Supporting Information

Novel ^{18}F-Labeled 1-Hydroxyanthraquinone Derivatives for Necrotic Myocardium Imaging

Ai-yan Ji,[†,‡,§] Qiao-mei Jin,[‡,§] Dong-jian Zhang,[‡,§] Hua Zhu,[∥] Chang Su,[†,‡,§] Xing-hua Duan,[†,‡,§] Li Bian,[‡,§,⊥] Zi-ping Sun,[▽] Yi-cheng Ni, [‡,§,#] Jian Zhang,*[,‡,§] Zhi Yang,*[,∥] Zhi-qi Yin,*[,†]

[†]Department of Natural Medicinal Chemistry & Jiangsu Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, Jiangsu, China

[‡]Laboratory of Translational Medicine, Jiangsu Province Academy of Traditional Chinese Medicine, Nanjing 210028, Jiangsu, China

[§]Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, Nanjing 210028, Jiangsu, China

[∥]Key Laboratory of Carcinogenesis and Translational Research, Department of Nuclear Medicine, Peking University Cancer Hospital & Institute, Beijing 100142, China

[⊥]College of Pharmacy, Nanjing University of Chinese Medicine, Nanjing, Jiangsu 210023, China

[▽]Radiation Medical Institute, Shandong Academy of Medical Sciences, Jinan 250062, Shandong, China

[#] Theragnostic Laboratory, Campus Gasthuisberg, KU Leuven, 3000 Leuven, Belgium

1. Materials and Methods

General. [^{18}F]F^{-} was obtained from the PET Center of Peking University Cancer Hospital & Institute (BeiJing, China). No-carrier-added [^{18}F]F- was trapped on a QMA cartridge (Waters) and eluted with 0.1 mL of K_2CO_3 solution (30 mg/mL in H_2O) combined with 1 mL of Kryptofix2.2.2. solution (Sigma-Aldrich) (13 mg/mL in acetonitrile). All reagents and solvents in the synthesis purchased from commercial suppliers and were used without further purification unless otherwise indicated. All reaction mixtures were monitored using thin layer chromatography (TLC) on silica gel 60 F_{254} plates (Merck, Germany), and visualized by ultraviolet (UV) irradiation at 254 nm. The synthesized compounds were purified by silica gel chromatography. Sep-Pak Plus C18 cartridges (Part No. WAT020515, Waters, USA) were preconditioned with ethanol (10 mL) and water (10 mL) before use. The ^{1}H NMR spectra were obtained at 300 or 500 MHz on Bruker spectrometer in $CDCl_3$ or DMSO-d_6 solutions at room temperature with TMS as an internal standard. ^{13}C NMR spectra were recorded on a 75 or 125 MHz spectrometer (Bruker). Chemical shifts were reported as δ values relative to the internal TMS. Coupling constants were reported in hertz. Multiplicity is defined by s (singlet), d (doublet), t (triplet), and m (multiplet). Kunming mice (16 − 20 g) and Sprague−Dawley (SD) rats (280 − 300 g) were furnished by Experimental Animal Center, Peking University Cancer Hospital & Institute, (Beijing, China). All animal experimental protocols and cares were approved and supervised by the Animal Affairs Committee of the Peking University Cancer Hospital & Institute. Reversed-phase high performance liquid chromatography

(RP-HPLC) was performed on Agilent Technologies 1200 series (USA) equipped with both a UV absorption detector and a B-Fc 1000 HPLC radioactivity detector obtained from Bioscan (USA). The RP-HPLC column was ZorBox Alltima C18 analytical column (Agela Technologies, 250 mm × 4.6 mm, 5 μm). The column was eluted with acetonitrile (containig 0.1% trifluoroacetic acid) and water (containig 0.1% trifluoroacetic acid) (v/v = 48:52) at a flow rate of 1.0 mL/min under the column temperature of 25°C.

Calf thymus DNA (Ct-DNA) and ethidium bromide (EB) were purchased from Sigma Aldrich, USA. Stock solution of ^{19}F-FA3OP was prepared in dimethyl sulfoxide (DMSO). Ct-DNA was prepared freshly in Tris-HCl buffer, pH 7.35. The purity of the DNA was checked by observing the ratio of the absorbance at 260/280 nm. The solution gave a ratio of > 1.8 at A_{260}/A_{280}, which indicated that DNA was sufficiently free from protein. The concentration of DNA in stock solution was determined by UV absorption at 260 nm using a molar absorption coefficient ε_{260} = 6600 L mol^{-1} cm^{-1}. Stock solutions were stored at 4°C and used after no more than 4 days. DNA in this work is double stranded DNA unless it is especially noted clearly.

2. Chemistry

2,2′-Oxybis(ethane-2,1-diyl)Bis(4-methylbenzenesulfonate) (C1). C1 was synthesized according to the literature[9]. Briefly, KOH (4.78 g, 85 mmol) was added to a solution of *p*-Toluenesulfonyl chloride (3.8 g, 20 mmol) and 2, 2′-oxidiethanol (1 g, 9.4 mmol) in CH_2Cl_2 (55 mL) with stirring in an ice bath for 8 h. Then CH_2Cl_2 (40 mL) and ice-water (50 mL) were added to the reaction mixture. The organic layer was separated and the water layer was extracted with CH_2Cl_2 (2 × 60 mL). The organic layer was dried with anhydrous $MgSO_4$. After removal of the solvent, the residue was purified by recrystallization in anhydrous MeOH to give **C1** (3.6 g, 85.7%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83 (d, *J* = 7.5 Hz, 4H), 7.40 (d, *J* = 7.5 Hz, 4H), 4.14 (t, *J* = 4.5 Hz, 4H), 3.66 (t, *J* = 4.3 Hz, 4H), 2.5 (s, 6H).

(Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl)-Bis(4-methyl-benzenesulfonate) (C2). The same reaction as described above to prepare **C1** was used, and **C2** was obtained as a white crystal (3.28 g, 65.1%). ^{1}H NMR (500 MHz,$CDCl_3$): δ 7.84 (d, *J* = 8.5 Hz, 4H), 7.39 (d, *J* = 8.5 Hz, 4H), 4.19 (t, *J* = 5.0Hz, 4H), 3.70 (t, *J* = 5.0 Hz, 4H), 3.57 (s, 4H), 2.49 (s, 6H).

2-((9,10-dioxo-9,10-dihydroanthracen-1-yl)oxy)ethyl-4-methyl-benzenesulfonate (OTs-A1OP). To a solution of 1-hydroxyanthraquinone (500 mg, 2.2 mmol) in DMF (25 mL) was added 1,2-bis (tosyloxy)ethane (1.1 g, 3.0 mmol) and NaH (60% dispersion in mineral oil, 30 mg, 1.25 mmol) with stirring at room temperature for 3 days. After 3 days, the reaction was not complete under the TLC monitoring. To help drive the reaction, the flask was placed in an oil bath for an additional 4 days at 45°C.

After reacting completely, the ethyl acetate (150 mL) and water (250 mL) were added to the reaction mixture. The organic layer was separated and the water layer was extracted with ethyl acetate (50 mL) three times. The organic layer was dried with anhydrous $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography (petroleum ether/ethyl acetate, 3/1) to give **OTs-A1OP** as a yellow solid (188.4 mg, 20.2%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23 (t, ArH, 2H), 8.01 (d, ArH, 1H), 7.76 (m, ArH, 5H), 7.30 (m, ArH, 3H), 4.51 (t, *J* = 4.5 Hz, 2H), 4.40 (t, *J* = 4.5 Hz, 2H), 2.39 (s, 3H). ESI-MS calculated for $C_{23}H_{18}O_6S$: 422.1; Found 445.1 $[M+Na]^+$.

2-(2-((9,10-dioxo-9,10-dihydroanthracen-1-yl)oxy)ethoxy)-ethyl-4-methylbenzen-esulfonate (OTs-A2OP). The same reaction as described above to prepare **OTs-A1OP** was used, and **OTs-A2OP** was obtained as a yellow solid (308.2 mg, 29.5%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.24 (d, ArH, 2H), 7.99 (d, ArH, 1H), 7.73 (m, ArH, 5H), 7.30 (m, ArH, 3H), 4.25 (m, 4H), 3.96 (m, 4H), 2.40 (s, 3H). ESI-MS calculated for $C_{25}H_{22}O_7S$: 466.1; Found 489.2 $[M+Na]^+$.

2-(2-(2-((9,10-dioxo-9,10-dihydroanthracen-1-yl)oxy)ethoxy)ethoxy)ethyl-4-methylbenzenesulfonate (OTs-A3OP). The same reaction as described above to prepare **OTs-A1OP** was used, and **OTs-A3OP** was obtained as a yellow solid (225.4 mg, 19.8%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.24 (m, ArH, 2H), 7.98 (d, ArH, 1H), 7.73 (m, 5H), 7.33 (m, 3H), 4.32 (t, *J* = 4.8 Hz, 2H), 4.18 (t, *J* = 4.8 Hz, 2H), 4.01 (t, *J* = 4.5 Hz, 2H), 3.74 (m, 6H), 2.42 (s, 3H). ESI-MS calculated for $C_{27}H_{26}O_8S$: 510.1; Found 533.2 $[M+Na]^+$.

