

LUMINESCENT CYCLOMETALATED
IRIDIUM(III) POLYPYRIDINE
COMPLEXES AS BIOMOLECULAR
AND CELLULAR PROBES

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Luminescent Cyclometalated Iridium(III)
Polypyridine Complexes as Biomolecular and
Cellular Probes

發光性環金屬化三價銦多吡啶絡合物作為
生物分子和細胞探測器

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Abstract

A series of luminescent cyclometalated iridium(III) dipyridoquinoxaline complexes $[\text{Ir}(\text{N}^{\wedge}\text{C})_2(\text{N}^{\wedge}\text{N})](\text{PF}_6)$ ($\text{HN}^{\wedge}\text{C} = \text{Hppz}, \text{Hbzq}, \text{Hpq}$; $\text{N}^{\wedge}\text{N} = \text{dpq}$ and dpqa) has been synthesized and characterized. The X-ray crystal structure of $[\text{Ir}(\text{pq})_2(\text{dpq})](\text{PF}_6)$ has been determined. Cyclic voltammetric studies revealed a reversible or quasi-reversible iridium(IV/III) oxidation couple at ca. +1.13 to +1.32 V and a reversible diimine reduction couple at ca. -1.10 to -1.29 V vs. SCE. Upon photoexcitation, all the complexes displayed intense and long-lived green to orange triplet metal-to-ligand charge-transfer ($^3\text{MLCT}$) ($d\pi(\text{Ir}) \rightarrow \pi^*(\text{dpq}$ or $\text{dpqa})$) emission in aprotic organic solvents at room temperature and in low-temperature glass. In aqueous solution, these complexes were only weakly emissive or even non-emissive. The lipophilicity of all the complexes has been determined by reversed-phase HPLC. The cytotoxicity of these iridium(III) complexes towards the human cervix epithelioid carcinoma (HeLa) and the Madin-Darby canine kidney (MDCK) cell lines has been evaluated by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cellular uptake of the complexes by MDCK cells has been examined by laser-scanning confocal microscopy. Most importantly, apparent nucleolar staining was observed after the cells were treated by the complexes. Additionally, the interactions of these complexes with proteins, DNA, and RNA have been studied by emission titrations and SDS-PAGE gel staining. The results revealed that the complexes bound to the hydrophobic pockets of proteins, intercalated into the base-pairs of double-stranded DNA, but did not

appear to interact with RNA.

Luminescent dendritic cyclometalated iridium(III) polypyridine complexes $[\{\text{Ir}(\text{N}^{\wedge}\text{C})_2\}_n(\text{bpy-}n)](\text{PF}_6)_n$ ($\text{N}^{\wedge}\text{C} = \text{Hppy}, \text{Hppq}; n = 8, 4, 3$) have been designed and synthesized. The properties of these dendrimers have been compared to those of their monomeric counterparts $[\text{Ir}(\text{N}^{\wedge}\text{C})_2(\text{bpy-}1)](\text{PF}_6)$ ($\text{N}^{\wedge}\text{C} = \text{Hppy}, \text{Hppq}$). Cyclic voltammetric studies revealed that the iridium(IV/III) oxidation and bpy-based reduction occurred at ca. +1.24 to +1.29 V and -1.21 to -1.27 V vs. SCE, respectively, for all the complexes. The molar absorptivity of the dendritic iridium(III) complexes is approximately proportional to the number of $[\text{Ir}(\text{N}^{\wedge}\text{C})_2(\text{N}^{\wedge}\text{N})]$ moieties in one complex molecule. However, the emission lifetimes and quantum yields are relatively independent on the number of $[\text{Ir}(\text{N}^{\wedge}\text{C})_2(\text{N}^{\wedge}\text{N})]$ units, suggesting negligible electronic communications between these units. Upon photoexcitation, the complexes displayed $^3\text{MLCT}$ ($d\pi(\text{Ir}) \rightarrow \pi^*(\text{bpy-}n)$) emission. The interaction of these complexes with plasmid DNA has been investigated by agarose gel retardation assays. The results showed that the dendritic iridium(III) complexes, unlike their monomeric counterparts, bound to the plasmid and the interaction is electrostatic in nature. The lipophilicity of all the complexes has been determined by reversed-phase HPLC. Additionally, the cellular uptake of the complexes has been examined by ICP-MS, laser-scanning confocal microscopy, and flow cytometry. Upon internalization, all the complexes were localized in the perinuclear region, forming very sharp luminescent rings surrounding the nuclei. Interestingly, in addition to these rings, HeLa cells treated with the dendritic iridium(III) complexes showed specific labeled compartments, which have been identified to be the Golgi apparatus.

