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Luminescent Rhenium(I) Polypyridine Complexes Appended with an α-D-Glucose Moiety as Novel Biomolecular and Cellular Probes

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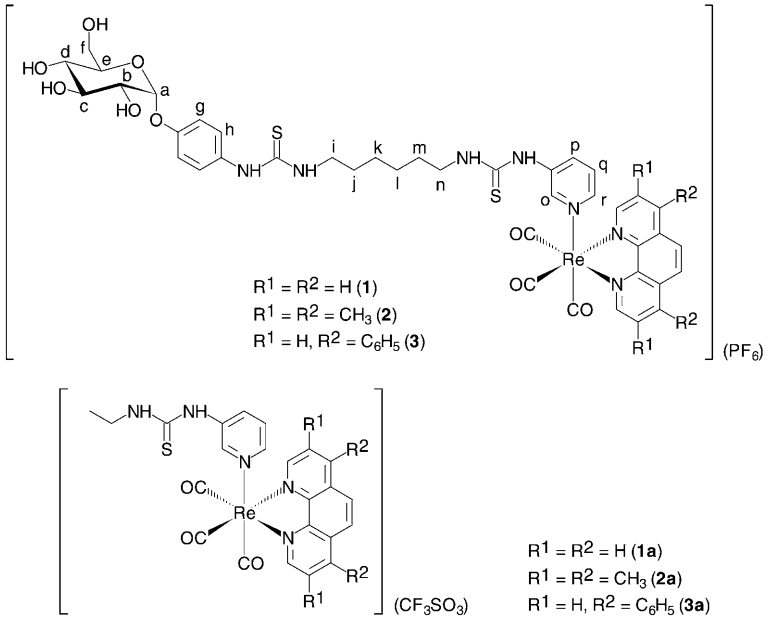
Glucose is the most important carbohydrate in cellular metabolism and an energy source for the growth of cells.[1] One of the most characteristic phenotypes of rapidly growing cancer cells is their propensity to catabolize glucose at high rates, possibly due to the overexpression of glucose transporters (GLUTs).[2] Thus, the in vitro and in vivo monitoring of glucose utilization in cancer cells has attracted much attention. Different reporting and therapeutic units such as radioactive labels,[3a] IRDye 800CW,[3b] organic fluorophores,[3c,d] and two-photon dyes[3e] have been conjugated to glucose or 2-deoxyglucose for the diagnosis and treatment of various tumors or cancers. Despite the development of these reagents, the possibility of using luminescent transition-metal glucose conjugates as glucose-uptake tracers and cancer cell imaging reagents has not been explored.[4] With our ongoing interest in luminescent rhenium(I) polypyridine complexes as biological probes,[5] we envisage that modification of these complexes with an α-D-glucose pendant will generate useful luminescent probes for biomolecules and cancer cells.



Scheme 1. Structures of the rhenium(I) polypyridine complexes.

Herein we report three rhenium(I) polypyridine glucose complexes $[Re(N^N)(CO)_3(py\text{-}3\text{-}glu)](PF_6)$ (py-3-glu = 3-(*N*-(6-(*N*′-(4-(α-D-glucopyranosyl)phenyl)thioureidyl)hexyl)thioureidyl)pyridine, N^N = 1,10-phenanthroline (phen) (**1**), 3,4,7,8-tetramethyl-1,10-phenanthroline (Me_4-phen) (**2**), 4,7-diphenyl-1,10-phenanthroline (Ph_2-phen) (**3**)) and their glucose-free counterparts $[Re(N^N)(CO)_3(py\text{-}3\text{-}Et)](CF_3SO_3)$ (py-3-Et = 3-(ethylthioureidyl)pyridine, N^N = phen (**1a**), Me_4-phen (**2a**), Ph_2-phen (**3a**)) (Scheme 1). The glucose complexes were synthesized from the addition reaction of the isothiocyanate complexes $[Re(N^N)(CO)_3(py\text{-}3\text{-}NCS)](PF_6)$[5a] with AcO-glu-$C_6$-$NH_2$ in acetone, followed by deacetylation (see the Supporting Information, Schemes S1 and S2). All the complexes were characterized by ^{1}H NMR spectroscopy, positive-ion ESI-MS, and IR spectroscopy and gave satisfactory elemental analyses (see the Supporting Information). Upon irradiation, the complexes exhibited intense and long-lived green-to-yellow triplet metal-to-ligand charge-transfer (MLCT; dπ(Re)→π*(N^N)) emission (see the Supporting Information, Table S1).[6] The structured emission band and very long lifetime of complex **2** in alcohol glass at 77 K are probably due to the involvement of ^{3}IL (π→π*) (Me_4-phen) character in its emissive state.[7]

