Supplementary information related to the article: Chukwunonso K. Nwabufo, Omozojie P. Aigbogun, Kevin J.H Allen, Madeline N. Owens, Jeremy S. Lee, Christopher P. Phenix & Ed S. Krol (2021). Employing in vitro metabolism to guide design of F-labelled PET probes of novel α-synuclein binding bifunctional compounds, Xenobiotica, 51:8, 885-900, 10.1080/00498254.2021.1943566.

Supplementary Information

Employing *in vitro* metabolism to guide design of F-labeled PET probes of novel α-Synuclein binding bifunctional compounds

Table S1. Neutral losses observed from the tandem mass spectrometry of ^{19}F-[C_8-6-C_8], ^{19}F-[C_8-6-N] and ^{19}F-[C_8-6-I]

	^{19}F-[C_8-6-C_8]	**^{19}F-[C_8-6-N]**	**^{19}F-[C_8-6-I]**
Neutral losses (Da)	20 (HF) 44 (CO_2) 27 (CN) 57 (C_2H_3NO) 194 ($C_8H_{10}N_4O_2$) 232 ($C_{11}H_{12}N_4O_2$) 260 ($C_{13}H_{16}N_4O_2$) 303 ($C_{15}H_{21}N_5O_2$) 2 (H_2)	20 (HF) 57 (C_2H_3NO) 69 (C_4H_7N) 79 (C_5H_5N) 105 (C_7H_7N) 119 (C_8H_9N) 126 ($C_7H_{14}N_2$) 131 (C_9H_9) 148 ($C_{11}H_{18}N_2$) 319 ($C_{16}H_{25}N_5O_2$) 331 ($C_{17}H_{25}N_5O_2$) 345 ($C_{18}H_{27}N_5O_2$) 2 (H_2)	20 (HF) 116 (C_9H_8) 339 ($C_{16}H_{26}FN_5O_2$)

Table S2 HPLC-high-resolution and tandem mass spectrometric (LC-QqTOF) data for C_8-6-I and C_8-6-N metabolites detected in human, mouse, and rat liver microsomes (HLM, MLM, and RLM).

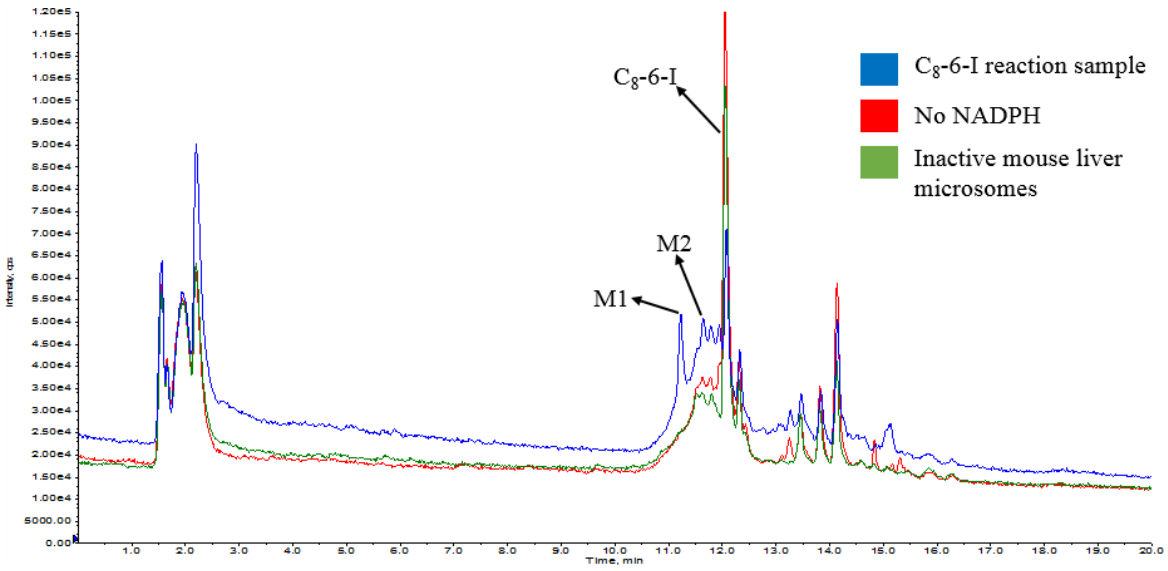
Metabolite Designation	**Metabolic Reaction**	**Retention Time (min)**	**Molecular Formular**	**Exact Mass (*m/z*)**	**Mass Error(ppm)**	**Parent Compound**	**Matrices**
M1	De-alkylation	11.23	$C_{14}H_{24}N_5O_2$	294.1924	0.000	C_8-6-I	HLM, MLM, RLM
M2	Hydroxylation	11.64	$C_{23}H_{32}N_5O_3$	426.2499	6.8035	C_8-6-I	HLM, MLM, RLM
M3	De-alkylation	11.25	$C_{14}H_{24}N_5O_2$	294.1924	0.0000	C_8-6-N	HLM, MLM, RLM
M4	Hydroxylation	11.53	$C_{23}H_{33}N_6O_3$	441.2608	6.1188	C_8-6-N	HLM, MLM, RLM

Table S3 HPLC-tandem mass spectrometric (LC-QqLIT) data for C_8-6-I, ^{19}F-[C_8-6-I], ^{19}F-[C_8-6-C_8] and ^{19}F-[C_8-6-N] metabolites detected in human, mouse, and rat liver microsomes (HLM, MLM, and RLM).

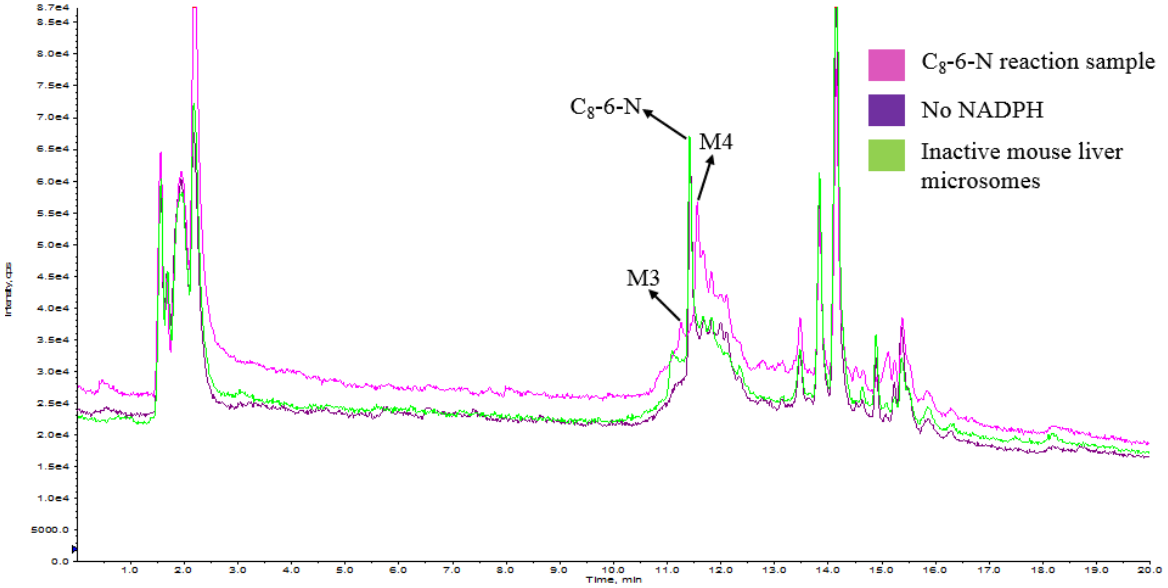
Metabolite Designation	**Metabolic Reaction**	**Retention Time (min)**	**Molecular Formular**	**Exact Mass (*m/z*)**	**Parent Compound**	**Matrices**
M1	De-alkylation	9.02	$C_{14}H_{24}N_50_2$	294.1924	C_8-6-I	HLM, MLM, RLM
M2	Hydroxylation	9.66	$C_{23}H_{32}N_50_3$	426.2499	C_8-6-I	HLM, MLM, RLM
M5A, M5B	N-demethylation	9.90	$C_{22}H_{29}N_5O_2$	396.2429	C_8-6-I	HLM, MLM, RLM
M6	De-alkylation	9.51	$C_{16}H_{26}FN_5O_2$	340.1871	^{19}F-[C_8-6-I]	HLM MLM, RLM

M7A, M7B	Hydroxylation	9.96	$C_{25}H_{34}FN_5O_3$	472.2808	^{19}F-[C_8-6-I]	HLM MLM, RLM
M8A, M8B	N-demethylation	10.12	$C_{24}H_{32}FN_5O_3$	442.2252	^{19}F-[C_8-6-I]	HLM MLM, RLM
M9	Hydroxylation	10.88	$C_{24}H_{35}FN_8O_5$	533.2789	^{19}F-[C_8-6-C_8]	HLM, MLM, RLM
M10	De-alkylation	9.51	$C_{16}H_{26}FN_5O_2$	340.2291	^{19}F-[C_8-6-N]	HLM, MLM, RLM
M11	Hydroxylation	10.19	$C_{25}H_{35}FN_6O_3$	487.3347	^{19}F-[C_8-6-N]	HLM, MLM, RLM

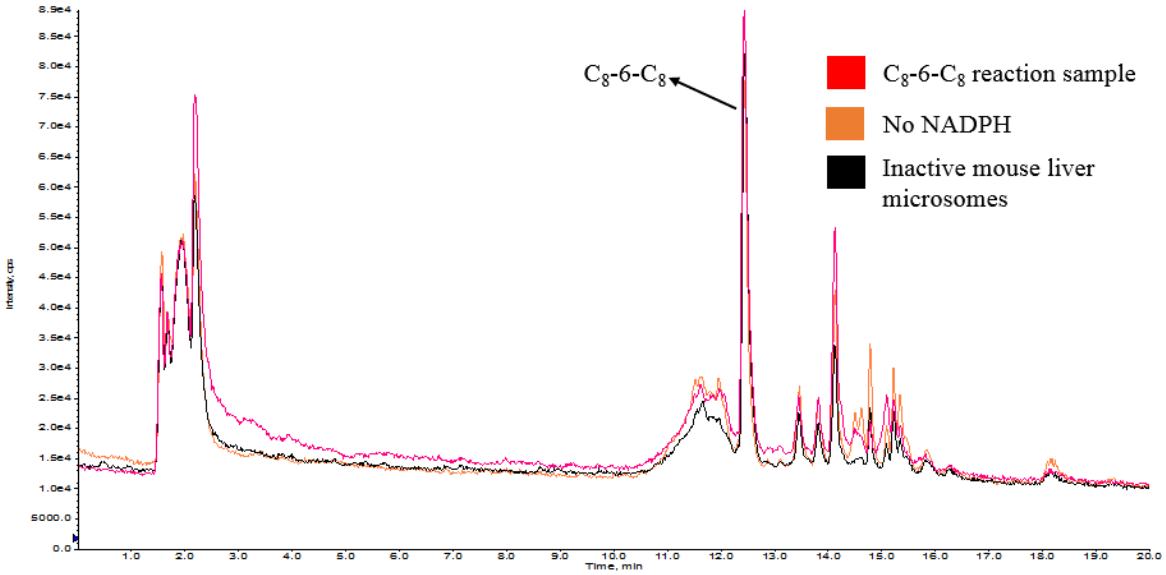
A


C8-6-I
M2
M1
C8-6-I reaction sample
No NADPH
Inactive mouse liver microsomes
Intensity, cps
Time, min

B


C8-6-N
M4
M3
C8-6-N reaction sample
No NADPH
Inactive mouse liver microsomes
Intensity, cps
Time, min

C


C8-6-C8
C8-6-C8 reaction sample
No NADPH
Inactive mouse liver microsomes
Intensity, cps
Time, min

D

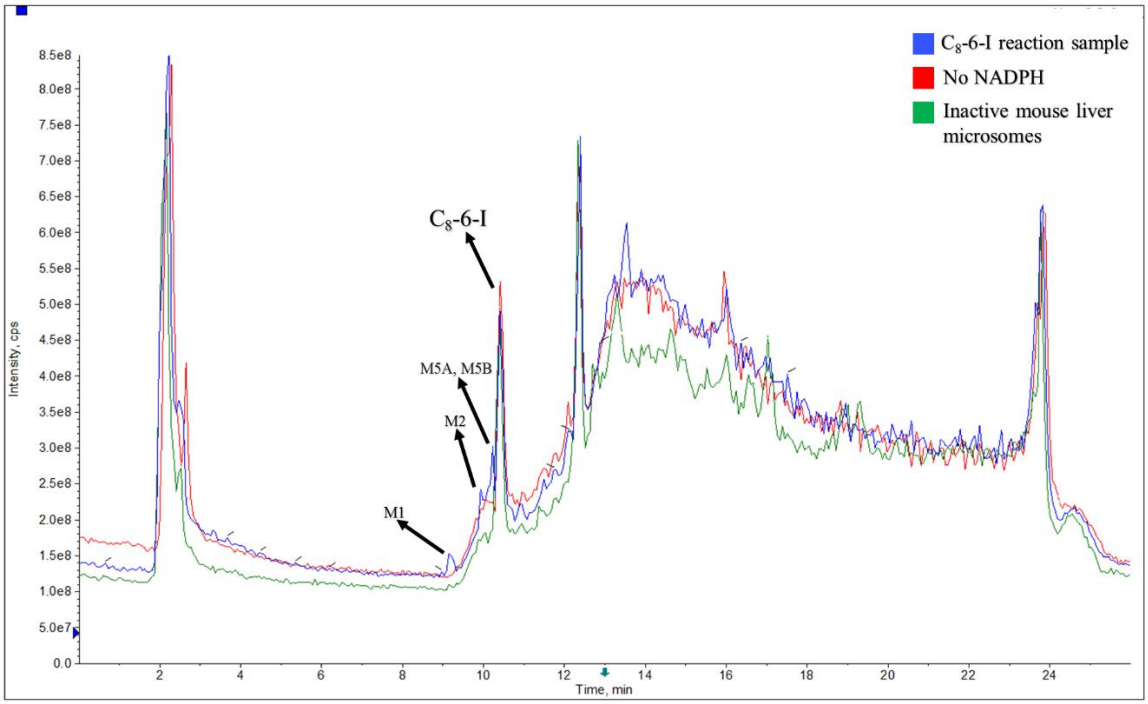
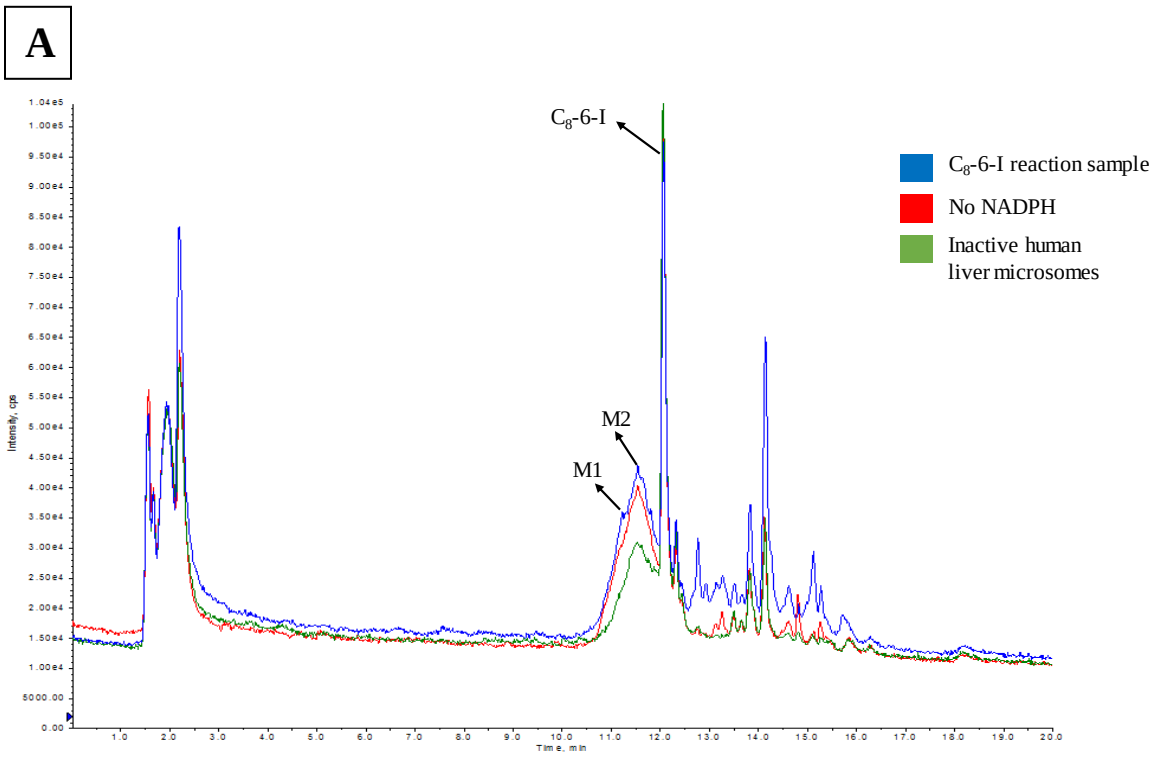
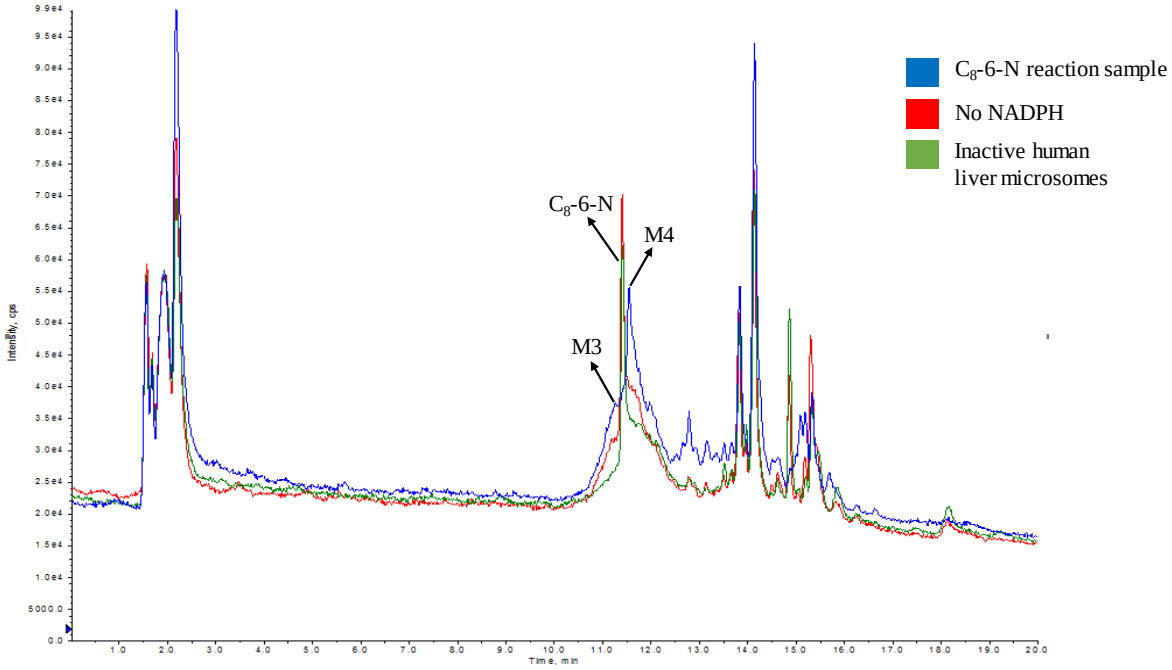

C8-6-I reaction sample
No NADPH
Inactive mouse liver microsomes
C8-6-I
M5A, M5B
M2
M1
Intensity, cps
Time, min

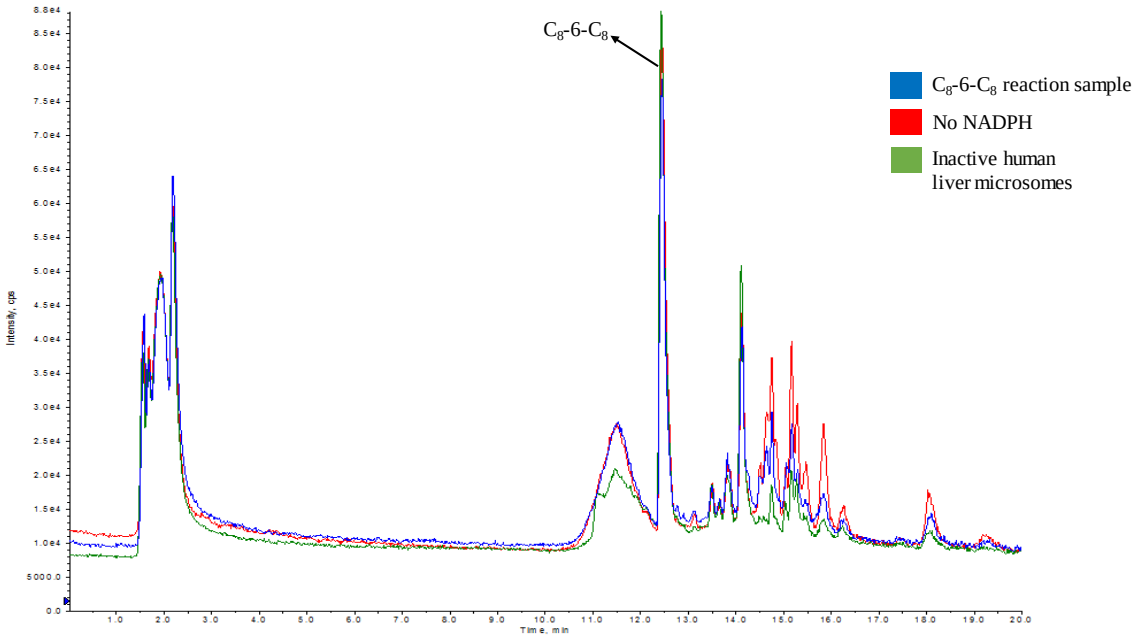
Figure S1. Total ion chromatogram (ESI in positive mode) of LC-QqTOF-MS analysis of a 50 µL injection of 15 µM samples from *in vitro* mouse liver microsomal metabolism of C_8-6-I (A), C_8-6-N (B), and C_8-6-C_8 (C) and LC-QqLIT-MS analysis of a 10 µL injection of 15 µM C_8-6-I (D) from *in vitro* mouse liver microsomal metabolism.