Furthermore, the cytotoxicity of these iridium(III) complexes towards the HeLa cell line has been evaluated by the MTT assay.

Three luminescent cyclometalated iridium(III) bis-biotin complexes $[\text{Ir}(\text{ppy-C6-Biotin})_2(\text{bpy})](\text{PF}_6)$, $[\text{Ir}(\text{ppy})_2(\text{bpy-C2-Biotin}_2)](\text{PF}_6)$, and $[\text{Ir}(\text{ppy})_2(\text{bpy-C2-C6-Biotin}_2)](\text{PF}_6)$ and one tris-biotin complex $[\text{Ir}(\text{ppy-C6-Biotin})_2(\text{bpy-C6-Biotin})](\text{PF}_6)$ have been synthesized and characterized. The biotin-free complex $[\text{Ir}(\text{ppy})_2(\text{bpy-C4})](\text{PF}_6)$ has also been prepared for comparison studies. Cyclic voltammetric studies revealed iridium(IV/III) oxidations at ca. +1.21 to +1.29 V and diimine-based reductions at ca. -1.07 to -1.39 V vs. SCE. Upon photoexcitation, all the complexes displayed intense and long-lived orange-yellow to red $^3\text{MLCT}$ ($d\pi(\text{Ir}) \rightarrow \pi^*(\text{N}^{\wedge}\text{N})$) emission in fluid solutions at room temperature and in low-temperature glass. The interactions of the bis-biotin and tris-biotin complexes with avidin have been studied by 4'-hydroxyazobenzene-2-carboxylic acid (HABA) assays, emission titrations, and dissociation assays. The possibility of these complexes as crosslinkers for avidin has been examined by microscopy studies using avidin-conjugated green fluorescent microspheres and size-exclusion HPLC analysis. Utilization of these luminescent iridium(III) biotin complexes in signal amplification has been demonstrated using avidin-coated nonfluorescent microspheres. Additionally, the lipophilicity of all the complexes has been determined by reversed-phase HPLC. The cytotoxicity of these iridium(III) complexes towards the HeLa cell line has been evaluated by the MTT assay. Furthermore, the cellular uptake of the complexes has been examined by ICP-MS, laser-scanning confocal microscopy, and flow cytometry.

A novel dual-emissive cyclometalated iridium(III) polypyridine complex $[\text{Ir}(\text{ppy-C4})_2(\text{bpy-et})](\text{PF}_6)$ has been synthesized and characterized. Upon irradiation, this complex exhibited intense and long-lived luminescence in fluid solutions at 298 K and alcohol glass at 77 K. Interestingly, it showed dual emission in fluid solutions at room temperature, with a high-energy (HE) structured band at ca. 500 nm ($\tau_0 = 1.1 - 2.5 \mu\text{s}$) and a low-energy (LE) broad band/shoulder at ca. 593 – 619 nm ($\tau_0 = 0.1 - 0.3 \mu\text{s}$). The HE and LE features have been assigned to $^3\text{IL} (\pi \rightarrow \pi^*)$ (bpy-et) and triplet charge-transfer $^3\text{CT} (d\pi(\text{Ir})/\text{amine}/\pi(\text{ppy-C4}) \rightarrow \pi^*(\text{bpy-et}))$ excited states, respectively. Biotin, estradiol, and an aliphatic C18 chain have been incorporated into the bipyridine ligand of the complex to afford novel luminescent biological probes for avidin, estrogen receptor α , and human serum albumin, respectively. The resulting complexes showed similar dual-emissive properties. Upon binding to their corresponding specific receptors, all these dual-emitting complexes showed a significant change in their emission profile and spectral characteristics. Complexes, $[\text{Ir}(\text{ppy-C4})_2(\text{N}^{\wedge}\text{N})](\text{PF}_6)$ ($\text{N}^{\wedge}\text{N} = \text{Me}_2\text{-bpy}$, phen, $\text{Me}_4\text{-phen}$, $\text{Ph}_2\text{-phen}$) with common diimine ligands have been prepared to investigate the dual emissive properties. By introducing a phenyl ring to the cyclometalating ligand, another series of cyclometalated iridium(III) polypyridine complexes $[\text{Ir}(\text{pppy-C4})_2(\text{N}^{\wedge}\text{N})](\text{PF}_6)$ ($\text{N}^{\wedge}\text{N} = \text{Me}_2\text{-bpy}$, phen, $\text{Me}_4\text{-phen}$, $\text{Ph}_2\text{-phen}$, bpy-et, bpy-C6-biotin) has been designed, synthesized, and characterized. Their electrochemical and photophysical properties have been studied and compared to their amine-free counterparts $[\text{Ir}(\text{pppy})_2(\text{N}^{\wedge}\text{N})](\text{PF}_6)$ ($\text{N}^{\wedge}\text{N} = \text{Me}_2\text{-bpy}$, phen, $\text{Me}_4\text{-phen}$, $\text{Ph}_2\text{-phen}$, bpy-et, bpy-C6-biotin). The cytotoxicity of all the complexes has been examined by the MTT assay.

