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Exploratory Process Development of a Novel Diacylglycerol Acyltransferase-1 (DGAT-1) Inhibitor

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Supporting Information

ABSTRACT: A practical large-scale synthesis was developed for **1**, a DGAT-1 inhibitor, involving an aza-Michael reaction, amidation, Dieckman cyclization, and conjugate addition of cyanamide followed by cyclization, to form the fused 4-amino-7,8-dihydropyrido[4,3-*d*]pyrimidin-5-one scaffold. The enabled process presented here substantially improved safety (in particular, due to eliminating a nitration step and optimizing a high-energy intermediate step), reproducibility, and scalability, resulting in delivery of a multikilogram quantity of the API with high purity. The controls of API quality and particle size were also discussed.

INTRODUCTION

Enzyme acyl-CoA:diacylglycerol acyltransferase-1 (DGAT-1) catalyzes the rate-limiting step in triglyceride synthesis. It has recently emerged as an attractive target for therapeutic intervention in the treatment of Type II diabetes and obesity.[1] As part of the program aimed at finding an orally active API, a series of highly potent and selective DGAT-1 inhibitors was recently discovered carrying a novel 4-amino-7,8-dihydropyrido[4,3-*d*]pyrimidin-5-one scaffold.[2] Compound **A** (Figure 1) was first identified as a promising lead to inhibit the DGAT-1-mediated pathway. Afterwards, analogue **1** demonstrated a better biological profile and a wider therapeutic index (TI) and was therefore selected for clinical development. Due to the structural similarity of these two compounds, useful synthetic solutions initially found for compound **A** were adapted to expedite scale-up development of **1** with the aim of quick delivery for regulatory toxicity and early clinical studies. Overall results of this work evidenced the efficiency of the general synthetic strategy originally employed by our medicinal chemists. Herein we describe the early process development work that allowed for successful transition from gram-scale laboratory preparation to multikilogram production of **1** in a kilo lab.

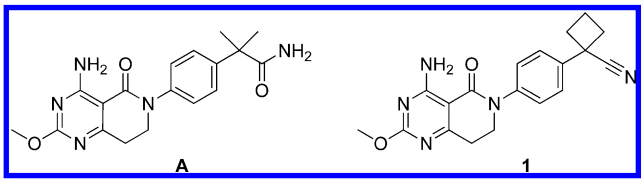

Figure 1. Structures of compounds **A** and **1**.

RESULTS AND DISCUSSION

Synthetic Route Selection. The synthesis outlined in Scheme 1 was the original discovery route to compound **1** that was prepared as part of 4-amino-7,8-dihydropyrido[4,3-*d*]-pyrimidin-5-one series of DGAT-1 inhibitors. This strategy, after thorough investigation of several modes of retrosynthetic bond disconnection, proved to be reliable and versatile in expansive analogue production. This synthesis started with aza-Michael reaction of aniline **2** to ethyl acrylate, followed by N-acylation with cyanoacetic acid, leading to intermediate **3**, which was converted to the key vinylogous carbamic acid **4** through a Dieckmann cyclization.[3] Upon conversion of **4** to the corresponding chloride **5** and further to vinyl methylcarbamate **6**, the latter was reacted with cyanamide under basic conditions to afford the penultimate intermediate **7**. The 2-methoxy-4-aminopyrimidine system formation, leading to target **1**, was effected by acid-catalyzed addition of methanol.[4] While many well thought-out solutions were involved in the overall discovery route design, especially the efficient building of the 4-amino-7,8-dihydropyrido[4,3-*d*]pyrimidin-5-one scaffold, multikilogram delivery of API required further investigation to ensure high purity and yield as well as to enable suitable process safety, reproducibility, and operational efficiency on a kilogram scale. The API quality control, including impurity profile, residual solvent content, polymorph forms, and particle size distribution, was also addressed.

Preparation of Aniline 2. 1-Aminophenyl-cyclobutanecarbonitrile **2** was synthesized by reduction of the corresponding nitro compound **8**, which itself was prepared via two different pathways as depicted in Scheme 2. In the original medicinal chemistry approach, nitration of 1-phenyl-cyclobutanecarbonitrile **9** with potassium nitrate in concentrated sulfuric acid at 0 °C cleanly afforded **8** in 90% yield. However, these reaction conditions were expected to have a potential run-away hazard during scale-up, as a delayed sharp exothermic event occurred on a 10 g scale. In an effort to find homogeneous conditions suitable for a safer flow process, we unsuccessfully examined a milder variant of batch nitration, in particular using a solution of 70% aqueous nitric acid in acetic acid at 70 °C. We also envisioned that **8** could be prepared by cycloalkylation[5] of readily available nitrile **10**, which already carried the nitro

