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NIR-FLUORESCENT ETHYL 4,7-BIS(5-ARYLTHIOPHEN-2-YL)-1,2,5-OXADIAZOLO[3,4-*c*]PYRIDINE-6-CARBOXYLATE

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Abstract – Ethyl 4,7-bis(5-bromothiophen-2-yl)-1,2,5-oxadiazolo[3,4-*c*]pyridine-6-carboxylate **2** reacted with phenyl-, 4-methylphenyl-, 4-methoxyphenyl-, 3,4-dimethoxyphenyl-, 1-naphthyl-, and 2-naphthyl-boronic acid to give the corresponding π-extended 4,7-bis(5-arylthiophen-2-yl) derivatives **3a–f**, which emitted fluorescence at around 700 nm in DMSO solution. The 4-methoxy and 2-naphthyl derivatives **(3c and 3f)** emitted fluorescence at 729 nm (13717 cm^{-1}, Φ = 0.02) and 714 nm (14005 cm^{-1} and Φ = 0.11), respectively, with a large Stokes shift (184 and 182 nm for **3c**; 4631 cm^{-1} and 4791 cm^{-1} for **3f**).

INTRODUCTION

A wide variety of fluorescent dyes have been developed for biological applications; for example, Cy3 and Cy5 are used in DNA microarray studies,[1] while Alexa fluor dyes[2] and FITC dyes are used in immunohistochemistry. Near-infrared fluorescent (NIRF) dyes, in particular, have gained importance for the visualization of abnormal tissues *in vivo*. NIR fluorescence is unaffected by

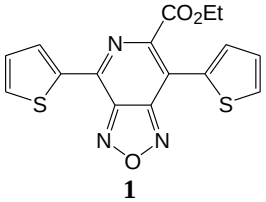

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Figure 1

the autofluorescence arising from red blood cells or organelles, at wavelengths of 650 nm or shorter, and is hence clearly observed in *in vivo* systems. The NIR wavelength region is called as optical window in biological tissue.[3,4]

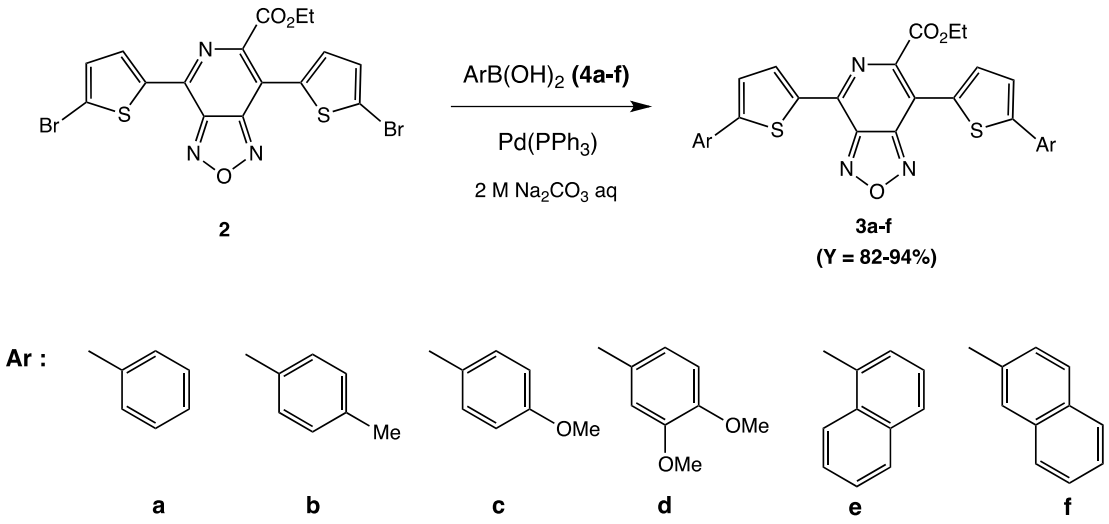
Introduction of aryl groups at the 5-position of the two thiophen rings of the orange fluorescent 4,7-di(thiophen-2-yl)-1,2,5-oxadiazolo[3,4-*c*]pyridine **1** (Figure 1) causes a long-wavelength shift of the fluorescence by 46–78 nm, because of extension of the π-chromophore in the thiophen rings; the 5-arylthiophen-2-yl derivatives emit fluorescence in the 546–647 nm region.[5]

The present article discusses the effect of π-chromophore extension on the absorption and emission spectra of ethyl 4,7-bis(5-arylthiophen-2-yl)-1,2,5-oxadiazolo[3,4-*c*]pyridine-6-carboxylates **3**, which were prepared by Suzuki coupling[6] of bis(5-bromothiophen-2-yl) derivatives **2** with the corresponding arylboronic acids **4**.

