Synthesis and Characterization of the Diastereomers Λ- and Δ-[Ru(bpy)$_2$-(m-bpy-Gly-L-His-L-Lys)]Cl$_2$ − ^{1}H NMR Studies on Their Interactions with the Deoxynucleotide Duplex d[(5′-CGCGAATTCGCG-3′)$_2$]

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The synthesis and characterization of the diastereomeric complexes Λ- and Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$, (GHK = glycine-L-histidine-L-lysine, m-bpy = 4|-methyl-2,2|-bipyridine) as well as their binding properties to the deoxynucleotide duplex d(5′-CGCGAATTCGCG-3′)$_2$ studied by means of NMR, ESI-MS and CD spectroscopy, are reported. The ROESY spectrum of Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ shows intramolecular cross peaks between the bpy H3 or H3′ protons and the aromatic H2 and H5 of the histidine imidazole ring, indicating that the peptide adopts an orientation with the imidazole ring close to the bpy ligand, possibly interacting by π-stacking. The absence of intramolecular cross peaks between the peptide and the bipyridine ligands in the ROESY spectrum of Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ on the other hand, shows that in this case the peptide is far from the two bpy ligands, having a different orientation from the Λ isomer. The isomers interact with the oligonucleotide duplex differently. Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ binds in the oligonucleotide major groove close to the central part of the sequence. Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ binds, probably non selectively, approaching the helix from the minor groove. It can be concluded that the peptide (GHK) binding leads the rest of the isomer to interact with the oligonucleotide in the case of the Δ isomer.

1. Introduction

The idea that low-molecular mass organic compounds (e.g., DNA targeting drugs) may bind selectively to DNA was raised several years ago.[1] There is evidence for ligands exhibiting antiviral activity and antineoplastic properties[2] or catalyzing the hydrolysis of DNA phosphodiesteric bonds (chemical nucleases).[3] The ways that such molecules bind to DNA are known and include covalent and noncovalent binding (intercalation, groove binding and electrostatic interactions). Some of them bind in specific domains of the DNA (sequence selectivity) or selectively from the major or minor groove depending on the relation of ligand−DNA recognition. Consequently, the structure of the incoming molecule is important due to different dimensions of the two DNA grooves when it approaches the bases. From the point of view of inorganic chemistry it is interesting to study the binding properties of the metal complexes of such ligands that may potentially combine the properties of the metal and the ligand. For instance the complex [Pt(net)$_2$(NH$_3$)$_2$]$^{2+}$ preserves the selective binding of netropsin (net) and binds in the AT rich region from the minor groove but induces DNA-cleavage near the binding sites upon X-ray irradiation.[4] On the other hand, the complex of the peptide GGKGGG with Cu^{2+} conjugated to netropsin binds to DNA in the same manner as the antibiotic alone, i.e. from the minor groove, assisted by the complex−peptide moiety through electrostatic and van der Waals interactions.[5,6] This compound generates oxygen active species upon activation by a mild oxidizer and induces cleavage in a specific DNA sequence region. Using photophysical techniques, Barton and co-workers have shown DNA sequence recognition by the metal peptide complex [Rh(phi)$_2$(phen′)]$^{3+}$ (phen′ = 1,10 phenanthroline conjugated with a 14/peptide and phi = phenanthrenequinonediamine) which binds at the 5′-ACAA-3′ region.[7] It is worth mentioning that the complex of Cu^{2+} with bis(salicylideneethylenediamine) (salen) conjugated with distamycine (DNA minor groove binder in AT rich regions) binds rather selectively to DNA. Upon activation, however, the cleavage is nonspecific.[8]

Ruthenium polypyridine complexes are undoubtedly the main class of molecules which may bind to DNA through intercalation, inducing cleavage of the DNA backbone through photoxidation.[9] The sequence specificity of these complexes has been investigated with known sequence oligonucleotides, mainly by means of NMR techniques, but no general rules about such selectivity have been

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suggested.[10−15] Our efforts are focused on the design and synthesis of ruthenium polypyridyl complexes able to recognize and bind to a specific DNA sequence. Peptides are important biological ligands having the functional groups essential for the formation of sequence specific contacts such as hydrogen bonds and van der Waals contacts. Hence, the incorporation of a peptide sequence may be an asset in the design of sequence specific binders. Our design of such complexes involves a Ru^{II} ion surrounded by polypyridine ligands, one of which is conjugated to the peptide sequence (Figure 1).

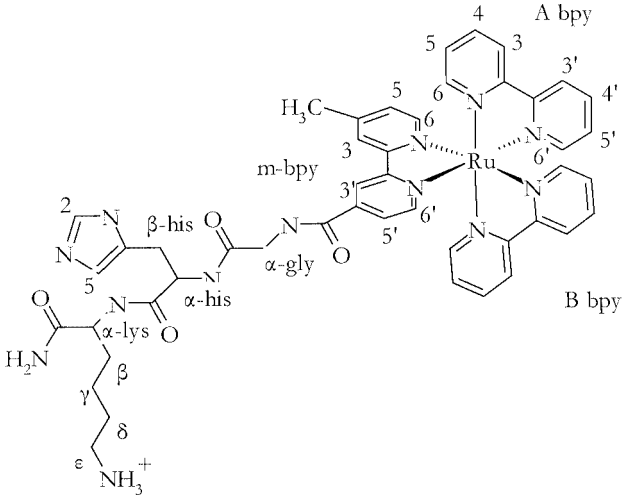


Figure 1. Structure of the complex Δ-$[Ru(bpy)_2(m\text{-}bpy\text{-}GHK)]^{2+}$ with atom numbering

Recently, we have focused our attention on the tripeptide Gly-His-Lys (GHK) related to GGH. Its Cu^{2+} complex conjugated with netropsin contributes to the binding reaction with the DNA from the minor groove in AT rich domains.[5,6] The DNA binding properties of the complex we designed were tested on the spectroscopically and crystallographically characterized Dickerson−Drew dodecamer, d(5′-CGCGAATTCGCG-3′)$_2$ which forms a self-complementary duplex the structure of which is known.[16,17] This paper describes the synthesis and characterization of the diastereomeric complexes Λ- and Δ-$[Ru(bpy)_2(m\text{-}bpy\text{-}GHK)]Cl_2$, derived from the compounds Λ- and Δ- *cis*-$[Ru(bpy)_2(py)_2]A$ (where A = *O*,*O*′-dibenzoyltartaric acid)[18,19] by a substitution of the two py molecules with the chelate ligand m-bpy-COOH followed by a solid-phase peptide synthesis. This was also our first attempt to investigate, by means of NMR, ESI-MS and CD spectroscopic techniques, their binding properties with the deoxynucleotide duplex d(5′-CGCGAATTCGCG-3′)$_2$. The possible selective cleavage of the oligonucleotide by these complexes is under investigation and the results will appear in a later publication.

