Characterization of a Unique Co-Crystal of the BACE1 inhibitor Verubecestat and a reaction intermediate: Implications to the Development of a Commercial Manufacturing Process

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S1: Process Description and NMR Data for 1-pTSA and Verubecestat (1)



1-pTSA:

Stage 1: To reactor R1 was charged (*R*)-*N*-(3-(2-amino-1-(N-methylsulfamoyl)propan-2-yl)-4-fluorophenyl)-5-fluoropicolinamide (7, 100 g, 1.0 equivalent), 2-methyltetrahydrofuran (500 mL), water (50 mL), sodium bicarbonate (22.9 g, 1.05 equivalents) and cyanogen bromide (5 M in acetonitrile, 54.6 mL, 1.05 equivalents). The reaction mixture was heated to an internal temperature of 45 to 50 °C and aged for 24 hours. The batch was cooled to between 20 and 25 °C and then aqueous sodium thiosulfate (10% w/w, 250 mL, 0.6 equivalents) was charged. The mixture was aged for 30 minutes and then the aqueous layer discarded. The resulting organic layer was washed with aqueous sodium chloride (15% w/w, 250 mL).

Stage 2: To reactor R1 was charged aqueous sodium hydroxide (1 M, 260 mL), maintaining the internal temperature below 25 °C. The batch was aged for 3 hours and the organic and aqueous phases were allowed to split. The aqueous layer was discarded.

Stage 3: To reactor R1 was charged 2-methyltetrahydrofuran to dilute the product concentration to approximately 125 g/L. The contents of R1 were distilled at constant volume by adding 2-methyltetrahydrofuran until the measured KF was between 4000 and 8000 ppm, maintaining the internal temperature below 50 °C. The mixture was then heated to between 50 and 55 °C and seeded with **1-pTSA** (1 g). To reactor R2 was charged 2-methyltetrahydrofuran (200 mL) and *p*-toluenesulfonic acid monohydrate (54.4 g, 1.1 equivalents). Approximately 5% of the solution in R2 was transferred to R1 and the slurry aged for 1 hour. The remaining solution in R2 was transferred to R1 over 4 to 6 hours, and the crystallization aged for an additional hour at 50 to 55 °C. The batch was cooled to between 20 and 25 °C over 2 hours and aged for 1 hour. The solids were filtered, washed twice with 2-methyltetrahydrofuran (200 mL), and dried under vacuum at

40 to 50 °C. The typical purity of the isolated solids by HPLC area percent is greater than 99%, and the typical isolated yield is 90%.

¹H NMR (500 MHz, DMSO-*d*₆) δ 10.81 (s, 1H), 8.75 (s, 1H), 8.63 (s, 2H), 8.25 (dd, *J* = 8.7, 4.5 Hz, 1H), 8.09 – 7.89 (m, 3H), 7.49 (d, *J* = 7.8 Hz, 2H), 7.37 – 7.22 (m, 1H), 7.09 (d, *J* = 7.7 Hz, 2H), 4.54 (s, 2H), 3.24 (s, 3H), 2.24 (s, 3H), 1.84 (s, 3H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 162.20, 161.38 (d, *J* = 257.03 Hz), 155.69 (d, *J* = 243.0 Hz), 154.06, 146.94 (d, *J* = 3.6 Hz), 145.25, 138.56, 137.33 (d, *J* = 25.2 Hz), 135.36 (d, *J* = 2.2 Hz), 128.64, 128.49 (d, *J* = 11.6 Hz), 125.92, 125.39 (d, *J* = 15.5 Hz), 125.30 (d, *J* = 2.5 Hz), 122.76 (d, *J* = 8.7 Hz), 119.76 (d, *J* = 2.8 Hz), 117.24 (d, *J* = 24.2 Hz), 56.50 (d, *J* = 3.0 Hz), 54.60 (d, *J* = 5.1 Hz), 29.57, 27.66, 21.19; HRMS [M+H] (C₁₇H₁₈F₂N₅O₃S) calc. 410.1098, obs. 410.1104.

