Luminescent Tricarbonylrhenium(I) Dipyridoquinoxaline Indole Complexes as Sensitive Probes for Indole-Binding Proteins

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Received January 20, 2007

Luminescent tricarbonylrhenium(I) dipyridoquinoxaline indole complexes $[Re(N-N)(CO)_3(L)](CF_3SO_3)$ (N−N = dipyrido[3,2-*f*:2′,3′-*h*]quinoxaline (dpq), L = *N*-(3-pyridoyl)tryptamine (py-3-$CONHC_2H_4$-indole) (**1a**), *N*-(*N*-(3-pyridoyl)-6-aminohexanoyl)tryptamine (py-3-$CONHC_5H_{10}CONHC_2H_4$-indole) (**1b**); N−N = 2-(*n*-butylamido)dipyrido[3,2-*f*:2′,3′-*h*]quinoxaline (dpqa), L = py-3-$CONHC_2H_4$-indole (**2a**), py-3-$CONHC_5H_{10}CONHC_2H_4$-indole (**2b**)) and their indole-free counterparts $[Re(N-N)(CO)_3(py\text{-}3\text{-}CONH\text{-}Et)](CF_3SO_3)$ (py-3-CONH-Et = *N*-ethyl-(3-pyridyl)formamide; N−N = dpq (**1c**), dpqa (**2c**)) have been synthesized and characterized. The crystal structure of a related complex, $[Re(dpqa)(CO)_3(pyridine)](PF_6)$, has also been studied. All the complexes exhibited triplet metal-to-ligand charge-transfer (^{3}MLCT) ($d\pi(Re) \rightarrow \pi^*(N-N)$) emission in fluid solutions at 298 K and in alcohol glass at 77 K. The emission quantum yields of the complexes were reduced upon changing from CH_2Cl_2 to aqueous buffer. The reduction was much more significant for the dpqa complexes than the dpq complexes due to the hydrogen-bonding interaction of the amide substituent of the dpqa ligand with water molecules. The interactions of these tricarbonylrhenium(I) complexes with indole-binding proteins including bovine serum albumin and tryptophanase have been studied by emission titrations and inhibition assays, respectively.

Introduction

The important physiological activities of indole and its derivatives have been the subject of many studies.[1] Various approaches have been adopted to examine the substrate-binding properties of the biological receptors of indole compounds. For example, radioactive indole derivatives,[2] indole−biotin conjugates,[3] and the fluorescence properties of indole compounds[4] have been utilized. In view of the rich photophysical properties of tricarbonylrhenium(I) complexes[5−24] and our interest in organometallic and inorganic metal complexes as biological probes and labels,[24−27] we have designed luminescent rhenium(I)[24d,h] and ruthenium(II)[25b] polypyridine indole complexes and redox-active ferrocene indole conjugates[27c] and studied their protein-binding behavior by optical and electrochemical methods. Our current target is to design luminescent probes that show higher protein-induced emission enhancement factors upon biological binding. Recently, Kelly and co-workers have reported the interesting DNA-binding properties of ruthenium(II) amidodipyrido[3,2-*f*:2′,3′-*h*]quinoxaline (amido-dpq) com-

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(1) (a) Ishida, T.; Hamada, M.; Inoue, M.; Wakahara, A. *Chem. Pharm. Bull.* **1990**, *38*, 851. (b) Bartel, B. *Annu. Rev. Plant Physiol.* **1997**, *48*, 51.

(2) (a) Bilang, J.; Macdonald, H.; King, P. J.; Sturm, A. *Plant Physiol.* **1993**, *102*, 29. (b) Zettl, R.; Schell, J.; Palme, K. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 689.

(3) Dolušić, E.; Kowalczyk, M.; Magnus, V.; Sandberg, G.; Normanly, J. *Bioconjugate Chem.* **2001**, *12*, 152.

(4) (a) Schore, N. E.; Turro, N. J. *J. Am. Chem. Soc.* **1975**, *97*, 2488. (b) Mazzini, A.; Cavatorta, P.; Iori, M.; Favilla, R.; Sartor, G. *Biophys. Chem.* **1992**, *42*, 101.

(5) (a) Wrighton, M. S.; Morse, D. L. *J. Am. Chem. Soc.* **1974**, *96*, 998. (b) Giordano, P. J.; Wrighton, M. S. *J. Am. Chem. Soc.* **1979**, *101*, 2888.

(6) (a) Connick, W. B.; Di Bilio, A. J.; Hill, M. G.; Winkler, J. R.; Gray, H. B. *Inorg. Chim. Acta* **1995**, *240*, 169. (b) Wenger, O. S.; Henling, L. M.; Day, M. W.; Winkler, J. R.; Gray, H. B. *Inorg. Chem.* **2004**, *43*, 2043. (c) Dunn, A. R.; Belliston-Bittner, W.; Winkler, J. R.; Getzoff, E. D.; Stuehr, D. J.; Gray, H. B. *J. Am. Chem. Soc.* **2005**, *127*, 5169.

(7) (a) Yam, V. W.-W.; Lo, K. K.-W.; Cheung, K.-K.; Kong, R. Y.-C. *J. Chem. Soc., Chem. Commun.* **1995**, 1191. (b) Yam, V. W.-W.; Lo, K. K.-W.; Cheung, K.-K.; Kong, R. Y.-C. *J. Chem. Soc., Dalton Trans.* **1997**, 2067. (c) Wong, K. M.-C.; Li, W.-P.; Cheung, K.-K.; Yam, V. W.-W. *New J. Chem.* **2005**, *29*, 165.

(8) (a) Guo, X.-Q.; Castellano, F. N.; Li, L.; Szmacinski, H.; Lakowicz, J. R.; Sipior, J. *Anal. Biochem.* **1997**, *254*, 179. (b) Guo, X.-Q.; Castellano, F. N.; Li, L.; Lakowicz, J. R. *Anal. Chem.* **1998**, *70*, 632. (c) Shen, Y.; Maliwal, B. P.; Lakowicz, J. R. *J. Fluoresc.* **2001**, *11*, 315.

(9) (a) van Outersterp, J. W. M.; Stufkens, D. J.; Fraanje, J.; Goubitz, K.; Vlček, A., Jr. *Inorg. Chem.* **1995**, *34*, 4756. (b) van Outersterp, J. W. M.; Stufkens, D. J.; Vlček, A., Jr. *Inorg. Chem.* **1995**, *34*, 5183. (c) Rossenaar, B. D.; Stufkens, D. J.; Vlček, A., Jr. *Inorg. Chim. Acta* **1996**, *247*, 247. (d) Gabrielsson, A.; Blanco-Rodríguez, A. M.; Matousek, P.; Towrie, M.; Vlček, A., Jr. *Organometallics* **2006**, *25*, 2148.

(10) (a) Stoeffler, H. D.; Thornton, N. B.; Temkin, S. L.; Schanze, K. S. *J. Am. Chem. Soc.* **1995**, *117*, 7119. (b) Lucia, L. A.; Abboud, K.; Schanze, K. S. *Inorg. Chem.* **1997**, *36*, 6224. (c) Walters, K. A.; Dattelbaum, D. M.; Ley, K. D.; Schoonover, J. R.; Meyer, T. J.; Schanze, K. S. *Chem. Commun.* **2001**, 1834. (d) Walters, K. A.; Ley, K. D.; Cavalaheiro, C. S. P.; Miller, S. E.; Gosztola, D.; Wasielewski, M. R.; Bussandri, A. P.; van Willigen, H.; Schanze, K. S. *J. Am. Chem. Soc.* **2001**, *123*, 8329.

(11) (a) van Wallendael, S.; Shaver, R. J.; Rillema, D. P.; Yoblinski, B. J.; Stathis, M.; Guarr, T. F. *Inorg. Chem.* **1990**, *29*, 1761. (b) Wallace, L.; Jackman, D. C.; Rillema, D. P.; Merkert, J. W. *Inorg. Chem.* **1995**, *34*, 5210. (c) Xue, W.-M.; Goswami, N.; Eichhorn, D. M.; Orizondo, P. L.; Rillema, D. P. *Inorg. Chem.* **2000**, *39*, 4460. (d) Villegas, J. M.; Stoyanov, S. R.; Huang, W.; Rillema, D. P. *Dalton Trans.* **2005**, 1042. (e) Stoyanov, S. R.; Villegas, J. M.; Cruz, A. J.; Lockyear, L. L.; Reibenspies, J. H.; Rillema, D. P. *J. Chem. Theory Comput.* **2005**, *1*, 95.

(12) (a) Sacksteder, L.; Zipp, A. P.; Brown, E. A.; Streich, J.; Demas, J. N.; DeGraff, B. A. *Inorg. Chem.* **1990**, *29*, 4335. (b) Zipp, A. P.; Sacksteder, L.; Streich, J.; Cook, A.; Demas, J. N.; DeGraff, B. A. *Inorg. Chem.* **1993**, *32*, 5629. (c) Sacksteder, L.; Lee, M.; Demas, J. N.; DeGraff, B. A. *J. Am. Chem. Soc.* **1993**, *115*, 8230. (d) Kneas, K. A.; Xu, W.; Demas, J. N.; DeGraff, B. A.; Zipp, A. P. *J. Fluoresc.* **1998**, *8*, 295.

10.1021/om0700617 CCC: $37.00
Publication on Web 06/01/2007

plexes.[28] We have also employed rhenium(I),[24i] ruthenium(II),[25c] and iridium(III)[26h] complexes containing related ligands in the development of more sensitive luminescent probes for avidin. We envisage that incorporating an amido-dpq ligand into a tricarbonylrhenium(I) indole complex would produce an interesting probe for indole-binding proteins.

