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Fe(III)-Induced FRET-On: Energy Transfer from Rhodamine 6G to Nile Red

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The design of fluorescent chemosensors is mainly based on photo-induced electron/energy transfer (PET),[1] metal-ligand charge transfer (MLCT),[2] intramolecular charge transfer (ICT),[3] excimer/exciplex formation,[4] imine isomerization,[5] chelation-enhanced fluorescence (CHEF),[6] and fluorescence resonance energy transfer (FRET).[7] Among them, at present, the FRET is an active field in chemistry and biochemistry due to its potential practical benefits in cell physiology, optical therapy, as well as selective and sensitive sensing toward target molecular or ionic species. FRET arises from an excited-state energy interaction between two fluorophores in which a donor fluorophore, in its excited-state, transfers energy to an acceptor fluorephore. Accordingly, it is strongly influenced by the distance between donor and acceptor, the extent of spectral overlap between donor emission and acceptor absorption spectrum (Figure 1).

As a fluorophore unit, rhodamine 6G has attracted considerable interest from chemists on account of its excellent photophysical properties. The spirolactam ring form of rhodamine derivatives is non-fluorescent and colorless, whereas its ring-opened form gives a strong fluorescence and a pink color. In general, a rhodamine derivative shows a pink color change and strong fluorescence upon addition of specific metal ion or in acidic condition. Thereby, the rhodamine fluorophore can be an ideal framework to construct OFF-ON FRET system for the specific metal ion or pH variation.[8]

For the FRET-based chemosensors for specific metal ions, we have previously reported a donor (pyrene, dansyl, or coumarin)-acceptor (rhodamine) fluorophore in conjunction with a spacer tris(2-aminoethyl)-amine (tren).[9] The tren-spaced rhodamine

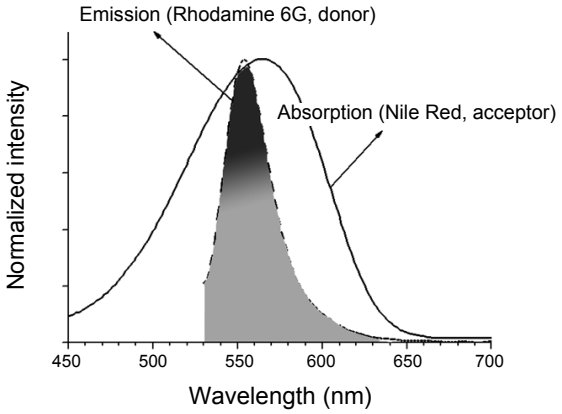
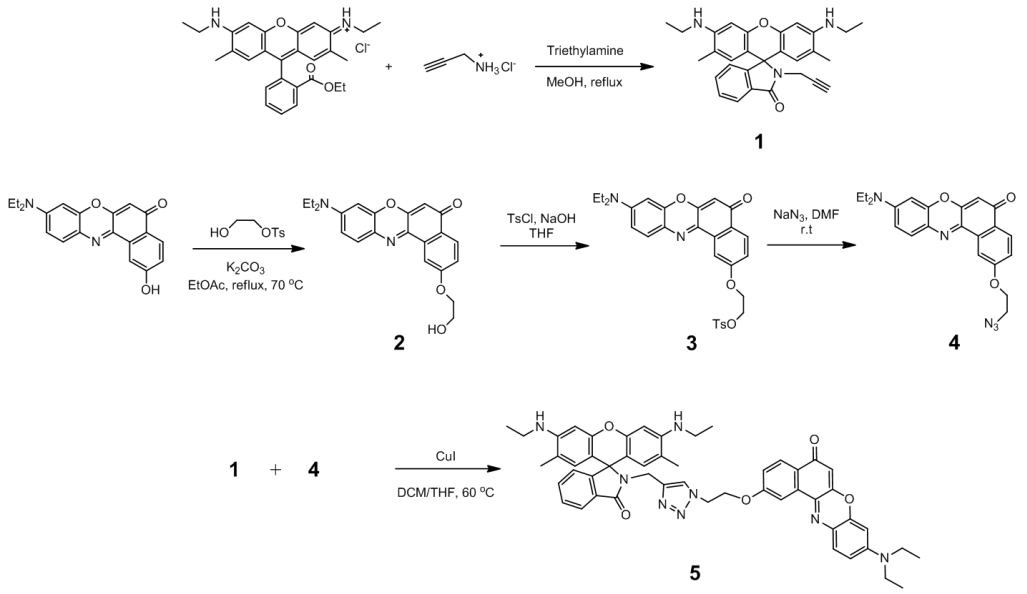


Figure 1. The normalized spectral overlap for FRET between Rhodamine 6G and Nile Red.