RESULTS AND DISCUSSION

1. Preparation of 4,7-bis(5-arylthiophen-2-yl)-1,2,5-oxadiazolo[3,4-*c*]pyridines 3

The desired π-extended 5-arylthiophen-2-yl derivatives **3a–f** were prepared in high yields by the Suzuki coupling of bromothienyl compound **2**[5] with arylboronic acids **4a–f** (Scheme 1).



Scheme 1

The yield of 4,7-diphenyl derivative **3a** was improved (from 67%[5] to 91%) when the reaction was performed using a smaller amount of aq. 2 M sodium carbonate and a greater amount of benzene, with effective stirring of the reaction mixture. This procedure was successfully applied for the preparation of substituted-phenyl derivatives **3b–d** (82–94% yields) and naphthyl derivatives **3e–f** (83–90% yields).

2. Absorption and fluorescence spectra

The spectral properties of **3** were studied in $CHCl_3$ and DMSO (Tables 1, 2 and Figures 2, 3). The

absorption spectra of the derivatives with electron-donating methyl and methoxy groups **3b–d** and the derivative with a π-extended 2-naphthyl compound **3f** showed long-wavelength shift (10–41 nm) as compared with the spectrum of the phenyl derivative **3a**.

Table 1. Absorption and fluorescence spectral data of **3** in $CHCl_3$

Compound	Absorption in $CHCl_3$ [a]			Emission in $CHCl_3$ [b, c]			Stokes shift	
	λ_{max} [nm]	ν [cm^{-1}]	ε [$M^{-1}cm^{-1}$]	λ_{max} [nm]	ν [cm^{-1}]	Φ	[nm]	[cm^{-1}]
3a	517	19342	29000	673	14859	0.31	156	4484
3b	527	18975	30900	688	14535	0.24	161	4440
3c	543	18416	31600	712	14045	0.13	169	4371
3d	547	18282	31300	723	13831	0.08	176	4450
3e	504	19841	28600	676	14793	0.29	172	5048
3f	530	18868	36400	692	14451	0.28	162	4417

a) Conc. = 1.5-3.5 x 10^{-5} M. b) Conc. = 1.0 x 10^{-6} M.
c) Emission spectra were obtained from excitation of the molecules at the λ_{max} observed in the UV/Vis spectra.

Table 2. Absorption and fluorescence spectral data of **3** in DMSO

Compound	Absorption[a*]			Emission[b, c]			Stokes shift	
	λ_{max} [nm]	ν [cm^{-1}]	ε [$M^{-1}cm^{-1}$]	λ_{max} [nm]	ν [cm^{-1}]	Φ	[nm]	[cm^{-1}]
3a	515	19417	32300	693	14430	0.16	178	4987
3b	526	19011	34700	709	14104	0.1	183	4907
3c	545	18349	36100	729	13717	0.02	184	4631
3d	556	17986	35800	Not-FL	—	—	—	—
3e	500	20000	28700	709	14104	0.05	209	5896
3f	532	18797	39900	714	14006	0.11	182	4791

a*) Conc. = 1.4-3.0 x 10^{-6} M. b) Conc. = 1.0 x 10^{-6} M.
c) Emission spectra were obtained from excitation of the molecules at the λ_{max} observed in the UV/Vis spectra.

