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Process Research and Kilogram Synthesis of an Investigational, Potent MEK Inhibitor

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ABSTRACT: TAK-733 (**1**) is an investigational, novel MEK kinase inhibitor that bears a 6-fluoropyridopyrimidone core. Process research of **1** was conducted, and an efficient, scalable route was developed. The key intermediate, a multisubstituted fluoropyridone, was formed in one pot via a three-step cascade reaction: condensation between α-fluoromalonate and malononitrile, methyl amide formation, and intramolecular cyclization. Chlorination of the hydroxyl functionality and cyclization with formic acid provided the desired pyridopyrimidone core in high yield. Subsequent *N*-alkylation with the nosylate of (*R*)-glycerol acetonide and displacement of the chlorine with 2-fluoro-4-iodoaniline proceeded successfully with good yields. Final acid-catalyzed deprotection of the acetonide functionality followed by a controlled crystallization protocol afforded the active pharmaceutical ingredient (API) with the desired polymorph. Compared to the initial synthesis, this route was more concise (six steps compared to the original nine steps), and the overall yield was improved significantly (from 3% to 25%). These improvements allowed for production of multikilograms of **1**.

1. INTRODUCTION

MEK kinases (mitogen-activated protein kinase kinases, also known as MAPK/ERK kinases) regulate the RAF−MEK−ERK pathway that mediates proliferative and anti-apoptotic signaling factors which promote tumor growth, progression, and metastasis.[1] This pathway is inappropriately activated in 30% of human cancers.[2] Therefore, there have been considerable research activities in targeting the inhibition of MEK as a potential strategy to provide cancer treatment. Recently, several MEK inhibitors have been reported to be in phase I and phase II clinical trials for evaluation in oncology.[3] TAK-733 (**1**) was discovered in our research laboratories as a potent and selective ATP-noncompetitive investigational MEK inhibitor.[4] It has demonstrated effective anticancer activity in mouse xenograft models of human cancers including melanoma, colorectal, NSCLC, pancreatic, and breast cancer. This compound is currently in phase I clinical trials for the treatment of cancer. In order to support the preclinical and clinical studies, an efficient, scalable, and economical synthetic route was needed.

2. DISCUSSION AND RESULTS

2.1. Initial Chemistry Synthesis. TAK-733 (**1**) was first prepared from 6-(methylamino)pyrimidin-4(3*H*)-one (**2**) in nine steps in 2.9% overall yield (Scheme 1).[4] Benzyl protection of **2** followed by high-temperature cyclization with diethylmalonate[5] afforded the bicyclic pyridopyrimidone intermediate **4** in moderate yield. Chlorination of **4** and subsequent C−N coupling with 2-fluoro-4-nitroaniline under Buchwald conditions installed the aniline moiety. Then, nitro reduction of **5** and Sandmeyer reaction gave the corresponding iodide **6**. Next, alkylation of **6** with (*S*)-4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane (**7**) and acidic deprotection provided the penultimate intermediate **8**. Finally, fluorination of **8** gave the desired product TAK-733 (**1**) in low yield. While this route enabled the production of gram quantities of **1**, there were several opportunities for improvement including high-temperature conditions to form the bicyclic core **4**, a low-yielding final fluorination step, and the high cost of two starting materials **2** and **7**. In addition, multiple chromatographic purifications were required along the synthetic pathway.

2.2. Synthetic Strategy of the Development Route. After careful evaluation of the initial synthesis, we considered that a new synthetic strategy was desired for larger-scale synthesis (Scheme 2). First of all, the low-yielding fluorination step in the initial route significantly decreased the synthetic efficiency. In addition, the high-temperature condition to form the pyridone moiety in the initial synthesis would not be convenient for the development process. This transformation involved ketene formation, and it is well-known that the generation of ketene from its malonate precursor usually requires reaction temperature above 200 °C.[5a] Thus, it would be difficult to form the desired pyridone ring from the pyrimidone intermediate **2**. Finally, it was also necessary to simplify the fluoroiodoaniline installation process in the downstream chemistry.

We envisioned and implemented an alternative strategy that would incorporate the fluorine at position C-6 from a commercially available material to give fluoropyridone ring **9** first, followed by low-temperature formation of the bicyclic pyridopyrimidone core **10** (Scheme 2).[6] Preparation of **9** was supported by a similar pyridine synthesis reported by

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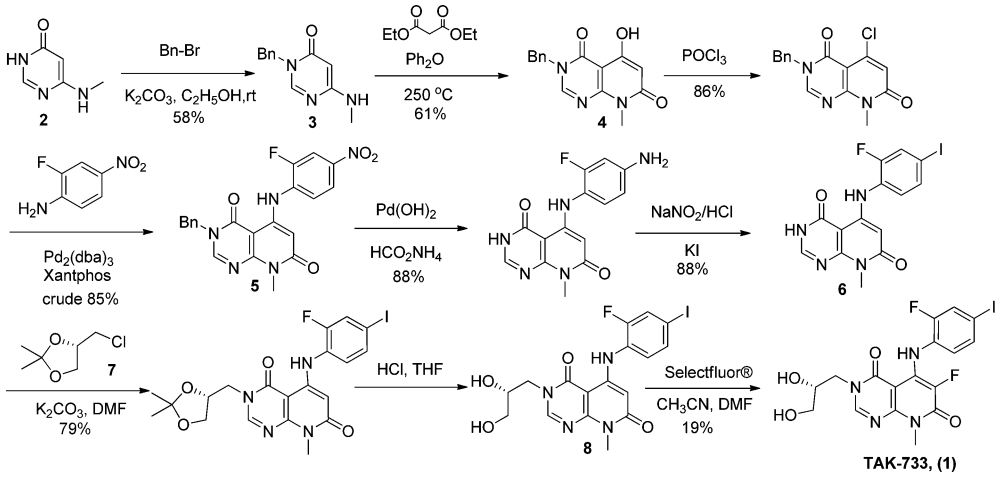
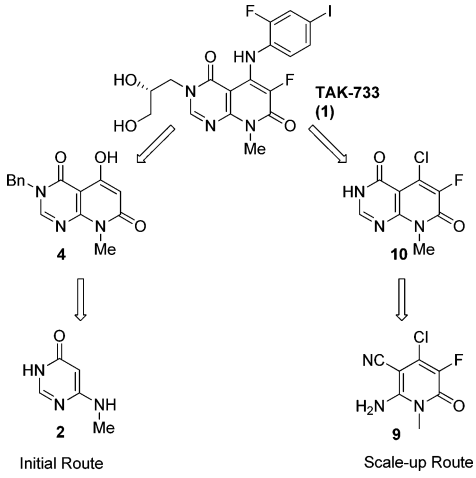
Scheme 1. Initial synthesis of TAK-733 (1)



