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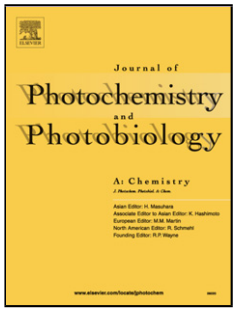

Title: Unsymmetric indolylmaleimides: synthesis, photophysical properties and amyloid detection

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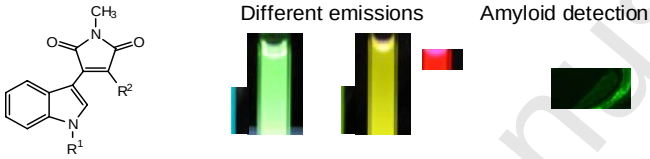
Graphical abstract

Some unsymmetric indolylmaleimides have large Stokes shifts of more than 120 nm, fluorescence maxima emission wavelengths of more than 550 nm and different emissions under UV irradiation at 365 nm. 3-(1*H*-Indol-3-yl)-1-methyl-4-phenyl-1*H*-pyrrole-2,5-dione detects amyloid fibrils in senile systemic amyloidosis.



Highlights

- Synthesis of various unsymmetric indolylmaleimides
- Unsymmetric indolylmaleimides having large Stokes shifts and different fluorescence emissions
- Unsymmetric indolylmaleimide detecting amyloid fibrils in senile systemic amyloidosis

Unsymmetric indolylmaleimides: synthesis, photophysical properties and amyloid detection

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ABSTRACT

Various unsymmetric indolylmaleimides were synthesized. Their photophysical properties and affinities for amyloid fibrils were evaluated. Some unsymmetric indolylmaleimides have large Stokes shifts of more than 120 nm, fluorescence maxima emission wavelengths of more than 550 nm and different emissions under UV irradiation at 365 nm. From the results of histopathologic methods using stains, 3-(1*H*-Indol-3-yl)-1-methyl-4-phenyl-1*H*-pyrrole-2,5-dione has high selectivity for amyloid fibrils in senile systemic amyloidosis.

Keywords: Fluorescence, Indolylmaleimide, π-conjugation, Amyloid

1. Introduction

Recently, indolylmaleimide (IM) derivatives have been a focus in the development of anticancer medicines based on inhibition of protein kinases [1–4]. Furthermore, IM compounds have been evaluated as luminescent materials for organic light-emitting diodes [5,6]. Some research groups [7–10], including us [11–14], have studied the fluorescence (FL) and chemiluminescence properties of symmetric bisindolylmaleimides. However, the potential use of IM derivatives in clinical diagnosis has been unexplored. There have been two studies on the FL properties of unsymmetric IMs [6,7]. A monosubstituted IM compound is obtained from the reaction of indolylmagnesium bromide and 2,3-dibromo-*N*-methylmaleimide [15]. The resulting monosubstituted IM is readily transformed to unsymmetric disubstituted IMs through Suzuki-Miyaura coupling. The advantage of this synthetic method is that various substituents, including fluorophores, can be introduced at the C=C bond of the maleimide moiety. When the indolylmaleimides are modified with a functional group possessing high affinity for a specific analyte, the utility of the unsymmetric IM derivatives as FL probes is drastically increased.

Amyloidosis is a disorder of protein metabolism in which normally soluble autologous proteins are deposited in tissues as abnormal insoluble fibrils, which cause structural and functional disruptions [16–21]. Among several histopathologic methods using stains, Congo red staining is one of the most popular detection methods of amyloid deposits in tissues [22,23]. However, Congo red-stained histochemical specimens are not always easily interpreted. In previous studies, styrylbenzene derivatives exhibited high selectivity for amyloid fibrils in systemic amyloidoses [24–27]. For example, (*trans*, *trans*)-1-bromo-2,5-bis-(3-hydroxycarbonyl-4-

hydroxy)styrylbenzene (BSB) was developed for in vivo detection of amyloid deposits in patients with various systemic and localized forms of amyloidosis [25]. Styrylbenzene derivatives such as BSB have π–conjugated structure. In this study, we designed and synthesized various π–conjugated unsymmetric indolylmaleimides, cleared their photophysical properties and carefully checked the reactivity of the compounds with amyloid fibrils.

2. Experimental

2.1. Chemicals

Ethylmagnesium bromide (tetrahydrofuran solution), *trans*-2-phenylvinyl boronic acid, 3-quinine boronic acid, 2,2'-bithiophene-5,5'-diboronic acid bis(pinacol)ester, 4-formylphenylboronic acid, pyrene-1-boronic acid, 9*H*-carbazole-2-boronic acid pinacol ester and 4,4'-biphenyl-diboronic acid were purchased from Aldrich (Milwaukee, USA). Indole, sodium hydride, phenylboronic acid, palladium(II) acetate, tetrakis(triphenylphosphine)palladium(0), naphthalene-2-boronic acid, sodium methoxide, di-*tert*-butyl dicarbonate, 4-bromomethyl-7-methoxycoumarin and 4-dimethylaminopyridine were purchased from Wako Chemicals (Osaka, Japan). Diethyl(4-iodobenzyl)phophonate and rhodamin B were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Cesium fluoride was purchased from Nakalai Tesque Inc. (Kyoto, Japan). 3-Bromo-4-(1*H*-indol-3-yl)-1-methyl-1*H*-pyrrole-2,5-dione and *tert*-butyl 3-(4-bromo-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)-1*H*-indole-1-carboxylate were prepared by the reported method [15]. All other chemicals and solvents were of analytical reagent grade.

2.2. Apparatus

The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125.7 MHz) spectra were obtained using a Varian UNITY plus (USA) spectrometer. The HR ESI-TOF-MS spectra of compounds **1**–**13** were obtained using a Bruker/micrOTOF II (Karlsruhe, Germany). The FAB MS spectra were obtained using a JEOL JMS-HX110A (Tokyo, Japan). The absorbance and fluorescence spectra of compounds **1**–**13** were obtained using a Jasco V-530 absorptiometer and FP-6500 fluorometer (Tokyo, Japan). The slit widths at the excitation and emission of the fluorometer were 5 nm. All the FL spectra were corrected.

2.3. Synthesis and characterization

All reactions were done under an atmosphere of inert gas.

*2.3.1. 3-(1H-indol-3-yl)-1-methyl-4-phenyl-1H-pyrrole-2,5-dione (**1**)*

3-Bromo-4-(1*H*-indol-3-yl)-1-methyl-1*H*-pyrrole-2,5-dione (0.12 g, 0.39 mmol), phenylboronic acid (0.1 g, 0.82 mmol) and K_2CO_3 (0.08 g, 0.58 mmol) were added to dioxane-H_2O (28 mL, 6:1). Palladium(II) acetate (0.06 g, 0.27 mmol) was then added to the mixture, and the solution was refluxed for 17 h. Ethyl acetate (200 mL), 1 M aqHCl (100 mL) and 10% (w/v) aqNaCl (100 mL) were added to the mixture. The organic layer was dried with anhydrous Na_2SO_4. The filtrate was concentrated and purified by column chromatography ($CHCl_3$:CH_3OH=20:1) to produce compound **1** as a red solid (0.04 g, 33% yield). M.p. 203–205 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S$=O) 3.03

(s, 3H, CH_3), 6.3–6.32 (d, J = 8 Hz, 1H, ArH), 6.65–6.68 (t, J = 8 Hz, 1H, ArH), 7.03–7.06 (t, J = 8 Hz, 1H, ArH), 7.31–7.36 (m, 3H, ArH), 7.39–7.43 (m, 3H, ArH), 8 (s, 1H, ArH), 11.9 (s, 1H, indole NH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S$=O) 24.0, 104.1, 112.1, 119.7, 121.1, 122.0, 123.8, 128.0, 128.6, 129.5, 130.6, 131.1, 132.0, 136.5, 171.0, 171.3. HRMS-ESI (m/z) calculated for $C_{19}H_{14}N_2O_2$ $[M + Na]^+$ 325.0953, found 325.0952.

