Electronic Supplementary Material (ESI) for Chemical Communications

Rapid aqueous [^{18}F]-labeling of a bodipy dye for positron emission tomography/fluorescence dual modality imaging

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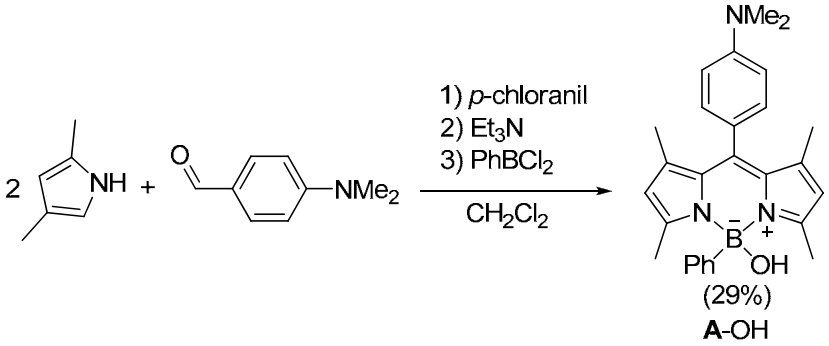
SUPPORTING INFORMATION

This document includes:

Materials and Methods

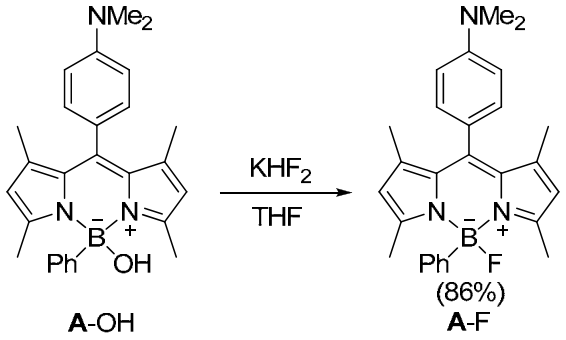
General considerations. 4-dimethylaminobenzaldehyde, *p*-chloranil, and phenylboron dichloride were purchased from Aldrich. 2,4-dimethylpyrrole was purchased from TCI. All preparations were carried out under an atmosphere of dry N_2 employing either a glove box or standard Schlenk techniques. Solvents were dried by passing through an alumina column (CH_2Cl_2) or refluxing under N_2 over Na/K (Et_3N). NMR spectra were recorded on a Varian Unity Inova 400 NMR and an Inova 500 NMR spectrometer at ambient temperature. Chemical shifts are given in ppm, and are referenced to residual ^1H and ^{13}C solvent signals and external neat BF_3-Et_2O for ^{11}B and ^{19}F. Electrospray mass spectra were acquired on a MDS Sciex API QStar Pulsar. The spray voltage was 4.5 kV. All spectra were obtained in positive mode from CH_3CN. HPLC analyses were carried out on a analytical reversed-phase high performance liquid chromatography (HPLC) system equipped with a dual UV absorbance detector (Waters 2487) using a phenomenex C18 RP (250 x 4.6 mm 5 micron). The flow was 1 mL/min, with the mobile phase starting from 95% solvent A (0.1% TFA in water) and 5% solvent B (0.1% TFA in acetonitrile) (0-2 min), followed by a gradient mobile phase to 5% solvent A and 95% solvent B at 22 min. The radioactivity was detected by a model of Ludlum 2200 single-channel radiation detector. The stability study was performed using the same HPLC condition.



