NMR Evidence for Major Groove Binding of Both Λ- and Δ-[Ru(bpy)$_2$(pqx)](PF$_6$)$_2$ to the Oligonucleotide d(CGCGAATTCGCG)$_2$

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The enantiomeric complexes Λ- and Δ-[Ru(bpy)$_2$(pqx)](PF$_6$)$_2$ [pqx = 2-(2′-pyridyl)quinoxaline] were synthesized and characterized by various spectroscopic techniques. Their binding properties to the oligonucleotide duplex d(CGCGAATTCGCG)$_2$ were subsequently studied by NMR spectroscopy. 1D and 2D NMR techniques provided evidence for major-groove binding of both enantiomers with the oligonucleotide. The Δ-[Ru(bpy)$_2$(pqx)](PF$_6$)$_2$ enantiomer binds more deeply enantiomer to the oligonucleotide than the Λone. The quinoxalinic moiety of the pqx ligand faces the major groove in the Δ complex and is oriented at the external side of the adduct in the Λ complex. Both complexes show some affinity for the T8C9 base sequence of the dodecanucleotide helix.

Indroduction

The binding properties of transition metal tris-chelate complexes with DNA have been extensively studied in recent years.[1,2] One of the pioneering works was the report by Barton et al. pointing out the remarkable difference in the binding strength of the enantiomers of [Zn(phen)$_3$]$^{2+}$ with DNA.[3] The interactions between CT-DNA and the enantiomeric ruthenium complexes Λ- and Δ-[Ru(phen)$_3$]$^{2+}$ have been studied by several methods, with controversial conclusions. Several studies led to the conclusion that the Δ-isomer either intercalates between the base pairs,[4−7] or is partially inserted,[8−10] or it binds to DNA in various ways[11,12] (e.g. in the major[8,10] or in the minor groove[13,14]). On the other hand Λ-[Ru(phen)$_3$]$^{2+}$ binds to DNA either by electrostatic forces,[4,5] or surface-bound interactions,[6,12] and with weak binding in the minor[13,14] or the major groove.[8,10] 2,2′-Bipyridine has been used as a ligand less often than 1,10-phenanthroline in tris-chelate complexes of Ru^{II} in studies of interactions with DNA due to the lower affinity of this ligand to bind to the DNA helix. Based on photophysical results, it has been suggested that Δ-[Ru(bpy)$_3$]$^{2+}$ binds weakly with DNA either by intercalation or electrostatic interactions.[4,5,15] It has also been reported that no enantiomeric discrimination is apparent between the binding of Λ- and Δ-[Ru(bpy)$_3$]$^{2+}$ with DNA.[16]

The interaction of the mixed-ligand complex [Ru(bpy)$_2$(phen)]$^{2+}$ with DNA has been studied by linear dichroism spectroscopy with the aim of investigating the role of each ligand involved in the complex. The results indicate that the complex shows a similar binding geometry to [Ru(phen)$_3$]$^{2+}$ in which one phen ligand faces the DNA base pairs, almost as if it were intercalated nearly parallel to them.[9] A similar mixed-ligand complex, [Ru(bpy)$_2$(ppz)]$^{2+}$ (ppz = 4,7-phenanthrolino[6,5-*b*]pyrazine, a non-classical intercalative ligand), which is longer than phen by a six-membered fused ring, shows that both Λ- and Δ-enantiomers bind in the major groove of DNA by partial intercalation of the ppz ligand. The bpy ligands are not capable of inducing intercalation and the enantioselectivity is maintained by the different interactions of the Δ- and Λ-enantiomers with the DNA major groove.[17] In an attempt to contribute to the better understanding of how these mixed-ligand complexes bind to DNA, we have synthesized, characterized and studied the binding properties of the enantiomeric complexes Λ- and Δ-[Ru(bpy)$_2$(pqx)]$^{2+}$ [pqx = 2-(2′-pyridyl)quinoxaline], to the oligonucleotide duplex d(5′-CGCGAATTCGCG-3′)$_2$ mainly by NMR spectroscopy. The ligand pqx was chosen based on the following criteria: (i) it has an asymmetric nature that could be extended to the two bpy ligands of the complex, making them magnetically distinct; (ii) it is more similar to the structure of bpy than phen, even though it contains a six-membered fused aromatic ring on one side of the molecule.

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Results and Discussion

Synthesis, Characterization and Solution Structure of the Enantiomers

rac-[Ru(bpy)$_2$(pqx)](PF$_6$)$_2$ has been synthesized previously by a one-step reaction between *cis*-[RuCl$_2$(bpy)$_2$] and pqx,[18] although the enantiomeric resolution by chromatographic techniques was unsuccessful. This is the reason our synthesis of the enantiomers was based on the optically pure precursors Δ- and Λ-[Ru(bpy)$_2$(py)$_2$]A·12H$_2$O (A = *O*,*O*′-dibenzoyltartaric acid), by replacing the two pyridine molecules by the bidentate ligand 2-(2′-pyridyl)quinoxaline (pqx) in ethylene glycol/DMSO (9:1). Since the substitution of the pyridines by pqx requires high temperature (ca. 160 °C), a stream of argon was used to prevent the oxidation of Ru^{II}. Isolation of the final products was achieved after addition of NH_4PF_6.

The CD spectra of Λ- and Δ-[Ru(bpy)$_2$(pqx)](PF$_6$)$_2$ are mirror images of each other and exhibit absorptions at λ_{max} ($\Delta\varepsilon$) = 463.7 nm (−1.6 м$^{-1}$cm^{-1}), 419.2 (+ 9.5), 402.3 (+9.1), 376.2 (−4.2) and 361.6 (-4.8) for the Δ-enantiomer and at 464.2 (+1.6), 419. 5 (−9.4), 403.8 (−9.0), 377.0 (+ 4.2) and 362.4 (+ 4.8) for the Λ-enantiomer (Figure 1). These assignments were made by comparison with similar complexes of known configuration.[19,20]

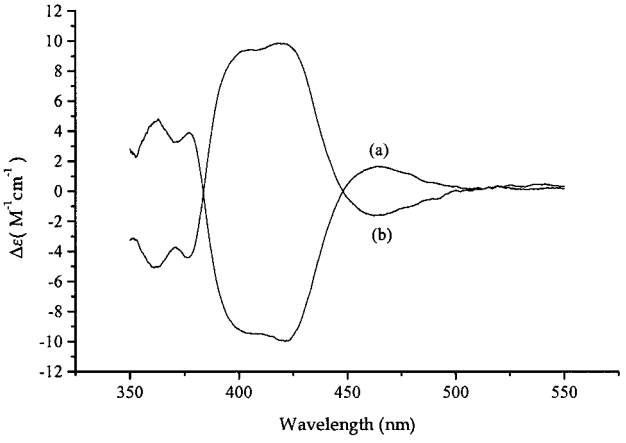

Figure 1. The CD spectra of: (a) Λ-[Ru(bpy)$_2$(pqx)](PF$_6$)$_2$ and (b) Δ-[Ru(bpy)$_2$(pqx)](PF$_6$)$_2$ in the region 350−550 nm

The ^{1}H and ^{13}C NMR resonances of the enantiomers were assigned assisted by 2D [^{1}H,^{1}H] DQF-COSY and 2D [^{1}H,^{13}C] HMBC/HMQC experiments. Figure 2 shows the structures of Λ- and Δ-[Ru(bpy)$_2$(pqx)](PF$_6$)$_2$ with atom labeling. Table 1 and 2 present chemical shift data for the enantiomeric complexes.