*2.3.2. 3-(1H-indol-3-yl)-1-methyl-4-styryl-1H-pyrrole-2,5-dione (**2**)*

Tert-butyl 3-(4-bromo-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)-1*H*-indole-1-carboxylate (0.4 g, 1 mmol), *trans*-2-phenylvinyl boronic acid (0.22 g, 1.49 mmol) and K_2CO_3 (0.14 g, 1 mmol) were added to dioxane-H_2O (30 mL, 4:1). Tetrakis(triphenylphosphine)palladium (0) (0.12 g, 0.1 mmol) was then added to the mixture, and the solution was refluxed for 16.5 h. Ethyl acetate (200 mL), 0.5 M aqHCl (100 mL) and 10% (w/v) aqNaCl (100 mL) were added to the mixture. The organic layer was dried with anhydrous $MgSO_4$. The filtrate was concentrated and purified by column chromatography (ethyl acetate:hexane=1:2→$CHCl_3$:CH_3OH=20:1) to produce compound **2** as a red solid (0.03 g, 9% yield). M.p. 166–168 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S$=O) 3 (s, 3H, CH_3), 7.1–7.13 (t, J = 7 Hz, 1H, ArH), 7.2–7.24 (m, 2H, ArH), 7.29–7.37 (m, 3H, ArH), 7.48–7.53 (m, 3H, ArH), 7.62–7.64 (d, J = 8 Hz, 1H, ArH), 7.79–7.82 (d, J = 16.5 Hz, 1H, CH=CH), 7.88–7.89 (d, J = 2.5 Hz, 1H, ArH), 12 (s, 1H, indole NH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S$=O) 23.7, 104.8, 112.5, 118.7, 120.5, 122.4, 125.3, 126.3, 126.6, 128.9, 129.0, 130.3, 131.7, 135.9, 136.6, 136.7, 170.6, 171.0. HRMS-ESI (m/z) calculated for $C_{21}H_{16}N_2O_2$ $[M + Na]^+$ 351.1109, found 351.1106.

*2.3.3. 3-(1H-indol-3-yl)-1-methyl-4-(naphthalen-2-yl)-1H-pyrrole-2,5-dione (**3**)*

3-Bromo-4-(1*H*-indol-3-yl)-1-methyl-1*H*-pyrrole-2,5-dione (0.12 g, 0.39 mmol), naphthalene-2-boronic acid (0.14 g, 0.8 mmol) and K_2CO_3 (0.08 g, 0.58 mmol) were added to dioxane-H_2O (28 mL, 6:1). Palladium(II) acetate (0.06 g, 0.27 mmol) was added to the mixture, and the solution was refluxed for 15 h. Ethyl acetate (200 mL), 1 M aqHCl (100 mL) and 10% (w/v) aqNaCl (100 mL) were added to the mixture. The organic layer was dried with anhydrous Na_2SO_4. The filtrate was concentrated and purified by column chromatography ($CHCl_3$:CH_3OH=20:1) to produce compound **3** as a red solid (0.1 g, 71% yield). M.p. 230–232 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S$=O) 3.06 (s, 3H, CH_3), 6.35–6.36 (d, J = 8.5 Hz, 1H, ArH), 6.51–6.54 (t, J = 8 Hz, 1H, ArH), 7–7.01 (m, 1H, ArH), 7.4–7.43 (m, 2H, ArH), 7.48–7.55 (m, 2H, ArH), 7.77–7.79 (d, J = 8.5 Hz, 1H, ArH), 7.83–7.85 (d, J = 8 Hz, 1H, ArH), 7.87–7.88 (d, J = 8 Hz, 1H, ArH), 8.02 (s, 1H, ArH), 8.09 (s, 1H, ArH), 11.94 (s, 1H, indole NH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S$=O) 24.0 (CH_3), 104.2, 112.1, 119.7, 120.9, 122.0, 124.0, 126.4, 126.6, 126.8, 127.3, 127.5, 127.8, 128.1, 128.3, 129.2, 131.1, 132.2, 132.3, 132.5, 136.5, 171.0, 171.3. HRMS-ESI (m/z) calculated for $C_{23}H_{16}N_2O_2$ $[M + Na]^+$ 375.1109, found 375.1109.

*2.3.4. 3-(1H-indol-3-yl)-1-methyl-4-(quinolin-3-yl)-1H-pyrrole-2,5-dione (**4**)*

Tert-butyl 3-(4-bromo-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)-1*H*-indole-1-carboxylate (0.6 g, 1.5 mmol), 3-quinoline boronic acid (0.35 g, 2.25 mmol) and Na_2CO_3 (1.2 g, 15.5 mmol) were added to dioxane-H_2O (120 mL, 4:1). Tetrakis(triphenylphosphine)palladium(0) (0.25 g, 0.22 mmol) was then added to the mixture, and the solution was refluxed for 20.5 h. Ethyl acetate (300 mL) and H_2O (200 mL) were added to the mixture. The organic layer was dried with anhydrous $MgSO_4$.

The filtrate was concentrated and purified by column chromatography (ethyl acetate:hexane=4:5) to produce compound **4** as a red solid (0.29 g, 56% yield). M.p. 249–251 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S=O$) 3.08 (s, 3H, CH_3), 6.37–6.38 (d, J = 8 Hz, 1H, ArH), 6.56–6.59 (m, 1H, ArH), 7.01–7.04 (m, 1H, ArH), 7.45–7.47 (d, J = 8 Hz, 1H, ArH), 7.59–7.63 (m, 1H, ArH), 7.76–7.8 (m, 1H, ArH), 7.95–7.97 (m, 2H, ArH), 8.08 (s, 1H, ArH), 8.48 (s, 1H, ArH), 8.73 (s, 1H, ArH), 12.04 (s, 1H, indole NH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S=O$) 24.1, 104.1, 112.4, 120.1, 120.6, 122.2, 123.7, 124.2, 124.6, 126.9, 127.1, 128.6, 128.7, 130.3, 131.7, 133.1, 136.2, 136.7, 146.6, 150.1, 170.7, 171.1. HRMS-ESI (m/z) calculated for $C_{22}H_{15}N_3O_2$ $[M + Na]^+$ 376.1062, found 376.1051.

*2.3.5. 3-([2,2'-Bithiophen]-5-yl)-4-(1H-indol-3-yl)-1-methyl-1H-pyrrole-2,5-dione (**5**)*

Tert-butyl 3-(4-bromo-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)-1*H*-indole-1-carboxylate (0.21 g, 0.52 mmol) was added to dioxane (40 mL), and then tetrakis(triphenylphosphine)palladium(0) (0.06 g, 0.052 mmol) was added to the mixture. The solution was stirred for 10 min. 2,2'-Bithiophene-5,5'-diboronic acid bis(pinacol)ester (0.13 g, 0.3 mmol) and cesium fluoride (0.47 g, 3 mmol) were added to the mixture. The mixture was refluxed for 22 h. The solution was then filtered by celite, and ethyl acetate (200 mL) and H_2O (200 mL) were added to the filtrate. The organic layer was dried with anhydrous $MgSO_4$. The filtrate was concentrated and purified by column chromatography (ethyl acetate:hexane=2:5) to produce *tert*-butyl 3-(4-([2,2'-bithiophen]-5-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)-1*H*-indole-1-carboxylate as a red solid (0.05 g, 20% yield). M.p. 114–116 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S=O$) 1.67 (s, 9H, *t*-Bu), 3.04 (s, 3H, CH_3), 7.09–7.11 (m, 2H, ArH), 7.16–7.19 (m, 1H, ArH), 7.24–7.26 (m, 2H, ArH), 7.37–7.40 (m, 2H, ArH), 7.57–7.58

(d, J = 5 Hz, 1H, ArH), 8.01 (s, 1H, ArH), 8.15–8.17 (d, J = 8 Hz, 1H, ArH). FAB MS (m/z) $[M]^+$ 490.08.

