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The First Water-Soluble Hexarhenium Cluster Complexes with a Heterocyclic Ligand Environment: Synthesis, Luminescence, and Biological Properties

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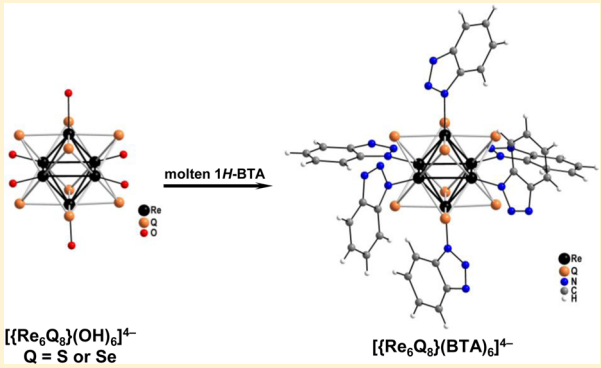
Supporting Information

ABSTRACT: The hexarhenium cluster complexes with benzotriazolate apical ligands $[\{Re_6(\mu_3\text{-}Q)_8\}(BTA)_6]^{4-}$ (Q = S, Se; BTA = benzotriazolate ion) were obtained by the reaction of $[\{Re_6(\mu_3\text{-}Q)_8\}(OH)_6]^{4-}$ with molten 1*H*-BTA (1*H*-benzotriazole). The clusters were crystallized as potassium salts and characterized by X-ray single-crystal diffraction, elemental analyses, and UV−vis and luminescence spectroscopy. In addition, their cellular uptake and toxicity were evaluated. It was found that both clusters exhibited luminescence with high lifetimes and quantum yield values; they were taken up by the cells illuminating them under UV irradiation and, at the same time, did not exhibit acute cytotoxic effects.



INTRODUCTION

Complexes with the general formula $[\{Re_6(\mu_3\text{-}Q)_8\}L_6]^n$ contain the robust cluster core $\{Re_6(\mu_3\text{-}Q)_8\}^{2+}$ with a nearly regular Re_6 octahedron residing inside a Q_8 cube (Q = S^{2-}, Se^{2-}) (Figure 1a,b). In addition, each rhenium atom is coordinated by an outer L ligand (Figure 1c). The cluster core is responsible for phosphorescence in the red and near-infrared regions with a microsecond excited-state lifetime upon UV−visible excitation in both the solid state and solution.[1] The photoluminescence in the ∼550−1000 nm emission window is of special interest for bioimaging and biolabeling, since it corresponds to minimum absorption and low autofluorescence intensity from a biological tissue.[2] At the same time, it has been shown that such clusters upon photoexcitation efficiently generate singlet oxygen.[3] Thus, complexes based on a $\{Re_6(\mu_3\text{-}Q)_8\}^{2+}$ core (Q = S, Se) have been considered as promising phosphorescent dyes for live cell imaging and photosensitizers for photodynamic therapy (PDT).[3,4]

However, for biomedical applications the hexarhenium complexes should pass through the cell membrane and, if necessary, penetrate into the nucleus. The desired effects can be achieved by tuning outer ligands of the cluster complexes to create a water-soluble unit also possessing the property of lipophilicity: i.e., an amphiphilic unit.

Earlier, we have demonstrated the influence of conjugation of the amphiphilic diblock copolymer MPEG550-CH_2CONH-GlyPheLeuGlyPheLeu-COOH to a rhenium cluster on its permeation through the cell membrane.[4b] It was shown that, in contrast to the hexahydroxo anionic cluster complex $[\{Re_6(\mu_3\text{-}S)_8\}(OH)_6]^{4-}$ which did not permeate through the cell membrane due to a high repulsive force, the cluster−copolymer conjugate $[\{Re_6(\mu_3\text{-}S)_8\}(OH)_5$(OOC-LeuPheGlyLeuPheGly-NHOCCH$_2$-MPEG550)$]^{4-}$ passed through the cell membrane and was found to be distributed throughout the cytoplasm and nucleus. However, with the cluster complexes $[\{Re_6(\mu_3\text{-}S)_8\}(RCOO)_6]^{4-}$ (R = H, CH_3) it was shown that the carboxylate ligands are labile in aqueous solutions, being gradually substituted by water molecules.[1j,5] The lability limits the use of aqueous solutions of anionic carboxylate clusters such as $[\{Re_6(\mu_3\text{-}Q)_8\}(RCOO)_6]^{4-}$ due to the formation of poorly soluble neutral aqua hydroxo complexes $[\{Re_6(\mu_3\text{-}Q)_8\}(H_2O)_4(OH)_2]$.

In this paper, octahedral rhenium cluster complexes with a pseudoamphiphilic heterocyclic organic ligand, the benzotriazolate ion, are described for the first time. The synthesis and

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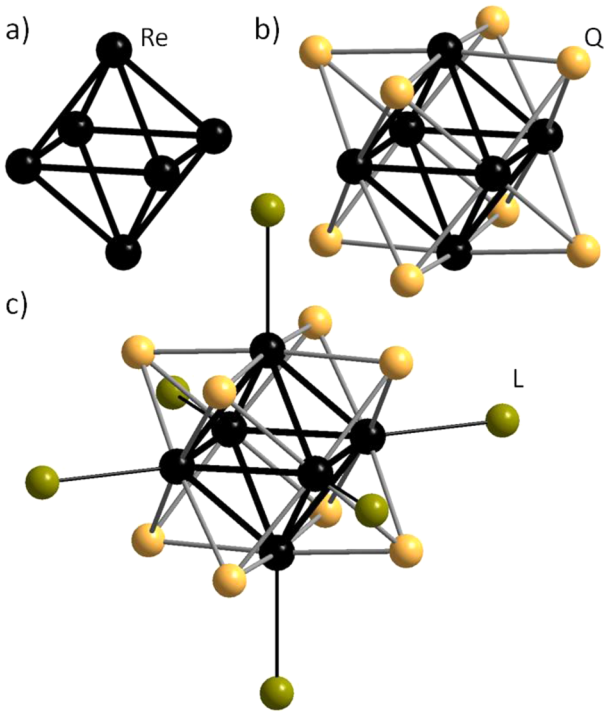

Figure 1. Detailed representation of a hexarhenium chalcogenide cluster complex: (a) Re_6 metallocluster; (b) $\{Re_6(\mu_3\text{-}Q)_8\}^{2+}$ cluster core; (c) $[\{Re_6(\mu_3\text{-}Q)_8\}L_6]^n$ cluster complex.

structural and analytical characterization of two new complex compounds, namely $K_4[\{Re_6(\mu_3\text{-}S)_8\}(BTA)_6]\cdot 3.5EtOH\cdot 4H_2O$ (K_4-**1**-3.5EtOH·$4H_2O$) and $K_{2.75}H_{1.25}[\{Re_6(\mu_3\text{-}Se)_8\}(BTA)_6]\cdot 3EtOH\cdot 7H_2O$ ($K_{2.75}H_{1.25}$-**2**-3EtOH·$7H_2O$), are considered. The luminescence properties, cytotoxicity, and cellular uptake behavior of the complexes are also studied.