The ^{1}H and ^{13}C NMR spectra of both enantiomers show resolved signals for each of the bpy ligands, indicating that they are magnetically inequivalent. In addition, separate signals for the constituents of the bpy ligands (i.e. the pyridine rings) were observed. Sixteen proton and twenty carbon signals were observed in total, corresponding to all the proton and carbon nuclei of the two bpy ligands of each

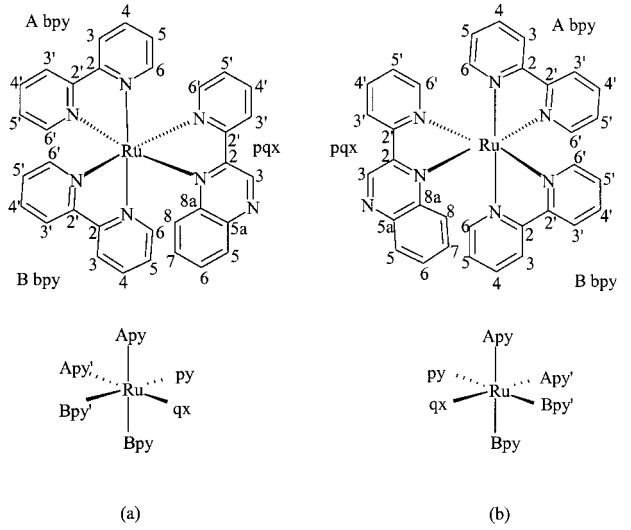

Figure 2. Structures of the enantiomeric complexes Λ- and Δ-[Ru(bpy)$_2$(pqx)]$^{2+}$, including atom numbering

enantiomer, which were fully assigned. This observation shows that the influence of the asymmetric nature of the pqx ligand on the magnetic environment of the two bpy molecules is important, in contrast to what was suggested for other similar complexes.[20−22] Since in the cases of Λ- and Δ-[Ru(bpy)$_2$(pqx)]$^{2+}$ the bpy ligands are magnetically discernible (probably due to the high magnetic field), it is necessary to define the relative location of the two bpy molecules towards pqx. In an attempt to determine the complete assignments of the enantiomers ^{13}C NMR spectroscopy was used to evaluate the Coordination Induced Shifts (CIS = $\delta_{complexed} - \delta_{free}$) and 2D [^{1}H^{1}H] ROE spectroscopy to investigate internuclear distances of less than 5 Å between the ligand protons. In general, the major effects affecting the CIS are: (i) the σ-withdrawal of electron density of the carbon nuclei through the nitrogen lone-pair upon coordination, causing downfield shifts, (ii) the π-back donation from the metal back-bonding to the π* orbitals of the ligand, causing downfield shifts, and (iii) the location of a carbon nucleus close to a high-electron-density area, such as an aromatic ring, causing dramatic upfield shifts. Figure 3 presents the CIS for all carbon atoms of the pyridine rings (py) of the complex Ru(bpy)$_3^{2+}$ together with the CIS of the pyridine rings of the two bpy (Abpy and Bbpy) and the pqx ligand of the complex Δ-[Ru(bpy)$_2$(pqx)]$^{2+}$ (see Figure 2). In the case of Ru(bpy)$_3^{2+}$ all six pyridine rings are magnetically equivalent, with the planes of the bipyridine rings perpendicular to each other. Also, the six pyridine rings are opposite to each other in the coordination sphere of the ruthenium.

As seen in Figure 2, the planes of the pyridine rings of Apy and Bpy, which occupy the axial sites of the complex, are perpendicular to the py and qx moieties of the pqx respectively. Since the quinoxalinic part of pqx is a more extended aromatic system (two fused six-membered aromatic rings) than a single pyridine ring, it is expected to provide a lower electron density to the C$_6$ atom of the per-

Table 1. ^{13}C NMR[a] chemical shifts in ppm for the complex $[Ru(bpy)_2(pqx)](PF_6)_2$ in $CDCl_3$ at 298 K; CIS indicates the Coordination Induced Shifts ($CIS = \delta_{complexed} - \delta_{free}$)

Carbon[a]	$[Ru(bpy)_2(pqx)](PF_6)_2$	CIS
A bpy C2	157.24	+1.79
A bpy C3	125.75	+5.24
A bpy C4	139.29	+2.56
A bpy C5	128.72	+4.99
A bpy C6	151.86	+2.94
A bpy C2′	157.20	+1.75
A bpy C3′	125.76	+5.25
A bpy C4′	139.71	+2.98
A bpy C5′	129.17	+5.44
A bpy C6′	151.99	+3.07
B bpy C2	157.35	+1.90
B bpy C3	125.51	+5.00
B bpy C4	138.88	+2.15
B bpy C5	129.16	+5.43
B bpy C6	154.50	+5.58
B bpy C2′	157.16	+1.71
B bpy C3′	125.12	+4.61
B bpy C4′	139.17	+2.44
B bpy C5′	128.95	+5.22
B bpy C6′	151.89	+2.97
pqx C2	154.81	+5.37
pqx C3	144.93	+0.70
pqx C5a	144.55	+2.71
pqx C5	125.18	−4.53
pqx C6	132.57	+2.47
pqx C7	133.15	+3.10
pqx C8	131.50	+2.17
pqx C8a	143.71	+1.17
pqx C2′	157.45	+2.94
pqx C3′	128.15	+6.02
pqx C4′	139.56	+2.46
pqx C5′	129.27	+4.67
pqx C6′	152.70	+2.58

[a] NMR spectroscopic data at 600 MHz in $CDCl_3$ at 298 K (in ppm): (i) 2,2′-bipyridine: C2 = 155.45, C3 = 120.51, C4 = 136.73, C5 = 123.73, C6 = 148.92; (ii) ligand pqx: C2 = 149.44, C3 = 144.23, C5a= 141.84, C5 = 129.71, C6 = 130.10, C7 = 130.05, C8 = 129.33, C8a = 142.54, C2′ = 154.51, C3′ = 122.13, C4′ = 137.10, C5′ = 124.60, C6′ = 150.12; (iii) $[Ru(bpy)_3]^{2+}$: C2 = 156.50, C3 = 124.80, C4 = 138.00, C5 = 127.80, C6 = 150.80.

pendicularly located pyridine ring. Consequently, the CIS value of this nucleus differs from those of the other five C_6 and $C_{6'}$ atoms because it is less shielded, causing the higher downfield shift. Indeed, the C_6 of the Bpy ring shifts downfield by 5.60 ppm, about two times more than the C_6 of the other pyridine rings, indicating that Bpy is the one located vertically with respect to the quinoxaline ring system.

