Donor–acceptor–acceptor-type near-infrared fluorophores that contain dithienophosphole oxide and boryl groups: effect of the boryl group on the nonradiative decay†

Yoshiaki Sugihara,†a Naoto Inai,†a Masayasu Taki,lb,c Thomas Baumgartner,lb,c Ryosuke Kawakami,ld Takashi Saitou,dl Takeshi Imamura,dl Takeshi Yanai,lb,‡a and Shigehiro Yamaguchi lb,∗ab

The use of donor–π–acceptor (D–π–A) skeletons is an effective strategy for the design of fluorophores with red-shifted emission. In particular, the use of amino and boryl moieties as the electron-donating and -accepting groups, respectively, can produce dyes that exhibit high fluorescence and solvatochromism. Herein, we introduce a dithienophosphole P-oxide scaffold as an acceptor–spacer to produce a boryl- and amino-substituted donor–acceptor–acceptor (D–A–A) π-system. The thus obtained fluorophores exhibit emission in the near-infrared (NIR) region, while maintaining high fluorescence quantum yields even in polar solvents (e.g. λem = 704 nm and ΦF = 0.69 in CH3CN). A comparison of these compounds with their formyl- or cyano-substituted counterparts demonstrated the importance of the boryl group for generating intense emission. The differences among these electron-accepting substituents were examined in detail using theoretical calculations, which revealed the crucial role of the boryl group in lowering the nonradiative decay rate constant by decreasing the non-adiabatic coupling in the internal conversion process. The D–A–A framework was further fine-tuned to improve the photostability. One of these D–A–A dyes was successfully used in bioimaging to visualize the blood vessels of Japanese medaka larvae and mouse brain.

Introduction

Organic π-conjugated compounds with a donor–π–acceptor (D–π–A) framework have attracted substantial attention due to their wide range of applications.1–4 The absorption and emission properties of D–π–A compounds can be tuned by structural modifications of the individual components. By using an appropriate combination of donor, π-spacer, and acceptor moieties, even near-infrared (NIR) emission can be attained. Such NIR-emissive compounds are promising materials for NIR-emissive OLEDs,5,10 biosensing applications,11 and deep-tissue bioimaging given the high biopermeability of the NIR light.12,13

Even though various D–π–A-type fluorophores that exhibit NIR emission have been developed so far, their fluorescence quantum yields (ΦF) tend to decrease drastically in polar solvents. However, D–π–A compounds that contain a diarylboronyl moiety as the electron-accepting group represent one exception to this trend.5–8 In 1972, Williams and co-workers reported a simple p-(dimesitylboroyl)-substituted triphenylamine as the first example of a boron-based D–π–A-type fluorophore.14 Since then, boryl-substituted D–π–A dyes have been extensively studied in order to explore their potential utility as nonlinear optical materials,5,16 two-photon-emissive materials,17 anion sensors,18 and bioimaging.19 These compounds often exhibit high ΦF values even in polar media, despite the significant red-shift of their emission bands.5,6 Two moieties in this type of molecular frameworks can be modified to obtain NIR emission: the aryl group on the boron atom and the π-spacer. For example, in 2015, Marder and co-workers reported D–π–A compounds with an electron-withdrawing perfluorophenyl or 3,5-(CF3)2C6H3 group at the para position of the
aryl group on the boron atom (Fig. 1).\textsuperscript{26} In combination with the strong donor julolidine, the compounds showed NIR fluorescence in CH$_2$CN with maximum emission wavelengths ($\lambda_{em}$) around 745 nm. On the other hand, in 2014, Zhao and coworkers reported a fluorophore that employed 2,1,3-benzothiadiazole as additional electron-accepting $\pi$-spacer, emitting in the far-red to NIR region ($\lambda_{em} = 669$ nm) in CH$_2$CN (Fig. 1).\textsuperscript{21} Misra and co-workers achieved a further red-shifted emission ($\lambda_{em} = 692$ nm; $\Phi_f = 0.27$ in CH$_2$Cl$_2$) by insertion of acetylene spacers.\textsuperscript{22} Such donor–acceptor–acceptor (D–A–A) type structures should thus be beneficial for achieving red-shifted emission.