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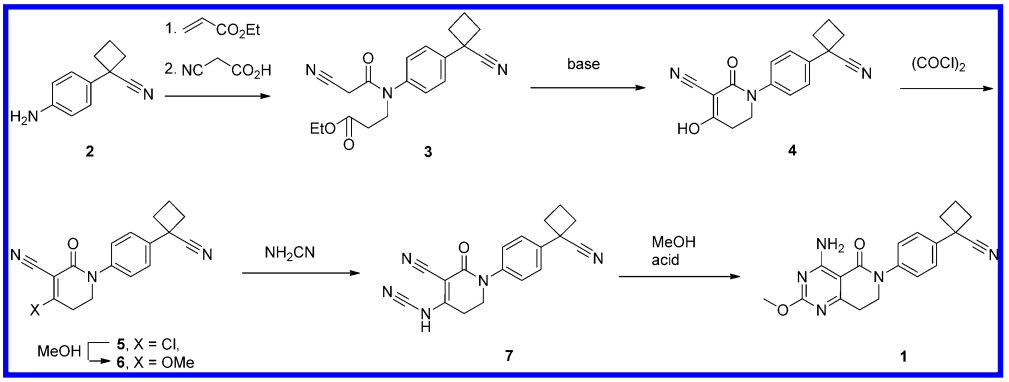
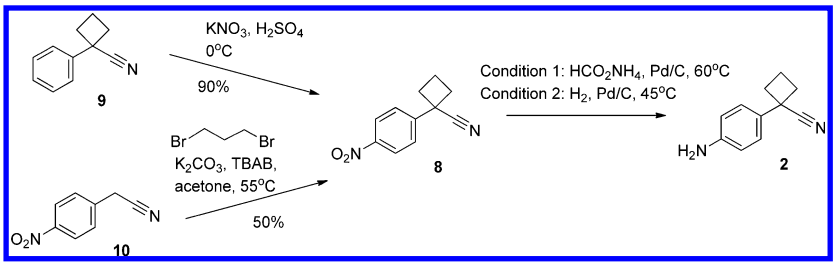
Scheme 1. Medicinal Chemistry synthetic route to target 1



Scheme 2. Synthesis of the starting aniline 2



group. After screening a number of alkylating reactants, bases, solvents, and phase transfer catalysts at various temperatures, optimal conditions were found that employed 1.2 equiv of 1,3-dibromopropane, 2.6 equiv of potassium carbonate, and 0.1 equiv of tetrabutylammonium bromide (TBAB) in acetone at 55 °C. The reaction profile showed ~80% of **8** with low levels of three side products (Figure 2). Purification via silica gel plug

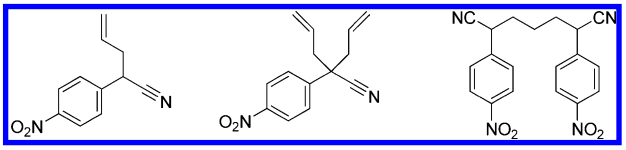


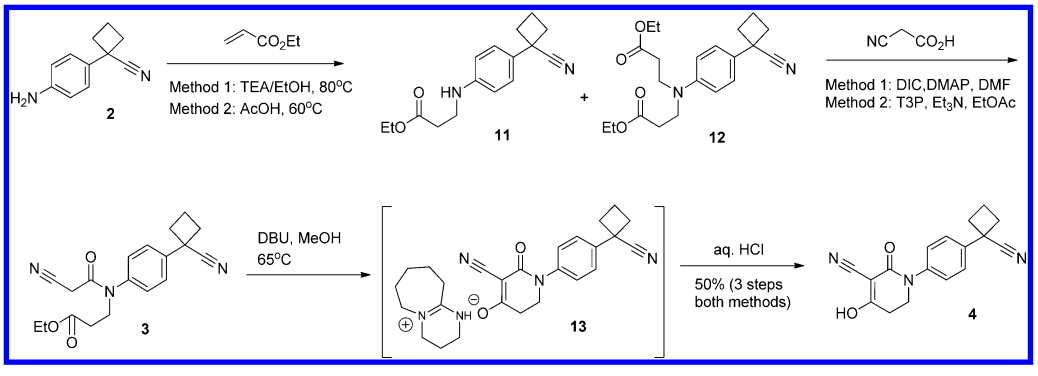
Figure 2. Byproducts of cyclobutanation.

filtration, followed by recrystallization afforded **8** in 49–56% yields with >95% purity (HPLC). Even though the cyclobutanation approach gave lower throughput, it was still selected for scale-up due to safety considerations, and 8.5 kg of **8** was successfully produced.

In the original discovery synthesis, transfer hydrogenation with ammonium formate and dry 10% palladium on carbon cleanly converted **8** to **2**. Our safety protocol for scale-up, however, required using wet palladium on carbon (containing 50% of water), which unexpectedly led to significantly more byproducts. Additionally, formation of ammonium formate in the reflux condenser under the reaction conditions (70 °C) became a serious safety issue. Other transfer hydrogenation reagents, e.g., formic acid and cyclohexadiene, did not improve the reaction profile. Instead, the conventional hydrogenation with hydrogen gas (30–50 psi) in the presence of wet 5% palladium on carbon (10 wt % loading) in methanol cleanly converted **8** to aniline **2** at a temperature above 45 °C. The exothermic process was controlled between 45 to 60 °C by the adjusting hydrogen feeding rate, agitation speed, and cooling capacity, and aniline **2** was obtained in 98% yield.