The σ- and π-back donations are two factors related to the bond strength that may also affect the CIS of the carbon nuclei of the pyridine rings. For both enantiomers the pyridine rings Apy′ and Bpy′ are located opposite the quinoxaline (qx) and pyridine (py) moieties of pqx, respectively, while the pyridine rings Apy and Bpy are opposite to each other (see Figure 2). Thus, only one of the five (py) rings seems to differ (slightly) from the others with respect to the σ- and π-back donation to the ruthenium coordination sphere. This is the Apy′ opposite to the (qx) moiety of the

Table 2. ^1H NMR chemical shifts in ppm of the free $[Ru(bpy)_2(pqx)](PF_6)_2$ complexes, identical in both Δ- and Λ-enantiomers and in the presence of the oligonucleotide duplex $[d(CGCGAATTCGCG)_2]$ in D_2O (100 mM buffer phosphate pH 7.01) at 298 K in a 1:1 molar ratio; all shifts are referred to HOD at $\delta = 4.69$ ppm

Complex proton	$[Ru(bpy)_2(pqx)](PF_6)_2$	Δ-Isomer	Λ-Isomer
A bpy H3	8.47	−0.06	−0.05
A bpy H3′	8.46	−0.06	−0.05
A bpy H4	7.97	−0.05	−0.03
A bpy H4′	7.98	−0.02	−0.02
A bpy H5	7.25	−0.07	−0.14
A bpy H5′	7.21	−0.15	−0.13
A bpy H6	7.54	−0.38	−0.38
A bpy H6′	7.57	−0.32	−0.35
B bpy H3	8.28	−0.07	−0.05
B bpy H3′	8.19	−0.07	−0.07
B bpy H4	7.95	−0.14	−0.14
B bpy H4′	7.81	−0.02	−0.03
B bpy H5	7.16	−0.04	−0.01
B bpy H5′	7.16	−0.02	−0.01
B bpy H6	7.53	−0.08	−0.08
B bpy H6′	7.28	−0.11	−0.07
pqx H3	9.51	−0.20	−0.15
pqx H3′	8.66	−0.15	−0.10
pqx H4′	8.03	−0.03	−0.03
pqx H5′	7.31	+0.04	+0.04
pqx H6′	7.64	−0.07	−0.05
pqx H5	7.85	−0.14	−0.14
pqx H8	7.57	−0.17	−0.17
pqx H6	7.32	−0.01	−0.01
pqx H7	7.17	−0.15	−0.19

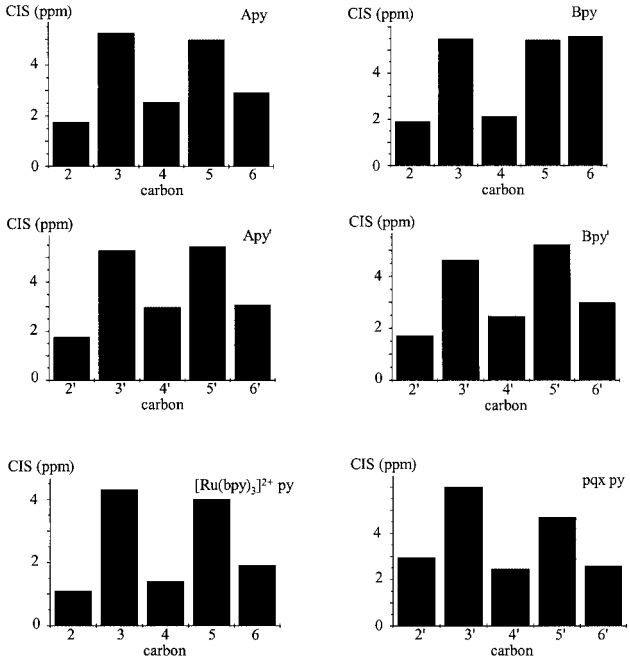

Figure 3. The patterns of CIS of the carbons for all the pyridine rings of the complex and the pyridine ring (all equivalent) of the complex $[Ru(bpy)_3]^{2+}$.

pqx. Supposing that the Ru−N(py) bond strength differs from that of Ru−N(qx), the bond opposite to them will also differ. Consequently, the Apy′ with the higher downfield shifts of its carbon atoms (see Table 1) is probably located opposite the qx in the meridian plane of the ruthenium octahedron.

2D (^{1}H,^{1}H) ROESY spectroscopy was also used to confirm the above structure. Figure 4 shows the aromatic region of the ROESY spectrum of Δ-[Ru(bpy)$_2$(pqx)]$^{2+}$ at τ_m = 80 ms at a concentration of 5 mM and a temperature of 298 K. Note that three ROESY spectra were acquired with different mixing times of 60, 80 and 120 ms to select the optimum value for the intramolecular (^{1}H,^{1}H) correlation (r = 5 Å). The low concentration was necessary to avoid intermolecular contacts due to π-π stacking effects between the aromatic rings of the ligands of neighboring ions of the complex Δ-[Ru(bpy)$_2$(pqx)]$^{2+}$.[21]

Many intramolecular cross peaks were observed and were assigned to internuclei spin-spin interactions between the neighboring protons of the aromatic rings of the ligands, together with cross-peaks between the ring protons. A very clear cross-peak appears between H4′ of the pqx ligand and H6 of the Bbpy ligand, showing that these protons are close to each other. Additionally, the observation of an interligand cross-peak between H6 of Abpy and H6 of pqx confirms the structure shown in Figure 2. In this structure the plane of the Bpy′ ring is located perpendicular to the pyridine moiety of the pqx ligand and the Apy ring is also vertically located to the quinoxalinic ring system of the pqx ligand.

Interactions of the Enantiomers with d(CGCGAATTCGCG)$_2$

The addition of the Λ- or Δ-[Ru(bpy)$_2$(pqx)]$^{2+}$ enantiomer to the oligonucleotide duplex d(CGCGAATTCGCG)$_2$ causes changes in the proton chemical shifts of both enantiomers and the oligonucleotide; these are listed in Table 2 and 3.

