Fluorescence and chemiluminescence properties of indolylmaleimides: experimental and theoretical studies†‡

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Various indolylmaleimides (IMs) were synthesized, and their fluorescence (FL) and chemiluminescence (CL) were measured. The substitution at the 2-position of the indole ring and the 3- or 4-position of the maleimide moiety caused an obvious change in the FL and CL of the IMs. An almost on–off switching of the FL of the IMs was observed. The intramolecular charge transfer from the indole moiety to the maleimide moiety occurred in 3-(1H-3-indolyl)-2,5-dihydro-1H-2,5-pyrroledione. In the FL of the IMs, CASPT2 calculations showed deprotonation of the NH group of the indole ring and the maleimide moiety at the excited state. The C=C bond in the maleimide moiety was needed for strong CL in the IMs without substitution at the 2-position of the indole ring. The relationships between the FL or CL properties and the structures of the IMs were clarified. These results provide significant information on the rational design of IMs as FL and CL probes.

Introduction

Various fluorescence (FL), chemiluminescence (CL) and bioluminescence (BL) compounds have been developed for labeling reagents[1–5] and probes in living cells.[6–10] The derivatives of fluorescein,[11–14] coumarin,[15–18] luminol,[19–24] acridinium ester,[25–28] luciferin[29–32] and green fluorescent protein[33–37] are useful luminescent compounds for highly sensitive and selective FL, CL and BL methods. Recently, FL and CL probes have been designed and synthesized on the basis of energy transfer (ET),[38–41] photoinduced electron transfer (PeT)[42–46] and charge transfer (CT).[47–50] Especially, the on–off switching mechanism of FL probes utilizing these mechanisms was applied to various FL methods.[51–53] The development of CL probes having on–off switching has been needed for a wide range of applications of CL methods. Therefore, clarification of the FL and CL properties and the luminescent mechanism of FL and CL compounds is very informative for the development of FL and CL properties and the luminescence mechanism of FL and CL compounds is very informative for the

development of FL and CL probes. The relationship between the FL or CL properties and the structure of FL and CL compounds should be clarified.

Indole derivatives are known as FL, CL and BL compounds.[54–59] Recently, indolylmaleimides (IMs) composed of an indole moiety and a maleimide moiety have been focused on for their luminescence properties[60–65] and their usage for organic light-emitting diodes.[66–68] However, the essential factors in molecular design for useful FL or CL probes of IMs have not been clarified. When the factors are found, the efficiency of the development of FL or CL probes of IMs can be increased. We synthesized various IMs and measured the FL and CL. Very interesting FL and CL properties were obtained. In this study, we found the essential factors determining the FL and CL properties of IMs which provide significant information on developing FL and CL probes of IMs efficiently. A clear relationship was found between the structure and the FL or CL properties of IMs. Furthermore, a theoretical study was performed to clarify the FL properties of the IMs by CASPT2 calculations.

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Experimental

General methods

^{1}H-NMR spectra were obtained in DMSO-d_6 as the solvent using a Varian UNITY plus (USA) spectrometer at 500 MHz. FAB MS spectra were obtained using a JEOL JMS 600 (Japan). Absorption and fluorescence spectra were obtained using a Jasco V-530 absorptiometer and an FP-6500 fluorometer (Tokyo, Japan). All FL spectra were corrected. A Lumat LB 9501 (Berthold, Wildbad, Germany) was used to measure the CL in a round-bottom glass tube (75 × 12 mm id). Deionized and distilled water purified by a Millie-Gradient

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(Japan Millipore, Tokyo, Japan) was used. All other chemicals and solvents were of analytical reagent grade.

Syntheses

2-Naphthyl indole,[69] compounds 1,[70,71] 2,[71] 4,[71] 7[71] and 13[72] were synthesized by the reported method.

3-Bromo-4-(2-naphthyl-1H-3-indolyl)-2,5-dihydro-1H-pyrroledione (3). To stirred toluene–THF (15 mL, 2 : 1, v/v) was added 2-naphthyl indole (0.2 g, 0.8 mmol); then CH_3MgCl (0.5 mL, 1.5 mmol, 3M in THF) was added dropwise and the solution was stirred at 60 °C for 1 h. Dibromomaleimide (0.105 g, 0.4 mmol) in toluene–THF (4 mL, 1 : 1, v/v) was added to the solution, and the mixture was refluxed for 48 h. EtOAc (300 mL) was added to the reaction mixture, and the organic layer was washed with 1 M HCl (100 mL), H_2O (100 mL) and 10% aqueous NaCl (100 mL), and dried with anhydrous $MgSO_4$. The filtrate was concentrated and the resulting precipitates were purified by column chromatography (silica gel, EtOAc–hexane = 5 : 3, v/v) to give 3 (0.05 g, 15%). Found: C, 63.46; H, 4.02; N, 6.02. $C_{22}H_{13}BrN_2O_2$ requires C, 63.33; H, 3.14; N, 6.71%; mp 135–138 °C; ^{1}H-NMR(DMSO$-d_6$) 7.10–7.14 (m, 1H, ArH), 7.21–7.24 (m, 1H, ArH), 7.50–7.60 (m, 4H, ArH), 7.69–7.7 (d, $J = 8$ Hz, 1H, ArH), 7.92–8.01 (m, 3H, ArH), 8.12 (s, 1H, ArH), 11.29 (s, 1H, maleimide NH), 12.25 (s, 1H, indole NH); m/z (FAB MS) 417.02 $[M]^+$.

3-(2-Phenyl-1H-3-indolyl)-2,5-pyrrolidinedione (5). CH_3OH (120 mL) was added to compound 2 (0.27 g, 0.74 mmol) and Pd-black (0.6 g). The mixture was stirred under hydrogen gas for 19 h. The filtrate was concentrated in $vacuo$, and the resulting precipitates were purified by column chromatography (silica gel, $CHCl_3$: CH_3OH = 20 : 1 → EtOAc :hexane = 2 : 3, v/v) to give 5 (0.04 g, 19%). Found: C, 73.47; H, 5.18; N, 9.11. $C_{18}H_{14}N_2O_2·0.25H_2O$ requires C, 73.33; H, 4.95; N, 9.50%; mp 295–297 °C; ^{1}H-NMR(DMSO$-d_6$) 2.75–2.80 (dd, $J = 5$ Hz, 1H), 3.16–3.21 (dd, $J = 10$ Hz, 1H), 4.39–4.42 (dd, $J = 5.5$ Hz, 1H), 6.99–7.02 (t, $J = 7$ Hz, 1H, ArH), 7.11–7.14 (t, $J = 7$ Hz, 1H, ArH), 7.19–7.20 (d, $J = 8$ Hz, 1H, ArH), 7.39–7.45 (m, 2H, ArH), 7.51–7.54 (t, $J = 8$ Hz, 2H, ArH), 7.63–7.64 (d, $J = 7$ Hz, 2H, ArH), 11.39 (s, 1H, maleimide NH), 11.43 (s, 1H, indole NH); m/z (FAB MS) 290.2 $[M]^+$.

3-(2-Naphthyl-1H-3-indolyl)-2,5-pyrrolidinedione (6). DMF (3 mL) was added to compound 3 (0.03 g, 0.072 mmol) and 10% Pd–C (0.03 g). The mixture was stirred under hydrogen gas (750 psi) for 72 h. The filtrate was concentrated in $vacuo$, and EtOAc (30 mL) and H_2O (30 mL) were added to the resulting precipitates; the organic layer was dried with anhydrous $MgSO_4$ and the filtrate was concentrated. The resulting precipitates were purified by column chromatography (silica gel, EtOAc–hexane = 3 : 5, v/v) to give 6 (0.01 g, 41.7%). ^{1}H-NMR(DMSO$-d_6$) 2.79–2.84 (dd, $J = 5.5$ Hz, 1H), 4.52–4.55 (dd, $J = 5.5$ Hz, 1H), 7.01–7.04 (t, $J = 8$ Hz, 1H, ArH), 7.14–7.16 (t, $J = 7$ Hz, 1H, ArH), 7.23–7.25 (d, $J = 8$ Hz, 1H, ArH), 7.43–7.44 (d, $J = 8$ Hz, 1H, ArH), 7.56–7.60 (m, 2H, ArH), 7.77–7.79 (dd, $J = 2$ Hz, 1H, ArH), 7.97–8.00 (m, 2H, ArH), 8.05–8.07 (d, $J = 8.5$ Hz, 1H, ArH), 8.14

(s, 1H, ArH), 11.45 (s, 1H, maleimide NH), 11.53 (s, 1H, indole NH); m/z (FAB MS) 340.15 $[M]^+$.