Since the lectin concanavalin A (Con A) binds α-D-mannopyranoside and α-D-glucopyranoside,[8] the possible use of the glucose complexes **1**–**3** as a luminescent sensor for this lectin has been investigated.[9] Upon addition of Con A to a

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Table 1. Results of emission titrations of complexes **1–3** with Con A in potassium phosphate buffer (10 mM) at pH 8.5/methanol (9:1, v/v) with $CaCl_2$ (0.1 mM) and $MnCl_2$ (0.1 mM) at 298 K.

Complex	I/I_o[a]	τ_o [μs][b]	τ [μs][b]	K_a [M^{-1}][c]	n_H[c]
1	1.8	0.39	1.15 (19%), 0.22 (81%)	4.5×10^5	2.0
2	2.0	0.64	4.32 (44%), 0.93 (56%)	5.8×10^5	2.3
3	3.2	0.40	2.04 (33%), 0.40 (67%)	4.4×10^5	2.5

[a] I_o and I are the emission intensities of the complex (5 μM) in the presence of 0 and 5.5 μM Con A, respectively. [b] τ_o and τ are the emission lifetimes of the complex (5 μM) in the presence of 0 and 5.5 μM Con A, respectively. [c] Binding constants and Hill coefficients as determined by the Hill equation.

Table 2. Lipophilicity, cellular uptake, and IC_{50} values of complexes **1–3**, **1a–3a**, and cisplatin.

Complex	Log $P_{o/w}$	Amount of complex [fmol][a]	IC_{50} [μM][b]
1	0.57	1.10±0.09	>150
2	2.73	2.15±0.03	90.0±7.6
3	3.44	3.02±0.10	68.9±2.3
1a	0.77	1.64±0.13	22.8±5.2
2a	2.94	4.73±0.22	7.7±0.6
3a	4.03	6.61±0.33	2.8±0.4
cisplatin	−2.30[c]	N.A.	27.6±1.8

[a] Amounts of rhenium associated with an average HeLa cell upon incubation with the complexes (100 μM) in a glucose-free medium at 37 °C for 5 min as determined by ICP-MS. [b] HeLa cells, incubation in high glucose Dulbecco's modified Eagle's medium (DMEM) for 48 h. [c] See ref. [24].

buffer solution of the complexes, the emission intensities were enhanced by ≈1.8 to 3.2 fold (Table 1) and the emission maxima were blueshifted by ≈5 to 15 nm. The emission decay became biexponential with components of 0.22 to 0.93 μs and 1.15 to 4.32 μs (Table 1). The emission titration curves for complexes **3** and **3a** with Con A are shown in Figure S1 (see the Supporting Information). Since the glucose-free complexes **1a–3a** did not give similar observations, it is likely that the changes exhibited by the glucose complexes originated from the increased hydrophobicity and rigidity of the local environment of the complexes after binding to Con A. The binding has been analyzed by the Hill equation and the binding constants ($K_a=4.4\times10^5$ to $5.8\times10^5\,M^{-1}$, Table 1) are comparable to that of a ruthenium(II) glucose complex Λ-[Ru(α-Glc-3-bpy)$_3$]Cl_2 ($K_a=9.5\times10^5\,M^{-1}$)[8b] but one order of magnitude larger than that of *p*-nitrophenyl-α-D-glucopyranoside ($K_a=1\times10^4\,M^{-1}$).[10] The higher binding affinity has been attributed to the relatively hydrophobic rhenium(I) polypyridine units.

