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Rhenium(I) polypyridine dibenzocyclooctyne complexes as phosphorescent bioorthogonal probes: Synthesis, characterization, emissive behavior, and biolabeling properties

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ABSTRACT

We report the development of rhenium(I) polypyridine complexes appended with a dibenzocyclooctyne (DIBO) moiety as bioorthogonal probes for azide-modified biomolecules. Three phosphorescent rhenium(I) polypyridine DIBO complexes [Re(N^N)(CO)$_3$(py-C6-DIBO)][CF_3SO_3] (py-C6-DIBO = 3-(*N*-(6-(3,4:7,8-dibenzocyclooctyne-5-oxycarbonylamino)hexyl)aminocarbonyl)pyridine; N^N = 1,10-phenanthroline (phen) (**1a**), 3,4,7,8-tetramethyl-1,10-phenanthroline (Me_4-phen) (**2a**), 4,7-diphenyl-1,10-phenanthroline (Ph_2-phen) (**3a**)) and their DIBO-free counterparts [Re(N^N)(CO)$_3$(py-C6-BOC)][CF_3SO_3] (py-C6-BOC = 3-(*N*-(6-(*tert*-butoxycarbonylamino)hexyl) aminocarbonyl)pyridine; N^N = phen (**1b**), Me_4-phen (**2b**), Ph_2-phen (**3b**)) were synthesized and characterized. Upon photoexcitation, all the complexes displayed intense and long-lived yellow triplet metal-to-ligand charge-transfer (^{3}MLCT) (dπ(Re) → π*(N^N)) emission. The DIBO complexes underwent facile reactions with benzyl azide in methanol at 298 K with second-order rate constants (k_2) in the range of 0.077 to 0.091 M^{-1} s^{-1}. As revealed from SDS-PAGE analysis, the DIBO complexes can selectively label azide-modified proteins and the resulting bioconjugates displayed strong phosphorescence upon photoexcitation. Results of inductively coupled plasma mass spectrometry (ICP-MS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays indicated that the DIBO complexes accumulated in Chinese Hamster Ovary (CHO) cells with considerable cytotoxic activity. Upon incubation of CHO cells with these complexes, relatively weak intracellular emission was observed. In contrast, upon pretreatment of the cells with 1,3,4,6-tetra-*O*-acetyl-*N*-azidoacetyl-D-mannosamine (Ac_4ManNAz), intense emission was observed from the cell membrane and some internal compartments. The results suggest that the DIBO complexes are promising candidates for imaging azide-labeled biomolecules.

1. Introduction

The discovery of green fluorescent protein enables the visualization of protein dynamics in living systems through a genetic tagging method [1]. However, this strategy is not applicable to the study of non-protein biomolecules such as nucleic acids, glycans, lipids, and small-molecule metabolites. To overcome this limitation, a bioorthogonal chemistry approach has been designed as a versatile method to study these biomolecules in their native environments. A number of chemical reporters and their specific probes for bioorthogonal reactions have been identified and successfully applied in biological studies [2,3]. Among different bioorthogonal reactions, the strain-promoted alkyne-azide cycloaddition (SPAAC) between azide and strained alkyne is one of the most extensively used reactions in bioorthogonal chemistry [4,5]. Azide is a particularly useful handle because of its small size, non-native nature, and inertness toward biomolecules; for example, 1,3,4,6-tetra-*O*-acetyl-*N*-azidoacetyl-D-mannosamine (Ac_4ManNAz) is a very commonly used unnatural sugar derivative [6,7]. Upon cellular uptake, the acetyl groups are removed by carboxyesterases and the mannose derivative is metabolized by glycosyltransferases, and eventually expressed as an end-group of a glycan chain, leaving the azide group unmodified. Thus, the azide group is able to undergo specific reactions with bioorthogonal probes such as a strained alkyne. To date, the design of bioorthogonal probes to trace azide-labeled biomolecules has been focused on affinity tags [8–11], fluorescent organic dyes [12–15], and luminescent quantum dots [16]. Recently, we have prepared iridium(III) dibenzocyclooctyne (DIBO) complexes as the first class of transition metal-based bioorthogonal probes [17]. In view of the interesting photophysical and biological properties of rhenium(I) polypyridine complexes [18], we anticipate that the modification of these complexes with a DIBO moiety will generate a new class of phosphorescent bioorthogonal reagents for various biological applications. Herein, we report the synthesis and characterization of three phosphorescent rhenium(I) polypyridine

