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Efficient DNA Binding by Optically Pure Ruthenium Tris(bipyridyl) Complexes Incorporating Carboxylic Functionalities. Solution and Structural Analysis

Régis Caspar,[†] Léna Musatkina,[‡] Alexander Tatosyan,[‡] Hani Amouri,*[,†] Michel Gruselle,[†] Carine Guyard-Duhayon,[†] Raphaël Duval,[§] and Christine Cordier*[,||]

ITODYS, UMR CNRS 7086, Université Paris 7, Denis Diderot, 1 rue Guy de la Brosse, 75005 Paris, France, Laboratoire de Chimie Inorganique et Matériaux Moléculaires, UMR CNRS 7071, Université Paris 6, Pierre et Marie Curie, 4 place Jussieu, case 42, 75252 Paris Cedex 05, France, Laboratory of Cellular and Viral Oncogenes Regulation, Cancer Research Centre Moscow, Russian Academy of Medical Science, Moscow, Russia, and Chirosep, Parc de la Boissière, 76170 La Frenaye, France

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Herein, we report on the binding of optically pure ruthenium complexes Δ- or Λ-$[Ru(bpy)_2(L–L)][PF_6]_2$ [L–L = Hcmbpy = 4-carboxy-4′-methyl-2,2′-bipyridine (**1**), L–L = H_2dcbpy = 4,4′-dicarboxy-2,2′-bipyridine (**2**)] to DNA. The binding constants of the two enantiomeric Δ-**1** and Λ-**1** complexes to DNA were estimated from titration monitored by ^{1}H NMR spectroscopy. 2D transferred NOESY (TRNOESY) experiments support the conclusion that Δ-**1** and Λ-**1** bind to DNA and that an intermediate-to-fast exchange occurs between bound and free Ru(II) complex. Further, evidence for enantioselective DNA cleavage by Δ-**2** is provided by means of gel electrophoresis performed in the presence and in the absence of light; in contrast, the Λ-**2** enantiomer does not. The IR spectrum of enantiomer Δ-**2** (or Λ-**2**) compared to that of the racemate (*rac*-**2**) gives evidence that, in the latter form, the enantiomers are strongly associated. Moreover the X-ray structure of *rac*-**2** was also determined and exhibits as an outstanding feature the formation of a one-dimensional supramolecular species in which the cohesion of the system is maintained by strong hydrogen bonding between carboxylic acid groups of enantiomers Δ-**2** and Λ-**2** (cationic parts) with d(O···O) = 2.6 Å in agreement with the infrared results. The conclusion that can be drawn from IR and X-ray spectroscopies together is that the self-association in *rac*-**2** is strong.

Introduction

Polypyridyl ruthenium complexes are known to be interesting nonradioactive probes for structure elucidation of nucleic acids.[1] Furthermore, these complexes are used for many purposes such as the design and development of synthetic restriction enzymes, new drugs, DNA footprinting agents, etc.,[1h,i] because of their potential to bind DNA via a multitude of interactions and to cleave the duplex by virtue of their intrinsic chemical, electrochemical, and photochemical reactivities. More recently, efforts have been devoted to the study of the stereochemistry of these complexes as a result of the different interactions that can arise when enantiomeric forms (Δ or Λ) interact with a variety of chiral biological molecules.[2,3]

* To whom correspondence should be addressed. E-mail: amouri@ccr.jussieu.fr (H.A.), cordier@itodys.jussieu.fr (C.C.).
† Université Paris 6.
‡ Cancer Research Centre Moscow.
§ Chirosep.
|| Université Paris 7.

(1) (a) Barton, J. K. *Science* **1986**, *233*, 727. (b) Hartshorn, R.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 5919. (c) Friedman, A. E.; Chambron, J.-C.; Sauvage, J.-P.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 4960. (d) J. K. Barton, A. T. Danishefsky, J. M. Goldberg, *J. Am. Chem. Soc.* **1984**, *106*, 2172. (e) Hiort, C.; Lincoln, P.; Norden, B. *J. Am. Chem. Soc.* **1993**, *115*, 3448. (f) Shimidzu, T.; Iyoda, T.; Izaki, K. *J. Phys. Chem.* **1985**, *89*, 642. (g) Terpetschnig, E.; Szmacinski, H.; Malak, H.; Lakowicz, J. R. *Biophys. J.* **1995**, *68*, 342. (h) Murphy, C. J.; Barton, J. K. *Methods Enzymol.* **1993**, *226*, 576. (i) Kelley, S. O.; Barton, J. K. In *Metal Ions in Biological Systems*; Sigel, A., Sigel, H., Eds.; Marcel Dekker: New York, 1998; Vol. 26, p 211.
(2) (a) Shulman, A.; Dwyer, F. P. *Chelating Agents and Metal Chelates*; Dwyer, F. P., Mellor, D. P., Eds.; Academic Press: New York, 1964; pp 383−439. (b) Rutherford, T. J.; Pellegrini, P. A.; Aldrich-Wright, J.; Junk, P. C.; Keene, F. R. *Eur. J. Inorg. Chem.* **1998**, 1677 and references therein. (c) Kirsch-De Mesmaeker, A.; Lecomte, J.-P.; Kelly, J. M. *Top. Curr. Chem.* **1996**, *177*, 25.

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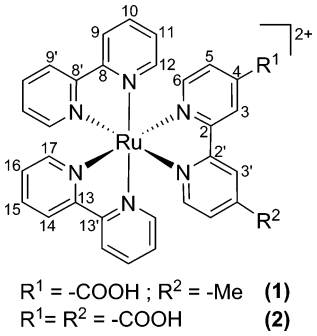
Chart 1. Atom Labeling[a]



R^1 = -COOH ; R^2 = -Me **(1)**

R^1= R^2 = -COOH **(2)**

[a] Λ-**2** cationic part represented.