EXPERIMENTAL SECTION

Materials and Syntheses. All reagents were used as purchased. $K_4[\{Re_6(\mu_3\text{-}Q)_8\}(OH)_6]\cdot 8H_2O$ (Q = S, Se) were prepared according to the described procedure.[6] The C, H, N, S elemental analyses were performed with a EuroEA3000 (Eurovector) analyzer. Energy dispersive spectroscopy (EDS) was performed on an EDAX equipped (JEOL EX-23000BU) JEOL JSM-6700F field emission scanning electron microscope. Infrared spectra were measured on KBr pellets with a Scimitar FTS 2000 spectrometer. Absorption spectroscopy was conducted using a U-3300 spectrophotometer (Hitachi).

For emission measurements, the powdered samples K_4-**1**-3.5EtOH·$4H_2O$ and $K_{2.75}H_{1.25}$-**2**-3EtOH·$7H_2O$ were placed between two nonfluorescent glass plates. The absorbance of aqueous and acetonitrile solutions was set to <0.1 at 355 nm. The solutions were poured into quartz cuvettes. For deaeration, an Ar-gas stream was purged through the solutions for 30 min, and then the cuvettes were sealed. The measurements were carried out at 298 K. The samples were excited by 355 nm laser pulses (6 ns duration, LOTIS TII, LS-2137/3). The corrected emission spectra were recorded on a red-light-sensitive multichannel photodetector (Hamamatsu Photonics, PMA-12). For emission decay measurements, the emission was analyzed by a streakscope system (Hamamatsu Photonics, C4334 and C5094). The emission quantum yields (Φ_{em}) for the aerated and deaerated solutions were estimated by using $(Bu_4N)_4[Re_6S_8Cl_6]$ as a standard: Φ_{em} = 0.039 in deaerated acetonitrile.[1b] The emission quantum yields of the powdered samples were determined by an Absolute Photo-Luminescence Quantum Yield Measurement System (Hamamatsu Photonics, C9920-03), which comprised an excitation xenon light

Table 1. Crystallographic Data for K_4-1-3.5EtOH·$4H_2O$ and $K_{2.75}H_{1.25}$-2-3EtOH·$7H_2O$

	K_4-**1**-3.5EtOH·$4H_2O$	$K_{2.75}H_{1.25}$-**2**-3EtOH·$7H_2O$
empirical formula	$C_{43}H_{53}K_4N_{18}O_{7.5}Re_6S_8$	$C_{42}H_{57.25}K_{2.75}N_{18}O_{10}Re_6Se_8$
formula wt	2472.11	2830.71
cryst syst	triclinic	monoclinic
space group	$P\bar{1}$	$P2_1/c$
a (Å)	11.8923(4)	15.3922(4)
b (Å)	13.1430(4)	21.7391(5)
c (Å)	13.7070(4)	23.2431(6)
α (deg)	64.751(1)	92.9350(10)
β (deg)	66.522(1)	
γ (deg)	75.384(1)	
V (Å^3)	1768.52(10)	7767.2(3)
Z	1	4
ρ_{calcd} (g/cm^3)	2.321	2.421
μ (mm^{-1})	10.754	13.264
F(000)	1151	5158
cryst size (mm^3)	0.09 × 0.07 × 0.05	0.20 × 0.14 × 0.10
θ range (deg)	1.72−27.57	1.75−27.14
index ranges	$-15 \le h \le 11$	$-19 \le h \le 18$
	$-16 \le k \le 17$	$-27 \le k \le 20$
	$-17 \le l \le 17$	$-28 \le l \le 29$
no. of rflns collected	15636	58707
no. of indep rflns	8133 (R(int) = 0.0213)	17134 (R(int) = 0.0415)
T_{max}; T_{min}	0.6154; 0.4445	0.3505; 0.1767
no. of data/restraints/params	8133/2/406	17134/17/821
goodness of fit on F^2	1.050	1.140
R1; wR2 ($I > 2\sigma(I)$)	0.0307; 0.0753	0.0387; 0.1186
R1; wR2 (all data)	0.0443; 0.0792	0.0743; 0.1318
largest diff peak and hole (e/Å^3)	1.524 and −2.110	2.651 and −1.609

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source (the excitation wavelength was 380 nm), an integrating sphere, and a red-sensitive multichannel photodetector (Hamamatsu Photonics, PMA-12).

Preparation of $K_4[\{Re_6(\mu_3\text{-}S)_8\}(BTA)_6]\cdot 3.5EtOH\cdot 4H_2O$ (K_4-1-3.5EtOH·4H_2O) and $K_{2.75}H_{1.25}[\{Re_6(\mu_3\text{-}Se)_8\}(BTA)_6]\cdot 3EtOH\cdot 7H_2O$ ($K_{2.75}H_{1.25}$-2-3EtOH·7H_2O). Synthetic procedures for both compounds were the same. For the preparation of K_4-**1**-3.5EtOH·4H_2O, $K_4[\{Re_6(\mu_3\text{-}S)_8\}(OH)_6]\cdot 8H_2O$ (200 mg, 0.113 mmol) and 1*H*-benzotriazole (200 mg, 1.679 mmol) were heated in a sealed glass tube at 150 °C for 48 h. After the reaction mixture was cooled, it was dissolved in 100 mL of hot ethanol and filtered. The solution was evaporated at 70 °C until the formation of an orange crystalline precipitate. The precipitate was filtered and dried in air. Yield: 253 mg (91%). The compound $K_{2.75}H_{1.25}$-**2**-3EtOH·7H_2O was prepared in the same way: $K_4[\{Re_6(\mu_3\text{-}Se)_8\}(OH)_6]\cdot 8H_2O$ (200 mg, 0.093 mmol) was heated with 1*H*-benzotriazole (200 mg, 1.679 mmol). Yield: 230 mg (88%). Anal. Calcd for $C_{43}H_{53}K_4N_{18}O_{7.5}Re_6S_8$ (K_4-**1**-3.5EtOH·4H_2O): C, 20.9; H, 2.2; N, 10.2; S, 10.4. Found: C, 20.2; H, 1.8; N, 10.5; S, 10.6. Anal. Calcd for $C_{42}H_{57.25}K_{2.75}N_{18}O_{10}Re_6Se_8$ ($K_{2.75}H_{1.25}$-**2**-3EtOH·7H_2O): C, 17.8; H, 2.0; N, 8.9. Found: C, 17.9; H, 1.9; N, 8.8. EDS shows the following: K:Re:S ratio of 4.2:6:8.1 for $C_{43}H_{53}K_4N_{18}O_{7.5}Re_6S_8$ (**1**); K:Re:Se ratio of 2.6:6:7.9 for $C_{42}H_{57.25}K_{2.75}N_{18}O_{10}Re_6Se_8$ (**2**). UV−vis in MeCN, λ_{max}, nm (ε, mol^{-1} dm^3 cm^{-1}): 330 (sh, 27850), 297 (sh, 44800), 279 (sh, 59150), 273 (sh, 62700), 250 (sh, 66600), 211 (205000) for **1**; 340 (sh, 15750), 310 (sh, 28150), 282 (sh, 63250), 276 (64500), 258 (sh, 72700), 251 (74950), 211 (sh, 200550) for **2**. UV−vis in 0.1 M aqueous solution of KOH, λ_{max}, nm (ε, mol^{-1} dm^3 cm^{-1}): 330 (sh, 25450), 297 (sh, 42500), 279 (sh, 60100), 274 (61950) for **1**; 340 (sh, 13050), 310 (sh, 24950), 282 (sh, 66050), 274 (70950) for **2**. The IR spectra (400−4000 cm^{-1}) of compounds **1** and **2** in KBr pellets show all of the peaks expected for benzotriazole ligands. Crystals suitable for X-ray structure determination were separated from the precipitate formed in an ethanol solution during evaporation.

