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Notes

Antihypertensive Effects of 5-(4-Nitrobenzenediazo)-8-benzenesulfonamidoquinaldine in Spontaneously Hypertensive Rats†

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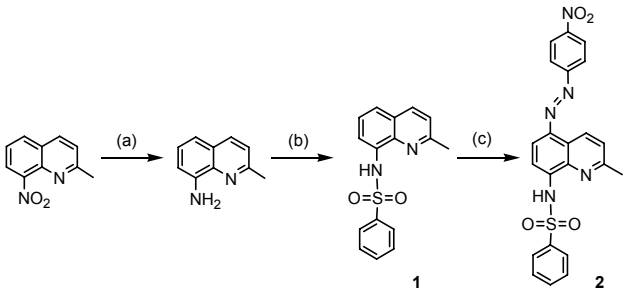
Hypertension is the major risk factor for cardiovascular events and the progression of cardiovascular disease. The renin-angiotensin-aldosterone system (RAAS) and angiotensin II have important roles in the regulation of systolic blood pressure and the prognosis of hypertension. Angiotensin converting enzyme (ACE) is widely distributed not only in the cardiovascular system, but also in various tissues (e.g. kidney, aorta, serum, lung, and heart).[1] In vasculature, ACE promotes cellular proliferation in kidney, it causes angiotensin II production, causing retention of water, sodium, which play important roles in long-term stabilization of hypertension.[2] Several reports have shown the ability of angiotensin-converting enzyme (ACE) inhibitors to prevent or reverse the cardiovascular changes (vascular smooth muscle hypertrophy, collagen synthesis, endotherial dysfunction).[3-5] Zinc (II) ion is important for protein synthesis and the regulation of cell production in the immune system.[6-8] In the Zinc-deprived rats model, Reeves et al. showed that there is a positive correlation between zinc(II) ion concentration and ACE activity.[9] However, no information is available for the relationship of zinc(II)-chelating agent (**2**) and anti hypertensive effect *in vivo*.

As a part of our research projects, these finding has prompted us to apply a zinc(II)-chelating agent (**2**) for antihypertensive effects utilizing the inhibition of angiotensin converting enzyme (ACE) activity in spontaneously hypertensive rats (SHR) model.

Fluorescent molecules with intramolecular charge transfer (ICT) characters have been widely developed for ionic sensing, molecular switching and fluorescent labelling due to their longer emission wavelength and sensitive spectral changes.[10] Herein, we have synthesized an effective ICT compound, 5-(4-nitrobenzenediazo)-8-benzenesulfonamidoquinaldine (**2**) for Zn^{2+} complexation. The benzenesulfonamidoquinaldine scaffold provides not only an excellent fluorophore candidate but also a pseudocavity for cation complexation. In **2**, the fluorophore itself acts as an intrinsic binding site, which may remarkably affect fluorescent characters when bound with cations. 4-Nitrobenzenediazo moiety is conjugated to the fluorophore to increase ICT character. To this report, we describe the actual chelation ability of **2** towards Zn^{2+} ion elucidated by UV-vis and fluorescence spectra as well as its ACE activity controls *in vivo*/*vitro* for an antihypertensive effect.



Scheme 1. Synthetic pathways of **1** and **2**. (a) H_2/Pd-C/Dioxane/12 h, (b) Benzenesulfonyl chloride/Pyridine/0 °C/5 h, (c) *p*-Nitrobenzenediazoniumtetrafluoroborate/Pyridine/THF/0 °C/12 h

Compound **2** was synthesized from 8-nitroquinaldine *via* a three-step procedure (reduction, sulfonamidation and diazotization) in *ca.* 18% overall yield (see Experimental Section).

The absorption spectra of free **2** give a characteristic ICT band centered at 407 nm. When Zn^{2+} was gradually added, the λ_{max} displayed a 38 nm red shift with a well-defined isosbestic point at 423 nm (Figure 1a). When Zn^{2+} was gradually added, the λ_{max} bathochromically shifted by 36 nm to 436 nm and the solution color changed from yellow to red. In addition, as depicted in Figure 1b, molecule **2** is non-fluorescent whereas it gives a strong emission upon the addition of Zn^{2+}. The inset of Figure 1b shows that the addition of only 0.2 equiv of Zn^{2+} increases the fluorescence by 90% and the fluorescence reaches a plateau at 0.5 equiv of Zn^{2+}. The corresponding calculated association constant of **2** for Zn^{2+} was $4.27 \pm 0.03 \times 10^7$ M^{-1}. This demonstrates that **2** is a very sensitive and selective zinc-chelating agent.

