

Naphthalimide-Based Fluorescent Dyes: Impact of Extension of π -Conjugation and Introduction of an Electron-Donating Moiety on the Photophysical Properties

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Abstract

Concerning a series of naphthalimide-based fluorescence dyes in which the π -system is extended with oligothiophene units, it has been revealed that the absorption and fluorescence maxima can be tuned over ca. 100 nm and ca. 180 nm range by extending π -conjugation, respectively. The effects of the solvent on the fluorescence quantum yield depend on the conjugation length. For the same series but with an electron-donating moiety (push-pull type dyes), the absorption and fluorescence maxima are less dependent on the conjugation length. The fluorescence quantum yields of the push-pull type dyes are large in toluene (>0.3) but extremely low in DMSO. These results will be a guide for the design of naphthalimide-based sensors and probes.

Keywords: Fluorescent dye | Naphthalimide | π-Conjugation

1. Introduction

Fluorescent dyes represent an important class of compounds for applications to fluorescent sensors and probes. Fluorescent dyes based on 1,8-naphthalimide have been attracting particularly intense attention as manifested in a surge of reports aiming at applications to DNA-binding agents,^{1,2} pH indicators,³ sensors for various cationic and anionic species,^{3–5} and emitters for organic light-emitting diodes.^{6,7} As the π -system of naphthalimide is relatively small, it absorbs at ca. 340 nm in the UV region,⁸ although substitution by an amino group at the 4position brings the absorption to ca. 420 nm. It is desirable to bring the absorption further towards the red to allow visible light excitation/detection and prepare a multiple color set of dyes to allow simultaneous quantification of multiple analytes and ratiometric analysis as well as Förster-type resonance energy transfer (FRET) applications. There are two typical ways to shift the absorption/fluorescence wavelengths towards the red. One is to extend the π -conjugation. The other is to introduce an electron-donating or electron-withdrawing moiety. Introduction of both electron-donating and electronwithdrawing moieties to make the molecule a push-pull type is particularly effective in red-shifting the electronic transitions. To apply the latter strategy to the naphthalimide derivatives, introducing an electron-donating moiety via a π -bridge to the naphthalimide framework suffices as the naphthalimide unit serves an electron withdrawing group.

With these backgrounds in mind, we need to know how much the absorption and fluorescence are red-shifted by π extension and/or introduction of an electron-donating moiety as a practical guide for the design of elaborate multicolor sensors. While photophysical properties have been extensively investigated for the parent naphthalimide and its derivatives, in which substituents are directly introduced onto the naphthalene core,^{9–16} less is known for π -extended naphthalimide derivatives. We have chosen a thiophene group, which is chemically stable and has attracted attention as a functional π -system for optoelectronic applications,^{17–19} as a component for the π bridge. The choice of thiophene as a π -bridge may be justified in light of the purpose of bringing the optical transitions to longer wavelength regions because oligothiophenes are among π -conjugated oligomers that show the lowest-energy absorption. For example, the absorption energy of oligothiophenes²⁰ are the lowest when compared to oligophenylenes,²¹ oligopyrroles,²² and oligoynes²³ with the same numbers of monomeric units. We report herein the preparation and photophysics of naphthalimide derivatives which are π -extended with oligo-



Chart 1. Naphthalimide-based fluorescent dyes with extended π -conjugation through oligothiophenes without (upper series) and with (lower series) an electron-donating moiety.

thiophene units with and without an electron-donating group (dialkylamino groups). Chart 1 displays the naphthalimide derivatives which have been analyzed in the present study. The compounds with two (T₂ dyes: Ph-T₂-NI and PyPh-T₂-NI) and four (T₄ dyes: Ph-T₄-NI, PyPh-T₄-NI, and EH₂NPh-T₄-NI) thiophene units in the chart have been prepared in this study, while the data on the dyes without the thiophene unit (T₀ dye: Ph-NI (with R^{3 24} or R^{4 25}) are taken from the literature and those on the dye with one thiophene unit (T₁ dye: Ph-T₁-NI) are reported previously by us.²⁶

2. Experimental

2.1 Physical Measurements. ¹H NMR spectra were recorded on a ECX-400 spectrometer. Tetramethylsilane was used as an internal standard for chemical shifts ($\delta = 0$). Atmospheric pressure chemical ionization high-resolution mass spectra (APCI–HRMS) were recorded using an Agilent G1969A system for CHCl₃ solutions. Elemental analysis was performed by the Center for Creative Materials Research, College of Science and Technology, Nihon University.

Absorption spectra were measured using a Shimadzu UV-2400PC spectrometer. Fluorescence spectra were measured using a JACSO FP-8600 spectrometer. Spectral correction of the excitation optics was performed with a SID-844 calibrated detector and spectral correction of the emission optics was performed with a ESC-842 secondary standard light source such that the intensity corresponds to the relative number of photons per unit wavelength. Fluorescence lifetimes were obtained using Hamamatsu Quantaurus-Tau C11367 instrument.

