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Synthesis of a new fluorine-18-labeled bexarotene analogue for PET imaging of retinoid X receptor

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Cancer

ABSTRACT

The reference standard 2-fluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoic acid was synthesized from 2,5-dimethyl-2,5-hexanediol and 2-fluoro-4-methylbenzoic acid in 10 steps with 3% overall chemical yield. The precursor 2-nitro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoic acid was synthesized from 2,5-dimethyl-2,5-hexanediol and dimethyl-2-nitroterephthalate in seven steps with 2% overall chemical yield. The target tracer 2-[^{18}F]fluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoic acid was synthesized from its nitro-precursor by the nucleophilic substitution with K[^{18}F]F/Kryptofix 2.2.2 and isolated by HPLC combined with solid-phase extraction (SPE) purification in 20–30% radiochemical yield with 37–370 GBq/μmol specific activity at end of bombardment (EOB).

Retinoid X receptor (RXR) is a member of nuclear hormone receptor family proteins, and it is closely linked to the apolipoprotein E (APOE), a cholesterol transport protein.[1] RXR plays important roles in the regulation of cellular processes, including transcription of genes, differentiation, and proliferation, and thus it is associated with various uncontrolled cellular proliferation diseases such as cancers, noninsulin-dependent diabetes mellitus (NIDDM) and Alzheimer's disease (AD).[2,3] Allelic variation in APOE gene is the most influential genetic risk factor for sporadic AD.[4] Bexarotene (tradenamed Targretin, binding affinity K_i to RXR 21 nM) is a selective RXR agonist approved by the U.S. Food and Drug Administration (FDA) as an anticancer drug, and it is being explored as a potential drug against Alzheimer's disease (AD) in 3 murine models of AD, because recent reports suggest stimulation of the RXR facilitates β-amyloid clearance across the blood–brain barrier (BBB), although the results remain controversial.[4–13] RXR is an attractive target for the development of therapeutic agents, a novel series of bexarotene analogues have been recently developed as selective RXR agonists, and representative compound 2-fluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoic acid (**15**) exhibited higher binding affinity to RXR (K_i 12 nM) than its parent compound bexarotene.[2] RXR is also a promising target for the development of diagnostic biomarkers. We are interested in the development of AD imaging agents for biomedical imaging technique positron emission tomography (PET). Previous works have targeted β-amyloid and tau-protein, which have resulted in a number of β-amyloid PET tracers such as [^{11}C]PIB[14] and [^{18}F]Amyvid (formerly known as [^{18}F]AV-45),[15] and tau PET tracers such as [^{18}F]-T808,[16] [^{18}F]-T807 ([^{18}F]AV1451)[17] and [^{11}C]PBB3,[18] as indicated in Figure 1. These tracers are currently in different stages of clinical development and commercialization. In this Letter, we target RXR and APOE, and develop a fluorine-18-labeled bexarotene analogue, 2-[^{18}F]fluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoic acid ([^{18}F]**15**), as a new potential PET agent for imaging of RXR and APOE in cancer and AD.

The precursor 2-nitro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoic acid (**14**) and reference standard **15** were synthesized according to the reported procedures with modifications.[2,19,20]

As shown in Scheme 1, 2,5-dimetnyl-2,5-hexanediol was treated with concentrated HCl to give 2,5-dichloro-2,5-dimethylhexane (**1**) in 68% yield. Aluminum trichloride catalyzed Friedel–Crafts alkylation of toluene with dihalide **1** in CH_2Cl_2 provided 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene (**2**) in 85% yield.

As indicated in Scheme 2, partial hydrolysis of dimethyl-2-nitroterephthalate with 1 N NaOH aqueous solution in dioxane

