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^{18}F-Labeled BODIPY Dye: A Potential Prosthetic Group for Brain Hybrid PET/Optical Imaging Agents

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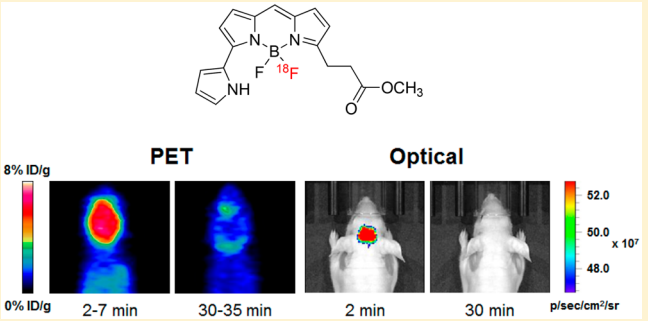
Supporting Information

ABSTRACT: There are few hybrid positron emission tomography (PET)/fluorescence imaging agents available for brain imaging. For this purpose, BODIPY dye is very attractive because one of its fluorine atoms can be readily exchanged with ^{18}F, and it can be modified to produce red-shifted fluorescence. In this study, therefore, we synthesized and investigated a ^{18}F-labeled red-shifted BODIPY dye as a prosthetic group for brain hybrid PET/optical imaging agents and determined the optimal dose of this radioligand for hybrid imaging. The red-shifted BODIPY dye (**1**) was synthesized, and one of its fluorine atoms was exchanged with ^{18}F using $SnCl_4$ in high yield. Partition coefficients of ^{18}F-labeled BODIPY dye ([^{18}F]**1**) and **1** were measured using its radioactivity and fluorescence, respectively, which were shown to be suitable for brain penetration. Optimal dose for hybrid imaging was determined by analysis of PET/CT and optical images of Balb/C nude mice injected with [^{18}F]**1** and **1**, respectively. Hybrid PET/optical images of mice injected with optimal dose of [^{18}F]**1** showed strong radioactivity and fluorescence signal in the brain at 2 min after injection, with rapid clearance by 30 min. Tissue distribution data confirmed the in vivo and ex vivo PET/optical imaging data, indicating desirable brain pharmacokinetics of the radioligand. Taken together, the results of this study suggest that [^{18}F]**1** can be widely used as a prosthetic group for brain hybrid PET/optical imaging agents.



KEYWORDS: *BODIPY, ^{18}F, PET/optical, brain, prosthetic group, optimal dose*

INTRODUCTION

Nucleophilic substitution reaction is commonly used for synthesis of ^{18}F-labeled compounds by substituting sulfonate esters, halides, etc with ^{18}F. Isotopic exchange reaction is also used, particularly for preparation of radioiodinated compounds such as [^{123}I]MIBG and [^{131}I]MIBG.[1−3] This reaction produces the radiolabeled compounds in high radiochemical yields but with low molar activity. Recently, several groups reported ^{18}F-labeling methods of BODIPY dyes, which are BF_2 complexes of dipyrrole derivatives. BODIPY dye has been used as a fluorescent prosthetic group for conjugation to biologically active molecules because it has unique characteristics, such as neutral nature, photostability, fluorescence in diverse regions of the spectrum.[4−6] Therefore, the ^{18}F/^{19}F exchange on one of the fluorine atoms of BODIPY dye allows extension of its application in fluorescence imaging to versatile hybrid imaging fields.

Hybrid PET/optical imaging has been the focus of considerable interest in recent years.[7−9] While PET imaging provides sensitive and quantitative information, optical imaging gives real-time information with high sensitivity. Moreover, PET radioligands can be easily translated for clinical use, and optical imaging dyes can be used for intraoperative image-guided surgery. Therefore, hybrid PET/optical imaging can provide complementary information by compensating the drawbacks of each imaging modality.

The first ^{18}F-labeling of a BODIPY dye was carried out using a compound where a difluoroboron moiety was replaced with a hydroxo(phenyl)boron moiety; this precursor was labeled with ^{18}F in aqueous solution, which had been heated with KHF_2 (70 °C, 10 min), at room temperature for 15 min under acidic conditions, and the radiochemical yield was 22 ± 3%.[10] Under no-carrier-added conditions, the precursor (0.85 μmol) was activated using trimethylsilyl trifluoromethanesulfonate (TMSOTf) and then reacted with *n*-Bu_4N[^{18}F]F at 60 °C for 5 min to produce [^{18}F]fluoro(phenyl)boron-containing BODIPY dye in 61% radiochemical yield.[10] Another group substituted one of the fluorine atoms of BODIPY dye with 4-

