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Synthesis, Characterization, Photophysical Properties, and Biological Labeling Studies of a Series of Luminescent Rhenium(I) Polypyridine Maleimide Complexes

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We report the synthesis, characterization, and photophysical properties of a series of rhenium(I) polypyridine maleimide complexes [Re(N–N)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$) [N–N = 1,10-phenanthroline, phen (**1**), 2,9-dimethyl-1,10-phenanthroline, 2,9-Me$_2$-phen (**2**), 3,4,7,8-tetramethyl-1,10-phenanthroline, 3,4,7,8-Me$_4$-phen (**3**), 4,7-diphenyl-1,10-phenanthroline, 4,7-Ph$_2$-phen (**4**), 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline, 2,9-Me$_2$-4,7-Ph$_2$-phen (**5**), 2,2′-biquinonine, biq (**6**); py-3-mal = *N*-(3-pyridyl)maleimide]. The X-ray crystal structure of complex **2** has been investigated. Upon excitation, the complexes exhibit intense and long-lived photoluminescence in fluid solutions at 298 K. The emission wavelengths range from 514 to 654 nm, and the emission lifetimes fall in the microsecond time scale. The luminescence is assigned to originate from a metal-to-ligand charge-transfer MLCT [dπ(Re) → π*(diimine)] triplet excited state. As the maleimide group can react with the sulfhydryl group to form a stable thioether moiety, these complexes have been used as thiol-specific luminescent labels for a thiolated oligonucleotide, glutathione, and bovine serum albumin and human serum albumin. The photoluminescence properties of the labeled biological species have also been investigated.

Introduction

The maleimide moiety possesses an activated carbon−carbon double bond and is well-known to undergo specific alkylation reactions with the sulfhydryl group to form stable thioethers.[1] The maleimide functional group has been linked to lipids for anchoring of polypeptides to hydrophobic membranes,[2] anticancer drugs for conjugation to thiolated carrier proteins[3] and redox-active agents for providing electrochemical properties to cysteine-containing oligopeptides and metalloproteins.[4] Fluorescent organic compounds such as fluorescein,[5] eosin,[6] and acridine[7] have also been functionalized with a maleimide group to produce site-specific fluorescent labeling reagents. Of particular interest is *N*-(1-pyrenyl)maleimide since its excimer fluorescence properties enable the label to be utilized as a probe for proximity and conformational changes of proteins.[8]

On the other hand, labeling of biological species with fluorescent markers is a very common procedure nowadays.[9] However, designs of biological labels rely essentially on

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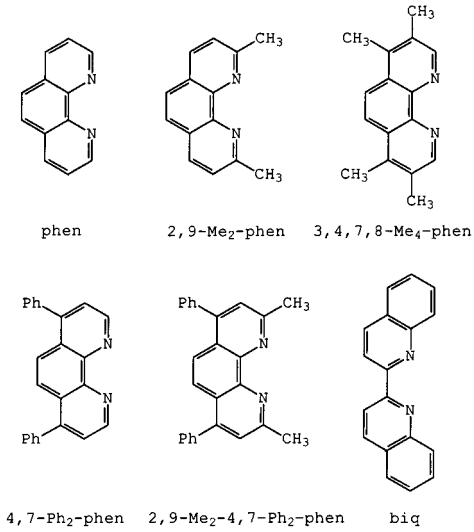
fluorescent organic dyes while relatively less attention has been paid on inorganic transition metal complexes.[10] Despite being commonly used, organic labeling reagents have several disadvantages such as short emission lifetimes, strong pH dependence, self-quenching effects, and low photostability. In this regard, rhenium(I) polypyridine complexes are very promising candidates for biological labeling in view of their remarkable luminescence properties.[11−16] Recently, utilization of luminescent rhenium(I) polypyridine complexes as a nucleic acid[13a] and uracil-dimer[13b] photocleavage agent and a DNA metallointercalator[14] has been reported. The strong photooxidizing properties of these complexes have also been employed in studying electron tunneling in metalloproteins.[15] In addition, luminescent rhenium(I) complexes have also been used as anisotropy probes for protein hydrodynamics.[16]

We anticipate that the photoluminescence of rhenium(I) polypyridine complexes can be exploited in the development of luminescent biological labeling reagents for the following reasons. First, the use of various diimine ligands for the rhenium(I) complexes allows control on the metal-to-ligand charge-transfer (MLCT) emission energy and thereby offers a series of multicolor luminescent labeling reagents. Also, large Stokes' shifts are usually observed for these complexes, which can minimize self-quenching effects commonly encountered in multiple labeling of biomolecules with fluorescent organic dyes.[17] Moreover, the long emission lifetimes of rhenium(I) polypyridine complexes, compared to those of organic fluorophores, could be applied in time-resolved detection techniques that can provide improved sensitivity.[18]

Chart 1. Structures of Diimine Ligands of Complexes **1**−**6**



We report here the synthesis and characterization of a series of new rhenium(I) polypyridine complexes containing a maleimide moiety, $[Re(N-N)(CO)_3(py\text{-}3\text{-}mal)](CF_3SO_3)$ [N−N = 1,10-phenanthroline, phen (**1**), 2,9-dimethyl-1,10-phenanthroline, 2,9-Me_2-phen (**2**), 3,4,7,8-tetramethyl-1,10-phenanthroline, 3,4,7,8-Me_4-phen (**3**), 4,7-diphenyl-1,10-phenanthroline, 4,7-Ph_2-phen (**4**), 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline, 2,9-Me_2-4,7-Ph_2-phen (**5**), 2,2′-biquinonine, biq (**6**); py-3-mal = *N*-(3-pyridyl)maleimide]. The structures of the diimine ligands are shown in Chart 1. The X-ray crystal structure of complex **2** has also been investigated. The photophysical properties of these luminescent complexes and their conjugation to different biological molecules with the sulfhydryl group have also been studied.