1-(2-fluoroethoxy)anthracene-9,10-dione ([^{19}F]FA1OP). The same reaction as described above to prepare **OTs-A1OP** was used, and **[^{19}F]FA1OP** was obtained as a yellow solid (52 mg, 43.2%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.13 (m, ArH, 2H), 7.88 (m, ArH, 4H), 7.60 (m, ArH, 1H), 4.83 (dt, *J* = 48 Hz, 3.0 Hz, 2H), 4.49 (t, *J* =3.0 Hz,1H), 4.39 (t, *J* = 3.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): 182.76, 180.95, 158.87, 135.32, 135.02, 134.56, 134.50, 133.50, 131.91, 126.58, 126.06, 121.11, 120.52, 119.50, 82.04 (d, *J* = 166.5 Hz), 68.75 (d, *J* = 19.5 Hz). HRMS (EI) calculated for $C_{16}H_{11}O_3F$: 270.0692; Found 293.0593 $[M+Na]^+$.

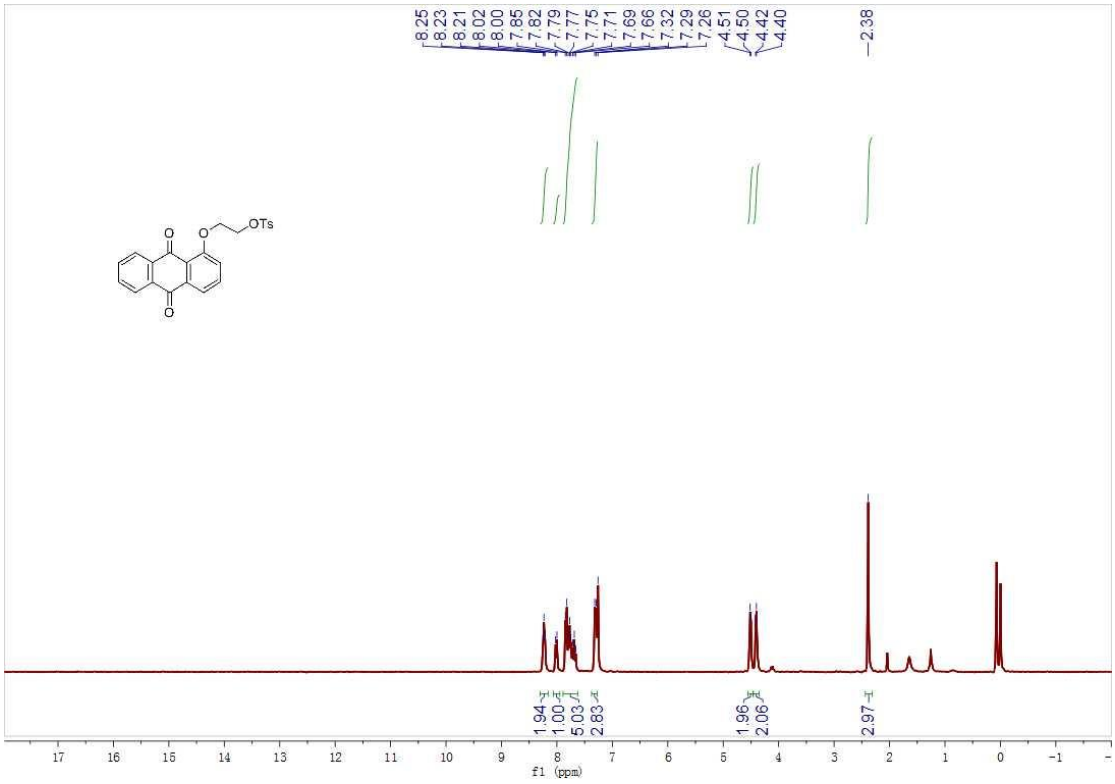
1-(2-(2-fluoroethoxy)ethoxy)anthracene-9,10-dione ([^{19}F]FA2OP). Intermediate **[^{19}F]FA2OP** was synthesized according to the literature[10]. Briefly, to a solution of **OTs-A2OP** (100 mg, 5 mmol) in dry THF (5 mL) was added anhydrous TBAF (1 mL, 1 mmol, 1.0 M in THF). The solution was stirred at 70°C for 8 h. After removal of solvent, the residue was purified by silica gel chromatography to give **[^{19}F]FA2OP** as a yellow solid (31 mg, 46%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.16 (m, ArH, 2H), 7.89 (m, ArH, 4H), 7.63 (m, ArH, 1H), 4.60 (dt, *J* = 45 Hz, 5.0 Hz, 2H), 4.35 (t, *J* = 5.0 Hz, 2H), 3.94 (m, 3H), 3.87 (t, *J* = 5.0 Hz, 1H).^{13}C NMR (125 MHz, DMSO-d_6): 183.22, 181.49, 159.67, 135.80, 135.47, 135.02, 135.02, 133.96, 132.41, 127.07, 126.54, 121.45, 120.90, 119.71, 83.61 (d, *J* = 140 Hz), 70.55 (d, *J* = 18.75 Hz), 69.70, 69.31. HRMS (EI) calculated for $C_{18}H_{15}O_4F$: 314.0954; Found 337.0859 $[M+Na]^+$.

1-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)anthracene-9,10-dione ([^{19}F]FA3OP). The same reaction as described above to prepare **[^{19}F]FA2OP** was used, and**[^{19}F]FA3OP** was obtained as a yellow solid (37 mg, 52.7%). ^{1}H NMR (500 MHz, DMSO-d_6): δ

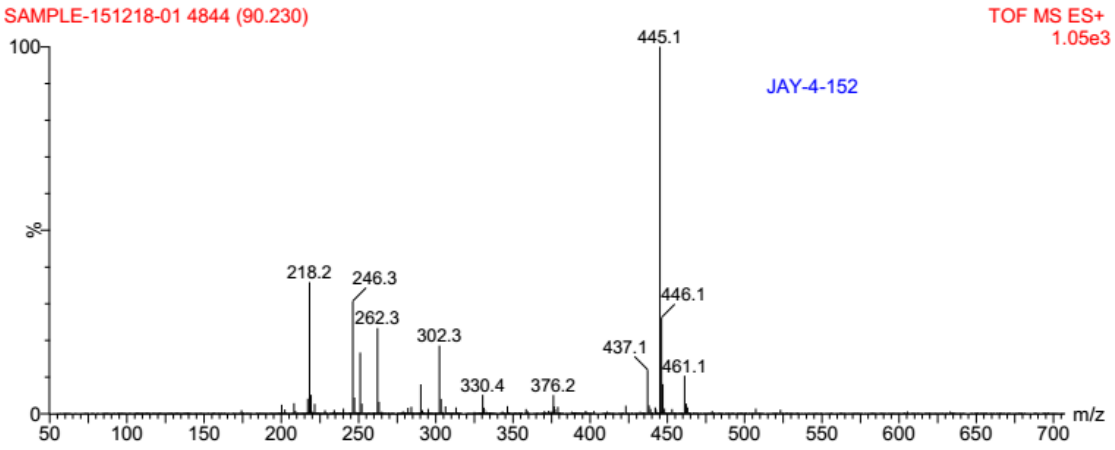
8.16 (m, ArH, 2H), 7.89 (m, ArH, 4H), 7.63 (m, ArH, 1H), 4.53 (dt, *J* = 50 Hz, 5 Hz, 2H), 4.33 (t, *J* = 5 Hz, 2H), 3.90 (t, *J* = 5 Hz, 2H), 3.70 (m, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): 182.71, 180.94, 159.20, 135.25, 134.93, 134.50, 134.50, 133.42, 131.88, 126.52, 126.01, 120.93, 120.43, 119.14, 82.95 (d, *J* = 140 Hz), 70.13, 69.81, 69.60 (d, *J* = 18.75 Hz), 69.10, 68.76. HRMS (EI) calculated for $C_{20}H_{19}O_5F$: 358.1217; Found 381.1121 $[M+Na]^+$.

