DOI: 10.1002/chem.200601581

Design and Non-Covalent DNA Binding of Platinum(II) Metallacalix[4]arenes

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Dedicated to Professor Bernhard Lippert on the occasion of his 60th birthday

Abstract: A set of cyclic tetranuclear complexes of the metallacalix[4]arene type with formula $[\{Pt(en)(L)\}_4]^{4+}$ (en = ethylenediamine; **2**: LH = 5-chloro-2-hydroxypyrimidine (5-Cl-Hpymo); **3**: LH = 5-bromo-2-hydroxypyrimidine (5-Br-Hpymo); **4**: LH = 5-iodo-2-hydroxypyrimidine (5-I-Hpymo)) have been obtained from the reaction between *cis*-protected square-planar $[Pt(en)(H_2O)_2]^{2+}$ metal entities and LH in aqueous media. Additionally, the binding properties of **2**, **3**, **4** and their congener $[\{Pt(en)(L)\}_4]^{4+}$ (**1**: LH = 2-hydroxypyrimidine (Hpymo)) with calf thymus-DNA (ct-DNA) have been studied by using different techniques including circular and linear dichroism (CD and LD, respectively) and UV-visible absorbance spectroscopies, gel electrophoresis, fluorescence competitive-binding studies and atomic force microscopy (AFM). The results are consistent with significant non-covalent interactions taking place between the polynuclear cyclic species and ct-DNA. Moreover, gel electrophoresis, linear dichroism titrations and AFM images of ct-DNA with metallacalixarenes show ct-DNA coiling at low metallacalixarene concentrations and upon subsequent increments in metallacalixarene concentration ct-DNA can be seen to uncoil with concomitant formation of long and inflexible ct-DNA structures.

Keywords: atomic force microscopy · binding studies · DNA · metallacalixarenes · platinum

Introduction

The mechanism of interaction of classical DNA-targeting metal-based drugs generally involves covalent binding to nucleobase moieties and a low degree of selectivity.[1] Therefore, there is a considerable interest in the design of new DNA-binding metal-based drugs that exhibit enhanced selectivity and novel interaction modes, for instance non-covalent interactions that mimic the interaction mode of proteins.[2,3] Recently, a hexacationic ammonium-functionalised bis-calixarene has been observed to bind DNA and has been proposed to do so through binding in the major groove.[4] Metallacalix[*n*]arenes are a class of container molecules that are structurally and functionally related to classical organic calixarenes in which the methylene and phenol rings have been respectively replaced by a metal fragment and a bent nitrogen heterocycle. Like their organic analogues, these systems are able to give a rich variety of host–guest interactions, which include metal-ion, anion and ion-pair recognition.[5] In this regard, we and others have shown that their cationic nature leads to a concomitant high affinity for

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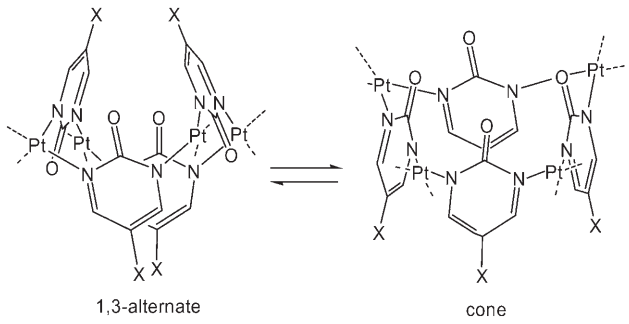
anionic substrates.[5–7] In particular, we have observed that metallacalixarenes are especially suited for supramolecular interactions with mononucleotides[8] with some examples of enantioselective recognition processes.[9] These previous results have prompted us to investigate the possible use of metallacalixarenes as selective DNA binding agents. Thus, in this contribution, we describe the non-covalent DNA binding properties of a series of platinum(II)-based metallacalix[4]arenes (Scheme 1).



Scheme 1. Metallacalix[4]arenes $[\{Pt(en)(5\text{-X-pymo})\}_4]^{4+}$ (en = ethylenediamine; **1**: X = H; **2**: X = Cl, **3**: X = Br, **4**: X = I) in their 1,3-alternate and cone conformations. Intermediate conformers are also possible.

