CHANGE IN THE FLUORESCENCE OF 1,1′-DIARYL-2,2′-BIIMIDAZOLES UPON THE ADDITION OF ACID

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Abstract – The optical properties of a series of 1,1′-diaryl-2,2′-biimidazoles were examined. Different transitions were observed in their fluorescence spectra upon changing the electronic properties of the phenyl ring at the C1 and C1′ positions. The presence of a formyl group on the phenyl ring results in fluorescence via a CT transition with a solvent effect. A bathochromic change was observed when HCl was added to a solution of compound bearing a methoxy group in CH2Cl2, whereas a hypsochromic change was observed in compound bearing a formyl group. These observations were attributed to the protonation, which causes a characteristic change in their biimidazole moieties.

Fluorescence technology has been the focus of research attention due to its wide range of applications in various fields such as biological probes, environmental sensors, and chemical sensors.1 Because pH plays an important role in cellular functions, various pH sensors have been reported with turn-on/off abilities.2 Fluorescence with different colors under various pH conditions is also an efficient strategy to achieve clear sensing.3 By changing the optical properties under acidic conditions, an imidazole ring is an attractive structure due to its basicity and aromaticity. A pH sensor with fluorescence change has been reported using a compound constructed from coumarin and benzimidazole moieties.4 We have previously investigated a variety of fluorophores based on 2,2′-biimidazole and 2,2′-bibenzimidazole structures.5-8 We also revealed that imidazole-based fluorophores retain their fluorescence properties upon introduction of an alkyl group on the sp²-hybridized nitrogen atom.9,10 When investigating the influence of different substituents at the C1 position of the imidazole ring on their fluorescence properties, we observed a unique change in the fluorescence peak upon the addition of HCl. Herein, we report the optical properties of a series of 1,1′-diaryl-2,2′-biimidazoles (I). We found that an opposite change was observed in the different compounds upon the addition of HCl (Scheme 1).
Compounds 1a–c were synthesized via a Cu-mediated coupling reaction of their corresponding 2-iodoimidazole derivatives using the method reported by Park and Alper (Scheme 2). During the synthesis of the compound bearing a formyl group at the C1 and C1′ positions (1c), the coupling reaction did not proceed when using the acetal precursor. However, 1c was successfully obtained in the coupling reaction using the free formyl group in the precursor, which was obtained after deprotection of the acetal group. It would be same reason why the Ullmann coupling is accelerated by introducing electron-withdrawing group in the pyrrole system.

The absorption and fluorescence spectra of 1a–c were measured in CH$_2$Cl$_2$ and MeCN, and the results are summarized in Table 1 and Figure 1. A shoulder peak was observed at around the maximum absorption peak although the peak top of 1a could not be observed under the measuring conditions used. The absorption peaks were shifted to longer wavelengths upon introducing a substituent on the phenyl ring (entry 1 vs. 3 and 5). There was no solvent effect observed in the absorption spectra for each compound studied. In their fluorescence spectra, similar wavelengths were observed for 1a and 1b (entries 1–4) without solvent effect, which correspond to the π-π* transition. A longer wavelength was observed for compound 1c compared to that for 1a and 1b (entry 1 and 3 vs. 5). In addition, a solvent effect was observed in 1c; the polar solvent (MeCN) led to a longer wavelength when compared to the less polar solvent (CH$_2$Cl$_2$) (entries 5 and 6). These results suggest that the fluorescence peak observed for 1c is
derived from a CT transition. In addition, a decrease in the quantum yield of 1c was observed compared to that of 1a and 1b. It would be also supported by the emission from CT transition on 1c.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Solvent</th>
<th>$\lambda_{\text{abs}}$ (nm) $[\varepsilon$ (M$^{-1}$ cm$^{-1}$)]$^a$</th>
<th>$\lambda_{\text{em}}$ (nm)$^b$ $[\Phi_F]^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>CH$_2$Cl$_2$</td>
<td>255(sh) [7,800]</td>
<td>361 [0.19]</td>
</tr>
<tr>
<td>2</td>
<td>1a</td>
<td>MeCN</td>
<td>253(sh) [9,000]</td>
<td>361$^d$ [0.18]</td>
</tr>
<tr>
<td>3</td>
<td>1b</td>
<td>CH$_2$Cl$_2$</td>
<td>234 [24,200], 269(sh) [10,100]</td>
<td>365 [0.10]</td>
</tr>
<tr>
<td>4</td>
<td>1b</td>
<td>MeCN</td>
<td>232 [23,400], 270(sh) [9,200]</td>
<td>366 [0.13]</td>
</tr>
<tr>
<td>5</td>
<td>1c</td>
<td>CH$_2$Cl$_2$</td>
<td>258 [27,400], 281(sh) [15,600]</td>
<td>462$^e$ [0.02]</td>
</tr>
<tr>
<td>6</td>
<td>1c</td>
<td>MeCN</td>
<td>256 [26,700], 280(sh) [16,600]</td>
<td>476$^e$ [0.06]</td>
</tr>
</tbody>
</table>

