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Tetraaza[14]macrocyclic Transition Metal Complexes as DNA Intercalators

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Six tetraazamacrocyclic copper(II) and nickel(II) complexes have been synthesised and their interactions with double-stranded DNA (calf-thymus DNA) have been studied using circular and linear dichroism, as well as other spectroscopic methods. The ability of the studied complexes to intercalate between the DNA base-pairs has been demonstrated qualitatively and confirmed by determining the stoichiometry and association constants values that were found to be between 8.5×10^3 and 2.8×10^4. The nickel(II) complexes, being more electron-deficient, seem to have higher binding abilities than guests containing the copper ion. It has been also shown that not only CD, but also linear dichroism, can be a very useful tool for qualitative DNA interactions studies.

Introduction

There is a large number of DNA intercalators being extensively studied recently.[1] Their ability to inhibit the synthesis of nucleic acids in living organisms, and thus the potential to act as anticancer agents, is one of the most important reasons why they have lately attracted much attention from researchers.[2,3] A vast majority of intercalator molecules contain aromatic multi-ring π-acceptor components (e.g. dipyridophenazine,[4] phenanthrolino[5,6-*b*]hexaazatriphenylene[5] and pyrene[6] derivatives) able to π-stack between the base-pairs. The systems are often linked to functional groups, which may be involved in many kinds of interactions, such as van der Waals forces, hydrogen-bonding, as well as electrostatic and dipolar interactions, since they affect the DNA binding strength. Some of intercalators can act as photoactive[7-9] or redox[10,11] centres, enabling the use of such compounds for an effective detection of nucleic acids, that can be utilised in various areas, particularly in medicine and pharmaceutical industry.[1,10]

Thus it is very important to be able to modify various aspects of molecular properties accurately and in a controlled manner so the studied systems could interact with biomolecules in a desired way, allowing at the same time to track changes resulting from the interactions. A great versatility in terms of the properties and their modification opportunities is usually offered by transition metal complexes of organic ligands.

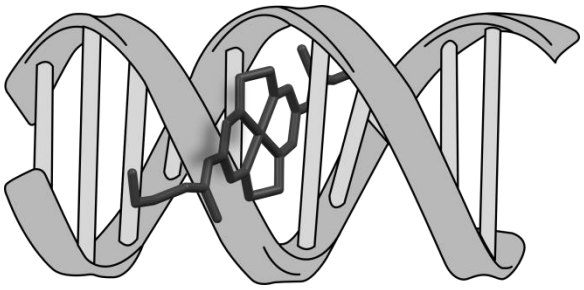
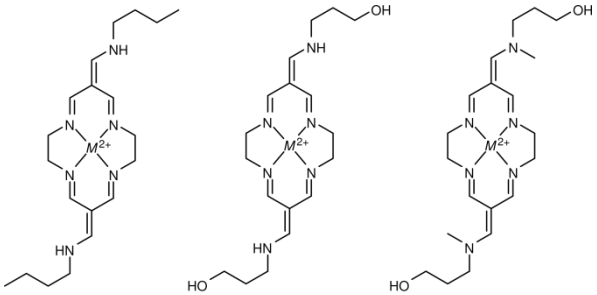


Figure 1 Schematic representation of a cyclidene intercalating into DNA.



Scheme 1 Complexes selected for the interaction studies. The butylamine derivatives were used as chlorides, while others (dihydroxy derivatives) studied as hexafluorophosphates.

Cyclidenes[12,13] – being a representative example of metal complexes – combine the ease of synthesis and modification with rich electrochemistry and spectroscopic properties. They are water-stable π-electron rich tetraazamacrocyclic systems (also called Jäger complexes), that during the last decade they have been used by the group of Korybut-Daszkiewicz as building blocks for the synthesis of a range of macrocycles, including linear compounds, oligomacrocycles,[14,15] catenanes,[16,17] pseudorotaxanes[18] and rotaxanes.[19] It has been demonstrated

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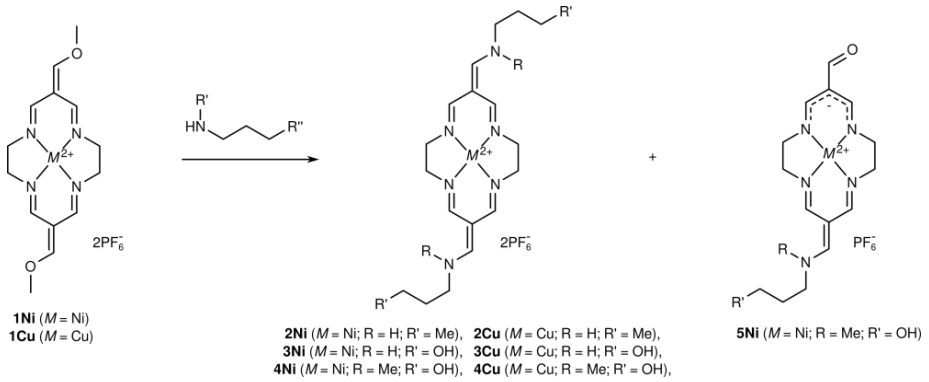

Scheme 2 The synthesis starting from *O*-methylated complexes.

that some of those can act as molecular devices,[17] while others are able to recognise small aromatic systems.[20]

The 14-membered cyclidene units are relatively easy to obtain[21-23] and able to coordinate various transition metal ions, such as copper(II), nickel(II), but also iron(II), cobalt(II, III), palladium(II) and platinum(II, IV).[13,23] The rings are π-electron rich and almost ideally planar. As a consequence, the π-π-stacking can be observed in most of X-ray structures of cyclidene derivatives[14,23-26]

The solubility of the complexes is determined by functional groups attached to the macrocycle and also depends on the type of counterion used for the crystallisation. However, small monomacrocyclic cyclidenes are usually well soluble in water. Moreover, they are fully stable either in aqueous solutions or in common organic solvents, particularly as most extensively studied copper(II) and nickel(II) complexes. The dissociation to metal cation and free tetraaza[14]macrocyclic ligand was never observed in nickel(II) and copper(II) cyclidenes, as confirmed many times using NMR and mass spectrometry.[14-22] The removal of the metal ion can only occur in the presence of strong acids in dry solvents. Moreover, a large number of heterodinuclear bismacrocyclic complexes, containing both copper and nickel ion in one molecule have been previously obtained and neither the presence of free ligand in solution nor metal scrambling was observed.

