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Synthesis, Photophysical and Electrochemical Properties, and Protein-Binding Studies of Luminescent Cyclometalated Iridium(III) Bipyridine Estradiol Conjugates

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Abstract: A new series of luminescent cyclometalated iridium(III) bipyridine estradiol conjugates $[Ir(N\text{-}C)_2(N\text{-}N)](PF_6)$ (N-N = 5-(4-(17α-ethynylestradiolyl)phenyl)-2,2′-bipyridine, bpy-est, HN-C = 2-phenylpyridine, Hppy (**1a**), 1-phenylpyrazole, Hppz (**2a**), 7,8-benzoquinoline, Hbzq (**3a**), 2-phenylquinoline, Hpq (**4a**), 2-((1,1′-biphenyl)-4-yl)-benzothiazole, Hbsb (**5a**); N-N = 4-(*N*-(6-(4-(17α-ethynylestradiolyl)benzoylamino)hexyl)aminocarbonyl)-4′-methyl-2,2′-bipyridine, bpy-C6-est, HN-C = Hppy (**1b**), Hppz (**2b**), Hbzq (**3b**), Hpq (**4b**), Hbsb (**5b**)) was synthesized, characterized, and their photophysical and electrochemical properties studied. Upon photoexcitation, all the complexes displayed intense and long-lived emission in fluid solutions at 298 K and in low-temperature glass. The emission of complexes **1a**–**3a** and **1b**–**3b** was assigned to a triplet metal-to-ligand charge-transfer (^{3}MLCT) ($d\pi(Ir)\rightarrow\pi^*$(bpy-est and N-C$^-$)) state mixed with some triplet intraligand (^{3}IL) ($\pi\rightarrow\pi^*$) (N-C$^-$ and N-N) character. However, the emissive states of the pq$^-$ and bsb$^-$ complexes **4a**, **4b**, **5a**, and **5b** showed substantial ^{3}IL ($\pi\rightarrow\pi^*$) (pq$^-$/bsb$^-$) character. The lipophilicity of all the complexes was determined by reversed-phase HPLC. Upon binding to estrogen receptor α, all of these iridium(III) estradiol conjugates exhibited emission enhancement and lifetime extension, rendering them a novel series of luminescent probes for this receptor.

Keywords: estrogen · iridium · luminescence · N ligands · probes

Introduction

Estradiol is the most potent natural estrogen responsible for the development and maintenance of the secondary sexual characteristics and functions of the reproductive system in females.[1] The physiological effects of estradiol are triggered by its binding to estrogen receptors.[2] Owing to the important roles of both estradiol and its receptor in female physiology, the design of new biological probes for estradiol-binding proteins has attracted much attention.[3–16] Radioactive hormone derivatives, such as those containing tritium[3,4] and technetium,[5,6] are the most frequently used reagents. However, the potential health hazards of these reagents and the long experimental time required have prompted the search for nonisotopic reagents. Different estradiol derivatives equipped with reporters such as organic fluorophores,[7–10] biotin,[10–12] transition-metal complexes,[13,14] and organometallic compounds[15] have been reported. In particular, a wide range of estradiol derivatives containing chromium, rhenium, technetium, cobalt, iron, and manganese carbonyl complexes have been investigated.[5,15a,c,d,f–h] Recently, we studied the interactions of luminescent rhenium(I) polypyridine estradiol complexes with estrogen receptors; binding of the complexes to the biomolecules leads to an enhancement of their emission intensities of between five- and sixfold.[16] Our current aim is to design new probes with more favorable emission properties and higher sensitivity. In view of the interesting emissive behavior of iridium(III) polypyridine complexes,[17–33] in particular their high environment-sensitivity, diverse emissive-state character due to a wide choice of both cyclometalating and diimine ligands, and our interest in using these complexes as biological labels and probes,[33] we believe that cyclometalated iridium(III) polypyridine estradiol conjugates have a high potential to serve as new luminescent probes for estrogen receptors.

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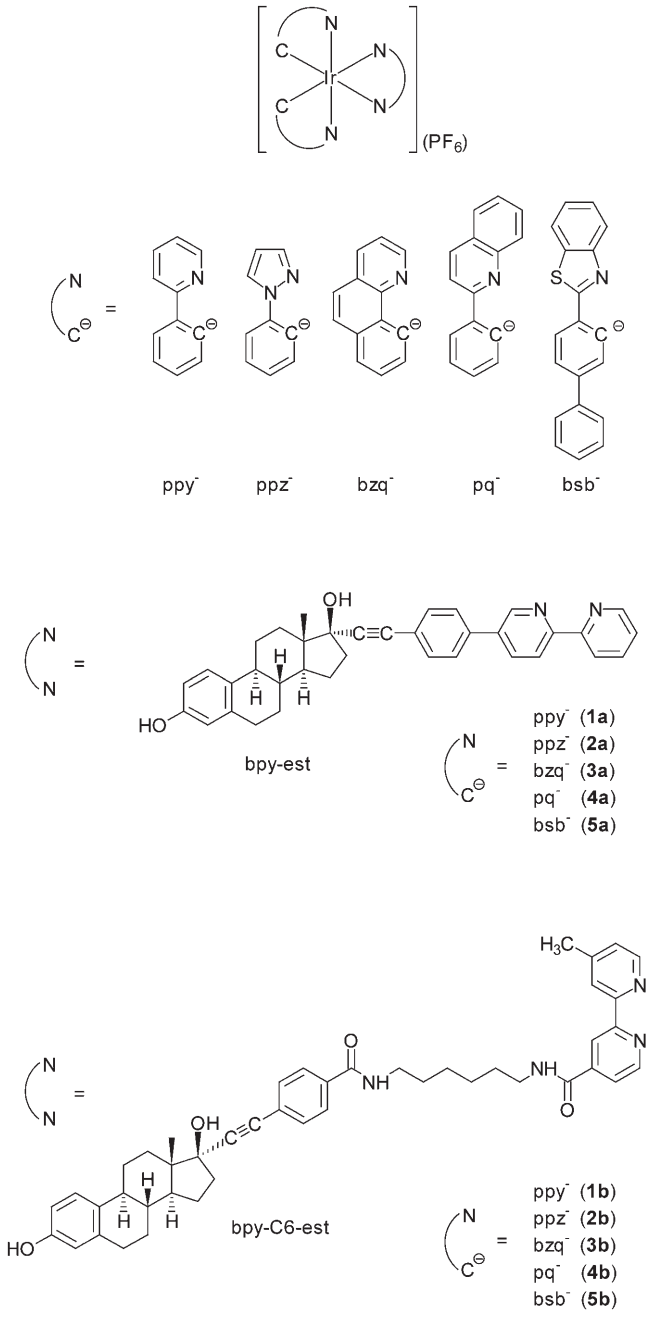
Here we report the synthesis, characterization, and photophysical and electrochemical properties of a series of luminescent cyclometalated iridium(III) bipyridine estradiol complexes $[Ir(N\text{-}C)_2(N\text{-}N)](PF_6)$ (N-N = 5-(4-(17α-ethynylestradiolyl)phenyl)-2,2′-bipyridine, bpy-est, HN-C = 2-phenylpyridine, Hppy (**1a**), 1-phenylpyrazole, Hppz (**2a**), 7,8-benzoquinoline, Hbzq (**3a**), 2-phenylquinoline, Hpq (**4a**), 2-((1,1′-biphenyl)-4-yl)benzothiazole, Hbsb (**5a**); N-N = 4-(*N*-(6-(4-(17α-ethynylestradiolyl)benzoylamino)hexyl)aminocarbonyl)-4′-methyl-2,2′-bipyridine, bpy-C6-est, HN-C = Hppy (**1b**), Hppz (**2b**), Hbzq (**3b**), Hpq (**4b**), Hbsb (**5b**)) (Scheme 1). The lipophilicity of all the conjugates was determined by reversed-phase HPLC. Additionally, the binding of the conjugates to estrogen receptor α (ERα) was investigated by emission titration experiments. To increase the ERα-induced emission enhancement, ferricyanide was used as a quencher to suppress the emission intensities of the free complexes, resulting in more sensitive responses to protein binding.



Scheme 1. Complexes **1a**–**5a** and **1b**–**5b**.

Results and Discussion

Synthesis: The estradiol-containing bipyridine ligands bpy-est and bpy-C6-est were synthesized from Sonogashira reaction of 17α-ethynylestradiol with 5-(4-bromophenyl)-2,2′-bipyridine and 4-(*N*-(6-(4-iodobenzoylamino)hexyl)aminocarbonyl)-4′-methyl-2,2′-bipyridine, respectively. All the iridium(III) estradiol complexes were obtained from the reaction of $[Ir_2(N\text{-}C)_4Cl_2]$ with bpy-est or bpy-C6-est in refluxing methanol/dichloromethane, followed by anion exchange to the PF_6^- salts and purification by column chromatography and recrystallization. They were isolated as air-stable yellow to orange-red crystals. The complexes were characterized by ^1H NMR spectroscopy, positive-ion ESI-MS, and IR spectroscopy and gave satisfactory microanalysis.

Electronic absorption spectroscopy: The electronic absorption spectral data of the complexes are listed in Table 1. The electronic absorption spectra of complexes **1a**–**5a** and **1b**–**5b** in CH_2Cl_2 at 298 K are shown in Figures 1 and 2, respectively. All the complexes showed intense absorption bands and shoulders at about 251–340 nm (ε in the order of $10^4\,dm^3\,mol^{-1}\,cm^{-1}$) and less intense bands and shoulders at >345 nm, which were assigned to spin-allowed intraligand (^{1}IL) (π→π*) (N-N and N-C$^-$) and metal-to-ligand charge transfer (^{1}MLCT) (dπ(Ir)→π*(N-N and N-C$^-$)) transitions, respectively.[17b,c,18–20,21a,c,d,22a,c,23b,c,24a,25–27,28b,29–31,32b,33] However, the mixing of ligand-to-ligand charge transfer (^{1}LLCT) (π-(N-C$^-$)→π*(N-N))[20b–d,21c,d,23b,25b,28b] and sigma-bond-to-ligand charge transfer (^{1}SBLCT) (σ(Ir-C)→π*(N-N))[18,19d,20,25b,27b] character into the CT transitions cannot be completely excluded. All the complexes also exhibited weak absorption tailing at about 450–550 nm that was assigned to spin-forbidden ^{3}MLCT (dπ(Ir)→π*(N-N and N-C$^-$)) transitions.[19a,b,20a,c,d,21d,23b,c,24a,25,26a,27a,29,30a,31a,b,33]

Interestingly, the bpy-est complexes **1a**–**5a** displayed more intense absorption at about 333–340 nm than the bpy-C6-est complexes **1b**–**5b** (Figures 1 and 2). These absorption features are mainly associated with the bpy-est ligand because the uncoordinated ligand also showed an intense absorption band in a similar region.

Luminescence properties: Upon photoexcitation, all the complexes exhibited intense and long-lived greenish-yellow to orange emission in fluid solutions under ambient conditions and in low-temperature glass. The photophysical data of the complexes are summarized in Table 2. The emission spectra of complexes **2a**, **2b**, **5a**, and **5b** in CH_2Cl_2 at 298 K

Table 1. Electronic absorption spectral data of the iridium(III) estradiol complexes at 298 K.

