ORGANIC PROCESS RESEARCH & DEVELOPMENT
OPR&D

pubs.acs.org/OPRD

Article

Practical and Scalable Manufacturing Process for Plasma Kallikrein Inhibitor ASP5069

Shun Hirasawa* and Yoshinori Kohmura

Cite This: https://dx.doi.org/10.1021/acs.oprd.0c00291

Read Online

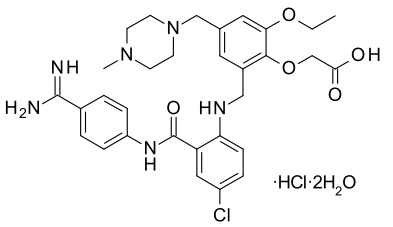
ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The plasma kallikrein inhibitor ASP5069 is a promising drug candidate for the treatment of edema and hematoma and for the prevention of bleeding during surgery. Here, we report the development of a practical and scalable process for manufacturing ASP5069 that features a convergent synthetic approach, suppression of impurity formation, effective purification of the amine compound by extraction, improved reproducibility of the reductive amination reaction, and a drying process for the dihydrate form. This process was successfully used to prepare >100 g of ASP5069.

KEYWORDS: *ASP5069, plasma kallikrein inhibitor, Mannich reaction, reductive amination, dihydrate form*

INTRODUCTION

In the kallikrein−kinin system, the serine protease plasma kallikrein cleaves high-molecular-weight kininogen to release the active peptide bradykinin.[1] Bradykinin is a vasodilator that contributes to the activation of inflammation and production of pain and may mediate hereditary angioedema (HAE).[2] Additionally, plasma kallikrein is implicated in the expansion of chronic subdural hematoma[3] and hyperglycemia-induced cerebral hematoma,[4] and fibrinolysis.[5] The plasma kallikrein inhibitor ASP5069 (**1**·HCl·2H_2O)—a carboxylic acid derivative having three aromatic rings, amidine, and piperazine (Figure 1)—is therefore a promising drug candidate for the treatment of edema and hematoma and for the prevention of bleeding during surgery.[6]



ASP5069 (1·HCl·2H_2O)

Figure 1. Structure of ASP5069 (**1**·HCl·2H_2O).

Scheme 1 shows the discovery route for **1**·TFA,[6] which comprises a convergent synthetic approach featuring reductive amination of aldehyde **3** and aniline **5**. Mannich reaction of **2** with methylpiperazine and formaldehyde, followed by alkylation with *tert*-butyl bromoacetate, yielded aldehyde **3**. Aniline **5** was synthesized from **4** using the following three-step sequence: Boc protection, amidation with 5-chloro-2-nitorobenzoic acid, and hydrogenation of the nitro group. Reductive amination of **3** and **5** was performed using sodium triacetoxyborohydride in acetic acid to give **6**. Finally, deprotection of Boc and *tert*-butyl groups by trifluoroacetic acid (TFA) gave **1**·TFA.

