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Kilogram Synthesis of a Second-Generation LFA-1/ICAM Inhibitor

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Abstract:

The process development and the kilogram-scale synthesis of BMS-688521 (1) are described. The synthesis features a highly efficient telescoped sequence which utilizes previously described spirocyclic hydantoin (4b) to produce the final intermediate via an SN_{AR} reaction. A final deprotection step affords BMS-688521 (1) in high quality with an overall yield of 65% from the key intermediate, spirocyclic hydantoin (4b).

Introduction

A key feature of the intercellular immune response is that cytokine stimulated cells express LFA-1 (leukocyte function-associated antigen-1) on their surface. LFA-1 then interacts with ICAM (intercellular adhesion molecule), which is found on the surfaces of both leukocytes and endothelium. This interaction facilitates T-cell adhesion and migration through the blood vessel wall to the inflamed area.[1] Small molecules which successfully inhibit the LFA-1/ICAM interaction have potential as drugs for the treatment of a variety of autoimmune and inflammatory diseases such as rheumatoid arthritis and psoriasis.[2,3] The LFA-1 receptor antagonist, BMS-688521, **1**, was a second generation molecule selected for clinical development and we required a synthesis that would reliably generate kilogram quantities of API. This contribution details the identification and development of a synthesis which enabled the realization of this goal.

Results and Discussions

The original synthesis of **1** by our Discovery colleagues provided the initial quantities required for some of the preclinical development studies (Scheme 1).[4] The generation of *tert*-butyl nicotinic ester **3** was achieved by formation of the acid chloride via the use of thionyl chloride and subsequent treatment with *tert*-butanol. Following the workup, the reaction mixture was dried with Na_2SO_4 and concentrated via rotovap to afford **3** as a yellow solid. Treating the spirocyclic hydantoin **4a** with a slight excess of **3** at 112 °C for 18 h in the presence of DIPEA afforded the desired N-alkylated product **5a.** Addition of a solution of **5a** into cold H_2O resulted in precipitation of a crude solid which was subsequently chromatographed to remove 1−3% of the isopropyl ester analogue **6** (Figure 1). Impurity **6** resulted from low levels of isopropanol present in the *tert*-butanol used to prepare **3**.[5] Purified **5a** was then deprotected utilizing a 1:1 trifluoroacetic acid:dichloromethane solvent system, and after an extractive workup, was concentrated to dryness. The crude material was subsequently dissolved in hot chloroform and the desired product **1** was isolated in two crops. Unfortunately residual chloroform (0.04%) made this material unsuitable for toxicology studies.

A modified synthesis of **1** (Scheme 2) was developed by our Discovery scale-up group colleagues to address the isopropyl ester impurity as well as the residual chloroform issue in order to supply multigram quantities for preclinical development studies.[6] In this modified approach, the 6-chloronicotinic acid **2** was protected *in situ* with TMSCl, and the resulting intermediate **7** was coupled with hydantoin **4a**. The reaction mixture containing intermediate **8** was treated with MeOH, and crude **1** was directly isolated from the reaction mixture upon addition of water. Crude **1** was recrystallized from EtOH/H_2O to afford the desired **1** in high purity and yield (99.5 HPLC area percent purity, >99.9% ee, and 89% overall yield).

While the modified approach was attractive for producing multigram quantities of API quickly, we had concerns that the extensive telescope which affords **1** directly provides little control over any potential impurities that might be introduced from the raw materials. From an impurity control perspective,

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(1) For a discussion on the inhibition of LFA-1/ICAM-1as an approach to treating autoimmune diseases see: Yusuf-Makagiansar, H.; Anderson, M. E.; Yakovleva, T. V.; Murray, J. S.; Siahaan, T. *J. Med. Res. Rev.* **2002**, *22*, 146.

(2) For a discussion of therapeutic options for treatment of psoriasis, see: Gottlieb, A. B. *J. Acad. Dermatol* **2005**, *53*, S3. Larson, R. S.; Davis, T.; Bologa, C.; Semenuk, G.; Vijayan, S.; Li, Y.; Oprea, T.; Chigaev, A.; Buranda, T.; Wagner, C. R.; Sklar, L. A.

(3) For other small-molecule LFA-1/ICAM-1 antagonists as potential drugs please see: (a) Pei, Z.; Xin, Z.; Liu, G.; Li, Y.; Reilly, E. B.; Lubbers, N. L.; Huth, J. R.; Link, J. T.; von Geldern, T. W.; Cox, B. F.; Leitza, S.; Gao, Y.; Marsh, K. C.; DeVries, P.; Okasinski, G. F. *J. Med. Chem.* **2001**, *44*, 2913. (b) Liu, G.; Huth, J. R.; Olejniczak, E. T.; Mendoza, R.; DeVries, P.; Leitza, S.; Reilly, E. B.; Olasinski, G. F.; Fesik, S. W.; von Geldern, T. W. *J. Med. Chem.* **2001**, *44*, 1202. (c) Wu, J.-P.; Emeigh, J.; Gao, D. A.; Goldberg, D. R.; Kuzmich, D.; Miao, C.; Potocki, I.; Qian, K. C.; Sorcek, R. J.; Jeanfavre, D. D.; Kishimoto, K.; Mainolfi, E. A.; Nabozny, G.; Peng, C.; Reilly, P; Rothlein, R.; Sellati, R. H.; Woska, J. R.; Chen, S.; Gunn, J. A.; O'Brien, D.; Norris, S. H.; Kelly, T. A. *J. Med. Chem.* **2004**, *47*, 5356. (d) Last-Barney, K.; Davidson, W.; Cardozo, M.; Frye, L. L.; Grygon, C. a.; Hopkins, J. L.; Jeanfavre, D. D.; Pav, S.; Qian, C.; Stevenson, J. M.; Tong, L.; Zindell, R.; Kelly, T. A. *J. Am. Chem. Soc.* **2001**, *123*, 5643. (e) Wang, G. T.; Wang, S.; Gentles, R.; Sowin, T.; Leitza, S.; Reilly, E. B.; von Geldern, T. W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 195. (f) Wattanasin, S.; Albert, R.; Ehrhardt, C.; Roche, D.; Savio, M.; Hommel, U.; Welzenbach, K.; Weitz-Schmidt, G. *Bioorg. Med. Chem. Lett.* **2003**, *12*, 499.

(4) Watterson, S. H.; Xiao, Z.; Dodd, D. S.; Tortolani, D. R.; Vaccaro, W.; Potin, D.; Launay, M.; Stetsko, D. K.; Skala, S.; Davis, P. M.; Lee, D.; Yang, X.; McIntyre, K. W.; Balimane, R.; Patel, K.; Yang, Z.; Marathe, P.; Kadiyala, P.; Tebben, A. J.; Sheriff, S.; Chang, C. Y. Y.; Ziemba, T.; Zhang, H.; Chen, B.-C.; DelMonte, A. J.; Aranibar, N.; McKinnon, M.; Barrish, J. C.; Suchard, S. J.; Dhar, T. G. M. *J. Med. Chem.* **2010**, *53*, 3814.

