Synthesis Protectin D1 Analogs: Novel Pro-resolution and Radiotracer Agents

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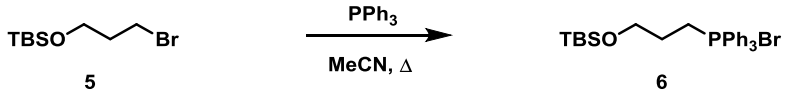
General Information

All commercially available reagents and solvents were used in the form they were supplied without any further purification. The stated yields are based on isolated material. Melting points were measured using a Barnstead Electrothermal IA9200 melting point apparatus. The melting points are uncorrected. Thin layer chromatography was performed on silica gel 60 F_{254} aluminum-backed plates fabricated by Merck. Flash column chromatography was performed on silica gel 60 (40 - 63 μm) fabricated by Merck. NMR spectra were recorded on a Bruker AVII DPX 300 spectrometer at 300 MHz, Bruker AVII 400 or a Bruker AVIII HD 400 spectrometer at 400 MHz or a Bruker AVII600 spectrometer at 600 MHz for ^{1}H NMR, and at 75 MHz, 100 MHz or 150 MHz respectively for ^{13}C NMR. Coupling constants (*J*) are reported in hertz and chemical shifts are reported in parts per million (δ) relative to the central residual protium solvent resonance in ^{1}H NMR ($CDCl_3$ = δ 7.27, $(CD_3)_2CO$ = δ 2.05 and CD_3OD = δ 3.31) and the central carbon solvent resonance in ^{13}C NMR ($CDCl_3$ = δ 77.00 ppm, CD_3OD = δ 49.00). Mass spectra were recorded at 70 eV on Micromass Prospec Q or Micromass QTOF 2W spectrometer using ESI as the method of ionization. High resolution mass spectra were recorded at 70 eV on Micromass Prospec Q or Micromass QTOF 2W spectrometer using ESI as the method of ionization. Optical rotations were measured using a 0.7 mL cell with a 1.0 dm path length on an Anton Paar MCP 100 polarimeter. HPLC analyses were performed using a C_{18} stationary phase (Eclipse XDB-C18, 4.6 x 250 mm, particle size 5 μm, from Agilent Technologies), applying the conditions stated. The UV/Vis spectrum from 190-900 nm was recorded using a Agilent Technologies Cary 8485 UV-VIS spectrophotometer using quartz cuvettes.

Bromo(3-((*tert*-butyldimethylsilyl)oxy)propyl)triphenyl-l5-phosphane (6)

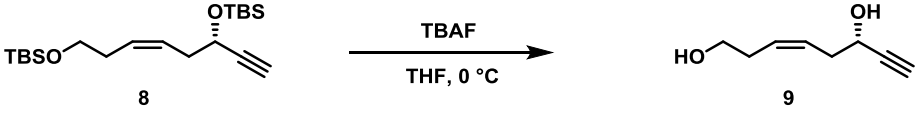


Bromide **5** (1.00 g, 3.94 mmol, 1.00 equiv.) was dissolved in dry MeCN (15 mL) and stirred under argon. Triphenylphosphine (2.63 g, 10.05 mmol, 2.55 equiv.) was added and the reaction mixture was heated to reflux for 12 h. The reaction mixture was concentrated *in vacuo*. The crude product was purified by column chromatography on silica (CH_2Cl_2/MeOH 95:5) to afford the title compound **8** as a white salt. Yield: 2.03 g (83%). All spectroscopic and physical data were in full agreement with those reported in the literature.[1] **TLC** (CH_2Cl_2/MeOH 95:5): $\boldsymbol{R_f}$ = 0.26. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 7.89 – 7.75 (m, 9H), 7.74 – 7.64 (m, 6H), 3.96 – 3.80 (m, 4H), 1.97 – 1.83 (m, 2H), 0.85 (s, 9H), 0.03 (s, 6H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 135.1 (d, $^{4'}J_{CP}$ = 3.0 Hz), 133.9 (d, $^{3'}J_{CP}$ = 9.9 Hz), 130.6 (d, $^{2'}J_{CP}$ = 12.5 Hz), 118.6 (d, $^{1'}J_{CP}$ = 86.1 Hz), 61.9 (d, ^{3}J = 16.9 Hz), 26.2 (d, $^{2}J_{CP}$ = 4.1 Hz), 26.1 (3C), 19.1 (d, $^{1}J_{CP}$ = 52.6 Hz), 18.3, 5.2 (2C). **Mp**. 136.7 – 138.9 ˚C.

(*S*,*Z*)-5-Ethynyl-2,2,3,3,12,12,13,13-octamethyl-4,11-dioxa-3,12-disilatetradec-7-ene (8)

Wittig salt **6** (374 mg, 0.654 mmol, 1.00 equiv.) was dissolved in THF (8.80 mL) and HMPA (0.88 mL), cooled to −78 °C and added NaHMDS (0.6 M in toluene, 1.09 mL, 0.654 mmol, 1.00 equiv.). The reaction mixture was stirred for 45 min before aldehyde **7**[2] (200 mg, 65.5 µmol, 1.00 equiv.) in THF (0.88 mL) was added dropwise. The reaction was stirred for 1 h at −78 °C and then the reaction mixture was allowed to slowly warm to 0 °C. Phosphate buffer (4.7 mL, pH = 7.2) was added to quench the reaction and the aq. phase was extracted with Et_2O (2 × 4.0 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (heptane/EtOAc 95:5, $KMnO_4$ stain) to afford compound **8** as a pale yellow oil. Yield: 188 mg (78%). **TLC** (hexanes/EtOAc 95:5, CAM stain): **Rf** = 0.17. $\boldsymbol{[\alpha]_D^{20}}$= -15.0 (c = 1.25, $CHCl_3$; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 5.59 – 5.49 (m, 2H), 4.34 (td, *J* = 6.5, 2.1 Hz, 1H), 3.61 (t, *J* = 7.0 Hz, 2H), 2.45 (t, *J* = 6.0 Hz, 2H), 2.38 (d, *J* = 2.1 Hz, 1H), 2.30 (q, *J* = 6.7 Hz, 2H), 0.90 (s, 9H), 0.89 (s, 9H), 0.13 (s, 3H), 0.11 (s, 3H), 0.07 (s, 3H), 0.05 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 128.7, 126.3, 85.5, 72.3, 63.0, 62.8, 36.8, 31.5, 26.1 (3C), 25.9 (3C), 18.5, 18.4, -4.5, -4.9, -5.1 (2C). **HRMS** (TOF ES+): Exact mass calculated for $C_{20}H_{40}O_2Si_2Na$ $[M+Na]^+$: 391,2464, found 391.2474.

(*S*,*Z*)-oct-3-en-7-yne-1,6-diol (9)



TBAF (2.10 mL 1.0 M in THF, 2.10 mmol, 5.00 equiv.) was added to a solution of **8** (155 mg, 0.420 mmol, 1.00 equiv.) in THF (5.0 mL) at 0 °C. The reaction was stirred for 20 h before it was quenched with phosphate buffer (pH = 7.2, 3.0 mL). Brine (5 mL) and CH_2Cl_2 (10 mL) were added and the phases were separated. The aq. phase

was extracted with CH_2Cl_2 (2 x 10 mL). The combined organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by column chromatography on silica (hexanes/EtOAc 4:6) to afford the title compound **9** as a pale yellow oil. **Yield**: 54 mg (92%). All spectroscopic and physical data were in full agreement with those reported in the literature.[3] **TLC** (hexanes/EtOAc 4:6, CAM stain): $\boldsymbol{R_f}$ = 0.17. $\boldsymbol{[\alpha]_D^{20}}$ = -9 (c = 0.2, MeOH). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.72 – 5.58 (m, 2H), 4.46 – 4.41 (m, 1H), 3.67 (t, *J* = 5.9 Hz, 2H), 3.46 (bs, 1H), 2.60 (bs, 1H), 2.55 – 2.49 (m, 2H), 2.46 (d, *J* = 2.2 Hz, 1H), 2.38 – 2.31 (m, 2H); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 130.3, 126.9, 84.6, 73.0, 61.7, 61.4, 35.4, 30.7.

Conditions for HPLC analyses

Conditions I used for analyses of compounds 4, 11 and 12:

HPLC analyses for chemical purities were performed on an Agilent Technologies 1200 Series instrument with diode array detector set at 254 nm and equipped with a C18 stationary phase (Eclipse XDB-C18 5 µm 4.6 × 150 mm) using MeOH:H_2O (65:35) as eluent with flow at 1.0 mL/min.

Conditions II used for analyses of compound 4:

HPLC analyses for analysis of compound **4** in connection with radiolabelling experiments were performed on an Agilent Technologies 1200 Series instrument with diode array detector set at 271 nm and an ACE C18-AR column (3 µm, 50 × 4.6 mm) at a flow rate 1 mL/min.

Conditions III used for radiochemical HPLC analyses of compounds 4 and [^{18}F]4:

Radiochemical HPLC analyses were performed on an Agilent Technologies 1200 Series instrument coupled a Gabi star NaI-radiodetector and were performed using an isocratic eluent of H_2O:MeOH (30:70) with diode array detector set at 27 nm and an ACE C18-AR column (3 µm, 50 × 4.6 mm) at a flow rate 1 mL/min.