To develop NIR-fluorescent dyes, we have focused our attention on the modification of the $\pi$-spacer by introducing a phosphine oxide group. We envisioned that the P(=O) group could bathochromically shift the emission by lowering the LUMO energy level due to the $\pi^*-\pi^*$ interaction\textsuperscript{23,24} as well as the inherent inductive electron-withdrawing effect. The profound effect of introducing a phosphine oxide group has already been documented for several fluorescent dyes. For example, fluorescein, a xanthene dye, exhibits a fluorescence maximum at 510 nm, while phospha-fluorescein, a phosphine-oxide-containing fluorescein analogue, exhibits an emission maximum of 656 nm.\textsuperscript{25} This example aptly illustrates the substantial impact of the phosphine oxide group on the electronic structure.

In this context, dithieno[3,2-b:2',3'-d]phosphole P-oxide represents an attractive scaffold. Various dithienophosphole derivatives have already been developed by expanding its $\pi$-skeleton or modifying the Paryl groups.\textsuperscript{26,27} While dithienophosphole P-oxide itself exhibits an emission maximum at 453 nm in CH$_2$Cl$_2$, its emission wavelength can be red-shifted by introducing electron-donating triphenylamine moieties at its termini.\textsuperscript{28} The incorporation of the stronger donor moiety aminothiophene further red-shifts the emission to 657 nm with a moderate fluorescence quantum yield ($\Phi_f = 0.39$) in CH$_2$Cl$_2$ (Fig. 1).\textsuperscript{29}

In this article, we report the design and synthesis of boryl-substituted D–A–A-type fluorophores, which contain a dithienophosphole P-oxide scaffold as an additional acceptor moiety, as highly emissive NIR-fluorescent dyes (Fig. 1). The phosphine oxide group was expected to red-shift the emission band, improve the photostability of the dye, and afford a more rigid molecular structure, which would suppress the non-radiative decay process and thus improve the quantum yield. A series of the D–A–A type dyes 1a–1f that bear various aryl groups on the boron atom, phosphorus-substitution patterns, and electron-donating moieties was synthesized, and their substituent effects were studied in-depth in order to accomplish intense NIR emission and high photostability. A comparative study with other D–A–A type analogues 2 and 3 with different acceptor units in place of the boryl group (Fig. 1) revealed the important role of a terminal boryl group to suppress the non-radiative decay process, resulting in the high fluorescence quantum yields, according to excited-state theoretical calculations. To demonstrate the utility of such dyes, 1c was employed for fluorescence imaging in vivo.

Results and discussion

Compounds 1a–1f were synthesized using 2,6-dibromodithienophosphole P-oxide 4 as the key precursor (Scheme 1). The crucial step in the synthesis of 1a–1e is the monofunctionalization of 4. Its reduction with trichlorosilane, followed by treatment with 1.1 equiv. of $n$-BuLi produced mainly the mono-lithiated product, which was successively treated with Mes$_3$BF. Further oxidation with pyridinium chlorochromate (PCC) or sulfur afforded 5 or 6 in 24% and 18% overall yield, respectively. Suzuki–Miyaura cross-coupling of 5 or 6 with the corresponding amino-substituted arylboronic acid or boronic ester afforded 1a–1d in moderate yields. The phosphonium derivative 1e was obtained by the reduction of 1a, followed by methylation of the P centre with MeOTf. Subsequent recrystallization from a hexane/CHCl$_3$ mixed solvent afforded 1e.

For the synthesis of 1f, bearing a bulky tri(4-butylyphenyl) group on the boron atom, mono-arylation of the key precursor 4 was employed. Specifically, the Suzuki–Miyaura cross-coupling of 4 with 1.0 equiv. of diphenylaminophenylboronic acid furnished 7, which was subsequently coupled with boryl-arylboronic ester 8, obtained in situ from the direct borylation of the corresponding thiophenol boronic precursor using an Ir catalyst, to generate 1f (for details, see the ESI†). Compounds 1a–1f are sufficiently stable to be handled in air without any special precautions.