In aspect of metal ion selectivity of **2** with respect to the fluorescence enhancement, **2** shows a remarkable Zn^{2+} ion selecti-

†This paper is dedicated to Professor Hasuck Kim for his outstanding contribution to electrochemistry and analytical chemistry.

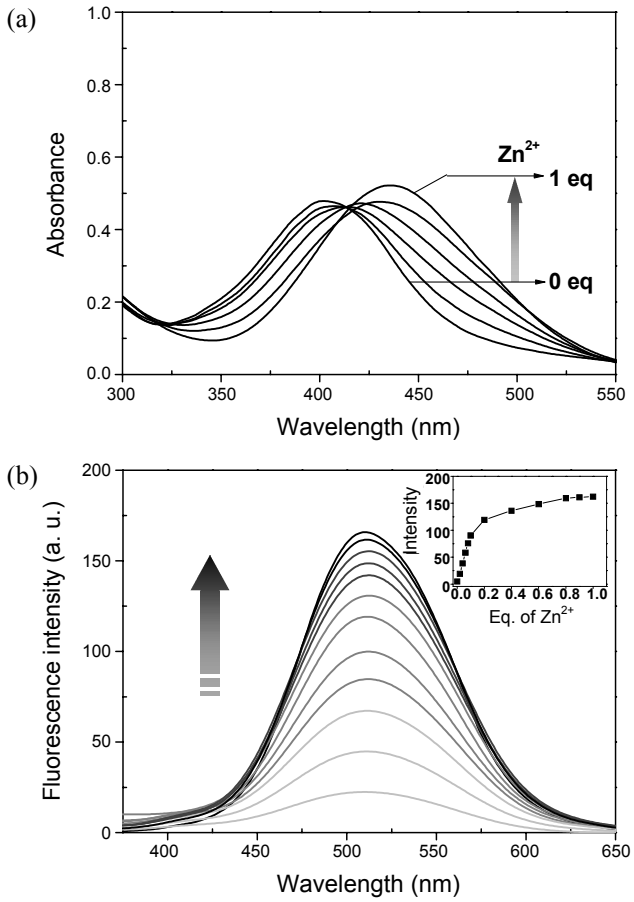


Figure 1. (a) UV-vis and (b) fluorescence spectra of **2** (20 μM and 10 μM, respectively) upon addition of different concentrations of $ZnCl_2$ (0, 2, 4, 8, 12, 16 and 20 μM, respectively) in CH_3CN/H_2O solution (1:1, v/v). Excitation at 365 nm; Inset 2b: fluorescence intensity of **2** at 513 nm *vs.* equiv of Zn^{2+}.

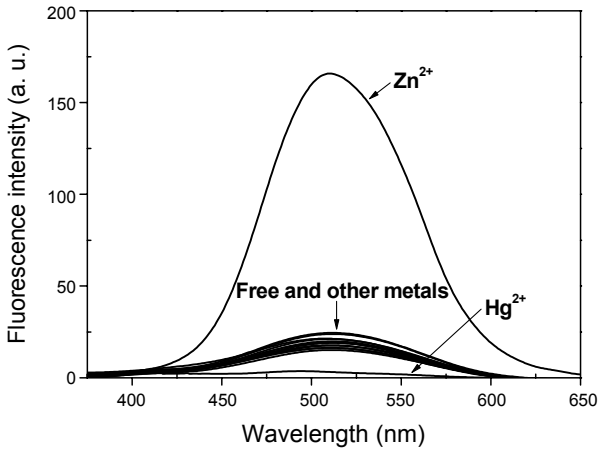


Figure 2. Fluorescence responses of **2** (10 μM) in CH_3CN/H_2O solution (1/1, v/v) at pH 7.4 (0.02 M HEPES) in the presence of (a) chloride salts of various metal cations (Li^+, Rb^+, Cs^+, Sr^{2+}, Ba^{2+}, Cu^{2+}, Cd^{2+}, Hg^{2+} and Zn^{2+} (100 μM, respectively) and Na^+, K^+, Ca^{2+} and Mg^{2+} (5 mM, respectively)). Excitation at 365 nm.