Tert-butyl 3-(4-([2,2'-bithiophen]-5-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)-1*H*-indole-1-carboxylate (0.05 g, 0.061 mmol) was added to CH_2Cl_2 (30 mL). The solution was stirred at 0 ^oC for 15 min, and then trifluoroacetic acid (3 mL) was added to the solution. The mixture was stirred at 0 °C for 20 min and stirred at an ambient temperature for 4 h. Ethyl acetate (150 mL), 1 M aq$NaHCO_3$ (50 mL) and 1 M aqK_2CO_3 (100 mL) were added to the mixture. The organic layer was dried with anhydrous $MgSO_4$. The filtrate was concentrated and purified by column chromatography (ethyl acetate:hexane=2:5) to produce compound **5** as a red solid (0.03 g, 79% yield). M.p. 218–220 °C. ^1H NMR (500 MHz, $(CD_3)_2S{=}O$) 3.03 (s, 3H, CH_3), 6.87–6.89 (d, J = 8 Hz, 1H, ArH), 6.97–6.99 (t, J = 7 Hz, 1H, ArH), 7.08–7.12 (m, 2H, ArH), 7.15–7.19 (m, 1H, ArH), 7.23–7.24 (d, J = 4 Hz, 1H, ArH), 7.34–7.35 (dd, J = 1 Hz, 4 Hz, 1H, ArH), 7.51–7.55 (m, 2H, ArH), 7.91–7.92 (d, J = 3 Hz, 1H, ArH), 11.96 (s, 1H, indole NH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S{=}O$) 24.1, 103.9, 112.5, 120.0, 121.5, 122.1, 123.6, 123.8, 124.8, 127.7, 128.5, 128.6, 129.2, 136.0, 136.6, 140.4, 170.7, 170.8. HRMS-ESI (m/z) calculated for $C_{21}H_{14}N_2O_2S_2$ $[M + Na]^+$ 413.0394, found 413.0393.

*2.3.6. 3-(1H-indol-3-yl)-1-methyl-4-(4-styrylphenyl)-1H-pyrrole-2,5-dione (**6**)*

3-Bromo-4-(1*H*-indol-3-yl)-1-methyl-1*H*-pyrrole-2,5-dione (0.12 g, 0.39 mmol), 4-formylphenylboronic acid (0.12 g, 0.8 mmol) and K_2CO_3 (0.08 g, 0.58 mmol) were added to dioxane-H_2O (30 mL, 5:1). Palladium(II) acetate (0.034 g, 0.15 mmol) was then added to the mixture, and the solution was refluxed for 5 h. Ethyl acetate (200 mL), 0.5 M aqHCl (100 mL) and 10% (w/v) aqNaCl (100 mL) were added to the

mixture. The organic layer was dried with anhydrous Na_2SO_4. The filtrate was concentrated and purified by column chromatography ($CHCl_3$:CH_3OH=20:1) to produce 4-(4-(1*H*-indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-1-pyrrol-3-yl) benzaldehyde as a red solid (0.09 g, 69% yield). ^{1}H NMR (500 MHz, $(CD_3)_2S$=O) 3.04 (s, 3H, CH_3), 6.26–6.28 (d, J = 8 Hz, 1H, ArH), 6.68–6.71 (t, J = 7.5 Hz, 1H, ArH), 7.05–7.08 (t, J = 8.5 Hz, 1H, ArH), 7.44–7.46 (d, J = 8 Hz, 1H, ArH), 7.6–7.61 (d, J = 8.5 Hz, 2H, ArH), 7.85–7.87 (d, J = 8 Hz, 2H, ArH), 8.07–8.08 (d, J = 3 Hz, 1H, ArH), 10 (s, 1H, CHO), 12.04 (s, 1H, indole NH).

4-(4-(1*H*-indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-1-pyrrol-3-yl)benz-aldehyde (0.14 g, 0.42 mmol) and diethyl benzylphophonate (0.2 ml, 0.96 mmol) were added to DMF (2 mL). The solution was stirred at 0 °C for 15 min. Sodium methoxide (28% in methanol solution, 0.1 ml, 0.52 mmol) was added the solution and the mixture was stirred at 90 °C for 18 hr. Ethyl acetate (200 mL) and H_2O (150 mL) were added to the mixture. The organic layer was dried with anhydrous Na_2SO_4. The filtrate was concentrated and purified by column chromatography (ethyl acetate:hexane=1:1) to produce compound **6** as a red solid (0.01 g, 6% yield).

M.p. 248–250 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S$=O) 3.04 (s, 3H, CH_3), 6.44–6.45 (d, J = 8 Hz, 1H, ArH), 6.7–6.73 (t, J = 7 Hz, 1H, ArH), 7.04–7.07 (t, J = 7 Hz, 1H, ArH), 7.25–7.29 (m, 2H, ArH), 7.31–7.41 (m, 3H, ArH), 7.43–7.45 (m, 3H, ArH), 7.55–7.6 (m, 4H, ArH), 8 (s, 1H, ArH), 11.9 (s, 1H, indole NH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S$=O) 24.0, 104.3, 112.2, 119.8, 121.3, 122.1, 123.8, 126.1, 126.6, 127.6, 127.8, 128.1, 128.7, 129.4, 129.5, 129.7, 129.8, 131.1, 131.5, 136.6, 136.8, 137.3, 171.1, 171.3. HRMS-ESI (m/z) calculated for $C_{27}H_{20}N_2O_2$ $[M + Na]^+$ 427.1422, found 427.1420.

*2.3.7. 3-(4-(2-([1,1'-Biphenyl]-4-yl)vinyl)phenyl)-4-(1H-indol-3-yl)-1-methyl-1H-pyrrole-2,5-dione (**7**)*

Diethyl(4-iodobenzyl)phophonate (0.12 ml, 0.52 mmol), phenylboronic acid (0.1 g, 0.82 mmol), K_2CO_3 (0.1 g, 0.72 mmol) and palladium acetate(II) (0.03 g, 0.13 mmol) were added to DMF (3 mL). The solution was stirred at 110 °C for 13 h and cooled to room temperature. 4-(4-(1*H*-indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-1-pyrrol-3-yl)benzaldehyde (0.12 g, 0.36 mmol) and sodium methoxide (28% in methanol solution, 0.2 mL, 1 mmol) were added the solution and the mixture was stirred at 90 °C for 3 hr. $CHCl_3$ (200 mL), 1 M aqHCl (100 mL) and 10% (w/v) aqNaCl (100 mL) were added to the mixture. The organic layer was dried with anhydrous Na_2SO_4. The filtrate was concentrated and purified by flash column chromatography ($CHCl_3$:CH_3OH=40:1) to produce compound **7** as a red solid (0.05 g, 20% yield). M.p. > 300 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S$=O) 3.04 (s, 3H, CH_3), 6.45–6.46 (d, J = 8 Hz, 1H, ArH), 6.71–6.74 (t, J = 7.5 Hz, 1H, ArH), 7.05–7.08 (t, J = 7.5 Hz, 1H, ArH), 7.31–7.37 (m, 3H, ArH), 7.44–7.48 (m, 5H, ArH), 7.58–7.59 (d, J = 8.5 Hz, 2H, ArH), 7.69–7.71 (d, J = 8 Hz, 6H, ArH), 8.01 (s, 1H, ArH), 11.93 (s, 1H,indole NH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S$=O) 24.0, 104.3, 112.2, 119.8, 121.2, 122.1, 123.8, 126.1, 126.4, 126.9, 127.1, 127.4, 127.6, 127.9, 128.8, 128.9, 129.7, 129.8, 131.1, 131.5, 136.0, 136.6, 137.3, 139.3, 139.5, 171.0, 171.3. HRMS-ESI (m/z) calculated for $C_{33}H_{24}N_2O_2$ $[M + Na]^+$ 503.1735, found 503.1726.