The formyl- and cyano-substituted analogues 2 and 3 were synthesized as reference compounds (vide infra via the monolithiation of 4; i.e., following the reduction of 4 and its...
Cyclohexane 458 3.56 532 3037 0.81 2.6 0.62
1a

(l) MeOTf, CH2Cl2, rt; (d) (1) ethylene glycol, TsOH/C140
4-(Ph2N)C6H4B(OH)2, 4-(Et2N)C6H4B(OH)2, or 2-(Ph2N)-5-(pin)B;
(e) H2NOH
thiophene, Pd(PPh3)4, K2CO3, toluene, 110°C

Scheme 1 Synthesis of compounds 1a–1f, 2, and 3. Reagents and
conditions: (a) (1) HSiCl3, toluene, rt; (2) n-BuLi, THF, −78°C; (3)
Mes2BF or DMF, THF, −78°C to rt; (4) PCC, S8, or H2O2, CH2Cl2, rt; (b)
4-(Ph2NC6H4H2)B(OH)2, 4-(Et2NCH2)B(OH)2, or 2-(Ph2N)-5-(pin)B-
thiophene, Pd(PPh3)4, K2CO3, toluene, 110°C; (c) (1) HSiCl3, toluene, rt;
(2) MeOTf, CH2Cl2, rt; (d) (1) ethylene glycol, TsOH·H2O, benzene,
100°C; (2) 4-(Ph2NC6H4H2)B(OH)2, Pd(PPh3)4, Na2CO3, toluene/H2O, (3)
HCl or H2O, benzene, Pd(PPh3)4, Na2CO3, toluene/H2O, (3)
HCl or H2O, benzene, Pd(PPh3)4, Na2CO3, toluene/H2O, (3)
HCl or H2O, benzene, Pd(PPh3)4, Na2CO3, toluene/H2O, (3)

Photophysical properties of D–A–A dye 1a
Initially, we evaluated the photophysical properties of 1a,
summarized in Table 1, and the UV–vis absorption and
fluorescence spectra of 1a in various solvents are shown in Fig. 2a.
Regardless of the solvent used, 1a showed absorption maxima
(λabs) at 458–466 nm with molar absorption coefficients
>30 000 M⁻¹ cm⁻¹. In contrast, the fluorescence spectra of 1a
exhibited significant solvatochroism. While 1a showed an
emission maximum (λem) at 532 nm with a Stokes shift of
3037 cm⁻¹ in nonpolar cyclohexane, a significant bathochromic
shift (λem = 665 nm) with a large Stokes shift (6607 cm⁻¹) was
observed in polar CH3CN. This result demonstrates that 1a
exhibits a strong intramolecular charge-transfer (ICT) character
in the excited state, which is a typical feature of D–π–A fluore-
rophores. The ICT character of 1a is also reflected in a large
dipole moment in the excited state (μE = 20.6 D), which was
estimated using the Lippert–Mataga equation (Fig. S8 and Table
S2†). Notably, 1a maintained a high fluorescence quantum yield
in polar solvents (e.g., in CH3CN: ΦF = 0.59) despite its large μE
value. This behaviour differs significantly from that commonly
seen in D–π–A-type fluorescent dyes.

Effects of the boryl group on the photophysical properties
To investigate the origin of the high fluorescence quantum yield
of 1a, especially in polar solvents, its photophysical properties

Table 1 Photophysical properties of D–A–A-type dyes 1a–1f and reference compounds 2 and 3 in various solvents

| Compound | Solvent | λabs (nm) | ε (10⁴ M⁻¹ cm⁻¹) | λem (nm) | Stokes shift (cm⁻¹) | ΦF | k (10⁻⁸ s⁻¹) | kwa (10⁻⁸ s⁻¹) |
|----------|---------|-----------|-----------------|--------|-------------------|-----|-------------|---------------|
| 1a       | Cyclohexane | 458       | 3.56            | 532    | 3037              | 0.81| 2.6         | 0.62          |
|          | Toluene  | 466       | 3.43            | 566    | 3791              | 0.90| 2.8         | 0.31          |
| 1b       | Cyclohexane | 470       | 3.26            | 540    | 2738              | 0.81| 2.3         | 0.54          |
|          | CH3CN    | 487       | 3.28            | 695    | 6145              | 0.72| 1.5         | 0.59          |
| 1c       | Cyclohexane | 486       | 3.14            | 570    | 3032              | 0.65| 1.9         | 0.99          |
|          | CH3CN    | 477       | 2.77            | 704    | 6760              | 0.67| 1.5         | 0.74          |
| 1d       | Cyclohexane | 476       | 3.07            | 567    | 3372              | 0.86| 2.4         | 0.39          |
|          | CH3CN    | 475       | 2.77            | 699    | 6848              | 0.69| 1.5         | 0.67          |
| 1e       | Cyclohexane | 488       | —               | 617    | 4284              | 0.62| n.d          | n.d           |
|          | CH3CN    | 480       | 2.67            | 748    | 7464              | 0.07| n.d         | n.d           |
| 1f       | Cyclohexane | 470       | 4.47            | 550    | 3095              | 0.44| 2.0         | 2.6           |
|          | CH3CN    | 474       | 4.70            | 651    | 5736              | 0.71| 1.8         | 0.72          |
| 2        | Cyclohexane | 463       | 2.44            | 537    | 2976              | 0.60| 1.5         | 1.0           |
|          | CH3CN    | 464       | 2.70            | 709    | 7447              | 0.08| 0.94        | 11            |
| 3        | Cyclohexane | 453       | —               | 548    | 3827              | 0.59| 1.4         | 0.98          |
|          | CH3CN    | 452       | 2.46            | 681    | 7440              | 0.26| 0.95        | 2.7           |

