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SYNTHESIS OF (PYRIDIN-2-YL)HYDRAZONE RHENIUM(I) TRICARBONYL COMPLEXES THAT EXHIBIT pH-SENSITIVE FLUORESCENCE

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There is great potential for novel diagnostic and therapeutic agents that combine multiple imaging modalities. The combination of optical and radionuclide imaging systems is particularly promising for the improved management of cancer. We describe a new heteroorganometallacyclic system containing the (2-pyridyl)hydrazono-Re(CO)$_3$ that exhibits pH-sensitive photophysical properties. This optical reporter group was easily incorporated into a novel synthetic probe for targeting estrogen receptors in cancer. The efficient complexation conditions are expected to be compatible with ^{99m}Tc-radiolabeling to obtain dual function imaging probes.

Keywords: chelate, estrogen, hydrazone, tricarbonylrhenium, fluorescence.

The presence of an organometallic component in a heterocyclic system contributes important structural characteristics and chemical reactivity, and many complexes exhibit interesting and useful physical, chemical, and spectroscopic properties. There is great interest in the development of multimodal imaging probes, for example identifying moieties able to combine optical and radionuclide-based imaging, particularly for medical applications such as cancer diagnosis and treatment [1, 2]. In pursuit of our interest in radiopharmaceuticals containing technetium and rhenium for diagnostic imaging and therapy of breast cancer, we have recently developed a series of water-stable 2-pyridylhydrazine chelates of Re(CO)$_3$ and the important isostructural γ-emitting radionuclide ^{99m}Tc(CO)$_3$ complexes for single photon emission computed tomography (SPECT) imaging [3-6]. While the pyridylhydrazine chelates we developed were not fluorescent, we recently discovered a promising new class of fluorescent dyes possessing a triazaborolopyridinium heterocyclic core derived from hydrazones [7]. These 2-hydrazinylpyridine dyes possess structural rigidity due to the 5-membered boron-containing ring that was essential for fluorescent emission. The corresponding hydrazones are non-emissive, and the planarity of the extended conjugated system enables structure-based variation of photophysical properties. In order to evaluate the potential for organometallic 2-pyridylhydrazones to function as optical imaging probes, we initiated this study, focusing on metallacyclic systems containing the [Re(CO)$_3$]$^+$ complex. We predicted that the d^6 electron configuration and octahedral geometry of the tricarbonylrhenium(I) metal center would form emissive complexes with coordinating hydrazone ligands. Herein we describe the synthesis and characterization

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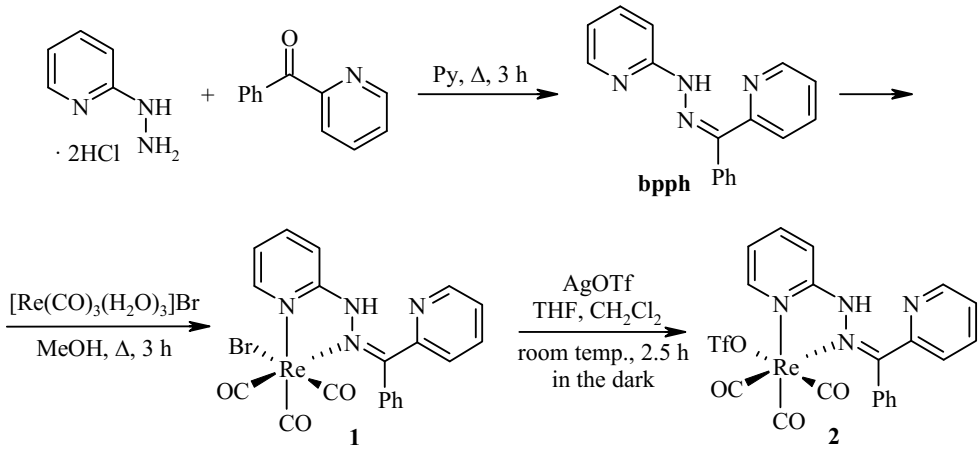
of a new class of (2-pyridyl)hydrazonotricarbonylrhenium(I) complexes that exhibit pH-dependent photophysical properties, and the incorporation of this moiety into a steroidal derivative designed for targeting estrogen receptors.

Hydrazones are effective chelates for complexation with a variety of metals such as Pd, Cu, In, and Zn, and many of these complexes exhibit fluorescent or luminescent properties that can be utilized for applications in molecular imaging. Bidentate polypyridine-type donor ligands such as 2,2'-bipyridine and 1,10-phenanthroline yield photoluminescent $Re(CO)_3$/$^{99m}Tc(CO)_3$ complexes [8-15]. We expected that bidentate (2-pyridyl)hydrazono ligands would form stable $Re(CO)_3$-complexes with potential for optical imaging applications.

The condensation of 2-benzoylpyridine with (2-pyridyl)hydrazine dihydrochloride in pyridine gave the desired hydrazone ligand 2-{(*Z*)-phenyl[2-(pyridin-2-yl)hydrazinylidene]methyl}pyridine, which we refer to as **bpph**. This compound was previously prepared using acid catalyzed conditions [16], and was also found to have antitumor activity [17]. The tricarbonylrhenium(I) aqua complex precursor $[Re(CO)_3(H_2O)_3]Br$ was prepared following the literature procedure [18]. The reaction between **bpph** and rhenium aqua complex in refluxing methanol provided orange complex *fac*-$Re(CO)_3$(bpph)Br (**1**). The yield and ease of purification of complex **1** was superior using $[Re(CO)_3(H_2O)_3]Br$ aqua complex compared with $(NEt_4)_2[ReBr_3(CO)_3]$ under these conditions [18-33].