The ^{1}H NMR spectra of the adduct of the Δ- or Λ-enantiomer to the oligonucleotide duplex at a 0.5:1 and 1:1 molar ratio at 298 K show only one set of resonances, indicating a fast exchange.[22]

A good indicator of which groove of the oligonucleotide the enantiomer binds to is the observed change in the chemical shifts of specific protons of the oligonucleotide duplex. The protons AH8 and GH8, CH6/H5, sugar H2′and H2′′ located in the major groove and AH2 and sugar H1′in the minor groove are mostly affected by this binding. The influence of the high electron density of the ligand aromatic protons causes upfield shifts of these protons. For both the Λ- and the Δ-enantiomer, only very small changes for all these protons (−0.02 to +0.02 ppm) were observed, showing that the stacking between the DNA base rings remains intact keeping the B-DNA structure. A π-type stacking interaction between the bases and the aromatic ligands of the enantiomers has to be excluded due to the

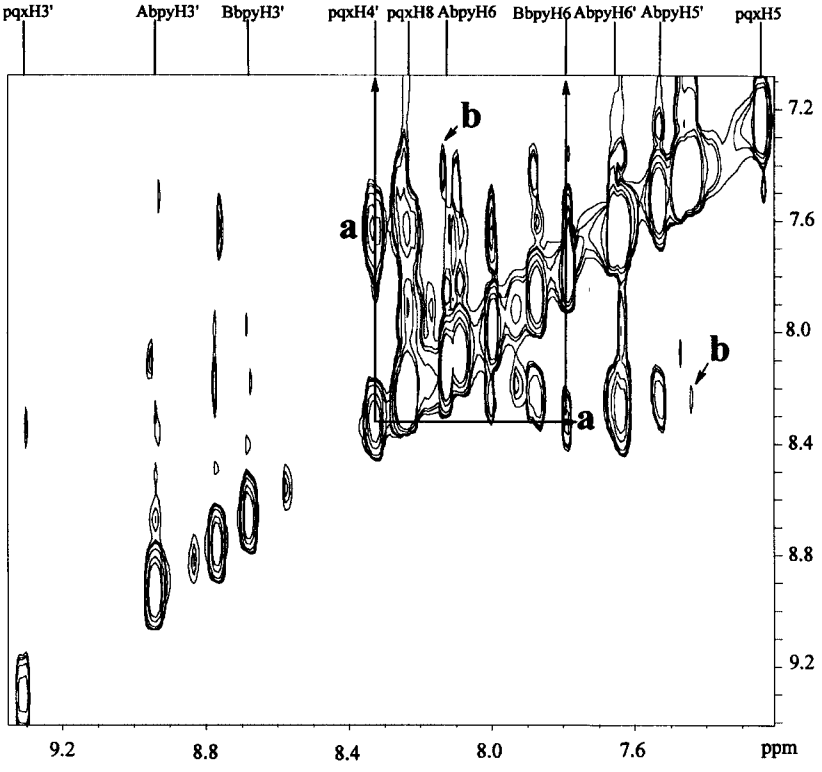

Figure 4. Part of the ROESY spectrum (τ_m = 80 ms) of Δ-[Ru(bpy)$_2$(pqx)](PF$_6$)$_2$; the labelled cross peaks correspond to ROE conducts assigned to the protons pqxH4′ and BbpyH6 (a) and pqxH6 and AbpyH6 (b), confirming the suggested structure of Figure 2

Table 3. Chemical shift changes of the oligonucleotide protons upon addition of the Ru enantiomers under the same conditions; the changes indicate the difference between the bound and free DNA ($\delta_B - \delta_F$); all shifts are referred to HOD at $\delta = 4.69$ ppm

	H6/H8	H2/H5/CH3	H1′	H2′	H2′′
C1	7.49	5.76	5.62	1.83	2.27
Δ-	−0.01	−0.04	−0.03	−0.04	−0.01
Λ-	−0.02	−0.02	−0.04	−0.07	−0.01
G2	7.82		5.75	2.52	2.58
Δ-	−0.02		0.00	0.00	+0.01
Λ-	−003		−0.05	−0.01	+0.02
C3	7.20	5.23	5.46	1.77	2.20
Δ-	−0.00	0.00	0.00	−0.05	−0.06
Λ-	−0.02	+0.01	+0.01	−0.05	−0.07
G4	7.72		5.31	2.61	2.63
Δ-	0.00		−0.01	−0.05	−0.10
Λ-	−0.01		−0.02	−0.05	−0.10
A5	7.98	7.10	5.87	2.62	2.80
Δ-	0.00	−0.01	−0.01	−0.01	0.00
Λ-	−0.01	−0.01	−0.01	−0.01	0.00
A6	7.98	7.49	6.03	2.44	2.81
Δ-	0.00	−0.01	0.00	−0.01	−0.02
Λ-	−0.01	−0.01	0.00	−0.01	−0.02
T7	6.98	1.13	5.78	1.85	2.44
Δ-	0.00	+0.01	0.00	0.00	0.00
Λ-	0.00	+0.01	+0.01	0.00	+0.10
T8	7.24	1.39	5.97	2.04	2.42
Δ-	−0.02	−0.01	0.00	0.00	−0.01
Λ-	+0.02	0.00	−0.02	0.00	0.00
C9	7.33	5.49	5.53	1.93	2.29
Δ-	0.01	+0.01	0.00	0.00	−0.01
Λ-	−0.01	0.00	−0.03	−0.01	0.00
G10	7.78		5.72	2.51	2.57
Δ-	0.00		+0.01	0.00	+0.01
Λ-	0.00		0.00	0.00	+0.01
C11	7.14	5.30	5.62	1.76	2.14
Δ-	0.00	+0.01	−0.03	+0.03	+0.02
Λ-	0.00	0.00	−0.03	0.00	+0.02
G12	7.80		6.01	2.25	2.49
Δ-	−0.02		−0.04	−0.04	−0.02
Λ-	−0.02		−0.05	−0.02	0.00

small upfield shifts of the protons of the oligonucleotide bases. Even though the environment of the bases in DNA is already rich in electron density, since they are stacked over the *z* axis itself with a separation of 3.4 Å, intercalators cause upfield shifts of about −0.2 ppm in the base protons by increasing their electron density still further.[23]

Small changes in the chemical shifts for the pqx ligand protons in both the Δ- and Λ-$Ru(bpy)_2(pqx)]^{2+}$ enantiomers, ranging from +0.04 to −0.20 ppm, were also observed, thus excluding any possibility for intercalation. It is known that for an intercalating aromatic ring, which stacks above and below the ring planes of a DNA base-pair, a dramatic upfield shift of −0.5 to −1.0 ppm typically occurs.[24] The magnitude of the upfield shift depends on the relative position of the protons of the intercalating ligand and the center of the base plane. Classical intercalators, like dppz, cause higher upfield shifts of the protons that are located between the DNA bases, by about −0.80 ppm[22] in the complex Δ-$[Ru(phen)_2(dppz)]^{2+}$ and by -0.67 ppm in the complex Δ-$[Ru(Me_2phen)_2(dppz)]^{2+}$.[23] As for the other two bipyridine ligands (Abpy and Bbpy), the most affected protons are H6 and H6′ of Abpy, which shift upfield by -0.32 and -0.38 ppm, respectively, for both the Δ- and the Λ-enantiomer. These values are not large enough to allow us to conclude that this ligand intercalates between the base pairs. Upfield shifts of the order of −0.2 to −0.4 ppm have been observed for the proton signals of ligands that are either partially inserted between the DNA bases,[25,26] or associate to the DNA grooves (e.g. 1,10-phenanthroline). Both Λ- and Δ-$[Ru(phen)_3]^{2+}$ bind to the minor groove of the oligonucleotide[13,14] $d(CGCGATCGCG)_2$ and cause upfield changes of about −0.3 ppm in some of the phen proton signals.[14] It is worth mentioning that in both enantiomers the influence of the binding causes similar changes to the proton chemical shifts.