2.2 Computational Details. Density functional theory (DFT) calculations were performed on Gaussian09 (Revision $(E.01)^{27}$ interfaced with GaussView 5.0 running on an iMac computer. The combination of the BMK functional²⁸ and the 6-31G* basis set was used throughout the study. The imide \mathbb{R}^n group (n = 1-4, see Chart 1) was replaced by a hydrogen atom in computation. The polarizable continuum model²⁹ as the self-consistent reaction field model (SCRF) was used with toluene and DMSO as solvents. First, geometry optimization was performed for each molecule in each solvent model. It was confirmed that reasonable conformations were obtained by comparing conformations among different molecules (see Table S1) and with the related molecules in the literature.³⁰ The transition energies and the oscillator strength were calculated by the time-dependent density functional theory (TDDFT) using the same functional and basis set for the optimized structures.

2.3 Synthesis of Novel Compounds. Recycle GPC was conducted, using CHCl₃ as the eluent, on a Japan Analytical Industry LC-9201 system equipped with Jaigel-2H and Jaigel-1H columns in tandem, with exclusion limits of 5000 and 1000, respectively. Chemicals for synthesis were used as received unless otherwise noted. Silica gel TLC plates (60 F₂₅₄) were purchased from Merck. Silica gel for column chromatography (Silica Gel 60 N, spherical, neutral, 40–50 μ m) was purchased from Kanto Chemical. The preparation of Ph-T₁-NI was reported previously.²⁶ The novel fluorescent dyes were prepared according to the synthetic route shown in Scheme 1. Compounds 1,³¹ 7a,³² 5c³³ were prepared according to the literature.

Compound 3: An argon-purged solution of K₂CO₃ (92 mg, 0.82 mmol) in a mixture of H₂O (1.5 mL) and 1,4-dioxane (2 mL) was added to a flask containing bromonaphthalimide 1 (179 mg, 0.410 mmol), bithiophene boronic acid pinacol ester 2 (179 mg, 0.612 mmol), and Pd(PPh₃)₄ (47 mg, 0.041 mmol) and the mixture was refluxed under argon for 17 h. The solvent was evaporated and the residue was chromatographed (SiO₂, CHCl₃) and washed with hexane to afford an orange solid (161 mg, 0.309 mmol, 76%). TLC (SiO₂, CHCl₃): Rf = 0.47. ¹H NMR (CDCl₃): δ (ppm) = 1.16 (12H, d, J = 6.9 Hz), 2.7– 2.8 (2H, m), 7.09 (1H, dd, J = 3.7, 1.4 Hz), 7.3–7.4 (6H, m), 7.45 (1H, t, J = 7.8 Hz), 7.84 (1H, dd, J = 8.4, 1.1 Hz), 7.90 (1H, d, J = 7.8 Hz), 8.68 (1H, d, J = 7.3 Hz), 8.73 (1H, dd, J = 7.3 Hz), 8.73 (1H, dd, J = 7.8 Hz), 8.73J = 7.3, 1.4 Hz), 8.82 (1H, dd, J = 8.4, 1.1 Hz). APCI-HRMS (CHCl₃): m/z calcd for C₃₂H₂₈NO₂S₂ ([MH]⁺), 522.1556; found, 522.1565.

Compound 4: AcOH (170 µL, 2.97 mmol) and NBS (= *N*-bromosuccinimide, 101 mg, 0.567 mmol) were added to a CHCl₃ solution (1.7 mL) of **3** (297 mg, 0.569 mmol) and the mixture was stirred at 0 °C for 2 h. The solution was washed with H₂O (4 × 3 mL) and an aqueous NaCl solution, dried over Na₂SO₄, and evaporated to afford yellow powder (332 mg, 0.553 mmol, 97%). TLC (SiO₂, CHCl₃): Rf = 0.47. ¹H NMR (CDCl₃): δ (ppm) = 1.17 (12H, d, *J* = 6.9 Hz), 2.7–2.8 (2H,



Scheme 1. Preparation of naphthalimide-based fluorescent dyes π -extended with thiophene units. The compounds in parentheses were not isolated.

m), 7.04 (2H, s), 7.2–7.4 (4H, m), 7.48 (1H, t, J = 7.8 Hz), 7.84 (1H, dd, J = 7.3, 0.92 Hz), 7.88 (1H, d, J = 7.8 Hz), 8.68 (1H, d, J = 7.3 Hz), 8.7–8.8 (2H, m). APCI-HRMS (CHCl₃): m/z calcd for C₃₂H₂₇BrNO₂S₂ ([MH]⁺), 600.0661 and 602.0641; found, 600.0652 and 602.0638.