In order to evaluate any potential effects of the halide ligand on the physicochemical and optical properties of the complexes, we required the synthesis of a complex analogous to compound **1** possessing a weaker coordinating monoanionic ligand. The corresponding triflate complex **2** was obtained by treating the bromide complex **1** with silver trifluoromethanesulfonate in a mixed solvent containing CH_2Cl_2 and THF at room temperature. Generally, bromide replacement reactions in $Re(CO)_3Br$ organometallic complexes require more forcing conditions [33]; however, this procedure was mild and resulted in near quantitative conversion of complex **1** to **2**. Aqueous solutions of both the bromide and triflate complexes were prepared by first dissolving the complexes in polar solvents such as methanol or ethanol, then diluting with water to ≥10% MeOH (EtOH) solution, in which the complexes remained soluble. Comparison of the ^1H and ^{13}C NMR spectra of complexes **1** and **2** revealed no significant differences associated with the different monoanionic ligands.

Crystals of complex **1** were obtained by slow evaporation of a saturated solution in ethanol at room temperature. Complex **2** was recrystallized by slow diffusion of diethyl ether vapor into a saturated solution in chloroform at -4°C. Single crystals were obtained for structure determination by X-ray crystallography. The structures of complexes **1**, **2** are shown in Figs. 1 and 2, respectively. The rhenium(I) center of both complexes adopted a slightly distorted octahedral geometry, with the π-acidic CO ligands arranged in a *facial* orientation. The 2-pyridyl group is nearly coplanar with the coordinated pyridyl moiety, allowing intramolecular H-bonding between the hydrazino NH proton and N atom in pendant *Z*-(2-pyridyl) group in both complexes **1** and **2**. The

nearly coplanar orientation of the pyridyl–hydrazone–pyridyl enables extended π-conjugation, providing thermodynamic stability for the complex. The phenyl groups were oriented orthogonally relative to the bidentate coordinated hydrazinopyridine cycle in both complexes.

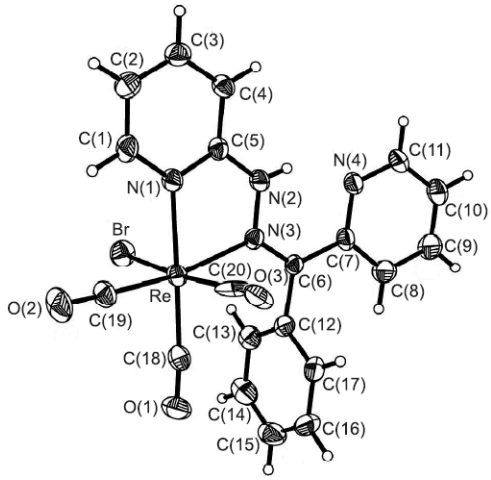


Fig. 1. X-ray ORTEP rendition of complex **1**. Thermal ellipsoids are shown at the 50% probability level.

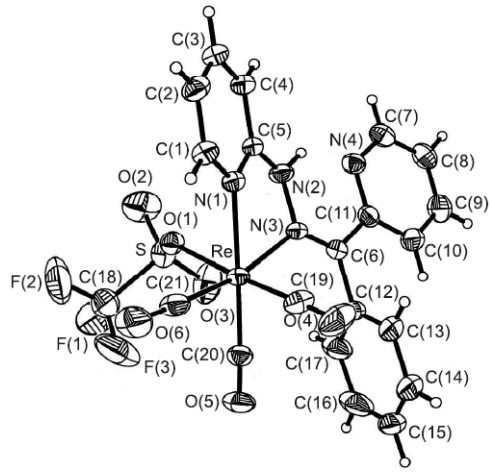


Fig. 2. X-ray ORTEP rendition of complex **2**. Thermal ellipsoids are shown at the 50% probability level.

The photophysical properties of complexes **1** and **2** were determined over a pH range between 4.50-8.75 (20 μM, 10% MeOH in phosphate buffered saline (PBS); pH 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 7.50, 8.00, 8.50, 8.75). The effect of pH on the absorption profiles of complexes **1** and **2** is shown in Figure 3. Both complexes demonstrated similar absorption spectral effects; the metal-to-ligand charge transfer absorption band at λ_{max} 373 nm exhibited a bathochromic shift to 451 nm with increasing pH. The extinction coefficients (ε) for complexes **1** and **2** at pH 4.5 (λ_{max} 373 nm) were $1.34 \cdot 10^4$ and $1.06 \cdot 10^4$, respectively. At pH 8.75 the λ_{max} value increases to 451 nm, and the corresponding values for ε of complexes **1** and **2** were $0.87 \cdot 10^4$ and $0.66 \cdot 10^4$. In both spectra, isobestic points were observed.

The emission spectra of complexes **1** and **2** were measured over the pH range between 4.50-8.75 with the excitation at λ_{ex} 451 nm. The samples were prepared in the same manner as described above, and emission signals were observed from 460 to 800 nm. The cumulative emission spectra of complexes **1** and **2** at pH 4.50-8.75 are shown in Figure 4. In both cases similar emission spectra were observed, the intensity increasing with the pH. The excitation of both complexes at the higher energy absorption frequency 373 nm did not produce observable emission over the range of pH tested (data not shown). The changes in fluorescent intensity at λ_{emi} 610 nm of complexes **1** and

2 with varying pH are shown in Fig. 5. Both curves fit the logarithmic equation $y = c / (1 + ae^{(-bx)})$, and the second derivative of the equation gives the inflection point. The inflection point corresponds to the pK_a of the excited state. The excited state pK_a values for complexes **1** and **2** are 6.96 and 6.83, respectively.

