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Communication

Development of a Scalable Method for Manufacturing the Central Core of CD73 Inhibitor AB680

Jeremy Fournier,* Xuelei Yan, Anh T. Tran, Rebecca L. Grange, Steven D. Jacob, Jaroslaw Kalisiak, Kenneth V. Lawson, Eric F. Connor, Manmohan R. Leleti, and Jay P. Powers

Cite This: https://dx.doi.org/10.1021/acs.oprd.0c00469

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ABSTRACT: AB680 is a highly potent CD73 small molecule inhibitor discovered and developed by Arcus Biosciences, currently in clinical trials for the treatment of pancreatic cancer. Here, we report the development of a scalable and practical method for the manufacturing of the azaindazole central core. This synthesis features an *N*-oxide formation followed by an α-chlorination with $POCl_3$ leading to the formation of 4,6-dichloro-1*H*-pyrazolo[3,4-*b*]pyridine **1** in high yield and 99.5% UV purity. This method was successfully performed on multikilogram scale to support the synthesis of AB680.

KEYWORDS: *AB680, CD73, chlorination, azaindazole*

INTRODUCTION

A high concentration of adenosine in the tumor microenvironment, caused by high cellular turnover, immunogenic cell death due to chemotherapy, and a variety of nonlytic mechanisms, has been known to suppress immune response via inhibition of immune cell activation. In fact, cell death generates extracellular adenosine triphosphate (ATP) which is converted to adenosine monophosphate (AMP) by ecto-nucleoside CD39, and then to adenosine by ecto-5′-nucleotidase CD73.[1−4] The adenosine produced in the tumor microenvironment suppresses the immune response of T cells, NK cells, dendritic cells, and macrophages through activation of $A_{2a}R$ and $A_{2b}R$ receptors.[5] As CD73 has been found to be overexpressed in different tumor types,[6] we developed an exceptionally potent small molecule inhibitor (AB680, K_i = 5 pM),[7] currently in clinical trials in cancer patients (Figure 1). In order to support ongoing clinical trials,

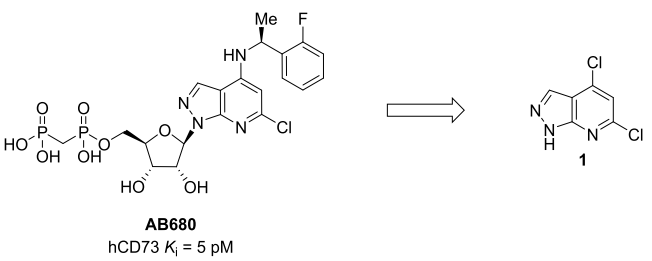


Figure 1. Structure of CD73 inhibitor AB680.

we developed a manufacturing process and this contribution describes the challenges we encountered in the large scale synthesis of the azaindazole central core.