Complex	Medium	λ_{abs} [nm] (ε [$dm^3 mol^{-1} cm^{-1}$])
1a	CH_2Cl_2	260 (52,995), 270 sh (50,925), 314 sh (31,390), 339 (34,840), 400 sh (5,485), 469 sh (785)
	CH_3CN	259 (58,235), 269 (53,705), 313 sh (37,855), 334 (40,555), 406 sh (4,340), 468 sh (850)
1b	CH_2Cl_2	268 (76,485), 337 sh (10,800), 361 sh (8,865), 381 sh (8,115), 419 sh (3,455), 471 sh (1,040)
	CH_3CN	264 (78,570), 317 sh (19,315), 335 sh (10,560), 380 sh (7,465), 411 sh (3,705), 475 sh (875)
2a	CH_2Cl_2	261 (37,230), 315 sh (23,730), 339 (30,185), 458 sh (665)
	CH_3CN	257 (37,190), 316 sh (27,605), 334 (32,320), 443 sh (650)
2b	CH_2Cl_2	261 (54,460), 317 sh (15,630), 353 sh (7,170), 453 sh (910)
	CH_3CN	260 (56,270), 315 sh (16,455), 347 sh (3,470), 443 sh (935)
3a	CH_2Cl_2	251 sh (45,930), 257 (48,480), 315 sh (27,270), 333 (32,135), 415 sh (4,440)
	CH_3CN	252 sh (48,975), 259 (50,370), 316 sh (32,530), 340 (36,380), 416 sh (4,115)
3b	CH_2Cl_2	260 (55,145), 286 sh (30,680), 322 sh (15,655), 360 sh (9,035), 418 sh (3,930), 490 sh (575)
	CH_3CN	251 sh (49,270), 259 (51,260), 281 sh (30,585), 317 sh (15,190), 351 sh (9,060), 414 sh (3,330)
4a	CH_2Cl_2	262 (37,660), 281 (38,045), 337 (31,830), 438 sh (3,765), 527 sh (565)
	CH_3CN	261 (33,595), 278 (33,095), 333 (28,515), 435 (2,925), 522 sh (285)
4b	CH_2Cl_2	264 (55,280), 282 sh (51,420), 320 sh (18,940), 337 (18,655), 438 (3,670), 522 sh (570)
	CH_3CN	263 (59,935), 281 sh (52,030), 315 sh (19,270), 337 (18,640), 435 (3,985), 516 (495)
5a	CH_2Cl_2	271 (54,335), 338 (96,030), 385 sh (26,435), 426 (12,825), 442 sh (12,165)
	CH_3CN	263 (55,830), 334 (103,280), 389 sh (19,170), 426 sh (11,320), 447 (9,360)
5b	CH_2Cl_2	267 (65,860), 322 sh (53,960), 337 (61,870), 389 sh (21,385), 423 (11,930), 441 sh (11,365)
	CH_3CN	263 (68,105), 321 sh (55,915), 334 (61,870), 389 sh (18,860), 422 (10,910), 440 sh (10,229)

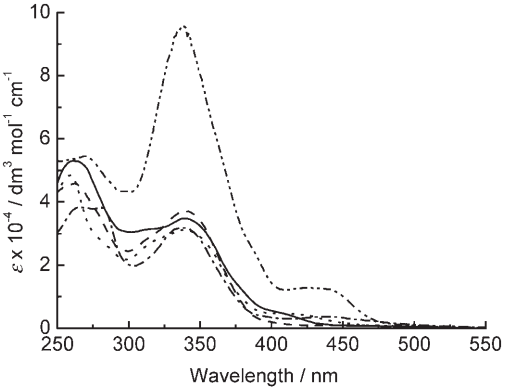


Figure 1. Electronic absorption spectra of complexes **1a** (——), **2a** (-----), **3a** (•••••), **4a** (–•–•), and **5a** (-··) in CH_2Cl_2 at 298 K.

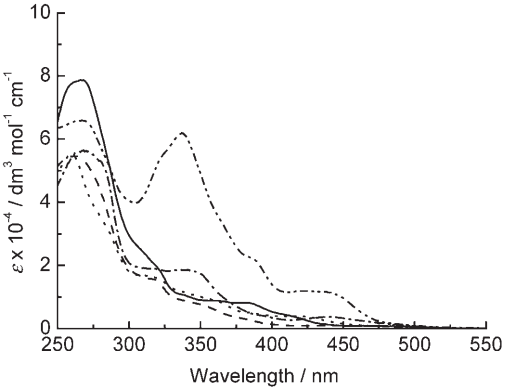


Figure 2. Electronic absorption spectra of complexes **1b** (——), **2b** (-----), **3b** (•••••), **4b** (–•–•), and **5b** (-··) in CH_2Cl_2 at 298 K.

and in low-temperature alcohol glass are shown in Figure 3. According to previous studies on the luminescence properties of cyclometalated iridium(III) polypyridine complexes $[Ir(N\text{-}C)_2(N\text{-}N)]^+$,[17b,c,18a,19d,20,22,23,25b,26b,30,31,33] the emission usually originates from a ^{3}MLCT state that may be mixed with some ^{3}LLCT[20b–d,21c,d,23b,25b,28b] or ^{3}SBLCT[18,19d,20,25b,27b] character in some cases. If more-conjugated cyclometalating ligands such as pq^-[19a,33b,c] and bsb^-[33d] are used, the emissive state involves predominant ^{3}IL ($\pi\rightarrow\pi^*$) (pq^- or bsb^-) character. Thus, the complexes in this work can be tentatively grouped into two classes: Class I includes complexes **1a**–**3a** and **1b**–**3b**, whose emission energies, lifetimes, and band shapes in fluid solutions at 298 K are very similar to those of common cyclometalated iridium(III) polypyridine complexes. The emission was assigned to a ^{3}MLCT ($d\pi(Ir)\rightarrow\pi^*$(N-N and N-C^-)) emissive state. This assignment is supported by the reduced emission lifetimes and quantum yields upon changing the solvent from CH_2Cl_2 to more polar CH_3CN and aqueous buffer. Interestingly, the bpy-est complexes **1a**–**3a** showed substantially longer emission lifetimes than their bpy-C6-est counterparts, complexes **1b**–**3b** (Table 2), suggesting the presence of ^{3}IL ($\pi\rightarrow\pi^*$) (bpy-est) character in the emissive states of complexes **1a**–**3a** due to the electron-withdrawing phenylacetylene substituent on the bipyridine ligand.[19c,20a,21a,c,d,22a,c,23b,c,25b,26,27a,b,29a,b,30a,b,33] Class II includes the four pq^- and bsb^- complexes. Among them, complexes **4a**, **5a**, and **5b** exhibited structured emission spectra and very long emission lifetimes ($\tau_o \approx 1.4$–5.5 μs) in fluid solutions at 298 K (Table 2), indicating that their emissive states should possess substantial ^{3}IL ($\pi\rightarrow\pi^*$) (pq^- or bsb^-) character. Similar features have been observed in related iridium(III)-pq[19a,33b,c] and -bsb systems.[33d] Unexpectedly, the emission properties of complex **4b** are very different from those of its bpy-est counterpart and other iridium(III)-pq complexes reported in the literature.[19a,33b,c] First, the emission spectrum of this complex did not display rich structural features. Second, the emission quantum yield showed strong dependence on the polarity of solvents. Additionally, the emission lifetimes (<1 μs) were noticeably

Table 2. Photophysical data of the iridium(III) estradiol complexes in deaerated solutions at 298 K and alcohol glass at 77 K.

Complex	Medium (T [K])	λ_{em} [nm]	τ_o [μs]	Φ_{em}
1a	CH_2Cl_2 (298)	596	0.74	0.22
	CH_3CN (298)	605	0.39	0.091
	buffer[a] (298)	578	0.15	0.038
	glass[b] (77)	530 (max), 565, 617 sh	5.56	
1b	CH_2Cl_2 (298)	598	0.40	0.14
	CH_3CN (298)	604	0.24	0.058
	buffer[a] (298)	590	0.12	0.0060
	glass[b] (77)	538, 569 sh	5.00	
2a	CH_2Cl_2 (298)	566	1.38	0.40
	CH_3CN (298)	577	0.68	0.16
	buffer[a] (298)	540 sh, 571	0.18	0.092
	glass[b] (77)	521 (max), 561, 608 sh	10.25	
2b	CH_2Cl_2 (298)	577	0.68	0.30
	CH_3CN (298)	588	0.31	0.11
	buffer[a] (298)	574	0.13	0.013
	glass[b] (77)	520, 556 sh	5.34	
3a	CH_2Cl_2 (298)	599	0.66	0.18
	CH_3CN (298)	608	0.33	0.070
	buffer[a] (298)	581	0.15	0.064
	glass[b] (77)	528 (max), 562, 617 sh	9.85 (25%), 3.57 (75%)	
3b	CH_2Cl_2 (298)	601	0.34	0.11
	CH_3CN (298)	606	0.18	0.054
	buffer[a] (298)	592	0.11	0.0061
	glass[b] (77)	535, 568 sh	4.45	
4a	CH_2Cl_2 (298)	564, 602 sh	1.66	0.30
	CH_3CN (298)	564, 602 sh	1.40	0.29
	buffer[a] (298)	570, 606 sh	0.38	0.23
	glass[b] (77)	545 (max), 587, 639 sh	5.65	
4b	CH_2Cl_2 (298)	574	0.76	0.30
	CH_3CN (298)	582	0.56	0.12
	buffer[a] (298)	582	0.20	0.011
	glass[b] (77)	539 (max), 582, 635 sh	5.05	
5a	CH_2Cl_2 (298)	553, 594 sh, 646 sh	5.49	0.39
	CH_3CN (298)	554, 596 sh, 644 sh	4.93	0.31
	buffer[a] (298)	562 (max), 601, 648 sh	0.28	0.30
	glass[b] (77)	543 (max), 588, 643	9.72	
5b	CH_2Cl_2 (298)	554, 595 sh, 645 sh	4.56	0.51
	CH_3CN (298)	554, 595 sh, 645 sh	4.02	0.24
	buffer[a] (298)	563, 601 sh, 645 sh	0.20	0.10
	glass[b] (77)	541 (max), 585, 638	9.28	

[a] Potassium phosphate (50 mM, pH 7.4) containing 10% MeOH, except for the quantum yield measurements in which 70% MeOH was used (MeOH was used for solubility reasons). [b] EtOH/MeOH (4:1).

shorter than those of other iridium(III)-pq complexes.[19a,33b,c] On the basis of these observations, we argue that the emissive state of this complex possesses predominant ^{3}MLCT ($d\pi(Ir)\rightarrow\pi^*$(bpy-C6-est)) character. A shift of excited-state character from the expected ^{3}IL to ^{3}MLCT of this iridium-(III)-pq bipyridine complex is probably a consequence of the electron-withdrawing amide substituent that stabilizes the π^* orbitals of the diimine ligand.

Upon cooling to 77 K, Class I complexes **1a**–**3a** and **1b**–**3b** showed significant blue shifts in their emission maxima (Table 2 and Figure 3). The emission was assigned to an excited state of ^{3}MLCT ($d\pi(Ir)\rightarrow\pi^*$(N-N and N-C$^-$)) character. Again, the rich vibronic structures of the emission bands and long emission lifetimes of complexes **1a**–**3a** relative to those of the bpy-C6-est analogues, complexes **1b**–**3b** (Table 2 and Figure 3), reveal that the excited states of these complexes should bear a high parentage of ^{3}IL ($\pi\rightarrow\pi^*$) (bpy-est) character. Complex **3a** showed bi-exponential decay with lifetimes of about 10 and 4 μs that are possibly associated with ^{3}MLCT ($d\pi(Ir)\rightarrow\pi^*$(bzq$^-$)) and ^{3}MLCT ($d\pi(Ir)\rightarrow\pi^*$(bpy-est)) excited states, respectively, with reference to related complexes in the literature.[17b,c,18a,19d,20,23b,30a,33] Class II complexes **4a**, **4b**, **5a**, and **5b** showed a highly structured emission band and a much smaller blue shift upon cooling to 77 K (Table 2 and Figure 3). It is conceivable that the emission is derived from a ^{3}IL ($\pi\rightarrow\pi^*$) (pq$^-$ or bsb$^-$) state that may be mixed with some CT character.[19a,33b–d]

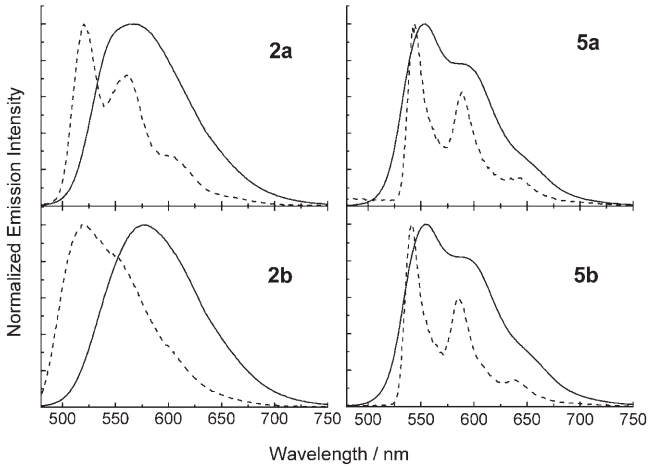


Figure 3. Emission spectra of complexes **2a**, **2b**, **5a**, and **5b** in CH_2Cl_2 at 298 K (——) and in EtOH/MeOH (4:1) at 77 K (-----).