vity over other metal ions (Figure 2). Even under physiological conditions in such highly concentrated Na^+, K^+, Ca^{2+} and Mg^{2+} (~5.0 mM) solution,[11] we could not observe any significant

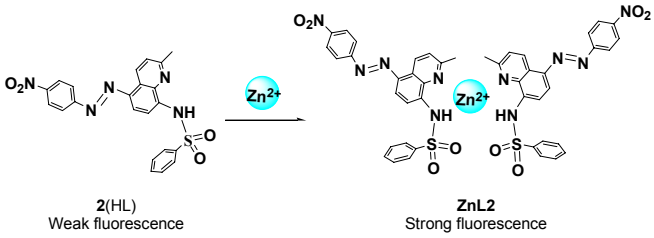


Figure 3. Complexation mechanism of **2** for Zn^{2+} ion.

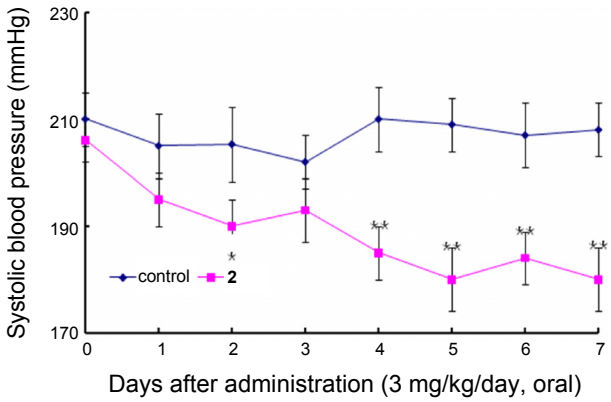


Figure 4. Antihypertensive effects of the **2** on the SHR. Each group was treated with the indicated doses of the **2** for 7 days. The values are mean ± SD (n = 5). *denote a significant difference compared with the measurement before administration of **2**. (*$p < 0.05$). **denote a significant difference compared with the control-SHR group (*$p < 0.05$).

fluorescence changes but almost the same fluorescence intensity as that of **2** itself. Interestingly, on the other hand, **2** was found to be quenched by the addition of Hg^{2+} ion. The fluorescence changes upon Hg^{2+} ion binding, however, can be ignorable in biological environment because the intracellular concentration of free Zn^{2+} is much higher than that of Hg^{2+}. A Job's plot analysis exhibited a maximum at 0.33 mol fraction of Zn^{2+}, indicating the formation of 2:1 complex between **2**(HL) and Zn^{2+}. The FAB-MS analysis with the result of m/z = 958 (**ZnL2**) gives further solid evidence of 2:1 stoichiometry. According to Job's plot and mass spectral data, we herewith suggest the complexation mechanism of the **2** for Zn^{2+} ion as shown in Figure 3.

Zinc(II) ion is a divalent positive ion which influences DNA synthesis, micro-channel polymerization, gene manifestation, cell apoptosis, and the immune system, as well as the activities of carbonic anhydrase and matrix metalloproteinase.[12-15] Zinc(II) also plays a neurotransmitter role and is known to be an element that participates in central nervous system-related diseases such as Alzheimer's and epilepsy.[16-18] Recently, candidates of new drug substances, which control various diseases, are being developed through Zinc(II) chelation. Among these substances are anticancer drugs, circulating systemic disease treatments, and Alzheimer's treatments.[19-21] We have screened the mentioned possibilities of physiological activities of **2**. Among those possibilities, we have discovered the antihypertensive activity of **2** with the spontaneously hypertensive rat model system.