Results and Discussion

The novel metallacalix[4]arenes $[\{Pt(en)(5\text{-X-pymo})\}_4](NO_3)_4$ (**2**, **3**, and **4**; see Scheme 1) have been obtained by the reaction of an aqueous solution of $[Pt(en)(H_2O)_2](NO_3)_2$ with the corresponding 5-halo-2-hydroxypyrimidine (5-X-pymoH) derivative to afford, in a single step, the thermodynamically favoured tetranuclear metallamacrocycles. From analysis of the ^{1}H NMR spectroscopy data, it can be deduced that the reaction is quantitative and diagnostic of the N1,N3 bridging coordination mode of 5-X-pymo derivatives, with retention of the original equivalence of H4 and H4′ protons in the 5-X-pymo moieties in a similar way to $[\{Pt(en)(pymo)\}_4](NO_3)_4$ (**1**), which has previously been fully characterised by using single-crystal X-ray crystallography.[10] The most remarkable feature of the ^{1}H NMR spectra is that the H4,H4′ resonances are shifted downfield on passing from the chloro-substituted pymo derivative (δ = 8.43 ppm), to the bromo (δ = 8.49 ppm) and iodo (δ = 8.53 ppm) derivatives, as expected. The ^{1}H NMR spectra also show that the compounds do not decompose in aqueous solution even during long storage periods and over a wide range of pH (1–13), which is in agreement with the highly inert nature of the Pt–N bonds. Variable-temperature ^{1}H NMR experiments (22–85 °C) have been performed for species **1**, **2**, **3** and **4** to explore their conformational flexibility. The results are consistent with conformational flexibility for species **1** and **2** with a unique set of signals observed throughout the whole temperature range. By contrast, above 50 °C, species **3** and **4** show new sets of downfield shifted signals, which agree with a constricted conformational flexibility between the plausible conformers (i.e., 1,2-alternate, 1,3-alternate, partial cone and cone)[10] with predominance of the 1,3-alternate conformer at low temperatures and a mixture of the 1,3-alternate, cone and other intermediate conformers at higher temperatures (Figure 1, Scheme 1). The

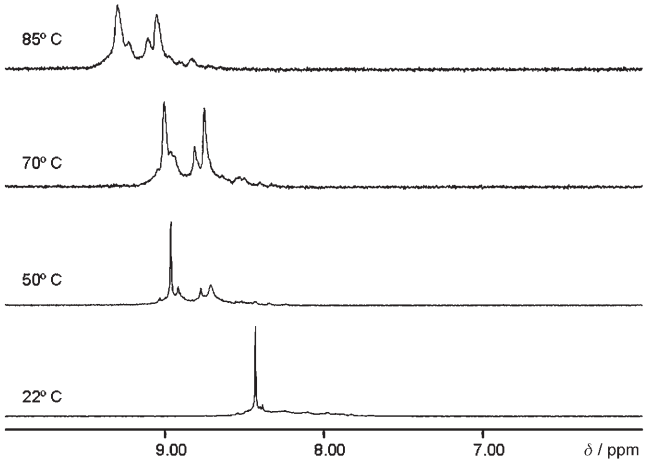


Figure 1. Variable-temperature ^{1}H NMR spectra of **3** in the aromatic region. At high temperature the low- and high-field signals are attributed to the 1,3-alternate and the cone conformer, respectively. The intermediate signals are related to the other conformers (i.e., 1,2-alternate and partial cone).

different behaviour of **1** and **2** relative to **3** and **4** can be attributed to the bulk and heavy nature of the Br and I substituents. The electronic spectra of **1**–**4** each show a broad ligand-to-ligand absorption band in the 300–390 nm region, centred at 323 nm for **1** (ε = 14400 $mol^{-1}dm^3cm^{-1}$), 348 nm for **2** (ε = 34000 $mol^{-1}dm^3cm^{-1}$), 350 nm for **3** (ε = 27500 $mol^{-1}dm^3cm^{-1}$), and 358 nm for **4** (ε = 13000 $mol^{-1}dm^3cm^{-1}$).

DNA binding studies: UV-visible absorbance, circular dichroism (CD) and flow linear dichroism (LD) titration experiments between the compounds and calf thymus DNA (ct-DNA) have been carried out in aqueous solution with NaCl (20 mM) and sodium cacodylate buffer (1 mM) while keeping the ct-DNA concentration constant (300 μM).