3. ^{1}H-NMR and ESI-MS for precursors

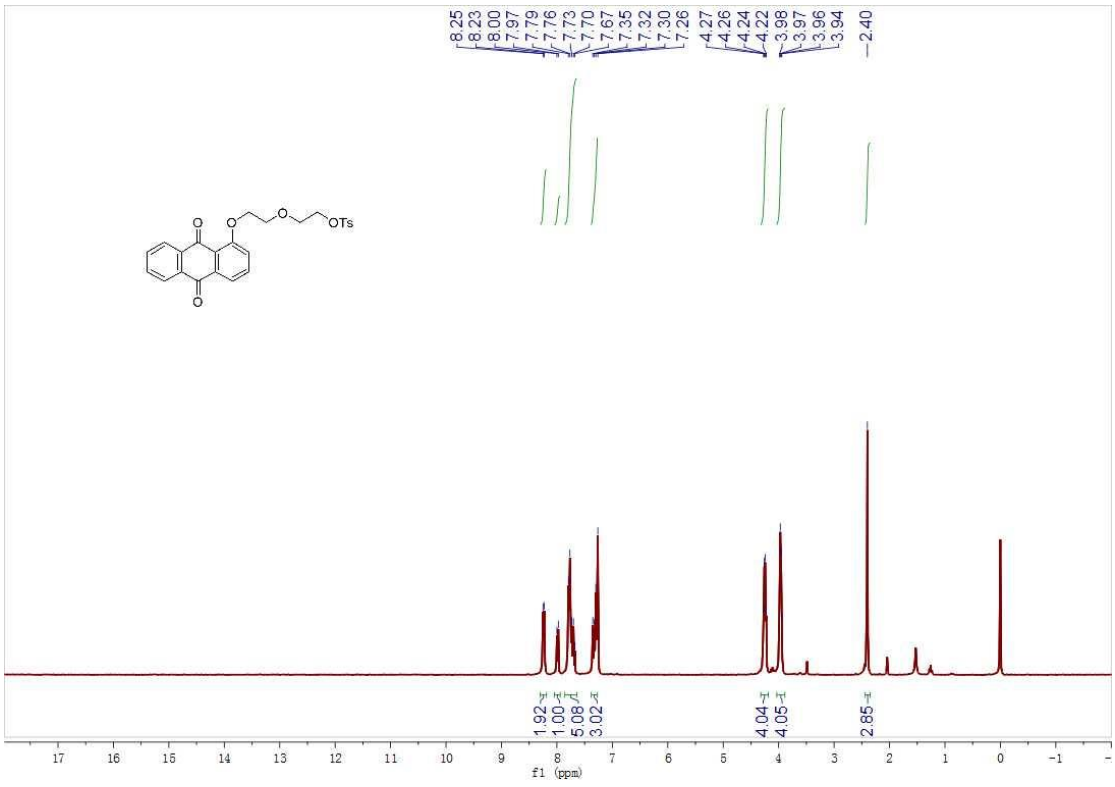
^{1}H-NMR for compound **OTs-A1OP**



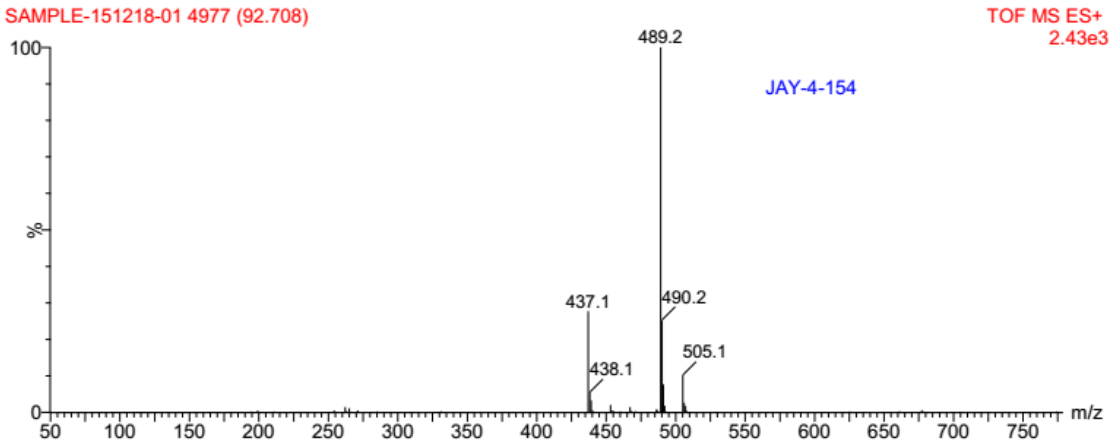
ESI-MS for compound **OTs-A1OP**



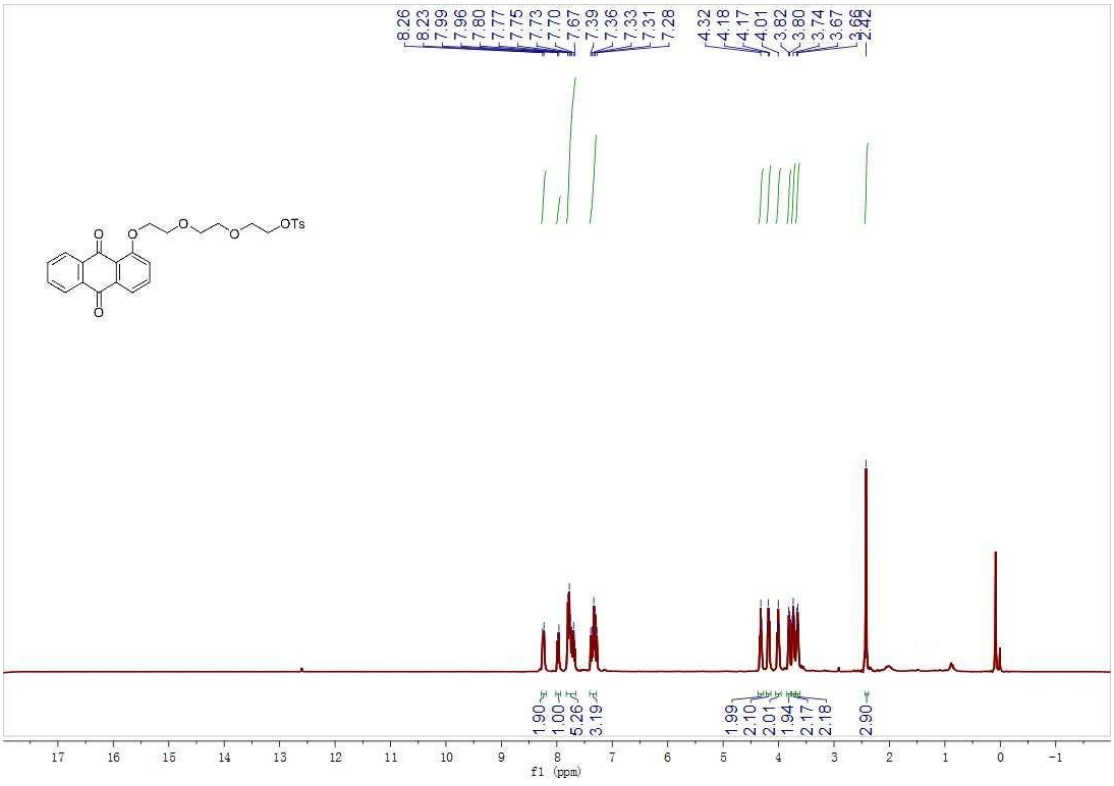
^{1}H-NMR for compound **OTs-A2OP**



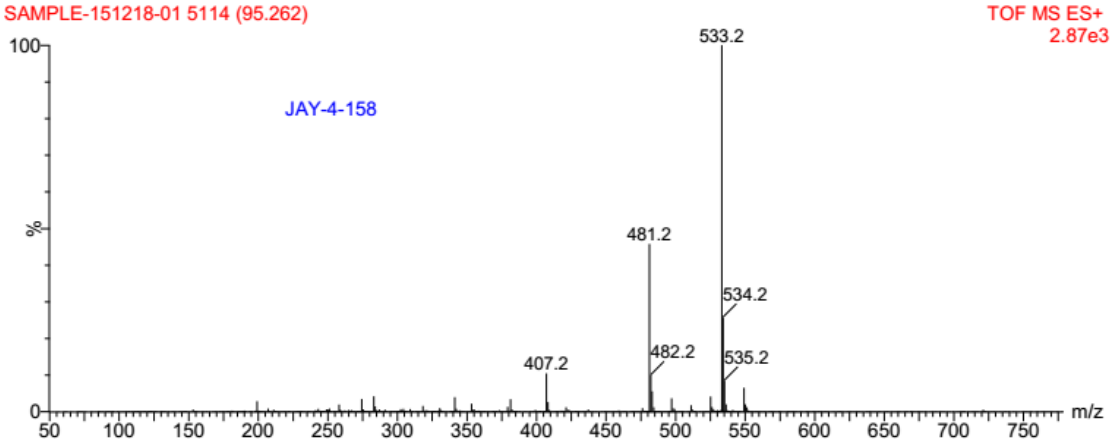
ESI-MS for compound **OTs-A2OP**



^{1}H-NMR for compound **OTs-A3OP**

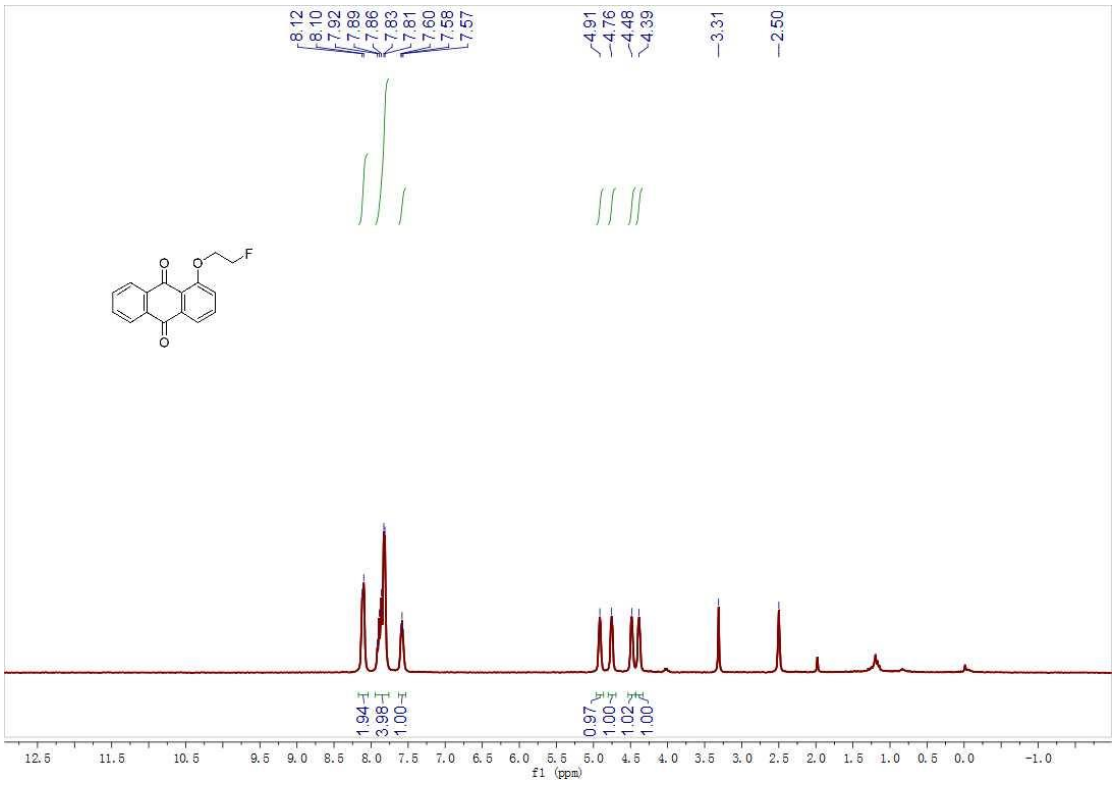


ESI-MS for compound **OTs-A3OP**

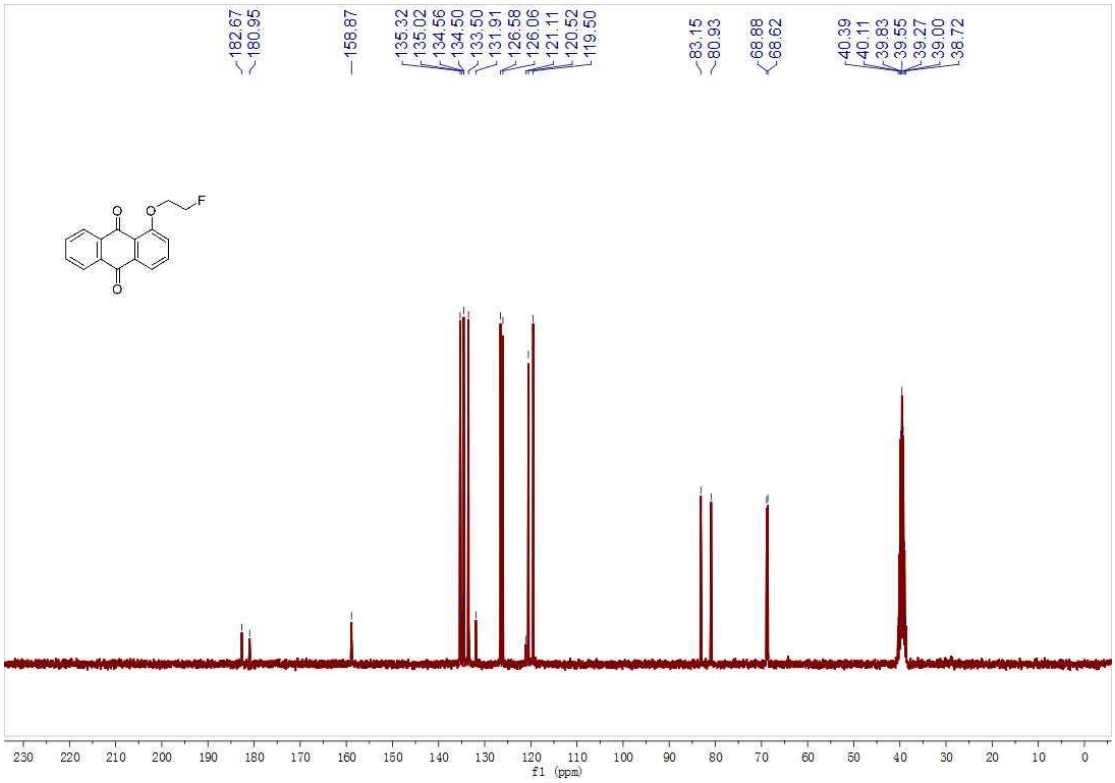