Preparation of $[\{Re_6(\mu_3\text{-}S)_8\}(1H\text{-}BTA)_4(BTA)_2]$ (H_4-1) and $[\{Re_6(\mu_3\text{-}Se)_8\}(1H\text{-}BTA)_4(BTA)_2]$ (H_4-2). The compounds were quantitatively precipitated as orange powders by acidifying the aqueous solution of K_4-**1**-3.5EtOH·4H_2O or $K_{2.75}H_{1.25}$-**2**-3EtOH·7H_2O, respectively, with 0.1 M HCl until the pH was equal to ∼2. The powders were separated by centrifugation and then dried in an oven at 110 °C for 1 h. Anal. Calcd for $C_{36}H_{28}N_{18}Re_6S_8$ (H_4-**1**): C, 20.7; H, 1.4; N, 12.1; S, 12.3. Found: C, 21.1; H, 1.6; N, 12.5; S, 11.8. Anal. Calcd for $C_{36}H_{28}N_{18}Re_6Se_8$ (H_4-**2**): C, 17.6; H, 1.1; N, 10.2. Found: C, 18.0; H, 1.4; N, 10.5. EDS shows the absence of potassium and chlorine in samples of H_4-**1** and H_4-**2**.

Titration. H_4-**1** (130 mg, 0.0625 mmol) or H_4-**2** (154 mg, 0.0625 mmol) was added to 30.0 mL of degassed water. The titration measurements were performed with an 0.084 M aqueous solution of KOH using a Hanna HI 9024 pH meter. The pH meter was calibrated at pH 9.18 and 6.86 by using standard titrants: aqueous solutions of $Na_2B_4O_7\cdot 10H_2O$ and KH_2PO_4, respectively.

Single-Crystal Diffraction. The single-crystal X-ray diffraction data were collected using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) at 150(2) K on a Bruker APEX DUO diffractometer (K_4-**1**-3.5EtOH·4H_2O) and a Bruker Nonius X8 APEX diffractometer ($K_{2.75}H_{1.25}$-**2**-3EtOH·7H_2O) equipped with a 4K CCD area detector. The φ-scan technique was employed to measure the intensities. Absorption corrections were applied empirically using the SADABS program.[7] The structures were solved by the direct methods of difference Fourier synthesis and then refined by the full-matrix least-squares method using the SHELXTL package.[8] The atomic thermal parameters for non-hydrogen atoms were refined anisotropically. The positions of hydrogen atoms of the benzotriazole ligands and ethanol molecules of crystallization (except for the hydroxyl groups) were calculated in accordance with their geometric conditions and refined using the rigid-body approximation (riding model). The hydrogen atoms for crystallization water molecules and the hydroxyl groups of ethanol were not localized. More experimental details are given in Table 1. Table S1 in Supporting Information gives selected interatomic distances and angles for **1** and **2**. CCDC-987781 (K_4-**1**-3.5EtOH·4H_2O) and CCDC-987780 ($K_{2.75}H_{1.25}$-**2**-3EtOH·7H_2O) contain supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Cell Culture. Human larynx carcinoma cell line (Hep2) was purchased from the State Research Center of Virology and Biotechnology VECTOR and cultured in Eagle's Minimum Essential Medium (EMEM, pH ∼7.4) supplemented with a 10% fetal bovine serum under a humidified atmosphere (5% CO_2 plus 95% air) at 37 °C.

Confocal Microscopy. Hep2 cells were seeded on slides (1.5×10^5 cells/slide) and incubated overnight at 37 °C under a 5% CO_2 atmosphere. The medium was then replaced with a fresh medium containing rhenium cluster complexes (12.5, 25, or 50 μM) and incubated for 24 h. Finally, the cells were washed twice with phosphate buffered saline (PBS), fixed in 4% paraformaldehyde, and visualized by using a Zeiss LSM 510 confocal microscope (Carl Zeiss Inc., Jena, Germany) equipped with a laser diode (405 nm) for fluorescence and with a 100× λ-blu corrected oil immersion objective. The images were obtained and analyzed with ZEN 2009 software. Each experiment was repeated three times on separate days.

Flow Cytometry. The cells (10^6 cells/mL) were incubated overnight at 37 °C under a 5% CO_2 atmosphere, and the cellular uptake of rhenium clusters was measured by incubating the cells with a given concentration of rhenium cluster complexes (12.5, 25, or 50 μM) for 24 h. The cells were washed twice with PBS, harvested by trypsinization, and then suspended in PBS containing a 0.1% bovine serum albumin and NaN_3. The accumulation of rhenium clusters in the cells was analyzed by using a FACSCanto II (Becton Dickinson). The cells incubated in the absence of rhenium clusters were also used as a control. All of the data were the mean fluorescence obtained from a population of 10000 cells. The experiments were carried out in triplicate and expressed as the percentage of viable cells compared to the control group.

Cell Proliferation/Viability. The percentage of cell proliferation/viability was measured using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. The Hep2 cells were seeded into 96-well plates at 5×10^4 cells/well in a medium containing K_4-**1**-3.5EtOH·4H_2O or $K_{2.75}H_{1.25}$-**2**-3EtOH·7H_2O with concentrations from 3.125 to 400 μM and then incubated for 72 h under a 5% CO_2 atmosphere. The effect of the cluster complexes on the cell proliferation/viability was also determined using the MTT assay. Ten microliters of the MTT solution with a concentration of 5 mg/mL was added to each well, and the plates were incubated for 4 h and then solubilized with a dimethyl sulfoxide solution. The optical density was measured with a plate reader (Sunrise, TECAN) at 620 nm. The experiment was repeated three times on separate days.

Singlet Oxygen Generation. The singlet oxygen generation was investigated as follows: a solution of K_4-**1**-3.5EtOH·4H_2O (5 mg, 0.002 mmol) or $K_{2.75}H_{1.25}$-**2**-3EtOH·7H_2O (6 mg, 0.002 mmol) and 2,3-diphenyl-*p*-dioxene[9] (48 mg, 0.2 mmol) in 1 mL of acetone-d_6 in a conventional NMR tube was saturated with oxygen for 10 min and then irradiated with a DRSh-500 mercury lamp with a filtered light wavelength longer than 400 nm for 2 h. ^{1}H NMR (200 MHz) spectra were collected on a Bruker Avance 200 NMR spectrometer before and after irradiation of the samples.