Ph-T₂-NI: An argon-purged solution of K_2CO_3 (56 mg, 0.50 mmol) in a mixture of H_2O (750 µL) and 1,4-dioxane (3 mL) was added to a flask containing bromobithienvlnaphthalimide 4 (121 mg, 0.201 mmol), phenylboronic acid (38 mg, 0.41 mmol), and Pd(PPh₃)₄ (10 mg, 0.0087 mmol) and the mixture was refluxed under argon overnight. The reaction mixture was extracted with CHCl₃, which was dried over Na₂SO₄ and evaporated. The residue was chromatographed (SiO₂, CHCl₃/ hexane = 2:1) to collect an overlapped band containing a byproduct. The collected material was crystallized from CHCl₃ and 1-propanol to afford orange powder (98 mg, 0.16 mmol, 80%). TLC (SiO₂, CHCl₃): Rf = 0.62. ¹H NMR (CDCl₃): δ (ppm) = 1.17 (12H, d, J = 6.9 Hz), 2.7–2.8 (2H, m), 7.2–7.4 (7H, m), 7.40–7.45 (2H, m), 7.48 (1H, t, J = 7.8 Hz), 7.6–7.7 (2H, m), 7.85 (1H, dd, J = 8.7, 1.3 Hz), 7.91 (1H, d, J = 7.3Hz), 8.69 (1H, d, J = 7.8 Hz), 8.73 (1H, dd, J = 7.1, 1.1 Hz), 8.82 (1H, dd, J = 8.7, 0.9 Hz). APCI-HRMS (CHCl₃): m/zcalcd for C₃₈H₃₂NO₂S₂ ([MH]⁺), 598.1869; found, 598.1909. Analysis: calcd for C₃₈H₃₁NO₂S₂, C 76.35, H 5.23, N 2.34%; found, C 76.20, H 5.12, N, 2.22%.

Compound 6b: An argon-purged 1,4-dioxane (30 mL) was added to a flask containing bromophenylpyrrolidine **5b** (722 mg, 3.19 mmol), (BPin)₂ (= bis(pinacolato)diboron (1.21 g, 4.78 mmol), AcOK (939 mg, 9.56 mmol), and PdCl₂(dppf) (dppf = 1,1'-bis(diphenylphosphino)ferrocene, 79 mg, 0.097 mmol) and the mixture was refluxed under argon for 14 h. The reaction mixture was extracted with hexane, which was dried over Na₂SO₄ and evaporated. The obtained residue was chromatographed (SiO₂, hexane/AcOEt = 17:3) to afford black-red needles (575 mg, 2.10 mmol, 66%). TLC (SiO₂, CHCl₃): Rf = 0.60. ¹H NMR (CDCl₃): δ (ppm) = 1.32 (12H, s), 1.9–2.1 (4H, m), 3.2–3.4 (4H, m), 6.53 (2H, dd, *J* = 6.5, 1.8 Hz), 7.67 (2H, dd, *J* = 6.5, 1.8 Hz). APCI-MS (CHCl₃): *m/z* calcd for C₁₆H₂₅BNO₂ ([MH]⁺), 274.1973; found, 274.2256.

PyPh-T₂-NI: An argon-purged solution of K₂CO₃ (210 mg, 1.52 mmol) in a mixture of H₂O (1 mL) and 1,4-dioxane (4 mL) was added to a flask containing bromobithienylnaphthalimide 4 (232 mg, 0.386 mmol), boronic acid pinacol ester 6b (101 mg, 0.370 mmol), and Pd(PPh₃)₄ (18 mg, 0.015 mmol) and the mixture was refluxed under argon for 16 h. The reaction mixture was extracted with CHCl₃, which was dried over Na₂SO₄ and evaporated. The residue was chromatographed (SiO₂, CHCl₃/ hexane = 2:1) to collect an overlapped band containing a byproduct. The collected material was precipitated from CHCl₃ and hexane to afford red powder (969 mg, 0.15 mmol, 39%). TLC (SiO₂, CHCl₃): Rf = 0.66. ¹H NMR (CDCl₃): δ (ppm) = 1.17 (12H, d, J = 6.9 Hz), 2.0–2.1 (4H, m), 2.7–2.8 (2H, m), 3.3–3.4 (4H, m), 6.59 (2H, d, J = 8.7 Hz), 7.11 (1H, t, J = 3.7 Hz), 7.24 (1H, d, J = 3.7 Hz), 7.3–7.4 (4H, m), 7.4–7.5 (3H, m), 7.85 (1H, dd, J = 7.3, 1.4 Hz), 7.90 (1H, d, J = 7.8 Hz), 8.68 (1H, d, J = 7.8 Hz), 8.73 (1H, dd, J = 7.3, 0.9 Hz), 8.83 (1H, dd, J = 8.7, 1.4 Hz). APCI-HRMS (CHCl₃): m/z calcd for C₄₂H₃₉N₂O₂S₂ ([MH]⁺), 667.2447; found, 667.2404.