3-(2-Phenyl-1H-3-indolyl)-2,5-dihydro-1H-2,5-pyrroledione (8). To stirred benzene (100 mL) were added compound 5 (0.1 g, 0.34 mmol), DDQ (0.1 g, 0.44 mmol) and p-TsOH·H_2O (0.1 g, 0.53 mmol), and the mixture was refluxed for 4 h. EtOAc (200 mL) and 10% aqueous NaCl (100 mL) were added to the solution, and the organic layer was extracted and concentrated. The resulting precipitate was purified by column chromatography (silica gel, hexane–EtOAc = 1 : 1, v/v) to give 8 (0.04 g, 40.4%). Found: C, 74.77; H, 4.17; N, 9.68. $C_{18}H_{12}N_2O_2$ requires C, 74.99; H, 4.20; N, 9.72%; mp 290–291 °C; ^{1}H-NMR(DMSO$-d_6$) 6.67 (s, 1H, Olefin H), 7.11–7.14 (t, $J = 7$ Hz, 1H, ArH), 7.19–7.22 (t, $J = 7$ Hz, 1H, ArH), 7.37–7.40 (t, $J = 7.5$ Hz, 1H, ArH), 7.44–7.47 (t, $J = 7.5$ Hz, 3H, ArH), 7.53–7.54 (d, $J = 7$ Hz, 2H, ArH), 7.64–7.66 (d, $J = 8$ Hz, 1H, ArH), 10.72 (s, 1H, maleimide NH), 12.02 (s, 1H, indole NH); m/z (FAB MS) 289.2 $[M + H]^+$.

3-(3-indolyl)-4-(1-phenyl)-1H-pyrrole-2,5-dione (9)[73]. To stirred dioxane-H_2O (24 mL, 5 : 1, v/v) were added compound 1 (0.44 g, 1.51 mmol), K_2CO_3 (0.31 g, 2.25 mmol) and phenyl-boronic acid (0.28 g, 2.3 mmol), and N_2 gas was bubbled into the mixture. $Pd(OAc)_2$ (0.1 g, 0.45 mmol) was added to the solution, and the mixture was refluxed for 23 h. The organic layer was extracted with EtOAc and concentrated. The resulting precipitate was purified by column chromatography (silica gel, hexane–EtOAc = 5 : 3, v/v) to give 9 (0.3 g, 69%). Found: C, 74.60; H, 4.11; N, 9.59. $C_{18}H_{12}N_2O_2$ requires C, 74.99; H, 4.20; N, 9.72%; mp 263–264 °C; ^{1}H-NMR(DMSO$-d_6$) 6.30–6.31 (d, $J = 8$ Hz, 1H, ArH), 6.64–6.67 (m, 1H, ArH), 7.01–7.05 (m, 1H, ArH), 7.29–7.35 (m, 3H, ArH), 7.37–7.41 (m, 3H, ArH), 7.962–7.968 (d, $J = 3$ Hz, 1H, ArH), 11.0 (s, 1H, maleimide NH), 11.87 (s, 1H, indole NH); m/z (FAB MS) 289.1 $[M + H]^+$.

3-(3-indolyl)-4-(2-naphthyl)-1H-pyrrole-2,5-dione (10)[73]. To stirred dioxane-H_2O (24 mL, 5 : 1, v/v) were added compound 1 (0.44 g, 1.51 mmol), K_2CO_3 (0.31 g, 2.25 mmol) and 2-naphthaleneboronic acid (0.4 g, 2.3 mmol), and N_2 gas was bubbled into the solution. $Pd(OAc)_2$ (0.1 g, 0.45 mmol) was added to the solution, and the mixture was refluxed for 24 h. The organic layer was extracted with EtOAc and concentrated. The resulting precipitate was purified by column chromatography (silica gel, hexane–EtOAc = 5 : 3, v/v) to give 10 (0.11 g, 21.6%). Found: C, 77.63; H, 4.12; N, 8.20. $C_{22}H_{14}N_2O_2$ requires C, 78.09; H, 4.17; N, 8.28%; mp 287–289 °C; ^{1}H-NMR(DMSO$-d_6$) 6.34–6.36 (d, $J = 8.5$ Hz, 1H, ArH), 6.50–6.53 (t, $J = 8$ Hz, 1H, ArH), 6.96–6.99 (t, $J = 8$ Hz, 1H, ArH), 7.38–7.41 (m, 2H, ArH), 7.47–7.54 (m, 2H, ArH), 7.75–7.77 (d, $J = 8.5$ Hz, 1H, ArH), 7.83–7.84 (d, $J = 8$ Hz, 1H, ArH), 7.86–7.87 (d, $J = 8$ Hz, 1H, ArH), 7.985–7.99 (d, $J = 2.5$ Hz, 1H, ArH), 8.07 (s, 1H, ArH), 11.07 (s, 1H, maleimide NH), 11.9 (s, 1H, indole NH); m/z (FAB MS) 339.1 $[M + H]^+$.

3-Phenyl-4-(1H-3-indolyl)-2,5-pyrrolidinedione (11). DMF (3 mL) was added to compound 9 (0.069 g, 0.24 mmol) and

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10% Pd–C (0.04 g). The mixture was stirred under hydrogen gas (150 psi) for 92 h. The filtrate was concentrated *in vacuo*, EtOAc (100 mL) and H_2O (100 mL) were added to the resulting precipitates, and the organic layer was concentrated. The resulting precipitates were purified by column chromatography (silica gel, EtOAc–hexane = 1 : 1, v/v) to give **11**(0.05 g, 72%). mp 105–107 °C; ^{1}H-NMR(DMSO-d_6) 4.35–4.36 (d, $J = 7.5$ Hz, 1H), 4.5–4.51 (d, $J = 7.5$ Hz, 1H), 6.92–6.95 (t, $J = 8$ Hz, 1H), 7.05–7.08 (t, $J = 8$ Hz, 1H, ArH), 7.24–7.36 (m, 8H, ArH), 11.0 (s, 1H, maleimide NH), 11.46 (s, 1H, indole NH); m/z (FAB MS) 290.11 [M]$^+$.

3-Naphthyl-4-(1H-3-indolyl)-2,5-pyrrolidinedione (12). DMF (3 mL) was added to compound **10** (0.033 g, 0.1 mmol) and 10% Pd–C (0.025 g). The mixture was stirred under hydrogen gas (150 psi) for 92 h. The filtrate was concentrated *in vacuo*, EtOAc (100 mL) and H_2O (100 mL) were added to the resulting precipitates, and the organic layer was concentrated. The resulting precipitates were purified by column chromatography (silica gel, EtOAc–hexane = 1 : 1, v/v) to give **12** (0.02 g, 61%). mp 115–117 °C; ^{1}H-NMR(DMSO-d_6) 4.51–4.53 (d, $J = 7.5$ Hz, 1H), 4.62–4.64 (d, $J = 7.5$ Hz, 1H), 6.91–6.94 (t, $J = 7$ Hz, 1H), 7.04–7.07 (t, $J = 7$ Hz, 1H, ArH), 7.33–7.38 (m, 3H, ArH), 7.47–7.53 (m, 3H, ArH), 7.82–7.89 (m, 4H, ArH), 11.01 (s, 1H, maleimide NH), 11.54 (s, 1H, indole NH); m/z (FAB MS) 341.14 [M + H]$^+$.

(Z)-2-(1H-3-methylindole)-1,4-dimethylbutenedioic acid (14). To stirred THF (30 mL) was added NaH (60% in oil, 0.2 g, 5 mmol). The solution was stirred at 0 °C and methyl diethylphosphonoacetate (1 mL, 5.3 mmol) was added into the solution. After the production of H_2 gas ended, a THF (40 mL) solution of compound **13** (0.55 g, 2.5 mmol) was added to the mixture. The reaction mixture was stirred at ambient temperature for 5 h. EtOAc (200 mL) and H_2O (150 mL) were added to the solution, and the organic layer was extracted and washed with H_2O (100 mL). The organic layer was dried with anhydrous $MgSO_4$, the filtrate was concentrated and the resulting precipitate was purified using column chromatography (silica gel, EtOAc–hexane = 1 : 1, v/v) to give **14** (0.27 g, 39.1%). ^{1}H-NMR(DMSO-d_6) 3.58 (s, 3H, –OCH$_3$), 3.63 (s, 3H, –OCH$_3$), 3.77 (s, 2H, –CH$_2$–), 5.86 (s, 1H, olefin CH–), 6.96–6.99 (t, $J = 7.5$ Hz, 1H,ArH), 7.06–7.09 (t, $J = 7$ Hz, 1H, ArH), 7.18–7.19 (d, $J = 2.5$ Hz, 1H, ArH), 7.35–7.36 (d, $J = 8$ Hz, 1H, ArH), 7.41–7.43 (d, $J = 8$ Hz, 1H, ArH), 10.94 (s, 1H, indole NH); m/z (FAB MS) 273.18 [M]$^+$.

3-(1H-3-methylindolyl)-2,5-dihydro-1H-2,5-pyrroledione (15). A pressure reaction vessel (stainless Parr bomb) was charged with compound **14** (0.2 g, 0.73 mmol) and ethylene glycol (20 mL). The vessel was sealed and charged with ammonia gas (0.2 MPa). The reaction mixture was stirred at 100 °C (oil bath) for 6 h and then returned to room temperature. Diethylether (200 mL × 3) and H_2O (150 mL) were added to the solution, and the organic layer was extracted. The organic layer was dried with anhydrous $MgSO_4$, the filtrate was concentrated and the resulting precipitate was purified by column chromatography (silica gel, CHCl$_3$: CH$_3$OH = 10 : 1, v/v) to give **15** (0.05 g, 30.1%). Found: C, 65.23; H, 4.56; N, 11.19.