2. Results and Discussion

2.1. Synthesis and Characterization of the $[Ru(bpy)_2(m\text{-}bpy\text{-}GHK)]Cl_2$ Isomers

2.1.1. Synthesis

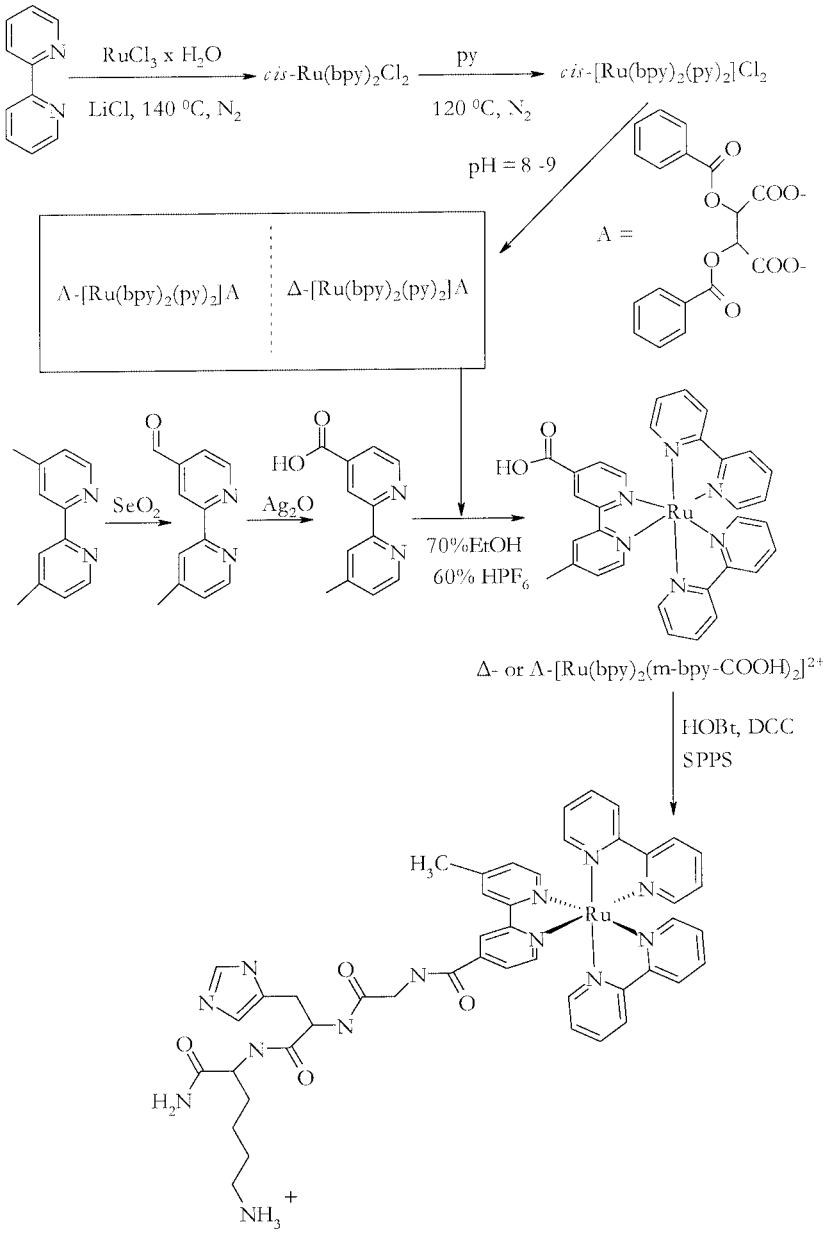
The synthesis of the diastereomers Λ- and Δ-$[Ru(bpy)_2(m\text{-}bpy\text{-}GHK)]Cl_2$ was based on the enantiomerically pure complexes Λ- and Δ-*cis*-$[Ru(bpy)_2(py)_2]^{2+}$ as building blocks. These complexes were used as precursors for the synthesis of the tris(chelates) Λ- and Δ-$[Ru(bpy)_2(m\text{-}bpy\text{-}COOH)]^{2+}$.[20,21] Both pyridine molecules in the complex *cis*-$[Ru(bpy)_2(py)_2]^{2+}$ were substituted by the chelate ligand m-bpy-COOH (m-bpy-COOH = 4′-methyl-2,2′-bipyridine-4-carboxylic acid), the optical purity being retained in the resultant compounds. The carboxyl group of the ligand (m-bpy-COOH) remained free due to the higher propensity of the phenanthroline nitrogen donor atoms over the oxygen atoms for coordination to the Ru^{II} ion[24] assisted by the formation of a very stable five-membered chelate ring. These enantiomers were isolated as their $[PF_6]^-$ salts by the addition of HPF_6 (pH = 1) thus keeping the carboxyl group protonated. The tripeptide (GHK) was then conjugated to the m-bpy-COOH molecule through the solid state method SPPS, using the 2-chlorotrityl-H-Fmoc-resin by activation of the carboxyl group with succinic acid and DCC.[25] The isomers were isolated as TFA salts and then converted in to their water-soluble chlorides with LiCl in acetone.[15] The total procedure is detailed in Scheme 1.

2.1.2. Electrospray Ionization Mass Spectrometry

Positive electrospray spectra for both Λ- and Δ-$[Ru(bpy)_2(m\text{-}bpy\text{-}GHK)]Cl_2$ in aqueous media were recorded using the time-of-flight method to register all ions simultaneously without scanning.[26] Both spectra were generally the same, showing mainly two peaks of high intensity at 474.58 and 316.74 *m*/*z* arising from $[RuC_{46}H_{49}N_{13}O_4]^+$ (948.30 *m*/*z*) at doubly and triply charged states correspondingly. The very weak intensity of the peak at *m*/*z* = 948.30 indicates that the abundance of the peptide which is deprotonated both to the lysine terminal amino group and probably to any peptidic NH group or to the terminal amide group $[Ru(bpy)_2(m\text{-}bpy\text{-}CO\text{-}Gly\text{-}His\text{-}LysCONH_2)H^+]^+$ is very low. Indeed, a relatively high intensity peak that was observed at 474.58 *m*/*z* may be assigned to the doubly charged cation $[Ru(bpy)_2(m\text{-}bpy\text{-}CO\text{-}Gly\text{-}His\text{-}LysCONH_2)]^{2+}$ with all the peptidic NH groups being protonated. Finally the main peak of the spectrum at *m*/*z* = 316.74 can be assigned to the triply charged cation $[Ru(bpy)_2(m\text{-}bpy\text{-}CO\text{-}Gly\text{-}His\text{-}LysCONH_2)]^{3+}$ where the lysine terminal amino group (p*K*a = 10.4) [27] is protonated (Figure 2).

2.1.3. Circular Dichroism Spectroscopy

The CD spectra of the Δ- and Λ-diastereomers are not mirror images of each other as expected (Figure 3) since the presence of another chiral center in the peptide moiety



Scheme 1

besides the metal results in diastereomers. Assignments were made by comparison with similar complexes of known configuration.[20,28] The spectra of the isomers exhibit $\lambda_{max.}$ (nm) with $\Delta\varepsilon$ ($M^{-1}cm^{-1}$) at 479 nm (+6.7), 422 (−9.6), 295 nm (+90.1), 278 (−36.9) 226 nm (−13.5), 205 nm (+11.7), 195 nm (−14.8) for the Λ isomer and at 479 nm (− 4.6) 421.5 nm (+6.4), 295 nm (−60.8), 278 nm (+22.6), 225 nm (+5.2), 204 nm (−18.6), 190 nm (−11.5) for the Δ isomer.

2.1.4. NMR Spectroscopy

^{1}H NMR resonances of the diastereomers Λ- and Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ were assigned based on homo and heteronuclear 2-D COSY and TOCSY experiments. In the spectra of both isomers only one set of proton signals for the two bpy ligands were observed. The influence of the third non-symmetric ligand m-bpy-GHK on the bpy proton signals is insignificant and the differences in their magnetic environments are non-detectable. Similar behavior was also observed in the complexes [Ru(bpy)$_2$(HPIP)]$^{2+}$ [15] and [Ru(bpy)$_2$(pqx)]$^{2+}$,[29] where HPIP {HPIP is 2-(2-hydroxyphenyl)imidazo[4,5-*f*][1,10] phenanthroline} and pqx [pqx is 2-(2′-pyridyl)quinoxaline] are non-symmetric chelate ligands. On the other hand, the ligand m-bpy-GHK exhibits one set of proton resonances for each pyridine ring which differ significantly from each other. The pyridine ring, which is substituted by a methyl group at the 4th position, exhibits similar chemical shifts with the pyridine residues of the other two bpy ligands with the protons at the neighbor-

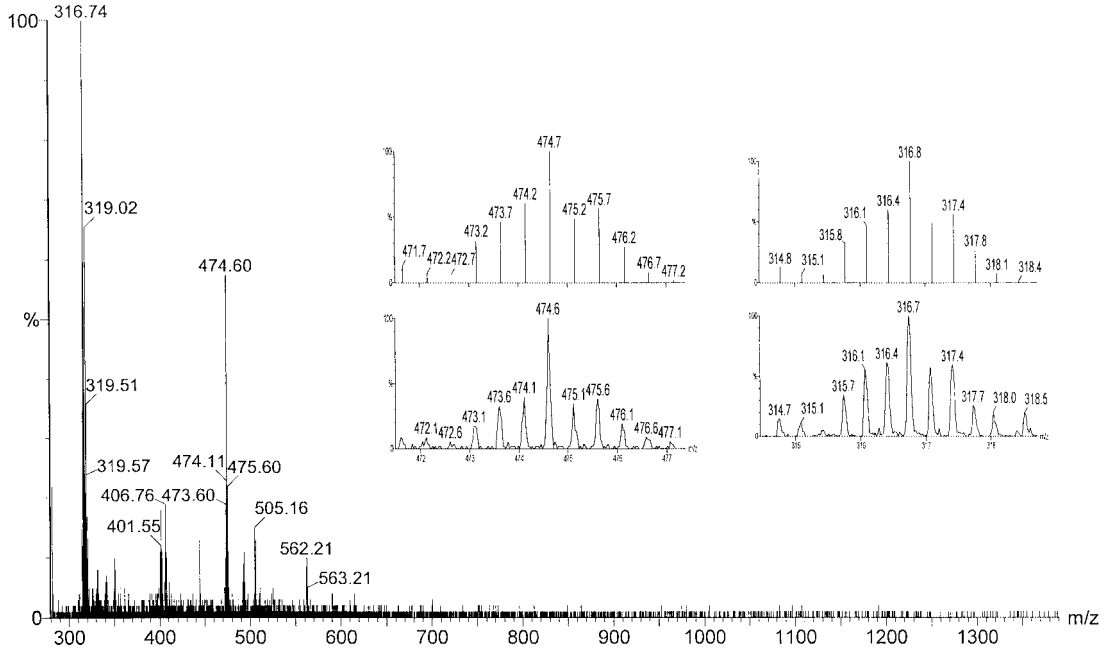


Figure 2. A typical electrospray mass scan of Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ in the positive mode. Insets, the calculated spectrum in comparison with the observed peaks for the cations Λ-[Ru(bpy)$_2$(m-bpy-GHK + H$^+$)]$^{3+}$ (*m*/*z* 316.74) and Λ-[Ru(bpy)$_2$(m-bpy-GHK)]$^{2+}$ (*m*/*z* 474.58)