a Only the longest absorption maximum wavelengths are shown. b Absolute fluorescence quantum yields were determined by a calibrated integrating sphere system within ±3% error. c Not determined due to poor solubility.
were compared to those of reference compounds 2 and 3, which contain a formyl and cyano group instead of the boryl group, respectively. While cyano analogue 3 showed a maximum emission wavelength slightly longer than that of 1a in CH$_3$CN, formyl analogue 2 exhibited a more red-shifted emission ($\Delta\lambda = 44$ nm; Fig. 2b). Moreover, 2 and 3 also showed significant solvent effects, i.e., large dipole moments (2: $\mu_r = 23.8$ D; 3: $\mu_r = 21.0$ D) were estimated using the Lippert–Mataga equation (Fig. S8†), which suggests strong ICT character in the excited state, akin to that of 1a. However, their fluorescence quantum yields decreased drastically in polar solvents such as CH$_3$CN (2: $\Phi_F = 0.08$; 3: $\Phi_F = 0.26$), which is typical for D–π–A-type dyes.

To gain further insight into the features of boryl-substituted fluorescent dye 1a, we examined its excited-state dynamics in terms of its radiative ($k_r$) and nonradiative ($k_{nr}$) decay rate constants from the excited singlet state ($S_1$). These values are determined by the $\Phi_F$ values and the fluorescence lifetimes $\tau$. For 1a, 2, and 3, the $k_r$ values decrease with increasing solvent polarity (Table 1). Boryl derivative 1a exhibits a higher $k_r$ value than 2 or 3 in CH$_3$CN, which is at least partially responsible for its higher $\Phi_F$ value. Moreover, 1a shows the lowest $k_{nr}$ value in CHCl$_3$, which increases slightly in CH$_3$CN. Importantly, the $k_{nr}$ value of 1a in CH$_3$CN is $1.0 \times 10^8$ s$^{-1}$, while those of 2 and 3 are beyond $10^8$ s$^{-1}$. These results indicate that not only the higher $k_r$ value, but also the suppressed $k_{nr}$ value are responsible for the high fluorescence quantum yield of 1a in polar solvents.

Theoretical examination of the effects of the boryl group

A theoretical analysis of the photophysical properties of the diarylboryl and dithienophosphole-based D–A–A type molecules was carried out using time-dependent density functional theory (TD-DFT). As the characteristic effects of the boryl group in 1a on $\Phi_F$ were experimentally observed in comparison with reference compounds 2 and 3, this computational study was focused on the elucidation of the role of the boryl group in increasing $\Phi_F$. The solvent effects were considered using the polarizable continuum model (PCM) to verify their impact on $\Phi_F$. The calculations were designed to identify the major factors that determine $k_r$ and $k_{nr}$ (for the details of the computational calculations, see the ESI†).

First, the radiative transition relevant to the $k_r$ value was examined based on the TD-DFT results in $S_0$ and $S_1$ (Fig. 3). Boryl derivative 1a exhibits a slightly higher oscillator strength ($f = 1.19$) for the Franck–Condon transition from $S_0$ to $S_1$ (Fig. 3). This result is consistent with the fact that 1a exhibits a $\sim$50% higher molar absorption coefficient than 2 and 3. Notably, the $f$ value for the vertical transition from $S_0$ to $S_1$ for 1a with the optimized $S_1$ geometry is also higher than those for 2 and 3 (1a: $f = 1.42$; 2: 1.34; 3: 1.26, Fig. S13†). As $k_r$ is proportional to $r^2f$, where $r$ is the wave number of the emission, the higher $f$ value in 1a should be partially responsible for the higher $k_r$ value relative to those of 2 and 3.