Preparation of Vinylogous Carbamic Acid 4. A telescoped three-step conversion was initially developed for a multigram scale to make the key intermediate **4** as shown in Scheme 3 (Method 1). Aniline **2** was reacted with excess ethyl acrylate (2 equiv) through an aza-Michael reaction in

Scheme 3. Telescoped Route to Key Intermediate 4



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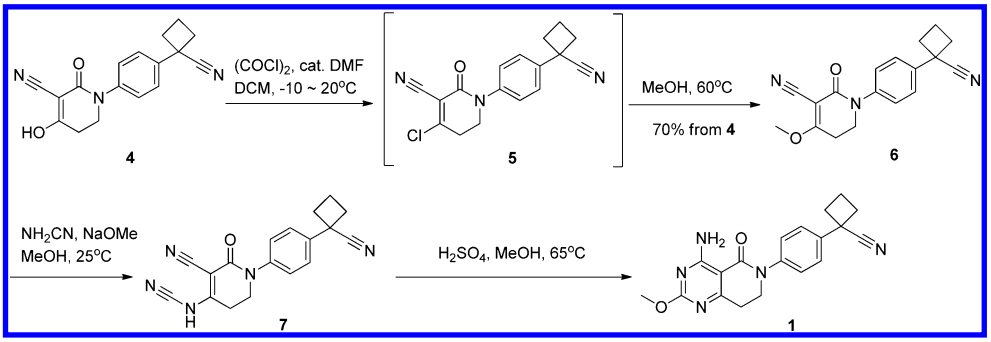
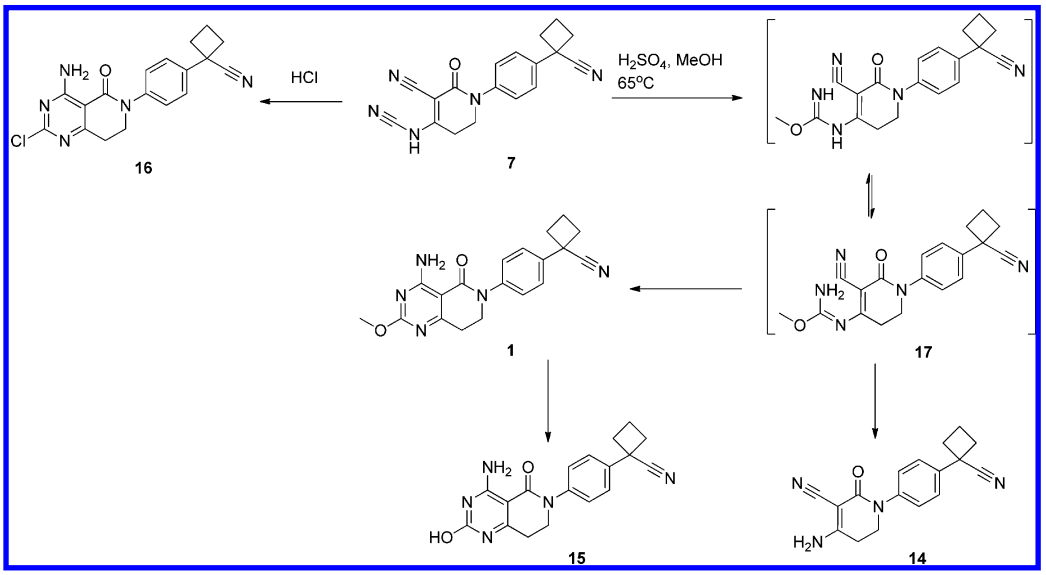
Scheme 4. Synthesis of 1



Scheme 5. Formation of 1 and impurities



triethylamine and ethanol at 80 °C to give a mixture of the desired monoalkylated **11** and dialkylated side product **12** in ~4:1 ratio. Without purification, this mixture was subjected to two sequential chemical conversions: *N*-acylation with cyanoacetic acid in the presence of *N,N′*-diisopropylcarbodiimide (DIC) and DMAP in DMF to give intermediate **3**, and then an intramolecular Dieckmann-type cyclization with DBU in THF to afford key intermediate **4**. An efficient isolation protocol that utilized the acidic nature of vinylogous carbamic acid **4** was developed as follows. Partition of the reaction mixture between water and ethyl acetate kept most lipophilic impurities, including **12**, in the organic layer, while extracting **4** into the aqueous phase as the DBU salt **13**. Acidification of the aqueous phase with hydrochloric acid resulted in precipitation of **4** in 96.2% purity (HPLC). Using this method resulted in ~10 g of intermediate **4** prepared in 50% yield over three steps.