Synthesis of 10-dimethyl-aminophenyl-5-hydroxyl-5-phenyl-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (A-OH)

2,4-dimethylpyrrole (298 mg, 3.13 mmol) was dissolved in 300 mL of dichloromethane. To this solution, 4-dimethylaminobenzaldehyde (381 mg, 2.55 mmol) was added two drops of trifluoroacetic acid. The resulting solution became dark red, and was stirred for three hours at room temperature. The red solution was then treated with *p*-chloranil (491 mg, 2.00 mmol) in dichloromethane (250 mL) and stirred for 15 minutes. Dry triethylamine (1.0 mL, 13.6 mmol) was then added followed by dropwise addition of phenylboron dichloride (1.49 g, 9.38 mmol) in Et_2O (10 mL) which resulted in a green fluorescent solution. The solution was stirred overnight then quenched with water (2 × 300 mL). After each wash the organic layer was separated and then dried over $MgSO_4$. The solvent was removed *in vacuo* and then chromatographed on silica eluting with chloroform until all of the green fluorescent material had eluted (followed using a hand-held UV lamp). The solvent was again removed under reduced pressure. This residue was subjected to column chromatography over a small column of silica gel using toluene:hexanes (80:20 v/v) as the eluent (followed using a UV lamp). The fractions with the green fluorescence were combined and the solvent removed to afford the desired product (**A**-OH) as an orange solid (310 mg, 29% yield). **^{1}H NMR** (399.59 MHz; $CDCl_3$): δ 1.49 (s, 6H, dipyrrin-C*H*$_3$), 2.17 (s, 6H,

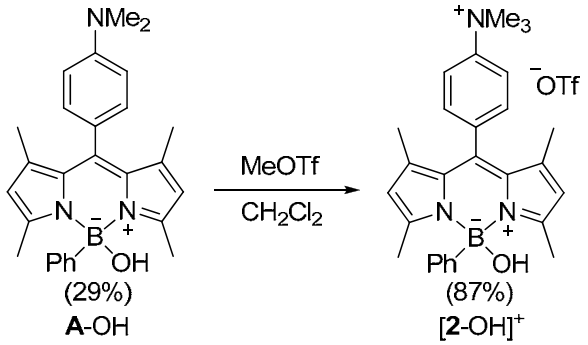
dipyrrin-CH_3), 3.02 (s, 6H, N-CH_3), 5.85 (s, 2H, dipyrrin-C*H*), 6.80 (t, 2H, ^3J = 8.5 Hz, phenyl-C*H*), 7.09-7.25 (m, 5H, phenyl-C*H*), 7.42 (d, 2H, ^3J = 7.0 Hz, phenyl-C*H*). **^{13}C{^{1}H} NMR** (100. 45 MHz, $CDCl_3$): δ 16.36, 16.73, 17.74, 122.60, 126.47, 126.93, 131.45, 133.29, 140.24, 141.38, 154.73. B-*C* peak not observed. **^{11}B{^{1}H} NMR** (128.20 MHz, $CDCl_3$): δ 2.53.



A-OH **A**-F

Synthesis of 10-dimethyl-aminophenyl-5-fluoro-5-phenyl-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (A-F)

A THF (5 mL) solution of **A**-OH (100 mg, 0.236 mmol) was treated with KHF_2 (111 mg, 1.417 mmol) and stirred for 24 hours. The reaction mixture was then quenched with water (10 mL) and extracted with dichloromethane (3 × 5 mL). The organic layers were combined, dried over $MgSO_4$, and filtered. The solvent was removed under reduced pressure and the residue was recrystallized at -40 °C from EtOAc (5 mL) to afford the desired product (**A**-F) as a bright orange crystalline solid (86 mg, 86% yield). **^{1}H NMR** (399.59 MHz, $CDCl_3$): δ 1.49 (s, 6H, dipyrrin-CH_3), 2.16 (s, 6H, dipyrrin-CH_3), 3.02 (s, 6H, N-CH_3), 5.85 (s, 2H, dipyrrin-C*H*), 6.80 (t, 2H, ^3J = 8.5 Hz, phenyl-C*H*), 7.09-7.25 (m, 5H, phenyl-C*H*), 7.42 (d, 2H, ^3J = 7.0 Hz, phenyl-C*H*). **^{13}C{^{1}H} NMR** (100. 45 MHz, $CDCl_3$): δ 16.36, 16.73, 17.74, 122.60, 126.47, 126.93, 131.45, 133.29, 140.24, 141.38, 154.73. B-*C* peak not observed. **^{19}F{^{1}H} NMR** (375.97 MHz, $CDCl_3$): δ -173.9. **^{11}B{^{1}H} NMR** (128.20 MHz, $CDCl_3$): δ 2.51.