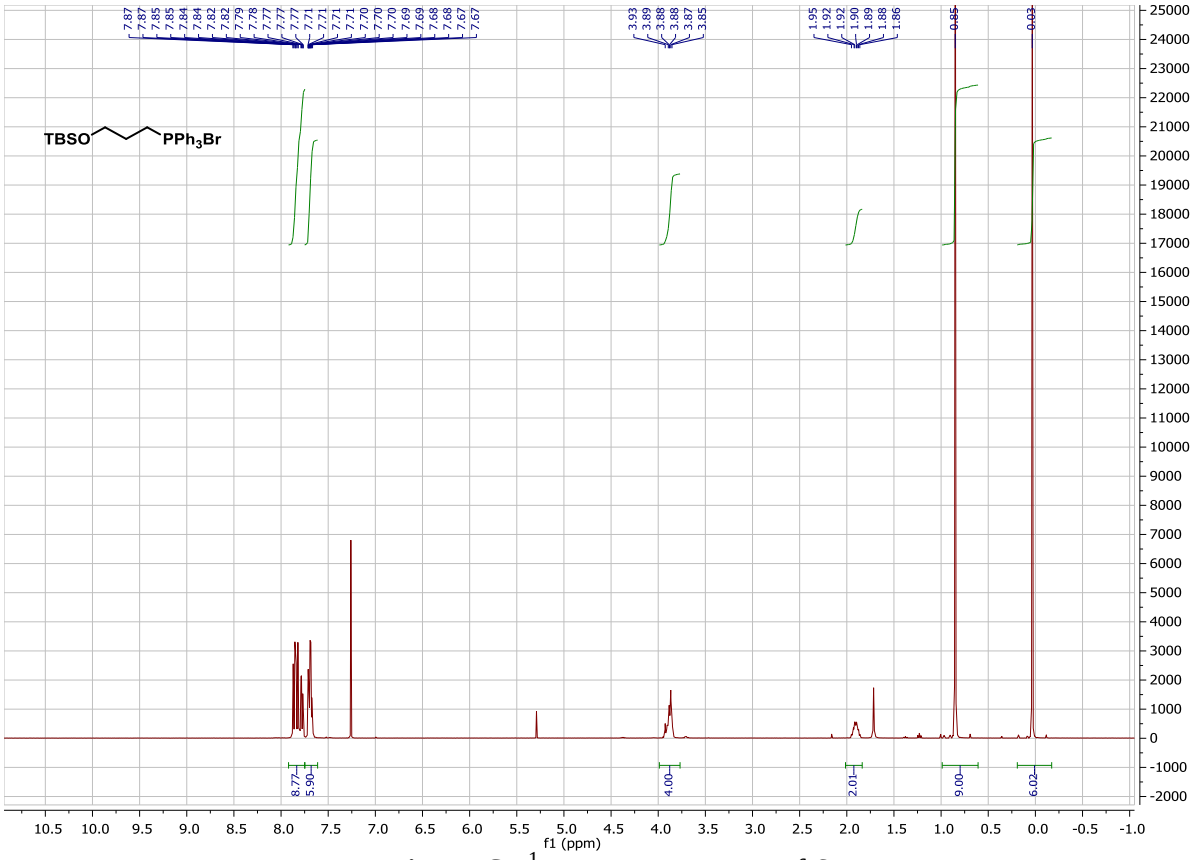


Figure S1 ^{1}H-NMR spectrum of **6**.

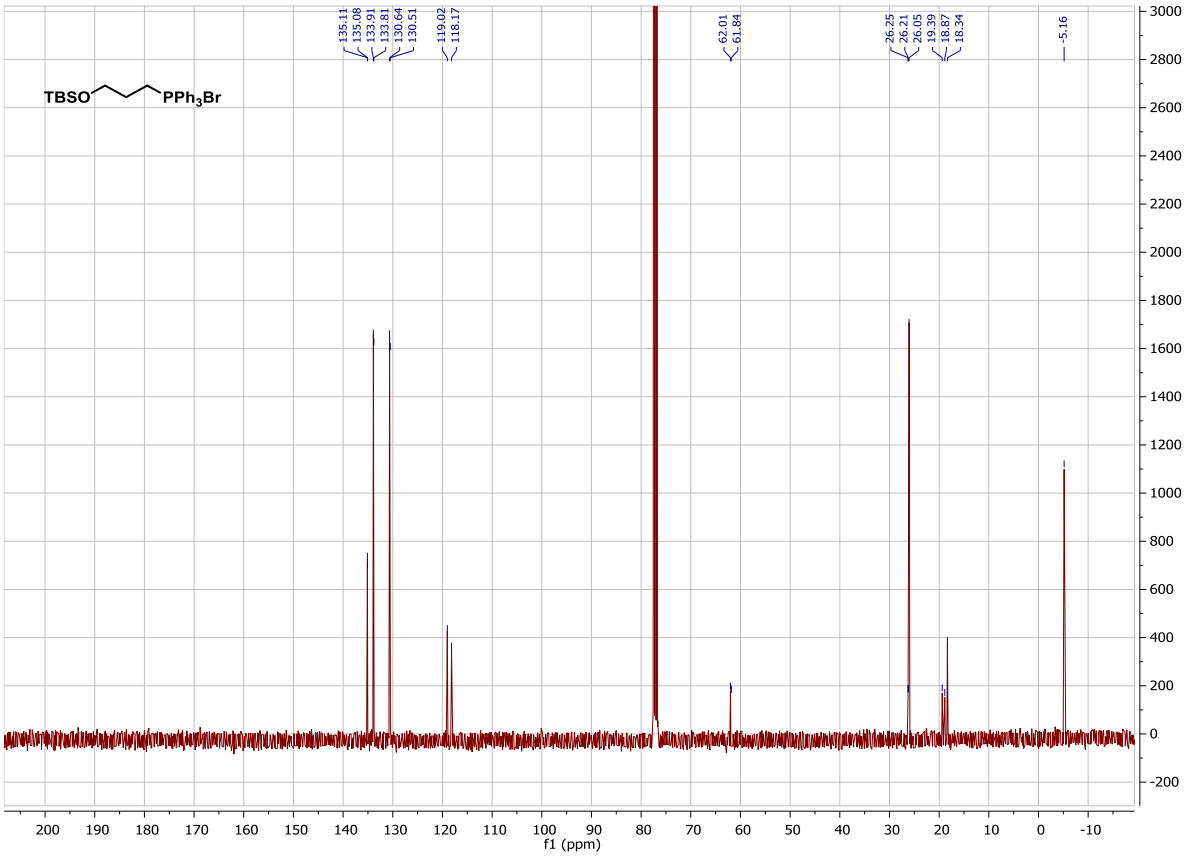


Figure S2 ^{13}C-NMR spectrum of **6**.

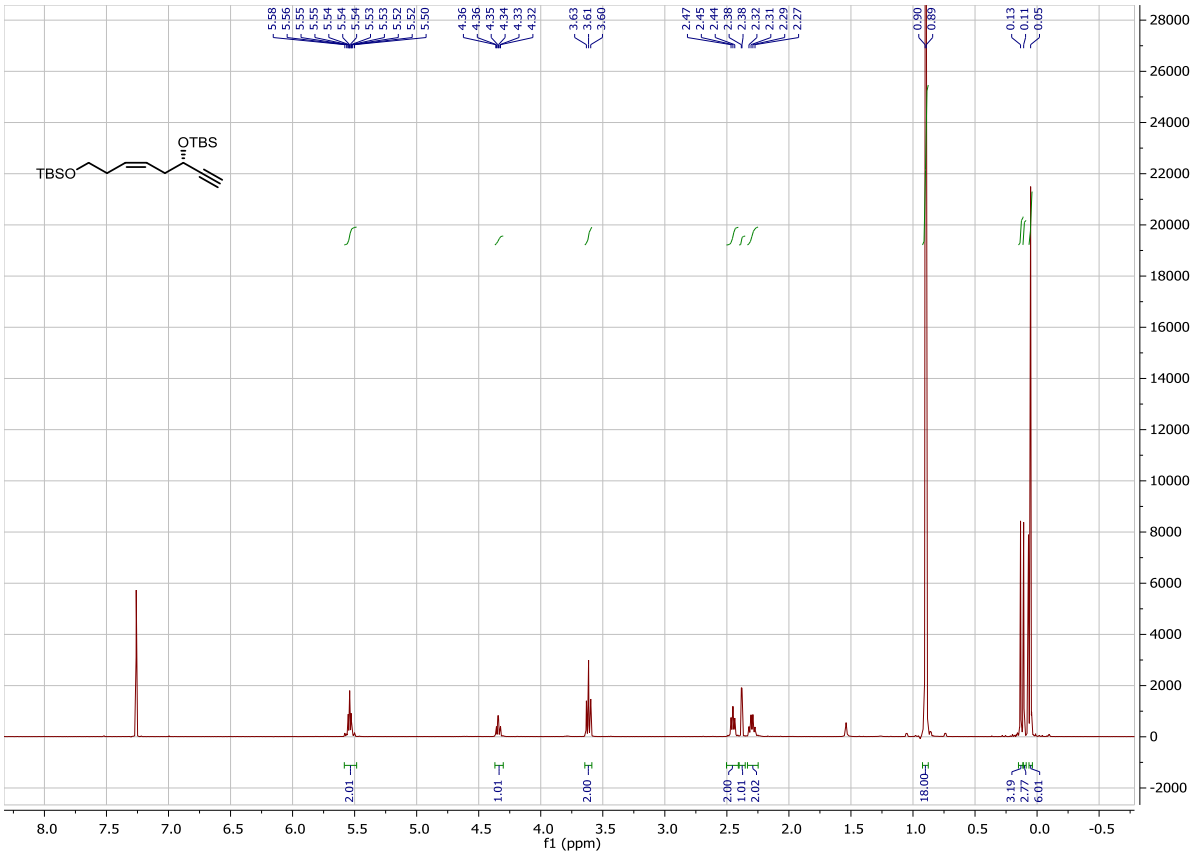


Figure S3 ^{1}H-NMR spectrum of **8**.