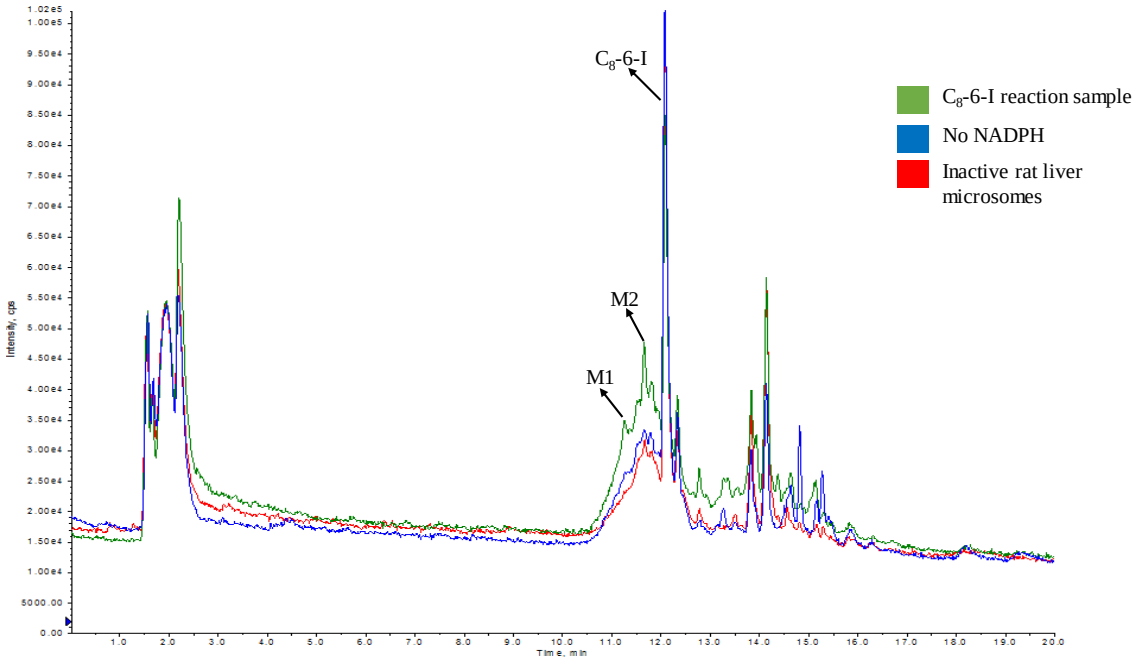
B


C8-6-N reaction sample
No NADPH
Inactive human liver microsomes
C8-6-N
M4
M3
Intensity, cps
Time, min

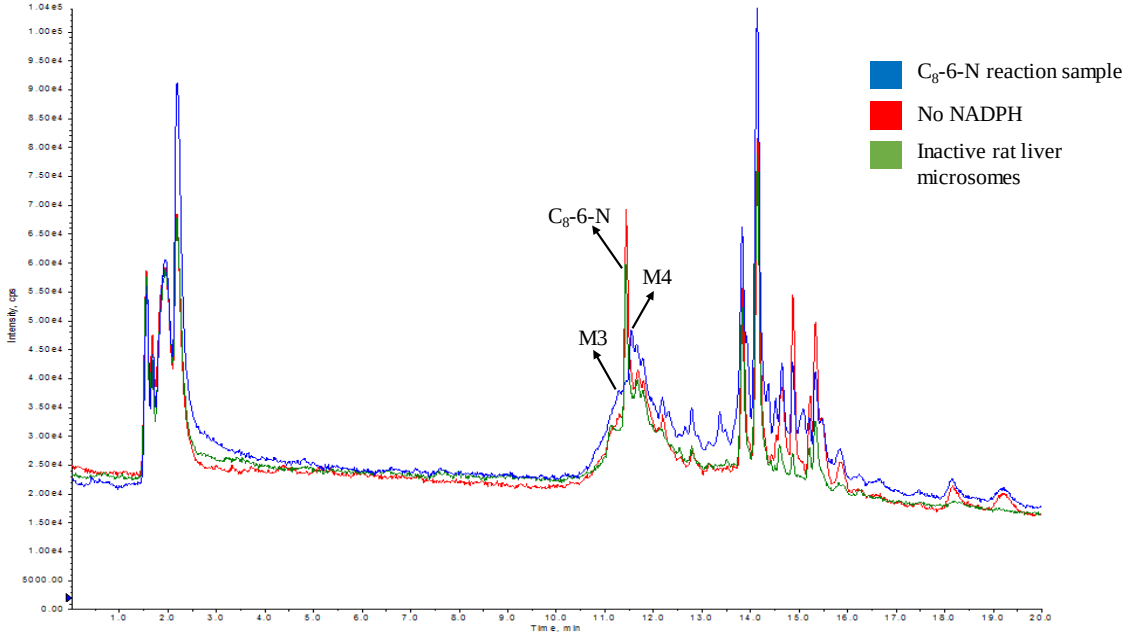
C


C8-6-C8
C8-6-C8 reaction sample
No NADPH
Inactive human liver microsomes
Intensity, cps
Time, min

D


C8-6-I
M2
M1
C8-6-I reaction sample
No NADPH
Inactive rat liver microsomes
Intensity, cps
Time, min

E


C8-6-N
M4
M3
C8-6-N reaction sample
No NADPH
Inactive rat liver microsomes
Intensity, cps
Time, min

F

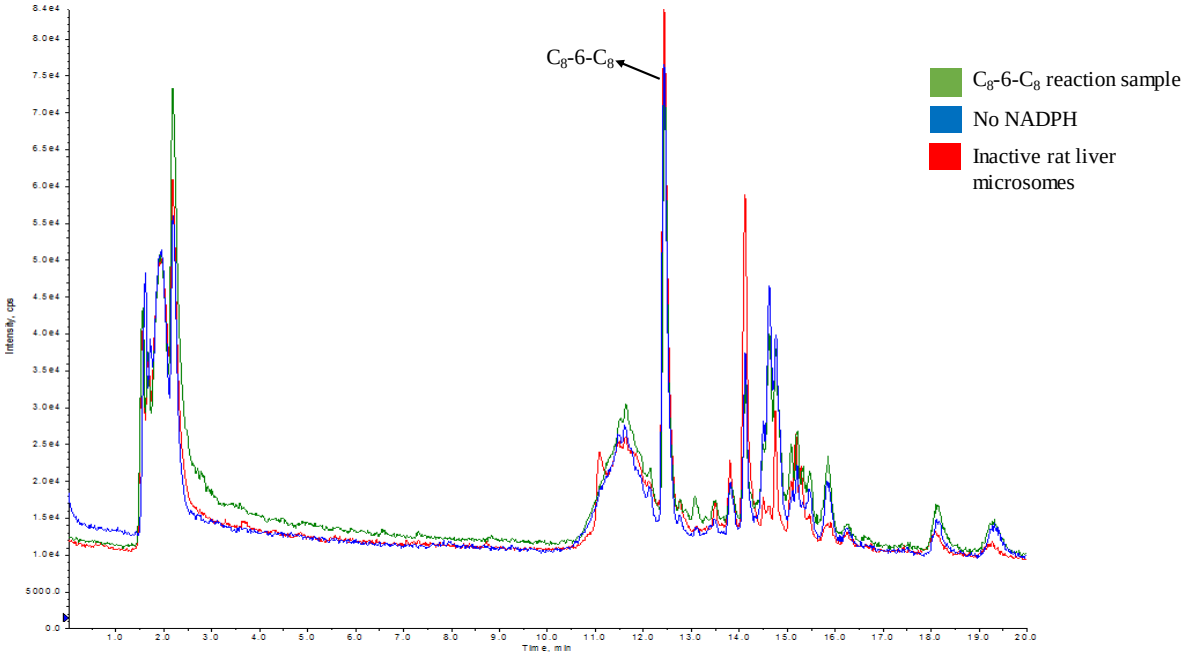
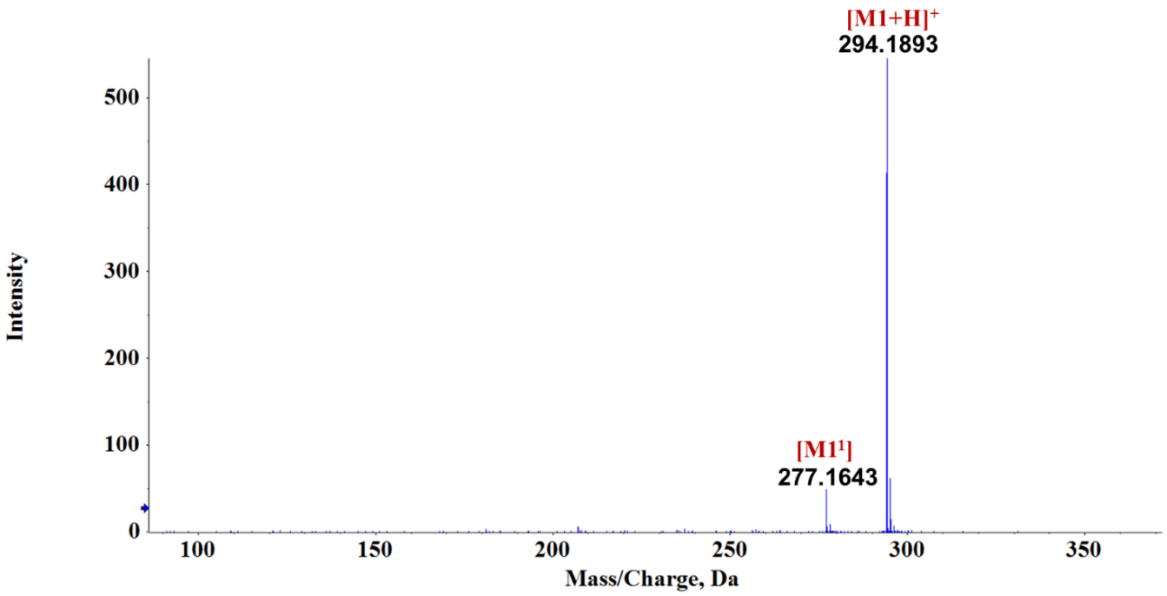
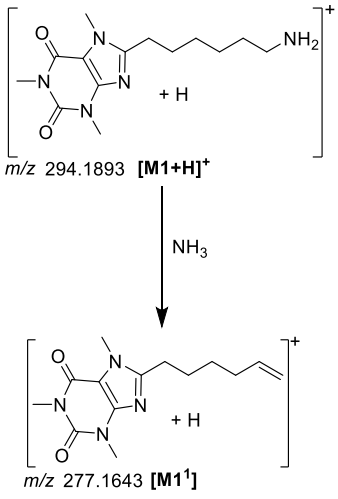


Figure S2. Total ion chromatogram of LC-MS analysis of samples from *in vitro* human liver microsomal metabolism of C_8-6-I (A), C_8-6-N (B), C_8-6-C_8 (C), and *in vitro* rat liver microsomal metabolism of C_8-6-I (D), C_8-6-N (E), C_8-6-C_8 (F).

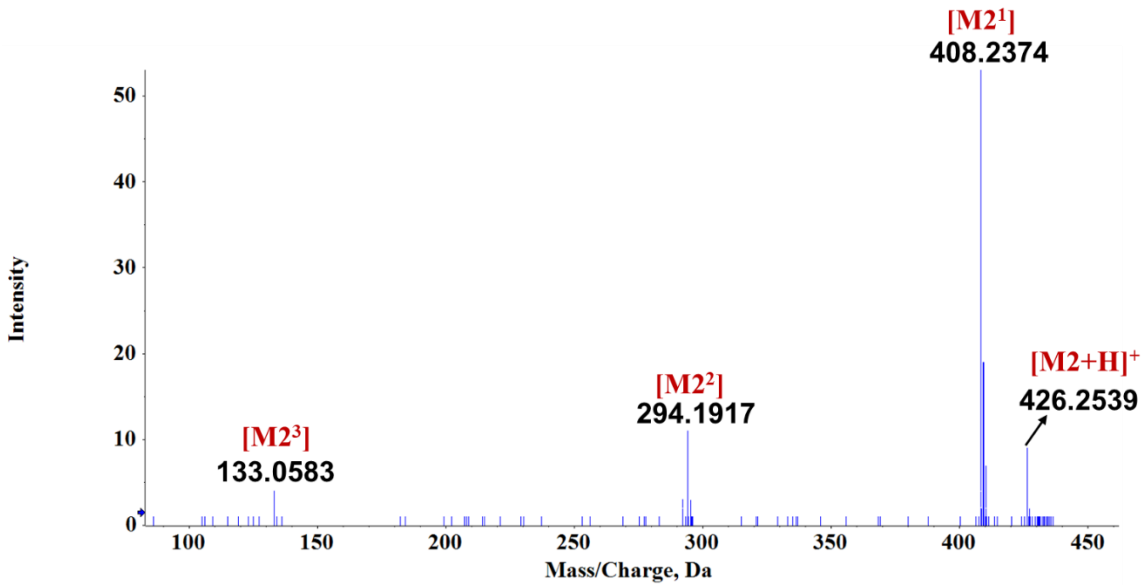
A


[M1+H]+
294.1893
[M1¹]
277.1643
Intensity
Mass/Charge, Da
0
100
200
300
400
500
100
150
200
250
300
350

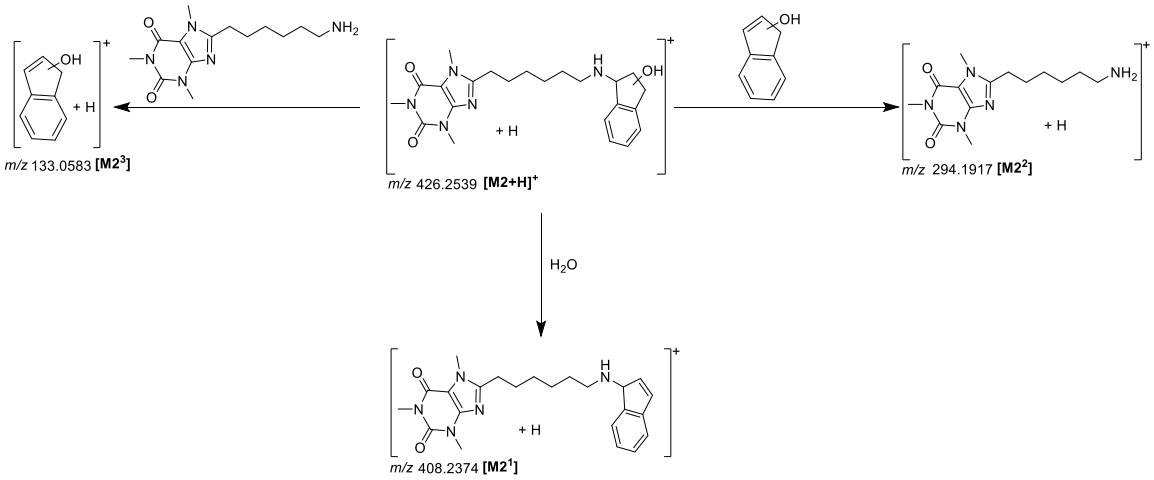
B


NH2
+ H
m/z 294.1893 [M1+H]+
NH3
+ H
m/z 277.1643 [M1¹]

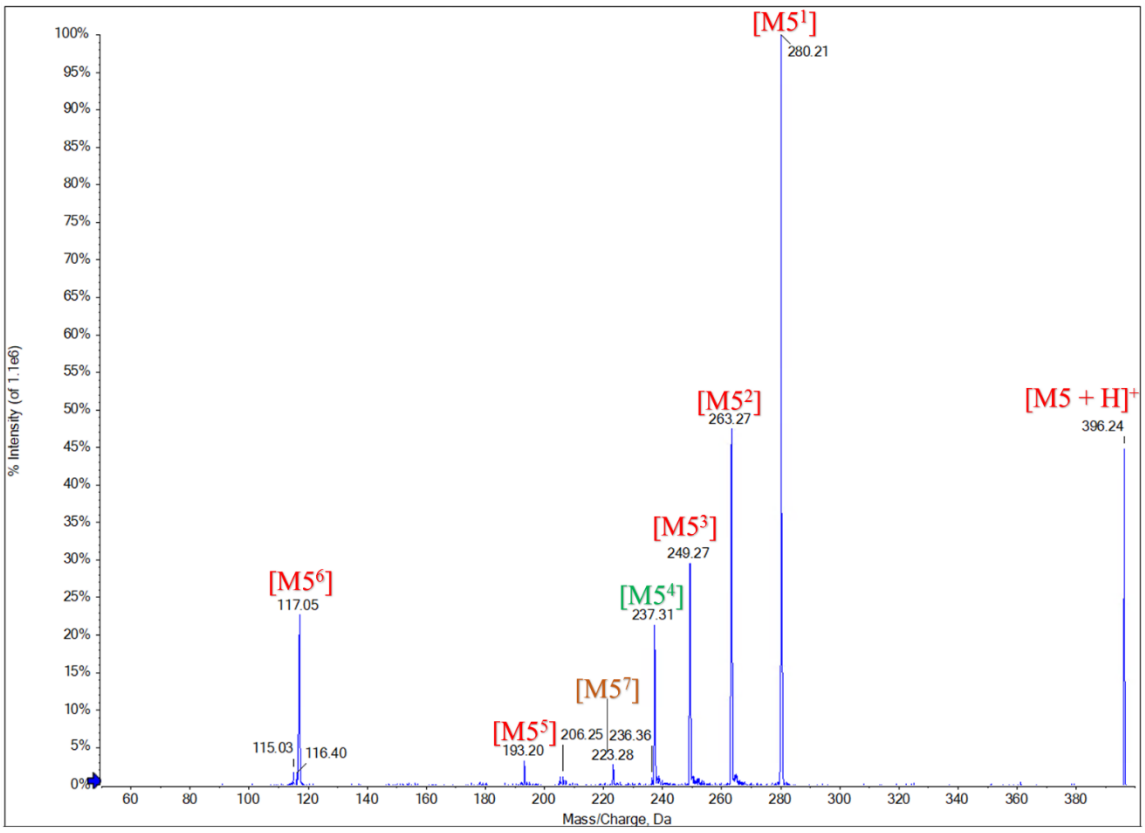
C


[M2¹]
408.2374
[M2+H]⁺
426.2539
[M2²]
294.1917
[M2³]
133.0583
Intensity
Mass/Charge, Da
0
10
20
30
40
50
100
150
200
250
300
350
400
450

D


OH
+ H
NH2
m/z 133.0583 [M2³]
m/z 426.2539 [M2+H]⁺
m/z 294.1917 [M2²]
H2O
m/z 408.2374 [M2¹]

E


[M5¹]
280.21
[M5²]
263.27
[M5 + H]⁺
396.24
[M5³]
249.27
[M5⁶]
117.05
[M5⁴]
237.31
[M5⁷]
[M5⁵]
193.20
206.25
236.36
223.28
115.03
116.40
% Intensity (of 1.1e6)
Mass/Charge, Da

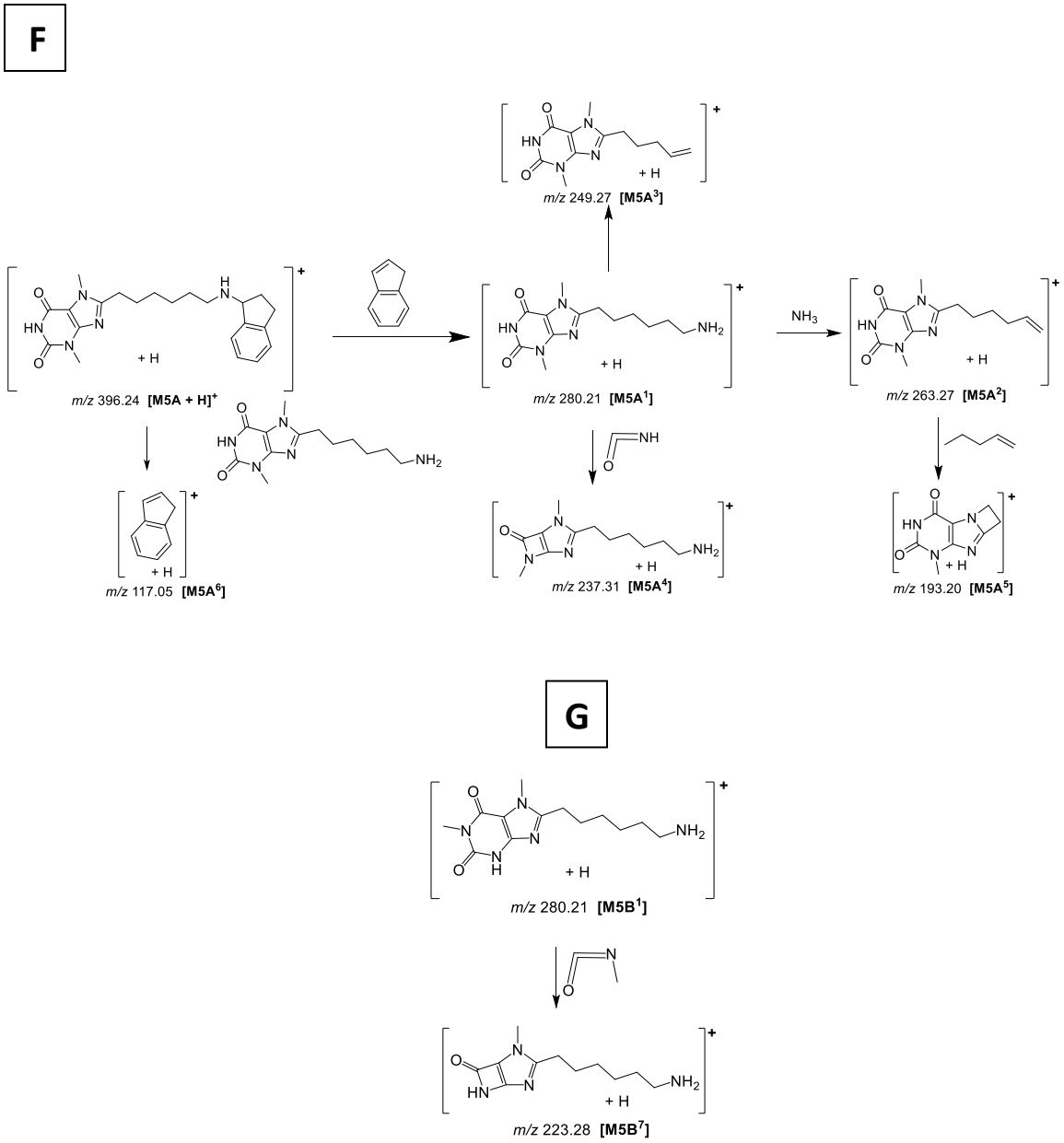
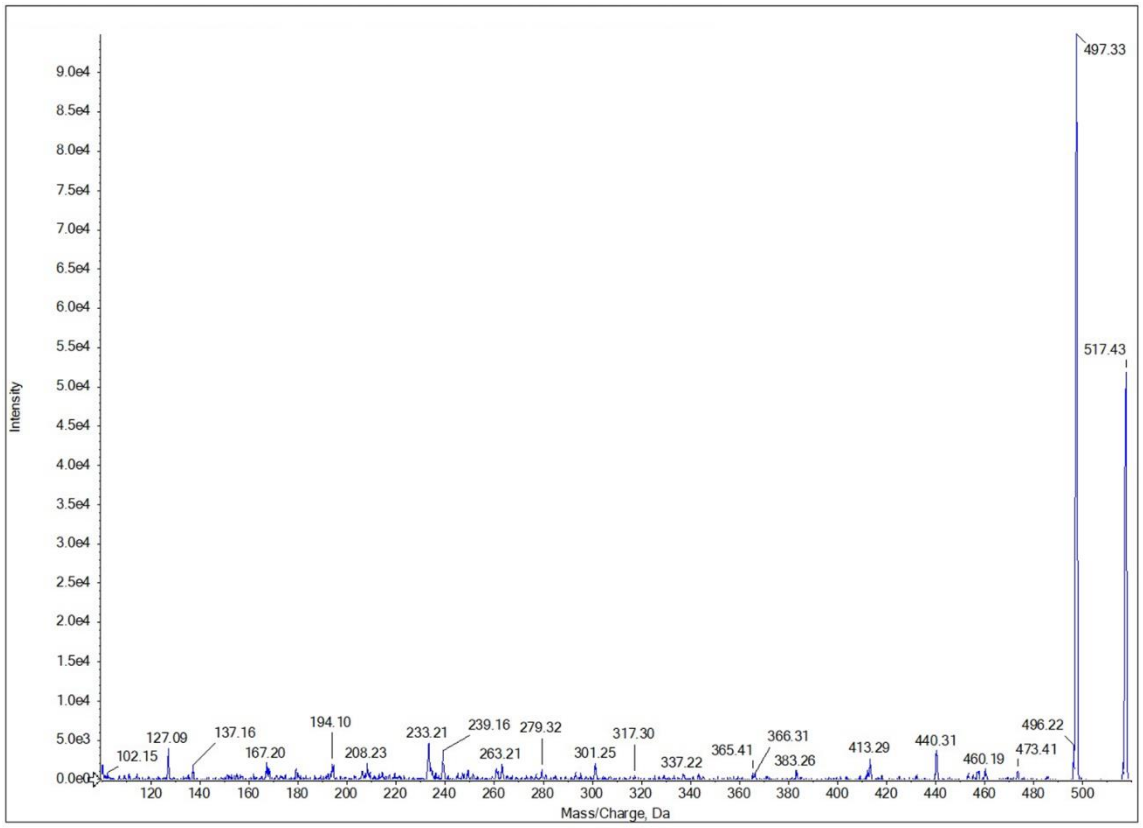


Figure S3. The ESI-QToF-MS/MS spectrum for C_8-6-I metabolites M1(A), M2(C) and the proposed fragmentation pathway for M1(B), M2(D). The ESIQqLIT-MS/MS spectrum of C_8-6-I metabolites M5A and M5B (E), and the proposed fragmentation pathway for M5A (F) and M5B (G) ESI was performed in positive mode.

