# Synthesis, Characterization, and Properties of Luminescent Organoiridium(III) Polypyridine Complexes Appended with an Alkyl Chain and Their Interactions with Lipid Bilayers, Surfactants, and Living Cells

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A series of new luminescent organoiridium(III) polypyridine complexes $[Ir(N{-}C)_2(N{-}N)](PF_6)$ (HN−C = 2-phenylpyridine, Hppy, N−N = 4-*n*-octadecylaminocarbonyl-4′-methyl-2,2′-bipyridine (Me-bpy-CONH-$C_{18}H_{37}$) (**1a**), 4-*n*-decylaminocarbonyl-4′-methyl-2,2′-bipyridine (Me-bpy-CONH-$C_{10}H_{21}$) (**1b**), 4-ethylaminocarbonyl-4′-methyl-2,2′-bipyridine (Me-bpy-CONH-$C_2H_5$) (**1c**); HN−C = 1-phenylpyrazole, Hppz, N−N = Me-bpy-CONH-$C_{18}H_{37}$ (**2a**), Me-bpy-CONH-$C_{10}H_{21}$ (**2b**), Me-bpy-CONH-$C_2H_5$ (**2c**); HN−C = 2-phenylquinoline, Hpq, N−N = Me-bpy-CONH-$C_{18}H_{37}$ (**3a**), Me-bpy-CONH-$C_{10}H_{21}$ (**3b**), Me-bpy-CONH-$C_2H_5$ (**3c**)) bearing an alkyl pendant have been synthesized and characterized. The photophysical and electrochemical properties of these complexes have been investigated. Upon irradiation, all the complexes exhibited intense and long-lived luminescence in homogeneous fluid solutions at 298 K and in alcohol glass at 77 K. The emission has been assigned to a triplet metal-to-ligand charge-transfer ($^3$MLCT) (d$\pi$(Ir) → $\pi^*$(diimine)) excited state. The emissive states of the pq complexes **3a**−**c** are probably mixed with some triplet intraligand ($^3$IL) ($\pi \rightarrow \pi^*$) (pq) character. All the complexes have been incorporated into phospholipid vesicles composed of 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), and the resulting liposomes have been examined by cryogenic transmission electron microscopy (cryo-TEM) and luminescence spectroscopy. Also, the emission properties of the complexes in aqueous solutions containing the surfactants sodium dodecylsulfate (SDS), Triton X-100 (TX), and cetyltrimethylammonium bromide (CTAB) have been studied. The lipophilicity of the complexes has been determined by reversed-phase HPLC, and the log $P_{o/w}$ values were dependent on the cyclometalating and diimine ligands. Additionally, the cytotoxicity of these organoiridium(III) complexes toward the human cervix epithelioid carcinoma (HeLa) cell line has been evaluated by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay. Furthermore, the cellular uptake of all the complexes by HeLa cells has been examined by flow cytometry and laser-scanning confocal microscopy.

## Introduction

Hydrophobic interactions occur in many essential life processes such as the regulated transport of substances in fluids and their mediated exchange across the cell membrane by a great variety of biological carriers. The development of lipophilic probes to study these systems including lipoproteins and natural lipid bilayers, together with artificial liposomes as stimulated models, has thus been growing. In this context, fluorescent lipophilic probes have been derived from most well-known organic fluorophores such as anthracene,[1a,b] fluorene,[1c] and pyrene.[1d,e] The structures of these reagents resemble those of naturally occurring lipids such as fatty acids, phospholipids, and triglycerides. Since labeled lipids also interact innately with hydrophobic biological systems, fluorophores of their structural analogues can be embedded into different regions of these systems and thus act as fluorescent reporters of their local environments. Consequently, these probes have been commonly utilized in biological studies, for instance, to examine various biological organizations such as natural membranes,[2a,b] fatty acid-binding proteins,[2c] and specific lipid classes in living cells.[2d] They have also been used to investigate a diverse assortment of biological events such as membrane fusion processes,[3a] lipid diffusion,[3b] lipid−protein interactions,[3c] and the triacylglycerol transport between plasma lipoproteins.[3d] Although parallel studies of transition metal complexes as lipophilic biological probes have not been widely reported, interactions between transition metal complexes and surfactants have received much attention.[4] Additionally, surfactant molecules have been incorporated into luminescent transition metal complexes and the

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(1) (a) Wootan, M. G.; Bass, N. M.; Bernlohr, D. A.; Storch, J. *Biochemistry* **1990**, *29*, 9305. (b) Abrams, F. S.; Chattopadhyay, A.; London, E. *Biochemistry* **1992**, *31*, 5322. (c) Lala, A. K.; Koppaka, V. *Biochemistry* **1992**, *31*, 5586. (d) Galla, H.-J.; Sackmann, E. *J. Am. Chem. Soc.* **1975**, *97*, 4114. (e) Stegmann, T.; Schoen, P.; Bron, R.; Wey, J.; Bartoldus, I.; Ortiz, A.; Nieva, J.-L.; Wilschut, J. *Biochemistry* **1993**, *32*, 11330.

(2) (a) Asuncion-Punzalan, E.; Kachel, K.; London, E. *Biochemistry* **1998**, *37*, 4603. (b) Kaiser, R. D.; London, E. *Biochemistry* **1998**, *37*, 8180. (c) Prior, A.; Jones, J. T.; Blok, V. C.; Beauchamp, J.; McDermott, L.; Cooper, A.; Kennedy, M. W. *Biochem. J.* **2001**, *356*, 387. (d) Rogers, R. A.; Jack, R. M.; Furlong, S. T. *J. Cell Sci.* **1993**, *106*, 485.

(3) (a) Struck, D. K.; Hoekstra, D.; Pagano, R. E. *Biochemistry* **1981**, *20*, 4093. (b) Hwang, J.; Gheber, L. A.; Margolis, L.; Edidin, M. *Biophys. J.* **1998**, *74*, 2184. (c) Keller, R. C. A.; ten Berge, D.; Nouwen, N.; Snel, M. M. E.; Tommassen, J.; Marsh, D.; de Kruijff, B. *Biochemistry* **1996**, *35*, 3063. (d) Main, L. A.; Okumura-Noji, K.; Ohnishi, T.; Yokoyama, S. *J. Biochem.* **1998**, *124*, 237.

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properties of these derivatives have been examined.[5] In view of the environment-sensitive emission of organometallic and inorganic metal complexes and their longer emission lifetimes compared to organic fluorophores, a systematic design of related compounds promisingly affords an alternative discernible detection of hydrophobic interactions in biological systems.

The photophysics and photochemistry of many luminescent organoiridium(III) polypyridine complexes have been well documented.[6–23] On account of their typically excellent luminescence quantum yields and flexibility for emission tuning, these remarkable emitters have found enormous applications as light-emitting devices,[10c,13c,15,16b,18a,22a,b] photocatalysts,[22b,c] and sensors[16c,22b] in various fields. Our interest is to design and utilize these organometallic compounds as luminescent probes and labels for biological applications.[23] By incorporating a hydrocarbon chain into emissive organoiridium(III) polypyridine complexes, we envisage that a new class of luminescent sensors for probing hydrophobic biological interactions can be developed. In addition to their intense and long-lived emission, luminescent organoiridium(III) polypyridine complexes possess advantages over other inorganic probes including their high structural variation, rich emissive-state character, and a wider range of emission energy. Herein, we report the synthesis and characterization of a series of luminescent organoiridium(III) polypyridine complexes $[Ir(N{-}C)_2(N{-}N)](PF_6)$ (HN−C = 2-phenylpyridine, Hppy, N−N = 4-*n*-octadecylaminocarbonyl-4′-methyl-2,2′-bipyridine (Me-bpy-CONH-$C_{18}H_{37}$) (**1a**), 4-*n*-decylaminocarbonyl-4′-methyl-2,2′-bipyridine (Me-bpy-CONH-$C_{10}H_{21}$) (**1b**), 4-ethylaminocarbonyl-4′-methyl-2,2′-bipyridine (Me-bpy-CONH-$C_2H_5$) (**1c**); HN−C = 1-phenylpyrazole, Hppz, N−N = Me-bpy-CONH-$C_{18}H_{37}$ (**2a**), Me-bpy-CONH-$C_{10}H_{21}$ (**2b**), Me-bpy-CONH-$C_2H_5$ (**2c**); HN−C = 2-phenylquinoline, Hpq, N−N = Me-bpy-CONH-$C_{18}H_{37}$ (**3a**), Me-bpy-CONH-$C_{10}H_{21}$ (**3b**), Me-bpy-CONH-$C_2H_5$ (**3c**)) appended with an alkyl chain. The structures of these complexes are illustrated in Chart 1. Their electronic absorption, photophysical, and electrochemical properties have been investigated. These complexes have been incorporated into lipid vesicles composed of 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), and the resulting liposomes have been examined by cryogenic transmission electron microscopy (cryo-TEM) and luminescence spectroscopy. Also, the emission properties of the complexes in aqueous solutions

(4) (a) Hauenstein, B. L., Jr.; Dressick, W. J.; Buell, S. L.; Demas, J. N.; DeGraff, B. A. *J. Am. Chem. Soc.* **1983**, *105*, 4251. (b) Thorp, H. H.; Kumar, C. V.; Turro, N. J.; Gray, H. B. *J. Am. Chem. Soc.* **1989**, *111*, 4364. (c) Chambron, J.-C.; Sauvage, J.-P. *Chem. Phys. Lett.* **1991**, *182*, 603. (d) Arkin, M. R.; Stemp, E. D. A.; Turro, C.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1996**, *118*, 2267. (e) Hackett, J. W., II; Turro, C. *Inorg. Chem.* **1998**, *37*, 2039. (f) Wu, L.-Z.; Cheung, T.-C.; Che, C.-M.; Cheung, K.-K.; Lam, M. H.-W. *Chem. Commun.* **1998**, 1127. (g) Jain, A.; Xu, W.; Demas, J. N.; DeGraff, B. A. *Inorg. Chem.* **1998**, *37*, 1876. (h) García-Fresnadillo, D.; Orellana, G. *Helv. Chim. Acta* **2001**, *84*, 2708.