The FimH adhesin of *E. coli* type 1 pili is able to bind D-mannosides and D-glucosides by virtue of a receptor-binding domain.[11] Two *E. coli* strains ORN178 and ORN208 have been used in this work to study the possible binding of the glucose complexes to FimH. The ORN178 strain expresses wild-type type 1 pili that exhibit monosaccharide-binding properties, whereas the ORN208 strain is deficient of the FimH gene and expresses abnormal type 1 pili that do not show similar behavior.[12] Both *E. coli* strains were incubated with the glucose complex **3** for 3 h and then imaged with laser-scanning confocal microscopy. The emission intensity of the ORN178 strain was 7.7 times that of ORN208 ($n=30$) (See the Supporting Information, Figure S2). This observation has been ascribed to the binding of the glucose pendant of the complex to the lectin expressed on the ORN178 strain. Both strains were also stained by the glucose-free complex **3a** but the emission intensities were very weak and indistinguishable (See the Supporting Information, Figure S2), which further supports the specific binding of the glucose moiety of complex **3** to FimH on the ORN178 strain.

The lipophilicity (log$P_{o/w}$) of all the complexes has been measured by reversed-phase HPLC. The log$P_{o/w}$ values followed the orders: **1**<**2**<**3** and **1a**<**2a**<**3a** (Table 2), which are in accordance with the hydrophobic character of the diimine ligands (phen<Me$_4$-phen<Ph$_2$-phen). Interestingly, the glucose moiety reduced the log$P_{o/w}$ values of complexes **1–3** by ≈0.20 to 0.59 units with respect to the glucose-free analogues **1a–3a**. The somewhat high lipophilicity of complexes **1–3** despite the polar glucose unit is probably a consequence of the hydrophobic C6 spacer-arm. We have investigated the cellular uptake properties of the complexes ([Re]=100 μM with HeLa cells at 37 °C for 5 min) and their cytotoxicity by ICP-MS and MTT assays, respectively. The amounts of rhenium taken up by an average HeLa cell were in the femtomole scale (Table 2), which is comparable to the results from other cellular uptake studies.[13] The intracellular amounts in both series of rhenium complexes followed the orders: **1**<**2**<**3** and **1a**<**2a**<**3a**, which are in accordance with their lipophilicity and cytotoxicity (Table 2). Since the glucose complexes **1–3** showed lower cytotoxicity compared to their glucose-free counterparts complexes **1a–3a** (Table 2), the incorporation of a glucose unit renders the complexes more biocompatible.

To study the possible role of GLUTs on the internalization of the glucose complexes, the cellular uptake properties of complexes **3** and **3a** towards two transformed cell lines, HeLa and human breast adenocarcinoma (MCF-7), and two non-transformed cell lines, human embryonic kidney cells (HEK293T) and mouse embryonic fibroblast (NIH/3T3), have been studied. In general, the intracellular amounts of the glucose complex **3** were lower than those of the glucose-free complex **3a** among the cell lines studied (Table 3), which is attributable to the lower lipophilicity of the former complex. Interestingly, the uptake of complex **3** by HeLa

Table 3. Cellular uptake and IC_{50} values of complexes **3** and **3a** towards different cell lines.

Cell line	Amount [fmol][a]		IC_{50} [μM][b]	
	Complex **3**	Complex **3a**	Complex **3**	Complex **3a**
HeLa	2.92±0.36	6.29±0.06	62.9±1.7	3.20±0.70
MCF-7	2.33±0.37	4.30±0.31	20.3±2.0	2.94±0.14
HEK293T	0.86±0.04	3.73±0.68	40.9±1.6	4.94±0.54
NIH/3T3	0.33±0.03	4.43±0.08	>150	2.80±0.56

[a] [Re]=100 μM, incubation in glucose-free DMEM at 37 °C for 5 min. [b] Incubation in high glucose DMEM for 48 h.

and MCF-7 cells was at least 2.7 times that of HEK293T and NIH/3T3 cells. This is possibly a result of the overexpression of GLUTs in the transformed cell lines (HeLa and MCF-7) rather than the non-transformed cell lines (HEK293T and NIH/3T3).[2] In the case of complex **3a**, the difference in cellular uptake efficiency between the transformed and non-transformed cell lines was much smaller (Table 3) and the intracellular amount of rhenium in HeLa cells was slightly higher than those in the other three cell lines. Also, the cytotoxicity of complexes **3** and **3a** did not show any dependence on the cell types (Table 3).