Electrochemical properties: The electrochemical properties of all the iridium(III) estradiol complexes were studied by cyclic voltammetry. The electrochemical data are listed in Table 3. Complexes **1a**–**4a** and **1b**–**4b** showed an irreversible wave at about +1.17 to +1.23 V versus a saturated calomel electrode (SCE). This was assigned to the oxidation of the estradiol moiety because a wave at a similar potential was observed for free estradiol under the same experimental conditions. Complexes **1a**, **1b**, **2a**, **2b**, **4a**, and **4b** exhibited a quasireversible couple at about +1.25 to +1.33 V, attributable to a metal-centered iridium(IV/III) oxidation process.[18a,19d,20,22a,c,23b,c,25b,30b,33] This iridium-oxidation couple did not appear in the cyclic voltammograms of complexes **3a** and **3b**; probably it is embedded in the estradiol-based irreversible oxidation wave. Interestingly, complexes **5a** and **5b** are different from the other complexes in that they displayed an irreversible oxidation wave at about +1.3 V and a quasireversible couple at about +1.5 V versus SCE. Although the first irreversible oxidation wave occurred at a potential similar to that of the iridium(IV/III) oxidation, its

Table 3. Electrochemical data of the iridium(III) estradiol complexes.[a]

Complex	Oxidation, $E_{1/2}$ or E_a [V]	Reduction, $E_{1/2}$ or E_c [V]
1a	+1.17,[b] +1.25[c]	−1.30, −1.77,[b] −1.90,[c] −2.26,[c] −2.52[c]
1b	+1.19,[b] +1.27[c]	−1.26, −1.90,[c] −2.19,[c] −2.45,[c] −2.62[b]
2a	+1.23,[b] +1.32[c]	−1.31, −1.74,[b] −1.88,[c] −2.36,[c] −2.52[b]
2b	+1.21,[b] +1.33[c]	−1.29, −2.00,[b] −2.17,[b] −2.60[b]
3a	+1.20[b]	−1.29, −1.65,[b] −2.22,[c] −2.36[b]
3b	+1.17[b]	−1.25, −2.28,[c] −2.64[c]
4a	+1.23,[b] +1.31[c]	−1.29, −1.91,[c] −2.35[c]
4b	+1.19,[b] +1.32[c]	−1.26, −1.97,[c] −2.12,[c] −2.23,[b] −2.59[c]
5a	+1.32,[b] +1.49[c]	−1.22, −1.72,[c] −1.95,[c] −2.15,[c] −2.29[c]
5b	+1.32,[b] +1.45[c]	−1.20, −1.78,[c] −1.95,[c] −2.19,[c] −2.56[c]

[a] In CH_3CN (0.1 mol dm^{-3} nBu$_4$NPF$_6$) at 298 K, glassy carbon electrode, sweep rate 100 mV s^{-1}, all potentials versus SCE. [b] Irreversible waves. [c] Quasireversible couples.

irreversible nature and the finding of a similar irreversible wave for the free Hbsb ligand imply that it is associated with the oxidation of the coordinated bsb$^-$ ligands.[33d] The estradiol-based oxidation wave was not observable and is probably unresolved with this bsb$^-$-based signal. The second oxidation couple at about +1.45 to +1.49 V was tentatively assigned to iridium(IV/III) oxidation.[33d] This potential is more positive than that of common cyclometalated iridium(III) polypyridine complexes due to the strong π-accepting properties of the conjugated bsb$^-$ ligand. The first reduction couples of all the iridium(III) estradiol complexes appeared at about −1.20 to −1.31 V and are reversible in nature. They were tentatively assigned to the reduction of the coordinated diimine ligands.[18a,19d,20,22a,c,23b,c,25b,30b,33] The reduction waves at more negative potentials are highly irreversible, and were ascribed to the reduction of the diimine and the cyclometalating ligands.

Lipophilicity: The lipophilicity of an estradiol derivative is an important property with regard to the ability of the compound to permeate biological membranes.[34] It is commonly estimated by the partition coefficient ($P_{o/w}$) of the derivative in *n*-octanol/water.[35] The lipophilicity ($\log P_{o/w}$ values) of the iridium(III) estradiol complexes and their estradiol-free bipyridine counterparts, [Ir(N-C)$_2$(bpy)](PF$_6$) (HN-C = Hppy (**1c**), Hppz (**2c**), Hbzq (**3c**), Hpq (**4c**), Hbsb (**5c**), bpy = 2,2′-bipyridine), and 17α-ethynylestradiol was determined and the data are listed in Table 4. The lipophilicity of all the estradiol complexes (from 4.61 to 10.15) is significantly higher than that of 17α-ethynylestradiol (3.20), even though they all carry a formal cationic charge. The lipophilicity of the estradiol-free complexes **1c**–**5c** is lower than those of their estradiol-containing counterparts by about 3.6–4.7 units, reflecting the hydrophobic nature of the estradiol moiety and the linkers. The $\log P_{o/w}$ values of the iridium(III) complexes depend strongly on the cyclometalating ligands, and the observed order (ppz$^-$ < ppy$^-$ < bzq$^-$ < pq$^-$ ≪ bsb$^-$) is in accordance with the hydrophobic nature of the cyclometalating ligands. It can be seen that the introduction of a fused-benzene or phenyl ring to the cyclometalating ligand (for example, from ppy$^-$ to bzq$^-$ or pq$^-$) has substantially increased the lipophilicity of the iridium(III) complex. Interestingly, complex **1a** shows higher lipophilicity than its bpy-C6-est counterpart complex **1b**, even though the latter includes a C6 spacer arm. This could be attributed to the very nonpolar bpy-est ligand and the additional polar amide moieties of the bpy-C6-est ligand. Nevertheless, the difference is negligible for the bzq$^-$ and bsb$^-$ complexes because their lipophilicity is dominated by the hydrophobic cyclometalating ligands.

Table 4. Lipophilicity ($\log P_{o/w}$) of the iridium(III) complexes and 17α-ethynylestradiol.

Compound	$\log P_{o/w}$
1a	5.56
1b	5.06
1c	1.12
2a	4.79
2b	4.61
2c	1.00
3a	6.10
3b	6.09
3c	2.39
4a	6.75
4b	6.37
4c	2.46
5a	10.15
5b	10.11
5c	5.48
17α-ethynylestradiol	3.20

Emission titrations: The ERα-binding properties of all the complexes were investigated by emission titrations using the receptor as the titrant. All the estradiol complexes displayed emission enhancement in the presence of ERα. As an example, the emission spectral traces of complex **4a** upon addition of the receptor are shown in Figure 4. The emission intensities of the estradiol complexes were increased about 1.3

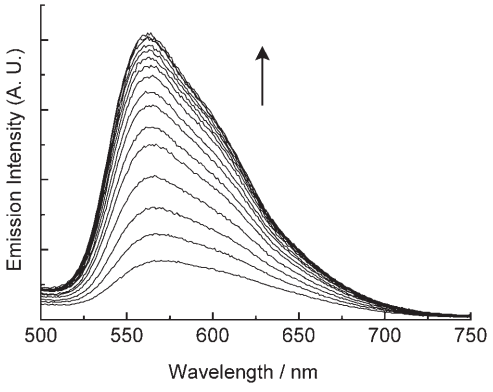


Figure 4. Emission spectral traces of complex **4a** in potassium phosphate buffer (50 mM, pH 7.4)/methanol (9:1) at 298 K in the presence of 0–375 nM ERα.

to 4.8 times (Table 5). Although the emission wavelengths did not change substantially, the emission lifetimes were extended about 2.4 to 7.0 times (Table 5). We ascribed these changes in photophysical properties to the specific binding of the estradiol moieties of the complexes to ERα because most of the estradiol-free control complexes **1c**–**5c** did not give similar observations (Table 5; see below for exceptions). To illustrate this, the emission titration curves for complexes **4a**, **4b**, and **4c** are shown in Figure 5. The specificity of the binding was supported by the fact that similar changes were absent when excess estradiol was present in the titration mixture from the outset ($I/I_o < 1.1$). We attributed the protein-induced emission enhancement and lifetime elongation of the estradiol complexes **1a**–**5a** and **1b**–**5b** to the increase in hydrophobicity and rigidity of their local environment on the basis of the photophysical data of the complexes in solvents of different polarities (Table 2). Interestingly, the control bzq$^-$ complex **3c** and bsb$^-$ complex **5c** also showed small emission enhancement in the presence of ERα ($I/I_o = 1.2$ and 1.3, respectively; $\tau/\tau_o = 1.2$ in both cases, Table 2). To study further the protein-binding specificity of all the complexes, bovine serum album (BSA) was used as a model protein and the titrations were performed under the same experimental conditions. The majority of the estradiol complexes did not show noticeable changes in the presence of BSA (data not shown), suggesting that the ERα-induced emission changes are due to specific binding of the complexes to the receptor. The only exception is the control complex **5c** that showed increased emission intensity and lifetime upon addition of BSA ($I/I_o = 1.60$; $\tau/\tau_o = 1.24$). From these results, we tentatively assigned the emission enhancement and lifetime elongation shown by complexes **3c** and **5c** in the presence of ERα to nonspecific binding of the complexes to the protein. The binding is likely to be hydrophobic in nature given the high lipophilicity of the complexes, in particular the bsb$^-$ conjugates (Table 4). The binding constants (K_a) of all the iridium(III) estradiol complexes to ERα were determined by the Hill equation.[36] The Hill plot for complex **4a** is shown in Figure 6 as an example. The binding parameters of all the iridium(III) estradiol complexes are listed in Table 5. Similar to other estradiol systems,[3,8,37] positive cooperativity was observed for the binding of the estradiol 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 range from 1.0 to $2.1\times10^7\,M^{-1}$, which are smaller than that of unmodified estradiol ($K_a = 5\times10^9\,M^{-1}$).[38] The reduced binding affinity is probably a consequence of the bulky [Ir(N-C)$_2$(N-N)]$^+$ moieties that increase the steric hindrance between the complexes and the receptor. The binding of the bpy-est complexes to ERα is slightly weaker than that of the bpy-C6-est complexes (Table 5) due to the lack of C6 spacer arms. Nevertheless, the binding constants of all the complexes are comparable to or larger than those of other related estradiol derivatives such as 17α-[(L)Re(CO)$_3$]-estra-

Table 5. Emission titration results and parameters for the binding of the iridium(III) complexes to ERα in potassium phosphate buffer (50 mM, pH 7.4)/methanol (9:1) at 298 K.

Complex	I/I_o[a]	τ_o[b] [μs]	τ[b] [μs]	K_a [M^{-1}]	n_H
1a	1.8	<0.1	0.50	1.2×10^7	1.6
1b	1.3	<0.1	0.28	1.5×10^7	1.9
1c	1.0	<0.1	<0.1		
2a	2.3	0.10	0.67	1.3×10^7	1.7
2b	1.7	<0.1	0.33	2.0×10^7	2.2
2c	1.0	<0.1	<0.1		
3a	3.6	<0.1	0.41	1.0×10^7	1.1
3b	2.4	<0.1	0.35	1.7×10^7	1.6
3c	1.2	<0.1	0.12		
4a	4.8	0.25	1.45	1.0×10^7	1.6
4b	2.0	0.11	0.77	1.6×10^7	1.2
4c	1.0	0.57	0.59		
5a	2.2	0.10	0.28	1.7×10^7	1.2
5b	1.9	0.10	0.24	2.1×10^7	2.1
5c	1.3	1.00	1.24		

[a] I_o and I are the emission intensities of the complexes (5 μM) in the absence and presence (375 nM), respectively, of ERα. [b] τ_o and τ are the emission lifetimes of the complexes (5 μM) in the absence and presence (375 nM), respectively, of ERα.

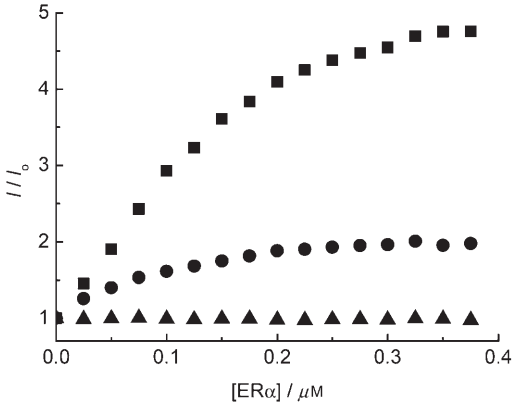


Figure 5. Luminescence titration curves for the titrations of complexes **4a** (■), **4b** (●), and **4c** (▲) (5 μM). The emission intensities of complexes **4a**, **4b**, and **4c** were monitored at 566, 584, and 560 nm, respectively.

