

# Long-Lived Luminescent Re(I) Complexes Containing *cis*-Carbonyl and Bidentate Phosphine Ligands

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Highly emissive Re(I) complexes, hydrophobic *cis*-Re(CO)<sub>2</sub>(*c*-dppene)(dpphen) and water-soluble *cis*-Re(CO)<sub>2</sub>(*c*-dppene)(SO<sub>3</sub>-dpphen) with a negative charge, were synthesized [where *c*-dppene is *cis*-(bis(diphenylphosphino)ethylene, dpphen is 4,7-diphenyl-1,10-phenanthroline, and SO<sub>3</sub>-dpphen is its disulfonate derivative]. These Re(I) complexes have significantly higher molar absorption in the 350- to 490-nm region compared to their tricarbonyl Re(I) counterparts and their emission spectra are similar to those of Ru(II) polypyridyl complexes. The luminescence lifetimes of these Re(I) complexes approach 10 μs in the absence of oxygen in both aqueous and less polar solvents. The complexes have limiting anisotropy values exceeding 0.3. As both ligands, the diimine and the bidentate phosphine, can be modified by adding different substituents, it should be possible to make *cis*-Re(CO)<sub>2</sub>(diimine)(P-P) derivatives which are capable of labeling biomacromolecules for biochemical and biophysical studies.

**KEY WORDS:** *cis*-Bicarbonyl Re(I) complexes; microsecond lifetime; anisotropy; metal–ligand complexes.

## INTRODUCTION

Luminescent probes based on metal-to-ligand charge transfer (MLCT)<sup>3</sup> excited states, i.e., Ru(II) polypyridyl and tricarbonyl Re(I) complexes, have shown great potential for biophysical and biochemical research (Refs. 1 and 2 and references therein). The main attributes which separate these luminophores from the great majority of organic fluorophores are their submicro- to microsecond lifetimes in solution, large Stoke's shifts in emission, and remarkable photochemical stability (see reviews in Refs. 3 and 4). The long-lived excited states coupled with their intrinsic polarization have opened possibilities of performing polarization-based immunoassays of high molecular weight species [5,6], characterizing hydrodynamics

of large macromolecules [6–8], and studying microsecond events such as diffusion in membranes [8,9].

Re(I) tricarbonyl diimine complexes [(Re(CO)<sub>3</sub>(diimine)(L)]<sup>+</sup>, where L is a monodentate ligand which can be a pyridine or an acetonitrile, show higher fundamental anisotropies compared to Ru(II) polypyridyl complexes [10,13]. The main disadvantage of Re(I) complexes is the lack of significant absorption beyond 400 nm. Their molar extinction values are less than 4000 M<sup>-1</sup> cm<sup>-1</sup> at 370–380 nm. The typical molar extinctions are about 15,000 M<sup>-1</sup> cm<sup>-1</sup> around 450 nm in the case of Ru(II) complexes. Recently Sullivan and co-workers [11] reported a new class of luminescent Re(I) complexes, *cis*-

<sup>3</sup> Abbreviations used: Re-1, *cis*-Re(CO)<sub>2</sub>(*c*-dppene)(dpphen); Re-2, *cis*-Re(CO)<sub>2</sub>(*c*-dppene)(SO<sub>3</sub>-dpphen); Re-3, *fac*-Re(CO)<sub>3</sub>(dmdpphen)(py-COOH); *c*-dppene, *cis*-(bis(diphenylphosphino)ethylene; dpphen, 4,7-diphenyl-1,10-phenanthroline; dmdpphen, 2,9-dimethyl,4,7-diphenyl-1,10-phenanthroline; MLC, metal–ligand complex; phen, 1,10-phenanthroline; QY, quantum yield; SO<sub>3</sub>-dpphen, bathophenanthroline disulfonic acid; DPPC, dipalmitoyl phosphatidylcholine; MLCT, metal-to-ligand charge transfer.

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$[\text{Re}(\text{CO})_2(\text{N}-\text{N})(\text{P}-\text{P})]^+$ , where P-P is a bidentate phosphine, *cis*-(bis(diphenylphosphino)ethylene) (*c*-dppene), and N-N is a polypyridine chelate, either 2,2'-bipyridine or 1,10-phenanthroline. These novel *cis*-P-P Re complexes emit almost 60 nm to the red, with substantial emission at wavelengths longer than 700 nm, and still have microsecond lifetimes. They also have significantly higher absorptivity beyond 400 nm compared to tricarbonyl Re MLCs.