4. ^{1}H-NMR, ^{13}C-NMR and HRMS for ^{19}F target compounds

^{1}H-NMR for compound **[^{19}F]FA1OP**



^{13}C-NMR for compound **[^{19}F]FA1OP**

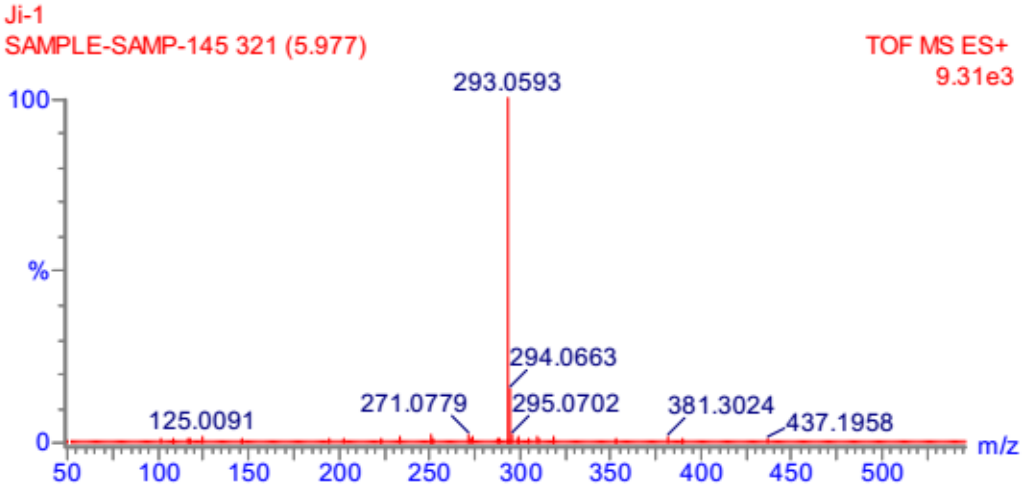


HRMS for compound **[^{19}F]FA1OP**

Elemental Composition Report

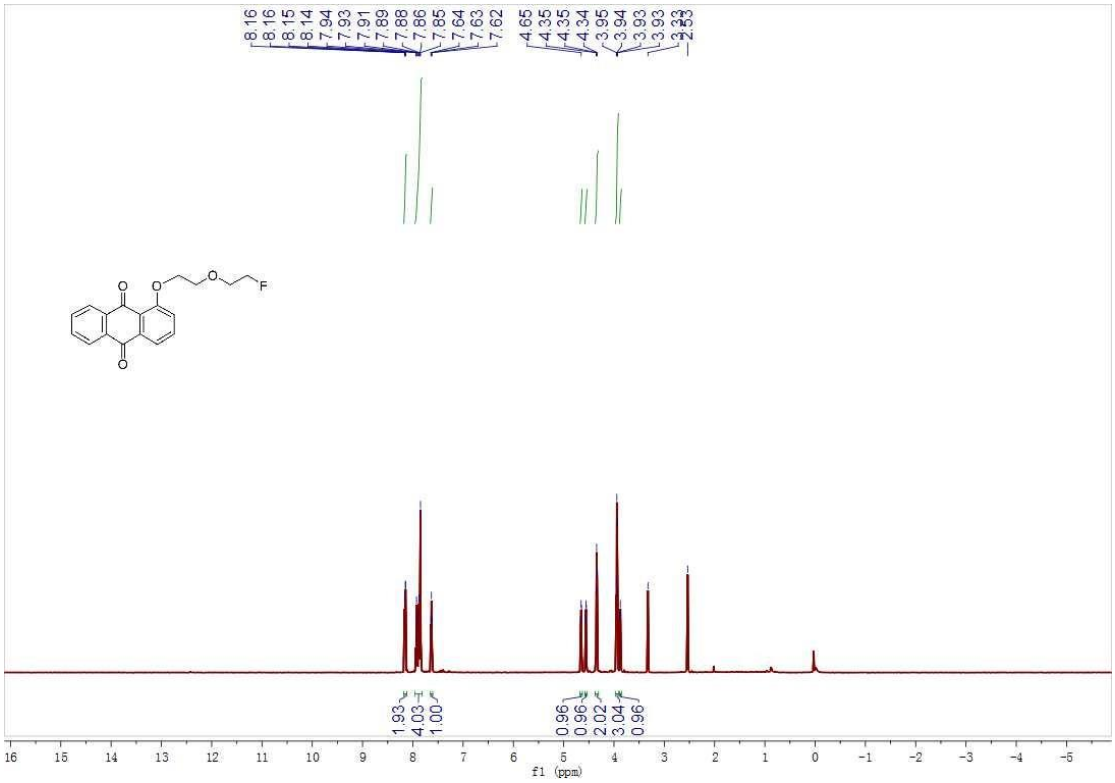
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 20.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
500 formula(e) evaluated with 3 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-50 H: 0-100 N: 0-5 O: 0-5 Na: 0-1 F: 0-1