(5) (a) Sprintschnik, G.; Sprintschnik, H. W.; Kirsch, P. P.; Whitten, D. G. *J. Am. Chem. Soc.* **1977**, *99*, 4947. (b) Gaines, G. L., Jr. *Inorg. Chem.* **1980**, *19*, 1710. (c) Reitz, G. A.; Demas, J. N.; DeGraff, B. A.; Stephens, E. M. *J. Am. Chem. Soc.* **1988**, *110*, 5051. (d) Sacksteder, L.; Demas, J. N.; DeGraff, B. A. *Inorg. Chem.* **1989**, *28*, 1787. (e) Li, L.; Szmacinski, H.; Lakowicz, J. R. *Anal. Biochem.* **1997**, *244*, 80. (f) Yam, V. W.-W.; Lau, V. C.-Y.; Wang, K.-Z.; Cheung, K.-K.; Huang, C.-H. *J. Mater. Chem.* **1998**, *8*, 89. (g) Yam, V. W.-W.; Yang, Y.; Zhang, J.; Chu, B. W.-K.; Zhu, N. *Organometallics* **2001**, *20*, 4911. (h) Zhang, J.; Chu, B. W.-K.; Zhu, N.; Yam, V. W.-W. *Organometallics* **2007**, *26*, 5423. (i) Fiore, G. L.; Edwards, J. M.; Payne, S. J.; Klinkenberg, J. L.; Gioeli, D. G.; Demas, J. N.; Fraser, C. L. *Biomacromolecules* **2007**, *8*, 2829.

(6) (a) Sprouse, S.; King, K. A.; Spellane, P. J.; Watts, R. J. *J. Am. Chem. Soc.* **1984**, *106*, 6647. (b) Wilde, A. P.; Watts, R. J. *J. Phys. Chem.* **1991**, *95*, 622. (c) Wilde, A. P.; King, K. A.; Watts, R. J. *J. Phys. Chem.* **1991**, *95*, 629.

(7) (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.

(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.; Flamigni, L.; Encinas, S.; Barigelletti, F. *Chem. Soc. Rev.* **2000**, *29*, 385. (c) Auffrant, A.; Barbieri, A.; Barigelletti, F.; Lacour, J.; Mobian, P.; Collin, J.-P.; Sauvage, J.-P.; Ventura, B. *Inorg. Chem.* **2007**, *46*, 6911.

(9) (a) Neve, F.; Crispini, A.; Campagna, S.; Serroni, S. *Inorg. Chem.* **1999**, *38*, 2250. (b) Neve, F.; La Deda, M.; Crispini, A.; Bellusci, A.; Puntoriero, F.; Campagna, S. *Organometallics* **2004**, *23*, 5856. (c) Neve, F.; La Deda, M.; Puntoriero, F.; Campagna, S. *Inorg. Chim. Acta* **2006**, *359*, 1666.

(10) (a) 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. (b) Sajoto, T.; Djurovich, P. I.; Tamayo, A.; Yousufuddin, M.; Bau, R.; Thompson, M. E. *Inorg. Chem.* **2005**, *44*, 7992. (c) Tamayo, A. B.; Garon, S.; Sajoto, T.; Djurovich, P. I.; Tsyba, I. M.; Bau, R.; Thompson, M. E. *Inorg. Chem.* **2005**, *44*, 8723.

(11) (a) Wilkinson, A. J.; Goeta, A. E.; Foster, C. E.; Williams, J. A. G. *Inorg. Chem.* **2004**, *43*, 6513. (b) Wilkinson, A. J.; Puschmann, H.; Howard, J. A. K.; Foster, C. E.; Williams, J. A. G. *Inorg. Chem.* **2006**, *45*, 8685. (c) Williams, J. A. G.; Wilkinson, A. J.; Whittle, V. L. *Dalton Trans.* **2008**, 2081.

(12) (a) Polson, M.; Fracasso, S.; Bertolasi, V.; Ravaglia, M.; Scandola, F. *Inorg. Chem.* **2004**, *43*, 1950. (b) Polson, M.; Ravaglia, M.; Fracasso, S.; Garavelli, M.; Scandola, F. *Inorg. Chem.* **2005**, *44*, 1282.

(13) (a) Coppo, P.; Duati, M.; Kozhevnikov, V. N.; Hofstraat, J. W.; De Cola, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 1806. (b) Avilov, I.; Minoofar, P.; Cornil, J.; De Cola, L. *J. Am. Chem. Soc.* **2007**, *129*, 8247. (c) Orselli, E.; Kottas, G. S.; Konradsson, A. E.; Coppo, P.; Frohlich, R.; Frtshlich, R.; De Cola, L.; van Dijken, A.; Buchel, M.; Borner, H. *Inorg. Chem.* **2007**, *46*, 11082.

(14) (a) Yutaka, T.; Obara, S.; Ogawa, S.; Nozaki, K.; Ikeda, N.; Ohno, T.; Ishii, Y.; Sakai, K.; Haga, M. *Inorg. Chem.* **2005**, *44*, 4737. (b) Obara, S.; Itabashi, M.; Okuda, F.; Tamaki, S.; Tanabe, Y.; Ishii, Y.; Nozaki, K.; Haga, M. *Inorg. Chem.* **2006**, *45*, 8907.

(15) (a) Hwang, F.-M.; Chen, H.-Y.; Chen, P.-S.; Liu, C.-S.; Chi, Y.; Shu, C.-F.; Wu, F.-L.; Chou, P.-T.; Peng, S.-M.; Lee, G.-H. *Inorg. Chem.* **2005**, *44*, 1344. (b) Li, H.-C.; Chou, P.-T.; Hu, Y.-H.; Cheng, Y.-M.; Liu, R.-S. *Organometallics* **2005**, *24*, 1329. (c) Chang, C.-J.; Yang, C.-H.; Chen, K.; Chi, Y.; Shu, C.-F.; Ho, M.-L.; Yeh, Y.-S.; Chou, P.-T. *Dalton Trans.* **2007**, 1881.

(16) (a) Zhao, Q.; Liu, S.; Shi, M.; Wang, C.; Yu, M.; Li, L.; Li, F.; Yi, T.; Huang, C. *Inorg. Chem.* **2006**, *45*, 6152. (b) Li, X.; Chen, Z.; Zhao, Q.; Shen, L.; Li, F.; Yi, T.; Cao, Y.; Huang, C. *Inorg. Chem.* **2007**, *46*, 5518. (c) Zhao, Q.; Cao, T.; Li, F.; Li, X.; Jing, H.; Yi, T.; Huang, C. *Organometallics* **2007**, *26*, 2077.

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

(18) (a) Yersin, H. *Top. Curr. Chem.* **2004**, *241*, 1. (b) Breu, J.; Stössel, P.; Schrader, S.; Starukhin, A.; Finkenzeller, W. J.; Yersin, H. *Chem. Mater.* **2005**, *17*, 1745.

(19) (a) Holder, E.; Marin, V.; Meier, M. A. R.; Schubert, U. S. *Macromol. Rapid Commun.* **2004**, *25*, 1491. (b) Holder, E.; Marin, V.; Alexeev, A.; Schubert, U. S. *J. Polym. Sci. Pol. Chem.* **2005**, *43*, 2765.

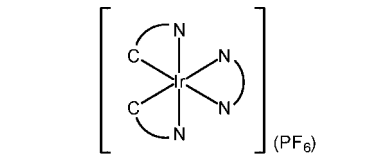
(20) (a) Maestri, M.; Balzani, V.; Deuschel-Cornioley, C.; von Zelewsky, A. *Adv. Photochem.* **1992**, *17*, 1. (b) Schaffner-Hamann, C.; von Zelewsky, A.; Barbieri, A.; Barigelletti, F.; Muller, G.; Riehl, J. P.; Neels, A. *J. Am. Chem. Soc.* **2004**, *126*, 9339.

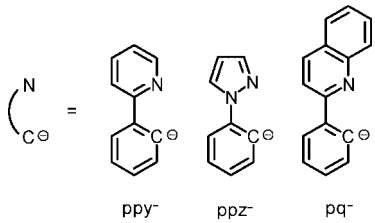
(21) (a) Ayala, N. P.; Flynn, C. M.; Sacksteder, L.; Demas, J. N.; DeGraff, B. A. *J. Am. Chem. Soc.* **1990**, *112*, 3837. (b) van Diemen, J. H.; Haasnoot, J. G.; Hage, R.; Müller, E.; Reedijk, J. *Inorg. Chim. Acta* **1991**, *181*, 245. (c) van Diemen, J. H.; Hage, R.; Haasnoot, J. G.; Lempers, H. E. B.; Reedijk, J.; Vos, J. G.; De Cola, L.; Barigelletti, F.; Balzani, V. *Inorg. Chem.* **1992**, *31*, 3518.

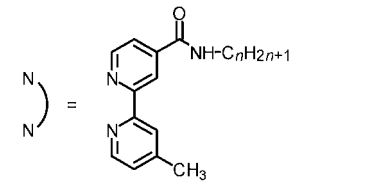
(22) (a) Slinker, J. D.; Gorodetsky, A. A.; Lowry, M. S.; Wang, J.; Parker, S.; Rohl, R.; Bernhard, S.; Malliaras, G. G. *J. Am. Chem. Soc.* **2004**, *126*, 2763. (b) Lowry, M. S.; Bernhard, S. *Chem.−Eur. J.* **2006**, *12*, 7970. (c) McDaniel, N. D.; Coughlin, F. J.; Tinker, L. L.; Bernhard, S. *J. Am. Chem. Soc.* **2008**, *130*, 210.