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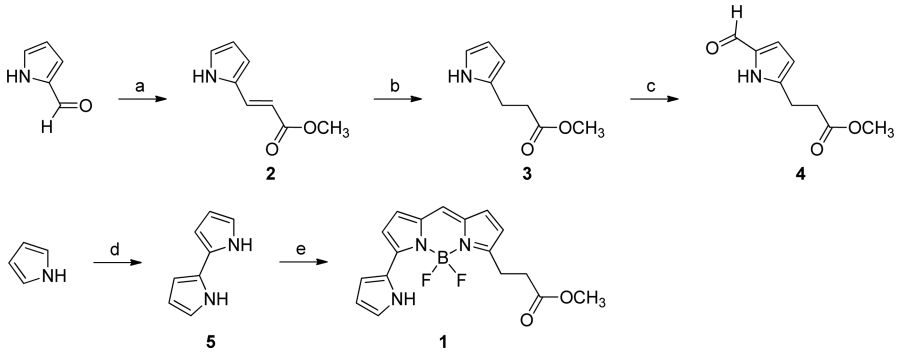
Scheme 1. Synthesis of BODIPY Dye 1[a]



[a]Reagents and conditions: (a) PPh_3=$CHCOOCH_3$, benzene, rt, 3 h; (b) 10% Pd/C, H_2, CH_3OH, 3 h; (c) $POCl_3$, DMF, $(CH_2)_2Cl_2$, reflux, 15 min, then $NaOAc{\cdot}3H_2O$, reflux, 15 min; (d) PIFA, TMSBr, CH_2Cl_2, −78 °C, 1 h; (e) **4**, $POCl_3$, CH_2Cl_2, rt, 5.5 h, then DIPEA, $BF_3{\cdot}OEt_2$, 2 h.

dimethylaminopyridine and then carried out ^{18}F-labeling under harsh conditions, which resulted in 67.6 ± 22.9% radiochemical yield.[11] They further found the mild conditions for ^{18}F/^{19}F exchange reaction; one of the fluorine atoms of BODIPY dye was substituted with a triflate group using TMSOTf, which was stable under organic base such as 2,6-lutidine or *N*,*N*-diisopropylethylamine (DIPEA), and then the triflate-activated BODIPY dye was easily converted into ^{18}F-labeled compound at room temperature within 1 min.[11] Using a much smaller amount of the precursor (0.01 μmol vs 0.25 μmol), ^{18}F-labeled BODIPY dye was achieved in higher radiochemical yield (66.7% vs 37%) and with higher molar activity (35.52 GBq/μmol vs 2.59 GBq/μmol). In order to facilitate ^{18}F-labeling of BODIPY dyes, Liu et al. tried diverse Lewis acids and found that $SnCl_4$ was the choice of Lewis acid for ^{18}F/^{19}F isotopic exchange (room temperature, 10 min), which resulted in 79% labeling yield with an estimated molar activity of 1.30 ± 0.37 GBq/μmol.[12] To extend the application of ^{18}F-labeled BODIPY dyes for biological use, ^{18}F-labeled ammonium BODIPY dyes were synthesized for myocardial perfusion imaging due to their lipophilic cationic properties,[13,14] and ^{18}F-labeled BODIPY dyes were conjugated to biomolecules such as peptides and antibodies as well as small molecules for tumor imaging.[11,12,15,16] Technically, ^{18}F-labeled BODIPY dye enables hybrid PET/fluorescence imaging, and moreover, it has advantages as the hybrid imaging agent, because ^{18}F-labeled BODIPY dye and its nonradioactive form are the same compound with the difference of one neutron.

There are few ^{18}F-labeled dyes available for brain hybrid PET/optical imaging. Brain imaging agents should meet important criteria, such as size, lipophilicity, and brain permeability. Particularly, red-shifted fluorescence dyes are required for brain imaging due to the presence of the skull; however, there are few fluorescent dyes that can cross the blood-brain barrier (BBB). In this context, ^{18}F-labeled red-shifted BOIDPY dyes that are small in size and have appropriate lipophilicity may have potential for brain hybrid PET/optical imaging. In addition, optimal dose suitable for hybrid PET/optical imaging has never been determined.

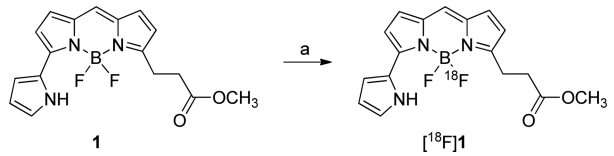
In the present study, therefore, we synthesized and evaluated ^{18}F-labeled red-shifted BODIPY dye as a prosthetic group for brain hybrid PET/optical imaging agents, and determined the optimal dose for brain hybrid imaging.

RESULTS AND DISCUSSION

BODIPY dye (**1**) was synthesized on a large scale from commercially available reagents in five steps (Scheme 1 and Figures S1−S4 in the Supporting Information). Briefly, compound **4** was synthesized in three steps via Horner−Wadsworth−Emmons reaction, catalytic hydrogenation, and Vilsmeier−Haack formylation. Reaction of pyrrole carboxaldehye with methyl(triphenylphosphoranylidene)acetate in benzene at room temperature provided the alkene **2** in 63% yield. The *E*-configuration of **2** was confirmed by large coupling constants (15.9 Hz) of vinylic protons on ^{1}H NMR spectrum. Compound **2** was converted into compound **3** under a conventional catalytic hydrogenation condition (Pd/C, H_2).[17] Formylation of the pyrrole **3** to afford compound **4** was achieved in 92% yield using Vilsmeier reagent (DMF and $POCl_3$) in 1,2-dichloroethane.[18] 2,2′-Bipyrrole (**5**) was prepared using the reported method.[19] Condensation of **4** with **5** under an acid-catalyzed condition and subsequent complexation with boron trifluoride provided the final BODIPY dye **1** in 36% yield.