Scheme 2. Synthetic strategy comparison between the initial and the development routes



Kantlehner and co-workers.[7] Furthermore, we also simplified the fluoroiodoaniline installation process by eliminating the use of palladium.

2.3. Novel Methodology in Preparation of 6-Fluoropyridopyrimidone Core 10. Literature reports provided examples of the synthesis of multisubstituted pyridine-2-thiones from ketene-*S*,*N*-acetals and malononitrile.[8] Several synthetic approaches utilizing α-fluorocarbonyl derivatives and malononitrile as building blocks were explored, and one such approach was demonstrated to be an efficient and scalable route. In this approach, the key accomplishment was the successful establishment of the 6-fluoropyridopyrimidone core **10** (Scheme 3). Condensation between dimethyl α-fluoro malonate (**11**) and malononitrile followed by methyl amide formation and intramolecular cyclization afforded the densely functionalized 5-fluoropyridone product **12** in one pot. Pyridopyrimidone core **10** was subsequently synthesized in high yield via chlorination and cyclization with formic acid.

A key step in this newly developed synthesis was the effective formation of the pyridone intermediate **12**. This one-pot transformation included three cascade reactions (Table 1). Dimethyl α-fluoro malonate (**11**) was first condensed with malononitrile under basic conditions to form intermediate **13**. Treatment of **13** with methyl amine provided methylamide **14**.

Scheme 3. Synthesis of 6-fluoropyridopyrimidone core 10

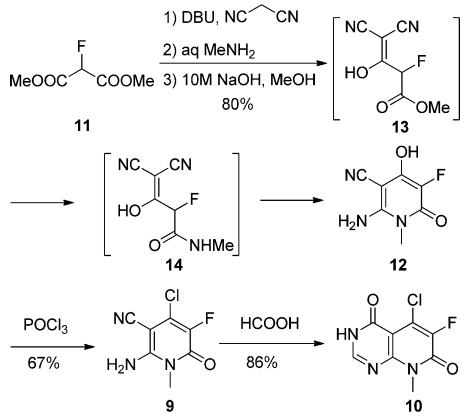
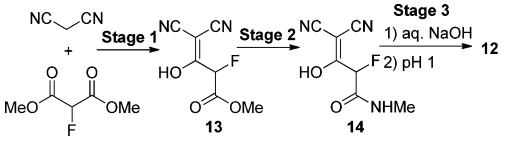


Table 1. Three-step, one-pot synthesis of 12



stage 1 base/solvent	stage 2 $MeNH_2$/solvent	yield (%)
NaOMe/MeOH	MeOH	~35
NaOMe/THF	MeOH	no pdt
NaOMe/DMF	THF	no pdt
KO*t*Bu/THF	MeOH	no pdt
KO*t*Bu/*t*BuOH	MeOH	no pdt
Et_3N/THF	MeOH	no rxn
DBU/THF	MeOH	80
DBU/THF	H_2O	80

Then base-promoted intramolecular cyclization of **14** afforded the desired product **12**. Base screening indicated that nucleophilic bases such as NaOMe readily reacted with malononitrile to form methyl imidate and caused low or no yields of product **12**. Furthermore, among non-nucleophilic bases DBU was found to be the best choice. When KO*t*-Bu was used, decomposition of fluoro malonate was observed. On the other hand, Et_3N did not promote any reaction. These results indicated that the pK_a of the base needed to be similar to those of the two substrates. In addition, it was observed that a proton source was necessary to induce the amide formation and

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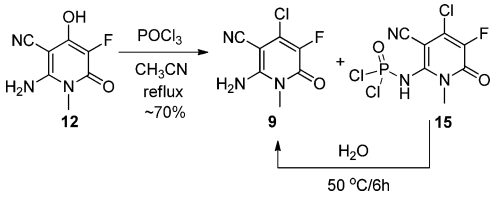
subsequent intramolecular cyclization. When THF or DMF was used without the addition of a proton source in the second step (water or alcohol as cosolvent), intermediate **14** failed to form. Once cyclization reached completion, the reaction mixture was acidified with concentrated HCl to pH 1−2, at which point product **12** precipitated and was isolated by filtration in good purity (>99%) and yield (80%).

Chlorination of **12** was carried out using $POCl_3$ (3 equiv) in CH_3CN at reflux temperature. Upon reaction completion, the reaction mixture was quenched by ice−water. Product **9** was precipitated and collected by filtration (Scheme 4). Upon

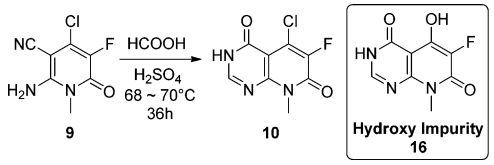
Scheme 4. Chlorination studies



scaling, it was observed that varying amounts of the phosphoramidic dichloride byproduct **15** were present in the filtrate and the isolated yield was also decreased (from 60% down to 45%). As suggested in the literature,[9] the filtrate was reheated to 55 °C for 5 h in order to hydrolyze the phosphoramidic dichloride moiety. During heating, a second crop of product **9** precipitated and was collected by filtration again. The combined yield was 67% on kilogram scale, and both crops of the product were isolated in high purity (>98%).

