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Iridium(III) *bis*-Nitrone Complexes as Innovative Phosphorogenic Reagents for Bioimaging and Phototherapeutics

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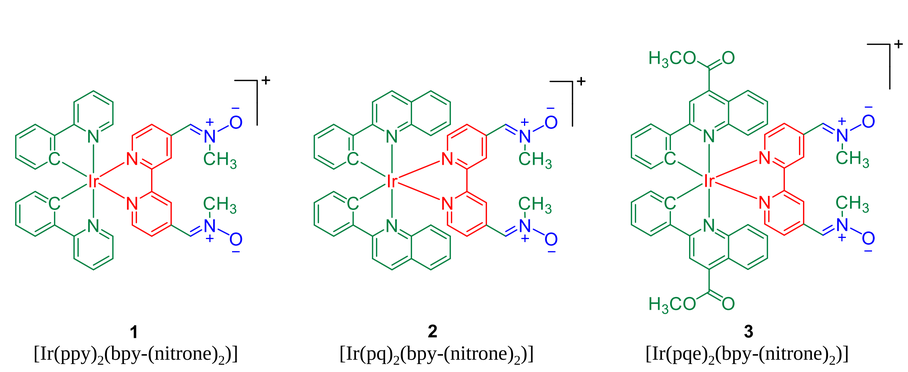
Introduction

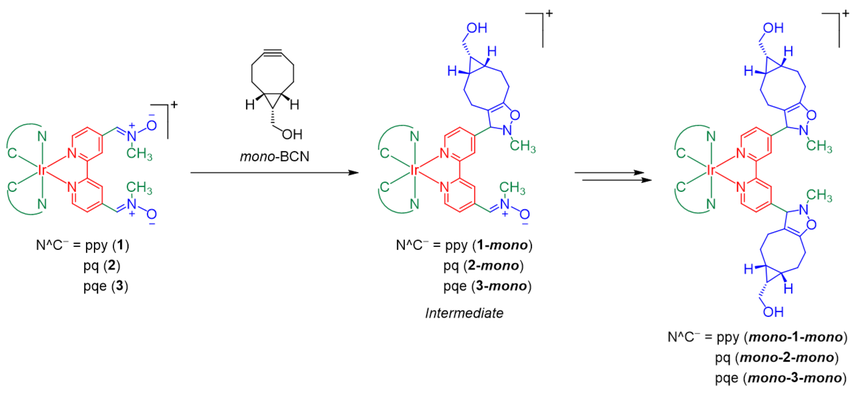
Bioorthogonal chemical reactions have emerged as useful tools for labeling biomolecules in living systems. Nitrones belong to a relatively new bioorthogonal system that has demonstrated high versatility and applicability. These compounds show high reactivity toward strained cyclooctynes via the strain-promoted alkyne-nitrone cycloaddition (SPANC) reaction. Transition metal complexes containing a nitrone moiety are weakly emissive due to the efficient non-radiative decay caused by the photoinduced C=N isomerization, but exhibit intense and long-lived emission upon reaction with strained alkynes such as (1*R*,8*S*,9*s*)-bicyclo[6.1.0]nonyne (BCN).

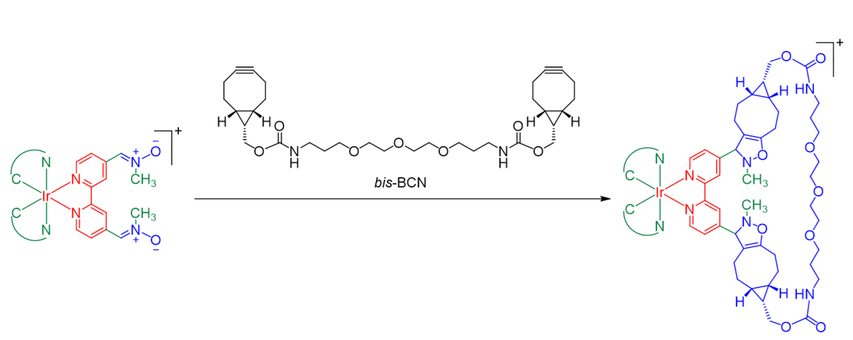
In this work, three cyclometalated iridium(III) complexes functionalized with two nitrone units were designed as novel phosphorogenic bioorthogonal reagents for bioimaging and phototherapeutics.