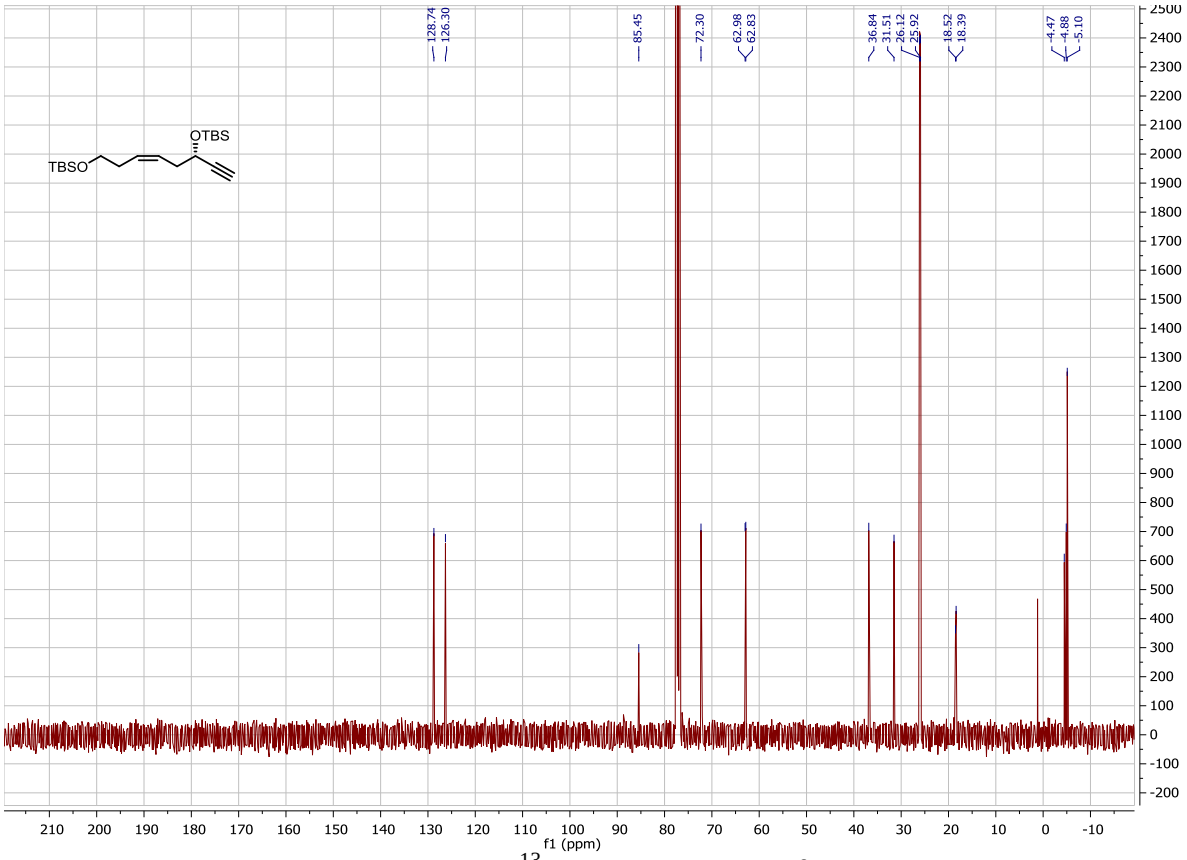


Figure S4 ^{13}C-NMR spectrum of **8**.

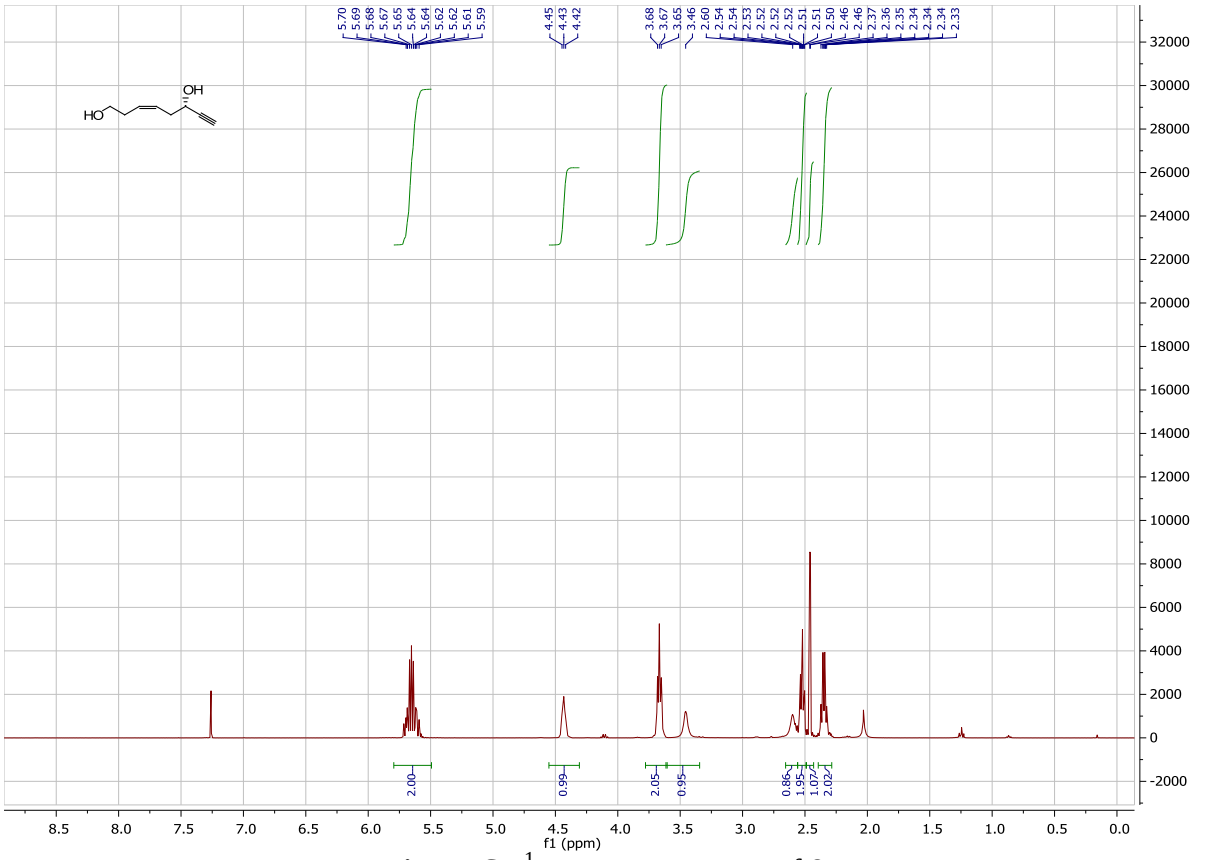


Figure S5 ^{1}H-NMR spectrum of **9**.