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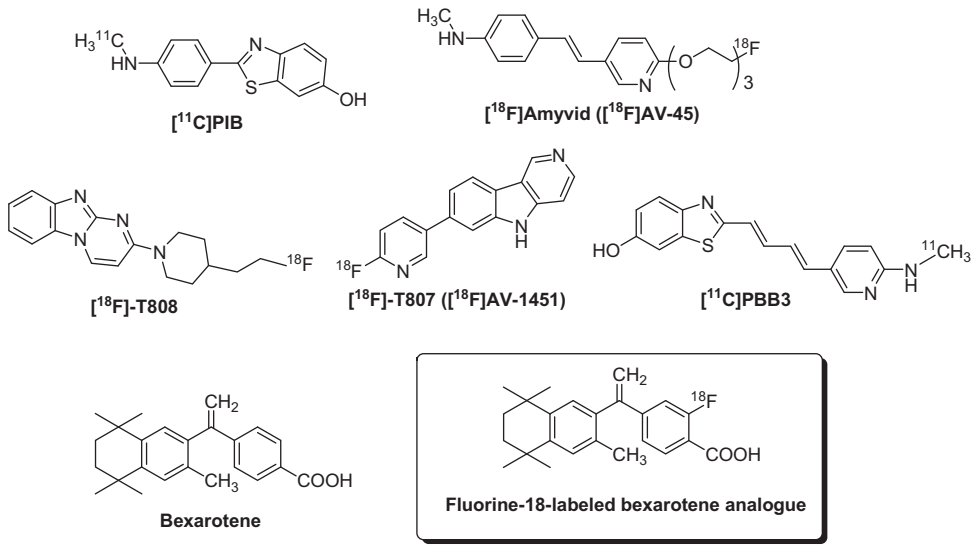
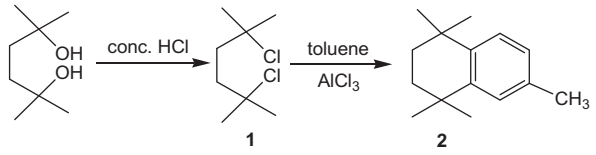
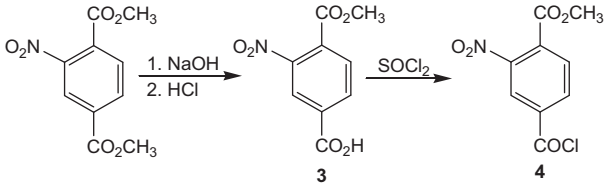


Figure 1. Chemical structure of [^{11}C]PIB, [^{18}F]Amyvid ([^{18}F]AV-45), [^{18}F]-T808, [^{18}F]-T807 ([^{18}F]AV-1451), [^{11}C]PBB3 and fluorine-18-labeled bexarotene analogue.



Scheme 1. Synthesis of an intermediate **2**.



Scheme 2. Synthesis of an intermediate **4**.

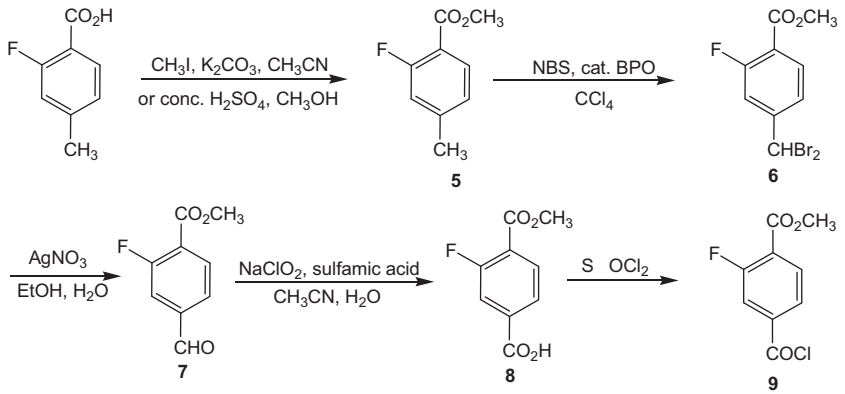
afforded monoacid ester **3** in 51% yield.[21] Transformation of acid **3** to its corresponding acid chloride **4** was accomplished with thionyl chloride in 98% yield.

According to the reported procedure by Kishida and co-workers,[22,23] we prepared methyl 4-(chlorocarbonyl)-2-fluorobenzoate (**9**) in five steps with experimental details as outlined in Scheme 3. Starting from commercially available 2-fluoro-4-methylbenzoic acid, it was esterified to methyl benzoate **5** with CH_3I in the presence of K_2CO_3 in acetonitrile in 79% yield. Another esterification method was achieved by treatment 2-fluoro-4-methylbenzoic acid with MeOH in the presence of concentrated H_2SO_4 as catalyst to afford **5** in 90% yield. Bromination of methyl ester **5** with N-bromosuccinimide (NBS) and catalytic amount of benzoylperoxide (BPO) in CCl_4 to provide dibromides **6**, which was subsequently treated with silver nitrate in EtOH and water to afford aldehyde **7** in 53% yield. Oxidation of aldehyde **7** with sodium hypochlorite in the presence of sulfamic acid in acetonitrile and water to achieve acid **8** in 85% yield, which was converted to acid chloride **9** with thionyl chloride in 97% yield.