Preparation of the pyrimidone ring was accomplished using a slightly modified literature procedure.[10] Chloride **9** (Scheme 5)

Scheme 5. Preparation of pyridopyrimidone 10

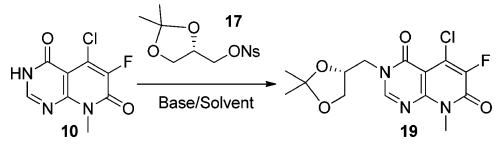


was first suspended in formic acid (42 equiv) and heated to provide a solution. Addition of 9 N sulfuric acid promoted the nitrile hydrolysis, formamide condensation, and subsequent cyclization to give product **10**. After the reaction reached completion, the mixture was cooled and diluted with ice-cold water and filtered to afford the desired bicyclic product in >85% yield and >98% purity. The major impurity in the isolated product was found to be **16** according to LC/MS data. We discovered that the use of high-grade formic acid (99%) and 96+% sulfuric acid could minimize the water content of the reaction mixture and resulted in better control of impurity **16**, giving <3% of **16** in the completed reaction mixture and <2% in the isolated product.

2.4. Process Development of Downstream Chemistry. After detailed process development of **10** was accomplished, the research focus shifted to downstream chemistry. The first challenge was to replace the expensive alkyl chloride **7** with a cheaper starting material (Scheme 1). Furthermore, the reaction did not go to completion upon scale-up. The second challenge was to simplify the three-step installation of 2-fluoro-4-iodoaniline on the bicyclic core. This transformation was not efficient and involved unsafe diazotization. Thus, a more effective direct aniline displacement needed to be developed. Finally, multiple polymorphic forms were isolated from both the initial synthesis and polymorph screening studies.[11] Therefore, it was necessary to investigate the final deprotection step carefully to control the formation of desired polymorph form A.

After thorough process development, alternative and less expensive acetonide-protected (*R*)-glycerol nosylate **17** was found to be suitable for the alkylation.[12] Due to the poor solubility of substrate **10**, DMA and THF were used initially in order to achieve higher conversion (Table 2). Since nosylate **17**

Table 2. Alkylation of the pyridopyrimidine core 10 with nosylate 17



base/solvent	temp (°C)	time (h)	conversion (%)	*N*-/*O*-alkylation
LiHMDS/DMA	60	27	96	82/18
KtOBu/DMA	60	27	79	79/21
K_2CO_3/DMA	60	27	61	79/21
NaHMDS/THF	60	30	<5	N/A
NaHMDS/DMA	60	30	91	81/19
KHMDS/THF	60	30	<5	N/A

was found to be unstable above 60 °C in DMA, initial reaction condition screening was carried out at 60 °C. Among the limited bases screened, LiHMDS (1.1 equiv) in DMA gave complete consumption of the starting material **10**. Under these conditions, only 1.1 equiv of nosylate **17** was needed to drive the alkylation to completion. However, the reaction generated a significant amount of the undesired *O*-alkylation product **18** (~20%) along with the desired *N*-alkylation product **19**. During aqueous workup, a mixture of these two isomers was isolated as a solid. Then, pure *N*-isomer **19** was obtained by recrystallization in isopropyl acetate (overall isolated yield 53%, HPLC purity >99.5%).

Further optimization was carried out to improve regioselectivity in the alkylation reaction. With DBU as base, the isomeric ratio was improved to 10:1 in favor of the desired product **19** when alcohols were used as solvents (Table 3). On the other hand, a new impurity which was formed from solvent alkylation at levels between 5−15% was observed but was readily purged during workup. While MeOH afforded the greatest regioselectivity, it also led to the formation of up to 15% of methylation side product.[13] When IPA was used as solvent, 8:1 regioselectivity was maintained with only ~8% of the analogous isopropyl impurity. Therefore, IPA was selected as the optimal solvent. In addition to DBU, a few amine bases such as triethylamine, Hunig's base, and imidazole were also tested and showed similar selectivity. Although using some of these amines minimized the alkylation impurity, a significant drop in purity of the isolated product (from >97% to 90%) occurred with the current workup procedure. Therefore, DBU was eventually selected as the optimal base for the manufacturing runs. As a result of the reduced amount of **18** in the reaction mixture, the recrystallization was eliminated

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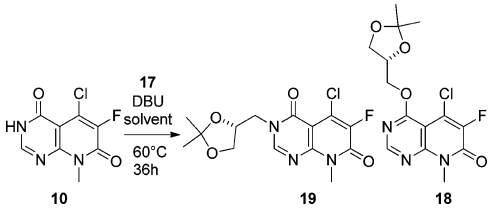
Table 3. Alkylation of the pyridopyrimidine core 10 with DBU as base

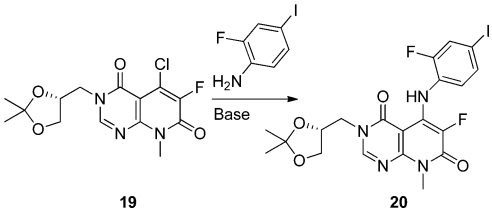


solvent	ratio of **19**/**18**
THF	6:1
ACN	6:1
acetone	6:1
IPA	8:1
MeOH	10:1
t-BuOH	6:1
s-BuOH	8:1

from the workup, and the desired product **19** was obtained in comparable quality and >70% isolated yield.

