Luminescent Cyclometalated Iridium(III) Arylbenzothiazole Biotin Complexes

Kenneth Kam-Wing Lo,* Chi-Kwan Li, and Jason Shing-Yip Lau

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

Received April 25, 2005

A series of luminescent cyclometalated iridium(III) arylbenzothiazole biotin complexes, $[Ir(N{-}C)_2L](PF_6)$ (N−CH = 2-phenylbenzothiazole, Hbt (**1**); 2-((1,1′-biphenyl)-4-yl)benzothiazole, Hbsb (**2**); 2-(2-thienyl)benzothiazole, Hbtth (**3**); 2-(1-naphthyl)benzothiazole, Hbsn (**4**); L = 4-(*N*-((6-biotinamido)hexyl)amido)-4′-methyl-2,2′-bipyridine), have been synthesized and characterized. The photophysical and electrochemical properties of these complexes have been investigated. Upon excitation, these complexes displayed intense and long-lived luminescence in fluid solutions at 298 K and in low-temperature glass. The emission is derived from an ^{3}IL ($\pi \rightarrow \pi^*$) (N−C$^-$) excited state, probably with some mixing of ^{3}MLCT ($d\pi(Ir) \rightarrow \pi^*(N{-}C^-)$) character. The avidin-binding properties of these complexes have been investigated by luminescence titrations and competitive assays using native biotin. Upon binding to avidin, these complexes showed significant emission enhancement and lifetime extension. On the basis of these observations, homogeneous competitive assays for biotin and resonance-energy transfer (RET) quenching using modified avidin have been studied.

Introduction

Owing to the extremely high affinity of biotin toward avidin (first dissociation constant, K_d = ca. 10^{-15} M),[1] biotinylated biomolecules are commonly recognized by fluorophore-labeled avidin molecules.[2] Since avidin has four biotin-binding sites, it can act as a bridge and biotinylated biomolecules can be recognized by biotin-reporter conjugates. However, this strategy does not apply to fluorescent biotin conjugates because they suffer from severe self-quenching upon binding to avidin.[3] We have recently shown that luminescent transition metal biotin complexes that show large Stokes' shifts could be used as an alternative.[4]

Cyclometalated iridium(III) polypyridine complexes have attracted much attention because of their interesting photophysical properties.[5−14] Such remarkable properties render these complexes useful candidates in applications such as organic light-emitting devices,[9] luminescent sensors,[7b,12] and artificial photosynthetic sysytems.[8b−e] In view of this, we have recently designed a series of luminescent cyclometalated iridium(III) polypyridine biotin complexes as probes for avidin.[4c] The complexes display enhanced emission intensities and extended lifetimes upon binding to avidin. However, the emission maxima of these complexes are somewhat confined to ca. 550 to 590 nm, limiting their use as multicolor probes. The reason is that the excited states

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

(1) (a) Wilchek, M.; Bayer, E. A. *Anal. Biochem*. **1988**, *171*, 1. (b) Wilchek, M.; Bayer, E. A. *Methods Enzymol*. **1990**, *184*, 123. (c) Hofstetter, H.; Morpurgol, M.; Hofstetter, O.; Bayer, E. A.; Wilchek, M. *Anal. Biochem*. **2000**, *284*, 354.

(2) (a) Levine, L. M.; Michener, M. L.; Toth, M. V.; Holwerda, B. C. *Anal. Biochem*. **1997**, *247*, 83. (b) Malicka, J.; Gryczynski, I.; Gryczynski, Z.; Lakowicz, J. R. *Anal. Biochem*. **2003**, *315*, 57.

(3) (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.

(4) (a) Lo, K. K.-W.; Hui, W.-K.; Ng, D. C.-M. *J. Am. Chem. Soc.* **2002**, *124*, 9344. (b) Lo, K. K.-W.; Tsang, K. H.-K. *Organometallics* **2004**, *23*, 3062. (c) Lo, K. K.-W.; Chan, J. S.-W.; Lui, L.-H.; Chung, C.-K. *Organometallics* **2004**, *23*, 3108. (d) Lo, K. K.-W.; Lee, T. K.-M. *Inorg. Chem*. **2004**, *43*, 5275. (e) Lo, K. K.-W.; Hui, W.-K. *Inorg. Chem*. **2005**, *44*, 1992.

(5) (a) Sprouse, S.; King, K. A.; Spellane, P. J.; Watts, R. J. *J. Am. Chem. Soc*. **1984**, *106*, 6647. (b) Ohsawa, Y.; Sprouse, S.; King, K. A.; DeArmond, M. K.; Hanck, K. W.; Watts, R. J. *J. Phys. Chem*. **1987**, *91*, 1047. (c) Garces, F. O.; King, K. A.; Watts, R. J. *Inorg. Chem*. **1988**, *27*, 3464.

(6) Colombo, M. G.; Hauser, A.; Güdel, H. U. *Top. Curr. Chem*. **1994**, *171*, 143.

(7) (a) Mamo, A.; Stefio, I.; Parisi, M. F.; Credi, A.; Venturi, M.; Di Pietro, C.; Campagna, S. *Inorg. Chem*. **1997**, *36*, 5947. (b) Di Marco, G.; Lanza, M.; Mamo, A.; Stefio, I.; Di Pietro, C.; Romeo, G.; Campagna, S. *Anal. Chem*. **1998**, *70*, 5019. (c) Neve, F.; Crispini, A.; Campagna, S.; Serroni, S. *Inorg. Chem*. **1999**, *38*, 2250. (d) Neve, F.; Crispini, A.; Serroni, S.; Loiseau, F.; Campagna, S. *Inorg. Chem*. **2001**, *40*, 1093.

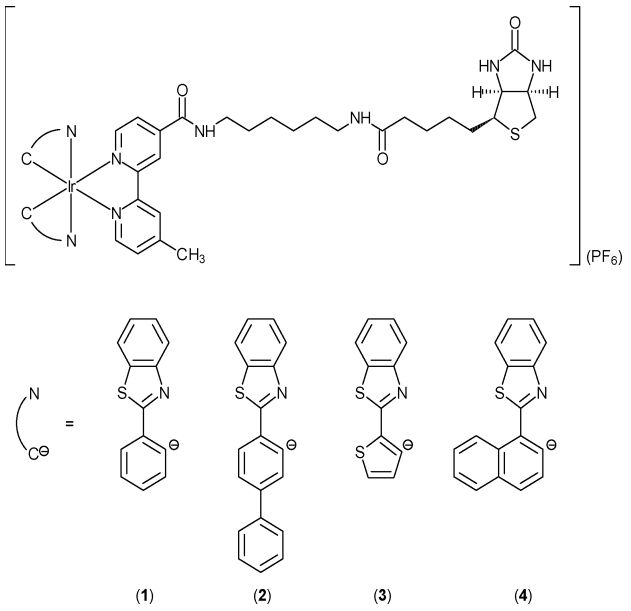
(8) (a) Collin, J.-P.; Dixon, I. M.; Sauvage, J.-P.; Williams, J. A. G.; Barigelletti, F.; Flamigni, L. *J. Am. Chem. Soc*. **1999**, *121*, 5009. (b) Dixon, I. M.; Collin, J.-P.; Sauvage, J.-P.; Barigelletti, F.; Flamigni, L. *Angew. Chem., Int. Ed*. **2000**, *39*, 1292. (c) Dixon, I. M.; Collin, J.-P.; Sauvage, J.-P.; Flamigni, L.; Encinas, S.; Barigelletti, F. *Chem. Soc. Rev*. **2000**, *29*, 385. (d) Dixon, I. M.; Collin, J.-P.; Sauvage, J.-P.; Flamigni, L. *Inorg. Chem*. **2001**, *40*, 5507. (e) Baranoff, E.; Collin, J.-P.; Flamigni, L.; Sauvage, J.-P. *Chem. Soc. Rev*. **2004**, *33*, 147.