(5) Due to the higher level of reactivity of isopropanol relative to that of *tert*-butanol, the isopropyl impurity **6** is enriched to a higher level than the level of isopropanol observed in the *tert*-butanol.

(6) Zhang, H.; Watterson, S. H.; Xiao, Z.; Dhar, T. G. M.; Balasubramanian, B.; Barrish, J. C.; Chen, B.-C. *Org. Process Res. Dev.* **2010**, *14*, 936.

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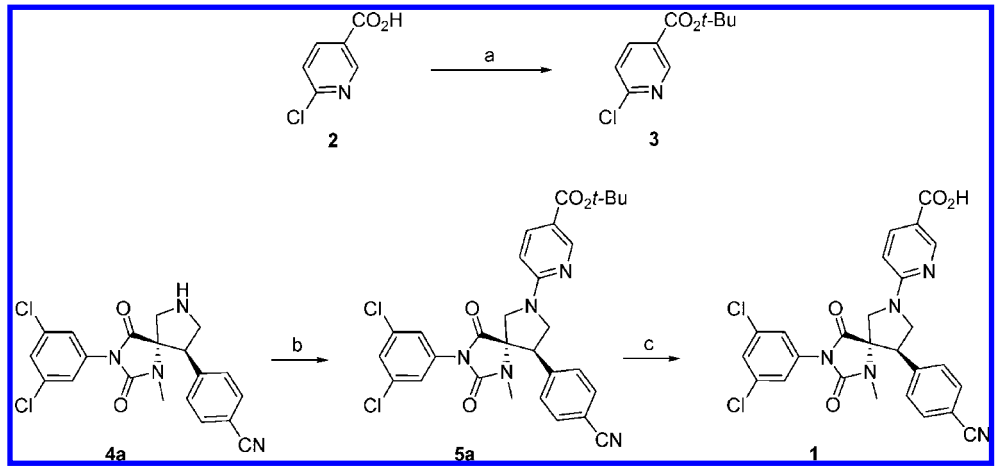
Scheme 1. **Discovery synthesis of 1**[a]



[a] Reagents and conditions: (a) $SOCl_2$; *t*-BuOH, CH_2Cl_2, Et_3N, DMAP, 70%; (b) **3**, DIPEA, DMA, 112 °C, 77%; (c) TFA, DCM, 86%.

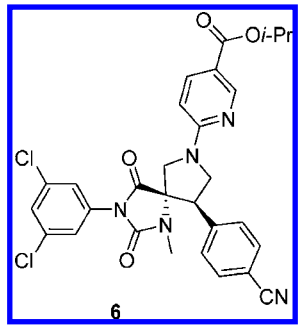


Figure 1. **Impurity observed during the esterification of 5a.**

we considered the original Discovery approach, in which **5a** was isolated and deprotected in a final step, to be a more attractive strategy. However, the pursuit of this approach required addressing the specific issues of the isopropyl ester impurity and the identification of a chloroform-free crystallization process to the API. In general, the identification of a robust and scalable process required additional efforts to determine the optimal reagents,[7] reaction conditions, and isolation procedures, with focus on avoiding chromatography, isolation of amorphous solids, recrystallizations, and two-crop isolations.

Preparation and Isolation of *tert*-Butyl Nicotinic Ester (3). An alternative approach for the preparation of **3** which completely circumvents the issue of the isopropanol contamination of *tert*-butanol was identified. A variant of the previously reported procedure[8] utilizing 2.25 equiv of di-*tert*-butyl dicarbonate (Boc_2O) in THF with catalytic (7 mol %) dimethylaminopyridine (DMAP) afforded conversion of 6-chloronicotinic acid **2** to the desired *tert*-butyl protected **3** with very low impurity levels (Scheme 3). A basic extraction was utilized to quench residual Boc_2O and remove low levels of unreacted 6-chloronicotinic acid **2**. To safely scale this process we conducted a detailed study to fully understand both the heat flow as well as the rate of CO_2 off-gassing. No unusual thermochemical properties were observed, and calorimetry indicated the reaction was endothermic (155 KJ/mol) with an adiabatic temperature change of −37 °C. During the *tert*-butyl ester formation there is potential for the generation of 4.5 equiv of CO_2 gas. Figure 2 shows the off-gassing profile for an experiment utilizing 53.0 g of **2**. A total of 32.6 L of CO_2 was measured, corresponding to 88% of the theoretical amount. Interestingly, the gas evolution consisted of several phases. The first was a non-dose-controlled release (∼0.6 L/min) with an induction period, followed by a dose-controlled release (∼1.2 L/min) during addition of the first equivalent of Boc_2O solution.[9] The rate of gas evolution decreased during the addition of the final 1.25 equiv of Boc_2O. Based on this data, the maximum off-gassing rate for a batch utilizing 1.8 kg of **2** as input was calculated to be 41 L/min for a similar Boc_2O solution feed rate. This off-gassing rate was well within the design basis of the process equipment planned for use in our pilot plant.[10]

To address the non-dose-controlled release of CO_2 at the beginning of the reaction, an initial charge of ∼10% of the Boc_2O/THF solution was performed. After the initial gas evolution phase was over, the remainder of the Boc_2O/THF solution was added in an addition-controlled fashion. The higher temperature of the reaction (55−60 °C) combined with the subsequent aqueous alkaline washes ensured very little dissolved CO_2 would be present after the workup.

With confidence in the safety of the reaction, our efforts were then focused on the isolation protocol. Our initial attempts to isolate **3** as a solid only afforded amorphous materials. Eventually, through the use of high-throughput crystallization experiments, we found that crystalline material could be obtained from an IPA/water mixture. However, we quickly realized that drying and handling of crystalline **3** on large scale would pose a challenge due to its low melting point (54 °C). While much of the early development work for the subsequent SN_{AR} step was

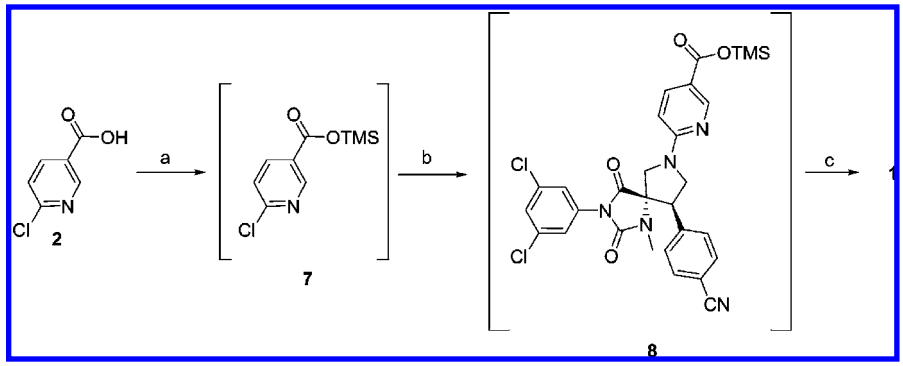
(7) Identification of alternative reagents for the *tert*-butyl ester formation was desired to avoid the use of the very odiferous thionyl chloride. Ideally, an alternative procedure would also obviate the need to source ultra-pure *tert*-butanol which was free of isopropanol.

