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Design and photochemical tuning of Ir(III) and Ru(II) photoprobes

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Bonne lecture à tous !

To my mother

*« If one way be better than another, that you may be sure is nature's
way »*

Aristotle

List of abbreviations

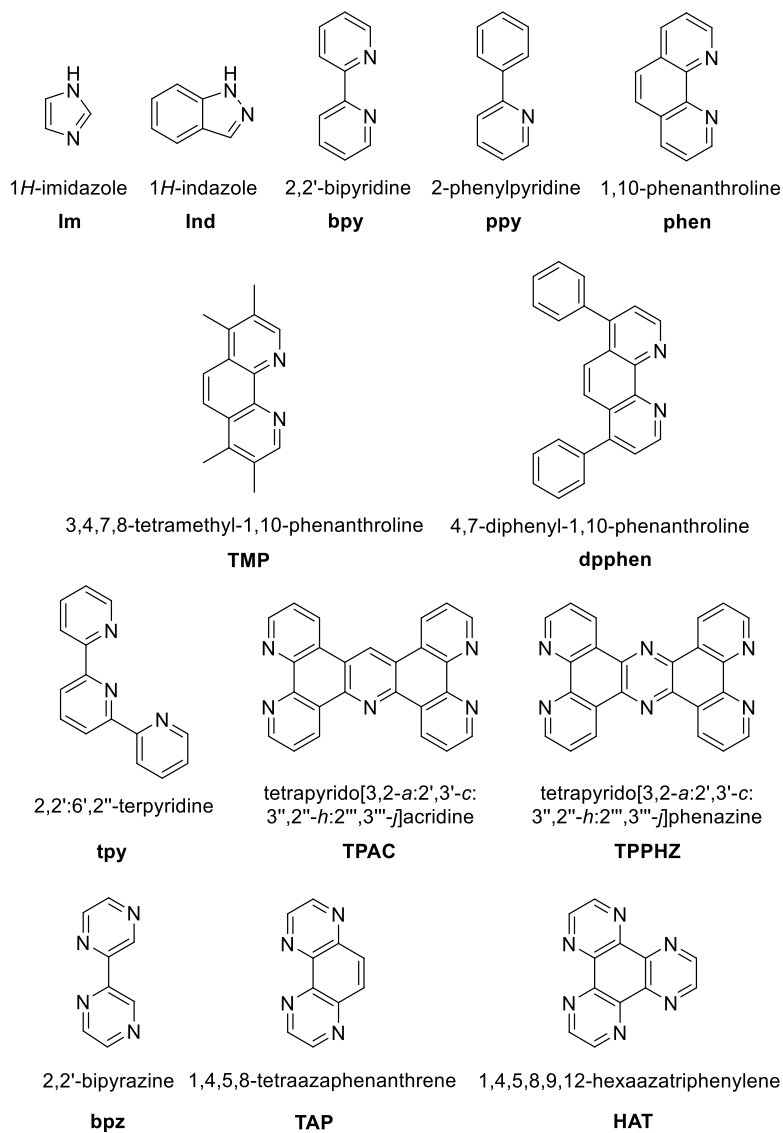
A	adenine
Å	angstrom
A.U.	arbitrary unit
Ac	acetyl
AP	apurinic/apyrimidinic site
C	cytosine
bdppz	benzo[<i>h</i>]dipyrido[3,2- <i>a</i> :2',3'- <i>c</i>]phenazine
BET	back electron transfer
bpy	2,2'-bipyridine
bpz	2,2'-bipyrazine
chrysi	5,6-chrysenequinone diimine
CT-DNA	calf thymus DNA
DCM	dichloromethane
DFT	Density Functional Theory
dGMP	deoxyguanosine-5'-monophosphate
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dpac	dipyrido-[3,2- <i>a</i> :2',3'- <i>c</i>]acridine
dpacF ₂	7,8-difluorodipyrido[3,2- <i>a</i> :2',3'- <i>c</i>]acridine
dpacF ₄	6,7,8,9-tetrafluorodipyrido[3,2- <i>a</i> :2',3'- <i>c</i>]acridine
dpphen	4,7-diphenyl-1,10-phenanthroline
dppz	dipyrido-[3,2- <i>a</i> :2',3'- <i>c</i>]phenazine

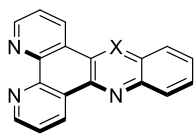
ε	molar extinction coefficient
EB	ethidium bromide
em	emission
EPR	electronic paramagnetic resonance
ER	endoplasmic reticulum
ESI	electrospray ionization
Et	ethyl
ex	excitation
G	guanine
G4	G-quadruplex
GSH	glutathione
h	hour
HAT	1,4,5,8,9,12-hexaazatriphenylene
HOMO	Highest Occupied Molecular Orbital
HRMS	high resolution mass spectrometry
IC	internal conversion
Im	<i>1H</i> -imidazole
Ind	<i>1H</i> -indazole
IR	infrared
ISC	Inter System Crossing
K_{aff}	binding constant
k_r	radiative rate constant
k_{nr}	non-radiative rate-constant
λ	wavelength
LC	Ligand-Centered

LL	bidentate ligand
LLCT	Ligand-to-Ligand Charge transfer
LUMO	Lowest Unoccupied Molecular Orbital
MC	Metal-Centered
Me	methyl
min	minute
MLCT	Metal-to-Ligand Charge transfer
MLLCT	Metal-Ligand-to-Ligand Charge transfer
MMR	mismatch repair machinery
MO	molecular orbital
NIR	near infrared
NMR	nuclear magnetic resonance
npp	naphtho[1,2- <i>b</i>]pyrido[3,2- <i>f</i>][1,7]phenanthroline
PBS	phosphate buffer saline
PCET	proton-coupled electron transfer
PDT	photodynamic therapy
PEG	polyethylene glycol
Φ	luminescence quantum yield
phen	1,10-phenanthroline
PIET	photo-induced electron transfer
ppm	part per million
ppy	2-phenylpyridine
PS	photosensitizer
Q	quencher
RT	room temperature

SCE	saturated calomel electrode
SS-DNA	salmon sperm DNA
T	thymine
τ	Excited state lifetime
TA	transient absorption
TAP	1,4,5,8-tetraazaphenanthrene
TD-DFT	Time Dependent-Density Functional Theory
TFA	trifluoroacetic acid
TMP	3,4,7,8-tetramethyl-1,10-phenanthroline
TPAC	tetrapyrido[3,2- <i>a</i> :2',3'- <i>c</i> :3'',2''- <i>h</i> :2''',3'''- <i>j</i>]acridine
TPPHZ	tetrapyrido[3,2- <i>a</i> :2',3'- <i>c</i> :3'',2''- <i>h</i> :2''',3'''- <i>j</i>]phenazine
tpy	2,2';6',2''-terpyridine
UV	ultraviolet
Vis	visible
VR	vibrational relaxation

Structure of the ligands involved in the manuscript



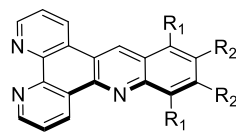


X = CH: dipyrido[3,2-*a*:2',3'-*c*]acridine

X = N: dipyrido[3,2-*a*:2',3'-*c*]phenazine

dpac (X = CH)

dppz (X = N)

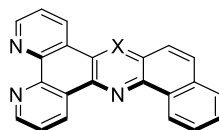


7,8-difluorodipyrido[3,2-*a*:2',3'-*c*]acridine

6,7,8,9-tetrafluorodipyrido[3,2-*a*:2',3'-*c*]acridine

dpacF₂ (R₁=H, R₂=F)

dpacF₄ (R₁=R₂=F)



X = CH: naphtho[1,2-*b*]pyrido[3,2-*f*][1,7]phenanthroline

X = N: benzo[*h*]dipyrido[3,2-*a*:2',3'-*c*]phenazine

npp (X = CH)

bdppz (X = N)

List of publications

“Design and Photophysical Studies of Acridine-Based Ru^{II} Complexes for Applications as DNA Photoprobes”

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“A new ER-specific photosensitizer unravels 1O₂-driven protein oxidation and inhibition of deubiquitinases as a generic mechanism for cancer PDT”

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Table of content

Chapter 1 – General introduction.....	1
1. DNA genesis – a story about mutating singularity.....	3
2. Targeting DNA: drug design infancy	12
2.1. The first discoveries: organic chemists on track	12
2.2. Blended science: a tasty mix	14
2.2.1. <i>Elements of structure and geometry</i>	15
2.2.2. <i>Binding to DNA – a matter of geometry and chemical properties</i>	16
2.2.3. <i>Attractive compounds for attractive applications.....</i>	19
2.2.4. <i>DNA-binding transition metal complexes.....</i>	23
3. Ru(II) complexes – Photophysics and photochemistry	28
3.1. Geometry and orbitals	28
3.2. Absorption of light	29
3.2.1. <i>Basic principles</i>	29
3.2.2. <i>Possible electronic transitions.....</i>	31
3.2.3. <i>The Beer-Lambert Law</i>	33
3.2.4. <i>Selection rules.....</i>	34
3.2.5. <i>Deactivation of excited complexes.....</i>	35
3.2.6. <i>Lifetimes and quantum yield.....</i>	37
3.2.7. <i>Case of [Ru(bpy)₃]²⁺</i>	38
3.2.8. <i>Redox properties</i>	40
Chapter 2 – Objectives of the Ph.D. thesis	43
1. Objectives and strategies	45
2. Content of the manuscript	50

Chapter 3 – Design and photophysical studies of acridine-based Ru(II) complexes for application as DNA photoprobes	53
1. Foreword	55
1.1. Introduction	55
1.1.1. <i>Photophysics in the presence of DNA</i>	56
1.1.2. <i>DNA binding of Ru-DPPZ</i>	62
1.2. Objectives.....	64
2. Preliminary results.....	66
2.1. Synthesis of the dpac ligand and its fluorine analogs	66
2.1.1. <i>Synthesis of dpac through Friedländer annulation</i> ..	66
2.1.2. <i>Synthesis of dpac through cycloaddition reaction</i>	70
2.1.3. <i>Synthesis of dpac through intermolecular condensation</i>	71
2.1.4. <i>Synthesis of dpac through intramolecular condensation</i>	73
2.1.5. <i>Synthesis of dpac through intermolecular cyclisation</i> ..	74
3. Content of the publication	75
3.1. Introduction	76
3.2. Results and discussion	79
3.2.1. <i>Synthesis</i>	79
3.2.2. <i>Electrochemistry</i>	80
3.2.3. <i>Absorption spectra and computational studies</i>	85
3.2.4. <i>Emission spectra, lifetimes, quantum yields and computational studies</i>	89
3.2.5. <i>Photophysical behaviour in the presence of DNA and polynucleotides</i>	94
3.3. Conclusions.....	98

3.4.	Experimental section	99
3.4.1.	<i>Materials and instrumentation</i>	99
3.4.2.	<i>Synthetic procedures and characterization of dpac ligands and respective Ru(II) complexes</i>	101
3.5.	Associated content	112
3.5.1.	<i>Absorption spectra of the ligands</i>	112
3.5.2.	<i>Absorption and emission spectra of the Ru(II) complexes</i>	114
3.5.3.	<i>Emission spectra of the Ru(II) complexes at 77K...</i>	115
3.5.4.	<i>Quantum yields of the Ru(II) complexes</i>	116
3.5.5.	<i>HRMS spectra and data</i>	117
3.5.6.	<i>Computational data</i>	123
Chapter 4 – Towards mismatched DNA photoprobes: “elbow-shaped” Ru(II) complexes		131
1.	Introduction	137
1.1.	Mismatched DNA targeting	137
2.	Objectives	141
3.	Results	142
3.1.	Synthetic procedures	142
3.2.	Electrochemistry	143
3.3.	Absorption spectra	147
3.4.	Emission spectra, lifetimes, quantum yields	149
3.5.	Photophysical behavior in the presence of DNA	154
3.6.	Photophysical behaviour in the presence of mismatched DNA	156
4.	Conclusions	159
5.	Experimental section	160

5.1. Synthetic procedures and characterization of “elbow-shaped” ligands and respective bpy-based Ru(II) complexes.....	160
6. Associated content.....	166
6.1. Absorption spectra of the ligands.....	166
6.2. Absorption and emission spectra of the Ru(II) complexes ..	167
6.3. Emission spectra of the Ru(II) complexes at 77 K	168
6.4. HRMS spectra and data.....	169

Chapter 5 – Photo-induced electron transfer between Ru(II) complexes and polynucleotides..... 165

1. Introduction	175
1.1. Luminescence quenching processes.....	175
1.1.1. <i>Dynamic quenching</i>	177
1.1.2. <i>Static quenching</i>	178
1.1.3. <i>Simultaneous static and dynamic quenching</i>	179
1.2. Photo-induced electron transfer	181
1.2.1. <i>Basic principles</i>	181
1.2.2. <i>Photo-induced electron transfer with polynucleotides .</i>	182
1.2.2.1. DNA and oligonucleotides.....	182
1.2.2.2. G-quadruplexes (G4).....	186
1.3. Objectives.....	188
2. Main topics addressed in the publication	189
3. Content of the publication	190
3.1. Results.....	190
3.1.1. <i>Synthesis</i>	190

3.1.2.	<i>Electrochemistry</i>	192
3.1.3.	<i>Absorption</i>	193
3.1.4.	<i>Emission</i>	195
3.1.5.	<i>Photophysical behavior in the presence of DNA</i>	199
3.1.6.	<i>Photophysical behavior in the presence of mismatched DNA</i>	203
3.1.7.	<i>Complement to the Article: Transient absorption (TA) spectroscopy studies</i>	205
3.1.8.	<i>Complement to the Article: Photoreactivity in the presence of G-quadruplexes</i>	214
3.2.	<i>Conclusions</i>	216
3.3.	<i>Experimental section</i>	218
3.3.1.	<i>Synthetic procedures and characterization of Ru(II) complexes</i>	218
3.4.	<i>Associated content</i>	227
3.4.1.	<i>Absorption and emission spectra of the Ru(II) complexes</i>	227
3.4.2.	<i>HRMS spectra</i>	229
3.4.3.	<i>Cyclovoltammograms</i>	232
 Chapter 6 – Tuning of the photochemical properties: Design of Ir(III) complexes as DNA photoprobes		
1.	<i>Introduction</i>	237
1.1.	<i>Photophysics and photochemistry</i>	237
1.1.1.	<i>Geometry and orbitals</i>	237
1.1.2.	<i>Absorption of light</i>	239
1.1.3.	<i>Emission of light</i>	241
1.1.4.	<i>Redox properties</i>	242

1.2.	Ir(III) complexes as biomolecular probes	242
1.2.1.	<i>Non-covalent DNA probes</i>	243
1.2.2.	<i>Cellular probes</i>	245
2.	Tuning of Ir(III) DNA photoprobes	247
3.	Content of the publication	248
3.1.	Introduction	249
3.2.	Results	251
3.2.1.	<i>Synthesis</i>	251
3.2.2.	<i>Electrochemistry</i>	253
3.2.3.	<i>Absorption</i>	260
3.2.4.	<i>Emission</i>	262
3.2.5.	<i>Photophysical studies in the presence of DNA</i>	266
3.3.	Conclusions	268
3.4.	Experimental section	270
3.4.1.	<i>Materials and instrumentation</i>	270
3.4.2.	<i>Synthetic procedures and characterization of Ir(III) complexes</i>	271
3.5.	Associated content	283
3.5.1.	<i>HRMS data</i>	283
Chapter 7 – Conclusions and prospects		289
1.	Conclusions	291
2.	Prospects	295
Chapter 8 – Instrumental techniques		303
1.	Nuclear Magnetic Resonance (^1H NMR)	305
2.	Mass spectrometry	305
3.	UV-visible absorption spectroscopy	305

4. Determination of molar extinction coefficient	305
5. Emission spectroscopy	306
6. Luminescence lifetimes	306
7. Determination of quantum yield.....	307
8. Cyclic voltammetry	307
9. (TD-)DFT calculations	307
10. Surface Plasmon Resonance measurements.....	308
11. Transient absorption measurements	309
Chapter 9 – Annexes	313
Additional chapter: Photophysical and photochemical studies of a new organic photosensitizer for cancer photodynamic therapy.....	315
1. Introduction.....	315
1.1. Cancer fundamentals	315
1.1.1. <i>Cancer – A matter of cells dysfunction</i>	315
1.1.2. <i>Origins</i>	317
1.1.3. <i>Treatments</i>	317
1.2. Photodynamic therapy (PDT).....	318
1.2.1. <i>Basic principles</i>	318
1.2.2. <i>Requirements</i>	318
1.3. Objectives	324
2. Results and discussion	325
3. Conclusions.....	330
Publications	331
Chapter 10 – References	361

Abstract

Species integrity is warranted by the information that is carried by DNA. The diversity allowed by Nature accounts for the substantial role of this biopolymer, notably for evolution. While DNA mutations are essential for species survival, they lie also at the basis of the triggering of some genetic diseases and, in some cases, cancer. Consequently, Nature has developed its own repair mechanisms to correct DNA defects but some of them can escape the control processes. The detection, and ultimately the correction of these defects is thus of high relevance for diagnostic and therapeutic applications.

In this context, the development of luminescent environment-sensitive probes able to target DNA defects, such as single-base mismatches, is promising. Transition metal complexes represent a particularly interesting choice due to their outstanding DNA binding and photochemical properties.

During this Ph.D. thesis, we aimed to tune the DNA binding and photochemical properties of transition metal complexes photoprobes by modifying either the ligands or the nature of the metal cation. The synthesized Ru(II) and Ir(III) derivatives have shown strong affinity for DNA, and selective targeting of mismatched DNA has been achieved by using dissymmetric ligands. In addition, tuning of the photoreactivity of these complexes has been achieved, allowing DNA photooxidation. Finally, the environment-sensitive properties of an organic phenazine derivative proved to be crucial for PDT.

Résumé

L'intégrité des espèces est garantie par les informations contenues dans l'ADN. La diversité permise par la Nature montre le rôle substantiel de ce biopolymère, notamment pour l'évolution. Tandis que les mutations génétiques au sein de l'ADN s'avèrent essentielles pour la survie des espèces, elles sont aussi à l'origine du déclenchement de maladies génétiques et, dans certains cas, de cancers. En conséquence, la Nature a développé ses propres mécanismes de réparation pour corriger les défauts au sein de la structure d'ADN. Cependant, certains de ces défauts parviennent à échapper aux processus de contrôle. La détection, et *in fine* la correction de ces défauts relèvent d'une importance capitale à des fins diagnostiques et thérapeutiques.

Dans ce contexte, le développement de sondes dont les propriétés de luminescence sont sensibles à l'environnement qui sont capables de cibler les défauts au sein de l'ADN, tels que les bases mal appariées, s'avère prometteur. Les complexes de métaux de transition sont particulièrement intéressants à ces fins grâce à leurs remarquables propriétés photochimiques et à leur aptitude à interagir avec l'ADN.

Lors de cette thèse de doctorat, nous avons décidé d'ajuster les propriétés photochimiques et le mode d'interaction avec l'ADN de complexes de métaux de transition en modifiant soit les ligands, soit le cation métallique, afin d'améliorer leur comportement en tant que photosondes à ADN. Les dérivés de Ru(II) et d'Ir(III) synthétisés ont montré une forte affinité pour l'ADN, ainsi qu'un ciblage sélectif des bases mal appariées lors de l'utilisation de ligands dissymétriques. De plus, la modification de la photoréactivité de ces complexes a permis d'obtenir des composés photooxydants envers l'ADN. Finalement, les propriétés sensibles à l'environnement d'un dérivé organique de phénazine se sont avérées cruciales pour la photothérapie dynamique.

Chapter 1

General introduction

1. DNA genesis – a story about mutating singularity

Each species on Earth is unique. At the origin of each singularity lies a molecule called deoxyribonucleic acid (DNA), a biopolymer that carries all the genetic information and which thereby acts as a warrant for the species integrity. DNA was fortuitously discovered in the late 1860's by Friedrich Miescher, a Swiss physiological chemist. Miescher's plan was to isolate and identify the protein components of leukocytes, white blood cells, by recovering used, pus-coated bandages from a local surgical clinic. But the isolated substance, coming from the cell nuclei, soon revealed unexpected chemical properties, as a high phosphorous content and resistance to protein digestion.¹ Miescher called this new substance "nuclein", because he found it inside the nuclei of human white blood cells. This term was later changed to "nucleic acid" and then to "DNA".

The interest for DNA grew up the following decades. Several details about the biomolecule were divulged during the 20th century. Notably, Phoebus Levene, a Russian biochemist, proposed in 1919 that nucleic acids were made of nucleotides, and that each nucleotide was in turn made of a base, a phosphate group and a sugar derivative (Figure 1.1).² This work was expanded during the 1950's by Erwin Chargaff, an Austrian biochemist, who discovered that the nucleotide composition of DNA varies among species and that there are two types of bases: purines (A and G) and pyrimidines (C and T) (Figure 1.1). He also noticed that the total amount of purines is usually nearly equal and the same for pyrimidines (known nowadays as "Chargaff's rule").³

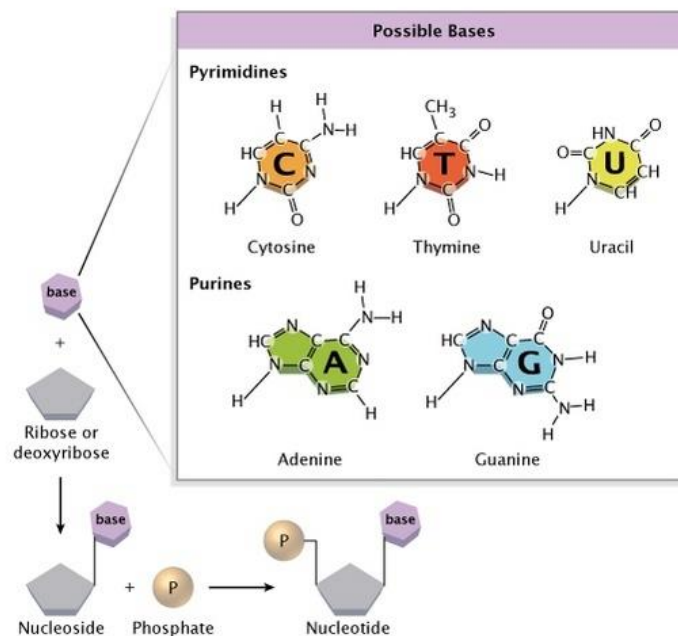


Figure 1.1. Schematic representation of a nucleotide. Uracil is solely found in RNA structures.⁴

Chargaff's observations combined with pioneering X-ray crystallography work by Rosalind Franklin and Maurice Wilkins, both English researchers, paved the way for Watson and Crick's double-helix model for the 3D structure of DNA in 1953.⁵ This proposal would not have been possible without Linus Pauling's work in the USA about 3D molecular assembly. Excepting some minor modifications, the main features of Watson and Crick model remain the same today (Figure 1.2):

(I) DNA displays a double-stranded helix, with the two strands connected by hydrogen bonds. The nucleotides are linked to each other by their phosphate groups, which bind the 3' end of a ribose derivative to the 5' end of the next ribose derivative. Adenine bases are paired with thymines, and cytosines are paired with guanines, consistently to Chargaff's rule. The sequence of the bases determines which biological instructions are contained in a DNA strand ;

(II) DNA can adopt several conformations, most of them being right-handed (A- and B-DNA), and one left-handed (Z-DNA) ;

(III) The DNA double helix is anti-parallel, which means that the 5' end of one strand is paired with the 3' end of its complementary strand (and *vice versa*) ;

(IV) The outer edges of the bases are available for potential hydrogen bonding, thus providing access to the DNA for other molecules (proteins, ...).

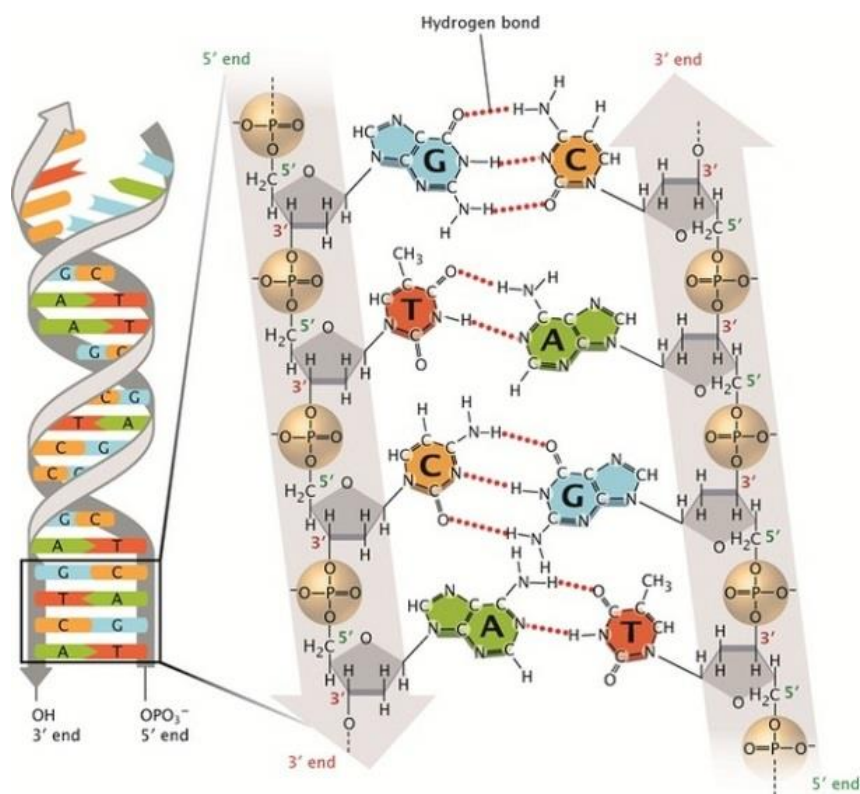


Figure 1.2. Detailed schematic representation of B-DNA.⁴

One of the supplementary information scientists have worked out from Watson and Crick's model is through the identification of different conformations of the double helix. The most common conformation in most living cells is known as B-DNA, as depicted on Figure 1.2. Surrounding its central base stack, the sugar-phosphate

backbone forms two distinct grooves, a wide major groove and a narrow minor groove, the minor one being two times narrower than the major one. The diameter of the B-DNA helix is approximately 2 nm and the distance between two stacked base pairs is about 0.34 nm (Figure 1.3). This latter distance corresponds to the sum of the Van der Waals radii of the carbon atoms from the upper and lower base pairs. For comparison purpose, proteins diameter is usually of about 3-10 nm (Figure 1.4). The two other conformations are A-DNA, a right-handed shorter and wider form found in dehydrated samples of DNA and rarely under normal physiological conditions, and Z-DNA, a left-handed conformation occasionally existing under specific biological activity conditions.

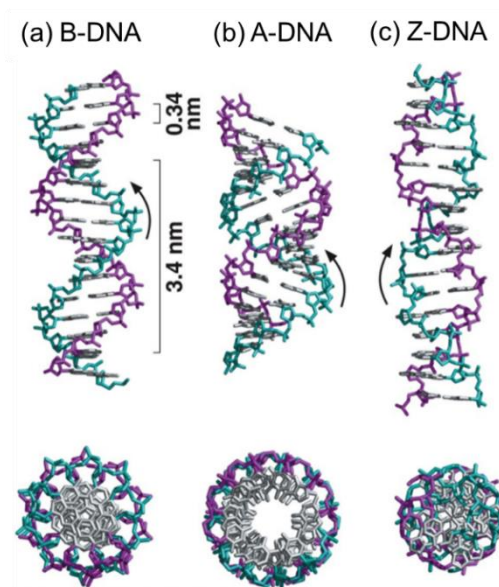


Figure 1.3. Schematic representation of the most common DNA conformations (a) B-DNA, b) A-DNA and c) Z-DNA). Top: perpendicular view; bottom: top view, with respect to the base pair stack.

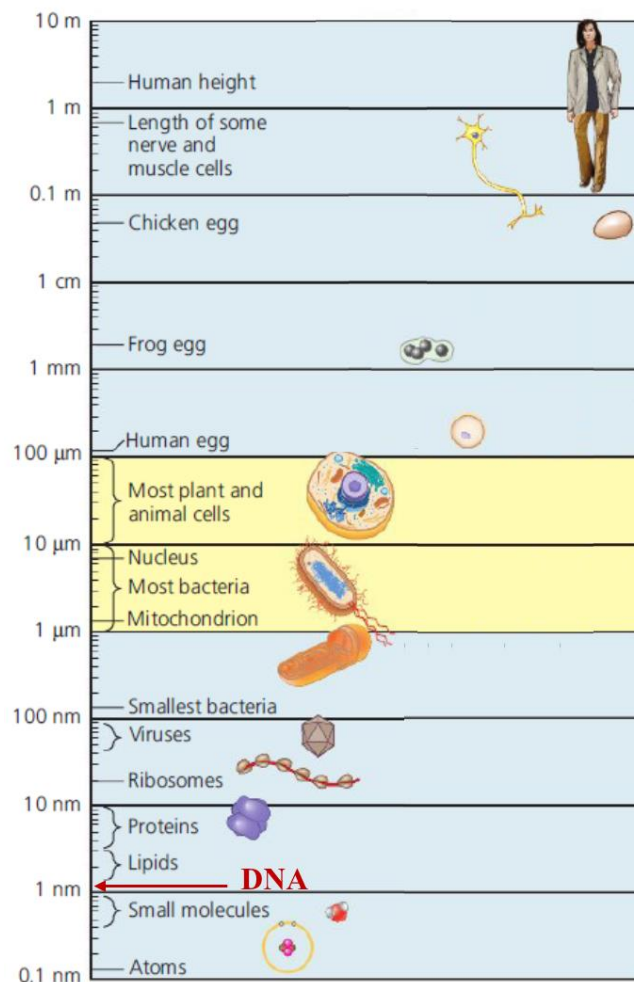


Figure 1.4. Scale length for some biological species. The yellow region indicates usual cells diameter.⁶

DNA also displays non-canonical conformations and some of them have known, or putative, biologically interesting function. Mismatches, bulges, abasic sites, hairpins and G-quadruplexes are among the most studied (Figure 1.5). Note this manuscript will only discuss about structures found in animal cells as solely DNA from animal origin will be used in our experiments (*vide infra*).

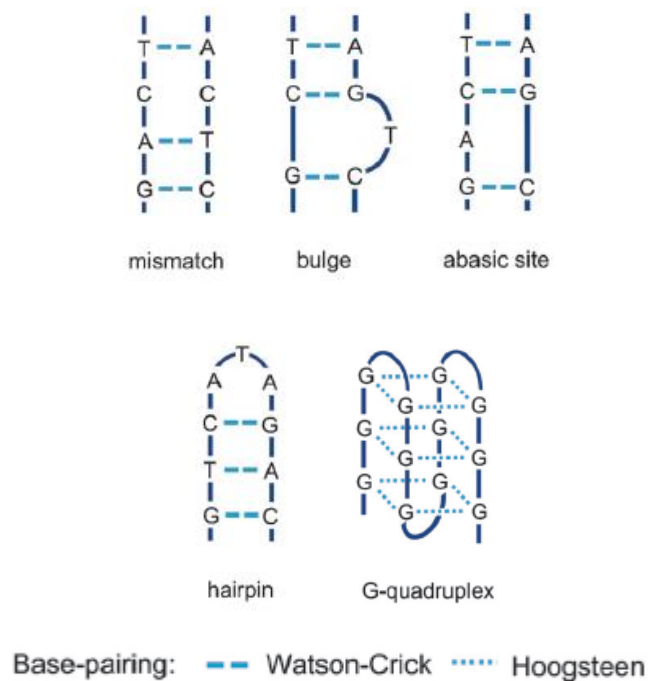


Figure 1.5. Schematic examples of non-canonical DNA structures.⁷

DNA base mismatches are DNA defects occurring when two non-complementary bases are aligned in the same base-pair step of DNA duplex.⁸ These occur in the cell as a result of errors during replication or exposure to genotoxic agents (energetic electromagnetic radiations, oxidizing or alkylating agents, ...).⁹ A mismatch is qualified as “transition” when formed by non-complementary purine-pyrimidine (GT or AC) bases, and “transversion” in the case of purine-purine (AA, GG or GA) or pyrimidine-pyrimidine (TT, CC or TC) bases. Depending on the nature of the mismatch, significant changes in the structure and stability of DNA at the mismatched site may occur (see selected examples on Figure 1.6).

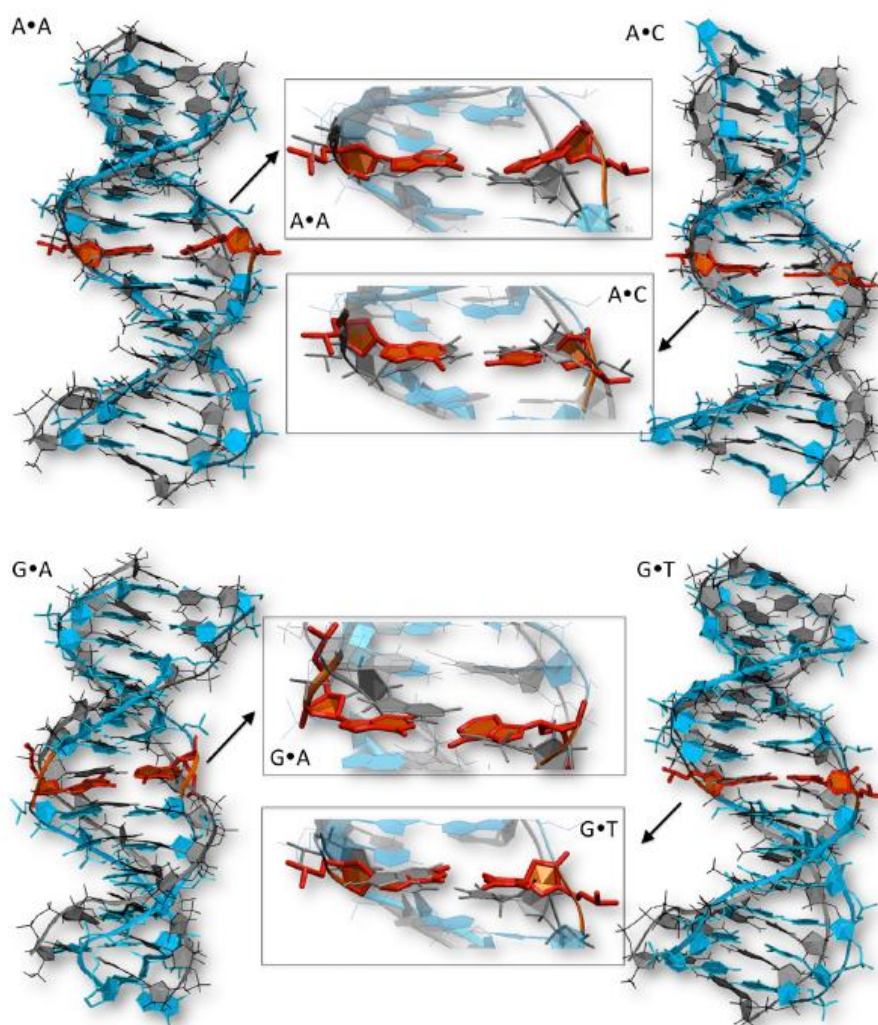


Figure 1.6. Superimposition of selected mismatches (A·A, A·C, G·A, G·T, T·T) structures over the canonical A:T oligomer. The oligomer with the MM is shown in blue ribbon, while the canonical structure is shown in grey. The mismatched site is highlighted in red.⁸

Among others, the structure of GT mismatches was extensively studied as they are regularly produced in living systems by spontaneous deamination or due to DNA replication errors. The deamination process is often mutagenic because the amino group in pyrimidine and purine bases, while serving as hydrogen donors for Watson-Crick base pairing, is replaced by carbonyl functions thus acting as hydrogen acceptors. Consequently, the GT mismatch

features two hydrogen bonds between the imino protons and carbonyl oxygen of guanine and thymine, respectively. While a little effect on the global helical structure or backbone conformation is observed in that case, the guanine is shifted towards the minor groove and thymine is rejected into the major groove with respect to the complementary bases. In contrast, AC mismatches are among the least stable ones. These occurs for example upon tautomerization of cytosine, leading to the possibility of pairing with an adenine moiety.

The thermodynamic stability of mismatched base pairs varies significantly, depending on the nature of the mismatched bases as well as their sequence context. In general, the following trend is observed with respect to the base pair stability: $CC \leq AC \leq TC < AA \leq TT < GA \leq GT < GG < AT < GC$.¹⁰ This sequence suggests on the one hand that guanine is the most promiscuous base, forming the strongest Watson-Crick base pair and the most stable mismatches. On the other hand, cytosine is the most discriminating base since it forms the strongest Watson-Crick base pair but the three weakest mismatches. The flanking pairs play an important role in mismatch stability, with GC base pairs generally leading to more stable mismatches than AT pairs.

Bulges arise in DNA from combination between two imperfectly complementary sequences, leading to a structure where a duplex section is distorted due to the presence of unmatched bases on one strand. These regions of the duplexes, then destabilized, are usually exploited for specific recognition with proteins.^{11,12}

Abasic sites, also called AP sites for apurinic/apyrimidinic sites, are places in DNA bearing neither a purine nor a pyrimidine base. These non-canonical structures, formed by spontaneous depurination or following DNA damage during the base excision repair process¹³ are daily estimated to 10,000 apurinic sites and 500 apyrimidinic sites in a cell under physiological conditions.¹⁴ It is estimated that each cell permanently contains between 50,000 and 200,000 abasic sites, depending on the nature of the tissues.^{15,16} If left uncorrected, abasic sites could lead to various issues such as mutagenesis.¹⁷

Hairpins are stem-loop structures in which intramolecular base pairings occur. These are for example found in single-stranded DNA. Hairpins structures are formed when a sequence can fold back on itself to form a paired double helix, ending in an unpaired loop.

G-quadruplexes (G4) are DNA secondary structures formed in guanine-rich sequences, consisting of square-planar arrangements of four guanine residues.¹⁸⁻²⁰ These are stabilized by Hoogsteen base pairing and monovalent cations, and can be intra- or intermolecular. G4 are mainly found in telomeres and promoter regions of the genome, especially in proto-oncogenes.^{21,22}

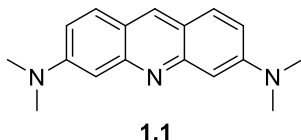
DNA being considered as “the library of the cell”²³ because it is simultaneously storing and dispensing the information required for ensuring life, Nature has developed several ways to maintain its integrity, like the mismatch repair machinery (MMR) to repair mismatches in the DNA sequence.^{24,25} However, the DNA repair mechanisms of the cells can be somehow damaged or disabled, increasing the likelihood of harmful transformations in the genome. Indeed, even if mutations serve evolution, they are also the causes of the triggering of genetic diseases in some cases. They can for example help a species to adapt to particular environment variations, but also lead to Huntington disease, disabling nervous system and producing shaking, depression and apathy. This may occur when a person inherits two copies of a mutated gene, leading to the production of an aberrant protein. Being composed of three billion base pairs, the average mutation rate of the human genome was estimated at around one mutation every 30 million bases, or in other words to 100-200 mutations per diploid genome per generation.²⁶ Due to their potential harmful effect on the genome integrity, non-canonical DNA structures represent important targets for molecular recognition and diagnostic applications.

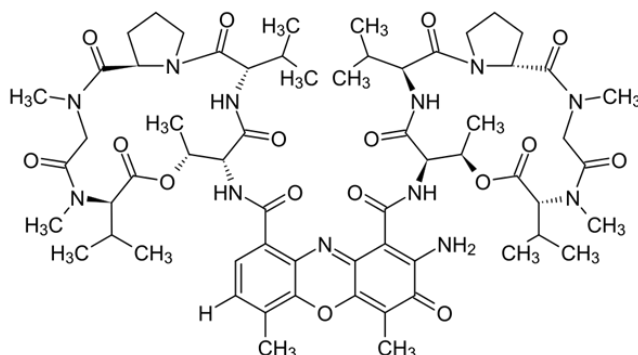
2. Targeting DNA: drug design infancy

2.1. The first discoveries: organic chemists on track

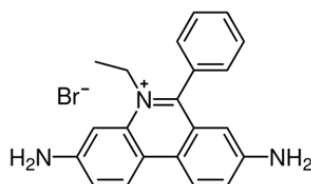
The design of DNA interacting compounds as drug candidate started more than fifty years ago, with the paper of L.S. Lerman dealing with the intercalation of compounds in the DNA double helix in 1961.²⁷ Dyes, typically aminoacridines derivatives, were used by histologists since late 1890's at the origin of chemotherapy to stain cells and tissues sections. Among them, acridine orange (**1.1**, Figure 1.7) displays evidence of defined fluorescence for nucleus and cytoplasm.^{28,29} For the first time, it was shown that particular dyes can interact in different ways with polynucleotides, paving the way for the concept of molecular recognition.³⁰ These observations, together with the proven antiseptic and antimalarial activity of aminoacridines,³¹ led to the connection between cell targeting and biological activity. Another acridine derivative, actinomycin D (**1.2**, Figure 1.7), had been extensively studied between the 1940's and the 1960's. It was the first antibiotic to exhibit strong activity towards cancerous cells, being recognized at that time as the most potent anticancer agent available. Actinomycin D is indeed able to inhibit the nucleic acid synthesis in cancerous cells through selective inhibition of DNA polymerase, the enzyme responsible of the transcription of the genetic material. The reversible interaction between actinomycin D and the enzyme is responsible for the specificity of the recognition.³²

In the late 1940's, acridine isomers such as phenanthridine derivatives displayed high biological activity. Among them, ethidium bromide (**1.3**, Figure 1.7) is the archetype compound. It was profusely used as an antibacterial and antiviral agent,^{33,34} consequently to the inhibition of nucleic acid synthesis through the binding of **1.3** to DNA, as for actinomycin D.





1.2



1.3

Figure 1.7. Structure of acridine orange (1.1), actinomycin D (1.2) and ethidium bromide (1.3).

The interaction between aminoacridines or phenanthridines and DNA was proposed to be intercalative, leading to the intercalation of the acridine moiety between the base pairs of the double helix.²⁷ This binding mode is stabilized by π -stacking between the DNA base pairs and the aromatic rings of the binder, which is thus favored by the presence of an extended fused aromatic structure and by the hydrophobic effect. The latter consists in the removal of water molecules from the solvation sphere of the binding site and the binder through its intercalation in DNA, leading to an increase of the global entropy of the system.³⁵ The intercalation binding mode can be evidenced by the increase of the viscosity of the binder-DNA solution³⁶ caused by a lengthening of the helix,³⁷ by ^1H NMR NOE experiments,³⁸ by X-ray diffraction³⁹ and atomic force microscopy.⁴⁰

2.2. Blended science: a tasty mix

For the last forty years, another class of DNA binders has been the focus of growing interest, which are transition metal complexes. Even if confined to coordination sites in proteins or cofactors in Nature, these compounds offer numerous advantages for their use as DNA interacting agents. First and foremost, transition metal complexes are modular systems in essence. The anchoring site is made from a metal cation, defining the coordination geometry of its ligands. The choice of the ligands and the metal allows the tuning of the three-dimensional properties of the complex, leading to variation of the binding and recognition properties with duplex DNA. Second, through the association of a metal cation and ligands, each possessing their own properties, new properties emerge from the resulting assembly. Typically, the photophysical and electrochemical behavior of the complex is dramatically altered.

In order to make suitable DNA binding agents, we have decided some important features are required:

- (1) the structure needs to be rigid to interact with DNA in a defined geometry;
- (2) the stability of the species is adequate in aqueous medium and into cells;
- (3) the compounds exhibit suitable photophysical and photochemical properties allowing their easy monitoring in the absence and in the presence of DNA.

In that context, transition metal complexes represent compounds of choice. Indeed, robust scaffolds may be obtained through the chelation of ligands on metal cations. Among the different possibilities, d^6 octahedral and d^8 square-planar complexes are particularly stable. These geometries imply electronic configurations where all the electrons are paired and where the addition or a removal of a single electron would have a destabilizing effect.⁴¹

2.2.1. Elements of structure and geometry

By far the most common, complexes displaying an octahedral geometry lie at the basis of the concepts of coordination chemistry. In that case, the metal center is coordinated to six coordination sites shared between several ligands, each of them possessing one (monodentate), two (bidentate), or more sites (Figure 1.8).

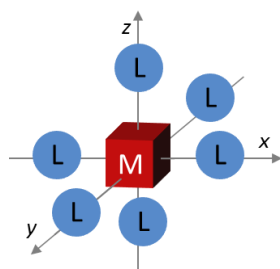


Figure 1.8. Schematic representation of an octahedral transition metal complex in a three dimensional axis system. M stands for metal and L for chelating site.

The chelation of the metal center by the ligands is achieved by two contributing components: a donation of electron density from the chelating sites to the metal, and a back-donation of electron density from the metal to the chelating sites. Such a process requires positive orbital overlap between the chelating sites and the metal. In the case of bidentate ligands, the octahedral geometry of these complexes provide helical chirality, leading to a mixture of two enantiomers Δ and Λ (Figure 1.9).⁴¹

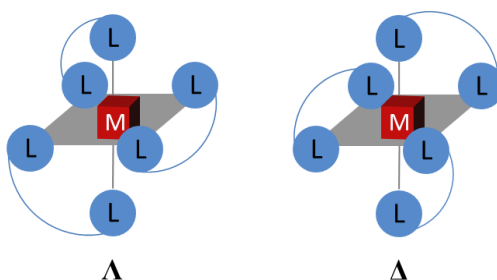


Figure 1.9. Schematic representation of Δ (left) and Λ (right) enantiomers for octahedral transition metal complexes bearing three bidentate ligands (depicted as L^2).

2.2.2. Binding to DNA – a matter of geometry and chemical properties

Octahedral and square-planar transition metal complexes featuring robust scaffolds, these compounds display suitable properties to interact with DNA. The helical structure of DNA offers several possibilities for binding of transition metal complexes, some being irreversible, and other reversible.

a) Irreversible binding

Irreversible bonds can be formed between complexes and phosphodiester backbone, sugar residues or nucleobases from DNA. Alkylating agents and some platinum complexes, which will be discussed further, belongs to this category. The induction of DNA damages through irreversible binding of a compound lies at the basis of chemotherapy (*vide infra*).

b) Reversible binding

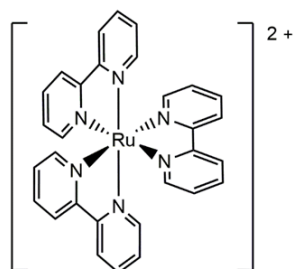
Irreversible binding to DNA is not a prerequisite to induce DNA dysfunction. Indeed, numerous examples have been described in the literature where a non-covalent binding between a transition metal complex and the DNA molecule leads to DNA damage.⁴² The use of complexes allows a fine tuning of the interaction through a control of the hydrophobicity, binding affinity, ... Three types of reversible interactions takes place (Figure 1.10):



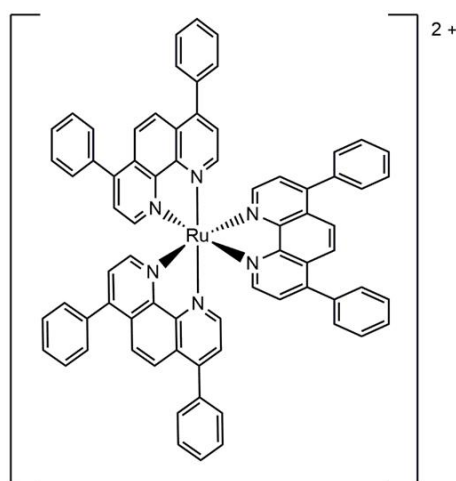
Figure 1.10. Schematic representation of reversible DNA interactions with transition metal complexes. From left to right: groove binding, intercalation and insertion.²³

(I) Groove binding

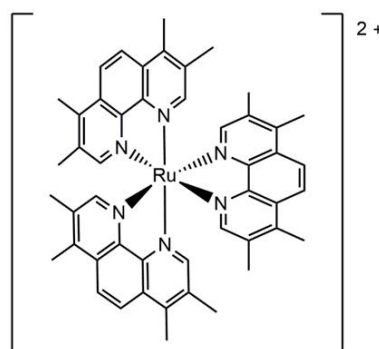
The adsorption of complexes into minor and major grooves of the double helix involves a combination of hydrogen bonds, van der Waals, hydrophobic and electrostatic interactions. Groove binders can exhibit strong sequence and chiral specificity due to their high covering of the base pairs and high binding affinity ($K_{\text{aff}} \sim 10^3$ - 10^5 L/mol). Some complexes displaying DNA groove binding are $[\text{Ru}(\text{bpy})_3]^{2+}$ (**1.4**, bpy = 2,2'-bipyridine), $[\text{Ru}(\text{dpphen})_3]^{2+}$ (**1.5**, dpphen = 4,7-diphenyl-1,10-phenanthroline),⁴³ **1.6**, $[\text{Ru}(\text{TMP})_3]^{2+}$ (TMP = 3,4,7,8-tetramethyl-1,10-phenanthroline)⁴⁴ and $[(\text{Ru}(\text{bpy})_3)_2(\text{tpphz})]^{4+}$ (**1.7**, tpphz = tetrapyrido[3,2-*a*:2',3'-*c*:3'',2''-*h*:2''',3'''-*j*]phenazine) (Figure 1.12).⁴⁵



1.4



1.5



1.6

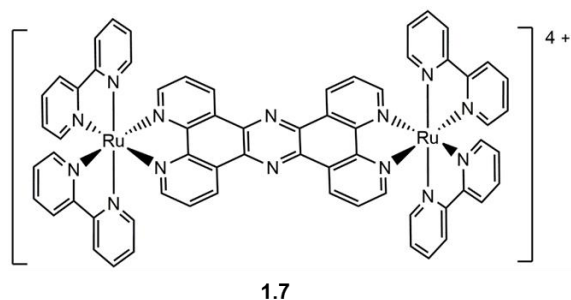


Figure 1.12. Structures of $[\text{Ru}(\text{bpy})_3]^{2+}$ (**1.4**), $[\text{Ru}(\text{dpphen})_3]^{2+}$ (**1.5**), $[\text{Ru}(\text{TMP})_3]^{2+}$ (**1.6**) and $[(\text{Ru}(\text{bpy})_2)_2(\text{tpphz})]^{4+}$ (**1.7**).

(II) Intercalation

Intercalating compounds are made of planar aromatic derivatives which find space between the stacking of adjacent base pairs. The binding mode involves a π -overlap between the nucleic acids and the binder, together with hydrogen bonds, Van der Waals, hydrophobic and electrostatic interactions. The intercalation of complexes into the DNA leads to a partial unwinding and lengthening of the double helix. By far the most studied type of interaction, numerous examples have been described in the literature. The archetypical intercalating complexes are $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ (**1.8**, dppz = dipyrido-[3,2- α :2',3'- c]-phenazine, Figure 1.13) and $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ (**1.9**, phen = 1,10-phenanthroline, Figure 1.13), which bind DNA with very high affinity ($K_{\text{aff}} \sim 10^6$ L/mol) and display intense luminescence at the same time.^{46,47}

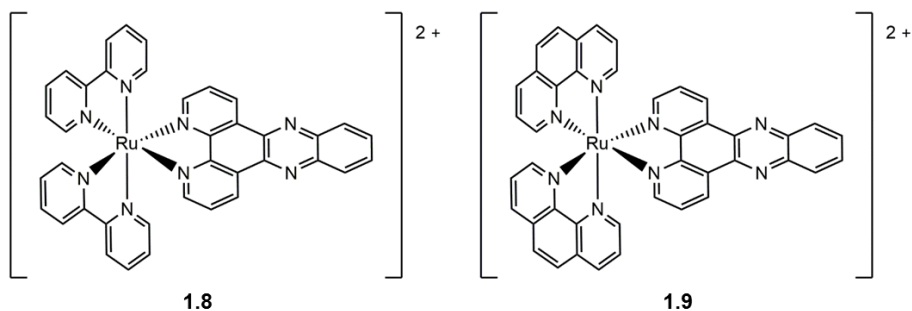


Figure 1.13. Structures of $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ (**1.8**) and $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ (**1.9**).

(III) Insertion

Inserting transition metal complexes, like intercalating ones, contain a planar aromatic ligand which is able to stack between adjacent base pairs. However, insertors eject the bases of a base-pair while their aromatic ligand takes place inside the double helix. This binding mode arises in the presence of DNA damages (i.e. DNA mismatches) and high binding affinities are often observed ($K_{\text{aff}} \sim 10^5$ - 10^6 L/mol).⁴⁸

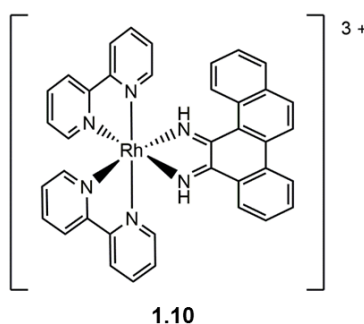


Figure 1.14. Structure of $[\text{Rh}(\text{bpy})_2\text{chrysi}]^{3+}$ (**1.10**, chrysi = 5,6-chrysenequinone diimine).

2.2.3. Attractive compounds for attractive applications

Transition metal complexes displaying robust scaffolds and being able to interact with DNA, the choice of the ligands and of the metal center will aim to allow easy monitoring of the properties of the resulting complexes. The modularity of the properties of these complexes makes them ideal biologically relevant structures. For many years, the development of new complexes for biomedical applications has attracted growing interest, and some allow the treatment of intractable pathologies until then.^{49,50} Possible biomedical applications of metal complexes include:

a) contrasting agents for Magnetic Resonance Imaging (MRI)

MRI is a non-invasive imaging technology that produces three dimensional detailed anatomical images without the use of damaging radiation.⁵¹ It is often used for disease detection, diagnosis and treatment monitoring. It is based on the detection of changes in the direction of the rotational axis of protons found in the water that makes up living tissues, after magnetic excitation. By using MRI, the patients may receive contrasting agents, often containing Gd^{3+} (**1.11**, Figure 1.15)⁵² or Fe^{3+} ions, in order to increase the speed at which protons realign with the magnetic field. The faster the protons realign, the brighter the image.

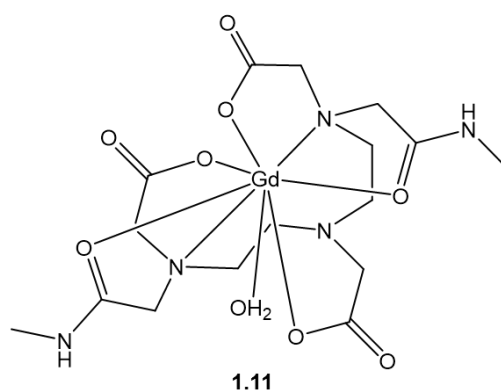


Figure 1.15. Structure of Gadodiamide MRI agent (**1.11**).

b) Chemotherapeutics

Chemotherapy is a cancer treatment that uses chemical compounds to kill cancer cells. It may be given to patients with a curative or palliative intent. Chemotherapeutic agents oxaliplatin (**1.12**, Figure 1.16) and satraplatin (**1.13**, Figure 1.16) are generally cytotoxic: they kill cells that divide rapidly, one of the main characteristic of cancer cells. New generations of chemotherapeutics target proteins that are abnormally expressed in cancer cells and that are essential for their growth, referring then to “targeted therapy”.^{53,54} Chemotherapy may be for example used in conjunction with surgery

and radiotherapy to treat cancer. Note that chemotherapy can also be used to treat other diseases, such as Crohn's disease⁵⁵ and psoriasis.⁵⁶

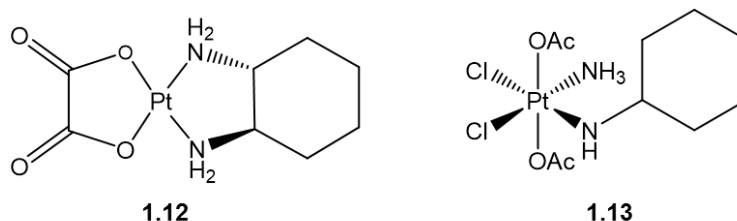


Figure 1.16. Structures of oxaliplatin (**1.12**) and satraplatin (**1.13**).

The use of chemotherapeutics features however some drawbacks. Side effects include damage to healthy cells that divide quickly, like cancer cells, leading i.e. to hair loss, nausea and mouth sores. To overcome these problems, the last decades have seen the emergence of chemotherapeutics agents that exhibit cytotoxic activity exclusively upon light exposure. This treatment is called photodynamic therapy (PDT).

c) photosensitizers for photodynamic therapy

PDT consists in the use of photosensitizers (PS) that induce the inhibition of tumor growth *via* the formation of prooxidant species.⁵⁷⁻⁵⁹ The first step is the generation of excited PS through light irradiation. The second step can arise according to two types of mechanism (Figure 1.17). The first one (type I photosensitization) involves direct electron transfer (that is to say, a redox reaction) between the excited PS and a surrounding molecule to produce ion radicals, the reaction of which with triplet O_2 (3O_2) leads to the formation of superoxide anion ($O_2^{\cdot-}$) and, consecutively, to hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\cdot}$). The second mechanism (type II photosensitization) refers to the direct energy transfer from the PS in the excited state to ground state 3O_2 to form singlet oxygen (1O_2). Both type I and type II processes can occur at the same time, but the second one is dominant for most PS.^{60,61}

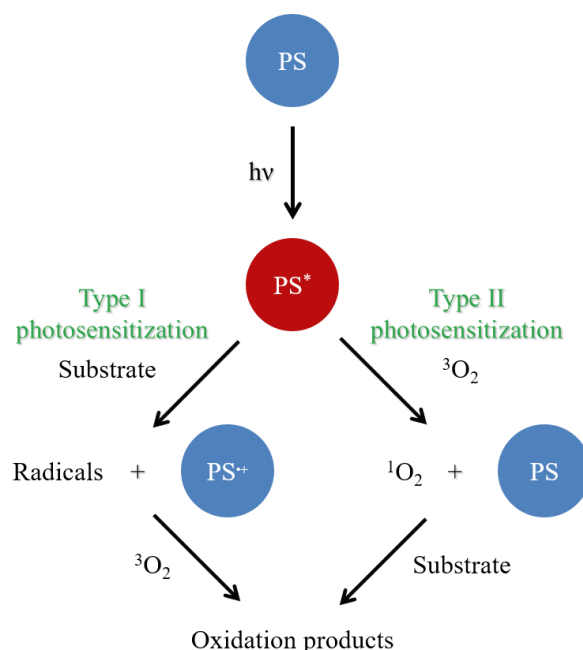


Figure 1.17. Schematic representation of the two types of mechanisms arising in PDT.

Many PS are based on porphyrin cores bearing transition metals because these compounds absorb near infrared radiations (NIR, from about 700 to 900 nm) and are able to lead to the formation of high levels of ¹O₂.^{62,63} NIR electromagnetic waves are attractive for biomedical applications due to their higher penetration into biological tissues with respect to higher energy wavelengths, allowing to deliver as much light energy as possible to the desired location.⁶⁴

The importance of DNA to cellular function is directing increasing research focus on transition metal complexes as potential PDT agents based on their excited state reactions with DNA. These efforts have given rise to unprecedented discoveries involving direct oxidation of DNA bases upon light irradiation.⁶⁵⁻⁶⁷ Such photo-induced processes will be discussed later.

2.2.4. DNA-binding transition metal complexes

a) The first discoveries

The development of DNA binding agents, and specially DNA defects, has been the subject of numerous studies for the last decades. The seminal work on interactions between DNA and metallic compounds dealt mainly with metal-aqua ions,⁶⁸ and complexes bearing open coordination sites.⁶⁹ The first metal complexes which display DNA-interaction appeared in the 1950's, particularly with the work of Dwyer featuring biologically active metal polypyridyl complexes towards DNA.⁷⁰ Among others, it has been shown Ru(II) and Ni(II)-based complexes bearing bidentate ligands present antiviral and bacteriostatic activities (**1.14-1.15**, Figure 1.18).

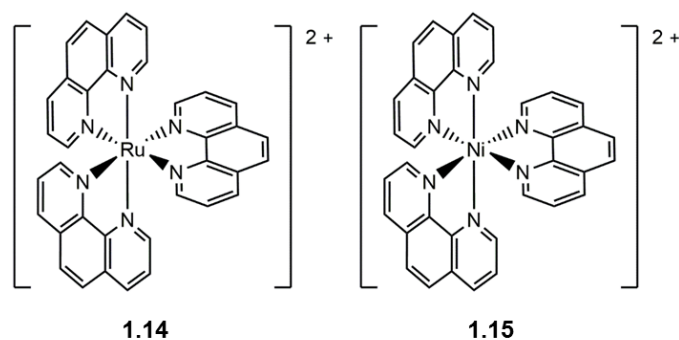


Figure 1.18. Structures of [Ru(phen)₃]²⁺ (**1.14**) and [Ni(phen)₃]²⁺ (**1.15**).

b) The promising platinum complexes

In the mid-1970's, Lippard observed non-covalent binding of Pt(II) complexes, [Pt(tpy)Cl]⁺ (**1.16**, tpy = 2,2';6',2''-terpyridine, Figure 1.19) and [Pt(tpy)(SCH₂CH₂OH)]⁺ (**1.17**, Figure 1.19), with nucleic acids.⁷¹ The intercalation of these complexes in the DNA double helix, evidenced by X-ray diffraction, arises every 10 Å, and leads to a partial unwinding of the double helix.^{72,73} Following the use of tpy as planar ligand, other Pt(II) complexes bearing bpy and phen have displayed intercalative DNA binding mode with high binding constants (in the range 10⁴-10⁵ M⁻¹).⁷⁴

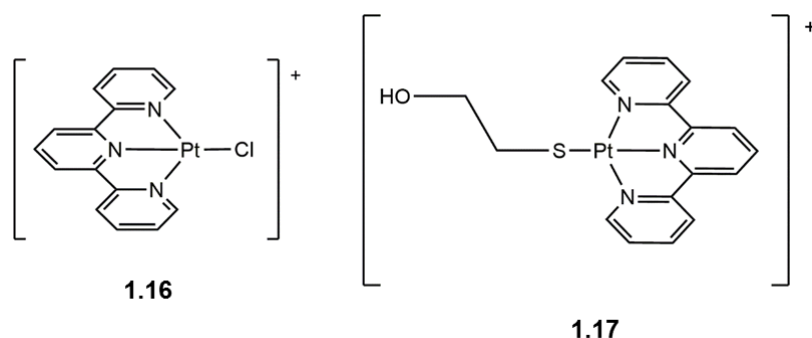


Figure 1.19. Structures of $[Pt(tpy)Cl]^+$ (**1.16**) and $[Pt(tpy)(SCH_2CH_2OH)]^+$ (**1.17**).

The most remarkable breakthrough in the search of biologically active transition metal complexes was definitely the discovery of *cis*-platin (= *cis*-diaminedichloroplatinum(II), **1.18**, Figure 1.20) by Rosenberg.⁷⁵ This Pt(II) complex has been one of the most widely used anticancer agent, which was then derivatized to optimize its biological properties, leading for example to the introduction of carboplatin (= *cis*-diamine(1,1-cyclobutanedicarboxylato)platinum(II))⁷⁶ (**1.19**, Figure 1.20) and oxaliplatin⁷⁷ (**1.12**, Figure 1.16). Once located inside the cell, *cis*-platin and its analogs are hydrolyzed to give mono- and bis-aqua intermediates which then bind irreversibly to DNA through chelation of the platinum to two successive purine residues (Figure 1.21). The coordination of the two nucleobases gives rise to the formation of inter- or intrastrand cross-linking, leading to a distortion of the double helix which prevent the replication and transcription processes (Figure 1.22).⁷⁸

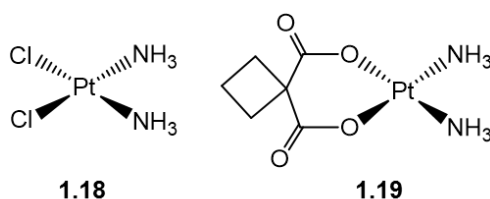


Figure 1.20. Structures of *cis*-platin (**1.18**) and carboplatin (**1.19**).

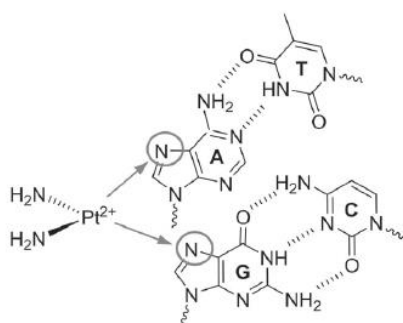


Figure 1.21. Binding of *cis*-platin to purine residues.⁷

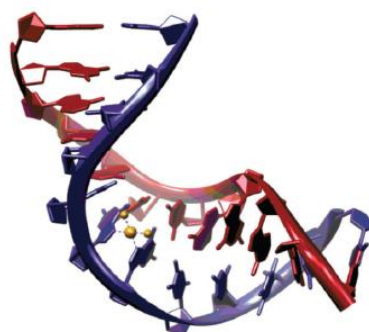


Figure 1.22. Schematic representation of the intrastrand crosslink between *cis*-platin and the N7 atoms of two adjacent guanines. Platinum atom and ammonia molecules are depicted in yellow.⁷

Despite its outstanding properties, the use of *cis*-platin lead to substantial secondary effects. The major one is its inability to target cancerous cells vs healthy ones. Other secondary effects include protein activity troubles, such as proteins overexpression, and cell resistance.⁷⁹ Resistance mechanisms that limit the extent of DNA damages by *cis*-platin include reduced drug uptake, increased drug inactivation, and increased DNA adduct repair.⁸⁰ These arise as a consequence of intracellular changes that either prevent *cis*-platin from interacting with DNA, interfere with DNA damage signals from activating the apoptotic machinery, or both.⁸⁰ While several resistance mechanisms usually take place at the same time, let's cite for example

the inactivation of *cis*-platin by sulfur-containing compounds, such as glutathione.⁸¹ *Cis*-platin derivatives are still used nowadays to treat among others brain⁸² and lung cancer.⁸³

c) The advent of ruthenium complexes

The interesting biological activity of *cis*-platin on one hand, and its limitations on the other hand have paved the way for the development of new compounds displaying improved therapeutic properties, and more generally biological activity. Another approach that appeared to reach these objectives is the use of other transition metals. In this context, the emergence in the late 1970's of Ru(II) and Ru(III) complexes proved to be a breakthrough.^{84,85} The first which was biologically relevant is *cis*-[Ru(DMSO)₄Cl₂] (**1.20**, Figure 1.23). This complex displays similar antitumoral activity than *cis*-platin towards several types of tumors, but leads to considerably less damages to intestinal tissues⁸⁶ mainly due to its higher water solubility.^{87,88}

Further development of these complexes to increase their biological activity involved Ru(III) complexes bearing heteroaromatic ligands. Notably, *trans*-[RuCl₄(Ind)₂]IndH (Ind = *1H*-indazole, **1.21**, Figure 1.23) and *trans*-[RuCl₄(DMSO)(Im)]ImH (Im = *1H*-imidazole, **1.22**, Figure 1.23) exhibit enhanced antitumor activity.^{69,89} Possible reasons for this are (1) the decrease in the rate of chloride aquation due the π -acceptor effect of the aromatic ligand, increasing the effective charge on the metal center, (2) increased hydrophobic or intercalative interactions with DNA, which may facilitate covalent binding, (3) geometric effects exerted by the ligands, which may facilitate (or inhibit) protein binding to the nucleic acid.⁹⁰ These Ru(III) complexes are reduced to Ru(II) *in vivo* and display important antimetastatic activity.⁹¹ The active species being Ru(II) derivatives, many research groups then focused on the synthesis and the study of Ru(II) complexes.

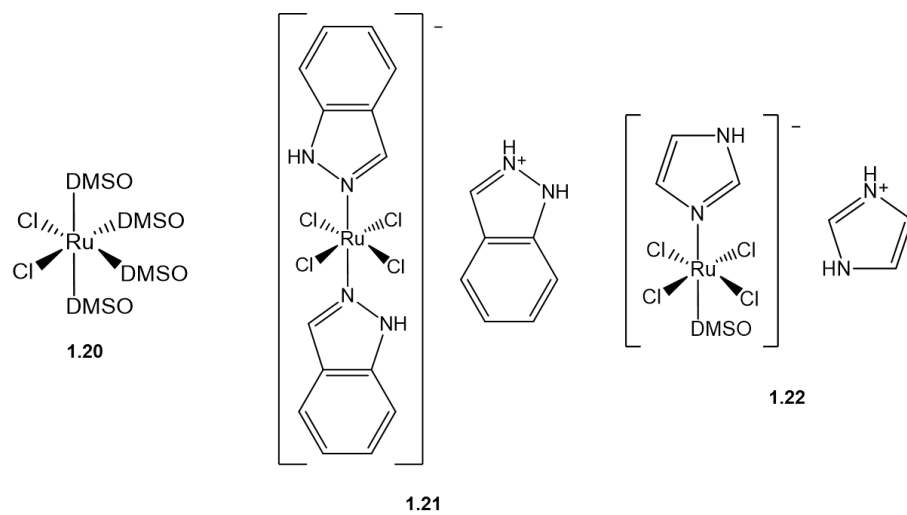


Figure 1.23. Structures of $\text{cis-}[\text{Ru}(\text{DMSO})_4\text{Cl}_2]$ (**1.20**), $\text{trans-}[\text{RuCl}_4(\text{Ind})_2]\text{IndH}$ (**1.21**) and $[\text{RuCl}_4(\text{DMSO})(\text{Im})]\text{ImH}$ (**1.22**).

3. Ru(II) complexes – Photophysics and photochemistry

The understanding of the outstanding properties of the Ru(II) complexes required some theoretical considerations. As this manuscript will only involve polypyridyl octahedral transition metal complexes, we will limit our discussion on the fundamentals of photophysics and photochemistry to this class of compounds in the next paragraphs.

3.1. Geometry and orbitals

As mentioned earlier, transition metal complexes are supramolecules and display, as a consequence of the association between the metal cation and the ligands, other properties than the ones of the different partners involved. The metal is coordinated to several ligands, possessing one (monodentate), two (bidentate), or more chelating sites. The octahedral geometry of these complexes provides helical chirality, leading to a mixture of two enantiomers Δ and Λ (Figure 1.9).

Polypyridyl octahedral transition metal complexes are d^6 systems for which the complexation of the cation by three polypyridinic bidentate ligands involve a coordinative part and a retro-coordinative part. The coordinative part implies the σ -donation of six non-bonding lone pairs from the nitrogen atoms of the ligands to the empty orbitals of the metal, whereas the retro-coordinative part concerns the partial delocalization of electrons from the metal center to the empty π^* orbitals of the polypyridyl ligands.⁴¹ The representation of the linear combination of the molecular orbitals for this type of complex leads to a simplified diagram (Figure 1.24). The octahedral field generated by the chelating ligands splits the contribution of the d atomic orbitals from the metal into bonding $d\pi$ and anti-bonding $d\sigma^*$ molecular orbitals, whereas the orbitals from the ligands, *i.e.* σ , π and π^* molecular orbitals, are globally stabilized. The Highest Occupied Molecular Orbital (HOMO) is located on the metal-centered $d\pi$ molecular orbital, and the Lowest Unoccupied Molecular Orbital (LUMO) on the ligand-centered π^* molecular orbital. These two

electronic levels lies at the basis of the photophysical and photochemical properties of the complex.

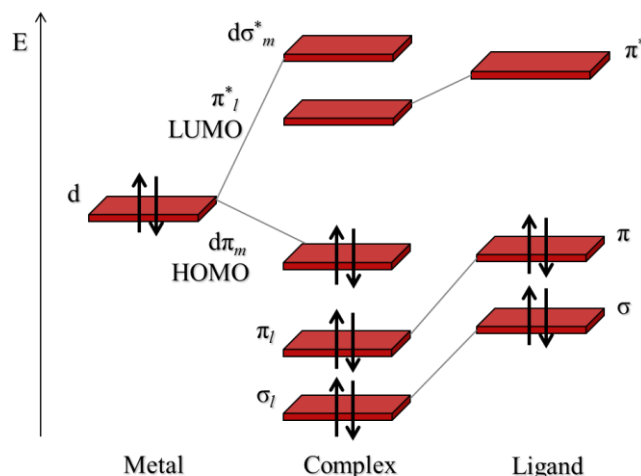


Figure 1.24. Molecular orbital diagram for polypyridyl octahedral transition metal complexes. The combination of the atomic orbitals of the metal with the molecular orbitals of the ligand lead to the formation of molecular orbitals for the complex. d , σ , π , $d\pi$, π^* and σ^* stands for the nature of the orbitals involved; m and l subscripts depict the major contribution to the orbital, m standing for metal and l for ligand.

3.2. Absorption of light

3.2.1. Basic principles

Polypyridyl transition metal complexes bearing chromophoric ligands, these species are able to absorb light irradiation. When such compounds are exposed to light having an energy that matches a possible electronic transition, some of the light energy will be absorbed as the electron is promoted to a higher energy orbital. In other words, the absorption of a photon leads to the promotion of an electron from a fully occupied orbital to an unoccupied orbital, going from a ground state to an excited state. While the exact energy differences between the orbitals depends on the atoms involved and the nature of the molecular orbitals, most of the electronic transitions from σ -bonding orbitals are too high in energy to be monitored by UV-visible light absorption.

The spin multiplicity of the states proves to be essential to electronic transitions. In the ground state, the HOMO is occupied by two electrons with opposite spins (paired electrons). The spin multiplicity equals to unity and the state is called *singlet* (S_0). Upon light irradiation, a promotion from one of these two paired electrons to an upper energy electronic level may occur. The spin of the promoted electron remains in principle unchanged so that the spin multiplicity of the generated excited state still equals unity. Consequently, both ground and excited states are singlet states. Depending on the excited state involved, the multiplicity will be noted S_1 , S_2 , S_3 , ... (Figure 1.25). The corresponding transition is termed singlet-singlet transition.

Upon population of S_n ($n = 1, 2, 3, \dots$) excited states, vibrational relaxation and internal conversion of the excited state occur. The first involves the return from vibrational excited states to the vibrational ground state, and the latter is the relaxation between electronic states of same multiplicity, *i.e.* for the relaxation of excited states S_2 , S_3 , ... to S_1 . As a result, the emission of a molecule exclusively arises from the lowest excited states to their ground states (Kasha's rule).⁹²

The energies of the orbitals involved in electronic transitions having fixed values, it would be expected that absorption peaks in UV-visible spectroscopy are depicted by sharp peaks. However this is rarely the case. Instead, broad absorption bands are commonly observed. This is because of the presence of vibrational and rotational energy levels which are available at each electronic energy level. The electronic transitions can occur to and from the different vibrational levels (Figure 1.25), resulting in peak broadening.

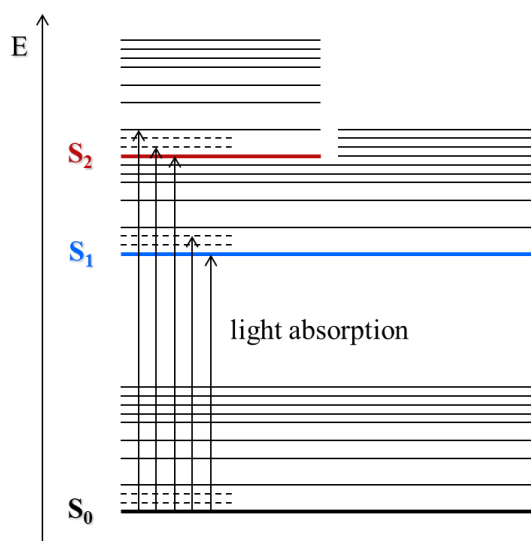


Figure 1.25. Diagram of possible electronic transitions upon absorption of light. Full lines stands for vibrational levels, and dashed lines for rotational levels.

3.2.2. Possible electronic transitions

The absorption of UV-visible light by transition metal complexes can lead to various electronic transitions.⁹³ Among them (Figure 1.26, L = ligand and M = metal):

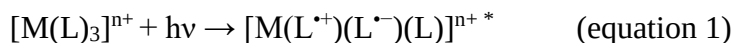
a) Ligand Centered transition (LC)

LC transitions involve electronic promotion from the bonding π_l orbital to the antibonding π_l^* orbital, both being located on the same ligand. These transitions require generally near-UV radiation and their molar extinction coefficients are at the magnitude of 10^4 - 10^5 L/(mol.cm). The LC bands being practically not sensitive to the complexation of the ligand to the metal, they are found in the UV spectrum of the free ligand nearly at the same wavelength and intensity.

b) Ligand-to-Ligand Charge transfer (LLCT)

These electronic transitions implies the promotion of an electron from a bonding π_l orbital of a particular ligand to an antibonding π_l^* orbital of another ligand (equation 1). Such transition

leads to the formation of a dipole in the excited state. LLCT transitions are observed in the visible part of the electromagnetic spectrum. Their molar extinction coefficients are about 10^4 L/(mol.cm).

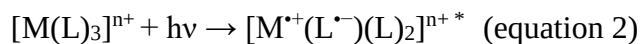


c) Metal Centered transition (MC)

MC transitions concern the excitation of a $d\pi_m$ electron to the do_m^* orbital. The wavelength of such transitions lies in the UV-visible range. These bands are difficult to observe because of their low molar extinction coefficients, typically around 100 L/(mol.cm).

d) Metal-to-Ligand Charge transfer transitions (MLCT)

MLCT bands implies the transition of an electron located on the $d\pi_m$ orbital of the metal to the antibonding π_l^* orbital of the ligand (HOMO $\rightarrow$ LUMO transition). Visible light irradiation leads to their observation and their molar extinction coefficients are usually at the magnitude 10^4 L/(mol.cm). MLCT transitions can be seen as intramolecular redox processes (equation 2). For that reason, a modification of the polarity of the complex arises following MLCT transition:



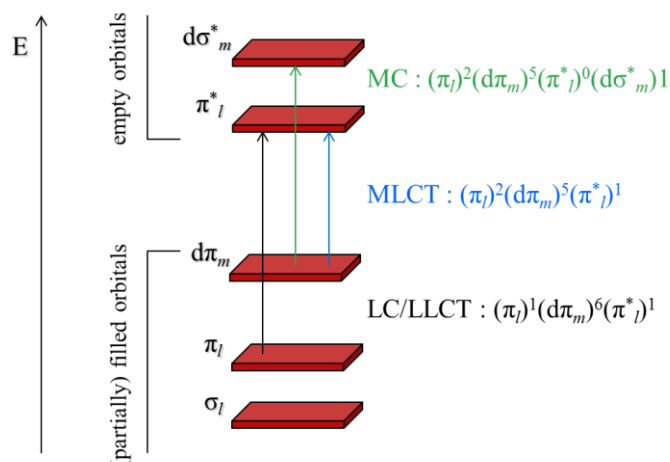


Figure 1.26. Schematic representation of the different electronic transitions arising upon light activation for polypyridyl octahedral transition metal complexes. σ , π , $d\pi$, π^* and σ^* stands for the nature of the orbitals involved; m and l subscripts depict the major contribution to the orbital, m standing for metal and l for ligand; LC = ligand centered transition, LLCT, Ligand-to-Ligand Charge transfer, MLCT = Metal-to-Ligand Charge transfer transition, MC = metal centered transition.

3.2.3. The Beer-Lambert Law

The Beer-Lambert law indicates absorption is proportional to the concentration of absorbing molecules and that the fraction of radiation absorbed is independent of the intensity of the radiation. The Beer-Lambert Law is thus defined as:

$$A(\lambda) = \log \frac{I_{\lambda}^0}{I_{\lambda}} = \varepsilon(\lambda) d C \quad (\text{equation 1})$$

where $A(\lambda)$ is the absorbance, I_{λ}^0 and I_{λ} are the light intensities of the incident and transmitted beams, respectively, $\varepsilon(\lambda)$ is the molar extinction coefficient (L/(mol.cm)), d is the absorption path length (cm) and C is the concentration of the compound in the sample (mol/L). The molar extinction coefficient ε is independent of the concentration and the path length, whereas absorption depends upon both. The other main parameter is the wavelength at which maximum absorption occurs, λ .

3.2.4. Selection rules

The absorption of light by chromophores may lead to various electronic transitions, showing the wealth of photophysics. Nevertheless, not all electronic transitions give rise to absorption bands. Two major selection rules govern electronic transitions:

a) Spin selection rule

Following absorption of light, singlet excited states may undergo conversion to another excited state where the two electrons bears the same spin multiplicity (Figure 1.27). Such a state, of multiplicity of 3, is called a *triplet* excited state. This conversion will be discussed later. According to Hund's Rule, the triplet excited state has a lower energy than that of the corresponding singlet state.

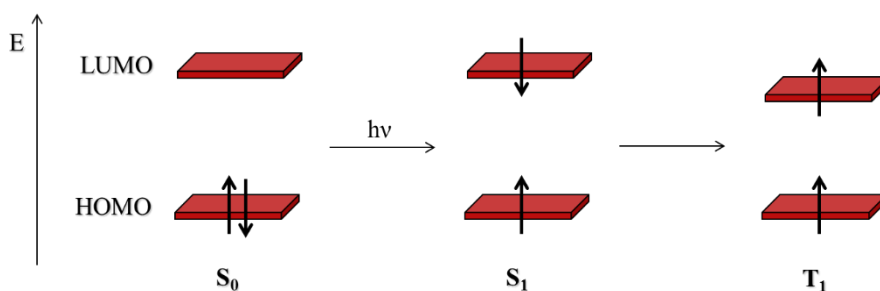


Figure 1.27. Upon absorption of light, the singlet ground state leads to the formation of a singlet excited state, which converts into a triplet excited state.

The spin selection rule states electronic transitions between states having the same spin multiplicity are allowed, whereas those between states of different multiplicity are forbidden. Therefore, the electronic absorption bands observed in the UV–visible spectrum of a molecule would display bands corresponding to $S_0 \rightarrow S_n$ or $T_0 \rightarrow T_n$ ($n = 1, 2, 3, \dots$) transitions.⁹⁴

b) Orbital symmetry selection rule (Laporte rule)

For metal complexes, which usually are highly symmetric species, symmetry selection rules can also play a role in determining the intensity of the absorption bands. Electronic transitions are indeed forbidden between orbitals of the same subshell, meaning a change of

polarity is required ($\Delta l = \pm 1$ where l is the orbital quantum number). Detailed considerations of the Laporte rule are beyond the scope of this introduction but note in some cases, forbidden electronic transitions may occur, notably if a heavy atom such as a transition metal is present. Three factors allow to circumvent the rule:⁹⁴

- the spin-orbit coupling which causes shifts in electronic energy levels due to electromagnetic interaction between the spin of the electron and the magnetic field generated by the orbit of the electron around the nucleus;

- the vibronic coupling: an octahedral complex may have vibrations causing the loss of the symmetry, making non-allowed transitions possible;

- orbitals from π -acceptor and π -donor ligands can mix with the d orbitals of the metal so transitions are no longer purely from the same subshell.

3.2.5. Deactivation of excited complexes

Following excitation through absorption of light, polypyridyl octahedral transition metal complexes can go back to their ground state according to several pathways (Figure 1.28). In that case, ultrafast dynamic processes occur, associated with a temporal evolution of the Franck-Condon excited state to the lowest-lying excited state. Due to the presence of a transition metal cation, fast intersystem crossing takes place, leading to $S_1 \rightarrow T_1$ electronic transition. Relaxation of the excited state of such species arises then from the T_1 state.^{95,96}

a) radiative deactivation

This pathway gives rise to luminescence of the complex in the visible wavelengths. The luminescence originates from the relaxation of the lowest lying excited states to the ground state. In that particular case, it is characterized by a radiative deactivation rate constant k_r close to 10^5 s^{-1} and by a quantum yield of about 1-10 %. This phenomenon is generally a minor contributor to the deactivation processes which take place.

b) non-radiative deactivation

Vibrational relaxation as well as internal conversion imply at some point the transfer of some energy to the surrounding medium. These processes are characterized by a non-radiative deactivation rate constant k_{nr} and usually determines the excited state lifetime of the complex.

Another non-radiative deactivation mechanism involves the shift of the population from the $^3\text{MLCT}$ to the ^3MC excited state. The latter corresponds to a distorted conformation along the nuclear coordinate's axis. This shift happens through the gain of thermal energy and generally leads to the photo-dechelation of one of the ligands, accompanied by non-radiative relaxation.

c) electron transfer

Photo-induced electron transfer (PIET) may occur when the lowest excited state of the complex lives long enough to encounter other molecules and it displays suitable properties to act as electron donor or acceptor (only its role as electron donor is depicted on Figure 1.28). The electrochemical properties of this class of compounds will be discussed later in this chapter.

d) energy transfer

The deactivation of the excited state of the complex can also happens through energy transfer to another molecule, for example $^3\text{O}_2$, which role has already been discussed earlier in this manuscript for the use of metal complexes as photosensitizers for PDT.

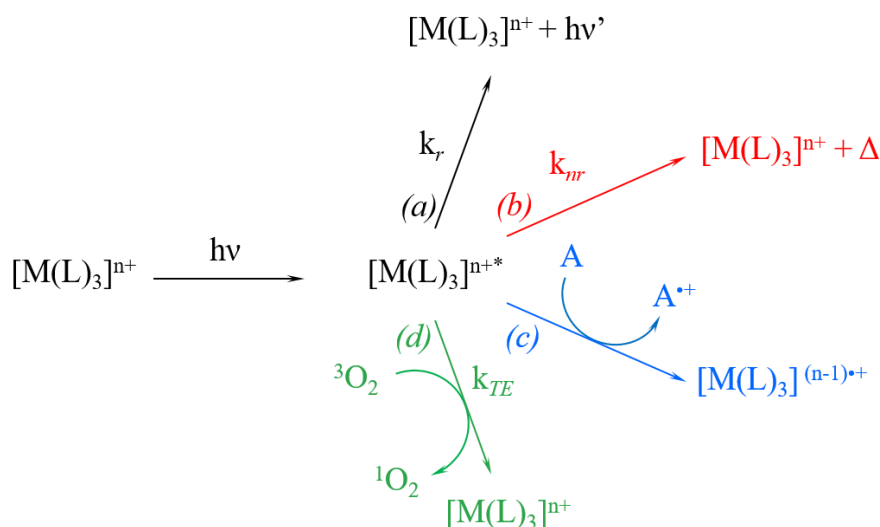


Figure 1.28. Possible deactivation pathways of an excited transition metal complex: (a) radiative deactivation, (b) non-radiative deactivation, (c) electron transfer and (d) energy transfer. M = metal center and L = bidentate chelating ligand.

3.2.6. Lifetimes and quantum yield

a) Excited state lifetimes

As mentioned in previous sections, S_1 and T_1 excited states are those involved in radiative deactivation processes. Notably, the excited state lifetime, τ , is among the most important parameter related to excited states. Its value depends on the different rate constants associated with processes leading to the disappearance of excited molecules. Upon light irradiation, the disappearance rate of an excited compound A^* can be written as:

$$-\frac{d[A^*]}{dt} = (k_r + k_{nr})[A^*] \quad (\text{equation 2})$$

where k_r is the radiative rate constant, and k_{nr} the sum of all the non-radiative rate constants. Integration of equation 2 in function of time yields:

$$[A^*]_t = [A^*]_0 e^{-(k_r + k_{nr})t} = [A^*]_0 e^{\left(-\frac{t}{\tau}\right)} \quad (\text{equation 3})$$

with $\tau = \frac{1}{k_r + k_{nr}}$ and is the excited state lifetime.

It is worthwhile mentioning the excited state lifetime can be measured experimentally. The time-dependent luminescence intensity I_t is directly connected to the population of the luminescent excited state:

$$I_t = k_r [A^*]_t = k_r [A^*]_0 e\left(\frac{-t}{\tau}\right) \quad (\text{equation 4})$$

The excited state lifetime is one of the most important parameter of a luminescent compound as it defines the time scale when observation of dynamic processes can be possible. No accurate information on processes occurring at time-scales shorter than about $\tau/100$ or longer than about 10τ can be obtained, whereas at intermediate times, the time evolution of these processes can be monitored. For organic molecules, singlet and triplet excited state lifetimes are respectively from 10 ps to 100 ns, and from 1 μ s to 1 s.

b) Quantum yield

By definition, the luminescence quantum yield, Φ , is the percentage of excited molecules that emits photons when returning to the ground state S_0 , or in other words, the ratio of the number of emitted photons to the number of absorbed photons:

$$\Phi = \frac{k_r}{k_r + k_{nr}} = k_r \tau \quad (\text{equation 5})$$

3.2.7. Case of $[Ru(bpy)_3]^{2+}$

The photophysical and photochemical properties of transition metal complexes depend on the relative energies of its excited states and, particularly, to the nature of their lowest excited state. The excited states energy diagram relies on the nature of the ligands and of the metal cation. Judicious selection of ligands and of the metal cation thus allows the tuning of the photophysical and photochemical properties of the complex as a whole.

Among the plethora of Ru(II) complexes that have been described in the literature, $[Ru(bpy)_3]^{2+}$ (**1.4**, Figure 1.11) is considered as a reference for photophysical considerations. The study of its properties have led to a substantial amount of publications,⁹⁷⁻⁹⁹ and paved the way for the photophysical characterization of the Ru(II) complexes that will be discussed hereafter. Indeed, most polypyridyl Ru(II)

complexes obey to a simple photophysical scheme, similar to the one which have been established for $[\text{Ru}(\text{bpy})_3]^{2+}$ (Figure 1.29).^{93,100} The absorption of a photon leads to the formation of $^1\text{MLCT}$ excited states, which then deactivates rapidly in a set of $^3\text{MLCT}$ states, the lowest excited states in energy.¹⁰¹ This happens through an inter system crossing (ISC) in a fast time scale (approximately 300 femtoseconds)⁹⁵ with a quantum yield close to unity in the case of $[\text{Ru}(\text{bpy})_3]^{2+}$.¹⁰²

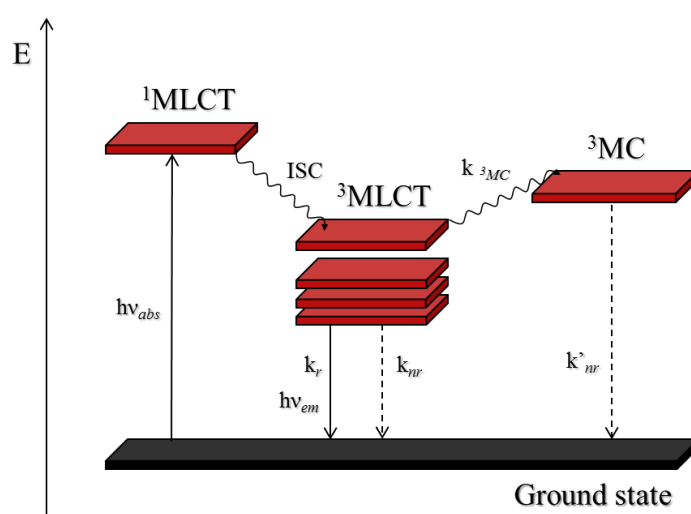


Figure 1.29. Representation of the photophysical scheme for polypyridyl $\text{Ru}(\text{II})$ complexes bearing bidentate chelating ligands, such as $[\text{Ru}(\text{bpy})_3]^{2+}$ (**1.4**).

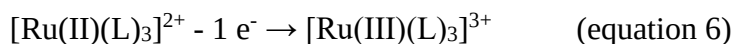
3.2.8. Redox properties

The deactivation pathway involving electron transfer from or to the metal complexes relies on their redox properties. These need to be distinguished in the excited state from the ground state:

a) redox properties in the ground state

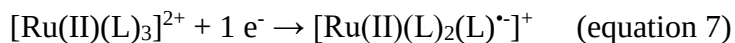
(I) Oxidation process

The oxidation of most polypyridyl Ru(II) complexes, for which an MLCT excited state is formed, corresponds to the loss of a $d\pi_m$ electron, the HOMO of the system.¹⁰² As discussed earlier, this orbital is located on the metal cation and the oxidation of these complexes refers then to the Ru(II)/Ru(III) redox couple (equation 6, L stands for bidentate chelating ligand). The oxidation potential depends on the nature of the chelating ligands: a strong π -acceptor ligand decreases the energy of the $d\pi_m$ orbital and thus increases the oxidation potential of the complex.



(II) Reduction process

The reduction process involves the gain of one electron on the LUMO of the system, that is a π^*_l orbital for polypyridyl Ru(II) complexes (equation 7). This being localized on one of the ligands, the addition of one electron will take place on the most electron acceptor ligand if they are different. Consequently to the presence of three bidentate ligands, several reduction reactions happen consecutively.



b) redox properties in the excited state

Polypyridyl Ru(II) complexes display stronger oxidizing and reducing power in the excited state. This behavior has been ascribed to the nature of the orbitals involved in their redox processes (Figure 1.30).¹⁰² Indeed, upon light irradiation, one electron is

promoted from the HOMO ($d\pi_m$) to the LUMO (π_l^*) of the system. Such change in the electronic configuration leads to enhanced oxidative power by accepting one electron from an external donor into the $d\pi_m$ orbital instead of the π_l^* in the ground state. Analogously, enhanced reductive power is observed with respect to the ground state by the removal of the promoted electron located on the π_l^* orbital.

The excited state oxidation and reduction potentials can be estimated from the ground state potentials and the 0-0 transition energy (E_{0-0} , equations 8 and 9).¹⁰³ E_{0-0} corresponds to the energy difference between the ground singlet state and the lowest singlet excited state and is generally estimated by the emission energy ($\Delta E_{\text{max emission}}$) of the complexes.⁹⁷ The $e^2/\epsilon d$ term corresponds to a coulombic correction due to attraction between the radical species obtained upon electron transfer. This term is often neglected because of the relatively high dielectric constant (ϵ) from the solvent. The $\Delta E_{\text{max emission}}$ values are lower limits since the luminescence energy contains a contribution from vibrational distortion between ground and excited states. The ground and excited state potentials are displayed on Figure 1.20 for $[\text{Ru}(\text{bpy})_3]^{2+}$.

$$E_{\text{ox}}^* = E_{\text{ox}} - \Delta E_{0-0} - e^2/\epsilon d \approx E_{\text{ox}} - \Delta E_{\text{max emission}} - e^2/\epsilon d \quad (\text{equation 8})$$

$$E_{\text{red}}^* = E_{\text{red}} + \Delta E_{0-0} - e^2/\epsilon d \approx E_{\text{red}} + \Delta E_{\text{max emission}} - e^2/\epsilon d \quad (\text{equation 9})$$

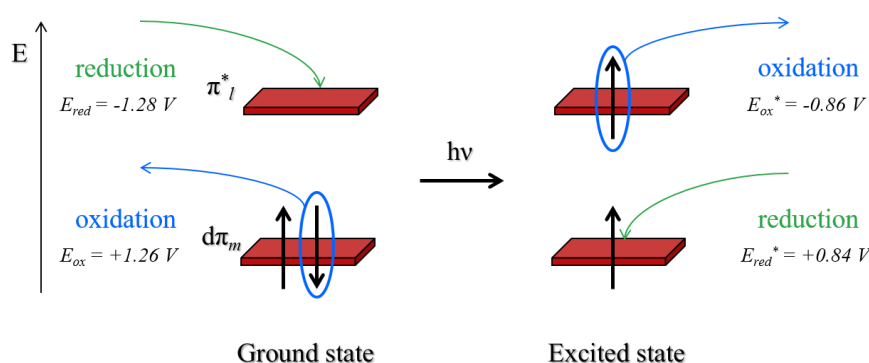


Figure 1.30. Schematic representation of enhanced redox properties upon light irradiation. Values are given for $[\text{Ru}(\text{bpy})_3]^{2+}$ in acetonitrile. All potentials are given in V/ECS.

Chapter 2

Objectives of the Ph.D. thesis

1. Objectives and strategies

DNA mutations are needs for evolution. Yet, in some cases, they are also responsible for triggering harmful transformations. To prevent these mutations, DNA has developed its own repair machinery. However, some mutations can escape this control process and ultimately be at the origin of cancers. The design of dyes able to probe DNA and, in addition, specifically detect mutations and defects represents therefore a high interest for diagnostic and therapeutic applications. In this context, the development of environment-sensitive compounds is attractive. Consequently, it is first required to design compounds which are able to interact with DNA and whose properties can be easily and quickly monitored. Secondly, the compounds need to be able to selectively probe DNA defects with respect to correctly matched base pairs.

As described in the introduction, acridine derivatives have been widely used as DNA photoprobes since the late 19th century. These dyes have been used by histologists to stain cells and tissues sections, lying at the origin of chemotherapy. In the middle of the 20th century, a new class of DNA photoprobes emerged, *i.e.* transition metal complexes. These compounds offer numerous advantages with respect to their purely organic predecessors, such as the modularity of their structural, photophysical and photochemical properties. Among them, polypyridyl Ru(II) complexes became widely used, in particular thanks to the description of the “light switch” effect observed for the $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ (dppz = dipyrindo[3,2-*a*:2',3'-*c*]phenazine, Figure 2.1). This complex can be used as a photoprobe for DNA, since, while the complex is not emissive in water, its luminescence is turned on when interacting with DNA. Extensive studies have been achieved for the development of metal complexes able to selectively recognize mismatched sites in DNA. Even if some phenazine based complexes were shown to discriminate complementary and mismatched hairpin sequences,^{7,104-106} their acridine counterparts have surprisingly received much less attention. As both nitrogen of dppz-based Ru(II) complexes account for the photochemical properties observed for the species alone and in the presence of DNA, the removal of one of them

is expected to lead to dramatic changes in the photophysics of the resulting complexes (Figure 2.1). Indeed, previous studies on acridine-based ligands and the corresponding complexes showed that the acridine core induces notable modifications on the behaviour of the resulting complex as compared to the phenazine-based equivalent.¹⁰⁷⁻¹⁰⁹

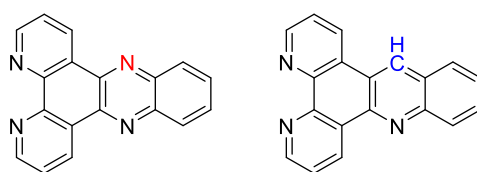


Figure 2.1. Structures of dppz (= dipyrido[3,2-*a*:2',3'-*c*]phenazine) (left) and its acridine analog, dpac (= dipyrido[3,2-*a*:2',3'-*c*]acridine) (right).

In this context, the main goal of this Ph.D. thesis is to shed some light on the photochemical tuning allowed by the modification of (I) the core and (II) the shape of the extended ligand, (III) the nature of the ancillary ligands, and (IV) by changing the nature of the metal cation for the design of DNA photoprobes.

In order to get Ru(II) complexes targeting DNA, associated with a defined binding mode as well as a good affinity for DNA, we designed firstly a linear extended planar aromatic ligand based on an acridine core and its subsequent complexation on a Ru(II) metallic center (Figure 2.2, $R_1 = R_2 = H$). The study of the optical behavior of this complex alone in solution allowed us to establish a photophysical model. Once its characterization has been achieved, studies of the interaction between the complex and well- or mismatched DNA were performed.

Secondly, fluorine analogs of the dpac ligand have been prepared, together with their corresponding Ru(II) complexes (Figure 2.2, $R_1 = H$, $R_2 = F$ and $R_1 = R_2 = F$). The replacement of the two or four terminal hydrogen atoms of the dpac ligand by fluorines is expected to lead to a modification of the properties of the resulting Ru(II) complexes in the absence and in the presence of DNA. Indeed, the terminal part of the extended planar ligand being intercalated in the base pairs stack, its chemical modification might have an influence on

the binding affinity of the complexes towards DNA thanks to the tuning of their hydrophobicity and their sensitivity to the local DNA micro-environment.

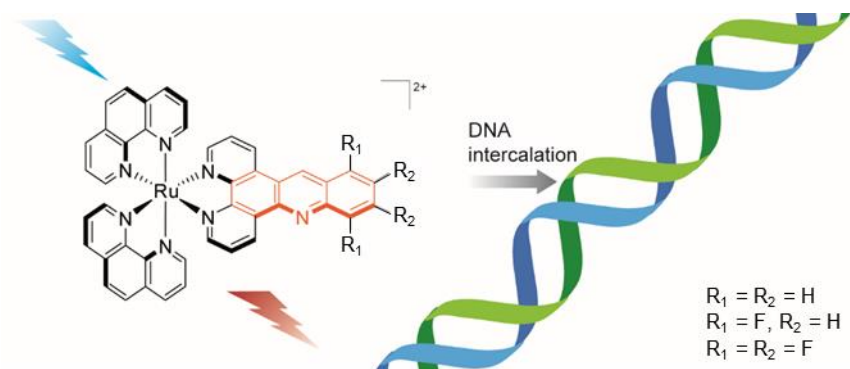


Figure 2.2. Structure of the acridine-based Ru(II) complexes as DNA photoprobes.