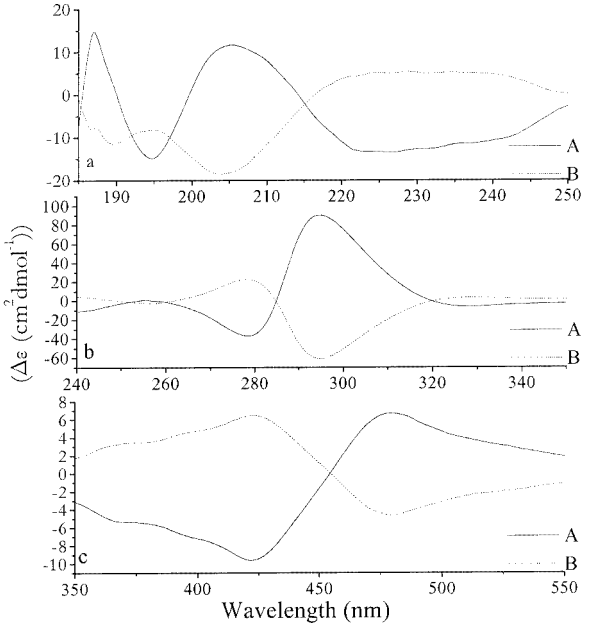


Figure 3. The CD spectra of A = Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ and B = Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ in the region (a) (190−250 nm), (b) (240−350 nm) and (c) (350−550 nm)

ing 3rd and 5th positions shifted upfield only by δ = −0.06 and −0.04 ppm respectively. In contrast, the protons H3′ and H5′ of the other pyridine ring of the m-bpy group are shifted downfield by about δ = +0.29 ppm (H3′) and δ = +0.78 ppm (H5′), indicating that the CO-GHK substitution at the 4th position provides a deshielding effect on these protons reducing the electron density of this pyridine ring.[30]

The chemical shifts of the peptide protons are similar for the diastereomers Λ- and Δ-[Ru(bpy)$_2$ (m-bpy-GHK)]Cl$_2$, differing only slightly (about ±0.01 ppm). The one exception being H2 of the histidine imidazole ring of the Λ- isomer which is shifted upfield by δ = −0.16 ppm compared with the shift of the equivalent resonance in the Δ isomer. This effect probably arises from a different configuration of the peptide in the isomers, with the histidine's imidazole ring being closer to the aromatic pyridine moieties in the case of the Λ isomer (Figure 4). A qualitative approach to the observed shielding by −0.16 ppm based on a method proposed by Cheney results in a distance of about 4 to 5 Å between the plane of one of the pyridine rings and H2 of the imidazole.[31] The ROE spectra of both isomers are similar as the cross peaks in the ROE map indicate. These can be assigned to dipolar interactions between the pyridine ring protons of rings on the same or adjacent metal bipyridine units. Additionally, two new cross peaks were observed in the spectrum of the Λ isomer (Figure 4), which we have assigned to the interaction of the bpy H3 or H3′ protons with both the aromatic protons H2 and H5 of the histidine imidazole ring. This indicates that it has a different orientation in the two diastereomers. Such a geometry will direct the imidazole ring in the case of Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ close to the bpy ligand possibly resulting in a π-stacking interaction. This would not be the case in the Δ isomer (Figure 4).

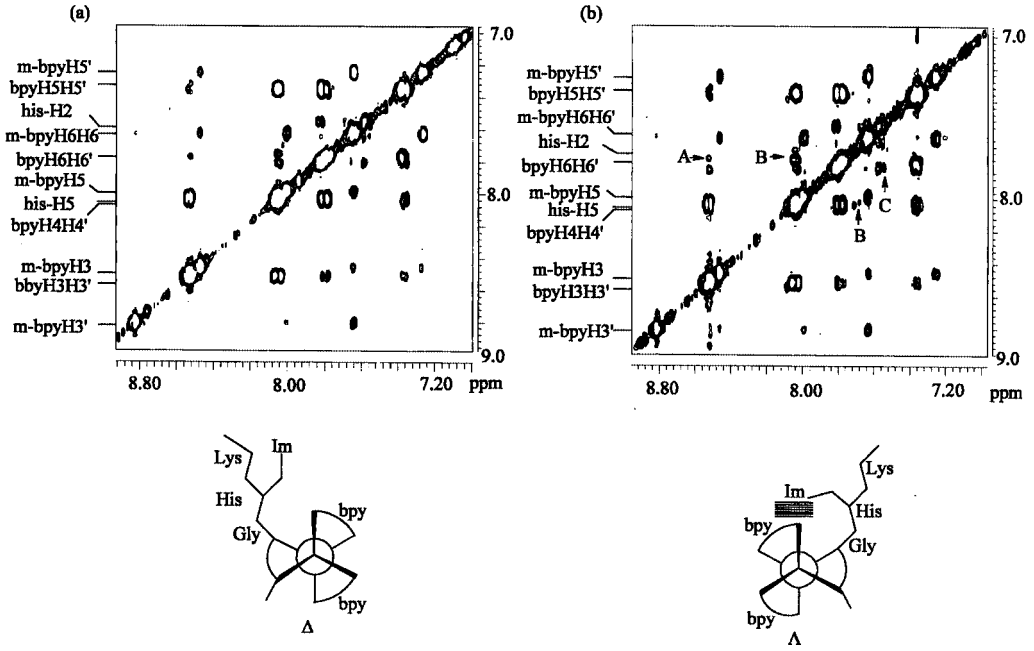


Figure 4. (a) Part of the ROESY spectrum (τ_m = 100 ms) of Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$, following a qualitative model of the peptide orientation. (b) The same part of the ROESY spectrum under the same conditions for Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$, following a qualitative model of the peptide orientation. The labeled cross peaks correspond to ROE contacts assigned to the protons: (A) hisH2-bpyH3H3′, (B) hisH2-bpyH4H4′, (C) hisH5-bpyH6H6′

2.2. Interactions of the Diastereomers [Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ with the DNA Dodecamer Duplex [d(5′-CGCGAATTCGCG-3′)$_2$]

2.2.1 Assignments of the Proton Resonances of [d(5′-CGCGAATTCGCG-3′)$_2$]

1-D-NOE (Nuclear Overhauser Effect) experiments in 90% H_2O/10% D_2O were used to assign the amino and the imino resonances of the duplex. Five signals in the imino region (δ = 14 ppm) of the NMR spectrum of the dodecanucleotide were observed, indicating that only the terminal C···G bases do not form stable Watson−Crick hydrogen bonding. The two most downfield shifted resonances were identified as T7N3-H and T8N3-H in the A···T pairs and the other three as G2N1-H, G10N1-H and G4N1-H in the G···C pairs, typical for B-type DNA structures in aqueous solution.[32] In addition, the signals of the hydrogen-bonded exocyclic amino groups of the purines and pyrimidines were observed in the region of δ = −9.5 ppm, in contrast to the resonances of the non hydrogen-bonded amino groups C1-NH_2 and G12-NH_2 located at a higher field (δ ≈ 6 ppm).