(9) (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. (d) Tamayo, A. B.; Alleyne, B. D.; Djurovich, P. I.; Lamansky, S.; Tsyba, I.; Ho, N. N.; Bau, R.; Thompson, M. E. *J. Am. Chem. Soc*. **2003**, *125*, 7377. (e) Li, J.; Djurovich, P. I.; Alleyne, B. D.; Tsyba, I.; Ho, N. N.; Bau, R.; Thompson, M. E. *Polyhedron* **2004**, *23*, 419.

(10) (a) Plummer, E. A.; Hofstraat, J. W.; De Cola, L. *Dalton Trans.* **2003**, 2080. (b) Haider, J. M.; Williams, R. M.; De Cola, L.; Pikramenou, Z. *Angew. Chem., Int. Ed*. **2003**, *42*, 1830. (c) Coppo, P.; Plummerab, E. A.; De Cola, L. *Chem. Commun*. **2004**, 1774.

10.1021/om050327e CCC: $30.25
Publication on Web 08/06/2005

Chart 1. Structures of Complexes 1−4



of these complexes are essentially triplet metal-to-ligand charge-transfer ^{3}MLCT (dπ(Ir) → π^*(N−N)) in nature. Thus, changing the cyclometalating ligand cannot significantly alter the emission energy of the system. Recent studies have shown that the introduction of arylbenzothiazole ligands to the iridium(III) center can give efficient triplet intraligand ^{3}IL ($\pi \rightarrow \pi^*$) (arylbenzothiazole) emitters that show very intense and long-lived emission.[9a−c,14] In fact, the use of such hydrophobic ligands is attractive because we have noticed that more hydrophobic transition metal biotin conjugates can provide higher degrees of emission enhancement.[4] In consideration of these factors, we have designed a new series of luminescent cyclometalated iridium(III) arylbenzothiazole biotin complexes, $[Ir(N{-}C)_2L](PF_6)$ (N−CH = 2-phenylbenzothiazole, Hbt (**1**); 2-((1,1′-biphenyl)-4-yl)benzothiazole, Hbsb (**2**); 2-(2-thienyl)benzothiazole, Hbtth (**3**); 2-(1-naphthyl)benzothiazole, Hbsn (**4**); L = 4-(*N*-((6-biotinamido)hexyl)amido)-4′-methyl-2,2′-bipyridine). The structures of these complexes are shown in Chart 1. The photophysical, electrochemical, and avidin-binding properties of these luminescent complexes are described in this article.

Experimental Section

Materials and Reagents. All syntheses were carried out under an atmosphere of nitrogen. Solvents were purified by reported procedures.[15] $IrCl_3{\cdot}3H_2O$ (Aldrich), KPF_6 (Aldrich), Hbt (Acros), HABA (Sigma), avidin (Calbiochem), and Malachite Green isothiocyanate (Molecular Probes) were used as received. The ligands Hbsb, Hbtth, and Hbsn[16] and all the dimers $[Ir_2(N{-}C)_4Cl_2]$[5] were prepared according to reported procedures. Size-exclusion columns (NAP-5 and PD-10) and YM-30 centricons were purchased from Pharmacia and Amicon, respectively. Instruments for the characterization, photophysical, and electrochemical measurements have been described previously.[4] Luminescence quantum yields were measured using the optically dilute method[17] with an aerated aqueous solution of $[Ru(bpy)_3]Cl_2$ ($\Phi = 0.028$)[18] as the standard solution.

4-(*N*-((6-Biotinamido)hexyl)amido)-4′-methyl-2,2′-bipyridine) (L).[4d] A mixture of succinimidyl-4-carboxy-4′-methyl-2,2′-bipyridine[19] (103 mg, 0.33 mmol), *N*-biotinyl-1,6-diaminohexane[20] (113 mg, 0.33 mmol), and triethylamine (1 mL, 7.12 mmol) in 10 mL of DMF was stirred at room temperature for 12 h. The yellow solution was evaporated under vacuum to give a brownish yellow solid. The solid was washed with chloroform and then recrystallized from methanol/diethyl ether to give yellow crystals. Yield: 100 mg (58%). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K, relative to TMS): δ 8.90 (t, J = 5.4 Hz, 1H, bpy-4-CON*H*), 8.78 (d, J = 4.7 Hz, 1H, H6 of pyridyl ring), 8.73 (s, 1H, H3 of pyridyl ring), 8.56 (d, J = 4.7 Hz, 1H, H6′ of pyridyl ring), 8.25 (s, 1H, H3′ of pyridyl ring), 7.78−7.74 (m, 2H, H5 of pyridyl ring and N*H*-biotin), 7.31 (d, J = 4.7 Hz, 1H, H5′ of pyridyl ring), 6.43 (s, 1H, NH of biotin), 6.36 (s, 1H, NH of biotin), 4.32−4.22 (m, 1H, NCH of biotin), 4.15−4.05 (m, 1H, NCH of biotin), 3.29−3.25 (m, 2H, bpy-4-CONHC*H*$_2$), 3.10−2.92 (m, 3H, SCH of biotin and C*H*$_2$NH-biotin), 2.78 (dd, J_{gem} = 12.3 Hz, J_{vic} = 4.8 Hz, 1H, SCH of biotin), 2.54 (d, J_{gem} = 12.3 Hz, 1H, SCH of biotin), 2.41 (s, 3H, CH_3 on C4′ of pyridyl ring), 2.02 (t, J = 6.9 Hz, 2H, COC*H*$_2$C$_3$H$_6$ of biotin), 1.67−1.12 (m, 14H, NHCH$_2$C$_4$*H*$_8$CH$_2$-NH and COCH$_2$C$_3$*H*$_6$ of biotin). IR (KBr): ν 3298 (br, NH), 1696 cm^{-1} (s, C=O). Positive-ion ESI-MS: *m/z* 539 $\{M + H^+\}^+$, 561 $\{M + Na^+\}^+$. UV/vis (DMSO): λ_{abs}/nm (ϵ/dm^3 mol^{-1} cm^{-1}) 284 (11 165), 307 sh (5785).

$[Ir(bt)_2(L)](PF_6)$ (1). A mixture of $[Ir_2(bt)_4Cl_2]$ (100 mg, 0.08 mmol) and L (83 mg, 0.16 mmol) in 30 mL of dichloromethane/2-ethoxyethanol (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 acetonitrile/diethyl ether afforded complex **1** as yellow crystals. Yield: 154 mg (75%). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K, relative to TMS): δ 9.36−9.22 (m, 2H, bpy-4-CON*H* and H3 of pyridyl ring of L), 8.94 (s, 1H, H3′ of pyridyl ring of L), 8.25 (d, J = 8.5 Hz, 2H, H4 of benzothiazole ring of bt$^-$), 8.14 (d, J = 5.9 Hz, 1H, H6 of pyridyl ring of L), 8.07−8.01 (m, 3H, H5 of pyridyl ring of L, and H6 of phenyl ring of bt$^-$), 7.87 (d, J = 5.6 Hz, 1H, H6′ of pyridyl ring of bt$^-$), 7.79 (t, J = 5.6 Hz, 1H, N*H*-biotin of L), 7.65 (d, J = 5.6 Hz, 1H, H5′ of pyridyl ring of L), 7.43 (t, J = 7.6 Hz, 2H, H5 of benzothiazole ring of bt$^-$), 7.24−7.11 (m, 4H, H6 of benzothiazole ring and H5 of phenyl ring of bt$^-$), 6.93 (dt, J = 7.5 Hz, J = 1.4 Hz, 2H, H4 of phenyl ring of bt$^-$), 6.45 (s, 1H, NH of biotin of L), 6.40 (s, 1H, NH of biotin of L), 6.29 (t, J = 7.9 Hz, 2H, H3 of phenyl ring of bt$^-$), 6.15 (t, J = 7.6 Hz, 2H, H7 of benzothiazole ring of bt$^-$), 4.32−4.27 (m, 1H, NCH of biotin of L), 4.13−4.09 (m,