Next, we examined the non-radiative transition relevant to the $k_{nr}$ value, which may occur through various decay processes. The possible pathways include the internal
conversion (IC) from $S_1$ to $S_0$ and/or the intersystem crossing (ISC) to the triplet states. We performed rate-constant calculations on the $S_1 \rightarrow S_0$ IC process and the $S_1 \rightarrow T_2$ ISC process; hereafter, their rates are denoted as $k_{IC}$ and $k_{ISC}$, respectively. With the relaxed $S_1$ geometry, $S_1$ and $T_2$ lie closely in energy and largely away from other states, which were thus neglected (Fig. S14†). The $k_{IC}$ and $k_{ISC}$ of 1a in CH$_3$CN were estimated to be $1.4 \times 10^8$ and $1.0 \times 10^7$ [s$^{-1}$], respectively, using MOMAP-2020A program (for details, see the ESI†). This implies that the main nonradiative decay pathway of these dyes in CH$_3$CN is the $S_1 \rightarrow S_0$ IC.

To discuss the relative trend of the experimentally obtained $k_{nr}$ values among 1a, 2, and 3, which are assumed to be mainly $k_{IC}$, the normal-mode contribution of the nonadiabatic coupling (NAC) calculated with the $S_1$-optimized geometry was analysed as shown in Fig. 4a. For all compounds, the normal mode with a frequency of ca. 1570 cm$^{-1}$ was found to have one of the largest NAC values (Fig. S15†). These modes were attributed mainly to the C5–C6 and C7–C8 stretching vibration in the bithiophene moiety of the dithienophosphole scaffold (for the atom labelling, see Fig. 4b). Because the largest geometry changes occur in the C5–C6 bond length between the optimized structures of $S_0$ and $S_1$, while they are nearly identical among 1a, 2, and 3 (Fig. S16†), this quinoidal mode should thus make the largest contribution to $k_{IC}$ through large nonadiabatic interstate coupling.

The NAC values along with the quinoidal mode were calculated to be 113.9 cm$^{-1}$, 124.6 cm$^{-1}$ and 120.8 cm$^{-1}$ in CH$_3$CN for 1a, 2, and 3, respectively. Assuming that this mode is the promoting mode of the $S_1 \rightarrow S_0$ IC and vibrational terms are comparable among these dye molecules, the $k_{IC}$ should be proportional to the square of NAC along with this mode. The square of these NAC values provides the following relation: $1a > 3 > 2$ (Fig. 4c). This trend qualitatively matches the relative magnitude of the experimental $k_{nr}$ among the three compounds in CH$_3$CN. For the CH$_3$CN solutions, we can thus deduce that the substituent-dependence of $k_{IC}$, which almost coincides with $k_{nr}$, is determined by the NACs associated with the quinoidal vibration.

The spin–orbit coupling (SOC) between $S_1$ and $T_2$ was also computed. In the procedure here, SOC was treated as a constant. This means that $k_{ISC}$ is proportional to the square of SOC when vibrational terms are comparable among these dye molecules. Compound 1a has a smaller square of the SOC than the other molecules (Fig. 4c), which implies that 1a has smaller $k_{ISC}$ than the others.

Considering that the experimentally obtained $k_{nr}$ for 2 was about 10 times larger than that for 1a, our prediction might underestimate the interstate interaction of 2. This seemingly arises from the limitation of our model, where the terms that can be important in the calculation of $k_{ISC}$ with small direct SOC (see the ESI† for details) were neglected.

Thus, the question of why boryl derivative 1a exhibits a larger $\Phi_P$ ($= k_e(k_r + k_{nr})$) compared to 2 and 3 can be partially addressed by our theoretical model, which shows that 1a in $S_1$ undergoes decay processes with a larger radiative decay rate constant $k_r$ and a smaller non-radiative decay rate constant $k_{nr}$. The latter can be rationalized in terms of a suppression of the nonadiabatic IC process with the smaller NACs and the ISC process with smaller SOC.