Scheme 1. Synthetic route of **5**

molecules show FRET 'OFF→ON' behavior upon binding of specific metal ion. More recently, FRET-based fluorescent tags of biomolecules by using click chemistry have been designed by Kele *et al.*[10] The click reaction occurs between an azide and a terminal alkyne in the presence of Cu(I) catalyst, producing 1,4-disubstituted 1,2,3-triazoles in quantitative yields.[11] The high specificity, compatibility with water, and high yielding nature of this reaction makes it potentially applicable for a variety of *in vivo* applications. In addition, the triazole moiety formed by the click reaction was also utilized as binding sites for specific metal ions.[12]

Keeping the aforementioned research in mind, we herein report the synthesis of a new triazole-based compound (**5**) bearing rhodamine 6G (energy donor) and nile red (energy acceptor) fluorophore as a FRET 'OFF→ON' chemosensor.

Compound **5** was prepared by the overall synthetic routes outlined in Scheme 1. This is based on two reaction partners, namely rhodamine-alkyne (**1**) and nile-red-azide (**4**). Rhodamine 6G was treated with propargylamine and triethylamine in methanol to give rhodamine-alkyne (**1**) as a colorless solid in 27% yield. Synthesis of **4**, functionalized with azide group, started from nile-red which was treated with monotosylated ethylene glycol in the presence of K_2CO_3 in DMF/ethyl acetate (v/v, 1:1) to give **2** in 37 % yield. Subsequently, **2** was treated with TsCl and NaOH in THF to afford **3** in 64% yield. Substitution reaction by NaN_3 in DMF gave nile-red-azide (**4**) in 24% yield. Compound **5** as a target was then obtained in 19% yield *via* click reaction by reacting **1** with **4** in the presence of CuI in DCM/THF (v/v, 1:1). The overall chemical structures of compounds **1-5** were confirmed by ^{1}H-NMR, ^{13}C-NMR spectroscopy, and FAB-MS (experimental section). Formation of the spirolactam ring of the rhodamine unit in **1** and **5** was confirmed by the ^{13}C-NMR spectra where the spirolactam quaternary carbon peak (~ 65 ppm) is present.

To get an insight into the FRET system working in **5**, we have investigated the absorption and fluorescence changes upon addition of the chloride salts of various metal ions including Li(I), Na(I), K(I), Cs(I), Mg(II), Ca(II), Sr(II), Ba(II), Mn(II), Cu(II), Zn(II), Cd(II), Co(II), Ni(II), Hg(II), Pb(II), Fe(II), and Fe(III) in CH_3CN/H_2O (1:1). In Figure 2, compound **5** shows absorption band at 564 nm and emission band at 636 nm, corresponding to Nile red component. The ring closed rhodamine part (energy donor) in **5** absorbs the UV light at ~300 nm, but no emission band observed (non-fluorescent). Accordingly, it is obvious that the FRET system of **5** from Rhodamine 6G to Nile red cannot be operated (FRET-Off).

On the other hand, unlike other metal ions, upon addition of Fe(III) ion, the solution of **5** displayed new strong absorption band at 526 nm and emission band at 552 nm, respectively. Both UV-vis and fluorescence emission results indicate that the binding interaction between **5** and Fe(III) ion induces the ring-opening of spirolactam of rhodamine **5**. Notably, in regard of FRET, the resulting emission (552 nm) spectrum of **5**•Fe(III) complex overlaps well with the absorption band of the Nile red (564 nm), fulfilling a favorable FRET condition. We then found that the fluorescence intensity of Nile red (energy acceptor) at 636 nm was also increased upon the Fe(III) ion complexation with irradiation at 526 nm as shown in Figure 2(b).