As an example, in a previous work,[4] we established that each enantiomer of the pairs Δ- and Λ-$[Ru(bpy)_2(Hcmbpy)][PF_6]_2$ (Δ-**1** and Λ-**1**) or Δ- and Λ-$[Ru(bpy)_2(H_2dcbpy)][PF_6]_2$ (Δ-**2** and Λ-**2**) (Chart 1) shows strong, but quite different, affinities for the Δ-Trisphat anion [Trisphat = tris(tetrachlorobenzenediolato)phosphate(V)]. Ion pairing is controlled by π-stacking interactions and favors the homochiral pairing relative to the heterochiral one. In this paper, we highlight the finding that the DNA recognition by the dicarboxylic ruthenium salts strongly differs from that displayed by the monocarboxylic ruthenium salts. Further, we address the question of determining the stereospecificity of the binding of these chiral ruthenium bipyridyl complexes to DNA, which could be viewed as a chiral "supermolecule". Binding constants for the two enantiomers Δ-**1** and Λ-**1** are estimated from the ^{1}H NMR chemical shift variation of the resonances attributed to the ruthenium system upon DNA addition. Also, transferred NOESY (TRNOESY) experiments are used to study the Δ-**1**−DNA and Λ-**1**−DNA interactions.[5] Gel electrophoresis experiments are utilized to investigate the interactions of Δ-**2** and Λ-**2** with linear DNA. Evidence for a chiral recognition of linear DNA by the dicarboxylic metallic species is found even in the absence of light. Further, the X-ray structure of *rac*-**2** was determined and exhibits as an outstanding feature the formation of a one-dimensional supramolecular species in which the cohesion of the system is maintained by strong hydrogen bonding between carboxylic acid groups of enantiomers Δ-**2** and Λ-**2** with *d*(O···O) = 2.6 Å. To the best of our knowledge, no structural studies have previously been reported on analogous systems. Interestingly, *rac*-**2** in solution does not cleave linear DNA, whereas the Δ-**2** enantiomer does. A complete solution and structural analysis of these ruthenium polypyridyl complexes is included.

Results and Discussion

Δ- and Λ-Monocarboxylic Tris(bipyridyl) Ruthenium Bind DNA. NMR Investigation. Recently, Metcalfe and Thomas published a review in which they largely describe the binding modes of the ruthenium polypyridyl cations (i.e., $[Ru(phen)_3]^{2+}$, $[Ru(dpphen)_3]^{2+}$, and $[Ru(bpy)_2(dppz)]^{2+}$) to B-DNA. In addition to the intercalation of a planar aromatic ligand into the DNA base stack, other nonintercalative binding modes such as groove binding and surface binding, among others, are proposed.[6] Among the polypyridyl ruthenium salts, it was stated by Barton and colleagues that the binding of $[Ru(bpy)_3]^{2+}$ to DNA is negligible either by intercalation or by electrostatic interaction.[7] Here, we assume that the incorporation of a carboxylic group will favor the DNA−Ru assembly, and we have investigated this issue using NOESY experiments. All NMR measurements were run in a mixture of phosphate-buffered D_2O with a few drops of CD_3CN to ensure the complete solubility of our compounds. Because of their molecular weights (~1000 g·mol^{-1}), the free Δ-**1** and Λ-**1** enantiomers typically display weak and positive NOE cross-peaks in solution (2D NOESY experiments at 500 MHz with a short mixing time).[5] Now, when transferred NOESY (TRNOESY) spectra are recorded for a Ru/DNA concentration ratio of ~6500/1 (Ru = Δ-**1** or Λ-**1**), the cross-peaks are strong and negative (Figure 1). The phase inversion of the intra-monocarboxylic system NOEs indicates that the correlation time of the ruthenium complex is modified by the presence of DNA, thus reaching the macromolecular correlation time domain. The observation of antiphase cross-peaks for the sodium propionate chosen as the internal reference (50 μL of a solution of sodium propionate 0.1 M was added to the NMR sample) precludes an artifact due either to the NMR data processing or to the high viscosity of the medium (see Figure S1 in Supporting Information). This result confirms the association between Δ-**1** (or Λ-**1**) and DNA. This is also in agreement with a fast (or intermediate) exchange process (fast or intermediate on the time scale of spin−lattice relaxation rate) between the free and bound metallic species.

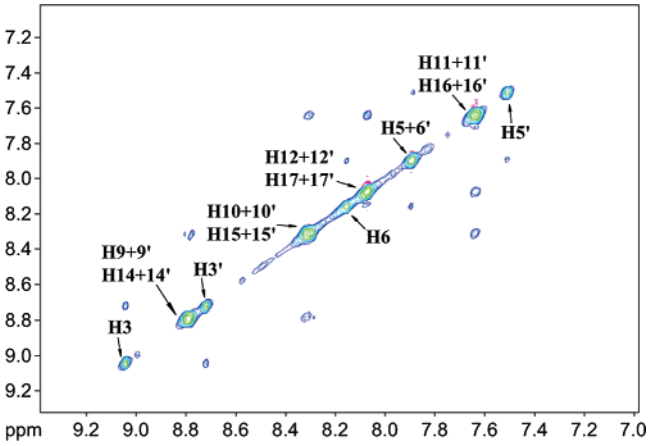


Figure 1. Transferred NOESY subspectrum of the cation Δ-$[Ru(bpy)_2(Hcmbpy)]^{2+}$ (Δ-**1**) + purified linear DNA ($[\Delta\text{-}\mathbf{1}]/[DNA]_{strand} \approx 6500$) showing the Δ-**1** intramolecular dipolar connectivities. Particularly, the strong negative NOE cross-peaks mark the formation of the Δ-**1**−Δ-**DNA** binding. [500 MHz, D_2O + a few drops of CD_3CN (phosphate buffer, I = 50 mM, pH = 7.2 + 1 mM EDTA), T = 303 K, mixing time = 200 ms.]

(3) (a) Wärnmark, K.; Thomas, J. A.; Heyke, O.; Lehn, J.-M. *Chem. Commun.* **1996**, 701. (b) Wärnmark, K.; Heyke, O.; Thomas, J. A.; Lehn, J.-M. *Chem. Commun.* **1996**, 2603.
(4) Caspar, R.; Amouri, H.; Gruselle, M.; Cordier, C.; Malézieux, B.; Duval, R.; Lévêque, H. *Eur. J. Inorg. Chem.* **2003**, 499.
(5) (a) Campbell, A. P.; Sykes, B. D. *J. Magn. Reson.* **1991**, *93*, 77. (b) Bertho, G.; Gharbi-Benarous, J.; Ladam, P.; Delaforge, M.; Girault, J.-P. *Bioorg. Med. Chem.* **1998**, *6*, 209.
(6) Metcalfe, C.; Thomas, J. A. *Chem. Soc. Rev.* **2003**, *32*, 215.
(7) Kumar, C. V.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc.* **1985**, *107*, 5518.