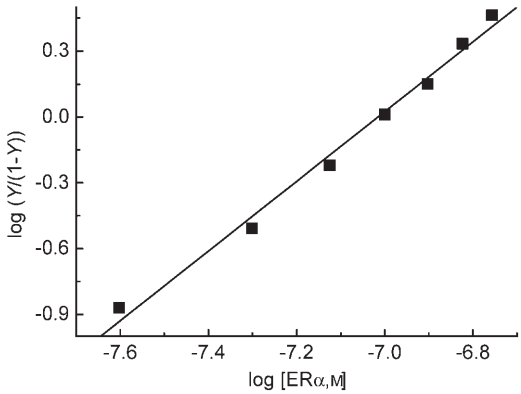


Figure 6. Hill plot of $\log(Y/(1-Y))$ versus log[ERα, M] for complex **4a**.

diol (L = 4′,4′-bis(ethanethio)-4′-carboxybutyn-1′-yl, 6′,6′-bis(ethanethio)-6′-carboxyhexyn-1′-yl; $K_a = 1.3$ and $1.1 \times 10^7\,M^{-1}$, respectively),[5] 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 $2.5 \times 10^6\,M^{-1}$, respectively),[6] and [Re(N-N)(CO)$_3$(py-C6-est)](CF$_3$SO$_3$) (py-C6-est = 4-(*N*-(6-(4-(17α-ethynylestradiolyl)benzoylamino)hexanoyl)aminomethyl)pyridine) (1.5 to $2.0 \times 10^7\,M^{-1}$).[16]

The intrinsic increase in emission intensities of the complexes upon binding to the receptor is moderate (about 1.3 to 4.8 times, Table 5). To develop homogeneous assays for ERα, it is desirable to maximize the difference in emission intensities between the free and protein-bound forms of the iridium(III) estradiol complexes. We anticipate that the use of a quencher will improve the luminescence response if it preferentially quenches the free form of the complexes rather than the ERα-bound form. We selected ferricyanide, $[Fe(CN)_6]^{3-}$, as the quencher because it can effectively quench the emission of common d^6 metal polypyridine complexes.[39] The emission intensity quenching of the iridium(III) estradiol complexes by $[Fe(CN)_6]^{3-}$ was studied in aerated buffer solutions in the absence and presence (375 nM) of ERα. The Stern–Volmer constants (K_{SV}) are listed in Table 6. In the absence of the protein, the emission of all the iridium(III) estradiol complexes was significantly quenched by $[Fe(CN)_6]^{3-}$, with K_{SV} ranging from 9.1×10^4 to $7.4 \times 10^5\,M^{-1}$ (Table 6). Interestingly, in the presence of ERα, the emission quenching became much less effective, and the K_{SV} values were reduced by two to three orders of magnitude, ranging from 4.3×10^2 to $2.4 \times 10^3\,M^{-1}$ (Table 6). We attribute the lower emission-quenching efficiency to the specific binding of the iridium(III) estradiol complexes to ERα because when excess unmodified estradiol was initially present, the emission quenching was similar to that in the absence of the protein. It is likely that immobilization of the complexes to the protein matrix renders the quenching by $[Fe(CN)_6]^{3-}$ more difficult. Based on these observations, we repeated the emission titration experiments using $[Fe(CN)_6]^{3-}$ (100 μM) as a quencher in the solution. Remarkably, in the presence of the quencher, all the iridium(III) estradiol complexes exhibited much more significant ERα-induced emission-intensity enhancement (Table 6). As examples, the emission spectra of complexes **1a**, **2a**, **3a**, and **4a** in the absence and presence of ERα are shown in Figure 7.

Table 6. Stern–Volmer constants for the emission quenching of the iridium(III) estradiol complexes by $[Fe(CN)_6]^{3-}$ in the absence and presence of ERα, and emission-enhancement factors of iridium(III) estradiol complexes upon binding to ERα in the presence of $[Fe(CN)_6]^{3-}$.

Complex	K_{SV}[a] [M^{-1}]	K_{SV}[b] [M^{-1}]	I/I_o[c]
1a	1.4×10^5	1.1×10^3	17.2
1b	7.4×10^5	2.4×10^3	7.7
2a	1.1×10^5	8.3×10^2	16.0
2b	5.4×10^5	1.7×10^3	8.5
3a	1.2×10^5	1.3×10^3	12.7
3b	6.1×10^5	2.3×10^3	10.4
4a	1.6×10^5	4.3×10^2	48.7
4b	5.3×10^5	2.0×10^3	16.4
5a	9.1×10^4	7.7×10^2	14.4
5b	2.7×10^5	1.1×10^3	10.2

[a] [ERα] = 0 nM. [b] [ERα] = 375 nM. [c] I_o and I are the emission intensities of the complexes (5 μM) in the absence and presence (375 nM), respectively, of ERα in potassium phosphate buffer (50 mM, pH 7.4)/methanol (9:1) at 298 K containing 100 μM $[Fe(CN)_6]^{3-}$.

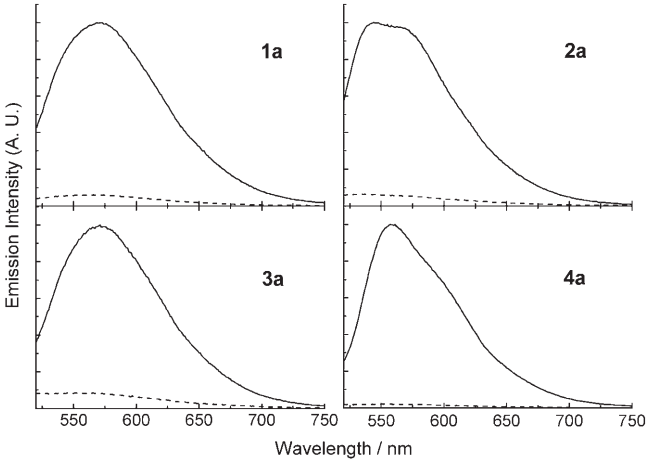


Figure 7. Emission spectra of complexes **1a**, **2a**, **3a**, and **4a** in the absence (-----) and presence (——) (375 nM) of ERα in potassium phosphate buffer (50 mM, pH 7.4)/methanol (9:1) at 298 K containing 100 μM $[Fe(CN)_6]^{3-}$.

The emission intensities of all the complexes were increased by at least 7.7 times (Table 6), which is larger than those of the rhenium(I) estradiol complexes that we reported recently.[16] In particular, due to the largest difference in K_{SV} in the absence and presence of ERα, the emission intensity of complex **4a** was substantially increased by about 49 times upon binding to the biological receptor (Table 6 and Figure 7), rendering it to serve as a "light switch" for this protein. These interesting changes in the photophysical properties of the luminescent iridium(III) estradiol complexes upon binding to ERα, together with the reasonably strong binding affinities, indicate that they act as effective luminescent probes for this biological receptor and are useful in the development of new homogeneous bioassays.

Conclusion

In this work, a series of luminescent cyclometalated iridium(III) bipyridine estradiol conjugates was designed. Their photophysical and electrochemical properties and lipophilicity were studied. All the complexes exhibited rich photoluminescence behavior. The most important result is that all these iridium(III) estradiol conjugates displayed emission enhancement and lifetime extension upon binding to ERα. The binding is specific as revealed by results from the control experiments. When ferricyanide was used as a quencher,

the increase of emission intensities of all the estradiol complexes upon binding to ERα was much more significant. From these results we can conclude that these iridium(III) estradiol conjugates are promising candidates in the development of novel biosensors for receptors, related estrogen-binding proteins, and endocrine disruptors.

Experimental Section

Materials and synthesis: All solvents were of analytical reagent grade and purified according to standard procedures. $IrCl_3 \cdot 3H_2O$, Hppy, Hppz, Hbzq, Hpq, 17α-ethynylestradiol, triphenylphosphine, 1-octanol, 4-methoxyaniline, 4-methoxyphenol, phenol, acetophenone, naphthalene, *tert*-butylbenzene, anthracene, and pyrene were purchased from Aldrich. *N*-Boc-1,6-diaminohexane hydrochloride (Boc = *tert*-butyl oxycarbonyl), 2-aminothiophenol, and 4-biphenylcarboxaldehyde were purchased from International Laboratory. Palladium(II) chloride, copper(I) iodide, 4-iodobenzoic acid, *N*-hydroxysuccinimide, *N*,*N*′-dicyclohexylcarbodiimide, KPF_6, and $K_3[Fe(CN)_6]$ were purchased from Acros. $K_3[Fe(CN)_6]$ was recrystallized from hot water and dried in a vacuum desiccator thoroughly before use. 5-(4-Bromophenyl)-2,2′-bipyridine was obtained from Wako. Succinimidyl-4-carboxy-4′-methyl-2,2′-bipyridine,[40] 4-iodobenzoic acid *N*-hydroxysuccinimidyl ester,[41] Hbsb,[42] and all the precursor complexes $[Ir_2(N\text{-}C)_4Cl_2]$[17a] were prepared according to reported procedures. Lamb uteri cytosol was used as the source of ERα.[15] 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.[15,43]

Synthesis of bpy-est: 17α-Ethynylestradiol (444 mg, 1.50 mmol) was added as a solid to a mixture of 5-(4-bromophenyl)-2,2′-bipyridine (449 mg, 1.50 mmol), $[Pd(PPh_3)_2Cl_2]$ (21 mg, 30 nmol), and CuI (11 mg, 60 nmol) suspended in diethylamine (25 mL) under an inert atmosphere of nitrogen. A black solid formed immediately. The mixture was stirred at RT for 10 min and then refluxed for 12 h. After cooling to RT, the mixture was filtered and the filtrate was evaporated to dryness to give a grey solid that was washed with a small amount of cold CH_2Cl_2. Recrystallization of the crude product from MeOH/diethyl ether afforded bpy-est as off-white crystals. Yield: 229 mg (29 %). ^{1}H NMR (500 MHz, [D$_6$]acetone, 243 K, TMS): $\delta = 9.03$ (s, 1H; H6 pyridyl ring), 8.71 (d, $J = 4.3$ Hz, 1H; H6′ pyridyl ring), 8.58 (d, $J = 7.9$ Hz, 1H; H3 pyridyl ring), 8.54–8.52 (m, 2H; 3-OH estradiol, H3′ pyridyl ring), 8.28 (d, $J = 8.5$ Hz, 1H; H4 pyridyl ring), 7.98 (t, $J = 7.7$ Hz, 1H; H4′ pyridyl ring), 7.86 (d, $J = 7.6$ Hz, 2H; H3, H5 phenyl ring), 7.62 (d, $J = 6.9$ Hz, 2H; H2, H6 phenyl ring), 7.48 (t, $J = 6.2$ Hz, 1H; H5′ pyridyl ring), 7.14 (d, $J = 8.1$ Hz, 1H; H1 estradiol), 6.59 (d, $J = 8.4$ Hz, 1H; H2 estradiol), 6.51 (s, 1H; H4 estradiol), 5.73 (s, 1H; 17-OH estradiol), 2.78–2.70 (m, 2H; H6 estradiol), 2.39–2.16 (m, 4H; $H9_\alpha$, $H11_\alpha$, $H12_\beta$, $H16_\alpha$ estradiol), 1.86–1.80 (m, 5H; $H7_\beta$, $H8_\beta$, $H11_\beta$, $H15_\alpha$, $H16_\beta$ estradiol), 1.48–1.28 (m, 4H; $H7_\alpha$, $H12_\alpha$, $H14_\alpha$, $H15_\beta$ estradiol), 0.93 ppm (s, 3H; CH_3 estradiol); IR (KBr): $\tilde{\nu} =$ 3421 (O-H), 2919 (C-H), 2863 cm^{-1} (C-H); ESI-MS (positive mode): m/z: 527 [M^++H].

***N*-(4-Iodobenzoyl)-1,6-diaminohexane**: A mixture of 4-iodobenzoic acid *N*-hydroxysuccinimidyl ester (480 mg, 1.39 mmol), *N*-boc-1,6-diaminohexane hydrochloride (420 mg, 1.66 mmol), and triethylamine (10 mL) in DMF (80 mL) was stirred under an inert atmosphere of nitrogen at RT for 12 h. The solution was evaporated to dryness to give a white solid that was then washed with diethyl ether. The solid was dissolved in CH_2Cl_2 (20 mL) and the mixture was filtered. The filtrate was evaporated to dryness to give a white solid. The solid was dissolved in trifluoroacetic acid (5 mL) and the solution was stirred at RT for 12 h. The solution was evaporated to dryness to give a white solid. The product was recrystallized from CH_2Cl_2/diethyl ether to give white crystals (390 mg, 81 %). ESI-MS (positive mode): m/z: 347 [M^++H].

4-(*N*-(6-(4-Iodobenzoylamino)hexyl)aminocarbonyl)-4′-methyl-2,2′-bipyridine: A mixture of *N*-(4-iodobenzoyl)-1,6-diaminohexane (390 mg, 1.13 mmol), succinimidyl-4-carboxy-4′-methyl-2,2′-bipyridine (350 mg, 1.13 mmol), and triethylamine (5 mL) in DMF (40 mL) was stirred under nitrogen at RT for 12 h. The clear yellow solution was evaporated to dryness under vacuum to give a yellow solid. The product was recrystallized from MeOH/diethyl ether to give white crystals. Yield: 260 mg (43 %). ESI-MS (positive mode): m/z: 543 [M^++H].