Structure of the complexes



Bioorthogonal reaction with *mono*-BCN and *bis*-BCN





mono-BCN = (1*R*,8*S*,9*s*)-bicyclo[6.1.0]non-4-yn-9-ylmethanol
bis-BCN = 1,13-bis((1*R*,8*S*,9*s*)-bicyclo[6.1.0]non-4-yn-9-ylmethyloxycarbonylamino)-4,7,10-trioxatridecane

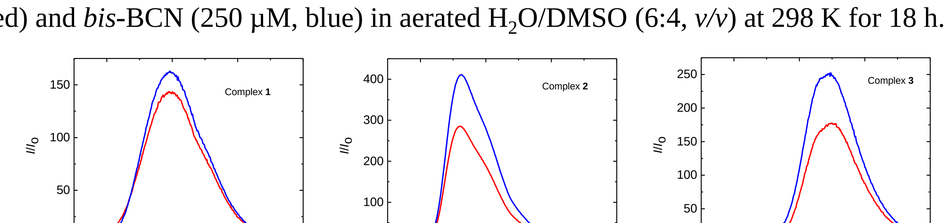
Bioorthogonal reactivity toward strained alkynes

Emission maxima (λ_{em}), emission enhancement factors (I/I_o), emission lifetimes (τ), and second-order rate constants (k_2) of complexes **1** – **3**, bpy-(nitrone)$_2$ (10 μM) upon reaction with *mono*-BCN and *bis*-BCN in aerated H_2O/DMSO (6:4, *v/v*) at 298 K.

Compound	+ *mono*-BCN				+ *bis*-BCN			
	λ_{em}/nm	I/I_o[a]	τ/μs	k_2/M^{-1} s^{-1}	λ_{em}/nm	I/I_o[a]	τ/μs	k_2/M^{-1} s^{-1}
1	596	143.6	0.09	0.26	596	163.0	0.10	1.57
2	560	272.9	0.51	0.46	563	411.1	0.82	5.78
3	650	202.6	0.24	0.94	648	252.5	0.31	6.72
bpy-(nitrone)$_2$	*N/A*	*N/A*	*N/A*	0.07	*N/A*	*N/A*	*N/A*	0.51

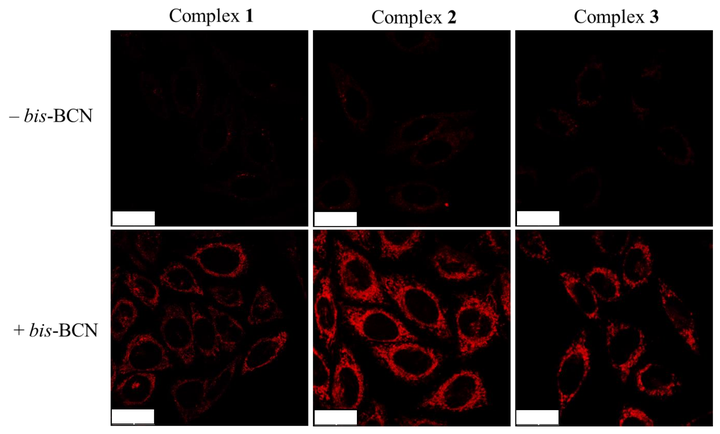
[a] I_o and I are the emission intensities of complexes **1** – **3** (10 μM) in the absence and presence of *mono*-BCN (500 μM) and *bis*-BCN (250 μM), respectively; incubation time = 18 h.

Emission spectra of complexes **1** – **3** (10 μM) in the absence (black) and upon incubation of *mono*-BCN (500 μM, red) and *bis*-BCN (250 μM, blue) in aerated H_2O/DMSO (6:4, *v/v*) at 298 K for 18 h.