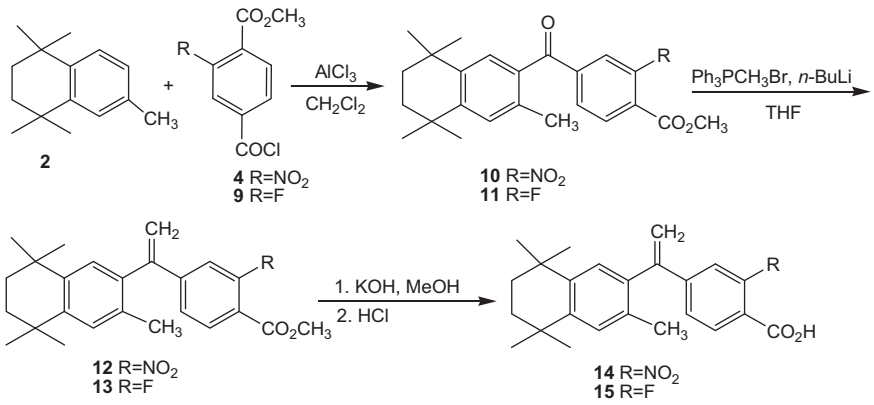
As shown in Scheme 4, aluminum trichloride catalyzed Friedel–Crafts acylation of **2** with acid chloride **4** or **9** in CH_2Cl_2 provided ketones **10** and **11** in 88% and 84% yield, respectively. Ketones **10** and **11** were converted to alkene esters **12** and **13** in 7% and 19% yield, respectively, via Wittig reaction using triphenylphosphonium methylide in THF. The esters **12** and **13** were saponified with 5 N KOH aqueous solution in MeOH, followed by acidification with 2 N HCl aqueous solution to give precursor **14** and standard **15** in 92% and 89% yield, respectively.

The overall chemical yield for the precursor **14** and standard **15** was 3% and 2%, respectively. The main reason resulted in the low overall chemical yields is that the Wittig reaction to convert ketones **10** and **11** to corresponding alkenes **12** and **13** generated multiple undesired side-products, and was difficult to separate desired products **12** and **13** from the reaction mixtures, subsequently the yield for this key step was low (7% and 19%), which significantly contributed to low overall chemical yield for the precursor and standard. Currently there is no available method to increase the yield of this specific step, Wittig reaction, to synthesize **12** and **13**. To improve the overall chemical yield for the precursor and standard, new synthetic approaches need to be developed.

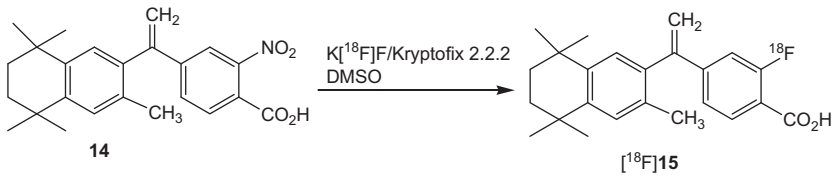
Synthesis of the target tracer [^{18}F]**15** is indicated in Scheme 5. The nitro-precursor **14** was labeled with K[^{18}F]F/Kryptofix 2.2.2 through nucleophilic substitution at 140 °C and isolated by a semi-preparative reverse-phase (RP) high performance liquid chromatography (HPLC) method with C-18 column and a solid-phase extraction SPE method with a C-18 Plus Sep-Pak cartridge (a second purification or isolation process)[24–27] to produce the corresponding pure radiolabeled compound [^{18}F]**15** in 20–30% radiochemical yield, decay-corrected to end of bombardment (EOB), based on H[^{18}F]F. Addition of $NaHCO_3$ to quench the radiolabeling reaction and to dilute the radiolabeling mixture prior to the injection onto the semi-preparative HPLC column for purification gave better separation of labeled product [^{18}F]**15** from its corresponding nitro-precursor **14** and unreacted [^{18}F]fluoride.[24–27] The radiosynthesis was performed using a self-designed automated multi-purpose [^{18}F]-radiosynthesis module.[28–30] The overall synthesis, purification and formulation time was 50–60 min from EOB. The specific activity (SA) was 37–370 GBq/μmol at EOB. SA is defined as the radioactivity per unit mass of a radionuclide or a labeled compound. SA (MBq/mg) = $3.13 \times 10^9/A \times t_{1/2}$, where A is the mass number of the radionuclide, and $t_{1/2}$ is the half-life in hours of the radionuclide. For fluorine-18, carrier-free ^{18}F, maximum (theoretical) ^{18}F SA = 63,381 GBq/μmol.[31] Actual SA of ^{18}F-tracers in our PET chemistry facility is depended on two parts: (1) carrier from the ^{18}F target during the production of H[^{18}F]F, the target we use is Siemens RDS-111 Eclipse cyclotron ^{18}F target; and (2) carrier from



Scheme 3. Synthesis of an intermediate **9**.



Scheme 4. Synthesis of precursor **14** and reference standard **15**.



