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Enabled Process To Synthesize Monobactam 1 for Early Development

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ABSTRACT: An efficient process to synthesize Monobactam **1** from intermediates **2**, **3**, and **4** was developed. The process was initially enabled to deliver 500 g of **1** for exploratory toxicity studies and further improved to facilitate kilogram-scale production of the active pharmaceutical ingredient with enhanced quality and throughput. Highlights of the process encompass utilizing 1,1′-carbonyldiimidazole to construct a urea linker with good selectivity, oxidative cleavage to remove a 2,4-dimethoxybenzyl protecting group, two activation methods to form an amide bond, a high yielding reaction to introduce *N*-sulfonic moiety, and a global deprotection to remove all protecting groups. Practical procedures were developed to isolate intermediates en route by adding a concentrated substrate to antisolvents to obtain filterable amorphous solids with partial purification. Amberchrom CG161M resin was applied not only as a "resin-capture−release" tool to remove the bulk amount of water but also as an effective method to purify **1**. Finally, a process isolating **1** pentahydrate (**1**·$5H_2O$) as a crystalline solid from the acidic aqueous solution was developed based on zwitterionic crystallization methodology.

KEYWORDS: *unsymmetrical urea, oxidative DMB cleavage, amide bond formation, global deprotection, Amberchrom CG161M, resin-capture−release, zwitterionic crystallization*

INTRODUCTION

Monobactam **1**[1] is a promising antibacterial candidate with a unique pyridone-conjugated monocyclic β-lactam structure for the treatment of life-threatening infections caused by Gram-negative pathogens with multidrug-resistant strains, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*. To fast-track this candidate for early development, we were initially requested to prepare 500 g of **1** for exploratory toxicity studies, followed by additional multikilograms of the active pharmaceutical ingredient (API) for regulatory toxicity studies and Phase I clinical trials. As described in the previous papers,[1,2] the original synthesis was unable to meet bulk API demands due to high step count and extremely poor throughput (∼0.2% overall yield). Therefore, a more concise and efficient synthesis to **1** was needed. Previously, we reported a process to prepare kilogram quantities of monocyclic β-lactam core (**2**).[2] Herein, we describe the process development to synthesize **1** from **2**, **3** (the "left-hand" thiazole piece, LHP),[3] and **4** (the "right-hand" pyridone piece, RHP).[1]

DISCUSSION AND RESULTS

Route Selection. The new synthetic sequence was designed with orthogonal considerations in mind, which utilized intermediates **2**, **3**, and **4** with minimal protecting group manipulation. In recognizing the *t*-butyl ester of **3** being relatively labile to acidic conditions and having the new core (**2**) bearing the 4-aminomethyl moiety in hand, a logical approach assembling the target molecule was first constructing the urea linker between **2** and **4**, followed by forming the amide bond with **3**, then introducing the *N*-sulfonic acid moiety, and finally removing all protecting groups to deliver monobactam **1** (Scheme 1).

Urea Formation. Among many activating reagents used to construct an unsymmetrical urea,[4] 1,1′-carbonyldiimidazole (CDI) is the most safe and nontoxic one.[5] Initial tests revealed that the reaction of CDI with **4** in dichloromethane (Scheme 2) offered a superior profile to form the carbamoylimidazole intermediate compared to the reaction with **2**. It was also found that using a free base of **4** in anhydrous conditions was required to obtain clean and stable carbamoylimidazole **10**. The optimal molar ratio of CDI, **4**, and **2** was then identified at 1.5:1:1, respectively, that provided >90% of pure desired urea **7** with low levels of unreacted **2** (<2%) and **4** (<1%), and side symmetric ureas **8** (∼5%) and **9** (∼2%) (Figure 1). Thus, neutralizing the mesylate salt of **4** with K_3PO_4 in water and dichloromethane, followed by drying the organic layer with magnesium sulfate, offered an anhydrous solution of free base **4** in dichloromethane (<0.2% wt. of water), which was slowly added to a solution of CDI (1.5 equiv) in dichloromethane at 20 °C to form **10**. A stress test indicated that **10** in the solution was stable for at least 24 h at 20 °C. Due to the slow reaction of **10** with amine **2**, adding dimethylformamide (DMF, ∼2 vol) and increasing the temperature to 40 °C were needed to accelerate the formation of urea **7**. With no success in attempting crystallization, precipitation through reverse addition was applied to isolate amorphous **7** with partial purification. Thus, after solvent replacement with ethyl acetate, washes with aqueous citric acid and aqueous NaCl purged most **2**, **4**, and DMF. The slow addition of the organic layer to *n*-heptane precipitated **7**, which was easy to filter. On a 6 kg scale, **7** (95.9% pure) was obtained in a 94% yield. The main impurities were **8** (2.5%) and **9** (1.3%).

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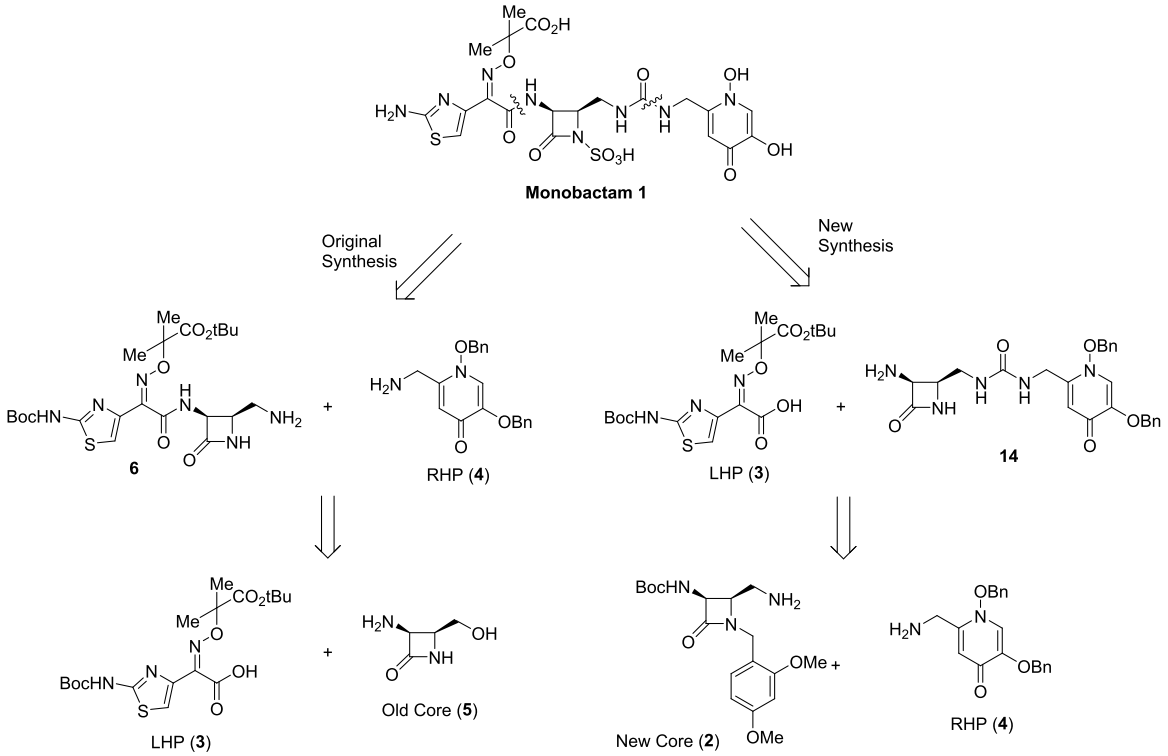
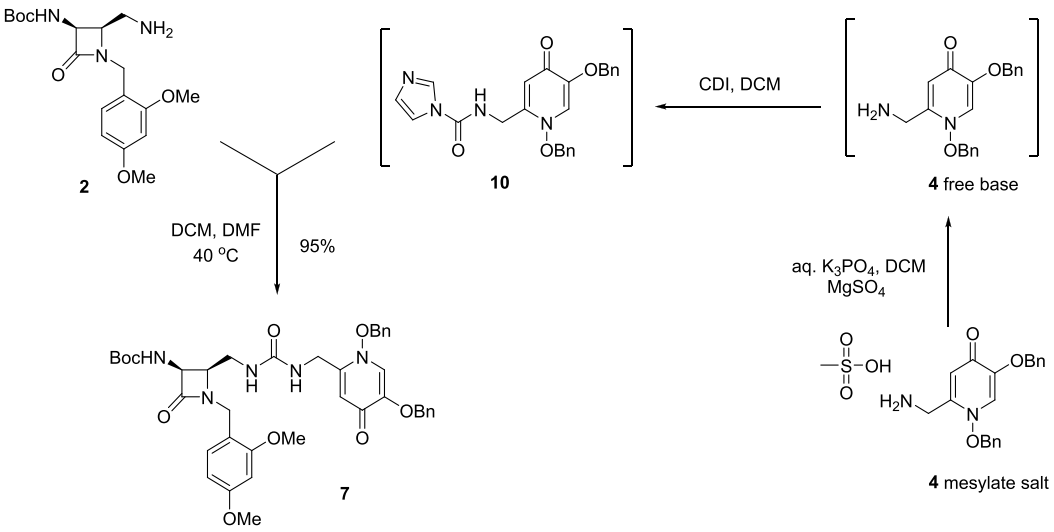
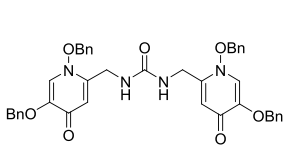
Scheme 1. Comparison of Bond Disconnections between Original and New Syntheses toward 1



Scheme 2. Formation of Urea 7 through CDI Activation





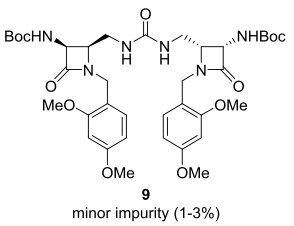


Figure 1. Structures of side symmetric ureas.