2.5. Preparation of Penultimate Intermediate 20. A palladium-catalyzed coupling was used to install 2-fluoro-4-iodoaniline (Scheme 6) in initial attempts. We then decided to explore the possibility of a base-promoted direct displacement reaction. Our initial studies revealed that the reaction proceeded successfully without the use of palladium catalysis. Two equivalents of strong base were needed to drive the reaction to completion. Presumably, one equivalent of base was needed to neutralize the HCl formed in the reaction, and the other equivalent was to deprotonate a sufficient amount of 2-fluoro-4-iodoaniline. In the end, the second full equivalent of base deprotonated the −*NH* functionality of product **20** due to its lower pK_a compared to that of 2-fluoro-4-iodoaniline. Among bases and solvents screened, LiHMDS/THF was found to give the best result. It was also observed that accurate stoichiometric control of the base was the key to the successful aniline displacement since two major impurities were observed upon scale-up when more than 2 equiv of base was dosed.[14] In the early phase of development, the level of each impurity could reach as high as 10%. Removal of these two impurities relied on recrystallization of the crude product using DMA and water. After systematic condition adjustment, it was found that the usage of 1.95 equiv of LiHMDS minimizes the impurity formation and ensures the completion of the reaction. Titration of LiHMDS before each use ensured the accurate dose of the base. Other factors that could impact the level of impurities were also investigated. Among them, high agitation rate was found to be the key to avoid high local concentrations of base. Another factor impacting the impurity profile was found to be the addition temperature which was optimal between −5 and 0 °C. Under the optimized conditions, chloride **19** and fluoroiodoaniline (1.0 equiv) were first dissolved in anhydrous THF and cooled to −5−0 °C, LiHMDS/THF (~1.95 equiv) was added in a controlled manner so that the internal temperature was maintained between −5−0 °C. The reaction was slowly warmed to room temperature. Upon reaction completion, MeOH/water workup followed by pH adjustment, precipitation, and filtration led to the isolation of product **20** as a light-brown solid (>97% purity) in >85% yield. The product isolated from these optimized conditions was pure enough for use in the next synthetic step.

Scheme 6. Preparation of penultimate intermediate 20



2.6. Synthesis of TAK-733 (1, Form A). In the first development process, the crude penultimate precursor **20** was dissolved in DCM and washed twice with aqueous AcOH solution before inline filtration. This procedure was to remove inorganic impurities and residual DMA prior to the final deprotection step. After polish filtration and solvent swap to ethanol, 9 N sulfuric acid was added at elevated temperature to induce the deprotection. TAK-733 (**1**) precipitated from the reaction mixture and was isolated by simple filtration with good purity (>98%). On the basis of the polymorph studies, the crude product was usually a mixture of amorphous solid and undesired Form H according to DSC and XRPD analyses.[15] Subsequent polymorph conversion studies showed that this crystal mixture could be readily converted to the desired Form A in alcoholic solvents when heated above 45 °C. Upon scaling, the crude TAK-733 solid was suspended in MeOH and heated to 55 °C for 24 h. After form conversion was confirmed by DSC and XRPD spectra (Figure 1), the final active pharmaceutical ingredient (API) (**1**, Form A) was isolated by filtration.

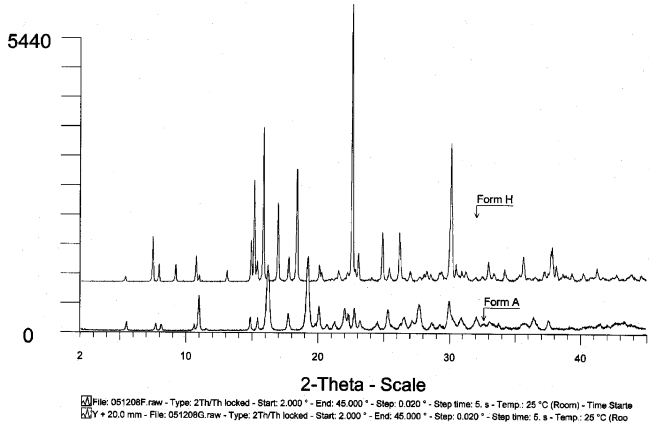


Figure 1. XRPD spectra comparison between TAK-733 Forms A and H.

A much improved process was developed later that enabled a direct isolation of TAK-733 as Form A without an additional form conversion step (Scheme 7). In this process, penultimate intermediate **20** was dissolved in NMP/MeOH. The deprotection was completed in 3 h with addition of conc. HCl. Adjustment of the NMP/MeOH ratio set TAK-733 (**1**) into the metastable zone before Form A seeds were added. Further slow addition of MeOH as an anti-solvent at 50 °C and cooling to 5 °C resulted in steady growth of Form A. The suspension was cooled to 5 °C before filtration. In a recent 10 kg GMP production, the API was obtained as the desired Form A with high yield (86%) and excellent purity (>99.5% both chemical and chiral).

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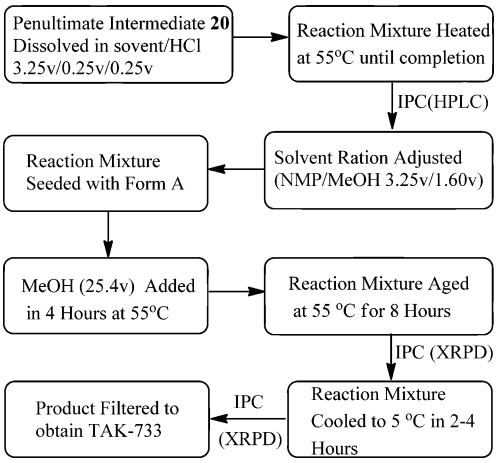
Scheme 7. Process flow diagram of the final step synthesis



3. CONCLUSION

An efficient and scalable synthetic route of a potent MEK inhibitor, TAK-733 (**1**), was developed (Scheme 8).[6] The highlights of the synthesis included (1) a novel three-step, one-pot synthesis of highly substituted fluoropyridone; (2) a highly chemoselective alkylation of pyridopyrimidone core; and (3) a simplified installation of the fluoroiodoaniline. In addition, a robust process was developed to control the final API polymorph. This new synthetic route allowed for the effective incorporation of fluorine, decreased cost-of-goods, and significantly improved overall yield. The synthesis was successfully carried out in several GMP campaigns on up to 10-kg scale.