(23) (a) Lo, K. K.-W.; Ng, D. C.-M.; Chung, C.-K. *Organometallics* **2001**, *20*, 4999. (b) Lo, K. K.-W.; Chung, C.-K.; Zhu, N. *Chem.−Eur. J.* **2003**, *9*, 475. (c) 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. (d) Lo, K. K.-W.; Chan, J. S.-W.; Lui, L.-H.; Chung, C.-K. *Organometallics* **2004**, *23*, 3108. (e) Lo, K. K.-W.; Li, C.-K.; Lau, J. S.-Y. *Organometallics* **2005**, *24*, 4594. (f) Lo, K. K.-W.; Chung, C.-K.; Zhu, N. *Chem.−Eur. J.* **2006**, *12*, 1500. (g) Lo, K. K.-W.; Lau, J. S.-Y. *Inorg. Chem.* **2007**, *46*, 700. (h) Lo, K. K.-W.; Zhang, K. Y.; Chung, C.-K.; Kwok, K. Y. *Chem.−Eur. J.* **2007**, *13*, 7110. (i) Lo, K. K.-W.; Zhang, K. Y.; Leung, S.-K.; Tang, M.-C. *Angew. Chem., Int. Ed.* **2008**, *47*, 2213.

**Chart 1. Structures of Complexes**




*n* = 18 (Me-bpy-CONH-$C_{18}H_{37}$)
10 (Me-bpy-CONH-$C_{10}H_{21}$)
2 (Me-bpy-CONH-$C_2H_5$)

| | N–N | | |
|---|---|---|---|
| N–C⁻ | *n* = 18 | *n* = 10 | *n* = 2 |
| ppy⁻ | **1a** | **1b** | **1c** |
| ppz⁻ | **2a** | **2b** | **2c** |
| pq⁻ | **3a** | **3b** | **3c** |

containing the surfactants sodium dodecylsulfate (SDS), Triton X-100 (TX), and cetyltrimethylammonium bromide (CTAB) have been studied. Additionally, the lipophilicity and the cytotoxicity of these organoiridium(III) complexes toward the human cervix epithelioid carcinoma (HeLa) cell line has been assessed by reversed-phase HPLC and the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay, respectively. Furthermore, the cellular uptake of these organometallic compounds by HeLa cells has been investigated by flow cytometry and laser-scanning confocal microscopy.

## Experimental Section

**Materials and Synthesis.** All solvents were of analytical reagent grade and purified according to published procedures.[24] All buffer components were of biological grade and used as received. Iridium(III) chloride hydrate (Aldrich), 2-phenylpyridine (Aldrich), 1-phenylpyrazole (Aldrich), 2-phenylquinoline (Aldrich), 4,4′-dimethyl-2,2′-bipyridine (Aldrich), selenium(IV) oxide (Aldrich), silver nitrate (Acros), *N*-hydroxysuccinimide (Acros), *N*,*N*′-dicyclohexylcarbodiimide (Acros), *n*-octadecylamine (Acros), *n*-decylamine (MP Biomedicals), ethylamine (2 M in THF) (International Laboratory), potassium hexafluorophosphate (Acros), DSPC (Sigma), SDS (Acros), TX (Sigma), CTAB (Aldrich), cisplatin (Acros), and MTT (Sigma) were used without further purification. $[Ir_2(N{-}C)_4Cl_2]$,[6a] 4′-methyl-2,2′-bipyridine-4-carboxylic acid *N*-hydroxysuccinimide ester,[25a] and the diimine ligands[25b] were prepared as described previously.

HeLa cells were obtained from the American Type Culture Collection. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA, and penicillin/streptomycin were purchased from Invitrogen. The growth medium for cell culture contained DMEM with 10% FBS and 1% penicillin/streptomycin.

**Synthesis of the Organoiridium(III) Complexes.** A mixture of $[Ir_2(N{-}C)_4Cl_2]$[6a] (0.06 mmol) and the diimine ligand[25b] (0.12 mmol) in 20 mL of $CH_2Cl_2$/MeOH (1:1 v/v) was heated at reflux under nitrogen for 4 h. The solution was then cooled to room temperature and $KPF_6$ (0.12 mmol) was added to the solution. The mixture was then evaporated to dryness. The solid was dissolved in $CH_2Cl_2$ and purified by column chromatography on silica gel. The product was eluted with a mixture of $CH_2Cl_2$ and acetone, and it was subsequently recrystallized from a mixture of acetone and diethyl ether.

**[Ir(ppy)$_2$(Me-bpy-CONH-$C_{18}H_{37}$)](PF$_6$) (1a).** Complex **1a** was isolated as yellow crystals. Yield: 91 mg (80%). $^1$H NMR (300 MHz, acetone-$d_6$, 298 K, TMS): $\delta$ 9.06 (s, 1H, H3 of bpy), 8.83 (s, 1H, H3′ of bpy), 8.26−8.17 (m, 4H, CON*H*, H6 of bpy, and H3 of pyridyl ring of ppy), 7.99−7.82 (m, 8H, H5 and H6′ of bpy, H3 of phenyl ring of ppy, and H4 and H6 of pyridyl ring of ppy), 7.58 (d, 1H, $J$ = 5.9 Hz, H5′ of bpy), 7.18−7.15 (m, 2H, H5 of pyridyl ring of ppy), 7.06−7.01 (m, 2H, H4 of phenyl ring of ppy), 6.93−6.88 (m, 2H, H5 of phenyl ring of ppy), 6.35−6.31 (m, 2H, H6 of phenyl ring of ppy), 3.45−3.39 (m, 2H, CONHC$H_2$), 2.63 (s, 3H, $CH_3$ on C4′ of bpy), 1.60−1.58 (m, 2H, CONHCH$_2$C$H_2$), 1.33−1.26 (m, 30H, CONHCH$_2$CH$_2$C$_{15}H_{30}$), 0.86 (t, 3H, $J$ = 6.3 Hz, CONHCH$_2$CH$_2$C$_{15}$H$_{30}$C$H_3$). IR (KBr) $\nu$/cm$^{-1}$: 3436 (s, br, NH), 1668 (s, C═O), 846 (s, $PF_6^-$). Positive-ion ESI-MS ion cluster at $m/z$ 966 {[Ir(ppy)$_2$(Me-bpy-CONH-$C_{18}H_{37}$)]$^+$}$^+$. Anal. Calcd for $C_{52}H_{63}N_5OPF_6Ir$: C, 56.20; H, 5.71; N, 6.30. Found: C, 56.30; H, 5.50; N, 6.60.

**[Ir(ppy)$_2$(Me-bpy-CONH-$C_{10}H_{21}$)](PF$_6$) (1b).** Complex **1b** was isolated as yellow crystals. Yield: 82 mg (76%). $^1$H NMR (300 MHz, acetone-$d_6$, 298 K, TMS): $\delta$ 9.08 (s, 1H, H3 of bpy), 8.83 (s, 1H, H3′ of bpy), 8.26−8.17 (m, 4H, CON*H*, H6 of bpy, and H3 of pyridyl ring of ppy), 7.99−7.84 (m, 8H, H5 and H6′ of bpy, H3 of phenyl ring of ppy, and H4 and H6 of pyridyl ring of ppy), 7.56 (d, 1H, $J$ = 5.0 Hz, H5′ of bpy), 7.15−7.06 (m, 2H, H5 of pyridyl ring of ppy), 7.04−7.01 (m, 2H, H4 of phenyl ring of ppy), 6.92−6.88 (m, 2H, H5 of phenyl ring of ppy), 6.36−6.31 (m, 2H, H6 of phenyl ring of ppy), 3.43−3.39 (m, 2H, CONHC$H_2$), 2.63 (s, 3H, $CH_3$ on C4′ of bpy), 1.61−1.58 (m, 2H, CONHCH$_2$C$H_2$), 1.44−1.11 (m, 14H, CONHCH$_2$CH$_2$C$_7H_{14}$), 0.86 (t, 3H, $J$ = 6.7 Hz, CONHCH$_2$CH$_2$C$_7$H$_{14}$C$H_3$). IR (KBr) $\nu$/cm$^{-1}$: 3436 (s, br, NH), 1668 (s, C═O), 845 (s, $PF_6^-$). Positive-ion ESI-MS ion cluster at $m/z$ 854 {[Ir(ppy)$_2$(Me-bpy-CONH-$C_{10}H_{21}$)]$^+$}$^+$. Anal. Calcd for $C_{44}H_{47}N_5OPF_6Ir\cdot 0.5((CH_3)_2CO)$: C, 53.16; H, 4.90; N, 6.81. Found: C, 53.43; H, 4.77; N, 7.10.

**[Ir(ppy)$_2$(Me-bpy-CONH-$C_2H_5$)](PF$_6$) (1c).** Complex **1c** was isolated as yellow crystals. Yield: 62 mg (65%). $^1$H NMR (300 MHz, acetone-$d_6$, 298 K, TMS): $\delta$ 9.06 (s, 1H, H3 of bpy), 8.82 (s, 1H, H3′ of bpy), 8.24−8.19 (m, 4H, CON*H*, H6 of bpy, and H3 of pyridyl ring of ppy), 8.00−7.83 (m, 8H, H5 and H6′ of bpy, H3 of phenyl ring of ppy, and H4 and H6 of pyridyl ring of ppy), 7.58 (d, 1H $J$ = 6.2 Hz, H5′ of bpy), 7.15−7.06 (m, 2H, H5 of pyridyl ring of ppy), 7.04−7.01 (m, 2H, H4 of phenyl ring of ppy), 6.92−6.88 (m, 2H, H5 of phenyl ring of ppy), 6.34−6.31 (m, 2H, H6 of phenyl ring of ppy), 3.45−3.39 (m, 2H, CONHC$H_2$), 2.64 (s, 3H, $CH_3$ on C4′ of bpy), 1.21 (t, 3H, $J$ = 3.4 Hz, CONHCH$_2$C$H_3$). IR (KBr) $\nu$/cm$^{-1}$: 3423 (s, br, NH), 1665 (s, C═O), 845 (s, $PF_6^-$). Positive-ion ESI-MS ion cluster at $m/z$ 742 {[Ir(ppy)$_2$(Me-bpy-CONH-$C_2H_5$)]$^+$}$^+$. Anal. Calcd for $C_{36}H_{31}N_5OPF_6Ir\cdot 0.5((CH_3)_2CO)$: C, 49.18; H, 3.74; N, 7.65. Found: C, 49.17; H, 4.04; N, 7.45.