Removal of 2,4-dimethoxybenzyl (DMB) and *N*-Boc Groups. Treating 7 with strong acids, such as trifluoroacetic acid (TFA), HCl, MsOH, and TsOH, only cleaved the Boc group and left the 2,4-dimethoxybenzyl (DMB) group intact. Consequently, a two-step deprotection approach that removed first DMB group through oxidation and then *N*-Boc group by acid was utilized. On finding that reaction 7 with ceric ammonium nitrate in aqueous acetonitrile gave only a 3:1 mixture of desired **11** and side amide **12** (Figure 2),[6] oxidative DMB cleavage with potassium persulfate in the presence of K_2HPO_4 in aqueous acetonitrile[7] was tried, and a complete reaction was achieved at 70 °C without forming **12** (Scheme 3). Further studies revealed that using 4 equiv of potassium persulfate and 4.5 equiv of K_2HPO_4 to set the initial pH in 6−7 range and keeping the temperature around 70 °C were essential for both reaction initiation and fast progress. Under these conditions, the reaction was complete in 3 h when the in situ yield was measured as 65% and the pH of the reaction

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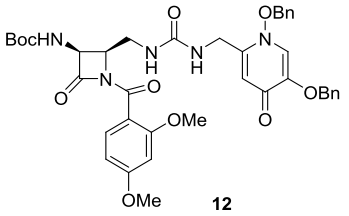


Figure 2. Structure of side amide **12**.

mixture was read at 6.3. However, extending the reaction time resulted in yield decrease and pH drop (52% yield and pH 4 in 6 h; 13% yield and pH 2 in 18 h). In contrast, periodically adding K_2HPO_4 to maintain pH in 6−7 significantly slowed down the product degradation, and the in situ yield was 62% after 18 h. With this knowledge on the process stability, the scale-up of this oxidative cleavage was set up by mixing **7**, potassium persulfate (4.0 equiv), and K_2HPO_4 (4.5 equiv) in 2:1 acetonitrile and water. The pH was continuously monitored during the reaction at 70 °C. When the pH dropped to 6.3, the aqueous K_2HPO_4 solution was fed to maintain the pH between 6.3 and 7.0. As oxygen gas was a byproduct of the reaction, high nitrogen sweep was applied over the course to keep the oxygen level <6% in the headspace to meet our safety requirements (<12%). After the reaction completion in 2 h, most inorganic precipitates were removed by filtration and the organic solvent was switched from acetonitrile to ethyl acetate to crystallize crude **11**. Further trituration in biphasic ethyl acetate and water purged more inorganic salts and organic impurities, such as aldehyde **13** and urea **8**. Sufficient drying of **11** to residual water <0.5% was required for the next step. On a 4 kg scale, **11** (96.6% pure) was obtained in a 61% yield.

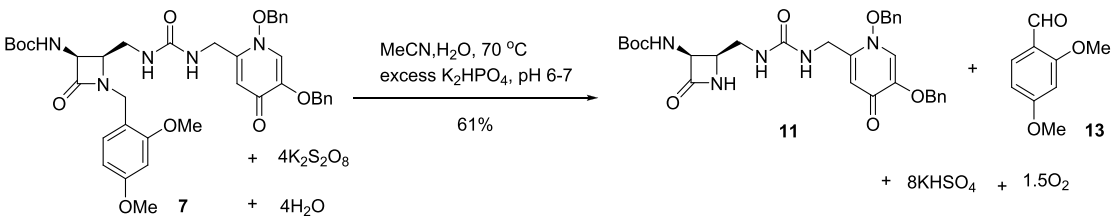
The main challenge to deprotect *N*-Boc from **11** was that the procedure called for concentrated strong acid, which caused degradation of product **14** over time. Initial experiments revealed that neat TFA gave a superior reaction profile at low temperature compared to HCl in dioxane or TFA in dichloromethane. Reacting with large excess (∼40 equiv) of TFA in the presence of anisole (2 equiv) as a cation scavenger[8] at −10 to −5 °C for 2−4 h was concluded as the optimal condition, which offered a typical reaction profile with ∼90% of **14** and ∼10% of the proposed side product **15** (Scheme 4). Liquid chromatography−mass spectrometry inference indicated that **14** could further react with TFA to form **16**, and **15** to **17**. Due to the poor stability of **14** in strong acid at elevated temperature, isolating **14** on scale was initially problematic with significant product degradation in removing TFA under vacuum distillation. This challenge was overcome by implementing a precipitation protocol without heat. Consequently, the reaction solution in TFA was slowly added to a cooled suspension of diatomaceous earth (220 wt % to **11**) in methyl *tert*-butyl ether (MTBE) to precipitate crude **14** with diatomaceous earth as a well-behaved mixed solid. Further trituration in MTBE purged more TFA and therefore improved the stability of the mixed solid, which could be stored at ambient temperature for a couple of weeks with little purity loss. Using this technique, 3.1 kg of **14** (86.5% pure, loaded with 8.2 kg of diatomaceous earth) was isolated in an 81% yield on scale.

Formation of Amide 19. With **14** (∼27 wt % loaded with diatomaceous earth) in hand, we developed two procedures to construct the amide bond. The first method was through succinimidyl ester **18**,[9] a stable white solid obtained in an 85% yield by treating **3** with *N*-hydroxysuccinimide and *N*,*N*′-diisopropylcarbodiimide (DIC) in dichloromethane. The reaction of **18** and **14** at a 1:1 molar ratio in the presence of DMAP (2.5 equiv) and molecular sieves in acetonitrile at 40 °C for 20 h formed amide **19** with >95% conversion (Scheme 5, Method A). Workup through pH adjustment with aqueous citric acid, solvent exchange to ethyl acetate, filtration to remove diatomaceous earth and molecular sieves, partial concentration, and $MgSO_4$ drying offered a solution of **19** in ethyl acetate, which was slowly added to *n*-heptane to precipitate amorphous crude **19** (81.0% pure) in an 83% yield. As crystallization attempts were fruitless, silica gel chromatography was used for the first delivery. Thus, 2.35 kg of purified amorphous **19** (97.8% pure) was obtained in a 64% yield on a 3 kg scale by precipitation from ethyl acetate and *n*-heptane.

An alternative approach to amide **19** (Scheme 5, Method B), which provided a better reaction profile than Method A, entailed partially purifying **14** with basic aluminum oxide and in situ activating acid **3** with 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT). Thus, treating **14** (as mixed solid with diatomaceous earth) with *N*-methylmorpholine (NMM) in acetonitrile, followed by filtering and switching solvent to DMF, afforded a neutralized **14** solution in DMF. Subjecting this solution through a short column of basic aluminum oxide with a DMF rinse produced a ∼95% pure **14** solution in DMF, while leaving most side products **15, 16, 17**, and other small unidentified impurities on the column. On the other side, the in situ activation of **3** (∼0.85 equiv) to **20** was achieved by reacting with CDMT (0.85 equiv) and NMM (1.7 equiv)[10] in ethyl acetate at 20 °C for 2 h. At that point, the partially purified **14** solution in DMF was added in for amidation, which took 16 h at 20 °C to complete. After aqueous washes to purge DMF and concentration of the organic layer, the slow addition of the solution in ethyl acetate to *n*-heptane precipitated amorphous **19** (96.1% pure) in a 69% yield. This process, which circumvented the chromatography, was successfully demonstrated on a 100 g scale.