The X-Ray studies of tetraaza[14]macrocyclic complexes show that the charge is not located on the metal ion only but is delocalised over all sp^2 atoms, up to the exocyclic nitrogen atoms (if deprotonated). As a result, the ring becomes relatively hydrophobic, what can be observed in catenanes, where the benzene ring of the crown ether moiety is located in the cavity between two opposite cyclidene rings.[16,17] Very close analogues of the studied compounds, namely 16-membered cyclidenes, do also form a hydrophobic cavity, that is in aqueous media able to host aliphatic and aromatic alcohols. NMR relaxation techniques indicated their hydroxyl groups remaining outside the cavity[27-30] which also confirms the hydrophobic nature of cyclidene rings. It is also worth mentioning, that the copper ion tends very weakly to coordinate ligands axially, while nickel(II) complexes seem to be square-planar only, as observed in cyclidenes' X-ray structures solved so far.

All the above-mentioned features, along with the ability to tune up their properties easily, either by functionalising[20] or, as already noted, by coordinating different metal ions, make cyclidene derivatives very promising, not only as DNA intercalators, but also in terms of future analytical and medical applications. Additionally, the accessibility of various oxidation states of the metal centre may also be very useful, especially for applications that involve electrochemical processes. There are few examples of recently reported DNA interaction studies carried out using similar tetraazamacrocyclic derivatives, confirming their ability to intercalate between the base-pairs.[31,32] However, the chemical structure of those compounds, and thus synthetic procedures, along with the functionalisation capabilities, is far more complicated if compared to complexes presented here, which makes the latter and their derivatives easily available and much cheaper.

In this paper the synthesis and DNA binding properties of certain cyclidene copper(II) and nickel(II) complexes is described. We studied the binding using various spectroscopic methods including circular dichroism, as well as linear dichroism,[33,34] which is a polarised light absorbance technique applied to oriented molecules, particularly useful in analyses of biomaterial systems.[35]

Results and Discussion

Synthesis and characterisation

It has been reported earlier[15,20,36] that the coordination of various metal ions together with the presence of methyl substituent at the exocyclic nitrogen atom[20] can visibly change the π-accepting properties of a cyclidene system. Furthermore, introducing the hydroxyl group at the terminal position of each aliphatic chain should modify both the complex solubility in water and the affinity to π-donors, including DNA. We therefore functionalised the nickel(II) and copper(II) cyclidene units using three different amines: *n*-butylamine, 3-aminopropan-1-ol and *N*-methyl-3-aminopropan-1-ol to test these hypotheses. The synthesis was carried out in an analogous way to the methods used for the synthesis of other cyclidene systems that were obtained previously by the group of Korybut-Daszkiewicz.[14-17,31] The complexes were separated using reversed-phase silica gel and crystallised from water/acetonitrile mixture with an excess of ammonium hexafluorophosphate upon slow evaporation of solvents. The corresponding chlorides were precipitated from acetonitrile solutions of hexafluorophosphate salts by adding a large excess of anhydrous tetrabutylammonium chloride. The chloride salts of **2Cu** and **2Ni** were obtained in order to increase the solubility

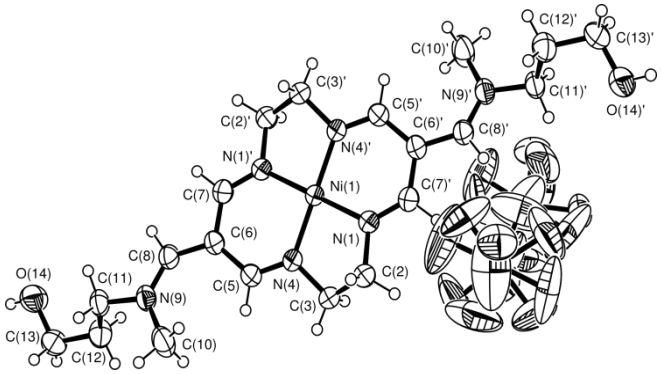

Figure 2 X-ray structure of compound **4Ni**.

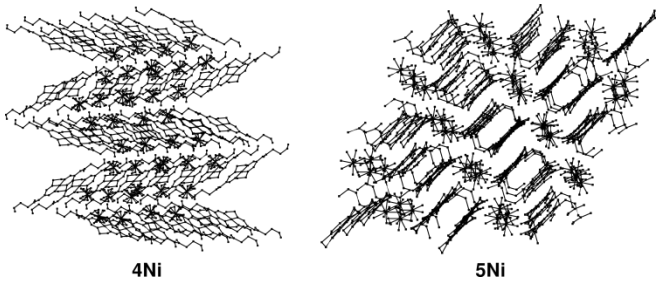

Figure 3 A view of the crystal packing of **4Ni** (down c crystallographic axis) and **5Ni** (down b axis) complexes. Hydrogen atoms were omitted for clarity.

of the complexes in water. The hexafluorophosphates of cyclidenes containing terminal hydroxyl group (**3Ni**, **3Cu**, **4Ni** and **4Cu**) were well soluble in water, and therefore their conversion into corresponding chlorides prior the interaction studies was not necessary.

X-ray crystallography

Only **4Ni** complex and its by-product (**5Ni**) gave crystals of a quality sufficient for data collection.

4Ni (Fig. 2). Unlike the most of π-electron rich tetraazamacrocycles that were previously studied, no face-to-face orientation of cyclidene rings, as well as no π-stacking can be observed in the structure of **4Ni** (Fig. 3). Cyclidene systems form a herringbone-pattern with an angle enclosed by the normals to the ring planes of 76.5°. Although there are two hydroxyl groups in the compound, there are no hydrogen bonds in its crystal structure. Each hydroxyl group interacts weakly with a fluoride atom (D···A distance of 2.947 Å) of hexafluorophosphate and with exocyclic methyl group (D···A distance of 3.539 Å).

5Ni. An interesting feature is that pairs of **5Ni** (Fig. 4) molecules tend to form box-like structures (Fig. 5), similarly to those observed in the case of bis-macrocycles' published earlier.[20,36–38] The two macrocyclic rings of each such pair are face-to-face oriented and bridged via intermolecular hydrogen bond between O(16) and O(23) with D···A distance of 2.742 Å. The distance between two mean-planes within the dimer, defined by 14-membered ring atoms of each cyclidene unit is equal to 3.747 Å, while the corresponding nickel···nickel distance value is 4.302 Å. These distances are visibly shorter than those observed between cyclidene moieties, being covalently bridged by very short linkers (propylenediamine[33] and N,N-methylpropylenediamine[20]

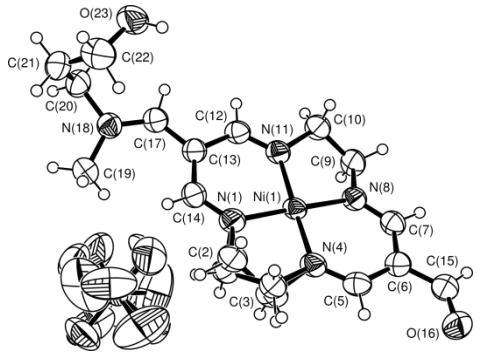

Figure 4 **5Ni** complex crystallographic structure.