*2.3.8. 3-(1H-indol-3-yl)-1-methyl-4-(4-(4-styryl)styryl)phenyl)-1H-pyrrole-2,5-dione (**8**)*

Diethyl(4-iodobenzyl)phophonate (0.12 mL, 0.52 mmol), *trans*-2-phenylvinyl-boronic acid (0.12 g, 0.81 mmol), K_2CO_3 (0.1 g, 0.72 mmol) and palladium acetate(II)

(0.03 g, 0.13 mmol) were added to DMF (2.5 mL). The solution was stirred at 110 °C for 18 h and returned to room temperature. 4-(4-(1*H*-indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-1-pyrrol-3-yl)benzaldehyde (0.1 g, 0.3 mmol) in DMF solution (2 mL) and sodium methoxide (28% in methanol solution, 0.2 mL, 1 mmol) were added to the solution and the mixture was stirred at 90 °C for 2.5 hr. $CHCl_3$ (200 mL), 1 M aqHCl (100 mL) and 10% (w/v) aqNaCl (100 mL) were added to the mixture. The organic layer was dried with anhydrous Na_2SO_4. The filtrate was concentrated and purified by flash column chromatography ($CHCl_3$:CH_3OH=40:1) to produce compound **8** as a red solid (0.01 g, 4% yield). M.p. > 300 °C. ^{1}HNMR (500 MHz, $(CD_3)_2S=O$) 3.04 (s, 3H, CH_3), 6.44–6.46 (d, J = 8.5 Hz, 1H, ArH), 6.71–6.74 (t, J = 7.5 Hz, 1H, ArH), 7.05–7.08 (t, J = 8 Hz, 1H, ArH), 7.23–7.34 (m, 6H, ArH), 7.36–7.39 (t, J = 8 Hz, 2H, ArH), 7.44–7.45 (d, J = 8.5 Hz, 3H, ArH), 7.56–7.61 (m, 7H, ArH), 8.01 (s, 1H, ArH), 11.93 (s, 1H, indole NH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S=O$) 24.0, 104.3, 119.8, 121.3, 122.1, 123.8, 126.1, 126.5, 126.9, 127.0, 127.6, 127.9, 128.5, 128.7, 129.7, 129.8, 131.5, 136.2, 136.6, 137.0, 137.4, 171.1, 171.3. HRMS-ESI (m/z) calculated for $C_{35}H_{26}N_2O_2$ $[M - H]^-$ 505.1916, found 505.1894.

*2.3.9. 3-(1-Ethyl-1H-indol-3-yl)-1-methyl-4-(4-4-(styryl)styryl)phenyl)-1H-pyrrole-2,5-dione (**9**)*

Compound **8** (0.01 g, 0.02 mmol) was added to DMF (3 mL). The solution was stirred at 0 °C for 15 min. Sodium hydride (60%, 2 mg, 0.05 mmol) was added to the solution and the mixture was stirred at room temperature for 20 min and returned to 0 °C. Iodoethane (5 μL, 0.063 mmol) was added to the solution and the mixture was stirred at room temperature for 1.5 hr. The mixture was concentrated and purified by flash column chromatography ($CHCl_3$) to produce compound **9** as a red solid (0.01 g,

94% yield). M.p. > 300 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S=O$) 1.43–1.46 (t, J = 7 Hz, 3H, CH_3), 3.04 (s, 3H, CH_3), 4.32–4.37 (q, J = 7 Hz, 2H, CH_2), 6.45–6.47 (d, J = 7.5 Hz, 1H, ArH), 6.75–6.77 (t, J = 7.5 Hz, 1H, ArH), 7.1–7.13 (t, J = 6.5 Hz, 1H, ArH), 7.27–7.29 (m, 5H, ArH), 7.36–7.39 (t, J = 7.5 Hz, 2H, ArH), 7.43–7.45 (d, J = 8.5 Hz, 2H, ArH), 7.54–7.61 (m, 9H, ArH), 8.1 (s, 1H, ArH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S=O$) 15.2, 24.0, 103.6, 124.4, 126.1, 126.5, 126.9, 127.0, 127.5, 127.9, 128.7, 129.7, 129.8, 133.2, 136.1, 136.6, 137.0, 171.0, 171.3. HRMS-ESI (m/z) calculated for $C_{37}H_{30}N_2O_2$ $[M + Na]^+$ 557.2205, found 557.2195.

*2.3.10. 3-(1H-indol-3-yl)-1-methyl-4-(pyren-1-yl)-1H-pyrrole-2,5-dione (**10**)*

Tert-butyl 3-(4-bromo-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)-1*H*-indole-1-carboxylate (0.13 g, 0.32 mmol), pyrene-1-boronic acid (0.08 g, 0.32 mmol) and K_2CO_3 (0.044 g, 0.32 mmol) were added to dioxane-H_2O (20 mL, 4:1). Tetrakis(triphenylphosphine)palladium(0) (0.08 g, 0.07 mmol) was then added to the mixture, and the solution was refluxed for 16 h. Ethyl acetate (100 mL), 0.5 M aqHCl (100 mL) and 10% (w/v) aqNaCl (100 mL) were added to the mixture. The organic layer was dried with anhydrous $MgSO_4$. The filtrate was concentrated and purified by column chromatography (ethyl acetate:hexane=1:2) to produce compound **10** as a red solid (0.07 g, 50% yield). M.p. 295–297 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S=O$) 3.13 (s, 3H, CH_3), 5.92–5.93 (d, J = 8.5 Hz, 1H, ArH), 6.10–6.14 (m, 1H, ArH), 6.73–6.77 (m, 1H, ArH), 7.24–7.25 (d, J = 8.5 Hz, 1H, ArH), 7.99–8.07 (m, 5H, ArH), 8.20–8.26 (m, 3H, ArH), 8.30–8.33 (dd, J = 4 Hz, 7.5 Hz, 2H, ArH), 11.84 (s, 1H, indole NH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S=O$) 24.2, 105.1, 111.9, 119.6, 120.0, 121.8, 123.6, 123.7, 124.5, 125.2, 125.4, 125.5, 126.3, 126.4, 127.2, 127.3, 127.8, 128.0, 128.4, 129.1, 130.2, 130.6, 131.0, 131.5, 135.2, 136.2, 171.1, 171.5. HRMS-ESI (m/z) calculated for

$C_{29}H_{18}N_2O_2$ $[M + Na]^+$ 449.1266, found 449.1257.