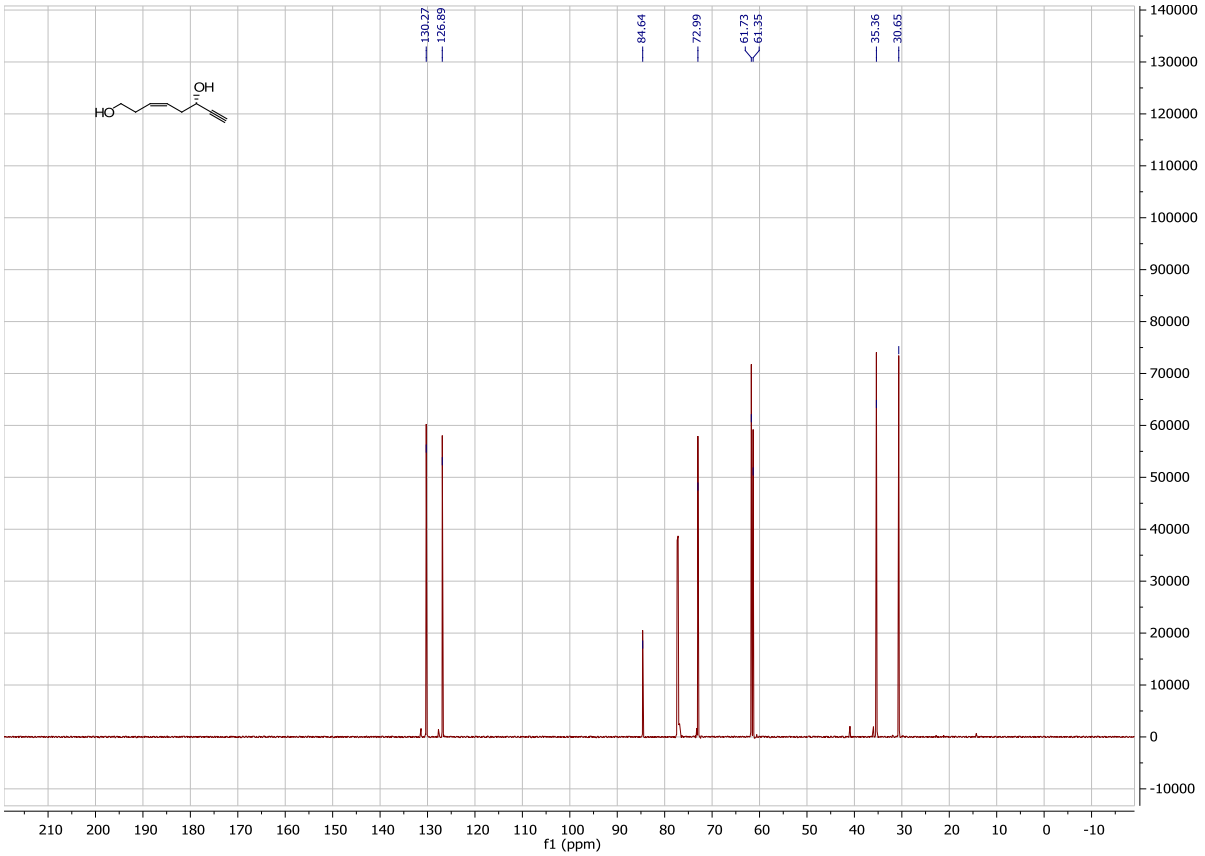


Figure S6 ^{13}C-NMR spectrum of **9**.

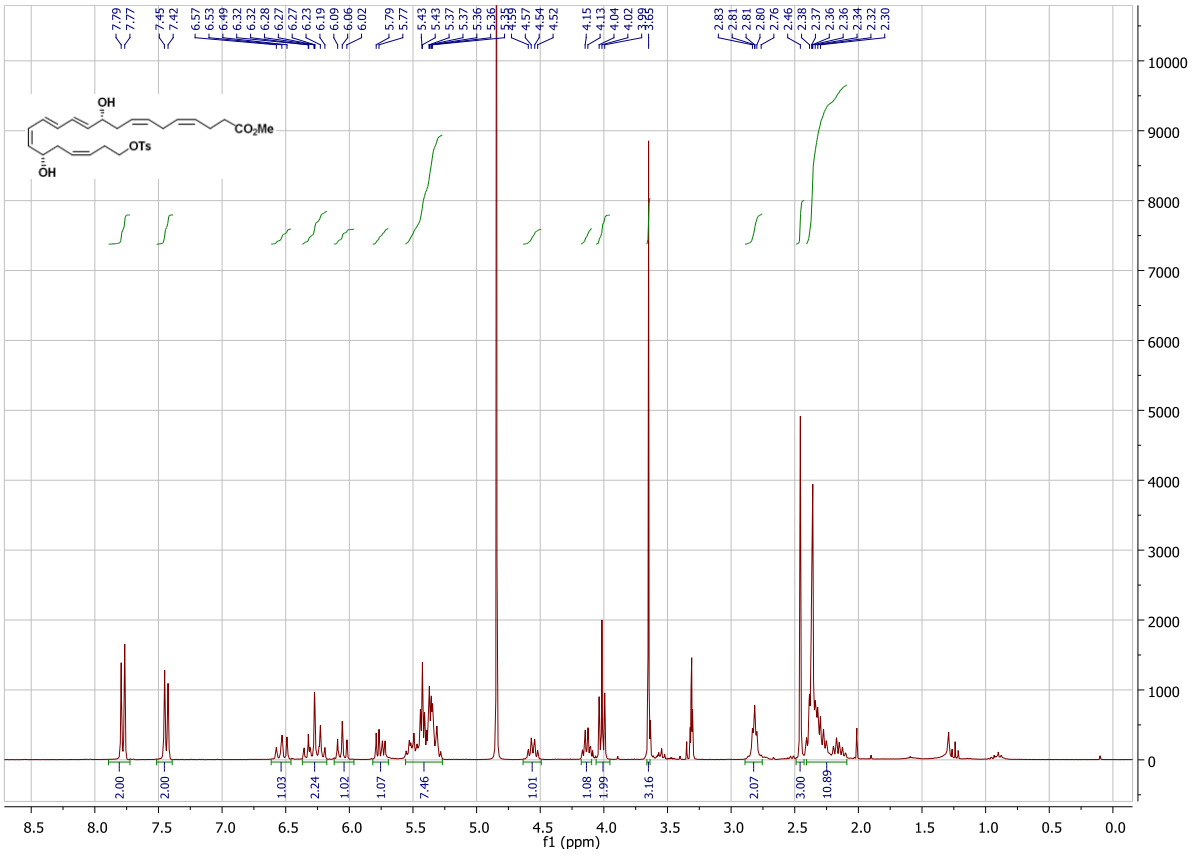


Figure S7 ^{1}H-NMR spectrum of methyl ester **12**.

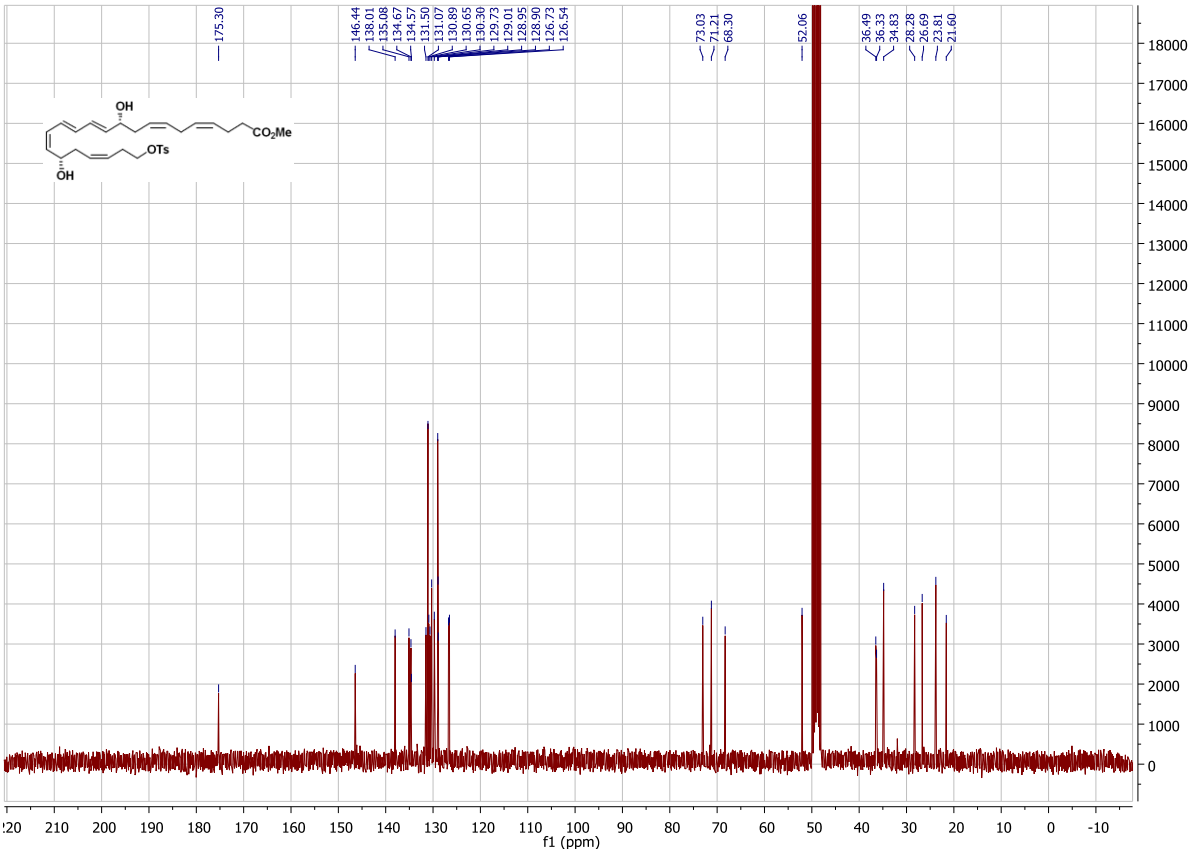


Figure S8 ^{13}C-NMR spectrum of methyl ester **12**.

