Inorg. Chem. **2007**, *46*, 11075–11081

Selective Phosphorescence Chemosensor for Homocysteine Based on an Iridium(III) Complex

Huili Chen,[†,‡] Qiang Zhao,[†] Yanbo Wu,[‡] Fuyou Li,*[,†] Hong Yang,[†] Tao Yi,[†] and Chunhui Huang*[,†]

Department of Chemistry and Laboratory of Advanced Materials, Fudan University, Shanghai 200433, P. R. China, and Institute of Molecular Science, Key Laboratory of Chemical Biology and Molecular Engineering of Education Ministry, Shanxi University, Taiyuan 030006, P. R. China

Received June 3, 2007

A new homocysteine-selective sensor based on the iridium(III) complex $Ir(pba)_2(acac)$ (Hpba = 4-(2-pyridyl)-benzaldehyde; acac = acetylacetone) was synthesized, and its' photophysical properties were studied. Upon the addition of homocysteine (Hcy) to a semi-aqueous solution of $Ir(pba)_2(acac)$, a color change from orange to yellow and a luminescent variation from deep red to green were evident to the naked eye. The blue-shift of the absorption spectrum and enhancement of the phosphorescence emission upon the addition of Hcy can be attributed to the formation of a thiazinane group by selective reaction of the aldehyde group of $Ir(pba)_2(acac)$ with Hcy, which was confirmed by ^1H NMR studies. Importantly, $Ir(pba)_2(acac)$ shows uniquely luminescent recognition of Hcy over other amino acids (including cysteine) and thiol-related peptides (reduced glutathione), in agreement with the higher luminescent quantum yield of the adduct of $Ir(pba)_2(acac)$ with Hcy (0.038) compared with that of the adduct with Cys (~0.002). Both surface charge analysis and the electrochemical measurement indicated that a photoinduced electron-transfer process for $Ir(pba)_2(acac)$–Cys might be responsible for the high specificity of $Ir(pba)_2(acac)$ toward Hcy over Cys.

Introduction

As an important amino acid containing a free thiol moiety, homocysteine (Hcy) has several important roles within physiological matrices.[1] In fact, it was recently discovered that there is a link between folate homeostasis and homocysteine metabolism which is very important in various types of vascular disease[2,3] and in renal diseases.[3,4] At elevated levels in plasma, Hcy is a risk factor for Alzheimer's[5] and cardiovascular diseases.[6] Methods of direct detection of Hcy are usually hampered by interference from structurally related molecules such as cysteine (Cys) and glutathione (GSH), and Hcy analyses are thus performed in conjunction with chromatographic separations or immunoassays.[1c] Recently, some chromophores were developed as chemodosimeters for Hcy and Cys.[7–9] For example, Strongin's group has reported on a xanthene dye containing aldehyde that afforded thiazolidines or thiazinanes by reaction with Cys or Hcy,

* To whom correspondence should be addressed. E-mail: fyli@fudan.edu.cn (F.Y.L.), chhuang@pku.edu.cn (C.H.H). Fax: 86-21-55664621. Tel: 86-21-55664185.

† Fudan University.

‡ Shanxi University.

(1) For recent review see: (a) Wood, Z. A.; Schröder, E.; Harris, J. R.; Poole, L. B. *Trends Biochem. Sci.* **2003**, *28*, 32. (b) *Homocysteine in Health and Disease*; Carmel, R.; Jacobsen, D. W., Eds.; Cambridge University Press: Cambridge, U.K., 2001. (c) Nekrassova, O.; Lawrence, N. S.; Compton, R. G. *Talanta* **2003**, *60*, 1085.

(2) Fowler, B. *Kidney Int.* **2001**, *59*, S221.

(3) Brown, B. A.; Marx, J. L.; Ward, T. P.; Hollifield, R. D.; Dick, J. S.; Brozetti, J. J.; Howard, R. S.; Thach, A. B. *Ophthalmology* **2002**, *109*, 287.

(4) Guldener, C. V.; Stam, F.; Stehouwer, C. D. A. *Kidney Int.* **2001**, *59*, S234.

(5) Seshadri, S.; Beiser, A.; Selhub, J.; Jacques, P. F.; Rosenberg, I. H.; D'Agostino, R. B.; Wilson, P. W. F. Wolf, P. A. *N. Engl. J. Med.* **2002**, *346*, 476.

(6) Review: Refsum, H.; Ueland, P. M.; Nygård, O.; Vollset, S. E. *Annu. Rev. Med.* **1989**, *49*, 31.

(7) (a) Rusin, O.; Luce, N. N. S.; Agbaria, R. A.; Escobedo, J. O.; Jiang, S.; Warner, I. M.; Dawan, F. B.; Lian, K.; Strongin, R. M. *J. Am. Chem. Soc.* **2004**, *126*, 438. (b) Wang, W. H.; Rusin, O.; Xu, X. Y.; Kim, K. K.; Escobedo, J. O.; Fakayode, S. O.; Fletcher, K. A.; Lowry, M.; Schowalter, C. M.; Lawrence, C. M.; Fronczek, F. R.; Warner, I. M.; Strongin, R. M. *J. Am. Chem. Soc.* **2005**, *127*, 15949. (c) Wang, W. H.; Escobedo, J. O.; Lawrence, C. M.; Strongin, R. M. *J. Am. Chem. Soc.* **2004**, *126*, 3400.