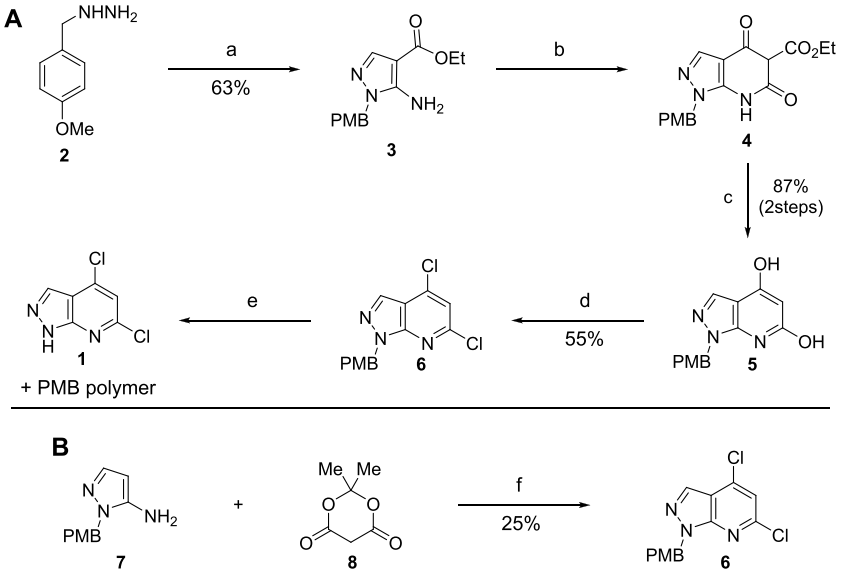
RESULTS AND DISCUSSION

Our first generation synthesis (Scheme 1A) of 4,6-dichloro-1*H*-pyrazolo[3,4-*b*]pyridine **1** started with the formation of amino-pyrrazole **3** by reacting [(4-methoxyphenyl)methyl]-hydrazine and ethyl (ethoxymethylene)cyanoacetate in 63% yield.[8] Next, pyrrazole **3** was subjected to a condensation reaction to form β-keto ester **4**, which was then decarboxylated under basic conditions to provide pyrazolopyridine **5** in 87% yield over two steps. Compound **5** was then chlorinated in the presence of phenylphosphonic dichloride[9] at 170 °C followed by PMB deprotection in neat TFA to afford 4,6-dichloro-1*H*-pyrazolo[3,4-*b*]pyridine **1**. We were also able to develop a one-pot construction of the pyrazolo-pyridine heterocycle by reacting amino-pyrazole **7** and Meldrum's acid in the presence of $POCl_3$ and $BnEt_3N^+Cl^-$, allowing us to isolate the PMB-protected dichloro heterocycle **6** in 25% yield (Scheme 1B).[10] Although these synthetic approaches have been shown to be robust enough to provide multigrams of material to support SAR studies and the discovery of AB680, several problems associated with these synthetic sequences emerged during the development of a process friendly route. First, the chlorination reaction in the first route was carried out at very high temperature (170 °C) and yields were not always consistent. Second the TFA mediated PMB deprotection generated a large amount of a polymer which was not easily removable without the use of column chromatography.

In order to overcome these challenges, we developed a second generation synthesis of heterocycle **1** which we believed would be more process friendly and would allow us to provide kilograms of material to support the ongoing clinical trials. The initial plan was to start with readily available 7-azaindole (Scheme 2) and perform two *N*-oxidation/

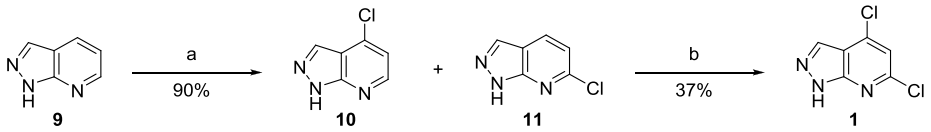
Received: October 19, 2020

Scheme 1. First Generation Synthesis of 4,6-Dichloro-1*H*-pyrazolo[3,4-*b*]pyridine 1



(a) ethyl (ethoxymethylene)cyanoacetate, EtOH, reflux; (b) diethyl malonate, NaOEt, EtOH, reflux; (c) 15% $NaOH_{aq}$, reflux; (d) $PhPOCl_2$, 170 °C; (e) TFA, 60 °C; (f) $POCl_3$, $BnEt_3N^+Cl^-$, 100 °C.

