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Bimodal Chemiluminescence of 8-Chlorostyryl-6-phenylethynylimidazopyrazinone: Large Bathochromic Shift Caused by a Styryl Group at 8-Position[1]

Hideshi Nakamura,* Chun Wu, Daisuke Takeuchi, and Akio Murai

Division of Chemistry, Graduate School of Science, Hokkaido University, Sapporo 060, Japan.

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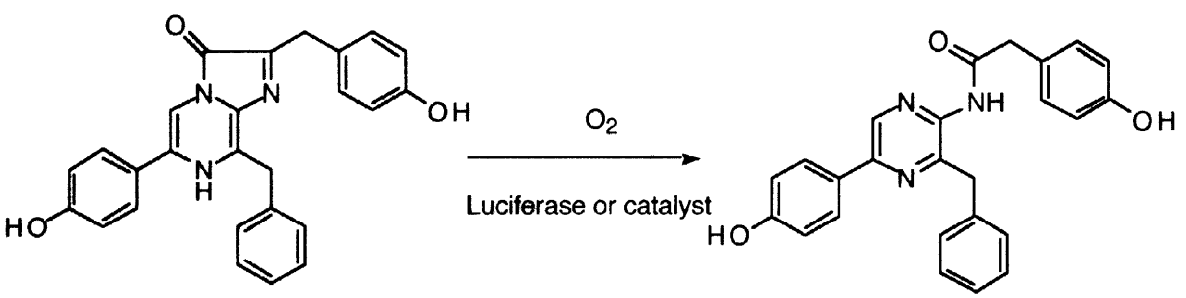
Abstract: 3,7-Dihydro-8-chlorostyryl-6-phenylethynyl-2-imidazo[1,2-*a*]pyrazin-3-one (**5a**) prepared from 2-amino-3,5-diphenylethynylpyrazine by condensation with methylglyoxal showed bimodal luminescence in DMSO, i.e., orange-colored luminescence under acidic to neutral conditions and yellow-colored luminescence under basic conditions. The large bathochromic shift of chemiluminescence caused by a styryl group at the 8 position was established by synthesizing 3,7-dihydro-8-styryl-6-phenylethynyl-2-imidazo[1,2-*a*]pyrazin-3-one (**5b**).

Chemi- and bioluminescences are now widely used as highly sensitive methods to detect chemicals such as superoxide, Ca^{++}, ATP, steroid hormones, proteineous enzymes, and nucleotides having a specific sequence.[2] Their high sensitivity allows the monitoring of transient biological events including Ca wave in a single cell and gene expression. Recently, we have developed a new synthetic approach to 2-amino-3,5-disubstituted pyrazines from commercially available 2-aminopyrazine which allows us to make various types of bioluminescent and/or chemiluminescent 6,8-disubstituted imidazo[1,2-*a*]pyrazin-3-ones including coelenterazine (**1**, chromogenic compound of a photoprotein, aequorin from a jellyfish *Aequorea aequorea*).[2] Coelenterazine also widely distributes among marine organisms even in non-bioluminescent invertebrates. Coelenterazine is oxidized by luciferase or apoaequorin under aerobic conditions and blue light emission occurs from an amide anion of coelenteramide (**2**). Several attempts to control the color of luminescence were made by structural modification of either coelenterazine[3a] or proteins.[3b]



Colenterazine (**1**) Coelenteramide (**2**)

During our course of studies on chemi- and bioluminescent imidazopyrazinones having a conjugated chromophore at the 6- and/or 8-position,[4] it was found that a cyclization reaction of 2-amino-3,5-diphenylethynylpyrazine (**4**)[5] with methylglyoxal in HCl-EtOH gave an imidazopyrazinone which showed bimodal chemiluminescence in DMSO, i.e., orange-colored luminescence under acidic to neutral conditions and yellow-colored luminescence under basic conditions. Imidazopyrazinone usually emits blue to yellow light from an excited singlet state of the corresponding amide anion under both acidic and basic conditions. Here we would like to describe the structure and chemiluminescence of the new type of imidazopyrazinone.