Structural modification to achieve NIR emission
To accomplish a more red-shifted emission, we modified the donor moiety, π-spacer, and/or the phosphorus moiety of the D–A–A framework (Fig. 2c). The photophysical properties of the corresponding compounds 1b–1e are summarized in Table 1 (for the full data in various solvents, see Table S1†). Compound 1b, which contains ethyl groups in place of phenyl groups on the amino moiety, and 1c, which contains a thiophene π-spacer, exhibited more red-shifted emission bands than 1a in CH$_3$CN due to their enhanced electron-donating character (1b: $\lambda_{em} = 695$ nm; 1c: $\lambda_{em} = 699$ nm). Compound 1d, in which the P=O bond of 1e is replaced with a P=S bond, exhibited a comparable emission to that of 1e. Although 1e, with a quarternized P centre, showed the longest wavelength emission ($\lambda_{em} = 748$ nm in CH$_3$CN), its $\Phi_P$ value was low (0.07). Notably, all these derivatives, except for 1e, retained high quantum yields even in CH$_3$CN, despite their long-wavelength emission.

© 2021 The Author(s). Published by the Royal Society of Chemistry
Evaluation of the photostability

The introduction of additional heteroatoms can be expected to affect the photostability of the present compounds. Therefore, the photostability of 1a, 3, and reference compound 10 (Fig. 5a), which does not contain a P(=O)Ph group, was evaluated in degassed CH₂CN under irradiation from a 449 nm high-power LED lamp equipped with a 450/10 nm hard-coated bandpass filter. We have previously demonstrated that the introduction of a bulky substituent on the B atom results in increased photostability. Accordingly, we designed 1f, which should exhibit improved photostability relative to 1a, on account of the bulky substituent at the boron atom, and evaluated its photophysical properties. To quantitatively evaluate the photostability of these compounds, we attempted to determine their total quantum yield of photodecomposition (Φdec), which is defined as the sum of the quantum yields of all photoreactions producing products under light irradiation. The Φdec values estimated were considered to be the minimum possible values, as Φdec is underestimated when the decomposition products absorb light at the monitored wavelength.

The results of the irradiation experiments are shown in Fig. 5b. While the change in absorbance over time was almost linear for 3 and 1f, 1a and 10 showed non-linear behaviour, probably due to the influence of the absorption of the photodecomposed product(s). Although the Φdec values for 1a and 10 cannot be determined quantitatively, given that a linear slope value is required for the calculation, a qualitative comparison demonstrates that 1a is substantially more photostable than 10, confirming that the introduction of the P(=O)Ph group improves the photostability of the fluorophore. Furthermore, bulky aryl-substituted 1f showed improved photostability (Φdec = 5.8 × 10⁻⁶). This value approaches that of 3 (2.7 × 10⁻⁴) and is two orders of magnitude smaller than that of Alexa Fluor 488 (2.3 × 10⁻⁴ in DMSO/buffer = 7/3), which is widely used as a representative photostable dye in bioimaging. However, it should be noted here that these values cannot be compared directly as different solvents were used in the measurements.

The improved photostability of 1f suggests that the steric congestion around the boron atom greatly enhances its photostability.

Application to bioimaging

The bright far-red to NIR emission of the D–A–A dyes 1, even in polar solvents, suggest promising potential for fluorescence imaging of biological samples. To examine the utility of these dyes in such applications, 1c was employed as a representative example. Prior to conducting the imaging experiments, we confirmed the solubility of 1c in phosphate-buffered saline (PBS; pH = 7.4) containing 2% bovine serum albumin (BSA), which is the most abundant protein in the blood plasma, by dynamic light scattering (DLS) measurements (Fig. S17†). The solution of 1c in the presence of BSA only showed a peak comparable to that of a solution of BSA without 1c, indicating that 1c was solved under these conditions. Moreover, the solution exhibited red fluorescence with a λem of 632 nm (Fig. S18†), which is slightly shorter than that in CHCl₃. This fact implies that 1c is bound to the hydrophobic pocket of BSA.

With these results in hand, we tested 1c in two kinds of imaging. First, we used 1c for the in vivo imaging of blood vessels in Japanese medaka (Oryzias latipes) larvae one week after hatching. For that purpose, a solution of 1c in DMSO (<1 μL, 1 mM) was directly injected into the peritoneal cavity of the fish using a microinjection system. The fish was then placed into water with 0.3% salinity and cultured for 1 h. We conducted a whole-body imaging analysis of the larvae using a confocal microscopy system (λex = 488 nm; emission collection: 570–620 nm). Fig. 6a shows a 3D image of the fish that was reconstructed by combining five 1272.8 μm × 1272.8 μm × 918 μm images; as shown, the blood vessels are clearly visible in the living fish. This result suggests that 1c is rapidly absorbed into the bloodstream, where it most likely binds to proteins such as albumin.

