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Development and Large-Scale Preparation of an Oral TACE Inhibitor

Scott A. Savage,* Robert E. Waltermire, Silvio Campagna, Shailendra Bordawekar, and Joan Dalla Riva Toma

Bristol-Myers Squibb Company, Research and Development, One Squibb Drive, New Brunswick, New Jersey 08903, U.S.A.

Abstract:

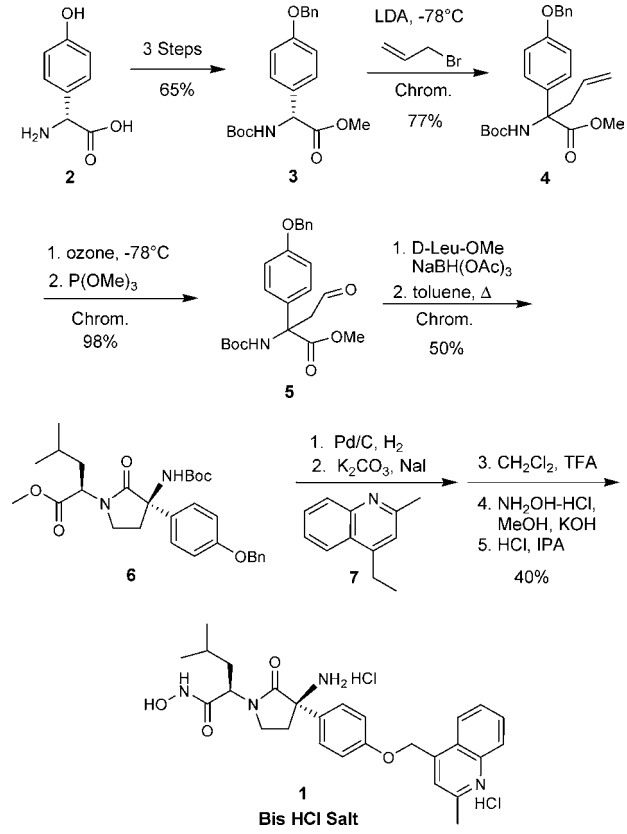
An efficient, expedient synthesis of BMS-561392, 1, which enabled rapid delivery of drug substance for clinical development is described. The key features of the synthesis include an efficient synthesis of a phenolic α,α-disubstituted amino ester via carbon alkylation without protection of the phenol, an effective enzymatic resolution of this racemic amino ester, and a process for the preparation of a hydroxamic acid drug substance with undetectable levels of hydroxylamine.

Introduction

Hydroxamic acid, **1**, is a potent, selective TNF-α convertase enzyme (TACE) inhibitor with appropriate attributes for development including excellent pharmacokinetics (PK) in multiple species and efficacy in preclinical models of rheumatoid arthritis.[1] From a development perspective, a small-molecule, orally active, TACE inhibitor was considered to be a potential high-impact medicine. Thus, from a process research and development perspective we sought to prepare BMS-561392, **1**, as rapidly as possible to try and limit the impact of drug substance availability on the rate of development. The primary challenges of a rapid delivery were synthesis of the quaternary stereogenic center[2] and delivery of drug substance with no more than trace levels of the mutagen hydroxylamine.

The initial goal of the project team was to rapidly identify an enantioselective route to BMS-561392, **1**, that was practical, scalable, and reasonably cost-effective. A preliminary delivery was rapidly needed to produce supplies for toxicology studies, and ultimately larger quantities were required for phase I/II studies. Our Discovery colleagues provided a 12-step linear synthesis, centered around forming the γ-lactam core from a phenyl glycine derivative **5** and D-leucine methyl ester[1] (Scheme 1). The resulting diastereomers were then chromatographically separated to give **6**. This synthesis was appealing from a process perspective due to the straightforward approach along with the availability and extremely low cost of the starting material D-4-hydroxyphenyl glycine, **2**. We took advantage of this and devised a similar synthesis that hinged upon an enantioselective preparation of a compound resembling **4** and provided key intermediates as crystalline solids to facilitate purification.[3]

Scheme 1. **Original synthesis of BMS-561392**



Results and Discussion

Our group focused on the set of retrosynthetic disconnects shown in Scheme 2. The goal was to identify an

* Author for correspondence. E-mail: scott.savage@bms.com.

(1) (a) Maduskuie, T. P., Jr.; Duan, J.; Mercer, S. E. (Dupont Pharmaceuticals Company, U.S.A.). Novel Lactam Metalloprotease Inhibitors. PCT Int. Appl. WO 2002/004416 A2, 20020117, 2002. (b) Duan, J. J.-W.; Chen, L.; Lu, Z.; Wasserman, Z. R.; Maduskuie, T. P.; Liu, R.-Q.; Covington, M. B.; Vaddi, K. G.; Qian, M.; Voss, M. E.; Xue, C.-B.; Hardman, K. D.; Ribadeneira, M. D.; Newton, R. C.; Magolda, R. L.; Christ, D. D.; Decicco, C. P. *Abstracts of Papers*; 224th ACS National Meeting, Boston, MA, U.S.A., August 18−22, 2002, American Chemical Society: Washington, DC, 2002, MEDI-426.

(2) For reviews and syntheses of related tetra-substituted amino acids see: (a) Cativiela, C.; Diaz-De-Villegas, M. D. *Tetrahedron: Asymmetry* **1998**, *9*, 3517–3599. (b) Ma, D.; Tian, H. *Tetrahedron: Asymmetry* **1996**, *7*, 1567–1570.

(3) Waltermire, R. E.; Savage, S. A.; Campagna, S.; Magnus, N. A.; Confalone, P. N.; Yates, M.; Meloni, D. J. (Bristol-Myers Squibb Company, U.S.A.). Asymmetric synthesis of aminopyrrolidinones. PCT Int. Appl. WO 2003/104220, A1 20031218 , 2003, and U.S. Patent 6,770,763, B2 20040803.

(4) (a) Vedejs, E.; Fields, S. C.; Schrimpf, M. R. *J. Am. Chem. Soc.* **1993**, *115*, 11612–11613. (b) Seebach, D.; Boes, M.; Naef, R.; Schweizer, W. B. *J. Am. Chem. Soc.* **1983**, *105*, 5390–5398. (c) Belokon, Y. N.; Kochetkov, K. A.; Churkina, T. D.; Ikonnikov, N. S.; Chesnokov, A. A.; Larionov, O. V.; Parmar, V. S.; Kumar, R.; Kagan, H. B. *Tetrahedron: Asymmetry* **1998**, *9*, 851–857. (d) Ooi, T.; Kameda, M.; Maruoka, K. *J. Am. Chem. Soc.* **1999**, *121*, 6519–6520. (e) Horikawa, M.; Busch-Petersen, J.; Corey, E. J. *Tetrahedron Lett.* **1999**, *40*, 3843–3846.

(5) Jacques, J.; Collet, A.; Wilen, S. H. *Enantiomers, Racemates, and Resolutions*; John Wiley: New York, 1981.