To utilize these luminescent properties of this class of novel rhenium complexes for biochemical research, we synthesized two *cis*-P-P Re(I) complexes: A hydrophobic complex, *cis*-Re(CO)<sub>2</sub>(*c*-dppene)(dpphen) (Re-1), and a more polar and water-soluble, negatively charged complex, *cis*-Re(CO)<sub>2</sub>(*c*-dppene)(SO<sub>3</sub>-dpphen) (Re-2). We used as the diimine ligand either hydrophobic dpphen or its water-soluble disulfonated derivative, SO<sub>3</sub>-dpphen (see Scheme I). In both Ru and Re MLCs substitutions of the phen ligand with dpphen has been shown to increase the quantum yields and the lifetimes [4]. We therefore reasoned that a similar replacement in one of the Re complexes made by Sullivan and co-workers should result in a longer-lived Re(I) MLC than the parent *cis*-Re(CO)<sub>2</sub>(*c*-dppene)(phen). This communication describes the luminescence properties of these two Re(I) complexes including their limiting anisotropy and lifetimes in solution.

## EXPERIMENTAL

All the chemicals were obtained from Aldrich/Sigma and used as such unless noted otherwise. The absorption spectra were taken on a HP8453 diode array spectrophotometer. The steady-state fluorescence measurements were carried out with a Cary Eclipse fluorescence spectrophotometer with the slits set at 5 nm. The absorbance of the solutions was near 0.05 OD. The lifetimes were measured on a ISS K2 frequency domain instrument with

a blue LED light source in a setup similar to that used by Sipior *et al.* [12]. The excitation was selected through a 450-nm-centered bandpass filter, while the emission was observed through a bandpass filter centered at 605 nm. A rhodamine B aqueous solution was used as a reference, with a lifetime of 1.68 ns. To remove dissolved oxygen, nitrogen was flushed through a sealed cuvette for 40 min for aqueous solutions, 30 min for acetonitrile solutions, and 20 min for dichloromethane solutions. We did not attempt to eliminate the last traces of oxygen, though we expect solutions to be essentially free of dissolved oxygen. The quantum yields (QY) were measured using Ru(bpy)<sub>3</sub> as a reference.

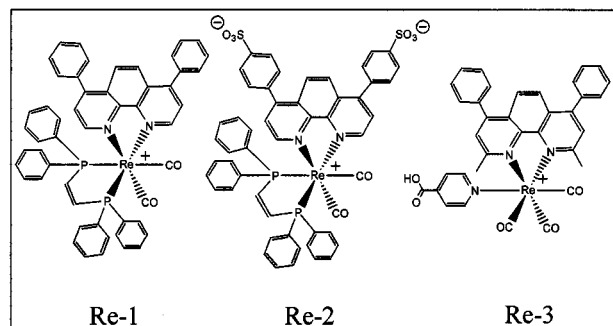
The Re-1 and Re-2 (Scheme I) complexes were synthesized following the procedures of Sullivan and co-workers [11]. The precursor of these complexes, *fac*-Re(CO)<sub>3</sub>(*c*-dppene)Cl, was prepared by refluxing Re(CO)<sub>5</sub>Cl and 1 equiv *c*-dppene in toluene under nitrogen overnight and collected as a white powder after the solvent was removed under reduced pressure. The IR spectrum of the product in dichloromethane has three strong CO stretches, at 2034, 1958, and 1910 cm<sup>-1</sup>. Then *fac*-Re(CO)<sub>3</sub>(*c*-dppene)Cl, along with 1.2 equiv thallium PF<sub>6</sub> and 1.3 equiv SO<sub>3</sub>-dpphen (sodium salt) or dpphen, in 1,2-dichlorobenzene was heated to reflux in dark under nitrogen for 8 h. Chalky-white thallium chloride was removed via a filtration through celite. After the solvent was removed, the residue was loaded on a short silica gel column (eluent: CH<sub>2</sub>Cl<sub>2</sub>/MeOH). The product was collected as a yellow-orange band eluted from the column. The symmetric and asymmetric CO stretches of the *cis*-dicarbonyl in dichloromethane were found at 1958 and 1888 cm<sup>-1</sup>. The procedure for making the tricarbonyl Re-3 has been published elsewhere [10].

The MLC Re-1 is quite hydrophobic and shows limited aqueous solubility. However, due to the presence of two sulfonates, Re-2 has a better water solubility. We could obtain about 250 μM Re-2 solution in a buffer. Re-2 also has an overall negative charge, while Re-1 is positively charged.

FAB MS (*m/z*): Re-1 (M<sup>+</sup>), 971.2; Re-2 (MH<sup>+</sup>), 1153.2.