**[Ir(ppz)$_2$(Me-bpy-CONH-$C_{18}H_{37}$)](PF$_6$) (2a).** Complex **2a** was isolated as yellow crystals. Yield: 78 mg (66%). $^1$H NMR (300 MHz, acetone-$d_6$, 298 K, TMS): $\delta$ 9.06 (s, 1H, H3 of bpy), 8.81 (s, 1H, H3′ of bpy), 8.73−8.71 (m, 2H, H5 of pyrazole ring of

(24) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*; Pergamon: Oxford, 1997.

ppz), 8.35−8.33 (m, 1H, H6 of bpy), 8.27−8.25 (m, 1H, CON*H*), 8.10−8.08 (m, 1H, H5 of bpy), 7.99 (d, 1H, $J$ = 5.6 Hz, H6′ of bpy), 7.64 (d, 2H, $J$ = 7.3 Hz, H3 of phenyl ring of ppz), 7.58 (d, 1H, $J$ = 5.6 Hz, H5′ of bpy), 7.35−7.25 (m, 2H, H3 of pyrazole ring of ppz), 7.08−7.03 (m, 2H, H4 of phenyl ring of ppz), 6.89−6.84 (m, 2H, H5 of phenyl ring of ppz), 6.70−6.69 (m, 2H, H4 of pyrazole ring of ppz), 6.35−6.30 (m, 2H, H6 of phenyl ring of ppz), 3.45−3.41 (m, 2H, CONHC$H_2$), 2.66 (s, 3H, $CH_3$ on C4′ of bpy), 1.61−1.59 (m, 2H, CONHCH$_2$C$H_2$), 1.33−1.27 (m, 30H, CONHCH$_2$CH$_2$C$_{15}H_{30}$), 0.86 (t, 3H, $J$ = 6.0 Hz, CONHCH$_2$-CH$_2$C$_{15}$H$_{30}$C$H_3$). IR (KBr) $\nu$/cm$^{-1}$: 3426 (s, br, NH), 1670 (s, C═O), 845 (s, $PF_6^-$). Positive-ion ESI-MS ion cluster at $m/z$ 944 {[Ir(ppz)$_2$(Me-bpy-CONH-C$_{18}$H$_{37}$)]$^+$}$^+$. Anal. Calcd for $C_{48}H_{61}N_7$-$OPF_6Ir \cdot 0.5H_2O$: C, 52.49; H, 5.69; N, 8.93. Found: C, 52.49; H, 5.63; N, 8.83.

**[Ir(ppz)$_2$(Me-bpy-CONH-C$_{10}$H$_{21}$)](PF$_6$) (2b).** Complex **2b** was isolated as yellow crystals. Yield: 79 mg (75%). $^1$H NMR (300 MHz, acetone-$d_6$, 298 K, TMS): $\delta$ 9.10 (s, 1H, H3 of bpy), 8.84 (s, 1H, H3′ of bpy), 8.72−8.71 (m, 2H, H5 of pyrazole ring of ppz), 8.34 (d, 1H, $J$ = 5.6 Hz, H6 of bpy), 8.27−8.25 (m, 1H, CON*H*), 8.09 (d, 1H, $J$ = 5.6 Hz, H5 of bpy), 8.00 (d, 1H, $J$ = 5.6 Hz, H6′ of bpy), 7.63 (d, 2H, $J$ = 7.9 Hz, H3 of phenyl ring of ppz), 7.59−7.57 (m, 1H, H5′ of bpy), 7.35−7.25 (m, 2H, H3 of pyrazole ring of ppz), 7.08−7.03 (m, 2H, H4 of phenyl ring of ppz), 6.89−6.84 (m, 2H, H5 of phenyl ring of ppz), 6.70−6.69 (m, 2H, H4 of pyrazole ring of ppz), 6.35−6.30 (m, 2H, H6 of phenyl ring of ppz), 3.45−3.42 (m, 2H, CONHC$H_2$), 2.65 (s, 3H, $CH_3$ on C4′ of bpy), 1.61−1.59 (m, 2H, CONHCH$_2$C$H_2$), 1.33−1.21 (m, 14H, CONHCH$_2$CH$_2$C$_7$H$_{14}$), 0.85 (t, 3H, $J$ = 7.3 Hz, CONHCH$_2$-CH$_2$C$_7$H$_{14}$C$H_3$). IR (KBr) $\nu$/cm$^{-1}$: 3421 (s, br, NH), 1665 (s, C═O), 846 (s, $PF_6^-$). Positive-ion ESI-MS ion cluster at $m/z$ 832 {[Ir(ppz)$_2$(Me-bpy-CONH-C$_{10}$H$_{21}$)]$^+$}$^+$. Anal. Calcd for $C_{40}H_{45}N_7OPF_6Ir$: C, 49.17; H, 4.64; N, 10.04. Found: C, 48.94; H, 4.58; N, 10.01.

**[Ir(ppz)$_2$(Me-bpy-CONH-C$_2$H$_5$)](PF$_6$) (2c).** Complex **2c** was isolated as yellow crystals. Yield: 63 mg (68%). $^1$H NMR (300 MHz, acetone-$d_6$, 298 K, TMS): $\delta$ 9.04 (s, 1H, H3 of bpy), 8.80 (s, 1H, H3′ of bpy), 8.72−8.71 (m, 2H, H5 of pyrazole ring of ppz), 8.34−8.33 (m, 1H, H6 of bpy), 8.25−8.20 (m, 1H, CON*H*), 8.09−8.07 (m, 1H, H5 of bpy), 7.98 (d, 1H, $J$ = 5.9 Hz, H6′ of bpy), 7.63 (d, 2H, $J$ = 7.9 Hz, H3 of phenyl ring of ppz), 7.59 (m, 1H, H5′ of bpy), 7.35−7.24 (m, 2H, H3 of pyrazole ring of ppz), 7.07−7.02 (m, 2H, H4 of phenyl ring of ppz), 6.88−6.83 (m, 2H, H5 of phenyl ring of ppz), 6.69−6.68 (m, 2H, H4 of pyrazole ring of ppz), 6.34−6.29 (m, 2H, H6 of phenyl ring of ppz), 3.50−3.41 (m, 2H, CONHC$H_2$), 2.64 (s, 3H, $CH_3$ on C4′ of bpy), 1.19 (t, 3H, $J$ = 7.3 Hz, CONHCH$_2$C$H_3$). IR (KBr) $\nu$/cm$^{-1}$: 3440 (s, br, NH), 1663 (s, C═O), 846 (s, $PF_6^-$). Positive-ion ESI-MS ion cluster at $m/z$ 720 {[Ir(ppz)$_2$(Me-bpy-CONH-C$_2$H$_5$)]$^+$}$^+$. Anal. Calcd for $C_{32}H_{29}N_7OPF_6Ir \cdot 0.5H_2O$: C, 43.98; H, 3.46; N, 11.22. Found: C, 43.92; H, 3.50; N, 11.51.

**[Ir(pq)$_2$(Me-bpy-CONH-C$_{18}$H$_{37}$)](PF$_6$) (3a).** Complex **3a** was isolated as red crystals. Yield: 110 mg (84%). $^1$H NMR (300 MHz, acetone-$d_6$, 298 K, TMS): $\delta$ 8.67 (s, 1H, H3 of bpy), 8.53−8.52 (m, 4H, H3 of phenyl ring of pq and H3 of quinoline ring of pq), 8.45 (d, 1H, $J$ = 5.9 Hz, H6 of bpy), 8.39 (s, 1H, H3′ of bpy), 8.24 (d, 2H, $J$ = 7.9 Hz, H4 of quinoline ring of pq), 8.19 (d, 1H, $J$ = 5.6 Hz, H5 of bpy), 8.16−8.12 (m, 1H, CON*H*), 8.00−7.98 (m, 1H, H6′ of bpy), 7.92 (t, 2H, $J$ = 8.1 Hz, H8 of quinoline ring of pq), 7.57 (d, 1H, $J$ = 5.9 Hz, H5′ of bpy), 7.47−7.40 (m, 4H, H5 and H7 of quinoline ring of pq), 7.20−7.10 (m, 4H, H4 of phenyl ring of pq and H6 of quinoline ring of pq), 6.82 (t, 2H, $J$ = 7.3 Hz, H5 of phenyl ring of pq), 6.56−6.55 (m, 2H, H6 of phenyl ring of pq), 3.37−3.30 (m, 2H, CONHC$H_2$), 2.49 (s, 3H, $CH_3$ on C4′ of bpy), 1.56−1.52 (m, 2H, CONHCH$_2$C$H_2$), 1.33−1.25 (m, 30H, CONHCH$_2$CH$_2$C$_{15}H_{30}$), 0.86 (t, 3H, $J$ = 6.6 Hz, CONHCH$_2$CH$_2$C$_{15}$H$_{30}$C$H_3$). IR (KBr) $\nu$/cm$^{-1}$: 3439 (s, br, NH), 1668 (s, C═O), 847 (s, $PF_6^-$). Positive-ion ESI-MS ion cluster at $m/z$ 1066 {[Ir(pq)$_2$(Me-bpy-CONH-C$_{18}$H$_{37}$)]$^+$}$^+$. Anal. Calcd for $C_{60}H_{67}N_5OPF_6Ir \cdot 0.5H_2O$: C, 59.05; H, 5.62; N, 5.74. Found: C, 58.79; H, 5.40; N, 5.67.