Then, we performed deep imaging of the blood vessels in mouse brain using two-photon excitation microscopy. Immediately after administering 100 μL of 1c (0.9 mM in PBS containing 18% DMSO and 1.6% BSA) to a mouse via intravenous injection, images were recorded through an open-skull window at a two-photon excitation wavelength of 880 nm (Fig. 6b). Notably, in addition to emission of the dye in the bloodstream in the red region (λem = 601–657 nm), some blood vessels also exhibited green fluorescence in the 500–550 nm region, which indicates that the microenvironment of the dye is considerably different from that in CHCl₃.
hydrophobic. Although the identity of the biological component that is stained with \textit{ic} to produce this green fluorescence has not yet been clarified, its observation suggests that the hydrophobic dye may have detached from the albumin and then adsorbed on the hydrophobic region of the blood vessel wall.

Conclusions

We have developed a series of diarylboryl and dithienophosphole \(P\)-oxide-containing D-A-A-type fluorophores, which exhibit far-red to NIR emissions. Thus, the tuning of the acceptor spacer is a complementary useful strategy for gaining such red-shifted emissions with the conventional modification of the terminal acceptor boryl group in the D-\(\pi\)-A scaffolds.\textsuperscript{20} Unlike analogues that bear formyl or cyano groups in place of the boryl group, the boryl-substituted D-A-A-type fluorophores retain a high fluorescence quantum yield even in polar solvents such as CH\(_3\)CN. An analysis of the excited-state dynamics revealed that the higher \(k_s\) and the lower \(k_{nr}\) values of the developed dyes are responsible for the high quantum yields. TD-DFT calculations demonstrated that the higher \(k_s\) values can be attributed to higher oscillator strengths for the electronic transition from \(S_1\) to \(S_0\). In terms of nonradiative decay, the internal conversion from \(S_1\) to \(S_0\) is likely a major pathway, in which the boryl group contributes to decreasing the non-adiabatic coupling associated with the quinoidal stretching mode of the dithienophosphole oxide moiety. This result should provide an important insight into the effect of the boryl groups in the widely studied boron-based D-\(\pi\)-A fluorophores. The introduction of the \(P(=O)\)Ph group also greatly influences the photostability of the dyes; the presence of bulky substituents on the boron atom improves the photostability. Moreover, one of the synthesized dyes, \textit{ic}, was successfully applied for whole-body imaging of blood vessels and two-photon bioimaging.

Author contributions

S. Y. and Y. S. conceived the idea. Y. S. synthesized all the compounds and evaluated their properties. N. I. and T. Y. conducted the theoretical calculations. M. T., R. K., T. S., and T. I. conducted imaging experiments. S. Y. and T. B. discussed the \(\pi\)-electron systems studied. Y. S., N. I., M. T., T. Y., and S. Y. wrote the manuscript, and all authors discussed and commented on the manuscript. S. Y. directed the project.

Ethical statement

All animal experiments were approved by the Ethics Committee for Animal Experiments of Ehime University (No5-RE-4-16). The experimental procedures we employed were conducted in accordance with the approved guidelines.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by MEXT/JSPS KAKENHI Grant Numbers JP18H03909 and JP18H05261 (to S. Y.), JP16H06280 (ABIS), JP15H05952 (to T. I. and T. S.), JP19K12218 and JP20H05038 (to T. S.) and by AMED Grant Number JP20gm1210001 (to T. I.). This work was also funded by Nakanishi Foundation for Advancement of Measuring Technologies in Biomedical Engineering, and Kato Memorial Bioscience Foundation (to T. S.). ItBm is supported by the World Premier International Research Center (WPI) Initiative, Japan. T. B. thanks the Canada Research Chairs program for support.