$C_{13}H_{10}N_2O_2$·0.75H_2O requires C, 65.13; H, 4.83; N, 11.68%; mp 260–262 °C; ^{1}H-NMR(DMSO−d_6) 3.5 (s, 2H, –CH$_2$–), 7.13–7.21(m, 2H, ArH), 7.45–7.46 (d, $J = 7.5$ Hz, 1H, Olefin CH–), 7.68 (s, 1H, ArH), 7.78–7.82 (m, 2H, ArH), 11.13 (s, 1H, maleimide NH), 11.9 (s, 1H, indole NH); m/z (FAB MS) 226.13 [M]$^+$.

Chemiluminescence measurement

To 200 μL of 10 μM compounds **1–12** or **15** in CH$_3$CN was added 100 μL of 1–250 mM NaOH. After allowing the solution to stand for 20 s, the CL reaction was initiated by the addition of 100 μL of 10–750 mM H_2O_2. The CL emission was measured for 10 min, and the integral photon counts were used to estimate the CL intensities.

Cyclic voltammetry

Cyclic voltammetry was performed on a CV-50W electrochemical analyzer (BAS). A three-electrode arrangement in a single cell was used for the measurement: a Pt wire as the auxiliary electrode, a GC electrode as the working electrode, and an Ag/Ag$^+$ electrode as the reference electrode. The sample solution (10 mM) contained 0.1 M tetrabutyl-ammonium perchlorate as a supporting electrolyte in DMF, and argon was bubbled into the solution for 10 min before each measurement.

The confirmation of compound 16

To stirred CH$_3$CN (20 mL) was added compound **7** (0.011 g, 0.052 mmol) and 500 mM aqNaOH (5 mL), and the solution was stirred at ambient temperature for 5 min. 500 mM aqH$_2$O$_2$ (5 mL) was added to the solution, and the mixture was stirred for 2 h. Furthermore, 500 mM aqH$_2$O$_2$ (5 mL) was added to the solution, and the solution was stirred at ambient temperature for 40 min. To the solution was added ethyl acetate (100 mL) and H_2O (100 mL); the aqueous layer was then lyophilized. The resulting precipitate was purified by column chromatography (ODS, methanol–H_2O = 1 : 9, v/v) to give compound **16**. mp >300 °C; ^{1}H-NMR(D$_2$O) 6.75–6.82 (m, 2H,ArH), 7.23–7.26 (m, 1H, ArH), 7.64–7.66 (d, 1H, $J = 8$ Hz, ArH), 8.41 (s, 2H, CHO); m/z (FAB MS) 329 [M + 3H_2O]$^+$.

Computational methods

The molecular structures were completely optimized by the multi-reference perturbation calculations with the second order Rayleight-Schödinger perturbation theory (RS2). The complete active space was employed as CAS (6, 5), which consists of the 5 MOs with 6 electrons. The main electron configuration of the S_0 state of compound **7** is written as: **IM** ($1^1A(S_0)$): (inner occupied orbitals)^{106}(53a)^2(54a)^2(55a)^2 (56a)^0(57a)^0. Dunning's cc-pVDZ[74] (correlation consistent, polarized valence, double ζ) basis set was used. All of the *ab initio* calculations were performed using the electronic structure program MOLPRO[75] at the super-computer (Primergy RX200S3, Fujitsu) in the Research Institute for Information Technology, Kyushu University, Japan. Each structure optimization of the IM derivatives required 3 weeks of CPU time to obtain the converged results.
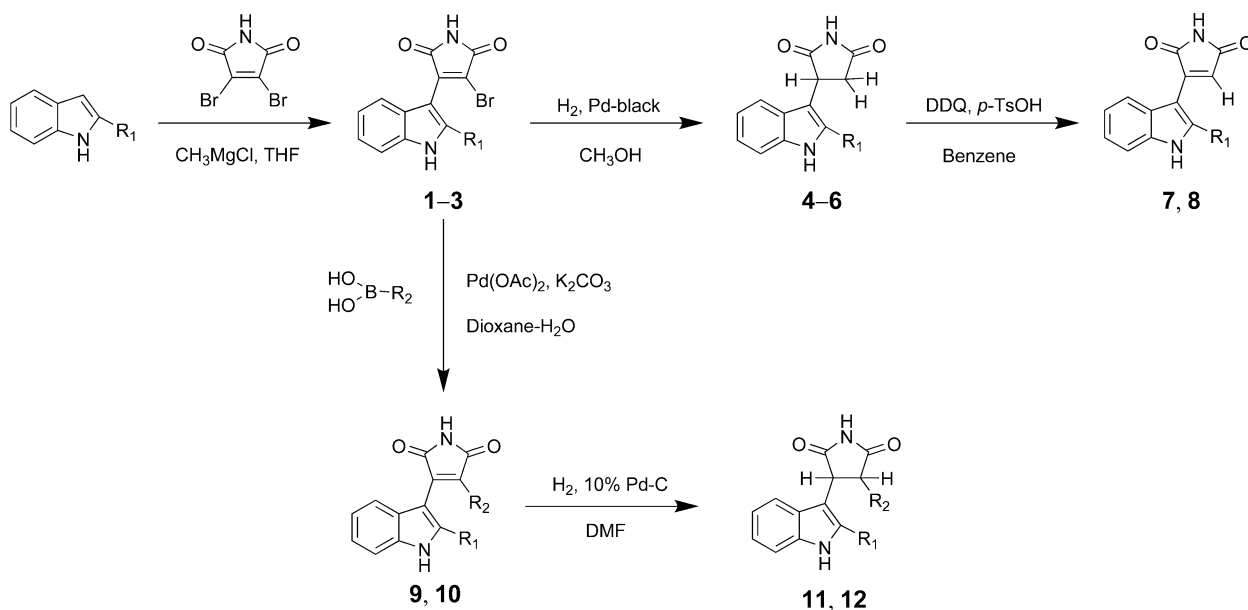
Results and discussion

Synthesis of indolylmaleimides

A series of indolylmaleimides **1–12** was prepared from indole or a 2-arylindole as shown in Scheme 1. After the starting materials were deprotonated with methyl magnesium chloride, the resulting metalloindoles were converted into compounds **1–3** through the 1,4-addition to dibromo-maleimide and subsequent elimination of hydrogen bromide. The treatment of **1–3** with hydrogen in the presence of the palladium catalyst afforded the debrominated succinimides **4–6**. The reduction of **3** required a high pressure of H_2 (750 psi) for the production of **6**, while **1** and **2** can be hydrogenated at atmospheric pressure.
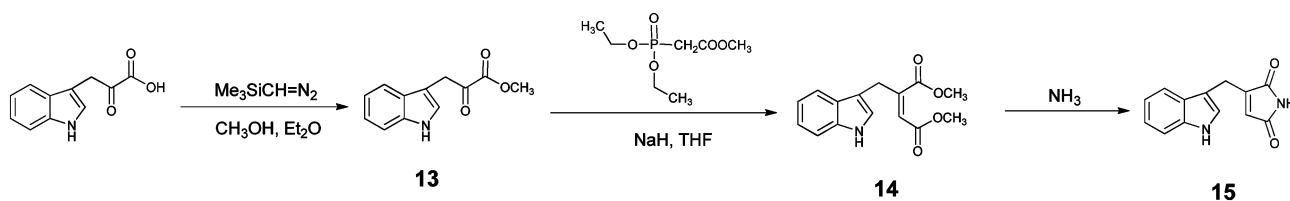
The succinimides **4** and **5** were oxidized into the debrominated maleimides **7** and **8** by DDQ, respectively. The bromo- group of **1** was transformed into a phenyl or 2-naphthyl group by Suzuki-Miyaura coupling.[76–79] The arylated maleimides **9** and **10** were saturated with hydrogen, yielding the arylated succinimides **11** and **12**.

Compound **15**, in which a methylene was inserted between the maleimide and indole moieties, was designed for evaluating the effect of the conjugation between the maleimide and indolyl moieties on the FL property. The methylene-inserted indolylmaleimide **15** was synthesized from 3-(3-indolyl)pyruvic acid (Scheme 2). After the pyruvic acid was esterified with (trimethylsilyl)diazomethane, the resulting **13** was subjected to

Scheme 1 Synthesis of compounds **1–12**.

compound	R$_1$	compound	R$_1$	compound	R$_1$	R$_2$
1	H	5	(phenyl)	9	H	(phenyl)
2	(phenyl)	6	(naphthyl)	10	H	(naphthyl)
3	(naphthyl)	7	H	11	H	(phenyl)
4	H	8	(phenyl)	12	H	(naphthyl)

Scheme 2 Synthesis of compounds **13–15**.