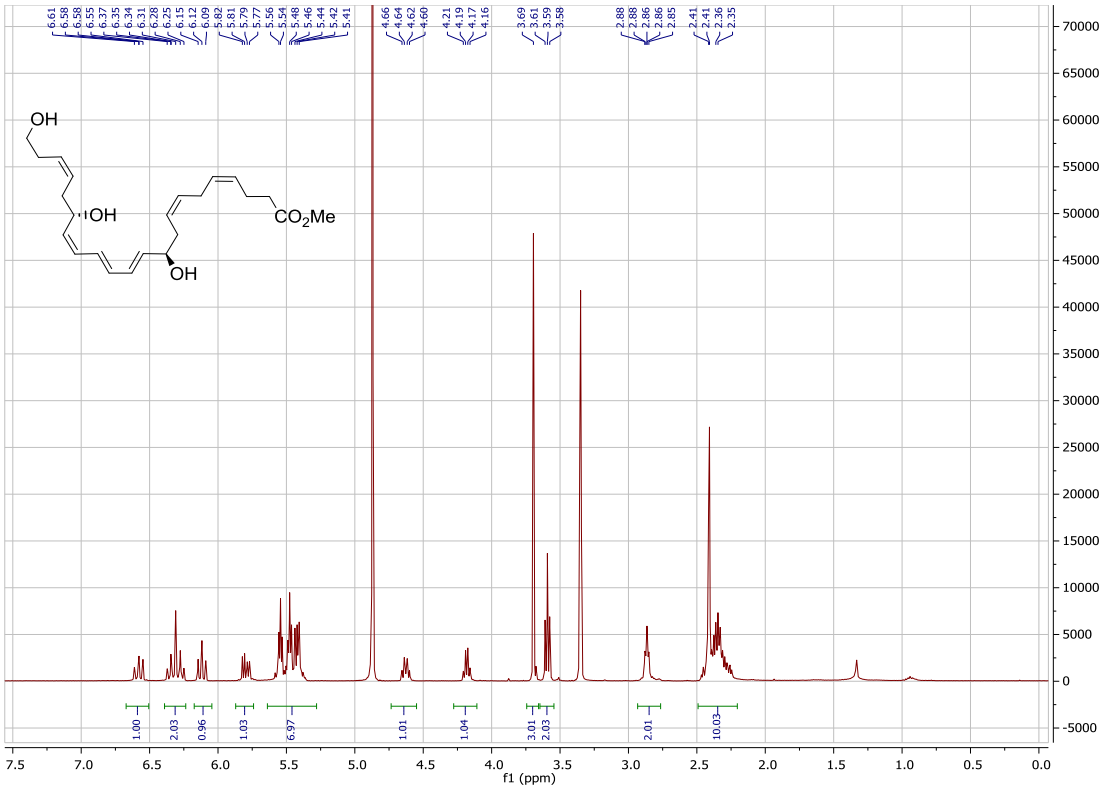


Figure S9 ^{1}H-NMR spectrum of methyl ester **11**. See reference 4 for comparison.

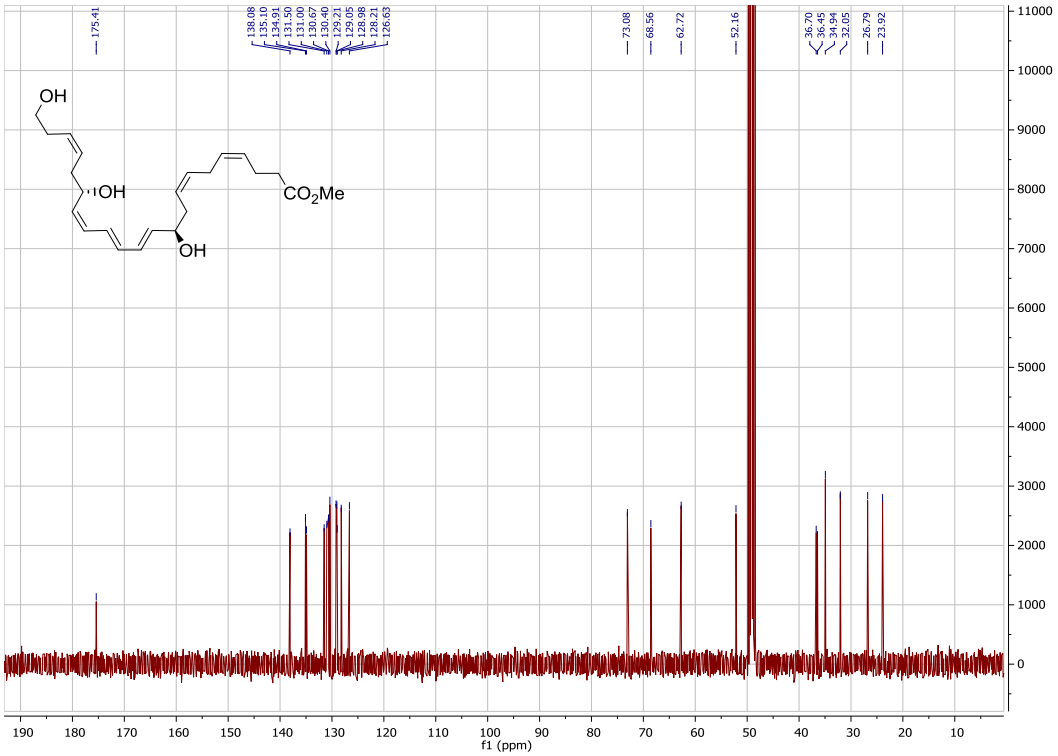


Figure S10 ^{13}C-NMR spectrum of methyl ester **11**. See reference 4 for comparison.

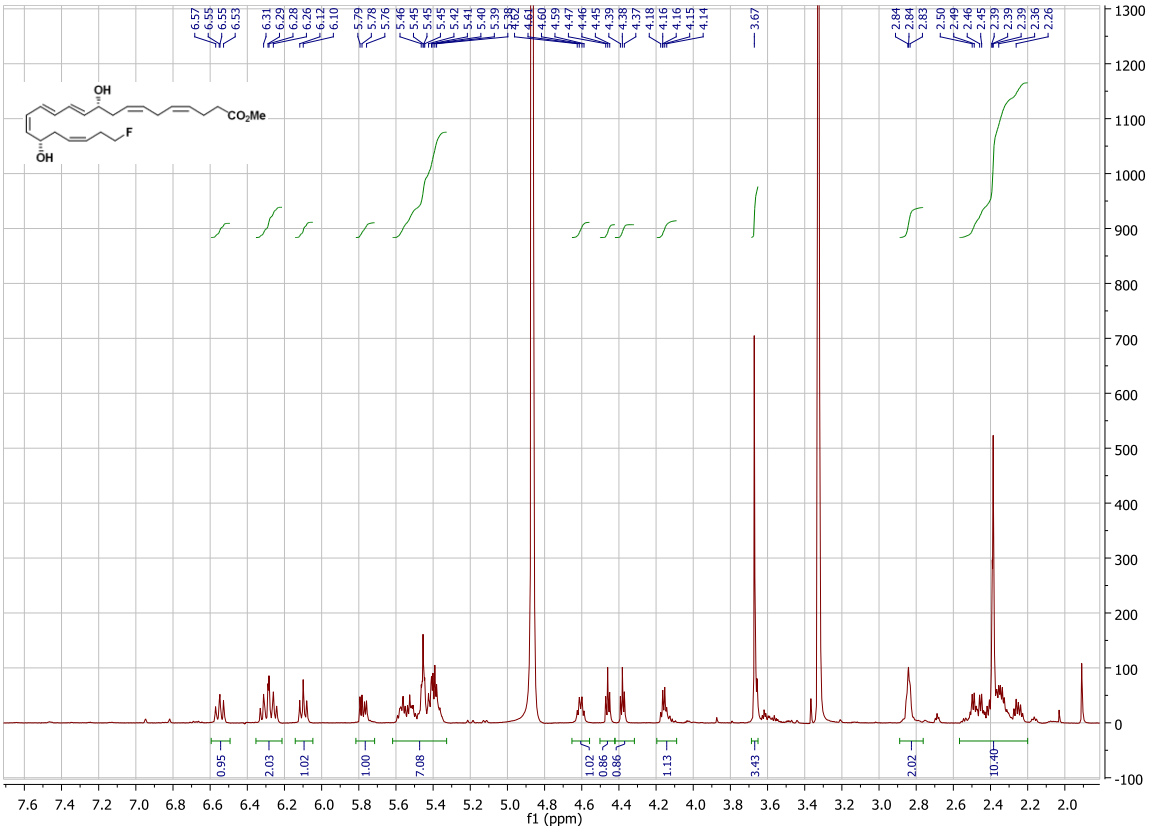


Figure S11 ^{1}H-NMR spectrum of 22-F-PD1-ME (**4**).