As one of the major problems that arise when choosing DNA as a target is the lack of selectivity for defects with respect to complementary DNA, Ru(II) complexes bearing so-called “elbow-shaped” extended planar ligands have also been designed. Due to their particular shape, a greater sensitivity of the properties of the respective Ru(II) complexes to their environment is expected (Figure 2.3).

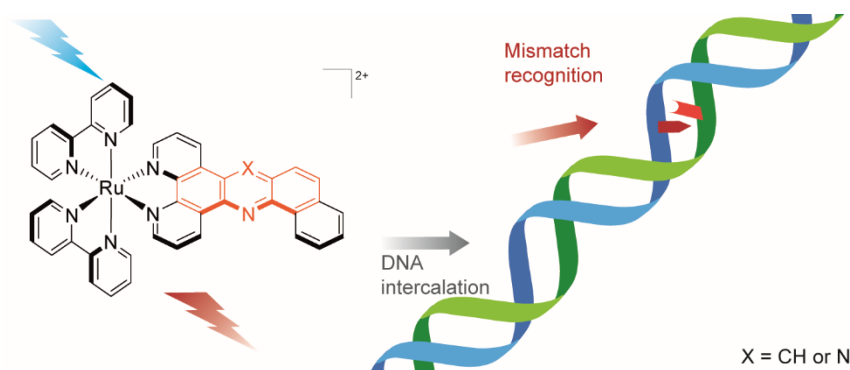


Figure 2.3. Structure of the “elbow-shaped” Ru(II) complexes as selective DNA photoprobes.

Thanks to their avid intercalation into the DNA base pairs stack and some specificity for mismatched sites, we decided to exploit the DNA

binding properties of acridine and phenazine Ru(II) complexes to develop new photoreactive complexes by replacing their ancillary bpy ligands by π -deficient bpz (= 2,2'-bipyrazine, Figure 2.4). For several years, such Ru(II) complexes have been developed in order to react with DNA upon irradiation. It has indeed been shown in the literature that Ru(II) complexes containing at least two π -deficient ligands such as TAP (= 1,4,5,8-tetraazaphenanthrene, Figure 2.4) and bpz are able to trigger oxidation of guanine moieties under light irradiation. The photochemical properties of these Ru(II) complexes have therefore been investigated in the presence of G-containing DNA sequences.

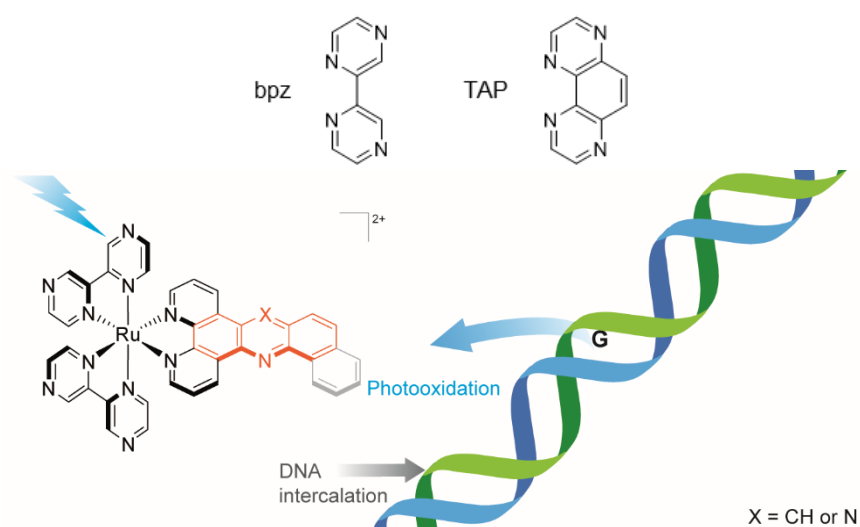


Figure 2.4. Structure of (top) the 2,2'-bipyrazine and 1,4,5,8-tetraazaphenanthrene ligands and of (bottom) photoreactive “elbow-shaped” bpz-based Ru(II) complexes.

Having demonstrated Ru(II) complexes bearing acridine and phenazine extended planar ligands can be photophysically tuned to demonstrate promising abilities as photoprobes for DNA mismatches, we were then interested in the study of the effect of the replacement of the Ru(II) metal cation by an Ir(III) center. The choice of this metal arises from the easier tunability of the photophysical and photochemical properties of the corresponding complexes with respect to Ru(II) analogs. The design of linear and “elbow-shaped” Ir(III)

complexes (Figure 2.5) as selective mismatched DNA photoprobes has thus been investigated.

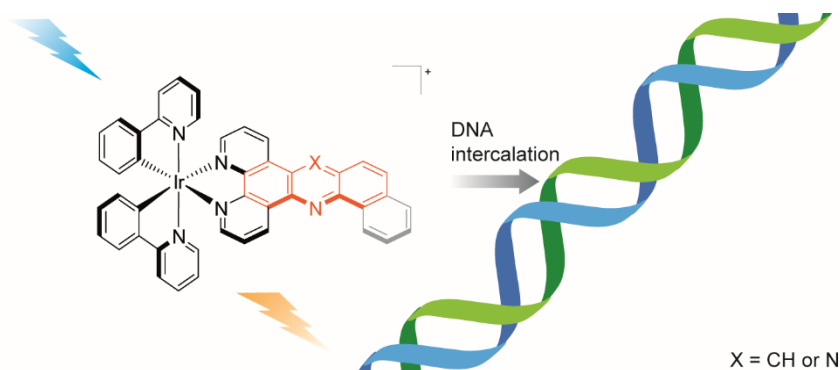


Figure 2.5. Structure of the DNA binding linear and “elbow-shaped” Ir(III) complexes.

Aditiionally, a new phenazine-based photosensitizer for cancer photodynamic therapy has been designed by the group of Prof. O. Riant. Biological studies performed by Adan PINTO (Prof. O. Feron) in the presence of cancerous cells demonstrate particular cytotoxicity under light irradiation (Figure 2.6). We aimed to give insights into the mechanism involved for the observed photodynamic process, leading to a detailed understanding of the mechanism of action of the photosensitizer in the vicinity of cancerous cells.

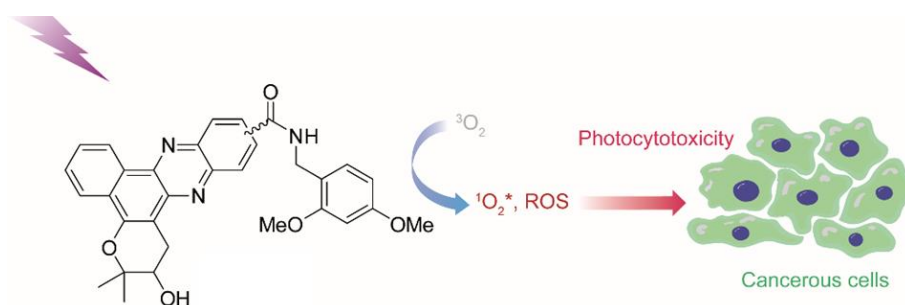


Figure 2.6. Phototoxic activity of a new phenazine-based photosensitizer for cancer photodynamic therapy. ROS = reactive oxygen species.

2. Content of the manuscript

The results presented in this Ph.D. thesis have been divided into five chapters. Due to the relatively different topics treated in each one, each chapter contains its own introduction.

Chapter 3 describes an extensive theoretical and experimental study on acridine-based complexes and analyzes the influence of the acridine core on the photophysical properties and DNA interactions of the resulting Ru(II) complexes compared to their phenazine counterparts. The ability of the acridine derivatives to selectively probe DNA mismatches has also been investigated.

In chapter 4, the photophysical study of “elbow-shaped” Ru(II) complexes has been achieved in order to selectively probe mismatched *versus* complementary DNA sequences.

Chapter 5 involves the photochemical tuning of the linear and “elbow-shaped” Ru(II) complexes upon replacement of the ancillary ligands by π -deficient ones, obtaining photoreactive species.

The chapter 6 describes the Ir(III) counterparts of the Ru(II) complexes presented above.

The chapter 7 gathers the conclusions and prospects of this Ph.D. thesis manuscript.

Finally, the chapters 8 and 9 detail the instrumental techniques as well as some annexes, respectively, including an additional chapter dealing with the photophysical study of a new photosensitizer for cancer photodynamic therapy, giving insights into the mechanism of action *in cellulo*.

The different chapters have been the subject of publications. Chapters 3 and 6 gathered the results obtained as published, while chapters 4 and 5 have been modified in order to facilitate the reading of this research work. In some cases, relevant data stored in Supporting Information for publication have been integrated in the chapters. For sake of clarity, the experimental section is presented at the end of each chapter. Note that isomery effect has not been studied in this

manuscript and that, unless explicitly mentioned, mixture of isomers are referenced by the name of the complexes.

Chapter 3

Design and photophysical studies
of acridine-based
Ru(II) complexes for application
as DNA photoprobes

1. Foreword

1.1. Introduction

As briefly stated in the general introduction, polypyridyl Ru(II) complexes have been the subject of considerable interest for the past decades. They appeared to display relevant properties for biological applications. Since the early 1960's, $[\text{Ru}(\text{bpy})_3]^{2+}$ has been one of the most studied Ru(II) complexes. Its unique chemical stability, redox properties and excited state reactivity has attracted attention on some several hundreds of its derivatives.^{95,102,110} The great interest generated by the study of this class of compounds has stimulated the development of areas such as photocatalysis or photochemistry. However, the relatively weak binding of $[\text{Ru}(\text{bpy})_3]^{2+}$ and its analogs appeared insufficient for the broader application of the Ru(II) complexes as DNA probes.

Based on this pioneering work, Chambron and Sauvage reported the application of a novel Ru(II) complex, $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ (dppz = dipyrido[3,2-*a*:2,3-*c*]phenazine) (Figure 3.1), which acts as a molecular “light switch” for DNA. This complex shows negligible luminescence in aqueous solution at ambient temperature but displays strong luminescence in the presence of DNA.^{46,111} The presence of the extended dppz ligand allows the complex to intercalate into the base pairs stack. Since, as many applications require that the complexes bind to DNA in an defined mode, much work has been done on modifying the different ligands to improve the DNA binding properties.^{23,105,112}

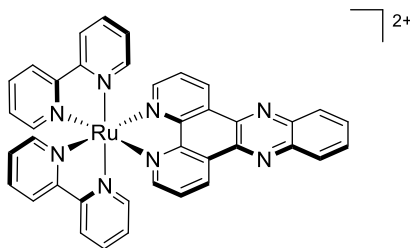


Figure 3.1. Structure of $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$.

1.1.1. Photophysics in the presence of DNA

The conception of new Ru(II) complexes to probe DNA is based on earlier studies on polypyridyl Ru(II) derivatives. In this area, the complexes bearing the dppz ligand gave rise to high interest for the last twenty-five years. Indeed, $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ and $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ (both complexes will be noted **Ru-DPPZ** for the rest of the manuscript) bind avidly to DNA ($K_{\text{aff}} \approx 10^6 \text{ L.mol}^{-1}$) and display DNA “light switch” behavior (Figure 3.2).⁴⁶ The reason for this unusual observation lies in the electronic properties of the complex and the intercalation of the dppz ligand in the base-pair stack.

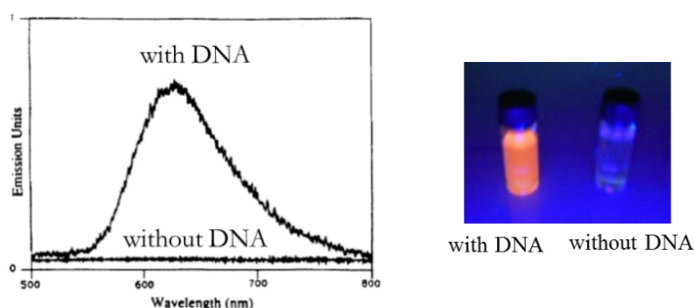


Figure 3.2. Emission spectrum of **Ru-DPPZ** in aqueous buffer in the absence and in the presence of DNA oligonucleotides⁴⁶ (left), and experimental view of the light-switch effect (right).

The unusual photophysical properties of the dppz-based complexes has been the subject of much debate in the literature for the last decades. In the late 1990's, Barbara *et al.* suggest the presence of two close-lying $^3\text{MLCT}$ states¹¹³ ($^3\text{MLCT}'$ and $^3\text{MLCT}''$), which relative energies are sensitive to the polarity of the solvent. Time-resolved and steady-state spectroscopy data indicate both $^3\text{MLCT}$ states involve the dppz extended planar ligand, differing by their charge distribution (Figure 3.3). It was proposed that decay following excitation shifts rapidly ($\sim 3 \text{ ps}$) from $^3\text{MLCT}'$ to $^3\text{MLCT}''$ which is largely non-radiative in water (“dark state”). In a less polar solvent such as acetonitrile, the $^3\text{MLCT}''$ excited state is higher in energy compared to that of $^3\text{MLCT}'$ and thus unpopulated, leading to radiative deactivation (“luminescent state”).^{113,114}

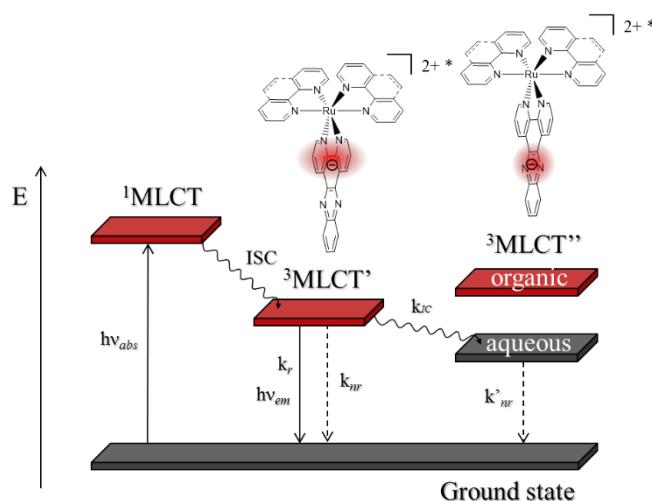


Figure 3.3. Simplified photophysical scheme for **Ru-DPPZ**. The $^3\text{MLCT}'$ excited converts by internal conversion to $^3\text{MLCT}''$. The latter is higher in energy of that of $^3\text{MLCT}'$ in organic medium, whereas it is stabilized in aqueous conditions. Both $^3\text{MLCT}$ excited states differ by their charge distribution on the extended dppz ligand.¹¹³

It has been proposed the fast non-radiative decay of **Ru-DPPZ** is driven by differential hydrogen bonding of the excited and ground states and solvent polarity. Consequently, the interplay between the two excited states differing by their distribution of charge on the dppz ligand is extremely sensitive to the local environment. While the luminescence of the “free” complex in aqueous medium is quenched, its luminescence is restored upon intercalation in the DNA base pairs stack due to the protection of the phenazine moiety by the double helix, preventing the formation of Hydrogen bonds in the excited state.^{113,115}

At the beginning of the 21st century, temperature-dependent luminescence lifetime measurements in various solvents performed by the group of Papanikolas proved to be inconsistent with the state reversal description of the light-switch behavior by Barbara *et al.* Instead, temperature-dependent luminescence lifetime studies in various solvents suggest the non-radiative $^3\text{MLCT}$ excited state (“dark state”) is lower in energy compared to radiative $^3\text{MLCT}$ (“bright state”) both in organic and aqueous media (Figure 3.4).¹¹⁶

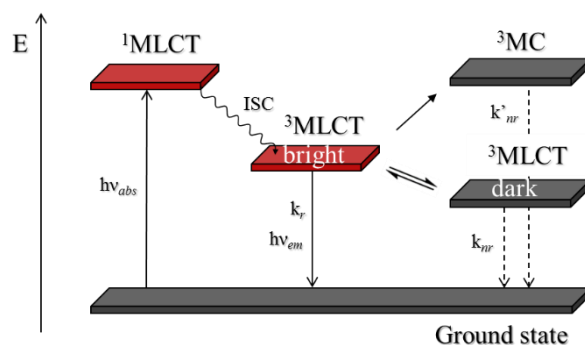


Figure 3.4. Simplified photophysical scheme for **Ru-DPPZ**. The luminescent $^3\text{MLCT}'$ state lies in equilibrium with the dark $^3\text{MLCT}''$ state. The latter is lower in energy than $^3\text{MLCT}'$ both in organic and aqueous media.¹¹⁶

With comparison with $[\text{Ru}(\text{bpy})_3]^{2+}$ data, the temperature dependence of the excited lifetime of **Ru-DPPZ** indicates the luminescent state bears an MLCT character involving the bpy fragment of the dppz ligand. Indeed, upon increase of the temperature, a maximum of luminescence lifetime is observed (Figure 3.5, up). The increase of luminescence lifetime before ~ 270 K indicates the interplay between a low-lying non-radiative and a radiative excited state. The decrease beyond ~ 270 K being similar for $[\text{Ru}(\text{bpy})_3]^{2+}$, excited states of same nature are suggested, corresponding to the shift of the electronic population from the bright $^3\text{MLCT}$ state to the non-radiative ^3MC state (see Chapter 1). These observations indicate the dark state is the lowest lying excited state, both in organic and aqueous media (Figure 3.4). As an increase of temperature is required to shift the electronic population from the dark state to the luminescent state, the “light switch” behavior has been ascribed as resulting from a dynamic equilibrium between an enthalpically favored dark state and an entropically favored luminescent state.^{116,117} Measurements performed in various solvents suggesting the dark state has a greater degree of Charge transfer character than the luminescent state, it was assigned to an MLCT state in which the promoted electron is localized on the extended portion (*i.e.* the phenazine moiety) of the dppz ligand.

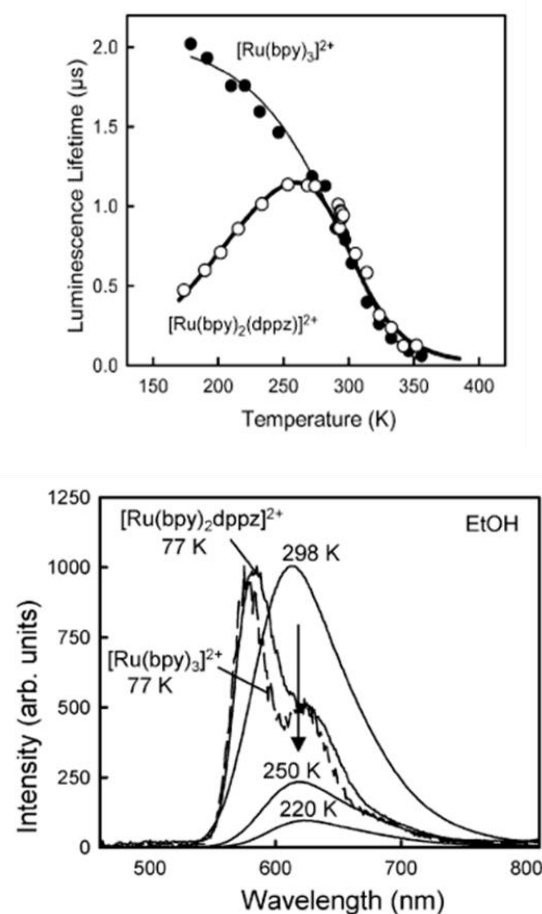


Figure 3.5. Up: Temperature-dependent luminescence lifetimes for $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ (open circles) and $[\text{Ru}(\text{bpy})_3]^{2+}$ (full circles) in ethanol. Bottom: Emission spectra of $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ and $[\text{Ru}(\text{bpy})_3]^{2+}$ at various temperature.¹¹⁷

The equilibrium between the bright and the dark $^3\text{MLCT}$ excited states corresponds thus to the transfer of the photo-excited electron between the proximal and distal fragments of dppz. The measurement in rigid matrix (at 77 K, Figure 3.5, down) confirms the Charge transfer in the excited state as the solvent is frozen in a configuration appropriate for the ground state in a way no rearrangement of the solvent occurs to stabilize the electronic configuration in the excited state.

The photophysics of **Ru-DPPZ** has been further investigated by the group of Lincoln. Kinetic and thermodynamic studies suggest the “light switch” effect of this complex is ascribed to the presence of

several $^3\text{MLCT}$ excited states in equilibrium assigned to non-, mono and dihydrogen bonded species by solvent molecules.¹¹⁸ The associated photophysical model indicates there is, after light excitation, the formation of a mixture of charge-separated states (MLCT states) where the electron is first localized in a π^* orbital either on the bpy/phen ancillary ligands or on the bpy/phen portion of the dppz ligand (state A – Figure 3.6¹¹⁸). This state converts then to another MLCT state where the electron is localized solely on the dppz ligand (state B). Depending on the medium, organization of solvent molecules able to form hydrogen bonds to one or both phenazine nitrogens leads to states C and D. State D being associated with lower entropy, the equilibrium shifts back to state C at higher temperature.¹¹⁸ In aqueous conditions, water quenches this excited state through the formation of hydrogen bonds with the phenazine nitrogen atoms. While when the dppz is protected from water, as upon intercalation into DNA, the luminescence of the complex is restored as in organic medium.^{119,120}

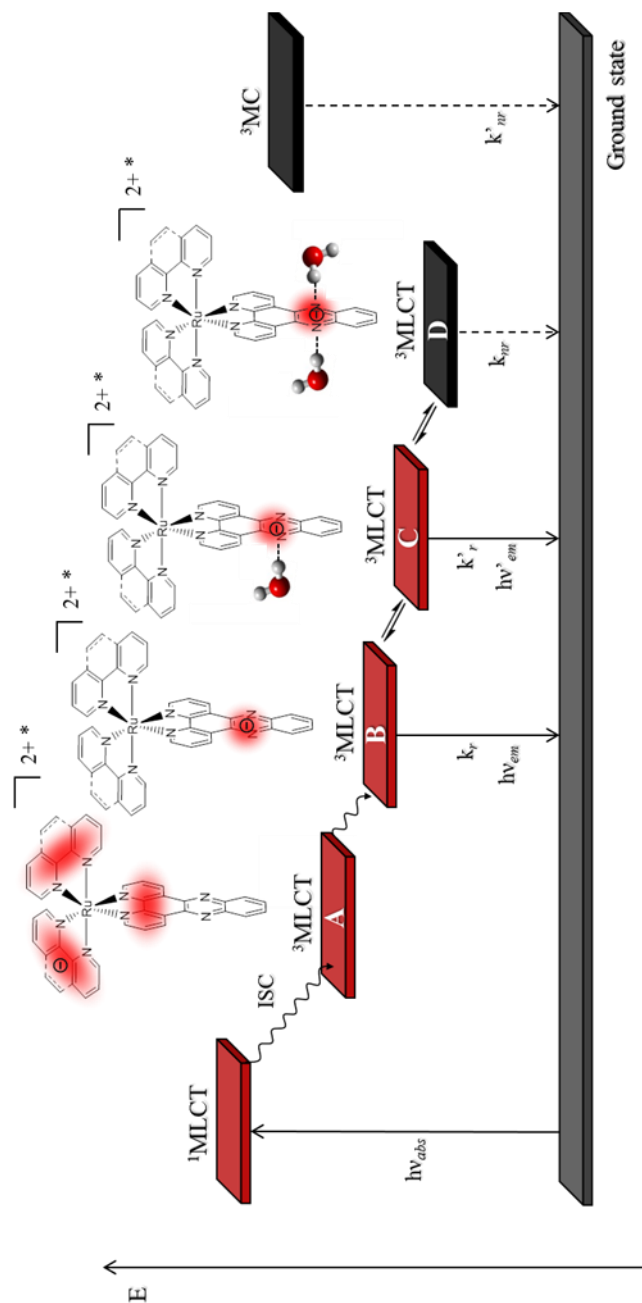


Figure 3.6. Simplified photophysical scheme of **Ru-DPPZ**. Upon excitation, the promoted electron is distributed over the three ligands (state A). This state converts to a non-hydrogen-bonded $^3\text{MLCT}$ state (state B) where the electron is localized on the phenazine moiety of the dppz ligand. State B lies in equilibrium with the mono-hydrogen-bonded excited state (state C), itself in equilibrium with the bis-hydrogen-bonded excited state (state D).

1.1.2. DNA binding of **Ru-DPPZ**

The exact binding mode of **Ru-DPPZ** towards DNA has been the subject of much debate in the literature. Pioneering work by Lincoln indicates the presence of two distinct luminescence lifetimes for the complex in the presence of DNA, suggesting binding in micro-environment with different solvent accessibility.¹²¹⁻¹²³ It was first suggested that the dppz-based complexes intercalate *via* the major groove with two different geometries, either perpendicular or parallel to the base pairs axis.¹²⁴ Further studies revealed a strong sensitivity of the excited state lifetime to the concentration of the complex in the presence of oligonucleotides,¹²³ suggesting either the presence of different orientations within the major groove⁴⁷ or isolated or contiguous binding in the minor groove.¹²² Recent crystallographic data rather indicate the presence of two distinct intercalation binding modes, one being “symmetric”, and the other “angled”.³⁹ Each of them exhibits sequence preference and defined geometry (Figure 3.7).

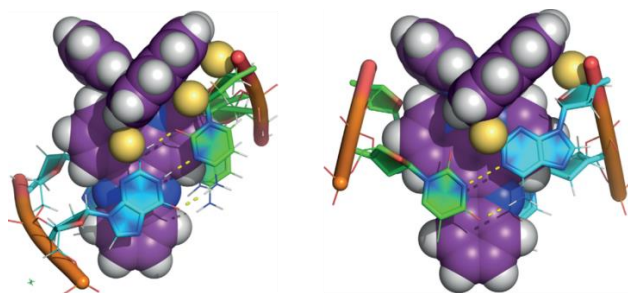


Figure 3.7. Representation of the two intercalative binding modes of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ in $\text{d}(\text{CCGGTACCGG})_2$. Left: angled intercalation geometry between CC/GG base pairs, displaying stacking between the upper CG base pair and the dppz ligand. Right: symmetrical intercalation geometry between TA base pairs.³⁹

The angled intercalation of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ into the base pairs stack creates inequivalence between the two ancillary phen ligands. One is shown forming close contacts with the DNA bases and backbone, while the other one remains free. Interestingly, the latter is able to semi-intercalate into a second duplex. The symmetrical intercalative binding mode leads to a deep insertion of the dppz ligand between the base pairs stack, inducing particular enantiomeric and step specificity

due to interactions between the ancillary ligands and the DNA backbone.³⁹

The luminescent features of **Ru-DPPZ** were also investigated in the presence of oligonucleotides containing single base mismatches or an abasic site.¹⁰⁶ Interestingly, **Ru-DPPZ** exhibit significant luminescent sensitivity to these non-canonical DNA structures with respect to the complementary oligonucleotides (Figure 3.8). In the presence of a single base mismatch, strong luminescent enhancement occurs for the Δ isomer (see Chapter 1), while the Λ isomer shows higher luminescence when bound to an oligonucleotide containing an abasic site. Studies with hairpins containing a variable mismatch site show correlation between the level of luminescent enhancement and the thermodynamic stability associated with the mismatch (CT, AC and CC being the less stable mismatches).^{106,125}

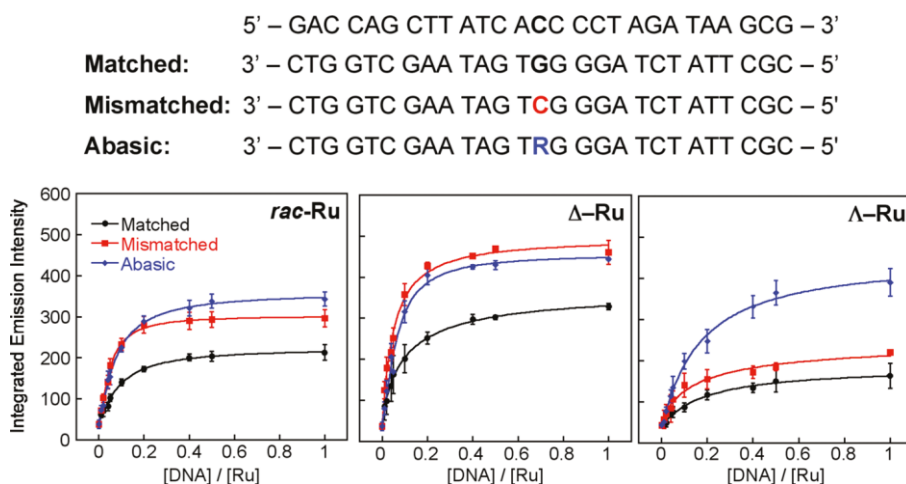


Figure 3.8. Luminescence titrations of $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ with mismatch-containing oligonucleotide. Top: DNA sequences of matched, mismatched and abasic 27-mer duplex DNA (R denotes a tetrahydrofuranly abasic site). Bottom: plots of the integrated emission intensity ($\lambda_{\text{ex}} = 440 \text{ nm}$) of *rac*- (left), Δ - (middle), and Λ -complex (right) upon increasing the concentration of DNA in 50 mM NaCl, 5 mM Tris, pH 7.5.¹⁰⁶

This behavior is similar to that observed for a bulky rhodium complex, $[\text{Rh}(\text{bpy})_2\text{chrysi}]^{3+}$ (chrysi = 5,6-chrysenequinone diimine), that binds mismatched DNA sites through insertion. This complex binds DNA from the minor groove side, ejecting the mismatched bases into the major groove (Figure 3.9).^{23,126,127}

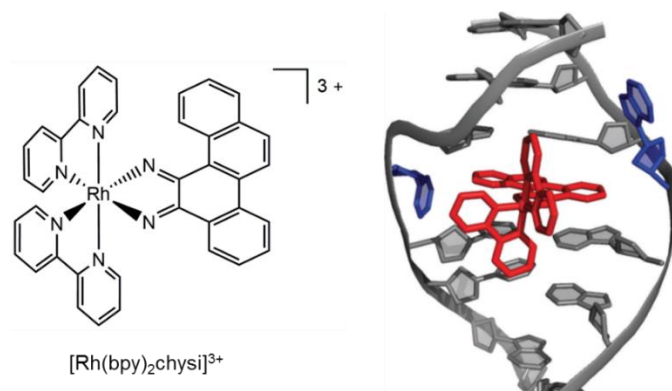


Figure 3.9. Structure of $\Delta\text{-}[\text{Rh}(\text{bpy})_2(\text{chrysi})]^{3+}$ (left) bound to an AC Mismatch (right). The rhodium complex (red) inserts into the DNA from the minor groove and ejects the mismatched bases (blue) into the major groove.¹²⁶

1.2. Objectives

Ru-DPPZ complexes are chemicals of great interest due to their outstanding photochemical behavior. Their environment-sensitive luminescence properties make them potent candidates as photoprobes for DNA topologies and mismatches. While complexes based on dppz derivatives and other ligands incorporating a phenazine core received a lot of attention, fewer studies were reported on complexes based on ligand incorporating an acridine core. Studies on the tpac ligand (tpac = tetrapyrido[3,2-*a*:2',3'-*c*:3'',2''-*h*:2''',3'''-*j*]acridine)¹⁰⁷ and the corresponding complexes^{108,109} showed that the acridine core induces notable modifications on the behaviour of the resulting complex as compared to the phenazine-based equivalent. Analogous to dppz, the dpac ligand (dpac = dipyrido[3,2-*a*:2',3'-*c*]acridine) bears one less non-chelating nitrogen atom, leading thus to the possible formation of only one Hydrogen bond with solvent

molecules in the excited state, and consequently to potential photochemical differences with dppz analogs.

In this context, our work focuses on the synthesis of a series of extended planar aromatic ligands based on an acridine core (dpac; dpacF₂ = 7,8-difluorodipyrido[3,2-*a*:2',3'-*c*]acridine; dpacF₄ = 6,7,8,9-tetrafluorodipyrido[3,2-*a*:2',3'-*c*]acridine) and the photophysical study of their respective Ru(II) complexes (**Ru-DPAC** for [Ru(phen)₂dpac]²⁺; **Ru-DPACF₂** for [Ru(phen)₂dpacF₂]²⁺; **Ru-DPACF₄** for [Ru(phen)₂dpacF₄]²⁺) both in the absence and in the presence of complementary and single-base mismatched DNA (Figure 3.10).

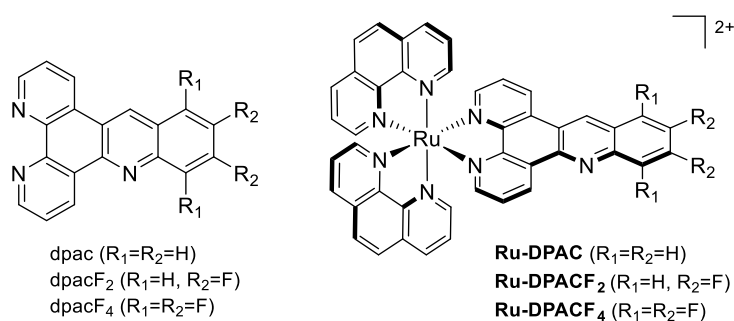


Figure 3.10. Structures of acridine-based ligands and corresponding Ru(II) complexes.

The photophysical results have been supported by theoretical calculations performed by Thomas AUVRAY, Ph.D. student in the group of Prof. G.S. HANAN (Université de Montréal, Canada). This chapter of the Ph.D. thesis has been the subject of a publication which manuscript is included hereafter.

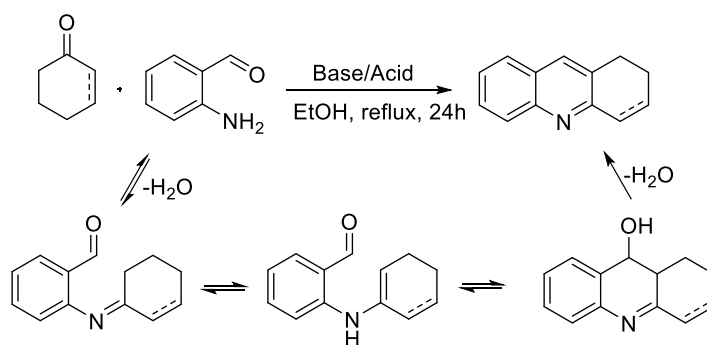
2. Preliminary results

2.1. Synthesis of the dpac ligand and its fluorine analogs

Acridine based ligands can be obtained through numerous synthetic pathways, such as Skraup cyclization,¹²⁸ self-condensation, organometallic couplings,¹²⁹ and cycloadditions.^{130,131} Some of them have been envisaged to prepare the dpac ligand and will be successively discussed hereafter.

2.1.1. Synthesis of dpac through Friedländer annulation

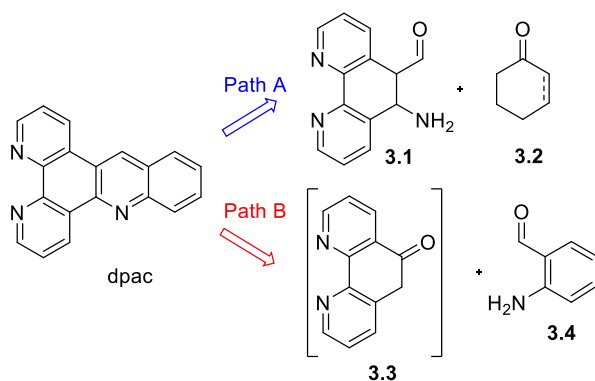
Friedländer annulation is one of the simplest methods to prepare quinoline or acridine derivatives.¹³² Typically, this reaction implies base- or acid-promoted condensation of an aromatic 2-amino-substituted carbonyl derivative, such as aldehyde or ketone, with a substituted carbonyl derivative containing an α -methylene group (Scheme 3.1). Friedländer annulations are generally carried out either by refluxing an aqueous or alcoholic solution of the reactants in the presence of a base or acid.



Scheme 3.1. General mechanism of acridine precursors synthesis by Friedländer annulation.

In the frame of the synthesis of dpac ligand, both reactants involved can act as the aromatic 2-amino-substituted carbonyl derivative or as the substituted carbonyl derivative containing an α -methylene group. As a consequence, two synthetic strategies were proposed (Scheme

3.2), noted paths A and B. Path A implies the condensation of 6-amino-5,6-dihydro-1,10-phenanthroline-5-carbaldehyde (**3.1**) with cyclohexanone or cyclohex-2-en-1-one (**3.2**) (followed by an oxidation step to lead to dpac), while path B requires 1,10-phenanthroline-5-one (**3.3**) and 2-aminobenzaldehyde (**3.4**).

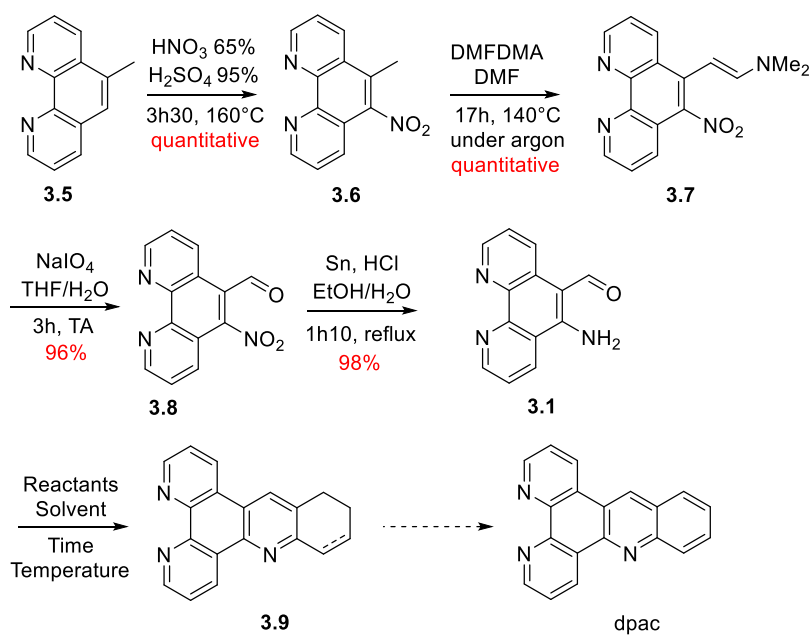


Scheme 3.2. Proposed retrosynthetic pathways to achieve dpac synthesis through Friedländer annulation.

a) Path A

Cyclohexanone and cyclohex-2-en-1-one being commercially available, the preparation of the 6-amino-5,6-dihydro-1,10-phenanthroline-5-carbaldehyde derivative (**3.1**) is required.¹³³ We proposed a six steps synthetic pathway to prepare the dpac ligand (Scheme 3.3). The first step involves the nitration of 5-methyl-1,10-phenanthroline (**3.5**), followed by the formation of the enamine (**3.7**). Oxidation of the enamine and reduction of the nitro group lead then to intermediate **3.1**. The Friedländer annulation step was first performed with cyclohexanone (Table 3.1), and lead to a product showing an unclear ¹H-NMR spectrum. Mass spectrometry of this product leads to the observation of a single peak at $m/z = 279.11$, instead of expected 285.13. Different reaction conditions have been investigated, such as the use of more reactive carbonyl derivatives (aldehydes) with the aim of getting insights on the reactivity of **3.1**. In order to favor the pyridine ring formation, we also introduce a small and cheap organocatalyst, *i.e.* *L*-proline, which has demonstrated high efficiency

for Friedländer annulation under smooth conditions.¹³⁴ However, the formation of an unknown product was observed, except when using aldehydes for which the reactant **3.1** was recovered.



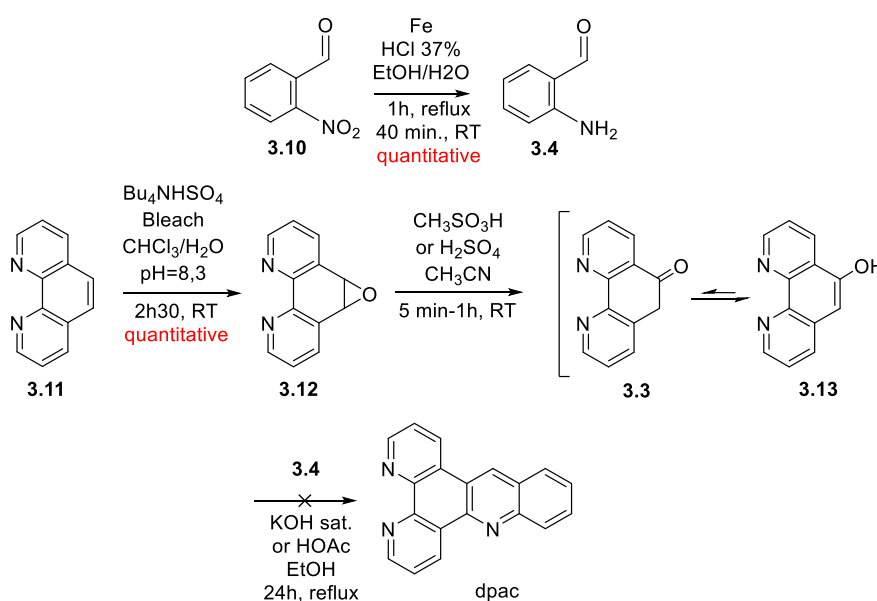
Scheme 3.3. Synthetic strategy to dpac through Friedländer annulation (Path A).

Entry	Carbonyl derivative	Base	Solvent	Catalyst	T [° C]	Time	Observations
1	cyclohexanone	KOH	EtOH	/	78	48h	Unknown product
2	cyclohexanone	KOH	EtOH	/	78	24h	Unknown product
3	acetaldehyde	KOH	EtOH	/	65	5h	Starting material recovered
4	butyraldehyde	KOH	EtOH	/	78	4h	Starting material recovered
5	cyclohexanone	NaOH	EtOH	/	78	1h10	Unknown product
6	cyclohexanone	/	EtOH	<i>L</i> -proline	78	26h	Unknown product
7	cyclohexanone	/	EtOH	<i>L</i> -proline	78	18h	Unknown product
8	cyclohexanone	/	EtOH	<i>L</i> -proline	78	4h	Unknown product
9	cyclohexanone	/	THF	<i>L</i> -proline	RT	48h	Unknown product
10	2-cyclohexen-1-one	/	EtOH	<i>L</i> -proline	78	4h	Unknown product
11	2-cyclohexen-1-one	/	EtOH	<i>L</i> -proline	78	18h	Unknown product

Table 3.1. Reaction conditions investigated for Path A synthetic scheme.

b) Path B

The second synthetic route envisaged required the preparation of 1,10-phenanthroline-5-one (**3.3**) and 2-aminobenzaldehyde (**3.4**). These two are readily obtained from commercially available 1,10-phenanthroline (**3.11**) and 2-nitrobenzaldehyde (**3.10**) (Scheme 3.4).¹³⁴ The Friedländer annulation step didn't lead to the formation of the expected product, probably because of the disfavored equilibrium between the ketone (**3.3**) and its enol counterpart (**3.13**).



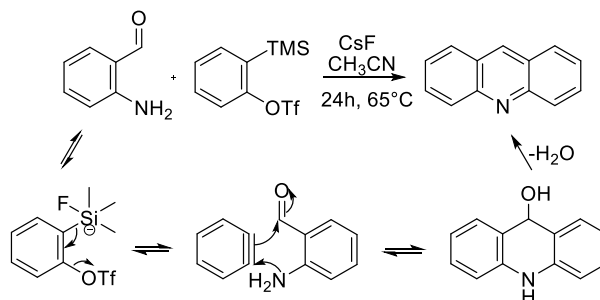
Scheme 3.4. Synthetic strategy to dpac through Friedländer annulation (Path B).

2.1.2. Synthesis of dpac through cycloaddition reaction

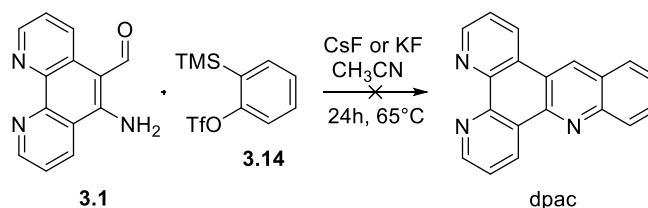
Larock published in 2010 an efficient method to prepare substituted acridines.¹³⁰ It consists in the reaction of 2-aminoaryl ketones/aldehydes with arynes, generated by the treatment of *o*-(trimethylsilyl)aryl triflates derivatives with cesium fluoride (Scheme 3.5). This [4+2] annulation uses mild conditions and allows therefore the presence of various substituents on both reactants.

To achieve the synthesis of dpac, compound **3.1** and commercially available *o*-(trimethylsilyl)phenyl triflate (**3.14**) were refluxed in acetonitrile (Scheme 3.6). Unfortunately, starting material **3.1** was

recovered, probably because of its poor nucleophilicity due to the presence of the two electron-withdrawing pyridine rings.



Scheme 3.5. General mechanism of acridine synthesis by [4+2] annulation.

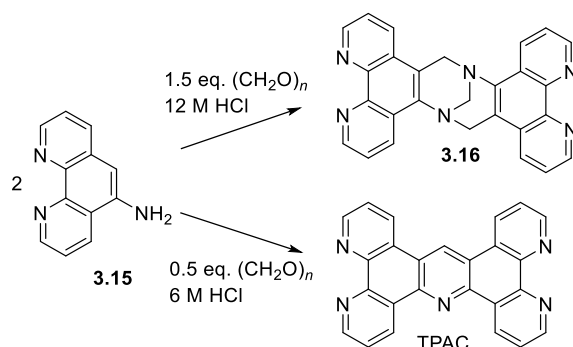


Scheme 3.6. dpac synthesis through [4+2] annulation.

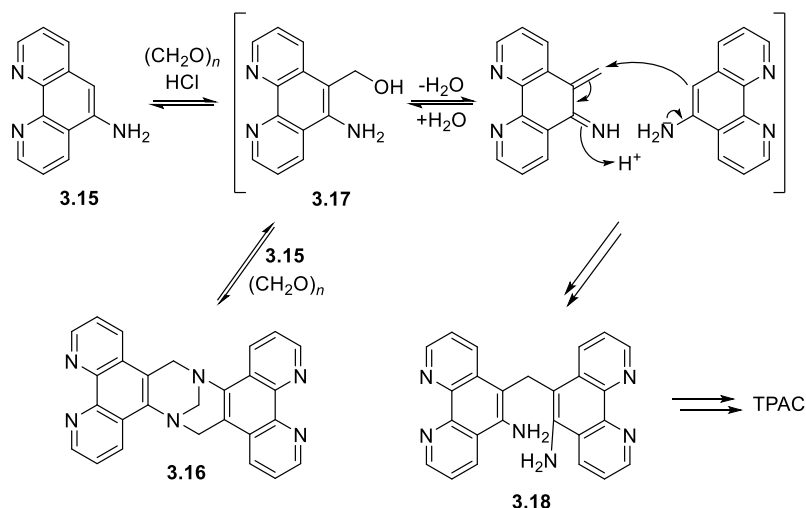
2.1.3. Synthesis of dpac through intermolecular condensation

In 2002, the TPAC ligand was synthesized for the first time, using a Tröger base modified synthetic scheme.¹⁰⁷ It was shown in the literature that the reaction of 3-aminoacridine with paraformaldehyde in acidic medium strongly depends on the acid concentration and on the stoichiometry in paraformaldehyde. In strong acids, and in the presence of excess of paraformaldehyde, the heterocyclic analogue of Tröger's base **3.16** is formed, but in 6 M hydrochloric acid and in the presence of excess of amine, the reaction leads to the formation of the triazaheptacycle TPAC (shown for 5-amino-1,10-phenanthroline (**3.15**) as starting material in Scheme 3.7). The proposed mechanism (Scheme 3.8) involved electrophilic substitution on the aromatic amine **3.15** with paraformaldehyde, giving 3-amino-4-hydroxymethyl derivative **3.17**. In the presence of excess paraformaldehyde, the reaction yields the Tröger's base **3.16**, but in the presence of excess aromatic amine, methylene-

bis(arylamine) intermediate **3.18** is produced. The formation of tpac results from cyclization and oxidation of this intermediate.

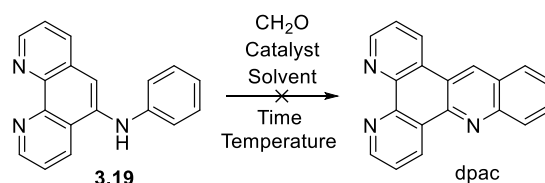


Scheme 3.7. Tröger base and TPAC synthetic scheme.



Scheme 3.8. Proposed mechanism for Tröger base and TPAC synthesis.

The dpac ligand being structurally similar to TPAC, we expected that the intermolecular condensation between *N*-phenyl-1,10-phenanthroline-5-amine (**3.19**) and formaldehyde would allow the formation of dpac (Scheme 3.9 and Table 3.2). Yet, this synthetic strategy proved to be unsuccessful to reach this goal. The reason could come from a too poor nucleophilicity from **3.19**, preventing the aromatic electrophilic substitution with formaldehyde.



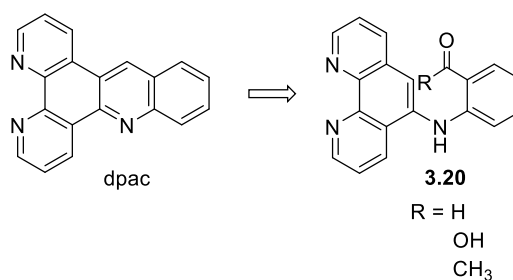
Scheme 3.9. Inter-molecular condensation to dpac.

Entry	Solvent	Catalyst	T [°C]	Time	Isolated product
1	EtOH	HOAc	78	24h	starting material
2	EtOH	<i>L</i> -Proline	78	48h	starting material
3	EtOH	<i>L</i> -Proline and HOAc	78	24h	starting material

Table 3.2. Reaction conditions investigated for intermolecular condensation synthetic scheme.

2.1.4. Synthesis of dpac through intramolecular condensation

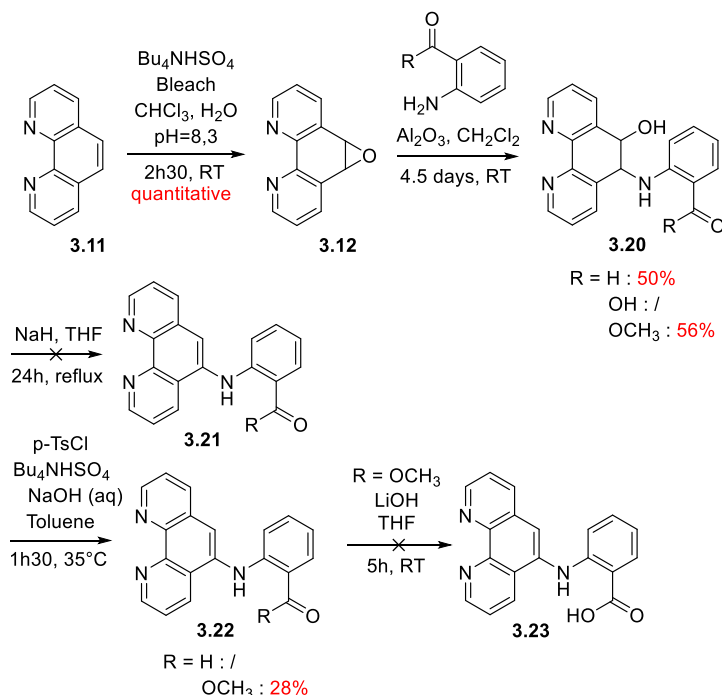
As the nucleophilicity of 1,10-phenanthroline derivatives seem to be the reason of our failures, we envisaged to prepare an analogue to *N*-phenyl-1,10-phenanthroline-5-amine (**3.19**) bearing a carbonyl derivative (Scheme 3.10).



Scheme 3.10. Retrosynthetic scheme to prepare dpac through intramolecular condensation.

Such a strategy first required the formation of 5,6-epoxy-1,10-phenanthroline (**3.12**), followed by its opening by the suitable aniline derivative (Scheme 3.11). Elimination and intramolecular

condensation of **3.20** are expected to lead to the formation of dpac.¹³⁵ Unfortunately, when R = OH the opening of the epoxide leads to the formation of 5,6-dihydro-1,10-phenanthroline-5,6-diol. Whatever was the nature of the R group, elimination using NaH lead to the recovery of 5-amino-1,10-phenanthroline. Milder conditions allowed the formation of the desired compound for R = OCH₃, but the saponification step appeared to cleave the aniline fragment, leading to 5-amino-1,10-phenanthroline (**3.11**).



Scheme 3.11. Synthesis of dpac through intramolecular condensation.

2.1.5. Synthesis of dpac through intermolecular cyclisation

A Tröger's base modified synthetic scheme, derived from Scheme 3.8, was found to be successful and allowed us to obtain the dpac and its fluoro derivatives with high yields. The synthetic methodology consists in a cyclization between 5-amino-1,10-phenanthroline and respective *o*-aminobenzyl alcohols.¹⁰⁷ Subsequent complexation of the dpac derivatives with Ru(II) precursor leads to the formation of corresponding complexes, according to previously

described methodologies.¹⁰⁹ These syntheses are described in the next section, together with the full photochemical characterization of the ligands and complexes. Experimental results are supported by DFT and TD-DFT calculations. Application of the resulting complexes as DNA and mismatch-containing oligonucleotides photoprobes has been investigated and lead to the publication of an Article.

3. Content of the publication

Journal

European Journal of Inorganic Chemistry, **2016**, 22, 3649-3658
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Title

Design and photophysical studies of acridine-based Ru(II) complexes for application as DNA photoprobes

Authors

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Abstract

A series of new extended planar aromatic ligands based on an acridine core (**dpac** = dipyrido[3,2-*a*:2',3'-*c*]acridine ; **dpacF₂** = 7,8-difluorodipyrido[3,2-*a*:2',3'-*c*]acridine ; **dpacF₄** = 6,7,8,9-tetrafluorodipyrido[3,2-*a*:2',3'-*c*]acridine) and their respective Ru(II) complexes were synthesized and extensively studied. The photophysical and theoretical studies revealed that **Ru-DPAC** and **Ru-DPACF₂** allow emission from a $^3\text{MLCT}_{\text{phen/dpac}}$ excited state at 597 and 604 nm, respectively, while the lowest excited state for **Ru-DPACF₄** shifts from a bright $^3\text{MLCT}_{\text{phen/dpac}}$ state towards a poorly luminescent $^3\text{MLCT}_{\text{dpac}}$ state ($\lambda_{\text{em}} = 609$ nm) due to the formation of hydrogen bonds with water molecules in the excited state. These complexes exhibit up to sevenfold luminescence enhancement in the presence of complementary and mismatched DNA, demonstrating a strong binding to polynucleotides. The results emphasized the interest of Ru(II) complexes bearing acridine derivatives as DNA interacting agents, as a complement to the well-known phenazine-based derivatives.

3.1. Introduction

Transition metal complexes are of great interest in molecular biology and biochemistry.^{49,50,136} In addition to being present as coordination sites in proteins or cofactors in Nature, these compounds offer numerous advantages for their use as photoprobes or photoreagents, like DNA interacting agents. Indeed, the great modularity of the properties of the transition metal complexes makes them ideal in this scope. Polypyridyl Ru(II) complexes have been the subject of considerable interest for the past decades. Even though numerous publications describe their use as catalysts,^{137,138} or energy conversion and storage devices,¹³⁹⁻¹⁴¹ these Ru(II) complexes appeared to be relevant in the context of biological applications as sensing^{7,104,105} and therapeutic agents as well.^{142,143} Among them, Ru(II) complexes bearing the intercalating dppz ligand (dppz = dipyrido[3,2-*a*:2',3'-*c*]phenazine) have been studied for the last twenty-five years. Indeed, $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ and $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$

bind avidly to DNA ($K_{\text{aff}} \approx 10^6 \text{ L.mol}^{-1}$) and display intense luminescence in the presence of DNA, whereas the luminescence of the complex alone is switched off in aqueous media.⁴⁶ This “light switch” behaviour has been ascribed to the population of a mixture of excited Metal-to-Ligand Charge transfer states (MLCT states) where the electron located on the dppz ligand is either proximal or distal from the metallic center.^{113,116,144,145} In water, a quenching occurs *via* the formation of hydrogen bonds with the phenazine core of the dppz, while upon intercalation into DNA, the luminescence of the complex is restored, as observed in organic media.¹²⁰ In addition, studies with mismatch containing DNA show correlation between the luminescence increase of $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ and the mismatch thermodynamic stability.¹⁰⁶ These environment-sensitive optical properties render dppz-based Ru(II) complexes potent candidates as photoprobes for different DNA topologies.

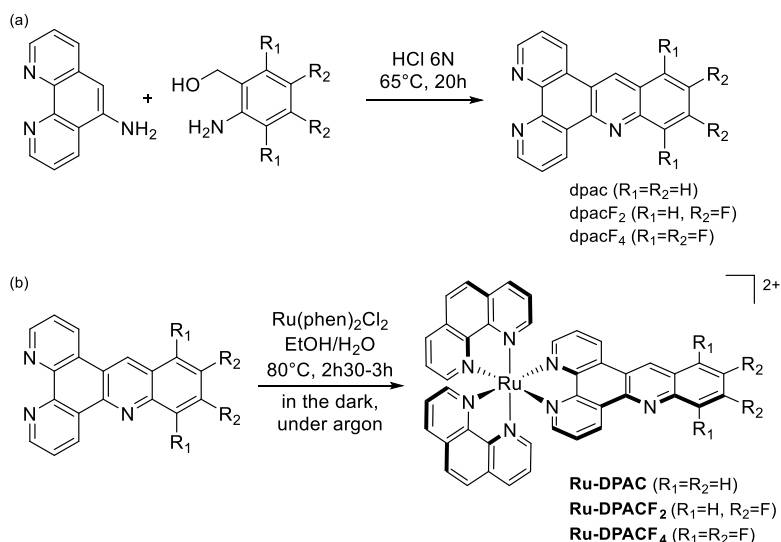


Figure 3.11. Synthetic scheme of dpac ligands and their corresponding Ru(II) complexes.

While complexes based on dppz derivatives and other ligands incorporating a phenazine core received a lot of attention, fewer studies were reported on complexes based on ligand incorporating an

acridine core. Studies on the tpac ligand (tpac = tetrapyrido[3,2-*a*:2',3'-*c*:3'',2''-*h*:2''',3'''-*j*]acridine)¹⁰⁷ and the corresponding complexes^{108,109} showed that the acridine core induces notable modifications on the behaviour of the resulting complex as compared to the phenazine-based equivalent. Analogous to dppz, the dpac ligand (**dpac** = dipyrdo[3,2-*a*:2',3'-*c*]acridine) and its Ru(II) complex have been only recently reported and the latter was shown to be a potent nicotinamide (NAD) derivative for electron storage system.¹⁴⁶ However, its photophysical properties were not fully investigated and its interaction with DNA was not studied. Furthermore, as dpac bears one less non-chelating nitrogen atom than dppz, only one hydrogen bond with solvent molecules in the excited state would be possible, suggesting potential differences with dppz-based Ru(II) complexes.

In this context, our work focuses on the synthesis of a series of extended planar aromatic ligands based on an acridine core (**dpac** = dipyrdo[3,2-*a*:2',3'-*c*]acridine; **dpacF₂** = 7,8-difluorodipyrdo[3,2-*a*:2',3'-*c*]acridine; **dpacF₄** = 6,7,8,9-tetrafluorodipyrdo[3,2-*a*:2',3'-*c*]acridine), and the study of their respective Ru(II) complexes (**Ru-DPAC** for [Ru(phen)₂dpac]²⁺; **Ru-DPACF₂** for [Ru(phen)₂dpacF₂]²⁺; **Ru-DPACF₄** for [Ru(phen)₂dpacF₄]²⁺) (Figure 3.11). In this paper, we report an extensive theoretical and experimental study on these complexes and analyse the influence of the acridine core on the photophysical properties and DNA interactions of the resulting Ru(II) complexes compared to dppz-based complex. We also investigate the selectivity for **Ru-DPAC** to probe DNA mismatches.

3.2. Results and discussion

3.2.1. Synthesis

The acridine core can be obtained through numerous synthetic pathways, such as Skraup cyclization,¹⁴⁷ self-condensation,¹⁰⁷ Cu(0), Pd(0) or Zn(II)-based organometallic couplings,^{129,148-150} and cycloadditions.^{130,131} In our case, a Tröger's base modified synthetic scheme was found to be successful and allowed us to obtain dpac and its fluoro derivatives with high yields (Figure 3.11). The synthetic methodology consisted in a cyclization between 5-amino-1,10-phenanthroline and the appropriate *o*-aminobenzyl alcohols.^{107,151}

The corresponding Ru(II) complexes were synthesized by the direct chelation of the extended ligand onto a Ru(II) precursor (Figure 3.11) using methodologies previously described for similar compounds. All the intermediate and final compounds were protected from direct light during the synthesis and purification steps to prevent photochemical degradation. The reactions were performed under argon to avoid any oxidation of the metal center.

The complexes were fully characterized by HRMS and ¹H NMR spectroscopy (see the Experimental Section and associated content). All the multiplicity and coupling constants cannot be determined due to the superposition of several signals and/or lack of fine resolution for some peaks in the ¹H NMR spectra. The ¹H NMR spectra show unambiguously the absence of symmetry elements for all the complexes, which induces the non-equivalence of (i) the protons on the extended planar ligand and (ii) the ancillary ligands. The ¹H NMR spectra are sensitive to concentration as some shifts occurred upon change in the concentration. For solubility purposes, all the complexes were converted to the chloride salt for experiments conducted in water or buffer or to the hexafluorophosphate salt for studies in organic solvents.

3.2.2. Electrochemistry

The electrochemical data of the three complexes were obtained by cyclic voltammetry in dry deoxygenated MeCN or *N,N*-dimethylformamide (Table 3.3 and Figures 3.12-3.14). The three complexes display a one-electron reversible oxidation wave at a potential close to that of the $\text{Ru}^{2+}/\text{Ru}^{3+}$ oxidation of $[\text{Ru}(\text{phen})_3]^{2+}$.¹⁰² This similarity between the oxidation potentials of all these complexes confirms the metal-based nature of the process ($\text{Ru}^{2+}/\text{Ru}^{3+}$ redox couple) irrespective of the substitution on the extended planar ligand. Three reduction waves are monitored for each complex. From the comparison with the reduction potentials of $[\text{Ru}(\text{phen})_3]^{2+}$ (-1.30 V vs Ag/AgCl),¹¹¹ and $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ (noted **Ru-DPPZ**) (-0.95 V vs Ag/AgCl),¹¹¹ the first reduction of the five complexes is attributed to the addition of one electron on the extended planar ligand. The anodic shift observed for **Ru-DPACF₄** (-0.97 V vs Ag/AgCl) indicates a greater stabilization of the π^* orbital centered on the dpacF₄ ligand with respect to that of **Ru-DPAC** and **Ru-DPACF₂** respectively. The next two reduction waves are attributed to successive reduction of the two ancillary phen ligands. The potentials in the excited state (namely, E_{ox}^* and E_{red}^*) for the three complexes have been estimated from the ground state redox potentials and the energy of the excited state corresponding to the maximum of the emission spectrum at 298 K (see hereafter). Not surprisingly, **Ru-DPACF₄** ($E_{\text{red}}^* = +1.07$ V vs Ag/AgCl) is the most oxidizing complex in the excited state.

	$E_{1/2 \text{ ox}}^a$ [V vs Ag/AgCl]	$E_{1/2 \text{ red}}^{a,b}$ [V vs Ag/AgCl]	$\lambda_{\text{max, em}}^a$ [nm]	$E_{\text{ox}}^*{}^c$ [V vs Ag/AgCl]	$E_{\text{red}}^*{}^d$ [V vs Ag/AgCl]
[Ru(phen) ₃] ²⁺	1.32	-1.30 -1.47 -1.68	604	-0.73	0.70
Ru-DPPZ ⁶⁵	1.35	-0.95 -1.39	618	-0.67	1.02
Ru-DPAC	1.35	-1.22 -1.35 -1.66	597	-0.73	0.86
Ru-DPACF₂	1.35	-1.15 -1.34 -1.62	604	-0.71	0.91
Ru-DPACF₄	1.35	-0.97 -1.30 -1.52	609	-0.69	1.07

Table 3.3. Potentials for the oxidation ($E_{1/2 \text{ ox}}$) and reduction ($E_{1/2 \text{ red}}$) of **Ru-DPAC**, **Ru-DPACF₂** and **Ru-DPACF₄**. ^a Measured in dry acetonitrile. ^b The reversibility of the reduction processes being not able to determine due to adsorption of the reduced species on the electrode surface, $E_{1/2}$ has been estimated from the reduction peak. ^c The excited state oxidation potential was estimated from the ground state oxidation potential and the energy of emission maximum with the equation $E_{\text{ox}}^* = E_{1/2 \text{ ox}} - E_{0-0} \approx E_{1/2 \text{ ox}} - \Delta E_{\lambda_{\text{max, em}}}$. ^d The excited state reduction potential was estimated from the ground state reduction potential and the energy of emission maximum with the equation $E_{\text{red}}^* = E_{1/2 \text{ red}} + E_{0-0} \approx E_{1/2 \text{ red}} + \Delta E_{\lambda_{\text{max, em}}}$.

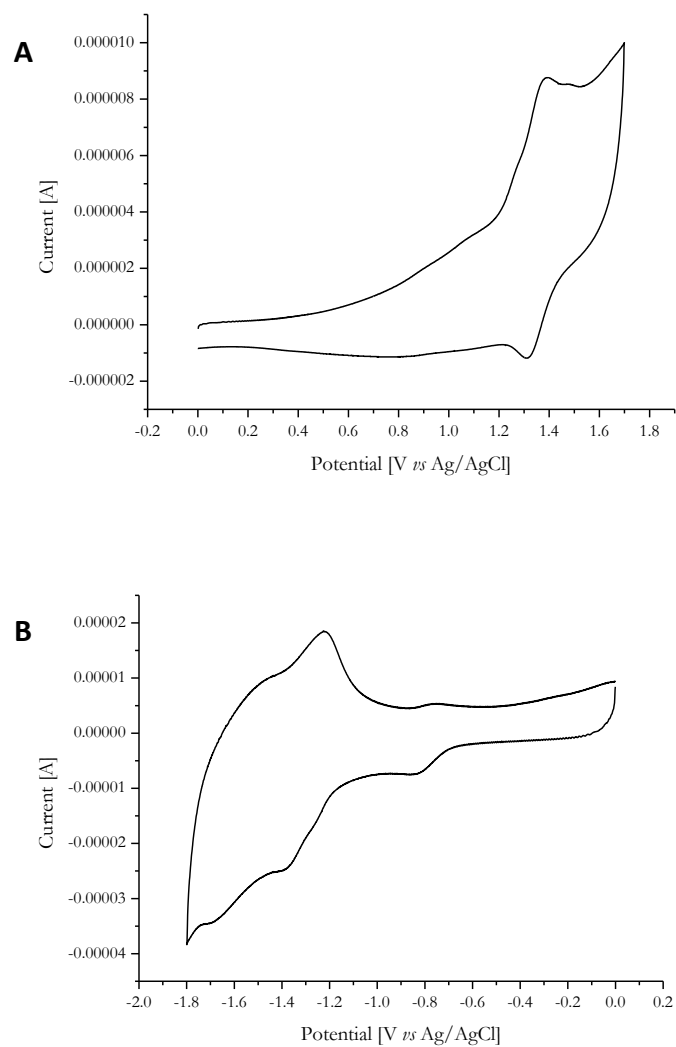


Figure 3.12. Cyclic voltammograms of **Ru-DPAC** recorded in dry acetonitrile under argon, with a sweep rate of 0.3 V/s, at room temperature. The concentration of the complex is $8 \cdot 10^{-4}$ mol/L, with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte. (A) Positive polarization; (B) negative polarization.

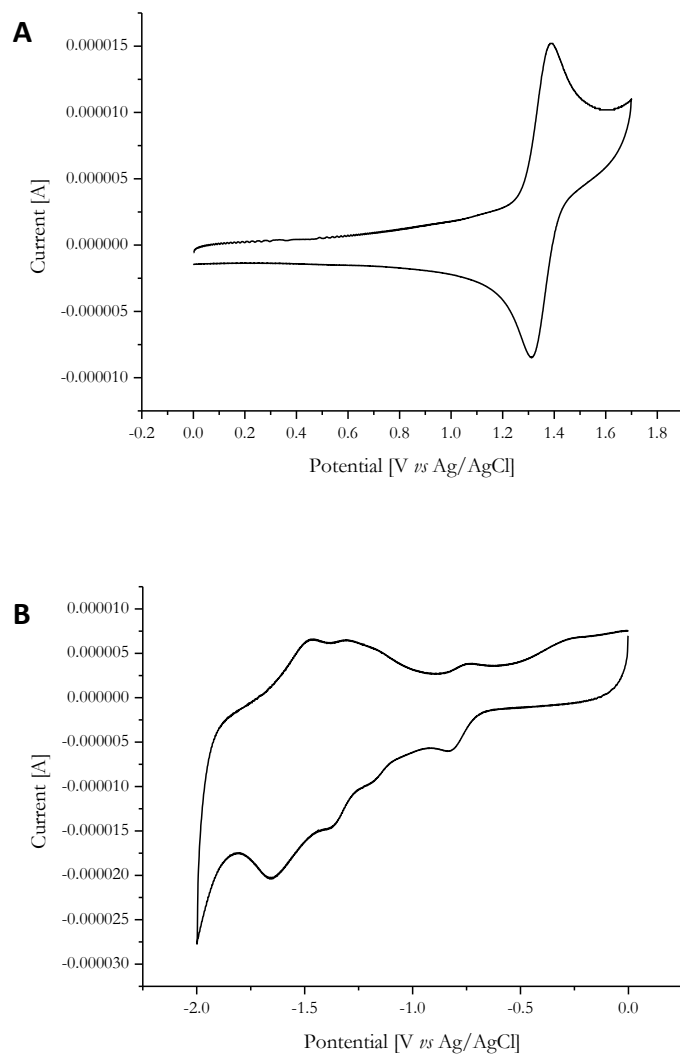


Figure 3.13. Cyclic voltammograms of **Ru-DPACF₂** recorded in dry acetonitrile under argon, with a sweep rate of 0.3 V/s, at room temperature. The concentration of the complex is 8.10^{-4} mol/L, with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte. (A) Positive polarization; (B) negative polarization.

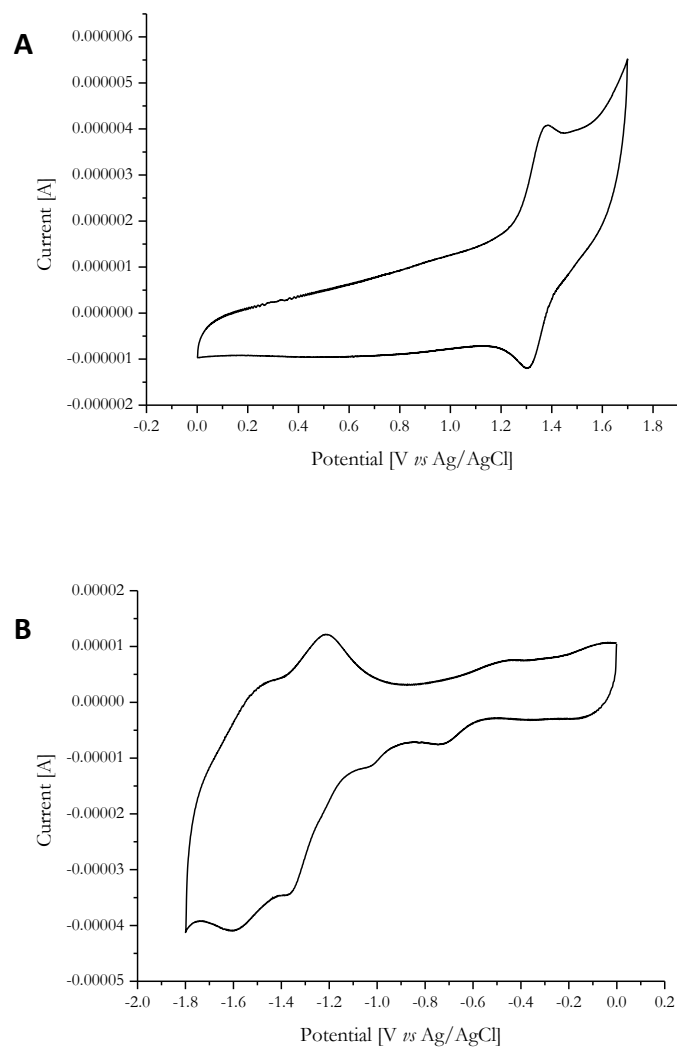


Figure 3.14. Cyclic voltammograms of **Ru-DPACF₄** recorded in dry acetonitrile under argon, with a sweep rate of 0.3 V/s, at room temperature. The concentration of the complex is $8 \cdot 10^{-4}$ mol/L, with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte. (A) Positive polarization; (B) negative polarization.

3.2.3. Absorption spectra and computational studies

The absorption data at ambient temperature in water and acetonitrile, under air and under argon for the three complexes, as well as for other complexes for comparison purposes, are listed in Table 3.4. Absorption spectra for all complexes in acetonitrile are depicted in Figure 3.15 a. The absorption of Ru(II) complexes generally corresponds to the superposition of different transitions involving either the ligands or both the metal and the ligand.⁹³ In the present case, comparison with literature and absorption spectra of the free ligands allowed us to assign the intense absorption ($\epsilon \geq 10^5 \text{ M}^{-1}\text{cm}^{-1}$) bands in the UV region to ligand centered (LC) transitions and the ones in the visible region ($\epsilon \approx 10^4 \text{ M}^{-1}\text{cm}^{-1}$ around $\lambda \approx 400 \text{ nm}$) to Metal-to-Ligand Charge transfer (MLCT) transitions, where L=phen ($^1\text{MLCT}_{\text{phen}}$) and dpac ($^1\text{MLCT}_{\text{dpac}}$).

In the case of **Ru-DPPZ**, the absorption of a photon leads to the formation of a charge separated state $\text{Ru}^{3+}/\text{dppz}^{\bullet-}$ *i.e.* in which an electron is transferred from the metal center towards either the phen or phenazine part of dppz ligand depending on the environment of the complex.^{47,152} This accounts for the peculiar photophysical behavior of **Ru-DPPZ**. In our case, exchanging the phenazine core by an acridine, *i.e.* replacing a nitrogen atom by a carbon atom, should influence the electronic transitions involved in the formation of the excited state. Strikingly, solely a small bathochromic shift ($\Delta\lambda = 8 \text{ nm}$) of the MLCT band is observed for **Ru-DPAC**, **Ru-DPACF₂** and **Ru-DPACF₄** compared to **Ru-DPPZ**.

	Absorbance λ_{max} [nm] (ϵ [$10^4 \text{ M}^{-1}\text{cm}^{-1}$]) ^a	
	MeCN	H ₂ O
[Ru(phen) ₃] ²⁺	418, 447 (1.84)	419, 447 (1.83)
Ru-DPPZ ⁶⁵	221, 264, 370, 440 (2.34)	220, 264, 368, 440
Ru-DPAC	224 (15.1), 264 (16.3), 280 (11.6), 448 (2.62)	224 (15.1), 264 (16.2), 280 (11.6), 448 (2.62)
Ru-DPACF₂	223 (16.2), 264 (19.0), 278 (14.4), 448 (2.88)	223 (16.1), 264 (19.0), 278 (14.3), 448 (2.88)
Ru-DPACF₄	221 (17.1), 264 (18.9), 279 (14.0), 448 (2.87)	221 (17.0), 264 (18.9), 279 (14.1), 448 (2.88)

Table 3.4. Absorption data in CH₃CN and H₂O at 298K in aerated or deaerated solution for **Ru-DPAC**, **Ru-DPACF₂** and **Ru-DPACF₄**. ^a Measurements made with solutions $1.10^{-5} \text{ mol.L}^{-1}$ in complex at room temperature.

In order to get more information on the photophysical scheme of our complexes, **Ru-DPAC**, **Ru-DPACF₂** and **Ru-DPACF₄** as well as **Ru-DPPZ** are studied computationally. The ground and vertical excited states electronic structures are investigated by the means of DFT/TD-DFT calculations using the hybrid PBE0 functional and the LanL2DZ basis set. Solvent (MeCN or H₂O) is included by a conductor-like polarizable continuum model.¹⁵³ With this system, the first runs of calculations performed with water did not account for the experimental differences when going from acetonitrile to water. A similar observation was made by Daniel *et al.* who overcome the limitation of the continuum model that does not describe specific interactions, such as nitrogen–hydrogen bond interactions, by using a discrete model.¹⁵⁴ Water molecules are thus added without any constraint on their position before geometry optimization and calculations.

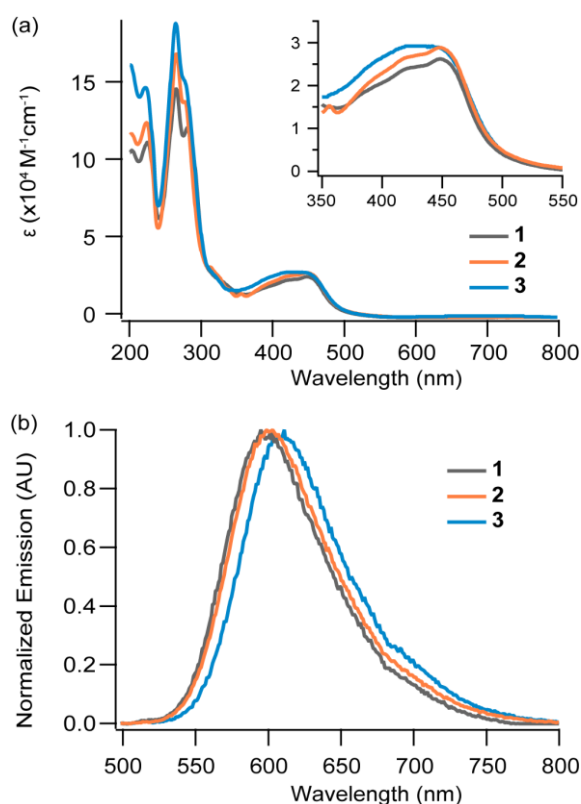


Figure 3.15. (a) Absorption and (b) emission spectra ($\lambda_{\text{exc}} = 448 \text{ nm}$) of **Ru-DPAC** (grey), **Ru-DPACF₂** (orange) and **Ru-DPACF₄** (blue) in acetonitrile under air at room temperature. (a-inset) : absorption bands corresponding to MLCT transitions.

The energy diagram for the different compounds is presented in Figure 3.16 (further details on electronic structures can be found in Tables 3.8-3.13). As convention for the rest of this manuscript, the electron-accepting portion of the extended ligands is underlined in order to differentiate the two moieties which are suggested to participate in the relaxation processes. The contributions of each moiety of the complexes are depicted in different colors; in particular, the phenanthroline part of the extended ligand (dpac, green) and the terminal fragment (dpac/dppz, orange) are differentiated in order to discuss their respective contribution. Regardless of the solvent, the introduction of electron-withdrawing fluoride modifies the electronic repartition in the ground state. If it has only little effect on the Ru

(red) and ancillary phen (blue) centered orbitals, it strongly stabilizes the levels centered on the dpac/dppz ligand. Besides, as the number of fluoride increases, the dpac contribution increases in the LUMO while the dpac contribution increases in the LUMO+1.

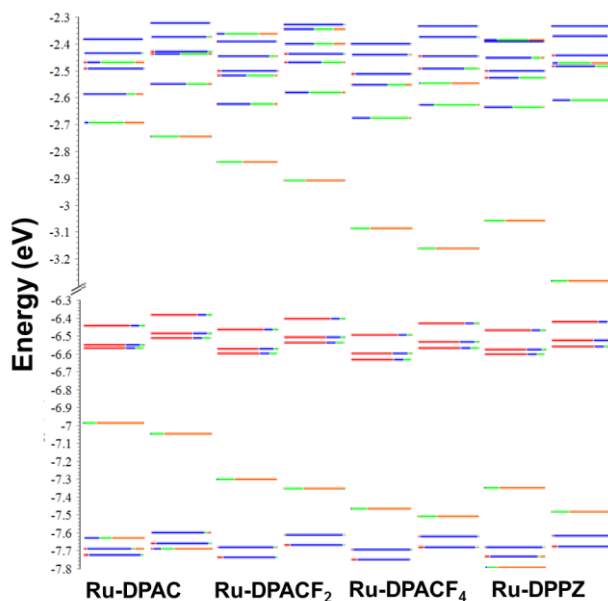


Figure 3.16. Energy diagram of **Ru-DPAC**, **Ru-DPACF₂** and **Ru-DPACF₄** and **Ru-DPPZ**. Fragments contributions are reported with different colours: Ru in red, ancillary phen in blue, dpac/dppz in green, dpac/dppz in orange. Solvent used: left = MeCN, right = H₂O.

The experimental absorption spectra matched well with the calculated transitions, allowing us to attribute the different transitions using natural transitions orbitals (NTOs)¹⁵⁵ (see associated content, Figures 3.36-3.38). When comparing the absorption spectra of the different complexes in acetonitrile, one can observe that there are no major differences in the position and intensities of the absorption maxima. Indeed, in all cases the ¹MLCT_{phen} are the main transitions in the visible part of the spectra (400-550 nm); transitions involving the ¹MLCT_{dpac} and ¹MLCT_{dpac/dppz} of lower intensities are red-shifted as the number of fluoride increases but does not affect the final absorption spectra. A similar explanation can be used to account for

the similarity between the absorption spectra in acetonitrile and water. As the polarity of the solvent increases, the levels centered on the metal and ancillary phen are equally destabilized, leaving the $^1\text{MLCT}_{\text{phen}}$ unchanged while the dpac/dppz orbitals are stabilized, leading to a red-shift of the $^1\text{MLCT}_{\text{dpac/dppz}}$ and $^1\text{MLCT}_{\text{dpac/dppz}}$ transitions. These shifts are however small, as seen in Table 3.4.

3.2.4. Emission spectra, lifetimes, quantum yields and computational studies

All our complexes display broad unstructured emission (Figure 3.15 b), typical of a $^3\text{MLCT}$ -type excited state, both in organic solvent and water. The luminescence lifetimes and the quantum yield of luminescence for each complex are measured in water and acetonitrile under inert atmosphere (Table 3.5). The positive solvatochromic effect ($\Delta\lambda \approx +20$ nm when going from DCM to water – data not shown), the large k_r values and the hypsochromic shift of the emission band at 77 K (see associated content) confirm the charge transfer character of the excited state. It is worth mentioning that, in air-equilibrated solvents, a decrease in the luminescence intensity with respect to degassed solutions is systematically observed, indicating a quenching by oxygen (see associated content). We expect that, as for other Ru(II) complexes, an efficient photosensitization of $^1\text{O}_2$ by the excited Ru(II) complexes is occurring.¹⁵⁶ Other quenching mechanisms like electron transfer to form superoxide anion can also occurs. Nevertheless, the determination of the quantum yield of singlet-oxygen photosensitization is beyond the scope of this study.

	Emission $\lambda_{em\ max}$ [nm]		Emission λ_{max} at 77 K [nm]	$\Phi_{em}^{a, c, d}$		τ_{em} [ns] ^d		k_r [$10^3\ s^{-1}$] ^d		k_{nr} [$10^5\ s^{-1}$] ^d	
	MeCN	H ₂ O		MeCN	H ₂ O	MeCN	H ₂ O	MeCN	H ₂ O	MeCN	H ₂ O
[Ru(phen) ₃] ²⁺	604	606	564 ^[36]	0.028	0.072	460	920	60.9	75.0	21	9.7
Ru-DPPZ ^[30]	618	- [*]	580 ^[7d]	0.033	- [*]	663	- [*]	49.8	- [*]	15	- [*]
Ru-DPAC	597	604	567	0.038	0.066	710	999	53.5	66.1	14	9.4
Ru-DPACF₂	604	607	573	0.052	0.189	875	958	59.4	197.3	11	8.5
Ru-DPACF₄	609	611	568	0.084	0.050	915	238	91.8	210.1	10	40

Table 3.3. Emission data for **Ru-DPAC**, **Ru-DPACF₂** and **Ru-DPACF₄**. ^a Measurements made with solutions $1.10^{-5}\ mol\ L^{-1}$ in complex at 298 K. ^b Measurements made under ambient air conditions. ^c The quantum yield of luminescence is calculated relative to the one of [Ru(bpy)₃]²⁺ in an aerated aqueous solution ($\Phi_{em}^{air} = 0.028$)^[28]. ^d Quantum yield of luminescence, luminescence lifetimes and radiative rate constants are determined under an argon atmosphere. Errors estimated to 10%. ^{*} No luminescence was observed in H₂O.

The emission maxima are almost identical for **Ru-DPAC** and **Ru-DPACF₂**; the addition of the two fluorine atoms onto the dpac ligand has thus only a small influence on the energy difference between the ³MLCT and the ground state, while this energy decreases for **Ru-DPACF₄**. This observation is in agreement with the decrease in energy of the π^* orbital centered on the extended planar ligand going to a more electron-withdrawing core. Interestingly, none of these complexes behave like **Ru-DPPZ**, which is known as a “light switch”.

Numerous experimental and theoretical studies have been devoted to understand the “light switch” effect.^{104,118,144,157} It is now commonly accepted that this quenching is caused by hydrogen-bonding with water molecules, leading to the stabilization of dark states, such as ³MLCT_{dppz} and ³LC_{dppz}, the latter being the lowest triplet state (summarized in Figure 3.17 c).¹¹⁸ Our calculations lead to similar result with a change in the nature of the lowest Metal-to-Ligand Charge transfer state from a bright ³MLCT_{phen} in acetonitrile toward a more stabilized dark ³MLCT_{dppz} in water.

In the case of our complexes, **Ru-DPAC** and **Ru-DPACF₂** do not present a light switch behaviour while **Ru-DPACF₄** partially does as its luminescence is notably reduced in water, indicated by its smaller quantum yield and larger k_{nr} value in water. Once again, computational studies are used to gain some information in order to explain this behaviour and compare it with **Ru-DPPZ**. The qualitative results obtained for the triplet TD-DFT calculations are used to draw photophysical schemes presented in Figure 3.17 for each situation. The computational studies show that ³LC states centered on dpac/dppz ligands have a lower energy than ³MLCT states for all complexes. The presence of such ³LC states was also reported in computational studies realized previously.^{154,157,158} Nevertheless, the experimental characteristics of emission clearly indicate that the luminescence of all Ru(II) complexes originates from ³MLCT states and that low-lying ³LC states are not likely to play a role in the deactivation of ¹MLCT states generated upon light irradiation (see Table 3.5, larger quantum yield of luminescence for complexes **Ru-DPAC**, **Ru-DPACF₂** and

Ru-DPACF₄ in MeCN compared to [Ru(phen)₃]²⁺). Therefore, we focus our discussion on the ³MLCT manifold which accounts for the photophysical behaviour of our complexes.

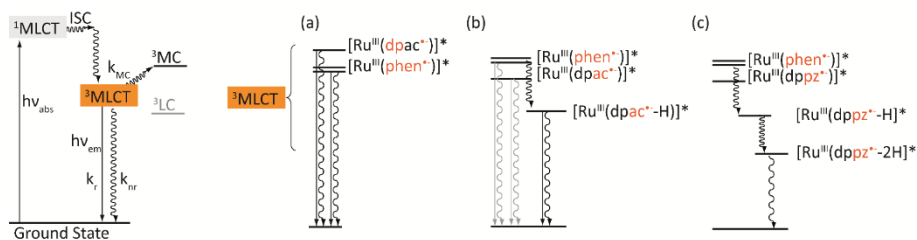


Figure 3.17. Representation of the photophysical scheme for dpac-based complexes in water. The deactivation of ³MLCT states is emphasized on the right part of the figure for (a) **Ru-DPAC** and **Ru-DPACF₂**, (b) **Ru-DPACF₄**, and (c) **Ru-DPPZ** in water. The photophysical scheme for all four complexes in MeCN can be described as in (a). Solid arrows stand for radiative wavy arrows stand for non-radiative transitions (ISC, IC or VR).

In acetonitrile, all compounds follow the scheme 3.17 a. After an ultrafast ISC^{159,160} from ¹MLCT states generated upon absorption of photon, ³MLCT states are populated corresponding to charge transfer from the Ru(II) center towards either the ancillary phen or the phen part of the planar ligand (dpac or dppz). The similar energies of these emissive ³MLCT states explain the similarity of the luminescence maxima observed for all complexes.

In water, complexes **Ru-DPAC** and **Ru-DPACF₂** present a stronger emission and are longer lived (see Table 3.5). Calculations show that the relative energy of each ³MLCT state is not significantly modified and the fragment contributions in each triplet state remain similar as in MeCN; hence these compounds behave like [Ru(phen)₃]²⁺ and exhibit an increased luminescence in water compared to organic solvent. **Ru-DPAC** and **Ru-DPACF₂** follow then the same photophysical scheme in water than in MeCN (scheme 3.17 a). In contrast to **Ru-DPPZ** where lowest excited states imply the phenazine core of the dppz, the acridine core in **Ru-DPAC** and **Ru-DPACF₂** seems not to be involved and ³MLCT remains localized on the ancillary ligands or the phen moiety of the dpac ligands. We thus propose for these complexes that

their lowest excited states are luminescent $^3\text{MLCT}_{\text{phen/dpac}}$. $^3\text{MLCT}_{\text{dpac}}$ excited states are assumed to be higher in energy than the $^3\text{MLCT}_{\text{phen/dpac}}$ ones for these two complexes (in contrast to $^3\text{MLCT}_{\text{dppz}}$ for **Ru-DPPZ**), suggesting the acridine core disfavours the charge separation process at the excited state in **Ru-DPAC** and **Ru-DPACF₂**. A similar behaviour has been previously observed for TPAC-based mono- and di-nuclear Ru(II) complexes, for which the electrochemical and spectroscopic data indicate that the lowest MLCT transition is a $^3\text{MLCT}_{\text{tpac}}$ one and that dinucleation affects very slightly these properties. This suggests that the two metallic centres of the dinuclear TPAC-based Ru(II) complexes behave independently in spite of the conjugated TPAC bridging ligand.^{108,109,161}

In opposition to the former ones, **Ru-DPACF₄** displays a much shorter luminescence lifetime and lower quantum yield in water than in acetonitrile (Table 3.5). For this complex, we assume that upon absorption of light, a $^1\text{MLCT}_{\text{phen}}$ is formed, which rapidly undergoes an ISC to luminescent $^3\text{MLCT}_{\text{phen/dpac}}$. Further relaxation occurs on the acridine moiety of the extended planar ligand, leading to the formation of $^3\text{MLCT}_{\text{dpac}}$ excited state. In water, there is an interconversion between two excited states: one assigned to the non-hydrogen bonded excited state dpac , and the other one to a mono-hydrogen bonded excited state (depicted as dpac-H , in scheme 3.17 b). The presence of the dpac-H excited state is suggested by the shorter excited state lifetime of this complex in water. In a similar way than described for **Ru-DPPZ**,¹¹³ we proposed that the excited state localized on dpac , which is higher in energy in aprotic solvents, is stabilized when involved in a hydrogen bond and thus becomes dominant in water. Indeed, protic solvents would stabilize the charge-separated excited state due to the formation of hydrogen bond with the free nitrogen atom of the dpacF_4 ligand. The partial quenching of luminescence of **Ru-DPACF₄** lies in agreement with the model proposed by Lincoln and co-workers for which two Hydrogen bonds are required for the full luminescence quenching of the excited state for **Ru-DPPZ** in water. The photophysical behaviour of **Ru-DPACF₄** is thus

contrasting with the photophysical properties of **Ru-DPAC** and **Ru-DPACF₂**, which are similar to the ones of [Ru(phen)₃]²⁺.

3.2.5. Photophysical behaviour in the presence of DNA and polynucleotides

As mentioned in the introduction, Ru(II) complexes are relevant tools to probe DNA. The influence of hydrogen bonds with water on the photophysics of the complex is one of the key parameters in this context, but not the only one at stake. With the notable exception of some photoreactive complexes,^{142,143} polypyridyl Ru(II) complexes are more luminescent in the presence of DNA. This enhancement of luminescence is due to the association of the complex with DNA, which protects the excited complex from quenching or non-radiative deactivations. If complexes possess one extended planar ligand, a specific interaction is observed with DNA, where the complex is intercalated inside the bases stack. This intercalation mode generally leads to strong association ($K_{\text{aff}} \approx 10^6 \text{ L.mol}^{-1}$) and an intense luminescence enhancement of the excited intercalated complex in comparison to the free complex left in aqueous media.^{111,120,145,162} Interestingly, **Ru-DPPZ** is able to distinguish matched and single-base mismatched sites in oligonucleotides. This small sensitivity of luminescence enhancement in the presence of a mismatch can be correlated to its thermodynamic stability.¹⁰⁶ We thus investigate the ability of our new complexes to probe DNA and some particular topologies, like different single-base mismatches.

In the presence of increasing concentration of DNA (CT-DNA and SS-DNA), the luminescence of our three dpac complexes is enhanced (Figure 3.18). This increase of luminescence for the three complexes is strong and occurs even at a very low ratio of DNA per complex, suggesting a high binding affinity of the complexes for these two DNAs. In order to determine a binding affinity for these equilibria, we used a modified McGhee-von Hippel equation¹⁶³ as expression for the luminescence intensity as function of ratio of Ru(II) per DNA (I/I_0 vs $[\text{DNA}]/[\text{complex}]$, with I_0 , the intensity of luminescence in the absence of DNA and $[\text{DNA}]$ expressed in base-pair equivalents, see

Table 3.6 and Figure 3.18). The luminescence enhancement observed presumably results from a decrease of the non-radiative deactivation processes of the $^3\text{MLCT}$ excited states. This protection is induced by the double helix microenvironment which protects the excited complexes from quenching by oxygen. Our extended planar ligands being similar to dppz regarding its structure, we assume our complexes also intercalate into the DNA strands, which is consistent with the binding values obtained.

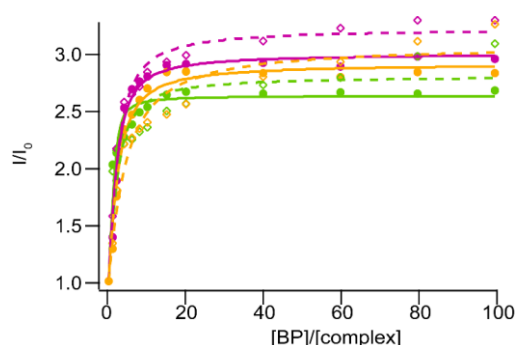


Figure 3.18. Steady-state luminescence titration ($\lambda_{\text{exc}} = 448 \text{ nm}$) of **Ru-DPAC** (green), **Ru-DPACF₂** (purple) and **Ru-DPACF₄** (orange) with SS-DNA (full circles) and CT-DNA (empty diamonds). Measurements are performed using $10 \mu\text{M}$ of complex in Tris.HCl buffer 50 mM , NaCl 50 mM at pH 7.4 under ambient air conditions. The fitted curves are obtained using a modified McGhee-Von Hippel equation and drawn as solid and dashed curves for SS- and CT-DNA respectively.

	SS-DNA		CT-DNA	
	$K_{\text{aff}} [\text{L.mol}^{-1}]^a$	$I/I_0 \text{ max}$	$K_{\text{aff}} [\text{L.mol}^{-1}]^a$	$I/I_0 \text{ max}$
Ru-DPAC	$1.8 \cdot 10^6$	2.6	$4.2 \cdot 10^5$	2.8
Ru-DPACF₂	$4.8 \cdot 10^5$	3.0	$3.7 \cdot 10^5$	3.2
Ru-DPACF₄	$3.6 \cdot 10^5$	2.9	$1.5 \cdot 10^5$	3.1

Table 3.6. Binding affinity constants calculated for **Ru-DPAC**, **Ru-DPACF₂** and **Ru-DPACF₄** with SS-DNA and CT-DNA. ^a Binding constant are obtained using a McGhee-von Hippel type equation; the binding site was fixed to two base pairs per complex (best fit). Errors estimated to 5%.

Even if these two natural DNAs contain nearly the same G-C base pairs content (43% for SS-DNA vs 42% for CT-DNA)¹⁶⁴ the luminescence behaviour is different for the complexes **Ru-DPAC**, **Ru-DPACF₂** and **Ru-DPACF₄** in the presence of each DNA. Such observation could arise from the different topologies of these two DNAs: CT-DNA features a long and well-defined scaffold (> 10,000 base pairs),¹⁶⁵ while SS-DNA is made of shorter (~ 2,000 base pairs)¹⁶⁶ and poorly defined sequences. The interaction between the complexes and the DNA sequences depends on the local environment encountered by the intercalated complex^{66,67}; the heterogeneity of these environments results in small but noticeable differences in the optical properties of the complexes. The binding curves obtained are thus affected by this heterogeneity, but overall the binding constants calculated are in good agreement with an intercalation mode of interaction between each of our complexes and DNA. In the case of SS-DNA, the emission intensity increases quickly to reach a plateau value, between 2.6 and 2.9 for the three complexes, the fluorinated analogues **Ru-DPACF₂** and **Ru-DPACF₄** displaying slightly higher I/I_0 values than the non-fluorinated complex **Ru-DPAC**.

We also investigated the ability of **Ru-DPAC** to detect base mismatches in short oligonucleotides by using a series of hairpin oligonucleotide containing a mismatch near the center of the duplex (see sequences in Figure 3.19). As shown in Figure 3.20, an attenuation of the luminescence is observed for the mismatched hairpins with respect to the complementary one. No differential luminescence intensity for the nature of the mismatch occurs. The absence of specificity for the nature of the mismatch sites, and, more surprisingly, the lower luminescence enhancement of the complex in the presence of mismatched hairpin compared to the complementary one lead us to the conclusion that **Ru-DPAC** is less effectively intercalated between the mismatched base pair stack. This less effective intercalation results in an increased exposure to oxygen with respect to the complementary sites, a looser fit of the complex within the mismatch site that favors non-radiative relaxation pathways of the excited state, leading to a decreased luminescence and/or a lower

binding affinity for these sites compared to complementary sites. Since a saturation of the interaction between the complex and the hairpins cannot be obtained, it is difficult to differentiate the processes at stake in this case. The behaviour observed for **Ru-DPAC** differs from the one reported for the **Ru-DPPZ**¹⁰⁶ in the same conditions. This fundamental difference underlines the complexity of designing complexes able to probe mismatched sites in DNA.

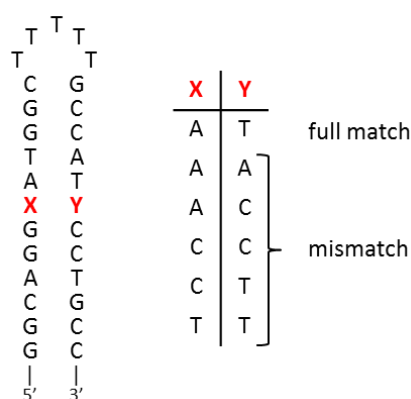


Figure 3.19. Hairpin oligonucleotides sequences used for titrations.

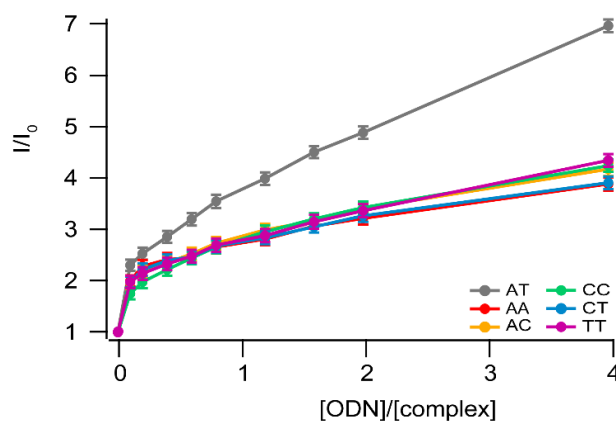


Figure 3.20. Steady-state luminescence titration of **Ru-DPAC** with a series of small hairpins oligonucleotides which possess (or not) a mismatched base pair. Measurements are performed using 1 μ M of complex in Tris.HCl buffer 5 mM, NaCl 1 mM, pH 7.5 under ambient air conditions.