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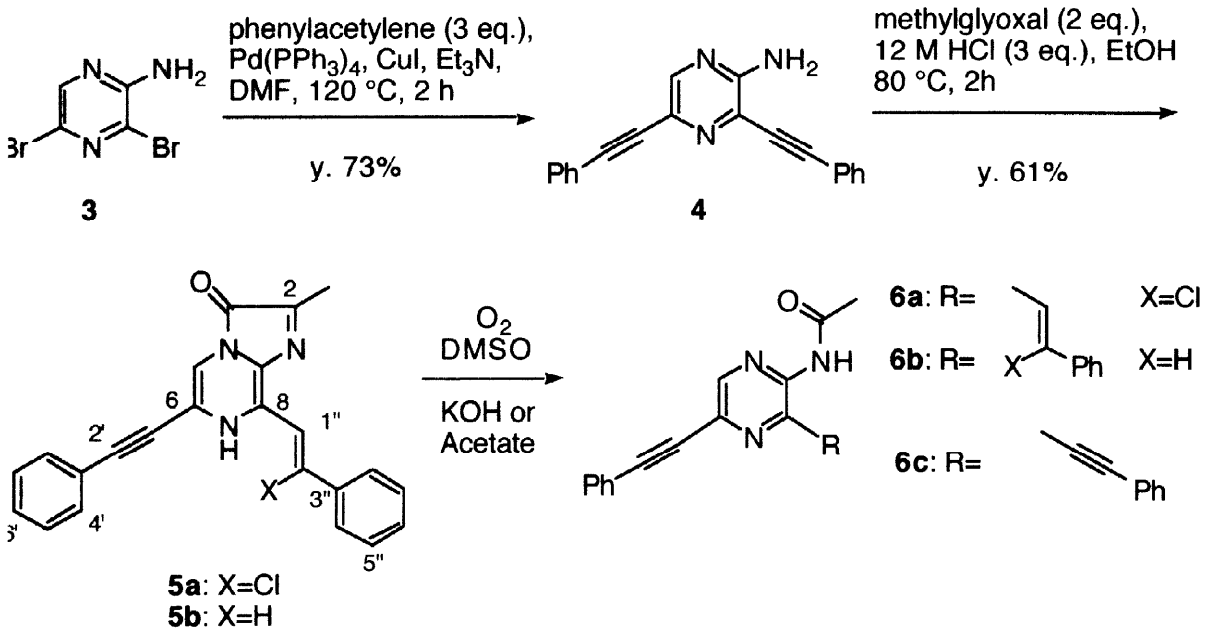
The bimodal chemiluminescent product was crystallized from a reaction mixture of the condensation of **4** with methylglyoxal as yellow amorphous solids, mp 132-133 °C. The structure was elucidated as **5a**[6] rather than 6,8-diphenylethynylimidazopyrazinone on the basis of its spectral data. Addition of HCl to a carbon-carbon triple bond was evident by HR-EIMS *m/z* 385.1007 (M^+) (Calcd for $C_{23}H_{16}N_3OCl$ 385.0981), coupled with existence of an olefinic proton signal at δ 7.79 in the ^{1}H-NMR spectrum (9:1 $CDCl_3$-CD_3OD containing trace amoutt of 12 M HCl). The newly formed double bond adopted *Z*-configuration which was determined by NOE experiments (4"-H to 1"-H). The position of the phenylethynyl substituent was established by HMBC spectrum (a cross peak between 5-H and C1'). Under a condensation reaction, a chloride ion attacked a C2" carbon of the triple bond activated by an imidazopyrazinone group and the thermodynamically stable double bond was formed through a chloro-allene intermediate.

Scheme 1.



Under neutral to acidic conditions, **5a** showed luminescence at λmax 590 nm. From the spent solution at the relatively higher concentration of **5a** (10^{-4} M) used, the expected product **6a**[7] was obtained in 41% yield. Like other imidazopyrazinone the light was supposed to be emitted from an amide anion of **6a**; however, it was not possible to measure the fluorescence spectrum of the amide anion in DMSO because of rapid elimination of HCl to afford an amide anion of **6c**.[8]

In contrast to orange-colored luminescence of **5a** under acidic to neutral conditions in DMSO, addition of 1 M KOH triggered yellow-colored luminescence whose spectrum was superimposable to the fluorescence spectrum of the spent solution. From the reaction mixture (10^{-4} M), an amide (**6c**) was isolated in 61% yield along with **4** (35% yield). The fluorescence spectrum of **6c** prepared from **4** with Ac_2O-Py in DMSO containing 1 M KOH is identical with the above chemiluminescence spectrum, suggesting that the chemiluminescence of **5a** under basic conditions was due to radiation from the excited singlet state of the amide anion of **6c** which formed **5a** by HCl elimination during the chemiluminescent process.