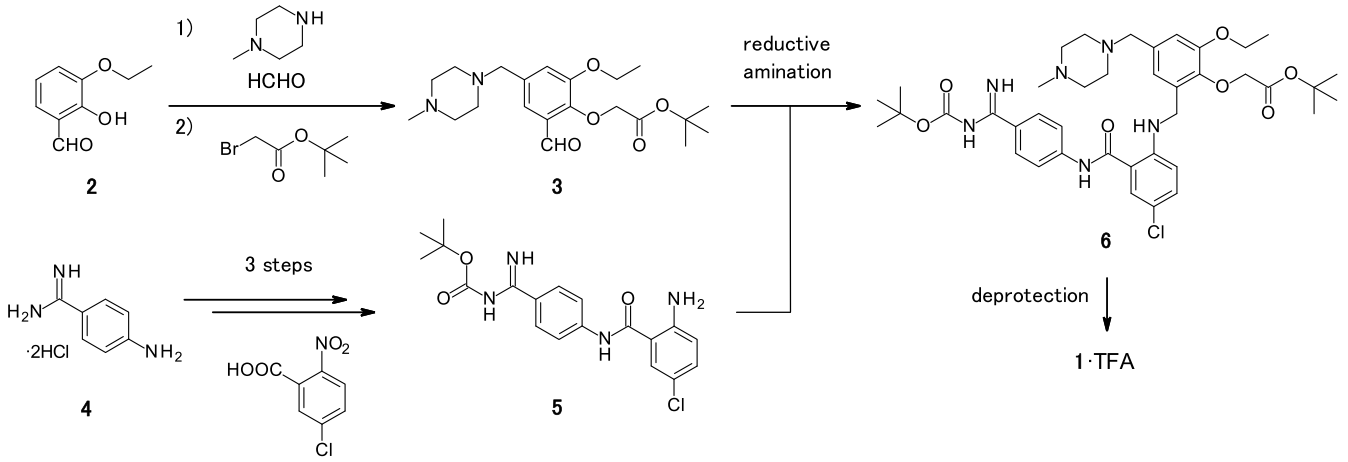
After salt screening of **1** for the development form, monohydrochloride dihydrate (**1**·HCl·2H_2O) was selected. Because multi-kilograms of **1**·HCl·2H_2O are required for preclinical and clinical studies, the development of a scalable and practical preparation process was needed. To determine the scale-up synthetic route, we evaluated two alternative approaches to obtain **6** (Scheme 2). Route A involved reductive amination of **3** and 5-chloroanthranilic acid, followed by amidation with the aniline **4**, to give the intermediate **6**. However, the reductive amination conditions did not give the compound **7**. Route B was possibly a one-step shorter synthetic method for obtaining the compound **5** because the coupling of anthranilic acid[7] or isatoic anhydride[7d,8] with various amines is widely known. In our case, however, the target **5** was not obtained under a variety of conditions. Thus, the failure of the alternative approaches and the convergent and simple nature of the discovery approach suggested that the latter may be suitable for scale-up. However, several issues within the discovery procedure would make it difficult to manufacture multi-kilograms of **1**·HCl·2H_2O, including the use of column chromatography purification, poor reproducibility, and safety and environmental concerns. The challenges we faced in solving these problems are described in detail below.

RESULTS AND DISCUSSION

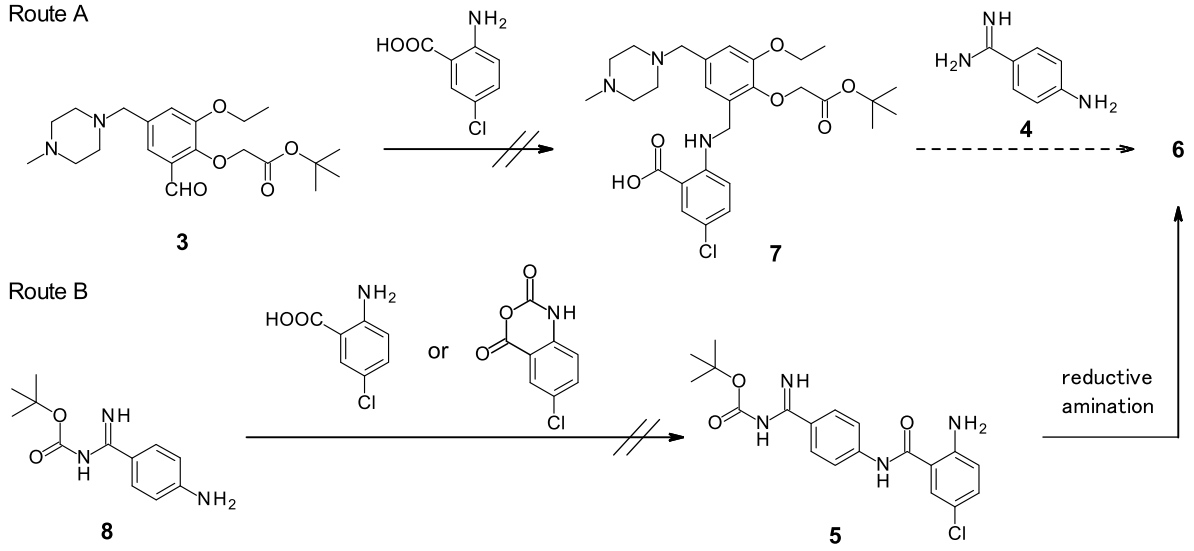
Mannich Reaction and Alkylation. In the Mannich reaction of 3-ethoxysalicylaldehyde (**2**) with methylpiperazine and formaldehyde in ethanol solvent under discovery