$^a$ Concentration: 3.0 $\times$ 10$^{-5}$ M. $^b$ Concentration: 3.0 $\times$ 10$^{-6}$ M. Excited at $\lambda_{\text{abs}}$(sh). $^c$ Determined using $p$-terphenyl in cyclohexane as a standard ($\Phi_F = 0.87$) excited at 265 nm. $^d$ Concentration: 3.0 $\times$ 10$^{-7}$ M. Excited at $\lambda_{\text{abs}}$(sh). $^e$ Concentration: 3.0 $\times$ 10$^{-5}$ M. Excited at $\lambda_{\text{abs}}$.

Figure 1. Absorption (plane line) and fluorescence (dashed line) spectra recorded for a) 1a, b) 1b, and c) 1c in CH$_2$Cl$_2$ (blue line) and MeCN (red line)
We examined the changes in the fluorescence spectra that occurred upon the addition of HCl to compounds 1a–c in CH2Cl2. A specific peak change was obtained when a solution of HCl in CH2Cl2 was added to 1a–c in CH2Cl2 (Figure 2).

![Figure 2](image_url)

**Figure 2.** Changes in the fluorescence spectra recorded for a) 1a (3.0 × 10⁻⁶ M, excited at λ_{abs(sh)}), b) 1b (3.0 × 10⁻⁶ M, excited at λ_{abs(sh)}), and 1c (3.0 × 10⁻⁵ M, excited at λ_{abs}) upon the addition of HCl

For compound 1a, a peak shift was observed from 361 to 381 nm along with a decrease in the peak intensity (Figure 2a). An increase in the intensity was observed upon the introduction of an excess of HCl after reaching 381 nm (1a:HCl = 1:27 vs. 1:40 and 1:53). No isofluorescence point was observed upon the addition of HCl. Thus, this change was attributed to several states, such as the single and double protonation of the imidazole rings in 1a. In the case of 1b, a peak change from 365 to 440 nm was
observed, and not a peak shift (Figure 2b). Upon the addition of HCl, a broadened spectrum with two peak tops (365 and 440 nm) was obtained in the case of 1b·HCl = 1:3. Moreover, a decrease in the intensity of the peak observed at 440 nm occurred upon the addition of excess HCl (1b·HCl = 1:5 vs. 1:27 and 1:53) accompanied by a decrease in the peak intensity at 365 nm. During the changes in the spectra recorded for 1b, no isofluorescence point was observed. The change in 1c showed an opposite result to that found for 1b. A peak at the shorter wavelength of 389 nm was observed upon the addition of HCl (Figure 2c). The intensity of the original peak (462 nm) decreased upon the introduction of HCl. In addition, one isofluorescence point was observed at 392 nm, which suggests that this change was caused by the equilibrium formed between two states. As mentioned above, 1c shows a solvent effect in the fluorescence spectra. Upon the addition of HCl, the polarity of the solution will be changed to become more polar. However, a bathochromic shift was observed in 1c when polar MeCN was used as the solvent (Table 1, entries 5 and 6). Thus, the spectral change observed in this experiment was not caused by the change in the solvent polarity, but by the acidity of HCl.