^{18}F/^{19}F exchange reaction on **1** was carried out as described in the literature with slight modifications;[12] lesser amount of **1** (0.25 μmol) and higher amount of $SnCl_4$ (6 μmol) were used (Scheme 2). Radiochemical yield of [^{18}F]**1** increased from 15−20% to 70−80% based on TLC analysis when the mole ratio of $SnCl_4$ to **1** increased from 12 to 24. The radiochemical yield after HPLC purification was 35−50%. It is expected that the radiochemical yield would increase if the radioligand is purified using a sep-pak cartridge rather than HPLC. Molar activity of [^{18}F]**1** was 0.8−1 GBq/μmol when **1** was labeled with 370 MBq of ^{18}F. Radioligand with high molar activity is required for PET imaging of low density targets in the brain, such as receptors. For this purpose, molar activity higher than 37 GBq/μmol is generally required for the radioligand.[20] As

Scheme 2. Synthesis of [^{18}F]1[a]



[a]Reagents and conditions: (a) *n*-Bu_4N[^{18}F]F, $SnCl_4$, acetonitrile, rt, 10 min.

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reported in the literature, the amount of starting ^{18}F and the molar activity of ^{18}F-labeled BODIPY dye are linearly correlated.[21] As such, it was reported that isotopic exchange on BODIPY dye using increased amount of starting radioactivity (1850 MBq) and low amount of precursor (0.04 μmol) elevated estimated molar activity of the product to approximately 42 GBq/μmol.[12] Similar results were also reported in that use of smaller amount of BODIPY dye (0.01 μmol) provided the ^{18}F-labeled product with high molar activity (35.52 GBq/μmol).[11] Therefore, ^{18}F-labeled BODIPY dye can be synthesized at this high molar activity by using higher amount of ^{18}F and reduced amount of **1**. In contrast, imaging of high density targets can be achieved using ^{18}F-labeled BODIPY dye with lower molar activity.

BODIPY dye **1** displayed an excitation peak at 580 nm and an emission peak at 590 nm in methanol (Figure 1), which was

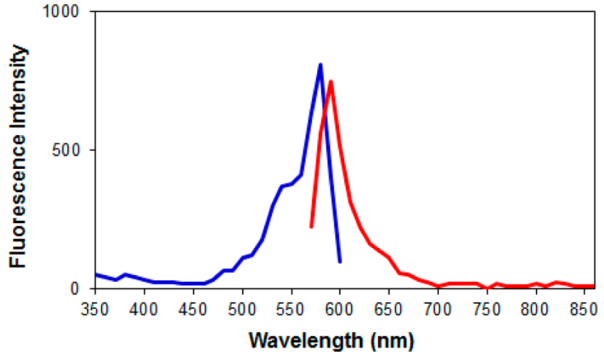

Figure 1. Excitation and emission spectra of **1** (1 μM) in methanol.

shown to be suitable for brain optical imaging. In order to predict brain permeability, partition coefficients of [^{18}F]**1** and **1** were measured using radioactivity and fluorescence, which were within the same range. Log *P* value of [^{18}F]**1** was 1.96 ± 0.01. Partition coefficient of **1** was carefully measured because fluorescence properties are solvent-dependent. Therefore, we re-extracted the water layer with 1-octanol after each extraction of **1** with a mixture of 1-octanol and water. Log *P* value was then calculated from the fluorescence data of **1** in the same solvents. Log *P* value of **1** was 1.94 ± 0.07. The log *P* values obtained using radioactivity and fluorescence were also close to the Clog *P* value (1.79). Brain imaging agents should be lipophilic enough to penetrate across the BBB (log *P* values of 0.1−3.5).[22] [^{18}F]**1** used in this study was shown to be suitable for penetration through the BBB.

The BODIPY dye has intrinsic fluorescence, and one of its fluorine atoms can be readily exchanged with ^{18}F, which enables PET and fluorescence imaging using the same compound. As a result, ^{18}F-labeled BODIPY dye always contains nonradioactive **1**, which gives lower molar activity compared with other radioligands synthesized by nucleophilic radiofluorination. In this study, therefore, we determined the optimal dose of [^{18}F]**1** suitable for brain hybrid PET/optical imaging.

