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Efficient synthesis of fluorescent-PET probes based on [^{18}F]bodipy dye

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Materials and Methods

The synthetic and radiosynthetic work was carried out with the following equipment and methods. The syringe filter and polyethersulfone membranes (pore size, 0.22 μm; diameter, 13 mm) were obtained from Nalge Nunc International (Rochester, NY). Analytical reversed-phase high-performance liquid chromatography (HPLC) was accomplished on a Waters 515 chromatography system with a Waters 2487 dual λ absorbance detector and model 2200 scaler-ratemeter radiation detector from Ludlum Measurements, Inc. (Sweetwater, TX). Empower 2 software from Waters Corporation (Milford, MA) was used to record chromatograms. HPLC was performed on a phenomenex Luna 5μ C18 column (250 × 4.6 mm). The flow was 1 mL/min, with the mobile phase starting from 80% solvent A (0.1% TFA in water) and 20% solvent B (0.1% TFA in MeCN) (0–2 min) to 5% solvent A and 95% solvent B at 22 min. ^{1}H, and 13C spectra were recorded on 400 MHz Varian NMR spectrometers. 1H NMR chemical shifts were determined relative to trace amount solvent as the internal standard. Mass spectra were recorded on a Bruker 300-MS TQ mass spectrometer in the EI mode.

Syntheses of BODIPY®FL-RGD and BODIPY®R6G-RGD$_2$

Commercially available BODIPY®FL NHS ester (1.5 μmol) in 50 μL DMSO, *c*(RGDyK) (0.66 μmol, denoted as RGD) and *N*,*N*-diisopropylethylamine (5 μL) were mixed together. After 2 h incubation at room tempature, the reaction mixture was subjected to HPLC purification. BODIPY®FL-RGD (Rt = 16.9 min) was obtained in 81% yield. The final product was characterized by Thermo LTQ FT mass spectrometry (m/z 942.4 for $[MH]^+$, $C_{45}H_{55}BF_2N_{11}O_9$, calculated $[MH]^+$: 942.4).

Commercially available BODIPY®R6G NHS ester (1.5 μmol) in 50 μL DMSO, PEGylated *c*(RGDyK) dimer (1.0 μmol, denoted as RGD_2) and *N*,*N*-diisopropylethylamine (5 μL) were mixed together. After 2 h incubation at room temperature, the reaction mixture was subjected to HPLC purification. BODIPY®R6G-RGD_2 (Rt = 18.5 min) was obtained in 73% yield. The final product was characterized by Thermo LTQ FT mass spectrometry (m/z 2069.1 for $[MH]^+$, $C_{96}H_{130}BF_2N_{24}O_{25}$, calculated $[MH]^+$: 2069.0). ^{1}H NMR (400 MHz, DMSO) δ 1.01 (br, 1H), 1.17-1.48 (m, 18H), 1.69-1.71 (m, 3H), 2.02-2.08 (m, 3H), 2.34-2.45 (m, 2H), 2.66-2.75 (m, 5H), 2.94-3.25 (m, 11H), 4.49 (s, 2H), 4.57-4.59 (m, 2H), 6.36 (br, 1H), 6.61-6.63 (m, 4H), 6.90-6.92 (m, 4H), 7.01-7.57 (m, 15H), 7.74-7.95 (m, 6H), 8.03-8.05 (m, 5H), 8.16-8.19 (m, 1H), 8.28 (br, 2H), 11.33 (s, 1H), 12.50 (s, 1H).

The NMR and MS of BODIPY-PACMA31 and BAP-1

4,4-Difluoro-3-{(*E*)-{2-(4-dimethylaminophenyl)ethenyl}}-1-methyl-4-bora-3a,4a-diaza-s-indacene (BAP-1). ^{1}H NMR (400 MHz, DMSO) δ 2.27 (s, 3H), 2.99 (s, 6H), 6.42 (dd, *J* = 3.8, 2.2 Hz, 1H), 6.77 (d, *J* = 9.0 Hz, 2H), 6.94 (d, *J* = 3.7 Hz, 1H), 7.06 (s, 1H), 7.16 (d, *J*= 15.9 Hz, 1H), 7.48 (d, *J* = 9.0 Hz, 1H), 7.50 (m, 2H), 7.66 (d, *J* = 16.0 Hz, 1H), 7.60 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δ 11.6, 40.2, 111.9, 112.1, 112.7, 116.0, 118.5, 121.9, 123.3, 124.4, 130.5, 132.8, 136.2, 138.6, 143.6, 146.1, 152.5, 160.8. MS (EI) *m/z* 351.3 (M^+). Chemical Formula: $C_{20}H_{20}BF_2N_3$, calculated molecular weight: 351.2.