4. EXPERIMENTAL SECTION

4.1. General. LiHMDS/THF was tested first on small scale to determine the accurate potency. Assay yields were obtained using analytical standards prepared by recrystallization or preparative chromatography. All isolated yields reflect correction for purity based on HPLC assays. Polymorph forms were characterized by X-ray diffraction. The peaks were measured using a powder diffractometer equipped with a copper source, primary beam monochromator, and position sensitive detector. The incident beam was collimated using a 1° divergence slit. The source was operated at 40 kV and 30 mA. X-ray powder diffraction data were collected from 3° to 45°, a step width of 0.04°. The spectrometer was well calibrated with a silicon standard.

4.2. HPLC Methods. *4.2.1. HPLC Purity of TAK-733 (**1**, Area %).* Phenomenex Luna C8(2) 100 Å, 3 μm, 4.6 mm × 150 mm, gradient elution from 95:5 to 35:65 0.1% H_3PO_4 in water/0.1% H_3PO_4 in MeCN over 20 min, from 35:65 to 5:95 0.1% H_3PO_4 in water/0.1% H_3PO_4 in MeCN over 5 min, isocratic elution with 5:95 0.1% H_3PO_4 in water/0.1% H_3PO_4 in MeCN over 2 min, 1.0 mL/min flow at 40 °C with detection at 285 nm. HPLC retention times: **9** = 10.5 min, **10** = 7.8 min, **17** = 18.5 min, **19** = 14.3 min, 2-fluoro-4-iodoaniline =17.6 min, **20** = 21.4 min, TAK-733 (**1**) = 15.3 min.

*4.2.2. Chiral HPLC method for TAK-733 (**1**).* Chiralpak AS-RH, 5 μm, 150 mm × 4.6 mm, isocratic elution with 50:40:10 20 mM NH_4OAc/MeCN/2-propanol over 10 min, 0.8 mL/min flow at 25 °C with detection at 285 nm. HPLC retention times: TAK-733 (**1**) = 4.7 min, enantiomer of **1** = 3.9 min.

*4.2.3. Chiral HPLC Method for **19**.* Chiralpak AD-RH, 150 mm × 4.6 mm, 5 μm, isocratic elution with 55:43:2 water/MeCN/2-propanol over 15 min, 1.0 mL/min flow at 35 °C with detection at 240 nm. HPLC retention times: **19** = 8.9 min, enantiomer of **19** = 11.1 min.

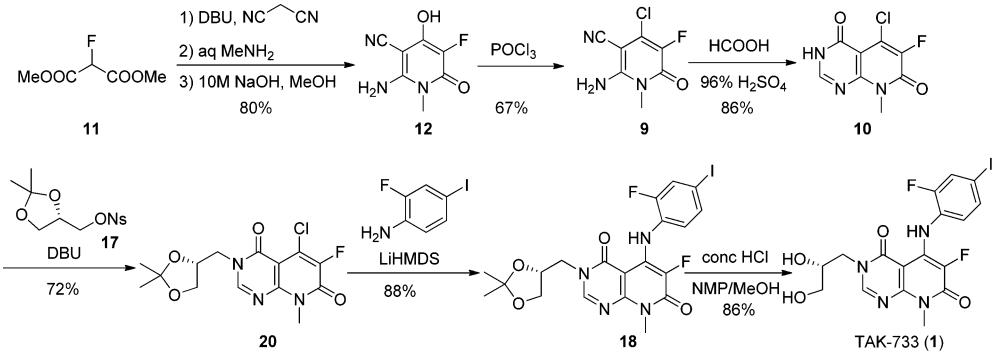
*4.2.4. Chiral HPLC Method for **20**.* Chiralpak OJ-RH, 150 mm × 4.6 mm, 5 μm, isocratic elution with 40:60 water/MeCN over 15 min, 1.0 mL/min flow at 35 °C with detection at 285 nm. HPLC retention times: **20** = 8.6 min, enantiomer of **20** = 7.9 min.

*4.2.5. Chiral HPLC Method for **17**.* Chiralpak AD-RH, 150 mm × 4.6 mm, 5 μm, isocratic elution with 40:60 water/MeCN over 20 min, 0.8 mL/min flow at 40 °C with detection at 254 nm. HPLC retention times: **17** = 12.8 min, enantiomer of **17** = 11.7 min.

*4.2.6. HPLC Method for Monitoring the Formation of Intermediate **13** and **14**.* Phenomenex Luna C8, 3 μm, 4.6 mm× 150 mm, gradient elution from 95:5 to 10:90 0.05% H_3PO_4 in water/MeCN over 9 min, isocratic elution with 10:90 0.05% H_3PO_4 in water/MeCN over 2.5 min, isocratic elution with 95:5 0.05% H_3PO_4 in water/MeCN over 2 min, 1.0 mL/min flow at 40 °C with detection at 225 and 250 nm. HPLC retention times: **13** = 6.8 min, **14** = 6.0 min.