The intermolecular NOEs observed between the Λ- or Δ-enantiomer and $d(CGCGAATTCGCG)_2$ are listed in Table 4. Figure 5 and 6 show portions of the NOESY spectra of the Λ- or Δ-$[Ru(bpy)_2(pqx)]^{2+}$/$d(CGCGAATTCGCG)_2$ adduct at 298 K.

Table 4. Intermolecular NOEs observed between Δ- and Λ-$[Ru(bpy)_2(pqx)]^{2+}$ and the oligonucleotide duplex $d(CGCGAATTCGCG)_2$ at molar ratio 1:1 and temperature 298 K

Λ-$[Ru(bpy)_2(pqx)]^{2+}$	Δ-$[Ru(bpy)_2(pqx)]^{2+}$
T8H6 AbpyH3′	T8H6 AbpyH3′
T8H6 BbpyH3	T8H6 BbpyH4
T8H6 pqxH6′	T8H6 BbpyH6
H5pqx C9H2′′	T8H6 pqxH8
H5pqx G10H2′′	C9H6 pqxH8
	C9H6 BbpyH4
	G10H2′′ pqxH5
	C9H6 BbpyH3

In both NOE maps of the adducts the sequential NOEs arising from the inter- and intramolecular contacts between the oligonucleotide protons remain intact, indicating that the B-type conformation is kept upon interaction with Λ- or Δ-$[Ru(bpy)_2(pqx)]^{2+}$. This observation is consistent with a groove or a weak type of binding rather than with an intercalation, as this would cause significant perturbation in the DNA helix. Small differences in the cross-peak intensities of the duplex indicate insignificant changes of the oligonucleotide structure following interaction with the Ru enantiomers.

In the case of the Λ-isomer, a few cross-peaks with weak to medium intensities are observed. In the aromatic region, three intramolecular NOEs appear between the complex protons AbpyH3, BbpyH3 and pqxH6′, and T8H6. Since additional cross-peaks between the aromatic protons of bases C9 and T7 (neighboring to T8) and the enantiomer are not detected, the binding with intercalation above or below the T8 plane must be excluded. Taking into account that two more intermolecular cross-peaks between C9H2′′ and G10H2′′ and pqxH5 are observed, we can propose that

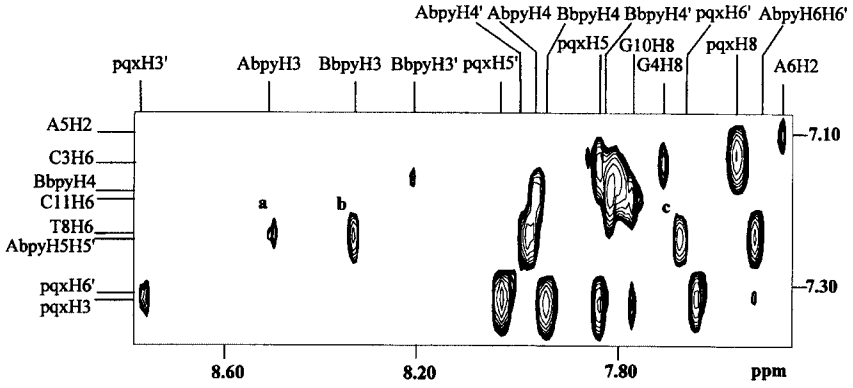

Figure 5. Part of the ^{1}H NOESY spectrum (τ_m = 350 ms) of the Λ-[Ru(bpy)$_2$(pqx)](PF$_6$)$_2$·[d(CGCGAATTCGCG)$_2$] adduct (1:1 molar ratio) at 298 K with the assignments of the aromatic protons; the labelled cross-peaks correspond to NOE conducts between the protons T8H6 and AbpyH3′ (a), T8H6 and BbpyH3 (b), and T8H6 and pqxH6′ (c)

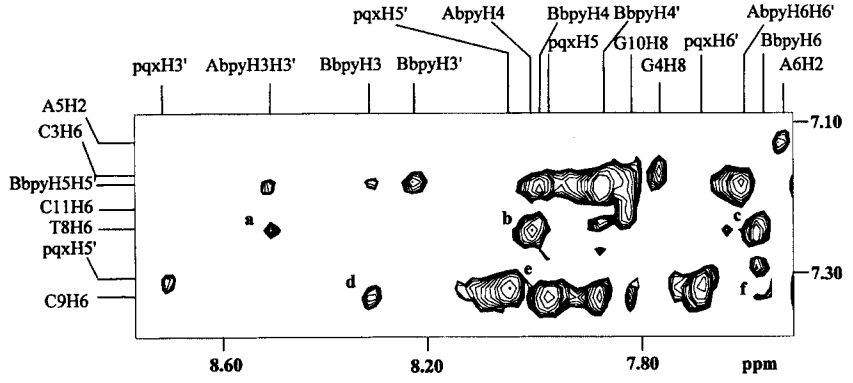

Figure 6. Part of the ^{1}H NOESY spectrum (τ_m = 350 ms) of the Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$·[d(CGCGAATTCGCG)$_2$] adduct (1:1 molar ratio) at 298 K with the assignments of the aromatic protons; the labelled cross-peaks correspond to NOEs between the protons T8H6 and AbpyH3 (a), T8H6 and BbpyH4 (b), T8H6 and BbpyH6 (c), C9H6 and BbpyH3 (d), C9H6 and BbpyH4 (e), and C9H6 and pqxH8 (f)

the complex binds in the major groove of the oligonucleotide where all these protons are located. The absence of cross-peaks between the complex protons and the sugar H1′ proton, or the adenine ring protons H2, located in the minor groove support the suggestion of binding in the major groove, close to the base T8. All five observed intermolecular NOEs cannot arise from the same binding state of the complex to the oligonucleotide due to the fast exchange kinetics of the system but, fortunately, they are located in the same part of the oligonucleotide duplex. While binding is unambiguous for the region -T8-C9- of the d(CGCGAATTCGCG)$_2$ complex, it is difficult to suggest which of the three ligands (Abpy, Bbpy, or pqx) binds to the oligonucleotide. All five observed NOEs should belong to different binding states (different ligands) — T8H6 pqxH6 and T8H6 BbpyH3. In an attempt to understand how the enantiomer binds to the oligonucleotide, a binding model generated by manually docking the complex close to the T8 of the major groove of the helix was obtained. Rotation of the complex in the range of ±15° in its *x*, *y*, *z* axis, produced a superimposed structure that satisfied all the observed NOE cross-peaks. In general, the obtained structures show that the complex binds mainly through the ligand Abpy in the major groove of the helix while the pyridine rings Bpy of the ligand Bbpy and py of the ligand pqx, are oriented along the DNA *z* axis. The quinoxaline moiety of the pqx ligand occupies the external side of the Λ- [Ru(bpy)$_2$(pqx)]$^{2+}$/d(CGCGAATTCGCG)$_2$ adduct (Figure 7).

