

# Rational Design of a Highly Reactive Ratiometric Fluorescent Probe for Cyanide

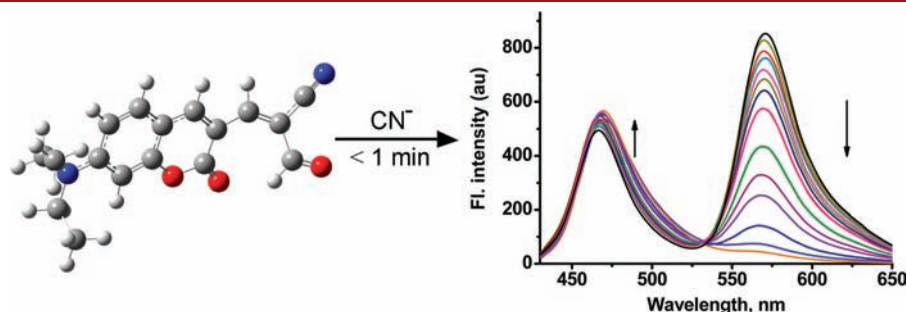
Lin Yuan, Weiyang Lin,\* Yueting Yang, Jizeng Song, and Jiaoliang Wang

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, Hunan 410082, People's Republic of China

weiyanglin@hnu.cn

Received May 23, 2011

## ABSTRACT



A novel highly reactive ratiometric fluorescent cyanide probe was judiciously designed based on 2-formylacrylonitrile moiety as a new cyanide reaction site. A DFT study was conducted to rationalize the extremely high reactivity nature of the ratiometric fluorescent cyanide probe.

Cyanide is a basic component in the preparation of a wide variety of products ranging from plastic, fibers, gold, dyes, chelating agents for water treatment, to pharmaceuticals.<sup>1</sup> However, the widespread use of

cyanide presents a major health risk to human due to its high toxicity.<sup>1a,2</sup> Cyanide is a potent inhibitor for some metallo-enzymes and non-metallo-enzymes.<sup>2,3</sup> This leads to diseases in the vascular, cardiac, visual, endocrine, central nervous, and metabolic systems.<sup>3</sup> Thus, the development of fluorescent probes for cyanide is very important.

A diverse array of fluorescent cyanide probes have been constructed based on reversible binding and reaction-based approaches.<sup>3–5</sup> However, some of them have delayed response time and require high equivalents (100 or higher) of cyanide to reach a maximal fluorescent signal. Furthermore, some of them are based on fluorescence measurement at a single wavelength, which may be influenced by variations in the sample environment. By contrast, ratiometric fluorescent probes allow the measurement of emission intensities at two wavelengths, which should provide a built-in correction for environmental effects.<sup>6</sup> Therefore, it is of high interest to develop new

(1) (a) Acheampong, M. A.; Meulepasa, R. J. W.; Lens, P. N. L. *J. Chem. Technol. Biotechnol.* **2010**, *85*, 590. (b) Young, C.; Tidwell, L.; Anderson, C. *Cyanide: Social, Industrial and Economic Aspects*; Minerals, Metals, and Materials Society: Warrendale, PA, 2001.

(2) Kulig, K. W. *Cyanide Toxicity*; U.S. Department of Health and Human Services: Atlanta, GA, 1991.

(3) Xu, Z.; Chen, X.; Kim, H. N.; Yoon, J. *Chem. Soc. Rev.* **2010**, *39*, 127.

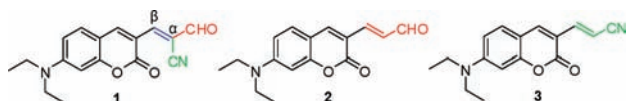
(4) For some examples, see: (a) Odago, M. O.; Colabello, D. M.; Lees, A. J. *Tetrahedron* **2010**, *66*, 7465. (b) Saha, S.; Ghosh, A.; Mahato, P.; Mishra, S.; Mishra, S. K.; Suresh, E.; Das, S.; Das, A. *Org. Lett.* **2010**, *12*, 3406.