**[Ir(pq)$_2$(Me-bpy-CONH-C$_{10}$H$_{21}$)](PF$_6$) (3b).** Complex **3b** was isolated as red crystals. Yield: 97 mg (82%). $^1$H NMR (300 MHz, acetone-$d_6$, 298 K, TMS): $\delta$ 8.68 (s, 1H, H3 of bpy), 8.53−8.52 (m, 4H, H3 of phenyl ring of pq and H3 of quinoline ring of pq), 8.45 (d, 1H, $J$ = 5.3 Hz, H6 of bpy), 8.40 (s, 1H, H3′ of bpy), 8.24 (d, 2H, $J$ = 7.9 Hz, H4 of quinoline ring of pq), 8.19 (d, 1H, $J$ = 5.9 Hz, H5 of bpy), 8.16−8.12 (m, 1H, CON*H*), 8.00−7.98 (m, 1H, H6′ of bpy), 7.93 (t, 2H, $J$ = 8.5 Hz, H8 of quinoline ring of pq), 7.57 (d, 1H, $J$ = 5.6 Hz, H5′ of bpy), 7.47−7.40 (m, 4H, H5 and H7 of quinoline ring of pq), 7.20−7.09 (m, 4H, H4 of phenyl ring of pq and H6 of quinoline ring of pq), 6.83 (t, 2H, $J$ = 6.7 Hz, H5 of phenyl ring of pq), 6.57−6.52 (m, 2H, H6 of phenyl ring of pq), 3.37−3.30 (m, 2H, CONHC$H_2$), 2.49 (s, 3H, $CH_3$ on C4′ of bpy), 1.56−1.50 (m, 2H, CONHCH$_2$C$H_2$), 1.30−1.24 (m, 14H, CONHCH$_2$CH$_2$C$_7$H$_{14}$), 0.84 (t, 3H, $J$ = 6.3 Hz, CONHCH$_2$CH$_2$C$_7$H$_{14}$C$H_3$). IR (KBr) $\nu$/cm$^{-1}$: 3427 (s, br, NH), 1666 (s, C═O), 847 (s, $PF_6^-$). Positive-ion ESI-MS ion cluster at $m/z$ 954 {[Ir(pq)$_2$(Me-bpy-CONH-C$_{10}$H$_{21}$)]$^+$}$^+$. Anal. Calcd for $C_{52}H_{51}N_5OPF_6Ir \cdot H_2O$: C, 55.90; H, 4.78; N, 6.27. Found: C, 55.83; H, 4.66; N, 6.19.

**[Ir(pq)$_2$(Me-bpy-CONH-C$_2$H$_5$)](PF$_6$) (3c).** Complex **3c** was isolated as red crystals. Yield: 84 mg (79%). $^1$H NMR (300 MHz, acetone-$d_6$, 298 K, TMS): $\delta$ 8.69 (s, 1H, H3 of bpy), 8.54−8.53 (m, 4H, H3 of phenyl ring of pq and H3 of quinoline ring of pq), 8.46 (d, 1H, $J$ = 5.3 Hz, H6 of bpy), 8.41 (s, 1H, H3′ of bpy), 8.17−8.09 (m, 3H, CON*H* and H4 of quinoline ring of pq), 8.01−7.91 (m, 4H, H5 and H6′ of bpy and H8 of quinoline ring of pq), 7.58 (d, 1H, $J$ = 6.2 Hz, H5 of bpy), 7.49−7.41 (m, 4H, H5 and H7 of quinoline ring of pq), 7.21−7.12 (m, 4H, H4 of phenyl ring of pq and H6 of quinoline ring of pq), 6.84 (t, 2H, $J$ = 7.0 Hz, H5 of phenyl ring of pq), 6.57−6.52 (m, 2H, H5 of phenyl ring of pq), 3.52−3.41 (m, 2H, CONHC$H_2$), 2.51 (s, 3H, $CH_3$ on C4′ of bpy), 1.14 (t, 3H, $J$ = 7.2 Hz, CONHCH$_2$C$H_3$). IR (KBr) $\nu$/cm$^{-1}$: 3421 (s, br, NH), 1665 (s, C═O), 847 (s, $PF_6^-$). Positive-ion ESI-MS ion cluster at $m/z$ 842 {[Ir(pq)$_2$(Me-bpy-CONH-C$_2$H$_5$)]$^+$}$^+$. Anal. Calcd for $C_{44}H_{35}N_5OPF_6Ir \cdot (CH_3)_2CO \cdot 0.5H_2O$: C, 53.56; H, 4.02; N, 6.64. Found: C, 53.51; H, 4.18; N, 6.67.

**Physical Measurements and Instrumentation.** The equipment for characterization and photophysical and electrochemical studies has been described previously[23b] except that the emission lifetimes were measured using a SPEX FluoroLog 3-TCSPC spectrophotometer in the Fast MCS mode with a NanoLED N-340 as the excitation source. Luminescence quantum yields of the complexes were measured using the optically dilute method[26a] with an aerated aqueous solution of [Ru(bpy)$_3$]Cl$_2$ ($\Phi_{em}$ = 0.028, $\lambda_{ex}$ = 455 nm)[26b] as the standard solution. All the emission spectra were uncorrected for instrumental responses.

**Preparation of Ir/DSPC Liposomes.**[27] In a typical procedure, DSPC (1 mg, 1.26 $\mu$mol) in 100 $\mu$L of $CHCl_3$ and the iridium(III) complex (0.13 $\mu$mol) in 100 $\mu$L of $CHCl_3$ were mixed thoroughly in a glass test tube. The solvent was then removed under a stream of dry nitrogen. The resulting thin film was further dried under vacuum for 12 h. Ultrapure Milli-Q water (1 mL) was added to the dried lipid film, and the suspension was sonicated at 37 °C for 1 h. The solution was extruded through a polycarbonate polymer membrane filter (Alltech) with a pore size of 0.45 $\mu$m and then used for microscopy and emission studies.

(25) (a) Telser, J.; Cruickshank, K. A.; Schanze, K. S.; Netzel, T. L. *J. Am. Chem. Soc.* **1989**, *111*, 7221. (b) Lo, K. K.-W.; Lee, T. K.-M.; Zhang, K. Y. *Inorg. Chim. Acta* **2006**, *359*, 1845.

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

(27) Hermanson, G. T. *Bioconjugate Techniques*; Academic Press: San Diego, CA, 1996; p 528.

**Cryo-TEM Studies.** The electron microscopy investigations were performed with an FEI/Philips Tecnai 12 BioTWIN transmission electron microscope, operating at 80 kV. Specimens were prepared by a blotting procedure at room temperature. In a typical procedure, the sample dispersion (10 $\mu$L) was deposited on an electron microscopy copper grid coated by a Lacey Formva/Carbon film. Excess solution was removed by means of a filter paper. The sample was then plunged into liquid propane, and the vitrified sample was transferred to the microscope under liquid nitrogen, where the specimen temperature was maintained at 103 K.

**Determination of Lipophilicity.** The lipophilicity of the complexes, which is referred to as log $P_{o/w}$ ($P_{o/w}$ = *n*-octan-1-ol/water partition coefficient), was determined from the log $k'_w$ values ($k'_w$ = chromatographic capacity factor at 100% aqueous solution). Detailed procedures for the determination of lipophilicity have been described previously.[28]

**Cytotoxicity Assays.**[29] HeLa cells were seeded in a 96-well flat-bottomed microplate (10 000 cells/well) in growth medium (100 $\mu$L) and incubated at 37 °C under a 5% $CO_2$ atmosphere for 24 h. Detailed procedures for the cytotoxicity assays have been described previously.[30] The absorbance of the solutions at 570 nm was measured with a SPECTRAmax 340 microplate reader (Molecular Devices Corp., Sunnyvale, CA). The $IC_{50}$ values of the complexes were determined from dose dependence of surviving cells after exposure to the complexes for 48 h.

**Flow Cytometry.** HeLa cells in growth medium (100 000 cells $mL^{-1}$) were seeded in a 35 mm tissue culture dish and incubated at 37 °C under a 5% $CO_2$ atmosphere for 48 h. The culture medium was removed and replaced with medium/DMSO (99:1, v/v) containing the organoiridium(III) complexes at a concentration of 5 $\mu$M. After incubation for 1 h, the medium was removed and the cell layer was washed gently with PBS (1 mL × 3). The cell layer was then trypsinized and added up to a final volume of 3 mL with PBS. The samples were analyzed by a FACSCalibur flow cytometer (Becton, Dickinson and Co., Franklin Lakes, NJ). The cell samples were excited with an argon laser at 488 nm, and the emission was monitored at 585 ± 21 nm. The number of cells analyzed for each sample was between ca. 9000 and 10 000.

**Live-Cell Confocal Imaging.** HeLa cells were grown on sterile glass coverslips in a 35 mm tissue culture dish. The sample preparation procedure was similar to that of the flow cytometry. After washing with PBS, the coverslips were mounted onto slides for measurements. Imaging was performed using a confocal microscope (Carl Zeiss, LSM510) with an excitation wavelength at 488 nm. The emission was measured using a long-pass filter at 505 nm.

## Results and Discussion

**Synthesis.** The diimine ligands were prepared from the reactions of 4′-methyl-2,2′-bipyridine-4-carboxylic acid *N*-hydroxysuccinimide ester with the amines *n*-octadecylamine, *n*-decylamine, or ethylamine in $CH_2Cl_2$ at room temperature. The organoiridium(III) complexes were prepared, in moderate yields, from the reactions of $[Ir_2(N{-}C)_4Cl_2]$ with the diimine ligands Me-bpy-CONH-$C_{18}H_{37}$, Me-bpy-CONH-$C_{10}H_{21}$, or Me-bpy-CONH-$C_2H_5$ in refluxing $CH_2Cl_2$/MeOH, followed by anion exchange with $KPF_6$, chromatographic purification on silica gel, and recrystallization from a mixture of acetone and diethyl ether. All the complexes were characterized by $^1$H NMR, positive-ion ESI-MS, and IR and gave satisfactory elemental analyses.

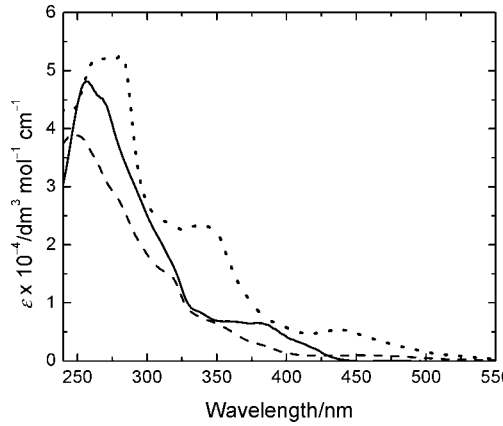



**Figure 1.** Electronic absorption spectra of complexes **1a** (—), **2a** (---), and **3a** (•••) in $CH_2Cl_2$ at 298 K.

