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Ring Opening of Epoxides with [^{18}F]FeF Species to Produce [^{18}F]Fluorohydrin PET Imaging Agents

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Contents

1. General Considerations, Methods and Materials

^{1}H NMR spectra were obtained on a Varian 400 MHz MR NMR (399.7 MHz for 1H, 100.5 MHz for ^{13}C and 376.1 MHz for 19F) and Varian 500 MHz VNMRS (499.5 MHz for 1H, 125.6 MHz for ^{13}C) spectrometers. Chemical shifts are reported in parts per million (ppm) and referenced to tetramethylsilane as in internal standard (^{1}H: $\delta = 0.00$) or residual solvent peak ($CDCl_3$: 1H: $\delta = 7.26$ ppm, ^{13}C: $\delta = 77.16$ ppm). ^{19}F NMR spectra are referenced to an external standard trichlorofluoromethane ($CFCl_3$: $\delta = 0.00$ ppm for ^{19}F). NMR spectra were recorded at room temperature. The abbreviations for ^{1}H and ^{19}F multiplicities are reported as follows: singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), doublet of doublets of doublets (ddd), doublet of triplets (dt), triplet of doublets (td), and multiplet (m). Broad signals are indicated by "br". Coupling constants (J) are reported in hertz (Hz). High-resolution mass spectra were recorded on a Micromass AutoSpec Ultima Magnetic Sector mass spectrometer. Thin layer chromatography (TLC) was performed on Merck KGaA. pre-coated TLC Silica gel 60 F254 plates. Flash column chromatography was conducted using a Biotage Isolera Prime system with SNAP KP-Sil column cartridges (10 g, 25 g, 100 g). Glass backed thin layer chromatography (TLC) plates coated with silica gel 60F254 were used for normal- and radio-TLC analysis and were purchased from EMD-Millipore. Normal TLC plates were visualized with $KMnO_4$ or anisaldehyde stain.

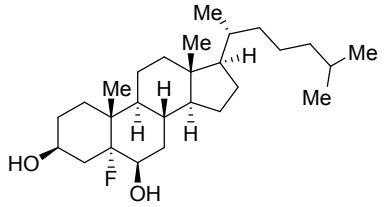
Reagents were purchased from Sigma Aldrich, Alfa Aesar, Oakwood, Fisher Scientific, EMD Millipore Corporation and Acros Organics. Ultrapure water was obtained from a Millipore MilliQ Gradient A10 system. Sterile vials were purchased from Hollister-Stier.

Safety and hazards: All hazardous laboratory chemicals were used by trained personnel under the supervision of Environmental Health and Safety at the University of Michigan.

Caution! Proper precautions must be used when handling anhydrous HF and its complexes such as pyridinium poly(hydrogen fluoride). Hydrogen fluoride and its complexes are extremely corrosive to human tissue, and contact with skin will result in painful, slow-healing burns. Laboratory work with HF and its complexes should be conducted only in an efficient hood, with the operator wearing a full-face shield and suitable protective clothing. See G. A. Olah and M. Watkins, *Org. Synth.,* **1978**, *58*, 75 and C. M. Sharts and W. A. Sheppard, *Org. React.*, **1974**, *21*(192), 220-223.

2. Experimental Procedures and Characterisation Data

(3*S*, 5*R*, 6*R*, 8*S*, 9*S*, 10*R*, 13*R*, 14*S*, 17*R*)-5-fluoro-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)hexadecahydro-1*H*-cyclopenta[a]phenanthrene-3,6-diol (1a)



A 15 mL falcon tube was charged with 5,6-epoxycholesterol[1] (201 mg, 0.50 mmol ; (5α,6α):(5β,6β) = 11:89) and DCM (1.5 mL) was added. The resulting solution was cooled in an ice-bath and HF/pyridine 65-70 %w/w (140 μL, 5 mmol) was added in one portion after which the cloudy mixture was vigorously stirred at 0 °C for 60 min. The crude reaction mixture was carefully poured into a mixture of ice and sat. $NaHCO_3$ solution (25 mL) and extracted with DCM (3 x 15 mL). The organic layers were washed with brine (25 mL), dried over Na_2SO_4, and concentrated in vacuo. Automated silica gel column chromatography (KP-Sil 10g column, eluent DCM/MeOH 97:3) gave the title compound as a white solid (81 mg, 38%). **(^{1}H, 400 MHz, $CDCl_3$)** δ 4.08 – 3.95 (m, 1H), 3.73 (br. s, 1H), 2.20 – 1.96 (m, 2H), 1.85 (tt, *J* = 13.8, 4.6 Hz, 3H), 1.76 – 1.44 (m, 11H), 1.44 – 1.20 (m, 5H), 1.19 – 1.03 (m, 10H), 0.90

(d, J = 6.4 Hz, 3H), 0.86 (dd, J = 6.6, 2.4 Hz, 6H), 0.68 (s, 3H); **^{13}C NMR (126 MHz, $CDCl_3$)** δ 99.1 (d, J = 166.7 Hz), 73.2 (d, J = 35.5 Hz), 67.4 (d, J = 4.3 Hz), 56.2, 55.7, 45.9, 42.7, 39.8, 39.5, 39.2 (d, J = 20.7 Hz), 38.3, 38.2, 36.1, 35.8, 34.9, 32.3, 30.6, 29.7, 28.1 (d, J = 23.2 Hz), 24.1, 23.8, 22.8, 22.5, 20.9, 18.7, 16.6 (d, J = 7.0 Hz), 12.1; **^{19}F NMR, 376 MHz, $CDCl_3$)** δ -159.8 (br. d, J = 43.3 Hz). **IR (neat)** ν 3558, 3425, 2936, 2865, 1042 ; HRMS (ESI) for $C_{27}H_{47}FNaO_2$ $[M+Na]^+$ requires 445.3452 found 445.4351; Mp: 177-178°C (dec.).

The trans opening of the epoxide was confirmed by treatment of **1a** with KOtBu, described by Henbest and Wrigley,[2] as follows :
To a vial under argon charged with (3*S*, 5*R*, 6*R*, 8*S*, 9*S*, 10*R*, 13*R*, 14*S*, 17*R*)-5-fluoro-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)hexadecahydro-1*H*-cyclopenta[a]phenanthrene-3,6-diol **1a** (20 mg, 0.047 mmol) and KOtBu (45 mg, 0.40 mmol) was added tBuOH (0.40 mL) and the resulting yellow mixture was stirred overnight (16 hrs) at room temperature. The reaction was quenched with sat. NH_4Cl solution (10 mL) and extracted with Et_2O (3 x 10 mL). The organic layers were washed with H_2O (10 mL), dried (Na_2SO_4), filtered and evaporated to give a colorless oil (18 mg). The ^{1}H NMR showed 5β,6β-epoxycholesterol as the main product, with the characteristic signal from the 6α-hydrogen at 3.05 ppm (d, J = 2.6 Hz).[1b] Only a very small signal (approx. 1%) was found for the 6β-hydrogen of 5α,6α-epoxycholesterol at 2.89 ppm (d, J = 4.4 Hz).[1b] The formation of 5β,6β-epoxycholesterol confirms the proposed structure of **1a**.

[1] a) Syamala, S.M.; Das, J.; Baskaran, S.; Chandrasekaran, S. *J. Org. Chem.* **1992**, *57*, 1928-1930; b) Bisogno, F.R.; Orden, A.A.; Pranzoni, C.A.; Cifuente, D.A.; Giordano, O.S.; Sanz, M. K. *Steroids*, **2007**, *72*, 643-652.
[2] Henbest, H.B.; Wrigley, T.I. *J. Chem. Soc.* **1957**, 4756-4758.

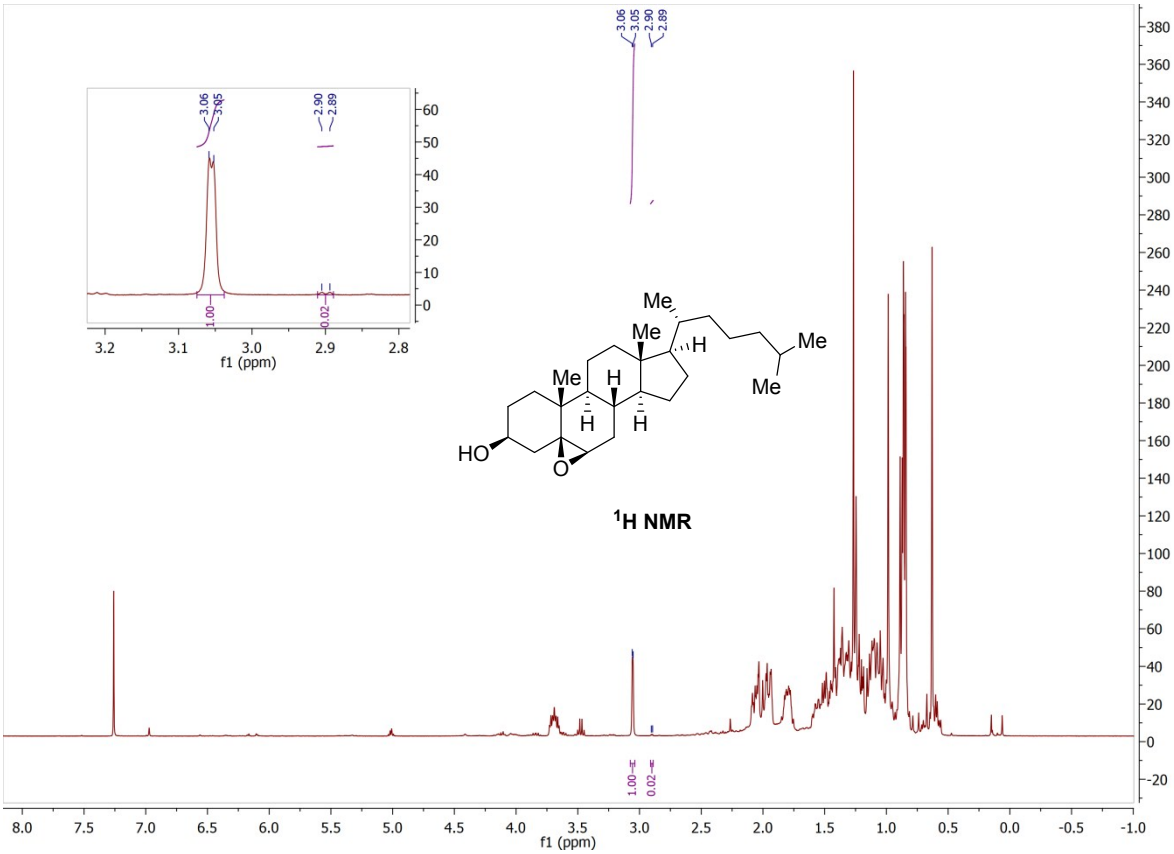
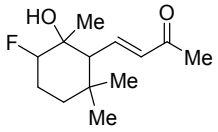


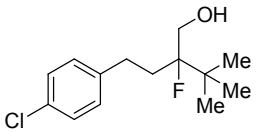
Figure S1. ^{1}H NMR of the colorless oil obtained after treatment of **1a** with KOtBu in tBuOH.

(*E*)-4-(3-fluoro-2-hydroxy-2,6,6-trimethylcyclohexyl)but-3-en-2-one (2a)



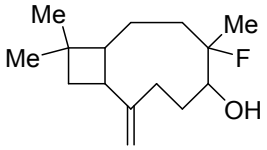
To a solution of 4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)-3-buten-2-one (technical grade, 104 mg, 0.50 mmol) in $CHCl_3$ (3.8 mL) in a 15 mL falcon tube was added $Fe(acac)_3$ (177 mg, 0.50 mmol) giving a dark-red solution. HF/pyridine (65-70 %w/w, 83.8 µl, 3.0 mmol) was added in one portion, upon which the solution turned dark brown followed by a gradual change to a pale brown light suspension. The mixture was stirred for 75 min at room temperature, after which it was quenched with a solution of 0.1M $(NH_4)_2EDTA$ in H_2O and extracted with DCM (3 x 15 mL). The organic layers were washed with sat. $NaHCO_3$ (25 mL), brine (25 mL), dried over Na_2SO_4 and dry-loaded onto Celite®. Automated silica gel column chromatography (KP-Sil 10g column, eluent : gradient EtOAc/hexane 9:1 to 8:2) provided the title compound as a white solid (66 mg, 0.28 mmol, 58% yield). **(^{1}H, 500 MHz, $CDCl_3$)** δ 6.94 (dd, J = 16.1, 10.6, 1H), 6.11 (d, J = 16.1 Hz, 1H), 4.31 (ddd, J = 47.4, 3.6, 1.8 Hz, 1H), 2.30 (s, 3H), 2.14 (dtdd, J = 46.7, 14.7, 4.2, 2.0 Hz, 1H), 2.05 (dd, J = 10.5, 2.2 Hz, 1H), 1.83 (ddq, J = 14.4, 10.4, 3.4 Hz, 1H), 1.61 (td, J = 14.0, 3.8 Hz, 2H), 1.36 – 1.23 (m, 2H), 1.19 (d, J = 2.9 Hz, 3H), 1.06 (s, 3H), 0.83 (s, 3H); **(^{13}C, 125 MHz, $CDCl_3$)** δ 198.3, 145.3, 135.1, 94.2 (d, J = 176.2 Hz), 71.7 (d, J = 22.7 Hz), 53.5 (d, J = 1.6 Hz), 33.72 (d, J = 2.0 Hz), 33.67, 32.1, 27.7 (d, J = 2.3 Hz), 27.2, 23.1 (d, J = 20.7 Hz), 21.8; **(^{19}F NMR, 376 MHz, $CDCl_3$)** δ -188.2 (ttt, J = 47.1, 11.5, 2.7 Hz, 1F) ; **IR (neat)** ν 3467, 2958, 2923, 1660, 1630, 1257, 955; HRMS (ESI) for $C_{13}H_{21}FNaO_2$ $[M+Na]^+$ requires 251.1418 found 251.1423; Mp: 101 – 103 °C.

2-(4-chlorophenethyl)-2-fluoro-3,3-dimethylbutan-1-ol (3a)



To a solution of 2-*tert*-butyl-2-[2-(4-chlorophenyl)ethyl]oxirane (0.24g, 1.0 mmol) in $CHCl_3$ (7.5 mL) in a 45 mL falcon tube was added $Fe(acac)_3$ (353 mg, 1.0 mmol) giving a dark-red solution. HF/pyridine (65-70 %w/w, 168 µl, 6.0 mmol) was added in one portion, upon which the solution turned dark brown followed by a gradual change to a pale yellow light suspension. The mixture was stirred for 90 min at room temperature, after which it was quenched with a solution of 0.1M $(NH_4)_2EDTA$ in H_2O and extracted with DCM (3 x 15 mL). The organic layers were washed with sat. $NaHCO_3$ (25 mL), brine (25 mL), dried over Na_2SO_4 and evaporated *in vacuo* to give a yellow oil. Automated silica gel column chromatography (KP-Sil 10g column, eluent : gradient EtOAc/hexane 9:1 to 8:2) provided the title compound as a colorless oil (40 mg, 15% yield). **(^{1}H, 500 MHz, $CDCl_3$)** δ 7.20 – 7.16 (m, 2H), 7.10 – 7.05 (m, 2H), 3.85 (td, J = 11.9, 4.3 Hz, 1H), 3.78 – 3.68 (m, 1H), 2.78 – 2.63 (m, 2H), 2.05 – 1.85 (m, 2H), 1.52 – 1.57(m, OH), 0.95 (d, J = 1.1 Hz, 9H); **(^{13}C, 126 MHz, $CDCl_3$)** δ 141.1, 131.6, 129.7, 128.5, 100.4 (d, J = 177.2 Hz), 63.5 (d, J = 28.6 Hz), 37.3 (d, J = 21.5 Hz), 33.8 (d, J = 22.8 Hz), 30.0 (d, J = 8.7 Hz), 25.5 (d, J = 5.2 Hz); **(^{19}F NMR, 376 MHz, $CDCl_3$)** δ -173.5 – -173.8 (m, 1F). **IR (neat)** ν 3405, 2965, 2876, 1491, 1092, 1041, 1014, 806; HRMS (ESI) for $C_{13}H_{21}FNaO_2$ $[M+Na]^+$ requires 281.1079 found 281.082.

6-fluoro-6,10,10-trimethyl-2-methylenebicyclo[7.2.0]undecan-5-ol (4a)



To a solution of (-)-caryophyllene oxide (0.22 g, 1.0 mmol) in $CHCl_3$ (7.5 mL) in a 45 mL falcon tube was added $Fe(acac)_3$ (353 mg, 1.0 mmol) giving a dark-red solution. HF/pyridine (65-70 %w/w, 168 µl, 6.0 mmol) was added in one portion, upon which the solution turned dark brown followed by a gradual change to a pale yellow light suspension. The mixture was stirred for 60 min at room temperature, after which it was quenched with a solution of 0.1M $(NH_4)_2EDTA$ in H_2O and extracted with DCM (3 x 15 mL). The organic layers were washed with sat. $NaHCO_3$ (25 mL), brine (25 mL), dried over Na_2SO_4 and dry-loaded onto Celite®. Automated silica gel column chromatography (KP-Sil 25g column, eluent : gradient EtOAc/hexane 95:5 to 9:1) provided the title compound as a colorless oil (24 mg, 10% yield). **(^{1}H, 500 MHz, $CDCl_3$)** δ 4.94 (br. d, J = 7.2 Hz, 2H), 3.87 – 3.81 (m, 1H), 2.49 – 2.40 (dtd, J = 14.0, 7.0, 6.6, 2.2 Hz, 1H), 2.40 – 2.31 (m, 1H), 2.20 (br. d, J = 5.8 Hz, 1H), 2.08 – 1.91 (m, 2H), 1.83 – 1.56 (m, 8H), 1.38 – 1.23 (m, 5H), 0.99 (dd, J = 15.2, 2.4 Hz, 6H); **(^{13}C, 125 MHz, $CDCl_3$)** δ 151.5, 110.8, 100.5 (d, J = 164.6 Hz), 72.0 (d, J = 20.1 Hz), 56.6, 42.1, 38.5 (d, J = 23.8 Hz), 36.2, 34.3, 34.1, 31.4 (d, J = 5.8 Hz), 30.0, 22.7 (d, J = 9.0 Hz), 22.0, 18.9, 18.7; **(^{19}F NMR, 470 MHz, $CDCl_3$)** δ -141.2 – -141.7 (m, 1F). **IR (neat)** ν 3452, 2929, 2862, 1634, 1082, 884; HRMS (ESI) for $C_{13}H_{21}FNaO_2$ $[M+Na]^+$ requires 263.1782 found 263.1786.

3. Radiochemistry

3.1 General Experimental Information

Unless otherwise stated, reagents and solvents were commercially available and used without further purification: sodium chloride, 0.9% USP, and sterile water for injection, USP, were purchased from Hospira; ethanol was purchased from American Regent; HPLC grade acetonitrile was purchased from Fisher Scientific. Other synthesis components were obtained as follows: sterile filters were obtained from Millipore; sterile product vials were purchased from Hollister-Stier; QMA-light and Alumina Sep-Paks were purchased from Waters Corporation. QMA-light Carb Sep-Paks were prepared by flushing with 10 mL of water. Alumnia neutral Sep-Paks were flushed with 10 mL of CH_3CN prior to use. Analytical HPLC was performed using a Shimadzu LC-2010A HT system equipped with a Bioscan B-FC-1000 radiation detector and a UV detector.