Experimental Section

Materials and Reagents. All solvents were of analytical reagent grade and purified according to literature procedures.[19] $Re(CO)_5Cl$, 3-aminopyridine, maleic anhydride, and all the diimine ligands were purchased from Aldrich and were used without purification. A thiolated universal M13 reverse sequencing primer, **M13-R** [5′-$HS(CH_2)_6$-AACAGCTATGACCATG-3′] (Sigma-Genosys), glutathione, reduced form (Sigma), bovine serum albumin (Calbiochem), and human serum albumin, fraction V (Calbiochem), were used as received. All buffer components were of molecular biology grade and used without purification.

***N*-(3-Pyridyl)maleamic Acid.** Maleic anhydride (4.42 g, 45.1 mmol) in 20 mL of ice-cold THF was added to 3-aminopyridine (3.54 g, 37.6 mmol) in 20 mL of ice-cold THF. The mixture was stirred for 1 h at 4 °C. The pale yellow solid formed was collected by filtration and washed with THF and air-dried (6.72 g, yield =

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94%). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K, TMS): δ 10.57 (s, 1H, COOH), 8.76 (d, 1H, J = 1.8 Hz, H2 of pyridine), 8.30 (dd, 1H, J = 4.7 and 1.5 Hz, H6 of pyridine), 8.07 (ddd, 1H, J = 8.2, 2.3 and 1.5 Hz, H4 of pyridine), 7.38 (ddd, 1H, J = 8.2, 4.7 and 0.6 Hz, H5 of pyridine), 6.50 (d, 1H, J = 12.0 Hz, NHCOC*H*═CHCOOH), 6.34 (d, 1H, J = 12.0 Hz, NHCOCH═C*H*COOH). IR (KBr) (ν/cm^{-1}): 2526 (br, OH), 1703 (m, C═O), 1566 (m, C═C). Positive-ion ESI-MS: m/z at 193, $\{M + H^+\}^+$.

***N*-(3-Pyridyl)maleimide, py-3-mal.** Acetic anhydride (20 mL) and ammonium acetate (3 g) were added to *N*-(3-pyridyl)maleamic acid (1.0 g, 5.2 mmol), and the mixture was heated at 100 °C for 10 min. The solution was then cooled and added into 100 mL of cold water. The solution was neutralized to pH 7 with $NaHCO_3$, and the product was extracted with ethyl acetate. The organic solution was collected, washed with saturated $NaHCO_3$, brine, and water, and dried over anhydrous magnesium sulfate. The product was purified by column chromatography (silica gel) using ethyl acetate as the eluent (396 mg, yield = 44%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 8.65 (m, 1H, H2 of pyridine), 8.58 (dd, 1H, J = 4.9 and 1.6 Hz, H6 of pyridine), 7.84 (ddd, 1H, J = 8.2, 2.5 and 1.6 Hz, H4 of pyridine), 7.52 (ddd, 1H, J = 8.2, 4.7 and 0.8 Hz, H5 of pyridine), 7.10 (s, 2H, maleimide H's). IR (KBr) (ν/cm^{-1}): 1713 (s, C═O), 1583 (m, C═C). Positive-ion ESI-MS: m/z at 175, $\{M + H^+\}^+$.

[Re(phen)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$) (1). An anhydrous THF solution of [Re(phen)(CO)$_3$(CH$_3$CN)](CF$_3$SO$_3$) (416.4 mg, 0.65 mmol) and py-3-mal (112.9 mg, 0.65 mmol) was refluxed under nitrogen in the dark for 4 h. After which the solution was evaporated to dryness and the yellow solid was dissolved in CH_2Cl_2 and purified by column chromatography on silica gel. The product was eluted with CH_2Cl_2/acetone (1:1 v/v). Slow diffusion of diethyl ether vapor into a concentrated CH_2Cl_2/acetone solution of the complex afforded [Re(phen)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$) as yellow crystals. Yield: 286.6 mg, 57%. ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 9.88 (d, 2H, J = 5.2 Hz, H2 and H9 of phen), 9.12 (d, 2H, J = 8.2 Hz, H4 and H7 of phen), 8.70 (m, 1H, H6 of py-3-mal), 8.63 (d, 1H, J = 2.2 Hz, H2 of py-3-mal), 8.39 (s, 2H, H5 and H6 of phen), 8.35 (dd, 2H, J = 8.5 and 5.2 Hz, H3 and H8 of phen), 7.99 (m, 1H, H4 of py-3-mal), 7.53 (dd, 1H, J = 8.2 and 5.5 Hz, H5 of py-3-mal), 7.05 (s, 2H, maleimide H's). Anal. Calcd for $C_{25}H_{14}N_4O_8SF_3Re$: C, 38.81; H, 1.82; N, 7.24. Found: C, 38.66; H, 1.80; N, 7.42. IR (KBr) (ν/cm^{-1}): 2039 (s, C≡O), 1929 (s, C≡O), 1720 (s, C═O, maleimide), 1158 (m, $CF_3SO_3^-$), 1027 (m, $CF_3SO_3^-$). Positive-ion ESI-MS: m/z at 624, $\{[Re(bpy)(CO)_3(py\text{-}3\text{-}mal)]\}^+$, and 450, $\{[Re(bpy)(CO)_3]\}^+$.

