Figures and tables are 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.

Table 1. Percentage of parent compound remaining after 1 h incubation in HLM, RLM and MLM

Parent Compounds	**% of Parent Compound Remaining after 1 h incubation**			**Metabolic Reaction**
	HLM	**RLM**	**MLM**	
C_8-6-C_8	100	100	100	No metabolite detected
^{19}F-[C_8-6-C_8]	90.9	61.6	83.3	Propyl fluoride hydroxylation
C_8-6-I	36.2	62.4	32.7	Amino-indan hydroxylation, N-dealkylation, N3-demethylation and N1-demethylation
^{19}F-[C_8-6-I]	77.6	57.4	50.3	Amino-indan hydroxylation, Propyl fluoride hydroxylation N-dealkylation, N3-demethylation and N1-demethylation
C_8-6-N	NIL	NIL	NIL	Nicotine hydroxylation, N-dealkylation
^{19}F-[C_8-6-N]	43.1	52.8	63.2	Nicotine hydroxylation, N-dealkylation

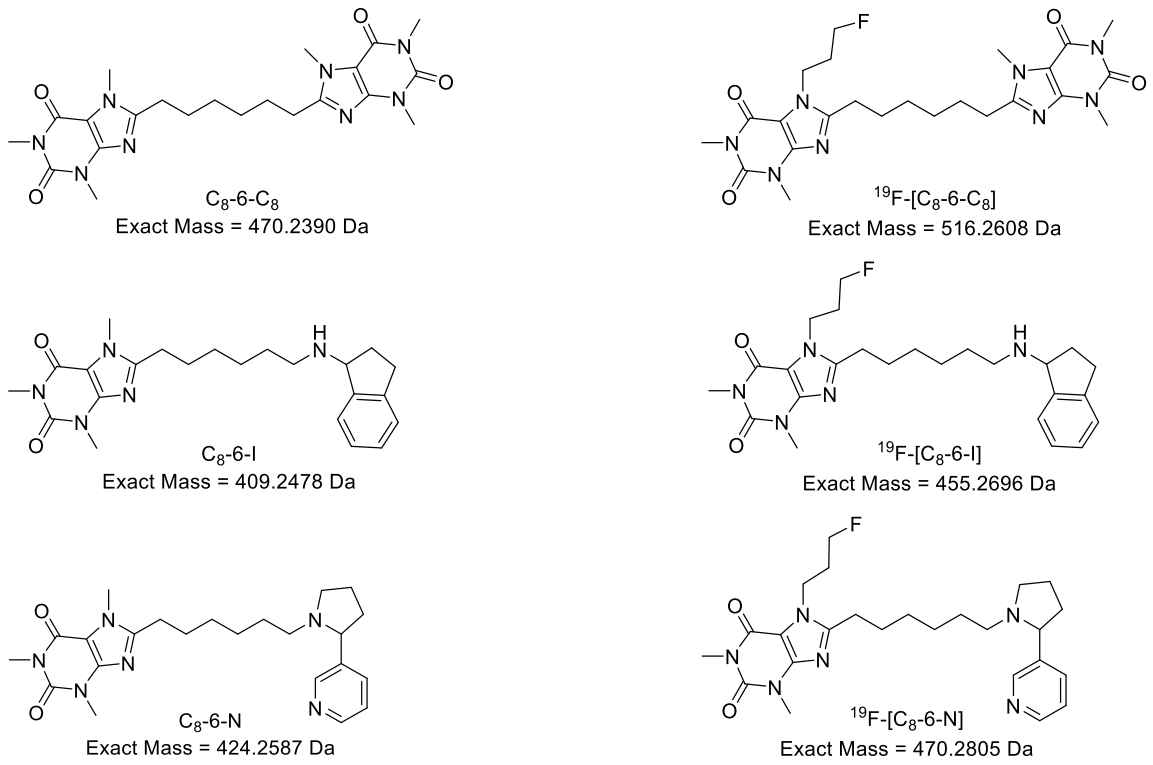
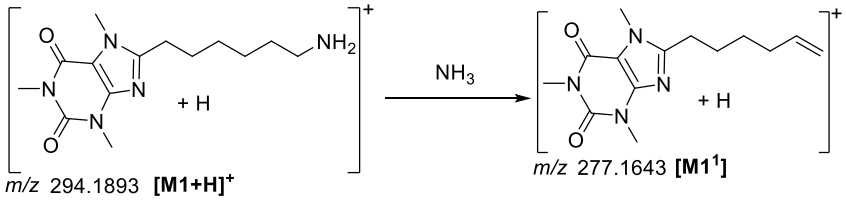


Figure 1. Structure of novel bifunctional compounds and N7-propyl-fluoro analogues.