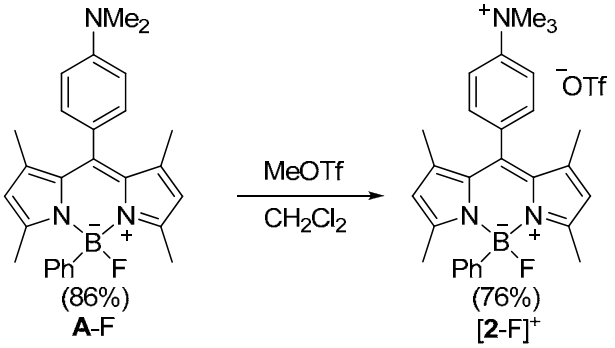


A-OH [**2**-OH]$^+$

Synthesis of [2-OH][OTf]

To a dichloromethane (5 mL) solution of **A**-OH (80 mg, 0.189 mmol) was added a dichloromethane (2 mL) solution of methyl trifluoromethanesulfonate (19.2 mg, 0.246 mmol) dropwise. The formation of an orange precipitate was observed after stirring for 15 min. This solid was collected by filtration and washed with hexane (20 mL) to yield a pure sample of [**2**-OH][OTf] (97 mg, 87%). **^{1}H NMR** (499.43 MHz, CD_3CN): δ 1.38 (s, 6H, dipyrrin-CH_3), 2.20 (s,

6H, dipyrrin-CH_3), 3.60 (s, 9H, N-CH_3), 5.96 (s, 2H, dipyrrin-C*H*), 7.11 (t, 1H, ^3J = 7.2 Hz, phenyl-C*H*), 7.18 (t, 2H, ^3J = 7.1 Hz, phenyl-C*H*), 7.41 (d, 2H, ^3J = 7.7 Hz, phenyl-C*H*), 7.71 (dd, 2H, ^3J = 38.9, 8.1 Hz, phenyl-C*H*), 7.93 (t, 2H, ^3J = 10.1 Hz, phenyl-C*H*). **^{13}C{^{1}H} NMR** (125.59 MHz, CD_3CN): δ 15.0, 15.9, 58.2, 121.9, 122.0, 122.1, 126.8, 127.8, 131.7, 132.8, 132.9, 133.0, 139.2, 140.5, 141.6, 148.3, 156.8. **^{19}F{^{1}H} NMR** (469.87 MHz, CD_3CN): δ -78.1. **^{11}B{^{1}H} NMR** (128.20 MHz, CD_3CN): δ 2.70. HRMS (ESI^+) calcd for [**2**-OH]$^+$ ($C_{28}H_{33}BN_3O^+$): 438.2711, found: 438.2735.



Synthesis of [2-F][OTf]