Abbreviations

ABC	avidin-biotin complex
acac	2,4-pentanedionate
AM-carboxy-H ₂ DCFDA	6-carboxy-2',7'-dichlorodihydrofluorescein iacetate, di(acetoxymethyl ester)
BNCT	boron neutron capture therapy
BODIPY	4,4-difluoro-4-bora-3a,4a-diaza-s-indacene
bpm	2,2'-bipyrimidine
bpy	2,2'-bipyridine
bpy-C10	4- <i>n</i> -decylaminocarbonyl-4'-methyl-2,2'-bipyridine
bpy-C18	4- <i>n</i> -octadecylaminocarbonyl-4'-methyl-2,2'- bipyridine
bpy-C2-biotin	4-(<i>N</i> -(2-(biotinamido)ethyl)aminomethyl)-4'- methyl-2,2'-bipyridine
bpy-C2-biotin ₂	4,4'-bis((2-(biotinamido)ethyl)aminocarbonyl)- 2,2'-bipyridine
bpy-C2-C6-biotin ₂	4,4'-bis((2-((6-(biotinamido)hexanoyl)amino) ethyl)aminocarbonyl)-2,2'-bipyridine
bpy-C4	4,4'-bis(<i>n</i> -butylaminocarbonyl)-2,2'-bipyridine
bpy-C6-biotin	4-(<i>N</i> -((6-biotinamido)hexyl)aminocarbonyl)-4'- methyl-2,2'-bipyridine
bpy-C6-estradiol	4-(<i>N</i> -(6-(4-(17 α -ethynylestradiolyl)benzoylamino) hexyl)aminocarbonyl))-4'-methyl-2,2'-bipyridine

bpy-C6-indole	4-((5-((2-(indol-3-yl)ethyl)aminocarbonyl)pentyl)aminocarbonyl)-4'-methyl-2,-2'-bipyridine
bpy-et	4-ethylaminocarbonyl-4'-methyl-2,2'-bipyridine
bpy-indole	4-((2-(indol-3-yl)ethyl)aminocarbonyl)-4'-methyl-2,2'-bipyridine
bpy-NHS	4-succinimidyl-carboxy-4'-methyl-2,2'-bipyridine
bpy-Ph-estradiol	5-(4-(17 α -ethynylestradiolyl)phenyl)-2,2'-bipyridine
bpy-TEG-biotin	4-((13-biotinamido-4,7,10-trioxa)tridecylaminocarbonyl)-4'-methyl-2,2'-bipyridine
BRAB	bridged avidin-biotin
BSA	bovine serum albumin
^t Butpy	4'-(3,5-di- <i>tert</i> -butylphenyl)-2,2':6',2''-terpyridine
CCCP	3-chlorophenylhydrazone
β -CD	β -cyclodextrin
chrysi	5,6-chrysenediimine
cryo-TEM	cryogenic transmission electron microscopy
CT	charge-transfer
DAPI	4',6-diamidino-2-phenylindole
DFT	density functional theory
DHR	dihydrorhodamine 123
DMEM	Dulbecco's modified Eagle's medium
dmpp	(<i>Z</i>)-4-(4-(dimethylamino)benzylidene)-3-methyl-1-(pyridin-2-yl)-1 <i>H</i> -pyrazol-5-(4 <i>H</i>)-one
dnb	3,5-dinitrobenzoate
DPA	2,2'-dipicolylamine