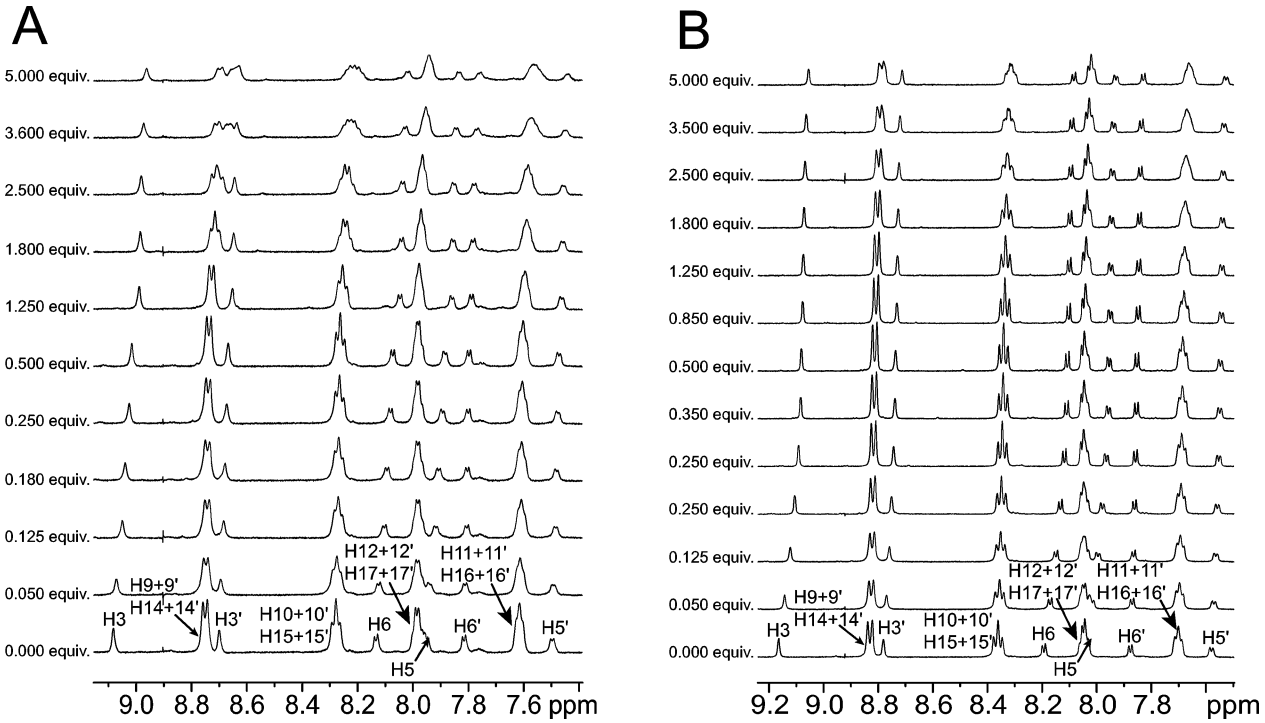


Figure 2. Section of the ^{1}H NMR spectra of (A) Δ-**1** and (B) Λ-**1** upon sequential addition of sonicated herring DNA from 0 to 5 equiv (DNA base pairs). (The slight shift of the signals observed at 0 equiv from the A spectrum to the B spectrum is due to a slight difference of the sample concentration after direct filtration in the NMR tube.)

The gradual variation of the proton chemical shifts, upon titration by sonicated herring DNA, precludes slow exchange kinetics for the two enantiomers Δ-**1** and Λ-**1** (1D NMR experiments) even when the DNA/Ru ratio is equal to 5 (7.5 μmol DNA base pairs for 1.5 μmol Δ-**1** or Λ-**1**), (Figure 2). Because a significant broadening of the signals is observed for the highest DNA/Ru ratios and the sample solution is known to be homogeneous, we attribute this phenomenon to an intermediate exchange kinetics for the binding of the two enantiomers to DNA. The presence of a small amount of CD_3CN (few drops) in the NMR sample precludes the aggregation of the ruthenium species as well as their precipitation, even for the highest DNA volume added. It is clear from the two series of data that both metallic enantiomers bind to DNA. Indeed, (i) the shielding of the ruthenium species protons, compatible with a ring current effect from the DNA Watson−Crick base pairs over the protons of the ruthenium salts, and (ii) the observed intermediate exchange binding kinetics have been reported by others as the spectral changes inherent to binding process.[8] However, the invariance of the DNA melting temperature (T_m) in the presence and in the absence of the ruthenium compounds precludes the intercalation mode as the binding process (T_m measurements were performed by using the standard UV protocol). A detailed examination of the NMR spectra shows that many signals of Δ-**1** depart from their initial shape and gradually split upon DNA addition [for example, signals (H9, H9′, H14, H14′) or signals (H10, H10′, H15, H15′)]. This reflects the loss of the C_2 symmetry of the Ru(bpy)$_2$ moiety when Δ-1 is bound to the DNA helix. In contrast, for Λ-**1**, this signal pattern is left unchanged upon DNA addition. In previous work[4] where the Δ-**1** and Λ-**1** enantiomers were allowed to interact with the Δ-Trisphat anion, known as a chiral shift reagent of D_3 symmetry, we showed that homochiral associations are favored relative to heterochiral pairing. For such assemblies involving two small ions, because the electrostatic interactions are similar in the two pairs (Δ−Δ and Λ−Δ), only the stacking strength differentiates the two pairings. Although there is no direct link between the previous work on Trisphat anion and the current studies on DNA, yet we would like to draw attention to the fact that, in both studies, optically pure ruthenium complexes were analyzed in the presence of chiral anion systems (Δ-Trisphat and right-handed DNA).

When proton chemical shifts are plotted as a function of DNA concentration, two groups of protons are easily distinguishable (Figure 3). The group (H3, H5, and H6) is the most affected by DNA addition. These protons are all located on the pyridine bearing the −COOH function.[9] Therefore, we propose this region of the metallic species to be the putative binding site to DNA. Large upfield shifts (0.5−1 ppm) of phen and dpq[10] protons upon DNA binding have been reported previously.[11] In contrast, in this work, the shielding of the H3, H5, and H6 protons is weaker, thus

(8) (a) Collins, J. G.; Aldrich-Wright, J. R.; Greguric, I.; Pellegrini, P. P. *Inorg. Chem.* **1999**, *38*, 5502. (b) Dupureur, C. M.; Barton, J. K. *J. Am. Chem. Soc.*1994, *116*, 10286.

(9) We note that, at pH = 7.2 (D_2O, phosphate buffer), the acidic proton cannot be observed.

(10) phen = phenanthroline ligand, dpq = dipyrido[3,2-*f*:2′,3′-*h*]quinoxaline ligand.

(11) (a) Rehmann, J. P.; Barton, J. K. *Biochemistry* **1990**, *29*, 1701. (b) Eriksson, M.; Leijon, M.; Hiort, C.; Norden, B.; Graeslund, A. *Biochemistry* **1994**, *33*, 5031. (c) Collins, J. G.; Sleeman, A. D.; Aldrich-Wright, J. R.; Greguric, I.; Hambley, T. W. *Inorg. Chem.* **1998**, *37*, 3133.