[Re(2,9-Me$_2$-phen)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$) (2). The synthetic procedure is similar to that for **1** except that [Re(2,9-Me$_2$-phen)(CO)$_3$(CH$_3$CN)](CF$_3$SO$_3$) (434.6 mg, 0.65 mmol) was used. **2** was isolated as yellow crystals. Yield: 354.3 mg, 68%. ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 8.86 (d, 2H, J = 8.5 Hz, H4 and H7 of 2,9-Me$_2$-phen), 8.29−8.26 (m, 3H, H3 and H8 of 2,9-Me$_2$-phen, and H2 of py-3-mal), 8.24−8.22 (m, 1H, H6 of py-3-mal), 8.16 (s, 2H, H5 and H6 of 2,9-Me$_2$-phen), 8.08−7.94 (m, 1H, H4 of py-3-mal), 7.43 (dd, 1H, J = 8.0 and 5.2 Hz, H5 of py-3-mal), 7.04 (s, 2H, maleimide H's), 3.49 (s, 6H, Me of 2,9-Me$_2$-phen). Anal. Calcd for $C_{27}H_{18}N_4O_8SF_3Re$: C, 40.45; H, 2.26; N, 6.99. Found: C, 40.51; H, 2.30; N 6.87. IR (KBr) (ν/cm^{-1}): 2032 (s, C≡O), 1934 (s, C≡O), 1723 (s, C═O, maleimide), 1154 (m, $CF_3SO_3^-$), 1031 (m, $CF_3SO_3^-$). Positive-ion ESI-MS: m/z at 652, $\{[Re(2,9\text{-}Me_2\text{-}phen)(CO)_3(py\text{-}3\text{-}mal)]\}^+$.

[Re(3,4,7,8-Me$_4$-phen)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$) (3). The synthetic procedure is similar to that for **1** except that [Re(3,4,7,8-Me$_4$-phen)(CO)$_3$(CH$_3$CN)](CF$_3$SO$_3$) (452.8 mg, 0.65 mmol) was used. **3** was isolated as pale yellow crystals. Yield: 312.8 mg, 58%. ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 9.62 (s, 2H, H2 and H9 of 3,4,7,8-Me$_4$-phen), 8.69 (d, 1H, J = 2.1 Hz, H2 of py-3-mal), 8.67 (d, 1H, J = 5.6 Hz, H6 of py-3-mal), 8.47 (s, 2H, H5 and H6 of 3,4,7,8-Me$_4$-phen), 7.98−7.95 (m, 1H, H4 of py-3-mal), 7.48 (dd, 1H, J = 8.5 and 5.6 Hz, H5 of py-3-mal), 7.08 (s, 2H, maleimide H's), 2.94 (s, 6H, Me at C4 and C7 of 3,4,7,8-Me$_4$-phen), 2.80 (s, 6H, Me at C3 and C8 of 3,4,7,8-Me$_4$-phen). Anal. Calcd for $C_{29}H_{22}N_4O_8SF_3Re$: C, 41.98; H, 2.67; N, 6.75. Found: C, 42.07; H, 2.58; N, 6.60. IR (KBr) (ν/cm^{-1}): 2032 (s, C≡O), 1921 (s, C≡O), 1722 (s, C═O, maleimide), 1160 (m, $CF_3SO_3^-$), 1033 (m, $CF_3SO_3^-$). Positive-ion ESI-MS: m/z at 680, $\{[Re(3,4,7,8\text{-}Me_4\text{-}phen)(CO)_3(py\text{-}3\text{-}mal)]\}^+$.

[Re(4,7-Ph$_2$-phen)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$) (4). The synthetic procedure is similar to that for **1** except that [Re(4,7-Ph$_2$-phen)(CO)$_3$(CH$_3$CN)](CF$_3$SO$_3$) (515.3 mg, 0.65 mmol) was used. **4** was isolated as orange-yellow crystals. Yield: 385.1 mg, 64%. ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 9.94 (d, 2H, J = 5.3 Hz, H2 and H9 of 4,7-Ph$_2$-phen), 8.85−8.84 (m, 1H, H6 of py-3-mal), 8.60 (d, 1H, J = 1.8 Hz, H2 of py-3-mal), 8.29 (d, 2H, J = 5.6 Hz, H3 and H8 of 4,7-Ph$_2$-phen), 8.25 (s, 2H, H5 and H6 of 4,7-Ph$_2$-phen), 8.04−8.00 (m, 1H, H4 of py-3-mal), 7.77−7.69 (m, 10H, Ph of 4,7-Ph$_2$-phen), 7.51 (dd, 1H, J = 8.2 and 5.6 Hz, H5 of py-3-mal), 7.07 (s, 2H, maleimide H's). Anal. Calcd for $C_{37}H_{22}N_4O_8SF_3Re$: C, 48.00; H, 2.40; N, 6.05. Found: C, 48.13; H, 2.67; N, 5.95. IR (KBr) (ν/cm^{-1}): 2033 (s, C≡O), 1923 (s, C≡O), 1722 (s, C═O, malemide), 1154 (m, $CF_3SO_3^-$), 1031 (m, $CF_3SO_3^-$). Positive-ion ESI-MS: m/z at 776, $\{[Re(4,7\text{-}Ph_2\text{-}phen)(CO)_3(py\text{-}3\text{-}mal)]\}^+$.

[Re(2,9-Me$_2$-4,7-Ph$_2$-phen)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$) (5). The synthetic procedure is similar to that for **1** except that [Re(2,9-Me$_2$-4,7-Ph$_2$-phen)(CO)$_3$(CH$_3$CN)](CF$_3$SO$_3$) (533.5 mg, 0.65 mmol) was used. **5** was isolated as yellow crystals. Yield: 403.0 mg, 65%. ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 8.33−8.29 (m, 2H, H2 and H6 of py-3-mal), 8.26 (s, 2H, H3 and H8 of 2,9-Me$_2$-4,7-Ph$_2$-phen), 7.99 (s, 2H, H5 and H6 of 2,9-Me$_2$-4,7-Ph$_2$-phen), 7.98−7.95 (m, 1H, H4 of py-3-mal), 7.70−7.64 (m, 10H, Ph of 2,9-Me$_2$-4,7-Ph$_2$-phen), 7.47 (dd, 1H, J = 5.9 and 8.2 Hz, H5 of py-3-mal), 7.06 (s, 2H, maleimide H's), 3.57 (s, 6H, Me of 2,9-Me$_2$-4,7-Ph$_2$-phen). Anal. Calcd for $C_{39}H_{26}N_4O_8SF_3Re$: C, 49.11; H, 2.75; N, 5.87. Found: C, 49.37; H, 2.71; N, 5.99. IR (KBr) (ν/cm^{-1}): 2030 (s, C≡O), 1922 (s, C≡O), 1721 (s, C═O, malemide), 1148 (m, $CF_3SO_3^-$), 1031 (m, $CF_3SO_3^-$). Positive-ion ESI-MS: m/z at 804, $\{[Re(2,9\text{-}Me_2\text{-}4,7\text{-}Ph_2\text{-}phen)(CO)_3(py\text{-}3\text{-}mal)]\}^+$.