(5) For some examples, see: (a) Tetilla, M. A.; Aragoni, M. C.; Arca, M.; Caltagirone, C.; Bazzicalupi, C.; Bencini, A.; Garau, A.; Isaia, F.; Laguna, A.; Lippolis, V.; Meli, V. *Chem. Commun.* **2011**, *47*, 3805. (b) Wang, J.; Ha, C.-S. *Tetrahedron* **2010**, *66*, 1846. (c) Jamkratoke, M.; Ruangpornvisuti, V.; Tumcharern, G.; Tuntulani, T.; Tomapatnanaget, B. *J. Org. Chem.* **2009**, *74*, 3919. (d) Cho, D.-G.; Kim, J. H.; Sessler, J. L. *J. Am. Chem. Soc.* **2008**, *130*, 12163. (e) Tomasulo, M.; Sortino, S.; White, A. J. P.; Raymo, F. M. *J. Org. Chem.* **2006**, *71*, 744. (f) Ekmekci, Z.; Yilmaz, M. D.; Akkaya, E. U. *Org. Lett.* **2008**, *10*, 461. (g) Jo, J.; Lee, D. *J. Am. Chem. Soc.* **2009**, *131*, 16283. (h) Shiraishi, Y.; Sumiya, S.; Hirai, T. *Chem. Commun.* **2011**, *47*, 4953. (i) Lee, K.-S.; Kim, H.-J.; Kim, G.-H.; Shin, I.; Hong, J.-I. *Org. Lett.* **2008**, *10*, 49. (j) Kim, G.-J.; Kim, H.-J. *Tetrahedron Lett.* **2010**, *51*, 185.

(6) For some examples, see: (a) Tsien, R. Y.; Harootunian, A. T. *Cell Calcium* **1990**, *11*, 93. (b) Demchenko, A. P. *J. Fluoresc.* **2010**, *20*, 1099. (c) Bao, Y.; Liu, B.; Wang, H.; Tian, J.; Bai, R. *Chem. Commun.* **2011**, *47*, 3957. (d) Fu, L.; Jiang, F.-L.; Fortin, D.; Harvey, P. D.; Liu, Y. *Chem. Commun.*, 2011, DOI: 10.1039/c1cc10784d.

ratiometric fluorescent probes for cyanide, in particular, with rapid response and high sensitivity.

Herein, we describe the rational design of compound **1** (Figure 1) as a new ratiometric fluorescent probe for cyanide based on a novel  $\text{CN}^-$  reaction site, 2-formylacrylonitrile moiety. Importantly, probe **1** displays a very fast response ( $< 1$  min) to cyanide at room temperature, and a maximal ratiometric fluorescent signal is achieved in the presence of only 5 equiv of cyanide.



**Figure 1.** Structures of the new ratiometric fluorescent probe **1** and the control compounds **2** and **3**.

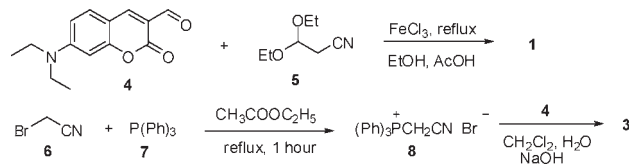
In this work, 7-diethylaminocoumarin was selected as the fluorescent signal reporting unit in light of its high photostability, large Stokes shift, and emission in the visible region.<sup>7</sup> Furthermore, diethylamino moiety is a strong electron-donating group and thus is suitable as a donor in an intramolecular charge transfer (ICT) system. To effectively manipulate an ICT process, we then judiciously placed an acrylaldehyde moiety on the 3-position of the coumarin dye. This may afford compound **2** (Figure 1). We envisioned that there is a strong ICT process in the free compound **2**. However, the ICT will be blocked upon addition of  $\text{CN}^-$  to the  $\beta$ -carbon of the acrylaldehyde moiety due to breaking of the conjugation. The drastic change in ICT efficiency should elicit a ratiometric optical response. Thus, we preliminarily tested the possibility of compound **2** as a ratiometric fluorescent probe for cyanide. Indeed,  $\text{CN}^-$  can induce a marked blue shift in the absorption spectra of compound **2** as shown in Figure S2a in the Supporting Information. However, like some reaction-based probes for  $\text{CN}^-$ , compound **2** responded to  $\text{CN}^-$  relatively sluggishly. The reaction was not complete after incubation of compound **2** ( $10 \mu\text{M}$ ) with  $\text{CN}^-$  (5 equiv) for over 1 h (Figure S2a and its inset).