4.3. 2-Amino-5-fluoro-4-hydroxy-1-methyl-6-oxo-1,6-dihydropyridine-3-carbonitrile (12). Dimethyl α-fluoro malonate (**11**, 3.00 kg, 20.0 mol) and malononitrile (1.32 kg, 20.0 mol) were dissolved in THF (15 L) and cooled to −25 ± 5 °C. DBU (6.09 L, 39.9 mol) was added over 5 h at <10 °C. Then the reaction mixture was allowed to slowly warm to rt over 2 h and stirred for another 16 h. The reaction progress was monitored with HPLC (Method 4.2.6.). Aqueous methylamine (40%, 14.1 L, 133 mol) was added dropwise, and the reaction

Scheme 8. Process route of TAK-733 (1)



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mixture was stirred for another 2 h at rt. Then 10 N NaOH (3.0 L, 30 mol) was added, and the reaction was stirred at rt for 5 h. The methylamine and THF were removed by rotavap with a 35 °C bath under 600−650 mmHg vacuum. The resulting mixture was cooled to 0 ± 5 °C followed by pH adjustment to ∼1−2 using conc. HCl (8.50 L). The resulting solid was collected by filtration, washed with water (2 × 6.0 L), and dried in vacuum oven at 60 °C until the moisture content of the product was below 10% by KF. Product **12** was obtained as a light-brown solid (3.00 kg, 80% yield, 99.6% purity, moisture content 9.28 wt %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.71 (s, 1H), 7.29 (s, 2H), 3.27 (s, 3H); ^{13}C NMR (100 MHz, DMSD-d_6) δ 154.7 (d, J = 21.9 Hz), 153.1, 151.4 (d, J = 13.2 Hz), 129.5 (d, J = 211 Hz), 115.4 (d, J = 3.7 Hz), 63.2 (d, J = 2.9 Hz), 28.7; ^{19}F NMR (376 MHz, DMSD-d_6) δ −178.9; MS (M + H)$^+$ m/z calcd 184.0, found 184.0.

4.4. 2-Amino-4-chloro-5-fluoro-1-methyl-6-oxo-1,6-dihydropyridine-3-carbonitrile (9). Compound **12** (3.00 kg, 16.4 mol) was mixed with anhydrous CH_3CN (30.0 L) before $POCl_3$ (7.54 kg, 49.2 mol) was added very slowly through addition funnel to ensure the internal temperature at <50 °C. After addition was completed, the reaction mixture was heated to reflux (70−74 °C) for 3−5 h until **12** was consumed completely (monitored by HPLC). After the reaction was completed, the reaction mixture was cooled to rt before slowly being added to ice−water (45.0 L, 10 volumes) with stirring over 45 min. During this process, product precipitated out. The resulting solid was collected by filtration and washed with water (15.0 L). The first crop of the product was dried in a vacuum oven at 60 °C overnight to provide the desired product **14** as a light-brown solid (1.80 kg, 55%, HPLC purity 99.8%). The mother liquor (containing 38% byproduct according to HPLC) and the wash water were then combined and transferred to a reactor (equipped with condenser under N_2) and heated to 55 °C for 5 h until most of the impurity peak had disappeared (<5%). The mixture was then cooled to rt before being filtered, washed with CH_3CN/H_2O (1v:1v, 20 L), and dried over vacuum at 60 °C overnight to provide the second crop of **9** as a light-brown solid (0.41 kg, 12% yield, 98% purity). HPLC retention time (Method 4.2.1.): 10.5 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (s, 2H). 3.33 (s, 3H); ^{13}C NMR (100 MHz, DMSD-d_6) δ 154.2, 153.3 (d, J = 17.6 Hz), 138.5 (d, J = 227.6 Hz), 128.9 (d, J = 16.8 Hz), 115.3, 68.1, 29.7 (d, J = 1.4 Hz); ^{19}F NMR (376 MHz, DMSD-d_6) δ −152.0; MS (M + H)$^+$ m/z calcd 202.0, found 202.0.

4.5. 5-Chloro-6-fluoro-8-methylpyrido[2,3-*d*]pyrimidine-4,7(3*H*,8*H*)-dione (10). Compound **9** (8.10 kg, 40.2 mol) was added to the reactor followed by 99% formic acid (77.4 kg, 1.68 kmol). Stirring started, and the reaction mixture was heated to 80 °C (bath temperature) until **9** was dissolved completely. Then 96+% H_2SO_4 (56.5 kg) was added slowly through addition funnel at <60 °C. After addition, the reaction solution was stirred at 75 ± 5 °C for 20 h and monitored by HPLC. After the reaction was complete (level of **9** <4% according to HPLC assay), the reaction mixture was cooled at <5 °C before ice−water (80 kg) was charged slowly with stirring while maintaining the internal temperature <15 °C. During this process, the product precipitated out. After completion of water addition, the whole mixture was stirred for another 30 min at 10 ± 10 °C. The mixture was then filtered, washed with H_2O (2 × 40 kg), and dried in the vacuum oven at 55 °C until <0.5% of water was detected by KF. This provided **10** as yellow solid (7.90 kg, 85.6% yield, 98.2% purity) HPLC retention time (Method 4.2.1.): 7.8 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (s, 1H), 8.35 (s, 1H). 3.61 (s, 3H); ^{13}C NMR (100 MHz, DMSD-d_6) δ 157.7 (d, J = 4.4 Hz), 154.6 (d, J = 26.4 Hz), 151.2 (d, J = 2.2 Hz), 149.9, 146.4 (d, J = 242 Hz), 125.0 (d, J = 16.9 Hz), 99.94, 29.8; ^{19}F NMR (376 MHz, DMSD-d_6) δ −133.8; MS (M + H)$^+$ m/z calcd 230.0, found 230.0.