Optical imaging was performed in hairless Balb/C nude mice. Strong fluorescence signal was detected in mouse brain at 2 min after injection of **1**, which demonstrated penetration of **1** across the BBB as well as penetration of fluorescence signal through the intact skull, and then it faded rapidly within 30 min after injection (Figure 2). Because **1** is a fluorescent dye without conjugating with a targeting compound, it is

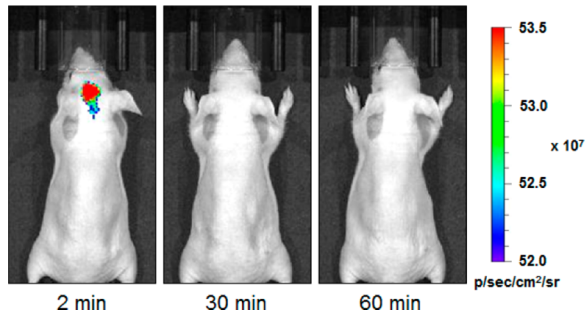

Figure 2. Optical images of Balb/C nude mice obtained at 2, 30, and 60 min after injection of **1** (16 μg) (excitation, 570 nm; emission, 640 nm). Strong fluorescence signal was detected in the mouse brain at 2 min, with fast clearance by 30 min.

expected that the dye penetrated across the BBB and then was rapidly washed out. This result showed the desirable brain pharmacokinetics of **1**. Optimal dose of **1** for optical imaging was also investigated; a dose higher than 16 μg (46.6 nmol) per mouse was required, and a lower dose than this (12 μg; 35.0 nmol) did not show sufficient fluorescence signal in mouse brain.

For PET/CT imaging, [^{18}F]**1** with relatively high molar activity (0.8−1 GBq/μmol) was injected into a Balb/C nude mouse. Sagittal and coronal PET/CT images showed high radioactivity accumulation in the brain at 2 min after injection, with fast wash-out within 30 min after injection, indicating desirable brain pharmacokinetics of the radioligand (Figures 3A and 3B). PET ROI analysis of the brain revealed 4.6% ID/g at 2 min, 1.2% ID/g at 30 min, and 0.51% ID/g at 60 min. Persistent retention of high radioactivity was detected in the liver, kidneys, and intestines. The mouse was also subjected to optical imaging, but no fluorescence signal was detected in the mouse brain (Figure 3C), which is not surprising because [^{18}F]**1** (4.8 MBq) containing 1.8 μg (5.25 nmol) of **1** was injected into the mouse, and a dose higher than 16 μg was determined as the optimal dose for optical imaging. This result demonstrated that [^{18}F]**1** with relatively high molar activity did not provide hybrid imaging of mouse brain. These results suggest that [^{18}F]**1** with molar activity of approximately 0.1 GBq/μmol is suitable for brain hybrid PET/optical imaging of mice.

Hybrid PET/optical imaging of mice was then performed using the optimal dose of [^{18}F]**1**(Figure 4). PET images of mice showed high radioactivity accumulation in the brain, liver, kidney, and intestines at 2 min after injection, and the radioactivity uptake in the brain disappeared at 30 min (Figure 4A). PET ROI analysis of the brain revealed % ID/g values of 4.7 ± 0.2 at 2 min and 1.1 ± 0.1 at 30 min, which indicates fast clearance from mouse brain. Optical images of the mice at 2 min demonstrated strong fluorescence signal in the brain, which decreased rapidly within 30 min (Figure 4B). Due to the limited penetration depth of the fluorescence imaging, other organs were not clearly detected.

Ex vivo imaging was also performed on major tissues obtained at 2 and 30 min after injection of [^{18}F]**1** into mice. PET images showed that strong radioactivity uptake was detected in the brain at 2 min after injection, and the uptake decreased to the background level at 30 min (Figure 5A). High radioactivity uptake was also detected in the liver, kidney, and small intestine at 2 min, and the uptake was retained in the liver, but a much stronger signal was detected in the small

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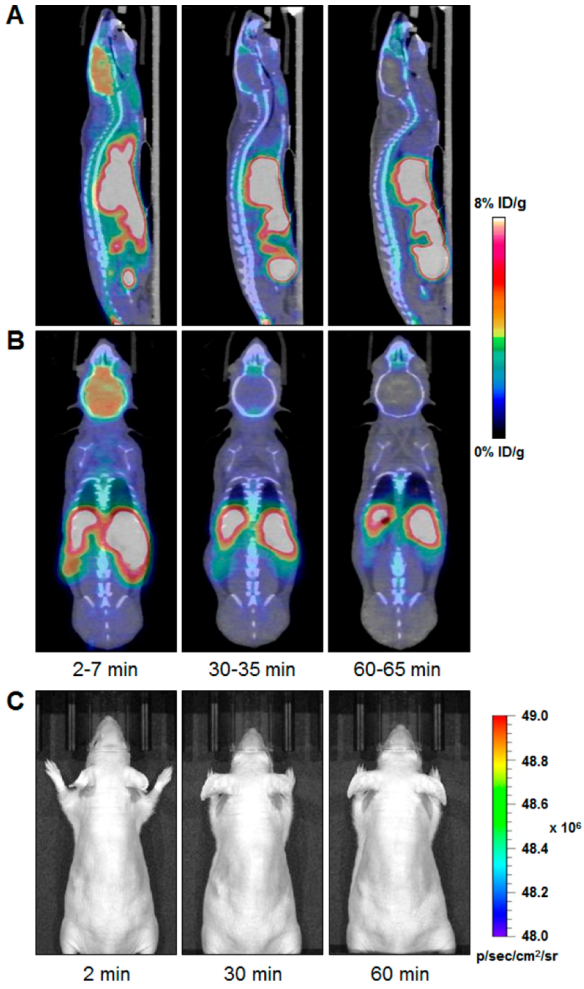

Figure 3. MicroPET/CT and optical images of Balb/C nude mice acquired after injection of [^{18}F]**1** (molar activity, 0.8−1 GBq/μmol). Sagittal (A) and coronal (B) PET/CT images acquired for 5 min at 2, 30, and 60 min after injection showed high radioactivity accumulation in the mouse brain at 2 min after injection of the radioligand, with rapid wash-out by 30 min, and no fluorescence signal was detected in the mouse brain at any time points (C).