Ph-T₄-NI: A 2.5 M hexane solution of *n*-BuLi (0.20 mL, 0.49 mmol) was added dropwise to a dry THF solution (5 mL) of phenylbithiophene 7a (119 mg, 0.49 mmol) at -78 °C and the mixture was stirred for 3 h. SnBu₃Cl (0.15 mL, 0.54 mmol) was added and the reaction mixture was stirred at room temperature for 1 h. H₂O (2 mL) was added to the reaction mixture followed by extraction with CHCl₃, which was dried over Na₂SO₄ and evaporated. A solution of this crude tributylstannyl derivative of phenylbithiophene (8a) (315 mg), which was found unstable toward SiO₂ and used without further purification, and bromobithienvlnaphthalimide 4 (320 mg, 0.533 mmol) and Pd(PPh₃)₄ (70 mg, 0.060 mmol) in dry THF (20 mL) was refluxed under argon for 22 h. The precipitate formed was collected and washed with CHCl₃. A crystallization procedure for the precipitate from CHCl₃ and hexane afforded a red powdery solid (159 mg, 0.209 mmol, 43%). TLC (SiO₂, CHCl₃): Rf = 0.48. ¹H NMR (CDCl₃): δ (ppm) = 1.18 (12H, d, J = 6.9 Hz), 2.7–2.8 (2H, m), 7.1–7.2 (4H, m), 7.23 (1H, d, J = 3.7 Hz), 7.3–7.4 (6H, m), 7.41 (1H, dd, J = 7.8, 7.3 Hz), 7.49 (1H, t, J = 7.8 Hz), 7.63 (2H, dd, J = 8.3, 1.4 Hz), 7.86 (1H, dd, J = 8.3, 0.9 Hz), 7.91 (1H, d, J = 7.8 Hz), 8.69 (1H, d, J = 7.2 Hz), 8.74 (1H, dd, J = 7.2, 1.1 Hz), 8.81 (1H, d, J = 8.3, 1.1 Hz). APCI-HRMS (CHCl₃): m/z calcd for C₄₆H₃₆NO₂S₄ ([MH]⁺), 762.1623; found, 762.1691.

Compound 7c: A solution of K₂CO₃ (340 mg, 2.46 mmol) in a mixture of H₂O (5 mL) and 1,4-dioxane (15 mL) was added to a flask containing bithiopheneboronic acid pinacol ester 2 (525 mg, 1.80 mmol) and *p*-bromoaniline derivative 5c (501 mg, 1.80 mmol). After the solution was purged with N_2 , $Pd(Ph_3P)_4$ (56 mg, 0.048 mmol) was added and the mixture was refluxed under N₂ for 16 h. The reaction mixture, after H₂O (15 mL) was added, was extracted with EtOAc, which was dried over Na₂SO₄ and evaporated. The residue was chromatographed (SiO₂, hexane) to afford yellow powder (299 mg, 0.620 mmol, 49%). TLC (SiO₂, hexane):Rf = 0.21. ¹H NMR $(CDCl_3): \delta$ (ppm) = 0.8–1.0 (12H, m), 1.1–1.4 (14H, m), 1.7– 1.9 (2H, m), 3.1-3.4 (4H, m), 6.9-7.1 (2H, m), 7.09 (1H, d, J = 3.7 Hz, 7.14 (1H, d, J = 3.2 Hz), 7.16 (1H, d, J = 5.0 Hz), 7.43 (2H, d, J = 9.2 Hz). APCI-HRMS (CHCl₃): m/z calcd for C₃₀H₄₄NS₂ ([MH]⁺), 482.2910; found, 482.3218.

A hexane solution of n-BuLi (2.5 M, EH₂NPh-T₄-NI: 0.25 mL, 0.62 mmol) was added dropwise to a solution of the bithiophene derivative 7c (225 mg, 0.467 mmol) in dry THF (8 mL) at $-78 \degree \text{C}$ under N₂ and the reaction mixture was stirred for 1 h. SnBu₃Cl (0.13 mL, 0.47 mmol) was added and the reaction mixture was stirred at room temperature for a day. To the reaction mixture containing tributylstannyl derivative 8c was added bromobithienylnaphthalimide 4 (281 mg, 0.467 mmol) and Pd(PPh₃)₄ (56 mg, 0.048 mmol) and the mixture was refluxed under N2 for 20 h. The reaction mixture was extracted with CHCl₃, which was dried over Na₂SO₄ and evaporated. The residue was chromatographed (SiO₂, CHCl₃) and reprecipitated from CHCl₃ and hexane to afford red powder (71 mg, 0.071 mmol, 15% from 7c and 4). TLC (SiO₂, CHCl₃): Rf = 0.53. ¹H NMR (CDCl₃): δ (ppm) = 0.8–1.0 (12H, m), 1.17 (12H, d, J = 6.8 Hz), 1.2-1.4 (16H, m), 1.7-1.9 (16H, m),2.7–2.8 (2H, m), 3.1–3.4 (4H, m), 6.66 (2H, d, J = 8.7 Hz), 7.05 (1H, d, J = 3.7 Hz), 7.08 (1H, d, J = 3.7 Hz), 7.1–7.2 (3H, m), 7.21 (1H, d, J = 3.7 Hz), 7.33 (4H, dd, J = 7.8, 3.7 Hz),

7.4–7.5 (3H, m), 7.85 (1H, dd, J = 7.3, 0.9 Hz), 7.90 (1H, d, J = 7.3 Hz), 8.68 (1H, d, J = 7.8 Hz), 8.73 (1H, dd, J = 7.3, 0.9 Hz), 8.80 (1H, dd, J = 8.5, 0.9 Hz). APCI-HRMS (CHCl₃): m/z calcd for C₆₂H₆₉N₂O₂S₄ ([MH]⁺), 1001.4236; found, 1001.4121. Analysis: calcd for C₆₂H₆₈N₂O₂S₄•H₂O, C 73.04, H 6.92, N 2.75%; found, C 73.26, H 6.67, N, 2.78%.