The UV-visible titration experiments of ct-DNA with **1** (see the Supporting Information) show no significant changes in the 300–390 nm ligand-centred complex bands on addition of the ct-DNA. This is consistent with the structure of the metallacalixarene remaining unchanged and thus implies that if there is an interaction of the tetracationic species **1** with ct-DNA it should be of supramolecular nature rather than covalent DNA platination. This is consistent with the chemical inertness of the Pt–N bonds in the **1**–**4** series, which prevent any covalent binding to ct-DNA.[11] The region in which the ct-DNA absorbs changes in intensity as the titration experiment progresses. The variation in the intensity of this ct-DNA band can be correlated with the changes in base–base stacking. Thus, at low concentrations

of metallacalixarene there is a decrease in the intensity of the band associated with the ct-DNA, which appears to exclude intercalation as a binding mode at low metallacalixarene loading. Intercalation reduces the flexibility of the ct-DNA and is usually associated with an increase in the 260 nm absorption band, but at low concentrations of metallacalixarene a certain degree of coiling can be induced by supramolecular interactions.

To gain further information, we also recorded CD and LD spectra. The complexes **1** to **4** have no intrinsic CD signals as they are achiral. Any CD signals that arise in the spectroscopic regions of the complex are therefore a consequence of their interaction with the ct-DNA. Linear dichroism (LD) is the difference in absorption of linearly polarised light both parallel and perpendicular to a chosen plane and can be used to probe the orientation of molecules. Long molecules, such as DNA (minimum length of ≈250 base pairs) can, in a flow Couette cell, be orientated through viscous drag.[2] The linearly polarised light is incident radial to the flow cell and perpendicular to the flow direction. Small unbound molecules are not orientated in the experiment and show no signal. Similarly molecules bound randomly to the ct-DNA show no signal. However, molecules bound in a specific orientation with respect to the ct-DNA will show a signal. Complexes **1**–**4** are too small to be orientated and thus show no intrinsic signal. Any signals that arise in the spectroscopic regions of the complex after the addition of ct-DNA, therefore, indicate binding of the complex to the ct-DNA in a specific orientation(s).

For each of the metallacalix[4]arenes **1**–**4** we observed bands in the 300–390 nm region in both the LD (Figure 2) and CD spectra (see the Supporting Information). These results indicate that each of these complexes binds to ct-DNA and does so in a specific orientation(s), not randomly.[2] Moreover, the band that arises in the 350–370 nm region suggests that the pyrimidine heterocycles might be orientated less than 54.7° from the helix axis. This often means that these systems are slotted in a groove, which from size considerations should be the ct-DNA major groove.[4] The ct-DNA LD bands (220–300 nm) confirm that the ct-DNA remains in the B-DNA conformation, however, some structural changes in ct-DNA are suggested by the decrease in the ct-DNA LD band at 260 nm upon metallacalix[4]arene addition. The ct-DNA LD signal is consistent with a non-intercalative mode of interaction (intercalation reduces the flexibility of the ct-DNA and is usually associated with an increase in the 260 nm LD band). Indeed at low loading there is very little change in the ct-DNA LD signal. This contrasts with the dramatic loss of ct-DNA LD signal seen when tetracationic supramolecular cylinders bind and coil ct-DNA even at very low loading.[2] The results are similar along the **1**–**4** series, however, these conclusions are hampered for compounds **3** and **4** owing to some degree of precipitation.

Fluorescence competitive-binding assays have also been performed with ethidium bromide (EB) to evaluate the strength of the interaction of the metallacalixarenes **1** to **4** with ct-DNA (Figure 3). It should be noted that in contrast