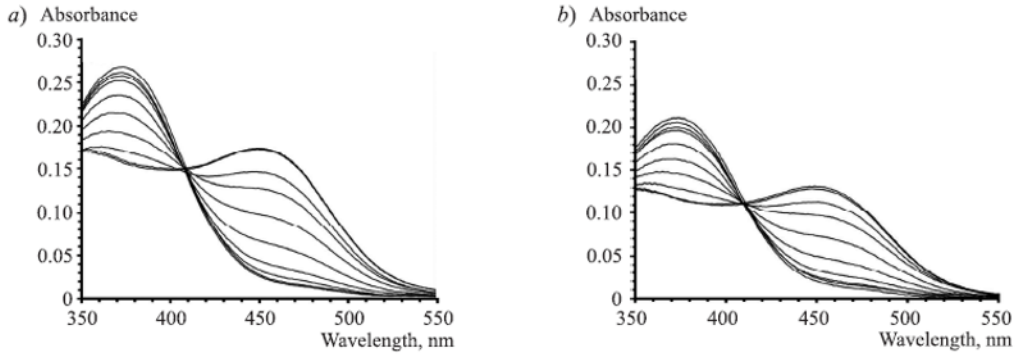


Fig. 3. Dependence of absorption spectra on pH. Enhanced absorption was observed when pH buffer was increased from 4.50 to 8.75 for complex **1** (*a*) and complex **2** (*b*). Different lines represent each specific pH buffer tested, with the maximum long wave length absorbance occurring at the highest pH.

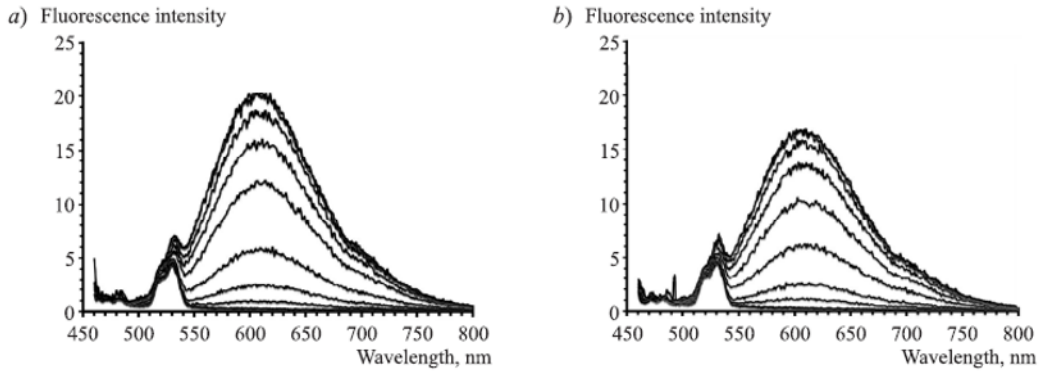


Fig. 4. Dependence of emission spectra on pH for complex **1** (*a*) and complex **2** (*b*) at 451 nm. Different lines represent each specific pH buffer tested (from 4.50 to 8.75), with the maximum long wavelength emission occurring at the highest pH.

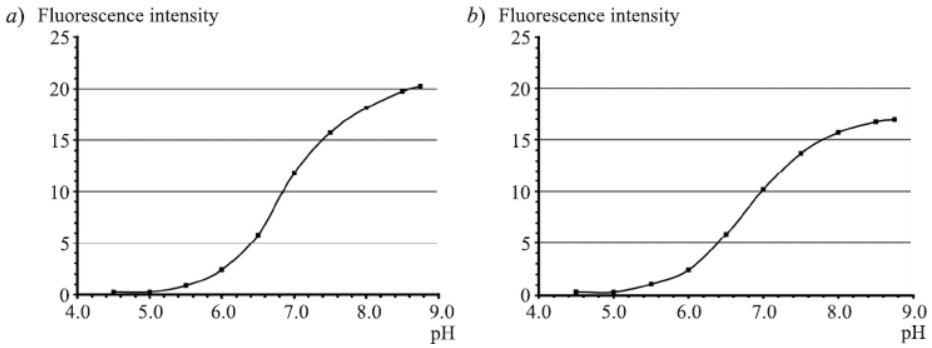
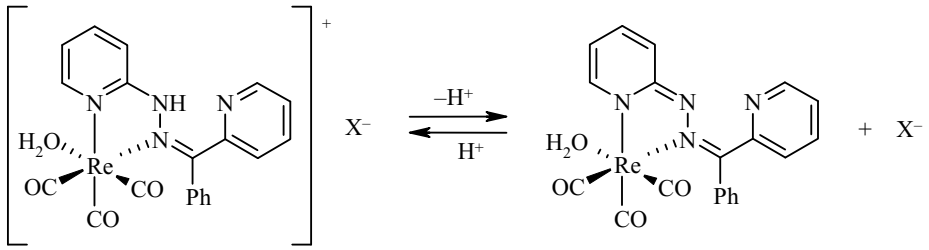


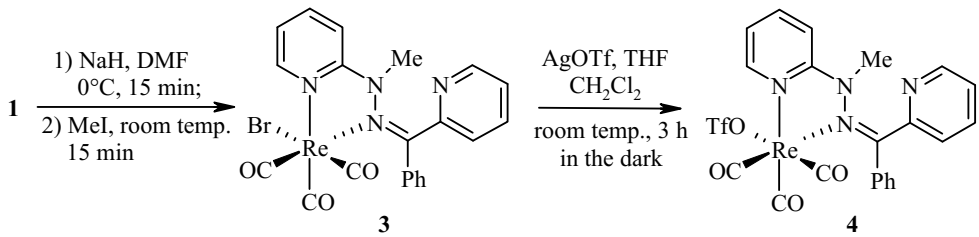
Fig. 5. Dependence of fluorescence emission intensity on pH. Excitation at 451 nm of complex **1** (*a*) and complex **2** (*b*) was measured, and the fluorescent intensity at λ_{emi} 610 nm was plotted against pH to produce the curves shown. Each data point on the curve corresponds to the emission intensity at that specific pH buffer value.