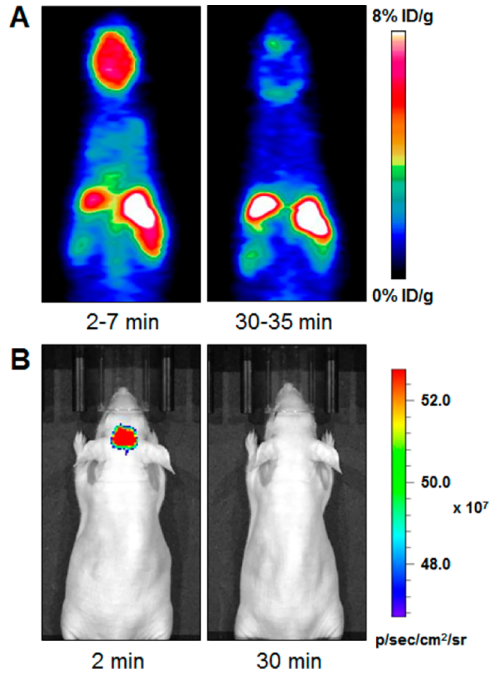

Figure 4. Hybrid PET/optical images of nude mice obtained after injection of [^{18}F]**1** (molar activity, 0.1 GBq/μmol). Static PET images were acquired for 5 min at 2 and 30 min after injection (A) and optical images were acquired for 10 s at the same time points (B).

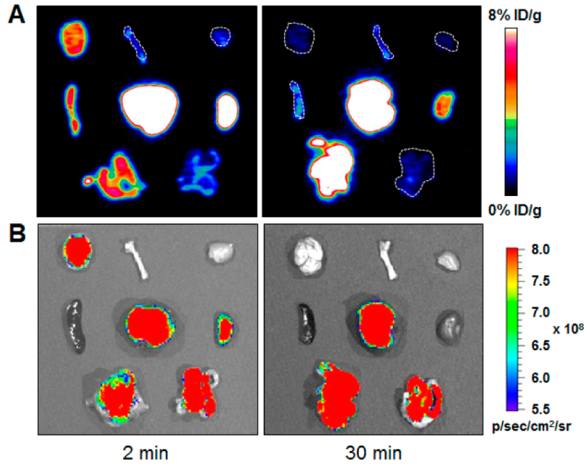

Figure 5. Ex vivo PET (A) and optical (B) images of major tissues obtained at 2 and 30 min after injection of [^{18}F]**1** into mice. Major tissues: (top row) brain, femur, and muscle; (middle row) spleen, liver, and kidney; (bottom row) small and large intestines.

intestine at 30 min. This result suggests excretion of the radioligand through the liver and intestines. Ex vivo optical images confirmed ex vivo PET images: strong fluorescence signal at 2 min and no measurable signal at 30 min in the brain (Figure 5B).

Tissue distribution data demonstrated high radioactivity accumulation of [^{18}F]**1** in the mouse brainat 2 min after injection, with rapid wash-out by 30 min (Table 1); the respective brain uptakes at 2 and 30 min were 4.51 ± 0.43% ID/g and 0.83 ± 0.20% ID/g (***P < 0.001) with a high 2 min-to-30 min uptake ratio of 5.43 , indicating good penetration of the radioligand through the BBB and favorable brain pharmacokinetics.[22,23] This result meets the requirements for brain PET imaging agents such as for diagnosis of Alzheimer's disease: high initial brain uptake at 2 min and fast wash-out at 30 min in normal mice.[23−25] [^{18}F]**1** was excreted through the liver and intestines. Low and constant femur uptake indicated little metabolic defluorination of the radioligand, which is consistent with the reported results showing no measurable radioactivity uptake in the bone even at 4 h after injection.[10,11] These data confirmed the in vivo and ex vivo PET/optical imaging data.

There are few brain hybrid PET/optical imaging agents because most fluorescent dyes cannot cross the BBB. In this context, ^{18}F-labeled BODIPY dyes have great advantages over other fluorescent dyes. The BODIPY dye we used in this study is relatively small compared to other BODIPY dyes and also red-shifted. In addition, the propionyl side chain is short and flexible. There are a few studies on BODIPY dyes developed for brain imaging. One study compared the ex vivo optical images of the brain of a transgenic mouse (Tg2576) injected with BODIPY dye with those of an age-matched wild-type mouse.[6] In another study, the brain tissue sections were obtained after injection of BODIPY dye into mice and then