3.2 Elution Experiments

3.2.1 Preparation of aqueous Fluorine-18 for elution studies

All loading operations were conducted under an ambient atmosphere and argon was used a pressurizing gas during automated sample transfer. Fluorine-18 was produced by the $^{18}O(p, n)^{18}F$ nuclear reaction using a GE PETTrace cyclotron (a 55 µA beam for 15 sec generated approx. 30 mCi (1.1 GBq) of fluorine-18) and delivered to a GE TRACERLab FX_{FN} automated radiochemist synthesis module in a 2.5 mL bolus of $[^{18}O]H_2O$ which was collected in a 10 mL sterile vial. Aqueous fluorine-18 produced this way was diluted with Milli-Q water to ca. 1 – 3 mCi/mL (37 – 110 MBq/mL) prior to use in fluorine-18 elution studies.

3.2.2 General Elution Procedure using solutions of acid in H_2O.

Aqueous fluorine-18 (0.5 mL, approx. 0.5 – 1.5 mCi (18.5 – 55.5 MBq)) was passed through a Waters QMA SepPak Light Carb cartridge (Waters, order# WAT023525 ; activated with 10 mL H_2O) followed by H_2O (2 mL) and air (2 mL), and the activity on the QMA was determined using a Capintec® dose calibrator. [^{18}F]HF was then eluted from the QMA into a 4 mL vial with a solution of acid in H_2O (1 mL) followed by air (5 mL). The activity of the 4 mL vial (eluate) and the QMA (residual QMA activity, [^{18}F]F$^-$) were measured using a Capintec® dose calibrator.

3.2.3 General Elution Procedure using solutions of acid in organic solvent.

Aqueous fluorine-18 (0.5 mL, approx. 0.5 – 1.5 mCi (18.5 – 55.5 MBq)) was passed through a Waters QMA SepPak Light Carb cartridge (Waters, order# WAT023525 ; activated with 10 mL H_2O) followed by H_2O (2 mL), air (2 mL) and organic solvent (3 mL), and the activity on the QMA was determined using a Capintec® dose calibrator (Initial QMA activity). [^{18}F]HF was then eluted from the QMA into a 4 mL vial with a solution of acid in organic solvent (1 mL) followed by air (5 mL). The activity of the 4 mL vial (eluate activity, [^{18}F]HF) and the QMA (residual QMA activity, [^{18}F]F$^-$) were measured using Capintec® dose calibrator.

Activity data from these elution procedures was used to calculate the percentage of [^{18}F]HF recovery as follows :

$$\%[^{18}F]HF\ recovery = \frac{Eluate\ activity}{(Eluate\ activity + Residual\ QMA\ activity)}$$

[^{18}F]HF recovery is equal to elution efficiency.

3.2.4 Elution Study Results using $MeSO_3H$ in H_2O

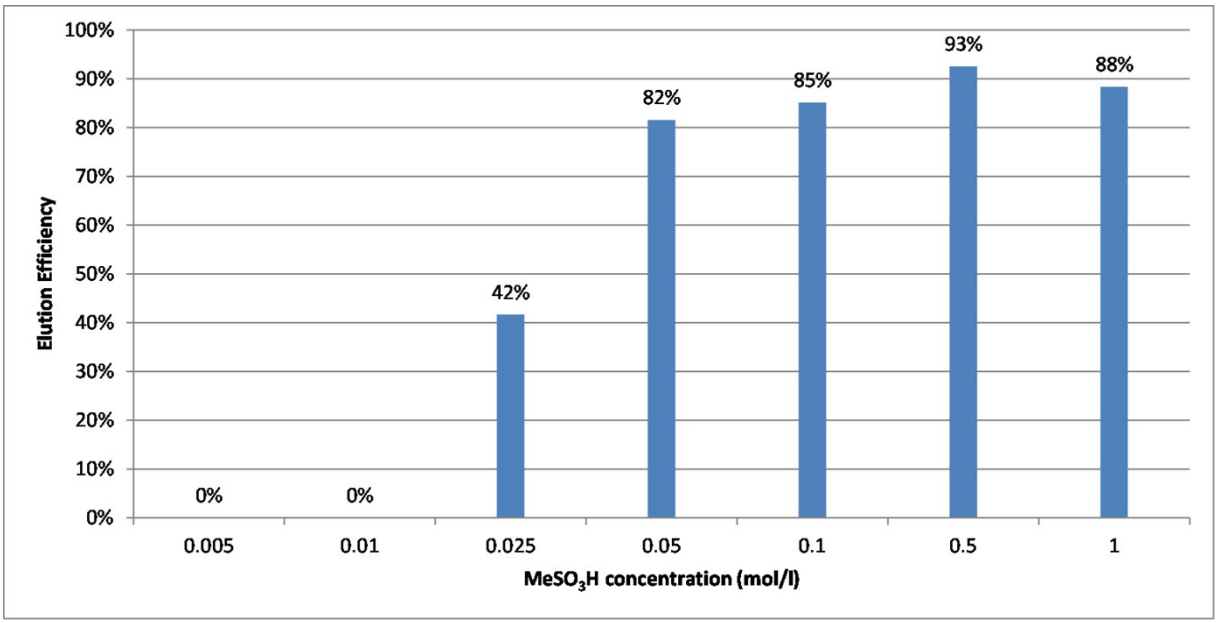


Figure S2. [^{18}F]HF recovery from elution of [^{18}F]F$^-$ with $MeSO_3H$ in H_2O

[$MeSO_3H$] in H_2O (mol/l)	pH	QMA initial		QMA residual		Eluate		[^{18}F]HF recovery
		µCi	MBq	µCi	MBq	µCi	MBq	
0.01	2.00	687	25.4	685	25.3	0	0	0%
0.025	1.60	1040	38.5	578	21.4	413	15.3	42%
0.05	1.30	1120	41.4	199	7.4	880	32.6	82%
0.10	1.00	1120	41.4	157	5.8	900	33.3	85%
0.50	0.30	1140	42.2	84	3.1	1037	38.4	93%
1.00	0.00	1040	38.5	118	4.4	895	33.1	88%

Table S1. Determination of [^{18}F]HF recovery from elution of [^{18}F]F^- with $MeSO_3H$ in H_2O.

3.2.5 Elution Study Results using acids of different strengths

Eluent (as 0.50 M solution in H_2O)	pKa	QMA initial		QMA residual		Eluate		[^{18}F]HF recovery
		µCi	MBq	µCi	MBq	µCi	MBq	
$MeSO_3H$	-2.60	1140	42.2	84	3.1	1037	38.4	93%
p-TosOH	-0.51	1280	47.4	113	4.2	1080	40.0	91%
CF_3CO_2H	-0.25	788	29.2	73	2.7	670	24.8	90%
CCl_3CO_2H	0.65	1270	47.0	235	8.7	1010	37.4	81%
$(CO_2H)_2$	1.23	690	25.5	109	4.0	571	21.1	83%
$CHCl_2CO_2H$	1.29	1650	61.1	423	15.7	1170	43.3	71%
$KHSO_4$	1.99	1260	46.6	162	6.0	990	36.6	79%
$(NH_4)_2EDTA$	2.00	1270	47.0	92	3.4	1130	41.8	89%
H_3PO_4	2.12	785	29.0	93	3.4	676	25.0	86%
CH_2ClCO_2H	2.86	1320	48.8	540	20.0	741	27.4	56%
Citric acid	3.13	1230	45.5	758	28.0	439	16.2	36%
2-OH-isobutyric acid	3.86	1294	47.9	1193	44.1	78	2.9	6%
Ascorbic acid	4.17	1190	44.0	1040	38.5	136	5.0	11%
CH_3COOH[a]	4.86	654	24.2	628	23.2	0	0	0%

[a] Neat AcOH (0.5 mL) was used to elute the QMA

Table S2. Determination of [^{18}F]HF recovery from elution of [^{18}F]F^- with a 0.5M solution of acids of various strength in H_2O (**Blue** = monoprotic acids ; **Red** = diprotic acids; **Green** = Triprotic Acids; **Yellow** = Tetraprotic acids)

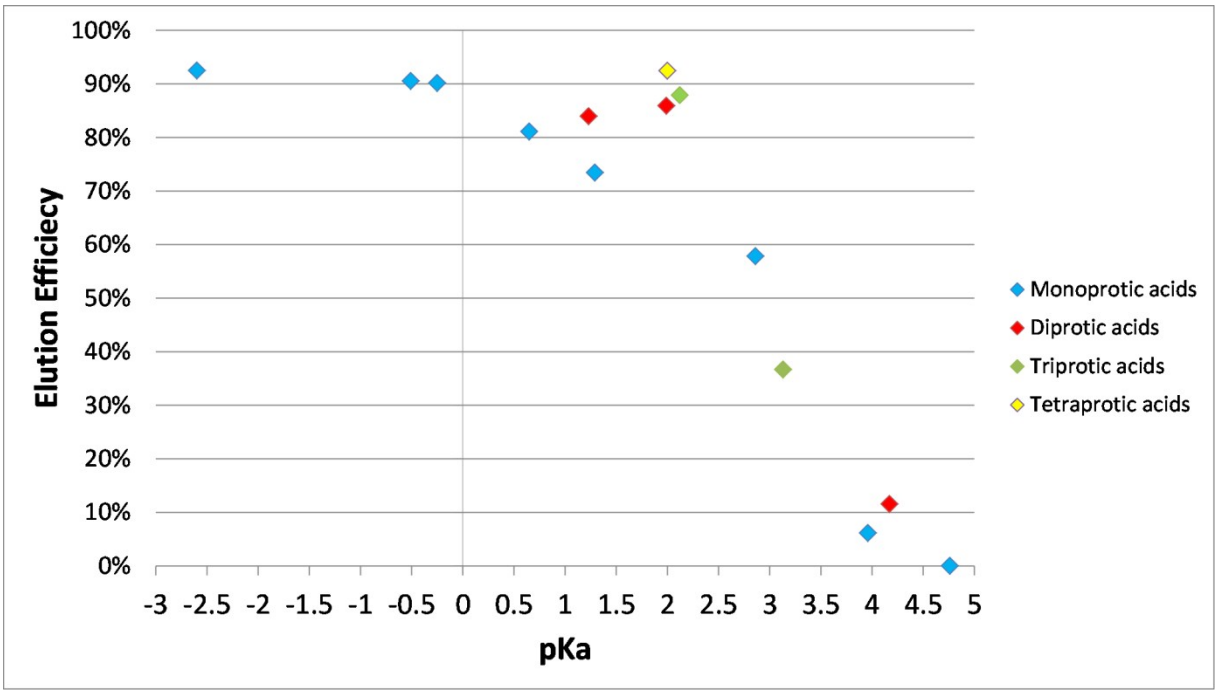


Figure S3. Elution efficiency ([^{18}F]HF recovery) results using 0.5M solutions in H_2O of acids of various strength.

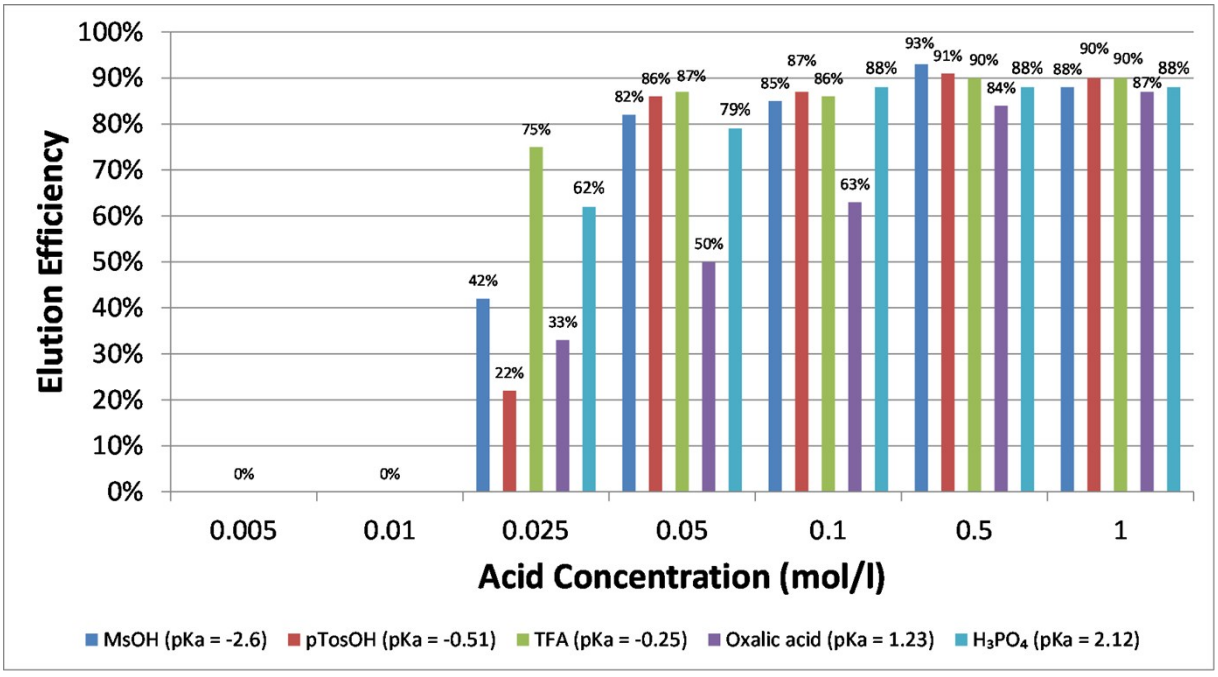


Figure S4. Elution efficiency ([^{18}F]HF recovery) results using solutions of different concentrations of acids.

3.2.6 Elution results using solutions of $MeSO_3H$ in various organic solvents

[$MeSO_3H$] in DMA (mol/l)	QMA initial		QMA residual		Eluate		[^{18}F]HF recovery
	µCi	MBq	µCi	MBq	µCi	MBq	
0.01	1040	37.5	1030	38.1	0	0	0%
0.025	1020	37.4	870	32.2	131	4.8	13%
0.05	1070	38.5	465	17.2	551	20.4	54%
0.10	876	32.4	273	10.1	569	21.1	68%
0.50	890	32.9	106	3.9	766	28.3	88%
1.00	860	31.8	117	4.3	720	26.6	86%

Table S3. Determination of [^{18}F]HF recovery from elution of [^{18}F]F^- with $MeSO_3H$ in DMA.

[$MeSO_3H$] in DMF (mol/l)	QMA initial		QMA residual		Eluate		[^{18}F]HF recovery
	µCi	MBq	µCi	MBq	µCi	MBq	
0.01	1170	43.3	1160	42.9	0	0	0%
0.025	1217	45.0	589	21.8	575	21.3	49%
0.05	1340	49.6	418	15.5	885	32.7	68%
0.10	1220	45.1	371	13.7	751	27.8	67%
0.50	1370	50.7	168	6.2	1153	42.7	87%
1.00	1270	47.0	202	7.5	980	36.3	83%

Table S4. Determination of [^{18}F]HF recovery from elution of [^{18}F]F^- with $MeSO_3H$ in DMF.

[$MeSO_3H$] in EtOH (mol/l)	QMA initial		QMA residual		Eluate		[^{18}F]HF recovery
	µCi	MBq	µCi	MBq	µCi	MBq	
0.01	1060	39.2	1050	38.9	0	0	0%
0.025	1110	41.1	960	35.5	113	4.2	11%
0.05	1180	43.7	472	17.5	684	25.3	59%
0.10	942	34.9	291	10.8	612	22.6	68%
0.50	1200	44.4	309	11.4	853	31.6	73%
1.00	960	35.5	137	5.1	751	27.8	85%

Table S5. Determination of [^{18}F]HF recovery from elution of [^{18}F]F^- with $MeSO_3H$ in EtOH.

[$MeSO_3H$] in CH_3CN (mol/l)	QMA initial		QMA residual		Eluate		[^{18}F]HF recovery
	µCi	MBq	µCi	MBq	µCi	MBq	
0.01	850	31.5	850	31.5	0	0	0%
0.025	940	34.8	930	34.4	0	0	0%
0.05	940	34.8	910	33.7	20	0.7	2%
0.10	970	35.9	730	27.0	201	7.4	22%
0.50	1060	39.2	722	26.7	310	11.5	30%
1.00	950	35.2	734	27.2	189	7.0	20%

Table S6. Determination of [^{18}F]HF recovery from elution of [^{18}F]F^- with $MeSO_3H$ in CH_3CN.

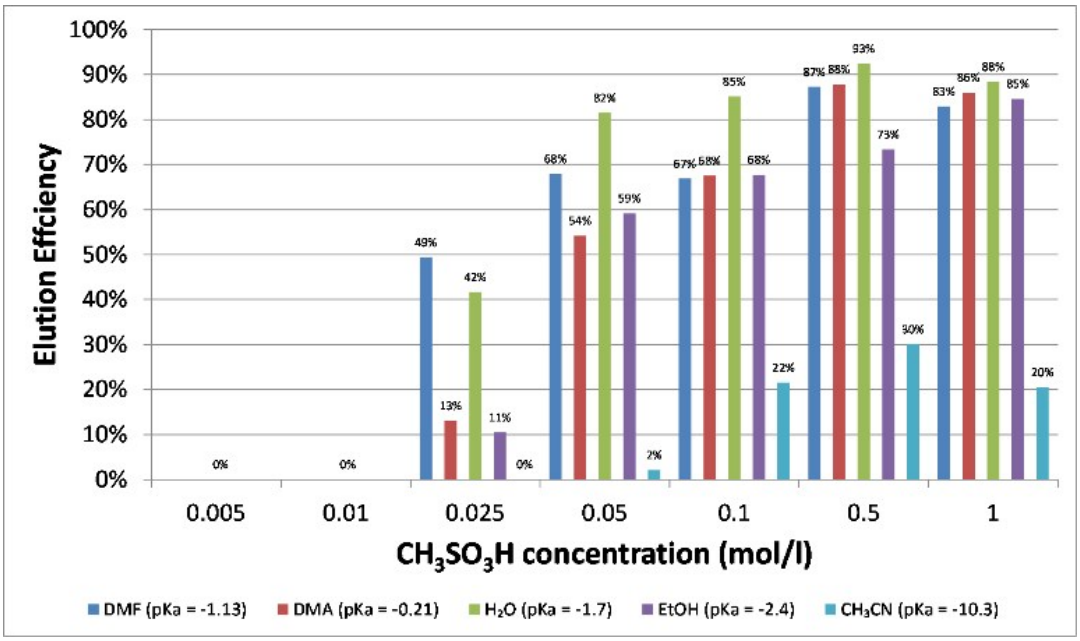


Figure S5: Elution efficiency versus molarity of methanesulfonic acid for five commonly used radiofluorination solvents.

3.3 ^{18}F-Fluorination/ring opening of epoxides

[^{18}F]1a, [^{18}F]2a, [^{18}F]3a and [^{18}F]4a were prepared using a TRACERLab FX_{FN} automated radiochemistry synthesis module (General Electric, GE) in standard configuration using a glassy carbon reactor.

