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Practical Synthesis of a Cathepsin S Inhibitor: Route Identification, Purification Strategies, and Serendipitous Discovery of a Crystalline Salt Form

Xiaohu Deng,* Jimmy T. Liang, Matthew Peterson,† Raymond Rynberg, Eugene Cheung,‡ and Neelakandha S. Mani

Johnson & Johnson Pharmaceutical Research & Development LLC, 3210 Merryfield Row, San Diego, California 92121. †*Current address: Amgen Inc., 360 Binney St, Cambridge, MA 02139.* ‡*TransForm Pharmaceuticals, 29 Hartwell Avenue, Lexington, MA 02421.*

xdeng@its.jnj.com

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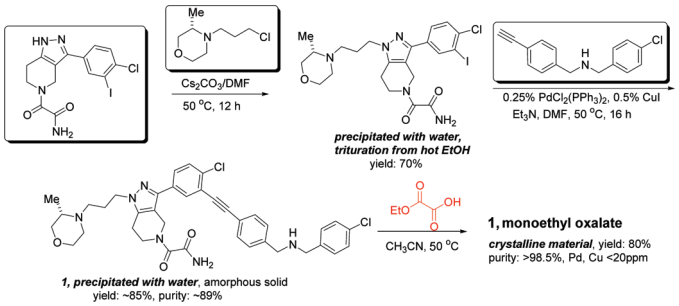

A "redox economical" strategy resulted in a concise, modular synthesis of compound **1**, a potent Cathepsin S inhibitor. Starting from three building blocks, crude drug substance was prepared in a two-step sequence in high yield. Efficient purification of the crude drug substance was accomplished via the formation of an unusual monoethyl oxalate salt.

Introduction

Cathepsin S is a cysteine protease of the papain superfamily. Unlike the ubiquitous housekeeping enzymes cathepsins B, D, and L, cathepsin S is mainly expressed in antigen presenting cells such as dendritic cells, B cells, and macrophages. It is believed that cathepsin S is involved in antigen-presentation to the cell surface for recognition by the CD4+ T cells, which in turn triggers an immune response.[1] Therefore, cathepsin S inhibitors have been proposed to be potential therapeutics for a range of immunological disorders and have been intensively pursued by many major pharmaceutical companies.[2] Most cathepsin S inhibitors discovered earlier were peptidic compounds that covalently bind to the cysteine at the active site. In 2004, our laboratories disclosed the first highly potent nonpeptidic, noncovalent cathepsin S inhibitor.[3] Subsequent medicinal chemistry efforts[4] identified compound **1** as a potent inhibitor of cathepsin S. For the purpose of further pharmacologically profiling this compound, a practical, large-scale synthesis was required.

(1) (a) Villandangos, J. A.; Bryant, R. A. R.; Deussing, J.; Driessen, C.; Lennon-Dumenil, A-M; Riese, R. J.; Roth, W.; Saftig, P.; Shi, G-P; Chapman, H. A.; Peters, C.; Ploegh, H. L. *Immunol. Rev.* **1999**, *172*, 109–120. (b) Nakagawa, T. Y.; Rudensky, A. Y. *Immunol. Rev.* **1999**, *172*, 121–129. (c) Hsing, L. C.; Rudensky, A. Y. *Immunol. Rev.* **2005**, *207*, 229–241.

(2) (a) Kim, W.; Kang, K. *Expert Opin. Ther. Pat.* **2002**, *12*, 419–432. (b) Leroy, V.; Thruairatnam, S. *Expert Opin. Ther. Pat.* **2004**, *14*, 301–311. (c) Thrumond, R. L.; Sun, S.; Karlsson, L.; Edwards, J. P. *Curr. Opin. Invest. Drugs* **2005**, *6*, 473–482. (d) Link, J. O.; Zipfel, S. *Curr. Opin. Drug Discovery Dev.* **2006**, *9*, 471–482. (e) Gupta, S.; Kumar, S. R.; Dastidar, S.; Ray, A. *Expert Opin. Ther. Targets* **2008**, *12*, 291–299.

(3) (a) Thurmond, R. L.; Beavers, M. P.; Cai, H.; Meduna, S. P.; Gustin, D. J.; Sun, S.; Almond, H. J.; Karlsson, L.; Edwards, J. P. *J. Med. Chem.* **2004**, *47*, 4799–4801. (b) Thurmond, R. L.; Sun, S.; Sehon, C. A.; Baker, S. M.; Cai, H.; Gu, Y.; Jiang, W.; Riley, J. P.; Williams, K. N.; Edwards, J. P.; Karlsson, L. *J. Pharm. Exp. Ther.* **2004**, *308*, 268–276.

(4) (a) Gustin, D. J.; Sehon, C. A.; Wei, J.; Cai, H.; Meduna, S. P.; Khatuya, H.; Sun, S.; Gu, Y.; Jiang, W.; Thurmond, R. L.; Karlsson, L.; Edwards, J. P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1687–1691. (b) Grice, C. A.; Tays, K.; Khatuya, H.; Gustin, D. J.; Butler, C. R.; Wei, J.; Sehon, C. A.; Sun, S.; Gu, Y.; Jiang, W.; Thurmond, R. L.; Karlsson, L.; Edwards, J. P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2209–2212. (c) Wei, J.; Pio, B. A.; Cai, H.; Meduna, S. P.; Sun, S.; Gu, Y.; Jiang, W.; Thurmond, R. L.; Karlsson, L.; Edwards, J. P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5525–5528. (d) Ameriks, M. K.; Axe, F. U.; Bembenek, S. D.; Edwards, J. P.; Gu, Y.; Karlsson, L.; Randal, M.; Sun, S.; Thurmond, R. L.; Zhu, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6131–6134. (e) Ameriks, M. K.; Cai, H.; Edwards, J. P.; Gebauer, D.; Gleason, E.; Gu, Y.; Karlsson, L.; Nguyen, S.; Sun, S.; Thurmond, R. L.; Zhu, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6135–6139.

