Luminescent Tricarbonylrhenium(I) Polypyridine Estradiol Conjugates: Synthesis, Crystal Structure, and Photophysical, Electrochemical, and Protein-Binding Properties

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A series of luminescent tricarbonylrhenium(I) polypyridine estradiol complexes $[Re(N-N)(CO)_3(L)](CF_3SO_3)$ (N−N = 1,10-phenanthroline (phen), L = 4-(17α-ethynylestradiolyl)pyridine (py-est) (**1a**), 4-(*N*-(6-(4-(17α-ethynylestradiolyl)benzoylamino)hexanoyl)aminomethyl)pyridine (py-C6-est) (**1b**); N−N = 3,4,7,8-tetramethyl-1,10-phenanthroline (Me_4-phen), L = py-est (**2a**), py-C6-est (**2b**); N−N = 4,7-diphenyl-1,10-phenanthroline (Ph_2-phen), L = py-est (**3a**), py-C6-est (**3b**)) have been synthesized and characterized. The X-ray crystal structure of complex **1a** has also been investigated. The photophysical and electrochemical properties of all the complexes have been studied. Upon irradiation, most of the complexes exhibited intense and long-lived triplet metal-to-ligand charge-transfer (^{3}MLCT) (dπ(Re) → π^*(N−N)) emission in fluid solutions at 298 K and in low-temperature glass. The excited states of the Me_4-phen complexes possessed some triplet intraligand ^{3}IL character. The lipophilicity of all the complexes has been determined by reversed-phase HPLC. In addition, the binding of these complexes to estrogen receptor α has been investigated by emission titrations.

Introduction

The role of estrogen receptors in hormone-dependent breast cancer is the focus of many clinical studies because the receptor content gives the most accurate index of the cancer.[1] Estrogen receptors are considered to be target proteins of a number of compounds known as endocrine disruptors, some of which are environmental pollutants and hazardous to humans and animals.[2] The design of probes for these proteins has relied mainly on radioactive estrogen derivatives such as those containing tritium[3] and technetium[4,5] because of their high detection sensitivity. Estrogen molecules labeled with biotin,[6,7] organometallics,[4,5,8−10] transition metal complexes,[11,12] and organic fluorophores[13−15] have also been reported. However, the possibility of using luminescent organotransition metal complexes as probes for estrogen receptors has not been explored. This, together with our recent interest in employing luminescent organotransition metal complexes as biological labels and probes,[16] has prompted us to functionalize a potent estrogen, 17α-ethynylestradiol, with a pyridine ring and coordinate this ligand to luminescent tricarbonylrhenium(I) diimine moieties to give new estradiol conjugates.

This paper describes the synthesis, characterization, X-ray crystal structure, and photophysical and electrochemical properties of a series of luminescent tricarbonylrhenium(I) polypyridine

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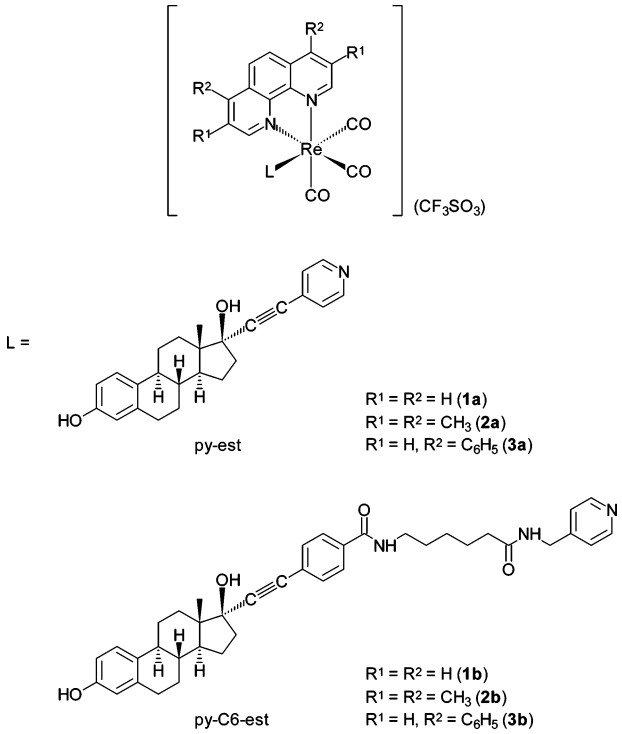
Chart 1. Structures of Tricarbonylrhenium(I) Polypyridine Estradiol Complexes



estradiol complexes $[Re(N-N)(CO)_3(L)](CF_3SO_3)$ (N−N = 1,10-phenanthroline (phen), L = 4-(17α-ethynylestradiolyl)-pyridine (py-est) (**1a**), 4-(*N*-(6-(4-(17α-ethynylestradiolyl)-benzoylamino)hexanoyl)aminomethyl)pyridine (py-C6-est) (**1b**); N−N = 3,4,7,8-tetramethyl-1,10-phenanthroline (Me_4-phen), L = py-est (**2a**), py-C6-est (**2b**); N−N = 4,7-diphenyl-1,10-phenanthroline (Ph_2-phen), L = py-est (**3a**), py-C6-est (**3b**)) (Chart 1). The lipophilicity and estrogen receptor-binding properties of these complexes have also been examined.

Experimental Section

Materials and Reagents. All solvents were of analytical grade. Bromopentacarbonylrhenium(I), diimine ligands, standards for lipophilicity measurements, 1-octanol, 17α-ethynylestradiol, and triphenylphosphine were purchased from Aldrich and used without purification. 6-Aminocaproic acid, *N*-hydroxysuccinimide, *N*,*N*′-dicyclohexylcarbodiimide, 4-iodobenzoic acid, 3-morpholinopropanesulfonic acid (MOPS), palladium(II) chloride, 2-mercaptoethanol, copper(I) iodide, and 4-bromopyridine hydrochloride were purchased from Acros and used without purification. Diethylamine (Sigma) was freshly distilled over KOH under nitrogen before use. Autoclaved Milli-Q water was used for the preparation of the aqueous solutions. Lamb uteri cytosol was used as the source of estrogen receptor α (ERα). The organ tissues obtained from the HKSAR slaughterhouse in Sheung Shui, New Territories, Hong Kong, were frozen immediately after isolation and stored at −70 °C prior to purification. ERα was purified and quantitated according to reported procedures.[10,17]

4-(17α-Ethynylestradiolyl)pyridine, py-est. 17α-Ethynylestradiol (469 mg, 1.58 mmol) was added as a solid to a solution of 4-bromopyridine hydrochloride (305 mg, 1.58 mmol), $Pd(PPh_3)_2Cl_2$ (22 mg, 2 mol %), and CuI (12 mg, 4 mol %) in 25 mL of diethylamine under an inert atmosphere of nitrogen. The solution was stirred at room temperature. A white solid was formed after ca. 10 min. The mixture was then refluxed for 12 h. The solid was then removed by filtration, and the filtrate was evaporated to dryness to give a yellow solid, which was washed with a small amount of cold CH_2Cl_2. Recrystallization of the crude product from acetone/diethyl ether afforded py-est as pale yellow crystals. Yield: 278 mg (47%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 8.56 (d, 2H, J = 4.1 Hz; H2 and H6 of pyridine), 8.05 (s, 1H; 3-OH of estradiol), 7.37 (d, 2H, J = 4.7 Hz; H3 and H5 of pyridine), 7.12 (d, 1H, J = 8.5 Hz; H1 of estradiol), 6.60 (d, 1H, J = 8.5 Hz; H2 of estradiol), 6.54 (s, 1H; H4 of estradiol), 4.73 (s, 1H; 17-OH of estradiol), 2.82−2.76 (m, 2H; H6 of estradiol), 2.40−2.14 (m, 4H; H9α, H11α, H12β, and H16α of estradiol), 2.00−1.79 (m, 5H; H7β, H8β, H11β, H15α, and H16β of estradiol), 1.56−1.27 (m, 4H; H7α, H12α, H14α, and H15β of estradiol), 0.96 (s, 3H; CH_3 of estradiol). IR (KBr) ν/cm^{-1}: 3467 (s, OH), 2925 (s, CH), 2868 (s, CH), 2223 (w, C≡C). Positive-ion ESI-MS: m/z = 374 $[M]^+$.