3.3.1 ^{18}F-Production Curve

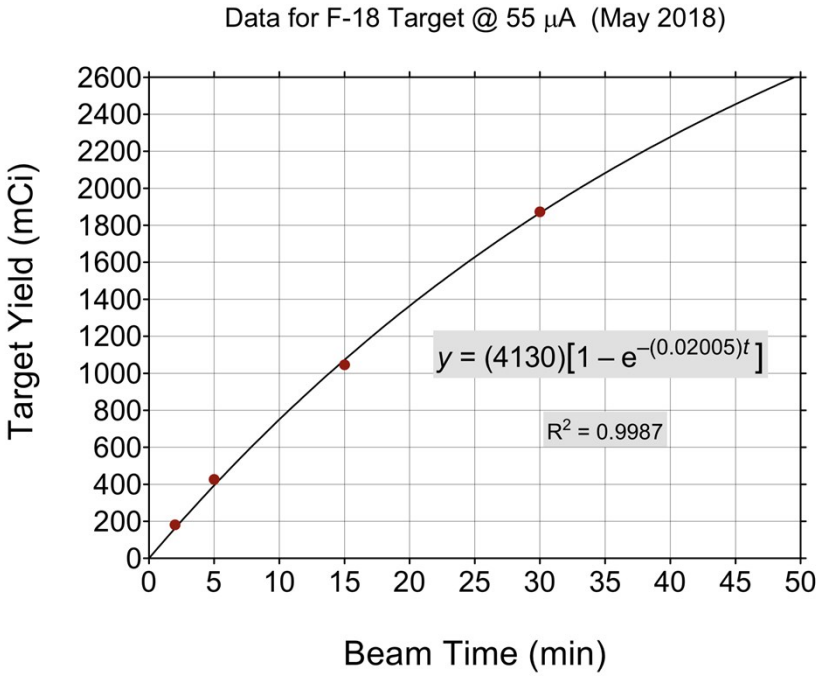
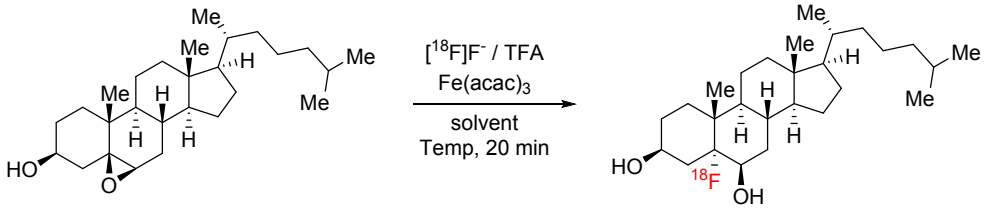


Figure S6: ^{18}F-Production Curve at 55 μA

3.3.2 Reaction Optimization General Procedure

Fluorine-18 was produced by the $^{18}O(p, n)^{18}F$ nuclear reaction using a GE PETTrace cyclotron (a 55 µA beam for 1 minute generated approx. 104 mCi (3.8 GBq) of fluorine-18) and delivered to a GE TRACERLab FX_{FN} automated radiochemistry synthesis module in a 2.5 mL bolus of $[^{18}O]H_2O$ followed by trapping on a Waters QMA SepPak Light Carb cartridge (Waters, order# WAT023525 ; activated with 10 mL H_2O) as $[^{18}F]F^-$ to remove $[^{18}O]H_2O$ and other impurities. This was followed by elution (as $[^{18}F]HF$) with a solution of acid in CH_3CN/H_2O 4:1 (500 µL) from vial 1 into the reactor, which had been charged with $Fe(acac)_3$ (0.04 mmol, 14 mg). The reactor was then pressurized with argon to approx. 200 kPa (by opening valve 20 for 3 s) and heated at 80 °C for 10 min to allow capture of $[^{18}F]HF$ by $Fe(acac)_3$. The resulting mixture was azeotropically dried at 100 °C under vacuum for 5 min, followed by heating with vacuum and argon flow for another 5 min. The reactor was then cooled to 60 °C using compressed air, and a solution of epoxide (0.04 mmol) in dioxane (500 µL) was added from vial 3 using argon push gas. The reactor was heated to the desired reaction temperature and stirred for 20 min under autogenous pressure. After cooling to 50 °C using compressed air, a solution of EtOH:H_2O (4:1, 3.5 mL) for **[^{18}F]1a** or 50% CH_3CN/10mM NH_4OAc (3.5 mL) containing $(NH_4)_2EDTA$ (28 mg, 0.08 mmol) for **[^{18}F]2a** was added to the reactor from vial 6 using push gas. The content of the reactor was then pushed with argon through a Waters Al_2O_3 N SepPak Light (activated with 4 mL EtOH or 4 mL CH_3CN) into a 20 mL vial for analysis. The activity in the collected fraction was measured using a Capintec® dose calibrator and an aliquot of the collected fraction was analyzed by radio-HPLC to determine product identity and calculate radiochemical conversion. Radiochemical conversion (RCC) was calculated by dividing the activity in the collected fraction by the starting activity (104 mCi) and multiplying with the percentage of radiochemical purity, and was non decay corrected.

Note : analysis with radioTLC was attempted but found to be unreliable, as a blank reaction (no epoxide added) showed non-baseline radioactive peaks (eluent DCM/MeOH 9/1 or EtOAc/Hexane 50/50) that would interfere with RCC measurement.



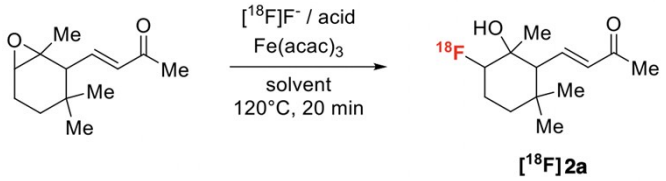
5α,6α/5β,6β = 11:89

[^{18}F]1a

Entry	Solvent	Acid	Temp.	RCC [^{18}F]1a[a,b]
1	dioxane	TFA (0.50 M)	100 °C	5 %
2	dioxane	TFA (0.12 M)	100 °C	0.2 %
3	DMF	TFA (0.50 M)	100 °C	0 %
4	dioxane	TFA (0.50 M)	80 °C	1 %
5	dioxane	TFA (0.50 M)	120 °C	22 %
6	dioxane	TFA (0.50 M)	140 °C	<0.1%

a) Calculated from the HPLC radiotrace as described in the general procedure ; b) Phenomenex Luna C18(2) 250x4.6mm 5µ, eluent = 100% CH_3CN for 10 min followed by 50% CH_3CN/H_2O for 10 min and equilibration back to 100% CH_3CN for 10 min, flowrate = 2 ml/min.

Table S7. Reaction optimization using **[^{18}F]1a**

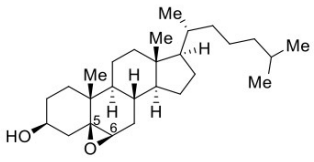
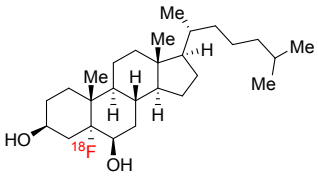
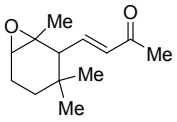
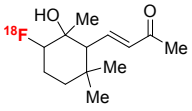
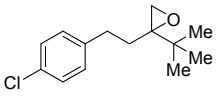
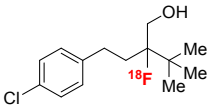
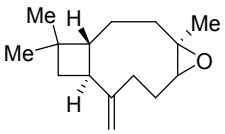
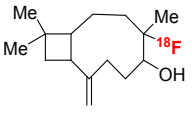


Entry	Additive	Acid	Solvent	Temp.	RCC [18F]2a[a,b]
1	$Fe(acac)_3$	TFA (0.50 M)	dioxane	120 °C	4 %
2	$Fe(acac)_3$	TFA (0.40 M)	dioxane	120 °C	2.8 %
3	$Fe(acac)_3$	TFA (0.30 M)	dioxane	120 °C	1.8 %
4	$Fe(acac)_3$	TFA (0.50 M)	CH_3CN	120 °C	<0.1 %
5	$Fe(acac)_3$	TFA (0.50 M)	DME	120 °C	0.5 %
6	$Fe(acac)_3$	TFA (0.50 M)	Sulfolane/ dioxane 9:1	120 °C	1 %

a) Calculated from the HPLC radiotrace as described in the general procedure ; b) Phenomenex Synergi Hydro RP 250x4.6mm, eluent = 50% CH_3CN/10mM NH_4OAc, flowrate = 1 ml/min.

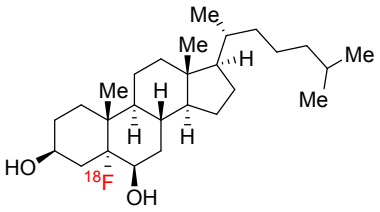
Table S8. Reaction optimization using **[18F]2a**

3.3.3 Isolation Procedures

Entry[a]	Substrate	Product	Isolated Activity		RCP
			GBq	mCi	
1	 (1)	 (1a)	7.83 ± 0.87 (*n* = 5)	212 ± 23 (*n* = 5)	99%
2	 (2)	 (2a)	3.57 ± 0.39 (*n* = 2)	97 ± 11 (*n* = 2)	99%
3	 (3)	 (3a)	0.54 ± 0.23 (*n* = 3)	15 ± 7 (*n* = 3)	94%
4	 (4)	 (4a)	0.90 ± 0.11 (*n* = 3)	24 ± 3 (*n* = 3)	99%

[a] Cyclotron produced fluorine-18 (approx.. 66.6 Gbq, 1.8 Ci), elution solution = TFA in CH_3CN/H_2O 4:1 (0.5M, 1.0 mL), substrate (0.04 mmol), $Fe(acac)_3$ (0.08 mmol) in dioxane (0.5 mL) stirred at 120°C for 20 min.

(3*S*,5*R*,6*R*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-5-[^{18}F]-fluoro-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)hexadecahydro-1H-cyclopenta[a]phenanthrene-3,6-diol ([^{18}F]1a)



Fluorine-18 was produced by the $^{18}O(p, n)^{18}F$ nuclear reaction using a GE PETTrace cyclotron (a 55 µA beam for 30 minutes generated approx. 1.8 Ci (66.6 GBq) of fluorine-18) and delivered to a GE TRACERLab FX_{FN} automated radiochemistry synthesis module in a 2.5 mL bolus of $[^{18}O]H_2O$ followed by trapping on a Waters QMA SepPak Light Carb cartridge (Waters, order# WAT023525 ; activated with 10 mL H_2O) as $[^{18}F]F^-$ to remove $[^{18}O]H_2O$ and other impurities. This was followed by elution (as $[^{18}F]HF$) with a solution of TFA in CH_3CN/H_2O 4:1 (0.5 M, 500 µL) from vial 1 into the reactor, which had been charged with $Fe(acac)_3$ (0.04 mmol, 14 mg). The reactor was then pressurized with argon to approx. 200 kPa (by opening valve 20 for 3 s) and heated at 80 °C for 10 min to allow capture of $[^{18}F]HF$ by $Fe(acac)_3$. The resulting mixture was azeotropically dried at 100 °C under vacuum for 5 min, followed by heating with vacuum and argon flow for another 5 min. The reactor was then cooled to 60 °C using compressed air, and a solution of (5β,6β)-epoxycholesterol (**1**)[1] (0.04 mmol, 18 mg; ratio (5α,6α) : (5β,6β) = 11:89) in dioxane (500 µL) was added from vial 3 using argon push gas. The reactor was heated to 120 °C and stirred for 20 min under autogenous pressure. After cooling to 50 °C using compressed air, a solution of EtOH:H_2O (4:1, 3.5 mL) was added to the reactor from vial 6 using push gas. The content of the reactor was then pushed with argon through a Waters Al_2O_3 N SepPak Light (activated with 4 mL EtOH) into the intermediate vial and loaded onto a semi-prep HPLC column (Agilent Eclipse XDB 250x9.4mm 5µ, eluent = 80% EtOH/H_2O, flowrate = 3 mL/min) for purification. The fraction at Rt = 24.1 – 26.4 min was collected to give **[^{18}F]1a**. An aliquot of the collected fraction was analyzed by radio-HPLC (Phenomenex Luna C18(2) 250x4.6mm 5µ, eluent = 100% CH_3CN, flowrate = 2 ml/min) to determine radiochemical identity and purity.

Run	Isolated activity [^{18}F]1a		Radiochemical purity
	mCi	GBq	
1	189.6	7.01	99%
2	251	9.28	99%
3	202	7.47	99%
4	205	7.58	99%
5	211	7.81	99%

Table S9. Isolated activities of **[^{18}F]1a**.

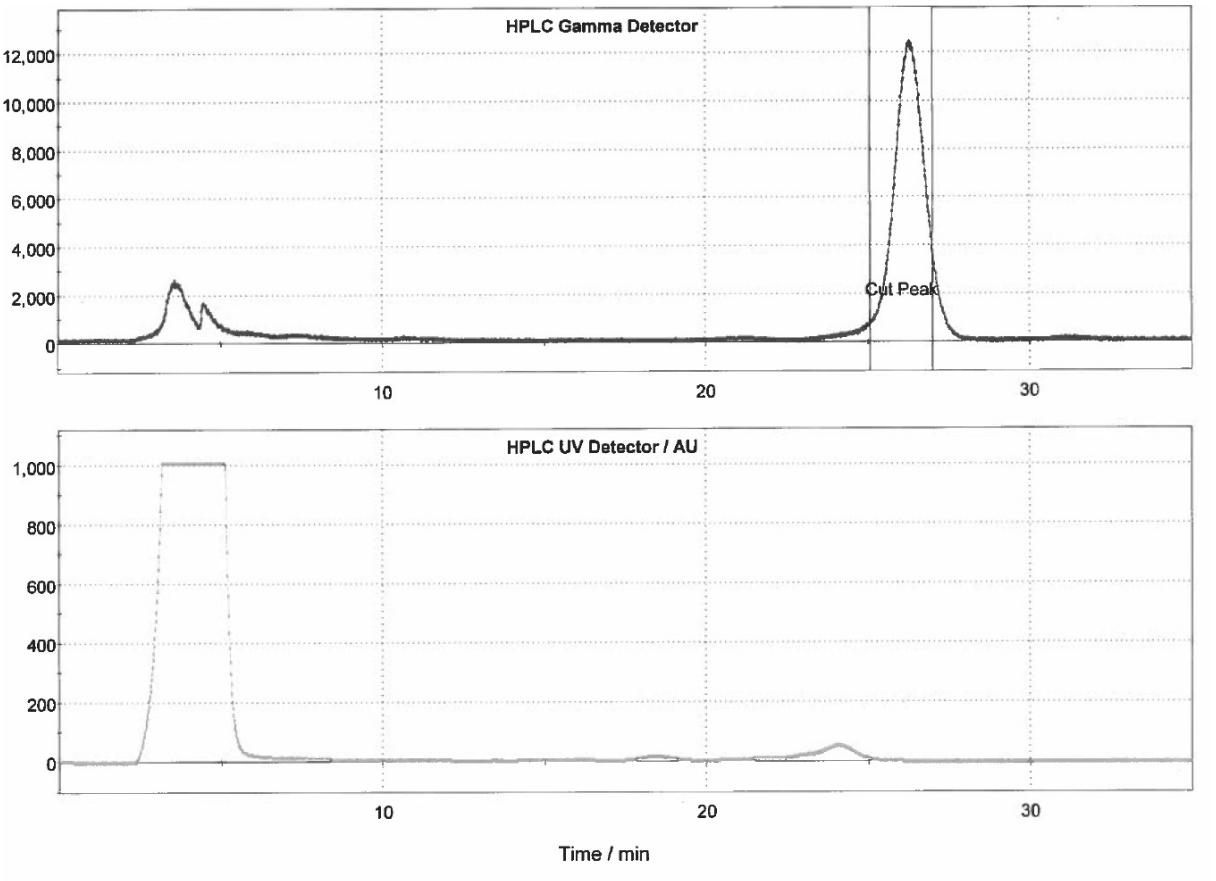


Figure S7. Crude prep HPLC radiotrace (top) and UV trace (254 nm, bottom) of **[^{18}F]1a**