3.3. Conclusions

We successfully synthesized and characterized new Ru(II) complexes having extended planar ligands based on an acridine core. Their spectroscopic and electrochemical data revealed that **Ru-DPAC** and **Ru-DPACF₂** allow emission from a $^3\text{MLCT}_{\text{phen/dpac}}$ excited state, which explain the similarity of the luminescence maxima. As only minor reorganizations of the different triplet excited states of **Ru-DPAC** and **Ru-DPACF₂** occur in water, these compounds behave like $[\text{Ru}(\text{phen})_3]^{2+}$ and exhibit an increased luminescence in this solvent. However, the lowest $^3\text{MLCT}$ for **Ru-DPACF₄** change from a bright $^3\text{MLCT}_{\text{phen/dpac}}$ state towards a poorly luminescent $^3\text{MLCT}_{\text{dpac}}$ state. The stabilization of this state against the other $^3\text{MLCT}$ states occurs in water via the establishment of a hydrogen bond; the relative population of excited state is then shifted to this state and emissive $^3\text{MLCT}_{\text{phen/dpac}}$ states are less populated, yielding a reduction of the luminescence of **Ru-DPACF₄** in water compared to organic solvents.

Studies of these acridine-based complexes in the presence of DNA demonstrate that they strongly bind to polynucleotides and that this association, attributed to an intercalation within the base pairs stacking, is accompanied by an enhancement of their luminescence. This enhancement is presumably due to a decrease of the non-radiative deactivation processes of the $^3\text{MLCT}$ excited state of complexes intercalated in the double helix, where the microenvironment protects them from quenching by oxygen. In the case of **Ru-DPACF₄**, an inhibition of hydrogen bonds between the tetrafluoro-acridine and water is likely to be responsible for the luminescence enhancement upon intercalation through DNA; this process is the same as the one responsible for the light-switching behavior of the well-known **Ru-DPPZ** complex. **Ru-DPAC** shows differential luminescence intensity for matched and mismatched hairpins, but no discrimination for the nature of the mismatch site. We suggest that a looser fit to mismatched containing hairpins lies at the basis of the observed luminescence behaviour of this complex, resulting in an increased exposure to oxygen with respect to the

complementary sites. The design of extended planar ligands allowing better differential interactions between their corresponding Ru(II) complexes and polynucleotides is currently under investigation in our group.

3.4. Experimental section

3.4.1. Materials and instrumentation

[Ru(phen)₂Cl₂]¹⁶⁷ has been synthesized according to previously described literature protocol. All solvents and reagents for the synthesis are of reagent grade and are used without any further purification. All solvents for the spectroscopic and electrochemical measurements are of spectroscopic grade. Water is purified with a Millipore Milli-Q system. Calf thymus DNA Type I (CT-DNA) and Salmon sperm DNA (SS-DNA) were purchased from Sigma-Aldrich. Hairpins ODN were purchased from Eurogentec. DNA and ODN concentrations are determined spectroscopically ($\lambda_{260\text{ nm}} = 6600\text{ M}^{-1}\text{ cm}^{-1}/\text{bp}$ for CT-DNA and SS-DNA^{168,169}; $\lambda_{260\text{ nm}} = 260000\text{ M}^{-1}\text{ cm}^{-1}$ for ODN-AT, 264900 $\text{M}^{-1}\text{ cm}^{-1}$ for ODN-AA, 253300 $\text{M}^{-1}\text{ cm}^{-1}$ for ODN-CC, 259100 $\text{M}^{-1}\text{ cm}^{-1}$ for ODN-AC, 254200 $\text{M}^{-1}\text{ cm}^{-1}$ for ODN-CT, 257500 $\text{M}^{-1}\text{ cm}^{-1}$ for ODN-TT). The molar extinction coefficient of hairpin oligonucleotides are calculated values based on the base content of each sequence. ¹H NMR experiments are performed in CDCl₃, CD₃OD or CD₃CN on a Bruker AC-300 Avance II (300 MHz) or on a Bruker AM-500 (500 MHz) at 20°C. The chemical shifts (given in ppm) are measured vs the residual peak of the solvent as the internal standard. High-resolution mass spectrometry (HRMS) spectra are recorded on a Q-Exactive orbitrap from ThermoFisher using reserpine as internal standard. Samples are ionized by electrospray ionization (ESI; capillary temperature = 320°C, vaporizer temperature = 320°C, sheath gas flow rate = 5 mL/min). UV-vis absorption spectra are recorded on a Shimadzu UV-1700. Room temperature fluorescence spectra are recorded on a Varian Cary Eclipse instrument. Luminescence intensity at 77K is recorded on a FluoroLog3 FL3-22 from Jobin Yvon equipped with an 18V 450W

Xenon Short Arc lamp and an R928P photomultiplier, using an Oxford Instrument Optistat DN nitrogen cryostat controlled by an Oxford Intelligent Temperature Controller (ITC503S) instrument. For the luminescence lifetime measurements as a function of temperature, the pulsed excitation source was a pulsed laser Nd:YAG Q-switched laser (Continuum Inc.) frequency-tripled (355 nm) coupled with an optical parametric oscillator (Continuum Inc.) covering the wavelengths region 410-2300 nm with a maximum pulse energy from 10 to 120 mJ depending on the wavelength. The emission was detected perpendicularly by a photomultiplier (R928, Hamamatsu). The signal was recorded with a digital oscilloscope (HP 54200A), connected through the IEEE488 interface to a personal computer, and was averaged over at least 16 shots. The emission wavelength was selected via a grating Czerny-Turner monochromator (Spectra Pro 2300i, Acton Research Corp.) Cyclic voltammetry is carried out in a one-compartment cell, using a glassy carbon disk working electrode (approximate area = 0.03 cm²), a platinum wire counter electrode, and an Ag/AgCl reference electrode. The potential of the working electrode is controlled by an Autolab PGSTAT 100 potentiostat through a PC interface. The cyclic voltammograms are recorded with a sweep rate of 300 mV s⁻¹, in dried acetonitrile or N,N-dimethylformamide (Sigma-Aldrich, HPLC grade). The concentration of the complexes is 8.10⁻⁴ mol/L, with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte. Before each measurement, the samples are purged by nitrogen. The molar absorption coefficients are determined by ¹H NMR using 2,2'-((perfluoroethane-1,2-diyl)bis(oxy))bis(2,2-difluoroethan-1-ol) as reference. DFT calculations are performed using Gaussian 09.¹⁷⁰ The hybrid functional PBE0 is used to carry the DFT¹⁷¹⁻¹⁷⁴ and TD-DFT¹⁷⁵⁻¹⁷⁷ calculations in combination with the LanL2DZ¹⁷⁸⁻¹⁸¹ basis set for all atoms. Geometry optimizations are conducted without symmetry constraints. Frequency calculations on each optimized structure confirmed that energy minima is reached. The energy, oscillator strength, and related MO contributions for the 100 lowest singlet–singlet and 10 lowest singlet–triplet excitations for the S₀-optimized geometry are obtained respectively from the TD-DFT/singlets and the

TD-DFT/triplets output files. One should note that the PBE0 functional, while providing the correct excitation energies is not able to fully reproduce their relative intensity. This behavior has already been noticed in the literature in the case of even simpler compounds and different functional and more elaborated approaches have been used in cases where the band shape is of interest which was actually not the case of the present study.¹⁸² GaussView3.0.9¹⁸³, GaussSum 3.0¹⁸⁴ and Chemissian 4.38¹⁸⁵ software are used for data analysis, visualization and surface plots. Calculations are performed in MeCN or H₂O solution by use of the conductor-like polarized continuum (CPCM) solvation model as implemented in Gaussian 09.¹⁷⁰ Because the continuum model could not describe specific interactions such as nitrogen–hydrogen bond interactions, a discrete model is also used in an approach similar to what Daniel *et al.* have recently reported.¹⁵⁴

3.4.2. Synthetic procedures and characterization of *dpac* ligands and respective Ru(II) complexes

2-aminobenzyl alcohol. To a solution of anthranilic acid (617.3 mg, 4.50 mmol) in dry Et₂O (30.0 mL) is added dropwise a solution of LiAlH₄ in dry Et₂O (300.0 mg in 7.5 mL). The mixture is then stirred at reflux (35°C) for 1h. After cooling to room temperature, EtOAc (25.0 mL) is added, followed by a solution of NaOH 2M until effervescence stops, and MilliQ water (40.0 mL). The organic phase is separated and the aqueous phase is extracted with Et₂O (3x 30.0 mL). The combined organic phases are dried over MgSO₄, evaporated and dried under vacuum. The desired compound is obtained as a beige solid. Yield: 93%. ¹H NMR (300 MHz, CDCl₃): δ 7.15 (ddd, J_{c-d} = 8.0 Hz, J_{c-b} = 8.0 Hz, J_{c-a} = 1.6 Hz, 1H, H_c), 7.07 (dd, J_{a-b} = 7.6 Hz, J_{a-c} = 1.6 Hz, 1H, H_a), 6.70-6.75 (m, 2H, H_b and H_d), 4.70 (s, 2H, CH₂), 3.19 (br, 2H, NH₂).

dpac. 5-amino-1,10-phenanthroline (183.0 mg, 0.938 mmol) and 2-aminobenzyl alcohol (115.6 mg, 0.938 mmol) are suspended in 6 N HCl (3.0 mL). The reaction mixture is stirred at reflux (65°C) for 20h and, after cooling to room temperature, the reaction is quenched by addition of NH₃.H₂O until reaching pH 9. The orange precipitate is

filtered and purified by neutral alumina column of chromatography (eluent: acetone/methanol gradient, from 100/0 to 90/10), yielding the targeted compound as a dark orange solid. Yield: 93%. ^1H NMR (500 MHz, CD_3OD): δ 9.77 (dd, $J_{c-b} = 8.3$ Hz, $J_{c-a} = 1.8$ Hz, 1H, H_c), 9.68 (s, 1H, H_d), 9.30 (dd, $J_{i-j} = 8.3$ Hz, $J_{i-k} = 1.5$ Hz, 1H, H_i), 9.14 (dd, $J_{a-b} = 4.5$ Hz, $J_{a-c} = 1.8$ Hz, 1H, H_a), 9.08 (dd, $J_{k-j} = 4.5$ Hz, $J_{k-i} = 1.6$ Hz, 1H, H_k), 8.34 (d, H_e , $J_{e-f} = 8.7$ Hz, 1H), 8.26 (d, H_h , $J_{h-g} = 7.6$ Hz, 1H, H_h), 7.95 (dd, $J_{f-e} = 8.5$ Hz, $J_{f-g} = 6.9$ Hz, 1H, H_f), 7.89 (2dd, $J_{b-c} = J_{j-i} = 8.0$ Hz, $J_{b-a} = J_{j-k} = 4.5$ Hz, 2H, H_b and H_j), 7.76 (t, $J_{g-h} = 7.2$ Hz, 1H, H_g). MS (APCI, +c): calc. 282.10 for $\text{C}_{19}\text{H}_{12}\text{N}_3$, found 282.27 ($\text{M}+\text{H}^+$).

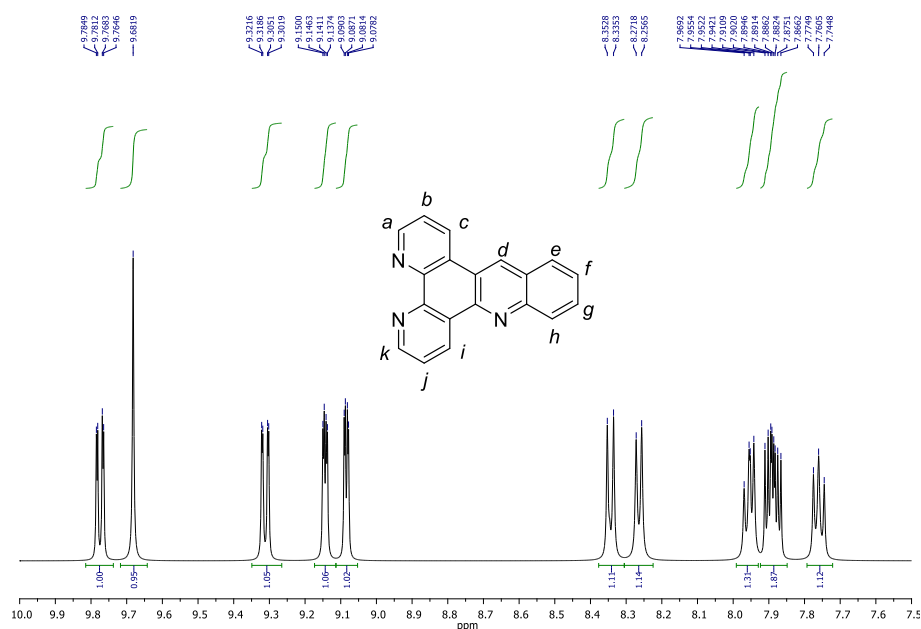


Figure 3.21. ^1H -NMR spectrum for dpac (500 MHz, CD_3OD).

(2-amino-4,5-difluorophenyl)methanol. To a solution of 4,5-difluoroanthranilic acid (200.0 mg, 1.155 mmol) in dry Et_2O (8.0 mL) is added dropwise a solution of LiAlH_4 in dry Et_2O (78.5 mg in 2.0 mL). The mixture is then stirred at reflux (35°C) for 1h15. After cooling to room temperature, EtOAc (10.0 mL) is added, followed by a solution of NaOH 2M until effervescence stops, and MilliQ water (15.0 mL). The organic phase is separated and the aqueous phase is extracted with Et_2O (3x 10.0 mL). The combined organic phases are

dried over MgSO_4 , evaporated and dried under vacuum. The desired compound is obtained as a flaky yellowish-brownish solid. Yield: 91%. ^1H NMR (300 MHz, CDCl_3): δ 6.92 (dd, $J_{a-b} = 10.5$ Hz, $J_{a-c} = 8.6$ Hz, 1H, H_a), 6.50 (dd, $J_{d-c} = 11.8$ Hz, $J_{d-b} = 6.8$ Hz, 1H, H_d), 4.61 (s, 2H, CH_2), 4.12 (br, 2H, NH_2).

dpacF2. 5-amino-1,10-phenanthroline (50.0 mg, 0.256 mmol) and (2-amino-4,5-difluorophenyl)methanol (40.8 mg, 0.256 mmol) are suspended in 6 N HCl (1.0 mL). The reaction mixture is stirred at reflux (65°C) for 20h and, after cooling to room temperature, the reaction is quenched by addition of $\text{NH}_3\cdot\text{H}_2\text{O}$ until reaching pH 9. The orange-red precipitate is filtered and purified by neutral alumina column of chromatography (eluent: acetone/methanol gradient, from 100/0 to 90/10), yielding the targeted compound as an orange solid. Yield: 98%. ^1H NMR (300 MHz, CDCl_3): δ 9.70 (dd, $J_{c-b} = 8.2$ Hz, $J_{c-a} = 1.9$ Hz, 1H, H_c), 9.27 (dd, $J_{a-b} = 4.5$ Hz, $J_{a-c} = 1.8$ Hz, 1H, H_a), 9.25 (s, 1H, H_d), 9.23 (dd, $J_{k-j} = 4.4$ Hz, $J_{k-i} = 1.5$ Hz, 1H, H_k), 8.96 (dd, $J_{i-j} = 8.3$ Hz, $J_{i-k} = 1.6$ Hz, 1H, H_i), 8.10 (dd, $J_{e-f} = 10.9$ Hz, $J_{e-g} = 7.6$ Hz, 1H, H_e), 7.78 (m, 3H, H_b , H_j and H_h). MS (APCI, +c): calc. 318.08 for $\text{C}_{19}\text{H}_{10}\text{F}_2\text{N}_3$, found 318.55 ($\text{M}+\text{H}^+$).

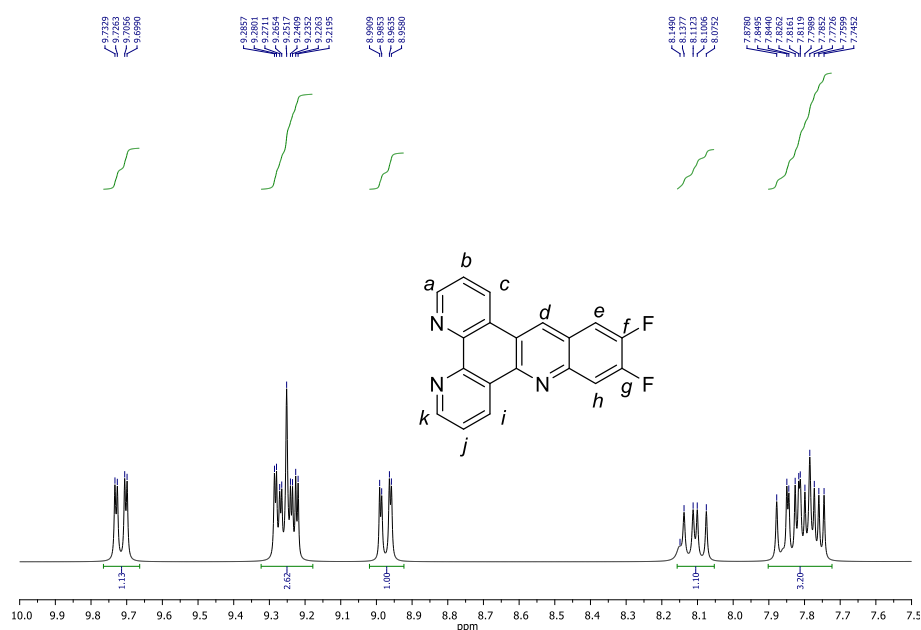


Figure 3.22. ^1H -NMR spectrum for dpacF₂ (300 MHz, CDCl_3).

(2-amino-3,4,5,6-tetrafluorophenyl)methanol. To a solution of 2-amino-3,4,5,6-tetrafluorobenzoic acid (200.0 mg, 0.9565 mmol) in dry Et_2O (8.0 mL) is added dropwise a solution of LiAlH_4 in dry Et_2O (76.5 mg in 2.0 mL). The mixture is then stirred at reflux (35°C) for 2h10. After cooling to room temperature, EtOAc (10.0 mL) is added, followed by a solution of NaOH 2M until effervescence stops, and MilliQ water (15.0 mL). The organic phase is separated and the aqueous phase is extracted with Et_2O (3x 10.0 mL). The combined organic phases are dried over MgSO_4 , evaporated and dried under vacuum. The desired compound is obtained as a beige solid. Yield: 93%. ^1H NMR (300 MHz, CDCl_3): δ 4.78 (d, $J_{\text{CH}_2-\text{F}} = 1.95$ Hz, 2H, CH_2), 3.63 (br, 2H, NH_2).

dpacF₄. 5-amino-1,10-phenanthroline (167.1 mg, 0.8568 mmol) and (2-amino-3,4,5,6-tetrafluorophenyl)methanol (167.1 mg, 0.8568 mmol) are suspended in 6 N HCl (2.0 mL). The reaction mixture is stirred at reflux (65°C) for 20h and, after cooling to room temperature, the reaction is quenched by addition of $\text{NH}_3 \cdot \text{H}_2\text{O}$ until reaching pH 9.

The orange-red precipitate is filtered and purified by neutral alumina column of chromatography (eluent: acetone/methanol gradient, from 100/0 to 90/10). After evaporation of the solvents, the residue is washed with chloroform. The precipitate is filtered and the filtrate is evaporated yielding the targeted compound as an orange-yellow solid. Yield: 94%. ^1H NMR (300 MHz, CDCl_3): δ 9.77 (dd, $J_{c-b} = 8.1$ Hz, $J_{c-a} = 1.7$ Hz, 1H, H_c), 9.55 (s, 1H, H_d), 9.31 (dd, $J_{a-b} = 4.5$ Hz, $J_{a-c} = 1.7$ Hz, 1H, H_a), 9.27 (dd, $J_{k-j} = 4.4$ Hz, $J_{k-i} = 1.5$ Hz, 1H, H_k), 9.05 (dd, $J_{i-j} = 8.2$ Hz, $J_{i-k} = 1.5$ Hz, 1H, H_i), 7.83 (m, 2H, H_b and H_j). MS (APCI, $+c$): calc. 354.07 for $\text{C}_{19}\text{H}_8\text{F}_4\text{N}_3$, found 354.31 ($\text{M}+\text{H}^+$).

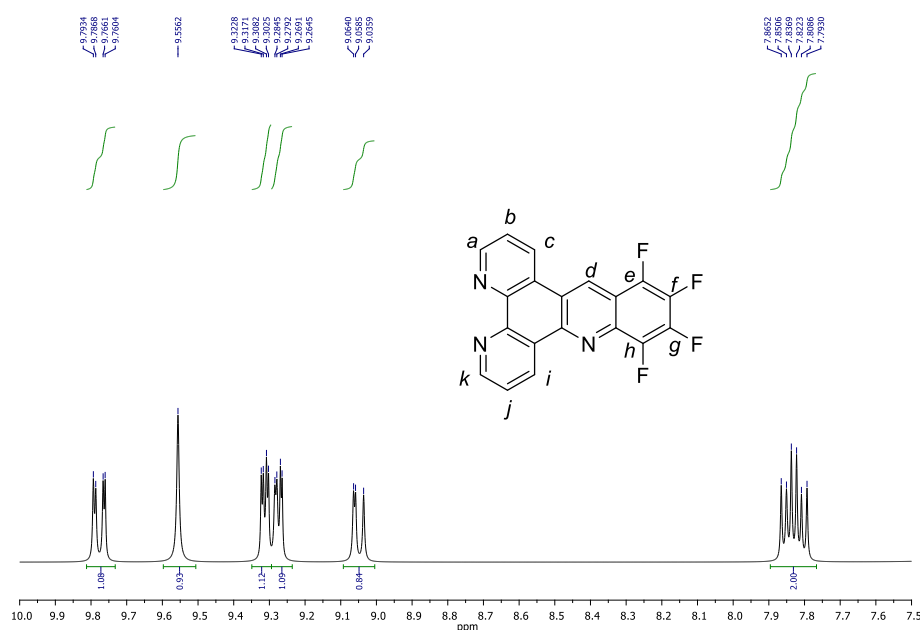


Figure 3.23. ^1H -NMR spectrum for dpacF₄ (300 MHz, CDCl_3).

Ru-DPAC. One equivalent of dichloro precursor $\text{Ru}(\text{phen})_2\text{Cl}_2$ (10.0 mg, 0.0188 mmol) is mixed with 1.2 equivalent of dpac (6.9 mg, 0.024 mmol) in a mixture of absolute ethanol/water (50/50, 2.0 mL). The reaction mixture is stirred under an argon atmosphere at reflux (80°C) and is followed by absorption spectroscopy. After 3 hours, the

medium is concentrated and addition of small portions of NH_4PF_6 and drops of water yielded a red precipitate. After centrifugation, the solid is washed with excess water. After drying under vacuum, the orange-red residue is purified by preparative plate of chromatography (silica, eluent: $\text{CH}_3\text{CN}/\text{H}_2\text{O}/\text{saturated aqueous NH}_4\text{Cl}$ 4/3/1, $R_f = 0.30$). The counter-anion exchange from PF_6 to Cl is performed by eluting a solution of complex in a mixture $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ on Sephadex DEAE A25. ^1H NMR (PF_6 salt, 500 MHz, CD_3CN): δ (ppm), 9.80 (s, 1H, H_d), 9.73 (dd, $J_{i-j} = 8.2$ Hz, $J_{i-k} = 1.3$ Hz, 1H, H_i), 9.25 (dd, $J_{c-b} = 8.4$ Hz, $J_{c-a} = 1.2$ Hz, 1H, H_c), 8.61 (2dd, $J_{4-3} = J_{7-8} = 8.7$ Hz, $J_{4-2} = J_{7-9} = 1.2$ Hz, 4H, $\text{H}_4, \text{H}_4', \text{H}_7, \text{H}_7'$), 8.41 (d, $J_{f-e} = 8.6$ Hz, 1H, H_f), 8.35 (d, $J_{g-h} = 8.2$ Hz, 1H, H_g), 8.25 (AB system, 4H, $\text{H}_5, \text{H}_5', \text{H}_6, \text{H}_6'$), 8.20 (dd, $J_{a-b} = 5.3$ Hz, $J_{a-c} = 1.2$ Hz, 1H, H_a), 8.19 (dd, $J_{k-j} = 5.3$ Hz, $J_{k-i} = 1.3$ Hz, 1H, H_k), 8.03 (m, 5H, $\text{H}_2, \text{H}_2', \text{H}_9, \text{H}_9', \text{H}_h$), 7.87 (d, $J_{e-f} = 8.6$ Hz, 1H, H_e), 7.64 (m, 6H, $\text{H}_3, \text{H}_3', \text{H}_8, \text{H}_8', \text{H}_b, \text{H}_j$). HRMS (ESI, +p): calc. 888.10078 for $[\text{C}_{43}\text{H}_{27}\text{N}_7\text{F}_6\text{PRu}]^+$, found 888.10167 ($[\text{M-PF}_6]^-$) and calc. 371.56802 for $[\text{C}_{43}\text{H}_{27}\text{N}_7\text{Ru}]^{2+}$, found 371.56835 ($[\text{M-2PF}_6]^{-2+}$).

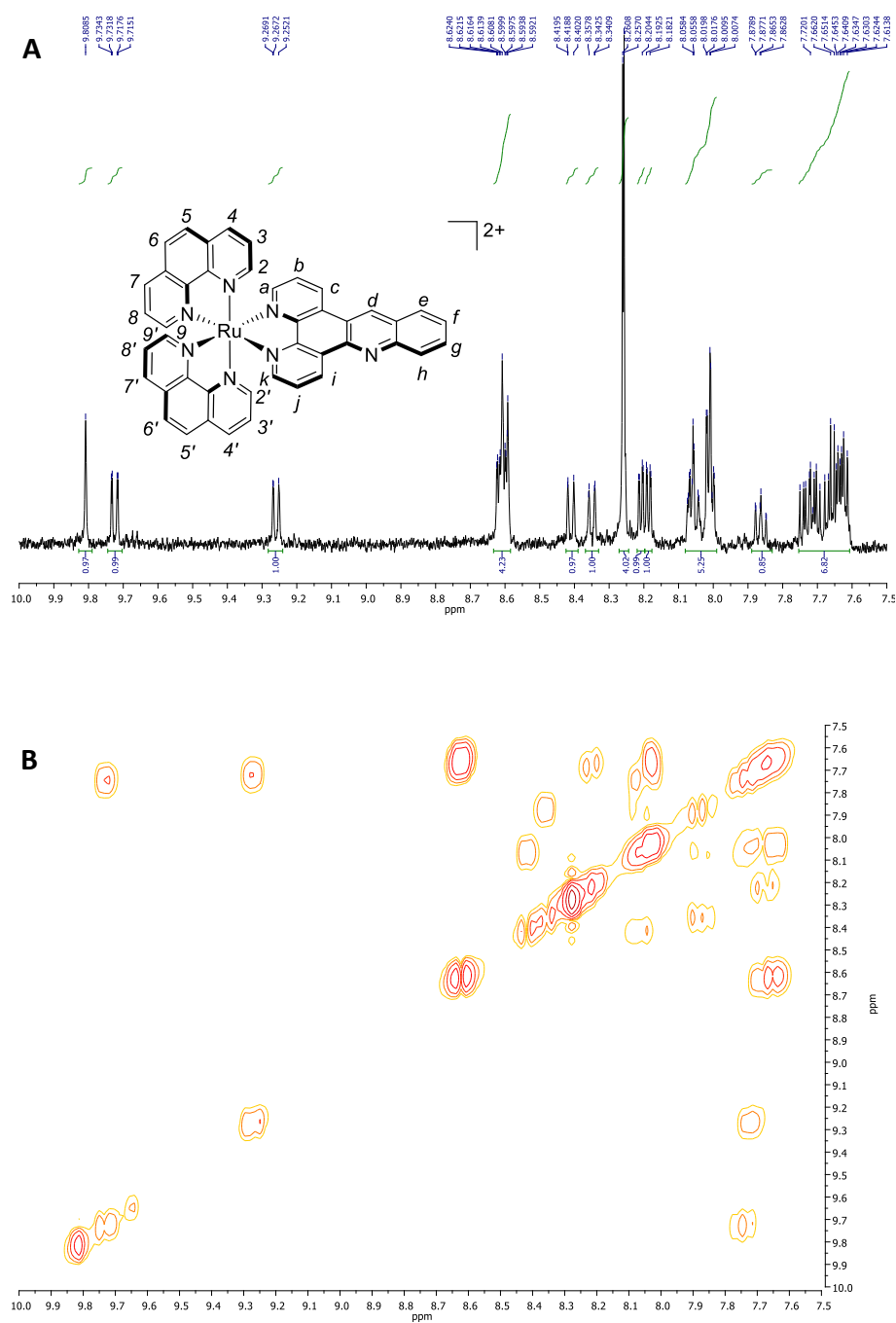


Figure 3.24. (A) 1D and (B) 2D ^1H -NMR spectra for **Ru-DPAC** (500 MHz, CD_3CN).

Ru-DPACF₂. One equivalent of dichloro precursor Ru(phen)₂Cl₂ (10.0 mg, 0.0188 mmol) is mixed with 1.5 equivalent of dpacF₂ (8.9 mg, 0.028 mmol) in a mixture of absolute ethanol/water (50/50, 2.0 mL). The reaction mixture is stirred under an argon atmosphere at reflux (80°C) and is followed by absorption spectroscopy. After 3 hours, the medium is concentrated and addition of small portions of NH₄PF₆ and drops of water yielded a red precipitate. After centrifugation, the solid is washed with excess water. After drying under vacuum, the orange-red residue is purified by preparative plate of chromatography (silica, eluent: CH₃CN/H₂O/saturated aqueous NH₄Cl 4/3/1, R_f = 0.28). The counter-anion exchange from PF₆ to Cl is performed by eluting a solution of complex in a mixture CH₃CN/H₂O on Sephadex DEAE A25. ¹H NMR (PF₆ salt, 500 MHz, CD₃CN): δ (ppm), 9.77 (s, 1H, H_d), 9.67 (dd, *J*_{i-j} = 8.2 Hz, *J*_{i-k} = 1.3 Hz, 1H, H_i), 9.22 (dd, *J*_{c-b} = 8.5 Hz, *J*_{c-a} = 1.1 Hz, 1H, H_c), 8.61 (2dd, *J*₄₋₃ = *J*₇₋₈ = 8.5 Hz, *J*₄₋₂ = *J*₇₋₉ = 1.3 Hz, 4H, H₄, H_{4'}, H₇, H_{7'}), 8.26 (AB system, 4H, H₅, H_{5'}, H₆, H_{6'}), 8.17 (m, 4H, H_e, H_b, H₂, H_{2'}), 8.06 (dd, *J*_{k-j} = 5.3 Hz, *J*_{k-i} = 1.3 Hz, 1H, H_k), 8.01 (m, 3H, H_a, H₉, H_{9'}), 7.72 (2dd, *J*_{3-4/3'-4'} = *J*_{8-7/8'-7'} = 8.3 Hz, *J*_{3-2/3'-2'} = *J*_{8-9/8'-9'} = 5.4 Hz, 2H, H_{3/3'}, H_{8/8'}), 7.63 (m, 4H, H_{3/3'}, H_{8/8'}, H_b, H_j). HRMS (ESI, +p): calc. 924.08193 for [C₄₃H₂₅N₇F₈PRu]⁺, found 924.08240 ([M-PF₆]⁺) and calc. 389.55860 for [C₄₃H₂₅N₇F₂Ru]²⁺, found 389.55897 ([M-2PF₆]²⁺).

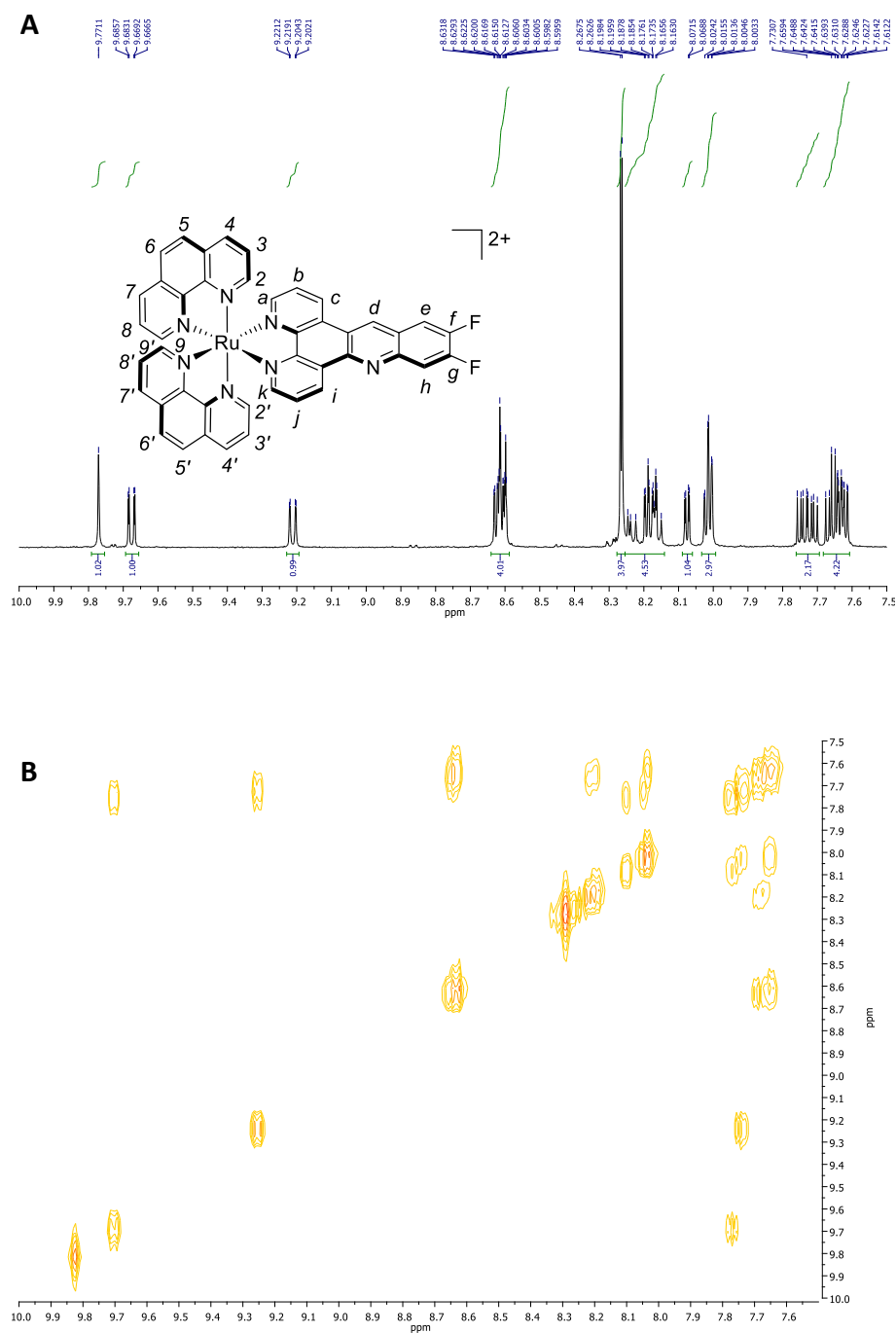


Figure 3.25. (A) 1D and (B) 2D ¹H-NMR spectra for **Ru-DPACF₂** (500 MHz, CD₃CN).

Ru-DPACF₄. One equivalent of dichloro precursor Ru(phen)₂Cl₂ (11.6 mg, 0.0218 mmol) is mixed with 1.3 equivalent of dpacF₄ (10.0 mg, 0.0283 mmol) in a mixture of absolute ethanol/water (50/50, 2.0 mL). The reaction mixture is stirred under an argon atmosphere at reflux (80°C) and is followed by absorption spectroscopy. After 2 hours and a half, the medium is concentrated and addition of small portions of NH₄PF₆ and drops of water yielded a red precipitate. After centrifugation, the solid is washed with excess water. After drying under vacuum, the orange-red residue is purified by HPLC (XBridge C18 10x100 mm, 5 µm, gradient from H₂O/CH₃CN 90/10 + 0.1% TFA to CH₃CN + 0.1% TFA, elution time: 3.07 min). The counter-anion exchange from PF₆ to Cl is performed by eluting a solution of complex in a mixture CH₃CN/H₂O on Sephadex DEAE A25. ¹H NMR (PF₆ salt, 500 MHz, CD₃CN): δ (ppm), 9.97 (s, 1H, H_d), 9.68 (dd, *J*_{i-j} = 8.3 Hz, *J*_{i-k} = 1.3 Hz, 1H, H_i), 9.33 (dd, *J*_{c-b} = 7.5 Hz, *J*_{c-a} = 1.0 Hz, 1H, H_c), 8.61 (2dd, *J*₄₋₃ = *J*₇₋₈ = 8.6 Hz, *J*₄₋₂ = *J*₇₋₉ = 1.2 Hz, 4H, H₄, H_{4'}, H₇, H_{7'}), 8.26 (AB system, 4H, H₅, H_{5'}, H₆, H_{6'}), 8.19 (dd, *J*₂₋₃ = 5.2 Hz, *J*₂₋₄ = 1.2 Hz, 1H, H_{2/2'}), 8.16 (dd, *J*₉₋₈ = 5.3 Hz, *J*₉₋₇ = 1.2 Hz, 1H, H_{9/9'}), 8.10 (dd, *J*_{k-j} = 5.3 Hz, *J*_{k-i} = 1.3 Hz, 1H, H_k), 8.04 (dd, *J*_{a-b} = 5.4 Hz, *J*_{a-c} = 1.0 Hz, 1H, H_a), 8.00 (m, 2H, H_{2/2'}, H_{9/9'}), 7.73 (2dd, *J*_{b-a} = *J*_{j-k} = 5.4 Hz, *J*_{b-c} = *J*_{j-i} = 8.3 Hz, 2H, H_b, H_j), 7.65 (m, 4H, H₃, H_{3'}, H₈, H_{8'}). HRMS (ESI, +p): calc. 960.06309 for [C₄₃H₂₃N₇F₁₀PRu]⁺, found 960.06347 ([M-PF₆]⁺) and calc. 407.54918 for [C₄₃H₂₃N₇F₄Ru]²⁺, found 407.54953 ([M-2PF₆]²⁺).

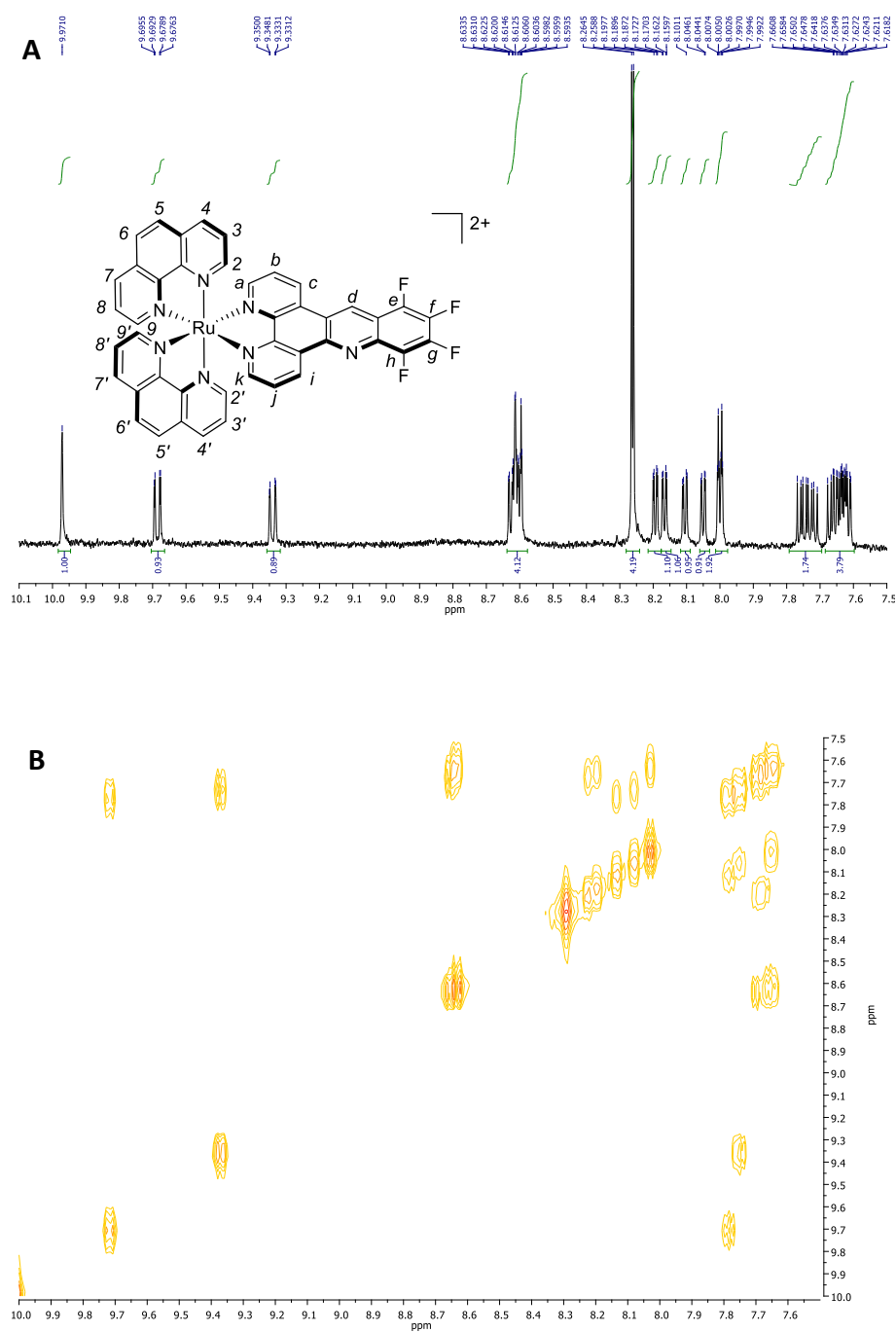
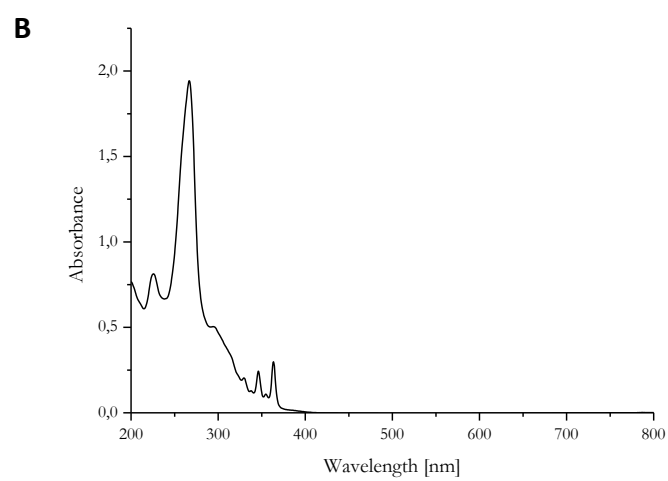
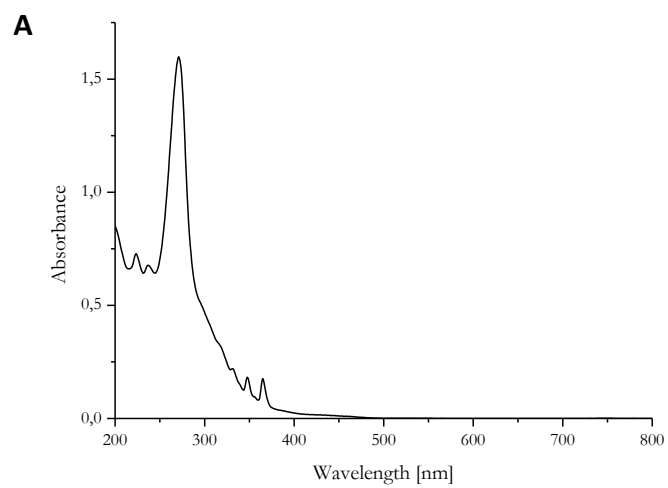


Figure 3.26. (A) 1D and (B) 2D ¹H-NMR spectra for **Ru-DPACF₄** (500 MHz, CD₃CN).

3.5. Associated content

3.5.1. *Absorption spectra of the ligands*



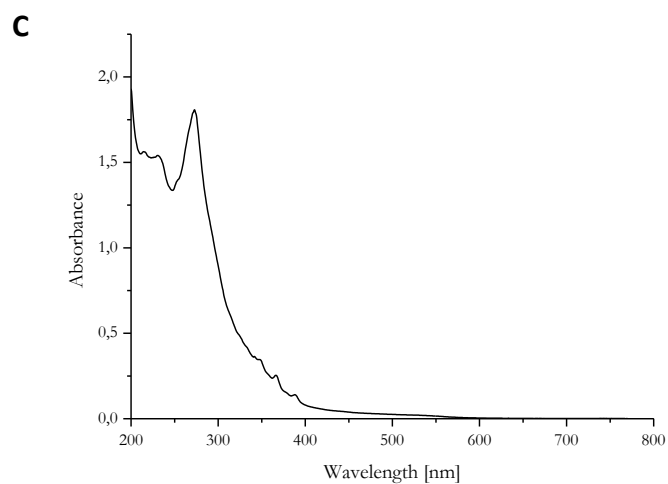
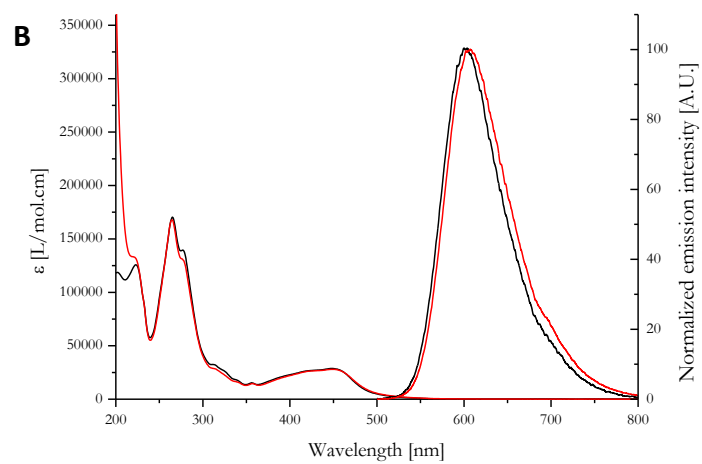
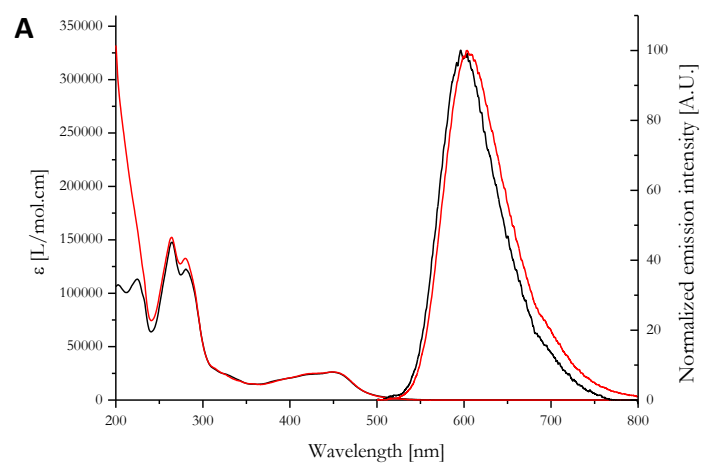


Figure 3.27. Absorption spectra under air in acetonitrile for (A) dpac, (B) dpacF₂ and (C) dpacF₄.

3.5.2. Absorption and emission spectra of the Ru(II) complexes



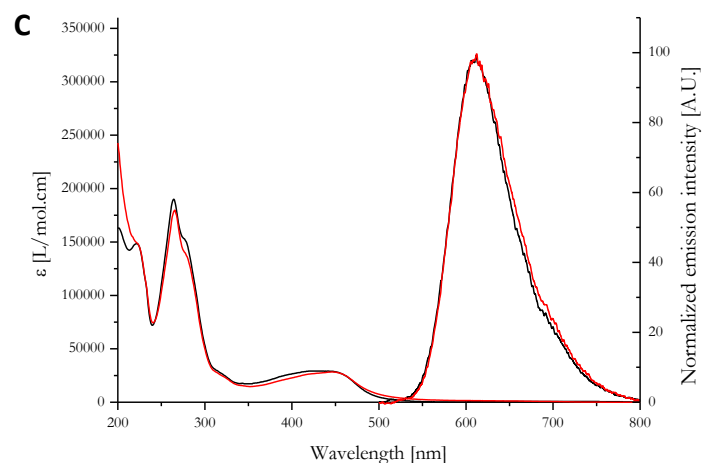


Figure 3.28. Absorption and emission spectra under air in acetonitrile (black) and in water (red) for (A) **Ru-DPAC**, (B) **Ru-DPACF₂** and (C) **Ru-DPACF₄**.

3.5.3. Emission spectra of the Ru(II) complexes at 77K

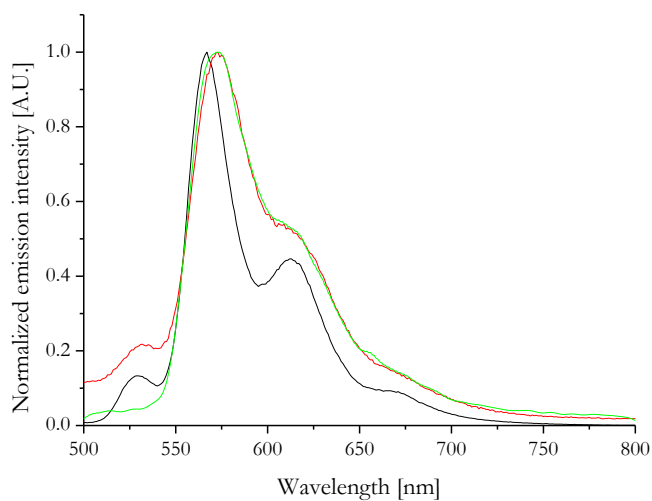


Figure 3.29. Emission spectra at 77K in EtOH/MeOH 4/1 (v/v) rigid matrix for **Ru-DPAC** (black), **Ru-DPACF₂** (red) and **Ru-DPACF₄** (green).

3.5.4. Quantum yields of the Ru(II) complexes

Complex	Atm.	Φ		$k_q(\text{O}_2) [\text{M}^{-1}\text{s}^{-1}]$	
		CH ₃ CN	H ₂ O	CH ₃ CN	H ₂ O
[Ru(phen) ₂ dpac] ²⁺	Air	0.014	0.028	9.98 10 ⁸	5.43 10 ⁹
	Argon	0.038	0.066		
[Ru(phen) ₂ dpac-F ₂] ²⁺	Air	0.029	0.091	3.74 10 ⁸	4.50 10 ⁹
	Argon	0.052	0.189		
[Ru(phen) ₂ dpac-F ₄] ²⁺	Air	0.014	0.034	2.26 10 ⁹	7.91 10 ⁹
	Argon	0.084	0.050		

Table 3.7. Quantum yields for **1-3** in acetonitrile and in water, under air and under argon, and k_q values for oxygen quenching in air-saturated solution. Concentrations in oxygen are considered to be equal to 2.42 10⁻³ mol/L ¹⁸⁶ and 2.5 10⁻⁴ mol/L ¹⁸⁷ in air-saturated acetonitrile and water respectively.

3.5.5. HRMS spectra and data

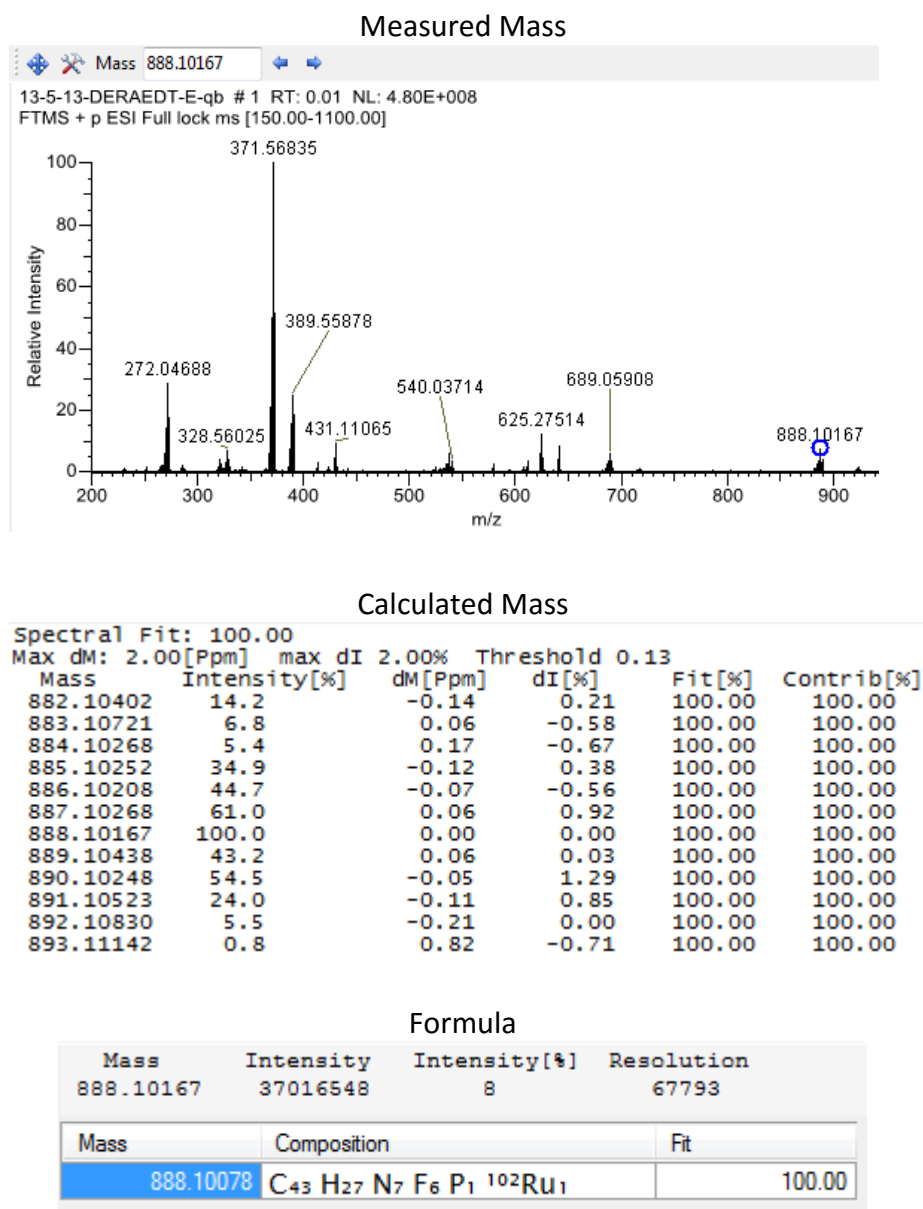
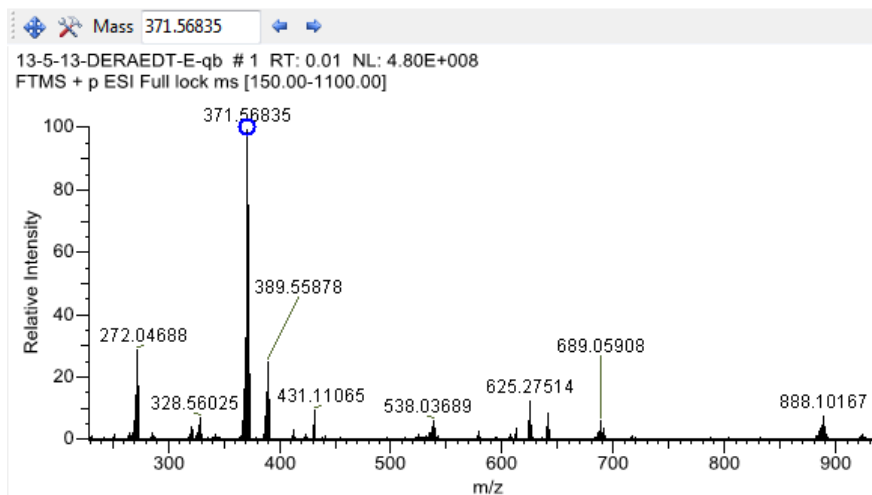


Figure 3.30. HRMS data for **Ru-DPAC** ([M-PF₆]⁺).

Measured Mass



Calculated Mass

Spectral Fit: 100.00
Max dM: 2.00[Ppm] max dI 2.00% Threshold 0.01

Mass	Intensity[%]	dM[Ppm]	dI[%]	Fit[%]	Contrib[%]
368.56965	16.0	0.66	-3.06	100.00	100.00
369.07132	7.5	0.74	-1.52	100.00	100.00
369.56854	5.5	0.79	-1.48	100.00	100.00
369.57281	1.8	1.24	-1.19	100.00	100.00
370.06887	38.6	0.54	-6.00	100.00	100.00
370.56827	41.5	1.66	0.94	100.00	100.00
371.06888	64.3	0.45	-7.12	100.00	100.00
371.56821	100.0	0.35	0.00	100.00	100.00
372.06977	44.9	-0.61	-5.18	100.00	100.00
372.56864	56.4	-0.19	-4.51	100.00	100.00
373.07025	25.7	-1.27	-4.82	100.00	100.00
373.57190	5.9	-1.21	-1.24	100.00	100.00
374.07356	0.9	-1.23	-0.21	100.00	100.00

Formula

Mass	Intensity	Intensity[%]	Resolution
371.56835	480348864	100	110055

Mass	Composition	Fit
371.56802	C ₄₃ H ₂₇ N ₇ ¹⁰² Ru ₁	100.00

Figure 3.31. HRMS data for **Ru-DPAC** ([M-2(PF₆)⁻]⁺⁺).

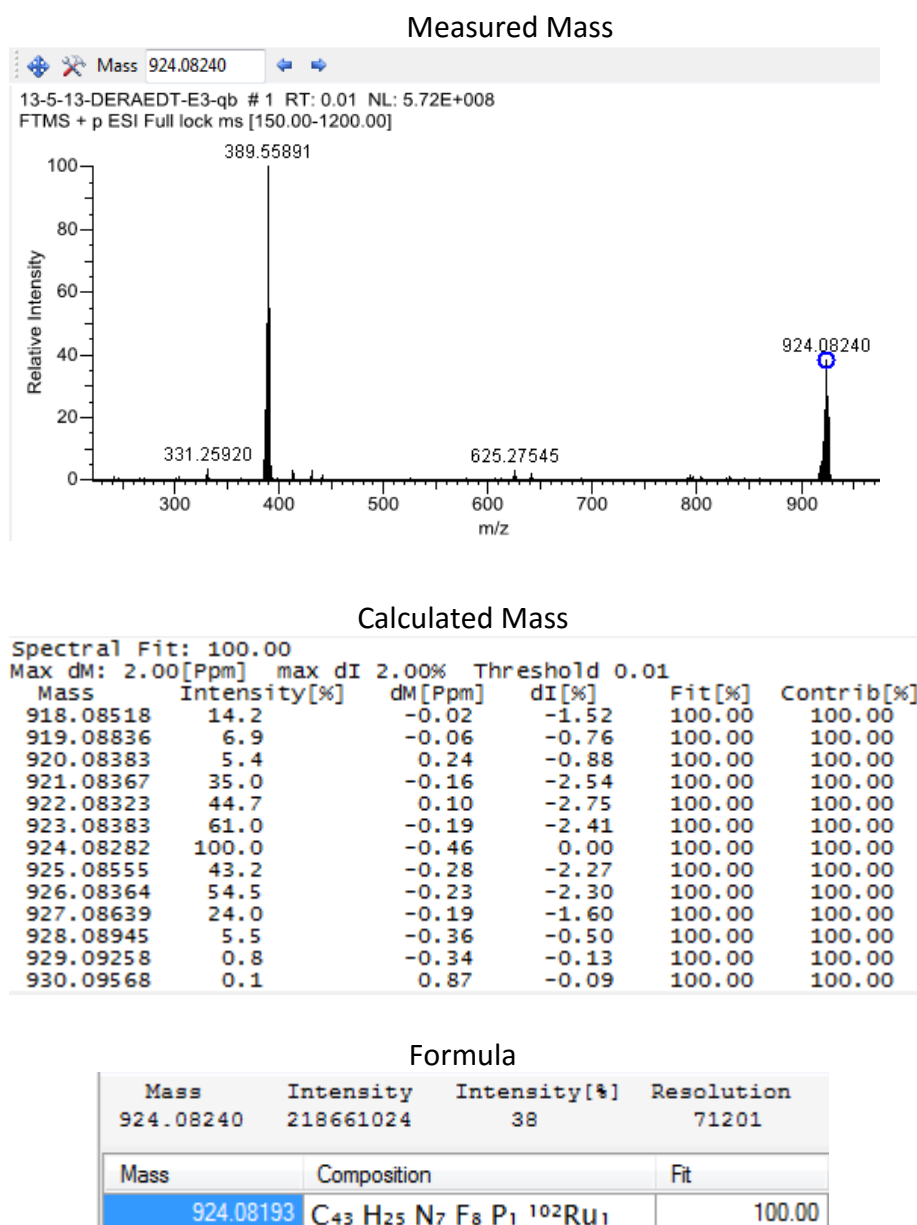
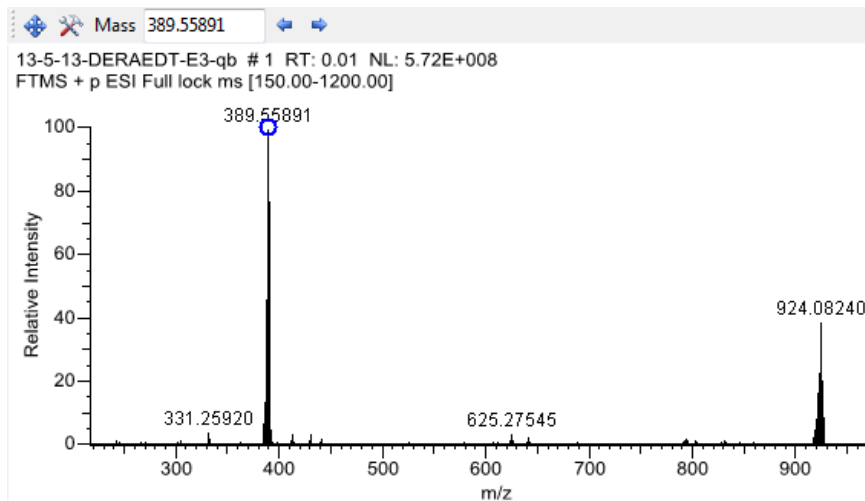


Figure 3.32. HRMS data for **Ru-DPACF₂** ([M-PF₆]⁺).

Measured Mass



Calculated Mass

Spectral Fit: 100.00					
Max dM: 2.00[Ppm] max dI 2.00% Threshold 0.00					
Mass	Intensity[%]	dM[Ppm]	dI[%]	Fit[%]	Contrib[%]
386.56023	15.7	1.66	-2.66	100.00	100.00
387.06189	7.4	1.62	-1.53	100.00	100.00
387.55913	5.5	2.09	-1.67	100.00	100.00
388.05946	38.0	1.64	-5.54	100.00	100.00
388.55893	42.0	2.22	3.48	100.00	100.00
389.05947	63.8	0.85	-4.36	100.00	100.00
389.55883	100.0	0.22	0.00	100.00	100.00
390.06036	44.6	-1.61	-2.47	100.00	100.00
390.55925	56.0	-1.16	-1.54	100.00	100.00
391.06083	25.4	-2.44	-2.99	100.00	100.00
391.56247	5.8	-2.48	-1.33	100.00	100.00
392.06412	0.9	-2.35	-0.26	100.00	100.00
392.56577	0.1	-1.46	-0.07	100.00	100.00

Formula

Mass	Intensity	Intensity[%]	Resolution
389.55891	571997120	100	106499

Mass	Composition	Fit
389.55860	C ₄₃ H ₂₅ N ₇ F ₂ ¹⁰² Ru ₁	100.00

Figure 3.33. HRMS data for **Ru-DPACF₂** ([M-2(PF₆)]⁺⁺).

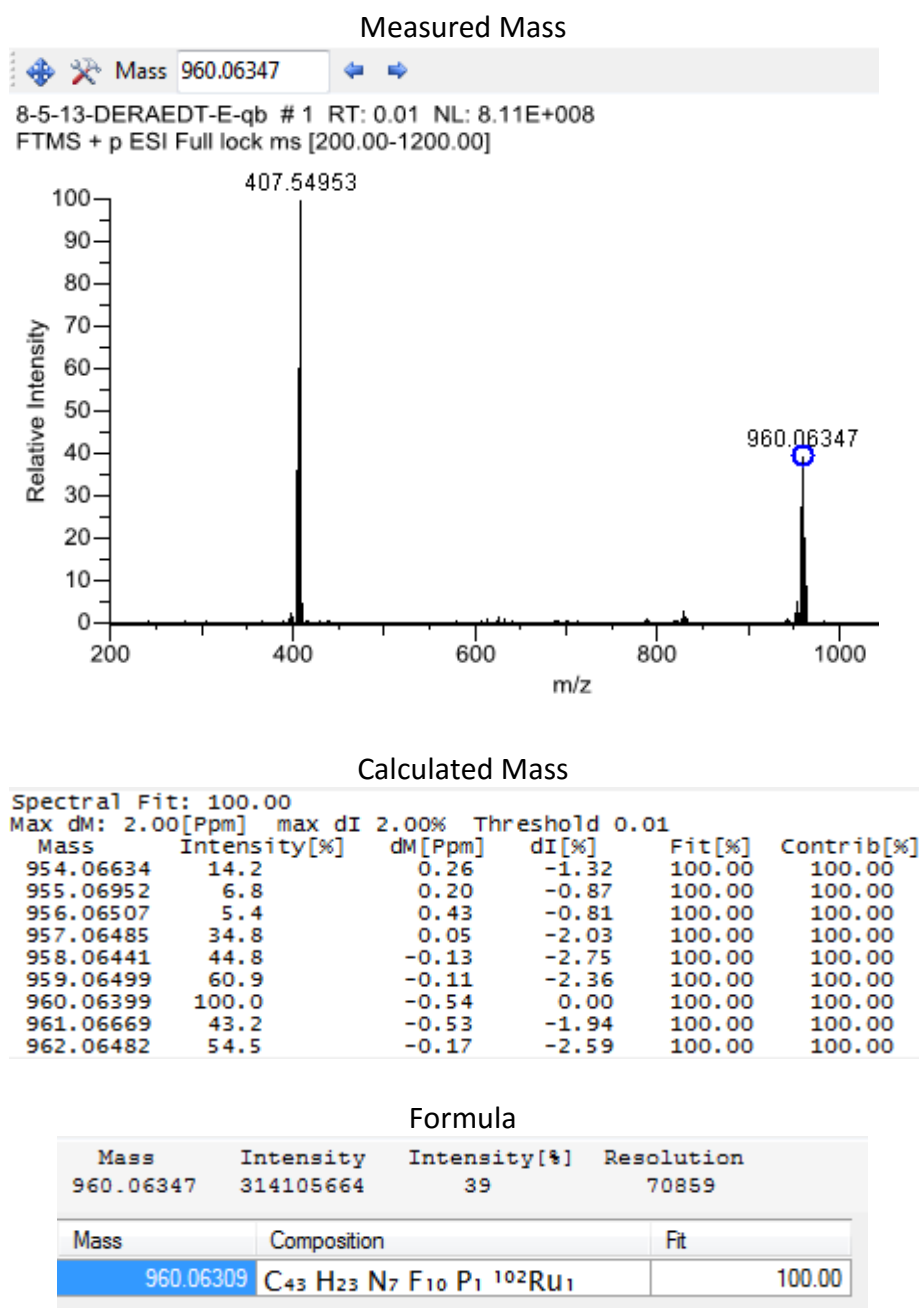
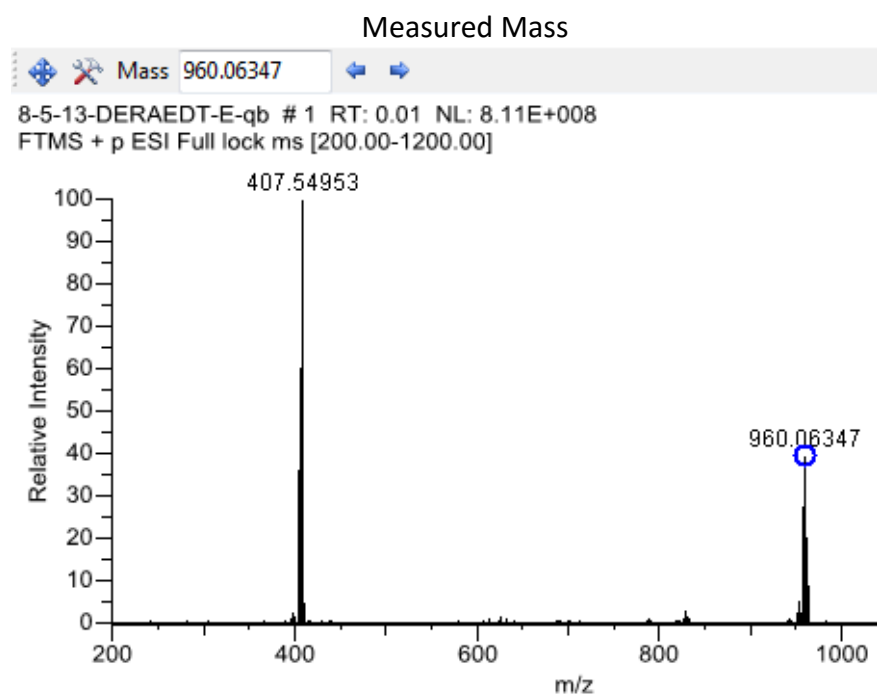


Figure 3.34. HRMS data for **Ru-DPACF₄** ([M-PF₆]⁺).



Calculated Mass

Spectral Fit: 100.00
Max dM: 2.00[Ppm] max dI 2.00% Threshold 0.00

Mass	Intensity[%]	dM[Ppm]	dI[%]	Fit[%]	Contrib[%]
404.55080	15.5	2.03	-2.50	100.00	100.00
405.05245	7.3	1.88	-1.36	100.00	100.00
405.54973	5.4	2.31	-1.67	100.00	100.00
406.05004	37.5	2.07	-5.29	100.00	100.00
406.54959	42.6	2.20	2.78	100.00	100.00
407.05006	63.3	0.99	-3.73	100.00	100.00
407.54945	100.0	0.18	0.00	100.00	100.00
408.05095	44.3	-1.70	-2.48	100.00	100.00
408.54986	55.6	-1.22	-1.96	100.00	100.00

Formula

Mass	Intensity	Intensity[%]	Resolution
407.54953	810640768	100	101822

Mass	Composition	Fit
407.54918	C ₄₃ H ₂₃ N ₇ F ₄ ¹⁰² Ru ₁	100.00

Figure 3.35. HRMS data for **Ru-DPACF₄** ([M-2(PF₆)]⁺⁺).

3.5.6. Computational data

Orbitals	Contributions (%)	Orbitals	Contributions (%)
LUMO+4	Ru = 1 phen = 95 <u>dpac</u> = 3 <u>dpac</u> = 1	HOMO	Ru = 82 phen = 12 <u>dpac</u> = 6 <u>dpac</u> = 0
LUMO+3	Ru = 6 phen = 22 <u>dpac</u> = 60 <u>dpac</u> = 12	HOMO-1	Ru = 70 phen = 26 <u>dpac</u> = 4 <u>dpac</u> = 0
LUMO+2	Ru = 6 phen = 93 <u>dpac</u> = 1 <u>dpac</u> = 0	HOMO-2	Ru = 71 phen = 14 <u>dpac</u> = 14 <u>dpac</u> = 1
LUMO+1	Ru = 0 phen = 72 <u>dpac</u> = 15 <u>dpac</u> = 13	HOMO-3	Ru = 0 phen = 0 <u>dpac</u> = 26 <u>dpac</u> = 74
LUMO	Ru = 0 phen = 6 <u>dpac</u> = 56 <u>dpac</u> = 38	HOMO-4	Ru = 0 phen = 34 <u>dpac</u> = 33 <u>dpac</u> = 33

Table 3.8. Representation of the orbitals LUMO+4 to HOMO-4 with the contributions of the different fragments of **Ru-DPAC** modeled in MeCN.

Orbitals	Contributions (%)	Orbitals	Contributions (%)
LUMO+4	Ru = 2 phen = 86 <u>dpac</u> = 10 <u>dpac</u> = 2	HOMO	Ru = 82 phen = 12 <u>dpac</u> = 6 <u>dpac</u> = 0
LUMO+3	Ru = 6 phen = 93 <u>dpac</u> = 1 <u>dpac</u> = 0	HOMO-1	Ru = 70 phen = 26 <u>dpac</u> = 4 <u>dpac</u> = 0
LUMO+2	Ru = 6 phen = 42 <u>dpac</u> = 48 <u>dpac</u> = 4	HOMO-2	Ru = 71 phen = 14 <u>dpac</u> = 14 <u>dpac</u> = 1
LUMO+1	Ru = 0 phen = 56 <u>dpac</u> = 38 <u>dpac</u> = 6	HOMO-3	Ru = 0 phen = 0 <u>dpac</u> = 34 <u>dpac</u> = 66
LUMO	Ru = 0 phen = 1 <u>dpac</u> = 56 <u>dpac</u> = 43	HOMO-4	Ru = 2 phen = 92 <u>dpac</u> = 4 <u>dpac</u> = 2

Table 3.9. Representation of the orbitals LUMO+4 to HOMO-4 with the contributions of the different fragments of **Ru-DPACF₂** modeled in MeCN.

Orbitals	Contributions (%)	Orbitals	Contributions (%)
LUMO+4	Ru = 0 phen = 94 <u>dpac</u> = 6 <u>dpac</u> = 0	HOMO	Ru = 82 phen = 12 <u>dpac</u> = 6 <u>dpac</u> = 0
LUMO+3	Ru = 5 phen = 58 <u>dpac</u> = 33 <u>dpac</u> = 4	HOMO-1	Ru = 70 phen = 27 <u>dpac</u> = 3 <u>dpac</u> = 0
LUMO+2	Ru = 2 phen = 15 <u>dpac</u> = 65 <u>dpac</u> = 18	HOMO-2	Ru = 72 phen = 14 <u>dpac</u> = 13 <u>dpac</u> = 1
LUMO+1	Ru = 1 phen = 31 <u>dpac</u> = 66 <u>dpac</u> = 3	HOMO-3	Ru = 0 phen = 0 <u>dpac</u> = 33 <u>dpac</u> = 67
LUMO	Ru = 0 phen = 0 <u>dpac</u> = 36 <u>dpac</u> = 64	HOMO-4	Ru = 3 phen = 96 <u>dpac</u> = 1 <u>dpac</u> = 0

Table 3.10. Representation of the orbitals LUMO+4 to HOMO-4 with the contributions of the different fragments of **Ru-DPACF₄** modeled in MeCN.

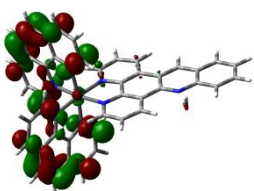
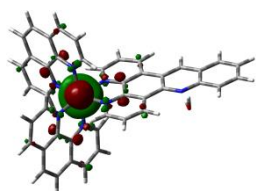
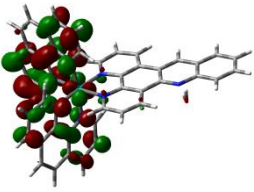
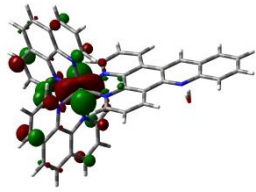
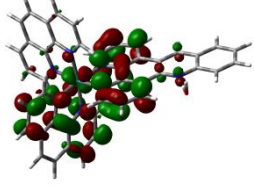
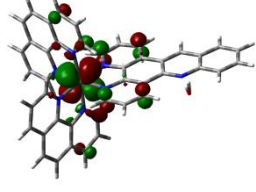
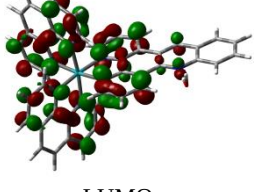
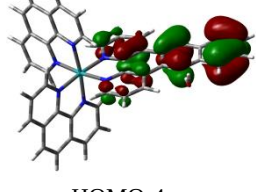
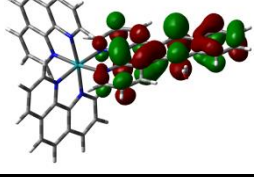
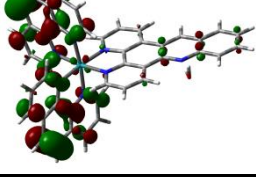
Orbitals	Contributions (%)	Orbitals	Contributions (%)
LUMO+4		HOMO	
	Ru = 1 phen = 92 <u>dpac</u> = 6 <u>dpac</u> = 1		Ru = 82 phen = 12 <u>dpac</u> = 6 <u>dpac</u> = 0
LUMO+3		HOMO-1	
	Ru = 6 phen = 90 <u>dpac</u> = 4 <u>dpac</u> = 0		Ru = 70 phen = 26 <u>dpac</u> = 4 <u>dpac</u> = 0
LUMO+2		HOMO-2	
	Ru = 6 phen = 42 <u>dpac</u> = 48 <u>dpac</u> = 4		Ru = 71 phen = 14 <u>dpac</u> = 14 <u>dpac</u> = 1
LUMO+1		HOMO-3	
	Ru = 0 phen = 60 <u>dpac</u> = 34 <u>dpac</u> = 6		Ru = 0 phen = 0 <u>dpac</u> = 26 <u>dpac</u> = 74
LUMO		HOMO-4	
	Ru = 0 phen = 1 <u>dpac</u> = 46 <u>dpac</u> = 53		Ru = 1 phen = 89 <u>dpac</u> = 6 <u>dpac</u> = 4

Table 3.11. Representation of the orbitals LUMO+4 to HOMO-4 with the contributions of the different fragments of **Ru-DPAC** modeled in H₂O.

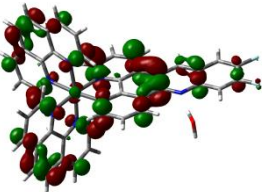
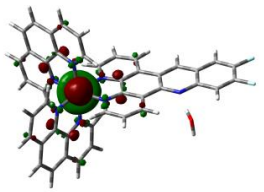
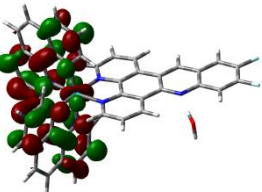
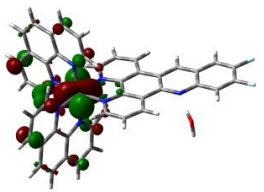
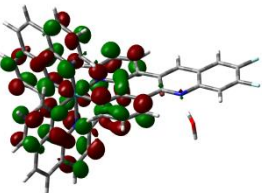
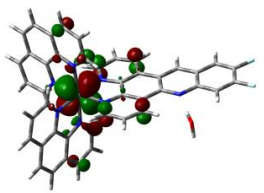
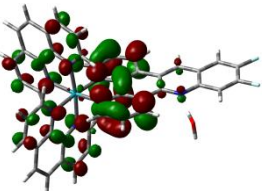
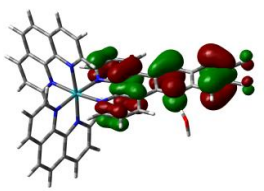
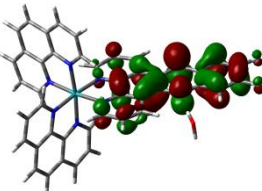
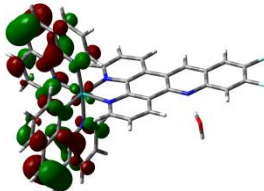
Orbitals	Contributions (%)	Orbitals	Contributions (%)
LUMO+4		HOMO	
	Ru = 2 phen = 50 <u>dpac</u> = 39 <u>dpac</u> = 9		Ru = 82 phen = 12 <u>dpac</u> = 6 <u>dpac</u> = 0
LUMO+3		HOMO-1	
	Ru = 1 phen = 93 <u>dpac</u> = 6 <u>dpac</u> = 0		Ru = 70 phen = 26 <u>dpac</u> = 4 <u>dpac</u> = 0
LUMO+2		HOMO-2	
	Ru = 6 phen = 59 <u>dpac</u> = 34 <u>dpac</u> = 1		Ru = 71 phen = 14 <u>dpac</u> = 14 <u>dpac</u> = 1
LUMO+1		HOMO-3	
	Ru = 1 phen = 40 <u>dpac</u> = 56 <u>dpac</u> = 3		Ru = 0 phen = 1 <u>dpac</u> = 35 <u>dpac</u> = 64
LUMO		HOMO-4	
	Ru = 0 phen = 0 <u>dpac</u> = 38 <u>dpac</u> = 62		Ru = 2 phen = 95 <u>dpac</u> = 2 <u>dpac</u> = 1

Table 3.12. Representation of the orbitals LUMO+4 to HOMO-4 with the contributions of the different fragments of **Ru-DPACF₂** modeled in H₂O.

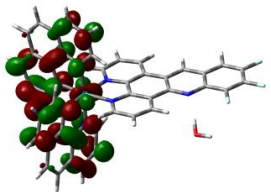
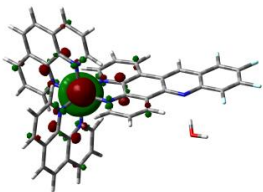
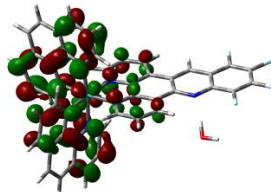
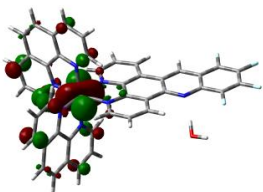
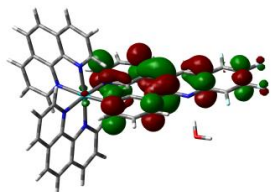
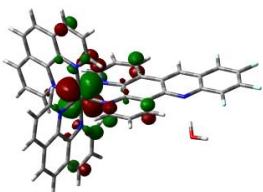
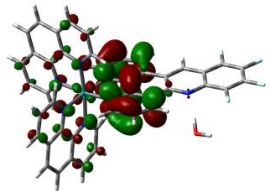
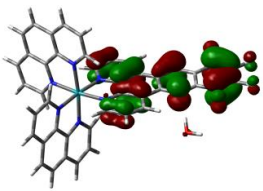
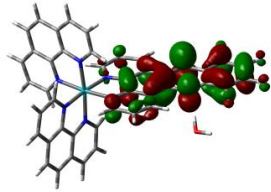
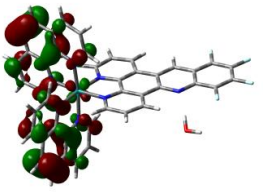
Orbitals	Contributions (%)	Orbitals	Contributions (%)
LUMO+4		HOMO	
	Ru = 6 phen = 93 <u>dpac</u> = 1 dpac = 0		Ru = 82 phen = 12 <u>dpac</u> = 6 dpac = 0
LUMO+3		HOMO-1	
	Ru = 5 phen = 76 <u>dpac</u> = 19 dpac = 0		Ru = 69 phen = 27 <u>dpac</u> = 4 dpac = 0
LUMO+2		HOMO-2	
	Ru = 1 phen = 2 <u>dpac</u> = 73 dpac = 24		Ru = 72 phen = 14 <u>dpac</u> = 13 dpac = 1
LUMO+1		HOMO-3	
	Ru = 2 phen = 23 <u>dpac</u> = 73 dpac = 2		Ru = 0 phen = 2 <u>dpac</u> = 35 dpac = 63
LUMO		HOMO-4	
	Ru = 0 phen = 0 <u>dpac</u> = 35 dpac = 65		Ru = 3 phen = 96 <u>dpac</u> = 1 dpac = 0

Table 3.13. Representation of the orbitals LUMO+4 to HOMO-4 with the contributions of the different fragments of **Ru-DPACF₄** modeled in H₂O.

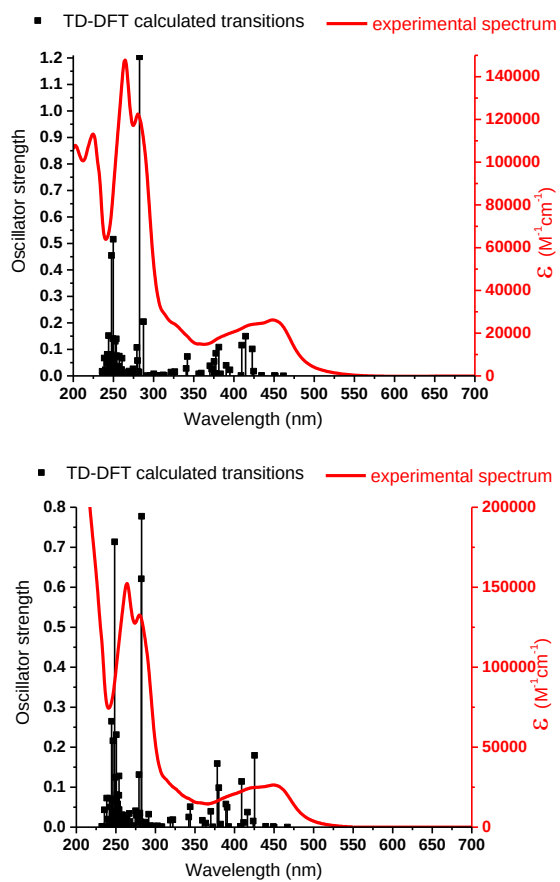


Figure 3.36. Experimental and calculated transitions for **Ru-DPAC** in MeCN (left) and H₂O (right).

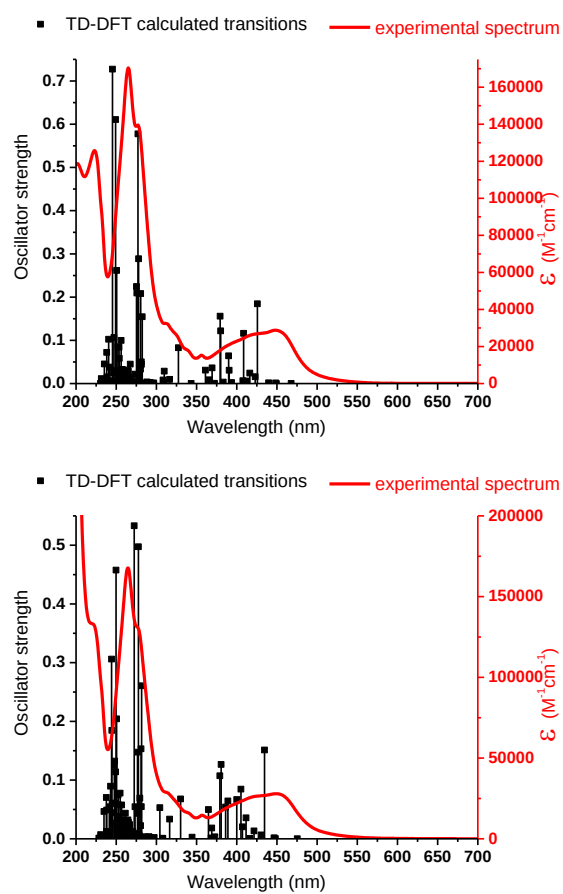


Figure 3.37. Experimental and calculated transitions for **Ru-DPACF₂** in MeCN (left) and H₂O (right).

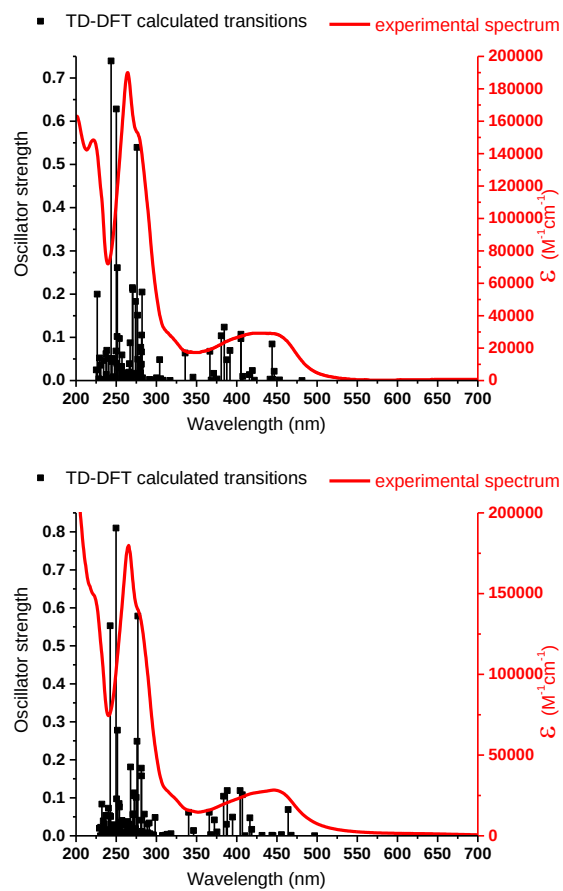


Figure 3.38. Experimental and calculated transitions for **Ru-DPACF₄** in MeCN (left) and H₂O (right).

Compound	λ (nm) in MeCN	Character (%) in MeCN	λ (nm) in H ₂ O	Character (%) in H ₂ O
Ru-DPAC		³ IL 76		³ IL 75
	567	³ MLCT _{dpac} 12	566	³ MLCT _{dpac} 12
		³ MLCT _{dpac} 9		³ MLCT _{dpac} 9
	524	³ MLCT _{phen} 97	522	³ MLCT _{phen} 95
	518	³ MLCT _{phen} 94	517	³ MLCT _{phen} 92
		³ MLCT _{dpac} 50		³ MLCT _{dpac} 60
Ru-DPACF ₂	500	³ MLCT _{phen} 47	502	³ MLCT _{phen} 32
		³ IL 13		³ IL 5
	496	³ MLCT _{dpac} 77	500	³ MLCT _{dpac} 63
		³ IL 66		³ IL 62
	556	³ MLCT _{dpac} 19	555	³ MLCT _{dpac} 22
		³ MLCT _{dpac} 11		³ MLCT _{dpac} 11
Ru-DPACF ₄	522	³ MLCT _{phen} 97	521	³ MLCT _{phen} 97
	516	³ MLCT _{phen} 92	515	³ MLCT _{phen} 89
		³ MLCT _{dpac} 57		³ IL 5
	501	³ MLCT _{phen} 39	503	³ MLCT _{dpac} 61
		³ MLCT _{dpac} 71		³ MLCT _{dpac} 36
	497	³ IL 19	501	³ MLCT _{dpac} 63
Ru-DPACF ₄		³ IL 72		³ MLCT _{phen} 18
	572	³ MLCT _{dpac} 14	573	³ MLCT _{dpac} 15
		³ MLCT _{dpac} 10		³ IL 71
	519	³ MLCT _{phen} 95	521	³ MLCT _{dpac} 16
		³ MLCT _{dpac} 57		³ MLCT _{dpac} 10
	516	³ MLCT _{phen} 25	518	³ MLCT _{dpac} 86
Ru-DPPZ		³ IL 8		³ MLCT _{phen} 8
	507	³ MLCT _{dpac} 63	509	³ MLCT _{phen} 93
		³ MLCT _{phen} 11		³ MLCT _{dpac} 49
	503	³ MLCT _{dpac} 16	506	³ MLCT _{phen} 43
		³ MLCT _{dpac} 74		³ MLCT _{dpac} 79
		³ MLCT _{phen} 23		³ MLCT _{phen} 19
Ru-DPPZ		³ IL 79		³ IL 81
	581	³ MLCT _{dppz} 18	595	³ MLCT _{dppz} 9
		³ MLCT _{phen} 96		³ MLCT _{dppz} 8
	521	³ MLCT _{dppz} 53	534	³ MLCT _{dppz} 94
	517	³ MLCT _{phen} 39	519	³ MLCT _{phen} 94
		³ MLCT _{dppz} 74		³ MLCT _{phen} 95
Ru-DPPZ	505	³ MLCT _{phen} 7	512	³ MLCT _{phen} 95
		³ MLCT _{dppz} 8		³ MLCT _{dppz} 86
	501	³ MLCT _{dppz} 61	511	³ MLCT _{dppz} 11
		³ MLCT _{phen} 31		³ MLCT _{phen} 11

Table 3.14. Low-lying triplet excited states of **Ru-DPAC**, **Ru-DPACF₂**, **Ru-DPACF₄** and **Ru-DPPZ** computed on the electronic ground state optimized geometry at the TD-DFT level in acetonitrile (CPCM) and water (discrete model+CPCM). The values corresponding to the water environment (CPCM only) are not reported since they are nearly identical to the data obtained for acetonitrile. Contributions were obtained from the Natural Transitions Orbitals analysis.

Chapter 4

Towards mismatched DNA
photoprobes: “elbow-shaped”
Ru(II) complexes

1. Introduction

In the previous chapter, we have demonstrated Ru(II) complexes bearing acridine derivatives display suitable photophysical properties to act as DNA photoprobes. An enhancement of luminescence is observed for **Ru-DPAC**, **Ru-DPACF₂** and **Ru-DPACF₄** with increasing concentration of DNA, associated to a high binding affinity. The linear acridine moiety of the dpac ligand is suggested to account for the lack of sensitivity to the nature of the mismatch in the hairpin oligonucleotides through a looser fit into mismatched sequences. As a result, we were interested in the design of new ligands able to discriminate complementary and single-base mismatched hairpins, and to exhibit differential luminescence behavior with respect to the nature of the mismatched site.

1.1. Mismatched DNA targeting

Since the discovery of the DNA double helix structure, the processes involved in the defense of its integrity as data storage system has been the focus of much attention. Even if the replication machinery that copies the DNA molecule by pairing A with T and G with C makes only one mistake in about thirty million steps, thousands of mismatches may be present for example in the case of the human genome composed of $3 \cdot 10^9$ base pairs. In addition, the genetic material is surrounded by mitochondria which produce reactive oxygen species. A part of these ROS may react with DNA leading to a wide range of oxidative degradation products. DNA can also be degraded by exposure to UV irradiation, which is part of the natural electromagnetic spectrum of sunlight, resulting in DNA photolesions. By nature, all of these DNA defects represent a loss of genetic information. In other words, these events rule out the ability of DNA to code for a human being. Based on these considerations, pioneering work on the design of DNA mismatches repair mechanisms has been achieved by Modrich, Lindahl and Sancar, the 2015 Nobel Prize in Chemistry recipients. They found once a lesion is detected, the repair systems trigger a series of events that finally lead

to the removal of the lesion from the DNA duplex and the restoration of the genetic information. In particular, Modrich focused on mismatch repair processes, which are able to remove errors generated during replication of the DNA by DNA polymerase.²⁵ This type of DNA damage often occurs due to the possible tautomerization of the base. Indeed, DNA bases can exist in different tautomeric structures, displaying different Hydrogen bonding donating or accepting ability. If they appear inside the DNA polymerase during the replication process, they can lead to the selection of a wrong counterbase during the copying reaction. For example, cytosine may tautomerizes to its imino form which is able to pair with an adenine base, leading to the formation of a C:A mismatch (Figure 4.1).²⁴

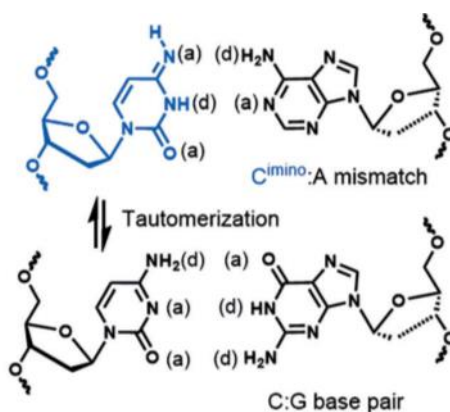


Figure 4.1. Cytosine nucleobase may undergo tautomerization, leading to the possibility of pairing with an adenine moiety.²⁴

At some point, the DNA repair processes need to specifically target the defective sites to be able to remove or replace the incorrect base. Such a performance is effectively achieved by Nature, even if all the mechanisms involved are not fully understood. Recent efforts in this area suggest the key to this efficiency is likely to lie in the structural features of the mismatch recognition by enzymes, which include (i) rapid sliding of the enzyme along the DNA duplex, (ii) structural heterogeneity of the duplex at the mismatch sites and (iii) conformational change of the enzyme initiating the repair process.¹⁸⁸

Aside from these progresses in understanding the mechanism of DNA repair processes, the last decades have witnessed the development of small molecules which, like the repair systems, bind with high affinity to DNA mismatched sites, while having much lower affinity to complementary DNA base pairs. As mentioned in the introduction, the design of DNA targeting agents is a well-established research area and leads to the discovery of numerous drugs or drug candidates (*e.g.* cis-platin, ethidium bromide, actinomycin D, ...). While the presence of DNA mismatches may lead to harmful diseases (*e.g.* cancer) if left uncorrected, selective detection of these defects is of high interest for the development of diagnostic and potential therapeutic tools. Despite much efforts, poor selectivity for binding to DNA mismatched sites is often observed. In that context, $[\text{Rh}(\text{bpy})_2\text{chrysi}]^{3+}$ appeared as a major breakthrough as it binds selectively mismatched DNA sites (at least 1000 times tighter than complementary base pairs) through insertion from the minor groove, ejecting the mismatched bases into the major groove (Figure 3.9).^{23,126} This mismatch-specific targeting has been associated with the thermodynamic destabilization due to the presence of the single-base mismatch.^{189,190} The presence of the bulky non-linear aromatic chrysi ligand renders the stacking of the Rh(III) complex between complementary base pairs difficult. Instead, this complex is able to insert more easily at the destabilized mismatched site within the DNA.¹⁹⁰

In parallel to the development of Rh(III) mismatch-selective metalloinsertors, numerous ruthenium(II) complexes have been studied due to their interesting luminescent properties. As stated in Chapter 3, **Ru-DPPZ** complexes exhibit a more pronounced light-switch effect in the case of mismatched-DNA with respect to complementary base pairs (Figure 3.8).¹⁰⁶ In order to favor the binding of Ru(II) complexes to mismatched sites, Barton *et al.* synthesized a series of **Ru-DPPZ** analogues using bulkier ligands (cite for example $[\text{Ru}(\text{bpy})_2(\text{dppn})]^{2+}$ (dppn = benzo[*i*]dipyrido[3,2-*a*:20,30-*c*]phenazine), $[\text{Ru}(\text{bpy})_2(\text{tactp})]^{2+}$ (tactp = 4,5,9,18-tetraazachryseno[9,10-*b*]triphenylene), $[\text{Ru}(\text{bpy})_2(\text{chrysi})]^{2+}$, Figure

4.2).^{191,192} Although preferential binding of these complexes to CC mismatches has been observed for in DNA, no efficient luminescent mismatch detection could be achieved due to their unfavorable luminescent properties. Thus, $[\text{Ru}(\text{bpy})_2(\text{dppn})]^{2+}$ and $[\text{Ru}(\text{bpy})_2(\text{tactp})]^{2+}$ are both luminescent in the presence of matched and mismatched DNA, while $[\text{Ru}(\text{bpy})_2(\text{chrysi})]^{2+}$ displays no luminescence in aqueous solutions at room temperature in the absence or in the presence of DNA. These observations suggest that structural modifications of the dppz ligand dramatically alter the luminescent sensing properties of DNA mismatches with Ru(II) complexes.