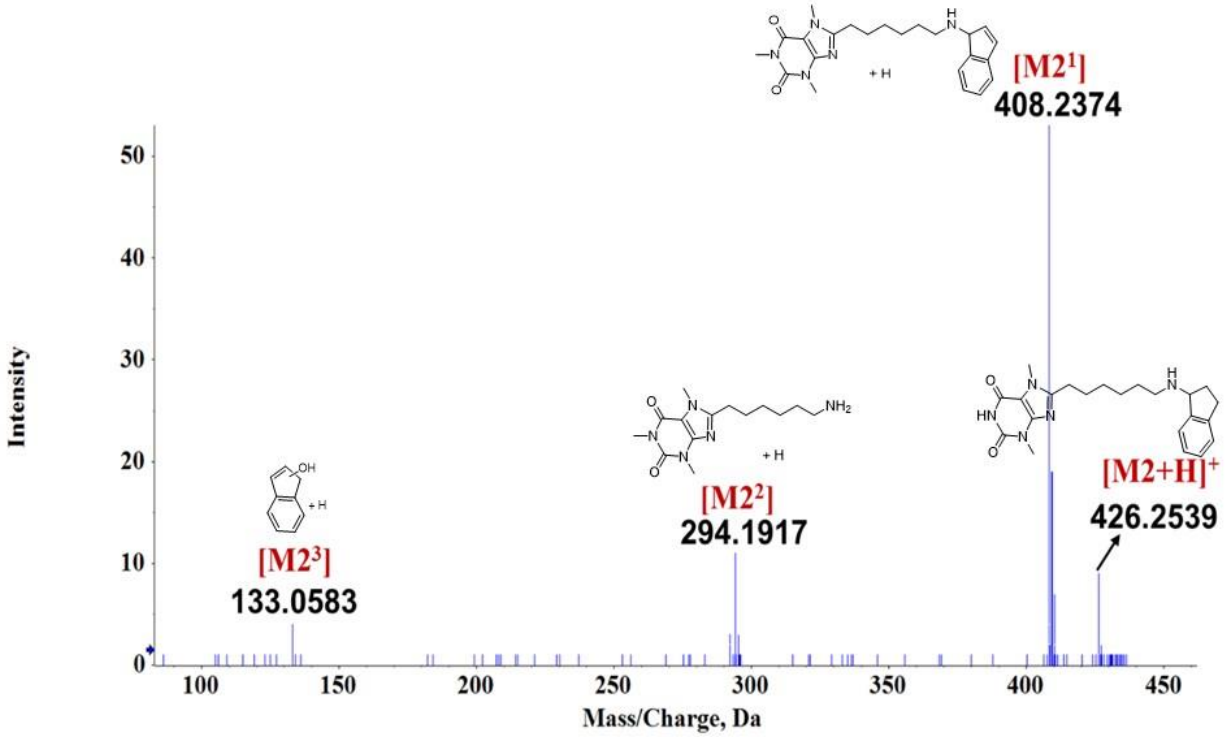
A



m/z 294.1893 **[M1+H]$^+$**

m/z 277.1643 **[M1^1]**

B



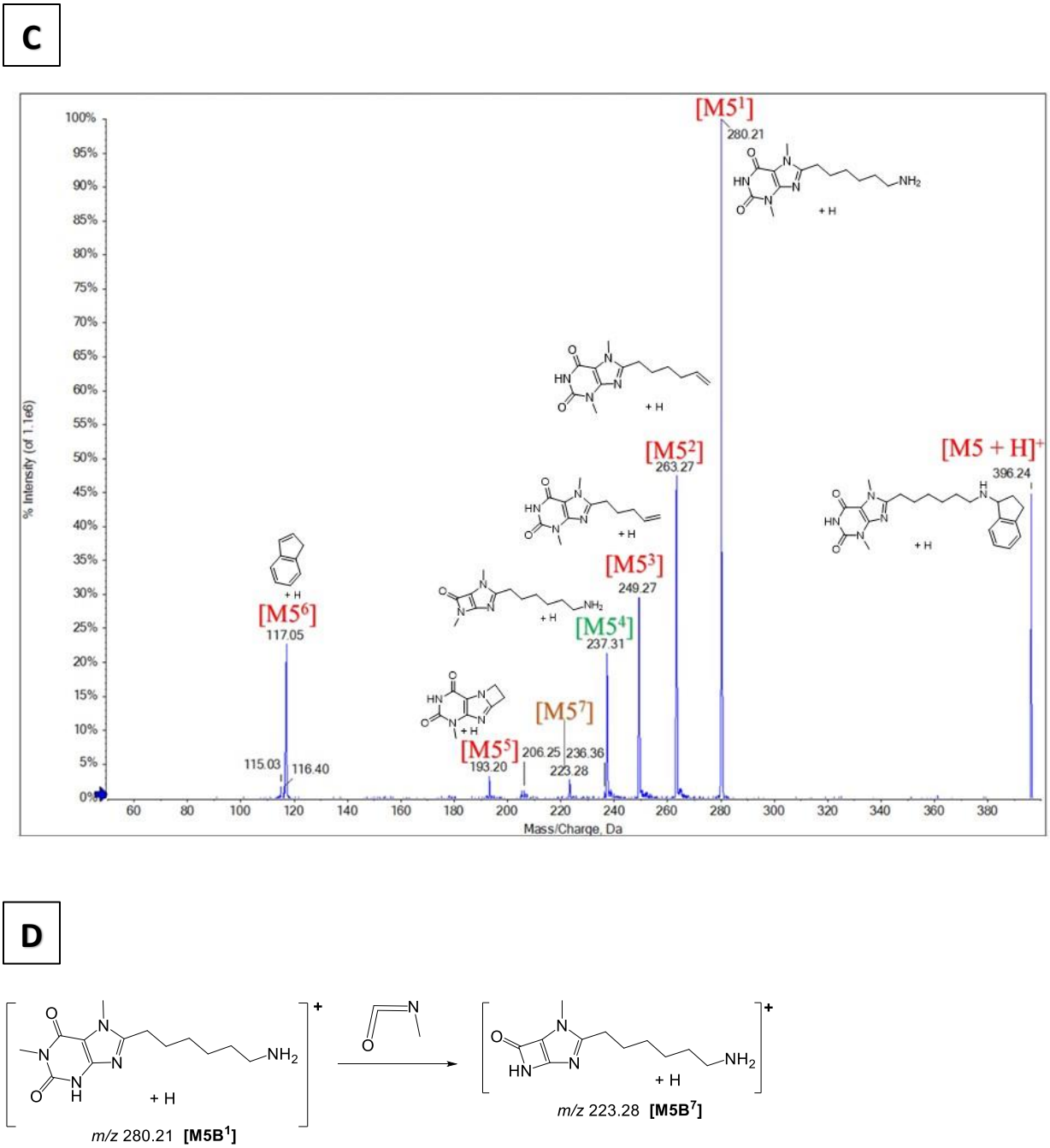


Figure 2. (A) Proposed fragmentation pathway for C_8-6-I metabolite M1. (B) The ESI-QToF-MS/MS spectrum and the proposed fragments for C_8-6-I metabolite M2. (C) The ESI-QToF-MS/MS spectrum and the proposed fragments for C_8-6-I metabolites M5. (D) Proposed fragmentation pathway for C_8-6-I metabolite M5B. ESI was performed in positive mode.

