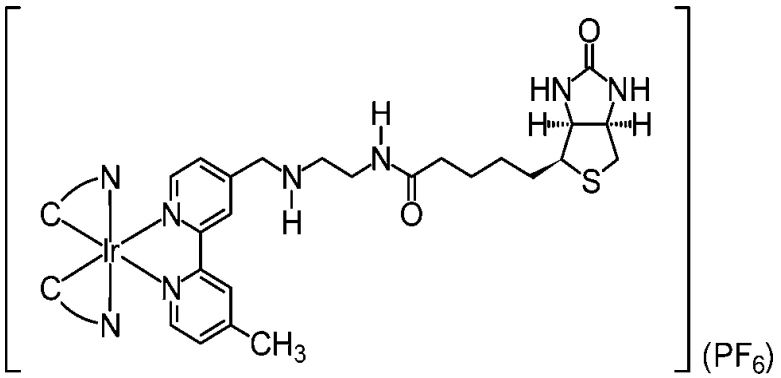
Article

Novel Luminescent Cyclometalated Iridium(III) Diimine Complexes That Contain a Biotin Moiety

Kenneth Kam-Wing Lo, Joe Sai-Wan Chan, Lok-Hei Lui, and Chi-Keung Chung

Organometallics, **2004**, 23 (13), 3108-3116 • DOI: 10.1021/om0499355



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 16 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Articles

Novel Luminescent Cyclometalated Iridium(III) Diimine Complexes That Contain a Biotin Moiety

Kenneth Kam-Wing Lo,* Joe Sai-Wan Chan, Lok-Hei Lui, and Chi-Keung Chung

Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, People's Republic of China

Received January 26, 2004

We report the synthesis and photophysical and electrochemical properties of a series of cyclometalated iridium(III) diimine complexes equipped with a biotin moiety [Ir(N−C)$_2$(bpy-en-biotin)](PF$_6$) (HN−C = 2-phenylpyridine, Hppy (**1**); 2-(4-methylphenyl)pyridine, Hmppy (**2**); 1-phenylpyrazole, Hppz (**3**); 3-methyl-1-phenylpyrazole, Hmppz (**4**); 7,8-benzoquinoline, Hbzq (**5**); 2-phenylquinoline, Hpq (**6**); bpy-en-biotin = 4-(*N*-((2-biotinamido)ethyl)aminomethyl)-4′-methyl-2,2′-bipyridine). Upon photoexcitation, complexes **1**−**6** display intense and long-lived emission in fluid solutions at 298 K and in low-temperature glass. The emission is assigned to a triplet metal-to-ligand charge-transfer (^{3}MLCT) (dπ(Ir) → π*(bpy-en-biotin)) excited state. However, the excited state of complex **6** is likely to possess substantial triplet intraligand (^{3}IL) ($\pi \rightarrow \pi^*$) (pq$^-$) character. We have studied the binding of these iridium(III) biotin complexes to avidin by 4′-hydroxyazobenzene-2-carboxylic acid (HABA) assays, luminescence titrations, and competitive assays using native biotin. Homogeneous competitive assays for biotin have also been designed.

Introduction

Biotin (vitamin H) shows an extremely high affinity to the glycoprotein avidin (first dissociation constant, K_d = ca. 10^{-15} M). Owing to the strong binding interaction, the avidin−biotin system has been widely utilized in bioanalytical applications.[1] Biomolecules can be modified with a wide range of biotinylating reagents and can then be selectively recognized by avidin molecules labeled with fluorophores or enzymes.[2,3] In theory, since avidin has four biotin-binding sites, it can be used as a bridge and the biotinylated biomolecules can be recognized by fluorophore−biotin conjugates. However, this approach is not feasible because fluorescent biotin molecules suffer from efficient self-quenching due to resonance-energy transfer (RET) upon binding to avidin, unless long spacers are present between the fluorophore and biotin.[4]

Recently, we reported the use of luminescent rhenium(I) polypyridine biotin conjugates as luminescent probes for avidin.[5] Due to the large Stokes shift of these complexes, emission quenching was not observed when they bind to avidin. Emission enhancement actually occurred after the complexes bind to the hydrophobic biotin-binding sites of the protein. We envisage that other luminescent metal biotin conjugates would behave similarly. In view of the interesting luminescence properties of iridium(III) polypyridine complexes,[6−19] and our recent interest in using these complexes as biological labeling reagents,[19] we have designed a new

* To whom correspondence should be addressed. Fax: (852) 2788 7406. Tel: (852) 2788 7231. E-mail: bhkenlo@cityu.edu.hk.

(1) Wilchek, M.; Bayer, E. A. *Methods in Enzymology*; Academic Press: San Diego, 1990; Vol. 184.

(2) Christopoulos, T. K.; Diamandis, E. P. *Immunoassay*; Academic Press: San Diego, CA, 1996; p 227.

(3) Hermanson, G. T. *Bioconjugate Techniques*; Academic Press: San Diego, CA, 1996.

(4) (a) Gruber, H. J.; Marek, M.; Schindler, H.; Kaiser, K. *Bioconjugate Chem.* **1997**, *8*, 552. (b) Marek, M.; Kaiser, K.; Gruber, H. J. *Bioconjugate Chem.* **1997**, *8*, 560. (c) Kada, G.; Falk, H.; Gruber, H. J. *Biochim. Biophys. Acta* **1999**, *1427*, 33. (d) Kada, G.; Kaiser, K.; Falk, H.; Gruber, H. J. *Biochim. Biophys. Acta* **1999**, *1427*, 44. (e) Gruber, H. J.; Hahn, C. D.; Kada, G.; Riener, C. K.; Harms, G. S.; Ahrer, W.; Dax, T. G.; Knaus, H.-G. *Bioconjugate Chem.* **2000**, *11*, 696.

(5) Lo, K. K. W.; Hui, W. K.; Ng, D. C. M. *J. Am. Chem. Soc.* **2002**, *124*, 9344.

(6) (a) Ohsawa, Y.; Sprouse, S.; King, K. A.; DeArmond, M. K.; Hanck, K. W.; Watts, R. J. *J. Phys. Chem.* **1987**, *91*, 1047. (b) Garces, F. O.; King, K. A.; Watts, R. J. *Inorg. Chem.* **1988**, *27*, 3464. (c) Wilde, A. P.; Watts, R. J. *J. Phys. Chem.* **1991**, *95*, 622. (d) Wilde, A. P.; King, K. A.; Watts, R. J. *J. Phys. Chem.* **1991**, *95*, 629.

(7) (a) Serroni, S.; Juris, A.; Campagna, S.; Venturi, M.; Denti, G.; Balzani, V. *J. Am. Chem. Soc.* **1994**, *116*, 9086. (b) Calogero, G.; Giuffrida, G.; Serroni, S.; Ricevuto, V.; Campagna, S. *Inorg. Chem.* **1995**, *34*, 541. (c) Di Marco, G.; Lanza, M.; Mamo, A.; Stefio, I.; Di Pietro, C.; Romeo, G.; Campagna, S. *Anal. Chem.* **1998**, *70*, 5019. (d) Griffiths, P. M.; Loiseau, F.; Puntoriero, F.; Serroni, S.; Campagna, S. *Chem. Commun.* **2000**, 2297.

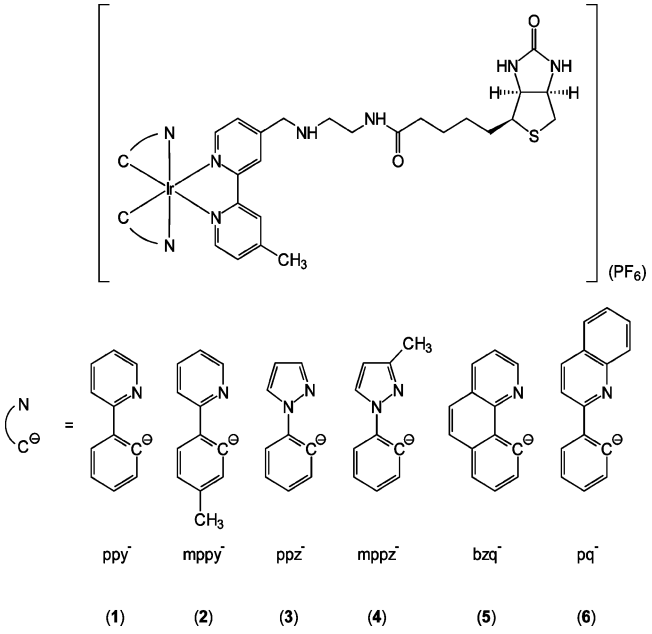
(8) (a) Vogler, L. M.; Scott, B.; Brewer, K. J. *Inorg. Chem.* **1993**, *32*, 898. (b) Bridgewater, J. S.; Vogler, L. M.; Molnar, S. M.; Brewer, K. J. *Inorg. Chim. Acta* **1993**, *208*, 179. (c) Molnar, S. M.; Nallas, G.; Bridgewater, J. S.; Brewer, K. J. *J. Am. Chem. Soc.* **1994**, *116*, 5206.