6-(4-Iodobenzoylamino)hexanoic Acid *N*-Hydroxysuccinimidyl Ester. 6-(4-Iodobenzoylamino)hexanoic acid[18] (563 mg, 1.56 mmol) was dissolved in 4 mL of hot *N*-methyl-2-pyrrolidone under an inert atmosphere of nitrogen. After the solution was cooled to room temperature, a mixture of *N*-hydroxysuccinimide (219 mg, 1.90 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (360 mg, 1.75 mmol) in 2 mL of *N*-methyl-2-pyrrolidone was added. The suspension was stirred under nitrogen at room temperature for 12 h. After the white precipitate (dicyclohexylurea) was removed by filtration, the product was precipitated by addition of 500 mL of a mixture of petroleum ether and diethyl ether (7/3 v/v) and then collected by filtration. The white solid was washed with diethyl ether and dried in vacuo. Yield: 479 mg (67%). IR (KBr) ν/cm^{-1}: 3344 (m, NH), 2929 (m, CH), 2863 (s, CH), 1623 (w, C═O). Positive-ion ESI-MS: m/z = 459 $[M]^+$.

4-(*N*-(6-(4-Iodobenzoylamino)hexanoyl)aminomethyl)-pyridine. A mixture of 4-aminomethylpyridine (136 mg, 1.26 mmol) and triethylamine (0.59 mL, 4.17 mmol) in 2 mL of DMF was added to a solution of 6-(4-iodobenzoylamino)hexanoic acid *N*-hydroxysuccinimidyl ester (479 mg, 1.04 mmol) in 10 mL of DMF. The mixture was stirred under an inert atmosphere of nitrogen at room temperature for 12 h. The solution was then evaporated to dryness under reduced pressure to give a yellow solid. The crude product was purified by column chromatography on silica gel. The desired product was eluted with acetone. Yield: 398 mg (84%). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K, TMS): δ 8.51−8.41 (m, 4H; H2 and H6 of pyridine, py-CH_2NH, and py-CH_2-$NHCOC_5H_{10}NH$), 7.82 (d, 2H, J = 8.5 Hz; H2 and H6 of phenyl ring), 7.60 (d, 2H, J = 8.5 Hz; H3 and H5 of phenyl ring), 7.20 (d, 2H, J = 6.2 Hz; H3 and H5 of pyridine), 4.25 (d, 2H, J = 5.9 Hz; py-CH_2NH), 3.24−3.20 (m, 2H; $CH_2NHCOC_6H_4$−I), 2.16 (t, 2H, J = 7.0 Hz; py-$CH_2NHCOCH_2$), 1.29−1.27 (m, 6H; py-CH_2-$NHCOCH_2C_3H_6CH_2$). IR (KBr) ν/cm^{-1}: 3318 (m, NH), 3252 (m, OH), 3067 (m, CH), 2929 (m, CH_3), 2847 (m, CH_3). Positive-ion ESI-MS: m/z = 451 $[M]^+$.

4-(*N*-(6-(4-(17α-Ethynylestradiolyl)benzoylamino)hexanoyl)-aminomethyl)pyridine, py-C6-est. 17α-Ethynylestradiol (306 mg, 1.04 mmol) was added as a solid to a solution of 4-(*N*-(6-(4-iodobenzoylamino)hexanoyl)aminomethyl)pyridine (444 mg, 0.94 mmol), $Pd(PPh_3)_2Cl_2$ (26 mg, 4 mol %), and CuI (14 mg, 8 mol %) in 30 mL of DMF/diethylamine (5/1 v/v) under an inert atmosphere of nitrogen. The solution was stirred at room temperature. A white solid was formed after ca. 10 min. The mixture was then heated at 60 °C for 12 h. The solid was then removed by filtration, and the filtrate was evaporated to dryness under reduced pressure to give a yellow solid, which was washed with a small amount of MeOH. Recrystallization of the crude product from hot MeOH afforded py-C6-est as pale yellow crystals. Yield: 390 mg (67%). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K, TMS): δ 9.00 (s, 1H; 3-OH of estradiol), 8.52 (t, 1H, J = 5.3 Hz; py-CH_2NH), 8.48−8.43 (m, 3H; py-$CH_2NHCOC_5H_{10}NH$ and H2 and H6 of pyridine),

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7.81 (d, 2H, $J = 8.2$ Hz; H2 and H6 of phenyl ring), 7.47 (d, 2H, $J = 7.9$ Hz; H3 and H5 of phenyl ring), 7.29 (s, br, 2H; H3 and H5 of pyridine), 7.05 (d, 1H, $J = 8.2$ Hz; H1 of estradiol), 6.49 (d, 1H, $J = 7.9$ Hz; H2 of estradiol), 6.42 (s, 1H; H4 of estradiol), 5.53 (s, 1H; 17-OH of estradiol), 4.20 (s, 2H; py-CH_2NH), 3.27−3.21 (m, 2H; CH_2NHCO−C_6H_4), 2.71−2.65 (m, 2H; H6 of estradiol), 2.25−2.07 (m, 6H; py-CH_2NHCOCH_2 and H9$_\alpha$, H11$_\alpha$, H12$_\beta$, and H16$_\alpha$ of estradiol), 1.94−1.67 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, and H16$_\beta$ of estradiol), 1.52−1.13 (m, 10H; py-CH_2-NHCOC$H_2C_3H_6CH_2$ and H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, and H15$_\beta$ of estradiol), 0.79 (s, 3H; CH_3 of estradiol). IR (KBr) ν/cm^{-1}: 3257 (s, OH), 2925 (s, CH), 2847 (m, CH), 2269 (w, C≡C). Positive-ion ESI-MS: $m/z = 620$ [M]$^+$.

Synthesis of the Tricarbonylrhenium(I) Complexes. A mixture of [Re(N−N)(CO)$_3$(CH_3CN)](CF_3SO_3)[19] (0.23 mmol) and an equimolar amount of py-est or py-C6-est in anhydrous THF was refluxed under nitrogen in the dark for 12 h. The mixture was evaporated to dryness to give a yellow solid. The complex was then purified by column chromatography on alumina. The desired product was eluted with acetone/methanol (3/1 v/v). Recrystallization of the crude product from acetonitrile/diethyl ether or methanol/diethyl ether afforded the complex as yellow crystals.