N-{2-[(2-{[2-[2,4-dimethoxy(propioloyl)anilino]-2-(2-thienyl)acetyl]amino}acetyl)amino]ethyl carbamoyl} phenyl-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-s-indacene (BODIPY-PACMA31). ^{1}H NMR (500 MHz, DMSO) δ 1.29 (s, 6H), 2.42 (s, 6H), 2.63 (s, 1H), 4.47 (m, 1H), 6.03 (m, 1H), 6.16 (m, 2H), 6.33-6.37 (m, 2H), 6.76 (m, 1H), 6.87 (m, 1H), 7.28-7.31 (m,

2H), 7.41-7.43 (m, 2H), 7.79 (m, 1H), 7.97-7.99 (m, 2H), 8.55-8.63 (m, 1H). MS (EI) *m/z* 794.2 (M^+), 795.4 ($[M+H]^+$). Chemical Formula ($[M+H]^+$): $C_{41}H_{42}BF_2N_6O_6S$, calculated molecular weight: 795.3.

Radiolabeling

[^{18}F]Fluoride was produced by the $^{18}O(p,n)^{18}F$ reaction in [^{18}O]water using a CTI/Siemens RDS112 11MeV cyclotron. [^{18}F]fluoride was trapped by a QMA cartridge and eluted off with tetrabutylammonium bicarbonate (TBAB) into a 5-mL V-vial (Type I Borosilicate), followed by azeotropic drying with anhydrous MeCN. [^{18}F]fluoride was then dissolved in anhydrous MeCN for the labeling reactions.

BODIPY®FL-RGD (0.2 μmol) in 50 μ anhydrous DMSO was mixed with chlorides shown in Table 1. After violently shaking for 1 min, the resulting solution was added [^{18}F]fluoride (10 ± 3 mCi) in MeCN. After shaking at room temperature for 10 min, an aliquot of the reaction mixture (50–100 μCi) was collected for HPLC analysis. Integration of the radio-chromatogram was used for the yield calculation.

[^{18}F]BODIPY®R6G, [^{18}F]BODIPY-PACMA31, and [^{18}F]BAP-1 utilized the same protocol for [^{18}F]fluorination. As an example, BODIPY®R6G (0.2 μmol) in 50 μl anhydrous MeCN was added to $SnCl_4$ (3.0 μmol). The resulting solution was then mixed with [^{18}F]fluoride (10 ± 3 mCi). After shaking at room temperature for 10 min, an aliquot of the reaction mixture (50–100 μCi) was collected for HPLC analysis. Integration of the radio-chromatogram was used for the yield calculation.

For the synthesis of [^{18}F]BODIPY®R6G-RGD_2, the HPLC eluent containing [^{18}F]BODIPY®R6G was collected and the solvent was removed under reduced pressure.

[^{18}F]BODIPY®R6G was azeotropically dried twice with anhydrous MeCN under reduced pressure at 85 °C. Then [^{18}F]BODIPY®R6G (~2 mCi) was taken with 100 µl MeCN and added to 0.2 µmol RGD_2 in 50 µl DMSO. After addition of 10 µl diisopropylethylamine, the reaction remained at 55 °C with slightly shaking. After 15 min, the reaction was quenched with 5% acetic acid and purified with HPLC. After removing the HPLC solvent from [^{18}F]BODIPY®R6G-RGD_2 by rotary evaporation and the activity was reconstituted in 1 mL phosphate-buffered saline (PBS) and passed through a 0.22 µm syringe filter for *in vivo* animal experiments.