(8) Goossen, L. J.; Döhring, A. *Synlett* **2004**, *2*, 263.

(9) To the best of our knowledge, this mechanism has not been studied in detail. Our hypothesis regarding the induction period is that at the beginning of the reaction the majority of the DMAP is not initially available to enter the catalytic cycle as it forms a salt with the nicotinic acid.

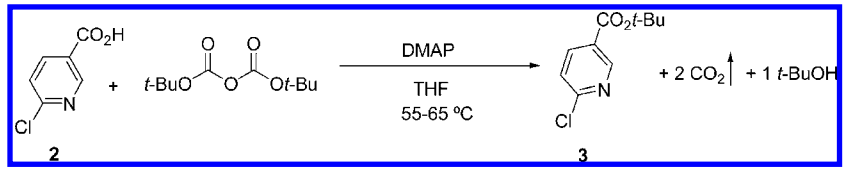
(10) This calculation must be performed for any changes to scale or equipment to ensure the equipment can tolerate the maximum off-gassing rate. In practice, 2.3 equiv of BOC_2O was utilized on scale-up with a molar feed rate of 0.163 mol/min. Since the gas evolution was addition controlled, the maximum potential offgassing rate was ∼7.4 L/min.

Scheme 2. **Discovery-modified synthesis of 1[a]**



[a] Reagents and conditions: (a) $(CH_3)_3SiNHSi(CH_3)_3$, cat. TMSCl, 78 °C, 3 h, 100%; (b) **4a**, DMAP, DMA, DIPEA, 95 °C, 18 h; (c) MeOH, 25 °C, 2 h, 98%.

Scheme 3. **Synthesis of *tert*-butyl nicotinic ester**



performed with isolated **3**, its low melting point was an intrinsic liability which could not be avoided and would complicate all handling efforts. This suggested that the best approach ultimately would involve a telescoped process in which **3** was not isolated, but rather directly coupled with spirocyclic hydantoin **4a**.

Early Development of the Penultimate Step. The free base spirocyclic hydantoin **4a** was initially utilized in the Discovery synthesis for the synthesis of gram-scale quantities of API. We planned to ultimately use the hemi (+)-di-*p*-toluyl-D-tartaric acid ((+)-DTTA) salt **4b** directly as it was readily available from a prior large-scale synthesis.[11] However, to simplify our initial lab development work, we initially used the free base spirocyclic hydantoin **4a.** To access gram quantities of the freebase **4a**, the DTTA salt **4b** was dissolved in 10 mL/g THF/MTBE (1:1) and extracted with a 5 mL/g of 1 M NaOH solution to remove the 0.5 equiv of the DTTA.[12] Following a 5 mL/g aqueous wash, the organic layer was concentrated, and **4a** was isolated as the free base (Scheme 4).

Addition of solid *tert*-butyl nicotinic ester **3** to **4a** with 2.5 equiv of DIPEA and heating the reaction at 112 °C for 18 h in dimethylacetamide (DMA) afforded the desired product. However, the acetyl impurity **9** which results from nucleophilic attack of **4a** on DMA was also observed (Figure 3).[13] NMP was identified as an ideal solvent as it afforded reactions free of impurity **9**. Further development work indicated that low water content of the NMP (KF < 0.3%) was critical as higher water levels afforded increased levels of impurity **10** resulting from epimerization of the benzylic center (Figure 3).[14] We also found 1.4 equiv of *tert*-butyl nicotinic ester **3** was optimal, as this

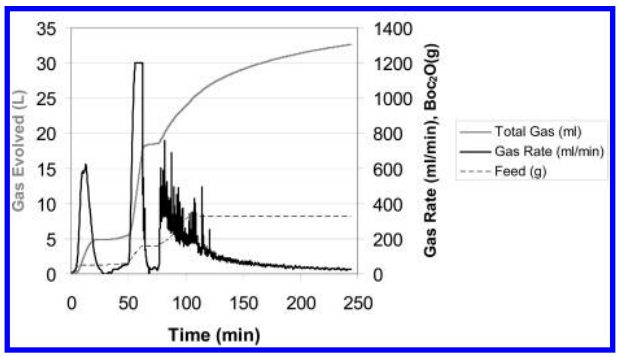


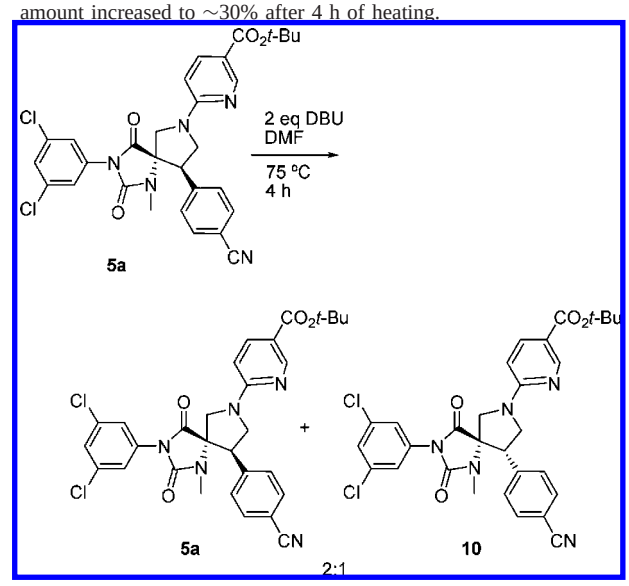
Figure 2. **Lab-scale off-gassing experiment.**

(11) The DTTA salt **4b** was readily available as it was a common intermediate with BMS-578101, a first-generation molecule. For a description of its synthesis, see: DelMonte, A. J.; Waltermire, R. E.; Fan, Y.; McLeod, D. D.; Gao, Z.; Gesenberg, K. D.; Girard, K. P.; Rosingana, M.; Wang, X.; Kuehne-Willmore, J.; Braem, A. D.; Castoro, J. *Org. Process Res. Dev.* **2010**, *14*, 553.

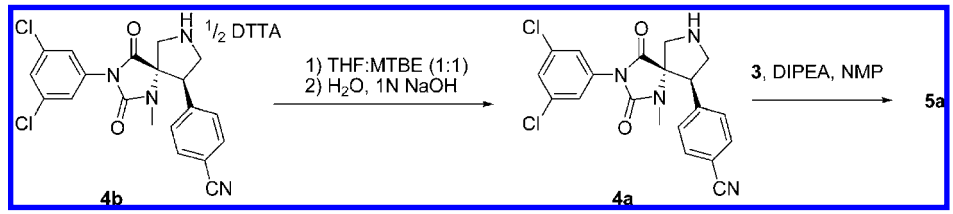
(12) Throughout this publication mL/g refers to mL of solvent to grams of input material.

