Transformation of the Manufacturing Process from Discovery to Kilogram Scale for AWZ1066S, A Highly Specific Anti-*Wolbachia* Drug Candidate for a Short-Course Treatment of Filariasis

W. David Hong[†], Paul M. O'Neill[†], Mark J. Taylor[‡], Joseph D. Turner[‡], Stephen A. Ward[‡], Fabian Gusovsky[§], Farid Benayoud[∥], Nao Shibuguchi[⊥], Dixit Girish[#], Francis Gerard Fang[∥], Ravi Kumar Talabhakthla[#], Anand Vaddi[#], Chiranjeevi Challa[#], Karri Satya Ammi Reddy[#], Vijay Kalla[#], Sugandham Srinivasa Rao[#], Durga Mahesh Kumar Nagireddi[#], Jayesh Patel[#], Anil Shahaji Khile[#*]*

[†] Department of Chemistry, University of Liverpool, L69 7ZD Liverpool, United Kingdom

[‡] Research Centre for Drugs and Diagnostics, Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, L3 5QA Liverpool, United Kingdom;

[§] G2D2 Eisai Inc. 35 Cambridgepark Drive, Cambridge MA 02140, USA;

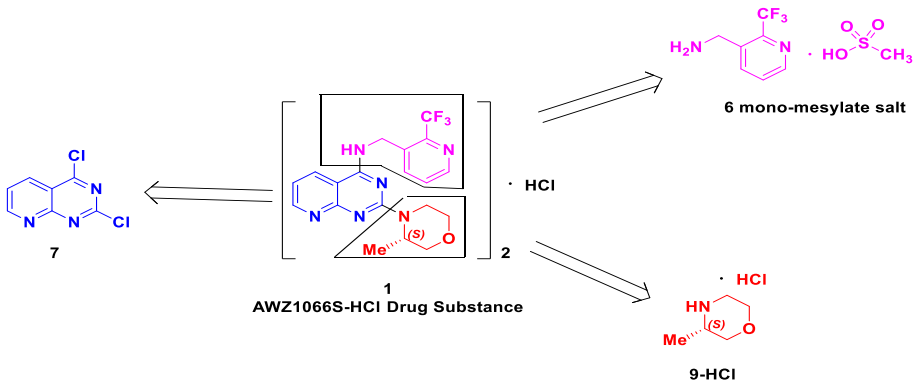
[∥] Eisai AiM Institute, Eisai, Inc., Andover, MA 01810;

[⊥] Analytical Research, Pharmaceutical Science & Technology, CFU, Medicine Development Center, Eisai Co. Ltd., 5-1-3-Tokodai, Tsukuba-shi, Ibaraki 300-2635, Japan;

[#] Eisai Pharmaceuticals India Pvt.Ltd. Ramky Pharma City, (SEZ), Plot Nos.96, 97, 98,124 &126, Parawada – 531019, Visakhapatnam, Andhra Pradesh, India;

*Email: a-khile@eisai.co.in

*Email: j2-patel@eisai.co.in



ABSTRACT.

Anti-*Wolbachia* therapy has been clinically proven to be a safe approach for the treatment for onchocerciasis and lymphatic filariasis. AWZ1066S, a first-in-class highly specific anti-*Wolbachia* drug candidate developed for a short course treatment of human filariasis, has advanced into clinical development. An improved, cost efficient and scalable process for the manufacture of this clinical candidate is described. Presented herein is the process development work for the active pharmaceutical ingredient (API) and its two key starting materials [2-(trifluoromethyl)-3-pyridyl]methanamine and (*S*)-3-methylmorpholine, starting from 2,4-dichloropyrido[2,3-d]pyrimidine, which is capable of delivering high purity (>99%) API consistently. The optimized

production route was used in the manufacture of the clinical candidate at kilogram scale to support ongoing clinical development.

KEYWORDS: Anti-*Wolbachia*, filariasis, AWZ1066S, trifluoromethyl pyridine derivative, chiral morpholine analogue.

INTRODUCTION.

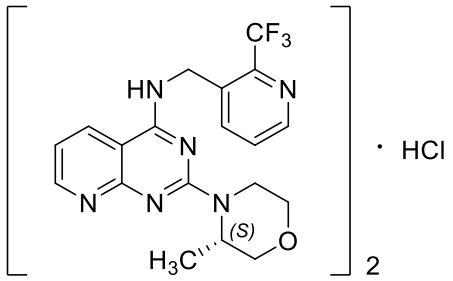
Lymphatic filariasis (LF, the cause of elephantiasis) and onchocerciasis (the cause of river blindness) are leading causes of global morbidity affecting 86 million people.[1] Current treatments principally target the microfilarial (larval) stage of the parasites, which demands a prolonged annual/biannual treatment timeframe over several years. This prolonged treatment delivery period introduces compliance and logistical challenges, which impact on achieving the elimination goals. Furthermore, current anti-filarial drugs such as diethylcarbamazine (DEC) and ivermectin (IVM) cannot be used in specific geographic regions where co-infections with *Loa loa* (*L. loa*) occur, as the rapid killing of *L. loa* microfilaria can cause severe life-threatening adverse events.[2] It is the growing evidence of resistance to IVM[3-4] and safety constraints in areas co-endemic with *L. loa* that has re-focused efforts to develop new macrofilaricidal drugs and drug regimens to accelerate elimination goals within existing timeframes. The WHO roadmap for NTDs has identified the development of a safe macrofilaricide as a critical priority to achieve the elimination goals.[5]

A validated approach for delivering macrofilaricidal activity is to target the *Wolbachia* bacterial endosymbiont present in the causative nematode parasites. This approach is supported by multiple clinical trials conducted over the past 15 years.[6-8] These bacteria are essential for multiple components of the nematodes biological processes including larval growth, development, embryogenesis and ultimately survival of the adult worm, making *Wolbachia* a valuable

chemotherapeutic target. Removal of *Wolbachia* results in permanent sterilization and the protracted death of the adult worm over 18-24 months, delivering an excellent safety profile. Furthermore, by targeting *Wolbachia*, off target concerns, such as co-infections with *L. loa* which do not carry *Wolbachia,* are not affected, thus eliminating the risk of severe adverse events in relation to *L. loa* co-infection.[9]

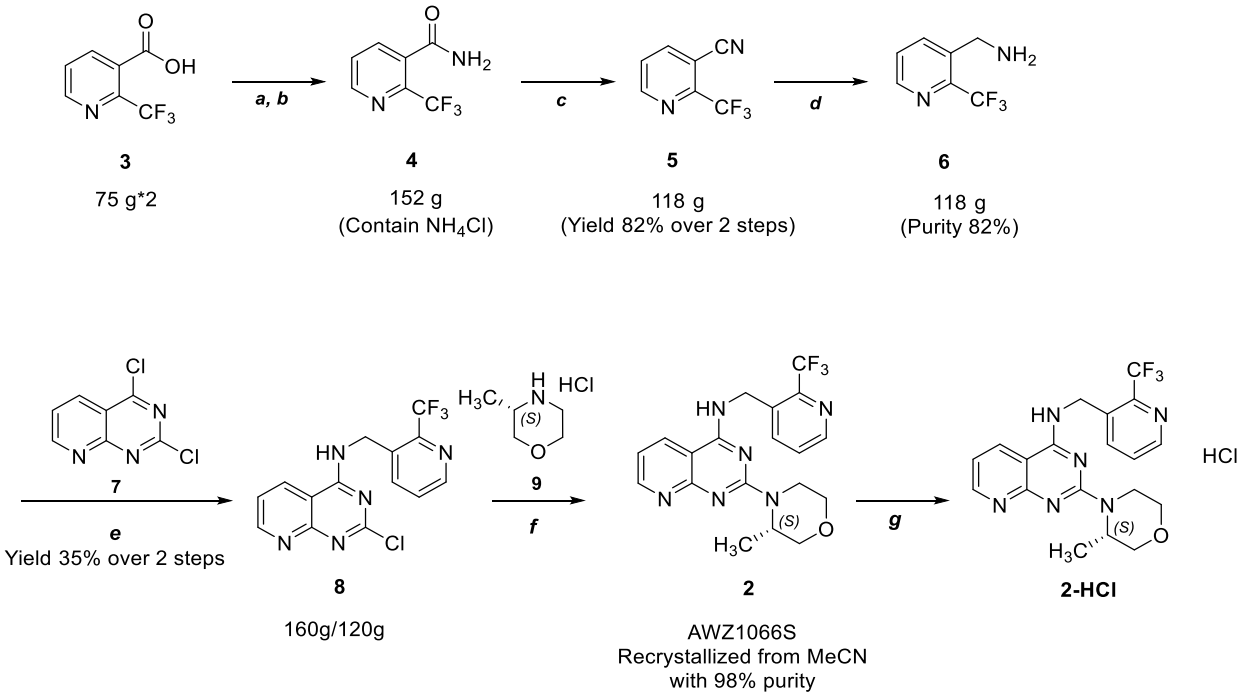
Anti-*Wolbachia* therapy delivers safe macrofilaricidal activity with superior therapeutic outcomes compared to all standard anti-filarial treatments, with the added benefit of substantial improvements in clinical pathology. These outcomes can be achieved in principle with existing registered drugs, e.g., doxycycline (DOX), however, these drugs have well known limiting safety profiles restricting their scale-up in target populations. Through the anti-*Wolbachia* (A·WOL) consortium, led by an industrial-academic partnership between Eisai, University of Liverpool and Liverpool School of Tropical Medicine, AWZ1066S (**Figure 1)** has been selected as a *Wolbachia* targeting macrofilaricide molecule capable of delivering a short oral treatment (≤ 7days) in multiple preclinical animal models. This is a first-in-class clinical candidate specifically designed to target *Wolbachia*.[10-11]

At the late-lead optimization stage, a five-step synthetic route (**Scheme 1**) was established to provide around 100 g (per batch) scale of the final product in a research synthetic laboratory setting.[10] Even though this route provided required quantities of API for pre-clinical studies, the route of synthesis involved the usage of undesirable reagents i.e. oxalyl chloride, trifluoroacetic anhydride, Raney nickel, and workup/purification conditions, i.e. trituration, that were not suitable for further scale-up production process. To overcome these challenges a phase-appropriate fit-for-purpose process that would allow expeditious scale-up to multi-kilograms of **1** was developed and is reported here.



1
AWZ1066S-HCl Drug Substance

Figure 1: Sturcture of AWZ1066S-HCl Drug Substance (**1**)



Scheme 1. Laboratory Synthetic Route for Anti-*Wolbachia* Clinical Candidate (**2-HCl**)

Reagents and conditions: (a) Oxalyl chloride; $(COCl)_2$, DMF, DCM, 0-15 °C (b) Ammonia, THF/DCM, 0 °C (c) Trifluoroacetic anhydride (TFAA), triethyl amine (TEA), DCM, 0-30 °C (d) H_2, Raney Ni, MeOH, NH_3-H2O (5/1), 20-30 °C (e) TEA, THF, 20-30 °C (f) *N*,*N*-Diisopropylethylamine (DIEA), DMSO, 90 °C , 12 hours (g) Acetone, 4N HCl in 1,4-dioxane.

