Supplemental material to:

Biotransformation of two β-secretase Inhibitors Including Ring Opening and contraction of a Pyrimidine Ring

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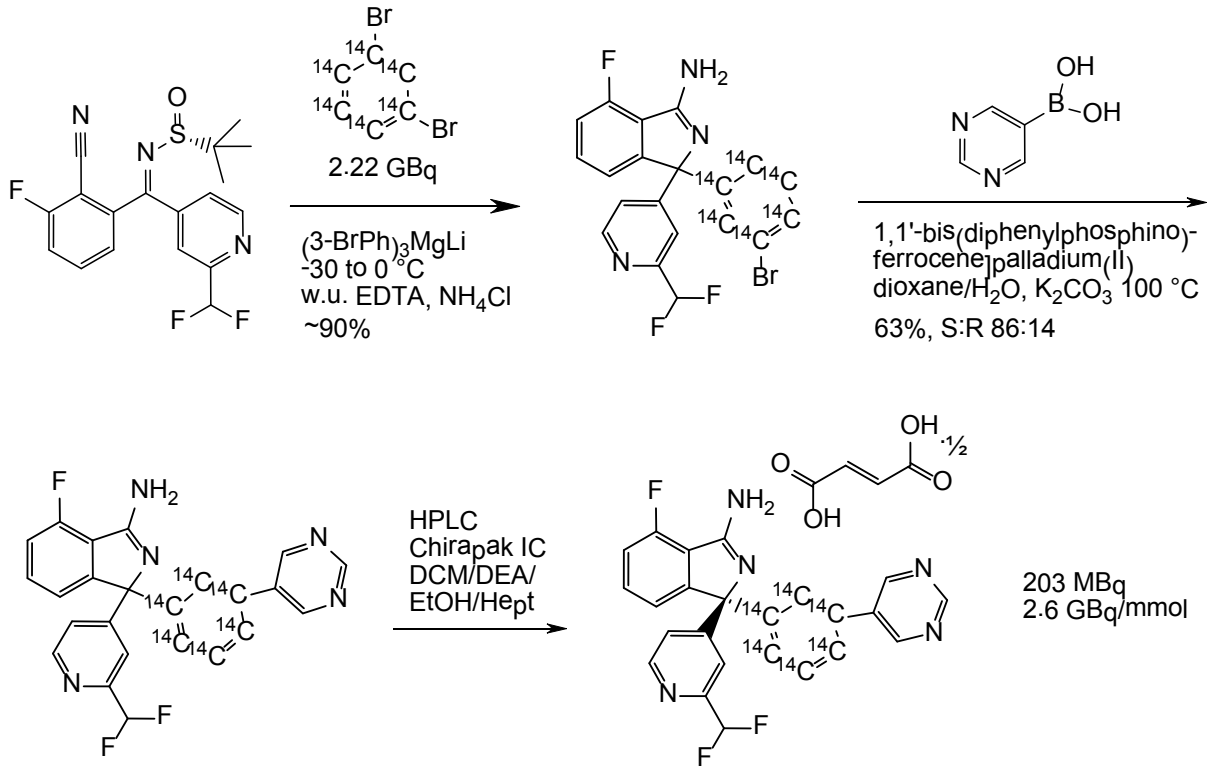
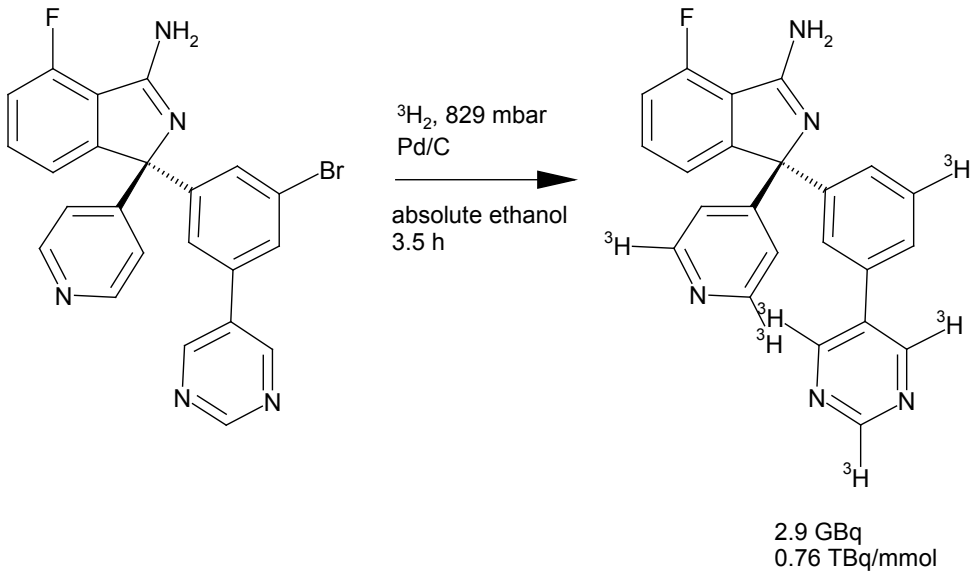
Drug metabolism and Disposition