(13) The same impurity was also observed during the Discovery development work (see ref. 6).

(14) With similar substrates (see ref 11), it was observed that the benzylic center could epimerize under basic conditions. To test this, ~50 mg of the penultimate **5a** (one diastereomer) was heated in 0.75 mL DMF-d_7 in the presence of 2 equiv of DBU. After 2 h, NMR and HPLC indicated ~15% formation of the undesired diastereomer **10**. This amount increased to ~30% after 4 h of heating.



Scheme 4. **Synthesis of 5a from 4b**



moderate excess helped drive the reaction to completion. However, it was clear that we would require a strategy to remove this excess reagent upon reaction completion in order to facilitate isolation of the product. In our early development efforts, a small sample of pure SN_{AR} product **5a** was isolated by chromatography and subjected to a high-throughput salt screen, resulting in the identification of a crystalline *p*-toluenesulfonic acid (*p*-TSA) salt **5b.** With these encouraging results for each component of the overall reaction sequence, free-basing of **4b**, the SN_{AR} reaction conditions, and the isolation of product as a crystalline salt **5b**, we focused on the development of a telescoped process using a solution of **3** and the DTTA salt **4b**.

Development of a Telescoped Process for the Production of 5b. Since the low melting point of the *tert*-butyl nicotinic ester **3** was anticipated to complicate isolation and drying of crystalline material on scale, it was highly desirable to develop a telescoped process. One of the primary objectives in developing an efficient telescoped process is the careful integration of the steps so the number of unit operations (i.e., extractions, distillations, etc.) is minimized. We chose to perform the *tert*-butyl ester protection under the Boc_2O conditions as previously described, followed by addition of MTBE and aqueous NaOH. Since a freebasing of the spirocyclic hydantion DTTA salt **4b** to **4a** was ultimately required, we saw an opportunity to reduce the number of overall extractions by charging **4b** directly to the basic mixture.[15] After a short period of stirring, we continued with the extraction. This approach allowed us to quench any excess Boc_2O while simultaneously removing the DTTA and any residual nicotinic acid **2**. The next step was to take this MTBE/THF solution and solvent exchange to NMP which was the preferred solvent for the SN_{AR} reaction. While solvent exchanges can sometimes be time-consuming, this solvent exchange to a much higher-boiling solvent was quite facile. More importantly, it afforded a clear processing advantage as it was effective at affording a very dry solution via azeotropic water removal from the reaction mixture, thus reducing the probability of forming impurity **10**. The SN_{AR} reaction performed as expected, but we still required a strategy for removing 0.4 equiv of excess **3** from the reaction mixture. We found we could capitalize on the NMP solvent system and the nonpolar

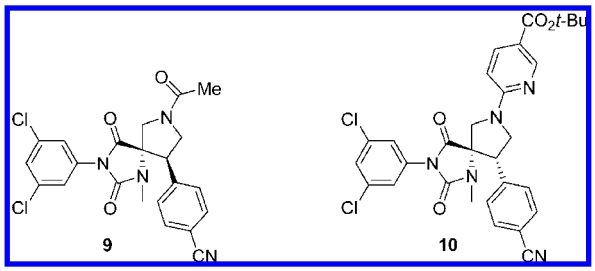


Figure 3. **Impurities formed in the SN_{AR} step.**

nature of **3** and simply employ a heptane/cyclohexane extraction of the NMP solution to remove the majority of the residual *tert*-butyl nicotinic ester **3**. MTBE was then charged to the reaction mixture, and the NMP was removed by several aqueous extractions. After azeotropic drying, *p*-TSA was charged followed by isolation of the desired compound **5b** (Scheme 5). This process was performed on a 6-kg scale in two batches, affording material in good yield and quality (88.7% and 81.8% yield, with both batches >98.0 HPLC area percent purity).[16]

The Hydrolysis of 5b to API. The first step was to remove the *p*-TSA via a basic extraction of an MTBE/THF mixture containing **5b**. A variety of reagents and reaction conditions for the ester hydrolysis of **5a** were rapidly screened, and ultimately a mixture of concentrated HCl (0.94 mL/g) and AcOH (1.75 mL/g) in toluene was selected, affording the hydrolyzed product in 3−4 h at 55−65 °C. The reaction mixture was washed with aqueous sodium chloride and sodium hydroxide and subsequently solvent exchanged to EtOH. Seeding and addition of heptane as an antisolvent afforded **1** as the desired neat form (Scheme 6). Rapid development of this process and execution on a 5-kg scale afforded 2.2 kg of API in 66.2% yield, >99.9% ee and 98.0 HPLC area percent purity with a D90 = 36 μm (after milling).[17]

During the 5-kg scale-up we identified two opportunities for improvement. The first was related to the purity of the product **1** (98.0 AP) which was lower than desired. While several impurities (**11**−**13**) were previously observed in our lab runs (Figure 4), the *tert*-butylamide impurity **11** was present at a much higher level (1 AP) than desired. Our hypothesis is that impurity **11** results from a Ritter reaction[18] in which there is nucleophilic attack of the cyano nitrogen on the isobutyl cation byproduct from the acidic deprotection of the *tert*-butyl ester.

The second opportunity for improvement was related to the crystal morphology and particle size of isolated **1**. The current procedure involved a solvent exchange to EtOH generating a supersaturated solution. Uncontrolled crystallization afforded a previously unseen crystal morphology which, after SEM (Figure 5), was described as hard, round agglomerates with a D90 of ~200 μm. Either due to their hardness or spherical shape, our attempts to significantly reduce the particle size by

(15) The successful quench of Boc_2O was evidenced by absence of the *tert*-butyl carboxylate of **4a** which could be observed if **4b** was charged to the mixture immediately.

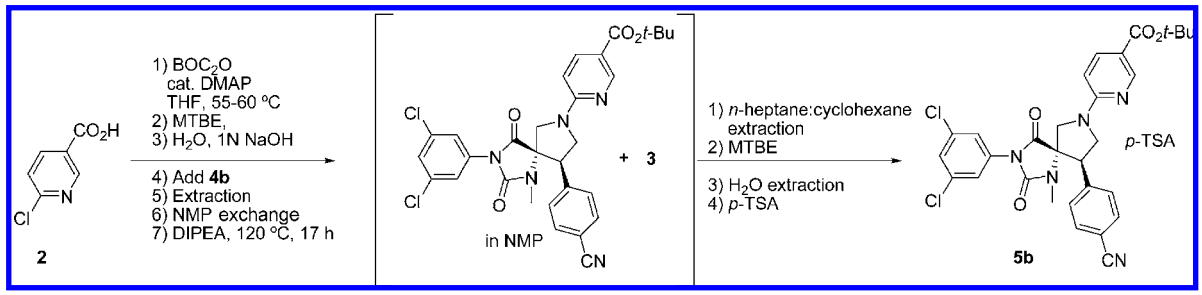
(16) Yields reported are corrected yields, and the HPLC area percent purity reported is the combined HPLC area percent purity for the spirocyclic hydantoin **5a** and *p*-TSA.