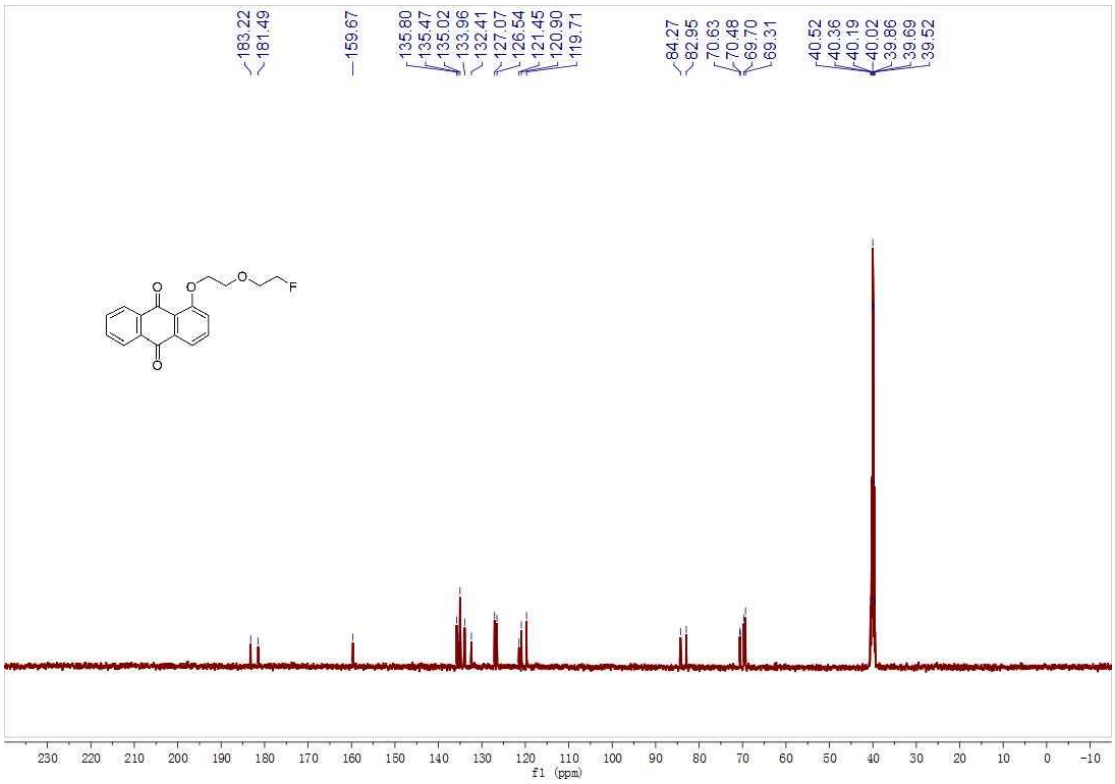


Minimum:				-1.5		
Maximum:		5.0	5.0	20.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
293.0593	293.0590	0.3	1.0	10.5	42.7	C16 H11 O3 Na F
	293.0578	1.5	5.1	14.5	162.9	C19 H10 O2 Na
	293.0603	-1.0	-3.4	17.5	287.4	C21 H9 O2

^{1}H-NMR for compound **[^{19}F]FA2OP**



^{13}C-NMR for compound **[^{19}F]FA2OP**

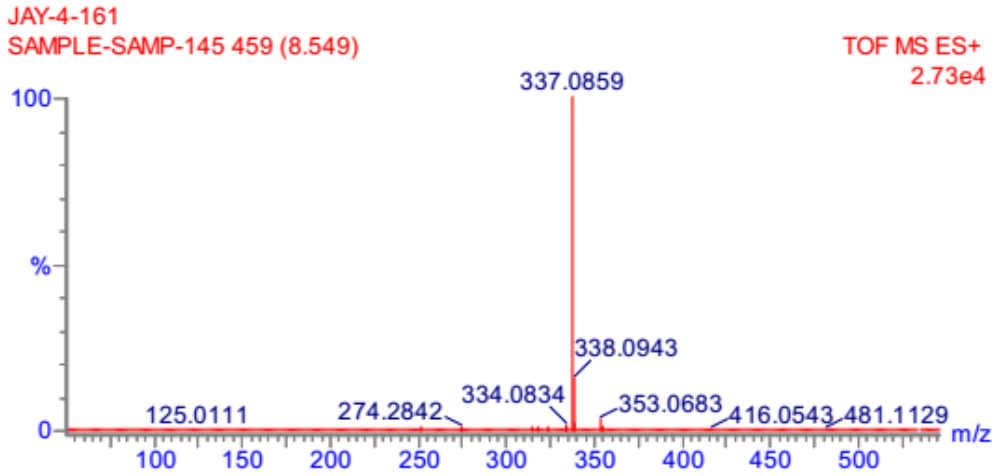


HRMS for compound **[^{19}F]FA2OP**

Elemental Composition Report

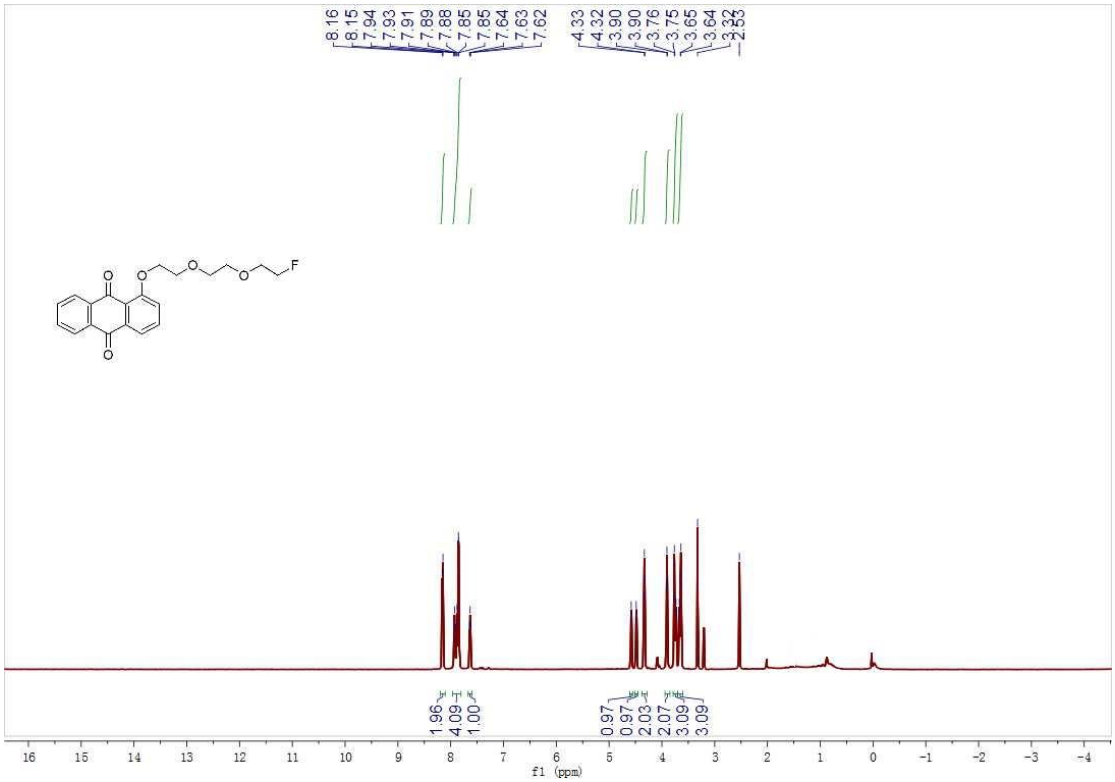
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 20.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
576 formula(e) evaluated with 3 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-50 H: 0-100 N: 0-5 O: 0-5 Na: 0-1 F: 0-1