*2.3.11. Tert-butyl 3-(1-methyl-2,5-dioxo-4-(pyren-1-yl)-2,5-dihydro-1H-pyrrol-3-yl)-1H-indole-1-carboxylate (**11**)*

Compound **10** (0.05 g, 0.12 mmol), 4-dimethylaminopyridine (0.01 g, 0.08 mmol) and Di-*tert*-butyl dicarbonate (0.1 ml, 0.42 mmol) were added to THF (10 mL). The solution was stirred at 0 °C for 1.5 h. Ethyl acetate (100 mL) and H_2O (100 mL) were added to the mixture. The organic layer was dried with anhydrous Na_2SO_4. The filtrate was concentrated and purified by column chromatography (ethyl acetate: hexane=1:2) to produce compound **11** as a red solid (0.05 g, 83% yield). M.p. 173–175 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S{=}O$) 1.54 (s, 9H, *t*-butyl), 3.16 (s, 3H, CH_3), 6.27–6.28 (d, J = 8 Hz, 1H, ArH), 6.4–6.43 (t, J = 8 Hz, 1H, ArH), 6.96–6.99 (t, J = 8.5 Hz, 1H, ArH), 7.88–7.9 (d, J = 8.5 Hz, 1H, ArH), 8.02–8.04 (d, J = 7.5 Hz, 1H, ArH), 8.06–8.08 (d, J = 7 Hz, 2H, ArH), 8.09–8.11 (d, J = 9.5 Hz, 1H, ArH), 8.2–8.22 (d, J = 9.5 Hz, 1H, ArH), 8.25–8.27 (d, J = 8.5 Hz, 2H, ArH), 8.31–8.32 (d, J = 8 Hz, 1H, ArH), 8.33–8.34 (d, J = 7.5 Hz, 1H, ArH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S{=}O$) 24.4, 27.4, 84.9, 109.6, 114.6, 120.5, 122.4, 123.4, 123.7, 124.5, 124.6, 125.0, 125.7, 125.8, 126.5, 126.7, 127.2, 127.7, 128.1, 128.4, 129.0, 129.5, 130.1, 130.6, 131.5, 133.1, 134.4, 148.3, 170.3, 170.9. HRMS-ESI (m/z) calculated for $C_{34}H_{26}N_2O_4$ $[M + Na]^+$ 549.1790, found 549.1776.

*2.3.12. 3-(9H-carbazol-2-yl)-4-(1H-indol-3-yl)-1-methyl-1H-pyrrole-2,5-dione (**12**)*

Tert-butyl 3-(4-bromo-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)-1*H*-indole-1-carboxylate (0.51 g, 1.26 mmol), 9*H*-carbazole-2-boronic acid pinacol ester (0.62 g, 1.9 mmol) and K_2CO_3 (0.35 g, 2.53 mmol) were added to dioxane-H_2O (50 mL, 4:1),

and then tetrakis(triphenylphosphine)palladium(0) (0.16 g, 0.14 mmol) was added to the mixture. The solution was refluxed for 16 h. Ethyl acetate (200 mL), 0.5 M aqHCl (100 mL) and 10% (w/v) aqNaCl (100 mL) were added to the mixture. The organic layer was dried with anhydrous $MgSO_4$. The filtrate was concentrated and purified by column chromatography (ethyl acetate:hexane=4:5) to produce compound **12** as a red solid (0.25 g, 51% yield). M.p. 293–295 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S$=O) 3.06 (s, 3H, CH_3), 6.32–6.34 (d, J = 8 Hz, 1H, ArH), 6.51–6.54 (m, 1H, ArH), 6.96–6.99 (m, 1H, ArH), 7.13–7.2 (m, 2H, ArH), 7.37–7.44 (m, 3H, ArH), 7.64 (s, 1H, ArH), 7.99–8.02 (m, 2H, ArH), 8.07–8.09 (d, J = 7.5 Hz, 1H, ArH), 11.24 (s, 1H, carbazole NH), 11.88 (s, 1H, indole NH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S$=O) 24.0, 104.5, 111.0, 112.1, 112.2, 118.7, 119.6, 120.2, 120.4, 121.2, 121.9, 122.0, 122.6, 124.0, 126.0, 127.5, 129.2, 130.9, 131.2, 136.5, 139.2, 140.3, 171.2, 171.6. HRMS-ESI (m/z) calculated for $C_{25}H_{17}N_3O_2$ $[M + Na]^+$ 414.1218, found 414.1204.

*2.3.13. Tert-butyl 2-(4-(1-(tert-butoxycarbonyl)-1H-indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-9H-carbazole-9-carboxylate (**13**)*

Compound **12** (0.04 g, 1 mmol), 4-dimethylaminopyridine (0.03 g, 0.25 mmol) and Di-*tert*-butyl dicarbonate (0.45 mL, 1.9 mmol) were added to THF (10 mL). The solution was stirred at 0 °C for 1.5 h. Ethyl acetate (150 mL) and H_2O (100 mL) were added to the mixture. The organic layer was dried with anhydrous Na_2SO_4. The filtrate was concentrated and purified by column chromatography (ethyl acetate: hexane=1:2) to produce compound **13** as a red solid (0.06 g, 100% yield). M.p. 140–142 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S$=O) 1.42 (s, 9H, *t*-butyl), 1.67 (s, 9H, *t*-butyl), 3.09 (s, 3H, CH_3), 6.44–6.46 (d, J =8 Hz, 1H, ArH), 6.75–6.78 (t, J = 7.5 Hz, 1H, ArH), 7.18–7.21 (t, J = 8 Hz, 1H, ArH), 7.39–7.42 (t, J = 7.5 Hz, 1H, ArH),

7.52–7.55 (t, *J* = 8 Hz, 1H, ArH), 7.56–7.58 (d, *J* = 8 Hz, 1H, ArH), 8.07–8.08 (d, *J* = 8 Hz, 1H, ArH), 8.14 (s, 1H, ArH), 8.16–8.18 (d, *J* = 8.5 Hz, 2H, ArH), 8.22–8.24 (d, *J* = 8.5 Hz, 1H, ArH), 8.25 (s, 1H, ArH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S{=}O$) 24.2, 26.8, 27.4, 27.6, 31.3, 84.0, 84.9, 109.1, 114.9, 115.8, 117.3, 119.9, 120.5, 121.4, 122.6, 123.5, 124.4, 124.9, 125.9, 126.1, 128.1, 129.1, 129.3, 134.1, 134.8, 136.8, 138.5, 148.5, 149.9, 170.2, 170.7. HRMS-ESI (m/z) calculated for $C_{35}H_{33}N_3O_6$ $[M + Na]^+$ 614.2267, found 614.2267.

2.4. Histochemical analysis

BSB is π–conjugated molecule and have styrylbenzene moieties. Unsymmetric indolylmaleimides (compounds **1**–**4**, compounds **6**–**8**), which had aromatic moiety such as styrylbenzene, were selected to evaluate the selectivity and sensitivity for amyloid fibrils. To evaluate the selectivity and sensitivity for amyloid fibrils, we used tissue samples of patients with senile systemic amyloidosis (SSA) caused by deposition of wild-type transthyretin (WT-TTR) [28]. Each tissue sample was obtained by biopsy or autopsy from the abdominal wall of patients with senile systemic amyloidosis. Sections were cut 3 μm thick from paraffin-embedded blocks of tissue and were stained with alkaline Congo red. Congo red reactivity was confirmed under polarized light. For the staining of our new compounds, sections were immersed in a solution containing 0.01% of each compound and 50% ethanol for 30 minutes. Then, the sections were quickly differentiated in saturated Li_2CO_3 and rinsed with 50% ethanol before examination by fluorescence.