Verubecestat (1):

To reactor R1 was charged **1-pTSA** (100 g, 1.0 equivalents) and ethyl acetate (1000 mL). Aqueous potassium carbonate (1 M, 413 mL, 2.4 equivalents) was then charged and the mixture aged for 1 hour. The aqueous layer was discarded and additional aqueous potassium carbonate (1 M, 413 mL, 2.4 equivalents) was then charged and the mixture aged for 30 minutes. The aqueous layer was discarded and the organic layer washed with water (300 mL). The organic layer was concentrated to a target volume of 480 mL, maintaining the batch temperature below 35 °C. The contents of R1 were distilled at constant volume by adding ethyl acetate until the measured KF was less than 2000 ppm, maintaining the internal temperature below 35 °C. The batch was heated to between 68 and 72 °C to dissolve any remaining solids and then cooled to between 43 and 49 °C. The batch was seeded with **1** (3 g) and the resulting seed bed aged for 2 hours. To R1 was charged *n*-heptane (650 mL) over 10 hours, and then the crystallization cooled to between 15 and 25 °C over 2 hours. The solids were filtered, washed with a 1:2 mixture of ethyl acetate and *n*-heptane (300 mL) followed by *n*-heptane (300 mL), and then dried under vacuum at 35 °C.

¹H NMR (500 MHz, DMSO-d₆) δ 10.50 (s, 1H), 8.73 (d, J = 2.8 Hz, 1H), 8.23 (dd, J = 13.5 Hz, 17.6 Hz, 1H), 7.95-8.00 (m, 2H), 7.79 (ddd, J = 3.2, 4.0, 7.1 Hz, 1H), 5.97 (s, 2H), 3.76 (dd, J = 14, 18.5 Hz, 2H), 3.31(s, 1H), 3.10 (s, 3H), 1.61 (s, 3H); ¹³C NMR (500 MHz, DMSO-d₆) δ 161.82, 161.35, 159.77, 156.90, 154.98, 146.62, 146.59, 146.51, 136.88, 136.68, 134.03, 133.83, 133.73, 124.85, 124.70, 124.61, 124.57, 120.85, 115.94, 115.74, 56.54, 54.16, 29.49, 29.28; HRMS [M+H] (C₁₇H₁₇F₂N₅O₃S) calc. 410.1098, obs. 410.1105.





S2. Crystal Data and Structure Refinement for Compound 1-HBr (CCDC 1816468)

A single crystal grown from acetonitrile and isopropylacetate by slow cooling was selected for single crystal X-ray data analysis. The crystal was a small colorless block with dimensions of 0.10 mm x 0.08 mm x 0.08mm. Data collection was performed on a Bruker Apex II system at 100K. The unit cell was determined to be monoclinic in space group P2₁ and the structure contained one molecule of the protonated organic moiety and one bromide anion in the asymmetric unit. Crystallographic data is summarized in Table S2.1. Absolute configuration was determined by resonant scattering effects in diffraction measurements on the crystal and confirmed that the stereochemistry at stereogenic centre was *R*. Figure S2.1 shows a thermal ellipsoid representation of Compound **1-HBr** with thermal ellipsoids set at the 50% probability level. Coordinates, refinement details and structure factors have been deposited with the Cambridge Crystallographic Data Centre (CCDC 1816468).

Figure S2.1: Thermal ellipsoid representation of Compound **1-HBr** with thermal ellipsoids set at the 50% probability level.



Identification code	mdk070	
Empirical formula	C17 H18 Br F2 N5 O3 S	
Formula weight	490.33	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	P2 ₁	
Unit cell dimensions	a = 6.5900(3) Å	α= 90°.
	b = 21.4362(9) Å	β=104.0671(13)°.
	c = 6.8762(3) Å	$\gamma = 90^{\circ}$.
Volume	942.23(7) Å ³	
Z	2	
Density (calculated)	1.728 g/cm ³	
Absorption coefficient	4.475 mm ⁻¹	
F(000)	496	
Crystal size	0.10 x 0.08 x 0.08 mm ³	
Theta range for data collection	4.125 to 66.590°.	
Index ranges	-7<=h<=7, -25<=k<=25, -6<=l	<=8
Reflections collected	11766	
Independent reflections	3291 [R(int) = 0.0307]	
Completeness to theta = 66.590°	99.8 %	
Absorption correction	Semi-empirical from equivalent	its
Max. and min. transmission	0.699 and 0.638	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	3291 / 1 / 276	
Goodness-of-fit on F ²	1.020	
Final R indices [I>2sigma(I)]	R1 = 0.0205, wR2 = 0.0551	
R indices (all data)	R1 = 0.0206, wR2 = 0.0552	
Absolute structure parameter	-0.018(7)	
Largest diff. peak and hole	0.381 and -0.215 e.Å ⁻³	

Table S2.1. Crystal Data and Structure Refinement for 1-HBr (CCDC 1816468)

S3. Crystal Data and Structure Refinement for Compound 2-HBr Acetonitrile Solvate (CCDC 1816469)