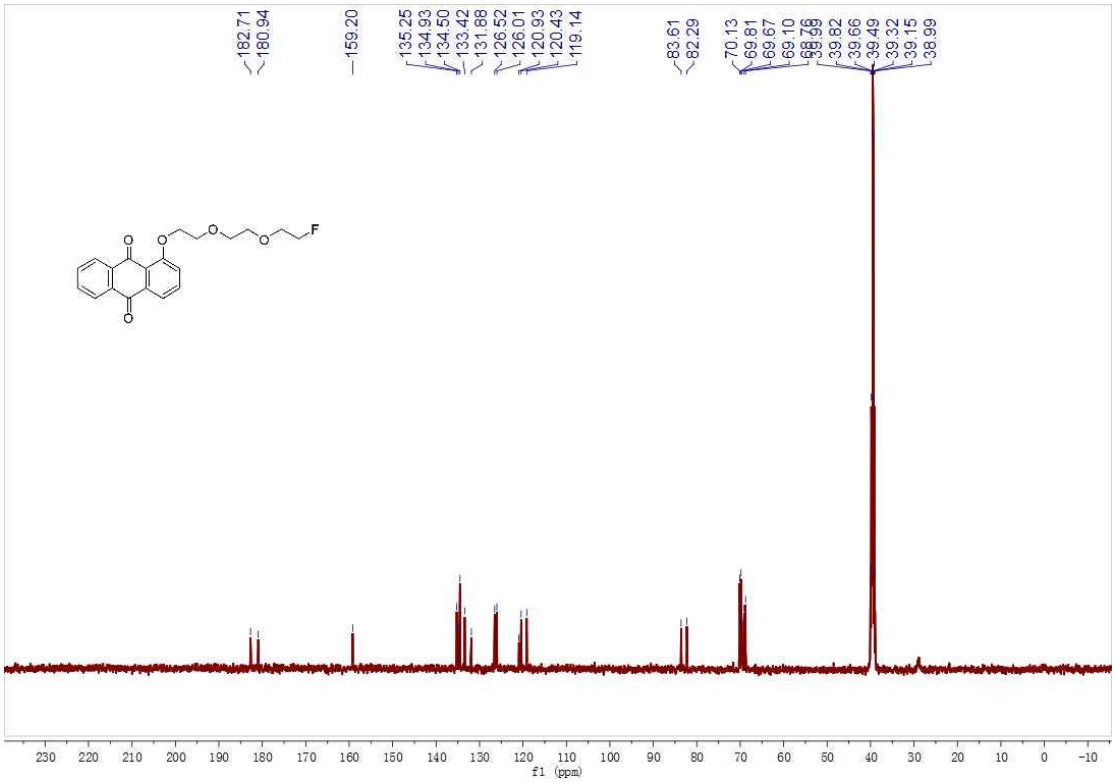


Minimum:				-1.5						
Maximum:		5.0	5.0	20.0						
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula				
337.0859	337.0852	0.7	2.1	10.5	229.3	C18	H15	O4	Na	F
	337.0865	-0.6	-1.8	15.5	399.0	C19	H11	N4	Na	F
	337.0865	-0.6	-1.8	17.5	848.3	C23	H13	O3		

^{1}H-NMR for compound **[^{19}F]FA3OP**



^{13}C-NMR for compound **[^{19}F]FA3OP**

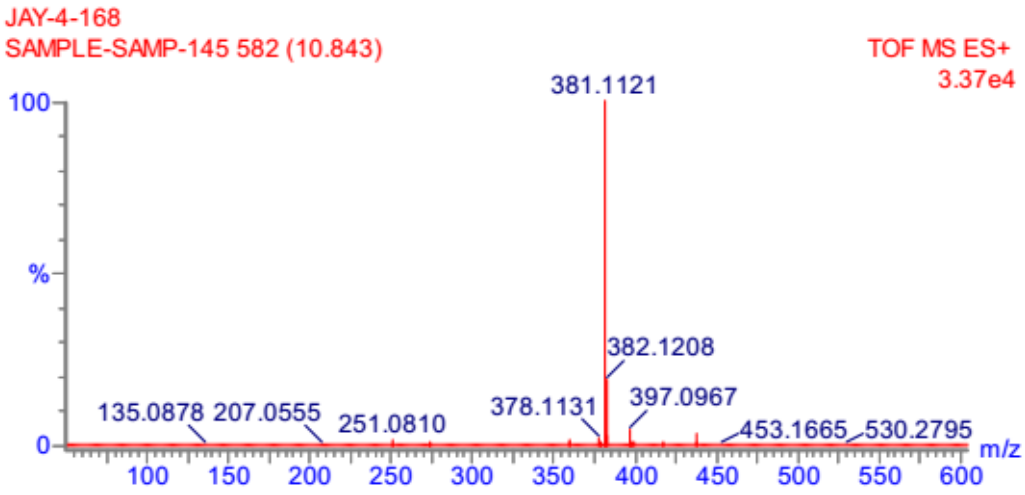


HRMS for compound **[^{19}F]FA3OP**

Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 20.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
913 formula(e) evaluated with 6 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-50 H: 0-100 N: 0-5 O: 0-8 Na: 0-1 F: 0-1



Minimum:				-1.5							
Maximum:		5.0	5.0	20.0							
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula					
381.1121	381.1114	0.7	1.8	10.5	199.3	C20	H19	O5	Na	F	
	381.1128	-0.7	-1.8	15.5	304.2	C21	H15	N4	O	Na	F
	381.1138	-1.7	-4.5	13.5	414.1	C22	H18	O5	F		
	381.1103	1.8	4.7	14.5	509.5	C23	H18	O4	Na		
	381.1116	0.5	1.3	19.5	680.0	C24	H14	N4	Na		
	381.1127	-0.6	-1.6	17.5	819.4	C25	H17	O4			

5. Radiochemistry

The labeling procedures of [^{18}F]FA1OP, [^{18}F]FA2OP, and [^{18}F]FA3OP were shown in Scheme 1. [^{18}F]Fluoride (about 3700 MBq) trapped on a QMA cartridge was eluted with 1 mL of Kryptofix222/K_2CO_3 solution. The solvent was removed at 100℃ under a stream of nitrogen gas. The residue was azeotropically dried with 1 mL of anhydrous acetonitrile twice at 100℃ under a stream of nitrogen gas. A solution of the tosylate precursors (1.0 mg) in CH_3CN (1 mL) were added to the reaction vessel containing the $^{18}F^-$ activity, respectively. The mixture was heated at 95℃ for 20 min. Then water (20 mL) was added after the mixture was cooled to room temperature. The reaction solution was passed through a Sep-Pak Plus C18 cartridge. Then the cartridge was washed with 15 mL of water, and the labeled compound was eluted with 1 mL of ethanol. After the removal of ethanol with flowing nitrogen, the residue was dissolved in DMF and subjected to analytical HPLC (Agela Technologies, ZorBox SB-C18 analytical column, 250 mm × 4.6 mm, 5 μm) for prep purification. The HPLC fraction was concentrated with flowing nitrogen at 100°C firstly and the dried product was redissolved in 80% ethanol solution and diluted with saline (about 2% ethanol solution) for the follow-up experiments. The final radiochemical purity was determined by reinjection of the product onto a radio-HPLC column. Specific activity was estimated by comparing the UV peak intensity of purified ^{18}F-labeled compounds with reference nonradioactive compounds (Figure S1).

6. Octanol–Water Partition Coefficient

To a solution of octanol (500 μL) and PBS (500 μL, 0.01 M, PH 7.4) (obtained from saturated octanol-PBS solution), [^{18}F]FA1OP, [^{18}F]FA2OP, and [^{18}F]FA3OP (10 μL, 185 KBq) were added, respectively. The resulting solution was vortexed at room temperature and centrifuged at 3000 rpm for 5 min. Aliquots of 10 μL were taken from both octanol and saline phases, and the activity was measured on a 2470 Wizard automatic γ-counter (Perkin-Elmer). The experiment was performed in triplicate. The octanol/buffer partition coefficient P was calculated as P = (cpm in the organic phase)/(cpm in the aqueous phase). The partition coefficient (P) was calculated as Log P.

7. In vitro stability study

Three radiotracers were tested in 80% ethanol solution at room temperature for 5 h. Then radiotracers (0.1 mL) which were diluted by saline (about 2% ethanol solution) were tested in 0.9 mL rat serum at 37°C for 6 h. Serum proteins were precipitated by adding 200 μL of acetonitrile and were removed by centrifugation. Radiochemical purity was determined by radio-HPLC.

8. Animal Models of Necrosis

Mice Model of Muscular Necrosis. Each mouse was intramuscularly injected with 0.1 mL absolute alcohol in the left hind limb to induce muscular necrosis.