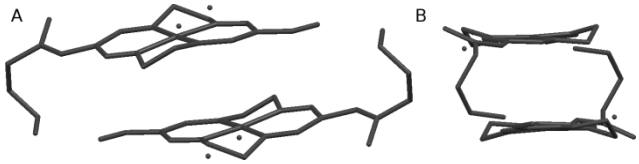

Figure 5 Dimer-like alignment of **5Ni** molecules –views down crystallographic a (A), and b (B) axis.

derivatives), where the intramolecular distance between cyclidene ring mean planes vary from 3.803 Å to 3.937 Å, and metal···metal distance is of 4.355 Å to 4.464 Å. Moreover, the analogous distances observed in crystal structures of donor-acceptor complexes are also slightly longer than for **5Ni**, with the corresponding minimum values of 4.577 Å (metal...metal) and 3.751 Å (between the mean-planes).[39] In the crystal structure, cyclidene rings are packed approximately parallel, however face-to-face alignment can only be observed within hydrogen bond-bridged pairs mentioned above.

Crystallographic data (excluding structural factors) for the structures of complexes **4Ni** and **5Ni** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre and allocated the deposition numbers CCDC 979762 and CCDC 979763 respectively. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EW, U.K. (Fax: Int code + (1223)336-033. E-mail: deposit@ccdc.cam.ac.uk).

Binding studies

Linear dichroism was used to probe the binding of the complexes with DNA. The cyclidenes themselves show no LD (except for a slight scattering at low wavelength), however the spectra of their mixtures with calf-thymus DNA show relatively strong bands (Fig. 6 and 7) at the wavelengths where the complexes absorb light (Fig. 8) and DNA does not. Since the linear dichroism can only be observed if molecules are oriented this indicates clearly that the investigated complexes bind to DNA. Moreover, the complexes have LD bands at longer wavelength than the complex absorbance in the absence of DNA. Since LD only reports signals for bound ligands, this suggests the molecules bound to DNA are experiencing π-stacking and thus intercalating (similar effects were observed in CD and UV/Vis experiments discussed later in this article). This is consistent with the sign of the LD of the observed MLCT transitions (assigned as according to literature[40] data for similar complexes), that is negative for **3Ni**

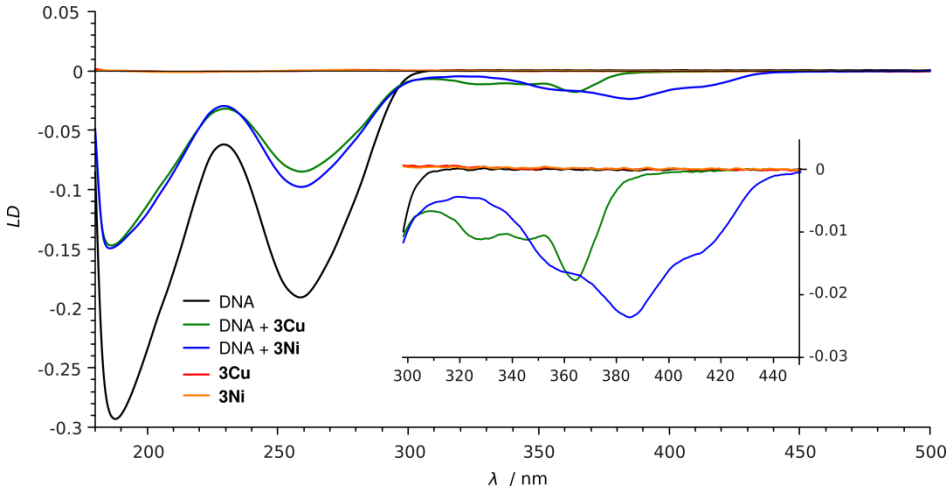

Figure 6 LD spectra of: **3Ni** (5×10^{-4} M), **3Cu** (5×10^{-4} M), ct-DNA (1×10^{-3} M), equivoluminal mixtures of the above ct-DNA and complex solutions (blue and green curve respectively).

and **3Cu** across the entire spectrum indicating the plane of the metal complexes is perpendicular to the helix axis of the DNA.

When we come to consider interactions with DNA of **2Ni** and **2Cu** from the data shown in Figure 7 we get a more complicated picture. The LD sign is negative from 350 nm upwards for **2Ni** and from 360 nm for **2Cu**. The positive LD at lower wavelengths, which is probably a minimum in the bound spectrum if intercalation dominates, suggests that some of molecules are groove bound. There are at least two binding modes then, where one involves a shift to longer wavelength and negative LD sign, which is consistent with intercalation.

In the DNA region of the spectrum the absorbance is dominated by the DNA signal. The *LD* of the DNA with **2Ni** and **2Cu** complexes has the same shape as the DNA alone but the spectrum of the mixture containing **2Ni** is visibly reduced in magnitude (Fig. 7, blue curve), which indicates that **2Ni** bends the DNA or make it significantly more flexible – this suggests groove binding not intercalation which stiffens the DNA. Such a reduction in the intensity may not indicate the binding strength directly, however it demonstrates that interaction with the DNA is particularly favourable for **2Ni** in aqueous media, especially as according to earlier studies,[20] the nickel cyclidenes can interact with π-donors stronger. Higher affinity to the DNA base pairs may additionally be supported by slightly higher hydrophobicity of the nickel complex in comparison to the copper derivative, which may be particularly important for groove binding. At the same time, in the mixed sample (DNA, **2Ni** and **2Cu**; Fig. 7, grey curve), **2Ni** influences visibly stronger on the metal complex absorbance region of the *LD* spectrum than analogous copper derivative. The DNA *LD* signals intensity is however only slightly reduced. Since both complexes intercalate and both are most likely able to undergo groove binding, it seems like either the groove binding occurs within different sites of the DNA or the nickel complex intercalation dominates the interaction.