[Re(phen)(CO)$_3$(py-est)](CF_3SO_3) (1a). Yield: 104 mg (46%). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K, TMS): δ 9.76 (d, 2H, $J =$ 4.4 Hz; H2 and H9 of phen), 9.07−9.05 (m, 3H; H4 and H7 of phen and 3-OH of estradiol), 8.39 (d, 2H, $J = 6.7$ Hz; H2 and H6 of pyridine), 8.33 (s, 2H; H5 and H6 of phen), 8.26 (dd, 2H, $J =$ 8.5 and 5.0 Hz; H3 and H8 of phen), 7.28 (d, 2H, $J = 6.5$ Hz; H3 and H5 of pyridine), 7.00 (d, 1H, $J = 8.5$ Hz; H1 of estradiol), 6.48 (d, 1H, $J = 8.5$ Hz; H2 of estradiol), 6.47 (s, 1H; H4 of estradiol), 5.66 (s, 1H; 17-OH of estradiol), 2.74−2.66 (m, 2H; H6 of estradiol), 2.29−1.89 (m, 4H; H9$_\alpha$, H11$_\alpha$, H12$_\beta$, and H16$_\alpha$ of estradiol), 1.89−1.49 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, and H16$_\beta$ of estradiol), 1.49−1.10 (m, 4H; H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, and H15$_\beta$ of estradiol), 0.75 (s, 3H; CH_3 of estradiol). Anal. Calcd for $C_{41}H_{35}N_3F_3O_8SRe\cdot CH_3CN\cdot H_2O$: C, 50.04; H, 3.90; N, 5.43. Found: C, 50.09; H, 4.05; N, 5.46. IR (KBr) ν/cm^{-1}: 3447 (m, OH), 2935 (w, CH), 2863 (w, CH), 2213 (w, C≡C), 2034 (s, C≡O), 1911 (s, C≡O), 1153 (m, $CF_3SO_3^-$), 1024 (m, $CF_3SO_3^-$). Positive-ion ESI-MS: $m/z = 824$ [M − $CF_3SO_3^-$]$^+$.

[Re(phen)(CO)$_3$(py-C6-est)](CF_3SO_3) (1b). Yield: 98 mg (35%). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K, TMS): δ 9.73 (d, 2H, $J = 3.8$ Hz; H2 and H9 of phen), 9.02−8.99 (m, 3H; H4 and H7 of phen and 3-OH of estradiol), 8.49 (t, 1H, $J = 5.3$ Hz; py-CH_2N*H*), 8.36 (d, 2H, $J = 6.5$ Hz; H2 and H6 of pyridine), 8.28 (s, 2H; H5 and H6 of phen), 8.24−8.19 (m, 3H; H3 and H8 of phen and py-CH_2NHCOC$_5$H$_{10}$N*H*), 7.80 (d, 2H, $J = 8.5$ Hz; H2 and H6 of phenyl ring), 7.47 (d, 2H, $J = 8.2$ Hz; H3 and H5 of phenyl ring), 7.09 (d, 2H, $J = 6.7$ Hz; H3 and H5 of pyridine), 7.05 (d, 1H, $J = 9.4$ Hz; H1 of estradiol), 6.48 (d, 1H, $J = 8.5$ Hz; H2 of estradiol), 6.40 (s, 1H; H4 of estradiol), 5.52 (s, 1H; 17-OH of estradiol), 4.09 (d, 2H, $J = 5.3$ Hz; py-CH_2NH), 3.20−3.14 (m, 2H; CH_2NHCO−C_6H_4), 2.73−2.65 (m, 2H; H6 of estradiol), 2.42−2.02 (m, 6H; py-CH_2NHCOCH_2 and H9$_\alpha$, H11$_\alpha$, H12$_\beta$, and H16$_\alpha$ of estradiol), 1.95−1.75 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, and H16$_\beta$ of estradiol), 1.46−1.05 (m, 10H; py-CH_2NHCOC$H_2C_3H_6CH_2$ and H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, and H15$_\beta$ of estradiol), 0.80 (s, 3H; CH_3 of estradiol). Anal. Calcd for $C_{55}H_{53}N_5F_3O_{10}SRe\cdot CH_3CN$: C, 54.32; H, 4.48; N, 6.67. Found: C, 54.46; H, 4.72; N, 6.94. IR (KBr) ν/cm^{-1}: 3349 (br, OH), 2914 (m, CH), 2852 (m, CH), 2038 (s, C≡O), 1920 (s, C≡O), 1168 (m, $CF_3SO_3^-$), 1029 (m, $CF_3SO_3^-$). Positive-ion ESI-MS: $m/z = 1070$ [M − $CF_3SO_3^-$]$^+$.

[Re(Me$_4$-phen)(CO)$_3$(py-est)](CF_3SO_3) (2a). Yield: 105 mg (43%). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K, TMS): δ 9.46 (s, 2H; H2 and H9 of Me$_4$-phen), 9.04 (s, 1H; 3-OH of estradiol), 8.48 (d, 2H, $J = 6.7$ Hz; H2 and H6 of pyridine), 8.40 (s, 2H; H5 and H6 of Me$_4$-phen), 7.28 (d, 2H, $J = 6.7$ Hz; H3 and H5 of pyridine), 6.98 (d, 1H, $J = 8.2$ Hz; H1 of estradiol), 6.48 (d, 1H, $J = 8.5$ Hz; H2 of estradiol), 6.41 (s, 1H; H4 of estradiol), 5.63 (s, 1H; 17-OH of estradiol), 2.83 (s, 6H; CH_3 at C4 and C7 of Me$_4$-phen), 2.74−2.60 (m, 8H; CH_3 at C3 and C8 of Me$_4$-phen and H6 of estradiol), 2.27−1.88 (m, 4H; H9$_\alpha$, H11$_\alpha$, H12$_\beta$, and H16$_\alpha$ of estradiol), 1.88−1.42 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, and H16$_\beta$ of estradiol), 1.42−1.06 (m, 4H; H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, and H15$_\beta$ of estradiol), 0.74 (s, 3H; CH_3 of estradiol). Anal. Calcd for $C_{45}H_{43}N_3F_3O_8SRe\cdot CH_3CN$: C, 52.75; H, 4.33; N, 5.24. Found: C, 52.82; H, 4.49; N, 5.06%. IR (KBr) ν/cm^{-1}: 3421 (br, OH), 2930 (w, CH), 2863 (w, CH), 2217 (w, C≡C), 2034 (s, C≡O), 1916 (s, C≡O), 1158 (m, $CF_3SO_3^-$), 1030 (m, $CF_3SO_3^-$). Positive-ion ESI-MS: $m/z = 881$ [M − $CF_3SO_3^-$]$^+$.

[Re(Me$_4$-phen)(CO)$_3$(py-C6-est)](CF_3SO_3) (2b). Yield: 105 mg (36%). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K, TMS): δ 9.47 (s, 2H; H2 and H9 of Me$_4$-phen), 9.01 (s, 1H; 3-OH of estradiol), 8.49−8.48 (m, 3H; H2 and H6 of pyridine and py-CH_2N*H*), 8.36 (s, 2H; H5 and H6 of Me$_4$-phen), 8.32 (t, 1H, $J = 6.1$ Hz; py-CH_2NHCOC$_5$H$_{10}$N*H*), 7.79 (d, 2H, $J = 8.5$ Hz; H2 and H6 of phenyl ring), 7.47 (d, 2H, $J = 8.5$ Hz; H3 and H5 of phenyl ring), 7.11 (d, 2H, $J = 6.7$ Hz; H3 and H5 of pyridine), 7.05 (d, 1H, $J =$ 9.1 Hz; H1 of estradiol), 6.49 (d, 1H, $J = 8.5$ Hz; H2 of estradiol), 6.40 (s, 1H; H4 of estradiol), 5.53 (s, 1H; 17-OH of estradiol), 4.10 (d, 2H, $J = 4.4$ Hz; py-CH_2NH), 3.21−3.12 (m, 2H; CH_2-NHCO−C_6H_4), 2.79 (s, 6H; CH_3 at C4 and C7 of Me$_4$-phen), 2.74−2.59 (m, 8H; CH_3 at C3 and C8 of Me$_4$-phen and H6 of estradiol), 2.25−2.05 (m, 6H; py-CH_2NHCOCH_2 and H9$_\alpha$, H11$_\alpha$, H12$_\beta$, and H16$_\alpha$ of estradiol), 1.95−1.67 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, and H16$_\beta$ of estradiol), 1.42−1.12 (m, 10H; py-CH_2NHCOC$H_2C_3H_6$-CH_2 and H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, and H15$_\beta$ of estradiol), 0.80 (s, 3H; CH_3 of estradiol). Anal. Calcd for $C_{59}H_{61}N_5F_3O_{10}SRe\cdot CH_3OH\cdot H_2O$: C, 54.37; H, 5.09; N, 5.28. Found: C, 54.17; H, 5.29; N, 5.46. IR (KBr) ν/cm^{-1}: 3370 (w, OH), 2924 (w, CH), 2863 (w, CH), 2030 (s, C≡O), 1917 (s, C≡O), 1157 (m, $CF_3SO_3^-$), 1029 (m, $CF_3SO_3^-$). Positive-ion ESI-MS: $m/z = 1126$ [M − $CF_3SO_3^-$]$^+$.