## RESULTS AND DISCUSSION

Figure 1 shows the absorption and emission spectra of these three Re complexes in acetonitrile. The spectra are characterized by a ligand-centered transition around 285–290 nm and a MLCT band as a shoulder at higher wavelengths. Compared to Re-3, the MLCT absorption bands of both Re-1 and Re-2 complexes which have the



Scheme 1.

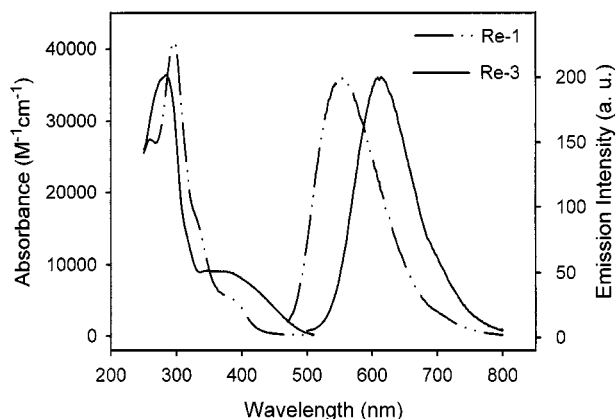


Fig. 1. Absorption and emission spectra of *cis*-P-P Re(I) and Re-3 complexes in acetonitrile.

*cis*-P-P ligand extend to wavelengths longer by about 50 nm, reaching almost to 500 nm. Also, the molar absorption of the *cis*-P-P Re(I) complexes at around 380 nm are at least twice that for a similar tricarbonyl Re-3. A molar extinction coefficient of  $8800 \text{ M}^{-1} \text{ cm}^{-1}$  at 390 nm was reported in the case of *cis*-Re(CO)<sub>2</sub>(*c*-dppene)(phen)<sup>+</sup> [11], while it is around  $4500 \text{ M}^{-1} \text{ cm}^{-1}$  at 350 nm for a typical tricarbonyl Re(I) complex. The absorption and emission spectra of Re-2 are similar to those for Re-1 (results not shown). This suggests that the presence of a sulfonate group at the *para* position of the phenyl ring of dpphen has no effect on the MLCT transitions of the Re(I) complexes. Compared to the tricarbonyl Re(I) complex, the emission maxima of both the Re-1 and the Re-2 complexes are red-shifted by about 60 nm, to around 610 nm. Also, a significant portion of their emission is present beyond 700 nm. In fact these emission spectra more closely resemble that for Ru(II) polypyridyl complexes. From practical considerations, these *cis*-P-P Re(I) complexes can be easily excited with blue LED light sources with output in the 370- to 450-nm region. In contrast, it would be very difficult to excite Re-3 complex at wavelengths higher than 400 nm. These spectral shifts should make these *cis*-P-P Re(I) complexes a new useful series of probes for biochemical and biophysical applications.

The excitation anisotropies of both *cis*-P-P Re(I) complexes at  $-55^\circ\text{C}$  in a glycerol/DMF (7:3) mixture are presented in Fig. 2. We used DMF due to the nonpolar nature of the Re-1 complex. As the presence of DMF will lower the glycerol viscosity, it is conceivable that under these experimental conditions the molecular motions do not freeze completely and the absolute values for anisotropy may be somewhat higher. The anisotropy values for the MLCT band of both the complexes

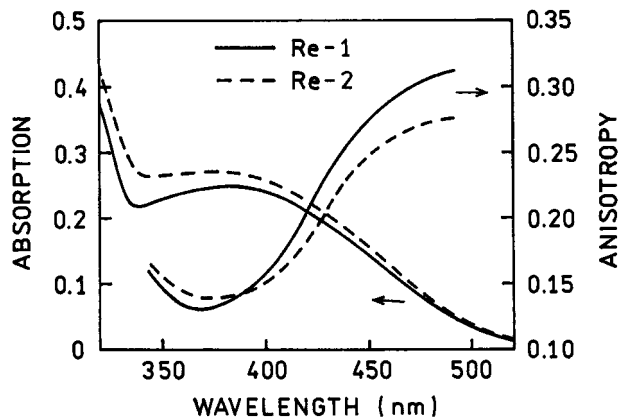


Fig. 2. Absorption and excitation anisotropy spectra of *cis*-P-P Re(I) complexes in glycerol:DMF (7:3) at  $-55^\circ\text{C}$ . The emission was observed at 600 nm.

increased gradually from about 0.13 around 350 nm to about 0.3 near 480 nm and beyond. These values are similar to those that have been observed with tricarbonyl Re complexes [10,13]. These results demonstrate that it is possible to obtain high anisotropy emission from the *cis*-P-P Re complexes with simple light sources such as LED emitting around 450 nm.