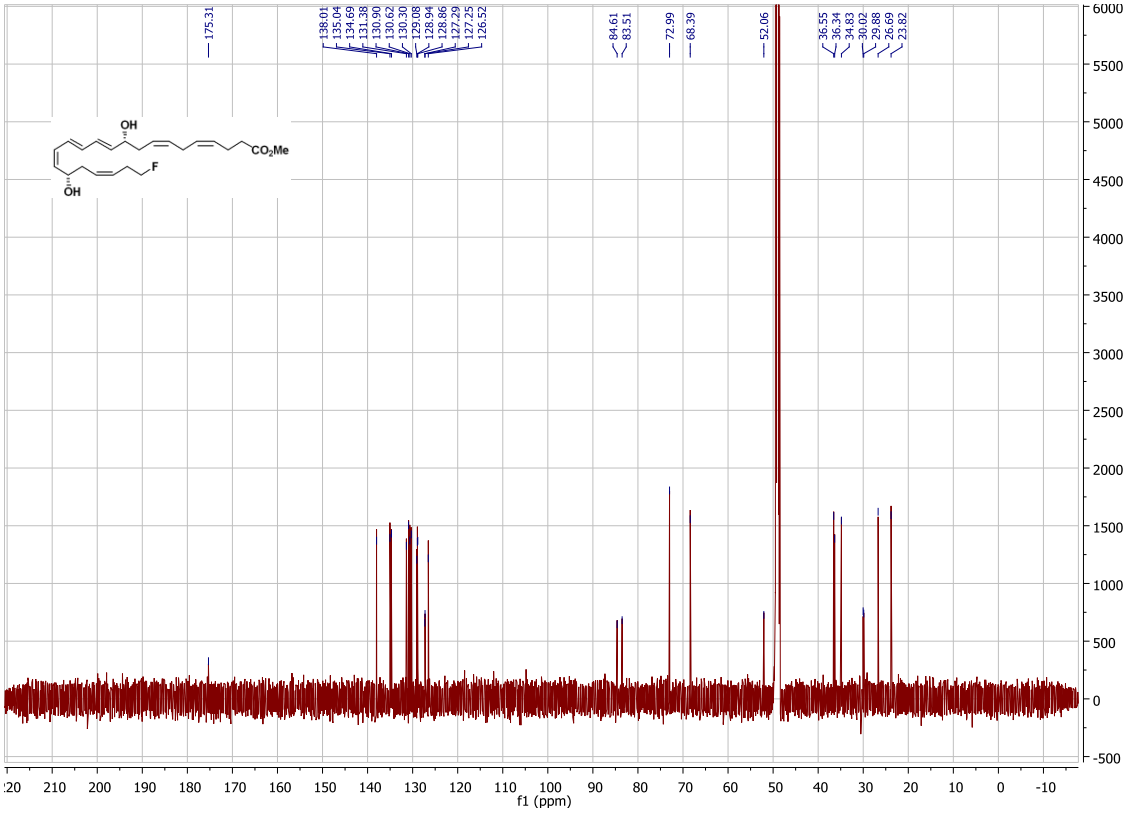
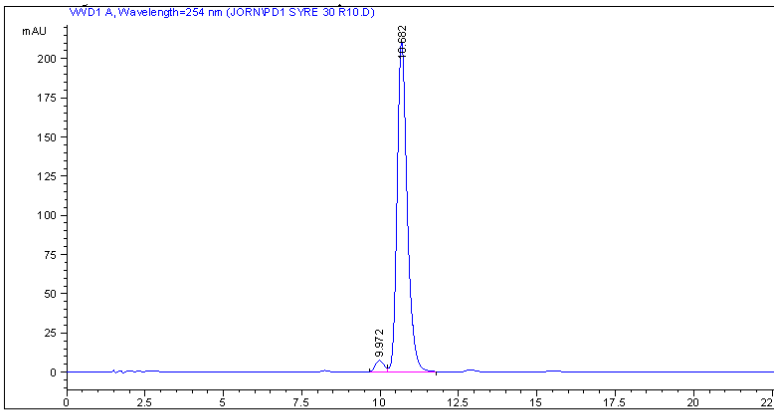


Figure S12 ^{13}C-NMR spectrum of 22-F-PD1-ME (**4**).

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Dilution              :      1.0000
Sample Amount         :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
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   2  10.682 MM T  0.4291 4714.59863  210.86153  97.1043

Totals :                  4855.19153  218.05592
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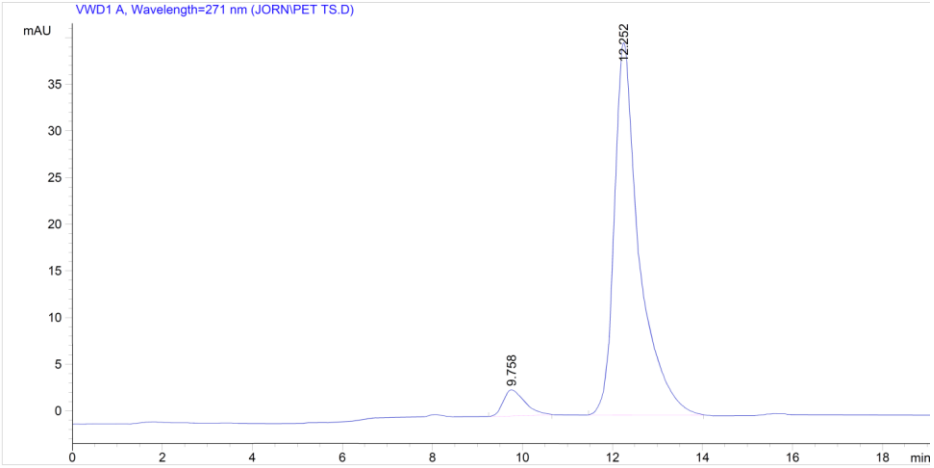
Figure S13 HPLC chromatogram of methyl ester **11** using conditions I**.**

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Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=271 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s   [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1   9.758 BB    0.5009   93.51983    2.80662   5.7729
   2  12.252 BB    0.5495 1526.45715   39.91669  94.2271

Totals :                  1619.97698   42.72331

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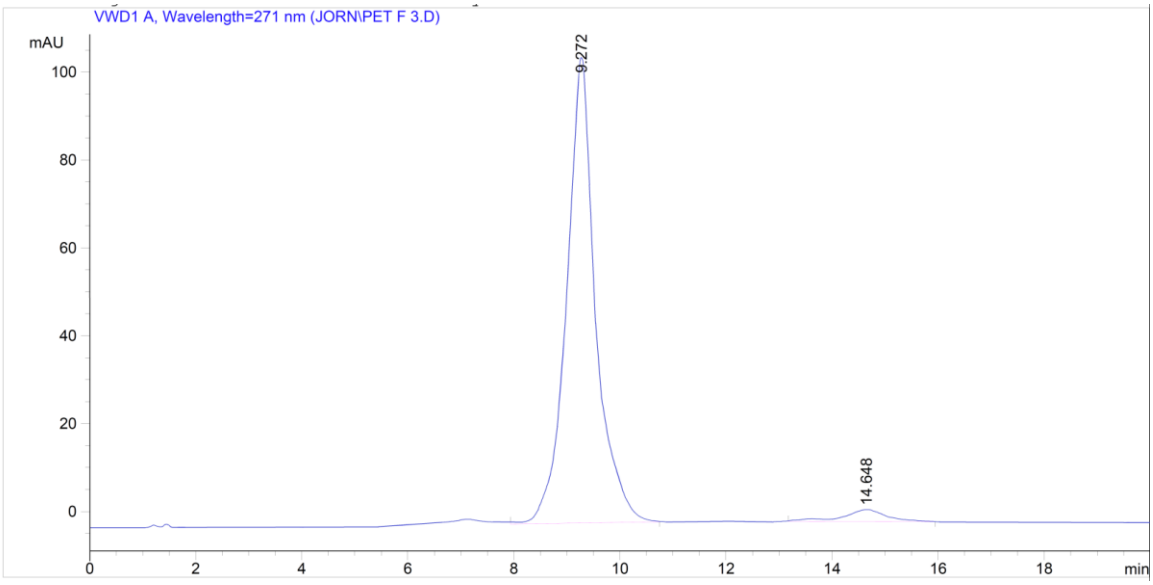
Figure S14 HPLC chromatogram of methyl ester **12** using conditions I.

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Dilution              :      1.0000
Sample Amount         :      1.00000  [ng/ul]    (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=271 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1   9.272 VB    0.5347 3995.23657  105.92757  96.1854
   2  14.648 BB    0.8145  158.44466    2.73383   3.8146

Totals :                  4153.68123  108.66139


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Figure S15 HPLC chromatogram of 22-F-PD1-ME (**4**) using conditions I.

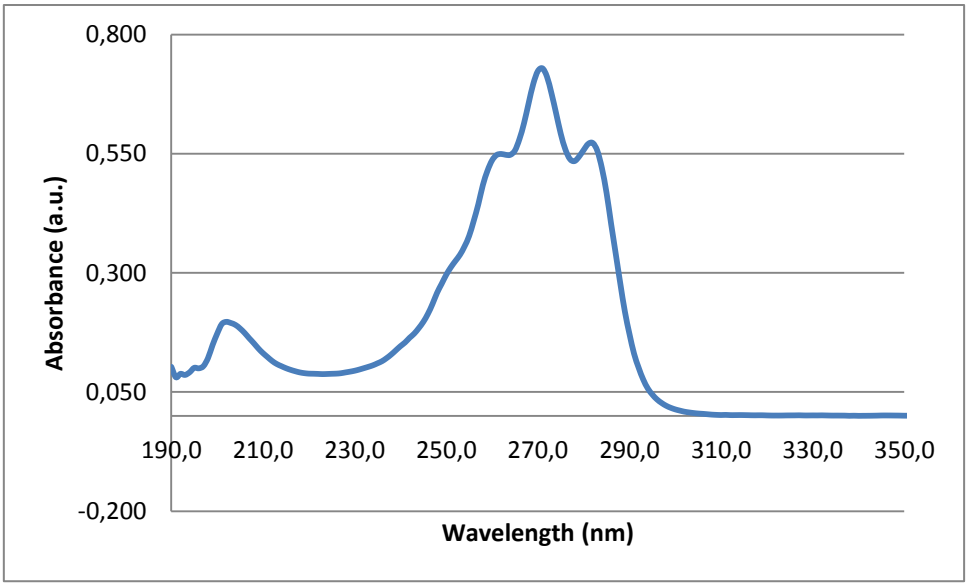


Figure S16 UV/Vis chromatogram of 22-F-PD1-ME (**4**).