[Re(Ph$_2$-phen)(CO)$_3$(py-est)](CF_3SO_3) (3a). Yield: 136 mg (47%). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K, TMS): δ 9.81 (s, 2H; H2 and H9 of Ph$_2$-phen), 9.02 (s, 1H; 3-OH of estradiol), 8.53 (d, 2H, $J = 6.7$ Hz; H2 and H6 of pyridine), 8.21 (d, 2H, $J = 5.3$ Hz; H3 and H8 of Ph$_2$-phen), 8.15 (s, 2H; H5 and H6 of Ph$_2$-phen), 7.68 (s, 10H; C_6H_5 of Ph$_2$-phen), 7.37 (d, 2H, $J = 6.7$ Hz; H3 and H5 of pyridine), 6.98 (d, 1H, $J = 8.5$ Hz; H1 of estradiol), 6.47 (d, 1H, $J = 8.2$ Hz; H2 of estradiol), 6.40 (s, 1H; H4 of estradiol), 5.67 (s, 1H; 17-OH of estradiol), 2.73−2.64 (m, 2H; H6 of estradiol), 2.27−1.89 (m, 4H; H9$_\alpha$, H11$_\alpha$, H12$_\beta$, and H16$_\alpha$ of estradiol), 1.89−1.50 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, and H16$_\beta$ of estradiol), 1.50−1.07 (m, 4H; H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, and H15$_\beta$ of estradiol), 0.75 (s, 3H; CH_3 of estradiol). Anal. Calcd for $C_{53}H_{43}N_3F_3O_8SRe\cdot CH_3CN$: C, 56.64; H, 3.97; N, 4.80. Found: C, 56.67; H, 4.27; N, 4.75. IR (KBr) ν/cm^{-1}: 3417 (br, OH), 2930 (m, CH), 2863 (m, CH), 2217 (w, C≡C), 2028 (s, C≡O), 1916 (s, C≡O), 1147 (m, $CF_3SO_3^-$), 1030 (m, $CF_3SO_3^-$). Positive-ion ESI-MS: $m/z = 977$ [M − $CF_3SO_3^-$]$^+$.

[Re(Ph$_2$-phen)(CO)$_3$(py-C6-est)](CF_3SO_3) (3b). Yield: 98 mg (35%). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K, TMS): δ 9.79 (d, 2H, $J = 5.6$ Hz; H2 and H9 of Ph$_2$-phen), 9.01 (s, 1H; 3-OH of estradiol), 8.53−8.48 (m, 3H; H2 and H6 of pyridine and py-CH_2N*H*), 8.35 (t, 1H, $J = 5.1$ Hz; py-CH_2NHCOC$_5$H$_{10}$N*H*), 8.17 (d, 2H, $J = 5.6$ Hz; H3 and H8 of Ph$_2$-phen), 8.12 (s, 2H; H5 and H6 of Ph$_2$-phen), 7.77 (d, 2H, $J = 8.5$ Hz; H2 and H6 of phenyl ring), 7.67 (s, 10H; C_5H_6 of Ph$_2$-phen), 7.45 (d, 2H, $J = 8.5$ Hz; H3 and H5 of phenyl ring), 7.20 (d, 2H, $J = 6.7$ Hz; H3 and H5 of pyridine), 7.04 (d, 1H, $J = 8.5$ Hz; H1 of estradiol), 6.48 (d, 1H, $J = 8.5$ Hz; H2 of estradiol), 6.41 (s, 1H; H4 of estradiol), 5.51 (s, 1H; 17-OH of estradiol), 4.15 (d, 2H, $J = 5.6$ Hz; py-

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CH_2NH), 3.20−3.13 (m, 2H; CH_2NHCO−C_6H_4), 2.72−2.70 (m, 2H; H6 of estradiol), 2.35−2.04 (m, 6H; py-CH_2NHCOCH_2 and H9$_\alpha$, H11$_\alpha$, H12$_\beta$, and H16$_\alpha$ of estradiol), 1.94−1.66 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, and H16$_\beta$ of estradiol), 1.42−1.05 (m, 10H; py-CH_2NHCO$CH_2C_3H_6CH_2$ and H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, and H15$_\beta$ of estradiol), 0.80 (s, 3H; CH_3 of estradiol). Anal. Calcd for $C_{67}H_{61}N_5F_3O_{10}SRe\cdot 2H_2O$: C, 57.17; H, 4.65; N, 4.98. Found: C, 57.11; H, 4.92; N, 5.01. IR (KBr) ν/cm^{-1}: 3380 (m, OH), 2929 (m, CH_3), 2852 (m, CH_3), 2023 (m, C≡O), 1910 (m, C≡O), 1163 (m, $CF_3SO_3^-$), 1069 (m, $CF_3SO_3^-$). Positive-ion ESI-MS: m/z = 1222 [M − $CF_3SO_3^-$]$^+$.

X-ray Structural Analysis for Complex 1a. Single crystals of the complex suitable for X-ray crystallographic studies were obtained by layering petroleum ether on a mixture of acetone and methanol solution of the complex. A crystal of dimensions 0.4 × 0.25 × 0.15 mm mounted in a glass capillary was used for data collection at −10 °C on a MAR diffractometer with a 300 mm image plate detector using graphite-monochromatized Mo Kα radiation (λ = 0.71073 Å). Data collection was made with 2° oscillation steps of φ, 7 min exposure time, and scanner distance at 120 mm. The number of images collected was 100. The images were interpreted and intensities integrated using the program DENZO.[20] The structure was solved by direct methods employing the program SHELXS-97[21] on a PC. Rhenium and many non-hydrogen atoms were located according to direct methods. The positions of other non-hydrogen atoms were found after successful refinement by full-matrix least-squares using the program SHELXL-97.[22] One $CF_3SO_3^-$ anion was located. There existed positions for solvent molecules, which were temporarily solved by employing half acetone and half methanol solvent molecules. Restraints were applied to assume the bond lengths or distances of O−C, O═C, C−C, and 1,3-C···C to be ca. 1.50(2), 1.25(2), 1.53(2), and 2.65(4) Å, respectively. The flatness of the carbon and oxygen atoms of acetone was also assumed. The absolute structure was assisted by the Flack absolute structure parameter, which was equal to −0.011(6). One crystallographic asymmetric unit consisted of one formula unit, including one anion and two halves of solvent molecules occupying the same space. In the final stage of least-squares refinement, non-hydrogen atoms of solvent molecules were refined isotropically; other non-hydrogen atoms were refined anisotropically. Hydrogen atoms except those on solvent molecules were generated by the program SHELXL 97. The positions of hydrogen atoms were calculated on the basis of a riding mode with thermal parameters equal to 1.2 times that of the associated carbon atoms and were used in the calculation of final *R*-indices.

Physical Measurements, Instrumentation, Determination of Lipophilicity, and Emission Titrations. The equipment for characterization and photophysical and electrochemical measurements has been described previously.[16c] Luminescence quantum yields of the complexes were measured by the optically dilute method[23] using an aerated aqueous solution of $[Ru(bpy)_3]Cl_2$ (Φ = 0.028)[24] as the standard solution.