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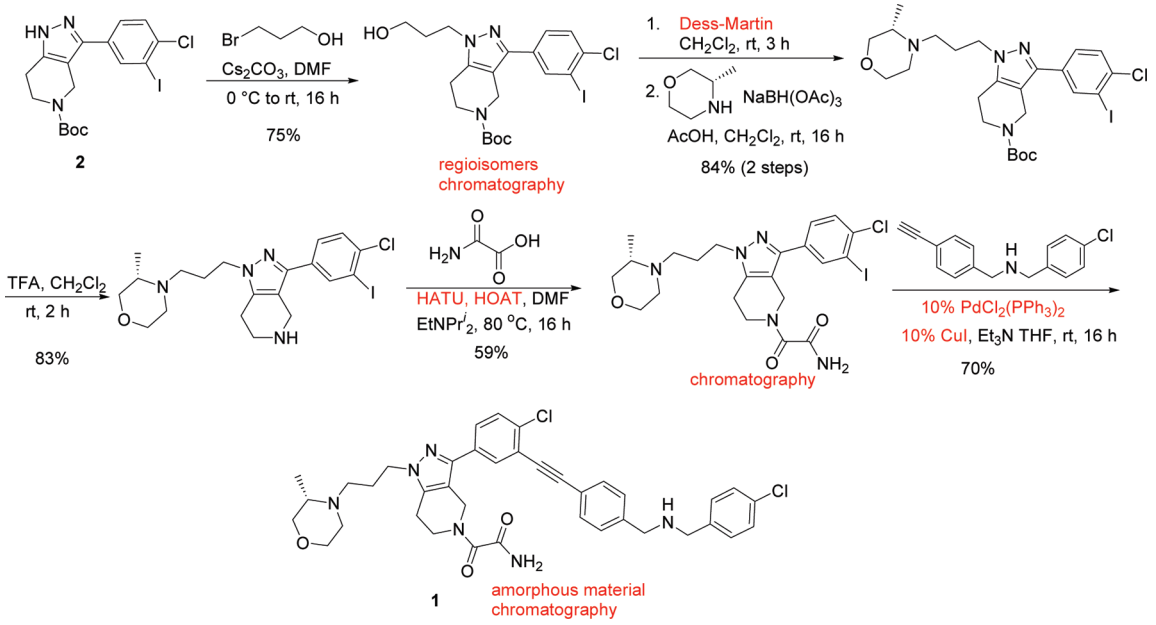
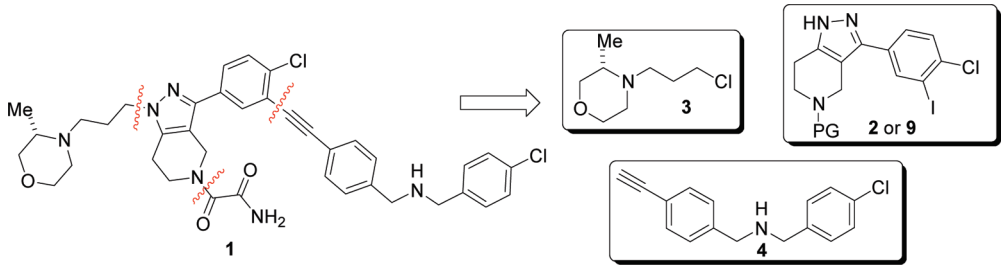
SCHEME 1. Original Synthesis of Compound 1



SCHEME 2. Modular "Redox Economical" Approach



The original synthesis of compound **1** involved a 7-step sequence (Scheme 1) from compound **2**, which had been prepared previously in a three-step, one-pot process (see Experimental Section for details). Because this synthesis of **1** was originally tailored to facilitate analogue production, it did not capitalize on opportunities for convergence. We hoped to address this issue in our revised route. In addition, from economical and environmental points of view, we planned to avoid expensive, environment-unfriendly reagents such as the Dess−Martin reagent and HATU. Finally, we deemed it critical to minimize unit operations and eliminate column chromatographic purification; because of its high molecular weight (699 Da) and polarity, purification of the final drug substance **1** was particularly challenging. Herein, we report our endeavor toward the efficient synthesis of drug substance **1**, which features a modular, two-step route from three readily available building blocks. The purification of the final drug substance was accomplished through the formation of an unusual monoethyl oxalate salt. Overall, this synthesis is operationally simple, high-yielding, chromatography-free, and amenable for large-scale production.

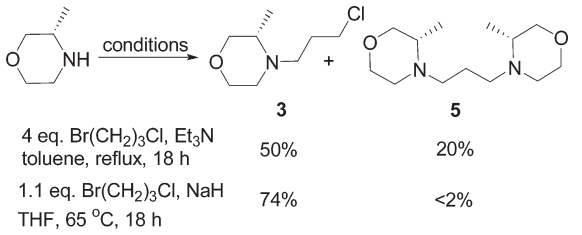
Results and Discussion

Hendrickson has formalized the concept of an "ideal synthesis" as one that "creates a complex skeleton ... in a sequence only of successive construction reactions involving no intermediary refunctionalizations ...".[5] To approach that, the practitioners of industrial process research must strive to minimize oxidation state manipulations. This concept has been further delineated recently by Baran and Hoffmann, who coined the term "redox economy".[6] In the original synthesis of **1**, two chemical steps were devoted to counterproductive oxidation state adjustments with expensive $NaBH(OAc)_3$ and the Dess−Martin reagent. In addition, the sequence was performed in a linear fashion starting from the rather expensive advanced intermediate **2**. Strategically, a modular approach from optimal building blocks would be more efficient and economical. To that end, we devised a new retro-synthetic analysis (Scheme 2).

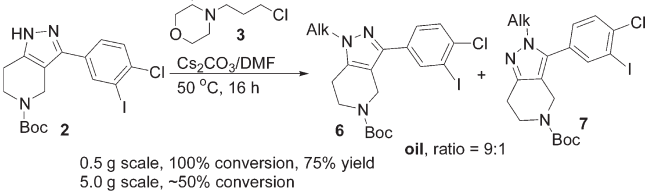
To access the fully elaborated left-hand building block **3**, we chose the readily available and inexpensive 1-bromo-3-chloropropane as the starting material with the hope that the reactivity difference of bromide and chloride would provide the requisite chemoselectivity (Scheme 3). The oxidation state and functionality of this building block were keys to the potential for the realization of redox economy. Unfortunately, a number of literature procedures[7] afforded a mixture

(5) Hendrickson, J. B. *J. Am. Chem. Soc.* **1975**, *97*, 5784–5800.
(6) Burns, N. Z.; Baran, P. S.; Hoffmann, R. W. *Angew. Chem., Int. Ed.* **2009**, *48*, 2854–2867.
(7) (a) Murty, M. S. R.; Jyothirmai, B.; Krishna, P. R.; Yadav, J. S. *Synth. Commun.* **2003**, *33*, 2483–2486. (b) Wang, J-Q; Gao, M.; Miller, K. D.; Sledge, G. W.; Zheng, Q-H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4102–4106.

SCHEME 3. Synthesis of Compound 3



SCHEME 4. Alkylation of Compound 2 with 3

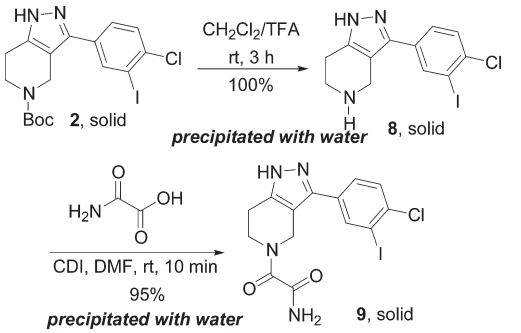


of **3** and dialkylation product **5** even in the presence of a large excess of 1-bromo-3-chloropropane. The low selectivity was probably due to the high reaction temperature required for the alkylation of sterically hindered 2-methylmorpholine. To address this problem, we treated 2-methylmorpholine with NaH to effect complete deprotonation. Subsequent addition of equimolar 1-bromo-2-chloropropane and warming to 65 °C gave rise to the desired product **3** with effective suppression of the dialkylation pathway. A 100-g scale reaction was performed to provide **3** in 74% yield, and no column chromatographic purification was needed.

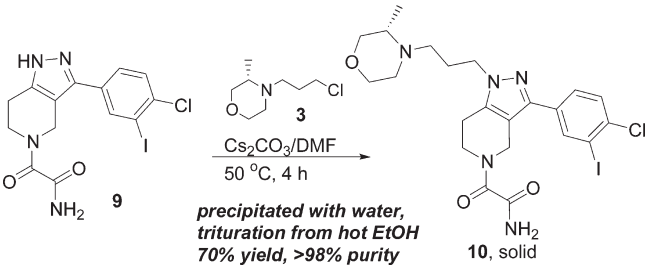
The alkylation reaction of compound **2** with **3** was then investigated. It is well-known in the literature,[8] and from our own experience, that N^1:N^2 regioselectivity is always an issue upon alkylation of 1-*H*-pyrazoles. Indeed, on a 0.5-g scale test run, the alkylation reaction provided a mixture of **6** and **7** in a 9:1 ratio and 75% yield (Scheme 4). A similar regioisomeric ratio was observed in the reaction between compound **2** and 3-bromo-propanol in the original synthesis. Our past experience with the regioisomeric pyrazole products[9] has taught us that recrystallization often provides an effective purification strategy. Unfortunately, in this case, efforts to generate crystalline solids of either regioisomer **6** or **7** were unsuccessful. More problematically, on a 5-g scale, the reaction stalled at ~50% conversion. Addition of excess reagents (**3** and base) and increase of reaction temperature failed to drive the reaction to completion. The cause of this scale-up issue is not fully understood.