***N*-Sulfonation to 21.** Converting **19** to **21** with 10 equiv of sulfur trioxide−DMF complex in DMF[11] was fast at ambient

Scheme 3. Balanced Equation of Oxidative DMB Cleavage To Prepare 11



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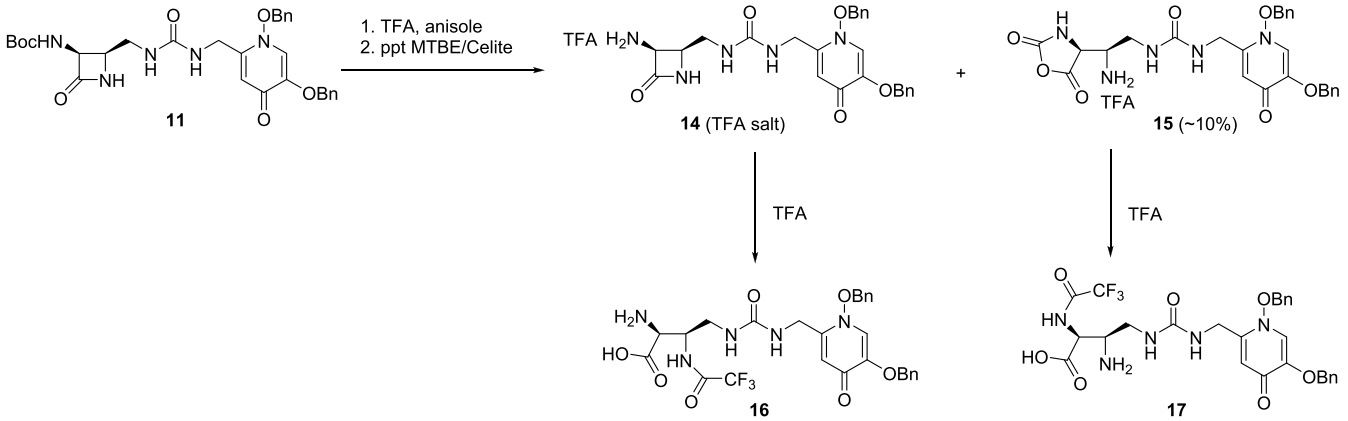
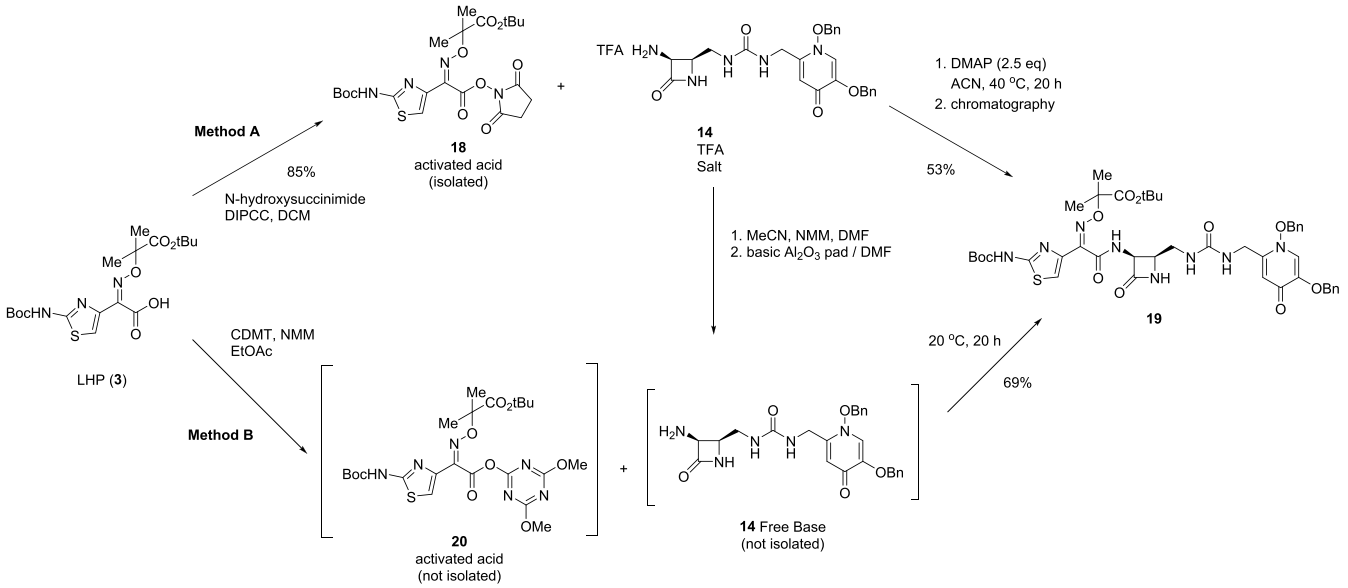
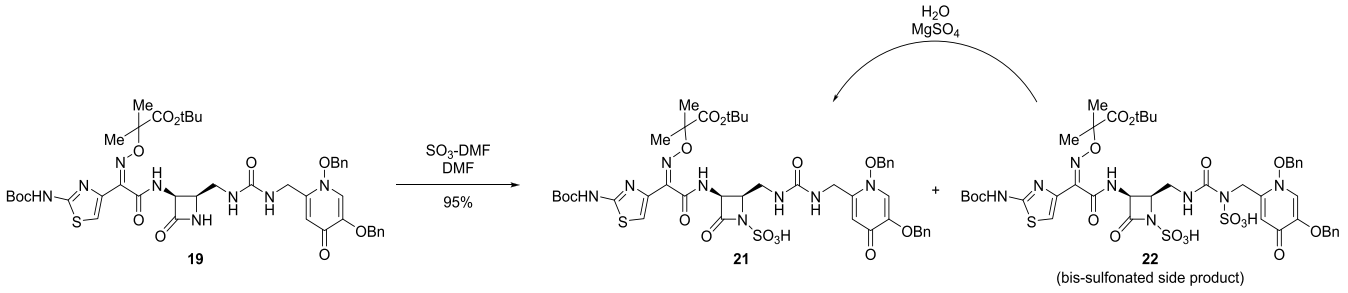
Scheme 4. *N*-Boc Deprotection of 11 and Proposed Side Products



Scheme 5. Methods A and B to Amide 19



Scheme 6. *N*-Sulfonation to 21



temperature (Scheme 6), and the reaction was complete in less than 30 min with forming side bis-sulfonated product **22** (2–15%). An attempt to eliminate the formation of **22** by lowering the amount of the sulfonating reagent to <8 equiv only resulted in an incomplete conversion. Fortunately, **22** could be cleanly converted to **21** in the workup by treating the mixture with $MgSO_4$ in the presence of a small amount of water.[12] Thus, quenching the reaction to a biphasic mixture of ethyl acetate and aqueous NaCl, removing DMF by aqueous washes, and drying the wet organic layer with $MgSO_4$ under vigorous agitation for 1 h converted **22** to **21**. Concentrating the filtrates and then adding MTBE precipitated amorphous **21** (89.8% pure) in an 87% yield on a 2.3 kg scale.

Global Deprotection To Target 1. For the final step removing all protecting groups to form **1** (Scheme 7), two-step deprotection approaches were initially explored. Approach 1 was able to deprotect *N*-Boc and *t*-butyl groups by treating **21** with HCl in dioxane to give intermediate **23**, which surprisingly would not undergo debenzylation under standard hydrogenation conditions. In contrast, the reversed sequence (Approach 2) offered a smooth debenzylation under hydrogenation with Pd/C; however, *N*-Boc and *t*-butyl deprotection

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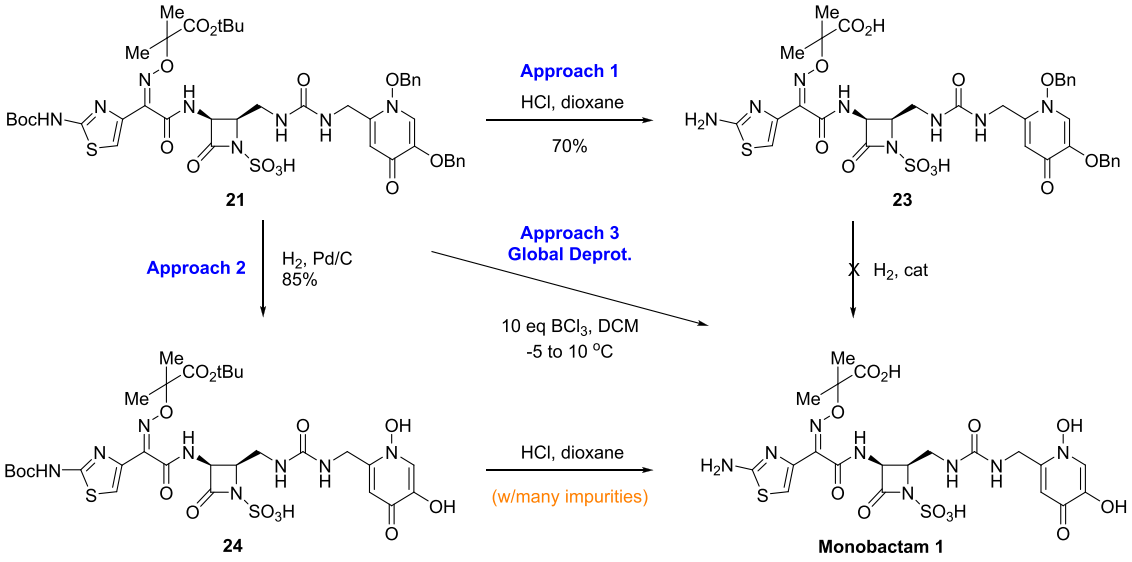
Scheme 7. Deprotection Strategy to Monobactam 1