The lipophilicity, which is referred to as log $P_{o/w}$ (where $P_{o/w}$ = 1-octanol/water partition coefficient), of the complexes was determined from the log k'_w values (k'_w = chromatographic capacity factor at 100% aqueous solution). The log k'_w values were determined by reversed-phase HPLC on a C-18 column according to the method described by Minick.[25] The HPLC system consisted of a Waters 600 pump (Milford, MA) equipped with Rheodyne 7725i injector (Rohnert Park, CA) with a 20 μL sample loop. The column was an Ultrasphere ODS column (250 mm × 4.6 mm, Beckman, Fullerton, CA). The UV detector (Waters 996 photodiode array) was set at 280 nm. The organic portion of the mobile phase was composed of methanol and 0.25% (v/v) 1-octanol. The aqueous portion was 0.02 M MOPS buffer and 0.15% (v/v) *n*-decylamine, adjusted to pH 7.4 with NaOH and then saturated with 1-octanol. The volumetric flow rate was 1 mL min^{-1}. The complexes were dissolved in methanol at a concentration of 100 μg mL^{-1}, and a volume of 20 μL was injected into the column. The k' values are defined as $(t_R - t_o)/t_o$, where t_R and t_o are the retention times of the complex and nonretained species (solvent), respectively. The log k'_w values were obtained from linear extrapolation of log k' vs ϕ methanol (ϕ = volume fractions of methanol) data acquired in the region 0.60 ≤ ϕ methanol ≤ 0.75. The log $P_{o/w}$ values of the complexes were then determined from a standard curve (log $P_{o/w}$ vs log k'_w) constructed with data of standard compounds including 4-methoxyaniline, 4-methoxyphenol, phenol, acetophenone, naphthalene, *tert*-butylbenzene, anthracene, and pyrene with experimentally verified partition coefficients.

In the emission titrations, the tricarbonylrhenium(I) polypyridine estradiol complex or estradiol-free complex $[Re(N-N)(CO)_3(py)](CF_3SO_3)$ (py = pyridine; N−N = phen (**1c**), Me_4-phen (**2c**), Ph_2-phen (**3c**))[26] (4 nmol) in 200 μL of MeOH was added to a solution of ERα in potassium phosphate buffer solutions (1.8 mL). The concentrations of ERα varied from 25 to 250 nM. The mixtures were incubated in the dark at room temperature for 3 h. The emission spectra of the solutions were then measured.

The Hill equation was used to determine the binding constants (K_a) of the complexes to ERα:[27]

$$\log\left(\frac{Y}{1-Y}\right) = n_H \log[\text{ER}\alpha] + n_H \log K_a$$

where $Y = (I_{obs} - I_{min})/(I_{max} - I_{min})$; I_{obs}, I_{min}, and I_{max} are the emission intensities of the apparent, free, and bound forms of the tricarbonylrhenium(I) complex, respectively; n_H and K_a are the Hill coefficient and binding constant, respectively.

Results and Discussion

Synthesis. In this work, the 17α-position of estradiol was chosen as the site for modification because (i) the starting material, 17α-ethynylestradiol, is commercially available and (ii) substitution at this position does not significantly lower the binding affinity of the estradiol derivatives to estrogen receptors.[8b] The ligands py-est and py-C6-est were synthesized from the standard Sonogashira reactions of 17α-ethynylestradiol with the halo-aromatics 4-bromopyridine and 4-(*N*-(6-(4-iodobenzoylamino)hexanoyl)aminomethyl)pyridine, respectively, in diethylamine. The luminescent tricarbonylrhenium(I) polypyridine estradiol complexes were prepared from the reactions of $[Re(N-N)(CO)_3(CH_3CN)](CF_3SO_3)$[19] with the corresponding pyridine-estradiol ligands in refluxing THF, followed by column chromatographic purification and recrystallization. All the new complexes were characterized by ^{1}H NMR spectroscopy, positive-ion ESI-MS, and IR spectroscopy and gave satisfactory elemental analyses.

Crystal Structure Determination. The perspective view of the cation of complex **1a** is depicted in Figure 1. The crystal data and selected bond lengths and angles are listed in Tables 1 and 2, respectively. The rhenium(I) center of complex **1a**

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(21) SHELXS-97: Sheldrick, G. M. *Programs for Crystal Structure Analysis* (*Release 97-2*); University of Göttingen: Göttingen, Germany.
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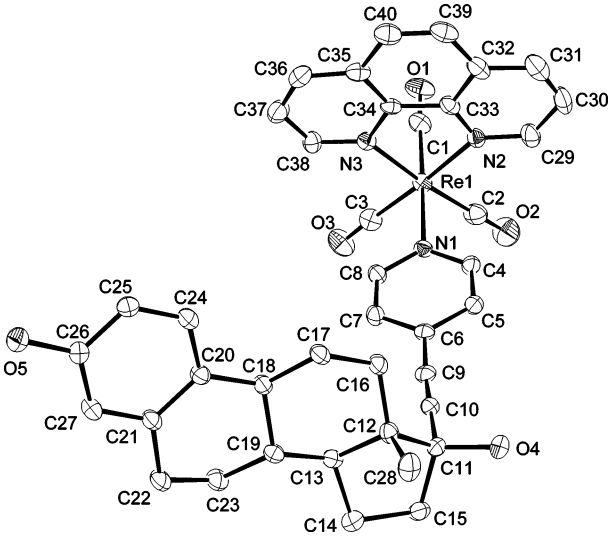

Figure 1. Perspective drawing of the cation of complex **1a** with the atomic numbering scheme. Thermal ellipsoids are set at the 20% probability level. Hydrogen atoms are omitted for clarity.

Table 1. Crystal Data and Summary of Data Collection and Refinement for Complex 1a

formula	$C_{43}H_{40}F_3N_3O_9ReS$
fw	1018.04
cryst size (mm^3)	0.4 × 0.25 × 0.15
T (K)	263 (2)
cryst syst	orthorhombic
space group	$P2_12_12_1$
a (Å)	10.977(2)
b (Å)	19.155(4)
c (Å)	20.287(4)
V ($Å^3$)	4265.6(15)
Z	4
ρ_{calcd} (g cm^{-3})	1.585
μ (mm^{-1})	2.969
F(000)	2036
θ range (deg)	2.01−25.66
index ranges	$-13 \le h \le 13$ $-23 \le k \le 23$ $-24 \le l \le 24$
no. of unique data/restraints/params	8004/6/541
R_{int}[a]	0.0471
GOF on F^2 [b]	0.947
R_1, wR_2 ($I > 2\sigma(I)$)[c]	0.0283, 0.0611
R_1, wR_2 (all data)	0.0408, 0.0648
largest diff peak/hole (e $Å^{-3}$)	0.616, −0.492

[a] $R_{int} = \sum|F_o^2 - F_o^2(\text{mean})|/\sum[F_o^2]$. [b] GOF $= S = \{\sum[w(F_o^2 - F_c^2)^2]/(n-p)\}^{1/2}$, where n is the number of reflections and p is the total number of parameters refined. The weighting scheme is $w = 1/[\sigma^2(F_o^2) + (aP)^2 + (bP)]$, where P is $[2F_c^2 + \max(F_o^2,0)]/3$, $a = 0.0047$, and $b = 0.0$. [c] $R_1 = \sum||F_o| - |F_c||/\sum|F_o|$, $wR_2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]\}^{1/2}$.