This strategy was adopted for scale-up; enabling work was focused on improving process safety and efficiency and led to conditions outlined as Method 2 (Scheme 3). Ethyl acrylate is known to undergo polymerization at elevated temperature,[6] and therefore excess reagent should be minimized. The use of acetic acid as the reaction solvent successfully reduced the amount of ethyl acrylate required for the reaction to 1.1 equiv and lowered the reaction temperature to 60 °C. The new conditions resulted in a mixture of **11**, **12**, and **2** with the ratio of 15:1:1. The original *N*-acylation using *N,N′*-diisopropylcarbodiimide (DIC) as the amide coupling reagent generated diisopropyl urea, which was difficult to purge and complicated intermediate isolation in the downstream process. T3P (propanephosphonic acid anhydride, cyclic trimer, 50% solution in ethyl acetate)[7,8] was found to be an excellent substitute. Reaction of crude **11** with cyanoacetic acid in the presence of T3P and TEA in EtOAc and MTBE afforded crude **3** with >90% HPLC purity. Dieckmann-type condensation of crude **3** was effected by DBU in refluxing THF. Solvent switch from THF to EtOAc, followed by water extraction, effectively removed **12** and other lipophilic impurities, and product **4** was precipitated by adjusting the pH of the aqueous layer with hydrochloric acid to pH < 2. Trituration with methanol enhanced the purity of **4** to >98%. The yields were in the range of 50−54% over three steps on a kilogram scale.

Building the Pyrimidine Ring To Form Target 1. Ten grams of **1** was quickly prepared for early biological tests (Scheme 4). *O*-Methylation of **4** was carried out as two transformations in one pot: chlorination by Vilsmeier method[9] with oxalyl chloride in the presence of catalytic DMF (10 mol %) in dichloromethane at −10 to 20 °C led to vinylogous acid chloride **5**; methanolysis of the latter at 60 °C provided vinylogous methyl carbamate **6** in ~70% yield. Reaction of **6** with cyanamide in the presence of sodium methoxide in methanol afforded the penultimate compound **7**, which precipitated during the reaction and was isolated by filtration in 90% yield. While direct conversion of **5** to **7** was possible, this reaction was lower yielding, and hydrolysis of **5** to the

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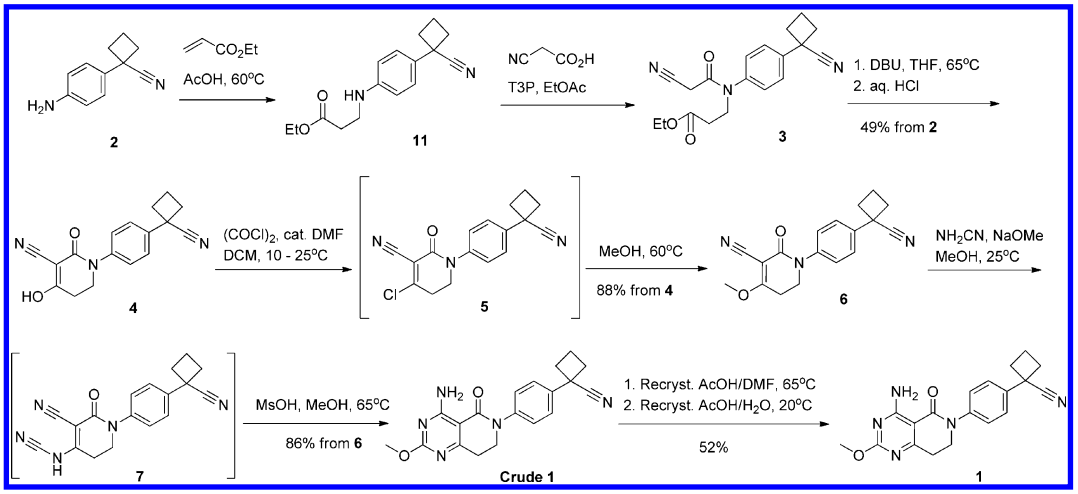
Scheme 6. Enabled process for 1



starting material **4** was observed. Treatment of **7** with hot methanol in the presence of 1 equiv of sulfuric acid generated *O*-methylisourea **17**, which cyclized in situ to form the desired 2-methoxy-4-aminopyrimidine system (Scheme 5). Crude **1** was precipitated out by neutralizing the reaction mixture with 1 N KOH. The isolated crude **1** was typically contaminated with 10−20% of each of the side products **14** and **15**.

The strategy described above was adopted with the following modifications to improve process safety and scale-up feasibility. The rate of off-gassing during the chlorination stage was controlled by increasing the initial temperature from −10 to 10 °C, reducing the amount of DMF to 5% mol equiv and adding oxalyl chloride (1.2 equiv) at a rate that allowed maintaining the reaction temperature below 15 °C. A scrubber of aqueous NaOH was also introduced to trap hydrogen chloride. Dilution of the reaction mixture with methanol at 35 °C for 20 h smoothly converted **5** to **6**. Removal of dichloromethane by evaporation resulted in full precipitation of **6** and 5.2 kg (largest-scale run) of this material was obtained as a white powder in 88% yield with 96.2% HPLC purity (Scheme 6).