Moreover, with this orientation the complex has an approximate height along the *z* DNA axis of approximately 11.5 Å. This is defined as the distance between the Bbpy H4 and the H4 of the pyridine ring of the pqx ligand, which is closer to the dimensions of the major groove (11.6 Å width × 8.5 Å depth), than those of the minor (6.0 Å width × 8.2 Å depth) in the B-DNA structure.[27] In this way the depth of the insertion is also defined. A ligand (L) with an extended aromatic fused rings' system in its Ru^{II} complexes of general formulae [Ru(phen)$_2$(L)]$^{2+}$, intercalates between the DNA bases with significant perturbation of the DNA structure, thereby affecting the dimentions of the grooves. Such complexes (e.g. Δ-[Ru(Me$_2$phen)$_2$(dppz)]$^{2+}$,[23] Δ-[Ru(phen)$_2$(L)]$^{2+}$ L = dipyrido[3,2-*a*:2′,3′-*c*][6,7,8,9]tetrahydrophenazine,[28] etc.) bind to the minor groove following

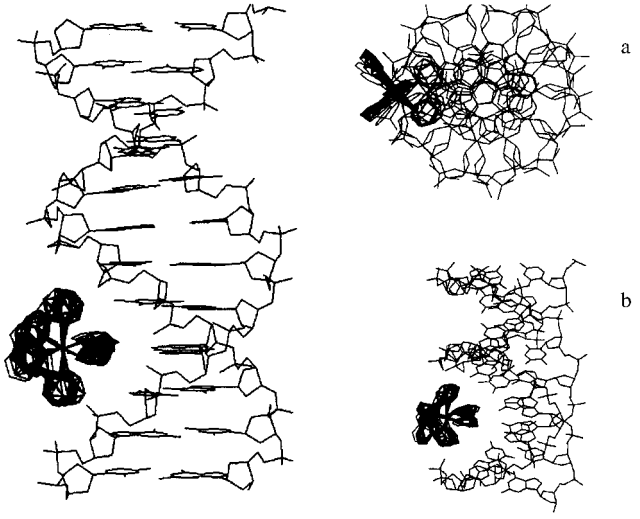

Figure 7. A molecular model of the interactions between Λ-$[Ru(bpy)_2(pqx)]^{2+}$ and $d(CGCGAATTCGCG)_2$ reflects qualitatively the binding of the complex to the oligonucleotide; inset: (a) top view along the *z*-axis showing the complex orientation with only the Abpy ligand facing the oligonucleotide; (b) a view across the major groove showing the complex orientation

the intercalating ligand which prefers the narrow minor groove and is stabilized by van der Waals interactions. On the other hand, complexes without a "leader" intercalative ligand such as $[Ru(bpy)_3]^{2+}$ or $[Ru(phen)_3]^{2+}$ bind without intercalation. Based on photophysical results it has been suggested that $[Ru(bpy)_3]^{2+}$ binds to DNA weakly[15] without intercalation and its electrostatic interactions with the DNA phosphates are ambiguous. Minor-[13,14] or major-groove[8,10] binding has been found for the related complex $[Ru(phen)_3]^{2+}$ for both Λ- and Δ-enantiomers by spectroscopic techniques. Λ-$[Ru(phen)_3]^{2+}$ was found to interact with the DNA minor groove only with one phenanthroline molecule such as in the case of Λ-$[Ru(bpy)_2(pqx)]^{2+}$ that interact only with the Abpy. Hiort et al. have suggested a major-groove binding for both enantiomers of the complex $[Ru(phen)_3]^{2+}$ based on LD studies.[10] Major-groove binding was also observed for both enantiomers of the complex $[Ru(bpy)_2(ppz)]^{2+}$, where the ligand ppz was deeply inserted between the base pairs.[17] In the case of Λ-$[Ru(bpy)_2(pqx)]^{2+}$, which clearly prefers the major groove, the 2,2′-bipyridine ligand seems to have a lower affinity than 1,10-phenanthroline, with van der Waals interactions with the DNA mainly taking place from the minor groove. On the other hand, electrostatic interactions that probably coexist in our case take place through the phosphates in the major groove. Additionally, the bpy ligands provide less steric hindrance than phenanthroline. These are probably the reasons why Λ-$[Ru(bpy)_2(pqx)]^{2+}$ prefers the major groove.

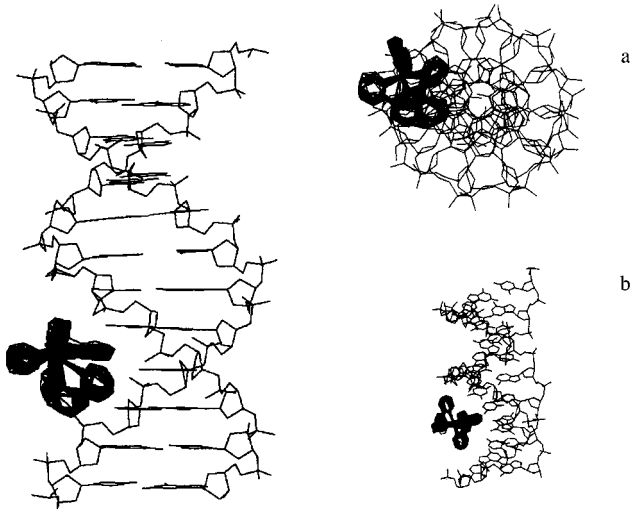

Figure 8. A molecular model of the interactions between Δ-$[Ru(bpy)_2(pqx)]^{2+}$ and $d(CGCGAATTCGCG)_2$ reflects qualitatively the binding of the complex to the oligonucleotide; inset: (a) top view along the *z*-axis showing that the complex inserts both the Bbpy and the pqx ligands into the major groove of the helix; (b) a view across the major groove showing the complex orientation

In the case of the Δ-isomer many more cross-peaks are observed, all of which are located at the same part of the oligonucleotide close to the bases T8 and C9, as in the case of the Λ- isomer. The protons that are involved in these intramolecular NOEs are all located in the major groove of the helix. This observation is in agreement with the results of 1D ^1H NMR spectra where the chemical-shift changes are very similar for both enanatiomers. However, some specific cross-peaks between the Δ-enantiomeric protons and the base ring protons, particularly in neighboring bases (e.g. the pqxH8 with T8H6 and C9H6), indicate that the complex is inserted deeply into the major groove. Due to the fast kinetics of the binding it is not possible to assign all the observed cross-peaks to one binding state, but, as shown in Figure 8, all the generated structures (according to the binding model procedure) place the Bpy of Bbpy and the quinoxalinic part of the pqx ligand close to the bases in the depth of the major groove. The Abpy ligand is located on the external part of the adduct without being in contact with the oligonucleotide helix.