A


Intensity
Mass/Charge, Da
497.33
517.43
102.15
127.09
137.16
167.20
194.10
208.23
233.21
239.16
263.21
279.32
301.25
317.30
337.22
365.41
366.31
383.26
413.29
440.31
460.19
473.41
496.22

B

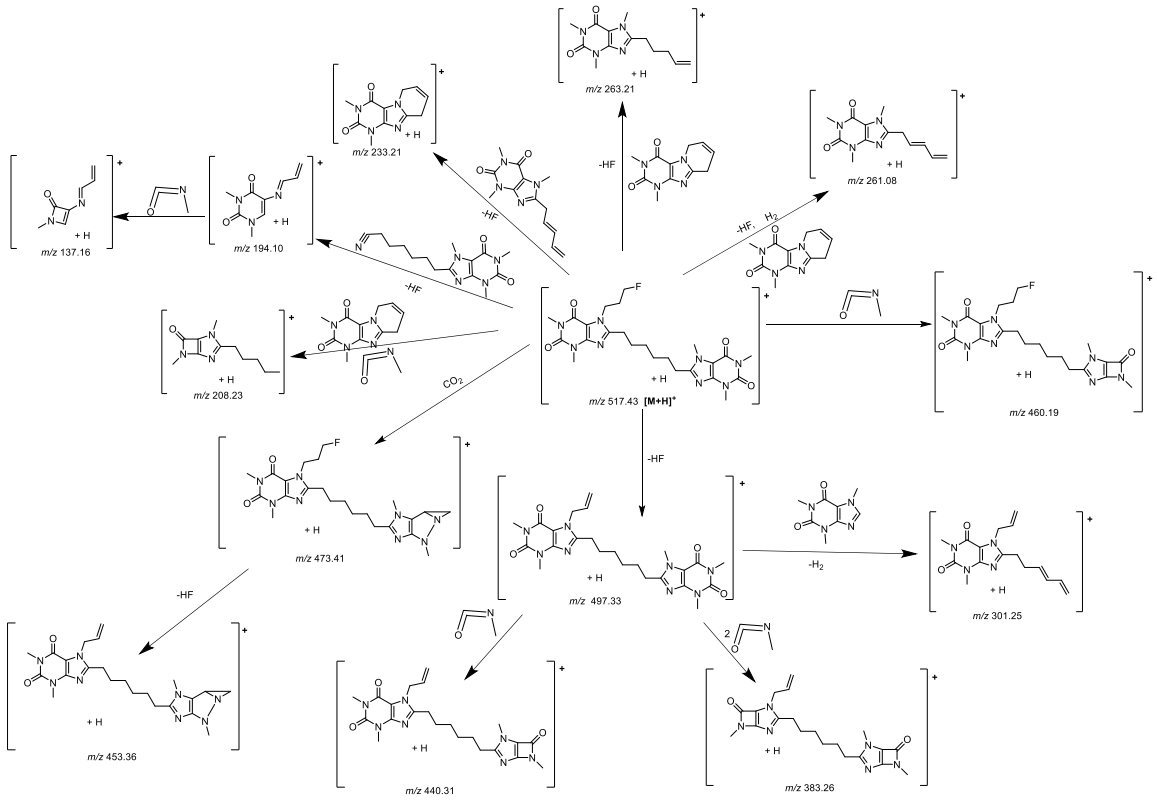
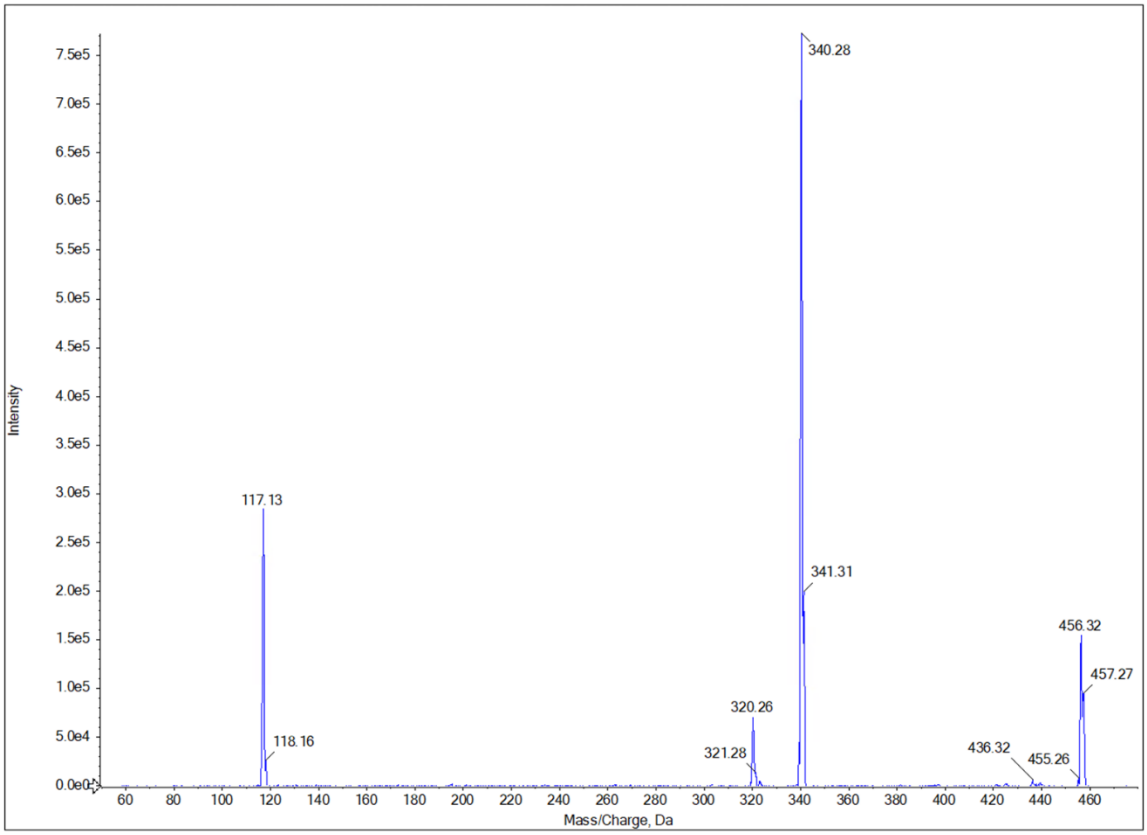


Figure S4. MS/MS spectrum (A) and proposed fragmentation pathway (B) of ^{19}F-[C_8-6-C_8].

A


Intensity
7.5e5
7.0e5
6.5e5
6.0e5
5.5e5
5.0e5
4.5e5
4.0e5
3.5e5
3.0e5
2.5e5
2.0e5
1.5e5
1.0e5
5.0e4
0.0e0
340.28
117.13
341.31
456.32
457.27
320.26
118.16
321.28
436.32
455.26
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460
Mass/Charge, Da

B

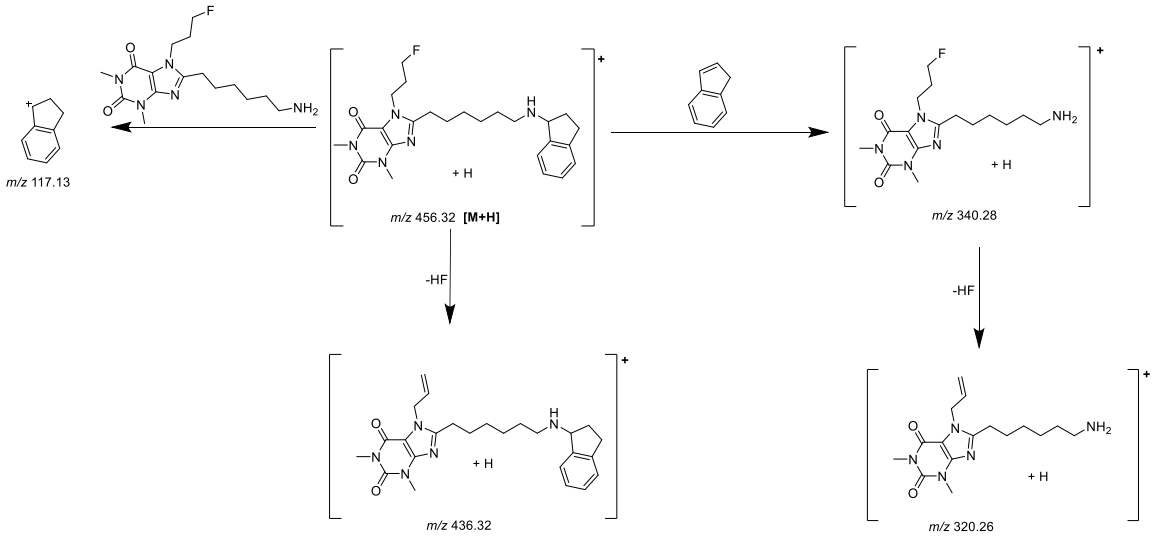
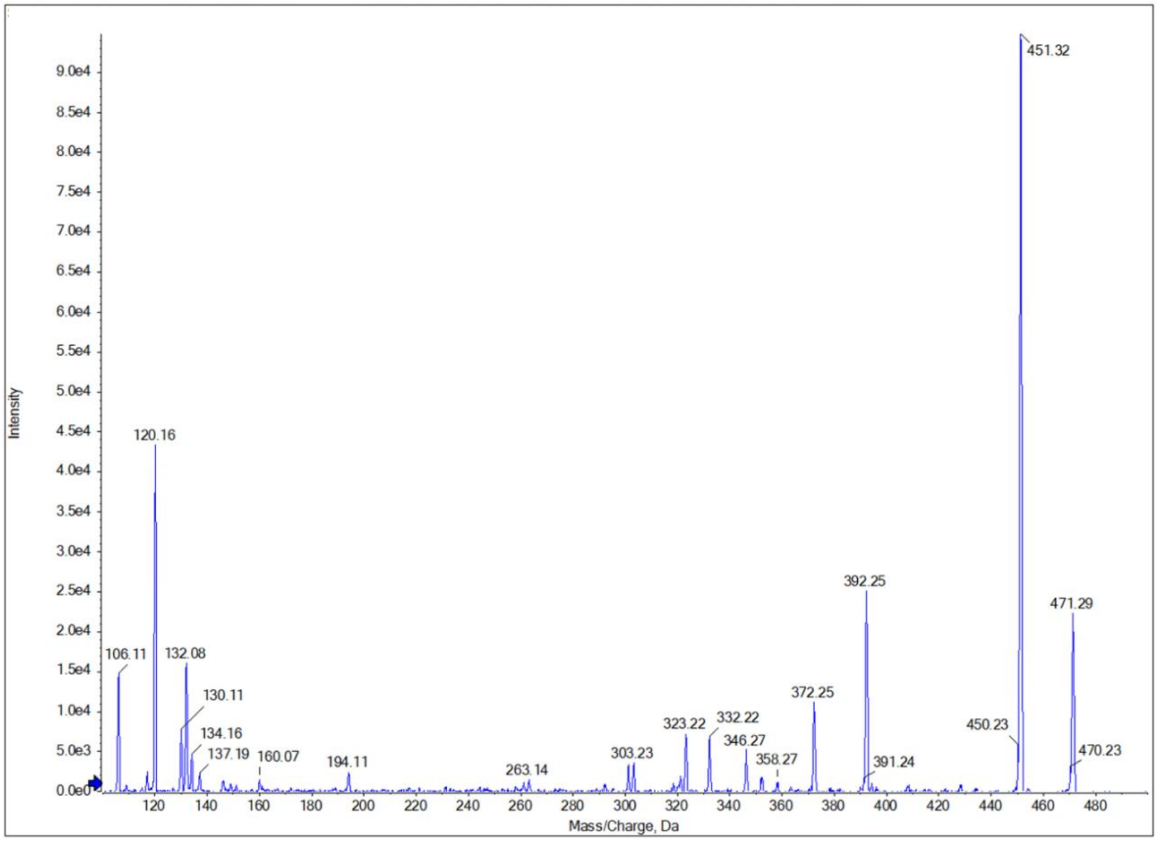


Figure S5. MS/MS spectrum (A) and proposed fragmentation pathway (B) of ^{19}F-[C_8-6-I].

A


Intensity
Mass/Charge, Da
451.32
120.16
392.25
471.29
106.11
132.08
130.11
372.25
323.22
332.22
450.23
134.16
346.27
137.19
160.07
358.27
470.23
194.11
303.23
391.24
263.14

B

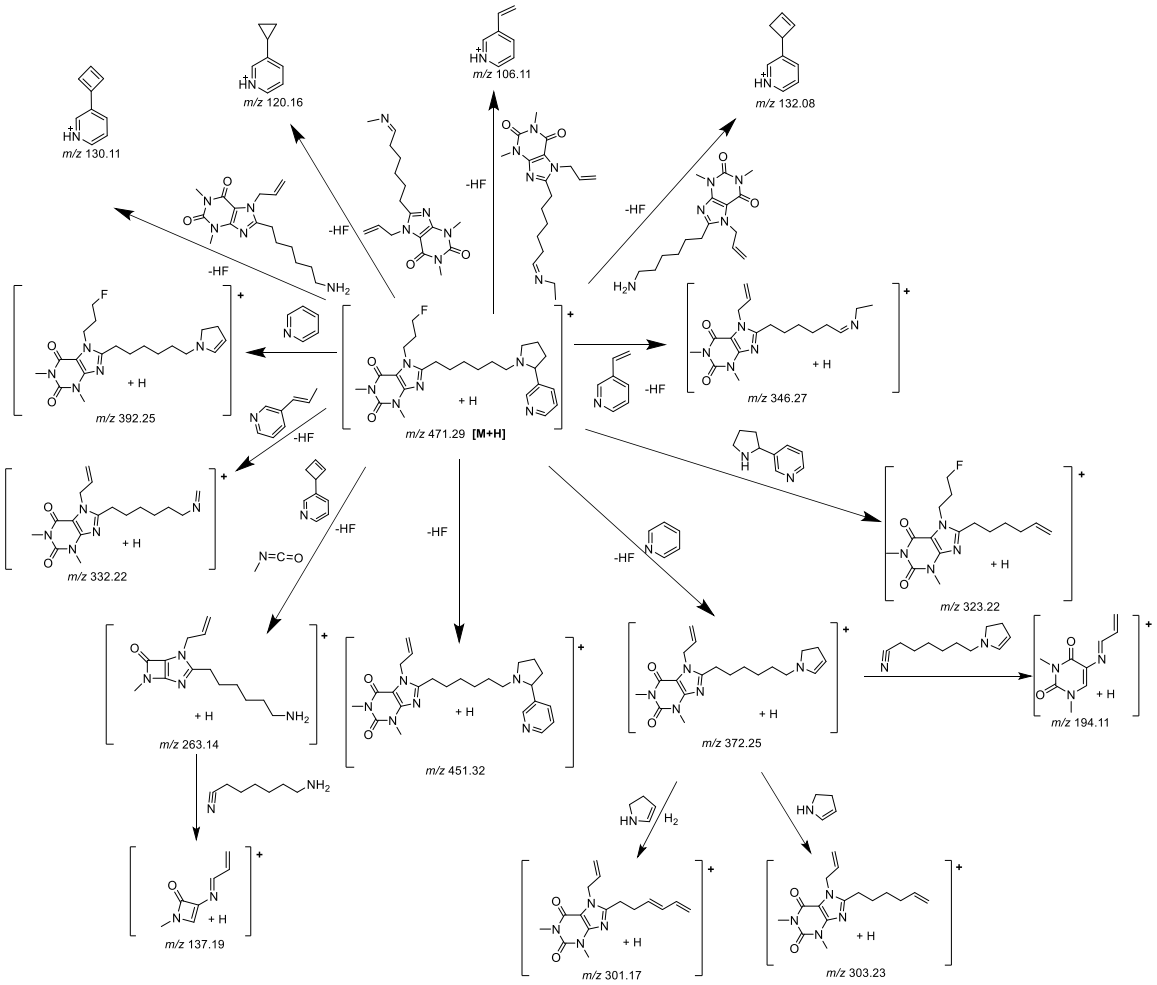
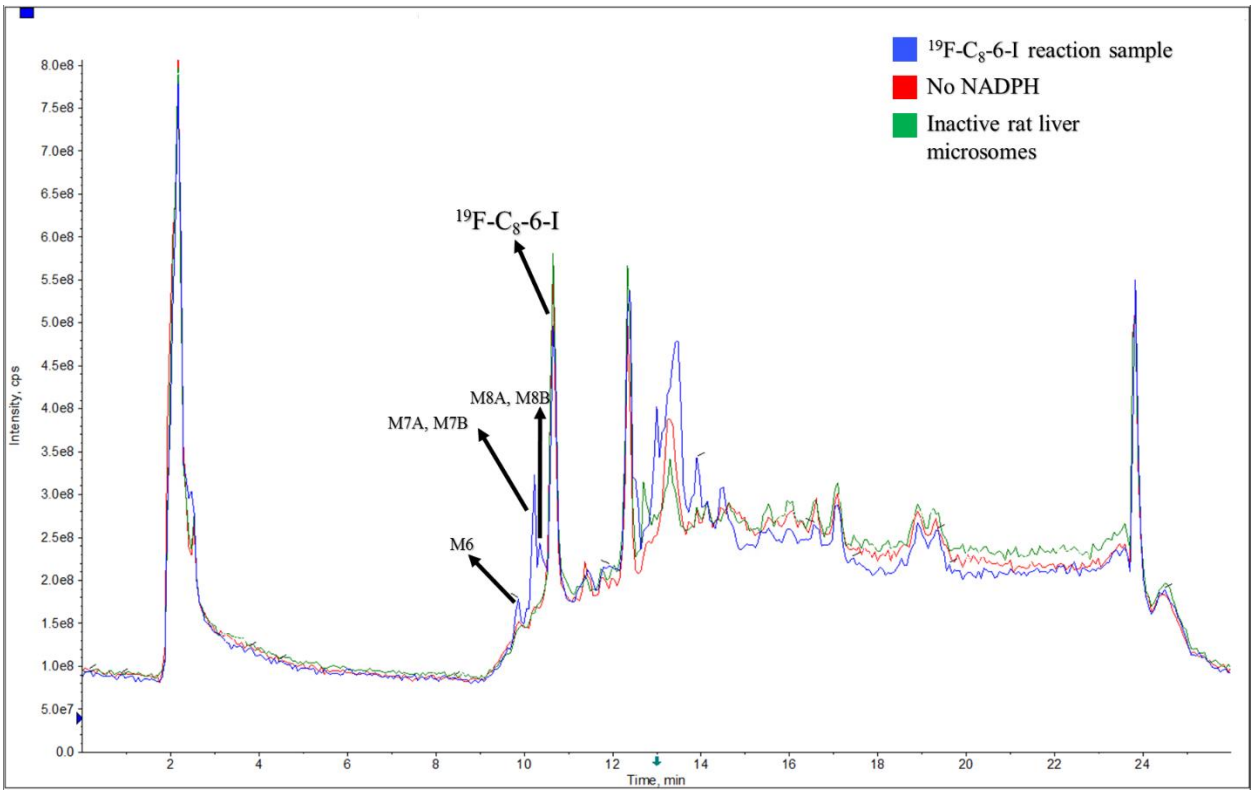
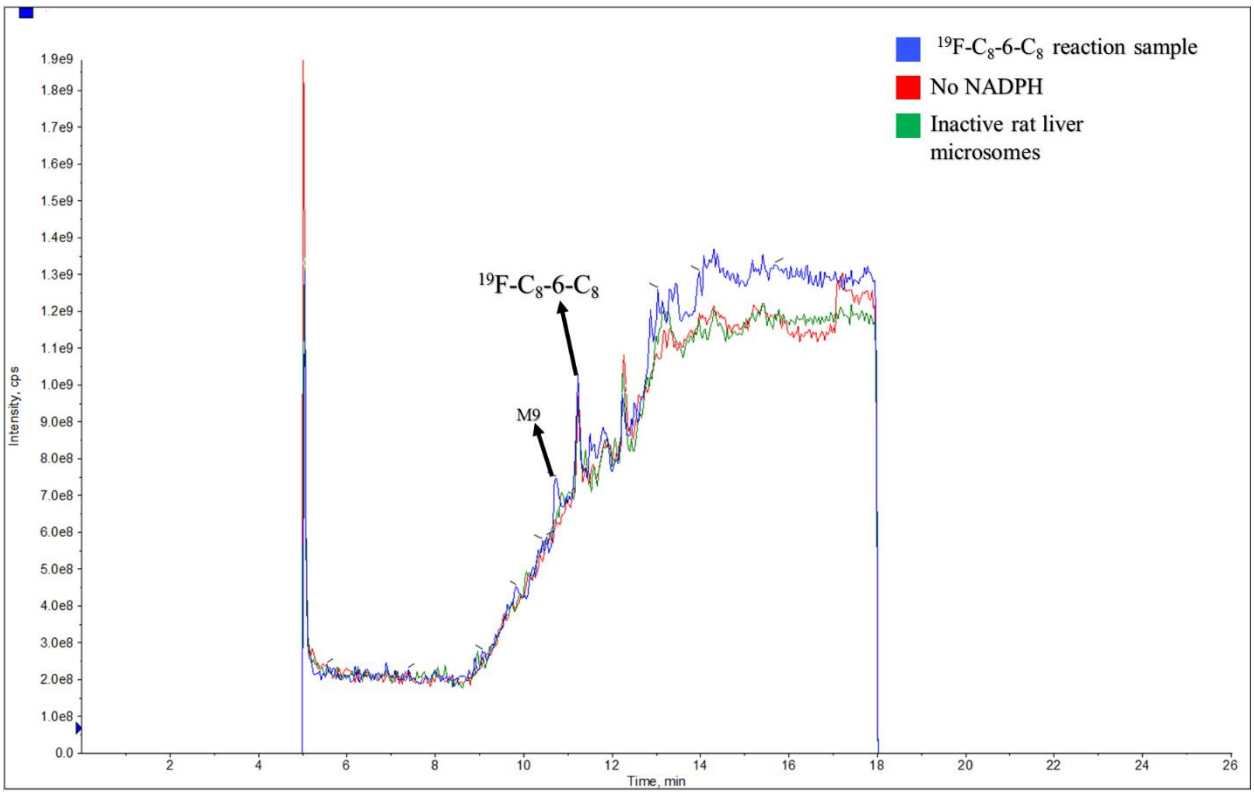


Figure S6. MS/MS spectrum (A) and proposed fragmentation pathway (B) of ^{19}F-[C_8-6-N].

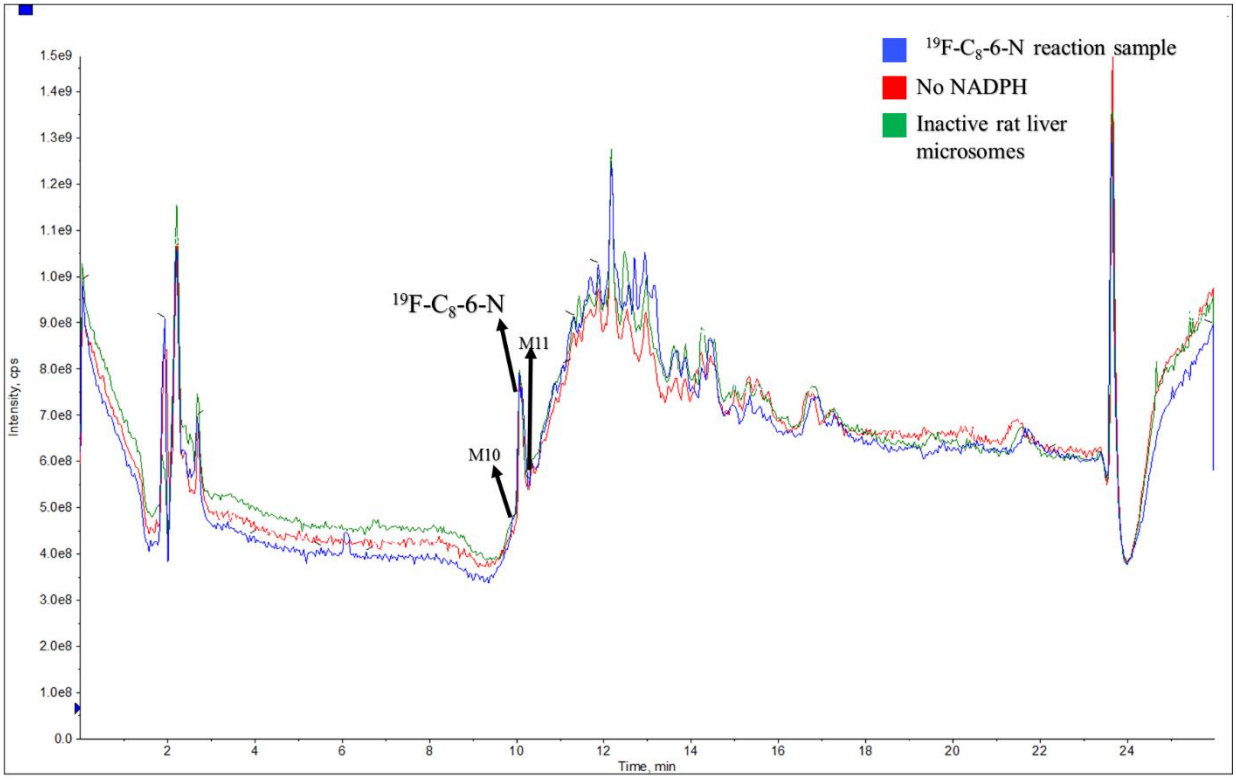
A


^{19}F-C$_8$-6-I reaction sample
No NADPH
Inactive rat liver microsomes
^{19}F-C$_8$-6-I
M8A, M8B
M7A, M7B
M6
Intensity, cps
Time, min

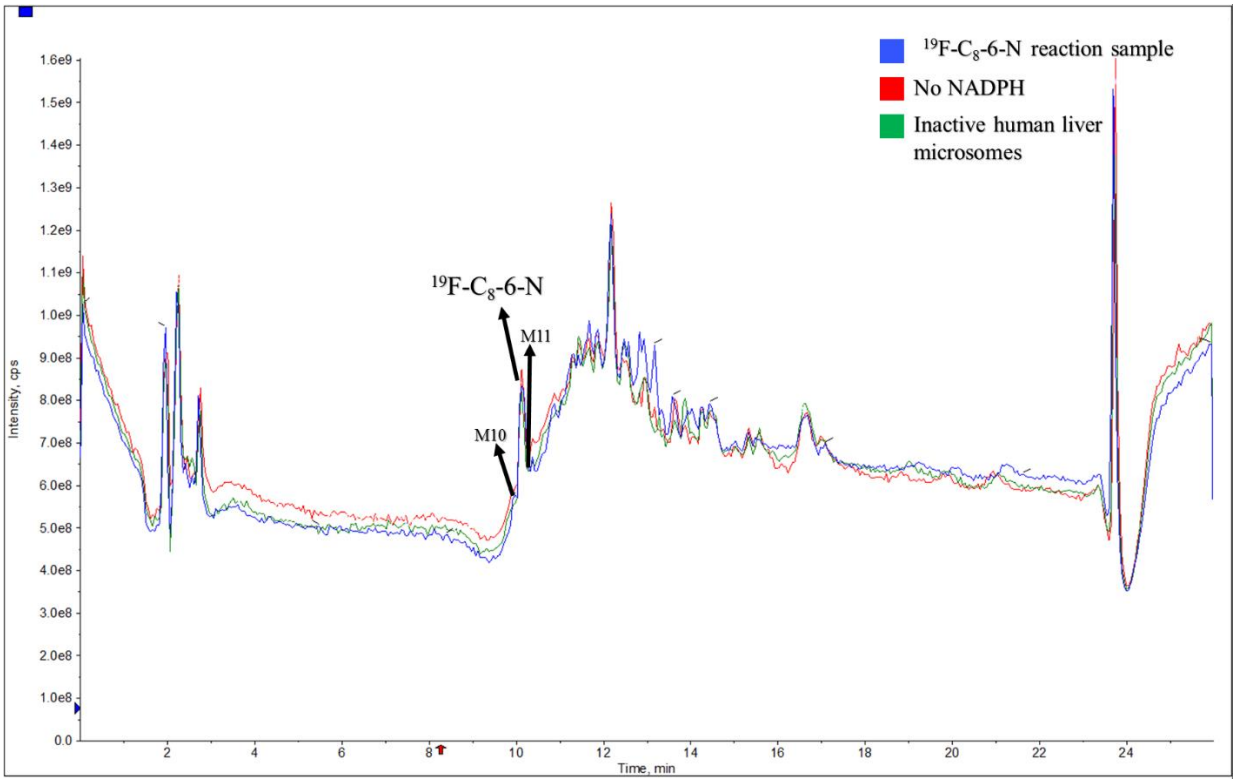
B


^{19}F-C_8-6-C_8 reaction sample
No NADPH
Inactive rat liver microsomes
^{19}F-C_8-6-C_8
M9
Intensity, cps
Time, min
1.9e9
1.8e9
1.7e9
1.6e9
1.5e9
1.4e9
1.3e9
1.2e9
1.1e9
1.0e9
9.0e8
8.0e8
7.0e8
6.0e8
5.0e8
4.0e8
3.0e8
2.0e8
1.0e8
0.0
2
4
6
8
10
12
14
16
18
20
22
24
26