Clearly, to improve the sensitivity, it is necessary to enhance the reactivity of the probe to cyanide. We reasoned that this could be accomplished by increasing the electrophilicity of the  $\beta$ -carbon. With this in mind, we decided to introduce a cyano moiety, a strong electron-withdrawing group,<sup>8</sup> on the  $\alpha$ -carbon to afford probe **1** (Figure 1). Another key advantage of the incorporation of the cyano moiety is to provide a 2-formylacrylonitrile moiety, a dual  $\alpha,\beta$ -unsaturated system, to be attacked by  $\text{CN}^-$ . One system is the acrylaldehyde moiety (as shown in

red in Figure 1), and the other is the acrylonitrile moiety (as shown in green in Figure 1). Thus, we anticipated that probe **1** should be highly reactive to  $\text{CN}^-$ . To the best of our knowledge, the 2-formylacrylonitrile moiety has not been previously exploited as a cyanide reaction site for design of fluorescent probes.

Compound **1** was readily prepared in one step by condensation of coumarinyl aldehyde **4** with 3,3-diethoxypropionitrile **5** in the presence of  $\text{FeCl}_3$  (Scheme 1).<sup>9</sup> The synthesis of control compound **2** was based on a reported procedure.<sup>7b</sup> The control compound **3** was made in two steps (Scheme 1). Treatment of 2-bromoacetonitrile **6** with  $\text{Ph}_3\text{P}$  **7** in ethyl acetate afforded the Wittig reagent **8**, which was then reacted with coumarinyl aldehyde **4** to give compound **3**. The products were well characterized by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, MS, and HRMS.

### Scheme 1. Synthesis of Probe **1** and the Control Compound **3**



The absorption spectrum of probe **1** exhibits a main absorption peak at 523 nm (Figure S1a), which is notably red-shifted relative to that of compounds **2** (451 nm) and **3** (442 nm), indicating that indeed 2-formylacrylonitrile is a stronger electron-withdrawing group than acrylaldehyde or acrylonitrile, as anticipated.

The time-dependent changes in the absorption spectra of probe **1** ( $10 \mu\text{M}$ ) upon reaction with  $\text{CN}^-$  (5 equiv) is shown in Figure 2. Upon introduction of  $\text{CN}^-$ , a new peak at 363 nm appears immediately accompanied with a drastic decrease in the peak at 523 nm. Three well-defined isosbestic points at 417, 323, and 272 nm, respectively, are observed.<sup>10</sup> Notably, the main absorption peak at 523 nm completely disappears in less than 1 min, indicating that probe **1** is much more reactive than compounds **2** (Figure S2a) and **3** (Figure S2b) under the same conditions, as judiciously designed. Importantly, only 5 equiv of cyanide was used in the assay. By contrast, some fluorescent cyanide probes require high equivalents (100 or higher) of cyanide to reach reaction completion under prolonged incubation. Thus, high reactivity is the unique feature of probe **1** when compared to compounds **2**, **3**, and some known fluorescent cyanide probes.

To shed light on the highly reactive nature of probe **1**, the electrophilic Fukui function from density functional reactivity theory (DFRT)<sup>11</sup> was performed to estimate the

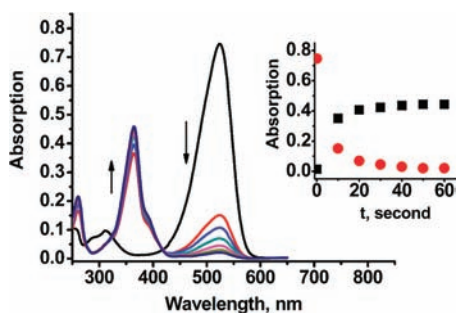
(7) (a) Dong, Y.; Li, J.; Jiang, X.; Song, F.; Cheng, Y.; Zhu, C. *Org. Lett.* **2011**, *13*, 2252. (b) Yuan, L.; Lin, W.; Yang, Y. *Chem. Commun.* **2011**, *47*, 6275. (c) Voutsadaki, S.; Tsikalas, G. K.; Klontzas, E.; Froudakis, G. E.; Katerinopoulos, H. E. *Chem. Commun.* **2010**, *46*, 3292.