In this paper, we report the synthesis and characterization of luminescent tricarbonylrhenium(I) dipyridoquinoxaline indole complexes $[Re(N-N)(CO)_3(L)](CF_3SO_3)$ (N−N = dipyrido[3,2-*f*:2′,3′-*h*]quinoxaline (dpq), L = *N*-(3-pyridoyl)tryptamine (py-3-$CONHC_2H_4$-indole) (**1a**), *N*-(*N*-(3-pyridoyl)-6-aminohexanoyl)tryptamine, (py-3-$CONHC_5H_{10}CONHC_2H_4$-indole) (**1b**); N−N = 2-(*n*-butylamido)dipyrido[3,2-*f*:2′,3′-*h*]quinoxaline (dpqa), L = py-3-$CONHC_2H_4$-indole (**2a**), py-3-$CONHC_5H_{10}CONHC_2H_4$-indole (**2b**)) and their indole-free counterparts $[Re(N-N)(CO)_3$(py-3-CONH-Et)$](CF_3SO_3)$ (py-3-CONH-Et = *N*-ethyl-(3-pyridyl)formamide; N−N = dpq (**1c**), dpqa (**2c**)). The structures of these rhenium(I) dipyridoquinoxaline complexes are shown in Chart 1. The crystal structure of a related complex, $[Re(dpqa)(CO)_3(pyridine)](PF_6)$, has also been studied. The photophysical and electrochemical properties of complexes **1a**−**1c** and **2a**−**2c** have been examined. Additionally, the interactions of these tricarbonylrhenium(I) dipyridoquinoxaline complexes with indole-binding proteins including bovine serum albumin (BSA) and tryptophanase (TPase) have been studied by emission titrations and enzyme inhibition assays, respectively. The most important result is that all the indole complexes showed emission enhancement and lifetime extension upon binding to BSA; the emission enhancement was very significant due to the use of dipyridoquinoxaline ligands.

Experimental Section

Materials and Synthesis. All solvents were of analytical reagent grade. $Re(CO)_5Cl$ (Aldrich), tryptamine (Aldrich), BSA (Calbiochem), β-nicotinamide adenine dinucleotide, disodium salt (NADH) (Calbiochem), TPase (Sigma), and pyridoxal 5-phosphate (Sigma) were used without purification. Nicotinic acid *N*-hydroxysuccinimidyl ester,[29a] dpq,[29b] dpqa,[24i] py-3-$CONHC_2H_4$-indole,[24d,h] py-3-$CONHC_5H_{10}CONHC_2H_4$-indole,[24d,h] py-3-CONH-Et,[24d,h] $[Re(N-N)(CO)_3(CH_3CN)](CF_3SO_3)$ (N−N = dpq, dpqa),[24g,i] and $[Re(dpqa)(CO)_3(pyridine)](PF_6)$[24i] were prepared as described previously.

Synthesis of Complexes. A mixture of $[Re(N-N)(CO)_3(CH_3CN)](CF_3SO_3)$ (0.29 mmol) and the pyridine ligand (0.35 mmol) in THF (20 mL) was refluxed under nitrogen for 12 h. The mixture was evaporated to dryness to give a yellow solid, which was subsequently recrystallized from a mixture of acetone, acetonitrile, and diethyl ether.

$[Re(dpq)(CO)_3$(py-3-$CONHC_2H_4$-indole)$](CF_3SO_3)$ (1a). Complex **1a** was isolated as yellow crystals. Yield: 160 mg (60%). ^1H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.03 (d, 3H, J = 5.3 Hz, H4 and H4′ of pyrido rings of dpq and NH of indole), 9.93 (d, 2H, J = 8.5 Hz, H6 and H6′ of pyrido rings of dpq), 9.33 (s, 2H, H2 and H3 of dpq), 8.96 (s, 1H, H2 of pyridine), 8.76 (d, 1H,

(13) (a) Hino, J. K.; Ciana, L. D.; Dressick, W. J.; Sullivan, B. P. *Inorg. Chem.* **1992**, *31*, 1072. (b) Shen, Y.; Sullivan, B. P. *Inorg. Chem.* **1995**, *34*, 6235. (c) Schutte, E.; Helms, J. B.; Woessner, S. M.; Bowen, J.; Sullivan, B. P. *Inorg. Chem.* **1998**, *37*, 2618. (d) Smithback, J. L.; Helms, J. B.; Schutte, E.; Woessner, S. M.; Sullivan, B. P. *Inorg. Chem.* **2006**, *45*, 2163.

(14) (a) Worl, L. A.; Duesing, R.; Chen, P.; Della Ciana, L.; Meyer, T. J. *J. Chem. Soc., Dalton Trans.* **1991**, 849. (b) Mecklenburg, S. L.; Opperman, K. A.; Chen, P.; Meyer, T. J. *J. Phys. Chem.* **1996**, *100*, 15145. (c) López, R.; Leiva, A. M.; Zuloaga, F.; Leob, B.; Norambuena, E.; Omberg, K. M.; Schoonover, J. R.; Striplin, D.; Devenney, M.; Meyer, T. J. *Inorg. Chem.* **1999**, *38*, 2924. (d) Claude, J. P.; Omberg, K. M.; Williams, D. S.; Meyer, T. J. *J. Phys. Chem. A* **2002**, *106*, 7795.

(15) (a) Sun, S.-S.; Lees, A. J. *J. Am. Chem. Soc.* **2000**, *122*, 8956. (b) Sun, S.-S.; Lees, A. J. *Coord. Chem. Rev.* **2002**, *230*, 171. (c) Sun, S.-S.; Lees, A. J. *Organometallics* **2002**, *21*, 39. (d) Sun, S.-S.; Lees, A. J.; Zavalij, P. Y. *Inorg. Chem.* **2003**, *42*, 3445.

(16) (a) Moya, S. A.; Guerrero, J.; Pastene, R.; Schmidt, R.; Sariego, R.; Sartori, R.; Sanz-Aparicio, J.; Fonseca, I.; Martínez-Ripoll, M. *Inorg. Chem.* **1994**, *33*, 2341. (b) Moya, S. A.; Guerrero, J.; Pastene, R.; Pardey, A. J.; Baricelli, P. *Polyhedron* **1998**, *17*, 2289. (c) Guerrero, J.; Piro, O. E.; Wolcan, E.; Feliz, M. R.; Ferraudi, G.; Moya, S. A. *Organometallics* **2001**, *20*, 2842.

(17) (a) Juris, A.; Campagna, S.; Bidd, I.; Lehn, J.-M.; Ziessel, R. *Inorg. Chem.* **1988**, *27*, 4007. (b) Ziessel, R.; Juris, A.; Venturi, M. *Chem. Commun.* **1997**, 1593. (c) Goeb, S.; De Nicola, A.; Ziessel, R.; Sabatini, C.; Barbieri, A.; Barigelletti, F. *Inorg. Chem.* **2006**, *45*, 1173.

(18) (a) Lin, R.; Guarr, T. F.; Duesing, R. *Inorg. Chem.* **1990**, *29*, 4169. (b) Yoblinski, B. J.; Stathis, M.; Guarr, T. F. *Inorg. Chem.* **1992**, *31*, 5. (c) Lin, R.; Fu, Y.; Brock, C. P.; Guarr, T. F. *Inorg. Chem.* **1992**, *31*, 4346.

(19) (a) Wei, L.; Babich, J.; Eckelman, W. C.; Zubieta, J. *Inorg. Chem.* **2005**, *44*, 2198. (b) Banerjee, S. R.; Schaffer, P.; Babich, J.; Valliant, J. F.; Zubieta, J. *Dalton Trans.* **2005**, 3886. (c) Wei, L.; Babich, J.; Zubieta, J. *Inorg. Chim. Acta* **2005**, *358*, 3691. (d) James, S.; Maresca, K. P.; Babich, J. W.; Valliant, J. F.; Doering, L.; Zubieta, J. *Bioconjugate Chem.* **2006**, *17*, 590.

(20) (a) Metcalfe, C.; Webb, M.; Thomas, J. A. *Chem. Commun.* **2002**, 2026. (b) Metcalfe, C.; Spey, S.; Adams, H.; Thomas, J. A. *J. Chem. Soc., Dalton Trans.* **2002**, 4732. (c) de Wolf, P.; Heath, S. L.; Thomas, J. A. *Inorg. Chim. Acta* **2003**, *355*, 280.

(21) (a) Walters, K. A.; Kim, Y.-J.; Hupp, J. T. *Inorg. Chem.* **2002**, *41*, 2909. (b) Dinolfo, P. H.; Benkstein, K. D.; Stern, C. L.; Hupp, J. T. *Inorg. Chem.* **2005**, *44*, 8707. (c) She, C.; Anderson, N. A.; Guo, J.; Liu, F.; Goh, W.-H.; Chen, D.-T.; Mohler, D. L.; Tian, Z.-Q.; Hupp, J. T.; Lian, T. *J. Phys. Chem. B* **2005**, *109*, 19345.

(22) (a) Dyer, J.; Grills, D. C.; Matousek, P.; Parker, A. W.; Towrie, M.; Weinstein, J. A.; George, M. W. *Chem. Commun.* **2002**, 872. (b) Dyer, J.; Blau, W. J.; Coates, C. G.; Creely, C. M.; Gavey, J. D.; George, M. W.; Grills, D. C.; Hudson, S.; Kelly, J. M.; Matousek, P.; McGarvey, J. J.; McMaster, J.; Parker, A. W.; Towrie, M.; Weinstein, J. A. *Photochem. Photobiol. Sci.* **2003**, *2*, 542.