To a dichloromethane (5 mL) solution of **A**-F (63 mg, 0.148 mmol) was added a dichloromethane (2 mL) solution of methyl trifluoromethanesulfonate (15 mg, 0.193 mmol) dropewise. The formation of an orange precipitate was observed after stirring for 15 min. This solid was collected by filtration and washed with hexane (20 mL) to yield a pure sample of [**2**-F][OTf] (66 mg, 76%). **^{1}H NMR** (499.43 MHz, MeOD): δ 1.42 (s, 6H, dipyrrin-CH_3), 2.14 (s, 6H, dipyrrin-CH_3), 3.76 (s, 9H, N-CH_3), 5.99 (s, 2H, dipyrrin-C*H*), 7.12 (t, 1H, ^3J = 7.4 Hz, phenyl-C*H*), 7.18 (t, 2H, ^3J = 6.9 Hz, phenyl-C*H*), 7.36 (d, 2H, ^3J = 7.0 Hz, phenyl-C*H*), 7.75 (dd, 2H, ^3J = 31.0, 8.5 Hz, phenyl-C*H*), 8.15 (t, 2H, ^3J = 10.2 Hz, phenyl-C*H*). **^{13}C{^{1}H} NMR** (125.59 MHz, MeOD): δ 15.0, 15.4, 57.9, 122.4, 122.5, 122.9, 127.4, 128.0, 131.8, 132.1, 132.3, 132.8, 139.5, 140.7, 142.8, 149.4, 157.7. **^{19}F{^{1}H} NMR** (469.87 MHz, MeOD): δ -78.1, -172.8. **^{11}B{^{1}H} NMR** (128.20 MHz, MeOD): δ 3.04. HRMS (ESI^+) calcd for [**2**-F]$^+$ ($C_{28}H_{33}BN_3O^+$): 440.2668, found: 440.2679.

NMR study of $[2\text{-OH}]^+$ vs KHF_2

Figure S1(a) shows the ^{19}F NMR spectrum of $[\mathbf{2}\text{-OH}]^+$ in acidic D_2O/MeOD (v/v = 1/1) solution. Upon the addition of KHF_2, a signal appeared at -173 ppm within 2 min (Figure S1(b)), indicating the formation of $[\mathbf{2}\text{-F}]^+$.

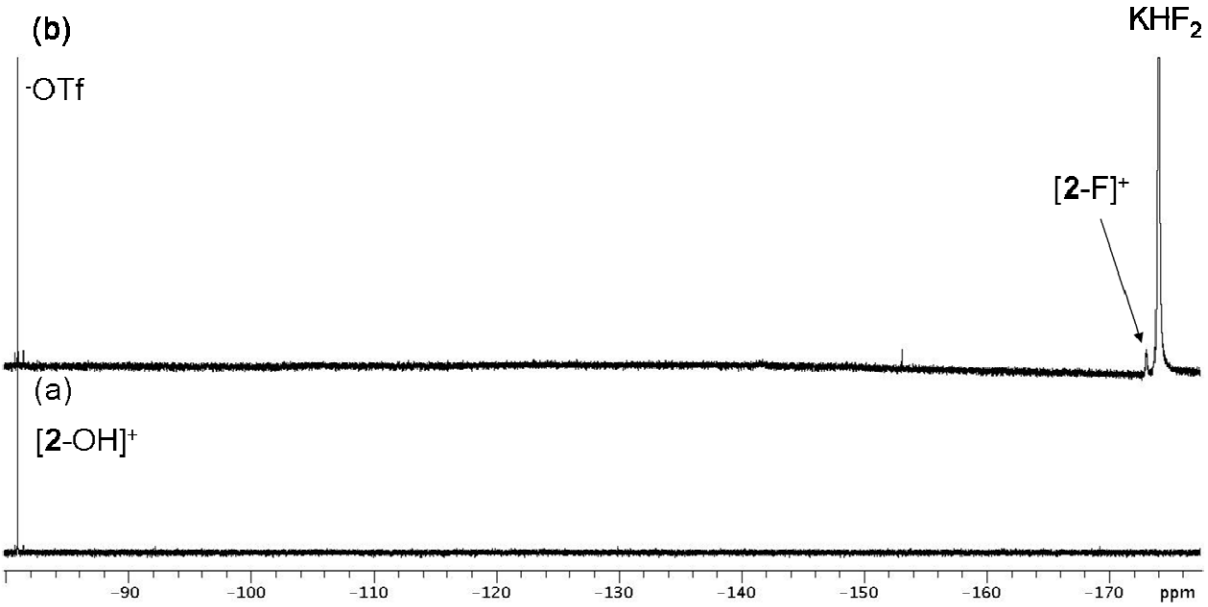


Figure S1. ^{19}F NMR spectra of $[\mathbf{2}\text{-OH}]^+$ in a 0.95 M DCl solution (D_2O/MeOD = 1/1) (a) without KHF_2 and (b) with excess KHF_2.