Induced dichroism can also be observed when circularly polarised light is used, although the investigated metal complexes are themselves achiral. The CD spectra of DNA mixtures with the studied complexes show strong positive bands in the ligand absorption regions (Fig. 9). These bands appear at longer wavelengths than the absorption maxima observed in UV/Vis absorbance spectra of the studied compounds without addition of DNA. Similar to the LD results, the induced signals of nickel complexes dominate CD spectra of mixtures containing the DNA and the complexes with two different metal ions (dotted curve, Fig. 9). Moreover, the addition of the complexes to the DNA causes shifting of the bands in the DNA region towards longer wavelengths, which cannot unambiguously be identified as an effect of the intercalation, however confirms that the studied compounds interact with the DNA.

The binding stoichiometry was determined by carrying out UV/Visible absorbance titrations with the mole fractions varied while keeping the total amount (concentration) of interacting components constant, that is often called the Job's method or the method of continuous variations.[40,41] Although the LD experiments suggest more than one mode of binding for **2Ni** and **2Cu**, the titrations and their analysis were carried out with the assumption that interaction ratio is equal to one guest molecule per two base-pairs, thus in accordance to the neighbour-exclusion binding model.[42] The sum of metal complex concentration and doubled DNA base-pair (*i.e.* 4 DNA bases) concentration was then kept constant. In the resulting Job plots (Fig. 10) of n-butyl derivatives, peaks were found at the molar fraction values of $x \approx 0.67$ and 0.6 for **2Ni** and **2Cu** respectively (with theoretical values of 0.67 for 2:1 and 0.5 for 1:1 ratio). This means the stoichiometry is close to 2:1, which is equivalent to one macrocycle per one base-

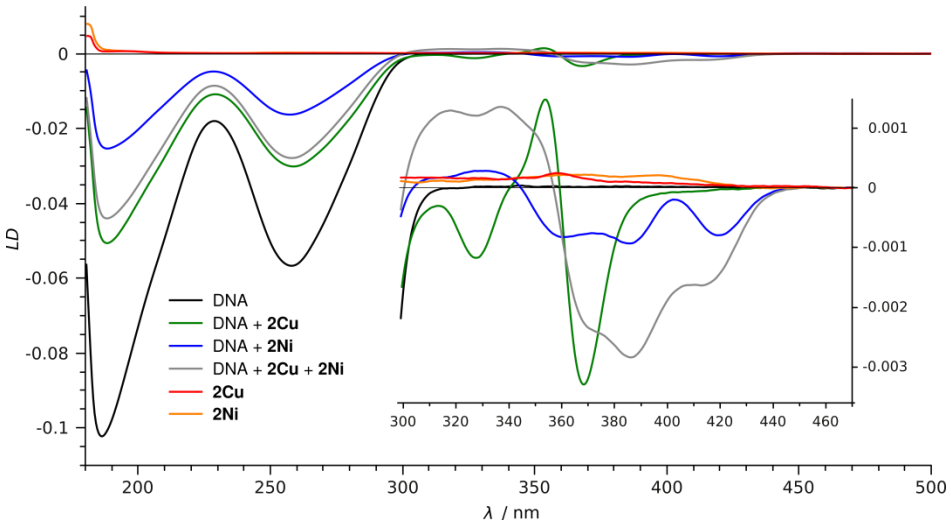

Figure 7 LD spectra of: **2Ni** (1×10^{-3} M), **2Cu** (1×10^{-3} M), ct-DNA (1×10^{-3} M), equivoluminal mixtures of the above ct-DNA and complex solutions (blue and green curve respectively) 2:1:1 mixture of ct-DNA, **2Ni** and **2Cu** solutions (grey curve).

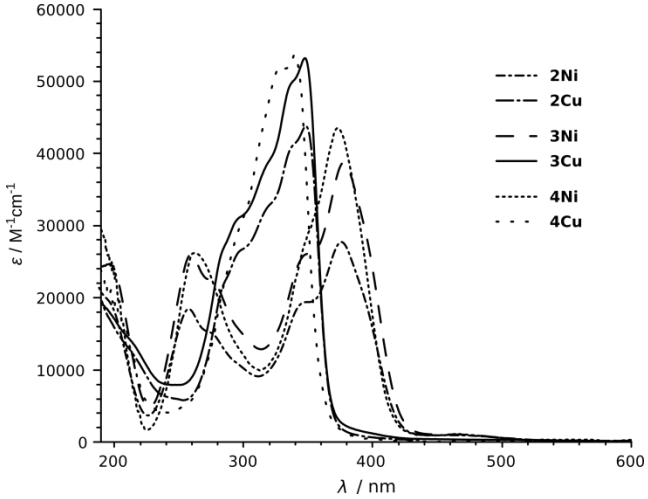

Figure 8 An overlay of absorption spectra of the complexes dissolved in water (c = 3 × 10^{-5} M, l = 1 cm).

pair. This is an extremely dense binding ratio and given the predominance of nearest neighbour exclusion in intercalative binding requires at least some groove binding as suggested by the LD. The remaining guests show binding with 1:1 ratio, for **4Ni** and **4Cu**, or nearly 1:1 stoichiometry, for **3Ni** and **3Cu** – two base pairs per one guest molecule in accord with nearest neighbour exclusion intercalative binding.

The presence of calf thymus DNA in the solution of the studied complexes is associated with a moderate hypochromic effect. The red shift values of 3 – 3.5 nm (Table 1) are common for all compounds under investigation and similar to shifts observed for *phen* and *DIP* based intercalating metal complexes.[44] The red-shift magnitude is often correlated with a relative DNA binding strength and is caused by the stacking interactions of intercalating molecules with the base pairs.[44,45] However, for the studied compounds, only the red-shift value for **4Ni** is distinctly different from the others. In order to determine the actual binding strength a series of UV/Vis titrations were carried out.