Analysis of the DSC showed that the penultimate intermediate **7** had an energy potential of −510.4 J/g with an onset temperature of 91 °C. Our safety policy required at least 100 °C difference between the process temperature and the DSC onset temperature for scale-up of high-energy compounds, so it was decided not to isolate intermediate **7**. Thus, base-promoted cyanamidation and acid-promoted cyclization were carried out in one pot (Scheme 6). In addition, excess cyanamide was minimized as the reagent had a very high energy potential (−1461.7 J/g) with low onset temperature of 108.5 °C. Compound **6** was cleanly converted to **7** in 2 h at 20 °C with 1.1 equiv of cyanamide and 1.2 equiv of sodium methoxide in methanol. The reaction mixture was then subjected to strong acid at elevated temperature to effect the cyclization. Similar to the original synthesis, sulfuric acid (2.5 mol equiv) afforded a relatively clean cyclized target product **1** (with ∼75% HPLC purity). However, the original isolation protocol did not work well in the new process because neutralization with aqueous NaOH or KOH resulted in a large amount of sodium or potassium sulfates that coprecipitated with **1** as a very fine solid and made the product filtration extremely slow. Methanesulfonic acid (2.8 mol equiv) was found to be more effective for the cyclization and generated a slightly better reaction profile. It was critical that sodium and potassium methanesulfonates had good solubility in aqueous methanol and remained soluble after neutralization with aqueous KOH. When sulfuric acid was replaced with methanesulfonic acid (Scheme 6), the filtration was dramatically improved and 5.1 kg of crude **1** was obtained by this method in 86% yield with 81% HPLC purity mostly associated with two major impurities **14** and **15** at ∼7% each. It should be noted that weaker acids, such as trifluoroacetic, phosphoric, *p*-toluenesulfonic, acetic, and citric acids, resulted in low conversion; hydrochloric acid led predominantly to chlorinated product **16** (Scheme 5)

Purification of 1 To Meet the Purity Requirement for Clinical Use. Target **1** exhibited a unique solubility profile. The drug substance was very soluble in acetic acid, partially soluble in hot DMF, DMAc or DMSO, but poorly soluble in other organic solvents and water. The original two-step purification protocol afforded **1** in 20−40% yields on gram scale: trituration with hot methanol mainly purged off **14** to enhance the purity to ∼85%, which was followed by recrystallization in hot aqueous acetic acid to further upgrade purity to >98% with <1% of **15**. The latter operation purged most impurities but also hydrolyzed the labile methoxy group to convert a small amount of **1** back to side product **15** at elevated temperature. Consequently, both yield and purity varied dramatically from batch to batch, especially during scale-up syntheses. All attempts to recrystallize crude **1** in acetic acid and water at ambient temperature resulted in unacceptable purity enhancement. The need for an effective purification protocol made it necessary to thoroughly investigate new conditions. Compound **1** had a good solubility response to temperature in mixtures of 5−20% acetic acid with other polar solvents, such as MeOH, EtOH, acetone, MeTHF, DMF, and DMAc. In these mixtures, it dissolved at 60−70 °C and crystallized out when cooled. However, only a combination of acetic acid with DMF or DMAc demonstrated efficiency to purge impurities. The final process involved dissolving crude **1** in DMF and acetic acid at 65 °C, followed by cooling to −20 °C at rate of 1 °C per minute and cold filtration. The purified **1** was obtained in 56% yield as a white powder with 99.3% HPLC purity and no single impurity over 0.2%. Raising the final temperature to 0 °C resulted in an additional ∼10% loss in yield. The main drawback of this purification was that the isolated **1** contained high level of residual DMF (>0.5 wt %), which was impossible to lower by drying, probably due to formation of a solvate.

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In order to purge DMF to acceptable ICH levels for clinical use,[10] recrystallization in an acetic acid/water mixture at ambient temperature was applied. It should also be pointed out that only one polymorph, Form I, was obtained in all conditions of recrystallization. However, the particle size was typically too large to get adequate dissolution and content uniformity for regulatory toxicity studies and clinical trials due to its low water solubility (0.01 mg/mL at pH 6.5). Therefore, high-shear, wet milling technology was adopted in the final recrystallization to achieve the recommended particle size D[4,3] < 30 μm and D[v, 0.95] < 60 μm. The above purified **1** was dissolved in acetic acid (3v) at 35 °C, filtered through 0.5 μm in-line filter and cooled to 20 °C. Addition of water (12v) over 1.5 h resulted in crystallization of **1**. The slurry was further cooled to 10 °C and wet-milled. As a result, 2.6 kg of target **1** was obtained in 93% recovery with 99.63% HPLC purity. The particle size distribution met the recommended standard with D[4,3] = 25 μm and D[v, 0.95] = 58 μm.

CONCLUSIONS

A novel DGAT-1 inhibitor **1** was synthesized from aniline **2** through an efficient Dieckmann-type cyclization to form vinylogous carbamic acid **4**, followed by cyanamide incorporation and acid-promoted cyclization. The process was developed for scale-up by telescoping many conversions and designing safety into the process. Compared to the original Medicinal Chemistry synthesis, the overall safety, robustness, and reproducibility were significantly improved; HPLC purity of the final product was increased from 99.08% to 99.63%. The enabled process, summarized in Scheme 6 provided 2.6 kg of API in overall 24% yield, whereas the best yield achieved in the original synthesis was 14%. The particle size distribution was also controlled through wet milling.