Bpy-C6-est: 17α-Ethynylestradiol (70 mg, 0.24 mmol) was added as a solid to a mixture of 4-(*N*-(6-(4-iodobenzoylamino)hexyl)aminocarbonyl)-4′-methyl-2,2′-bipyridine (130 mg, 0.24 mmol), $[Pd(PPh_3)_2Cl_2]$ (3.4 mg, 4.8 nmol), and CuI (1.8 mg, 9.6 nmol) suspended in diethylamine (5 mL) and DMF (15 mL) under an inert atmosphere of nitrogen. A black solid was formed immediately. The mixture was stirred at RT for 10 min and then refluxed for 12 h. After cooling to RT, the mixture was filtered and the filtrate was evaporated to dryness to give a brown solid that was washed with CH_2Cl_2 and diethyl ether. Recrystallization of the crude product from MeOH/diethyl ether afforded bpy-C6-est as pale-yellow crystals. Yield: 100 mg (59 %). ^{1}H NMR (500 MHz, [D$_6$]acetone, 243 K, TMS): $\delta = 9.19$ (s, 1H; H3 pyridyl ring), 9.05 (br s, 1H; bpy-4-CON*H*), 8.93 (s, 1H; H3′ pyridyl ring), 8.81 (d, $J = 5.0$ Hz, 1H; H6 pyridyl ring), 8.64–9.62 (m, 1H; H6′ pyridyl ring), 8.59 (d, $J = 5.0$ Hz, 1H; H5 pyridyl ring), 8.35 (s, 1H; 3-OH estradiol), 7.93–7.91 (m, 3H; H5′ pyridyl ring, H2, H6 phenyl ring), 7.54 (d, $J = 8.2$ Hz, 2H; H3, H5 phenyl ring), 7.34 (br s, 1H; bpy-4-CONHC$_6$H$_{12}$N*H*), 7.08 (d, $J = 8.7$ Hz, 1H; H1 estradiol), 6.54 (d, $J = 8.7$ Hz, 1H; H2 estradiol), 6.46 (s, 1H; H4 estradiol), 5.60 (s, 1H; 17-OH estradiol), 3.37–3.31 (m, 4H; NHCH_2C$_4$H$_8$CH_2NH), 2.77–2.67 (m, 2H; H6 estradiol), 2.46 (s, 3H; CH_3 pyridyl ring), 2.35–2.12 (m, 4H; $H9_\alpha$, $H11_\alpha$, $H12_\beta$, $H16_\alpha$ estradiol), 2.00–1.79 (m, 5H; $H7_\beta$, $H8_\beta$, $H11_\beta$, $H15_\alpha$, $H16_\beta$ estradiol), 1.59–1.29 (m, 12H; NHCH$_2$C$_4$*H*$_8$CH$_2$NH, $H7_\alpha$, $H12_\alpha$, $H14_\alpha$, $H15_\beta$ estradiol), 0.86 ppm (s, 3H; CH_3 estradiol); IR (KBr): $\tilde{\nu} = 3400$ (O-H), 3290 (N-H), 2919 (C-H), 2852 (C-H), 1653 cm^{-1} (C=O); ESI-MS (positive mode): m/z: 711 [M^++H].

[Ir(N-C)$_2$(N-N)](PF$_6$): A mixture of $[Ir_2(N\text{-}C)_4Cl_2]$ (0.03 mmol) and bpy-est or bpy-C6-est (0.06 mmol) in methanol/dichloromethane (30 mL, 1:1) was refluxed under an inert atmosphere of nitrogen in the dark for 4 h. The yellow solution was then cooled to RT and KPF_6 (0.07 mmol) was added. The mixture was then evaporated to dryness. The solid was dissolved in dichloromethane and purified by column chromatography on silica gel. The desired product was eluted with dichloromethane/acetone (1:1) and recrystallized from acetone/diethyl ether.

[Ir(ppy)$_2$(bpy-est)](PF$_6$) (1a): Complex **1a** was isolated as yellow crystals in 72 % yield. ^{1}H NMR (300 MHz, [D$_6$]acetone, 298 K, TMS): $\delta = 8.93$–8.88 (m, 2H; H3, H3′ pyridyl rings bpy-est), 8.58 (dd, $J = 8.5$, 2.3 Hz, 1H; H6′ pyridyl ring bpy-est), 8.35–8.22 (m, 4H; H3 pyridyl ring ppy$^-$, H6 pyridyl ring bpy-est, 3-OH estradiol), 8.14 (dd, $J = 5.6$, 0.9 Hz, 1H; H4 pyridyl ring bpy-est), 8.04–7.88 (m, 7H; H3 phenyl ring ppy$^-$, H4, H6 pyridyl ring ppy$^-$, H4′ pyridyl ring bpy-est), 7.74 (ddd, $J = 7.6$, 5.6, 1.2 Hz, 1H; H5′ pyridyl ring bpy-est), 7.52 (d, $J = 8.5$ Hz, 2H; H3, H5 phenyl ring bpy-est), 7.42 (d, $J = 8.5$ Hz, 2H; H2, H6 phenyl ring bpy-est), 7.21–7.03 (m, 5H; H4 phenyl ring ppy$^-$, H5 pyridyl ring ppy$^-$, H1 estradiol), 6.99–6.92 (m, 2H; H5 phenyl ring ppy$^-$), 6.61 (dd, $J = 8.5$, 2.6 Hz, 1H; H2 estradiol), 6.54 (d, $J = 2.6$ Hz, 1H; H4 estradiol), 6.44–6.38 (m, 2H; H6 phenyl ring ppy$^-$), 4.57 (s, 1H; 17-OH estradiol), 2.82–2.77 (m, 2H; H6 estradiol), 2.40–2.09 (m, 4H; $H9_\alpha$, $H11_\alpha$, $H12_\beta$, $H16_\alpha$ estradiol), 2.01–1.76 (m, 5H; $H7_\beta$, $H8_\beta$, $H11_\beta$, $H15_\alpha$, $H16_\beta$ estradiol), 1.54–1.29 (m, 4H; $H7_\alpha$, $H12_\alpha$, $H14_\alpha$, $H15_\beta$ estradiol), 0.95 ppm (s, 3H; CH_3 estradiol); IR (KBr): $\tilde{\nu} = 3423$ (O-H), 2925 (C-H), 2868 (C-H), 845 cm^{-1} (PF_6^-); ESI-MS (positive mode): m/z: 1026 [M^+] [Ir(ppy)$_2$(bpy-est)]$^+$; elemental analysis calcd (%) for $IrC_{58}H_{50}N_4O_2PF_6 \cdot 2H_2O$: C 57.66, H 4.50, N 4.64; found: C 57.60, H 4.43, N 4.66.

[Ir(ppy)$_2$(bpy-C6-est)](PF$_6$) (1b): Complex **1b** was isolated as yellow crystals in 66 % yield. ^{1}H NMR (300 MHz, [D$_6$]acetone, 298 K, TMS): $\delta =$ 9.14 (s, 1H; H3 pyridyl ring bpy-C6-est), 8.80 (s, 1H; H3′ pyridyl ring bpy-C6-est), 8.40 (t, $J = 5.5$ Hz, 1H; bpy-4-CON*H*), 8.24 (d, $J = 7.7$ Hz, 2H; H3 pyridyl ring ppy$^-$), 8.18 (d, $J = 5.6$ Hz, 1H; H6 pyridyl ring bpy-C6-est), 8.11 (s, 1H; 3-OH estradiol), 8.04–8.02 (m, 2H; H4 pyridyl ring ppy$^-$), 7.99–7.82 (m, 9H; H3 phenyl ring ppy$^-$, H6 pyridyl ring ppy$^-$, H5,

H6′ pyridyl rings bpy-C6-est, H2, H6 phenyl ring bpy-C6-est, bpy-4-CONHC$_6$H$_{12}$N*H*), 7.56 (d, J=5.6 Hz, 1H; H5′ pyridyl ring bpy-C6-est), 7.48 (d, J=8.1 Hz, 2H; H3, H5 phenyl ring bpy-C6-est), 7.16–7.10 (m, 3H; H5 pyridyl ring ppy$^-$, H1 estradiol), 7.07–7.02 (m, 2H; H4 phenyl ring ppy$^-$), 6.94–6.90 (m, 2H; H5 phenyl ring ppy$^-$), 6.60 (dd, J=8.1, 2.3 Hz, 1H; H2 estradiol), 6.54 (d, J=2.3 Hz, 1H; H4 estradiol), 6.36–6.31 (m, 2H; H6 phenyl ring ppy$^-$), 4.63 (s, 1H; 17-OH estradiol), 3.46–3.39 (m, 4H; NHC*H*$_2$C$_4$H$_8$C*H*$_2$NH), 2.82–2.76 (m, 2H; H6 estradiol), 2.58 (s, 3H; CH$_3$ pyridyl ring bpy-C6-est), 2.34–2.15 (m, 4H; H9$_\alpha$, H11$_\alpha$, H12$_\beta$, H16$_\alpha$ estradiol), 1.93–1.82 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, H16$_\beta$ estradiol), 1.62–1.29 (m, 12H; NHCH$_2$C$_4$*H*$_8$CH$_2$NH, H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, H15$_\beta$ estradiol), 0.96 ppm (s, 3H; CH$_3$ estradiol); IR (KBr): $\tilde{\nu}$=3420 (O-H), 3288 (N-H), 2923 (C-H), 2864 (C-H), 1676 (C=O), 843 cm^{-1} (PF$_6^-$); ESI-MS (positive mode): m/z: 1210 [M^+] [Ir(ppy)$_2$(bpy-C6-est)]$^+$; elemental analysis calcd (%) for IrC$_{67}$H$_{66}$N$_6$O$_4$PF$_6$·H$_2$O: C 58.55, H 4.99, N 6.11; found: C 58.30, H 4.87, N 6.04.

[Ir(ppz)$_2$(bpy-est)](PF$_6$) (2a): Complex **2a** was isolated as pale-yellow crystals in 83% yield. ^{1}H NMR (300 MHz, [D$_6$]acetone, 298 K, TMS): δ= 8.92–8.87 (m, 2H; H3, H3′ pyridyl rings bpy-est), 8.73 (d, J=2.6 Hz, 1H; H5 pyrazole ring ppz$^-$), 8.70 (d, J=3.0 Hz, 1H; H5 pyrazole ring ppz$^-$), 8.58 (dd, J=8.5, 2.6 Hz, 1H; H6′ pyridyl ring bpy-est), 8.46 (d, J=1.7 Hz, 1H; H6 pyridyl ring bpy-est), 8.36–8.28 (m 2H; H4, H4′ pyridyl rings bpy-est), 8.03 (s, 1H; 3-OH estradiol), 7.76–7.71 (m, 1H; H5′ pyridyl ring bpy-est), 7.68–7.63 (m, 2H; H3 phenyl ring ppz$^-$), 7.54–7.47 (m, 4H; H2, H3, H5, H6 phenyl ring bpy-C6-est), 7.45 (d, J=2.6 Hz, 1H; H3 pyrazole ring ppz$^-$), 7.31 (d, J=2.1 Hz, 1H; H3 pyrazole ring ppz$^-$), 7.13–7.05 (m, 3H; H4 phenyl ring ppz$^-$, H1 estradiol), 6.94–6.86 (m, 2H; H5 phenyl ring ppz$^-$), 6.71–6.69 (m, 2H; H4 pyrazole ring ppz$^-$), 6.61 (dd, J=8.5, 2.6 Hz, 1H; H2 estradiol), 6.54 (d, J=2.6 Hz, 1H; H4 estradiol), 6.44 (dd, J=7.3, 1.3 Hz, 1H; H6 phenyl ring ppz$^-$), 6.38 (dd, J=7.3, 1.3 Hz, 1H; H6 phenyl ring ppz$^-$), 4.58 (s, 1H; 17-OH estradiol), 2.82–2.76 (m, 2H; H6 estradiol), 2.38–2.13 (m, 4H; H9$_\alpha$, H11$_\alpha$, H12$_\beta$, H16$_\alpha$ estradiol), 1.99–1.78 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, H16$_\beta$ estradiol), 1.50–1.29 (m, 4H; H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, H15$_\beta$ estradiol), 0.95 ppm (s, 3H; CH$_3$ estradiol); IR (KBr): $\tilde{\nu}$=3425 (O-H), 2918 (C-H), 2854 (C-H), 850 cm^{-1} (PF$_6^-$); ESI-MS (positive mode): m/z: 1004 [M^+] [Ir(ppz)$_2$(bpy-est)]$^+$; elemental analysis calcd (%) for IrC$_{54}$H$_{48}$N$_6$O$_2$PF$_6$·2H$_2$O: C 54.68, H 4.42, N 7.08; found: C 54.58, H 4.44, N 7.10.