Abbrevations

DEA, diethylamine; DME, dimethoxyethane; DMF, N,N-dimethylformamide; DMSO, dimethyl sulfoxide; OAc, acetate; rt, room temperature; sat., saturated; SFC, supercritical fluid chromatography



Scheme 1. Synthesis of [^{14}C]AZD3839 hemifumarate from [U-^{14}C]-1,3-dibromobenzene [Malmquist J (2011) [U-^{14}C]- and [$^{13}C_6$]-1,3-dibromobenzene; useful precursors with a rare substitution pattern in the synthesis of isotopologs. *J Label Compd Radiopharm* **54:**408–410].



Scheme 2. Synthesis of [^{3}H]-(1*S*)-4-fluoro-1-(pyridin-4-yl)-1-(3-(pyrimidin-5-yl)phenyl)-1*H*-isoindol-3-amine prepared from a brominated compound synthesized in a similar way as for the unlabeled material [Swahn BM, Kolmodin K, Karlström S, et al., (2012b) Design and Synthesis of β-Site Amyloid Precursor Protein Cleaving Enzyme (BACE1) Inhibitors with in vivo brain reduction of β-amyloid peptides. *J Med Chem* **55:**9346-9361.] The labeled positions were identified by running ^{1}H and ^{3}H NMR experiments (Figure S1).

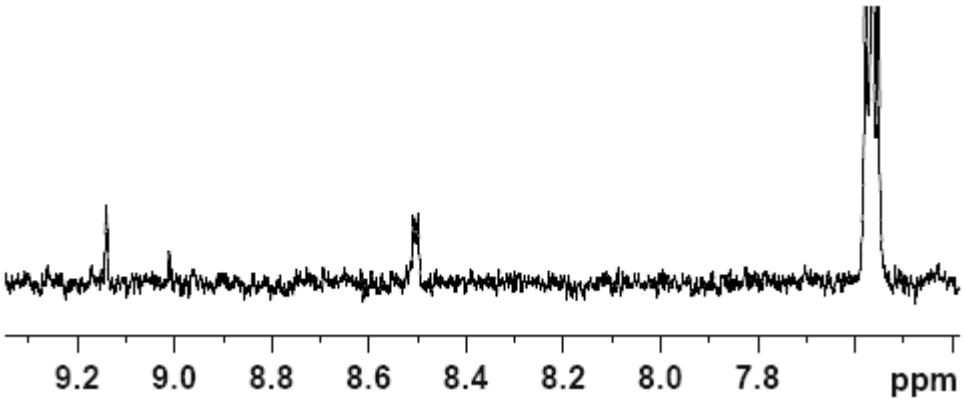
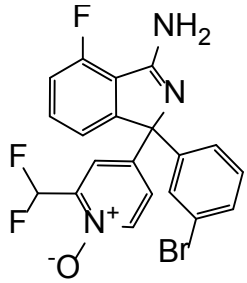


Figure 1. ^{3}H-NMR of 75 MBq [^{3}H]-(*S*)- 1-pyridin-4-yl-4-fluoro-1-(3-(pyrimidin-5-yl)phenyl)-1*H*-isoindol-3-amine at 140 μM in CD_3OD.

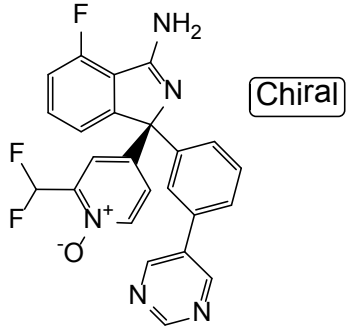
Synthesis of metabolite M1 and M2

4-(3-amino-1-(3-bromophenyl)-4-fluoro-1H-isoindol-1-yl)-2-(difluoromethyl)pyridine 1-oxide



Hydrogen peroxide (0.598 mL, 5.86 mmol) was added to 1-(3-bromophenyl)-1-(2-(difluoromethyl)pyridin-4-yl)-4-fluoro-1H-isoindol-3-amine (a 84:16 ratio of the S and R- isomers) (422 mg, 0.98 mmol) in acetic acid (10 mL). The reaction mixture was stirred at 60 ºC for three days after three days at rt the mixture was diluted with K_2CO_3 (aq sat) and ethyl acetate, the phases were separated. NaCl (s) was added and the aqueous phase was extracted twice with ethyl acetate. The combined organics were dried (Na_2SO_4), filtered and concentrated. Purification by flash silica gel chromatography using a gradient of chloroform/ methanol 40:1-30:1-20:1 gave the title compound (125 mg) in 29 % yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 6.81 (br. s., 2 H), 7.16 (t, *J*=53.28 Hz, 1 H), 7.25 - 7.38 (m, 3 H), 7.42 - 7.53 (m, 4 H), 7.58 (td, *J*=7.88, 4.73 Hz, 1 H), 7.69 (d, *J*=7.57 Hz, 1 H), 8.29 (d, *J*=6.94 Hz, 1 H); MS (ES+) *m/z* 448 $[M+1]^+$.