(9) (a) Didier, P.; Ortmans, I.; Kirsch-De Mesmaeker, A.; Watts, R. J. *Inorg. Chem.* **1993**, *32*, 5239. (b) Ortmans, I.; Didier, P.; Kirsch-De Mesmaeker, A. *Inorg. Chem.* **1995**, *34*, 3695.

(10) (a) Collin, J.-P.; Dixon, I. M.; Sauvage, J.-P.; Williams, J. A. G.; Barigelletti, F.; Flamigni, L. *J. Am. Chem. Soc.* **1999**, *121*, 5009. (b) Dixon, I. M.; Collin, J.-P.; Sauvage, J.-P.; Flamigni, L.; Encinas, S.; Barigelletti, F. *Chem. Soc. Rev.* **2000**, *29*, 385. (c) Dixon, I. M.; Collin, J.-P.; Sauvage, J.-P.; Barigelletti, F.; Flamigni, L. *Angew. Chem., Int. Ed.* **2000**, *39*, 1292. (d) Flamigni, L.; Dixon, I. M.; Collin, J.-P.; Sauvage, J.-P. *Chem. Commun.* **2000**, 2479.

10.1021/om0499355 CCC: $27.50
Publication on Web 05/13/2004

Chart 1. Structures of Complexes 1−6



series of luminescent cyclometalated iridium(III) polypyridine complexes containing a biotin moiety, $[Ir(N{-}C)_2(bpy\text{-}en\text{-}biotin)](PF_6)$ (HN−C = 2-phenylpyridine, Hppy (**1**); 2-(4-methylphenyl)pyridine, Hmppy (**2**); 1-phenylpyrazole, Hppz (**3**); 3-methyl-1-phenylpyrazole, Hmppz (**4**); 7,8-benzoquinoline, Hbzq (**5**); 2-phenylquinoline, Hpq (**6**); bpy-en-biotin = 4-(*N*-((2-biotinamido)ethyl)-aminomethyl)-4′-methyl-2,2′-bipyridine). The structures of the complexes are shown in Chart 1. The synthesis, characterization, photophysical and electrochemical properties, and the avidin-binding properties of these conjugates are described here.

Results and Discussion

Synthesis. The biotin-containing ligand bpy-en-biotin is synthesized from the reaction of biotinylethylenediamine[20] with 4-formyl-4′-methyl-2,2′-bipyridine[21] in refluxing ethanol, followed by reduction with sodium borohydride. Complexes **1**−**6** are obtained from the reaction of $[Ir_2(N{-}C)_4Cl_2]$ with bpy-en-biotin in refluxing methanol/dichloromethane. The complexes are converted to the PF_6^- salt, purified by column chromatography and recrystallization, and finally isolated as air-stable orange-yellow crystals. All the complexes are characterized by ^{1}H NMR spectroscopy, positive-ion ESI-MS, and IR spectroscopy and give satisfactory microanalysis.

Table 1. Electronic Absorption Spectral Data for Complexes 1−6 and $[Ir(ppy)_2(bpy)](PF_6)$ at 298 K

complex	medium	λ_{abs}/nm (ϵ/dm^3 mol^{-1} cm^{-1})
1	CH_3CN	257 (41,585), 267 sh (38,455), 309 sh (16,965), 343 sh (7160), 381 sh (4395), 418 sh (2515), 468 sh (540)
	MeOH	257 (41,190), 269 sh (38,420), 310 sh (17,700), 340 sh (7865), 379 sh (4970), 414 sh (2890), 469 sh (650)
2	CH_3CN	259 (52,200), 272 sh (50,950), 311 sh (24,115), 381 sh (6475), 410 sh (4085), 473 sh (690)
	MeOH	258 (45,340), 271 (45,600), 310 sh (22,470), 379 sh (6255), 413 sh (3570), 472 sh (695)
3	CH_3CN	251 sh (28,450), 309 sh (10,900), 433 sh (590)
	MeOH	250 sh (37,210), 310 sh (13,800), 437 sh (590)
4	CH_3CN	252 (25,550), 309 sh (9995), 348 sh (3980), 436 sh (385)
	MeOH	249 (26,855), 310 sh (10,535), 353 sh (4005), 451 sh (465)
5	CH_3CN	251 (42,630), 260 sh (39,565), 310 (16,685), 354 sh (9695), 413 (3900)
	MeOH	252 (34,700), 310 (13,710), 346 sh (9515), 416 (3225)
6	CH_3CN	235 sh (32,610), 268 (39,870), 310 sh (17,265), 336 (18,015), 349 sh (15,960), 436 (4035), 524 sh (355)
	MeOH	236 sh (33,710), 259 (37,930), 281 (38,415), 307 sh (18,285), 337 (18,055), 350 sh (16,035), 397 sh (3325), 439 (3980), 525 sh (340)
$[Ir(ppy)_2(bpy)](PF_6)$	CH_3CN	255 (45,230), 265 sh (43,475), 274 sh (37,405), 311 sh (19,300), 337 sh (8620), 376 sh (5600), 411 sh (3315), 465 sh (670)
	MeOH	255 (36,030), 266 sh (35,310), 310 sh (16,575), 337 sh (7235), 378 sh (4810), 412 sh (2810), 465 sh (620)

Electronic Absorption Spectroscopy. The electronic absorption spectral data of complexes **1**−**6** and the model complex $[Ir(ppy)_2(bpy)](PF_6)$ in CH_3CN and MeOH are listed in Table 1. The absorption spectra of complexes **1** and **6** in CH_3CN are shown in Figure 1. Complexes **1**−**6** show intense absorption bands and shoulders at ca. 235−311 nm (ϵ on the order of 10^4 dm^3 mol^{-1} cm^{-1}). With reference to the absorption studies of related cyclometalated iridium(III) systems,[6a−c,7,9,11d,15a,16,19a,c,d] these absorption bands are attributed to spin-allowed intraligand (^{1}IL) ($\pi \rightarrow \pi^*$) (bpy-en-biotin and N−C$^-$) transitions. The spectra of the complexes also display less intense absorption shoulders

(11) (a) Colombo, M. G.; Güdel, H. U. *Inorg. Chem.* **1993**, *32*, 3081. (b) Colombo, M. G.; Hauser, A.; Güdel, H. U. *Inorg. Chem.* **1993**, *32*, 3088. (c) Colombo, M. G.; Brunold, T. C.; Riedener, T.; Güdel, H. U.; Förtsch, M.; Bürgi, H.-B. *Inorg. Chem.* **1994**, *33*, 545. (d) Colombo, M. G.; Hauser, A.; Güdel, H. U. *Top. Curr. Chem.* **1994**, *171*, 143.

(12) (a) Lamansky, S.; Djurovich, P.; Murphy, D.; Abdel-Razzaq, F.; Kwong, R.; Tsyba, I.; Bortz, M.; Mui, B.; Bau, R.; Thompson, M. E. *Inorg. Chem.* **2001**, *40*, 1704. (b) Lamansky, S.; Djurovich, P.; Murphy, D.; Abdel-Razzaq, F.; Lee, H.-E.; Adachi, C.; Burrows, P. E.; Forrest, S. R.; Thompson, M. E. *J. Am. Chem. Soc.* **2001**, *123*, 4304. (c) Gao, R.; Ho, D. G.; Hernandez, B.; Selke, M.; Murphy, D.; Djurovich, P. I.; Thompson, M. E. *J. Am. Chem. Soc.* **2002**, *124*, 14828.

(13) (a) Licini, M.; Williams, J. A. G. *Chem. Commun.* **1999**, 1943. (b) Goodall, W.; Williams, J. A. G. *J. Chem. Soc., Dalton Trans.* **2000**, 2893.

(14) Ayala, N. P.; Flynn, C. M., Jr.; Sacksteder, L.; Demas, J. N.; DeGraff, B. A. *J. Am. Chem. Soc.* **1990**, *112*, 3837.

(15) (a) van Diemen, J. H.; Haasnoot, J. G.; Hage, R.; Müller, E.; Reedijk, J. *Inorg. Chim. Acta* **1991**, *181*, 245. (b) van Diemen, J. H.; Hage, R.; Haasnoot, J. G.; Lempers, H. E. B.; Reedijk, J.; Vos, J. G.; De Cola, L.; Barigelletti, F.; Balzani, V. *Inorg. Chem.* **1992**, *31*, 3518.

(16) Maestri, M.; Balzani, V.; Deuschel-Cornioley, C.; von Zelewsky, A. *Adv. Photochem.* **1992**, *17*, 1.

(17) DeRosa, M. C.; Mosher, P. J.; Yap, G. P. A.; Focsaneanu, K.-S.; Crutchley, R. J.; Evans, C. E. B. *Inorg. Chem.* **2003**, *42*, 4864.

(18) Tsuboyama, A.; Iwawaki, H.; Furugori, M.; Mukaide, T.; Kamatani, J.; Igawa, S.; Moriyama, T.; Miura, S.; Takiguchi, T.; Okada, S.; Hoshino, M.; Ueno, K. *J. Am. Chem. Soc.* **2003**, *125*, 12971.