C


^{19}F-C$_8$-6-N reaction sample
No NADPH
Inactive rat liver microsomes
^{19}F-C$_8$-6-N
M11
M10
Intensity, cps
Time, min
1.5e9
1.4e9
1.3e9
1.2e9
1.1e9
1.0e9
9.0e8
8.0e8
7.0e8
6.0e8
5.0e8
4.0e8
3.0e8
2.0e8
1.0e8
0.0
2
4
6
8
10
12
14
16
18
20
22
24

D


^{19}F-C_8-6-N reaction sample
No NADPH
Inactive human liver microsomes
^{19}F-C_8-6-N
M11
M10
Intensity, cps
Time, min
1.6e9
1.5e9
1.4e9
1.3e9
1.2e9
1.1e9
1.0e9
9.0e8
8.0e8
7.0e8
6.0e8
5.0e8
4.0e8
3.0e8
2.0e8
1.0e8
0.0
2
4
6
8
10
12
14
16
18
20
22
24

E

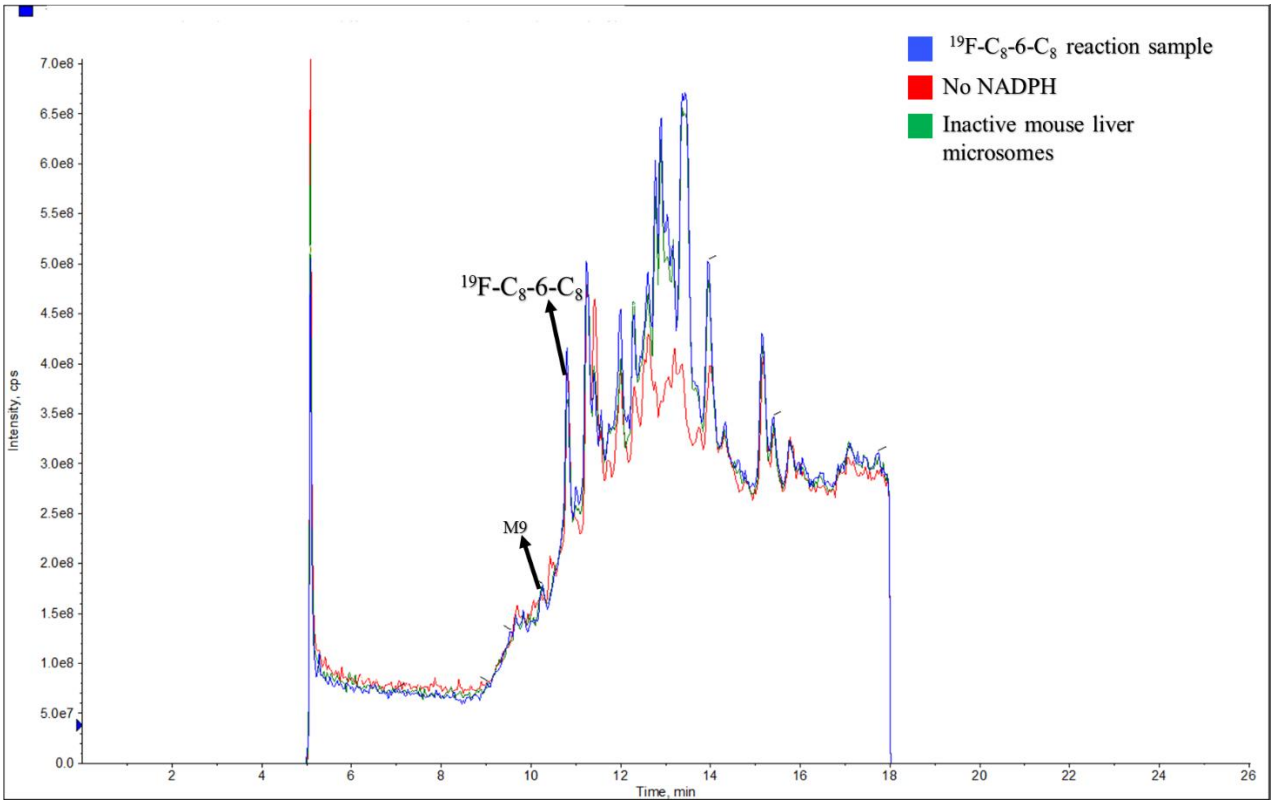
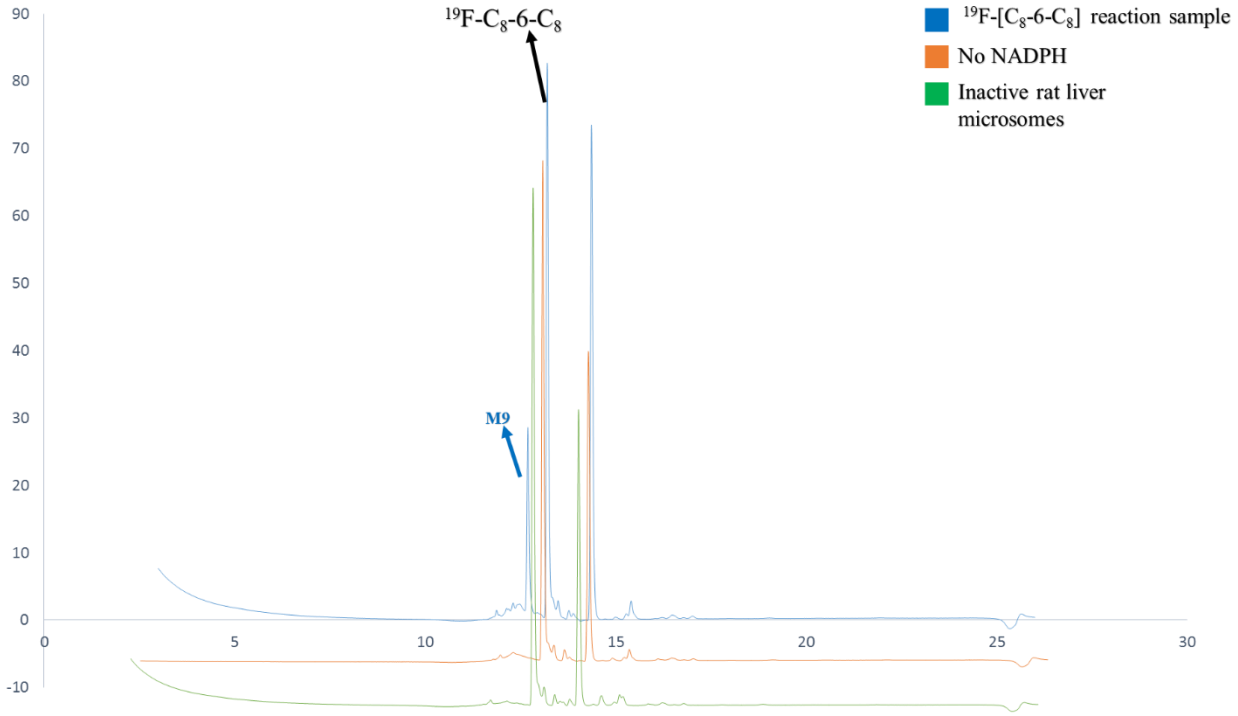
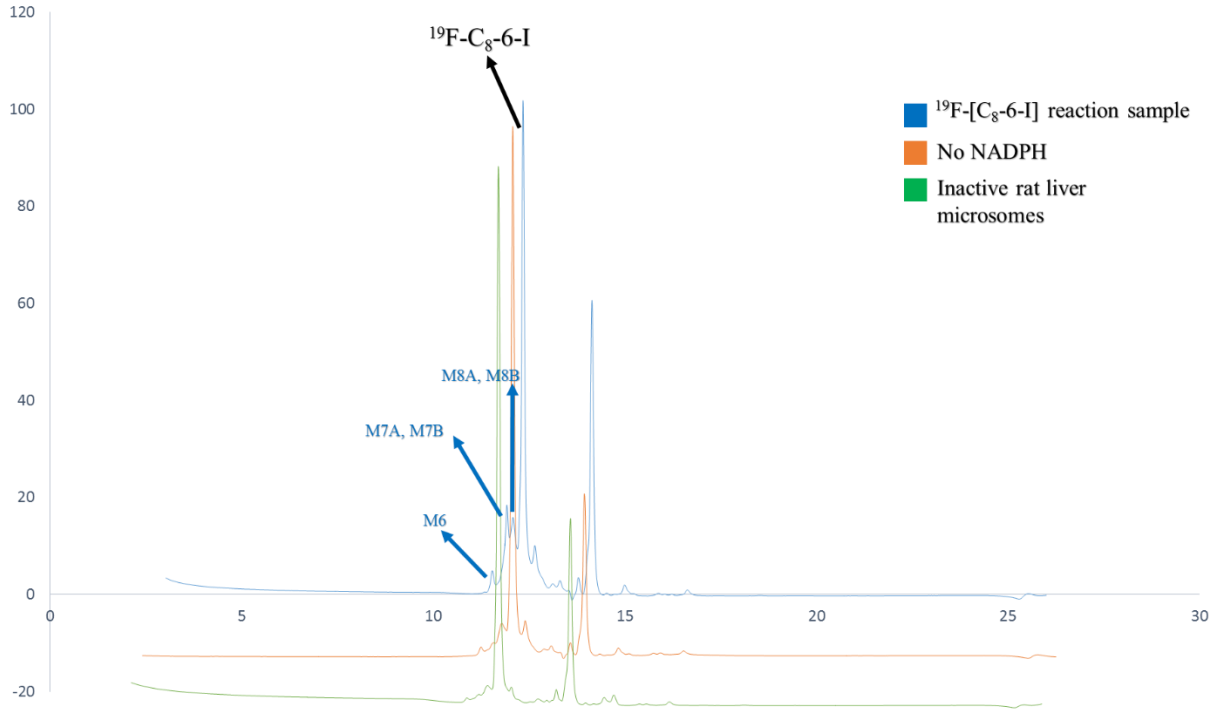


Figure S7. Total ion chromatogram of LC-MS analysis of samples from *in vitro* rat liver microsomal metabolism of ^{19}F-[C$_8$-6-I] (A) ^{19}F-[C$_8$-6-C$_8$] (B) ^{19}F-[C$_8$-6-N] (C), *in vitro* human liver microsomal metabolism of ^{19}F-[C$_8$-6-N] (D), and *in vitro* mouse liver microsomal metabolism of ^{19}F-[C$_8$-6-C$_8$] (E)

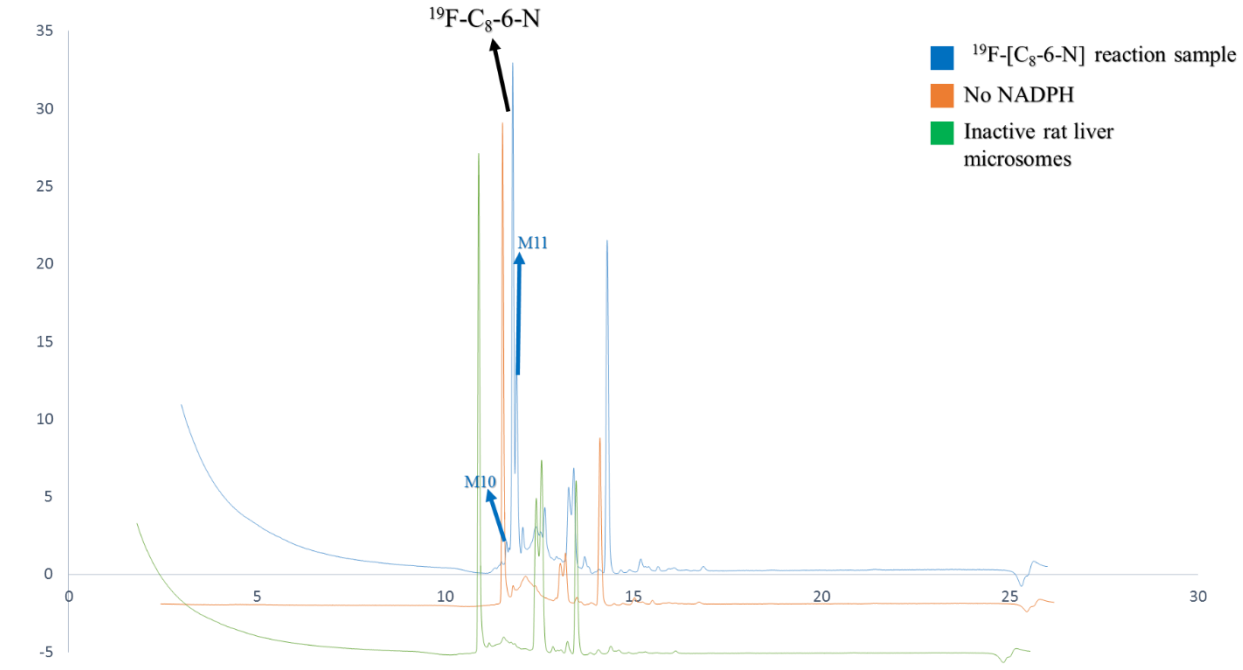
A


^{19}F-C_8-6-C_8
^{19}F-[C_8-6-C_8] reaction sample
No NADPH
Inactive rat liver microsomes
M9
90
80
70
60
50
40
30
20
10
0
-10
0
5
10
15
20
25
30

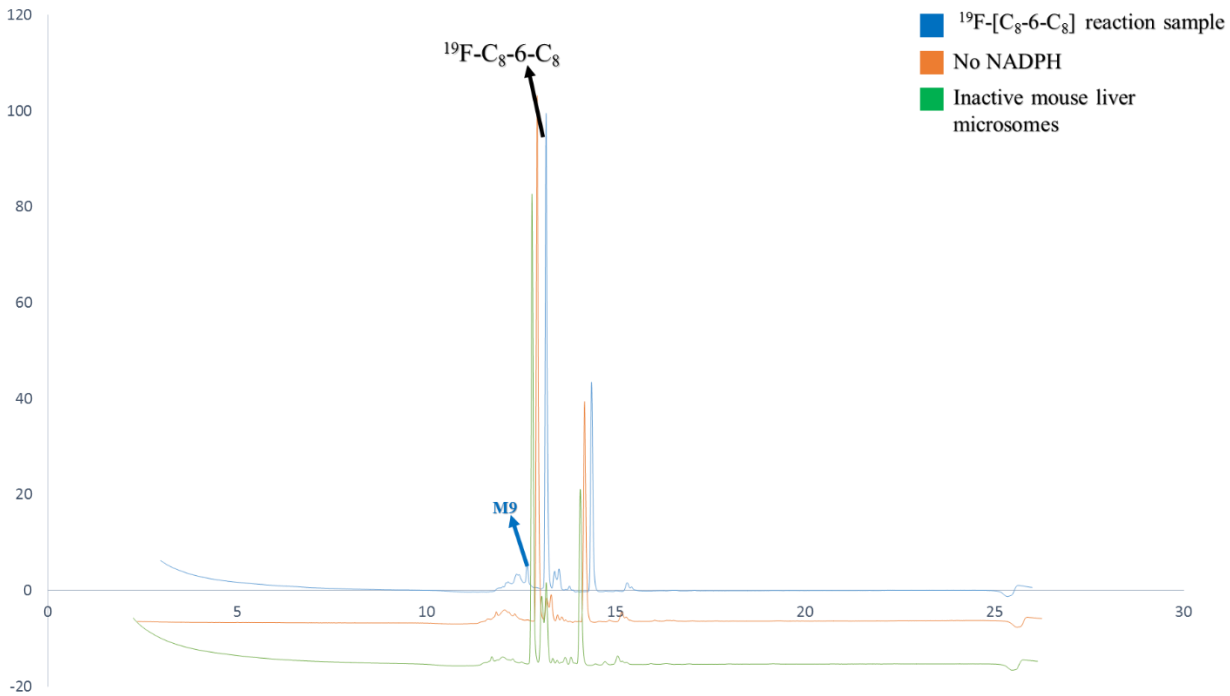
B


120
100
80
60
40
20
0
-20
0
5
10
15
20
25
30
^{19}F-C_8-6-I
M8A, M8B
M7A, M7B
M6
^{19}F-[C_8-6-I] reaction sample
No NADPH
Inactive rat liver microsomes

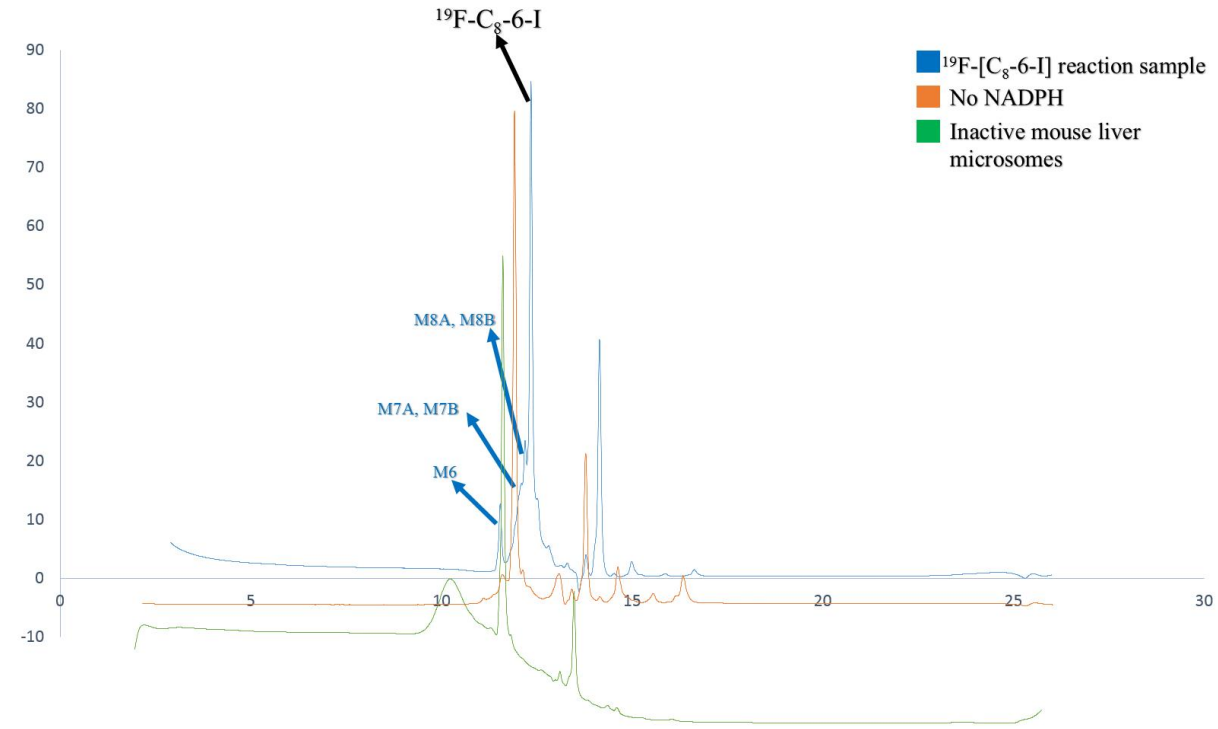
C


^{19}F-C_8-6-N
^{19}F-[C_8-6-N] reaction sample
No NADPH
Inactive rat liver microsomes
M11
M10
35
30
25
20
15
10
5
0
-5
0
5
10
15
20
25
30

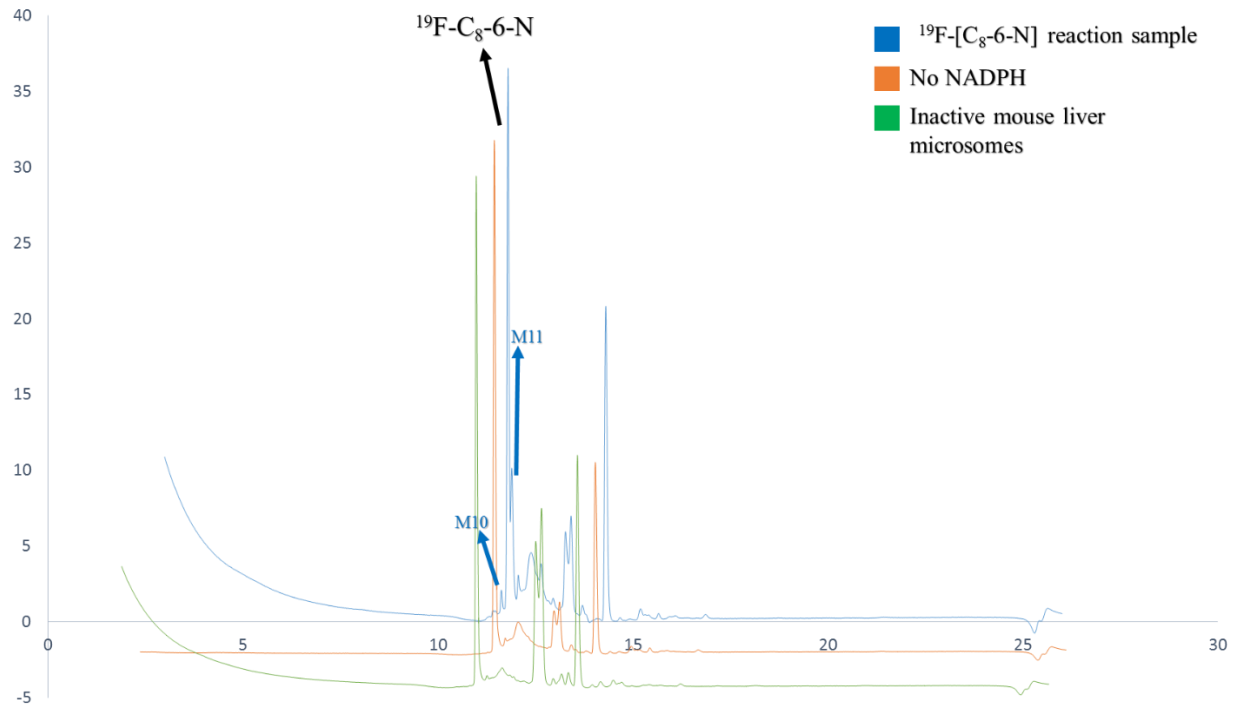
D


120
100
80
60
40
20
0
-20
0
5
10
15
20
25
30
^{19}F-C_8-6-C_8
M9
^{19}F-[C_8-6-C_8] reaction sample
No NADPH
Inactive mouse liver microsomes

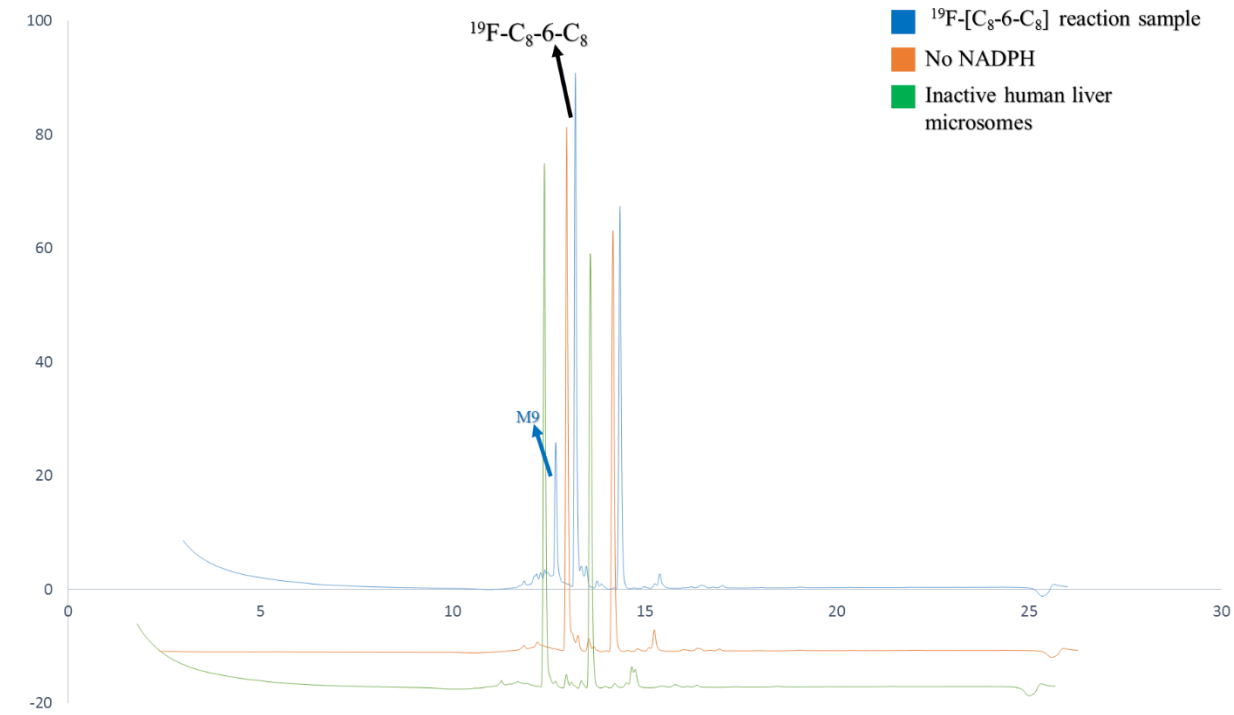
E


^{19}F-C_8-6-I
^{19}F-[C_8-6-I] reaction sample
No NADPH
Inactive mouse liver microsomes
M8A, M8B
M7A, M7B
M6
90
80
70
60
50
40
30
20
10
0
-10
0
5
10
15
20
25
30