3. Results and discussions

*3.1. Synthesis of unsymmetric maleimides (compounds **1**–**13**)*

The expansion of π-conjugation has a major impact on the FL property of FL compounds [29–31]. Various π-conjugated polymers are designed and synthesized to produce particularly desirable emissions. In this study, we developed unsymmetric IMs that exhibit large Stokes shift and FL maxima emission wavelengths of more than 550 nm. To achieve this, various aromatic groups such as naphthyl, carbazolyl and pyrenyl were introduced to maleimide moiety. Furthermore, unsymmetric IMs having bithiophene and 4-phenylstylyl moieties were synthesized to achieve a longer shift in the FL emission maximum wavelength. Unsymmetric IMs were synthesized as shown in Scheme 1. After indole was metalated with ethylmagnesium bromide, the resulting indolylmagnesium was coupled with another substrate (halide) to give 3-Bromo-4- (1*H*-indol-3-yl)-1-methyl-1*H*-pyrrole-2,5-dione. The remaining bromo group of 3-Bromo-4-(1*H*-indol-3-yl)-1-methyl-1*H*-pyrrole-2,5-dione was transformed by arylboronic acids (Scheme 1(a)) through Suzuki-Miyaura coupling. This synthetic method of unsymmetric IMs is thus facile and promising. A Wittig-Horner reaction was used to introduce the 4-phenylstylyl moiety (Scheme 1(b)). Table 1 shows the unsymmetric IMs prepared through the above methods.

3.2 Absorption and emission studies

All compounds **1**–**13** were dissolved in DMF and CH_3CN. The absorption and FL spectra of compounds **1**–**13** were measured in DMF and CH_3CN. The absorption spectra of compounds **1**, **2**, **3**, **5**, **8** and **12** in DMF are shown in Fig. 1. The absorption

maxima wavelengths of most IMs were over 430 nm, and the absorption maximum wavelength of compound **5** was approximately 500 nm, the largest value among compounds **1**–**13**. The FL excitation and emission spectra of compounds **1**, **2**, **3**, **5**, **8** and **12** in DMF were obtained (Fig. S1). The FL emission spectra of compounds **1**, **2**, **3**, **5**, **8** and **12** in DMF are shown in Fig. 2. The FL emission maximum wavelength of compound **5** in DMF was 597 nm and red FL emission was observed. Apparently, the introduction of the benzene, naphthalene, styrylbenzene and bithiophene into the maleimide moiety shifted the FL emission maxima to longer wavelengths. The expansion of π–conjugation should cause an obvious change in the FL properties of IMs. The Stokes shifts of compounds **7**–**13** were more than 100 nm (Table 2). The introduction of pyrene caused the largest Stokes shift. In DMF, the FL intensity of compound **13** was the strongest. The FL quantum yield of compound **3** in DMF was higher than those of other compounds. Compound **10** has a pyrene in the structure. When compound **10** was excited at 342 nm in DMF, the excimer FL was observed (Fig. S2). The optical properties of compounds **1**–**13** in CH_3CN (Table S1) were similar with that of compounds **1**–**13** in DMF. The FL emission of compounds **3**, **13**, **7**, **9** and **5** exhibited different emissions under UV irradiation at 365 nm (Fig. 3).

3.3 Histochemical analysis

Congo red appears orange-red and produces apple-green birefringence under polarized light (Fig. 4 (A), (B)). Compound **1** showed the highest strength of fluorescence with amyloid deposited the tissues in a patient with SSA in our compounds (Fig. 4 (C)). Unsymmetric indolylmaleimide such as compound **1** was useful compound to detect SSA amyloid fibrils. The fibril in SSA amyloid is derived from

normal transthyretin [33]. The FL intensity of compound **1** increased apparently in the presence of transthyretin fibrils (Fig. S4). A few of aggregation-induced emission (AIE) phenomena were found. For example, quinolinemalononitrile exhibits extraordinary self-assembly property and strong emission in H_2O-tetrahydrofuran (THF) solution [34]. When 1,2-Bis[4-(3-sulfonatopropoxy)phenyl]-1,2-diphenyl-ethene salt (BSPOTPE) was mixed with performed insulin fibrils, BSPOTPE emitted a strong green light [35]. The absorption and FL spectra of compound **1** were measured in H_2O-THF solution, however, the FL emission enhancement were not observed (Fig. S5). Compound **1** did not exhibit AIE phenomenon in H_2O-THF solution. The FL intensity of compound **1** in THF was much stronger than that of compound **1** in CH_3CN and DMF (Fig. S6). Thus, the FL intensity of compound **1** increased in a hydrophobic solvent. Compound **1** binds to SSA amyloid, the FL intensity of compound **1** may increase under highly hydrophobic environment.

4. Conclusions

Various fluorescent unsymmetric indolylmaleimides were synthesized in a facile manner. The introduction of various aromatic compounds into the maleimide moiety in unsymmetric indolylmaleimides produced a large Stokes shift and exhibited different emissions under UV irradiation at 365 nm. We showed that unsymmetric indolylmaleimides can be used for detecting amyloid fibrils. These results should provide significant information for the development of useful fluorescent unsymmetric indolylmaleimides.

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AppendixA. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/

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Figure legends

Scheme 1. Synthesis of unsymmetric indolylmaleimides.

Fig. 1. The absorption spectra of compounds **1**, **2**, **3**, **5**, **8** and **12** in DMF. The concentration of compounds **1**, **2**, **3**, **5**, **8** and **12** in DMF was 10 μM.

Fig. 2. The fluorescence emission spectra of compounds **1**, **2**, **3**, **5**, **8** and **12** in DMF. The concentration of compounds **1**, **2**, **3**, **5**, **8** and **12** in DMF was 10 μM. The excitation wavelengths of compounds **1**, **2**, **3**, **5**, **8** and **12** were 439, 492, 432, 513, 451 and 454 nm.

Fig. 3. The emission color of the compounds **3**, **13**, **7**, **9** and **5** in DMF under UV irradiation at 365 nm.

Fig. 4. Congo red, compound **1**, compound **4** and compound **8** staining of blood vessels in abdominal fat tissue in an 81-year-old male SSA patient. (A) Congo red staining (B) Congo red staining (polarized light) (C) compound **1** staining (D) compound **4** staining (E) compound **8** staining. Scale bars = 200 μm.

Table 1. Structure of unsymmetric indolylmaleimides.

Table2. Optical properties of compounds **1**–**13** in DMF. Absorption maximum wavelength (λ_{abs}), molar absorption coefficients (ε), excitation maximum wavelength (λ_{ex}), emission maximum wavelength (λ_{em}) and FL quantum yields (Φ_F).

[a.] The concentration of compounds **1**–**13** in DMF is 10 μM. [b.] Ref. [32].