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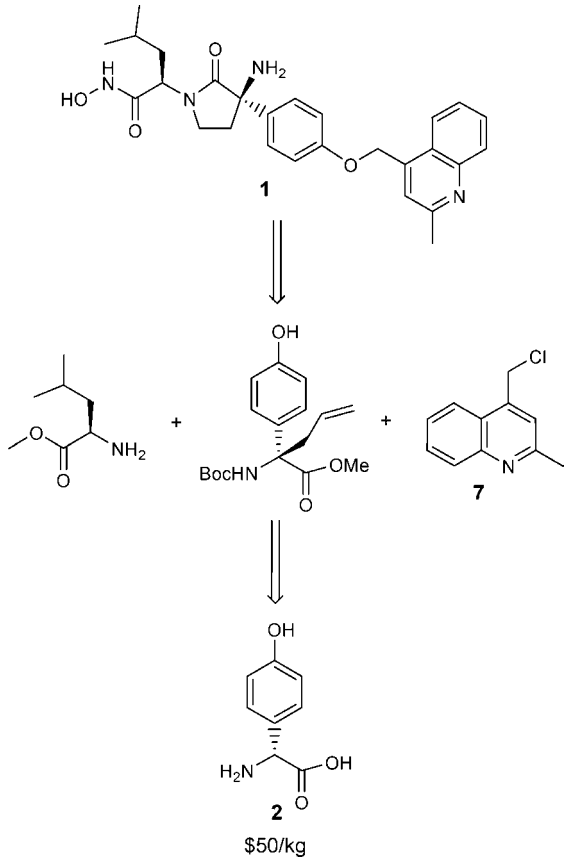
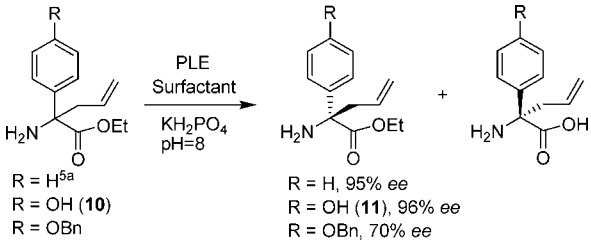
Scheme 2. **Retrosynthetic analysis**



asymmetric route to compounds similar to **4** while avoiding benzyl protection and deprotection of the phenol. Three approaches to form chiral α,α-disubstituted amino esters were investigated in parallel: (1) asymmetric alkylations of D-4-hydroxyphenyl glycine derivatives,[4] (2) traditional salt resolutions of α-substituted D-4-hydroxyphenyl glycine,[5] and (3) enzymatic resolutions of α-substituted D-4-hydroxyphenyl glycine esters.[6]

The most promising lead came from a reported enzymatic resolution with pig liver esterase (PLE), whereby Van Betsbrugge et al. demonstrated the successful enzymatic hydrolysis of α-allylphenyl glycine ethyl ester[6] (Scheme 3). By driving the hydrolysis of the racemic

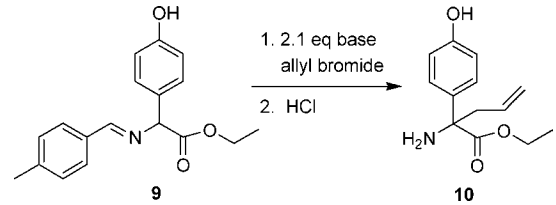
Scheme 3. **Enzymatic resolution of α-allylphenyl glycine derivatives**



ester to 57% conversion, they obtained the *R*-ester with 95% ee. Our initial screen of the enzymatic hydrolysis of 4-hydroxyphenyl glycine ethyl ester with PLE gave similar results of 88% ee crude ester and 96% ee crystallized product. The benzyl protected phenol was less effective in the enzymatic hydrolysis, affording only 70% ee. The benzyl group significantly lowered the substrate's aqueous solubility and consequently the reactivity in the enzymatic hydrolysis. These results were appealing as we hoped to avoid phenol protection and deprotection. In addition, there were obvious cost benefits of alkylating the phenol with 4-chloromethyl-2-methyl quinoline, **7**, after the resolution. The enzymatic resolution *via* hydrolysis of **10** to **11** with PLE[7] became the cornerstone of our new synthesis of BMS-561392.

Enantioselective Synthesis of αα-Disubstituted Amino Ester 12. Having identified an effective enzymatic resolution of **10**, we began development work towards its efficient preparation. The Fischer esterification of D-4-hydroxyphenyl glycine, **2**, was straightforward using methanesulfonic acid (MSA) and ethanol to afford the ethyl ester **8** as the corresponding MSA salt.[8] The free amine **8** was crystallized upon neutralization of the reaction mixture with aqueous sodium hydroxide and was isolated in 92% yield. Imine formation was chosen to protect the amine during alkylation as our discovery colleagues observed that allylation of the corresponding Boc-protected phenyl glycinate **2** was not selective and resulted in a mixture of C- and N-alkylation. The crystalline *p*-tolualdehyde-protected imine **9** was prepared in 97% yield by Schiff base formation by way of azeotropic distillation of water using toluene. This allowed for a single solvent for both the reaction and the crystallization. The presence of the unprotected phenol necessitated double deprotonation to form both the phenolate and enolate ions with the expectation that the enolate anion would be more reactive towards an allyl electrophile. Considering that competitive allylation of the phenol oxygen versus the enolate carbon could be an issue, we selected a lithium base to exploit the advantage of the strong Li−O bond[9] to minimize allyl ether formation. The initial condition of 2.1 equiv of lithium bis(dimethylsilyl)amide (LiHMDS) and 1.05 equiv of allyl bromide was successful with >99% conversion and almost none of the allyl ether byproduct (Scheme 4). After *in situ* hydrolytic

Scheme 4. **Enolate alkylation in the presence of a phenol**



cleavage of the imine, the product **10** was crystallized as the MSA salt in 80% yield. Lithium *tert*-butoxide (LiOt-Bu) was shown to be an equally effective base. On the basis of a significantly lower cost, lithium *tert*-butoxide was selected over

(6) PLE had previously been shown to be effective for the resolution of analogues of **10**. Van Betsbrugge, J.; Tourwe, D.; Kaptein, B.; Kierkels, H.; Broxterman, R. *Tetrahedron* **1997**, *53*, 9233–9240. Moorlag, H.; Kellogg, R. M. *Tetrahedron: Asymmetry* **1991**, *2*, 705–720.

(7) This work was conducted before BSE/TSE issues became a concern. If this process is to be utilized for commercial manufacture, a further screen of enzymes will be conducted to identify one lacking this concern.

(8) Wasserman, H. H.; Hlasta, D. J.; Tremper, A. W.; Wu, J. S. *J. Org. Chem.* **1981**, *46*, 2999–3011.

(9) Arnett, E. M.; Small, L. E. *J. Am. Chem. Soc.* **1977**, *99*, 808–816.

LiHMDS for scale-up. The use of LiHMDS or LiOt-Bu allowed the allyl bromide to be charged prior to the formation of the bis-lithiate with no impact on the level of phenol alkylation. This was important on scale, as the bis-lithiate was crystalline and would form a thick slurry in tetrahydrofuran at the optimal reaction concentration. Charging allyl bromide prior to LiOt-Bu avoided pre-formation of the enolate which provided a significant reduction in utilization of tetrahydrofuran. This change resulted in an increase in volume efficiency and a decrease in yield loss to aqueous waste streams. Experiments using potassium *tert*-butoxide (KOt-Bu) were conducted to further explore the effect of counterion on alkylation selectivity. As expected, the carbon versus oxygen selectivity was significantly reduced, as was the overall robustness of the allylation due to tighter temperature constraints and the requirement to pre-form the potassium enolate prior to the addition of allyl bromide.