Scheme 5. Synthesis of target tracer [^{18}F]**15**.

the ^{18}F radiolabeled precursor during the production of K[^{18}F]F/Kryptofix 2.2.2 by azeotropic distillation in our [^{18}F]-radiosynthesis module. Our study has proved that the maximum in-target SA for our ^{18}F-tracers is ~370 GBq/μmol at EOB produced in our cyclotron and ^{18}F-radiosynthesis unit. The SA for our ^{18}F-tracers usually ranges from 37 to 370 GBq/μmol at EOB according to our previous works for the ^{18}F-tracers produced in this facility, including [^{18}F]Fallypride, [^{18}F]PBR06, [^{18}F]FEDAA1106, [^{18}F]-T808, etc.[24–27,30] Theoretically, all ^{18}F-tracers have same SA, and actual SA of ^{18}F-tracers is mainly related to the type of cyclotron (^{18}F targetry conditions) and synthetic module.[31] The SA of the title tracer was in the range of 37–370 GBq/μmol at EOB, which was similar to the values previously reported by our lab.[24–27,30] Although this SA range is big, it is normal, because with ^{18}F-tracers where 'cold' fluorine (^{19}F) can be introduced at various points in the radiosynthesis, SA can vary by several orders of magnitude between syntheses of the same tracer even at the same institution.[31] SA is arguably one of the most important parameters in radiopharmaceutical development, consequently various techniques have been developed to increase SA.[31] No-carrier-added [^{18}F]fluoride ion in [^{18}O]water was trapped without a QMA cartridge. This way[24–27,30,32] significantly increased the SA of the prepared F-18 labeled product. As indicated in the literature,[32] when the cyclotron-produced [^{18}F]fluoride ion was dried without the use of a cartridge, but through cycles of evaporation with added acetonitrile, the SA of the prepared [^{18}F]**15** was substantially higher, and was similar to that we have achieved in the radiosynthesis of other F-18 tracers such as [^{18}F]fallypride and [^{18}F]PBR06 previously reported.[24–27,30] The reason was that there was a low-level contamination of QMA anionic resins with fluoride ion.[32] The amounts of the nitro-precursor used were ~1 mg. Small amount of the precursor was used for radiolabeling instead of large amount of the precursor (3 mg) reported in the literature,[16,17] which improved the chemical purity of the final tracer solution. A large amount of precursor would increase the radiochemical yield, but decrease the chemical purity of [^{18}F]**15** tracer solution due to precursor contamination. In addition, a large amount of precursor would also decrease the SA of final labeled product due to potential F-18/F-19 exchange during the radiolabeling. The reaction solvent and temperature were either CH_3CN/120 °C or DMSO/140 °C. Radiolabeling procedure with DMSO at 140 °C resulted in higher radiochemical yield.[24–27,30,33] To our F-18 labeling experiences on

F-18 tracers,[24–27,30] although the HPLC systems we employed have shown good separation of products from precursors, there always was a co-elution of the F-18 labeled product with its corresponding precursor from the HPLC column, very tiny amount of the precursor (0.1–0.4 μg/mL) contaminating the tracer solution. [^{18}F]**15** was also in the same situation. Therefore, we used a C-18 Plus Sep-Pak cartridge for this purpose to further remove the precursor and most of possible non-radiolabeled undesired side-products. A C-18 Plus Sep-Pak cartridge instead of rotatory evaporation was used to significantly improve the chemical purity of the tracer solution.[24–27,30] In this study, the Sep-Pak purification further increased the chemical purity more than 10%.[24–27,30] Chemical purity and radiochemical purity were determined by analytical HPLC.[34] The chemical purity of nitro-precursor **14** and fluoro-standard **15** was >93% determined by HPLC through UV flow detector. The radiochemical purity of the target tracer [^{18}F]**15** was >98% determined by radio-HPLC through γ-ray (PIN diode) flow detector.

The experimental details and characterization data for compounds **1**–**15** and for the tracer [^{18}F]**15** are given.[35]

In summary, a practical synthetic route to nitro-precursor, fluoro-standard and a fluorine-18-labeled bexarotene analogue [^{18}F]**15** has been developed. An automated self-designed multi-purpose [^{18}F]-radiosynthesis module for the synthesis of [^{18}F]**15** has been built. The radiosynthesis employed nucleophilic substitution of the nitro-precursor with K[^{18}F]F/Kryptofix 2.2.2. The target tracer was isolated and purified by a semi-preparative HPLC combined with SPE procedure in relatively high radiochemical yields, shortened overall synthesis time, and high specific activity and radiochemical purity. New and improved results in the synthetic methodology, radiolabeling, preparative separation and analytical details for nitro-precursor, fluoro-standard and a fluorine-18-labeled bexarotene analogue have been presented. These methods are efficient and convenient. It is anticipated that the approaches for the design, synthesis and automation of new tracer, authentic standard and radiolabeling precursor, and improvements to increase radiochemical yield, chemical purity and specific activity of the tracer described here can be applied with advantage to the synthesis of other ^{18}F-radiotracers for PET imaging. These results facilitate the potential preclinical and clinical PET studies of a fluorine-18-labeled bexarotene analogue for imaging of RXR and APOE in cancer and AD in animals and humans.