To be consistent with the groove dimentions, this deeper insertion of the Δ- than the Λ-isomer means that the internal *z* axis of the complex is not parallel to the *z* axis of the helix but is slightly bent. In this orientation the complex interacts with the major groove of the oligonucleotide with the two ligands without causing a significant distortion of the DNA structure. Based on intramolecular NOE contacts, it has been suggested that the complex Δ-$[Ru(phen)_3]^{2+}$ binds to $d(CGCGATCGCG)_2$ by insertion of two phenanthroline molecules into the minor groove of the central AT part of the oligonucleotide.[13,14] This suggestion seems to be in contrast with our observation that the complex binds to the major groove. However, the complex $[Ru(bpy)_2(pqx)]^{2+}$ shows an affinity for the oligonucleotide T8C9 base sequence and not for the central AATT region, and this is probably the reason why the approach of the helix is from the major groove. In general, the central AATT region of $d(CGCGAATTCGCG)_2$ is preferred by molecules that bind from the minor groove.[29,30]

Conclusions

In both enantiomers Λ- and Δ-[Ru(bpy)$_2$(pqx)]$^{2+}$ the asymmetric nature of the pqx ligand causes different magnetic environments of the two bpy ligands and allows the distinction of Abpy and Bbpy. The pqx ligand is not a classical intercalator and its DNA binding properties were previously unexplored. The determination of the complete assignment of these complexes allows us to understand better their binding properties with the oligonucleotide d(CGCGAATTCGCG)$_2$. The main conclusions are summarized below:

(i) As indicated by NMR spectroscopy, both enantiomers bind non-intercalatively in the d(CGCGAATTCGCG)$_2$ major groove close to the T8C9 sequence without significant perturbation of the helix structure. Evidence for a similar binding of the enantiomers comes from the similar changes in the proton chemical shifts of the adducts, and the NOE results.

(ii) Δ-[Ru(bpy)$_2$(pqx)]$^{2+}$ binds more deeply than Λ-[Ru(bpy)$_2$(pqx)]$^{2+}$, with two ligands (pqx and Bbpy) facing the DNA groove while the Λ isomer only has one (Abpy).

(iii) The quinoxalinic moiety of the pqx ligand in the case of Δ-[Ru(bpy)$_2$(pqx)]$^{2+}$ contributes to the interaction with the oligonucleotide while in the case of the Λ-enantiomer it is located in the external part of the adduct.

Experimental Section

Materials: All chemicals and solvents were of reagent grade and were used without further purification. The deoxynucleotide d(5′-CGCGAATTCGCG-3′) was purchased from OSWEL DNA Service (University of Southampton, UK) and purified by chromatography on a 120 × 2.5 cm Sephadex G-25 superfine column, using distilled water as eluent and lyophilized to dryness. The complexes *cis*-[RuCl$_2$(bpy)$_2$][31] and Λ- and Δ-[Ru(bpy)$_2$(py)$_2$]A·12H$_2$O (where A = *O*,*O*′-dibenzoyltartaric acid),[32,33] and the ligand 2-(2′-pyridyl)quinoxaline (pqx) were synthesised according to literature procedures.[34]

Instrumentation: Elemental analyses for C, H and N were performed using a Carlo−Erba 1108 apparatus. CD spectra were recorded on a Jasco J-720 spectropolarimeter. A 2-cm path length cuvette was used for the region 350−500 nm. All NMR spectroscopic data were acquired at 150.92 and 201.21 MHz for ^{13}C and 600.13 and 800.13 MHz for ^{1}H transmitter frequencies on AVANCE 600 and 800 MHz Bruker spectrometers. 1D (^{1}H and ^{13}C) spectra for both enantiomers were recorded at 298 K in either $CDCl_3$ or D_2O. In addition 2D (^{1}H,^{1}H) DQF-COSY, 2D (^{1}H,^{13}C) HMBC/HMQC and 2D (^{1}H,^{1}H) ROESY spectra were acquired in $CDCl_3$ on the AVANCE 600 and (^{1}H,^{1}H) DQF-COSY in D_2O on the 800 MHz instrument, at 298 K. In the case of (^{1}H,^{13}C) HMBC/HMQC spectra, a total of 2048 complex points in t_2 were collected for each of 320 t_1 increments. (^{1}H,^{1}H) ROESY spectra were collected at mixing times (τ_m) between 60 and 120 ms with the pulse program roesyetgp.[35,36] 2D (^{1}H,^{13}C) HMBC spectra were obtained using the inv4gplplrnd pulse sequence, optimized for long-range couplings, with a low-pass J-filter to suppress one-bond correlation. The AVANCE 800 instrument was used mainly to acquire the spectra of the [d(CGCGAATTCGCG)$_2$]/[Ru(bpy)$_2$(pqx)]$^{2+}$ adducts in D_2O at 298 K. (^{1}H,^{1}H) DQF-COSY spectra were recorded using the cosydfesgpph26 pulse programe, while the water suppression was achieved using excitation sculpting with gradients allowing for presaturation during relaxation delay in cases of radiation damping phasecycle.[37] The (^{1}H,^{1}H) NOESY spectra were collected in 4096 complex points in t_2 for each of 512 t_1 increments at two mixing times (τ_m) of 350 and 400 ms with the noesyesgpph26 phase-sensitive pulse programme using the TPPI method.[38] Finally the (^{1}H,^{13}C) HMQC spectra were collected in 2048 complex points in t_2 for each of 128 t_1 increments with the pulse sequence inv4prtp with decoupling during acquisition.[39] The NMR spectroscopic data were processed using the Bruker Xwin NMR Ver. 2.6 software.

Sample Preparation: In each oligonucleotide sample the amounts were estimated by weighing and the concentrations were determined from the absorption at 260 nm.[40] In all NMR experiments a 100 mM Na_2HPO_4/NaH_2PO_4, pH 7.00 (uncorrected for isotope effects) buffer was used. The lyophilized samples were dissolved in D_2O (99.9%) and lyophilized again to dryness. These samples were finally dissolved in 0.4 mL of D_2O (99.96%) and transferred to 5 mm NMR tubes. The 1D ^{1}H NMR spectra were recorded on samples with a concentration of about 50 OD_{260} units while the 2D NOE experiments were performed with more-concentrated samples (ca. 200 OD_{260}). No internal chemical-shift reference was added to the samples.

Molecular Modeling: Both Λ- and Δ-[Ru(bpy)$_2$(pqx)](PF$_6$)$_2$ were constructed manually from the X-ray atomic coordinates of the closely related complexes [Ru(bpy)$_2$(py)$_2$]$^{2+}$.[33] Each of the above complexes was docked manually at the major groove of the dodecanucleotide close to bases T8C9. The enantiomers were rotated along the axis *x*, *y*, *z*, to produce superimposed structures. This average structure satisfied all the observed NOE interatomic distances between the protons of the complex and the dodecanucleotide. The DNA helix was in the B-type configuration and was produced by the database of the molecular software program HyperChem. v. 6.0. Graphical outputs were obtained with the RasMol v2.6 software.