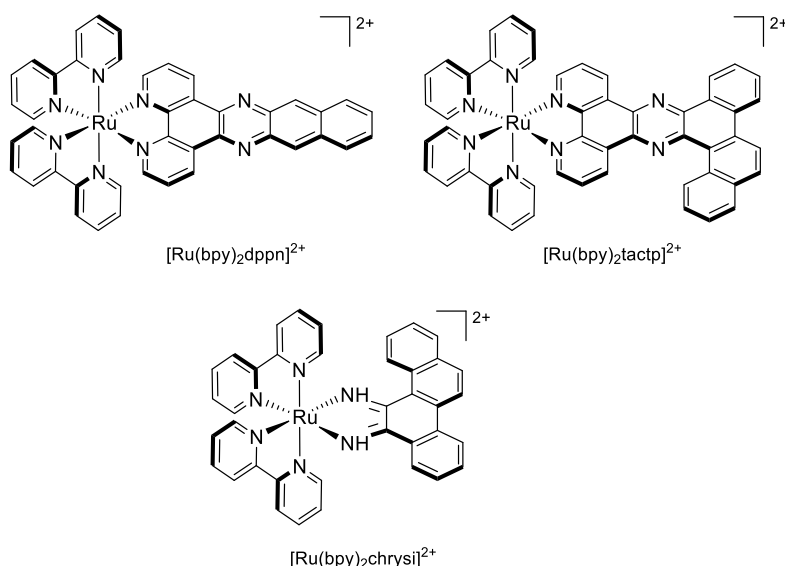


Figure 4.2. Structure of $[\text{Ru}(\text{bpy})_2\text{dppn}]^{2+}$, $[\text{Ru}(\text{bpy})_2\text{tactp}]^{2+}$ and $[\text{Ru}(\text{bpy})_2\text{chrysi}]^{2+}$.

In the frame of the design of mismatch-selective DNA binders, transition metal complexes represent thus useful and valuable tools. Indeed, the ability to detect point mutations in DNA and to be able to directly monitor the binding properties is of utmost importance in the field of molecular genetics, as these mutations may be related to the triggering of some types of cancer.¹⁹³ The development of transition metal mismatched DNA photoprobes can allow sensitive diagnosis of diseases and, in particular, could be of considerable benefit for prevention and prediction of cancer susceptibility. Therefore, the

development of Ru(II) complexes being able to specifically target mismatched DNA is of high significance for applications in biochemistry area.

2. Objectives

Transition metal complexes attract increasing interest for the preparation of selective mismatched DNA binders which allow straightforward monitoring. The design of such diagnostic and/or therapeutic agents takes advantage of the powerful photophysics and photochemistry of this class of compounds.

For that purpose, “elbow-shaped” ligands (npp = naphtho[1,2-*b*]pyrido[3,2-*f*][1,7]phenanthroline and bdppz = benzo[*h*]dipyrido[3,2-*a*:2',3'-*c*]phenazine) have been synthesized, as well as their corresponding Ru(II) complexes (**Ru-NPP** for [Ru(bpy)₂NPP]²⁺ and **Ru-BDPPZ** for [Ru(bpy)₂bdppz]²⁺, Figure 4.3). The use of an acridine and a phenazine core respectively will give us information about the effect of the substitution of one atom of the extended planar ligand on the photophysics for the complexes alone, and in the presence of DNA. The significance of this study is supported by our work in the previous chapter for the linear derivatives. We suggest the non-linear structure of the intercalating ligand would allow to probe selectively different DNA non-canonical structures, as observed for [Rh(bpy)₂chrysi]³⁺.

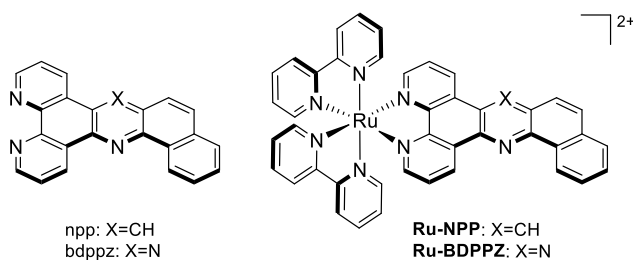


Figure 4.3. Structure of npp and bdppz ligands (left), and of **Ru-NPP** and **Ru-BDPPZ** (right).

The study of the photophysical properties of the two “elbow-shaped” complexes both in the absence and in the presence of DNA has been investigated and described in a publication, together with the results from the next chapter. For the sake of clarity, only the results dealing with the above mentioned Ru(II) mentioned will be discussed hereafter.

3. Results

3.1. Synthetic procedures

A Tröger’s base modified synthetic scheme was found to be successful and allowed us to obtain npp.^{107,151,194} Bdppz is obtained through condensation between 1,10-phenanthroline-5,6-dione and 1,2-diaminonaphthalene.

The corresponding Ru(II) complexes are synthesized by the direct chelation of the extended ligand onto the corresponding Ru(II) precursor (Figure 4.4) using methodologies previously described for similar compounds. All intermediates and final compounds are protected from direct light during the synthesis and purification steps to prevent photochemical degradation. The reactions are performed under argon to avoid any oxidation of the metal center.

The complexes are fully characterized by HRMS and ¹H NMR spectroscopy (see the Experimental section and Associated contents). All the multiplicity and coupling constants cannot be determined due to the superposition of several signals and/or lack of fine resolution for some peaks in the ¹H NMR spectra. The ¹H NMR shows unambiguously the absence of symmetry elements for all the complexes, which induces the non-equivalence of (i) the protons on the extended planar ligand and (ii) the ancillary ligands. The ¹H NMR spectra are sensitive to concentration as some shifts occurred in the ¹H NMR spectra upon change in the concentration (data not shown). For solubility purposes, all the complexes are converted to the chloride

salt for experiments conducted in water or buffer or to the hexafluorophosphate salt for studies in organic solvents.

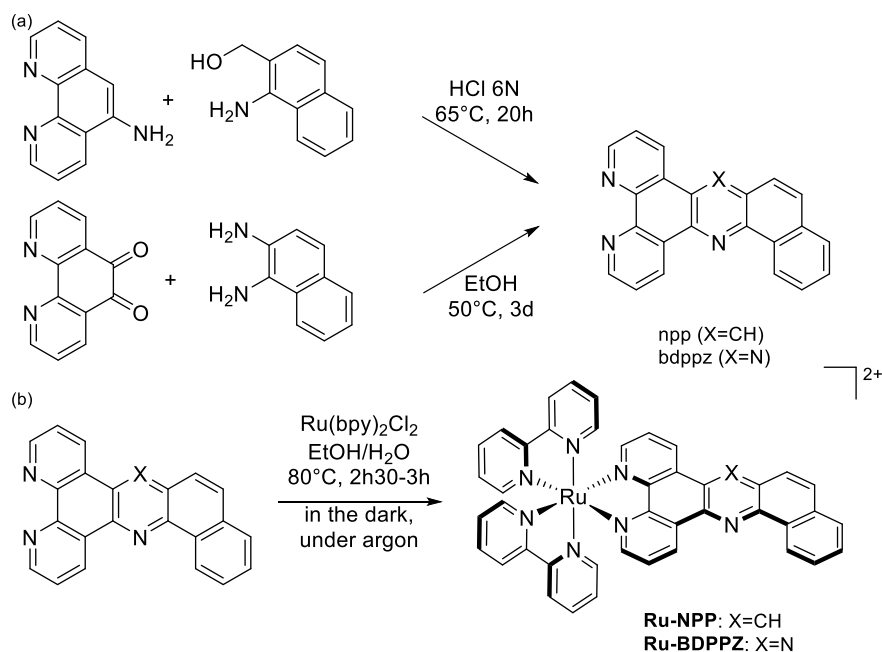


Figure 4.4. Synthetic scheme for npp and bdppz ligands, and their corresponding Ru(II) complexes, **Ru-NPP** and **Ru-BDPPZ**.

3.2. Electrochemistry

The electrochemical data of the two complexes are obtained by cyclic voltammetry in dry deoxygenated MeCN or N,N-dimethylformamide (Table 4.1, Figures 4.5 and 4.6). Both complexes display a one-electron reversible oxidation wave at a potential close to that of the Ru²⁺/Ru³⁺ oxidation of [Ru(bpy)₃]²⁺¹⁰² and [Ru(phen)₂dpac]²⁺ (noted **Ru-DPAC**) (1.34-1.35 V vs Ag/AgCl).¹⁹⁴ The nature of the extended ligand does not really affect the oxidation potential of all complexes.

Three reduction waves are monitored for the two complexes. From the comparison with the reduction potentials of [Ru(bpy)₃]²⁺ (-1.28 V vs Ag/AgCl)¹¹¹ and [Ru(bpy)₂dppz]²⁺ (noted **Ru-DPPZ**) (-0.95 V vs

Ag/AgCl),¹¹¹ the first reduction of the two “elbow-shaped” complexes is attributed to the addition of one electron on npp or bdppz ligand, respectively. The anodic shift observed for **Ru-BDPPZ** (-1.02 V vs Ag/AgCl) indicates a greater stabilization of the LUMO with respect to that of **Ru-DPAC** and **Ru-NPP** respectively, indicating that the π^* orbital is centered on the bdppz ligand. The next two reduction waves may be attributed to successive reduction of the two ancillary bpy ligands due to the similarity of the potentials with $[\text{Ru}(\text{bpy})_3]^{2+}$ for both complexes.

Complex	$E_{1/2 \text{ ox}}^a$ [V vs Ag/AgCl]	$E_{1/2 \text{ red}}^b$ [V vs Ag/AgCl]	$\lambda_{\text{max, em}}^a$ [nm]	E_{ox}^{*c} [V vs Ag/AgCl]	E_{red}^{*d} [V vs Ag/AgCl]
$[\text{Ru}(\text{bpy})_3]^{2+ \text{ 111}}$	1.34	-1.28 -1.47 -1.71	604	-0.71	0.77
Ru-DPPZ ¹¹¹	1.29	-0.97 -1.39 -1.62	610	-0.75	1.07
Ru-DPAC	1.35	-1.22 -1.35 -1.66	604	-0.71	0.84
Ru-NPP	1.39	-1.23 -1.38 -1.60	606	-0.66	0.82
Ru-BDPPZ	1.36	-1.02 -1.37 -1.61	620	-0.64	0.98

Table 4.1. Potentials for the oxidation ($E_{1/2 \text{ ox}}$) and reduction ($E_{1/2 \text{ red}}$) of **Ru-NPP** and **Ru-BDPPZ**. ^a Measured in dry acetonitrile. ^b Measured in dry N,N-dimethylformamide. ^c The excited state oxidation potential was estimated from the ground state oxidation potential and the energy of emission maximum with the equation $E_{\text{ox}}^* = E_{1/2 \text{ ox}} - E_{0-0} \approx E_{1/2 \text{ ox}} - \Delta E_{\lambda_{\text{max, em}}}$. ^d The excited state reduction potential was estimated from the ground state reduction potential and the energy of emission maximum with the equation $E_{\text{red}}^* = E_{1/2 \text{ red}} + E_{0-0} \approx E_{1/2 \text{ red}} + \Delta E_{\lambda_{\text{max, em}}}$.

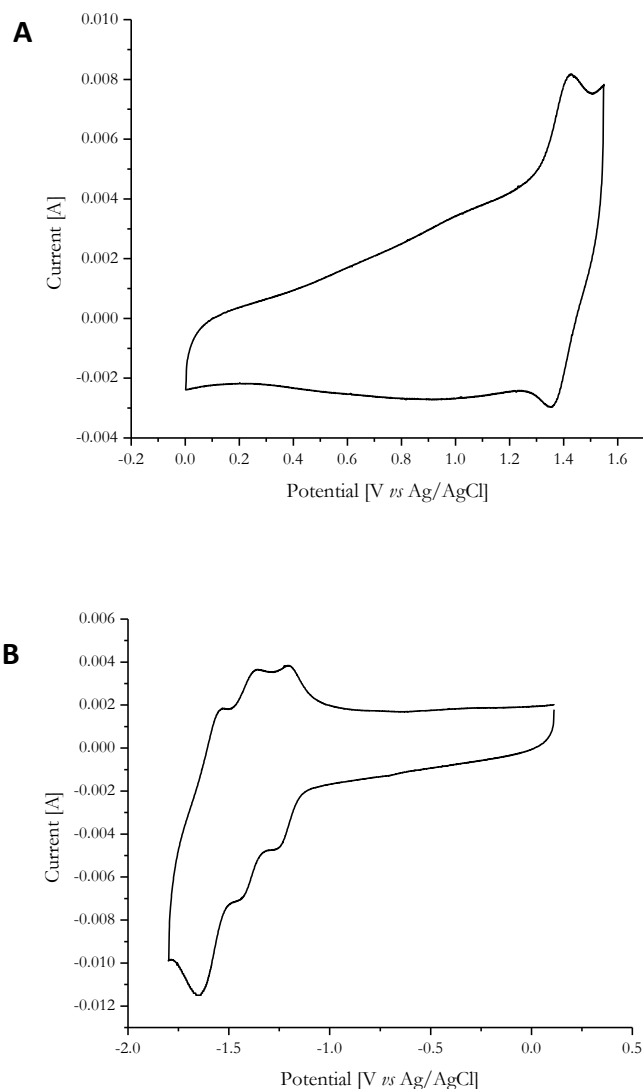


Figure 4.5. Cyclic voltammograms of **Ru-NPP** recorded in dry acetonitrile under argon, with a sweep rate of 0.3 V/s, at room temperature. The concentration of the complexes is 8.10^{-4} mol/L, with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte. (A) Positive polarization ($\Delta E_p = 64$ mV); (B) negative polarization ($\Delta E_{p1} = 56$ mV, $\Delta E_{p2} = 67$ mV, $\Delta E_{p3} = 93$ mV).

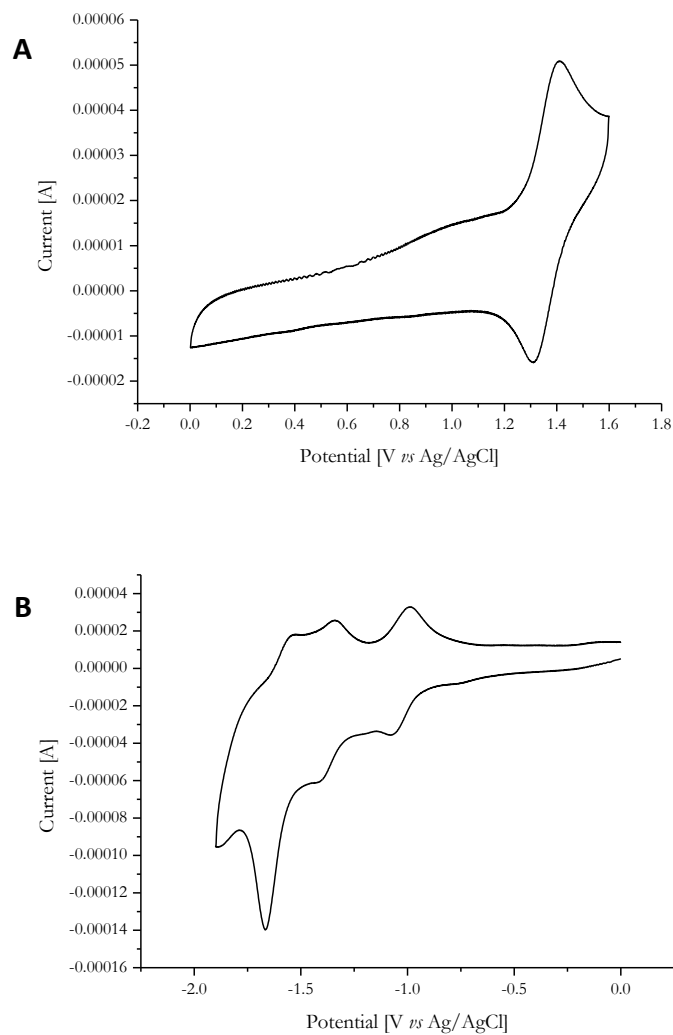


Figure 4.6. Cyclic voltammograms of **Ru-BDPPZ** recorded in dry acetonitrile under argon, with a sweep rate of 0.3 V/s, at room temperature. The concentration of the complexes is 8.10^{-4} mol/L, with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte. (A) Positive polarization ($\Delta E_p = 68$ mV); (B) negative polarization ($\Delta E_{p1} = 72$ mV, $\Delta E_{p2} = 67$ mV, $\Delta E_{p3} = 120$ mV).

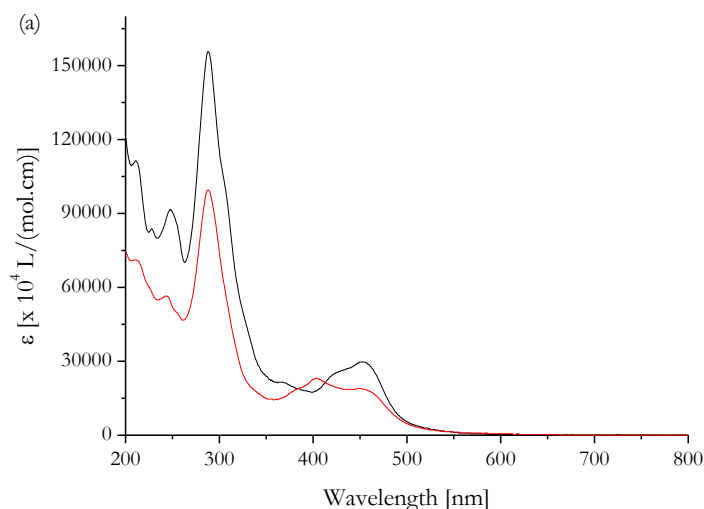
3.3. Absorption spectra

The absorption data in water and acetonitrile under ambient air conditions for the two complexes, as well as for other complexes for comparison purposes, are listed in Table 4.2. Absorption spectra for the two complexes in acetonitrile are depicted in Figure 4.7 a (see annexes for absorption spectra in water). In our case, the comparison with literature and absorption spectra of the free ligands (see annexes) allowed to assign the intense absorption ($\epsilon \geq 10^5 \text{ M}^{-1}\text{cm}^{-1}$, *ca.* 300 nm) bands in the UV region to ligand centered (LC) transitions and the ones in the visible region ($\epsilon \approx 10^4 \text{ M}^{-1}\text{cm}^{-1}$ around $\lambda \approx 400\text{-}450 \text{ nm}$) to Metal-to-Ligand Charge transfer (MLCT) transitions $\text{Ru} \rightarrow \text{L/L}'$, where L = bpy ($^1\text{MLCT}_{\text{bpy}}$) and L' = npp/bdppz ($^1\text{MLCT}_{\text{npp/bdppz}}$),

Complex	Absorbance λ_{max} [nm] ($\epsilon [10^4 \text{ M}^{-1}\text{cm}^{-1}]$) ^a	
	CH ₃ CN	H ₂ O
[Ru(bpy) ₃] ²⁺ ¹¹¹	250 (2.51), 285 (8.71), 323 (sh), 345 (sh), 452 (1.45)	250, 286, 322, 345, 451
Ru-DPPZ ¹¹¹	255 (4.18), 284 (9.36), 315 (sh), 342 (sh), 352 (sh), 357 (1.56), 366 (1.55), 448 (1.57)	255, 283, 315, 341, 352, 357, 365, 448
Ru-DPAC	224 (15.1), 264 (16.3), 280 (11.6), 448 (2.62)	224, 264 (16.2), 280, 448 (2.62)
Ru-NPP	210 (11.1), 248 (9.21) 287 (15.6), 457 (2.94)	210, 248, 287 (15.6), 457 (2.93)
Ru-BDPPZ	210 (7.10), 243 (5.64), 287 (9.96), 404 (2.32), 450 (1.89)	210, 243, 287 (9.96), 404, 448 (1.87)

Table 4.2. Absorption data in CH₃CN and H₂O under ambient air conditions for **Ru-NPP** and **Ru-BDPPZ**. ^a Measurements made with solutions $1.10^{-5} \text{ mol.L}^{-1}$ in complex at room temperature.

It has been shown in the literature that absorption of photons by **Ru-DPPZ** leads to the formation of a charge separated state $\text{Ru}^{3+}/\text{dppz}^{\bullet-}$ in which an electron is transferred from the metal center either towards the bpy ligands and the bpy portion of the dppz ligand, or towards the phenazine portion of the dppz ligand, depending on the environment of the complex.^{47,152} Such behavior accounts for the unusual photophysical properties of **Ru-DPPZ**. We previously reported that the replacement of a nitrogen atom of the phenazine moiety of dppz by a carbon atom strongly modifies the excited state properties of the resulting Ru(II) complex.¹⁹⁴ The increased conjugation provided by the addition of an aromatic ring in npp and bdppz compared to dpac and dppz respectively strikingly leads to small changes in the absorption spectra. Indeed, solely a small bathochromic shift (*ca.* 2-9 nm) of the MLCT band is observed for **Ru-NPP** and **Ru-BDPPZ** compared to **Ru-DPAC** and **Ru-DPPZ** respectively.



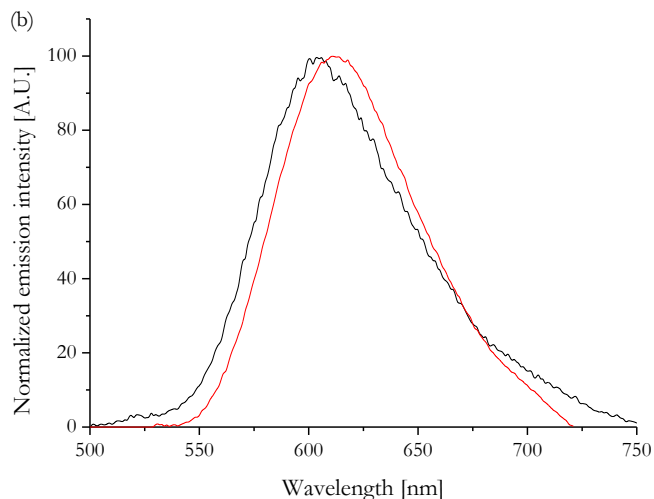


Figure 4.7. (a) Absorption and (b) emission spectra of **Ru-NPP** (black) and **Ru-BDPPZ** (red) in acetonitrile under air at room temperature.

3.4. Emission spectra, lifetimes, quantum yields

Complexes **Ru-NPP** and **Ru-BDPPZ** display broad unstructured emission both in organic solvent and water (Figure 4.7 b and associated content), suggesting a $^3\text{MLCT}$ -type excited state. The excited state lifetimes and luminescence quantum yield for each complex are measured in acetonitrile and water under an argon atmosphere (Table 4.3). The large k_r values ($> 10^4 \text{ s}^{-1}$), the positive solvatochromic effect ($\Delta\lambda \approx +2\text{-}9 \text{ nm}$ when going from MeCN to water) and the hypsochromic shift of the emission band at 77 K confirm the formation a dipole at the excited state, consequently to a Charge Transfer excited state. A decrease in the luminescence intensity in air-equilibrated solvents with respect to argon-purged solutions is observed in all cases, indicating a photosensitization of $^3\text{O}_2$ by the excited Ru(II) complexes (data not shown). The energy of luminescence decreases from **Ru-NPP** to **Ru-BDPPZ**, in agreement with the stabilization of the π^* orbital centered on the extended planar ligand going from npp to the more electron-withdrawing bdppz ligand. Interestingly, complex **Ru-BDPPZ** displays a low quantum yield but

does not behave like its analogous **Ru-DPPZ**, which is known as a “light switch”. Numerous experimental and theoretical studies have been devoted to understand the “light switching” effect.^{104,118,144,157} It is now commonly accepted that luminescence quenching in aqueous media is caused by hydrogen bonding with two water molecules, leading to the stabilization of dark states being a $^3\text{MLCT}_{\text{dppz}}$ and a $^3\text{LC}_{\text{dppz}}$, the latter being the lowest triplet state.¹⁹⁵ The residual luminescence in water observed for **Ru-BDPPZ** could be explained by a probable inhibition of the second Hydrogen bond between water and the non-coordinated nitrogen atom of the bdppz ligand, cramped by its “elbow-shaped” structure. Similar trend is indeed observed for **Ru-NPP** in comparison with **Ru-DPAC**. The former exhibits higher quantum yield than its linear equivalent (see Chapter 3), probably due to the inhibition of solvent interactions with the non-chelating nitrogen of the acridine moiety.

Complex	Emission λ_{max} [nm] ^a		Emission λ_{max} at 77 K [nm]		Φ_{em} ^{b, c}	τ_{em} [ns] ^b		k_r [10^3 s ⁻¹]		k_{nr} [10^5 s ⁻¹]	
	CH ₃ CN	H ₂ O	EtOH/MeOH 4/1			CH ₃ CN	H ₂ O	CH ₃ CN	H ₂ O	CH ₃ CN	H ₂ O
[Ru(bpy) ₃] ²⁺ ⁹⁸	604	604	578		0.062	0.042	855	630	77	69	15.7
Ru-DPPZ	610	-*	581		0.031	-*	692	-*	44.8	-*	-*
Ru-DPAC ¹⁹⁴	597	604	567		0.038	0.066	710	999	53.5	66.1	9.35
Ru-NPP	604	606	577		0.083	0.092	702	750	118.2	122.7	12.1
Ru-BDPPZ	611	620	580		0.068	0.0015	940 (+2% 5.5)	372 (+13% 9.4)	72.3	4.1	27.3

Table 4.3. Emission data in CH₃CN and H₂O at 298 K and 77 K for **Ru-NPP** and **Ru-BDPPZ**. ^a Measurements made with solutions 1.10⁻⁵ mol.L⁻¹ in complex under air. ^b Measurements made with solutions 1.10⁻⁵ mol.L⁻¹ in complex under Argon. ^c Measured relative to [Ru(bpy)₃]²⁺ in an aerated aqueous solution ($\Phi_{em}^{air} = 0.028$)¹⁰². * No luminescence is observed in H₂O.

On the basis on our emission data and results from Chapter 3, we suggest both complexes follow different photophysical schemes. In the case of **Ru-NPP**, $^1\text{MLCT}$ states are generated upon light irradiation which rapidly undergo fast ISC to form a $^3\text{MLCT}$ state involving the ancillary bpy ligands ($[\text{Ru}^{\text{III}}(\text{bpy}^{\bullet})]^*$ – Figure 4.8). As suggested by electrochemical data, further relaxation occurs to a luminescent excited state localized on the bpy fragment of the extended planar ligand ($[\text{Ru}^{\text{III}}(\text{npp}^{\bullet})]^*$). The “elbow-shape” of the npp ligand disfavoring solvent interactions with the non-chelating nitrogen atom in the excited state, no further stabilization of the excited states towards the extended portion of npp may occur in that case.

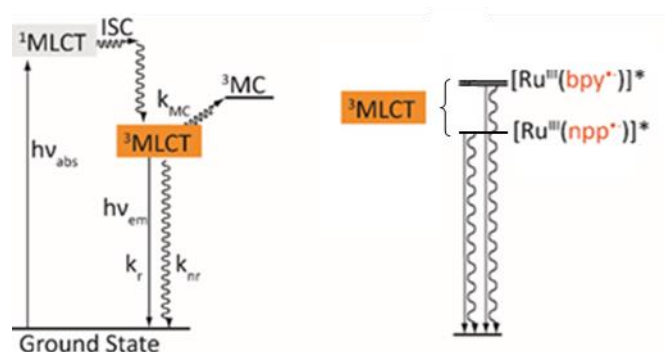


Figure 4.8. Simplified representation of the photophysical scheme for **Ru-NPP** both in acetonitrile and in water. The deactivation of $^3\text{MLCT}$ states is emphasized on the right part of the figure for the complex. Solid arrows stand for radiative transitions, wedged arrows stand for non-radiative transitions (ISC, IC or VR).

In opposition to **Ru-NPP**, a different photophysical scheme is required to rationalize the luminescence behavior of **Ru-BDPPZ**. Analogously to **Ru-DPPZ** (see Chapter 3), several $^3\text{MLCT}$ states are required to explain the experimental data. At first, after ISC^{159,160} from $^1\text{MLCT}$ excited states, a $^3\text{MLCT}$ state involving the ancillary bpy ligands ($[\text{Ru}^{\text{III}}(\text{bpy}^{\bullet})]^*$ – Figure 4.9) is formed. Further relaxation occurs towards a luminescent $^3\text{MLCT}$ state involving the bpy fragment from bdppz ($[\text{Ru}^{\text{III}}(\text{bdppz}^{\bullet})]^*$). Subsequent stabilization then occurs on the phenazine moiety of the ligand, leading to the formation

of a delocalized $[\text{Ru}^{\text{III}}(\text{bdppz}^{\bullet-})]^*$ excited state. Further interconversions from this state may ultimately arise in aqueous media: one to a luminescent mono-hydrogen bonded excited state $[\text{Ru}^{\text{III}}(\text{bdppz}^{\bullet-}\text{-H})]^*$, and another one to a non-luminescent bis-hydrogen bonded excited state $[\text{Ru}^{\text{III}}(\text{bdppz}^{\bullet-}\text{-2H})]^*$ (Figure 4.9).¹¹³ The presence of the latter is suggested by the partial luminescence quenching of **Ru-BDPPZ** in water. However, the formation of the second hydrogen bond, responsible for the extinction of the luminescence, is disfavored due to the “elbow-shape” of the ligand. Such proposal lies in agreement with the model of Lincoln and co-workers for which two hydrogen bonds are required for the complete luminescence quenching of **Ru-DPPZ** in water.¹¹⁸ These results highlight the importance of the central ring of the extended planar ligands on the resulting photophysical properties of the corresponding Ru(II) complexes.

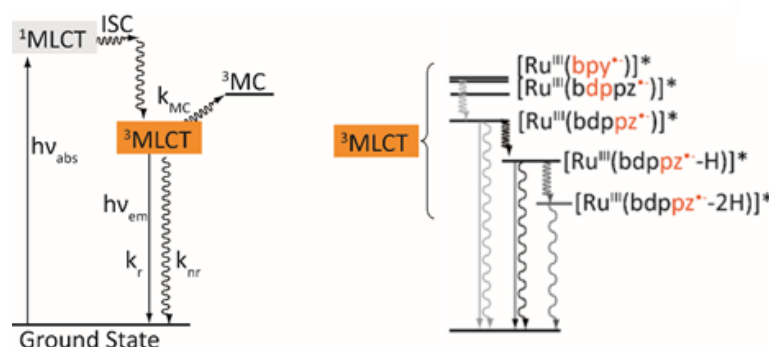


Figure 4.9. Simplified representation of the photophysical scheme for **Ru-BDPPZ** in water. The deactivation of $^3\text{MLCT}$ states is emphasized on the right part of the figure for the complex. Solid arrows stand for radiative transitions, wedged arrows stand for non-radiative transitions (ISC, IC or VR).

3.5. Photophysical behavior in the presence of DNA

Ru(II) complexes have been extensively studied as DNA photoprobes and photoreagents.^{142,143} When used as probes, the luminescence of the complexes is enhanced in the presence of DNA, as a result of the interaction of the complex with DNA which decrease or inhibit some non-radiative deactivations pathways. In particular, very sensitive binding to DNA could be observed for Ru(II) complexes bearing an extended planar ligand, which is intercalated between the DNA base pairs. Usually, this binding mode is accompanied by strong emission increase in aqueous medium and is associated with strong binding affinity ($K_{\text{aff}} \approx 10^6 \text{ L.mol}^{-1}$) between DNA and the complex.^{111,120,145,162} In that context, pioneering work by Barton *et al.* has shown **Ru-DPPZ** exhibits a luminescent-sensitive behavior in the presence of oligonucleotides.⁴⁶ Our complexes being structurally similar to the latter, we have investigated the luminescence behavior of our two “elbow-shaped” Ru(II) complexes in presence of SS- and CT-DNA.

DNA titrations experiments show luminescence enhancement for both complexes in the presence of increasing concentration of DNA (CT-DNA and SS-DNA) (Figure 4.10). The strong and fast emission increase suggests a high binding affinity of the complexes for these two DNAs. Binding affinity values have thus been calculated using a modified McGhee-von Hippel equation¹⁶³ as expression for the luminescence intensity as function of ratio of Ru(II) complex per DNA base pair (I/I_0 vs $[\text{DNA}]/[\text{complex}]$, with I_0 , the intensity of luminescence in the absence of DNA and $[\text{DNA}]$ expressed in base-pair equivalents, see Table 4.4 and Figure 4.10). Such behavior is ascribed to the inhibition of the non-radiative deactivation processes of the ³MLCT excited states upon binding to DNA, either by protection of the complexes which avoid luminescence quenching by oxygen, or by protection from interactions with solvent molecules. Consequently, the enhancement of luminescence is much stronger with **Ru-BDPPZ** than **Ru-NPP**, thanks to the efficient protection of

the two nitrogen atoms of the bdppz ligand made by the DNA base pairs stack. The DNA binding thus prevents the luminescence quenching by inhibiting the formation of the two Hydrogen bonds in the excited state. Due to the elongated planar structure of npp and bdppz and consistently with the binding values obtained, we assume our complexes intercalate into the DNA strands as other **Ru-DPPZ** analogues.

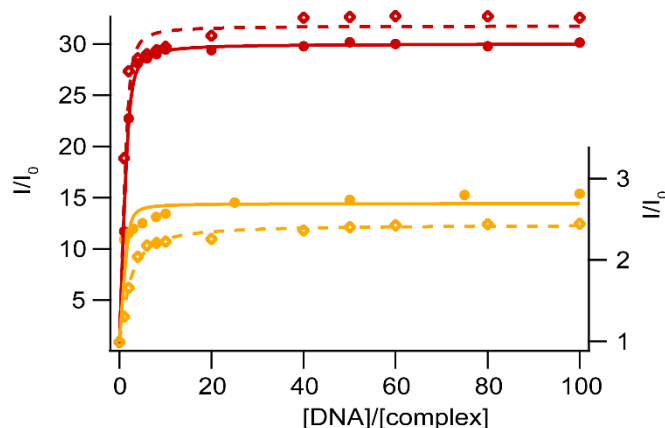


Figure 4.10. Steady-state luminescence titration of **Ru-NPP** (yellow – right axis) and **Ru-BDPPZ** (red – left axis) with SS-DNA (full circles) and CT-DNA (empty diamonds). Measurements are performed using 10 μ M of complex in TrisHCl buffer 50 mM, NaCl 50 mM at pH 7.4 under ambient air conditions. The fitted curves are obtained using a modified McGhee-Von Hippel equation and drawn as solid and dashed curves for SS- and CT-DNA respectively.

	SS-DNA		CT-DNA	
	$K_{aff} (M^{-1})^a$	I/I_0 max	$K_{aff} (M^{-1})^a$	I/I_0 max
Ru-NPP	$7.95 \cdot 10^5$	2.70	$8.31 \cdot 10^4$	2.44
Ru-BDPPZ	$5.06 \cdot 10^5$	30.1	$6.86 \cdot 10^5$	31.8

Table 4.4. Binding affinity constants calculated for **Ru-NPP** and **Ru-BDPPZ** with SS-DNA and CT-DNA. ^a Binding constant are obtained using a McGhee-von Hippel type equation. The binding site is fixed to two base pairs per complex (best fit). Errors estimated to 5%.

3.6. Photophysical behaviour in the presence of mismatched DNA

As discussed in the introduction, a Ru(II) complex which would specifically associate to mismatches in DNA would be very valuable in order to detect these mutations in the genetic material. **Ru-DPPZ** has been shown to be able to sense single-base mismatch with a moderate selectivity.¹⁰⁶ In that case, the recognition of the mismatches is correlated to their relative thermodynamic stability.^{106,191,192} Since then, other Ru(II) complexes where the intercalating ligand has been modified were studied.^{191,192} An enhanced selectivity can be achieved for some of them, but these complexes were not or poorly luminescent.

We have demonstrated in Chapter 3 that Ru(II) linear dpac-complexes are unfortunately unable to probe mismatch site.¹⁹⁴ The elbow-shaped structure of our npp and bdppz ligands is of great interest in order to obtain such selectivity for DNA mismatches as dissymmetry of the inserted ligand proved to be useful in this context for other Ru(II) complexes.¹⁹² The ability of **Ru-NPP** and **Ru-BDPPZ** to detect single-base mismatches in short oligonucleotides was thus investigated using a series of hairpin oligonucleotides containing a mismatch near the center of the duplex (see sequences in Figure 4.11).

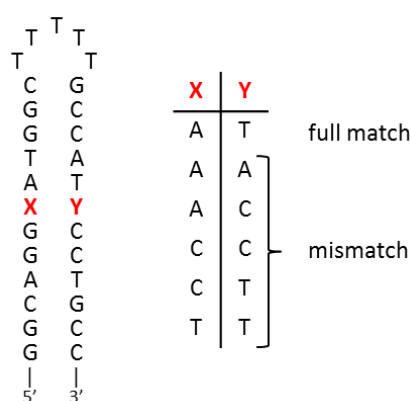


Figure 4.11. Hairpin oligonucleotides sequences used for titrations.

Both complexes bearing “elbow-shaped” intercalating ligand show distinct luminescence intensities with mismatched sequences as compared to complementary one (Figure 4.12). Interestingly, while the strongest luminescence enhancement is observed for the complementary hairpin for **Ru-NPP**, an opposite behavior occurs for **Ru-BDPPZ**.

The luminescence decrease observed for **Ru-NPP** in the presence of mismatched hairpins with respect to the complementary one could be ascribed to a less effective interaction of this complex for the mismatched base pairs (either a weaker binding and/or a less efficient protection from non-radiative deactivation pathway), leading to increased exposure to oxygen and solvent molecules with respect to the complementary sites. Similar behavior has been observed for linear **Ru-DPAC**, as a result of a looser fit of the complex within mismatched sites¹²⁵ favoring non-radiative deactivations of the excited state.¹⁹⁴

In contrast, the luminescence of complex **Ru-BDPPZ** is greatly enhanced while the complex is in the presence of mismatched sequences, compared to the complementary hairpin. In addition, the greater the thermodynamic instability of the mismatched site, the greater the luminescence enhancement is observed. Such a similar trend has previously been reported for **Ru-DPPZ** and $[\text{Rh}(\text{bpy})_2\text{chrysi}]^{3+}$, for which binding to mismatched sites occurs *via* insertion with ejection of the mismatched bases towards the DNA groove.^{48,106,189,190} The enhancement of the luminescence behavior, observed for **Ru-BDPPZ** and reported for **Ru-DPPZ** and its analogues, is due to the deeper packing allowed by the mismatched site of the hairpin, and thus better protection of the complex from the solvent with respect to the corresponding complementary site. As a result, non-radiative relaxation pathways of the excited state of **Ru-BDPPZ** are disfavored in the presence of mismatched containing hairpins. Since this particular photophysical behavior is observed for

this complex, we suggest it binds mismatched hairpins through metallo-insertion, probably associated with favorable interactions between hairpin residues and the phenazine portion of the ligand.

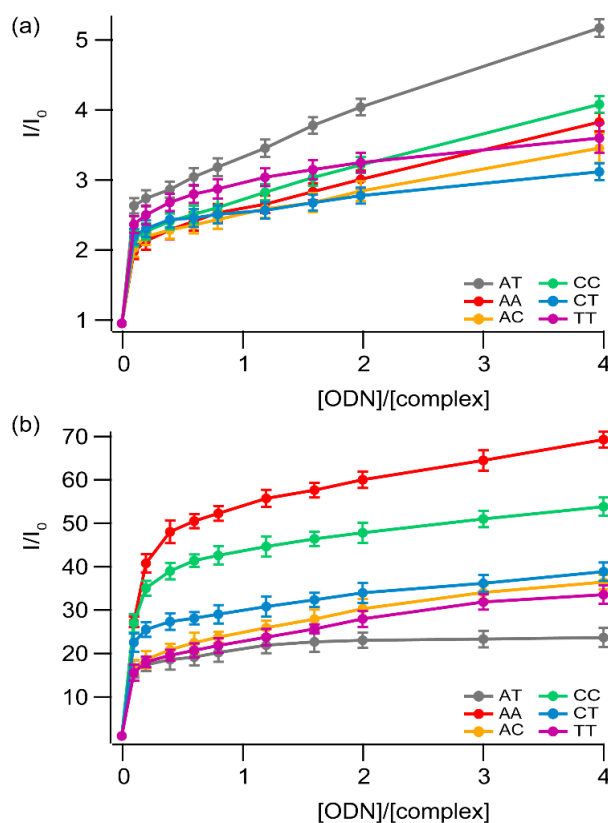


Figure 4.12. Steady-state luminescence titration of (a) **Ru-NPP** and (b) **Ru-BDPPZ** with a series of small hairpins oligonucleotides which possess (or not) a mismatched base pair. Measurements are performed using 1 μM of complex in TrisHCl buffer 5 mM, NaCl 1 mM, pH 7.5 under ambient air conditions.

The strong differential luminescence behavior of **Ru-BDPPZ** in the presence of mismatched hairpin versus the complementary duplex correspond to a 3.5-fold enhancement for CC mismatches compared to well-match, which is a better enhancement than the one observed using only the more favorable Δ stereoisomer of **Ru-DPPZ**¹⁰⁶ in the same conditions. Using a modified McGhee-von Hippel equation to get an insight of the binding affinity of **Ru-BDPPZ** for the different

mismatches, we can estimate that the complex has a one-order higher affinity for the mismatch site compared to well-match ones ($\sim 10^7$ vs $3.88 \cdot 10^6$ L/mol). Consequently, the luminescence enhancement is not only due to a better protection of the excited complex inserted at the mismatched site, but also to a greater affinity of the complex for insertion compared to intercalation within DNA.

4. Conclusions

New “elbow-shaped” Ru(II) complexes bearing acridine- and phenazine-based extended planar ligands have been prepared. The electrochemical and photophysical studies indicate that the lowest $^3\text{MLCT}$ excited state for $[\text{Ru}(\text{bpy})_2\text{npp}]^{2+}$, **Ru-NPP**, is located on the bpy fragment of the npp ligand. The replacement of a central carbon atom in npp for a nitrogen to obtain bdppz has a strong effect on the electrochemical and photophysical properties of the resulting Ru(II) complex, as **Ru-BDPPZ** is poorly luminescent in water. An excited state located on the phenazine moiety of the bdppz ligand and involved in two Hydrogen bonds is proposed to account for its partial luminescence quenching in aqueous medium. The residual luminescence observed in aqueous medium (in contrast to the non-luminescent well-known **Ru-DPPZ**) is rationalized by the inhibition of the second Hydrogen bond between water molecules and the second nitrogen atom of the phenazine core thanks to the “elbow-shape” of bdppz.

Studies of these two complexes in the presence of DNA suggest avid binding to polynucleotides and protection of the excited complexes from non-radiative deactivation pathways upon intercalation into the base pairs stack. The stronger enhancement of emission for **Ru-BDPPZ** with respect to **Ru-NPP** is attributed to the prevention of the two Hydrogen bonds in the excited state, the second one being responsible for the complete luminescence quenching.

The behavior of our complexes was also investigated in the presence of single-base mismatch-containing hairpins. While a small difference in luminescence of **Ru-NPP** in the presence of mismatched hairpins compared to complementary might be due to poor protection of the excited state of **Ru-NPP** from the non-radiative relaxation pathways, a strong differential luminescence behavior in the presence of mismatched hairpin versus the complementary duplex occurs for **Ru-BDPPZ**. In the presence of CC mismatches, a 3.5-fold enhancement of the luminescence is observed, compared to the intercalation with complementary sequence. This enhancement for the racemic mixture of **Ru-BDPPZ** is more important than the ratio reported for Δ -**Ru-DPPZ**. Like this latter one, **Ru-BDPPZ** is suggested to bind mismatched sites via metallo-insertion. This tight fit interaction leads to better protection of the complex from the solvent compared to intercalation, and is supported by the correlation observed with the relative thermodynamic stability of the mismatch. We propose favorable interactions between the phenazine portion of bdppz with hairpin residues lie at the basis of the stronger binding of **Ru-BDPPZ** to mismatched hairpins, and consequently of the luminescence sensitivity for the mismatched sites. The “elbow-shaped” complex **Ru-BDPPZ** might therefore represent a promising candidate for the development of DNA mismatches selective photoprobes.

5. Experimental section

5.1. Synthetic procedures and characterization of “elbow-shaped” ligands and respective bpy-based Ru(II) complexes

npp. 5-amino-1,10-phenanthroline (100.0 mg, 0.5773 mmol) and (1-aminonaphthalen-2-yl)methanol (112.6 mg, 0.5773 mmol) are suspended in 6 N HCl (1.6 mL). The reaction mixture is stirred at reflux (65°C) for 20h and, after cooling to room temperature, the reaction is quenched by addition of NH₃.H₂O until reaching pH 9. The

orange-red precipitate is filtered and purified by neutral alumina column of chromatography (eluent: dichloromethane/methanol gradient, from 100/0 to 90/10). No further purification is performed and the isolated material was used as such for the next step.

bdppz. 1,10-phenanthroline-5,6-dione (369.8 mg, 1.759 mmol) and 1,2-diaminonaphtalene (411.0 mg, 2.111 mmol) are stirred at 50°C for three days in ethanol (150.0 mL). The reaction mixture is recrystallized in absolute ethanol and the light brown solid formed is filtered and washed with cold ethanol. The residue is dried under vacuum. Further addition of TFA 1% (aq) lead to the precipitation of the targeted compound as a dark yellow solid. Yield: 86%. ^1H NMR (500 MHz, CDCl_3): δ 9.75 (dd, 1H, H_c , $J_{c-b} = 8.1$ Hz, $J_{c-a} = 1.7$ Hz), 9.61 (dd, 1H, H_k , $J_{k-l} = 8.1$ Hz, $J_{k-m} = 1.7$ Hz), 9.47 (dd, 1H, H_j , $J_{j-i} = 7.4$ Hz, $J_{j-h} = 1.3$ Hz), 9.28 (m, 2H, H_a and H_a'), 8.10 (2d, 2H, H_e , H_f , $J_{e-f} = 9.1$ Hz, $J_{f-e} = 9.1$ Hz), 7.98 (dd, 1H, H_g , $J_{g-h} = 7.3$ Hz, $J_{g-i} = 1.4$ Hz), 7.83 (m, 4H, H_b , H_h , H_i , H_l). HRMS (APCI, +p): calc. 333.11402 for $\text{C}_{22}\text{H}_{13}\text{N}_4$, found 333.11347 ($\text{M}+\text{H}^+$).

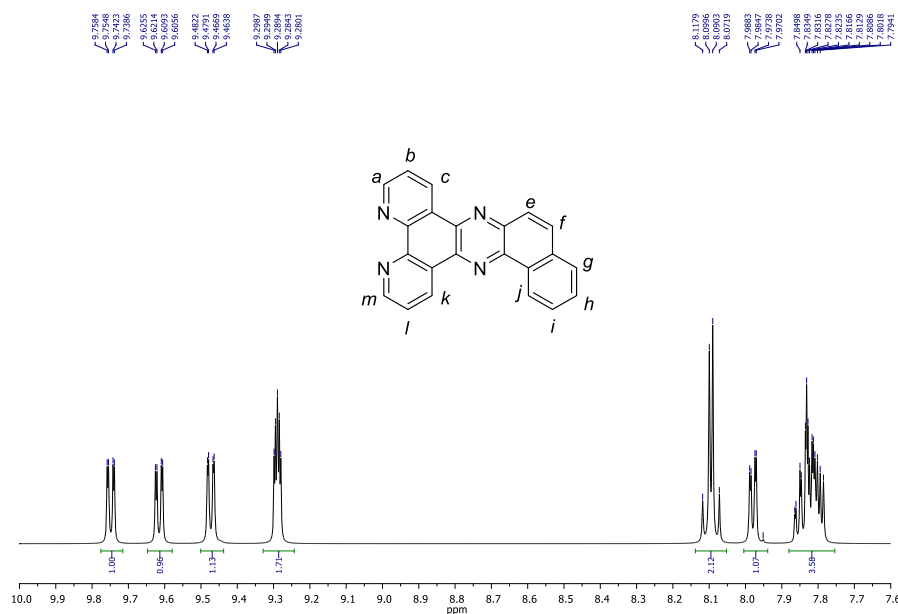


Figure 4.13. ^1H NMR spectrum for the bdppz ligand (500 MHz, CDCl_3).

[Ru(bpy)₂npp]²⁺. One equivalent of dichloro precursor Ru(bpy)₂Cl₂ (30.0 mg, 0.06194 mmol) is mixed with 1.1 equivalent of npp (22.6 mg, 0.06813 mmol) in a mixture of absolute ethanol/water (50/50, 5 mL). The reaction mixture is stirred under an argon atmosphere at reflux (80°C) and is followed by absorption spectroscopy. After 3 hours, the medium is concentrated and addition of small portions of NH₄PF₆ and drops of water yielded a red precipitate. After centrifugation, the solid is washed with excess water. After drying under vacuum, the orange-red residue is purified by preparative plate of chromatography (silica, eluent: CH₃CN/H₂O/saturated aqueous NH₄Cl 4/4/1, R_f = 0.95). The counter-anion exchange from PF₆⁻ to Cl⁻ is performed by eluting a solution of complex in a mixture CH₃CN/H₂O on Sephadex DEAE A25. ¹H NMR (PF₆ salt, 300 MHz, (CD₃CN): δ (ppm), 10.07 (dd, 1H, H_k, J_{k-l} = 8.3 Hz, J_{k-m} = 1.3 Hz), 9.77 (s, 1H, H_d), 9.71 (dd, 1H, H_j), 9.34 (dd, 1H, H_c, J_{c-b} = 8.2, J_{c-a} = 1.7 Hz), 8.53 (m, 4H, H₅, H_{5'}, H₆, H_{6'}), 8.15 (m, 7H, H₄, H_{4'}, H_a, H_e, H_g, H_i, H_m), 8.00 (m, 2H, H₇, H_{7'}), 7.90 (m, 6H, H_b, H_f, H_j, H_l, H₂, H_{2'}), 7.71 (m, 2H, H₉, H_{9'}), 7.48 (dd, 2H, H₃, H_{3'}), 7.25 (m, 2H, H₈, H_{8'}). HRMS (ESI, +p): calc. 890.11643 for [C₄₃H₂₉N₇F₆PRu]⁺, found 890.11728 ([M-PF₆]⁺) and calc. 372.57585 for [C₄₃H₂₉N₇Ru]²⁺, found 372.57616 ([M-2PF₆]²⁺).

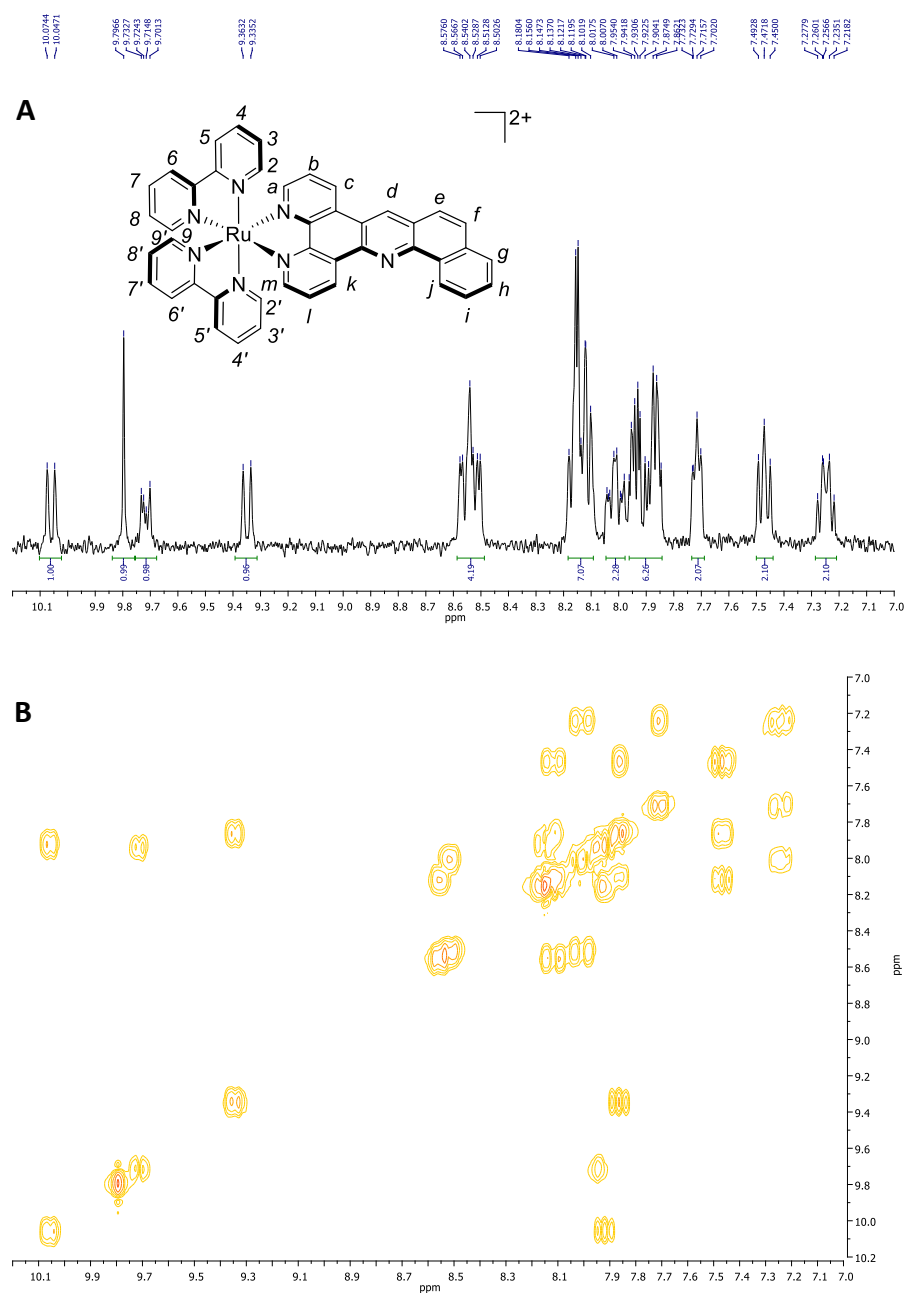


Figure 4.14. (A) 1D and (B) 2D ^1H NMR COSY spectra for **Ru-NPP** (500 MHz, CD_3CN).

[Ru(bpy)₂bdppz]²⁺. One equivalent of Ru(bpy)₂Cl₂ (40.0 mg, 0.04376 mmol) is mixed with 1.6 equivalent of bdppz (23.2 mg, 0.07002 mmol) in a mixture of absolute ethanol/acetonitrile (50/50, 4 mL). The reaction mixture is stirred under an argon atmosphere at reflux (80°C) and followed by absorption spectroscopy and thin layer chromatography (silica, eluent: CH₃CN/H₂O/saturated aqueous NH₄Cl 4/5/1, R_f = 0.25). After 3 hours, the medium is concentrated and addition of small portions of NH₄PF₆ and drops of water yielded a brown-red precipitate. After centrifugation, the solid is washed with excess water. After drying under vacuum, the residue is purified by preparative plate of chromatography (silica, eluent: CH₃CN/H₂O/saturated aqueous NH₄Cl 4/5/1, R_f = 0.25). The counter-anion exchange from PF₆⁻ to Cl⁻ is performed by eluting a solution of complex in a mixture CH₃CN/H₂O on Sephadex DEAE A25. ¹H NMR (PF₆ salt, 500 MHz, (CD₃CN): δ (ppm), 9.92 (dd, 1H, H_k, J_{k-l} = 8.3 Hz, J_{k-m} = 1.3 Hz), 9.70 (dd, 1H, H_c, J_{c-b} = 8.2, J_{c-a} = 1.3 Hz), 9.62 (dd, 1H, H_j, J_{j-i} = 7.3, J_{j-h} = 2.2 Hz), 8.56 (d, 2H, H₅, H_{5'}, J₅₋₄ = J_{5'-4'} = 8.3 Hz), 8.53 (dd, 2H, H₆, H_{6'}, J₆₋₇ = J_{6'-7'} = 8.3 Hz), 8.39 (d, 1H, H_e, J_{e-f} = 9.2 Hz), 8.23 (d, 1H, H_f, J_{f-e} = 9.2 Hz), 8.21 (m, 2H, H_g, H_m), 8.12 (m, 2H, H₄, H_{4'}), 8.03 (dd, 2H, H₇, H_{7'}, J₇₋₈ = 7.85 Hz, J₇₋₉ = 1.5 Hz), 7.98 (2dd, 2H, H_h, H_i), 7.93 (2dd, 2H, H_b, H_l, J_{b-c} = J_{l-k} = 8.3 Hz, J_{b-a} = J_{l-m} = 5.3 Hz), 7.88 (dd, 2H, H₂, H_{2'}, J₂₋₃ = J_{2'-3'} = 5.5 Hz, J₂₋₄ = J_{2'-4'} = 1.3 Hz), 7.74 (dd, 2H, H₉, H_{9'}, J₉₋₈ = J_{9'-8'} = 5.2 Hz, J₉₋₇ = J_{9'-7'} = 1.4 Hz), 7.48 (m, 2H, H₃, H_{3'}), 7.26 (dd, 2H, H₈, H_{8'}, J₈₋₆ = J_{8'-6'} = 1.3 Hz). HRMS (ESI, +p): calc. 891.11167 for [C₄₂H₂₈N₈F₆PRu]⁺, found 891.11251 ([M-PF₆]⁺) and calc. 373.07347 for [C₄₂H₂₈N₈Ru]²⁺, found 373.07391 ([M-2PF₆]²⁺).

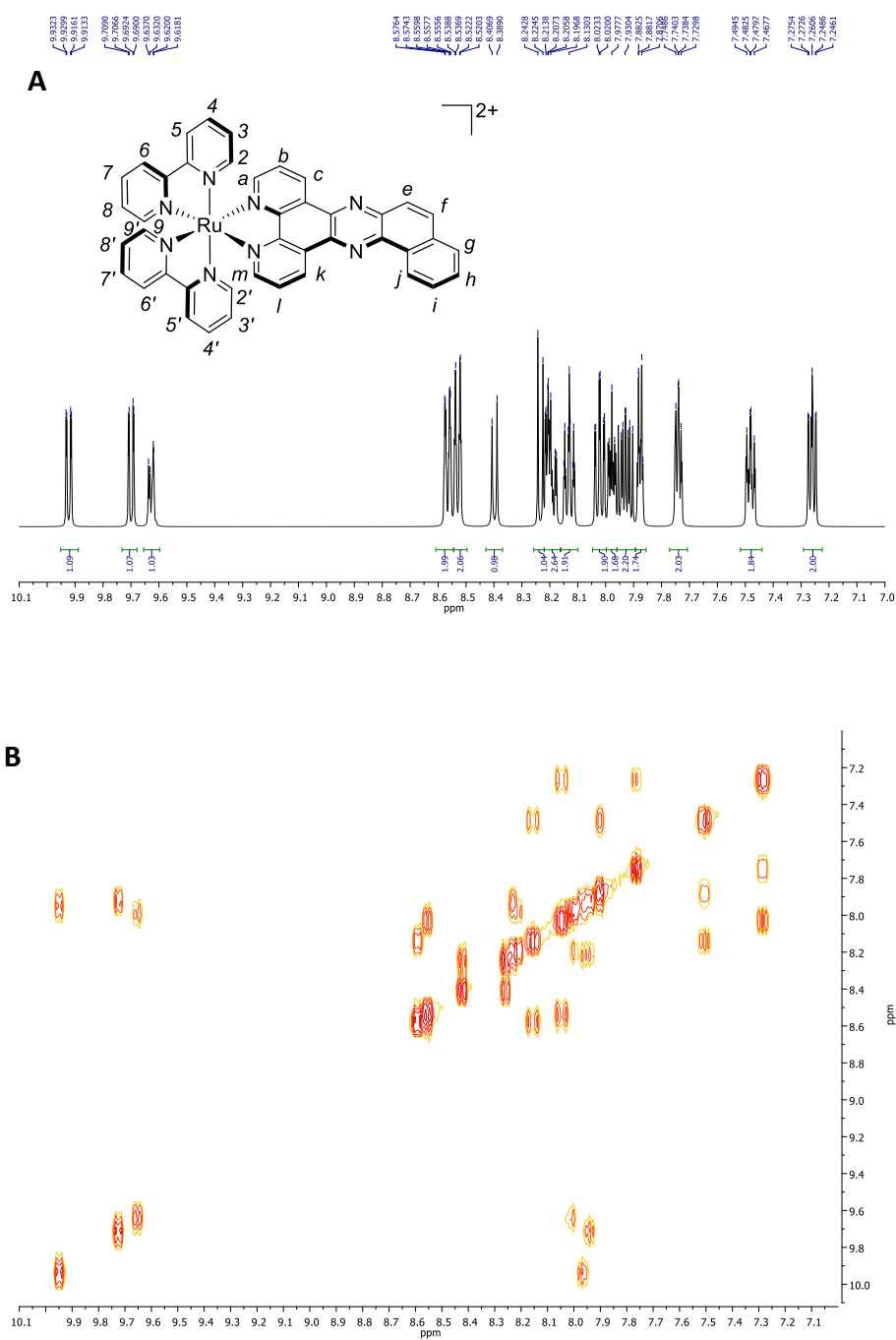


Figure 4.15. (A) 1D and (B) 2D ^1H NMR COSY spectra for **Ru-BDPPZ** (500 MHz, CD_3CN).

6. Associated content

6.1. Absorption spectra of the ligands

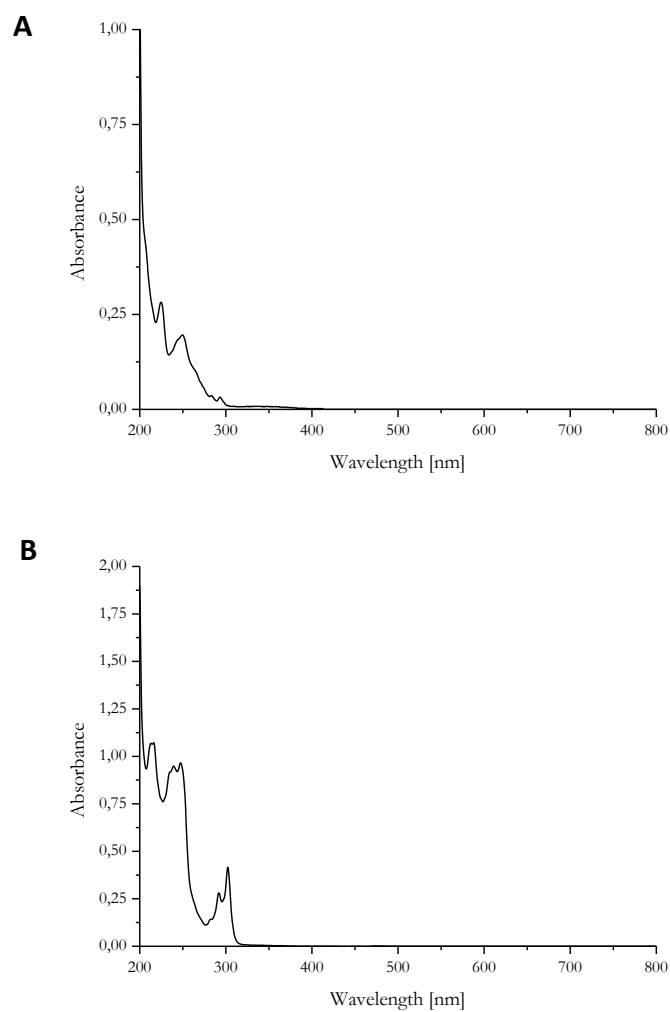


Figure 4.16. Absorption spectra under air in acetonitrile for (A) **Ru-NPP** and (B) **Ru-BDPPZ**.

6.2. Absorption and emission spectra of the Ru(II) complexes

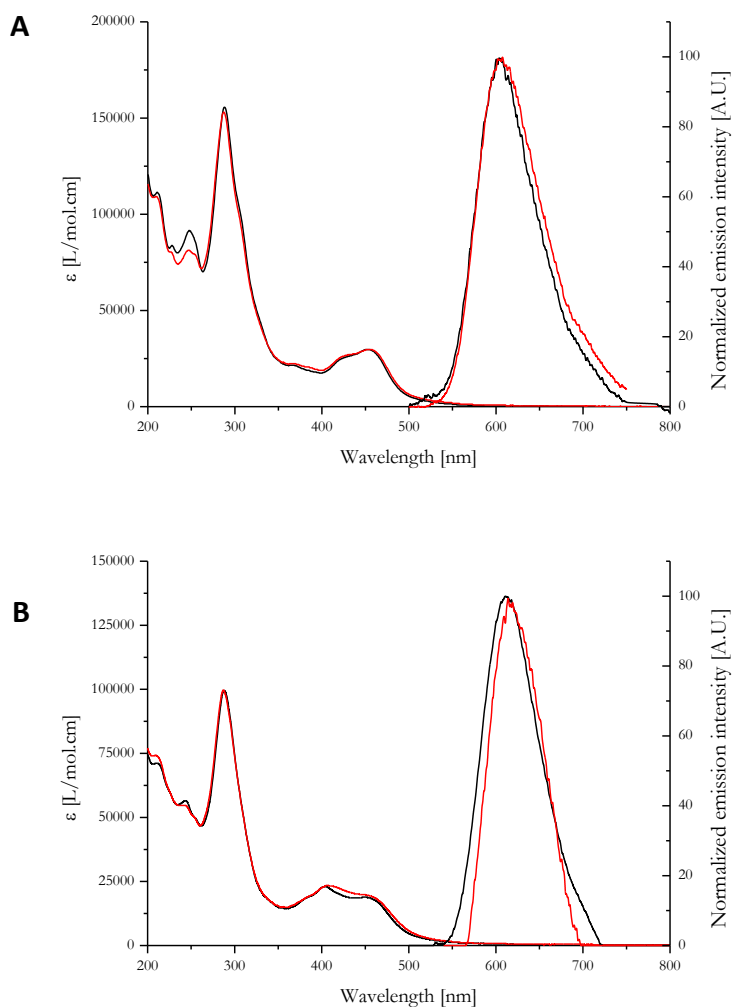


Figure 4.17. Absorption and emission spectra under air in acetonitrile (black) and in water (red) for (A) **Ru-NPP** and (B) **Ru-BDPPZ**.

6.3. Emission spectra of the Ru(II) complexes at 77 K

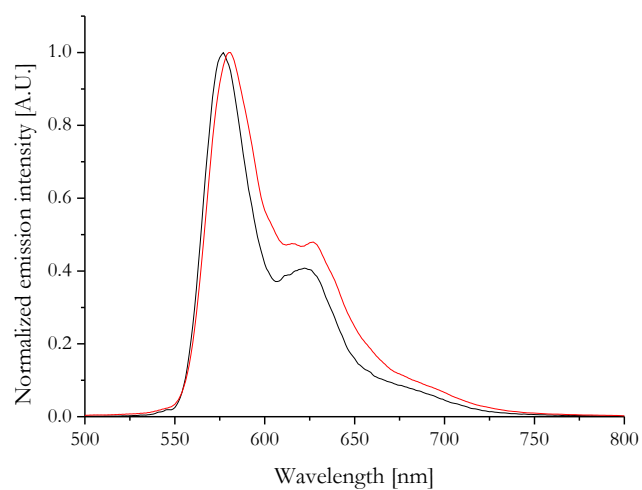


Figure 4.18. Emission spectra at 77 K in EtOH/MeOH 4/1 (v/v) rigid matrix for **Ru-NPP** (black) and **Ru-BDPPZ** (red).

6.4. HRMS spectra and data

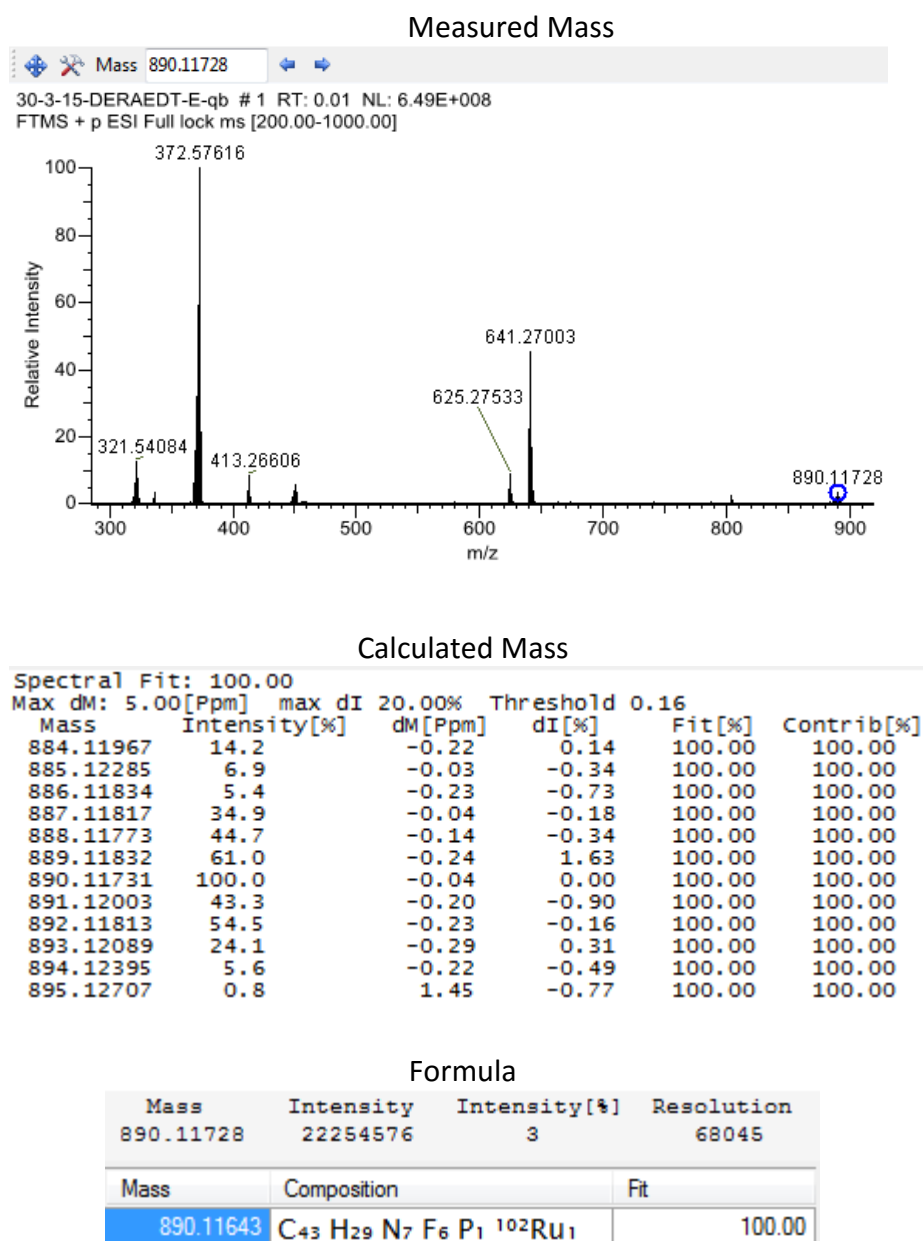
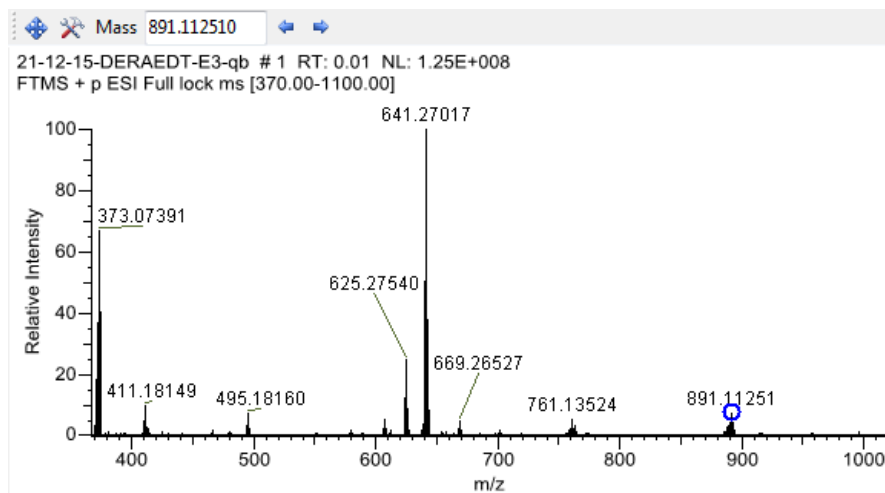


Figure 4.19. HRMS data for **Ru-NPP** ([M-PF₆]⁺).

Measured Mass



Calculated Mass

Spectral Fit: 100.00
Max dM: 5.00[Ppm] max dI 20.00% Threshold 0.09

Mass	Intensity[%]	dM[Ppm]	dI[%]	Fit[%]	Contrib[%]
885.11492	14.3	-0.08	-0.01	100.00	100.00
886.11808	6.7	-0.07	-0.40	100.00	100.00
887.11355	5.4	0.01	-0.78	100.00	100.00
888.11343	35.0	-0.10	0.73	100.00	100.00
889.11294	44.7	-0.17	-1.82	100.00	100.00
890.11355	60.8	-0.08	-0.96	100.00	100.00
891.11255	100.0	-0.04	0.00	100.00	100.00
892.11523	42.5	-0.22	-0.11	100.00	100.00
893.11335	54.5	-0.02	-0.71	100.00	100.00
894.11609	23.6	-0.03	-0.13	100.00	100.00
895.11913	5.4	-0.06	-0.34	100.00	100.00
896.12220	0.8	1.18	-0.47	100.00	100.00

Formula

Mass	Intensity	Intensity[%]	Resolution
891.112510	9291717	7	67521

Mass	Composition	Fit
891.111675	C ₄₂ H ₂₈ N ₈ F ₆ P ₁ ¹⁰² Ru ₁	100.00

Figure 4.20. HRMS data for Ru-BDPPZ ([M-PF₆]⁺).

Chapter 5

Photo-induced electron transfer between Ru(II) complexes and polynucleotides

1. Introduction

As mentioned in the introduction, the excited states of Ru(II) polypyridyl complexes could be deactivated according to different pathways. Among them, photo-induced reactions such as photo-induced electron transfer can lead to partial or total luminescence quenching, if the excited state of the complex is luminescent. The main possible intermolecular processes responsible for luminescence quenching of an excited molecule A^* by a quencher B are gathered in Table 5.1.⁹⁴ It should be noted that intramolecular processes such as electron or proton transfer, isomerizations can also give rise to luminescence quenching. However, solely intermolecular reactions will be discussed in this chapter.

Photophysical process	Reaction
Electron transfer	$A^* + B \rightleftharpoons A^+ + B^-$
Proton transfer	$AH^* + B \rightleftharpoons A^{\cdot-} + BH^+$
Energy transfer	$A^* + B \rightleftharpoons A + B^*$
Excimer formation	$A^* + A \rightleftharpoons (AA)^*$
Exciplex formation	$A^* + B \rightleftharpoons (AB)^*$

Table 5.1. Main photophysical processes responsible for luminescence quenching between two molecules (A and B). The corresponding excited states are depicted with a star. In the cases mentioned here, B acts a quencher (except for the excimers).

1.1. Luminescence quenching processes

The different intermolecular photo-induced reactions which may arise between an excited luminescent molecule A^* and B (Table 5.1), regardless the intrinsic deactivation of A^* , can be differentiated by the experimental conditions in which they occur:

- first case: B, the quencher, is in large excess compared to A, so that there is a high probability that A and B are associated to each other at the time of excitation (Figure 5.1). No mutual

approach during the excited state lifetime of A^* is thus required. In that case, the luminescence quenching is called static and is characterized by a pseudo-first order rate constant, and the photo-induced reaction takes place under non-diffusive conditions.

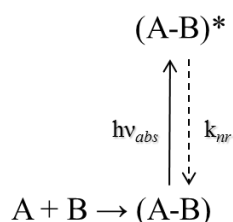


Figure 5.1. Schematic reactions occurring for static quenching, k_{nr} being the non-radiative deactivation constant of the (A-B)* system.

- b) second case: if B is not in large excess compared to A but the excited state lifetime of A* is too short or the medium is too viscous, a long-range (up to 20 nm)⁹⁴ quenching of the luminescence may occur. Such case can be observed for long-range non-radiative energy transfer (see section 1.2.2.2., Chapter 7).
- c) third case: when B is not in large excess compared to A and the excited state lifetime of A* is long enough, a mutual approach between A and B is possible after excitation of A (Figure 5.2). This could lead to a so-called dynamic quenching, characterized by a diffusion step (determining or not). It should be noted that static quenching may occur in addition to dynamic quenching at higher concentrations of quencher B.

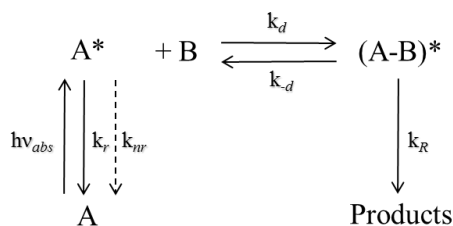


Figure 5.2. Schematic reactions occurring for dynamic quenching, k_r and k_{nr} being respectively the radiative and non-radiative rate constants of the luminescent molecule A, k_d the diffusion rate constant for the approach between A and B and k_R the reaction rate constant for various subsequent reactions, such as electron transfer.

While our studies will only involve transition metal complexes displaying relatively long lifetime and a non-viscous medium, dynamic and static quenching will be the two main luminescence quenching processes that may occur. In this context, we will focus on them in this section.

1.1.1. Dynamic quenching

Photo-induced processes leading to dynamic quenching have been first described by Stern and Volmer.¹⁹⁶ The kinetic processes that take place are depicted on Figure 5.3.

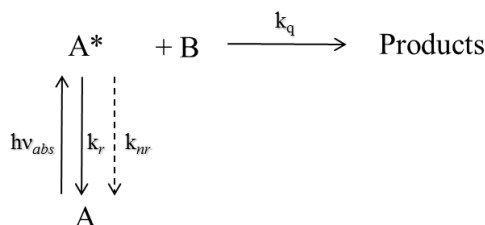


Figure 5.3. Schematic reactions occurring for dynamic quenching, k_r and k_{nr} being respectively the radiative and non-radiative rate constants of the luminescent molecule A and k_q the bimolecular quenching constant.

According to this simplified scheme, Stern and Volmer proposed the ratio of the luminescence intensity of A in the absence and in the presence of B is function of the concentration of the quencher:

$$\frac{I_0}{I} = 1 + k_q \tau_0 [B] \quad (\text{equation 1})$$

where I_0 and I are respectively the emission intensity in the absence and in the presence of B, and τ_0 the excited state lifetime of A in the absence of B. The dynamic bimolecular quenching constant can thus be obtained by plotting I_0/I as a function of the concentration in quencher B (Figure 5.4).

It is noteworthy to mention the bimolecular quenching constant k_q includes two processes (Figures 5.2 and 5.3): (i) the formation of the (A-B)* complex, characterized by k_d and k_{-d} rate constants, and (ii) the rate constant of the subsequent reaction between A and B in the association complex (k_R). In that case, two situations may arise:

- a) the bimolecular dynamic quenching process is diffusion-limited ($k_R \gg k_d$); thus $k_q \approx k_d$. In non-viscous medium, a k_q value close to 10^9 – $10^{10} \text{ M}^{-1} \text{ s}^{-1}$ indicates an efficient luminescence quenching which is limited by the diffusion of the species.
- b) the bimolecular process is not diffusion-limited ($k_R \approx k_d$), and $k_q = pk_d$ where p is the efficiency of the reaction.

1.1.2. Static quenching

When the luminescence quenching is not diffusion-dependent, static quenching may occur. The static quenching involves either the formation of a non-luminescent complex in the ground state or takes place when B and A* are not able to diffuse towards each other. This behavior may be observed in viscous media or in rigid matrices.

The formation of a non-luminescent ground state complex (A-B) implies the observed luminescence is solely due to luminescent molecules A which are not associated with B at the time of the excitation. Consequently, the excited state lifetime of uncomplexed A is not affected, but the overall emission will decrease because one part of A is bound to B and it does not contribute to the luminescence anymore. The emission behavior can thus be related to the concentration of B using the complex equilibrium constant:

$$K_s = \frac{[(A-B)]}{[A][B]} \text{ where } [A]_0 = [A] + [(A-B)] \quad (\text{equation 2})$$

where $[A]_0$ is the total concentration of A and K_s is the static quenching constant. Therefore,

$$\frac{[A]}{[A]_0} = \frac{1}{1+K_s [B]} \quad (\text{equation 3})$$

Considering the luminescence intensities are proportional to the concentrations (valid in dilute solutions), equation 3 can be rewritten:

$$\frac{I_0}{I} = 1 + K_s [B] \quad (\text{equation 4})$$

The static quenching constant K_s can thus be obtained by plotting I_0/I as a function of the concentration in quencher B (Figure 5.4).

1.1.3. Simultaneous static and dynamic quenching

Static and dynamic quenching may occur simultaneously, resulting in a deviation of the plot of I_0/I against $[B]$ from linearity.⁹⁴ In that case, a fraction of the luminescence quenching occurs under diffusion-controlled conditions, while the other fraction is ascribed to the formation of a non-luminescent complex in the ground state (Figure 5.4).¹⁹⁷

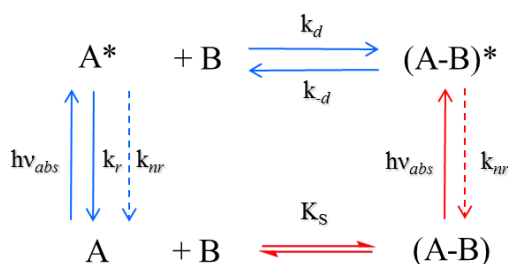


Figure 5.4. Schematic processes occurring in the case of simultaneous dynamic (blue arrows) and static (red arrows) quenching mechanisms, k_r and k_{nr} being respectively the radiative and non-radiative rate constants of the luminescent molecule A, k_d the diffusion rate constant for the approach between A and B, and K_s the static quenching constant.

The kinetics of these two simultaneous processes can be described as the multiplication of the ratio I_0/I obtained for dynamic quenching by the fraction of “free” luminescent molecules. Thus, from equations 1 and 4:

$$\frac{I_0}{I} = (1 + k_q \tau_0 [B])(1 + K_s[B]) \quad (\text{equation 5})$$

which can be rewritten:

$$\frac{I_0}{I} = 1 + (k_q \tau_0 + K_s)[B] + (k_q \tau_0 + K_s)[B]^2 \quad (\text{equation 6})$$

Consequently, an upward curvature is observed when plotting I_0/I against $[B]$. The kinetic constants K_q and K_s can be obtained by curve fitting using equation 6 (Figure 5.5).

Note other models have been developed for systems involving less specific or weaker interactions between A and B, or having a non-equimolar stoichiometry.⁹⁴ These models will not be treated in this manuscript.

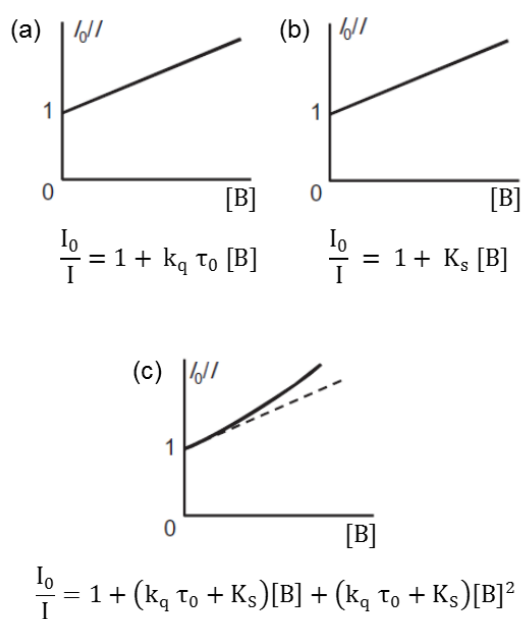


Figure 5.5. Stern-Volmer-like plots obtained for (a) dynamic quenching, (b) static quenching and (c) simultaneous dynamic and static quenching with the formation of a non-luminescent ground state complex.⁹⁴

1.2. Photo-induced electron transfer

1.2.1. Basic principles

Photo-induced electron transfer is receiving a lot of attention nowadays for a wide range of applications, such as natural and artificial photosynthesis.¹⁹⁸⁻²⁰⁰ This process involves an electron transfer from a molecule to another taking place only upon light irradiation. Both oxidative and reductive photo-induced charge transfer may happen depending on the nature of the species involved (Figure 5.6). Briefly, the electron transfer may occur either from an excited donor (D^*) to an acceptor (C) (oxidative photo-induced electron transfer) or from the donor (D) to the excited acceptor (C^*) (reductive photo-induced electron transfer). In the case of d^6 transition metal complexes, only their lowest-lying excited state is involved, which is triplet in nature (see Chapter 1). The driving force (ΔG_{ET}) for the electron transfer can be estimated by the Rehm-Weller equation¹⁰³ (equation 7) according to electrochemical and spectroscopic data of studied species in a given solvent:

$$\Delta G_{ET} = (E_{D^{+}/D} - E_{C/C^{\bullet-}}) - \Delta E_{0-0} - e^2/\epsilon d \quad (\text{equation 7})$$

where $E_{D^{+}/D}$ and $E_{C/C^{\bullet-}}$ are the reduction potentials, ΔE_{0-0} is the energy transition between the lowest vibrational states of the excited and ground states and $e^2/\epsilon d$ term corresponds to a coulombic correction due to attraction between the radical species obtained upon electron transfer. This last term is negligible in polar solvents due to their high dielectric constant (ϵ) values.

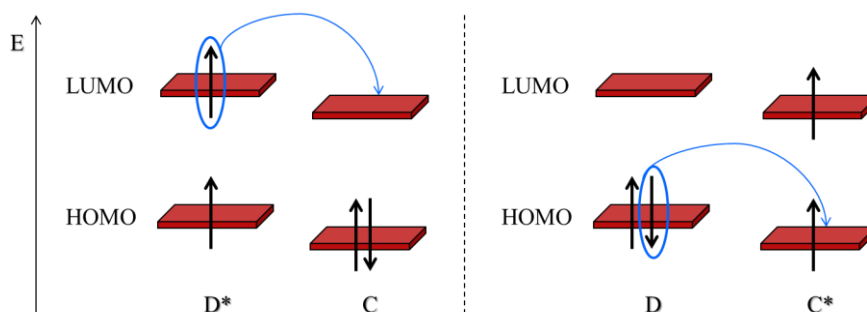


Figure 5.6. Schematic photo-induced electron transfer processes.

1.2.2. Photo-induced electron transfer with polynucleotides

1.2.2.1. DNA and oligonucleotides

Due to their relatively good photo-stability, long excited state lifetime and redox tunability, transition metal complexes are particularly interesting compounds to undergo photo-induced electron transfer. Following the discovery of $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ and its DNA photoprobe properties, therapeutic studies have shown that this complex is able to inject positive charges (holes) in DNA. However, it happens only after excitation of the complex by a laser pulse and oxidation by an external compound, such as $[\text{Ru}(\text{NH}_3)_6]^{3+}$, in order to generate *in situ* the highly oxidizing Ru(III) species.²⁰¹ Without the use of the so-called “flash-quench technique”, the excited state of the complex is not enough oxidizing to induce the electron transfer from DNA bases. Nevertheless, as Ru(III) complexes are poorly stable and the need to generate *in situ* the active species prevent from using this technique *in vivo*.

A convenient and easy way to increase the oxidation power of Ru(II) complexes is to use highly π -deficient polyaaromatic ligands, that is by increasing the number of non-chelating nitrogen atoms. Indeed, bpz, TAP and HAT ligands have been successfully used to confer high oxidizing properties to the resulting complex, being thus able to oxidize rather poor electron donors such as nucleotides, and more precisely guanine.²⁰²⁻²⁰⁴ Increasing the number of these strong π -acceptor ligands leads to an increase of the excited state reduction potential. Photo-induced charge transfer with polynucleotides could be useful in biological diagnostic tests and for gene phototherapy.

$[\text{Ru}(\text{bpy})_n(\text{bpz}/\text{TAP}/\text{HAT})_{3-n}]^{2+}$ or $[\text{Ru}(\text{phen})_n(\text{bpz}/\text{TAP}/\text{HAT})_{3-n}]^{2+}$ are however not really suitable for photo-induced electron transfer studies. Indeed, these complexes display a similar geometry than that of $[\text{Ru}(\text{phen})_3]^{2+}$ and probably interact in the same way with DNA and oligonucleotides. Depending on its concentration, it has been shown $[\text{Ru}(\text{phen})_3]^{2+}$ binds to DNA through electrostatic interactions,

adsorption or semi-intercalation.²⁰⁵ This mixture of binding modes makes detailed studies of this range of complexes quite difficult.

In order to have (i) a defined binding geometry, (ii) a good affinity towards DNA and (iii) a high photo-oxidizing power, dppz-based Ru(II) complexes bearing bpz, TAP or HAT ligands have been designed during the last two decades.⁶⁵ For example, it has been shown that the kinetic behavior of transient species from $[\text{Ru}(\text{TAP})_2\text{dppz}]^{2+}$ (Figure 5.6) intercalated into DNA is strongly different from that of the free complex in solution.^{202,206}

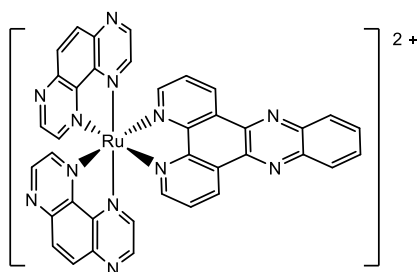


Figure 5.6. Structure of $[\text{Ru}(\text{TAP})_2\text{dppz}]^{2+}$.

Transient absorption (TA) spectra of this complex in aqueous buffer show a strong bleaching between 450 and 500 nm, as well as weaker positive absorption above 500 nm (Figure 5.7).²⁰² This result suggests a transient species having a lifetime greater than 10 ns is formed, consistent with luminescence lifetime and resonance Raman studies showing the MLCT excited state of this complex features a lifetime of 820 ns in aerated aqueous solution.^{65,206} In contrast, $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ displays two excited state lifetimes in aqueous solution, one of 3 ps, the other of 250 ps.^{113,145,206} In the case of $[\text{Ru}(\text{TAP})_2\text{dppz}]^{2+}$, a very different behavior is observed with TA measurements when the complex is in the presence of polynucleotides. For example, with $[\text{poly}(\text{dG-dC})]_2$, the absorption increases exponentially between 500 and 550 nm, with a lifetime of 506 ps,²⁰² consistent with the formation of the reduced complex $[\text{Ru}(\text{TAP})_2\text{dppz}]^+$ as a result of electron transfer from a guanine base.

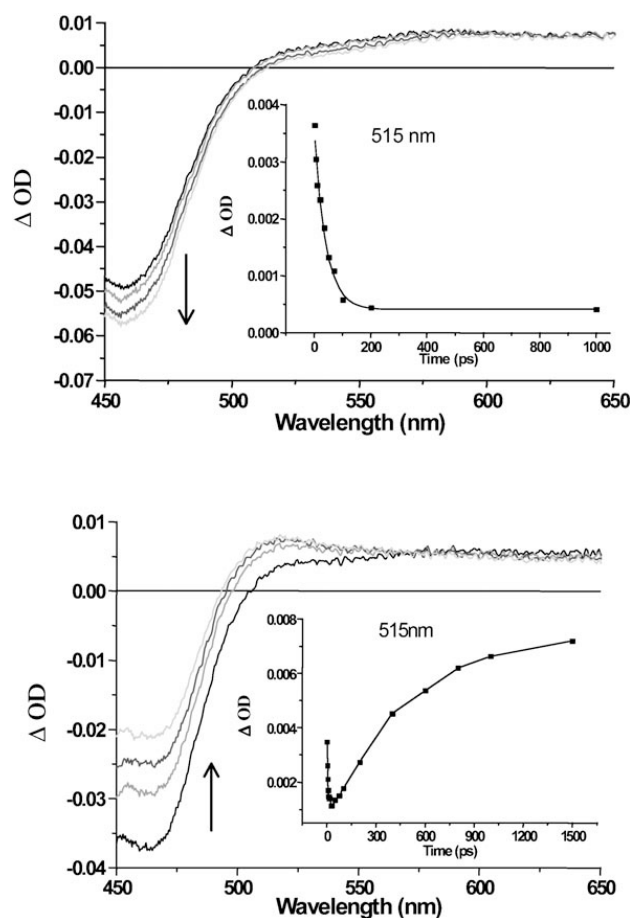


Figure 5.7. Picosecond TA spectra of $[\text{Ru}(\text{TAP})_2(\text{dppz})]^{2+}$ in 10 mM PBS in the absence (top) and in the presence (bottom) of $[\text{poly}(\text{dG-dC})]_2$. Traces at 10, 20, 70 and 1000 ps (from top to bottom) in the top part of the graphic and at 100, 600, 1000 and 1500 ps (from bottom to top) in bottom part of the graphic are shown. Insets: kinetics at 515 nm.²⁰²

The observation of a solvent isotope effect, *i.e.* switching from H_2O to D_2O , suggests the oxidation process involves simultaneous transfer of a proton (or deuteron in D_2O) and electron, that is Proton-Coupled Electron Transfer (PCET).^{65,207} It was indeed observed the rates of the oxidation process and subsequent reverse reaction (Back Electron Transfer, BET) are both slower in D_2O .^{112,202} It has been proposed in that case there is formation of a guanine radical, guanine being the

strongest reducing base, with proton transfer to the matched cytosine (Figure 5.8 and Scheme 5.1).²⁰⁸

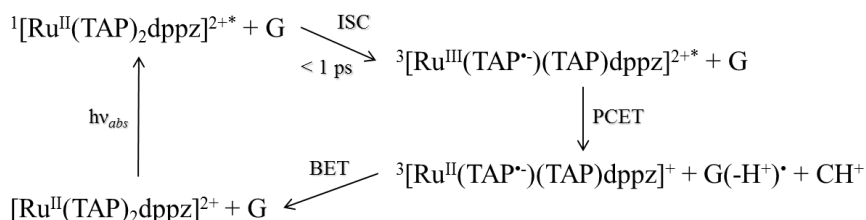
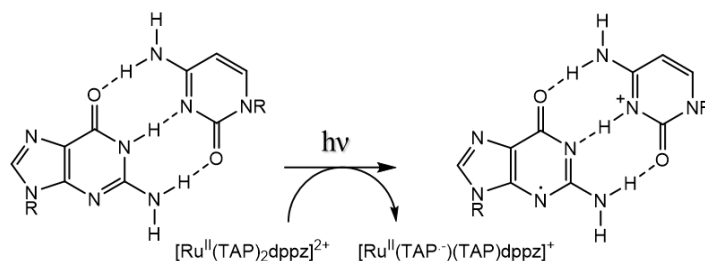


Figure 5.8. Formation of the MLCT excited state of $[\text{Ru}(\text{TAP})_2(\text{dppz})]^{2+}$ and ET from G to form $\text{G}(-\text{H}^{\bullet})^{\bullet}$ via PCET.



Scheme 5.1. Prototropic equilibrium of proton transfer in one electron oxidized G-C base pair. One electron oxidation of G-C base pair and proton transfer from G to C can occur from step wise or concerted PCET within the DNA duplex.

It has been shown in the literature that in some cases, depending on the choice of the ligands, DNA-strand cleavage or covalent bond formation between one or two guanine bases and reduced complex occur under irradiation.^{112,209,210} It should be noted that the use of intercalating ligands such as dppz or TPAC requires higher loading of Ru(II) complexes to observe adducts formation. Indeed, the mobility of these complexes is restricted due to their intercalation into the base pair stack, leading to higher concentrations of complexes to saturate the polynucleotides which is a prerequisite to photo-induce damages.²¹⁰

Recently, mono- and dinuclear Ru(II) complexes have exhibited the formation of covalent adducts with guanine bases of oligonucleotides

in duplexes or quadruplex forms.^{108,161} Such properties could be exploited for phototherapies by preventing gene transcription under light irradiation, leading then to cell death.²¹¹

1.2.2.2. G-quadruplexes (G4)

G-tetrads are structures consisting of four guanine bases bound together in a Hoogsteen fashion which are stabilized by monovalent metal cations (Figure 5.9). The four guanine bases of these tetrads can join from multiple strands or from the same strand, depending on the conformation (Figure 5.10).²¹² The tetrads may stack on each other like base pairs in DNA, creating three-dimensional structures known as G-quadruplexes. While these are non-canonical structures of DNA, more than 700,000 sequences have been identified within the human genome that have the potential to form G4, particularly in promoter regions and telomeres.^{213,214}

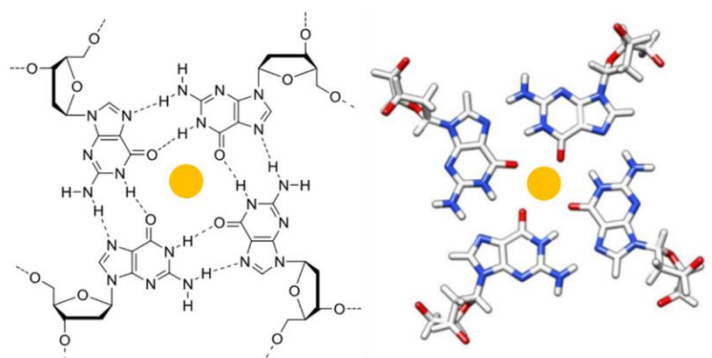


Figure 5.9. Structure (left) and molecular representation (right) of a G-tetrad. Metal cation is depicted in yellow, nitrogen in blue, oxygen in red, carbon and hydrogen in white.

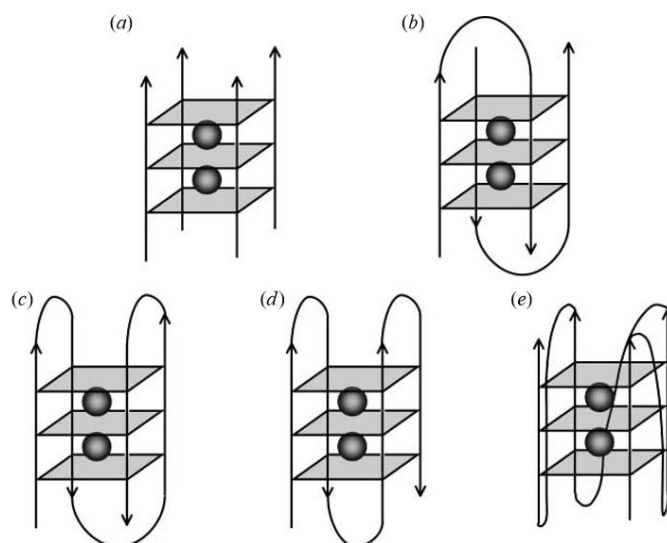


Figure 5.10. Schematic possible folding of G4: (a) four-stranded parallel, (b) bimolecular antiparallel, (c) and (d) monomolecular antiparallel, and (e) monomolecular parallel. Many sequences can also form mixed structures or are polymorphic.

It has been shown the proliferation of over 85% of cancerous cells rely on the regular extension of their telomeric sequences *via* overexpression of telomerase.²¹⁵ Nevertheless, it has been showed the presence of G4 structures within this DNA region could inhibit the action of the telomerase, preventing cancer cell proliferation. In this context, the stabilization of G-quadruplexes represents a novel approach widely studied due to high potential for chemotherapeutic applications. Until now, this approach has been successful *in vitro*.²¹⁶

Due to the stacked arrangement of the guanine residues in G4, insertion between base pairs is rather unlikely. Instead, planar aromatic compounds are able to stack at either end of the quadruplex scaffold. For that purpose, Ru(II) complexes have emerged as potent G4 binders due to their stacking ability into polynucleotides.²¹⁷⁻²¹⁹ For example, while $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ acts as a molecular switch for CT-DNA and different G4 structures without any selectivity, it has been shown recently that the replacement of a terminal hydrogen of the

dppz ligand by a bromine significantly enhanced the binding selectivity of the complex towards parallel intermolecular G4.²²⁰ Structural modifications upon binding to the quadruplexes, such as changes in folding, have been hypothesized to lie at the basis of the quadruplex selectivity.

Another example involves dinuclear Ru(II) complexes, such as $\Delta\Delta$ - and $\Lambda\Lambda$ - $[(\text{Ru}(\text{phen})_2)_2(\text{TPPHZ})]^{4+}$ which have demonstrated the ability to stack within the ends of a G4 structure with high affinity.²²¹ A chiral specificity is observed as the $\Lambda\Lambda$ - isomer has been reported to bind approximately forty times stronger than the $\Delta\Delta$ - isomer, accompanied by a strong luminescence enhancement. In that case, NMR-based modelling studies show the $\Lambda\Lambda$ - isomer fits more effectively into the diagonal loop than the lateral one (Figure 5.11). G-quadruplexes being mainly found in telomeres and proto-oncogenes, the development of new photoprobes targeting these structures is of high interest nowadays.