Table 2. Selected Bond Lengths (Å) and Bond Angles (Deg) for Complex 1a

Re(1)−N(1)	2.210(4)	Re(1)−N(2)	2.175(4)
Re(1)−N(3)	2.180(4)	Re(1)−C(1)	1.936(7)
Re(1)−C(2)	1.919(7)	Re(1)−C(3)	1.913(7)
C(1)−Re(1)−C(2)	91.5(2)	C(1)−Re(1)−C(3)	89.8(3)
C(1)−Re(1)−N(1)	175.2(2)	C(1)−Re(1)−N(2)	90.3(2)
C(1)−Re(1)−N(3)	91.08(19)	C(2)−Re(1)−C(3)	88.3(3)
C(2)−Re(1)−N(1)	91.02(19)	C(2)−Re(1)−N(2)	97.84(19)
C(2)−Re(1)−N(3)	172.98(19)	C(3)−Re(1)−N(1)	94.4(2)
C(3)−Re(1)−N(2)	173.9(2)	C(3)−Re(1)−N(3)	98.2(2)
N(1)−Re(1)−N(2)	85.34(15)	N(1)−Re(1)−N(3)	85.94(14)
N(2)−Re(1)−N(3)	75.62(16)	C(6)−C(9)−C(10)	173.8(6)
C(9)−C(11)−C(10)	178.6(6)		

adopted a distorted octahedral coordination geometry, and the carbonyls were arranged in a *facial* orientation. The Re−N bond lengths (from 2.175(4) to 2.210(4) Å) and the bite angle of the phen ligand (75.62(16)°) are comparable to those of related tricarbonylrhenium(I) diimine systems.[16a,e,28−31] The steroidal skeletal arrangement is similar to that of other estradiol derivatives; for example, the C(11)−O(4) (1.443(6) Å) and C(26)−O(5) (1.368(6) Å) bond lengths of complex **1a** are very similar to those of 11β-(chloromethyl)-17α-[(C≡CC_5H_4)Re(CO)$_3$]estradiol (1.46(2) and 1.40(2) Å),[8b] 4-fluoro-17β-estradiol (1.435 and 1.367 Å),[32] and 17β-estradiol (1.424 and 1.387 Å).[33] The ethynyl linkage of complex **1a** was slightly bent, as indicated by the bond angles C(6)−C(9)−C(10) (173.8(6)°) and C(9)−C(10)−C(11) (178.6(6)°). Similar observations have been made in other organometallic estradiol complexes such as 11β-(chloromethyl)-17α-[(C≡CC_5H_4)Re(CO)$_3$]estradiol (171.7(27)° and 176.8(27)°)[8b] and 17α-[(C≡CC_5H_4)Ru]Cp-estradiol (177.0° and 174.7°).[8c]

Table 3. Electronic Absorption Spectral Data of Tricarbonylrhenium(I) Polypyridine Estradiol Complexes at 298 K

complex	medium	λ_{abs}/nm (ϵ/dm^3 mol^{-1} cm^{-1})
1a	CH_2Cl_2	258 sh (30 046), 276 (32 475), 298 sh (19 350), 336 sh (8585), 382 sh (3420)
	CH_3CN	257 sh (29 390), 275 (33 180), 304 sh (16 440), 326 sh (9605), 370 sh (3315)
1b	CH_2Cl_2	260 sh (41 710), 275 (43 600), 337 sh (4520), 391 sh (2850)
	CH_3CN	254 sh (38 960), 270 (46 765), 330 sh (4955), 381 sh (2685)
2a	CH_2Cl_2	257 (31 050), 281 (35 570), 325 sh (10 155), 385 sh (2600)
	CH_3CN	250 (33 450), 280 (37 170), 325 sh (13 390), 373 sh (2940)
2b	CH_2Cl_2	252 sh (38 635), 281 (44 860), 326 sh (8835), 371 sh (3560)
	CH_3CN	250 sh (42 120), 277 (47 995), 326 sh (9370), 368 sh (3340)
3a	CH_2Cl_2	267 (28 860), 289 (34 920), 344 sh (13 500), 399 sh (5395)
	CH_3CN	261 (27 975), 290 (36 205), 340 sh (12 150), 385 sh (5140)
3b	CH_2Cl_2	266 (47 410), 300 sh (41 105), 343 sh (12 815), 408 sh (4960)
	CH_3CN	266 (47 755), 286 sh (43 430), 339 sh (12 625), 387 sh (5780)

Electronic Absorption and Emission Properties. The electronic absorption spectral data of all the complexes are summarized in Table 3. The electronic absorption spectrum of complex **2a** in CH_3CN at 298 K is shown in Figure 2. The absorption spectra of all the complexes are similar, with strong spin-allowed intraligand (^{1}IL) ($\pi \rightarrow \pi^*$) (diimine and pyridine ligands) absorption bands at ca. 250−325 nm and less intense spin-allowed metal-to-ligand charge-transfer (^{1}MLCT) (dπ(Re) $\rightarrow$ π^*(N−N)) absorption shoulders at ca. 326−408

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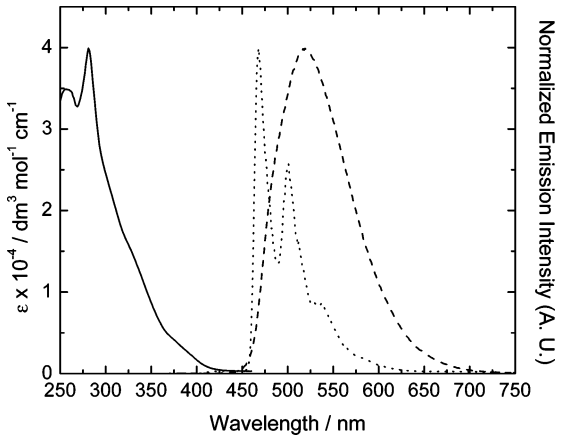

Figure 2. Electronic absorption (—) and emission (- - -) spectra of complex **2a** in CH_3CN at 298 K and in EtOH/MeOH (···) (4/1 v/v) at 77 K.

nm.[16a−f,h,19,26,28−31,34−41] However, it is likely that the absorption shoulders of Ph_2-phen complexes **3a** and **3b** at ca. 339 to 344 nm possess some ^{1}IL character because of the electron-withdrawing phenyl substituents of Ph_2-phen. Accordingly, the ^{1}MLCT (dπ(Re) → π^*(Ph_2-phen)) transitions of the Ph_2-phen complexes **3a** and **3b** are red-shifted compared to those of the phen and Me_4-phen complexes (Table 3).

Upon photoexcitation, all the complexes displayed intense and long-lived green to orange-yellow luminescence in fluid solutions at 298 K and in low-temperature alcohol glass. The photophysical data are listed in Table 4. The emission spectra of complex **2a** in CH_3CN at 298 K and in EtOH/MeOH (4/1 v/v) at 77 K are shown in Figure 2. In fluid solutions at 298 K, the emission energy of these tricarbonylrhenium(I) polypyridine estradiol complexes is similar to that of their estradiol-free analogues.[16a,b,d−f,h,19,26,28,29b,30a,34,35,36b,37,38a,39−41] The emission origin of the complexes is assigned to a ^{3}MLCT (dπ(Re) → π^*(N−N)) excited state.[16a,b,d−f,h,19,26,28,29b,30a,34,35,36b,37,38a,39−41] However, the structural features and long excited-state lifetimes of the Me_4-phen complexes **2a** and **2b** in solutions at room temperature (Table 4) suggest that the emissive state of these complexes exhibits substantial ^{3}IL (π → π^*) (Me_4-phen) character. Similar assignments have been made for related Me_4-phen complexes;[16a,b,d,e,34,41b] for example, the (i) insensitivity of the excited-state lifetime of $[Re(Me_4\text{-phen})(CO)_3(py)]^+$ to the solvent environment and (ii) its different temperature dependence from that of other $[Re(N{-}N)(CO)_3(py)]^+$ complexes were ascribed to the contribution of a ^{3}IL state.[26] In another study, calculations of the complexes $[M(CO)_4(N{-}N)]$ (M = Cr, W; N−N = phen, Me_4-phen) showed that the LUMOs of the phen and Me_4-phen complexes are of b_1 and a_2 symmetry, respectively.[42] The exceptionally long excited-state lifetime of $[W(CO)_4(Me_4\text{-phen})]$ as compared to $[W(CO)_4(phen)]$ was attributed to the different contributions of individual orbital excitations to the excited states involved.