(19) (a) Lo, K. K. W.; Ng, D. C. M.; Chung, C. K. *Organometallics* **2001**, *20*, 4999. (b) Lo, K. K. W.; Chung, C. K.; Ng, D. C. M.; Zhu, N. *New J. Chem.* **2002**, *26*, 81. (c) Lo, K. K. W.; Chung, C. K.; Zhu, N. *Chem. Eur. J.* **2003**, *9*, 475. (d) Lo, K. K. W.; Chung, C. K.; Lee, T. K. M.; Lui, L. H.; Tsang, K. H. K.; Zhu, N. *Inorg. Chem.* **2003**, *42*, 6886.

(20) Garlick, R. K.; Giese, R. W. *J. Biol. Chem.* **1988**, *263*, 210.

(21) Peek, B. M.; Ross, G. T.; Edwards, S. W.; Meyer, G. J.; Meyer, T. J.; Erickson, B. W. *Int. J. Pept. Protein Res.* **1991**, *38*, 114.

Table 2. Emission Data of Complexes 1−6 and $[Ir(ppy)_2(bpy)](PF_6)$[a]

complex	medium (*T*/K)	λ_{em}/nm	τ_o/μs	Φ_{em}
1	CH_3CN (298)	576	0.48	0.16
	MeOH (298)	577	0.36	0.11
	H_2O/DMSO (298)[b]	584	0.21	0.035
	Glass (77)[c]	473 sh, 506, 537 sh	4.74	
2	CH_3CN (298)	587	0.35	0.081
	MeOH (298)	580	0.24	0.065
	H_2O/DMSO (298)[b]	589	0.17	0.053
	glass (77)[c]	475 sh, 513, 542 sh	4.57	
3	CH_3CN (298)	560	0.92	0.19
	MeOH (298)	561	0.62	0.11
	H_2O/DMSO (298)[b]	567	0.28	0.12
	glass (77)[c]	496, 527 sh	5.47	
4	CH_3CN (298)	574	0.39	0.093
	MeOH (298)	577	0.28	0.079
	H_2O/DMSO (298)[b]	576	0.17	0.058
	Glass (77)[c]	509, 538 sh	4.38	
5	CH_3CN (298)	577	0.47	0.12
	MeOH (298)	580	0.29	0.054
	H_2O/DMSO (298)[b]	583	0.17	0.054
	glass (77)[c]	502 (max), 541, 583 sh	41.36 (33%), 5.12 (67%)	
6	CH_3CN (298)	554, 605 sh	2.95	0.37
	MeOH (298)	551, 605 sh	2.90	0.37
	H_2O/DMSO (298)[b]	559, 604 sh	1.88	0.24
	glass (77)[c]	540 (max), 582, 632 sh	4.78	
$[Ir(ppy)_2(bpy)](PF_6)$	CH_3CN (298)	588	0.37	0.093
	MeOH (298)	588	0.28	0.087
	glass (77)[c]	474 sh, 515, 549 sh	5.08	

[a] Estimated errors on emission wavelengths = ±1 nm, emission lifetimes = ±10%, emission quantum yields = ±20%. [b] H_2O/DMSO (1:1 v/v). [c] EtOH/MeOH (4:1 v/v).

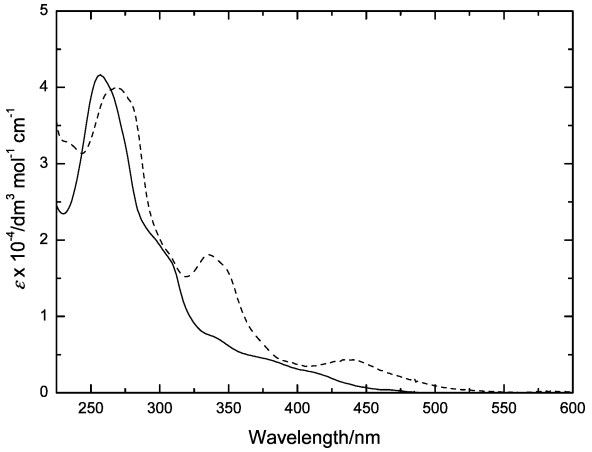


Figure 1. Electronic absorption spectra of complexes **1** (—) and **6** (- - -) in CH_3CN at 298 K.

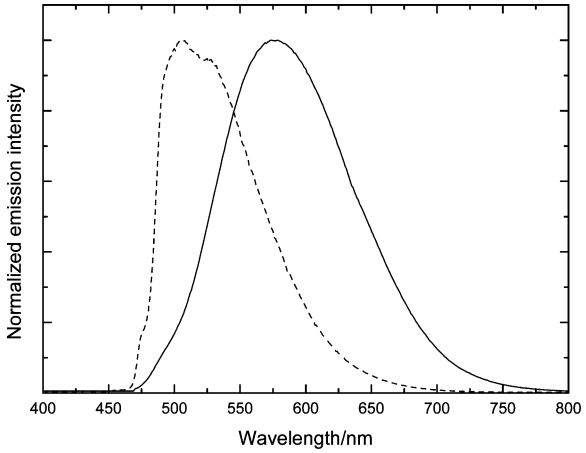


Figure 2. Emission spectra of complex **1** in CH_3CN at 298 K (—) and in EtOH/MeOH (4:1 v/v) at 77 K (- - -).

at ca. 340−418 nm. These shoulders are assigned to spin-allowed metal-to-ligand charge-transfer (^{1}MLCT) (dπ(Ir) → π*(bpy-en-biotin and N−C$^-$)) transitions.[6a−c,7,9,11d,15a,16,19a,c,d] However, owing to the extended π-conjugation of the pq$^-$ ligand, the lowest-energy ^{1}IL (π → π*) (pq$^-$) absorption of complex **6** extends to ca. 350 nm and the ^{1}MLCT (dπ(Ir) → π*(bpy-en-biotin and N−C$^-$)) absorption features occur at further lower energy (ca. 397−439 nm) (Figure 1). In addition, all the complexes show weak absorption tails at ca. 450−550 nm, which are attributable to spin-forbidden ^{3}MLCT (dπ(Ir) → π*(bpy-en-biotin and N−C$^-$)) transitions. It is interesting to note that the absorption bands and shoulders of complex **1** closely resemble those of the model complex (Table 1), indicating that there are no interactions between the iridium chromophore and the biotin unit.

Luminescence Properties. Upon excitation, all the complexes show intense and long-lived orange to green-ish-yellow luminescence in fluid solutions under ambient conditions and in low-temperature glass. The emission data of the iridium(III) biotin complexes and the model complex $[Ir(ppy)_2(bpy)](PF_6)$ are collected in Table 2. The emission spectra of complex **1** in CH_3CN at 298 K and in low-temperature glass are shown in Figure 2. In fluid solutions at room temperature, complexes **1**−**6** emit at ca. 551−589 nm, and the emission is assigned to an ^{3}MLCT (dπ(Ir) → π*(bpy-en-biotin)) excited state.[6,7b,9a,10b,11a,b,d,15a,19a,d] This assignment is supported by the observations that complex **1** emits at higher energy than the model complex (Table 2) because the electron-donating methyl and aminomethyl substituents of bpy-en-biotin would destabilize the π* orbitals of this diimine ligand and thus lead to higher ^{3}MLCT (dπ(Ir) → π*(N−N)) emission energy for complex **1**. The ^{3}MLCT assignment is further supported by the findings that complexes **1** and **3** emit at slightly higher energy than complexes **2** and **4**, respectively (Table 2). It is likely

Table 3. Electrochemical Data and E^{00} Values for Complexes 1−6 and $[Ir(ppy)_2(bpy)](PF_6)$ at 298 K[a]

	ground state		excited state		
complex	oxidation, $E_{1/2}$ or E_a/V	reduction, $E_{1/2}$ or E_c/V	$E°(Ir^{2+/+*})$/V	$E°(Ir^{+*/0})$/V	E^{00}/eV
1	+1.24, +1.07[b]	−1.47, −1.95,[b] −2.18,[c] −2.50[b]	−1.22	+0.99	2.46
2	+1.19, +1.09[b]	−1.47, −2.01,[b] −2.31,[b] −2.59[b]	−1.23	+0.95	2.42
3	+1.34, +1.11[b]	−1.45,[c] −2.78[b]	−1.17	+1.06	2.51
4	+1.28, +1.07[b]	−1.48, −2.03,[b] −2.72[b]	−1.16	+0.96	2.44
5	+1.18,[c] +1.00[b]	−1.46, −1.94,[b] −2.27[b]	−1.30	+1.02	2.48
6	+1.28, +1.13[b]	−1.47, −1.79,[b] −1.96,[c] −2.30[b]	−1.02	+0.83	2.30
$[Ir(ppy)_2(bpy)](PF_6)$	+1.24	−1.40, −2.01,[b] −2.19, −2.51,[b]	−1.17	+1.01	2.41

[a] In CH_3CN (0.1 M TBAP), glassy carbon electrode, sweep rate 0.1 V s^{-1}, all potentials vs SCE. [b] Irreversible wave. [c] Quasi-reversible wave.