[Re(biq)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$) (6). The synthetic procedure is similar to that for **1** except that [Re(biq)(CO)$_3$(CH$_3$CN)](CF$_3$SO$_3$) (465.8 mg, 0.65 mmol) was used. **6** was isolated as orange crystals. Yield: 309.3 mg, 56%. ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 9.12 (d, 2H, J = 8.8 Hz, H4 and H4′ of biq), 9.01 (d, 2H, J = 8.8 Hz, H8 and H8′ of biq), 8.86 (d, 2H, J = 8.8 Hz, H3 and H3′ of biq), 8.40 (d, 2H, J = 8.0 Hz, H5 and H5′ of biq), 8.35−8.29 (m, 2H, H7 and H7′ of biq), 8.09−8.03 (m, 3H, H6 and H6′ of biq, and H4 of py-3-mal), 7.89 (d, 1H, J = 2.5 Hz, H2 of py-3-mal), 7.68−7.67 (m, 1H, H6 of py-3-mal), 7.41 (dd, 1H, J = 8.2 and 5.5 Hz, H5 of py-3-mal), 6.94 (s, 2H, maleimide H's). Anal. Calcd for $C_{31}H_{18}N_4O_8SF_3Re$: C, 43.82; H, 2.14; N, 6.59. Found: C, 43.80; H, 2.23; N, 6.38. IR (KBr) (ν/cm^{-1}): 2031 (s, C≡O), 1918 (s, C≡O), 1721 (s, C═O, maleimide), 1145 (m, $CF_3SO_3^-$), 1032 (m, $CF_3SO_3^-$). Positive-ion ESI-MS: m/z at 700, $\{[Re(biq)(CO)_3(py\text{-}3\text{-}mal)]\}^+$.

Labeling of Single-Stranded DNA with Complex 1. In a typical labeling reaction, complex **1** (0.7 mg, 0.90 μmol) in 50 μL

anhydrous DMSO was added to a thiolated universal M13 reverse sequencing primer **M13-R** (10 nmol) in 450 μL of 50 mM potassium phosphate buffer pH 7.4. The mixture was stirred gently in the dark at RT (room temperature) for 24 h. The solid residue was removed by centrifugation. To the supernatant, 50 μL of NaOAc (3.0 M, pH 5.2) and 900 μL of 2-propanol were added. The labeled DNA was collected by centrifugation, and the pellet was washed with 70% aqueous EtOH and twice with absolute EtOH and then dried in vacuo. The labeled oligonucleotide was further purified by RP-HPLC with CH_3CN (5−50% over 50 min.) and 0.1 M triethylammonium acetate pH 7.0 as the mobile phase at a flow rate of 1 mL min^{-1}.

Labeling of Glutathione (γ-Glu-Cys-Gly) (GSH) with Complex 1. Complex **1** (1.8 mg, 2.32 μmol) in 50 μL anhydrous DMSO was added to glutathione (0.2 mg, 0.65 μmol) dissolved in 100 μL of 50 mM potassium phosphate buffer, pH 7.4. The mixture was stirred gently in the dark at RT for 24 h. The solid residue was removed by centrifugation. The excess label was removed by washing the aqueous solution repeatedly with ethyl acetate, and the labeled peptide was further purified by HPLC.

Labeling of Bovine Serum Albumin (BSA) and Human Serum Albumin (HSA) with Complex 1. Complex **1** (1.3 mg, 1.67 μmol) in 20 μL of anhydrous DMSO was added to BSA or HSA (1.32 mg, 20 nmol) dissolved in 180 μL of 50 mM potassium phosphate buffer, pH 7.4. The mixture was incubated in the dark at RT for 12 h. The solid residue was removed by centrifugation. The supernatant was diluted to 1 mL with 50 mM Tris-HCl, pH 7.4 and loaded onto a PD-10 column (Pharmacia) that had been equilibrated with the same buffer. The first band that came out from the column with intense yellow luminescence was collected, and the solution was concentrated with a YM-30 centricon (Amicon). The labeled protein was further purified by HPLC equipped with a size-exclusion column. The mobile phase was 50 mM Tris-HCl, pH 7.4, at a flow rate of 1 mL min^{-1}.