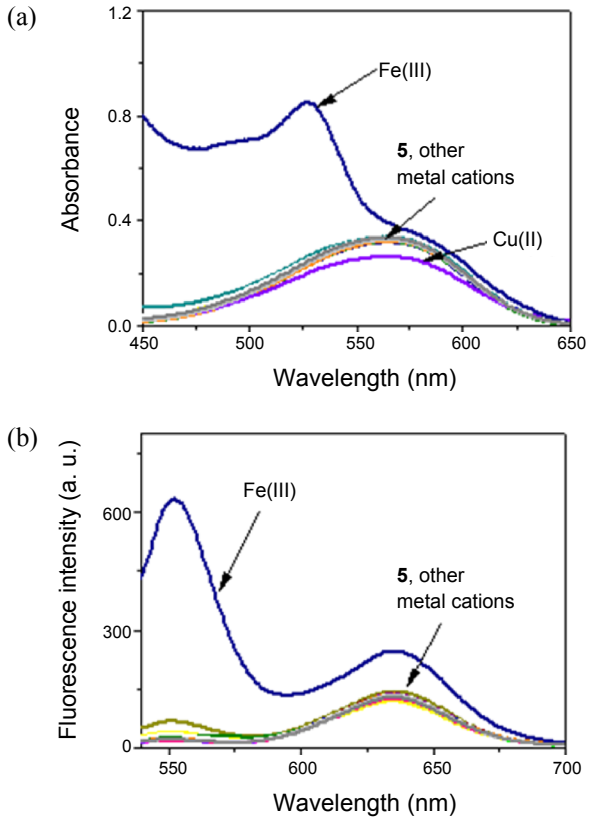


Figure 2. Absorption (a) and fluorescence (b) spectra of **5** (10 μM) upon addition of the chloride salts of various metal ions including Li(I), Na(I), K(I), Cs(I), Mg(II), Ca(II), Sr(II), Ba(II), Mn(II), Cu(II), Zn(II), Cd(II), Co(II), Ni(II), Hg(II), Pb(II), Fe(II), and Fe(III) in CH_3CN/H_2O (v/v, 1 :1) with an excitation at 526 nm. Excitation/emission slits = 3/5.

Figure 3 gives detailed fluorescence changes of **5** upon gradual addition of Fe(III) ion (up to 500 equiv). The titration of Fe(III) ion induces a new emission band at 552 nm (Rhodamine) and enhances the fluorescence intensity at 636 nm (Nile red). However, in this case, although there is a FRET pattern in this system upon addition of Fe(III), a ratiometric fluorescence change could not be seen probably due to marked emission enhancement at 552 nm upon Fe(III) ion binding.

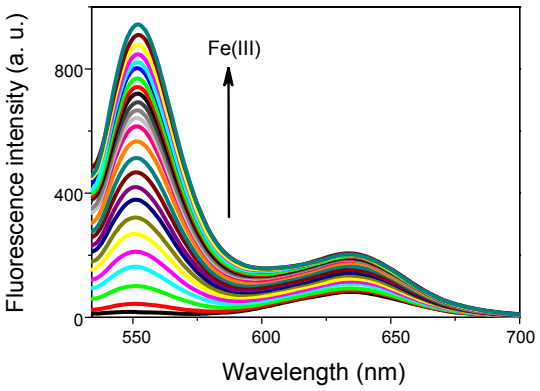


Figure 3. Fluorescence spectra of **5** (10 μM) upon addition of increasing concentrations of $FeCl_3$ (0 ~ 500 equiv) in CH_3CN/H_2O (v/v, 1 :1) with an excitation at 526 nm. Excitation/emission slits = 3/5.

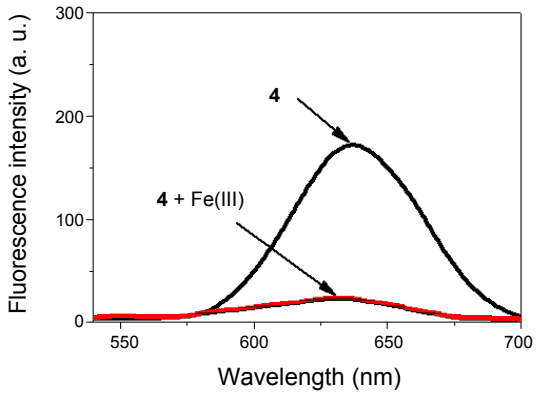


Figure 4. Fluorescence spectra of **4** (10 μM) in the absence and presence of Fe(III) ions in CH_3CN/H_2O (v/v, 1 :1) with an excitation at 526 nm. Excitation/emission slits = 3/5.