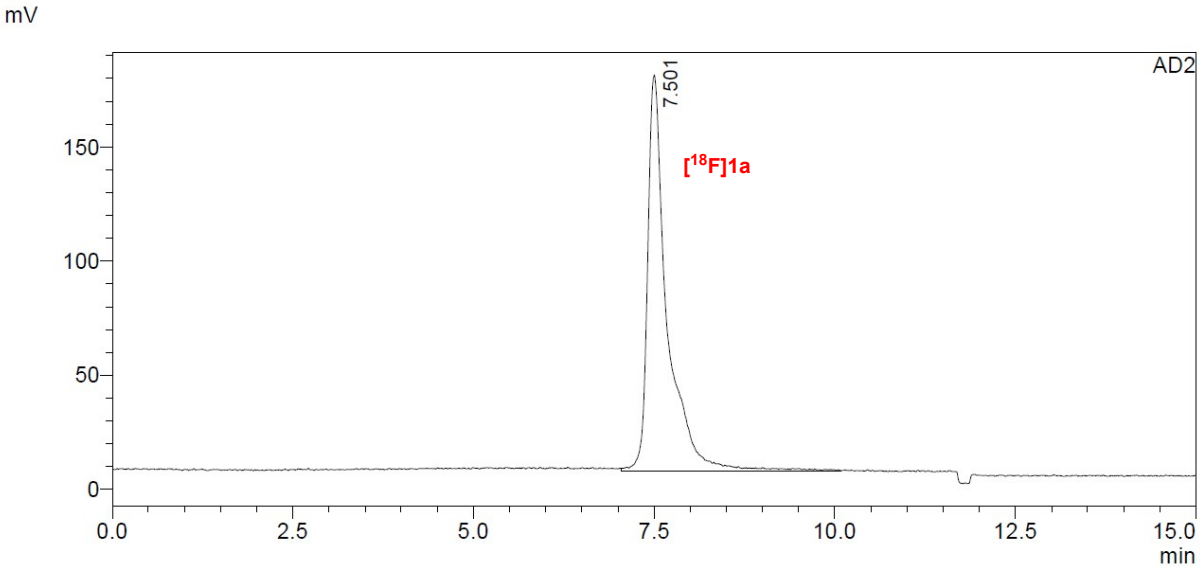


Figure S8. HPLC radiotrace of collected fraction of **[^{18}F]1a**

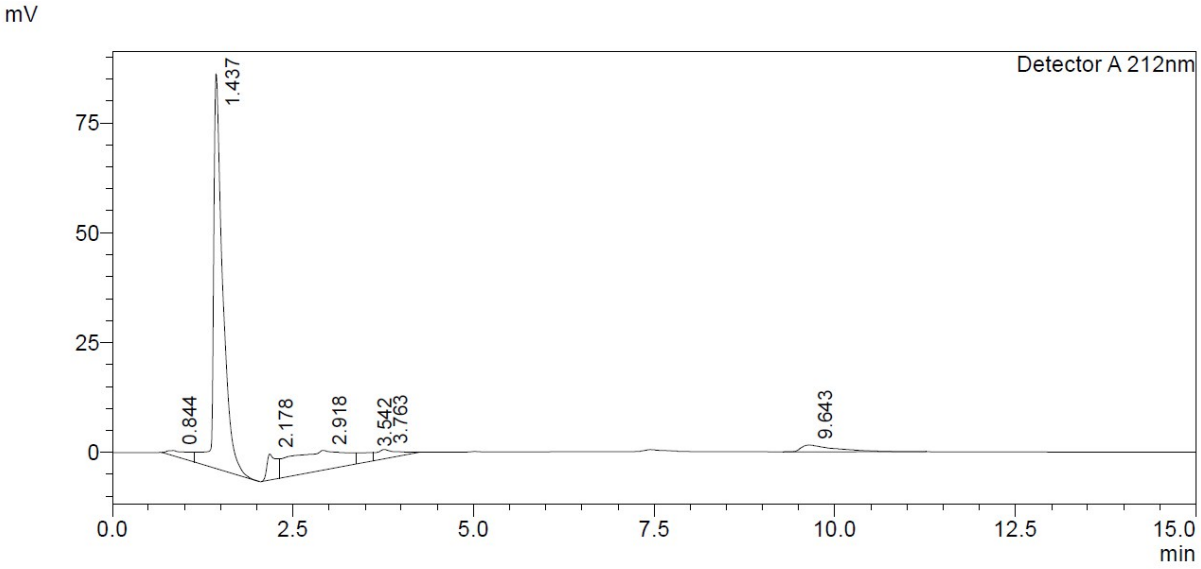


Figure S9. HPLC UV trace (212 nm) of collected fraction of **[^{18}F]1a**

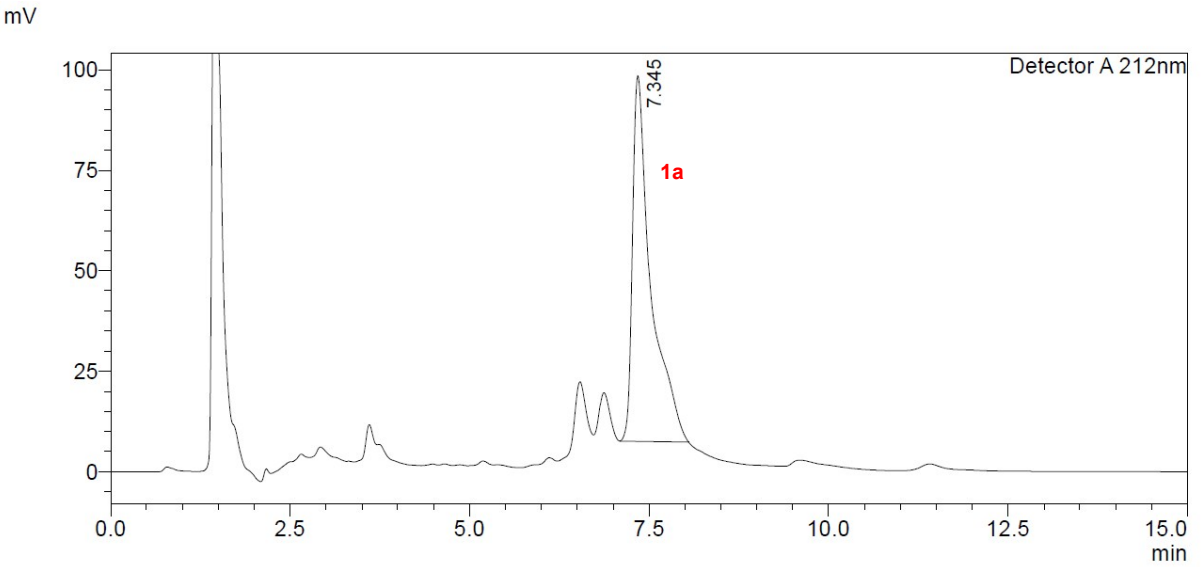
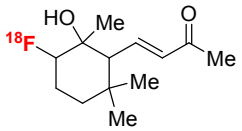


Figure S10. HPLC UV trace (254 nm) of collected fraction of **[^{18}F]1a** spiked with **1a** (Rt = 7.34 min)

(*E*)-4-(3-[^{18}F]-fluoro-2-hydroxy-2,6,6-trimethylcyclohexyl)but-3-en-2-one ([^{18}F]2a)



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by heating with vacuum and argon flow for another 5 min. The reactor was then cooled to 60 °C using compressed air, and a solution of 4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)-3-buten-2-one (**2**) (technical grade, 8 mg, 0.04 mmol) in dioxane (500 μL) was added from vial 3 using argon push gas. The reactor was heated to 120 °C and stirred for 20 min under autogenous pressure. After cooling to 50 °C using compressed air, a solution of 40% CH_3CN/10mM NH4OAc (3.5 mL) containing $(NH_4)_2EDTA$ (28 mg, 0.08 mmol) was added to the reactor from vial 6 using push gas. The content of the reactor was then loaded onto a semi-prep HPLC column (Agilent Eclipse XDB 250x9.4mm 5μ, eluent = 40% CH_3CN/10mM NH4OAc, flowrate = 4 mL/min) for purification. The fraction at Rt = 17.5 – 20.1 min was collected to give **[^{18}F]2a**. An aliquot of the collected fraction was analyzed by radio-HPLC (Phenomenex Synergi Hydro RP 250x4.6mm, eluent = 50% CH_3CN/10mM NH_4OAc, flowrate = 1 ml/min) to determine radiochemical identity and purity.

Run	Isolated activity [^{18}F]2a		Radiochemical purity
	mCi	GBq	
1	104.8	3.88	96%
2	89.1	3.30	99%

Table S10. Isolated activities of **[^{18}F]2a**

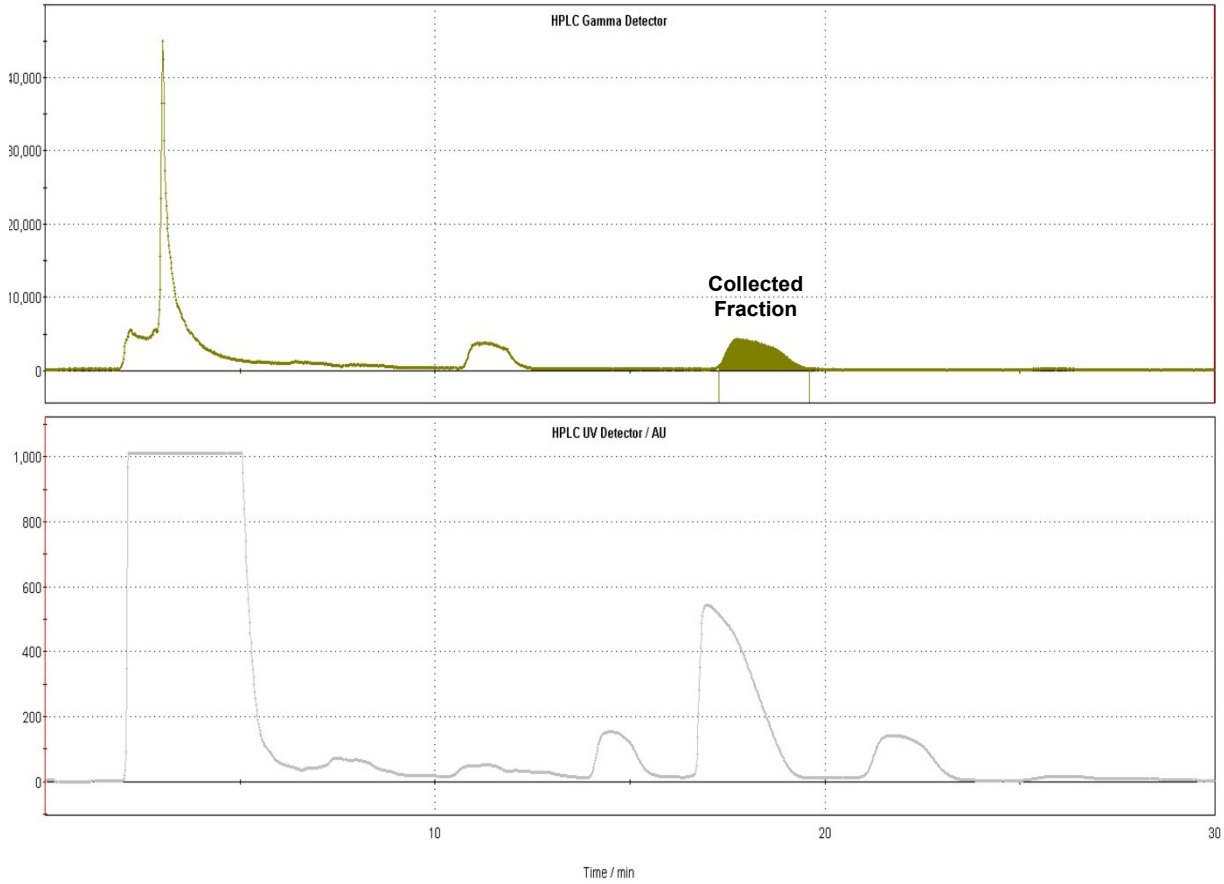


Figure S11. Crude prep HPLC radiotrace (top) and UV trace (254 nm, bottom) of **[^{18}F]2a**

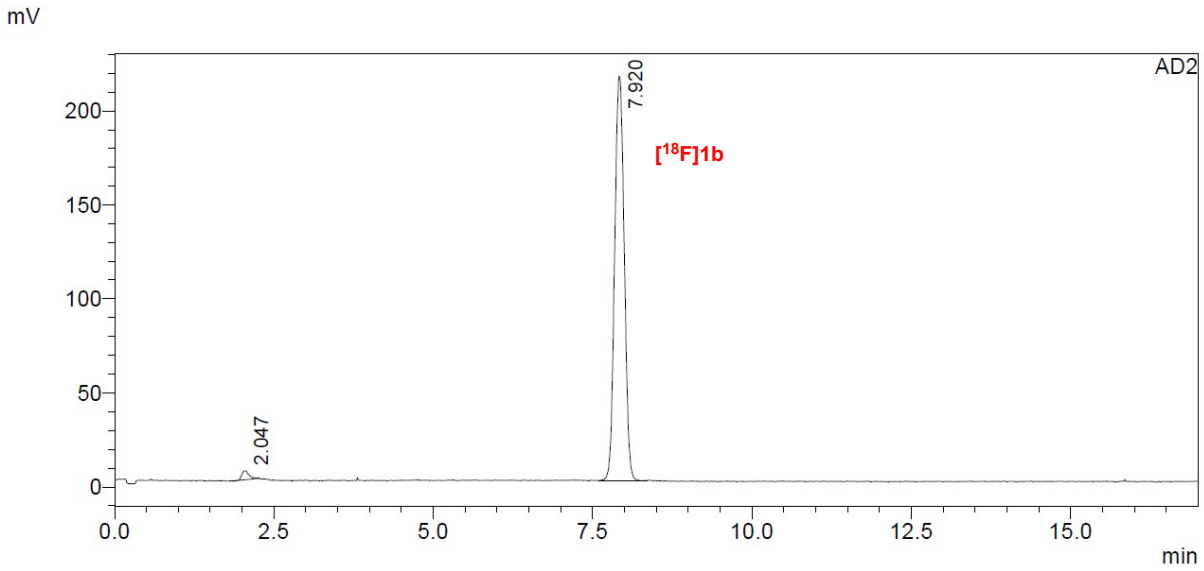


Figure S12. HPLC radiotrace of collected fraction of **[^{18}F]2a**

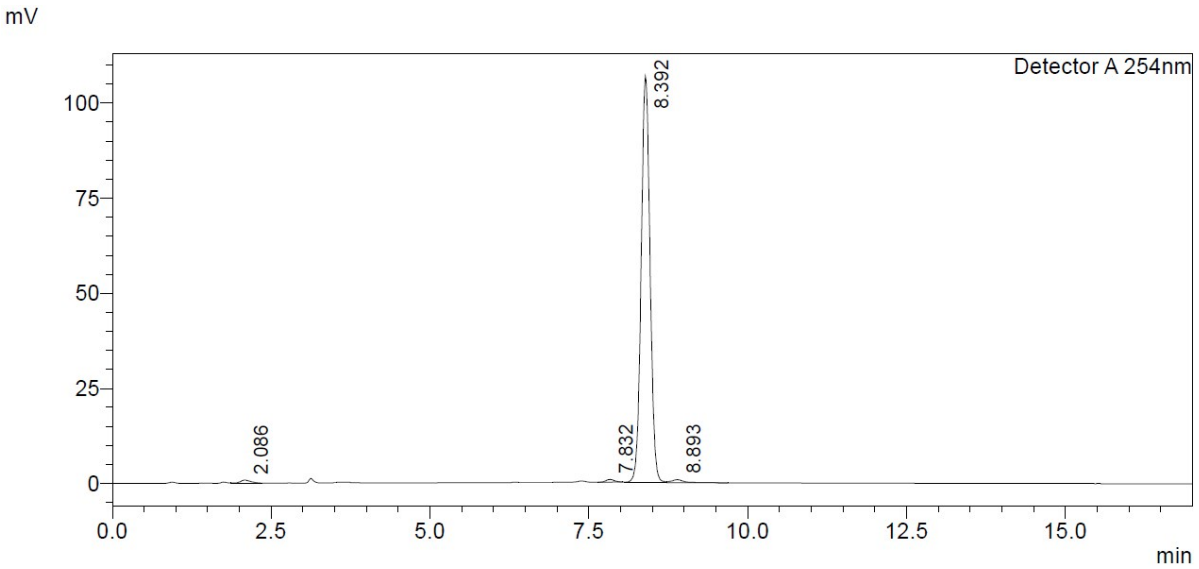


Figure S13. HPLC UV trace (254 nm) of collected fraction of **[^{18}F]2a**

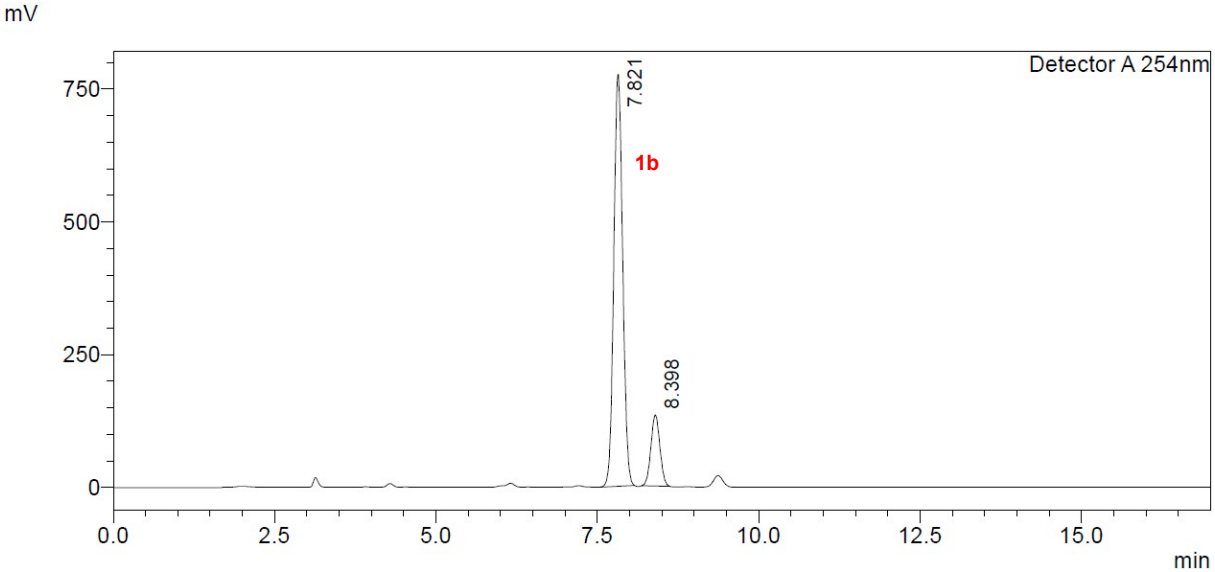
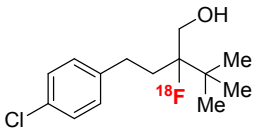


Figure S14. HPLC UV trace (254 nm) of collected fraction of **[^{18}F]2a** spiked with **2a** (Rt = 7.82 min)

2-(4-chlorophenethyl)-2-[^{18}F]-fluoro-3,3-dimethylbutan-1-ol ([^{18}F]3a)



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Run	Isolated activity [^{18}F]3a		Radiochemical purity
	mCi	GBq	
1	7.6	0.28	89%
2	16.6	0.61	94%
3	19.6	0.73	94%

Table S11. Isolated activities of **[^{18}F]3a**.

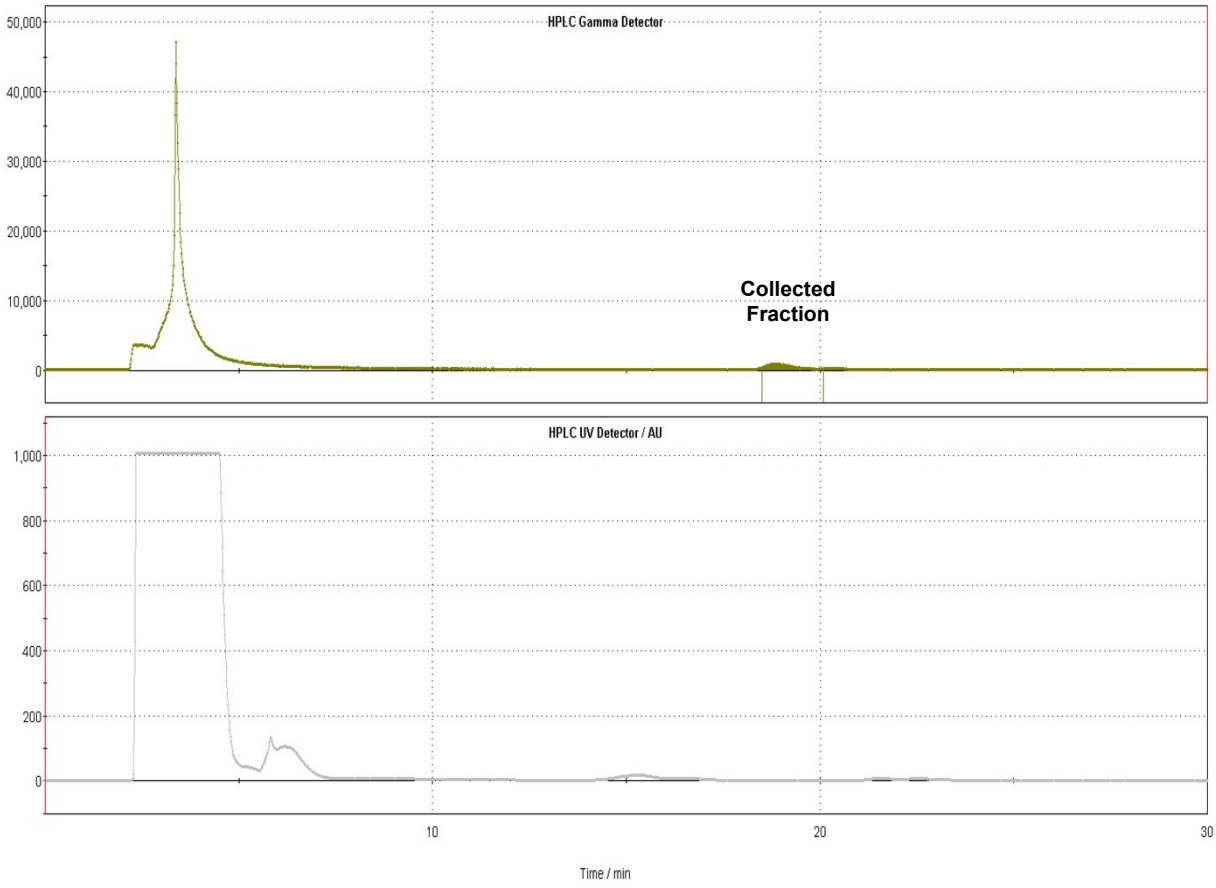


Figure S15. Crude prep HPLC radiotrace (top) and UV trace (254 nm, bottom) of **[^{18}F]3a**