The similarity of the absorption and emission spectra for both complexes demonstrates that the identity of the anionic ligand (bromide/trifluoromethylsulfonate) has a minimal effect on the photophysical properties. Dissociative substitution of the anionic ligand in both complexes with water in aqueous media would produce identical cationic species as shown below. The observed pH dependence of the absorption/emission properties can be rationalized by focusing on the exchangeable hydrazono NH proton and the effect that removing this proton has on the extended conjugation of these complexes. The X-ray structures demonstrated the planarity of the N–H bond and the pendant *Z*-(2-pyridyl) cycle, which are consistent with the stabilization of this conformation through intramolecular H-bonding. At low pH these species absorb at higher energy (λ_{max} 373 nm) and are non-emissive. Deprotonation of the hydrazono NH group at higher pH results in extended conjugation that would shift the absorption to lower energy (λ_{max} 451 nm), and excitation at this wavelength then results in the observed emission. These results are consistent with the electron withdrawing capacity of the tricarbonylrhenium(I) core, and the experimentally determined p*K*a values for both complexes **1** and **2** are very similar (p*K*a 6.96 and 6.83).



X = Br, OTf

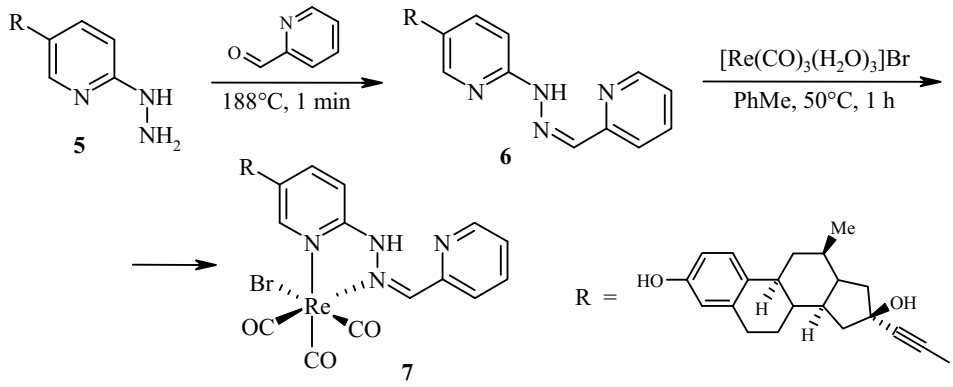
In order to further evaluate this model for the observed pH sensitivity we derivatized the hydrazono NH group of complex **1** by *N*-methylation. This modification was introduced in order to provide analogous complexes lacking an exchangeable NH proton for comparison. Deprotonation with sodium hydride in DMF, followed by *N*-methylation with iodomethane, gave complex **3**. The corresponding triflate complex **4** was synthesized by ligand exchange between the bromide complex **3** and silver triflate. The ^{1}H NMR signal of the introduced methyl group was observed at 3.15 ppm; it demonstrates that *N*-methylation occurred at the hydrazono NH position and not at the pendant pyridyl group (expected 4.2-4.5 ppm). The absorption spectra for the *N*-methylated complexes **3** and **4** exhibited λ_{max} 373 nm and were unaffected by changes in pH. The extinction coefficients of complexes **3** and **4** at λ_{max} 373 nm were 0.58(±0.01)·10^4 and 0.62(±0.01)·10^4, respectively. The *N*-methylated complexes **3** and **4** were not fluorescent. These results are consistent with the proposed model for emission that requires deprotonation of the hydrazono NH group, since the *N*-methylated complexes lack an exchangeable proton in that position.



3 **4**

In order to evaluate a potential biological probe incorporating the (2-pyridyl)hydrazono-Re(CO)$_3$ moiety, we synthesized a derivative of the potent natural estrogen 17β-estradiol. We have previously synthesized the starting hydrazine **5** [3], and considering the lipophilicity of the steroid scaffold, we selected picolinaldehyde rather than 2-benzoylpyridine for construction of the hydrazone, to minimize the overall hydrophobicity and maintain the aqueous solubility of the resulting imaging probe. The neat hydrazine **5** and picolinaldehyde were

heated under vacuum to produce the desired hydrazone **6**. The complexation of compound **6** with $Re(CO)_3^+$ ion was performed in toluene and provided complex **7** in good yield. The probe of complex **7** was characterized spectroscopically and exhibited an absorption coefficient ε 8650 $M^{-1}\cdot cm^{-1}$ for λ_{max} at the longest wavelength 420 nm in PBS at pH ≥ 7. Excitation of complex **7** at 420 nm results in an emission band at 576 nm (Fig. 6) with quantum yield Φ_f 0.01 and fluorescence lifetime 9.0 μs.



The probe of complex **7** was compared with the reported luminescent 1,10-phenanthroline–estradiol–$Re(CO)_3^+$ conjugate developed by Lo and co-workers [34]. This complex exhibited λ_{max} 370 nm in MeCN, with an absorption coefficient ε 3315. This complex produced an emission at 550 nm with quantum yield Φ_f 0.06 and fluorescent lifetime τ_o 0.93 μs in PBS. In comparison, complex **7** undergoes excitation at a longer wavelength; this is advantageous for biological applications, and although the observed quantum yield for complex **7** was lower, the longer fluorescence lifetime provides other potential advantages for molecular imaging applications.