under acidic conditions were not clean. On the other hand, strong Lewis acids, such as BCl_3 and BBr_3, have been widely used to cleave *O*-benzyl, carbamates, and ester bonds[1,13] and would provide an ideal one-step "global deprotection" in our case if successful. Indeed, treating **21** with excess BCl_3 in dichloromethane at −5−10 °C cleaved all protecting groups (e.g., *t*-butyl ester, *N*-Boc, and two *O*-benzyl groups) within 1 h. An optimal amount of 10 equiv of BCl_3 ensured reaction completion without significant impact to the product stability and afforded ∼65% pure **1** with numerous minor side products, including monobactam ring-opened impurity (**25**, Figure 3). Although the product directly precipitated out, the

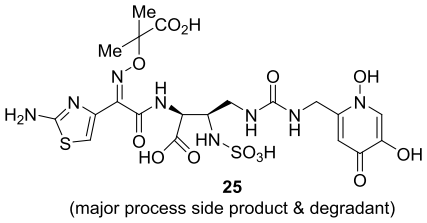


Figure 3. Structure of side product **25**.

reaction mixture was treated with a solution of trifluoroethanol (45 equiv) in MTBE in an attempt to quench excess BCl_3 and break up **1**−boron complex before filtration.[14] The collected solid was set in filter under nitrogen-blowing for a long time and then triturated in MTBE to further remove acidic volatiles. Thus, a 2.27 kg-scale reaction afforded 1.7 kg of crude **1** (63% pure) with a 115% mass recovery for the first scale-up. Further experiments turned out that the reverse addition of the **21** solution in dichloromethane to a precooled (0−10 °C) 1 M BCl_3 solution in *n*-heptane provided a slightly cleaner reaction with improved product stability that eliminated the need of trifluoroethanol treatment. Thus, the reaction slurry was directly filtered, and the collected solid was blown with nitrogen for 16 h producing crude **1** (∼70% pure) with a ∼150% mass recovery on a multiple 10 g scale.

Purification of 1 for the First Delivery. The first delivery of monobactam **1** targeted 500 g of the API with a purity acceptance of >85% for exploratory toxicity studies. While this was unusual, the project team recognized the need for qualifying impurities at elevated levels as **1** being a lack of stability and crystallinity at the outset. Thus, little effort was placed on obtaining crystalline API at that time; instead, a reverse-phase chromatography was chosen to accomplish the API delivery with adequate purity. With the siderophore (*N*-hydroxypyridone) liberated at this stage, it was important to use iron-free solvents, reagents, and equipment going-forward to avoid chelation with iron, resulting in red to pink product **1** discoloration.[1a] Preparing the feed solution for the chromatography was challenging due to the residual boron occluded in crude **1** that resulted in foaming and decreasing product purity. The slow addition of crude **1** to a mixture of DMF and 1 M aqueous ammonium bicarbonate solution, followed by filtration, gave a feed mixture (pH ∼4), which was subjected to a Kromasil C-18 column (9 kg) for purification using acetonitrile and water (w/0.2% formic acid) as eluent. The product fractions were concentrated to remove acetonitrile under 30 °C. To minimize the degradation of **1**, a "resin-capture−release" technique using Amberchrom CG161M resin[15] was applied at this point to remove the bulk amount of water without heating.[16] Thus, the aqueous residue was passed through the resin column with a water wash to capture product **1** on the resin, which was then released by a wash with 9:1 (v/v) MeOH/water (60 L). Slowly adding the product fractions (20.4 kg) to stirred ethyl acetate (240 kg) at 2−8 °C resulted in the precipitation of **1**. This technique provided 551 g of **1** (88.2% pure) as a white amorphous powder in a 35% isolated yield. Both quantity and quality were adequate for the exploratory toxicity studies.

Crystallization Development of 1 for Further Scale-Up. With this "fit-for-purpose" procedure secured to prepare Monobactam **1**, our attention shifted to deliver 5 kg of **1** for the regulatory toxicity studies and Phase 1 clinical trials. Therefore, we needed to develop a practical purification procedure that could provide **1** with increased purity, ideally as a crystalline solid. To achieve this goal, we further purified a small amount of the exploratory toxicity lot (88.2% pure of **1**) by reverse-phase chromatography, CG161M resin treatment, and lyophilization. Pure amorphous **1** (∼2 g, 97.3% pure) was obtained to study stability and solubility in water under

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different pH values. Surprisingly, **1** is quite stable in the acidic aqueous solution from pH 1 to 4, and opening of the monobactam ring to generate **25** was relatively slow (Table 1).

Table 1. Stability of 1 in Water vs pH (22 °C)

pH	24 h purity of **1** (**25**)	48 h purity of **1** (**25**)	7 days purity of **1** (**25**)
1	95.84% (1.51%)	95.85% (1.62%)	94.17% (2.56%)
1.5	95.68% (1.46%)	95.55% (1.55%)	93.89% (2.19%)
2	95.62% (1.41%)	95.38% (1.44%)	92.92% (1.92%)
3	95.71% (1.41%)	95.47% (1.39%)	92.86% (1.85%)
4	95.60% (1.71%)	95.39% (1.73%)	93.37% (2.09%)
T = 0 control	97.25% (1.19%)		

On the other side, the aqueous solubility of **1** quickly decreased from pH 4 to 3 and stayed flat at ∼2 mg/mL in the range of pH 1−3 (Figure 4). This solubility pattern is likely

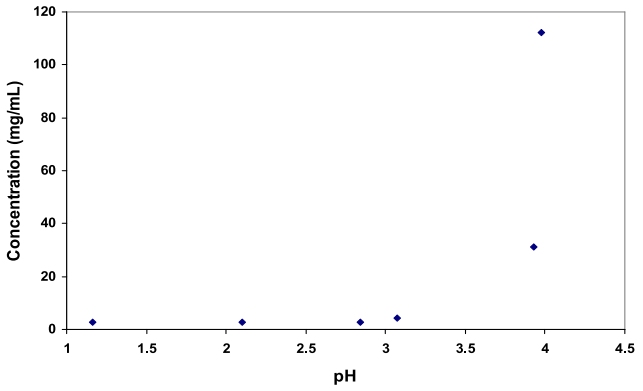

Figure 4. Solubility of **1** in water vs pH (22 °C).

attributed to the zwitterionic nature of the molecule, which gave a calculated isoelectric point at pH 0.77.[17] With this knowledge, the crystallization of **1** was screened in buffered aqueous solutions at pH 0.7, 0.8, 0.9, 1.1, 1.3, 1.5, 2.0, and 2.5 (pH adjusted by various ratios of 1 N hydrochloric acid and 1 N KH_2PO_4 solution) in a 5 wt % concentration at 22 °C. After 48 h, crystalline solids were observed in two vials at pH 1.1 and 1.3, and the isolated white solid **1** owned the same polymorph (Form 1, see Figure 5) with ∼98% purity. KF measurement and elemental analysis reveal that Form 1 is Monobactam **1** pentahydrate (**1**·5H_2O).