Assignments of all non-exchangeable protons of the oligonucleotide were achieved by using the standard DQF COSY technique[33] and two-dimensional NOE spectroscopy.[34] Small differences in the proton assignments of the dodecamer exist between our findings and those reported in literature,[35] probably due to the slightly different conditions and the high field ^{1}H NMR (500 MHz) instrument used. In the NOESY map of [d(5′-CGCGAATTCGCG-3′)$_2$] the sequential assignments for the *n*H6/H8 → *n*H1′ => (*n* + 1)H6/H8 → (*n* + 1)H1′ (where the different arrows → and => denote *intra* and *inter* nucleotide distances-cross peaks, respectively) system

Table 1 ^{1}H NMR chemical shift data[a] for the diastereomers Λ- and Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ in the presence of the oligonucleotide [*d*(CGCGAATTCGCG)$_2$] at a molar ratio of 1:1

Proton[a]	Δ Isomer	Δ(δ)	Λ Isomer	Δ(δ)
bpy H3	8.34	−0.20	8.48	−0.06
bpy H3′	8.44	−0.10	8.48	−0.06
bpy H4	7.88	−0.17	8.01	−0.04
bpy H4′	7.97	−0.08	8.01	−0.04
bpy H5	7.33	−0.04	7.34	−0.04
bpy H5′	7.33	−0.04	7.34	−0.04
bpy H6	7.71	−0.09	7.75	−0.05
bpy H6′	7.71	−0.09	7.75	−0.05
m-bpy H6	7.59	−0.06	7.61	−0.04
m-bpy H6′	7.59	−0.06	7.61	−0.04
m-bpy H5	7.92	−0.08	7.94	−0.06
m-bpy H5′	7.22	−0.05	7.24	−0.03
m-bpy CH_3	2.49	−0.06	2.50	−0.04
m-bpy H3	8.76	−0.07	8.78	−0.05
m-bpy H3′	8.39	−0.09	8.42	−0.05
α-Gly	4.17	−0.00	4.16	−0.01
α-His	4.63	−0.04	4.65	−0.03
β-His	3.13	−0.02	3.15	−0.02
His H5	7.53	−0.04	7.54	−0.03
His H2	7.79	−0.04	7.80	+0.12
α-Lys	4.23	−0.02	4.25	−0.01
β-Lys	1.81	+0.01	1.82	+0.03
γ-Lys	1.37	+0.02	1.38	+0.04
δ-Lys	1.68	+0.01	1.66	−0.01
ε-Lys	2.88	−0.09	2.90	−0.07

[a] Concentration 10 mM, D_2O, 100 mM phosphate buffer (pH = 7.01), *T* = 303 K.

Table 2. ^{1}H NMR chemical shifts in ppm of free d(CGCGAATTCGCG)$_2$ and the chemical shift differences induced by the addition of Λ- or Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ at a molar ratio of 1:1 in 100 mM phosphate buffer (pH = 7.01)

	H8/H6	Λ Isomer	Δ Isomer	H2/H5	Λ Isomer	Δ Isomer	H1′	Λ Isomer	Δ Isomer	H2′′	Λ Isomer	Δ Isomer	H2′	Λ Isomer	Δ Isomer
C1	7.60	−0.01	+0.01	5.88	−0.02	−0.03	5.72	−0.02	−0.01	2.39	−0.01	−0.02	1.94	−	−
G2	7.93	+0.02	+0.02				5.88	−0.02	−0.03	2.68	0.00	0.00	2.62	0.00	0.00
C3	7.25	0.00	−0.01	5.35	−0.06	−0.07	5.56	+0.02	+0.03	2.26	+0.01	+0.01	1.83	0.00	+0.01
G4	7.83	0.00	0.00				5.42	−0.01	−0.01	2.75	−	−	2.63	0.00	−
A5	8.09	0.00	0.00	7.21	+0.05	−0.02	6.13	−0.05	−0.06	2.90	−0.11	+0.05	2.68	0.00	0.00
A6	8.09	0.00	0.00	7.60	−0.01	0.00	5.97	−0.02	−0.03	2.90	−0.11	+0.05	2.49	+0.01	+0.02
T7	7.09	+0.02	0.00				5.88	−0.02	−0.03	2.53	−0.01	−0.01	1.94	−	−
T8	7.35	+0.05	−0.02				6.12	−0.04	−0.01	2.53	−0.01	−0.01	2.14	0.00	+0.03
C9	7.44	+0.01	0.00	5.60	+0.01	+0.01	5.64	0.00	−0.02	2.43	−	+0.01	2.04	−0.01	−0.01
G10	7.89	+0.00	−0.01				5.83	+0.02	+0.01	2.68	+0.02	0.00	2.62	0.00	0.00
C11	7.31	−0.01	0.00	5.42	−0.01	−0.01	5.72	−0.02	−0.01	2.31	−0.01	−0.03	1.87	−0.01	−0.02
G12	7.92	+0.01	0.00				6.13	−0.05	−0.06	2.62	−0.01	0.00	2.36	−0.01	−0.01

were observed for all the bases of the sequence.[36,37] Moreover, NOE cross peaks from each H8/H6 to its own H2′ and H2′′ are consistent, as expected, with a B-type right-handed DNA duplex.[37]

2.2.2 1-D ^{1}H NMR Spectroscopy

Table 1 and 2 show the ^{1}H NMR chemical shift data of the free oligonucleotide, the complexes Δ- and Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ and the changes induced by addition of the isomers to the oligonucleotide.

The ^{1}H NMR spectra of the samples containing the oligonucleotide and the Δ or the Λ isomer in molar ratios of 1:0.5 and 1:1 show only one set of resonances in all cases. This behavior indicates fast exchange (on the NMR time scale at ambient temperature) between the bound form(s), the free oligonucleotide, and the metal complex.

Upon decreasing the temperature to 283 K, a slight broadening of the signals was observed indicating that the fast exchange changes slowly to intermediate. Moreover, decreasing the temperature close to the solvent's freezing point did not yield separate resonances (coalescence point). Under these conditions only average NMR results of the bound form(s) could be observed. This clearly means that the NMR observations do not necessarily belong to only one single species but may be the average of different bound forms in a non-specific binding of the diastereomers to the oligonucleotide. Each NMR signal in the fast exchange kinetics represents a statistical average for all species present and the extracting of specific information is difficult. However, the achievement of slow exchange conditions is rather scarce even for highly specific compounds such as distamycine.[38] In such cases, the NMR results can only be considered as a qualitative analysis of the binding. The majority of Ru^{II} polypyridine complexes bind to DNA with fast to intermediate kinetics and conformational predictions have been given using additional methods such as molecular mechanics (MM) and molecular dynamics (MD) calculations, coupled with some of the NOE results as distance constraints.[11−15,39,40]

Upfield changes in the bpy proton signals of both diastereomers were observed which were largest in the case of the Δ isomer. In particular, the protons located at the end of the one pyridine ring of bpy namely H3′ and H4′ were shifted by δ = − 0.19 and − 0.17 ppm respectively. These values, followed by smaller but significant changes in chemical shifts of all other bpy protons, indicate that the ligand clearly binds to the oligonucleotide without intercalating between the bases. Intercalation would cause large upfield changes in the resonances of the ligand protons (δ = 0.3−1.0 ppm) and significant broadening of the signals due to intermediate exchange.[11] The observed electronic shielding of the H3′ and H4′ bpy protons from the DNA bases is close to the lower limit for binding by intercalation, showing that the vertical distance between these protons and the base planes is not very high.[41] According to Pullman and co-workers, the induced shielding effect for a five or a six membered aromatic ring from the DNA bases is a function of distance.[41,42] The observed 0.2 ppm upfield shift corresponds to a vertical distance between the base planes and H3′/ H4′ of the bpy aromatic ring system of about 5 Å which is not enough to cause intercalation. These slight changes in the chemical shifts of the complex protons are more consistent with other modes of binding, such us groove binding. In general, molecules which bind in the DNA grooves interact with the edges of the bases causing zero or insignificant shielding of the molecule's protons.[43] Complexes with ligands similar to bpy, as in the case of [Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$ (μ-bpm = 2,2′-bipyrimidine), associate in the minor groove of the DNA and induce very small changes in the ligand proton resonances (less than 0.1 ppm).[14] Small changes were observed in the pyridyl and aliphatic proton chemical shifts for the complex Δ-*cis*-α-[Ru{*R*,*R*)-picchxnMe$_2$}(phen)]$^{2+}$ (< 0.2 ppm) which binds selectively at the minor groove of the oligonucleotide [d(CGCGATCGCG)$_2$] and the [d(ATATCGATAT)$_2$] duplex.[39] This type of DNA−metal complex interaction is also known for the complex [Ru(bpy)$_3$]$^{2+}$ which binds to the DNA without intercalation.[44] In the case of Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ the changes observed were in the range of δ = −0.06 to −0.03 ppm for the bpy protons, sug-

gesting a weak association with the oligonucleotide or a non-specific binding. The diffusion of shielding or deshielding effects induced by the interaction of the metal complex with DNA, for all proton signals of the Λ-[Ru(bpy)$_2$(m-bpy-GHK)] with approximately similar shifts, could be explained as being due to a non specific binding of this isomer.