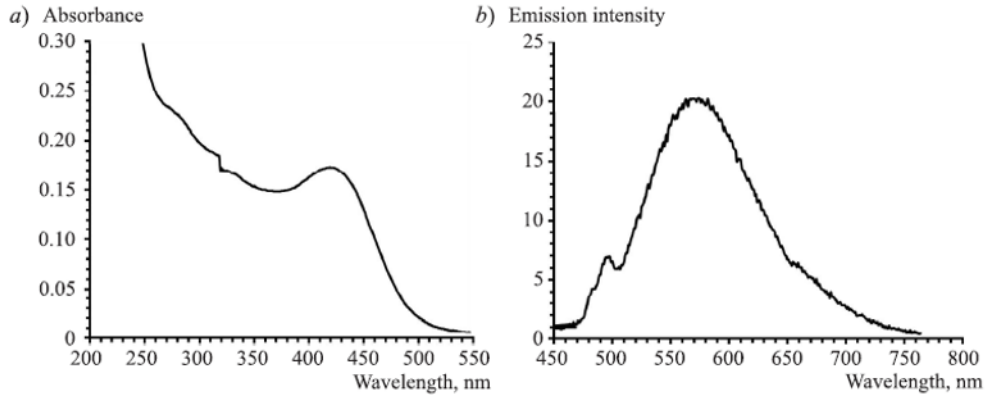


Fig. 6. Absorption (*a*) and emission (*b*) spectra of estrogen complex **7**. (Excitation of complex **7** was performed at 420 nm).

In conclusion, we have described the synthesis of a new heteroorganometallacyclic (2-pyridyl)-hydrazono-$Re(CO)_3$ system that exhibits pH sensitive absorption and fluorescence spectra. The key role of deprotonation of the hydrazono NH group in the bathochromic absorption shift and emission was demonstrated by comparison with *N*-methylated analogs that lack an exchangeable NH proton. The (2-pyridyl)hydrazono-$Re(CO)_3$ group can be readily incorporated into synthetic biological probes, as demonstrated here by the synthesis of the corresponding estrogen derivative. Continuing optimization of this new chelate system will include structural modifications seeking to enhance the long wavelength absorption and emission properties and increase the quantum yield, and initiating further studies to characterize the related ^{99m}Tc complexes as biological imaging probes.

EXPERIMENTAL

Absorption and emission spectra were recorded on a Cary 50 UV-Vis spectrophotometer and a Cary Eclipse fluorescence spectrophotometer, respectively, at room temperature. ^{1}H and ^{13}C NMR spectra of compound **6** were recorded on a Varian Unity 400 spectrometer (400 and 100 MHz, respectively) in acetone-d_6, internal standard TMS. ^{1}H, ^{13}C, and ^{19}F NMR spectra of the remaining compounds were recorded on a Varian Oxford EM-300 spectrometer (300 MHz for ^{1}H nuclei, 75 MHz for ^{13}C and ^{19}F nuclei) in DMSO-d_6 (^{13}C NMR spectrum of compound **1**), acetone-d_6 (compound **7**), and $CDCl_3$ (remaining compounds). Internal standards TMS (for ^{1}H and ^{13}C nuclei) and C_6F_6 (for ^{19}F nuclei). Analytical HPLC/mass spectra were recorded using a Waters 2695 system with a Waters 2996 Photodiode Array (PDA) and Micromass ZQ ESI-MS detection (cone voltage 62 V, capillary voltage 3 kV). The compound (1 mg/ml solution in MeCN, 20 μl) was injected into a Waters Symmetry® C_{18} 5 μm column (3.0×150 mm) eluted with 20-70% MeCN–H_2O. High-resolution mass spectra were obtained from the Mass Spectrometry Facility, University of California, Riverside. Elemental analyses were performed at Desert Analytics, Tucson. Melting points were obtained using an Electro Thermal MEL-TEMP instrument. 2-Benzoylpyridine was purchased from Acros Chemicals. 2-Hydrazinopyridine dihydrochloride and silver triflate were purchased from Aldrich. $Re(CO)_5Br$ was purchased from Strem Chemicals. The precursor, $[Re(CO)_3(H_2O)_3]Br$ was prepared from $Re(CO)_5Br$ by refluxing in water for 24 h [18]. All solvents used were of analytical grade purchased from Fisher Scientific. Toluene, CH_2Cl_2, DMF, and THF were dried and deoxygenated using a glass contour solvent system. All reactions were conducted in air at room temperature unless otherwise specified.

2-{(*Z*)-Phenyl[2-(pyridin-2-yl)hydrazinylidene]methyl}pyridine (bpph). (2-Pyridyl)hydrazine dihydrochloride (0.910 g, 5 mmol) and 2-benzoylpyridine (0.916 g, 5 mmol) were dissolved in pyridine (30 ml) and refluxed for 3 h. The solvent was removed under reduced pressure and the residue partitioned between EtOAc and saturated aq. $NaHCO_3$ solution. The organic layer was washed sequentially with H_2O and saturated aq. NaCl, dried over Na_2SO_4, filtered, and evaporated. Yield 1.18 g (86%), light-yellow crystals, mp 142-146°C (EtOH–H_2O) (mp 145-146°C [16]). IR spectrum, ν, cm^{-1}: 1594 (vs), 1436 (vs), 1137 (vs). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 13.10 (1H, s); 8.83 (1H, ddd, *J* = 4.9, *J* = 1.8, *J* = 0.9); 8.19 (1H, ddd, *J* = 5.0, *J* = 2.1, *J* = 0.9); 7.74 (1H, ddd, *J* = 7.8, *J* = 7.8, *J* = 1.9); 7.63-7.57 (3H, m); 7.50 (1H, ddd, *J* = 8.4, *J* = 1.0, *J* = 1.0); 7.45-7.28 (5H, m); 6.78 (1H, ddd, *J* = 7.0, *J* = 5.0, *J* = 1.2). ^{13}C NMR spectrum, δ, ppm: 157.2 (C); 153.4 (C); 148.4 (CH); 147.8 (CH); 139.8 (C); 138.9 (C); 137.9 (CH); 136.7 (CH); 128.8 (2CH); 128.3 (2CH); 128.1 (CH); 125.1 (CH); 122.9 (CH); 116.0 (CH); 107.6 (CH). Mass-spectrum, *m*/*z*: 275.17 $[M+H]^+$.