(8) (a) Zhang, D. Q; Zhang, M.; Liu, Z. Q.; Yu, M. X.; Li, F. Y.; Yi, T.; Huang, C. H. *Tetrahedron Lett.* **2006**, *47*, 7093. (b) Zhang, M.; Li, M. Y.; Zhao, Q.; Li, F. Y.; Zhang, D. Q.; Zhang, J. P.; Yi, T.; Huang, C. H. *Tetrahedron Lett.* **2007**, *48*, 2329. (c) Zhang, M.; Yu, M. X.; Li, F. Y.; Zhu, M. W.; Li, M. Y.; Gao, Y. H; Li, L.; Liu, Z. Q.; Zhang, J.; Zhang, D. Q.; Yi, T.; Huang, C. H. *J. Am. Chem. Soc.* **2007**, *129*, 10322.

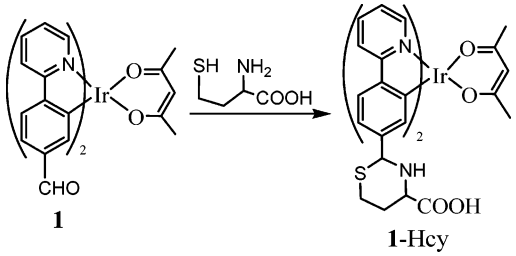
(9) Tanaka, F.; Mase, N.; Barbas, C. F., III. *Chem. Commun.* **2004**, *15*, 1762.

10.1021/ic7010887 CCC: $37.00 © 2007 American Chemical Society
Published on Web 11/29/2007

resulting in a decrease in fluorescence emission.[7a,b] Our group has developed a highly electron-deficient system as a turn-on fluorescence sensor for the intracellular imaging of Cys/Hcy in biological samples.[8c] However, to the best of our knowledge, no selective fluorescent recognition of Hcy over Cys and GSH has been explored to date, although Strongin's group[7c] developed a colorimetric method for the selective detection of Hcy at neutral pH.

The use of phosphorescent heavy-metal complexes as chemosensors[10−14] and biological labels[15] has recently attracted considerable interest, because of advantageous photophysical properties of heavy-metal complexes such as relatively long lifetimes and high stability compared with those of pure organic luminophores. As one of the best families of phosphorescent dyes, iridium(III) complexes used as phosphorescence chemosensors are still limited to the sensing of oxygen,[10] protons,[11] chloride ions,[12] and metal ions.[13,14] Recently, some cyclometalated iridium(III) complex containing an aldehyde group were reported as functional materials.[15−17] For example, Lo et al. reported that iridium(III) complexes with an aldehyde group could react with the amino group of amino acids under the reduction of sodium cyanoborohydride.[15] In the present study, based on the special reaction of the aldehyde group with β- and γ-aminoalkylthiol groups, a luminescent cyclometalated iridium(III) complex $Ir(pba)_2(acac)$ (acac = acetylacetone) (**1**) with an aldehyde group (Scheme 1) was designed and synthesized as a phosphorescent probe for Hcy and Cys. As expected, **1** could react with Hcy and Cys over other amino acids, as confirmed by absorption spectroscopy and ^{1}HNMR spectra. More interestingly, studies of the luminescent properties of **1** in DMSO-HEPES (pH 7.2, 9:1 v/v) (DMSO = dimethylsulfoxide) showed that only the addition of Hcy induced a large enhancement of the phosphorescent emission intensity at 525 nm, corresponding to an obvious variation in emission color from red to green. The high specificity toward Hcy over Cys and thiol-related peptides indicates that **1** is an ideal candidate for the selective optical sensing of Hcy.

Scheme 1. Chemical Structure of **1** and Possible Recognition Mechanism of **1** with Hcy



1 **1**-Hcy

Experimental Section

Materials. Acetylacetone (acac), 2-ethoxyethanol, 4-(2-pyridyl)-benzaldehyde (Hpba), and homocysteine (Hcy) were purchased from Acros. Other amino acids were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai). $IrCl_3 \cdot 3H_2O$ was an industrial product and used without further purification. Here, **1**-Hcy and **1**-Cys represent the adducts of **1** with homocysteine and cysteine, respectively.

Synthesis of Ir(pba)$_2$(acac) (1). Complex **1** was synthesized according to the previous literature.[18] A mixture of 2-ethoxyethanol and water (3:1 v/v) was added to a flask containing $IrCl_3 \cdot 3H_2O$ (1 mmol) and Hpba (2.5 mmol). The mixture was refluxed for 24 h. After cooling, a yellow solid precipitate was filtered to give crude cyclometalated iridium(III) chloro-bridged dimer. To the mixture of crude chloro-bridged dimer (0.2 mmol) and Na_2CO_3 (1.4 mmol), 2-ethoxyethanol and acac (0.5 mmol) were added and then the slurry was refluxed for 12 h. After cooling to room temperature, a red precipitate was collected by filtration and was chromatographed using CH_2Cl_2/Aceton (45:1 v/v) as an eluent to give **1** (red solid, yield 60%). ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 9.61 (s, 2H), 8.50 (d, J = 5.2 Hz, 2H), 8.35 (d, J = 8.0 Hz, 2H), 8.11−8.07 (m, 2H), 7.96 (d, J = 8.0 Hz, 2H), 7.58−7.54 (m, 2H), 7.33 (dd, J = 7.2 and 1.6 Hz, 2H), 6.57 (d, J = 1.6 Hz, 2H), 5.31 (s, 1H), 1.74 (s, 6H). IR (KBr, cm^{-1}): 1682 ($\nu_{HC=O}$), 2705 ($\nu_{HC=O}$). Anal. Calcd for $IrC_{29}H_{23}N_2O_4$: C, 53.12; H, 3.54; N, 4.27. Found: C, 53.39; H, 3.33; N, 4.10. Electrospray ionization MS (ESI-MS): m/z 656 (M^+).

Synthesis of 1-Hcy and 1-Cys. The mixture of **1** (1mmol) and Hcy (or Cys) (5 mmol) was stirred in CH_2Cl_2 and methanol (2:1 v/v) for 12 h. The yellow mixture was chromatographed on silica gel using CH_3OH/Et_3N (50:1 v/v) as an eluent to give **1**-Hcy or **1**-Cys.