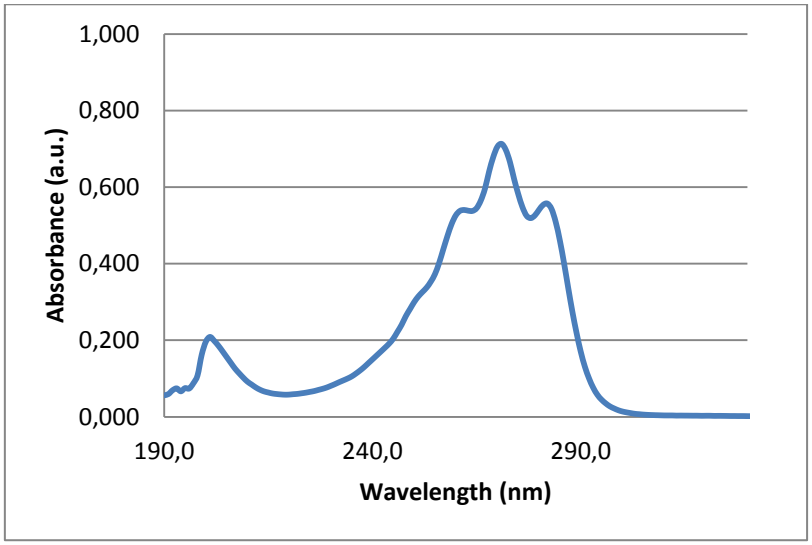
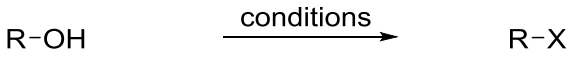
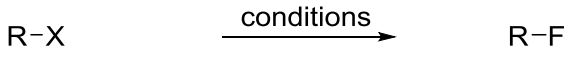


Figure S17 UV/Vis chromatogram of **11**. See reference 4 for comparison.

Table S1. Reaction conditions attempted in making 22-F-PD1-ME (**4**).



Entry	Conditions	X	Yield
1	MsCl, NEt_3, CH_2Cl_2	Ms	23
2	TsCl, pyridine	Ts	82
3	Tf_2O, lutidine, CH_2Cl_2	Tf	trace



Entry	X	Conditions	Yield
1	Ts	KF, DMSO	trace
2	Ts	KF, K222, MeCN	85
3	Ms	KF, K222, MeCN	46

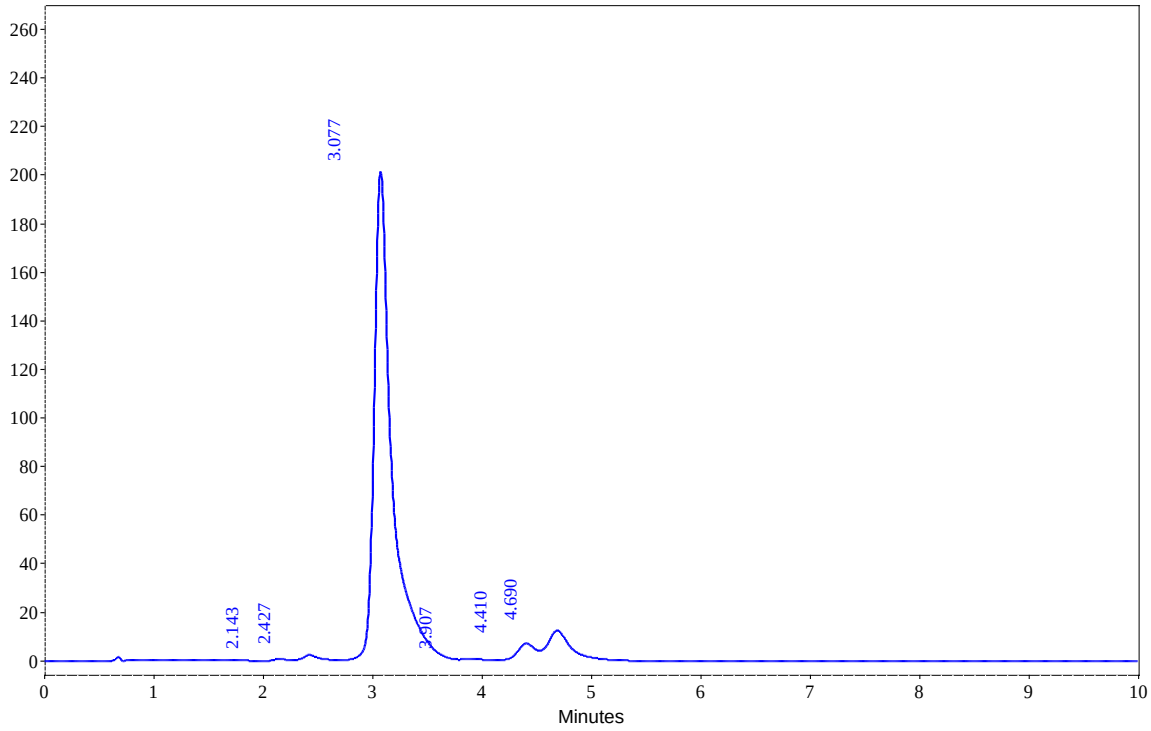


Figure S18 HPLC chromatogram of 22-F-PD1-ME (**4**) (271 nm) using conditions II**.**

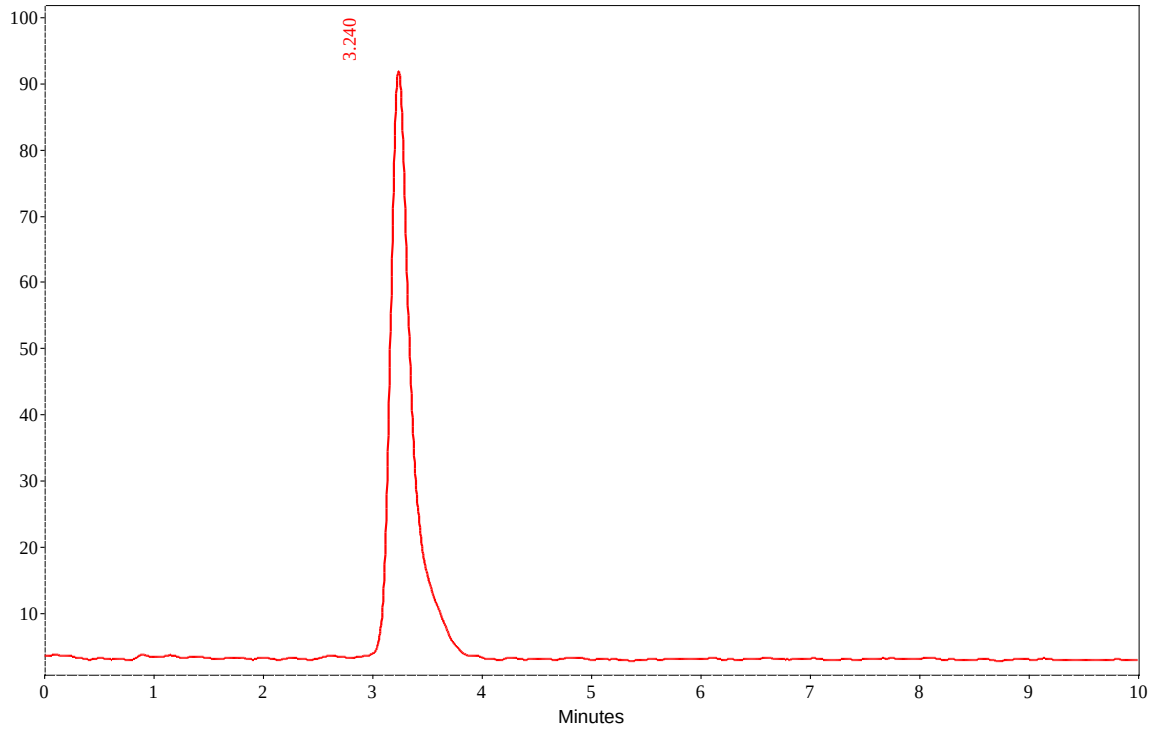


Figure S19 Radio-HPLC chromatogram of purified 22-[^{18}F]F-PD1-ME (**4**) using conditions III**.**

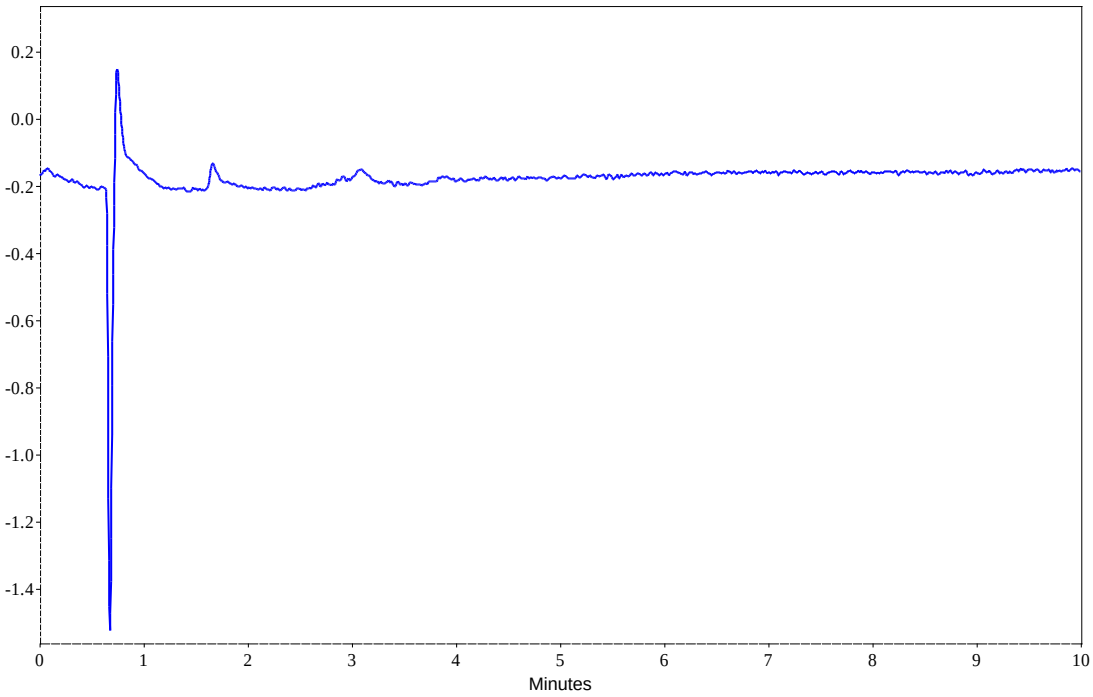


Figure S20 HPLC of purified 22-[^{18}F]F-PD1-ME (**4**) recorded at 271 nm using conditions III.