Crystal Structure Determination. Crystallographic data for complex **2**: $[(C_{26}H_{18}N_4O_5Re)^+(CF_3SO_3^-)]$, formula weight = 801.72, monoclinic, space group $P2_1/c$ (No. 14), a = 10.460(3) Å, b = 17.673(3) Å, c = 15.359(2) Å, β = 95.91(2)°, V = 2824.4(9) Å^3, Z = 4, D_c = 1.885 g cm^{-3}, μ(Mo Kα) = 44.55 cm^{-1}, F(000) = 1560, T = 301 K. A yellow crystal of dimensions 0.30 × 0.10 × 0.07 mm in a glass capillary was used for data collection at 28 °C on a Rigaku AFC7R diffractometer with graphite-monochromatized Mo Kα radiation (λ = 0.710 73 Å) using $\omega-2\theta$ scans with ω-scan angle (0.68 + 0.35 tan θ)° at a scan speed of 8.0 deg min^{-1} [up to 6 scans for reflection with $I < 15\sigma(I)$]. Unit-cell dimensions were determined on the basis of the setting angles of 25 reflections in the 2θ range of 28.4−32.5°. Intensity data (in the range of $2\theta_{max}$ = 52°; h, 0 to 12; k, 0 to 21; l, −18 to 18; 3 standard reflections measured after every 300 reflections showed decay of 2.66%) were corrected for Lorentz and polarization effects, and empirical absorption corrections were based on the ψ-scan of five strong reflections (minimum and maximum transmission factors 0.774 and 1.000). A total of 6052 reflections were measured, of which 5734 were unique and R_{int} = 0.020. A total of 3636 reflections with $I > 3\sigma(I)$ were considered observed and used in the structural analysis. The space group was uniquely determined on the basis of systematic absences, and the structure was solved by Patterson methods and expanded by Fourier methods (PATTY[20]) and refinement by full-matrix least-squares using the software package TeXsan[21] on a Silicon Graphics Indy computer. One crystallographic asymmetric unit consists of one formula unit. Two of the O atoms in the anion were disordered and were placed at three positions with O(7), O(7′), and O(8) having occupation numbers of 0.75, 0.45, and 0.8, respectively. In the least-squares refinement, 38 non-H atoms were refined anisotropically; the F and O atoms of the anion were refined isotropically, and 18 H atoms at calculated positions with thermal parameters equal to 1.3 times that of the attached C atoms were not refined. H atoms bonded to the disordered methyl C atom were not included in the calculation. Convergence for 371 variable parameters by least-squares refinement on F with $w = 4F_o^2/\sigma^2(F_o^2)$, where $\sigma^2(F_o^2) = [\sigma^2(I) + (0.025F_o^2)^2]$ for 3636 reflections with $I > 3\sigma(I)$ was reached at R = 0.038 and R_w = 0.050 with a goodness-of-fit of 1.94. $(\Delta/\sigma)_{max}$ = 0.05 except for the disordered O atoms. The final difference Fourier map was featureless, with maximum positive and negative peaks of 0.96 and 0.76 e Å^{-3}, respectively.

Photophysical Measurements. Electronic absorption and steady-state emission spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer and a Spex Fluorolog-2 model F 111 fluorescence spectrophotometer, respectively. Unless specified, all solutions for photophysical studies were degassed with no fewer than four successive freeze−pump−thaw cycles and stored in a 10-cm^3 round-bottomed flask equipped with a sidearm 1-cm fluorescence cuvette and sealed from the atmosphere by a Rotaflo HP6/6 quick-release Teflon stopper. Luminescence quantum yields were measured by the optical dilute method[22] using an aerated aqueous solution of $[Ru(bpy)_3]Cl_2$ (Φ = 0.028, excitation wavelength at 455 nm)[23] as the standard solution. The 355-nm output (third harmonic) of a Quanta-Ray Q-switched GCR-150-10 pulsed Nd:YAG laser was the excitation source for emission lifetime measurements. Luminescence decay signals from a Hamamatsu R928 photomultiplier tube were converted to potential changes by a 50-Ω load resistor and then recorded on a Tektronix model TDS 620A digital oscilloscope.

Results and Discussion

Synthesis and Characterization. The rhenium(I) polypyridine maleimide complexes **1**−**6** were obtained in moderate yields from the reactions of $[Re(N-N)(CO)_3(CH_3CN)](CF_3SO_3)$[11b] and the ligand py-3-mal. The complexes were characterized by ^{1}H NMR, positive-ion ESI-MS, and IR and gave satisfactory elemental analysis. The maleimide moiety of the complexes is characterized by a singlet at ca. δ 7.0 in the ^{1}H NMR spectra and a strong absorption at ca. 1722 cm^{-1} in the IR spectra. The complexes are air-stable, and the characterization data showed that their identities remained the same in a period of months when stored at −20 °C in the absence of moisture. However, it is well-known that the maleimide group is generally susceptible to hydrolysis.

Crystal Structure Determination. Single crystals of complex **2** were obtained by layering a concentrated acetone solution of the complex with a mixture of diethyl ether and petroleum ether. The perspective drawing of the complex cation of **2** with atomic numbering is depicted in Figure 1. Selected bond distances and angles are summarized in Table

(20) PATTY: Beurskens, P. T.; Admiral, G.; Beurskens, G.; Bosman, W. P.; Garcia-Granda, S.; Gould, R. O.; Smits, J. M. M.; Smykalla, C. *The DIRDIF program system*; Technical Report of the Crystallography Laboratory, University of Nijmegen: Nijmegen, The Netherlands, 1992.

(21) *TeXsan, Crystal Structure Analysis Package*; Molecular Structure Corp.: The Woodlands, TX, 1985 and 1992.

(22) Demas, J. N.; Crosby, G. A. *J. Phys. Chem.* **1971**, *75*, 991−1024.

(23) Nakamaru, K. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2697−2705.

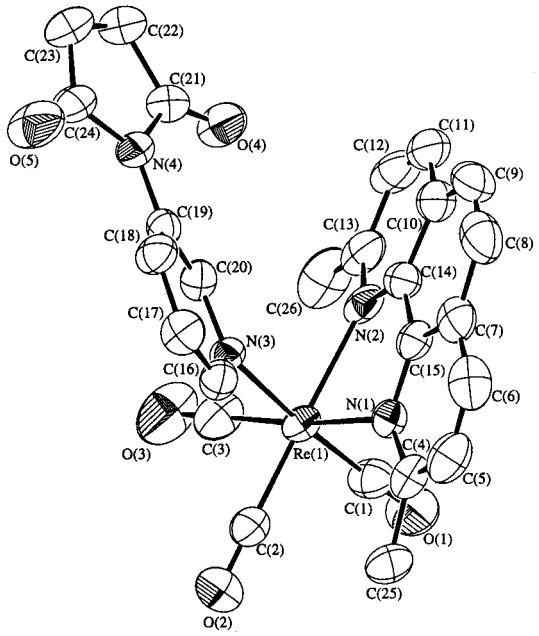


Figure 1. Perspective view of the complex cation of **2** with atomic numbering scheme. Hydrogen atoms have been omitted for clarity. Thermal ellipsoids are shown at the 50% probability level.