The aromatic protons of the m-bpy-GHK ligand show small upfield shifts (less than 0.1 ppm) for both isomers. In addition, the peptide protons were shifted upfield except the lysine side chain protons Hα (shifted upfield in both isomers), Hβ (shifted downfield in both isomers) and Hγ (shifted downfield in both isomers). The Hδ proton, which is very close to the terminal amino group, was shifted slightly downfield and upfield in the Δ and Λ isomers, respectively, indicating that it probably interacts either with the charged phosphate groups of the DNA or with the aromatic π system of the bpy ligands. Similar results were observed in the case of the binding of the tripeptide Lys-Tyr-Lys to the tetranucleotide [d(5′-GCGC-3′)]$_2$, where the positively charged amino group of the lysine side chain interacts electrostatically with the phosphodiester backbone of the DNA.[45] Furthermore, the binding of Lys-Trp-Lys with the DNA involves electrostatic interactions between the lysine residues and the DNA backbone.[46]

The influence of the binding of the Δ isomer on the oligonucleotide proton resonances is focused on the protons located at the major groove, namely H2′ and H2′′ of the sugar moieties which are shifted more than the minor groove proton H1′. The highest shifts were observed for A5 H2′′ and A6 H2′′ and for C3 H5. The base C3 is located opposite G10 and probably H5 is affected by a complex binding close to the T8C9 base sequence. Thus, the most affected part of the oligonucleotide is the region from A5 to G10 in the major groove.

In an attempt to further investigate the binding modes of the peptide moiety of the complexes to the synthetic DNA fragment, all spectra were recorded in 90% H_2O /10% D_2O in order to observe the exchangeable amino and imino protons. The assignments in Table 3 were aided by the use of

Table 3. ^{1}H NMR chemical shifts of the exchangeable protons of free d(CGCGAATTCGCG)$_2$, Λ- or Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ and their adducts at a 1:1 molar ratio in 100 mM phosphate buffer (pH = 7.01), in H_2O/D_2O, 9:1

	GNH1/TNH$_3$	CNH$_4$ [a]	CNH$_4$ [b]	Peptide NH	Lys-CONH$_2$
A5-T8					
Δ Free	13.78				
Δδ Δ isomer	0.05				
Δδ Λ isomer	0.03				
A6-T7					
Δ Free	13.65				
Δδ Δ isomer	0.04				
ΔΔ Λ isomer	0.01				
G2-C11					
Δ Free	13.02	6.55	8.37		
Δδ Δ isomer	0.02	0.11	0.02		
Δδ Λ isomer	0.00	0.10	0.02		
G10-C3					
Δ Free	12.88	6.42	8.41		
Δδ Δ isomer	0.01	0.07	0.03		
Δδ Λ isomer	0.00	0.09	0.02		
G4-C9					
Δ Free	12.68	6.80	8.40		
Δδ Δ isomer	0.01	0.08	0.03		
Δδ Λ isomer	−0.02	0.08	0.02		
Gly-NH					
Δ isomer					
Δ Free/Δδ bonded Λ isomer				8.19/0.00	
Δ Free/Δδ bonded				8.16/−0.01	
Lys-NH					
Δ isomer					
Δ Free/Δδ bonded Λ isomer				8.10/0.02	
Δ Free/Δδ bonded				8.21/0.04	
His-NH					
Δ isomer					
Δ Free/Δδ bonded Λ isomer				8.24/−0.13	
Δ Free/Δδ bonded				8.25/−0.13	
Lys-CONH$_2$					
Δ isomer					
Δ Free/Δδ bonded Λ isomer					7.16/0.04
Δ Free/Δδ bonded					7.17/−0.01

[a] Hydrogen bonded. [b] Not hydrogen-bonded.

NOE spectroscopy and by data available in the literature.[47−49]

The H8 proton (neighboring N7) of the guanine or the adenine residues of the oligonucleotide do not shift significantly in the presence of the diastereomers thus excluding any possibility of participation of this nitrogen in hydrogen bonding with the peptide complexes. O6 of guanine is also a good candidate but such interactions would affect the GO6···H4NC proton signal, which is not the case ($\Delta\delta$ = 0.02 to 0.03 ppm for both isomers). The other non-hydrogen bonded protons of the exocyclic amino group of C3, C9 and C11, (CN4-H) are shifted by about 0.1 ppm due to their interactions with both isomers (Figure 5). This downfield shift is significant but clearly not large enough to conclude that these protons participate in hydrogen bonding. This observation could, however, be due to hydrogen bonds which are bifurcated and longer than normal or indeed van der Waals interactions between the peptide residue of the complexes and the CN4-H protons of C3, C9 and C11.[50] A similar example is the interaction of the same DNA sequence with the drug *Distamycin A*, where small downfield shifts were observed for both protons of the adenine ring

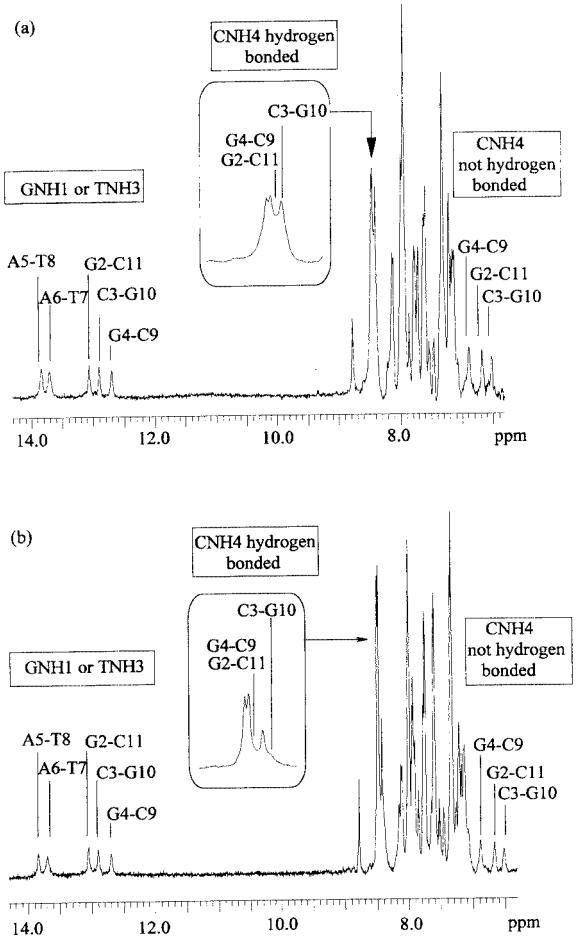


Figure 5. Part of the ^{1}H NMR spectra of the diastereoisomers showing the influence of (a) Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ and (b) Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ to the hydrogen bonds of GNH1, TNH3 and CNH4 in the base pairs CG and AT of the oligonucleotide duplex

and the NH of the drug's pyrrole groups[50] suggesting a groove interaction. The hydrogen-bonded proton of the AT base pair *A*N6-H···O4*T* does not seem to be affected by the binding because the neighboring proton signals of the thymine methyl group do not shift upfield. This excludes the possibility of interaction of these sites with the peptide. The A5H2 that shifts downfield by about 0.05 ppm reflects a possible association of the peptide with the A5N3 position.

Of the exchangeable peptide protons only the NH proton of the His group was shifted upfield by about −0.13 ppm in both diastereomers, suggesting that this site or the neighboring carbonyl (>C=O) participates in the binding of the complex to DNA.

2.2.3 2-D ^{1}H NMR Spectroscopy

Table 4 shows selected NOESY cross peaks observed between the isomers Λ- and Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ and [d(5′-CGCGAATTCGCG-3′)$_2$].

Table 4. Selective NOE cross peaks observed between the protons of the diastereomers Λ- and Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ and [d(5′-CGCGAATTCGCG-3′)$_2$] at a metal complex/duplex ratio of 1:1 in 100 mM phosphate buffer (pH = 7.01) at 303 K

Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$	Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$
(m-bpy-GHK)H3 → C9H2′′(W)	
(m-bpy-GHK)H3′ → C9H2′′ (S)	
(m-bpy-GHK)H5′ → C9H2′′ (S)	(m-bpy-GHK)H3′ → A6H1′(M)
	(m-bpy-GHK)H5′ → C9H1′ (W)
(bpy)H3′ → C9H2′′ (S)	
(bpy)H6H6′ → C1H2′ (M)	
(His)HA → A5H1′(W)	
	(Gly)NH → T8H1′(W)
(Lys)NH → A5A6H2′′ (M)	
(His)NH → T7T8H2′′ (M) → MeT8 (M)	

The NOE contacts, originating from the duplex conformation (B-form), are intact but the cross peak intensities of some of them are decreased indicating small perturbations of the helix conformation.[51] This observation allowed us to consider a "fix" B-DNA structure for the binding models. In addition, we observed a number of cross peaks between the ligand protons of the diastereomers. Intermolecular NOE effects were also observed between the diastereomeric complex protons and the dodecanucleotide (see Figure 6 and 7).