For the confirmation of antihypertensive effect, we measured

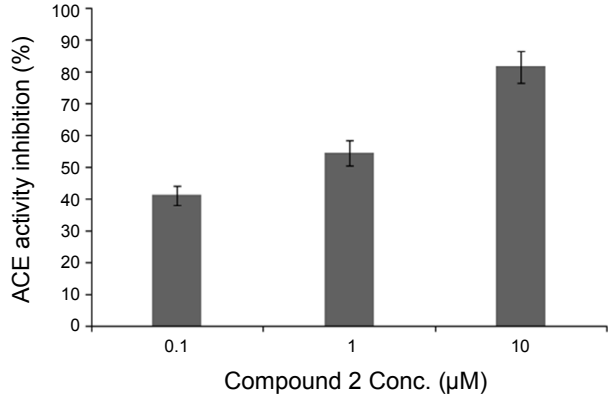


Figure 5. Percent inhibition of ACE activity in dose-dependent manner of **2**.

indirectly the systolic blood pressure of rat though the tail cuff method. Repeated oral administration was performed with using **2** for 7 days. Systolic blood pressure significantly decreased from 2 days to 7 days (Figure 4). In the **2**-treated SHR group, measurements before the administration showed significantly higher values compared with the 2-treated SHR ($p < 0.05$) on 7 days. Also, as shown in this figure, systolic blood pressure in the **2**-treated SHR group was significantly decreased compared with the control-treated SHR ($p < 0.05$) on 4 - 7 days.

To determine a possible correlation between ACE inhibition activity and **2**, the ACE inhibition activity was measured with *in vitro* ACE inhibition assay method. Figure 5 showed that the inhibitory effect of **2** on ACE activity was dose-dependent. IC50 of **2** on ACE activity was 0.36 uM.

In conclusion, we here presented synthetic methods of 5-(4-nitrobenzenediazo)-8-benzenesulfonamidoquinaldine (**2**) as a selective Zn^{2+} ion chelating agent. The selectivity and sensitivity of the **2** for Zn^{2+} ion over other metal ions were proven by UV absorption and fluorescence spectral changes. Biological assays illustrate that **2** can induce an antihypertensive effects through the inhibition of angiotensin converting enzyme (ACE) activity in spontaneously hypertensive rats (SHR) model. Therefore, these results may suggest that **2** can be much utilized as a novel antihypertensive drug.

Experimental Section

Synthesis.

8-Benzenesulfonamidoquinaldine (1): Benzenesulfonyl chloride (2.48 g, 14 mmol) was added dropwise to a cooled, stirred solution of 8-aminoquinaldine (1.85 g, 11.7 mmol) in 10 mL of pyridine and the mixture was stirred in an ice bath for 3 hours. Ice water was then added and the resulting precipitate was filtered, washed well with water, dried and dissolved in CH_2Cl_2. The resulting solution was washed with 1 M HCl and the aqueous phase was extracted with CH_2Cl_2 (300 mL). The organic layer was washed with water (2 × 300 mL) and dried over anhydrous $MgSO_4$. The crude product was further purified by column chromatography using ethyl acetate/hexane (1/3, v/v) as eluent on silica gel to give **1** in 83% yield. mp 158 - 159 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.23 (s, 1H), 7.98-7.74 (m, 4H), 7.44-7.25 (m, 6H), 2.69 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 159.7, 145.1, 141.0, 139.7, 138.8, 133.8, 129.0, 128.6, 128.0, 126.9, 125.2, 123.2, 122.8, 25.4. FAB-MS calc. for $C_{16}H_{15}N_2O_2S$ $[M+H]^+$ 299.0856, found 299.0836.

5-(4-Nitrobenzenediazo)-8-benzenesulfonamidoquinaldine (2): A solution of 8-benzenesulfonamidoquinaldine (1.0 g, 3.35 mmol)[22] and 4-nitrobenzenediazonium tetrafluoroborate (0.87 g, 3.6 mmol) in THF (100 mL) was stirred for 30 min at 0 °C, followed by the addition of pyridine (10 mL) dropwise with stirring for additional 12 h at 0 °C. The resulting solution was washed with water (300 mL) and extracted with CH_2Cl_2 (300 mL). The organic layer was washed with water (2 × 300 mL) and dried over anhydrous $MgSO_4$. The crude product was further purified by column chromatography using ethyl acetate/hexane (1/3, v/v) as eluent on silica gel to give **2** in 33% yield. mp 210 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.60 (s, 1H), 9.09 (d, J = 8.80 Hz, 1H), 8.35 (d, J = 7.20 Hz, 2H), 8.06-7.96 (m, 2H), 7.89 (d, J = 6.40 Hz, 2H), 7.50-7.24 (m, 4H), 2.77 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 158.8, 156.0, 148.5, 142.1, 139.2, 137.4, 137.3, 133.3, 132.4, 129.1, 128.2, 127.2, 125.4, 124.8, 124.4, 124.3, 113.5, 113.2, 25.2. FAB-MS calc. for $C_{22}H_{17}N_5O_4S$ [M+H]+ 448.1, found 448.0. Anal. Calcd for $C_{22}H_{17}N_5O_4S$: C, 59.05; H, 3.83; N, 15.65; O, 14.30; S, 7.17. Found: C, 59.07; H, 3.73.