MicroPET Imaging

Animal procedures were performed according to a protocol approved by the University of Southern California Institutional Animal Care and Use Committee. The detailed procedure was published previously.[1] In brief, each mouse was injected with 50 ± 10 µCi of the [^{18}F] probe via the tail vein. The imaging data were achieved with the mice under anesthesia using isoflurane (5% for induction and 2% for maintenance in 100% O_2). The regions of interest (ROIs) were converted to counts per gram per min based on the assumption of 1 g/mL tissue density. Dividing counts per gram per minute by injected dose gave the image ROI derived %ID/g values.

Fluorescence imaging

In vivo fluorescence imaging was performed using the Xenogen Lumina XR Imaging System and analyzed using the IVIS Living Imaging 3.0 software (Caliper Life Sciences, Alameda, CA, USA). A Cy5.5 filter set was used for acquiring the fluorescence of BODIPY®R6G-RGD_2 peptide. Identical illumination settings (lamp voltage, filters, f/stop, field

of views, binning) were used for acquiring all images. Fluorescence emission images were normalized and reported as photons per second per centimeter squared per steradian (p/s/cm^2/sr).

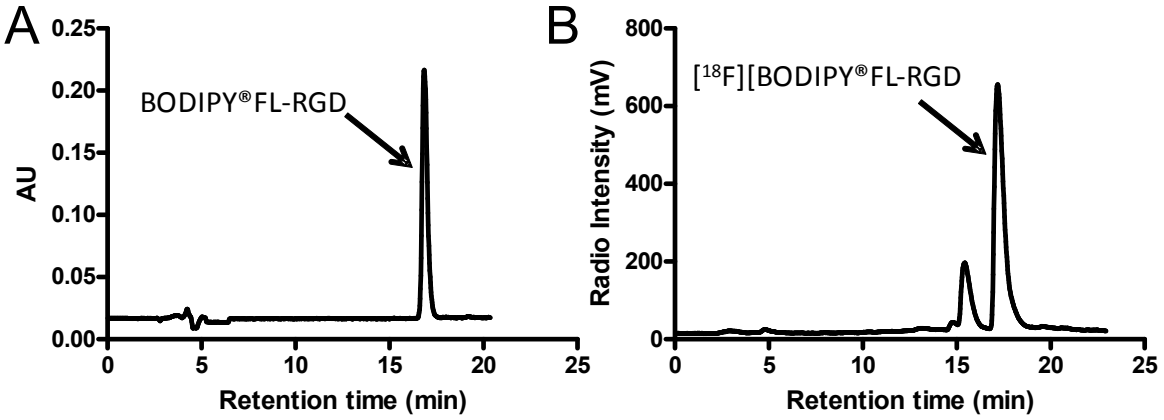

Figure 1. HPLC profiles. A. The UV trace of standard BODIPY®FL-RGD. B. The radio-trace of the crude reaction mixture of Entry 3 in Table 1.

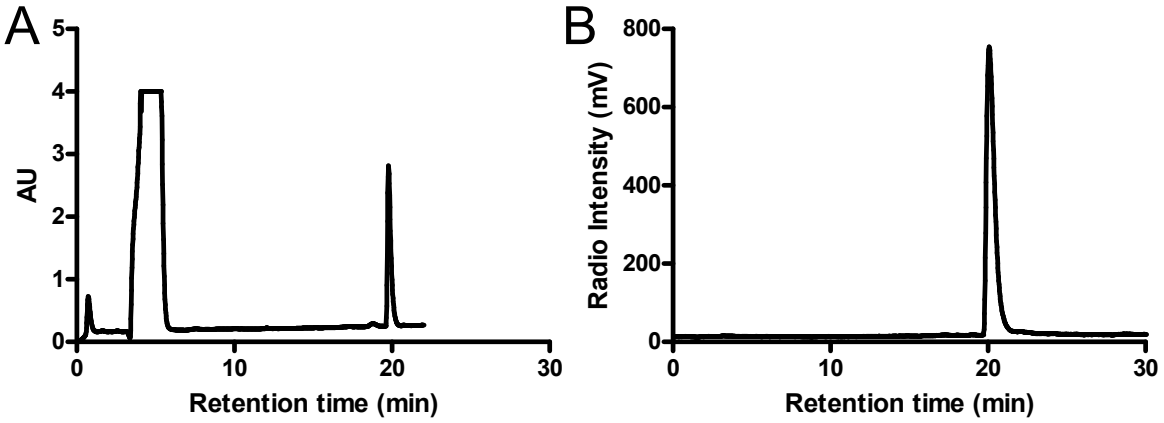

Figure 2. HPLC profiles. A. The UV trace of standard BODIPY-PACMA31. B. The radio-trace of the crude reaction mixture of [^{18}F]fluorination of BODIPY-PACMA31.