To circumvent these problems, we turned our attention to an alternative intermediate **9**. It was noticed that molecules with the oxalamide group tended to be highly crystalline solids. Besides the obvious operational advantages of solid intermediates in scale-up campaigns,[10] crystallization might also allow selective removal of the undesired N^2-alkylated byproduct. Therefore, we decided to prepare **9**, which was easily accomplished in two steps (Scheme 5). The deprotection of the Boc group of compound **2** proceeded uneventfully to afford **8** in quantitative yield. The isolation of **8** involved the simple addition of water followed by neutralization of TFA with NaOH. In the oxalamide formation step, an inexpensive coupling reagent, CDI, was used to replace the expensive HATU/HOAT used in the original synthesis. A water-miscible solvent, DMF, was purposely chosen, and compound **9** was directly precipitated in 95% yield by simply adding water to the reaction solution.

SCHEME 5. Synthesis of Compound 9



SCHEME 6. Synthesis of Compound 10



With **9** in hand, the alkylation reaction with **3** was investigated. Two regioisomers were again observed in a ~9:1 ratio favoring desired product **10** (Scheme 6). Gratifyingly, the desired regioisomer **10** was preferentially precipitated by addition of water to the DMF reaction solution. Subsequent trituration from hot EtOH provided pure, highly crystalline material in 70% yield. Thus, the difficult regioselectivity problem was solved with a simple purification strategy. It is noteworthy that no scale-up issues were observed in this case (up to 250 g scale).

Before the crucial Sonogashira cross-coupling reaction could be performed, a suitable large-scale synthesis of building block **4** was needed. Under various direct reductive amination conditions on 4-ethynyl-benzaldehyde with $NaBH(OAc)_3$, the desired product **4** was invariably contaminated with dialkylation product **11** (20−40%) (Scheme 7). The alternative stepwise reductive amination conditions (MeOH/$NaBH_4$), which usually result in enhanced selectivity,[11] also gave rise to significant quantities of **11**. Interestingly, upon TMS protection of the starting material, the two-step reductive amination procedure provided **4** exclusively, probably

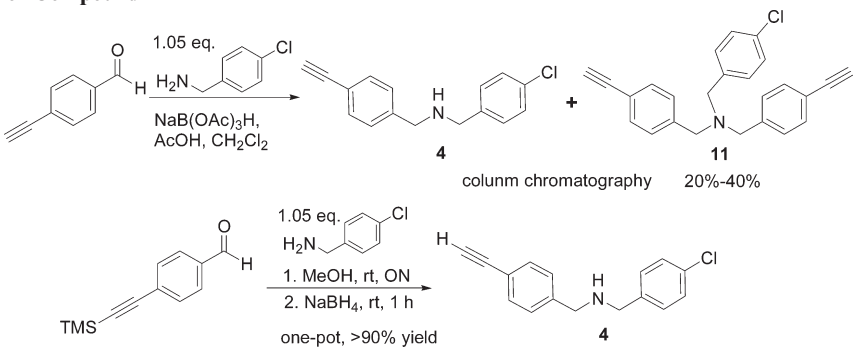
(8) (a) Meanwell, N. A.; Rosenfeld, M. J.; Wright, J. J. K.; Brassard, C. L.; Buchanan, J. O.; Federici, M. E.; Fleming, J. S.; Seiler, S. M. *J. Med. Chem.* **1992**, *35*, 389–397. (b) Wang, X.; Tan, J.; Zhang, L. *Org. Lett.* **2000**, *2*, 3107–3109.

(9) (a) Deng, X.; Mani, N. S. *Org. Lett.* **2006**, *8*, 3505–3508. (b) Liang, J. T.; Mani, N. S.; Jones, T. K. *J. Org. Chem.* **2007**, *72*, 8243–8350. (c) Deng, X.; Mani, N. S. *J. Org. Chem.* **2008**, *73*, 2412–2415. (d) Deng, X.; Mani, N. S. *Org. Lett.* **2008**, *10*, 1307–1310.

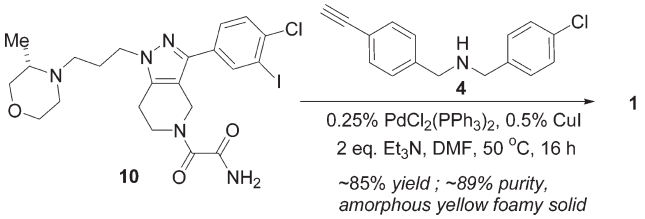
(10) Anderson N. G. *Practical Process Research & Development*; Academic Press: New York, 2000.

(11) Abdel-Magid, A. F.; Mehrman, S. J. *Org. Process. Res. Dev.* **2006**, *10*, 971–1031.

SCHEME 7. Synthesis of Compound 4



SCHEME 8. Synthesis of Compound 1

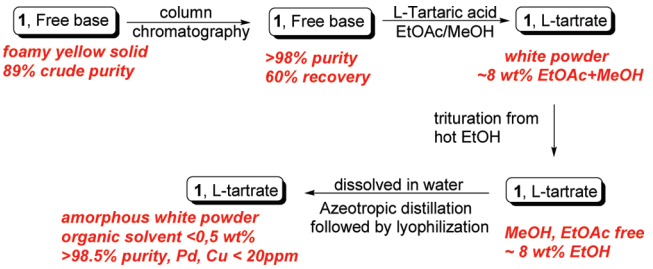


due to the increase in the rate of the imine reduction step.[12] It is worthwhile to note that removal of the TMS protecting group was achieved *in situ* upon the addition of $NaBH_4$, which made the sequence a highly efficient one-pot operation. Compound **4** was isolated in 90% yield, and no column chromatographic purification was needed.

With building blocks **4** and **10** in hand, the optimization of the Sonogashira cross-coupling reaction was studied. In the active pharmaceutical ingredient (API) production process, heavy metal contamination of the final drug substance is always a major concern. We quickly identified that the unusually high loading of Pd/Cu catalysts required in the original synthesis was the result of poor solubility of compound **10** in THF. Switching the solvent to DMF, the catalyst loading was successfully lowered to 0.25% Pd / 0.5% CuI (Scheme 8). At a slightly elevated temperature (50 °C), the Sonogashira reaction was complete in 16 h. Again, because of the use of water-miscible solvent DMF, crude **1** was precipitated by the addition of water in 85% yield and ~89% HPLC assay purity.