Compound 9: A solution of K_2CO_3 (259 mg, 1.88 mmol) in a mixture of H₂O (2 mL) and 1,4-dioxane (8 mL) was added to a flask containing bithiophene boronic acid pinacol ester 2 (402 mg, 1.38 mmol) and bromobithienylnaphthalimide 4 (284 mg, 0.474 mmol). After the mixture was purged with N₂, Pd(PPh₃)₄ (47 mg, 0.041 mmol) was added and the mixture was refluxed under N2 for 21 h. After H2O (15 mL) was added, the reaction mixture was extracted with CHCl₃, which was dried over Na₂SO₄ and evaporated. The residue was chromatographed (SiO₂, CHCl₃) to afford red powder (259 mg, 0.378 mmol, 86%). TLC (SiO₂, CHCl₃): Rf = 0.57. ¹H NMR (CDCl₃): δ (ppm) = 1.17 (12H, d, J = 6.9 Hz), 2.7–2.8 (2H, m), 7.05 (1H, dd, J = 1.8, 3.2 Hz), 7.1–7.2 (3H, m), 7.2–7.3 (3H, m), 7.3–7.4 (4H, m), 7.49 (1H, t, J = 7.8 Hz), 7.85 (1H, t, J = 7.8 Hz)dd, J = 7.3 Hz), 7.90 (1H, d, J = 7.8 Hz), 8.69 (1H, dd, J =7.8 Hz), 8.74 (1H, d, J = 0.9, 7.1 Hz), 8.80 (1H, d, J = 0.9, 8.7 Hz). APCI-HRMS (CHCl₃): m/z calcd for C₄₀H₃₂NO₂S₄ ([MH]⁺), 686.1310; found, 686.1574.

Compound 10: AcOH (100 µL, 1.8 mmol) and NBS (26 mg, 0.15 mmol) were added to a solution of terthienylnaphthalimide 9 (100 mg, 0.146 mmol) in CHCl₃ (10 mL) and the mixture was stirred at 0 °C for 1 h. The reaction mixture was washed with H_2O (4 × 3 mL) and an aqueous NaCl solution, dried over Na₂SO₄, and evaporated to afford red powder (105 mg, 0.137 mmol, 94%). TLC (SiO₂, CHCl₃): Rf = 0.57. ¹H NMR (CDCl₃): δ (ppm) = 1.17 (12H, d, J = 6.4 Hz), 2.7– 2.8 (2H, m), 6.94 (1H, d, J = 3.7 Hz), 7.00 (1H, d, J = 3.7 Hz), 7.05 (1H, d, J = 4.1 Hz), 7.12 (1H, d, J = 3.7 Hz), 7.15 (1H, d, J = 3.7 Hz, 7.22 (1H, d, J = 3.7 Hz), 7.3–7.4 (4H, m), 7.49 (1H, t, J = 7.8 Hz), 7.86 (1H, dd, J = 0.9, 7.3 Hz), 7.90 (1H, d, J = 0.9, 7J = 7.3 Hz), 8.69 (1H, d, J = 7.3 Hz), 8.74 (1H, dd, J = 0.9, 8.0 Hz), 8.78 (1H, dd, *J* = 0.9, 8.0 Hz). APCI-HRMS (CHCl₃): m/z calcd for C₄₀H₃₁BrNO₂S₄ ([MH]⁺), 764.0416 and 766.0395; found, 764.0537 and 766.0602.

PyPh-T₄-NI: A solution of K_2CO_3 (42 mg, 0.30 mmol) in a mixture of H₂O (5 mL), 1,4-dioxane (20 mL), and toluene (20 mL) was added to a flask containing boronic acid pinacol ester 6b (54 mg, 0.20 mmol) and bromoterthienylnaphthalimide 10 (100 mg, 0.13 mmol). After the mixture was purged with N_2 , Pd(PPh₃)₄ (10 mg, 0.087 mmol) was added and the mixture was refluxed under N₂ overnight. After H₂O (15 mL) was added, the reaction mixture was extracted with CHCl₃, which was dried over Na₂SO₄ and evaporated. The residue was chromatographed (SiO₂, CHCl₃) and the collected fraction was reprecipitated from CHCl₃ and hexane to afford red powder (55 mg, 0.066 mmol, 50%). TLC (SiO₂, CHCl₃): Rf = 0.40. ¹H NMR (CDCl₃): δ (ppm) = 1.17 (12H, d, J = 6.9 Hz), 2.0–2.1 (4H, m), 2.7–2.8 (2H, m), 3.3–3.4 (4H, m), 7.07 (2H, d, J = 3.7 Hz), 7.09 (1H, d, J = 3.7 Hz), 7.13 (1H, d, J = 1.4 Hz), 7.14 (1H, d, J = 1.4 Hz), 7.22 (1H, d, J = 3.7 Hz), 7.34 (4H, d, J = 7.8 Hz), 7.4–7.6 (3H, m), 7.86 (1H, dd, J = 0.9, 8.2 Hz), 7.91 (1H, d, J = 7.8 Hz), 8.69 (1H, d, J = 7.8 Hz), 8.74 (1H, dd, J = 0.9, 7.9 Hz), 8.81 (1H, dd, J = 0.9, 7.9 Hz). APCI-HRMS (CHCl₃): m/z calcd for $C_{50}H_{43}N_2O_2S_4$ ([MH]⁺), 831.2202; found, 831.2203.