[Ir(ppz)$_2$(bpy-C6-est)](PF$_6$) (2b): Complex **2b** was isolated as pale-yellow crystals in 69% yield. ^{1}H NMR (300 MHz, [D$_6$]acetone, 298 K, TMS): δ=9.13 (s, 1H; H3 pyridyl ring bpy-C6-est), 8.79 (s, 1H; H3′ pyridyl ring bpy-C6-est), 8.72 (d, J=2.1 Hz, 2H; H5 pyrazole ring ppz$^-$), 8.38–8.35 (m, 2H; bpy-4-CON*H*, H6 pyridyl ring bpy-C6-est), 8.10–8.12 (m, 3H; H5, H6′ pyridyl rings bpy-C6-est, 3-OH estradiol), 7.94 (t, J= 5.3 Hz, 1H; bpy-4-CONHC$_6$H$_{12}$N*H*), 7.86 (d, J=8.5 Hz, 2H; H2, H6 phenyl ring bpy-C6-est), 7.64 (d, J=7.3 Hz, 2H; H3 phenyl ring ppz$^-$), 7.57 (d, J=5.6 Hz, 1H; H5′ pyridyl ring bpy-C6-est), 7.49 (d, J=8.5 Hz, 2H; H3, H5 phenyl ring bpy-C6-est), 7.36 (d, J=1.8 Hz, 1H; H3 pyrazole ring ppz$^-$), 7.25 (d, J=1.8 Hz, 1H; H3 pyrazole ring ppz$^-$), 7.13–7.04 (m, 3H; H4 phenyl ring ppz$^-$, H1 estradiol), 6.90–6.85 (m, 2H; H5 phenyl ring ppz$^-$), 6.71–6.68 (m, 2H; H4 pyrazole ring ppz$^-$), 6.60 (dd, J=8.5, 2.8 Hz, 1H; H2 estradiol), 6.55 (d, J=2.8 Hz, 1H; H4 estradiol), 6.36–6.31 (m, 2H; H6 phenyl ring ppz$^-$), 4.58 (s, 1H; 17-OH estradiol), 3.50–3.40 (m, 4H; NHC*H*$_2$C$_4$H$_8$C*H*$_2$NH), 2.83–2.76 (m, 2H; H6 estradiol), 2.61 (s, 3H; CH$_3$ pyridyl ring bpy-C6-est), 2.39–2.14 (m, 4H; H9$_\alpha$, H11$_\alpha$, H12$_\beta$, H16$_\alpha$ estradiol), 1.93–1.79 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, H16$_\beta$ estradiol), 1.64–1.29 (m, 12H; NHCH$_2$C$_4$*H*$_8$CH$_2$NH, H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, H15$_\beta$ estradiol), 0.96 ppm (s, 3H; CH$_3$ estradiol); IR (KBr): $\tilde{\nu}$=3426 (O-H), 3279 (N-H), 2927 (C-H), 2853 (C-H), 1636 (C=O), 847 cm^{-1} (PF$_6^-$); ESI-MS (positive mode): m/z: 1188 [M^+] [Ir(ppz)$_2$(bpy-C6-est)]$^+$; elemental analysis calcd (%) for IrC$_{63}$H$_{64}$N$_8$O$_4$PF$_6$·1.5H$_2$O: C 55.58, H 4.96, N 8.23; found: C 55.60, H 4.89, N 8.32.

[Ir(bzq)$_2$(bpy-est)](PF$_6$) (3a): Complex **3a** was isolated as yellow crystals in 72% yield. ^{1}H NMR (300 MHz, [D$_6$]acetone, 298 K, TMS): δ=8.95–8.90 (m, 2H; H3, H3′ pyridyl rings of bpy-est), 8.58–8.46 (m, 3H; H4 bzq$^-$, H6, H6′ pyridyl rings bpy-est), 8.31–8.28 (m, 3H; H2 bzq$^-$, 3-OH estradiol), 8.11 (d, J=5.6 Hz, 1H; H4 pyridyl ring bpy-est), 8.02–7.84 (m, 5H; H5, H6 bzq$^-$, H4′ pyridyl ring bpy-est), 7.64–7.56 (m, 5H; H3, H7 bzq$^-$, H5′ pyridyl ring bpy-est), 7.42 (d, J=8.2 Hz, 2H; H3, H5 phenyl ring bpy-est), 7.26 (d, J=8.5 Hz, 2H; H2, H6 phenyl ring bpy-est), 7.22–7.18 (m, 2H; H8 bzq$^-$), 7.11 (d, J=8.2 Hz, 1H; H1 estradiol), 6.60 (d, J= 8.2 Hz, 1H; H2 estradiol), 6.54 (s, 1H; H4 estradiol), 6.47–6.42 (m, 2H; H9 bzq$^-$), 4.53 (s, 1H; 17-OH estradiol), 2.89–2.77 (m, 2H; H6 estradiol), 2.33–1.91 (m, 4H; H9$_\alpha$, H11$_\alpha$, H12$_\beta$, H16$_\alpha$ estradiol), 1.84–1.78 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, H16$_\beta$ estradiol), 1.49–1.37 (m, 4H; H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, H15$_\beta$ estradiol), 0.94 ppm (s, 3H; CH$_3$ estradiol); IR (KBr): $\tilde{\nu}$= 3421 (O-H), 2919 (C-H), 2858 (C-H), 835 cm^{-1} (PF$_6^-$); ESI-MS (positive mode): m/z: 1074 [M^+] [Ir(bzq)$_2$(bpy-est)]$^+$; elemental analysis calcd (%) for IrC$_{62}$H$_{50}$N$_4$O$_2$PF$_6$·2H$_2$O: C 59.27, H 4.33, N 4.46; found: C 59.14, H 4.43, N 4.29.

[Ir(bzq)$_2$(bpy-C6-est)](PF$_6$) (3b): Complex **3b** was isolated as yellow crystals in 70% yield. ^{1}H NMR (300 MHz, [D$_6$]acetone, 298 K, TMS): δ= 9.18 (s, 1H; H3 pyridyl ring bpy-C6-est), 8.85 (s, 1H; H3′ pyridyl ring bpy-C6-est), 8.56 (d, J=8.1 Hz, 2H; H4 bzq$^-$), 8.40–8.32 (m, 2H; bpy-4-CON*H*, H6 pyridyl ring bpy-C6-est), 8.26 (dd, J=5.6, 1.3 Hz, 1H; H6′ pyridyl ring bpy-C6-est), 8.18 (d, J=5.6 Hz, 1H; H5 pyridyl ring bpy-C6-est), 8.07 (s, 1H; 3-OH estradiol), 7.99 (d, J=7.7 Hz, 2H; H2 bzq$^-$), 7.95–7.87 (m, 5H; H5, H6 bzq$^-$, bpy-4-CONHC$_6$H$_{12}$N*H*), 7.84 (d, J= 8.5 Hz, 2H; H2, H6 phenyl ring bpy-C6-est), 7.62–7.55 (m, 4H; H3, H7 bzq$^-$), 7.49–7.46 (m, 3H; H3, H5 phenyl ring bpy-C6-est, H5′ pyridyl ring bpy-C6-est), 7.21–7.15 (m, 2H; H8 bzq$^-$), 7.11 (d, J=8.1 Hz, 1H; H1 estradiol), 6.60 (dd, J=8.5, 2.3 Hz, 1H; H2 estradiol), 6.55 (d, J=2.3 Hz, 1H; H4 estradiol), 6.37–6.33 (m, 2H; H9 bzq$^-$), 4.60 (s, 1H; 17-OH estradiol), 3.44–3.37 (m, 4H; NHC*H*$_2$C$_4$H$_8$C*H*$_2$NH), 2.79–2.75 (m, 2H; H6 estradiol), 2.57 (s, 3H; CH$_3$ pyridyl ring bpy-C6-est), 2.38–2.15 (m, 4H; H9$_\alpha$, H11$_\alpha$, H12$_\beta$, H16$_\alpha$ estradiol), 1.93–1.82 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, H16$_\beta$ estradiol), 1.63–1.29 (m, 12H; NHCH$_2$C$_4$*H*$_8$CH$_2$NH, H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, H15$_\beta$ estradiol), 0.96 ppm (s, 3H; CH$_3$ estradiol); IR (KBr): $\tilde{\nu}$=3416 (O-H), 3285 (N-H), 2930 (C-H), 2846 (C-H), 1647 (C=O), 849 cm^{-1} (PF$_6^-$); ESI-MS (positive mode): m/z: 1258 [M^+] [Ir(bzq)$_2$(bpy-C6-est)]$^+$; elemental analysis calcd (%) for IrC$_{66}$H$_{54}$N$_4$O$_2$PF$_6$·CH$_3$OH: C 60.20, H 4.91, N 5.85; found: C 60.26, H 4.85, N 5.63.

[Ir(pq)$_2$(bpy-est)](PF$_6$) (4a): Complex **4a** was isolated as orange crystals in 83% yield. ^{1}H NMR (300 MHz, [D$_6$]acetone, 298 K, TMS): δ=8.66–8.63 (m, 2H; H3, H3′ pyridyl rings bpy-est), 8.59–8.45 (m, 4H; H3 quinoline pq$^-$, H6, H6′ pyridyl rings bpy-est), 8.40–8.36 (m, 2H; H3 phenyl ring pq$^-$), 8.29 (d, J=7.0 Hz, 2H; H4 quinoline pq$^-$), 8.17–8.11 (m, 1H; H4 pyridyl ring bpy-est), 8.07 (s, 1H; 3-OH estradiol), 7.92 (dt, J=8.4, 1.5 Hz, 2H; H8 quinoline pq$^-$), 7.71 (t, J=7.6 Hz, 1H; H4′ pyridyl ring bpy-est), 7.61 (d, J=8.2 Hz, 2H; H3, H5 phenyl ring bpy-est), 7.56–7.41 (m, 7H; H5, H7 quinoline pq$^-$, H2, H6 phenyl ring bpy-est, H5′ pyridyl ring bpy-est), 7.24–7.12 (m, 5H; H4 phenyl ring pq$^-$, H6 quinoline pq$^-$, H1 estradiol), 6.89–6.82 (m, 2H; H5 phenyl ring pq$^-$), 6.66 (d, J=7.9 Hz, 1H; H2 estradiol), 6.62 (s, 1H; H4 estradiol), 6.59–6.55 (m, 2H; H6 phenyl ring pq$^-$), 4.62 (s, 1H; 17-OH estradiol), 2.84–2.79 (m, 2H; H6 estradiol), 2.39–2.16 (m, 4H; H9$_\alpha$, H11$_\alpha$, H12$_\beta$, H16$_\alpha$ estradiol), 2.02–1.83 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, H16$_\beta$ estradiol), 1.53–1.29 (m, 4H; H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, H15$_\beta$ estradiol), 0.98 ppm (s, 3H; CH$_3$ estradiol); IR (KBr): $\tilde{\nu}$=3420 (O-H), 2926 (C-H), 2853 (C-H), 848 cm^{-1} (PF$_6^-$); ESI-MS (positive mode): m/z: 1126 [M^+] [Ir(pq)$_2$(bpy-est)]$^+$; elemental analysis calcd (%) for IrC$_{66}$H$_{54}$N$_4$O$_2$PF$_6$·2H$_2$O: C 60.59, H 4.47, N 4.28; found: C 60.72, H 4.52, N 4.31.