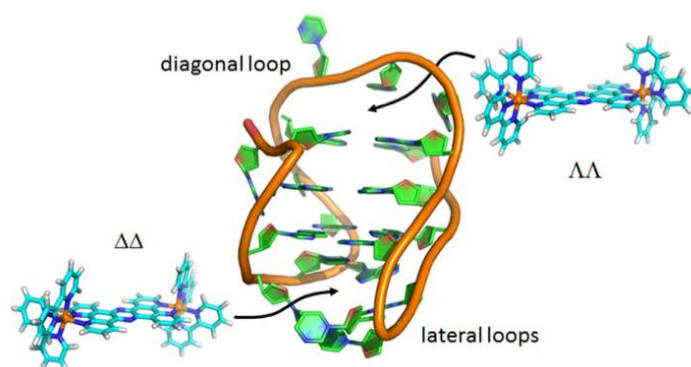


Figure 5.11. Molecular representation of the binding modes of $[(\text{Ru}(\text{phen})_2)_2(\text{tpphz})]^{4+}$ with the human telomere sequence $\text{d}[\text{AG}_3(\text{TTAG}_3)_3]$.²²²

1.3. Objectives

Having demonstrated Ru(II) complexes bearing dpac derivatives and “elbow-shaped” npp and bdppz ligands are able to bind complementary and mismatched DNA, we aimed to design analogous Ru(II) complexes bearing highly π -acceptor ancillary

ligands. We thus prepared $[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$ (Figure 5.12) to study its photochemical properties in the absence and in the presence of G-containing polynucleotides. Due to the replacement of the two ancillary ligands from phen (Chapter 3) to bpz, oxidation of guanine residues should be possible under light irradiation.

The “elbow-shaped” $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ and $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$ complexes (Figure 5.12) have also been prepared in order to investigate the effect of the modification of the shape of the extended planar ligand on the photophysical properties in different DNA topologies, such as DNA duplexes and G-quadruplexes.

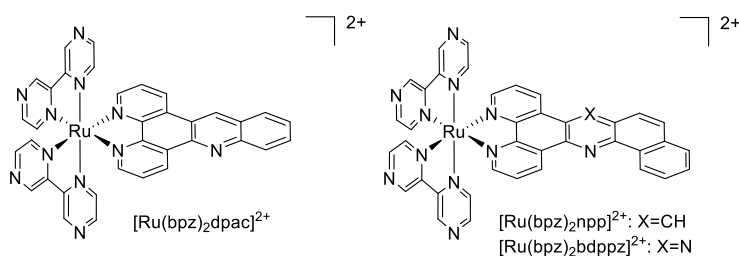


Figure 5.12. Structure of $[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$, $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ and $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$.

2. Main topics addressed in the publication

The results obtained in this chapter, together with the results from chapter 4 have been the subject of a publication. It consists in the photophysical and photochemical study of the “elbow-shaped” $[\text{Ru}(\text{bpy})_2\text{npp}]^{2+}$ and $[\text{Ru}(\text{bpy})_2\text{bdppz}]^{2+}$ complexes both in the absence and in the presence of DNA (Chapter 4). The results obtained demonstrate they strongly bind to polynucleotides and that they are able to discriminate between complementary and mismatched sequences. Noticeably, $[\text{Ru}(\text{bpy})_2\text{bdppz}]^{2+}$ exhibits strong differential luminescence behaviour in these environments that can be correlated to the nature and stability of the mismatch. Then, photo-reactive analogues of these complexes were prepared by replacing the ancillary bpy ligands by bpz ones. In that case, a photo-induced electron transfer from dGMP and polynucleotides towards the excited states of

the complexes is observed, emphasizing the role of Ru(II) complexes bearing acridine and phenazine derivatives as diagnostic and therapeutic agents. For clarity issues, only the results dealing with the above mentioned photoreactive Ru(II) mentioned will be treated hereafter. The next sections of this chapter correspond thus to an adapted version of the paper.

3. Content of the publication

Journal

Inorganic Chemistry Frontiers, submitted.

Title

“Elbow-shaped” Ru(II) complexes as photoprobes and photoreactants for DNA and mismatched DNA

Authors

Quentin DERAEDT, Lionel MARCELIS, Frédérique LOISEAU, Benjamin ELIAS

3.1. Results

3.1.1. Synthesis

The synthetic protocols for npp and bdppz have been reproduced as described in chapter 4 (see Figure 4.4).

The corresponding Ru(II) complexes are synthesized either by the direct chelation of the extended ligand for the acridine derivative or by condensation between 1,10-phenanthroline-5,6-dione and 1,2-diaminonaphthalene for bdppz onto the corresponding Ru(II) precursor (Figure 5.12) using methodologies previously described for similar compounds. All intermediates and final compounds are protected from direct light during the synthesis and purification steps to prevent photochemical degradation. The reactions are performed under argon to avoid any oxidation of the metal center.

The complexes are fully characterized by HRMS and ^1H NMR spectroscopy (see Experimental section). All the multiplicity and coupling constants cannot be determined due to the superposition of several signals and/or lack of fine resolution for some peaks in the ^1H NMR spectra. The ^1H NMR shows unambiguously the absence of symmetry elements for all the complexes, which induces the non-equivalence of (i) the protons on the extended planar ligand and (ii) the ancillary ligands. For solubility purposes, all the complexes are converted to the chloride salt for experiments conducted in water or buffer or to the hexafluorophosphate salt for studies in organic solvents.

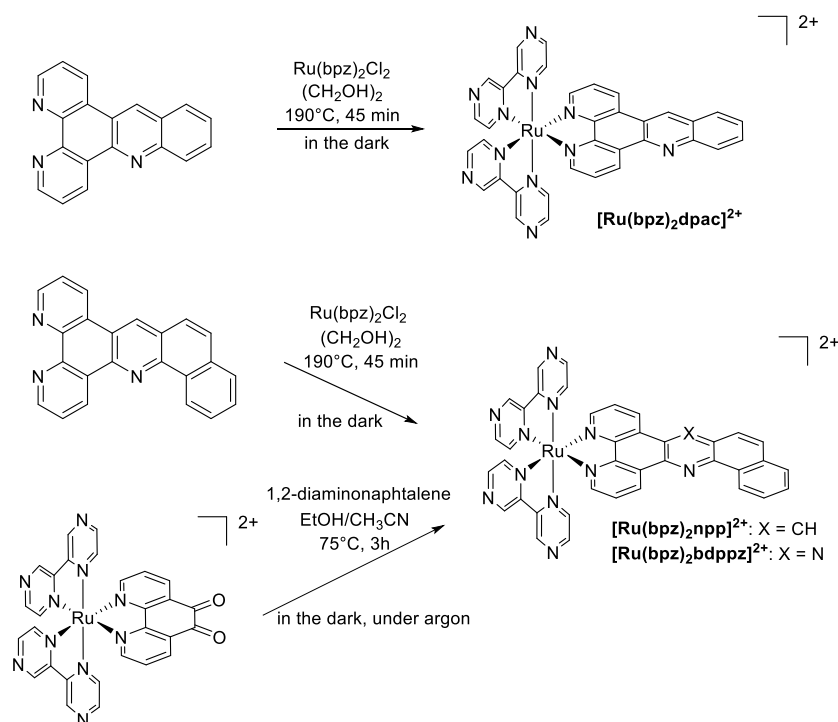


Figure 5.12. Synthetic scheme of bpz-based Ru(II) complexes.

3.1.2. Electrochemistry

The electrochemical data of the three complexes are obtained by cyclic voltammetry in dry deoxygenated MeCN or N,N-dimethylformamide (Table 5.2). While bpy/phen-based Ru(II) counterparts display a one-electron reversible oxidation wave at a potential close to that of the $\text{Ru}^{2+}/\text{Ru}^{3+}$ oxidation of $[\text{Ru}(\text{bpy})_3]^{2+}$ ¹⁰² and $[\text{Ru}(\text{phen})_2\text{dpac}]^{2+}$ (noted **Ru-DPAC**) (1.34-1.35 V vs Ag/AgCl),¹⁹⁴ a strong anodic shift is observed for the three bpz-based complexes (1.78-1.80 V vs Ag/AgCl) due to the presence of the strong π -acceptor bpz ancillary ligands. Indeed, the presence of electron-withdrawing ligands such as bpz stabilize the HOMO of these systems, which is localized on the metal cation. The nature of the extended ligand does not really affect the oxidation potential of these complexes.

Two reduction waves are monitored for $[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$, $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ and $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$. These are attributed to the successive reduction of each of the ancillary bpz ligands by comparison with the reduction potentials of $[\text{Ru}(\text{bpz})_3]^{2+}$ (-0.63 and -0.82 V vs Ag/AgCl),²²³ $[\text{Ru}(\text{bpz})_2\text{dppz}]^{2+}$ (-0.72 V vs Ag/AgCl)²²⁴ and their phen/bpy counterparts.

The potentials in the excited state in water (namely, E_{ox}^* and E_{red}^*) for all complexes have been estimated from the ground state redox potentials and the energy of the excited state corresponding to the maximum of the emission spectrum at 298 K in water. Not surprisingly, bpz-containing complexes are more oxidizing in the excited state than their bpy/phen analogs. These three complexes seem to be photo-oxidizing enough to be able to react with DNA under irradiation (*vide infra*).

Complex	$E_{1/2 \text{ ox}}^a$ [V vs Ag/AgCl]	$E_{1/2 \text{ red}}^b$ [V vs Ag/AgCl]			$E_{\text{ox}}^*{}^c$ [V vs Ag/AgCl]	$E_{\text{red}}^*{}^d$ [V vs Ag/AgCl]
		1	2	3		
[Ru(bpz) ₃] ²⁺ ⁹⁷	2.02	-0.63	-0.82	-1.09	-0.02	1.41
[Ru(bpz) ₂ dppz] ²⁺ ²²⁴	1.82	-0.72	-	-	-0.15	1.25
[Ru(bpz) ₂ dpac] ²⁺	1.78	-0.69	-0.83	-	-0.19	1.28
[Ru(bpz) ₂ npp] ²⁺	1.80	-0.69	-0.87	-	-0.17	1.28
[Ru(bpz) ₂ bdppz] ²⁺	1.80	-0.66	-0.87	-	-0.18	1.32

Table 5.2. Potentials for the oxidation ($E_{1/2 \text{ ox}}$) and reduction ($E_{1/2 \text{ red}}$) of [Ru(bpz)₂dpac]²⁺, [Ru(bpz)₂npp]²⁺ and [Ru(bpz)₂bdppz]²⁺. ^a Measured in dry acetonitrile. ^b Measured in dry N,N-dimethylformamide. ^c The excited state oxidation potential was estimated from the ground state oxidation potential and the energy of emission maximum with the equation $E_{\text{ox}}^* = E_{1/2 \text{ ox}} - E_{0-0} \approx E_{1/2 \text{ ox}} - \Delta E_{\lambda_{\text{max, em}}}$. ^d The excited state reduction potential was estimated from the ground state reduction potential and the energy of emission maximum with the equation $E_{\text{red}}^* = E_{1/2 \text{ red}} + E_{0-0} \approx E_{1/2 \text{ red}} + \Delta E_{\lambda_{\text{max, em}}}$. ΔE_p corresponds to the peak-to-peak separation.

3.1.3. Absorption

The absorption data in water and acetonitrile under ambient air conditions for the three complexes, as well as for other complexes for comparison purposes, are listed in Table 5.3. Absorption spectra in acetonitrile are depicted in Figure 5.13 (see associated content for absorption spectra in water). In our case, the comparison with literature and absorption spectra of the free ligands allowed to assign the intense absorption ($\epsilon \geq 10^5 \text{ M}^{-1}\text{cm}^{-1}$, *ca.* 300 nm) bands in the UV region to ligand centered (LC) transitions $\text{Ru} \rightarrow \text{L/L}'$, where $\text{L} = \text{bpz}$ (¹MLCT_{bpz}) and $\text{L}' = \text{dpac/npp/bdppz}$ (¹MLCT_{dpac/npp/bdppz}). We previously reported that the replacement of a nitrogen atom of the phenazine moiety of dppz by a carbon atom strongly modifies the excited state properties of the resulting Ru(II) complex.²²⁵ The MLCT absorption bands observed for [Ru(bpz)₂dpac]²⁺, [Ru(bpz)₂npp]²⁺ and [Ru(bpz)₂bdppz]²⁺ are notably more bathochromic than their bpy/phen analogs (shift of *ca.* 12-18 nm), as a result of the stabilization of the LUMO for these complexes. Indeed, the lowest energy transition for the three bpz-based complexes correspond to the promotion of an

electron from the metal center to one of the ancillary bpz ligand, the π^* orbital of which is more stabilized, leading to a $[\text{Ru}^{\text{III}}(\text{bpz}^*)(\text{bpz})\text{L}']^{2+}$ excited state. In that case, the most bathochromic transitions $^1\text{MLCT}_{\text{bpz}}$ are thus slightly affected upon change of the extended planar ligand, confirming the latter is not involved in these electronic transitions.

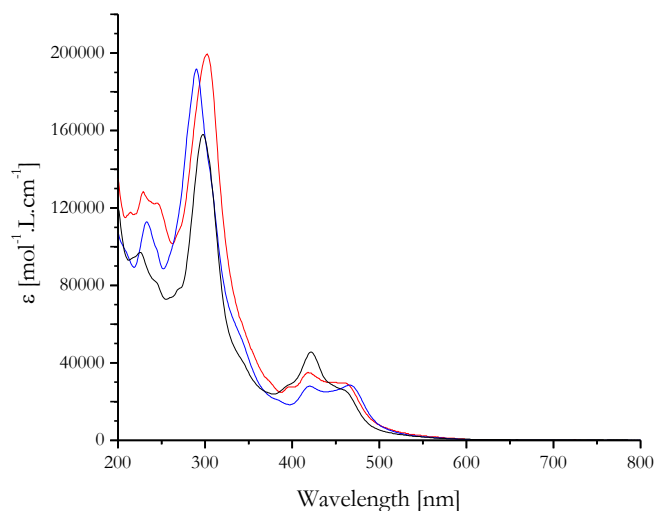


Figure 5.13. Absorption spectra of $[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$ (blue), $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ (red) and $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$ (black) in acetonitrile under air at room temperature.

Complex	Absorbance λ_{max} [nm] ($\epsilon / 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) ^a	
	CH ₃ CN	H ₂ O
[Ru(bpz) ₃] ²⁺ ⁹⁷	240 (2.0), 291 (5.1), 338, 415, 440 (1.3)	-
[Ru(bpz) ₂ dppz] ²⁺ ²²⁴	290 (7.87), 420 (1.59), 445 (1.45), 456 (1.45)	-
[Ru(bpz) ₂ dppz] ²⁺	233 (11.3), 290 (19.2), 418 (2.80), 466 (2.86)	233, 290, 418, 466
[Ru(bpz) ₂ npp] ²⁺	229 (12.8), 245 (12.2), 303 (19.9), 395 (2.75), 419 (3.50), 460 (2.99)	229, 245, 303, 395, 419, 460
[Ru(bpz) ₂ bdppz] ²⁺	226 (9.64), 246 (8.14), 299 (15.6), 396 (2.79), 419 (4.41), 460 (2.60)	226, 246, 299, 396, 419, 460

Table 5.3. Absorption data in CH₃CN and H₂O at 298K in aerated or deaerated solution for [Ru(bpz)₂dppz]²⁺, [Ru(bpz)₂npp]²⁺ and [Ru(bpz)₂bdppz]²⁺.^a Measurements made with solutions 1.10⁻⁵ mol.L⁻¹ in complex at room temperature.

3.1.4. Emission

The three bpz derivatives display broad unstructured emission both in organic solvent and water (Figure 5.14 and associated content to this chapter), suggesting a ³MLCT-type excited state. The excited state lifetimes and luminescence quantum yield for each complex are measured in acetonitrile and water under an argon atmosphere (Table 5.4). The large k_r values ($> 10^4 \text{ s}^{-1}$), the positive solvatochromic effect ($\Delta\lambda \approx +9\text{-}11 \text{ nm}$ when going from MeCN to water) and the hypsochromic shift of the emission band at 77 K confirm the formation a dipole at the excited state, consequently to a Charge Transfer excited state. A decrease in the luminescence intensity in air-equilibrated solvents with respect to argon-purged solutions is observed in all cases, indicating a possible photosensitization of ³O₂ by the excited Ru(II) complexes (data not shown).

The three complexes show almost identical emission maxima, which are very similar to the ones of the two references complexes [Ru(bpz)₃]²⁺ and [Ru(bpz)₂dppz]²⁺. The chemical modification of the

acridine core by addition of an aromatic ring and/or a nitrogen atom has thus only small influence on the energy difference between the triplet excited state ($^3\text{MLCT}$) and the ground state, as expected since the lowest $^3\text{MLCT}$ state involves bpz ligands (noted $^3\text{MLCT}_{\text{bpz}}$). Notably, the emission energy is decreased with respect to the phen/bpy analogs due to the decrease in energy of the π^* orbital involved in the radiative transition, also confirming the lowest excited state to be $^3\text{MLCT}_{\text{bpz}}$. Interestingly, $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$ displays higher quantum yield and excited state lifetimes than the acridine derivatives $[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$ and $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$. In addition, the quantum yield of these three complexes decreases when going from acetonitrile to water, indicating a partial quenching of the luminescence both by Hydrogen bonds formation with solvent molecules and strong stabilization of the $^3\text{MLCT}$ excited state resulting in higher non-radiative deactivation occurrence.^{100,226,227}

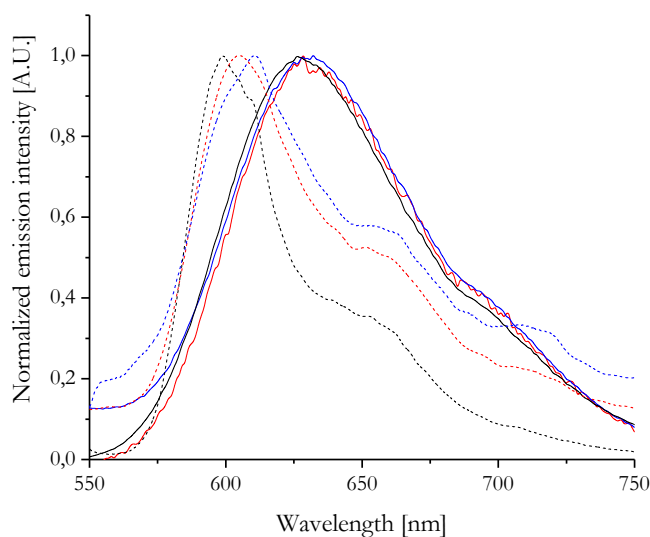


Figure 5.14. Emission spectra of $[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$ (blue), $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ (red) and $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$ (black) in acetonitrile under air at room temperature (solid lines) and at 77 K (dashed lines).

Figure 5.15 summarizes the photophysics of the complexes. The luminescence of the three bpz-based Ru(II) complexes arises definitely from an excited state of the same nature. The similar energies of their emissive excited states suggest emission from $^3\text{MLCT}_{\text{bpz}}$ states, corresponding to a charge transfer from the Ru(II) center towards one of the ancillary bpz. A bathochromic shift occurs in water due to the energy decrease of these $^3\text{MLCT}_{\text{bpz}}$ states in more polar solvent, caused by the stabilization of the dipole formed at the excited state. The extended planar ligand (dpac/npp/bdppz) does not seem to be involved in the lowest emissive state for these complexes.

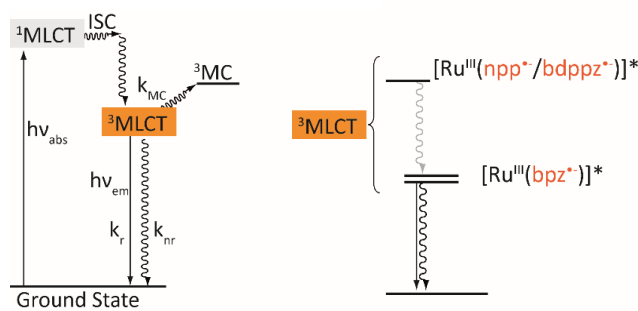


Figure 5.15. Photophysical scheme for $[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$, $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ and $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$. Solid arrows stand for radiative transitions, wedged arrows for non-radiative transitions.

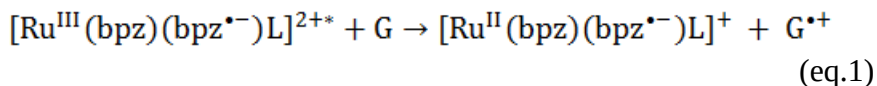
Complex	Emission λ_{max} [nm] ^a		Emission λ_{max} at 77 K [nm]		$\Phi_{em}^{a,b}$		τ_{em} [ns]		k_r [$10^3 s^{-1}$]	
	CH ₃ CN	H ₂ O	EtOH/ MeOH 4/1		CH ₃ CN	H ₂ O	CH ₃ CN	H ₂ O	CH ₃ CN	H ₂ O
							argon	argon		argon
[Ru(bpz) ₃] ²⁺ 228	614	621	586		-	-	785	1,040	-	-
[Ru(bpz) ₂ bdppz] ²⁺ 204	633	-	-		-	-	-	-	-	-
[Ru(bpz) ₂ dpac] ²⁺	632	643	606		0.111	0.035	466	825	238.2	75.1
[Ru(bpz) ₂ npp] ²⁺	632	643	610		0.057	0.015	471	790	121.2	31.8
[Ru(bpz) ₂ bdppz] ²⁺	628	639	599		0.305	0.092	522	1,565 (+0.5% 5.5)	584.3	58.8

Table 5.4. Emission data in CH₃CN and H₂O at 298 K and 77 K in aerated or deaerated solution for [Ru(bpz)₂dpac]²⁺, [Ru(bpz)₂npp]²⁺ and [Ru(bpz)₂bdppz]²⁺. ^a Measurements made with solutions 1.10^{-5} mol.L⁻¹ in complex. ^b Measured relative to [Ru(bpy)₃]²⁺ in an aerated aqueous solution ($\Phi_{em}^{air} = 0.028$).

3.1.5. Photophysical behavior in the presence of DNA

Consequently to the demonstrated high binding affinity of **Ru-NPP** and **Ru-BDPPZ** for DNA and their relevant application as DNA photoprobe (see Chapter 4), we investigated the behavior of their bpz counterparts, as well as the one of the new photoreactive **Ru-DPAC** analogue. As stated in the introduction, it has been shown in the literature that Ru(II) complexes bearing strongly π -deficient ligands induce oxidation of guanine bases upon irradiation due to their enhanced excited state reduction potential.^{65,202,206} Such properties could be exploited for phototherapies by inhibition of enzymes activity at the level of DNA,^{229,230} leading then to selective silencing of the expression of the targeted gene inside cell.^{231,232}

According to the excited state oxidation potentials of $[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$, $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ and $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$ (Table 5.2), a photo-induced electron transfer (PIET) is thermodynamically possible between the guanine and the excited complex (equation 1).



Indeed, although the oxidation potential of guanine is the subject of high debate in the literature, it is generally admitted that $E_{\text{G}^{\bullet+}/\text{G}}$ of isolated guanine is about + 1.10 V vs Ag/AgCl,^{233,234} and even less positive when stacked to other G nucleobases ($E_{\text{G}^{\bullet+}/\text{G}} [\text{poly}(\text{dG-dC})]_2 \sim + 0.85\text{-}0.93$ V vs Ag/AgCl).^{234,235} Considering the excited states reduction potentials of our Ru(II) complexes (Table 5.2), it is expected that the PIET (eq.1) process is exergonic for the three bpz complexes (Table 5.5).

	ΔG_{ET} (eV) ^a		k_q (M ⁻¹ .s ⁻¹) ^b
	dGMP	poly(dG-dC) ₂ ^c	
[Ru(bpz) ₂ dpac] ²⁺	- 0.18	- 0.43	5.06 10 ⁸
[Ru(bpz) ₂ npp] ²⁺	- 0.18	- 0.43	6.46 10 ⁸
[Ru(bpz) ₂ bdppz] ²⁺	- 0.22	- 0.47	6.04 10 ⁸

Table 5.5. Photoinduced ET between dGMP and [Ru(bpz)₂dpac]²⁺, [Ru(bpz)₂npp]²⁺ and [Ru(bpz)₂bdppz]²⁺. ^a Exergonicity of the ET between guanine and excited complex estimated as $\Delta G_{ET} = -nF (E_{Ru2+/Ru+} - E_{G+/G})$, with $E_{G+/G} = + 1.10$ V (dGMP) or $+ 0.85$ V (poly(dG-dC)₂) and $E_{Ru2+/Ru+} = E_{red}^*$ values in Table 1. ^b Kinetic constant of the quenching with dGMP, k_q , obtained using Stern-Volmer equation $I_0/I = 1 + k_q \tau_0 [dGMP]$; I_0/I (where I_0 is the luminescence of the complex in the absence of quencher, here dGMP, and I the luminescence in the presence of dGMP) as a function of the quencher concentration; τ_0 , the excited state lifetime of the complex in the absence of dGMP. ^c ΔG_{ET} values with [poly(dG-dC)₂] are given for comparison purpose in order to indicate the effect of the base pairs stack.

Luminescence quenching experiments have first been performed in the presence of deoxyguanosine-5'-monophosphate (dGMP); the luminescence of the three Ru(II) complexes is progressively quenched with increasing amounts of dGMP (Figure 5.16). A linear evolution is observed for Stern-Volmer in luminescence intensity for the three complexes, suggesting a pure dynamic quenching of the excited state of the three complexes by dGMP;⁹⁴ the obtained k_q values (Table 5.5) are close to the diffusion limit. The efficient quenching of the luminescence of the complexes by dGMP lies in agreement with a PIET process between the excited complexes and the guanine base.

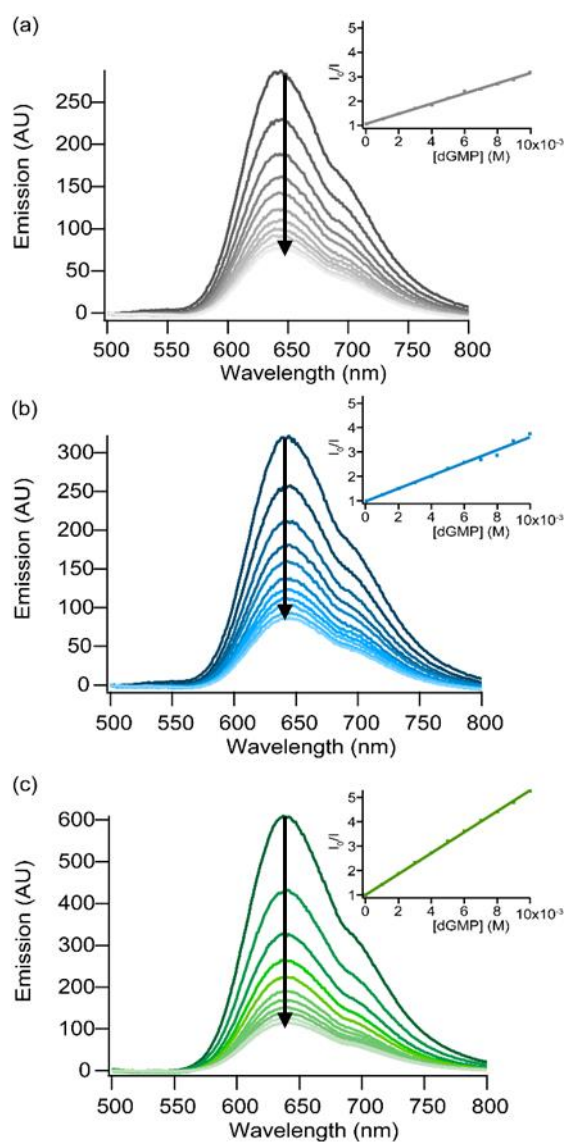


Figure 5.16. Evolution of the luminescence spectra of (a) $[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$ ($\lambda_{\text{ex}} = 466 \text{ nm}$), (b) $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ ($\lambda_{\text{ex}} = 460 \text{ nm}$) and (c) $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$ ($\lambda_{\text{ex}} = 419 \text{ nm}$) in the presence of increasing concentration of dGMP (dark to light). Measurements made in TRIS.HCl 5 mM, NaCl 50 mM, pH = 7.4, under ambient air conditions. Insets: Stern-Volmer plots obtained upon addition of dGMP for each complex.

Similar experiments have been conducted with Calf Thymus and Salmon Sperm DNA, known in the literature as model DNA's for such experiments, in order to probe the photoreactivity of the three complexes in the presence of polynucleotides. As expected the luminescence of these complexes is quenched in the presence of increasing concentrations of DNA (Figure 5.17), due to PIET with the guanine residues. The similar inhibition percentage for both types of DNA can be attributed to the similarity of GC content for these two DNA. Binding affinity values have been calculated using a modified McGhee-von Hippel equation¹⁶³ and are presented in Table 5.6. We can thus conclude that our complexes $[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$, $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ and $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$ can be efficiently used as photoreactive equivalents of their phen/bpy counterparts. If, among the latter, "elbow-shaped" ones revealed to have some selectivity for specific topologies in DNA, such as single-base mismatches, the utilization of their bpz equivalent will allow us to target these sites, with an equivalent specificity.

	SS-DNA		CT-DNA	
	$K_{\text{aff}} (\text{M}^{-1})^a$	$I/I_0 \text{ max}$	$K_{\text{aff}} (\text{M}^{-1})^a$	$I/I_0 \text{ max}$
$[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$	$5.69 \cdot 10^4$	0.47	$3.64 \cdot 10^4$	0.46
$[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$	$5.41 \cdot 10^4$	0.43	$4.50 \cdot 10^4$	0.44
$[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$	$9.83 \cdot 10^4$	0.36	$1.40 \cdot 10^5$	0.32

Table 5.6. Binding affinity constants calculated for **Ru-NPP** and **Ru-BDPPZ** with SS-DNA and CT-DNA. ^a Binding constant are obtained using a McGhee-von Hippel type equation; the binding site is fixed to two base pairs per complex (best fit). Errors estimated to 5%.

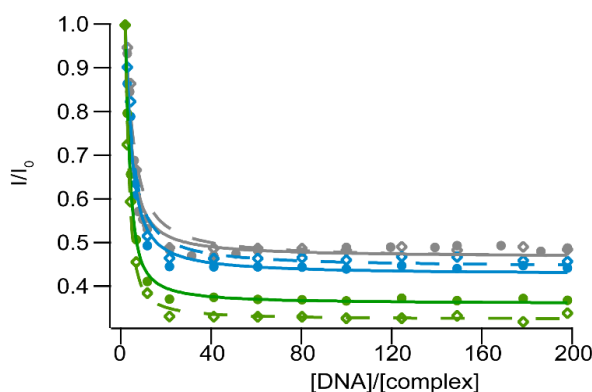


Figure 5.17. Steady-state luminescence titration of $[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$ (grey), $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ (blue) and $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$ (green) with SS-DNA (full circles) and CT-DNA (empty diamonds). Measurements are performed using $10\ \mu\text{M}$ of complex in Tris.HCl buffer $50\ \text{mM}$, NaCl $50\ \text{mM}$ at pH 7.4 under ambient air conditions. The fitted curves are obtained using a modified McGhee-Von Hippel equation and drawn as solid and dashed curves for SS- and CT-DNA respectively.

3.1.6. Photophysical behavior in the presence of mismatched DNA

As presented earlier, a Ru(II) complex which would specifically associate to mismatches in DNA would be very valuable in order to detect (photoprobe) and target (photoreactant) these mutations in the genetic material. **Ru-DPPZ** was shown to be able to sense single-base mismatch with a moderate specificity, in correlation with the relative thermodynamic stability of the sites.^{106,191,192} Since then, other Ru(II) complexes where the intercalating ligand^{191,192} or the ancillary ligands²³⁶ are modified were studied. An enhanced specificity can be achieved for some of them, but these complexes were no or poorly luminescent.

In chapter 3, we showed that Ru(II) linear dpac-complexes are unfortunately unable to probe mismatch site.¹⁹⁴ The “elbow-shaped” structure of our npp and bdppz ligands is thus of great interest in order to obtain such a specificity for DNA mismatches, as the dissymmetry of the inserted ligand proved to be useful in this context for other

Ru(II) complexes.¹⁹² The ability of the bpy-based “elbow-shaped” complexes to detect single-base mismatches in short oligonucleotides was thus investigated by using a series of hairpins oligonucleotide containing a mismatch near the center of the duplex (see sequences in Figure 4.6 and detailed results in Chapter 4). The strong differential luminescence behavior of $[\text{Ru}(\text{bpy})_2\text{bdppz}]^{2+}$ in the presence of mismatched hairpin vs the complementary duplex correspond to a 3.5-fold enhancement for CC mismatches compared to complementary sequence. This complex has a one-order higher affinity for the CC mismatch site compared to well-match sites, suggesting the luminescence enhancement is not only due to a better protection of the excited complex inserted at the mismatched site, but is also due to a greater affinity of the complex for insertion compared to intercalation within DNA.

The utilization of a photoreactive equivalent to induce photoreaction at these sites is thus relevant. Titrations realized with the photoactive equivalent of $[\text{Ru}(\text{bpy})_2\text{bdppz}]^{2+}$, *i.e.* $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$, demonstrate that the quenching, and thus the photo-induced electron transfer, is very efficient (Figure 5.18). Unfortunately, the hairpin sequence, used for comparison purpose with the literature, does not allow us to discriminate the ET process from complexes bound to the mismatched sites with complexes bound to adjacent complementary base pairs, as the PIET can occur with distant guanine residues. Nevertheless, based on the similar structure of $[\text{Ru}(\text{bpy})_2\text{bdppz}]^{2+}$ and $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$, and their similar behavior in binding complementary DNA, we can safely suggest that $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$ is preferentially associated with mismatched sites, and that a photo-induced ET will occur with guanine base in its immediate proximity.

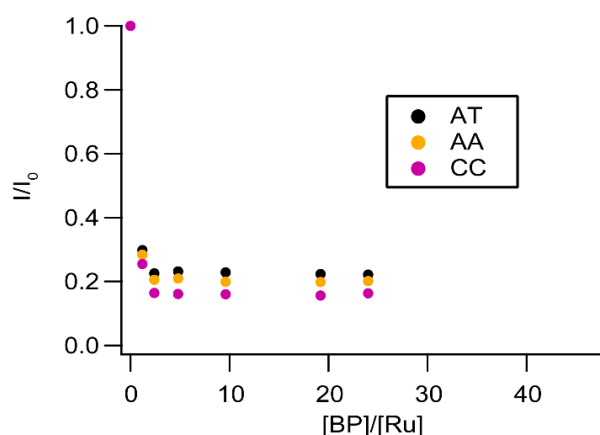


Figure 5.18. Steady-state luminescence titration of $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$ with hairpin oligonucleotides complementary (black) or containing either a AA (yellow) or a CC (purple) mismatched base pair. Measurements are performed using $1\ \mu\text{M}$ of complex in Tris.HCl buffer $5\ \text{mM}$, NaCl $1\ \text{mM}$, pH 7.5 under ambient air conditions. The fitted curves are obtained by global fitting on the whole data set.

3.1.7. Complement to the Article: Transient absorption (TA) spectroscopy studies

In order to get further insights into the kinetics associated with the luminescence quenching of our complexes by DNA, we have decided to study the processes that occur right after excitation of the complexes once intercalated into DNA. It consists in the recording of absorption spectra after irradiation of the DNA-complex system or complex alone by a short laser pulse ($80\ \text{fs}$). This technique gives access to the variations of the absorption spectrum (ΔOD) of the studied system as a function of time for each wavelength, giving information about the nature of the transient species and the kinetics associated with the processes that take place in a defined time window. In our case, femtosecond and nanosecond TA measurements have been performed, giving access to the kinetics of the formation of the excited state species and to the subsequent decay processes (from 0.5 to $1000\ \text{ps}$ for fs TA and from $30\ \text{ns}$ to hundreds of μs for ns TA). $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ has been chosen as model complex for these studies

and subsequent TA studies both in the absence and in the presence of SS-DNA in aqueous buffer have been realized. These experiments have been performed in Friedrich-Schiller-Universität Jena, in Jena (Germany), by Christian REICHARDT (Prof. Benjamin DIETZEK) in the frame of his Ph.D. thesis.

a) Complex in the absence of DNA

Femtosecond TA spectra (Figure 5.18 a) have been recorded between 10 and 9200 ps after a laser pulse at 403 nm. They are dominated by the bleaching of the ground state between 425 and 500 nm. Two positive absorption bands, located around 350 nm and beyond 660 nm are also detected. These signals are attributed to the absorption of the excited states formed after the laser pulse. The transient absorption observed above 600 nm is attributed to the absorption of the reduced bpz ancillary ligand, *i.e.* $\text{bpz}^{\bullet-}$, in agreement with spectroelectrochemical measurements performed by Julian SCHINDLER (Prof. Benjamin DIETZEK) and literature data for other complexes.^{66,202,237,238} Global kinetic analysis of the evolution of the spectra as function of time shows two distinct processes associated with lifetimes of 2.6 ps and 955 ps, respectively (Figure 5.18 b).

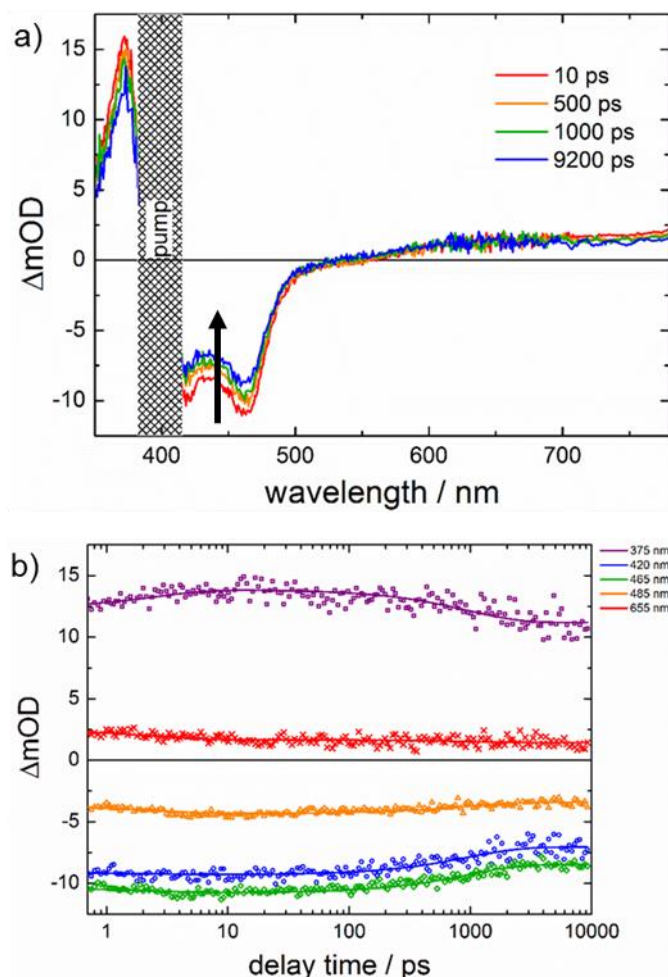


Figure 5.18. a) Femtosecond TA spectra of $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ at various delay time and b) evolution of the TA spectra at selected wavelengths between 0 and 10,000 ps after pump pulse ($\lambda_{\text{pump}} = 403 \text{ nm}$) in 10 μM of complex in Tris.HCl buffer 50 mM, NaCl 50 mM at pH 7.4 under ambient air conditions.

Nanosecond TA measurements have been recorded between 100 and 1000 ns after a laser pulse at 410 nm. Negative differential absorption signals are observed in the regions 425–500 nm, corresponding to recovery of the ground state and consistent with femtosecond data. The negative bands between 550 and 800 nm are consistent with emission for the complex. These two signals decay mono-exponentially and are associated with a lifetime of 449 ns (Figure 5.19

a). The lifetime value is similar to that obtained with luminescence lifetime measurement under the same conditions (381 ns, Figure 5.19 b).

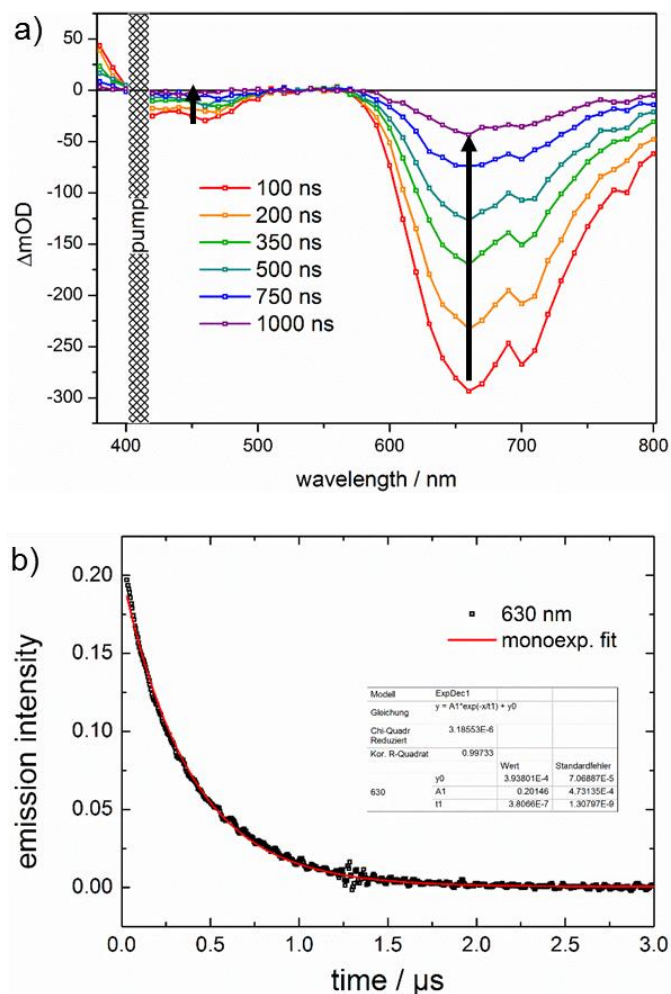


Figure 5.19. a) Spectra recorded on nanosecond TA experiment of $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ at selected delay time and b) luminescence decrease at 630 nm after pump pulse ($\lambda_{\text{pump}} = 410 \text{ nm}$) in $10 \mu\text{M}$ of complex in Tris.HCl buffer 50 mM, NaCl 50 mM at pH 7.4 under ambient air conditions.

As suggested by absorption and emission data discussed above, excitation of $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ at around 400 nm is expected to populate singlet MLCT excited states involving the bpz and the npp

ligands.^{237,238} As for other Ru(II) complexes, ultrafast intersystem crossing is expected to take place in less than one picosecond, due to the strong spin-orbit coupling induced by the presence of the heavy ruthenium metal center.^{160,239} The short lifetime component of 2.6 ps is thus suggested to account for internal conversion followed by internal vibrational relaxation (IVR) from different hot $^3\text{MLCT}$ excited states to the lowest $^3\text{MLCT}_{\text{bpz}}$ excited state (Figure 5.20).^{66,202}

The longer one (955 ps) is suggested to account for the rearrangement of solvent molecules around the complex and the formation (or reinforcement) of Hydrogen bonds with the $\text{bpz}^\bullet$ moieties (Figure 5.20), as it was proposed for $[\text{Ru}(\text{TAP})_3]^{2+}$.²⁴⁰ Similarly to $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$, this complex also possesses non-chelating nitrogen atoms, the basicity of which is enhanced compared to the ground state.²⁴¹ The enhanced basicity of the non-chelated nitrogen atoms of $\text{bpz}^\bullet$ will favor the formation of Hydrogen bonds with water/buffer molecules. Such assumption would require further investigation, such as TA measurements in deuterated buffer and/or in dry acetonitrile, which would slower or prevent this process.

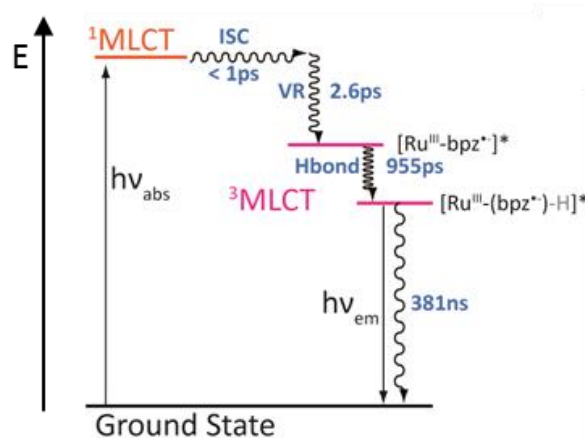


Figure 5.20. Simplified kinetic model for $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ in aqueous buffer in the absence of SS-DNA, as suggested by the TA measurements.

b) In the presence of SS-DNA

A similar set of nanosecond and femtosecond TA measurements were realized on $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ in the presence of SS-DNA. In the conditions used in this case, almost all of the complex is associated with DNA ($\sim 97\%$ according to the calculated binding constant (*vide supra*)). Nevertheless, the distribution of associated complex is not unique, as the photophysical and photochemical behavior of these intercalated complex will depend on the nature of the DNA bases at and near the intercalation site. Indeed, complexes laying very close to guanine moiety will be involved in photo-induced electron transfer and their lifetime will thus be shortened,^{65,66,238} while other complexes intercalated between AT base pairs will be protected from non-radiative quenching, and their respective lifetime will be enhanced.²³⁸ Notable differences are thus expected regarding the kinetics of the processes involved upon DNA binding.

Femtosecond TA spectra obtained for $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ in the presence of SS-DNA are shown in Figure 5.21 a. A global kinetic analysis of the signal (Figure 5.21 b) shows that two time processes associated with distinct lifetimes are present, *i.e.* 2.0 ps and 1375 ps respectively.

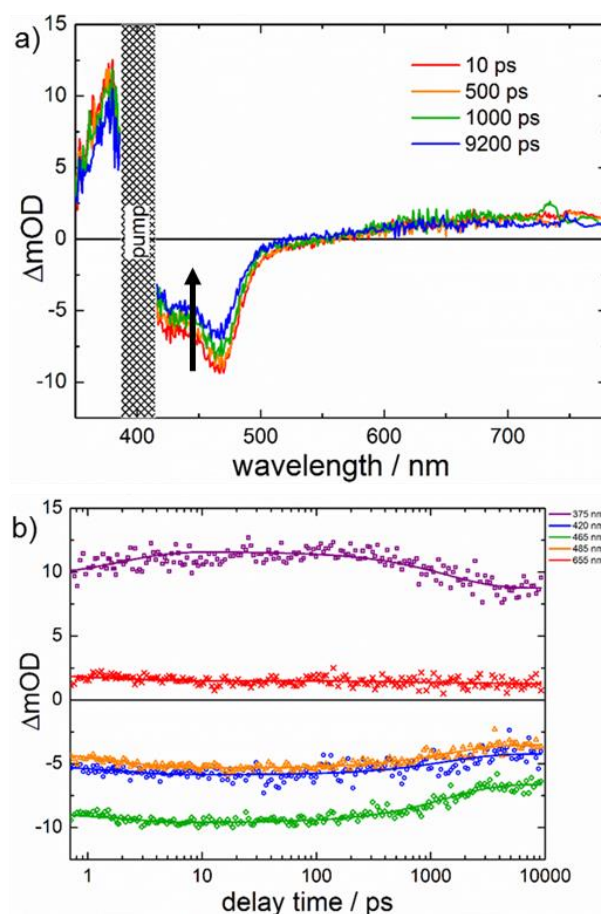


Figure 5.21. a) Femtosecond TA spectra of $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ in the presence of SS-DNA ($[\text{DNA base pair}]/[\text{Complex}] = 50/1$) at various delay time and b) evolution of the TA spectra at selected wavelengths between 0 and 10,000 ps after pump pulse ($\lambda_{\text{pump}} = 403 \text{ nm}$) in $10 \mu\text{M}$ of complex in Tris.HCl buffer 50 mM, NaCl 50 mM at pH 7.4 under ambient air conditions.

In nanosecond time regime, transient absorption spectra (Figure 5.22 a) are dominated by the recovery of the ground state between 425-500 nm and emission between 550-800 nm. These two signals decay as bi-exponential function, with two distinct lifetimes of 66 ns and 468 ns. These values are similar to that obtained with luminescence lifetime measurement under the same conditions (61 ns and 489 ns, Figure 5.22 b).

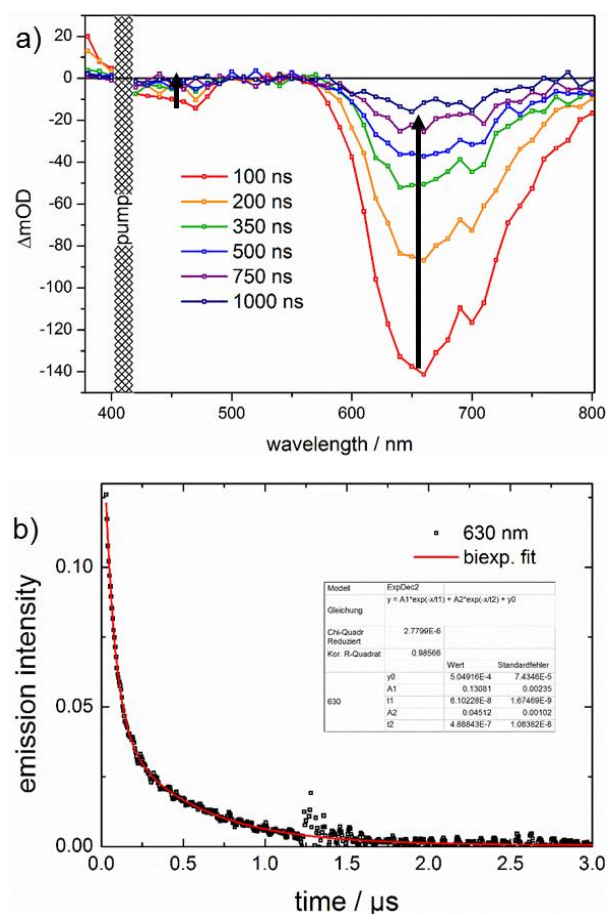


Figure 5.22. a) Spectra recorded on nanosecond TA experiment of $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ in the presence of SS-DNA ($[\text{DNA base pair}]/[\text{Complex}] = 50/1$) at selected delay time and b) luminescence decrease at 630 nm after pump pulse ($\lambda_{\text{pump}} = 410$ nm) in 10 μM of complex in Tris.HCl buffer 50 mM, NaCl 50 mM at pH 7.4 under ambient air conditions.

Like for the complex alone in solvent, the short lifetime component of 2.0 ps is associated to vibrational relaxation of hot triplet MLCT excited states to the lowest $^3\text{MLCT}_{\text{bpz}}$ excited state (Figure 5.23). The attribution of the long component observed in femtosecond experiments (1375 ps) is trickier. As the complexes are intercalated within DNA, the formation of hydrogen bonds is not expected since the complex is protected from the solvent. This component of 1375 ps could thus be attributed to an electron transfer between a guanine base

and an excited complex intercalated in a GC-site (Figure 5.23). Such quenching occurs at about 500 ps for $[\text{Ru}(\text{TAP})_2\text{dppz}]^{2+}$, for which a positive transient attributed to the mono-reduced species is observed around 510 nm.^{65,66,202,240} Unfortunately, such positive transient around 500-550 nm cannot be observed in our case. This could be explained by the fact that only the complexes intercalated in GC sites are quenched in this short time period, and complexes associated with AT sites are still excited. The observation of the positive absorption band of the mono-reduced complex would thus be prevented by the presence of the massive bleaching signal of the ground state of complexes that are not quenched in AT sites. The dramatic influence of the intercalation site on the photo-induced electron transfer between $[\text{Ru}(\text{TAP})_2\text{dppz}]^{2+}$ and guanine depending on the intercalation site was recently emphasized in literature.²³⁸ Further TA measurements with $[\text{poly}(\text{dG-dC})]_2$ are required to support this hypothesis. Note back electron transfer (BET) process is not observed in our case, probably because it occurs in a time regime which is not analyzed by our set-ups.

If our hypothesis is correct, in the nanosecond time scale, the only excited complexes remaining are the ones intercalated in AT sites. The decay of the signals observed in TA and time-resolved emission are characterized by two distinct lifetimes of 66 ns and 468 ns. We propose that the long component (468 ns) correspond to decay of complexes which are intercalated within an AT sites, protected from non-radiative quenching and that remain unquenched by neighboring guanine bases (Figure 5.23). This lifetime is longer than that of the complex alone in solution, consistently with protection *via* DNA intercalation.⁶⁵ In contrast, the short component (66 ns) could be attributed to the AT-intercalated complexes which are quenched by charge transfer to guanine residues in close proximity (Figure 5.23). Indeed, this lifetime is too short to be attributed to the decay of free complexes (*vide supra*), and cannot be due to BET process of quenched complexes at GC sites (between mono-reduced complex and

oxidized guanine), since the transient is observed in both transient absorption and emission.

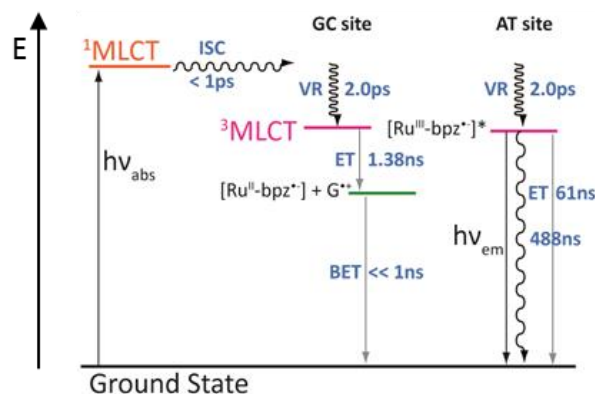


Figure 5.23. Simplified kinetic model for $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ in aqueous buffer in the presence of SS-DNA.

In conclusion, the TA measurements performed give some insights on the ultrafast processes that occur after laser pulse excitation of $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$. These constitutes preliminary experiments that need to be confirmed and more deeply analyzed by further transient techniques.

3.1.8. Complement to the Article: Photoreactivity in the presence of G-quadruplexes

Having demonstrated the potential of our complexes as DNA photoprobes, we were interested in investigating the possible selectivity for quadruplex vs duplex DNA topologies. For that purpose, binding affinity measurements of the linear dpac and “elbow-shaped” npp Ru(II) complexes for G-quadruplex structures were achieved by Laure BAR (M.S.) and Jérôme DEJEU (Associate Prof.) in the group of Prof. Eric DEFRANCQ (Département de Chimie Moléculaire, Université Grenoble-Alpes, Grenoble, France). The targeting of these scaffolds is of particular interest due to the stabilization offered by the interaction with extended planar structures

which could lead to inhibition of telomerase and consequently to prevention of cancer.

Two types of G-quadruplexes based on telomeric sequences have been used in these studies, and the results are compared with a GC rich hairpin duplex (Figure 5.24). The corresponding binding affinities are gathered in Table 5.7. Unfortunately, in our case, no selectivity was observed for quadruplexes vs duplexes structures. The lack of selectivity could be explained by the planarity of the extended planar ligands which have been used which is too high to be able to discriminate between these DNA topologies. The same behaviour has only recently been observed for $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$.²²⁰ In that case, selectivity occurs only upon substitution of the terminal position of the dppz ligand.

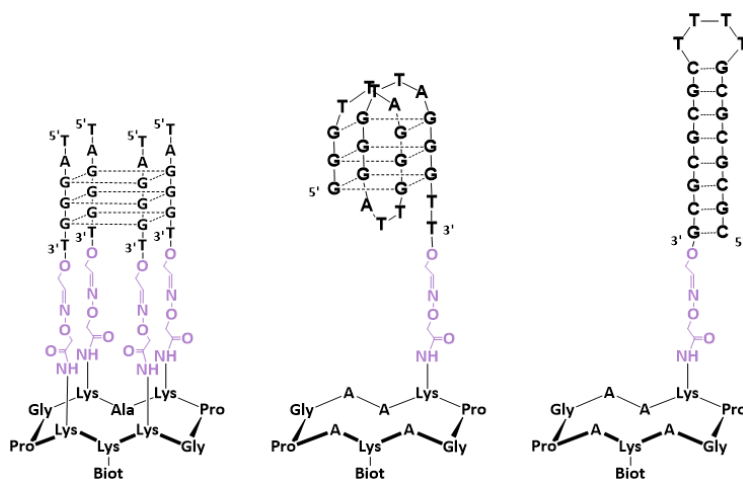


Figure 5.24. Structure of (from left to right) tetrameric and monomeric G-quadruplexes, and hairpin duplex.

	$K_{aff} [x10^6 \text{ L/mol}]$		
	Tetrameric G4	Monomeric G4	Hairpin
$[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$	1.4	3.2	0.4
$[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$	4.0	2.1	5.9

Table 5.7. Binding affinities of $[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$ and $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ for tetrameric and monomeric G-quadruplexes, and hairpin duplex. Values obtained by Surface Plasmon Resonance measurements.

3.2. Conclusions

Photooxidizing Ru(II) complexes $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ and $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$ have been successfully synthesized, together with their linear analogue $[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$. The presence of the two bpz ancillary ligands dramatically modify the electrochemical and photophysical properties of these new complexes compared to their bpy/phen counterparts; the excited state reduction potential is enhanced and is high enough to allow the three complexes to photo-oxidize the guanine nucleobase. Luminescence studies in the presence of dGMP and DNAs indicate that a photo-induced electron transfer is occurring between the guanine and the excited states of the complexes; indeed, a quenching of their luminescence is observed upon addition of dGMP or DNA. In the first case, Stern-Volmer plots indicate a dynamic quenching process by dGMP, associated with a quenching constant close to diffusion limit. In the presence of DNA, steady-state luminescence experiments indicate a high binding affinity of the three complexes for CT- and SS-DNA. The similar quenching percentage of the luminescence of these complexes by both types of DNAs is consistent with the similarity of the GC content in SS- and CT-DNA. Like their linear analogs, these three new bpz-based complexes are proposed to be interacting with DNA *via* intercalation.

Transient absorption measurements have been performed and allowed us to obtain kinetic data associated with deactivation processes for $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ both in the absence and in the presence of DNA.

Even though no direct evidence of electron transfer has been monitored, kinetic differences upon addition of DNA to the complex and comparison with literature data suggest on one hand protection of the complex by AT base-pairs and electron transfer from DNA for AT-bound complexes. Further TA measurements need however to be performed in order to confirm our kinetic analysis.

The enhancement of the binding affinity of $[\text{Ru}(\text{bpy})_2\text{bdppz}]^{2+}$ for CC mismatch compared to complementary sites indicating that the complex is not only better protected from deactivation when inserted, but also that insertion is favored over intercalation, its bpz analogue, $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$, can be used to efficiently photo-induce ET with guanine in close proximity with the mismatched site.

Unfortunately, the dpac and npp complexes seem to be unable to bind selectively to quadruplexes vs duplexes structures, probably due to the too planar surface of the intercalative ligands.

3.3. Experimental section

3.3.1. Synthetic procedures and characterization of Ru(II) complexes

Ru(bpz)₂Cl₂. The synthetic procedure used for the preparation of the Ru(II) precursor has been described elsewhere.²²³

RuCl₃ (220.0 mg, 1.059 mmol) and 2,2'-bipyrazine (335.0 mg, 2.118 mmol) were refluxed in DMF (30.0 mL) for 16h. After cooling to room temperature, the purple solution has been filtered and Et₂O (300 mL) was added to the filtrate. A black precipitate appeared, which is recovered by filtration and recrystallized in CH₃CN. The spectral properties of the product matched will literature data. ¹H NMR (500 MHz, CDCl₃): δ 10.26 (dd, 2H, H₆, J_{6-5} = 3.2 Hz, J_{6-3} = 1.3 Hz), 9.56 (d, 2H, H₃, J_{3-6} = 1.3 Hz), 9.39 (d, 2H, H_{3'}, $J_{3'-5'}$ = 1.3 Hz), 8.89 (d, 2H, H₅, J_{5-6} = 3.2 Hz), 8.25 (d, 2H, H_{5'}, $J_{5'-6'}$ = 3.3 Hz), 7.62 (dd, 2H, H_{6'}, $J_{6'-5'}$ = 3.4 Hz, $J_{6'-3'}$ = 1.2 Hz).

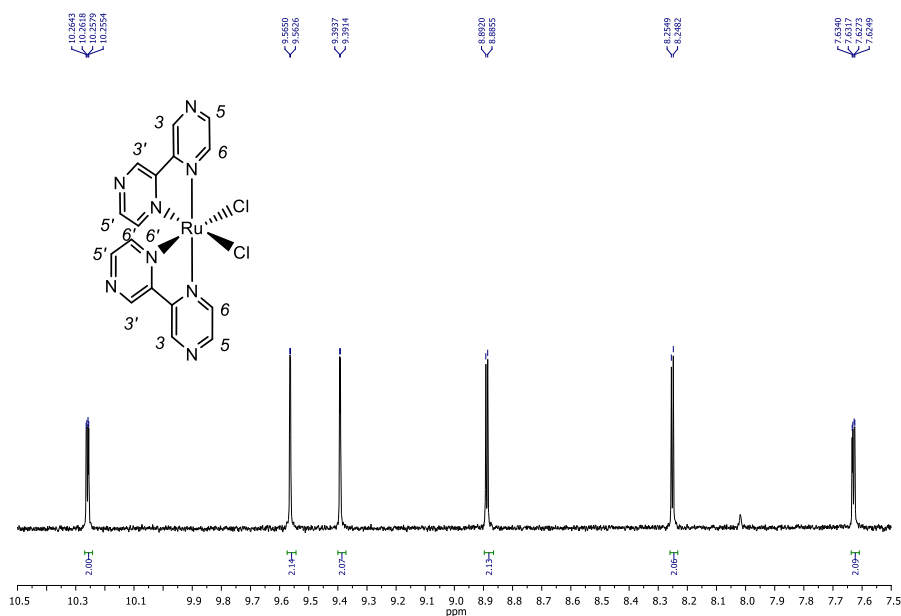


Figure 5.25. ¹H NMR spectrum (500 MHz, CDCl₃) of Ru(bpz)₂Cl₂.

$[Ru(bpz)_2dpac]^{2+}$. One equivalent of dichloro precursor $Ru(bpz)_2Cl_2$ (10.0 mg, 0.0203 mmol) was mixed with 2.0 equivalents of dpac (11.5 mg, 0.0406 mmol) in ethylene glycol (1.0 mL). The reaction mixture was stirred at reflux (197°C) and followed by absorption spectroscopy. After 1 hour, the reaction mixture was cooled at room temperature. After further cooling in an ice bath, addition of small portions of NH_4PF_6 and drops of water yielded a red precipitate. After centrifugation, the solid is washed with excess water. After drying under vacuum, the orange-red residue is purified by preparative plate of chromatography (silica, eluent: CH_3CN/H_2O /saturated aqueous NH_4Cl 4/4/1, $R_f = 0.60$). The counter-anion exchange from PF_6 to Cl is performed by eluting a solution of complex in a mixture CH_3CN/H_2O on Sephadex DEAE A25. 1H NMR (PF_6 salt, 300 MHz, CD_3CN): δ (ppm) 9.82 (m, 6H, $H_d, H_i, H_3, H_{3'}$), 9.38 (dd, 1H, H_c , $J_{c-b} = 8.5$ Hz, $J_{c-a} = 1.0$ Hz), 8.66 (d, 2H, H_5 or $H_{5'}$, J_{5-6} or $J_{5'-6'} = 3.2$ Hz), 8.46 (m, 3H, H_5 or $H_{5'}$ and H_h), 8.38 (d, 1H, H_e , $J_{e-f} = 8.3$ Hz), 8.07 (m, 3H, H_a, H_g, H_k), 7.99 (m, 2H, H_6 or $H_{6'}$), 7.91 (m, 3H, H_b, H_f, H_j), 7.78 (2dd, 2H, H_6 or $H_{6'}$). HRMS (ESI, +p): calc. 844.08177 for $[C_{35}H_{23}N_{11}F_6PRu]^+$, found 844.08212 ($[M-PF_6^-]^+$).

$[Ru(bpz)_2npp]^{2+}$. One equivalent of dichloro precursor $Ru(bpz)_2Cl_2$ (10.0 mg, 0.0203 mmol) was mixed with 2.0 equivalents of npp (13.5 mg, 0.0406 mmol) in ethylene glycol (1.0 mL). The reaction mixture was stirred at reflux (197°C) and followed by absorption spectroscopy. After 45 minutes, the reaction mixture was cooled at room temperature. After further cooling in an ice bath, addition of small portions of NH_4PF_6 and drops of water yielded a red precipitate. After centrifugation, the solid is washed with excess water. After drying under vacuum, the orange-red residue is purified by column of chromatography (silica, eluent: CH_3CN/H_2O /saturated aqueous NH_4Cl 4/4/1, $R_f = 0.57$). The counter-anion exchange from PF_6 to Cl is performed by eluting a solution of complex in a mixture CH_3CN/H_2O on Sephadex DEAE A25. 1H NMR (PF_6 salt, 300 MHz, CD_3CN): δ (ppm) 10.16 (dd, 1H, H_k , $J_{k-l} = 8.7$ Hz, $J_{k-m} = 1.1$ Hz), 9.80 (m, 5H, H_d , H_3 , $H_{3'}$), 9.72 (dd, 1H, H_j), 9.45 (d, 1H, H_c , $J_{c-b} = 8.1$ Hz), 8.67 (d, 2H, H_5 or $H_{5'}$, J_{5-6} or $J_{5'-6'} = 3.2$ Hz), 8.46 (dd, 2H, H_5 or $H_{5'}$, J_{5-6} or $J_{5'-6'} = 3.4$ Hz), 8.15 (m, 4H, H_e , H_g , H_i , H_m), 8.08 (d, 1H, H_a , $J_{a-b} = 5.0$ Hz), 7.96 (m, 6H, H_b , H_f , H_j , H_l , H_6 or $H_{6'}$), 7.78 (2dd, 2H, H_6 or $H_{6'}$). HRMS (ESI, +p): calc. 894.09742 for $[C_{39}H_{25}N_{11}F_6PRu]^+$, found 894.09853 ($[M-PF_6]^{+}$) and calc. 374.56689 for $[C_{39}H_{25}N_{11}Ru]^{2+}$, found 374.56663 ($[M-2PF_6]^{+}$).

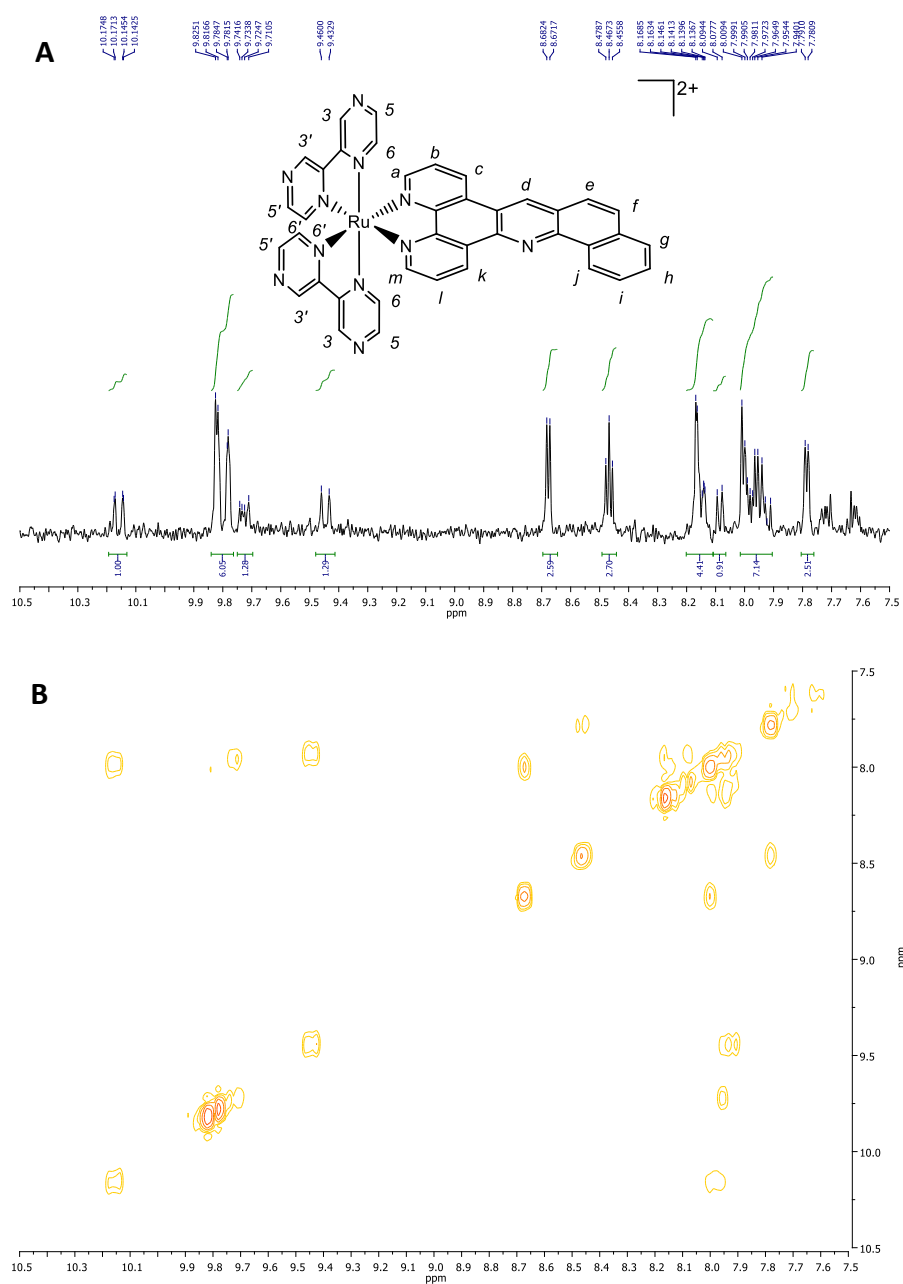


Figure 5.27. (A) 1D and (B) 2D ^1H NMR COSY spectrum (300 MHz, CD_3CN) of $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$.

$[Ru(bpz)_2(1,10\text{-phenanthroline-5,6-dione})]^{2+}$. One equivalent of dichloro precursor $Ru(bpz)_2Cl_2$ (50.0 mg, 0.1015 mmol) was mixed with 2.0 equivalents of 1,10-phenanthroline-5,6-dione (42.7 mg, 0.2031 mmol) in ethylene glycol (3.0 mL). The reaction mixture was stirred at 100°C under argon and followed by absorption spectroscopy. After 3 hours and a half, the reaction mixture was cooled at room temperature. After further cooling in an ice bath, addition of small portions of NH_4PF_6 and drops of water yielded a red precipitate. After centrifugation, the solid is washed with excess water. The orange-red residue is then dried under vacuum. The counter-anion exchange from PF_6 to Cl is performed by eluting a solution of complex in a mixture CH_3CN/H_2O on Sephadex DEAE A25. 1H NMR (PF_6 salt, 500 MHz, CD_3CN): δ (ppm) 9.78 (dd, 4H, $H_3, H_{3'}$, $J_{3-5} = J_{3'-5'} = 4.3$ Hz, $J_{3-6} = J_{3'-6'} = 1.2$ Hz), 8.64 (dd, 4H, $H_5, H_{5'}$, $J_{5-3} = J_{5'-3'} = 5.9$ Hz, $J_{5-6} = J_{5'-6'} = 3.2$ Hz), 8.61 (dd, 2H, H_c, H_d , $J_{c-b} = J_{d-e} = 8.0$ Hz, $J_{c-a} = J_{d-f} = 1.3$ Hz), 7.95 (dd, 2H, H_a, H_f , $J_{a-b} = J_{f-e} = 5.6$ Hz, $J_{a-c} = J_{f-d} = 1.3$ Hz), 7.89 (2dd, 4H, $H_6, H_{6'}$, $J_{6-5} = J_{6'-5'} = 3.2$ Hz, $J_{6-3} = J_{6'-3'} = 1.2$ Hz), 7.68 (dd, 2H, H_b, H_e , $J_{b-c} = J_{e-d} = 8.0$ Hz, $J_{b-a} = J_{e-f} = 5.7$ Hz). HRMS (ESI, +p): calc. 773.02940 for $[C_{28}H_{18}O_2N_{10}F_6PRu]^+$, found 773.02988 ($[M-PF_6]^{+}$).

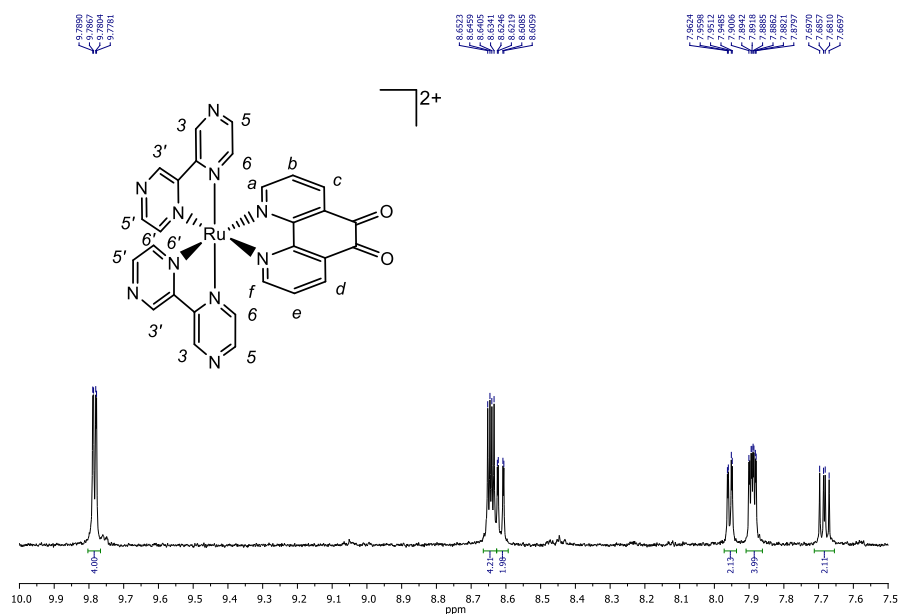


Figure 5.28. ^1H NMR spectrum (500 MHz, CD_3CN) of $[\text{Ru}(\text{bpz})_2(1,10\text{-phenanthroline-5,6-dione})]^{2+}$.

$[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$. One equivalent of $[\text{Ru}(\text{bpz})_2(1,10\text{-phenanthroline-5,6-dione})](\text{PF}_6)_2$ (46.6 mg, 0.05075 mmol) was mixed with 1.3 equivalent of 1,2-diaminonaphthalene (15.2 mg, 0.06597 mmol) in a $\text{EtOH}/\text{CH}_3\text{CN}$ mixture (50/50, 4.0 mL). The reaction mixture was stirred at reflux under argon and followed by absorption spectroscopy. After 3 hours, the reaction mixture was cooled at room temperature. After further cooling in an ice bath, addition of small portions of NH_4PF_6 and drops of water yielded a red precipitate. The organic solvents were evaporated under reduced pressure. After centrifugation, the solid is washed with excess water. After drying under vacuum, the orange-red residue is purified by preparative plate of chromatography (silica, eluent: $\text{CH}_3\text{CN}/\text{H}_2\text{O}/\text{saturated aqueous } \text{NH}_4\text{Cl}$ 4/4/1, $R_f = 0.35$). The counter-anion exchange from PF_6 to Cl is performed by eluting a solution of complex in a mixture $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ on Sephadex DEAE A25. ^1H NMR (PF_6 salt, 500 MHz, CD_3CN): δ (ppm) 10.08 (dd, 1H, H_j , $J_{j-k} = 8.3$ Hz, $J_{j-l} = 1.3$ Hz), 9.85 (m, 5H, H_c , H_3 , H_3' , $J_{c-a} = 1.3$ Hz), 9.70 (dd, 1H, H_j , $J_{i-g} = 2.4$ Hz),

8.70 (dd, 2H, H₅, $J_{5-6} = 3.2$ Hz, $J_{5-3} = 1.7$ Hz), 8.50 (d, 2H, H_{5'}, $J_{5'-6'} = 3.3$ Hz), 8.46 (d, 1H, H_d, $J_{d-e} = 9.2$ Hz), 8.3 (d, 1H, H_e, $J_{e-d} = 9.2$ Hz), 8.23 (m, 3H, H_a, H_f, H_l), 8.04 (m, 6H, H_b, H_k, H_g, H_h, H₆), 7.83 (2dd, 2H, H_{6'}, $J_{6'-5'} = 3.3$ Hz, $J_{6'-3'} = 1.2$ Hz). HRMS (ESI, +p): calc. 895.09267 for [C₃₈H₂₄N₁₂F₆PRu]⁺, found 895.09385 ([M-PF₆]⁺) and calc. 375.06452 for [C₃₈H₂₄N₁₂Ru]²⁺, found 375.06407 ([M-2PF₆]⁺).

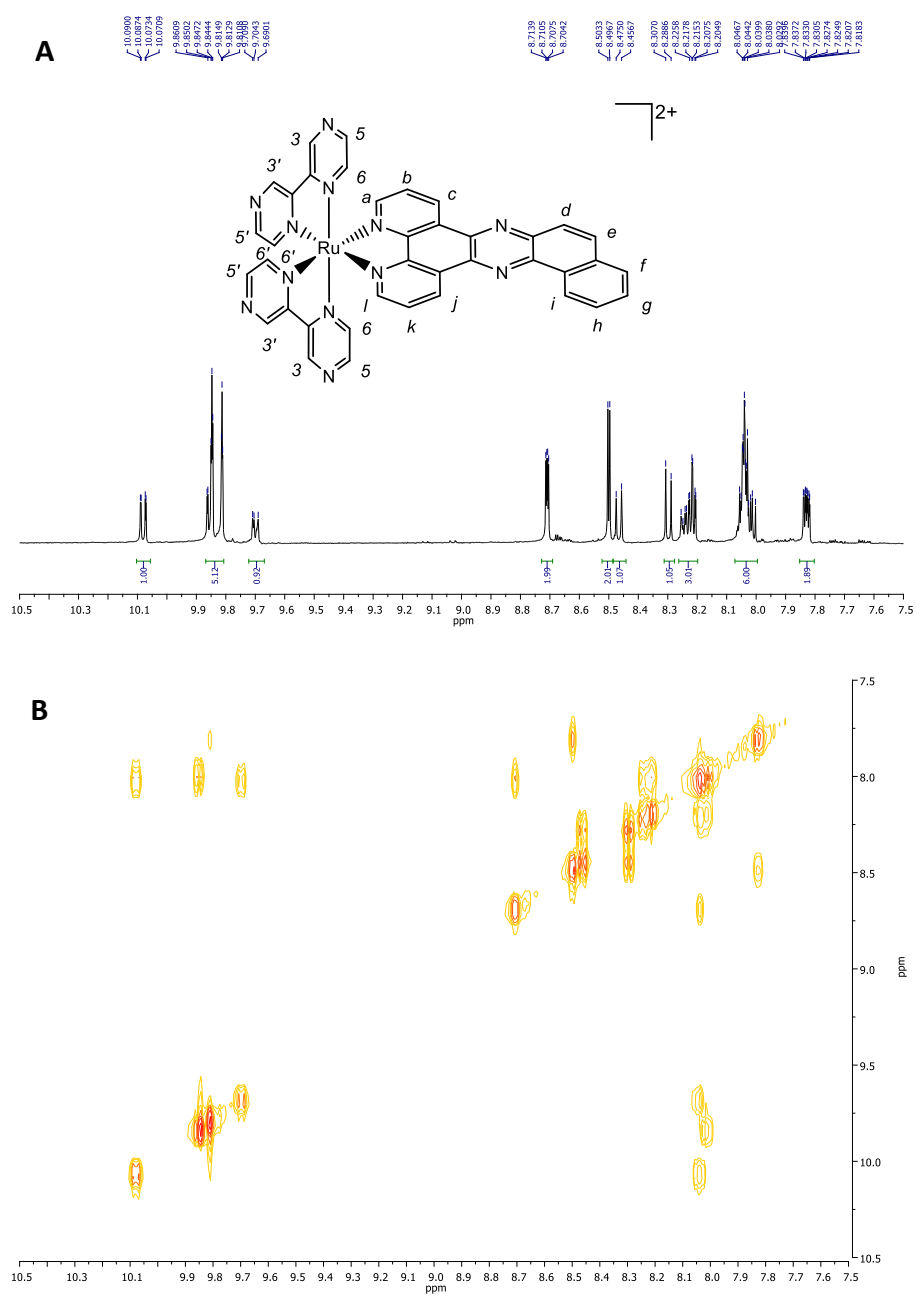
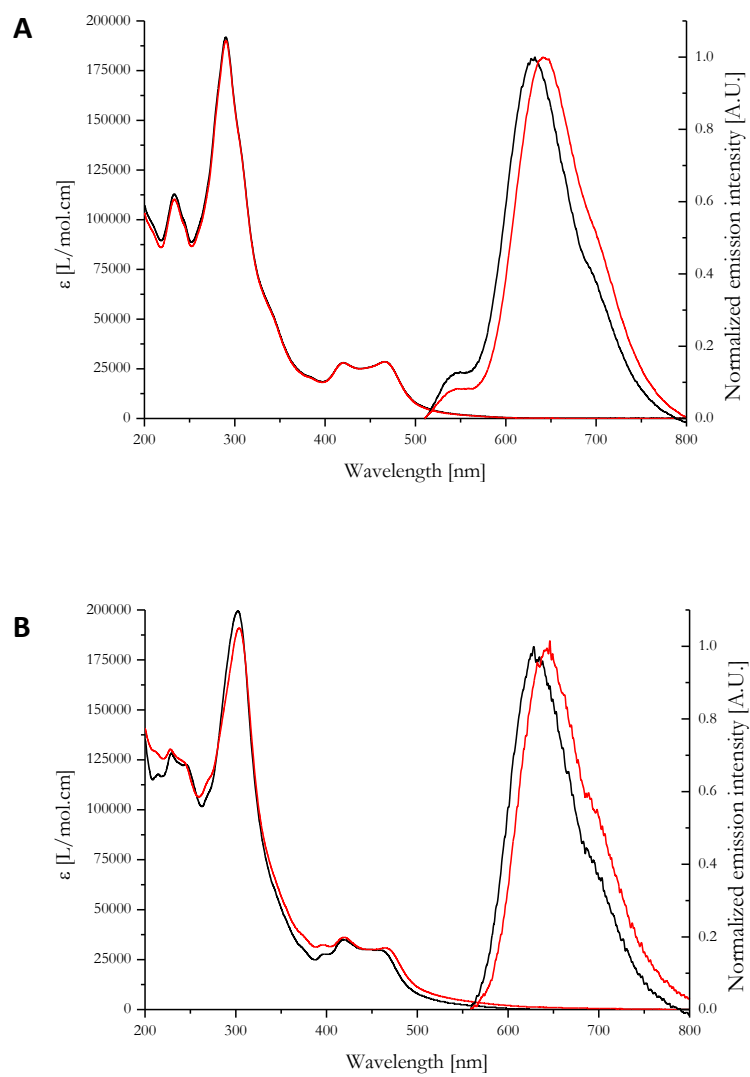


Figure 5.29. (A) 1D and (B) 2D ^1H NMR COSY spectrum (500 MHz, CD_3CN) of $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$.

3.4. Associated content

3.4.1. Absorption and emission spectra of the Ru(II) complexes



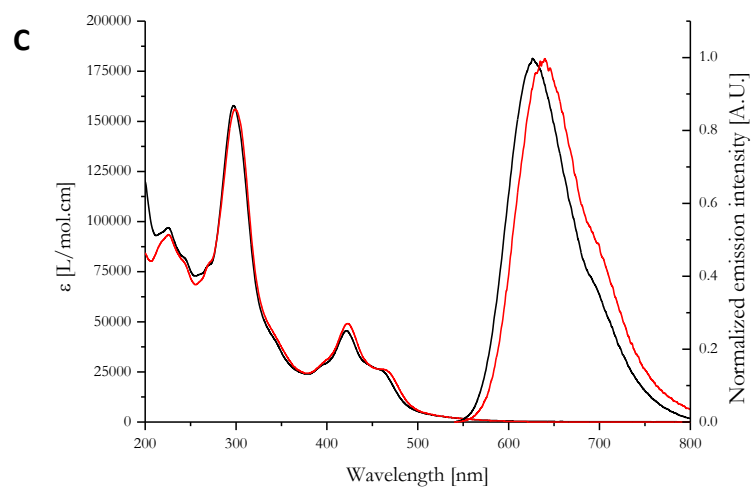


Figure 5.30. Absorption and emission spectra under air in acetonitrile (black) and in water (red) for (A) $[\text{Ru}(\text{bpz})_2\text{dpac}]^{2+}$, (B) $[\text{Ru}(\text{bpz})_2\text{npp}]^{2+}$ and (C) $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$.

3.4.2. HRMS spectra

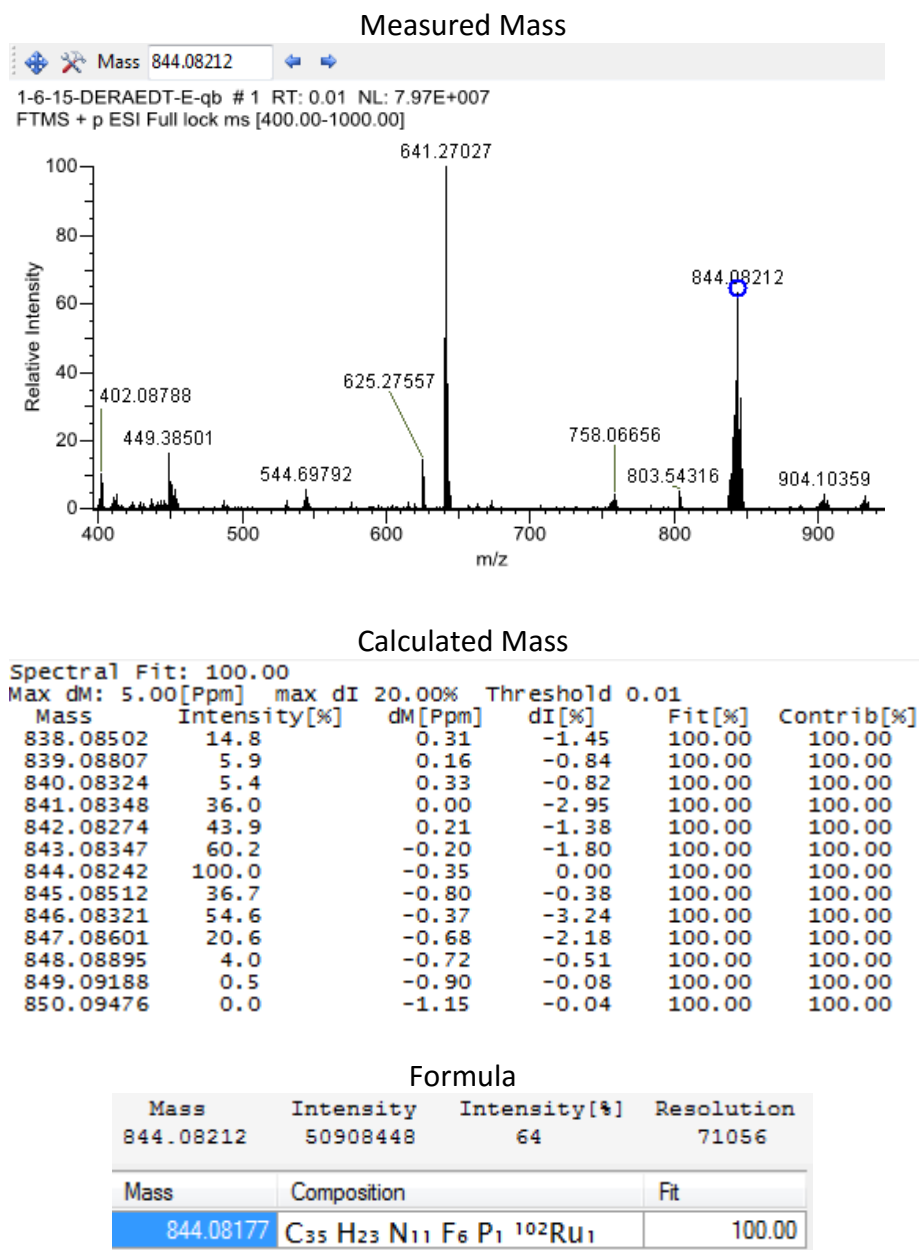
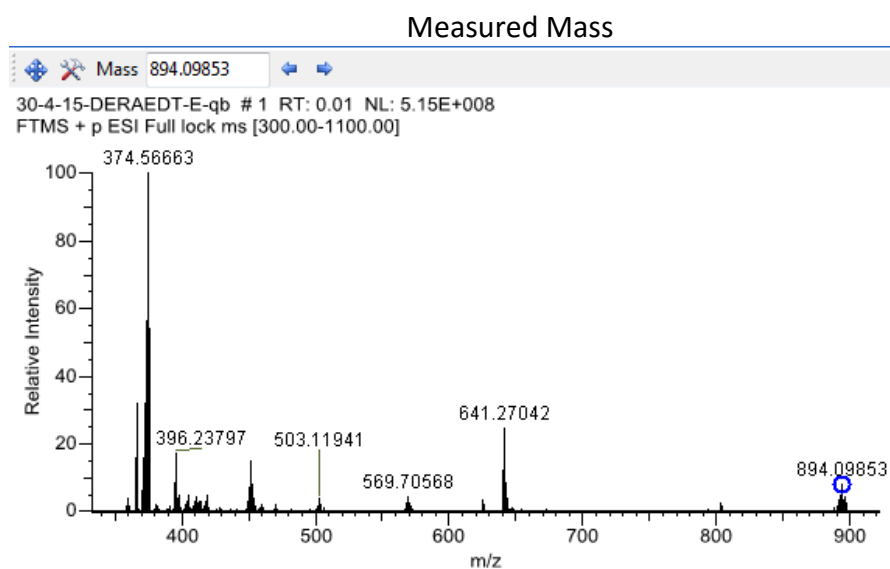


Figure 5.31. HRMS data for [Ru(bpz)₂dpac]²⁺ ([M-(PF₆)⁻]⁺).



Calculated Mass

Spectral Fit: 100.00
Max dM: 5.00[Ppm] max dI 20.00% Threshold 0.08

Mass	Intensity[%]	dM[Ppm]	dI[%]	Fit[%]	Contrib[%]
888.10067	14.4	0.29	-0.75	100.00	100.00
889.10373	6.4	0.14	-0.80	100.00	100.00
890.09913	5.5	0.55	-0.53	100.00	100.00
891.09915	35.3	0.18	-1.30	100.00	100.00
892.09856	44.4	0.42	-1.87	100.00	100.00
893.09921	60.5	0.14	0.55	100.00	100.00
894.09820	100.0	0.38	0.00	100.00	100.00
895.10084	40.3	0.17	0.23	100.00	100.00
896.09899	54.5	0.24	-2.46	100.00	100.00
897.10171	22.5	0.18	-0.99	100.00	100.00
898.10464	4.8	0.31	-0.70	100.00	100.00
899.10759	0.7	0.49	-0.41	100.00	100.00

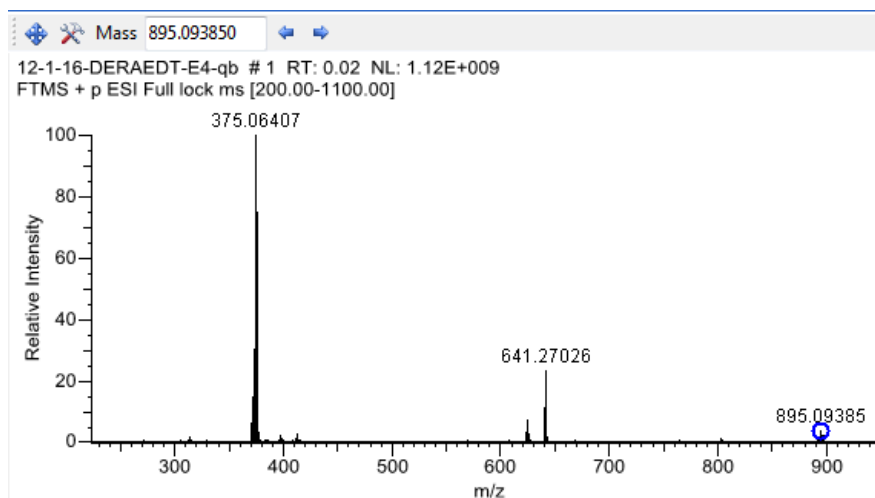
Formula

Mass	Intensity	Intensity[%]	Resolution
894.09853	41395020	8	69758

Mass	Composition	Fit
894.09742	C ₃₉ H ₂₅ N ₁₁ F ₆ P ₁ ¹⁰² Ru ₁	100.00

Figure 5.32. HRMS data for [Ru(bpz)₂npp]²⁺ ([M-(PF₆)⁻]⁺).

Measured Mass



Calculated Mass

Spectral Fit: 100.00
 Max dM: 5.00[Ppm] max dI 20.00% Threshold 0.10

Mass	Intensity[%]	dM[Ppm]	dI[%]	Fit[%]	Contrib[%]
889.09592	14.4	0.30	-2.87	100.00	100.00
890.09892	6.3	-0.15	-2.59	100.00	100.00
891.09445	5.5	-0.30	-1.61	100.00	100.00
892.09439	35.2	0.34	-1.84	100.00	100.00
893.09380	44.5	0.44	-2.61	100.00	100.00
894.09445	60.3	0.45	0.90	100.00	100.00
895.09344	100.0	0.45	0.00	100.00	100.00
896.09603	39.6	0.13	2.81	100.00	100.00
897.09424	54.5	0.45	-2.98	100.00	100.00
898.09690	22.1	0.20	-2.56	100.00	100.00
899.09977	4.6	0.14	-0.72	100.00	100.00
900.10264	0.6	1.67	-0.53	100.00	100.00

Formula

Mass	Intensity	Intensity[%]	Resolution
895.093850	44825764	4	66244

Mass	Composition	Fit
895.092671	C ₃₈ H ₂₄ N ₁₂ F ₆ P ₁ ¹⁰² Ru ₁	100.00

Figure 5.33. HRMS data for [Ru(bpz)₂bdppz]²⁺ ([M-(PF₆)⁻]⁺).

3.4.3. Cyclic voltammograms

Recorded in dry CH₃CN or DMF under nitrogen, with a sweep rate of 0.3 V/s, at room temperature. The concentration of the complexes is 8.10⁻⁴ mol/L, with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte.

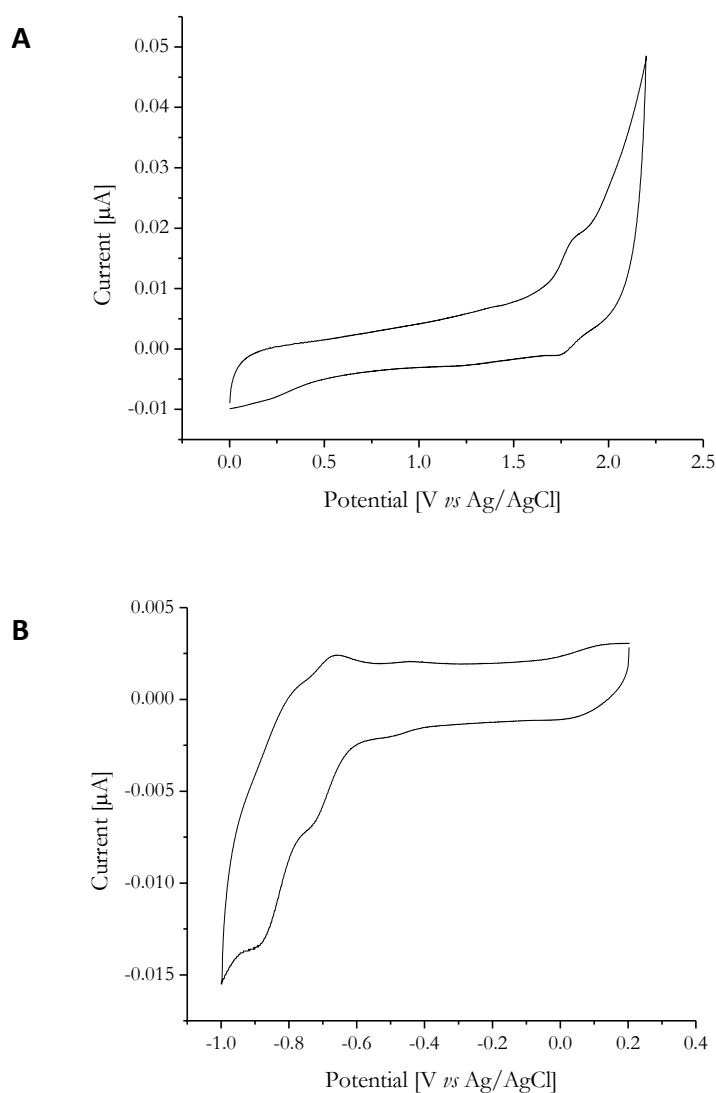


Figure 5.34. Cyclic voltammograms of [Ru(bpz)₂dpac]²⁺. (A) Positive polarization; (B) negative polarization.

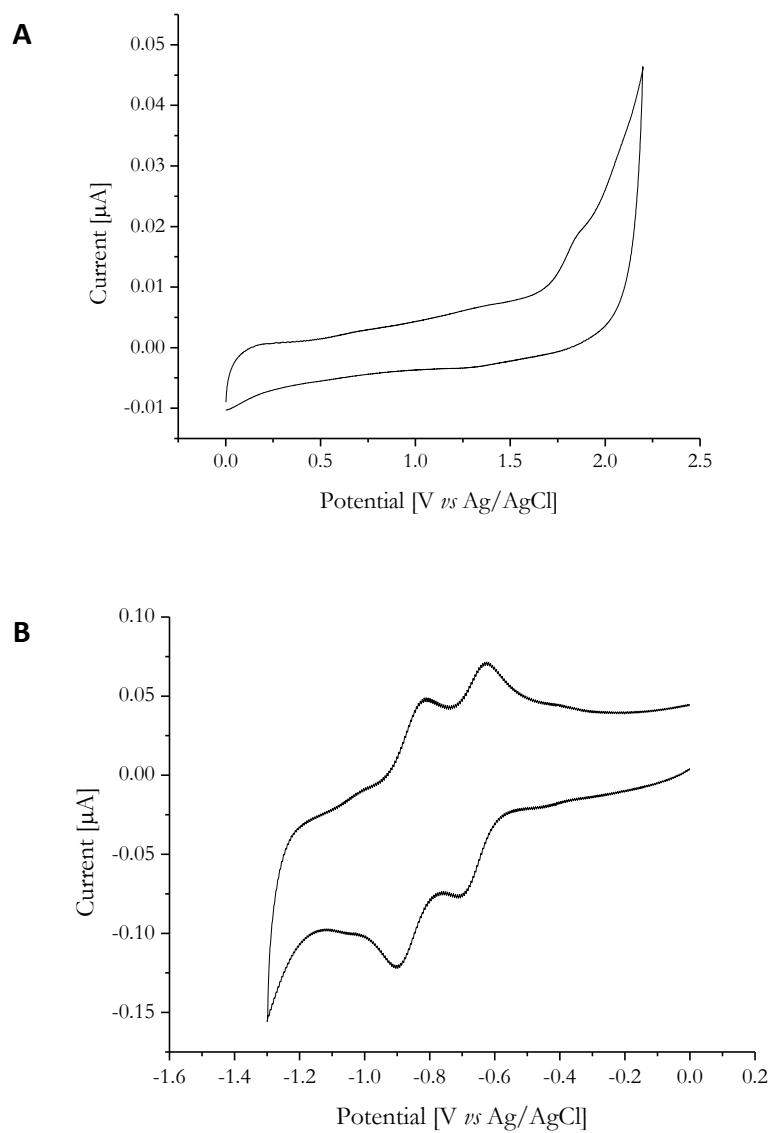


Figure 5.35. Cyclic voltammograms of [Ru(bpz)₂npp]²⁺. (A) Positive polarization; (B) negative polarization.