Table 1. Selected Geometric Data for Complex **2**

Bond Lengths (Å)			
Re(1)−N(1)	2.199(7)	Re(1)−N(2)	2.215(7)
Re(1)−N(3)	2.219(6)	Re(1)−C(1)	1.916(9)
Re(1)−C(2)	1.918(10)	Re(1)−C(3)	1.90(1)
O(4)−C(21)	1.20(1)	O(5)−C(24)	1.19(1)
C(22)−C(23)	1.32(1)		
Bond Angles (deg)			
N(1)−Re(1)−N(2)	75.5(3)	N(1)−Re(1)−N(3)	81.4(2)
N(1)−Re(1)−C(1)	95.8(3)	N(1)−Re(1)−C(2)	101.0(3)
N(1)−Re(1)−C(3)	172.5(3)	N(2)−Re(1)−N(3)	82.8(2)
N(2)−Re(1)−C(1)	93.8(3)	N(2)−Re(1)−C(2)	176.3(3)
N(2)−Re(1)−C(3)	101.2(4)	N(3)−Re(1)−C(1)	176.0(3)
N(3)−Re(1)−C(2)	95.5(3)	N(3)−Re(1)−C(3)	91.6(4)
C(1)−Re(1)−C(2)	87.9(4)	C(1)−Re(1)−C(3)	91.1(5)
C(2)−Re(1)−C(3)	82.1(5)		

1. The rhenium(I) center adopts a distorted octahedral coordination geometry, and the carbonyl groups are arranged in a *facial* orientation. The bond lengths and angles involving the rhenium(I) center are normal compared to those observed in related systems.[12,13a,b,15a] It is noteworthy that the dihedral angle between the ideal ring plane of the 2,9-Me_2-phen ligand and the C(2)−Re(1)−C(3) plane is ca. 154.1°. Deviation from 180° of the ideal case is probably a result of the steric requirements of the methyl groups. The geometric data of the maleimide group are similar to those of the unsubstituted maleimide ($C_4H_3NO_2$) molecule.[24] Owing to the bulkiness of the maleimide carbonyl groups, the maleimide ring establishes a dihedral angle of ca. 25.7° with the pyridyl ring.

Photophysical Properties. The complexes are soluble in common organic solvents such as dichloromethane, acetonitrile, and acetone, giving yellow to orange solutions. The electronic absorption spectral data for the complexes are listed in Table 2. The electronic absorption spectrum of **1** in CH_2Cl_2 at 298 K is shown in Figure 2 (spectrum a). The

(24) Cox, P. J.; Parker, S. F. *Acta Crystallogr. C* **1996**, *52*, 2578−2580.

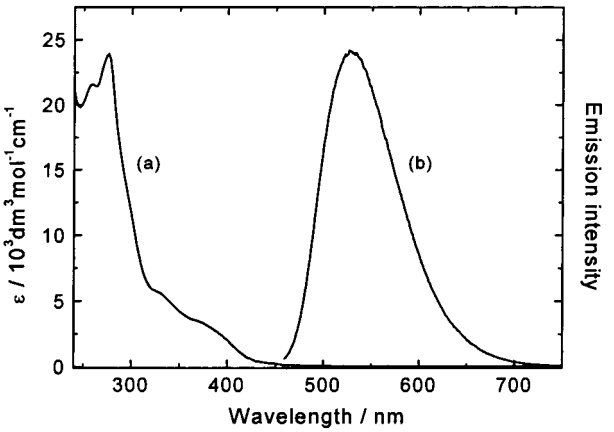


Figure 2. Electronic absorption (a) and emission (b) spectra of complex **1** in CH_2Cl_2 at 298 K.

Table 2. Electronic Absorption Spectral Data for Complexes **1**−**6** at 298 K

complex	medium	λ_{abs}/nm (ϵ/dm^3 mol^{-1} cm^{-1})
1	CH_2Cl_2	258 (20 745), 276 (23 930), 296 sh (13 385), 324 sh (5865), 364 sh (3725)
	CH_3CN	276 (24 605), 318 sh (6880), 362 sh (3555)
2	CH_2Cl_2	284 (22 585), 324 sh (8320), 368 sh (2425)
	CH_3CN	284 (24 645), 306 sh (14 880), 318 sh (10 395), 364 sh (2390)
3	CH_2Cl_2	250 sh (19 815), 282 (23 165), 324 sh (8670), 370 sh (2860)
	CH_3CN	250 sh (21 025), 282 (23 830), 316 sh (10 205), 370 sh (2490)
4	CH_2Cl_2	294 (29 160), 332 sh (12 900), 380 sh (6545)
	CH_3CN	260 sh (25 610), 292 (40 215), 324 sh (16 340), 374 sh (7415)
5	CH_2Cl_2	260 (21 085), 300 (31 915), 334 sh (13 255), 378 sh (4550)
	CH_3CN	254 (20 605), 298 (30 225), 332 sh (11 710), 374 sh (3965)
6	CH_2Cl_2	270 (32 570), 300 sh (11 450), 364 (13 985), 382 (19 870), 408 sh (3255)
	CH_3CN	270 (39 240), 298 sh (13 610), 360 (15 855), 378 (18 670), 406 sh (3600)