The development of the enzymatic resolution of **10** to **11** began by optimizing the conditions established by Van Betsbrugge et al. Since PLE is quite expensive, loading studies were conducted to identify the minimum quantity required for robust conversion (a range of 50−380 kU/mol of **10** was studied with a range of 130 to 150 kU/mol of **10** found to be optimal). Potassium dihydrogenphosphate (0.2 mol %) was used as the buffer (pH 8), and Triton-100X (2 wt %) was used as a surfactant to help facilitate phase interaction in this heterogeneous reaction. The crystallization was straightforward as enantiomerically pure **11** can also be conveniently isolated as an MSA salt with an upgrade in ee, using essentially the identical conditions as for the racemate **10**. As shown in Table 1 (batches 1−7), these conditions resulted in 18 h reaction times,

Table 1. **Enzymatic resolution of amino ester 10**

batch	input (kg)	PLE (kU/mol)	temp (°C)	surfactant	buffer	reaction time (h)	yield (%)	ee (%)
1	45.0	130	28	Triton	KH_2PO_4	18	34	95
2	45.0	130	28	Triton	KH_2PO_4	18	35	83
3	45.0	130	28	Triton	KH_2PO_4	18	37	88
4	45.0	130	28	Triton	KH_2PO_4	18	38	91
5	45.0	130	28	Triton	KH_2PO_4	18	35	81
6	45.0	130	28	Triton	KH_2PO_4	18	47	78
7	30.0	130	28	Triton	KH_2PO_4	18	41	96
8	12.0	130	28	Antarox	TRIS	18	38	93
9[a]	12.0	150	40	Antarox	TRIS	5	34	98
10	12.0	150	40	Antarox	TRIS	5	32	98
11	309	150	38	Antarox	TRIS	5	44	94
12	311	150	38	Antarox	TRIS	2.5	41	98
13	331	150	38	Antarox	TRIS	2.5	45	98
14	336	150	38	Antarox	TRIS	2.5	43	98
15	328	150	38	Antarox	TRIS	2.5	44	98

[a] Surfactant was increased from 2 to 5 wt % on subsequent batches.

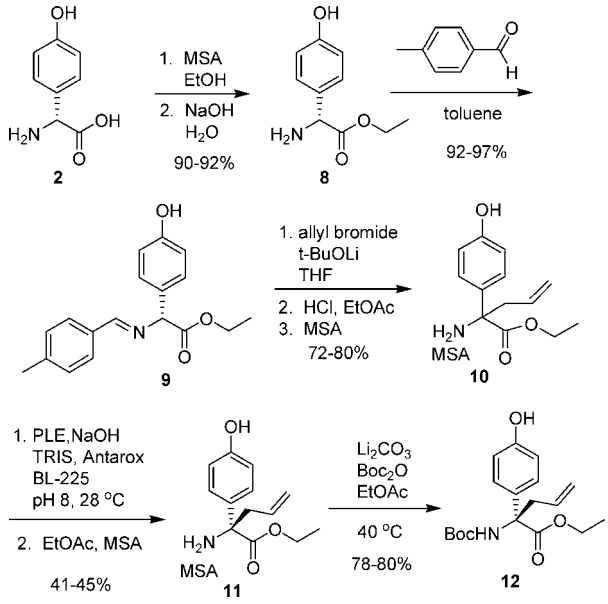
yields around 38%, and optical purities from 78 to 96% ee. This in-house campaign was effective in that the 113 kg of **11** prepared in batches 1−7, afforded >99.5% ee drug substance due to optical purity upgrades throughout the remainder of the synthesis. Before transferring this technology to a contract manufacturer, the opportunity was taken to further optimize the enzymatic hydrolysis. The issues focused on were the following: variable resolution results, long reaction times (18 h), and poor filtration of enzyme residue. The first challenge was to improve the heterogeneous nature of the reaction. Triton-100X surfactant produced foaming, leading to suspended substrate and ultimately variable conversion and enantiomeric excess. This problem was alleviated by switching to a nonfoaming surfactant (Antarox BL-225). In addition, Tris(hydroxymethyl)aminomethane (TRIS) buffer replaced potassium dihydrogenphosphate to improve the solubility of **10**. Enzyme loading and reaction temperature were reevaluated using these new reagents, the results of which are presented in Table 1 (batches 8−10). These changes resulted in a significant increase in the rate of reaction (decrease in reaction time in Table 1 batches 9−15). Looking to improve the speed of the biomass filtration, the effects of various Celite grades and loadings on filtration rates were studied. The results of these experiments showed that the addition of 10 wt %, based on reaction mass, of Celite 560 notably increased the flow rate of filtration to ∼2 L/min with a high recovery of ∼97% of the product. The final, and key change, was to heat the solution of **10** in water to 55 °C prior to pH adjustment and addition of the enzyme. Performing the neutralization of the methanesulfonic acid salt **10** at elevated temperature in the presence of surfactant was found to afford a stable emulsion. After cooling the emulsion to 38 °C, the enzyme was added. The culmination of these improvements was dramatic on production-scale (batches 11−15). The reaction time was decreased to 2.5 h, yields were increased to up to 45%, and the selectivity was consistently 98% ee.

The final step to prepare our desired intermediate, **12**, was to protect the amine in preparation for the ozonolysis and subsequent reductive amination with D-leucine. What was expected to be one of the simpler steps in the synthesis proved to be quite challenging due to the steric hindrance around the amine and the nucleophilicity of the phenol. Typical conditions for Boc protection using di-*tert*-butyl dicarbonate (Boc_2O) and triethylamine were extremely sluggish and nonselective for reaction at the amine vs the phenol.[10] When 4-(*N*,*N*-dimethylamino)pyridine was used to catalyze the N-acylation, the corresponding isocyanate was the predominant product. Potassium carbonate in THF and water was our first reasonable success using Boc_2O, but it was not possible to drive the reaction past 90% conversion without forming significant levels of the *tert*-butyl phenol carbonate of **12**. Drawing from our experience with the the synthesis of **10**, we postulated that lithium carbonate might decrease the reactivity of the phenol. The use of lithium carbonate in place of potassium carbonate was successful, allowing the use of excess Boc_2O at 40 °C to drive the reaction to >95% conversion while keeping the level of the *tert*-butyl phenol carbonate to <5%. Changing the reaction solvent from THF to EtOAc enabled an efficient phase separation after the reaction and an effective crystallization from EtOAc/heptane, which afforded an 80% yield of **12**.

Having developed a robust process for the synthesis of the chiral α,α-disubstituted amino ester, we scaled up in our pilot plant (73 kg **12** was produced) and ultimately at a contract manufacturer (405 kg was produced at ≥99% ee and ≥99% purity). It is important to note that all intermediates in the synthesis of **12** were isolated as crystalline solids (Scheme 5).

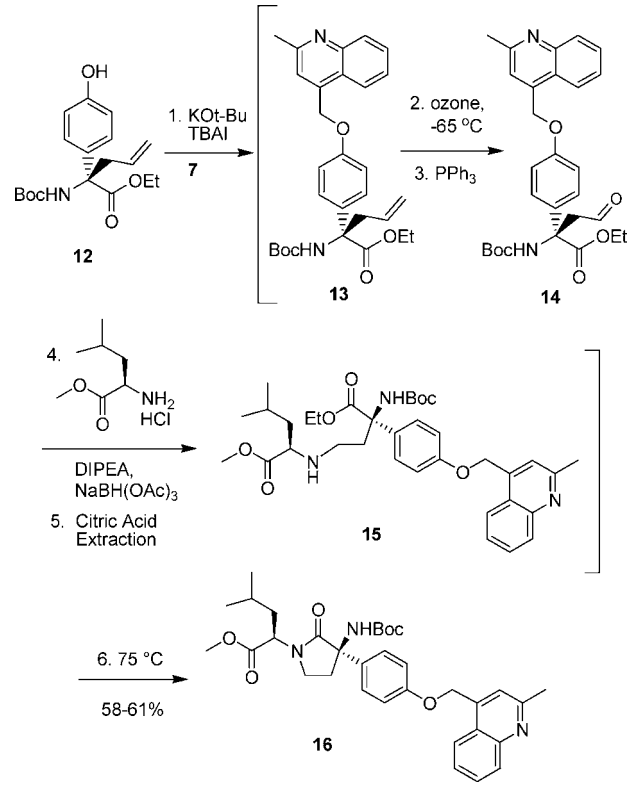
(10) Greene, T. W.; Wuts, P. G. M. *Protecting Groups in Organic Synthesis*; John Wiley: New York, 1999.