A summary of the QYs in the presence of oxygen and the luminescence lifetimes under several experimental conditions for both air-saturated and N<sub>2</sub>-purged samples is given in Table I. The QYs were less than 0.014 except for Re-2 in buffer in air-saturated samples and rather low for both complexes. We did not measure the QY in the absence of oxygen. However, from the extent of the increases seen in the lifetimes under nitrogen (see below), we expect these to be around 0.10–0.15. The value reported by Schutte *et al.* [11] is 0.12 in acetonitrile in

Table I. Room-Temperature Quantum Yields and Lifetimes of Re-1 and Re-2<sup>a</sup>

| Sample              | Re-1     |                   |                              | Re-2     |                   |                              |
|---------------------|----------|-------------------|------------------------------|----------|-------------------|------------------------------|
|                     | QY (air) | $\tau$ , air (ns) | $\tau$ , N <sub>2</sub> (ns) | QY (air) | $\tau$ , air (ns) | $\tau$ , N <sub>2</sub> (ns) |
| Hepes, pH 8         | ND       | ND                | ND                           | 0.032    | 4374              | ND                           |
| Hepes, 20% methanol | ND       | ND                | ND                           | 0.009    | 796               | ND                           |
| Hepes, 10 mM SDS    | 0.010    | 1,275             | 9,316                        | 0.011    | 1464              | 7,855                        |
| Acetonitrile        | 0.003    | 302               | 8,450                        | 0.003    | 343               | 7,836                        |
| Dichloromethane     | 0.014    | 972               | 8,864                        | 0.011    | 1114              | 10,550                       |
| Glycerol/DMF, 7:3   | ND       | 3,567             | ND                           | ND       | 3494              | ND                           |
| DPPC                | ND       | 2,206             | 7,501                        | ND       | ND                | ND                           |

<sup>a</sup> ND, not determined. Excitation was selected using a bandpass filter centered at 450 nm, and emission through a 605-nm-centered-bandpass filter. Ru(bpy)<sub>3</sub> was used as a reference for QY measurements.

the case of *cis*-Re(CO)<sub>2</sub>(*c*-dppene)(phen) in the absence of oxygen. The low QYs in the presence of oxygen are not surprising, as longer lifetimes are more susceptible to quenching by oxygen. The anomalously higher QY value seen in the case of the Re-2 complex in buffer may be due to aggregation, which shields the MLC from quenching by oxygen. The reason we suspect aggregation is the severe drop in intensity upon methanol or DMF addition, which will break up the aggregates. In fact we observed a concentration-dependent drop in the emission intensity for Re-2 upon the addition of up to 15% methanol or DMF (results not shown). It would seem that even after the introduction of highly charged/polar sulfonate groups on one side of the molecule, the presence of a highly hydrophobic bis-phosphine moiety on the other half of the molecule allows aggregation.

The intensity decays were essentially monoexponential, with the dominant component typically greater than 98% of the intensity. For the nonpolar Re-1, the room-temperature lifetimes were 300, 972, 1275, 2206, and 3567 ns in acetonitrile, dichloromethane, SDS micelles, glycerol/DMF mixture, and DPPC vesicles, respectively. Upon deoxygenation the lifetimes increased significantly and were between 7.5 and 9.3  $\mu$ s. For the more water-soluble Re-2, the room-temperature lifetimes in air-saturated samples were 343, 1114, 1464, and 3494 ns in acetonitrile, dichloromethane, SDS micelles, and a 7:3 glycerol/DMF mixture, respectively. Upon removal of oxygen the lifetimes increased to 7.9–10.5  $\mu$ s. Overall these results are similar to what was seen for Re-1. The presence of aggregation in aqueous solutions in the case of Re-2 is also suggested by the lifetime of 4.3  $\mu$ s, which decreased to 800 ns upon the addition of 20% methanol.

To summarize, we have synthesized a hydrophobic and a water-soluble Re(I) complex containing *cis*-bidentate phosphine along with either dpphen or SO<sub>3</sub>-dpphen. The complexes have lifetimes approaching 10  $\mu$ s in solution in the absence of oxygen. Other favorable properties are high anisotropy values, comparable to those seen in tricarbonyl Re(I) complexes, and significantly enhanced absorption beyond 400 nm. In fact both the absorption

and the emission begin to approach those seen in Ru(II) polypyridyl complexes. By converting sulfonate into sulfonyl chloride we can conjugate Re-2 MLCs with macromolecules. Similarly, it is also possible to make derivatives of phosphine which are more polar and capable of being converted into reactive conjugates using established chemistry [14].

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