B(M2), (S)-4-(3-amino-4-fluoro-1-(3-(pyrimidin-5-yl)phenyl)-1H-isoindol-1-yl)-2-(difluoromethyl)pyridine 1-oxide



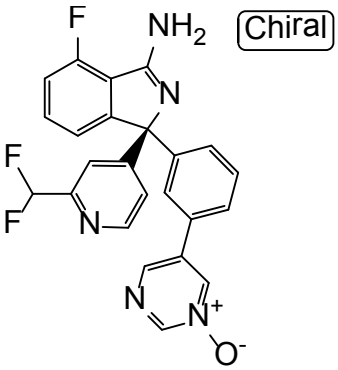
4-(3-Amino-1-(3-bromophenyl)-4-fluoro-1H-isoindol-1-yl)-2-(difluoromethyl)pyridine 1-oxide (60 mg, 0.13 mmol), pyrimidin-5-ylboronic acid (21.56 mg, 0.17 mmol), [1,1-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (9.79 mg, 0.01 mmol), potassium carbonate (2 M, aq.) (0.201 mL, 0.40 mmol) and DMF (2.1 mL) were added to a vial and microwaved for 20 min at

150 ºC. The mixture was diluted with brine, $NaHCO_3$ (aq. sat.) and ethyl acetate and the phases were separated. The aqueous phase was extracted twice with ethyl acetate, the combined organics were dried (Na_2SO_4), filtered through diatomaceous earth and concentrated. Purification by flash silica gel chromatography using a gradient of chloroform/ methanol 15:1-10:1-8:1 gave 4-(3-amino-4-fluoro-1-(3-(pyrimidin-5-yl)phenyl)-1H-isoindol-1-yl)-2-(difluoromethyl)pyridine 1-oxide (32.0 mg, 52.5 %). The material was, combined with 25 mg from a similar reaction, based on 4-(3-amino-1-(3-bromophenyl)-4-fluoro-1H-isoindol-1-yl)-2-(difluoromethyl)pyridine 1-oxide (50 mg, 0.11 mmol).

The enantiomers were separated by a SFC Berger Multigram II on a Chiralpak AD-H column (4.6*250 mm; 5 µm) using 20 % methanol + 0.1 % diethyl amine/ 80 % CO_2 as a mobile phase with a flow of 50 ml/min. The second eluting isomer was collected to give the title compound (27 mg) in 49 % yield, >99 % pure by UV and >99 % enantiomeric purity.

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 6.78 (br. s., 2 H), 7.16 (t, *J*=53.28 Hz,1 H), 7.25 - 7.34 (m, 1 H), 7.43 - 7.55 (m, 4 H), 7.58 (td, *J*=7.88, 4.73 Hz, 1 H), 7.66 - 7.73 (m, 2 H), 7.81 (d, *J*=7.57 Hz, 1 H), 8.29 (d, *J*=6.94 Hz, 1 H), 9.05 (s, 2 H), 9.18 (s, 1 H); MS (ES+) *m/z* 448 $[M+1]^+$

B(M1), (S)-5-(3-(3-amino-1-(2-(difluoromethyl)pyridin-4-yl)-4-fluoro-1H-isoindol-1-yl)phenyl)pyrimidine 1-oxide



1-(3-Bromophenyl)-1-(2-(difluoromethyl)pyridin-4-yl)-4-fluoro-1H-isoindol-3-amine (a 85:15 ratio of the S and R- isomers) (300 mg, 0.69 mmol), potassium acetate (136 mg, 1.39 mmol), 1,1'-bis(diphenylphosphino)ferrocenedichloro palladium(II) dichloromethane complex (56.7 mg, 0.07 mmol) and bis(pinacolato)diboron (194 mg, 0.76 mmol) in DME (10 mL) were microwaved for 30 min at 130 °C. 5-Bromopyrimidine 1-oxide (145 mg, 0.76 mmol) and water (3 mL) were added. The resulting mixture was microwaved for 50 min at 130 °C. The mixture was diluted with ethyl acetate and brine.