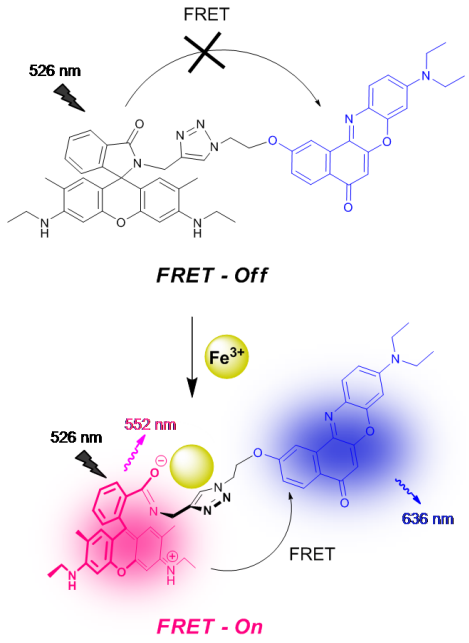


Figure 5. Fe(III)-induced FRET OFF-ON upon irradiation at 526 nm.

To more precisely understand the FRET system in 5, we tested the fluorescence changes of Nile red **4** having only acceptor part upon addition of Fe(III) ion (Figure 4). At the same condition to that of **5**, compound **4** showed fluorescence quenching in the presence of Fe(III) ion. Reason for the quenching mechanism of **4** with Fe(III) ion is not clear at this stage, but from this result we can notice that the increasing fluorescence at 636 nm in the titration spectra of **5** with addition of Fe(III) as seen in Figure 3 is solely attributable to the energy transfer from ring-opened rhodamine (donor) to Nile red (acceptor), that is FRET-On (Figure 5).

In summary, we have synthesized a new compound **5** containing both rhodamine 6G and Nile-red linked through triazole ring spacer prepared by click reaction. Upon addition of Fe(III), irradiation of absorption wavelength of ring-opened rhodamine **5** (energy donor) gives rise to FRET emission band (Nile red; energy acceptor).

Experimental Section

Preparation of 5. Copper iodide (15 mg) was added to propargyl Rhodamine 6G (**1**) (15 mg, 0.0372 mmol) and Nile Red azide (**4**) (16.7 mg, 0.0372 mmol) in 10 mL of CH_2Cl_2/THF (1:1) and the mixture heated at 60 °C for 4 h. The resulting solution was evaporated by rotary evaporation and dissolved in CH_2Cl_2 (50 mL). The organic layer was washed with water (300 mL), dried over anhydrous Na_2SO_4 and filtered. The residue was purified by column chromatography on silica gel, eluting with CH_2Cl_2-methanol (20:1) to give **5** (6 mg, 19%) as a dark purple solid. mp 174 °C. ^{1}H NMR (300 MHz) δ 8.21 (1H, d, J = 8.7 Hz), 8.01 (1H, d, J = 2.8 Hz), 7.97 (1H, dd, J_1 = 5.6 Hz, J_2 = 2.2 Hz), 7.60 (1H, d, J = 9.1 Hz), 7.46-7.43 (2H, m), 7.23 (1H, s), 7.14 (1H, dd, J_1 = 8.9 Hz, J_2 = 2.6 Hz), 7.04 (1H, dd, J_1 = 6.1 Hz, J_2 = 2.6 Hz), 6.65 (1H, dd, J_1 = 9.1 Hz, J_2 = 2.8 Hz), 6.45 (1H, d, J = 2.8 Hz), 6.32 (2H, s), 6.30 (1H, s), 6.10 (2H, s), 4.57 (2H, t, J = 5.5 Hz), 4.50 (2H, s), 4.41 (2H, t, J = 5.5 Hz), 3.47 (4H, q, J = 7.0 Hz), 3.18 (4H, m), 1.82 (6H, s), 1.27 (12H, m). ^{13}C NMR (100 MHz) δ 12.8, 14.97, 16.88, 35.2, 38.6, 45.3, 49.1, 65.2, 66.6, 96.5, 96.8, 105.5, 105.9, 106.5, 109.9, 117.8, 118.8, 123.2, 123.7, 124.0, 124.9, 126.5, 128.2, 128.3, 128.7, 130.9, 131.3, 132.9, 134.3, 139.7, 144.3, 147.1, 147.5, 151.1, 151.9, 152.3, 153.9, 160.6, 168.2, 183.3. FAB MS m/z (M^+) calcd 854.99, found 855.7.