DPAT	tyramine-derived 2,2'-dipicolylamine
dpb	2,3-bis(2-pyridyl)benzoquinoxaline
dpp	2,3-bis(2-pyridyl)pyrazine
dppa	dipyrido[3,2- <i>a</i> :2',3'- <i>c</i>]phenazine-11-carboxylic acid
dppb	dipyrido[3,2- <i>a</i> :2',3'- <i>c</i>]phenazin-11-ylphenylmethanone
dppm2	10-methyldipyrido[3,2- <i>a</i> :2',3'- <i>c</i>]phenazine
dppn	benzo[<i>l</i>]dipyrido[3,2- <i>a</i> :2',3'- <i>c</i>]phenazine
dppp2	pyrido[2',3':5,6]pyrazino[2,3- <i>f</i>]-1,10-phenanthroline
dppp3	pyrido[3',4':5,6]pyrazino[2,3- <i>f</i>]-1,10-phenanthroline
dppx	11,12-dimethyldipyrido[3,2- <i>a</i> :2',3'- <i>c</i>]phenazine
dppz	dipyrido[3,2- <i>a</i> :2',3'- <i>c</i>]phenazine
dppz-C2-biotin	11-((2-biotinamido)ethyl)aminocarbonyldipyrido[3,2- <i>a</i> :2',3'- <i>c</i>]-phenazine
dpq	dipyrido[3,2- <i>f</i> :2',3'- <i>h</i>]quinoxaline
dpqa	2- <i>n</i> -butylaminocarbonyldipyrido[3,2- <i>f</i> :2',3'- <i>h</i>]quinoxaline
dpq-C2-biotin	2-((2-biotinamido)ethyl)aminocarbonyldipyrido[3,2- <i>f</i> :2',3'- <i>h</i>]quinoxaline
dpq-C5	2- <i>n</i> -pentylaminocarbonyldipyrido[3,2- <i>f</i> :2',3'- <i>h</i>]quinoxaline
dpqu	2,3-bis(2-pyridyl)quinoxaline
ds-CT-DNA	double-stranded calf-thymus DNA
ds-DNA	double-stranded DNA
DSPC	1,2-distearoyl- <i>sn</i> -glycero-3-phosphocholine

DTPA	diethylenetriaminepentaacetic acid
<i>E. coli</i>	<i>Escherichia coli</i>
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EDTA	ethylenediaminetetraacetic acid
EPR	electron paramagnetic resonance
ER α	estrogen receptor α
ESI	electrospray ionization
Fast MCS	fast multi-channel scaling
FBS	fetal bovine serum
Fc	ferrocene
Fc ⁺	ferrocenium
Flrpic	iridium(III) bis((4,6-difluorophenyl)-pyridinato- <i>N,C</i> ^{2'})picolinate
gly	glycine
GT	gene therapy
H ₂ biim	2,2'-biimidazole
H ₂ DCFDA	2',7'-dichlorodihydrofluorescein diacetate
H ₂ dppy	2,6-diphenylpyridine
HABA	4'-hydroxyazobenzene-2-carboxylic acid
HAT	1,4,5,8,9,12-hexaazatriphenylene
Hazppz	3-(4-aza-15-crown-5-phenyl)pyridylpyrazole
Hbmpqpy	2,6-bis(7'-methyl-4'-phenyl-2'-quinolyl)pyridine
Hbppz	2-(5-((<i>tert</i> -butyl)-1 <i>H</i> -pyrazol-3-yl)pyridine
HBpq	2-(4-(dimesitylboryl)phenyl)quinoline

Hbsb	2-((1,1'-biphenyl)-4-yl)benzothiazole
Hbsn	2-(1-naphthyl)benzothiazole
Hbt	2-phenylbenzothiazole
Hbtp	2-(benzo[<i>b</i>]thiophen-2-yl)pyridine
Hbtth	2-(2-thienyl)benzothiazole
Hbzq	7,8-benzoquinoline
Hdbq	dibenzo[<i>f,h</i>]quinoxaline
Hdcbpy	4-carboxy-2,2'-bipyridine-4'-carboxylate
Hdfppy	2-(2,4-difluorophenyl)pyridine
Hdpyx	1,3-di(2-pyridyl)-4,6-dimethylbenzene
HE	high-energy
Hfppz	2-(5-(trifluoromethyl)-1 <i>H</i> -pyrazol-3-yl)pyridine
Hfptz	2-(5-(trifluoromethyl)-2 <i>H</i> -1,2,4-triazol-3-yl)pyridine
His	histidine
Hmppy	2-(4-methylphenyl)pyridine
Hmppz	3-methyl-1-phenylpyrazole
HOMO	highest occupied molecular orbital
Hpba	4-(2-pyridyl)benzaldehyde
Hdpdz	1-phenyl-3,5-dimethylpyrazole
Hpiq	1-phenyl-isoquinoline
HPLC	high-performance liquid chromatography
Hpppy	2-((1,1'-biphenyl)-4-yl)pyridine
Hpppy-C4	2-((4'-((<i>N-n</i> -butyl)aminomethyl)-1,1'-biphenyl)-4-yl)pyridine
Hpppy-CHO	2-((4'-carboxyaldehyde-1,1'-biphenyl)-4-yl)pyridine