RESULTS AND DISCUSSION

Synthesis and Structure. The hexahydroxo cluster complexes $K_4[\{Re_6(\mu_3\text{-}Q)_8\}(OH)_6]\cdot 8H_2O$ (Q = S, Se) were found to be convenient parent compounds for ligand substitution reactions. It was demonstrated that the OH^- groups could be substituted under various conditions by different inorganic and organic ligands, such as halides,[6,10] cyanide,[1n] azide,[1p] carboxylates,[1j−l,5] and pyridine derivatives.[1k,11] In fact, the possibility to controllably modify the ligand environment of the cluster complexes is a handy tool for

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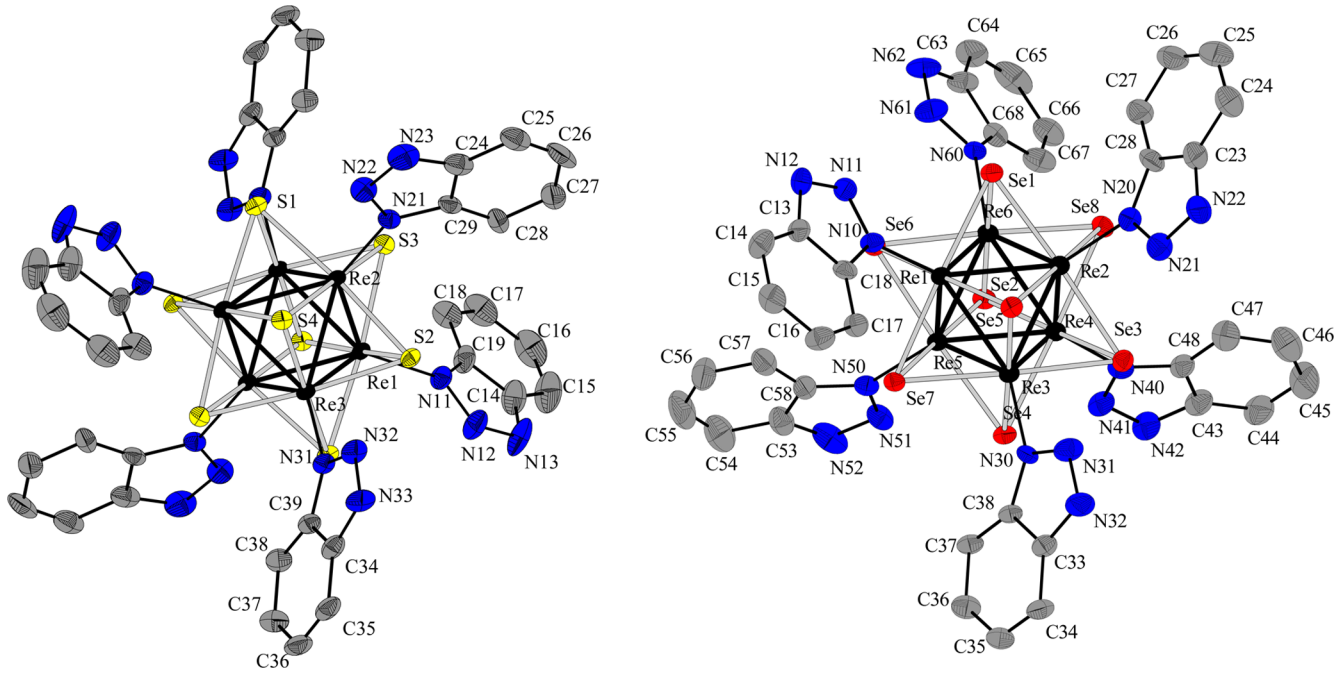

Figure 2. Structures of **1** (left) and **2** (right). Displacement ellipsoids of non-hydrogen atoms are drawn at the 50% probability level. H atoms of the BTA ligands are omitted for clarity.

Table 2. Interatomic Distances (Å) in K_4-1**-3.5EtOH·4H_2O, $K_{2.75}H_{1.25}$-**2**-3EtOH·7H_2O and Some Related $\{Re_6(\mu_3\text{-}Q)_8\}^{2+}$-Based Complexes with Five-Membered N-Heterocycles as Apical Ligands**

	d, Å		
compd	Re−Re	Re−(μ_3-Q)	Re−N
K_4-**1**-3.5EtOH·4H_2O	2.5870(3)−2.5945(3)	2.397(1)−2.416(1)	2.132(5)−2.142(5)
$K_{2.75}H_{1.25}$-**2**-3EtOH·7H_2O	2.6142(5)−2.6330(4)	2.5160(9)−2.5346(8)	2.141(7)−2.171(6)
$[\{Re_6S_8\}(3,5\text{-}Me_2PzH)_6]Br_2\cdot 2(3,5\text{-}Me_2PzH)$[4a]	2.5921(7)−2.6036(7)	2.396(2)−2.417(2)	2.164(7)−2.195(7)
$[\{Re_6Se_8\}(3,5\text{-}Me_2PzH)_6]Br_2\cdot 2(3,5\text{-}Me_2PzH)$[4a]	2.505(2)−2.532(2)	2.620(1)−2.637(1)	2.175(9)−2.198(9)
$[\{Re_6Se_8\}(PEt_3)_5(N_3C_2(COOMe_2)]BF_4$[15]	2.6205(4)−2.6564(4)	2.5036(7)−2.5255(7)	2.13(1)−2.18(1)
$[\{Re_6Se_8\}(PEt_3)_5(1,5\text{-}CH_3CN_4)]Cl\cdot 2CH_2Cl_2$[16]	2.6366(5)−2.6549(5)	2.511(1)−2.527(1)	2.143(8)

manipulation by their solubility, hydro- or lipophilicity, luminescence, etc.

For better cell membrane penetration, the complexes should possess the properties of both lipophilicity and water solubility. Thus, cluster complexes with amphiphilic outer ligands can enhance the cellular permeation capacity. In this paper, we synthesized two new cluster complexes with 1*H*-BTA molecules as outer ligands. 1*H*-Benzotriazole is a biopersistent substance soluble in water and, at the same time, in nonpolar solvents such as benzene and toluene, demonstrating, as mentioned above, properties that are important for potential medical applications: simultaneous hydrophilicity and lipophilicity. The reactions of hexahydroxo clusters $[\{Re_6(\mu_3\text{-}Q)_8\}(OH)_6]^{4-}$ with molten 1*H*-BTA lead to the substitution of all OH ligands by benzotriazole moieties. The resulting complexes $[\{Re_6(\mu_3\text{-}Q)_8\}(BTA)_6]^{4-}$ (Figure 2) were crystallized as salts of the compositions $K_4[\{Re_6(\mu_3\text{-}S)_8\}(BTA)_6]\cdot 3.5EtOH\cdot 4H_2O$ and $K_{2.75}H_{1.25}[\{Re_6(\mu_3\text{-}Se)_8\}(BTA)_6]\cdot 3EtOH\cdot 7H_2O$, whose structures were determined by an X-ray single-crystal analysis (Table 1). The crystal packings of K_4-**1**-3.5EtOH·4H_2O and $K_{2.75}H_{1.25}$-**2**-3EtOH·7H_2O are presented in the Supporting Information (Figures S1 and S2). The average distances within cluster units **1** and **2** are in good agreement with the literature data on $\{Re_6(\mu_3\text{-}Q)_8\}^{2+}$-based complexes with five-membered N-heterocycles as apical ligands (Table 2).

Since 1*H*-benzotriazole is a weak acid (pK_a = 8.2),[12] the compound $K_{2.75}H_{1.25}$-**2**-3EtOH·7H_2O can be considered as a cocrystallization of the two cluster anions $[\{Re_6(\mu_3\text{-}Se)_8\}(1H\text{-}BTA)(BTA)_5]^{3-}$ and $[\{Re_6(\mu_3\text{-}Se)_8\}(1H\text{-}BTA)_2(BTA)_4]^{2-}$ in the ratio 3:1 along with potassium cations and solvate molecules. The difference in the formulas K_4-**1**-3.5EtOH·4H_2O and $K_{2.75}H_{1.25}$-**2**-3EtOH·7H_2O can be explained by an assumption that the acidity of coordinated 1*H*-BTA increases due to the electron-accepting properties of the cluster core $\{Re_6(\mu_3\text{-}Q)_8\}^{2+}$ (Q = S, Se), where the sulfur-containing cluster core is a stronger acceptor than the core containing selenium. Therefore, the sulfide cluster complex is thought to be a stronger acid than the selenide complex, which explains the full dissociation of 1*H*-BTA ligands in **1** and their partial dissociation in **2**. To prove the assumption, aqueous solutions of H_4-**1** and H_4-**2** were titrated. In spite of the polybasicity of the studied cluster units, the titration curves show only one pronounced step (Figure S3, Supporting Information). However, the titration curve of H_4-**1** lies below that of H_4-**2**, which clearly confirms that the sulfide cluster complex is a stronger acid than the selenide complex.