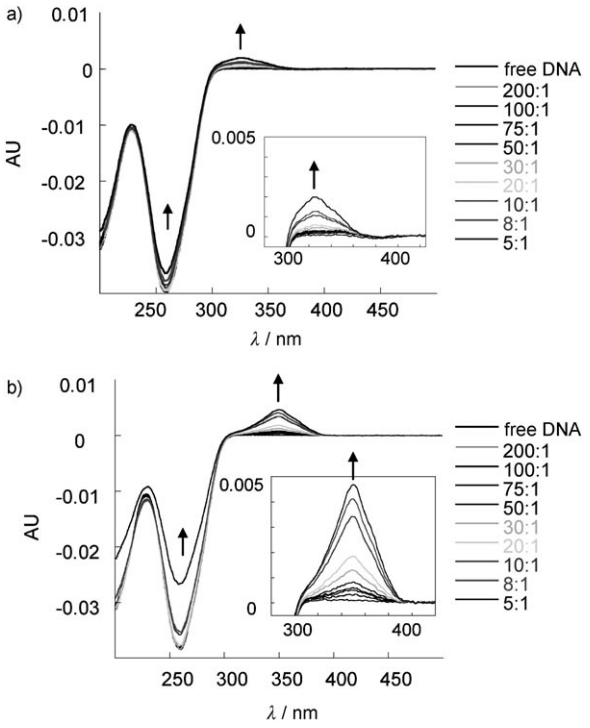


Figure 2. LD spectra of free ct-DNA (300 μM) and in the presence of a) **1** and b) **2**. ct-DNA/metallacalixarene mixing ratios range from 200:1 to 1:1. Inset enlargements ×4.

to previous experiments, precipitation does not take place in these experiments (where a lower ct-DNA concentration is used). Although, the UV-visible absorbance, LD and CD spectroscopic studies revealed that complexes **1** and **2** interact with ct-DNA, the strength of this interaction is not large enough to displace intercalated EB (Figure 3a), which is indicative of a lower association constant (K_{ass}) than that of EB. Complexes **3** and **4**, however, interact strongly with ct-DNA. This is manifested by the quenching of the band emission centred at 600 nm, which is associated with ct-DNA intercalated with EB, and is a consequence of the displacement of EB by the metallacalixarene (Figure 3b), which should be indicative of a association K_{ass} value in the same range as that of EB. It should also be noted that emission decreases upon complex addition until a metallacalixarene concentration of ≈2 μM (ratio ct-DNA base pairs/metallacalixarene, 3:1) is reached, indicating the saturation of ct-DNA binding sites and a high K_{ass} for **3** and **4** (Figure 3b) and in the Supporting Information). The different behaviour of **3** and **4** relative to **1** and **2** might be related to their different conformational behaviour (see NMR data discussed above). Thus, we presume that the interaction of the metallacalixarene with ct-DNA is enhanced by the fixation of a particular conformer of those possible for **3** and **4**, driven by the optimal fitting to the ct-DNA surface. The higher binding strength of **3** and **4** may also be the reason for their lower precipitation concentration from the absorbance, CD and LD experiments.

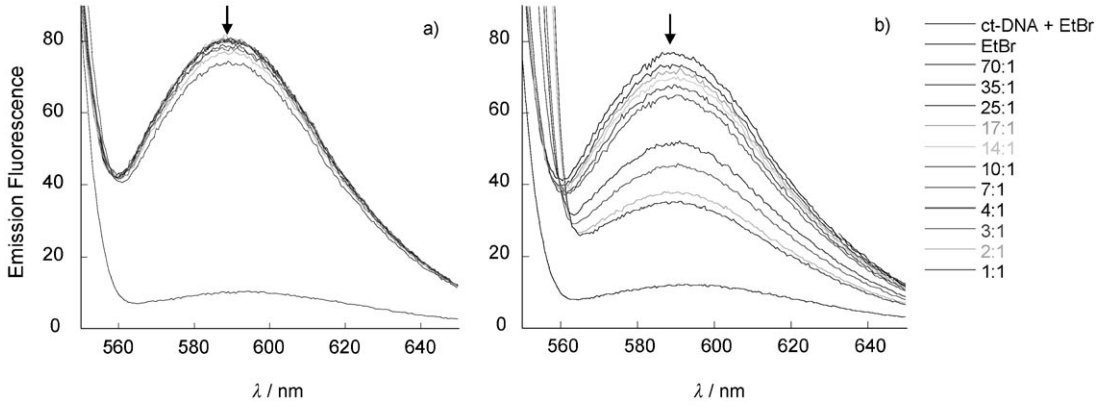


Figure 3. Fluorescence quenching of ethidium (5 μM) bromide and ct-DNA (4 μM) in the presence of a) **2** and b) **4**. ct-DNA base/metallacalixarene mixing ratios range from 70:1 to 1:1.