Scheme 5. **Synthesis of protected amino ester 12**



Reductive Amination and Cyclization to Lactam 17. An objective was to avoid the use of a benzyl-protected phenol in the ozonolysis step, and we were pleasantly surprised to find that installation of the quinoline group prior to the ozonolysis posed no reactivity issues. Analysis found no detectable oxidation of the quinoline nitrogen during ozonolysis. The synthesis of the Boc-protected lactam **16** was a four-step telescoped process, beginning with phenol alkylation in THF using potassium *tert*-butoxide, 4-chloromethyl-2-methyl quinoline, and 0.05 equiv of tetrabutylammonium iodide to afford **13**. After neutralization with aqueous acetic acid, the compound was extracted into EtOAc. An important part of this process was the subsequent use of an aqueous citric acid wash to remove residual 4-chloromethyl-2-methyl quinoline and its corresponding impurities. This step is necessary since **13** is not a crystalline compound. Originally, the ozonolysis was performed in methylene chloride at <−70 °C. In order to avoid using environmentally unfriendly chlorinated solvents, we examined alternative solvents and ultimately selected EtOAc as the solvent. We found the ozonolysis to be compatible with EtOAc and that it could be run at a slightly warmer temperature. The ozonolysis process entailed cooling a solution of **13** in EtOAc to −65 °C and introducing ozone subsurface. After reaction completion, nitrogen was introduced subsurface to purge excess ozone from the organic solution. Triphenylphosphine was added to reduce the intermediate ozonide to the desired aldehyde **14**. The EtOAc solution of **14** was cooled to −10 °C and treated with DIPEA, $NaBH(OAc)_3$, and D-leucine methyl ester to effect a reductive amination.[11] While the reaction was straightforward, the reductive amination process required an extensive workup due to the lack of crystallinity of aldehyde **14**, and to enable removal of the triphenylphosphine oxide byproduct and residual triphenylphosphine. It was critical to remove the triphenylphosphine and triphenylphosphine oxide as they were found to inhibit the crystallization of the lactam product **16**. The process we devised took advantage of the dibasic nature of the intermediate to extract **15** into water using 33 wt % citric acid. After phase separation, the acidic aqueous solution of **15** was basified to enable extraction into EtOAc. This allowed complete removal of all nonbasic byproduct. Care had to be taken to avoid heat during the reductive amination and workup, as any amine **15** that cyclized to lactam **16** was not extractable and would result in diminished yield. The reduction in efficiency during production was more than compensated by the purification provided by this extraction. As **16** is the only crystalline intermediate in this sequence, its crystallization was a critical aspect of this four-step telescoped process. The EtOAc solution of **15** was heated to reflux to convert the amine to the corresponding lactam, **16**, and a final solvent exchange to isopropanol (IPA) allowed for crystallization and isolation of **16** in 60% overall yield from **12** (Scheme 6).

Scheme 6. **Synthesis of lactam core to prepare 16**

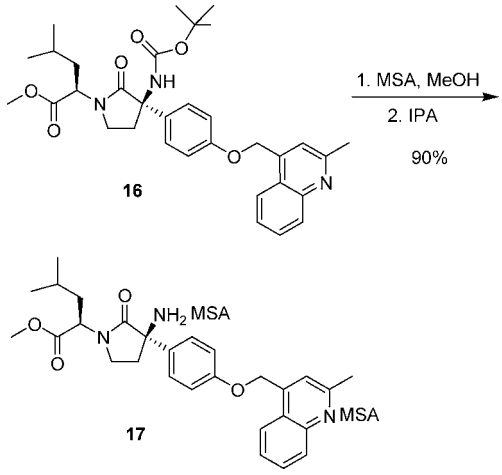


As is typical for pharmaceutical processes, the purity of the final intermediate was extremely important, and thus it was critical to have a robust process and crystallization. In our case, the deprotected lactam **17** fit this role perfectly as the simple Boc cleavage could be carried out easily under a variety of acidic conditions. This allowed for a thorough investigation of salt forms and reaction solvents. The acid selection was critical to provide a crystalline salt that gave the necessary purification while also having suitable filtration and handling properties. Again, methanesulfonic acid (MSA) was the chosen acid due to the excellent purification and solid-state properties of the bis-MSA salt **17**. Isopropanol was initially selected as the solvent as it provided a fast reaction, efficient impurity removal, and low product solubility (<0.5 wt %). As the purity of **16** improved over time, a consequence was that the bis-MSA salt **17** began crystallizing out of solution much earlier during the deprotection reaction. This resulted in a small amount (∼1.5%)

(11) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849–3862.

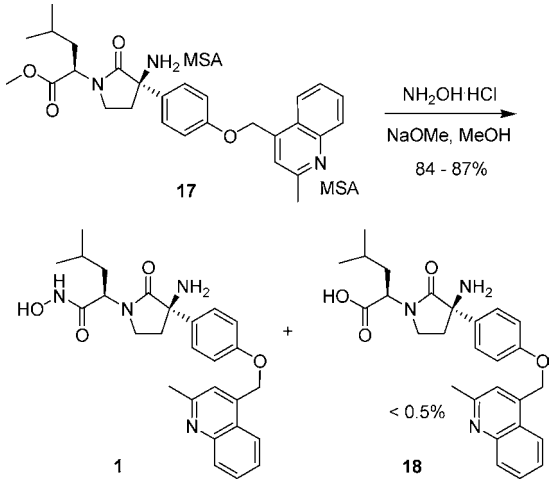
of the starting material **16** trapped within the crystals. The solution to this problem was to run the reaction in a minimal amount of methanol (3 kg MeOH/kg **16**) at 55 °C to keep the product in solution throughout the majority of the reaction. Isopropanol was added after the reaction to complete the crystallization. This modified procedure effectively converted all of the **16** to**17** without significantly increasing yield loss to the filtrate. A side benefit was that this new procedure eliminated the small amount of transesterification to the isopropyl ester formed during deprotection in isopropanol (Scheme 7).

Scheme 7. **Boc cleavage to form final intermediate 17**



Hydroxamic Acid Preparation and Purification. A major yield hindrance in the conversion of the methyl ester **17** to the hydroxamic acid **1** using hydroxylamine and potassium hydroxide was the competing hydrolysis to the corresponding carboxylic acid **18** (Scheme 8).[1] The solution to this problem

Scheme 8. **Hydroxamate formation to give target 1**



was to utilize sodium methoxide to neutralize the hydroxylamine hydrochloride salt.[12] This change, along with minimization of the water content of the reaction, was found to be effective at reducing ester hydrolysis, as the methoxide would redundantly react to afford the starting material **17**. A small amount of hydrolysis to **18** is unavoidable due to the small amount of water present in the hydroxylamine hydrochloride.

(12) Thouin, E.; Lubell, W. D. *Tetrahedron Lett.* **2000**, *41*, 457–460.