(8) (a) Tang, X.; Liu, W.; Wu, J.; Lee, C.-S.; You, J.; Wang, P. *J. Org. Chem.* **2010**, *75*, 7273. (b) Guo, Z.; Zhu, W.; Shen, L.; Tian, H. *Angew. Chem., Int. Ed.* **2007**, *46*, 5549.

(9) Maeda, S.; Horikawa, N.; Obora, Y.; Ishii, Y. *J. Org. Chem.* **2009**, *74*, 9558.

(10) Yin, S.; Leen, V.; Snick, S. V.; Boens, N.; Dehaen, W. *Chem. Commun.* **2010**, *46*, 6329.

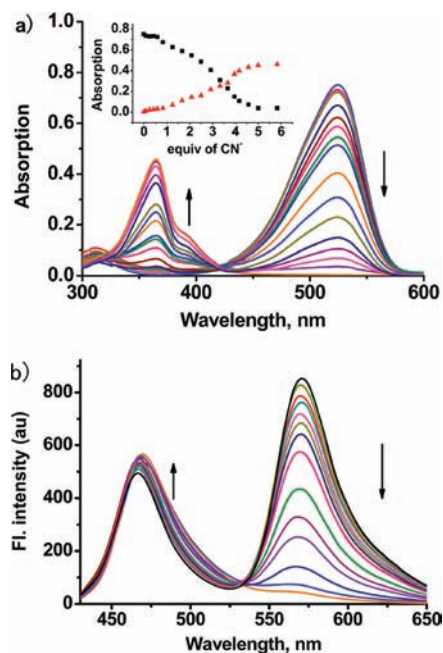
(11) (a) Liu, S.; Ess, D. H.; Schauer, C. K. *J. Phys. Chem. A* **2010**, *115*, 4738. (b) Liu, S. *Acta Phys. Chim. Sin.* **2009**, *25*, 590.



**Figure 2.** Time-dependent changes in the absorption spectra of probe **1** ( $10\ \mu\text{M}$ ) upon reaction with  $\text{CN}^-$  (5 equiv) in  $\text{CH}_3\text{CN}$ . The arrows indicate the change of incubation time from 0 to 60 s. Inset: Time-dependent absorption intensity of probe **1** at 523 nm (● in red) and 363 nm (■) in the presence of  $\text{CN}^-$  (5 equiv).

electrophilicity of the  $\beta$ -carbon in compounds **1**, **2**, and **3** using the B3LYP exchange functional, together with 6-31+G(d) basis sets with tight SCF convergence and ultrafine integration grids. The electronic structures of stationary points were analyzed by the natural bond orbital (NBO) method. All calculations were carried out with a suite of Gaussian 09 programs.<sup>12</sup> The calculations provide the electrophilicity of the  $\beta$ -carbon in compounds **1**, **2**, and **3** as 2.848978, 0.347214, and 0.284229 au, respectively. Thus, the  $\beta$ -carbon of probe **1** is much more electrophilic than that of compounds **2** and **3**, in good agreement with the observation that probe **1** is much more reactive than compounds **2** and **3**.

We then investigated the concentration-dependent changes in the absorption and fluorescence spectra upon incubation of probe **1** ( $10\ \mu\text{M}$ ) with  $\text{CN}^-$  (0–5 equiv) for 1 min. As displayed in Figure 3a, addition of  $\text{CN}^-$  induced a significant blue shift from 523 to 363 nm, and 5 equiv of cyanide is sufficient to drive the reaction to completion within 1 min, re-enforcing the high reactivity feature of the probe. Interestingly, the free probe **1** shows two characteristic fluorescence bands at 573 and 473 nm. Obviously, the emission band at 573 nm is attributed to the ICT band. However, the formation of the emission band at 473 nm is likely due to the steric hindrance between the 2-formylacrylonitrile moiety and the carbonyl group of the coumarin dye, which forces them to be slightly nonplanar and deconjugated, and thus the emission of the diethylamino coumarin dye at 473 nm is present. To get insight into the intriguing dual-emission feature of probe **1**, the structures of probe **1** and the control compounds **2** and **3** were optimized by DFT calculations.<sup>12</sup> As shown in Figure S3, the optimized structure of probe **1** has a dihedral angle about  $25^\circ$  between the coumarin ring and formylacrylonitrile moiety, whereas compounds **2** and **3** are essentially planar between the coumarin ring and the aldehyde (or cyano) moiety with a dihedral angle  $<0.1^\circ$ . Thus, the calculation results are consistent with the observation that



