 1145.
13. Maidwell, N. L.; Rezai, M. R.; Roeschlaub, C. A.; Sammes, P. G. *J. Chem. Soc., Perkin Trans. 1* **2000**, *10*, 1541.
14. Suh, D.; Chaires, J. B. *Bioorg. Med. Chem.* **1995**, *3*, 723.
15. Cohen, G.; Eisenberg, H. *Biopolymers* **1969**, *8*, 45.
16. (a) Drummond, D. S.; Pritchard, N. J.; Simpson-Gildemeister, V. F. W.; Peacocke, A. R. *Biopolymers* **1966**, *4*, 971; (b) Kubota, Y.; Hashimoto, K.; Fujita, K.; Wakita, M.; Miyanohana, E.; Fujisaki, Y. *Biochim. Biophys. Acta* **1977**, *478*, 23; (c) Chaires, J. B.; Leng, F.; Przewloka, T.; Fokt, I.; Ling, Y.-H.; Perez-Soler, R.; Priebe, W. *J. Med. Chem.* **1997**, *40*, 261.
17. (a) Huang, C.-H.; Mong, S.; Crooke, S. T. *Biochemistry* **1980**, *19*, 5537; (b) Wilson, W. D.; Keel, R. A.; Jones, R. L.; Mosher, C. W. *Nucleic Acids Res.* **1982**, *10*, 4093; (c) Leon, P.; Garbay-Jaureguiberry, C.; Lambert, B.; Le Pecq, J. B.; Roques, B. P. *J. Med. Chem.* **1988**, *31*, 1021; (d) Bordelon, J. A.; Feierabend, K. J.; Siddiqui, S. A.; Wright, L. L.; Petty, J. T. *J. Phys. Chem. B* **2002**, *106*, 4838; (e) Chan, H.-L.; Ma, D.-L.; Yang, M.; Che, C.-M. *J. Biol. Inorg. Chem.* **2003**, *8*, 761.
18. Ren, J.; Chaires, J. B. *Methods Enzymol.* **2001**, *340*, 99.
19. Chaires, J. B. *Current Protocols in Nucleic Acid Chemistry*; John Wiley & Sons: New York, 2002. pp 831–838.
20. Chaires, J. B. *Top. Curr. Chem.* **2005**, *253*, 33.
21. van Holde, K. E.; Johnson, W. C.; Ho, P. S. *Principles of Physical Biochemistry*; Prentice Hall: New Jersey, 1998.
22. Muller, W.; Crothers, D. M. *Eur. J. Biochem.* **1975**, *54*, 267.
23. Gude, L.; Fernandez, M.-J.; Grant, K. B.; Lorente, A. *Tetrahedron Lett.* **2002**, *43*, 4723.