3. Results and Discussion

The naphthalimide derivatives with two and four thiophene units were prepared through Suzuki-Miyaura coupling and Stille coupling reactions between the building blocks, *i.e.*, the naphthalimide part, the bithiophene unit, and the terminal phenyl or aliphatic amine-substituted phenyl group as the key steps, as shown in Scheme 1.

The absorption spectra for the dyes which have an extended π -system with thiophene units with or without an electrondonating moiety are shown in Figure 1. The solvents used are toluene as a representative of nonpolar solvents and DMSO as a representative of polar solvents. Before discussing the absorption features, it is important that the observed spectra represent the monomeric species in solution, since in general, it is known that some dye compounds tend to aggregate and show spectra distinct from those derived from monomeric species.³⁴ We have therefore investigated concentration dependence of the absorption spectra from 2 to 20 µM as shown in Figure S1. Some dyes strictly follow the Lambert-Beer law, while other dyes show slight deviations from linearity. However, the absorption maximum values are nearly independent of the concentration in any case and, therefore, we can safely assume that the values for absorption maxima represent those of monomeric species. On the other hand, the absorption coefficients for Ph-T₄-NI both in toluene and DMSO and PyPh-T₄-NI in DMSO are smaller than those of other compounds, as well as expectations from calculated oscillator strengths (vide infra). For the latter, while dissolving PyPh-T₄-NI in DMSO in concentrations of 2-20 µM gave apparently clear transparent solutions, some precipitates were observed upon allowing the solutions to stand still. Therefore, it is likely that PyPh-T₄-NI forms some aggregates in DMSO, which is supported by the observation that DMSO solutions of this compound do not follow the Lambert-Beer law (Figure S1, d, right). EH₂NPh-T₄-NI, which belongs to the same T_4 dye but with different terminal N,N-dialkyl moieties, is more soluble and exhibits a large absorption coefficient in DMSO as expected. In the meantime, we do not have a clear explanation of the low absorption coefficients for Ph-T₄-NI both in toluene and DMSO; these solutions follow the Lambert-Beer law (Figure S1, b).

A common feature to note in the range >300 nm is that the dyes with one and two thiophene units exhibit two-peaked absorption while dyes with four thiophene units exhibit a single extremely broad absorption peak. To assign these absorption peaks, DFT calculations were performed. The BMK functional²⁸ was used, because it is known that the most popular B3LYP functional underestimates the charge transfer transition energies^{35,36} and the BMK reasonably reproduced the transition energies for T₁ dyes.²⁶ The solvent effects were incorporated via the SCRF model. The substituents on the nitrogen of naphthalimide is replaced by a hydrogen atom to simplify the calculations, which may be justified because the imide nitrogen has little electron density both in the HOMOs and LUMOs. First, the structures were optimized and the results on the dihedral angles between neighboring aromatic rings are compiled in Table S1. For the bond between the naphthalimide unit and



Figure 1. Absorption spectra of naphthalimide dyes (10 μ M). The curves are experimental spectra (left axis) and the bars are calculated oscillator strengths (right axis). The calculations are based on TDDFT (BMK/6-31G* with SCRF). (a) Dyes without an electron-donating moiety in toluene. (b) Dyes with an electron-donating moiety in toluene. (c) Dyes without an electron-donating moiety in DMSO. (d) Dyes with and electron-donating moiety in DMSO.

the thiophene unit, the calculations show that a conformation in which the thiophene S atom is oriented away from the H5 of naphthalimide is more stable than a conformation in which the thiophene S is oriented oppositely. The average dihedral angle between the naphthalene ring and the directly connected thiophene ring for these compounds is 41.5° (standard deviation: 0.8°) which roughly agrees with those reported for 4thienylnaphthalimide moieties in other compounds.³⁰ The dihedral angles between one thiophene ring to the next are smaller with an average of 9° (standard deviation: 5°) and the average dihedral angle between the phenyl ring and the directly connected thiophene ring is 23° (standard deviation: 3°). The calculated frontier molecular orbitals (HOMO, LUMO, and LUMO+1) are displayed in Table S2.