RESULT AND DISCUSSIONS.

The original five-step synthesis[10] involved synthesis of [2-(trifluoromethyl)-3-pyridyl]methanamine **6**, starting from commercially available 2-(trifluoromethyl)nicotinic acid **3** via two steps. Further 2,4-dichloropyrido[2,3-d]pyrimidine **7** reacts with **6** via a regioselective S_NAr reaction using triethylamine as a base in tetrahydrofuran (THF)**,** to provide intermediate **8**. Intermediate **8** was further coupled with (*S*)-3-methylmorpholine **9** in presence of Hünig's base in dimethyl sulfoxide at elevated temperature to provide the free form **2**. Finally, the free form **2** was treated with 4 N hydrochloric acid in 1,4-dioxane and acetone to produce the mono hydrochloride salt, **2-HCl**.

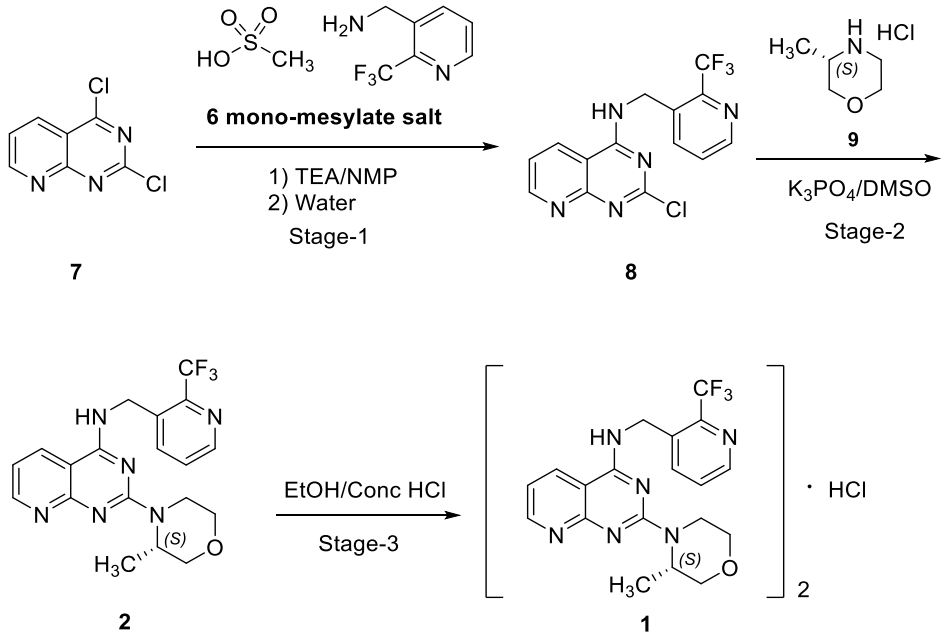
As part of an NCE (New Chemical Entity) process development program, an efficient, scalable, safe and cost effective route from gram scale to multi-kilogram quantities of **1** was required for consistent supply and manufacturing of clinical trial material (CTM). To achieve this, development studies were performed to improve the yield and quality of each stage (**Section A**) and to maintain overall low costs of the API. Key starting materials (**6** & **9**) processes were developed in-house and were transferred to CMOs to prepare multi-kilogram scale material within a cGMP environment (**Section B** & **C**).

Furthermore, the hemi-hydrochloride salt of AWZ1066S was selected over the mono hydrochloride salt of AWZ1066S based on the physicochemical assessments and stability of drug product.

Section A). *Development of API, AWZ1066S-HCl **(1)**.*

The second generation process consists of three stages (**Scheme 2**) to synthesize the API **1** using commercially available starting material, 2,4-dichloropyrido[2,3-d]pyrimidine **7**. The first two stages consist of two sequential S_NAr reactions to substitute the two chlorides with corresponding

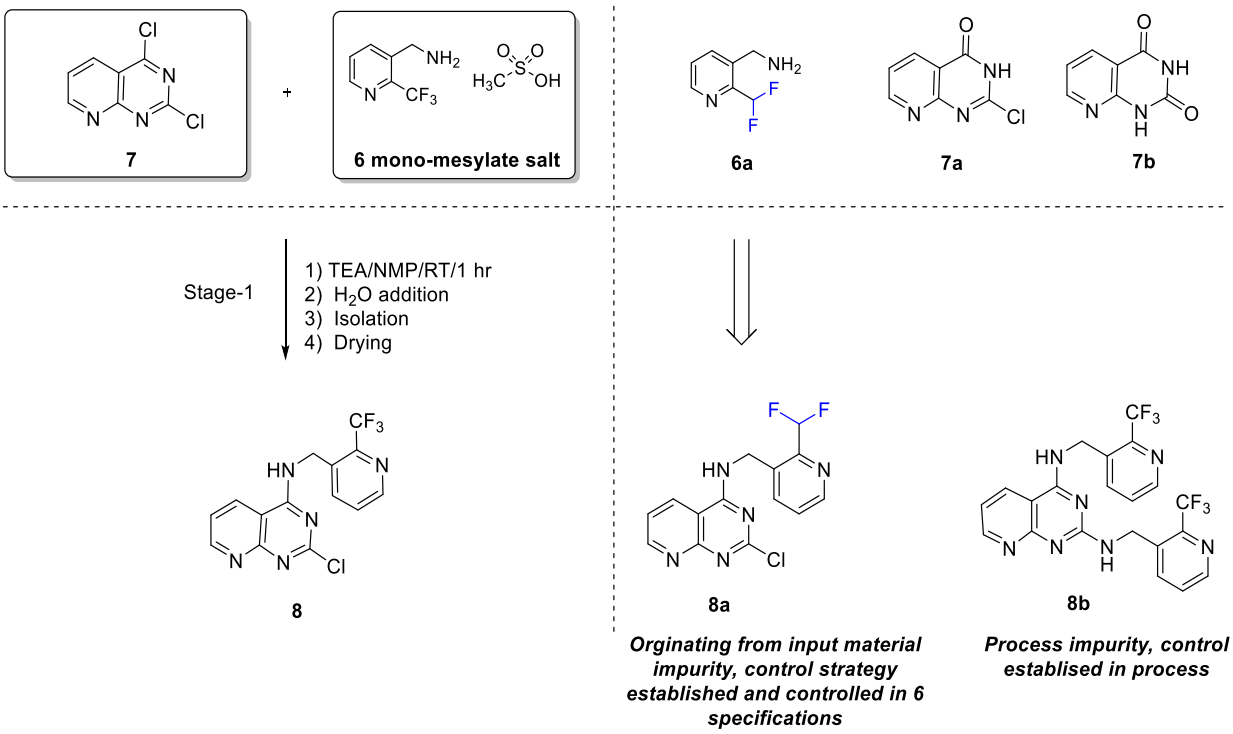
amine side-chains selectively, then follows by the final stage of converting the free form **2** to a hemi-hydrochloride salt, **1**.



Scheme 2. Evolution of AWZ1066S Synthesis

Process optimization of Stage 1 ***(8)***. The process was optimized through a combination of using triethylamine as a base in *N*-methyl-2-pyrrolidone (NMP), providing a good conversion of over 85% as compared to the original process. Due to the high product content, isolation of **8** was simplified by adding water as an anti-solvent without any further distillation or additional trituration, which resulted in improved yields (>85%) with high quality (>99 HPLC area %) product. The individual by-products/impurities observed in **8** were identified and the controls were established. The most commonly observed by-products are des-fluoro impurity **8a** and di-substituted impurity **8b**. By-product **8a** is generated from the reaction of impurity **6a** from the input CF_3 pyridine analogue **6**, therefore controls were established in the specifications of input material **6**. By-product **8b** is generating due to over reaction of **8** with unreacted input material **6**,

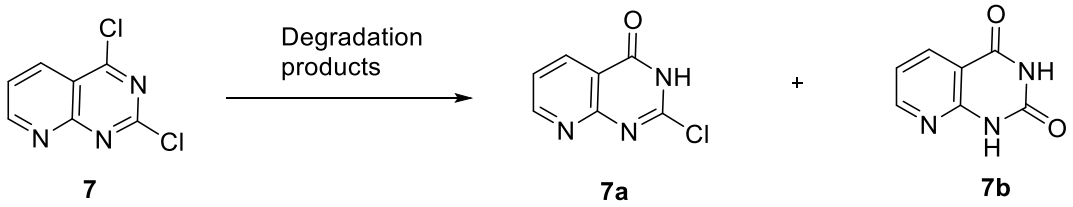
therefore controls were established by using stoichiometric amount of **6** against **7** after purity correction. The by-products/impurities and fate of impurities are elucidated in **Scheme 3.**



Scheme 3. Fate of Stage-1 impurities

During the pre-GMP execution, the stability of 2,4-dichloropyrido[2,3-d]pyrimidine **7** was one of the major concerns, as it tends to degrade through hydrolysis to 2-chloropyrido[2,3-*d*]pyrimidin-4(1*H*)-one **7a** and pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **7b**. This degradation was directly impacting the yield of Stage-1. Hence, 5 weeks' stability studies established for **7** at -15°C, 0-8°C & 20-30°C and found **7** is stable at -15°C (**Table 1**). While executing 15.0 kg GMP batch synthesis in the pilot plant, as per purity of **7**, 1.05 eq. of **6** was used to produce 22.1 kg of **8** with 86.76 % yield and 99.29 HPLC area %, while the formation of **8b** was restricted to 0.21 HPLC area %.

Table 1. Stability study data of **7**.