(a)

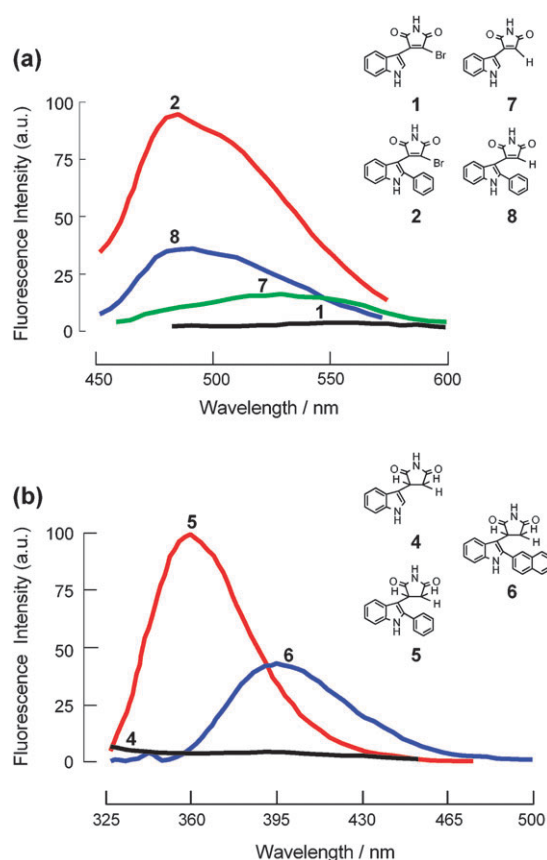Fig. 2   Molecular orbitals of compounds **4** and **7**.

Fig. 1 (a) Fluorescence spectra of **1,2**, **7** and **8** in CH$_3$CN. (b) Fluorescence spectra of **4–6** in CH$_3$CN. The numbers of the curves show the numbers of the compounds. The concentration of **2**, **8** and **4−6** was 0.1 μM. The concentration of **1** and **7** was 10 μM. The excitation maximum wavelengths of **1,2**, **7**, **8** and **4−6** were 415, 298, 396, 299, 279, 300 and 313 nm.
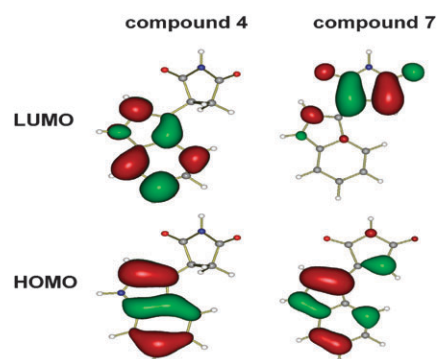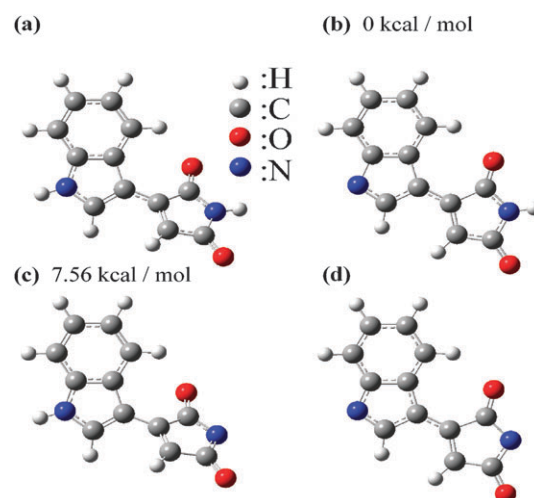
Fig. 3 Optimized geometries of indolylmaleimides (**IM**) obtained by CASPT2 /cc-pVDZ and their relative potential energy of the ground states. (a) neutral (**IM**) (b) monovalent anion by deprotonation of indole NH group (**IM$^{(-)}$**) (c) monovalent anion by deprotonation of maleimide NH group (**IM$^{(-)}$**) (d) divalent anion by deprotonation of indole and the maleimide NH groups (**IM$^{(2-)}$**).

Table 1   Fluorescence properties of compounds **1–12** and **15**

Compound[a]	λ_{abs}^{max}/nm	ε/M^{-1} cm^{-1}	λ_{em}^{max}/nm	Φ_f^b	Relative FL intensity[c]
1	415	9310	563		
2	298	21 150	484	0.24	630
	425	4380	487		230
3	303	30 950	539	0.20	530
	418	6220	478		160
4	279	6130	331		
5	300	19 560	367	0.64	2050
6	284	13 720	415		930
	313	14 030	415	0.43	890
7	270	8330	512		
	396	11 340	522		
8	299	22 920	486	0.08	250
	413	7000	472		100
9	418	9360	479	0.09	110
10	322	6590	476	0.46	420
	423	9830	484		190
11	279	2420	323		
12	273	9380	431	0.15	100
15	342	8220	429		

[a] The concentration was 0.1 μM in CH$_3$CN except for compounds **1**, **4**, **7**, **11** and **15** (10 μM). [b] The FL quantum yield of each compound was determined with reference to quinine bisulfate in 0.05 M H$_2$SO$_4$ aqueous solution (Φ_f = 0.51).[84] [c] The FL intensity of **12** in CH$_3$CN was taken as 100.

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the Horner–Emmons reaction with methyl diethylphosphono-acetate to yield a mixture of maleate **14** with its *E*-isomer. Compound **14** was separated from the undesired fumarate by silica gel column chromatography. The purified **14** in hand was transformed into the desired maleimide **15** through a treatment with pressurized NH_3 gas (29 psi).[80]

Fluorescence properties of indolylmaleimides

The FL spectra of the IMs were measured in CH_3CN, $CHCl_3$, CH_3OH, CH_3CH_2OH and DMF. A comparison of the FL spectra of **1**, **2**, **7** and **8** in CH_3CN is shown in Fig. 1(a). Apparently, the substitution of the phenyl group at the
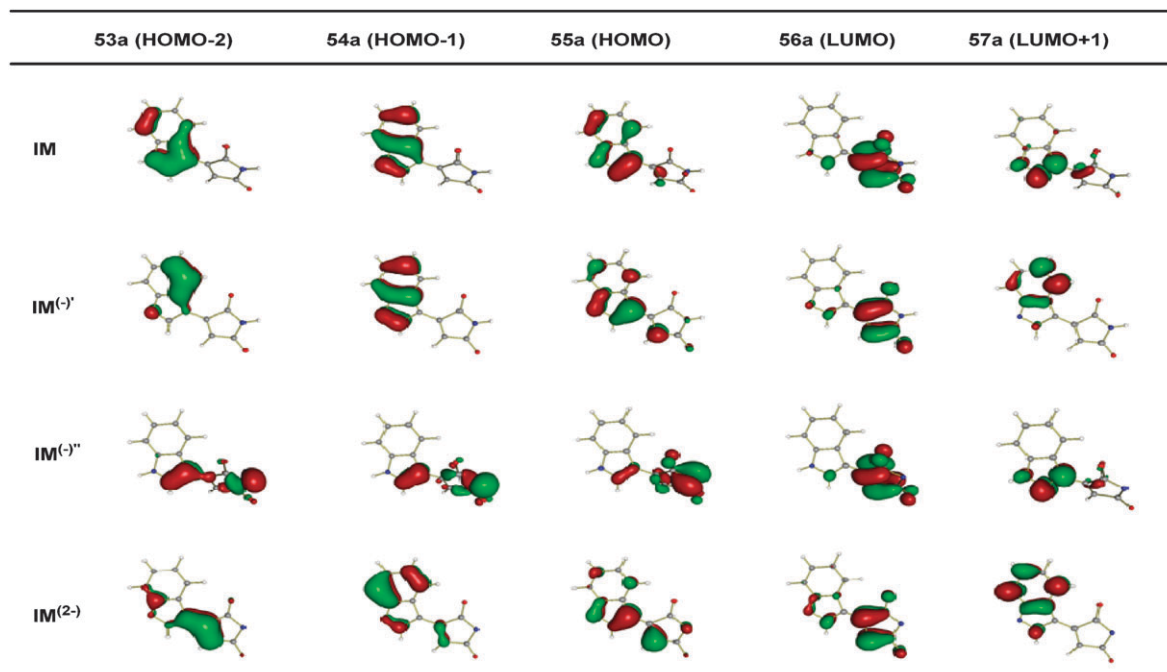