The presence of the pseudoamphiphilic outer ligands in **1** and **2** makes the complexes soluble in both water and polar organic solvents, such as ethanol, acetone, acetonitrile, etc. The complexes are well soluble in basic aqueous solutions, but the

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solubility is limited by pH: a decrease in pH below ~8 causes the formation of sparingly soluble orange amorphous precipitates that, according to elemental analyses, correspond to the formula $[\{Re_6(\mu_3\text{-}Q)_8\}(1H\text{-}BTA)_4(BTA)_2]$ (H_4-**1** or H_4-**2**).

Luminescence Properties. The luminescence properties of K_4-**1**-3.5EtOH·$4H_2O$ and $K_{2.75}H_{1.25}$-**2**-3EtOH·$7H_2O$ were studied in aqueous and acetonitrile solutions, as well as in the solid state. To investigate the luminescence in an aqueous medium, the samples were dissolved in a 0.1 M KOH aqueous solution (pH 13) to prevent protonation of the BTA ligands. The normalized emission spectra are shown in Figure 3 and

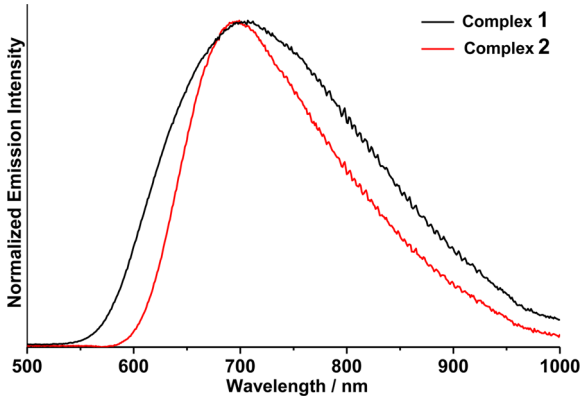


Figure 3. Emission spectra of **1** and **2** in basic aqueous solutions.

Figures S4 and S5 (Supporting Information), and the spectroscopic/photophysical parameters (emission maximum wavelengths λ_{em}, quantum yields Φ_{em}, and lifetimes τ_{em} with the corresponding amplitude A) are summarized in Table 3.

In the solid state, the emission decay profiles of K_4-**1**-3.5EtOH·$4H_2O$ and $K_{2.75}H_{1.25}$-**2**-3EtOH·$7H_2O$ were fitted by double-exponential functions with decay constants (τ_{em}) of 7.6 and 2.9 μs for the sulfide cluster and 7.7 and 1.8 μs for the selenide cluster, with emission quantum yields of 0.075 and 0.083, respectively. In deaerated acetonitrile solutions, complexes **1** and **2** showed single-exponential emission decays with higher emission lifetimes (18.1 and 19.6 μs, respectively), whereas the quantum yields were comparable to those of the corresponding solid samples (Table 3). It is worth noting that the lifetime and quantum yield values found for the solid samples and oxygen-free acetonitrile solutions of K_4-**1**-3.5EtOH·$4H_2O$ and $K_{2.75}H_{1.25}$-**2**-3EtOH·$7H_2O$ are among the highest values reported so far for hexarhenium chalcogenide cluster complexes (Table 3).

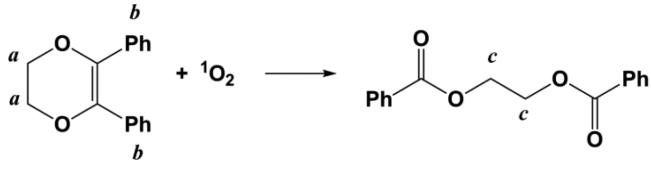
Complexes **1** and **2** in the aerated CH_3CN solutions showed significantly shorter lifetimes and smaller quantum yields in comparison with the deaerated solutions (Table 3), which explicitly indicates that the excited triplet states of **1** and **2** are efficiently quenched by dioxygen. The emission quenching is due to energy transfer from the excited cluster to dioxygen with the generation of singlet oxygen,[3] which is a requirement for photodynamic therapy agents (photosensitizers).[13] As was described before, the organic compound 2,3-diphenyl-*p*-dioxene is well-characterized as a singlet oxygen trap (Scheme 1), since the formation of ethylene glycol dibenzoate is easily discerned by means of ^{1}H NMR spectrometry.[3] Using it, we confirmed that the described complexes **1** and **2** also generate singlet oxygen as a result of the photoexcitation. Figure 4 demonstrates ^{1}H NMR spectra of acetone solutions of 2,3-

Table 3. Spectroscopic and Photophysical Parameters of K_4-1-3.5EtOH·$4H_2O$, $K_{2.75}H_{1.25}$-2-3EtOH·$7H_2O$, and Some Other Water-Soluble Hexarhenium Cluster Complexes