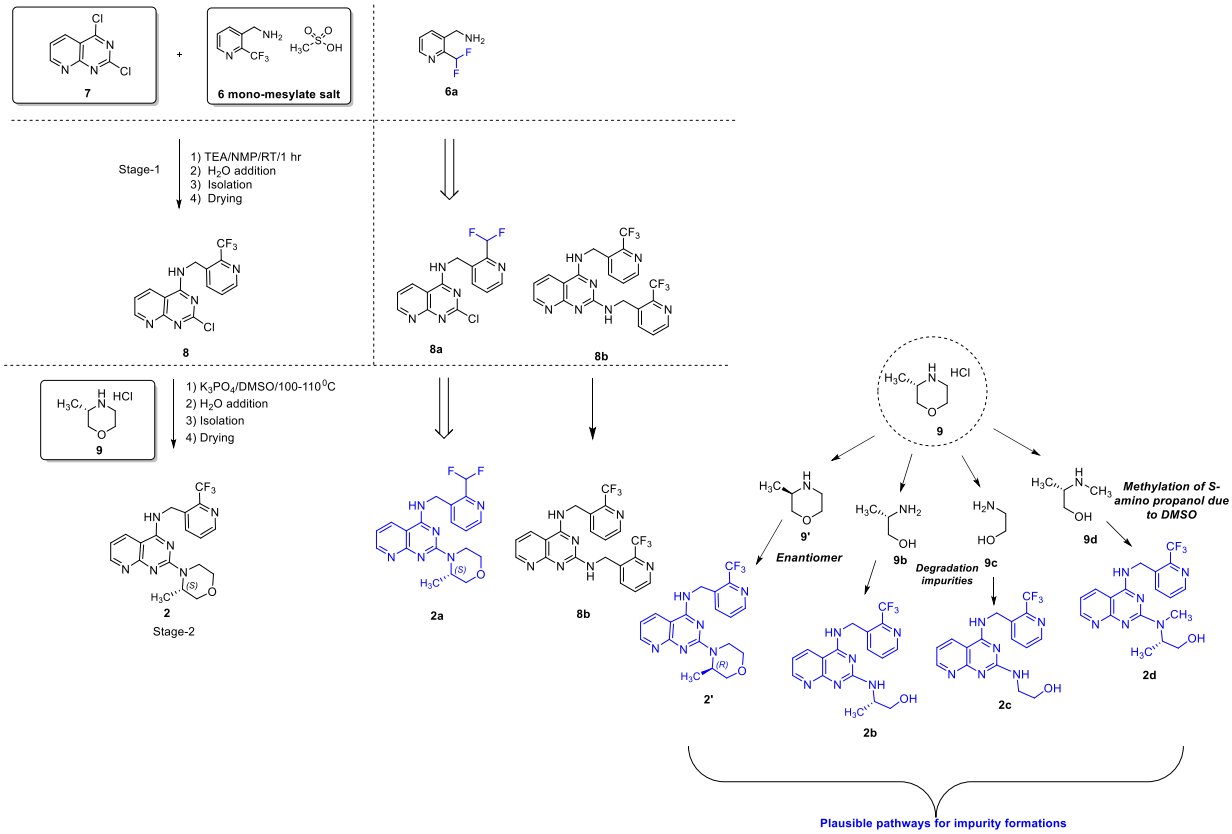


7 **7a** **7b**

Storage conditions	Duration (Weeks)	HPLC purity, area%		
		7a	**7b**	**7**
	0 (Initial assessments)	0.148	0.740	98.893
25-30 °C	2	0.690	1.134	97.884
	3	3.888	6.942	86.790
	5	7.230	12.310	78.887
2-8 °C	2	1.800	2.928	94.193
	3	2.311	3.662	92.720
	5	3.730	5.896	89.229
< -15 °C	2	1.727	2.961	94.229
	3	2.753	4.038	91.689
	5	2.080	3.299	93.207

Process Optimization of Stage 2 ***(2)****:* Several detailed conditions, such as different combinations of solvents (DMSO, NMP, DMAc, ACN), organic and inorganic bases (DIEPA, DBU, K_2CO_3, Na_2CO_3, $NaHCO_3$, K_3PO_4) and other reaction parameters, i.e. time and temperature to obtain **2** were explored. Amongst those conditions the best yield and purity for this reaction was obtained using tripotassium phosphate as base in DMSO. Using this combination, we consistently observed good conversion with over 95 HPLC area % and >85% yield which was superior compared to

earlier published. The individual by-products observed in **2** were identified and the controls were established. Impurity **8b** was carried forward from **8.** Impurities **2′, 2b**, **2c** and **2d** were impurities from (*S*)-3-methylmorpholine **9**. The by-products/impurities and fate of impurities are elucidated in **Scheme 4**.



Scheme 4. Fate of Stage-2 impurities

A process safety analysis was conducted for Stage-2, as there have been hazards reported with the decomposition of dimethyl sulfoxide (DMSO) in presence of bases.[12-13] The MTSR (maximum temperature of the synthesis reaction) was calculated for the reaction performed at 107 ºC using the heat of reaction measured with the reaction calorimeter (RC1e). The measured heat of reaction was -35.95 J/g of Step-1 (**8**) resulting an adiabatic temperature rise (Δt_{ad}) of 2.523 ºC. Thus, the calculated MTSR, which is the sum of process temperature and $\Delta t_{ad,}$ is 109.5 ºC. Through a series of experiments (please see additional information about the determination of T_{D24} in the

Supplementary Information), the T_{D24} for DMSO decomposition under Stage-2 conditions was determined as 133 ºC. The T_{D24} for the decomposition is 26 ºC above the reaction temperature and the Δt_{ad} of 2.5 ºC is not significant, so the process falls in the low criticality class 2.[14] (**Figure 2**) However, the effects of decomposition are found to be severe and could result in a major process safety incident. Thus, control measures were implemented to run the process safely on kilogram scale. The process temperature was limited to a maximum of 107 ºC keeping a cushion of 36 ºC to the onset temperature of decomposition (143 ºC). The reactor jacket is connected to a single fluid heat transfer system with the jacket temperature set to 110 ºC and the possibility of process temperature going beyond 110 ºC is eliminated.

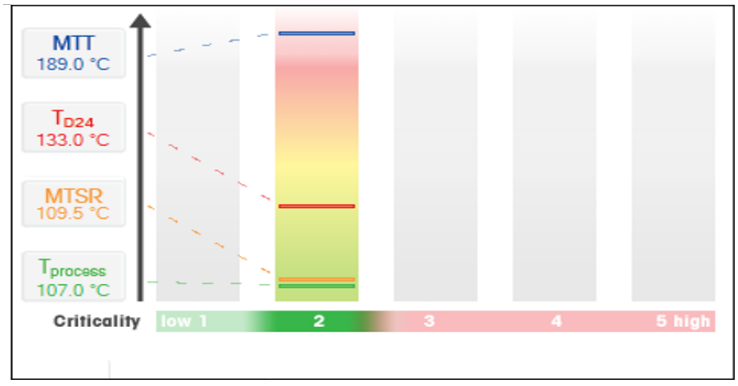


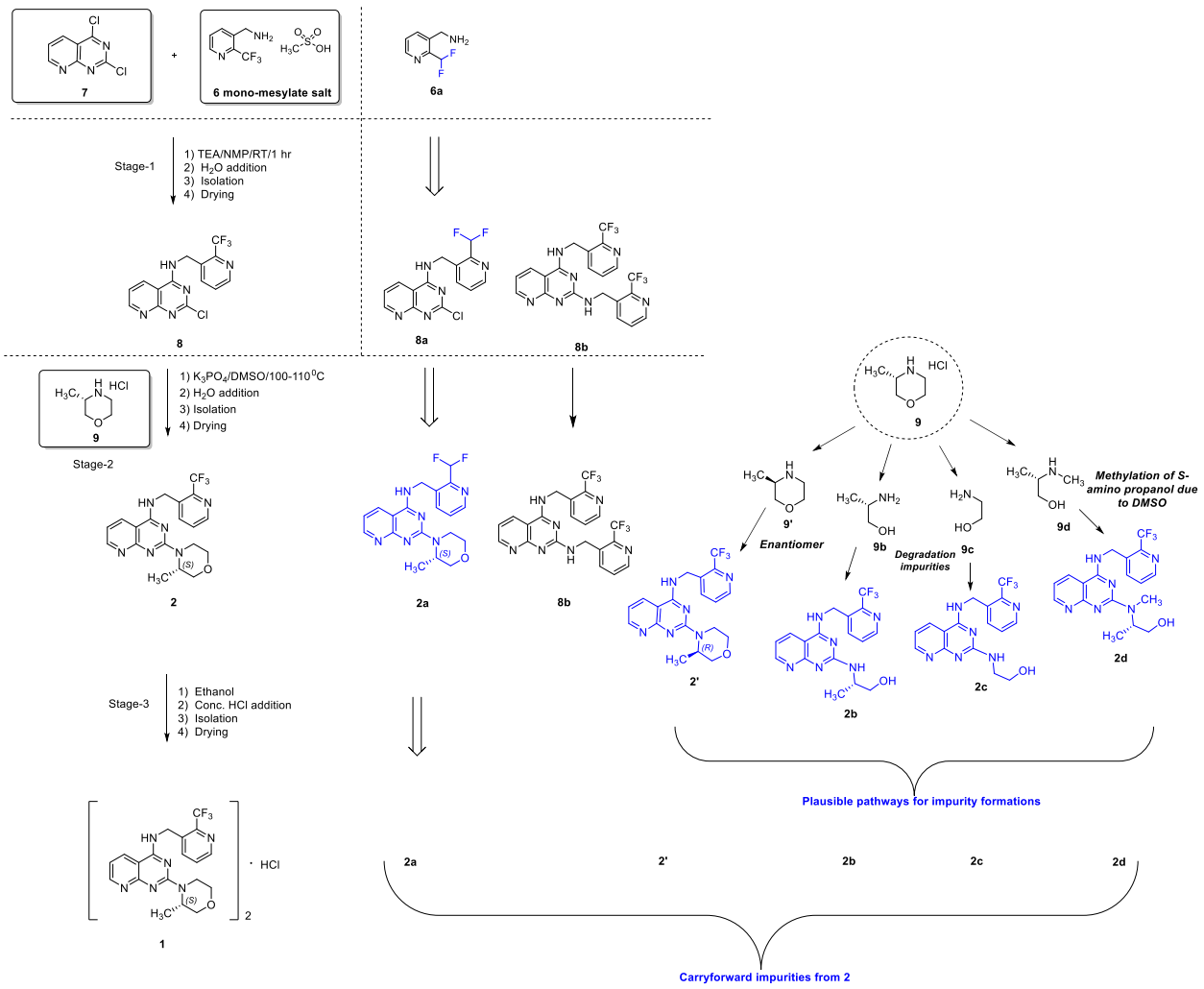
Figure 2. Criticality classification of Stage-2 reaction

Process Optimization of AWZ1066S-HCl ***(1)****, Stage-3:* For AWZ1066S API**,** a mono-hydrochloride salt **(2-HCl)** was used in the late discovery phase, however based on the physicochemical assessment and stability of drug product hemi-hydrochloride salt, API **(1)** was selected for clinical use. Thus, for **1**, a new process for hemi-hydrochloride salt formation was required.