The excited state of the py-C6-est complexes was expected to be longer-lived due to their more hydrophobic local environment associated with the longer spacer-arms. However, the py-C6-est complexes **1b**−**3b** actually displayed shorter excited-state lifetimes, especially in CH_2Cl_2, compared to their py-est counterparts (Table 4). There are two possible reasons for these interesting observations. First, the electron-withdrawing ethynyl substituent on the pyridine ligand of the py-est complexes reduces the nonradiative decay rates of the complexes, as observed in other tricarbonylrhenium(I) polypyridine systems.[39a] Second, it is possible that the longer and more flexible spacer-arms of the py-C6-est complexes facilitate their nonradiative decays, giving rise to a shorter-lived excited state.

Upon cooling to low temperature, the emission bands of the complexes were shifted to higher energy; the emission spectrum of complex **2a** at 77 K is shown in Figure 2 as an example. This rigidochromic shift is common to tricarbonylrhenium(I) diimine ^{3}MLCT emitters.[16c,e,h,19,37a,38a,39] The Me_4-phen complexes **2a** and **2b** displayed biexponential decays; the longer- and shorter-lived components are assigned to ^{3}IL (π → π^*) (Me_4-phen) and ^{3}MLCT (dπ(Re) → π^*(Me_4-phen)) excited states, respectively.[16c,e,h]

Electrochemical Properties. The cyclic voltammetric data are summarized in Table 5. The complexes displayed a reversible couple at ca. +1.70 V vs SCE, which is assigned to the rhenium(II/I) oxidation couple.[16c,e,h,28,29,35,38,39a,40a,41] Additionally, an irreversible wave appeared at ca. +1.33 to +1.39 V, which is assigned to the oxidation of the estradiol moiety of the pyridine ligands because similar waves at comparable potentials were observed for the uncoordinated ligands (at ca. +1.06 and +1.47 V for py-est and ca. + 1.41 V for py-C6-est). The first reduction waves of all the complexes (from ca. −1.12 to −1.42 V) are ascribed to the reduction of the diimine ligands.[16c,e,h,28,29,35,38,39a,40a,41] This assignment is supported by the fact that the electron-donating methyl substituents render the first reduction waves of the Me_4-phen complexes **2a** and **2b** to occur at a more negative potential (ca. −1.4 V) than those of the phen complexes **1a** and **1b** and Ph_2-phen complexes **3a** and **3b** (ca. −1.11 to −1.15 V).

Lipophilicity. The lipophilicity of estradiol derivatives is a good correlation with their in vivo uptake rate in fatty tissues and their nonspecific binding affinity to the receptor.[43] These two factors affect the in vivo distribution and transportation of estradiol derivatives. The log $P_{o/w}$ values of the current tricarbonylrhenium(I) polypyridine estradiol complexes and 17α-ethynylestradiol, determined by reversed-phase HPLC, are summarized in Table 6. Most of the complexes displayed larger log $P_{o/w}$ values than that of 17α-ethynylestradiol. We attribute these findings to the hydrophobic nature of the tricarbonylrhenium(I) polypyridine moieties despite their formal cationic charge. Note that the log $P_{o/w}$ values of the complexes follow the orders **1a** < **2a** < **3a** and **1b** < **2b** < **3b**, which is consistent with the hydrophobic character of the diimine ligands (phen <

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Table 4. Photophysical Data of Tricarbonylrhenium(I) Polypyridine Estradiol Complexes

complex	medium (T/K)	λ_{em}/nm	τ_o/μs	Φ
1a	CH_2Cl_2 (298)	540	2.17	0.20
	CH_3CN (298)	554	1.35	0.11
	buffer[a] (298)	550	0.93	0.060
	glass[b] (77)	461 sh, 499	11.74	
1b	CH_2Cl_2 (298)	541	1.02	0.14
	CH_3CN (298)	556	0.71	0.11
	buffer[a] (298)	550	0.89	0.012
	glass[b] (77)	465 sh, 497	11.43	
2a	CH_2Cl_2 (298)	490 sh, 515	8.37	0.067
	CH_3CN (298)	483 sh, 518	4.54	0.051
	buffer[a] (298)	491 sh, 526	3.17	0.017
	glass[b] (77)	468 (max), 501, 541 sh	113.18 (42%), 23.30 (58%)	
2b	CH_2Cl_2 (298)	489 sh, 524	4.54	0.051
	CH_3CN (298)	489 sh, 526	4.51	0.037
	buffer[a] (298)	494 sh, 533	2.89	0.0086
	glass[b] (77)	469 (max), 502, 537 sh	101.02 (47%), 20.29 (53%)	
3a	CH_2Cl_2 (298)	550	3.14	0.11
	CH_3CN (298)	565	2.60	0.094
	buffer[a] (298)	564	1.53	0.029
	glass[b] (77)	511, 547 sh	26.64	
3b	CH_2Cl_2 (298)	552	2.37	0.16
	CH_3CN (298)	569	2.27	0.14
	buffer[a] (298)	569	1.74	0.013
	glass[b] (77)	510, 554 sh	26.42	

[a] 50 mM potassium phosphate buffer pH 7.4 containing 30% MeOH (MeOH was used for solubility reasons). [b] EtOH/MeOH (4/1 v/v).

Table 5. Electrochemical Data of Tricarbonylrhenium(I) Polypyridine Estradiol Complexes[a]

complex	oxidation $E_{1/2}$ or E_a/V	reduction $E_{1/2}$ or E_c/V
1a	+1.39,[b] +1.74	−1.12,[c] −1.83,[b] −2.21[b]
1b	+1.33,[b] +1.72	−1.15,[c] −2.05,[b] −2.25[b]
2a	+1.39,[b] +1.68	−1.42,[b] −1.59,[c] −1.86,[b] −2.10[c]
2b	+1.37,[b] +1.67	−1.41,[c] −1.58,[b] −1.97,[c] −2.14[b]
3a	+1.35,[b] +1.71	−1.13,[c] −1.30,[c] −1.43,[c] −1.82,[c] −2.08[b]
3b	+1.34,[b] +1.70	−1.13, −1.34,[c] −1.45, −1.64,[c] −1.99[b]

[a] In CH_3CN (0.1 mol dm^{-3} $^nBu_4NPF_6$) at 298 K, glassy carbon electrode, sweep rate 100 mV s^{-1}, all potentials vs SCE. [b] Irreversible waves. [c] Quasi-reversible waves.