Glucose derivatives entering the cells through a GLUT-mediated glucose-uptake pathway are competitively inhibited by D-glucose but not by L-glucose.[3c–e] The possible involvement of GLUTs in the cellular uptake of complex **3** has been studied. HeLa cells were incubated with this complex (100 μM) for 5 min in the absence or presence of 5 to 50 mM D-glucose or L-glucose in a glucose-free medium and the intracellular rhenium associated with an average HeLa cell has been determined. The addition of D-glucose to the medium led to a decrease of cellular uptake of the complex, but L-glucose gave no effects (Figure 1, top and middle), which supports the argument that the internalization of this complex occurred by a GLUT-mediated uptake pathway. This is also in accordance with the finding that the cellular uptake of complex **3** by HeLa cells decreased with increasing concentration of 2-deoxyglucose in the medium (Figure 1, bottom) since 2-deoxyglucose is transported into the cells in a similar manner to D-glucose.[3a] On the contrary, the cellular uptake of the glucose-free complex **3a** did not show significant changes in the presence of D-glucose, L-glucose, or 2-deoxyglucose (Figure 1). All these results revealed that the glucose-dependent cellular uptake of complex **3** originated from the specific recognition of the D-glucose moiety of the complex by the cells. The possible involvement of GLUTs in the cellular uptake properties of complex **3** has been further examined. Since the expression of GLUTs in HeLa cells can be upregulated by addition of hypoxia-mimetic agents such as cobalt(II) chloride,[14] we have incubated HeLa cells with this salt (250 μM) in the growth medium for 2 h prior to treatment with complex **3** (100 μM) for 5 min. The results showed that the cellular uptake was ≈1.5 fold higher than that of the control in which cobalt(II) chloride was absent. Additionally, incubation of HeLa cells with two glucose-uptake inhibitors fasentin (80 μM) and cytochalasin B (10 μM)[15] for 1 h reduced the intracellular amounts of rhenium by ≈1.4 and 1.7 fold, respectively. In contrast, complex **3a** did not show significant changes in uptake efficiency upon addition of cobalt(II) chloride, fasentin, or cytochalasin B. These results support a GLUT-mediated transport pathway for the glucose complex.

Incubation of HeLa cells with complexes **3** and **3a** in a glucose-free medium at 4 °C resulted in reduction of the intracellular rhenium by ≈50 %. Thus, the complexes apparently entered the cells by an energy-dependent endocytosis-like pathway.[16] Additionally, treatment of the cells with sodium azide (3 mM) for 30 min at 37 °C before addition of