(11) (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.
(12) (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. (c) Leslie, W.; Batsanov, A. S.; Howard, J. A. K.; Williams, J. A. G. *Dalton Trans.* **2004**, 623.
(13) Polson, M.; Fracasso, S.; Bertolasi, V.; Ravaglia, M.; Scandola, F. *Inorg. Chem.* **2004**, *43*, 1950.
(14) Chang, W.-C.; Hu, A. T.; Duan, J.-P.; Rayabarapu, D. K.; Cheng, C.-H. *J. Organomet. Chem.* **2004**, *689*, 4882.
(15) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*; Pergamon: Oxford, 1997.
(16) (a) Capitan-Vallvey, L. F.; Espinosa, P. *Polyhedron* **1983**, *2*, 1147. (b) Deligeorgiev, T. G. *Dyes Pigm.* **1990**, *12*, 243. (c) Perry, R. J.; Wilson, B. D. *Organometallics* **1994**, *13*, 3346.
(17) Demas, J. N.; Crosby, G. A. *J. Phys. Chem.* **1971**, *75*, 991.
(18) Nakamaru, K. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2697.
(19) Telser, J.; Cruickshank, K. A.; Schanze K. S.; Netzel, T. L. *J. Am. Chem. Soc.* **1989**, *111*, 7221.
(20) Sabatino, G.; Chinol, M.; Paganelli, G.; Papi, S.; Chelli, M.; Leone, G.; Papini, A. M.; De Luca, A.; Ginanneschi, M. *J. Med. Chem.* **2003**, *46*, 3170.

1H, NCH of biotin of L), 3.32−3.27 (m, 2H, bpy-4-CONHCH_2 of L), 3.10−2.98 (m, 3H, SCH of biotin and CH_2NH-biotin of L), 2.80 (dd, J_{gem} = 12.5 Hz, J_{vic} = 5.1 Hz, 1H, SCH of biotin of L), 2.57−2.53 (m, 4H, SCH of biotin and CH_3 on C4′ of pyridyl ring of L), 2.03 (t, J = 7.2 Hz, 2H, COCH_2C$_3$H$_6$ of biotin of L), 1.53−1.28 (m, 14H, NHCH$_2$C$_4$$H_8CH_2$NH and COCH$_2C_3$$H_6$ of biotin of L). Anal. Calc for $C_{54}H_{54}F_6IrN_8O_3PS_3\cdot(C_2H_5)_2O\cdot 1/2CH_3CN$: C, 51.70; H, 4.82; N, 8.68. Found: C, 51.56; H, 4.92; N, 8.58. IR (KBr) ν 3432 (br, NH), 1696 (s, C=O), 835 cm^{-1} (s, PF_6^-). Positive-ion ESI-MS: m/z 1152 {[Ir(bt)$_2$(L)]}$^+$.

[Ir(bsb)$_2$(L)](PF$_6$) (2). The synthetic procedure was similar to that of complex **1** except that [Ir$_2$(bsb)$_4$Cl$_2$] (128 mg, 0.08 mmol) was used instead of [Ir$_2$(bt)$_4$Cl$_2$]. Recrystallization from acetonitrile/diethyl ether afforded complex **2** as yellow crystals. Yield: 182 mg (80%). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K, relative to TMS): δ 9.17 (s, 1H, H3 of pyridyl ring of L), 9.13 (t, J = 5.4 Hz, 1H, bpy-4-CONH of L), 8.92 (s, 1H, H3′ of pyridyl ring of L), 8.31−8.24 (m, 3H, H6 of pyridyl ring of L, and H4 of benzothiazole ring of bsb$^-$), 8.13−8.06 (m, 3H, H5 of pyridyl ring of L, and H5 of biphenyl ring of bsb$^-$), 7.98 (d, J = 5.9 Hz, 1H, H6′ of pyridyl ring of L), 7.78 (t, J = 7.9 Hz, 1H, NH-biotin of L), 7.69 (d, J = 5.6 Hz, 1H, H5′ of pyridyl ring of L), 7.48−7.43 (m, 4H, H2′ and H6′ of biphenyl ring of bsb$^-$), 7.29−7.22 (m, 8H, H5 of benzothiazole ring and H3′, H4′, and H5′ of biphenyl ring of bsb$^-$), 7.18−7.14 (m, 4H, H6 of benzothiazole ring and H6 of biphenyl ring of bsb$^-$), 6.48−6.40 (m, 4H, H2 of biphenyl ring of bsb$^-$ and NH of biotin of L), 6.23 (t, J = 7.0 Hz, 2H, H7 of benzothiazole ring of bsb$^-$), 4.31−4.29 (m, 1H, NCH of biotin of L), 4.27−4.11 (m, 1H, NCH of biotin of L), 3.35−3.30 (m, 2H, bpy-4-CONHCH_2 of L), 3.23−3.00 (m, 3H, SCH of biotin and CH_2NH-biotin of L), 2.80 (dd, J_{gem} = 12.0 Hz, J_{vic} = 5.0 Hz, 1H, SCH of biotin of L), 2.59−2.54 (m, 4H, SCH of biotin and CH_3 on C4′ of pyridyl ring of L), 2.02 (t, J = 7.2 Hz, 2H, COCH_2C$_3$H$_6$ of biotin of L), 1.53−1.29 (m, 14H, NHCH$_2$C$_4$$H_8CH_2$NH and COCH$_2C_3$$H_6$ of biotin of L). Anal. Calc for $C_{64}H_{62}F_6IrN_8O_3PS_3\cdot H_2O\cdot CH_3CN$: C, 53.43; H, 4.55; N, 8.50. Found: C, 53.26; H, 4.57; N, 8.77. IR (KBr) ν 3422 (br, NH), 1698 (s, C=O), 843 cm^{-1} (s, PF_6^-). Positive-ion ESI-MS: m/z 1279 {[Ir(bsb)$_2$(L)]}$^+$.

[Ir(btth)$_2$(L)](PF$_6$) (3). The synthetic procedure was similar to that of complex **1** except that [Ir$_2$(btth)$_4$Cl$_2$] (101 mg, 0.08 mmol) was used instead of [Ir$_2$(bt)$_4$Cl$_2$]. Recrystallization from acetonitrile/diethyl ether afforded complex **3** as orange crystals. Yield: 146 mg (70%). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K, relative to TMS): δ 9.09−9.06 (m, 2H, bpy-4-CONH and H3 of pyridyl ring of L), 8.85 (s, 1H, H3′ of pyridyl ring of L), 8.16−8.07 (m, 3H, H6 of pyridyl ring of L, and H4 of benzothiazole ring of btth$^-$), 7.86−7.77 (m, 5H, H5 and H6′ of pyridyl ring and NH-biotin of L, and H5 of thienyl ring of btth$^-$), 7.68 (d, J = 5.9 Hz, 1H, H5′ of pyridyl ring of L), 7.35 (t, J = 7.6 Hz, 2H, H5 of benzothiazole ring of btth$^-$), 7.17 (t, J = 7.8 Hz, 2H, H6 of benzothiazole ring of btth$^-$), 6.44 (s, 1H, NH of biotin of L), 6.39 (s, 1H, NH of biotin of L), 6.17 (d, J = 4.7 Hz, 1H, H4 of thienyl ring of btth$^-$), 6.14 (d, J = 4.7 Hz, 1H, H4 of thienyl ring of btth$^-$), 6.05 (t, J = 7.8 Hz, 2H, H7 of benzothiazole ring of btth$^-$), 4.31−4.27 (m, 1H, NCH of biotin of L), 4.12−4.08 (m, 1H, NCH of biotin of L), 3.32−3.26 (m, 2H, bpy-4-CONHCH_2 of L), 3.02−2.89 (m, 3H, SCH of biotin and CH_2NH-biotin of L), 2.80 (dd, J_{gem} = 12.6 Hz, J_{vic} = 5.0 Hz, 1H, SCH of biotin of L), 2.59−2.54 (m, 4H, SCH of biotin and CH_3 on C4′ of pyridyl ring of L), 2.03 (t, J = 7.2 Hz, 2H, COCH_2C$_3$H$_6$ of biotin of L), 1.53−1.23 (m, 14H, NHCH$_2$C$_4$$H_8$-CH$_2$NH and COCH$_2C_3$$H_6$ of biotin of L). Anal. Calc for $C_{50}H_{50}F_6IrN_8O_3PS_5\cdot H_2O\cdot 3/2CH_3CN$: C, 48.05; H, 4.59; N, 9.98. Found: C, 47.99; H, 4.29; N, 10.03. IR (KBr) ν 3442 (br, NH), 1690 (s, C=O), 840 cm^{-1} (s, PF_6^-). Positive-ion ESI-MS: m/z 1164 {[Ir(btth)$_2$(L)]}$^+$.