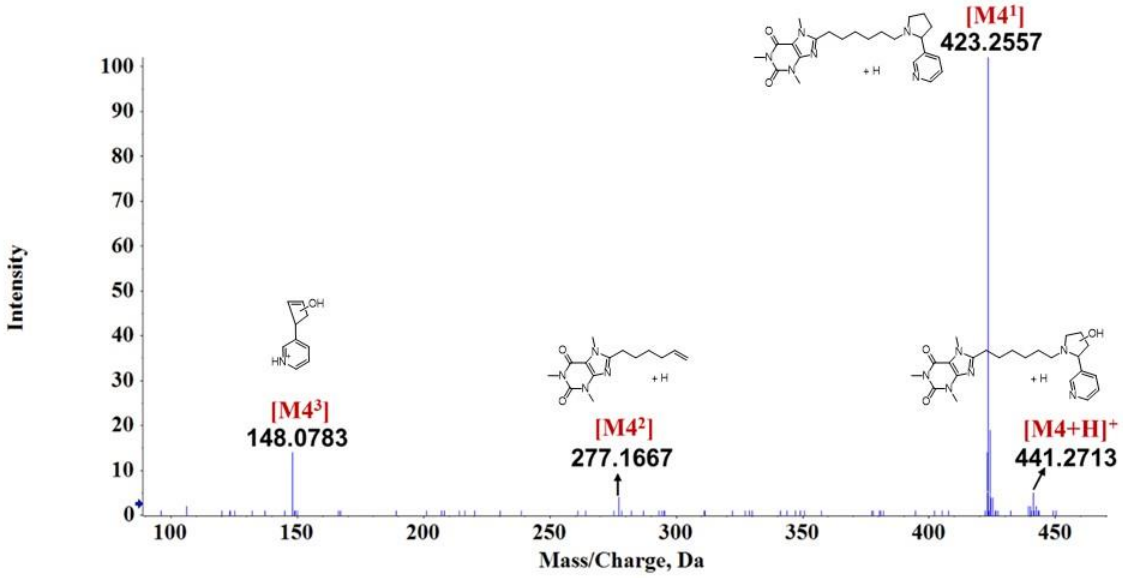
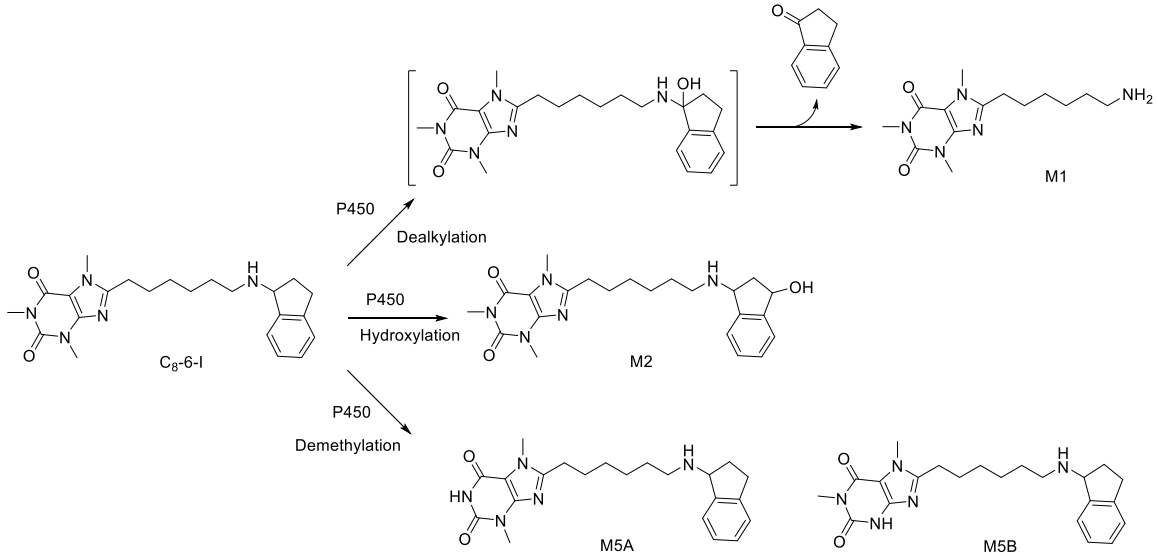