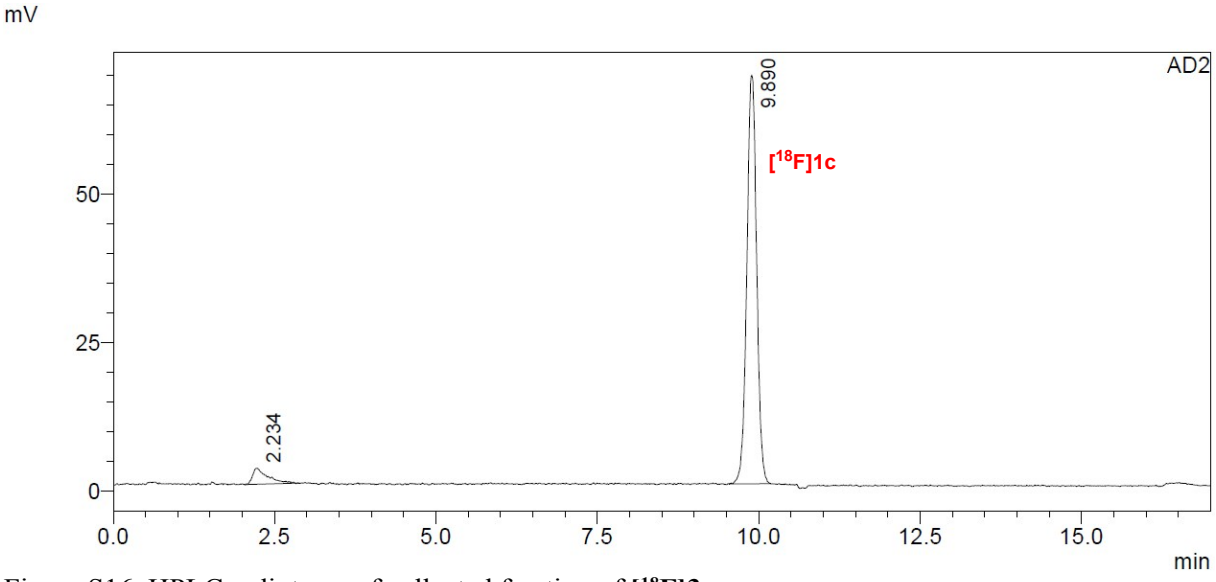


Figure S16. HPLC radiotrace of collected fraction of **[^{18}F]3a**

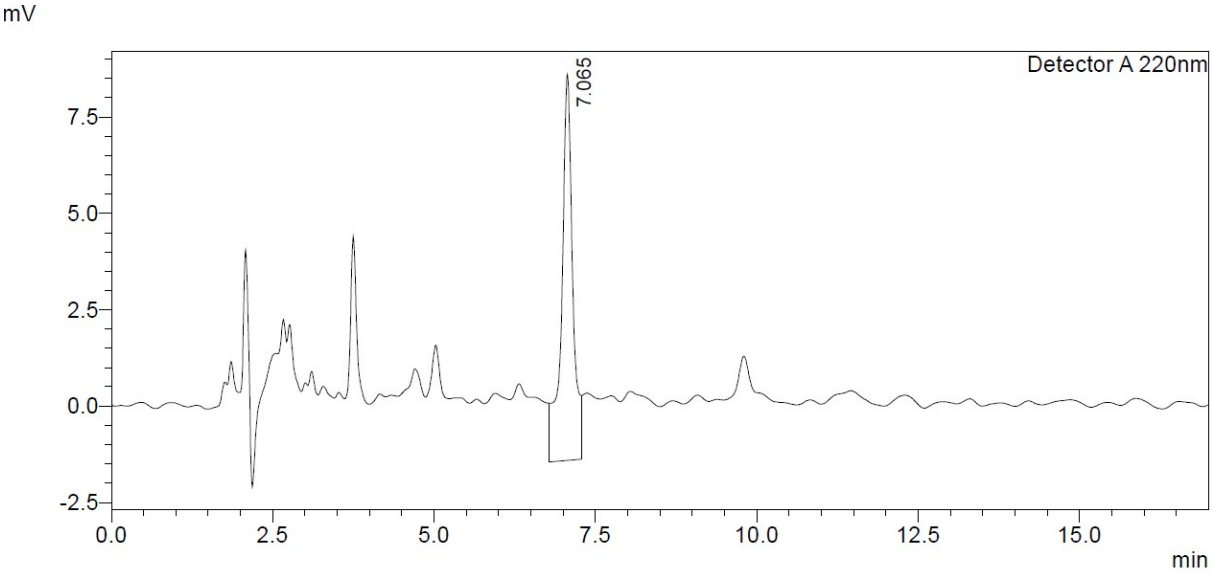


Figure S17. HPLC UV trace (220 nm) of collected fraction of **[^{18}F]3a**

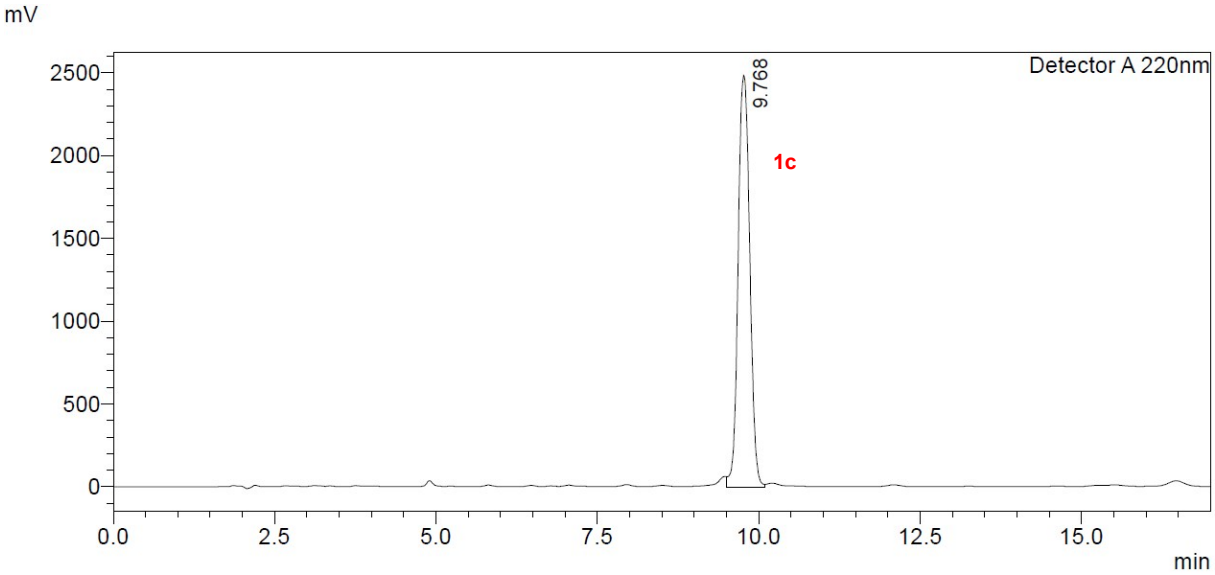
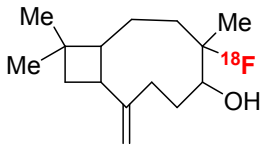


Figure S18. HPLC UV trace (220 nm) of collected fraction of **[^{18}F]3a** spiked with **3a** (Rt = 9.77 min)

6-[^{18}F]-fluoro-6,10,10-trimethyl-2-methylenebicyclo[7.2.0]undecan-5-ol, [^{18}F]4a



Fluorine-18 was produced by the ^{18}O(p, n)^{18}F nuclear reaction using a GE PETTrace cyclotron (a 55 µA beam for 30 minutes generated approx. 1.8 Ci (66.6 GBq) of fluorine-18) and delivered to a GE TRACERLab FX_{FN} automated radiochemistry synthesis module in a 2.5 mL bolus of [^{18}O]H_2O followed by trapping on a Waters QMA SepPak Light Carb cartridge (Waters, order# WAT023525 ; activated with 10 mL H_2O) as [^{18}F]F^- to remove [^{18}O]H_2O and other impurities. This was followed by elution (as [^{18}F]HF) with a solution of TFA in CH_3CN/H_2O 4:1 (0.5 M, 500 µL) from vial 1 into the reactor, which had been charged with $Fe(acac)_3$ (0.04 mmol, 14 mg). The reactor was then pressurized with argon to approx. 200 kPa (by opening valve 20 for 3 s) and heated at 80 °C for 10 min to allow capture of [^{18}F]HF by $Fe(acac)_3$. The resulting mixture was azeotropically dried at 100 °C under vacuum for 5 min, followed

by heating with vacuum and argon flow for another 5 min. The reactor was then cooled to 60 °C using compressed air, and a solution of (-)-caryophyllene oxide (**4**) (9 mg, 0.04 mmol) in dioxane (500 µL) was added from vial 3 using argon push gas. The reactor was heated to 120 °C and stirred for 20 min under autogenous pressure. After cooling to 50 °C using compressed air, a solution of 50% CH_3CN/10mM NH4OAc (3.5 mL) containing $(NH_4)_2EDTA$ (28 mg, 0.08 mmol) was added to the reactor from vial 6 using push gas. The content of the reactor was then loaded onto a semi-prep HPLC column (Agilent Eclipse XDB 250x9.4mm 5µ, eluent = 55% CH_3CN/H_2O, flowrate = 4 mL/min) for purification. The fraction at Rt = 20.3 – 22.2 min was collected to give **[^{18}F]4a**. An aliquot of the collected fraction was analyzed by radio-HPLC (Phenomenex Synergi Hydro RP 250x4.6mm, eluent = 60% CH_3CN/H_2O, flowrate = 1 ml/min) to determine radiochemical identity and purity.

Run	Isolated activity [^{18}F]4a		Radiochemical purity
	mCi	GBq	
1	25.5	0.94	99%
2	20.8	0.80	99%
3	26.3	0.97	91%

Table S12**.** Isolated activities of **[^{18}F]4a**.

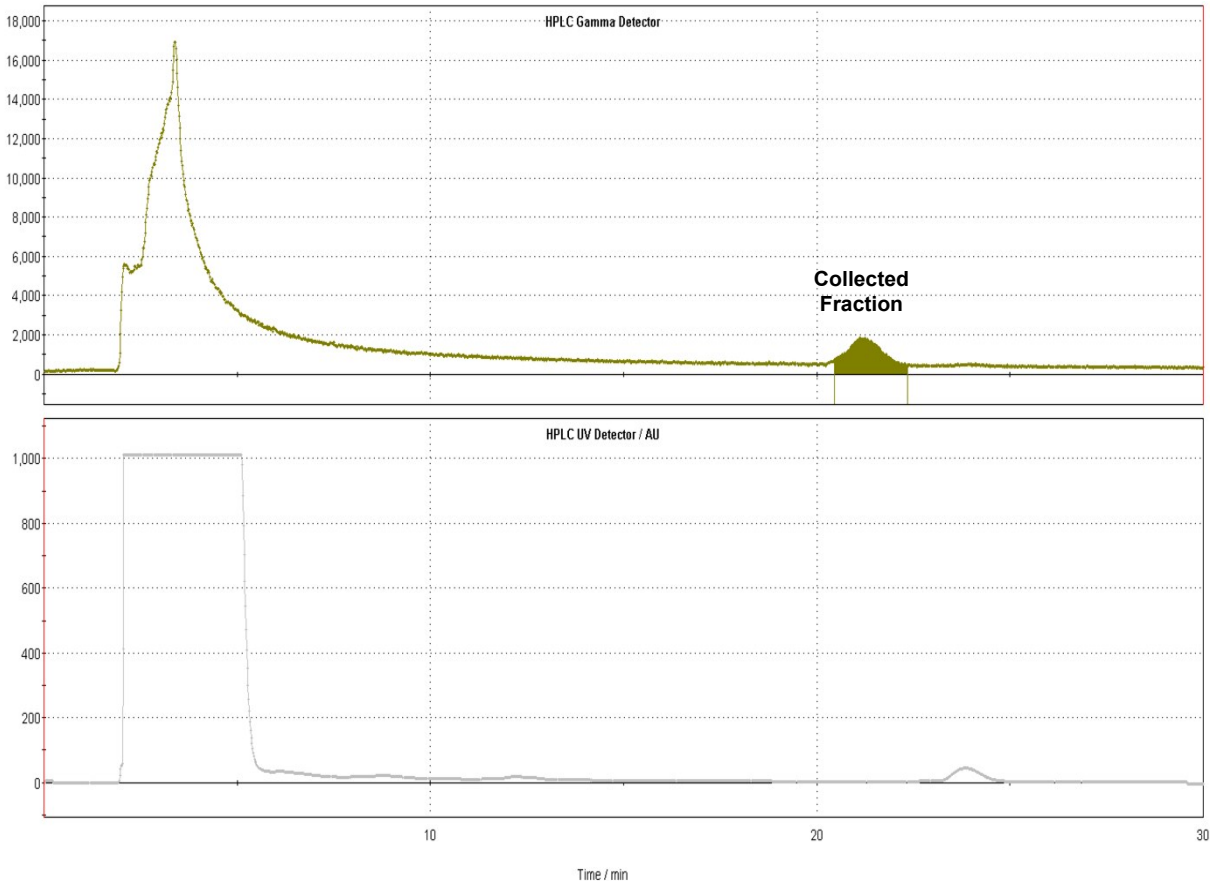


Figure S19. Crude prep HPLC radiotrace (top) and UV trace (254 nm, bottom) of **[^{18}F]4a**

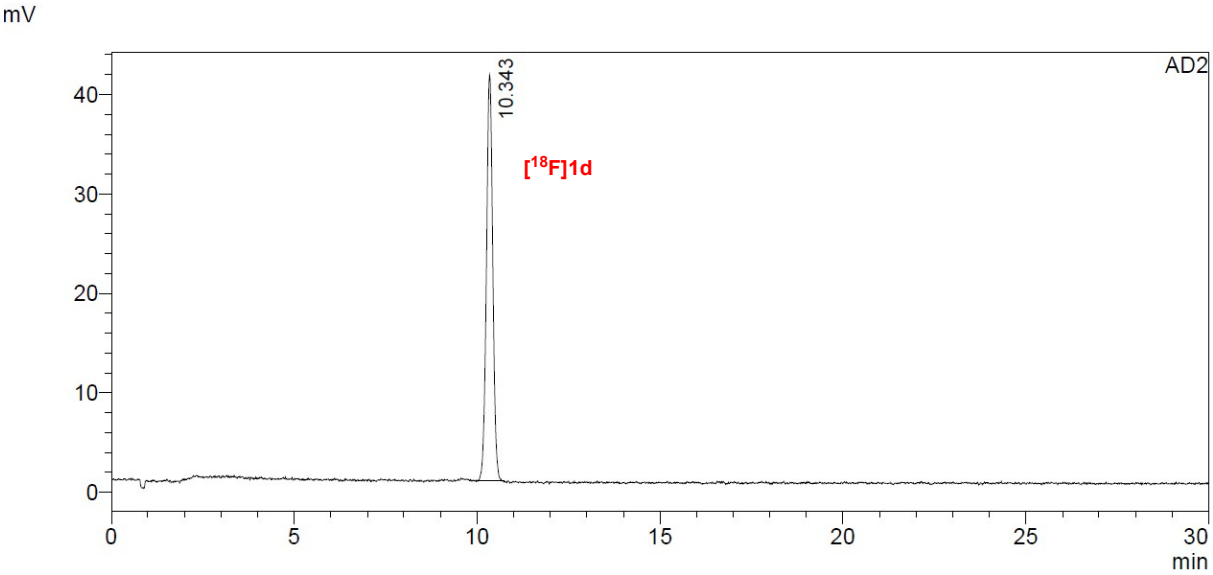


Figure S20. HPLC radiotrace of collected fraction of **[^{18}F]4a**.

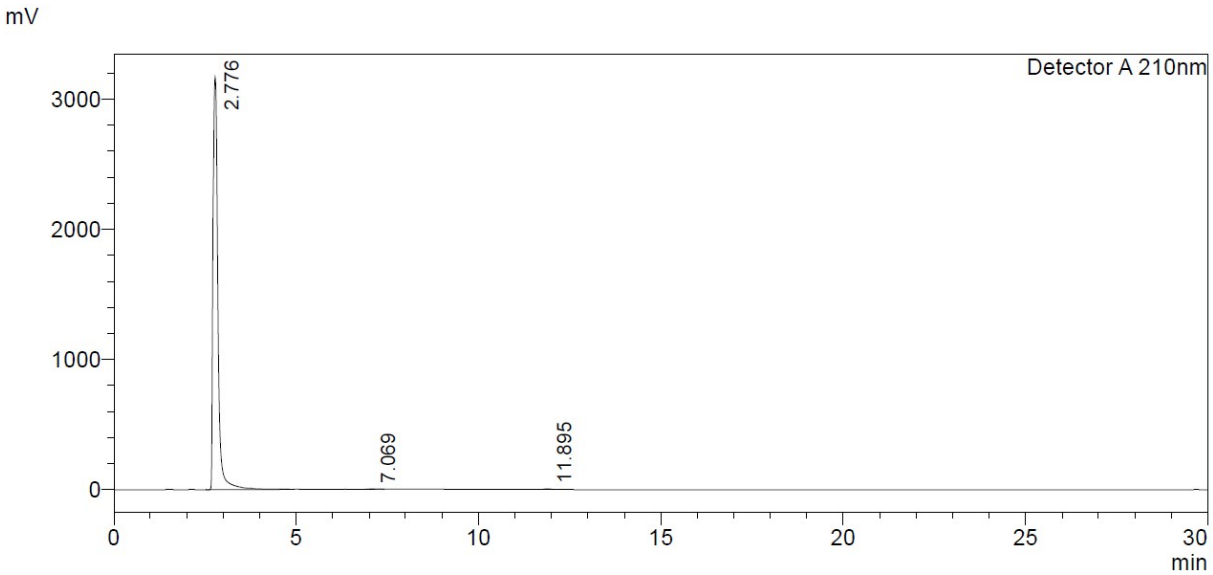


Figure S21. HPLC UV trace (210 nm) of collected fraction of **[^{18}F]4a**

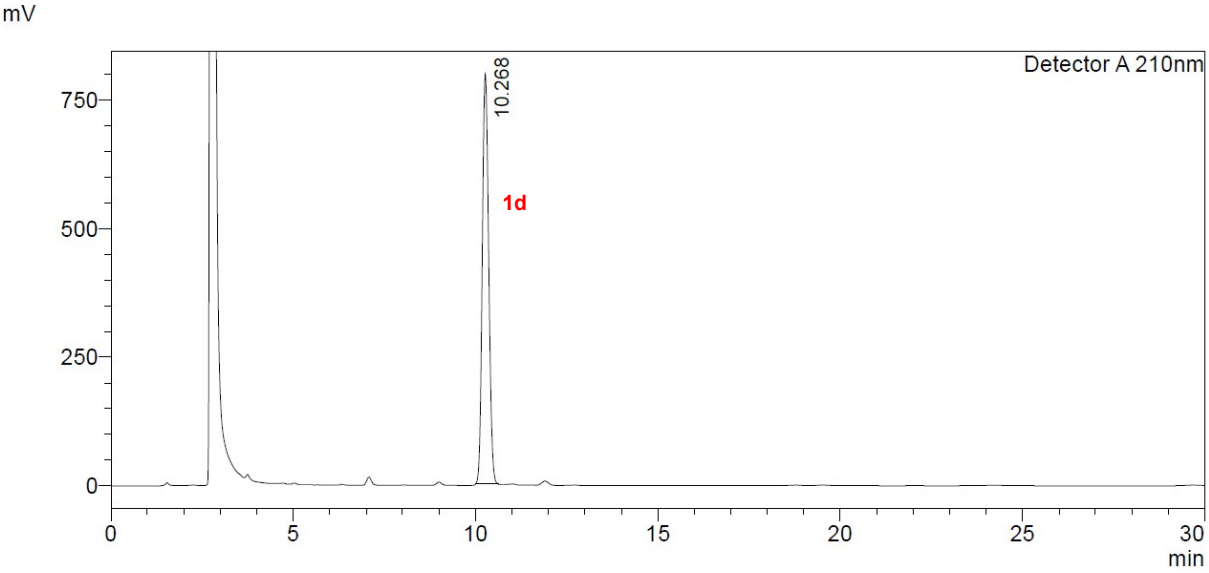


Figure S22. HPLC UV trace (210 nm) of collected fraction of **[^{18}F]4a** spiked with **4a** (Rt = 10.27 min)