In the case of Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$, distances of less than 5Å were observed between the aromatic protons of the ligands m-bpy and bpy with the H2′′ proton of C9 and T8 facing the major groove of the helix. The exchangeable NH protons of the peptide backbone, which extend from the 4th position of the ligand m-bpy, show cross peaks with protons that are also accessible from the major groove such as H2′′ of T7 and T8 and the thymine T8 methyl group. A few very weak intensity intermolecular NOE effects were observed between the lysine aliphatic side chain and the oligonucleotide protons indicating that this part of the peptide is located across the major groove of the helix.

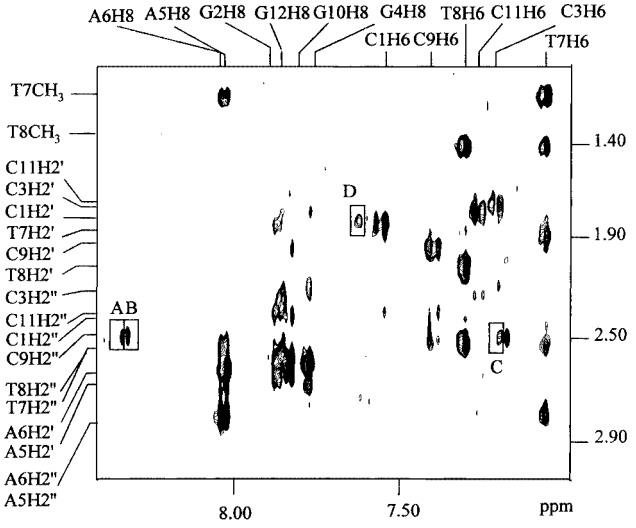


Figure 6. Part of the ^{1}H NOESY spectrum (τ_m = 400 ms) of the Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$−[d(CGCGAATTCGCG)$_2$] adduct at a 1:1 molar ratio at 303 K with the oligonucleotide assignments of the base's aromatic protons (F2) and the major groove sugar protons H2′ and H2′′ for the bases adenine, thymine and cytosine (F1). The labeled cross peaks correspond to NOE contacts between the protons: (A) m-bpyH3′-C9H2′′, (B) bpyH3−C9H2′′, (C) m-bpyH5′-C9H2′′ (D) bpyH6H6′-C1H2′

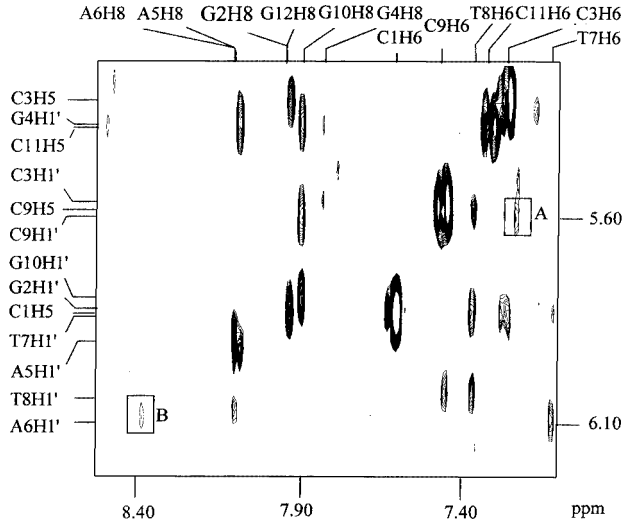


Figure 7. Part of the ^{1}H NOESY spectrum (τ_m = 400 ms) of the Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$−[d(CGCGAATTCGCG)$_2$] adduct at a 1:1 molar ratio at 303 K with the oligonucleotide assignments of the base's aromatic protons (F2) and the minor groove sugar proton H1′ (F1). The labeled cross peaks correspond to NOEs between the protons: (A) m-bpyH5′-C9H1′, (B) m-bpyH3′-A6H1′

On the other hand, the intermolecular NOE contacts between Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ and the oligonucleotide are significantly less than in the case of the Δ isomer, suggesting a looser or non-specific binding of the isomer. A few of these take place between the ligand m-bpy protons H5′and H3′ with C9H1′ and the peptide backbone Gly-NH with the sugar proton H1′ of the base T8, all located in the helix minor groove. In contrast to the Δ isomer, no cross peak was observed between the other two bpy ligand protons of the complex and the oligonucleotide indicating that only the m-bpy-GHK moiety of the complex is close to [d(5′-CGCGAATTCGCG-3′)$_2$].

2.2.4 Binding Models

It is not possible to develop quantitative 3-D models of the way the diastereomers bind to the oligonucleotide duplex in the Ru^{II}complex/d[(CGCGAATTCGCG)$_2$] due to the fast exchange kinetics of the system, which does not allow taking all NOE results as distance constraints. Taking in to account (i) that there is no significant perturbation to the DNA helix (according to NOE's and the *inter* and *intra*-strand connectivity of the oligonucleotide protons), (ii) that the geometry around the Ru^{II} coordination sphere is fixed and (iii) that using selective NOE's that are not geometrically in disagreement, we have constructed simple binding models that are consistent with the observations from 1-D and 2-D NMR spectroscopy (Figure 8 and 9). The complexes manually docked at distances are consistent with the inter-proton NOE in many different combinations and the geometries of the complexes were optimized with respect to the "freeze" geometry of the Ru^{II} coordination sphere (angles and bond lengths) and the DNA structure. The structures which are more consistent with the NMR observations are presented in Figure 8 and 9.

The molecular model of the Δ-[Ru(bpy)$_2$(m-bpy-GHK)]$^{2+}$/d[CGCGAATTCGCG)$_2$] system shows the two bpy ligands of the complex close to the DNA bases, in agreement with the observed ≈0.2 ppm upfield shift changes of H3′ and H4′ in the 1-D NMR spectra. The location of the complex is also consistent with the small changes in the aromatic proton chemical shifts of the m-bpy-GHK ligand, oriented out of the helix groove and with the observed upfield shifts of the protons A5A6H2′, A5A6H2′′ and C3H5 (opposite G10), since the complex is located close to this part of the oligonucleotide. In the model of the Λ isomer (Figure 9), the bpy ligands are far from the DNA bases and the terminal positive amino group of the lysine is close to DNA phosphates, as has already been suggested based on the 1-D NMR spectra. The resultant model located Λ-[Ru(bpy)$_2$(m-bpy-GHK)]$^{2+}$ at the helix minor groove.

It is known that ruthenium tris-chelates involving 2,2′-bipyridine or 1,10-phenanthroline ligands bind at the DNA grooves. For example [Ru(phen)$_3$]$^{2+}$ [10] binds in the minor groove and [Ru(bpy)$_2$(phen)]$^{2+}$ shows a similar binding geometry.[52] In all cases, however, where the Ru^{II} complex comprises an intercalative ligand [e.g. dipyridophenazine (dppz)] together with two bpy or phen ligands, the complex binds to DNA through intercalation of this ligand between the bases and leads the other two ligands into the oligonucleotide minor groove (e.g. Δ-[Ru(phen)$_2$(dpq)]$^{2+}$,[12] Δ-[Ru(Me$_2$phen)$_2$(dppz)]$^{2+}$ [13] and [{Ru(Me$_2$bpy)$_2$}(μ-bpy)]$^{4+}$ [14]). Sometimes the "leader" ligand intercalates from the major groove as in the case of Δ-[Ru(phen)$_2$(dppz)]$^{2+}$ leading the phenanthroline molecules to be located next to the major groove.[11] The questions which thus arises are, what makes an octahedral Ru^{II} com-