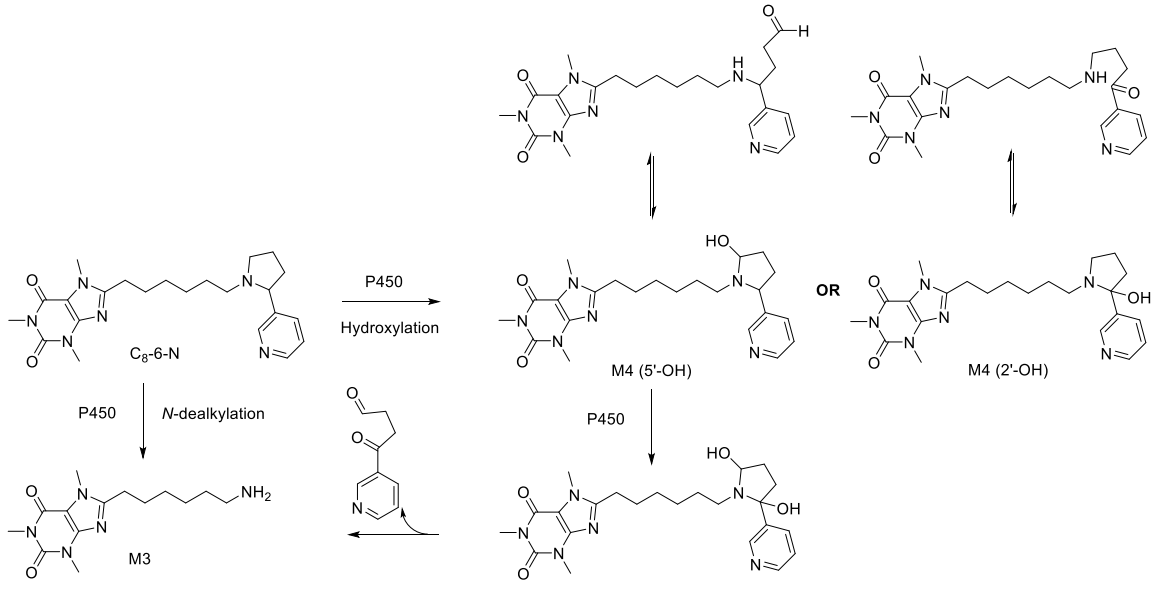