3.4 Calibration curves for determination of Molar Activity (A_m)

3.4.1 Molar activity of (*E*)-4-(3-[^{18}F]-fluoro-2-hydroxy-2,6,6-trimethylcyclohexyl)but-3-en-2-one, [^{18}F]2a

2a (mg/ml)	HPLC UV detector response (254 nm)		Mean response
0.0001	2485	2384	2434.5
0.001	20247	20191	20219
0.01	170969	170748	170858.5
0.1	1278260	1298653	1288456.5
1.0	9794765	9792904	9793834.5

Equation :
y = 5312443.9x
r^2 = 0.9988
(y =detector response, x = conc. of **2a** in mg/ml)

Calculation :

Detector response	Conc. mg/ml	Aliquot volume (mL)	2a (μmol)	Activity (mCi)	Activity (GBq)	RCP	A_m (GBq/ μmol)	A_m (Ci/ mmol)
6709	0.00126	0.5	0.00277	4.3	159.5	98%	56.5	1523

3.4.2 Molar Activity of (1*S*,9*R*)-6-[^{18}F]-fluoro-6,10,10-trimethyl-2-methylenebicyclo[7.2.0]undecan-5-ol, [^{18}F]4a

4a (mg/ml)	HPLC UV detector response (210 nm)
0.0001	577
0.001	6498
0.01	70202
0.1	746283
1.0	7087595

Equation :
$y = 7091302.5x$
$r^2 = 0.99997$
(y =detector response, x = conc. of **4a** in mg/ml)

Calculation :

Detector response	Conc. mg/ml	Collected volume (mL)	4a (μmol)	Activity (mCi)	Activity (GBq)	RCP	A_m (GBq/ μmol)	A_m (Ci/ mmol)
3166	0.00045	8	0.0149	25.5	946.05	99%	63.0	1699

3.5 Attempted synthesis of [^{18}F]2a using [^{18}F]KF/$K_{2.2.2}$

Fluorine-18 was produced by the $^{18}O(p, n)^{18}F$ nuclear reaction using a GE PETTrace cyclotron (a 55 μA beam for 30 minutes generated approx. 1.8 Ci (66.6 GBq) of fluorine-18) and delivered to a GE TRACERLab FX_{FN} automated radiochemistry synthesis module in 2.5 mL bolus of [^{18}O]H_2O followed by trapping on a Waters QMA SepPak Light Carb cartridge (Waters, order# WAT023525 ; activated with 10 mL H_2O) as [^{18}F]F^- to remove [^{18}O]H_2O and other impurities. This was followed by elution (as [^{18}F]KF) with a solution of Kryptofix $K_{2.2.2}$ (0.040 mmol, 15 mg) and K_2CO_3 (0.22 mmol, 3 mg) in CH_3CN/H_2O (4:1, 1 mL) from vial 1 into the reactor, which had been charged with $Fe(acac)_3$ (0.04 mmol, 14 mg). The resulting mixture was azeotropically dried at 100 °C under vacuum for 5 min, followed by heating with vacuum and argon flow for another 5 min. The reactor was then cooled to 60 °C using compressed air, and a solution of 4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)-3-buten-2-one (**2**) (technical grade, 8 mg, 0.04 mmol) in dioxane (500 μL) was added from vial 3 using argon push gas. The reactor was heated to 120 °C and stirred for 20 min under autogenous pressure. After cooling to 50 °C using compressed air, a solution of 40% CH_3CN/10mM NH_4OAc (3.5 mL) containing $(NH_4)_2EDTA$ (28 mg, 0.08 mmol) was added to the reactor from vial 6 using push gas. The content of the reactor was then loaded onto a semi-prep HPLC column (Agilent Eclipse XDB 250x9.4mm 5μ, eluent = 40% CH_3CN/10mM NH_4OAc, flowrate = 4 mL/min) for purification. Monitoring of the radioactive trace showed that the only radioactive fraction eluted with the solvent front, and no **[^{18}F]2a** was detected.

4. In vivo imaging experiments with 5-[^{18}F]fluoro-6-hydroxy-cholesterol ([^{18}F]1a)

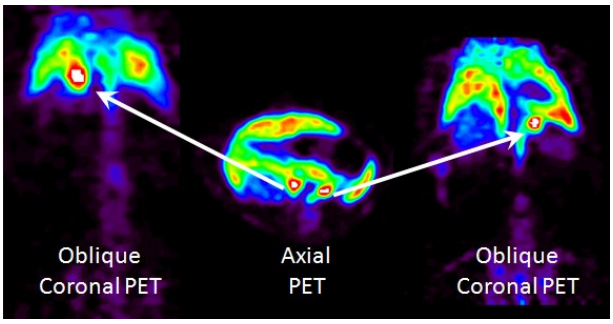
General considerations

All animal PET imaging experiments were conducted under the supervision of the University of Michigan and its Institutional Animal Care and Use Committee (IACUC) according to approved protocols and all applicable federal, state, local and institutional laws or guidelines governing animal research. Imaging studies were conducted using a Concorde Microsystems P4 PET scanner as described below.

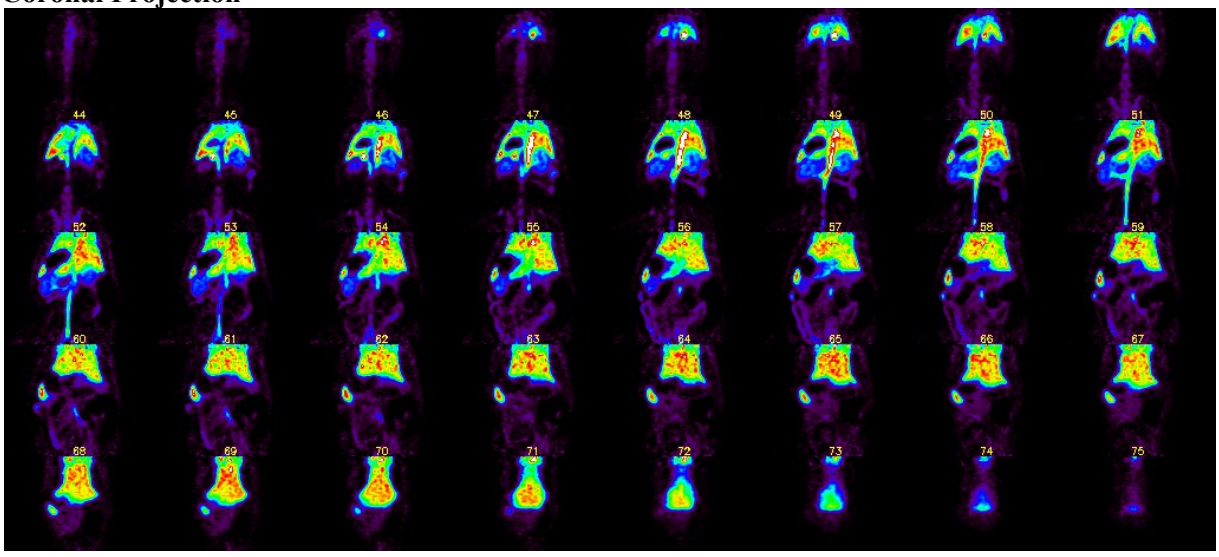
Rodent PET Imaging

Anesthesia was induced in a healthy female Sprague-Dawley rat (300 g) using isoflurane/O2, and anesthesia was maintained with 2-4% isoflurane/O2 throughout the imaging studies. Body temperature was maintained by a heating pad. Following a transmission scan for attenuation correction, the animals were injected (i.v. via intravenous tail vein; 19.9 MBq (0.537 mCi) in 24 µL) with [^{18}F]**1a** as a bolus over 1 min. Upon completion of the PET scans, emission data were corrected for decay, dead time and random coincidences before reconstruction using an iterative ordered subset expectation maximization–maximum a posteriori (MAP) method to generate reconstructed images.

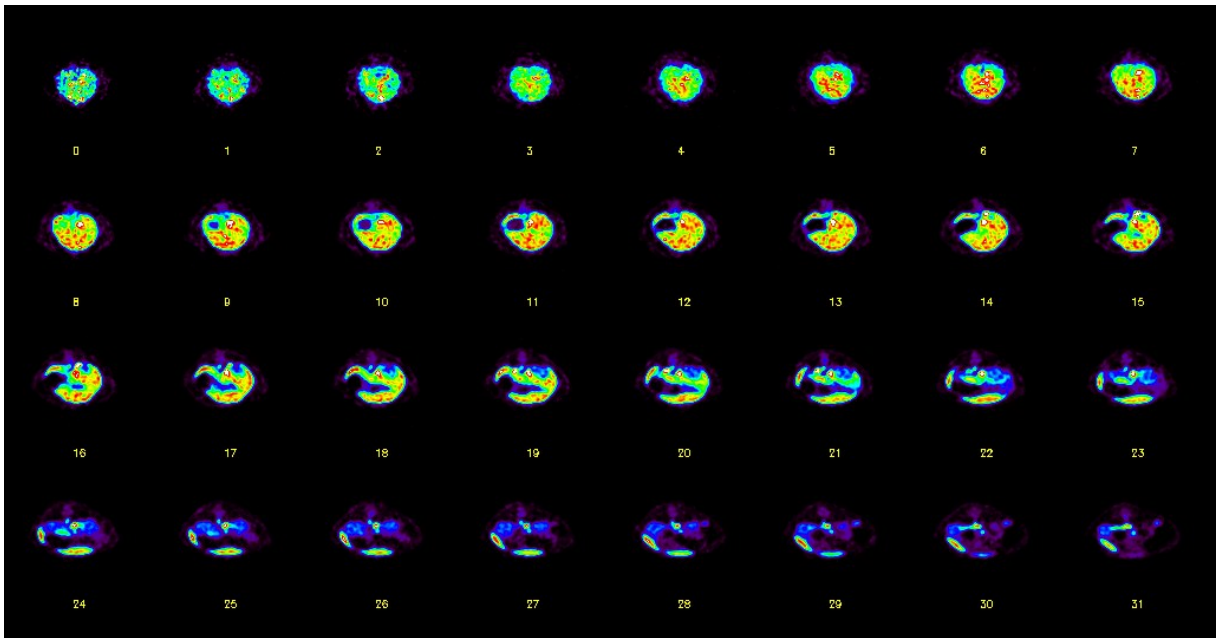
Adrenal Glands



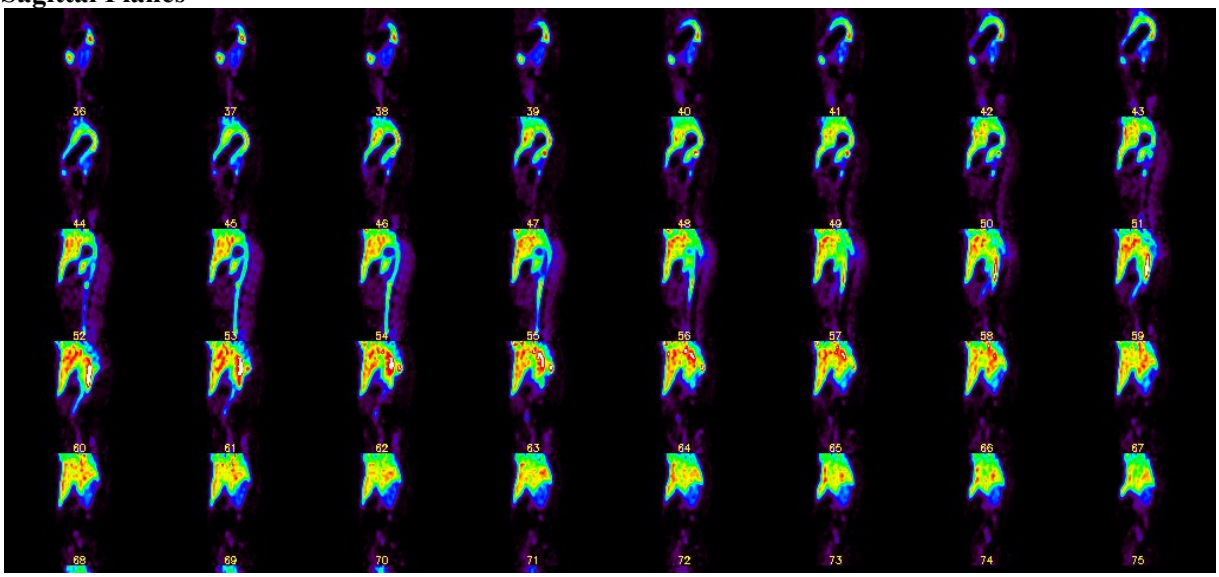
Coronal Projection



Transverse Planes



Sagittal Planes



Rodent Biodistribution Studies

Female Sprague-Dawley rats were injected (i.v. via intravenous tail vein) with [^{18}F]**1a** as a bolus. At given time points (10, 30, 60, 120, and 360 min) the animals were placed under anesthesia and sacrificed. The organs were removed, weighed and counted with a gamma counter to determine percent injected dose per gram of each organ of interest (%ID/gram organ).

Organ	%id/gram				
	10 min	30 min	60 min	120 min	360 min
ADRENAL	1.743	2.006	2.266	2.390	1.924
SPLEEN	0.797	1.409	2.193	2.325	1.684
LUNG	0.519	0.670	1.224	1.289	1.973
HEART	0.302	0.395	0.415	0.420	0.429
LIVER	0.205	0.151	0.450	0.734	0.931
THYROID	0.199	0.267	0.340	0.302	0.361
KIDNEY	0.177	0.274	0.334	0.362	0.477
OVARY	0.151	0.176	0.248	0.231	0.249
ANTERIOR PITUITARY	0.147	0.197	0.255	0.281	0.300
PANCREAS	0.111	0.170	0.193	0.184	0.269

Table S13. Biodistribution in %id/organ of **[^{18}F]1a** over various organs at multiple timepoints

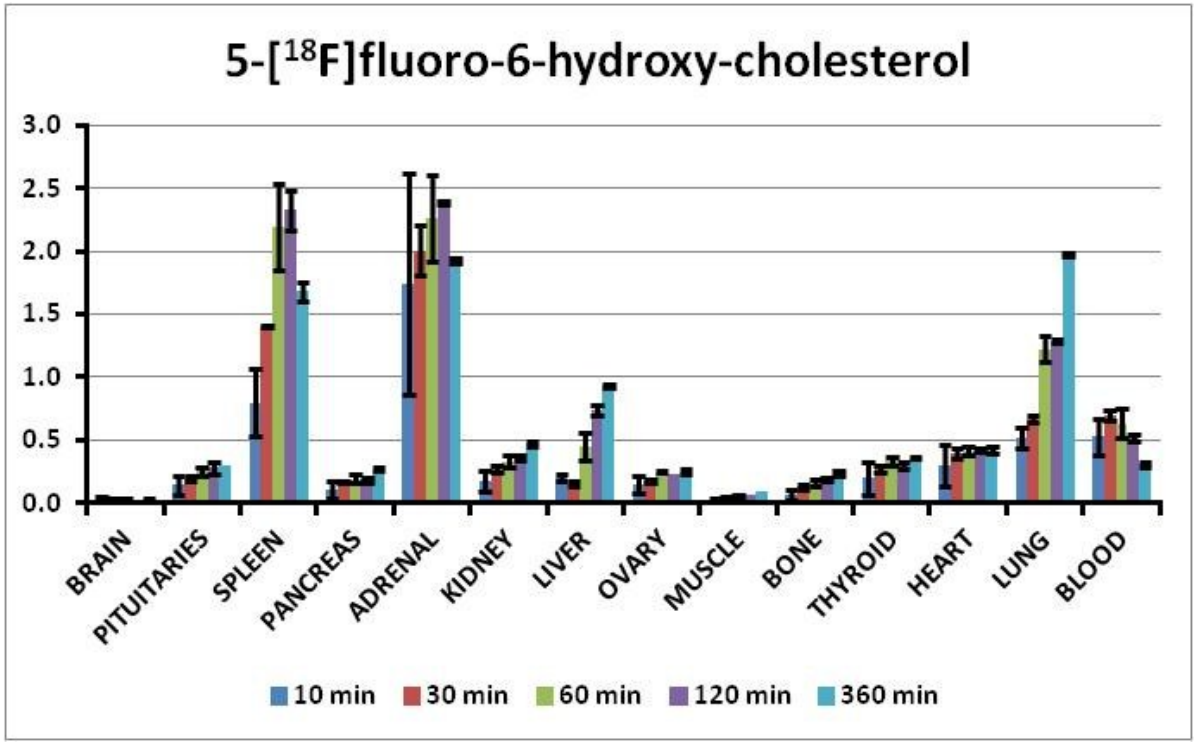
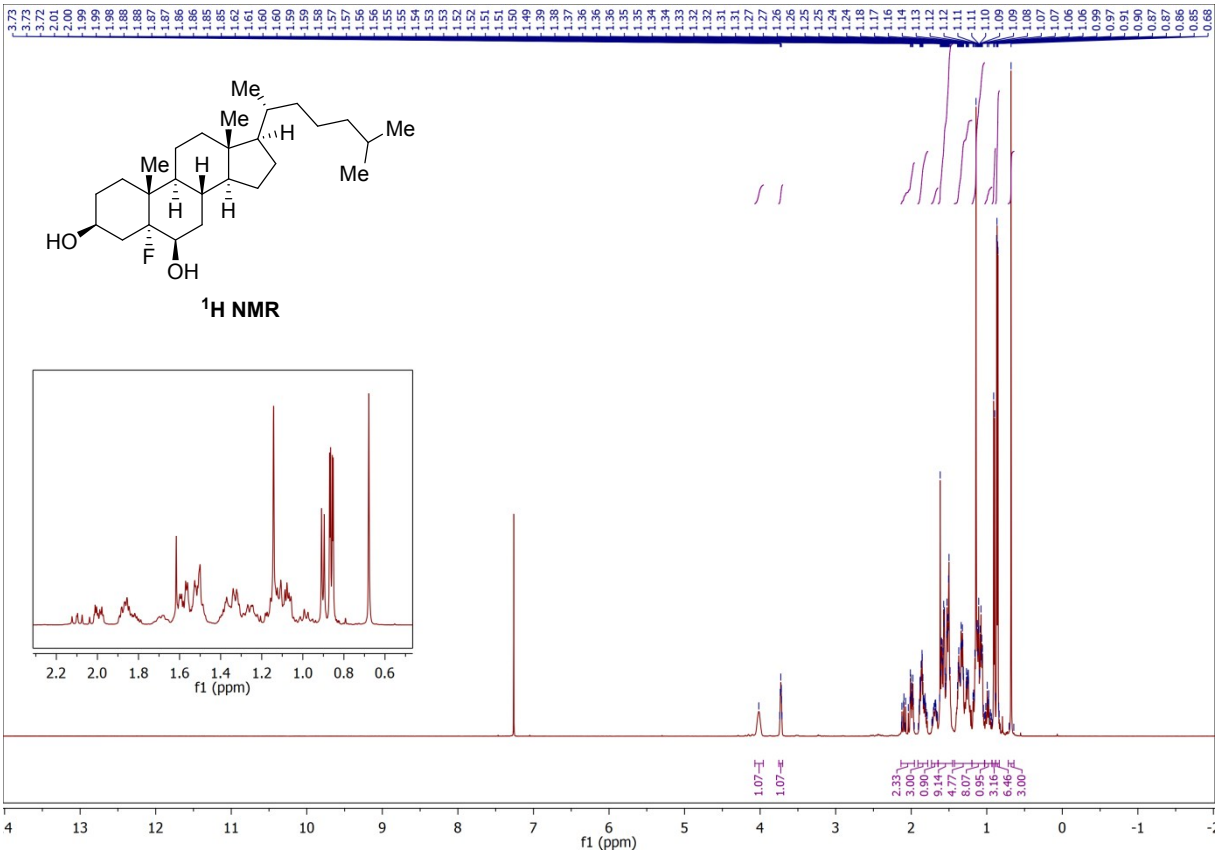