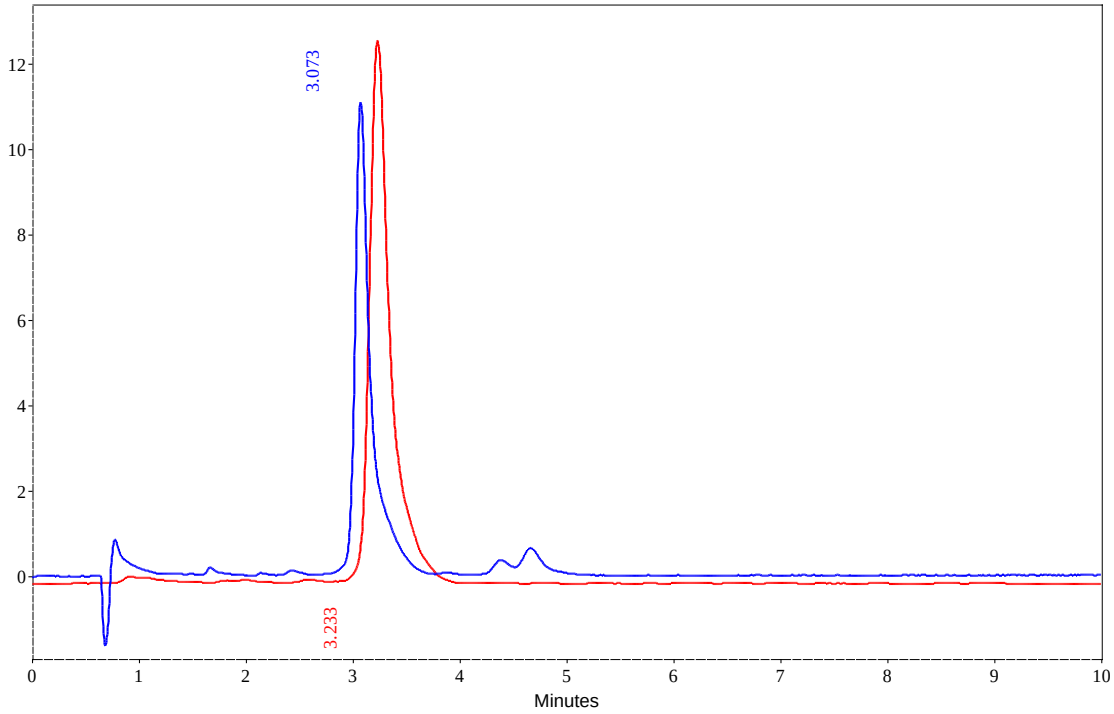


Figure S21 Radio-HPLC of purified purified 22-[^{18}F]F-PD1-ME (**4**) spiked with 22-F-PD1-ME (**4**) using conditions III. (Red trace; radioactive channel, blue trace; UV 271 nm.)

NB ! *The Radio-HPLC detector was connected in series after the UV detector accounting for the slight difference between retention times of ^{19}F- and ^{18}F-labeled species.*

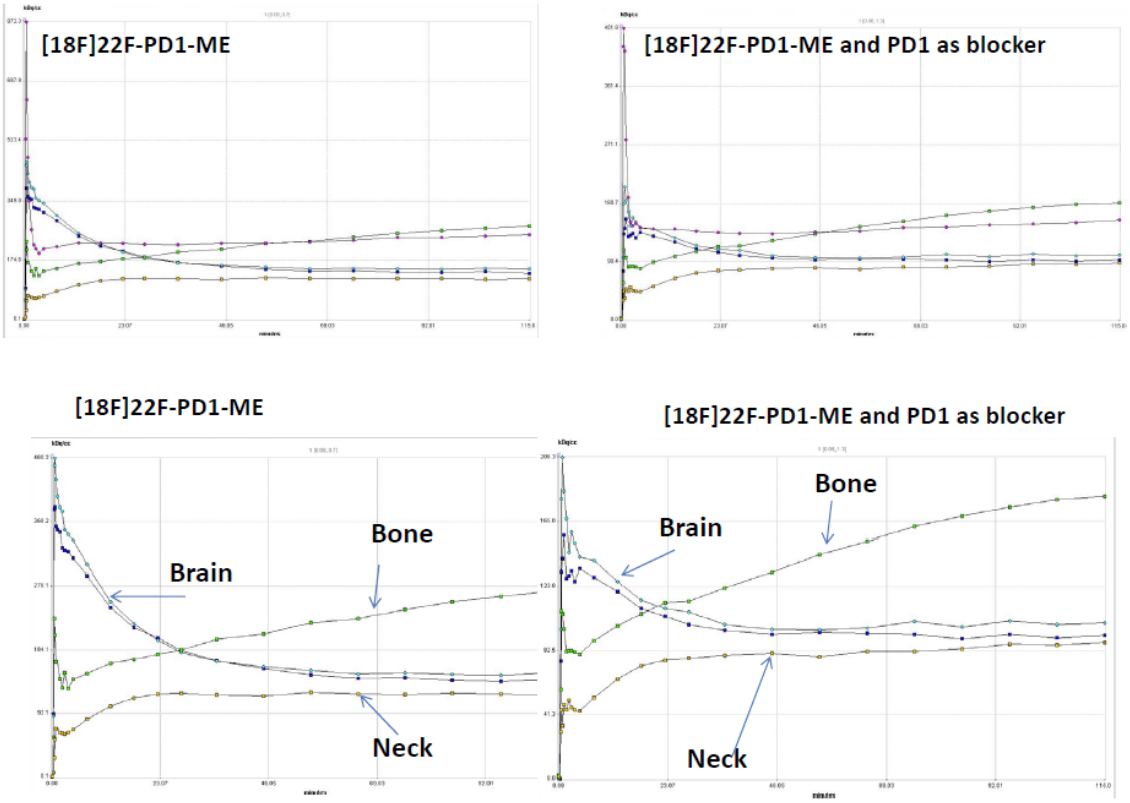


Figure S22 In vivo small animal PET imaging using PD1 as blocker.

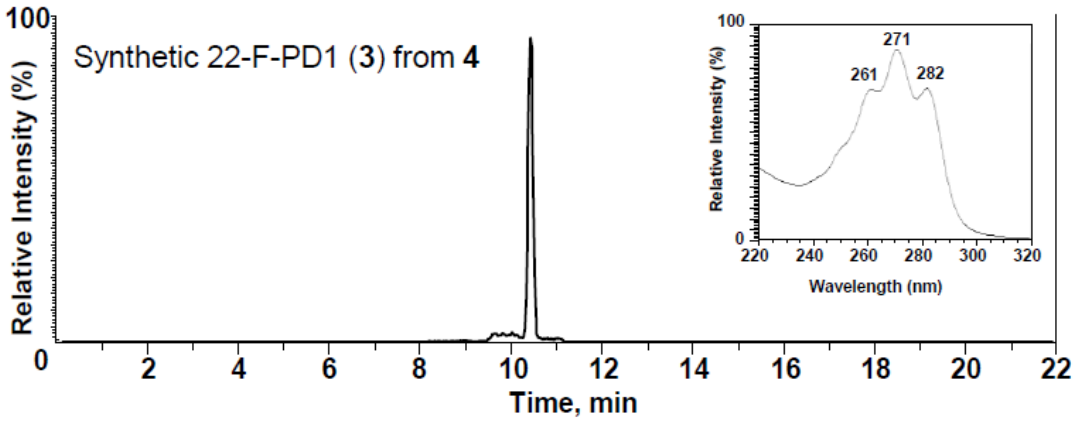


Figure S23 HPLC and UV Chromatograms of **3** used in biological evaluations.

References:

1. a) M. Cushman; M. W. Golebiewski; J. B. McMahon; R. W. Buckheit, Jr.; D. J. Clanton; O. Weislow; R. D. Haugwitz; J. P. Bader; L. Graham; W. G. Rice, *J. Med. Chem*. **1994**, *37*, 3040 b) N. Perlman; M. Livneh; A. Albeck, *Tetrahedron*, **2000**, *56*, 1505
2. J. E. Tungen, M. Aursnes, J. Dalli, C. N., Arnardottir, H., C. N. Serhan, T. V. Hansen, *Chem. Eur. J*., **2014**, *20*, 14575
3. J. E. Tungen, M. Aursnes, A. Vik, S. Ramon, R. A. Colas, J. Dalli, C. N. Serhan, T. V. Hansen, *J. Nat. Prod.* **2014**, ***77***, 2241.