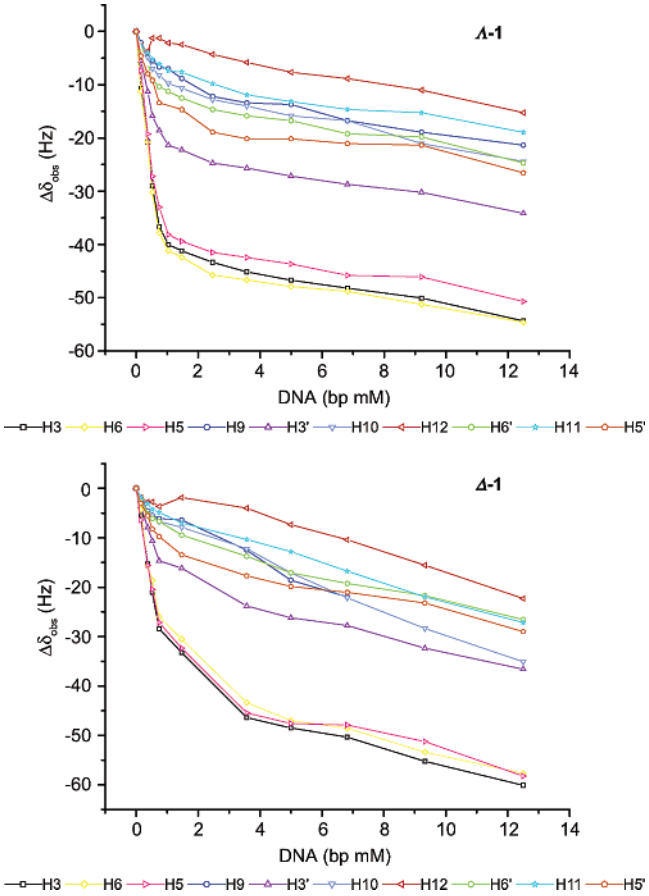


Figure 3. Chemical shift variation ($\Delta\delta_{obs} = \delta_{obs} - \delta_{Ruf}$) versus DNA concentration (millimoles of base pairs per liter) line plots for Δ-**1** and Λ-**1** protons.

supporting the idea of a weak association of Δ-**1** and Λ-**1** with the helix. Elsewhere, it has been reported that methyl groups on such bipyridyl ruthenium complexes are critical in eliciting different steric interactions with the walls of the DNA grooves.[12] For Δ-**1** and Λ-**1**, the methyl and −COOH share the same bipyridine unit, each group being located on one ring of the pair. Consequently, two effects can be used to monitor the Ru/DNA affinity: (i) the binding effect due to the carboxylate function and (ii) the −Me steric hindrance.

Binding Constant Determination. To quantify the Ru/DNA affinity, the binding constants K_b were calculated from the changes in the chemical shifts of the metallic compound signals recorded as a function of the DNA base pair concentration. The observed chemical shifts (δ_{obs}) are the weighted averages of the chemical shifts for the free (δ_{Ruf}) and bound (δ_{Rub}) Δ-**1** (or Λ-**1**) enantiomers under conditions of fast-to-intermediate exchange. In this study, the chosen DNA concentration unit is the base pair molarity.

Assuming a one-to-one binding stoichiometry such as

$$\mathrm{Ru} + \mathrm{DNA} \rightleftharpoons \mathrm{Ru{-}DNA}$$

(where Ru = Δ-**1** or Λ-**1**), it has been demonstrated that[13]

$$\Delta\delta_{obs} = (\delta_{Rub} - \delta_{Ruf}) \times \frac{([Ru]_t + [DNA]_t + K_d) - \sqrt{([Ru]_t + [DNA]_t + K_d)^2 - 4[Ru]_t[DNA]_t}}{2[Ru]_t} \quad (1)$$

where Rub and Ruf represent the bound and free forms of the ruthenium complex, respectively; $\Delta\delta_{obs} = \delta_{obs} - \delta_{Ruf}$ for selected protons of Δ-**1** (or Λ-**1**) in the presence of the DNA; $K_d = 1/K_b$, where K_d is the dissociation constant and K_b is the binding constant; and $[Ru]_t$ and $[DNA]_t$ are the total concentrations of Ru and DNA, respectively. $[Ru]_t$ is assumed to be constant (~3 mM) throughout the course of the titration (see Experimental Section). The chosen signals for the calculations were those corresponding to protons close to the putative binding site of the complex in question, i.e., H3, H5, and H6. The unknowns δ_{Rub} and K_b were determined by fitting the experimental values, $\Delta\delta_{obs}$, of the above-mentioned protons to the given equation (eq 1) using the nonlinear curve fitting routine of the program Origin (version 6.1, OriginLab Corporation, Northampton, MA), which uses the Levenberg−Marquardt algorithm (see Figure S2 in Supporting Information). The relative error on $\Delta\delta_{obs}$, calculated from the half-width at half-maximum of the line (singlet signal such as H3 was selected) reaches 5% owing to the broadening of the peaks (see Figure S3 in Supporting Information). It is satisfying to note that the postulated mathematical model (vide supra) fits accurately the experimental data. The good agreement between the binding constants K_b calculated from H3, H5 and H6 data confirmed the assumed 1:1 stoichiometry (see Table S1 in Supporting Information). The average values are $K_b = 0.91\ (\pm 0.08) \times 10^3\ M^{-1}$ and $2.21\ (\pm 0.10) \times 10^3\ M^{-1}$ for Δ-**1** and Λ-**1**, respectively. Some studies report affinity constants with the same magnitude but for metallic complexes containing ligands with a more extended π system.[14] For example, the K_b value is around $10^3\ M^{-1}$ for $[Rh(phen)_3]^{3+}$ [15] and for some $[Ru(R,R\text{-picchxnMe}_2)(\text{bidentate})]^{2+}$ derivatives.[16] Each time, the authors proposed intercalation as the binding mode. For this work, (i) the observation of an intermediate-to-fast exchange process between the free and bound metallic species and (ii) the determination of a low K_b value support a weak association of the monocarboxylic species where the carboxylated pyridine is involved in the binding site. Indeed, this carboxylic group would be predominant for Ru−DNA association given that the unsubstituted homologue, $[Ru(bpy)_3]^{2+}$, does not bind DNA, as shown by Barton and co-workers.[7]

Surprisingly, the Λ-**1** enantiomer binds to DNA 2.4 times more strongly than the Δ-**1** enantiomer. According to the

(12) Pellegrini, P. P.; Aldrich-Wright, J. R. *J. Chem Soc., Dalton Trans.* **2003**, 176.
(13) Connors, K. A. *Binding Constants: The Measurement of Molecular Complex Stability*; Wiley: New York, 1987; Chapter 5.
(14) Sorasaenee, K.; Fu, P. K.-L.; Angeles-Boza, A. M.; Dunbar, K. R.; Turro, C. *Inorg. Chem.* **2003**, *42*, 1267.
(15) Fu, P. K.-L.; Turro, C. *Chem. Commun.* **2001**, 279.
(16) (a) picchxnMe_2 = *N,N′*-dimethyl-*N,N′*-di(2-picolyl)-1,2-diaminocyclohexane. (b) Proudfoot, E. M.; Mackay, J. P.; Karuso, P. *Biochemistry* **2001**, *40*, 4867.