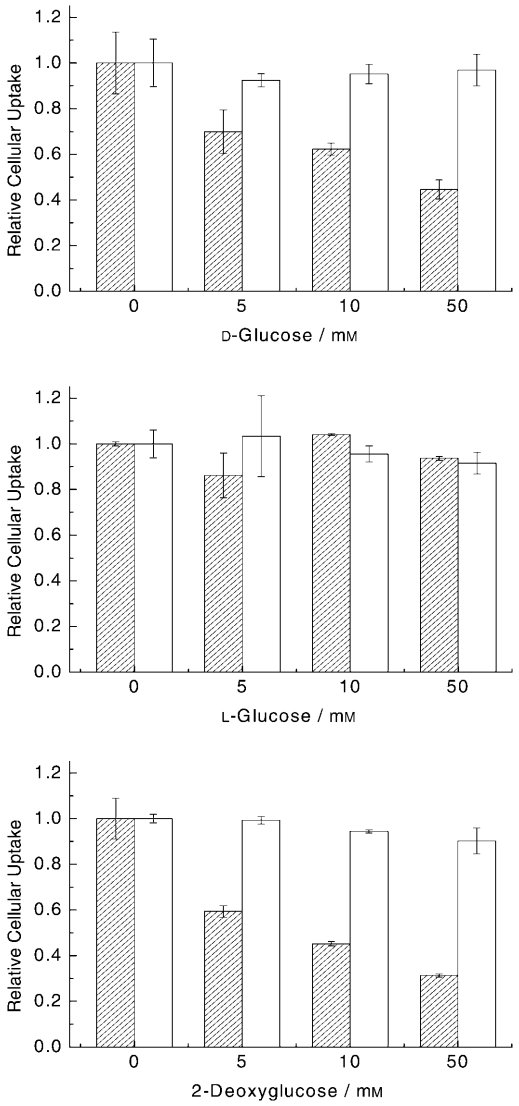

Figure 1. Relative cellular uptake of rhenium associated with an average HeLa cell upon incubation with complexes **3** (shaded) and **3a** (empty) (100 μM) at 37 °C for 5 min in a glucose-free medium containing various concentrations of D-glucose (top), L-glucose (middle), and 2-deoxyglucose (bottom) ($n=3$).

the glucose-free complex **3a** revealed a decrease of intracellular rhenium by ≈1.6 fold, most likely due to the inhibition of endocytic pathways by sodium azide through cellular energy depletion.[16] In sharp contrast, HeLa cells treated with sodium azide before incubation with the glucose complex **3** led to an *increase* of the intracellular rhenium by ≈2.5 fold. Since exposure of cells to azide is well known to cause an immediate inhibition of oxidative phosphorylation and a decline in cell ATP content resulting in a rapid stimulation of glucose transport,[14] the increased uptake strongly suggests that the internalization of complex **3** occurred through a glucose-specific transport system.

Taxol is a genotoxin and a mitotic inhibitor that is used to treat several types of cancers including ovarian, breast, and

non-small cell lung cancers.[17] Since genotoxic reagents are known to lower the cellular metabolic rate and reduce the glucose uptake in cancer cells,[18] the effects of taxol on the cellular uptake of complexes **3** and **3a** have been investigated. HeLa cells were incubated with taxol at various concentrations in the growth medium for 5 h, followed by treatment with the glucose complex **3** or the glucose-free complex **3a** (100 μM) for 5 min before the determination of cellular uptake efficiency (Figure 2). By using $\geq$125 nM of taxol, the intracellular amount of complex **3** in an average HeLa cell was reduced to ≈50% of that of the control (in

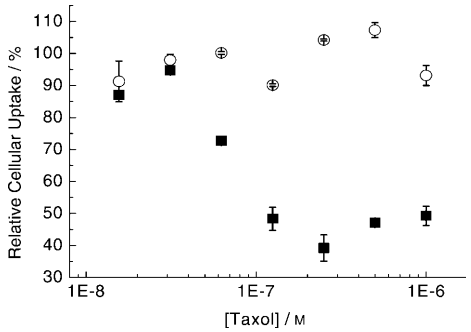

Figure 2. Relative cellular uptake of rhenium associated with an average HeLa cell upon incubation with 100 μM of complexes **3** (solid squares) and **3a** (empty circles) at 37 °C for 5 min after exposure to taxol at various concentrations for 5 h ($n=3$).

which taxol was absent). However, the presence of taxol did not cause a similar effect to the glucose-free complex **3a** (Figure 2). Thus, the incorporation of a glucose unit into the complex renders its uptake to be regulated by an anticancer reagent. Since the cellular uptake of the luminescent complex can be readily assessed by fluorescence spectroscopy and microscopy (see below), these findings could form the basis of new cell-viability assays.

The intracellular localization of complex **3** upon internalization by HeLa cells has been investigated by laser-scanning confocal microscopy. The complex was diffusely distributed in the cytoplasm with punctate staining (see the Supporting Information, Figure S3). The nucleus gave much weaker or no emission, indicative of negligible nuclear uptake. Similar intracellular distribution has been observed for other luminescent rhenium(I) polypyridine complexes.[5c,d] In addition to the perinuclear region, the complex was concentrated in specific compartments of the cells, which appeared to be the mitochondria. Thus, HeLa cells pretreated with MitoTracker Deep Red FM (100 nM, 20 min, $\lambda_{ex}=633$ nm), whose spectral properties do not interfere, were incubated with complex **3** (100 μM, 5 min, $\lambda_{ex}=405$ nm). The fluorescence staining pattern showed that the mitochondria of a typical HeLa cell have been co-stained by the fluorescent dye and the rhenium(I) complex (Figure 3). The intracellular localization of the complex **3** is different to that of its glucose-free complex **3a**, which did not show granular appearance, but a diffused staining of the whole cytoplasm.[5c] Thus, the glucose