(23) (a) Waterland, M. R.; Gordon, K. C.; McGarvey, J. J.; Jayaweera, P. M. *J. Chem. Soc., Dalton Trans.* **1998**, 609. (b) Lundin, N. J.; Walsh, P. J.; Howell, S. L.; McGarvey, J. J.; Blackman, A. G.; Gordon, K. C. *Inorg. Chem.* **2005**, *44*, 3551.

(24) (a) Lo, K. K.-W.; Ng, D. C.-M.; Hui, W.-K.; Cheung, K.-K. *J. Chem. Soc., Dalton Trans.* **2001**, 2634. (b) Lo, K. K.-W.; Hui, W.-K.; Ng, D. C.-M.; Cheung, K.-K. *Inorg. Chem.* **2002**, *41*, 40. (c) Lo, K. K.-W.; Hui, W.-K.; Ng, D. C.-M. *J. Am. Chem. Soc.* **2002**, *124*, 9344. (d) Lo, K. K.-W.; Tsang, K. H.-K.; Hui, W. K.; Zhu, N. *Chem. Commun.* **2003**, 2704. (e) Lo, K. K.-W.; Lau, J. S.-Y.; Fong, V. W.-Y.; Zhu, N. *Organometallics* **2004**, *23*, 1098. (f) Lo, K. K.-W.; Tsang, K. H.-K. *Organometallics* **2004**, *23*, 3062. (g) Lo, K. K.-W.; Hui, W. K. *Inorg. Chem.* **2005**, *44*, 1992. (h) Lo, K. K.-W.; Tsang, K. H.-K.; Hui, W.-K.; Zhu, N. *Inorg. Chem.* **2005**, *44*, 6100. (i) Lo, K. K.-W.; Tsang, K. H.-K.; Sze, K.-S. *Inorg. Chem.* **2006**, *45*, 1714. (j) Lo, K. K.-W.; Tsang, K. H.-K.; Zhu, N. *Organometallics* **2006**, *25*, 3220.

(25) (a) Lo, K. K.-W.; Lee, T. K.-M. *Inorg. Chem.* **2004**, *43*, 5275. (b) Lo, K. K.-W.; Lee, T. K.-M.; Zhang, K. Y. *Inorg. Chim. Acta* **2006**, *359*, 1845. (c) Lo, K. K.-W.; Lee, T. K.-M. *Inorg. Chim. Acta* **2007**, *360*, 293.

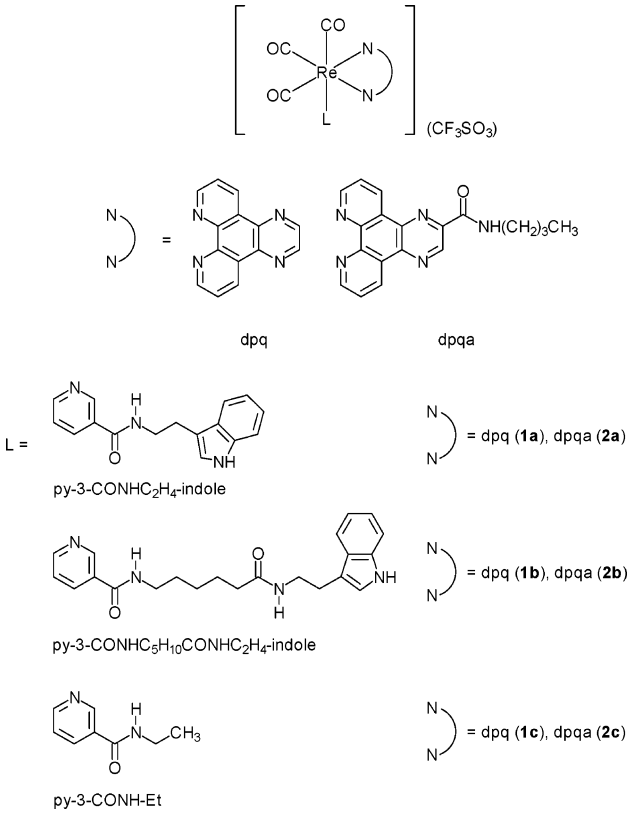
(26) (a) Lo, K. K.-W.; Ng, D. C.-M.; Chung, C.-K. *Organometallics* **2001**, *20*, 4999. (b) Lo, K. K.-W.; Chung, C.-K.; Ng, D. C.-M.; Zhu, N. *New J. Chem.* **2002**, *26*, 81. (c) Lo, K. K.-W.; Chung, C.-K.; Zhu, N. *Chem. Eur. J.* **2003**, *9*, 475. (d) Lo, K. K.-W.; Chung, C.-K.; Lee, T. K.-M.; Lui, L.-H.; Tsang, K. H.-K.; Zhu, N. *Inorg. Chem.* **2003**, *42*, 6886. (e) Lo, K. K.-W.; Chan, J. S.-W.; Chung, C.-K.; Tsang, V. W.-H.; Zhu, N. *Inorg. Chim. Acta* **2004**, *357*, 3109. (f) Lo, K. K.-W.; Chan, J. S.-W.; Lui, L.-H.; Chung, C.-K. *Organometallics* **2004**, *23*, 3108. (g) Lo, K. K.-W.; Li, C.-K.; Lau, J. S.-Y. *Organometallics* **2005**, *24*, 4594. (h) Lo, K. K.-W.; Chung, C.-K.; Zhu, N. *Chem.−Eur. J.* **2006**, *12*, 1500. (i) Lo, K. K.-W.; Lau, J. S.-Y.; Lo, D. K.-K.; Lo, L. T.-L. *Eur. J. Inorg. Chem.* **2006**, 4054. (j) Lo, K. K.-W.; Lau, J. S.-Y. *Inorg. Chem.* **2007**, *46*, 700.

(27) (a) Lo, K. K.-W.; Lau, J. S.-Y.; Ng, D. C.-M.; Zhu, N. *J. Chem. Soc., Dalton Trans.* **2002**, 1753. (b) Lo, K. K.-W.; Ng, D. C.-M.; Lau, J. S.-Y.; Wu, R. S.-S.; Lam, P. K.-S. *New J. Chem.* **2003**, *27*, 274. (c) Lo, K. K.-W.; Lau, J. S.-Y.; Zhu, N. *New J. Chem.* **2006**, *30*, 1567.

(28) (a) O'Donoghue, K. A.; Kelly, J. M.; Kruger, P. E. *Dalton Trans.* **2004**, 13. (b) O'Donoghue, K.; Penedo, J. C.; Kelly, J. M.; Kruger, P. E. *Dalton Trans.* **2005**, 1123.

(29) (a) Cohen, B. E.; Stoddard, B. L.; Koshland, D. E., Jr. *Biochemistry* **1997**, *36*, 9035. (b) Collins, J. G.; Sleeman, A. D.; Aldrich-Wright, J. R.; Greguric, I.; Hambley, T. W. *Inorg. Chem.* **1998**, *37*, 3133.

Chart 1. Structures of Tricarbonylrhenium(I) Dipyridoquinoxaline Complexes



J = 5.6 Hz, H6 of pyridine), 8.51 (dd, 2H, J = 8.2 and 5.3 Hz, H5 and H5′ of pyrido rings of dpq), 8.25 (d, 1H, J = 8.2 Hz, H4 of pyridine), 8.10 (s, br, 1H, py-3-CON*H*), 7.55 (d, 1H, J = 7.3 Hz, H4 of indole), 7.47−7.38 (m, 2H, H5 of pyridine and H7 of indole), 7.14−7.07 (m, 2H, H2 and H6 of indole), 6.99 (t, 1H, J = 7.5 Hz, H5 of indole), 3.62−3.56 (m, 2H, py-3-CONHC*H*$_2$CH$_2$), 2.95 (t, 2H, J = 7.6 Hz, py-3-CONHCH$_2$C*H*$_2$). IR (KBr) ν/cm^{-1}: 3314 (s, br, NH), 2033 (s, C≡O), 1919 (s, C≡O), 1654 (m, C═O), 1163 (s, $CF_3SO_3^-$), 1032 (s, $CF_3SO_3^-$). Positive-ion ESI-MS ion cluster at *m*/*z* 768 {[Re(dpq)(CO)$_3$(py-3-CONHC$_2$H$_4$-indole)]}$^+$. Anal. Calcd for $C_{34}H_{22}N_7O_7SF_3Re\cdot(CH_3)_2CO\cdot CH_3CN$: C, 46.15; H, 3.08; N, 11.04. Found: C, 46.35; H, 3.16; N, 10.94.