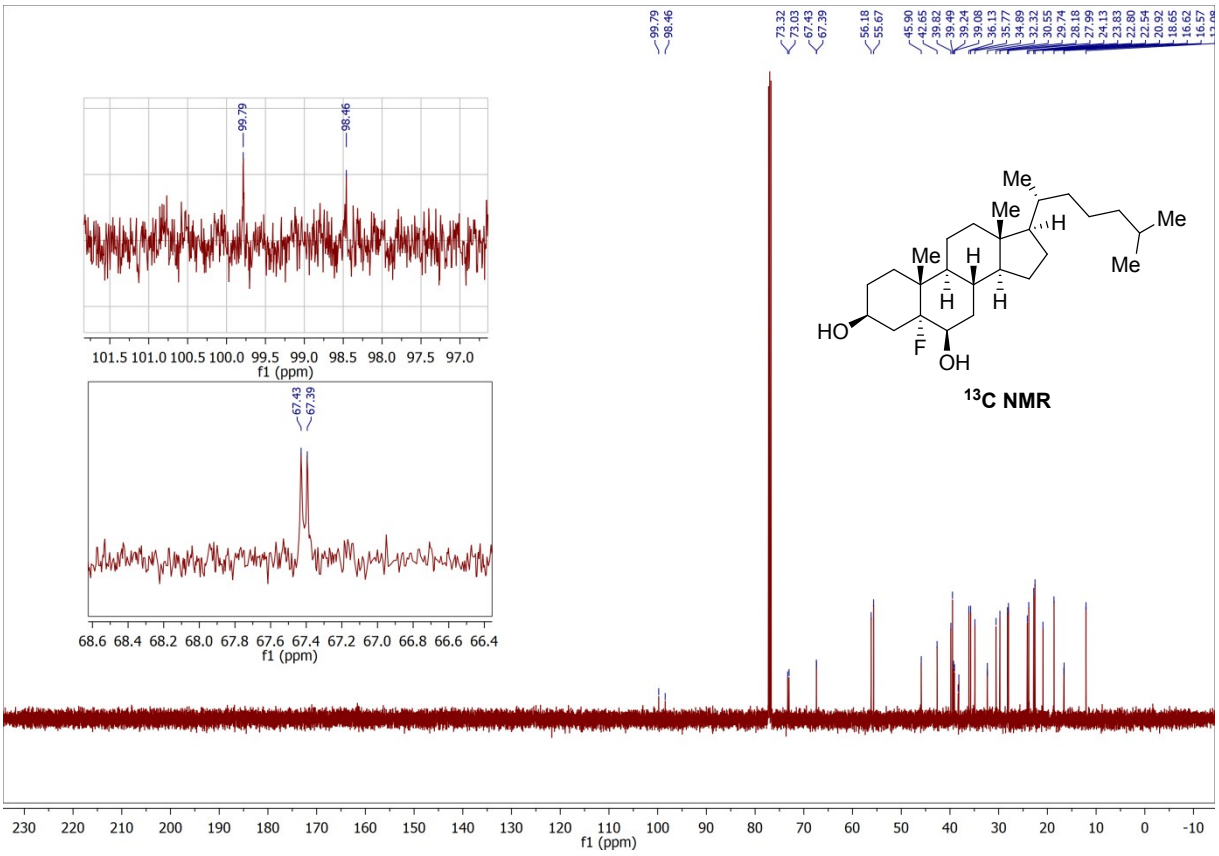
Figure S23: Biodistribution of **[^{18}F]1a** in Sprague-Dawley female rats at 10, 30, 60, 120, and 360 minutes. For each time point animals (n=2) were treated with **[^{18}F]1a** i.v., sacrificed, and organs were separated to determine distribution by gamma counting.

Biodistribution studies were conducted in female Sprague-Dawley rats with two animals for each of the six time points: 10, 30, 60, 120, and 360 min The biodistribution data shows the percent injected dose per gram (%ID/g) for each organ of interest. It is seen that adrenal uptake is very high compared to most other organs. The peak uptake of **[^{18}F]1a** of 3.3 % injected dose per gram (%ID/g) at 120 minutes with an adrenal to liver ratio of 3.3 at that time represented good signal to noise; since, the right adrenal is located just underneath the liver having a good adrenal to liver ratio is important for image analysis.

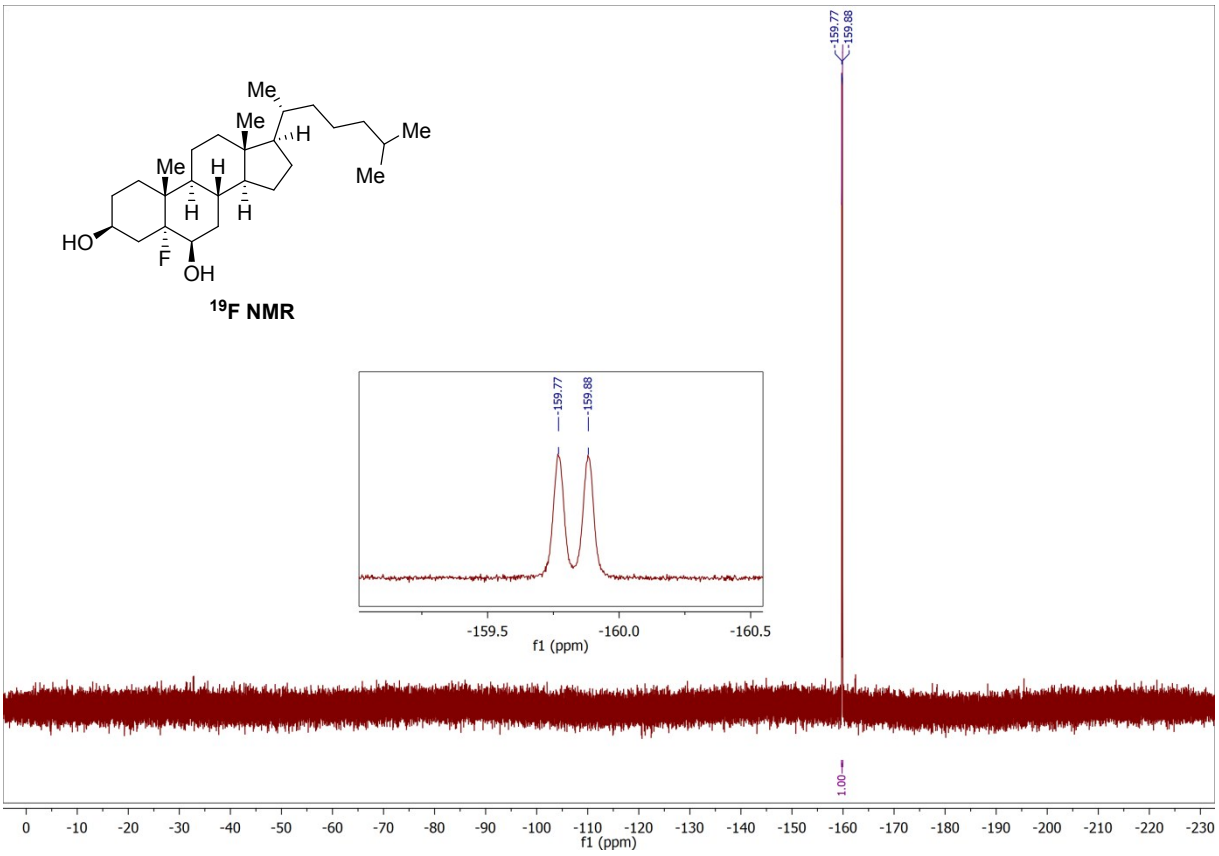
5. NMR Spectra

(3*S*, 5*R*, 6*R*, 8*S*, 9*S*, 10*R*, 13*R*, 14*S*, 17*R*)-5-fluoro-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)hexadecahydro-1*H*-cyclopenta[a]phenanthrene-3,6-diol (1a)