Figure 3. The ESI-QToF-MS/MS spectrum for C_8-6-N metabolite M4(A), and the proposed fragments for M4(B). ESI was performed in positive mode.

A


P450
Dealkylation
P450
Hydroxylation
P450
Demethylation
C8-6-I
M1
M2
M5A
M5B

B


P450
Hydroxylation
OR
C8-6-N
M4 (5'-OH)
M4 (2'-OH)
P450
N-dealkylation
P450
M3

C

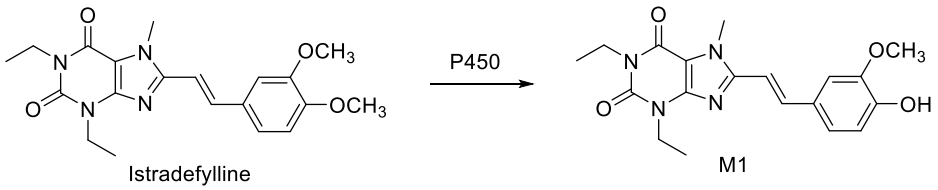


Figure 4. The proposed metabolic pathway for (A) C_8-6-I and (B) C_8-6-N in human, mouse, and rat liver microsomes. (C) Primary phase I metabolic pathway of Istradefylline.

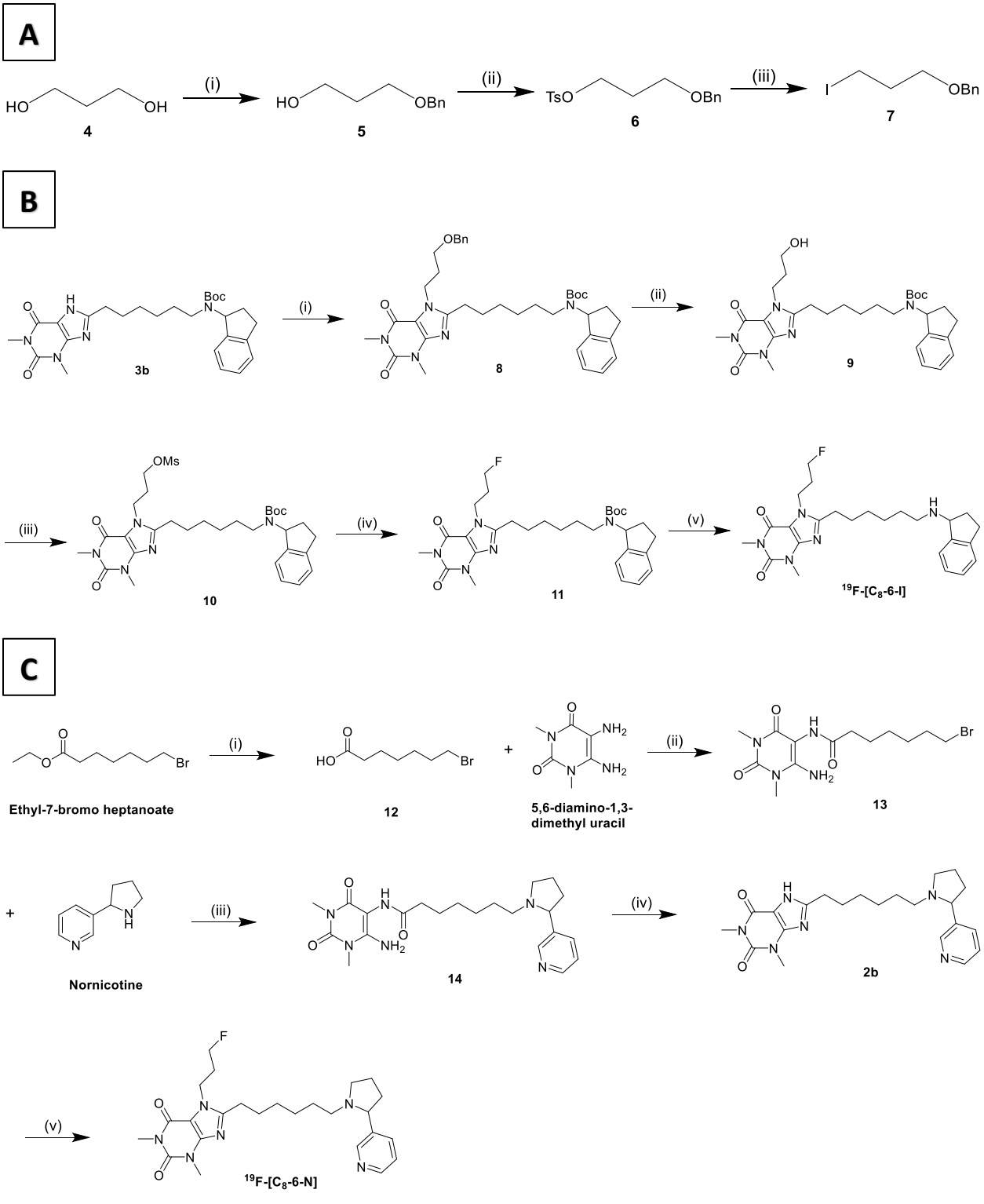


Figure 5. (A) Synthesis of 3-benzyloxy iodopropane. Reagents and conditions: (i) NaH, BnBr, 18 h, 0 °C – 100 °C, 100 %, (ii) TsCl, Et_3N, CH_2Cl_2, 12 h, room temp.,

72 %; (iii) NaI, Acetone, 9 h, room temp – reflux, 72 %. (B) Synthesis of ^{19}F-[C_8-6-I]. Reagents and conditions: (i) **7**, K_2CO_3, THF, 72 h, reflux, 69 %; (ii) 10 % Pd/C, THF, 9 h, room temp., 52 %; (iii) MsCl, Et_3N, CH_2Cl_2, 2 h, 0°C, 79 %; (iv) TBAF, CH_3CN, 0.5 h, 80°C, 73 %; (v) 4N HCl, CH_2Cl_2, 1.5 h, room temp, 65 %. (C) Synthesis of ^{19}F-[C_8-6-N]. Reagents and conditions: (i) 2N LiOH, CH_3OH, 1 h, room temp., 89 %; (ii) EDC.HCl, CH_3OH, 24 h, room temp, 45 %; (iii) DIPEA, CH_3CN, 21 h, 65 °C, 93 %; (iv) 10 % NaOH, CH_3OH, 18 h, 85 °C, 85 %; (v) 1-iodo-3-fluoro propane, Cs_2CO_3, THF, 22 h, reflux, 79 %.