[Re(dpq)(CO)$_3$(py-3-CONHC$_5$H$_{10}$CONHC$_2$H$_4$-indole)](CF$_3$SO$_3$) (1b). Complex **1b** was isolated as yellow crystals. Yield: 263 mg (88%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.04 (dd, 3H, J = 5.3 and 1.5 Hz, H4 and H4′ of pyrido rings of dpq and NH of indole), 9.93 (dd, 2H, J = 8.5 and 1.5 Hz, H6 and H6′ of pyrido rings of dpq), 9.31 (s, 2H, H2 and H3 of dpq), 8.93 (s, 1H, H2 of pyridine), 8.78 (d, 1H, J = 5.6 Hz, H6 of pyridine), 8.52 (dd, 2H, J = 8.5 and 5.3 Hz, H5 and H5′ of pyrido rings of dpq), 8.31 (d, 1H, J = 8.2 Hz, H4 of pyridine), 8.03 (s, br, 1H, py-3-CON*H*), 7.51 (d, 1H, J = 8.2 Hz, H4 of indole), 7.43 (dd, 1H, J = 8.2 and 5.6 Hz, H5 of pyridine), 7.35 (d, 1H, J = 8.2 Hz, H7 of indole), 7.12−7.04 (m, 3H, py-3-CONHC$_5$H$_{10}$CON*H* and H2 and H6 of indole), 6.94 (t, 1H, J = 7.0 Hz, H5 of indole), 3.48−3.38 (m, 4H, py-3-CONHC*H*$_2$C$_4$H$_8$CONHC*H*$_2$CH$_2$), 1.59−1.46 (m, 6H, py-3-CONHCH$_2$C$_3$*H*$_6$CH$_2$). IR (KBr) ν/cm^{-1}: 3322 (m, NH), 2034 (s, C≡O), 1923 (s, C≡O), 1654 (s, C═O), 1163 (s, $CF_3SO_3^-$), 1030 (s, $CF_3SO_3^-$). Positive-ion ESI-MS ion cluster at *m*/*z* 881 {[Re(dpq)(CO)$_3$(py-3-CONHC$_5$H$_{10}$CONHC$_2$H$_4$-indole)]}$^+$. Anal. Calcd for $C_{40}H_{34}N_8O_8SF_3Re\cdot(CH_3)_2CO\cdot CH_3CN$: C, 47.87; H, 3.84; N, 11.16. Found: C, 47.77; H, 3.88; N, 11.21.

[Re(dpq)(CO)$_3$(py-3-CONH-Et)](CF$_3$SO$_3$) (1c). Complex **1c** was isolated as yellow crystals. Yield: 88 mg (38%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.05 (d, 2H, J = 4.1 Hz, H4 and H4′ of pyrido rings of dpq), 9.94 (d, 2H, J = 8.2 Hz, H6 and H6′ of pyrido rings of dpq), 9.33 (s, 2H, H2 and H3 of dpq), 8.96 (s, 1H, H2 of pyridine), 8.76 (d, 1H, J = 5.9 Hz, H6 of pyridine), 8.53 (dd, 2H, J = 8.5 and 5.3 Hz, H5 and H5′ of pyrido rings of dpq), 8.28 (d, 1H, J = 8.2 Hz, H4 of pyridine), 8.06 (s, br, 1H, py-3-CON*H*), 7.44 (dd, 1H, J = 7.8 and 5.7 Hz, H5 of pyridine), 3.33−3.26 (m, 2H, py-3-CONHC*H*$_2$CH$_3$), 1.06 (t, 3H, J = 7.3 Hz, py-3-CONHCH$_2$C*H*$_3$). IR (KBr) ν/cm^{-1}: 3314 (s, br, NH), 2033 (s, C≡O), 1938 (s, C≡O), 1662 (m, C═O), 1156 (m, $CF_3SO_3^-$), 1032 (m, $CF_3SO_3^-$). Positive-ion ESI-MS ion cluster at *m*/*z* 652 {[Re(dpq)(CO)$_3$(py-3-CONH-Et)]}$^+$. Anal. Calcd for $C_{26}H_{18}N_6O_7SF_3Re$: C, 38.95; H, 2.26; N, 10.48. Found: C, 38.99; H, 2.36; N, 10.64.

[Re(dpqa)(CO)$_3$(py-3-CONHC$_2$H$_4$-indole)](CF$_3$SO$_3$) (2a). Complex **2a** was isolated as yellow crystals. Yield: 116 mg (39%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.19 (d, 1H, J = 8.5 Hz, H4′ of pyrido ring of dpqa), 10.06−10.02 (m, 3H, H6 and H6′ of pyrido rings of dpqa and NH of indole), 9.93 (d, 1H, J = 8.5 Hz, H4 of pyrido ring of dpqa), 9.79 (s, 1H, H3 of quinoxaline of dpqa), 9.13 (s, br, 1H, CON*H* of dpqa), 8.93 (s, 1H, H2 of pyridine), 8.76 (d, 1H, J = 5.3 Hz, H6 of pyridine), 8.55−8.46 (m, 2H, H5 and H5′ of pyrido rings of dpqa), 8.26 (d, 1H, J = 6.5 Hz, H4 of pyridine), 8.10 (s, br, 1H, py-3-CON*H*), 7.51 (d, 1H, J = 7.9 Hz, H4 of indole), 7.44 (t, 1H, J = 6.4 Hz, H5 of pyridine), 7.35 (d, 1H, J = 8.5 Hz, H7 of indole), 7.10−7.03 (m, 2H, H2 and H6 of indole), 6.95 (t, 1H, J = 7.3 Hz, H5 of indole), 3.61−3.50 (m, 4H, NHC*H*$_2$(CH$_2$)$_2$CH$_3$ and py-3-CONHC*H*$_2$CH$_2$), 2.93 (t, 2H, J = 7.3 Hz, py-3-CONHCH$_2$C*H*$_2$), 1.70−1.63 (m, 2H, NHCH$_2$C*H*$_2$-CH$_2$CH$_3$), 1.47−1.40 (m, 2H, NH(CH$_2$)$_2$C*H*$_2$CH$_3$), 0.98−0.93 (m, 3H, NH(CH$_2$)$_3$C*H*$_3$). IR (KBr) ν/cm^{-1}: 3442 (s, br, NH), 2036 (s, C≡O), 1919 (s, C≡O), 1654 (m, C═O), 1172 (s, $CF_3SO_3^-$), 1036 (s, $CF_3SO_3^-$). Positive-ion ESI-MS ion cluster at *m*/*z* 867 {[Re(dpqa)(CO)$_3$(py-3-CONHC$_2$H$_4$-indole)]}$^+$. Anal. Calcd for $C_{39}H_{32}N_8O_8SF_3Re\cdot CH_3CN$: C, 46.59; H, 3.34; N, 11.93. Found: C, 46.75; H, 3.27; N, 11.64.

[Re(dpqa)(CO)$_3$(py-3-CONHC$_5$H$_{10}$CONHC$_2$H$_4$-indole)](CF$_3$SO$_3$) (2b). Complex **2b** was isolated as yellow crystals. Yield: 198 mg (60%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.18 (d, 1H, J = 8.5 Hz, H4′ of pyrido ring of dpqa), 10.07−10.03 (m, 2H, H6 and H6′ of pyrido rings of dpqa), 9.97 (s, br, 1H, NH of indole), 9.92 (dd, 1H, J = 8.2 and 1.5 Hz, H4 of pyrido ring of dpqa), 9.77 (s, 1H, H3 of quinoxaline of dpqa), 9.18 (s, br, 1H, CON*H* of dpqa), 8.87 (s, 1H, H2 of pyridine), 8.81 (d, 1H, J = 5.6 Hz, H6 of pyridine), 8.55−8.48 (m, 2H, H5 and H5′ of pyrido rings of dpqa), 8.30 (d, 1H, J = 7.9 Hz, H4 of pyridine), 8.00 (s, br, 1H, py-3-CON*H*), 7.46−7.42 (m, 2H, H5 of pyridine and H4 of indole), 7.31 (d, 1H, J = 8.2 Hz, H7 of indole), 7.13 (s, br, 1H, py-3-CONHC$_5$H$_{10}$CON*H*C$_2$H$_4$-indole), 7.07−6.99 (m, 2H, and H2 and H6 of indole), 6.87 (t, 1H, J = 7.0 Hz, H5 of indole), 3.58−3.51 (m, 2H, NHC*H*$_2$(CH$_2$)$_2$CH$_3$), 3.47−3.37 (m, 4H, py-3-CONHC*H*$_2$C$_4$H$_8$CONHC*H*$_2$CH$_2$), 1.73−1.38 (m, 10H, NHCH$_2$-(C*H*$_2$)$_2$CH$_3$ and py-3-CONHCH$_2$C$_3$*H*$_6$CH$_2$), 0.96 (t, 3H, J = 7.3 Hz, NH(CH$_2$)$_3$C*H*$_3$). IR (KBr) ν/cm^{-1}: 3328 (s, br, NH), 2035 (s, C≡O), 1924 (s, C≡O), 1655 (s, C═O), 1164 (m, $CF_3SO_3^-$), 1030 (s, $CF_3SO_3^-$). Positive-ion ESI-MS ion cluster at *m*/*z* 981 {[Re(dpqa)(CO)$_3$(py-3-CONHC$_5$H$_{10}$CONHC$_2$H$_4$-indole)]}$^+$. Anal. Calcd for $C_{45}H_{43}N_9O_9SF_3Re\cdot(CH_3)_2CO\cdot CH_3CN$: C, 48.89; H, 4.27; N, 11.40. Found: C, 48.83; H, 4.43; N, 11.48.