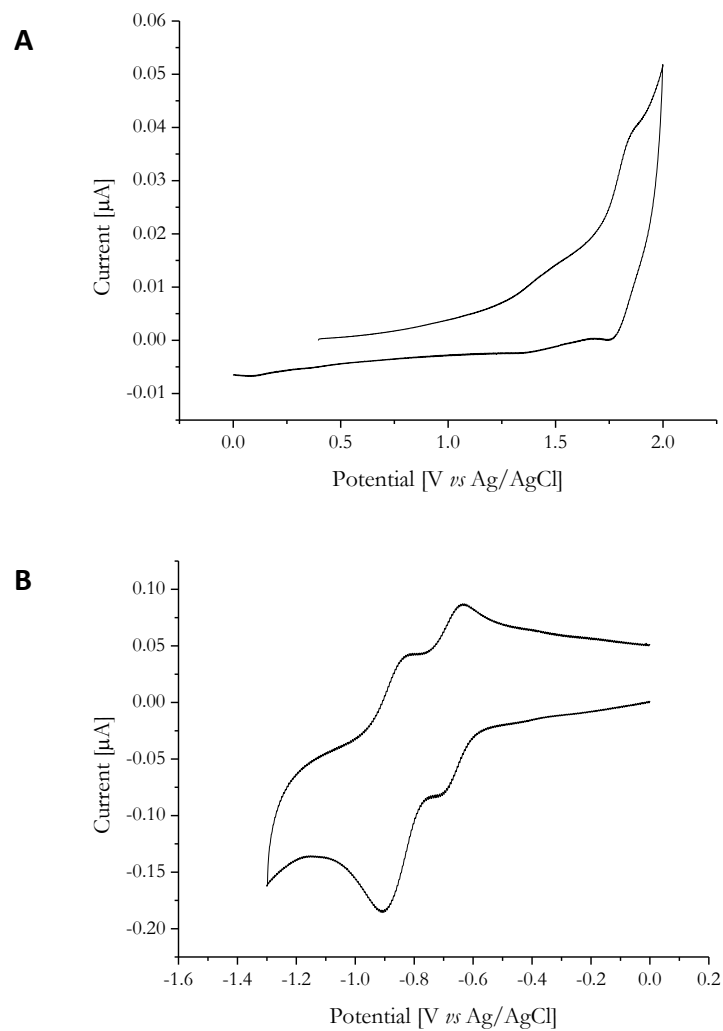


Figure 5.36. Cyclic voltammograms of $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$. (A) Positive polarization; (B) negative polarization

Chapter 6

Tuning of the photochemical
properties: Design of Ir(III)
complexes as DNA photoprobes

1. Introduction

We have shown in the previous chapters that Ru(II) complexes bearing acridine and phenazine extended planar ligands can be photophysically tuned to demonstrate promising abilities as photoprobes for DNA mismatches. For “elbow-shaped” derivatives, particularly, a noticeable differential luminescence behavior is observed that can be correlated to the nature and stability of the mismatch compared to complementary sequence.

In order to improve the photophysical properties of these complexes for their use as selective mismatched DNA photoprobe, we prepared their Ir(III) counterparts. Indeed, the photochemistry of Ir(III) complexes turns out to be more tunable than their Ru(II) analogs and these compounds display a more robust scaffold.²⁴² Switching from Ru(II) to Ir(III) thus appeared as an obvious continuation for our studies.

Concurrently to Ru(II) complexes chemistry and photophysical studies, Ir(III) complexes attracted increasing consideration since the beginning of the 21st century. Their inherent structural, photophysical and photochemical properties make them interesting compounds for a wide range of applications, from optically active materials²⁴³ to biological probes.²⁴⁴ As for Ru(II) complexes, the understanding of the properties of the Ir(III) complexes required some photophysical and photochemical considerations.

1.1. Photophysics and photochemistry

1.1.1. Geometry and orbitals

Ir(III) is a d⁶ metal cation. Its complexes share several properties with other d⁶ cations, such as Fe(II), Ru(II) and Os(II). The metal center is coordinated to several ligands, possessing one (monodentate), two (bidentate), or more chelating sites. All the Ir(III) complexes discussed herein will involve N-coordinating (polypyridyl)

and C-coordinating (cyclometalated) ligands. Interestingly, Ir(III) is able to coordinate up to three carbon atoms, what makes it, to the best of our knowledge, the only transition metal enabling tris-cyclometalated coordination (Figure 6.1).

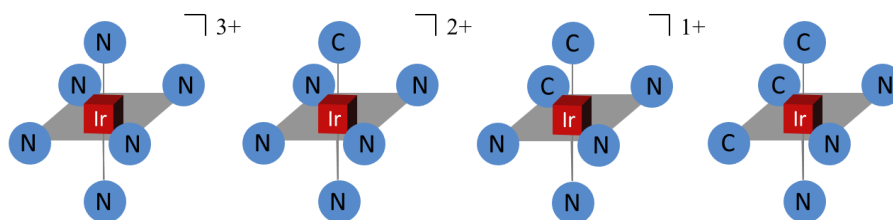


Figure 6.1. Simplified representation of the four possible coordinations of Ir(III) center by N- and C-coordinating ligands.

Up to now, bis-terdentate Ir(III) complexes have been extensively studied,^{245,246} mainly for applications such as light responsive bioconjugate²⁴⁷ or artificial photosynthesis^{200,248} due to their modular photophysical and photochemical properties.²⁴² However, the use of cyclometalated terdentate ligands can lead to the formation of a mixture of isomers due to the unpredictable position of the carbon atom(s) onto the coordination sphere of the Ir(III) center. In some cases, reversal of photophysical and photochemical properties can even be observed depending on the nature of the isomer. For that reason, we will focus on tris-bidentate Ir(III) complexes for which the photochemistry is less affected by the isomer structure. In addition, the position of each chelating carbon and nitrogen can be predicted by suitable synthetic protocols.

For tris-bidentate Ir(III) complexes bearing unsymmetrical ligands, the octahedral geometry leads to a mixture of four isomers, two of them being optical (Δ and Λ) and the two others being geometrical (*fac* and *mer*) isomers (Figure 6.2).

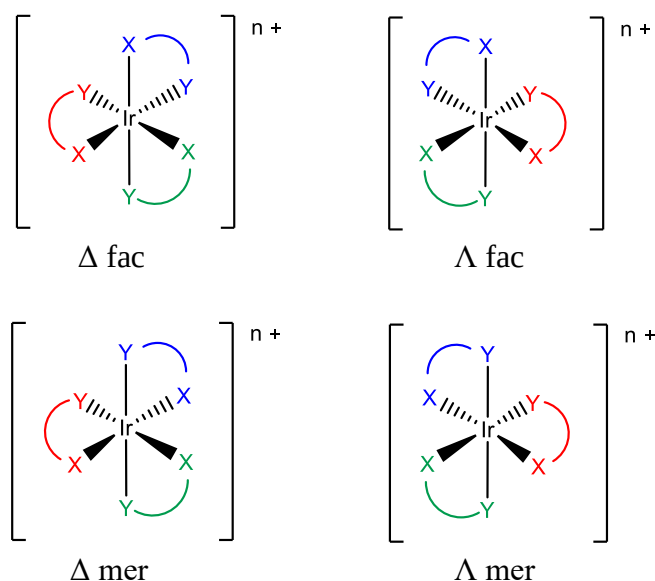


Figure 6.2. Scheme of the possible isomers of tris-bidentate Ir(III) complexes. X and Y are N- or C-coordinating atoms.

As bis-terdentate Ir(III) complexes photochemistry has been well described in the literature, the following considerations will treat these complexes and be further extrapolated to tris-bidentate analogs. For sake of clarity, the Ir(III) complexes are gathered on the basis of their carbon/nitrogen ratio (C/N) directly chelated onto the Ir(III) center, thus ranging from C/N = 0/6 to C/N = 3/3, whatever bidentate or terdentate are involved.

1.1.2. Absorption of light

Ir(III) complexes photophysics depend on the relative energy of the molecular orbitals, as for Ru(II) polypyridyl complexes. Together with LC, MC and MLCT transitions (see Figure 1.17, Chapter 1), LLCT transitions can be observed for Ir(III) complexes upon absorption of UV-visible light. Due to the relatively strong covalency of Ir-C bonds, the HOMO for cyclometalated complexes is rather made of an Ir-ligand fragment, leading to so-called “MLLCT” transitions (Metal-Ligand to Ligand Charge transfer).²⁴⁹

While the nature of the electronic transitions for Ru(II) complexes are often unambiguously defined, the absorption of light leads to a mixture of transitions (mostly LC/MLCT/LLCT) in the case of Ir(III) complexes.²⁴² Furthermore, through the appropriate choice of the ligands, the entire visible range can be covered, allowing a unique color tunability of Ir(III) complexes.

As a result of the unique ability of Ir(III) to coordinate up to three carbons, a single photophysical model can't be drawn up in this case. Indeed, the particular photophysics of bis-terdentate Ir(III) complexes induce reordering of the energy levels in the series IrN₆ to IrN₃C₃. This behavior has been reported for the first time in 1991 for an extended series of tris-cyclometalated Ir(III) complexes²⁵⁰ and emphasized the influence of the electron-withdrawing or donating character of various substituents on the ligands on the resulting photophysical and electrochemical properties of the complexes. Increasing the electron-donating ability of the substituent or ligand will destabilize the filled metal and ligand centered energy levels, strongly affecting the energy level diagram. This effect could be rationalized for the series IrN₆ to IrN₃C₃ considering the influence of the increased number of coordinating carbon atoms on the energies of metal and ligands orbitals (Figure 6.3). Note the use of bidentate ligands instead of terdentate ones may affect the relative energies of some states. Such studies are under investigation in our group.²⁵¹

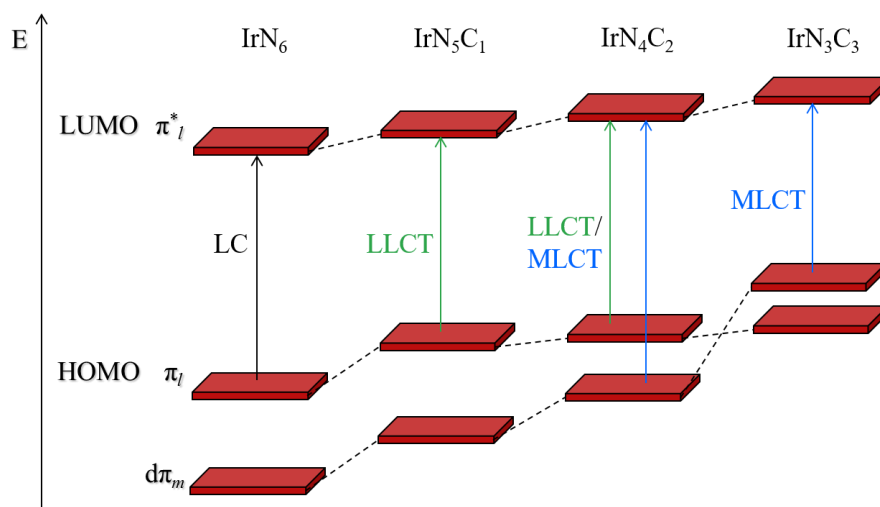


Figure 6.3. Representation of the relative energy levels of the orbitals for bis-terdentate (from left to right) IrN₆, IrN₅C₁, IrN₄ and IrN₃C₃, respectively. [adapt from ²⁴⁶]

1.1.3. Emission of light

As mentioned in the previous section, different orbitals are involved in the excited state depending on the nature of the ligands.

IrN₆ complexes display relatively narrow and well-resolved emission bands, as well as relatively long excited state lifetime and low radiative rate constant, which is characteristic for LC deactivation. Confirmation of this attribution has been made by luminescence measurements at low temperature. In this case, no shift of the emission λ_{max} and no vibrational bands appeared upon decrease of the temperature.²⁵²

IrN₅C₁ complexes, with respect to IrN₆, IrN₄C₂ and IrN₃C₃, are relatively poorly described in the literature. The LLCT character of their emission bands are mainly based on DFT calculations.²⁴⁵

IrN₄C₂ complexes are by far the most described Ir(III) complexes. The reason arises from their relative ease of synthesis and their absorption of light in the UV (LC transitions) and in the visible (mixture of MLCT and LLCT transitions) wavelengths. These properties make

them ideal candidates for applications such as photosensitizers²⁰⁰ or luminescent probes.^{253,254} The emission energy is decreased with respect to IrN₆ and IrN₅C₁ as a result of the destabilization of the HOMO of the system. Luminescence originates from a mixture of ³MLCT and ³LLCT excited states as seen by the large hypsochromic shift going from room temperature to 77 K.²⁵¹

IrN₃C₃ complexes display broad structureless emission band, typically observed for charge transfer states. The luminescence of these complexes is red-shifted compared to Ir(III) complexes featuring higher N/C ratio. Due to the destabilization of the HOMO through the incorporation of an additional coordinating carbon atom, these complexes show greater photo-decomposition in solution under light irradiation.²⁵⁵

1.1.4. Redox properties

As mentioned earlier, the nature of the HOMO and LUMO of the complexes is affected by the N/C ratio, such as no general statements can be made for Ir(III) complexes, unlike polypyridyl Ru(II) complexes. This is a consequence of the ease of photophysical tuning properties of the Ir(III) complexes. Nevertheless, one can admit that Ir(III) complexes are more easily reduced when the N/C ratio increases, and more easily oxidized when the N/C ratio decrease. This has been extensively studied for bis-terdentate Ir(III) complexes.^{255,256}

1.2. Ir(III) complexes as biomolecular probes

By far the most promising and studied for their high quantum yields and excited state lifetimes, IrN₄C₂ complexes are of particular interest for application as luminescent probes. These complexes are composed of two cyclometalated ligands (C[^]N) and one bearing two coordinating nitrogens (N[^]N). In this section, we will successively describe the use of these complexes as DNA and cellular probes.

1.2.1. Non-covalent DNA probes

Several bis-cyclometalated Ir(III) complexes have been designed for targeting DNA. Among them, those bearing an extended planar aromatic ligand such as dpq (= dipyrido[3,2-*f*:2',3'-*h*]-quinoxaline), dpqa (= 2-*n*-butylamidodipyrido[3,2-*f*:2',3'-*h*]quinoxaline), dppz or dppn (= benzo[*i*]dipyrido[3,2-*a*:2',3'-*c*]phenazine) (Figure 6.4) display luminescence enhancement when intercalated into DNA base pairs.^{244,253} Upon excitation, these complexes are strong emitters and exhibit long-lived ³MLLCT emission in aprotic organic solvents, while a (partial) luminescence quenching is observed in aqueous medium. This behavior has been ascribed, as for **Ru-DPPZ**, to the formation of two Hydrogen bonds in the excited state between the non-chelating nitrogen atoms of the phenazine or amide moiety and water molecules from the solvent (see Chapter 3). Luminescence titrations of these Ir(III) complexes with natural and synthetic oligonucleotides reveal strong luminescence enhancement in the presence of DNA or oligonucleotides. Note these studies have been performed in the presence of 30% of organic solvent (here methanol) for solubility issues.²⁵³

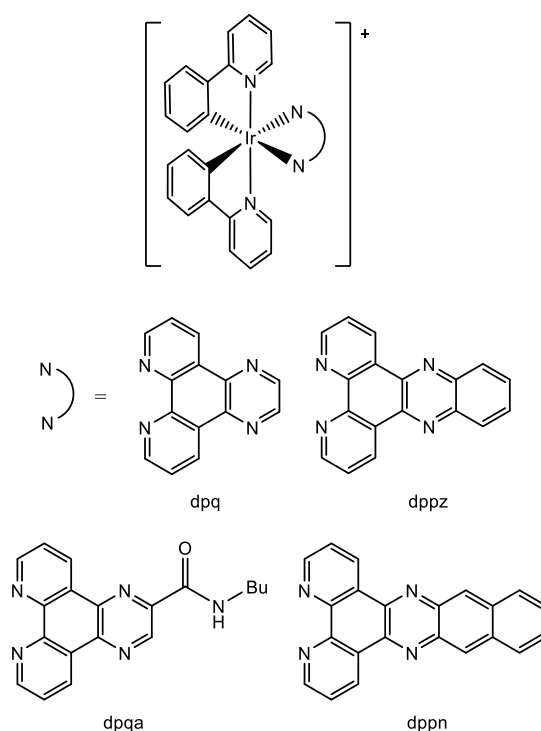


Figure 6.4. Structure of DNA-targeting Ir(III) complexes.

In 2015, a dppz-based IrN₆ complex, [Ir(bpy)₂dppz]³⁺ (Figure 6.5) have been reported for the first time.²⁵⁷ The replacement of two coordinating carbons by nitrogen atoms offers the advantages of increased water solubility and DNA binding affinity due to the higher formal charge of the resulting complex. The photophysical and photochemical studies performed until now suggest an oxidative quenching of its excited state by nucleobase from DNA. However, these results remain qualitative and no clear interpretation can be drawn up. These scaffolds still represent high potential for biological applications, such as photosensitizers for photodynamic therapy, given the tunable nature of the excited states of polypyridyl Ir(III) complexes.

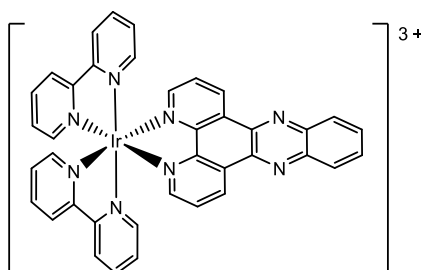


Figure 6.5. Structure $[\text{Ir}(\text{bpy})_2\text{dppz}]^{3+}$.

1.2.2. Cellular probes

Visualizing and tracking cells, tissues, organs and organisms is a growing interest area. Optical techniques represent attractive tools especially due to their relatively low cost and high detection sensitivities. Their highly tunable photophysical and photochemical properties renders transition metals complexes particularly interesting compounds for such applications. Notably, Ir(III) complexes have been used as intracellular probes thanks to their microenvironment-sensitive properties.

IrN_4C_2 complexes display appropriate features in this area due to their formal positive charge and choice of the extended ligand. Indeed, the former allows the attraction of the complexes by the negatively charged cell surface, and the later facilitates the diffusion into the cytoplasm through the control of the lipophilicity.²⁴⁴ Their photophysical tunability has led to the design of blue to red emitting $[\text{Ir}(\text{dfppy})_2\text{L}]^+$ (dfppy = 4',6'-difluorophenylpyridine, L = bidentate ligand) cellular probes ($\lambda_{\text{em}} \approx 457\text{-}632\text{ nm}$) (Figure 6.6).

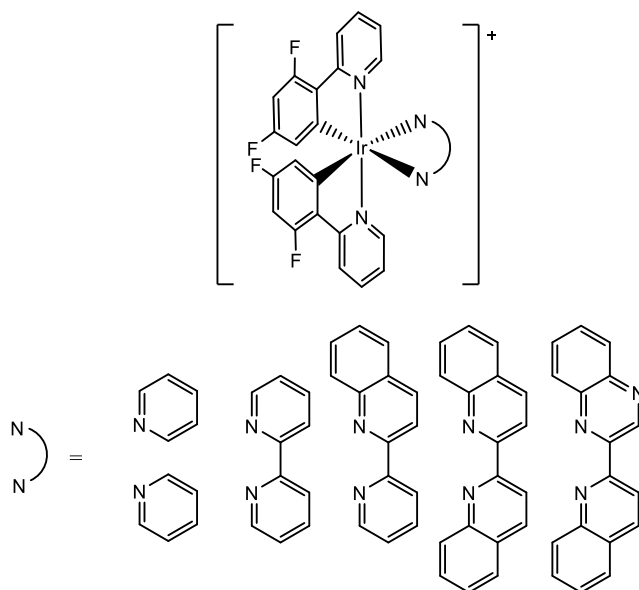


Figure 6.6. Structure of biologically relevant IrN₄C₂ complexes.

All these complexes are photo-stable, display ³MLLCT emission and are non-cytotoxic, which makes them suitable dyes for live cell imaging. Studies on the cellular uptake mechanism of such complexes suggest they enter the cells by both energy-requiring and diffusion-like pathways. Due to their high lipophilicity and large structures, most Ir(III) complexes do not target specific intracellular organelle neither show nuclear uptake. Recently, it has been shown that specific detection of organelles by IrN₄C₂ complexes is possible through rational design of their structures.²⁵⁸

Recently, new strategies emerge to increase the solubility of IrN₄C₂ complexes in aqueous medium and thus fully exploit the outstanding properties of such compounds as cellular probes. Derivatization of the N[^]N ligand for example with polyethylene glycol (PEG) groups led to an increased solubility in aqueous solutions (Figure 6.7).²⁵⁹ In addition, these PEG-derivatized Ir(III) complexes exhibit light-activated cytotoxicity, through generation of singlet oxygen in the excited state.²⁶⁰

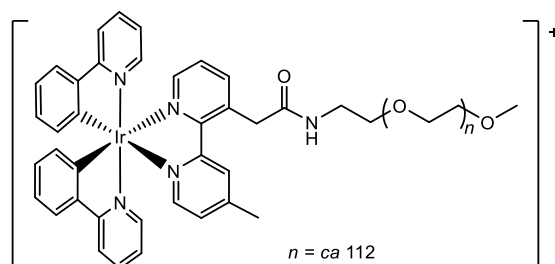


Figure 6.7. Example of PEG-derivatized IrN₄C₂ complexes.

2. Tuning of Ir(III) DNA photoprobes

We have previously showed that Ru(II) complexes bearing acridine extended planar ligands are able to act as a DNA probe under light irradiation. With the aim of further tuning the optical properties of these compounds, we propose, instead of modifying the chemical structure of either the ancillary or extended planar ligand, to replace the metal cation from Ru(II) to Ir(III). Indeed, as mentioned in the previous section, the photochemical properties of Ir(III) complexes are more tunable than their Ru(II) counterparts notably because of the inherent characteristics of the iridium(III) metal cation and of its ability to coordinate up to three C-coordinating ligands. In that context, the design of Ir(III) complexes as potential DNA photoprobes is attractive.

In this chapter, we aimed to study the photophysical and photochemical properties of a new acridine-based IrN₄C₂ Ir(III) complex, namely [Ir(ppy)₂dpac]⁺ (**Ir-DPAC**, Figure 6.8, section 3.1). The IrN₄C₂ scaffold has been chosen for its well-known properties under light irradiation which are suitable for the design of DNA photoprobes. Fluorine analogs, [Ir(ppy)₂dpacF₂]⁺ (**Ir-DPACF₂**) and [Ir(ppy)₂dpacF₄]⁺ (**Ir-DPACF₄**, Figure 6.8, section 3.1), have also been prepared in order to finely tune the properties of the resulting Ir(III) complexes.

In addition, to tune the DNA binding of the dpac-based Ir(III) complexes, the “elbow-shaped” [Ir(ppy)₂npp]⁺ (**Ir-NPP**, Figure 6.8,

section 3.1) Ir(III) complex has been prepared. The relevance of the use of this extended planar ligand has been established earlier in the case of Ru(II) counterparts (see chapter 4). For comparison purpose, its phenazine equivalent, $[\text{Ir}(\text{ppy})_2\text{bdppz}]^+$ (**Ir-BDPPZ**, Figure 6.8, section 3.1), has also been prepared. These complexes should exhibit higher binding sensitivity to their local micro-environment, leading to different excited state properties.

This chapter has been the subject of a publication which content is gathered hereafter.

3. Content of the publication

Journal

Journal of Fluorescence, ASAP Article
DOI:10.1007/s10895-016-1904-2

Title

Photochemical tuning of tris-bidentate acridine- and phenazine-based Ir(III) complexes

Authors

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Abstract

Five new Ir(III) complexes of the type $[\text{Ir}(\text{ppy})_2\text{L}]^+$ (where ppy = 2-phenylpyridine, L = bidentate extended planar ligand) bearing linear and elbow-shaped acridine- and phenazine-based extended planar aromatic ligands have been successfully synthesized and characterized. The electrochemical and photochemical studies revealed that all complexes allow emission in the range 589-601 nm from excited states corresponding to a charge transfer between an Ir-ppy fragment and the extended planar ligand. Luminescence quenching occurs in water for $[\text{Ir}(\text{ppy})_2\text{dpac}]^+$ (**Ir-DPAC**), $[\text{Ir}(\text{ppy})_2\text{dpacF}_2]^+$ (**Ir-DPACF₂**), $[\text{Ir}(\text{ppy})_2\text{dpacF}_4]^+$ (**Ir-DPACF₄**) and $[\text{Ir}(\text{ppy})_2\text{bdppz}]^+$ (**Ir-BDPPZ**), while solely partial quenching is observed for $[\text{Ir}(\text{ppy})_2\text{npp}]^+$ (**Ir-NPP**). This “light switch” effect has been ascribed to the possible formation of a non-emissive mono-hydrogen bonded excited state for the four complexes. The “elbow shape” of **Ir-NPP** is proposed to account for the prevention of the non-chelating nitrogen atom of the npp ligand to form hydrogen bond with solvent molecules. The results emphasized the potential of small chemical modifications of the extended planar ligand on the properties of the corresponding Ir(III) complexes. Their tunable properties make them ideal candidates for applications such as DNA photoprobes.

3.1. Introduction

Polypyridyl transition metal complexes bearing dppz (= dipyrido[3,2-*a*:2',3'-*c*]phenazine) derivatives as extended planar ligand has been the focus of much attention for the last decades.^{46,202,253,254} Notably, $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ and $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ (phen = 1,10-phenanthroline, bpy = 2,2'-bipyridine) display environment-sensitive luminescence properties which make them candidates as photoprobes for DNA topologies and mismatches.^{106,220} Indeed, these complexes exhibit luminescence quenching in aqueous media, which is then restored upon addition of DNA. The understanding of the under-lying photophysics of this “light switch” behavior received a lot of attention and lead to numerous

studies.^{113,116,144,261} The formation of hydrogen bonds between the nitrogen atoms from the phenazine core of the dppz with solvent molecules lies at the origin of the luminescence quenching process in the excited state. Upon binding to DNA, the luminescence of the complex is restored due to the inhibition of the formation of the Hydrogen bonds.¹⁴⁴ While Ru(II) complexes bearing dppz derivatives and other phenazine-based ligands received a lot of attention, complexes based on acridine ligands remain poorly described.^{225,262}

In that context, we recently published extensive photophysical studies on Ru(II) complexes based on dpac (= dipyrdo[3,2-*a*:2',3'-*c*]acridine) derivatives.^{225,263} These derivatives exhibit suitable optical properties for applications as DNA photoprobes. In addition, complexes bearing npp (= naphtho[1,2-*b*]pyrido[3,2-*f*][1,7]phenanthroline) and bdppz (= benzo[*h*]dipyrdo[3,2-*a*:2',3'-*c*]phenazine), qualified as “elbow-shaped” ligands, particularly show ability to discriminate between matched and mismatched DNA sequences.²⁶³

In order to tune the photochemical properties of these acridine-based complexes, we were interested in replacing the metal cation from Ru(II) to Ir(III). Indeed, the optical properties of Ir(III) complexes are more tunable than their Ru(II) counterparts.^{242,244,253} Notably, [Ir(ppy)₂L]⁺ (ppy = 2-phenylpyridine, L = bidentate N^N ligand) complexes have been the subject of increasing attention due to their suitable optical properties for application as DNA and oligonucleotides photoprobes. It has been shown that [Ir(ppy)₂dppz]⁺ exhibits luminescence enhancement in the presence of DNA, whereas its luminescence is quenched in the absence of genetic material. Such behavior has been ascribed to the formation of Hydrogen bonds with solvent molecules in the excited state, as for [Ru(phen)₂dppz]²⁺ and [Ru(bpy)₂dppz]²⁺, which are then prevented upon DNA binding.²⁵³ Analogous to dppz, the dpac ligand (Figure 6.8) bears one less non-chelating nitrogen atom, leading thus to the possible formation of only one Hydrogen bond with solvent molecules in the excited state, and consequently to potential differences with dppz-based complexes.

In this context, we prepared Ir(III) complexes bearing extended planar aromatic ligands based on an acridine core (dpac; dpacF₂ = 7,8-difluorodipyrido[3,2-*a*:2',3'-*c*]acridine; dpacF₄ = 6,7,8,9-tetrafluorodipyrido[3,2-*a*:2',3'-*c*]acridine). The study of the photochemical properties of the corresponding Ir(III) complexes ([Ir(ppy)₂dpac]⁺ (**Ir-DPAC**), [Ir(ppy)₂dpacF₂]⁺ (**Ir-DPACF₂**), [Ir(ppy)₂dpacF₄]⁺ (**Ir-DPACF₄**), Figure 6.8) both in the absence and in the presence of DNA has been achieved. In order to tune the DNA binding of these derivatives, the “elbow-shaped” [Ir(ppy)₂npp]⁺ (**Ir-NPP**) and [Ir(ppy)₂bdppz]⁺ (**Ir-BDPPZ**) complexes have also been prepared (Figure 6.8). In this paper, we report a detailed photochemical study of these five complexes and analyze the influence of the acridine core on the photophysical properties compared to the phenazine equivalents.

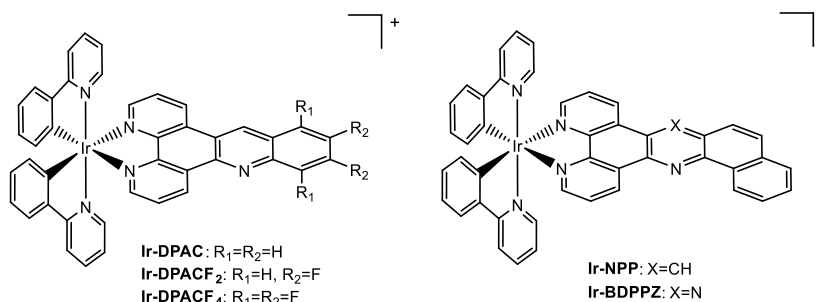


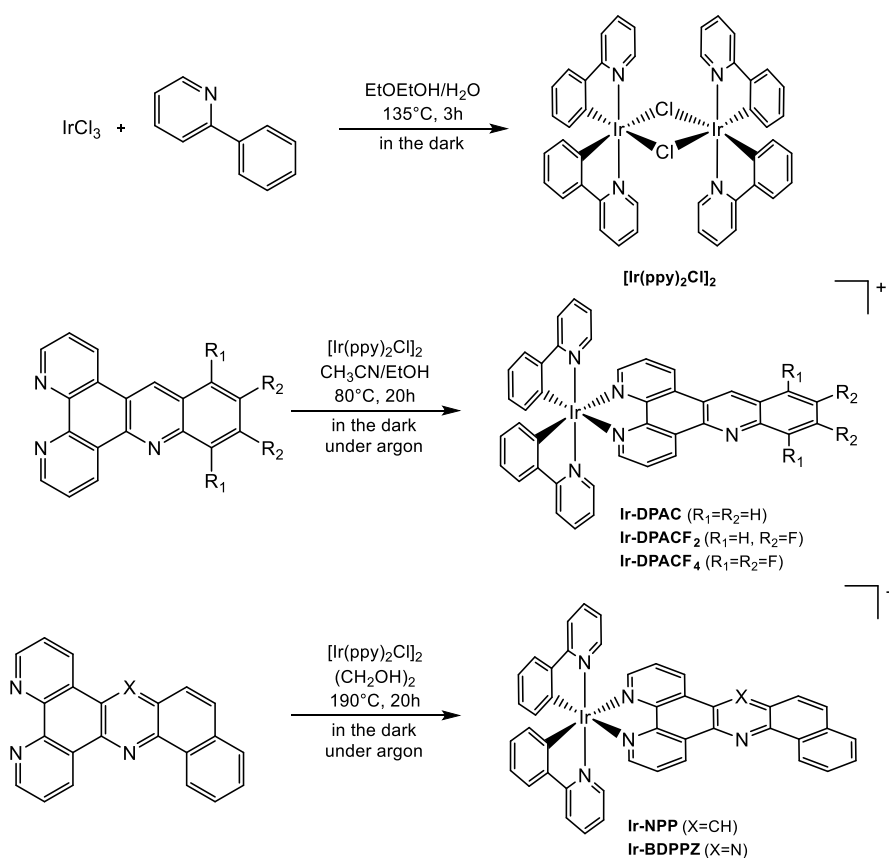
Figure 6.8. Structure of **Ir-DPAC**, **Ir-DPACF₂**, **Ir-DPACF₄** and “elbow-shaped” **Ir-NPP** and **Ir-BDPPZ** complexes.

3.2. Results

3.2.1. Synthesis

Acridine derivatives, *i.e.* dpac, dpacF₂, dpacF₄ and npp ligands, have been obtained through a Tröger's base modified synthetic scheme.^{107,151,225,263} Bdppz is obtained through condensation between 1,10-phenanthroline-5,6-dione and 1,2-diaminonaphthalene.²⁶³ Commercially available 2-phenylpyridine was used as ancillary ligands.

The corresponding Ir(III) complexes have been prepared by the direct chelation of the extended ligand onto a dichloro-bridged Ir(III) dimer (Scheme 6.1), [Ir(ppy)₂Cl]₂, using methodologies previously described for similar compounds.^{264,265} (stop at 268) All the intermediate and final compounds are protected from direct light during the synthesis and purification steps to prevent photochemical degradation. The reactions are performed under argon to avoid any side products due to the presence of oxygen.



Scheme 6.1. Synthetic scheme of **Ir-DPAC**, **Ir-DPACF₂**, **Ir-DPACF₄**, **Ir-NPP** and **Ir-BDPPZ**.

The complexes have been fully characterized by HRMS and ^1H NMR spectroscopy. All the multiplicity and coupling constants cannot be determined due to the superposition of several signals and/or lack of fine resolution for some peaks in the ^1H NMR spectra. The ^1H NMR

shows unambiguously the absence of symmetry elements for all the complexes, which induces the non-equivalence of (i) the protons on the extended planar ligand and (ii) the ancillary ligands. The ^1H NMR spectra does not seem sensitive to concentration as only minor changes occurred in the ^1H NMR spectra upon change in the concentration. For solubility purposes, all the complexes are converted to the chloride salt for experiments conducted in aqueous media, or to the hexafluorophosphate salt for studies in organic solvents.

3.2.2. Electrochemistry

The electrochemical data of the five Ir(III) complexes are obtained by cyclic voltammetry in dry deoxygenated CH_3CN or DMF (Table 6.1, Figures 6.9-6.13). For comparison purposes, the data of other complexes are also gathered in the table. All five complexes display a one-electron reversible oxidation wave at 1.30-1.35 V vs Ag/AgCl. This similarity between the oxidation potentials of all these complexes suggests a metal- and/or ancillary ligand-based nature of the oxidation process,^{253,266} irrespective of the substitution on the extended planar ligand. Indeed, while $[\text{Ir}(\text{bpy})_3]^{3+}$ displays a higher oxidation potential due to the stronger electron-withdrawing character of the three bpy ligands, it is the opposite for $[\text{Ir}(\text{ppy})_3]$. The ppy ligand, consequently to its C-coordinating atom, is strongly electron-donating and thus raises the level of the HOMO of the complex. Based on our data, we suggest, as others, it involves an Ir-ppy fragment due to the strong covalency of the carbon-iridium bond.^{249,265}

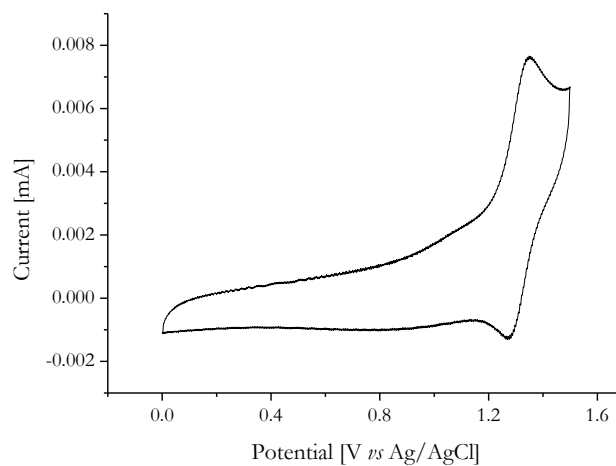
As described in the literature,^{253,266} reduction processes involve the different ligands, as noticeable shifts of the waves occur depending on the nature of the extended ligand. The first reduction wave is attributed to the reduction of the extended planar ligand. The more the electron-withdrawing ability of the ligand, the more anodic is the reduction potential. These observations suggest a stabilization of the LUMO of the respective complexes, in accordance with increased conjugation and/or electron-deficiency. The second reduction wave, as too anodic to involve the reduction of an ancillary ppy ligand, is

therefore attributed to the reduction of another fragment of the extended planar ligand.²⁵³ Excited state potentials for the five complexes have been estimated from the ground state redox potentials and the energy of the excited state corresponding to the maximum of the emission spectrum at 298 K.

Complex	$E_{1/2 \text{ ox}}^a$ [V vs Ag/AgCl]	$E_{1/2 \text{ red}}^b$ [V vs Ag/AgCl]			$E_{\text{ox}}^*{}^c$ [V vs Ag/AgCl]	$E_{\text{red}}^*{}^d$ [V vs Ag/AgCl]
		1	2	3		
$[\text{Ir}(\text{bpy})_3]^{3+}$ ^{a 267}	1.95	-0.98	-	-	-0.86	1.83
$[\text{Ir}(\text{ppy})_3]$ ^{a 267}	0.78	-2.22	-	-	-1.72	0.28
$[\text{Ir}(\text{ppy})_2\text{dppz}]^+$ ^{a 253}	1.31	-0.93	-1.45	-1.63	-0.66	1.04
Ir-DPAC	1.30	-1.09	-1.59	-	-0.79	1.00
Ir-DPACF₂	1.32	-1.01	-1.51	-	-0.76	1.07
Ir-DPACF₄	1.30	-0.82	-1.60	-	-0.77	1.25
Ir-NPP	1.35	-1.15	-1.69	-	-0.76	0.96
Ir-BDPPZ	1.35	-0.73	-0.83	-1.23	-0.72	1.34

Table 6.1. Potentials for the oxidation ($E_{1/2 \text{ ox}}$) and reduction ($E_{1/2 \text{ red}}$) of **Ir-DPAC**, **Ir-DPACF₂**, **Ir-DPACF₄**, **Ir-NPP** and **Ir-BDPPZ**. ^a Measured in dry acetonitrile. ^b Measured in dry *N,N*-dimethylformamide. ^c The excited state oxidation potential was estimated from the ground state oxidation potential and the energy of emission maximum with the equation $E_{\text{ox}}^* = E_{1/2 \text{ ox}} - E_{0-0} \approx E_{1/2 \text{ ox}} - \Delta E_{\lambda_{\text{max, em}}}$. ^d The excited state reduction potential was estimated from the ground state reduction potential and the energy of emission maximum with the equation $E_{\text{red}}^* = E_{1/2 \text{ red}} + E_{0-0} \approx E_{1/2 \text{ red}} + \Delta E_{\lambda_{\text{max, em}}}$. ΔE_p corresponds to the peak-to-peak separation.

A



B

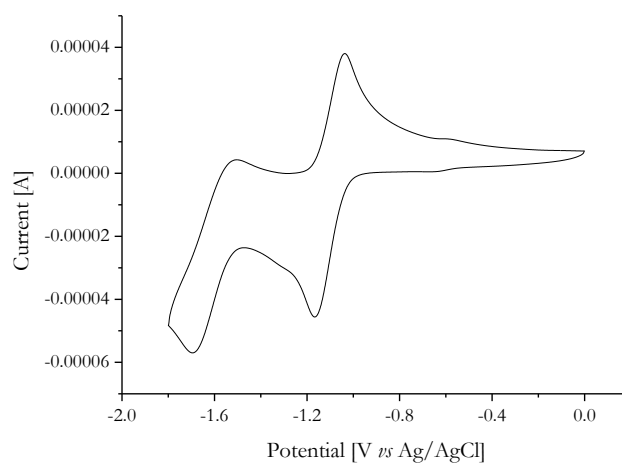


Figure 6.9. Cyclic voltammograms of **Ir-DPAC** recorded in dry CH_3CN or DMF under nitrogen, with a sweep rate of 0.15 V/s, at room temperature. The concentration of the complexes is $8 \cdot 10^{-4}$ mol/L, with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte. (A) Positive polarization; (B) negative polarization.

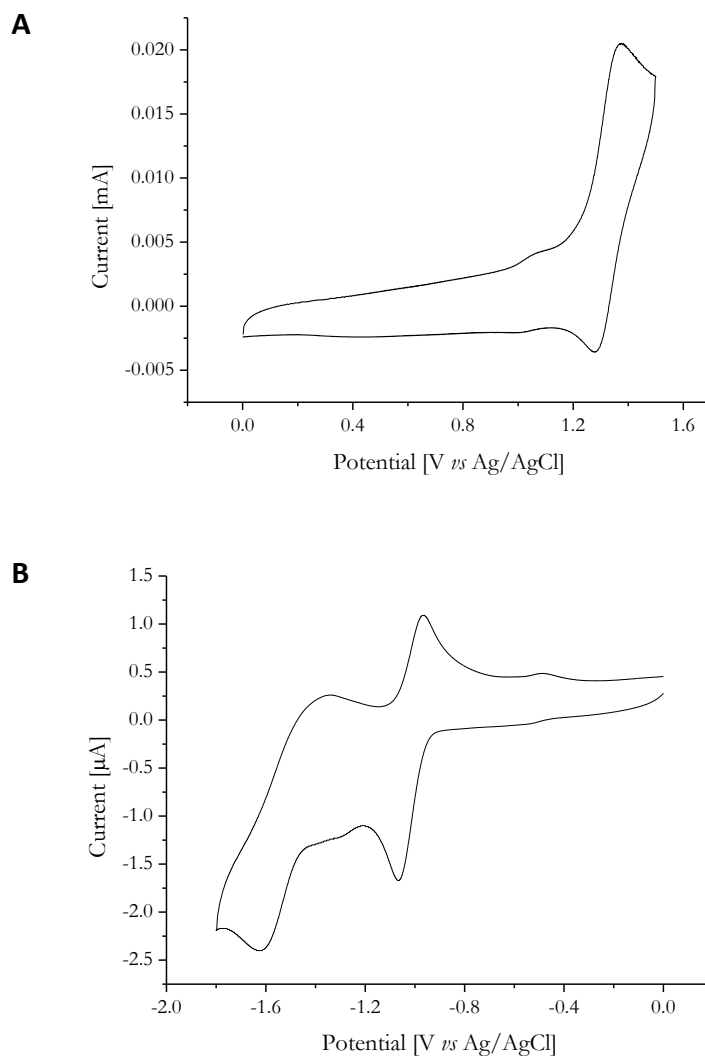


Figure 6.10. Cyclic voltammograms of **Ir-DPACF₂** recorded in dry CH₃CN or DMF under nitrogen, with a sweep rate of 0.15 V/s, at room temperature. The concentration of the complexes is 8.10⁻⁴ mol/L, with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte. (A) Positive polarization; (B) negative polarization.

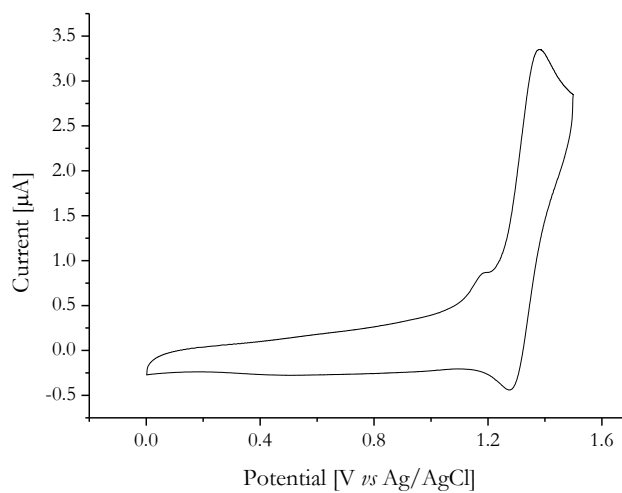
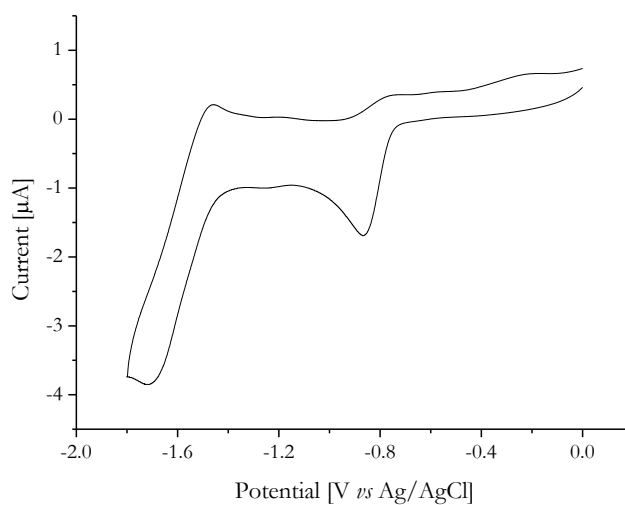
A**B**

Figure 6.11. Cyclic voltammograms of **Ir-DPACF₄** recorded in dry CH₃CN or DMF under nitrogen, with a sweep rate of 0.15 V/s, at room temperature. The concentration of the complexes is 8.10⁻⁴ mol/L, with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte. (A) Positive polarization; (B) negative polarization.

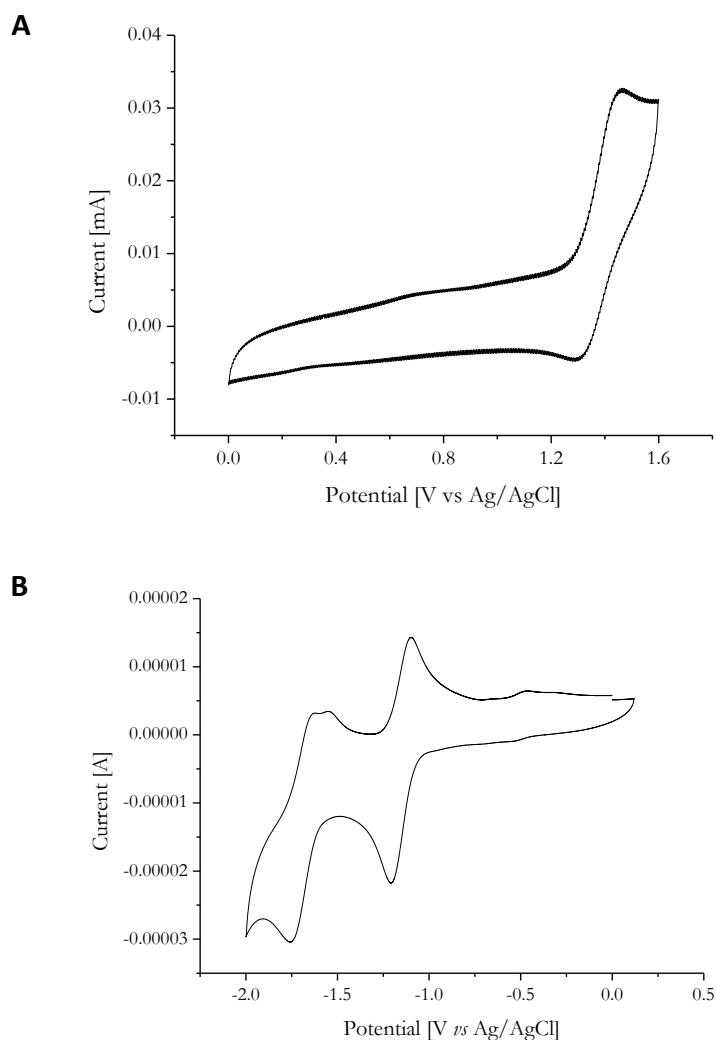


Figure 6.12. Cyclic voltammograms of **Ir-NPP** recorded in dry CH_3CN or DMF under nitrogen, with a sweep rate of 0.15 V/s, at room temperature. The concentration of the complexes is $8 \cdot 10^{-4}$ mol/L, with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte. (A) Positive polarization; (B) negative polarization.

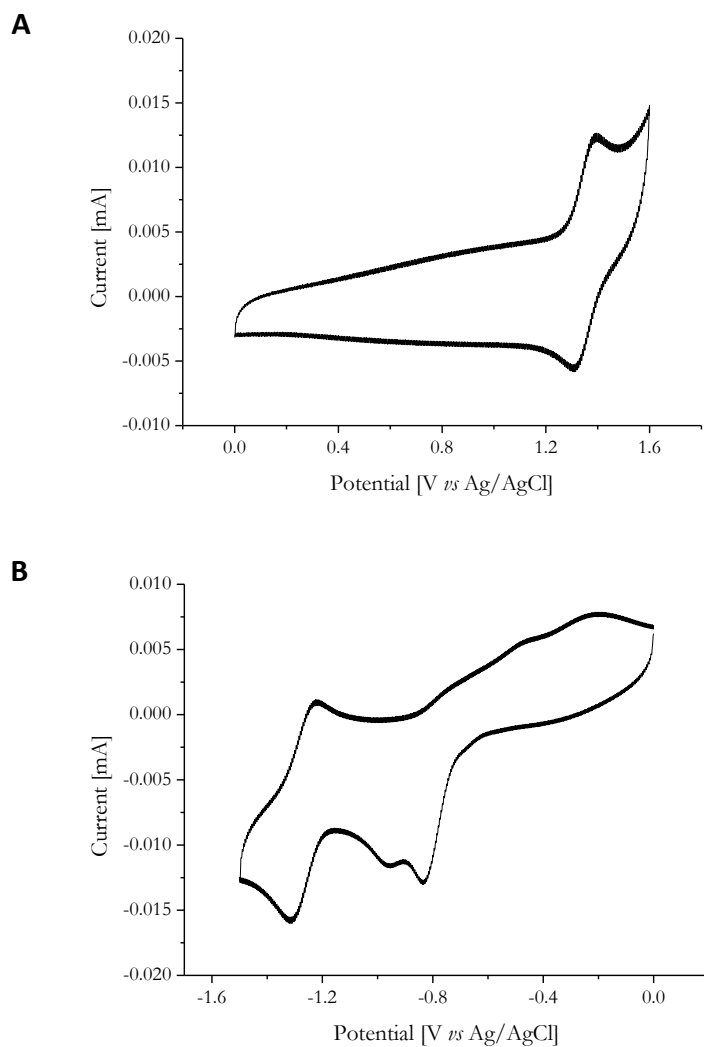


Figure 6.13. Cyclic voltammograms of **Ir-BDPPZ** recorded in dry CH₃CN or DMF under nitrogen, with a sweep rate of 0.15 V/s, at room temperature. The concentration of the complexes is 8.10⁻⁴ mol/L, with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte. (A) Positive polarization; (B) negative polarization.

3.2.3. Absorption

The absorption spectra under ambient air conditions in acetonitrile for the three complexes are depicted on Figure 6.14. Table 6.2 gathers the corresponding data, as well as data of other complexes for comparison purpose. As mentioned earlier in this chapter, different types of electronic transitions can be observed upon UV-visible light irradiation for Ir(III) complexes. Comparison with absorption spectra of the free ligands and literature data allowed to assign the intense absorption bands in the UV region to ligand centered transitions. Bands in the visible region probably arise from a mixture of $^1\text{MLCT}$ transitions $\text{Ir} \rightarrow \text{L}$, where L is the extended planar ligand and $^1\text{LLCT}$ transitions $\text{ppy} \rightarrow \text{L}$.

Complex	Absorbance λ_{max} [nm] ^a	
	CH ₃ CN	H ₂ O
$[\text{Ir}(\text{ppy})_2\text{dppz}]^{+253}$	275 (64.9), 334 (13.8), 364 (12.7), 385 (12.7), 468 (0.85)	-
Ir-DPAC	254 (45.5), 283 (63.2), 353 (10.7), 375 (9.48), 417 (5.06)	254, 283, 354, 375, 418
Ir-DPACF₂	252 (47.5), 275 (64.6), 348 (10.7), 371 (9.62), 412 (5.48)	252, 275, 348, 371, 412
Ir-DPACF₄	253 (48.1), 273 (63.6), 354 (11.2), 374 (5.50)	253, 273, 354, 374
Ir-NPP	264 (57.7), 302 (64.1), 362 (12.8), 389 (12.6), 408 (5.27)	264, 302, 352, 389, 408
Ir-BDPPZ	250 (45.1), 267 (52.4), 296 (65.5), 391 (19.2), 409 (24.9)	250, 267, 296, 391, 409

Table 6.2. Absorption data in CH₃CN and H₂O at 298K in aerated solution for complexes **Ir-DPAC**, **Ir-DPACF₂**, **Ir-DPACF₄**, **Ir-NPP** and **Ir-BDPPZ**. ^a Measurements made with solutions 1.10^{-5} mol.L⁻¹ in complex at room temperature.

Minor changes occur upon fluorination of the dpac ligand, indicating a possible low contribution of these ligands to the absorption spectra.

However, through comparison with **Ir-NPP** and **Ir-BDPPZ** and data from the literature,^{253,254} it is reasonable to assign the most bathochromic bands to a mixture of ¹MLCT and ¹LLCT transitions. These transitions are called MLLCT (Metal-Ligand to Ligand Charge Transfer), consequently to the suggested contribution of an Ir-ppy fragment to the HOMO.^{249,265} These transitions are called MLLCT (Metal-Ligand to Ligand Charge transfer). Our data suggest the LUMO of all our complexes is localized on a π^* orbital of the extended planar ligand due to the bathochromic shift observed for the more electron-withdrawing cores, lying in agreement with the electrochemical data.

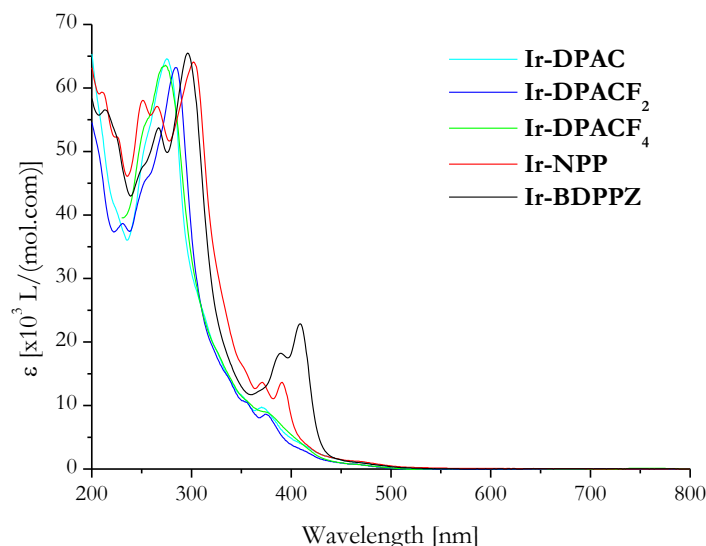


Figure 6.14. Absorption spectra of complexes **Ir-DPAC**, **Ir-DPACF₂**, **Ir-DPACF₄**, **Ir-NPP** and **Ir-BDPPZ** in acetonitrile under ambient air conditions.

3.2.4. Emission

All our complexes display broad unstructured emission (Figure 6.15), typical of a Charge transfer (CT)-type excited state (*i.e.* $^3\text{MLLCT}$, *vide infra*) for Ir(III) complexes, in organic solvent. Interestingly, only **Ir-NPP** emits in water. The luminescence lifetimes and the quantum yield of luminescence for each complex are measured in acetonitrile and in water for **Ir-NPP** under inert atmosphere (Table 6.3). The large k_r values ($> 10^4 \text{ s}^{-1}$) and the large hypsochromic shift of the emission band at 77 K (Figure 6.15) support the CT character of the excited state. In air-equilibrated solvents, a decrease in the luminescence intensity with respect to degassed solutions is observed, indicating a possible photosensitization of $^3\text{O}_2$ by the excited complexes (data not shown). Because all of our experiments have been carried out under the same conditions, the influence of oxygen is kept constant and is therefore not further investigated.

A small bathochromic shift of the luminescence is observed while increasing the number of fluorine atoms onto the dpac ligand (*i.e.* going from **Ir-DPAC** to **Ir-DPACF₄**), indicating the LUMO of these systems is a π^* orbital centered on the dpac moiety. Similar observations are made going from **Ir-NPP** to **Ir-BDPPZ**. Note the ancillary ppy ligands being much more electron-rich, their π^* orbital lies at higher energy.

Unlike $[\text{Ir}(\text{ppy})_2\text{dppz}]^+$, our five Ir(III) complexes show solvent-dependent emission data. The quenching of the luminescence in water has been attributed for $[\text{Ir}(\text{ppy})_2\text{dppz}]^+$ to the presence of hydrogen bonding interactions between the phenazine nitrogen atoms and water molecules.²⁵³ Similar interactions between the nitrogen atoms of the acridine or phenazine moiety and water molecules could lie at the basis of the inhibition of the luminescence for **Ir-DPAC**, **Ir-DPACF₂**, **Ir-DPACF₄** and **Ir-BDPPZ**. In that case, the low but still present emission of **Ir-NPP** in water may arise from the prevention of the Hydrogen bond to the nitrogen atom of the acridine due to the “elbow-shaped” structure of the npp ligand. This, together with the emission

data of the four other complexes, suggests only one hydrogen bond is required in the excited state to quench the luminescence of this series of Ir(III) complexes, while two are required for $[\text{Ru}(\text{phen/bpy})_2\text{dppz}]^{2+}$.^{113,116,144} This is supported by the luminescence lifetime and quantum yield data of **Ir-NPP** in water. Note the shorter component of the excited state lifetime could arise from self-aggregation of the complex in aqueous medium due to its low solubility.

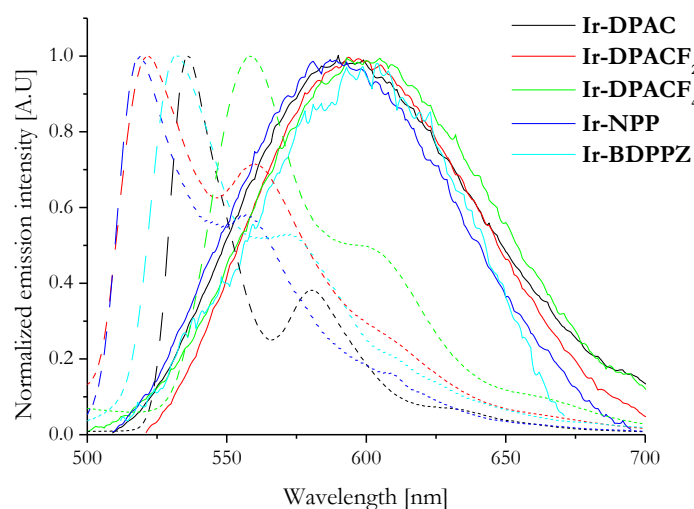


Figure 6.15. Emission spectra of complexes **Ir-DPAC** ($\lambda_{\text{exc}} = 417$ nm), **Ir-DPACF₂** ($\lambda_{\text{exc}} = 412$ nm), **Ir-DPACF₄** ($\lambda_{\text{exc}} = 374$ nm), **Ir-NPP** ($\lambda_{\text{exc}} = 408$ nm) and **Ir-BDPPZ** ($\lambda_{\text{exc}} = 409$ nm) in acetonitrile under ambient air conditions (solid lines) and at 77 K in deaerated rigid glassy matrix EtOH/MeOH 4/1 (dashed lines)

Complex	Emission									
	Emission λ_{max} [nm] ^a		λ_{max} at 77 K [nm]		Φ_{em} ^{a, b}		τ_{em} [ns]		k_r [10^3 s ⁻¹]	
	CH ₃ CN	H ₂ O	EtOH/ MeOH 4/1		CH ₃ CN	H ₂ O	CH ₃ CN	H ₂ O	CH ₃ CN	H ₂ O
[Ir(ppy) ₂ dppz] ⁺	630	- ^c	544		0.002	- ^c	77	- ^c	29.9	- ^c
Ir-DPAC	594	- ^c	536		0.039	- ^c	742	- ^c	52.6	- ^c
Ir-DPACF₂	597	- ^c	521		0.044	- ^c	347	- ^c	127	- ^c
Ir-DPACF₄	601	- ^c	558		0.009	- ^c	550	- ^c	16.4	- ^c
Ir-NPP	589	593	518		0.102	0.010	503	61.5 (+25% 7 ns)	203	163
Ir-BDPPZ	599	- ^c	531		0.019	- ^c	208.6	- ^c	91.1	- ^c

Table 6.3. Emission data in CH₃CN and H₂O at 298 K and 77 K in aerated or deaerated solution for complexes **Ir-DPAC** (λ_{exc} = 417 nm), **Ir-DPACF₂** (λ_{exc} = 412 nm), **Ir-DPACF₄** (λ_{exc} = 374 nm), **Ir-NPP** (λ_{exc} = 408 nm) and **Ir-BDPPZ** (λ_{exc} = 409 nm). ^a Measurements made with solutions 1.10⁻⁵ mol.L⁻¹ in complex. ^b Measured relative to [Ru(bpy)₃]²⁺ in an aerated aqueous solution (Φ_{em}^{air} = 0.028). ^c Non emissive.

Our data indicate the five complexes follow a similar photophysical scheme (Figure 6.16): (i) in aprotic medium, after an ultra-fast ISC^{159,160} from ¹MLLCT states generated upon absorption of light, the excited electrons shift towards ³MLLCT states, corresponding to a charge transfer between an Ir-ppy fragment and the extended planar ligand. The similar energies of these emissive states explain the similarity of the luminescence maxima observed for all complexes. (ii) In protic solvent, we assume, through comparison with TD-DFT and experimental data from literature for similar complexes,^{116,144,154,253,225} that upon absorption of light by complexes **Ir-DPAC**, **Ir-DPACF₂**, **Ir-DPACF₄** and **Ir-BDPPZ**, a mixture of ¹MLLCT is formed which rapidly undergoes an ISC to a luminescent ³MLLCT excited state, like it is the case in aprotic solvent. The presence of the electron delocalized on the dpac/bdppz ligand in this excited ³MLLCT state favors the formation of an hydrogen bond between solvent molecules and the free nitrogen from the extended planar ligand (depicted as dpac-H/bdppz-H, in Figure 6.16). The formation of this hydrogen bond in the excited state further stabilizes the ³MLLCT state. This stabilization and the presence of the Hydrogen bond is suggested to account for the luminescence quenching of these complexes in water. (iii) In opposition to the former ones, **Ir-NPP** exhibit solely partial luminescence quenching in protic medium. The same photophysical scheme in protic than in aprotic solvent is thus suggested as the formation of the only possible hydrogen bond in the excited state is disfavored by the structure of the npp ligand, as supported by luminescence lifetime data. Similar photophysical differences between dpac derivatives and “elbow-shaped” npp and bdppz ligands have already been observed in the literature for Ru(II) complexes.²²⁵

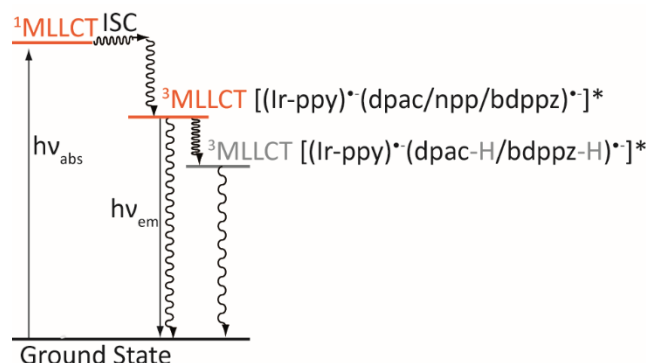


Figure 6.16. Photophysical scheme for complexes **Ir-DPAC**, **Ir-DPACF₂**, **Ir-DPACF₄**, **Ir-NPP** and **Ir-BDPPZ**. In aprotic medium, all complexes emit from a ³MLCT excited state involving the extended planar ligand. This state is stabilized in protic medium through the formation of a hydrogen bond, leading to a quenching of the luminescence for **Ir-DPAC**, **Ir-DPACF₂**, **Ir-DPACF₄** and **Ir-BDPPZ**. Solid arrows stand for radiative transitions and wedged arrows for non-radiative transitions.

3.2.5. Photophysical studies in the presence of DNA

DNA binding of transition metal complexes received much attention for the last decades. In particular, complexes containing phenazine- or pyrazine-based intercalating ligands have been the focus of many studies. While mainly Ru(II),^{42,144,206} Os(II)²⁶⁸ and Rh(III)⁴⁸ derivatives have been extensively studied, Ir(III) analogs have been relatively unexplored.^{253,254,269} Previous studies from our group show Ru(II) complexes bearing acridine cores display suitable optical properties for applications as DNA photoprobes.^{225,263}

Consequently, the behavior of our five Ir(III) complexes in the presence of DNA has been investigated. Surprisingly, all complexes turned out to be insoluble in aqueous buffer (TRIS.HCl 50 mM, NaCl 50 mM or TRIS.HCl 5 mM, NaCl 1 mM, pH 7.5). The presence of salts, and thus increased ionic strength with respect to pure water, seems to lead to self-aggregation of the complexes. Such behavior has already been observed for similar complexes, due to their low formal charge.^{253,265} As done by Lo et al.,²⁵³ 10% by volume of CH₃CN was

added to buffer solutions of complexes and polynucleotides in order to increase their solubility without leading to DNA denaturation. In that case, quantification of the interaction between the two partners was made possible thanks to residual luminescence of the complexes in the absence of polynucleotides. Strong luminescence increase is observed for all complexes upon addition of SS-DNA (Salmon Sperm DNA, Figure 6.17). As mentioned above, we suggest (partial) luminescence quenching occurs through Hydrogen bond formation in the excited state between the nitrogen atom of the acridine or phenazine moiety and water molecules for the five complexes. Upon intercalation of the extended ligand into the DNA base pairs stack,^{23,144} these are prevented, thus restoring the emission of the complexes. Such a “light switch” behavior has already been reported for dppz-based Ru(II) complexes.^{46,144} Our results highlights the potential of Ir(III) complexes as DNA photoprobes and preparation of buffer-soluble analogs are currently under investigation in our group.

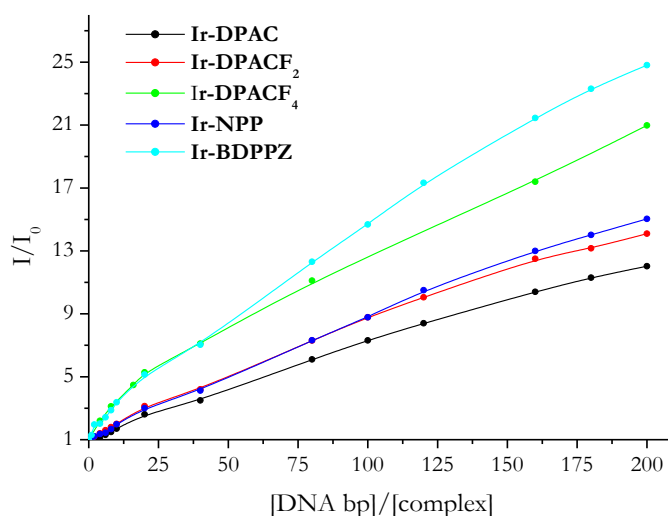


Figure 6.17. Steady-state luminescence titrations for **Ir-DPAC** ($\lambda_{\text{exc}} = 417$ nm), **Ir-DPACF₂** ($\lambda_{\text{exc}} = 412$ nm), **Ir-DPACF₄** ($\lambda_{\text{exc}} = 374$ nm), **Ir-NPP** ($\lambda_{\text{exc}} = 408$ nm) and **Ir-BDPPZ** ($\lambda_{\text{exc}} = 409$ nm) with increasing concentrations of SS-DNA.

Measurements performed using 1 μM of complex in TRIS.HCl 50 mM, NaCl 50 mM pH 7.4/CH₃CN 9/1 under ambient air conditions.

3.3. Conclusions

Five new Ir(III) complexes have been successfully synthesized and characterized. ^1H NMR and HRMS data confirms the existence of these Ir(III) complexes as salts.

Electrochemistry experiments show our complexes undergo Ir-ppy-based oxidation, whatever the nature of the extended planar ligand. The first two reduction waves clearly arise from the reduction of the extended planar ligand, which is much more electron-withdrawing than the ancillary ppy ligands. Indeed, the reduction of the latter takes place at potential < -2 V vs Ag/AgCl, suggesting the LUMO is located on dpac, npp or bdppz (π^* orbital) in the ground state.

Their absorption spectra are dominated by ^1LC transitions in the UV range. The most bathochromic bands are assigned to $^1\text{MLLCT}$ transitions, the π^* orbital of the extended planar ligand being stabilized either by increased conjugation or electron-withdrawing ability.

Emission data indicate the five complexes exhibit luminescence from $^3\text{MLLCT}$ states in acetonitrile, corresponding to a charge transfer between an Ir(III)-ppy fragment and the extended planar ligand. In water, further relaxation occurs on the acridine/phenazine moiety of the extended planar ligand to a non-emissive mono-hydrogen bonded excited state (dpac-H/bdppz-H) for **Ir-DPAC**, **Ir-DPACF₂**, **Ir-DPACF₄** and **Ir-BDPPZ**. A non-luminescent low-lying bdppz-H₂ excited state, where a second Hydrogen bond occurs for **Ir-BDPPZ** in the excited state, could exist but its formation is disfavored due to the “elbow shape” of the ligand. In sharp contrast to the former ones, **Ir-NPP** exhibit only partial luminescence quenching in aqueous medium. The same photophysical scheme in water than in CH₃CN is thus suggested as the formation of the only possible Hydrogen bond in the excited state is disfavored by the “elbow-shaped” structure of the npp ligand.

While the five Ir(III) complexes turned out to be insoluble in aqueous buffer, addition of 10% CH₃CN allows monitoring and quantification

of the interaction between the complexes with increasing concentrations of DNA. Our complexes display strong luminescence enhancement upon addition of DNA, probably due to the protection of the complexes from non-radiative deactivation pathways such as oxygen quenching and vibrational relaxations. The formation of Hydrogen bond in the excited state between the nitrogen atom of the acridine or phenazine moiety and water molecules for **Ir-DPAC**, **Ir-DPACF₂**, **Ir-DPACF₄** and **Ir-BDPPZ** could explain the luminescence quenching for the free complexes. Our results emphasized the role of Ir(III) complexes as DNA targeting agents and give photochemical insights on poorly described acridine-based Ir(III) complexes.

3.4. Experimental section

3.4.1. *Materials and instrumentation*

Dpac,²²⁵ dpacF₂,²²⁵ dpacF₄,²²⁵ npp²⁶³ and bdppz²⁶³ have been synthesized according to previously described literature protocols. All solvents and reagents for the synthesis are of reagent grade and are used without any further purification. All solvents for the spectroscopic and electrochemical measurements are of spectroscopic grade. Water is purified with a Millipore Milli-Q system. Salmon sperm DNA (SS-DNA) was purchased from Sigma-Aldrich. ¹H NMR experiments are performed in CDCl₃, CD₃OD or CD₃CN on a Bruker AC-300 Avance II (300 MHz) or on a Bruker AM-500 (500 MHz) at 20°C. The chemical shifts (given in ppm) are measured vs the residual peak of the solvent as the internal standard. High-resolution mass spectrometry (HRMS) spectra are recorded on a Q-Exactive orbitrap from ThermoFisher using reserpine as internal standard. Samples are ionized by electrospray ionization (ESI; capillary temperature = 320°C, vaporizer temperature = 320°C, sheath gas flow rate = 5 mL/min). UV-vis absorption spectra are recorded on a Shimadzu UV-1700. Room temperature fluorescence spectra are recorded on a Varian Cary Eclipse instrument. Luminescence intensity at 77 K is recorded on a FluoroLog3 FL3-22 from Jobin Yvon equipped with an 18V 450W Xenon Short Arc lamp and an R928P photomultiplier, using an Oxford Instrument Optistat DN nitrogen cryostat controlled by an Oxford Intelligent Temperature Controller (ITC503S) instrument. Luminescence lifetimes measurements were performed after irradiation at $\lambda = 400$ nm obtained by the second harmonic of a Titanium:Sapphire laser (picosecond Tsunami laser spectra physics 3950-M1BB+39868-03 pulse picker doubler) at a 80 kHz repetition rate. Fluotime 200 from AMS technologies was used for the decay acquisition. It consists of a GaAs microchannel plate photomultiplier tube (Hamamatsu model R3809U-50) followed by a time-correlated single photon counting system from Picoquant (PicoHarp300). The ultimate time resolution of the system is close to 30 ps. Luminescence

decays were analyzed with FLUOFIT software available from Picoquant. Cyclic voltammetry is carried out in a one-compartment cell, using a glassy carbon disk working electrode (approximate area = 0.03 cm²), a platinum wire counter electrode, and an Ag/AgCl reference electrode. The potential of the working electrode is controlled by an Autolab PGSTAT 100 potentiostat through a PC interface. The cyclic voltammograms are recorded with a sweep rate of 150 mV s⁻¹, in dried acetonitrile or N,N-dimethylformamide (Sigma-Aldrich, HPLC grade). The concentration of the complexes is 8.10⁻⁴ mol/L, with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte. Before each measurement, the samples are purged by nitrogen.

3.4.2. Synthetic procedures and characterization of Ir(III) complexes

[Ir(ppy)₂Cl]₂. One equivalent of (NH₄)₃IrCl₆ (40.0 mg, 0.0838 mmol) was mixed with 2.4 equivalents of 2-phenylpyridine (31.2 mg, 0.029 mL, 0.201 mmol) in a mixture of 2-ethoxyethanol/water (4.0 mL, 3/1 by volume). The reaction mixture was stirred at reflux (135°C) under argon. After 30 hours, the reaction mixture was cooled at room temperature and a yellow precipitate is formed. After centrifugation, the solid is washed two times with ethanol and two times with acetone. The residue is dissolved with dichloromethane and the mixture is centrifuged. The solid is washed three times with dichloromethane and the supernatant is each time placed in a round bottom flask. The solvent is removed under reduced pressure and the yellow precipitate formed is then dried under vacuum. ¹H NMR (500 MHz, CD₃CN): δ (ppm) 9.23 (dd, 4H, H₂, J₂₋₃ = 5.8 Hz, J₂₋₄ = 0.8 Hz), 7.85 (d, 4H, H₅, J₅₋₄ = 7.9 Hz), 7.72 (dd, 4H, H₄, J_{4-3/5} = 7.5 Hz, J₄₋₂ = 1.5 Hz), 7.47 (dd, 4H, H₆, J₆₋₇ = 7.8 Hz, J₆₋₈ = 1.2 Hz), 6.75 (m, 8H, H₃ and H₇, J₃₋₅ = 1.5 Hz, J_{7-6/8} = 7.4 Hz, J₇₋₉ = 1.1 Hz), 6.55 (dd, 4H, H₈, J₇₋₈ = 7.2 Hz, J₇₋₉ = 1.1 Hz), 5.92 (dd, 4H, H₉, J₉₋₈ = 7.8 Hz, J₉₋₇ = 0.8 Hz).

H₅, H_{5'}, J₅₋₄ = J_{5'-4'} = 5.9 Hz), 7.10 (dd, 2H, H₈, H_{8'}, J_{8-7/9} = J_{8'-7'/9'} = 7.8 Hz), 6.98 (ddd, 2H, H₇, H_{7'}, J_{7-6/8} = J_{7'-6'/8'} = 7.6 Hz), 6.90 (m, 2H, H₄, H_{4'}), 6.40 (d, 2H, H₆, H_{6'}, J₆₋₇ = J_{6'-7'} = 7.5 Hz). HRMS (ESI, +p): calc. 782.18902 for [C₄₁H₂₇N₅Ir]⁺, found 782.18843 ([M-PF₆]⁺).

$[Ir(ppy)_2dpacF_2]^+$. One equivalent of dichloro-bridged precursor $[Ir(ppy)_2Cl]_2$ (100.0 mg, 0.0932 mmol) was mixed with 2.5 equivalents of dpacF₂ (73.9 mg, 0.2330 mmol) in a mixture of acetonitrile/ethanol (15.0 mL, 4/1 v/v). The reaction mixture was stirred at reflux (80°C) under argon and followed by absorption spectroscopy. After 22 hours, the reaction mixture was cooled at room temperature. Addition of small portions of NH₄PF₆ and drops of water yielded an orange precipitate. After centrifugation, the solid is washed with excess water. After drying under vacuum, the residue is obtained pure. The counter-anion exchange from PF₆⁻ to Cl⁻ is performed by eluting a solution of complex in a mixture CH₃CN/H₂O on Sephadex DEAE A25. ¹H NMR (PF₆ salt, 500 MHz, CD₃CN): δ (ppm) 9.74 (dd, 2H, H_d, H_i, J_{i-k} = 1.5 Hz), 9.30 (dd, 1H, H_c, J_{c-b} = 8.5 Hz, J_{c-a} = 1.0 Hz), 8.36 (dd, 1H, H_k, J_{k-j} = 5.1 Hz, J_{k-i} = 1.5 Hz), 8.31 (dd, 1H, H_a, J_{a-b} = 5.1 Hz, J_{a-c} = 1.1 Hz), 8.19 (dd, 1H, H_e, J_{e-f} = 11.3 Hz, J_{e-g} = 7.8 Hz), 8.13 (dd, 1H, H_h, J_{h-g} = 11.3 Hz, J_{h-f} = 7.7 Hz), 8.09 (dd, 2H, H₃, H_{3'}, J₃₋₄ = J_{3'-4'} = 8.1 Hz, J₃₋₂ = J_{3'-2'} = 4.3 Hz), 7.94 (2dd, 2H, H_b, H_j, J_{b-c} = J_{j-i} = 8.1 Hz, J_{b-a} = J_{j-k} = 5.1 Hz), 7.86 (m, 2H, H₉, H_{9'}), 7.80 (m, 2H, H₂, H_{2'}), 7.61 (dd, 1H, H_{5'}, J_{5'-4'} = 5.3 Hz), 7.57 (dd, 1H, H₅, J₅₋₄ = 5.3 Hz), 7.10 (dd, 2H, H₈, H_{8'}, J_{8-7/9} = J_{8'-7'/9'} = 7.5 Hz), 6.98 (ddd, 2H, H₇, H_{7'}, J_{7-6/8} = J_{7'-6'/8'} = 7.5 Hz, J₇₋₉ = J_{7'-9'} = 1.2 Hz), 6.90 (m, 2H, H₄, H_{4'}), 6.39 (d, 2H, H₆, H_{6'}, J₆₋₇ = J_{6'-7'} = 7.6 Hz). HRMS (ESI, +p): calc. 818.17018 for [C₄₁H₂₅N₅F₂Ir]⁺, found 818.16908 ([M-PF₆]⁺).

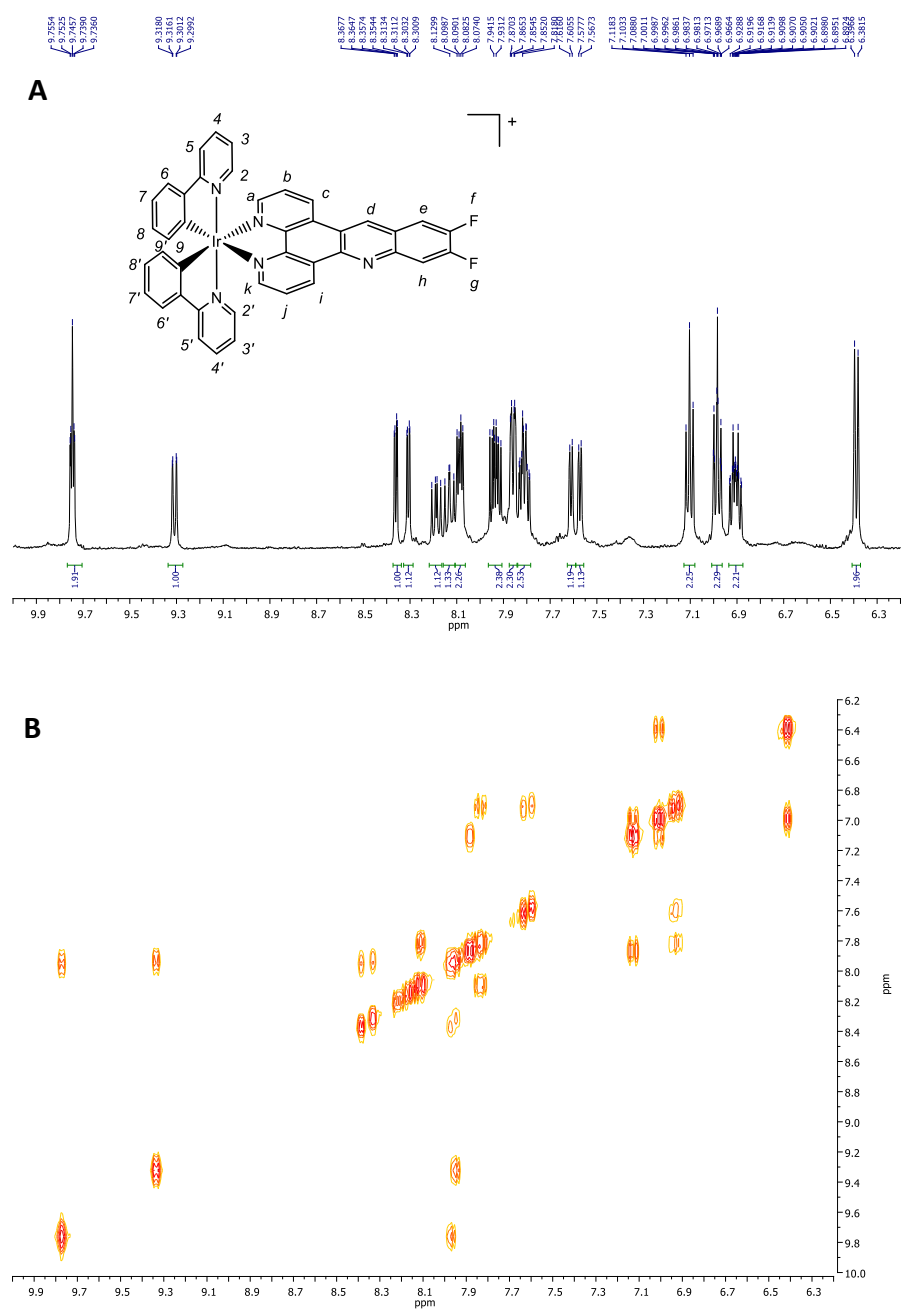


Figure 6.20. (A) 1D and (B) 2D ^1H - ^1H NMR spectra (500 MHz, CD_3CN) of **Ir-dpacF₂**.

$[Ir(ppy)_2dpacF_4]^+$. One equivalent of dichloro-bridged precursor $[Ir(ppy)_2Cl]_2$ (100.0 mg, 0.0932 mmol) was mixed with 2.5 equivalents of dpacF₄ (82.3 mg, 0.2330 mmol) in a mixture of acetonitrile/ethanol (15.0 mL, 4/1 v/v). The reaction mixture was stirred at reflux (80°C) under argon and followed by absorption spectroscopy. After 22 hours, the reaction mixture was cooled at room temperature. Addition of small portions of NH₄PF₆ and drops of water yielded an orange precipitate. After centrifugation, the solid is washed with excess water. After drying under vacuum, the residue is purified by column of chromatography (silica, eluent: CH₂Cl₂/CH₃OH 9/1, R_f = 0.62). The counter-anion exchange from PF₆⁻ to Cl⁻ is performed by eluting a solution of complex in a mixture CH₃CN/H₂O on Sephadex DEAE A25. ¹H NMR (PF₆ salt, 500 MHz, CD₃CN): δ (ppm) 9.95 (s, 1H, H_c), 9.80 (dd, 1H, H_i, J_{i-j} = 8.3 Hz, J_{i-k} = 1.5 Hz), 9.45 (dd, 1H, H_c, J_{c-b} = 8.5 Hz, J_{c-a} = 1.2 Hz), 8.40 (dd, 1H, H_k, J_{k-j} = 5.1 Hz, J_{k-i} = 1.5 Hz), 8.34 (dd, 1H, H_a, J_{a-b} = 5.1 Hz, J_{a-c} = 1.5 Hz), 8.09 (m, 2H, H₃, H_{3'}), 7.96 (2dd, 2H, H_b, H_j, J_{b-c} = J_{j-i} = 8.4 Hz, J_{b-a} = J_{j-k} = 5.1 Hz), 7.83 (m, 4H, H₂, H_{2'}, H₉, H_{9'}), 7.61 (dd, 1H, H_{5'}, J_{5'-4'} = 5.3 Hz, J_{5'-2'} = 0.8 Hz), 7.57 (dd, 1H, H₅, J₅₋₄ = 5.3 Hz, J₅₋₂ = 1.2 Hz), 7.10 (dd, 2H, H₈, H_{8'}, J_{8-7/9} = J_{8'-7'/9'} = 8.5 Hz), 6.97 (ddd, 2H, H₇, H_{7'}, J_{7-6/8} = J_{7'-6'/8'} = 7.5 Hz, J₇₋₉ = J_{7'-9'} = 1.5 Hz), 6.90 (m, 2H, H₄, H_{4'}), 6.38 (d, 2H, H₆, H_{6'}, J₆₋₇ = J_{6'-7'} = 7.5 Hz). HRMS (ESI, +p): calc. 854.15133 for [C₄₁H₂₃N₅F₄Ir]⁺, found 854.15123 ([M-PF₆]⁺).

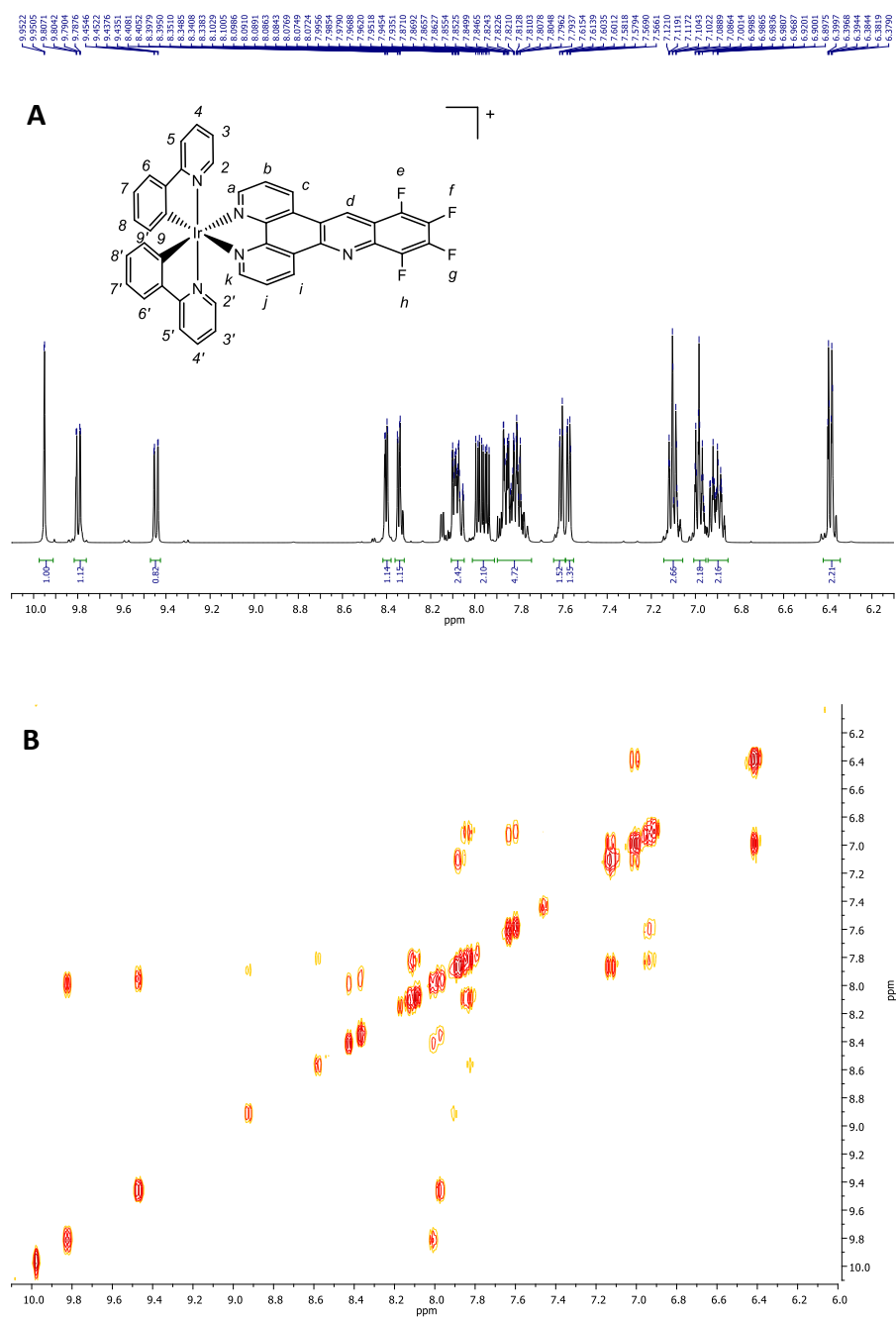


Figure 6.21. (A) 1D and (B) 2D ^1H - ^1H NMR spectra (500 MHz, CD_3CN) of **Ir-dpacF4**.

$[Ir(ppy)_2npp]^+$. One equivalent of dichloro-bridged precursor $[Ir(ppy)_2Cl]_2$ (50.0 mg, 0.0466 mmol) was mixed with 2.2 equivalents of npp (34.0 mg, 0.1025 mmol) in ethylene glycol (5.0 mL). The reaction mixture was stirred at 150°C under argon and followed by absorption spectroscopy. After 20 hours, the reaction mixture was cooled at room temperature. Addition of small portions of NH_4PF_6 and drops of water yielded an orange precipitate. After centrifugation, the solid is washed with excess diethylether. After drying under vacuum, the residue is purified by column of chromatography (silica, eluent: CH_2Cl_2/CH_3OH 9/1, $R_f = 0.32$). The counter-anion exchange from PF_6^- to Cl^- is performed by eluting a solution of complex in a mixture CH_3CN/H_2O on Sephadex DEAE A25. 1H NMR (PF_6^- salt, 500 MHz, CD_3CN): δ (ppm) 10.05 (dd, 1H, H_k , $J_{k-l} = 8.3$ Hz, $J_{k-m} = 1.4$ Hz), 9.67 (s, 1H, H_d), 9.60 (dd, 1H, H_j), 9.35 (dd, 1H, H_c , $J_{c-b} = 7.5$ Hz, $J_{c-a} = 1.0$ Hz), 8.39 (dd, 1H, H_m , $J_{m-l} = 5.1$ Hz, $J_{m-k} = 1.5$ Hz), 8.33 (dd, 1H, H_a , $J_{a-b} = 5.1$ Hz, $J_{a-c} = 1.5$ Hz), 8.07 (m, 4H, H_3 , $H_{3'}$, H_e , H_g), 7.98 (dd, 1H, H_l , $J_{l-k} = 8.3$ Hz, $J_{l-m} = 5.1$ Hz), 7.94 (dd, 1H, H_b , $J_{b-c} = 8.3$ Hz, $J_{b-a} = 5.1$ Hz), 7.87 (m, 4H, H_2 , H_9 , H_h , H_i), 7.81 (m, 3H, $H_{2'}$, $H_{9'}$, H_f), 7.61 (2dd, 2H, H_5 , $H_{5'}$, $J_{5-4} = J_{5'-4'} = 5.9$ Hz, $J_{5-2} = J_{5'-2'} = 0.7$ Hz), 7.12 (ddd, 2H, H_8 , $H_{8'}$, $J_{8-6} = J_{8'-6'} = 1.2$ Hz), 6.99 (ddd, 2H, H_7 , $H_{7'}$, $J_{7-6/8} = J_{7'-6'/8'} = 7.5$ Hz), 6.91 (m, 2H, H_4 , $H_{4'}$), 6.41 (d, 2H, H_6 , $H_{6'}$, $J_{6-7} = J_{6'-7'} = 7.1$ Hz). HRMS (ESI, +p): calc. 832.20467 for $[C_{45}H_{29}N_5Ir]^+$, found 832.20407 ($[M-PF_6^-]^+$).

$[Ir(ppy)_2bdppz]^+$. One equivalent of dichloro-bridged precursor $[Ir(ppy)_2Cl]_2$ (50.0 mg, 0.0466 mmol) was mixed with 2.2 equivalents of npp (34.1 mg, 0.1025 mmol) in ethylene glycol (5.0 mL). The reaction mixture was stirred at 150°C under argon and followed by absorption spectroscopy. After 20 hours, the reaction mixture was cooled at room temperature. Addition of small portions of NH_4PF_6 and drops of water yielded an orange precipitate. After centrifugation, the solid is washed with excess diethylether. After drying under vacuum, the residue is purified by column of chromatography (silica, eluent: CH_2Cl_2/CH_3OH 9/1, $R_f = 0.37$). The counter-anion exchange from PF_6^- to Cl^- is performed by eluting a solution of complex in a mixture CH_3CN/H_2O on Sephadex DEAE A25. 1H NMR (PF_6^- salt, 500 MHz, CD_3CN): δ (ppm) 9.97 (dd, 1H, H_k , $J_{k-l} = 8.3$ Hz, $J_{k-m} = 1.5$ Hz), 9.75 (dd, 1H, H_c , $J_{c-b} = 8.3$ Hz, $J_{c-a} = 1.5$ Hz), 9.56 (dd, 1H, H_j), 8.42 (dd, 2H, H_a , H_m , $J_{a-b} = J_{m-l} = 5.1$ Hz, $J_{a-c} = J_{m-k} = 1.5$ Hz), 8.35 (dd, 1H, H_e , $J_{e-f} = 9.2$ Hz), 8.16 (dd, 1H, H_f , $J_{f-e} = 9.2$ Hz), 8.14 (m, 1H, H_g), 8.10 (dd, 2H, H_5 , $H_{5'}$, $J_{5-4} = J_{5'-4'} = 8.3$ Hz, $J_{5-3} = J_{5'-3'} = 0.7$ Hz), 8.02 (2dd, 2H, H_b , H_l , $J_{b-c} = J_{l-k} = 8.3$ Hz, $J_{b-a} = J_{l-m} = 5.1$ Hz), 7.93 (m, 2H, H_h , H_i), 7.89 (ddd, 2H, H_9 , $H_{9'}$, $J_{9-8} = 7.9$ Hz), 7.82 (dd, 2H, H_4 , $H_{4'}$, $J_{4-3/5} = J_{4'-3'/5'} = 8.0$ Hz, $J_{4-2} = J_{4'-2'} = 1.5$ Hz), 7.62 (m, 2H, H_2 , $H_{2'}$), 7.12 (ddd, 2H, H_8 , $H_{8'}$), 7.00 (ddd, 2H, H_7 , $H_{7'}$), 6.92 (dd, 2H, H_3 , $H_{3'}$, $J_{3-5} = J_{3'-5'} = 1.3$ Hz), 6.42 (d, 2H, H_6 , $H_{6'}$, $J_{6-7} = J_{6'-7'} = 7.55$ Hz). HRMS (ESI, +p): calc. 833.19992 for $[C_{44}H_{28}N_6Ir]^+$, found 833.19941 ($[M-PF_6^-]^+$).

3.5. Associated content

3.5.1. HRMS data

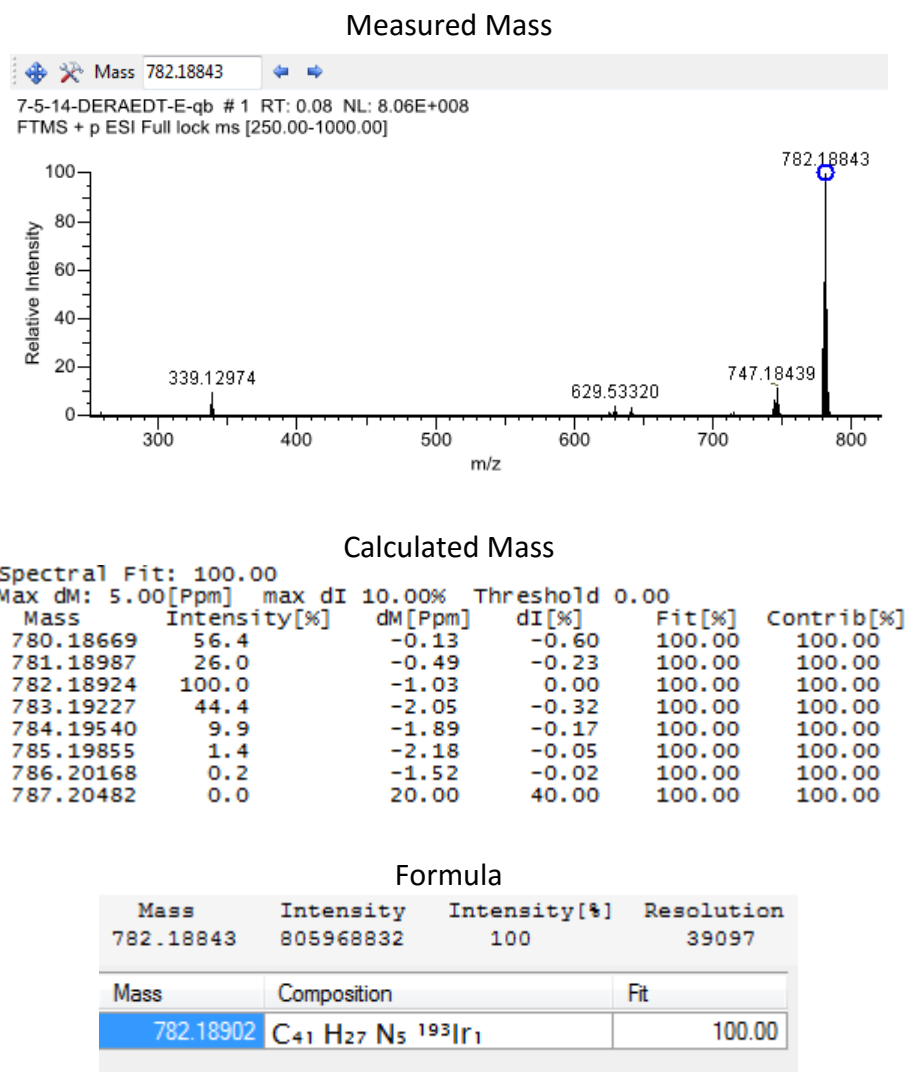


Figure 6.24. HRMS data for **Ir-DPAC** ([M-(PF₆)⁻]⁺).