To finish the production of the final API, we required a purification of crude **1**. This seemingly trivial task proved to be particularly challenging. Because of time constraints, we resorted to column chromatography to produce an early batch of the API (Scheme 9). Poor product recovery (~60%) was observed probably due to the high polarity and instability of the API on normal phase silica gel. As a result of its high water solubility, the L-tartrate salt of **1**[13] was identified as the potential salt form early. However, even under optimized salt formation conditions in a mixed EtOAc/MeOH solvent at 60 °C, the L-tartrate salt obtained was amorphous and hygroscopic and contained about 8 wt % solvent residues, which were extremely difficult to remove even

SCHEME 9. Production of Pure, Solvent-Free 1, L-Tartrate Salt



under high vacuum (0.1 Torr) at 70 °C for several days. Trituration in other solvents such as EtOH resulted in the trapping of the incoming solvent. Finally, azeotropic distillation of an aqueous solution of the L-tartrate salt successfully lowered the organic solvent residue level to < 0.5 wt %.[14] Subsequent lyophilization provided the final API as an amorphous white powder. This protocol was used to produce over 100 g of material for various animal studies (Scheme 9).

Unfortunately, this approach is not suitable for larger-scale production. For purification of API on large scale, recrystallization of free base or a salt remains the most practical option. Attempts at recrystallization or trituration of free base **1** with numerous combinations of common solvents (MeOH, EtOH, IPA, *t*-amylOH, EtOAc, CH_3CN, THF, 2-MeTHF, CH_2Cl_2, $CHCl_3$, hexanes, heptane toluene, acetone, MTBE, DMF, ^{i}PrOAc, 2-butanone, nitromethane, DME) failed to provide any crystalline material. Furthermore, improvements to the purity of **1** were minimal. This result did not come as a total surprise, because understandably, the two flexible side chains present in compound **1** would impede the formation of a rigid crystalline lattice. In the pharmaceutical industry, a common strategy to deal with amorphous drug substances is to form a salt, which may possess higher crystallinity, a desirable characteristic for drug development. Therefore, significant efforts were undertaken toward salt screening with both automated equipment and manual optimization. Over 30 pharmaceutically acceptable, structurally diverse acids[15] were investigated in various common solvents (Figure 1). In addition to the previously identified tartaric acid, only three other acids

(12) Abdel-Magid, A. F.; Harris, B. D.; Maryanoff, C. A. *Synlett* **1994**, 81–83.

(13) There was no different to use L- or D-tartaric acid. Naturally occurring L-tartaric acid was chosen for the cost reason.

(14) Direct lyophilization was not effective removing the organic solvent residues.

(15) *Pharmaceutical Salts: Properties, Selection, and Use*; Stahl, P. H., Wermuth, C. G., Eds.; Wiley-VCH: New York, 2008.

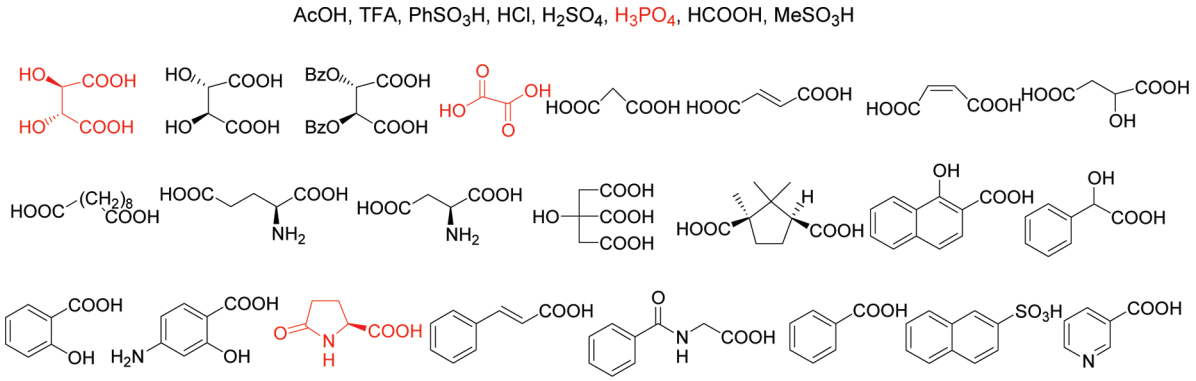
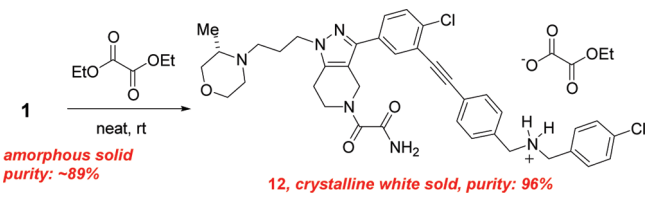


FIGURE 1. Common pharmaceutically accepted acids.

SCHEME 10. Formation of a Crystalline Solid



(H_3PO_4, oxalic acid, and 5-oxo-pyrrolidine-2-carboxylic acid) provided solid salts. Unfortunately, all four salts were amorphous and failed to reject the impurities.

Finally, a serendipitous breakthrough occurred during the study of the solubility of **1** in diethyl oxalate. This unorthodox "solvent" was purposefully chosen due to its structural similarity to the oxalamide side chain of **1**. When compound **1** was dissolved in diethyl oxalate, a white solid **12** precipitated almost immediately (Scheme 10). Optical microscope study of **12** (Figure 2) suggested a highly crystalline material; this crystallinity was confirmed by powder XRD analysis (Figure 3). More significantly, upon formation of **12**, the purity of the drug substance increased from 89% to 96% based on HPLC analysis.

Careful NMR studies revealed that crystalline material **12** was actually the monoethyl oxalate salt of **1**, which was unambiguously confirmed by single crystal X-ray crystallography (Figure 4). Apparently, diethyl oxalate was rapidly hydrolyzed under catalysis by **1** to generate monoethyl oxalate,[16] which was acidic enough to complex with compound **1** to form the salt **12**. Control experiments showed that compound **1** in >99.5% purity also afforded the same salt **12**, which ruled out the possibility of the impurities serving as the catalysts for hydrolysis. Interestingly, the same hydrolysis and salt formation was observed with $(PhCH_2)_2NH$ but NOT with intermediate **9**, suggesting that the secondary amine portion of **1** was responsible for the catalytic hydrolysis process. Extensive literature search revealed a single report of this unusual amine mediated hydrolysis of oxalate with *tert*-butylamine.[17] To confirm this theory, compound **1** was treated with preformed monoethyl oxalate, and the identical crystalline salt **12** was obtained.

Careful examination of the single crystal X-ray structural data suggested that both carbonyl groups and the length of its alkyl side chain were essential for the construction of the crystal lattice. To test the hypothesis, a number of 2-oxo-carboxylic acids (Figure 5) were investigated for the salt formation process. Indeed, other than monoethyl oxalate, only 2-oxo-pentanoic acid fits the structural requirements to provide a crystalline salt.

Encouraged by these results, the salt formation process was carefully optimized. It was found that in CH_3CN at 50 °C, addition of monoethyl oxalate to the crude **1** (89% purity) followed by slow cooling to room temperature produced the crystalline salt **12** in >98.5% purity. The salt

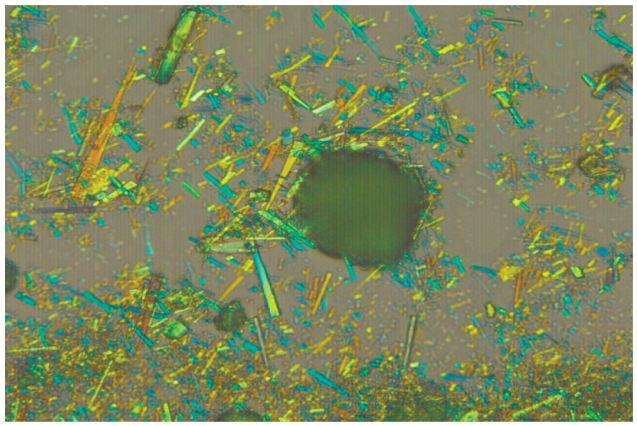


FIGURE 2. Microscope photo of crystal **12**.