Received: June 22, 2020

Scheme 1. Discovery Route for 1·TFA (ref 6)



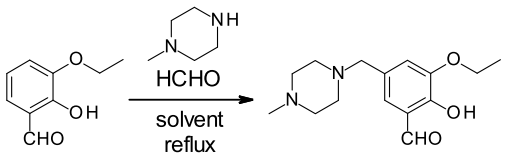
Scheme 2. Alternative Approaches to Obtain 6



conditions, 3.4 HPLC area percent (A %) of an unknown impurity was observed as a major impurity (Table 1, entry 1). Given that impurities derived from the unknown impurity remained in the product of the subsequent step, minimizing the formation of the unknown impurity was required.

We predicted that the nucleophilicity of the ethanol solvent may generate impurities. When the more sterically hindered solvent 2-propanol was used, the corresponding impurity was reduced to a very low level (0.7 A %) (entry 2). Because the purity of the resulting Mannich product **9** was sufficiently high without crystallization isolation, the reaction was telescoped to the next alkylation reaction.

Table 1. Solvent Study of Mannich Reaction



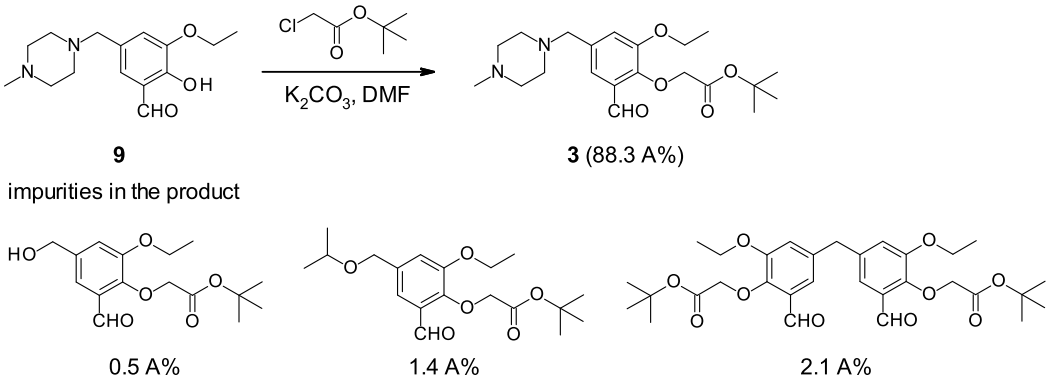
		HPLC A %		
entry	solvent	start 2	target 9	major impurity (HPLC retention time)[a]
1	ethanol	0.6	92.7	3.4 (9.5 min)
2	2-propanol	0.1	97.0	0.7 (14.9 min)

[a]HPLC method A.