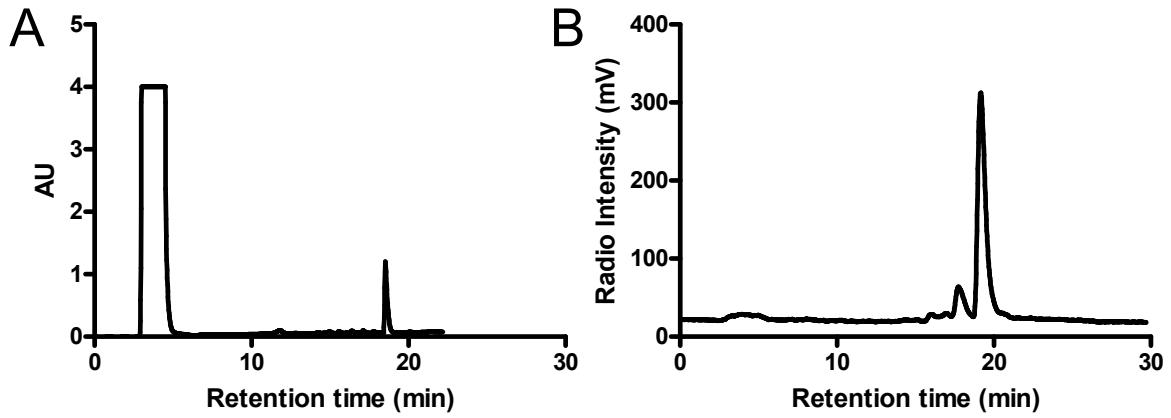

Figure 3. HPLC profiles. A. The UV trace of standard BODIPY@R6G. B. The radio trace of the crude reaction mixture of [^{18}F]fluorination of BODIPY@R6G.

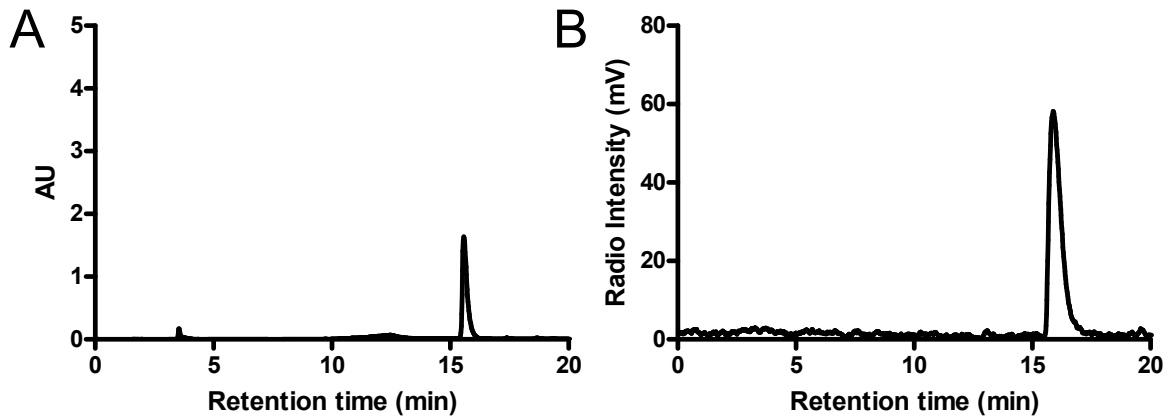

Figure 4. HPLC profiles. A. The UV trace of standard BAP-1. B. The radio trace of the crude reaction mixture of [^{18}F]fluorination of BAP-1.

Reference:

1. C. W. Huang, Z. Li, H. Cai, T. Shahinian and P. S. Conti, *Bioconjug Chem*, 2011, **22**, 256-263.