1-Hcy ($IrC_{37}H_{37}N_4O_6S_2$): yield, 16%. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 8.41 (2H), 8.12 (2H), 7.97 (2H), 7.70 (2H), 7.40 (2H), 6.96 (2H), 6.10 (2H), 5.25 (1H), 4.83 (2H), 3.42 (2H), 3.07 (2H), 2.76 (2H), 1.99 (4H), 1.71 (6H). ESI-MS: m/z 871 ($M - H_3O^+$). IR (KBr, cm^{-1}): 1600 and 1404 (ν_{COOH}), 2500−3300 (br, ν_{COOH}).

1-Cys ($IrC_{35}H_{33}N_4O_6S_2$): yield, 12%. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 8.40 (2H), 8.12 (2H), 7.91 (2H), 7.68 (2H),

(10) (a) Gao, R.; Ho, D. G.; Hernandez, B.; Selke, M.; Murphy, D.; Djurovich, P. L. *J. Am. Chem. Soc.* **2002**, *124*, 14828. (b) Brinas, R. P.; Troxler, T.; Hochstrasser, R. M.; Vinogradov, S. A. *J. Am. Chem. Soc.* **2005**, *127*, 11851.
(11) Licini, M.; Williams, J. A. G. *Chem. Commun.* **1999**, 1943.
(12) Goodall, W.; Williams, J. A. G. *J. Chem. Soc., Dalton Trans.* **2000**, 2893.
(13) Ho, M. L.; Hwang, F. M.; Chen, P. N.; Hu, Y. H.; Cheng, Y. M.; Chen, K. S.; Lee, G. H.; Chi, Y.; Chou, P. T. *Org. Biomol. Chem.* **2006**, *4*, 98.
(14) (a) Zhao, Q.; Cao, T. Y.; Li, F. Y.; Li, X. H.; Jing, H.; Yi, T.; Huang, C. H. *Organometallics* **2007**, *26*, 2077. (b) Zhao, Q.; Liu, S. J.; Shi, M.; Li, F. Y.; Jing, H.; Yi, T.; Huang, C. H. *Organometallics*, **2007**, *26*, 5922.
(15) (a) Lo, K. K. W; Chung, C. K.; Zhu, N. *Chem.—Eur. J.* **2003**, *9*, 475. (b) Lo, K. K. W; Chung, C. K.; Lee, T. K. Ming; Lui, L. H.; Tsang, K. H. K; Zhu, N. *Inorg. Chem.* **2003**, *42*, 6886. (c) Lo, K. K. W.; Lau, J. S. Y. *Inorg. Chem.* **2007**, *46*, 700. (d) Lo, K. K.-W.; Li, C.-K.; Lau, J. S.-Y. *Organometallics* **2005**, *24*, 4594. (e) Lo, K. K.-W.; Zhang, K. Y.; Chung, C.-K.; Kwok, K. Y. *Chem.—Eur. J.* **2007**, *13*, 7110. (f) Lo, K. K. W; Hui, W. K.; Chung, C. K.; Tsang, K. H. K.; Lee, T. K. M.; Li, C. K.; Lau, J. S. Y.; Ng, D. C. M. *Coord. Chem. Rev.* **2006**, *250*, 1724. (g) Lo, K. K. W; Chan, J. S. W.; Lui, L. H.; Chung, C. K. *Organometallics* **2004**, *23*, 3108.
(16) (a) Holder, E.; Langeveld, B. M. W.; Schubert, U. S. *Adv. Mater.* **2005**, *17*, 1109. (b) Holder, E.; Marin, V.; Meier, M. A. R.; Schubert, U. S. *Macromol. Rapid. Commun.* **2004**, *25*, 1491.
(17) (a) Hwang, F. M.; Chen, H. Y.; Chen, P. S.; Liu, C. S.; Chi, Y.; Shu, C. F.; Wu, F. I.; Chou, P. T.; Peng, S. M.; Lee, G. H. *Inorg. Chem.* **2005**, *44*, 1344. (b) Li, J.; Djurovich, P. I.; Alleyne, B. D.; Yousufuddin, M.; Ho, N. N.; Thomas, J. C.; Peters, J. C.; Bau, R.; Thompson, M. E. *Inorg. Chem.* **2005**, *44*, 1713.
(18) (a) Lamansky, S.; Djurovich, P.; Murphy, D.; Abdel-Razzaq, F.; Lee, H. E.; Adachi, C.; Burrows, P. E.; Forrest, S. R.; Thompson, M. E. *J. Am. Chem. Soc.* **2001**, *123*, 4304. (b) Zhao, Q.; Liu, S. J.; Shi, M.; Wang, C. M.; Yu, M. X.; Li, L.; Li, F. Y.; Yi, T.; Huang, C. H. *Inorg. Chem.* **2006**, *45*, 6152. (c) Zhao, Q.; Jiang, C.Y.; Shi, M.; Li, F.Y.; Yi, T.; Cao, Y.; Huang, C. H. *Organometallics* **2006**, *25*, 3631.

7.37 (2H), 6.88 (2H), 6.03 (2H), 5.22 (1H), 5.04 (2H), 4.01 (2H), 3.70 (2H), 2.84 (2H), 1.70 (6H). ESI-MS: *m/z* 843 ($M - H_3O^+$). IR (KBr, cm^{-1}): 1600 and 1395 (ν_{COOH}), 2500−3300 (br, ν_{COOH}).

Amino Acid Titration of 1. Spectrophotometric determination was performed in DMSO−HEPES buffer (50 mM, pH 7.2, 9:1 v/v). Typically, the samples containing different concentration of amino acids were kept at 37 °C overnight before the UV−vis absorption and photoluminescence spectra of the samples were recorded. For luminescence measurements, excitation was provided at 360 nm, and emission was collected from 450 to 700 nm.