[Ir(pq)$_2$(bpy-C6-est)](PF$_6$) (4b): Complex **4b** was isolated as orange crystals in 74% yield. ^{1}H NMR (300 MHz, [D$_6$]acetone, 298 K, TMS): δ= 8.75 (s, 1H; H3 pyridyl ring bpy-C6-est), 8.56–8.49 (m, 4H; H3 quinoline pq$^-$, H3′ pyridyl ring bpy-C6-est bpy-4-CON*H*), 8.45 (d, J=6.0 Hz, 1H; H6 pyridyl ring bpy-C6-est), 8.40 (s, 1H; 3-OH estradiol), 8.26–8.23 (m, 3H; H3 phenyl ring pq$^-$, H6′ pyridyl ring bpy-C6-est), 8.18 (d, J=6.0 Hz, 1H; H5 pyridyl ring bpy-C6-est), 8.05–8.02 (m, 2H; H4 quinoline pq$^-$), 7.94–7.88 (m, 3H; H8 quinoline pq$^-$, bpy-4-CONHC$_6$H$_{12}$N*H*), 7.82 (d, J= 8.5 Hz, 2H; H2, H6 phenyl ring bpy-C6-est), 7.55 (d, J=5.6 Hz, 1H; H5′ pyridyl ring bpy-C6-est), 7.46 (d, J=5.6 Hz, 2H; H3, H5 phenyl ring bpy-C6-est), 7.44–7.38 (m, 4H; H5, H7 quinoline pq$^-$), 7.20–7.09 (m, 5H; H4 phenyl ring pq$^-$, H6 quinoline pq$^-$, H1 estradiol), 6.85–6.81 (m, 2H; H5 phenyl ring pq$^-$), 6.63–6.53 (m, 4H; H6 phenyl ring pq$^-$, H2, H4 estradiol), 4.59 (s, 1H; 17-OH estradiol), 3.44–3.33 (m, 4H;

NHCH_2C$_4$H$_8$CH_2NH), 2.84–2.72 (m, 2H; H6 estradiol), 2.44 (s, 3H; CH_3 pyridyl ring bpy-C6-est), 2.39–2.15 (m, 4H; H9$_\alpha$, H11$_\alpha$, H12$_\beta$, H16$_\alpha$ estradiol), 1.92–1.82 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, H16$_\beta$ estradiol), 1.61–1.29 (m, 12H; NHCH$_2$C$_4$$H_8CH_2$NH, H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, H15$_\beta$ estradiol), 0.96 ppm (s, 3H; CH_3 estradiol); IR (KBr): $\tilde{\nu}$=3429 (O-H), 3273 (N-H), 2921 (C-H), 2863 (C-H), 1659 (C=O), 853 cm^{-1} (PF_6^-); ESI-MS (positive mode): m/z: 1310 [M^+] [Ir(pq)$_2$(bpy-C6-est)]$^+$; elemental analysis calcd (%) for $IrC_{75}H_{70}N_6O_4PF_6$·2H_2O: C 60.35, H 5.00, N 5.63; found: C 60.23, H 4.89, N 5.68.

[Ir(bsb)$_2$(bpy-est)](PF$_6$) (5a): Complex **5a** was isolated as orange-red crystals in 81% yield. ^{1}H NMR (300 MHz, [D$_6$]acetone, 298 K, TMS): δ= 8.94–8.89 (m, 2H; H3, H3′ pyridyl rings bpy-est), 8.62 (dd, J=8.3, 2.3 Hz, 1H; H6′ pyridyl ring bpy-est), 8.55 (d, J=2.1 Hz, 1H; H6 pyridyl ring bpy-est), 8.48 (dd, J=4.7, 2.1 Hz, 1H; H4 pyridyl ring bpy-est), 8.37 (dt, J=7.7, 1.7 Hz, 1H; H4′ pyridyl ring bpy-est), 8.23–8.19 (m, 2H; H4 benzothiazole ring bsb$^-$), 8.13 (d, J=7.7 Hz, 1H; H5 biphenyl ring bsb$^-$), 8.10 (d, J=7.7 Hz, 1H; H5 biphenyl ring bsb$^-$), 8.03 (s, 1H; 3-OH estradiol), 7.84 (dt, J=7.7, 1.3 Hz, 1H; H5′ pyridyl ring bpy-est), 7.53–7.42 (m, 8H; H2′, H6′ biphenyl ring bsb$^-$, H2, H3, H5, H6 phenyl ring bpy-est), 7.27–7.18 (m, 12H; H5, H6 benzothiazole ring bsb$^-$, H3′, H4′, H5′, H6 biphenyl rings bsb$^-$), 7.11 (d, J=8.1 Hz, 1H; H1 estradiol), 6.77–6.74 (m, 2H; H2 biphenyl ring bsb$^-$), 6.61 (dd, J=8.5, 3.0 Hz, 1H; H2 estradiol), 6.58 (d, J=8.1 Hz, 1H; H7 benzothiazole ring bsb$^-$), 6.54 (d, J= 2.6 Hz, 1H; H4 estradiol), 6.48 (d, J=8.1 Hz, 1H; H7 benzothiazole ring bsb$^-$), 4.56 (s, 1H; 17-OH estradiol), 2.83–2.76 (m, 2H; H6 estradiol), 2.38–2.13 (m, 4H; H9$_\alpha$, H11$_\alpha$, H12$_\beta$, H16$_\alpha$ estradiol), 1.96–1.78 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, H16$_\beta$ estradiol), 1.52–1.26 (m, 4H; H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, H15$_\beta$ estradiol), 0.94 ppm (s, 3H; CH_3 estradiol); IR (KBr): $\tilde{\nu}$= 3419 (O-H), 2926 (C-H), 2846 (C-H), 855 cm^{-1} (PF_6^-); ESI-MS (positive mode): m/z: 1290 [M^+] [Ir(bsb)$_2$(bpy-est)]$^+$; elemental analysis calcd (%) for $IrC_{74}H_{58}N_4O_2S_2PF_6$·1.5$H_2O$: C 60.73, H 4.20, N 3.83; found: C 60.55, H 4.28, N 4.00.

[Ir(bsb)$_2$(bpy-C6-est)](PF$_6$) (5b): Complex **5b** was isolated as orange-red crystals in 46% yield. ^{1}H NMR (300 MHz, [D$_6$]acetone, 298 K, TMS): δ= 9.17 (s, 1H; H3 pyridyl ring bpy-C6-est), 8.82 (s, 1H; H3′ pyridyl ring bpy-C6-est), 8.53 (d, J=5.6 Hz, 1H; H6 pyridyl ring bpy-C6-est), 8.38 (t, J=5.4 Hz, 1H; bpy-4-CONH), 8.26–8.19 (m, 3H; H4 benzothiazole ring bsb$^-$, H6′ pyridyl ring bpy-C6-est), 8.13 (d, J=4.7 Hz, 1H; H5 pyridyl ring bpy-C6-est), 8.07 (dd, J=6.7, 1.2 Hz, 2H; H5 biphenyl ring bsb$^-$), 8.02 (s, 1H; 3-OH estradiol), 7.93 (t, J=5.5 Hz, 1H; bpy-4-CONHC$_6$H$_{12}$NH), 7.83 (d, J=8.2 Hz, 2H; H2, H6 phenyl ring bpy-C6-est), 7.67 (d, J=5.4 Hz, 1H; H5′ pyridyl ring bpy-C6-est), 7.49–7.42 (m, 6H; H2′, H6′ biphenyl ring bsb$^-$, H3, H5 phenyl ring bpy-C6-est), 7.27–7.18 (m, 12H; H5, H6 benzothiazole ring bsb$^-$, H3′, H4′, H5′, H6 biphenyl rings bsb$^-$), 7.12 (d, J=8.2 Hz, 1H; H1 estradiol), 6.67 (dd, J=8.5, 1.8 Hz, 2H; H2 biphenyl ring bsb$^-$), 6.60 (dd, J=8.2, 2.3 Hz, 1H; H2 estradiol), 6.55 (d, J=2.3 Hz, 1H; H4 estradiol), 6.49 (d, J=8.2 Hz, 1H; H7 benzothiazole ring bsb$^-$), 6.43 (d, J=8.5 Hz, 1H; H7 benzothiazole ring bsb$^-$), 4.58 (s, 1H; 17-OH estradiol), 3.48–3.40 (m, 4H; NHCH_2C$_4$H$_8$CH_2NH), 2.82–2.75 (m, 2H; H6 estradiol), 2.61 (s, 3H; CH_3 pyridyl ring bpy-C6-est), 2.42–2.23 (m, 4H; H9$_\alpha$, H11$_\alpha$, H12$_\beta$, H16$_\alpha$ estradiol), 1.90–1.80 (m, 5H; H7$_\beta$, H8$_\beta$, H11$_\beta$, H15$_\alpha$, H16$_\beta$ estradiol), 1.67–1.33 (m, 12H; NHCH$_2$C$_4$$H_8CH_2$NH, H7$_\alpha$, H12$_\alpha$, H14$_\alpha$, H15$_\beta$ estradiol), 0.96 ppm (s, 3H; CH_3 estradiol); IR (KBr): $\tilde{\nu}$=3429 (O-H), 3278 (N-H), 2925 (C-H), 2857 (C-H), 1679 (C=O), 857 cm^{-1} (PF_6^-); ESI-MS (positive mode): m/z: 1474 [M^+] [Ir(bsb)$_2$(bpy-C6-est)]$^+$; elemental analysis calcd (%) for $IrC_{83}H_{74}N_6O_4S_2PF_6$·$CH_3OH$: C 61.04, H 4.76, N 5.08; found: C 61.17, H 4.65, N 5.14.

Physical measurements and instrumentation: Equipment for the characterization and photophysical, electrochemical, and lipophilicity measurements has been described previously.[16] Luminescence quantum yields were measured by the optically dilute method[44] using an aerated aqueous solution of [Ru(bpy)$_3$]Cl$_2$ (Φ=0.028) as the standard solution.[45]

Emission titrations: The iridium(III) complex (10 nmol) dissolved in methanol (200 μL) was added to a series of potassium phosphate buffer solutions (1.8 mL, 50 mM, pH 7.4) containing ERα. The concentration of ERα in the solutions ranged from 0 to 375 nM. The emission spectra of the solutions were measured. The binding constants (K_a) and Hill coefficients (n_H) of the iridium(III) estradiol complexes were determined from the Hill equation:[36]

$$\log((Y/(1-Y)) = n_H \log[\mathrm{ER}\alpha] + n_H \log K_a$$

$Y=(I_{obs}-I_{min})/(I_{max}-I_{min})$, in which I_{obs}, I_{min}, and I_{max} are the emission intensities of the apparent, free, and bound forms of the iridium(III) complex, respectively.

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[1] See, for example: a) C. Behl, *Estrogen—Mystery Drug for the Brain? The Neuroprotective Activities of the Female Sex Hormone*, Springer-Verlag, New York, **2001**; b) P. M. Parker, J. N. Parker, *Estradiol—A Medical Dictionary, Bibliography, and Annotated Research Guide to Internet References*, ICON Health Publications, San Diego, CA, **2004**.

[2] See, for example: a) E. von Angerer, *The Estrogen Receptor as a Target for Rational Drug Design*, Springer, Heidelberg, Germany, **1995**; b) F. F. Parl, *Estrogens, Estrogen Receptor, and Breast Cancer*, IOS Press, Amsterdam, Oxford, Tokyo, **2000**; c) S. Nilsson, J.-Å Gustafsson, *Crit. Rev. Biochem. Mol. Biol.* **2002**, *37*, 1–28.

[3] J. A. Schwartz, D. F. Skafar, *Biochemistry* **1994**, *33*, 13267—13273.

[4] P. Liang, B. Adhyaru, W. L. Pearson, K. R. Williams, *J. Chem. Educ.* **2006**, *83*, 294–295.

[5] L. G. Luyt, H. M. Bigott, M. J. Welch, J. A. Katzenellenbogen, *Bioorg. Med. Chem.* **2003**, *11*, 4977–4989.

[6] C. Cassino, E. Gabano, M. Ravera, G. Cravotto, G. Palmisano, A. Vessières, G. Jaouen, S. Mundwiler, R. Alberto, D. Osella, *Inorg. Chim. Acta* **2004**, *357*, 2157–2166.

[7] Y. J. Lee, A. C. Notides, Y.-G. Tsay, A. S. Kende, *Biochemistry* **1977**, *16*, 2896–2901.

[8] K. Ohno, T. Fukushima, T. Santa, N. Waizumi, H. Tokuyama, M. Maeda, K. Imai, *Anal. Chem.* **2002**, *74*, 4391–4396.

[9] K. Kuningas, T. Ukonaho, H. Pakkila, T. Rantanen, J. Rosenberg, T. Lovgren, T. Soukka, *Anal. Chem.* **2006**, *78*, 4690–4696.

[10] M. Adamczyk, Y.-Y. Chen, J. A. Moore, P. G. Mattingly, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1281–1284.

[11] H. Hauptmann, B. Paulus, T. Kaiser, P. B. Luppa, *Bioconjugate Chem.* **2000**, *11*, 537–548.

[12] L. Zhao, J.-M. Lin, Z. Li, X. Ying, *Anal. Chim. Acta* **2006**, *558*, 290–295.

[13] E. Gabano, C. Cassino, S. Bonetti, C. Prandi, D. Conangelo, A. Chiglia, D. Osella, *Org. Biomol. Chem.* **2005**, *3*, 3531–3539.

[14] a) C. Descôteaux, J. Provencher-Mandeville, I. Mathieu, V. Perron, S. K. Mandal, É. Asselin, G. Bérubé, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3927–3931; b) V. Gagnon, M.-E.ì St-Germain, C. Descôteaux, J. Provencher-Mandeville, S. Parent, S. K. Mandal, E. Asselin, G. Bérubé, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5919–5924.