standard relation $\Delta G_{obs}(313\ K) = -RT\ \ln(K_b)$, the observed binding free energies are favorable for both isomers, with a preference for the Λ compound. This specificity is unusual but has already been reported for a similar compound[12] (i.e., $[Ru(bpyMe_2)_2(phen)]^{2+}$) for which the Λ isomer binds to DNA more tightly. At first sight, this result contrasts with the proposal, which is that the Δ-Ru−Δ-DNA assembly would be more intimate than the Λ−Ru−Δ-DNA one.[1a] Nevertheless, the DNA binding thermodynamic results from a set of properties including hydrogen bonding, van der Waals interactions, electrostatic interactions, and "hydrophobic" interactions.[15] All of these terms are included in the relationship $\Delta G = \Delta H - T\Delta S$, where the binding free energy results from enthalpic and entropic terms. Haq et al.[17] have established a DNA binding thermodynamic profile for the $[Ru(phen)_2dppz]^{2+}$ compound.[18] In their hypothesis, the entropy associated with the heterochiral Λ−Ru−Δ-DNA assembly is higher than that of the homochiral assembly (Δ-Ru−Δ-DNA), and this supports a higher K_b value for the heterochiral association than for the homochiral association. We believe that this thermodynamic model might explain our results such as the apparent favorability of the Λ-**1** binding over the Δ-**1** one as shown by the respective K_b values.

Enantioselective Cleavage of Linear DNA by Δ-Dicarboxylic Tris(bipyridyl) Ruthenium. Gel Electrophoresis. Preliminary electrophoresis experiments (agarose gel) were run with dicarboxylic ruthenium compounds (*rac*-**2**, Δ-**2**, and Λ-**2**) mixed with intact plasmid DNA, present under its main forms: supercoiled, opened circular, and linear. Here, to point out the most interesting results, we restrict our electrophoresis study to double-stranded linear DNA in the presence of *rac*-**2**, Δ-**2**, and Λ-**2**. For this purpose, the linear form was obtained from pUST-1 plasmid DNA[19] by cleavage with the restriction enzyme Not1. The linear DNA (6587 base pairs) was purified by electroelution from agarose gel. A sample of 1 μL containing 0.5 μg of the prepared linear DNA was mixed at room temperature with the dicarboxylic ruthenium species at a final concentration of 1 mM (*rac*-**2**) or 0.5 mM (enantiomers) (see Experimental Section for additional details). As shown in Figure 4A, only the Δ-**2** species strongly affects the DNA migration, whereas the second isomer does not change it. This result suggests enantioselective recognition of DNA by these dicarboxylic ruthenium compounds. A DNA scission is suspected upon Δ-**2** treatment, in agreement with an increase in the migration rate.

When the migration pattern of the linear DNA (6587 base pairs) in the presence of the ruthenium species is compared to that of a linear DNA marker with various strand lengths, a smearing effect with limited size of cleaved DNA fragments is noticed (Figure 4B). This confirms the enantioselective cleavage of the linear DNA by Δ-**2**. Moreover, a

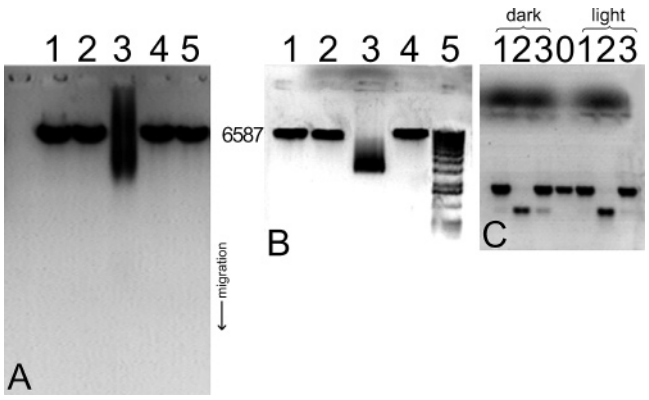


Figure 4. (A) Experiment performed in the presence of light. Lane 1: linear DNA. Lanes 2 and 5: linear DNA + *rac*-**2** at 1 mM. Lane 3: linear DNA+ Δ-**2** at 0.5 mM. Lane 4: linear DNA+ Λ-**2** at 0.5 mM. (B) Experiment performed in the presence of light. Lane 1: linear DNA (6587 base pairs), Lane 2: linear DNA + *rac*-**2** at 1 mM. Lane 3: linear DNA + Δ-**2** at 0.5 mM. Lane 4: linear DNA + Λ-**2** at 0.5 mM. Lane 5: linear DNA markers with various strand lengths. (C) Experiments with identical sampling conditions performed in the presence and in the absence of light. For both, DNA and ruthenium solutions were allowed to incubate 4 h. For the experiment in the absence of light, solutions were mixed under red light and incubated in the dark. Lane 0: linear DNA alone (6587 base pairs). Lane 1: linear DNA + *rac*-**2** at 1 mM. Lane 2: linear DNA + Δ-**2** at 0.5 mM. Lane 3: linear DNA + Λ-**2** at 0.5 mM.

selective cleavage with regard to the primary structure of the DNA sequence is suspected. Surprisingly, *rac*-**2**, which corresponds to a stoichiometric mixture of the two enantiomers, leaves the DNA intact. Both Λ-**2** and *rac*-**2** lead to identical DNA migration patterns. Ruthenium(II) polypyridyl complexes are known for their potential oxidative damage to DNA when they are photoexcited. To check the hypothesis that the DNA cleavage observed in this electrophoresis study could result from a photocleavage process in which only Δ-**2** was photosensitive, a series of experiments run in the *absence of light* was performed. Interestingly, the results in the absence of light reproduce those obtained in the presence of light (Figure 4C); namely, the Δ-**2** enantiomer has intrinsic reactivity as it cleaves the linear DNA form, whereas Λ-**2** and *rac*-**2** do not. For the latter species, we suspect that strong Δ-**2**−Λ-**2** pairing takes place, and thus, *rac*-**2** has to be regarded as an entity (vide infra IR and X-ray analysis on *rac*-**2**). To our knowledge, DNA cleavage by a ruthenium polypyridyl complex in the absence of light is a rare event.