The interactions of guest molecules (G) with calf-thymus DNA can be described as

$$\mathrm{DNA} + \mathrm{G} \rightleftarrows \mathrm{DNA} \cdot \mathrm{G}$$

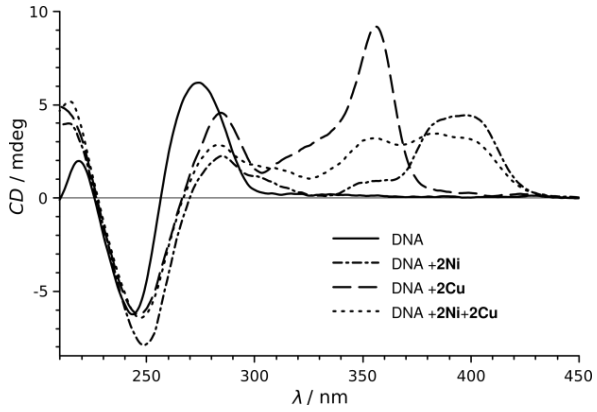

Figure 9 CD spectra of: ct-DNA (5 × 10^{-4} M), the mixture of **2Cu** and ct-DNA (both 5 × 10^{-4} M), the mixture of **2Ni** and ct-DNA (both 5 × 10^{-4} M), and 2:1:1 mixture of ct-DNA (5 × 10^{-4} M), **2Cu** (2.5 × 10^{-4} M) and **2Ni** (2.5 × 10^{-4} M). The actual concentrations of each component were given (ct-DNA conc. given in bp). All compounds were dissolved in water (l = 0.1 cm).

and the binding constant as

$$K = \frac{[\mathrm{DNA}]}{[\mathrm{DNA}][\mathrm{G}]}$$

Putting the concentration values from the Lambert–Beer law will lead to the following equation, according to methods reported in literature,[46-48]

$$\frac{[\mathrm{DNA}]}{\Delta\varepsilon_i} = \frac{1}{\Delta\varepsilon}[\mathrm{DNA}] + \frac{1}{\Delta\varepsilon K}$$

where $\Delta\varepsilon = \varepsilon_0 - \varepsilon_B$ and $\Delta\varepsilon_i = \varepsilon_0 - \varepsilon_i$. ε_0 is the extinction coefficient of free metal complex (at maxima near 380 nm for nickel, and 350 nm for copper derivatives), ε_B corresponds to fully bound guest, and ε_i is the absorption coefficient observed during titrations. The half-reciprocal plot of [DNA]/$\Delta\varepsilon_i$ vs. [DNA] is linear and the binding constant can be estimated from the ratio of the line slope to its *y*-intercept (Fig. 11).

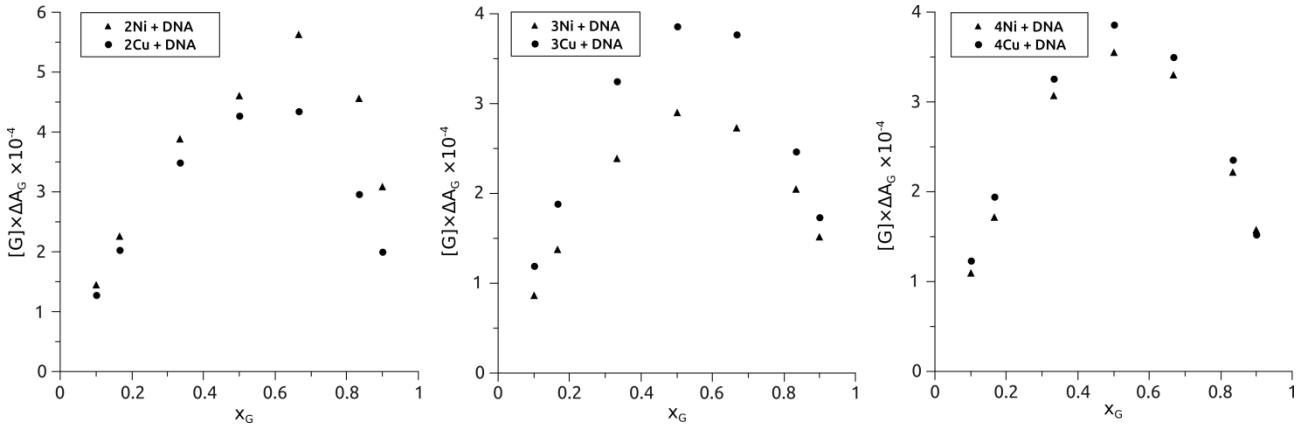

Figure 10 Job's plots based on UV titrations of nickel (▲) and copper (●) complexes' mixtures with calf-thymus DNA in total concentration of 5×10^{-4}.

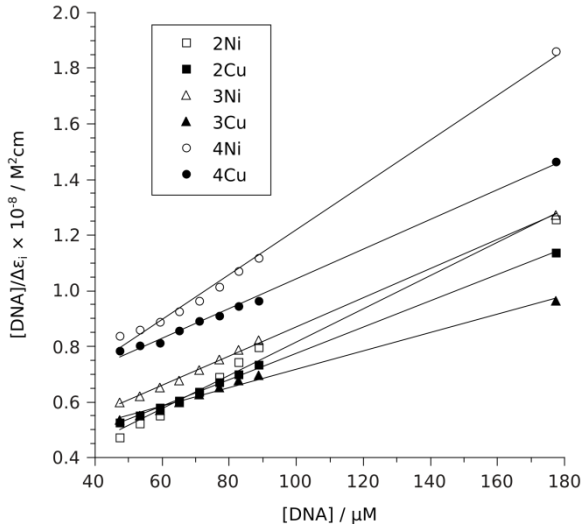

Figure 11 Half-reciprocal plot of [DNA]/$\Delta\varepsilon_i$ vs. DNA concentration (bp) for DNA mixtures with studied complexes (33.3 μM): **2Ni** (□), **2Cu** (■), **3Ni** (△), **3Cu**(▲), **4Ni**(○) and **4Cu**(●), where $\Delta\varepsilon_i$ is the difference between the initial extinction coefficient of a complex (ε_0) and observed during titrations (ε_i).

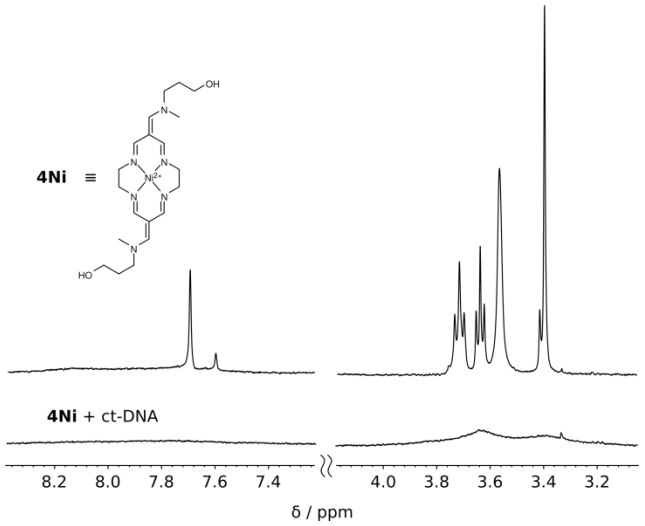

Figure 12 Spectra of **4Ni** (top) solution and its equivoluminal mixture with ct-DNA (bottom) solution. The stock solutions concentrations are 0.5 × 10^{-3} M for **4Ni** and 1 × 10^{-3} M (bp) for DNA. D_2O was used as a solvent.