The TDDFT calculations were done for these optimized structures employing the same functional and basis set. The transition wavelengths and oscillator strengths are indicated by the vertical bars in Figure 1 and main numerical values are collected in Table S3. It is seen from comparison that the calculations are in reasonable agreement with the experimental absorption spectra with slight overestimation of the transition energies for the T₁ and T₂ dyes and slight underestimation for the T_4 dyes. The calculations indicate that the lowest-energy transition corresponds mostly to the HOMO to LUMO transition. For every dye, the HOMO is distributed over the terminal phenyl group (including the amino nitrogen in the case of donor-containing dyes) and the oligothiophene units, while the LUMO is mainly distributed over the naphthalimide moiety. Thus, the HOMO to LUMO transitions are associated with the $\pi\pi^*$ transition involving charge transfer over large distances. The second lowest-energy absorption bands observed for Ph-T₂-NI and PyPh-T₂-NI correspond to the HOMO to LUMO+1 transition. These absorption bands also correspond to a $\pi\pi^*$ transition with a charge transfer nature because the LUMO+1 is distributed over the thiophene units admixed with some contribution from the naphthalimide moiety.

The fluorescence spectra are shown in Figure 2. Each of the dyes (10 µM) was excited at its lowest-energy absorption maxima and the relative number of photons per unit wavelength as measured are plotted as a qualitative guide for the trend in fluorescence intensity. The spectra for the dyes with an electron-donating moiety in DMSO are not included because the fluorescence is so weak that it was difficult to obtain reliable spectra. Photos of these dye solutions under UV lamp irradiations are shown in Figure S2. The fluorescence quantum yields together with other spectroscopic and photophysical data, including excited-state lifetimes (see Figure S3), radiative rate constants, and nonradiative rate constants, are compiled in Table 1. Some notable features are gleaned from these data on the excited states. For one, in the case of Ph-T₁-NI, the shortest derivative among those without an electron-donating moiety, the fluorescence intensity increases significantly on going from toluene to DMSO. However, it is found that this property cannot be generalized to the longer series of dyes; The fluorescence intensity of Ph-T₂-NI does not show a significant change and that of Ph-T₄-NI even decreases on going from toluene to DMSO. The major factor accounting for these behaviors is the changes in the rates of nonradiative decay. Upon going from toluene to DMSO, the nonradiative rate for Ph-T₁-NI decreases significantly, that for Ph-T2-NI decreases but by a less magnitude, and that for Ph-T₄-NI increases.

Another notable feature is that for those with an electrondonating moiety, the fluorescence quantum yields are high in toluene (0.29–0.56). However, these dyes exhibit very weak fluorescence in DMSO. We have previously shown that the



Figure 2. Fluorescence spectra of naphthalimide dyes (10 μ M) in toluene and DMSO. Each sample solution was excited at the lowest energy absorption peak. The vertical scale is common to all panels. (a) Dyes without an electron-donating moiety in toluene. (b) Dyes with an electron-donating moiety in toluene. (c) Dyes without an electron-donating moiety in DMSO.

nonradiative rate steeply increases as the orientation polarizability (Δf), which is a measure of solvent polarity, exceeds a certain value ($\Delta f \sim 0.2$) in the case of the compound in which a diphenylamino group is introduced at the para position of the phenyl group of Ph-T₁-NI (Ph₂NPh-T₁-NI).²⁶ This might account for the large difference in the fluorescence intensities in toluene ($\Delta f = 0.013$) and those in DMSO ($\Delta f = 0.263$), although the radiative and nonradiative rates in DMSO cannot be determined due to the poor fluorescence.

We are most interested in how much the absorption and fluorescence wavelengths are shifted upon extension of the π system and the introduction of an electron-donating moiety, because these are practical and critical information for the design of multicolored sensors and probes as described in the introduction. The effects of the number of thiophene units on the lowest-energy absorption and fluorescence peak positions are summarized in a wavenumber scale for the series of dyes without an electron-donating moiety in Figure 3a. The effects of further introduction of an electron-donating moiety are shown in Figure 3b.

The experimental absorption maxima for the series of dyes without an electron-donating moiety are red-shifted on going from the T_0 through T_2 dyes nearly as expected from calculations. The absorption maxima are further red-shifted on going from the T₂ to T₄ dyes but the magnitude of the shift is less than predicted from the calculations. As for the effects of the introduction of an electron-donating moiety, it is reported that the absorption maximum in toluene is shifted from 347 nm to 418 nm ($\Delta \bar{\nu} = 4900 \,\mathrm{cm}^{-1}$) upon going from Ph-NI to a donorcontaining counterpart in which a dimethylamino substituent is introduced at the para position of the phenyl group (M2NPh-NI).²⁵ Introduction of an electron-donating moiety to the T₂ dve results in red-shifted absorption by ca. 40 nm ($\Delta \bar{\nu} = ca$. 2000 cm⁻¹) both in toluene and in DMSO, the effect of which is much smaller than the corresponding modification to the T_0 dye (i.e., Ph-NI). The effects of the introduction of an electrondonating moiety to the T₄ dye is further diminished to $\Delta \bar{\nu} =$ 650-890 cm⁻¹ Indeed, the absorption maximum is *blue*-shifted on going from the donor-containing T_2 to T_4 dyes, while the TDDFT calculations indicate monotonic red-shifts. As the number of thiophene units are increased, the molecule has a greater number of rotatable bonds, which would result in a larger fraction of molecules taking diverse, twisted conformations, less π -conjugated and higher in energy, while the calculations presented here are based on the lowest-energy optimized conformations. This is consistent with the significantly broad absorption bands observed for the T₄ dyes, which may manifest diverse conformations present in the solutions.