Scheme 2. Double Oxidation/Chlorination Sequence

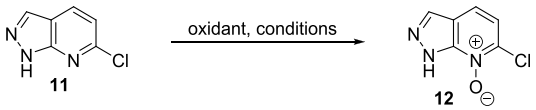


a) (i) mCPBA (1.2 equiv), NMP, rt; (ii) $POCl_3$ (4 equiv), DMF, rt; b) (i) mCPBA (1.2 equiv), NMP, rt, (ii) $POCl_3$ (4 equiv), CH_3CN, rt.

chlorination sequences to access 4,6-dichloro-1*H*-pyrazolo[3,4-*b*]pyridine **1** in four steps.[11] When performed on small scale, the two *N*-oxidation/chlorination sequences produced drastically different yields. Indeed, the oxidation of 7-azaindazole in the presence of *m*CPBA resulted in clean formation of the corresponding *N*-oxide which was further chlorinated with $POCl_3$ to provide a mixture of 4-chloro- and 6-chloro-azaindazole (**10** and **11**) in a 2:1 ratio and 90% overall yield. This mixture of compounds was resubjected to the same set of conditions which allowed us to isolate the desired 4,6-dichloro-1*H*-pyrazolo[3,4-*b*]pyridine **1** but in only 37% yield.

In order to identify the cause of the low yield observed, we carried out the *N*-oxidation/chlorination sequence on both monochloro azaindazoles separately. First, we focused our efforts on 6-chloroazaindazole derivative **11**, and we observed that the use of *m*CPBA oxidation conditions did not give satisfactory yields of the *N*-oxide product (Table 1, entry 1). Following this observation, we investigated other conditions that would allow us to get the *N*-oxide intermediate **12** in acceptable yield. First, changing the oxidant to H_2O_2 in water/acetic acid did not result in any conversion either (Table 1, entry 2). Interestingly, the use of urea·H_2O_2 in acetonitrile showed promise, as *N*-oxide **12** was formed in 70% conversion (Table 1, entry 3).[12] We then screened additional solvents to try to improve the conversion and found MTBE and DME to be the best solvents, affording >88% conversion (Table 1, entries 7−8). Although the conversion was excellent, the isolation of *N*-oxide **12** turned out to be challenging, which resulted in low overall yield. To overcome this issue, we tried

Table 1. Optimization of 6-Cl-Azaindazole Oxidation



Entry	Oxidant	Conditions	Conversion
1	*m*CPBA	AcOH, 80 °C	<5%
2	H_2O_2	H_2O, AcOH, 95 °C	<5%
3	Urea·H_2O_2, TFAA	CH_3CN, rt	70%
4	Urea·H_2O_2, TFAA	dioxane, rt	26%
5	Urea·H_2O_2, TFAA	THF, rt	21%
6	Urea·H_2O_2, TFAA	methanol, rt	<5%
7	Urea·H_2O_2, TFAA	MTBE, rt	88%
8	Urea·H_2O_2, TFAA	DME, rt	90%

to optimize a one-pot *N*-oxidation followed by chlorination, but to our disappointment this was unsuccessful.

On the other hand, when 4-chloro-azaindazole **10** was used as starting material, the oxidation/chlorination sequence proved to be very efficient, providing the desired dichloro-azaindazole **1** in 87% overall yield (Scheme 3).

Based on these results, we tried to optimize the first *N*-oxidation/chlorination sequence to favor the formation of 4-chloro-azaindazole **10** over 6-chloro-azaindazole **11** (see Table 2). The use of an alternate chlorine source like PCl_3, MsCl, or oxalyl chloride resulted in complex reaction mixtures (Table 2, entries 2−4), and varying the solvent did not allow us to obtain

https://dx.doi.org/10.1021/acs.oprd.0c00469
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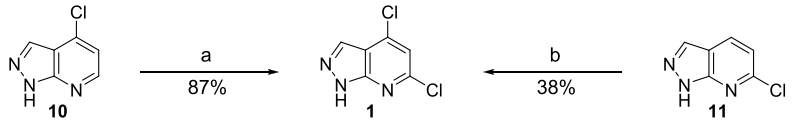
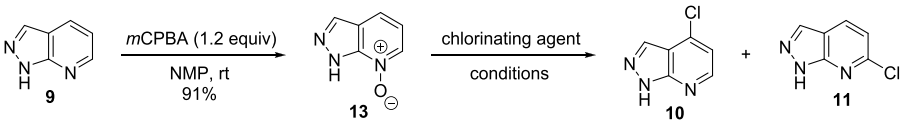
Scheme 3. Oxidation/Chlorination from Mono-chloro-azaindazoles