UV-vis and fluorescence measurements of [2-OH]$^+$ and [2-F]$^+$

UV-vis spectra were recorded on an Ocean Optics USB4000 spectrometer with an Ocean Optics ISS light source. Steady state emission spectra were collected at room temperature using a PTI QuantaMaster 4 fluorescence spectrophotometer equipped with a Model 810 PMT detector. The spectra of [**2**-OH]$^+$ and [**2**-F]$^+$ were measured in CH_2Cl_2 (Fig. S2). Quantum yields were measured using fluorescein as a standard in 0.1 M NaOH solution. Quantum yields obtained for [**2**-OH]$^+$ and [**2**-F]$^+$ are 12.1% and 14.3%, respectively.

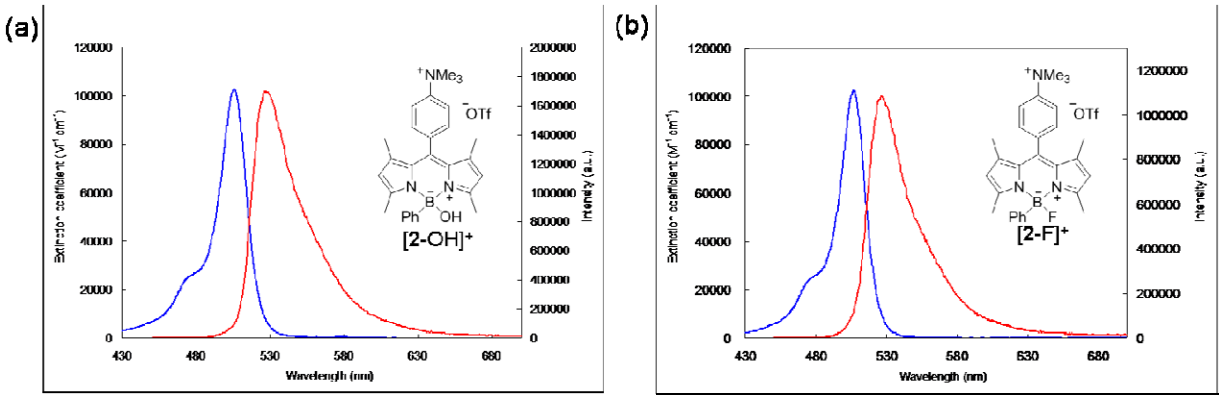


Figure S2. Absorption (blue) and emission (red) spectra of (a) [**2**-OH]$^+$ and (b) [**2**-F]$^+$ in CH_2Cl_2.

PBS stability

The [^{18}F]-**[2**-F]$^{+}$ *in vitro* stability was tested in 1 × PBS. Briefly, [^{18}F]-**[2**-F]$^{+}$ (about 500 μCi) was incubated in 1 × PBS at room temperature. At different time points (1 h, 3 h, and 6 h), an aliquot of [^{18}F]-**[2**-F]$^{+}$ solution was taken and analyzed by reverse-phase HPLC under identical conditions used for analyzing [^{19}F]-**[2**-F]$^{+}$ standard. The untouched compound ^{18}F-**2**-F was determined to be >99%, 97%, and 95% at 1 h, 3 h, and 6 h, respectively.