It had been observed that the Amberchrom CG161M resin was able to purge impurities and purify **1** if the ratio of water and methanol (or acetonitrile) was properly adjusted in the development of the resin-capture−release technique to remove water. Combining this observation and the crystallization knowledge, we developed a practical isolation protocol that utilizes CG161M resin to purify crude **1** and then crystallizes the final product (**1**·5H_2O) from a buffered aqueous solution at pH ∼1.12 on gram scale. Thus, the feed slurry was prepared by dissolving crude **1** (∼70% pure) in 1:2 water/acetonitrile, adjusting pH to ∼3 with aqueous sodium formate, adding the Amberchrom CG161M resin (100 wt %), and then removing acetonitrile under vacuum. The purification was then achieved by loading this feed onto an Amberchrom CG161M resin column (800 wt %) and eluting with 15:1 v/v of aqueous phosphate buffer (pH 3.1) and acetonitrile. The fractions containing >85% pure **1** were combined, concentrated to remove acetonitrile, and then loaded onto a second CG161M resin column (400 wt %) for the resin-capture−release manipulation. After water wash and nitrogen blow to remove the bulk amount of water and phosphate salts from the column, the captured product was eluted by 5:2 (v/v) acetonitrile/water (∼40 volumes). The removal of acetonitrile under vacuum generated a pH ∼3 aqueous solution of **1** (∼90% pure) in a ∼5 wt % concentration, which was set for crystallization. Adding 1 N hydrochloric acid to adjust the aqueous solution of **1** to pH 1.12, followed by stirring at 22 °C for 24 h with intermittently adding either dilute aqueous HCl or NaOH (as needed) to maintain the pH between 1.10 and 1.15, resulted in a white slurry. Filtration, followed by a water wash and drying at 25 °C under vacuum to a constant weight, produced crystalline **1**·5H_2O (Form 1, ∼94% pure). Triturating this solid in diluted aqueous hydrochloric acid at pH ∼1.12 upgraded the purity to 97.5% in the same form. The yield of the final step (from the global deprotection reaction to the crystallization) was 46%. Thus far, we enabled the synthesis with this purification/crystallization procedure.

CONCLUSIONS

A new synthesis of Monobactam **1** from three components (**2**, **3**, and **4**) was designed, and the corresponding process was developed as follows: forming the unsymmetrical urea linker

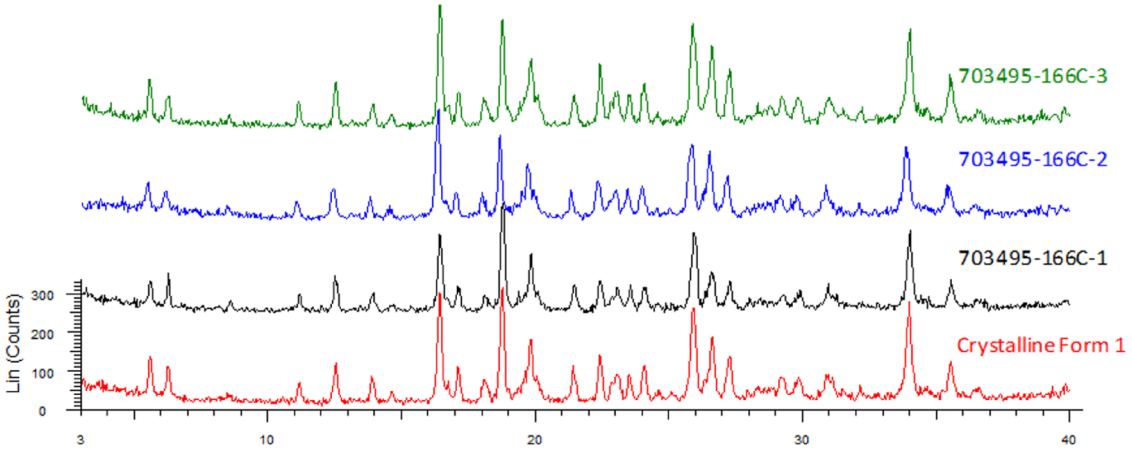

Figure 5. PXRD of **1**·5H_2O (Form 1).

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through CDI activation, removing the DMB group by oxidative cleavage, deprotecting *N*-Boc group with TFA, constructing the amide bond through two activation methods, introducing the *N*-sulfonic acid, and finally removing all remaining protecting groups in one step. In dealing with the intermediates lacking crystallinity, isolations with partial purification were frequently accomplished by precipitation en route. The Amberchrom CG161M resin was successfully used not only as a resin-capture−release technique to remove the bulk amount of water but also as an efficient purification tool to upgrade the API quality. More importantly, a process that isolated the API crystal as a pentahydrate form (**1**·$5H_2O$) from the acidic aqueous solution was developed based on studies of the stability and solubility of **1** at various pH values. The initial process work facilitated the delivery of 500 g of amorphous **1** for the exploratory toxicity studies, and the identification of the API crystallization process enabled the synthesis for further scale-up with improved quality, stability, and throughput.

EXPERIMENTAL SECTION

General. All commercially available materials and solvents were used as received, unless otherwise stated. All reactions were executed under a nitrogen atmosphere. Reaction temperatures were measured internally, unless indicated otherwise. Achiral high-performance liquid chromatography (HPLC) analyses were carried out using an XBridge C8 column (3.0 mm × 50 mm, 2.5 μm); column temperature 45 °C; flow rate 2.0 mL/min; detection UV 210 nm; mobile phase: acetonitrile (solvent A), 0.05% methanesulfonic acid in water with 10 mM sodium octylsulfonate (solvent B); linear gradient elution (12 min): 0−8.3 min increasing solvent A from 5% to 95, 8.3−10 min decreasing solvent A from 95 to 5%, and 10−12 min holding solvent A at 5%. Purity was reported as the area percentage of the main peak.

*Preparation of tert-Butyl ((2R,3S)-2-((3-((1,5-Bis-(benzyloxy)-4-oxo-1,4-dihydropyridin-2-yl)methyl)ureido)-methyl)-1-(2,4-dimethoxybenzyl)-4-oxoazetidin-3-yl)-carbamate (**7**).* To a 100 L reactor were charged K_3PO_4 (2.27 kg; 10.70 mol) and water (8.34 L). A suspension of **4** mesylate salt (3.28 kg; 7.13 mol) in dichloromethane (20.8 L) was added with a dichloromethane rinse (5.2 L) while maintaining the temperature between 15 and 25 °C. The mixture was stirred at 20 °C for 30 min. The bottom organic layer was separated, and the top aqueous layer was back-extracted with dichloromethane (13 L). The combined organic layers were dried with $MgSO_4$ (4.9 kg) and filtered with a dichloromethane rinse (3.2 L) to give a solution of **4** free base in dichloromethane (KF < 0.2%). This solution was added to a solution of CDI (1.70 kg, 10.50 mol) in dichloromethane (26 L) over 3 h with a dichloromethane rinse (5.0 L) while maintaining the temperature between 15 and 25 °C. The mixture was stirred at 20 °C for 1 h. The reaction completion was monitored by proton NMR. **2** (2.61 kg, 7.13 mol) was then added, followed by DMF (6.0 L). The mixture was stirred at 20 °C for 4 h and then at 40 °C for 17 h. Upon reaction completion, most of dichloromethane was distilled off under vacuum to a residual volume of ∼13 L. Ethyl acetate (56 L) was added. The mixture was washed with a 10 wt % aqueous citric acid solution (58 kg) and 5 wt % aqueous NaCl solution (2 × 58 kg). The organic layer was then dried with $MgSO_4$ (4.9 kg), and the filtrates were concentrated under vacuum at <35 °C to a residual volume of ∼28 L. The concentrated solution was added to a stirred *n*-heptane (108 L) over 30 min at 20 °C with ethyl acetate rinse (3 L). The white slurry was filtered with an *n*-heptane wash (6 L). The solid was dried at 50 °C under vacuum with nitrogen sweep over 6 h to afford **7** (4.90 kg, 6.71 mol) as a white powder in a 94.1% yield. HPLC purity: 95.9%. HRMS (ESI) *m*/*z*: calculated for $C_{39}H_{46}N_5O_9$ $[M + H]^+$ 728.3296; observed 728.3288. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.13 (s, 1H), 7.65 (d, J = 9.4 Hz, 1H), 7.54 (m, 2H), 7.38−7.47 (m, 8H), 7.10 (d, J = 8.2 Hz, 1H), 6.54 (m, 2H), 6.47 (dd, J = 8.2/2.3 Hz, 1H), 6.14 (m, 1H), 6.11 (s, 1H), 5.30 (s, 2H), 5.03 (s, 2H), 4.75 (dd, J = 9.4/4.3 Hz, 1H), 4.41 (d, J = 14.8 Hz, 1H), 4.22 (m, 1H), 4.02 (d, J = 14.8 Hz, 1H), 3.75 (s, 3H), 3.74 (s, 3H), 3.51 (m, 1H), 3.30 (m, 1H), 3.14 (m, 1H), 1.39 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.09, 166.96, 160.71, 158.47, 157.99, 155.61, 147.21, 146.91, 136.94, 133.36, 130.71, 130.06, 129.19, 128.91, 128.59, 123.77, 116.30, 111.17, 104.89, 98.84, 80.88, 79.13, 71.25, 58.71, 57.26, 55.91, 55.66, 37.69, 28.59.