[Re(dpqa)(CO)$_3$(py-3-CONH-Et)](CF$_3$SO$_3$) (2c). Complex **2c** was isolated as yellow crystals. Yield: 106 mg (41%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 10.22 (d, 1H, J = 8.5 Hz, H4′ of pyrido ring of dpqa), 10.06 (t, 2H, J = 5.3 Hz, H6 and H6′ of pyrido rings of dpqa), 9.96 (d, 1H, J = 8.5 Hz, H4 of pyrido ring of dpqa), 9.80 (s, 1H, H3 of quinoxaline of dpqa), 9.17 (s, br, 1H, CON*H* of dpqa), 8.93 (s, 1H, H2 of pyridine), 8.76 (d, 1H, J = 5.6 Hz, H6 of pyridine), 8.57−8.50 (m, 2H, H5 and H5′ of pyrido rings of dpqa), 8.27 (d, 1H, J = 7.0 Hz, H4 of pyridine),

8.01 (s, br, 1H, py-3-CON*H*), 7.46−7.42 (m, 1H, H5 of pyridine), 3.58−3.51 (m, 2H, NHCH_2(CH_2)$_2$$CH_3$), 3.33−3.24 (m, 2H, py-3-CONHC$H_2$$CH_3$), 1.73−1.63 (m, 2H, NHCH$_2$C$H_2$$CH_2$$CH_3$), 1.48−1.40 (m, 2H, NH($CH_2$)$_2CH_2$$CH_3$), 1.13−1.04 (m, 3H, py-3-CONHCH$_2$CH_3), 0.96 (t, 3H, J = 7.0 Hz NH(CH_2)$_3$CH_3). IR (KBr) ν/cm^{-1}: 3330 (s, br, NH), 2035 (s, C≡O), 1919 (s, C≡O), 1654 (m, C═O), 1164 (m, $CF_3SO_3^-$), 1030 (m, $CF_3SO_3^-$). Positive-ion ESI-MS ion cluster at m/z 752 {[Re(dpqa)(CO)$_3$(py-3-CONHEt)]}$^+$. Anal. Calcd for $C_{31}H_{27}N_7O_8SF_3Re$·0.25((CH_3)$_2$CO): C, 41.66; H, 3.14; N, 10.71. Found: C, 41.50; H, 3.38; N, 10.64.

Crystal Data of [Re(dpqa)(CO)$_3$(pyridine)](PF$_6$). Single crystals of the complex suitable for X-ray crystallographic studies were obtained by layering petroleum ether on a concentrated dichloromethane solution of the complex. A crystal of dimensions 0.6 × 0.15 × 0.1 mm^3 mounted in a glass capillary was used for data collection at −20 °C on a MAR diffractometer with a 300 mm image plate detector using graphite-monochromatized Mo Kα radiation (λ = 0.71073 Å). Data collection was made with a 2° oscillation step of φ, 10 min exposure time, and scanner distance at 120 mm. The number of images collected was 100. The images were interpreted and the intensities integrated using the program DENZO.[30] The structure was solved by direct methods employing the program SHELXS-97.[31] Rhenium and many non-hydrogen atoms were located according to the direct methods. The positions of the other non-hydrogen atoms were found after successful refinement by full-matrix least-squares using the program SHELXL-97.[31] One hexafluorophosphate anion and two dichloromethane solvent molecules were located. The anion was disordered in the mode of rotation along the F−P−F axis; two sets of positions of the other four fluoride atoms were located. The last three carbon atoms of the *n*-butyl group were also disordered; two sets of their positions were located, while restraints were applied to assume similar C−C bond lengths close to 1.55(2) Å. One crystallographic asymmetric unit consisted of one formula unit, including one hexafluorophosphate anion and two dichloromethane solvent molecules. In the final stage of least-squares refinement, the disordered fluoride and carbon atoms were refined isotropically; other non-hydrogen atoms were refined anisotropically. Hydrogen atoms were generated by the program SHELXL-97. The positions of hydrogen atoms were calculated on the basis of a riding mode with thermal parameters equal to 1.2 times that of the associated carbon atoms and participated in the calculation of final *R*-indices.

Instrumentation and Methods. Equipment for the characterization and photophysical and electrochemical studies has been described previously.[24e] Luminescence quantum yields were measured by the optically dilute method[32a] using an aerated aqueous solution of [Ru(bpy)$_3$]Cl$_2$ (Φ = 0.028) as the standard solution.[32b] Time-resolved emission spectra were recorded on an Oriel Instruments intensified charge-coupled device (ICCD) detector (Model DH520) and were analyzed using the software InstaSpec V. The excitation source is the same laser system as that used for lifetime measurement. The emission signal was collected by an optical fiber and dispersed onto the CCD detector with an Oriel MultiSpec 115 imaging spectrograph (Model 77480). A Stanford Research Systems delay generator (Model DG 535) was used to produce the transistor−transistor logic pulse required to operate the intensifier gating electronics in the detector head. The external trigger input of the delay generator was connected to the prepulse trigger output of the laser. The delay generator was controlled via an IBM AT APIB (IEEE 488) card interfaced with an IBM-compatible Pentium personal computer to control the width (5 μs) and delay (50 ns) of the TTL pulse. The system was operated at −15 °C by the single-stage system in order to reduce the dark current signal.

Self-Quenching Studies. The self-quenching rate constants (k_{sq}) and emission lifetimes at infinite dilution ($\tau_{i.d.}$) of the tricarbonylrhenium(I) indole complexes were determined using eq 1:

$$\frac{1}{\tau} = \frac{1}{\tau_{i.d.}} + k_{sq}[Re] \quad (1)$$

where τ is the emission lifetime of the complex at concentration [Re]. The slope and y-intercept of the linear fit of a plot of τ^{-1} vs [Re] gave k_{sq} and $\tau_{i.d.}{}^{-1}$, respectively.

Stern−Volmer Analysis. Stern−Volmer quenching was studied by emission lifetime measurements of the complex in CH_2Cl_2 in the presence of a quencher at a concentration [Q]. The data were treated by a Stern−Volmer fit as follows:

$$\frac{\tau_o}{\tau} = 1 + k_q\tau_o[Q] \quad (2)$$

where τ_o and τ are the excited-state lifetimes of the complex in the absence and presence of quencher, respectively, and k_q is the bimolecular quenching rate constant.

Emission Titrations with BSA. The tricarbonylrhenium(I) dipyridoquinoxaline complex (0.33 μmol) in 50 μL of 50 mM potassium phosphate buffer at pH 7.4/methanol solution (1:1, v/v) was added to a series of BSA solutions in phosphate buffer (450 μL). The concentrations of BSA varied from 1 × 10^{-4} to 1 × 10^{-3} M. The solutions were gently stirred at room temperature for 12 h in the dark. Time-resolved emission spectra of the solutions were then measured (width = 5 μs, and delay = 50 ns) in order to remove the background interference.

The binding constants K_a of the indole-containing complexes to BSA were determined using the Scatchard equation, eq 3:[33]

$$\frac{\upsilon}{[Re]_{free}} = nK_a - \upsilon K_a \quad (3)$$

where ν = [Re]$_{bound}$/[BSA]$_{total}$, [Re]$_{free}$ and [Re]$_{bound}$ are the concentrations of the free and bound forms of the complexes, respectively, n is the binding stoichiometry, and [BSA]$_{total}$ is the total concentration of BSA. [Re]$_{bound}$ was calculated using eq 4:

$$[Re]_{bound} = [Re]_{total}\frac{[1 - (I/I_o)]}{(1 - P)} \quad (4)$$

where [Re]$_{total}$ is the total concentration of complex, I and I_o are the emission intensities of the complex in the presence and absence of BSA, respectively, $P = (I/I_o)_{max}$, which can be obtained as 1/(y-intercept) from a linear fit of I/I_o vs [BSA]$^{-1}$.

TPase Inhibition Assays. The tricarbonylrhenium(I) dipyridoquinoxaline complex or free indole (0.10 μmol) dissolved in 100 μL of phosphate buffer solution/methanol (2:1 v/v) was added to a mixture of pyridoxal 5-phosphate (0.125 μmol), lactate dehydrogenase (8.225 U), NADH (0.70 μmol), and L-serine (from 100 to 800 mM) in 1.57 mL of 0.15 M potassium phosphate buffer at pH 8.0 in an absorption cuvette. The temperature of the solution was thermostated at 37 °C for 15 min. The conversion of L-serine to pyruvate was initiated by addition of TPase (12 U in 830 μL of phosphate buffer) to the mixture. The final concentration of the rhenium complex was 40 μM. The percentage of inhibition of TPase was determined by comparing the decrease of absorbance of the

(30) Otwinowski, Z.; Minor, W. *Methods in Enzymology*; Academic Press: San Diego, CA, 1997; Vol, 276, p 307.

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

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

(33) Scatchard, G.; Scheinberg, I. H.; Armstrong, S. H. *J. Am. Chem. Soc.* **1950**, *72*, 535.

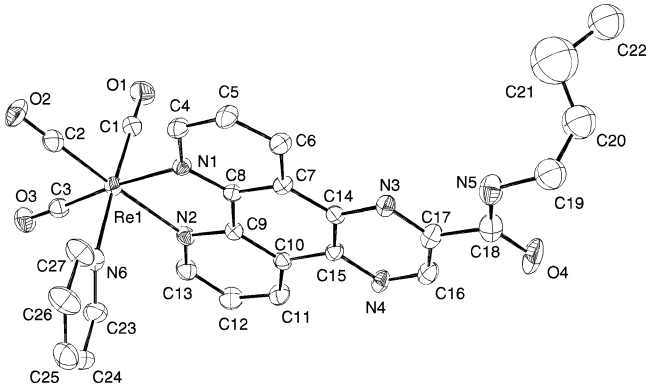


Figure 1. Perspective view of the complex cation $[Re(dpqa)(CO)_3(pyridine)]^+$ with atomic numbering. Hydrogen atoms have been omitted for clarity, and thermal ellipsoids are shown at the 20% probability level.