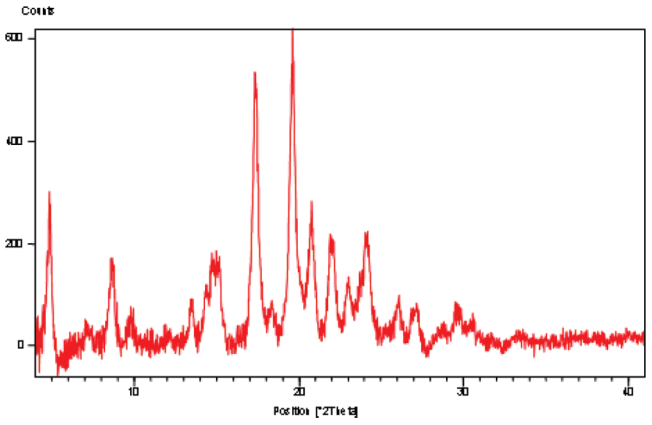


FIGURE 3. Powder X-ray diffraction pattern of crystal **12**.

(16) Presumably with water present in the solvent or in the air.
(17) Petyunin, G. P. *Farm. Zh.* (*Kiev*) **1980**, *3*, 40–42.

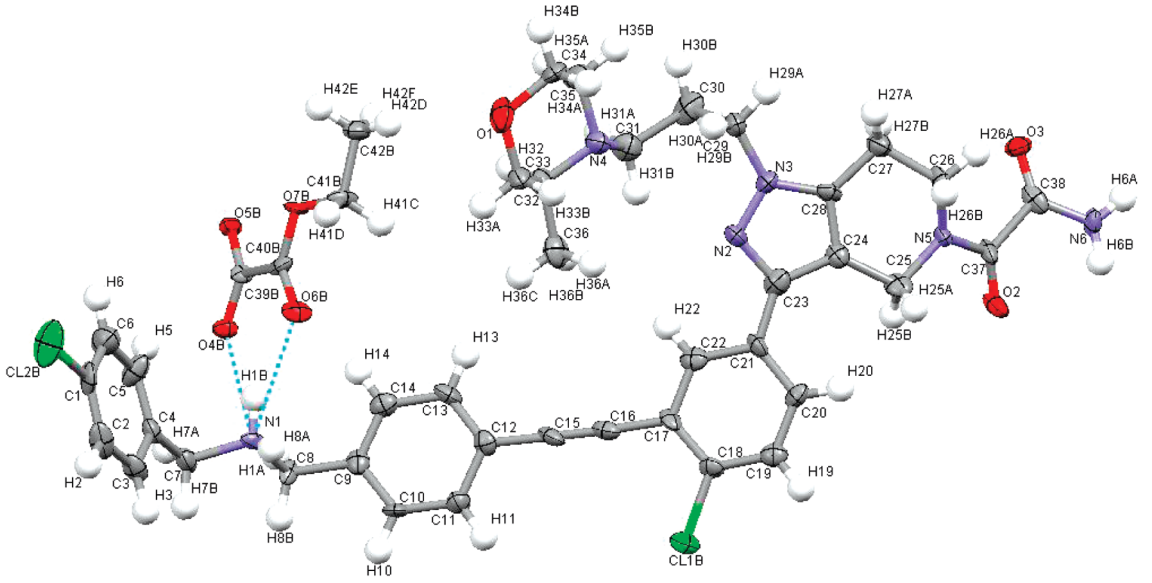


FIGURE 4. Single crystal X-ray structure of **12**.

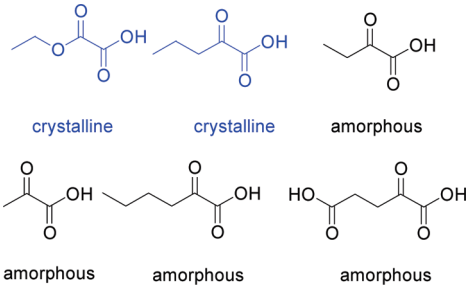


FIGURE 5. 2-Oxo-carboxylic acids.

12 could be directly used as the final API or easily converted to other suitable forms for future development.

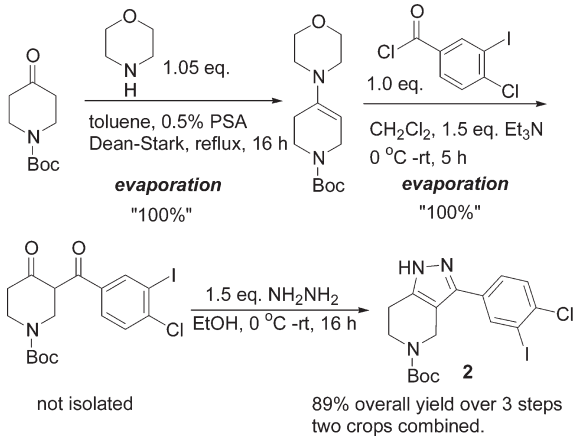
Conclusion

In summary, we devised a modular, redox economical synthesis of **1** from three readily available building blocks. This two-step synthesis is high-yielding, operationally simple, and amenable for large-scale manufacture. An unusual amine-mediated diethyloxalate hydrolysis reaction was observed. On the basis of these findings, salt formation with monoethyl oxalate provided an efficient purification method for the final drug substance.

Experimental Section

3-(4-Chloro-3-iodo-phenyl)-1,4,6,7-tetrahydro-pyrazolo[4,3-*c*]-pyridine-5-carboxylic Acid *tert*-Butyl Ester (2). Compound **2** was synthesized via a 3-step sequence in a one-pot fashion (Scheme 11). In a 3-L round-bottom flask equipped with a Dean−Stark trap, a reflux condenser, and an internal thermocouple were added sequentially *N*-Boc-piperidone (200 g, 1.0 mol, 1.0 equiv), toluene (2 L), morpholine (92 mL, 1.05 mol, 1.05 equiv), and *p*-toluenesulfonic acid (1.0 g, 0.005 mmol, 0.5% equiv). The reaction solution was refluxed under N_2 for 16 h (about 18 mL of water was collected). The solvent was evaporated to afford 4-morpholin-4-yl-3,6-dihydro-2*H*-pyridine-1-carboxylic acid *tert*-butyl ester, which was used directly in the next reaction (colorless oil, ~270 g, 100%).