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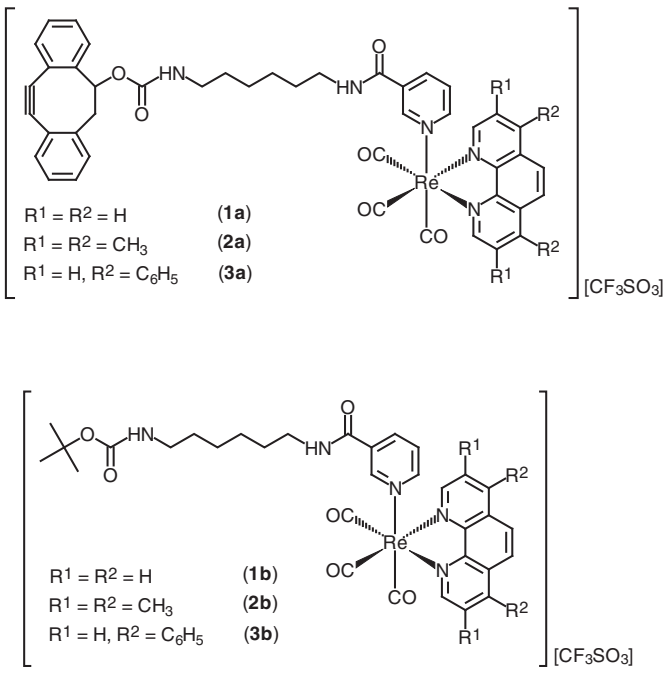
DIBO complexes [Re(N^N)(CO)$_3$(py-C6-DIBO)][CF_3SO_3] (py-C6-DIBO = 3-(*N*-(6-(3,4:7,8-dibenzocyclooctyne-5-oxycarbonylamino)hexyl)-aminocarbonyl)pyridine; N^N = 1,10-phenanthroline (phen) (**1a**), 3,4,7,8-tetramethyl-1,10-phenanthroline (Me_4-phen) (**2a**), 4,7-diphenyl-1,10-phenanthroline (Ph_2-phen) (**3a**)) and their DIBO-free counterparts [Re(N^N)(CO)$_3$(py-C6-BOC)][CF_3SO_3] (py-C6-BOC = 3-(*N*-(6-(*tert*-butoxycarbonylamino)hexyl)aminocarbonyl)pyridine; N^N = phen (**1b**), Me_4-phen (**2b**), Ph_2-phen (**3b**)) (Scheme 1). The spectroscopic and photophysical properties of the complexes were studied. The DIBO complexes underwent facile reactions with benzyl azide and the reaction kinetics were investigated. The bioorthogonal labeling properties of these complexes toward azide-modified bovine serum albumin (BSA), human serum albumin (HSA), and apo-transferrin (aTf) were examined. Additionally, the cellular uptake properties and cytotoxicity of these complexes were studied by inductively coupled plasma mass spectrometry (ICP-MS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays, respectively. Furthermore, the bioorthogonal labeling properties of one of the DIBO complexes were examined using Chinese Hamster Ovary (CHO) cells pretreated with Ac_4ManNAz.

2. Results and discussion

2.1. Design and synthesis of complexes

Regarding the design of the DIBO complexes **1a**–**3a**, the DIBO moiety was attached to the pyridine ligand through a hexyl spacer-arm to reduce the steric bulkiness of the rhenium(I) polypyridine unit. The pyridine ligand py-C6-DIBO was synthesized from the reaction of 3,4:7,8-dibenzocyclooctyn-5-yl 4-nitrophenyl carbonate with 3-(6-aminohexyl) aminocarbonylpyridine (py-C6-NH_2). The DIBO complexes **1a**–**3a** and the DIBO-free complexes **1b**–**3b** were obtained from the reaction of [Re(N^N)(CO)$_3$(CH_3CN)][CF_3SO_3] with the pyridine ligands py-C6-DIBO and py-C6-BOC, respectively, in refluxing THF. The samples were purified by column chromatography and recrystallization from CH_2Cl_2/diethyl ether. They were characterized by ^{1}H NMR, positive-ion ESI-MS, IR spectroscopy, and microanalysis. All the complexes were stable in air and soluble in most organic solvents such as CH_2Cl_2 and alcohols. One of the basic requirements for luminescent probes is their stability in the medium and environment where they are used [19]. Based on the ESI-MS data, all the complexes in this work remained stable in aqueous solutions (containing 1% DMSO for solubility reasons).



Scheme 1. Structures of the rhenium(I) DIBO complexes **1a**–**3a** and their DIBO-free counterparts complexes **1b**–**3b**.