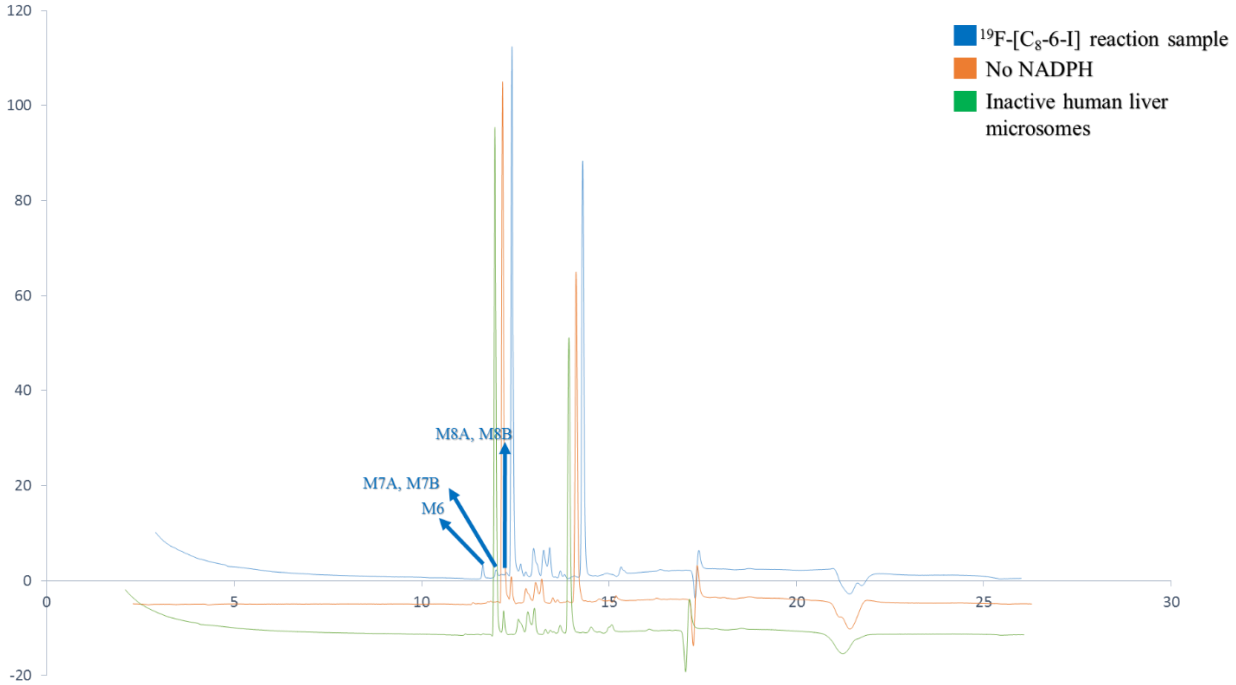
F


^{19}F-C_8-6-N
^{19}F-[C_8-6-N] reaction sample
No NADPH
Inactive mouse liver microsomes
M11
M10
40
35
30
25
20
15
10
5
0
-5
0
5
10
15
20
25
30

G


^{19}F-C_8-6-C_8
M9
^{19}F-[C_8-6-C_8] reaction sample
No NADPH
Inactive human liver microsomes
100
80
60
40
20
0
-20
0
5
10
15
20
25
30

H


120
100
80
60
40
20
0
-20
0
5
10
15
20
25
30
M8A, M8B
M7A, M7B
M6
^{19}F-[C_8-6-I] reaction sample
No NADPH
Inactive human liver microsomes

I

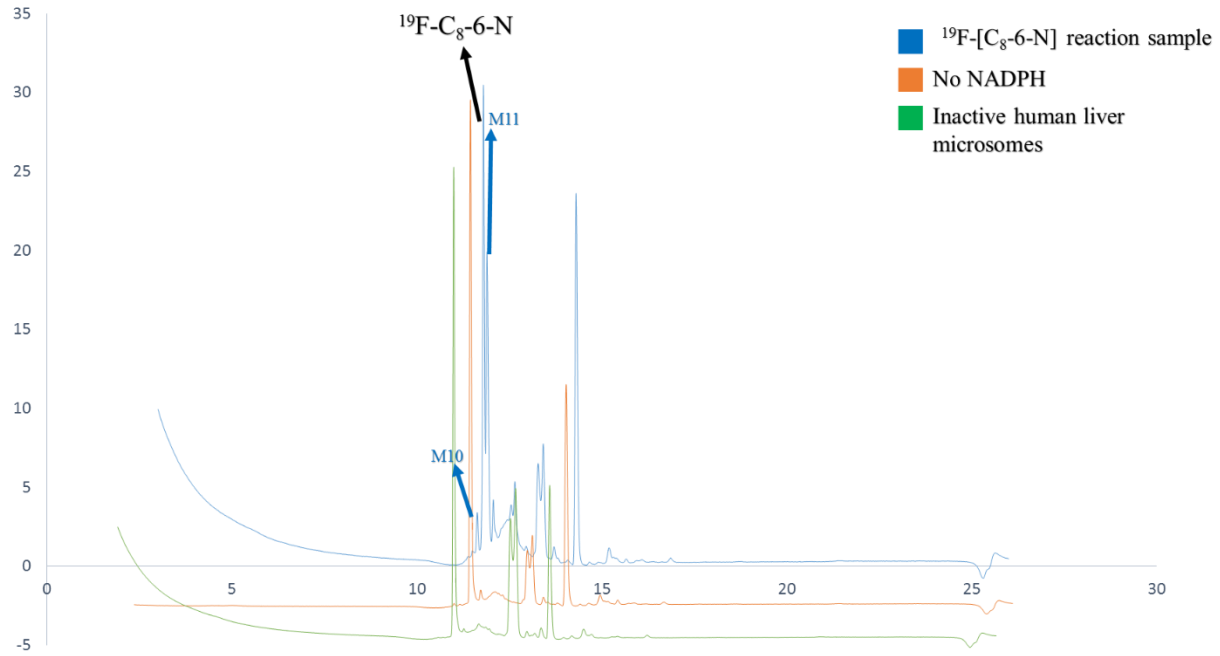
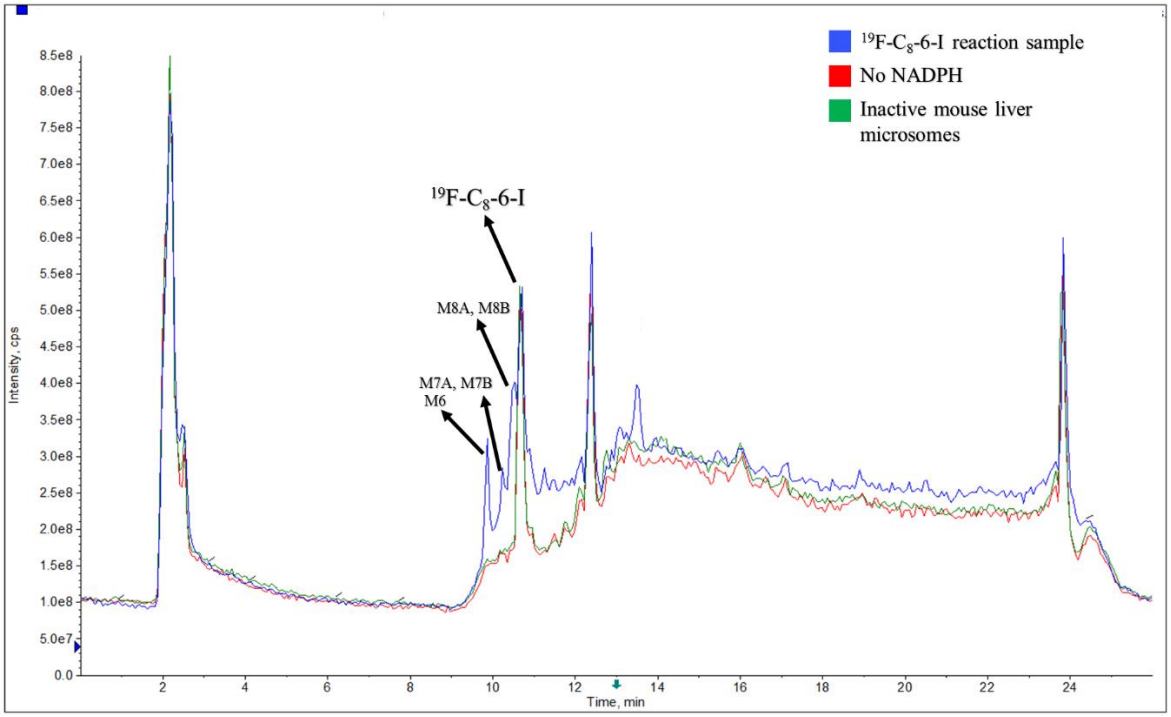
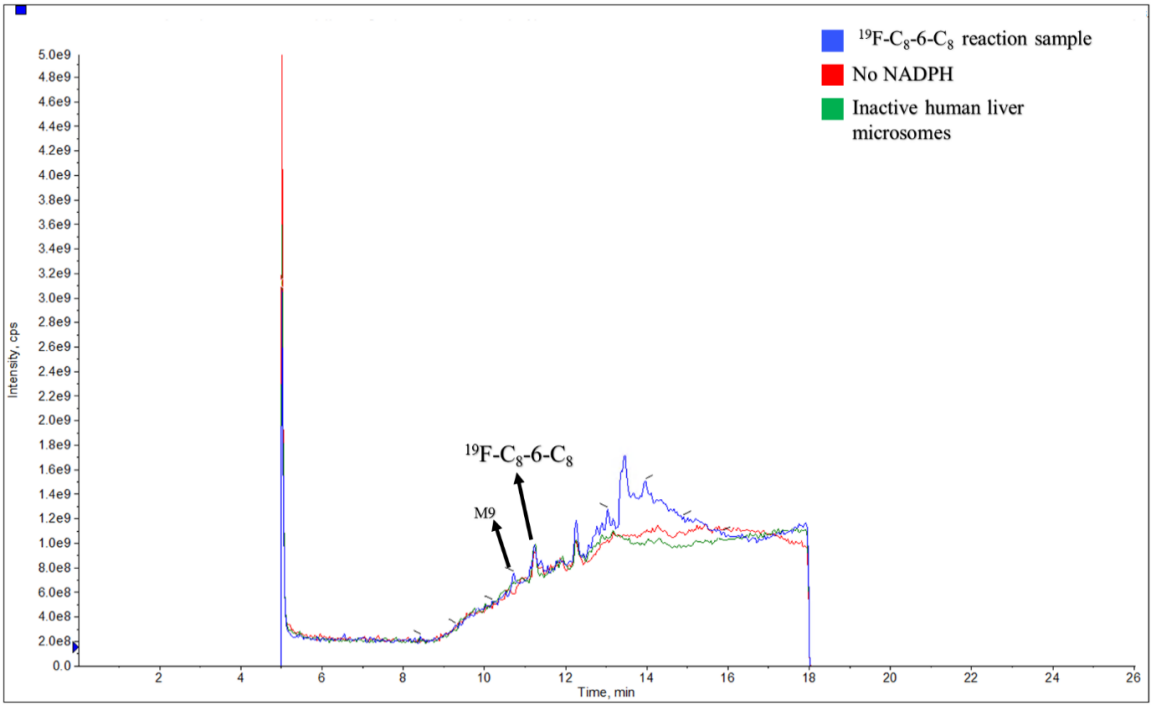


Figure S8. HPLC-UV chromatogram analysis of samples from *in vitro* rat liver microsomal metabolism of ^{19}F-[C$_8$-6-C$_8$] (A) ^{19}F-[C$_8$-6-I] (B) ^{19}F-[C$_8$-6-N] (C), *in vitro* mouse liver microsomal metabolism of ^{19}F-[C$_8$-6-C$_8$] (D) ^{19}F-[C$_8$-6-I] (E) ^{19}F-[C$_8$-6-N] (F), and *in vitro* human liver microsomal metabolism of ^{19}F-[C$_8$-6-C$_8$] (G) ^{19}F-[C$_8$-6-I] (H) and ^{19}F-[C$_8$-6-N] (I).

A


^{19}F-C$_8$-6-I reaction sample
No NADPH
Inactive mouse liver microsomes
^{19}F-C$_8$-6-I
M8A, M8B
M7A, M7B
M6
Intensity, cps
Time, min

B


^{19}F-C$_8$-6-C$_8$ reaction sample
No NADPH
Inactive human liver microsomes
^{19}F-C$_8$-6-C$_8$
M9
Intensity, cps
Time, min

C

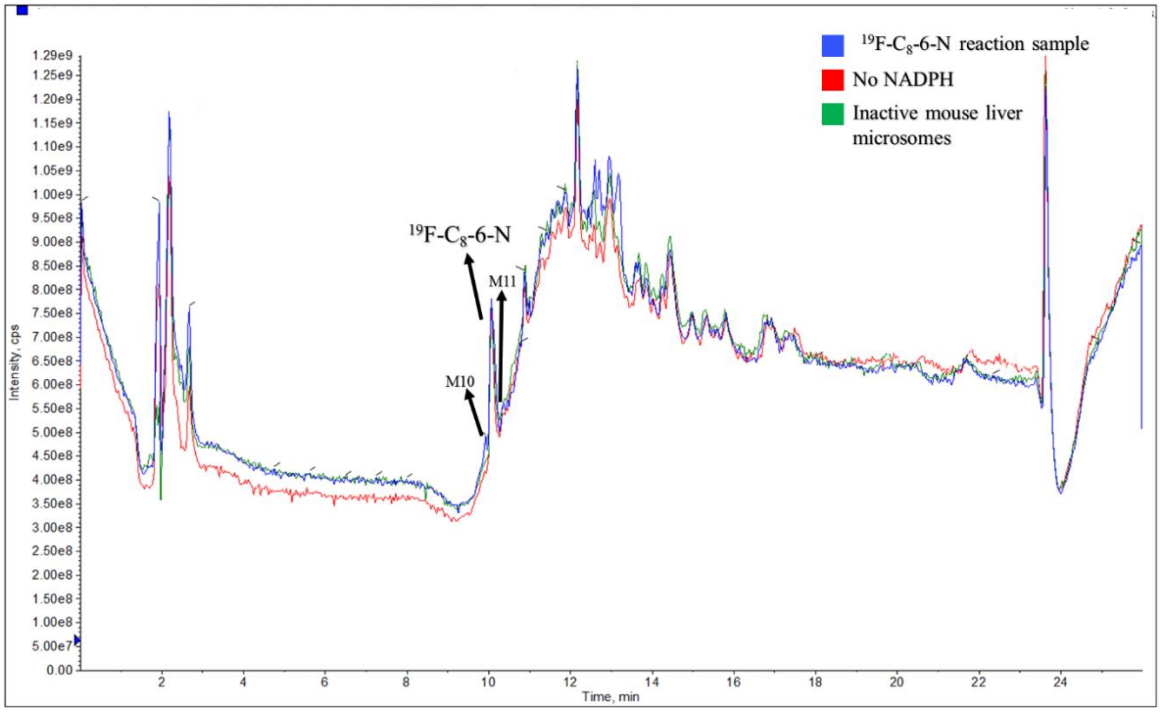
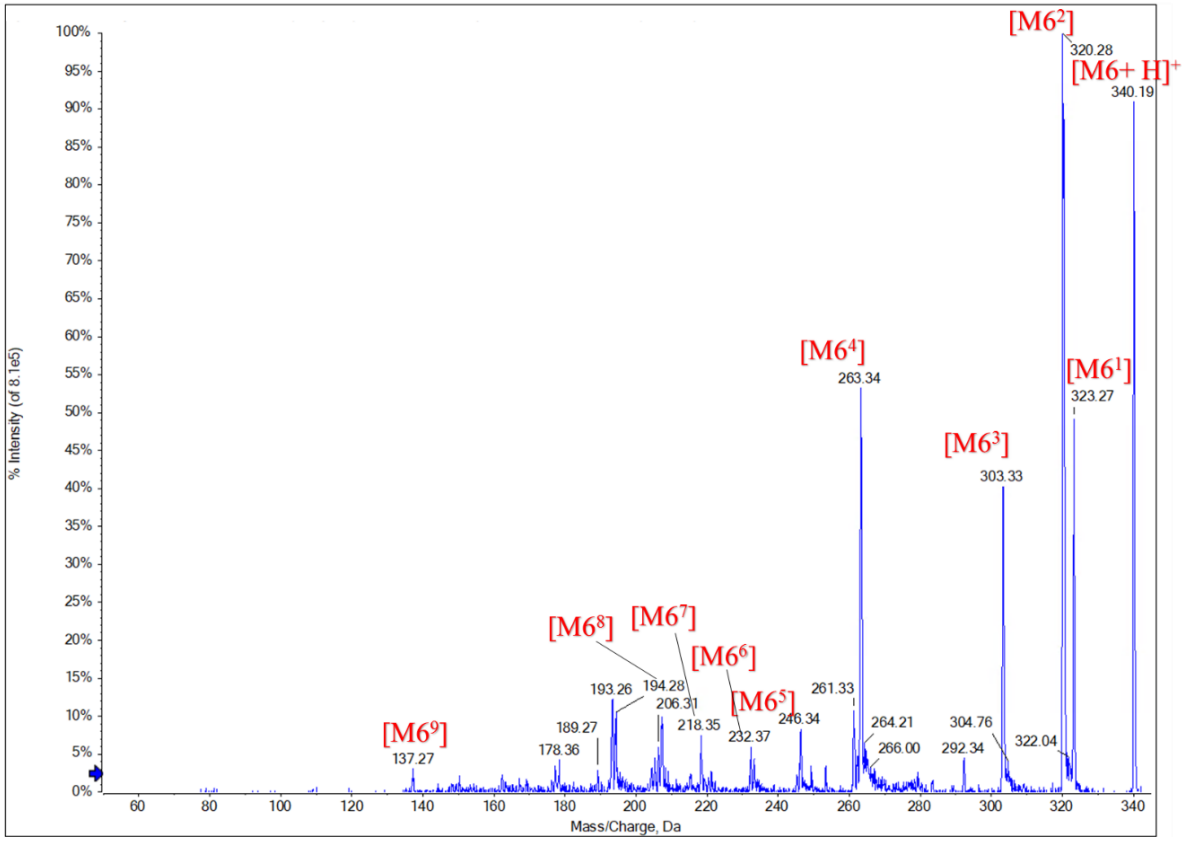
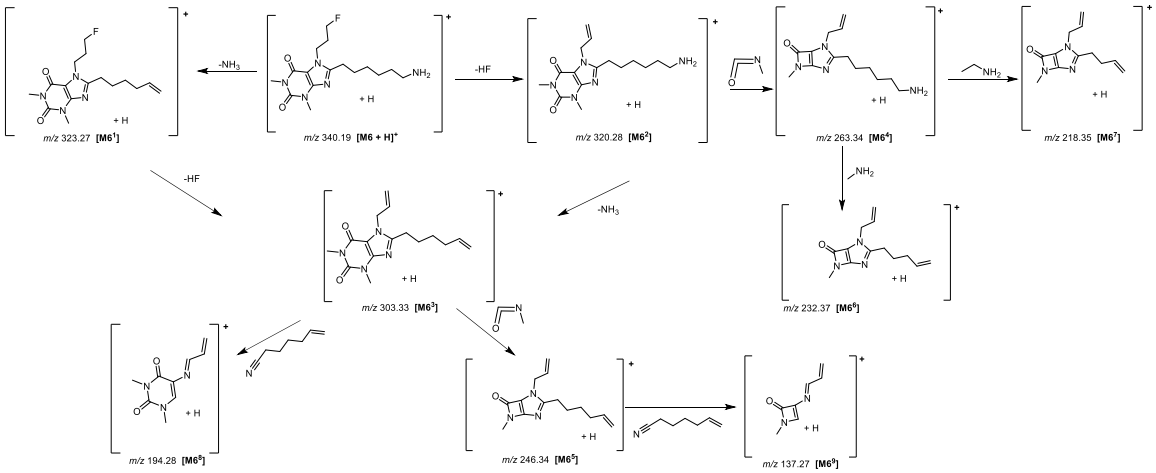


Figure S9. Total ion chromatogram (ESI in positive mode) of LC-QqLIT-MS analysis of a 10 μL injection of 15 μM samples from *in vitro* mouse liver microsomal metabolism of ^{19}F-[C$_8$-6-I] (A), *in vitro* human liver microsomal metabolism of ^{19}F-[C$_8$-6-C$_8$] (B) and *in vitro* mouse liver microsomal metabolism of ^{19}F-[C$_8$-6-N] (C).

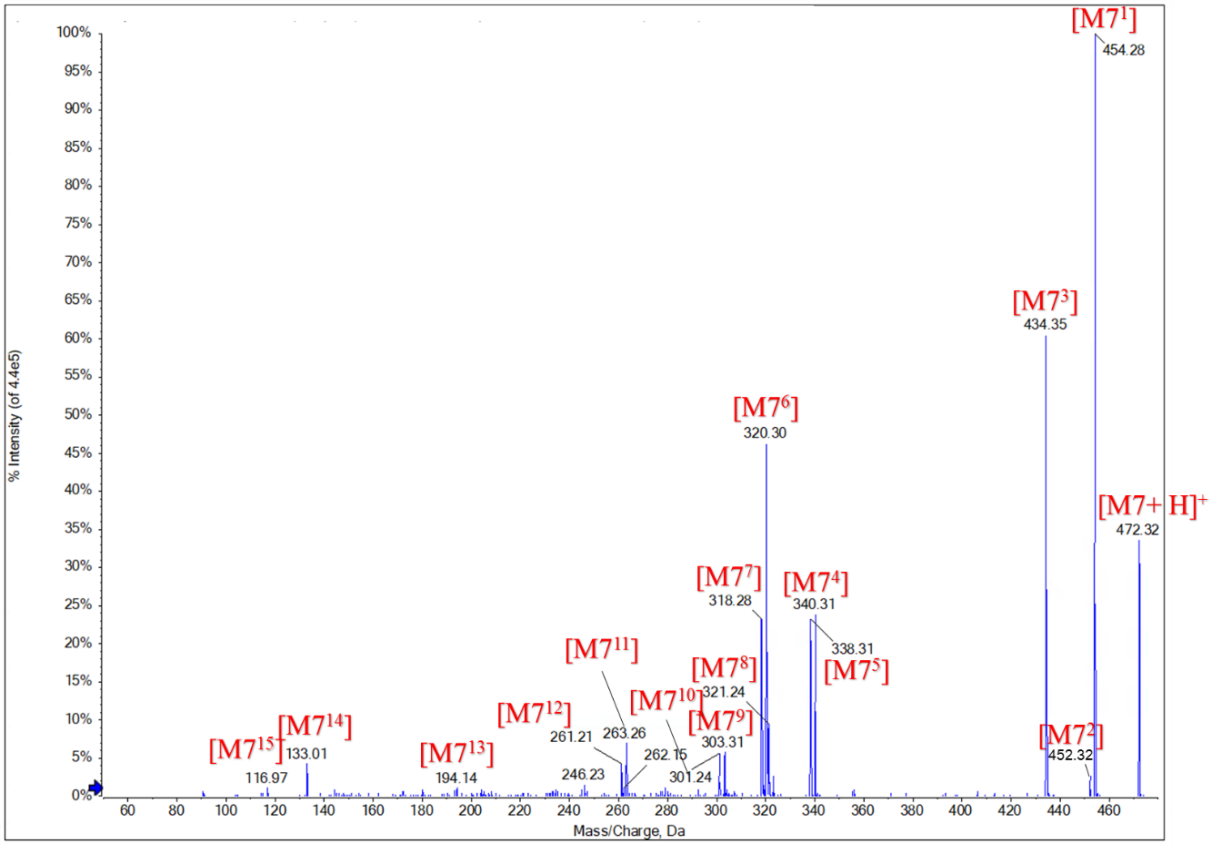
A


[M6²]
320.28
[M6+ H]⁺
340.19
[M6⁴]
263.34
[M6¹]
323.27
[M6³]
303.33
[M6⁸]
[M6⁷]
[M6⁶]
[M6⁵]
[M6⁹]
137.27
178.36
189.27
193.26
194.28
206.31
218.35
232.37
246.34
261.33
264.21
266.00
292.34
304.76
322.04
% Intensity (of 8.1e5)
Mass/Charge, Da