A number of solvents (acetone, isopropyl alcohol, methanol, and ethanol), hydrochloric acid source (hydrochloric acid solution in isopropyl alcohol and aqueous hydrochloric acid) were explored to optimize Stage 3 process. Among these conditions, using exact quantification (0.5

mole equivalent) of aqueous hydrochloric acid and ethanol as solvent affords good yield (>85%) and quality (purity >99 HPLC area %) with enhanced impurity control. Main carryforward impurities from **2** were observed in **1** and were identified, prepared and characterized. (**Scheme 5**)

The optimized process was implemented and produced required quantities of **1,** at an overall yield of 71%, for the toxicology batch and pre-GMP studies. The same process executed under GMP facility in the pilot plant accomplished delivery of 10.4 Kg of **1** with 99.84% HPLC area % and 49.99% of an overall yield from **7**. The final 3 steps in the lab based synthetic route have an overall yield of 66% for the mono-hydrochloride salt (**2-HCl**), however, these two processes are not directly comparable as the scale and the final product (HCl vs. hemi-HCl salts) are different in these two routes.



Scheme 5. Fate of Stage-3 impurities

Crystal structure of **1** showed one chloride atom (Cl59) and two free forms (**2**) in the asymmetric unit (**Figure 3**), indicating stoichiometry of hydrochloride and **2** in **1** is 1:2 (hemi-hydrochloride salt). The calculated x-ray diffraction pattern for crystal structure was similar to those of synthesized crystalline powder of **1**. This observation confirmed manufactured crystalline **1** is the desired hemi-hydrocholride salt. In addition, absolute configuration of the chiral center was determined to be *S*-configuration based on Flack parameter analysis (0.007(15)).

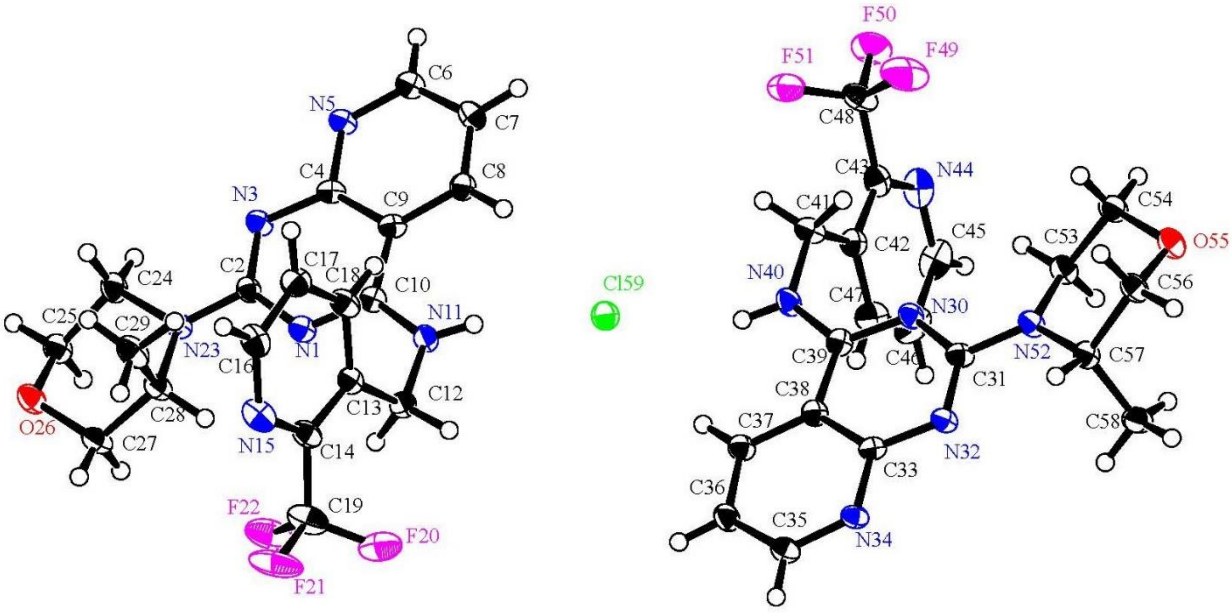


Figure 3: ORTEP representation of the asymmetric unit of **1** with thermal ellipsoids at the 50% probability level

Section B). *Development of key input material* ***6.***

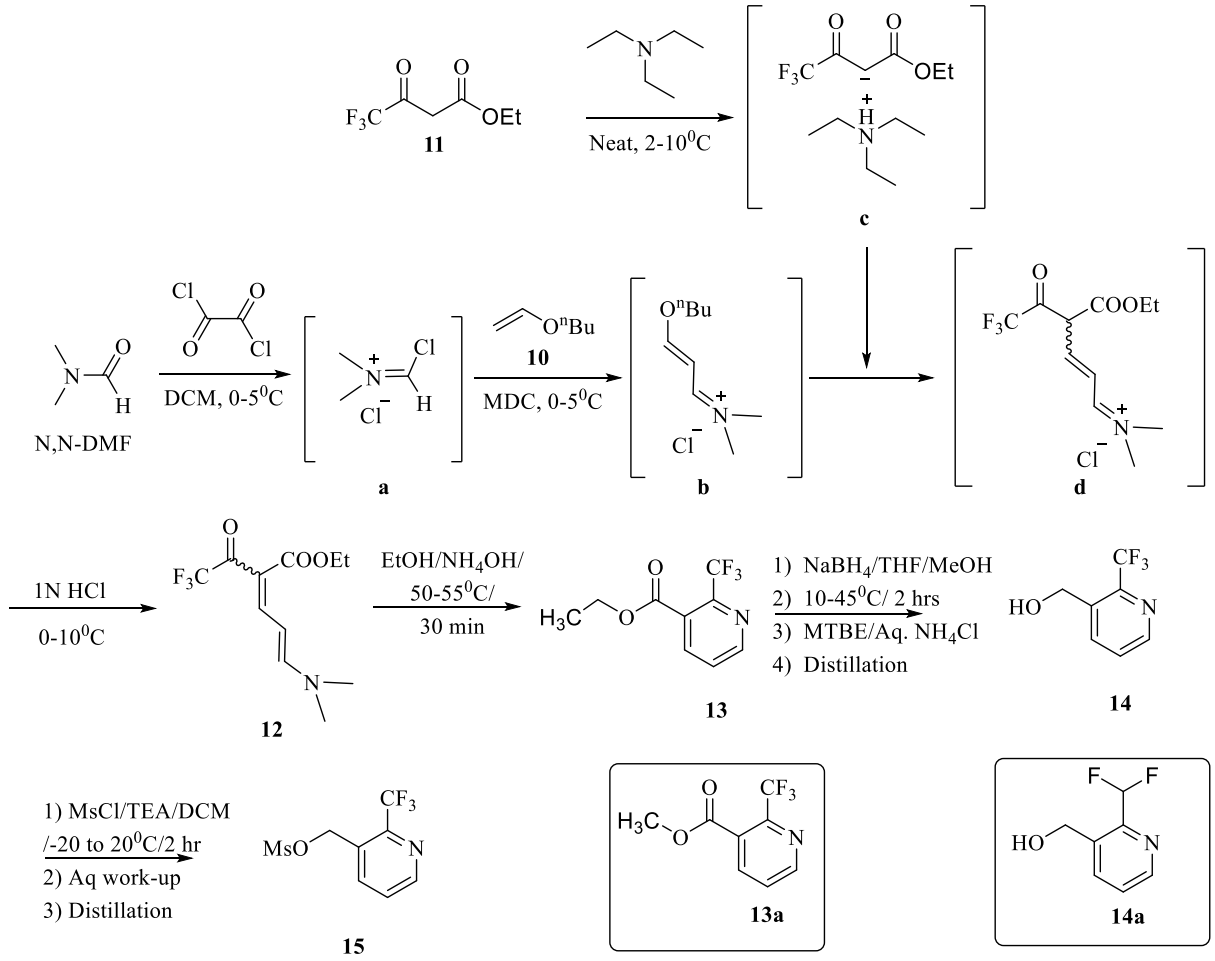
During the initial process development, to generate material for toxicology studies, intermediate **6,** prepared from CF_3 substituted alcohol **14**, was sourced from a CMO. Subsequently, when the availability of **14** was limited, costlier and low in quality from the supplier, an in-house synthesis starting with butyl vinyl ether **10** and ethyl 4,4,4-trifluoroethylacetoacetate (ETFAA) **11** was established as a robust process compared to the reported one.[15]

Among all the reactions for the preparation of intermediate **6** in multi-kilogram scale, Step 1 was found to be critical in terms of operations and reproducibility. Since it is a multicomponent reaction, establishing a reproduceable process from laboratory to plant scale was a big challenge. Initially, the published process[15] was adapted to generate reference standard. However, it involved the use of large volumes of dichloromethane, which was undesired in production. During

optimization dichloromethane volumes were reduced from twenty to nine volumes and exothermicity of the reaction was addressed by diluting reagents (oxalyl chloride and butyl vinyl ether) with dichloromethane. In the laboratory, equal amount of **12** by wt % with respect to wt % of ETFAA was produced consistently. Later this procedure was performed in our kilo-lab at up to 3 X 1.0 kg scale and yields ranged between 0.8 to 1.2 times to that of ETFAA wt % was observed and purity was intact (>98 HPLC area %). However, in the pilot plant at CMO for 10 kg of ETFAA input only 2.8 Kg of **12** was obtained. Various permutations and combinations (e.g. stoichiometry of reagents, reaction volumes, mode of additions, temperatures and time intervals) were investigated. From these trials, the addition of neat pre-mixture of ETFAA and triethylamine (TEA) **(c)** to the reaction mass containing *N*,*N*-dimethylamine adduct **b** (**Scheme 6**) was found to be an important step for producing the desired quality and quantity. This pre-mixture addition allows carbanion of ETFAA to react with adduct **b** spontaneously under dilutions and lead to the formation of desired compound with minimum degradation. The improvement of this modification reflected in consistent yields. In contrast, the regular approach consists of charging of ETFAA to the reaction mass containing **b** under dilution followed by drop wise addition of TEA to generate carbanion of ETFAA there by reacting with adduct **b** to produce **d**. During this process some unreacted ETFAA remained in the reaction mixture, which severely affects the outcome (material nature and yield) of **12** (Step 1). The modified process successfully implemented at CMO, and 71.7 Kg of **12** were produced from 66.0 Kg of input (ETFAA) in four batches with >99.6 HPLC area % and 75% yield. Cyclization of **12** using ammonium hydroxide yields ester **13** with >98 HPLC area % and 83% yield.

The conversion of ester **13** to alcohol **14** appeared to be straightforward. However, from laboratory to kilo scale, we observed delayed reaction conversion times, varying from 2 hours to

24 hours. This was addressed by dissolving ester **13** in methanol instead of THF and adding this to the mixture of sodium borohydride, THF and methanol in the reactor. This sequence of addition permitted reaction to be completed in a 2-hour period on pilot plant scale. Reaction monitoring during zero hours after addition showed methoxy ester **13a** (due to trans-esterification) <5 HPLC area % and des-fluoro impurity **14a** around 0.2 to 0.4 HPLC area %. From **14** to **15**, the mesylation was carried out smoothly without complication during scale up (**Scheme 6**). Thus, 44.8 Kg of **11** was converted into 43 kg of **15** with > 97 HPLC area % and 71% overall yield.