The reason for the changes observed in the fluorescence spectra of 1a–c upon the addition of HCl can be explained by the equilibrium formed between the protonated states (Scheme 3). In the case of 1a, three states will exist under acidic conditions; i.e. 1a, mono-protonated 1a·H⁺, and di-protonated 1a·2H⁺. If the fluorescence of 1a·H⁺ occurs at ~380 nm with small intensity, the influence of H⁺ will be observed such as the shift in the peaks with no isofluorescence point because of the slight change in the fluorescence of 1a and 1a·H⁺ (Scheme 3a). Unfortunately, we do not have any information in regard to the fluorescent properties of 1a·2H⁺. However, similar fluorescence to 1a·H⁺ will be plausible in 1a·2H⁺ because an increase in the peak intensity at ~380 nm was observed when excess HCl was added (1a·HCl = 1:40 and 1:53), which increased the amount of 1a·H⁺ and 1a·2H⁺. In the case of 1b, compound 1b·H⁺ bearing an electron-donating methoxy group can undergo a CT transition via electron transfer from the methoxyphenyl moiety to the protonated biimidazole moiety. Thus, the protonated state (1b·H⁺) will give a fluorescence peak originating from the CT transition at 440 nm. This is the reason for the drastic bathochromic shift observed upon the addition of HCl. Fluorescence quenching will occur via the formation of non-fluorescent 1b·2H⁺ when an excess amount of HCl was added (Scheme 3b). The hypsochromic shift will also be caused by the change in the transition observed in 1c. As mentioned above, the fluorescence of 1c originates from a CT transition, which is caused by electron-transfer from the electron-rich biimidazole moiety to the electron-deficient formylphenyl moiety. Upon protonation of the imidazole ring, the biimidazole moiety lacks electron-rich character to allow the π-π* transition to occur. Thus, this change in the transition led to the hypsochromic shift observed in the fluorescence peak. There was no equilibrium toward 1c·2H⁺ because of the existence of only one isofluorescence point.
Scheme 3. Plausible explanation for the changes observed in the fluorescence spectra of a) 1a, b) 1b, and c) 1c upon the addition of HCl

To support these hypotheses, especially for 1b and 1c, we performed TDDFT calculations to determine their excited states using the optimized structures obtained at the TDDFT/B3LYP/6-31+G** level of theory with the Gaussian 16 program (Figure 3).13,14 The HOMO of 1b was localized on the biimidazole moiety, whereas the LUMO of 1b was localized on the phenyl ring at the C1-position (Figure 3a). Thus, the HOMO-LUMO transition occurred via CT, which was not in accordance with the experimental result (i.e., no solvent effect was observed in the fluorescence spectra). We focused on the LUMO+1 state, whose orbital was localized on the biimidazole moiety. The HOMO-LUMO+1 transition can be explained by the fluorescence of 1b (π-π* transition). When protonation occurred, the HOMO and LUMO were localized on the methoxyphenyl and biimidazole moieties, respectively. The HOMO-LUMO transition in 1b•H+ corresponds to the CT transition. The overlap between HOMO and LUMO exists around biimidazole moiety (Figure 3a). The correlation with their energies was in good agreement with the change in the fluorescence peaks. The difference in the energy between the HOMO and LUMO+1 in 1b (4.3364 eV) was larger than that between the HOMO and LUMO in 1b•H+ (2.9524 eV) although the difference in the energy between the HOMO and LUMO in 1b (4.0330 eV) was also larger. In the case of
1c, the HOMO-LUMO transition supports the CT transition with the HOMO localized on the biimidazole moiety and the LUMO on the formylphenyl moiety. In LUMO, a little orbital was lied on C-N bonds at C2-N and N-C5. Thus, the transition would be occurred even in the twisted structure between donor and acceptor moieties (Figure 3b). Both the HOMO and LUMO of protonated 1c•H⁺ were on the formylphenyl moiety, but the LUMO was localized on a different part of the formylphenyl group compared to the HOMO; the HOMO-LUMO transition corresponds to the CT transition. The HOMO-1 orbital is localized on the same formylphenyl moiety as the HOMO. Thus, the HOMO-1-LUMO transition corresponds to the π-π* transition. The energetic relationship was in agreement with the experimental results. The difference in the HOMO and LUMO energies in 1c (2.8929 eV) was smaller than that between the HOMO-1 and LUMO (3.9532 eV). Other energy gaps in 1c were larger energy values than the value of 3.935 eV in 1c•H⁺, which means that those transition would be observed in shorter wavelength (see Figure S1). But the experimental result was opposite. Thus, the fluorescence of 1c was derived from the transition between HOMO and LUMO.