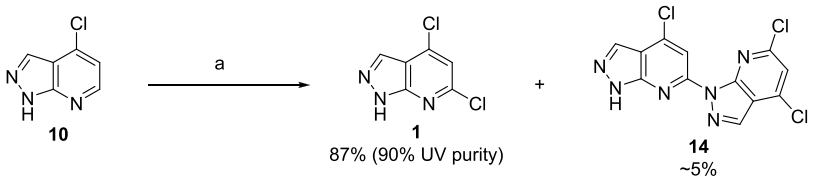
(a) (i) *m*CPBA, NMP, rt, (ii) $POCl_3$, CH_3CN, rt; (b) (i) urea.H_2O_2, DME, rt; (ii) $POCl_3$, CH_3CN, rt.

Table 2. Optimization of Oxidation/Chlorination Sequence



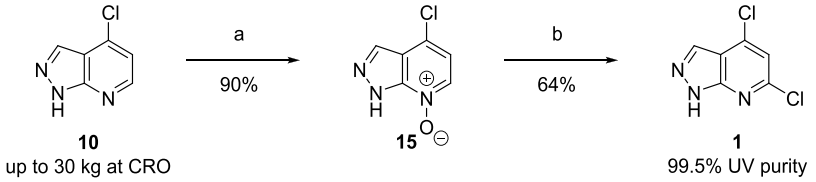
Entry	Chlorinating agent	Conditions	**10:11** ratio
1	$POCl_3$	CH_3CN, rt	1.2:1
2	PCl_3	CH_3CN, rt	complex mixture
3	MsCl	CH_3CN, 50 °C	complex mixture
4	Oxalyl chloride	CH_3CN, 50 °C	complex mixture
5	$POCl_3$	CH_2Cl_2, rt	1.5:1
6	$POCl_3$	Toluene, rt	1:1.5
7	$POCl_3$	DMF, rt	2:1
8	$POCl_3$	NMP, rt	1:1

Scheme 4. Impurity Profile of 4,6-Dichloro-1*H*-pyrazolo[3,4-*b*]pyridine 1



(a) (i) *m*CPBA, NMP, rt, (ii) $POCl_3$, CH_3CN, rt.

Scheme 5. Scale-up Synthesis of 4,6-Dichloro-1*H*-pyrazolo[3,4-*b*]pyridine 1



(a) (i) *m*CPBA (2.3 equiv), NMP, 0 °C to rt
(ii) $POCl_3$ (2 equiv), $BnEt_3N^+Cl^-$ (0.5 equiv), CH_3CN, 10-20 °C

sufficient selectivity to pursue this route from 7-azaindazole **9** (Table 2, entries 5−8).

However, as 4-chloroazaindazole **10** was commercially available and has shown high yields we decided to optimize that two-step sequence. There were mainly two issues that needed to be fixed to obtain high purity material (>99%) required for scale-up synthesis, as the final compound was contaminated with 5% of residual unreacted starting material and 5% of dimer **14**, the formation of which can be explained by the high concentration used in the reaction (Scheme 4).

First, the difference in pK_a between monocholoro-azaindazole **10** and dichloro-azaindazole **1** allowed us to remove unreacted starting material by filtration under acidic conditions. Concerning the elimination of the second impurity, we first tried to decrease the concentration of the reaction to avoid the formation of the dimer, or increase the amount of chlorinating agent, but the results were not conclusive. However, the use of a tetraalkylammonium salt as an additional chlorine source helped us eliminate the formation of dimer **14** and access 4,6-dichloro-1*H*-pyrazolo[3,4-*b*]pyridine **1** in high purity. As a result, we decided to use 2 equiv of $POCl_3$ and 0.5 equiv of $Bn(Et)_3N^+Cl^-$ as our final conditions for the chlorination step. This procedure was then transferred to a CRO which carried out the reaction on up to 30 kg, isolating the dichloroazaindazole **1** in 58% overall yield and 99.5% purity, which allowed us to support the phase 1 clinical trial of AB680 (Scheme 5).