Table 1. Summary results of interaction studies with ct-DNA.

guest	K_{assoc}	$logK_{assoc}$	*red shift (nm)*
2Ni	2.75 ± 0.30 × 10^4	4.44	3.0
2Cu	1.56 ± 0.03 × 10^4	4.19	3.5
3Ni	1.52 ± 0.03 × 10^4	4.18	3.5
3Cu	8.53 ± 0.37 × 10^3	3.93	3.5
4Ni	1.95 ± 0.10 × 10^4	4.29	3.5
4Cu	1.05 ± 0.03 × 10^4	4.02	3.0

The binding constants values (Table 1) are in the range of 8.5 × 10^3 up to 2.8 × 10^4, which is at least one order of magnitude lower than that of the best-known intercalator – ethidium bromide and its derivatives.[49] However, the constants are higher than those determined for Ru-bipy and Ru-phen[44] derivatives and similar to pyrene and anthracene intercalators[50-51] demonstrating, analogically to the studied complexes, a lack of positively charged supporting groups able to interact with the DNA's phosphates.

Relatively large errors obtained resulted from the inability to operate in concentrations higher than 1·10^{-3} mol·L^{-1}, which can cause the mixtures containing an excess of tetraazamacrocyclic complexes to precipitate. Since both components are soluble in water, it is likely that host-guest complex precipitation was observed as the DNA charge is neutralised by the ligands. Moreover, the precipitation was most noticeable in the case of **2Ni**, which, in addition to the determined binding constant and the effects observed using LD and CD, could be another confirmation of its highest affinity to the double-stranded calf-thymus DNA due in part to ist multiple binding modes.

Since cyclidene-based copper(II) complexes are paramagnetic only very broad signals corresponding to terminal groups can be observed in NMR thus interaction studies using this method are very difficult to carry out. However in the case of diamagnetic nickel(II) based cyclidenes NMR studies seem to be a good method for investigating their binding to the DNA. The ^{1}H NMR spectrum of **4Ni** in D_2O gives several well defined peaks with one sharp signal in the aromatic region and four signals between 3.3 and 3.8 ppm. Two additional peaks appear in the spectrum (at 7.60 ppm and 3.40 ppm) as a result of conformational isomerism involving rotation about the exocyclic =C−N bond, which is slow enough to see two rotamers at room temperature (the =C−N bond length is 1.302 Å according to X‑ray data, which is intermediate between characteristic values for single and double bond of such type). The addition of equal (according to the stoichiometry determined based on UV/Vis titrations) amount of double stranded calf-thymus DNA (Fig. 12) result in shifting and strong broadening of all 4Ni proton signals. Similar effects were observed by Korybut-Daszkiewicz as a result of π-stacking interactions between cyclidene-based mono- and bismacrocycles.[20] Furthermore, as reported by Schneider,[52] broadening of the ^{1}H NMR signals was observed in the spectra of selected bi- and tricyclic hetero- and non-heteroaromatic intercalators mixtures with calf thymus DNA. Such changes in the spectrum were classified as a result of intercalation if the signals' linewidth ($W_{½}$) was higher than 10 Hz. In the case of 4Ni in the presence of ct-DNA the proton signals are much broader than 10 Hz and the peaks are unresolved, which clearly indicates that intercalation is here a dominating binding mode.

Conclusions

As a result of the research presented here a series of transition metal complexes of macrocyclic Schiff bases was synthesised, isolated and characterised. Their ability to intercalate into the DNA duplex was confirmed qualitatively using linear and circular dichroism, as well as NMR. In case of both LD and CD techniques, a clear effect of induced dichroism was observed for the investigated compounds in the presence of DNA. Moreover, as a result of the DNA addition the guest's ^{1}H NMR signals broaden strongly. Additionally, it was found that the DNA base-pairs interact with the −OH functionalised complexes in ratio of approximately 2:1, while the stoichiometry for n-butyl derivatives is even close to 1:1, suggesting that groove binding may also have a contribution in the interactions, at least in case of **2Ni** and **2Cu**. Moreover, the determined constants, that are in the range of 8.5 × 10^3 (**3Cu**) to 2.8 × 10^4 (**2Ni**), as well as the adduct precipitation, suggest that more electron-deficient nickel(II) complexes intercalate visibly stronger than corresponding copper(II) derivatives.

Seeking new compounds able to bind to DNA and learning of models describing this kind of interactions may be very useful in the field of medical science. It is essential in terms of designing new anticancer drugs, but also other molecular systems, that could be particularly helpful in the area of diagnostics, as many intercalating molecules are recently tested for DNA sensing purposes. Therefore, very important is the synthesis of novel model compounds able to intercalate between the DNA base-pairs. In particular, transition metal complexes seem to be very promising, as their binding abilities can easily be tuned either by changing the metal's oxidation state or by replacing the metal ion as it has been demonstrated here, where some significant differences in binding are observed in LD spectra upon changing the metal ion. In order to further modify the properties of intercalating complexes we were able to slightly increase the cyclidene unit's hydrophobic character by methylating the exocyclic nitrogen atoms, as well as to change the hydrophillicity of guest molecules through introducing terminal hydroxyl groups. As a result, by tuning substituents remote from the metal centre we have been able to tune binding from purely intercalative to mixed intercalation/groove binding.

During further studies the number of model compounds will be increased. Modifications will involve the coordination of other metal ions as well as introducing additional functional groups allowing favourable interactions in the minor groove, in order to obtain more effective DNA intercalators. Moreover, intercalators showing high affinity to DNA will subsequently be used in cytotoxic activity studies. Additional studies will be carried out to describe the nature of the interactions, particularly the intercalation pattern.

Experimental Section

Materials and characterisation: **2Ni** and **2Cu** chlorides were synthesised following already published procedures.[45] All commercially available reagents were purchased and used without further purification. Solvents were purified and dried according to standard methods if necessary. Calf-thymus DNA sodium salt was purchased from Sigma-Aldrich.

^{1}H NMR analysis were carried out on either Varian Gemini 200 MHz or Varian Mercury 400 MHz spectrometer with CD3CN as a solvent. Elemental compositions (C, H and N) were determined using Elementar Vario El III analyser.