(17) A D90 < 60 μm was desired.

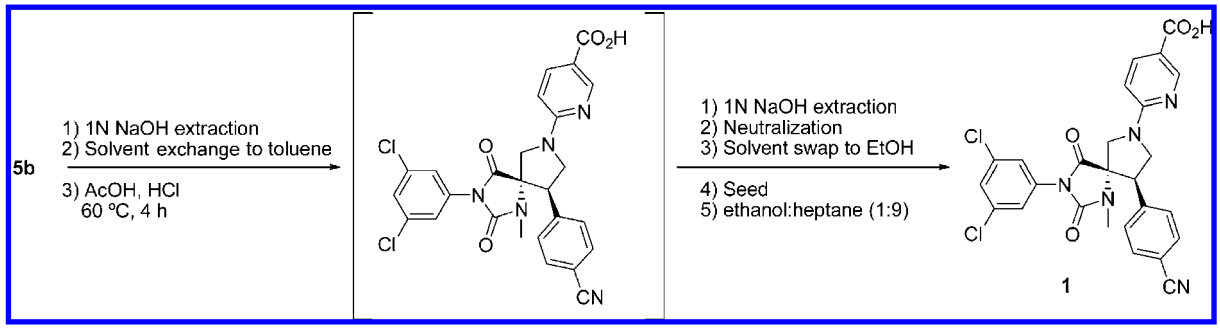
(18) For a review on the Ritter reaction see: Krimen, L. I.; Cota, D. J. *Org. React.* **1969**, *17*, 213.

(19) The high loss of **1** to mother liquor may have been due to the uncontrolled crystallization, the new morphology or the higher than previously observed level of impurities. It is notable that pXRD indicated **1** had the correct polymorphic form with little to no amorphous content.

Scheme 5. **Telescoped synthesis of 5b**



Scheme 6. **Hydrolysis of 5b to 1**



passing through a wet mill were unsuccessful. Even after two passes the D90 was only reduced to 130 μm. However, dry milling with a hammer mill was successful and after two passes a D90 of 36 μm was obtained. In addition to the unexpected loss of product due to dry milling (4.9%), there were also higher than anticipated quantities of **1** left in the mother liquor (~24.5%) resulting in the lower than expected yield.[19] Before proceeding with the next batch, we worked rapidly in the laboratory to increase our process knowledge to enable greater control of the process.

Development Work on the Hydrolysis Reaction. To address the higher than desired level of the *tert*-butyl amide impurity, we utilized DOE experimentation to investigate the reaction parameters and their interdependent effects on conversion and purity levels. We identified temperature, HCl concentration, and the two-way interaction between these factors as playing the biggest roles on reaction rate with increased temperature and increased HCl concentration affording the highest rate of conversion to product. These results are easily visualized through the use of a Pareto plot which uses a histogram to rank the variables from most impactful to least impactful (Figure 6). We then ran a central composite DOE which highlighted the effect of water concentration and HCl concentration on the impurity formation with focus on the *tert*-