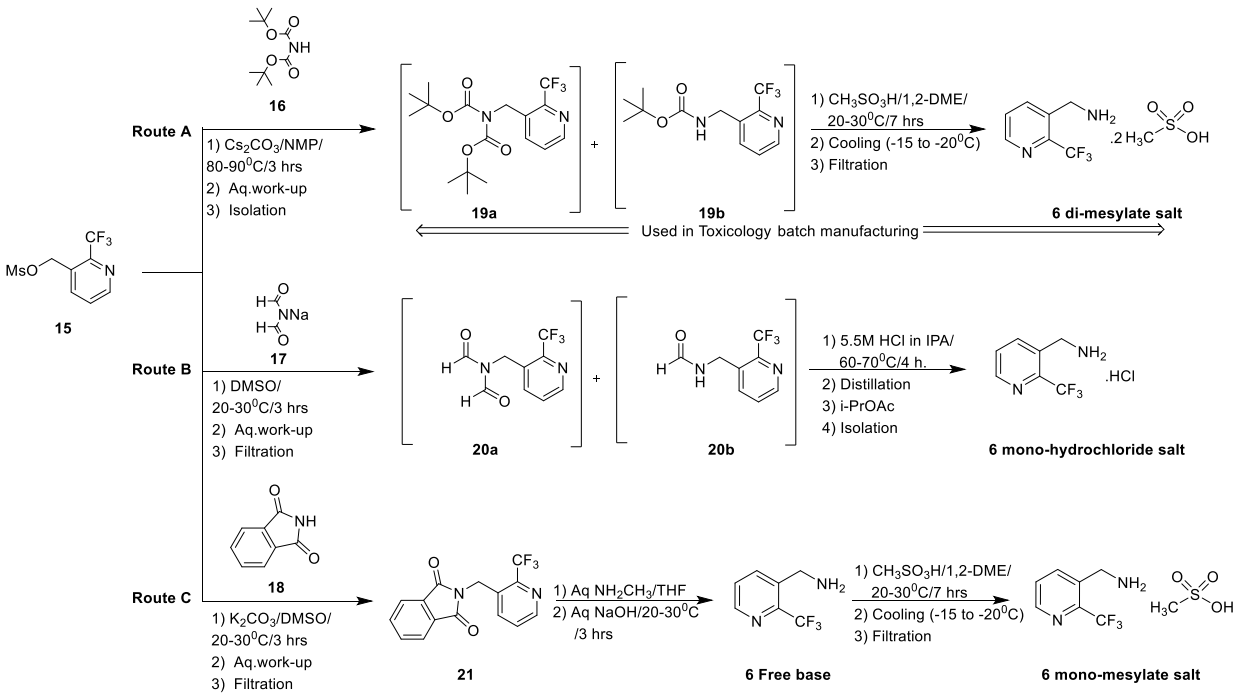
Scheme 6. Synthetic scheme of **15** from ethyl 4,4,4-trifluoroacetoacetate (ETFAA **11**)**.**

Substitution of the mesylate in **15** was completed by condensation with either di-*tert*-butyl-imino-dicarboxylate **16 (Scheme 7**, **Route A)** or sodium diformylamide **17 (Scheme 7, Route B)**.

For preparation of the Toxicology batch API, the intermediate was produced from **Route A**. However, both routes had their own drawbacks. In **Route A**, substitution of mesylate on **15** with **16** needs elevated temperatures (80-90°C), solvent extractions, distillations to purify the product (this material was a dark brown color oil with 85 to 95 HPLC area % consisting of mono (**19a**) and di-protected (**19b**) compounds). Subsequent treatment of **19b** with methanesulfonic acid (MSA) to produce intermediate **6** in its di-mesylate form gave an overall yield 44% from **15** and >99% HPLC area %. In **Route B**, to further enhance the purity at the protected stage, sodium diformylamide (**17**) was introduced and mono- (**20a**) and di-protected (**20b**) products were isolated as solid form with >95% HPLC area %. However, issues during hydrolysis, as the crude HCl salt of **6** needs trituration, to isolate fine solid, resulted in lower yields (59%).

To mitigate these issues, the inexpensive phthalimide group (**18**) was used (**Scheme 7**, **Route C**) and compound **21** was isolated as yellow solid with reduced impurities (>99 HPLC area %) from **15**. **21** on subsequent hydrolysis using aqueous methylamine solution provided the free base in a comfortable manner with mild reaction conditions (20-30°C). The free base obtained was treated with MSA to produce desired building block **6** as a mono mesylate salt.

During the initial process development to generate intermediate **6** via **Route A**, des-fluoro impurity **6a** (tox batch qualified with 0.73 HPLC area % in final API) was formed during sodium borohydride reduction of **13** to **14** at a level of 0.4 to 0.7 HPLC area % (**Scheme 6**) and this was the major concern for this route. However, following **Route C** protocol, around 50% of the des-fluoro impurity was purged in the phthalimide stage. Thus, for pre-GMP and GMP material supply, intermediate **6** was produced via **Route C** to meet our quality requirements.



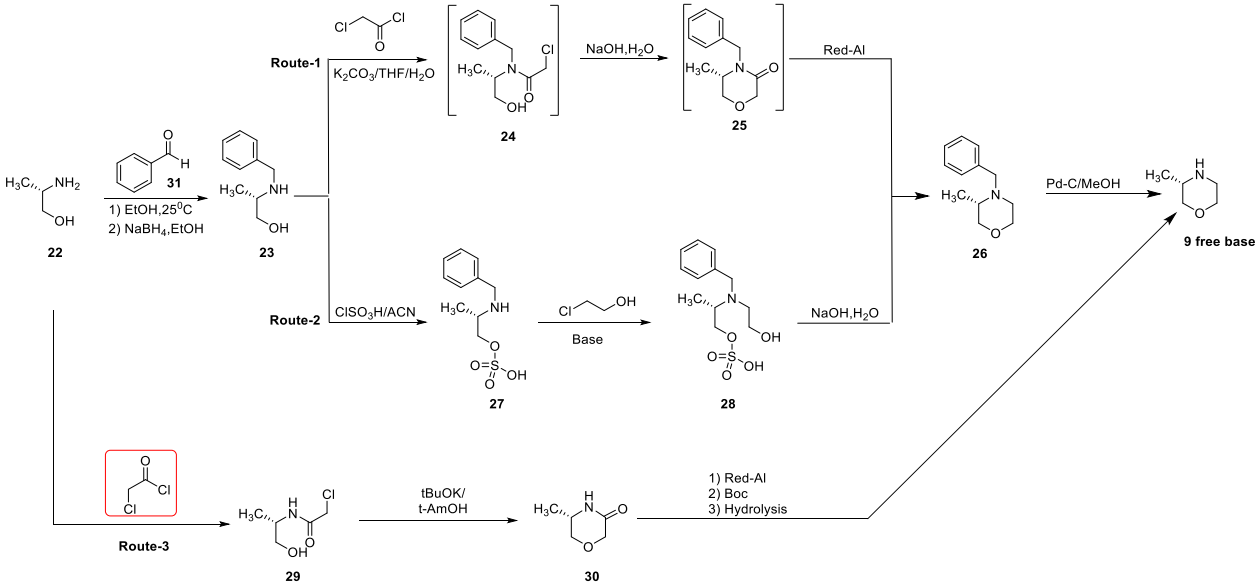
Scheme 7. Synthetic approaches to **6** from **15**.

Section C). *Process development for (S)-3-methylmorpholine (**9**):*

In order to establish a scalable path to **9**, three synthetic routes were investigated in parallel, as shown in **Scheme 8**. For **Route-1**, feasibility assessment was completed, and further development was initiated. During execution of **Route-2**, intermediate **23** and **27** was completed, however, the conversion rate of intermediate **28** was undesired**.** Therefore, **Route-2** was not considered further. **Route-3** feasibility also was completed. However, disadvantages were found with lower yields of

intermediate **30** and **9** free base as it was highly soluble in water. Therefore, **Route-2** and **Route-3** activities were stopped, and **Route-1** became the focus of the development.

Our initial evaluation of **Route-1** identified several concerns, such as, quality of intermediate **23**, **25** and **26** were less than optimal, with poor yields, difficulty in purifying and handling of intermediates **23** and **26** due to their liquid forms (**Scheme 8**).



Scheme 8. Proposed routes for (*S*)-3-methylmorpholine (**9**).

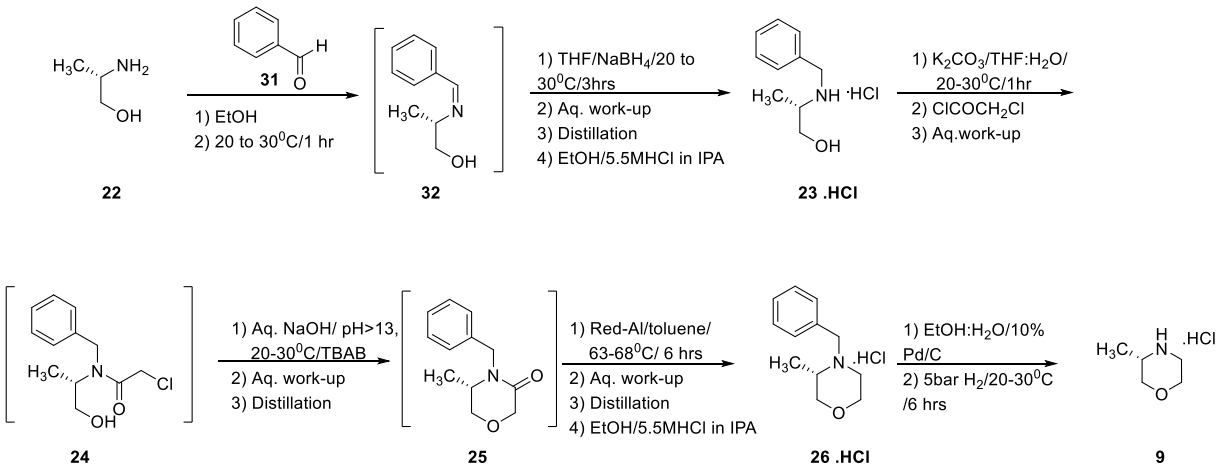
Optimization of Route 1: Preparation of **23** involves reductive amination of commercially available (*S*)-2-amino propanol (**22**) with benzaldehyde (**31**). Initially intermediate **23** was isolated as free base as per literature process.[16] However, purity ranges were between 80 to 85 HPLC area %. To enhance purity, the hydrochloride salt form of **23** was considered and it was prepared using molar solution of hydrochloric acid in isopropyl alcohol and ethanol as solvent. Following this procedure **23** was isolated as its HCl salt in kilo lab with purity >99 HPLC area % and >85% yield.