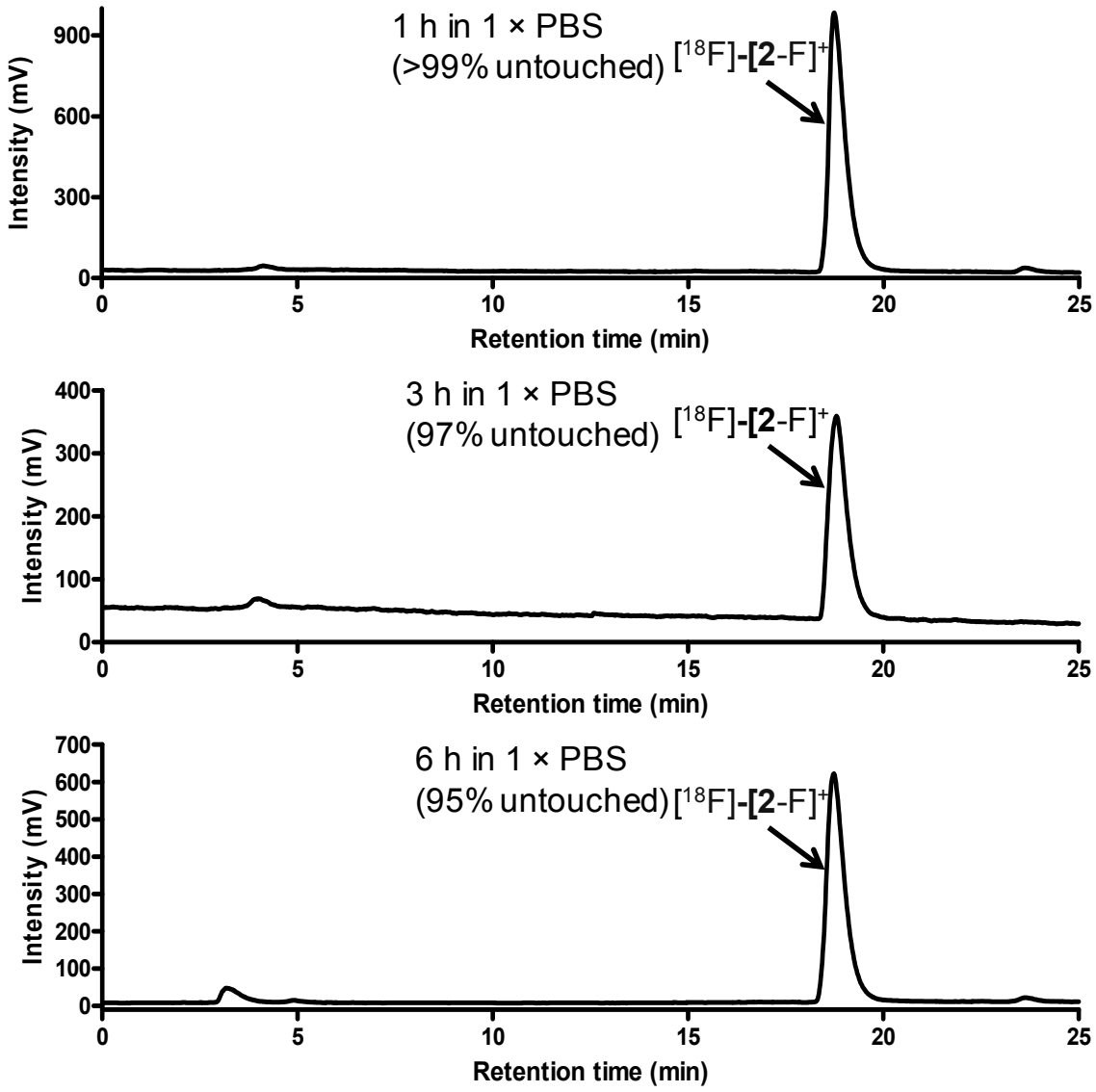
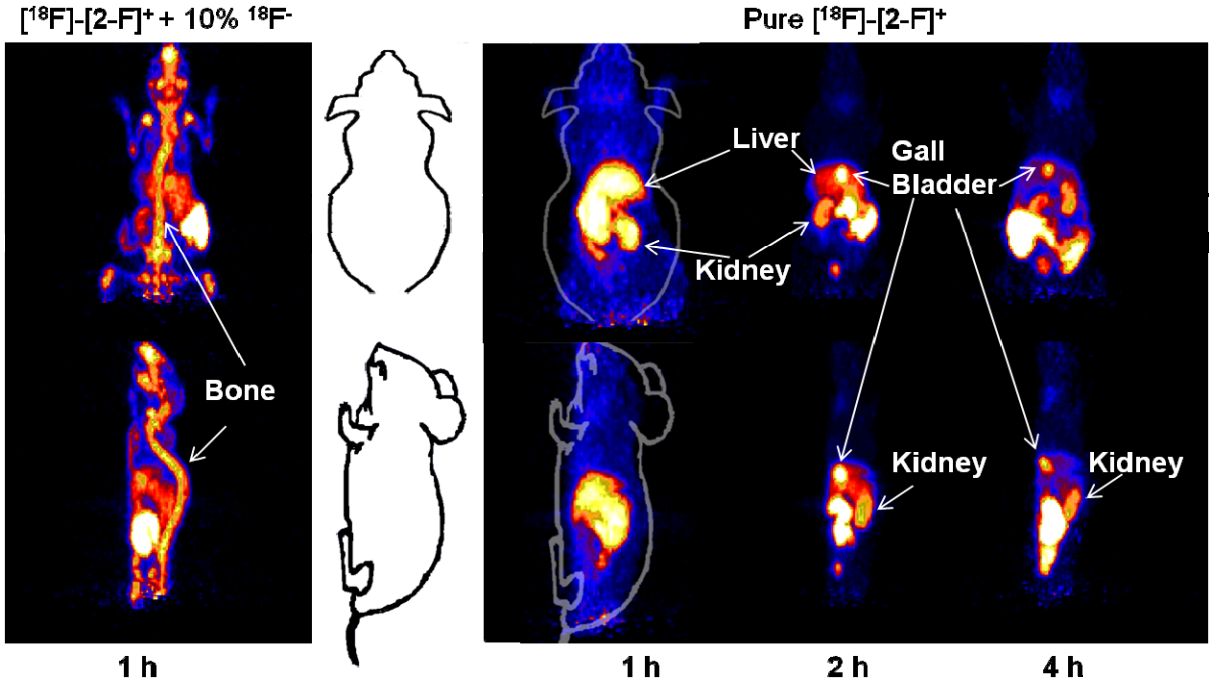