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Table 1. Tissue Distribution of [^{18}F]1 in Balb/C Nude Mice[a]

organ	2 min	30 min
blood	2.47 ± 0.21	1.01 ± 0.27
heart	10.88 ± 0.95	1.87 ± 0.84
lung	6.16 ± 0.44	1.70 ± 0.52
liver	10.23 ± 1.10	13.39 ± 1.16
spleen	5.66 ± 1.15	1.70 ± 0.45
kidney	14.63 ± 0.41	4.23 ± 1.16
small intestine	5.52 ± 0.48	16.22 ± 0.79
large intestine	1.74 ± 0.39	0.96 ± 0.73
muscle	0.64 ± 0.11	0.52 ± 0.16
femur	1.43 ± 0.18	1.26 ± 0.18
brain	4.51 ± 0.43	0.83 ± 0.20

[a]Values (% ID/g) are given as the mean ± SD of groups, n = 3 mice.

immunostained.[26] However, these data did not provide quantitative information on brain pharmacokinetics. In addition, there is a report on the radiolabeled BODIPY dye, which had poor brain permeability despite its suitable lipophilicity.[27] Therefore, the substituents on the BODIPY skeleton may play a major role in determining brain permeability of the compound.

To the best of our knowledge, this is the first study on dose optimization of radiolabeled fluorescent dye for brain hybrid PET/optical imaging. Although [^{18}F]**1** enables hybrid PET/optical imaging of mouse brain, single PET and optical imaging are also available using [^{18}F]**1** and **1**, respectively, particularly when high molar activity of the radioligand is required.

CONCLUSION

In this study, a novel ^{18}F-labeled BODIPY dye was synthesized in high yield and was shown to have good brain permeability in vitro and in vivo. Furthermore, hybrid PET/optical images demonstrated that the radioligand has favorable brain pharmacokinetics in vivo. Because ^{18}F-labeled and non-radioactive BODIPY dyes have the same chemical structures, with the difference of one neutron, the optimal dose of the radioligand for hybrid PET/optical imaging was determined by analyzing PET/CT and optical images of mouse brain. Taken together, these results suggest that [^{18}F]**1** can be widely used as a prosthetic group for developing various hybrid PET/optical imaging agents.

METHODS

Chemistry. Reagents were purchased from Merck (Darmstadt, Germany), TCI (Tokyo, Japan), and Alfa Aesar (Ward Hill, MA) and were used without further purification. Reaction progress was monitored by TLC analysis using a UV lamp and/or $KMnO_4$ staining. ^{1}H NMR spectra were recorded at room temperature on a Bruker BioSpin Avance 300 MHz spectrometer (Rheinstetten, Germany), and chemical shifts (δ) were reported as ppm downfield of tetramethylsilane as an internal standard. ^{13}C NMR spectrum was obtained using the same NMR spectrometer and was calibrated with $CDCl_3$ (δ = 77.16 ppm). Electrospray ionization (ESI) mass spectrum was obtained on an Agilent 6530 Accurate-Mass Q-TOF LC/MS system (Santa Clara, CA). The purity of compound **1** was measured using HPLC (Agilent 1260 Infinity) equipped with an analytical column (Phenomenex Gemini C18, 4.6 × 150 mm, 5 μm).

*Methyl (E)-3-(1H-Pyrrol-2-yl)acrylate (**2**).* To a solution of methyl(triphenylphosphoranylidene)acetate (14.3 g, 42.8 mmol) in benzene (45 mL) was added pyrrole-2-carboxaldehyde (2.03 g, 21.3 mmol) at room temperature. After stirring for 3 h, the organic layer was separated, and the aqueous phase was extracted with ethyl acetate. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (50:1 to 5:1 toluene−EtOAc) to afford compound **2** (2.03 g, 13.4 mmol, 63%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.31 (brs, 1H), 7.50 (d, J = 15.9 Hz, 1H), 6.83 (brs, 1H), 6.47 (brs, 1H), 6.18 (brd, J = 2.4 Hz, 1H), 6.00 (d, J = 15.9 Hz, 1H), 3.68 (s, 3H).

*Methyl 3-(1H-Pyrrol-2-yl)propanoate (**3**).* Compound **2** (2 g, 13.2 mmol) was dissolved in methanol (10 mL) and hydrogenated in a Parr hydrogenation apparatus using Pd/C under H_2 (1 bar). The reaction mixture was shaken at room temperature for 3 h. At the end of the reaction, the mixture was filtered over a bed of Celite and washed with methanol. The combined solution was concentrated in vacuo. The remaining residue was purified by flash column chromatography (110:1 CH_2Cl_2−CH_3OH) to provide compound **3** (1.86 g, 12.2 mmol, 92%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.61 (brs, 1H), 6.74−6.70 (m, 1H), 6.18 (dd, J = 2.9 and 5.8 Hz, 1H), 6.00−5.98 (m, 1H), 3.77 (s, 3H), 2.98 (t, J = 6.9 Hz, 2H), 2.71 (t, J = 6.9 Hz, 2H).