The biggest hurdle associated with the last step in our sequence was the removal of excess hydroxylamine. The limit determined in consultation with our drug safety evaluation group was to have <5 ppm hydroxylamine in the drug substance. This level was based both on review of the literature and most importantly on a decision to have undetectable levels with our current, best analytical methodology. At the time, our analytical group had developed an ion chromatography method that detected hydroxylamine in our compound with a detection limit of 4 ppm. The original synthesis of BMS-561392 used 10 equiv of hydroxylamine, and thus the removal of this potential impurity was a major concern. Using the modified procedure of sodium methoxide and hydroxylamine hydrochloride, we were able to reduce the requirements to 4 equiv of NH_2OH. The initial API was the bis-HCl salt of **1**, as shown in Figure 1. This target provided a significant challenge, as a portion of

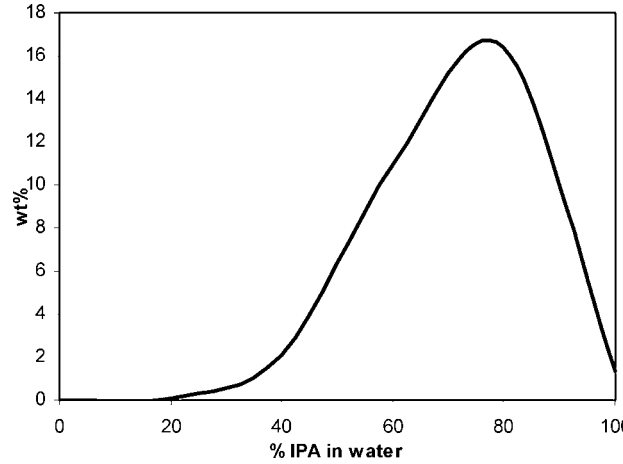


Figure 1. **Solubility of BMS-561392 in isopropanol and water mixtures.**

the residual hydroxylamine crystallized as the HCl salt during the bis-hydrochloride API formation. During our process, the product was extracted into EtOAc by adjusting the pH to neutral after the hydroxamate formation using HCl. After this aqueous workup, there was typically >1000 ppm NH_2OH in the organic stream. A crystallization study showed that the optimal HCl salt procedure could only tolerate ~30 ppm of hydroxylamine in solution prior to crystallization and still meet the limit. In order to achieve this 30 ppm level, four water washes were utilized after the initial aqueous workup to remove the excess hydroxylamine. Our first and only delivery of BMS-561392 bis-hydrochloride provided 1.6 kg for use in preclinical toxicology studies.

The bis-hydrochloride salt of **1** had significant issues with crystal form which were related to the release of hydroxylamine. The combination of its hygroscopicity (typically 5% water by KF) and acidic nature resulted in hydrolysis during both drying and storage to form detectable levels of hydroxylamine. Due to this issue, we began investigating other crystalline forms of **1**. Our solution came quickly, as we discovered the existence of a crystalline neutral form. This neutral form was found to have suitable properties for development, including excellent solid-state stability and PK in multiple species. This new final form not only solved all drying and storage issues, but afforded new process opportunities to remove hydroxylamine during

crystallization. Unlike hydroxylamine hydrochloride, hydroxylamine is soluble in alcohols as well as water. The crystalline free base of **1** was first discovered by crystallization from isopropanol, but it was slightly too soluble (~2 wt %) to be the process crystallization solvent. While looking for a suitable single solvent, as well as antisolvent combinations, an interesting trend was discovered. The solubility of **1** in IPA/water combinations exhibited nonlinear behavior whereby the product is not appreciably soluble in either single solvent, but was surprisingly very soluble (16 wt % at 20 °C) in 80 vol % isopropanol with 20% water (Figure 1).

Taking advantage of this solubility data, a new crystallization procedure was identified. As stated earlier, the product **1** was initially extracted into ethyl acetate. After solvent exchange from EtOAc to IPA, water was added to the desired 4:1 ratio to afford maximum solubility. This allowed **1** to be soluble in ~3.5 L/kg at 55−60 °C, improving the volume efficiency of our process. Water was then added to a ratio of 2:3, and seed crystals were added. After cooling to 45 °C, the remainder of the water was added to adjust to a final composition of 80% water and 20% isopropanol.

The discovery of the crystalline-free base of BMS-561392 and development of the isopropanol/water crystallization allowed for significant improvements in removal of residual hydroxylamine. The neutral form crystallization could tolerate as much as 200 ppm of residual hydroxylamine in the rich, organic stream, allowing the use of only two or three water washes after the reaction. Of equal importance was that this form does not liberate hydroxylamine upon drying or storage as did the bis-HCl salt. Overall our team prepared over 150 kg of BMS-561392 over a 2-year period, and all batches contained no detectable level of hydroxylamine.

Conclusions

From the inexpensive starting material, D-4-hydroxyphenyl glycine, the first enantioselective synthesis of the TACE inhibitor BMS-561392 was identified. The synthesis consists of a linear sequence of eleven chemical transformations with eight isolations of crystalline intermediates. The features of the synthesis include efficient synthesis of a phenolic α,α-disubstituted amino ester via carbon alkylation without protection of the phenol, an effective enzymatic resolution of this racemic amino ester, and a process for the preparation of a hydroxamic acid drug substance with undetectable levels of hydroxylamine. Discovery of the final form as neutral, stable, and crystalline proved very important to the development of **1**. The robustness of the processes are supported by multiple process demonstrations on manufacturing scale for early intermediates and pilot scale for the final intermediate and API. This efficient, expedient synthesis of **BMS-561392** enabled its rapid entry into clinical studies.

Experimental Section

(*R*)-Ethyl 2-Amino-2-(4-hydroxyphenyl)acetate (8). An inerted 300-gal reactor was charged with absolute ethanol (123 kg) and cooled to 10 °C. D-4-Hydroxyphenyl glycine (78 kg, 0.47 kmol) was added, followed by methanesulfonic acid (86 kg, 0.89 kmol, 1.93 equiv) at ≤78 °C. The reaction mass was heated to 78 °C and aged for 2 h. The reactor was cooled to 55 °C, and the reaction was determined complete by HPLC. The reactor was cooled to 40 °C and H_2O (468 L) was added. The reactor was cooled to 5 °C, and 17% aqueous sodium hydroxide (prepared from 142 L of H_2O, 71.3 kg of 50% sodium hydroxide {0.89 kmol, 1.93 equiv}) was slowly added over 2 h to provide a slurry of **8** at pH = 7.0 to 7.5. The slurry was stirred at 5 °C for 1 h. Ester **8** was isolated by centrifugation and washed with H_2O (3 × 106 kg). The solids were vacuum dried at 50−55 °C to a constant weight to yield 82 kg (90% yield) of a light-yellow crystalline solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.18 (d, J = 8.5 Hz, 2H), 6.73 (d, J = 8.6 Hz, 2H), 4.40 (s,1H), 4.15−3.95 (m, 2H), 1.12 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.7, 157.1, 131.7, 128.2, 115.4, 60.6, 58.0, 14.3. Analysis Calculated for $C_{10}H_{13}NO_3$: C, 61.53; H, 6.71; N, 7.18. Found: C, 61.54; H, 6.57; N, 7.11.

(*E*)-Ethyl 2-(4-Hydroxyphenyl)-2-(4-methylbenzylideneamino)acetate (9). An inerted 300-gal reactor was charged with toluene (685 kg) and cooled to 0 °C. Amine **8** (80 kg, 0.41 kmol) was added followed by *p*-tolualdehyde (51.4 kg, 0.43 kmol, 1.04 equiv) with a toluene (7 kg) flush. The reaction mass was heated to reflux (~90 °C) and distilled until approximately 180 kg of distillate was collected (~115 °C). Fresh toluene, equivalent in mass to the distillate, was added. The reaction mass was cooled to 85 °C and aged for 1 h, then cooled to 20 °C over 1 h and aged for 2 h. Imine **9** was isolated by centrifugation and washed with a toluene (200 kg)−heptane (157 kg) mixture in three portions. The product **9** was vacuum dried at 50 to 55 °C to a constant weight to yield 116 kg (95% yield) of a white to light-yellow crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.67 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 8.6 Hz, 2H), 7.16 (d, J = 8.0 Hz, 2H), 6.76 (d, J = 8.6 Hz, 2H), 5.13 (s, 1H), 4.25−4.11 (m, 2H), 2.33 (s, 3H), 1.20 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 164.0, 156.2, 141.8, 132.7, 129.3, 129.2, 129.1, 128.8, 115.9, 75.7, 61.6, 21.5, 14.0.