SCHEME 11. Synthesis of Compound 2



The enamine prepared above was dissolved in CH_2Cl_2 (1.6 L), and then Et_3N (209 mL, 1.5 mol, 1.5 equiv) was added. At 0 °C under N_2, a solution of 4-chloro-3-iodo-benzoyl chloride (290 g, 1.0 mol, 1.0 equiv) in CH_2Cl_2 (400 mL) was added over 30 min. The ice bath was then removed, and the reaction solution was stirred at room temperature for 3 h. All volatile solvents were removed under vacuum, and the residue was redissolved in EtOH (1.5 L). At 0 °C, anhydrous NH_2NH_2 (47 mL, 1.5 mol, 1.5 equiv) was added over 30 min. (exothermic reaction). The reaction solution was stirred at room temperature for 16 h. The precipitated white solid was collected by filtration and washed with cold EtOH to afford the pure compound **2** as a white solid (333 g, 0.73 mol, >95% purity, 73%). The mother liquor was concentrated and was partitioned between CH_2Cl_2/H_2O. The organic layer was washed with water, dried over Na_2SO_4, and concentrated. The crude product was recrystallized from hot CH_3CN to give another crop of the pure material (74 g, 0.16 mmol, 16%). The combined yield was 89% over three steps: mp 173 −175 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.16 (d, J = 1.8 Hz, 1H), 7.65 (d, J = 8.3 Hz, 1H), 7.58 (s, 1H), 4.56 (s, 2H), 3.63 (t, J = 5.8 Hz, 2H), 2.70 (t, J = 5.8 Hz, 2H), 1.42 (s, 9H). ^{13}C NMR (151 MHz, DMSO) δ 154.0, 136.6, 135.9, 129.6, 126.9, 110.1, 99.4, 79.1, 63.1, 42.5, 40.5, 27.9. (Due to the tautomeric nature of

this compound, a few carbon signals were undetectable under standard ^{13}C NMR conditions even at a 50 mg/mL concentration.) IR (neat, cm^{-1}): 3239 (m), 2978 (w), 1650 (s), 1428 (s). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{17}H_{19}N_3O_2ClI$ 460.0283, found 460.0265.

3-(4-Chloro-3-iodo-phenyl)-4,5,6,7-tetrahydro-1*H*-pyrazolo-[4,3-*c*]pyridine (8). In a 3-L round-bottom flask equipped with mechanical stirring and an internal thermocouple, compound **2** (260 g, 0.57 mol, 1.0 equiv) was suspended in CH_2Cl_2 (750 mL). TFA (250 mL) was added at room temperature over 20 min to form a clear solution. The reaction mixture was stirred at room temperature for 16 h. A lot of the TFA salt of compound **8** precipitated as a white solid. Water (2 L) was added, and then saturated NaOH aqueous solution was added to adjust to pH > 12. The mixture was stirred for 3 h. The white solid precipitated was collected by filtration, washed with water and dried in a vacuum oven to provide **8** as a white solid (205 g, 0.57 mol, 100%). This material was used in the next reaction without further purification. The compound was characterized as the TFA salt: mp 180 −182 °C. ^1H NMR (600 MHz, DMSO) δ 12.77 (s, 1H), 9.32 (s, 2H), 8.16 (d, J = 2.1 Hz, 1H), 7.66 (d, J = 8.3 Hz, 1H), 7.59 (dd, J = 8.3, 2.1 Hz, 1H), 4.40 (s, 2H), 3.43 (d, J = 5.3 Hz, 2H), 2.97 (t, J = 6.1 Hz, 2H). ^{13}C NMR (151 MHz, DMSO) δ 158.5 (q, J_{C-F} = 35.4 Hz, 1C), 140.9, 138.7, 136.9, 136.5, 132.0, 129.6, 127.3, 115.9 (q, J_{C-F} = 293.0 Hz, 1C), 106.1, 99.5, 40.5, 40.0, 19.0. IR (neat, cm^{-1}): 3231 (w), 3067 (w), 1754 (w), 1659 (s), 1437 (s). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{12}H_{11}N_3ClI$ 359.9759, found 359.9751.

2-[3-(4-Chloro-3-iodo-phenyl)-1,4,6,7-tetrahydro-pyrazolo-[4,3-*c*]pyridin-5-yl]-2-oxo-acetamide (9). In a 5-L flask equipped with mechanical stirring, CDI (110.5 g, 0.68 mol, 1.2 equiv) was suspended in DMF (1.5 L) and then oxalamic acid (60.7 g, 0.68 mol, 1.2 equiv) was added. After stirring at room temperature for 3 h, a nearly clear solution was formed. Compound **8** (205 g, 0.57 mol, 1.0 equiv) was added as a solid over 10 min. The reaction was complete in 30 min. Water (2.5 L) was added slowly over 2 h and stirred at room temperature overnight. The precipitated white solid was collected by filtration, washed with water and dried in a vacuum oven to provide **9** (231 g, 0.53 mol, 95%). This material was used in the next reaction without further purification: mp 235−237 °C. Two sets of NMR peaks were observed due to the tautomeric nature of this compound: ^1H NMR (600 MHz, DMSO) δ 13.02 (s, 1H), 8.20 (d, J = 2.0 Hz, 0.7H), 8.15 (s, 0.7H), 8.13 (dd, J = 2.0 Hz, 0.3H), 8.11 (s, 0.3H), 7.77 (s, 0.7H), 7.72 (s, 0.3H), 7.67 (s, 0.3H), 7.66 (s, 0.7H), 7.61 (dd, J = 8.4, 2.0 Hz, 0.7H), 7.50 (dd, J = 8.3, 2.0 Hz, 0.3H), 4.72 (s, 0.6H), 4.70 (s, 1.4H), 3.81 (t, J = 5.9 Hz, 0.6H), 3.73 (t, J = 5.7 Hz, 1.4H), 2.82 (t, J = 5.6 Hz, 1H), 2.75 (dd, J = 10.5, 4.6 Hz, 1H); ^{13}C NMR (151 MHz, DMSO) δ 165.6, 165.3, 164.5, 163.8, 136.7, 136.6, 136.1, 136.0, 129.6, 127.0, 126.9, 109.6, 109.2, 99.45, 99.37, 42.69, 42.62, 22.5, 21.4. (Due to the tautomeric nature of this compound, a few carbon signals were undetectable under standard ^{13}C NMR conditions even at a 50 mg/mL concentration.) IR (neat, cm^{-1}): 3333 (w), 3177 (w), 1671 (s), 1651 (s). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{14}H_{12}N_4O_2ClI$ 430.9766, found 430.9752.

4-(3-Chloro-propyl)-3-methyl-morpholine (3). In a 25-mL round-bottom flask with a magnetic stir bar, 3-methyl-morpholine (1 g, 9.9 mmol, 1.0 equiv) and 1-bromo-3-chloro-propane (3.1 g, 19.8 mmol, 2.0 equiv) were dissolved in THF (5 mL). NaH (0.79 g, 60 wt % in mineral oil, 2.0 equiv) was added in 2 portions. Mild bubbling occurred during the addition. The reaction slurry was heated to 65 °C for 18 h. GC/MS analysis indicated the completion of the reaction. The reaction solution was carefully quenched with addition of ice−water (40 mL) and then extracted with EtOAc (3 × 15 mL). The organic layers were combined and extracted with 1 mol/L HCl (40 mL).[18] The aqueous layer that contained compound **3** was cooled in an ice bath and basified with slow addition of NaOH pellets to pH 9−10, which was then extracted with EtOAc (3 × 15 mL). The combined organic layer was dried with Na_2SO_4 and concentrated to afford the title compound as a colorless oil (1.3 g, 7.4 mmol, 74%): ^1H NMR (500 MHz, $CDCl_3$) δ 3.78 (dtd, J = 11.3, 3.1, 0.9 Hz, 1H), 3.70−3.62 (m, 2H), 3.60 (t, J = 6.4 Hz, 2H), 3.23 (dd, J = 11.1, 9.0 Hz, 1H), 2.90 (dt, J = 13.0, 7.6 Hz, 1H), 2.72 (dt, J = 11.7, 2.8 Hz, 1H), 2.51−2.37 (m, 1H), 2.36 2.26 (m, 2H), 1.99−1.83 (m, 2H), 0.98 (d, J = 6.3 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 73.0, 67.4, 55.2, 51.3, 50.5, 43.1, 29.3, 14.1. HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_8H_{17}ClNO$ 178.0993, found 178.0992.