General Experimental Details. ^{1}H NMR spectra were recorded on a Varian spectrometer at 400 MHz. ESI-MS was measured on a Micromass LCTTM system. The UV−vis spectra were recorded on a Shimadzu UV-2550 spectrometer. Steady-state emission experiments at room temperature were measured on an Edinburgh Instruments LFS-920 spectrometer. Lifetime studies were performed with an Edinburgh FL 920 photocounting system with a hydrogen-filled lamp as the excitation source. The data were analyzed by iterative convolution of the luminescence decay profile with the instrument response function using a software package provided by Edinburgh Instruments. Luminescence quantum yields of **1**-Hcy and Ir(ppy)$_2$(acac) in air-equilibrated solution were measured with reference to rhodamine B ($\Phi_F = 0.69$ in ethanol),[19] and luminescence quantum yields of **1** and **1**-Cys were measured with reference to **1**-Hcy.

Energy Optimization. Geometric and energy optimizations were performed with the *Gaussian 03* program[20] based on the density functional theory (DFT) method. Becke's three-parameter hybrid functional with the Lee−Yang−Parr correlation functional (B3LYP)[21] was employed for all the calculations. The LANL2DZ basis set was used to treat the iridium atom, whereas the 3-21G* basis set was used to treat all other atoms. Once an optimized geometry was obtained, imaginary frequencies were checked at the same level by vibration analysis to verify the genuine minimum on the potential energy surfaces (PES). To verify the effect of the aldehyde group on photophysical properties, orbital analysis of the complex was also performed.

Results and Discussion

Photophysical Properties of 1. Absorption and room-temperature photoluminescence spectra of **1** are shown in Figure 1, with data listed in Table 1. The absorption spectrum of **1** shows intense high-energy absorption bands at approximately 280−350 nm (ϵ on the order of 10^4 dm^3 mol^{-1} cm^{-1}), and weaker absorption bands at >355 nm (ϵ on the order of 10^3 dm^3 mol^{-1} cm^{-1}) are assigned to intraligand ($\pi-\pi^*$) (pba^-) and spin-allowed metal-to-ligand charge-transfer (^{1}MLCT) ($d\pi(Ir)-\pi^*(pba^-)$) transitions, respectively, with reference to previous photophysical studies on related cyclometalated iridium(III) diimine systems.[15,22] In addition, weaker absorption tails toward the lower energy region (510−550 nm) in the absorption spectrum of **1** may be attributed to spin-forbidden ^{3}MLCT ($d\pi(Ir)-\pi^*(pba^-)$) transitions.

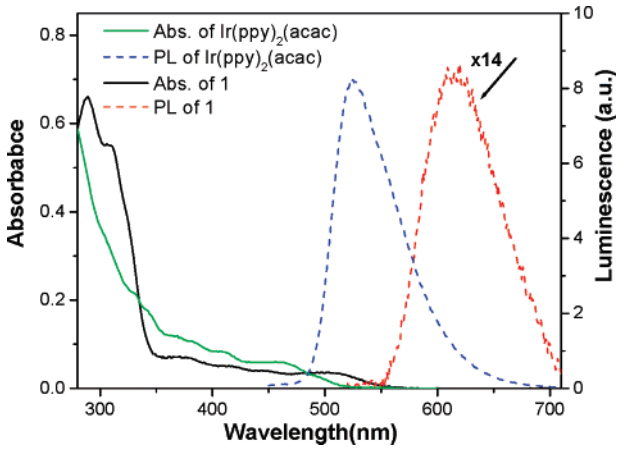


Figure 1. Absorption and emission spectra of **1** and Ir(ppy)$_2$(acac) (20 μM) in DMSO−HEPES buffer (50 mM, pH 7.2, 9:1 v/v).

Complex **1** shows a weak emission band peaked at 615 nm, and its' photoluminescent color is deep red. The luminescent quantum yield of **1** in air-equilibrated solution is ~0.003. The emission lifetime monitored at 615 nm in air-equilibrated solution at room temperature was measured as 106 ns, indicating the phosphorescent emission nature of **1**. Moreover, the photoluminescence emission band is broad and featureless, suggesting that emission originates primarily from the ^{3}MLCT state. As a comparison, we investigated the photophysical properties of a similar complex Ir(ppy)$_2$(acac)[18a] (ppy = 2-phenylpyridine) without an aldehyde group (see Figure 1 and Table 1). Interestingly, the maximal wavelengths for absorption ($\lambda_{max}^{abs} = 456$ nm) and emission ($\lambda_{max}^{em} = 525$ nm, $\Phi_{em} = 0.032$) of Ir(ppy)$_2$(acac) are blue-shifted compared with those of **1** ($\lambda_{max}^{abs} = 510$ nm, $\lambda_{max}^{em} = 615$ nm, and $\Phi_{em} = \sim 0.003$), which indicates that the introduction of an aldehyde moiety induces a decrease in luminescent quantum yield and an apparent red-shift in the absorption and emission spectra.