Fig. 4 Low-lying molecular orbitals (MOs) of IM, $IM^{(-)}$, $IM^{(-)}$ and $IM^{(2-)}$ at the optimized geometries of the S_0 state.
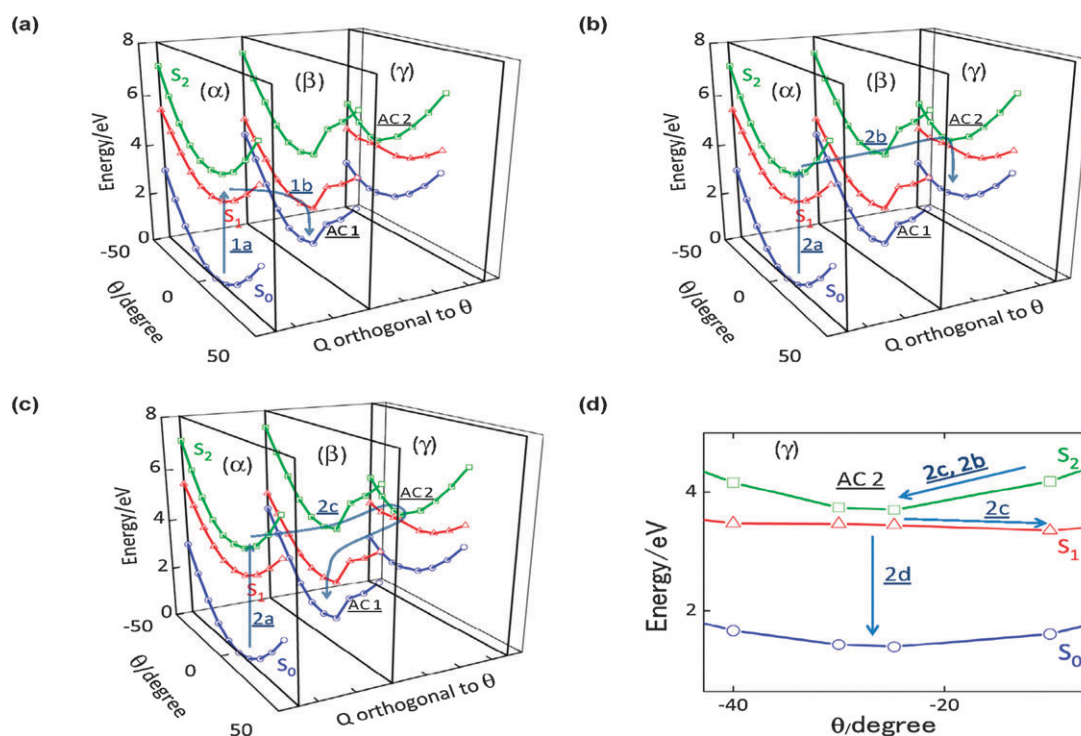

Fig. 5 Two-dimensional potential energy surfaces of $IM^{(2-)}$, as a function of the twisting angle (θ) between the indole and maleimide moieties and the orthogonal coordinates (**Q**) to the θ coordinate. Three potential curves on the three (Eθ)-planes ((α), (β), and (γ)) are based on the optimized geometries of the S_0, S_1, and S_2 states. "AC" indicates the position of the avoided curve crossing.

Table 2 Potential energies of the S_0, S_1 and S_2 states and the oscillator strengths and excitation energies for the electronic transitions of the neutral IM, two monodeprotonated IM anions (IM$^{(-)}$ and IM$^{(-)}$) and dideprotonated IM anion (IM$^{(2-)}$)

	Electronic state	E/cm^{-1}	Oscillator strength	λ/nm *threo.*	Exp.[a]
IM	S_0	0			
	S_1	31 464	0.2080	318	
	S_2	37 112	0.0663	269	
IM$^{(-)}$	S_0	0			
	S_1	22 921	0.4783	435	
	S_2	25 373	0.1101	393	
IM$^{(-)}$	S_0	0			
	S_1	25 871	0.01469	386	
	S_2	45 045	0.04113	222	
IM$^{(2-)}$	S_0	0			
	S_1	25 766	0.1826	388	396
	S_2	33 738	0.3559	296	270

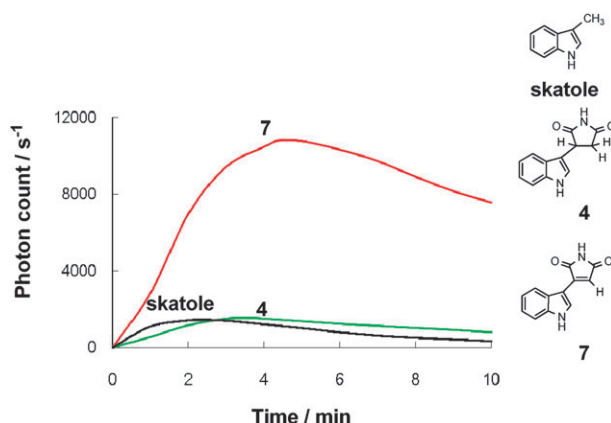[a] Experimental data in CH$_3$CN.

Fig. 7 Time course of chemiluminescence of skatole, **4** and **7**. The numbers of the curves show the numbers of the compounds. The concentration of skatole, **4** and **7** was 10 μM in CH$_3$CN.

2-position of the indole ring induced a shorter shift of the emission maximum wavelength and an increase in FL intensity. In CH$_3$CN, the FL intensities of **2** and **8** were a few hundred-fold stronger than those of **1** and **7**, respectively. As shown in Fig. 1(b), compound **4** (0.1 μM) did not fluoresce in CH$_3$CN, CH$_3$OH and DMF. This result inspired us in this study. In contrast, compounds **5** and **6** had strong FL intensity in CH$_3$CN and DMF. The substitution at the 2-position of the indole ring caused an obvious increase in the FL intensity. Almost an on–off switching of the FL was observed in **4–6**. The FL properties of **1–12** and **15** are summarized in Table 1. The emission maximum wavelength of **3** was 539 nm and was the longest among the IMs at 0.1 μM. The quantum yields (Φ_f) of compounds **5**, **6** and **10** were 0.64, 0.43 and 0.46, respectively, and were high. In the previous report, the quantum yield of 2-phenylindole was 0.86.[81] Compared with the Φ_f of **8** and 2-phenylindole, the introduction of maleimide caused a decrease in Φ_f. The FL intensity of **5** was the strongest among the IMs. The substituted group at the 2-position of the indole ring is important for the FL properties of the IMs. From a comparison of the FL properties of **7**, **9** and **10**, the effect of the introduction of a phenyl or naphthyl group to the 4-position of the maleimide moiety on the FL properties was estimated. The naphthyl group causes a large red shift of the emission maximum wavelength and an increase in the FL intensity. The emission maximum wavelengths of **9** and **10** were approximately 150 and 50 nm longer than those of **11** and **12**, respectively. The presence of the C=C bond of the maleimide moiety expands the π-conjugation. The C=C bond of the

maleimide moiety is needed for the longer shift of the emission maximum wavelengths of the IMs. The absorption maximum wavelength (396 nm) of **7** was long compared to that (342 nm) of **15** in CH$_3$CN. Furthermore, the emission maximum wavelength of **15** was approximately 100 nm longer than that of **7**. As described above, the presence of π-conjugation should cause an obvious change in FL properties. The binding of the indole ring and the maleimide moiety had a great effect on the FL of the IM. The FL intensity of most IMs in protic solvents such as CH$_3$OH and CH$_3$CH$_2$OH decreased. A large difference in FL intensities was observed in different solvents for the IMs. Some IMs should be useful for FL probes based on the on–off switching.

Ab initio MO CASPT2 calculations for indolylmaleimides

In order to clarify these obtained FL properties, *ab initio* molecular orbital (MO) and multi-reference second order perturbation (MRPT2 or CASPT2) methods were employed.[82,83] Natural molecular orbitals (MOs) of compounds **4** and **7** are shown in Fig. 2, where the MOs were obtained by CASSCF calculation. Both the HOMO and LUMO are localized in the indole moiety of compound **4**. In contrast, the LUMO in **7** was localized in the maleimide moiety; the photo-excitation of **7** would cause the electron transfer from the indole moiety to the maleimide moiety, which is an intramolecular charge transfer (CT). Our last study[65] suggests that the intramolecular CT process results in a twisting motion between these groups in bisindolyl-maleimide (BIM). Because the same process would be expected to occur even in the IM molecule, molecular
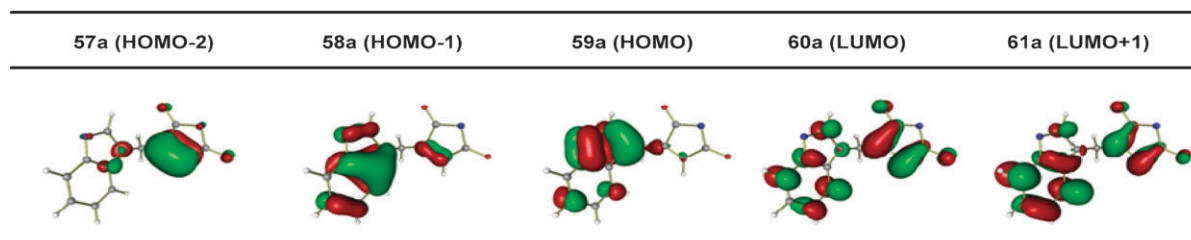

Fig. 6 Molecular orbitals of dideprotonated compound **15**.