3Ni, 6,13-[(3-hydroxypropylamino)methylidene]-1,4,8,11-tetraazacyclotetradeca-4,7,11,14-tetraene-(2-)-$\kappa^4N^{1,4,8,11}$]nickel(II) bis(hexafluorophosphate): An excess of 3-amino1-propanol (0.24 mL; 3.2 mmol) was added to a stirred solution of **1Ni** (0.2 g; 0.32 mmol) in 25 mL of dry acetonitrile . The mixture was stirred at room temperature for 24 h. Then 0.5 g of ammonium hexafluorophosphate and 100 mL of water with na addition of 0.5 mL of hydrochloric acid. The yellow precipitate was filtered off and dried. The product was recrystallised upon slow evaporation of solvent from CH3CN and water with an addition of ammonium hexafluorophosphate. The crystalline was filtered off, washed with small amount of cold water and dried under vacuo. Yield 34 % (0.08 g). Elemental analysis (%) calcd for $C_{18}H_{30}N_6O_2P_2F_{12}Ni$ (711.09): C 30.40, H 4.25, N 11.82; found: C 30.46, H 4.04, N 11.76; *MS* (ESI, CH_3CN, m/z): 210.1 ($[C_{18}H_{30}N_6O_2Ni]^{2+}$), 419.2 ($[C_{18}H_{30}N_6O_2Ni]^{2+} - H^+$), 565.1 ($[C_{18}H_{30}N_6O_2Ni]^{2+} + PF_6^-$); *IR* (nujol, cm^{-1}): 3387 m, 2728 w, 1669 s, 1624 m, 1461 s, 1377 s; ^{1}*H NMR* (CD_3CN, 500 MHz, ppm): 1.81 p (4H, J = 6.77 Hz, $-CH_2-$ β to $-NH-$); 2.77 v br (2H, −OH), 3.55 br (α-CH_2); 3.49 m (8H, $N-CH_2-CH_2-N$); 3.59 m (4H, γ-CH_2); 7.50 s (2H, HC=N); 7.66 s (2H, C=CH−N); 7.94 s (2H, HC=N); ^{13}*C NMR* (CD_3CN, 125 MHz, ppm): 32.7 (β-CH_2), 49.2 (α-CH_2), 59.3 (γ-CH_2), 59.5 ($N-CH_2-CH_2-N$), 60.4 ($N-CH_2-CH_2-N$), 104.1 (4° ring C), 155.0 (HC=N), 160.7 (HC=N), 164.0 (C=CH-N).

3Cu, 6,13-bis[(3-hydroxypropylamino)methylidene]-1,4,8,11tetraazacyclotetradeca-4,7,11,14-tetraene-(2–)-$\kappa^4N^{1,4,8,11}$]- copper(II) bis(hexafluorophosphate): The synthesis was carried out following the same procedure as shown above. Yield 46 %. Elemental analysis (%) calcd for $C_{18}H_{30}N_6O_2P_2F_{12}Cu$ (715.94): C 30.20, H 4.22, N 11.74; found: C 30.22, H 4.33, N 11.72; *MS* (ESI, CH_3CN, m/z): 212.6 ($[C_{18}H_{30}N_6O_2Cu]^{2+}$), 424.2 ($[C_{18}H_{30}N_6O_2Cu]^{2+} - H^+$), 570.1 ($[C_{18}H_{30}N_6O_2Ni]^{2+} + PF_6^-$); *IR* (nujol, cm^{-1}): 3377 m, 2726 w, 1668 s, 1617 m, 1463 s, 1376 s.

4Ni, 6,13-bis[(3-hydroxypropylmethylamino)methylidene]-1,4,8,11-tetraazacyclotetradeca-4,7,11,14-tetraene-(2–)-$\kappa^4N^{1,4,8,11}$]nickel(II) bis(hexafluorophosphate): 0.312 g (0.5 mmol) of 1Ni was dissolved in 15 mL of dry acetonitrile and 0.1 mL (1.05 mmol) of 3-hydroxypropylenemethylamine was added to the stirred solution. After 5 h the mixture was diluted with 60 mL of water and applied on a reversed phase silica gel (silanised) column. 20 % acetonitrile (aq.) with an addition of ammonium hexafluorophosphate (0.5 g/100 mL) was used as an eluent. The first fraction was collected and evaporated in order to remove the organic solvent. An excess of ammonium hexafluorophosphate (5 g) was added and the pure product crystallised in the fridge as yellow crystals. Yield 72 % (0.264 g). Elemental analysis (%) calcd for $C_{20}H_{34}N_6O_2NiP_2F_{12}$ (739.14): C 32.50, H 4.64, N 11.37; calcd for $C_{20}H_{34}N_6O_2NiP_2F_{12}\cdot H_2O$ (757.16): C 31.73, H 4.79, N 11.10; found: C 31.71, H 4.87, N 11.17; *MS* (ESI, CH_3CN, m/z): 224.3 ($[C_{20}H_{34}N_6O_2Ni]^{2+}$), 593.2 ($[C_{20}H_{34}N_6O_2Ni]^{2+} + PF_6^-$); *IR* (nujol, cm^{-1}): 3615 m, 1614 s, 1557 w; ^{1}*H NMR* (CD_3CN, 400 MHz, ppm): 1.87 p (4H, J = 6.10 Hz, $-CH_2-$ β to $-NCH_3$), 2.76 br t (2H, J = 4.50 Hz, −OH), 3.34 s (6H, $-CH_3$), 3.54 br m (γ-CH_2), 3.55 m (8H, $N-CH_2-CH_2-N$), 3.68 t (4H, J = 6.95 Hz, α-CH_2), 7.61 s (2H, N−HC=C), 7.90 v br s (4H, HC=N); ^{13}*C NMR* (CD_3CN, 50 MHz, ppm): 30.6 (β-CH_2), 41.1 (α-CH_2), 58.2 ($-CH_3$), 60.4 (γ-CH_2), 59.6 br ($N-CH_2-CH_2-N$), 104.3 (4° ring C), 162.8 (C=CH-N), HC=N not visible due to dynamic broadening.

4Cu, 6,13-bis[(3-hydroxypropylmethylamino)methylidene]-1,4,8,11-tetraazacyclotetradeca-4,7,11,14-tetraene-(2–)-$\kappa^4N^{1,4,8,11}$]copper(II) bis(heksafluorophosphate): The synthesis was carried out following the same procedure as shown above. Yield 74 %. Elemental analysis (%) calcd for $C_{20}H_{34}N_6O_2CuP_2F_{12}$ (744.00): C 32.29, H 4.61, N 11.30; found: C 32.37, H 4.61, N 11.67; *MS* (ESI, CH_3CN, m/z): 226.8 ($[C_{20}H_{34}N_6O_2Cu]^{2+}$), 598.1 ($[C_{20}H_{34}N_6O_2Cu]^{2+} + PF_6^-$); *IR* (nujol, cm^{-1}): 3590 m, 1606 s, 1571 m.