Notes and references

1 (a) H. Meier, Angew. Chem. Int. Ed., 2005, 44, 2482–2506; (b) F. Buret, RSC Adv., 2014, 4, 58826–58851.
2 (a) M. Kivala and F. Diederich, Acc. Chem. Res., 2009, 42, 235–248; (b) S. Kato and F. Diederich, Chem. Commun., 2010, 46, 1994–2006.
3 (a) M. Liang and J. Chen, Chem. Soc. Rev., 2013, 42, 3453–3488; (b) J.-M. Ji, H. Zhou and H. K. Kim, J. Mater. Chem. A, 2018, 6, 14318–14454; (c) L. Dou, Y. Liu, Z. Hong, G. Li and Y. Yang, Chem. Rev., 2015, 115, 12633–12665.
4 J. V. Jun, D. M. Chenoweth and E. J. Petersson, Org. Biomol. Chem., 2020, 18, 5747–7563.
5 (a) C. D. Entwistle and T. B. Marder, Angew. Chem. Int. Ed., 2002, 41, 2927–2931; (b) C. D. Entwistle and T. B. Marder, Chem. Mater., 2004, 16, 4574–4585; (c) L. Ji, S. Griesbeck and T. B. Marder, Chem. Sci., 2017, 8, 846–863.
6 Z. M. Hudson and S. Wang, Acc. Chem. Res., 2009, 42, 1584–1596.
7 S. Yamaguchi and A. Wakiyama, Pure Appl. Chem., 2006, 78, 1413–1424.
8 (a) F. Jäkle, Chem. Rev., 2010, 110(7), 3985–4022; (b) Y. Ren and F. Jäkle, Dalton Trans., 2016, 45, 13996–14007.
9 A. Zampetti, A. Minotto and F. Cacialli, Adv. Funct. Mater., 2019, 29, 1807623.
10 (a) Y. Yuan, Y. Hu, Y.-X. Zhang, J.-D. Lin, Y.-K. Wang, Z.-Q. Jiang, L.-S. Liao and S.-T. Lee, Adv. Funct. Mater., 2017, 27, 1700986; (b) D.-H. Kim, A. D’Aléò, X.-K. Chen, A. D. S. Sandanayaka, D. Yao, L. Zhao, T. Kominou, E. Zaborova, G. Canard, Y. Tsujiuchi, E. Choi, J. W. Wu, F. Fages, J.-L. Brédas, J.-C. Ribierre and C. Adachi, Nat. Photonics, 2018, 12, 98–104; (c) H. Ye, D. H. Kim, X. Chen, A. S. D. Sandanayaka, J. U. Kim, E. Zaborova, G. Canard, Y. Tsujiuchi, E. Y. Choi, J. W. Wu, F. Fages, J.-L. Brédas, A. D’Aléò, J.-C. Ribierre and C. Adachi, Chem. Mater., 2018, 30, 6702–6710; (d) D. G. Congrave, B. H. Drummond, P. J. Conaghan, H. Francis, S. T. E. Jones, C. P. Grey, N. C. Greenham, D. Credgington and H. Bronstein, J. Am. Chem. Soc., 2019, 141, 18390–18394; (e) J. Xue, Q. Liang, R. Wang, J. Hou, W. Li, Q. Peng, Z. Shuai and J. Qiao, Adv. Mater., 2019, 31, 1808242; (f) Q. Liang, J. Xue, J. Xue and J. Qiao, Chem. Commun., 2020, 56, 8988–8991.
11 T. Yamanaka, H. Nakanotani, S. Hara, T. Hirohata and C. Adachi, Appl. Phys. Express, 2017, 10, 074101.
38 T. Sumi, Y. Takagi, A. Yagi, M. Morimoto and M. Irie, *Chem. Commun.*, 2014, **50**, 3928–3930.

39 H. Sotome, T. Nagasaka, K. Une, C. Okui, Y. Ishibashi, K. Kamada, S. Kobatake, M. Irie and H. Miyasaka, *J. Phys. Chem. Lett.*, 2017, **8**, 3272–3276.

40 (a) Ž. Ban, S. Griesbeck, S. Tomić, J. Nitsch, T. B. Marder and I. Piantanida, *Chem.-Eur. J.*, 2020, **26**, 2195–2203; (b) H. Amini, Ž. Ban, M. Ferger, S. Lorenzen, F. Rauch, A. Friedrich, I. Crnolatac, A. Kendel, S. Miljanić, I. Piantanida and T. B. Marder, *Chem.-Eur. J.*, 2020, **26**, 6017–6028.