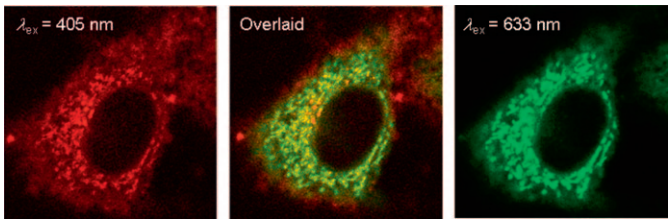

Figure 3. Laser-scanning confocal microscopy images of a HeLa cell upon incubation successively with MitoTracker Deep Red FM (100 nM, 20 min, $\lambda_{ex}=633$ nm) and complex **3** (100 μM, 5 min, $\lambda_{ex}=405$ nm) in a glucose-free medium at 37 °C.

pendant of complex **3** plays a role in the accumulation of the complex in mitochondria. Although a possible explanation is the binding of the glucose complex to hexokinases (the major proteins that phosphorylate glucose),[19] which strongly associate with mammalian mitochondria,[20] we did not observe phosphorylation of complex **3** in both in vitro and in vivo experiments involving the isolated enzyme and *E. coli*.[21] This is reasonable given the extremely rigid structure requirement of the enzyme for its substrates.[22] Nevertheless, other rhenium(I) polypyridine complexes have been reported to show similar mitochondria-targeting properties.[23]

Finally, we have examined the photostability of complex **3**. The 2-deoxyglucose analogue 2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-amino)-2-deoxy-D-glucose (2-NBDG) is a fluorescent indicator for direct glucose-uptake measurements and has been applied in tumor imaging and the examination of GLUT-related cell metabolism.[3c] However, the fast photobleaching rate of this organic compound limits its applications in prolonged exposure to the light source or time-lapse imaging experiments. We have compared the photostability of the glucose complex **3** and 2-NBDG. Confocal microscopy images and irradiation time-dependence emission intensity of HeLa cells treated with complex **3** (100 μM, 5 min, $\lambda_{ex}=405$ nm, 25 mW) or 2-NBDG (100 μM, 5 min, $\lambda_{ex}=488$ nm, 15 mW) are illustrated in Figure S4 (see the Supporting Information) and Figure 4, respectively. The emission intensity of 2-NBDG decreased much more rapidly compared to that of the glucose complex upon laser irradia-

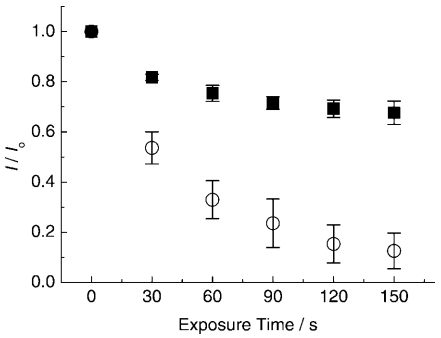

Figure 4. Irradiation time-dependence of the emission intensity of HeLa cells upon incubation with complex **3** (solid squares) and 2-NBDG (empty circles) under exposure to 405 nm (25 mW) and 488 nm (15 mW) laser excitation, respectively.

tion (Figure 4). After continuous exposure to 488 nm irradiation for 150 s, the emission intensity of 2-NBDG decreased to only ≈10% of its initial value, whereas the emission intensity of the glucose complex was maintained at ≈70% of its initial value after irradiation at a shorter wavelength (405 nm) for the same period (Figure 4). This much higher photostability renders this class of luminescent rhenium(I) glucose complexes excellent candidates for time-lapse cellular imaging applications.

In summary, we have designed three luminescent rhenium(I) polypyridine glucose complexes and investigated their photophysics, biomolecular binding, bacterial staining, and cellular-uptake properties. Various experimental results have indicated that GLUTs play a very important role in the cellular uptake of the complexes. We anticipate that these complexes can serve as new imaging reagents and glucose-uptake indicators for mammalian cells.

Acknowledgements

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