optimized geometries and potential energy curves as a function geometries and potential energy curves as a function of the twisting angle were computed in each of the ground and electronically excited states. On the other hand, the four candidate molecules in CH_3CN solution were considered here to assign the absorption and emission spectra of compound 7; the neutral IM, two monodeprotonated IM anions ($IM^{(-)}$ and $IM^{(-)}$) and dideprotonated IM anion ($IM^{(2-)}$) are shown in Fig. 3. In Table 2, vertical excitation energies and oscillator strengths based on experimental data in the CH_3CN solution are summarized, where the molecular geometries optimized in the S_0 state were used. The vertical excitation energy of the S_1–S_0 transition of neutral IM is higher than the experimental data, while that of the S_2–S_0 transition seems to be in good agreement with the experimental data. However, this is not a candidate because the corresponding oscillator strength is small (f_{theo} = 0.0663). One of the monodeprotonated IM anions ($IM^{(-)}$) would be assignable because of the good agreement with the vertical excitation energy of the S_1–S_0 transition. However, this is not a candidate in the solution either because of the high excitation energy of the S_2–S_0 transition. Finally, the molecular species in CH_3CN solution could be the dideprotonated IM anion ($IM^{(2-)}$). The experimental absorption maxima (λ_{max} = 270 and 396 nm) agree well with the vertical excitation energies (S_1–S_0, S_2–S_0; λ_{theo} = 296 and 388 nm). The molecular orbitals obtained by CASSCF calculations are plotted in Fig. 4. The first electronically excited state (S_1) of $IM^{(2-)}$ is characterized by the HOMO–LUMO single electron-excitation. The HOMO electrons are mainly distributed around the indole moiety but this is not a clear distribution. The LUMO electrons are distributed around the maleimide moiety. The electron-configuration of the second electronically excited state (S_2) is a (HOMO-1)-LUMO single electron-excitation, and more-over, the (HOMO-1) electrons are obviously distributed around the indole moiety. Thus, the intramolecular CT process from the indole moiety to the maleimide moiety could occur in the solution, as expected in our last paper.[65] Cyclic voltammetry (CV, v = 50 mV s^{-1}) was also performed for the electrochemical evaluation of 7. The CV of compound 7 showed reversible reduction processes. The indole and maleimide moieties should be electron donor and electron acceptor, respectively. The reduction peak potential of 7 was −1.26 V (vs Ag/AgCl) and was 0.19 V smaller than that of maleimide. In contrast, the reduction processes of 4 and 15 were not observed (ESI).† The absorption spectra of 7 in 0.5–2% (v/v) triethylamine-CH_3CN solution were similar to that of 7 in CH_3CN. The FL spectra of 7 in 1–10% (v/v) triethylamine-CH_3CN solution were similar to that of 7 in CH_3CN. Thus, anions of 7 should exist in the excited state in CH_3CN (ESI).† Here we consider the photo-emission process of $IM^{(2-)}$. The two dimensional potential energy surfaces ($E(\theta,\mathbf{Q}$; $\mathbf{Q}$ should be in bold print because of the vectors.)) as a function of the twisting angle (θ) between the indole and maleimide moieties and the orthogonal coordinates ($\mathbf{Q}$) to the θ coordinate are plotted in Fig. 5. The (α), (β), and (γ) panels show the potential energy curves based on the optimized geometries in S_0, S_1 and S_2, respectively; for example, in the (γ) panel, first we performed the molecular geometry optimization for the S_2

state, and then the potential energy curves as a function of θ are obtained by fixing $\mathbf{Q}$. The optimized angles in each of the electronic states are θ_{α} = 0°, θ_{β} = 0°, and θ_{γ} = 24.8°, respectively. The effective potential energy surface for the
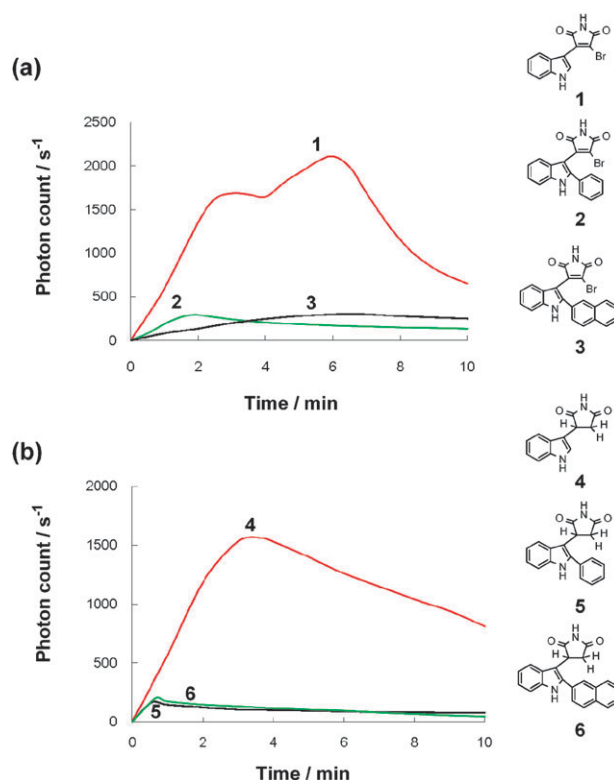

Fig. 8 (a) Time course of chemiluminescence of 1–3. (b) Time course of chemiluminescence of 4–6. The numbers of the curves show the numbers of the compounds. The concentration of 1–3 and 4–6 was 10 μM in CH_3CN.
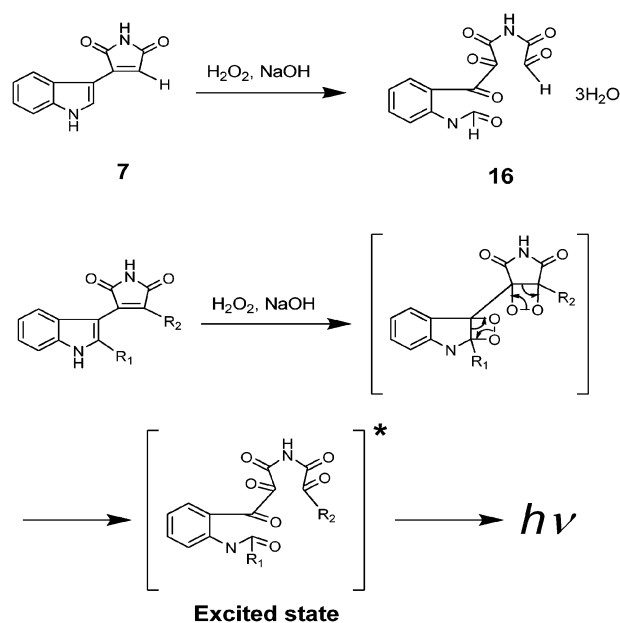

Fig. 9 The chemiluminescence reaction from compound 7 to 16 and the possible chemiluminescence mechanism of the IMs.

photo-emission process shown in Fig. 5 provides us with definite information on the reaction mechanism. Actually, we found that there may be three processes after the photo-absorption. The suggested processes in S_1 and S_2 are marked with the numbers **1** and **2**, respectively. Fig. 5(a) shows the process in S_1; after acquiring the photon (**1a**), the molecule starts to twist following the **1b** arrow and emits the photon ($\lambda_{theo} = 852$ nm) at the energy minimum in S_1. Thus, the emission would be expected by such a minimum energy path; however, this is inconsistent with the experimental data ($\lambda_{exp} = 522$ nm). Furthermore, the non-radiative process would occur by the non-adiabatic transition labeled **AC1** at around the same minimum, but the transition probability should be small because of the big energy difference between S_0 and S_1. On the other hand, the two optimized conformers retain the same molecular plane without any twisting, and the transition dipole moment of the S_1–S_0 transition at the minimum of the S_1 state is 5.66 Debye; this is sufficient to emit the photon. We therefore suggest the possibility of the photo-emission by the way of the energy-stabilization in S_1, which was shown in BIM.[65]

On the other hand, we could expect two processes in S_2, as shown in Fig. 5(b) and (c). The photo-excited molecule starts to twist following the **2b** and **2c** arrows and reaches the minimum caused by avoiding the crossing labeled **AC2**, and then the two processes in S_2 would occur; one is the non-adiabatic transition to the S_1 state and the molecule has the same process (**2c**) as described above, while the other is the emission from the minimum or the emission just after the non-adiabatic transition or both (**2b**). The **2b** and **2c** mechanisms are illustrated in Fig. 5(d). Because the S_1 and S_2 states lie in close proximity and these states are mixed around the minimum, we could expect the emission from this minimum even if considering Kasha's rule. The energy gap between the S_1 and S_0 states at this minimum is 18 622 cm^{-1} ($\lambda_{theo} = 536$ nm), although it is slightly higher than the experimental energy ($E_{exp} = 19\,708$ cm^{-1} ($\lambda_{exp} = 512$ nm)). As discussed in our last study,[65] we also suggest the possibility of photoemission by way of the twisting isomerization in the S_1 state, because the transition dipole moment is small (0.549 Debye) at the minimum energy point in S_2. Thus, IM$^{(2-)}$ would be regarded as the most likely molecule in the solution. To confirm this

prospect, *ab initio* molecular dynamics (*ab initio* MD) simulation would be a powerful tool, and we plan to perform such a calculation. We also suggest that compound **15** would be dideprotonated in solution, because the theoretical excitation energy for the S_1–S_0 transition is 29 498 cm^{-1} ($\lambda_{theo} = 339$ nm), which is in good agreement with the experimental data ($\lambda_{exp} = 342$ nm). The corresponding transition dipole moment (1.96 Debye) is also sufficient to allow the transition. The MOs of dideprotonated compound **15** are shown in Fig. 6. The significant feature of these MOs is that the electron density is no longer localized on the indole moiety or the maleimide moiety in 61a (LUMO) and 62a (LUMO + 1) orbitals, which are quite different from compound **7**. Finally, the intramolecular CT process would not be allowed in this molecule, because the electronic configuration obtained by the CASPT2 calculation shows that the S_1 state is characterized by HOMO–LUMO single electron-excitation. Furthermore, the S_2 state has a similar feature due to the configuration of HOMO-(LUMO + 1) excitation. Thus, we would miss the intramolecular CT process by introducing the CH$_2$ spacing between the indole moiety and the maleimide moiety.