(19) Casey, K. G.; Quitevis, E. L. *J. Phys. Chem.* **1998**, *92*, 6590.
(20) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision B.05; Gaussian, Inc.: Wallingford, CT, 2003.
(21) (a) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648. (b) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B*: *Condens. Matter Mater. Phys.* **1988**, *37*, 785. (c) Vosko, S. H.; Wilk, L.; Nusair, M. *Can. J. Phys.* **1980**, *58*, 1200.
(22) (a) Serroni, S.; Juris, A.; Campagna, S.; Venturi, M.; Denti, G.; Balzani, V. *J. Am. Chem. Soc.* **1994**, *116*, 9086. (b) Calogero, G.; Giuffrida, G.; Serroni, S.; Ricevuto, V.; Campagna, S. *Inorg. Chem.* **1995**, *34*, 541. (c) Mamo, A.; Stefio, I.; Parisi, M. F.; Credi, A.; Venturi, M.; Pietro, C. Di.; Campagna, S. *Inorg. Chem.* **1997**, *36*, 5947. (d) Marco, G. Di.; Lanza, M.; Mamo, A.; Stefio, I.; Pietro, C. Di; Romeo, G.; Campagna, S. *Anal. Chem.* **1998**, *70*, 5019. (e) Neve, F.; Crispini, A.; Campagna, S.; Serroni, S. *Inorg. Chem.* **1999**, *38*, 2250. (f) Griffiths, P. M.; Loiseau, F.; Puntoriero, F.; Serroni, S.; Campagna, S. *Chem. Commun.* **2000**, 2297. (g) Neve, F.; Crispini, A.; Loiseau, F.; Campagna, S. *J. Chem. Soc., Dalton Trans.* **2000**,1399. (h) Neve, F.; Crispini, A. *Eur. J. Inorg. Chem.* **2000**, 1039. (i) Neve, F.; Crispini, A.; Serroni, S.; Loiseau, F.; Campagna, S. *Inorg. Chem.* **2001**, *40*, 1093.

Table 1. Photophysical and Electrochemical Properties of the Iridium(III) Complexes

complex	λ_{abs} (nm) ($\epsilon \times 10^4$ dm^3 mol^{-1} cm^{-1})	$\lambda_{em}{}^a$ (nm)	τ^a (ns)	$\Phi_{em}{}^a$	$E_{ox}{}^b$ (V)	$E_{re}{}^b$ (V)	E^{00} (eV)	$E^0(Ir^{2+/+*})$ (V)
$Ir(ppy)_2(acac)$	284 (2.61), 331 (1.07), 340 (0.9), 367 (0.6), 384 (0.51), 409 (0.41), 456 (0.28), 495 (0.13)	525	667	0.032	0.63	−2.66		
1	289 (3.29), 308 (2.74), 367 (0.35), 414 (0.25), 447 (0.20), 510 (0.20)	615	106	~0.003	0.68[c]	−1.87[c], 2.06[c]	2.01	0.14
1-Hcy	284 (6.15), 306 (4.10), 360 (0.95), 412 (0.53), 450 (0.36)	525	750	0.038	0.85[d]	−1.90[d]	2.43	0.53
1-Cys	284 (6.23), 306 (5.20), 366 (0.93), 408 (0.78), 450 (0.33)	525	230	~0.002	0.55[d], 0.85[d]	−1.52[d]	2.42	0.90

[a] Measured in DMSO−HEPES buffer (50 mM, pH 7.2, 9:1 v/v) at a concentration of 2×10^{-5} mol L^{-1} at 298 K. The excitation wavelength was 360 nm for all complexes. [b]Measured in DMSO, scan rate of 0.1 V S^{-1}, all potentials versus $Ag/AgNO_3$ as a standard. [c] Reversible wave. [d] Quasi-reversible wave.

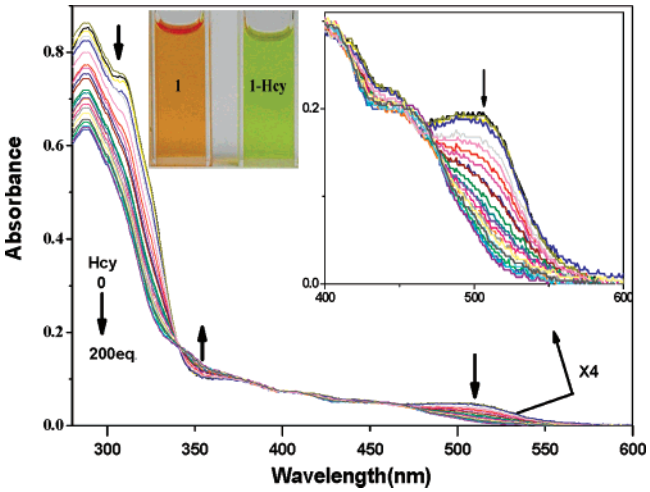


Figure 2. Changes in UV−vis absorption spectra of **1** (20 μM) in DMSO−HEPES buffer (50 mM, pH 7.2, 9:1 v/v) with various amounts of Hcy (0−4 mM). Inset: Color variation of **1** in the absence and presence of excessive Hcy.

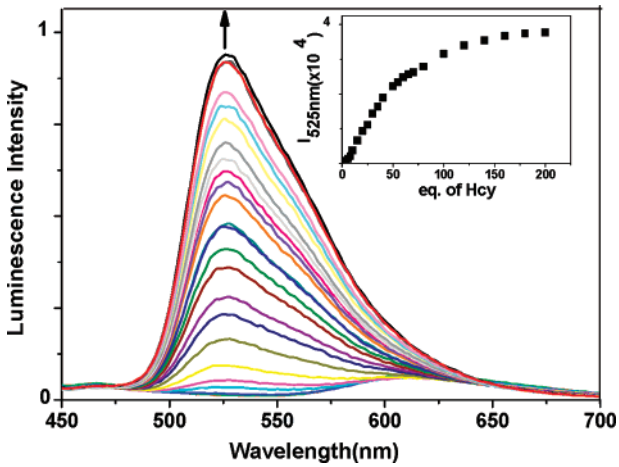


Figure 3. Changes in the phosphorescence emission spectra of **1** (20 μM) in DMSO−HEPES buffer (50 mM, pH 7.2, 9:1 v/v) with various amounts of Hcy (0−200 equiv) (λ_{ex} = 360 nm). Inset: titration curve of **1** with Hcy (0−200 equiv).