***fac*-Re(CO)$_3$(bpph)Br (1)**. A solution of $[Re(CO)_3(H_2O)_3]Br$ (0.304 g, 0.75 mmol) in MeOH (2 ml) was added to a soluion of hydrazone **bpph** (0.206 g, 0.75 mmol) in MeOH (10 ml) and refluxed for 3 h. The precipitate was filtered off, the filtrate was concentrated to provide a second crop, and the combined product was washed with a small portion of MeOH. Yield 0.327 g (70%), orange solid, mp 168-172°C (decomp.). IR spectrum, ν, cm^{-1}: 2022 (vs), 1914 (vs), 1888 (vs), 1618 (w). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 8.78 (1H, ddd, *J* = 3.7, *J* = 1.4, *J* = 0.7); 8.51 (1H, ddd, *J* = 4.4, *J* = 0.6, *J* = 0.3); 7.77 (1H, ddd, *J* = 6.2, *J* = 5.7, *J* = 1.4); 7.72 (1H, ddd, *J* = 6.5, *J* = 5.4, *J* = 1.3); 7.60-7.58 (5H, m); 7.47 (2H, ddd, *J* = 5.7, *J* = 3.7, *J* = 0.7); 7.07 (1H, ddd, *J* = 6.3, *J* = 0.7, *J* = 0.7); 6.88 (1H, ddd, *J* = 6.2, *J* = 0.7, *J* = 0.7); 6.86 (1H, ddd, *J* = 5.4, *J* = 4.4, *J* = 0.9). ^{13}C NMR spectrum, δ, ppm: 197.3 (CO); 192.1 (CO); 190.7 (CO); 155.8 (C); 151.5 (C); 151.1 (C); 149.3 (CH); 147.5 (CH); 140.3 (CH); 138.8 (CH); 138.7 (C); 130.3 (CH); 129.9 (CH); 129.2 (CH); 128.8 (2CH); 128.3 (CH); 125.9 (CH); 117.6 (CH); 109.9 (CH). Mass-spectrum, *m*/*z*: 625.05 $[M+H]^+$. Found, %: C 38.34; H 2.40; N 8.91. $C_{20}H_{14}BrN_4O_3Re$. Calculated, %: C 38.47; H 2.26; N 8.97.

***fac*-Re(CO)$_3$(bpph)OTf (2)**. A solution of AgOTf (0.039 g, 0.15 mmol) in dry THF (0.75 ml) was added dropwise to a solution of complex **1** (0.094 g, 0.15 mmol) in dry CH_2Cl_2 (7.50 ml). The reaction was stirred for 2.5 h in the dark at room temperature. The reaction was filtered through Celite to remove AgBr and concentrated under reduced pressure. The solid residue was dissolved in EtOAc and washed with H_2O. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Yield 0.103 g (99%),

orange solid, mp 165-170°C (decomp.). IR spectrum, ν, cm^{-1}: 2030 (vs), 1934 (vs), 1897 (vs). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 8.82 (1H, ddd, *J* = 4.8, *J* = 1.9, *J* = 0.9); 8.55 (1H, ddd, *J* = 5.9, *J* = 0.9, *J* = 0.6); 7.85-7.78 (2H, m); 7.67-7.58 (4H, m); 7.53 (2H, ddd, *J* = 7.7, *J* = 5.0, *J* = 1.2); 7.49 (1H, br. s); 7.10 (1H, ddd, *J* = 8.5, *J* = 0.9, *J* = 0.9); 6.98 (1H, ddd, *J* = 8.3, *J* = 0.9, *J* = 0.9); 6.92 (1H, ddd, *J* = 7.3, *J* = 6.0, *J* = 1.2). ^{13}C NMR spectrum, δ, ppm: 195.0 (CO); 192.7 (CO); 190.7 (CO); 156.7 (C); 152.2 (C); 151.4 (C); 150.4 (CH); 146.5 (CH); 140.5 (CH); 138.3 (CH); 138.1 (C); 130.9 (CH); 130.5 (CH); 130.1 (CH); 129.6 (CH); 129.5 (CH); 128.9 (CH); 125.8 (CH); 117.4 (CH); 109.2 (CH); 119.0 (q, *J* = 319, CF_3). ^{19}F NMR spectrum, δ, ppm: -78.1. Mass spectrum, *m*/*z*: 695.10 $[M+H]^+$. Found, %: C 36.20; H 2.21; N 7.82. $C_{21}H_{14}F_3N_4O_6ReS$. Calculated, %: C 36.36; H 2.03; N 8.08.