2-{3-(4-Chloro-3-iodo-phenyl)-1-[3-(3-methyl-morpholin-4-yl)-propyl]-1,4,6,7-tetrahydro-pyrazolo[4,3-*c*]pyridin-5-yl}-2-oxo-acetamide (10). In a 5-L flask equipped with mechanical stirring and an internal thermocouple, compound **9** (190 g, 0.44 mol, 1.0 equiv) was dissolved in DMF (1.8 L). Cs_2CO_3 (180 g, 0.55 mol, 1.25 equiv) and compound **3** (86 g, 0.48 mol, 1.1 equiv) were added sequentially. The reaction mixture was then stirred at 50 °C under N_2 for 4 h and cooled to room temperature. Water (3.5 L) was added slowly over 1 h, and the suspension was stirred at room temperature overnight. The white solid precipitated was collected by filtration, washed with water, and dried in a vacuum oven. The crude compound was triturated from hot EtOH (~1.5 L) to provide pure **10** as a white solid (176 g, 0.31 mol, 70%):[19] mp 189 −190 °C. (Two sets of NMR peaks were observed due to the tautomeric nature of this compound.) ^1H NMR (600 MHz, DMSO) δ 8.19 (d, J = 2.0 Hz, 0.7H), 8.14 (s, 0.7H), 8.13 (d, J = 2.1 Hz, 0.3H), 8.08 (s, 0.3H), 7.77 (s, 0.7H), 7.72 (s, 0.3H), 7.65 (s, 0.3H), 7.64 (s, 0.7H), 7.59 (dd, J = 8.4, 2.0 Hz, 0.7H), 7.48 (dd, J = 8.3, 2.1 Hz, 0.3H), 4.71 (s, 0.6H), 4.68 (s, 1.4H), 4.12−3.97 (m, 2H), 3.89−3.77 (m, 0.7H), 3.78−3.70 (m, 1.3H), 3.78−3.62 (m, 1H), 3.56 (dt, J = 10.9, 3.0 Hz, 1H), 3.47 (dt, J = 14.9, 2.8 Hz, 1H), 3.14−3.05 (m, 1H), 2.96−2.74 (m, 2H), 2.74−2.63 (m, 2H), 2.34−2.23 (m, 1H), 2.12 (ddd, J = 9.4, 5.7, 3.1 Hz, 2H), 1.92 (dq, J = 13.9, 7.1 Hz, 2H), 0.84 (d, J = 6.3 Hz, 3H); ^{13}C NMR (151 MHz, DMSO) δ 165.5, 165.1, 164.3, 163.7, 142.0, 141.7, 138.3, 137.9, 136.7, 136.6, 135.9, 135.8, 133.7, 133.6, 129.6, 126.9, 126.8, 110.2, 109.7, 99.35, 99.27, 72.0, 72.0, 66.5, 54.6, 50.2, 49.6, 42.6, 42.3, 38.0, 37.7, 25.8, 25.7, 22.1, 20.9, 13.3. IR (neat, cm^{-1}): 3328 (w), 3061 (w), 1694 (s), 1669 (s), 1637 (s), 1433 (s). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{22}H_{27}N_5O_3ClI$ 572.0920, found 572.0918.

(4-Chloro-benzyl)-(4-ethynyl-benzyl)-amine (4). 4-Trimethylsilanylethynyl-benzaldehyde (10 g, 49 mmol, 1.0 equiv) was dissolved in MeOH (200 mL), and 4-chlorobenzylamine (7.4 g, 52 mmol, 1.05 equiv) was added. After stirring at room temperature overnight, $NaBH_4$ (1.9 g, 49 mmol, 1.0 equiv) was added slowly. After stirring at room temperature for 1 h, 1 mol/L HCl aqueous solution was slowly added to quench the unreacted $NaBH_4$. MeOH was evaporated, and the residue was partitioned between CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. The organic layer was separated, dried over $MgSO_4$ and concentrated. The crude compound was passed through a short pad of silica gel to remove the baseline impurities with EtOAc/hexanes as eluent to provide **4** as a light yellow oil (11 g, 43 mmol, 90%): R_f = 0.54 (1:1 EtOAc/hexanes). ^1H NMR (600 MHz, $CDCl_3$) δ 7.47−7.44 (m, 2H), 7.32−7.26 (m, 6H), 4.70 (s, 0H), 3.78 (s, 2H), 3.75 (s, 2H), 3.06 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 141.1, 138.6, 132.7, 132.2, 129.5, 128.5, 128.0, 120.7, 83.6, 77.0, 52.7, 52.3. IR (neat, cm^{-1}): 3294 (m), 2911 (w), 2827 (w), 2106 (w), 1487 (s). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{16}H_{14}NCl$ 256.0888, found 256.0871.

(18) The small amount of unreacted 3-methyl-morpholine remained in the organic layer.

(19) The undesired regioisomer was completely rejected in this process.

2-{3-(4-Chloro-3-{4-[(4-chloro-benzylamino)-methyl]-phenyl-ethynyl}-phenyl)-1-[3-(3-methyl-morpholin-4-yl)-propyl]-1,4,6,7-tetrahydro-pyrazolo[4,3-*c*]pyridin-5-yl}-2-oxo-acetamide (1, free base). In a 5-L flask equipped with mechanical stirring and an internal thermocouple were added compound **10** (120 g, 0.21 mol, 1.0 equiv), compound **4** (56.3 g, 0.22 mol, 1.0 equiv), DMF (1.5 L), and Et_3N (120 mL, 0.84 mol, 4.0 equiv) sequentially. A stream of N_2 was bubbled into the solution for 15 min. A mixture of $Pd(PPh_3)_2Cl_2$ (0.37 g, 0.5 mmol, 0.0025 equiv) and CuI (0.2 g, 1.0 mmol, 0.005 equiv) was added as a solid under N_2. The solution was degassed with N_2 for another 10 min. The reaction solution was stirred at 50 °C overnight. The reaction solution was cooled to room temperature, and water (2 L) was added upon stirring. The liquid layer was decanted, and the oily precipitate was partitioned between EtOAc (2 L) and water (1 L) and saturated $NaHCO_3$ (500 mL). The organic layer was separated, dried over $MgSO_4$, and concentrated to provide the crude material as a foamy yellow solid (145 g, ~85% purity based on HPLC analysis). The crude material was purified on silica gel with 2 mol/L NH_3 in $MeOH/CH_2Cl_2$ as eluent to provide the drug substance in >98% purity:[20] mp 61−64 °C. Two sets of NMR peaks due to the tautomeric nature of this compound: ^{1}H NMR (600 MHz, DMSO) δ 8.17 (s, 0.7H), 8.12 (s, 0.3H), 7.90 (d, J = 1.9 Hz, 0.7H), 7.84 (d, J = 2.1 Hz, 0.3H), 7.81 (s, 0.7H), 7.75 (s, 0.3H), 7.67 (s, 0.3H), 7.65 (s, 0.7H), 7.63 (dd, J = 8.5, 2.0 Hz, 0.7H), 7.58 −7.54 (m, 2H), 7.52 (dd, J = 8.5, 2.1 Hz, 0.3H), 7.43 (d, J = 8.1 Hz, 2H), 7.40 −7.32 (s, 4H), 4.78 (s, 0.6H), 4.74 (s, 1.4H), 4.12−3.99 (m, 2H), 3.91−3.75 (m, 2H), 3.71 (s, 2H), 3.68−3.62 (m, 3H), 3.60−3.53 (m, 1H), 3.52−3.44 (m, 1H), 3.11 (dd, J = 10.8, 8.8 Hz, 1H), 2.87 (ddd, J = 33.1, 18.1, 10.4 Hz, 3H), 2.74−2.64 (m, 2H), 2.29 (dd, J = 10.0, 4.3 Hz, 1H), 2.19−2.06 (m, 2H), 1.94 (dt, J = 13.6, 6.8 Hz, 2H), 0.84 (d, J = 6.3 Hz, 3H); ^{13}C NMR (151 MHz, DMSO) δ 165.5, 165.2, 164.3, 163.7, 142.7, 142.43, 142.31, 142.28, 139.7, 138.2, 137.9, 133.17, 133.09, 132.63, 132.60, 131.3, 131.0, 129.82, 129.78, 129.73, 129.6, 128.2, 128.0, 127.15, 127.05, 122.50, 122.46, 119.78, 119.75, 110.2, 109.7, 94.66, 94.64, 85.44, 85.39, 72.07, 72.04, 66.6, 54.6, 51.7, 51.3, 50.2, 49.7, 46.3, 42.8, 42.4, 38.2, 37.8, 25.84, 25.79, 22.2, 21.0, 13.3. IR (neat, cm^{-1}): 2956 (w), 2843 (w), 2219 (w), 1694 (s), 1640 (s), 1452 (m). HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{38}H_{40}N_6O_3Cl_2$ 699.2612, found 699.2624.