CONCLUSION

In summary, we successfully developed a practical and scalable method for the synthesis of 4,6-dichloro-1*H*-pyrazolo[3,4-*b*]pyridine **1**, to support the scale-up synthesis of AB680. The method features an *N*-oxidation/chlorination two-step sequence that allowed us to access 4,6-dichloro-1*H*-pyrazolo[3,4-*b*]pyridine **1** in high yield and 99.5% UV purity, on up to 30 kg, without the use of column chromatography.

https://dx.doi.org/10.1021/acs.oprd.0c00469
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

EXPERIMENTAL SECTION

Ethyl 5-Amino-1-[(4-methoxyphenyl)methyl]-pyrazole-4-carboxylate (3). Ethyl (ethoxymethylene)cyanoacetate (50.5 g, 299.0 mmol, 1 equiv) was dissolved in anhydrous EtOH (350 mL), and then (4-methoxyphenyl)-methylhydrazine (50 g, 328.9 mmol, 1.1 equiv) was added. The reaction mixture was stirred under reflux overnight and then evaporated. The solid residue was washed with MTBE to give a white solid (55.5 g, 63%). ESI MS $[M + H]^+$ for $C_{14}H_{18}N_3O_3$, calcd 276.1, found 276.2. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.66 (s, 1H), 7.17−7.08 (m, 2H), 6.92−6.83 (m, 2H), 5.09 (s, 2H), 4.83 (s, 2H), 4.25 (qd, J = 2.1, 7.1 Hz, 2H), 3.79 (s, 3H), 1.32 (td, J = 2.2, 7.1 Hz, 3H).

Ethyl 1-[(4-Methoxyphenyl)methyl]-4,6-dioxo-7*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (4). Diethyl malonate (90 mL, 0.59 mol, 4 equiv) was dissolved in anhydrous EtOH (300 mL) and cooled to 0 °C (ice bath). A 21% solution of NaOEt in EtOH (220 mL, 0.59 mol, 4 equiv) was added dropwise (within 10 min), followed by the removal of the cooling bath, and the reaction was stirred at room temperature for 15 min. Ethyl 5-amino-1-[(4-methoxyphenyl)-methyl]pyrazole-4-carboxylate **3** (40.4 g, 147 mmol, 1 equiv) was added in portions (within 2 min), and the reaction mixture was stirred under reflux for 5 days and then evaporated. The residue was diluted with H_2O (1.2 L) and neutralized to pH ≈ 5 using AcOH. The product was filtered off, washed with H_2O (200 mL), dried under vacuum, and used without further purification.

1-[(4-Methoxyphenyl)methyl]pyrazolo[3,4-*b*]-pyridine-4,6-diol (5). Ethyl 1-[(4-methoxyphenyl)methyl]-4,6-dioxo-7*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate **4** (48.4 g, 141.1 mmol, 1 equiv) was dissolved in 15% aqueous NaOH (500 mL) and stirred under reflux for 5 h. The mixture was cooled to 0 °C and carefully neutralized with AcOH until pH ≈ 5. White solid was filtered off, washed with H_2O (100 mL), and dried under vacuum (38 g, 87% over 2 steps). ESI MS $[M + H]^+$ for $C_{14}H_{14}N_3O_3$, calcd 272.1, found 272.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.1 (s, 1H), 7.18−7.09 (m, 2H), 6.88−6.80 (m, 2H), 5.26 (s, 2H), 5.09 (s, 1H), 3.68 (s, 3H).