Subsequently, intermediate **23** was coupled with chloroacetyl chloride using aqueous solution of potassium carbonate and THF to obtain intermediate **24** *in situ*, followed by cyclization using aqueous sodium hydroxide solution to yield intermediate **25**. The reaction time for **25** from **24** was

shortened to 3 hours from >12 hours by the introduction of 0.1 eq of phase transfer catalyst, tetrabutylammonium bromide (TBAB) to promote the deprotonation of hydroxyl group in **24**, which led to desired consistency and reproducibility at kilogram scale.

Reduction of cyclic amide **25** was achieved using Red-Al in toluene to obtain intermediate **26**. Initially, the crude form of free base **26** was taken forward for de-benzylation. Since the free base **26** was isolated as crude after two *in situ* steps from **23** with insufficient purity (~90 HPLC area %), the HCl salt of 26 was considered. Eventually, **26-HCl** was taken forward for de-benzylation under Pd-C catalysis, used isopropyl alcohol and water mixture to address solubility of the salt. After reaction completion, reaction mass filtered and transferred through CUNO (3M™ Zeta Plus™ Activated Carbon Series Filter, R55SP) cartridge filter to control Pd content.

This optimized procedure (**Scheme 9**) was transferred to a CMO and has been used to manufacture 16.4 kg of (*S*)-3-methylmorpholine **9** with >99 GC area % and 69.0% of an overall yield.



Scheme 9. Optimized Synthesis of **9** (via **Route-1**).

Conclusion: In summary, improved synthetic procedures for both key starting materials (**6** & **9**) and drug substance **1** in a safe, cost effective and robust manner were established as part of a

(pre)clinical development program of anti-*Wolbachia* candidate, AWZ1066S. The new large-scale process solves many issues faced by earlier designed routes of intermediates **6** & **9** and drug substance **1**. With the optimized process, impurities were well controlled throughout the manufacturing process and API **1** was delivered with >99 HPLC area %. As a result of these changes, the overall process yield was improved to approx. 50% from the reported chemistry in the initial synthetic chemistry (**Scheme 1**)[10], resulting in a significant reduction in the projected costs of goods, which is critical for a drug candidate that is being developed for neglected tropical diseases.

ASSOCIATED CONTENT

Experimental Section:

NMR spectra were recorded on a BRUKER AVANCE-III NMR spectrometer. Chemical shifts are reported in parts per million (ppm). For ^{1}H NMR spectra ($CDCl_3$, CD_3OD, or $(CD_3)_2SO$), the residual solvent peak was used as the internal reference (7.26 ppm in $CDCl_3$; 3.31 ppm in CD_3OD; 2.50 ppm in $(CD_3)_2SO$), while the central solvent peak as the reference (77.0 ppm in $CDCl_3$; 49.0 ppm in CD_3OD; 39.5 ppm in $(CD_3)_2SO$) for ^{13}C NMR spectra. IR spectra were recorded on Shimadzu make IR Affinity-1 FT-IR spectrophotometer. High-resolution mass spectra (HRMS) were recorded on a Waters make Xevo G2-XS QT of mass spectrometer by electrospray ionization time of flight reflectron experiments. DSC recorded on Shimadzu make DSC-60 model. PXRD recorded on PANalytical make Empyrean model. Thermal analysis of reactions was carried out in a Mettler Toledo reaction calorimeter composed of a 2L glass reactor equipped with a glass temperature sensor, glass calibration heater and a pitched blade turbine with four blades. Thermal

stability of solid and liquid samples was carried out in Phi-TEC I, an adiabatic reaction calorimeter from HEL (Hazard Evaluation Laboratories).

2-Chloro-*N*-[2-(trifluoromethyl)pyridin-3-yl]methylpyrido[2,3-d]pyrimidin-4-amine (8):

To a mixture of **7** (1.80 kg, 8.99 mol, 1.00 eq.) and **6** mono-mesylate salt (2.49 kg, 9.18 mol, 1.02 eq.) in *N*-methyl-2-pyrrolidone (NMP) (14.40 L) was added triethylamine (2.27 kg, 22.43 mol, 2.50 eq.) at 20-30°C. The mixture was allowed to stir at 20-30 °C for 30 minutes at which point an HPLC sample determined completion of reaction. Upon reaction completion (0.1 HPLC area % of **7** relative to **8**; target <1.0%), water (28.8 L) was added to the reaction mass and the solids were filtered, washed the solids sequentially with water (2 X 3.6 L). The solid was dried under vacuum at 45-50 °C to afford compound **8** (2.62 kg, 86.0% yield, 99.18 HPLC area %) as pale brown solid. ^{1}H-NMR (400MHz, DMSO-d_6) δ9.56 (t, *J*=5 Hz, 1H), 9.01 (dd, *J*=4,2 Hz, 1H), 8.77 (dd, *J*=8,2 Hz, 1H), 8.65 (dd, *J*=5,1 Hz, 1H), 8.05 (dd, *J*=8,1 Hz, 1H), 7.67 (dd, *J*=8,5 Hz, 1H), 7.61 (dd, *J*=8,4 Hz, 1H), 4.93 (d, *J*=5 Hz, 2H); ^{13}C-NMR (100MHz, DMSO-d_6) δ 162.3, 159.9, 159.0, 156.7, 147.9, 143.8, 138.3, 133.3, 132.7, 127.5, 122.4, 122.1, 108.8, 40.4. HRMS (ESI) calcd for $C_{14}H_9Cl_{35}F_3N_5$ $[M+H]^+$ 340.0564 , found 340.0567.

2-[(*S*)-3-Methylmorpholin-4-yl]-*N*-[2-(trifluoromethyl)pyridin-3-yl]methylpyrido[2,3-*d*]pyrimidin-4-amine (2):

A mixture of compound **8** (2.20 kg, 6.47 mol, 1.00 eq.), **9** (1.07 kg, 7.77 mol, 1.20 eq.) and tripotassium phosphate (2.06 kg, 9.71 mol, 1.50 eq.) in *N*,*N*-dimethylsulfoxide (11.00 L) was heated to 105-110°C for 13 hours at which point an HPLC sample determined completion of reaction (0.6 HPLC area % of **8** relative to **2**; target <1.0 %). Upon completion of the reaction, the

reaction mass was cooled to 20-30°C and water (22.0 kg) was added. The obtained solids were collected by filtration, the solids were washed sequentially with water (2 X 4.4 L). The cake was dried under vacuum at 50-55 °C to yield Compound **2** (2.47 kg, 94% yield, 98.42 HPLC area %) as a pale brown solid.

2-[(*S*)-3-Methylmorpholin-4-yl]-*N*-[2-(trifluoromethyl)pyridin-3-yl]methylpyrido[2,3-*d*]pyrimidin-4-amine hemi-hydrochloride (1):

To a clean and dry reactor were charged compound **2** (2.30 kg, 5.69 mol, 1.0 eq.) and ethanol (23.0 L). The mixture was heated to 55-65 °C to form a clear solution. The reaction mass was passed through a carbon filter at 55-65 °C. This was then cooled to 40-45 °C. Concentrated hydrochloric acid was slowly added (0.28 kg, 2.84 mol, 0.50 eq.) to the reaction mass at 40-45 °C and the mixture was gradually cooled to 0-5 °C. The obtained precipitate was filtered and rinsed with ethanol twice (2 X 2.00 L). The wet cake was dried in vacuum at 50-60 °C to yield compound **1** (1.75 kg, 72.8% yield, 99.53 HPLC area %) as a yellow crystalline solid. ^{1}H-NMR (400MHz, MeOD-d_4) 8.66 (dd, *J*=5,2 Hz, 1H), 8.63 (dd, *J*=8,2 Hz, 1H), 8.56 (d, *J*=4 Hz, 1H), 7.99 (d, *J*=8 Hz, 1H), 7.58 (dd, *J*=8,5 Hz, 1H), 7.30 (dd, *J*=8,5 Hz, 1H), 4.99 (dd, *J*=41,16, 2H), 4.60 (d, *J*=5 Hz, 1H), 4.32 (dd, *J*=14,2 Hz, 1H), 3.88 (dd, *J*=11,4 Hz, 1H), 3.66 (d, *J*=12 Hz, 1H), 3.54 (dd, *J*=12,3 Hz, 1H), 3.41 (td, *J*=12,9,3 Hz, 1H), 3.21 (ddd, *J*=26,14,4 Hz, 1H), 1.07 (d, *J*=7 Hz, 3H); ^{13}C-NMR (100MHz, MeOD-d_4) 161.7, 160.5, 158.4, 152.7, 145.6, 138.4, 136.8, 134.9, 128.3, 123.8, 118.3, 108.4, 71.6, 67.7, 49.4, 41.9, 40.5, 14.5. HRMS (ESI) calcd for $C_{19}H_{19}F_3N_6O$ [M +H]$^+$ 404.1606 , found 405.1650.

(2Z,4Z)-ethyl 5-(dimethylamino)-2-(2,2,2-trifluoroacetyl)penta-2,4-dienoate (12):

To a clean and dry reactor charged *N*,*N*-dimethylformamide (10.32 kg, 141.22 mol; 1.3 eq.) and dichloromethane (DCM) (100.0 L). The clear solution was cooled to -10 °C under nitrogen atmosphere and was added a solution of oxalyl chloride (17.92 kg, 141.22 mol, 1.3 eq.) in DCM (40.0 L) over a period of 60 min. During the addition a white precipitate was formed. The reaction mixture was allowed to warm up to 25 °C and was stirred for 30 minutes. The reaction mixture was again cooled to -10 °C. To the reaction mixture was added a solution of **10** (28.30 kg, 282.43 mol, 2.6 eq.) in DCM (40.0 L) by keeping the internal temperature below 5 °C. The reaction mixture was allowed to warm up to 25 °C and was stirred for 30 minutes under nitrogen. The pale yellow homogeneous solution was cooled again to -10 °C. To the reaction mixture was added a pre-mixed solution of **11** (20.00 kg, 108.63 mol, 1.0 eq.) and triethylamine (31.30 kg, 309.60 mol, 2.85 eq.) [Preparation: To a clean and dry reactor were charged triethylamine (31.30 kg, 309.60 mol, 2.85 eq.) and cooled to 0°C. **11** (20.0 kg, 108.63 mol, 1.0 eq.) was added drop-wise keeping the internal temperature below 5 °C] was added by keeping the internal temperature below 0 °C. The yellow heterogeneous reaction mixture was stirred for 5 minutes. Where upon 1 *N* aqueous hydrochloric acid (150 L) was added to reaction mixture at below 8 °C. The resultant two phase mixture was separated; the aqueous phase was extracted with DCM (60.0 L). The combined organic phases were washed twice with 100.0 L of water. The organic layer was taken in a clean and dry reactor and solvent was removed under vacuum at below 35-40 °C to dryness. To the resulted red residue, *n*-heptane (100.0 L) was added and the obtained slurry was stirred for 45 minutes, the reaction mixture was cooled to -25 °C and filtered, washed with pre-cooled *n*-heptane (20.0 L) to afford title compound **12** as a light orange colored solid. The obtained wet cake was used directly in the next stage without drying (20.8 kg, 72.2 % Yield, LOD 2.25%, 99.79 HPLC area %).