IR Spectroscopy. The observation of (i) differences in the fingerprint region and (ii) a shift of the νC═O stretching frequency (7 cm^{-1}) in the IR spectra recorded for *rac*-**2** and Λ-**2** (or Δ-**2**) in the solid state (KBr pellet) indicates that the crystalline form of *rac*-**2** is a racemate and not a conglomerate.[20] In the racemate, the two enantiomers are associated by hydrogen-bonding interactions (vide infra). In contrast, the conglomerate would produce an IR spectrum that would be fully superposable on that of Λ-**2** (or Δ-**2**). This result supports a strong ion pairing between the

(17) Haq, I.; Lincoln, P.; Suh, D.; Norden, B.; Chowdhry, B. Z.; Chaires, J. B. *J. Am. Chem. Soc.* **1995**, *117*, 4788.
(18) dppz = dipyrido[3,2-*a*:2′3′-*c*]phenazine.
(19) pUST-1 (= 6587 bp) corresponds to the intact vector pUST (= 7352 bp) without the GFP gene. This vector is mentioned in: Gurova, K. V.; Hill, J. E.; Razorenova, O. V.; Chumakov, P. M.; Gudkov, A. V. *Cancer Res.* **2004**, *64*, 1951
(20) There are two types of crystalline racemates: *Conglomerates* "are [just] simple juxtapositions of crystals of the two enantiomers, which are relatively rare. *Racemic compounds*, by far the most frequently observed type, are cases whose crystals contain the two enantiomers in equal number". Jacques, J.; Collet, A.; Wilen, S. H. in *Enantiomers, Racemates, and Resolutions*.; Wiley-Interscience: New York, 1981; Chapter 1.

Table 1. Crystal Data and Structure Refinement for *rac*-**2**

formula	$C_{34}H_{27}F_{12}N_7O_4P_2Ru$
crystal class	monoclinic
space group	*C*2/*c*
a (Å)	18.354(3)
b (Å)	21.771(4)
c (Å)	20.752(5)
β (°)	110.837(9)
volume (Å^3)	7750(3)
Z	8
λ (Mo Kα) (Å)	0.710730
d_{calcd} (g·cm^{-3})	1.70
M (g·mol^{-1})	988.63
temperature (K)	173
size (mm)	0.16 × 0.22 × 0.50
$R = \|\|F_o - F_c\|\|/\sum F_o$	0.0804
$R_w = [\sum w(\|\|F_o - F_c\|\|)^2/\sum(wF_o^2)]^{1/2}$	0.0879
GOF	1.06

Table 2. Selected Interatomic Distances (Å) and Angles (deg) for the Cationic Part of *rac*-**2**

Ru(1)−N(1)	2.060(6)
Ru(1)−N(1′)	2.047(6)
Ru(1)−N(7)	2.054(6)
Ru(1)−N(7′)	2.052(6)
Ru(1)−N(13)	2.053(7)
Ru(1)−N(13′)	2.056(7)
C(19)−O(1)	1.27(1)
C(19)−O(2)	1.24(1)
C(19′)−O(1′)	1.19(1)
C(19′)−O(2′)	1.31(1)
O(1)−O(1)	2.43(1)
O(2)−O(2′)	2.60(1)
O(1)−C(19)−O(2)	125.8(7)
O(1′)−C(19′)−O(2′)	123.8(8)
(HOOC−pyridyl)−(pyridyl−COOH)[a]	17

[a] Denotes dihedral angles.

enantiomers in the racemate. Further, the IR spectra of *rac*-**2** recorded in solution (CD_2Cl_2/CD_3CN = 95/5) relative to the blank sample (solvent mixture) exhibit a broad peak around 3100 cm^{-1}. This band characterizes the H-bonded νO−H stretching mode for the acidic functional group. Moreover, the frequency of the νC═O stretching mode is slightly shifted from the value in the solid state, in agreement with intermolecular associations even in the solution phase. Finally, to confirm the above results, we decided to grow crystals of *rac*-**2**. After several attempts, suitable crystals of *rac*-**2** were obtained from the slow diffusion of toluene into a concentrated solution of CD_3CN/CH_2Cl_2, 5/95 (vide infra).

X-ray Molecular Structure of *rac*-2. Compound *rac*-**2** crystallizes in the monoclinic unit cell space group *C*2/*c*. Crystallographic data for *rac*-**2** are reported in Table 1. Selected bond distances and angles are given in Table 2 (see Figure 5a for the atom labeling scheme). A view of a dicationic species is shown in Figure 5a (enantiomer Λ-**2**). The ruthenium center has an octahedral geometry well-known for such compounds: the arrangement of the 4,4′-dicarboxy-2,2′-bipyridine ligand in the dicationic species is nearly planar, and the angle between the two pyridyl planes is 17°. Further there is a C_2 symmetry axis that passes through the ruthenium center and acts as a bisector to the C2···C2′ bond. The structure shows as an outstanding feature the formation of a double-chained 1D polymer, in which a simple Λ-**2** cationic subunit is connected to a Δ-**2** cationic subunit by two hydrogen bonds of equal distances, *d*(O2··· ·O2′) = 2.60 Å, thus forming a building block (Figure 5b). Eventually, these building blocks are enchained through strong intermolecular hydrogen bonding in an alternate fashion whether through the $\Lambda-\Lambda$ or $\Delta-\Delta$ end with O(1)···O(1) distance of 2.43 Å (Figure 5c). This bond length is remarkably short and can be compared to the O···O distance of 2.66 Å reported for a ruthenium polypyridyl complex incorporating a dicarboxybipyridine ligand.[21] The latter crystallizes with one molecule of water. One notes that, within the unit cell, subunits are chained in a homochiral mode ($\Delta-\Delta$ or $\Lambda-\Lambda$).

Only few ruthenium(II) 2,2′-bipyridine-4,4′-dicarboxylic acid complexes have been characterized by single-crystal X-ray diffraction measurements. To the best of our knowledge, none of the previous examples displayed a one-dimensional double-chained supramolecular structure. This is the first $[Ru(bpy)_2(H_2dcbpy)]^{2+}$ structure reported in the literature that shows clearly that, in *rac*-**2**, the Δ and Λ individual moieties are connected face-to-face by two hydrogen bonds and illustrate a striking example of chiral recognition (Figure 5c).

In short, our spectroscopic results (infrared and X-ray) in the solid state and in solution establish the existence of hydrogen bonds between $[Ru(bpy)_2(H_2dcbpy)][PF_6]_2$ moieties in the racemate. Interestingly, these results are parallel to those obtained in electrophoresis where DNA recognition by *rac*-**2** is nonexistent. Our ruthenium complexes with carboxylic functionalities, not investigated before, offer a convenient model and shed light on the behavior of such ruthenium complexes toward DNA recognition, an area of high complexity where several parameters intervene during the recognition process.