Ethyl 2-Amino-2-(4-hydroxyphenyl)pent-4-enoate Methane Sulfonate (10). An inerted 300-gal glass-lined reactor was charged with tetrahydrofuran (215.5 kg, THF) and cooled to −5 °C. Imine **9** (62.0 kg, 0.21 kmol) was added, followed by allyl bromide (26.7 kg, 0.22 kmol, 1.06 equiv) with a THF (2.5 kg) chase. The reactor was cooled to −5 °C and charged with 2 M lithium *tert*-butoxide in THF (192.6 kg, 0.44 kmol, 2.1 equiv) over 1 h at ≤5 °C. The reaction mass was sampled for conversion after 30 min and determined complete by HPLC (criteria ≥30:1 ratio of **10**:**9**). The reaction was quenched by adding 2 M aqueous hydrochloric acid (326 kg, 0.625 kmol, 3 equiv), pH = 1 after quench. Heptanes (107 kg) were added, and the layers were separated, retaining the product-rich aqueous phase. The pH of the aqueous phase was adjusted to 8−9 by adding a 16% aqueous sodium hydroxide solution (prepared by combining 32 kg of 50% aqueous sodium hydroxide {0.40 kmol, 1.9 equiv} and 62 kg of H_2O). Sodium chloride (31 kg) and ethyl acetate (369 kg) were added, and the layers were separated, retaining the product-rich organic phase. The batch was heated to reflux to distill off residual THF. The distillation was deemed complete when the batch temperature reached 78 °C. Ethyl acetate was used to adjust to a final volume of ~250

L. The batch was cooled to 60 °C, and 2-propanol (9.6 kg) was added. After cooling to 50 °C, methanesulfonic acid (20.1 kg, 0.21 kmol, 1 equiv) was added. The batch was then cooled to 0 °C and aged for 1 h. Product **10** was isolated by centrifugation and washed with ethyl acetate (2 × 56 kg). Sulfonate **10** was vacuum dried at 50−55 °C to a constant weight to afford 56 kg (80% yield) of a white to light yellow crystalline solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.90 (s, 1H), 8.83 (s, 3H), 7.29 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 8.8 Hz, 2H), 5.83−5.71 (m, 1H), 5.30 (d, J = 17.0 Hz, 1H), 5.24 (d, J = 10.1 Hz, 1H), 4.30−4.15 (m, 2H), 2.97 (dd, J = 6.8, 14.5 Hz, 2H), 2.35 (s, 3H), 1.18 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 171.4, 160.4, 131.1, 128.9, 127.1, 123.7, 117.4, 66.1, 64.7, 41.5, 40.0, 14.7.

(*R*)-Ethyl 2-Amino-2-(4-hydroxyphenyl)pent-4-enoate Methane Sulfonate (11). Racemate **10** (331.4 kg, 1.0 kmol), water (3164 L), tris(hydroxymethyl)aminomethane (20.7 kg, 0.17 kmol), and Antarox BL-225 (15 kg) were charged into a 3000-gal vessel. The pH of the solution was raised to 7.8−8.2 by adding 6 N NaOH solution (∼130 kg). The mixture was heated to 55 °C to consume the methanesulfonic acid, causing the starting material to oil out of solution. Utilizing a continuous pH monitor, the pH of the mixture was adjusted to 7.8−8.2 using 6 N NaOH (∼30 kg). The batch was cooled to 38 °C, and pig liver esterase (PLE, 3.5 kg) was added.[13] The solution was stirred while maintaining the desired pH range by adding 6 N NaOH as needed. After 2.5 h a sample was pulled to determine the enantiomeric excess of the desired amino ester (criteria ≥93% ee). After cooling to 20 °C, water (242 L) and ethyl acetate (2740 L) were added while continuing to monitor pH, adding 6 N NaOH (∼130 L) as necessary to keep the pH in range. The pH was adjusted to 9.5 to 9.8 using 6 N NaOH (∼90 kg), and Celite (390 kg) was then added to the mixture. The batch was filtered and the cake washed sequentially with ethyl acetate (635 L), water (120 L), and ethyl acetate (635 L). The combined filtrate and washes were recharged to the vessel and allowed to separate. The aqueous layer was removed and back extracted with ethyl acetate (1800 L). The combined organic layers were washed with a saturated aqueous sodium chloride solution (500 L). An atmospheric distillation was performed to reduce the solution to ∼1550 L. After cooling to 50 °C, isopropanol (45 kg) and methanesulfonic acid (MSA, 48.0 kg, 0.5 kmol) were added to form the desired salt. The resulting slurry was cooled to 10 °C and aged for one hour. The product was isolated by centrifugation and washed with ethyl acetate (700 L). Sulfonate **11** was vacuum dried at 50 °C to yield 148 kg (45% yield) of a white to light-yellow crystalline solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.90 (s, 1H), 8.83 (s, 3H), 7.29 (J = 8.8 Hz, 2H, d), 6.85 (d, J = 8.8 Hz, 2H), 5.83−5.71 (m, 1H), 5.30 (d, J = 17.0 Hz, 1H), 5.24 (d, J = 10.1 Hz, 1H), 4.30−4.15 (m, 2H), 2.97 (dd, J = 6.8, 14.5 Hz, 2H), 2.35 (s, 3H), 1.18 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 171.4, 160.4, 131.1, 128.9, 127.1, 123.7, 117.4, 66.1, 64.7, 41.5, 40.0, 14.7. Analysis calculated for $C_{14}H_{21}NO_6S$: C, 50.74; H, 6.39; N, 4.23; S, 9.68. Found: C, 50.43; H, 6.06; N, 4.08; S, 9.74.

(*R*)-Ethyl 2-(*tert*-Butoxycarbonyl)-2-(4-hydroxyphenyl)-pent-4-enoate (12). An inerted 300-gal glass-lined reactor was charged sequentially with **11** (55 kg, 0.17 kmol), lithium carbonate (26.8 kg, 0.36 kmol, 2.2 equiv), ethyl acetate (150 kg), di-*tert*-butyl dicarbonate (72.4 kg, 0.33 kmol, 1.95 equiv), and water (269 L). The batch was heated to 40 ± 2 °C and aged for 14 h. The reaction was sampled and determined complete by HPLC. (criteria: >20:1, **12**:**11**). After cooling the reaction mass to 20 °C, acetic acid was slowly charged (32.9 kg, 0.55 kmol, 3.2 equiv). The pH was checked and adjusted to <6.5 with acetic acid. The reaction was allowed to separate, and the aqueous layer was removed. The organics were washed with water (269 L). A vacuum distillation at 100 mmHg was used to remove ethyl acetate from the organics by reducing the volume to 400 L. The reaction was sampled for solvent composition (criteria: >95:1 heptane:ethyl acetate, <1 wt % **12** in supernatant). The resulting slurry was cooled to 20 °C. Product **12** was isolated by filtration on a nutsch and washed with heptane (2 × 75 kg). The solids were vacuum dried at 50−55 °C to a constant weight to yield 43 kg (78% yield) of a white to light-yellow crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, J = 8.7 Hz, 2H), 6.57 (d, J = 7.7 Hz, 2H), 6.19 (s, 1H), 5.75−5.58 (m, 1H), 5.18 (d, J = 14.0 Hz, 1H), 5.14 (d, J = 8.2, 1H), 4.25−4.00 (m, 2H), 3.48−3.30 (m, 1H), 3.25−3.10 (m, 1H), 1.44 (s br, 9H), 1.16 (t, J = 14.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 155.7, 154.0, 132.5, 130.6, 127.0, 119.4, 115.5, 79.9, 64.4, 62.2, 38.1, 28.4, 14.0. Analysis calculated for $C_{18}H_{25}NO_5$: C, 64.46; H, 7.51; N, 4.18. Found: C, 60.03; H, 7.38; N, 4.14.