[Ir(bsn)$_2$(L)](PF$_6$) (4). The synthetic procedure was similar to that of complex **1** except that [Ir$_2$(bsn)$_4$Cl$_2$] (115 mg, 0.08 mmol) was used instead of [Ir$_2$(bt)$_4$Cl$_2$]. Recrystallization from acetonitrile/diethyl ether afforded complex **4** as orange crystals. Yield: 162 mg (73%). ^{1}H NMR (300 MHz, DMSO-d_6, 298 K, relative to TMS): δ 9.09−9.04 (m, 2H, bpy-4-CONH and H3 of pyridyl ring of L), 8.85 (s, 1H, H3′ of pyridyl ring of L), 8.66 (d, J = 8.8 Hz, 2H, H8 of naphthyl ring of bsn$^-$), 8.33 (d, J = 8.5 Hz, 2H, H5 of naphthyl ring of bsn$^-$), 8.14 (d, J = 5.9 Hz, 1H, H6 of pyridyl ring of L), 7.96 (d, J = 5.9 Hz, 1H, H5 of pyridyl ring of L), 7.89−7.76 (m, 6H, H6′ of pyridyl ring and NH-biotin of L, and H4 of benzothiazole ring and H7 of naphthyl ring of bsn$^-$), 7.59−7.42 (m, 7H, H5′ of pyridyl ring of L, and H5 and H6 of benzothiazole ring and H6 of naphthyl ring of bsn$^-$), 7.24 (t, J = 7.8 Hz, 2H, H4 of naphthyl ring of bsn$^-$), 6.52 (t, J = 8.4 Hz, 2H, H7 of benzothiazole ring of bsn$^-$), 6.44 (s, 1H, NH of biotin), 6.39 (s, 1H, NH of biotin), 6.28 (q, J = 4.2 Hz, 2H, H3 of naphthyl ring of bsn$^-$), 4.31−4.27 (m, 1H, NCH of biotin of L), 4.11−4.08 (m, 1H, NCH of biotin of L), 3.32−3.22 (m, 2H, bpy-4-CONHCH_2 of L), 3.09−2.99 (m, 3H, SCH of biotin and CH_2NH-biotin of L), 2.79 (dd, J_{gem} = 12.5 Hz, J_{vic} = 5.1 Hz, 1H, SCH of biotin of L), 2.57−2.51 (m, 4H, SCH of biotin and CH_3 on C4′ of pyridyl ring of L), 2.02 (t, J = 7.3 Hz, 2H, COCH_2C$_3$H$_6$ of biotin of L), 1.51−1.23 (m, 14H, NHCH$_2$C$_4$$H_8CH_2$NH and COCH$_2C_3$$H_6$ of biotin of L). Anal. Calc for $C_{62}H_{58}F_6IrN_8O_3PS_3\cdot 2H_2O$: C, 51.98; H, 4.36; N, 7.82. Found: C, 51.68; H, 4.43; N, 7.59. IR (KBr) ν 3435 (br, NH), 1696 (s, C=O), 835 cm^{-1} (s, PF_6^-). Positive-ion ESI-MS: m/z 1252 {[Ir(bsn)$_2$(L)]}$^+$.

HABA Assays of Iridium(III)−Biotin Complexes. To a mixture (2 mL) of avidin (3.8 μM) and HABA (150.0 μM) in 50 mM potassium phosphate buffer pH 7.4 were added 5 μL aliquots of the iridium(III)−biotin complex (1.1 mM) in 1 min intervals. The absorbance of the solution at 500 nm was measured after each addition.

Emission Titrations. Avidin (0.67 μM) in 50 mM potassium phosphate buffer pH 7.4 (2 mL) was titrated with the iridium(III)−biotin complex (0.12 mM) by additions of 5 μL aliquots in 1 min intervals. The emission intensity of the solution was measured after each addition. 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 (67.0 μM).

Competitive Assays and Determination of First Dissociation Constants. In the association assay, avidin was added to a mixture of the iridium(III)−biotin complex and unmodified biotin. The concentrations of avidin, complex, and unmodified biotin were 7.0, 28.0, and 28.0 μM, respectively. The solution (250 μL) was incubated at room temperature for 1 h. Then, the solution was loaded onto a NAP-5 size-exclusion column that had been equilibrated with 50 mM potassium phosphate buffer pH 7.4. The first 1 mL of the eluted solution that contained avidin was collected, and its emission spectrum was measured. The emission intensity was compared to that of the control in which no unmodified biotin was used. In the dissociation assay, a mixture of the iridium(III)−biotin complex (28.0 μM) and avidin (7.0 μM) was incubated at room temperature for 1 h. Unmodified biotin was then added to the mixture to a concentration of 28.0 μM. The solution (250 μL) was incubated at room temperature for 1 h. Then, the solution was loaded onto a NAP-5 size-exclusion column that had been equilibrated with 50 mM potassium phosphate buffer at pH 7.4. The first 1 mL of the eluted solution that contained avidin was collected, and the emission intensity was compared to that of the control in which no unmodified biotin was used.

The first dissociation constants (K_d) were determined from the on-rate constants (k_{on}) and off-rate constants (k_{off}) of the binding of iridium(III)−biotin complexes toward avidin.[3b] In the association studies, avidin in 50 mM potassium phosphate buffer pH 7.4 was added to a solution of the iridium(III)−biotin complex. The emission intensity of the solution was monitored. The on-rate constant k_{on} for the binding of the iridium(III)−

Table 1. Electronic Absorption Spectral Data for Complexes 1−4 at 298 K

complex	medium	λ_{abs}/nm (ϵ/dm^3 mol^{-1} cm^{-1})
1	CH_3CN	255 (36 135), 269 sh (33 265), 279 sh (30 775), 310 (34 100), 327 (25 795), 352 sh (12 000), 373 (8875), 415 (6665), 440 sh (4885)
	CH_2Cl_2	257 (39 740), 271 sh (36 475), 282 sh (33 440), 313 (38 890), 320 (38 720), 352 sh (13 205), 373 (9615), 415 (6965), 440 sh (5355)
2	CH_3CN	253 sh (43 065), 265 sh (39 400), 281 (35 310), 300 sh (36 450), 324 sh (51 400), 335 (55 300), 360 sh (28 690), 380 sh (18 260), 426 (9810)
	CH_2Cl_2	254 sh (40 900), 267 sh (36 980), 282 (32 715), 300 sh (34 010), 324 sh (48 680), 337 (54 120), 360 sh (31 240), 380 sh (19 640), 428 (9910)
3	CH_3CN	261 (24 175), 273 sh (22 815), 290 sh (22 010), 315 (27 485), 330 sh (22 580), 355 sh (14 050), 380 sh (10 885), 437 sh (8560)
	CH_2Cl_2	263 (25 860), 273 sh (24 410), 293 sh (23 395), 319 (30 460), 330 sh (27 295), 356 sh (14 680), 380 sh (11 520), 442 sh (8700)
4	CH_3CN	244 (54 315), 272 (47 775), 292 sh (32 915), 315 (23 200), 347 (29 320), 378 sh (16 755), 405 (11 450), 443 (8360), 472 (7805)
	CH_2Cl_2	245 (57 550), 274 (51 970), 294 sh (34 930), 318 (24 100), 350 (31 430), 382 sh (17 900), 404 (13 085), 446 (9190), 472 (8930)

biotin complex to avidin was estimated from the following equation:[3b]

$$\frac{1}{F - F_4'} = \frac{1}{F_3' - F_4'} + \frac{1}{F_3' - F_4'}(a/V)k_{on}t$$

where F = emission intensity at time = t, F_3' = initial emission intensity, F_4' = final emission intensity, a = number of moles of avidin added, V = total volume of solution, and t = reaction time after avidin was added.