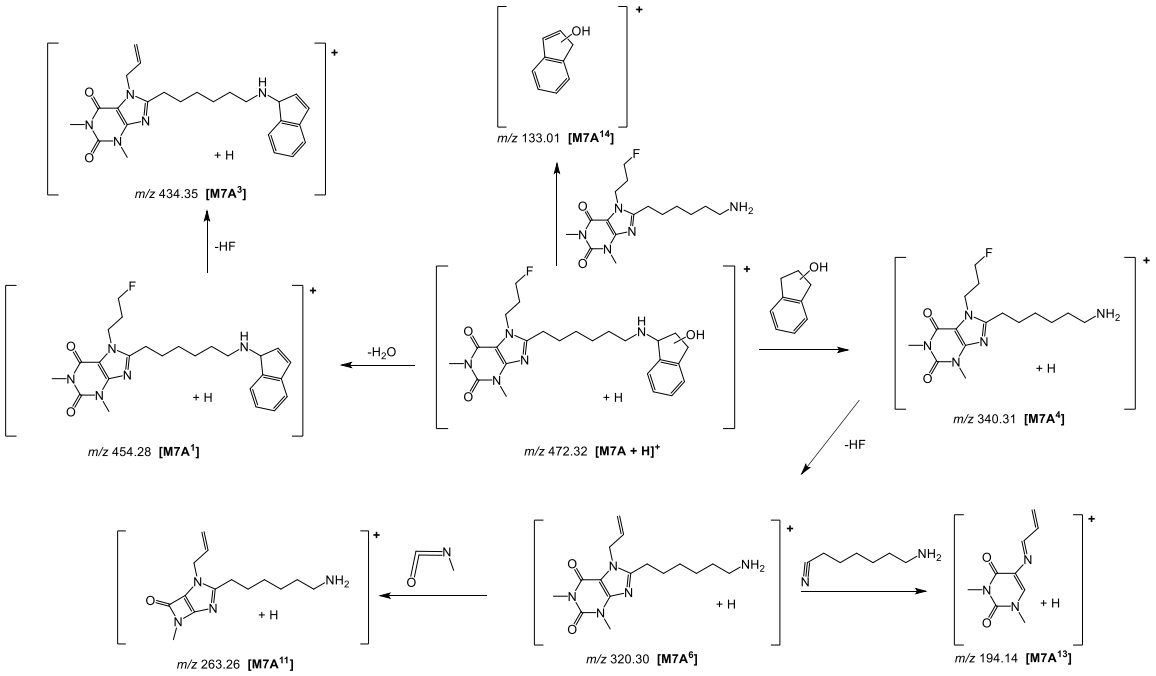
B


-NH3
-HF
m/z 323.27 [M6¹]
m/z 340.19 [M6 + H]⁺
m/z 320.28 [M6²]
m/z 263.34 [M6⁴]
m/z 218.35 [M6⁷]
m/z 303.33 [M6³]
m/z 232.37 [M6⁶]
m/z 194.28 [M6⁸]
m/z 246.34 [M6⁵]
m/z 137.27 [M6⁹]

C


[M7^1]
454.28
[M7^3]
434.35
[M7^6]
320.30
[M7+ H]^+
472.32
[M7^7]
318.28
[M7^4]
340.31
338.31
[M7^11]
[M7^8]
321.24
[M7^5]
[M7^12]
261.21
263.26
[M7^10]
[M7^9]
303.31
262.15
246.23
301.24
[M7^14]
[M7^15]
133.01
116.97
[M7^13]
194.14
[M7^2]
452.32
% Intensity (of 4.4e5)
Mass/Charge, Da

D


m/z 133.01 [M7A14]
m/z 434.35 [M7A3]
-HF
m/z 454.28 [M7A1]
-H2O
m/z 472.32 [M7A + H]+
m/z 340.31 [M7A4]
m/z 263.26 [M7A11]
m/z 320.30 [M7A6]
m/z 194.14 [M7A13]

E

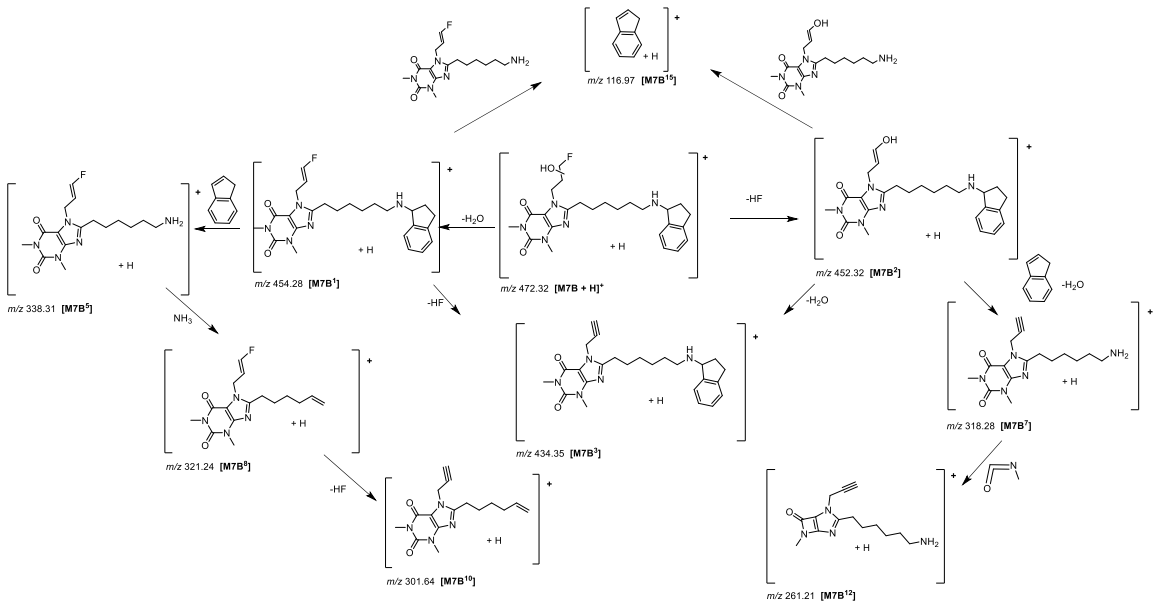
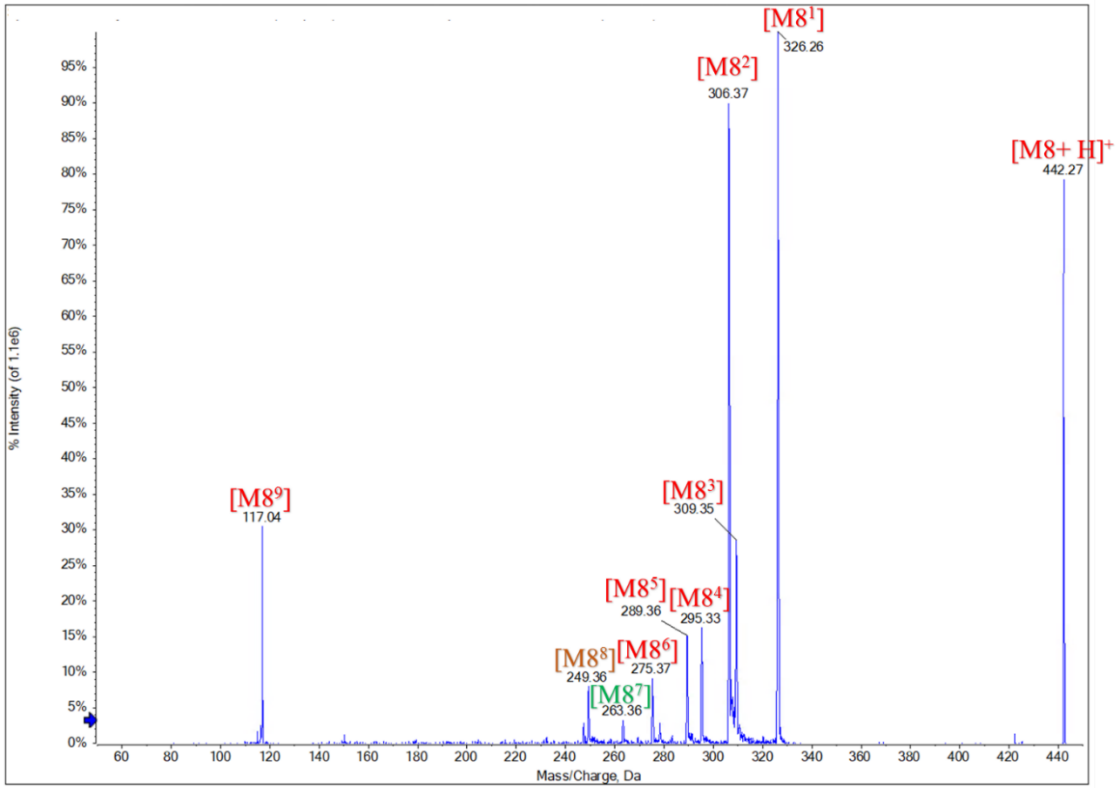
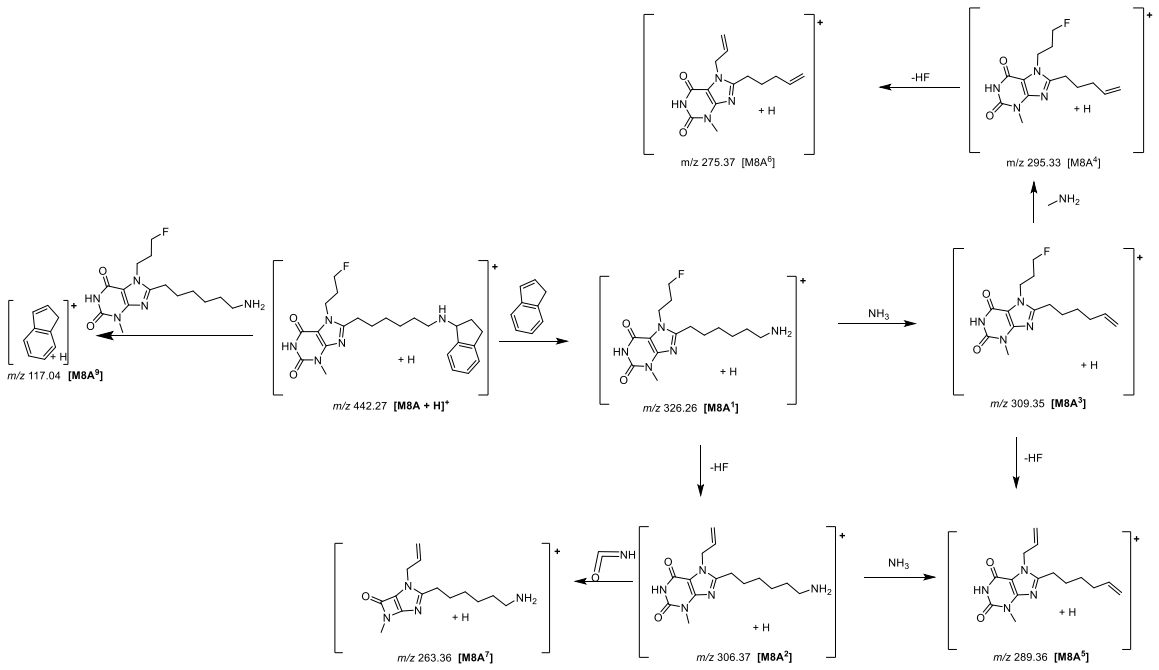

m/z 116.97 [M7B15]
m/z 338.31 [M7B5]
m/z 454.28 [M7B1]
m/z 472.32 [M7B + H]+
m/z 452.32 [M7B2]
m/z 321.24 [M7B8]
m/z 434.35 [M7B3]
m/z 318.28 [M7B7]
m/z 301.64 [M7B10]
m/z 261.21 [M7B12]

Figure S10.The ESI-QqLIT-MS/MS spectrum for ^{19}F-[C_8-6-I] metabolites M6(A), M7A and M7B(C) and the proposed fragmentation pathway for M6(B), M7A(D) and M7B(E). ESI was performed in positive mode.

A



B



C

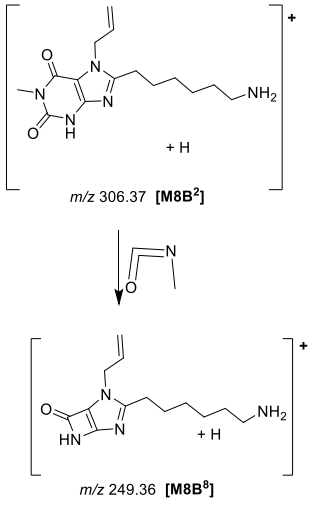
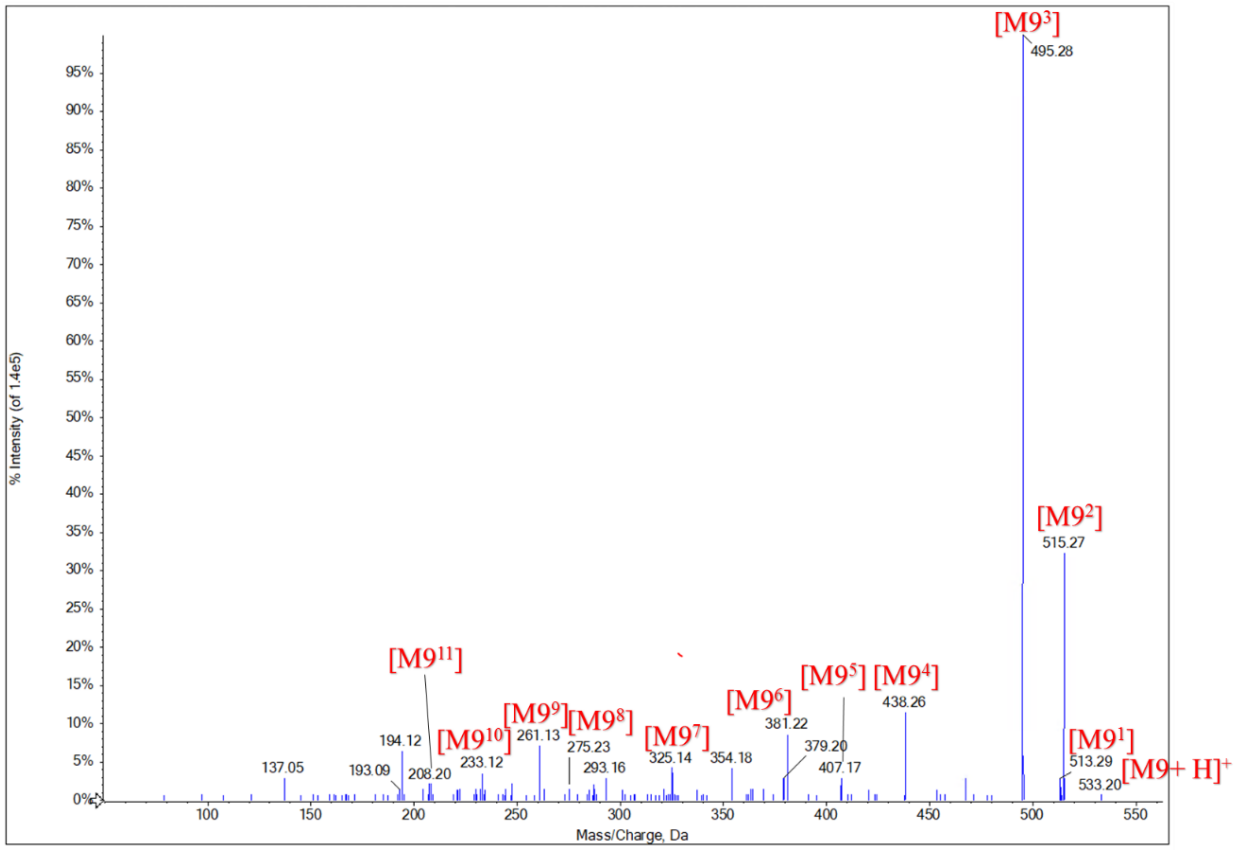


Figure S11.The ESI-QqLIT-MS/MS spectrum for ^{19}F-[C_8-6-I] metabolites M8A and M8B (A) and the proposed fragmentation pathway for M8A(B), and M8B(C). ESI was performed in positive mode.

A


[M9³]
495.28
[M9²]
515.27
[M9¹]
513.29
533.20
[M9+ H]⁺
[M9¹¹]
194.12
208.20
[M9¹⁰]
233.12
[M9⁹]
261.13
[M9⁸]
275.23
293.16
[M9⁷]
325.14
354.18
[M9⁶]
381.22
[M9⁵]
379.20
407.17
[M9⁴]
438.26
137.05
193.09
% Intensity (of 1.4e5)
Mass/Charge, Da

B

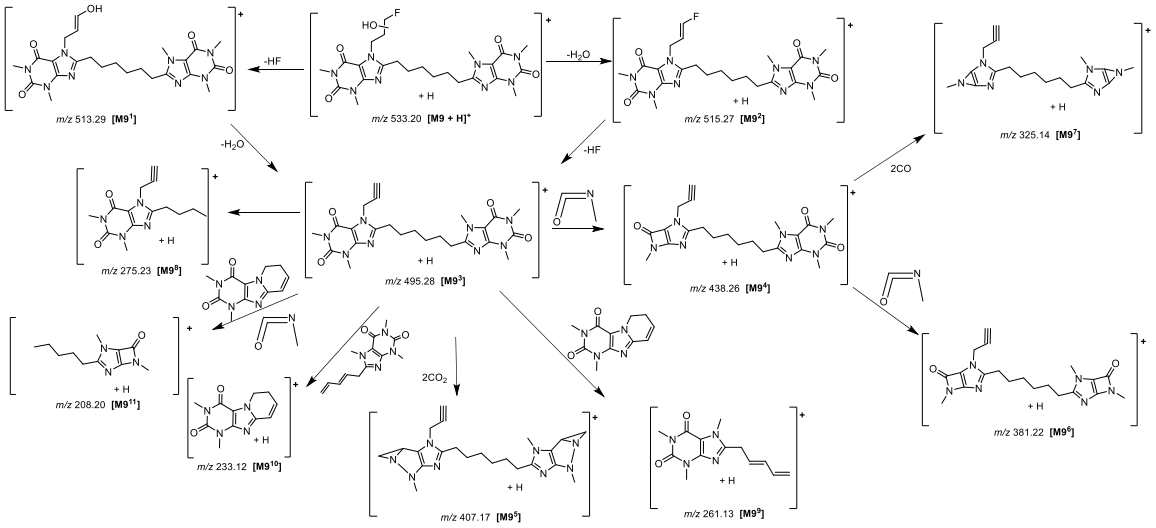
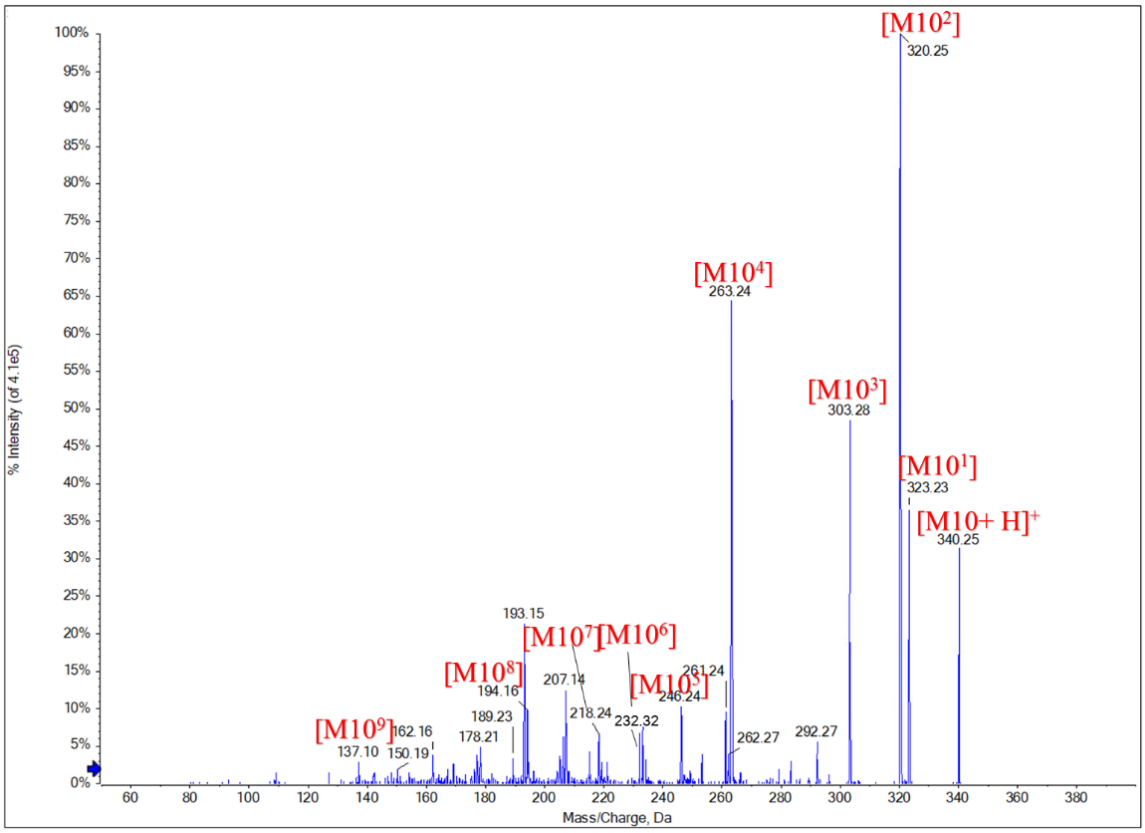
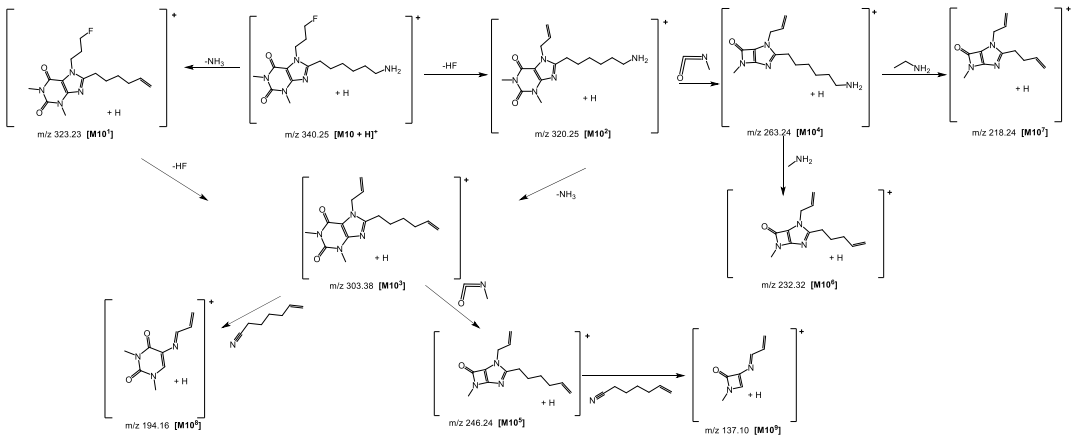


Figure S12.The ESI-QqLIT-MS/MS spectrum for ^{19}F-[C_8-6-C_8] metabolite M9(A) and the proposed fragmentation pathway for M9(B).