Compound 1, L-Tartrate. The free base **1** (100 g, 0.14 mol, 1.0 equiv, >98% purity) was dissolved in EtOAc (1.5 L). L-Tartaric acid (21.5, 0.14 mol, 1.0 equiv) dissolved in MeOH (150 mL) was added over 1 h. After stirring at room temperature for an additional 1 h, the precipitated solid was collected by filtration and washed with EtOAc under N_2.[21] This amorphous material contained ~8 wt % residue solvents (EtOAc and MeOH) based on ^{1}H NMR integration.[22] Azeotropic distillation of the aqueous solution (~500 mL water) at 50 °C under vacuum successfully lowered the organic solvent residue to <0.5 wt %. Subsequent lyophilization[23] of the aqueous solution afforded the pure **1**, L-tartrate salt as an amorphous white powder (97 g, 80%): mp 80−83 °C. Two sets of NMR peaks due to the tautomeric nature of this compound: ^{1}H NMR (600 MHz, DMSO) δ 8.16 (s, 0.7H), 8.11 (s, 0.3H), 7.89 (d, J = 1.9 Hz, 0.7H), 7.84 (d, J = 2.0 Hz, 0.3H), 7.79 (s, 0.7H), 7.73 (s, 0.3H), 7.71−7.59 (m, 3.7H), 7.56 −7.51 (m, 2.3H), 7.50−7.40 (m, 4H), 4.77 (s, 0.6H), 4.73 (s, 1.4H), 4.20 (s, 3H), 4.12−4.02 (m, 2H), 3.98 (s, 2H), 3.95 (s, 2H), 3.90−3.73 (m, 2H), 3.74−3.66 (m, 1H), 3.63−3.56 (m, 1H), 3.55 3.46 (m, 1H), 3.18 −3.10 (m, 1H), 2.99−2.68 (m, 4H), 2.45−2.35 (m, 1H), 2.26−2.16 (m, 2H), 2.04−1.90 (m, 2H), 0.88 (d, J = 6.3 Hz, 3H); ^{13}C NMR (151 MHz, DMSO) δ 173.7, 165.5, 165.1, 164.3, 163.7, 142.7, 142.4, 138.3, 138.0, 137.45, 137.38, 135.04, 135.00, 133.22, 133.14, 132.61, 132.58, 132.4, 131.5, 130.9, 129.87, 129.82, 129.76, 129.4, 128.2, 127.3, 127.2, 122.30, 122.25, 120.98, 120.93, 110.2, 109.7, 94.2, 85.99, 85.94, 71.9, 71.6, 66.2, 54.7, 50.4, 50.1, 49.6, 46.2, 42.7, 42.3, 38.2, 37.8, 25.5, 25.5, 22.1, 20.9, 13.0. IR (neat, cm^{-1}): 3009 (w), 2985 (w), 1691 (s), 1634 (s), 1592 (s), 1455 (m).

Compound 1, Monoethyl Oxalate (12). Two procedures were used to prepare the monoethyl oxalate salt. (A) Compound **1** (free base, 100 mg) was dissolved in 4 mL of CH_3CN at 50 °C. Diethyl oxalate was added dropwise until the solution became cloudy. The solution was left at 50 °C without stirring under N_2 for 16 h. The precipitated crystalline solid was collected by filtration and washed with CH_3CN to afford the title salt. (B) Compound **1** (free base, 1.5 g, 1.0 equiv) was dissolved in CH_3CN at 50 °C and then monoethyl oxalate (0.25 g, 1.0 equiv) was added. The solution was stirred at 50 °C for 16 h and cooled to room temperature. The precipitated crystalline solid was collected by filtration and washed with CH_3CN (1.27 g, 85%). Identical salts were obtained with both methods: mp 152.9 °C. Two sets of NMR peaks due to the tautomeric nature of this compound: ^{1}H NMR (600 MHz, DMSO) δ 8.16 (s, 0.7H), 8.12 (s, 0.3H), 7.90 (s, 0.7H), 7.84 (s, 0.3H), 7.80 (s, 0.7H), 7.73 (s, 0.3H), 7.71−7.52 (m, 6H), 7.53 −7.42 (m, 4H), 4.77 (s, 0.6H), 4.73 (s, 1.4H), 4.13 −4.04 (m, 6H), 4.02 (dd, J = 14.2, 7.1 Hz, 2H), 3.94−3.34 (m, 7H), 3.25−3.15 (m, 1H), 3.03−2.72 (m, 4H), 2.34 (br. s, 2H), 2.00 (br. s, 2H), 1.19 (t, J = 7.1 Hz, 3H), 0.93 (d, J = 6.3 Hz, 3H); ^{13}C NMR (151 MHz, DMSO) δ 165.9, 165.5, 165.2, 164.4, 163.8, 163.1, 142.8, 142.5, 138.4, 138.0, 133.3, 133.2, 133.0, 132.7, 132.6, 131.6, 131.4, 129.94, 129.88, 128.4, 127.5, 127.4, 122.3, 122.2, 121.53, 121.49, 110.3, 109.8, 94.1, 86.3, 71.3, 65.9, 62.6, 59.0, 55.0, 50.0, 49.64, 49.61, 46.2, 42.7, 42.4, 38.2, 37.8, 25.3, 22.2, 21.0, 14.1, 12.8. IR (neat, cm^{-1}): 3457 (w), 3009 (w), 2986 (w), 1724 (m), 1694 (s), 1644 (s), 1454 (m).

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Supporting Information Available: General experimental methods, ^{1}H and ^{13}C NMR spectra for compounds **1**−**4**, **8**−**10**, and **12** and chiral HPLC chromatograms of compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

(20) The recovery was ~60%. We also noticed slow decomposition of this material on silica gel during the chromatography process.

(21) The material is highly hygroscopic. Without N_2 flow, it gummed up upon filtration.

(22) Removal of the residue solvents was extremely difficult. High vacuum at 50 °C for a few days failed to reduce the residue solvent level. Trituration in various solvents (EtOH, THF, 2-methyl-THF, ^{i}PrOAc, etc.) resulted in the trapping of the coming solvents.

(23) Direct lyophilization was not effective on removing the residue organic solvents.