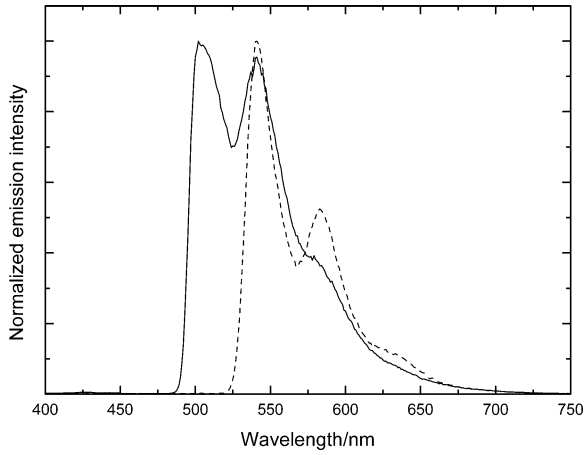


Figure 3. Emission spectra of complexes **5** (—) and **6** (- - -) in EtOH/MeOH (4:1 v/v) at 77 K.

that the electron-donating methyl groups of the cyclometalating ligands of complexes **2** ($mppy^-$) and **4** ($mppz^-$) render the iridium(III) centers more electron rich and thus stabilize the ^{3}MLCT ($d\pi(Ir) \rightarrow \pi^*$(bpy-en-biotin)) emissive states of these two complexes compared to those of complexes **1** and **3**, respectively. Complex **6** shows structured emission spectra and very long emission lifetimes (τ_o = ca. 2−3 μs) in fluid solutions at 298 K, suggesting that its emissive state possesses substantial ^{3}IL ($\pi \rightarrow \pi^*$) (pq^-) character. Similar observations have been made in related iridium(III)−pq systems.[12,19d] Nevertheless, the involvement of charge-transfer character cannot be excluded.

Upon cooling to 77 K, the emission maxima of most of the complexes show significant blue shifts (Table 2 and Figure 2). Interestingly, complexes **5** and **6** show vibronically structured emission spectra with progressional spacings of ca. 1332−1436 cm^{-1} (Figure 3), typical of ν(C···C) and ν(C···N) stretching. This suggests the involvement of the bzq^- and pq^- ligands in the emissive states of these two complexes. In general, the emission of complexes **1**−**4** in low-temperature glass is tentatively assigned to an excited state of ^{3}MLCT ($d\pi(Ir) \rightarrow \pi^*$(bpy-en-biotin)) character. This is supported by the observations that complex **1** emits at higher energy compared to the model complex $[Ir(ppy)_2(bpy)](PF_6)$ (Table 2). However, complex **5** displays biexponential decay with lifetimes of ca. 41 and 5 μs in low-temperature glass. The shorter-lived component is similar in magnitude to that of complexes **1**−**4** and is thus assigned to an ^{3}MLCT state associated with the bpy-en-biotin ligand. The longer-lived component is assigned to another ^{3}MLCT state associated with the bzq^- ligand.[6,7b,9,10b,15a,16,19a,d] Meanwhile, compared to complexes **1**−**5**, the emission maximum of complex **6** in alcohol shows an exceptionally small blue shift (ca. 370 cm^{-1}) upon cooling to 77 K. On the basis of this observation and the rich vibronic structures of the emission spectrum, we believe that the emissive state of complex **6** is predominantly ^{3}IL ($\pi \rightarrow \pi^*$) (pq^-) in character,[12a,19d] although the involvement of charge-transfer character cannot be excluded.

Electrochemical Properties. The electrochemical properties of the complexes have been studied by cyclic voltammetry. The electrochemical data are listed in Table 3. Complexes **1**−**6** show a reversible/quasi-reversible couple at ca. +1.18 to +1.34 V versus SCE, attributable to a metal-centered Ir(IV/III) oxidation process.[6a,b,7b,d,11a,15b,19a,c,d] A similar reversible oxidation couple is also observed for the model complex $[Ir(ppy)_2(bpy)](PF_6)$ at ca. +1.24 V versus SCE (Table 3). In addition, complexes **1**−**6** also display an irreversible wave at ca. +1.00 to +1.13 V, which is likely to arise from the oxidation of the coordinated bpy-en-biotin ligand because (i) the model complex does not reveal such an oxidation wave and (ii) an irreversible wave is noticed for the free ligand at ca. +1.54 V in CH_3CN/MeOH (95:5 v/v). Meanwhile, complexes **1**−**6** exhibit a reversible/quasi-reversible couple at ca. −1.47 V, which is assigned to reduction of the coordinated bpy-en-biotin ligand.[6a,b,7a,b,d,9,11a,15,16,19a,c,d] This assignment is substantiated by the fact that the model complex displays a similar bpy-based reduction couple at ca. −1.40 V, which is slightly less negative compared to those of complexes **1**−**6** due to the absence of the electron-donating methyl and aminomethyl substituents. In addition, reduction waves of highly irreversible nature are also observed at lower potentials for the complexes, which are assigned to the reduction of the diimine and the cyclometalating ligands.[6a,b,7a,b,d,9,11a,15,16,19a,c,d] The excited-state redox potentials of the complexes (Table 3) are determined from the ground-state redox potentials and the E^{00} energy, which is estimated from the emission maxima of the complexes at 77 K. The potentials $E°(Ir^{2+/+*})$ and $E°(Ir^{+*/0})$ vary from −1.02 to −1.30 V and from +0.83 to +1.06 V versus SCE, respectively, indicating that the excited complexes are mild reductants and oxidants.

HABA Assay of Iridium(III) Biotin Complexes. The binding of complexes **1**−**6** to avidin has been studied by the HABA assay. The assay is based on the competition between unmodified biotin or biotinylated species and the dye 4′-hydroxyazobenzene-2-carboxylic acid (HABA) on binding to avidin.[3,22] The binding of HABA to avidin is associated with an absorption feature at ca. 500 nm. Since the binding of HABA to avidin (first

(22) Green, N. M. *Adv. Protein Chem.* **1975**, *29*, 85.

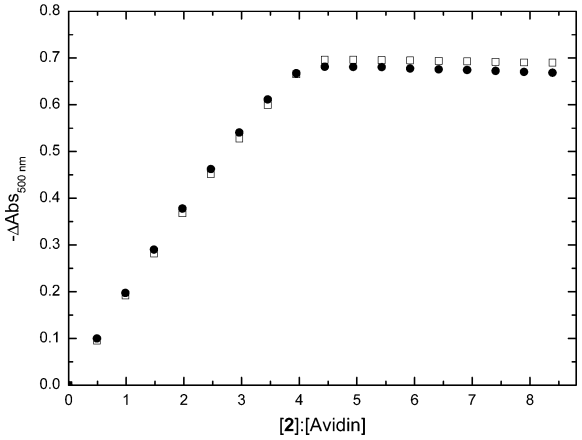


Figure 4. Spectroscopic titration curves for the titrations of the avidin−HABA complex with unmodified biotin (□) and complex **2** (●).

dissociation constant, $K_d = 6 \times 10^{-6}$ M) is much weaker than that of biotin ($K_d = 10^{-15}$ M), addition of biotin will displace the bound HABA molecules, leading to a decrease of the absorbance at 500 nm. In this work, addition of complexes **1**−**6** to a mixture of avidin and HABA results in a decrease in absorbance at 500 nm, suggesting the binding of the biotin moieties of the iridium(III) complexes to avidin. The spectroscopic titration curves for the titrations of avidin−HABA with free biotin and complex **2** are shown in Figure 4 as an example. The plots of $-\Delta Abs_{500\ nm}$ versus [Ir]:[avidin] for complexes **1**−**6** show that the equivalence points occur at [Ir]:[avidin] = from ca. 4.1 to 5.2. Assuming that avidin can only specifically bind the complexes at the four biotin-binding sites, the occurrence of the equivalence points at [Ir]:[avidin] > 4 suggests that the binding of these iridium(III) biotin complexes to avidin is not substantially stronger than that of HABA. To gain further insights into the avidin-binding properties of these complexes, luminescence titrations and association and dissociation assays are performed.

Luminescence Titrations and Association and Dissociation Assays. The binding of complexes **1**−**6** to avidin has been investigated by luminescence titrations using the complexes as the titrants.[4,5] The results are compared to two series of control titrations in which (i) avidin is absent and (ii) the avidin solution is saturated with excess unmodified biotin. The luminescence titrations of complexes **1** and **6** are shown in Figures 5 and 6, respectively. The luminescence titration results show that all the complexes display enhanced emission intensities in the presence of avidin. At the equivalence points, the emission intensities and lifetimes of the complexes increase by factors of ca. 1.5−3.3 (Table 4). These observations are in line with our earlier results on luminescent rhenium(I) polypyridine biotin conjugates.[5] We ascribe the emission enhancement and lifetime elongation to the binding of the biotin moieties of complexes **1**−**6** to the biotin-binding sites of avidin because no similar observations are noticed when excess unmodified biotin is initially present in the avidin solution (Table 4). It is likely that the observed enhancement is associated with the hydrophobicity of the biotin-binding sites of the avidin molecule, given that the emission quantum yields and lifetimes of the complexes are higher and longer in more nonpolar solvents (Table 2). While fluorophore−biotin conjugates exhibit significant emission quenching due to RET upon binding to avidin,[4] the iridium(III) biotin complexes in the current work do not suffer from self-quenching even when they are in close proximity. We reason that the insignificant overlap between absorption and emission

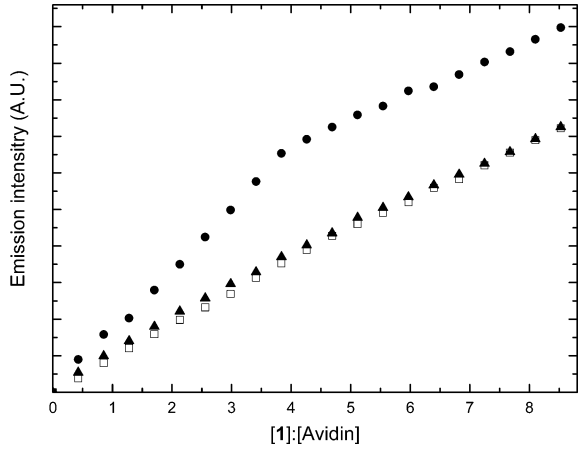


Figure 5. Luminescence titration curves for the titrations of (i) 3.8 μM avidin (●), (ii) 3.8 μM avidin and 380.0 μM unmodified biotin (▲), and (iii) a blank phosphate buffer solution (□) with complex **1**.