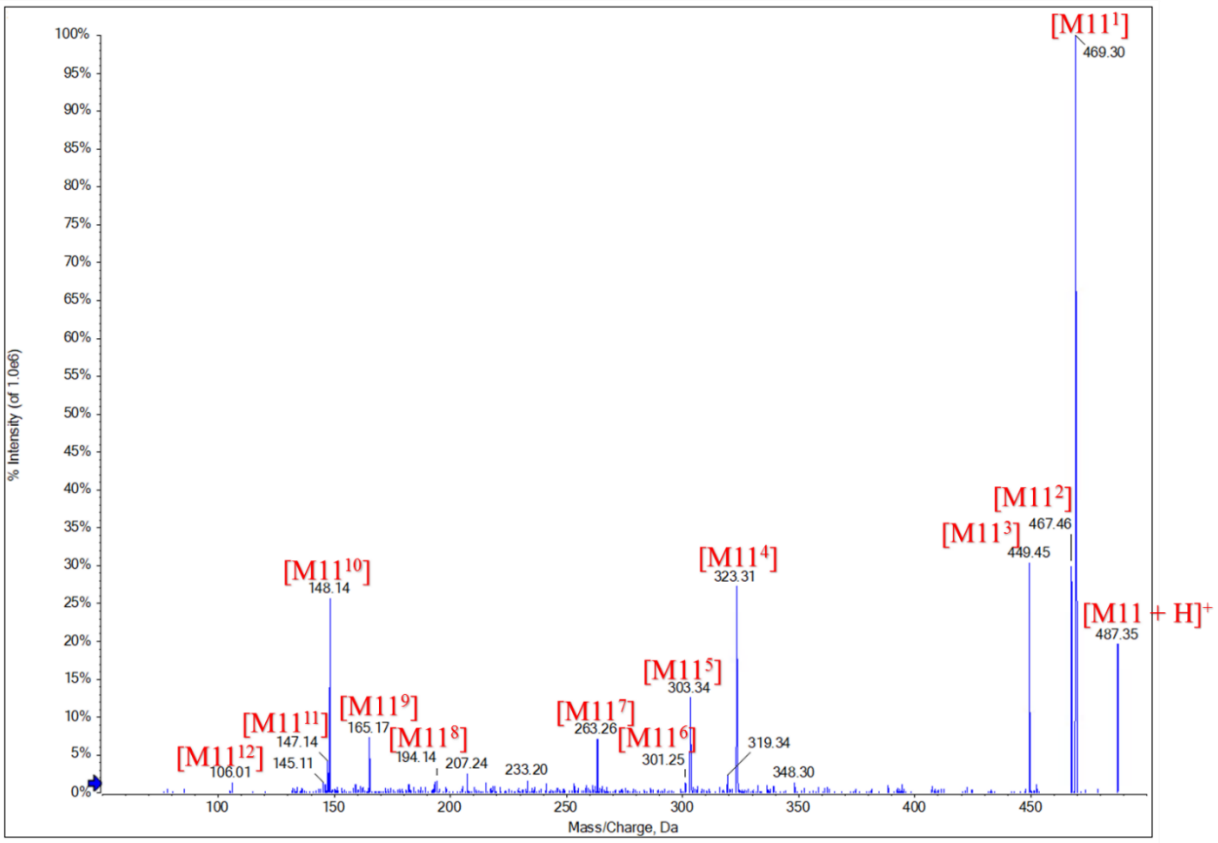
A


[M10²]
320.25
[M10⁴]
263.24
[M10³]
303.28
[M10¹]
323.23
[M10+ H]⁺
340.25
[M10⁹]
[M10⁸]
[M10⁷]
[M10⁶]
[M10⁵]
193.15
194.16
207.14
189.23
178.21
218.24
232.32
246.24
261.24
262.27
292.27
162.16
137.10
150.19
% Intensity (of 4.1e5)
Mass/Charge, Da

B


-NH3
-HF
m/z 323.23 [M10¹]
m/z 340.25 [M10 + H]⁺
m/z 320.25 [M10²]
m/z 263.24 [M10⁴]
m/z 218.24 [M10⁷]
m/z 303.38 [M10³]
m/z 232.32 [M10⁰]
m/z 194.16 [M10⁸]
m/z 246.24 [M10⁵]
m/z 137.10 [M10⁹]

C


[M11[1]]
469.30
[M11[2]]
467.46
[M11[3]]
449.45
[M11[4]]
323.31
[M11[5]]
303.34
[M11[6]]
301.25
[M11[7]]
263.26
[M11[8]]
194.14
[M11[9]]
165.17
[M11[10]]
148.14
[M11[11]]
147.14
[M11[12]]
106.01
145.11
207.24
233.20
319.34
348.30
[M11 + H]+
487.35
% Intensity (of 1.0e6)
Mass/Charge, Da

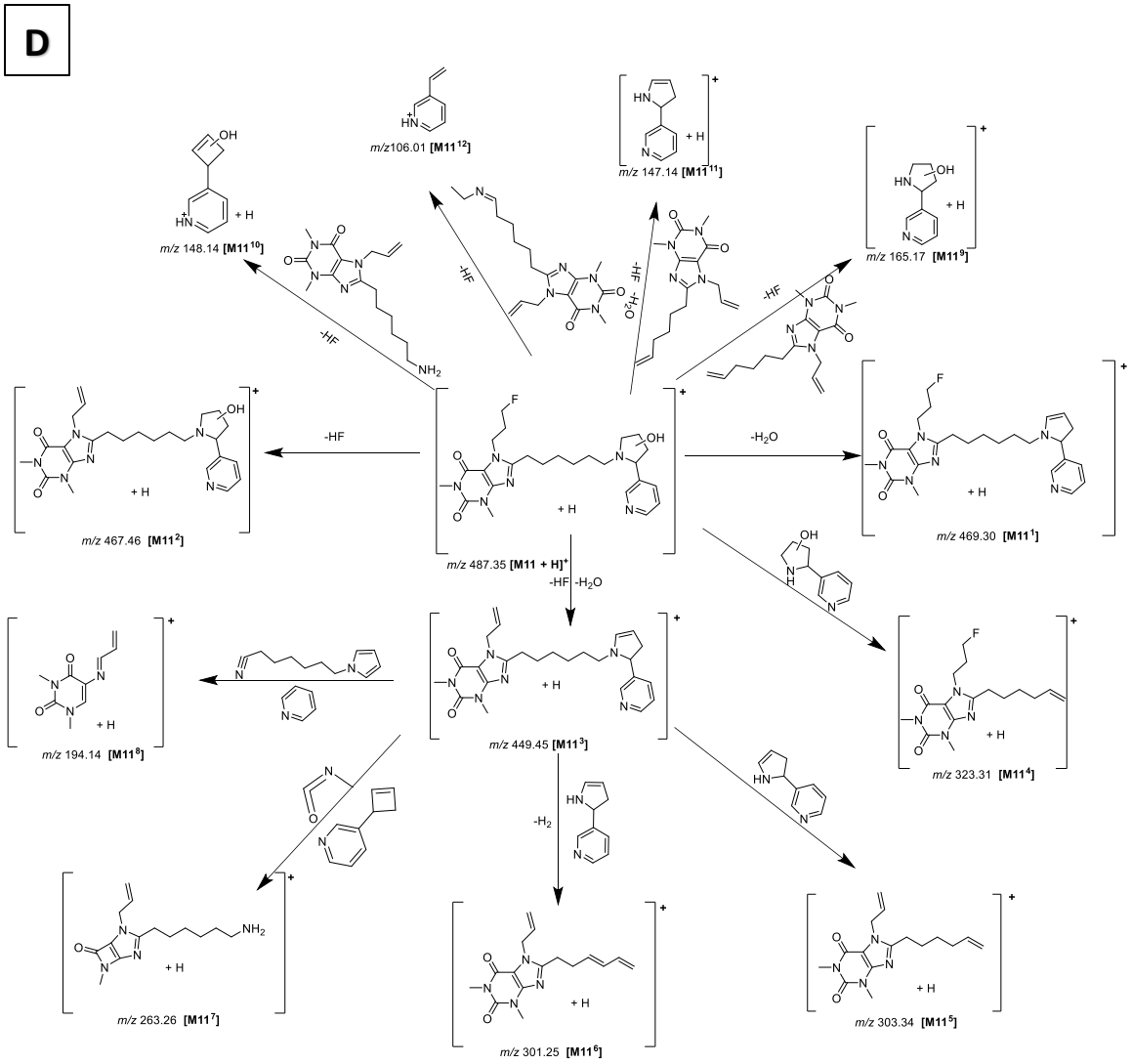


Figure S13. The ESI-QqLIT-MS/MS spectrum for ^{19}F-[C_8-6-N] metabolite M10 (A), M11 (C) and the proposed fragmentation pathway for M10 (B) and M11 (D).

Synthesis of Fluorinated Analogues

Chemistry. All chemicals were purchased from Sigma-Aldrich, Alfa-aesar, Chem-impex or Toronto research chemicals and used without further purification. Deionized water was obtained using a Millipore Q-POD Milli-Q 0.22 μm filter, 18 mΩ. **1b** and **3b** were synthesized using our previous method for making the non-fluorinated bifunctional compounds[1]. **5** and **6** were prepared from propan-1,3-diol using an established literature method employed for ethylene glycol[2] and **7** was prepared from **6** based on an established literature procedure[3].

Anhydrous reactions were run under (a positive pressure) dry N_2 atmosphere in flame-dried glasswares. Solvents were removed on a Büchi Rotary evaporator R-200, and Büchi v700 vacuum pump with attached v850 vacuum controller. Trace solvents were removed with an Edwards high vacuum pump. Reactions were monitored by aluminium TLC sheets coated with silica gel 60 $F_{254.}$ Visualization of compounds was observed under an ultraviolet lamp (254 nm) and treating the TLC plate with $KMnO_4$ and vanillin stains. TLC-ESI was carried out using a Plate Express Advion and ESI expression (ESI = 2 LPM, ASAP = 0 LPM, APCI = 4 LPM) reader to identify products on TLC. Flash Column Chromatography was performed with MERCK Silica gel 60 (0.040 – 0.063 mm; 230-400 mesh).

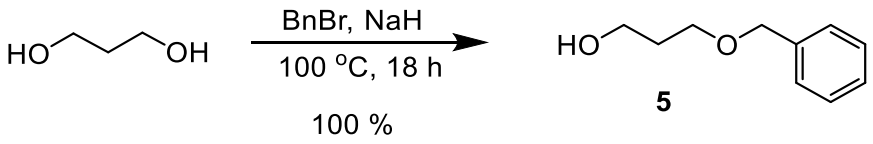
The NMR spectra data were recorded on a 500 MHz Bruker Avance NMR spectrometer in $CDCl_{3,}$ and CD_3OD, and data was processed using Topspin 3.5. All chemical shifts were reported as part per million (ppm) downfield from tetramethylsilane (δ = 0 ppm). The following calibrations were used: $CDCl_3$ δ = 7.26 and 77.16 ppm; and CD_3OD δ = 3.31 and 49.00 ppm. * denotes peak are split due to intermediate exchange (on the NMR time scale) between rotamers causing peak splitting and peak broadening. All coupling constants (*J*) are given in hertz (Hz). Multiplicity was

indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets; and br, broad signal. ^{1}H NMR spectra data are presented as follows: chemical shifts (multiplicity, coupling constants, and integration) and ^{13}C NMR presented as chemical shifts.

ESI-MS of final products were analyzed using an AB SCIEX 4000 Q TRAP hybrid triple quadrupole-linear ion trap mass spectrometer (QqLIT-MS) equipped with a turbo spray ESI source (AB SCIEX, Redwood City, CA, USA.).

Purity was determined on an Agilent 1200 HPLC using a Poroshell 120, EC-C18 2.7 µm (4.6 × 150 mm) column at 0.3 mL/min running a gradient from 90:10 H_2O/ACN (v/v) to 90:10 H_2O/ACN (v/v) (t = 5 minutes) to 10:90 H_2O/ACN (v/v) (t = 10 minutes) to 10:90 H_2O/ACN (t = 20 minutes) to 90:10 H_2O/ACN (t = 21 minutes) with mobile phase A (0.1 % formic acid + H_2O) and mobile phase B (0.1 % formic acid + ACN). The Agilent 1200 HPLC was equipped with a quaternary pump (G1311A), auto sampler (G1329A), column compartment (G1316A), diode array detector (G1315D), and fraction collector (G1364C).

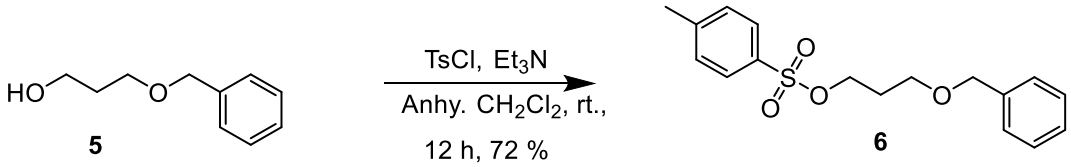
3-(benzyloxy)propan-1-ol, 5



Propane-1, 3-diol (6.47 g, 85.03 mmol) was placed in a round bottom flask and cooled to 0 °C. Sodium hydride (0.49 g, 20.42 mmol) was added in small batches to propane-1, 3-diol at 0 °C followed by dropwise addition of benzyl bromide (3.0 g, 17.54 mmol). The reaction mixture was then heated to 100 °C for the next 18 h. The mixture was allowed to cool to room temperature and

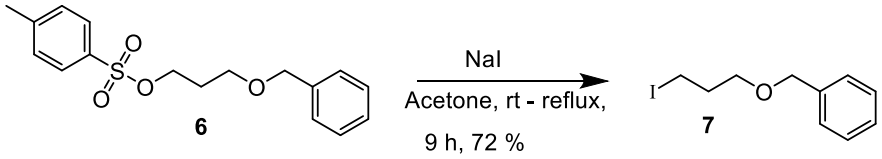
water (15 ml) was added to quench the reaction. This was stirred for the next 10 mins followed by extraction with dichloromethane (3 × 40 mL). The organic phases were combined together and washed with water (3 × 40 mL), dried over Mg_2SO_4, filtered and concentrated to giving **5** as a transparent oil. Yield 2.83 g (100 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37 – 7.37 (m, 5H), 4.53 (s, 2H), 3.80-3.78 (m, 2H), 3.67 (t, $^3J_{HH}$ = 5.74 Hz, 2H), 2.3 – 2.29 (br, 1H), 1.87 (quint, $^3J_{HH}$ = 5.72 Hz, 2H).

3- (benzyloxy)propyl 4-methylbenzene-1-sulfonate, 6



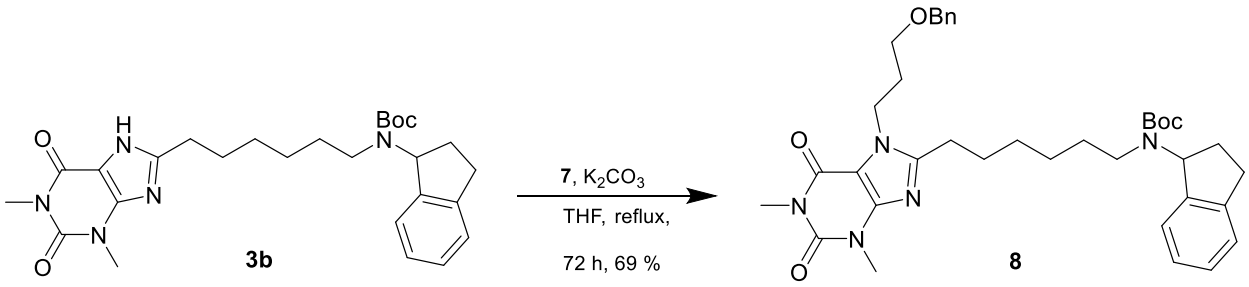
5 (2.80 g, 16.85 mmol) was dissolved in anhydrous dichloromethane (25 ml) under nitrogen and cooled to 0 ˚C. Triethylamine (4.70 ml, 33.70 mmol) was added dropwise to the **5** with stirring at 0 ˚C. Tosyl chloride (3.53 g, 18.53 mmol) was dissolved in anhydrous dichloromethane (35 mL) and added dropwise to the mixture at 0 ˚C. The reaction was allowed to rise to room temperature and stirred at room temperature for 12 h. The reaction was quenched with water (15 mL) and sodium bicarbonate (15 mL). The organic phase was collected and the aqueous phase was extracted with dichloromethane (3 × 40 mL). The organic extracts were combined together, dried over Mg_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by flash column chromatography using hexanes: ethyl acetate (8:2) giving **6** as a colorless oil which solidifies at -78ºC. Yield 3.9 g (72 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.80 – 7.78 (d, $^3J_{HH}$ = 8.30 Hz, 2H), 7.37 – 7.23 (m, 7H), 4.40 (s, 2H), 4.16 (t, $^3J_{HH}$ = 6.15 Hz, 2H), 3.50 (t, $^3J_{HH}$ = 5.95 Hz, 2H), 2.42 (s, 3H), 1.94 (quint, $^3J_{HH}$ = 6.11 Hz, 2H).

((3-iodopropoxy)methyl)benzene, 7



6 (1.89 g, 5.92 mmol) was placed in dry acetone (30 mL) under nitrogen. Sodium iodide (4.43 g, 29.8 mmol) was dissolved in dry acetone (25 mL) and added dropwise to **6**. The reaction was stirred at room temperature for 4 h and then at reflux at 70 ˚C for 5 h. The reaction mixture was allowed to cool to room temperature and quenched with water (20 mL). The mixture was then extracted with ethyl acetate (3 × 50 mL). The organic layers were combined and washed with 10 % sodium thiosulfate (20 mL), brine (20 mL), water (20 mL), then dried over Mg_2SO_4, filtered and concentrated under reduced pressure to give a crude yellow oil. The crude was purified by flash column chromatography using hexanes: ethyl acetate (9.8:0.2) giving **7**[3] as a lemon yellow oil. Yield 1.17 g (72 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37 – 7.27 (m, 5H), 4.52 (s, 2H), 3.54 (t, $^3J_{HH}$ = 5.82 Hz, 2H), 3.31 (t, $^3J_{HH}$ = 6.76 Hz, 2H), 2.42 (s, 3H), 2.09 (quint, $^3J_{HH}$ = 6.10 Hz, 2H).

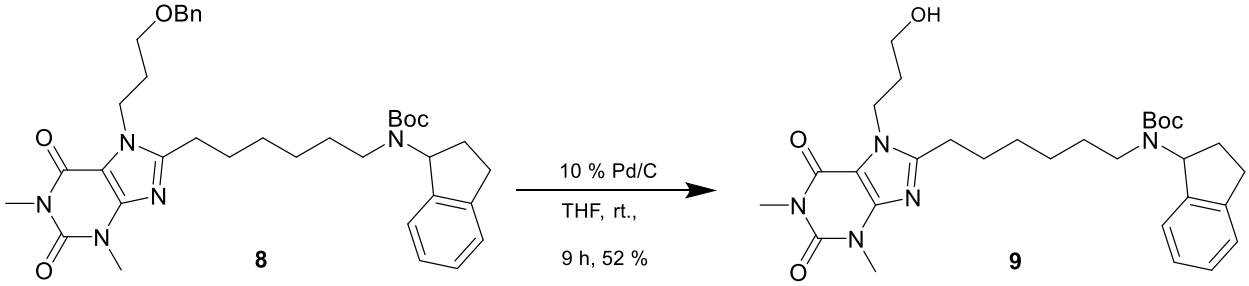
7-(3-(benzyloxy)propyl)-1,3-dimethyl-8-(N-Boc-N-ethylheptyl-2,3-dihydro-1*H*-indan-1-amine)-xanthine, 8



3b[1] (0.15 g, 0.30 mmol) and potassium carbonate (0.44 g, 3.18 mmol) were dissolved in anhydrous tetrahydrofuran (15 ml) under nitrogen. Compound **7** (0.17 g, 0.61 mmol) was dissolved in anhydrous tetrahydrofuran (6 ml) and added dropwise to **3b**. The reaction mixture was refluxed

under nitrogen at 75 °C for 72 h. The reaction was allowed to cool to room temperature and quenched with water (10 mL). The mixture was then extracted with dichloromethane (4 × 50 ml), the organic layers were combined, dried over Mg_2SO_4, filtered and concentrated under reduced pressure to give a crude oil. The crude was purified by flash column chromatography using ethyl acetate: hexanes (7: 3) giving **8** as a lemon yellow oil. Yield 0.135 g (69 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34 – 7.27 (m, 5H), 7.20 – 7.12 (m, 4H), 5.76* (br, 0.5H), 5.26* (br, 0.5H), 4.46 (s, 2H), 4.32 (t, $^3J_{HH}$ = 6.91 Hz, 2H), 3.55 (s, 3H), 3.45 (t, $^3J_{HH}$ = 5.54 Hz, 2H), 3.38 (s, 3H), 3.17* (br, 0.5H), 2.99 – 2.93 (m, 2H), 2.85 – 2.79 (m, 1H), 2.74* (br, 0.5H), 2.68 (t, $^3J_{HH}$ = 7.58 Hz, 2H), 2.37 (br, 1H), 2.13 – 2.08 (quint, $^3J_{HH}$ = 6.22 Hz, 2H), 2.04* (br, 0.5H), 1.91* (br, 0.5H), 1.66 – 1..32 (m, 17H).

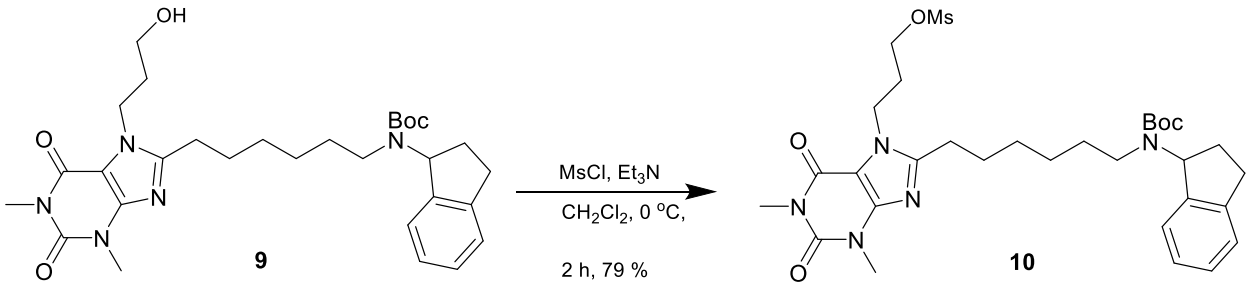
7-(3-hydroxypropyl)-1,3-dimethyl-8-(N-Boc-N-ethylheptyl-2,3-dihydro-1*H*-indan-1-amine)-xanthine, 9



10 % Pd/C (0.040 g) was added to **8** (0.131 g, 0.21 mmol) dissolved in tetrahydrofuran. The reaction mixture was stirred under hydrogen atmosphere (hydrogen balloon) at room temperature for 9 h. The mixture was filtered through a celite pad and concentrated under reduced pressure to give a crude oil. The crude was purified by flash column chromatography using ethyl acetate: hexanes (7: 3) giving **9** as a lemon yellow oil. Yield 0.058 g (52 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.21 – 7.11 (m, 4H), 5.75* (br, 0.5H), 5.27* (br, 0.5H), 4.38 (t, $^3J_{HH}$ = 6.11 Hz, 2H), 3.57 (br,

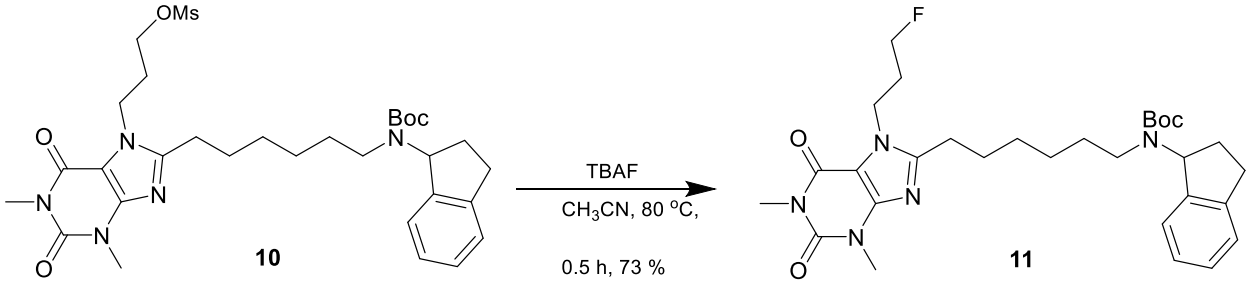
2H), 3.56 (s, 3H), 3.40 (s, 3H), 3.19* (br, 0.5H), 2.98 – 2.94 (m, 2H), 2.85 – 2.81 (m, 1H), 2.70 (br, 2H), 2.69* (br, 0.5H), 2.37 (br, 1H), 2.05* (br, 0.5H), 1.98 (br, 2H), 1.92* (br, 0.5H), 1.74 (br, 2H), 1.62 – 1.18 (m, 17H).

7-(3-((methylsulfonyl)oxy)propyl)-1,3-dimethyl-8-(N-Boc-N-ethylheptyl-2,3-dihydro-1*H*-indan-1-amine)-xanthine, 10



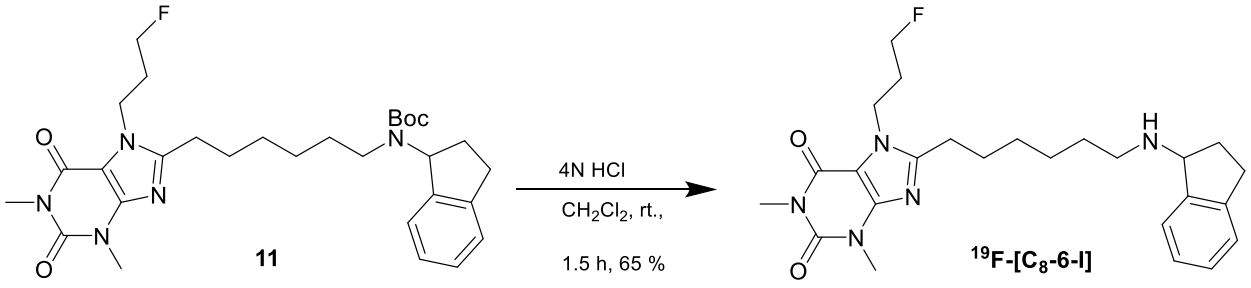
9 (0.058 g, 0.11 mmol) was dissolved in anhydrous dichloromethane (9 mL) under nitrogen. Triethylamine (0.029 mL, 0.21 mmol) was added and the mixture was cooled to 0 °C. Methanesulfonyl chloride (0.016 mL, 0.21 mmol) was added dropwise to the reaction mixture and stirred under nitrogen at 0 °C for 2 h. The reaction was quenched with water (4 mL) and transferred to a separatory funnel. The organic layer was collected and the aqueous layer extracted with dichloromethane (3 × 10 mL). The organic layers were combined, dried over Mg_2SO_4, filtered and concentrated under reduced pressure to give a crude oil. The crude was purified by flash column chromatography using ethyl acetate: hexanes (9: 1) giving **10** as a lemon yellow oil. Yield 0.052 g (79 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.20– 7.12 (m, 4H), 5.75* (br, 0.5H), 5.27* (br, 0.5H), 4.35 (t, $^3J_{HH}$ = 7.12 Hz, 2H), 4.27 (t, $^3J_{HH}$ = 5.68 Hz, 2H), 3.55 (s, 3H), 3.38 (s, 3H), 3.17* (br, 0.5H), 3.02 (s, 3H), 2.99 – 2.94 (m, 2H), 2.88 – 2.79 (m, 1H), 2.72* (br, 0.5H), 2.69 (t, $^3J_{HH}$ = 7.17 Hz, 2H), 2.38 (br, 1H), 2.28 (quint, $^3J_{HH}$ = 6.04 Hz, 2H), 2.06* (br, 0.5H), 1.92* (br, 0.5H), 1.71(br, 2H), 1.50 – 1.20 (m, 15H).

7-(3-fluoropropyl)-1,3-dimethyl-8-(N-Boc-N-ethylheptyl-2,3-dihydro-1*H*-indan-1-amine)-xanthine, 11



10 (0.052 g, 0.082 mmol), was dissolved in anhydrous acetonitrile (8 mL) under nitrogen. Tetra-n-butylammonium fluoride (1 M in THF, 0.25 mL, 0.25 mmol) was added dropwise to **10** and the mixture heated to 80 °C. The reaction mixture was stirred under nitrogen at 80 °C for 0.5 h. The reaction was allowed to cool to room temperature and quenched with water (5 mL). The mixture was then extracted with dichloromethane (3 × 25 mL). The organic layers were combined, dried over Mg_2SO_4, filtered and concentrated under reduced pressure to give a crude oil. The crude was purified by flash column chromatography using ethyl acetate: hexanes (7: 3) giving **11** as a lemon yellow oil. Yield 0.033 g (73 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.19 – 7.11 (m, 4H), 5.74* (br, 0.5H), 5.28* (br, 0.5H), 4.47 – 4.38 (dt, J = 5.32, 47.13 Hz, 2H), 4.33 (t, $^3J_{HH}$ = 6.97 Hz, 2H), 3.54 (s, 3H), 3.37 (s, 3H), 3.19* (br, 0.5H), 2.98 – 2.93 (m, 2H), 2.84 – 2.78 (m, 1H), 2.70* (br, 0.5H), 2.68 (t, $^3J_{HH}$ = 7.02 Hz, 2H), 2.37 (br, 1H), 2.25 – 2.15 (dquint, J = 6.65, 28.63 Hz, 2H), 2..05* (br, 0.5H), 1.90* (br, 0.5H), 1.69 (br, 2H), 1.59 – 1.17 (m, 15H).