Preparation of 1. Under nitrogen, a solution of rhodamine 6G (500 mg, 1.044 mmol), propargylamine hydrochloride (96 mg, 1.044 mmol), and triethylamine (5 mL) as a base in methanol (30 mL) was heated at 80 °C. After refluxing for 24 h, the resulting solution was evaporated by rotary evaporation and dissolved in CH_2Cl_2 (200 mL). The organic layer was washed with water (2 × 300 mL), dried over anhydrous Na_2SO_4 and filtered. The residue was purified by column chromatography on silica gel, eluting with ethyl acetate-hexane (1:3) to give **1** (125 mg, 27%) as a pale pink solid. mp 254 °C. ^{1}H NMR (300 MHz) δ 7.96-7.94 (1H, m), 7.46-7.42 (2H, m), 7.08-7.03 (1H, m), 6.36 (2H, s), 6.29 (2H, s), 3.93 (2H, d, J = 2 Hz), 3.54 (1H, br), 3.21 (4H, q, J = 7 Hz), 1.90 (6H, s), 1.32 (6H, t, J = 7.1 Hz). ^{13}C NMR (100 MHz) δ 14.7, 16.7, 28.6, 38.4, 65.0, 70.1, 78.3, 96.5, 105.3, 117.7, 123.1, 123.7, 128.0, 128.8, 130.2, 132.7, 147.4, 151.8, 154.0, 167.6. FAB MS m/z (M^+) calcd 451.56, found 452.3.

Preparation of 2. Under nitrogen, a solution of Nile Red[13] (240 mg, 0.711 mmol), monotosylated ethylene glycol[14] (153 mg, 0.711 mmol), and K_2CO_3 (100 mg, 0.723 mmol) in 30 mL of DMF/ethyl acetate (1:1) was heated at 80 °C. After refluxing for 12 h, the resulting solution was evaporated by rotary evaporation and dissolved in ethyl acetate (200 mL). The organic layer was washed with water (2 × 300 mL), dried over anhydrous $MgSO_4$ and filtered. The residue was purified by column chromatography on silica gel, eluting with ethyl acetate-hexane (1:2) to give **2** (100 mg, 37%) as dark purple solid. ^{1}H NMR (300 MHz) δ 8.23 (1H, d, J = 8.7 Hz), 8.06 (1H, d, J = 2.6 Hz), 7.58 (1H, d, J = 9.1 Hz), 7.19 (1H, dd, J_1 = 8.7 Hz, J_2 = 2.7 Hz), 6.66 (1H, dd, J_1 = 9.1 Hz, J_2 = 2.7 Hz), 6.45 (1H, d, J = 2.7 Hz), 6.31 (1H, s), 4.52 (2H, t, J = 4.5 Hz), 4.39 (2H, t, J = 4.5 Hz), 3.47 (4H, q, J = 7.1 Hz), 1.26 (6H, t, J = 7.1 Hz). ^{13}C NMR (100 MHz) δ 12.9, 45.3, 61.6, 69.8, 96.5, 105.4, 106.9,

109.8, 118.4, 124.9, 126.2, 128.1, 131.3, 134.3, 140.0, 147.1, 151.0, 152.3, 161.5, 183.4. FAB MS *m/z* (M^+) calcd 378.42, found 379.1.