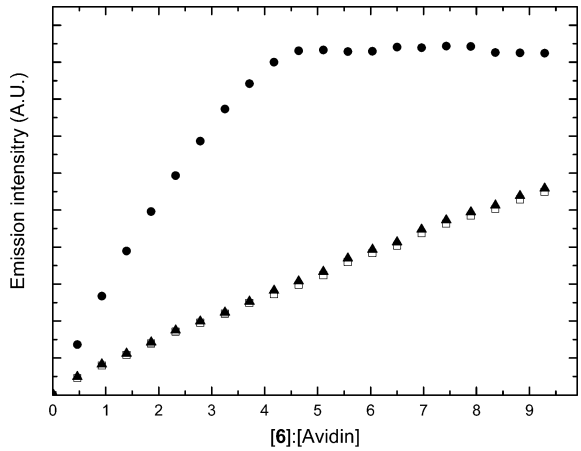


Figure 6. Luminescence titration curves for the titrations of (i) 3.8 μM avidin (●), (ii) 3.8 μM avidin and 380.0 μM unmodified biotin (▲), and (iii) a blank phosphate buffer solution (□) with complex **6**.

Table 4. Relative Emission Intensities and Emission Lifetimes of Complexes 1−6 in the Absence and Presence of Avidin (and Excess Biotin)[a]

complex	I (τ/ns)[b]	I (τ/ns)[c]	I (τ/ns)[d]
1	1.00 (90)	1.85 (140)	1.05 (82)
2	1.00 (83)	1.88 (148)	0.91 (87)
3	1.00 (71)	1.48 (232)	0.99 (79)
4	1.00 (59)	1.95 (166)	1.04 (65)
5	1.00 (73)	1.81 (147)	0.97 (80)
6	1.00 (1533)	3.29 (2343)	1.04 (1529)

[a] In aerated 50 mM potassium phosphate buffer pH 7.4 at 298 K. Degassed solutions were used for the emission lifetime measurements. Estimated errors on relative emission intensities = ±10%, emission lifetimes = ±10%. [b] [Ir] = 15.2 μM, [avidin] = 0 μM, [unmodified biotin] = 0 μM. [c] [Ir] = 15.2 μM, [avidin] = 3.8 μM, [unmodified biotin] = 0 μM. [d] [Ir] = 15.2 μM, [avidin] = 3.8 μM, [unmodified biotin] = 380.0 μM.

Table 5. First Dissociation Constants and Results of Association and Dissociation Assays for Complexes 1−6[a]

complex	K_d/M	association assay/ % avidin bound	dissociation assay/ % avidin bound
1	2.0×10^{-10}	25	32
2	5.6×10^{-10}	22	37
3	2.8×10^{-9}	23	34
4	6.8×10^{-9}	23	35
5	2.0×10^{-8}	15	19
6	3.2×10^{-9}	17	31

[a] In 50 mM potassium phosphate buffer pH 7.4 at 298 K.

spectra of the iridium(III) complexes disfavors the self-quenching effects due to RET. It is noteworthy that complex **6**, being more hydrophobic than the other complexes, exhibits a higher degree of emission enhancement after binding to avidin. Also, unlike the other complexes, beyond the equivalence point, the emission titration curve of this complex is not parallel to those of the two control solutions, and the emission intensity remains essentially constant (Figure 6). It is conceivable that these observations are related to the high hydrophobicity of this complex. In other words, the possibility of nonspecific interactions between the avidin-bound complex and excess complex **6** cannot be excluded.[5]

The first dissociation constants (K_d) of the complexes formed between avidin and complexes **1**−**6** have been determined from the emission titration experiments.[23] The K_d values of the complexes vary from ca. 2.0×10^{-10} to 2.0×10^{-8} M (Table 5), which are about 5−7 orders of magnitudes larger than that of the native biotin−avidin system.[1] It appears that the lack of a spacer-arm between the iridium(III) luminophore and the biotin moiety of complexes **1**−**6** leads to the diminished binding strength. The competitive binding of complexes **1**−**6** and native biotin to avidin has been investigated by association and dissociation assays.[4e,5,24] In the association assays, avidin is added to a mixture of the iridium(III) biotin complex and unmodified biotin. The solution is incubated at room temperature for 1 h and then loaded onto a size exclusion column. The eluted solution that contains free and iridium-bound avidin molecules is collected and its emission intensity measured. The intensity is compared to that of the control (in which unmodified biotin is absent) in order to determine the percentage of avidin molecules that are bound with the iridium(III) biotin complex. In the dissociation assays, a mixture of avidin and the iridium(III) biotin complex is first incubated at room temperature for 1 h, followed by addition of unmodified biotin and incubation for another hour. The percentage of iridium-bound avidin molecules is determined by the same method described above. The results (Table 5) show that ca. 15−37% avidin molecules remain bound by the complexes upon challenge by native biotin in the association and dissociation assays. Complex **5** reveals the lowest avidin-bound percentage (15 and 19% for the association and dissociation assays, respectively), which is in line with its largest K_d value (Table 5). These observations could be due to the steric hindrance of the coordinated bzq^- ligands.

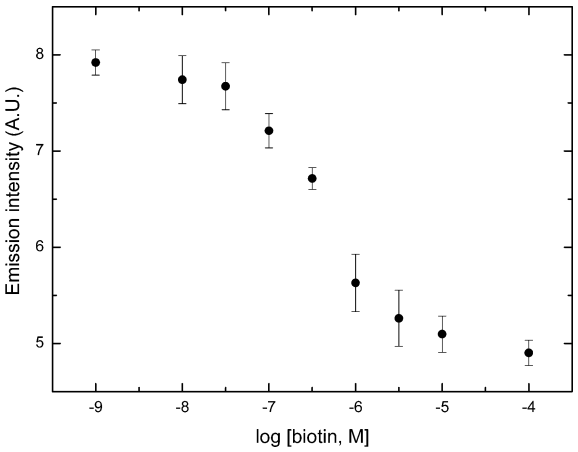


Figure 7. Homogeneous competitive assay for biotin using complex **1** and avidin. The emission intensity of the supernatant is an average of triplicate experiments ± 1 standard deviation.

Homogeneous Competitive Assays for Biotin. Since the emission intensities of the complexes are enhanced upon binding to avidin, these properties have been exploited in the design of a new homogeneous assay for biotin. The assay is based on the competition between complexes **1**−**6** and unmodified biotin on binding to avidin. In the assays, avidin is added to a solution of the iridium(III) biotin complex and the biotin analyte. After the solution is incubated at room temperature for 1 h, its emission intensity is measured. A lower biotin analyte concentration is expected to result in a higher degree of binding of the iridium(III) biotin complex to avidin, and hence a higher emission intensity. In our experiments, the emission of the solutions is measured over a biotin analyte concentration range from 1×10^{-4} to 1×10^{-9} M, and the results for the assay using complex **1** are shown in Figure 7. The concentration range of biotin that can be determined by this assay is between ca. $1 \times 10^{-7.5}$ and $1 \times 10^{-5.5}$ M. The lowest concentration of biotin analyte that gives a meaningful signal (ca. $1 \times 10^{-7.5}$ M) is about 1.5 order of magnitude lower than that of a heterogeneous competitive biotin assay we reported recently (ca. 1×10^{-6} M)[19c] but comparable to that of a competitive assay based on the change of electrode response of copper enhanced by a thiourea−biotin compound (ca. $1 \times 10^{7.54}$ M).[25] Our target is to design a system that can display a more significant change in emission properties in the presence of avidin and to develop related homogeneous and heterogeneous assays for biotinylated species.

Conclusion

A series of cyclometalated iridium(III) diimine complexes equipped with a biotin moiety have been synthesized and characterized, and their photophysical and electrochemical properties investigated. The emission data indicate that the emission of the complexes originates from an ^{3}MLCT ($d\pi$(Ir) → π^*(bpy-en-biotin)) excited state. However, the emissive state of complex **6**

(23) Wang, Z. X.; Kumar, N. R.; Srivastava, D. K. *Anal. Biochem.* **1992**, *206*, 376.