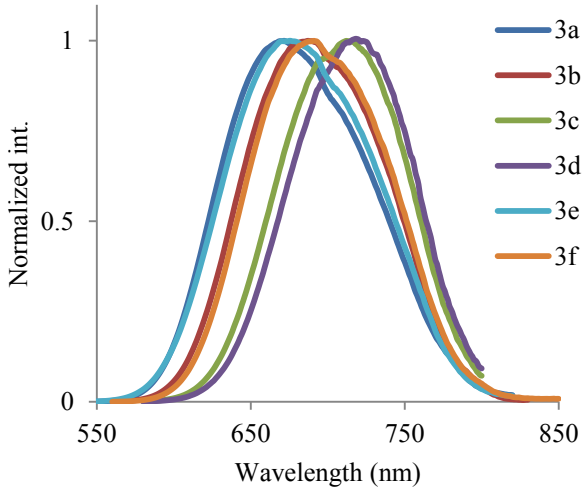


Figure 2. Fluorescence spectra of **3** in $CHCl_3$

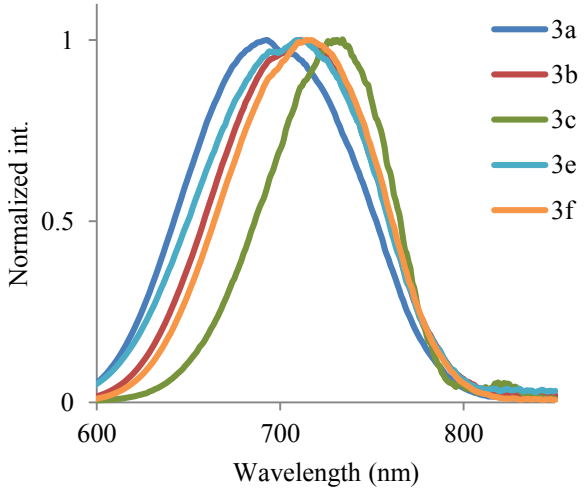


Figure 3. Fluorescence spectra of **3** in DMSO

Compounds **3b–d** emitted fluorescence in the 688–729 nm region. The methoxy group was effective in increasing the emission wavelength, but the quantum yield in this case was low. 4-Methoxy derivative **3c** emitted fluorescence at 712 nm (14045 cm^{-1}, $\Phi = 0.13$) in $CHCl_3$ and at 729 nm (13717 cm^{-1}, $\Phi = 0.02$) in DMSO. Dimethoxy derivative **3d** was weakly fluorescent (723 nm, 13831 cm^{-1} and $\Phi = 0.08$) in $CHCl_3$ and non-fluorescent in DMSO. Naphthyl compounds **3e–f** emitted fluorescence in the 676–714 nm regions, in $CHCl_3$ and DMSO. The emission properties of 2-naphthyl derivative **3f** were favorable. Due to steric reasons, it showed longer fluorescence emission spectra and larger quantum yield than 1-naphthyl isomer **(3e)**. 2-Naphthyl derivative **3f** emitted fluorescence at 692 nm (14450 cm^{-1} and $\Phi = 0.28$) in $CHCl_3$ and at 714 nm (14005 cm^{-1} and $\Phi = 0.11$) in DMSO, while 1-isomer **3e** emitted fluorescence at 676 nm (14792 cm^{-1}, $\Phi = 0.29$) in $CHCl_3$ and showed weak fluorescence at 709 nm (14104 cm^{-1}, $\Phi = 0.05$) in DMSO. Compounds **3** had characteristic large Stokes shifts (Tables 1 and 2),[5] indicative of a donor-acceptor-donor (D-A-D) structure.[7, 8] The solvent effect, which was more significant for emission than for absorption,[7, 8] suggested that the structure of **3** in the excited state was more polar than that in the ground state.[7-12] The solvent-chromophore interaction of D-A-D compounds **3** may be favored in the polar DMSO than in, $CHCl_3$, thus leading to reduced quantum yields in DMSO.

CONCLUSION

Suzuki coupling of bromothienyl compound **2** with various arylboronic acids **4a–f** gave π-extended 5-arylthiophen-2-yl derivatives **3a–f** in excellent yields (82–94%). In $CHCl_3$ and DMSO, compounds **3a–f** showed NIR fluorescence (673–729 nm) and large Stokes shifts. These properties of compounds **3** will be advantageous for application as fluorescent labeling reagents.

EXPERIMENTAL

General

Melting points were determined on a Yanaco micromelting point apparatus (MP 500P) and are reported as uncorrected values. ^1H NMR spectra were obtained on a JEOL JMS-70 (400 MHz) in $CDCl_3$ solution. MS spectra were obtained on a JEOL JMS-70 mass spectrometer. Elemental analyses were performed at the Elemental Analytical Center, Kyushu University. UV-VIS spectra were obtained on a JASCO V560-DS spectrometer. Fluorescence spectra were recorded on a HITACHI F-4500 spectrometer. Fluorescence quantum yields were measured on a HAMAMATSU C10027-02 photoluminescence spectrometer. Column chromatography was carried out on a silica gel column (KANTO C-60).