Rat Model of Myocardial Infarction and Reperfusion (MI/R). MI/R was conducted as previously described[7]. Briefly, male Sprague Dawley (SD) rats were anesthetized with 10% chloral hydrate, intubated and ventilated with a volume-cycled small-animal ventilator. A left thoracotomy was performed along the third and fourth intercostal space, followed by incision of the pericardium to fully expose the heart in thoracic cavity. The proximal left anterior descending coronary artery (LAD) was occluded by a single ligature at 1-2 mm below the junction of the pulmonary conus and the left atrial appendage. Sixty minutes after LAD occlusion, the suture end was removed to achieve coronary reperfusion**.** Sham-operated control rats were prepared in a similar manner without the LAD ligation.

*Biodistribution Studies of Mice***.** For biodistribution studies, Kunming mice of MN (n = 4/group) were intravenously administered with [^{18}F]FA2OP or [^{18}F]FA3OP under anesthesia and sacrificed at 30 min, 1 h and 2 h p.i., respectively. Organs of interest were removed, weighed, and counted for radioactivity using a γ counter. Uptake of the tracers was calculated as the percentage of the injected dose per gram of tissue (% ID/g) with the values expressed as mean ± standard deviation (SD). The decay-corrected activity per mass of tissue was calculated.

Small-Animal PET/CT Scanning. Sham-operated control rats and MI/R rats were anesthetized with isoflurane. MicroPET/CT (Inveon™, Siemens Medical Solutions,

USA, Inc.) studies in rats (n = 4) were carried out at 4 h after induction of MI/R with 8.9 ± 1.7MBq of [^{18}F]FDG to detect the myocardial zone with dynamic defect by 10 min static scans. Twenty-four hours after induction of MI/R, about 10 MBq of [^{18}F]FA3OP were injected intravenously. Dynamic MicroPET images were acquired for 60 min and reconstruction was done. Data were analyzed using PMOD2.7 software (Zurich, Switzerland), volumes of interest (VOIs) were defined on the summed images, and time-activity curves (TACs) were drawn.

Note that the isolated heart images of [^{18}F]FA3OP (1 h p.i.) were acquired from other microPET/CT (Super Argus, Sedecal, Spain, Inc.) with equipment failures. The heart was rinsed with 4°C saline to remove blood pool activity and placed on the PET/CT bed and imaged. Scanning involved 1 bed positions, with scanned for 10 min.

2, 3, 5-Triphenyl-2H-Tetrazolium Chloride (TTC) Staining. After the scan of PET, the isolated hearts from model rats were stained in a 2% buffered triphenyltetrazolium chloride (TTC) solution for 15 min at 37°C and digitally photographed for comparison with imaging−histochemistry later.

9. Interaction with DNA

UV–visible spectrophotometry. The UV spectra were recorded with a Cary 60 UV-visible spectrophotometer (Agilent Technologies Inc., California, USA) using a 1 cm × 1 cm quartz cuvettes. The spectra of [^{19}F]FA3OP and [^{19}F]FA3OP-Ct-DNA complex were recorded in the wavelength range of 200 – 800 nm. Experiment was carried out in the presence of fixed concentration of [^{19}F]FA3OP (2.79×10^{-4} M) in a total volume of 2 mL and titrated with varying concentration of DNA (0, 0.407, 1.58, 1.96, 2.33, 2.69, 3.04, 3.39, 3.74×10^{-4} M). The binding constant (K_b) was determined from the spectroscopic titration data using the following equation:

$$[DNA] / (\varepsilon_a - \varepsilon_f) = [DNA] / (\varepsilon_b - \varepsilon_f) + 1/K_b (\varepsilon_b - \varepsilon_f)$$

where [DNA] is the concentration of DNA in base pairs, the apparent absorption coefficient (ε_a) was obtained by calculating A_{obsd}/[compound]. The terms ε_f and ε_b correspond to the extinction coefficient of free (unbound) and the fully bound compounds, respectively.

Fluorescence quenching study. The fluorescence emission spectra were measured with Cary Elipse Fluorescence (Agilent Technologies Inc., California, USA) at 298 K in the wavelength range of 530-750 nm. Increasing concentrations of compound (0, 0.171, 0.339, 0.504, 0.824, 0.979, 1.43, 1.57×10^{-5} M) was micropipetted directly into a 1.0 cm quartz cell containing 1.30×10^{-5} M ethidium bromide (EB) and 5.20×10^{-5}M Ct-DNA (total volume 3 mL), and the reaction was performed at room temperature. The synchronous fluorescence spectra was recorded by scanning at excitation and emission wavelengths simultaneously. The spectrofluorometric measurements of all

samples were carried out over the excitation wavelength range of 530-750 nm. The EB-DNA system was excited at 530 nm, and the emission spectrum was taken at 603 nm. The relative binding of the compound with Ct-DNA was determined by using classical Stern−Volmer equation

$$F_0/F = 1 + K_{SV}[\text{compound}]$$

where F_0 and F are the fluorescence intensities in absence and presence of the quencher, K_{SV} is the Stern−Volmer quenching constant, and [compound] is the concentration of the compound.

Statistical Analysis. All quantitative results were expressed as mean ± standard deviation (SD). Between-group or data from tissues of interest were compared by one-way analysis of variance using Student's *t* test and $p < 0.05$ was considered statistically significant.

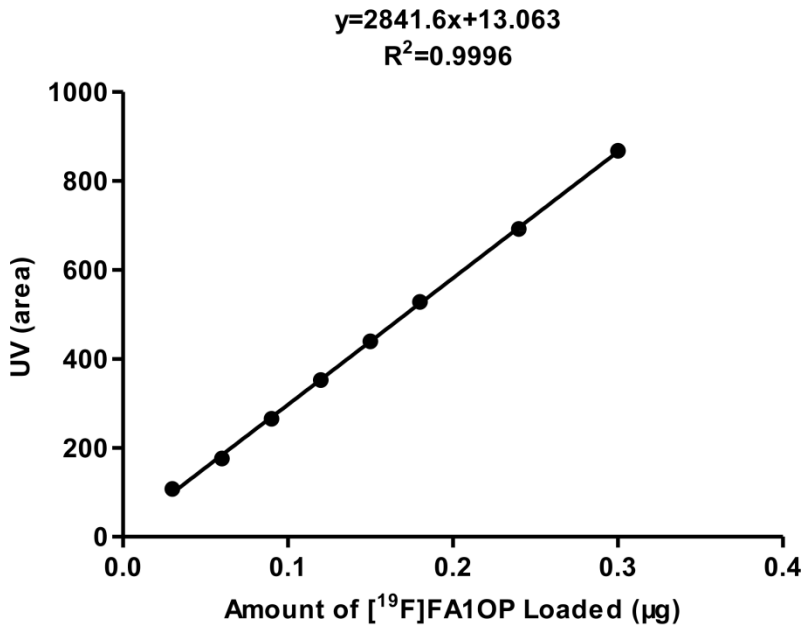


Figure S1. The HPLC quantitative analysis of [^{19}F]FA1OP in solution for specific activity calculation. The standard curve shows the relationship between UV area of HPLC and the mass of [^{19}F]FA1OP loaded.