4,6-Dichloro-1-[(4-methoxyphenyl)methyl]pyrazolo-[3,4-*b*]pyridine (6). 1-[(4-Methoxyphenyl)methyl]pyrazolo-[3,4-*b*]pyridine-4,6-diol **5** (38 g, 140.2 mmol, 1 equiv) and phenylphosphonic dichloride (79.5 mL, 560.8 mmol, 4 equiv) were stirred at 170 °C for 7 h, then cooled to ∼80 °C, and poured into vigorously stirred ice. Brown, sticky material precipitated that upon extensive stirring turned into a solid. The ice cold mixture was neutralized with concentrated aqueous NH_3 until pH ≈ 7, and the product was extracted using CH_2Cl_2 (2 × 400 mL). Combined organics were dried over $MgSO_4$, filtered, and evaporated to give product that was used without further purification (24 g, 55%). ESI MS $[M + H]^+$ for $C_{14}H_{12}Cl_2N_3O$, calcd 308.0, found 308.1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (s, 1H), 7.58 (s, 1H), 7.22−7.17 (m, 2H), 6.88−6.83 (m, 2H), 5.54 (s, 2H), 3.68 (s, 3H).

4,6-Dichloro-1*H*-pyrazolo[3,4-b]pyridine (1). 4,6-Dichloro-1-[(4-methoxyphenyl)methyl]pyrazolo[3,4-*b*]pyridine **6** (22 g, 71.4 mmol, 1 equiv) was dissolved in TFA (75 mL) and stirred at 60 °C for 12 h, then cooled down, and poured into H_2O (600 mL). A gray solid was filtered off, washed with saturated $NaHCO_3$ and then with H_2O, and dried under vacuum. ESI MS $[M + H]^+$ for $C_6H_4Cl_2N_3$, calcd 188.0, found 188.1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H), 7.53 (s, 1H).

4,6-Dichloro-1-[(4-methoxyphenyl)methyl]pyrazolo-[3,4-*b*]pyridine (6). To a mixture of 2-[(4-methoxyphenyl)-methyl]pyrazol-3-amine **7** (20 g, 98 mmol, 1 equiv) and Meldrum's acid **8** (14 g, 98 mmol, 1.0 equiv) in $POCl_3$ (91 mL, 980 mmol, 10 equiv) was added benzyltriethylammonium chloride (45 g, 196 mmol, 2 equiv), and the reaction mixture was heated to 110 °C for 16 h. At this point, the reaction mixture was cooled to rt and poured over crushed ice. The pH of the solution was carefully adjusted to 10 using 10 N NaOH solution and extracted into $CHCl_3$ (3 × 200 mL). Layers were separated, and combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. Solvent was removed in vacuo to give a crude residue that was purified by column chromatography (SiO_2, gradient 0% to 35% EtOAc in hexanes) to give intermediate **6** (7.55 g, 25%).

4,6-Dichloro-1*H*-pyrazolo[3,4-*b*]pyridine (1). To a mixture of 1*H*-Pyrazolo[3,4-*b*]pyridine **9** (4.75 g, 39.9 mmol, 1 equiv) in NMP (30 mL) was added *m*-CPBA (11.0 g, 47.9 mmol, 1.2 equiv) portionwise over 5 min. The reaction was stirred at rt for 1 h. Upon completion of reaction, MTBE (100 mL) was added and the mixture was stirred for 30 min at 0 °C. The reaction mixture was filtered, the filter cake was triturated with MTBE, and the resulting solid was dried under vacuum. The solid was dispersed in DMF (20 mL), and $POCl_3$ (10 mL, 107.3 mmol, 2.7 equiv) was added dropwise at 0 °C. The reaction was stirred at rt for 30 min. The reaction was then quenched with 20% $NaOH_{aq}$ until pH = 9 at 0 °C. The reaction mixture was diluted with water (20 mL) and filtered, and the filter cake was dried under vacuum to give a mixture of 4-chloro-1*H*-pyrazolo[3,4-*b*]pyridine **10** and 6-chloro-1*H*-pyrazolo[3,4-*b*]pyridine **11** (5.55 g, 90%) in a 2:1 ratio.