**Figure 3.** Concentration-dependent changes in absorption (a) and fluorescence (b) spectra of probe **1** ( $10\ \mu\text{M}$ ) upon gradual addition of  $\text{CN}^-$  (0–5 equiv) for 1 min in  $\text{CH}_3\text{CN}$  with excitation at 420 nm. Inset in panel a: Changes of absorption intensity of probe **1** at 523 nm (■) and 363 nm (▲ in red) in the presence of different equiv of  $\text{CN}^-$  (0–6 equiv).

compounds **2** and **3** only have an ICT emission band (Figure S1b). However, the dual fluorescence of compound **1** in polar solvents due to the TICT (twisted intramolecular charge transfer) state cannot be excluded.<sup>13</sup>

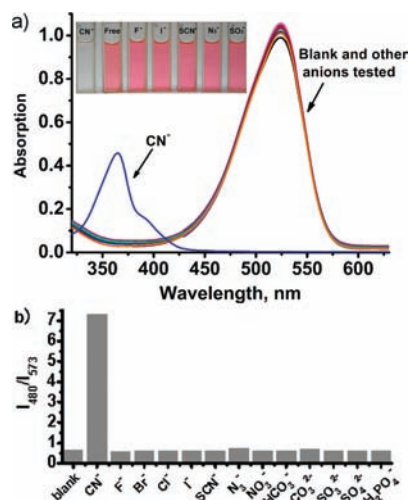
Upon gradual addition of  $\text{CN}^-$  to probe **1**, the intensity of the emission band at 473 nm is slightly increased with a minor shift and that of the emission band at 573 nm is significantly decreased (Figure 3b). There is a ca. 11.2-fold variation in the fluorescence ratio ( $I_{480}/I_{573}$ ) from 0.65 in the absence of  $\text{CN}^-$  to 7.3 in the presence of 5 equiv of  $\text{CN}^-$  (Figure 4b). The detection limit ( $S/N = 3$ ) was calculated to be 328 nM, superior or comparable to the known fluorescent cyanide probes.

To examine the selectivity, probe **1** was incubated with various anion species including  $\text{CN}^-$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{SCN}^-$ ,  $\text{N}_3^-$ ,  $\text{NO}_3^-$ ,  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{SO}_3^{2-}$ ,  $\text{SO}_4^{2-}$ , and  $\text{H}_2\text{PO}_4^-$ . Among various anion species tested, only  $\text{CN}^-$  responded to **1** with a large blue shift (Figure 4a) and a marked color change from pink to colorless (the inset in Figure 4a). Consistent with the results of the absorption spectra, the fluorescence ratio data ( $I_{480}/I_{573}$ ) reveal that probe **1** is highly selective to  $\text{CN}^-$  over various anion species tested (Figure 4b). We further examined the colorimetric and fluorescent ratio response of the probe toward  $\text{CN}^-$  in the presence of other potentially competing species. The other species only displayed minimum

(12) Frisch, M. J. et al. *GAUSSIAN 09*, revision A.01; Gaussian, Inc.: Pittsburgh, PA, 2009.

(13) Valeur, B. *Molecular Fluorescence: Principles and Applications*; Wiley-VCH: Weinheim, Germany, 2001; pp 62–66.