Gel electrophoresis experiments were performed on the ct-DNA/**1** system to follow the ct-DNA migration when it is bound to the complexes. The experiments show a smooth decrease in the ct-DNA migration rate for the ct-DNA/**1** ratios from 200:1 to 30:1 (see the Supporting Information). At higher complex concentrations the migration rate diminishes dramatically with no migration at all at ratios lower than 5:1. The smooth decrease in the ct-DNA migration rate can be attributed to the reduction of its negative charge as a consequence of the interaction with the tetracationic metallacalixarenes. However, the dramatic decrease of the ct-DNA migration rate at higher complex concentrations could be related to the formation of inflexible, long ct-DNA structures (see the AFM experiments below).

AFM studies: To clarify the concentration dependent ct-DNA conformational changes induced by the metallacalixarenes we have carried out atomic force microscopy (AFM) experiments at different ct-DNA/metallacalixarene mixing ratios.

An image of several molecules of calf thymus DNA adsorbed on a mica surface by using $MgCl_2$ is shown in Figure 4 (blank sample, see the Experimental Section for details). The molecule height is about 1 nm and has a persistence length of ≈50 nm, these dimensions are typical values for AFM measurements.[12,13] The length of the ct-DNA is not well defined, ranging from 300 to 2000 nm.

When ct-DNA was incubated with **1** at a mixing ratio of 30:1, we observed that on the mica surface and along the ct-DNA molecules, spots had appeared (Figure 5). These spots have been attributed to the metallacalixarenes. In this case (and in contrast to the 10:1 mixing ratio, see below) a certain degree of bending or coiling can be seen. In addition, the AFM topographic images show a marked drop of the persistence length with respect to free ct-DNA. This drop in the persistence length is also in agreement with some small amounts of bending or coiling, although no dramatic kinks or coils are observed; consistent with the LD results. Reductions in ct-DNA persistence length by multivalent cations are not unusual and have been attributed to the bending of the ct-DNA.[14] Finally, it should be noted that the height of the ct-DNA strands is in the typical 0.6–0.8 nm range, which

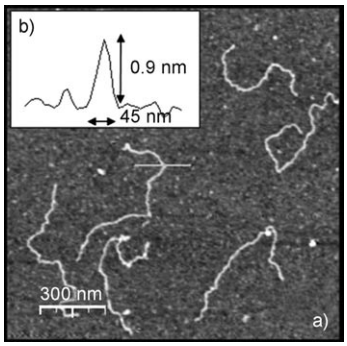


Figure 4. AFM topographic image showing ct-DNA double strands adsorbed on a mica substrate by using the classical method of adding $MgCl_2$ to a buffer solution with ct-DNA.

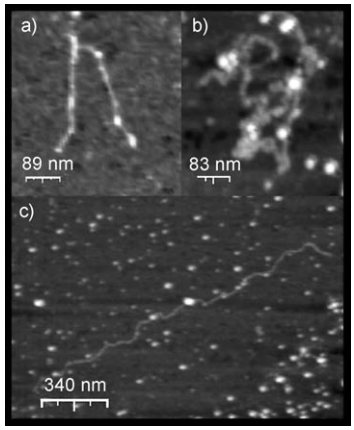


Figure 5. AFM topographic images of ct-DNA incubated with **1** (30:1 mixing ratio) adsorbed on mica. The white dots along the ct-DNA are attributed to ct-DNA/metallacalixarene supramolecular assemblies with a concomitant increase of the height of the strand from 0.6 nm to ≈2 nm.

increases to 2 nm in the regions where the spots attributed to metallacalixarenes bound to the ct-DNA are observed.