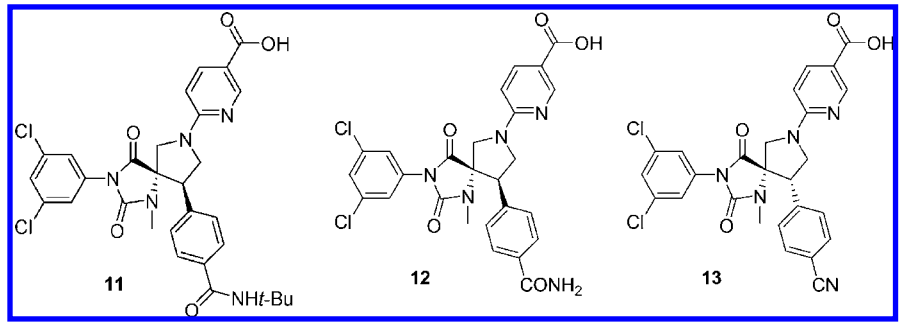


Figure 4. **Impurities formed in the hydrolysis step.**

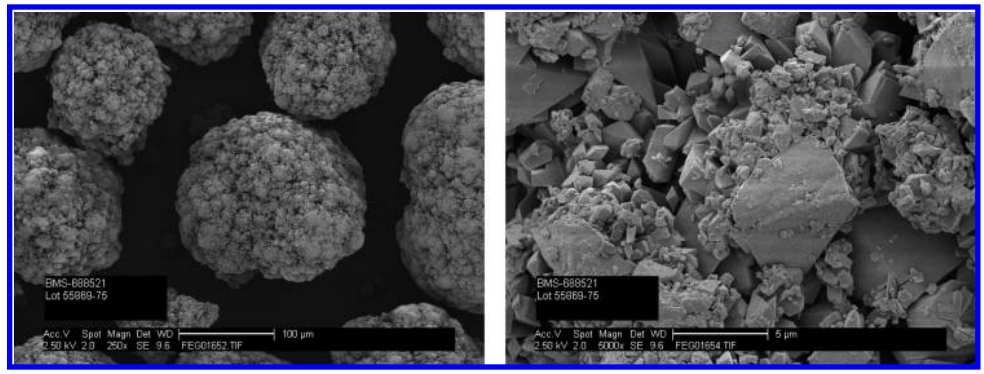


Figure 5. **SEM of premilled API (agglomerates and primary particles).**

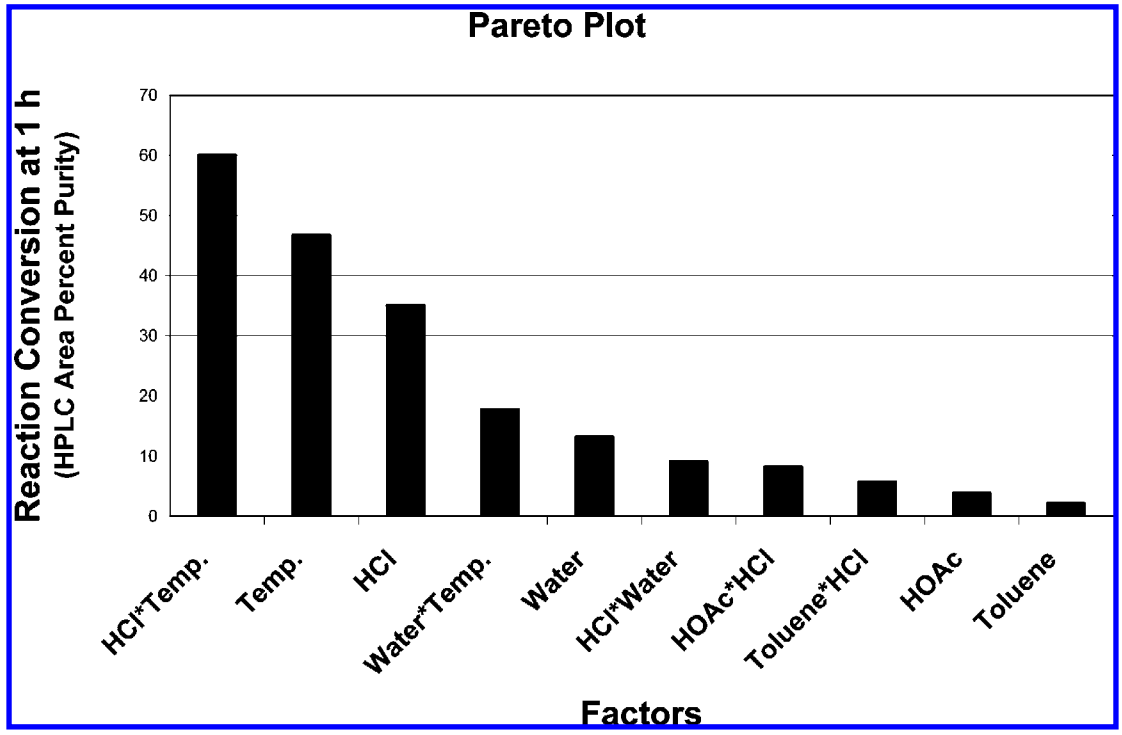


Figure 6. **DOE on the hydrolysis of 5b.**

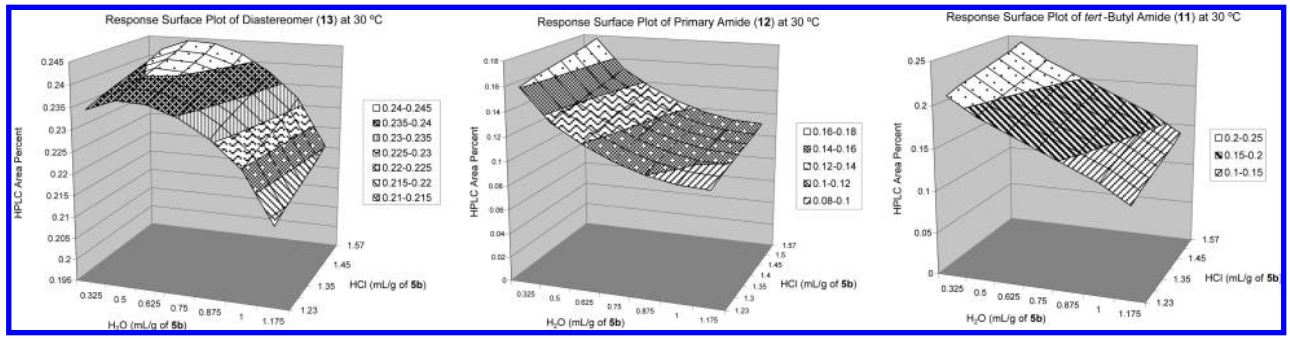


Figure 7. **Central composite DOE (effect of HCl and H_2O on impurities 11, 12, and 13).**

butyl amide impurity. A much cleaner reaction profile was obtained with increased water concentration and decreased HCl concentrations (Figure 7). Further lab work suggested modified conditions using AcOH (3.6 mL/g), HCl (0.91 mL/g), and H_2O (0.63 mL/g) in toluene at 30 °C. We were pleased to observe that under these new conditions, all of the impurities, including the *tert*-butyl amide impurity **11**, could be controlled to levels below 0.25 HPLC area percent purity (Figure 8).

Optimization of Particle Size and Morphology of 1. To better control the crystal morphology, we conducted further crystallization studies. During our lab work a new morphology was obtained in which the crystals formed fragile rods. We were very pleased when analysis by pXRD indicated we had still obtained the desired polymorphic form. By using the identical crystallization conditions previously employed, but seeding with crystals possessing this new morphology, we were able to generate fragile rods which were easily wet-milled and filtered well. This obviated the need for dry milling and the material loss associated with the additional handling.

Results from Batch 2. Incorporating all of these modifications allowed us to execute the second batch on 4.95 kg scale

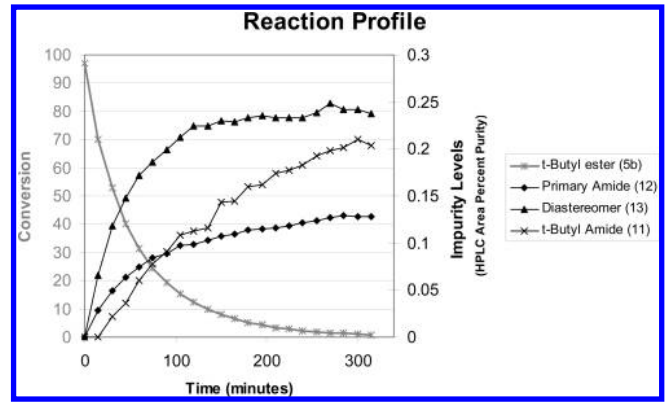
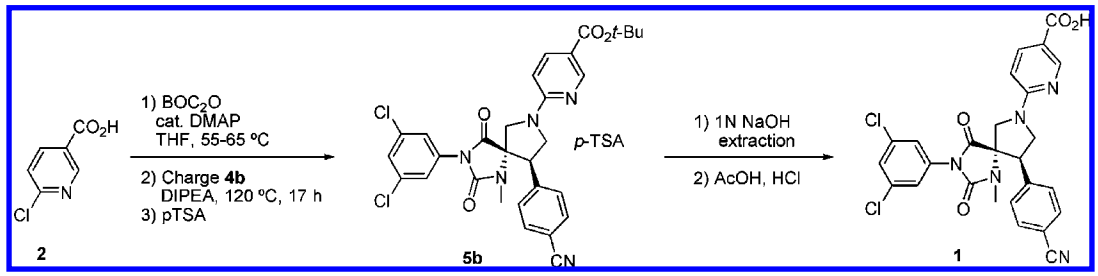


Figure 8. **Hydrolysis reaction profile under optimized conditions.**

with improved control. The second batch afforded **1** in 84.5% yield and 99.3 HPLC area percent purity, demonstrating significant improvements over batch 1 (66% yield and 98.0 HPLC area percent purity). In addition, the particle size was well controlled by wet milling with a D90 of 28 μm with no dry milling required, and chiral HPLC analysis indicated the material was >99.9% ee.

Scheme 7. **Efficient two-step synthesis of 1**



Conclusions

In summary, we have described an efficient, 2-step telescoped synthesis of BMS-688521 (**1**) from an available intermediate, spirocyclic hydantoin **4b**, as shown in Scheme 7. A highly efficient telescoped process was utilized to avoid handling and drying of a low-melting point solid as well as minimizing the overall number of unit operations. Only general purpose processing equipment was required, providing versatility in future manufacturing site selection. The overall process has been demonstrated on kilogram scale and is amenable for commercial-scale manufacture.