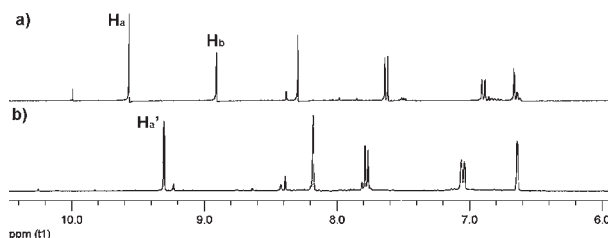


**Figure 4.** (a) Absorption spectra of probe **1** (10  $\mu$ M) in the presence of 5 equiv of various anion species ( $\text{CN}^-$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{SCN}^-$ ,  $\text{N}_3^-$ ,  $\text{NO}_3^-$ ,  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{SO}_3^{2-}$ ,  $\text{SO}_4^{2-}$ , and  $\text{H}_2\text{PO}_4^-$  as  $n\text{-Bu}_4\text{N}$  salts in  $\text{CH}_3\text{CN}$ ). Inset: Color of probe **1** in the absence or presence of various anion species; (b) emission ratio ( $I_{480}/I_{573}$ ) of probe **1** in the absence or presence of various species (5 equiv) in  $\text{CH}_3\text{CN}$  with excitation at 420 nm.

interference (Figure S4). This indicates that probe **1** is potentially useful for sensing  $\text{CN}^-$  in the presence of other related species.

A likely interaction mechanism of probe **1** with  $\text{CN}^-$  is shown in Figure S5. To shed light on the proposed mechanism,  $^1\text{H}$  NMR, mass spectroscopy, absorption spectroscopy, and DFT calculations were conducted. Addition of  $\text{CN}^-$  to the probe results in disappearance of the resonance signal of the double-bond proton  $\text{H}_b$  at around  $\delta = 8.91$  ppm (Figure 5), suggesting the formation of the **1**- $\text{CN}$  adduct. Furthermore, a major peak at  $m/z$  321.9, corresponding to  $[\text{1-CN-adduct-H}]^-$ , is observed in the ESI-MS spectrum (negative ion mode) (Figure S6) upon treatment of probe **1** with  $\text{CN}^-$ . This implies that a 1:1 adduct between probe **1** and  $\text{CN}^-$  is formed. However, the possibility of the interaction of probe **1** with  $\text{CN}^-$  to form a cyanohydrin adduct can be excluded. The maximal absorption wavelength of the cyanohydrin adduct should be comparable to compound **3** as they have the similar core structure (Figure S7). However, in fact, the **1**- $\text{CN}$  adduct displayed a large blue shift (78 nm) when compared with compound **3** (Figure S8). Furthermore, according to the

DFT calculations<sup>12</sup> for compound **1**, the electrophilicity (2.848978 au) of the  $\beta$ -carbon is larger than that (0.200041 au) of the carbon in carbonyl functional group, indicating that  $\beta$ -carbon is more susceptible to attack by  $\text{CN}^-$ . Thus, the results of  $^1\text{H}$  NMR, mass spectroscopic, absorption spectra, and calculation studies are in good agreement with the proposed interaction mechanism as shown in Figure S5.



**Figure 5.**  $^1\text{H}$  NMR spectral changes for probe **1** upon addition of  $\text{CN}^-$  (5 equiv). (a) Probe **1** only; (b) probe **1** and  $\text{CN}^-$  (5 equiv); [probe **1**] = 6 mM in  $\text{CD}_3\text{CN}$  at room temperature.

In summary, we have rationally designed and synthesized new ratiometric fluorescent cyanide probe **1** based on the novel  $\text{CN}^-$  reaction site, 2-formylacrylonitrile moiety. Ratiometric probe **1** displays a large emission ratio response (ca. 11.2-fold variation) to  $\text{CN}^-$ . Importantly, ratiometric probe **1** is highly reactive to  $\text{CN}^-$  with a very fast response ( $< 1$  min) to cyanide at room temperature, and a maximal fluorescent signal is achieved in the presence of only 5 equiv of cyanide. In addition, the unique feature of probe **1**, extremely high reactivity, is rationalized by DFT calculations. We expect that the 2-formylacrylonitrile moiety as a novel  $\text{CN}^-$  reaction site will be useful for development of other highly reactive fluorescent cyanide probes.

**Acknowledgment.** This research was supported by NSFC (20872032, 20972044), NCET (08-0175), the Doctoral Fund of Chinese Ministry of Education (No. 20100161110008).

**Supporting Information Available.** Experimental procedures, characterization data, and some spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.