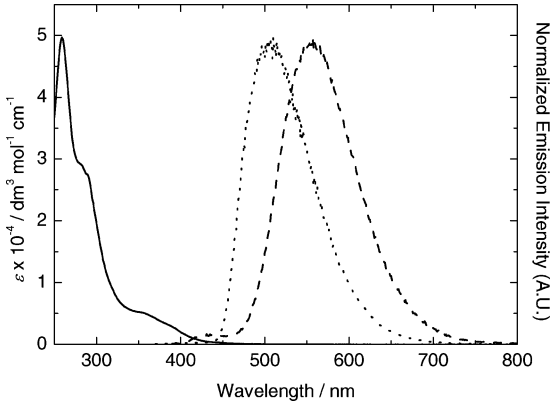


Figure 2. Electronic absorption spectrum of complex **1a** in CH_3CN at 298 K (—) and emission spectra of the same complex in CH_2Cl_2 at 298 K (- - -) and EtOH/MeOH (4:1, v/v) at 77 K (···).

Table 1. Crystal and Structure Determination Data of $[Re(dpqa)(CO)_3(pyridine)](PF_6)$

formula	$C_{29}H_{25}Cl_4F_6N_6O_4PRe$
fw	994.52
cryst size (mm^3)	0.6 × 0.15 × 0.1
T (K)	253
cryst syst	orthorhombic
space group	$P2_1/c$
a (Å)	10.632(2)
b (Å)	13.536(3)
c (Å)	25.590(5)
V ($Å^3$)	3682.8(13)
Z	4
ρ_{calcd} (g cm^{-3})	1.794
μ (mm^{-1})	3.707
$F(000)$	1940
θ range (deg)	2.19−25.59
index ranges	$-12 \le h \le 12$
	$-15 \le k \le 15$
	$-30 \le l \le 30$
no. of unique data/restraints/params	6557/11/448
R_{int}[a]	0.0555
GOF on F^2 [b]	0.882
R_1, wR_2 ($I > 2\sigma(I)$)[c]	0.0420, 0.1079
R_1, wR_2 (all data)	0.0863, 0.1177
largest diff peak/hole (e $Å^{-3}$)	1.143, −0.582

[a] $R_{int} = \sum|F_o^2 - F_o^2(mean)|/\sum[F_o^2]$. [b] GOF = $\{\sum[w(F_o^2 - F_c^2)^2]/(n - p)\}^{1/2}$, where n is the number of reflections and p is the total number of parameters refined. The weighting scheme is $w = 1/[\sigma^2(F_o^2) + (aP)^2 + (bP)]$, where P is $[2F_c^2 + \max(F_o^2,0)]/3$, $a = 0.0541$, and $b = 0.0$. [c] $R_1 = \sum||F_o| - |F_c||/\sum|F_o|$, $wR_2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]\}^{1/2}$.

solution at 340 nm to that of the control in which the rhenium(I) dipyridoquinoxaline complex or indole was absent.

Results and Discussion

Synthesis. The tricarbonylrhenium(I) dipyridoquinoxaline complexes were prepared, in moderate yields, from the reactions of $[Re(N-N)(CO)_3(CH_3CN)](CF_3SO_3)$ with the pyridine ligands py-3-$CONHC_2H_4$-indole, py-3-$CONHC_5H_{10}CONHC_2H_4$-indole, or py-3-CONH-Et in refluxing THF, followed by recrystallization from a mixture of acetone, acetonitrile, and diethyl ether. They were characterized by ^1H NMR, positive-ion ESI-MS, and IR and gave satisfactory elemental analyses.

Crystal Structure. The perspective view of the complex cation $[Re(dpqa)(CO)_3(pyridine)]^+$ is depicted in Figure 1. The crystal data and selected bond lengths and angles are listed in Tables 1 and 2, respectively. The rhenium(I) center of this complex adopted a distorted octahedral coordination geometry and the carbonyls were arranged in a *facial* orientation. The

Table 2. Selected Bond Lengths (Å) and Bond Angles (deg) for $[Re(dpqa)(CO)_3(pyridine)](PF_6)$

Re(1)−N(1)	2.193(5)	Re(1)−N(2)	2.207(6)
Re(1)−N(6)	2.195(6)	Re(1)−C(1)	1.934(10)
Re(1)−C(2)	1.939(10)	Re(1)−C(3)	1.911(8)
C(1)−Re(1)−C(2)	90.2(4)	C(1)−Re(1)−C(3)	87.9(3)
C(1)−Re(1)−N(1)	94.4(3)	C(1)−Re(1)−N(2)	92.8(3)
C(1)−Re(1)−N(6)	177.3(3)	C(2)−Re(1)−C(3)	85.5(3)
C(2)−Re(1)−N(1)	98.2(2)	C(2)−Re(1)−N(2)	172.8(2)
C(2)−Re(1)−N(6)	92.5(3)	C(3)−Re(1)−N(1)	175.6(3)
C(3)−Re(1)−N(2)	101.2(3)	C(3)−Re(1)−N(6)	92.4(3)
N(1)−Re(1)−N(2)	74.95(19)	N(1)−Re(1)−N(6)	85.2(2)
N(2)−Re(1)−N(6)	84.5(2)		

bond lengths of Re−N (2.193(5) to 2.207(6) Å) and Re−C (1.911(8) to 1.939(10) Å) and the bite angle of N(1)−Re−N(2) (74.95(19)°) of the complex are comparable to those of related tricarbonylrhenium(I) polypyridine systems.[6a,b,7,10b,11c,e,13d,14d,15d,16a,c,19a−c,21b,23b,24a,b,d,e,h,j] The amide plane of dpqa exhibited a dihedral angle of ca. 1.26° with the dipyridoquinoxaline plane, indicative of a high degree of planarity due to π-conjugation. The dihedral angle between the ideal ring plane of the dpqa ligand and the pyridine ligand of the same molecule is ca. 86.21°.

Electronic Absorption and Luminescence Properties. The electronic absorption spectral data of complexes **1a**−**1c** and **2a**−**2c** are listed in Table S1. The absorption spectrum of complex **1a** in CH_3CN at 298 K is shown in Figure 2. All the complexes showed intense absorption bands at ca. 259−298 nm (ϵ on the order of 10^4 dm^3 mol^{-1} cm^{-1}), which have been assigned to spin-allowed intraligand (^{1}IL) transitions ($\pi \rightarrow \pi^*$) (dpq/dpqa and pyridine ligands). The less intense absorption shoulders at ca. 355−393 nm have been assigned to spin-allowed metal-to-ligand charge-transfer (^{1}MLCT) ($d\pi(Re) \rightarrow \pi^*$(dpq/dpqa)) transitions.[5,6a,b,7a,c,8−10,11a,c−e,12,13a,c,d,14−16,17a,c,18b,c,19a,b,20−23,24a,b,e−j] As expected, the absorption characteristics of the indole complexes highly resembled those of their indole-free counterparts (Table S1) due to the less intense absorption of the indole and spacer arms.

Upon irradiation, all the complexes displayed orange-yellow to greenish-yellow luminescence both in fluid solutions at 298 K and in low-temperature alcohol glass. All the luminescence decay was single exponential. The emission data are summarized in Table 3. The emission spectra of complex **1a** in CH_2Cl_2 at 298 K and in ethanol/methanol glass at 77 K are shown in Figure 2. With reference to the photophysical studies of related tricarbonylrhenium(I) polypyridine systems, the emission of

Table 3. Photophysical Data of Complexes 1a−1c and 2a−2c

complex[a]	medium (*T*/K)	λ_{em}/nm	τ_o/μs	Φ
1a	CH_2Cl_2 (298)	556	0.76	0.021
	MeOH (298)	569	0.30	0.0041
	buffer[b] (298)	567	0.14	0.0023
	glass[c] (77)	507	6.42	
1b	CH_2Cl_2 (298)	550	0.80	0.018
	MeOH (298)	565	0.31	0.0046
	buffer[b] (298)	569	0.15	0.0025
	glass[c] (77)	506	6.61	
1c	CH_2Cl_2 (298)	547	1.25	0.417
	MeOH (298)	565	0.41	0.095
	buffer[b] (298)	568	0.21	0.048
	glass[c] (77)	502	6.82	
2a	CH_2Cl_2 (298)	558	0.43	0.015
	MeOH (298)	569	0.26	0.0069
	buffer[b] (298)	567	0.23	0.0019
	glass[c] (77)	508	5.92	
2b	CH_2Cl_2 (298)	556	0.51	0.015
	MeOH (298)	568	0.25	0.0036
	buffer[b] (298)	568	0.23	0.0012
	glass[c] (77)	505	5.83	
2c	CH_2Cl_2 (298)	552	0.99	0.315
	MeOH (298)	569	0.28	0.046
	buffer[b] (298)	569	0.22	0.0035
	glass[c] (77)	506	5.65	

[a] [Re] = 50 μM. [b] Potassium phosphate buffer at pH 7.4 containing 20% MeOH. [c] EtOH/MeOH (4:1, v/v).

these rhenium(I) dipyridoquinoxaline complexes has been assigned to a ^{3}MLCT (dπ(Re) → π*(dpq/dpqa)) excited state.[5,6a,b,7a,c,8−13,14a,b,d,15,16a,c,17,18,19a,b,d,20,21a,c,22−24] The slightly lower emission energy of the dpqa complexes compared to that of the dpq analogues (Table 3) is due to the electron-withdrawing amide group of the dpqa ligand, which stabilizes the π* orbitals of the dipyridoquinoxaline ligand and thus lowers the ^{3}MLCT emission energy. The emission of all the complexes in alcohol glass at 77 K occurred at higher energy owing to the rigidochromic effect (Table 3 and Figure 2), which is commonly observed in luminescent rhenium(I) polypyridine complexes.[5,7a,8a,9b,10d,11a,c−e,12a−c,14a,15b,17,24d−f,h−j] Similar to other luminescent rhenium(I) polypyridine complexes, all the current dipyridoquinoxaline complexes showed decreasing emission quantum yields upon increasing the polarity of the solvents. However, the reduction in the emission quantum yields of the dpqa complexes **2a**−**2c** upon changing the solvent from CH_2Cl_2 to aqueous buffer was larger than that of the dpq complexes **1a**−**1c** (Table 3). This significant change has been ascribed to hydrogen-bonding interactions of the amide group of the dpqa ligand with water molecules.[24i,25c,26h,28a]