Experimental Section

All reactions were performed under a nitrogen atmosphere. All reagents purchased from vendors were used as received unless otherwise indicated. Chiral analysis was conducted on a Shimadzu HPLC with a Chiracel AD or AD-H column unless stated otherwise. Proton and carbon NMR were run on a Bruker AC-300 at 300 MHz for proton and 75 MHz for carbon or on a Bruker AVANCE 400 at 400 MHz for proton and 100 MHz for carbon. The melting points were obtained with a Mettler-Toledo FP 62 melting point instrument by measurement of the change of luminous intensity during the melting process. Reported yields have not been corrected for impurity levels or moisture content.

Preparation of *tert*-Butyl 6-((5*S*,9*R*)-9-(4-cyanophenyl)-3-(3,5-dichlorophenyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)nicotinate p-TSA Salt (5b). To a reactor was charged 1.83 kg of **2** (11.62 mol, 1.00 equiv), 0.10 kg of DMAP (0.82 mol, 0.07 equiv) and 10.7 kg of THF. The reactor was heated to 62.5 °C. In a separate vessel the Boc_2O solution was prepared by dissolving 5.82 kg of Boc_2O (26.67 mol, 2.3 equiv) in 8.35 kg of THF. Approximately 10% of the Boc_2O solution was charged to the mixture of **2** at a rate of 44 g/min (addition time of ~0.5 h). The mixture was held for 40 min at 62.5 °C, and the remaining Boc_2O solution was charged at a rate of 87 g/min (addition time of ~2.5 h). The reaction was held for 2 h at 62.5 °C, and the conversion of **2** to **3** was monitored. Upon reaction completion, the mixture was cooled to 21 °C, and a mixture of THF/H_2O (3.9 kg:0.83 kg) was charged slowly to control gas evolution. The mixture was stirred 1 h, 28.7 kg of 1 M NaOH was charged, and the mixture was held for 20 min. To the reactor was charged 4.7 kg of **4b** and 20.4 kg of MTBE. The mixture was agitated 15 min and allowed to split 30 min, and the aqueous layer was discarded. A charge of 27.7 kg of H_2O was performed followed by agitation for 15 min. The phases were allowed to split 30 min, and the aqueous layer was discarded. The mixture was distilled at 60 °C to a volume of 25 L. MTBE (26.5 kg) was charged, and the mixture was distilled to a volume of 25 L. If the KF > 2.7%, MTBE was charged, and the distillation was repeated. NMP (21.4 kg), 16.1 kg of cyclohexane, and 1.2 kg of DIPEA was charged. The reaction mixture was distilled until the temperature reached 120 °C and no more THF or MTBE was collected. The mixture was held at reflux, and 1.8 kg of DIPEA was charged (total DIPEA charge of 3.0 kg). The mixture was held at reflux until full conversion was achieved (<3% of **4a** remaining), and then the mixture was cooled to 25 °C. To the reactor was charged 14.2 kg of heptane and 1.82 kg of cyclohexane. The mixture was agitated 15 min and allowed to split for 30 min, and the top heptane/cyclohexane layer was discarded. To the mixture was charged 14.2 kg of heptane and 1.82 kg of cyclohexane. The mixture was agitated 15 min and allowed to split 30 min, and the top heptane/cyclohexane layer was discarded. To the mixture was charged 23.4 kg of MTBE and 20.5 kg of H_2O. The mixture was agitated 15 min and allowed to split 30 min, and the bottom aqueous layer was discarded. To the mixture was charged 5.2 kg of MTBE and 20.5 kg of H_2O. The mixture was agitated 15 min and allowed to split 30 min, and the bottom aqueous layer was discarded. To the reactor was charged 20.5 kg of water, the mixture was agitated 15 min, the phases were allowed to split for 30 min, and the bottom aqueous layer was discarded. The mixture was filtered through a ZetaCarbon Pad (04701-R53SP) to remove color and particles. The pad and lines were rinsed with 8.1 kg of MTBE. The mixture was distilled at 55−62 °C to a volume of 30 L. (Note: If KF > 0.3%, recharge 25.1 kg of MTBE and distill to a volume of 30 L). To the reaction was charged 16.8 kg of MTBE and 8.6 kg of IPA. A solution of 1.45 kg of *p*-TSA and 17.3 kg of IPA was prepared and charged slowly to the reaction mixture. A seed mixture (29 g of seeds suspended in 0.5 kg of MTBE) was charged and the slurry was stirred for 30 min. The cake was filtered and washed in two portions with a solution of 7.1 kg of IPA and 6.8 kg of MTBE. A total of 5.59 kg of **5b** was obtained in >98 HPLC area percent purity and 88.7% yield as a white solid: [α] +58.1 (*c* 0.439, DMSO); IR (KBr) 3434 (s), 2974 (s), 1725 (s), 1650 (s), 1455 (s), 1307 (s), 1220 (s), 1163 (s), 1123 (s), 1039 (m) cm^{-1}; ^1H NMR (400 MHz, DMF) δ 8.74 (d, J = 1.8, 1H), 8.15−8.25 (m, 1H), 7.96 (d, J = 8.3 Hz, 2H), 7.67 (dd, J = 12.0, 8.3, 4H), 7.62 (t, J = 1.8, 1H), 7.18 (d, J = 12.0, 2H), 7.06 (dd, J = 8.3, 1H), 7.02 (d, J = 1.9, 2H), 4.25−4.62 (m, 5H), 3.80−3.93 (m, 1H), 3.03 (s, 3H), 2.32 (s, 3H), 1.59 (s, 9H); ^{13}C NMR (100 MHz, DMF) δ 171.7, 164.14, 162.67, 162.57, 162.38, 162.08, 153.76, 145.36, 139.55, 139.08, 134.67, 134.05, 132.98, 129.74, 128.66, 128.23, 126.12, 125.02, 118.62, 116.91, 112.11, 81.30, 71.93, 62.87,

49.45, 48.59, 46.34, 27.85, 25.41, 25.16, 20.71; Anal. Calcd for $C_{37}H_{35}Cl_2N_5O_7S \cdot 0.75H_2O$: C, 57.10; H, 4.82; N, 9.10; Found: C, 57.08; H, 4.87; N, 8.91.