EXPERIMENTAL SECTION

General. Liquid chromatography mass spectrometry (LCMS) was performed on an Agilent 1100 Series (Waters Atlantis C18 column, 4.6 mm × 50 mm, 5 μm; 95% water/acetonitrile linear gradient to 5% water/acetonitrile over 4 min, hold at 5% water/acetonitrile to 5 min, trifluoroacetic acid modifier (0.05%); flow rate = 2.0 mL/min). Reaction monitoring and purity of intermediates and the final compound were checked by HPLC in the following conditions: Column: Zorbax SB-CN, 5 μm, 4.6 mm × 150 mm; Column Temperature: 30 °C; Flow Rate: 2 mL/min; Detection: UV @ 210 nm; Mobile phase: A: 0.2% phosphoric acid in water, B: Acetonitrile; Linear Gradient: from 95% of A to 5% of A within 15 min. HPLC purity was reported at 210 nm wavelength.

1-(4-Nitrophenyl)cyclobutanecarbonitrile (8). A mixture of acetone (80 L), powdered potassium carbonate (15.35 kg, 111 mol), 2-(4-nitrophenyl)acetonitrile (**10**, 7.0 kg, 43.2 mol), 1,3-dibromopropane (5.13 L, 50.6 mol), and tetrabutylammonium bromide (1.0 kg, 3.1 mol) was stirred at 55 °C for 20 h. The reaction was cooled to 20 °C, and solids were filtered off with an acetone wash. The filtrates were concentrated in vacuo at 50 °C to ~20 L. The residual slurry was extracted with MTBE (3 × 12 L) at about 50 °C. The combined MTBE extracts were concentrated in vacuo at 50 °C, and the residue was loaded on a short 20 kg silica gel column. The column was eluted with a 1:2 mixture of ethyl acetate and heptane. The obtained solution was concentrated in vacuo until a precipitate began to form (residual volume was about 40 L). The slurry was cooled to 0 °C and stirred for 2 h. Solid was filtered off and dried in vacuo to obtain the title compound as a straw-colored solid (4.25 kg, 49%). HPLC purity was 95%. ^{1}H NMR ($CDCl_3$) δ 2.11−2.19 (m, 1H), 2.46−2.56 (m, 1H), 2.63−2.70 (m, 2H), 2.87−2.95 (m, 2H), 7.62 (d, J = 8.8 Hz, 2H), 8.27 (d, J = 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 17.2, 34.9, 40.2, 123.3, 124.4, 126.9, 146.8, 147.6; GC/MS: m/z = 202 (M).

1-(4-Aminophenyl)cyclobutanecarbonitrile (2). A 10 L autoclave reactor purged with nitrogen was charged with **8** (505 g, 2.5 mol), wet 5% palladium on carbon (8 g), and methanol (8 L). The reactor was pressurized with 50 psi of hydrogen. The mixture was agitated for 3 h under 45−55 psi of hydrogen gas, maintaining reaction temperature below 60 °C with external cooling. Upon cessation of hydrogen uptake, the reaction mixture was cooled to 20 °C. Hydrogen was vented and replaced with nitrogen. The catalyst was filtered off through Celite, and the filter cake was washed with methanol while being kept under flow of nitrogen. The combined filtrates were concentrated in vacuo to give the crude title compound as a colorless oily residue, which solidified upon standing (422 g, 98%). HPLC purity was over 95.8%. ^{1}H NMR spectrum and MS were consistent with the literature data.[2b,11] ^{1}H NMR ($CDCl_3$) δ 2.00−2.09 (m, 1H), 2.34−2.42 (m, 1H), 2.52−2.60 (m, 2H), 2.74−2.82 (m, 2H), 3.79 (br s, 2H), 6.70 (d, J = 8.80 Hz, 2H), 7.20 (d, J = 8.80 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 16.9, 34.8, 39.6, 115.3, 124.8, 126.6, 129.7, 145.8; LC/MS (API^+) m/z: 173.1 $[M + 1]^+$; mp 48−49 °C.

Ethyl 3-(4-(1-Cyanocyclobutyl)phenylamino)-propanoate (11). A mixture of **2** (7.16 kg, 41.58 mol), ethyl acrylate (4.9 L, 44.94 mol), and acetic acid (7.3 L, 127.7 mol) was stirred at 60 °C for 2 d. The mixture was cooled to 0 °C, and MTBE (50 L) was added. The obtained mixture was successively washed with 0.5 M hydrochloric acid (15 L), water (10 L), and 10% wt aqueous potassium bicarbonate (10 L). Each aqueous solution was sequentially extracted with an additional portion of MTBE (4 L). The combined organic layers were concentrated in vacuo to ~9 L of the residual volume.[12] This obtained solution of crude **11** was directly used in the next step without further purification (assumed 100% yield of **11** for calculations).