The emission quantum yields and lifetimes of the tricarbonylrhenium(I) indole complexes **1a**,**b** and **2a**,**b** were considerably lower and shorter than those of their indole-free counterparts **1c** and **2c** (Table 3). Also, contrary to the indole-free complexes, the emission lifetimes of the indole complexes exhibited concentration-dependence, indicative of a self-quenching process in which the ^{3}MLCT emission of the rhenium(I)-dipyridoquinoxaline moieties was quenched by the indole unit. The plot of τ^{-1} vs [Re] for complex **1a** is shown in Figure S1 as an example. The corrected self-quenching rate constants (k_{sq}') and emission lifetimes at infinite dilution ($\tau_{i.d.}$) of the indole-containing complexes in CH_2Cl_2 have been determined using eq 1, and the data are listed in Table S2. The lifetimes at infinite dilution (ca. 1 μs for complexes **1a**,**b** and 0.5 μs for complexes **2a**,**b**) are shorter than those of complexes **1c** and **2c** (Table 3), suggestive of some intramolecular quenching (intramolecular quenching rate constants estimated to be ca. 10^5 s^{-1}). Due to the lack of spectral overlap between the emission spectra of the indole-free complexes (**1c** and **2c**) and the absorption spectrum of indole, and the much higher triplet-state energy of indole (ca. 24 800 cm^{-1})[34] compared to the emission energy of these rhenium(I) indole complexes (ca. 17 921−18 281 cm^{-1} in CH_2Cl_2), it is unlikely that the quenching occurred via an energy-transfer mechanism. From the potentials of the dipyridoquinoxaline-based reduction of the indole-free complexes **1c** and **2c** (ca. −1.05 and −0.98 V vs SCE, respectively, see below) and the emission energies of these complexes in 77 K glass (E^{00} = 2.48 and 2.46 eV, respectively), the reduction potentials of the excited rhenium(I) complexes, $E^o[Re^{+}*^{/0}]$, have been estimated to be ca. +1.43 and +1.48 V vs SCE, respectively. On the basis of these potentials and the redox potential of indole ($E^o[indole^{+/0}]$ < +1.06 V vs SCE),[24d,h,25b,27c] reductive quenching of the excited complexes **1c** and **2c** by indole is favored by > 0.37 and 0.42 eV, respectively. Thus, it is likely that the emission quenching of the indole-containing complexes occurred via an electron-transfer mechanism (Re^{+}* + indole → Re^0 + $indole^{+\bullet}$). Interestingly, Gray and co-workers have demonstrated photoinduced electron transfer between tricarbonylrhenium(I) diimine wires and aromatic amino acid residues such as tryptophan in metalloproteins.[6a,c] We have performed Stern−Volmer analysis to study the emission quenching of the indole-free complexes **1c** and **2c** by unmodified indole. The Stern−Volmer plot for the emission quenching of complex **1c** by indole is shown in Figure S2. The corrected bimolecular quenching rate constants for complexes **1c** and **2c** have been determined to be 1.3 × 10^{10} and 1.9 × 10^{10} dm^3 mol^{-1} s^{-1}, respectively. These constants are comparable to the self-quenching rate constants of the indole complexes ((3.3−6.8) × 10^9 dm^3 mol^{-1} s^{-1}, Table S2), indicating that intermolecular electron transfer plays an important role in the self-quenching of the indole complexes.

The emission quantum yields of these dpq− and dpqa−indole complexes in aqueous buffer are not very different from each other compared to those of related dpq− and dpqa−biotin complexes we reported recently.[24g,i] For example, the emission quantum yields of the dpq−biotin complexes are about 1 order of magnitude higher than those of the dpqa−biotin complexes.[24g,i] The emission quantum yields of complexes **1a**,**b** and **2a**,**b** were, however, of the same order of magnitude. A possible reason is that the emission of the current dpq− and dpqa−indole complexes was substantially quenched by the indole moiety. On the basis of their high solvent-sensitivity, these rhenium(I) dipyridoquinoxaline indole complexes are promising luminescent probes for biological receptors since the substrate-binding pockets of many biological hosts are hydrophobic in nature.

Electrochemical Properties. The electrochemical properties of the tricarbonylrhenium(I) dipyridoquinoxaline complexes have been studied by cyclic voltammetry, and the electrochemical data are listed in Table 4. All the complexes displayed a quasi-reversible/irreversible rhenium(II/I) oxidation wave at ca. +1.8 V vs SCE.[6c,7c,11a,c−e,12a,13a,d,14,15c,16,17,18b,c,19a,20b,c,24d−f,h−j] The indole complexes exhibited an additional irreversible wave/quasi-reversible couple at ca. +1.1 V, which has been assigned to the oxidation of the indole unit.[24d,h,25b,27c] This assignment has been supported by the fact that similar waves at comparable potentials were observed for the uncoordinated ligands py-3-$CONHC_2H_4$-indole and py-3-$CONHC_5H_{10}CONHC_2H_4$-indole (ca. +1.1 and +1.3 V, respectively).[24d,h] The first

(34) (a) Klein, R.; Tatischeff, I.; Bazin, M.; Santua, R. *J. Phys. Chem.* **1981**, *85*, 670. (b) Kasama, K.; Takematsu, A.; Aral, S. *J. Phys. Chem.* **1982**, *86*, 2420.

Table 4. Electrochemical Data of Complexes 1a−1c and 2a−2c[a]

complex	oxidation, $E_{1/2}$ or E_a/V	reduction, $E_{1/2}$ or E_c/V
1a	+1.10,[b] +1.84[b]	−1.10,[b] −1.28,[c] −1.59,[c] −1.81[b]
1b	+1.06,[b] +1.83[b]	−1.08,[c] −1.29,[c] −1.59,[c] −1.81[b]
1c	+1.88[b]	−1.05,[c] −1.29,[c] −1.59,[c] −1.85[b]
2a	+1.06,[c] +1.87[b]	−0.97, −1.60,[c] −1.79,[b] −2.03[b]
2b	+1.09,[c] +1.81[b]	−0.97, −1.62,[c] −1.82,[b] −2.05[b]
2c	+1.85[c]	−0.98,[c] −1.59,[c] −1.78,[b] −2.03[b]

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

Table 5. Results of Titrations of Complexes 1a,b and 2a,b with BSA in 50 mM Potassium Phosphate Buffer at pH 7.4/MeOH (95:5, v/v) at 298 K

complex	I/I_o[a]	τ/τ_o[b]	K_a	n
1a	8.3	1.1	1.6×10^4	0.6
1b	8.0	1.1	3.5×10^4	0.5
2a	12.4	1.5	1.1×10^4	0.8
2b	13.4	1.3	3.3×10^4	0.6

[a] I_o and I are the emission intensities of the rhenium(I) indole complexes (0.22 mM) in the presence of 0 and 1 mM BSA, respectively. [b] τ_o and τ are the emission lifetimes of the rhenium(I) indole complexes in the presence of 0 and 1 mM BSA, respectively.

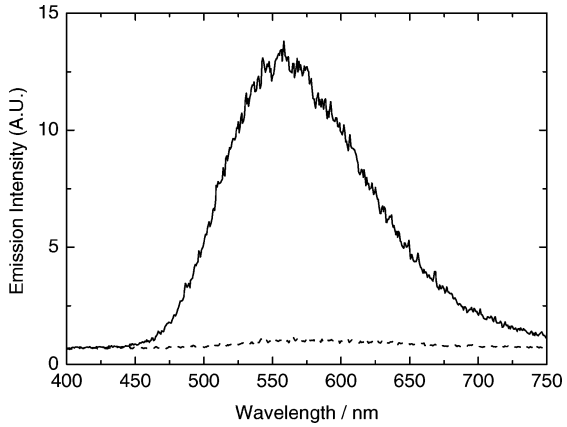


Figure 3. Time-resolved emission spectra of complex **2b** (0.22 mM) in the absence (- - -) and presence (—) of BSA (1 mM) in 50 mM potassium phosphate buffer at pH 7.4/MeOH (95:5, v/v) at 298 K.

reduction waves/couples for all the complexes have been attributed to the reduction of the dipyridoquinoxaline ligands.[7c,10b,11a,c−e,12a,13a,d,14,15c,16−18,19a,20b,c,23,24d−f,h−j] The first reduction of the dpqa complexes (ca. −1.0 V) appeared at a slightly less negative potential than that of their dpq counterparts (ca. −1.1 V), which can be accounted for by the electron-withdrawing amide substituent on the dpqa ligand. Additionally, all the complexes showed quasi-reversible/irreversible waves at more negative potentials. These features have been tentatively assigned to the reduction of the dipyridoquinoxaline ligands, except for those at ca. −1.6 V, which could be associated with the rhenium(I/0) couple.[16a,18c]

Emission Titrations with BSA. The possible binding of the current tricarbonylrhenium(I) dipyridoquinoxaline complexes to BSA, a common indole-binding protein, has been studied by emission titration experiments. Whereas the indole complexes did not exhibit any change in the visible region of their absorption spectra, their emission intensities were enhanced ca. 8.0 to 13.4 times in the presence of BSA (Table 5). The emission lifetimes were concomitantly extended ca. 1.1 to 1.5 times and