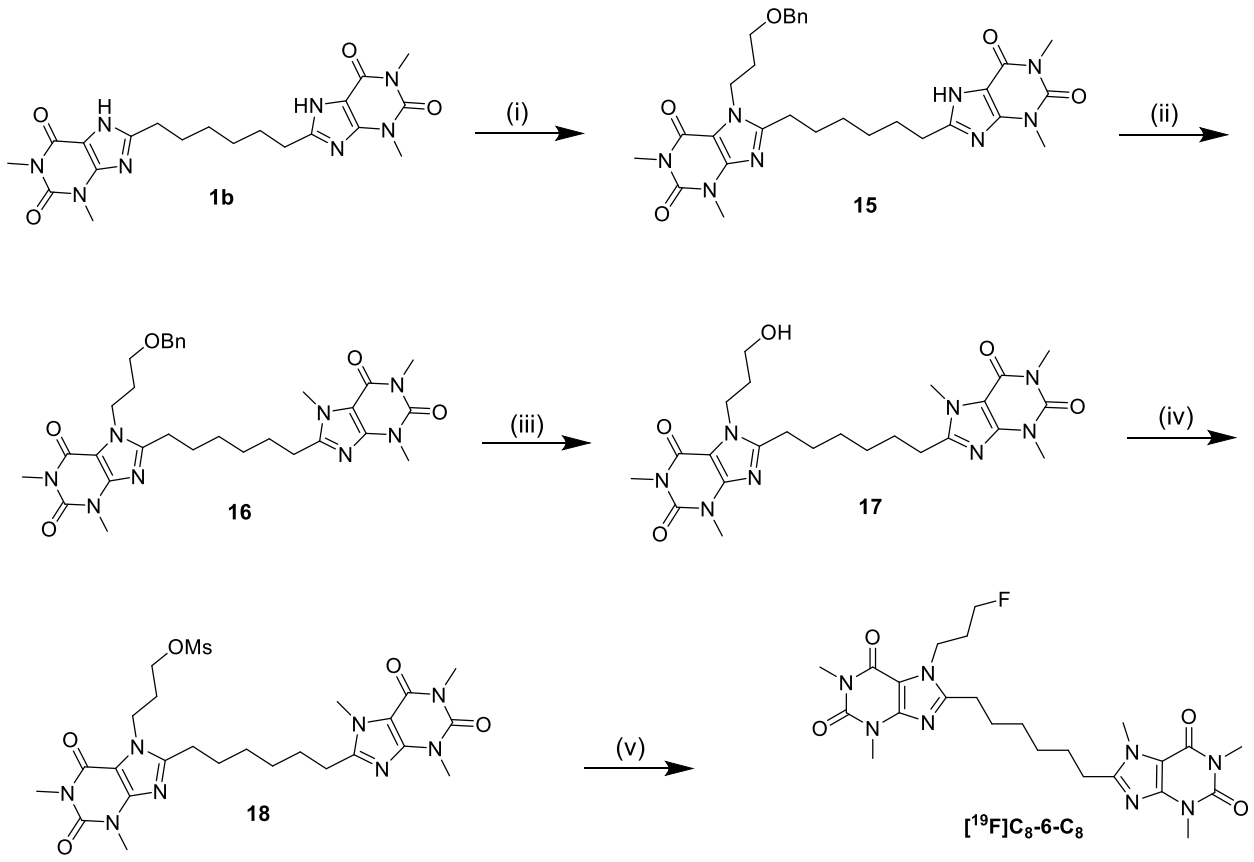
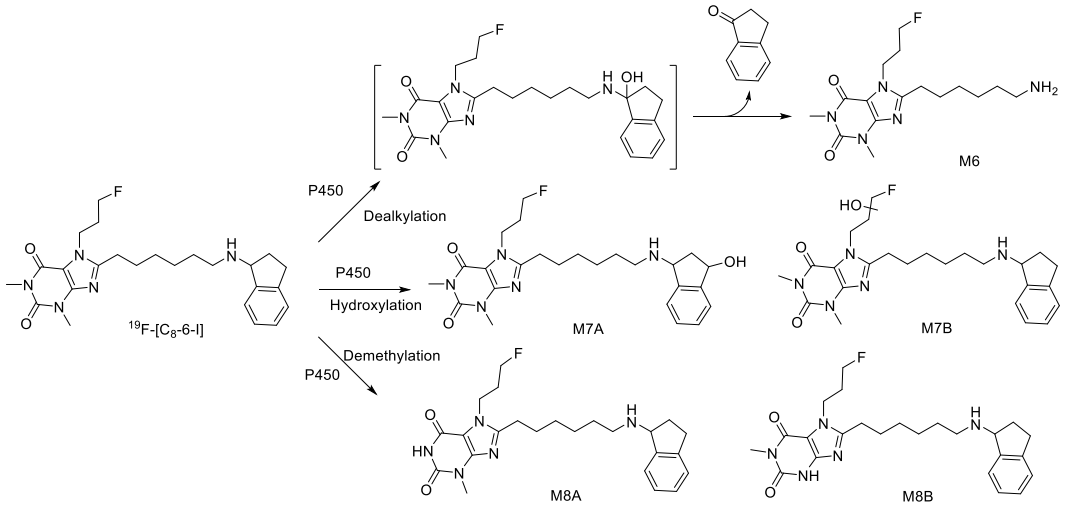
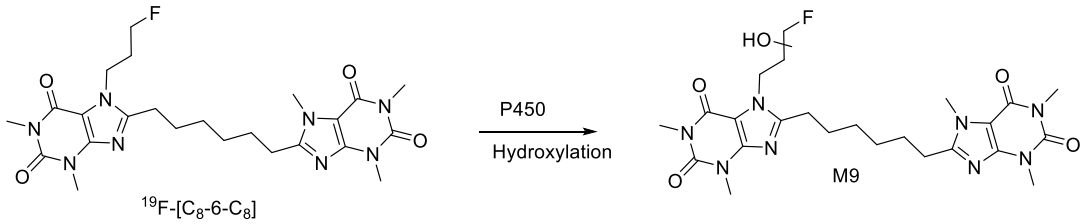