Preparation of 3. Under nitrogen, a solution of **2** (100 mg, 0.264 mmol), TsCl (55 mg, 0.288 mmol), and NaOH (15 mg, 0.375 mmol) in 10 mL of THF was stirred for 12 h. The resulting solution was evaporated by rotary evaporation and dissolved in CH_2Cl_2 (200 mL). The organic layer was washed with water (2 × 300 mL), dried over anhydrous $MgSO_4$ and filtered. The residue was purified by column chromatography on silica gel, eluting with ethyl acetate-hexane (1:3) to give **3** (90 mg, 64%) as dark purple solid. mp 184 °C. ^{1}H NMR (300 MHz) δ 8.18 (1H, d, J = 8.6 Hz), 7.94 (1H, d, J = 2.6 Hz), 7.84 (2H, d, J = 8.4 Hz), 7.60 (1H, d, J = 9.2 Hz), 7.34 (2H, d, J = 8.4 Hz), 7.01 (1H, dd, J_1 = 8.8 Hz, J_2 = 2.6 Hz), 6.67 (1H, dd, J_1 = 9.2 Hz, J_2 = 2.8 Hz), 6.46 (1H, d, J = 2.8 Hz), 6.30 (1H, s), 4.47 (2H, m), 4.37 (2H, m), 3.48 (4H, q, J = 7.2 Hz), 2.43 (3H, s), 1.27 (6H, t, J = 7.2 Hz). ^{13}C NMR (100 MHz) δ 12.6, 21.7, 45.1, 65.6, 68.0, 96.2, 105.2, 106.3, 109.6, 118.3, 124.7, 126.1, 127.8, 128.0, 129.9, 131.1, 132.8, 134.0, 145.1, 146.9, 150.8, 152.1, 160.5, 183.1. FAB MS *m/z* (M^+) calcd 532.61, found 533.3.

Preparation of 4. Under nitrogen, a solution of 3 (330 mg, 0.620 mmol) and sodium azide (60 mg, 0.929 mmol) in DMF was heated at 90 °C. After refluxing for 24 h, the resulting solution was evaporated by rotary evaporation and dissolved in ethyl acetate (300 mL). The organic layer was washed with water (2 × 300 mL), dried over anhydrous $MgSO_4$ and filtered. The residue was purified by column chromatography on silica gel, eluting with ethyl acetate-hexane (1:3) to give **4** (59 mg, 24%) as dark purple solid. mp 144 °C. ^{1}H NMR (300 MHz) δ 8.24 (1H, d, J = 8.7 Hz), 8.06 (1H, d, J = 2.7 Hz), 7.60 (1H, d, J = 9.0), 7.21 (1H, dd, J_1 = 8.7 Hz, J_2 = 2.7 Hz), 6.66 (1H, dd, J_1 = 9.0 Hz, J_2 = 2.8 Hz), 6.46 (1H, d, J = 2.8 Hz), 6.31 (1H, s), 4.38 (2H, t, J = 4.9 Hz), 3.70 (2H, t, J = 4.9 Hz), 3.48 (4H, q, J = 7.1 Hz), 1.27 (6H, t, J = 7.1 Hz). ^{13}C NMR (100 MHz) δ 12.6, 45.1, 50.1, 67.2, 96.2, 105.2, 106.3, 109.6, 118.4, 124.7, 126.1, 127.9, 131.1, 134.0, 139.6, 146.9, 150.8, 152.1, 160.8, 183.1. FAB MS *m/z* (M^+) calcd 404.43, found 404.0.