**Figure 3.** Related orbitals and energies of a) 1b and 1b•H⁺ and b) 1c and 1c•H⁺ calculated using the TDDFT/B3LYP/6-31+G** level of theory and the optimized structure obtained using the TDDFT method.
In summary, we have examined the optical properties of a series of 1,1′-diaryl-2,2′-biimidazoles (1). Their different transitions were observed using fluorescence spectroscopy upon changing the electronic properties of the phenyl ring at the C1 and C1′ positions, which were attributed to the electronic character of the biimidazole moiety. Furthermore, an opposite result was observed upon the addition of HCl when compared to the cases of 1b and 1c. A bathochromic shift was observed with 1b, but a hypsochromic shift was observed with 1c. This was attributed to the protonation of 1b and 1c, which causes a characteristic change in their biimidazole moieties. These results will provide information for the design of novel pH sensing materials.

EXPERIMENTAL
Melting points were determined with Yanaco MP-J3 and values were uncorrected. NMR spectra were recorded at 300 MHz (proton) (75 MHz (carbon-13)) on Bruker DPX-300 spectrometer and at 400 MHz (proton) (100 MHz (carbon-13)) on Bruker AVANCE III-400M. Chemical shifts (δ) of 1H NMR were expressed in parts per million downfield or upfield from tetramethylsilane in CDCl3 (δ = 0) as an internal standard. Multiplicities are indicated as s (singlet), d (doublet), bs (broadened singlet), m (multiplet) and coupling constants (J) are reported in hertz units. Chemical shifts (δ) of 13C NMR are expressed in parts per million downfield or upfield from CDCl3 (δ = 77.0) as an internal standard. Infrared (IR) spectra were recorded on a JASCO FT/IR-460 plus spectrometer. Mass spectra were carried out on a THERMO Fisher Exactive in Center for Analytical Instrumentation of Chiba University. UV-Vis spectra were measured with quartz cell (1 cm × 1 cm) on a JASCO V-570 spectrophotometer. Fluorescence spectra were measured with quartz cell (1 cm × 1 cm) on a JASCO FP-6600 spectrofluorometer. Analytical thin-layer chromatography (TLC) was performed on glass plates that had been pre-coated with SiO2 (0.25 mm layer thickness). Column chromatography was performed on 70–230 mesh SiO2. Anhydrous DMF was distilled from P2O5 under reduced pressure and was stored with MS 4 Å. All other commercially available materials were used without further purification. The reactions were performed under nitrogen or argon atmosphere otherwise noted. 1,1′-Diphenyl-2,2′-biimidazole (1a) was prepared according to the procedure mentioned in the literature.6

Synthesis of 1-(4-Methoxyphenyl)-1H-imidazole:15 Mixture of 4-iodoanisole (1.409 g, 6.02 mmol), 1,2,3-benzotriazole (7.4 mg, 0.06 mmol), Cul (69.8 mg, 0.367 mmol), and imidazole (0.408 g, 5.99 mmol) was dissolved in DMSO (6 mL). The mixture was stirred for 21 h at 110 °C. The resultant mixture was filtrated with a pad of Celite® and Floizil® with EtOAc (20 mL). The filtrate was added H2O (20 mL), and was extracted with EtOAc (20 mL × 3). The organic layer was washed with brine (20 mL), and was dried with MgSO4. After filtration and evaporation, the residue was subjected to column chromatography on SiO2 (EtOAc) to give 1-(4-methoxyphenyl)-1H-imidazole (0.801 g, 4.60 mmol, 77%) as pale yellow
solid: mp 58–60 °C [lit.: 16 60-61 °C], 1H NMR (300 MHz, CDCl3) δ = 3.85 (s, 3H), 6.98 (diffused d, J = 9.0 Hz, 2H), 7.18 (bs, 1H), 7.20 (bs, 1H), 7.30 (diffused d, J = 9.0 Hz, 2H), 7.76 (bs, 1H). 17