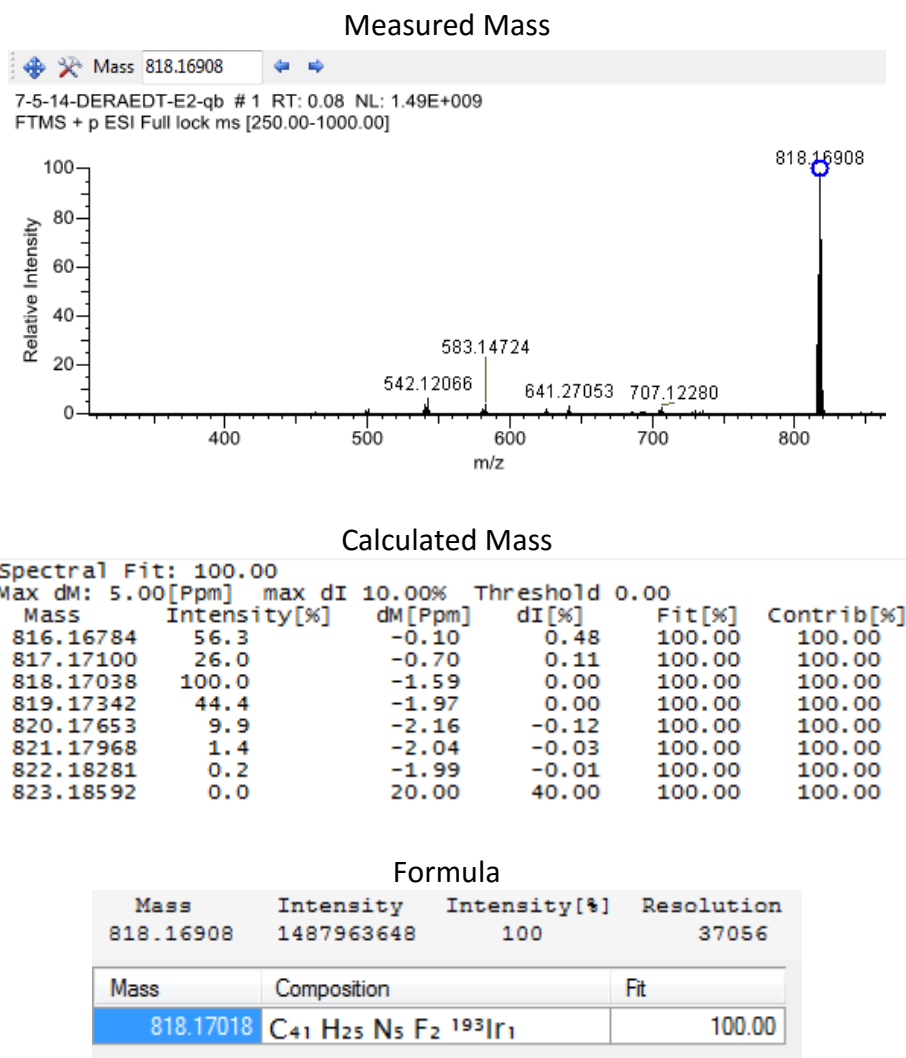
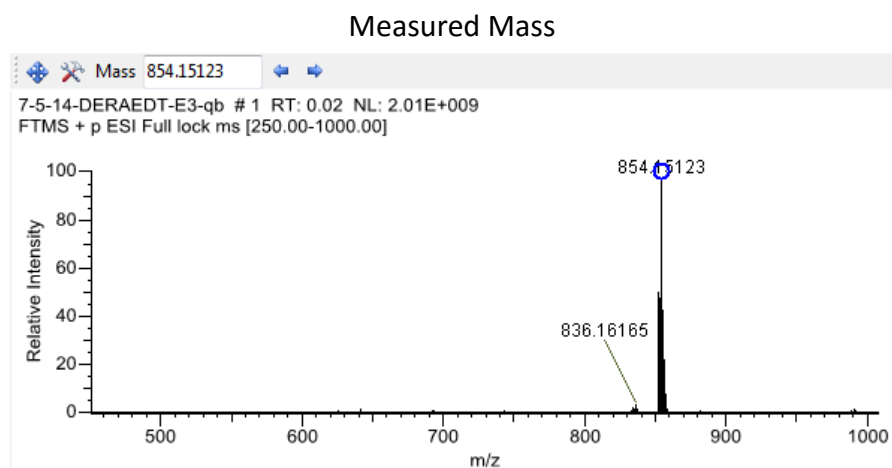


Figure 6.25. HRMS data for Ir-DPACF₂ ([M-(PF₆)⁺]).



Calculated Mass

Spectral Fit: 100.00
Max dM: 5.00[Ppm] max dI 10.00% Threshold 0.00

Mass	Intensity[%]	dM[Ppm]	dI[%]	Fit[%]	Contrib[%]
852.14900	56.3	0.24	-4.82	100.00	100.00
853.15217	25.9	-0.37	-2.37	100.00	100.00
854.15156	100.0	-0.39	0.00	100.00	100.00
855.15456	44.4	-0.78	-0.96	100.00	100.00
856.15768	9.9	4.73	13.00	100.00	100.00
857.16080	1.5	7.74	6.36	100.00	100.00
858.16395	0.2	8.02	1.49	100.00	100.00
859.16705	0.0	8.13	0.23	100.00	100.00

Formula

Mass	Intensity	Intensity[%]	Resolution
854.15123	2009121024	100	36303

Mass	Composition	Fit
854.15133	C ₄₁ H ₂₃ N ₅ F ₄ ¹⁹³ Ir ₁	100.00

Figure 6.26. HRMS data for Ir-DPACF₄ ([M-(PF₆)⁻]⁺).

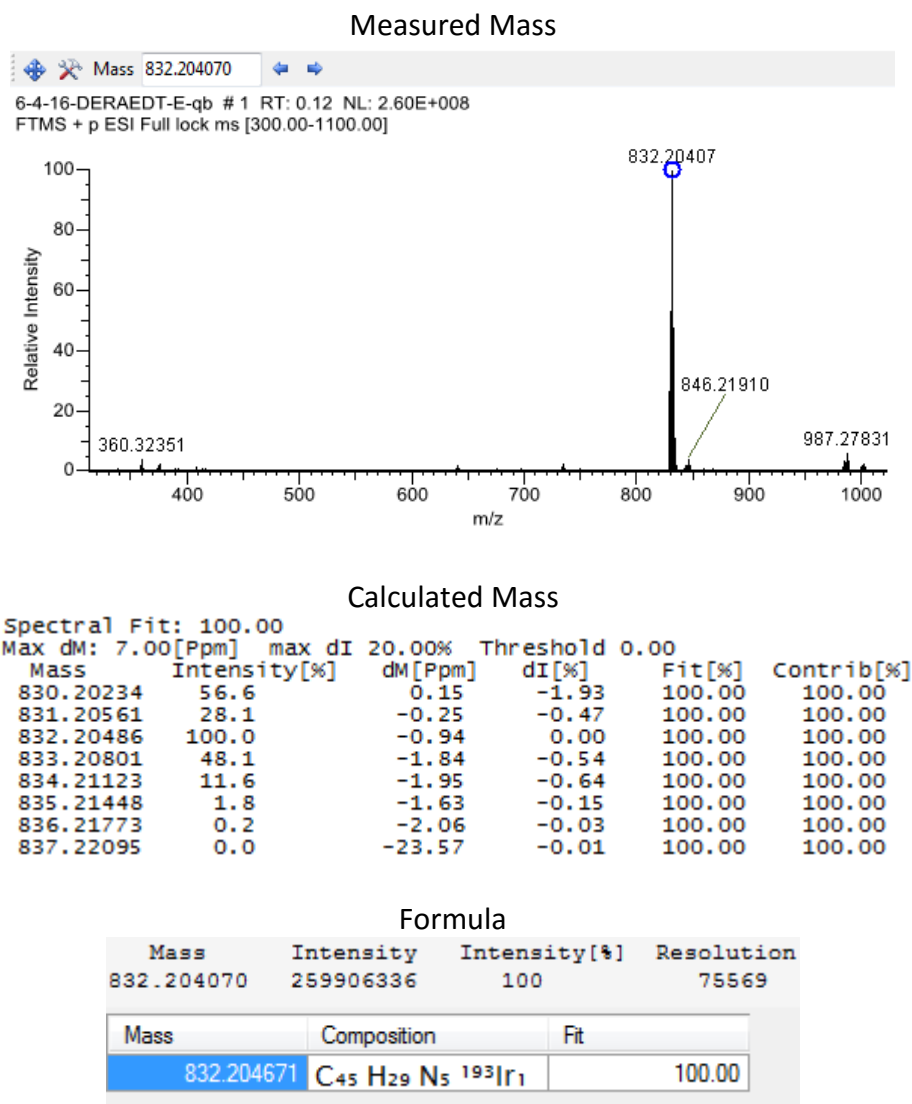
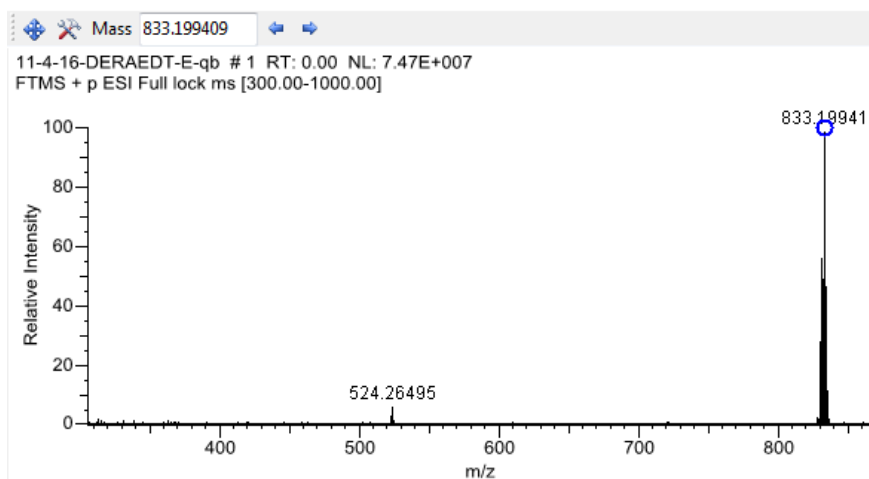


Figure 6.27. HRMS data for **Ir-NPP** ([M-(PF₆)]⁺).

Measured Mass



Calculated Mass

Spectral Fit: 100.00					
Max dM: 7.00[Ppm] max dI 20.00% Threshold 0.01					
Mass	Intensity[%]	dM[Ppm]	dI[%]	Fit[%]	Contrib[%]
831.19759	55.8	-0.25	0.13	100.00	100.00
832.20072	27.7	-0.62	0.04	100.00	100.00
833.20017	100.0	-0.91	0.00	100.00	100.00
834.20313	47.6	-1.83	-0.30	100.00	100.00
835.20622	11.4	-1.16	-0.08	100.00	100.00
836.20935	1.8	-1.16	-0.05	100.00	100.00
837.21245	0.2	-3.37	-0.02	100.00	100.00
838.21556	0.0	-21.65	0.14	100.00	100.00

Formula

Mass	Intensity	Intensity[%]	Resolution
833.199409	74742088	100	37551

Mass	Composition	Fit
833.199920	C ₄₄ H ₂₈ N ₆ ¹⁹³ Ir ₁	100.00

Figure 6.28. HRMS data for **Ir-BDPPZ** ([M-(PF₆)⁻]⁺).

Chapter 7

Conclusions and prospects

1. Conclusions

In this Ph.D. thesis, we were interested in the development of environment-sensitive dyes which are capable of probing DNA duplexes, and more specifically that target mismatched base pairs. We thus aimed to shed some light on the photochemical tuning allowed by the modification of the different components of the transition metal complex, *i.e.* (I) the core and (II) the shape of its DNA binding ligand, (III) the nature of the ancillary ligands, and (IV) by changing the nature of the metal cation. Such modifications were expected to tune the photochemical properties of the resulting complexes, leading to tunable DNA photoprobes.

We successfully synthesized and characterized Ru(II) complexes bearing linear extended planar ligands based on an acridine core. Tuning of the optical properties has been achieved by substituting the terminal atoms from the acridine ligand by fluorine atoms. The photophysical measurements performed have shown strong influence of small chemical modifications of the ligands. Indeed, while **Ru-DPAC** and **Ru-DPACF₂** allow emission from a ³MLCT excited state centered on the ancillary phen ligands or on the phen-portion of the acridine ligand both in organic and aqueous media, the substitution of dpac by four fluorine atoms shifts the electronic population towards a lower-lying poorly emissive ³MLCT excited state centered on the extended portion of the acridine ligand in water. The stabilization of this state compared to the other is believed to occur *via* the formation of Hydrogen bonds between the non-chelating nitrogen atom of the acridine moiety and water molecules from the solvent.

Having established the corresponding photophysical model for these acridine-based complexes, their interaction with matched and single-base mismatched DNA has been investigated. Strong binding of **Ru-DPAC** and its fluorine derivatives is observed, attributed to an intercalation binding mode within the base pairs stack. Their binding to DNA is accompanied by an enhancement of their luminescence,

presumably due to a decrease of the non-radiative deactivation processes of the emissive $^3\text{MLCT}$ excited state of complexes intercalated in the double helix. The inhibition of Hydrogen bonds between **Ru-DPACF₄** and solvent molecules is likely to be responsible for the luminescence enhancement upon intercalation through DNA, as suggested for **Ru-DPPZ**. In the presence of mismatched DNA, **Ru-DPAC** shows differential luminescence intensity for matched and mismatched sequences, but no discrimination for the nature of the mismatch site. A looser fit to mismatched hairpins could lie at the basis of the observed luminescence behaviour of this complex, resulting in an increased exposure to oxygen with respect to the complementary sites.

The results obtained in this chapter give insights on the photophysical properties of acridine-based Ru(II) complexes both in the absence and in the presence of matched and mismatched DNA. In addition, our results support the strong influence of small chemical modifications on the structure of the DNA interacting ligand on the resulting photophysical properties. However, the lack of selectivity for mismatched DNA exhort us to design extended planar ligands allowing better differential interactions between their corresponding Ru(II) complexes and DNA, *i.e.* in our case “elbow-shaped” derivatives.

In that case, the lowest $^3\text{MLCT}$ excited state for **Ru-NPP** is located on the bpy fragment of the npp ligand. In contrast, its phenazine equivalent **Ru-BDPPZ** is poorly luminescent in water, suggesting an excited state localized on the phenazine moiety of the bdppz ligand which is involved in one Hydrogen bond with solvent molecules. The formation of a second Hydrogen bond is prevented by the “elbow-structure” of the bdppz ligand. Again, avid binding to DNA and protection of the excited complexes from non-radiative deactivation pathways upon intercalation into the base pairs stack is observed for the two “elbow-shaped” complexes. In the presence of single-base mismatch-containing hairpins, while **Ru-NPP** is not able to clearly discriminate complementary and mismatched sequences, a strong differential luminescence behavior is shown for **Ru-BDPPZ**. We

suggest a different binding mode of the two complexes to the hairpins accounts for the observed luminescence behavior. Favorable interactions between the phenazine portion of the bdppz ligand with hairpins residues may occur for **Ru-BDPPZ**, leading to deeper packing and consequently to better protection from the environment once inserted into the base pairs stack with respect to **Ru-NPP**. Metallo-insertion binding mode for **Ru-BDPPZ** in mismatched sequences is supported by the correlation observed with the relative thermodynamic stability of the mismatch.

Having demonstrated the high binding affinity of our Ru(II) complexes bearing acridine and phenazine derivatives as extended planar ligands for polynucleotides, we have exploited their luminescence and DNA binding properties to develop their photoreactive analogues by using strong π -deficient ancillary ligands, *i.e.* bpz. These compounds showed strongly different electrochemical and photophysical properties compared to their bpy/phen counterparts. Notably, they are able to oxidize the guanine nucleobase under irradiation. Luminescence studies in the presence of guanine-containing polynucleotides (dGMP and DNA) suggest the presence of a photo-induced electron transfer between the guanine and the excited complexes through a dynamic quenching process. This photoreactivity can be used to efficiently photo-induce electron transfer with guanine moieties in close proximity with a mismatched site by using a mismatched-selective probe such as $[\text{Ru}(\text{bpz})_2\text{bdppz}]^{2+}$.

The photochemical tuning achieved by the modification of the different ligands has been then further investigated by replacing the metal cation from Ru(II) to Ir(III). The photochemical study of the related Ir(III) complexes indicates the presence of a low-lying excited state associated with the promotion of one electron from an Ir-ppy fragment to the extended planar ligand (*i.e.* MLLCT state). Interestingly, further relaxation towards a non-emissive mono-hydrogen bonded excited state occurs for **Ir-DPAC**, **Ir-DPACF₂**, **Ir-DPACF₄** and **Ir-BDPPZ** in water. The quenching of luminescence is however not observed in the case of **Ir-NPP**, probably arising from the “elbow-structure” of the extended ligand. Luminescence studies in

the presence of DNA was made possible by addition of 10% acetonitrile, solving solubility issues. While a strong luminescence enhancement is observed for all complexes, a “light switch” behavior is observed for **Ir-DPAC**, **Ir-DPACF₂**, **Ir-DPACF₄** and **Ir-BDPPZ**, indicating the protection of the complexes from non-radiative deactivation pathways accounts for the recovery of the luminescence.

In these chapters, we thus showed the effect of the modification of the different building blocks of transition metal complexes on the resulting photochemical properties both in the absence and in the presence of DNA. Our results clearly indicate strong photochemical tuning is achieved through small changes in the structure and shape of one of the partners. In addition, the data gathered in this manuscript give insights on the photochemical properties of poorly described acridine-based transition metal complexes. We suggest further development of such species could lead to potential diagnostic and therapeutic activity.

2. Prospects

In order to design selective mismatched DNA photoprobes for potential biological applications, we propose to modify the structure and the shape of the different ligands.

A first possibility envisaged is to prepare an “elbow-shaped” ligand similar to npp, where the extra benzene ring with respect to npp is on the opposite side from the non-chelating nitrogen atom (naphtho[2,1-*b*]pyrido[3,2-*f*][1,7]phenanthroline, **8.1**, Figure 8.1). The sensitivity of the resulting complex to its micro-environment would be increased due to the exposure of the nitrogen from the acridine moiety to solvent. The envisaged strategy would be a intermolecular condensation between 5-amino-1,10-phenanthroline (**3.15**) and (2-aminonaphthalen-1-yl)methanol (**8.2**). The latter can be obtained starting from 2-aminonaphthalene (**8.5**) and diethylketomalonate (**8.6**). In acidic conditions, these two partners lead to the formation of **8.4**.²⁷⁰ Ring opening can be performed upon treatment with hydrogen peroxide in the presence of an hydroxylated base, yielding 2-aminonaphthalene-1-carboxylic acid (**8.3**).²⁷⁰ Subsequent reduction with LiAlH₄ would allow the formation of **8.2**.

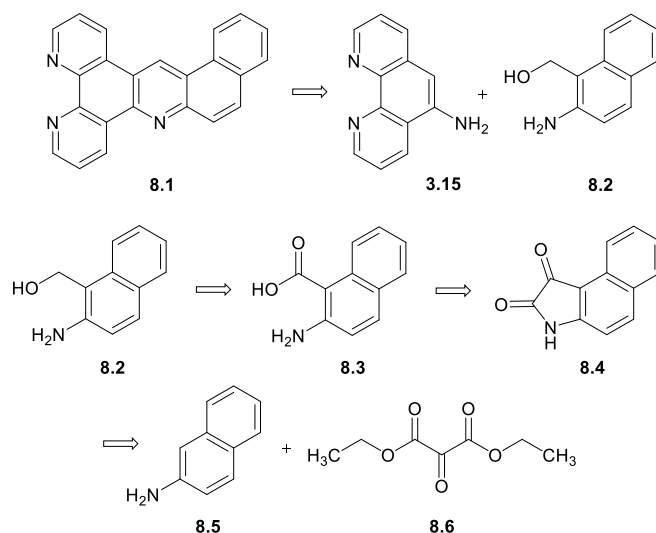


Figure 8.1. Retrosynthetic scheme of naphtho[2,1-*b*]pyrido[3,2-*f*][1,7]phenanthroline (**8.1**).

Another possibility is to use 3,4,7,8-tetramethyl-1,10-phenanthroline (TMP) as ancillary ligands. It has indeed recently been showed $[\text{Ru}(\text{TMP})_2\text{dppz}]^{2+}$ is able to specifically target single base mismatches in DNA, through minor groove metalloinsertion binding mode.²³⁶ In addition, this complex exhibit differential luminescence behavior which is correlated with the thermodynamic destabilization of the mismatch. Thus, combining the specific interaction of bdppz related Ru(II) complexes for mismatched DNA with the TMP ancillary ligands would further increase the selectivity of the corresponding Ru(II) complexes, representing potentially a new early cancer diagnostic tool for detecting deficiencies in mismatch repair processes (Figure 8.2).

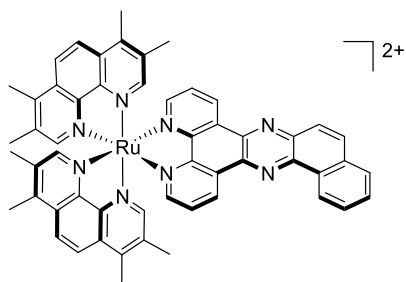


Figure 8.2. Structure of $[\text{Ru}(\text{TMP})_2\text{bdppz}]^{2+}$.

In order to increase the solubility of our Ir(III) complexes, several strategies could be envisaged. One particularly attractive because it both solves the solubility issues and increases the binding affinity of the complexes towards DNA is the design of IrN_6 (using bpy ancillary ligands, Figure 8.3) and IrN_5C_1 (using one bpy and one ppy-type ancillary ligands) counterparts. Indeed, the increase of the formal charge induces a stronger binding thanks to the higher contribution of electrostatic interactions between the positively charged complexes and the negatively charged polynucleotides.²⁵⁷ Preliminary attempts in the preparation of IrN_6 derivatives unfortunately failed to lead to the formation of the desired complexes (Figure 8.3). Spectroscopic studies of the isolated material suggest no complexation of the extended ligand occurs in our case. This might be due to the great inertness of the Ir(III) precursor which makes the synthesis of IrN_6 complexes very challenging.^{242,271}

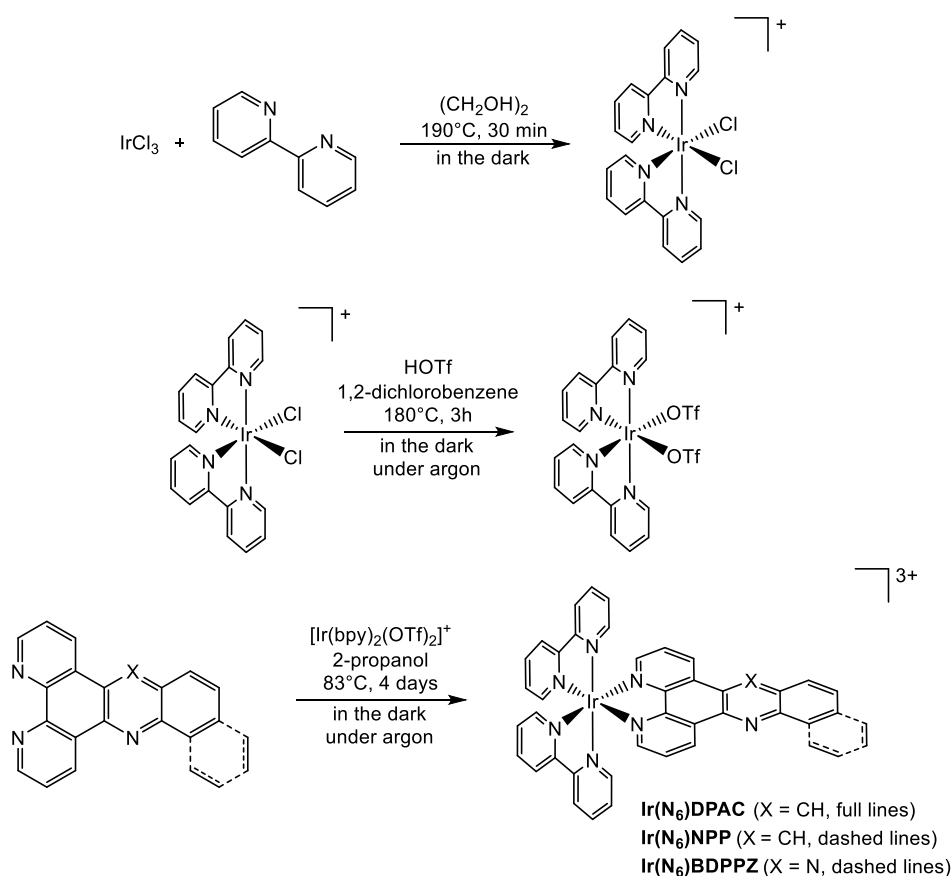


Figure 8.3. Synthetic scheme of IrN₆ complexes.

First attempts to prepare IrN₅C₁ derivatives imply an Ir(III) precursor analog to $[\text{Ir}(\text{bpy})_2\text{Cl}_2]^+$ where one of the bpy ligand displays a C[^]N coordinating fragment. This so-called “twisted” precursor^{272,273} (Figure 8.4) would allow, after complexation of the appropriate extended planar ligand, to increase the solubility of the resulting Ir(III) complexes in aqueous medium with respect to IrN₄C₁ derivatives. Unfortunately, this strategy lead to the recovery of the starting material after the complexation of the extended planar ligand step. As solely derivatives bearing less rigid ligands such as bpy has been reported in that case, we suggest the rigidity of dpac, npp and bdppz prevent their complexation to the Ir(III) center. The latter is indeed less tolerant than Ru(II) towards the rigidity of the ligands because of its relatively inert coordination sphere.

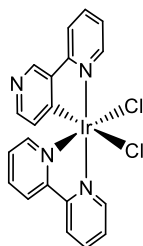


Figure 8.4. Structure of the twisted Ir(III) precursor, [Ir(bpy)(C^N-bpy)Cl₂].

As a result, the second envisaged strategy involves a less rigid extended planar ligand, i.e. a dpac analog where the two pyridinic complexing fragments are reduced (Figure 8.5). After successful synthesis of this reduced dpac ligand, its complexation to [Ir(bpy)(C^N-bpy)Cl₂] has yielded a mixture of products difficult to purify and poorly soluble, avoiding clear characterization. HRMS data indicate a possible re-oxidation of one of the reduced pyridinic ring. This work has been achieved by Justin WEYNAND, in the frame of his master thesis during the academic year 2014-2015.

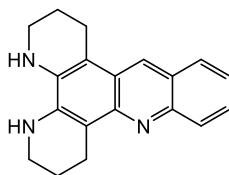


Figure 8.5. Structure of the targeted hydrogenated-dpac ligand.

Regarding the difficulties to prepare IrN₅C₁ derivatives with the twisted Ir(III) precursor, a solution would be to use the benzo[*a*]pyrido[2,3-*c*]acridine extended ligand (**8.6**, Figure 8.6). Indeed, this ligand differs from dpac solely by a C-coordinating atom instead of a nitrogen one, leading to a +2 formal charge for the resulting bpy-based Ir(III) complex. Further development of “elbow-shaped” analogs could be then envisaged in order to tune the DNA binding and photochemical properties. It could be obtained starting from benzo[*h*]quinoline (**8.11**) through a nitration step, following reduction to the corresponding amine **8.9** and intermolecular condensation with *o*-aminobenzylalcohol (**8.8**).

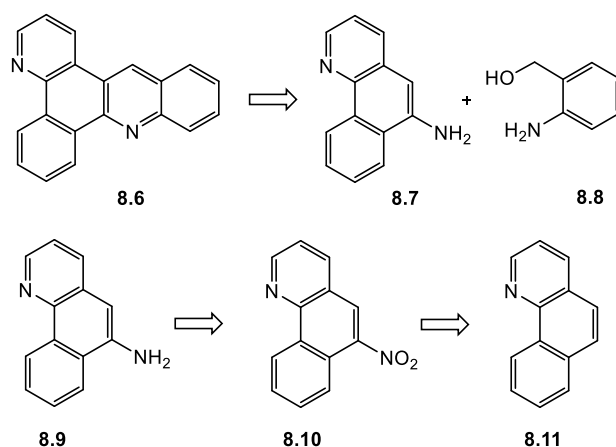


Figure 8.6. Retrosynthetic scheme of benzo[*a*]pyrido[2,3-*c*]acridine (**8.6**).

On the other hand, better understanding of the photochemical processes occurring for our bpz-based Ru(II)-complexes both in the absence and in the presence of matched and mismatched DNA and oligonucleotides would require further luminescence titrations studies and ultrafast transient absorption measurements. Notably, the identification of a PCET process and of the different transient species formed needs similar experiments to be realized in various solvents (*i.e.* in D₂O and dry MeCN). Ultrafast transient Infrared spectroscopy could also be used in order to determine the nature of the binding sites of our complexes into DNA or polynucleotides.

Finally, the scope of the extended ligands used in our Ph.D. thesis could be extended to the design of three-dimensional scaffolds such as metal-organic frameworks or artificial supramolecular antennas. Such supramolecular structures could thus benefit from the structural and electronic properties of the different ligands. The dpac-based scaffold **8.12** depicted on Figure 8.7 could be obtained through a Suzuki-Miyaura cross-coupling of the mononuclear precursor **8.13**, itself obtained after complexation of the extended planar brominated ligand **8.14** on an appropriate metallic cation. The brominated ligand has already been obtained through intermolecular condensation between 5-amino-1,10-phenanthroline (**3.15**) and (2-amino-4-bromophenyl)-

methanol (**8.15**), the latter coming from the reduction of the carboxylic acid precursor **8.16**.

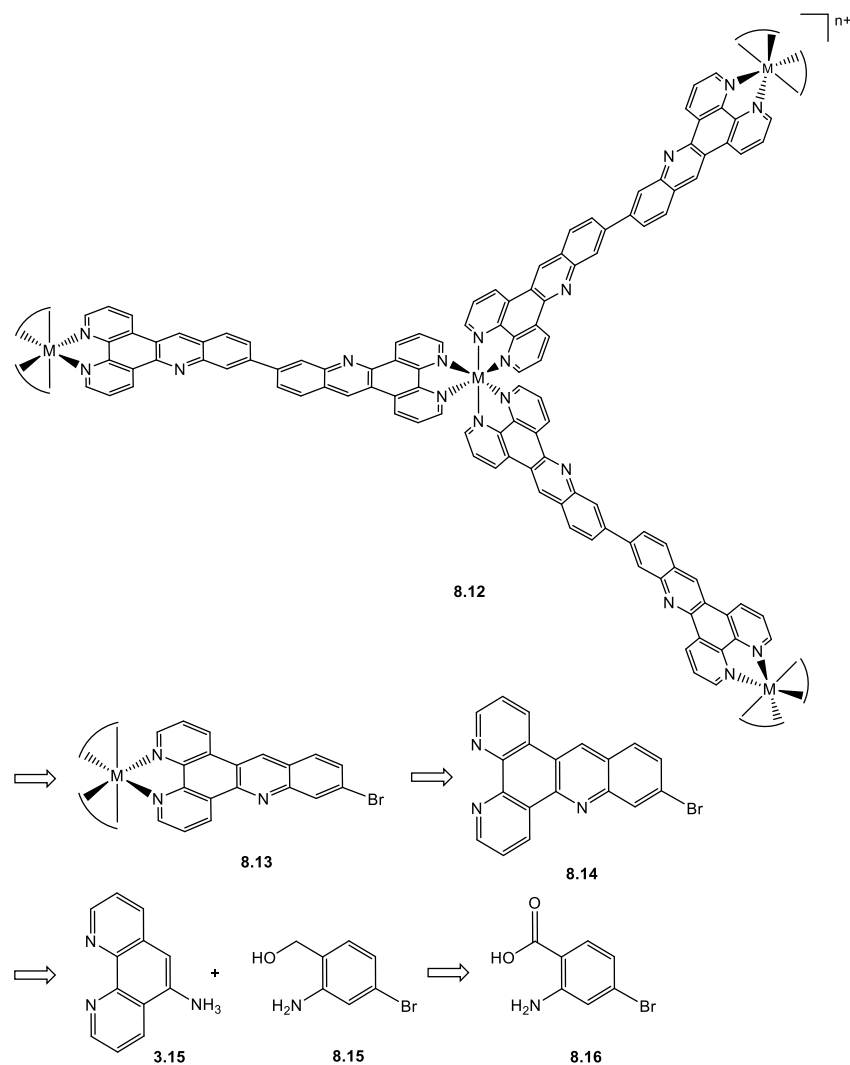


Figure 8.7. Retrosynthetic scheme of a dpac-based three-dimensional architecture.

Chapter 8

Instrumental techniques

1. Nuclear Magnetic Resonance (^1H NMR)

^1H NMR experiments are performed in CDCl_3 , CD_3OD or CD_3CN on a Bruker AC-300 Avance II (300 MHz) or on a Bruker AM-500 (500 MHz) at 20°C . The chemical shifts (given in ppm) are measured versus the residual peak of the solvent as the internal standard. The coupling constants (J) are given in Hertz. Multiplicity is given as follows: s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet, br = broad signal.

2. Mass spectrometry

High-resolution mass spectrometry (HRMS) are recorded on a Q-Exactive orbitrap from ThermoFisher using reserpine as internal standard. Samples are ionized by electrospray ionization (ESI; capillary temperature = 250°C , vaporizer temperature = 250°C , sheath gas flow rate = 20 ml/min).

3. UV-visible absorption spectroscopy

UV-vis absorption spectra are recorded on a Shimadzu UV-1700. Data were converted and transferred onto a PC. A halogen lamp from Narva-light (12 V/20 W) was used to generate visible range light, and a Shimadzu deuterium lamp was used for near-UV light.

4. Determination of molar extinction coefficient

Molar extinction coefficients were determined by measuring absorbance of different solutions of known complex concentrations in an appropriate solvent. The concentrations of the complexes were determined by weighting. The linear relation of the absorbance as a function of the concentration was used to obtain the molar extinction coefficients through its slope, according to the Beer-Lambert equation.

These values for complexes from Chapter 3 were determined by ^1H NMR using 2,2'-((perfluoroethane-1,2-diyl)bis(oxy))bis(2,2-difluoroethan-1-ol) as internal reference to calculate the complex concentrations.

5. Emission spectroscopy

Room temperature emission spectra were recorded on a Varian Cary Eclipse instrument. The excitation source was a Xenon lamp and a phototube was used for detection. Luminescence intensity at 77 K is recorded on a FluoroLog3 FL3-22 from Jobin Yvon equipped with an 18V 450W Xenon Short Arc lamp and an R928P photomultiplier, using an Oxford Instrument Optistat DN nitrogen cryostat controlled by an Oxford Intelligent Temperature Controller (ITC503S) instrument.

6. Luminescence lifetimes

For the luminescence lifetime measurements of complexes from Chapter 3, the pulsed excitation source was a pulsed laser Nd:YAG Q-switched laser (Continuum Inc.) frequency-tripled (355 nm) coupled with an optical parametric oscillator (Continuum Inc.) covering the wavelengths region 410-2300 nm with a maximum pulse energy from 10 to 120 mJ depending on the wavelength. The emission was detected perpendicularly by a photomultiplier (R928, Hamamatsu). The signal was recorded with a digital oscilloscope (HP 54200A), connected through the IEEE488 interface to a personal computer, and was averaged over at least 16 shots. The emission wavelength was selected via a grating Czerny-Turner monochromator (Spectra Pro 2300i, Acton Research Corp.).

For the others complexes, luminescence lifetimes measurements were performed after irradiation at $\lambda=400$ nm obtained by the second harmonic of a Titanium:Sapphire laser (picosecond Tsunami laser spectra physics 3950-M1BB+39868-03 pulse picker doubler) at a 80 kHz repetition rate. Fluotime 200 from AMS technologies was used for the decay acquisition. It consists of a GaAs microchannel plate photomultiplier tube (Hamamatsu model R3809U-50) followed by a time-correlated single photon counting system from Picoquant (PicoHarp300). The ultimate time resolution of the system is close to 30 ps. Luminescence decays were analyzed with FLUOFIT software available from Picoquant.

7. Determination of quantum yield

The luminescence quantum yield of a complex i , Φ_i , is determined by comparison with a reference complex, in our case $[\text{Ru}(\text{bpy})_3]^{2+}$ in water under air ($\Phi_{\text{ref}} = 0.028$).¹⁰² Φ_i is given by the equation:

$$\Phi_i = \Phi_{\text{ref}} \frac{A_{\text{ref}}}{A_i} \frac{I_i}{I_{\text{ref}}} \frac{\eta_i^2}{\eta_{\text{ref}}^2} \quad (\text{equation 9.1})$$

where A_{ref} and A_i are respectively the absorbance of the reference and of the complex i , I_i and I_{ref} are the integrated emission intensity of the complex i and the reference, and η_i and η_{ref} are the refractive index of the solvents.

8. Cyclic voltammetry

Cyclic voltammetry experiments were carried out in a one-compartment cell, using a glassy carbon disk working electrode (approximate area = 0.03 cm²), a platinum wire counter electrode, and an Ag/AgCl reference electrode. The potential of the working electrode is controlled by an Autolab PGSTAT 100 potentiostat through a PC interface. The cyclic voltammograms are recorded with a sweep rate of 150 or 300 mV s⁻¹, in dried acetonitrile (Sigma-Aldrich, HPLC grade). The concentration of the complexes is 8.10⁻⁴ mol/L, with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte. Before each measurement, the samples are purged by nitrogen.

9. (TD-)DFT calculations

DFT calculations are performed using Gaussian 09.¹⁷⁰ The hybrid functional PBE0 is used to carry the DFT¹⁷¹⁻¹⁷⁴ and TD-DFT¹⁷⁵⁻¹⁷⁷ calculations in combination with the LanL2DZ¹⁷⁸⁻¹⁸¹ basis set for all atoms. Geometry optimizations are conducted without symmetry constraints. Frequency calculations on each optimized structure confirmed that energy minima is reached. The energy, oscillator strength, and related MO contributions for the 100 lowest singlet–singlet and 10 lowest singlet–triplet excitations for the S_0 -optimized

geometry are obtained respectively from the TD-DFT/singlets and the TD-DFT/triplets output files. One should note that the PBE0 functional, while providing the correct excitation energies is not able to fully reproduce their relative intensity. This behavior has already been noticed in the literature in the case of even simpler compounds and different functional and more elaborated approaches have been used in cases where the band shape is of interest which was actually not the case of the present study.¹⁸² GaussView3.0.9¹⁸⁷, GaussSum 3.0¹⁸⁴ and Chemissian 4.38 software are used for data analysis, visualization and surface plots. Calculations are performed in MeCN or H₂O solution by use of the conductor-like polarized continuum (CPCM) solvation model as implemented in Gaussian 09.¹⁷⁰ Because the continuum model could not describe specific interactions such as nitrogen–hydrogen bond interactions, a discrete model is also used in an approach similar to what Daniel *et al.* have recently reported.¹⁵⁴

10. Surface Plasmon Resonance measurements

The SPR measurements were performed on a BIAcore T100 (BIAcore AB, Sweden) operated with BIAcore T100 Evaluation Software 1.1. The experiments were carried out at 25 °C, using as a working buffer an aqueous solution of 10 mM HEPES at a pH of 7.4, 100 mM NaCl, 100 mM KCl, and 3 mM EDTA supplemented by 0.05% v/v P20 surfactant. The hairpin duplex d(5'-GCGCGCGCTTTTGCGCGCGC-3') was immobilized on a Streptavidin-coated surface (SA sensor chip, BIAcore AB) via a peptidic scaffold possessing a biotin residue. This sequence was used as guanine-rich double-stranded DNA (ds-DNA). Binding experiments were conducted at 30 μ L min⁻¹ by the injection of the complexes dissolved in a buffer at concentrations from 50 to 1000 nM (injection time, 120 s; dissociation time, 500s). A non-modified channel was used as a reference. Curves obtained from the reference surface were subtracted from the curves recorded after host-guest recognition, allowing elimination of refractive index changes (due to buffer effects) and corrections of the nonspecific interaction of the complexes with the Streptavidin surface.

11. Transient absorption measurements

The sample was dissolved in buffer (5 mM Tris.HCl, 50 mM NaCl, pH = 7.4) and a ratio of 50:1 (SS-DNA:Ru) was prepared. The concentrations were 600 μ M in SS-DNA and 12 μ M in complex. All experiments were performed at constant room temperature (22 °C) and under aerated conditions. Absorption spectra were recorded prior and subsequent to all measurements to ensure photochemical stability of the samples. The time-resolved transient absorption set-up was based on an amplified Ti:Sapphire oscillator (Libra, Coherent Inc.) producing a pulse-train of 80 fs 950 mJ pulses centered at 800 nm with a repetition rate of 1 kHz. The pulses were split by means of a 50:50 beamsplitter and one of the individual portions was used to independently pump a non-collinear optical-parametric amplifier (TOPAS white, LightConversion Ltd.); the output of this was tuned between 500 and 700 nm and served as probe pulse in several experiments, while the other one was used to generate the pump pulse by second harmonic generation. For this reason the beam was focused in a 100 mm thick BBO crystal. In some experiments reported here, white light was used as probe. Therefore, 1% of the 800 nm fundamental beam was split away and focused into a Ti:Sapphire plate to generate a supercontinuum. A chopper was inserted into the pump path to block every second pump pulse. The probe pulses were delayed in time with respect to the arrival of the pump pulses at the sample position by means of a delay line and split by means of a 30:70 beamsplitter to obtain probe and reference pulses. The probe pulses were focused into the sample by means of a 500 mm focal-length spherical mirror, while the reference pulses were biasing the sample and were directed onto the detector. Probe pulses were spatially and temporally overlapped at the sample position with the pump pulses, which were focused into the sample by means of a 1000 mm focal-length quartz lens. The different focus conditions resulted in a larger focal spot of the pump beam as compared to the probe and ensured homogeneous excitation of the sample over the entire probe-focus volume. The polarization of the pump pulses was rotated to yield magic-angle polarization geometry. After the sample the pump pulse

was blocked, while the probe pulse is re-collimated by a second 500 mm focal-length spherical mirror and – in concert with the reference beam – was sent to the detection system, which was obtained from Pascher Instruments AB and allows for simultaneous spectrally resolved detection of probe and reference intensities on a double-stripe diode array, each stripe consisting of 512 individual diodes. The diode array was read-out with the repetition rate of the laser and the DA signal was calculated from the probe and reference intensities for a pair of measurements corresponding to pump-on and pump-off conditions. It was ensured that the energy of the pump pulses was kept well below 0.5 mJ, while typical probe intensities fall into the range of a few hundred nJ. Auto-correlation of the probe pulses and the experimental response function was recorded at the sample position in a 1 mm thick BBO crystal. From this data the temporal duration of the probe pulses and the experimental response function could be estimated to be in the order of 30–40 fs.

The fluorescence lifetimes were obtained by streak camera measurements. Therefore, a Ti:Sapphire Laser (Tsunami, Newport Spectra-Physics GmbH) was used as light source. Repetition rate was reduced to 4 kHz by a pulse selector (Model 3980, Newport Spectra-Physics GmbH) and afterwards the beam was frequency doubled in a second harmonic generator (Newport Spectra-Physics GmbH) to create the 435 nm pump beam. The emission was recorded using a CHROMEX 250IS spectrograph and a Hamamatsu HPD-TA streak-camera.

For femtosecond measurements, the complex was excited at 403 nm with a pulse energy of 0.4 μ J. The photo-induced dynamics were probed with a white-light supercontinuum. Samples were added to a quartz cell (1 mm path length), with the final concentrations of the complexes adjusted to yield an optical density of approximately 0.4 at the corresponding excitation wavelength. The data was chirp-corrected and subsequently analyzed by a global fitting routine using a sum of exponential functions.

Nanosecond transient absorption and emission lifetime measurements of the complex were performed in buffer in a sealed quartz cell with 1 cm path length ($OD = 0.5$ at 410 nm) on the set-up described above. Samples were excited with 10 ps pulses centered at 410 nm (pulse energy 0.2 mJ). For transient absorption measurements, the probe wavelength was scanned between 380 and 800 nm in steps of 10 nm, and a quantitative data analysis was based on a global fit of the data obtained at all probe- wavelengths. Emission lifetimes were recorded at 630 nm using the ns-transient absorption system without the probe light.

Chapter 9

Annexes

Additional chapter: Photophysical and photochemical studies of a new organic photosensitizer for cancer photodynamic therapy

1. Introduction

Up to now, only transition metal complexes have been discussed in this manuscript. Their photochemical properties and their use as DNA photoprobes or photoreactants have been extensively studied, paving the way for further application such as therapeutic tools. We were thus interested in the study of compounds of which therapeutic activity can be triggered by means of light irradiation. Photosensitizers for photodynamic therapy represent an obvious continuation for our Ph.D. thesis. In that context, we then investigated the photochemical properties of a purely organic photosensitizer designed by the group of Prof. O. Riant (Université catholique de Louvain, UCL) which PDT activity has been observed by the group of Prof. O. Feron (UCL).

1.1. Cancer fundamentals

1.1.1. Cancer – A matter of cells dysfunction

Every year, over twelve million people worldwide are diagnosed with cancer. Europe and North America together contribute about 40 % of this figure, while about 54 % of the cases arise from developing countries.²⁷⁴ Cancer is the name given to a collection of related diseases. In all types of cancer, some of the cells from the body begin to divide quickly without stopping and spread into surrounding tissues. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and divide to form new cells as the body needs them and when cells grow old or become damaged, they are replaced by new cells (Figure 7.1). However, when cancer develops, this orderly process breaks down. As cells become more and more abnormal, old or damaged cells survive

instead of dying, and new cells form when they are not needed. These new cells are able to divide quickly without stopping and may form growths called tumors. When cancerous tumors are malignant, they can spread into, or invade, nearby tissues, or travel to distant places in the body through the blood or the lymph system when the tumors break, forming new tumors far from the original one (metastasis).²⁷⁵ Cancer cells differ from healthy cells in many ways that allow them to grow out of control and become invasive. For example, whereas healthy cells grow into distinct cell types with specific functions, cancer cells do not. In addition, cancer cells are able to ignore signals that normally induce the stop of the cellular division processes or which lead to cell death, as well as to survive to the immune system activity.

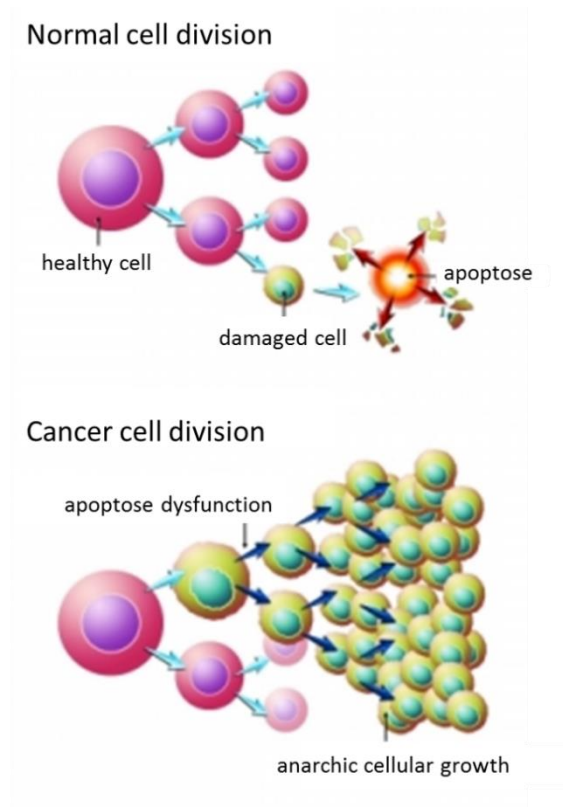


Figure 7.1. Differentiation between normal and cancer cell division processes.

1.1.2. Origins

Cancer is a genetic disease, meaning it is caused by alterations to genes, leading to cells dysfunction. These changes can be inherited by heredity or can also arise during a person's lifetime as a result of errors that occur as cells divide or because of damage to DNA caused by particular environmental exposures such as electromagnetic radiations (UV light) or harmful chemicals (alkylating agents). The genetic alterations that contribute to cancer tend to affect genes involved in cell growth and division, as well as DNA repair.

1.1.3. Treatments

More than 200 types of cancer exist and detailed considerations about them are beyond the scope of this manuscript. They are usually named according to the organ in which cancerous cells are located. The treatment of cancer will differ depending on its type and stage. The main cancer treatments used nowadays include:

- a) surgery: the use of surgery as cancer treatment consists in the surgical removal of cancer cells located in a defined area of the body. In some cases, the tumor is too close of important organs or tissues and only a part of it can be removed. Surgery is often used in combination with radiotherapy or chemotherapy.
- b) radiotherapy: radiotherapy uses high-energy rays to destroy cancer cells in a particular area. It can be given both externally and internally. External radiotherapy consists in the irradiation of the affected area with high-energy light rays, while internal radiotherapy involves having radioactive material injected inside the body. It is important to notice healthy cells can also be damaged by radiotherapy, causing side effects, such as skin reactions or hair loss.
- c) chemotherapy: this treatment involves the injection of harmful chemical agents into the human body. Its use is often required in order to control the development or to kill cancer cells.

Despite their valuable activity, chemotherapeutic agents features some drawbacks, such as severe damages to healthy cells. A few decades ago, chemotherapeutics that are activated upon light irradiation have been the subject of increasing interest due to their huge potential to overcome the limitations of the classical agents. This technique is called photodynamic therapy.

1.2. Photodynamic therapy (PDT)

1.2.1. *Basic principles*

Photodynamic therapy is a clinically used therapeutic technique involving the use of photoactivatable compounds as drugs. Such molecules are then called photosensitizers (PS). In the first step of the process, a photosensitizer is administered to the patient, either locally or systemically. After a delay (ranging from minutes to days) to allow for optimum biodistribution, the treatment site is irradiated with visible or NIR light. Light irradiation of the PS triggers photochemical reactions generating cytotoxic products towards the surrounding medium (see Figure 1.11). The main advantage of this technique is the possibility to focus on a particularly desired area of the body using the light beam, whereas classical chemotherapeutics are directly cytotoxic without requiring light activation.

1.2.2. *Requirements*

PDT required the use of an appropriate light source, the presence of a photosensitizer and a quencher, in the present case molecular oxygen.⁵⁹

1.2.2.1. The light source

The choice of the light source is based on several parameters. For example, it needs to deliver an appropriate energy to the PS (*i.e.* the PS needs to absorb the incident wavelengths) and depends on the targeted cells characteristics (size, location, accessibility). The clinical

efficiency of PDT is dependent on total light dose, light exposure time and light delivery mode. An important factor that needs to be taken into account is the penetration level of light into biological tissues. Indeed, blue light penetrates less efficiently, whereas red and infrared radiations penetrate more deeply. The region between 600 and 1200 nm is often called the optical window of tissue, or PDT window.

1.2.2.2. The photosensitizer

(I) Background

Most of the PS used in cancer PDT are based on a porphyrin core, similar to that of the protoporphyrin contained in hemoglobin. The ideal PS agent should be a single pure compound which allow quality control analysis with low manufacturing costs and good storage stability. It should also have a high molar extinction coefficient in the red region of the electromagnetic spectrum. The PS should have no dark toxicity and relatively rapid clearance from normal tissues, thereby minimizing phototoxic side effects. The cellular uptake of PS depends mainly on its charge, lipophobicity and size. However, these parameters also drive the elimination of the PS from the human body. As a result, hydrophobic PS show higher uptake but longer elimination time, leading to long-lasting photosensitivity for the body.

The first PS to be clinically employed for cancer PDT is Photofrin[®] (Figure 7.2), a water-soluble porphyrin derivative. Although it is still the most widely used PS, Photofrin[®] leads to some disadvantages, such as a long-lasting skin photosensitivity and a relatively low absorbance in the PDT window. The efficacy of this class of compounds has been improved by red-shifting the absorbance bands and increasing the molar extinction coefficient at these wavelengths. The development of PS that display all the ideal features has been the subject of increasing interest for the last decades. Second-generation PS and several hundred compounds have been proposed as potentially useful for anticancer PDT during that period, such as 5-aminolevulinic acid, a biosynthetic precursor of protoporphyrin.

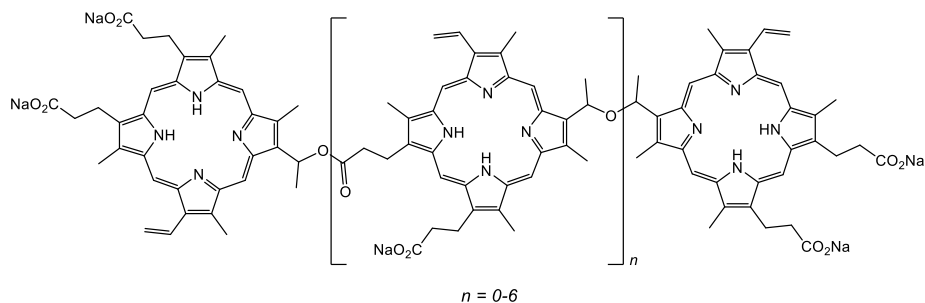


Figure 7.2. Structure of Photofrin®.

(II) Photophysics and photochemistry

Photosensitizers need to be photoactivatable compounds and photochemically active with surroundings molecules. They thus must display suitable photophysical and photochemical properties in order to observe PDT effects. Based on earlier photophysical considerations (see Chapter 1), absorption of light by PS leads to the formation a singlet excited state PS^* from a singlet ground state (Figure 7.3). Depending on its electronic properties, the excited PS may undergo an ISC to form a more stable triplet excited state, which may deactivate through radiative and/or non-radiative pathways. Among the latter, direct electron transfer between PS^* and a surrounding molecule (acting thus as a quencher) to produce ion radicals may arise, leading then to cellular damages. This mechanism is referred as type I photosensitization. The second mechanism (type II photosensitization) refers to the direct energy transfer from PS^* to a quencher (Q), itself causing damages once excited. Type II photosensitization appeared to be dominant for most cases.^{60,61}

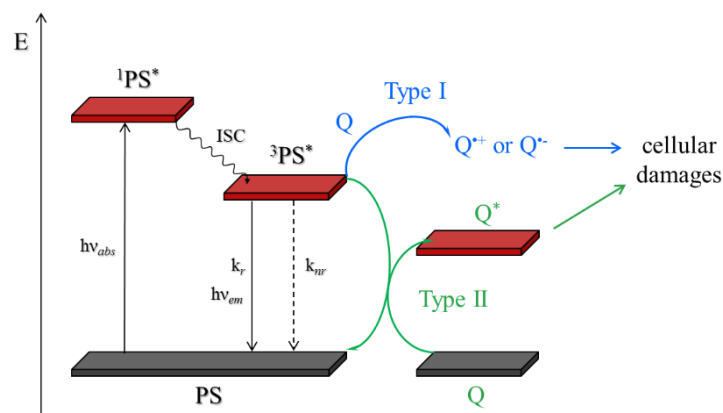


Figure 7.3. Photosensitization processes occurring in PDT. Light exposure takes a photosensitizer molecule (PS) from the ground singlet state to an excited singlet state ($^1\text{PS}^*$). The latter undergo intersystem crossing to an excited triplet state ($^3\text{PS}^*$) and then either lead to cellular damages via electron transfer (Type I mechanism) or energy transfer (Type II mechanism) with a surrounding molecule (Q).

The two types of mechanisms require either electron transfer (Type I), or energy transfer (Type II) processes in the excited state. While the fundamentals of excited state electron transfer has already been discussed in Chapter 6, no detailed considerations about excited state energy transfer has been treated yet.

Two mechanisms of excited state energy transfer have been described in the literature. They involve either excited state dipole coupling (Förster mechanism) or electron exchange (Dexter mechanism) between the excited donor (in the case of PDT, PS^*) and the quencher (Figure 7.4).

- a) Förster mechanism: it involves a quenching of PS^* by a quencher through space.⁹⁴ This mechanism takes place when PS^* is a dipole which in turn induces a dipole on the quencher. This coupling between the excited state dipoles leads to radiationless energy transfer from PS^* to the quencher. The efficiency of this mechanism is governed by the spectral overlap of PS^* emission and quencher absorption. Note Förster energy transfer solely occurs for singlet excited states

compounds. As coulombic interactions takes places, this mechanism may arise over a long distance ($\sim 100\text{-}200\text{ \AA}$).

- b) Dexter mechanism: in this mechanism, an electron exchange occurs between PS* and the quencher.⁹⁴ This implies an orbital coupling between the two partners, requiring their close vicinity ($\sim 10\text{-}20\text{ \AA}$). Dexter energy transfer does not obey the spin selection rule and is dominant when there is poor spectral overlap between PS* emission with the quencher absorption, such as for $^3\text{O}_2$ photosensitization.

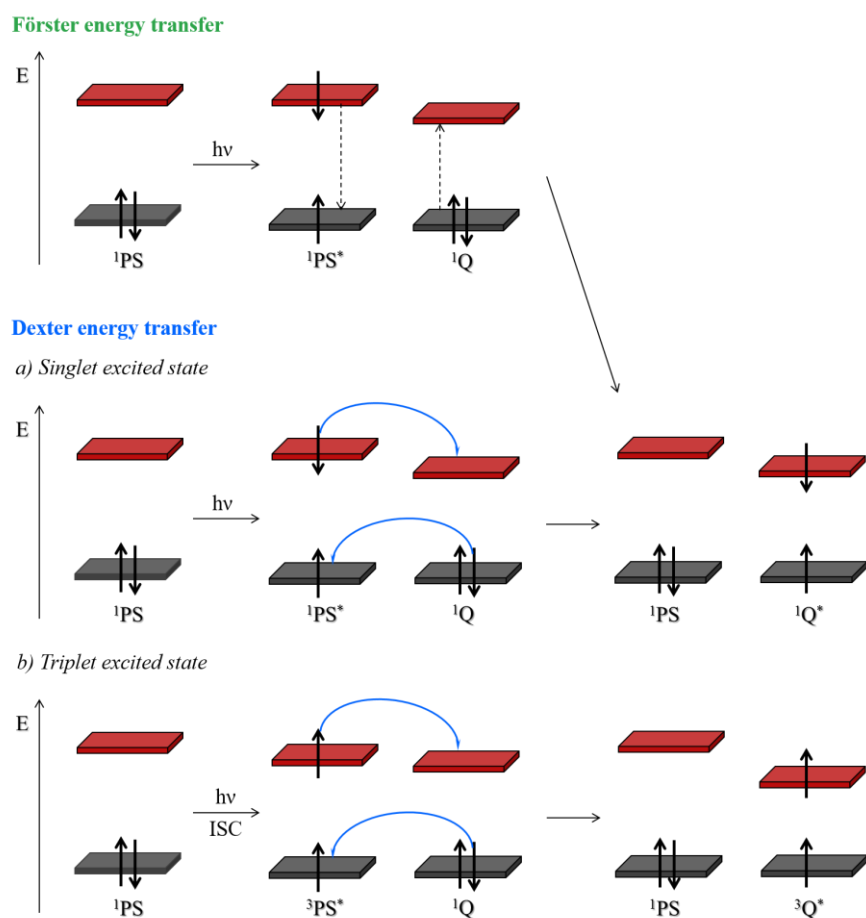
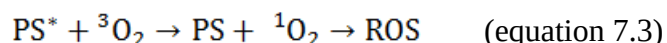
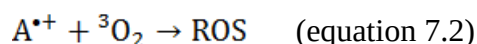
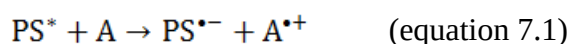


Figure 7.4. Simplified photophysical scheme of energy transfer mechanisms. Upon light irradiation, the photosensitizer (PS) can undergo Förster (up) or Dexter (bottom) energy transfer to a quencher (Q).

The study of the dynamics of the excited state energy transfer processes can be performed using Stern-Volmer kinetics analysis (See Chapter 6).

1.2.2.3. The role of oxygen

Together with the light source and the PS, the presence of oxygen is mandatory to induce PDT effects. Depending on the type of the photosensitization, oxygen ($^3\text{O}_2$) will react either with the ion radicals generated by electron transfer with PS^* (type I, equations 7.1 and 7.2), or directly with PS^* through an energy transfer mechanism obtaining $^1\text{O}_2$ (type II, equation 7.3), both types leading ultimately to the formation of reactive oxygen species (ROS).



Experimental and clinical studies revealed a critically low concentration of oxygen in solid tumors, as a result of their structurally and functionally disturbed environment, and of the deterioration of diffusion conditions.²⁷⁶ Such phenomenon is called hypoxia. Tumor hypoxia appears to be strongly associated with tumor propagation and resistance to therapy; consequently it has become a central issue in cancer treatment.²⁷⁷

1.3. Objectives

In order to study the effect of low O_2 concentrations, as usually encountered in tumors, on the PDT mechanisms, a new class of photosensitizers has been developed by the group of Prof. Olivier Riant from Université catholique de Louvain. These have been used to gain further insights on the intracellular targets of $^1\text{O}_2$ by the group of Prof. Olivier Feron from the same university. Among the plethora of available compounds, a phenazine derivative appeared particularly potent as it showed the larger difference of antiproliferative activity under hypoxia and light irradiation with respect to classical conditions (normoxia, in the dark). Our task was to investigate the photophysics and photochemistry of this new PDT photosensitizer, named **OR141** (Figure 7.4), in order to get

information about the mechanism involved in the PDT effect. This study, together with the biological consequences of irradiation of **OR141** in malignant cells, have been published in *Oncogene* (see Annexes).

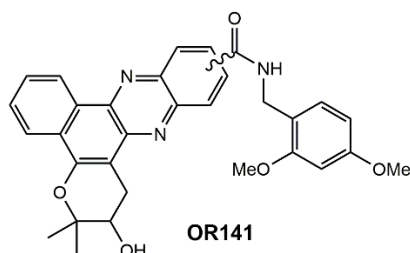


Figure 7.4. Structure of **OR141**.

2. Results and discussion

The discrimination between both types of PS sensitization mechanisms was achieved through optical measurements. Table 7.1 and Figure 7.5 gathers the absorption and emission data for **OR141** in various solvents. Absorption bands at *ca.* 309 nm ($\epsilon \sim 1300$ l/(mol.cm)) and *ca.* 450 nm ($\epsilon \sim 1000$ l/(mol.cm)) were observed. Upon excitation at 309 nm at room temperature, an intense unstructured emission was monitored. The excited state was shown to be short lived ($\tau < 8$ ns). The emission wavelength was blue shifted in less polar solvents or at 77 K ($\lambda_{\text{max}} = 485$ nm in EtOH/MeOH 4/1 rigid matrix), indicating that the excited state shows a greater dipolar moment compared with the ground state. Based on these experimental evidences and on literature data,²⁷⁸ the formation of excited **OR141** can be ascribed to a Charge Transfer process from an electron donor part to an electron acceptor part of the organic compound. Interestingly, no photosensitization of $^3\text{O}_2$ by **OR141** is observed under these conditions, suggesting no energy transfer with oxygen in the excited state (Type II photosensitization).

	Absorbance λ_{max} [nm] ($\epsilon / 10^3 \text{M}^{-1} \text{cm}^{-1}$) ^a	Emission λ_{max} [nm] ^{a, b}	Φ_{em} ^{a, b, c}	
	CH ₃ CN	CH ₃ CN	air	argon
PBS	309 (1.32), 458 (1.02)	525	0.041	0.044
PBS/DMSO 50/50	309, 345, 370, 441	527	0.176	0.176
DMSO	309, 372, 426	507	0.364	0.362
DCM	366, 426	482	0.237	0.208
EtOAc	345, 366, 415, 433	479	0.158	0.187

Table 7.1. Absorption and emission data for **OR141** in various solvents (PBS: phosphate buffer saline). ^a Measurements made with solutions $\sim 2.10^{-5} \text{ mol.L}^{-1}$ in **OR141**. The molar extinction coefficients were determined by weighing followed by absorption measurements. ^b $\lambda_{\text{ex}} = 309 \text{ nm}$ ^c Measured relative to an aerated solution of quinine sulphate 0.5 mol.L^{-1} in conc. H_2SO_4 ($\Phi_{\text{em}}^{\text{air}} = 0.60$).²⁷⁹

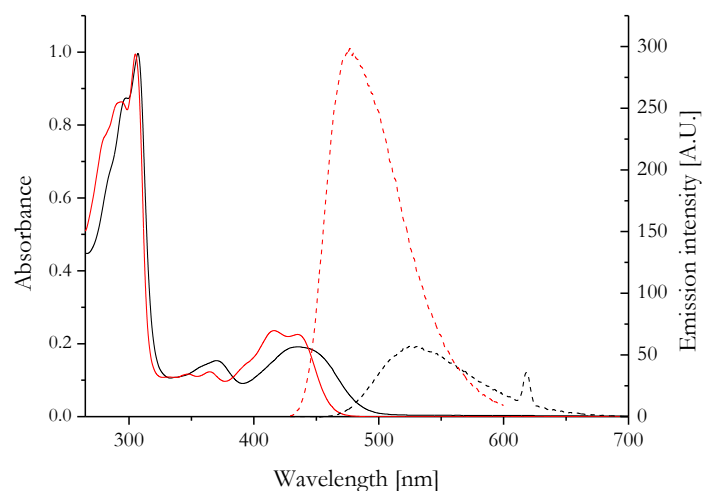


Figure 7.5. Absorption (full lines) and emission (dashed lines, $\lambda_{\text{ex}} = 309 \text{ nm}$) spectra of **OR141** in DMSO/PBS (50/50 v/v – black,) and in EtOAc (red).

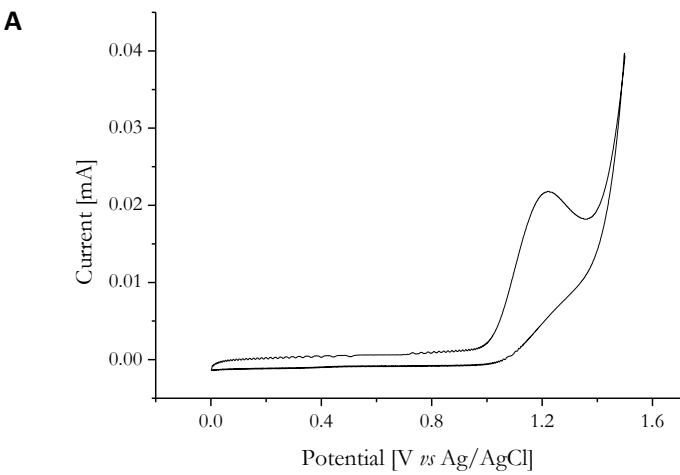
The nature of the excited state of **OR141** being determined and type II mechanism being discarded, we investigated the possibility of photo-induced electron transfer in the excited state (Type I photosensitization). Stern-Volmer titration involving the monitoring

of the luminescence behavior of **OR141** upon increased concentration of a biologically relevant quencher was achieved. Glutathione (GSH) has been chosen as quencher being a model reducing agent present in cancerous cells. Based on the reduction potential of **OR141** in the excited state (Table 7.2) and the oxidation potential of glutathione ($E_{\text{ox}} = -0.41 \text{ V vs Ag/AgCl}$),²⁸⁰ a photo-induced electron transfer could be expected (equation 7.4).



	$E_{1/2 \text{ ox}} \text{ (V)}$	$E_{1/2 \text{ red}} \text{ (V)}$		$E_{\text{ox}}^* \text{ (V)}^a$	$E_{\text{red}}^* \text{ (V)}^a$
		1	2		
OR141	1.10 (r)	-1.14 (r)	-1.69 (ir)	-1.61	1.36

Table 7.2. Oxidation ($E_{1/2 \text{ ox}}$) and reduction ($E_{1/2 \text{ red}}$) potentials of **OR141** determined by cyclic voltammetry, using a glassy carbon working electrode in dry deoxygenated DMF (r: reversible wave; ir: irreversible wave). All potentials have been determined using Ag/AgCl as a reference electrode. ^a The excited state oxidation (E_{ox}^*) and reduction (E_{red}^*) potentials were estimated from the ground state redox potentials, using the following equations $E_{\text{ox}}^* = E_{1/2 \text{ ox}} - E_{0-0}$, $E_{\text{red}}^* = E_{1/2 \text{ red}} + E_{0-0}$.⁹⁴



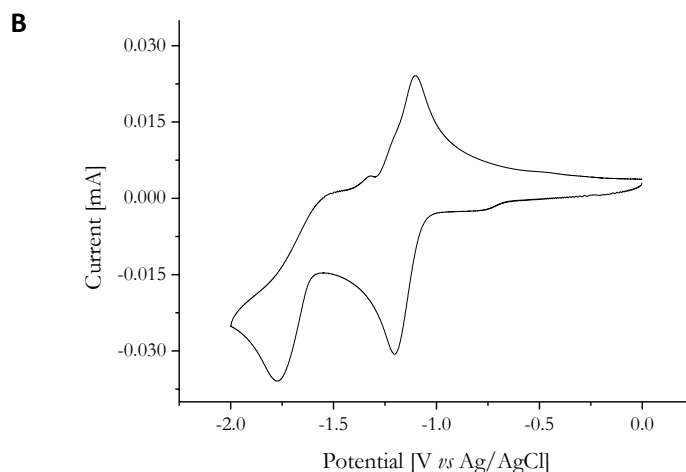
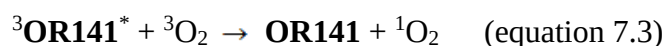
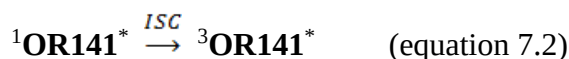


Figure 6.6. Cyclic voltammograms of **OR141** recorded in dry DMF under nitrogen, with a sweep rate of 0.1 V/s, at room temperature. The concentration of the compound is 8.10^{-4} mol/L, with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte. (A) Positive polarization; (B) negative polarization.

However, no fluorescence quenching was observed up to the addition of 10 mM glutathione (Figure 7.6) in line with the short lifetime of the singlet excited state of **OR141**, which deactivates before being reached by the quencher ($k_{\text{diff}} \sim 10^9 \text{ L.mol}^{-1}.\text{s}^{-1}$).⁹⁴ These results led us to the conclusion that the main photo-induced process occurring in the presence of excited **OR141** implies an intersystem crossing, converting the singlet excited state of **OR141** into a triplet excited state (equation 7.2), in turn able to photosensitize $^3\text{O}_2$ (equation 7.3), that is type II photosensitization. This mechanism, while discarded earlier in this section, may be restored in the close vicinity of biological targets, suggesting a complex medium effect on the properties of the photosensitizer. The presence of $^1\text{O}_2$ has been indeed observed *in vitro* by electronic paramagnetic resonance (EPR).



In parallel to our studies, the group of Prof. O. FERON has showed **OR141** combines a quasi-absence of cytotoxicity in the absence of light activation and a low IC_{50} (~ 10 - 100 nM according to the cell types and the assay) when activated. Off-targets are therefore less likely than with other PDT agents such as Photofrin[®] that inhibit tumor cell growth even in the absence of light. While type II mechanism is supported by EPR measurements both under normoxic and hypoxic conditions, photooxidation of various signaling proteins observed by the formation of high molecular weight protein complexes indicates the occurrence of type I mechanism. As a result, both types of photosensitizations are suggested to account for the photocytotoxic activity of **OR141**. In addition to these outstanding results, the accumulation of **OR141** in the endoplasmic reticulum (ER) leads to the inhibition of critical pathways involved in the adaptation of cells to hypoxia through the oxidation of ER-associated proteins.⁵⁷ Thus, the benzophenazine core of this PS represents a particularly suited class of compounds for future development of photodynamic drugs.

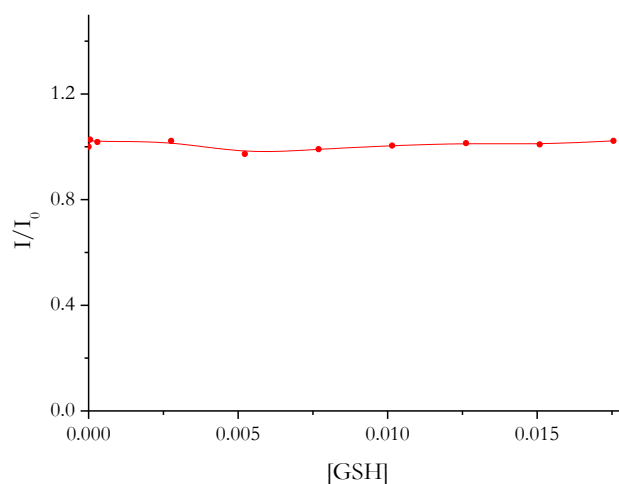


Figure 7.6. Stern-Volmer titration of **OR141** in the presence of increasing concentration of glutathione (GSH). Measurement made under argon in DMSO/PBS 50/50 (v/v), $\lambda_{\text{ex}} = 309$ nm, $[\text{OR141}] = 10^{-4}$ mol/L.

3. Conclusions

The photophysical and photochemical studies performed in our laboratory indicate **OR141*** can be ascribed to a triplet excited state resulting from a Charge Transfer state where an electron donor part of the compound promotes an electron to an electron acceptor part.

Environment-sensitive photochemical tuning is observed, making Types I and II photosensitizations, while not evidenced for **OR141** alone in solution, possible in biological media. These observations support the strong effect of the close environment on the photochemical properties of phenazine derivatives.

Publications



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DNA Photoprobes

Design and Photophysical Studies of Acridine-Based Ru^{II} Complexes for Applications as DNA Photoprobes

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Abstract: A series of new extended planar aromatic ligands based on an acridine core (dpac = dipyrrodo[3,2-*a*:2',3'-*c*]-acridine; dpacF₂ = 7,8-difluorodipyrrodo[3,2-*a*:2',3'-*c*]-acridine; dpacF₄ = 6,7,8,9-tetrafluorodipyrrodo[3,2-*a*:2',3'-*c*]-acridine) and their respective Ru^{II} complexes were synthesized and extensively studied. The photophysical and theoretical studies revealed that Ru-DPAC and Ru-DPACF₂ allow emission from a ³MLCT_{phen/dpac} excited state at 597 and 604 nm, respectively, whereas the lowest excited state for Ru-DPACF₄ shifts from a

bright ³MLCT_{phen/dpac} state towards a poorly luminescent ³MLCT_{dpac} state (λ_{em} = 609 nm) owing to the formation of hydrogen bonds with water molecules in the excited state. These complexes exhibit up to sevenfold luminescence enhancement in the presence of complementary and mismatched DNA, demonstrating a strong binding to polynucleotides. The results emphasize the interest in Ru^{II} complexes bearing acridine derivatives as DNA interacting agents as a complement to the well-known phenazine-based derivatives.

Introduction

Transition-metal complexes are of great interest in molecular biology and biochemistry.^[1] In addition to being present as coordination sites in proteins or cofactors in Nature, these compounds offer numerous advantages for their use as photoprobes or photoreagents, such as DNA interacting agents. Indeed, the great modularity of the properties of the transition-metal complexes makes them ideal in this scope. Polypyridyl Ru^{II} complexes have been the subject of considerable interest for the past few decades. Even though numerous publications describe their use as catalysts,^[2] or as energy conversion and storage devices,^[3] these Ru^{II} complexes also appear to be relevant in the context of biological applications such as sensing^[4] and therapeutic agents.^[5] Among them, Ru^{II} complexes bearing the intercalating dppz ligand (dppz = dipyrrodo[3,2-*a*:2',3'-*c*]phenazine) have been studied for the last twenty-five years. Indeed, [Ru(bpy)₂dppz]²⁺ and [Ru(phen)₂dppz]²⁺ (bpy = bipyridine, phen = phenanthroline) bind readily to DNA ($K_{\text{aff}} \approx 10^6 \text{ L mol}^{-1}$) and display intense luminescence in the presence of DNA, whereas the luminescence of the complex alone is switched off in aqueous media.^[6] This "light switch" behavior has been ascribed to the population of a mixture of excited

metal-to-ligand charge-transfer states (MLCT states) where the electron located on the dppz ligand is either proximal or distal from the metallic center.^[7] In water, a quenching occurs through the formation of hydrogen bonds with the phenazine core of the dppz, whereas upon intercalation into DNA, the luminescence of the complex is restored, as observed in organic media.^[8] In addition, studies with mismatch containing DNA show correlation between the luminescence increase of [Ru(bpy)₂dppz]²⁺ and the mismatch thermodynamic stability.^[9] These environment-sensitive optical properties render dppz-based Ru^{II} complexes potent candidates as photoprobes for different DNA topologies.

Although complexes based on dppz derivatives and other ligands incorporating a phenazine core have received a lot of attention, fewer studies have been reported on complexes based on ligands incorporating an acridine core. Studies on the tpac ligand (tpac = tetrapyrrodo[3,2-*a*:2',3'-*c*:3'',2''-*h*:2'',3''-*f*]acridine)^[10] and the corresponding complexes^[11] showed that the acridine core induces notable modifications to the behavior of the resulting complex compared with the phenazine-based equivalent. Analogous to dppz, the dpac ligand (dpac = dipyrrodo[3,2-*a*:2',3'-*c*]acridine) and its Ru^{II} complex have been only recently reported and the latter was shown to be a potent nicotinamide (NAD) derivative for electron storage systems.^[12] However, its photophysical properties were not fully investigated and its interaction with DNA was not studied. Furthermore, as dpac bears one less non-chelating nitrogen atom than dppz, only one hydrogen bond with solvent molecules in the excited state would be possible, suggesting potential differences with dppz-based Ru^{II} complexes.

In this context, our work focuses on the synthesis of a series of extended planar aromatic ligands based on the acridine core (dpac (4); dpacF₂ = 7,8-difluorodipyrrodo[3,2-*a*:2',3'-*c*]acridine (5);

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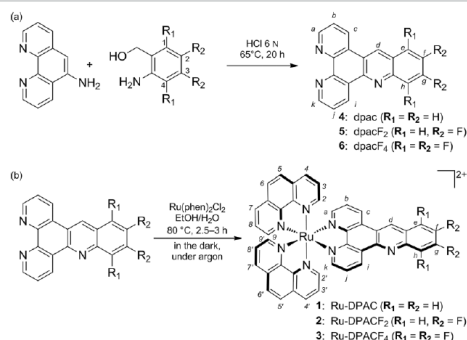


Figure 1. Synthetic scheme of dpac ligands and their corresponding Ru^{II} complexes.

$dpacF_4 = 6,7,8,9$ -tetrafluorodipyrido[3,2- a' :2',3'- c']acridine (6)), and the study of their respective Ru^{II} complexes ($Ru-DPAC$ for $[Ru(phen)_2dpac]^{2+}$ (1); $Ru-DPACF_2$ for $[Ru(phen)_2dpacF_2]^{2+}$ (2); $Ru-DPACF_4$ for $[Ru(phen)_2dpacF_4]^{2+}$ (3)) (Figure 1). In this paper, we report an extensive theoretical and experimental study on these complexes and analyze the influence of the acridine core on the photophysical properties and DNA interactions of the resulting Ru^{II} complexes compared with dppz-based complexes. We also investigate the selectivity for $Ru-DPAC$ to probe DNA mismatches.

Results and Discussion

Synthesis

The acridine core can be obtained through numerous synthetic pathways, such as Skraup cyclization,^[24] self-condensation,^[10] Cu^0 , Pd^0 , or Zn^0 -based organometallic couplings,^[25] and cyclo-additions.^[26] In our case, a Tröger's base modified synthetic scheme was found to be successful and allowed us to obtain dpac and its fluoro derivatives with high yields (Figure 1). The synthetic methodology consisted of a cyclization between 5-amino-1,10-phenanthroline and the appropriate o -aminobenzyl alcohols.^[10,27]

The corresponding Ru^{II} complexes were synthesized by the direct chelation of the extended ligand onto a Ru^{II} precursor (Figure 1) by using methodologies previously described for similar compounds. All the intermediate and final compounds were protected from direct light during the synthesis and purification steps to prevent photochemical degradation. The reactions were performed under argon to avoid any oxidation of the metal center.

The complexes were fully characterized by HRMS and 1H NMR spectroscopy (see the Experimental Section and the Supporting Information). Not all the multiplicity and coupling constants can be determined owing to the superposition of several

signals and/or lack of fine resolution for some peaks in the 1H NMR spectra. The 1H NMR spectra show unambiguously the absence of symmetry elements for all the complexes, which induces the non-equivalence of (i) the protons on the extended planar ligand and (ii) the ancillary ligands. The 1H NMR spectra are sensitive to concentration as some shifts occurred upon changes in the concentration. For solubility purposes, all the complexes were converted into the chloride salt for experiments conducted in water or buffer or to the hexafluorophosphate salt for studies in organic solvents.

Electrochemistry

The electrochemical data for the three complexes were obtained by cyclic voltammetry in dry deoxygenated MeCN or N,N -dimethylformamide (Table 1). Complexes 1–3 display a one-electron reversible oxidation wave at a potential close to that of the Ru^{2+}/Ru^{3+} oxidation of $[Ru(phen)_3]^{2+}$.^[28] This similarity between the oxidation potentials of all these complexes confirms the metal-based nature of the process (Ru^{2+}/Ru^{3+} redox couple) irrespective of the substitution on the extended planar ligand. Three reduction waves are monitored for each complex. From the comparison with the reduction potentials of $[Ru(phen)_3]^{2+}$ (–1.30 V vs. $Ag/AgCl$)^[29] and $[Ru(phen)_2dppz]^{2+}$ ($Ru-DPPZ$; –0.95 V vs. $Ag/AgCl$)^[29] the first reduction of the five complexes is attributed to the addition of one electron to the extended planar ligand. The anodic shift observed for $Ru-DPACF_4$ (–0.97 V vs. $Ag/AgCl$) indicates greater stabilization of the π^* orbital centered on the $dpacF_4$ ligand with respect to that of $Ru-DPAC$ and $Ru-DPACF_2$. The next two reduction waves are attributed to successive reduction of the two ancillary phen ligands. The potentials in the excited state (namely, E_{ox}^* and E_{red}^*) for the three complexes have been estimated from the ground-state redox potentials and the energy of the excited state corresponds to the maximum of the emission spectrum at 298 K (see below). Not surprisingly, $Ru-DPACF_4$ ($E_{red}^* =$

+1.07 V vs. Ag/AgCl) is the most oxidizing complex in the excited state.

Table 1. Potentials for the oxidation ($E_{1/2\text{ ox}}$) and reduction ($E_{1/2\text{ red}}$) of complexes 1–3.

	$E_{1/2\text{ ox}}^{[a]}$ [V vs. Ag/AgCl]	$E_{1/2\text{ red}}^{[a,b]}$ [V vs. Ag/AgCl]	$\lambda_{\text{max}}^{\text{em}}^{[c]}$ [nm]	$E_{\text{ox}}^{[c]}$ [V vs. Ag/AgCl]	$E_{\text{red}}^{[d]}$ [V vs. Ag/AgCl]
[Ru(phen)] $^{2+}$	1.32	-1.30 -1.47	604	-0.73	0.70
Ru-DPPZ $^{[30]}$	1.35	-0.95 -1.39	618	-0.67	1.02
Ru-DPAC	1.35	-1.22 -1.35	597	-0.73	0.86
Ru-DPACF $_2$	1.35	-1.66 -1.15	604	-0.71	0.91
Ru-DPACF $_4$	1.35	-1.34 -1.62 -0.97 -1.30 -1.52	609	-0.69	1.07

[a] Measured in dry acetonitrile. [b] The reversibility of the reduction processes being not able to be determined due to adsorption of the reduced species on the electrode surface, $E_{1/2}$ has been estimated from the reduction peak. [c] The excited-state oxidation potential was estimated from the ground-state oxidation potential and the energy of the emission maximum with the equation $E_{\text{ox}}^* = E_{1/2\text{ ox}} - E_{0-0} \approx E_{1/2\text{ ox}} - \Delta E_{\text{max, em}}$. [d] The excited-state reduction potential was estimated from the ground-state reduction potential and the energy of the emission maximum with the equation $E_{\text{red}}^* = E_{1/2\text{ red}} + E_{0-0} \approx E_{1/2\text{ red}} + \Delta E_{\text{max, em}}$.

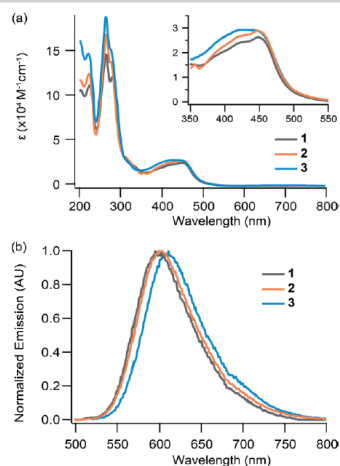


Figure 2. (a) Absorption and (b) emission spectra ($\lambda_{\text{exc}} = 448$ nm) of 1 (gray), 2 (orange), and 3 (blue) in acetonitrile under air at room temperature. Inset a: Absorption bands corresponding to MLCT transitions.

Absorption Spectra and Computational Studies

The absorption data at ambient temperature in water and acetonitrile, under air and under argon for the three complexes, as well as for other complexes for comparison purposes, are listed in Table 2. Absorption spectra for all complexes in acetonitrile are depicted in Figure 2, a. The absorption of Ru $^{\text{II}}$ complexes generally corresponds to the superposition of different transitions involving either the ligands or both the metal and the ligand. $^{[31]}$ In the present case, comparison with literature and absorption spectra of the free ligands allowed us to assign the intense absorption ($\epsilon \geq 10^5$ M $^{-1}$ cm $^{-1}$) bands in the UV region to ligand-centered (LC) transitions and the ones in the visible region ($\epsilon \approx 10^4$ M $^{-1}$ cm $^{-1}$ around $\lambda \approx 400$ nm) to metal-to-ligand charge-transfer (MLCT) transitions, where L = phen ($^1\text{MLCT}_{\text{phen}}$) and dpac ($^1\text{MLCT}_{\text{dpac}}$).

In the case of Ru-DPPZ, the absorption of a photon leads to the formation of a charge-separated state, Ru $^{3+}$ /dppz $^{-}$, in which an electron is transferred from the metal center towards either the phen or phenazine part of the dppz ligand depending on the environment of the complex. $^{[32]}$ This accounts for the peculiar photophysical behavior of Ru-DPPZ. In our case, exchanging the phenazine core with an acridine, that is, replacing a nitrogen atom with a carbon atom, should influence the electronic transitions involved in the formation of the excited state. Strikingly, a sole small bathochromic shift ($\Delta\lambda = 8$ nm) of the MLCT band is observed for 1–3 compared with Ru-DPPZ.

To get more information on the photophysical scheme of our complexes, 1–3 as well as Ru-DPPZ were studied computationally. The ground- and vertical excited-state electronic structures were investigated by means of DFT/time-dependent (TD)-DFT calculations by using the hybrid PBE0 functional and the

Table 2. Absorption data for complexes 1–3 in CH $_3$ CN and H $_2$ O at 298 K in aerated or deaerated solution.

	Absorbance λ_{max} [nm] (ϵ [10 4 M $^{-1}$ cm $^{-1}$]) $^{[a]}$	H $_2$ O
	MeCN	
[Ru(phen)] $^{2+}$	418, 447 (1.84)	419, 447 (1.83)
Ru-DPPZ $^{[30]}$	221, 264, 370, 440 (2.34)	220, 264, 368, 440
Ru-DPAC (1)	224 (15.1), 264 (16.3), 280 (11.6), 448 (2.62)	224 (15.1), 264 (16.2), 280 (11.6), 448 (2.62)
Ru-DPACF $_2$ (2)	223 (16.2), 264 (19.0), 278 (14.4), 448 (2.88)	223 (16.1), 264 (19.0), 278 (14.3), 448 (2.88)
Ru-DPACF $_4$ (3)	221 (17.1), 264 (18.9), 279 (14.0), 448 (2.87)	221 (17.0), 264 (18.9), 279 (14.1), 448 (2.88)

[a] Measurements made with solutions with 1×10^{-5} mol L $^{-1}$ of the complex at room temperature.

LanL2DZ basis set. The solvent (MeCN or H₂O) is included by a conductor-like polarizable continuum model.^[33] With this system, the first runs of calculations performed with water did not account for the experimental differences when going from acetonitrile to water. A similar observation was made by Daniel et al. who overcame the limitation of the continuum model, which does not describe specific interactions, such as nitrogen-hydrogen bond interactions, by using a discrete model.^[23] Water molecules are thus added without any constraint on their position before geometry optimization and calculations.

The energy diagram for the different compounds is presented in Figure 3 (further details on the electronic structures can be found in Tables S2–S7 in the Supporting Information). For the sake of clarity, throughout the manuscript the electron-accepting portion of the extended ligands is underlined to differentiate the two moieties that are suggested to participate in the relaxation processes. The contributions of each moiety of the complexes are depicted in different colors; in particular, the phenanthroline part of the extended ligand (dpac, green) and the terminal fragment (dpac/dppz, orange) are differentiated in order to discuss their respective contributions. Regardless of the solvent, the introduction of electron-withdrawing fluoride modifies the electronic repartition in the ground state. Although it has only a little effect on the Ru (red) and ancillary phen (blue) centered orbitals, it strongly stabilizes the levels centered on the dpac/dppz ligands. In addition, as the number

of fluoride atoms increases, the dpac contribution increases in the LUMO whereas the dpac contribution increases in the LUMO + 1.

The experimental absorption spectra matched well with the calculated transitions, allowing us to attribute the different transitions by using natural transitions orbitals (NTOs)^[34] (see Figures S19–S21). When comparing the absorption spectra of the different complexes in acetonitrile, one can observe that there are no major differences in the position and intensities of the absorption maxima. Indeed, in all cases the ¹MLCT_{phen} are the main transitions in the visible part of the spectra (400–550 nm); transitions involving the ¹MLCT_{dpac} and ¹MLCT_{dpac/dppz} of lower intensities are redshifted as the number of fluoride atoms increases, but this does not affect the final absorption spectra. A similar explanation can be used to account for the similarity between the absorption spectra in acetonitrile and water. As the polarity of the solvent increases, the levels centered on the metal and ancillary phen are equally destabilized, leaving the ¹MLCT_{phen} unchanged whereas the dpac/dppz orbitals are stabilized, leading to a redshift of the ¹MLCT_{dpac/dppz} and ¹MLCT_{dpac/dppz} transitions. These shifts are, however, small, as seen in Table 2.

Emission Spectra, Lifetimes, Quantum Yields, and Computational Studies

All our complexes display broad unstructured emission (Figure 2, b), typical of a ³MLCT-type excited state, both in organic solvent and water. The luminescence lifetimes and the quantum yields of luminescence for each complex were measured in water and acetonitrile under an inert atmosphere (Table 3). The large *k_r* values (> 10⁴ s^{−1}, Table 3), the positive solvatochromic effect (Δλ ≈ +20 nm when going from CH₂Cl₂ to water, data not shown) and the hypsochromic shift of the emission band at 77 K (see Figure S3) confirm the charge-transfer character of the excited state. It is worth mentioning that, in air-equilibrated solutions, a decrease in the luminescence intensity with respect to degassed solutions is systematically observed, indicating a quenching by oxygen (see Table S1). We expect that, as for other Ru^{II} complexes, an efficient photosensitization of ³O₂ by the excited Ru^{II} complexes is occurring.^[35] Other quenching mechanisms such as electron transfer to form superoxide anions may also occur, but are beyond the scope of this study.

The emission maxima are almost identical for Ru-DPAC and Ru-DPACF₂; the addition of the two fluoride atoms onto the

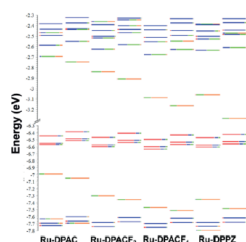


Figure 3. Energy diagram of the complexes 1–3 and Ru-DPPZ. Fragment contributions are reported with different colors: Ru in red, ancillary phen in blue, dpac/dppz in green, dpac/dppz in orange. Solvent used: left = MeCN, right = H₂O.

Table 3. Emission data for complexes 1–3.

	Emission λ _{em} max [nm] ^[a,b]		Emission λ _{max} at 77 K [nm] EtOH/MeOH, 4:1	Φ _{em} ^[a,c,d]		τ _{em} [ns] ^[d]		k _r [10 ³ s ^{−1}] ^[d]		k _i [10 ³ s ^{−1}] ^[d]	
	MeCN	H ₂ O		MeCN	H ₂ O	MeCN	H ₂ O	MeCN	H ₂ O	MeCN	H ₂ O
[Ru(phen)] ³⁺	604	606	564 ^[36]	0.028	0.072	460	920	60.9	75.0	21	9.7
Ru-DPPZ ^[30]	618	– ^a	580 ^[7d]	0.033	– ^a	663	– ^a	49.8	– ^a	15	– ^a
Ru-DPAC	597	604	567	0.038	0.066	710	999	53.5	66.1	14	9.4
Ru-DPACF ₂	604	607	573	0.052	0.189	875	958	59.4	197.3	11	8.5
Ru-DPACF ₄	609	611	568	0.084	0.050	915	238	91.8	210.1	10	40

[a] Measurements made with solutions with 1 × 10^{−5} mol L^{−1} of the complex. [b] Measurements made under ambient air conditions. [c] The quantum yield of luminescence is calculated relative to that of [Ru(bpy)₃]²⁺ in an aerated aqueous solution (Φ_{em}^{aq} = 0.028).^[36] [d] Quantum yield of luminescence, luminescence lifetimes and radiative rate constants are determined under an argon atmosphere. Errors estimated to 10 %. ^a No luminescence was observed in H₂O.

dppac ligand has thus only a small influence on the energy difference between the $^3\text{MLCT}$ and the ground state, whereas this energy decreases for Ru-DPACF_2 . This observation is in agreement with the decrease in energy of the π^* orbital centered on the extended planar ligand going to a more electron-withdrawing core. Interestingly, none of these complexes behave like Ru-DPPZ , which is known to have light-switching behavior.

Numerous experimental and theoretical studies have been devoted to understanding the light-switch effect.^[4b,7a,36] It is now commonly accepted that this quenching is caused by hydrogen-bonding with water molecules, leading to the stabilization of dark states, such as $^3\text{MLCT}_{\text{dppz}}$ and $^1\text{LC}_{\text{dppz}}$, the latter being the lowest triplet state (summarized in Figure 4, c).^[37,38] Our calculations led to similar results with a change in the nature of the lowest metal-to-ligand charge-transfer state from a bright $^3\text{MLCT}_{\text{phen}}$ state in acetonitrile to a more stabilized dark $^3\text{MLCT}_{\text{dppac}}$ state in water.

In the case of our complexes, Ru-DPAC and Ru-DPACF_2 do not present light-switching behavior whereas Ru-DPACF_4 partially does as its luminescence is notably reduced in water, as indicated by its smaller quantum yield and larger k_{nr} value in water. Once again, computational studies were used to gain some information to explain this behavior and compare it with Ru-DPPZ . The qualitative results obtained for the triplet TD-DFT calculations were used to draw the photophysical schemes presented in Figure 4 for each situation (for numeric values, see Table S8). The computational studies show that ^3LC states centered on dppac/dppz ligands have a lower energy than $^3\text{MLCT}$ states for all complexes. The presence of such ^3LC states was also reported in computational studies realized previously.^[23,35,39] Nevertheless, the experimental characteristics of emission clearly indicate that the luminescence of all Ru^{II} complexes originates from the $^3\text{MLCT}$ states and that low-lying ^3LC states are not likely to play a role in the deactivation of the $^1\text{MLCT}$ states generated upon light irradiation (see Table 3, larger quantum yield of luminescence for complexes 1–3 in MeCN compared with $[\text{Ru}(\text{phen})_3]^{2+}$). Therefore, we focus our discussion on the $^3\text{MLCT}$ manifold, which accounts for the photophysical behavior of our complexes.

In acetonitrile, all compounds follow similar trends as in Figure 4 (a). After an ultrafast ISC^[40] from $^1\text{MLCT}$ states generated upon absorption of photons, $^3\text{MLCT}$ states are populated corresponding to charge transfer from the Ru^{II} center towards either

the ancillary phen or the phen part of the planar ligand (dppac or dppz). The similar energies of these emissive $^3\text{MLCT}$ states explain the similarity of the luminescence maxima observed for all complexes.

In water, complexes 1–2 present a stronger emission and are longer lived (see Table 3). Calculations show that the relative energy of each $^3\text{MLCT}$ state is not significantly modified and the fragment contributions to each triplet state remain similar to those in MeCN; hence, these compounds behave like $[\text{Ru}(\text{phen})_3]^{2+}$ and exhibit an increased luminescence in water compared with organic solvent. Complexes 1–2 also follow the same photophysical scheme in water as in MeCN (Figure 4, a). In contrast to Ru-DPPZ , where the lowest excited states involve the phenazine core of the dppz, the acridine core in complexes 1–2 seems not to be involved and $^3\text{MLCT}$ remains localized on the ancillary ligands or the phen moiety of the dppac ligands. We thus propose for these complexes that their lowest excited states are luminescent $^3\text{MLCT}_{\text{phen/dppac}}$ states. The $^3\text{MLCT}_{\text{dppac}}$ excited states are assumed to be higher in energy than the $^3\text{MLCT}_{\text{phen/dppac}}$ ones for these two complexes (in contrast to $^3\text{MLCT}_{\text{dppz}}$ for Ru-DPPZ), suggesting the acridine core disfavors the charge-separation process in the excited state for 1–2. A similar behavior has been previously observed for TPAC-based mono- and dinuclear Ru^{II} complexes, for which the electrochemical and spectroscopic data indicate that the lowest MLCT transition is a $^3\text{MLCT}_{\text{tpac}}$ one and that dinucleation affects very slightly these properties. This suggests that the two metallic centers of the dinuclear TPAC-based Ru^{II} complexes behave independently in spite of the conjugated TPAC bridging ligand.^[11,41]

In opposition to the former examples, Ru-DPACF_4 displays a much shorter luminescence lifetime and lower quantum yield in water than in acetonitrile (Table 3). For this complex, we assume that upon absorption of light, a $^1\text{MLCT}_{\text{phen}}$ state is formed, which rapidly undergoes an ISC to a luminescent $^3\text{MLCT}_{\text{phen/dppac}}$ state. Further relaxation occurs on the acridine moiety of the extended planar ligand, leading to the formation of the $^3\text{MLCT}_{\text{dppac}}$ excited state. In water, there is an interconversion between two excited states: one assigned to the non-hydrogen-bonded excited state dppac, and the other one to a mono-hydrogen-bonded excited state (depicted as dppac-H, in Figure 4, b). The presence of the dppac-H excited state is suggested by the shorter excited state lifetime of this complex in

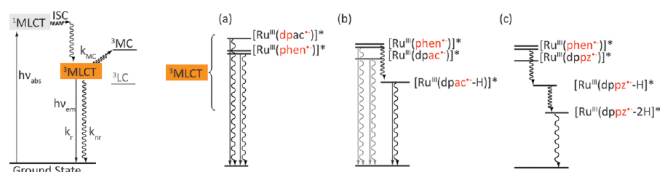


Figure 4. Representation of the photophysical scheme for dppac-based complexes in water. The deactivation of the $^3\text{MLCT}$ states is emphasized on the right part of the figure for (a) Ru-DPAC and Ru-DPACF_2 , (b) Ru-DPACF_4 , and (c) Ru-DPPZ in water. The photophysical scheme for all complexes (1–3 and Ru-DPPZ) in MeCN can be described as in (a). Solid arrows stand for radiative, wavy arrows stand for non-radiative transitions (intersystem crossing, internal conversion, or vibrational relaxation).

water. In a similar way as described for Ru-DPPZ,^[7b] we propose that the excited state localized on dpac, which is higher in energy in aprotic solvents, is stabilized when involved in a hydrogen bond and thus becomes dominant in water. Indeed, protic solvents would stabilize the charge-separated excited state owing to the formation of a hydrogen bond with the free nitrogen atom of the dpacF₄ ligand. The partial quenching of the luminescence of **3** is in agreement with the model proposed by Lincoln and co-workers for which two hydrogen bonds are required for the full luminescence quenching of the excited state of Ru-DPPZ in water. The photophysical behavior of Ru-DPACF₄ thus contrasts with the photophysical properties of Ru-DPAC and Ru-DPACF₂, which are similar to those of [Ru(phen)₃]²⁺.

Photophysical Behavior in the Presence of DNA and Polynucleotides

As mentioned in the Introduction, Ru^{II} complexes are relevant tools to probe DNA. The influence of hydrogen bonds with water on the photophysics of the complex is one of the key parameters in this context, but not the only one at stake. With the notable exception of some photoreactive complexes,^[5] polypyridyl Ru^{II} complexes are more luminescent in the presence of DNA. This enhancement of luminescence is due to the association of the complex with DNA, which protects the excited complex from quenching or non-radiative deactivations. If complexes possess one extended planar ligand, a specific interaction is observed with DNA, where the complex is intercalated inside the bases stack. This intercalation mode generally leads to strong association ($K_{\text{aff}} \approx 10^6 \text{ L mol}^{-1}$) and an intense luminescence enhancement of the excited intercalated complex in comparison to the "free" complex left in aqueous media.^[7c,8,29,42] Interestingly, Ru-DPPZ is able to distinguish matched and single-base mismatched sites in oligonucleotides. This small sensitivity of the luminescence enhancement in the presence of a mismatch can be correlated to its thermodynamic stability.^[9] We thus investigated the ability of our new complexes to probe DNA and some particular topologies, such as different single-base mismatches.

In the presence of increasing concentrations of DNA (CT-DNA and SS-DNA), the luminescence of the dpac complexes **1–3** is enhanced (Figure 5). This increase in luminescence for the three complexes is strong and occurs even at a very low ratio of DNA per complex, suggesting a high binding affinity of the complexes for these two DNA types. To determine the binding affinity for these equilibria, we used a modified McGhee–von Hippel equation^[43] as an expression for the luminescence intensity as a function of the ratio of Ru^{II} per DNA (I/I_0 vs. [DNA]/[complex]), with I_0 the intensity of luminescence in the absence of DNA and [DNA] expressed in base-pair equivalents, see Table 4 and Figure 5). The luminescence enhancement observed presumably results from a decrease in the non-radiative deactivation processes of the ³MLCT excited states. This protection is induced by the double helix microenvironment, which protects the excited complexes from quenching by oxygen. As our extended planar ligands are similar to dpz in terms of structure,

we assume our complexes also intercalate into the DNA strands, which is consistent with the binding values obtained.