In practice, F_4' was varied until the plot of $1/(F - F_4')$ vs t was linear. The y-intercept and slope of the linear fit gave $1/(F_3' - F_4')$ and $(F_3' - F_4')^{-1}(a/V)k_{on}$, respectively. Thus, the constant k_{on} can be determined.

The dissociation of the iridium(III)−biotin complex from the iridium(III)−avidin adduct was induced by addition of an excess of unmodified biotin. The subsequent decrease in emission intensity was interpreted in terms of a monomolecular reaction. The off-rate constant k_{off} was determined according to the following equation:[3b]

$$\frac{F - F_3}{F_4 - F_3} = -k_{off}t$$

where F = emission intensity at time = t, F_4 = initial emission intensity of the iridium(III)−avidin adduct, F_3 = emission intensity after dissociation of 1 equiv of iridium(III)−biotin, and t = reaction time after excess biotin is added.

The slope of the linear fit of $(F - F_3)/(F_4 - F_3)$ vs t gave the value $-k_{off}$.

The first dissociation constants K_d of the iridium(III)−biotin complexes were then calculated as k_{off}/k_{on}.

Homogeneous Competitive Assay for Biotin. Avidin in 50 mM potassium phosphate buffer at pH 7.4 was added to a mixture of complex **4** and biotin analyte. The concentrations of avidin and complex **4** were 0.87 and 3.5 μM, respectively, while that of biotin ranged from 1×10^{-3} to 1×10^{-9} M. The assay mixtures (2 mL) were incubated at room temperature for 30 min. The emission spectra of the solutions were then measured.

Conjugation of Malachite Green Isothiocyanate to Avidin. Malachite Green isothiocyanate (0.22 mg) in anhydrous DMSO (30 μL) was added to avidin (1.49 mg) dissolved in 270 μL of 50 mM carbonate buffer at pH 9. The solution was stirred gently at room temperature for 2 h. Then, the solution was diluted to 1 mL with 50 mM potassium phosphate buffer pH 7.4 and loaded onto a PD-10 column that had been equilibrated with the same buffer. The first green band that came out from the column was collected. The solution was concentrated by centrifugation with a YM-30 centricon.

Results and Discussion

Synthesis. The biotin-containing diimine ligand L was synthesized from the reaction of succinimidyl-4-carboxy-4′-methyl-2,2′-bipyridine[19] with *N*-biotinyl-1,6-diaminohexane[20] in DMF at room temperature. The luminescent iridium(III)−biotin complexes were prepared from the reaction of L with the corresponding dimers $[Ir_2(N{-}C)_4Cl_2]$ ($N{-}C^-$ = bt$^-$, bsb$^-$, btth$^-$, and bsn$^-$) in refluxing dichloromethane/2-ethoxyethanol (1:1 v/v), followed by metathesis with KPF_6. The complexes were then purified by column chromatography and finally recrystallized from acetonitrile/diethyl ether. All the complexes gave satisfactory elemental analysis and were characterized by ^{1}H NMR, IR, and positive-ion ESI-MS.

Electronic Absorption and Luminescence Properties. The electronic absorption spectral data for complexes **1**−**4** are summarized in Table 1. The electronic absorption spectra in dichloromethane at 298 K are shown in Figure 1. The electronic absorption spectra of complexes **1**−**3** showed intense high-energy absorption bands (ϵ on the order of 10^4 dm^3 mol^{-1} cm^{-1}) in the UV region at ca. 255−352 nm, ca. 253−360 nm, and ca. 261−356 nm, respectively. These bands are similar to those of the corresponding free cyclometalating and diimine ligands and are thus assigned to ^{1}IL ($\pi \rightarrow \pi^*$) ($N{-}C^-$ and L) transitions. In addition, less intense absorption bands in the visible region (> ca. 370 nm)

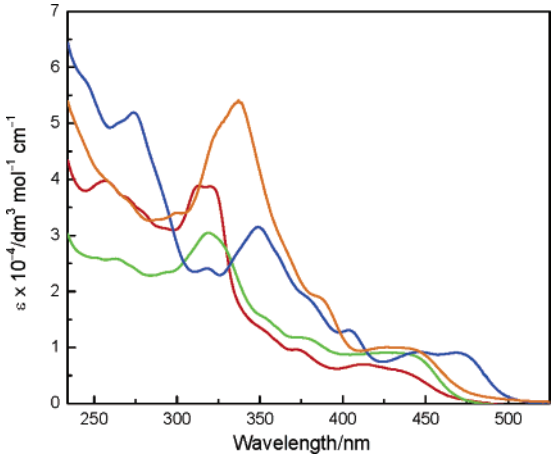


Figure 1. Electronic absorption spectra of complexes **1** (red), **2** (orange), **3** (green), and **4** (blue) in dichloromethane at 298 K.

Table 2. Photophysical Data for Complexes 1−4

complex	medium (*T*/K)	λ_{em}/nm	τ_o/μs	Φ
1	CH_2Cl_2 (298)	528, 563 (max), 612 sh	3.21	0.51
	CH_3CN (298)	528 sh, 570, 616 sh	1.31	0.33
	buffer[a] (298)	566, 612 sh	0.12	0.019
	glass[b] (77)	516 (max), 530 sh, 557, 574 sh, 604, 628 sh, 664 sh	5.68	
2	CH_2Cl_2 (298)	557 (max), 596, 638 sh	5.80	0.39
	CH_3CN (298)	556 (max), 595, 636 sh	5.84	0.40
	buffer[a] (298)	564 (max), 597, 637 sh	1.90	0.051
	glass[b] (77)	543 (max), 589, 640	8.25	
3	CH_2Cl_2 (298)	580 (max), 624, 685 sh	9.29	0.25
	CH_3CN (298)	580 (max), 624, 685 sh	8.53	0.23
	buffer[a] (298)	582 (max), 625, 685 sh	0.27	0.016
	glass[b] (77)	572 (max), 592, 622, 644 sh, 678	11.39	
4	CH_2Cl_2 (298)	598 (max), 648, 710 sh	4.53	0.11
	CH_3CN (298)	598 (max), 648, 710 sh	3.92	0.10
	buffer[a] (298)	598 (max), 648, 712 sh	1.91	0.014
	glass[b] (77)	590 (max), 608, 642, 664 sh, 702	6.42	

[a] Potassium phosphate buffer (50 mM, pH 7.4) containing 25% DMSO. [b] EtOH/MeOH (4:1 v/v).

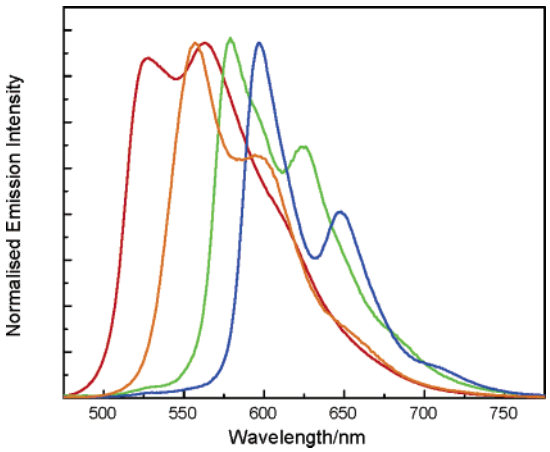


Figure 2. Emission spectra of complexes **1** (red), **2** (orange), **3** (green), and **4** (blue) in degassed dichloromethane at 298 K.

with tailing extending to ca. 500 nm were also observed. These low-energy absorption bands of complexes **1**−**3** are tentatively assigned to spin-allowed ^{1}MLCT (dπ(Ir) → π^*(N−C$^-$ and L)) transitions, and the absorption tails are assigned to spin-forbidden ^{3}MLCT (dπ(Ir) → π^*(N−C$^-$ and L)) transitions.[5,6a,b,d,7,9a,b,11] The electronic absorption spectrum of complex **4** also displayed similar ^{1}IL, ^{1}MLCT, and ^{3}MLCT absorption features. However, these absorption bands occurred at lower energy compared to those of complexes **1**−**3**, which could be accounted for by the higher π-conjugation of the bsn$^-$ ligand.