Synthesis of 1,1′-Bis(4-methoxylphenyl)-1H,1H'-2,2'-biimidazole (1b): To a solution of 1-(4-methoxyphenyl)-1H-imidazole (2.389 g, 13.7 mmol) in THF (33 mL) was dropwise added 1.6 M n-BuLi in n-hexane (9.3 mL, 15 mmol) at -30 °C. The mixture was stirred for 1 h at that temperature. To the reaction mixture was added I2 (4.519 g, 17.8 mmol) at that temperature. The mixture was gradually warmed up to room temperature and was stirred for 24 h. The resultant mixture was added saturated Na2S2O3 solution (50 mL) and H2O (20 mL), and was extracted with EtOAc (20 mL × 3). The organic layer was washed with brine (20 mL), and was dried with MgSO4. After filtration and evaporation, the obtained residue (0.806 g, 2.69 mmol estimated as 2-iodo-1-(4-methoxyphenyl)-1H-imidazole) was dissolved in DMF (10 mL). To the solution was added powder Cu (0.381 g, 6.00 matom-eq.). The mixture was stirred for 23 h at 90 °C. To the reaction mixture was added 28% NH4OH solution (80 mL) to dissolve powder Cu at room temperature. The resultant mixture was extracted with EtOAc (20 mL × 3). The organic layer was washed with brine (20 mL), and was dried with MgSO4. After filtration and evaporation, the residue was subjected to column chromatography on SiO2 (CHCl3 : MeOH = 10 : 1) to give 1,1′-bis(4-methoxylphenyl)-1H,1H'-2,2'-biimidazole (1b) (0.249 g, 0.719 mmol, 10% in two steps) as pale brown solid. To use optical measurement, the further purification was conducted by the recrystallization from CHCl3 and n-hexane: mp 180-181 °C [lit.: 18 191 °C (decomp.)], 1H NMR (300 MHz, CDCl3) δ = 3.79 (s, 6H), 6.73 (s, 4H), 7.02 (d, J = 1.1 Hz, 4H), 7.22 (d, J = 1.1 Hz, 4H). 18

Synthesis of 1-(4-(1,3-Dioxolan-2-yl)phenyl)-1H-imidazole: Mixture of CuI (0.380 g, 2.00 mmol), Cs2CO3 (6.519 g, 20.0 mmol), 4-bromobenzaldehyde (1.850 g, 10.0 mmol), and imidazole (0.955 g, 14.0 mmol) was dissolved in DMF (20 mL). After being bubbled with Ar for 30 min, the mixture was stirred for 23 h at 120 °C. The resultant mixture was filtrated with a pad of Celite® and Floizil® with EtOAc (20 mL). The filtrate was added H2O (20 mL), and was extracted with EtOAc (20 mL × 3). The organic layer was washed with brine (20 mL), and was dried with MgSO4. After filtration and evaporation, the residue was subjected to column chromatography on SiO2 (CHCl3 : MeOH = 10 : 1) to exclude starting 4-bromobenzaldehyde and imidazole. The obtained residue (0.850 g, 4.94 mmol estimated as 4-(1H-imidazol-1-yl)benzaldehyde was added ethylene glycol (0.920 g, 14.8 mmol), 4-tolylsulfonic acid monohydrate (0.211 g, 1.11 mmol), and toluene (20 mL). The mixture was refluxed for 24 h with Dean-Stark apparatus under refluxing conditions. To the resultant mixture was added saturated aqueous NaHCO3 solution (10 mL). The organic layer was washed with brine (20 mL), and was dried with MgSO4. After filtration and evaporation, the residue was subjected to column chromatography on SiO2 (CHCl3 : MeOH = 5 : 1) to give 1-(4-(1,3-dioxolan-2-yl)phenyl)-1H-imidazole (0.791 g, 3.66 mmol, 37% in two
steps) as pale yellow solid: mp 118-120 °C [lit.19 125–126 °C], 1H NMR (300 MHz, CDCl3) δ = 4.05-4.11 (m, 2H), 4.13-4.19 (m, 2H), 5.86 (s, 1H), 7.22 (s, 1H), 7.30 (s, 1H), 7.42 (diffused d, J = 8.6 Hz, 2H), 7.61 (diffused d, J = 8.4 Hz, 2H), 7.88 (s, 1H).