5Ni, 6-formyl-13-[(3-hydroxypropylmethylamino)methylidene]-1,4,8,11-tetraazacyclotetradeca-4,6,11,14-tetraenato-(–)-$\kappa^4N^{1,4,8,11}$]nickel(II) hexafluorophosphate: The complex was obtained as a by-product in the synthesis of **4Ni**. It was collected as the second fraction from the silica gel silanised column and isolated in an analogous way. Yield 0.018 g (5 %). *MS* (ESI, CH_3CN, m/z): 381.1 ($[C_{16}H_{24}N_5O_2Ni]^+$), 422.1 ($[C_{16}H_{24}N_5O_2Ni]^+ + C_2H_5OH$); Elemental analysis (%) calcd for $C_{16}H_{24}N_5O_2NiPF_6$ (522.05): C 36.81, H 4.63, N 13.42; found: C 36.59, H 4.70, N 13.45; *MS* (ESI, CH_3CN, m/z): 376.1 ($[C_{16}H_{24}N_5O_2Ni]^+$), 522.1 ($[C_{16}H_{24}N_5O_2Ni]^+ + H^+ + PF_6^-$); ^{1}*H NMR* ($CD_3CN$, 200 MHz, ppm): 1.99 p (2H, J = 5.80 Hz, $-CH_2-$ β to $-NCH_3$), 2.79 br t (1H, J = 4.50 Hz, −OH), 3.29 s (3H, $-CH_3$), 3.42 br m (γ-CH_2), 3.45 m (8H, $N-CH_2-CH_2-N$), 3.69 t (4H, J = 6.40 Hz, α-CH_2), 7.62 s (1H, N−HC=C), 7.45−7.90 v br (4H, C−HC=N); 9.19 s (1H, −HC=O).

Single Crystal X-ray diffraction: Compounds **4Ni** (yellow crystals) and **5Ni** (orange-yellow crystals) were measured at room temperature with a Bruker AXS single crystal diffractometer with an APEX II position-sensitive reader. The structure was solved by direct methods using SHELXL[53] and refined by full-matrix least-squares techniques against F_o^2. All non-hydrogen atoms were refined anisotropically and all hydrogen

atoms positions were found using difference Fourier map except those located by disordered ethylene bridges (C(2) and C(3), with site occupancy factors of 0.648 (20) and 0.352 (20)) in **5Ni** which were added to the structure in idealized geometrical positions with fixed thermal parameters.
Both measured complexes crystallised in the monoclinic system. No solvent molecules are found in the structures and only an appropriate number of hexafluorophosphate counter-ions surround both molecules. There are four molecules of 5Ni and two of 4Ni per unit cell, as well as four hexafluorophosphate ions in both structures.
In both structures each set of fluoride atoms from hexafluorophosphate anions was found to be disordered over two orientations around phosphorus atoms with site occupancy factors of 0.711 (8) and 0.289 (8) in **5Ni**, and with approximately equal population – 0.503 (9) and 0.497 (9) – in the case of **4Ni**. The crystal parameters and the refinement data are collected below (table 1).

Table 2. Crystal data and summary of the data collection and structure refinement detils for **4Ni** and **5Ni**.

Compound	**5Ni**	**4Ni**
Formula	$C_{16}H_{24}F_6N_5NiO_2P$	$C_{20}H_{34}F_{12}N_6NiO_2P_2$
Formula weight	522.05	739.14
Crystal system	monoclinic	monoclinic
Space group	*P* 2_1/n	*P* 2_1/c
Cell constants:		
a (Å)	12.4928(3)	9.4210(2)
b (Å)	13.6466(4)	16.9855(4)
c (Å)	13.4287(4)	10.1207(3)
α (◦)	90.00	90.00
β (◦)	114.1660(10)	113.5000(10)
γ (◦)	90.00	90.00
V (Å^3)	2088.75	1485.2
Molecules/unit cell	4	2
ρ_{calcd} (g cm^{-3})	1.660	1.653
Temperature (K)	296(2)	293(2)
Abs. coeff. (mm^{-1})	2.787	2.957
θ range for data collection (◦)	4.07 – 67.66	5.21 – 67.67
Index ranges	$-14 \le h \le 14$	$-8 \le h \le 11$
	$-15 \le k \le 16$	$-20 \le k \le 20$
	$-15 \le l \le 16$	$-12 \le l \le 12$
No. of reflections collected	13050	25888
No. of indep. Reflections	3702	2673
No. of observed reflections[a]	3255	2540
Refinement method	Full matrix on F^2	Full matrix on F^2
Data / restrains / parameters	3702 / 0 / 397	2673 / 0 / 319
gof on F2	1.039	1.035
R1 indices [I > 2σ(I)]	0.0449	0.0384
wR2 indices (all data)[b]	0.1267	0.1055
Weight parameters a / b	0.0730 / 0.9128	0.0584 / 0.8474
Largest peak/hole (e Å^{-3})	0.571 / -0.251	0.489 / -0.212

[a] $|F_0| > 4\sigma(|F_0|)$

[b] $w = 1/[\sigma^2(F_0^2) + (aP)^2 + bP], where\ P = (F_0^2 + 2F_c^2)/3$

Binding studies: For the interaction studies sodium salt of double-stranded calf thymus DNA (Sigma-Aldrich) was used. Calf-thymus DNA was dissolved in deionised water (Millipore Direct-Q, 18.2 MΩ·cm at 25°C). The DNA stock solutions were freshly prepared for each experiment in relatively high concentrations (ca. 0.9 g / 100 mL), that were calculated from ct-DNA solution absorption intensity at 260 nm using molar absorption coefficient of 6600 M^{-1}·cm^{-1}. For the titrations no buffer was added in order to prevent the complexes to precipitate. Prior to experiments the CD, LD and UV spectra of ct-DNA solutions were compared with those measured in buffered (containing 10 mM Tris) mixtures to make sure the double helix structure is fully reconstituted. The DNA solutions were then diluted with water in order to obtain concentrations suitable for further experiments.
Linear and circular dichroism experiments were carried out using Jasco J-815. For the LD measurements Dioptica Ultra Low Volume Couette Flow cell was attached to the instrument. UV/Vis spectra were collected on Jasco V-660. All titration experiments were run at room temperature.

Supporting Information (see footnote on the first page of this article):

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