Conclusion

In summary, we have studied the binding of DNA with optically pure ruthenium bipyridyl complexes incorporating carboxylic functions, which had never before been examined. Our preliminary results suggest that the carboxylic functions play an important role in the binding process, for instance, whereas the parent $[Ru(bpy)_3]^{2+}$ complex shows negligible binding to DNA, the Ru(II) monocarboxylic compounds Δ-**1** and Λ-**1** exhibit enhanced binding with K_b = 0.91 (±0.08) × 10^3 and 2.21 (±0.10) × 10^3 M^{-1}, respectively. Interestingly, the optically pure dicarboxylic Ru(II) complex Δ-**2** cleaves linear DNA in the presence and in the absence of light, thus precluding any photocleavage process. In contrast, Λ-**2** does not cleave DNA, as shown by electrophoresis experiments. Surprisingly, the *rac*-**2** is also inactive toward linear DNA binding in solution. X-ray analysis obtained on *rac*-**2** shows that self-assembly among individual $[Ru(bpy)_2(H_2dcbpy)][PF_6]_2$ moieties occurs. Infrared techniques also carried out on a sample of *rac*-**2** in a CD_2Cl_2/CD_3CN solution and in the solid state support the existence of hydrogen bonds between the carboxylic functionalities of the bipyridyl ligands. We are currently studying the potential binding sites

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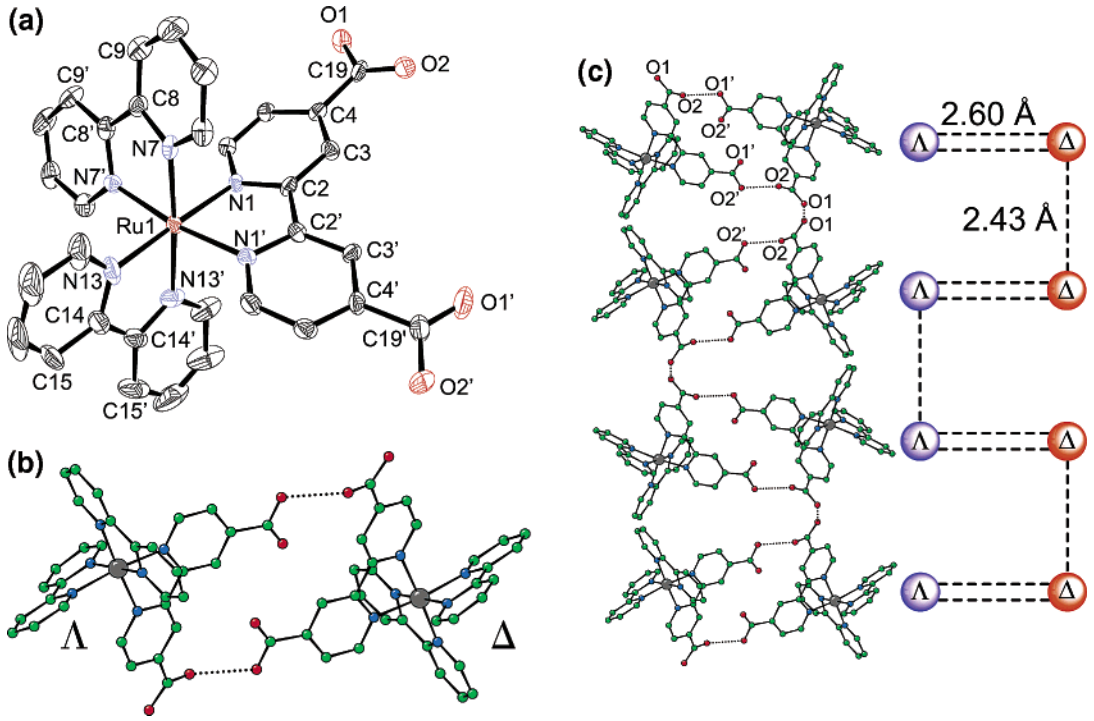

Figure 5. (a) X-ray molecular structure of *rac*-**2** with the atom numbering system; hydrogen atoms have been omitted for clarity. (b) View of Λ-**2** and Δ-**2** subunits (cationic part) showing the hydrogen-bonding connectivity. (c) Auto-assembly of (Λ-**2**, Δ-**2**) building blocks (cationic parts) through strong hydrogen bonding. Note that these bricks are enchained in an alternating manner, providing a double-chained 1D polymer.

for our model complexes using synthetic oligonucleotide, in the hopes of identifying both them and the cleavage sites of our chosen DNA.

Experimental Section

All solvents used were reagent grade or better. Deuterated solvents and commercially available reagents were used as received. ^{1}H NMR spectra were recorded on a Bruker AC-300 spectrometer and on a Bruker DRX-500 spectrometer equipped with a Silicon Graphics workstation (vide infra). Chemical shifts are reported in parts per million downfield from tetramethylsilane and are referenced to the residual hydrogen signal of deuterated solvents (CHD_2CN at 1.94 ppm for the titration and HOD at 4.8 ppm for the TRNOESY). IR spectra were recorded on a Bio-RAD FT-IR spectrophotometer as KBr pellets in the 4000−250 cm^{-1} region and on a Perkin-Elmer (Spectrum BX) FT-IR spectrometer for spectra performed in dried CD_2Cl_2/CD_3CN solution (using a 50-μm path-length cell with NaCl windows) in the 4000−1000 cm^{-1} region.

All NMR experiments concerning DNA were performed in buffered D_2O (deuterated phosphate buffer, pH = 7.2, I = 50 mM + 1 mM EDTA).

Purification and Resolution of the Enantiomers of the Ruthenium Complexes (Δ-1) or (Λ-1) and (Δ-2) or (Λ-2). Δ- or Λ-[Ru(bpy)$_2$(Hcmbpy)][PF$_6$]$_2$ (Δ-1 or Λ-1). Δ- or Λ-[Ru^{II}(bpy)$_2$(py)$_2$][*O*,*O*′-dibenzoyl-tartarate] (390 mg, 419 μmol), Hcmbpy (220 mg, 1 mmol, 2.38 equiv), sodium acetate (220 mg, 2.7 mmol, 5.65 equiv), water (3 mL), and ethylene glycol (13 mL) were heated at 120 °C for 1 night; the pH was then adjusted to 1 by addition of concentrated sulfuric acid. Water was added (30 mL). The mixture was filtered to remove the excess of free ligand. Then the product was precipitated by addition of saturated aqueous solution of NH_4PF_6, filtered on a fine porous glass (porosity 5) to give a red powder that was washed with water and dried under vacuum. Yield: 85% Spectroscopic data are similar to those reported for the racemic complexes.[4]

Δ- or Λ-[Ru(bpy)$_2$(H$_2$dcbpy)][PF$_6$]$_2$ (Δ-2 or Λ-2). These complexes were prepared in a similar way to that described for the mono-acid Δ- or Λ-[Ru(bpy)$_2$(Hcmbpy)](PF$_6$)$_2$ (Δ-**1**) or (Λ-**1**) but using the following reactants: Δ- or Λ-[Ru^{II}(bpy)$_2$(py)$_2$][*O*,*O*′-dibenzoyl-tartarate] (200 mg, 215 μmol), H2dcbpy (110 mg, 426 μmol, 1.98 equiv), sodium acetate (110 mg, 1.34 mmol, 6.23 equiv), water (3 mL), and ethylene glycol (13 mL). This compound was obtained as a purple powder, in a yield of 67%. Spectroscopic data are similar to those reported for the racemic compound.[4]

Gel Electrophoresis. Preparation of the Gel. Agarose was heated until it had completely melted and was dissolved in TAE buffer [tris(hydroxymethyl)aminomethane (40 mM), acetic acid (20 mM), ethylenediaminetetraacetic acid (1 mM); pH 8.0] to give a final concentration of 1% w/v.