Scheme 1

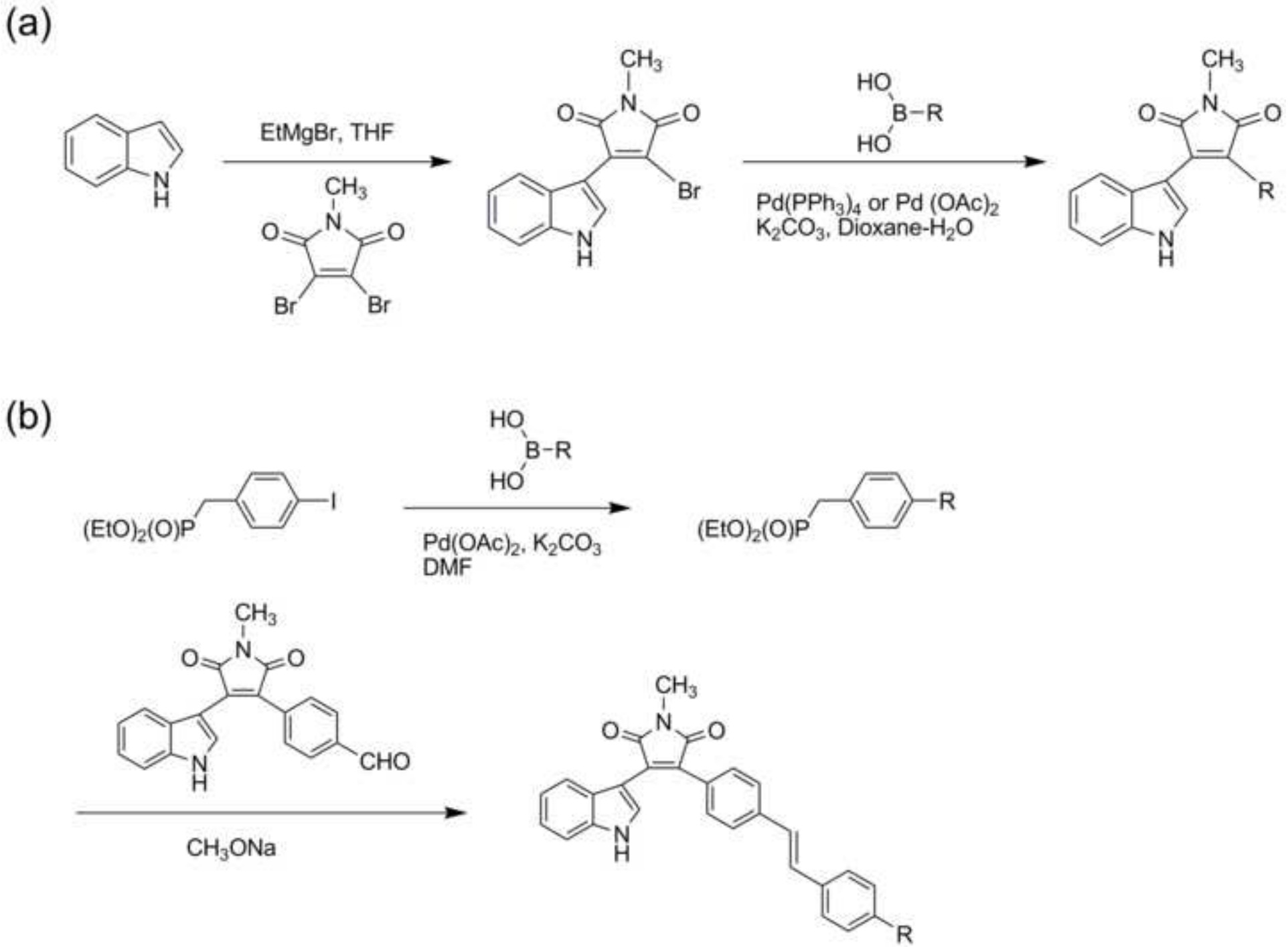

(a)
EtMgBr, THF
Br
Br
Pd(PPh3)4 or Pd (OAc)2
K2CO3, Dioxane-H2O
(b)
(EtO)2(O)P
Pd(OAc)2, K2CO3
DMF
CHO
CH3ONa