Figure S3. Stability studies of [^{18}F]-**[2**-F]$^{+}$ in 1 × PBS.

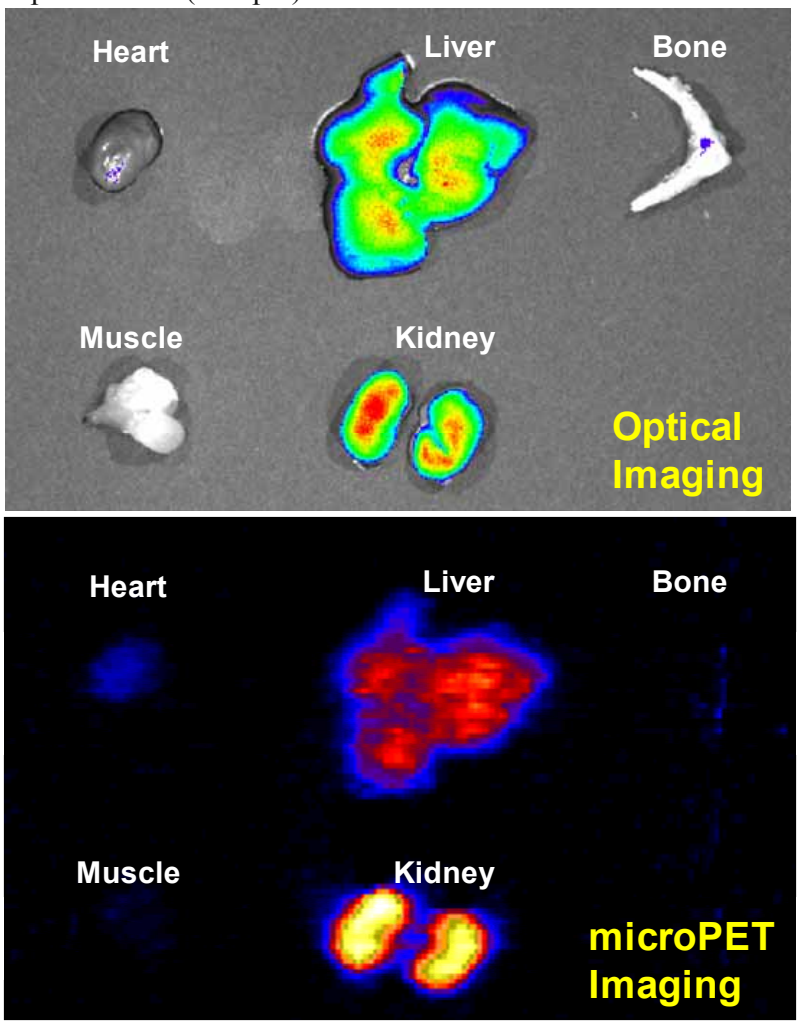
Conditions used to generate the MicroPET imaging shown in Figure 3