Ethyl 2-(trifluoromethyl)nicotinate (13):

To a clean and dry reactor **12** (35.0 kg. 132.0 mol, 1.0 *eq*.) was dissolved in a mixture of ethanol (105.0 L) and 25% aq. ammonia solution (105.0 L). The reaction mixture was heated to 55°C for 30 minutes. Upon the completion of reaction (0.05 HPLC area % of **12**, relative to **13**; target <1.0 %), the reaction mass was cooled to 20-30°C, whereupon *tert*-Butyl methyl ether and 10% aqueous sodium chloride solution (140.0 L) was added to reaction mixture. The resultant two phase mixture was separated, the aqueous phase was extracted with *tert*-Butyl methyl ether (175.0 L). The combined organic phases were washed with 10% aqueous sodium chloride solution (105.0 L). The organic layer was taken in a clean and dry reactor and solvent was removed under vacuum at below 40 °C to get **13** as a light yellow color oil (23.6 kg, 81.06 % yield, 98.60 HPLC area %).

(2-(trifluoromethyl)pyridin-3-yl)methanol (14):

To a clean and dry reactor was charged sodium borohydride (6.45 kg. 170.5 mol, 2.5 eq.) and tetrahydrofuran (75.0 L) under a nitrogen atmosphere. The reaction mixture was cooled to 0 °C and methanol (30.0 L) was added at below 10 °C. To the resulting mixture was added a solution of **13** (15.0 kg, 68.40 mol, 1.0 eq.) in methanol (30.0 L) at below 10 °C, the reaction mixture was allowed to stir at same temperature for 30 minutes after which it was gradually heated to 45 °C for 1 hour. Upon the completion of the reaction (0.5 HPLC area % of **13**, relative to **14**; target <1.0 %), the reaction mixture was cooled to 20 °C and was charged with *tert*-Butyl methyl ether (75.0 L). To the resulting mixture was added 12% aqueous ammonium chloride solution (150.0 L) at below 30°C and was allowed to stir for 45 minutes. The precipitated salts were filtered off and the resultant two phase mixture was separated; the aqueous phase was extracted twice with *tert*-Butyl methyl ether (2 X 30.0 L). The combined organic phases and solvent was distilled off under

vacuum at below 35 °C to afford **14** as a yellow color oil (10.50 kg 86.63 % Yield, 97.01 HPLC area %).

(2-(trifluoromethyl)pyridin-3-yl)methyl methanesulfonate (15):

To a clean and dry reactor were charged **14** (15.0 kg, 84.70 mol, 1.0 eq.) and dichloromethane (60.0 L). The reaction mixture was cooled to -25 °C and triethylamine (11.1 kg, 109.6 mol, 1.3 eq.) added. The reaction mass was cooled again to -25 °C and methanesulfonyl chloride (10.65 kg, 92.85 mol, 1.1 eq.) in dichloromethane (45.0 L) was added below -15 °C. The reaction mixture was allowed to stir at or below -15 °C for 1 hour. Upon reaction completion (0.6 HPLC area % of **14**, relative to **15**; target <1.0%), water (75.0 L) was added to the reaction mixture at below 30 °C and the resultant two phase mixture was separated the organic phase washed with water (75.0 L). The organic phase was charged to the reactor and solvent was distilled off under vacuum at below 40 °C to afford **15** as a yellow color oil (20.5 kg, 94.91 %, 97.58 HPLC area %).

2-((2-(trifluoromethyl)pyridin-3-yl)methyl)isoindoline-1,3-dione (21):

To a clean and dry reactor were charged **18** (12.48 kg, 84.34, 1.05 eq.), potassium carbonate (16.6 kg, 120.48mol, 1.5 eq.) and dimethyl sulfoxide (82.0 L) nitrogen atmosphere. The reaction mixture was cooled to 15 °C and a solution of **15** (20.5 kg, 80.32 mol. 1.0 eq.) in DMSO (82.0 L) was added. The reaction mass temperature was increased to 30 °C and stirred for 3 hours. Subsequently, the reaction mass was cooled to 0 °C and water (246.0 L) was added at below 35 °C. The reaction mixture was then cooled to 15 °C, allowed to stir for 1 hour and the solids were collected by filtration. The solids were sequentially washed with water (41.0 L) to afford **21** as yellow color solid. The wet cake was directly used in the next stage after applying loss on drying correction (25.1 kg, 79.67 % yield, LOD 21.91%, 99.71 HPLC area %).

(2-(trifluoromethyl)pyridin-3-yl)methanamine methanesulfonate (6 mono-mesylate salt):

To a clean and dry reactor were charged **21** (20.0 kg, 65.3 mol, 1.0 eq.), tetrahydrofuran (THF) (40.0 L) and 40% methylamine solution in water (40.0 L, 514.0 mol, 7.9 eq.). The reaction mixture was allowed to stir for 2 hours at 20-30 °C, at which point an HPLC sample determined complete reaction (0.05 HPLC area % of **21**, relative to *in situ* intermediate & title compound free base; target <1.0%). Upon completion of reaction, 10% aqueous sodium hydroxide solution (80.0 L) was added. The reaction mixture stirred for an additional 2 hours at 20-30 °C. Upon reaction completion (0.02 HPLC area % of *in situ* intermediate relative to **6**; target <1.0%), to the reaction mixture was added *tert*-butyl methyl ether (MTBE) (100.0 L). The resultant two phase mixture was separated, the aqueous phase was extracted twice with MTBE (2 X 50.0 L). The combined organic phases were dried over sodium sulfate and solvent was distilled off under vacuum at below 40 °C to get **6** as a yellow liquid. To **6**, 1,2-dimethoxyethane (30.0 L) was added, and the reaction mixture was cooled to 10 °C. To the reaction mixture, methanesulfonic acid (6.0 kg) was added at below 15 °C. The resulting slurry was gradually cooled to -20 °C and stirred for 1 hour. The obtained solids was filtered and rinsed with 1,2-dimethoxyethane (2 X 10.0 L). The wet cake was dried in vacuum at 50 °C to afford **6 mono-mesylate salt** as an off-white solid (12.8 kg, 71.91 % yield, 99.62 HPLC area %). ^{1}H-NMR (400MHz, DMSO-d_6) δ 8.74 (dd, *J*=5,1 Hz, 1H), 8.45 (brs, 3H Overlap), 8.19 (dd, *J*=8,1 Hz, 1H), 7.85 (dd, *J*=8,5 Hz, 1H), 4.25 (s, 2H), 2.35 (s, 3H); ^{13}C-NMR (100MHz, DMSO-d_6) δ 149.2, 144.3, 139.7, 128.8, 127.6, 122.0, 39.8, 37.9. HRMS (ESI) calcd for $C_7H_7F_3N_2$ $[M+H]^+$ 177.0595, found 177.0634.

(*S*)-2-(benzylamino)propan-1-ol hydrochloride (23):

To a clean and dry reactor was charged **22** (13.0 kg, 173.0 mol, 1.0 eq.) and ethanol (26.0 L). The reaction mixture was cooled to 0 °C and was added a solution of **31** (20.15 kg, 188.5 mol, 1.1

eq.) in ethanol (6.5 L) at below 10 °C. The reaction mixture temperature was raised to 25-30 °C and was allowed to stir for 3 hours (Limit: **31** NMT 10.0% by ^{1}H NMR in DMSO). Upon reaction completion the reaction mixture was cooled to 0 °C and was added to the solution of sodium borohydride (4.94 kg, 130.0 mol, 0.75 eq.) in tetrahydrofuran (THF) (26.0 L) at below 15 °C. The reaction mixture temperature was raised to 25 to 30 °C and was stirred for 8 hours at 20 to 30°C. Upon reaction completion (HPLC area % **32** relative to **23**; target <1%), the reaction mixture was cooled to 0 °C and was 2 N hydrochloric acid solution (7.8 L 36% hydrochloric acid in 39.0 L water) was added at below 25 °C. To the resulting mixture, isopropyl acetate (65.0 L) was charged and the obtained salts were filtered. The resultant two phase mixture was separated; the aqueous phase was extracted with isopropyl acetate (4 X 32.5 L). The combined organic phase was dried over anhydrous sodium sulfate (3.9 kg), filtered, and solvent was distilled off in such a way that the residue remains 3 to 4 volumes of **22** under vacuum at below 45 °C. After achieving reaction mass volume to 3-4, 5.5 M hydrochloric acid in isopropyl alcohol (39.0 L) was added to the reaction mixture at below 20 °C. Gradually the reaction mass was cooled to -25 °C and was stirred for 2 hours. The obtained precipitate was filtered, washed the cake with pre-cooled isopropyl acetate (19.5 L) and dried for 4 to 5 hours at 50-55 °C to afford **23-HCl** as a white solid (30.35 kg, 86.94 % yield, 99.63 HPLC area %).