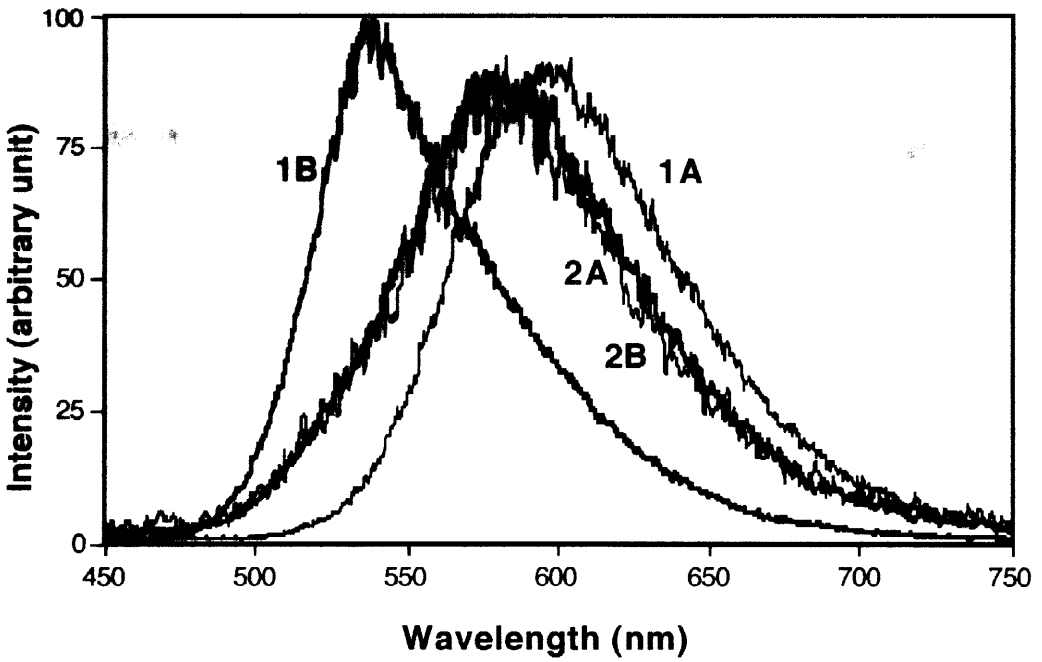


Fig. 1. Chemiluminescence spectra of **5a** (1) and **5b** (2) in DMSO with 1 M KOH (B) or with 0.1 M acetate buffer (A). The spectra were not corrected.

To confirm the large bathochromic shift caused by a chlorostyryl group at the 8-position of imidazopyrazinone, **5b** having a stable styryl chromophore was synthesized by a sequence of three step reactions from **3**: 1) Stille coupling with tributyl-*trans*-styrylstannane (y. 53%), 2) a second cross-coupling reaction with phenylacetylene (y. 90%), and 3) cyclization with methylglyoxal (y. 52%). Compound **5b**[9] emitted orange color under both basic and neutral conditions. The chemiluminescence spectra were superimposable to the fluorescence spectrum of an amide anion of **6b**[10] (DMSO-1 M KOH). These data suggested that a conjugated chromophore through a double bond to the pyrazine core at the 8-position causes a large bathochromic shift of luminescence color.

Table 1. Chemiluminescence properties of the imidazopyrazinones **5a** and **5b** in DMSO at 20 °C.[a]

	5a		**5b**	
	DMSO-KOH	DMSO-AcB	DMSO-KOH	DMSO-AcB
Relative light yield[b]	100	19	9	19
Relative rate ($t_{1/2max}$)	2 min	1 min	0.5 min	1 min
Emission maximum (λmax)	535 nm	590 nm	583 nm	583 nm

a) A 10^{-3} M methanol solution (30 μL) of **5a** and **5b** was added to DMSO (2 mL) and the luminescence was triggered by addition of DMSO containing 1 M KOH (10 μL) or 0.1 M acetate buffer (AcB) pH 6.5 (100 μL). b) Light yield and the emission spectra were not corrected.

Chemiluminescence of **5a** in DMSO-KOH was a slow reaction but the most efficient (Table 1) among the conditions examined, suggesting that a phenylalkynyl group at the 8-position may stabilize imidazopyrazinone against oxidation and increase efficiency of chemiluminescence reactions involved in the chemienergizing process. Bimodal luminescence as well as color variation may allow us to apply bio- and chemiluminescence for various purposes such as monitoring two concurrent events by different colors and monitoring the local pH

in a cell or in a droplet. Work on introducing a conjugated chromophore at the 8-position of imidazopyrazinone in a bioluminescent system is now progress at our laboratory.