intense absorptions at ca. 250−296 nm are assigned to intraligand (IL) transitions [$\pi \rightarrow \pi^*$ (bpy and py-3-mal)] since similar absorption bands are observed for the uncoordinated ligands. On the basis of previous spectroscopic studies of rhenium(I) polypyridine complexes,[11−16] the lower-energy absorption shoulders of **1** at ca. 324−364 nm with extinction coefficients in the order of 10^3 dm^3 mol^{-1} cm^{-1} are assigned to spin-allowed metal-to-ligand charge-transfer (MLCT) [dπ(Re) $\rightarrow$ π^*(bpy)] transitions. Similar spectroscopic assignments are made for the other complexes in this work. However, owing to the extended π-conjugation of the ligands 4,7-Ph_2-phen, 2,9-Me_2-4,7-Ph_2-phen, and biq, the intraligand absorptions of **4**−**6** are extended to a lower energy region (ca. 332−382 nm) and the ^{1}MLCT [dπ(Re) $\rightarrow$ π^*(diimine)] transitions of these complexes occur at a further lower energy (ca. 374−408 nm).

Excitation of complexes **1**−**6** at $\lambda_{ex} > 350$ nm gives rise to intense and long-lived green to orange-red luminescence. The photophysical data are listed in Table 3. As an example, complex **1** emits at 530 nm (Figure 2, spectrum b) with a lifetime of 2.30 μs in CH_2Cl_2 at 298 K. A shift of ca. 1.07 eV is observed from the absorption shoulder at ca. 364 nm

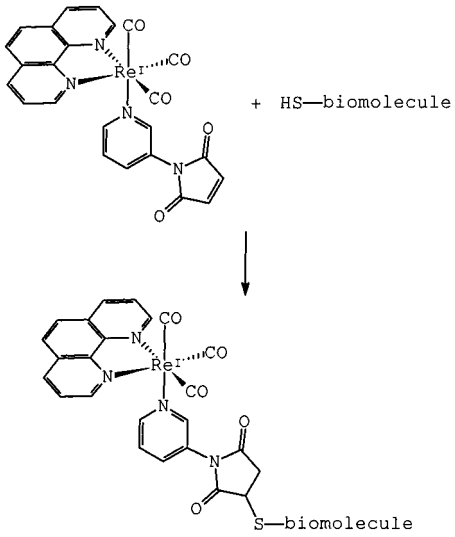
Table 3. Photophysical Data for Complexes **1**−**6** at 298 K

complex	medium	λ_{em}/nm	τ_o/μs	Φ
1	CH_2Cl_2	530	2.30	0.51
	acetone	546	1.84	
	CH_3CN	546	1.95 (5%), 0.19 (95%)	
2	CH_2Cl_2	518	1.34	0.43
	acetone	532	1.77	
	CH_3CN	532	2.08 (6%), 0.13 (94%)	
3	CH_2Cl_2	514	5.21	0.13
	acetone	518	2.45	
	CH_3CN	518	9.60 (70%), 1.64 (30%)	
4	CH_2Cl_2	542	7.42	0.53
	acetone	558	2.48	
	CH_3CN	558	5.81 (15%), 1.06 (85%)	
5	CH_2Cl_2	536	6.18	0.21
	acetone	544	3.43	
	CH_3CN	544	8.78 (15%), 1.55 (85%)	
6	CH_2Cl_2	632	0.10	0.0020
	acetone	654	0.05	
	CH_3CN	650	0.06	

to the emission maximum at ca. 530 nm. For **1**−**6**, the relatively long emission lifetimes (in the microsecond time scale), together with large absorption−emission shifts, indicate the phosphorescence nature of the emission. The emission energies of the complexes follow the order **3** > **2** > **1** ≈ **5** > **4** > **6**, which is in line with the π-accepting ability of the diimine ligands. The emissions of the complexes exhibit a red-shift upon changing the solvent from less polar CH_2Cl_2 to more polar acetone and CH_3CN. On the basis of the dependence between the emission energies of the complexes and the energy levels of the empty π^* orbitals of the diimine ligands, together with the observation of solvatochromic behavior, the photoluminescence of the complexes is assigned to associate with an MLCT [dπ(Re) → π^*(diimine)] triplet excited-state. Similar assignments have been made in other luminescent rhenium(I) polypyridine complexes.[11−16] The luminescence quantum yields of the complexes are comparable to those of related $[Re(N-N)(CO)_3(py)]^+$ complexes.[11b,e,g,16a]

Photophysical Properties of Bioconjugates. Since the maleimide group can react with the sulfhydryl group to form a stable thioether, the rhenium(I) polypyridine maleimide complexes in this work are designed to react specifically with sulfhydryl-containing biomolecules (Scheme 1). As an example, the thiolated M13 sequencing primer **M13-R** [5′-$HS(CH_2)_6$-AACAGCTATGACCATG-3′] has been labeled with complexes **1**−**6**. Although these rhenium(I) complexes are sparingly soluble in water, the DNA primers coupled with **1**−**6** are very soluble in water and aqueous buffers and exhibit intense and long-lived greenish yellow to orange luminescence upon photoexcitation (Table 4). The emission wavelengths are indistinguishable to those of the free labels in CH_3CN, and the trend of the emission energies of the labeled primers, **1-M13-R**−**6-M13-R**, is the same to that of the free complexes. This finding is important because it is our intention to use various diimine ligands to provide multicolor labeling reagents for biological substrates. In view of this observation, together with the microsecond time scale emission lifetimes, the emissive states of these labeled oligonucleotides should bear a high parentage of ^{3}MLCT [dπ(Re) → π^*(diimine)] character. The luminescence quantum yields of **1-M13-R**−**6-M13-R** in degassed and air-saturated Tris buffer are in line with those of other ruthenium(II)-labeled oligonucleotides.[10h,i] On the other hand, since the substituents at the *meta* positions of the pyridine ligand have only minor effects on the rhenium(I) diimine luminophore, a change from the maleimide group to a thioether is not expected to perturb significantly the MLCT emission behavior of the complex. Therefore, the small red-shifts and decreased lifetimes of the emissions of the free labels from CH_2Cl_2 to CH_3CN and to the labeled DNA in Tris buffer appear to result from the increasing polarity of the solvent environment (and the presence of highly polar phosphates of the oligonucleotides). Such an observation of solvatochromism has been observed for Re^I polypyridine MLCT emitters.[11a,c−f,12b] Nonetheless, given the strong photooxidizing behavior of rhenium(I) polypyridine complexes,[15] the decreased emission lifetimes may be a result of oxidation of guanines by the excited complexes.