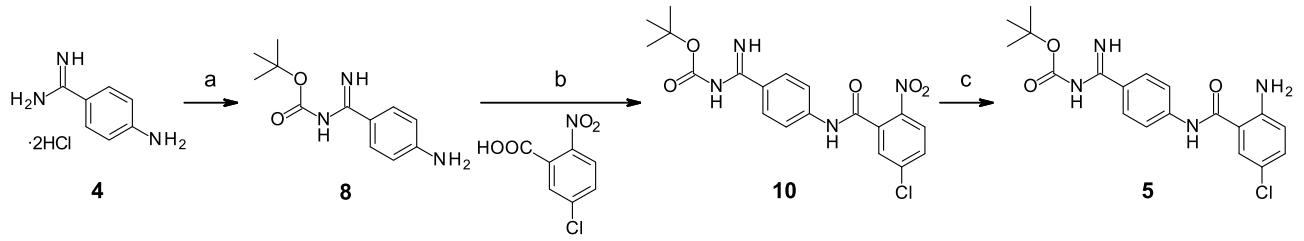
The yield of the target **3** was very low under discovery conditions because of the relatively high levels of an impurity (approximately 30 A %) formed from the alkylation of **9** with *tert*-butyl bromoacetate, and column chromatography purification was required to isolate **3**. We predicted that the impurity was a quaternary ammonium compound obtained from overalkylation of the piperazine nitrogen. To avoid the overalkylation, first, we attempted to inhibit the *N*-nucleophilicity by mixing the Lewis acid $ZnBr_2$ with **9** to form a metal complex with the piperazine moiety. Subsequent addition of *tert*-butyl bromoacetate yielded **3** with a reduced level of the overalkylated impurity (8 A %). While impurity formation was suppressed, there was room for improvement. Second, we attempted to use the harder electrophile *tert*-butyl chloroacetate[9] without a Lewis acid. As a result, the quaternary ammonium did not form and the two-step yield improved from 53% in the discovery method to 79%, as expected. However, small amounts of impurities derived from the Mannich

https://dx.doi.org/10.1021/acs.oprd.0c00291
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

Scheme 3. Alkylation of 9 with *tert*-Butyl Chloroacetate

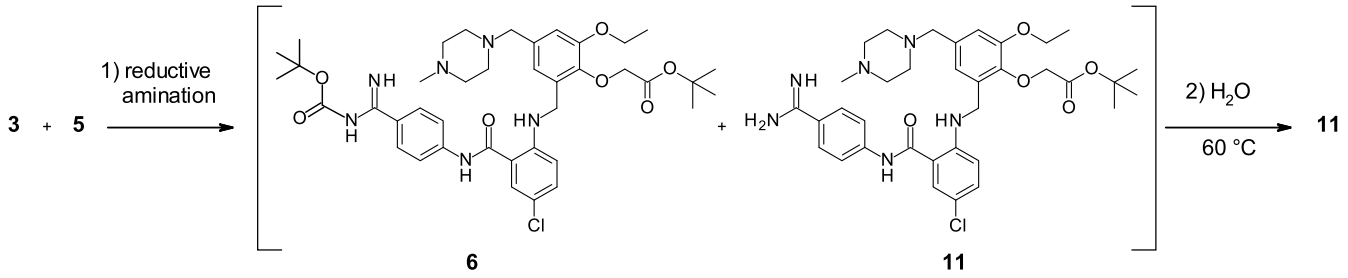


Scheme 4. Synthesis of Aniline 5 from Starting Material 4[a]



[a]Discovery conditions:[6] (a) 4 M aq NaOH, Boc_2O, 1,4-dioxane, 0 °C, 7 h, 89%; (b) 5-chloro-2-nitrobenzoic acid, $(COCl)_2$, DMF, DCE, rt, 3 h; concentration; then **8**, pyridine, MeCN, rt, 18 h, 76%; (c) H_2, Raney nickel, 1,4-dioxane, rt, 2 d, 96%. Scale-up conditions: (a) 5 M aq NaOH, Boc_2O, THF, 5 °C, 3 h, 97%; (b) 5-chloro-2-nitrobenzoic acid, $SOCl_2$, toluene, 100 °C, 6 h; concentration to 2 vol, 60 °C; then **8**, *i*-Pr_2NEt, THF, −10 °C, 2 h, 87%; (c) H_2, Raney nickel, DMF, H_2O, 30 °C, 16 h, 87%.