Acknowledgments

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34. Zheng, Q.-H.; Mock, B. H. *Biomed. Chromatogr.* **2005**, *19*, 671.
35. (a) *General:* All commercial reagents and solvents were purchased from Sigma–Aldrich and Fisher Scientific, and used without further purification. Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Bruker Avance II 500 MHz NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (ppm, δ scale) relative to internal standard TMS (δ 0.0), and coupling constants (J) were reported in hertz (Hz). Liquid chromatography–mass spectra (LC–MS) analysis was performed on an Agilent system, consisting of an 1100 series HPLC connected to a diode array detector and a 1946D mass spectrometer configured for positive-ion/negative-ion electrospray ionization. Chromatographic solvent proportions are indicated as volume: volume ratio. Thin-layer chromatography (TLC) was run using Analtech silica gel GF uniplates (5×10 cm^2). Plates were visualized under UV light. Preparative TLC was run using Analtech silica gel UV 254 plates (20×20 cm^2). Normal phase flash column chromatography was carried out on EM Science silica gel 60 (230–400 mesh) with a forced flow of the indicated solvent system in the proportions described below. All moisture- and air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. Analytical RP HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6×250 mm; mobile phase 25% EtOH/75% 0.1 N NH_4OAc; flow rate 1.0 mL/min; and UV (254 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative RP HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 12 nm, 10×250 mm; mobile phase 20% CH_3CN/80% 0.1 N NH_4OAc; 4.0 mL/min flow rate; UV (254 nm) and γ-ray (PIN diode) flow detectors. C18 Plus Sep-Pak cartridges were obtained from Waters Corporation (Milford, MA). Sterile Millex-FG 0.2 μm filter units were obtained from Millipore Corporation (Bedford, MA).

*(b) 2,5-Dichloro-2,5-dimethylhexane (**1**):* Concentrated HCl (400 mL) was added to 2,5-dimethyl-2,5-hexanediol (50.0 g, 432 mmol) dropwise with gentle swirling. The diol flakes gradually dissolved and a white precipitate formed simultaneously. The reaction mixture was stirred at room temperature (RT) for 2 h; the white precipitate was filtered off and was washed with water until pH ~7 followed by cold EtOH. The solid was dried under vacuum to afford **1** as a white solid (42.5 g, 68%), mp 63–65 C (lit.[2] 63–65.8 C, lit.[19] 63–65 °C). ^{1}H NMR ($CDCl_3$): δ 1.95 (s, 4H), 1.60 (s, 12H).

*(c) 1,1,4,4,6-Pentamethyl-1,2,3,4-tetrahydronaphthalene (**2**):* To a 1000 mL round-bottomed flask fitted with water condenser were added compound **1** (30.0 g, 165 mmol), toluene (35.1 mL, 330 mmol) and CH_2Cl_2 (150 mL). To this vigorously stirred solution was added aluminum chloride (1.92 g, 1.4 mmol) slowly in portionwise, which resulted in rapid evolution of gaseous hydrochloride acid. The reaction mixture was stirred at RT for 30 min followed by additional aluminum chloride (400 mg). After the reaction mixture was stirred and heated to reflux for 15 min, it was cooled in an ice bath and quenched with 20% HCl aqueous solution (150 mL). The mixture was extracted with hexanes; the combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by column chromatography with hexanes to afford **2** as a white solid (28.3 g, 85%), mp 34–35 °C (lit.[2] 34–36 °C). ^{1}H NMR ($CDCl_3$): δ 7.40 (d, J = 8.0 Hz, 1H), 7.31 (s, 1H), 7.14 (d, J = 8.0 Hz, 1H), 2.49 (s, 3H), 1.87 (s, 4H), 1.48(s, 6H), 1.47 (s, 6H).

*(d) 4-(Methoxycarbonyl)-3-nitrobenzoic acid (**3**):* To a stirred solution of dimethyl-2-nitroterephthalate (24.0 g, 100.4 mmol) in dioxane (200 mL) was added 1 N NaOH aqueous solution (100 mL) dropwise. The reaction mixture was stirred at RT overnight, water (200 mL) was added and the yellow solution was washed with diethyl ether (200 mL) twice. The aqueous layer was acidified with 1 N HCl aqueous solution (120 mL) until pH ~1, it was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was recrystallized in water to afford **3** as a white solid (11.6 g, 51%), mp 177–179 °C (lit.[2] 177–179 °C). ^{1}H NMR (DMSO-d_6): δ 13.9 (br s, 1H), 8.46 (d, J = 1.5 Hz, 1H), 8.34 (dd, J = 8.0, 1.5 Hz, 1H), 7.99 (d, J = 7.5 Hz, 1H), 3.89 (s, 3H).

*(e) Methyl 4-(chlorocarbonyl)-2-nitrobenzoate (**4**):* A suspension of compound **3** (10.0 g, 44.4 mmol) in thionyl chloride (100 mL, 1.37 mol) was stirred and heated to reflux for 4 h. Excess thionyl chloride was removed in vacuo, the residual was dissolved in benzene (30 mL) and evaporated to dryness three times to remove residual thionyl chloride. The crude product was dried under vacuum to afford **4** as a pale yellow solid (10.6 g, 98%), which was used directly for next step.