Scheme 1. Proposed Reaction between the Complex Cation of **1** and the Sulfhydryl Group of a Biomolecule



Other biomolecules, including a cysteine-containing peptide, glutathione (GSH), and proteins such as bovine serum albumin (BSA) and human serum albumin (HSA), have also been labeled with complex **1**. The UV−vis absorption spectra of these bioconjugates showed low-energy absorption shoulders at ca. 328 and 368 nm in Tris buffer, typical of ^{1}MLCT [dπ(Re) → π^*(phen)] transitions. According to the absorption spectral data, the rhenium:protein ratios have been determined to be ca. 4.0 and 4.8 for the conjugates **1-BSA** and **1-HSA**, respectively. Upon photoexcitation, the bioconjugates display intense and long-lived yellow luminescence in buffer solutions (Table 5). The emission wavelengths are indiscernible to that of the labeled oligonucleotide **1-M13-R**. The emission is also assigned to arise from an ^{3}MLCT [dπ(Re) → π^*(phen)] excited state. The luminescence quantum yields of these bioconjugates are similar in magnitude to those of other biomolecules labeled with luminescent rhenium(I) complexes.[16a] Concerning the emission lifetimes, while the bioconjugate **1-GSH** exhibits a single-exponential decay, both **1-BSA** and **1-HSA** show double-exponential decays with emission-lifetime components of ca. 1.1 and 0.2 μs. The observation of double-exponential and

Table 4. Photophysical Data for Labeled Oligonucleotides **1-M13-R**−**6-M13-R** in 50 mM Tris-HCl, pH 7.4, at 298 K

	degassed buffer			air-saturated buffer		
labeled oligonucleotide	λ_{em}/nm	τ_o/μs	Φ	λ_{em}/nm	τ/μs	Φ
1-M13-R	548	0.99 (18%), 0.31 (82%)	0.026	548	0.61 (37%), 0.23 (63%)	0.019
2-M13-R	536	1.46 (47%), 0.31 (53%)	0.020	536	1.29 (43%), 0.31 (57%)	0.016
3-M13-R	522	5.66 (67%), 0.74 (33%)	0.021	522	2.76 (59%), 0.28 (41%)	0.011
4-M13-R	558	2.72 (52%), 0.51 (48%)	0.022	558	2.07 (52%), 0.41 (48%)	0.016
5-M13-R	544	6.42 (58%), 0.88 (42%)	0.021	544	4.39 (56%), 0.96 (44%)	0.013
6-M13-R	636	0.12	0.0012	636	0.12	0.0010

Table 5. Photophysical Data for the Bioconjugates **1-GSH**, **1-BSA**, and **1-HSA** in 50 mM Tris-HCl, pH 7.4, at 298 K

	degassed buffer			air-saturated buffer		
bioconjugate	λ_{em}/nm	τ_o/μs	Φ	λ_{em}/nm	τ/μs	Φ
1-GSH	548	0.99	0.11	548	0.65	0.076
1-BSA	544	1.09 (49%), 0.19 (51%)	0.069	544	0.86 (47%), 0.18 (53%)	0.040
1-HSA	544	1.11 (48%), 0.20 (52%)	0.051	544	1.00 (43%), 0.26 (57%)	0.040

multiexponential emission decays is not uncommon for luminescent inorganic complexes attached to biomolecules.[16] On the other hand, the luminescence of the conjugates **1-GSH**, **1-BSA**, and **1-HSA** is quenched by oxygen molecules owing to the triplet character of the emissive states. However, it is interesting to note that for **1-GSH**, the emission quenching by oxygen is more significant ($k_q = 2.12 \times 10^9$ dm^3 mol^{-1} s^{-1}) than the labeled serum albumins (**1-BSA**, $k_q = 1.23 \times 10^9$ dm^3 mol^{-1} s^{-1}; **1-HSA**, $k_q = 7.66 \times 10^8$ dm^3 mol^{-1} s^{-1}). In view of the large difference in the molecular sizes of glutathione and the serum albumins, it appears that the labels coupled to BSA and HSA are well protected in the interior of the proteins and a lower exposure to the solvent surrounding could account for the less efficient quenching by the oxygen molecules. A similar observation has been noticed in other luminophore-labeled protein systems.[16] Nevertheless, the observations of emission lifetimes in the microsecond time scale and generally high luminescence quantum yields for the labeled DNA, peptide, and serum albumins suggest that these rhenium(I) maleimide complexes are promising candidates as thiol-specific labels in time-resolved bioassays.

Concluding Remarks

A series of new rhenium(I) polypyridine complexes containing a maleimide moiety has been synthesized and characterized, and their photophysical properties have been studied. The complexes have been employed as site-specific labels for biological species containing the sulfhydryl group. The labeled substrates exhibit long-lived and intense photoluminescence typical of rhenium(I) polypyridine ^{3}MLCT emissions. It is envisaged that these new thiol-specific labels can be applied in DNA hybridization assays, sequencing experiments, polymerase chain reactions, immunoassays, and probing the structures of biological substrates.

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Supporting Information Available: Tables giving atomic coordinates, anisotropic displacement parameters, and bond lengths and bond angles for complex **2** in a CIF file. This material is available free of charge via the Internet at http://pubs.acs.org.

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