Optical Response of 1 to Hcy. From the above result, it is evident that the existence of an electron-withdrawing group (CHO) significantly affects the photophysical properties of **1**. Selective reaction of the aldehyde moiety with β- and γ-aminoalkylthiol groups to form thiazolidines has been extensively applied in Cys and Hcy detection.[7a,b,8a,b,9] In the present study, we reasoned that the iridium(III) complex **1** with an aldehyde group also had a similar ability to recognize Cys and Hcy.

a. Electronic Absorption Spectroscopy. The recognition of Hcy by **1** was studied using a spectrophotometric absorption method. As shown in Figure 2, the addition of increasing amounts of Hcy to a solution of **1** led to obvious changes in its' absorption spectrum. In particular, the absorption band at 510 nm decreased gradually upon the addition of Hcy, resulting in a color change from orange to yellow (see Figure 2 inset), indicating that **1** could serve as a sensitive "naked eye" indicator of Hcy.

b. Photoluminescence Properties. It is well-known that emission spectroscopy is more sensitive to small changes that affect the electronic properties of molecules. Here, the ability of **1** to form a complex with Hcy was also investigated using a photoluminescent technique. The luminescence spectra of **1** upon treatment with Hcy are shown in Figure 3. Upon the addition of Hcy, a new emission band at 525 nm gradually appeared, corresponding to a blue-shift of approximately 90 nm and a change in emission color from deep red to green that could be observed by the naked eye (Figure 4).

Selective Optical Response of 1 to Various Amino Acids. High selectivity is necessary for an excellent chemosensor. The investigation of the selective response of **1** by photoluminescent spectroscopy was thus extended to other elementary amino acids. Upon the addition of amino acids such as L-histidine, L-leucine, L-asparagine, L-arginine, L-tyrosine, L-threonine, L-proline, L-isoleucine, L-tryptophan, L-methionine, L-valine, L-alanine, L-phenylalanine, L-glutamine, L-glutamic acid, L-serine, L-hydroxyproline, L-lysine, and L-glycine, no obvious changes was measured in the absorption and emission spectra (Supporting Information and Figure 4). Moreover, no obvious enhancement in phosphorescent emission of **1** was observed upon the addition of thiol-related peptides (such as reduced glutathione, GSH) (Figure 4).

Generally, methods for the direct detection of Hcy are usually hampered by interference from structurally related molecules,[1c] especially Cys, with high structural similarity. In the present study, upon the addition of Cys, the absorption band at 510 nm decreased, resulting in a color change from orange to yellow (Supporting Information). However, when an excess of Cys was added to a solution of **1**, weak enhancement (2-fold) of the luminescent intensity at 525 nm

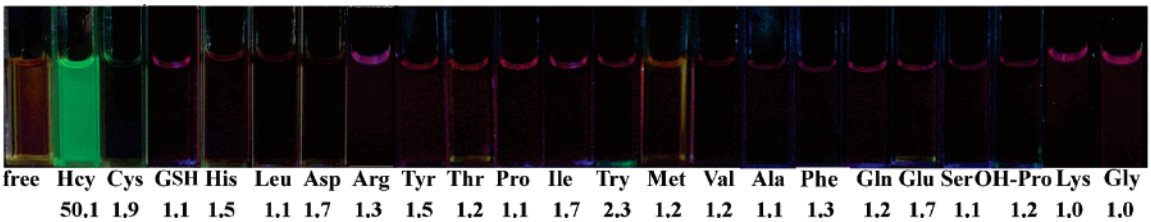


Figure 4. Phosphorescent emission observed from the solutions of **1** (20 μM) in the presence of different amino acids (4 mM). Excitation wavelength was 365 nm from a portable lamp. F_f/F_0 represents the final phosphorescence intensity (F_f) over the initial intensity (F_0) at 525 nm (λ_{ex} = 360 nm).

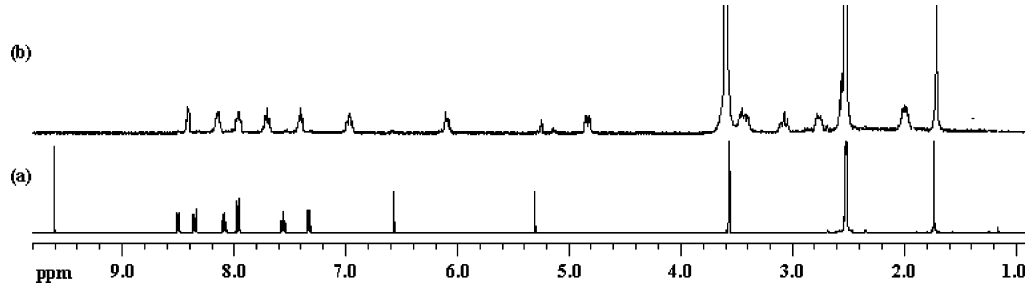


Figure 5. ^{1}H NMR spectra of **1** (a) and **1**-Hcy (b) in DMSO-d_6. The resonances centered at 9.61 and 4.84 ppm are assigned to the aldehyde hydrogens of **1** and the methine protons of the thiazolidine of **1**-Hcy, respectively.