*(f) Methyl 2-fluoro-4-methylbenzoate (**5**):* Method A: To a stirred mixture of 2-fluoro-4-methylbenzoic acid (2.0 g, 13.0 mmol), K_2CO_3 (3.6 g, 26.0 mmol) in acetonitrile (15 mL) was added CH_3I (1.6 mL, 25.6 mmol) dropwise. After the reaction mixture was heated to reflux overnight, the solvent was removed in vacuo. The residual was diluted with EtOAc, washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by column chromatography with hexanes/EtOAc (6:1) to afford **5** as a white solid (1.73 g, 79%), mp 51–53 °C. ^{1}H NMR ($CDCl_3$): δ 7.83 (t, J = 8.0 Hz, 1H), 7.00 (dd, J = 8.0, 1.0 Hz, 1H), 6.95 (d, J = 12.0 Hz, 1H), 3.91 (s, 3H), 2.39 (s, 3H). Method B: To a stirred solution of 2-fluoro-4-methylbenzoic acid (7.0 g, 45.4 mmol) in MeOH (100 mL) was added concentrated sulfuric acid (5 mL) dropwise. The reaction mixture was heated to reflux overnight; the solvent was removed in vacuo. The residual was diluted with EtOAc, washed with saturated $NaHCO_3$ aqueous solution and brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by column chromatography with hexanes/EtOAc (6:1) to afford **5** as a white solid (6.88 g, 90%). Analytical data were same as above.

*(g) Methyl 4-(dibromomethyl)-2-fluorobenzoate (**6**):* To a stirred solution of compound **5** (7.5 g, 44.6 mmol) in CCl_4 (60 mL) was added NBS (19.1 g, 107.1 mmol), followed by benzoylperoxide (721 mg, 2.23 mmol). After the reaction mixture was heated to reflux for 36 h, it was cooled to RT. The solid was filtered off and washed with CCl_4. The combined filtrates were concentrated in vacuo to afford the crude product **6**, which was dried under vacuum and used directly for next step.

*(h) Methyl 2-fluoro-4-formylbenzoate (**7**):* To a stirred and preheated solution (50 °C) of compound **6** (44.6 mmol) in EtOH (125 mL) was added silver nitrate (15.9 g, 93.7 mmol) in warm water (22 mL) dropwise at 50 °C. Upon addition of the silver nitrate solution, a green precipitate formed. After the reaction mixture was stirred at 50 °C for 1 h, it was cooled to RT and the green precipitate was filtered off. The filtrate was concentrated in vacuo, and extracted with EtOAc. The combined layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by column chromatography with hexanes/EtOAc (from 12:1 to 8:1) to afford **7** as a white solid (4.67 g, 53%), mp 75–76 C. ^{1}H NMR ($CDCl_3$): δ 10.05 (d, J = 1.5 Hz, 1H), 8.11 (t, J = 7.5 Hz, 1H), 7.73 (dd, J = 8.0, 1.5 Hz, 1H), 7.65 (dd, J = 10.0, 1.5 Hz, 1H), 3.98(s, 3H).

*(i) 3-Fluoro-4-(methoxycarbonyl)benzoic acid (**8**):* To a stirred solution of compound **7** (4.37 g, 24.0 mmol) and sulfamic acid (2.56 g, 26.4 mmol) in acetonitrile (30 mL) and water (15 mL) was added a solution of 80% $NaClO_2$ (2.9 g, 15.6 mmol) in water (15 mL) dropwise. After the reaction mixture was stirred at RT for 2 h, it was poured into saturated Na_2SO_3 solution (35 mL) and 1 N HCl aqueous solution (70 mL), and extracted with EtOAc. The combined layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was washed with small amount of hexanes/EtOAc (5:1) solution to afford **8** as a white solid (4.06 g, 85%), mp 211–212 C (lit.[2] 210–211 °C). ^{1}H NMR (DMSO-d_6): δ 13.65 (br s, 1H), 8.00 (t, J = 7.5 Hz, 1H), 7.86 (dd, J = 8.0, 1.0 Hz, 1H), 7.77 (dd, J = 11.5, 1.0 Hz, 1H), 3.89 (s, 3H).

*(j) Methyl 4-(chlorocarbonyl)-2-fluorobenzoate (**9**):* A suspension of compound **8** (3.0 g, 15.2 mmol) in thionyl chloride (30 mL, 411 mmol) was stirred and heated to reflux for 4 h. Excess thionyl chloride was removed in vacuo, the residual was dissolved in benzene (10 mL) and evaporated to dryness three times to remove residual thionyl chloride. The crude product was dried under vacuum to afford **9** as a white solid (3.18 g, 97%), which was used directly for next step.