Table 6. Log $P_{o/w}$ Values of Tricarbonylrhenium(I) Polypyridine Estradiol Complexes and 17α-Ethynylestradiol

complex	log $P_{o/w}$
1a	3.07
1b	4.34
2a	3.91
2b	5.44
3a	4.99
3b	6.48
17α-ethynylestradiol	3.20

Me_4-phen < Ph_2-phen). The presence of a spacer-arm in complexes **1b**−**3b** increased the log $P_{o/w}$ values by ca. 1.3 to 1.5. In general, the log $P_{o/w}$ values of the py-C6-est complexes (4.34 to 6.48) are comparable to those of other organometallic estradiol derivatives such as 17α-[(C≡$CCH_2N(CH_3)C_2H_4N(CH_3)_2$)$Pt(I)_2$]estradiol (4.02),[5] 17α-[(C≡CC_6H_5)$Cr(CO)_3$]estradiol (5.03),[8b] 17α-[(C≡CC_5H_4)$Re(CO)_3$]estradiol (5.31),[8b] and 7α-[$(C_6H_{12}SC_2H_4SCH_3)Re(CO)_3Br$]estradiol (6.29).[9]

Emission Titrations. To investigate the possible interactions of the current tricarbonylrhenium(I) polypyridine estradiol complexes with ERα, noncumulative emission titration experiments have been performed. In the presence of ERα, the emission intensities of the py-C6-est complexes **1b**−**3b** were enhanced and the emission maxima were blue-shifted. These are illustrated by the emission spectral traces of complex **1b** (Figure 3). The emission intensities of complexes **1b**, **2b**, and **3b** were increased by 5.06-, 6.08-, and 5.05-fold, respectively. The emission decays were biexponential. The longer-lived components varied from 1.3 to 4.6 μs, which are longer than the lifetimes of the free complexes (Table 7). The increase of emission intensities and excited-state lifetimes is ascribed to the binding of the estradiol moieties of the complexes to ERα because the estradiol-free complexes [Re(N−N)$(CO)_3$(py)](CF_3SO_3) (N−N = phen (**1c**), Me_4-phen (**2c**), Ph_2-phen (**3c**)) did not display similar observations. Also, when ERα that had been bound with excess estradiol was used, no significant changes were observed. The emission titration curves for complexes **1a**, **1b**, and **1c** with ERα are shown in Figure 4. The protein-induced emission enhancement and lifetime elongation of complexes **1b**−**3b** are due to the increase in hydropho-

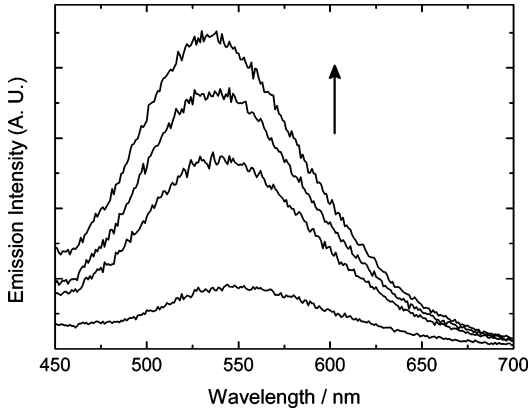

Figure 3. Emission spectral traces of complex **1b** in 50 mM potassium phosphate buffer at pH 7.4/methanol (9/1 v/v) at 298 K in the presence of 0, 50, 100, and 200 nM ERα.

Table 7. Results of Titrations of Complexes 1b−3b with ERα in Aerated 50 mM Potassium Phosphate Buffer at pH 7.4/Methanol (9/1 v/v) at 298 K

complex	I/I_o[a]	τ_o[b]	τ[b]	K_a/M^{-1}	n_H
1b	5.06	0.56	1.32 (14%), 0.25 (86%)	2.0×10^7	2.1
2b	6.08	1.30	4.60 (14%), 0.70 (86%)	1.5×10^7	2.1
3b	5.05	1.20	1.82 (14%), 0.26 (86%)	1.9×10^7	2.0

[a] I_o and I are the emission intensities of the complexes in the presence of 0 and 250 nM ERα, respectively. [b] τ_o and τ are the excited-state lifetimes of the complexes in the presence of 0 and 250 nM ERα, respectively.

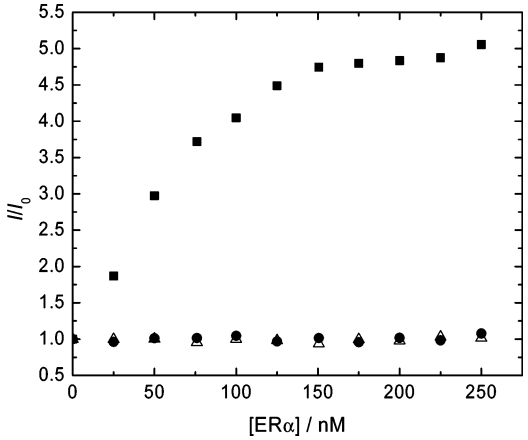

Figure 4. Emission titration curves for complexes **1a** (●), **1b** (■), and **1c** (△) with ERα. The emission intensities of the complexes were monitored at ca. 548 nm.

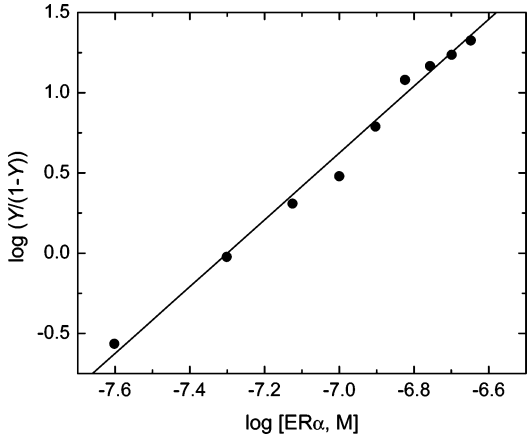

Figure 5. Plot of $\log(Y/(1 - Y))$ vs log [ERα] for complex **1b**.

bicity and rigidity of the local environment of the metal complexes because these complexes exhibited higher emission quantum yields, longer excited-state lifetimes, and shorter emission wavelengths in more nonpolar solvents (Table 4). The results are in agreement with those of the binding of tricarbonylrhenium(I) biotin and indole complexes to their respective biological receptors.[16b−e,h] The emission intensities of the py-est complexes **1a**−**3a** did not show significant changes in the presence of ERα. It is likely that the lack of a long spacer-arm substantially reduces the protein-binding affinity of these complexes.[16b−e,h]

The binding parameters of complexes **1b**−**3b** to ERα have been determined using the Hill equation.[27] The Hill plot for complex **1b** is shown in Figure 5. The binding constants (K_a) and Hill coefficients (n_H) are summarized in Table 7. Similar to other estradiol systems,[14,44] positive cooperativity was observed for the binding of the py-C6-est complexes to ERα, as evidenced by convex Scatchard plots (data not shown) and that the n_H values are >1. The binding constants of these complexes are smaller than that of unmodified estradiol ($K_a = 5 \times 10^9$ M).[45] The lower binding affinity may be a result of the bulkiness of tricarbonylrhenium(I) polypyridine moieties, which increases the steric hindrance between the complexes and the receptor. Nevertheless, these binding constants are comparable to or larger than those of other related estradiol systems such as 17α-[(L)Re(CO)$_3$]estradiol (L = 4′,4′-bis(ethanethio)-4′-carboxy-butyn-1′-yl, 6′,6′-bis(ethanethio)-6′-carboxyhexyn-1′-yl; $K_a = 1.3 \times 10^7$ and 1.1×10^7 M, respectively)[4] and 17α-[(C≡CCH$_2$N(CH$_3$)C$_2$H$_4$N(CH$_3$)$_2$)Pt(X)]estradiol (X = diiodide, malonato; $K_a = 1.0 \times 10^7$ and 2.5×10^6 M, respectively).[5]

Conclusion

In this work, a series of tricarbonylrhenium(I) polypyridine estradiol complexes have been synthesized and characterized, and their photophysical and electrochemical properties investigated. The lipophilicity and protein-binding properties of these complexes have also been studied. The py-C6-est complexes **1b**−**3b** showed emission enhancement, hypsochromic shifts in emission maxima, and lifetime elongation upon binding to ERα. Although many organometallic estradiol conjugates are known in the literature, the current tricarbonylrhenium(I) complexes are, to the best of our knowledge, the very first luminescent estradiol derivatives that function as probes for estrogen receptors. The design of other luminescent transition metal estrogen complexes is underway.

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Supporting Information Available: X-ray crystallographic data in CIF format for the structure determination of complex **1a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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