The previous mixture was dissolved in NMP (27 mL) and *m*-CPBA (10.8 g, 47.0 mmol, 1.3 equiv) portionwise over 5 min. The reaction was stirred at rt for 1 h, MTBE (100 mL) was added, and the mixture was stirred for 30 min at 0 °C. The reaction mixture was filtered, the filter cake was triturated with MTBE, and the solid was dried under vacuum. The resulting *N*-oxide intermediate (2.84 g, 16.7 mmol) was dispersed in CH_3CN (14 mL), and $POCl_3$ (6.2 mL, 67 mmol, 4 equiv) was added dropwise at 0 °C. The reaction was stirred at rt for 30 min. The reaction was then quenched with 20% $NaOH_{aq}$ until pH = 9 at 0 °C. The reaction mixture was diluted with water (20 mL) and filtered, and the filter cake was dried under vacuum to give 4,6-dichloro-1*H*-pyrazolo[3,4-*b*]pyridine **1** (2.49 g, 37%).

General Procedure for the Oxidation of 6-Chloro-1*H*-pyrazolo[3,4-*b*]pyridine (11). To a solution of trifluoroacetic anhydride (0.72 mL, 5.2 mmol, 4.0 equiv) in dimethoxymethane (1.9 mL) was added urea hydrogen peroxide (490 mg, 5.2 mmol, 4.0 equiv), and the reaction mixture was stirred at room temperature for 15 min. At this point, intermediate **11** (200 mg, 1.3 mmol, 1 equiv) was added in one portion and the reaction mixture was stirred at room temperature for an additional 2.5 h. The conversion was measured by LCMS.

*4-Chloro-1H-pyrazolo[3,4-b]pyridine **10** and 6-chloro-1H-pyrazolo[3,4-b]pyridine (**11**).* To a mixture of 1*H*-pyrazolo-[3,4-*b*]pyridine **9** (4.95 g, 41.6 mmol, 1 equiv) in NMP (32 mL) was added *m*-CPBA (11.5 g, 49.8 mmol, 1.2 equiv) portionwise over 5 min. The reaction was stirred at rt for 1 h. Upon completion of reaction, MTBE (100 mL) was added and

https://dx.doi.org/10.1021/acs.oprd.0c00469
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

the mixture was stirred for 30 min at 0 °C. The reaction mixture was filtered, the filter cake was triturated with MTBE, and the resulting solid was dried under vacuum to provide *N*-oxide **13** (5.10 g, 91%).

N-Oxide **13** (200 mg, 1.48 mmol, 1 equiv) was dispersed in solvent (1.5 mL), and the chlorinating reagent (5.92 mmol, 4 equiv) was added dropwise at 0 °C. The reaction mixture was then stirred at rt or 50 °C until consumption of starting material (see Table 2), quenched with 20% $NaOH_{aq}$, and extracted with EtOAc. The layers were separated, and the combined organic layers were washed with brine, evaporated, and dried over anhydrous Na_2SO_4. The ratio of 4-chloro-1*H*-pyrazolo[3,4-*b*]pyridine **10** and 6-chloro-1*H*-pyrazolo[3,4-*b*]-pyridine **11** was measured by ^1H NMR of the crude mixture.