Preparation of Linear DNA Sample. The plasmid pUST-1[19] was a generous gift of Dr. P. Chumakov. The linear form was obtained as result of intact DNA cleavage by the restriction enzyme Not1 and then separated in agarose gel and purified by electroelution. Total pUST-1 plasmid DNA was used as the starting material. The length of this DNA is 6587 base pairs. Linear DNA in solution (0.5 $\mu g \cdot \mu L^{-1}$, nuclease-free deionized water) was mixed with the metal complex in solution (nuclease-free deionized water + DMSO) and allowed to react at room temperature. Typically, to 1 μL of the linear DNA solution was first added 7 μL of nuclease-free deionized water; then, 2 μL of Λ-**2**, Δ-**2**, or *rac*-**2** dissolved in H_2O/DMSO (50/50) was added to the mixture. In this 10-μL final volume, the metallic enantiomer (or racemic) concentration was 0.5 mM (or 1 mM), and the DMSO concentration was 10%. Two series of experiments with identical sampling conditions were carried out in the presence and in the absence of light. Practically, for these latter experiments, DNA and ruthenium solutions were mixed under red light and incubated for 4 h in the dark.

Electrophoresis was carried out at room temperature at 60 V. The gels were removed from the horizontal electrophoresis apparatus and immersed for 10 min in ethidium bromide solution (1 μg·mL^{-1}). The DNA in the gel was visualized with UV light.

NMR Spectroscopy. For the spectra recorded with a Bruker DRX-500 spectrometer, equipped with a Silicon Graphics workstation, a 5-mm broadband probe with a shielded *z* gradient was used. The temperature was monitored with a BCU 05 temperature unit and fixed at 303 K (2D experiments) or 313 K (1D titration experiments). Data were processed with Silicon Graphics stations using GIFA (version 4.3).[22]

Titration. Each titration experiment was performed using an enantiomerically pure monocarboxylic tris(bipyridyl) ruthenium solution to which increments of stock DNA solution were added. For each enantiomer (Δ-**1** or Λ-**1**), 500 μL of a 3 mM solution were prepared in D_2O. For full solubility of the ruthenium complexes in aqueous medium, a few drops of CD_3CN were added. The stock DNA solution was made by dissolving herring sperm DNA fibers (purchased from Roche) in an aqueous phosphate buffer (pH = 7.2, I = 50 mM + 1 mM EDTA). This mixture was sonicated four times for 30 s each at 4 °C. The DNA concentration was measured by UV spectroscopy at λ = 260 nm (an OD of 1 corresponds to approximately 50 μg·mL^{-1} for double-stranded DNA, and the molar weight is 330 g per base mole).[23] Then, the stock DNA solution was lyophilized first from the aqueous buffer and then twice from 99.90% D_2O and finally dissolved in 100 μL of 99.95% D_2O. The latter D_2O volume was forced by the dual experimental requirements (i) to allow a good solubility of the herring DNA and (ii) to limit the variation of the total volume (Ru + DNA) throughout the course of the titration. 1D spectra were obtained in 48 scans of 16K data points over a 5.0 kHz spectral width. Presaturation (56 dB) of the HOD residual signal was performed. The free induction decays were processed using standard Fourier transformation.

TRNOESY. The NMR sample (670 μL in D_2O + few drops of CD_3CN) was prepared by mixing 1.8 mg of Δ-**1** (or Λ-**1**) with 0.6 mg of purified DNA (pUST-1 DNA, ~7000 base pair length) previously dissolved in the appropriate phosphate buffer (vide supra) and deuterium exchanged via two freeze-drying cycles. This Ru/DNA concentration ratio is large (~6500/1) with the DNA concentration expressed in terms of double-stranded DNA. Finally, 10 μL of a sodium propionate solution (0.1 M, D_2O) was added to the NMR sample as an internal reference for the NOE cross-peak phasing.

A set of 2D phase-sensitive TRNOESY spectra using the TPPI method were recorded with mixing times of 50, 80, 120, 150, and 200 ms over a single period. FIDs were acquired (32 scans) over 5.0 kHz into a 2K data block for 448 incremental values of the evolution time and a relaxation delay of 2 s. Water signal suppression was achieved by a low-power presaturation pulse (56 dB) during the relaxation delay and mixing time. Before 2D Fourier transformation, zero fillings were added in F_1 and a squared sine-bell window function was applied to both dimensions.

X-ray Structure of *rac*-2. Suitable crystals of *rac*-2 were obtained from the slow diffusion of toluene into a concentrated solution of CH_3CN/CH_2Cl_2 (5/95). The selected crystal was protected by paratone oil and Araldite and then mounted on the top of a glass rod. The data were collected at 173 K on a Nonius KappaCCD diffractometer with graphite-monochromated Mo Kα radiation. The Nonius Supergui program package was used for cell refinement and data collection. The structure was solved by direct methods and subsequent difference Fourier treatment and refined by full-matrix least-squares on F using the programs of the PC version of CRYSTALS.[24] All non-PF_6 molecules and non-hydrogen atoms were refined anisotropically. Hydrogen atoms were introduced in calculated positions in the last refinements and were allocated an overall refinable isotropic thermal parameter. Crystallographic data and collection parameters for *rac*-2 are listed in Table 1. Fractional parameters, anisotropic thermal parameters, and all bond lengths and angles can be found (CIF file) in the Supporting Information.

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Supporting Information Available: 2D-TRNOESY spectral data for Δ-**1** (Figure S1); curve fits for the H3, H5, and H6 protons of both Δ-**1** and Λ-**1** enantiomers (Figure S2) and their K_d calculation (Table S1); a plot indicating the error bars on $\Delta\delta_{obs}$ for the H3 proton of Λ-1 as well as the curve fit (Figure S3); crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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