*Methyl 3-(5-Formyl-1H-pyrrol-2-yl)propanoate (**4**).* To *N*,*N*-dimethylformamide (180 μL, 2.34 mmol) was added slowly phosphorus oxychloride (220 μL, 2.34 mmol) at 0 °C under an argon atmosphere. The solution was stirred for 15 min at the same temperature and then 1,2-dichloroethane (2 mL) was added. To this mixture was added slowly a solution of **3** (303 mg, 1.98 mmol) in 1,2-dichloroethane (8 mL) at 0 °C under an argon atmosphere. The reaction mixture was stirred under reflux for 15 min. After cooling to room temperature, a solution of sodium acetate trihydrate (890 mg, 6.5 mmol) in water (10 mL) was added to the reaction mixture and was allowed to stir under reflux for an additional 15 min. The reaction mixture was then cooled, the organic layer was separated, and the aqueous layer was extracted with chloroform. The combined organic layer was washed with saturated aqueous $NaHCO_3$ solution and then brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by flash column chromatography (50:1 to 5:1 toluene−EtOAc) to afford compound **4** (331 mg, 92%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.46 (brs, 1H), 9.38 (s, 1H), 6.89 (dd, J = 2.6 and 3.7 Hz, 1H), 6.11−6.07 (m, 1H), 3.70 (s, 3H), 3.02 (t, J = 7.2 Hz, 2H), 2.71 (t, J = 7.2 Hz, 2H).

*2,2′-Bipyrrole (**5**).* Compound **5** was synthesized as described in the literature.[19] Briefly, to a stirred solution of 1*H*-pyrrole (187 μL, 2.7 mmol) in dichloromethane (15 mL) at −78 °C, [bis-(trifluoroacetoxy)iodo]benzene (PIFA) (387 mg, 0.9 mmol) and trimethylsilyl bromide (TMSBr) (233 μL 1.8 mmol) were quickly added. The reaction mixture was stirred for 1 h and then stirred for another 10 min at room temperature after addition of saturated aqueous $NaHCO_3$ solution (~10 mL). The mixture was extracted with dichloromethane, and the organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (hexane−EtOAc) to give compound **5** (108 mg, 69%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23 (brs, 1H), 6.79−6.75 (m, 2H), 6.26−6.20 (m, 4H).

*4,4-Difluoro-3-(2-methoxycarbonylethyl)-5-(2-pyrrolyl)-4-bora-3a,4a-diaza-s-indacene (**1**).* To a solution of **4** (115 mg, 0.64 mmol) and **5** (84 mg, 0.64 mmol) in dichloromethane (10 mL) was added phosphorus oxychloride (60 μL, 0.65 mmol) at room temperature. After the reaction mixture was stirred at room temperature for 5.5 h, sequential additions of *N*,*N*-diisopropylethylamine (445 μL, 2.56 mmol) and boron trifluoride diethyletherate (325 μL, 2.59 mmol) were made. The reaction mixture was stirred at room temperature for an additional 2 h. The organic layer was washed with brine, separated, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash column chromatography (7:1 to 4:1 hexane−EtOAc) to give compound **1** (78 mg, 36%) as a dark-purple solid. An aliquot of **1** was analyzed by HPLC using a gradient from a 90:10 mixture to a 10:90 mixture of water and acetonitrile over 20 min, followed by isocratic program with a 10:90 mixture over 10 min at a flow rate of 1 mL/min. The eluent was monitored simultaneously using a UV detector (254 nm) and a visible light detector (588 nm). ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.42 (brs, 1H), 7.18 (brs, 1H), 7.05

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(d, J = 4.8 Hz, 1H), 6.99 (s, 2H), 6.87 (d, J = 4.5 Hz, 1H), 6.84 (d, J = 3.9 Hz, 1H), 6.42−6.34 (m, 1H), 6.28 (d, J = 3.9 Hz, 1H), 3.72 (s, 3H), 3.34 (t, J = 7.5 Hz, 2H), 2.80 (t, J = 7.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.1, 154.8, 150.7, 137.4, 133.4, 131.6, 126.1, 125.8, 123.7, 123.4, 120.4, 117.8, 116.1, 111.5, 51.8, 33.3, 24.0. ESI-HRMS (m/z) calcd for $C_{17}H_{16}BF_2N_3ONa^+$ $[M + Na]^+$, 366.1195; found, 366.1193; Purity (HPLC; t_R= 16.17 min) > 96% (Figures S1−S4 in the Supporting Information).

Radiochemistry. [^{18}F]Fluoride was produced by the $^{18}O(p,n)^{18}F$ reaction using a GE Healthcare PETtrace cyclotron (Uppsala, Sweden). TLC of radioligand was analyzed on a Bioscan radio-TLC scanner (Washington, DC). Purification and analysis of radioligand [^{18}F]**1** were performed using HPLC (Thermo Scientific, Waltham, MA) equipped with an analytical column (Phenomenex Gemini C18, 4.6 × 150 mm, 5 μm). Radioactivity was measured in a dose calibrator (Biodex Medical Systems, Shirley, NY).