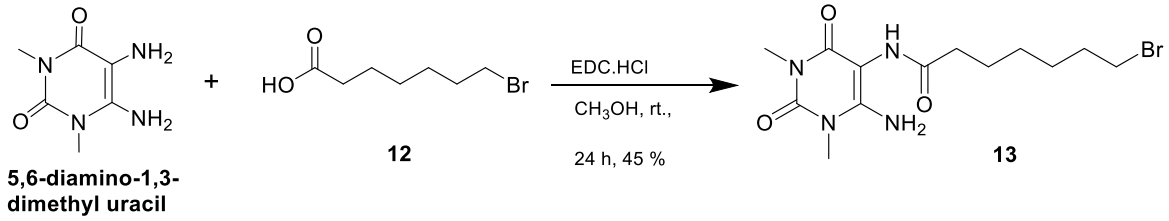
^{19}F-[C_8-6-I]



11 (0.032 g, 0.058 mmol) was dissolved in anhydrous acetonitrile (3 mL) under nitrogen. Hydrogen chloride (4 N in dioxane, 1 mL) was added dropwise to **11**. The reaction mixture was stirred at room temperature for 1.5 h. The reaction was quenched with saturated sodium bicarbonate (4 mL) and transferred to a separatory funnel. The organic layer was collected and the aqueous layer extracted with dichloromethane (3 × 20 mL). The organic layers were combined, dried over Mg_2SO_4, filtered and concentrated under reduced pressure to give a crude oil. The crude was purified by flash column chromatography using ethyl acetate: methanol (8: 2) giving **^{19}F-[C_8-6-I] as** a viscous lemon yellow oil. Yield 0.017 g (65 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.52 – 7.51 (d, 1H), 7.24 – 7.17 (m, 3H), 4.49 – 4.38 (dt, J = 5.36, 47.19 Hz, 2H), 4.45 – 4.42 (q, $^3J_{HH}$ = 4.93 Hz, 1H), 4.33 (t, $^3J_{HH}$ = 6.96 Hz, 2H), 3.55 (s, 3H), 3.38 (s, 3H), 3.13 – 3.07 (m, 1H), 2.87 - 2.81 (m, 1H), 2.77 – 2.63 (m, 4H), 2.43 – 2.36 (m, 1H), 2.27 – 2.17 (dquint, J = 6.58 , 22.85 Hz 2H), 2.13 – 2.07 (m, 1H), 1.76 – 1.66 (m, 4H), 1.39 – 1.37 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 155.33, 154.34, 151.72, 147.99, 144.44 (2 carbons), 128.67, 126.73, 125.33, 125.12, 106.261, 81.31, 80.00, 62.37, 45.24, 41.72, 31.80, 31.64, 30.67, 29.87, 29.08, 28.03, 27.91, 26.94, 26.52.

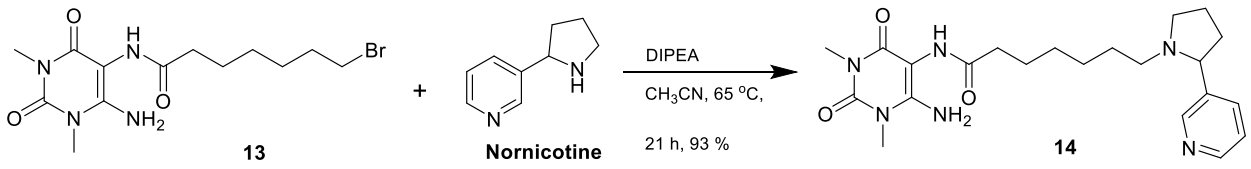
ESI-MS: 456.2803 ($[M+H]^+$, $C_{25}H_{34}FN_5O_2$; calculated: 456.2769). Purity (HPLC): ≥ 98 %, RT = 12.407 min

***N*-(6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-7-bromoheptanamide, 13**



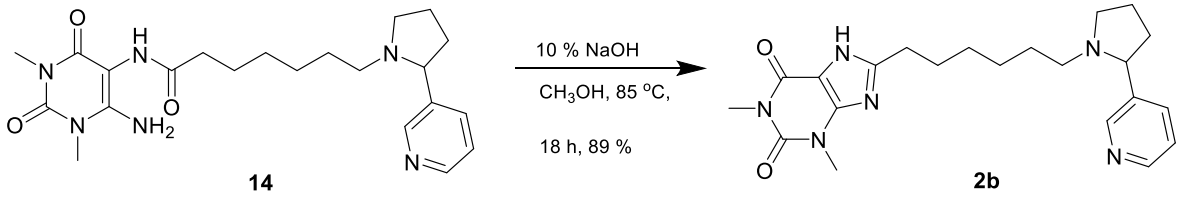
12 was prepared according to a method employed by Pierre *et al.* for hydrolysis of ethyl 7-bromoheptanoate[4] in 89 % yield. EDC.HCl (0.393 g, 2.05 mmol) was added to **12** (0.30 g, 1.44 mmol) dissolved in methanol (20 mL). The reaction mixture was stirred at room temperature for 0.75 h. 5, 6-Diamino-1, 3-dimethyl uracil (0.233 g, 1.37 mmol) was added to the reaction mixture and stirred at room temperature for the next 24 h. The reaction was concentrated under reduced pressure and purified by flash column chromatography using ethyl acetate: methanol (9: 1) giving **13** as a white solid. Yield 0.219 g (45 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.50 (br, 2H, NH_2) (3.48 (s, 3H), 3.41 (t, $^{3}J_{HH}$ = 6.78 Hz, 2H), 3.35 (s, 3H), 2.43 (t, $^{3}J_{HH}$ = 7.42 Hz, 2H), 1.87 (tt, $^{3}J_{HH}$ = 6.77 Hz, 2H), 1.73 (tt, $^{3}J_{HH}$ = 7.62 Hz, 2 H), 1.48 (tt, $^{3}J_{HH}$ = 6.84 Hz, 2H), 1.40 (tt, $^{3}J_{HH}$ = 7.71 Hz, 2H).

***N*-(6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-7-(2-(pyridin-3-yl)pyrrolidin-1-yl)heptanamide, 14**



Nornicotine (0.24 mL, 1.73 mmol) was dissolved in anhydrous acetonitrile (5 mL) under nitrogen. DIPEA (0.30 mL, 1.73 mmol) was added dropwise to nornicotine and stirred under nitrogen at room temperature for 1 h. **13** (0.209 g, 0.58 mmol) was dissolved in anhydrous acetonitrile (30 mL) and added dropwise to the reaction mixture. The reaction mixture was then stirred under nitrogen at 65 ^{o}C for 21 h. The reaction was allowed to cool to room temperature and quenched with saturated sodium bicarbonate (10 mL). The mixture was concentrated under reduced pressure and purified by flash column chromatography using ethyl acetate: methanol (6: 4) giving **14** as a light yellow oil. Yield 0.23 g (93 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.54 (d, $^{3}J_{HH}$ = 2.13 Hz, 1H), 8.46 (dt, *J* = 1.39, 4.88 Hz, 1H), 7.68 (ddd, *J* = 1.80, 7.84 Hz, 1H), 7.36 – 7.24 (ddd, *J* = 4.11, 7.58 Hz, 1H), 5.56 (s, 2H, NH_2), 3.47 (s, 3H), 3.32 (s, 3H), 3.32 – 3.29 (m, 1H), 3.24 (t, $^{3}J_{HH}$ = 8.22 Hz, 1H), 2.47 – 2.41 (m, 1H), 2.33 (t, $^{3}J_{HH}$ = 7.45 Hz, 2H), 2.23 – 2.13 (m, 2H), 2.07 – 2.02 (m, 1H), 1.97 – 1.88 (m, 1H), 1.85 – 1.77 (m, 1H), 1.69 – 1.60 (m, 3H), 1.44 – 1.35 (m, 2H), 1.32 – 1.19 (m, 4H).

1,3-dimethyl-8-(6-(2-(pyridin-3-yl)pyrrolidin-1-yl)hexyl)-xanthine, 2b

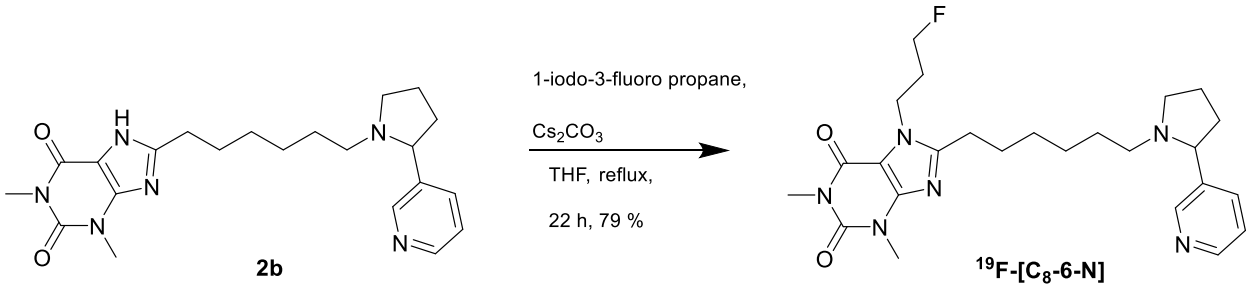


14 **2b**

14 (0.0072 g, 0.0168 mmol) was dissolved in methanol (4 mL) and 10 % sodium hydroxide in water (1 mL) was added. The reaction mixture was then refluxed at 85 ^{o}C for 18 h. The reaction was allowed to cool to room temperature and concentrated under reduced pressure to give crude. The crude was purified by flash column chromatography using ethyl acetate: methanol (7: 3) giving **2b** as a light yellow oil. Yield 0.0061 g (89 %); ^{1}H NMR (500 MHz, MeOD): δ 8.49 (d, *J*

= 2.03 Hz, 1H), 8.42 (dt, *J* = 1.47, 4.95 Hz, 1H), 7.84 (ddd, *J* = 1.85, 7.87 Hz, 1H), 7.39 (ddd, *J* = 4.91 Hz, 7.82 Hz, 1H), 3.53 (s, 3H), 3.38 – 3.36 (m, 2H), 3.35 (s, 3H), 2.72 (t, $^{3}J_{HH}$ = 7.47 Hz, 2H), 2.49 – 2.44 (m, 1H), 2.32 – 2.11 (m, 2H), 2.17 – 2.11 (m, 1H), 2.00 – 1.85 (m, 2H), 1.73 – 1.67 (m, 3H), 1.46 – 1.41 (m, 2H), 1.33 – 1.22 (m, 4H).

^{19}F-[C$_8$-6-N]

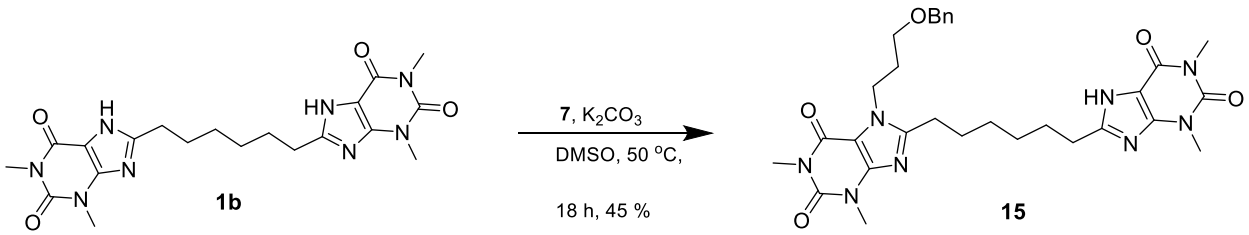


2b (0.0040 g, 0.0097 mmol) and caesium carbonate (0.0095 g, 0.0029 mmol) were dissolved in anhydrous tetrahydrofuran (2 mL) under nitrogen. 1-iodo-3-fluoro propane (0.012 g, 0.0061 mmol) was dissolved in tetrahydrofuran (1 mL) and added dropwise to **2b**. The reaction mixture was refluxed at 75 ^{o}C for 22 h. The reaction was allowed to cool to room temperature and quenched with water (1 mL). The mixture was then extracted with dichloromethane (3 × 6 mL). The organic layers were combined, dried over Mg_2SO_4, filtered and concentrated under reduced pressure to give a crude oil. The crude was purified by flash column chromatography using ethyl acetate: methanol (9: 1) giving **^{19}F-[C$_8$-6-N]** as a lemon yellow oil. Yield 0.0036 g (79 %); ^{1}H NMR (500 MHz, MeOD): δ 8.49 (d, *J* = 1.81 Hz, 1H), 8.40 (dt, *J* = 1.58, 4.89 Hz, 1H), 7.85 (ddd, *J* = 1.87, 3.74, 7.89 Hz, 1H), 7.39 (ddd, *J* = 4.77, 7.60 Hz, 1H), 4.51 – 4.39 (dt, $^{3}J_{HH}$ = 5.50 Hz, 2H), 4.39 (t, $^{3}J_{HH}$ = 7.03 Hz, 2H), 3.51 (s, 3H), 3.35 (m, 1H), 3.34 (s, 3H), 2.77 (t, $^{3}J_{HH}$ = 7.65 Hz, 2H), 2.49 – 2.44 (m, 1H), 2.30 – 2.10 (m, 5H), 1.98 – 1.85 (m, 2H), 1.78 – 1.65 (m, 3H), 1.47 – 1.41 (m, 2H), 1.36 – 1.26 (m, 5H); ^{13}C{^{1}H} NMR (MeOD, 125 MHz): δ 156.47, 156.36, 149.92, 149.04,

137.66, 125.47, 82.74, 81.43, 69.17, 55.59,54.85, 43.07, 35.95, 32.91, 32.76, 30.29, 30.21, 29.53, 28.92, 28.43, 28.20, 27.37, 23.61.

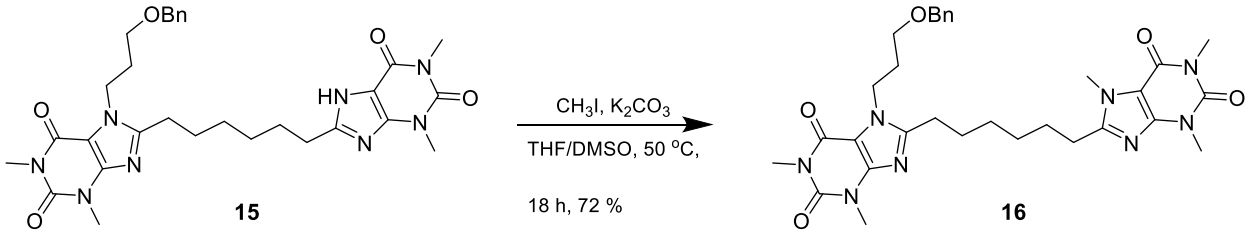
ESI-MS: 471.2566 ($[M+H]^+$, $C_{25}H_{35}FN_6O_2$; calculated: 471.2878). Purity (HPLC): ≥ 98 %, RT = 11.791 min

1-(7-(-3(benzyloxy)propyl)-1,3-dimethylxanthine)-6-(1,3-dimethylxanthine)-hexane, 15



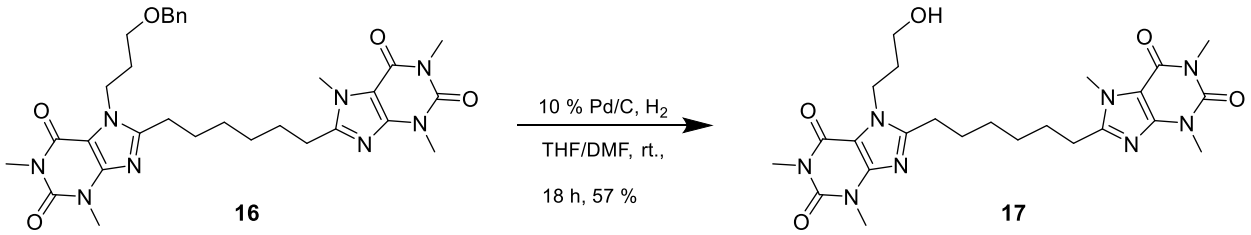
1b[1] (0.14 g, 0.31 mmol) and potassium carbonate (0.19 g, 1.38 mmol) were dissolved in anhydrous dimethylsulfoxide (10 mL) under nitrogen. Compound **7** (0.076 g, 0.28 mmol) was dissolved in anhydrous dimethylsulfoxide (3 mL) and added dropwise to **1b**. The reaction was then stirred under nitrogen at 50 °C for 18 h. The reaction was allowed to cool to room temperature and quenched with water (5 mL). The mixture was concentrated under reduced pressure using a rotary evaporator-high vacuum set-up to give a crude solid. The crude was purified by flash column chromatography using ethyl acetate: methanol (9.8: 0.2) giving **15** as a yellow solid. Yield 0.073 g (45 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33 – 7.27 (m, 5H), 4.47 (s, 2H), 4.34 (t, $^3J_{HH}$ =7.02 Hz, 2H), 3.60 (s, 3H), 3.54 (s, 3H), 3.49 (t, $^3J_{HH}$ =5.51 Hz, 2H), 3.43 (s, 3H), 3.38 (s, 3H), 2.81 (t, $^3J_{HH}$ =7.51 Hz, 2H), 2.73 (t, $^3J_{HH}$ = 7.64 Hz, 2H), 2.12 (quint, $^3J_{HH}$ = 6.22 Hz, 2H), 1.83 – 1.71 (m, 4H), 1,40 (m, 4H).

1-(7-(-3(benzyloxy)propyl)-1,3-dimethylxanthine)-6-(1,3,7-trimethylxanthine)-hexane, 16



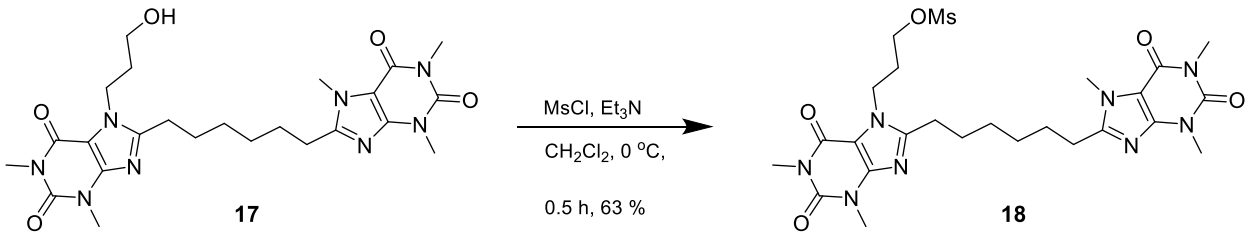
15 (0.071 mg, 0.12 mmol) and potassium carbonate (0.17 g, 1.26 mmol) were dissolved in anhydrous tetrahydrofuran: dimethylsulfoxide (8 mL: 1 mL) under nitrogen. Methyl iodide (0.080 mL, 1.26 mmol) was added dropwise to **15**. The reaction mixture was stirred under nitrogen at 50 ^{o}C for 18 h. The reaction was allowed to cool to room temperature and quenched with water (5 mL). The mixture was then extracted with ethyl acetate (3 × 30 mL), and washed with water (2 × 5 mL). The organic layers were combined, dried over Mg_2SO_4, filtered, and concentrated under reduced pressure to give a crude solid. The crude was purified by flash column chromatography using ethyl acetate: methanol (9.8: 0.2) giving **16** as a white solid. Yield 0.052 g (72 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34 – 7.27 (m, 5H), 4.47 (s, 2H), 4.34 (t, $^{3}J_{HH}$ = 6.95 Hz, 2H), 3.88 (s, 3H), 3.55 (s, 3H), 3.54 (s, 3H), 3.46 (t, $^{3}J_{HH}$ = 5.53 Hz, 2H), 3.38 (s, 3H), 3.37 (s, 3H), 2.73 (t, $^{3}J_{HH}$ = 7.65 Hz, 2H), 2.68 (t, $^{3}J_{HH}$ = 7.65 Hz, 2H), 2.12 (quint, $^{3}J_{HH}$ = 6.28 Hz, 2H), 1.77 – 1.71 (m, 4H), 1.41 – 1.39 (m, 4H).

1-(7-(-3-hydroxypropyl)-1,3-dimethylxanthine)-6-(1,3,7-trimethylxanthine)-hexane, 17



10 % Pd/C (0.020 g) was added to **16** (0.052 g, 0.086 mmol) dissolved in anhydrous tetrahydrofuran: dimethylformamide (5 mL: 1 mL). The reaction mixture was stirred under hydrogen atmosphere (hydrogen balloon) at room temperature for 18 h. The mixture was diluted with methanol and filtered through a celite pad. The mixture was concentrated under reduced pressure to give a crude solid. The crude was purified by flash column chromatography using ethyl acetate: methanol (9: 1) giving **17** as a white solid. Yield 0.025 g (57 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.39 (t, $^{3}J_{HH}$ = 6.32 Hz, 2H), 3.89 (s, 3H), 3.57 (t, $^{3}J_{HH}$ = 5.22 Hz, 2H), 3.55 (s, 3H), 3.53 (s, 3H), 3.38 (s, 3H), 3.37 (s, 3H), 2.75 – 2.70 (m, 4H), 1.81 - 1.75 (m, 4H), 1.47 – 1.46 (m, 4H).

1-(7-(3-((methylsulfonyl)oxy)propyl)-1,3-dimethylxanthine)-6-(1,3,7-trimethylxanthine)-hexane, 18

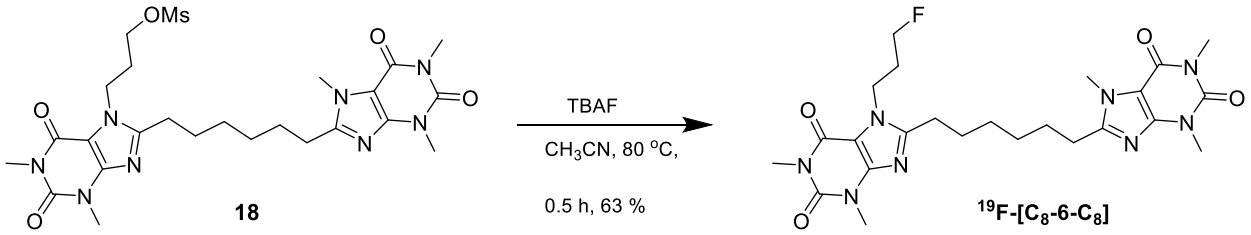


17 **18**

17 (0.024 g, 0.047 mmol) was dissolved in anhydrous dichloromethane (6 mL) under nitrogen. Triethylamine (0.013 mL, 0.093 mmol) was added and the mixture was cooled to 0 °C. Methanesulfonyl chloride (0.0070 mL, 0.93 mmol) was added dropwise to the reaction mixture and stirred under nitrogen at 0 °C for 0.5 h. The reaction was quenched with water (2 mL) and transferred to a separatory funnel. The organic layer was collected and the aqueous layer extracted with dichloromethane (3 × 15 mL). The organic layers were combined, dried over Mg_2SO_4, filtered and concentrated under reduced pressure to give a crude oil. The crude was purified by flash column chromatography using ethyl acetate: methanol (9: 1) giving **18** as a colorless oil. Yield

0.018 g (63 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.36 (t, $^{3}J_{HH}$ = 7.04 Hz, 2H), 4.27 (t, $^{3}J_{HH}$ = 5.67 Hz, 2H), 3.90 (s, 3H), 3.55 (s, 3H), 3.54 (s, 3H), 3.38 (s, 3H), 3.37 (s, 3H), 3.02 (s, 3H), 2.75 – 2.71 (m, 4H), 2.29 (quint, $^{3}J_{HH}$ = 5.97 Hz, 2H), 1.81 – 1.73 (m, 4H), 1.47 – 1.45 (m, 4H).

^{19}F-[C$_8$-6-C$_8$]



18 **^{19}F-[C$_8$-6-C$_8$]**

18 (0.018 g, 0.030 mmol), was dissolved in anhydrous acetonitrile (2 mL) under nitrogen. Tetra-n-butylammonium fluoride (1 M in THF, 0.090 mL, 0.090 mmol) was added dropwise to **18** and the mixture heated to 80 °C. The reaction mixture was stirred under nitrogen at 80 °C for 0.5 h. The reaction was allowed to cool to room temperature and quenched with water (2 mL). The mixture was then extracted with dichloromethane (3 × 10 mL). The organic layers were combined, dried over Mg_2SO_4, filtered and concentrated under reduced pressure to give a crude. The crude was purified by flash column chromatography using ethyl acetate: methanol (9.5: 0.5) giving **^{19}F-[C$_8$-6-C$_8$]** as a white solid. Yield 0.0097 g (63 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.50 – 4.38 (dt, *J* = 5.32, 47.06 Hz, 2H), 4.36 (t, $^{3}J_{HH}$ = 6.97 Hz, 2H), 3.90 (s, 3H), 3.55 (s, 3H), 3.54 (s, 3H), 3.39 (s, 3H), 3.38 (s, 3H), 2.75 – 2.71 (m, 4H), 2.29 – 2.18 (dquint, *J* = 6.63, 23.02 Hz, 2H), 1.81 – 1.73 (m, 4H), 1.47 – 1.45 (m, 4H); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 155.46, 155.04, 154.35, 154.28, 151.83, 151.80, 148.68, 148.10, 107.42, 106.64, 81.31, 80.02, 41.73, 31.84, 31.66, 29.89, 29.84, 29.15, 29.08, 28.06, 28.00, 27.90, 27.52, 26.88, 26.59.

ESI-MS: 517.3721 ($[M+H]^+$, $C_{24}H_{33}FN_8O_4$; calculated: 517.2682). Purity (HPLC): ≥ 98 %, RT = 13.19 min

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