Δ-[Ru(bpy)$_2$(pqx)](PF$_6$)$_2$: Δ-[Ru(bpy)$_2$(py)$_2$]A·12H$_2$O (0.1 mmol, 105 mg) was dissolved in 10 mL of a 9:1 mixture of ethylene glycol and DMSO and a solution of pqx (0.4 mmol, 83 mg) in the same solvent (5 mL) was added. The mixture was refluxed for 24 hours under argon (99.999%). After cooling to room temperature, 5 mL of water was added and the mixture filtered to remove the excess of pqx. A saturated solution of NH_4PF_6 (5 mL) was added to the remaining clear solution. A deep-red precipitate was obtained by cooling the solution to 4 °C overnight, and this was filtered and washed with cold water (2 × 5 mL) and diethyl ether (2 × 5 mL). The crude solid was dissolved in MeCN and purified on an activated aluminum oxide column (20 × 1 cm) using MeCN as eluent. The solvent (MeCN) was evaporated from the collected brown-orange band to yield orange needles of Δ-[Ru(bpy)$_2$(pqx)](PF$_6$)$_2$ (yield 65%). $C_{33}H_{25}F_{12}N_7P_2Ru$: calcd. C 43.5, H 2.7, N 10.8; found C 42.9, H 2.9, N 10.7. ^{1}H NMR ($CDCl_3$, 600 MHz, 298 K): δ = 10.19 (s, pqxH$_3$), 9.31 (d, $^{3}J_{H,H}$ = 8.3 Hz, pqxH$_{3'}$), 8.95 (d, $^{3}J_{H,H}$ = 6.7 Hz, AbpyH$_3$), 8.94 (d, $^{3}J_{H,H}$ = 6.7 Hz, AbpyH$_{3'}$), 8.77 (d, $^{3}J_{H,H}$ = 8.1 Hz, BbpyH$_3$), 8.68 (d, $^{3}J_{H,H}$ = 8.1 Hz, BbpyH$_{3'}$), 8.33 (t, $^{3}J_{H,H}$ = 7.8 Hz pqxH$_{4'}$), 8.26 (t, $^{3}J_{H,H}$ = 7.5 Hz, AbpyH$_{4'}$), 8.23 (t, $^{3}J_{H,H}$ = 7.8 Hz, BbpyH$_4$), 8.24 (d, $^{3}J_{H,H}$ = 8.1 Hz pqxH$_8$), 8.23 (t, $^{3}J_{H,H}$ = 7.8 Hz, AbpyH$_4$), 8.13 (d, $^{3}J_{H,H}$ = 5.3 Hz, AbpyH$_6$), 8.09 (t, $^{3}J_{H,H}$ = 7.5 Hz, BbpyH$_{4'}$), 8.00 (d, $^{3}J_{H,H}$ = 5.3 Hz pqxH$_{6'}$), 7.88 (t, $^{3}J_{H,H}$ = 7.5 Hz pqxH$_7$), 7.79 (d, $^{3}J_{H,H}$ = 5.3 Hz, BbpyH$_6$),

7.66 (d, $^3J_{H,H}$ = 5.3 Hz, AbpyH$_{6'}$), 7.64 (t, $^3J_{H,H}$ = 6.7 Hz, BbpyH$_5$), 7.64 (t, $^3J_{H,H}$ = 6.7 Hz, pqxH$_{5'}$), 7.54 (t, $^3J_{H,H}$ = 6.7 Hz, AbpyH$_{5'}$), 7.47 (t, $^3J_{H,H}$ = 6.7 Hz, BbpyH$_{5'}$), 7.45 (t, $^3J_{H,H}$ = 7.5 Hz AbpyH$_5$), 7.45 (d, $^3J_{H,H}$ = 5.3 Hz, BbpyH$_{6'}$), 7.44 (t, $^3J_{H,H}$ = 9.0 Hz, pqxH$_{6'}$), 7.25 (d, $^3J_{H,H}$ = 8.5 Hz, pqxH$_5$) ppm.

Λ-[Ru(bpy)$_2$(pqx)](PF$_6$)$_2$: A similar procedure was followed for this enantiomer starting with Λ-[Ru(bpy)$_2$(py)$_2$]A·12H$_2$O. $C_{33}H_{25}F_{12}N_7P_2Ru$: calcd. C 43.5, H 2.7, N 10.8; found C 43.4, H 2.6, N 10.7. ^{1}H NMR (CDCl$_3$, 600 MHz, 298 K): δ = 10.17 (s, pqxH$_3$), 9.31 (d, $^3J_{H,H}$ = 8.3 Hz pqxH$_{3'}$), 8.96 (d, $^3J_{H,H}$ = 6.7 Hz, AbpyH$_3$), 8.94 (d, $^3J_{H,H}$ = 6.7 Hz, AbpyH$_{3'}$), 8.78 (d, $^3J_{H,H}$ = 8.0 Hz, BbpyH$_3$), 8.69 (d, $^3J_{H,H}$ = 8.0 Hz, BbpyH$_{3'}$), 8.32 (t, $^3J_{H,H}$ = 7.8 Hz pqxH$_{4'}$), 8.27 (t, $^3J_{H,H}$ = 7.5 Hz, AbpyH$_{4'}$), 8.24 (d, $^3J_{H,H}$ = 8.0 Hz, pqxH$_8$), 8.24 (t, $^3J_{H,H}$ = 7.8 Hz, BbpyH$_4$), 8.22 (t, $^3J_{H,H}$ = 7.8 Hz, AbpyH$_4$), 8.12 (d, $^3J_{H,H}$ = 5.3 Hz, AbpyH$_6$), 8.09 (t, $^3J_{H,H}$ = 7.5 Hz BbpyH$_{4'}$), 8.00 (d, $^3J_{H,H}$ = 5.3 Hz, pqxH$_{6'}$), 7.88 (t, $^3J_{H,H}$ = 7.5 Hz, pqxH$_7$), 7.79 (d, $^3J_{H,H}$ = 5.3 Hz, BbpyH$_6$), 7.67 (d, $^3J_{H,H}$ = 5.2 Hz, AbpyH$_{6'}$), 7.64 (t, $^3J_{H,H}$ = 6.7 Hz, pqxH$_{5'}$), 7.64 (t, $^3J_{H,H}$ = 6.7 Hz, BbpyH$_5$), 7.54 (t, $^3J_{H,H}$ = 6.7 Hz, AbpyH$_{5'}$),7.46 (t, $^3J_{H,H}$ = 6.7 Hz, BbpyH$_{5'}$), 7.46 (d, $^3J_{H,H}$ = 5.3 Hz, BbpyH$_{6'}$), 7.45 (t, $^3J_{H,H}$ = 7.5 Hz, AbpyH$_5$), 7.43 (t, $^3J_{H,H}$ = 9.0 Hz, pqxH$_{6'}$), 7.25 (d, $^3J_{H,H}$ = 8.5 Hz, pqxH$_5$) ppm.

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