A single crystal grown from acetonitrile by slow cooling was selected for single crystal X-ray data analysis. The crystal was a small colorless block with dimensions of 0.03 mm x 0.03 mm x 0.15 mm. Data collection was performed on a Bruker Apex II system at 100K. The unit cell was determined to be triclinic in space group P1 and the structure contained four molecules of the protonated organic moiety, four bromide anions and four acetonitrile solvent molecules in the asymmetric unit. Crystallographic data is summarized in Table S3.1. Absolute configuration was determined by resonant scattering effects in diffraction measurements on the crystal and confirmed that the stereochemistry at stereogenic centre was *R*. Figure S3.1a shows a thermal ellipsoid representation of Compound **2-HBr** acetonitrile solvate with thermal ellipsoids set at the 50% probability level. Figure S3.1b shows a portion of the **2-HBr** acetonitrile solvate asymmetric unit contents. Coordinates, refinement details and structure factors have been deposited with the Cambridge Crystallographic Data Centre (CCDC 1816469).

Figure S3.1: Thermal ellipsoid representation of Compound **2-HBr** acetonitrile Solvate with thermal ellipsoids set at the 50% probability level.



Table S3.1 Crystal Data and Structure Refinement Compound for Compound 2-HBr Acetonitrile Solvate (CCDC 1816469)

Identification code	md1051		
Empirical formula	C18 H22 Br F2 N5 O3 S		
Formula weight	506.37		
Temperature	100(2) K		
Wavelength	1.54178 Å		
Crystal system	Triclinic		
Space group	P1		
Unit cell dimensions	a = 12.6507(16) Å	α= 118.192(4)°.	
	b = 13.7970(18) Å	$\beta = 96.596(4)^{\circ}$.	
	c = 14.0331(17) Å	$\gamma = 91.858(5)^{\circ}$.	
Volume	2134.0(5) Å ³		
Z	4		
Density (calculated)	1.576 g/cm ³		
Absorption coefficient	3.969 mm ⁻¹		
F(000)	1032		
Crystal size	0.15 x 0.03 x 0.03 mm ³		
Theta range for data collection	3.534 to 68.931°.		
Index ranges	-15<=h<=15, -16<=k<=1	16, -16<=l<=16	
Reflections collected	54946		
Independent reflections	15125 [R(int) = 0.0543]		
Completeness to theta = 68.250°	99.4 %		
Absorption correction	Semi-empirical from equ	ivalents	
Max. and min. transmission	0.889 and 0.717	0.889 and 0.717	
Refinement method	Full-matrix least-squares	on F ²	
Data / restraints / parameters	15125 / 3 / 1097		
Goodness-of-fit on F ²	1.029		
Final R indices [I>2sigma(I)]	R1 = 0.0490, wR2 = 0.14	405	
R indices (all data)	R1 = 0.0502, wR2 = 0.14	R1 = 0.0502, $wR2 = 0.1426$	
Absolute structure parameter	0.008(11)	0.008(11)	
Largest diff. peak and hole	1.548 and -0.570 e.Å ⁻³	1.548 and -0.570 e.Å ⁻³	

S4. Crystal Data and Structure Refinement for Cocrystal 1.HBr:2.HBr (CCDC 1816470)

A single crystal grown from acetonitrile by solvent evaporation was selected for single crystal X-ray data analysis. The crystal was a small colorless block with dimensions of 0.10 mm x 0.10 mm x 0.12 mm. Data collection was performed on a Bruker Apex II system at 100K. The unit cell was determined to be monoclinic in space group P2₁ and contained one molecule of pronated **1**, one molecule of pronated **2** and two bromide anions in the asymmetric unit. Crystallographic data is summarized in Table S4.1. Absolute configuration was determined by resonant scattering effects in diffraction measurements on the crystal and confirmed that the stereochemistry at stereogenic centres in both **1** and **2** was *R*. Figure S4.1 shows a thermal ellipsoid representation of cocrystal **1**.HBr:**2**.HBr with thermal ellipsoids set at the 50% probability level. Coordinates, refinement details and structure factors have been deposited with the Cambridge Crystallographic Data Centre (CCDC 1816470).

Figure S4.1: Thermal ellipsoid representation of Cocrystal **1.**HBr**:2.**HBr with thermal ellipsoids set at the 50% probability level.