*Synthesis of ^{18}F-Labeled BODIPY Dye ([^{18}F]**1**).* ^{18}F/^{19}F Exchange on BODIPY dye **1** was carried out using the method reported in the literature with slight modifications.[12] [^{18}F]Fluoride (170−450 MBq) was placed in a Vacutainer containing n-Bu_4NHCO_3. Three azeotropic distillations were then performed using 200−300 μL aliquots of CH_3CN at 90 °C (oil bath) under a gentle stream of N_2. The resulting n-Bu_4N[^{18}F]F (25 μL) was added to **1** (0.25 μmol), which had been mixed with $SnCl_4$ (6 μmol) dissolved in CH_3CN (40 μL). After the reaction mixture was stirred at room temperature for 10 min, it was purified by HPLC, and the eluent was monitored simultaneously, using UV (360 nm) and NaI(T1) radioactivity detectors. The desired product eluted between 18 and 19 min was concentrated under a stream of N_2.

Molar activity was determined by comparing UV peak area of the desired radioactive peak and those of different concentrations of nonradioactive standard **1** on HPLC using an analytical column eluted with a 40:60 mixture of water and acetonitrile at a flow rate of 1 mL/min. Identity of [^{18}F]**1** was confirmed by coelution of the radioligand with nonradioactive standard **1** when they were coinjected into the HPLC system.

Excitation and Emission Spectra. BODIPY dye **1** (1 μM) was dissolved in methanol, and its excitation and emission spectra were obtained using a Mithras^2 LB 943 monochromator multimode microplate reader (Berthold Technologies, Bad Wildbad, Germany).

Partition Coefficient Measurement. The partition coefficient of [^{18}F]**1** was measured according to the method described in the literature.[24] [^{18}F]**1** was dissolved in 1-octanol (1 mL) and diluted with water (1 mL). The solution was vortexed vigorously for 5 min and then centrifuged. The 1-octanol and aqueous layers were separated out, and 500 μL aliquot of each layer was removed and measured using a gamma counter. Samples from the 1-octanol and aqueous layers were repartitioned until consistent values were obtained. Similarly, the partition coefficient of BODIPY dye **1** (0.6−1 μM) was measured as described above. The 1-octanol and water layers were separated out, and then the water layer was vigorously re-extracted with 1-octanol. The fluorescence intensities of **1** from the resulting 1-octanol layers were measured at 590 nm using a Mithras^2 LB 943 monochromator multimode microplate reader. Log P was expressed as the logarithm of the ratio of the count per minute (or fluorescence) for 1-octanol versus that of water (or 1-octanol extract of water). Clog P of **1** was obtained using ChemDraw Ultra 12.0 software.

In Vivo Studies. Animal experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Samsung Medical Center. MicroPET and optical images of Balb/C nude mice were acquired at the Center for Molecular and Cellular Imaging using an Inveon microPET/CT scanner (Siemens Medical Solutions, Malvern, PA) and a Xenogen IVIS Spectrum (PerkinElmer), respectively. Tissue radioactivity was measured in a Wizard^2 automatic gamma counter (PerkinElmer, Waltham, MA).

Optical Imaging. BODIPY dye **1** was dissolved in 10% DMSO−saline and injected intravenously through a tail vein into 6-week-old Balb/C nude mice (male), which had been fed an alfalfa-free diet for 1 week prior to this experiment. Optical images were acquired for 10 s at 2, 30, and 60 min after injection (excitation, 570 nm; emission, 640 nm). Data are expressed as photons/s/cm^2/sr (sr: steradian).

MicroPET/CT Imaging. Radioligand [^{18}F]**1** was synthesized using 370 MBq of n-Bu_4N[^{18}F]F, whose molar activity was 0.8−1 GBq/μmol. [^{18}F]**1** dissolved in 10% DMSO−saline was injected intravenously into 6-week-old Balb/C nude mice through a tail vein. MicroPET/CT imaging of mice was performed at 2, 30, and 60 min after injection (5 min static PET scan followed by 20 min CT scan). Optical imaging of mice was performed for 10 s prior to each microPET/CT imaging. PET images were reconstructed using 3-D ordered subset expectation maximization and then processed using Siemens Inveon Research Workplace 4.2 (IRW 4.2). ROIs were drawn over the brain in the PET images, and the average signal levels in ROIs were measured.

Hybrid PET/Optical Imaging. Radiolabeled BODIPY dye ([^{18}F]**1**) was injected into Balb/C nude mice via a tail vein. MicroPET images were acquired for 5 min at 2 and 30 min after injection. Prior to each PET imaging, mice were subjected to optical imaging for 10 s (excitation, 570 nm; emission, 640 nm).

Ex Vivo Imaging. Mice were sacrificed at 2 and 30 min after injection of [^{18}F]**1**, and tissues of interest were excised and subjected to PET and optical imaging. Data are expressed as percent injected dose per gram of tissue (%ID/g) and as photons/s/cm^2/sr (sr: steradian), respectively.

Tissue Distribution Studies. [^{18}F]**1** was injected into Balb/C nude mice via a tail vein. Mice were sacrificed at 2 and 30 min after injection, and blood and major tissues were immediately separated, weighed, and counted.

Statistical Analysis. The results were analyzed using unpaired, two-tailed Student's t tests. Differences at the 95% confidence level (P < 0.05) were considered statistically significant.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.8b00480.

^{1}H and ^{13}C NMR spectra, HRMS spectrum, and HPLC chromatograms (PDF)

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Notes

The authors declare no competing financial interest.

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