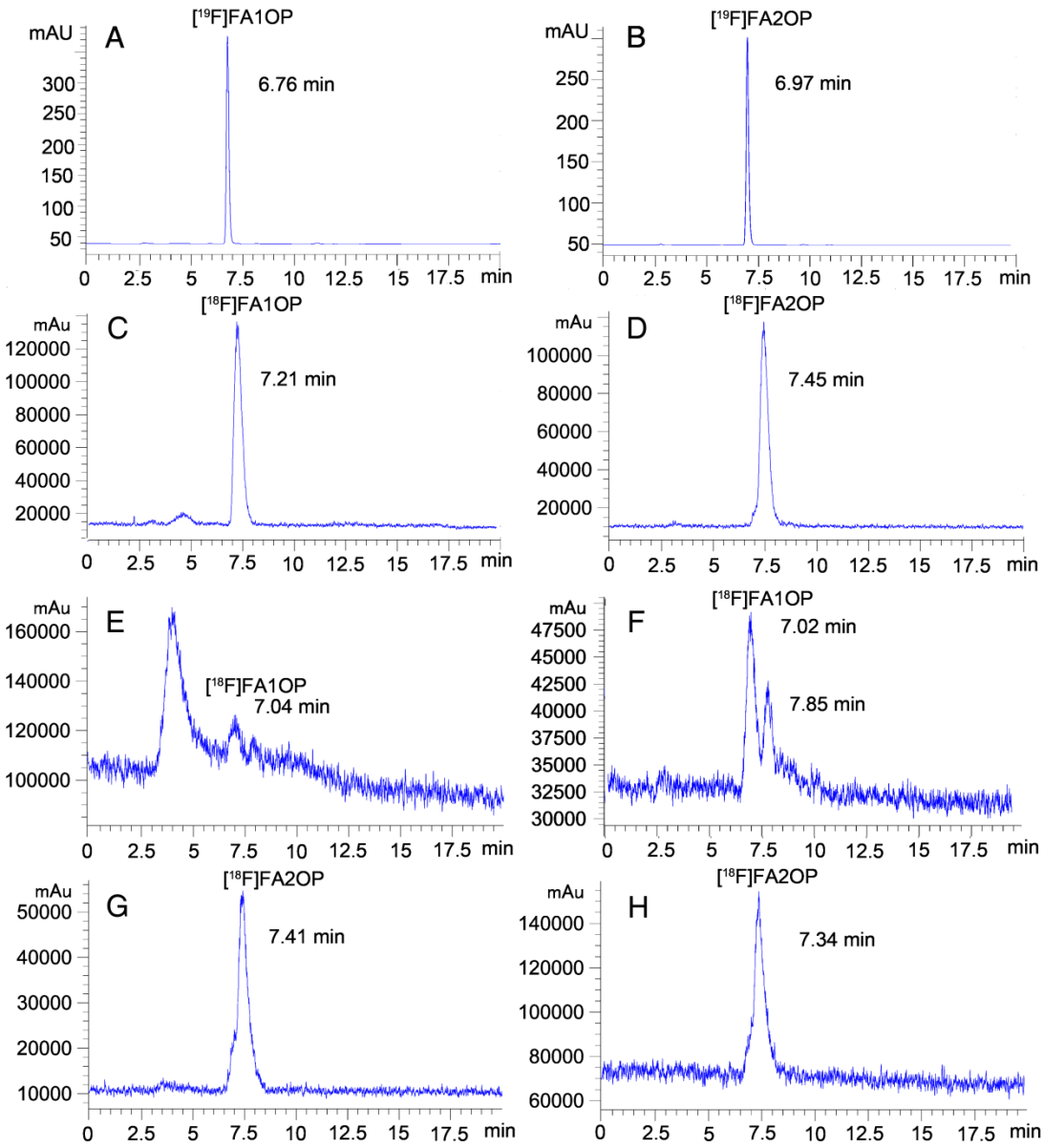


Figure S2. HPLC chromatograms of compounds [^{19}F]FA1OP (A), [^{19}F]FA2OP (B),and [^{18}F]FA1OP (C), [^{18}F]FA2OP (D), The radiochromatograms of [^{18}F]FA1OP after storage in rat serum at 37°C for 1 h, about only 10% of the total radioactivity was eluted at 7.04min (E), and [^{18}F]FA1OP after storage in 80% ethanol solution at room temperature for 1h, 66% of the total radioactivity was eluted at 7.02 min (F),and [^{18}F]FA2OP after storage in rat surem at 37°C for 6h, about 95% of the total radioactivity was eluted at 7.41 min (G), and [^{18}F]FA1OP after storage in 80% ethanol

solution at room temperature for 5h, over 95% of the total radioactivity was eluted at 7.34 min (H).

Table S1 Biodistribution and the Necrotic-to-Normal Tissue Ratios of [^{18}F]FA2OP and [^{18}F]FA3OP in Muscular Necrosis Models (n = 4/Group)

Tissue	30 min		60 min		120 min	
	[^{18}F]FA2OP	[^{18}F]FA3OP	[^{18}F]FA2OP	[^{18}F]FA3OP	[^{18}F]FA2OP	[^{18}F]FA3OP
Blood	4.99±0.47	7.63±0.48	3.78±0.06	2.96±0.22	2.31±0.02	2.26±0.17
Heart	4.56±0.29	5.42±0.40	4.00±0.15	2.21±0.16	2.41±0.09	1.72±0.26
Liver	5.16±0.81	6.87±0.07	2.97±0.02	2.53±0.14	2.19±0.04	1.93±0.29
Spleen	3.44±0.36	4.23±0.18	2.53±0.09	1.74±0.05	1.61±0.02	1.34±0.21
Lung	4.41±0.48	5.19±0.29	3.00±0.09	2.47±0.12	1.97±0.09	1.63±0.21
Kidney	3.69±0.18	5.35±0.32	2.90±0.05	2.27±0.01	1.62±0.01	1.56±0.18
Bone	4.32±0.16	5.41±0.33	4.42±0.17	3.78±0.10	3.97±0.62	3.65±0.20
Brain	2.91±0.70	3.34±0.13	2.03±0.06	1.37±0.02	1.38±0.16	1.01±0.09
Normal muscle	4.41±0.57	3.77±0.08	2.33±0.02	1.69±0.11	1.51±0.06	1.18±0.15
Necrotic muscle	5.30±0.16	5.49±0.20	4.24±0.27	4.23±0.13	2.57±0.06	2.32±0.19
Necrotic muscle/muscle	1.20±0.07	1.46±0.05	1.82±0.12	2.50±0.23	1.70±0.06	1.96±0.03

Data are presented as % ID/g tissue ± SD after intravenous injection of the tracer at 30, 60 and 120 min.

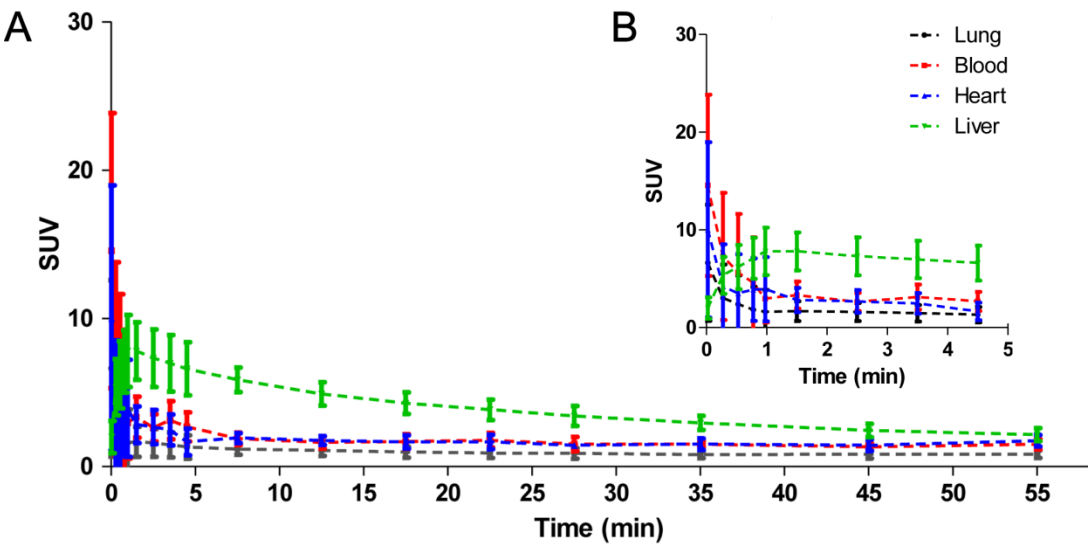


Figure S3. (A)Standard uptake values (SUVs) of [^{18}F]FA3OP in the blood, heart, lung and liver of normal rats. (B). Magnification of one segment of image A.

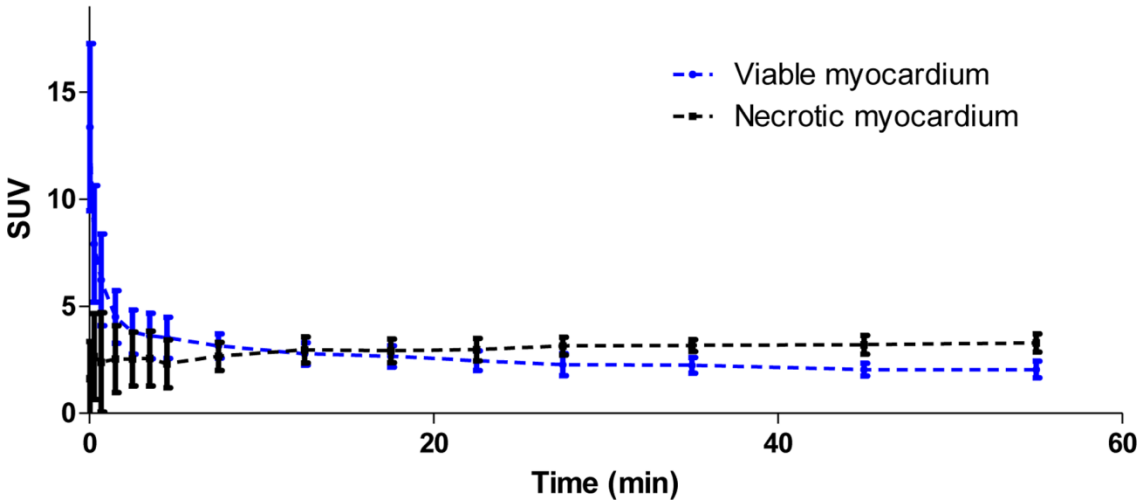


Figure S4. Standard uptake values (SUVs) of [^{18}F]FA3OP in the viable myocardium and necrotic myocardium of MI/R rats.