(24) Wilbur, D. S.; Pathare, P, M.; Hamlin, D. K.; Frownfelter, M. B.; Kegley, B. B.; Leung, W. Y.; Gee, K. R. *Bioconjugate Chem.* **2000**, *11*, 584.

(25) Kuramitz, H.; Natsui, J.; Tanaka, S.; Hasebe, K. *Electroanalysis* **2000**, *12*, 588.

is mixed with substantial ^{3}IL ($\pi \rightarrow \pi^*$) (pq^-) character. The binding of complexes **1**−**6** to avidin has been studied by HABA assays and luminescence titration experiments. First dissociation constants (K_d) of ca. 2.0 $\times 10^{-10}$ to 2.0 $\times 10^{-8}$ M have been determined for the avidin complexes of **1**−**6** from luminescence titrations. The binding of complexes to avidin with respect to native biotin has also been studied by competitive association and dissociation assays. The assay results show that 15−37% avidin molecules remain bound by the biotin complexes in the presence of native biotin. Homogeneous competitive assays for biotin have been developed using the complexes and avidin. The concentration range of biotin that can be determined by the assay using complex **1** is between ca. 1 $\times 10^{-7.5}$ and 1 $\times 10^{-5.5}$ M. In conclusion, we have demonstrated that new luminescent probes for avidin can be obtained by attaching a biotin moiety to cyclometalated iridium(III) diimine complexes. We anticipate that the avidin-binding properties of related luminescent biotin−transition metal complex conjugates can provide an insight into understanding protein−ligand interactions and can be utilized for the development of different bioanalytical applications.

Experimental Section

Materials and Synthesis. All solvents were of analytical reagent grade and purified according to the standard procedures.[26] The following chemicals were used without further purification: $IrCl_3 \cdot 3H_2O$ (Aldrich), Hppy (Aldrich), Hmppy (Aldrich), Hppz (Acros), Hmppz (Acros), Hbzq (Acros), Hpq (Acros), triethylamine (Acros), sodium borohydride (Acros), avidin (Calbiochem), and 4′-hydroxyazobenzene-2-carboxylic acid (HABA) (Sigma). The model complex $[Ir(ppy)_2(bpy)](PF_6)$ was synthesized according to a reported procedure.[6a]

Bpy-en-biotin. A solution of biotinylethylenediamine[20] (100 mg, 0.35 mmol), 4-formyl-4′-methyl-2,2′-bipyridine[21] (69 mg, 0.35 mmol), and 5 drops of triethylamine in 20 mL of ethanol was refluxed under an inert atmosphere of nitrogen for 3 h. The solution was then cooled to room temperature, and solid $NaBH_4$ (53 mg, 1.40 mmol) was added to the solution. The solution was then refluxed under an inert atmosphere of nitrogen for 24 h and then evaporated to dryness. The white solid was dissolved in 10 mL of ethanol/acetonitrile (4:1 v/v), filtered, and then evaporated to dryness. The white solid was then dissolved in methanol/acetone (1:1 v/v) and purified by column chromatography on silica gel. The desired product was eluted with methanol/acetone (4:1 v/v). Recrystallization from methanol/diethyl ether afforded bpy-en-biotin as a white solid. Yield: 67 mg (41%). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K, TMS): δ 8.58 (d, 1H, J = 5.0 Hz; H6′ of pyridyl ring), 8.54 (d, 1H, J = 4.7 Hz; H6 of pyridyl ring), 8.34 (s, 1H; H3′ of pyridyl ring), 8.24 (s, 1H; H3 of pyridyl ring), 7.80 (t, 1H, J = 5.9 Hz; $NH(CH_2)_2NHCO$), 7.40 (d, 1H, J = 5.0 Hz; H5′ of pyridyl ring), 7.29 (d, 1H, J = 5.0 Hz; H5 of pyridyl ring), 6.43 (s, 1H; NH of biotin), 6.37 (s, 1H; NH of biotin), 4.30−4.26 (m, 1H; NCH of biotin), 4.12−4.08 (m, 1H; NCH of biotin), 3.81 (d, 2H, J = 6.2 Hz; $CH_2NH(CH_2)_2NH$), 3.45−3.29 (m, 4H; $NH(CH_2)_2NH$), 3.10−3.03 (m, 1H; SCH of biotin), 2.82−2.73 (m, 2H; SCH of biotin), 2.42 (s, 3H; CH_3 on C4′ of pyridyl ring), 2.08−2.03 (m, 2H; $COCH_2$ of biotin), 1.61−1.24 (m, 6H; $COCH_2(CH_2)_3$ of biotin). IR (KBr): ν(cm^{-1}) 3240 (br, NH), 1696 (s, C=O). Positive-ion ESI-MS: m/z 469 $[M]^+$, 491 $[M + Na^+]^+$.

$[Ir(ppy)_2(bpy\text{-}en\text{-}biotin)](PF_6)$ (1). A mixture of $[Ir_2(ppy)_4Cl_2]$[6b] (75 mg, 0.07 mmol) and bpy-en-biotin (66 mg, 0.14 mmol) in 20 mL of methanol/dichloromethane (1:1 v/v) was refluxed under an inert atmosphere of nitrogen in the dark for 12 h. The yellow solution was then cooled to room temperature, and KPF_6 (26 mg, 0.14 mmol) was added to the solution. The mixture was stirred for 30 min at room temperature and then evaporated to dryness. The yellow solid was dissolved in acetonitrile and purified by column chromatography on alumina. The desired product was eluted with acetonitrile/methanol (1:1 v/v). Recrystallization from methanol/dichloromethane/diethyl ether afforded complex **1** as yellow crystals. Yield: 84 mg (54%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 8.85 (s, 1H; H3′ of pyridyl ring of bpy-en-biotin), 8.79 (s, 1H; H3 of pyridyl ring of bpy-en-biotin), 8.26−8.23 (m, 2H; H3 of pyridyl ring of ppy$^-$), 7.99−7.88 (m, 6H; H3 of phenyl ring of ppy$^-$, H4 of pyridyl ring of ppy$^-$, and H6 and H6′ of pyridyl ring of bpy-en-biotin), 7.84 (d, 1H, J = 4.7 Hz; H6 of pyridyl ring of ppy$^-$), 7.80 (d, 1H, J = 5.6 Hz; H6 of pyridyl ring of ppy$^-$), 7.67 (d, 1H, J = 5.6 Hz; H5′ of pyridyl ring of bpy-en-biotin), 7.53 (d, 1H, J = 5.6 Hz; H5 of pyridyl ring of bpy-en-biotin), 7.20−7.16 (m, 3H; H5 of pyridyl ring of ppy$^-$ and $NH(CH_2)_2NHCO$ of bpy-en-biotin), 7.06−7.01 (m, 2H; H4 of phenyl ring of ppy$^-$), 6.94−6.36 (m, 2H; H5 of phenyl ring of ppy$^-$), 6.36−6.33 (m, 2H; H6 of phenyl ring of ppy$^-$), 5.76 (s, 1H; NH of biotin), 5.64 (s, 1H; NH of biotin), 4.50−4.40 (m, 1H; NCH of biotin), 4.40−4.20 (m, 1H; NCH of biotin), 4.07 (s, 2H; $CH_2NH(CH_2)_2NH$ of bpy-en-biotin), 3.35−3.29 (m, 4H; $NH(CH_2)_2NH$ of bpy-en-biotin), 3.18−3.12 (m, 1H; SCH of biotin), 2.76 (t, 2H, J = 6.0 Hz; SCH of biotin), 2.62 (s, 3H; CH_3 on C4′ of pyridyl ring of bpy-en-biotin), 2.19−2.13 (t, 2H, J = 6.7 Hz; $COCH_2$ of biotin), 1.63−1.35 (m, 6H; $COCH_2(CH_2)_3$ of biotin). IR (KBr): ν(cm^{-1}) 3439 (br, NH), 1699 (s, C=O), 845 (s, PF_6^-). Positive-ion ESI-MS: m/z 969 $[M]^+$, 501 $[M - (bpy\text{-}en\text{-}biotin)]^+$. Anal. Calcd for $IrC_{46}H_{48}N_8O_2SPF_6 \cdot {}^1/_2H_2O$: C, 47.66; H, 4.61; N, 9.67. Found: C, 47.63; H, 4.68; N, 9.93.