sample	λ_{em}/nm	Φ_{em}[a]	τ_{em}/μs (A)
In Aqueous Solution			
1 (pH >6)	~705	0.014	2.6
2 (pH >6)	~700	0.018	3.4
$[\{Re_6S_8\}(HCOO)_6]^{4-}$ (pH ~7)[1j]	~672	0.028	3.1 (0.90), 0.30 (0.10)
$[\{Re_6S_8\}(CH_3COO)_6]^{4-}$ (pH ~7)[s]	~690	0.019	1.8 (0.90), 0.25 (0.10)
$[\{Re_6S_8\}(CN)_6]^{4-}$ (pH ~13, 7, or 1)[1a,q]	~720	0.009	1.2
$[\{Re_6Se_8\}(CN)_6]^{4-}$ (pH ~7)[1a]	~720	0.015	1.9
trans-$[\{Re_6S_8\}(CN)_4(OH)_2]^{4-}$ (pH ~13, 7 or 1)[1q]	~730	0.005	1.1
trans-$[\{Re_6Se_8\}(CN)_4(OH)_2]^{4-}$ (pH ~13)[1n]	~710	0.014	1.6
trans-$[\{Re_6Se_8\}(CN)_4(OH)_2]^{4-}$ (pH ~7)[1n]	~710	0.006	1.3 (0.33), 0.38 (0.67)
$[\{Re_6S_8\}(OH)_6]^{4-}$ (pH ~13)[1h]	~655	0.010	2.2
$[\{Re_6S_8\}(OH)_6]^{4-}$ (pH ~7)[1h]	~665	0.003	1.1
$[\{Re_6S_8\}(OH)_6]^{4-}$ (pH ~1)[1h]	~685	0.005	2.35
$[\{Re_6Se_8\}(OH)_6]^{4-}$ (pH ~13)[1h]	~660	0.017	1.8 (0.88), 0.45 (0.12)
$[\{Re_6Se_8\}(OH)_6]^{4-}$ (pH ~ 1)[1h]	~660	0.003	0.74 (0.70), 0.16 (0.30)
In Deaerated MeCN			
1	~715	0.072	18.1
2	~700	0.081	19.6
$[\{Re_6Se_8\}(N_3)_6]^{4-}$ [1p]	~690	0.04	14.3
$[\{Re_6S_8\}(CN)_6]^{4-}$ [1a]	~720	0.056	11.2
$[\{Re_6Se_8\}(CN)_6]^{4-}$ [1a]	~720	0.140	17.1
$[\{Re_6S_8\}(NCS)_6]^{4-}$ [1g]	~745	0.091	10.4
$[\{Re_6Se_8\}(NCS)_6]^{4-}$ [1g]	~730	0.15	11.8
$[\{Re_6S_8\}Cl_6]^{4-}$ [1b]	~770	0.039	6.3
$[\{Re_6S_8\}Br_6]^{4-}$ [1b]	~780	0.018	5.4
$[\{Re_6S_8\}I_6]^{4-}$ [1b]	~800	0.015	4.4
In Aerated MeCN			
1	~715	0.019	5.4
2	~700	0.002	1.1 (0.10), 0.6 (0.90)
In the Solid State			
K_4-**1**-3.5EtOH·$4H_2O$	~700	0.075	7.6 (0.65), 2.9 (0.35)
$K_{2.75}H_{1.25}$-**2**-3EtOH·$7H_2O$	~700	0.083	7.7 (0.50), 1.8 (0.50)
$K_4[\{Re_6S_8\}(HCOO)_6]$[1j]	~655	0.091	6.5 (0.73) 1.3 (0.27)
$K_4[\{Re_6S_8\}(CH_3COO)_6]\cdot 8H_2O$[s]	~670	0.074	
$K_4[\{Re_6Se_8\}(N_3)_6]\cdot 4H_2O$[1p]	~680	0.02	1.88 (0.03), 0.79 (0.19), 0.24 (0.78)
$Cs_{2.75}K_{1.25}[\{Re_6Se_8\}(CN)_4(OH)_2]\cdot H_2O$[1n]	~710	0.056	2.3 (0.62), 0.69 (0.38)

[a]Relative quantum yields for solutions and absolute yields for solid samples.

Scheme 1. Oxidation of 2,3-Diphenyl-*p*-dioxene by $^1O_2$



2,3-diphenyl-para-dioxene **ethylene glycol dibenzoate**

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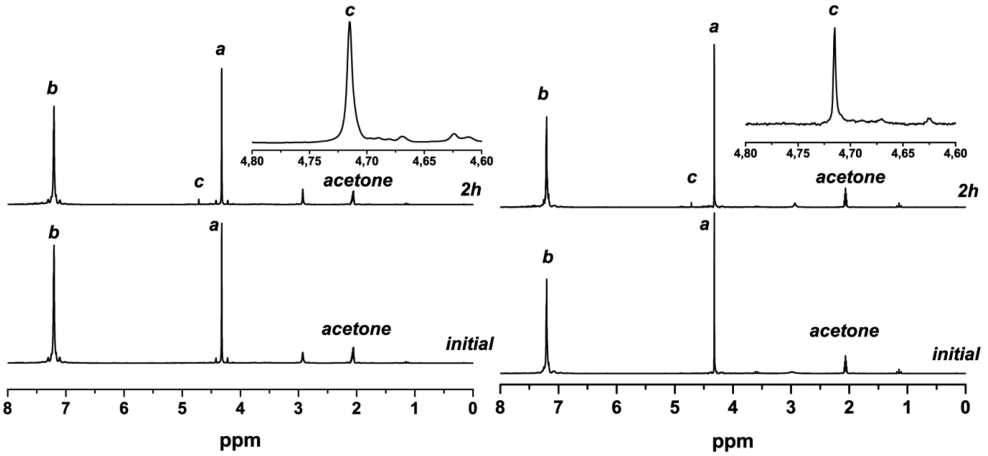

Figure 4. ^{1}H NMR spectra of 2,3-diphenyl-*p*-dioxene and cluster complexes **1** (left) and **2** (right) in oxygen-saturated acetone-d_6 before (lower spectrum) and after (upper spectrum) photoirradiation (***a***, resonance of $-CH_2CH_2-$ protons; ***b***, resonance of Ph protons). Inset: the resonance of methylene protons of ethylene glycol dibenzoate (***c***).

diphenyl-*p*-dioxene and cluster complexes **1** or **2** before and after photoirradiation. The appearance of a singlet peak at 4.72 ppm corresponding to the methylene protons of ethylene glycol dibenzoate, which is the product of oxidation of 2,3-diphenyl-*p*-dioxene by $^1O_2$, indicates the generation of singlet oxygen during photoirradiation of cluster-containing solutions.

The spectroscopic and photophysical parameters of complexes **1** and **2** in aerated and deaerated aqueous solutions were almost identical. However, the quantum yield and lifetime values in 0.1 M KOH solutions were respectively much lower and shorter than those found for deaerated acetonitrile solutions (Table 3). Similar degradation of luminescence in aqueous solution in comparison with that in organic solvents was also observed for cyanide hexarhenium cluster complexes (Table 3) and could be explained by the interactions of water molecules with the excited-state clusters through hydrogen bonding facilitating nonradiative decay[14] or, as was suggested before, by interactions of water molecules possibly with the capping chalcogenide ligands, providing a more efficient vibrational decay pathway of the excited state.[1a] Since in our recent studies it was shown that luminescence properties of aqueous solutions of the hexarhenium cluster complex can be either dependent (for example, $[\{Re_6(\mu_3\text{-}Q)_8\}(OH)_6]^{4-}$ and $[\{Re_6(\mu_3\text{-}Se)_8\}(CN)_4(OH)_2]^{4-}$) or independent on pH ($[\{Re_6(\mu_3\text{-}S)_8\}(CN)_6]^{4-}$ and *trans*-$[\{Re_6(\mu_3\text{-}S)_8\}(CN)_4(OH)_2]^{4-}$) (Table 3), we attempted to estimate a dependence of luminescence of aqueous solutions of **1** and **2** on pH. To that end, the compounds K_4-**1**-3.5EtOH·$4H_2O$ and $K_{2.75}H_{1.25}$-**2**-3EtOH·$7H_2O$ were dissolved in 0.1 M KOH to get solutions with absorbance <0.1 at 355 nm. Then the solutions were gradually acidified by 0.1 M HCl and studies of luminescence properties were iteratively carried out. It was found that luminescence spectra of the aqueous solutions of **1** and **2** as well as the corresponding Φ_{em} and τ_{em} values are almost unchanged if pH >6. At pH ∼6 precipitation was observed, and the colorless solution that separated from the precipitate was not emissive. In addition, luminescence of compounds K_4-**1**-3.5EtOH·$4H_2O$ and $K_{2.75}H_{1.25}$-**2**-3EtOH·$7H_2O$ in PBS, which is a buffer solution commonly used in biological research, was investigated and found to be identical with luminescence in aqueous solutions.