Hppy	2-phenylpyridine
Hppy-C4	2-(4-((<i>N-n</i> -butyl)aminomethyl)phenyl)pyridine
Hppy-C2-biotin	2-(4-(<i>N</i> -(2-(biotinamido)ethyl)aminomethyl)phenyl)pyridine
Hppy-C6-biotin	2-(4-(<i>N</i> -(6-(biotinamido)hexyl)aminomethyl)phenyl)pyridine
Hppz	1-phenylpyrazole
Hpq	2-phenylquinoline
HSA	human serum albumin
Hthq	2-(2-thienyl)quinoline
HUVEC	human umbilical vein endothelial cell
ICP-MS	inductively coupled plasma mass spectroscopy
IL	intraligand
ILCT	intraligand charge-transfer
IR	infra-red
LAB	labeled avidin-biotin
LE	low-energy
LED	light-emitting device
LLCT	ligand-to-ligand charge-transfer
LUMO	lowest unoccupied molecular orbital
Lys-Cys	lysine-cysteine
Mal	maleimide
MC	metal-centered
mcbpy	4'-methyl-2,2'-bipyridine-4-carboxylate
mCP	<i>N,N'</i> -dicarbazolyl-3,5-benzene

MDCK	Madin-Darby canine kidney
Me ₂ -bpy	4,4'-dimethyl-2,2'-bipyridine
2,9-Me ₂ -phen	2,9-dimethyl-1,10-phenanthroline
4,7-Me ₂ -phen	4,7-dimethyl-1,10-phenanthroline
5,6-Me ₂ -phen	5,6-dimethyl-1,10-phenanthroline
Me ₂ -Ph ₂ -phen	2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline
Me ₄ -phen	3,4,7,8-tetramethyl-1,10-phenanthroline
Metpy	4'-methyl-2,2':6',2''-terpyridine
MLCT	metal-to-ligand charge-transfer
MRI	magnetic resonance imaging
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NEM	<i>N</i> -ethylmaleimide
NHS	<i>N</i> -hydroxysuccinimide
NLCT	amine-to-ligand charge-transfer
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy
OLED	organic light-emitting diode
PAMAM	polyamidoamine
PBS	phosphate buffered saline
pDNA	plasmid DNA
PEG	poly(ethylene glycol)
Ph ₂ -bpy	4,4'-diphenyl-2,2'-bipyridine
Ph ₂ -phen	4,7-diphenyl-1,10-phenanthroline
phe	phenylalanine

phen	1,10-phenanthroline
phen-TU-et	<i>N</i> -ethyl- <i>N'</i> -1,10-phenanthroline-5-yl-thiourea
phen-TU-MeA	<i>N</i> -(1-adamantanemethyl)- <i>N'</i> -1,10-phenanthroline-5-yl-thiourea
phen-TU-Ph	<i>N</i> -phenyl- <i>N'</i> -1,10-phenanthroline-5-yl-thiourea
phi	9,10-phenanthrene diimine
PLED	polymer-based electrophosphorescent light-emitting diode
py	pyridine
py-biotin	4-(biotinamidomethyl)-pyridine
py-biotin-NCS	3-isothiocyanato-5-(<i>N</i> -((2-biotinamido)ethyl)aminocarbonyl)pyridine
py-C2-biotin	3-(<i>N</i> -((2-biotinamido)ethyl)aminocarbonyl)pyridine
py-C6-biotin	4-(<i>N</i> -((6-biotinamido)hexanoyl)aminomethyl)pyridine
py-C6-indole	<i>N</i> -(<i>N</i> -(3-pyridoyl)-6-aminohexanoyl)tryptamine
py-indole	<i>N</i> -(3-pyridoyl)tryptamine
py-TU-DPAT	3-(2-(4-hydroxy-3-(2,2'-dipicolylaminomethyl)phenyl)ethylthioureydyl)pyridine
quqo	2-(quinolin-2-yl)quinoxaline
r-[¹²⁵ I]SAv	radioactive recombinant streptavidin
RET	resonance-energy transfer
ROS	reactive oxygen species
r-SAv	recombinant streptavidin
SAv	streptavidin

SBLCT	sigma-bond-to-ligand charge-transfer
SCE	saturated calomel electrode
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SSCE	sodium saturated calomel electrode
TAE	Tris-acetate-EDTA
TBAH	tetrabutylammonium hydroxide
TBAP	tetra- <i>n</i> -butylammonium hexafluorophosphate
TCSPC	time-correlated single photon counting
TEG	triethylene glycol
TFA	trifluoroacetic acid
thipy	2-(2'-thienyl)pyridine
TMS	tetramethylsilane
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
trp	tryptophan
TTHA	tetraethylenetetraaminehexaacetic acid
ttpy	4'-tolyl-2,2':6',2''-terpyridine
UV	ultraviolet

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