Typical procedure for the preparation of 3

To a mixture of bis(5-bromothiophen-2-yl) derivative **2** (200 mg, 0.388 mmol) in benzene (8 mL) and phenylboronic acid **4a** (118 mg, 0.971 mmol) in EtOH (5 mL) were added aq. 2 M sodium carbonate (2

mL) and tetrakis(tripenylphosphine)palladium(0) (13.5 mg, 11.7 μmol). Then the mixture was heated under reflux for 2 h, cooled to rt, poured into water, extracted with $CHCl_3$, dried over magnesium sulfate, and evaporated in *vacuo*. The residue was column chromatographed (KANTO C-60, $CHCl_3$/hexane = 1:1, (v/v)) to give crude **3a**, which, on recrystallization from MeCN, afforded **ethyl 4,7-bis(5'-phenylthiophen-2-yl)-1,2,5-oxadiazolo[3,4-*c*]pyridine-6-carboxylate 3a** (180 mg, 91% yield) as dark red needles; mp 188–190 ºC (Lit.,[5] 188–190 ºC).

Ethyl 4,7-bis(5'-*p*-methylphenylthiophen-2-yl)-1,2,5-oxadiazolo[3,4-*c*]pyridine-6-carboxylate 3b: Yield 94%, dark green needles (EtOAc), mp 229–231 ºC; ^{1}H-NMR ($CDCl_3$) δ: 1.37 (t, 3H, *J* = 7.1 Hz), 2.39 (s, 3H), 2.40 (s, 3H), 4.50 (q, 2H, *J* = 7.1 Hz), 7.23 (dd, 4H, *J* = 7.8, 6.1 Hz), 7.36 (d, 1H, *J* = 3.9 Hz), 7.45 (d, 1H, *J* = 4.0 Hz), 7.56 (d, 2H, *J* = 8.2 Hz), 7.63 (d, 2H, *J* = 8.2 Hz), 7.72 (d, 1H, *J* = 3.9 Hz), 8.51 (d, 1H, *J* = 4.0 Hz); FAB-MS (3-nitrobenzyl alcohol): *m/z* 537 (M^+); *Anal*. Calcd for $C_{30}H_{23}N_3O_3S_2$: C, 67.02; H, 4.31; N, 7.82. Found; C, 67.18; H, 4.22; N, 7.99.

Ethyl 4,7-bis(5'-(4"-methoxyphenyl)thiophen-2-yl)-1,2,5-oxadiazolo[3,4-*c*]pyridine-6-carboxylate 3c: Yield 94%, black needles (EtOAc, mp 226–228 ºC; ^{1}H-NMR ($CDCl_3$) δ: 1.38 (t, 3H, *J* = 7.1 Hz), 3.86 (s, 3H), 3.87 (s, 3H), 4.50 (q, 2H, *J* = 7.1 Hz), 6.96 (dd, 4H, *J* = 8.8, 4.7 Hz), 7.29 (d, 1H, *J* = 3.9 Hz), 7.39 (d, 1H, *J* = 4.0 Hz), 7.60 (d, 2H, *J* = 8.8 Hz), 7.68 (d, 2H, *J* = 8.9 Hz), 7.71 (d, 1H, *J* = 3.9 Hz), 8.50 (d, 1H, *J* = 4.0 Hz); FAB-MS (3-nitrobenzyl alcohol): *m/z* 569 (M^+); *Anal*. Calcd for $C_{30}H_{23}N_3O_5S_2$: C, 63.25; H, 4.07; N, 7.38. Found; C, 63.01; H, 4.27; N, 7.55.

Ethyl 4,7-bis(5'-(3",4"-dimethoxyphenyl)thiophen-2-yl)-1,2,5-oxadiazolo[3,4-*c*]pyridine-6-carboxylate 3d: Yield 82%, dark violet needles (EtOAc), mp 186–188 ºC; ^{1}H-NMR ($CDCl_3$) δ: 1.38 (t, 3H, *J* = 7.1 Hz), 3.93 (s, 3H), 3.94 (s, 3H), 3.97 (s, 3H), 3.99 (s, 3H), 4.50 (q, 2H, *J* = 7.2 Hz), 6.91 (d, 1H, *J* = 4.3 Hz), 6.93 (d, 1H, *J* = 4.2 Hz), 7.15 (s, 1H), 7.21 (s, 1H), 7.24 (d, 1H, *J* = 8.3 Hz), 7.30 (d, 1H, *J* = 3.9 Hz), 7.33 (d, 1H, *J* = 8.4 Hz), 7.40 (d, 1H, *J* = 4.0 Hz), 7.70 (d, 1H, *J* = 3.9 Hz), 8.50 (d, 1H, *J* = 4.0 Hz); FAB-MS (3-nitrobenzyl alcohol): *m/z* 629 (M^+); *Anal*. Calcd for $C_{32}H_{27}N_3O_7S_2$: C, 61.04; H, 4.32; N, 6.67. Found; C, 60.76; H, 4.56; N, 6.88.