Absorption and fluorescence spectra. All UV-vis and fluorescence spectra were recorded with S-3100 spectrophotometer and RF-5301PC spectrophotometer, respectively. Stock solutions (1.00 mM) of the metal perchlorate salts were prepared in CH_3CN. Stock solutions of **2** (1.00 mM) were prepared in CH_3CN. For all measurements of fluorescence spectra, excitation was at 343 nm with excitation and emission slit widths at 3.0 and 5.0 nm. UV-vis and fluorescence titration experiments were performed using 20 μM and 10 μM of **2** in CH_3CN/H_2O solution (1/1, v/v) with varying concentrations of the metal chloride salts.

Measurement of systolic blood pressure in SHR. The animals used were male SHR (systolic blood pressure > 200 mmHg) in conventional conditions (Temp.: 25 °C, Humidity: 50%). These rats were fed with general diet during acclimation. Thereafter SHR were fed with **2** by oral administration for 7 consecutive days (3 mg/kg/day, oral, for 7 days). Systolic blood pressure was measured by tail cuff method using model 29 Pulse Amplifier (Flat-bed recorder & Amplifier operating instruments, USA).

***In vitro* ACE inhibition assay.** Angiotensin converting enzyme was from rabbit lung lyophilized powder and Hippuric acid-Histidine-Leucine (Hip-His-Leu), bradykinin and Epigallocatechin (EGCC) were purchased from Sigma Co.. The ACE assay of Cheung and Cushman was modified. The ACE activity inhibition (%) was determined by using the equation described below:

$$\text{ACE activity inhibition (\%)} = [1-\{(S-Sc)/(B-Bc)\}] \times 100$$

S = the absorbance at 228 nm in the presence of each sample
Sc = the absorbance as S except that enzyme reaction was quenched at zero time
B = the absorbance in the absence of the sample

Bc = the absorbance as S except that enzyme reaction was quenched at zero time

Data analysis. The results were expressed as means and standard deviations (S.D.). The comparison of the control-treated and compound **2** treated group in SHR was evaluated by Student's t-test.