In contrast to the lower loadings, if ct-DNA is incubated with larger loadings of metallacalixarenes (ct-DNA/**1** in a 10:1 ratio) the AFM topographic images show inflexible, long ct-DNA filaments with lengths often higher than 15 μm (Figure 6). Its persistence length is, on average, much longer

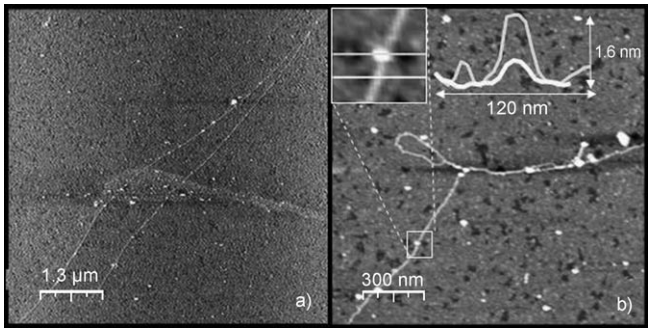


Figure 6. AFM topographic images of ct-DNA incubated with **1** (10:1 mixing ratio) and adsorbed on mica. Long structures with high persistence length are observed. a) ≈15 μm ct-DNA strands. b) ct-DNA strand with a typical height of 0.8 nm. The white dots along the ct-DNA are attributed to ct-DNA/metallacalixarene supramolecular assemblies.

than in blank ct-DNA. Strands of ct-DNA from these larger loadings had a height of 0.8 nm, which is not significantly different from the ct-DNA height observed in the blank sample seen in Figure 6b. The inset in Figure 6b shows a 2.5 nm high spot in the centre of a straight ct-DNA fibre. We attribute this spot to a metallacalixarene bound to the ct-DNA. In addition, similar spots are shown to be unspecifically adsorbed on a mica surface in Figure 6b.

The structural changes that occur when ct-DNA is incubated with **4** (ct-DNA/**4** in a 10:1 mixing ratio) are similar but more dramatic than the changes seen for ct-DNA/**1** (Figure 7 and Figure S3 in the Supporting Information). This is consistent with **4** having a higher affinity for ct-DNA than

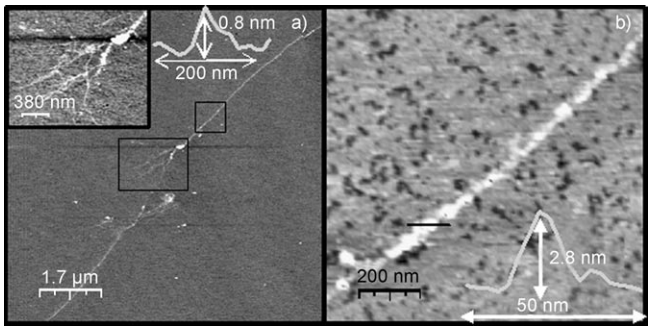


Figure 7. a) AFM topographic image of ct-DNA incubated with **4**. The inset shows several ct-DNA strands with a height of 0.8 nm indicating that the longer structure is a bundle with a height of 2.6 nm, much higher that the typical height measured with AFM for individual ct-DNA strands. b) A magnified image taken from the smaller square marked in a).

1, which matches the conclusions drawn from the spectroscopic studies. Very large structures can now be seen on the mica surface. These structures are likely formed by a mixture of ct-DNA double strands and metallacalixarenes that result in long fibres that in some cases (Figure 7a inset) give rise to large two-dimensional networks.

Shown in Figure 7b is an enlargement of the marked region of Figure 7a. In this image a quasi one-dimensional granular structure is clearly seen. The image suggests that ct-DNA/metallacalixarene assemblies give rise to the formation of long filaments of high persistence length. It is remarkable that in the central region of Figure 7a several strands of what are presumably individual ct-DNA molecules can be seen coming out from the fibre (see inset of Figure 7a).

Conclusions

We have observed clear non-covalent interactions between metallacalix[4]arenes and calf thymus DNA (ct-DNA). The non-covalent interactions were expected, based on the chemically inert nature of the platinum-containing metallacalix[4]arenes, and were deduced from the spectroscopic and AFM studies. The AFM images clearly show that the assayed compounds **1** and **4** interact with ct-DNA to produce supramolecular ct-DNA/metallacalixarene assemblies that induce conformational changes in the ct-DNA. This type of supramolecular interaction does not actively reduce the flexibility of the ct-DNA at low loadings (perhaps causing some small amounts of kinking or coiling), but this situation is reversed at higher loading with the formation of inflexible ct-DNA filaments. We presume that low concentrations of tetracationic metallacalixarene start to quench the ct-DNA charge and may enhance flexibility. This situation is reversed at higher complex concentrations, probably as a consequence of the occupation of a large number of ct-DNA binding sites, with a concomitant ct-DNA uncoiling, reduction in flexibility and subsequent aggregation into higher-order structures.