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Reference and Notes

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5. **4**: yellow powder: mp 157-158 °C; ^{1}H NMR (270 MHz, 9:1 $CDCl_3$-CD_3OD) δ 7.25–7.50 (6H, m), 7.50–7.65 (4H, m), 8.18 (1H, s); HR-EIMS *m/z* 295.1086 (M^+, Calcd for $C_{20}H_{13}N_3$ 295.1109)
6. **5a**: unstable HCl salts; ^{1}H NMR (400 MHz, 9:1 $CDCl_3$-CD_3OD-12 M HCl) δ 2.62 (3H, s, $2CH_3$), 7.42 (2H, m, 5"-H), 7.43 (1H, m, 6'-H), 7.46 (1H, m, 6"-H), 7.47 (2H, m, 5'-H), 7.64 (2H, m, 4'-H), 7.79 (1H, s, 1"-H), 8.01 (2H, m, 4"-H), 8.35 (1H, s, 5-H); ^{13}C NMR (100 MHz, 9:1 $CDCl_3$-CD_3OD-12 M HCl) δ 9.28 ($2CH_3$), 83.6 (C1'), 92.7 (C2'), 115.2 (C1"), 115.8 (C5), 119.7 (C2), 121.3 (C3'), 124.7 (C8), 127.1 (C6), 127.7 (C4"), 128.7 (C5"), 128.8 (C5'), 129.9 (C6'), 130.9 (C6"), 132.18 (C4'), 137.0 (C3"), 137.5 (C3), 141.7 (C9), 144.8 (C2"); HR-EIMS *m/z* 385.1007 (M^+, Calcd for $C_{23}H_{16}ON_3Cl$ 385.0981).
7. **6a**: yellow powder: mp 97-98 °C, ^{1}H-NMR (270 MHz, $CDCl_3$) δ 2.40 (3H, s), 7.16 (1H, s), 7.38-7.42 (6H, m,), 7.56-7.60 (2H, m), 7.72-7.76 (2H, m), 8.20 (1H, s, NH), 8.50 (1H, s); HR-EIMS *m/z* 373.098 (M^+, Calcd for $C_{22}H_{16}ON_3Cl$ 373.0982).
8. **6c**: yellow powder: mp 115-116°C, ^{1}H-NMR (270 MHz, $CDCl_3$) δ 2.55 (3H, s), 7.40-7.42 (6H, m), 7.55-7.60 (4H, m), 8.25 (1H, s, NH), 8.40 (1H, s); HR-EIMS *m/z* 337.1197 (M^+, Calcd for $C_{22}H_{15}ON_3$ 337.1215).
9. **5b**: brown solids; mp. 168-169 °C (decomposed); ^{1}H-NMR (9:1 $CDCl_3$-CD_3OD-12 M HCl) δ 2.62 (3H, s, $2CH_3$), 7.40-7.50 (6H, m), 7.72 (2H, d, *J*= 8 Hz, 4'-H), 7.82 (2H, d, *J*= 8 Hz, 4"-H), 7.86 (1H, d, *J*= 16 Hz), 8.23 (1H, s), 8.48 (1H, d, *J*= 16 Hz); HR-EIMS *m/z* 351.1352 (Calcd for $C_{23}H_{17}ON_3$ 351.1372). 2-amino-5-phenylethynyl-3-*trans*-styrylpyrazine (105 mg, 90% yield): yellow powder; mp 149-150°C; ^{1}H-NMR ($CDCl_3$) δ 4.88 (2H, s), 6.93 (1H, d, *J*= 16 Hz), 7.35-7.37 (6H, m), 7.58-7.60 (4H, m), 7.80 (2H, d, *J*= 16 Hz), 8.17 (1H, s); HR-EIMS *m/z* 297.1291 (Calcd for $C_{20}H_{15}N_3$ 297.1265). 2-amino-5-bromo-3-*trans*-styrylpyrazine (553 mg, 53% yield): yellow powder; mp 108-110°C, ^{1}H-NMR ($CDCl_3$) δ 4.68 (2H, s), 6.93 (1H, d, *J*= 16 Hz), 7.36-7.40 (3H, m), 7.58 (2H, d, *J*= 8 Hz), 7.76 (1H, d, *J*= 16 Hz), 8.00 (1H, s); HR-EIMS *m/z* 275.0070 (Calcd for $C_{12}H_{10}N_3{}^{79}Br$ 275.0058).
10. **6b**: yellow powder: mp 108-109°C, ^{1}H-NMR (270 MHz, $CDCl_3$) δ 2.37 (3H, s), 7.13 (1H, d, *J*= 16 Hz), 7.32-7.40 (6H, m), 7.58-7.65 (4H, m), 7.93 (1H, d, *J*= 16 Hz), 8.20 (1H, s), 8.37 (1H, s); HR-EIMS *m/z* 339.1372 (M^+, Calcd for $C_{22}H_{17}ON_3$ 339.1371).