***fac*-Re(CO)$_3$(*N*-Me-bpph)Br (3)**. A solution of complex **1** (0.312 g, 0.5 mmol) in dry DMF (4 ml) was added dropwise to a suspension of NaH (0.018 g, 0.75 mmol) in dry DMF (1 ml) at 0°C and stirred for 15 min. Two portions of MeI (47 μl each portion, 1.5 mmol) were added over 1 h at 0°C, and the mixture was stirred at ambient temperature for 5 h. The reaction mixture was quenched by addition of deionized water, extracted with EtOAc, and washed with deionized water followed by saturated aq. NaCl; the organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (40 g), eluent EtOAc–hexane, 3:1. Yield 0.276 g (87%), bright-yellow solid, mp not determined. IR spectrum, ν, cm^{-1}: 2021 (vs), 1918 (vs), 1890 (vs). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 8.72 (1H, ddd, *J* = 4.8, *J* = 1.8, *J* = 1.0); 8.16 (1H, ddd, *J* = 6.2, *J* = 2.1, *J* = 0.9); 7.93 (1H, ddd, *J* = 7.9, *J* = 1.2, *J* = 1.2); 7.84 (1H, ddd, *J* = 7.6, *J* = 7.6, *J* = 1.7); 7.79 (1H, ddd, *J* = 8.7, *J* = 7.2, *J* = 1.8); 7.56-7.45 (5H, m); 7.36 (1H, ddd, *J* = 7.5, *J* = 4.8, *J* = 1.3); 6.95 (1H, ddd, *J* = 7.2, *J* = 5.9, *J* = 1.2); 6.70 (1H, ddd, *J* = 8.5, *J* = 0.9, *J* = 0.9); 3.11 (3H, s). ^{13}C NMR spectrum, δ, ppm: 196.3 (CO); 192.9 (CO); 191.5 (CO); 169.8 (C); 158.4 (C); 155.9 (C); 150.8 (CH); 150.7 (CH); 140.0 (C); 139.9 (CH); 137.2 (CH); 131.5 (CH); 130.6 (2CH); 128.9 (2CH); 125.4 (CH); 124.7 (CH); 117.9 (CH); 110.1 (CH); 47.1 (CH_3). Mass-spectrum, *m*/*z*: 639.06 $[M+H]^+$.

***fac*-Re(CO)$_3$(*N*-Me-bpph)OTf (4)**. A solution of AgOTf (0.051 g, 0.2 mmol) in dry THF (1 ml) was added to complex **3** (0.128 g, 0.2 mmol) in dry CH_2Cl_2 (5 ml) and stirred for 3 h in the dark at room temperature. The reaction mixture was filtered through Celite to remove AgBr, and the filtrate was concentrated under reduced pressure. Yield 0.117 g (82%), orange solid, mp not determined. IR spectrum, ν, cm^{-1}: 2034 (vs), 1928 (vs), 1915 (vs). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 8.69 (1H, d, *J* = 5.0); 8.66 (1H, ddd, *J* = 5.8, *J* = 1.8, *J* = 0.9); 7.95 (1H, ddd, *J* = 7.8, *J* = 7.8, *J* = 1.8); 7.90 (1H, ddd, *J* = 8.7, *J* = 7.2, *J* = 1.8); 7.73 (1H, d, *J* = 7.9); 7.62-7.46 (6H, m); 7.05 (1H, ddd, *J* = 7.6, *J* = 6.2, *J* = 1.2); 6.77 (1H, ddd, *J* = 8.5, *J* = 0.9, *J* = 0.9); 3.15 (3H, s). ^{13}C NMR spectrum, δ, ppm: 194.5 (CO); 193.3 (CO); 192.0 (CO); 168.9 (C); 158.5 (C); 154.8 (C); 151.7 (CH); 151.3 (CH); 141.2 (CH); 138.9 (C); 138.4 (CH); 132.4 (CH); 130.6 (2CH); 129.3 (2CH); 125.9 (CH); 125.5 (CH); 118.9 (q, *J* = 319, CF_3); 118.1 (CH); 110.2 (CH); 46.8 (CH_3). ^{19}F NMR spectrum, δ, ppm: -78.1. Mass spectrum, *m*/*z*: 709.23 $[M+H]^+$.

(8*R*,9*S*,13*S*,14*S*,17*S*)-13-Methyl-17-({6-[(2*Z*)-2-(pyridin-2-ylmethylidene)hydrazinyl]pyridin-3-yl}-ethynyl)-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthrene-3,17-diol (6). Compound **5** (0.161 g, 0.40 mmol) synthesized following an established procedure [3] and pyridine-2-carbaldehyde (0.428 g, 4.00 mmol) were mixed and fused at 188°C for 1 min. The solid residue was purified by silica gel column chromatography, eluent 0-2% of MeOH in CH_2Cl_2. Yield 0.154 g (78%), yellow solid, mp 182-184°C. IR spectrum, ν, cm^{-1}: 3430 (b), 1601 (vs), 1473 (vs). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 10.35 (1H, br. s); 8.54 (1H, ddd, *J* = 5.0, *J* = 1.8, *J* = 1.0); 8.19 (1H, dd, *J* = 2.2, *J* = 0.8); 8.15 (1H, s); 8.04 (1H, ddd, *J* = 8.0, *J* = 1.0, *J* = 1.0); 7.94 (1H, br. s); 7.79 (1H, ddd, *J* = 7.7, *J* = 7.7, *J* = 1.8); 7.63 (1H, dd, *J* = 8.7, *J* = 2.3); 7.38 (1H, dd, *J* = 8.7, *J* = 0.8); 7.28 (1H, ddd, *J* = 7.4, *J* = 4.9, *J* = 1.2); 7.11 (1H, d, *J* = 8.5); 6.59 (1H, dd, *J* = 8.5, *J* = 2.6); 6.52 (1H, d, *J* = 2.6); 2.86 (1H, br. s); 2.82-2.71 (2H, m); 2.40-2.28 (2H, m); 2.19 (1H, dt, *J* = 11.0, *J* = 3.8); 2.12-2.06 (1H, m); 2.01 (1H, dt, *J* = 13.0, *J* = 4.3); 1.90-1.79 (4H, m); 1.50-1.27 (4H, m); 0.94 (3H, s). ^{13}C NMR spectrum, δ, ppm: 156.7 (C); 155.9 (C); 155.4 (CH); 151.6 (CH); 150.2 (CH); 141.6 (C); 141.2 (C); 138.4 (CH); 137.1 (C); 131.9 (CH); 127.1 (CH); 123.9 (CH); 120.0 (CH); 115.9 (CH); 113.6 (CH); 112.7 (C); 107.2 (C); 95.9 (C); 83.0 (C); 80.2 (CH_2); 50.7 (CH); 48.4 (CH); 44.6 (CH_2); 40.6 ($2CH_2$); 40.0 (C); 34.0 (CH);