The nude mice were imaged using a microPET R4 rodent model scanner (Concorde Microsystems, Knoxville, TN) in the prone position. The mice were injected with 50–80 μCi of [^{18}F]-**[2**-F]$^{+}$ via the tail vein. Multiple static scans were obtained at 1, 2, and 4 h post-injection after the mice were anesthetized with 2% isoflurane. The images were reconstructed by a two-dimensional ordered subsets expectation maximum algorithm. After each microPET scan, the images were displayed as 2-D projection to illustrate the whole-body distribution of the tracer. As shown below, significant amounts of bone uptake were observed when [^{18}F]-**[2**-F]$^{+}$ was co-injected with ~10% of free ^{18}F. However, no bone uptake even at 4 h post injection if pure [^{18}F]-**[2**-F]$^{+}$ was injected to the animal. This imaging result demonstrated that [^{18}F]-**[2**-F]$^{+}$ has reasonable stability *in vivo*.



Conditions used to generate the fluorescent imaging shown in Figure 4

To cross-evaluate the dual modality tracer ^{18}F-**2**-F, *ex vivo* fluorescence imaging was performed using a Lumina II small-animal imaging system (Xenogen, Alameda, CA). After the microPET imaging was done, the nude mouse was sacrificed. Major organs were collected and scanned with the microPET and Limina machines. Fluorescent images were acquired and analyzed using Living Image 2.5 software (Xenogen). The fluorescence images were acquired using a 2-s exposure time (f-stop 4).



Carrier-free radiofluorination of [2-OH]$^+$

In a typical experiment, [**2**-OH]$^+$ (500 µg, 0.85 µmol in 100 µL MeCN) was pretreated with TMSOTf (20 eq.) and then subsequently mixed with a MeCN solution (100 µL) of azeotropically dried [^{18}F]-TBAF (10 mCi). The reaction was allowed to proceed for 5 min at 60 °C. HPLC analysis indicated formation of [^{18}F]-[**2**-F]$^+$ (specific activity ≥ 1.4 Ci/µmol) in a 61%.

Radiolabeling of [**2**-OH]$^+$ with [^{18}F]-TBAF in MeCN without carrier.

Entries	Compound #	TMSOTf	Temp	Results
1	[**2**-OH]$^+$	None	Rt 15 min	Minimal
2	[**2**-OH]$^+$	None	75 °C 15 min	Minimal
3	[**2**-OH]$^+$	None	110 °C 15 min	Minimal
4	[**2**-OH]$^+$	0.6 equiv	60 °C 5 min	Yield < 1%
5	[**2**-OH]$^+$	20 equiv	Rt 5 min	30% yield*
6	[**2**-OH]$^+$	20 equiv	60 °C 5 min	61% yield*

* The yield was determined based on the HPLC integration.