Ethyl 3-(2-Cyano-*N*-(4-(1-cyanocyclobutyl)phenyl)-acetamido)propanoate (3). The above solution of crude **11** in MTBE (assumed 41.5 mol) was combined with ethyl acetate (40 L), cyanoacetic acid (3.2 kg, 37.6 mol), and triethylamine (12 L, 86.7 mol). The mixture was cooled to −5 °C and 1-propanephosphonic acid cyclic anhydride (T3P, 50% in ethyl acetate, 20 L, 34.0 mol) was added over 2 h while maintaining the temperature below 0 °C. After addition was complete, the reaction mixture was stirred at −5 °C for 1 h, then warmed to 20 °C in 2 h and stirred at this temperature for 18 h. The reaction mixture was diluted with ethyl acetate (40 L) and successively washed with 10% aqueous potassium bicarbonate (40 L), 0.5 M hydrochloric acid (20 L), and 25% wt sodium chloride (20 L). The organic layer was concentrated in vacuo to ~12 kg of the residual oil. This crude **3** was directly used in next step without further purification (assumed 100% yield of **3** for calculations).

1-(4-(1-Cyanocyclobutyl)phenyl)-4-hydroxy-2-oxo-1,2,5,6-tetrahydropyridine-3-carbonitrile (4). A mixture of the above crude **3** (assumed 41.5 mol), 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU, 5.6 L, 37.4 mol), and THF (80 L) was stirred at 60 °C for 1 h. The reaction mixture was cooled to 20 °C, then water (80 L) and ethyl acetate (60 L) were added

successively. The biphasic mixture was stirred for 30 min at 20 °C and the aqueous phase was separated and additionally washed with ethyl acetate (40 L). The two combined organic phases were back-extracted with water (40 L). The combined aqueous solution was heated to 30 °C, and 6 M hydrochloric acid (~8 L) was added over 2 h to reach pH = 1−2. The resulting slurry was cooled to 20 °C, stirred for 18 h, and then filtered. The filter cake was sequentially washed with water (6 L) and ethyl acetate (6 L) and dried under nitrogen blow for 18 h. The obtained solid (6.38 kg) was stirred in methanol (22 L) at 20 °C for 18 h and then filtered off, washed with methanol (2 L), and dried in vacuo at 50 °C for 18 h to obtain the title compound as a beige solid (5.80 kg, 49% over three steps from **2** to **4**). HPLC purity was 97.9%. ^{1}H NMR (DMSO-d_6) δ 1.92−2.04 (m, 1H), 2.17−2.31 (m, 1H), 2.54−2.64 (m, 2H), 2.66−2.75 (m, 2H), 2.80 (t, J = 6.7 Hz, 2H), 3.78 (t, J = 6.7 Hz, 2H), 7.31 (d, J = 8.6 Hz, 2H), 7.43 (d, J = 8.6 Hz, 2H). ^{13}C NMR (DMSO-d_6) δ 17.5, 29.8, 34.6, 46.2, 84.3, 115.6, 125.0, 126.2, 126.6, 137.3, 142.6, 163.1, 182.3. HRMS (m/z): calculated for $C_{17}H_{15}N_3O_2$, $[M + H]^+$ 294.1237; found 294.1242.

1-(4-(1-Cyanocyclobutyl)phenyl)-4-methoxy-2-oxo-1,2,5,6-tetrahydropyridine-3-carbonitrile (6). To a stirred, cooled to 10 °C mixture of dichloromethane (40 L) **4** (5.7 kg, 19.43 mol) and DMF (75 mL, 0.97 mol) was gradually added neat oxalyl chloride (2.96 kg, 23.32 mol), while maintaining the reaction temperature below 15 °C. After the addition was complete, the mixture was warmed to 20 °C and agitated at this temperature for 2 h. The initial insoluble solid dissolved, and in-process control indicated that all **4** was converted to **5**. Methanol (34 L) was added in 1 h, while maintaining the reaction temperature below 30 °C. The mixture was heated to 35 °C in 30 min and agitated at this temperature for 21 h. In-process control indicated that over 97% of **5** was converted to **6**. The solution was concentrated in vacuo to residual volume ~40 L. Methanol (40 L) was added, and the mixture was concentrated under vacuum again to residual volume ~40 L. Additional methanol (17 L) was added. The resulting slurry was agitated at 20 °C for 2 h and filtered. The filter cake was washed with methanol (11 L) and dried in a tray dryer under vacuum at 40 °C for 18 h to obtain the title compound **6** as a white powder (5.24 kg, 88%). HPLC purity was 96.2%. ^{1}H NMR (DMSO-d_6) δ 1.96−2.06 (m, 1 H), 2.22−2.33 (m, 1H), 2.57−2.66 (m, 2H), 2.70−2.79 (m, 2H), 3.07 (t, J = 6.6 Hz, 2H), 3.88 (t, J = 6.6 Hz, 2H), 4.05 (s, 3H), 7.38 (d, J = 8.3 Hz, 2H), 7.47 (d, J = 8.3 Hz, 2H). ^{13}C NMR (DMSO-d_6) δ 17.5, 25.9, 34.6, 46.0, 58.5, 86.6, 114.9, 125.0, 126.2, 126.6, 137.6, 142.2, 162.2, 183.4; HRMS (m/z): calculated for $C_{18}H_{17}N_3O_2$, $[M + H]^+$ 308.1394; found 308.1402.