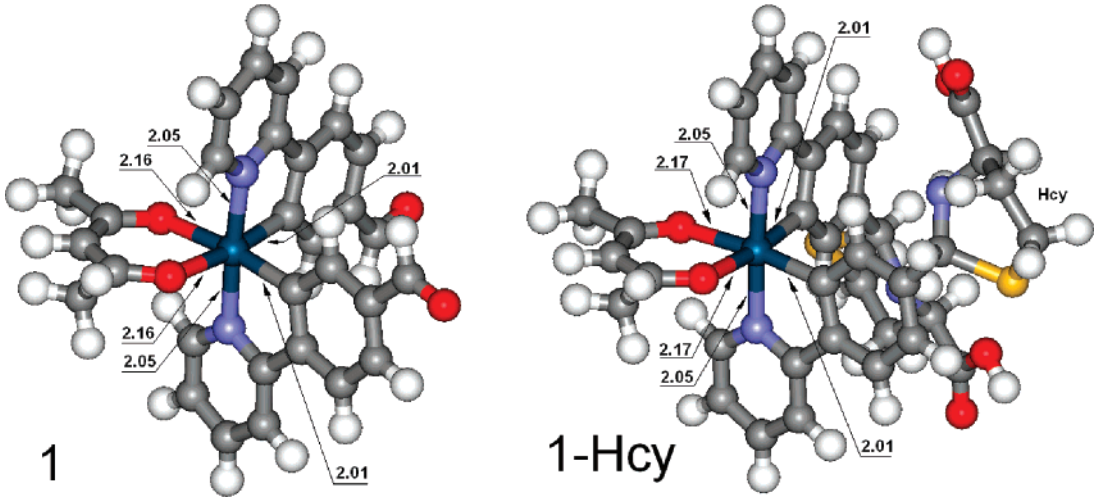


Figure 6. Optimized structures of **1** and **1**-Hcy with lengths of coordination bonds.

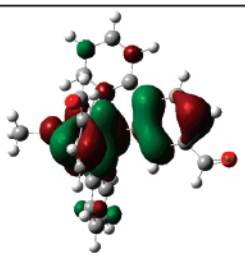
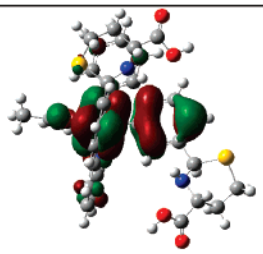
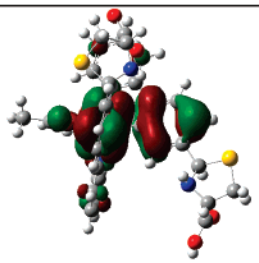
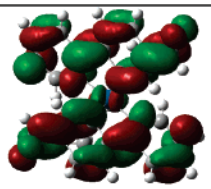
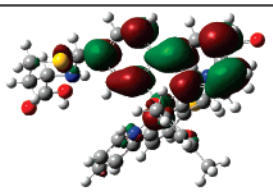
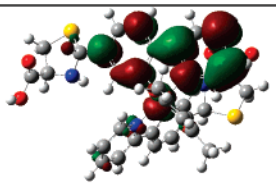
was measured, although the intensity of the emission band at 615 nm decreased 4-fold (Supporting Information). Together with the above phosphorescent response of **1** toward Hcy (Figure 4), we can conclude that **1** can selectively detect Hcy over other amino acids and thiol-related peptides. To the best of our knowledge, this is the first example of luminescent Hcy-selective sensors which can distinguish Hcy from Cys and thiol-related peptides with both emission shift and fluorescence intensity. Of course, DMSO−HEPES (9:1 v/v) is not biocompatible, and the requirement for 200 equiv of Hcy (4 mM) lowers the sensitivity of the system.

Photophysical and Electrochemical Properties of 1-Hcy and 1-Cys. To understand the difference in optical response of **1** to Hcy over Cys, the adducts **1**-Hcy and **1**-Cys were isolated and characterized by ^{1}H NMR experiments (Figure 5). For **1**-Hcy, the aldehyde resonance (9.61 ppm) of **1** disappeared and a new peak centered at 4.84 ppm appeared, assigned to the methine proton of the thiazolidine diastereometer.[7a,b,8a,b] This fact indicates that thiazolidine is formed by interaction of the aldehyde moiety with Hcy. With the disappearance of the strong electron-withdrawing group (CHO), the resonances corresponding to the phenyl group were shifted upfield.

^{1}H NMR experiments indicate that **1** can also react with Cys, which was confirmed by the absorption titration of **1** with Cys (Supporting Information). The luminescent lifetimes monitored at 525 nm were 750 and 230 ns for **1**-Hcy and **1**-Cys (Table 1), respectively. Such long lifetimes in air-equilibrated solution show that the excited states of **1**-Hcy and **1**-Cys have triplet character. Interesting, significant difference in luminescence properties between **1**-Cys and **1**-Hcy was observed (Table 1). The luminescent quantum yield of **1**-Hcy in air-equilibrated solution was measured to be 0.038 and was remarkably higher than that (~0.002) of **1**-Cys under the same conditions.

Moreover, the electrochemical properties of the complexes have been studied by cyclic voltammetry. The data are listed in Table 1. With reference to $Ag/AgNO_3$ as a standard, **1** shows a reversible couple at ca. 0.68 V that is attributable

Table 2. HOMO and LUMO Distributions of **1**, **1**-Hcy, and **1**-Cys

Complex	1	1-Hcy	1-Cys
HOMO			
LUMO			

to a metal-centered Ir(IV/III) oxidation process. Both **1**-Hcy and **1**-Cys exhibit an irreversible oxidation wave at ca. 0.85 V, which could be assigned to orbitals receiving a strong contribution from an iridium center and Ir−C$^-$ σ-bond orbitals simultaneously.[14,15] It is interesting to note that **1**-Cys shows another oxidation peak at 0.55 V attributed to the thiazolidine group (Supporting Information), compared to **1**-Hcy with a weak oxidation peak at 0.55 V. This fact indicated that the thiazolidine moiety of **1**-Cys is more easily oxidized than that of **1**-Hcy. Meanwhile, **1** exhibits two reversible couples at ca. −1.87 and −2.06 V assigned to reduction of the coordinated pba ligand.[15−17] **1**-Hcy and **1**-Cys exhibit a quasi-reversible couple at ca. −1.90 and −1.52 V, respectively, which is assigned to a reduction of the thiazolidine derivative. Thus, **1**-Cys is also more easily reduced.