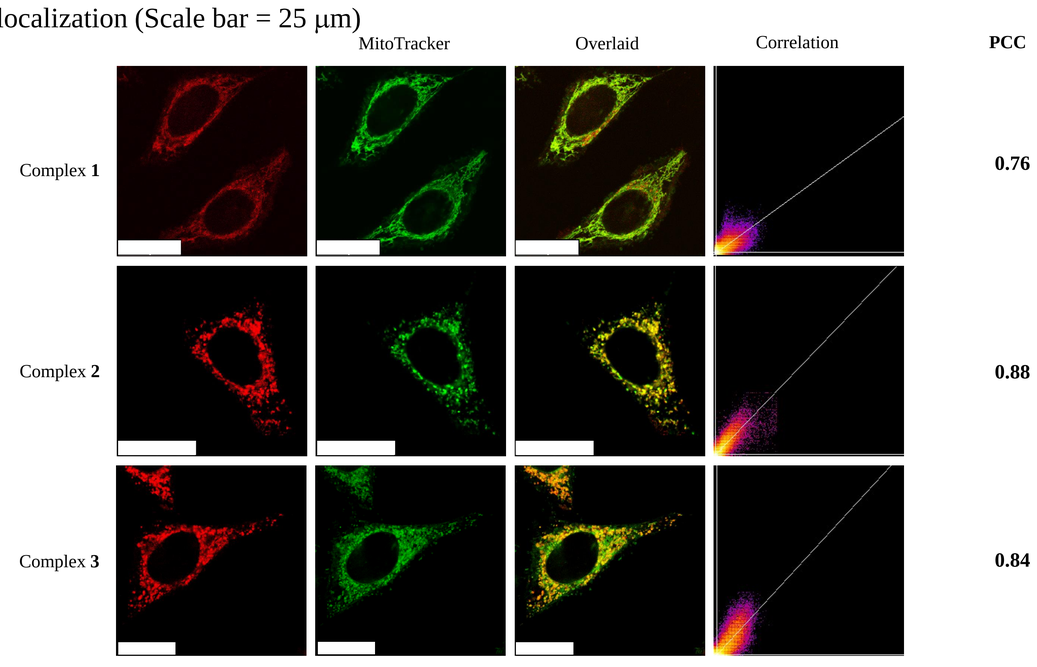


Cellular studies

Intracellular emission enhancement (Scale bar = 25 μm)



Intracellular localization (Scale bar = 25 μm)



(Photo)cytotoxicity of the complexes

Cytotoxicity of complexes **1** – **3** toward HeLa cells in the dark and upon irradiation at 450 nm (light dosage =14.6 mW cm^{-2}) for 10 min. PI is the ratio $IC_{50,dark}/IC_{50,light}$ under different conditions.[a]

Complex	– *bis*-BCN			+ *bis*-BCN[a] (50 μM)		
	$IC_{50,dark}$/μM	$IC_{50,light}$/μM	PI	$IC_{50,dark}$/μM	$IC_{50,light}$/μM	PI
1	> 100	86.49 ± 0.02	> 1.16	85.26 ± 2.51	23.52 ± 0.73	3.63
2	34.61 ± 0.53	5.33 ± 0.45	6.49	14.61 ± 0.41	0.067 ± 0.005	218.06
3	33.76 ± 0.40	1.15 ± 0.14	29.36	12.47 ± 0.29	0.060 ± 0.005	207.83
4	54.49 ± 1.71	1.16 ± 0.26	46.97	39.86 ± 1.20	0.050 ± 0.008	797.2

[a] Cells were treated with *bis*-BCN (50 μM, 1 h), followed by washing with PBS (100 μL × 1), and incubated with complexes for 3 h.

Conclusion

Three cyclometalated iridium(III) *bis*-nitrone complexes were prepared as novel phosphorogenic bioorthogonal reagents for bioimaging and phototherapeutics. Upon reaction with BCN substrates, the complexes were converted to isoxazoline derivatives, rendering the reaction mixture to display significant emission enhancement and lifetime extension. Notably, the complexes showed a higher reaction rate toward a *bis*-cyclooctyne derivative containing a TEG linker (*bis*-BCN) compared with its monomeric counterpart. The complexes exhibited high photocytotoxicity in *bis*-BCN-pretreated HeLa cells, which was attributed to the enhanced $^1O_2$ photosensitization upon elimination of the nitrone-associated quenching pathway. The crosslinking properties of the complexes mean that they are promising candidates for the construction of polymeric materials with intriguing photophysical and biological characteristics.

References

1. Lee, L. C.-C.; Lau, J. C.-W.; Liu, H.-W.; Lo, K. K.-W. *Angew. Chem. Int. Ed.* **2016**, *55*, 1046 – 1049.
2. Tang, T. S.-M.; Liu, H.-W.; Lo, K. K.-W. *Chem. Eur. J.* **2016**, *22*, 9649 – 9659.
3. Yip, A. M.-H.; Lai, C. K.-H.; Yiu, K. S.-M.; Lo, K. K.-W. *Angew. Chem. Int. Ed.* **2022**, *e202116078*.