The contrast with previous studies on the effects of tetracationic cylinders on ct-DNA is revealing. Both classes of agents are tetracations that have extensive π surfaces and both bind non-covalently to ct-DNA. The cylinders induce dramatic intramolecular ct-DNA coiling at both low and high loadings, whereas these metallacalixarene agents do not induce dramatic coiling effects but rather reduce the flexibility of and aggregate the ct-DNA. Tetracationic spermines condense, and may aggregate, ct-DNA but do not impart a reduction in flexibility or lead to fibre formation.

This not only highlights the importance of the precise cylindrical structure of the previously reported supramolecular ct-DNA-coiling agents, but implies that different supramolecular drug designs might be used to induce different DNA structural effects. It remains to be seen whether there could be any biological relevance or application of the DNA conformational changes induced by this kind of supramolec-

ular interaction described herein and this is the subject of planned future studies.

Experimental Section

Materials: 2-pyrimidinol·HCl (2-Hpymo·HCl) was purchased from Aldrich. 5-chloro-2-hydroxypyrimidine,[15] 5-bromo-2-hydroxypyrimidine,[16] 5-iodo-2-hydroxypyrimidine,[16] and [{Pt(en)(pymo)}$_4$] $(NO_3)_4$ (**1**)[10] were prepared according to literature methods.

Preparation of [{Pt(en)(L)}$_4$](NO$_3$)$_4$ (2: LH=5-Chloro-2-pyrimidinolate; 3: LH=5-Bromo-2-pyrimidinolate; 4: LH=5-Iodo-2-pyrimidinolate): [$PtCl_2$(en)] (1 mmol) was suspended in an aqueous solution of $AgNO_3$ (2 mmol, 25 mL). The suspension was stirred in the dark at 60 °C overnight. The resulting mixture was kept at 4 °C for some minutes before the AgCl precipitate was filtered off. The resulting filtrate was mixed with an aqueous solution of the corresponding HL (1 mmol in 30 mL), the pH was adjusted to 5.5 by means of NaOH (1 M) and the mixture was allowed to react at 60 °C for 5 h. The mixture was reduced to 10 mL by means of a rotary evaporator to afford the corresponding metallacalixarene.

Complex 2: Yield: 80 %; ^{1}H NMR (400 MHz, D_2O, 25 °C, tetramethylammonium tetrafluoroborate (TMA)): δ = 2.5–2.7 (m, 16H; en), 8.43 ppm (s, 8H; H4,H4′-5Cl-pymo); elemental analysis calcd (%) for $C_{24}N_{20}H_{40}O_{16}Pt_4Cl_4{\cdot}8H_2O$ (1930.96 g mol^{-1}): C 14.92, H 2.92, N 14.50; found: C 14.85, H 2.40, N 14.54.

Complex 3: Yield: 80 %; ^{1}H NMR (400 MHz, D_2O, 25 °C, TMA): δ = 2.5–2.7 (m, 16H; en), 8.49 ppm (s, 8H; H4,H4′-5Br-pymo); elemental analysis calcd (%) for $C_{24}N_{20}H_{40}O_{16}Pt_4Br_4{\cdot}8H_2O$ (2108.76 g mol^{-1}): C 13.67, H 2.67, N 13.28; found: C 13.50, H 2.39, N 13.15.

Complex 4: Yield: 75 %; ^{1}H NMR (400 MHz, D_2O, 25 °C, TMA): δ = 2.5–2.7 (m, 16H; en), 8.53 ppm (s, 8H; H4,H4′-5I-pymo); elemental analysis calcd (%) for $C_{24}N_{20}H_{40}O_{16}Pt_4I_4{\cdot}8H_2O$ (2296.77 g mol^{-1}): C 12.55, H 2.45, N 12.19; found: C 12.59, H 2.25, N 12.34.