For the series of dyes without an electron-donating moiety in toluene, the fluorescence maxima for the T_0 through T_4 dyes are red-shifted from 410 nm²⁵ to 588 nm and the red-shifting trend is still continuing. The same series of dyes behave differently in DMSO. The longest fluorescence wavelength is reached by the T_2 dye (Ph-T₂-NI) at 660 nm. The fluorescence peak wavelengths for the donor-containing T_2 and T_4 dyes in toluene are ca. 650 nm irrespective of the differences in the conjugation length.

4. Conclusion

The absorption maxima can range from 350 nm to 450 nm on the basis of the naphthalimide-based molecular design, in which the π -conjugated system is extended with the thiophene units. Introduction of an electron-donating moiety does redshift the absorption maxima for the shorter members but the effect decreases for the longer members, ending up with the maximum wavelength of 475 nm (Py-Ph2-NI). The fluorescence maxima of the series without an electron-donating moiety can be tuned over the 180 nm range from 410 nm²⁵ to 590 nm in toluene and can be tuned over the 100 nm range from 560 nm (Ph-T₁-NI) to 660 nm (Ph-T₂-NI) in DMSO. For the series with an electron-donating moiety, the fluorescence maxima are about 650 nm in toluene both for T_2 and T_4 dyes. These results will be a guide for the design of functional sensors and probes which exhibit absorption and fluorescence at desired wavelengths. Another new finding in this study concerns the fluorescence intensities or quantum yields, which are (i) for the dyes without an electron-donating moiety, the

Compounds	Solvents	$\lambda_{ m abs} / m nm$	$arepsilon imes 10^{-4}$ $/\mathrm{M}^{-1}\mathrm{cm}^{-1}$	$\lambda_{ m flu} / m nm$	$\Delta ar{ u}$ /cm ⁻¹	$\phi_{ m flu}$	τ /ns	$k_{ m r}$ $/{ m ns}^{-1}$	$k_{\rm nr}$ $/{\rm ns}^{-1}$
$Ph-T_1-NI^b$	Toluene	398	1.53	493	4841	0.06(1)	0.21(1)	0.29(10)	4.28(23)
	DMSO	405	1.68	557	6738	0.43(5)	2.62(5)	0.16(1)	0.22(1)
Ph-T ₂ -NI	Toluene	425	1.63	543	5113	0.06(2)	0.26(1)	0.29(6)	3.56(19)
	DMSO	435	1.62	660	7837	0.14(5)	0.88(1)	0.16(6)	0.97(6)
Ph-T ₄ -NI	Toluene	436	0.48	588 498	5929 2855	0.33(6)	0.89(1)	0.37(7)	0.76(7)
	DMSO	442	0.43	538 506	4037 2862	0.03(1)	0.80(1)	0.04(2)	1.22(2)
PyPh-T ₂ -NI	Toluene	466	2.62	647	6003	0.49(5)	2.51(3)	0.19(2)	0.20(2)
	DMSO	475	1.63	<i>c</i>	<i>c</i>	< 0.01	c	c	c
PyPh-T ₄ -NI	Toluene	450	4.28	648	6790	0.38(1)	1.01(1)	0.37(1)	0.76(1)
	DMSO	460	c	c	<u></u> c	c	c	c	c
EH ₂ NPh-T ₄ -NI	Toluene	453	3.94	650	6690	0.29(3)	0.92(18)	0.31(7)	0.78(16)
	DMSO	455	4.50	c	<u></u> c	< 0.01	c	c	c

Table 1. Photophysical characteristics of the naphthalimide-based fluorescence dyes with extended conjugation.^a

a) All measurements were done in air. Absorption and fluorescence spectra were measured at 25 °C and lifetimes were measured at room temperature. The values followed by a parenthesis are the averages of three measurements and the numbers in the parentheses are 68.3% confidence intervals. b) Data from the literature (T. Inari, M. Yamano, A. Hirano, K. Sugawa, J. Otsuki, *J. Phys. Chem. A* 2014, *118*, 5178). c) Difficult to determine due to poor solubility and/or poor fluorescence.



Figure 3. Dependence of lowest-energy absorption and fluorescence maxima on the number of thiophene units. Circles represent observed values and squares represent calculated values. Closed and open markers are for dyes without an electron-donating moiety and those with an electron-donating moiety, respectively. Color codes are as follows. Black: absorption in toluene; green: absorption in DMSO; red: fluorescence in toluene; orange: fluorescence in DMSO. Some markers are shifted horizontally to avoid overlapping. (a) Data for dyes without an electron donating moiety. (b) Comparison between the dyes with and without an electron-donating moiety.

solvent (polarity) effects are different depending on the number of thiophene units and (ii) for the dyes with an electrondonating moiety, the quantum yields in toluene is high (>0.3) but those in DMSO are extremely low. The latter property may find use in polarity sensitive fluorescent probes. We are now working on functionalization of the π -extended fluorescence dyes presented herein.

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Supporting Information

Supporting figures and tables and supporting data for novel compounds. This material is available on http://dx.doi.org/10.1246/bcsj.20180151.

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