Chemiluminescence properties of indolylmaleimides

The CL intensity of **7** was much stronger than that of 3-methylindole (skatole) and **4**, and a long-lasting CL was observed (Fig. 7). The time to reach the maximum photon count was 4−5 min after the addition of 50 mM NaOH and 50 mM H$_2$O$_2$. Furthermore, from a comparison of the CL intensity of **9–12**, the C=C bond in the maleimide moiety increased the CL intensity of the IMs. Compared with the CL intensity of **1–3**, the CL intensity of **1** was the strongest among them (Fig. 8(a)). The substitution of a phenyl or naphthyl group at the 2-position of the indole ring caused a decrease in the CL intensity. Similar results were obtained for the CL intensity of **4–6** (Fig. 8(b)). The CL properties of **1–12** and **15** are summarized in Table 3. The CL intensity of **7** was approximately 5-fold stronger than that of **15**. The π-conjugation by bonding the indole moiety and the maleimide moiety caused an increase in the CL intensity. Based on these experimental results, the relationship between CL intensity and the structure was clarified in the CL of the IMs. McCapra

Table 3 Chemiluminescence properties of compounds **1–12** and **15**

Compound^a	NaOH/mM	H$_2$O$_2$/mM	Integral photon count ($\times 10^4$)^b	Relative CL intensity^c
Skatole	400	25	47	100
1	5	250	79	168
2	25	250	9	19
3	25	500	11	23
4	2	50	63	134
5	10	100	4	9
6	10	250	4	9
7	50	50	490	1041
8	10	250	18	34
9	50	100	227	483
10	50	25	392	834
11	50	100	27	57
12	10	100	50	106
15	10	500	94	200

^{a} 10 μM in CH$_3$CN. ^{b} The integral photon count for 10 min. ^{c} The CL intensity of skatole in CH$_3$CN was taken as 100.

reported that the indole CL mechanism proceeds *via* the oxidation of C=C (dioxetane) with skatole.[85–88] From the investigation of products after the CL reaction of compound **7** using ^{1}H-NMR and FAB MS, compound **16** should be produced (Fig. 9). Compound **16** was a fluorescent compound (excitation maximum wavelength, 250 nm; emission maximum wavelength, 287 nm) (ESI).† Thus, the IMs having the C=C bond of the maleimide moiety also emitted light *via* dioxetane (Fig. 9). We proposed that the CL intensities were low because the FL intensities of FL compounds produced after CL reactions were weak. The CL reaction conditions (NaOH, H_2O_2 conc.) inducing the maximum CL intensity for the IMs are shown in Table 3. A large difference in CL intensities was observed for the IMs.This fact indicates that some IMs can be useful for the on–off switching of CL.

Conclusions

The synthesis of the IMs in this study was facile. Interesting FL and CL properties of various IMs were found. The introduction of a naphthyl or phenyl group at the 2-position in the indole ring and the 3- or 4-position in the maleimide moiety caused an obvious increase and decrease in the FL and CL of the IMs. The C=C bond in the maleimide moiety in the IMs without substitution at the 2-position of the indole ring was needed for strong CL. Significant information for the rational design of the IMs for FL and CL probes was provided.

Acknowledgements

References

1 M. S. T. Gonçalves, *Chem. Rev.*, 2009, **109**, 190.
2 T. Toyo'oka, *Modern Derivatization Methods for Separation Sciences*, John Wiley & Sons Ltd, CHichester, UK, 1999.
3 L. J. Kricka, *Anal. Chem.*, 1995, **67**, 499.
4 L. J. Kricka, *Anal. Chem.*, 1999, **71**, 305.
5 O. Shimomura, *Bioluminescence*, World Scientific Publishing Co., Pte., Ltd, Singapore, 2006.
6 T. Nagano and T. Yoshimura, *Chem. Rev.*, 2002, **102**, 1235.
7 A. Roda, M. Guardigli, P. Pasini, M. Mirasoli, E. Michelini and M. Musiani, *Anal. Chim. Acta*, 2005, **541**, 25.
8 K. E. Sapsford, L. Berti and I. L. Medintz, *Angew. Chem., Int. Ed.*, 2006, **45**, 4562.
9 T. Kogure, S. Karasawa, T. Araki, K. Saito, M. Kinjo and A. Miyawaki, *Nat. Biotechnol.*, 2006, **24**, 577.
10 C. Gota, K. Okabe, T. Funatsu, Y. Harada and S. Uchiyama, *J. Am. Chem. Soc.*, 2009, **131**, 2766.
11 T. Ueno, Y. Urano, K. Setsukinai, H. Takakusa, H. Kojima, K. Kikuchi, K. Ohkubo, S. Fukuzumi and T. Nagano, *J. Am. Chem. Soc.*, 2004, **126**, 14079.
12 T. Himeno, T. Ueno, Y. Urano, H. Kojima and T. Nagano, *Org. Lett.*, 2006, **8**, 5963.
13 L. D. Lavis, T.-Y. Chao and R. T. Raines, *ACS Chem. Biol.*, 2006, **1**, 252.
14 S. Izumi, Y. Urano, K. Hanaoka, T. Terai and T. Nagano, *J. Am. Chem. Soc.*, 2009, **131**, 10189.
15 A. Prasanna de Silva, H. Q. Nimal Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515.
16 J. S. Kim and D. T. Quang, *Chem. Rev.*, 2007, **107**, 3780.
17 H. S. Jung, P. S. Kwon, J. W. Lee, J. ll. Kim, C. S. Hong, J. W. Kim, S. Yan, J. Y. Lee, J. H. Lee, T. Joo and J. S. Kim, *J. Am. Chem. Soc.*, 2009, **131**, 2008.
18 S. Tsukiji, M. Miyagawa, Y. Takaoka, T. Tamura and I. Hamachi, *Nat. Chem. Biol.*, 2009, **5**, 341.
19 F. H. Stross and G. H. Branch, *J. Org. Chem.*, 1938, **3**, 385.
20 E. H. White and D. F. Roswell, *Acc. Chem. Res.*, 1970, **3**, 54.
21 T. P. Whitehead, G. H. G. Thorpe, T. J. N. Carter, C. Groucutt and L. J. Kricka, *Nature*, 1983, **305**, 158.
22 J.-M. Lin, X. Shan, S. Hanaoka and M. Yamada, *Anal. Chem.*, 2001, **73**, 5043.
23 K. Tsukagoshi, T. Nakamura and R. Nakajima, *Anal. Chem.*, 2002, **74**, 4109.
24 Y. Xiao, V. Pavlov, T. Niazov, A. Dishon, M. Kotler and I. Willner, *J. Am. Chem. Soc.*, 2004, **126**, 7430.
25 I. Weeks, M. Sturgess, R. C. Brown and J. S. Woodhead, *Methods Enzymol.*, 1986, **133**, 366.
26 M. L. Grayeski and M. A. Ruberto, *Anal. Chem.*, 1992, **64**, 2758.
27 H. Akhavan-Tafti, R. DeSilva, Z. Arghavani, R. A. Eickholt, R. S. Handley, B. A. Schoenfelner, K. Sugioka, Y. Sugioka and A. P. Schaap, *J. Org. Chem.*, 1998, **63**, 930.
28 K. C. Ahn, P. Lohstroh, N. A. Gee, B. Lasley and B. D. Hammock, *Anal. Chem.*, 2007, **79**, 8883.
29 B. Krishnan, J. D. Levine, M. K. S. Lynch, H. B. Dowse, P. Funes, J. C. Hall, P. E. Hardin and S. E. Dryer, *Nature*, 2001, **411**, 313.
30 L. R. Jones, E. A. Goun, R. Shinde, J. B. Rothbard, C. H. Contag and P. A. Wender, *J. Am. Chem. Soc.*, 2006, **128**, 6526.
31 C. Wu, K. Kawasaki, Y. Ogawa, Y. Yoshida, S. Ohgiya and Y. Ohmiya, *Anal. Chem.*, 2007, **79**, 1634.
32 Y. Zhang, G. J. Phillips, Q. Li and E. S. Yeung, *Anal. Chem.*, 2008, **80**, 9316.
33 O. Shimomura, *FEBS Lett.*, 1979, **104**, 220.
34 O. Shimomura, *Angew. Chem., Int. Ed.*, 2009, **48**, 5590.
35 A. Miyawaki, J. Llopis, R. Heim, J. M. McCaffery, J. A. Adams, M. Ikura and R. Y. Tsien, *Nature*, 1997, **388**, 882.
36 R. Y. Tsien, *Annu. Rev. Biochem.*, 1998, **67**, 509.
37 M. Zimmer, *Chem. Rev.*, 2002, **102**, 759.
38 D. T. Quang and J. S. Kim, *Chem. Rev.*, 2007, **107**, 3780.
39 O. Griesbeck and M. Mank, *Chem. Rev.*, 2008, **108**, 1550.
40 W. R. Dichtel, S. Hecht and J. M. J. Frechet, *Org. Lett.*, 2005, **7**, 4451.
41 J. Han, J. Jose, E. Mei and K. Burgess, *Angew. Chem., Int. Ed.*, 2007, **46**, 1684.
42 J. Yuasa and S. Fukuzumi, *J. Am. Chem. Soc.*, 2008, **130**, 566.
43 A. Loudet and K. Burgess, *Chem. Rev.*, 2007, **107**, 4891.
44 H. Sunahara, Y. Urano, H. Kojima and T. Nagano, *J. Am. Chem. Soc.*, 2007, **129**, 5597.
45 T. Miura, Y. Urano, K. Tanaka, T. Nagano, K. Ohkubo and S. Fukuzumi, *J. Am. Chem. Soc.*, 2003, **125**, 8666.
46 M. Onoda, S. Uchiyama, A. Endo, H. Tokuyama, T. Santa and K. Imai, *Org. Lett.*, 2003, **5**, 1459.
47 A. Wakamiya, K. Mori and S. Yamaguchi, *Angew. Chem., Int. Ed.*, 2007, **46**, 4273.
48 D. Srikun, E. W. Miller, D. W. Domaille and C. J. Chang, *J. Am. Chem. Soc.*, 2008, **130**, 4596.
49 G. Zhou, M. Baumgarten and K. Müllen, *J. Am. Chem. Soc.*, 2008, **130**, 12477.
50 Y. Liu, M. Han, H.-Y. Zhang, L.-X. Yang and W. Jiang, *Org. Lett.*, 2008, **10**, 2873.
51 X. Gao, Y. Zhang and B. Wang, *Org. Lett.*, 2003, **5**, 4615.