Upon irradiation, complexes **1**−**4** displayed intense green to red emission under ambient conditions and in alcohol glass at 77 K. The photophysical data are summarized in Table 2. The normalized emission spectra of complexes **1**−**4** in degassed dichloromethane at 298 K are shown in Figure 2. In solutions at 298 K, complexes **1**−**4** exhibited long-lived (in the microsecond time scale) and structured emission bands at ca. 528−712 nm. In view of very long emission lifetimes and rich structural features of the emission bands, the emission is assigned to an ^{3}IL (π → π^*) (N−C$^-$) excited state, perhaps with mixing of some ^{3}MLCT (dπ(Ir) → π^*(N−C$^-$)) character. This assignment is supported by the observations that complex **4**, which contained a more

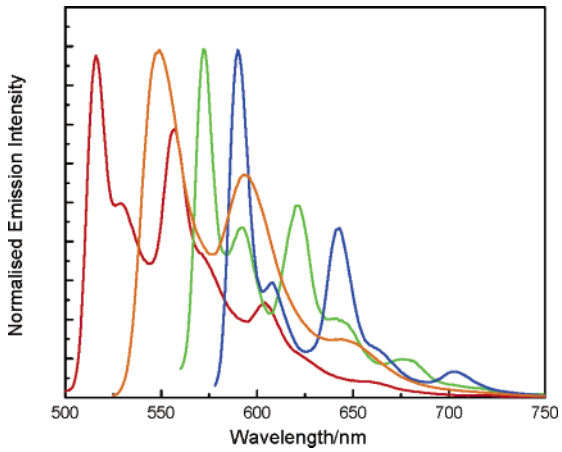


Figure 3. Emission spectra of complexes **1** (red), **2** (orange), **3** (green), and **4** (blue) in EtOH/MeOH (4:1 v/v) at 77 K.

Table 3. Electrochemical Data for Complexes 1−4 at 298 K[a]

complex	oxidation $E_{1/2}$ or E_a/V	reduction $E_{1/2}$/V
1	+1.28,[b] +1.41[c]	−1.22,[c] −1.87,[c] −2.00,[c] −2.37[c]
2	+1.26,[b] +1.42[c]	−1.24,[c] −1.72,[c] −1.96,[c] −2.28[c]
3	+1.27,[b] +1.40[c]	−1.19,[c] −1.87,[c] −2.11,[c] −2.32[c]
4	+1.37,[b] +1.47[c]	−1.20,[c] −1.68,[c] −1.93,[c] −2.41[c]

[a] In acetonitrile (0.1 mol dm^{-3} TBAP) at 298 K, glassy carbon electrode, sweep rate 0.1 V s^{-1}, all potentials vs SCE. [b] Irreversible wave. [c] Quasi-reversible wave.

conjugated bsn$^-$ ligand, emitted at lower energy than complex **1**, which had a less conjugated bt$^-$ ligand. Similar ^{3}IL emission has been commonly observed in related iridium(III) arylbenzothiazole complexes with acetylacetonate and other *O*,*O* and *O*,*N* ligands.[9,14] It is interesting to note that using arylbenzothiazole as the ligands, the emissive state of these cyclometalated iridium(III) polypyridine complexes shifted from ^{3}MLCT (dπ(Ir) → π^*(N−N)) to ^{3}IL (π → π^*) (N−C$^-$)/^{3}MLCT (dπ(Ir) → π^*(N−C$^-$)) in character. Similar observations have been made in the cyclometalated iridium(III) polypyridine complexes with a conjugated 2-phenylquinoline ligand.[4c,9b,c]

In alcohol glass at 77 K, complexes **1**−**4** also showed emission bands with very rich structural features (Figure 3). The emission maxima of complexes **1**−**4** appeared at ca. 516−590 nm, and other emission bands and shoulders occurred at longer wavelengths up to ca. 702 nm (Table 2). All the decays were single-exponential, and the lifetimes varied from 5.7 to 11.4 μs. These observations suggest that the emission of these complexes at 77 K was derived from an ^{3}IL (π → π^*) (N−C$^-$) emission state that may have some ^{3}MLCT (dπ(Ir) → π^*(N−C$^-$)) character. Similar assignments have been made in related systems.[4c,9]

Electrochemical Properties. The electrochemical properties of complexes **1**−**4** have been studied by cyclic voltammetry. The electrochemical data are listed in Table 3. Complexes **1**−**4** exhibited an irreversible oxidation wave at ca. +1.26 to +1.37 V and a quasi-reversible couple at ca. +1.40 to +1.47 V vs SCE. The observation of two oxidation features is rare for cyclometalated iridium(III) polypyridine complexes.[4c] Although the first irreversible oxidation wave occurred at a potential (ca. +1.26 to +1.37 V vs SCE) that is common for Ir(IV/III)

couples,[4c,5b,c,7c,d,9a−c,11] its irreversible nature and the observations of a similar irreversible wave for the free arylbenzothiazole ligands suggest that it could be associated with the oxidation of the cyclometalating ligands. The second oxidation features that appeared as a quasi-reversible couple at ca. +1.40 to +1.47 V are tentatively assigned to the Ir(IV/III) couple.[4c,5b,c,7c,d,9a−c,11] The occurrence of these couples at slightly more positive potentials than common cyclometalated iridium(III) polypyridine complexes is probably a result of the π-accepting properties of the conjugated arylbenzothiazole ligands. Complexes **1**−**4** showed a quasi-reversible couple at ca. −1.2 V, which is assigned to the reduction of the biotin-containing diimine ligand.[4c,5b,c,6,7a,c,8c,11a] This assignment is supported by the fact that these quasi-reversible couples appeared at very similar potentials for all four complexes. Several quasi-reversible couples at ca. −1.68 to −2.41 V were also noted in the cyclic voltammograms, which are assigned to the reduction of the diimine and the arylbenzothiazole ligands.[4c,5b,c,6,7a,c,8c,11a]

HABA Assay of Iridium(III)−Biotin Complexes. The avidin-binding properties of the iridium(III)−biotin complexes have been investigated by an assay that is based on the competition between unmodified biotin or biotinylated species and the dye 4′-hydroxyazobenzene-2-carboxylic acid (HABA) on binding to avidin.[21] The binding of HABA to avidin gives rise to an absorption feature at ca. 500 nm. Since the binding of HABA to avidin ($K_d = 6 \times 10^{-6}$ M) is much weaker than that of biotin (K_d = ca. 10^{-15} M), addition of biotin will replace the bound HABA molecules from the protein, leading to a decrease of the absorbance at 500 nm. In the current work, we observed that the absorbance of a solution of HABA and avidin at 500 nm decreased upon addition of the iridium(III)−biotin complexes. These findings indicated that the bound HABA molecules were replaced by the iridium(III)−biotin complexes. In other words, the biotin moiety of the complexes can specifically bind to the biotin-binding site of the protein. Unfortunately, precipitation occurred before the equivalence points were reached, which may be due to the highly hydrophobic nature of the cyclometalating ligands. To gain more insights into the avidin-binding properties of these complexes, luminescence titrations and competitive assays have been studied.