$[Ir(mppy)_2(bpy\text{-}en\text{-}biotin)](PF_6)$ (2). The synthetic procedure was similar to that of complex **1** except that $[Ir_2(mppy)_4Cl_2]$ (79 mg, 0.07 mmol) was used instead of $[Ir_2(ppy)_4Cl_2]$. Complex **2** was isolated as orange-yellow crystals. Yield: 60 mg (38%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 8.85 (s, 1H; H3′ of pyridyl ring of bpy-en-biotin), 8.78 (s, 1H; H3 of pyridyl ring of bpy-en-biotin), 8.19−8.14 (m, 2H; H3 of pyridyl ring of mppy$^-$), 7.97−7.80 (m, 5H; H3 of phenyl ring of mppy$^-$, H4 of pyridyl ring of mppy$^-$, and H6′ of pyridyl ring of bpy-en-biotin), 7.79−7.73 (m, 3H; H6 of pyridyl ring of mppy$^-$ and H6 of pyridyl ring of bpy-en-biotin), 7.66 (d, 1H, J = 5.3 Hz; H5′ of pyridyl ring of bpy-en-biotin), 7.52 (d, 1H, J = 6.5 Hz; H5 of pyridyl ring of bpy-en-biotin), 7.50−7.45 (m, 1H; $NH(CH_2)_2NHCO$ of bpy-en-biotin), 7.14−7.10 (m, 2H; H5 of pyridyl ring of mppy$^-$), 6.87 (d, 2H, J = 7.9 Hz; H4 of phenyl ring of mppy$^-$), 6.18 (s, 2H; H6 of phenyl ring of mppy$^-$), 5.97 (s, 1H; NH of biotin), 5.83 (s, 1H; NH of biotin), 4.90−4.45 (m, 1H; NCH of biotin), 4.31−4.27 (m, 1H; NCH of biotin), 4.06 (s, 2H; $CH_2NH(CH_2)_2NH$ of bpy-en-biotin), 3.37−3.20 (m, 5H; $NH(CH_2)_2NH$ of bpy-en-biotin and SCH of biotin), 3.11 (s, 6H; CH_3 of mppy$^-$), 2.76 (t, 2H, J = 6.2 Hz; SCH of biotin), 2.61 (s, 3H; CH_3 on C4′ of pyridyl ring of bpy-en-biotin), 2.20 − 2.18 (m, 2H; $COCH_2$ of biotin), 1.80−1.25 (m, 6H; $COCH_2(CH_2)_3$ of biotin). IR (KBr): ν(cm^{-1}) 3439 (br, NH), 1698 (s, C=O), 845 (s, PF_6^-). Positive-ion ESI-MS: m/z 946 $[M]^+$, 478 $[M - (bpy\text{-}en\text{-}biotin)]^+$. Anal. Calcd for $IrC_{48}H_{52}N_8O_2SPF_6 \cdot {}^1/_2H_2O$: C, 47.83; H, 4.93; N, 9.30. Found: C, 47.69; H, 4.72; N, 9.60.

$[Ir(ppz)_2(bpy\text{-}en\text{-}biotin)](PF_6)$ (3). The synthetic procedure was similar to that of complex **1** except that $[Ir_2(ppz)_4Cl_2]$ (72 mg, 0.07 mmol) was used instead of $[Ir_2(ppy)_4Cl_2]$. Complex **3** was isolated as orange-yellow crystals. Yield: 57 mg (37%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 8.82−8.70 (m, 4H; H5 of pyrazole ring of ppz$^-$, H3 and H3′ of pyridyl ring of bpy-en-biotin), 8.13−8.04 (m, 2H; H6 and H6′ of pyridyl ring of bpy-en-biotin), 7.68−7.62 (m, 3H; H3 of phenyl ring of ppz$^-$ and H5 of pyridyl ring of bpy-en-biotin), 7.53 (d, 1H, J = 5.0 Hz; H5′of pyridyl ring of bpy-en-biotin),

(26) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: New York, 1988.

7.25−7.22 (m, 3H, H3 of pyrazole ring of ppz⁻ and NH-$(CH_2)_2NHCO$ of bpy-en-biotin), 7.05 (t, 2H, J = 6.9 Hz; H4 of phenyl ring of ppz⁻), 6.86 (t, 2H; H5 of phenyl ring of ppz⁻), 6.71−6.69 (m, 1H; H3 of pyrazole ring of ppz⁻), 6.36−6.32 (m, 2H; H6 of phenyl ring of ppz⁻), 5.85 (s, 1H, NH of biotin), 5.70 (s, 1H; NH of biotin), 4.46−4.42 (m, 1H; NCH of biotin), 4.27−4.25 (m, 1H; NCH of biotin), 4.08 (s, 2H; $CH_2NH(CH_2)_2NH$ of bpy-en-biotin), 3.35−3.30 (m, 4H; $NH(CH_2)_2NH$ of bpy-en-biotin), 3.18−3.12 (m, 1H; SCH of biotin), 2.76 (t, 2H, J = 6.2 Hz; SCH of biotin), 2.17 (t, 2H, J = 7.3 Hz; $COCH_2$ of biotin), 1.72−1.37 (m, 6H; $COCH_2(CH_2)_3$ of biotin). IR (KBr): ν(cm^{-1}) 3444 (br, NH), 1697 (s, C═O), 844 (s, PF_6^-). Positive-ion ESI-MS: m/z 947 $[M]^+$, 479 $[M - (bpy\text{-}en\text{-}biotin)]^+$. Anal. Calcd for $IrC_{42}H_{46}N_{10}O_2SPF_6 \cdot CH_2Cl_2$: C, 43.87; H, 4.11; N, 11.90. Found: C, 43.94; H, 4.13; N, 11.83.

[Ir(mppz)$_2$(bpy-en-biotin)](PF$_6$) (4). The synthetic procedure was similar to that of complex **1** except that $[Ir_2(mppz)_4Cl_2]$ (76 mg, 0.07 mmol) was used instead of $[Ir_2(ppy)_4Cl_2]$. Complex **4** was isolated as orange-yellow crystals. Yield: 63 mg (40%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 8.86 (s, 1H; H3′ of pyridyl ring of bpy-en-biotin), 8.80 (s, 1H; H3 of pyridyl ring of bpy-en-biotin), 8.57 (d, 2H; J = 2.6 Hz; H5 of pyrazole ring of mppz⁻), 8.06 (d, 1H, J = 5.6 Hz; H6′of pyridyl ring of bpy-en-biotin), 8.01 (d, 1H, J = 5.6 Hz; H6 of pyridyl ring of bpy-en-biotin), 7.68 (d, 1H, J = 5.9 Hz; H5′ of pyridyl ring of bpy-en-biotin), 7.60−7.54 (m, 3H; H3 of phenyl ring of mppz⁻ and H5 of pyridyl ring of bpy-en-biotin), 7.25−7.18 (m, 1H; $NH(CH_2)_2NHCO$ of bpy-en-biotin), 7.04 (t, 2H, J = 7.6 Hz; H4 of phenyl ring of mppz⁻), 6.83 (t, 2H, J = 7.0 Hz; H5 of phenyl ring of mppz⁻), 6.50−6.49 (m, 2H; H4 of pyrazole ring of mppz⁻), 6.35 (dd, 2H, J = 6.3 and 2.6 Hz; H6 of phenyl ring of mppz⁻), 5.81 (s, 1H; NH of biotin), 5.66 (s, 1H; NH of biotin), 4.51−4.44 (m, 1H; NCH of biotin), 4.32−4.25 (m, 1H; NCH of biotin), 4.08 (s, 2H; $CH_2NH(CH_2)_2NH$ of bpy-en-biotin), 3.36−3.30 (m, 4H; $NH(CH_2)_2NH$ of bpy-en-biotin), 3.17−3.15 (m, 1H; SCH of biotin), 3.05 (s, 6H, CH_3 of mppz⁻), 2.76−2.63 (m, 5H; CH_3 on C4′ of pyridyl ring of bpy-en-biotin and SCH of biotin), 2.20−2.12 (m, 2H; $COCH_2$ of biotin), 1.69−1.40 (m, 6H; $COCH_2(CH_2)_3$ of biotin). IR (KBr): ν(cm^{-1}) 3441 (br, NH), 1699 (s, C═O), 844 (s, PF_6^-). Positive-ion ESI-MS: m/z 975 $[M]^+$, 507 $[M - (bpy\text{-}en\text{-}biotin)]^+$. Anal. Calcd for $IrC_{44}H_{50}N_{10}O_2SPF_6 \cdot 2H_2O$: C, 45.70; H, 4.70; N, 12.11. Found: C, 45.42; H, 4.62; N, 12.02.