52 M. Kamiya, H. Kobayashi, Y. Hama, Y. Koyama, M. Bernardo, T. Nagano, P. L. Choyke and Y. Urano, *J. Am. Chem. Soc.*, 2007, **129**, 3919.

53 J. Shaomin, J. Yang, Q. Yang, S. Liu, M. Chen and J. Zhao, *J. Org. Chem.*, 2009, **74**, 4855.

54 B. L. Van Duuren, *J. Org. Chem.*, 1961, **26**, 2954.

55 G. E. Philbrook, J. B. Ayers, J. F. Garst and J. R. Totter, *Photochem. Photobiol.*, 1965, **4**, 869.

56 H. Nakamura and T. Goto, *Photochem. Photobiol.*, 1979, **30**, 27.

57 M. Nakazono, Y. Sho and K. Zaitsu, *Anal. Sci.*, 2003, **19**, 123.

58 O. Shimomura, T. Goto and Y. Hirata, *Bull. Chem. Soc. Jpn.*, 1957, **30**, 929.

59 Y. Kishi, T. Goto, Y. Hirata, O. Shimomura and F. H. Johnson, *Tetrahedron Lett.*, 1966, **7**, 3427.

60 B. K. Kaletas, R. M. Williams, B. Konig and L. De Cola, *Chem. Commun.*, 2002, 776.

61 B. K. Kaletas, C. Mandl, G. van der Zwan, M. Fanti, F. Zerbetto, L. De Cola, B. König and R. M. Williams, *J. Phys. Chem. A*, 2005, **109**, 6440.

62 B. K. Kaletas, H. C. Joshi, G. van der Zwan, M. Fanti, F. Zerbetto, K. Goubitz, L. De Cola, B. König and R. M. Williams, *J. Phys. Chem. A*, 2005, **109**, 9443.

63 M. Nakazono, A. Uesaki and K. Zaitsu, *Talanta*, 2006, **70**, 128.

64 M. Nakazono, S. Nanbu, A. Uesaki, R. Kuwano, M. Kashiwabara and K. Zaitsu, *Org. Lett.*, 2007, **9**, 3583.

65 K. Saita, M. Nakazono, K. Zaitsu, S. Nanbu and H. Sekiya, *J. Phys. Chem. A*, 2009, **113**, 8213.

66 W.-C. Wu, H.-C. Yeh, L.-H. Chan and C.-T. Chen, *Adv. Mater.*, 2002, **14**, 1072.

67 C.-W. Chiu, T. J. Chow, C.-H. Chuen, H.-M. Lin and Y.-T. Tao, *Chem. Mater.*, 2003, **15**, 4527.

68 Z. Lin, Y.-S. Wen and T. J. Chow, *J. Mater. Chem.*, 2009, **19**, 5141.

69 M. T. Baumgartner, M. A. Nazareno, M. C. Murguia, A. B. Pierini and R. A. Rossi, *Synthesis*, 1999, 2053.

70 L. Salmon, V. Landry, O. Melnyk, L. Maes, C. Sergheraert and E. Davioud-Charvet, *Chem. Phar. Bull.*, 1998, **46**, 707.

71 S. Mahboobi, E. Eichhorn, A. P. Andreas, S. Sigurd Elz and U. Möllmann, *Eur. J. Med. Chem.*, 2006, **41**, 176.

72 T. Nishizawa, S. Grschow, D.-H. E. Jayamaha, C. Nishizawa-Harada and D. H. Sherman, *J. Am. Chem. Soc.*, 2006, **128**, 724.

73 C. Sanchez-Martinez, M. M. Faul, C. Shih, K. A. Sullivan, J. L. Grutsch, J. T. Cooper and S. P. Kolis, *J. Org. Chem.*, 2003, **68**, 8008.

74 D. E. Woon and T. H. Dunning Jr, *J. Chem. Phys.*, 1993, **98**, 1358.

75 H.-J. Werner, P. J. Knowles, R. Lindh, F. R. Manby, M. Schütz, P. Celani, T. Korona, G. Rauhut, R. D. Amos, A. Bernhardsson, A. Berning, D. L. Cooper, M. J. O. Deegan, A. J. Dobbyn, F. Eckert, C. Hampel, G. Hetzer, A. W. Lloyd, S. J. McNicholas, W. Meyer, M. E. Mura, A. Nicklass, P. Palmieri, R. Pitzer, U. Schumann, H. Stoll, A. J. Stone, R. Tarroni and T. Thorsteinsson, MOLPRO, version 2006.1, a package of *ab initio* programs (http://www.molpro.net).

76 N. Miyaura, K. Yamada and A. Suzuki, *Tetrahedron Lett.*, 1979, **20**, 3437.

77 N. Miyaura and A. Suzuki, *Chem. Rev.*, 1995, **95**, 2457.

78 T. E. Barder, S. D. Walker, J. R. Martinelli and S. L. Buchwald, *J. Am. Chem. Soc.*, 2005, **127**, 4685.

79 E. P. Gillis and M. D. Burke, *J. Am. Chem. Soc.*, 2007, **129**, 6716.

80 E. J. Hennessy and S. L. Buchwald, *J. Org. Chem.*, 2005, **70**, 7371.

81 I. B. Berlman, *Spectrochim. Acta, Part A*, 1971, **27**, 473.

82 H.-J. Werner and P. J. Knowles, *J. Chem. Phys.*, 1985, **82**, 5053.

83 P. J. Knowles and H.-J. Werner, *Chem. Phys. Lett.*, 1985, **115**, 259.

84 R. A. Velapoldi and H. H. Tφnnesen, *J. Fluoresc.*, 2004, **14**, 465.

85 F. McCapra and Y. C. Chang, *Chem. Commun.*, 1966, 522.

86 N. Sugiyama and M. Akutagawa, *Bull. Chem. Soc. Jpn.*, 1967, **40**, 240.

87 N. Sugiyama, M. Akutagawa, T. Gasha, Y. Saiga and H. Yamamoto, *Bull. Chem. Soc. Jpn.*, 1967, **40**, 347.

88 N. Sugiyama, H. Yamamaoto and Y. Omote, *Bull. Chem. Soc. Jpn.*, 1968, **41**, 1917.