**Electronic Absorption and Luminescence Properties in Homogeneous and Microhetereogeneous Solutions.** The electronic absorption spectral data of the complexes in $CH_2Cl_2$ and $CH_3CN$ at 298 K are listed in Table S1. The electronic absorption spectra of complexes **1a**−**3a** in $CH_2Cl_2$ are presented in Figure 1. With reference to previous photophysical studies on related iridium(III) polypyridine systems,[6–9,10c,11,12,13a,c,15a,c,16,17,18b,19,22a,b,23] the intense absorption features at ca. 246−344 nm ($\varepsilon$ on the order of $10^4$ $dm^3$ $mol^{-1}$ $cm^{-1}$) have been assigned to spin-allowed intraligand ($^1$IL) transitions ($\pi \rightarrow \pi^*$) (N−C and diimine). The moderately intense absorption bands at ca. 350−522 nm have been attributed to spin-allowed metal-to-ligand charge-transfer ($^1$MLCT) transition (d$\pi$(Ir) → $\pi^*$(diimine)). Since the incorporated alkyl chains should not play a significant role in absorption properties, the electronic absorption spectra of the complexes with the same cyclometalating ligand are very similar.

Photoexcitation of the complexes resulted in strong and long-lived greenish-yellow to orange luminescence in homogeneous fluid solutions under ambient conditions and in low-temperature alcohol glass. The photophysical data of the complexes are summarized in Table 1. The emission spectra of complexes **1a**−**3a** in $CH_2Cl_2$ are shown in Figure 2. All the complexes displayed large Stokes' shifts and long emission lifetimes (on the submicrosecond time scale), indicative of the phosphorescence nature of the emission. In general, all the complexes exhibited longer emission wavelengths, shorter emission lifetimes, and lower luminescence quantum yields in more polar media, and thus their emission has been tentatively assigned to an excited state of $^3$MLCT (d$\pi$(Ir) → $\pi^*$(diimine)) character.[6,8b,c,9–11,13–15,16a,17,19,20,21b,23a–d,f–i] This is in agreement with the observation that the emission energies of the ppz complexes **2a**−**c** are slightly higher than those of their ppy counterparts **1a**−**c** (Table 1). The reason is that the metal centers of complexes **2a**−**c** have a lower electron density as a result of the stronger electron-withdrawing pyrazole unit compared to the pyridine moiety of complexes **1a**−**c**. The emission wavelengths of the pq complexes **3a**−**c** are not very sensitive to solvent polarity. This is probably a result of mixing of some $^3$IL ($\pi \rightarrow \pi^*$) (pq) character into the emissive state.[23c,d,h] In alcohol glass at 77 K, all the complexes displayed intense and long-lived $^3$MLCT emission at higher energy, which is commonly observed for cyclometalated iridium(III) polypyridine systems.[6a,c,7a,8b,c,9–11,13c,14,16a,17,20,21b,23]

The photophysical properties of the complexes in microheterogeneous environments have been examined. Specifically, the complexes have been integrated into the model biological membrane composed of the phospholipid DSPC. Cryo-TEM has been employed to visualize the structures of the resulting assemblies. The cryo-TEM images of the Ir/DSPC liposomes

(28) Lo, K. K.-W.; Tsang, K. H.-K.; Zhu, N. *Organometallics* **2006**, *25*, 3220.
(29) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
(30) (a) Lo, K. K.-W.; Lee, T. K.-M.; Lau, J. S.-Y.; Poon, W.-L.; Cheng, S.-H. *Inorg. Chem.* **2008**, *47*, 200. (b) Lo, K. K.-W.; Louie, M.-W.; Sze, K.-S.; Lau, J. S.-Y. *Inorg. Chem.* **2008**, *47*, 602.

**Table 1. Photophysical Data of Complexes**

| complex | medium (*T*/K) | $\lambda_{em}$/nm | $\tau_o/\mu$s | Φ |
|---|---|---|---|---|
| **1a** | $CH_2Cl_2$ (298) | 608 | 0.39 | 0.11 |
| | $CH_3CN$ (298) | 614 | 0.24 | 0.068 |
| | MeOH/buffer[a] (298) | 623 | 0.046 | 0.0090 |
| | Ir/DSPC[b] (298) | 570 | 0.45 | [c] |
| | glass[d] (77) | 547, 561 sh | 4.35 | |
| **1b** | $CH_2Cl_2$ (298) | 607 | 0.39 | 0.13 |
| | $CH_3CN$ (298) | 613 | 0.23 | 0.061 |
| | MeOH/buffer[a] (298) | 626 | 0.040 | 0.0090 |
| | Ir/DSPC[b] (298) | 581 | 0.21 | [c] |
| | glass[d] (77) | 541, 560 sh | 4.80 | |
| **1c** | $CH_2Cl_2$ (298) | 609 | 0.40 | 0.13 |
| | $CH_3CN$ (298) | 613 | 0.23 | 0.058 |
| | MeOH/buffer[a] (298) | 626 | 0.078 | 0.0069 |
| | Ir/DSPC[b] (298) | 572 | 0.31 | [c] |
| | glass[d] (77) | 541, 569 sh | 4.80 | |
| **2a** | $CH_2Cl_2$ (298) | 586 | 0.60 | 0.25 |
| | $CH_3CN$ (298) | 599 | 0.29 | 0.10 |
| | MeOH/buffer[a] (298) | 609 | 0.058 | 0.018 |
| | Ir/DSPC[b] (298) | 570 | 0.27 | [c] |
| | glass[d] (77) | 532, 567 sh | 4.87 | |
| **2b** | $CH_2Cl_2$ (298) | 587 | 0.59 | 0.24 |
| | $CH_3CN$ (298) | 598 | 0.29 | 0.094 |
| | MeOH/buffer[a] (298) | 610 | 0.051 | 0.016 |
| | Ir/DSPC[b] (298) | 574 | 0.17 | [c] |
| | glass[d] (77) | 522, 561 sh | 5.10 | |
| **2c** | $CH_2Cl_2$ (298) | 585 | 0.59 | 0.16 |
| | $CH_3CN$ (298) | 598 | 0.29 | 0.093 |
| | MeOH/buffer[a] (298) | 610 | 0.054 | 0.014 |
| | Ir/DSPC[b] (298) | 595 | 0.26 | [c] |
| | glass[d] (77) | 524, 555 sh | 5.15 | |
| **3a** | $CH_2Cl_2$ (298) | 587 | 0.68 | 0.18 |
| | $CH_3CN$ (298) | 586 | 0.55 | 0.13 |
| | MeOH/buffer[a] (298) | 607 | 0.082 | 0.019 |
| | Ir/DSPC[b] (298) | 564 | 0.46 | [c] |
| | glass[d] (77) | 546 (max), 586 | 4.51 | |
| **3b** | $CH_2Cl_2$ (298) | 595 | 0.71 | 0.17 |
| | $CH_3CN$ (298) | 586 | 0.56 | 0.095 |
| | MeOH/buffer[a] (298) | 609 | 0.090 | 0.020 |
| | Ir/DSPC[b] (298) | 568 | 0.43 | [c] |
| | glass[d] (77) | 544 (max), 583 | 4.71 | |
| **3c** | $CH_2Cl_2$ (298) | 595 | 0.67 | 0.18 |
| | $CH_3CN$ (298) | 587 | 0.54 | 0.14 |
| | MeOH/buffer[a] (298) | 608 | 0.085 | 0.015 |
| | Ir/DSPC[b] (298) | 568 | 0.29 | [c] |
| | glass[d] (77) | 543 (max), 585 | 4.69 | |

[a] 50 mM potassium phosphate buffer pH 7.4 containing 70% MeOH. [b] In aerated aqueous solutions. [c] The concentration of metal complex incorporated in the sample was insufficient for accurate determination of emission quantum yields. [d] EtOH/MeOH (4:1 v/v).

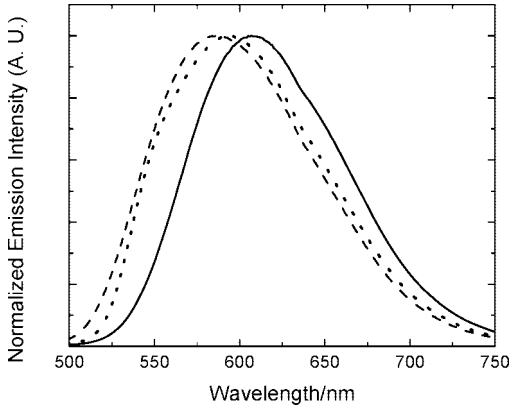



**Figure 2.** Emission spectra of complexes **1a** (—), **2a** (---), and **3a** (•••) in $CH_2Cl_2$ at 298 K.

obtained were reproducible, and typical images are depicted in Figure 3. The micrographs show the existence of polygonal vesicles, the majority of which had a diameter between 20 and

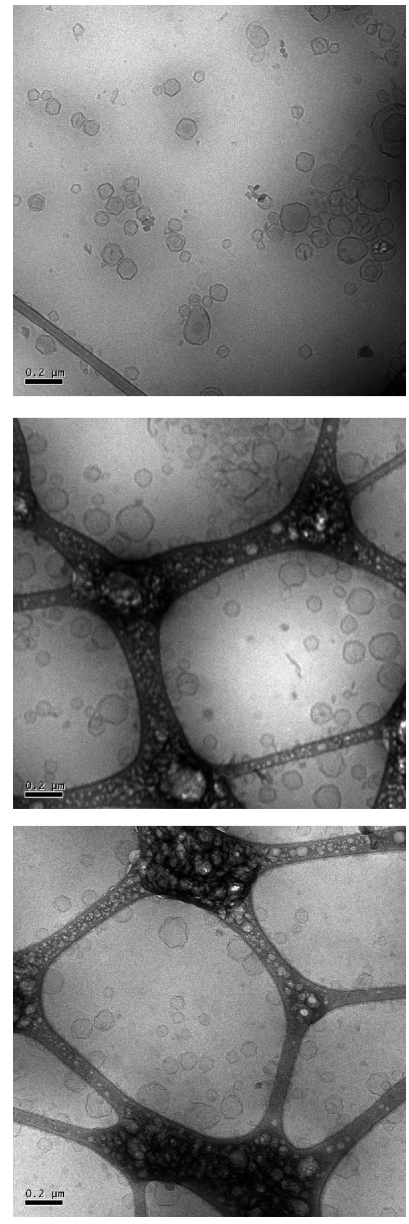



**Figure 3.** Cryo-TEM images of Ir/DSPC liposomes prepared with complexes **1a** (top), **2a** (middle), and **3a** (bottom).