Preparation of 6-((5*S*,9*R*)-9-(4-Cyanophenyl)-3-(3,5-dichlorophenyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)nicotinic Acid (1). To a reactor was charged 5.0 kg of **5b** (6.54 mol), 12.5 kg of THF, 28.4 kg of MTBE, and 26.0 kg of 1 M NaOH. The mixture was agitated 15 min and allowed to split for 30 min, and the bottom aqueous layer was discarded. To the reactor was charged 25 kg of H_2O, the mixture was agitated 15 min and allowed to split for 30 min, and the bottom aqueous layer was discarded. To the reactor was charged 9.75 kg of toluene, and the mixture was distilled until the batch temperature reached 85−90 °C. The reaction mixture was cooled to 25 °C and 9.2 kg of concentrated AcOH and 5.6 kg of concentrated HCl were charged while maintaining the reaction temperature <40 °C. The reaction was heated at 55−65 °C until <1% of **5b** remained. The reaction mixture was cooled to 5 °C and 26.7 kg THF, 16.8 kg 10 wt % brine solution was charged. A total of 6.5 kg of 10 N NaOH was then charged to adjust the pH to 4.5 (Note: AcOH can be used to lower pH if pH is accidentally adjusted too high). The mixture was agitated 15 min and allowed to split for 30 min, and the bottom aqueous layer was discarded. To the reaction mixture was charged 16.8 kg of 10 wt % brine solution and 26.0 kg of 2 N NaOH solution. The mixture was agitated 15 min and allowed to split for 30 min, and the bottom aqueous layer was charged. To the mixture was charged 11.25 kg of H_2O, and the pH was adjusted to 4.5 using $NaHCO_3$ solution (made from 0.94 kg $NaHCO_3$ and 17.8 kg H_2O). The mixture was agitated 15 min and allowed to split for 30 min, and the bottom aqueous layer was discarded. The batch was distilled until the reaction mixture was 82−90 °C. To the reactor was charged 13.34 kg of THF, and the KF was measured. (Note: If KF > 0.3%, the distillation and THF could be repeated). The mixture was cooled to 25 °C and passed through a 10 μm filter into a clean reactor. The reactor and filter were rinsed with 1 kg of THF. The mixture was distilled under vacuum until minimum volume or no more distillate was obtained. To the reactor was charged 23.7 kg of 200 proof ethanol, and the mixture was distilled. The ethanol charge and distillation were repeated. (Note: If the toluene content was >10%, the ethanol charge and distillation could be repeated.) The total volume was adjusted to 40 L and cooled to 60 °C. A seed solution (33 g of **1** in 0.8 kg of heptane and 0.2 kg of 200 proof ethanol) was charged and the line rinsed with 0.5 kg of 200 proof ethanol. The mixture was held at 60 °C until a slurry formed and the antisolvent solution (17.1 kg of heptane and 2.19 kg of ethanol) was slowly charged. The batch was cooled to 20 °C over 4 h. The slurry was passed through a wet mill with only minimal change to particle size. The cake was filtered and washed with 2 portions of wash solution (2.37 kg of ethanol and 18.47 kg of heptane). The solids were dried in a vacuum oven at 40−50 °C until the LOD was <1%. The isolate solids were then subjected to two passes through a hammer mill and D90 = 36 μm was achieved. In total, 2.17 kg of **1** was obtained in 98.0 HPLC area percent purity, >99.9% ee, and 66.2% yield (corrected for potency of input and product). The discrepancy between the observed yield (66.2%) and the expected yield (72%) was due to losses to the mother liquor (24.5%) as well as losses during the development and execution of the dry milling step (4.9%). ^{1}H NMR (400 MHz, DMF) δ 8.81 (d, J = 2.3, 1H), 8.13 (dd, J = 8.8, 2.3, 1H), 7.96 (d, J = 8.3 Hz, 2H), 7.66 (dd, J = 8.3, 2H), 7.61 (t, J = 1.9, 1H), 7.02 (d, J = 2.0, 2H), 6.78 (d, J = 8.6, 1H), 4.5 (d, J = 9.1, 1H), 4.24−4.42 (m, 5H), 4.16 (d, J = 12.1, 1H); ^{13}C NMR (100 MHz, DMF) δ 171.91, 167.19, 162.66, 162.07, 159.06, 153.81, 151.29, 140.07, 138.49, 134.66, 132.92, 129.76, 128.17, 125.02, 118.66, 115.66, 111.98, 105.97, 72.01, 49.08, 47.85, 46.54, 25.10; Anal. Calcd for $C_{26}H_{19}Cl_2N_5O_4S$: C, 58.22; H, 3.57; N, 13.05; Found: C, 58.02; H, 3.57; N, 12.81.

Modified Protocol for the Preparation of 6-((5*S*,9*R*)-9-(4-cyanophenyl)-3-(3,5-dichlorophenyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)nicotinic Acid (1). To a reactor was charged 4.95 kg of **5b** (6.47 mol), 12.4 kg of THF, 28.1 kg of MTBE, and 25.7 kg of 1 M NaOH. The mixture was agitated 15 min and allowed to split for 30 min, and the bottom aqueous layer was discarded. To the reactor was charged 24.8 kg of H_2O. The mixture was agitated 15 min and allowed to split for 30 min, and bottom aqueous layer was discarded. To the reactor was charged 9.66 kg of toluene, and the solution was distilled until the batch temperature reached 85−90 °C. The mixture was cooled to 25 °C, and 3.12 kg of H_2O, 18.46 kg of concentrated AcOH, and 5.44 kg of concentrated HCl were charged, maintaining the reaction temperature <30 °C. The mixture was heated at 30 °C until <1% of **5b** remained. The mixture was cooled to 5 °C, and 26.4 kg of THF and 7.2 kg of 10 wt % brine solution was charged. The pH was then adjusted to 4.5 with 16.3 kg of 10 N NaOH (Note: AcOH can be used to lower pH if pH is accidentally adjusted too high). The mixture was agitated 15 min and allowed to split for 30 min, and the bottom aqueous layer was discarded. To the reaction mixture 6.6 kg of 10 wt % brine solution and 25.7 kg of 2 N NaOH solution was charged. The mixture was agitated 15 min and allowed to split for 30 min, and the bottom aqueous layer was discarded. To the reaction mixture was charged 11.14 kg of H_2O, and the pH was adjusted to 4.5 using $NaHCO_3$ solution (0.928 kg $NaHCO_3$ and 17.63 kg H_2O). The reaction mixture was agitated 15 min and allowed to split for 30 min, and the bottom aqueous layer was discarded. The solution was distilled until the reaction mixture was 82−90 °C. To the reactor was charged 13.2 kg of THF, and the KF was measured. If KF > 0.3% the distillation and THF charge could be repeated. The mixture was cooled to 25 °C, passed through a 10 μm filter into a clean reactor, and the reactor and lines were rinsed with 1 kg of THF. The mixture was distilled under vacuum until minimum volume or no more distillate was obtained.

To the reactor was charged 23.4 kg of 200 proof ethanol and the mixture was distilled. The ethanol charge and distillation was repeated. If the toluene content was >10%, the ethanol charge and distillation could be repeated. The total volume was adjusted to 40 L, and the reaction mixture was cooled to 60 °C. Seeds were charged (33 g of **1** in 0.8 kg of heptane and 0.2 kg of 200 proof ethanol), and the line was rinsed with 0.5 kg of 200 proof ethanol. The mixture was held at 60 °C until a slurry formed and the antisolvent solution (16.9 kg of heptane and 2.17 kg of ethanol) was slowly charged. The batch was cooled to 20 °C over 4 h, and the slurry was wet-milled until the desired particle size was obtained (D90 < 60 μm). The cake was filtered and washed with two portions of wash mixture (2.34 kg of ethanol and 18.28 kg of heptane). The solids were dried in a vacuum oven at 40−50 °C until the LOD was <1%. A total of 2.655 kg was obtained in 85.4% yield (corrected for potency of input and product), >99.9% ee, and 99.8 HPLC area percent purity with a D90 of 28 μm.

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