Theoretical Calculations. To further understand the effect of the aldehyde (CHO) group on the photophysical properties, calculations based on DFT for **1** and **1**-Hcy were performed. Both complexes were confirmed to be genuine minima on the PES. The optimized structures are shown in Figure 6. Orbital analysis revealed that, for the two complexes, no obvious change was observed for the highest occupied molecular orbital (HOMO) distributions, and the HOMO primarily resided on the iridium center and phenyl part of the cyclometalated ligands, similar to most of the iridium(III) complexes.[16,17] However, the lowest unoccupied molecular orbital (LUMO) distributions of **1** and **1**-Hcy are strikingly different (Table 2). For **1**, the LUMO distribution is evenly localized on the two pba ligands. In contrast, the LUMO distribution of **1**-Hcy is partially located on the 2-phenylpyridine moiety of one ligand. As a result, the LUMO energy level of **1**-Hcy is higher. The HOMO−LUMO energy gaps were calculated as 3.21 and 3.46 eV for **1** and **1**-Hcy, respectively, which is in agreement with the remarkable blue-shift in the absorption and luminescence spectra of **1**-Hcy compared with those of **1**.

Mechanism of Hcy Sensing. It is interesting to note that the adduct **1**-Cys exhibited much lower luminescence quantum yield and shorter emission lifetime than **1** and **1**-Hcy with same MLCT units (Table 1). Since the triplet-state energy of the thiazolidine moiety of **1**-Cys is much higher than the emission energy of Ir(ppy)$_2$(acac) and there was no spectral overlap between the emission spectra of Ir(ppy)$_2$(acac) and the absorption spectrum of the thiazolidine moiety, it is unlikely that the quenching of **1**-Cys occurred via an energy-transfer mechanism.

Surface charge analysis based on DFT indicates that the charges on the S and N atoms of **1**-Cys are more negative than those of **1**-Hcy (Supporting Information). It can be expected that an intramolecular electron-transfer process might occur in the adduct **1**-Cys from the thiazolidine group to the excited complex, resulting in a weak MLCT photoluminescence of **1**-Cys.

Furthermore, the excited-state redox potentials $E°(Ir^{2+/+*})$ of the two complexes **1**-Hcy and **1**-Cys were determined from the ground-state redox potentials and the E^{00} energy, and the data was shown in Table 1. The emission maxima of **1**-Hcy and **1**-Cys at 77 K were 510 and 512 nm (Supporting Information), respectively, corresponding to the E^{00} energy gap[15f,23] of 2.43 and 2.42 eV for **1**-Hcy and **1**-Cys, respectively. From the reduction potentials of **1**-Hcy and **1**-Cys of −1.90 and −1.52 V, respectively, the potentials $E°(Ir^{2+/+*})$ of **1**-Hcy and **1**-Cys were estimated to be ca. +0.53 and +0.90 V, respectively. The difference between the potentials $E°(Ir^{2+/+*})$ and the redox potential of the thiazolidine moiety ($E°[\text{thiazolidine}^{+/0}] < +0.55$ V), were calculated to be ca. −0.02 and 0.45 eV for **1**-Hcy and **1**-Cys (Table 1), respectively. Therefore, reductive quenching of the excited complex by the thiazolidine moiety is favored for **1**-Cys. As a result, it is likely that the mechanism of the emission quenching of the adduct **1**-Cys is electron transfer in nature (Figure 7).

(23) Cummings, S. D.; Eisenberg, R. *J. Am. Chem. Soc.* **1996**, *118*, 1949.

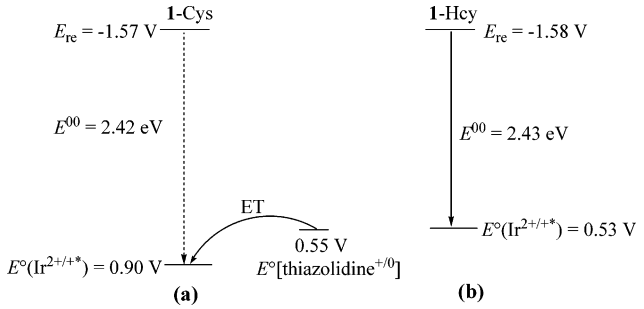


Figure 7. Possible mechanism of the emission of **1**-Cys (a) and **1**-Hcy (b).

Conclusions

In conclusion, we have presented a highly selective chemosensor for Hcy with emission enhancement based on the phosphorescent complex $Ir(pba)_2(acac)$ that contains an aldehyde group. In particular, the complex shows a change in phosphorescent emission visible to the naked eye on sensing Hcy; this is attributed to the reaction between Hcy and the aldehyde groups of cyclometalated ligands. To the best of our knowledge, this is the first report of a highly selective luminescent chemosensor for Hcy over other amino acids (including Cys) and thiol-related peptides (including GSH). This understanding of the Hcy sensing mechanism should help in the design of new phosphorescent probes based on iridium(III) complexes by the simple modification of the chemical structure of ligands to contain specific coordinating elements and in the exploration of new applications for chemosensors.

Acknowledgment. The authors are thankful for the financial support from the National Natural Science Foundation of China (20490210, 20501006, and 20775017), the National High Technology Program of China (2006AA03Z318), NCET-06-0353, and the Shanghai Science and Technology Community (05DJ14004 and 06QH14002).

Supporting Information Available: UV−vis and luminescence spectra upon treatment with other amino acids; luminescence spectra of **1**-Hcy and **1**-Cys at 77 K; CV curves, ^{1}H NMR, IR, and ESI spectra of **1**, **1**-Hcy, and **1**-Cys. This material is available free of charge via the Internet at http://pubs.acs.org.

IC7010887