140 nm. The polygonal shape of these structures has been ascribed to the fact that vesicle samples were prepared at 37 °C, a temperature significantly below the gel-to-liquid crystalline phase transition temperature ($T_m$) of DSPC (ca. 54 °C).[31] Interestingly, these vesicles are strongly emissive in aqueous solution. Since the free complexes were insoluble in neat water and DSPC itself is nonemissive in the visible region, the observed luminescence of the samples in the aqueous phase should originate from the organoiridium(III) complexes incorporated into the vesicles. The emission lifetime decay curves were all single-exponential. It is noteworthy that the emission wavelengths of the vesicles are substantially shorter than those of the samples in fluid solutions; their emission lifetimes are also very long even in aerated aqueous solutions (Table 1). These observations indicate that the local environment of the luminophores is considerably hydrophobic. It is conceivable that the complexes are embedded in the hydrophobic region of DSPC

(31) Otake, K.; Shimomura, T.; Goto, T.; Imura, T.; Furuya, T.; Yoda, S.; Takebayashi, Y.; Sakai, H.; Abe, M. *Langmuir* **2006**, *22*, 2543.

**Table 2. Photophysical Data of Complexes in Aerated Aqueous Solutions at 298 K[a]**

| complex | medium | $\lambda_{em}$/nm | $\tau_o/\mu$s | $I/I_o$[b] |
|---|---|---|---|---|
| **1a** | $H_2O$ | 587 | 0.39 | 1.00 |
| | SDS[c] | 584 | 0.43 | 1.45 |
| | TX[d] | 618 | 0.064 | 0.15 |
| | CTAB[e] | 588 | 0.37 | 1.59 |
| **1b** | $H_2O$ | 590 | 0.21 | 1.00 |
| | SDS[c] | 583 | 0.22 | 1.46 |
| | TX[d] | 620 | 0.061 | 0.21 |
| | CTAB[e] | 609 | 0.059 | 0.26 |
| **1c** | $H_2O$ | 633 | [f] | 1.00 |
| | SDS[c] | 619 | 0.042 | 9.33 |
| | TX[d] | 620 | 0.057 | 20.18 |
| | CTAB[e] | 605 | 0.074 | 3.92 |
| **2a** | $H_2O$ | 573 | 0.53 | 1.00 |
| | SDS[c] | 571 | 0.57 | 1.10 |
| | TX[d] | 606 | 0.079 | 0.088 |
| | CTAB[e] | 574 | 0.52 | 1.30 |
| **2b** | $H_2O$ | 575 | 0.37 | 1.00 |
| | SDS[c] | 570 | 0.35 | 1.28 |
| | TX[d] | 606 | 0.077 | 0.18 |
| | CTAB[e] | 595 | 0.067 | 0.17 |
| **2c** | $H_2O$ | 620 | [f] | 1.00 |
| | SDS[c] | 604 | 0.043 | 17.91 |
| | TX[d] | 607 | 0.067 | 23.18 |
| | CTAB[e] | 606 | 0.092 | 4.49 |
| **3a** | $H_2O$ | 568 sh, 599 | 0.095 | 1.00 |
| | SDS[c] | 572, 604 sh | 0.086 | 2.46 |
| | TX[d] | 571, 606 sh | 0.21 | 4.01 |
| | CTAB[e] | 566 sh, 600 | 0.26 | 2.57 |
| **3b** | $H_2O$ | 578 | 0.039 | 1.00 |
| | SDS[c] | 569, 601 sh | 0.074 | 3.69 |
| | TX[d] | 572, 608 sh | 0.20 | 20.25 |
| | CTAB[e] | 568, 607 sh | 0.16 | 5.28 |
| **3c** | $H_2O$ | 593 | 0.12 | 1.00 |
| | SDS[c] | 572, 603 sh | 0.099 | 6.37 |
| | TX[d] | 568, 605 sh | 0.20 | 10.14 |
| | CTAB[e] | 568, 607 sh | 0.17 | 4.80 |

[a] [Ir] = 10 $\mu$M in $H_2O$ containing 1% MeOH. [b] $I_o$ and $I$ are the emission intensities of the complexes in $H_2O$ and the micellar medium, respectively. [c] [SDS] = 18 mM. [d] [TX] = 2 mM. [e] [CTAB] = 1.8 mM. [f] The emission was too weak for accurate determination of $\tau_o$.

vesicles via interactions of the hydrocarbon chains and/or the hydrophobic cyclometalating ligands.

The luminescence properties of the complexes in aerated 1% aqueous methanol in the absence and presence of surfactants (above their critical micelle concentrations) have been investigated. The photophysical data are presented in Table 2. Similar to the case of the vesicles, the luminescence decay of the complexes in these surfactant solutions all followed single-exponential kinetics. In 1% aqueous methanol, the luminescence of the C18 and C10 complexes **1a**,**b** and **2a**,**b** occurred at higher energy with longer lifetimes compared to that of their C2 counterparts complexes **1c** and **2c**, respectively (Table 2). Also, the emission intensities of complexes **1a** and **1b** are ca. 362 and 171 times that of complex **1c**, whereas those of complexes **2a** and **2b** are ca. 456 and 188 times that of complex **2c**. We reason that, in such a highly polar medium, the hydrocarbon pendants of complexes **1a**,**b** and **2a**,**b** wrap the complex cores, resulting in a hydrophobic local environment.[5c] This is not possible for complexes **1c** and **2c** owing to the lack of long aliphatic chains. In the presence of SDS micelles, these six complexes showed a small to moderate blue-shift in their emission maxima, suggestive of a more nonpolar local environment (Table 2). The emission intensities of complexes **1c** and **2c** increased dramatically ($I/I_o$ = 9.33 and 17.91, respectively), whereas the effect is insignificant for the long-chain complexes **1a**,**b** and **2a**,**b** ($I/I_o$ = 1.10 to 1.46) (Table 2). It is likely that the C2 complexes interact with the micelles and are located in a more nonpolar environment. A similar observation has been made with other luminescent transition metal complexes.[4] A comparable change was not observed for the C18 and C10 complexes owing to their intrinsically hydrophobic environment contributed by their aliphatic pendants. Interestingly, in the presence of the neutral surfactant TX, the emission bands of complexes **1a**,**b** and **2a**,**b** showed a red-shift, and the emission intensities and lifetimes were reduced significantly (Table 2). This can be attributed to the TX-induced "unwrapping" of the complexes and the resulting reduction of local hydrophobicity of the luminophores. On the contrary, the emission bands of both complexes **1c** and **2c** displayed a blue-shift and the emission became more intense and longer-lived (Table 2). On the basis of these changes, it is likely that TX provides a more nonpolar environment to these two C2 complexes in aqueous solutions.[4e] It is noteworthy that, after TX is added, the emission wavelengths and lifetimes are very similar for complexes **1a**−**c** and **2a**−**c**, respectively. Additionally, we have studied the effects of the cationic micelles formed by CTAB on the complexes. While the emission properties of the C18 complexes **1a** and **2a** did not show significant changes, the emission of the C10 complexes **1b** and **2b** was quenched ($I/I_o$ = 0.26 and 0.17, respectively) by the cationic micelles, and the emission bands also exhibited a red-shift (Table 2). It is probable that the C18 complexes do not interact significantly with the micelles, whereas complexes **1b** and **2b** are unwrapped by the micelles. However, the reason for this difference is unknown at this stage. Again, both complexes **1c** and **2c** showed a blue-shift in their emission maxima, and the emission became stronger and longer-lived in the presence of the CTAB micelles. These findings are similar to those observed in the cases of SDS and TX and have been ascribed to localization of the complexes in the hydrophobic regions of the micelles. In most cases, the pq complexes **3a**−**c** showed similar changes in their emission properties upon addition of the three micelles. The most noticeable difference is that all these pq complexes displayed emission enhancement in the presence of all three micelles. Also, the effects of the aliphatic chains on the responses of the complexes toward the micelles are smaller, which could be accounted for by the relatively nonpolar $Ir(pq)_2$ core.