(*R*)-Ethyl 2-(*tert*-Butoxycarbonyl)-2-(4-((2-methylquinolin-4-yl)methoxy)phenyl)-4-oxobutanoate (14). A 400-L inerted glass-lined reactor was charged with THF (150 kg) followed by potassium *tert*-butoxide (48.2 kg, 0.43 kmol, 1.2 equiv) and agitated for 30 min. A 3000-L inerted glass-lined reactor was charged with **12** (120 kg, 0.358 kmol) and THF (532 kg). While stirring, the solution of **12** was cooled to 5 °C, and the prepared potassium *tert*-butoxide solution was charged slowly, maintaining the batch temperature below 10 °C. The reactor contents were mixed for 15 min at 5 °C. Then **7** (68.5 kg charge corrected for purity, 0.357 kmol, 1.0 equiv) was charged, followed by tetrabutylammonium iodide (6.6 kg, 17.9 mol, 0.05 equiv). After the addition, the reactor contents were heated to 40−45 °C for 2 h. The reaction was sampled, and HPLC was used to determine completion (criteria: >30:1 **13**:**12**). Once complete, the reactor contents were cooled to 20−25 °C. Water (725 L) was then charged, followed by glacial acetic acid (18.2 kg, 0.286 kmol, 0.8 equiv) and ethyl acetate (690 L). The reactor contents were mixed for 20 min and the layers allowed to separate. The aqueous phase was removed. Aqueous citric acid solution (728 kg, prepared from 630 L of water and 98 kg of citric acid monohydrate {0.46 kmol, 1.3 equiv}) was charged to the organic phase. The reactor contents were mixed for 10 min followed by layer separation. The aqueous layer was discarded. The organic phase was washed with an aqueous

(13) This preparation was conducted in the late 1990s using PLE certified to originate from food-grade animals. As the issue of TSE/BSE emerged, a new synthesis that eliminated the need for PLE was devised to support further clinical studies. This new synthesis will be the subject of a future communication.

sodium chloride solution (720 kg of 10 wt %). To the organic phase, water (690 L) was added, followed by 50 wt % aqueous NaOH solution to adjust the pH to between 6.5 and 7.5 (typically 50 L). After the pH adjustment, the layers were separated, and the aqueous layer was removed. The organic phase was washed again with aqueous sodium chloride (720 kg of 10 wt %). The organic layer was distilled down to ~600 L, and ethyl acetate (~1200 L) was charged at a rate such that the volume remained at ~600 L. The organics were sampled, and GC was used to determine completion (criteria: < 0.5% THF) The solution was transferred to an inerted 5000-L Hastelloy reactor and diluted with ethyl acetate (1400 L). The reaction was cooled to −65 to −70 °C. The oxygen flow on the ozone generator was set to 100 Nm^3/h, and oxygen was introduced subsurface to the reactor. The ozone generator was turned on, and a stream of ozone (18.4 kg, 0.383 kmol, 1.07 equiv) and oxygen was introduced subsurface to the reactor over a period of ~4 h (target 5 kg of O_3/h). The ozone generator was switched off, and the reactor was purged with oxygen, followed by nitrogen to purge oxygen and unreacted ozone. The reaction mass was sampled for completion (criteria: area ratio of **13** to ozonide ≤ 0.2). Once reaction completion was established, a 0 °C, ethyl acetate solution of triphenylphosphine (113 kg, 0.430 kmol, 1.20 equiv in 500 L of ethyl acetate) was added to the ozonide solution. The reaction was warmed to 0 °C over 4 h and held for 12 h at 0−10 °C. The reaction was sampled for completion (criteria: area ratio of ozonide to **14** ≤ 0.2). Once the reduction was complete, the organic phase was washed with water (980 L). The resulting aldehyde **14** in ethyl acetate was transferred to a 5000-L glass-lined reactor for the synthesis of **15**. The solution was cooled to −10 °C. Diisopropylethylamine (60.1 kg, 0.465 kmol, 1.3 equiv) and D-leucine methyl ester hydrochloride (84.5 kg 0.465 kmol, 1.3 equiv) were added. The reactor contents were aged at 0 to 5 °C for 1 h, and then sodium triacetoxyborohydride (110 kg 0.519 kmol, 1.45 equiv) was added. The reaction mass was aged at 0 ± 5 °C for 2 h and sampled for completion (criteria: conversion of **14** to **15** ≥ 99.8 by area %). Once the reaction was deemed complete, the excess sodium triacetoxyborohydride was quenched by adding water (2000 kg). The aqueous and organic phases were separated. Toluene (150 kg) was added to the organic phase, and it was then extracted with 28 wt % aqueous citric acid four times (1000 L, 720 L, 720 L, 720 L). The organic layer was discarded, and the aqueous citric acid extracts were combined in a second 5000-L vessel. Ethyl acetate (788 L) and toluene (75 kg) were added as a wash, and the aqueous layer of **15** was transferred back to the original glass-lined reactor. EtOAc (550 L) was added to the aqueous phase. The mixture was cooled to 5 ± 5 °C, and then 30% aqueous sodium hydroxide (~900 kg) was added to adjust to a pH range of 4−5. The layers were separated (the organic phase was retained), and the aqueous phase was extracted with EtOAc (790 L). The second EtOAc extraction was combined with the first, and the organics were washed with water (500 L). EtOAc (1550 L) was added, and the solution was heated to reflux. An initial azeotropic distillation was performed to remove water and raise the bp to ~77 °C by concentrating to 700 L. The solution was held at reflux for 8 h, and the reaction was sampled for completion (criteria: ≥ 99.8% conversion of **15** to **16**). A constant volume distillation was then initiated where isopropanol (~2000 L) was added at approximately the same rate of distillation to keep the total volume of the solution at ~700 L. The organic phase was sampled and analyzed for EtOAc/IPA ratio (criteria: <0.2% EtOAc). The solution was then cooled gradually to 5 °C, at a rate of 10 °C/h. Seed crystals (0.5 kg) were added to induce crystallization, and the resulting slurry was aged at 5 °C for 2 h. The product was filtered by centrifugation and washed with cold isopropanol (2 × 125 L). The product **16** was dried in a conical dryer at 40 °C to a constant weight to yield 121.5 kg (59%) of a white to off-white, crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.72 (dd, J_1 = 8.3, J_2 = 8.3 Hz, 1H), 7.53 (dd, J_1 = 8.3, J_2 = 8.3 Hz, 1H), 7.48 (s, 1H), 7.46 (d, J = 9.2 Hz, 2H), 7.00 (d, J = 9.2 Hz, 2H), 5.64 (s, 1H), 5.49 (s, 2H), 4.93 (s,Br, 1H), 3.58 (s, 3H), 3.40 (m, 2H), 2.89 (t, 1H), 2.77 (s, 1H), 2.76 (s, 3H), 1.81−1.72 (m, 2H), 1.58 (m, 1H), 1.42 (s, 9H), 0.97 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 173.7, 171.4, 158.9, 157.9, 154.7, 147.7, 142.0, 133.2, 129.4, 127.3, 126.1, 124.1, 122.6, 120.3, 114.9, 66.8, 63.2, 52.4, 52.1, 40.5, 36.9, 28.3, 25.4, 24.6, 23.3, 21.1. MS (DCI/NH_3): m/z: 576.3 (M + 1), 520.2, 459.1. Analysis Calculated for $C_{33}H_{41}N_3O_6$: C, 68.85; H, 7.18; N, 7.30. Found: C, 68.69; H, 7.06; N, 7.14.