*Preparation of tert-Butyl ((2R,3S)-2-((3-((1,5-Bis-(benzyloxy)-4-oxo-1,4-dihydropyridin-2-yl)methyl)ureido)-methyl)-4-oxoazetidin-3-yl)carbamate (**11**).* To a 200 L reactor, equipped with a pH probe, a dip pipe, and a pH titrator, were charged acetonitrile (70.0 L), **7** (4.67 kg, 6.41 mol), potassium persulfate (6.94 kg, 25.7 mol), K_2HPO_4 (5.03 kg, 28.9 mol), and water (37.3 L). After setting the nitrogen purge rate at >2 standard cubic feet per minute, the mixture was heated to 72 °C over 30 min. The mixture was stirred at 70−72 °C for ∼2 h for reaction completion. During the period, part (as needed) of a stock solution of K_2HPO_4 (5.50 kg, 28.9 mol) in water (64.3 L) was added in a combination manner from the stock tank (main) and the pH titrator (supplemental) to maintain the pH of the reaction mixture in the range of 6.3−7.0 when the pH was dropped to 6.3. The fact that the pH stopped dropping indicated the reaction completion, which was further confirmed by HPLC analysis. The mixture was cooled to 20 °C over 30 min and filtered with acetonitrile washes (2 × 10 L) to remove most inorganic precipitates. The combined filtrates were concentrated under vacuum at ∼35 °C to a residual volume of ∼83 L. Water (23.3 L) and ethyl acetate (56.0 L) were added. The mixture was concentrated again under vacuum at ∼40 °C to a residual volume of ∼98 L. The mixture was then cooled to 20 °C, and ethyl acetate (93.4 L) was added. The resulting slurry was stirred for 20 min and then filtered. The cake was washed with water (37.3 L) and ethyl acetate (18.7 L) and dried at 60 °C under vacuum with nitrogen sweep for 8 h to give crude **11** (3.08 kg). This crude was charged back to the reactor, followed by the addition of water (30.8 L) and ethyl acetate (30.8 L). The slurry was stirred at 20 °C for 1 h and filtered with a wash of water (5 L) and ethyl acetate (10 L). The solid was dried at 60 °C under vacuum with nitrogen sweep for 12 h to afford **11** (2.24 kg, 3.88 mol) as an off-white powder in a 61% yield. HPLC purity: 96.6%. HRMS (ESI) *m*/*z*: calculated for $C_{30}H_{36}N_5O_7$ $[M + H]^+$ 578.2615; observed 578.2601. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.21 (s, 1H), 8.00 (s, 1H), 7.63 (d, J = 9.8 Hz, 1H), 7.32−7.57 (m, 10H), 6.51 (m, 1H), 6.11 (m, 1H), 5.96 (s, 1H), 5.26 (s, 2H), 5.00 (s, 2H), 4.81 (dd, J = 9.4/4.4 Hz, 1H), 4.19 (m, 1H), 3.65 (m,1H), 3.16 (m, 2H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.19, 168.28, 158.14, 155.63, 147.07, 146.83, 137.14, 133.48, 130.68, 129.99, 129.18, 128.89, 128.55, 128.51, 123.37, 111.27, 80.62, 79.06, 71.08, 59.52, 53.27, 41.43, 37.59, 28.61.

Preparation of 1-(((2R,3S)-3-Amino-4-oxoazetidin-2-yl)-methyl)-3-((1,5-bis(benzyloxy)-4-oxo-1,4-dihydropyridin-2-

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*yl)methyl)urea Trifluoroacetic Acid Salt (**14**).* To a 100 L reactor were charged trifluoroacetic acid (36.81 kg, 322.9 mol) and anisole (8.05 kg, 56.0 mol). The solution was cooled to −7 °C. **11** (3.73 kg; 6.46 mol) was added. The mixture was stirred at −5 to −10 °C for 3 h. Upon reaction completion, the mixture was slowly transferred to a cold (10 °C) mixture of diatomaceous earth (8.21 kg) in MTBE (112.0 L) in a 200 L reactor while maintaining the temperature in the receiving reactor around 10 °C. The slurry was stirred at 10 °C for 30 min and filtered. The cake was washed with MTBE (69.4 L) and dried with nitrogen-blowing for 1 h. This crude material was charged back to the 200 L reactor and stirred with MTBE (112 L) at 20 °C for 30 min and then filtered with an MTBE wash (69.4 L). The solid was dried with nitrogen-blowing for 12 h to a constant weight to give a mixed white solid (11.32 kg), which contained **14** (3.11 kg, 5.26 mol) and diatomaceous earth (8.21 kg) in an 81% yield. HPLC purity: 86.5%. MS (ESI) *m/z*: calculated for $C_{25}H_{28}N_5O_5$ $[M + H]^+$ 478.2090; observed 478.2. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.81 (brs, 2H), 8.70 (s, 1H), 8.43 (s, 1H), 7.34−7.54 (m, 10H), 6.97 (t, J = 6.0 Hz, 1H), 6.82 (t, J = 5.7 Hz, 1H), 6.41 (s, 1H), 5.36 (s, 2H), 5.09 (s, 2H), 4.47 (m, 1H), 4.28 (m, 2H), 3.73 (m, 1H), 3.36 (m, 1H), 3.22 (m, 1H).

*Preparation of (Z)-2-(((1-(2-((tert-Butoxycarbonyl)-amino)thiazol-4-yl)-2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethylidene)amino)oxy)-2-methylpropanoic Acid (**18**).* To a 20 L reactor were charged dichloromethane (11.5 L), **3** (1.15 kg, 2.68 mol), and *N*-hydroxysuccinimide (0.35 kg, 3.04 mol). The solution was cooled to 2 °C. *N*,*N*′-Diisopropylcarbodiimide (0.37 kg, 2.93 mol) was added over 30 min while maintaining the temperature between 0 and 10 °C. The mixture was warmed to 22 °C and stirred for 3 h. Upon reaction completion, the mixture was filtered through a 0.5 μm polypropylene cartridge filter with a dichloromethane rinse (2.0 L). The filtrates were concentrated under vacuum at <35 °C to a residual volume of ∼4 L. Methanol (12.0 L) was added, and the mixture was concentrated under vacuum at <40 °C to a residual volume of ∼6.5 L. The mixture was then cooled to 20 °C and stirred for 1 h. The slurry was filtered with a methanol wash (2.30 L). The solid was dried at 40 °C under vacuum with nitrogen sweep for 8 h to afford **18** (1.198 kg, 2.27 mol) as an off-white powder in an 85.0% yield. HPLC purity: 98.1%. HRMS (ESI) *m/z*: calculated for $C_{22}H_{31}N_4O_9S$ $[M + H]^+$ 527.1812; observed 527.1824. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.97 (s, 1H), 7.57 (s, 1H), 2.90 (s, 4H), 1.49 (s, 6H), 1.48 (s, 9H), 1.37 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.79, 170.16, 162.10, 158.66, 153.51, 143.50, 139.98, 117.57, 83.77, 82.07, 81.23, 28.29, 28.05, 26.16, 23.89.

*Preparation of 2-((((Z)-2-(((2R,3S)-2-((3-((1,5-Bis-(benzyloxy)-4-oxo-1,4-dihydropyridin-2-yl)methyl)ureido)-methyl)-4-oxoazetidin-3-yl)amino)-1-(2-((tert-butoxycarbonyl)amino)thiazol-4-yl)-2-oxoethylidene)-amino)oxy)-2-methylpropanoic Acid (**19**): Method A through Succinimidyl Ester Activation.* To a 75 L reactor were charged acetonitrile (21.2 L) and the mixed solid (5.51 kg), which contained **14** (1.51 kg, 2.56 mol) and diatomaceous earth (4.00 kg). Molecular sieves (3Å, 3.03 kg), **18** (1.38 kg, 2.62 mol), and DMAP (0.80 kg, 6.56 mol) were then added. The mixture was heated to 40 °C and stirred for 16 h. Upon reaction completion, the mixture was cooled to 20 °C. The aqueous citric acid solution (2 wt %, 13 kg) was added to adjust the pH to ∼6. The mixture was concentrated under vacuum at ∼35 °C to a residual volume of ∼18 L. Ethyl acetate (22.0 L) was added, and the mixture was filtered with a wash of ethyl acetate (20 L) and water (7 L). The organic layer of the combined filtrates was separated, washed with a 10 wt % aqueous citric acid solution (17 kg) and 5 wt % aqueous NaCl solution (2 × 10 kg), and then dried with $MgSO_4$ (3.0 kg). The mixture was filtered with an ethyl acetate wash (10 L). The combined filtrates were concentrated under vacuum at ∼30 °C to a residual volume of ∼7 L. This solution was added to *n*-heptane (36 L) over 30 min. The slurry was stirred at 20 °C for 1 h and filtered with a wash of 4:1 (v/v) heptane and ethyl acetate (10 L). The solid was dried at 40 °C under vacuum with nitrogen sweep for 8 h to afford crude **19** (1.92 kg, 81.0% HPLC purity) as a tan-colored powder in an 83% yield.