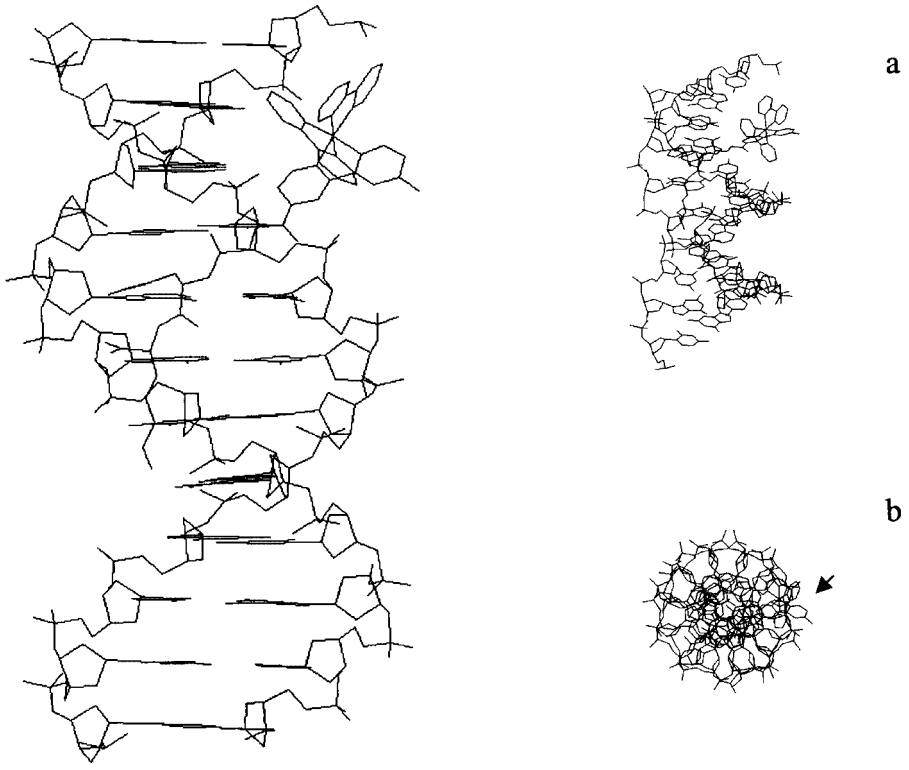


Figure 8. A molecular model of the interactions between Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ and [d(CGCGAATTCGCG)$_2$] reflects the average binding of the complex to the oligonucleotide. In the insets (a) a view across the major groove showing the complex orientation with the peptide back bone into the helix groove (b) top view along the *z*-axis showing a complex orientation where the peptide is bound to the oligonucleotide

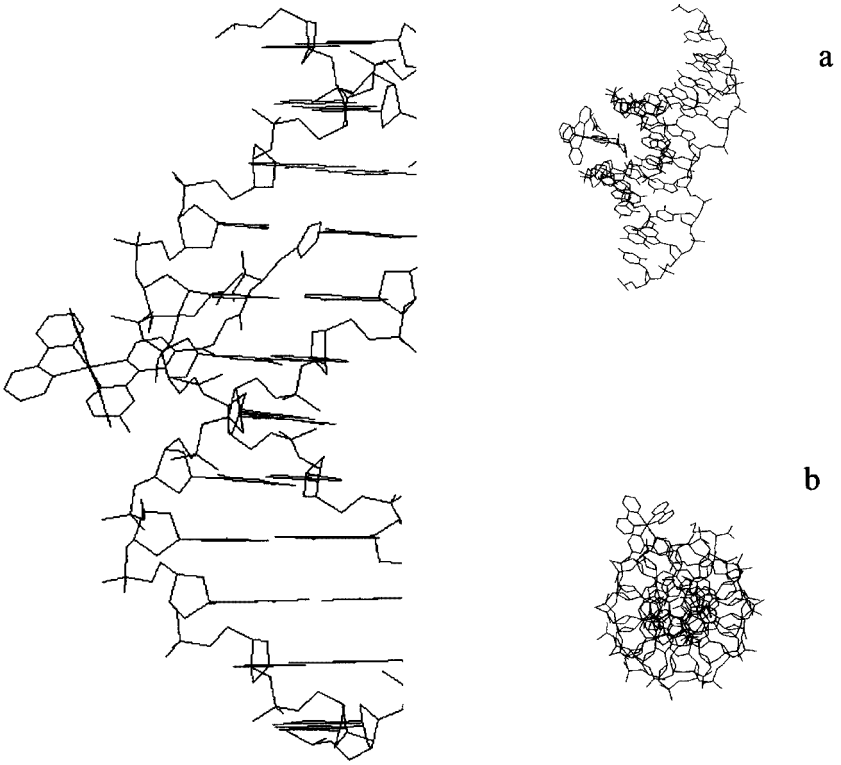


Figure 9. A molecular model of the interactions between Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ and the [d(CGCGAATTCGCG)$_2$] reflects the average binding of the complex to the oligonucleotide. In the insets (a) a view across the minor groove showing the complex orientation in the minor groove (b) top view along the *z*-axis showing the complex oriented with the m-bpy ligand towards to the helix

plex approach from the minor or the major groove of the DNA helix? Is the intercalative ligand responsible for this?

In the case of Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ a non-intercalative and flexible ligand (m-bpy-GHK) which binds to the DNA major groove has been used, showing that it is probably the peptide which leads the other two bpy ligands of the isomer to interact with the major groove. In other words, the peptide affinity for the major groove is higher than that of Δ-[Ru(bpy)$_2$] for the minor. In contrast, the same ligand does not follow the same way of binding in the case of the Λ isomer. For this complex, the different orientation of the peptide (see Figure 4) from that in the Δ isomer gives the main role to the bpy ligands that lead the complex into the minor groove. In particular, in this context, the major groove binding of Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ is consistent with (i) the relative dimensions of the complex and the oligonucleotide major groove (estimated dimensions of the complex are 22 Å long and 11 Å wide, while the size of the major groove is 11.6 Å × 8.5 Å and the minor is 6.0 Å × 8.2 Å [53]), (ii) the formation of hydrogen bonds between the peptide backbone and the non-hydrogen bonded protons of the cytidine exocyclic NH_2 group, which was deduced from the 1-D NMR spectra, can be explained by an approach of the oligonucleotide by the complexes from the major groove, (iii) the fact that the major groove is rich in negatively charged phosphates and for this reason it prefers the positively charged cationic complex and the lysine's terminal NH_3^+ group as well. It seems that the peptide determines the way that the complex binds to the oligonucleotide followed by a possible binding of the other ligands.

In the case of the Λ isomer, weak intensity cross-peaks were observed only between the glycine NH with the H1′ of T8 and H3′/H5′, located on the one pyridine ring of the m-bpy ligand, with the A6 and C9H1′, indicating that only the m-bpy ligand of the complex interacts with the oligonucleotide probably from the minor groove.

3. Conclusion

In summary, it is concluded from the 1-D and 2-D NMR spectroscopic data that (i) the diastereomers bind to the oligonucleotide differently. The Δ isomer binds from the helix major groove, close to the central part of the sequence while the peptide moiety extends across the major groove from the base T8 to C11. The Λ isomer probably binds non-selectively, approaching the helix from the minor groove. (ii) The peptide binding, it would appear, leads the rest of the Δ isomer to interact with the oligonucleotide and thus may explain why the two bpy ligands were found to be located in the major groove in the case of the Δ isomer. The detection of sequence selectivity binding requires further studies with different DNA sequences. This work together with photo cleavage experiments of the oligonucleotides by such complexes is in progress.

4. Experimental Section

4.1. Starting 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 (University of Southampton U.K.) and purified by chromatography on a 120 × 2.5 cm Sephadex G-25 superfine column, using distilled water as the eluent and lyophilized to dryness. The complexes *cis*-[Ru(bpy)$_2$Cl$_2$][18] Λ- and Δ-[Ru(bpy)$_2$(py)$_2$]A (where A = *O*,*O*′-dibenzoyltartaric acid) ,[19,20] and the ligand 4′-methyl-2,2′-bipyridine-4-carboxylic acid (m-bpy-COOH) were synthesized according to literature.[21]

4.2. Synthesis of the Peptide Conjugated Ru^{II} Complexes

Λ-, Δ-[Ru(bpy)$_2$(m-bpy-COOH)](PF$_6$)$_2$: Solid Λ- or Δ-[Ru(bpy)$_2$(py)$_2$]A (0.38 g) and m-bpy-COOH (0.105 g) were suspended in 70% ethanol in water (10 mL) and the mixture was heated to reflux overnight. The reaction mixture was cooled to room temperature and the solvent was reduced with a rotary evaporator to approximately 3 mL. After standing overnight, the mixture was filtered and the remaining solid was washed with water. The filtrate was acidified to pH = 1 by dropwise addition of aqueous 60% HPF_6 with vigorous stirring. The solid was collected on a fine-porosity glass filter, washed with water (2 × 5 mL), diethyl ether (2 × 5 mL) and oven dried at 110 °C overnight to provide pure Λ- or Δ-[Ru(bpy)$_2$(m-bpy-COOH)](PF$_6$)$_2$ as a red solid. Yield 0.30 g (82%). **Δ-[Ru(bpy)$_2$(m-bpy-COOH)](PF$_6$)$_2$:** $C_{32}H_{26}F_{12}N_6O_2P_2Ru$: calcd. C 41.9, H 2.9, N 9.2; found C 41.2, H 2.9, N 9.3. **Λ-[Ru(bpy)$_2$(m-bpy-COOH)](PF$_6$)$_2$:** $C_{32}H_{26}F_{12}N_6O_2P_2Ru$: calcd. C 41.9, H 2.9, N 9.2; found C 41.1, H 2.9, N 9.2.