*(k) Methyl 2-nitro-4-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalene-2-carbonyl)benzoate (**10**):* To a 250 mL round-bottomed flask fitted with water condenser were added compound **2** (6.0 g, 29.7 mmol), compound **4** (7.57 g, 31.16 mmol) and CH_2Cl_2 (60 mL). To this vigorously stirred solution, aluminum chloride (11.64 g, 87.3 mmol) was added slowly in portionwise which resulted in rapid evolution of gaseous hydrochloride acid. The reaction mixture was stirred at RT for 5 min followed by refluxing for an additional 30 min to give a red solution. After the mixture was poured into ice and acidified with 2 N HCl aqueous solution (55 mL), it was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by column chromatography with hexanes/EtOAc (from 50:3 to 50:5) to afford **10** as a pale yellow solid (10.7 g, 88%), mp 115–116 °C (lit.[2] 115–116 °C). ^{1}H NMR ($CDCl_3$): δ 8.33 (d, J = 1.5 Hz, 1H), 8.10 (dd, J = 8.0, 1.5 Hz, 1H), 7.83 (d, J = 8.0, 1.0 Hz, 1H), 7.26 (s, 1H), 7.25 (s, 1H), 3.97 (s, 3H), 2.38 (s, 3H), 1.70 (s, 4H), 1.32 (s, 6H), 1.21 (s, 6H).

*(l) Methyl 2-fluoro-4-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalene-2-carbonyl)benzoate (**11**):* To a 100 mL round-bottomed flask fitted with water condenser were compound **2** (2.03 g, 10.1 mmol), compound **9** (2.0 g, 9.3 mmol) and CH_2Cl_2 (25 mL). To this vigorously stirred solution, aluminum chloride (3.03 g, 22.7 mmol) was added slowly in portionwise which resulted in rapid evolution of gaseous hydrochloride acid. The reaction mixture was stirred at RT for 5 min followed by refluxing for an additional 30 min to give a red solution. After the mixture was poured into ice and acidified with 2 N HCl aqueous solution (15 mL), it was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by column chromatography with hexanes/EtOAc (25:2) to afford **11** as a white solid (2.99 g, 84%), mp 105–107 °C (lit.[2] 105–106 °C). ^{1}H NMR ($CDCl_3$): δ 8.01 (t, J = 7.5 Hz, 1H), 7.60 (dd, J = 8.0, 1.5 Hz, 1H), 7.57 (dd, J = 10.5, 1.0 Hz, 1H), 7.26 (s, 1H), 7.22 (s, 1H), 3.97 (s, 3H), 2.35 (s, 3H), 1.70 (s, 4H), 1.32 (s, 6H), 1.21 (s, 6H).

*(m) Methyl 2-nitro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoate (**12**):* To a suspension of methyltriphenylphosphonium bromide (3.0 g, 8.4 mmol) in THF (5 mL) was added a 2.5 M solution of *n*-butyl lithium in hexanes (4.5 mL, 3.72 mmol). The red solution was stirred at RT for 30 min to give a triphenylphosphonium methylide solution. The triphenylphosphonium methylide solution was added to a solution of compound **10** (2.24 g, 5.46 mmol) in THF (10 mL) dropwise. After the reaction mixture was stirred at RT for 5 h, it was pour into ice water and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by column chromatography with hexanes/EtOAc (10:1) followed by preparative TLC plates with hexanes/EtOAc (4:1) to afford **12** as a pale yellow solid (147 mg, 7%). ^{1}H NMR ($CDCl_3$): δ 7.77 (d, J = 2.0 Hz, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.50 (dd, J = 8.0, 1.5 Hz, 1H), 7.10 (s, 1H), 7.09 (s, 1H), 5.88 (s, 1H), 5.43 (s, 1H), 1.96 (s, 3H), 1.70 (s, 4H), 1.31 (s, 6H), 1.27 (s, 6H).

*(n) Methyl 2-fluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoate (**13**):* To a suspension of methyltriphenylphosphonium bromide (1.0 g, 2.8 mmol) in THF (2 mL) was added a 2.5 M solution of *n*-butyl lithium in hexanes (1.5 mL, 3.72 mmol). The red solution was stirred at RT for 30 min to give a triphenylphosphoniu methylide solution. The triphenylphosphoniu methylide solution was added to a solution of compound **11** (694 mg, 1.82 mmol) in THF (3 mL) dropwise. After the reaction mixture was stirred at RT for 1 h, it was pour into ice water and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by preparative TLC plates with hexanes/EtOAc (97:3) to afford **13** as a white solid (130 mg, 19%), mp 131–133 °C (lit.[2] 130–132 C). ^{1}H NMR ($CDCl_3$): δ 7.86 (t, J = 8.0 Hz, 1H), 7.13 (dd, J = 8.0, 1.5 Hz, 1H), 7.10 (s, 1H), 7.08 (s, 1H), 7.01 (dd, J = 12.5, 1.5 Hz, 1H), 5.82 (s, 1H), 5.35 (s, 1H), 3.92 (s, 3H), 1.95 (s, 3H), 1.70 (s, 4H), 1.30 (s, 6H), 1.27 (s, 6H).