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References

1. (a) Gunnlaugsson, T.; Davis, A. P.; O'Brien, J. E.; Glynn, M. *Org. Lett.* **2002**, *4*, 2449. (b) Kim, S. K.; Yoon, J. *Chem. Commun.* **2002**, 770.
2. Beer, P. D. *Acc. Chem. Res.* **1998**, *31*, 71.
3. (a) Xu, Z.; Xiao, Y.; Qian, X.; Cui, J.; Cui, D. *Org. Lett.* **2005**, *7*, 889. (b) Lee, M. H.; Lee, S. W.; Kim, S. H.; Kang, C.; Kim, J. S. *Org. Lett.* **2009**, *11*, 2101. (c) Quang, D. T.; Kim, J. S. *Chem. Rev.* **2010**, *110*, 6280.
4. (a) Nishizawa, S.; Kato, Y.; Teramae, N. *J. Am. Chem. Soc.* **1999**, *121*, 9463. (b) Wu, J.-S.; Zhou, J.-H.; Wang, P.-F.; Zhang, X.-H.; Wu, S. K. *Org. Lett.* **2005**, *7*, 2133. (c) Schazmann, B.; Alhashimy, N.; Diamond, D. *J. Am. Chem. Soc*. **2006**, *128*, 8607.
5. Wu, J.-S.; Liu, W.-M.; Zhuang, X.-Q.; Wang, F.; Wang, P. F.; Tao, S. L.; Zhang, X.-H.; Wu, S.-K.; Lee, S.-T. *Org. Lett.* **2007**, *9*, 33.
6. Lim, N. C.; Schuster, J. V.; Porto, M. C.; Tanudra, M. A.; Yao, L.; Freake, H. C.; Bruckner, C. *Inorg. Chem.* **2005**, *44*, 2018.
7. (a) Serin, J. M.; Brousmiche, D. W.; Frechet, J. M. J. *J. Am. Chem. Soc.* **2002**, *124*, 11848. (b) Albers, A. E.; Okreglak, V. S.; Chang, C. J. *J. Am. Chem. Soc.* **2006**, *128*, 9640. (c) Lee, S. H.; Kim, S. K.; Bok, J. H.; Lee, S. H.; Yoon, J.; Lee, K.; Kim, J. S. *Tetrahedron Lett.* **2005**, *46*, 8163. (d) Dichtel, W. R.; Serin, J. M.; Edder, C.; Frechet, J. M. J.; Matuszewski, M.; Tan, L.-S.; Ohulchanskyy, T. Y.; Prasad, P. N. *J. Am. Chem. Soc.* **2004**, *126*, 5380.
8. (a) Kim, H. N.; Lee, M. H.; Kim, H. J.; Kim, J. S.; Yoon, J. *Chem. Soc. Rev.* **2008**, *37*, 1465. (b) Lee, M. H.; Kim, S. H.; Lee, Y. H.; Giap, T. V.; Kang, C.; Kim, J. S. *Chem. Comm.* **2010**, *46*, 1407. (c) Lee, M. H.; Wu, J. S.; Lee, J. W.; Kim, J. S. *Org. Lett*. **2007**, *9*, 2501.
9. (a) Lee, Y. H.; Lee, M. H.; Zhang, J. F.; Kim, J. S. *J. Org. Chem.* **2010**, *75*, 7159. (b) Lee, M. H.; Kim, H. J.; Yoon, S.; Park, N.; Kim, J. S. *Org. Lett.* **2008**, *10*, 213. (c) Lee, S. Y.; Kim, H. J.; Wu, J.-S.; No, K.; Kim J. S. *Tetrahedron Lett.* **2008**, *49*, 6141. (d) Othman, A. B.; Lee, J. W.; Wu, J.-S.; Kim, J. S.; Abidi, R.; Thuery, P.; Strub, J. M.; Dorsselaer, A. V.; Vicens, J. *J. Org. Chem.* **2007**, *72*, 7634.
10. Kele, P.; Mezö, G.; Achatz, D.; Wolfbeis, O. S. *Angew. Chem. Int. Ed.* **2009**, *48*, 344.
11. (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem.* **2001**, *113*, 2056; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004. (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem.* **2002**, *114*, 2708; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596.
12. (a) Kim, S. H.; Choi, H. S.; Kim, J.; Lee, S. J.; Quang, D. T.; Kim, J. S. *Org. Lett.* **2010**, *12*, 560. (b) Park, S. Y.; Yoon, J. H.; Hong, C. S.; Kim, J. S.; Matthews, S. E.; Souane, R.; Vicens, J. *J. Org. Chem.* **2008**, *73*, 8212.
13. (a) Nagy, K.; Göktürk, S.; Biczók, L. *J. Phys. Chem. A* **2003**, *107*, 8784. (b) Briggs, M. S. J.; Bruce, I.; Miller, J. N.; Moody, C. J.; Simmonds, A. C.; Swann, E. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1051.
14. Bradshaw, J. S.; Krakowiak, K. E.; Lindh, G. C.; Izatt, R. M. *Tetrahedron* **1987**, *43*, 4271.