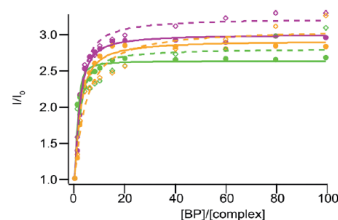


Figure 5. Steady-state luminescence titration ($\lambda_{\text{exc}} = 448 \text{ nm}$) of **1** (green), **2** (purple), and **3** (orange) with SS-DNA (full circles) and CT-DNA (empty diamonds). Measurements were performed by using $10 \mu\text{M}$ of complex in 50 mM Tris-HCl buffer, with 50 mM NaCl at pH 7.4 under ambient air conditions. The fitted curves were obtained by using a modified McGhee–von Hippel equation and drawn as solid and dashed curves for SS- and CT-DNA, respectively.

Table 4. Binding affinity constants calculated for complexes **1–3** with SS-DNA and CT-DNA.

	SS-DNA $K_{\text{aff}} [\text{L mol}^{-1}]^{\text{[a]}}$	$I/I_0 \text{ max}$	CT-DNA $K_{\text{aff}} [\text{L mol}^{-1}]^{\text{[a]}}$	$I/I_0 \text{ max}$
Ru-DPAC	1.8×10^6	2.6	4.2×10^5	2.8
Ru-DPACF ₂	4.8×10^5	3.0	3.7×10^5	3.2
Ru-DPACF ₄	3.5×10^5	2.9	1.5×10^5	3.1

[a] Binding constant were obtained by using a McGhee–von Hippel-type equation; the binding site was fixed to two base pairs per complex (best fit). Errors estimated to 5 %.

Even if these two natural DNAs contain nearly the same content of G–C base pairs (43 % for SS-DNA vs. 42 % for CT-DNA),^[44] the luminescence behavior is slightly different for the complexes **1–3** in the presence of each DNA. Such an observation could arise from the different topologies of these two DNAs: CT-DNA features a long and well-defined scaffold (> 10 000 base pairs)^[45] whereas SS-DNA is made of shorter (ca. 2000 base pairs)^[46] and poorly defined sequences. The interaction between the complexes and the DNA sequences depends on the local environment encountered by the intercalated complex;^[47] the heterogeneity of these environments results in small but noticeable differences in the optical properties of the complexes. The binding curves obtained are thus affected by this heterogeneity, but overall the binding constants calculated are in good agreement with an intercalation mode of interaction between each of our complexes and DNA.

In the case of SS-DNA, the emission intensity increases quickly to reach a plateau value, between 2.6 and 2.9 for the three complexes, the fluorinated analogs **2** and **3** display slightly higher I/I_0 values than the non-fluorinated complex **1**.

We also investigated the ability of Ru-DPAC to detect base mismatches in short oligonucleotides by using a series of hairpins oligonucleotide containing a mismatch near the center of the duplex (see sequences in Figure 6). As shown in Figure 7, an attenuation of the luminescence is observed for the mismatched hairpins with respect to the complementary one. No

differential luminescence intensity occurs related to the nature of the mismatch. The absence of specificity for the nature of the mismatch sites, and, more surprisingly, the lower luminescence enhancement of the complex in the presence of mismatched hairpins compared with the complementary one led us to the conclusion that Ru-DPAC is less effectively intercalated between the mismatched base pair stack. This less effective intercalation results in increased exposure to oxygen with respect to the complementary sites; a looser fit of the complex within the mismatch site favors non-radiative relaxation pathways of the excited state, leading to a decreased luminescence and/or a lower binding affinity for these sites compared with complementary sites. As saturation of the interaction between the complex and the hairpins cannot be obtained, it is difficult to differentiate the processes at stake in this case. The behavior observed for Ru-DPAC differs from the one reported for Ru-DPPZ^[3] under the same conditions. This fundamental difference underlines the complexity of designing complexes able to probe mismatched sites in DNA.

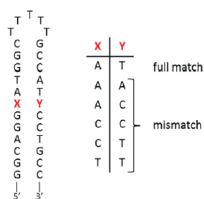


Figure 6. Hairpin oligonucleotide sequences used for titrations.

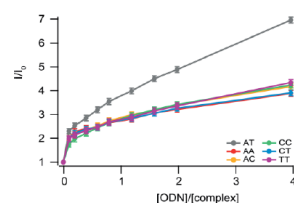


Figure 7. Oligonucleotides possessing (or not) a mismatched base pair. Measurements were performed by using 1 μ M of complex in 5 mM Tris-HCl buffer, with 1 mM NaCl, at pH 7.5 under ambient air conditions.

Conclusions

We successfully synthesized and characterized new Ru^{II} complexes with extended planar ligands based on an acridine core. Their spectroscopic and electrochemical data revealed that 1–2 allow emission from an ³MLCT_{phen/dpac} excited state, which explain the similarity of the luminescence maxima. As only minor reorganizations of the different triplet excited states of 1–2 occur in water, these compounds behave like [Ru(phen)₃]²⁺ and

exhibit increased luminescence in this solvent. However, the lowest ³MLCT state for Ru-DPACF₄ changes from a bright ³MLCT_{phen/dpac} state to a poorly luminescent ³MLCT_{dpac} state. The stabilization of this state compared with the other ³MLCT states occurs in water through the establishment of a hydrogen bond; the relative population of the excited state is then shifted to this state and the emissive ³MLCT_{phen/dpac} states are less populated, yielding a reduction of the luminescence of Ru-DPACF₄ in water compared with organic solvents.

Studies of these acridine-based complexes in the presence of DNA demonstrate that they strongly bind to polynucleotides and that this association, attributed to an intercalation within the base pair stacking, is accompanied by an enhancement in their luminescence. This enhancement is presumably due to a decrease in the non-radiative deactivation processes of the ³MLCT excited state of complexes intercalated in the double helix, where the microenvironment protects them from quenching by oxygen. In the case of Ru-DPACF₄, an inhibition of hydrogen bonds between the tetrafluoroacridine and water is likely to be responsible for the luminescence enhancement upon intercalation through DNA; this process is the same as the one responsible for the light-switching behavior of the well-known Ru-DPPZ complex. Ru-DPAC shows differential luminescence intensity for matched and mismatched hairpins, but no discrimination for the nature of the mismatch site. We suggest that a looser fit to the mismatched containing hairpins lies at the basis of the observed luminescence behavior of this complex, resulting in an increased exposure to oxygen with respect to the complementary sites. The design of extended planar ligands allowing better differential interactions between their corresponding Ru^{II} complexes and polynucleotides is currently under investigation in our group.

Experimental Section

Materials and Instrumentation: [Ru(phen)₃Cl₂]^[13] was synthesized according to a previously described literature protocol. All solvents and reagents for the synthesis were of reagent grade and were used without any further purification. All solvents for the spectroscopic and electrochemical measurements were of spectroscopic grade. Water was purified with a Millipore Milli-Q system. Calf thymus DNA type I (CT-DNA) and salmon sperm DNA (SS-DNA) were purchased from Sigma-Aldrich. Hairpin oligonucleotides (ODNs) were purchased from Eurogentec. DNA and ODN concentrations were determined spectroscopically ($\lambda_{260\text{ nm}} = 6600\text{ M}^{-1}\text{ cm}^{-1}$ /bp for CT-DNA and SS-DNA;^[14] $\lambda_{260\text{ nm}} = 260\,000\text{ M}^{-1}\text{ cm}^{-1}$ for ODN-AT, 264\,900 $\text{M}^{-1}\text{ cm}^{-1}$ for ODN-AA, 253\,300 $\text{M}^{-1}\text{ cm}^{-1}$ for ODN-CC, 259\,100 $\text{M}^{-1}\text{ cm}^{-1}$ for ODN-AC, 254\,200 $\text{M}^{-1}\text{ cm}^{-1}$ for ODN-CT, and 257\,500 $\text{M}^{-1}\text{ cm}^{-1}$ for ODN-TT). The molar extinction coefficients of the hairpin oligonucleotides were calculated based on the base content of each sequence. ¹H NMR experiments were performed in CDCl₃, CD₃OD, or CD₃CN with a Bruker AC-300 Avance II (300 MHz) or a Bruker AM-500 (500 MHz) at 20 °C. The chemical shifts (given in ppm) are measured versus the residual peak of the solvent as the internal standard. High-resolution mass spectrometry (HRMS) spectra were recorded with a Q-Exactive orbitrap from Thermo-Fisher by using reserpine as the internal standard. Samples were ionized by electrospray ionization (ESI); capillary temperature = 320 °C, vaporizer temperature = 320 °C, sheath gas flow rate = 5 mL

min⁻¹). UV/Vis absorption spectra were recorded with a Shimadzu UV-1700. Room temperature fluorescence spectra were recorded with a Varian Cary Eclipse instrument. Luminescence intensity at 77 K was recorded with a FluoroLog3 FL3-22 from Jobin Yvon equipped with an 18 V 450 W Xenon Short Arc lamp and an R928P photomultiplier, using an Oxford Instrument Optistat DN nitrogen cryostat controlled by an Oxford Intelligent Temperature Controller (ITC503S) instrument. For the luminescence lifetime measurements as a function of temperature, the pulsed excitation source was a pulsed laser Nd:YAG Q-switched laser (Continuum Inc.) frequency-tripled (355 nm) coupled with an optical parametric oscillator (Continuum Inc.) covering the wavelength region 410–2300 nm with a maximum pulse energy from 10 to 120 mJ depending on the wavelength. The emission was detected perpendicularly by a photomultiplier (R928, Hamamatsu). The signal was recorded with a digital oscilloscope (HP 54200A), connected through the IEEE488 interface to a personal computer, and was averaged over at least 16 shots. The emission wavelength was selected by a grating Czerny–Turner monochromator (Spectra Pro 2300i, Acton Research Corp.). Cyclic voltammetry was carried out in a one-compartment cell, using a glassy carbon disk working electrode (approximate area = 0.03 cm²), a platinum wire counter electrode, and an Ag/AgCl reference electrode (salt bridge: 3 mol L⁻¹ NaCl/saturated AgCl). The potential of the working electrode was controlled by an Autolab PGSTAT 100 potentiostat through a PC interface. The cyclic voltammograms were recorded with a sweep rate of 300 mV s⁻¹, in dried acetonitrile or *N,N*-dimethylformamide (Sigma-Aldrich, HPLC grade). The concentration of the complexes was 8 × 10⁻⁴ mol L⁻¹, with 0.1 mol L⁻¹ tetrabutylammonium perchlorate as supporting electrolyte. Before each measurement, the samples were purged with nitrogen. The molar absorption coefficients were determined by ¹H NMR spectroscopy by using 2,2'-(perfluoroethane-1,2-diyl)bis(oxy)bis(2,2-difluoroethan-1-ol) as a reference. DFT calculations were performed by using Gaussian 09.^[15] The hybrid functional PBE0 was used to carry out the DFT^[16] and TD-DFT^[17] calculations in combination with the LanL2DZ^[18] basis set for all atoms. Geometry optimizations were conducted without symmetry constraints. Frequency calculations on each optimized structure confirmed that the energy minima was reached. The energy, oscillator strength, and related MO contributions for the 100 lowest singlet-singlet and ten lowest singlet-triplet excitations for the S₀-optimized geometry were obtained, respectively, from the TD-DFT/singlets and the TD-DFT/triplets output files. One should note that the PBE0 functional, although providing the correct excitation energies, is not able to fully reproduce their relative intensities. This behavior has already been noticed in the literature in the case of even simpler compounds and different functionals and more elaborated approaches have been used in cases where the band shape is of interest, which was actually not the case in the present study.^[19] Gauss-View 3.0.9,^[20] GaussSum 3.0,^[21] and Chemission 4.38^[22] software were used for data analysis, visualization, and surface plots. Calculations were performed in MeCN or H₂O solution by use of the conductor-like polarized continuum (CPCM) solvation model as implemented in Gaussian 09.^[15] Because the continuum model could not describe specific interactions such as nitrogen–hydrogen-bond interactions, a discrete model is also used in an approach similar to that reported by Daniel et al. recently.^[23]

Synthetic Procedures and Characterization of dpac Ligands and Respective Ru^{II} Complexes

2-Aminobenzyl Alcohol: A solution of LiAlH₄ in dry Et₂O (300.0 mg in 7.5 mL) was added dropwise to a solution of anthranilic acid (617.3 mg, 4.50 mmol) in dry Et₂O (30 mL). The mixture was then stirred at reflux (35 °C) for 1 h. After cooling to room temperature,

EtOAc (25 mL) was added, followed by a solution of NaOH 2 M until effervescence stops, and MilliQ water (40 mL). The organic phase was separated and the aqueous phase was extracted with Et₂O (3 × 30 mL). The combined organic phases were dried with MgSO₄, evaporated, and dried under vacuum. The desired compound was obtained as a beige solid, yield 93 %. ¹H NMR (300 MHz, CDCl₃): δ = 7.15 (ddd, *J*_{c-d} = 8.0, *J*_{c-b} = 8.0, *J*_{c-a} = 1.6 Hz, 1 H, H_c), 7.07 (dd, *J*_{a-b} = 7.6, *J*_{a-c} = 1.6 Hz, 1 H, H_a), 6.70–6.75 (m, 2 H, H_b and H_d), 4.70 (s, 2 H, CH₂), 3.19 (br, 2 H, NH₂) ppm.

dpac (4): 5-Amino-1,10-phenanthroline (183.0 mg, 0.938 mmol) and 2-aminobenzyl alcohol (115.6 mg, 0.938 mmol) were suspended in 6 N HCl (3.0 mL). The reaction mixture was stirred at reflux (65 °C) for 20 h and, after cooling to room temperature, the reaction was quenched by addition of NH₃·H₂O until reaching pH 9. The orange precipitate was filtered and purified by using a neutral alumina column for chromatography (eluent: acetone/methanol gradient, from 100:0 to 90:10), yielding the targeted compound as a dark-orange solid, yield 93 %. ¹H NMR (500 MHz, CD₃OD): δ = 9.77 (dd, *J*_{c-b} = 8.3, *J*_{c-a} = 1.8 Hz, 1 H, H_c), 9.68 (s, 1 H, H_d), 9.30 (dd, *J*_d = 8.3, *J*_{d-a} = 1.5 Hz, 1 H, H_d), 9.14 (dd, *J*_{a-b} = 4.5, *J*_{a-c} = 1.8 Hz, 1 H, H_a), 9.08 (dd, *J*_a = 4.5, *J*_{a-c} = 1.6 Hz, 1 H, H_a), 8.34 (d, *J*_{e-f} = 8.7 Hz, 1 H, H_e), 8.26 (d, *J*_{e-g} = 7.6 Hz, 1 H, H_e), 7.95 (dd, *J*_{f-g} = 8.5, *J*_{f-h} = 6.9 Hz, 1 H, H_f), 7.89 (2dd, *J*_{b-c} = *J*_i = 8.0, *J*_{b-a} = *J*_k = 4.5 Hz, 2 H, H_b and H_j), 7.76 (t, *J*_{g-h} = 7.2 Hz, 1 H, H_g) ppm. MS (APCI, +c): calcd. 282.10 for C₁₉H₁₂N₃, found 282.27 [M + H]⁺.

(2-Amino-4,5-difluorophenyl)methanol: A solution of LiAlH₄ in dry Et₂O (78.5 mg in 2.0 mL) was added dropwise to a solution of 4,5-difluoroanthranilic acid (200.0 mg, 1.155 mmol) in dry Et₂O (8.0 mL). The mixture was then stirred at reflux (35 °C) for 1 h 15 min. After cooling to room temperature, EtOAc (10 mL) was added, followed by a solution of NaOH (2 M) until effervescence stopped, and MilliQ water (15 mL). The organic phase was separated and the aqueous phase was extracted with Et₂O (3 × 10 mL). The combined organic phases were dried with MgSO₄, evaporated, and dried under vacuum. The desired compound was obtained as a flaky yellowish-brownish solid, yield 91 %. ¹H NMR (300 MHz, CDCl₃): δ = 6.92 (dd, *J*_{a-b} = 10.5, *J*_{a-c} = 8.6 Hz, 1 H, H_a), 6.50 (dd, *J*_{a-c} = 11.8, *J*_{a-b} = 6.8 Hz, 1 H, H_a), 4.61 (s, 2 H, CH₂), 4.12 (br, 2 H, NH₂) ppm.

dpacF₂ (5): 5-Amino-1,10-phenanthroline (50.0 mg, 0.256 mmol) and (2-amino-4,5-difluorophenyl)methanol (40.8 mg, 0.256 mmol) were suspended in 6 N HCl (1.0 mL). The reaction mixture was stirred at reflux (65 °C) for 20 h and, after cooling to room temperature, the reaction was quenched by addition of NH₃·H₂O until reaching pH 9. The orange-red precipitate was filtered and purified by using a neutral alumina column for chromatography (eluent: acetone/methanol gradient, from 100:0 to 90:10), yielding the targeted compound as an orange solid, yield 98 %. ¹H NMR (300 MHz, CDCl₃): δ = 9.70 (dd, *J*_{c-b} = 8.2, *J*_{c-a} = 1.9 Hz, 1 H, H_c), 9.27 (dd, *J*_{a-b} = 4.5, *J*_{a-c} = 1.8 Hz, 1 H, H_a), 9.25 (s, 1 H, H_d), 9.23 (dd, *J*_d = 4.4, *J*_{d-a} = 1.5 Hz, 1 H, H_d), 8.96 (dd, *J*_{e-f} = 8.3, *J*_{e-g} = 1.6 Hz, 1 H, H_e), 8.10 (dd, *J*_{e-f} = 10.9, *J*_{e-g} = 7.6 Hz, 1 H, H_e), 7.78 (m, 3 H, H_b, H_j and H_k) ppm. MS (APCI, +c): calcd. 318.08 for C₁₉H₁₀F₂N₃, found 318.55 [M + H]⁺.

(2-Amino-3,4,5,6-tetrafluorophenyl)methanol: A solution of LiAlH₄ in dry Et₂O (76.5 mg in 2.0 mL) was added dropwise to a solution of 2-amino-3,4,5,6-tetrafluorobenzoic acid (200.0 mg, 0.9565 mmol) in dry Et₂O (8.0 mL). The mixture was then stirred at reflux (35 °C) for 2 h 10 min. After cooling to room temperature, EtOAc (10 mL) was added, followed by a solution of NaOH (2 M) until effervescence stopped, and MilliQ water (15 mL). The organic phase was separated and the aqueous phase was extracted with Et₂O (3 × 10 mL). The combined organic phases were dried with MgSO₄, evaporated, and dried under vacuum. The desired com-

924.08193 for $[\text{C}_{43}\text{H}_{25}\text{N}_7\text{F}_8\text{PRu}]^+$, found 924.08240 ($[\text{M} - \text{PF}_6]^-$) and calcd. 389.55860 for $[\text{C}_{43}\text{H}_{25}\text{N}_7\text{F}_2\text{Ru}]^{2+}$, found 389.55897 ($[\text{M} - 2\text{PF}_6]^{-2+}$).

dpacF₆ (6): 5-Amino-1,10-phenanthroline (167.1 mg, 0.8568 mmol) and (2-amino-3,4,5,6-tetrafluorophenyl)methanol (167.1 mg, 0.8568 mmol) were suspended in 6 N HCl (2.0 mL). The reaction mixture was stirred at reflux (60 °C) for 2.0 h. After cooling to room temperature, methanol was quenched by addition of 10 mL MeOH. The resulting orange-red precipitate was added to NH₃·H₂O until reaching pH 7. The orange-red precipitate was filtered and purified by using a neutral alumina column for chromatography (eluent: acetone/methanol gradient, from 100:0 to 90:10). After evaporation of the solvents, the residue was washed with chloroform. The precipitate was filtered and the filtrate was evaporated, yielding the targeted compound as an orange-yellow solid, yield 94 %. ¹H NMR (300 MHz, CDCl₃): δ = 9.77 (dd, *J*_{HH} = 8.1, *J*_{HF} = 1.7 Hz, 1 H, H₉), 9.55 (s, 1 H, H₈), 9.31 (dd, *J*_{HH} = 4.5, *J*_{HF} = 1.7 Hz, 1 H, H₇), 9.27 (dd, *J*_{HH} = 4.4, *J*_{HF} = 1.7 Hz, 1 H, H₆), 9.05 (dd, *J*_{HH} = 8.2, *J*_{HF} = 1.5 Hz, 1 H, H₄), 7.83 (m, 2 H, H₃ and H₅) ppm. MS (APCI, calc): 354.07 for C₁₂H₆F₄N₂, found 354.13 [M + H]⁺.

Ru-DPAFC (3): One equivalent of dichloro precursor Ru(pheh)₂Cl₂ (11.6 mg, 0.0218 mmol) was mixed with 1.3 equiv. of dpacF₂ (6) (10.0 mg, 0.0283 mmol) in a mixture of absolute ethanol/water (50:50, 2.0 mL). The reaction mixture was stirred under an argon atmosphere at reflux (80 °C) and the reaction was followed by absorption spectroscopy. After 2 h 30 min, the medium was concentrated and addition of small portions of NH₄PF₆ and drops of water yielded a red precipitate. After centrifugation, the solid was washed with excess water. After drying under vacuum, the orange-red residue was purified by HPLC (XBridge C18 100 × 100 mm, 5 μm, gradient from H₂O/CH₃CN, 90:10 + 0.1% TFA to CH₃CN + 0.1% TFA, elution time: 3.07 min). The counter-anion exchange from PF₆ to Cl was performed by eluting a solution of the complex in a mixture of CH₃CN/H₂O on Sephadex DEAE A25. ¹H NMR (PF₆ salt, 5.00 MHz, CD₃CN): δ = 9.97 (s, 1 H, H_A), 9.68 (dd, 1 H, δ _A), 8.3, 8.3, 8.3, 8.3, 1.2 Hz, 1 H, H_A), 9.33 (dd, $J_{AB} = 7.5$ Hz, 1.0 Hz, 1 H, H_A), 8.61 (2dd, $J_{AB} = J_{AC} = 8.6$, $J_{BC} = 1.2$, 2 H, 4 H, H_A, H_B, H_C, H_D), 8.26 (ab system, 4 H, H_B, H_C, H_D, 8.1), 8.19 (dd, $J_{AB} = 5.2$, $J_{AC} = 1.2$ Hz, 1 H, H_A), 8.12 (dd, $J_{AB} = 5.3$, $J_{BC} = 1.2$ Hz, 1 H, H_B), 8.10 (dd, $J_{AB} = 5.3$, $J_{BC} = 1.3$ Hz, 1 H, H_A), 8.04 (dd, $J_{AB} = 5.4$, $J_{AC} = 1.0$ Hz, 1 H, H_A), 8.00 (m, 2 H, H_B, H_C), 7.73 (7dd, $J_{AB} = 5.4$, $J_{BC} = 1.3$ Hz, 8.3 Hz, 2 H, H_A), 7.65 (m, 4 H, H_B, H_C, H_D, 8.1) ppm. HRMS (ESI⁺): calcd. 960.06309 for [C₃₂H₂₈N₆Fe₁₀P₁₀Ru]⁺, found 960.06347 (M - PF₆)⁺; calcd. 947.54918 for [C₃₂H₂₈N₆Fe₁₀Ru]²⁺, found 947.54953 (M - 2 PF₆)²⁺.

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Ru-DPAC2 (2): One equivalent of dichloro precursor Ru(phen)₂Cl₂ (10.0 mg, 0.0188 mmol) was mixed with 1.5 equiv. of dpac2 (5) (8.9 mg, 0.028 mmol) in a mixture of absolute ethanol/water (50:50, 2 mL). The mixture was stirred under vacuum under an argon atmosphere, then reflux (80 °C) and the reaction was followed by absorption spectroscopy. After 31 min the medium was concentrated by absorption of small portions of NH₄PF₆ and drops of water yielded a red precipitate. After centrifugation, the solid was washed with excess water. After drying under vacuum, the orange-red residue was purified by preparative plate chromatography (silica, eluent: CH₂Cl₂/CH₂O/saturated aqueous NH₄Cl 4:3:1, R_f = 0.28). The counter-anion exchange from PF₆ to Cl was performed by eluting a solution of the complex in a mixture of CH₂Cl₂/H₂O on Sephadex DEAE A25 ⁺ HM (PF₆ salt, 500 MHz; CD₃CO₂H δ = 9.77 (1 H, H₁), 9.67 (dd, 1 H = 8.2, J_{H1}, 1.3 Hz, 1 H, H₂), 9.22 (dd, $J_{\text{C-H}}$ = 8.5, C₂H₂ = 1.1 Hz, H₃), 8.61 (dd, 1.3 Hz, 1 H, H₄) = 8.5, J_{H4}, 1.3 Hz, 4 H, H₅^H, H₅^H, H₇^H, H₇^H), 8.26 (AB system, 4 H, H₅^H, H₅^H, H₆^H, H₆^H), 8.17 (m, 4 H, H₆^H, H₆^H, H₇^H, H₇^H), 8.06 (dd, 1 H = 5.3, J_{H4} = 1.3 Hz, 1 H, H₄), 8.01 (m, 3 H, H₄, H₅^H, H₅^H), 7.72 (2 d, $J_{\text{H-H}}$ = 4, $J_{\text{H-H}}$ = 8.3, $J_{\text{H-H}}$ = 5.4 Hz, 2 H, H₃^H), 7.63 (m, 4 H, H₃^H, H₃^H, H₄^H, H₄^H), ppm. HMRS (ESI, +): calcd.

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Photochemical Tuning of Tris-Bidentate Acridine- and Phenazine-Based Ir(III) Complexes

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Abstract Five new Ir(III) complexes of the type $[\text{Ir}(\text{ppy})_2\text{L}]^+$ (where ppy = 2-phenylpyridine, L = bidentate N^N ligand) bearing linear and elbow-shaped acridine- and phenazine-based extended planar aromatic ligands have been successfully synthesized and characterized. The electrochemical and photochemical studies revealed that all complexes allow emission in the range 589–601 nm from excited states corresponding to a charge transfer between an Ir-ppy fragment and the extended planar ligand. Luminescence quenching occurs in water for $[\text{Ir}(\text{ppy})_2\text{dpac}]^+$ (Ir-DPAC), $[\text{Ir}(\text{ppy})_2\text{dpacF}_2]^+$ (Ir-DPACF₂), $[\text{Ir}(\text{ppy})_2\text{dpacF}_4]^+$ (Ir-DPACF₄) and $[\text{Ir}(\text{ppy})_2\text{bdppz}]^+$ (Ir-BDPPZ), while solely partial quenching is observed for $[\text{Ir}(\text{ppy})_2\text{npp}]^+$ (Ir-NPP). This “light-switch” effect has been ascribed to the possible formation of a non-emissive mono-hydrogen-bonded excited state for the four complexes. The “elbow shaped” of Ir-NPP is believed to prevent the non-chelating nitrogen atom of the npp ligand to form H-bond with solvent molecules. The results emphasized the potential of small chemical modifications of the extended planar ligand on the properties of the corresponding Ir(III) complexes. Their tunable properties make them ideal candidates for applications such as DNA photoprobes.

Keywords Iridium · Acridine · Polypyridyl complexes · Luminescence

Introduction

Polypyridyl transition metal complexes bearing dpz (= dipyrrodo[3,2-*a*:2',3'-*c*]phenazine) derivatives as extended planar ligand has been the focus of much attention for the last decades [1–4]. Notably, $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ and $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ (phen = 1,10-phenanthroline, bpy = 2,2'-bipyridine) display environment-sensitive luminescence properties which make them candidates as photoprobes for DNA topologies and mismatches [5, 6]. Indeed, these complexes exhibit luminescence quenching in aqueous media, which is then restored upon addition of DNA. The understanding of the under-lying photophysics of this “light switch” behavior received a lot of attention and lead to numerous studies [7–10]. The formation of hydrogen bonds between the nitrogen atoms from the phenazine core of the dpz with solvent molecules lies at the origin of the luminescence quenching process in the excited state. Upon binding to DNA, the luminescence of the complex is restored due to the inhibition of the formation of the H-bonds [9]. While Ru(II) complexes bearing dpz derivatives and other phenazine-based ligands received a lot of attention, complexes based on acridine ligands remain poorly described [11, 12].

In that context, we recently published extensive photophysical studies on Ru(II) complexes based on dpac (= dipyrrodo[3,2-*a*:2',3'-*c*]acridine) derivatives [12, 13]. These derivatives exhibit suitable optical properties for applications as DNA photoprobes. In addition, complexes bearing npp (= naphtho[1,2-*b*]pyrido[3,2-*f*][1,

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7]phenanthroline) and bdppz (= benzo[*h*]dipyrido[3,2-*a*:2',3'-*c*]phenazine), qualified as “elbow-shaped” ligands, particularly show ability to discriminate between matched and mismatched DNA sequences [13].

In order to tune the photochemical properties of these acridine-based complexes, we were interested in replacing the metal cation from Ru(II) to Ir(III). Indeed, the optical properties of Ir(III) complexes are more tunable than their Ru(II) counterparts [2, 14, 15]. Notably, $[\text{Ir}(\text{ppy})_2\text{L}]^+$ (ppy = 2-phenylpyridine, L = bidentate N²N ligand) complexes have been the subject of increasing attention due to their suitable optical properties for application as DNA and oligonucleotides photoprobes. It has been shown that $[\text{Ir}(\text{ppy})_2\text{dppz}]^+$ exhibits luminescence enhancement in the presence of DNA, whereas its luminescence is quenched in the absence of genetic material. Such behavior has been ascribed to the formation of H-bonds with solvent molecules in the excited state, as for $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ and $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$, which are then prevented upon DNA binding [2]. Analogous to dppz, the dpac ligand (Fig. 1) bears one less non-chelating nitrogen atom, leading thus to the possible formation of only one H-bond with solvent molecules in the excited state, and consequently to potential differences with dppz-based complexes.

In this context, we prepared Ir(III) complexes bearing extended planar aromatic ligands based on an acridine core (dpac; dpacF_2 = 7,8-difluorodipyrido[3,2-*a*:2',3'-*c*]acridine; dpacF_4 = 6,7,8,9-tetrafluorodipyrido[3,2-*a*:2',3'-*c*]acridine). The study of the photochemical properties of the corresponding Ir(III) complexes ($[\text{Ir}(\text{ppy})_2\text{dpac}]^+$ (Ir-DPAC), $[\text{Ir}(\text{ppy})_2\text{dpacF}_2]^+$ (Ir-DPACF₂), $[\text{Ir}(\text{ppy})_2\text{dpacF}_4]^+$ (Ir-DPACF₄), Fig. 1) both in the absence and in the presence of DNA has been achieved. In order to tune the DNA binding of these derivatives, the “elbow-shaped” $[\text{Ir}(\text{ppy})_2\text{npp}]^+$ (Ir-NPP) and $[\text{Ir}(\text{ppy})_2\text{bdppz}]^+$ (Ir-BDPPZ) complexes have also been prepared (Fig. 1). In this paper, we report a detailed photochemical study of these five complexes and analyze the influence of the acridine core on the photophysical properties compared to the phenazine equivalents.

Results

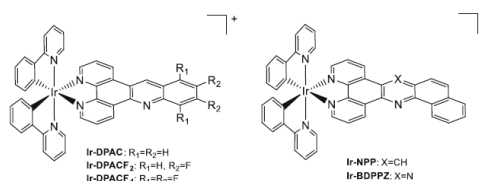
Synthesis Acridine derivatives, i.e. dpac, dpacF_2 , dpacF_4 and npp ligands, have been obtained through a Tröger's base modified synthetic scheme [12, 13]. Bdppz is obtained through condensation between 1,10-phenanthroline-5,6-dione and 1,2-diaminonaphthalene [13]. Commercially available 2-phenylpyridine was used as ancillary ligands.

The corresponding Ir(III) complexes have been prepared by the direct chelation of the extended ligand onto a dichloro-bridged Ir(III) dimer (Scheme 1), $[\text{Ir}(\text{ppy})_2\text{Cl}]_2$, using methodologies previously described for similar compounds [16, 17]. All the intermediate and final compounds are protected from direct light during the synthesis and purification steps to prevent photochemical degradation. The reactions are performed under argon to avoid any side products due to the presence of oxygen.

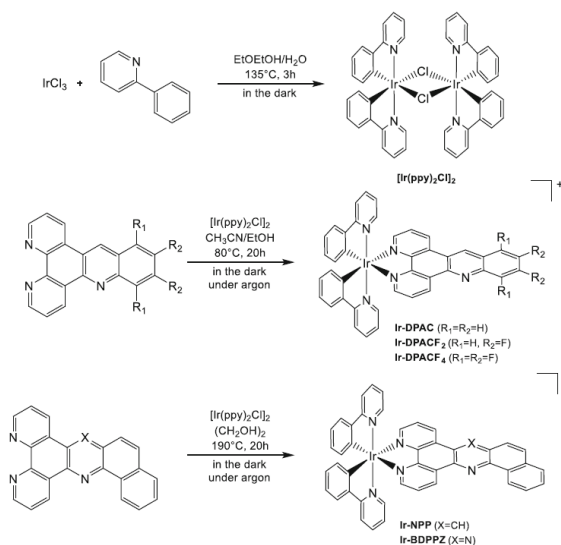
The complexes have been fully characterized by HRMS and ¹H NMR spectroscopy. All the multiplicity and coupling constants cannot be determined due to the superposition of several signals and/or lack of fine resolution for some peaks in the ¹H NMR spectra. The ¹H NMR shows unambiguously the absence of symmetry elements for all the complexes, which induces the non-equivalence of (i) the protons on the extended planar ligand and (ii) the ancillary ligands. The ¹H NMR spectra does not seem sensitive to concentration as only minor changes occurred in the ¹H NMR spectra upon change in the concentration. For solubility purposes, all the complexes are converted to the chloride salt for experiments conducted in aqueous media, or to the hexafluorophosphate salt for studies in organic solvents.

Electrochemistry The electrochemical data of the five Ir(III) complexes are obtained by cyclic voltammetry in dry deoxygenated CH₃CN or DMF (Table 1). For comparison purposes, the data of other complexes are also gathered in the table. All five complexes display a one-electron reversible oxidation wave at 1.30–1.35 V vs Ag/AgCl. This similarity between the oxidation potentials of all these complexes suggests a metal- and/or ancillary ligand-based nature of the oxidation process [2, 18], irrespective of the substitution on the extended planar ligand.

Fig. 1 Structure of Ir-DPAC, Ir-DPACF₂, Ir-DPACF₄, Ir-NPP and Ir-BDPPZ complexes



Scheme 1 Synthetic scheme of Ir-DPAC, Ir-DPACF₂, Ir-DPACF₄, Ir-NPP and Ir-BDPPZ



Indeed, while $[\text{Ir}(\text{ppy})_3]^{3+}$ displays a higher oxidation potential due to the stronger electron-withdrawing character of the three bpy ligands, it is the opposite for $[\text{Ir}(\text{ppy})_3]$. The ppy ligand, consequently to its C-coordinating atom,

is strongly electron-donating and thus raises the level of the HOMO of the complex. Based on our data, we suggest, as others, it involves an Ir-ppy fragment due to the strong covalency of the carbon-iridium bond [17, 19].

Table 1 Potentials for the oxidation ($E_{1/2 \text{ ox}}$) and reduction ($E_{1/2 \text{ red}}$) of Ir-DPAC, Ir-DPACF₂, Ir-DPACF₄, Ir-NPP and Ir-BDPPZ

Complex	$E_{1/2 \text{ ox}}^a$ [V vs Ag/AgCl]	$E_{1/2 \text{ red}}^b$ [V vs Ag/AgCl]			E_{ox}^{*c} [V vs Ag/AgCl]	E_{red}^{*d} [V vs Ag/AgCl]
		1	2	3		
$[\text{Ir}(\text{bpy})_3]^{3+}$ ^a [20]	1.95	-0.98	-	-	-0.86	1.83
$[\text{Ir}(\text{ppy})_3]$ ^a [20]	0.78	-2.22	-	-	-1.72	0.28
$[\text{Ir}(\text{ppy})_2\text{dppz}]^+$ ^a [2]	1.31	-0.93	-1.45	-1.63	-0.66	1.04
Ir-DPAC	1.30	-1.09	-1.59	-	-0.79	1.00
Ir-DPACF ₂	1.32	-1.01	-1.51	-	-0.76	1.07
Ir-DPACF ₄	1.30	-0.82*	-1.60	-	-0.77	1.25
Ir-NPP	1.35	-1.15	-1.69	-	-0.76	0.96
Ir-BDPPZ	1.35	-0.73*	-0.83*	-1.23	-0.72	1.34

^a Measured in dry acetonitrile. ^b Measured in dry *N,N*-dimethylformamide. ^c The excited state oxidation potential was estimated from the ground state oxidation potential and the energy of emission maximum with the equation $E_{\text{ox}}^* = E_{1/2 \text{ ox}} - E_{0-0} \approx E_{1/2 \text{ ox}} - \Delta E_{\text{max, em}}$. ^d The excited state reduction potential was estimated from the ground state reduction potential and the energy of emission maximum with the equation $E_{\text{red}}^* = E_{1/2 \text{ red}} + E_{0-0} \approx E_{1/2 \text{ red}} + \Delta E_{\text{max, em}}$. * Irreversible wave

As described in the literature [2, 18], reduction processes involve the different ligands, as noticeable shifts of the waves occur depending on the nature of the extended ligand. The first reduction wave is attributed to the reduction of the extended planar ligand. The more the electron-withdrawing ability of the ligand, the more anodic is the reduction potential. These observations suggest a stabilization of the LUMO of the respective complexes, in accordance with increased conjugation and/or electron-deficiency. The second reduction wave, as too anodic to involve the reduction of an ancillary ppy ligand, is therefore attributed to the reduction of another fragment of the extended planar ligand [2]. Excited state potentials for the five complexes have been estimated from the ground-state redox potentials and the energy of the excited state corresponding to the maximum of the emission spectrum at 298 K.

Absorption The absorption spectra under ambient air conditions in acetonitrile for the three complexes are depicted on Fig. 2. Table 2 gathers the corresponding data, as well as data of other complexes for comparison purpose. As mentioned earlier in this chapter, different types of electronic transitions can be observed upon UV-visible light irradiation for Ir(III) complexes. Comparison with absorption spectra of the free ligands and literature data allowed to assign the intense absorption bands in the UV region to ligand centered transitions. Bands in the visible region probably arise from a mixture of $^1\text{MLCT}$ transitions $\text{Ir} \rightarrow \text{L}$, where L is the extended planar ligand and $^1\text{LLCT}$ transitions $\text{ppy} \rightarrow \text{L}$.

Minor changes occur upon fluorination of the dpac ligand, indicating a possible low contribution of these

ligands to the absorption spectra. However, through comparison with **Ir-NPP** and **Ir-BDPPZ** and data from the literature [2, 4], it is reasonable to assign the most bathochromic bands to a mixture of $^1\text{MLCT}$ and $^1\text{LLCT}$ transitions. These transitions are called $^1\text{MLLCT}$ (Metal-Ligand to Ligand Charge Transfer), consequently to the suggested contribution of an Ir-ppy fragment to the HOMO [17, 19]. Our data suggest the LUMO of all our complexes is localized on a π^* orbital of the extended planar ligand due to the bathochromic shift observed for the more electron-withdrawing cores, lying in agreement with the electrochemical data.

Emission All our complexes display broad unstructured emission (Fig. 3), typical of a Charge Transfer (CT)-type excited state (i.e. $^3\text{MLLCT}$, vide infra) for Ir(III) complexes, in organic solvent. Interestingly, only **Ir-NPP** emits in water. The luminescence lifetimes and the quantum yield of luminescence for each complex are measured in acetonitrile and in water for **Ir-NPP** under inert atmosphere (Table 3). The large k_r values ($> 10^4 \text{ s}^{-1}$) and the large hypsochromic shift of the emission band at 77 K (Fig. 3) support the CT character of the excited state. In air-equilibrated solvents, a decrease in the luminescence intensity with respect to degassed solutions is observed, indicating a potential photosensitization of $^3\text{O}_2$ by the excited complexes (data not shown). Because all of our experiments have been carried out under the same conditions, the influence of oxygen is kept constant and is therefore not further investigated.

A small bathochromic shift of the luminescence is observed while increasing the number of fluorine atoms onto the dpac ligand (i.e. going from **Ir-DPAC** to **Ir-DPACF₂**), indicating the LUMO of these systems is a π^* orbital centered on the dpac moiety. Similar observations are made going from **Ir-NPP** to **Ir-BDPPZ**. Note the ancillary ppy ligands being much more electron-rich, their π^* orbital lies at higher energy.

Unlike $[\text{Ir}(\text{ppy})_2\text{dppz}]^+$, our five Ir(III) complexes show solvent-dependent emission data. The quenching of the luminescence in water has been attributed for $[\text{Ir}(\text{ppy})_2\text{dppz}]^+$ to the presence of hydrogen-bonding interactions between the phenazine nitrogen atoms and water molecules [2]. Similar interactions between the nitrogen atoms of the acridine or phenazine moiety and water molecules could lie at the basis of the inhibition of the luminescence for **Ir-DPAC**, **Ir-DPACF₂**, **Ir-DPACF₄** and **Ir-BDPPZ**. In that case, the low but still present emission of **Ir-NPP** in water may arise from the prevention of the H-bond to the nitrogen atom of the acridine due to the “elbow-shaped” structure of

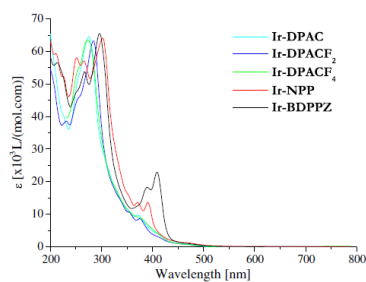


Fig. 2 Absorption spectra of complexes **Ir-DPAC**, **Ir-DPACF₂**, **Ir-DPACF₄**, **Ir-NPP** and **Ir-BDPPZ** in acetonitrile under ambient air conditions

Table 2 Absorption data in CH₃CN and H₂O at 298 K in aerated solution for complexes Ir-DPAC, Ir-DPACF₂, Ir-DPACF₄, Ir-NPP and Ir-BDPPZ. ^aMeasurements made with solutions 1.10^{−5} mol.L^{−1} in complex at room temperature

Complex	Absorbance λ_{max} [nm] (ϵ [L(mol.cm)]) ^a	
	CH ₃ CN	H ₂ O
[Ir(ppy) ₂ dppz] ⁺ [2]	275 (64.9), 334 (13.8), 364 (12.7), 385 (12.7), 468 (0.85)	-
Ir-DPAC	254 (45.5), 283 (63.2), 353 (10.7), 375 (9.48), 417 (5.06)	254, 283, 354, 375, 418
Ir-DPACF₂	252 (47.5), 275 (64.6), 348 (10.7), 371 (9.62), 412 (5.48)	252, 275, 348, 371, 412
Ir-DPACF₄	253 (48.1), 273 (63.6), 354 (11.2), 374 (5.50)	253, 273, 354, 374
Ir-NPP	264 (57.7), 302 (64.1), 362 (12.8), 389 (12.6), 408 (5.27)	264, 302, 352, 389, 408
Ir-BDPPZ	250 (45.1), 267 (52.4), 296 (65.5), 391 (19.2), 409 (24.9)	250, 267, 296, 391, 409

the npp ligand. This, together with the emission data of the four other complexes, suggests only one hydrogen bond is required in the excited state to quench the luminescence of this series of Ir(III) complexes, while two are required for [Ru(phen/bpy)₂dppz]²⁺ [7–9]. This is supported by the luminescence lifetime and quantum yield data of Ir-NPP in water. Note the shorter component of the excited state lifetime could arise from self-aggregation of the complex in aqueous medium due to its low solubility.

Our data indicate the five complexes follow a similar photophysical scheme (Fig. 4): (i) in aprotic medium, after an ultra-fast ISC [21, 22] from ¹MLLCT states generated upon absorption of light, the excited electrons shift towards ³MLLCT states, corresponding to a charge transfer between an Ir-ppy fragment and the extended planar ligand. The similar energies of these emissive states explain the similarity of the luminescence maxima observed for all complexes. (ii) In protic solvent, we

assume, through comparison with TD-DFT and experimental data from literature for similar complexes [2, 8, 9, 12, 23], that upon absorption of light by complexes Ir-DPAC, Ir-DPACF₂, Ir-DPACF₄ and Ir-BDPPZ, a mixture of ¹MLLCT is formed which rapidly undergoes an ISC to a luminescent ³MLLCT excited state, like it is the case in aprotic solvent. The presence of the electron delocalized on the dpac/bdppz ligand in this excited ³MLLCT state favors the formation of an H-bond between solvent molecules and the free nitrogen from the extended planar ligand (depicted as dpac-H/bdppz-H, in Fig. 4). The formation of this H-bond in the excited state further stabilizes the ³MLLCT state. This stabilization and the presence of the H-bond is suggested to account for the luminescence quenching of these complexes in water. (iii) In opposition to the former ones, Ir-NPP exhibit solely partial luminescence quenching in protic medium. The same photophysical scheme in protic than in aprotic solvent is thus suggested as the formation of the only possible H-bond in the excited state is disfavored by the structure of the npp ligand, as supported by luminescence lifetime data. Similar photophysical differences between dpac derivatives and “elbow-shaped” npp and bdppz ligands have already been observed in the literature for Ru(II) complexes [12, 13].

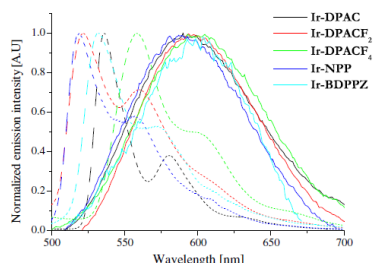


Fig. 3 Emission spectra of complexes Ir-DPAC (λ_{exc} = 417 nm), Ir-DPACF₂ (λ_{exc} = 412 nm), Ir-DPACF₄ (λ_{exc} = 374 nm), Ir-NPP (λ_{exc} = 408 nm) and Ir-BDPPZ (λ_{exc} = 409 nm) in acetonitrile under ambient air conditions (solid lines) and at 77 K in deaerated rigid glassy matrix EtOH/MeOH 4/1 (dashed lines)

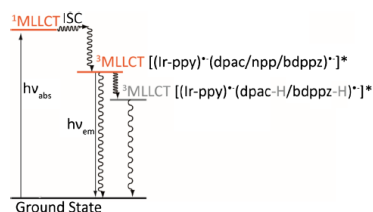
Photophysical Studies in the Presence of DNA DNA binding of transition metal complexes received much attention for the last decades. In particular, complexes containing phenazine- or pyrazine-based intercalating ligands have been the focus of many studies. While mainly Ru(II) [9, 24, 25], Os(II) [26] and Rh(III) [27] derivatives have been extensively studied, Ir(III) analogs have been relatively unexplored [2, 4, 27]. Previous studies from our group show Ru(II) complexes bearing acridine cores display suitable optical properties for applications as DNA photoprobes [12, 13].

Table 3 Emission data in CH₃CN and H₂O at 298 K and 77 K in aerated or deaerated solution for complexes Ir-DPAC ($\lambda_{\text{exc}} = 417$ nm), Ir-DPACF₂ ($\lambda_{\text{exc}} = 412$ nm), Ir-DPACF₄ ($\lambda_{\text{exc}} = 374$ nm), Ir-NPP ($\lambda_{\text{exc}} = 408$ nm) and Ir-BDPPZ ($\lambda_{\text{exc}} = 409$ nm)

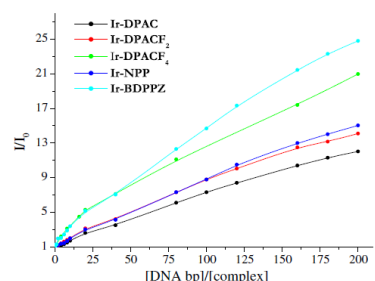
Complex	Emission λ_{max} [nm] ^a		Emission λ_{max} at 77 K [nm]		ϕ_{em} ^{a, b}		τ_{em} [ns]		k_f [10^3 s^{-1}]	
	CH ₃ CN	H ₂ O	EtOH/ MeOH 4/1		CH ₃ CN	H ₂ O	CH ₃ CN	H ₂ O	CH ₃ CN	H ₂ O
	air	air	argon		argon		argon		argon	
[Ir(ppy) ₂ dppz] ⁺ [2]	630	- ^c	544		0.002	- ^c	77	- ^c	29.9	- ^c
Ir-DPAC	594	- ^c	536		0.039	- ^c	742	- ^c	52.6	- ^c
Ir-DPACF ₂	597	- ^c	521		0.044	- ^c	347	- ^c	127	- ^c
Ir-DPACF ₄	601	- ^c	558		0.009	- ^c	550	- ^c	16.4	- ^c
Ir-NPP	589	593	518		0.102	0.010	503	61.5 (+25 % 7 ns)	203	163
Ir-BDPPZ	599	- ^c	531		0.019	- ^c	208.6	- ^c	91.1	- ^c

^a Measurements made with solutions $1.10^{-5} \text{ mol L}^{-1}$ in complex. ^b Measured relative to [Ru(bpy)₃]²⁺ in an aerated aqueous solution ($\phi_{\text{em}}^{\text{air}} = 0.028$). ^c Non emissive

Consequently, the behavior of our five Ir(III) complexes in the presence of DNA has been investigated. Surprisingly, all complexes turned out to be insoluble in aqueous buffer (TRIS.HCl 50 mM, NaCl 50 mM or TRIS.HCl 5 mM, NaCl 1 mM, pH 7.5). The presence of salts, and thus increased ionic strength with respect to pure water, seems to lead to self-aggregation of the complexes. Such behavior has already been observed for similar complexes, due to their low formal charge [2, 17]. As done by Lo et al. [2], 10 % by volume of CH₃CN was added to buffer solutions of complexes and polynucleotides in order to increase their solubility without leading to DNA denaturation. In that case, quantification of the interaction between the two partners was made possible thanks to residual luminescence of the complexes in the absence of polynucleotides.

**Fig. 4** Photophysical scheme for complexes Ir-DPAC, Ir-DPACF₂, Ir-DPACF₄, Ir-NPP and Ir-BDPPZ. In aprotic medium, all complexes emit from a ³MLCT excited state involving the extended planar ligand. This state is stabilized in protic medium through the formation of an H-bond, leading to a quenching of the luminescence for Ir-DPAC, Ir-DPACF₂, Ir-DPACF₄ and Ir-BDPPZ. Solid arrows stand for radiative transitions and wedged arrows for non-radiative transitions

Strong luminescence increase is observed for all complexes upon addition of SS-DNA (Salmon Sperm DNA, Fig. 5). As mentioned above, we suggest (partial) luminescence quenching occurs through H-bond formation in the excited state between the nitrogen atom of the acridine or phenazine moiety and water molecules for the five complexes. Upon intercalation of the extended ligand into the DNA base pairs stack [9, 28], these are prevented, thus restoring the emission of the complexes. Such a “light switch” behavior has already been reported for dppz-based Ru(II) complexes [1, 9]. Our results highlights the potential of Ir(III) complexes as DNA photoprobes and preparation of buffer-soluble analogs are currently under investigation in our group.

**Fig. 5** Steady-state luminescence titrations for Ir-DPAC ($\lambda_{\text{exc}} = 417$ nm), Ir-DPACF₂ ($\lambda_{\text{exc}} = 412$ nm), Ir-DPACF₄ ($\lambda_{\text{exc}} = 374$ nm), Ir-NPP ($\lambda_{\text{exc}} = 408$ nm) and Ir-BDPPZ ($\lambda_{\text{exc}} = 409$ nm) with increasing concentrations of SS-DNA. Measurements performed using $1 \mu\text{M}$ of complex in TRIS.HCl 50 mM, NaCl 50 mM pH 7.4/CH₃CN 9/1 under ambient air conditions

Conclusions

Five new Ir(III) complexes have been successfully synthesized and characterized. ^1H NMR and HRMS data confirms the existence of these Ir(III) complexes as salts.

Electrochemistry experiments show our complexes undergo Ir-ppy-based oxidation, whatever the nature of the extended planar ligand. The first two reduction waves clearly arise from the reduction of the extended planar ligand, which is much more electron-withdrawing than the ancillary ppy ligands. Indeed, the reduction of the latter takes place at potential $< -2\text{ V vs Ag/AgCl}$, suggesting the LUMO is located on dpac, npp or bdppz (π^* orbital) in the ground state.

Their absorption spectra are dominated by ^1LC transitions in the UV range. The most bathochromic bands are assigned to $^1\text{MLCT}$ transitions, the π^* orbital of the extended planar ligand being stabilized either by increased conjugation or electron-withdrawing ability.

Emission data indicate the five complexes exhibit luminescence from $^3\text{MLCT}$ states in acetonitrile, corresponding to a charge transfer between an Ir(III)-ppy fragment and the extended planar ligand. In water, further relaxation occurs on the acridine/phenazine moiety of the extended planar ligand to a non-emissive monohydrogen bonded excited state (dpac-H/bdppz-H) for Ir-DPAC, Ir-DPACF₂, Ir-DPACF₄ and Ir-BDPPZ. A non-luminescent low-lying bdppz-H₂ excited state, where a second H-bond occurs for Ir-BDPPZ in the excited state, could exist but its formation is disfavored due to the “elbow shape” of the ligand. In sharp contrast to the former ones, Ir-NPP exhibit only partial luminescence quenching in aqueous medium. The same photophysical scheme in water than in CH₃CN is thus suggested as the formation of the only possible H-bond in the excited state is disfavored by the “elbow-shaped” structure of the npp ligand.

While the five Ir(III) complexes turned out to be insoluble in aqueous buffer, addition of 10 % CH₃CN allows monitoring and quantification of the interaction between the complexes with increasing concentrations of DNA. Our complexes display strong luminescence enhancement upon addition of DNA, probably due to the protection of the complexes from non-radiative deactivation pathways such as oxygen quenching and vibrational relaxations. The formation of H-bond in the excited state between the nitrogen atom of the acridine or phenazine moiety and water molecules for Ir-DPAC, Ir-DPACF₂, Ir-DPACF₄ and Ir-BDPPZ could explain the luminescence quenching for the free complexes. Our results emphasized the role of Ir(III) complexes as DNA targeting agents and give photochemical insights on poorly described acridine-based Ir(III) complexes.

Experimental Section

Materials and Instrumentation Dpac, [12] dpacF₂, [12] dpacF₄, [12] npp [13] and bdppz [13] have been synthesized according to previously described literature protocols. All solvents and reagents for the synthesis are of reagent grade and are used without any further purification. All solvents for the spectroscopic and electrochemical measurements are of spectroscopic grade. Water is purified with a Millipore Milli-Q system. Salmon sperm DNA (SS-DNA) was purchased from Sigma-Aldrich. The DNA concentration (expressed in base pair units) is determined spectroscopically ($\epsilon_{260} = 6,600\text{ L/(mol.cm) / base pair}$) [29]. ^1H NMR experiments are performed in CDCl₃, CD₃OD or CD₃CN on a Bruker AC-300 Avance II (300 MHz) or on a Bruker AM-500 (500 MHz) at 20 °C. The chemical shifts (given in ppm) are measured vs the residual peak of the solvent as the internal standard. High-resolution mass spectrometry (HRMS) spectra are recorded on a Q-Exactive orbitrap from ThermoFisher using reserpine as internal standard. Samples are ionized by electrospray ionization (ESI; capillary temperature = 320 °C, vaporizer temperature = 320 °C, sheath gas flow rate = 5 mL/min). UV-vis absorption spectra are recorded on a Shimadzu UV-1700. Room temperature fluorescence spectra are recorded on a Varian Cary Eclipse instrument. Luminescence intensity at 77 K is recorded on a FluoroLog3 FL3-22 from Jobin Yvon equipped with an 18 V 450 W Xenon Short Arc lamp and an R928P photomultiplier, using an Oxford Instrument Optistat DN nitrogen cryostat controlled by an Oxford Intelligent Temperature Controller (ITC503S) instrument. Luminescence lifetimes measurements were performed after irradiation at $\lambda = 400\text{ nm}$ obtained by the second harmonic of a Titanium:Sapphire laser (picosecond Tsunami laser spectra physics 3950-M1BB + 39868-03 pulse picker doubler) at a 200 kHz repetition rate. Fluotime 200 from AMS technologies was used for the decay acquisition. It consists of a GaAs microchannel plate photomultiplier tube (Hamamatsu model R3809U-50) followed by a time-correlated single photon counting system from Picoquant (PicoHarp300). The ultimate time resolution of the system is close to 30 ps. Luminescence decays were analyzed with FLUOFIT software available from Picoquant. Cyclic voltammetry is carried out in a one-compartment cell, using a glassy carbon disk working electrode (approximate area = 0.03 cm²), a platinum wire counter electrode, and an Ag/AgCl reference electrode. The potential of the working electrode is controlled by an Autolab PGSTAT 100 potentiostat through a PC interface. The

cyclic voltammograms, measuring the current as a function of the applied potential, are recorded with a sweep rate of 150 mV s^{-1} , in dried acetonitrile or *N,N*-dimethylformamide (Sigma-Aldrich, HPLC grade). The concentration of the complexes is 8.10^{-4} mol/L , with 0.1 mol/L tetrabutylammonium perchlorate as supporting electrolyte. Before each measurement, the samples are purged by nitrogen. The excited state potentials have been estimated using the energy of the emission maximum, $\Delta E_{\text{max, em}}$, according to the Rehm-Weller equation [30].

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

A new ER-specific photosensitizer unravels $^1\text{O}_2$ -driven protein oxidation and inhibition of deubiquitinases as a generic mechanism for cancer PDT

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Photosensitizers (PS) are ideally devoid of any activity in the absence of photoactivation, and rely on molecular oxygen for the formation of singlet oxygen ($^1\text{O}_2$) to produce cellular damage. Off-targets and tumor hypoxia therefore represent obstacles for the use of PS for cancer photodynamic therapy. Herein, we describe the characterization of OR141, a benzophenazine compound identified through a phenotypic screening for its capacity to be strictly activated by light and to kill a large variety of tumor cells under both normoxia and hypoxia. This new class of PS unraveled an unsuspected common mechanism of action for PS that involves the combined inhibition of the mammalian target of rapamycin (mTOR) signaling pathway and proteasomal deubiquitinases (DUBs) USP14 and UCH37. Oxidation of mTOR and other endoplasmic reticulum (ER)-associated proteins drives the early formation of high molecular weight (MW) complexes of multimeric proteins, the concomitant blockade of DUBs preventing their degradation and precipitating cell death. Furthermore, we validated the antitumor effects of OR141 *in vivo* and documented its highly selective accumulation in the ER, further increasing the ER stress resulting from $^1\text{O}_2$ generation upon light activation.

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INTRODUCTION

Photodynamic therapy of cancer consists in the use of photosensitizers (PS) that induce the inhibition of tumor growth.^{1,2} Three conditions are usually described to support the anticancer activity of PS: accumulation of the PS in tumors, the need of local illumination and the presence of molecular oxygen. Although the latter may represent a limitation in the hypoxic environment of many tumors, this issue is usually underestimated in the studies documenting the use of photodynamic therapy (PDT) to treat cancer. Oxygen, more exactly triplet O_2 (which is the normal unexcited state of O_2), is however needed for PS to generate pro-oxidant entities. Light irradiation of PS generates an excited state singlet in nature that, in turn, usually relaxes to a triplet excited state via an intersystem crossing pathway. A first mechanism (type I sensitization) involves direct electron transfer between the excited PS and a surrounding molecule to produce ion radicals, the reaction of which with triplet O_2 ($^3\text{O}_2$) leading to superoxide anion ($\text{O}_2^{\cdot -}$) and consecutively to hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\text{OH}^\cdot$). A second possibility (type II sensitization) refers to the direct transfer of energy from the PS in the triplet excited state to ground state $^3\text{O}_2$ to form singlet oxygen ($^1\text{O}_2$). Both type I and type II processes can occur at the same time, but the second is dominant for most PS.^{3,4} An unanswered question is thus to determine whether the low $p\text{O}_2$ present in tumors

interferes with these mechanisms and whether it introduces some discrepancies between the available PS.

Another related issue is the nature of the PS-induced oxidizing events that trigger the antitumor effects. Indeed, while pro-inflammatory processes associated with the oxidative stress that results from photosensitization are proposed to account for the inhibition of tumor growth upon PDT,^{4,5} how this is related to cell death and what are the early oxidized targets remain largely unknown. Even when damages to the tumor vasculature are proposed to actively participate in the observed antitumor effects,² the mechanisms leading to endothelial cell death were not extensively addressed. Different insights on the likely targets of PS can however be derived from the characteristics of the $^1\text{O}_2$ action. *First*, the high reactivity of $^1\text{O}_2$ limits the extent of the pro-oxidant effects to a subset of intracellular targets located in close proximity with the PS when activated by light. The lifetime of $^1\text{O}_2$ is estimated in the μs range inside the cells, meaning reactivity limited to a diffusion diameter of 10–20 nm. *Second*, proteins are major biological targets for oxidative damage within cells because of their high abundance (65–70% of the dry mass) and rapid rates of reaction with $^1\text{O}_2$.⁶ *Third*, the consequences of protein oxidation by $^1\text{O}_2$ may guide the search for specific signaling pathways that are altered in response to PS. Kinetics data indicate that $^1\text{O}_2$ reacts with a considerable selectivity, with Trp, Tyr, His, Met and Cys.⁶ Furthermore, $^1\text{O}_2$ can generate high yields of hydroperoxides on

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proteins; and these species are capable of propagating oxidative damage specific for thiol-dependent species. Formation of high molecular weight (MW) aggregates via intermolecular disulfide cross-linking⁷ and inactivation of active cysteine proteins such as cysteine proteases⁸ can therefore be anticipated.

In the current study, we aimed to identify a new family of PS taking into account the need for such compounds to work under low pO₂ as usually encountered in tumors, and to use these compounds to better understand the downstream intracellular targets of ¹O₂ formed upon PS activation.

RESULTS

Identification of a new class of PS

As hypoxia may in theory alter the capacity of PS to generate ¹O₂, we launched a drug discovery campaign aiming to identify new PS able to exert growth inhibitory effects even under minimal amount of O₂ (1% O₂). Another major criteria was to exclude the existence of off-targets as reported with many existing PS. We thus chose to compare the antiproliferative effects of several hundreds of molecules under hypoxia and light activation vs normoxic/dark conditions. The initial screening was performed on proliferating endothelial cells (instead of tumor cells) in order to exclude a bias in the identification of compounds active on specific mutation-driven pathways in cancer cells. This strategy led to the identification of benzophenazines as a family of compounds exhibiting an up to 1000-fold higher selectivity for the hypoxia/light conditions. To better evaluate this new class of PS, we chose to focus on OR141 (Figure 1a), the compound exhibiting the largest difference in IC₅₀ between hypoxia/light and normoxia/dark conditions in proliferating endothelial cells (Figure 1b). A dose-dependent activation of caspase-3/7 confirmed the rapid cytotoxic effects of photoactivated OR141 (Figure 1c); and the preferential effect of OR141 under light was further documented in a large variety of cancer and non-cancer cells as shown by comparing the IC₅₀ values between hypoxia/light and normoxia/dark conditions (Figure 1d). To further prove that the mechanism of action of OR141 required photodynamic activation, we repeated these experiments by treating both hypoxic and normoxic HeLa cervix cancer cells in stricter dark conditions (that is, any possible interfering light sources were switched off). In these conditions, OR141 barely inhibited cell proliferation, thereby excluding any significant effect of the non-photoactivated compound on prosurvival pathways triggered by hypoxia (Figure 1e). These effects contrasted with two well-known PS, namely Verteporfin (VP) and Methylene Blue (MB), that both showed growth inhibitory effects in the absence of light, confirming the existence of off-targets for these compounds (Figure 1e).⁹ From these experiments, we could calculate that the mean ratios of selectivity with or without light activation amounted to ~10⁴ for OR141, ~10³ for VP and ~10¹ for MB. A third established PS 5-ALA did not show light-independent effects (Figure 1e), but exhibited a more than 4 logs of magnitude higher IC₅₀ than OR141 in the presence of light (Figure 1f).

OR141 photophysics and photochemistry

To gain insights into the photophysics of OR141, optical measurements were achieved. Supplementary Table 1 gathers the absorption and emission data for OR141 in various solvents. Absorption bands at ca. 310 nm ($\epsilon \sim 1300$ l/mol/cm) and ca. 450 nm ($\epsilon \sim 1000$ l/mol/cm) were observed. Upon excitation at 310 nm at room temperature, an intense unstructured emission was monitored. The excited state was shown to be short lived ($\tau < 8$ ns). The emission wavelength was blue shifted in less polar solvents or at 77 K ($\lambda_{\text{max}} = 485$ nm in EtOH/MeOH 4/1 rigid matrix), indicating that the excited state shows a greater dipolar moment compared with the ground state. Based on these experimental

evidence and on literature data,¹⁰ the formation of excited OR141 can be ascribed to a singlet-singlet spin-allowed $\pi\text{-}\pi^*$ transition or a Charge Transfer process from an electron donor part to an electron acceptor part of the organic compound. A photoinduced electron transfer between the excited state and a biologically relevant quencher (Q) (type I sensitization) was further excluded (see Supplementary Table 2). This analysis led us to the conclusion that the main photoinduced process occurring in the presence of excited OR141 implies an intersystem crossing, converting the singlet excited state of OR141 into a triplet excited state (¹OR141* $\rightarrow$ ³OR141*), in turn able to photosensitize triplet molecular oxygen (³O₂ $\rightarrow$ ¹O₂ + ³O₂).

To prove the formation of ¹O₂, we used electronic paramagnetic resonance (EPR) with 2,2,6,6-tetramethyl-4-piperidine (TMPD) as a spin trap (Figures 2a and b). TMPD reacts with ¹O₂ to produce 2,2,6,6-tetramethyl-4-piperidone-1-oxyl (TEMPO or 4-Oxo-TEMPO) that can be monitored by EPR at room temperature.¹¹ The EPR signal of TEMPO was significantly increased after addition of OR141 in the presence of light, confirming the production of ¹O₂ (Figures 2a and b). This signal was reverted by adding sodium azide, a well-described ¹O₂ scavenger (Figures 2a and b).¹² Production of ¹O₂ by light-activated OR141 was also confirmed by another method using *trans*-1-(2-Methoxyvinyl) Pyrene (MVP)¹³ that specifically displays chemiluminescence in the presence of ¹O₂ (Figure 2c). Using MVP, OR141-induced ¹O₂ generation was also compared with that resulting from VP and MB in either normoxic or hypoxic conditions. The results showed that OR141 was less dependent on O₂ than the two other PS as it produced almost as much ¹O₂ in the presence of 1 or 21% O₂ (Figure 2c). Importantly, the production of ¹O₂ upon OR141 exposure was confirmed in the presence of cells (Figure 2d).

Light-activated OR141 and other PS block critical pathways involved in the adaptation of cells to hypoxia

To understand the mechanism of action of photoactivated OR141 (termed OR141* in the rest of this manuscript), we focused on major pathways known to have key roles under hypoxia.^{13–16} First, we found that 3 h of treatment was sufficient to prevent the expression of the hypoxia-induced transcription factor (HIF-1 α) in both endothelial cells and cancer cells (Figure 3a and Supplementary Figure 1a) as well its transcriptional activity using a PGK1-HRE reporter plasmid (Supplementary Figure 1b). Of note, we also found that in endothelial cells, the expression of HIF-2 α was also completely inhibited following OR141* exposure (Figure 3b). We then examined the status of mammalian target of rapamycin (mTOR) that is known to regulate HIF-1 α abundance and to be potentially deregulated under hypoxia to support cell growth.¹⁶ We found that mTOR was activated under not only 21% O₂ but also 1% O₂, as evidenced by the phosphorylation of p70S6K, a kinase acting downstream mTOR (Figure 3c). Interestingly, we found that under both pO₂ conditions, mTOR activity was dramatically inhibited by OR141*, as shown by a net reduction in phospho-p70S6K (Figure 3c). Also, we observed a shift of the mTOR immunoblot signal to a high MW band when cells were exposed to OR141* (Figure 3c). Finally, as the combination of hypoxia and cell proliferation makes tumor cells especially prone to endoplasmic reticulum (ER) stress,^{14–18} we also focused on the unfolded protein response (UPR) known to alleviate this stress through the activation, *inter alia*, of the PERK/eIF2/CHOP cascade. An increase in the phosphorylation of eIF2 α (an immediate downstream effector of PERK) and a net induction of CHOP (that mediates apoptosis downstream of PERK/eIF2) were observed as soon as 3 h after cell exposure to OR141* (Figure 3d). Of note, the changes in phosphorylation reported above could not be related to a dramatic ATP breakdown as the stress kinase p38 was consistently found activated (phosphorylated) in response to OR141* (Supplementary Figure 1c).

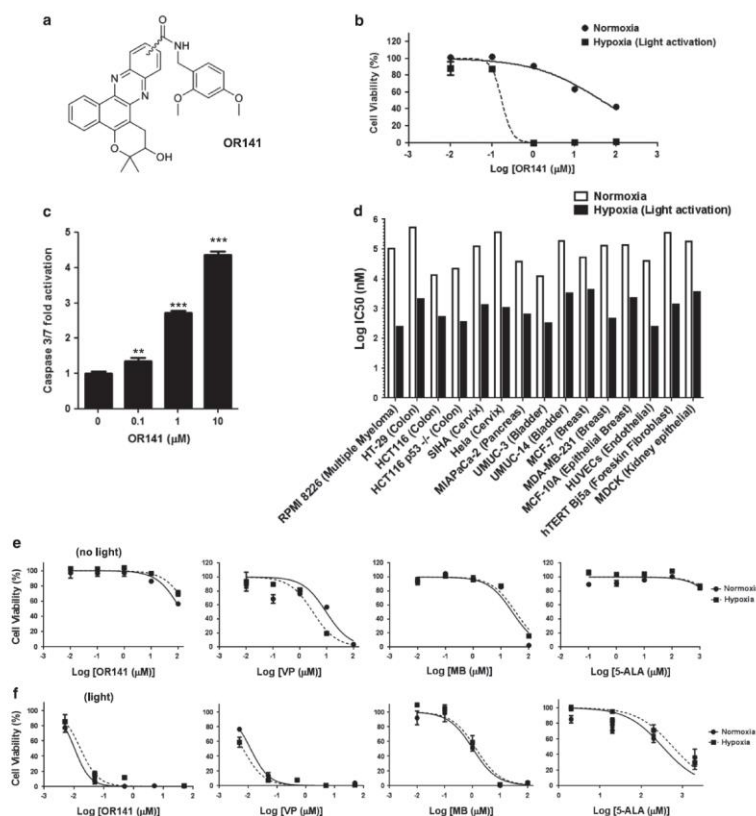


Figure 1. Validation of OR141 as a selective PS. **(a)** Molecular structure of OR141. **(b)** Endothelial cell growth inhibition following exposure to OR141, in the presence of light under hypoxia (1% O₂) or in the dark under normoxia, for 48 h (*n* = 5). **(c)** Caspase-3/7 cleavage observed in response to 8-h exposure to light-activated OR141 under hypoxia (*n* = 3, ***P* < 0.01, ****P* < 0.001). **(d)** IC₅₀ values, expressed as the log IC₅₀ (nM), related to the growth inhibitory effects of OR141 48-h treatment of the indicated tumor and non-tumor cells; light-activated/hypoxia conditions were compared with dark/normoxia conditions (*n* = 5). **(e)** Effects of OR141, VP, MB and 5-ALA on HeLa cervix cancer cell growth for 24 h (*n* = 5) **(e)** in the strict absence of illumination or **(f)** upon light activation; in both conditions, cells were either maintained in normoxia (21% O₂) or incubated under hypoxia (1% O₂).

Since the formation of the mTOR high MW complex occurred before the decrease in HIF-1α abundance (Supplementary Figure 1d) and could result from protein oxidation leading to ER stress/UPR, we further focused on the blockade of the mTOR pathway as a major triggering event in the OR141* inhibitory growth effects downstream of ¹O₂ generation. We used cancer cell

extracts (instead of intact cells as in Figure 3c) to document that cell integrity was not required for OR141* to induce the formation of high MW mTOR complexes and that the process was highly sensitive to the reducing agent dithiothreitol (DTT) (Figure 3e). Similar results were obtained using purified mTOR exposed to photoactivated OR141* (Figure 3f), indicating that the high MW

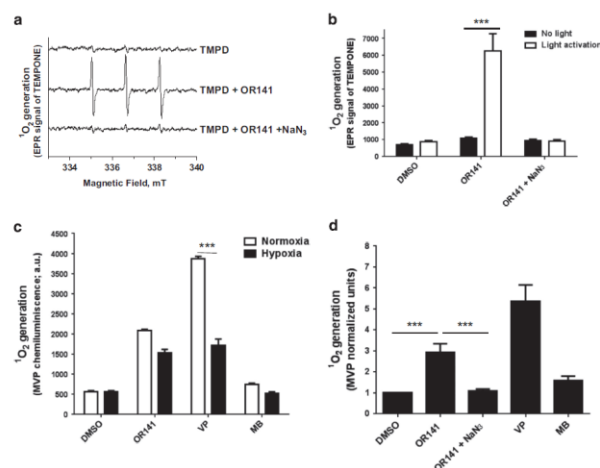


Figure 2. Production of singlet oxygen (1O_2) by light-activated OR141 and other PS. EPR detection of 1O_2 after reaction of light-activated OR141 with the TMPD probe. **(a)** Typical EPR spectra of nitroxide radical detected in the reactive medium containing TMPD (100 mM) and OR141 (50 mM), and illuminated or not with light, in the presence (or not) of NaN₃ (50 mM). The intensity of first hyperfine component was used for the EPR signal quantitation. **(b)** Derived amount of 1O_2 produced in the indicated conditions ($n=3$, *** $P < 0.001$); sodium azide (NaN₃) was used as a specific scavenger of 1O_2 . Chemiluminescence detection of 1O_2 after reaction of the indicated PS with MVP **(c)** in *in vitro* (cell-free) under normoxia or hypoxia and **(d)** in HeLa cancer cells ($n=4$, *** $P < 0.001$).

mTOR band could result from the multimerization of mTOR itself. Importantly, we showed that not only the OR141-dependent mTOR high MW shift but also the induction of BiP, a marker of UPR activation, was prevented in the presence of DTT or the antioxidant compound Ebselen (Figure 3g); the latter largely prevented cell death upon OR141* exposure (Figure 3h). Finally, we showed that light-activated VP, MB and 5-ALA dose-dependently produced the exact same effects as OR141*, namely mTOR high MW complex formation, a reduction in HIF-1 α abundance and the induction of BiP (Figure 3i). Note that for Figure 3i, cells were collected after only 1 h exposure to the different PS.

Photooxidation of proteins by OR141 induces polyubiquitination and inhibition of the proteasomal deubiquitinase activity. The ER stress and the formation of high MW complexes in response to the oxidation process induced by OR141* led us to postulate that the ubiquitin-proteasome system was stimulated in these conditions. The extent of ubiquitinated proteins was indeed dose-dependently increased upon exposure to OR141* (Figure 4a) in a DTT-sensitive manner (Figure 4b). Using cells expressing myc-tagged mTOR and HA-tagged ubiquitin, we confirmed in co-immunoprecipitation experiments that high MW mTOR complex was ubiquitinated (Figure 4c). As an inhibition of the proteasome could account for these effects, we then examined the effect of OR141* on proteasomal activity and found that contrary to the *bona fide* proteasome inhibitor bortezomib, OR141* failed to inhibit any of the three protease-like activities (Figure 4d). OR141 actually stimulated the caspase-like and (to a lesser extent) chymotrypsin-like activities of purified proteasome fractions

(Figure 4d). As an increase in the extent of ubiquitinated proteins can also arise from the inhibition of deubiquitinase (DUB) activity, we investigated the effects of OR141* on the proteasome-associated DUBs. As a first support to this hypothesis, we found that OR141* prevented the cleavage of the ubiquitin-aminoluciferin (Ub-AML) reagent by DUBs present into purified 19S proteasome (Figure 4e). We then used a more elaborated technology based on the use of HA-Ub-V5, a DUB active site directed probe. Immunoblot experiments using anti-HA antibodies showed that OR141* inhibited the activity of DUBs present in purified proteasome fractions (Figure 4f), the MWs of which corresponding to the predicted sizes of two of the three proteasomal DUBs USP14 and UCH37 (Figure 4f); the cysteine peptidase inhibitor N-ethylmaleimide (NEM) was used as a positive control in these experiments. Similar results were obtained with full cell lysates; and the identity of USP14 and UCH37 was confirmed using specific antibodies (Supplementary Figure 2a). As USP14 and UCH37 contain active cysteines prone to direct oxidation, we examined the possible formation of DUBs multi-protein complexes as described above for mTOR. We found indeed that a fraction of USP14 and UCH37 exhibited a net shift to high MW in a DTT-sensitive manner when using purified proteasomes (Figures 4g and h) or full cell lysates (Supplementary Figure 2b and c). Note that light-activated VP, MB and 5-ALA had the same oxidizing effects as OR141* on these two DUBs (Supplementary Figure 2b and c).

OR141 targets the ER

To examine the nature of the proteins that were targeted by OR141* under hypoxic conditions, we first collected the high MW

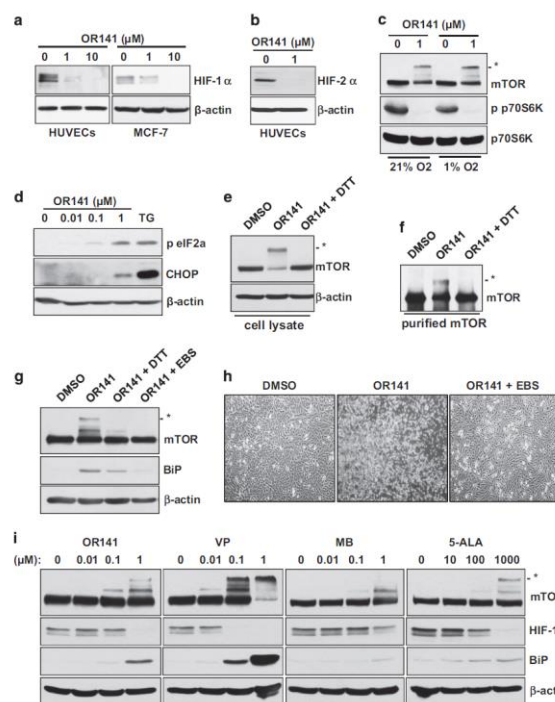


Figure 3. Light-activated OR141 and other PS block HIF-1α and mTOR pathways under hypoxia and induce the formation of high MW protein complexes. Endothelial (human umbilical vein endothelial cells, HUVECs) and cancer (MCF7) cells were treated under hypoxia with the indicated concentration of light-activated OR141; note that for the purpose of the immunoblotting (IB) experiments, short exposure times (i.e., 1–3 h) were preferred to avoid the confusing late effects of dying cells. Representative immunoblots of (a) HIF-1α, (b) HIF-2α, (c) mTOR, p70S6K and phospho-p70S6K and (d) the UPR markers phospho-eIF2α and CHOP; thapsigargin (TG) treatment was used as a positive control for UPR activation. * denotes high MW complex detection. As starting materials for mTOR IB, we also used HeLa cancer cells (e) crude extracts and (f) purified mTOR (obtained from mTOR immunoprecipitation (IP)) to study the effect of the reducing agent DTT (10 mM) on the formation of mTOR high MW complexes in response to 10 μM light-activated OR141 treatment (2 h, 37 °C). The effects of DTT and/or the cysteine modifier Ebselen (EBS; 20 μM) were also evaluated in endothelial cells (g) on the formation of high MW mTOR complex and the inhibition of BiP induction and (h) on the cell viability in response to 10 μM OR141 treatment for 2 and 24 h, respectively. (i) The effects of 1-h treatment with VP, MB and 5-ALA on mTOR high MW complex formation, HIF-1α and BiP expression was compared with those of OR141 in hypoxic HeLa cells (for 5-ALA, cells were incubated for 4 h in the dark prior light activation). All the experiments described in this figure were repeated at least three times with similar results; β-actin IB detection was used as a loading control.

mTOR band obtained after SDS-PAGE (Figure 5a). Most of the proteins identified by MS (Supplementary Table 3) were either residents of the ER or secreted proteins trafficking via the ER (Figure 5a); we confirmed the presence of some of them (for example, PDIA3 and vWF) in high MW complexes by immunoblotting (Figure 5b). In a second set of experiments, we used a methodology based on the immune-affinity purification of trypsin-digested proteins with anti-GG antibodies (Figure 5c). These antibodies recognized specifically diglycyl modified lysines, an

ubiquitin remnant detectable after trypsin digestion.¹⁹ Eluted peptides were subjected to tandem mass spectrometry (LC-MS/MS) for quantitative sequence and site identification (Supplementary Tables 4 and 5). Enrichment clusters were generated using bioinformatics tools for peptides that were significantly ubiquitinated. The majority of the enriched clusters had a direct relationship with the ER such as the ER/Redox, ER/Vesicles, Lipid Metabolism, Endomembrane System, Intracellular Protein Transport and Nucleotide-binding/Ion Transport clusters (Figure 5d). The

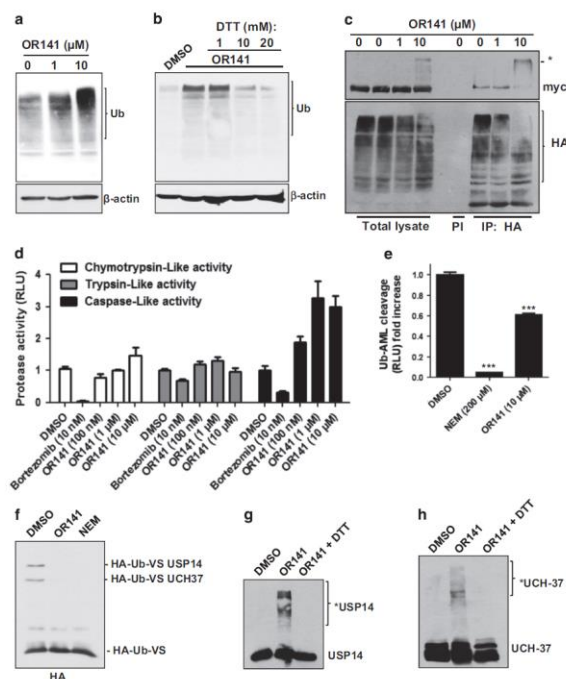


Figure 4. Light-activated OR141 induces the accumulation of ubiquitinated proteins via the inhibition of proteasomal DUBs. (a) Immunoblot (b) of ubiquitin conjugates in cancer cells (HeLa) treated with the indicated concentrations of light-activated OR141 for 3 h in hypoxia. (b) Effect of increasing concentrations of DTT on the formation of ubiquitin conjugates after incubation with light-activated OR141 (1 μM) for 2 h in hypoxic HeLa cells; β-actin IB detection was used as a loading control. (c) HEK 293 T cells were transfected with HA-tagged ubiquitin and myc-mTOR encoding plasmids and treated with light-activated OR141 for 3 h. Typical anti-myc and anti-HA immunoblots of either total cell lysates or following immunoprecipitation (IP) with anti-HA antibodies; pre-immune (PI) serum was used as a control IP. Note the detection of high MW mTOR complex in lysates and in HA-ubiquitin IP at 10 μM OR141*. (d) Effect of the indicated concentrations of OR141* on the proteasome enzymatic activity in endothelial cells (3 h treatment under hypoxia); the proteasome inhibitor bortezomib was used as a positive control. Data correspond to three independent experiments. (e) Inhibition of Ub-AML cleavage by purified 19S proteasome (5 nm) after treatment with 10 μM OR141*; the general cysteine peptidase inhibitor NEM (200 μM) was used as a control (n=3, ***P<0.001). (f) Purified preparations of 26 S proteasome pre-exposed for 30 min at 37 °C to OR141* (50 μM) or NEM (200 μM) were incubated with HA-Ub-V5 (1 μM) for 1 h at 37 °C. Proteasomal DUBs were detected by immunoblotting with an anti-HA antibody. Two bands corresponding to the predicted MW of USP14 and UCH37 (56 and 37.6 kDa, respectively) were found in control conditions but not in the OR141* and NEM conditions. Purified preparations of proteasome 19 S (50 nm) were also incubated for 2 h at 37 °C with OR141* (10 μM) with or without DTT (10 mM). Immunoblot detection of (g) USP14 and (h) UCH37; note the effects of DTT on the formation of high MW complexes. All the experiments described in this figure were repeated at least three times with similar results.

intimate relationship between OR141* effects and the ER led us to exploit the autofluorescence of OR141* to evaluate its intracellular distribution. We found an exquisite perinuclear location of OR141* (Figure 5e), as confirmed by the perfect match with an ER-specific fluorescent probe (Figure 5f). As another evidence of the ER specificity of action of OR141*, we failed to identify DNA breaks in cells exposed to OR141* (Supplementary Figure 2d), thereby excluding significant nucleus accumulation.

Light-activated OR141 inhibits tumor growth in mice

Finally, to prove the *in vivo* efficacy of OR141*, we used a human colon carcinoma xenograft model, a tumor model known to give

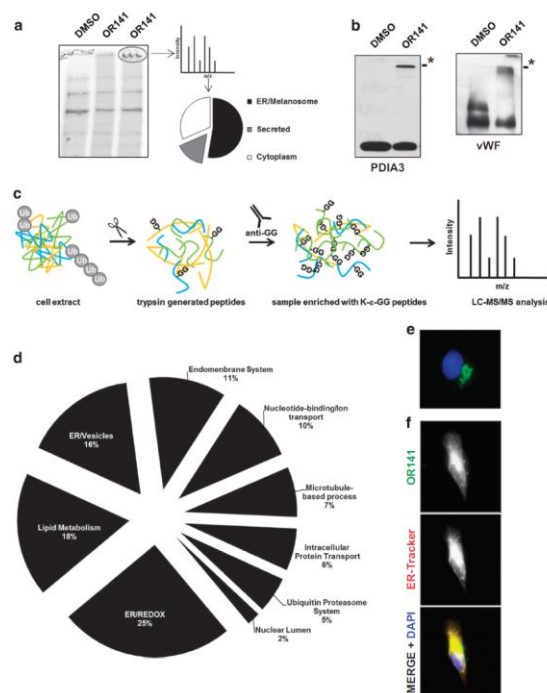


Figure 5. OR141-induced high MW adducts containing a majority of ER-related proteins. **(a)** High MW complexes formed after endothelial cell exposure to 10 μ M OR141* (3 h, under hypoxia) were resolved by SDS-PAGE, stained with Coomassie Blue and picked for protein identification via MS analysis (see Supplementary Table 3) and clustering according to their intracellular location. **(b)** The presence of some of the proteins identified in **(a)**, namely PDIA3 and vWF, were validated in immunoblot **(b)** experiments using specific antibodies. *denotes high MW complex detection. **(c)** Extracts of endothelial and tumor cells (treated as in **a**) were digested with trypsin, and ubiquitinated peptides were subjected to immune-affinity purification (IAP) with anti-GG antibodies. Eluted material was subjected to tandem mass spectrometry (LC-MS/MS) for qualitative sequence and site identification (see Supplementary Tables 4 and 5). **(d)** Enrichment clusters (derived using DAVID software) of ubiquitinated proteins identified in **(c)**. **(e)** Perinuclear location of OR141* in SiHa cancer cells as detected by autofluorescence. **(f)** Co-localization of OR141* and ER tracker as determined by fluorescence microscopy in SiHa cancer cells; merged image also shows DAPI counterstaining.

rise to hypoxia in mice, as previously reported in our laboratory and in others.^{20,21} Pharmacokinetic studies first showed that the C_{max} was reached after 4 h (Figure 6a); we chose this time point to illuminate tumors. Mice were treated or not with OR141 and exposed either to the environment illumination or to an optic fiber delivering white light (5 mm distance from the tumor). A dramatic inhibitory effect on the growth of tumors was reached when OR141 administration was combined every 4 days with direct light illumination of the tumors for 15 min (Figure 6b). To confirm the mechanism of action identified *in vitro*, we also collected tumors post-treatment and validated the accumulation of a large pool of poly-ubiquitinated proteins in the tumors from mice exposed to

OR141* and the inhibition of the mTOR-dependent S6RP and HIF-1 α signaling pathways (Figure 6c).

DISCUSSION

Cancer PDT is described as a modality that induces apoptosis/necrosis in tumors through a specific increase in local oxidative stress upon PS photoactivation. In this study, we identified a new class of photoactivatable compounds that led us to unravel an unsuspected mechanism of action mediating the effects of PS downstream of the generation of 1O_2 . We showed that the mTOR signaling pathway is inhibited by PS, concomitantly to

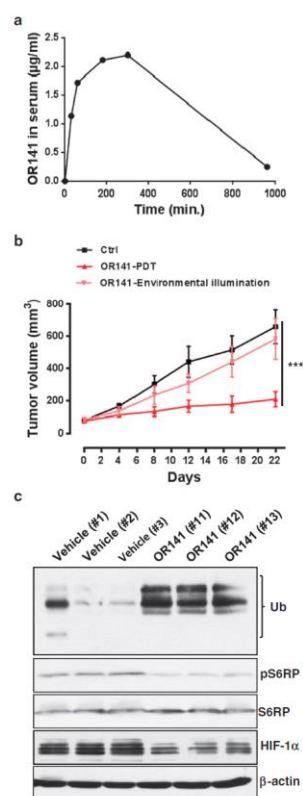


Figure 6. Light-activated OR141 inhibits tumor growth *in vivo*. (a) Evolution of the serum OR141 concentration in mice intraperitoneally (i.p.) injected with 10 mg/kg OR141. (b) Time course of the tumor growth in mice bearing subcutaneous HCT116 human tumor xenografts; mice were i.p. injected every 4 days with vehicle or with OR141 and either maintained under environmental light illumination or exposed to an optic fiber delivering white light at the tumor site for 15 min (exactly 4 h after OR141 administration). ****P* < 0.001. (c) Representative immunoblot of ubiquitin conjugates (Ub), (phospho)-S6RP and HIF-1α in HCT116 tumors removed from mice treated with either vehicle or OR141* (*n* = 3).

proteasomal DUBs USP14 and UCH37. These effects result from the PS-induced protein oxidation leading to the formation of high MW complexes made of poly-ubiquitinated multimeric proteins. Although the integrity of various signaling proteins was altered

upon PS-induced oxidation, inhibition of mTOR and proteasomal DUBs was identified as major events precipitating the antiproliferative effects of PS.

The roles of proteasomal DUBs are to mediate stepwise removal of ubiquitin from the substrates by disassembling the chain from its distal end before proteasomal degradation. Thus, the inhibition of the proteasomal DUBs USP14 and UCH37 allows us to understand how OR141* treatment may rapidly lead to the accumulation of poly-ubiquitinated proteins to an extent incompatible with the degradation capacity of the proteasomal machinery. Interestingly, USP14 and UCH37 are cysteine proteases and thus highly susceptible to react with ¹O₂ while RPN11, the third proteasomal DUB that we did not find to be inhibited by OR141*, is not a cysteine protease but a Zn-dependent metalloprotease. Oxidation of active cysteines by ¹O₂ leads to the formation of intra- and intermolecular disulfide bonds.⁸ Although the former may account for the inhibition of the catalytic activity of enzymes²² as observed for proteasomal DUBs (see Figures 4e and h and Supplementary Figures 2a and c), the intermolecular disulfide bonds can drive the multimerization of proteins leading to the formation of high MW complexes^{23,24} as observed in the current study for mTOR (see Figures 3c–g and i). It should be noted that multimers of mTORs were already reported to form under physiological conditions.^{25,26} In our hands, the detection of high MW mTOR complexes (including from purified mTOR preparations) in denaturing SDS-polyacrylamide gels and in a DTT-sensitive manner indicated that OR141* and the other tested PS stabilized mTOR multimers through the formation of (covalent) disulfide bonds. We also showed that these complexes of oxidized proteins also concern other proteins including PERK (Figure 3d) and IRE1a (not shown) as well as proteins listed in Supplementary Table 3. Oxidation of (multimeric) proteins in response to PS exposure is prone to rapidly lead to accumulation of ubiquitinated proteins as observed in the current study (Figures 4a and c), a phenomenon further exacerbated by the inhibition of proteasomal DUBs (see Figures 4e and f).

Our study also points out that the hypoxic environment of tumors does not represent a limitation to PDT despite two expected hurdles. First, PDT is usually claimed to require the presence O₂ to be efficient. In this study, we showed that a moderate level of hypoxia (that is, 1% O₂), as reported in most tumors, is sufficient to drive the formation of ¹O₂ in response to PS activation. Second, although, physiologically, mTOR-driven mRNA translation processes are proposed to be inhibited under hypoxia to spare energy, dysregulation of the mTOR pathway can occur in tumor cells to support proliferation under hypoxia,^{27,28} and account for the mTOR-driven expression of HIF-1α. In this study, we used p70S6K and S6RP as reporters of the mTOR activity and documented that this pathway was active under hypoxia (Figure 3c) and inhibited by PS*. Also, when mTOR was pharmacologically inhibited or genetically silenced, tumor cell growth was similarly inhibited under normoxia and hypoxia (Supplementary Figure 3), an effect further reinforced by proteasomal DUB inhibition or knockdown (Supplementary Figure 3). The net reduction in the expression of HIF-1α in response to OR141* that we observed both *in vitro* and *in vivo* could also directly arise from the inhibition of mTOR. We found indeed that under hypoxia, mTOR inhibition by OR141* occurred before the reduction in HIF-1α abundance and that HIF-1α translation, but not its stability was actually inhibited (Supplementary Figure 1a). As the HIF transcription factors drive the expression of programs of genes to allow cell adaptation to low O₂, the blockade of HIF-1α (and HIF-2α) also contributes to the mTOR-driven antiproliferative effects of PS observed under hypoxia.

The OR141 benzophenazine presents a specific profile making this compound family particularly suited for future development of a new class of photodynamic drugs. First of all, OR141 combines

a quasi-absence of cytotoxicity in the absence of light activation and a low IC_{50} (≈ 10 – 100 nM according to the cell types and the assay) when activated. Off-targets are therefore less likely than with VP or MB that both inhibit tumor cell growth even in the absence of light. Second, the capacity to produce 1O_2 is equivalent in normoxia and hypoxia contrary to VP, which presents a 60% reduction in the capacity to generate 1O_2 when pO_2 is reduced to 1% (Figure 2c). Third, OR141 has a high tropism for the ER as proven by its strict accumulation in the ER and the large set of ER residents or ER-related proteins among the ubiquitinated (oxidized) proteins.

More generally, OR141 and other PS actually appear as activators of the so-called ER-associated protein degradation (ERAD).^{22,29} This process describes the proteolytic pathways that degrade damaged or misfolded ER proteins after retrotranslocation.³⁰ ERAD pathways involve the ubiquitin-proteasome system to execute degradation. The ubiquitination of the ERAD substrates has been proposed to occur at the cytoplasmic side of the ER where E3 ubiquitin ligase could be part of the retrotranslocation channel.²⁹ In this context, the recruitment of the 26S proteasome and associated DUBs to the ER was recently reported³¹ and could account for the exposure of USP14 and UCH37 to the oxidative effects of ER-enriched OR141*. mTOR itself was reported to be concentrated into the ER³² and could therefore account for a preferential inhibition of this critical pathway upon PS exposure.

In conclusion, our work with OR141 led us to identify for the first time that the early steps driving the effects of commonly used PS, in particular under hypoxia, include the inhibition of both the mTOR/HIF pathway and the proteasomal DUB activity. More particularly, the high tropism of OR141 for the ER compartment together with the lack of off-targets when not activated by light position this compound family as PS particularly suited to stimulate ERAD in tumors and exert potent and selective tumor growth inhibitory effects.

MATERIALS AND METHODS

Cell culture and reagents

All tumor cell lines were acquired from ATCC where they are regularly authenticated by short tandem repeat profiling, and human umbilical vein endothelial cells were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cells were used within 3 months after resuscitation of frozen aliquots and regularly checked for mycoplasma contamination. Cell growth was analyzed in colorimetric assays using either MTT (Sigma-Aldrich) or PrestoBlue (Invitrogen, Waltham, MA, USA) reagents; and apoptosis was determined with the Caspase-Glo 3/7 assay (Promega, Fitchburg, WI, USA) according to the supplier's instructions. For imaging, cells were seeded in eight-chamber slides (Labtek, Roskilde, Denmark) and fixed with 4% formaldehyde before staining. Transfections were performed with Lipofectamine (Life Technologies, Carlsbad, CA, USA) following the manufacturer's recommendations. Chemicals were purchased from Sigma-Aldrich, unless indicated otherwise. Immunoblotting and immunoprecipitations were performed as previously described.^{33,34}

Singlet oxygen measurement

The EPR spectroscopy was used for detection of 1O_2 *in vitro*. The technique allowed quantitative detection of the stable paramagnetic adduct of oxidation of the EPR silent, diamagnetic spin probe, TMPD by 1O_2 at room temperature.^{35–37} Alternatively, 1O_2 production was measured by using the chemiluminescent probe MVP (Life Technologies) that specifically reacts with 1O_2 and no other oxygen species.³⁷

Protein ubiquitination assay

Cells were washed with ice-cold PBS and lysed with RIPA buffer containing protease and phosphatase inhibitors; in some experiments, cells were pre-exposed to 5 mM NEM (Sigma-Aldrich). Ubiquitinated proteins were immunoprecipitated from cell lysates with anti-ubiquitin antibodies

(Zymed, South San Francisco, CA, USA) and collected via protein G-agarose magnetic beads (Life Technologies).

Proteasome and DUB activity assays

Proteasome and DUB activities were measured using dedicated Glo Cell-Based Reagents and a Glomax microplate reader (Promega) following the manufacturer's instructions; for the DUB assay, Ub-AML (Boston Biochem, Cambridge, MA, USA) was used as a substrate instead of using the provided substrate because of the OR141 autofluorescence. The labeling of active DUBs in cell lysates or in purified 26S proteasomes was performed using the active site molecular probe HA-UbVs (Boston Biochem).³⁸

Identification of ubiquitinated proteins by quantitative diglyc proteomics

Treated human umbilical vein endothelial cells and MDA-MB-231 cells were lysed with 20 mM urea lysis buffer. Proteins were reduced, alkylated and digested with trypsin-TPCK (Worthington, Lakewood, NJ, USA) followed by peptide purification by reversed-phase, solid-phase extraction on Sep-Pak column (Waters Corporation, Milford, MA, USA). Ubiquitinated peptides were purified by immunoaffinity with PTMScan ubiquitin remnant motif (K-e-GG) antibody conjugated to protein A (Cell Signaling, Danvers, MA, USA). Reversed-phase purification was performed on StageTips C18 (Thermo Scientific, Waltham, MA, USA) to desalt and separate peptides from antibody before concentrating the enriched peptides for LC-MS/MS analysis.

Mouse tumors and treatment

All the experiments involving mice received the approval of the University Ethic Committee (approval ID 2012/UCL/MD005), and were carried out according to National Animal Care regulations. Eight-week-old NMRI nude mice (Elevage Janvier, LeGenest-St-Isle, France) were injected subcutaneously with 1×10^6 HCT116 tumor cells in the right dorsal flank. Tumors were allowed to reach a minimal diameter of 100 mm³, and the animals (randomly distributed in groups of six mice) were then injected intraperitoneally every 4 days with vehicle (Solutol/DMSO/NaCl 0.9%), OR141 (40 mg/kg) and OR141-PDT (40 mg/kg+15 min light activation (4 h post injection) using a 150-W light source KL 5000 LCD; Schott AG, Mainz, Germany). Blood collection was used to determine serum OR141 concentrations by HPLC-UV in isocratic mode on a RP-18e 250 mm $\times$ 4 mm LiChroCART column (Merck Millipore, Darmstadt, Germany). Tumor sizes were tracked by a blinded operator with an electronic caliper and when collected, tumor samples were frozen in liquid nitrogen.

Statistical analyses

Data are expressed as mean $\pm$ s.e.m.; a minimum of three independent experiments were carried out for each experimental condition. The statistical significance between treatments was determined by Student's two-tailed *t*-test when comparing two groups and one-way analysis of variance (ANOVA, Bonferroni's *post hoc* test) when comparing multiple groups. $P < 0.05$ was considered as statistically significant. All data were analyzed with the GraphPad Prism 5.0 software (San Diego, CA, USA).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on the Oncogene website (<http://www.nature.com/onc>)

Chapter 10

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