*(o) 2-Nitro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoic acid (**14**):* To a stirred solution of compound **12** (100 mg, 0.25 mmol) in MeOH (3 mL) was added 5 N KOH aqueous solution (0.12 mL, 0.61 mmol). After the reaction mixture was stirred and heated to reflux for 2 h, it was cooled and acidified with 2 N HCl aqueous solution, and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was triturated with cold hexanes, and filtered. The solid was washed with small amount of cold hexanes to afford **14** as a pale yellow solid (89.1 mg, 92%), mp 210–212 °C (lit.[2] 212–214 °C). ^{1}H NMR ($CDCl_3$): δ 7.85 (d, J = 8.5 Hz, 1H), 7.70 (d, J = 1.0 Hz, 1H), 7.52 (dd, J = 8.0, 1.5 Hz, 1H), 7.11 (s, 1H), 7.10 (s, 1H), 5.91(s, 1H), 5.46 (s, 1H), 1.97 (s, 3H), 1.70 (s, 4H), 1.31 (s, 6H), 1.27 (s, 6H). LC–MS (ESI, m/z): calcd for $C_{24}H_{31}N_2O_4$ ($[M+NH_4]^+$) 411.2, found 411.0; calcd for $C_{24}H_{26}NO_4$ ($[M-H]^-$) 392.2, found 392.0.

*(p) 2-Fluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoic acid (**15**):* To a stirred suspension of compound **13** (50 mg, 0.13 mmol) in MeOH (1 mL) was added 5 N KOH aqueous solution (0.07 mL, 0.33 mmol). After the reaction mixture was stirred and heated to reflux for 2 h, it was cooled and acidified with 2 N HCl aqueous solution, and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was washed with small amount of cold hexanes to afford **15** as a white solid (42.8 mg, 89%), mp 212–214 °C (lit.[2] 212–214 °C). ^{1}H NMR ($CDCl_3$): δ 7.95 (t, J = 8.0 Hz, 1H), 7.17 (dd, J = 8.0, 1.5 Hz, 1H), 7.10 (s, 1H), 7.09 (s, 1H), 7.05 (dd, J = 12.0, 1.5 Hz, 1H), 5.85 (s, 1H), 5.39 (s, 1H), 1.96 (s, 3H), 1.70 (s, 4H), 1.31 (s, 6H), 1.28 (s, 6H). LC–MS (ESI, m/z): calcd for $C_{24}H_{31}FNO_2$ ($[M+NH_4]^+$) 384.2, found 384.0; calcd for $C_{24}H_{26}FO_2$ ($[M-H]^-$) 365.2, found 365.0.

*(q) 2-[^{18}F]fluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoic acid ([^{18}F]**15**):* No-carrier-added (NCA) aqueous H[^{18}F]F was produced by ^{18}O(p,n)^{18}F nuclear reaction using a Siemens Eclipse RDS-111 cyclotron by irradiation of $H_2{}^{18}O$ (2.5 mL). H[^{18}F]F (7.4–18.5 GBq) in [^{18}O]water plus 0.1 mL K_2CO_3 solution (1.7 mg) and Kryptofix 2.2.2 (10 mg) in 1.0 mL CH_3CN with additional 1 mL CH_3CN were placed in the fluorination reaction vial (10-mL V-vial) and repeated azeotropic distillation (17 min) was performed at 110 °C to remove water and to form the anhydrous K[^{18}F]F-Kryptofix 2.2.2 complex. The precursor **14** (1 mg) dissolved in DMSO (1.0 mL) was introduced to the reaction vessel and heated at 140 °C for 15 min for radiofluorination. The contents of the reaction vial were cooled down to ~100 °C and diluted with 0.1 M $NaHCO_3$ (1 mL), and injected onto the semi-preparative HPLC column with 3 mL injection loop for purification. The product fraction was collected in a recovery vial containing 30 mL water. The diluted tracer solution was then passed through a C-18 Sep-Pak Plus cartridge, and washed with water (5 mL × 4). The cartridge was eluted with EtOH (1 mL × 2) to release [^{18}F]**15**, followed by saline (10 mL). The eluted product was then sterile-filtered through a Millex-FG 0.2 μm membrane into a sterile vial. Total radioactivity was assayed and total volume was noted for tracer dose dispensing. Retention times in the semi-preparative HPLC system were: t_R **14** = 12.03 min, t_R **15** = 8.12 min, t_R [^{18}F]**15** = 8.12 min. Retention times in the analytical HPLC system were: t_R **14** = 7.89 min, t_R **15** = 5.61 min, t_R [^{18}F]**15** = 5.61 min. The decay corrected radiochemical yields of [^{18}F]**15** were 20–30%.