Figure 6. Synthesis of ^{19}F-[C_8-6-C_8]. Reagents and conditions: (i) **7**, K_2CO_3, DMSO, 18 h, 50 °C, 45 %; (ii) CH_3I, K_2CO_3, THF/DMSO, 18 h, 50 °C, 72 %; (iii) 10 % Pd/C, H_2, THF/DMF, 18 h, room temp, 57 %; (iv) MsCl, Et_3N, CH_2Cl_2, 0.5 h, 0 °C, 63 %; (v) TBAF, CH_3CN, 0.5 h, 80 °C, 63%.

A


P450
Dealkylation
P450
Hydroxylation
Demethylation
P450
^{19}F-[C$_8$-6-I]
M6
M7A
M7B
M8A
M8B

B


P450
Hydroxylation
^{19}F-[C$_8$-6-C$_8$]
M9

C

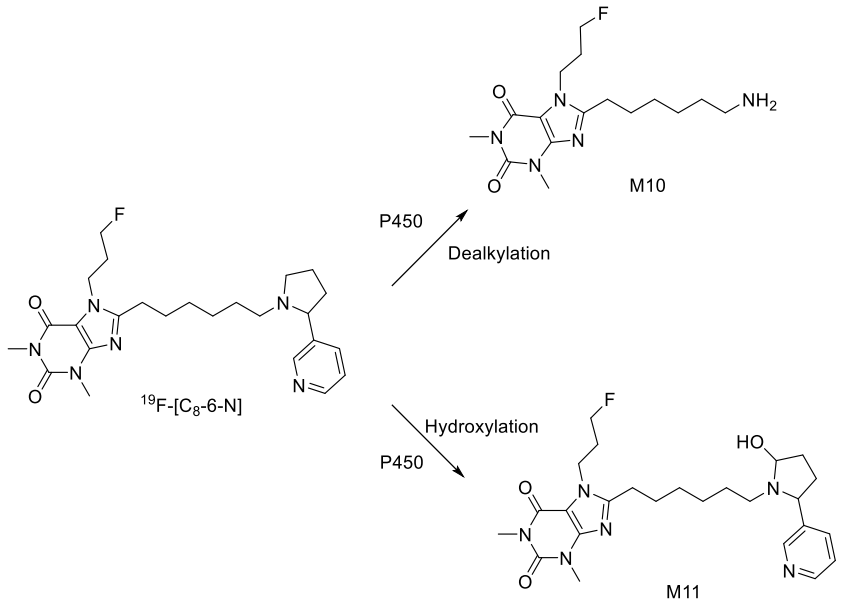


Figure 7. The proposed metabolic pathway for ^{19}F-[C_8-6-I] (A), ^{19}F-[C_8-6-C_8] (B) and ^{19}F-[C_8-6-N] (C) in human, mouse, and rat liver microsomes.