The aqueous phase was extracted twice with ethyl acetate, the combined organics were dried (Na_2SO_4), filtered through diatomaceous earth and concentrated. Purification by flash silica gel chromatography using a gradient of chloroform/methanol 10:1-8:1-6:1 gave 5-(3-(3-amino-1-(2-(difluoromethyl)pyridin-4-yl)-4-fluoro-1H-isoindol-1-yl)phenyl)pyrimidine 1-oxide (131 mg) in 36 % yield. The enantiomers were separated by a SFC Berger Multigram II on a Chiralpak AD-H column (4.6*250 mm; 5 μm) using 30 % methanol + 0.1 % diethyl amine/ 70 % CO_2 as a mobile phase with a flow of 50 ml/min. The second eluting isomer was collected to give the title compound (44 mg) in 33 % yield, 99 % pure by UV and >99 % enantiomeric purity.

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 6.76 (br. s., 2 H), 6.92 (t, *J*=55.02, 1 H), 7.27 - 7.34 (m, 1 H), 7.45 - 7.53 (m, 3 H), 7.54 - 7.60 (m, 2 H), 7.66 (s, 1 H), 7.71 (dt, *J*=7.17, 1.62 Hz, 1 H), 7.85 (d, *J*=7.57 Hz, 1 H), 8.53 (d, *J*=1.89 Hz, 1 H), 8.59 (d, *J*=5.04 Hz, 1 H), 8.89 (t, *J*=1.89 Hz, 1 H), 9.05 (d, *J*=1.89 Hz, 1 H); MS (ES+) *m/z* 448 $[M+1]^+$.

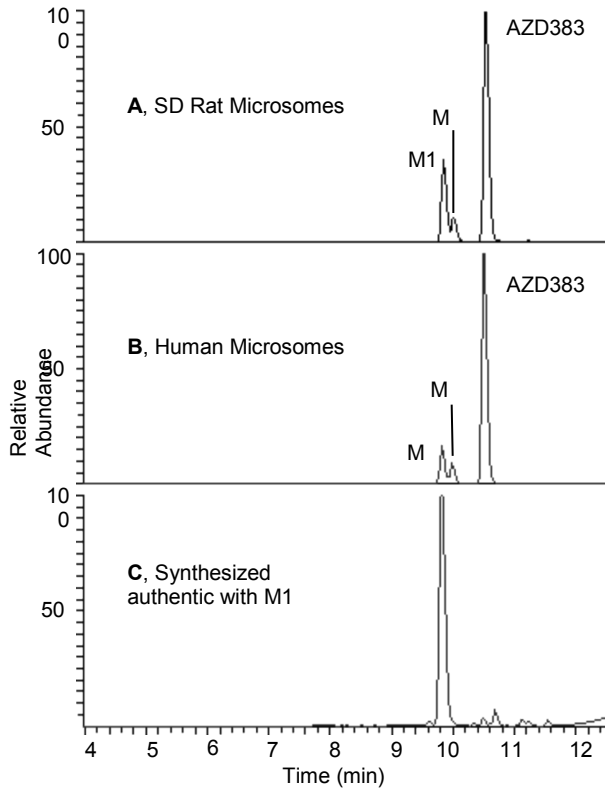


Figure 2. Metabolic profiling of AZD3839 in microsomes. A) Rat microsomes **B)** Human microsomes. **C)** Synthesized M1. LC-MS chromatograms are showing the MDF scan. Incubations were conducted at 1 µM substrate concentration for 30 min. Proposed structures of identified metabolites are depicted in Figure 8 and Table 2. Relative abundance values are presented for each individual panel.

Table 1. Summary of detected metabolites in excreta expressed as average fractions of the total administered dose of [^{3}H]-(S)-25 to male Sprague Dawley rats (30 µmol/kg, 50 MBq/kg).

	Fraction of dose (%)			
M#	Urine 0-24 h	Feces 0-48 h	Bile 0-6 h p.o.	i.v.
(S)-25	0.3			0.2
M1a	2		1.7	22
M2a	0.4			0.7
M4a			0.4	0
M5a			1.8	4.7
M6a			0.8	2.0
M7a			1.6	4.4
M8a			7.3	20
M9a	1.3		0.5	1.2
Total	4	94	14	55

Table 2. Summary of detected metabolites in excreta expressed as average fractions of the total administered dose of [^{14}C]-AZD3839 to male Han Wistar rats (58 µmol/kg, 9.25 MBq/kg).

	Fraction of dose (%)		
M#	Urine 0-24 h	Feces 0-48 h	Bile 0-48 h p.o.
AZD3839	0.35		
M1	2.2		
M2	3.4		4.0
M9	1.0		21
M11			4.8
M12			3.1
M17			2.6
M19			0.7
M23			3.1
M26			3.5
M31			1.5
M32			1.0
M34			1.7
M35			0.8
Total	6.9	91	49