Synthesis of 4,4’-(1H,1’H-(2,2’-Biimidazole)-1,1'-diyl)dibenzaldehyde (1c): To a mixture of 1-(4-(1,3-dioxolan-2-yl)phenyl)-1H-imidazole (1.155 g, 5.34 mmol) and N,N,N’,N’-tetramethylene-1,2-diamine (TMEDA) (0.9 mL, 6.04 mmol) in THF (13 mL) was dropwise added 1.6 M n-BuLi in n-hexane (3.6 mL, 5.76 mmol) at -30 °C. The mixture was stirred for 1 h at that temperature. To the reaction mixture was added I2 (1.751 g, 6.90 mmol) at that temperature. The mixture was gradually warmed up to room temperature and was stirred for 19 h. The resultant mixture was added saturated aqueous Na2S2O3 solution (50 mL) and H2O (20 mL), and was extracted with EtOAc (20 mL × 3). The organic layer was washed with brine (20 mL), and was dried with MgSO4. After filtration and evaporation, the residue was subjected to column chromatography on SiO2 (n-hexane : EtOAc = 1 : 1) to give 1-(4-(1,3-dioxolan-2-yl)phenyl)-2-iodo-1H-imidazole (0.775 g, 2.27 mmol, 43%). To 1-(4-(1,3-dioxolan-2-yl)phenyl)-2-iodo-1H-imidazole (0.775 g, 2.27 mmol) was added 1 M aqueous HCl (30 mL). The reaction mixture was stirred for 20 h at room temperature. The resultant mixture was neutralized by saturated aqueous NaHCO3 solution. The mixture was extracted with EtOAc (20 mL × 3). The organic layer was washed with brine (20 mL), and was dried with MgSO4. After filtration and evaporation, the residue was obtained as crude 4-(2-iodo-1H-imidazol-1-yl)benzaldehyde (0.522 g). To a solution of crude 4-(2-iodo-1H-imidazol-1-yl)benzaldehyde (0.522 g, 1.75 mmol estimation) was dissolved in DMF (2 mL). To the solution was added powder Cu (0.334 g, 5.26 matom-eq.). The mixture was stirred for 23 h at 90 °C. To the reaction mixture was added 28% NH4OH solution (20 mL) to dissolve powder Cu at room temperature. The resultant mixture was extracted with EtOAc (20 mL × 3). The organic layer was washed with brine (20 mL), and was dried with MgSO4. After filtration and evaporation, the residue was subjected to column chromatography on SiO2 (CHCl3 : MeOH = 10 : 1) to give 4,4’-(1H,1’H-(2,2’-biimidazole)-1,1’-diyl)dibenzaldehyde (1c) (91.4 mg, 0.266 mmol, 12% in two steps) as white solid.

Procedure for the Addition of HCl in Fluorescence Spectra: A solution of HCl in CH2Cl2 (2.4 × 10⁻⁴ M) was prepared by 1.0 μL of concentrated HCl (12 M) and 50 mL of CH2Cl2. And a solution of
compound in CH$_2$Cl$_2$ (1a, 9.0 × 10$^{-6}$ M; 1b, 9.0 × 10$^{-6}$ M; 1c, 9.0 × 10$^{-5}$ M) was also prepared. In a quartz cell (1 cm × 1 cm), to a mixture of the solution of compound (1.0 mL) and the solution of HCl (corresponding amount) was added CH$_2$Cl$_2$ to reach to 3 mL of total amount. The mixture was directly measured with the spectrofluorometer. Each sample was freshly prepared before measurement.

REFERENCES AND NOTES


14. We could not obtain the optimized structure for $1\text{a}\cdot\text{H}^+$. Therefore, we were unable to explain the phenomena for $1\text{a}$.