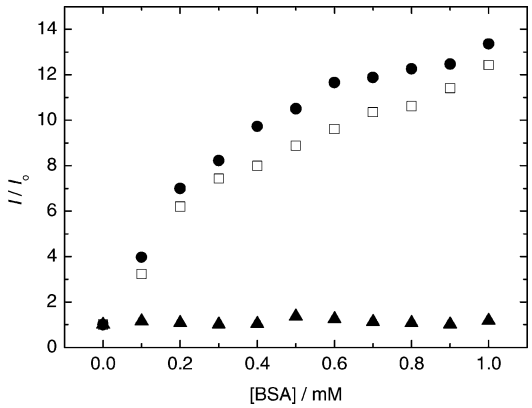


Figure 4. Emission titration curves for complexes **2a** (□), **2b** (●), and **2c** (▲) (0.22 mM) with BSA. I and I_o are the emission intensities of the solutions in the presence and absence of BSA, respectively.

the decay remained single-exponential.[35] The time-resolved emission spectral traces of complex **2b** upon addition of BSA are shown in Figure 3. The emission titration curves for complexes **2a**−**2c** with BSA are illustrated in Figure 4. The changes of photophysical properties have been attributed to the specific binding of the indole moiety of the complexes to the protein because the indole-free complexes **1c** and **2c** did not give similar observations. The increase of emission intensities and lifetimes of the indole complexes should be closely related to the higher hydrophobicity and rigidity of their local surroundings upon binding to the protein. Another possible explanation is that intermolecular electron transfer between the rhenium(I) indole complexes was inhibited upon the protein-binding event. Importantly, the emission enhancement factors of these complexes are higher than those of related rhenium(I) indole complexes we reported previously.[24d,h] The main reason is that the emission of these free tricarbonylrhenium(I) dipyridoquinoxaline indole complexes in aqueous buffer was very weak. In particular, the dpqa−indole complexes showed higher BSA-induced enhancement factors compared to the dpq complexes **1a**,**b** because of the hydrogen bonding between the amide substituent and water molecules. The binding constants (K_a) and binding stoichiometries (n) determined from Scatchard analyses are listed in Table 5. These values are of the same order of magnitude as tryptamine ($K_a = 1.1 \times 10^4$ M^{-1}, $n = 1$),[36] indole-3-acetic acid ($K_a = 1.7 \times 10^4$ M^{-1}, $n = 1$),[36] 5-hydroxyindole-3-acetic acid ($K_a = 2.0 \times 10^4$ M^{-1}, $n = 1$),[36] a platinum(II) poly(ethylene glycol) complex ($K_a = 2.7 \times 10^4$ M^{-1}, $n = 1$),[37] and other rhenium(I) indole complexes ($K_a = 1 \times 10^4$ M^{-1}, $n = 0.5$ to 0.8).[24d,h] The binding constants of complexes **1a** and **2a** are smaller than those of complexes **1b** and **2b**, indicating that a longer spacer arm can alleviate the steric hindrance between the tricarbonylrhenium(I) dipyridoquinoxaline units and the protein.

(35) The unparallel increase of emission intensities and lifetimes of the complexes upon binding to BSA was a result of a change of the radiative decay rate constant, k_r. Actually, the reduction of emission quantum yields and lifetimes of the complexes from CH_2Cl_2 to buffer solutions was different (Table 3). These results indicate that the k_r values of the complexes were different in various solvents (for example, the k_r value of complex **1c** in buffer was 70% of that in CH_2Cl_2, while the k_r value of complex **2c** in buffer was only 5% of that in CH_2Cl_2). We do not fully understand the reasons for this change in k_r in different solvents or upon the protein-binding event. However, it is possible that the interaction of the quinoxaline nitrogens of the diimine ligands with protons in buffer leads to static emission quenching of the complexes, giving rise to a greater extent of decrease of emission intensities compared to emission lifetimes.

(36) Okabe, N.; Adachi, K. *Chem. Pharm. Bull.* **1992**, *40*, 499.
(37) Che, C.-M.; Zhang, J.-L.; Lin, L.-R. *Chem. Commun.* **2002**, 2556.

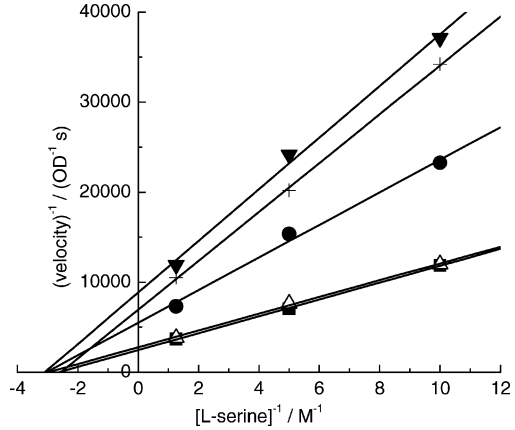


Figure 5. Plots of υ^{-1} vs [L-serine]$^{-1}$ for the TPase inhibition assays, in which complexes **1a** (+), **1b** (▼), and **1c** (■), indole (●), and no inhibitor (△) were used.

Table 6. Percentage Inhibition of TPase Activity by Complexes 1a−1c and 2a−2c, and Indole at [L-serine] = 800 mM and Michaelis Constants for the TPase Assays in 0.15 mM Potassium Phosphate Buffer at pH 8.0 at 310 K

compound	inhibition/%[a]	K_m/mM[a]
1a	64 (1.5)	389 (26.8)
1b	68 (2.4)	323 (37.7)
1c	0 (3.0)	381 (39.0)
2a	64 (2.1)	388 (29.1)
2b	65 (0.7)	298 (28.9)
2c	44 (0.6)	307 (23.9)
indole	48 (2.1)	328 (34.6)

[a] Standard deviations are shown in parentheses ($n = 3$).

TPase Inhibition Assays. TPase catalyzes a variety of α,β-elimination and β-replacement reactions of amino acid substrates in the presence of the cofactor pyridoxal 5-phosphate.[38] It is known that indole can inhibit the activity of this enzyme.[38] To study the possible binding of the current tricarbonylrhenium(I) indole complexes to this enzyme, inhibition assays based on the conversion of L-serine to pyruvate have been studied.[38] The formation of pyruvate was coupled to the lactate dehydrogenase/NADH system and hence could be reflected by a decrease of the absorbance at 340 nm due to the consumption of NADH. At [L-serine] = 800 mM, unmodified indole inhibited the enzyme activity by ca. 48% under the experimental conditions employed. The rhenium(I) indole complexes caused a decrease of the enzyme activity by ca. 64−68% (Table 6). It is conceivable that the inhibition resulted from binding of the indole moieties of the complexes to the enzyme. Whereas the control complex **1c** did not show any inhibition, complex **2c** caused 44% inhibition of the enzyme activity. We cannot exclude the possibility that the amide substituent of the dpqa ligand also interacts with TPase. The Michaelis constants (K_m) for the enzyme activity have been determined from plots of the linear transform of the Michaelis−Menten equation.[39] The plots of υ^{-1} vs [L-serine]$^{-1}$ for the TPase inhibition assays for complexes **1a**−**1c** are shown in Figure 5. The K_m values of the complexes ranged from 298 to 389 mM (Table 6). The similar values reveal that the inhibition of the TPase-catalyzed conversion of L-serine by the indole complexes occurred in a noncompetitive fashion. For this reason, the length of the spacer arms of the complexes plays only a minor role in the binding of substrate to the enzyme.

Summary

A series of luminescent tricarbonylrhenium(I) dipyridoquinoxaline indole complexes have been synthesized and characterized, and their photophysical and electrochemical properties have been investigated. The binding of the indole complexes to BSA and TPase has been studied by emission titrations and enzyme inhibition assays, respectively. Importantly, the emission intensities of the indole complexes were enhanced upon binding to BSA. The use of the dpqa ligand gave higher BSA-induced emission enhancement factors compared to the dpq complexes due to the extremely weak emission of the free complex as a result of the hydrogen-bonding interactions between the amide substituent and water molecules. The changes in the photophysical properties are reasonably large for the purpose of homogeneous binding assays. We anticipate that sensitive biological probes can be developed using related organometallic luminophore−quencher conjugates.

Acknowledgment. We thank the Hong Kong Research Grants Council (Project No. CityU 101704) and City University of Hong Kong (Project No. 7001985) for financial support. K.-S.S. and K.H.-K.T. acknowledge the receipt of a Postgraduate Studentship administered by the City University of Hong Kong. We thank Dr. Julie-Anne Stevenson for reading this manuscript.

Supporting Information Available: X-ray data (CIF) of [Re(dpqa)(CO)$_3$(pyridine)](PF$_6$), electronic absorption spectral data of complexes **1a**−**1c** and **2a**−**2c** at 298 K, corrected self-quenching rate constants and emission lifetimes at infinite dilution of complexes **1a**,**b** and **2a**,**b** in CH_2Cl_2 at 298 K, a plot of τ^{-1} vs [Re] for complex **1a** in CH_2Cl_2 at 298 K, and a Stern−Volmer plot for the emission quenching of complex **1c** by indole in CH_2Cl_2 at 298 K. This material is available free of charge via the Internet at http://pubs.acs.org.

OM0700617

(38) Morino, Y.; Snell, E. E. *J. Biol. Chem.* **1967**, *242*, 2793.

(39) Atkins, G. L.; Nimmo, I. A. *Biochem. J.* **1973**, *135*, 779.