Emission Titrations. The avidin-binding properties of the iridium(III)−biotin complexes have been investigated by luminescence titrations using the complexes as the titrants. The emission titration curves for complexes **1**−**4** are illustrated in Figures 4−7, respectively. Similar to other luminescent transition metal biotin complexes,[4] all the current iridium(III)−biotin complexes displayed enhanced emission intensities and extended emission lifetimes in the presence of avidin (Table 4). Results of the control experiments confirmed that these changes of emission properties were a consequence of the binding of the biotin moieties of the complexes to avidin. It is interesting to note that under our experimental conditions, the equivalence points of the titrations varied from ca. 5.1 to 8.5 for complexes **1**−**4**. The possibility of nonspecific binding is excluded on the basis of the results of the control experiments. The occurrence of equivalence points beyond the expected stoichiometry ([biotin]:[avidin] = 4) suggests that the concentrations of the complexes used in these titration experiments were not substantially larger than the K_d values of these systems. Unfortunately, higher concentrations of complexes could not be employed in these titrations due to precipitation problems.

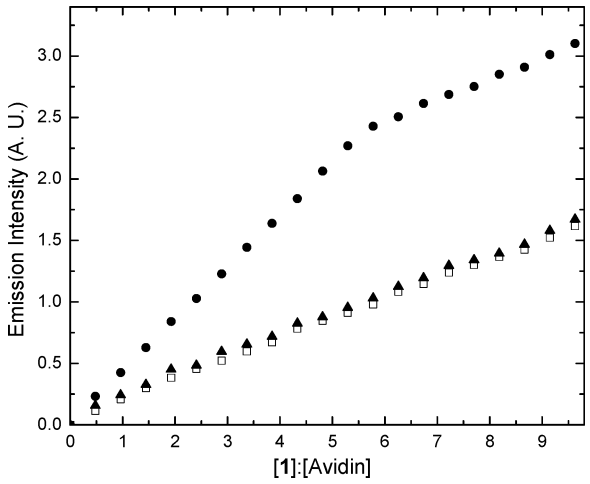


Figure 4. Luminescence titration curves for the titrations of (i) 0.67 μM avidin (●), (ii) 0.67 μM avidin and 67.0 μM unmodified biotin (▲), and (iii) a blank phosphate buffer solution (□) with complex **1**.

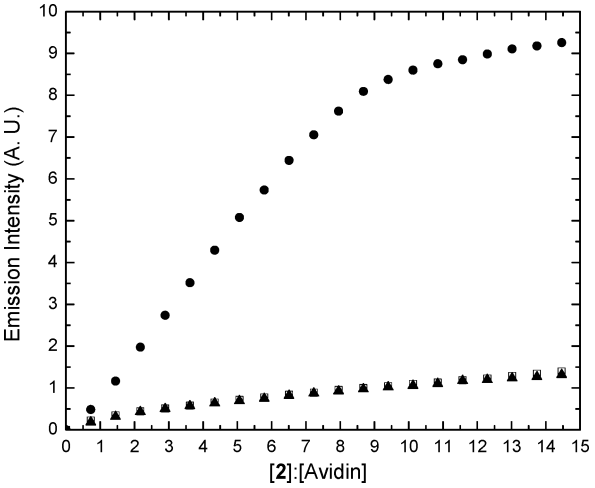


Figure 5. Luminescence titration curves for the titrations of (i) 0.67 μM avidin (●), (ii) 0.67 μM avidin and 67.0 μM unmodified biotin (▲), and (iii) a blank phosphate buffer solution (□) with complex **2**.

Since the emission quantum yields and lifetimes of complexes **1**−**4** decreased with increasing solvent polarity (Table 2), the avidin-induced emission enhancement and lifetime extension should be closely related to the increase in the hydrophobicity of the local environment of the complexes after they bind to avidin.[4] In line with this argument, it is important to note that the more hydrophobic biphenyl complex **2** and naphthyl complex **4** showed very significant emission enhancement (8.1- and 5.8-fold, respectively, Table 4), which are among the highest enhancement factors that have been observed.[4] Thus, employing more hydrophobic luminescent biotin conjugates provides higher detection sensitivity,

(21) (a) Green, N. M. *Biochem. J.* **1965**, *94*, 23C. (b) Green, N. M. *Adv. Protein Chem.* **1975**, *29*, 85. (c) Hermanson, G. T. *Bioconjugate Techniques*; Academic Press: San Diego, CA, 1996.

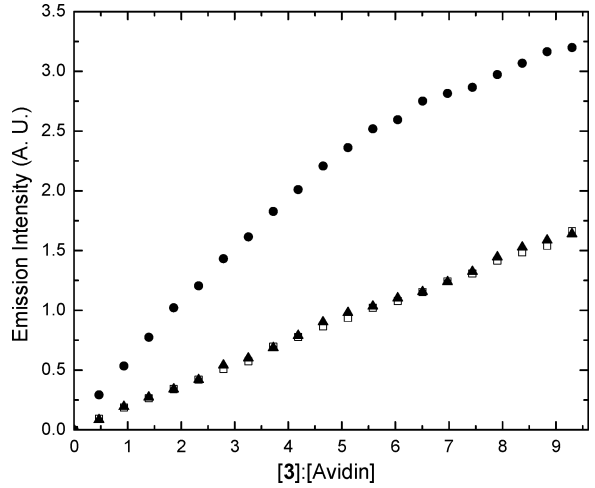


Figure 6. Luminescence titration curves for the titrations of (i) 0.67 μM avidin (●), (ii) 0.67 μM avidin and 67.0 μM unmodified biotin (▲), and (iii) a blank phosphate buffer solution (□) with complex **3**.

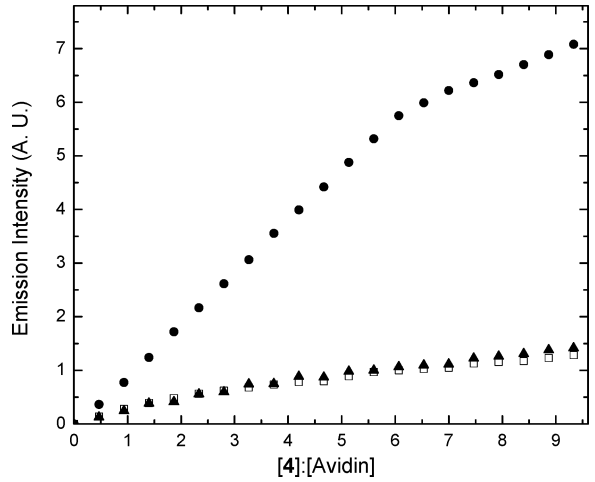


Figure 7. Luminescence titration curves for the titrations of (i) 0.67 μM avidin (●), (ii) 0.67 μM avidin and 67.0 μM unmodified biotin (▲), and (iii) a blank phosphate buffer solution (□) with complex **4**.

Table 4. Relative Emission Intensities and Lifetimes of Complexes 1−4 in the Absence and Presence of Avidin (and excess biotin) in Aerated 50 mM Potassium Phosphate Buffer pH 7.4 at 298 K

complex	I (τ/μs)[a]	I (τ/μs)[b]	I (τ/μs)[c]
1[d]	1.00 (0.051)	2.38 (0.12)	1.03 (0.049)
2[e]	1.00 (0.39)	8.09 (1.94)	0.97 (0.48)
3[f]	1.00 (0.12)	2.47 (0.45)	1.02 (0.13)
4[g]	1.00 (1.24)	5.84 (1.83)	1.05 (1.00)

[a] Iridium(III) complex only. [b] [avidin] = 0.67 μM. [c] [avidin] = 0.67 μM, [unmodified biotin] = 67.0 μM. [d] [**1**] = 3.7 μM. [e] [**2**] = 4.1 μM. [f] [**3**] = 3.4 μM. [g] [**4**] = 5.7 μM.

although lower solubility may be a concern. Apart from the hydrophobicity factor, we believe that the emission-enhancement could also be related to the increased rigidity of the surroundings of the iridium(III) complexes after they bind to avidin. This increase in rigidity is expected to result in lower nonradiative decay rates for the complexes, and hence enhanced emission intensities and longer lifetimes.