1-(4-(4-Amino-2-methoxy-5-oxo-7,8-dihydropyrido-[4,3-*d*]pyrimidin-6(5*H*)-yl)phenyl)-cyclobutanecarbonitrile (crude 1). To a stirred mixture of methanol (61.5 L), **6** (5.21 kg, 16.96 mol), and cyanamide (790 g, 18.82 mol) was added over 30 min a 25% solution of sodium methoxide in methanol (4.4 kg, 20.35 mol) while maintaining the reaction temperature below 25 °C. The mixture was agitated at 20 °C for 2 h. In-process control indicated that over 98% of **6** was converted to **7**. Methanesulfonic acid (4.56 kg, 47.48 mol) was added over 30 min while maintaining the reaction temperature below 30 °C. The temperature was raised to 50 °C in 2 h, and the mixture agitated at this temperature for 18 h. In-process control indicated that over 98% of **7** was consumed. The thin slurry was cooled to 20 °C. Water (20 L) was slowly added, followed by 34% wt aqueous KOH (7.9 kg, 47.48 mol) to reach pH > 10 while maintaining the reaction temperature below 25 °C. The resulting slurry was agitated at 20 °C for 2 h and filtered. The filter cake was washed with 3:1 (v/v) of methanol and water (20 L) and water (14 L), and then dried in a tray dryer under vacuum at 55 °C for 18 h to obtain the crude title product **1** as an off-white powder (5.07 kg, 86%). HPLC purity was 86.7%, associated with 4.2% of **14** and 5.3% of **15**.

Purification of Crude 1-(4-(4-Amino-2-methoxy-5-oxo-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl)-phenyl)cyclobutanecarbonitrile (1). A mixture of DMF (32 L), acetic acid (1.6 L), and **crude 1** (5.04 kg) was heated to 70 °C over 30 min and agitated at this temperature for 15 min. The resulting solution was cooled to −20 °C at a rate of one degree per minute. The slurry was agitated at this temperature for 1 h and then filtered through a Nutsche filter. The cake was washed with methanol (10 L) and blown with nitrogen flow for 3 h. [Note: By adding water (90 L) to the mother liquor, an additional 1.88 kg of a second crop of **1** with 90% HPLC purity was obtained as an off-white powder in 37% yield.] In-process control indicated HPLC purity 99.3%, associated with 0.28% of **14** and 0.14% of **15**. The entire solid was charged back to the 100 L reactor, and acetic acid (13.4 L) was added. The mixture was heated to 35 °C until all solids dissolved. The solution was transferred through 0.5 μm in-line filter into a reactor, which had been set up under speck-free conditions, connected with Silverson 200HLS high shear wet mill (HSWM) with Lasentech FBRM probe and preheated to 35 °C. Acetic acid (2 L) was used to rinse the first reactor. The combined solution was cooled to 20 °C. Water (61.5 L) was added slowly through 0.5 μm in-line filter over 90 min. The resulting slurry was agitated at 20 °C for 1 h and cooled to 10 °C. The slurry was circulated to HSWM with square-holed stator at RPM 5400 for 2 h at 10 °C until meeting particle size target. The milled slurry was filtered through a Nutsche filter. The cake was washed with water (10 L) and dried in a tray dryer under vacuum at 60 °C for 18 h to obtain the title compound **1** as a white powder (2.61 kg, 51.8%). HPLC purity was 99.63%, associated with 0.16% of **14** and 0.13% of **15**. Particle Size: D[4, 3] = 25 μm, D[v, 0.95] = 58 μm. Residual Solvents: acetic acid 0.4 wt %, water 0.1 wt % and DMF <0.1 wt %. ^{1}H NMR (DMSO-d_6) δ 1.93−2.05 (m, 1H), 2.18−2.32 (m, 1H), 2.55−2.65 (m, 2H), 2.68−2.77 (m, 2H), 2.93 (t, J = 6.7 Hz, 2H), 3.83 (s, 3H), 3.88 (t, J = 6.7 Hz, 2H), 7.39 (d, J = 8.6 Hz, 2H), 7.46 (d, J = 8.6 Hz, 2H), 7.78 (d, J = 3.9 Hz, 1H), 8.32 (d, J = 3.9 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ 17.5, 31.4, 34.6, 47.5, 54.9, 98.8, 125.0, 126.6, 126.7, 137.7, 142.8, 164.9, 165.3, 165.9, 171.0; HRMS (m/z): calculated for $C_{19}H_{19}N_5O_2$, $[M + H]^+$ 350.1612; found 350.1620. Elemental analysis: calculated for $C_{19}H_{19}N_5O_2$: C 65.32, H 5.48, N 20.04; found: C 65.40, H 5.45, N 20.16.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra of **1** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.

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