Fig. 1

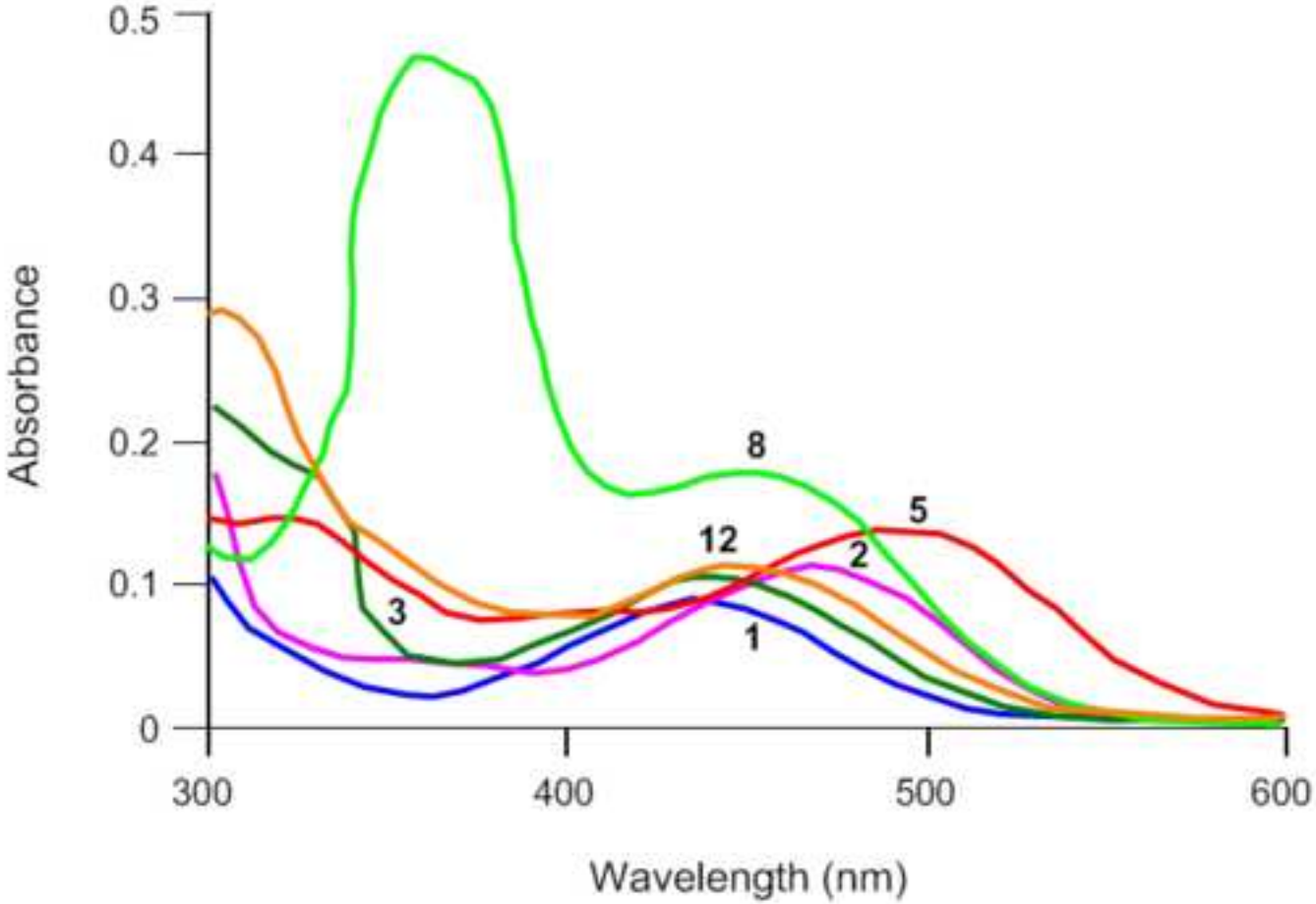

0.5
0.4
0.3
0.2
0.1
0
Absorbance
300
400
500
600
Wavelength (nm)
8
5
12
2
3
1

Fig. 2

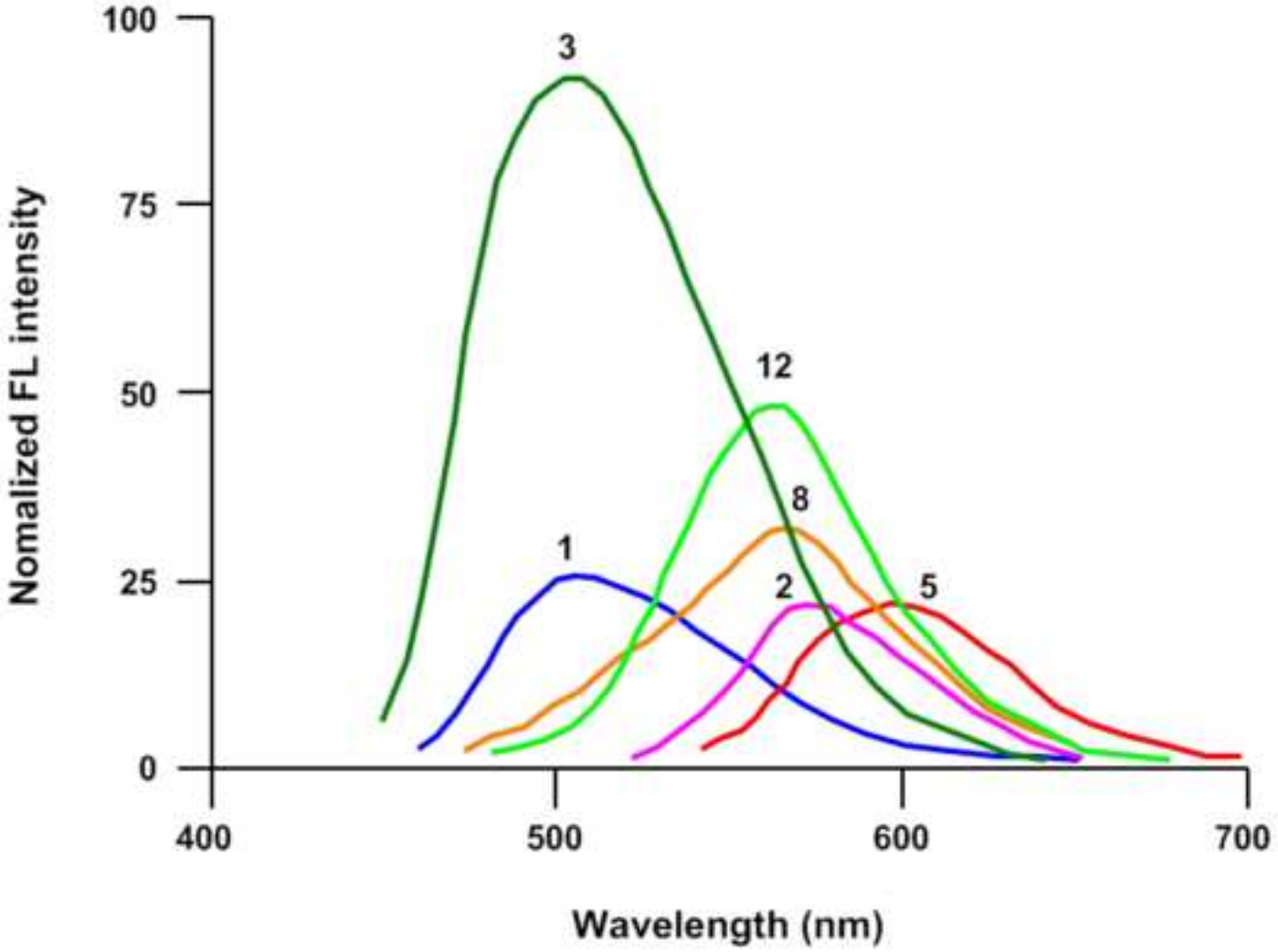

100
75
50
25
0
Nomalized FL intensity
400
500
600
700
Wavelength (nm)
3
12
8
1
2
5

Fig. 3

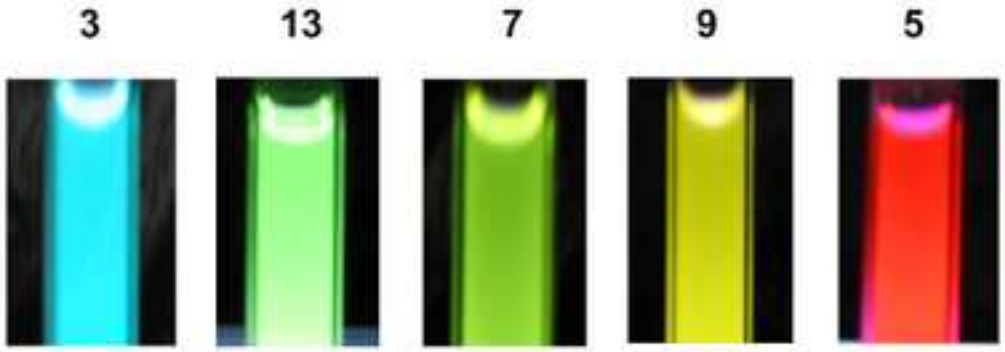
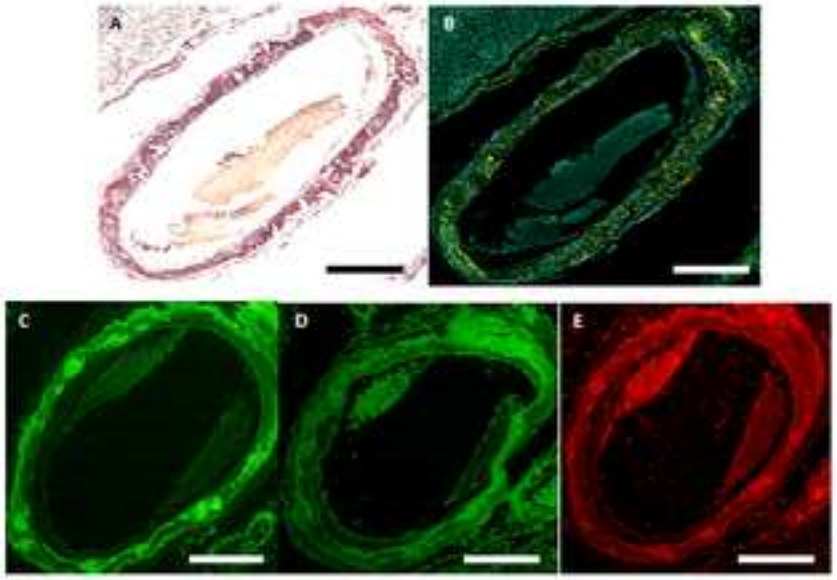
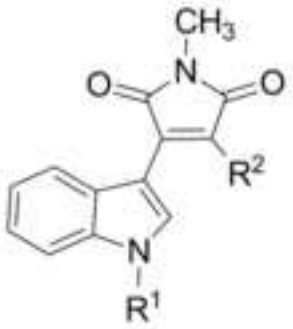
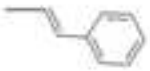
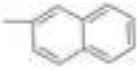
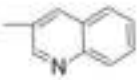
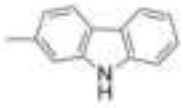
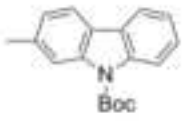


Fig. 4





Compound	R^1	R^2	Compound	R^1	R^2
1	H		**8**	H	
2	H		**9**	CH_3CH_2	
3	H		**10**	H	
4	H		**11**	Boc	
5	H		**12**	H	
6	H		**13**	Boc	
7	H				

Compound[a]	λabs (nm)	ε ($M^{-1}cm^{-1}$)	λex (nm)	λem (nm)	Stokes shift (nm)	Φ_F[b]
1	436	8500	439	506	67	0.09
2	467	10920	492	573	81	0.02
3	440	10240	432	507	75	0.3
4	448	10110	381	471	90	0.04
5	491	13860	513	597	84	0.01
6	450	11480	451	522	71	0.06
7	453	16810	453	555	102	0.04
8	451	17870	451	567	116	0.03
9	459	19130	459	564	105	0.06
10	439	11240	440	571	131	0.09
11	404	8800	433	573	140	0.14
12	448	11000	454	563	109	0.06
13	404	7260	404	536	132	0.13