28.2 (CH_2); 27.4 (CH_2); 23.6 (CH_2); 13.4 (CH_3). Found, *m*/*z*: 493.2600 $[M+H]^+$. $C_{31}H_{33}N_4O_2$. Calculated, *m*/*z*: 493.2598 $[M+H]^+$.

***fac*-Re(CO)$_3$[6]Br (7)**. The ligand **6** (49.3 mg, 0.10 mmol) and $[Re(CO)_3(H_2O)_3]Br$ (81 mg, 0.20 mmol) were dissolved in abs. PhMe (13 ml). The mixture was heated at 50°C for 1 h. The solid residue was washed with a minimum amount of hot PhMe followed by deionized water. Yield 0.062 g (74%), orange solid, mp 191-193°C (decomp.). IR spectrum, ν, cm^{-1}: 3430 (b), 2022 (vs), 1909 (vs), 1605 (w). ^1H NMR spectrum, δ, ppm (*J*, Hz): 8.69 (1H, d, *J* = 5.1); 8.25 (1H, d, *J* = 1.5); 8.23 (1H, br. s); 8.14 (1H, br. s); 8.12 (1H, dt, *J* = 7.9, *J* = 1.5); 7.76 (1H, dd, *J* = 8.7, *J* = 2.2); 7.56 (1H, ddd, *J* = 5.7, *J* = 5.7, *J* = 2.6); 7.42 (1H, d, *J* = 8.6); 7.24-7.09 (3H, m); 6.59 (1H, dd, *J* = 8.3, *J* = 2.6); 6.52 (1H, d, *J* = 2.2); 2.85-2.68 (2H, m); 2.39-2.33 (2H, m); 2.22-2.09 (2H, m); 2.00-1.76 (5H, m); 1.53-1.23 (5H, m); 0.94 (3H, s). ^{13}C NMR spectrum, δ, ppm: 197.7 (CO); 196.4 (CO); 188.4 (CO); 156.6 (C); 155.8 (CH); 153.5 (C); 152.6 (C); 141.7 (CH); 141.2 (CH); 140.4 (CH); 138.4 (C); 131.9 (CH); 129.7 (C); 129.0 (CH); 128.1 (CH); 127.6 (CH); 127.0 (CH); 126.1 (CH); 115.9 (CH); 113.6 (C); 97.6 (C); 82.2 (C); 80.2 (C); 50.7 (2CH); 48.4 (CH_2); 44.6 (CH); 40.6 (C); 39.9 (CH_2); 28.1 (CH_2); 27.4 (CH_2); 23.6 (CH_2); 21.4 (CH_2); 13.4 (CH_3). Mass-spectrum, *m*/*z*: 843.43 $[M+H]^+$.

X-ray Structural Study of Complexes 1 and 2. Structure determination for complexes **1** and **2** was performed on a Bruker X8 Apex2 CCD-based X-ray diffractometer equipped with an Oxford Cryostream 700 low-temperature device and normal focus Mo-target X-ray tube (λ 0.71073 Å) operating at 1500 W power (50 kV, 30 mA) at 223(2) K. The refinement was performed with the method of full-matrix least squares on F^2 using the SHELXL-97 program [35]. Crystals of complex **1** ($C_{20}H_{14}BrN_4O_3Re$, *M* 624.46) are monoclinic, unit cell dimension: *a* 11.0340(5), *b* 15.8582(6), *c* 11.9476(5) Å; α 90.0000, β 108.7900(10), γ 90.0000°; space group *P*2(1)/*n*; *V* 1979.17(14) $Å^3$; *Z* 4; d_{calc} 2.096 g/cm^3; ε 8.188 mm^{-1}; *F*(000) 1184; *R* 0.0427 for 7588 independent reflections. The intensities of 7835 independent reflections were measured to $2\theta_{max}$ 66.39°. Crystals of complex **2** ($C_{21}H_{14}F_3N_4O_6ReS$, *M* 693.63) are monoclinic, unit cell dimension: *a* 11.0133(2), *b* 11.7028(3) Å, *c* 18.3260(4) Å; α 90.0000, β 100.8140(10), γ 90.0000°; space group *P*2(1)/*n*; *V* 2320.03(9) $Å^3$; *Z* 4, d_{calc} 1.986 mg/m^3; ε 5.398 mm^{-1}, *F*(000) 1336; *R* 0.0351 for 8913 independent reflections. The intensities of 9286 independent reflections were measured to $2\theta_{max}$ 66.50°. Crystallographic data for complexes **1** and **2** have been deposited at the Cambridge Crystallographic Data Center (deposits CCDC 983500 and CCDC 983501, respectively).

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