(S)-4-benzyl-3-methylmorpholine hydrochloride (26):

To a clean and dry reactor was charged **23-HCl** (15.0 kg, 74.4 mol, 1.0 eq.) in THF (52.5 L). To the reaction mixture, a solution of potassium carbonate (37.5 kg, 271.5 mol, 3.65 eq.) in water (90.0 L) was added, and the reaction mixture was stirred for 30 minutes at 25 °C. To the resulting mixture, a solution of chloroacetyl chloride (14.25 kg, 127.0 mol, 1.7 eq.) in THF (7.5 L) was added and the reaction mixture was allowed to stir for 1 hour at 25-30 °C. Upon reaction

completion (0.03 HPLC area % **23**, relative to **24**; target <1%), an aqueous solution of sodium hydroxide (16.05 kg of sodium hydroxide in 16.05 kg of water) was added along with tetrabutylammonium bromide (2.40 kg) at 25 °C. The resulting reaction mixture was stirred for 4 hours at 25-30 °C. Upon reaction completion (0.02 HPLC area % **24**, relative to **25**; target <1%), two phase mixture was separated; the aqueous phase was extracted twice with toluene (2 X 75.0 L). The combined organic phase was washed with water (45.0 L) and solvent was removed from organic phase under vacuum at below 45 °C. To the obtained residue, toluene (15.0 L) was charged and was removed under vacuum at below 45 °C. To the obtained residue, **25** was added toluene (90.0 L). The resulting solution was cooled to below -20 °C and was added sodium bis (2-methoxyethoxy)aluminumhydride (70% solution in toluene) (Red-Al, 45.0 L, 160.5 mol, 2.14 eq.) slowly for a period of 2 hours at below 5 °C. The reaction mixture temperature was raised to 65 °C and was allowed to stir for 6 hours at 65-70 °C. Upon reaction completion (0.02 HPLC area % **25**, relative to **26**; target NMT 1.0%), the reaction mixture was cooled to -25 °C and was added 1 N aqueous sodium hydroxide solution (300.0 L) over a period of 60-120 minutes at below 5 °C. The resulting two-phase mixture was separated; the aqueous phase was extracted with toluene (45.0 L). The combined organic phase was distilled off under vacuum at below 45 °C. To the obtained residue, ethanol (15.0 L) was charged and was removed under vacuum at below 45 °C. To the obtained residue, **26** free base, ethanol (60.0 L) and 5.5 M hydrochloric acid in isopropyl alcohol was added to the reaction mixture at below 25 °C. Gradually the reaction mass was cooled to -25 °C and was stirred for 2 hours. The precipitate was obtained by filtration, the cake was washed with pre-cooled ethanol (15.0 L) and was dried under vacuum at 50 °C to yield **26-HCl** as white crystalline solid (14.7 kg, 86.79 % yield, 99.13 HPLC area %).

(*S*)-3-methylmorpholine hydrochloride (9-HCl):

Compound **26-HCl** (14.2 kg), isopropyl alcohol (114.0 L), and water (14.2 L) and 10% Pd/C (0.426 kg) were charged to a jacketed vessel equipped with stirrer and temperature probe. The reactor was sequentially purged to 0.5 bar with nitrogen (3 times) and then hydrogen. The vessel was then applied with 5 bars of hydrogen pressure. The reaction mixture was maintained for 4 hours at 30 °C and 5 bars of hydrogen pressure. Upon reaction completion (0.04 GC area % **26**, relative to **9**; target <1%), hydrogen gas was released and was replaced with nitrogen atmosphere. Reaction mixture was filtered through hyflo-bed under nitrogen atmosphere and the filter cake was washed twice with isopropyl alcohol (2 X 42.0 L). The obtained filtrate was charged to the clean and dry reactor, circulated through CUNO filter and passed through celite bed. The solvent was distilled off under vacuum at below 50 °C. The residue was azeotroped with isopropyl alcohol (14.0 L) and *tert*-butyl methyl ether (1.0 L) to obtain a semi-solid. The resulting semi-solid was triturated in *tert*-butyl methyl ether (50.0 L) and the solids were filtered under nitrogen atmosphere and dried under vacuum at 60–65 °C to afford **9** as an off-white solid (7.9 kg, 92.06% yield, 99.49 GC area %). ^{1}H-NMR (400MHz, DMSO-d_6) δ 9.74 (brs, 2H overlap), 3.89 (m, 2H), 3.85 (dd, *J*=7,4 Hz, 1H), 3.59 (dd, *J*=12.10 Hz, 1H), 3.33 (dddd, *J*=12,12,6,3 Hz, 1H), 3.22, (dt, *J*=13.2 Hz, 1H), 3.07, (ddd, *J*=8,7,5 Hz, 1H), 1.31 (d, *J*=7 Hz, 3H); ^{13}C-NMR (100MHz, Acetonitrile-d_3) δ 69.9, 64.0, 51.8, 43.7, 14.5. HRMS (ESI): calculated for $C_5H_{11}NO$ $[M+H]^+$ 102.0874 , found 102.0912.

Supporting Information

Spectral data for compounds in **Scheme 2**. HPLC conditions (chemical purity), FT-IR, ^{1}H-NMR, ^{13}C-NMR, HRMS and PXRD for **8**, HPLC conditions (chemical purity), FT-IR and PXRD for **2**,

FT-IR, ^{1}H-NMR, ^{13}C-NMR, HRMS, PXRD, DSC and HPLC conditions (chemical purity), for **1**, HPLC conditions (chemical purity), FT-IR, ^{1}H-NMR, ^{13}C-NMR and HRMS for **6** and GC conditions , FT-IR, ^{1}H-NMR, ^{13}C-NMR and HRMS for **9**. Safety evaluations of the Stage-2 S_NAr substitution conditions.

AUTHOR INFORMATION

Corresponding Author

*Email: a-khile@eisai.co.in

*Email: j2-patel@eisai.co.in

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding Sources

This work was supported by grants from the U.K. Medical Research Council (MR/R025401/1) and the Global Health Innovation Technology (GHIT) Fund (G2019-202R1).

Notes

The authors declare no competing financial interest.

REFERENCES

1. Disease, G. B. D.; Injury, I.; Prevalence, C., Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* **2018,** *392* (10159), 1789-1858.

2. Boussinesq, M.; Gardon, J.; Gardon-Wendel, N.; Chippaux, J. P., Clinical picture, epidemiology and outcome of Loa-associated serious adverse events related to mass ivermectin treatment of onchocerciasis in Cameroon. *Filaria J* **2003,** *2 Suppl 1* (Suppl 1), S4.
3. Doyle, S. R.; Bourguinat, C.; Nana-Djeunga, H. C.; Kengne-Ouafo, J. A.; Pion, S. D. S.; Bopda, J.; Kamgno, J.; Wanji, S.; Che, H.; Kuesel, A. C.; Walker, M.; Basanez, M. G.; Boakye, D. A.; Osei-Atweneboana, M. Y.; Boussinesq, M.; Prichard, R. K.; Grant, W. N., Genome-wide analysis of ivermectin response by Onchocerca volvulus reveals that genetic drift and soft selective sweeps contribute to loss of drug sensitivity. *PLoS Negl Trop Dis* **2017,** *11* (7), e0005816.
4. Osei-Atweneboana, M. Y.; Awadzi, K.; Attah, S. K.; Boakye, D. A.; Gyapong, J. O.; Prichard, R. K., Phenotypic evidence of emerging ivermectin resistance in Onchocerca volvulus. *PLoS Negl Trop Dis* **2011,** *5* (3), e998.
5. Organization, W. H. *Ending the Neglect to Attain the Sustainable Development Goals – A Road Map for Neglected Tropical Diseases 2021–2030*; 2020.
6. Hoerauf, A.; Specht, S.; Buttner, M.; Pfarr, K.; Mand, S.; Fimmers, R.; Marfo-Debrekyei, Y.; Konadu, P.; Debrah, A. Y.; Bandi, C.; Brattig, N.; Albers, A.; Larbi, J.; Batsa, L.; Taylor, M. J.; Adjei, O.; Buttner, D. W., Wolbachia endobacteria depletion by doxycycline as antifilarial therapy has macrofilaricidal activity in onchocerciasis: a randomized placebo-controlled study. *Med Microbiol Immunol* **2008,** *197* (3), 295-311.
7. Taylor, M. J.; Makunde, W. H.; McGarry, H. F.; Turner, J. D.; Mand, S.; Hoerauf, A., Macrofilaricidal activity after doxycycline treatment of Wuchereria bancrofti: a double-blind, randomised placebo-controlled trial. *Lancet* **2005,** *365* (9477), 2116-21.
8. Turner, J. D.; Tendongfor, N.; Esum, M.; Johnston, K. L.; Langley, R. S.; Ford, L.; Faragher, B.; Specht, S.; Mand, S.; Hoerauf, A.; Enyong, P.; Wanji, S.; Taylor, M. J., Macrofilaricidal activity after doxycycline only treatment of Onchocerca volvulus in an area of Loa loa co-endemicity: a randomized controlled trial. *PLoS Negl Trop Dis* **2010,** *4* (4), e660.
9. Taylor, M. J.; Hoerauf, A.; Townson, S.; Slatko, B. E.; Ward, S. A., Anti-Wolbachia drug discovery and development: safe macrofilaricides for onchocerciasis and lymphatic filariasis. *Parasitology* **2014,** *141* (1), 119-27.
10. Hong, W. D.; Benayoud, F.; Nixon, G. L.; Ford, L.; Johnston, K. L.; Clare, R. H.; Cassidy, A.; Cook, D. A. N.; Siu, A.; Shiotani, M.; Webborn, P. J. H.; Kavanagh, S.; Aljayyoussi, G.; Murphy, E.; Steven, A.; Archer, J.; Struever, D.; Frohberger, S. J.; Ehrens, A.; Hubner, M. P.; Hoerauf, A.; Roberts, A. P.; Hubbard, A. T. M.; Tate, E. W.; Serwa, R. A.; Leung, S. C.; Qie, L.; Berry, N. G.; Gusovsky, F.; Hemingway, J.; Turner, J. D.; Taylor, M. J.; Ward, S. A.; O'Neill, P. M., AWZ1066S, a highly specific anti-Wolbachia drug candidate for a short-course treatment of filariasis. *Proc Natl Acad Sci U S A* **2019,** *116* (4), 1414-1419.
11. Johnston, K. L.; Hong, W. D.; Turner, J. D.; O'Neill, P. M.; Ward, S. A.; Taylor, M. J., Anti-Wolbachia drugs for filariasis. *Trends Parasitol* **2021,** *37* (12), 1068-1081.
12. Wang, Z.; Richter, S. M.; Gates, B. D.; Grieme, T. A., Safety Concerns in a Pharmaceutical Manufacturing Process Using Dimethyl Sulfoxide (DMSO) as a Solvent. *Organic Process Research & Development* **2012,** *16* (12), 1994-2000.
13. Yang, Q.; Sheng, M.; Li, X.; Tucker, C.; Vásquez Céspedes, S.; Webb, N. J.; Whiteker, G. T.; Yu, J., Potential Explosion Hazards Associated with the Autocatalytic Thermal Decomposition of Dimethyl Sulfoxide and Its Mixtures. *Organic Process Research & Development* **2020,** *24* (6), 916-939.
14. Stoessel, F., *Thermal safety of chemical processes: risk assessment and process design*. John Wiley & Sons: 2021.

15. Kiss, L. E.; Ferreira, H. S.; Learmonth, D. A., Efficient Synthesis of 2-(Trifluoromethyl)nicotinic Acid Derivatives from Simple Fluorinated Precursors. *Organic Letters* **2008,** *10* (9), 1835-1837.
16. Dugar, S.; Sharma, A.; Kuila, B.; Mahajan, D.; Dwivedi, S.; Tripathi, V., A concise and efficient synthesis of substituted morpholines. *Synthesis* **2015,** *47* (05), 712-720.