4.6. (*S*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 4-nitrobenzenesulfonate (17). (*R*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methanol (6.80 kg, 51.5 mol) was dissolved in ethyl acetate (35.4 kg) followed by the addition of triethylamine (6.50 kg, 64.3 mol). After the solution was cooled to 5 ± 5 °C, a solution of nosyl chloride (11.9 kg, 53.70 mol) in ethyl acetate (30 kg) was added through addition funnel at <10 °C. Upon addition completion, the reaction mixture was warmed to rt. After 1 h, the reaction was completed according to HPLC analyses. Water (40 kg) was added, and the mixture was stirred for 15 min before the organic layer was separated, washed with water (2 × 40 kg) and brine (40 kg), and dried over sodium sulfate (10 kg). The dried solution was then filtered and partially distilled down to 46 L over 1 h. The temperature of the distillation was kept <35 °C, and the vacuum was kept between 667 and 677 mmHg. Subsequently, heptane (14 kg) was added, and the mixture was further distilled down to about 34 L total volume under the same conditions over 2 h. Finally, more heptane (27 kg) was added, and the mixture was condensed down to ∼49 L total over 2 h before being cooled to 10 ± 5 °C with stirring. During this process, white solid precipitated from the solution. The resulting solid was collected by filtration, rinsed with heptane (2 × 5.0 kg), and dried under vacuum at <30 °C to yield the desired nosylate as a white solid (12.7 kg, 77.8% yield, 99.5% purity, and 99.5% ee). Data of **17**: HPLC retention time (Method 4.2.1.): 18.5 min; chiral HPLC retention times (Method 4.2.5.): **17** = 12.8 min, enantiomer of **17** = 11.7 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (d, 2H, J = 8.8 Hz), 8.20 (d, 2H, J = 8.8 Hz), 4.28 (m, 1H), 4.25 (dd, 1H, J = 10.8, 3.2 Hz), 4.08 (dd, 1H, J = 11.2, 7.2 Hz), 3.96 (dd, 1H, J = 8.8, 6.8 Hz), 3.63 (dd, 1H, J = 8.4, 5.2 Hz), 1.22 (s, 3H), 1.22 (s, 3H); MS (M + H)$^+$ m/z calcd 318.1, found 318.0.

4.7. (*R*)-5-Chloro-3-((2,2-dimethyl-1,3-dioxolan-4-yl)-methyl)-6-fluoro-8-methylpyrido[2,3-*d*]pyrimidine-4,7-(3*H*,8*H*)-dione (19). The reaction mixture of **10** (9.46 kg, 41.2 mol), **17** (15.6 kg, 49.2 mol), DBU (9.75 L, 65.3 mol) in IPA (42.2 L) was stirred at 63 °C for 63 h. HPLC analysis indicated <2% **10** remaining. The reaction mixture was cooled to 50 °C followed by slow addition of *i*-PrOAc (7.57 L) over 1 h. Then water (95.6 L) was slowly added at 50 °C. The resulting suspension was stirred at 50 °C for 30 min before being cooled to rt. The mixture was filtered, and the isolated solid was rinsed with water (3 × 18.9 L). This product **19** was collected [after drying to a water content ≤0.30% under vacuum in a Nutsche filter drier (≤50 °C)] as an off-white solid (9.3 kg, 66% yield, 99% pure). HPLC retention time (Method 4.2.1.): 14.3 min; chiral HPLC retention times (Method 4.2.3.): **19** = 8.9 min, enantiomer of **19** = 11.1 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 1H), 4.38 (m, 1H), 4.22 (dd, 1H, J = 13.6, 3.6 Hz), 4.07 (dd,1H, J = 8.4, 6.4 Hz), 3.94 (dd, 1H, J = 13.6, 7.6 Hz), 3.76 (dd, 1H, J = 8.8, 5.2 Hz), 3.61 (s, 3H), 1.37 (s, 3H), 1.24 (s, 3H); ^{13}C NMR (100 MHz, DMSD-d_6) δ 157.0 (d, J = 3.6 Hz), 154.5 (d, J = 25.6 Hz), 152.4, 150.5 (d, J = 2.2 Hz), 146.8 (d, J = 243 Hz), 124.9 (d, J = 17.6 Hz), 109.1, 99.1, 72.3, 66.1, 48.8, 29.7, 26.5, 25.0; HRMS (QSTAR) (M + H)$^+$ m/z calcd 344.0808, found 344.0799. Data of *O*-alkylated isomer **18**: ^{1}H

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NMR (400 MHz, DMSO-d_6) δ 8.76 (s, 1H), 4.57 (d, 2H, J = 4.8 Hz), 4.51 (quintet, 1H, J = 5.2 Hz), 4.13 (dd, 1H, J = 8.4, 6.8 Hz), 3.88 (dd, 1H, J = 8.0, 6.0 Hz), 3.66 (s, 3H), 1.35 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (100 MHz, DMSD-d_6) δ 165.5 (d, J = 5.8 Hz), 156.4, 154.8 (d, J = 26.4), 152.1, 147.8 (d, J = 248 Hz), 121.8 (d, J = 18.3 Hz), 108.9, 98.0, 72.9, 67.9, 65.7, 29.4, 26.6, 25.2; ^{19}F NMR (376 MHz, DMSD-d_6) δ −126.8; HRMS (QSTAR) (M + H)$^+$ m/z calcd 344.0808, found 344.0799.