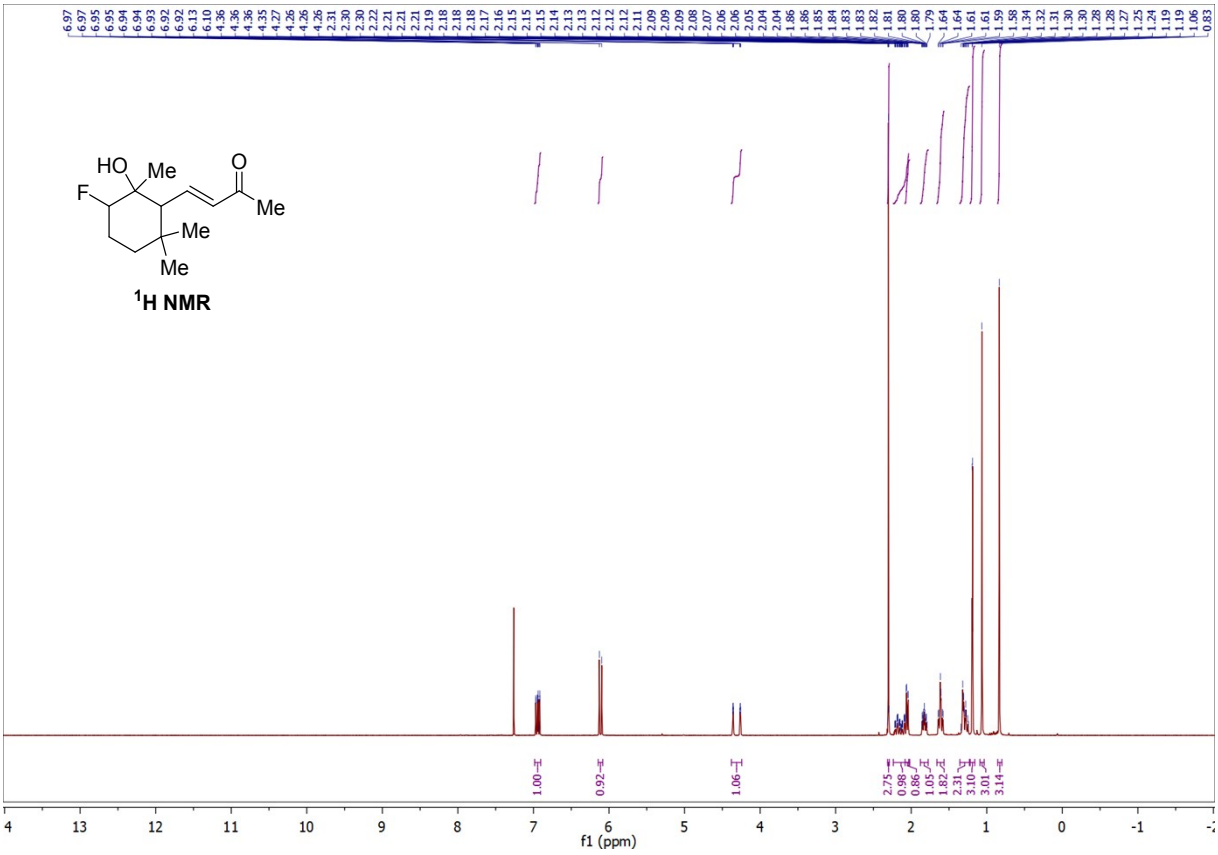


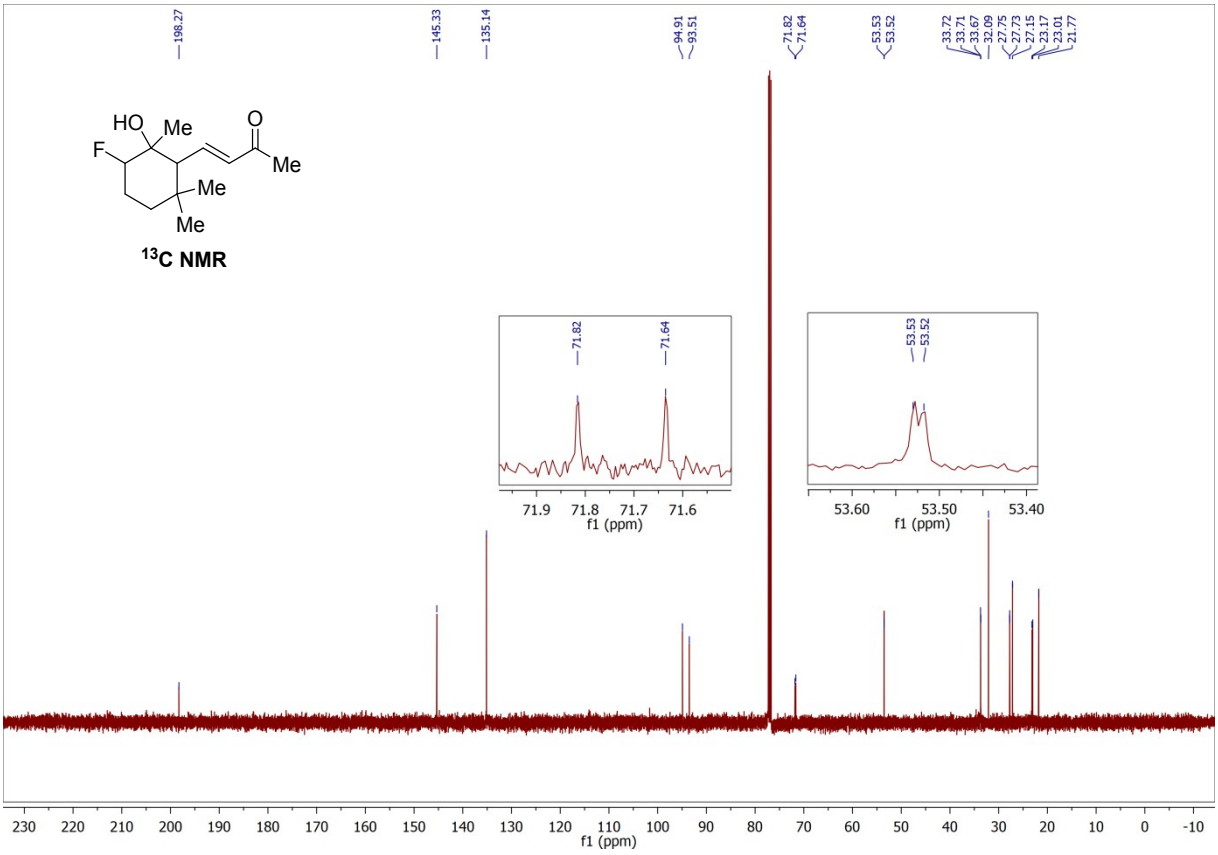
^{13}C NMR

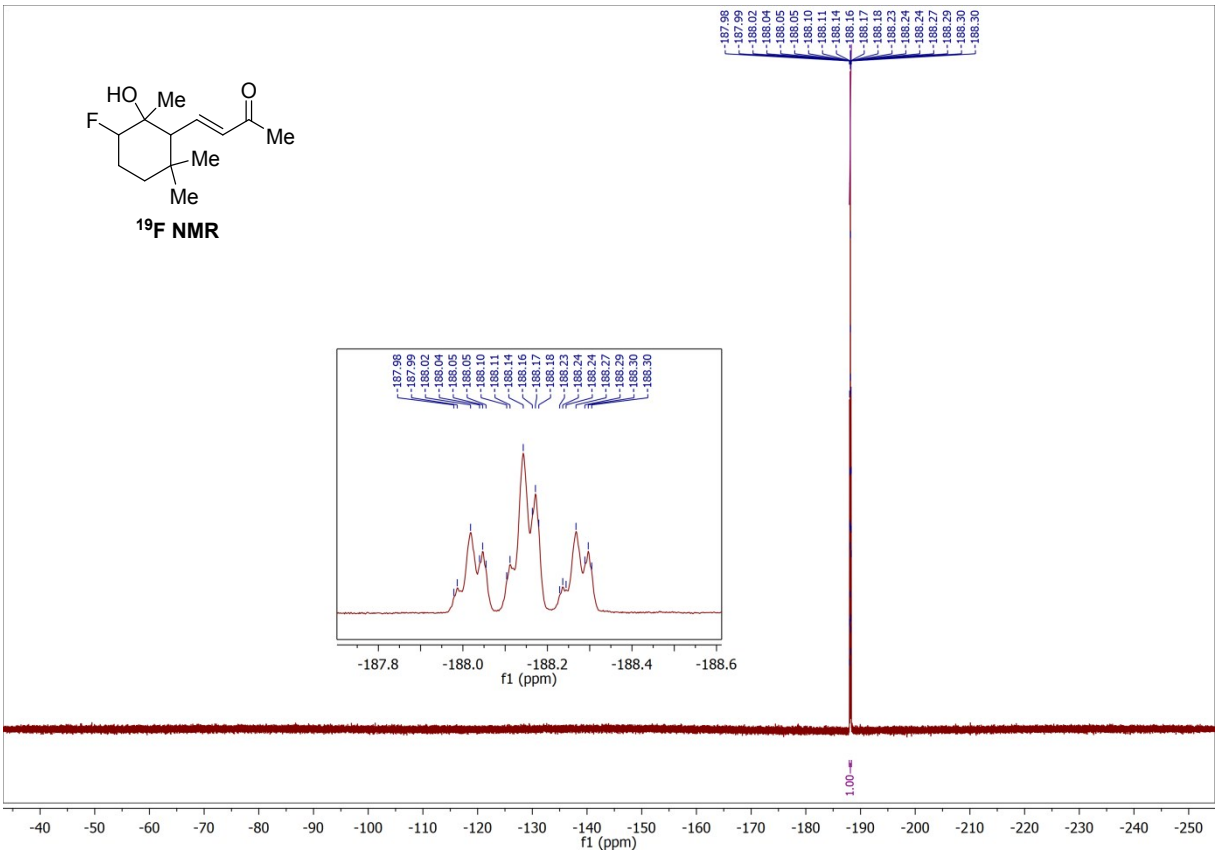


^{19}F NMR

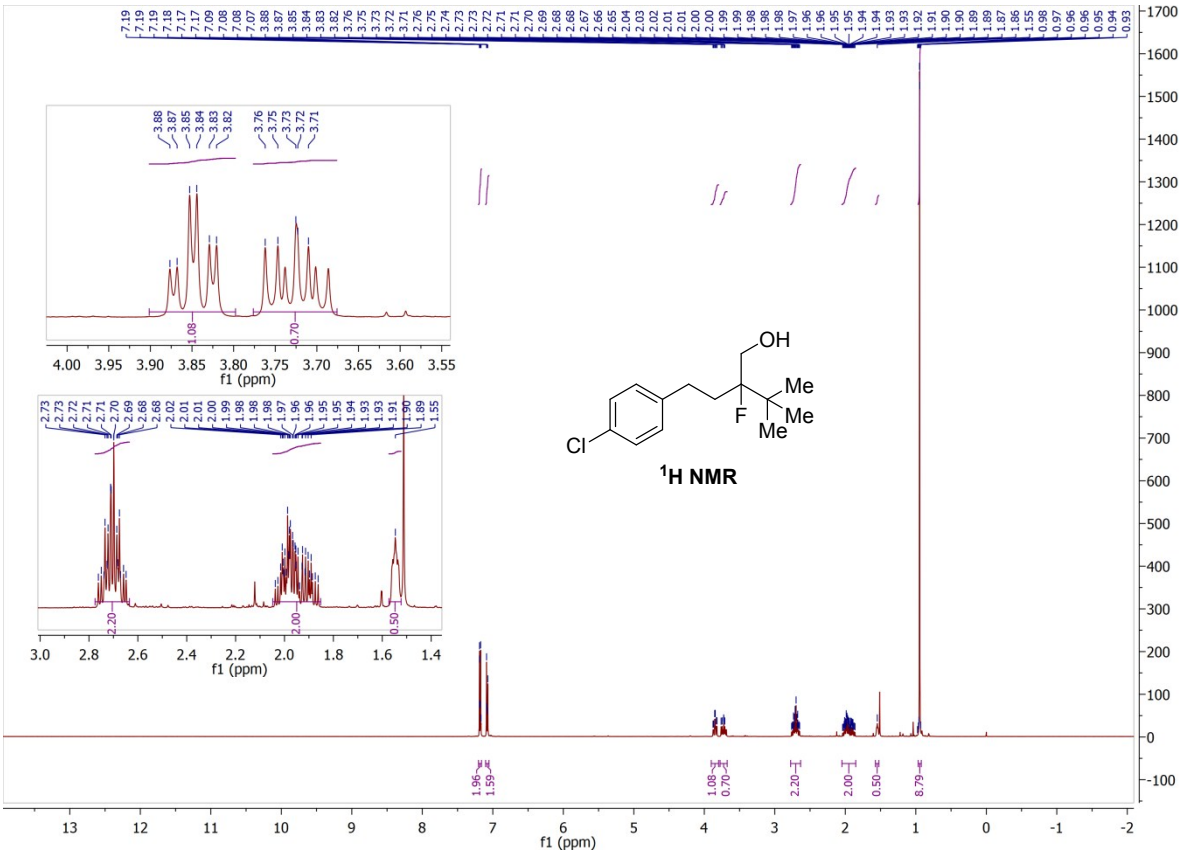
(*E*)-4-(3-fluoro-2-hydroxy-2,6,6-trimethylcyclohexyl)but-3-en-2-one (2a)

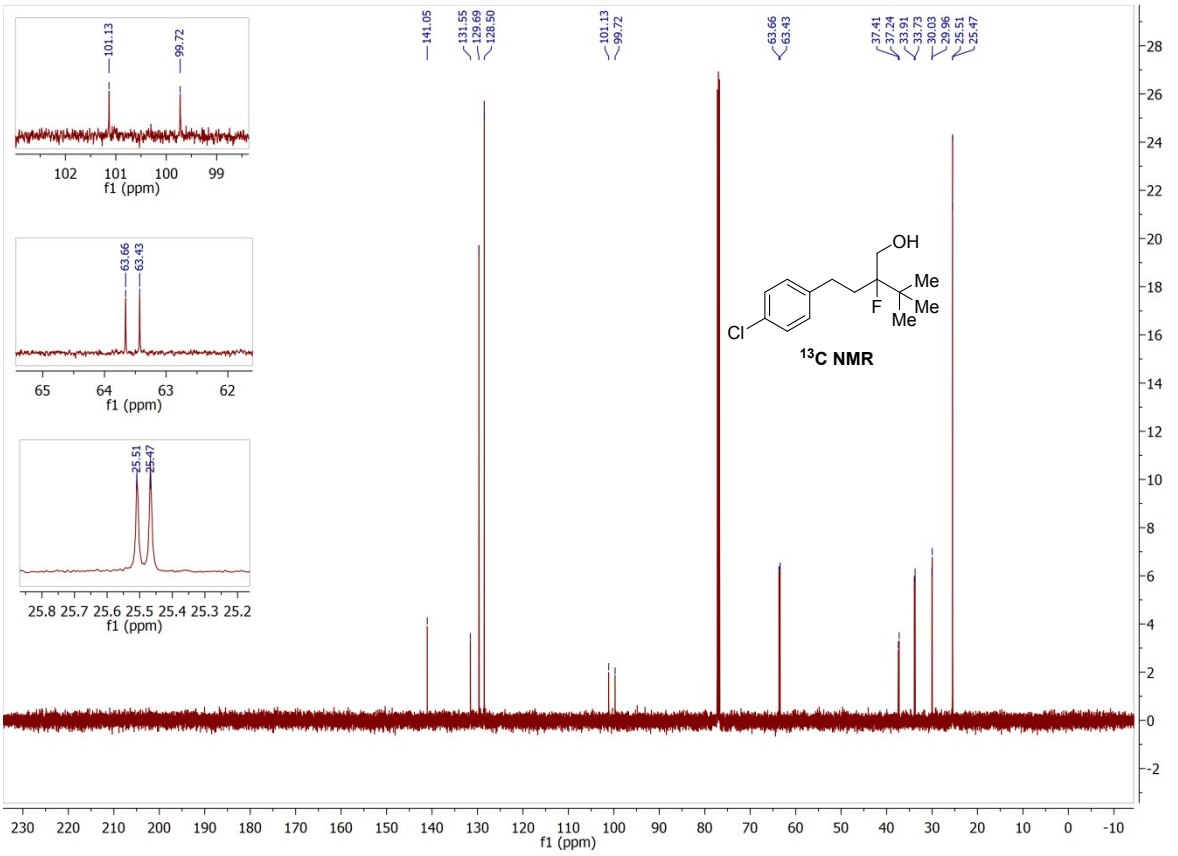






2-(4-chlorophenethyl)-2-fluoro-3,3-dimethylbutan-1-ol (3a)

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F
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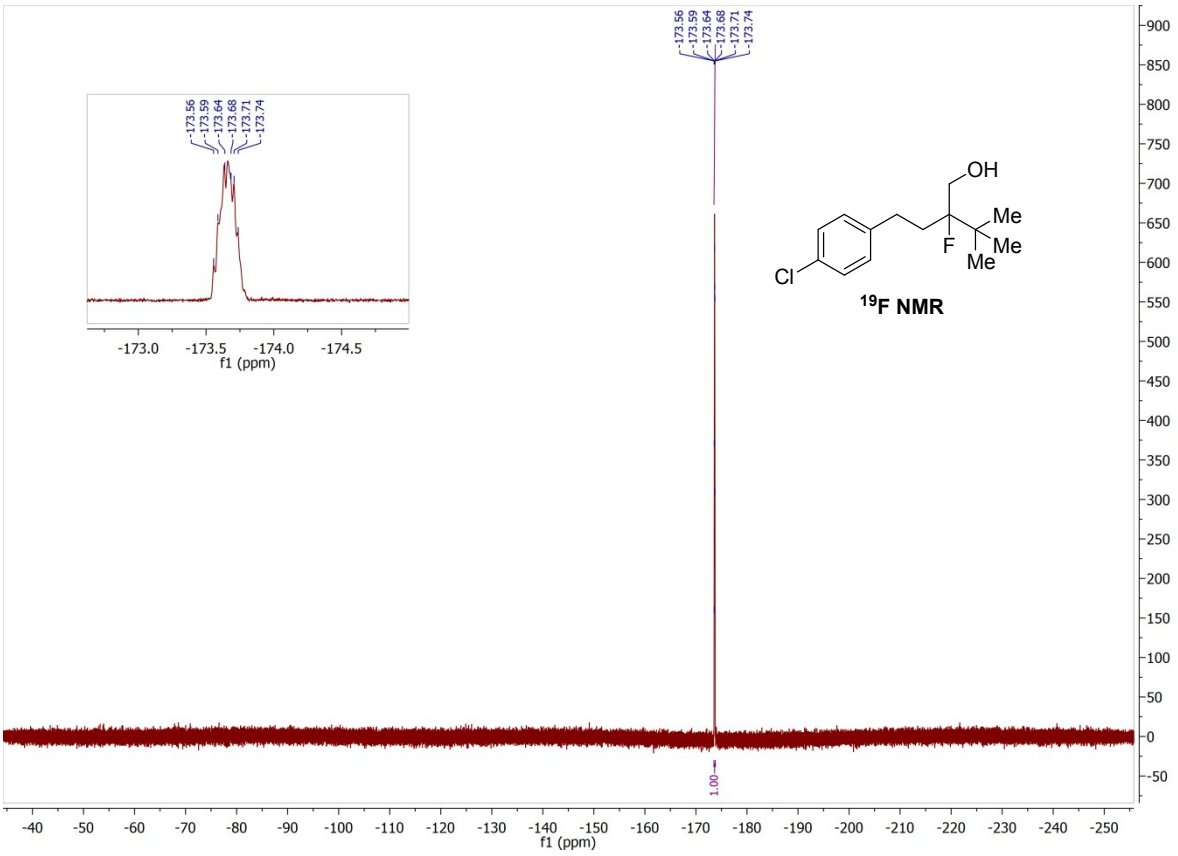
Cl
13C NMR
f1 (ppm)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Cl
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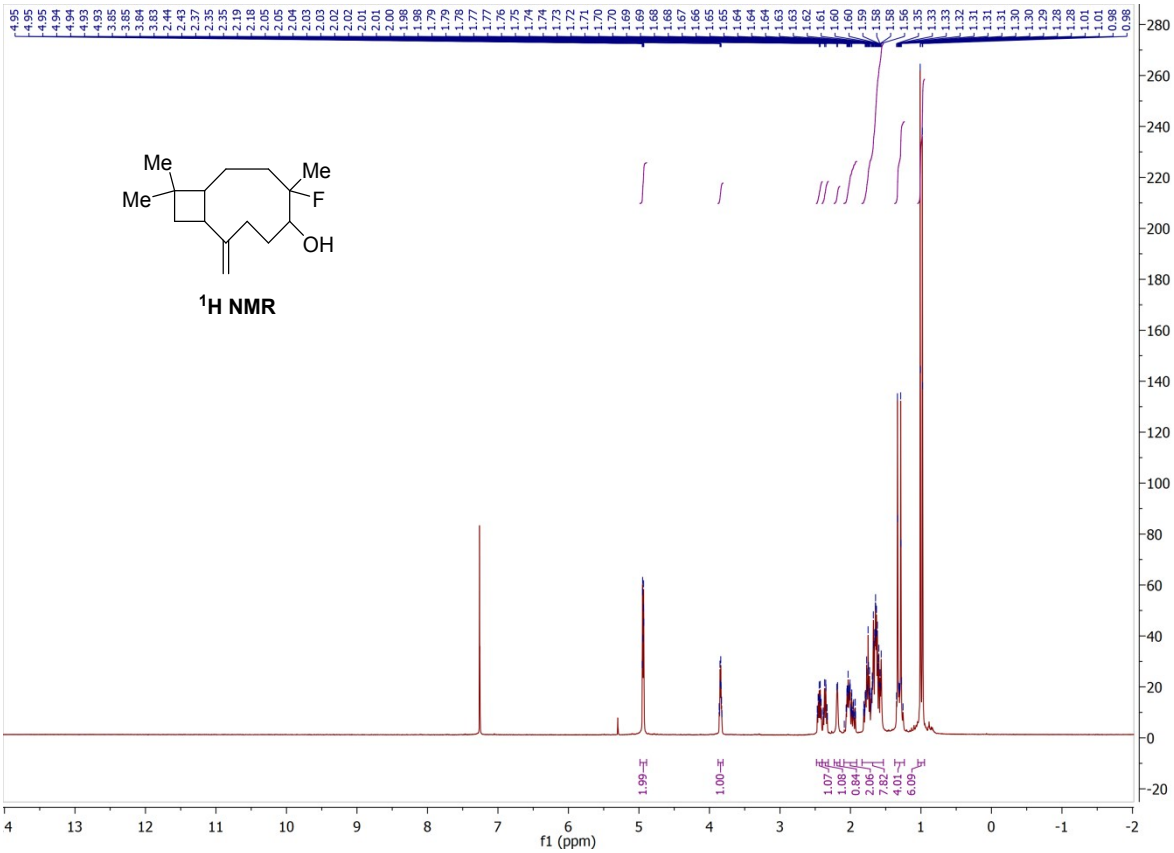
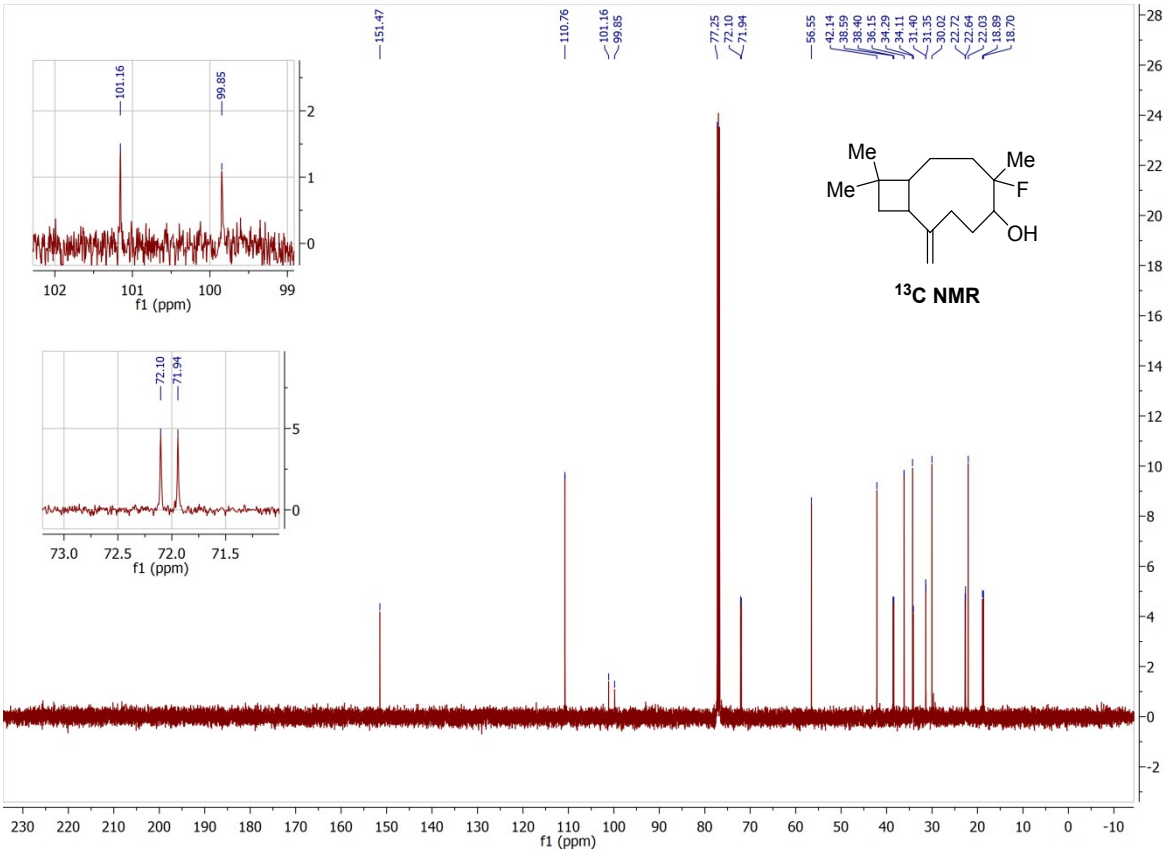
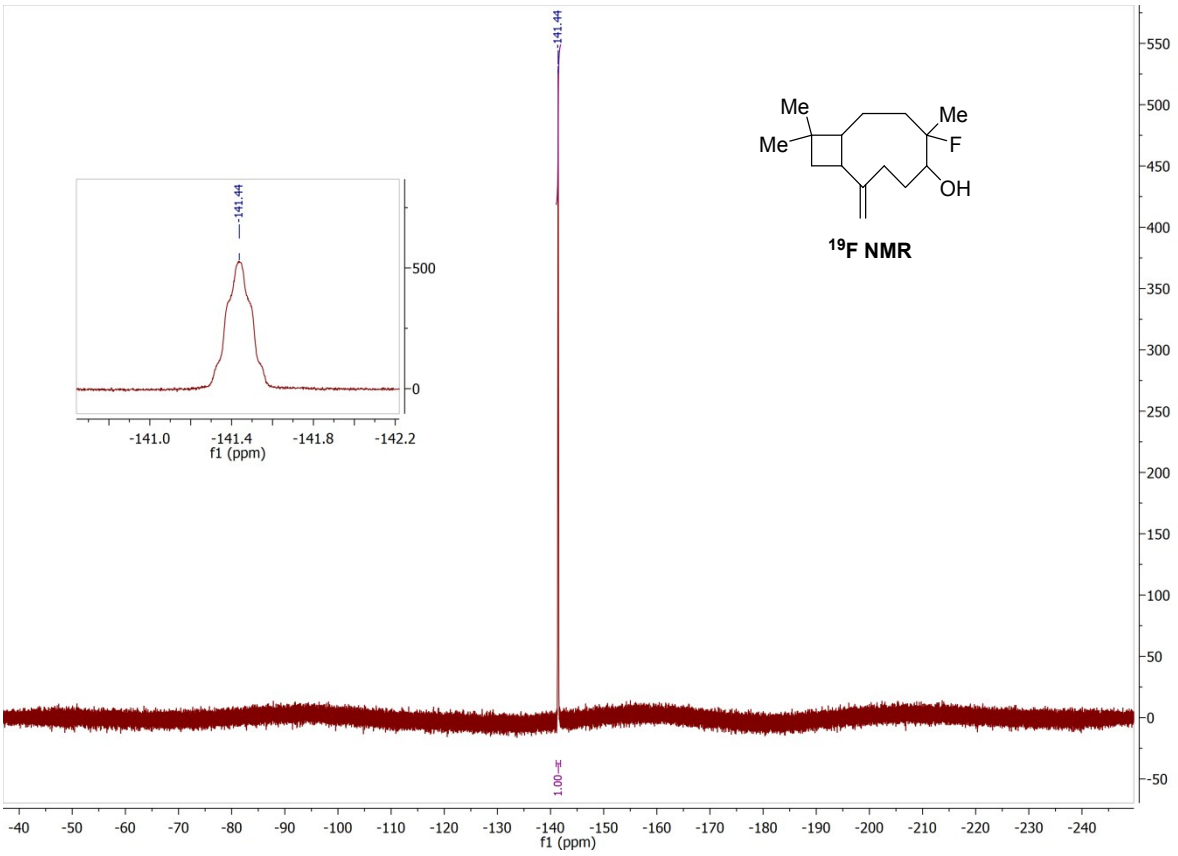
f1 (ppm)

6-Fluoro-6,10,10-trimethyl-2-methylenebicyclo[7.2.0]undecan-5-ol (4a)



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f1 (ppm)