Biological Properties. It is obvious that, to be applied as photosensitizers or bioimaging agents, the compounds should have low toxicity and be taken up by cells. Therefore, we evaluated the potential toxicity of the rhenium clusters for Hep2 cells. The *in vitro* cytotoxicity assay in the cell culture was intended to be a model for cell injury that may follow drug administration *in vivo*. The effects of the clusters on the cell proliferation and viability were evaluated by the MTT assay. The MTT colorimetric assay is a generally accepted method of determining viable cell numbers in proliferation and cytotoxicity studies. This assay is based on the cleavage of the yellow tetrazolium salt (MTT) to form a soluble blue formazan product by mitochondrial enzymes, and the produced formazan amount is directly proportional to the number of living (not dead) cells present during the MTT exposure. Neither cluster had significant toxic effects on the proliferation or viability of Hep2 cells in a concentration of up to 50 μM (Figure 5). The

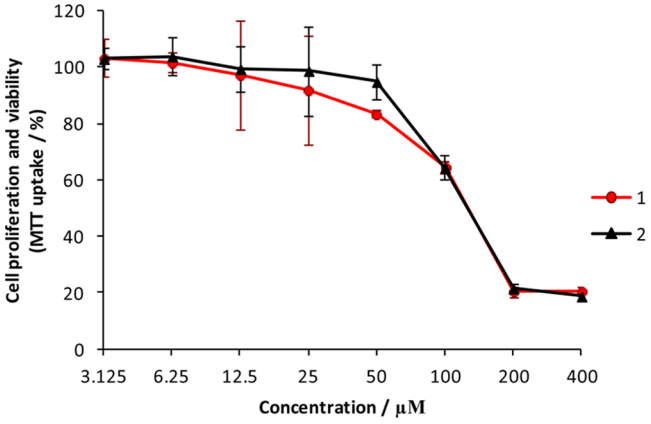

Figure 5. Effect of rhenium clusters on proliferation and viability of Hep2 cells measured by MTT.

in vitro cytotoxicity assays on model cell lines (Hep2) were used to evaluate the acceptable limit dosage compatible with *in vivo* parenteral administration. The half-maximum inhibitory concentrations (IC_{50}) of the S and Se clusters were found to be close to each other: 123.7 ± 0.8 and 122.6 ± 2.1 μM, respectively. However, it is likely that the toxic concentration of the rhenium clusters above 100 μM is too high to be used in practical biomedical applications.

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Experiments on the cellular uptake were also carried out on Hep2 cells for 12.5, 25, and 50 μM solutions of K_4-**1**-3.5EtOH·$4H_2O$ and $K_{2.75}H_{1.25}$-**2**-3EtOH·$7H_2O$. Confocal luminescence microscopy studies showed that both complexes were taken up by the cells. Irrespective of the concentration complexes **1** and **2** were localized in the cell cytoplasm in a punctate manner as spherelike agglomerates close to the nucleus. On the basis of the shape, size, and location of the agglomerates (Figure 6 and Figure S6 (Supporting Information)), it is supposed that the clusters are accumulated in the Golgi apparatus and parts of the endoplasmic reticulum.

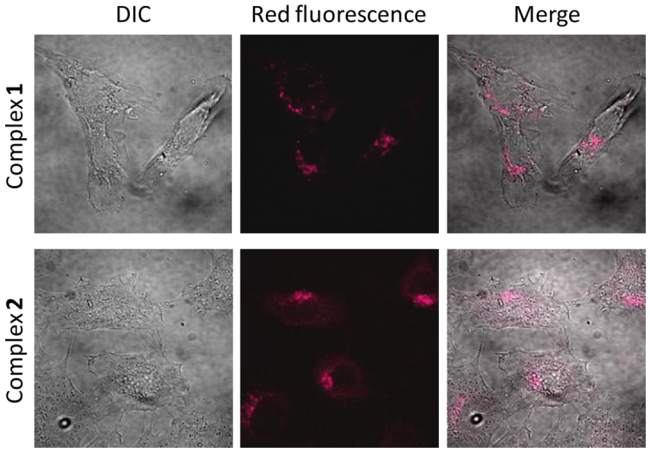

Figure 6. Confocal microscopic images of Hep2 cells incubated with 12.5 μM solutions of complexes **1** (top) and **2** (bottom). DIC denotes the differential interference contrast.

Since the therapeutic effects depend on the efficiency of cellular uptake of a drug, quantitative analysis of the cellular uptake of the rhenium clusters was carried out. According to the experiments on cellular uptake using flow cytometry, the luminescence intensity of the cluster-treated cells was found to be much higher than that of the untreated control cells. The luminescence intensity increased with an increase in the concentration of the cluster: i.e., both clusters were taken up by the cells in a dose-dependent manner (Figure S7 (Supporting Information)).

It is worthy of note that, despite the aforementioned formation of poorly soluble compounds H_4-**1** and H_4-**2** caused by a decrease of pH below ~8, during the studies of the biological properties using solutions with pH ~7.4 (EMEM and PBS) precipitation of the cluster complexes at the concentrations used (up to 400 μM) was not observed.

CONCLUSIONS

To summarize, we have succeeded in preparing the first water-soluble hexarhenium cluster complexes with a heterocyclic outer ligand environment $[\{Re_6(\mu_3\text{-}Q)_8\}(BTA)_6]^{4-}$ (Q = S, Se). The complexes exhibit red phosphorescence with lifetime and quantum yield values among the highest reported so far for chalcogenide cluster complexes, which makes them promising bioimaging and PDT agents. To prove the potential, the cellular uptake and toxicity were evaluated for the complexes. It was found that the both clusters were taken up by the cells and, at the same time, did not exhibit acute cytotoxic effects at the concentration level of practical biological applications. The complexes $[\{Re_6(\mu_3\text{-}S)_8\}(BTA)_6]^{4-}$ and $[\{Re_6(\mu_3\text{-}Se)_8\}(BTA)_6]^{4-}$ were localized in the cell cytoplasm. Thus, it has been shown that the pseudoamphiphilic ligand environment allows the cluster complexes to smoothly penetrate through the cell membrane. The preparation of such complexes may be the first step toward the creation of a new class of NIR phosphorescent dyes based on hexanuclear metal clusters.