Large Scale Synthesis of 4,6-Dichloro-1H-pyrazolo[3,4-b]pyridine (1). To a 1000 L reactor was charged NMP (400.0 kg) and 4-chloro-1*H*-pyrazolo[3,4-*b*]pyridine **10** (30.0 kg, 195.4 mol, 1 equiv). *m*-CPBA (77.5 kg, 449.4 mol, 2.3 equiv) was added portionwise below 20 °C. The reaction was stirred at 20 °C for 20 h. Upon completion of reaction, MTBE (440.0 kg) was added and the mixture was stirred for 1 h. The reaction mixture was filtered, the filter cake was triturated with MTBE (150.0 kg), the resulting slurry was filtered, and the filter cake was washed with MTBE (150.0 kg). The filter cake was dried at 45−50 °C to give *N*-oxide **15** (30.0 kg, 90.0% yield, 97.2 by HPLC) as a yellow solid. To a 500 L reactor were charged CH_3CN (265.0 kg), *N*-oxide **15** (28.0 kg, 165.1 mol, 1 equiv), and $Bn(Et)_3Cl$ (18.8 kg, 82.6 mol, 0.5 equiv). $POCl_3$ (51.3 kg, 330.2 mol, 2 equiv) was added below 20 °C. The reaction was stirred at 10−20 °C for 2 h. Upon completion of reaction, water (280.0 kg) was added below 20 °C and then stirred for 2 h. The reaction mixture was filtered. The filter cake was triturated with sat. $NaHCO_3$ solution (300.0 kg) and then diluted with water (280.0 kg), the resulting slurry was filtered, and the filter cake was dried at 45−50 °C to give crude product (23.0 kg) which was dissolved in EA (180.0 kg) at reflux and then filtered. The filtrate was concentrated to approximately 80 L and heptane was added (70.0 kg), the resulting mixture was cooled to 0−5 °C and filtered, and the filtered cake was dried to offer 4,6-dichloro-1*H*-pyrazolo[3,4-*b*]pyridine **1** (20.0 kg, 64.4% yield, 99.7% by HPLC) as an off-white solid.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.0c00469.

General information, ^1H and ^{13}C NMR spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Jeremy Fournier − *Arcus Biosciences, Inc., Hayward, California 94545, United States;* orcid.org/0000-0003-3162-3588; Email: jfournier@arcusbio.com

Authors

Xuelei Yan − *Arcus Biosciences, Inc., Hayward, California 94545, United States*

Anh T. Tran − *Arcus Biosciences, Inc., Hayward, California 94545, United States*

Rebecca L. Grange − *Arcus Biosciences, Inc., Hayward, California 94545, United States*

Steven D. Jacob − *Arcus Biosciences, Inc., Hayward, California 94545, United States*

Jaroslaw Kalisiak − *Arcus Biosciences, Inc., Hayward, California 94545, United States*

Kenneth V. Lawson − *Arcus Biosciences, Inc., Hayward, California 94545, United States;* orcid.org/0000-0001-5094-6337

Eric F. Connor − *Arcus Biosciences, Inc., Hayward, California 94545, United States*

Manmohan R. Leleti − *Arcus Biosciences, Inc., Hayward, California 94545, United States*

Jay P. Powers − *Arcus Biosciences, Inc., Hayward, California 94545, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.oprd.0c00469

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

ADO, adenosine monophosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; CD73, cluster of differentiation 73, ecto-5′-nucleotidase; CD39, cluster of differentiation 39; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; NMP, *N*-methylpyrrolidone; *p*TsOH, *para*-toluenesulfonic acid; $T_{1/2}$, half-life; TFA, trifluoroacetic acid; TFAA, trifluoroacetic anhydride; THF, tetrahydrofuran; PMB, *para*-methoxybenzyl; SAR, structure−activity relationship; EtOH, ethanol; *m*CPBA, *meta*-chloroperoxybenzoic acid; DME, 1,2-dimethoxyethane; MTBE, methyl *tert*-butyl ether; AcOH, acetic acid; MsCl, methanesulfonyl chloride; rt, room temperature; EtOAc, ethyl acetate.

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https://dx.doi.org/10.1021/acs.oprd.0c00469
Org. Process Res. Dev. XXXX, XXX, XXX−XXX