Purification of 19 by Chromatography. A dynamic axial compression column was packed with 85 kg of silica gel by slurry in 2:1 (v/v) ethyl acetate-toluene and then equilibrated with ethyl acetate (153 kg). The feed solution was prepared by dissolving crude **19** (3.68 kg) in ethyl acetate (10.0 kg) in a vessel at 20 °C. The entire feed was loaded onto the equilibrated column with an ethyl acetate rinse (3.9 kg). The column was eluted first with ethyl acetate (172 kg), followed by 10:1 (w/w) ethyl acetate−isopropanol (355 kg) and then 5:3 (w/w) ethyl acetate−isopropanol (673 kg). The flow rate was 3 L/min. The product was collected between 163 and 656 kg of the strongest mobile phase. An online UV detector set at 290 nm was used to monitor the elution and determine cut points. All fractions were analyzed by offline HPLC. Product-containing cuts were combined and concentrated via vacuum distillation to a low stir volume. The partially concentrated solution was transferred to a rotary evaporator and further concentrated to dryness. The solids were redissolved in ethyl acetate (9.0 kg). The concentrate was transferred to a pressure canister and then added to *n*-heptane (62 kg) in a stirred vessel over 50 min at 20−25 °C with an ethyl acetate rinse (4.0 kg) to precipitate the product. The product slurry was stirred for 1 h at 20−25 °C and filtered on a pressure filter with a wash of *n*-heptane (5.9 kg) and ethyl acetate (1.3 kg). The solid was dried in the filter by nitrogen-blowing over 24 h to a constant weight. Purified **19** (2.35 kg) was obtained as an off-white powder in a 64% yield. HPLC purity: 97.8%. HRMS (ESI) *m/z*: calculated for $C_{43}H_{53}N_8O_{11}S$ $[M + H]^+$ 889.3555; observed 889.3534. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.85 (brs, 1H), 9.27 (d, J = 8.6 Hz, 1H), 8.41 (s, 1H), 8.09 (s, 1H), 7.54 (m, 2H), 7.32−7.48 (m, 8H), 7.29 (s, 1H), 6.67 (brs, 1H), 6.32 (brs, 1H), 6.08 (s, 1H), 5.29 (s, 2H), 5.19 (m, 1H), 5.03 (s, 2H), 4.24 (m, 2H), 3.78 (m,1H), 3.38 (m,1H), 3.19 (m, 1H), 1.43 (s, 9H), 1.41 (s, 6H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.38, 170.34, 167.06, 162.66, 160.96, 158.39, 153.45, 149.53, 147.03, 146.94, 142.47, 136.98, 133.37, 130.71, 130.04, 129.18, 128.91, 128.57, 123.69, 113.86, 111.19, 82.84, 81.75, 80.92, 80.83, 71.21, 57.64, 53.47, 42.04, 37.68, 28.28, 28.10, 24.27, 24.18.

*Preparation of 2-((((Z)-2-(((2R,3S)-2-((3-((1,5-Bis-(benzyloxy)-4-oxo-1,4-dihydropyridin-2-yl)methyl)ureido)-methyl)-4-oxoazetidin-3-yl)amino)-1-(2-((tert-butoxycarbonyl)amino)thiazol-4-yl)-2-oxoethylidene)-amino)oxy)-2-methylpropanoic Acid (**19**): Method B through CDMT In Situ Activation.* To a 1 L round-bottom flask were charged acetonitrile (664 mL) and the mixed solid (251.9 g), which contained **14** (∼69.2 g, 0.117 mol) and diatomaceous earth (∼182.7 g). The mixture was stirred at 20 °C for 15 min and then filtered with acetonitrile rinses (2 ×

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330 mL). To the combined filtrates collected in a 2 L round-bottom flask were added DMF (330 mL) and *N*-methylmorpholine (38.7 mL, 0.35 mol). The mixture was concentrated using a rotary evaporator under vacuum at <45 °C to a residual volume of ∼450 mL. A basic aluminum oxide column (207 g, 50 mm D × 120 mm H) was packed with a DMF rinse (200 mL). The above crude **14** solution in DMF was loaded on top and eluted with DMF (500 mL). The filtrates collected were **14** free base solution in DMF.

To a 3 L three-neck round-bottom flask were charged **3** (42.18 g, 98.22 mmol), CDMT (17.24 g, 98.22 mmol), and ethyl acetate (664 mL). *N*-Methylmorpholine (23.2 mL, 210.5 mmol) was added. The mixture was stirred at 20 °C for 60 min. The reaction completion was monitored by HPLC with benzylamine derivatization. The above **14** free base solution in DMF was added over 15 min via addition funnel. The mixture was stirred at 20 °C for 16 h. Upon reaction completion, ethyl acetate (1 L) was added. The mixture was washed with a 5 wt % aqueous NaCl solution (830 mL), and the aqueous layer was back-extracted with ethyl acetate (1 L). The combined organic layers were then washed with a 5 wt % aqueous NaCl solution (2 × 830 mL), 10 wt % aqueous citric acid solution (830 mL), 10 wt % aqueous K_3PO_4 solution (830 mL), and saturated aqueous NaCl solution (830 mL). The organic layer was then concentrated using a rotary evaporator at ∼50 °C to a residual weight of ∼300 g, which was filtered with ethyl acetate rinses (2 × 25 mL). The combined filtrates were added slowly to *n*-heptane (1.5 L) over 30 min. The slurry was stirred at 20 °C for 1 h and filtered with a wash of 5:1 (v/v) *n*-heptane and ethyl acetate (400 mL). The solid was dried under vacuum at 50 °C for 15 h to afford **19** (72.36 g, 81.39 mmol) as a light tan powder in a 69% yield. HPLC purity: 96.1%.

*Preparation of (2R,3S)-2-((3-((1,5-Bis(benzyloxy)-4-oxo-1,4-dihydropyridin-2-yl)methyl)ureido)methyl)-3-((Z)-2-(((1-(tert-butoxy)-2-methyl-1-oxopropan-2-yl)oxy)imino)-2-(2-((tert-butoxycarbonyl)amino)thiazol-4-yl)acetamido)-4-oxoazetidine-1-sulfonic Acid (**21**).* To a 100 L reactor were charged DMF (23.0 L) and **19** (2.30 kg, 2.59 mol). The solution was cooled to 15 °C, and sulfur trioxide−DMF complex (3.96 kg, 25.87 mol) was added. The mixture was stirred at 20 °C for 30 min. Upon reaction completion, the mixture was cooled to 0 °C and transferred with a DMF rinse (2.3 L) to a precooled (2 °C) mixture of ethyl acetate (69 L) and 5 wt % aqueous NaCl solution (46 kg) in a 200 L reactor over 30 min. The organic layer was separated, and the aqueous layer was back-extracted with ethyl acetate (23 L). The combined organic layers were washed with a 5 wt % aqueous NaCl solution (3 × 46 kg) and then saturated aqueous NaCl solution (26 kg). $MgSO_4$ (6.90 kg) was added to the organic layer, and the mixture was stirred for 1 h (for both drying and converting **22** to **21**). Upon reaction completion, the mixture was filtered with an ethyl acetate rinse (15 L). The combined filtrates were concentrated under vacuum at ∼30 °C to a residual volume of ∼8 L. MTBE (69 L) was added to over 1 h. The slurry was stirred at 20 °C for 1 h and filtered with an MTBE wash (9.2 L). Nitrogen-blowing the cake for 12 h afforded **21** (2.19 kg, 2.26 mol) as an off-white powder in an 87% yield. HPLC purity: 89.8%. MS (ESI) *m*/*z*: calculated for $C_{43}H_{53}N_8O_{14}S_2$ $[M + H]^+$ 969.3123; observed 969.3. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.27 (d, J = 8.8 Hz, 1H), 8.86 (s, 1H), 7.57 (dd, J = 5.7/2.0 Hz, 2H), 7.36−7.50 (m, 10H), 7.26 (s, 1H), 6.91 (s, 1H), 6.38 (m, 1H), 5.49 (s, 2H), 5.20 (s, 2H), 5.19 (m, 1H), 4.38 (m, 2H), 3.93 (m,1H), 3.82 (m,1H), 3.10 (m, 1H), 1.41 (s, 9H), 1.38 (s, 6H), 1.36 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.39, 163.96, 162.83, 162.55, 160.69, 158.14, 153.39, 149.31, 149.10, 146.04, 142.47, 135.83, 132.60, 130.97, 130.42, 129.30, 129.08, 128.99, 128.80, 126.18, 113.56, 110.70, 82.90, 82.44, 81.81, 80.86, 72.20, 59.24, 56.03, 41.67, 38.29, 28.26, 28.09, 24.24, 24.21.