Λ-, Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$: Solid-phase peptide synthesis was used for peptide conjugation. The Fmoc strategy was followed using 2-chlorotrityl-H-Linker-Fmoc resin as the solid support. Piperidine was used as a deprotection reagent. DCC and HOBt were used as coupling reagents. The cleavage from the resin and the final deprotection of peptide side chain protecting groups were performed in one step by shaking with a 90% TFA solution in DMF. Anisol was used as a scavenger. The final products Λ- or Δ-[Ru(bpy)$_2$m-GHL]$^{2+}$ were isolated as trifuoroacetate salts which are insoluble in water. The trifluoroacetate anion was replaced by a chloride anion by mixing an acetone solution of the ruthenium peptide derivative with an excess of LiCl. [Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ precipitated as an orange water soluble solid. The product was purified by reverse-phase HPLC using a DIONEX chromatograph and a Waters C18 column. Chromatography solvents were HPLC grade acetonitrile and water both containing 0.1% TFA. Yield approximately 30%. **Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$:** ^{1}H NMR (500 MHz, D_2O, 10 mM, in 100 mM phosphate buffer, pH = 7.01, 303 K): δ = 8.83 (s, 1 H, m-bpy H3′), 8.54 (d, $^3J_{H,H}$ = 8.1 Hz, 4 H, bpy H3H3′), 8.48 (s, 1 H, m-bpy H3), 8.05 (t, $^3J_{H,H}$ = 7.5 Hz, 4 H, bpy H4H4′), 8.00 (d, $^3J_{H,H}$ = 5.8 Hz, 1 H, m-bpy H5), 7.79 (s, 1 H, His H2), 7.80 (d, $^3J_{H,H}$ = 5.7 Hz, 4 H, bpy H6H6′), 7.37 (t, $^3J_{H,H}$ = 6.3 Hz, 4 H, bpy H5H5′), 7.65 (d, $^3J_{H,H}$ = 5.8 Hz, 2 H, m-bpy H6H6′), 7.53 (s, 1 H, His H5), 7.27 (d, $^3J_{H,H}$ = 5.8 Hz, 1 H, m-bpy H5′), 4.63 (d, $^3J_{H,H}$ = 4.1 Hz, 1 H, His *a*-CH), 4.25 (d, $^3J_{H,H}$ = 4.2 Hz, 1 H, Lys *a*-CH), 4.17 (d, $^3J_{H,H}$ = 4.2 Hz, 1 H, Gly *a*-CH), 2.88 (t, $^3J_{H,H}$ = 7.5 Hz, 2 H, Lys ε-CH), 3.15 (m, $^3J_{H,H}$ = 7.5 Hz, 2 H, His β-CH), 2.49 (s, 3 H, m-bpy CH_3), 1.80 (m, 2 H, Lys β-CH), 1.67 (t, $^3J_{H,H}$ = 6.3 Hz, 2 H, Lys δ-CH), 1.37 (m, 2 H, Lys γ-CH), ppm. **Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$:** ^{1}H NMR (500 MHz, D_2O, 10 mM, in 100 mM phosphate buffer, pH = 7.01, 303 K): δ = 8.83 (s, 1 H, m-bpy H3′), 8.54 (d, $^3J_{H,H}$ = 8.6 Hz, 4 H, bpy H3H3′),

8.47 (s, 1 H, m-bpy H3), 8.05 (t, $^3J_{H,H}$ = 7.5 Hz, 4 H, bpy H4H4′), 8.00 (d, $^3J_{H,H}$ = 5.8 Hz, 1 H, m-bpy H5), 7.80 (d, $^3J_{H,H}$ = 5.7 Hz, 4 H, bpy H6H6′), 7.80 (s, 1 H, His H2), 7.65 (d, $^3J_{H,H}$ = 5.8 Hz, 2 H, m-bpy H6H6′), 7.54 (s, 1 H, His H5), 7.38 (t, $^3J_{H,H}$ = 6.3 Hz, 4 H, bpy H5H5′), 7.27 (d, $^3J_{H,H}$ = 5.8 Hz, 1 H, m-bpy H5′), 4.65 (d, $^3J_{H,H}$ = 5.0 Hz, 1 H, His *a*-CH), 4.26 (d, $^3J_{H,H}$ = 4.2 Hz, 1 H, Lys *a*-CH), 4.17 (d, $^3J_{H,H}$ = 4.6 Hz, 1 H, Gly *a*-CH), 3.17 (m, $^3J_{H,H}$ = 7.6 Hz, 2 H, His β-CH), 2.90 (t, $^3J_{H,H}$ = 7.5 Hz, 2 H, Lys ε-CH), 2.54 (s, 3 H, m-bpy CH_3), 1.82 (m, 2 H, Lys β-CH), 1.67 (t, $^3J_{H,H}$ = 6.3 Hz, 2 H, Lys δ-CH), 1.38 (m, 2 H, Lys γ-CH) ppm. **Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$:** $C_{45}H_{49}F_{12}N_{13}O_4P_2Ru$: calcd. C 48.8, H 4.4, N 16.4; found C 49.0, H 4.5, N 16.4. **Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$:** $C_{45}H_{49}F_{12}N_{13}O_4P_2Ru$: calcd. C 48.8, H 4.4, N 16.4; found C 48.7, H 4.6, N 16.3.

4.3. Physical Measurements

4.3.1 NMR Spectroscopy

Measurements were made with a Varian Unity-500 MHz instrument. 1-D ^{1}H NMR spectra were recorded at 303 K into 4096 data points, with a 6024 Hz spectral width after 128 transients. The water resonance was suppressed with a time-shared Redfield "2-1-4"-pulse. ^{1}H NOESY spectra were recorded in phase sensitive mode with a total 2048 × 256 points for a mixing time of 200 to 400ms, while ^{1}H ROESY spectra had mixing times (τ_m) of 60−120 ms. ^{1}H DQFCOSY spectra were collected using the TPPI method with a spectral width of 3125 Hz with a total of 2048 × 256 points and a relaxation delay of 1.5 s.

The amounts of the oligonucleotide were estimated by weighing, and the concentration of the sample was determined using its absorption at 260 nm .[22] In all NMR experiments carried out, a 100 mM Na_2HPO_4/NaH_2PO_4 (pH = 7.00) buffer was used. The lyophilized samples were dissolved in D_2O (99.96) and lyophilized again to dryness. These samples were finally dissolved in D_2O (99.996, 0.4 mL) and transferred to 5 mm NMR tubes.

The 1-D ^{1}H NMR spectra were recorded with sample concentration ≈ 100 OD_{260} units while 2-D NOE experiments were carried out in more concentrated samples (≈ 300 OD_{260}). ^{1}H NMR spectra of the labile oligonucleotide protons were recorded in 90% H_2O 10% D_2O (field-frequency lock). No internal chemical shift reference was added to the samples.

4.3.2 Electrospray Mass Spectrometry

ESI mass spectra were acquired with a Micromass Q-Tof quadrupole MS instrument. All spectra were recorded in TOF (time of flight) mode with a source temperature of 80 °C, and low cone voltage (ΔCS = 100−120V) to obtain gentle interface conditions.

4.3.3 CD spectroscopy

CD spectra were recorded with a Jasco J-720 spectropolarimeter. A 0.1-cm path length cuvette was used for the 190−250 nm region while a 2-cm path length cuvette was used for the 250−540 nm region. The blank was subtracted from the CD spectrum of the sample. Smoothing of the curves was performed using the Adjacent Averaging method from Microcal Origin 6.0.

4.4. Molecular Modeling

The 3-D binding models were constructed using the HyperChem. v. 6.0 molecular modeling software. The model of the oligonucleotide [d(CGCGAATTCGCG)$_2$] duplex was based on a standard B-type structure with a C2′ *endo* sugar conformation using the database of the program. The Ru^{II} complex was constructed in two steps. Firstly, the coordination sphere of the ruthenium was constructed using crystal structure data of similar complexes [23] and secondly, the peptide was conjugated at the 4th position of the ligand m-bpy. The complex was then manually docked close to the oligonucleotide duplex with respect to the observed NOE contacts, which were inserted as distance restraints. The NOE cross peaks were classified into three categories: weak (5 Å), medium (4 Å) and strong (3 Å) relative to the volume integral of the cytosine CH5/CH6 cross peak. Selective NOEs from the nine observations (Table 4) for Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ were inserted as distance restraints and the complexes were selectively and briefly energy minimized in the presence of the oligonucleotide using the AMBER force field. Several structures from different combinations of the intermolecular NOEs were observed and the most consistent one with the NMR spectroscopic data was presented as a possible binding model of the complex to the oligonucleotide duplex. In the case of Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$, a similar procedure was followed and a structure that was more consistent with the NMR observations was maintained. Graphical outputs were obtained from the selected structures with the RasMol v. 2.6 software.

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