Competitive Assays and First Dissociation Constants. The competition between the iridium(III)−biotin complexes and native biotin to avidin has been studied by competitive association and dissociation assays.[3,22] In the association assays, the iridium(III)−biotin complexes competed with biotin on binding to avidin, while in the dissociation assays, the complexes bound to avidin were challenged by addition of biotin. The percentage of the labeled avidin molecules was determined by emission measurements with reference to control experiments. The results of the association and dissociation assays are shown in Table 5. The percentage values of avidin that remained bound by complexes **1** and **3** were larger than those of complexes **2** and **4** in both assays. It appears that these observations were due to the relatively bulky biphenyl and naphthyl groups of the cyclometalating ligands of complexes **2** and **4**, respectively. The first dissociation constants, K_d, have been estimated from the on-rate constants (k_{on}) and off-rate constants (k_{off}) of the binding of iridium(III)−biotin complexes to avidin molecules (K_d was defined as k_{off}/k_{on}).[3b,23] The K_d values ranged from 7.5×10^{-9} to 1.8×10^{-8} M (Table 5), which were about 6 to 7 orders of magnitude larger than that of the native biotin−avidin system (K_d = ca. 10^{-15} M).[1] It is conceivable that the weaker binding of these complexes toward avidin than that of native biotin is due to the bulkiness of the cyclometalated iridium(III)−biotin complexes, which is also in line with the results from the association and dissociation assays.

Table 5. Results of Competitive Association and Dissociation Assays and First Dissociation Constants for the Iridium−Avidin Adducts in 50 mM Potassium Phosphate Buffer pH 7.4 at 298 K

complex	association assay/ % avidin bound	dissociation assay/ % avidin bound	K_d/M
1	37	70	7.5×10^{-9}
2	16	49	1.8×10^{-8}
3	43	65	8.3×10^{-9}
4	22	53	1.0×10^{-8}

Homogeneous Competitive Assay for Biotin. In view of the significant emission enhancement exhibited by the iridium(III)−biotin complexes when they bind to avidin, a homogeneous assay for biotin has been developed. The assay was based on the competition between native biotin and the iridium(III)−biotin on binding to avidin. In the assay, avidin was added to a mixture of native biotin and complex **4**. After incubation at room temperature, the emission intensity of the solution was measured. At lower native biotin concentration, a higher degree of binding of complex **4** occurs, and thus a higher emission intensity is expected. The emission of the solutions was measured over a biotin analyte concentration range from 1×10^{-3} to 1×10^{-9} M. The results of the assay are shown in Figure 8. The concentration range of biotin that can be measured by this assay was from ca. 1×10^{-5} to 1×10^{-7} M. The lowest biotin analyte concentration (ca. 1×10^{-7} M) that gave a meaningful signal was slightly higher than the competitive biotin assay we reported on other cyclometalated iridium(III) complexes.[4c]

(22) 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.
(23) Wang, Z. X.; Kumar, N. R.; Srivastava, D. K. *Anal. Biochem.* **1992**, *206*, 376.

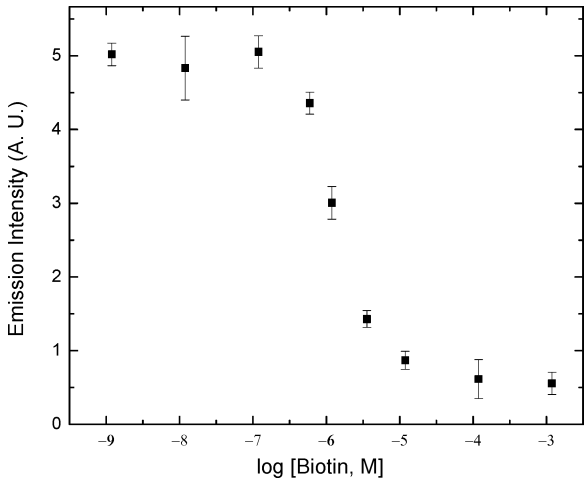


Figure 8. Homogeneous competitive assay for biotin using complex **4** and avidin. The emission intensity of the supernatant was an average of triplicate experiments ±1 standard deviation.

Quenching Studies Using Avidin Modified by Malachite Green. The possibility of utilizing the current iridium(III) complexes as probes for biotin and biotinylated species on the basis of the resonance-energy transfer (RET) principle has been investigated. To maximize the Förster distance, Malachite Green-modified avidin (MG-Av) was used as the energy acceptor given the considerable overlap between the absorption band of the dye ($\lambda_{abs} = 629$ nm) and the emission bands of the donor, complex **3** ($\lambda_{em} = 582$ (max), 625, and 685 (sh) nm). On the basis of the spectral data of the donor and acceptor, a Förster distance of ca. 38.5 Å has been estimated. We found that the emission intensity of complex **3** (3.7 μM) in degassed buffer was enhanced by 20-fold (Figure 9) and the lifetime was extended from 0.27 to 1.82 μs in the presence of avidin (1.4 μM). However, the emission intensity of the complex only increased by 1.5-fold and the lifetime was reduced from 0.27 to 0.15 μs when MG-Av was used instead of avidin. It is likely that the lower emission intensity and shorter emission lifetime were a consequence of the binding of the complex to MG-Av, leading to shorter distances between the donor and the acceptor molecules. As a result, emission quenching due to RET occurred. This is supported by the fact that no such decrease in emission lifetime ($\tau = 0.29$ μs) was observed when MG-Av was preblocked with unmodified biotin (370 μM). The results of these experiments could form the basis of detection of biotin and biotinylated molecules using emission lifetime measurements.

Summary. In this work, a series of luminescent iridium(III) arylbenzothiazole biotin complexes have been synthesized and characterized. The photophysical and electrochemical properties of these complexes have been investigated. Unlike other common luminescent cyclometalated iridium(III) polypyridine complexes, the lowest-energy emission of these complexes was derived from an ^{3}IL ($\pi \rightarrow \pi^*$) (N−C$^-$) state that may have some ^{3}MLCT (dπ(Ir) $\rightarrow$ π^*(N−C$^-$)) character. The use of various arylbenzothiazoles as the cyclometalating ligands enabled the emission of the complexes to occur in a wider wavelength range, from ca. 528 to 712 nm in fluid solutions at 298 K. The avidin−biotin binding properties of these complexes have been studied by emission titrations and various assays. Similar to other luminescent transition metal biotin complexes, all these iridium(III) arylbenzothiazole biotin complexes exhibited emission enhancement and extension of emission lifetimes. Most importantly, the more hydrophobic biphenyl and naphthyl complexes showed remarkably high emission enhancement factors upon binding to avidin, rendering them useful probes for this protein. The designs of related luminescent probes for avidin and related proteins are underway.

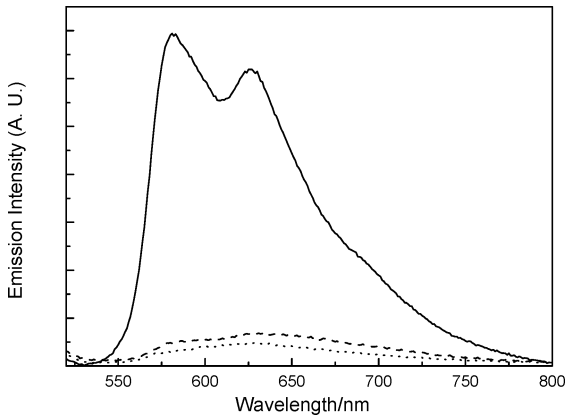


Figure 9. Emission spectra of complex **3** (3.7 μM) in a degassed (i) 1.4 μM avidin solution (—), (ii) 1.4 μM MG-Av solution (- - -), and (iii) blank phosphate buffer solution (···) at 298 K.

Acknowledgment. We thank the Hong Kong Research Grants Council (Project No. CityU 101603) and City University of Hong Kong (Project No. 7001632) for financial support. J.S.-Y.L. acknowledges the receipt of a Postgraduate Studentship and a Research Tuition Scholarship, both administered by the City University of Hong Kong. K.K.-W.L. thanks the Faculty of Science and Engineering for financial support (Young/Junior Scholars Scheme).

Note Added after ASAP Publication. In the version of this paper posted on the Web August 6, 2005, the first two entries in column 2 of Table 2 for each of the four compounds were transposed. The version of this table that now appears is correct.

OM050327E