Identification code	md1045	
Empirical formula	C33 H37 Br2 F4 N9 O6 S2	
Formula weight	955.65	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	P2 ₁	
Unit cell dimensions	a = 6.9896(7) Å	α= 90°.
	b = 22.398(2) Å	β=94.093(3)°.
	c = 12.1743(12) Å	$\gamma = 90^{\circ}$.
Volume	1901.1(3) Å ³	
Z	2	
Density (calculated)	1.669 g/cm ³	
Absorption coefficient	4.408 mm ⁻¹	
F(000)	968	
Crystal size	0.12 x 0.10 x 0.10 mm ³	
Theta range for data collection	3.640 to 68.325°.	
Index ranges	-7<=h<=8, -26<=k<=26, -14<=	=1<=14
Reflections collected	25823	
Independent reflections	6904 [R(int) = 0.0319]	
Completeness to theta = 68.250°	99.7 %	
Absorption correction	Semi-empirical from equivaler	nts
Max. and min. transmission	0.644 and 0.526	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	6904 / 1 / 528	
Goodness-of-fit on F ²	1.022	
Final R indices [I>2sigma(I)]	R1 = 0.0187, wR2 = 0.0466	
R indices (all data)	R1 = 0.0189, w $R2 = 0.0468$	
Absolute structure parameter	-0.014(5)	
Largest diff. peak and hole	0.174 and -0.324 e.Å ⁻³	

Table S4.1. Crystal Data and Structure Refinement for Cocrystal 1.HBr:2.HBr (CCDC 1816470)

S.6: Description of Analytical Methods

S.6.1 HPLC Method

Instrument	Agilent 1100 HPLC systems	s or equivale	ent HPLC systems
Column	Agilent Poroshell 120 EC-C18 2.7um 150x4.6mm or		
	equivalent		
Column Temperature	40 °C		
Flow Rate	1.2 mL/min		
Injection Volume	5 μL		
Detector	UV at 220 nm		
Run Time	13 min, 5 min post time		
Mobile Phase A	0.1 % (v/v) perchloric acid i	n water	
Mobile Phase B	Acetonitrile		
Gradient table:			
	Time (min)	A%	В%
	0.0	90	10
	6	25	75
	10	10	90
	13	10	90
	13.1	90	10

S.6.2: Heavy Metals and Residue on Ignition by Atomic Spectroscopy

A. <u>Reagents</u>

- Concentrated nitric acid (70%, trace metal grade or better).
- NIST traceable (or equivalent) single and/or mixed-element stock standard solutions containing 69 elements
- Deionized water [Milli-Q water system (or equivalent), Millipore Corporation, Bedford, MA]
- B. <u>Solutions</u>

<u>80% nitric acid</u> - Dilute 80 mL concentrated nitric acid to 100 mL with deionized water. Mix well.

C. <u>Operating Conditions</u>

The Perkin Elmer Elan 6000 Inductively Coupled Plasma Mass Spectrometer (or equivalent ICP-MS spectrometer at optimized conditions) was used and the operating conditions are given below:

RF Power:	1300 W
Coolant argon flow	15.0 L/min
Auxiliary argon flow:	1 L/min
Nebulizer argon flow:	0.80- 1.06 L/min
Sample introduction system:	Cross flow nebulizer with Scott spray
	chamber or equivalent
Operating frequency:	40 MHz
Sample uptake rate:	1.5 mL/min
Detector mode:	Dual mode
Sampler/Skimmer cones:	Platinum
Scanning mode:	Peak hopping
Number of points per peak:	1
Dwell time:	15 ms
Sweeps per reading:	40
Number of replicate:	2

The Perkin Elmer Optima 3300 DV ICP-AES Spectrometer (or equivalent ICP-AES spectrometer at optimized conditions) was used and the operating conditions are given below:

RF Power	1350 Watts
Coolant argon flow	15.0 L/min
Auxiliary gas Flow:	0.5 L/min
Nebulizer gas Flow:	0.8 L/min
Sample introduction system:	Cross flow nebulizer with Scott spray
	chamber or equivalent
Operating frequency:	40 MHz
Resolution:	Normal
Replicates:	3
Plasma View:	Axial
Sample Flow Rate:	1.5 mL/min
Sample Flush Rate:	3.0 mL/min
Read Delay Time (sec):	30

S.6.3: XRPD Method:

The xray samples were prepared on zero background silicon holders and analyzed using Cu Kalpha radiation between 2° and 40° 2-theta.

S.6.4: DSC method

The DSC was run using approximately 5mg of sample in a covered Al pan at a heating rate of $10^{\circ}/\text{min}$.

Below are the DSC data for 1-HBr, The Unknown Solids, second production batch and 2-HBr