ASSOCIATED CONTENT

Supporting Information

Figures, a table, and CIF files giving crystallographic data, selected interatomic distances and angles, crystal packing of compounds, titration curves, photoemission spectra, confocal microscopic images, flow cytometric data, absorption and excitation spectra, and emission decay profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) (a) Yoshimura, T.; Ishizaka, S.; Sasaki, Y.; Kim, H.-B.; Kitamura, N.; Naumov, N. G.; Sokolov, M. N.; Fedorov, V. E. *Chem. Lett.* **1999**, 1121. (b) Yoshimura, T.; Ishizaka, S.; Umakoshi, K.; Sasaki, Y.; Kim, H.-B.; Kitamura, N. *Chem. Lett.* **1999**, 697. (c) Yoshimura, T.; Umakoshi, K.; Sasaki, Y.; Ishizaka, S.; Kim, H.-B.; Kitamura, N. *Inorg. Chem.* **2000**, *39*, 1765. (d) Chen, Z.-N.; Yoshimura, T.; Abe, M.; Sasaki, Y.; Ishizaka, S.; Kim, H.-B.; Kitamura, N. *Angew. Chem., Int. Ed.* **2001**, *40*, 239. (e) Chen, Z.-N.; Yoshimura, T.; Abe, M.; Tsuge, K.; Sasaki, Y.; Ishizaka, S.; Kim, H.-B.; Kitamura, N. *Chem. Eur. J.* **2001**, *7*, 4447. (f) Gray, T. G.; Rudzinski, C. M.; Meyer, E. E.; Holm, R. H.; Nocera, D. G. *J. Am. Chem. Soc.* **2003**, *125*, 4755. (g) Yoshimura, T.; Chen, Z. N.; Itasaka, A.; Abe, M.; Sasaki, Y.; Ishizaka, S.; Kitamura, N.; Yarovoi, S. S.; Solodovnikov, S. F.; Fedorov, V. E. *Inorg. Chem.* **2003**, *42*, 4857. (h) Brylev, K. A.; Mironov, Y. V.; Yarovoi, S. S.; Naumov, N. G.; Fedorov, V. E.; Kim, S.-J.; Kitamura, N.; Kuwahara, Y.; Yamada, K.; Ishizaka, S.; Sasaki, Y. *Inorg. Chem.* **2007**, *46*, 7414. (i) Shestopalov, M. A.; Mironov, Y. V.; Brylev, K. A.; Kozlova, S. G.; Fedorov, V. E.; Spies, H.; Pietzsch, H.-J.; Stephan, H.; Geipel, G.; Bernhard, G. *J. Am. Chem. Soc.* **2007**, *129*, 3714. (j) Brylev, K. A.; Mironov, Y. V.; Kozlova, S. G.; Fedorov, V. E.; Kim, S.-J.; Pietzsch, H.-J.; Stephan, H.; Ito, A.; Ishizaka, S.; Kitamura, N. *Inorg. Chem.* **2009**, *48*, 2309. (k) Dorson, F.; Molard, Y.; Cordier, S.; Fabre, B.; Efremova, O.; Rondeau, D.; Mironov, Y.; Circu, V.; Naumov, N.; Perrin, C. *Dalton Trans.* **2009**, 1297. (l) Molard, Y.; Dorson, F.; Brylev, K. A.; Shestopalov, M. A.; Le Gal, Y.; Cordier, S.; Mironov, Y. V.; Kitamura, N.; Perrin, C. *Chem. Eur. J.* **2010**, *16*, 5613. (m) Szczepura, L. F.; Cedeno, D. L.; Johnson, D. B.; McDonald, R.; Knott, S. A.; Jeans, K. M.; Durham, J. L. *Inorg. Chem.* **2010**, *49*, 11386. (n) Mironov, Y. V.; Brylev, K. A.; Kim, S.-J.; Kozlova, S. G.; Kitamura, N.; Fedorov, V. E. *Inorg. Chim. Acta* **2011**, *370*, 363. (o) Yoshimura, T.; Ishizaka, S.; Kashiwa, T.; Ito, A.; Sakuda, E.; Shinohara, A.; Kitamura, N. *Inorg. Chem.* **2011**, *50*, 9918. (p) Gandubert, A.; Brylev, K. A.; Nguyen, T. T.; Naumov, N. G.;

Kitamura, N.; Molard, Y.; Gautier, R.; Cordier, S. *Z. Anorg. Allg. Chem.* **2013**, *639*, 1756. (q) Ledneva, A. Y.; Brylev, K. A.; Smolentsev, A. I.; Mironov, Y. V.; Molard, Y.; Cordier, S.; Kitamura, N.; Naumov, N. G. *Polyhedron* **2014**, *67*, 351. (r) Gray, T. G.; Rudzinski, C. M.; Nocera, D. G.; Holm, R. H. *Inorg. Chem.* **1999**, *38*, 5932. (s) Guilbaud, C.; Deluzet, A.; Domercq, B.; Molinie, P.; Coulon, C.; Boubekeur, K.; Batail, P. *Chem. Commun.* **1999**, 1867. (t) Gabriel, J. C. P.; Boubekeur, K.; Uriel, S.; Batail, P. *Chem. Rev.* **2001**, *101*, 2037. (u) Cordier, S.; Molard, Y.; Brylev, K. A.; Mironov, Y. V.; Grasset, F.; Fabre, B.; Naumov, N. G. *J. Cluster Sci.* **2014**, DOI: 10.1007/s10876-014-0734-0.

(2) Day, R. N.; Davidson, M. W. *Chem. Soc. Rev.* **2009**, *38*, 2887.

(3) Gao, L.; Peay, M. A.; Gray, T. G. *Chem. Mater.* **2010**, *22*, 6240.

(4) (a) Mironov, Y. V.; Brylev, K. A.; Shestopalov, M. A.; Yarovoi, S. S.; Fedorov, V. E.; Spies, H.; Pietzsch, H.-J.; Stephan, H.; Geipel, G.; Bernhard, G.; Kraus, W. *Inorg. Chim. Acta* **2006**, *359*, 1129. (b) Choi, S.-J.; Brylev, K. A.; Xu, J.-Z.; Mironov, Y. V.; Fedorov, V. E.; Sohn, Y. S.; Kim, S.-J.; Choy, J.-H. *J. Inorg. Biochem.* **2008**, *102*, 1991. (c) Echeverria, C.; Becerra, A.; Nunez-Villena, F.; Munoz-Castro, A.; Stehberg, J.; Zheng, Z. P.; Arratia-Perez, R.; Simon, F.; Ramirez-Tagle, R. *New J. Chem.* **2012**, *36*, 927. (d) Brylev, K. A.; Shestopalov, M. A.; Khripko, O. P.; Trunova, V. A.; Zvereva, V. V.; Wang, C.-C.; Mironov, Y. V.; Fedorov, V. E. *Bull. Exp. Biol. Med.* **2013**, *155*, 741.

(5) Brylev, K. A.; Mironov, Y. V.; Fedorov, V. E.; Kim, S.-J.; Pietzsch, H.-J.; Stephan, H.; Ito, A.; Kitamura, N. *Inorg. Chim. Acta* **2010**, *363*, 2686.

(6) Yarovoi, S. S.; Mironov, Y. V.; Naumov, D. Y.; Gatilov, Y. V.; Kozlova, S. G.; Kim, S.-J.; Fedorov, V. E. *Eur. J. Inorg. Chem.* **2005**, 3945.

(7) *APEX2 (Version 2012.2-0), SAINT (Version 8.18c), and SADABS (Version 2008/1)*; Bruker Advanced X-ray Solutions, Madison, WI, USA, 2000−2012.

(8) Sheldrick, G. M. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **2008**, *64*, 112.

(9) Summerbell, R. K.; Berger, D. R. *J. Am. Chem. Soc.* **1959**, *81*, 633.

(10) Yarovoi, S. S.; Mironov, Y. V.; Solodovnikov, S. F.; Naumov, D. Y.; Moroz, N. K.; Kozlova, S. G.; Simon, A.; Fedorov, V. E. *Chem. Commun.* **2005**, 719.

(11) Ledneva, A. Y.; Naumov, N. G.; Virovets, A. V.; Cordier, S.; Molard, Y. *J. Struct. Chem.* **2012**, *53*, 132.

(12) Katritzky, A. R.; Rachwal, S.; Hitchings, G. J. *Tetrahedron* **1991**, *47*, 2683.

(13) Weishaupt, K. R.; Gomer, C. J.; Dougherty, T. J. *Cancer Res.* **1976**, *36*, 2326.

(14) Ghoneim, N.; Suppan, P. *Solvatochromism*; Royal Society of Chemistry: Cambridge, England, 1997.

(15) Knott, S. A.; Templeton, J. N.; Durham, J. L.; Howard, A. M.; McDonald, R.; Szczepura, L. F. *Dalton Trans.* **2013**, *42*, 8132.

(16) Szczepura, L. F.; Oh, M. K.; Knott, S. A. *Chem. Commun.* **2007**, 4617.