(*R*)-Methyl 2-((*R*)-3-Amino-3-(4-((2-methylquinolin-4-yl)-methoxy)phenyl)-2-oxopyrrolidin-1-yl)-4-methylpentanoate Bis-MSA Salt (17). To an inerted 200-gal glass-lined reactor was charged **16** (30 kg, 0.052 kmol) and methanol (90 kg). Methanesulfonic acid (5.8 kg, 0.06 kmol, 1.15 equiv) was gradually charged at 25 °C. The batch was heated slowly to 55 °C. Additional methanesulfonic acid (5.8 kg, 0.06 kmol, 1.15 equiv) was added gradually over 30 min at 55 °C. The reactor contents were aged at 55 °C for 2 h and sampled for reaction completion (HPLC criteria: <0.5 area % **16**). Isopropanol (214 kg) was charged at 55 °C, and then the batch was cooled to 15 °C at a rate of 1 °C/min and aged at 15 °C for 1 h. The product **17** was isolated on a centrifuge, and the cake was washed with isopropanol (3 × 50 kg). The solids were dried under vacuum at 50 °C to a constant weight to yield 34.9 kg (90%) of a white crystalline solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.07 (s, 4H), 8.51 (d, J = 8.4 Hz, 1H), 8.32 (d, J = 8.6 Hz, 1H), 8.14 (s, 1H), 8.14 (t, J = 8.6 Hz, 1H), 7.96 (t, J = 8.6 Hz, 1H), 7.61 (d, J = 8.9 Hz, 2H), 7.39 (d, J = 9.0 Hz, 2H), 5.94 (s, 2H), 4.80 (dd, J_1 = 4.0 Hz, J_2 = 11.3 Hz, 1H), 3.62 (s, 3H), 3.51 (t, J = 9.5 Hz, 1H), 3.15−3.28 (m, 1H), 3.01 (s, 3H), 2.65−2.75 (m, 1H), 2.48−2.60 (m, 2H), 2.43 (s, 6H), 1.85 (t, J = 12.2 Hz, 1H), 1.52−1.71 (m, 2H), 0.96 (d, J = 6.3 Hz, 3H), 0.93 (d, J = 6.10 Hz, 3H). ^{13}C NMR (100

MHz, DMSO-d_6) δ 172.9, 172.5, 160.6, 159.7, 156.5, 139.1, 136.1, 131.2, 130.0, 128.9, 126.3, 126.0, 122.1, 117.2, 67.8, 64.3, 53.4, 50.1, 41.5, 40.1, 38.1, 33.8, 26.2, 24.0, 22.0, 21.5. Analysis Calculated for $C_{30}H_{41}N_3O_{10}S_2$: C, 53.96; H, 6.19; N, 6.29; S, 9.60. Found: C, 53.83; H, 5.97; N, 6.15; S, 9.76.

(*R*)-2-((*R*)-3-Amino-3-(4-((2-methylquinolin-4-yl)methoxy)phenyl)-2-oxopyrrolidin-1-yl)-*N*-hydroxy-4-methylpentanamide (1). To an inerted 30-gal glass-lined reactor was charged hydroxylamine hydrochloride (10 kg, 0.14 kmol, 4.7 equiv) and methanol (15.6 kg). The batch temperature was set to 50 °C, and a 25 wt % solution of sodium methoxide in methanol (64.6 kg, 16.15 kg, 0.3 kmol, 10 equiv) was charged followed by a methanol rinse of the charging line. The reactor contents were heated to 55 °C and aged for 15 min. The batch was then cooled to 25 °C and filtered through a 36 in. nutsch filter using a polypropylene filter bag. The filtrate was collected in a 100-gal glass-lined reactor and cooled to 10 °C. Final intermediate **17** (20 kg, 0.03 kmol) was added, and the batch was warmed to 25 °C for 1 h. The reactor contents were sampled for reaction completion (HPLC criteria: >99.5 area % **1**). Once the reaction was deemed complete, ~55 kg of 2 N HCl solution (prepared using 126 kg of purified water and 30 kg of concentrated HCl) was added, and the reaction mass was sampled for pH measurement (acceptance criteria: pH $\approx$ 7.0). The batch was vacuum distilled at ~35 °C to remove ~20 L of methanol. The batch temperature was set to 25 °C, and the batch was charged with purified water (75 kg) followed by ethyl acetate (135 kg) and mixed for 15 min, and the layers were separated. The organic phase was washed three times with purified water (120 kg, 120 kg, 75 kg). The batch was vacuum distilled at 35 °C to remove ~70 kg of solvent (ethyl acetate, methanol). Isopropanol (62 kg) was charged, and the batch was vacuum distilled at 35 °C to remove 55 kg of solvent (ethyl acetate/isopropanol azeotrope). Isopropanol (55 kg) and purified water (20 kg) were charged, and the batch was vacuum distilled at 35 °C to remove 25 kg of solvent (water/isopropanol azeotrope). The batch was sampled for analysis (criteria: <5 area % ethyl acetate, ~4:1 isopropanol:water volume ratio, 10 L of isopropanol/water per kg of **1**. The batch temperature was set to 40 °C, and the reactor contents were discharged to a clean Teflon drum. The contents of the Teflon drum were transferred to the cleaned reactor through a cartridge filter, followed by an isopropanol chase (0.5 kg). The batch was vacuum distilled at 35 °C to remove 35 kg of solvent (isopropanol/water azeotrope). After the distillation, the batch temperature was set to 55 °C, and purified water (50 kg) was charged through a cartridge filter over 30 min. The batch was heated to 60 °C, aged for 15 min, and then cooled to 55 °C in 30 min. At 55 °C, a slurry of milled seeds of **1** (1 L, ~200 g of solids) in 1:4 isopropanol/water (volume ratio) was charged. The batch was cooled from 55 to 45 °C in 90 min. At 45 °C, purified water (67 kg) was charged through a cartridge filter gradually over a period of 3.5 h. The batch was cooled from 45 to 20 °C in 2 h, aged for 30 min, and filtered through a 36 in. nutsch filter using a Dacron filter bag. The filter cake was washed three times with a mixture of isopropanol/water (first wash: 38 kg of water, 8 kg of isopropanol; second and third washes: 19 kg of water, 4 kg of isopropanol). The product **1** was dried in a tray dryer under vacuum at 50 °C to a constant weight to yield 12.4 kg (87%) of a white, crystalline solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.86 (s, 1H), 8.92 (s, 1H), 8.10 (dd, $J_1 = 8.3$ Hz, $J_2 = 0.8$ Hz, 1H), 7.97 (dd, $J_1 = 8.3$ Hz, $J_2 = 0.8$ Hz, 1H), 7.74 (ddd, $J_1 = 8.3$ Hz, $J_2 = 8.3$ Hz, $J_3 = 1.2$ Hz, 1H), 7.58 (ddd, $J_1 = 8.3$ Hz, $J_2 = 8.3$ Hz, $J_e = 1.2$ Hz, 1H), 7.54 (s, 1H), 7.36 (d, $J = 8.9$ Hz, 2H), 7.07 (d, $J = 9.1$ Hz, 2H), 5.57 (s, 1H), 4.55 (dd, $J_1 = 9.1$ Hz, $J_2 = 5.8$ Hz, 1H), 3.45−3.31 (m, 2H), 2.66 (s, 3H), 2.20 (bs, 2H), 2.18−2.01 (m, 2H), 1.71−1.62 (m, 1H), 1.57−1.43 (m, 2H), 0.93 (d, $J = 6.3$ Hz, 3H), 0.90 (d, $J = 6.3$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.6, 166.7, 158.5, 156.9, 147.3, 142.3, 137.4, 129.3, 128.8, 127.0, 125.8, 123.9, 123.6, 120.1, 114.3, 66.2, 62.1, 50.1, 39.8, 37.6, 36.5, 24.9, 24.1, 22.8, 21.9. Analysis Calculated for $C_{27}H_{32}N_4O_4$: C, 68.05; H, 6.78; N, 11.76. Found: C, 68.00; H, 6.77; N, 11.77.

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