2.2. Photophysical properties

The electronic absorption spectral data of the complexes are summarized in Table 1. All the complexes displayed intense spin-allowed intraligand (^{1}IL) ($\pi \rightarrow \pi^*$) (N^N and pyridine ligands) absorption bands at ca. 250–338 nm and metal-to-ligand charge-transfer (^{1}MLCT) ($d\pi(Re) \rightarrow \pi^*$(N^N)) absorption shoulders at ca. 366–394 nm [20–49]. It is noteworthy that the DIBO complexes revealed an additional absorption shoulder at ca. 305 nm, which was assigned to a ($\pi \rightarrow \pi^*$) transition of the DIBO moiety.

Upon photoexcitation, all the complexes showed intense and long-lived green to yellow emission. The photophysical data are summarized in Table 2 and the emission spectra of the DIBO complexes **1a**–**3a** in CH_3CN at 298 K are shown in Fig. 1. In fluid solutions at 298 K, most of the complexes exhibited increased emission energy, quantum yields, and lifetimes on changing the solvent from the more polar CH_3CN to the less polar CH_2Cl_2. These findings, together with the dependence of the emission energy on the π^* orbital energy level of the N^N ligands, point to an emissive state of triplet metal-to-ligand charge-transfer (^{3}MLCT) ($d\pi(Re) \rightarrow \pi^*$(N^N)) character. However, the structural features and long emission lifetimes of the Me_4-phen complexes in fluid solutions under ambient conditions (Table 2) suggest the involvement of triplet intraligand (^{3}IL) ($\pi \rightarrow \pi^*$) (Me_4-phen) character in their emissive states.

2.3. Reaction with benzyl azide

The azide-targeting properties of the DIBO complexes were examined using benzyl azide as a model substrate (Scheme 2). The SPAAC reaction kinetics of the DIBO complexes with benzyl azide in methanol at 298 K were studied by monitoring the exponential decay of the absorption feature of DIBO at ca. 305 nm and the results are summarized in Table 3. The formation of the triazole complexes was confirmed by electrospray ionization mass spectrometry (ESI-MS) (Table 4) and the plots of the pseudo first-order rate constants (k_{obs}) of the DIBO complexes versus the concentrations of benzyl azide are shown in Fig. 2. The second-order rate constants (k_2) were determined to range from 0.077 to 0.091 M^{-1} s^{-1} (Table 3), which are comparable to those of other DIBO-azide systems such as dibenzocyclooctynol (k_2 = 0.057 M^{-1} s^{-1}) [10] and [Ir(ppy-COOH)$_2$(bpy-TEG-DIBO)][PF_6] (k_2 = 0.069 M^{-1} s^{-1}) [17]. This result reflects that the bulky rhenium(I) polypyridine unit did not

Table 1
Electronic absorption spectral data of rhenium(I) polypyridine complexes at 298 K.

Complex	Solvent	λ_{abs} [nm] (ε [M^{-1} cm^{-1}])
1a	CH_2Cl_2	257 sh (30 365), 277 (38 725), 288 sh (34 460), 306 (25 385), 334 sh (5940), 375 sh (4005)
	CH_3CN	259 sh (31 165), 274 (41 920), 285 sh (38 860), 304 (29 245), 330 sh (6655), 367 sh (3970)
1b	CH_2Cl_2	257 sh (27 585), 277 (29 790), 295 sh (16 155), 334 sh (6140), 380 sh (4085)
	CH_3CN	254 sh (22 710), 274 (26 840), 290 sh (15 775), 325 sh (6225), 368 sh (3560)
2a	CH_2Cl_2	254 (33 715), 282 (46 375), 306 (32 055), 328 sh (10 840), 369 sh (4245)
	CH_3CN	250 (34 945), 286 (46 340), 304 (33 300), 320 sh (12 260), 366 sh (3710)
2b	CH_2Cl_2	251 (30 520), 282 (33 815), 325 sh (12 610), 373 sh (4305)
	CH_3CN	247 (33 530), 280 (35 020), 313 sh (14 755), 366 sh (3880)
3a	CH_2Cl_2	276 sh (44 430), 289 (57 335), 304 sh (46 980), 338 sh (16 265), 384 sh (8970)
	CH_3CN	264 sh (37 375), 288 (58 395), 303 sh (45 995), 334 sh (16 780), 382 sh (7995)
3b	CH_2Cl_2	271 sh (34 445), 294 (49 710), 337 sh (20 650), 394 sh (9245)
	CH_3CN	258 sh (35 105), 291 (51 485), 334 sh (20 495), 386 sh (9295)