**Table 3. Electrochemical Data of Complexes[a]**

| complex | oxidation $E_{1/2}$ or $E_a$/V | reduction $E_{1/2}$ or $E_c$/V |
|---|---|---|
| **1a** | +1.26 | −1.28, −1.85,[b] −2.17,[c] −2.48[b] |
| **1b** | +1.25 | −1.28, −1.86,[b] −2.17,[c] −2.50[b] |
| **1c** | +1.26 | −1.28, −1.84,[b] −2.27,[b] −2.48[b] |
| **2a** | +1.32 | −1.30, −1.86,[b] −2.41[b] |
| **2b** | +1.32 | −1.30, −1.87,[b] −2.44[b] |
| **2c** | +1.32 | −1.30, −1.93,[b] −2.44[b] |
| **3a** | +1.27 | −1.31, −1.82,[b] −2.01,[b] −2.27[b] |
| **3b** | +1.27 | −1.31, −1.82,[b] −2.01,[b] −2.26[b] |
| **3c** | +1.28 | −1.28, −1.72,[b] −1.93,[b] −2.22[b] |

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

**Electrochemical Properties.** The electrochemical properties of the complexes have been studied by cyclic voltammetry. The electrochemical data of the complexes are collected in Table 3. All the complexes showed a reversible iridium(IV/III) couple at ca. +1.3 V vs SCE, which is similar to related cyclometalated iridium(III) polypyridine complexes.[7,8b,9,10,11c,12,13b,c,15a,c,16,19b,20,21b,c,22c,23a–h] The metal-centered oxidation of the ppz complexes **2a**−**c** occurred at slightly higher potentials than that of the ppy and pq complexes, which is in line with their higher $^3$MLCT emission energies. Another reversible couple occurred at ca. −1.3 V vs SCE, which has been ascribed to the reduction of the diimine

**Table 4. Log $P_{o/w}$ Values of Complexes**

| complex | log $P_{o/w}$ |
|---|---|
| **1a** | 8.79 |
| **1b** | 4.42 |
| **1c** | 0.44 |
| **2a** | 6.52 |
| **2b** | 3.94 |
| **2c** | −0.34 |
| **3a** | 9.89 |
| **3b** | 5.34 |
| **3c** | 2.01 |

**Table 5. Cytotoxicity ($IC_{50}$, 48 h) of Complexes and Cisplatin toward the HeLa Cell Line**

| complex | $IC_{50}/\mu M$ |
|---|---|
| **1a** | 12.0 ± 0.8 |
| **1b** | 2.0 ± 0.4 |
| **1c** | 18.1 ± 2.3 |
| **2a** | 7.5 ± 1.0 |
| **2b** | 2.0 ± 0.2 |
| **2c** | 27.7 ± 1.4 |
| **3a** | [a] |
| **3b** | 2.3 ± 0.4 |
| **3c** | 3.8 ± 0.5 |
| cisplatin | 18.1 ± 1.5 |

[a] Not determined due to possible aggregation or precipitation of complex under the conditions employed in the assays.

ligands. As expected, the diimine-based reduction shows no significant dependence on the chain length of the hydrocarbon substituent on the diimine. Additionally, quasi-reversible couples and irreversible waves occurred at more negative potentials, and they have been assigned to the reduction of the cyclometalating ligands.

**Lipophilicity.** The cellular and in vivo tissue-uptake selectivity and characteristics of probes and therapeutic reagents can be estimated by their lipophilicity.[32] This is commonly referred to as the *n*-octan-1-ol/water partition coefficients (expressed in log $P_{o/w}$) of the compounds, which are readily determined by reversed-phase HPLC.[28] The log $P_{o/w}$ values of the organoiridium(III) complexes are listed in Table 4. The lipophilicity of the complexes can be substantially enhanced by lengthening the alkyl chains, as revealed by the significantly larger log $P_{o/w}$ values of the C18 complexes **1a**−**3a** (from 6.52 to 9.89) compared to those of the C2 complexes **1c**−**3c** (from −0.34 to 2.01). Moreover, the log $P_{o/w}$ values of the complexes followed the order ppz < ppy < pq, which is in accordance with the hydrophobic character of the ligands. The incorporation of longer alkyl chains and more hydrophobic cyclometalating ligands may facilitate the tissue and cellular uptake of these complexes, although the solubility of the complexes in aqueous solution may present a problem.

**Cytotoxicity.** The cytotoxicity of the complexes has been studied by the MTT assay using HeLa cells as the model cell line.[29] The $IC_{50}$ values have been determined from the dose dependence of surviving HeLa cells after their exposure to the complexes for 48 h. The cytotoxicity data of the complexes are shown in Table 5. The dose dependence of surviving HeLa cells with respect to the ppz complexes **2a**−**c** is illustrated in Figure 4. Interestingly, the C10 complexes **1b**−**3b** exhibited the highest cytotoxicity among the complexes studied, with the $IC_{50}$ values being almost 10 times lower than that of cisplatin. Although we expect that the cytotoxicity of the C18 complexes **1a**−**3a** would be similarly high, the $IC_{50}$ values of complexes **1a** and **2a** are comparable to those of the relatively noncytotoxic C2 complexes **1c** and **2c**. Additionally, the $IC_{50}$ values of the pq complexes **3b** and **3c** are small, suggesting high cytotoxicity related to the use of this cyclometalating ligand. We do not understand the apparent incongruity in cytotoxicity of complex **3a** (Table 5). Since this complex is highly hydrophobic and has the largest molecular size among the complexes studied, probably it cannot enter the cells effectively due to aggregation or precipitation under the conditions we employed in the MTT assays.

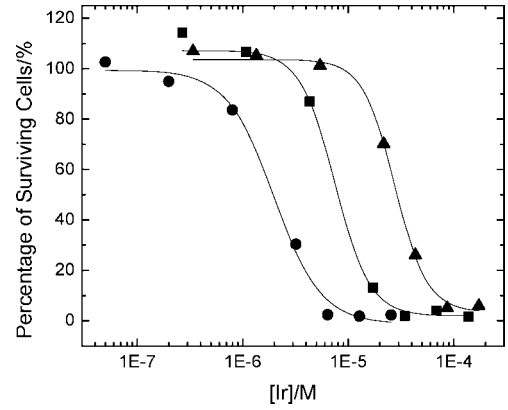



**Figure 4.** Dose dependence of surviving HeLa cells after exposure to complexes **2a** (■), **2b** (●), and **2c** (▲) for 48 h.

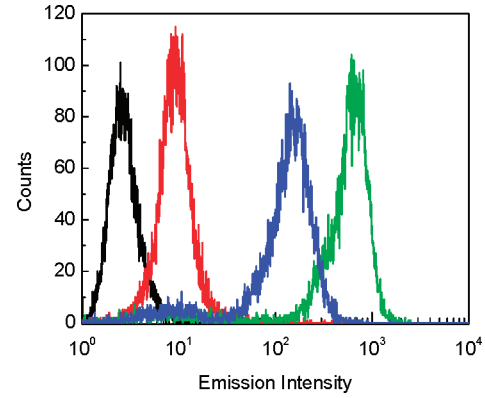



**Figure 5.** Results of flow cytometry of HeLa cells incubated with blank medium (black), **3a** (red), **3b** (green), and **3c** (blue) (5 $\mu$M) for 1 h.

**Flow Cytometry.** The cellular uptake characteristics of the complexes have been investigated using flow cytometry. The results of the flow cytometric studies for complexes **3a**−**c** are shown in Figure 5 as an example. Upon excitation at 488 nm, all the cell samples loaded with the organoiridium(III) complexes displayed higher emission intensities compared to the autofluorescence of untreated HeLa cells, reflecting the efficient internalization of the complexes by the cells. Interestingly, the emission intensities of the cells treated with complexes follow the order **3b** > **3c** > **3a** (Figure 5). Since (i) the emission wavelengths and intensities of complexes **3a**−**c** are comparable (Table 1), (ii) the responses of the complexes toward hydrophobic micellar media are similar (Table 2), and (iii) the complexes are localized in a similar region in the cells (see below), the trend observed in the flow cytometric measurements can be correlated to the cellular uptake efficiencies of the complexes. Thus, these results illustrate that although efficient internalization of the complexes is supposed to be assisted by their high lipophilicity, complex **3a**, being the most lipophilic complex among the three, showed the lowest cellular uptake

(32) VanBrocklin, H. F.; Liu, A.; Welch, M. J.; O'Neil, J. P.; Katzenellenbogen, J. A. *Steroids* **1994**, *59*, 34.

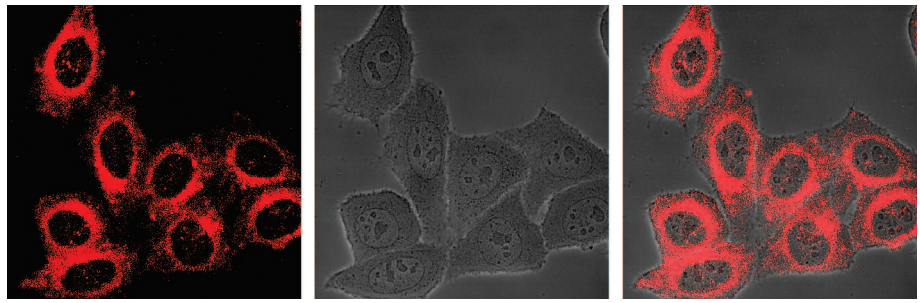


**Figure 6.** Fluorescence (left), brightfield (middle), and overlaid (right) images of HeLa cells incubated with complex **3a** (5 $\mu$M) at 37 °C for 5 h.

efficiency. This could be due to its largest molecular size or possible self-aggregation.

**Live-Cell Confocal Imaging.** The cellular uptake characteristics of the complexes have been investigated using laser-scanning confocal microscopy. The possibility of utilizing the organoiridium(III) complexes as luminescent probes for live-cell imaging has been examined using **3a** as an example. The laser-scanning confocal microscopy images of **3a** are displayed in Figure 6. Incubation of HeLa cells with the complex at 37 °C under a 5% $CO_2$ atmosphere for 5 h led to efficient interiorization of the complex, as observed by laser-scanning confocal microscopy with an excitation wavelength at 488 nm. It is noteworthy that most of the complex molecules were distributed inside the cytoplasm with a lower extent of nuclear uptake, as revealed by the much more weakly stained nucleus. Importantly, a higher degree of localization of the complexes in the perinuclear region is likely to result from the interactions of the complex molecules with hydrophobic organelles such as endoplasmic reticulum, mitochondria, and Golgi apparatus. When the cells were incubated at 4 °C, no interiorization was observed, implying that the uptake of the complex and its subsequent localization are due to energy-requiring processes such as endocytosis.[33] Related work on cellular uptake of organoiridium(III) polypyridine complexes is in progress.

**Summary.** In this work, a series of organoiridium(III) polypyridine complexes equipped with an alkyl pendant have been synthesized and characterized, and their photophysical and electrochemical properties investigated. The lipophilicity of these complexes and their cytotoxicity toward the HeLa cell line have also been studied. The successful preparation of Ir/DSPC liposomes and cellular uptake of the complexes suggest that these complexes are potential luminescent probes for hydrophobic biological entities and live-cell imaging agents.

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**Supporting Information Available:** Electronic absorption spectral data of all the complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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(33) Reaven, E.; Tsai, L.; Azhar, S. *J. Biol. Chem.* **1996**, *271*, 16208.