DNA binding studies: Calf-thymus DNA (ct-DNA) was purchased from Sigma/Aldrich. The ct-DNA was dissolved in water without any further purification and kept frozen until the day of the experiment. The ct-DNA concentration (moles of bases per litre) was determined spectroscopically by using the molar extinction coefficients at the maximum of the long-wavelength absorbance (ct-DNA ε_{258} = 6600 cm^{-1} mol^{-1} dm^3). Concentrations of stock solutions of the metallacalix[4]arenes were determined from accurately weighed samples of this material. A stock sodium cacodylate buffer (100 mM) was prepared by mixing a 50 mL solution of sodium cacodylate (0.2 M, 4.24 g of $Na(CH_2)_2AsO_2{\cdot}3H_2O$ in 100 mL) with 9.3 mL of hydrochloric acid (0.2 M), and diluting to a total of 100 mL. Stock solutions of the metallacalix[4]arene (500 μM) and ct-DNA (300 μM) were prepared by using sodium cacodylic buffer (1 mM) and NaCl (20 mM). Spectroscopic titration series experiments keeping the ct-DNA concentration constant were undertaken by adding the salt, buffer, water and metallacalix[4]arene to the ct-DNA. The circular dichroism (CD) spectra were produced by using a Jasco J-715 spectropolarimeter. Linear dichroism (LD) experiments were performed in a Jasco J-715 spectropolarimeter adapted for LD using a 1 mm pathlength Couette flow cell.

Ethidium bromide (EB) displacement by the metallacalix[4]arenes was calculated by measuring the quenching of the EB fluorescence as it leaves the protection of the ct-DNA.

A ct-DNA/salts/buffer solution with EB (ct-DNA/EB 4:5, 4 μM:5 μM) was prepared. The emission spectrum was recorded as a function of metallacalix[4]arene concentration by using a Perkin–Elmer Luminescence spectrometer (LS50b) and the metallacalix[4]arene concentration was slowly increased for ct-DNA/metal-complex ratios from 70:1 to 1:1 keeping the ct-DNA and EB concentrations constant. After each addition the fluorescence and UV-visible spectra were recorded (parameters: emission: 600 nm; excitation: 540 nm; excitation slit: 10.0 nm; emission slit: 15.0 nm).

The gel electrophoresis was done using a HE99X Max submarine electrophoresis tray and EPS 3051 power supply from Amersham, UK. The power used was 7.5 V per cm of gel and run in 1X TAE buffer. The gel was 1 % agarose in 1X TAE buffer. The dye mixture was 0.5 % bromophenol blue and 0.5 % xylene cyanole in 30 % glycerol/70 % water. It was developed by soaking for 30 min in a bath of ethidium bromide (0.05 μg mL^{-1}), followed by 5 min in a magnesium chloride (0.01 M) and irradiated at 312 nm for visualisation.

Atomic force microscopy (AFM):

Adsorption of blank calf thymus DNA: Samples were prepared by depositing a drop (10 μL) of ct-DNA solution (0.001 g L^{-1}) containing $MgCl_2$ (4 mM) onto a recently cleaved muscovite mica sheet (Electron Microscopy Sciences). After adsorption for 2 min at room temperature, the samples were gently rinsed with Milli-Q quality water and dried with nitrogen.

*Incubation of ct-DNA with **1a** and **4***: Solutions of ct-DNA (0.01 g L)$^{-1}$ were incubated at 25 °C with **1a** and **4** (10 basepairs/metallacalixarene) for one hour.

Adsorption of ct-DNA–platinumcalix[4]arenes: The ct-DNA–metallacalixarene solutions were diluted 10 times. A drop of these solutions (10 μL) was deposited onto a recently cleaved sheet of mica for 2 min. The sample was then rinsed and dried as described above.

AFM imaging: AFM images were acquired in dynamic mode using a Nanotec Electronica system (http://www.nanotec.es).[17] Olympus cantilevers were used with a nominal force constant of 0.75 N m^{-1}.

Acknowledgements

The authors acknowledge the Spanish Ministry of Education and Science for grants CTQ2005-00329/BQU, MAT2004-05589-C02-01/02 and NISAN/IST-2001-38052 and for PhD fellowships (M.A.G.). We also thank Junta de Andalucía and COST-D20 Action for funding. This work was conducted within the framework of COST D20 (metal complexes in anti-cancer and anti-viral therapy) and working group D20/0010/02 (non-covalent DNA recognition), and supported an exchange of researchers (M.A.G.).

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Received: November 6, 2006
Published online: April 30, 2007