Ethyl 4,7-bis(5'-(1"-naphthyl)thiophen-2-yl)-1,2,5-oxadiazolo[3,4-*c*]pyridine-6-carboxylate 3e: Yield 90%, Red needles (MeCN), mp 204–207 ºC; ^{1}H-NMR ($CDCl_3$) δ: 1.39 (t, 3H, *J* = 7.1 Hz), 4.50 (q, 2H, *J* = 7.2 Hz), 7.38 (d, 2H, *J* = 3.8 Hz), 7.46 (d, 2H, *J* = 3.9 Hz), 7.52–7.58 (m, 6H), 7.65–7.71 (m, 2H), 7.89 (d, 1H, *J* = 3.8 Hz), 7.91–7.96 (m, 4H), 8.29–8.33 (m, 2H), 8.65 (d, 1H, *J* = 3.9 Hz); FAB-MS (3-nitrobenzyl alcohol): *m/z* 609 (M^+); *Anal*. Calcd for $C_{36}H_{23}N_3O_3S_2$: C, 70.91; H, 3.80; N, 6.89. Found; C, 70.93; H, 3.81; N, 6.89.

Ethyl 4,7-bis(5'-(2"-naphthyl)thiophen-2-yl)-1,2,5-oxadiazolo[3,4-*c*]pyridine-6-carboxylate 3f: Yield 83%, dark green needles (EtOAc), mp 232–235 ºC; ^{1}H-NMR ($CDCl_3$) δ: 1.41 (t, 3H, *J* = 7.1 Hz), 4.54 (q, 2H, *J* = 7.2 Hz), 7.50–7.55 (m, 5H), 7.64 (d, 2H, *J* = 4.2 Hz), 7.78–7.92 (m, 9H), 8.14 (s, 1H), 8.23 (s,

1H), 8.57 (d, 1H, J = 4.0 Hz); FAB-MS (3-nitrobenzyl alcohol): m/z 609 (M^+); *Anal*. Calcd for $C_{36}H_{23}N_3O_3S_2$: C, 70.91; H, 3.80; N, 6.89. Found; C, 70.88; H, 3.83; N, 6.84.

REFERENCES

1. J. L. DeRisi, V. R. Iyer, and P. O. Brown, *Science*, 1997, **278**, 680.
2. R. Haugland, 'The Molecular Probes® Handbook, A Guide to Fluorescent Probes and Labeling Technologies', 10th, ed. by life technologies, Inc., California, 2005.
3. H. Kojima, *Yakugaku Zasshi*, 2008, 128, 1653.
4. R. Weissleder, *Nat. Biotech.*, 2001, 19, 316.
5. H. Gorohmaru, T. Thiemann, T. Sawada, K. Takahashi, K. Nishi-i, N. Ochi, Y. Kosugi, and S. Mataka, *Heterocycles*, 2002, **56**, 421.
6. N. Miyaura and A. Suzuki, *Chem. Rev.*, 1995, **95**, 2457.
7. H. Hiyoshi, H. Kumagai, H. Ooi, T. Sonoda, and S. Mataka, *Heterocycles*, 2007, **72**, 231.
8. R. Nandy and S. Sankararaman, *Beilstein J. Org. Chem.*, 2010, **6**, 992.
9. J. A. Marsden, J. J. Miller, L. D. Shirtcliff, and M. M. Haley, *J. Am. Chem. Soc.*, 2005, **127**, 2464.
10. A. B. J. Parusel, K. Rechthaler, D. Piorun, A. Danel, K. Khatchatryan, K. Rotkiewicz, and G. Kohler1, *J. Fluoresc.*, 1998, **8**, 375.
11. R. Letrun, M. Koch, M. L. Dekhtyar, V. V. Kurdyukov, A. I. Tolmachev, W Rettig, and E. Vauthey, *J. Phys. Chem. A*, 2013, **117**, 13112.
12. C. Cao, X. Liu, Q. Qiao, M. Zhao, W. Yin, D. Mao, H. Zhang, and Z. Xu, *Chem. Comm.*, 2014, **50**, 15811.