[Ir(bzq)$_2$(bpy-en-biotin)](PF$_6$) (5). The synthetic procedure was similar to that of complex **1** except that $[Ir_2(bzq)_4Cl_2]$ (82 mg, 0.07 mmol) was used instead of $[Ir_2(ppy)_4Cl_2]$. Complex **5** was isolated as orange-yellow crystals. Yield: 70 mg (44%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 8.87 (s, 1H; H3′ of pyridyl ring of bpy-en-biotin), 8.82 (s, 1H; H3 of pyridyl ring of bpy-en-biotin), 8.56 (d, 2H, J = 8.2 Hz; H4 of bzq⁻), 8.27 (d, 1H, J = 5.6 Hz; H6′of pyridyl ring of bpy-en-biotin), 8.23 (d, 1H, J = 4.4 Hz; H6 of pyridyl ring of bpy-en-biotin), 8.03−7.80 (m, 8H; H2, H5 and H6 of bzq⁻, and H5 and H5′ of pyridyl ring of bpy-en-biotin), 7.64−7.53 (m, 4H; H3 and H7 of bzq⁻), 7.42 (d, 1H, J = 5.6 Hz; $NH(CH_2)_2NHCO$ of bpy-en-biotin), 7.20−7.14 (m, 2H; H8 of bzq⁻), 6.36 (dd, 2H, J = 6.7 and 3.8 Hz; H9 of bzq⁻), 5.77 (s, 1H; NH of biotin), 5.63 (s, 1H; NH of biotin), 4.46−4.39 (m, 1H; NCH of biotin), 4.29−4.20 (m, 1H; NCH of biotin), 4.04 (s, 2H; $CH_2NH(CH_2)_2NH$ of bpy-en-biotin), 3.32−3.25 (m, 4H; $NH(CH_2)_2NH$ of bpy-en-biotin), 3.15−3.10 (m, 1H; SCH of biotin), 2.74 (t, 2H, J = 6.2 Hz; SCH of biotin), 2.60 (s, 3H; CH_3 on C4′ of pyridyl ring of bpy-en-biotin), 2.17−2.11 (m, 2H; $COCH_2$ of biotin), 1.67−1.37 (m, 6H; $COCH_2(CH_2)_3$ of bpy-en-biotin). IR (KBr): ν(cm^{-1}) 3439 (br, NH), 1698 (s, C═O), 847 (s, PF_6^-). Positive-ion ESI-MS: m/z 1017 $[M]^+$, 549 $[M - (bpy\text{-}en\text{-}biotin)]^+$. Anal. Calcd for $IrC_{50}H_{48}N_8O_2SPF_6 \cdot 3H_2O$: C, 49.38; H, 4.48; N, 9.21. Found: C, 49.34; H, 4.69; N, 9.41.

[Ir(pq)$_2$(bpy-en-biotin)](PF$_6$) (6). The synthetic procedure was similar to that of complex **1** except that $[Ir_2(pq)_4Cl_2]$ (89 mg, 0.07 mmol) was used instead of $[Ir_2(ppy)_4Cl_2]$. Complex **6** was isolated as orange crystals. Yield: 70 mg (42%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 8.60−8.40 (m, 4H; H3 of quinoline of pq⁻, H3 and H3′ of pyridyl ring of bpy-en-biotin), 8.44 (s, 1H; H6′ of pyridyl ring of bpy-en-biotin), 8.37 (s, 1H; H6 of pyridyl ring of bpy-en-biotin), 8.27−8.20 (m, 2H; H3 of phenyl ring of pq⁻), 8.16 (d, 2H, J = 5.6 Hz; H4 of quinoline of pq⁻), 7.95 (d, 2H, J = 8.2 Hz; H8 of quinoline of pq⁻), 7.67 (d, 1H, J = 4.4 Hz; H5′ of pyridyl ring of bpy-en-biotin), 7.53 (d, 1H, J = 5.6 Hz; H5 of pyridyl ring of bpy-en-biotin), 7.49−7.40 (m, 4H; H5 and H7 of quinoline of pq⁻), 7.22−7.11 (m, 5H; H4 of phenyl ring of pq⁻, H6 of quinoline of pq⁻, and NH-$(CH_2)_2NHCO$ of bpy-en-biotin), 6.82 (t, 2H, J = 7.5 Hz; H5 of phenyl ring of pq⁻), 6.56 (d, 2H, J = 7.6 Hz; H6 of phenyl ring of pq⁻), 5.85 (s, 1H; NH of biotin), 5.71 (s, 1H; NH of biotin), 4.50−4.40 (m, 1H; NCH of biotin), 4.35−4.23 (m, 1H; NCH of biotin), 3.94 (s, 2H; $CH_2NH(CH_2)_2NH$ of bpy-en-biotin), 3.31−3.21 (m, 4H; $NH(CH_2)_2NH$ of bpy-en-biotin), 3.18−3.13 (m, 1H; SCH of biotin), 2.69−2.59 (m, 2H; SCH of biotin), 2.49 (s, 3H; CH_3 on C4′ of pyridyl ring of bpy-en-biotin), 2.18−2.10 (m, 2H; $COCH_2$ of biotin), 1.64−1.31 (m, 6H; $COCH_2(CH_2)_3$ of biotin). IR (KBr): ν(cm^{-1}) 3441 (br, NH), 1670 (s, C═O), 844 (s, PF_6^-). Positive-ion ESI-MS: m/z 1069 $[M]^+$, 601 $[M - (bpy\text{-}en\text{-}biotin)]^+$. Anal. Calcd for $IrC_{54}H_{52}N_8O_2SPF_6 \cdot {}^1/_2CH_3OH \cdot H_2O$: C, 52.44; H, 4.52; N, 8.98. Found: C, 52.73; H, 4.83; N, 9.18.

HABA Assays of Iridium(III) Biotin Complexes. In a typical experiment, aliquots (5 μL) of the iridium(III) biotin complex (1.1 mM) were added cumulatively to a mixture of avidin (7.6 μM) and HABA (0.3 mM) in 50 mM potassium phosphate buffer pH 7.4 (2 mL) at 1 min intervals. The absorption spectrum of the solution was measured. The binding of the iridium(III) biotin complex to avidin was indicated by the decrease of the absorbance at 500 nm due to the displacement of HABA from the avidin by the iridium(III) biotin complex. By plotting $-\Delta A_{500\ nm}$ versus [Ir]:[avidin], the equivalence points of the titrations were determined.

Luminescence Titrations. In a typical procedure, aliquots (5 μL) of the iridium(III) biotin complex (0.55 mM) were added cumulatively to avidin (3.8 μM) in 2 mL of 50 mM potassium phosphate buffer pH 7.4 at 1 min intervals. The emission intensity of the solution was then measured. The titration results were compared to two sets of control titrations in which (i) avidin was absent and (ii) the avidin solution was saturated with excess biotin.

First dissociation constants K_d of the adducts formed from the iridium(III) biotin complexes and avidin were determined on the basis of a noncooperative protein−ligand (P−L$_n$) binding model using the following equation:[23]

$$K_d = \frac{(n[P]_0 - \alpha[L]_0)^2}{(1 - \alpha^2)([L]_0 - n[P]_0)}$$

where n is the binding stoichiometry, which is taken as 4 for the complexes in this work; $[P]_0$ and $[L]_0$ are the initial concentrations of the protein and the stock metal complex concentration, respectively; and $\alpha = V_c^*/V_0$, where V_c^* and V_0 are the volume of the metal complex solution added to attain the maximum observed changes in the spectroscopic signal and the initial volume of the protein solution, respectively.

Association and Dissociation Assays. The competition between the iridium(III) biotin complexes and unmodified biotin on binding to avidin was investigated by competitive association and dissociation assays. In the association assays, avidin was added to a mixture of the iridium(III) biotin complex (16 μM) and unmodified biotin (16 μM) to a concentration of 4 μM. The solution was incubated at room temperature for 1 h and then diluted to 500 μL. The solution was then loaded onto a PD-10 size exclusion column (Pharmacia) that had been equilibrated with 50 mM potassium phosphate buffer pH 7.4. The first 5 mL of the eluted solution that contained avidin was collected, and the emission intensity of

this solution was measured. The emission intensity was compared to that of the control, in which unmodified biotin was absent. In the dissociation assay, a mixture of avidin (4 μM) and the iridium(III) biotin complex (16 μM) was incubated at room temperature for 1 h. Then unmodified biotin was added to the solution to a concentration of 16 μM, and the mixture was incubated for 1 h. The mixture was then diluted to 500 μL and loaded onto a PD-10 size exclusion column (Pharmacia) that had been equilibrated with 50 mM potassium phosphate buffer pH 7.4. The first 5 mL of the eluted solution that contained avidin was collected, and the emission intensity of this solution was measured. The emission intensity was compared to that of the control, in which unmodified biotin was absent.

Homogeneous Competitive Assays for Biotin. In a typical experiment, a biotin analyte solution (500 μL) in 50 mM potassium phosphate buffer pH 7.4 was mixed with the iridium(III) biotin complex (20 μL, 0.15 mM) dissolved in DMSO. Avidin (100 μL, 7.6 μM) in 50 mM potassium phosphate buffer pH 7.4 was then added to the solution. The final concentration of the biotin analyte in the solutions ranged from 1×10^{-4} to 1×10^{-9} M. The solutions were incubated at room temperature for 1 h, and their emission intensity was then measured.

Physical Measurements and Instrumentation. All physical measurements and instrumentations have been described previously.[19d] Luminescence quantum yields were measured using the optically dilute method[27] with an aerated aqueous solution of $[Ru(bpy)_3]Cl_2$ ($\Phi = 0.028$)[28] as the standard solution.

Acknowledgment. We thank the Hong Kong Research Grants Council (Project No. CityU 101603) for financial support. C.K.C. acknowledges the receipt of a postgraduate studentship and a Research Tuition scholarship, both administered by the City University of Hong Kong. We are grateful to Professor V. W. W. Yam of The University of Hong Kong for access to the equipment for photophysical measurements.

Supporting Information Available: Electronic absorption and emission spectra of complexes **1**−**6** in CH_3CN at 298 K; HABA assays for complexes **1**, **3**−**6**; emission titration results for complexes **2**−**5**; and homogeneous competitive assays for biotin using complexes **2**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

OM0499355

(27) Demas, J. N.; Crosby, G. A. *J. Phys. Chem.* **1971**, *75*, 991.
(28) Nakamaru, K. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2697.