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References

1. Sharifi, A. M.; Akbarloo, N.; Heshmatian, B.; Ziai, A. *Pharmacol. Res.* **2003**, *47*, 201-209.
2. Brewster, U. C.; Perazella, M. A. *Am. J. Med.* **2004**, *116*, 263-272.
3. Owens, G. K. *Hypertension* **1987**, *9*, 178-187.
4. Albaladejo, P.; Bouaziz, H.; Duriez, M.; Gohlke, P.; Levy, B. I.; Safar, M. E.; Benetos, A. *Hypertension* **1994**, *23*, 74-82.
5. Clozel, M.; Kuhn, H.; Baumgartner, H. R. *J. Cardiovasc. Pharmacol.* **1993**, *22*, 15-18.
6. Kaya, S.; Keçeci, T.; Haliloğlu, S. *Res. Vet. Sci.* **2001**, *71*, 135-139.
7. Scrimgeour, A. G.; Stahl, C. H.; McClung, J. P.; Marchitelli, L. J.; Young, A. J. *J. Nutr. Biochem*. **2007**, *18*, 813-819.
8. El Hendy, H. A.; Yousef, M. I.; Abo El-Naga, N. I. *Toxicology* **2001**, *167*, 163-170.
9. Reeves, P. G.; O'Dell, B. L. *Clin. Chem.* **1985**, *31*, 581-584.
10. (a) Taki, M.; Wolford, J. L.; O'Halloran, T. V. *J. Am. Chem. Soc.* **2004**, *126*, 712-713. (b) Rurack, K.; Kollmannsberger, M.; Daub, J. *Angew. Chem., Int. Ed.* **2001**, *40*, 385-387. (c) Peng, X.; Du, J.; Fan, J.; Wang, J.; Wu, Y.; Zhao, J.; Sun, S.; Xu, T. *J. Am. Chem. Soc.* **2007**, *129*, 1500-1501. (d) Wang, J. B.; Qian, X. F.; Cui, J. N. *J. Org. Chem.* **2006**, *71*, 4308. (e) Coskun, A.; Akkaya, E. U. *J. Am. Chem. Soc.* **2005**, *127*, 10464. (f) Kim, S. K.; Bok, J. H.; Bartsch, R. A.; Lee, J. Y.; Kim, J. S. *Org. Lett.* **2005**, *7*, 4839-4842. (g) Kim, J. S.; Quang, D. T. *Chem. Rev.* **2007**, *107*, 3780-3799. (h) Kim, S. K.; Lee, S. H.; Lee, J. Y.; Bartsch, R. A.; Kim, J. S. *J. Am. Chem. Soc.* **2004**, *126*, 16499-16506. (i) Jung, H. S.; Kwon, P. S.; Lee, J. W.; Kim, J. I.; Hong, C. S.; Kim, J. W.; Yan, S.; Lee, J. Y.; Lee, J. H.; Joo, T.; Kim, J. S. *J. Am. Chem. Soc.* **2009**, *131*, 2008-2012. (j) Lee, M. H.; Wu, J.-S.; Lee, J. W.; Jung, J. H.; Kim, J. S. *Org. Lett.* **2007**, *9*, 2501-2504.
11. Rae, T. D.; Schmidt, P. J.; Pufahl, R. A.; Culotta, V. C.; O'Halloran, T. V. *Science* **1999**, *284*, 805-808.
12. Srivastava, V.; Rawall, S.; Vijayan, V. K.; Khanna, M. *Indian J. Med. Res.* **2009**, *129*, 579-586.
13. Prasad, A. S.; Beck, F. W.; Snell, D. C.; Kucuk, O. *Nutr. Cancer* **2009**, *61*, 879-887.
14. Tatard, V. M.; Xiang, C.; Biegel, J. A.; Dahmane, N. *Cancer Res.* **2010**, *70*, 1236-1246.
15. Freitas, M.; Porto, G.; Lima, J. L.; Fernandes, E. *Biometals* **2010**, *23*, 31-41.
16. Miller, Y.; Ma, B.; Nussinov, R. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 9490-9495.
17. Miura, T.; Yoda, M.; Tsutsumi, C.; Murayama, K.; Takeuchi, H. *Yakugaku Zasshi.* **2010**, *130*, 495-501.
18. Lakatos, A.; Zsigó, E.; Hollender, D.; Nagy, N. V.; Fülöp, L.; Simon, D.; Bozsó, Z.; Kiss, T. *Dalton Trans.* **2010**, *39*, 1302-1315.
19. Baum, L.; Chan, I. H.; Cheung, S. K.; Goggins, W. B.; Mok, V.; Lam, L.; Leung, V.; Hui, E.; Ng, C.; Woo, J.; Chiu, H. F.; Zee, B. C.; Cheng, W.; Chan, M. H.; Szeto, S.; Lui, V.; Tsoh, J.; Bush, A. I.; Lam, C. W.; Kwok, T. *Biometals* **2010**, *23*, 173-179.
20. Mo, Z. Y.; Zhu, Y. Z.; Zhu, H. L.; Fan, J. B.; Chen, J.; Liang, Y. *J. Biol. Chem.* **2009**, *284*, 34648-34657.
21. Ding, W. Q.; Lind, S. E. *UBMB Life* **2009**, *61*, 1013-1018.
22. Lee, J. W.; Jung, H. S.; Kwon, P. S.; Kim, J. W.; Bartsch, R. A.; Kim, Y.; Kim, S.-J.; Kim, J. S. *Org. Lett.* **2008**, *10*, 3801-3804.