Preparation of 2-((((Z)-1-(2-Aminothiazol-4-yl)-2-(((2R,3S)-2-((3-((1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)methyl)ureido)methyl)-4-oxo-1-sulfoazetidin-3-yl)-amino)-2-oxoethylidene)amino)oxy)-2-methylpropanoic Acid (Crude 1): Using 1 M BCl_3 Solution in Dichloromethane. To a 200 L reactor were charged dichloromethane (45.4 L) and **21** (2.27 kg, 2.34 mol). The solution was concentrated under vacuum at ∼18 °C to a residual volume of ∼24 L to reach KF < 0.1%. The mixture was then cooled to −5 °C, and the reactor was connected to an aqueous NaOH scrubber. The BCl_3 solution (1 M) in dichloromethane (34.20 kg, 23.42 mol) was added over 60 min while maintaining the temperature between −5 and 5 °C. The mixture was warmed to 15 °C over 45 min and stirred for 30 min. Upon reaction completion, the mixture was cooled to −15 °C. A precooled (−15 °C) solution of trifluoroethanol (10.55 kg, 105.41 mol) in MTBE (45.4 L) was added over 30 min while maintaining the temperature below −10 °C. The slurry was warmed to 0 °C over 15 min, stirred for 30 min, and then filtered with an MTBE wash (45.4 L). The cake was dried with nitrogen-blowing for 3 h and then charged back to the reactor. MTBE (45.4 L) was added. The slurry was stirred at 20 °C for 1 h and filtered with an MTBE wash (20 L). The cake was dried with nitrogen-blowing for 12 h to afford crude **1** (1.72 kg, 63% HPLC purity) as an off-white powder with a 115% mass recovery.

Purification of 1 by Reversed-Phase Chromatography. A dynamic axial compression column was packed with 9 kg of Kromasil C-18 by slurry in isopropanol, rinsed with purified water (low iron content, 40 L), and equilibrated with mobile phase (acetonitrile-purified water and 0.2% formic acid modifier, 145 kg). The column feed solution was prepared by dissolving crude **1** (1.70 kg) in 1 M aqueous ammonium bicarbonate (10.4 L) and DMF (2.2 L) in a stirred vessel placed in an ice bath, and the solution was filtered. For each run, the feed solution (148 mL) was loaded onto the column, and the column was eluted with the mobile phase (acetonitrile-purified water and 0.2% formic acid modifier) using a reverse-phase gradient algorithm. The flow rate was 4.2 L/min. An online UV detector set at 320 nm was used to monitor the elution and determine cut points. All fractions were analyzed by offline HPLC. Product-containing cuts were combined in a stirred vessel and concentrated via vacuum distillation until acetonitrile was removed.

A dynamic axial compression column was packed with 22 L of the Amberchrom CG161M resin by slurry in 4:1 (v/v) ethanol−water and rinsed with methanol (60 L) and then purified water (60 L). The product-containing partial concentrate was loaded onto the column. The column was washed with purified water, purged with nitrogen to remove free water, and eluted with 9:1 (v/v) methanol−purified water (60 L). Product-containing cuts (20.4 kg) were combined in a pressure canister and then added to ethyl acetate (240 kg) with a methanol rinse (4.0 kg) in a stirred vessel over 60 min at 2−8 °C to precipitate the product. Additional ethyl acetate (63 kg) was added, and the slurry was stirred for 40 min at 2−8 °C and filtered. The filter cake was washed with ethyl acetate (2 × 9.0

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kg) and blown with nitrogen for 8 h. The solid was dried at 30 °C under vacuum with nitrogen sweep for 8 h to afford **1** (551 g, 0.814 mol, 88.2% HPLC purity) as a white powder in a 34.8% yield.

Preparation of 2-((((Z)-1-(2-Aminothiazol-4-yl)-2-(((2R,3S)-2-((3-((1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)methyl)ureido)methyl)-4-oxo-1-sulfoazetidin-3-yl)-amino)-2-oxoethylidene)amino)oxy)-2-methylpropanoic Acid Crude (1): Using 1 M BCl_3 Solution in n-Heptane. To a 250 mL three-necked round-bottom flask was charged 1 M BCl_3 solution in *n*-heptane (74.3 mL, 74.3 mmol). The solution was cooled to 10 °C. A solution of **21** (9.00 g, 9.30 mmol) in dichloromethane (90 mL) was added via addition funnel over 15 min while maintaining the temperature between 8 and 12 °C. The mixture was stirred at 10 °C for 30 min. Upon reaction completion, the slurry was filtered under nitrogen pressure and washed by 1:1 dichloromethane and *n*-heptane three times (3 × 90 mL). The cake was dried with nitrogen-blowing for 16 h to afford 70% pure crude **1** (8.84 g) as a yellow powder with a 150% mass recovery. The material was stored in a refrigerator for use.

Purification of 1 through Amberchrom CG161M Resin Column and Crystallization of 1·5H_2O. To a 250 mL round-bottom flask were charged crude **1** (5.60 g, 70% purity, estimated ∼4 mmol) and acetonitrile (60 mL). The slurry was cooled to 10 °C with ice/water bath. Water (30 mL) was added slowly, followed by sodium formate (3.40 g, 50 mmol) portion-wise to adjust the pH to 2.9−3.1 while controlling the temperature below 20 °C. The Amberchrom CG161M resin (7.2 g, prewashed with water) was added. Acetonitrile was removed by a rotary evaporator with bath temperature <30 °C. The brown slurry was loaded onto an Amberchrom CG161M resin column (60 g, 32 mm D × 110 mm H) with a water rinse (200 mL). The column was then eluted with 2.8 L of a 15:1 (v/v) aqueous phosphate buffer (pH 3.1, prepared by dissolving 1.0 g of KH_2PO_4 and 60 mg of 85% phosphoric acid to 1 L water) and acetonitrile. The fractions that contained >85% pure 1 (∼1.1 L) were combined. Acetonitrile was removed by a rotary evaporator with bath temperature <30 °C, and the aqueous solution (∼91% purity) was loaded onto an Amberchrom CG161M resin column (30 g, 32 mm D × 55 mm H) and washed with water (150 mL). The column was blown with nitrogen for 10 min and then eluted with 5:2 (v/v) acetonitrile and water (330 mL). The filtrates were concentrated to a residual volume of ∼70 mL by a rotary evaporator with bath temperature <35 °C. Hydrochloric acid (1 N, 5 mL) was added slowly to adjust the pH to a constant 1.12. The seed was added, and the mixture was stirred at 21 °C for 24 h while an additional amount (∼2 mL in total) of 1 N hydrochloric acid was added intermittently to maintain pH ∼1.12. The resulting slurry was filtered with water rinses (2 × 4 mL). After drying at 25 °C for 48 h, 94.5% pure **1·5H_2O** (2.04 g) was obtained as an off-white powder. This **1·5H_2O** and water (30 mL) were added to a 100 mL round-bottom flask, and the pH of the slurry was measured at 2.23. Hydrochloric acid (1 N, 3.2 mL) was added slowly to adjust the pH to a constant 1.12. The slurry was stirred at 21 °C for 24 h and filtered with water rinses (2 × 4 mL). After drying at 25 °C for 48 h, **1·5H_2O** (Form 1, 1.96 g, 2.71 mmol) was obtained as an off-white powder in a 45.8% yield (from reaction to trituration). HPLC purity: 97.45% (with 0.62% of 25). HRMS (ESI) *m/z*: calculated for $C_{20}H_{25}N_8O_{12}S_2$ $[M + H]^+$ 633.1033; observed 633.1045. ^1H NMR (400 MHz, DMSO-d_6) δ 9.22 (d, J = 8.8 Hz, 1H), 8.18 (s, 1H), 7.37 (brs, 1H), 7.22 (brs, 1H), 7.00 (s, 1H), 6.74 (s, 1H), 6.34 (m, 1H), 5.17 (dd, J = 8.7/5.8 Hz, 1H), 4.33 (m, 2H), 3.95 (m, 1H), 3.63 (m, 1H), 3.22 (m, 1H), 1.39 (s, 3H), 1.37 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.18, 169.32, 162.85, 162.59, 158.24, 157.54, 148.93, 145.74, 144.57, 141.51, 127.90, 110.14, 109.76, 82.39, 58.99, 56.15, 41.19, 38.64, 24.38, 24.25. KF: calculated for **1·5H_2O**, 12.4 wt % of water; found 12.1 wt %. Elemental analysis: calculated for **1·5H_2O**, C: 33.24%, H: 4.74%, N: 15.51%; found C: 32.86%, H: 4.69%, N: 15.38%. PXRD: Form 1.

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Notes

The authors declare no competing financial interest.

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