4.8. (*R*)-3-((2,2-Dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-(2-fluoro-4-iodophenylamino)-8-methylpyrido-[2,3-*d*]pyrimidine-4,7(3*H*,8*H*)-dione (20). To a mixture of **19** (9.3 kg, 27 mol) and 2-fluoro-4-iodoaniline (6.5 kg, 27 mol) in THF (48.92 L) was added LiHMDS (0.99 M in THF, 47.2 kg, 54 mol) while keeping the internal temperature between −5 and 0 °C. After addition, the reaction mixture was stirred at −5 to 0 °C for 1 h before it was warmed to rt with stirring overnight. HPLC analysis indicated <2% **19** remaining. A premixed solution of MeOH/water (9.3 L/9.3 L) was slowly added at rt. During the addition, a suspension formed. Then 1 N HCl in 1:1 MeOH/water was slowly added while keeping the internal temperature below rt until the pH was adjusted to 7.5−6.5. The resulting suspension was stirred at rt for 4 h before filtration. The filter cake was rinsed with water/MeOH (3v:1v) (3 × 46.2 L). Product **20** was collected as an off-white solid (13.6 kg, 93% yield, 99% pure) after drying to a water content of ≤0.30% under vacuum in a Nutsche filter (≤50 °C). HPLC retention times (Method 4.2.1.): **20** = 21.4 min, 2-fluoro-4-iodoaniline = 17.6 min; chiral HPLC retention times (Method 4.2.4.): **20** = 8.6 min, enantiomer of **20** = 7.9 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (s, 1H), 8.60 (s, 1H), 7.68 (d, 1H, J = 10.4, 2.0 Hz), 7.52 (d, 1H, J = 8.8 Hz), 6.96 (td, 1H, J = 8.8, 6.0 Hz), 4.41 (m, 1H), 4.25 (dd, 1H, J = 13.6, 3.6 Hz), 4.06 (dd, 1H, J = 8.8, 6.4 Hz), 3.98 (dd, 1H, J = 13.6, 7.6 Hz), 3.78 (dd, 1H, J = 9.2, 5.6 Hz), 3.58 (s, 3H), 1.37 (s, 3H), 1.23 (s, 3H); ^{13}C NMR (100 MHz, DMSD-d_6) δ 161.1, 155.7 (d, J = 10.1 Hz), 154.3 (d, J = 218 Hz), 150.5, 134.6, (d, J = 199 Hz), 133.7 (d, J = 7.0 Hz), 133.1, 127.8 (d, J = 8.0 Hz), 125.3, 124.0, 123.8, 109.1, 95.1 (d, J = 4.4 Hz), 87.1 (d, J = 7.3 Hz), 72.2, 66.1, 48.9, 28.8, 26.6, 25.0; ^{19}F NMR (376 MHz, DMSD-d_6) δ −124.5, −149.3; MS (M + H)$^+$ m/z calcd 545.0, found 545.0.

4.9. (*R*)-3-(2,3-Dihydroxypropyl)-6-fluoro-5-(2-fluoro-4-iodophenylamino)-8-methylpyrido[2,3-*d*]pyrimidine-4,7(3*H*,8*H*)-dione (TAK-733, 1, Form A). A mixture of **20** (13.6 kg, 25.0 mol), NMP (44.0 L), MeOH (3.45 L), and conc. HCl (4.08 kg) was heated at 55 °C until a solution was formed. After stirring at 55 °C for 2 h, HPLC analysis indicated <1% **18** remaining. The reaction mixture went through polish filtration and was heated back to 55 °C. MeOH (27 L) was added followed by TAK-733 seed crystals (131 g, Form A). MeOH (334 L) was slowly added over 3 h. After addition of MeOH, the resulting suspension was held at 55 °C for 2 h and cooled down to 0 °C in 4 h. After holding at 0 °C for 9 h, the solid was filtered and rinsed with MeOH (3 × 60 L). The final API **1** was obtained as a white solid (10.6 kg, 84% yield, >99% purity) after drying to a methanol content of ≤3000 ppm and a NMP content of ≤530 ppm under vacuum in a Nutsche filter drier (≤50 °C). HPLC retention time (Method 4.2.1.): 15.3 min; chiral HPLC retention times (Method 4.2.2.): **1** = 4.7 min, enantiomer of **1** = 3.9 min; chiral purity (>99.5% ee according to chiral HPLC); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.24 (s, 1H), 8.52 (s, 1H), 7.69 (dd, 1H, J = 10.4, 1.8 Hz), 7.52 (d, 1H, J = 8.6 Hz), 6.98 (m, 1H), 5.14 (br s, 1H), 4.83 (br s, 1H), 4.32 (dd, 1H, J = 12.9, 2.5 Hz), 3.76 (m, 1H), 3.67 (dd, 1H, J = 13.1, 12.9 Hz), 3.58 (s, 3H), 3.46 (ddd, 1H, J = 10.9, 5.3, 5.1 Hz), 3.38 (m, 1H); ^{13}C NMR (100 MHz, DMSD-d_6) δ 161.3 (d, J = 4.0 Hz), 155.6 (d, J = 22.8 Hz), 154.6 (d, J = 250 Hz), 152.0, 150.6, 134.3 (d, J = 231 Hz), 133.8 (d, J = 7.1 Hz), 133.1 (d, J = 3.0 Hz), 127.8 (d, J = 10.3 Hz), 125.3 (d, J = 7.0 Hz), 123.9 (d, J = 21.5 Hz), 95.0 (d, J = 4.0 Hz), 87.1 (d, J = 7.8 Hz), 68.0, 63.8, 50.1, 28.8; ^{19}F NMR (376 MHz, DMSD-d_6) δ −124.4, −149.8; MS (M + H)$^+$ m/z calcd 505.0, found 505.0; Form A was found to have the following peaks in degrees 2θ, rounded to 2 significant figures (relative intensity): 11.03 (34%), 15.88 (15%), 16.26 (100%), 19.32 (90%), 20.11 (15%), 22.16 (23%), 26.66 (17%), 27.84 (33%), and 30.18 (17%).

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Notes

The authors declare no competing financial interest.

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(13) LC/MS and MS/MS analyses indicated that this impurity is either the *N*-methylated or *O*-methylated pyridopyrimidone core. The mechanism of this byproduct formation is still not clear.

(14) Preliminary LC/MS analysis showed that these two impurities contained the same molecular weight (twice as the mass of desired product **20**) and fragmentation pattern; thus, they could be the diastereo-dimers of the product.

(15) On gram-scale synthesis, Form H was usually observed as the single polymorph form isolated directly from reaction mixture. Upon scale-up (a few hundred grams scale), the mixture of amorphous solid and Form H was observed.