[15] a) A. Vessières, S. Top, A. A. Ismail, I. S. Butler, M. Louer, G. Jaouen, *Biochemistry* **1988**, *27*, 6659–6666; b) D. Vichard, M. Gruselle, H. El Amouri, G. Jaouen, J. Vaissermann, *Organometallics* **1992**, *11*, 2952–2956; c) H. El Amouri, A. Vessières, D. Vichard, S. Top, M. Gruselle, G. Jaouen, *J. Med. Chem.* **1992**, *35*, 3130–3135; d) D. Osella, G. Dutto, G. Jaouen, A. Vessières, P. R. Raithby, L. De Benedetto, M. J. McGlinchey, *Organometallics* **1993**, *12*, 4545–4552; e) D. Vichard, M. Gruselle, G. Jaouen, M. N. Nefedova, I. A. Mamedyarova, V. I. Sokolov, J. Vaissermann, *J. Organomet. Chem.* **1994**, *484*, 1–8; f) S. Top, H. El Hafa, A. Vessières, J. Quivy, J. Vaissermann, D. W. Hughes, M. J. McGlinchey, J.-P. Mornon, E. Thoreau, G. Jaouen, *J. Am. Chem. Soc.* **1995**, *117*, 8372–8380; g) S. Top, H. El

Hafa, A. Vessières, M. Huché, J. Vaissermann, G. Jaouen, *Chem. Eur. J.* **2002**, *8*, 5241–5249; h) B. Ferber, S. Top, A. Vessières, R. Welter, G. Jaouen, *Organometallics* **2006**, *25*, 5730–5739.
[16] K. K.-W. Lo, K. H.-K. Tsang, N. Zhu, *Organometallics* **2006**, *25*, 3220–3227.
[17] a) S. Sprouse, K. A. King, P. J. Spellane, R. J. Watts, *J. Am. Chem. Soc.* **1984**, *106*, 6647–6653; b) A. P. Wilde, R. J. Watts, *J. Phys. Chem.* **1991**, *95*, 622–629; c) A. P. Wilde, K. A. King, R. J. Watts, *J. Phys. Chem.* **1991**, *95*, 629–634.
[18] a) P. Didier, I. Ortmans, A. Kirsch-De Mesmaeker, R. J. Watts, *Inorg. Chem.* **1993**, *32*, 5239–5245; b) I. Ortmans, P. Didier, A. Kirsch-De Mesmaeker, *Inorg. Chem.* **1995**, *34*, 3695–3704.
[19] a) S. Lamansky, P. Djurovich, D. Murphy, F. Abdel-Razzaq, R. Kwong, I. Tsyba, M. Bortz, B. Mui, R. Bau, M. E. Thompson, *Inorg, Inorg. Chem.* **2001**, *40*, 1704–1711; b) A. B. Tamayo, B. D. Alleyne, P. I. Djurovich, S. Lamansky, I. Tsyba, N. N. Ho, R. Bau, M. E. Thompson, *J. Am. Chem. Soc.* **2003**, *125*, 7377–7387; c) J. Li, P. I. Djurovich, B. D. Alleyne, M. Yousufuddin, N. N. Ho, J. C. Thomas, J. C. Peters, R. Bau, M. E. Thompson, *Inorg. Chem.* **2005**, *44*, 1713–1727; d) A. B. Tamayo, S. Garon, T. Sajoto, P. I. Djurovich, I. M. Tsyba, R. Bau, M. E. Thompson, *Inorg. Chem.* **2005**, *44*, 8723–8732.
[20] a) F. Neve, A. Crispini, S. Campagna, S. Serroni, *Inorg. Chem.* **1999**, *38*, 2250–2258; b) F. Neve, A. Crispini, S. Serroni, F. Loiseau, S. Campagna, *Inorg. Chem.* **2001**, *40*, 1093–1101; c) F. Neve, M. La Deda, A. Crispini, A. Bellusci, F. Puntoriero, S. Campagna, *Organometallics* **2004**, *23*, 5856–5863; d) F. Neve, M. La Deda, F. Puntoriero, S. Campagna, *Inorg. Chim. Acta* **2006**, *359*, 1666–1672.
[21] a) M. Licini, J. A. G. Williams, *Chem. Commun.* **1999**, 1943–1944; b) W. Goodall, J. A. G. Williams, *J. Chem. Soc. Dalton Trans.* **2000**, 2893–2895; c) A. J. Wilkinson, A. E. Goeta, C. E. Foster, J. A. G. Williams, *Inorg. Chem.* **2004**, *43*, 6513–6515; d) A. J. Wilkinson, H. Puschmann, J. A. K. Howard, C. E. Foster, J. A. G. Williams, *Inorg. Chem.* **2006**, *45*, 8685–8699.
[22] a) J. D. Slinker, A. A. Gorodetsky, M. S. Lowry, J. Wang, S. Parker, R. Rohl, S. Bernhard, G. G. Malliaras, *J. Am. Chem. Soc.* **2004**, *126*, 2763–2767; b) M. S. Lowry, W. R. Hudson, R. A. Pascal, Jr., S. Bernhard, *J. Am. Chem. Soc.* **2004**, *126*, 14129–14135; c) M. S. Lowry, S. Bernhard, *Chem. Eur. J.* **2006**, *12*, 7970–7977.
[23] a) S.-J. Yeh, M.-F. Wu, C.-T. Chen, Y.-H. Song, Y. Chi, M.-H. Ho, S.-F. Hsu, C. H. Chen, *Adv. Mater.* **2005**, *17*, 285–289; b) C.-H. Yang, S.-W. Li, Y. Chi, Y.-M. Cheng, Y.-S. Yeh, P.-T. Chou, G.-H. Lee, C.-H. Wang, C.-F. Shu, *Inorg. Chem.* **2005**, *44*, 7770–7780; c) F.-M. Hwang, H.-Y. Chen, P.-S. Chen, C.-S. Liu, Y. Chi, C.-F. Shu, F.-I. Wu, P.-T. Chou, S.-M. Peng, G.-H. Lee, *Inorg. Chem.* **2005**, *44*, 1344–1353.
[24] a) M. C. Tseng, W. L. Su, Y. C. Yu, S. P. Wang, W. L. Huang, *Inorg. Chim. Acta* **2006**, *359*, 4144–4148; b) C.-H. Yang, W.-L. Su, K.-H. Fang, S.-P. Wang, I.-W. Sun, *Organometallics* **2006**, *25*, 4514–4519.
[25] a) Q. Zhao, C.-Y. Jiang, M. Shi, F.-Y. Li, T. Yi, Y. Cao, C.-H. Huang, *Organometallics* **2006**, *25*, 3631–3638; b) Q. Zhao, S. Liu, M. Shi, C. Wang, M. Yu, L. Li, F. Li, T. Yi, C. Huang, *Inorg. Chem.* **2006**, *45*, 6152–6160.
[26] a) M. G. Colombo, T. C. Brunold, T. Riedener, H. U. Güdel, M. Förtsch, H.-B. Bürgi, *Inorg. Chem.* **1994**, *33*, 545–550; b) M. G. Colombo, A. Hauser, H. U. Güdel, *Top. Curr. Chem.* **1994**, *171*, 143–171.
[27] a) J.-P. Collin, I. M. Dixon, J.-P. Sauvage, J. A. G. Williams, F. Barigelletti, L. Flamigni, *J. Am. Chem. Soc.* **1999**, *121*, 5009–5016; b) I. M. Dixon, J.-P. Collin, J.-P. Sauvage, L. Flamigni, S. Encinas, F. Barigelletti, *Chem. Soc. Rev.* **2000**, *29*, 385–391; c) E. Baranoff, J.-P. Collin, L. Flamigni, J.-P. Sauvage, *Chem. Soc. Rev.* **2004**, *33*, 147–155; d) L. Flamigni, E. Baranoff, J.-P. Collin, J.-P. Sauvage, *Chem. Eur. J.* **2006**, *12*, 6592–6606.
[28] a) M. Polson, S. Fracasso, V. Bertolasi, M. Ravaglia, F. Scandola, *Inorg. Chem.* **2004**, *43*, 1950–1956; b) M. Polson, M. Ravaglia, S. Fracasso, M. Garavelli, F. Scandola, *Inorg. Chem.* **2005**, *44*, 1282–1289.
[29] a) T. Yutaka, S. Obara, S. Ogawa, K. Nozaki, N. Ikeda, T. Ohno, Y. Ishii, K. Sakai, M. Haga, *Inorg. Chem.* **2005**, *44*, 4737–4746; b) S. Obara, M. Itabashi, F. Okuda, S. Tamaki, Y. Tanabe, Y. Ishii, K. Nozaki, M. Haga, *Inorg. Chem.* **2006**, *45*, 8907–8921.
[30] a) E. A. Plummer, J. W. Hofstraat, L. De Cola, *Dalton Trans.* **2003**, 2080–2084; b) P. Coppo, E. A. Plummer, L. De Cola, *Chem. Commun.* **2004**, 1774–1775; c) P. Coppo, M. Duati, V. N. Kozhevnikov, J. W. Hofstraat, L. De Cola, *Angew. Chem.* **2005**, *117*, 1840–1844; *Angew. Chem. Int. Ed.* **2005**, *44*, 1806–1810.
[31] a) E. Holder, V. Marin, M. A. R. Meier, U. S. Schubert, *Macromol. Rapid Commun.* **2004**, *25*, 1491–1496; b) E. Holder, V. Marin, A. Alexeev, U. S. Schubert, *J. Polym. Sci. Polym. Chem.* **2005**, *43*, 2765–2776; c) A. Winter, C. Ulbricht, E. Holder, N. Risch, U. S. Schubert, *Aust. J. Chem.* **2006**, *59*, 773–782.
[32] a) H. Yersin, *Top. Curr. Chem.* **2004**, *241*, 1–26; b) J. Breu, P. Stössel, S. Schrader, A. Starukhin, W. J. Finkenzeller, H. Yersin, *Chem. Mater.* **2005**, *17*, 1745–1752.
[33] a) K. K.-W. Lo, C.-K. Chung, N. Zhu, *Chem. Eur. J.* **2003**, *9*, 475–483; b) K. K.-W. Lo, C.-K. Chung, T. K.-M. Lee, L.-H. Lui, K. H.-K. Tsang, N. Zhu, *Inorg. Chem.* **2003**, *42*, 6886–6897; c) K. K.-W. Lo, J. S.-W. Chan, L.-H. Lui, C.-K. Chung, *Organometallics* **2004**, *23*, 3108–3116; d) K. K.-W. Lo, C.-K. Li, J. S.-Y. Lau, *Organometallics* **2005**, *24*, 4594–4601; e) K. K.-W. Lo, C.-K. Chung, N. Zhu, *Chem. Eur. J.* **2006**, *12*, 1500–1512.
[34] H. F. VanBrocklin, A. Liu, M. J. Welch, J. P. O'Neil, J. A. Katzenellenbogen, *Steroids* **1994**, *59*, 34–45.
[35] D. J. Minick, J. H. Frenz, M. A. Patrick, D. A. Brent, *J. Med. Chem.* **1988**, *31*, 1923–1933.
[36] Y. Yamada, K. Matsuura, K. Kobayashi, *Bioorg. Med. Chem.* **2005**, *13*, 1913–1922.
[37] J. A. Schwartz, D. F. Skafar, *Biochemistry* **1993**, *32*, 10109–10115.
[38] J. A. Katzenellenbogen, H. J. Johnson, Jr., H. N. Myers, *Biochemistry* **1973**, *12*, 4085–4092.
[39] a) K. Kalyanasundaram, *Photochemistry of Polypyridine and Porphyrin Complexes*, Academic Press, San Diego, **1992**; b) A. Juris, M. T. Gandolfi, M. F. Manfrin, V. Balzani, *J. Am. Chem. Soc.* **1976**, *98*, 1047–1048; c) J. N. Demas, J. W. Addington, S. H. Peterson, E. W. Harris, *J. Phys. Chem.* **1977**, *81*, 1039–1043; d) A. Juris, M. F. Manfrin, M. Maestri, N. Serpone, *Inorg. Chem.* **1978**, *17*, 2258–2261.
[40] J. Telser, K. A. Cruickshank, K. S. Schanze, T. L. Netzel, *J. Am. Chem. Soc.* **1989**, *111*, 7221–7226.
[41] E. B. Araujo, J. S. Santos, M. T. Colturato, E. Muramoto, C. P. G. Silva, *Appl. Radiat. Isot.* **2003**, *58*, 667–673.
[42] L. F. Capitán-Vallvey, P. Espinosa, *Polyhedron* **1983**, *2*, 1147–1153.
[43] M. M. Bradford, *Anal. Biochem.* **1976**, *72*, 248–254.
[44] J. N. Demas, G. A. Crosby, *J. Phys. Chem.* **1971**, *75*, 991–1024.
[45] K. Nakamura, *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2697–2705.

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