Scheme 5. Reductive Amination and Boc Deprotection to Converge to Amidine 11



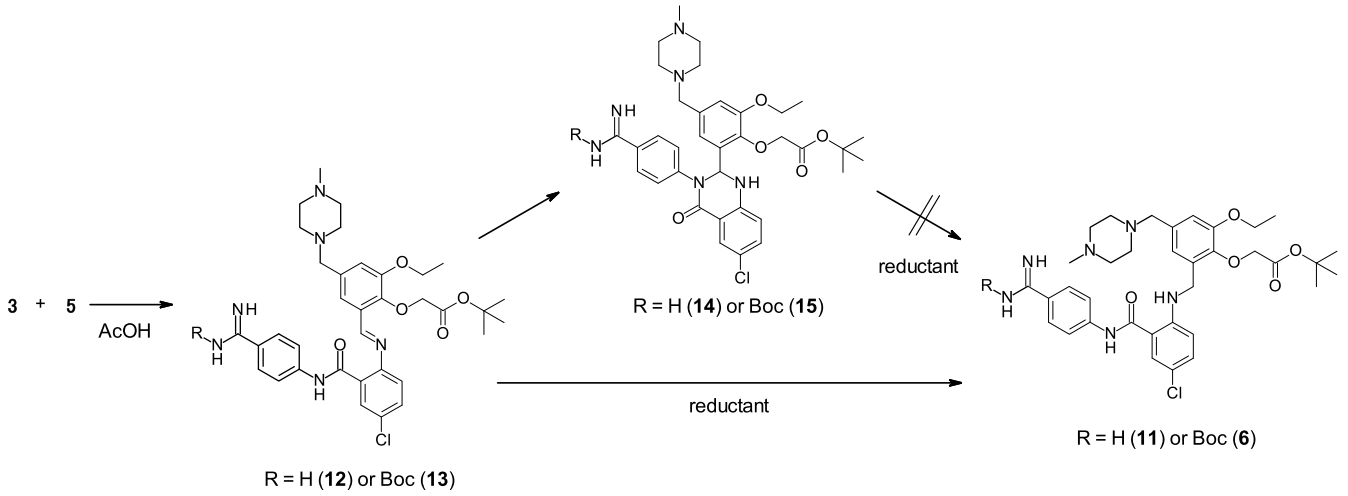
reaction contaminated the product, as shown in Scheme 3. These neutral impurities could be removed to the organic layer by extraction of the target **3** with an acidic aqueous solution. Subsequently, basification of the aqueous extract and extraction of **3** with an organic solvent (isopropyl acetate (*i*-PrOAc)) gave pure **3** in high quality (98.7 A %). This Mannich reaction−alkylation sequence was successfully performed on a 100 g scale.

Preparation of 5. Aniline **5** was prepared from amidine **4** (Scheme 4). The discovery method for **5** required improvements to safety and environment-related processes. In the Boc protection of **4**, use of 1,4-dioxane needed to be eliminated for scale-up because of its suspected carcinogenicity.[10] Tetrahydrofuran (THF) was found to be a good alternative and Boc-amidine **8** was obtained in 97% yield. In the discovery method, oxalyl chloride was used to activate 5-chloro-2-nitorobenzoic acid and the subsequent amidation was performed in 1,2-dichloroethane (DCE). Because oxalyl chloride induces the formation of carbon monoxide, it needed to be avoided. DCE is inappropriate for scale-up because of potential mutagenic and environmental impact.[10] This problem was solved by replacing oxalyl chloride with thionyl chloride and DCE with toluene, which gave amide **10** in 87% yield. A previous report suggested that isolated 2-nitrobenzoyl chloride decomposes violently in the presence of heat, suggesting the need to use a diluted solution.[11] In the case of our 5-chloro-2-nitorobenzoyl chloride intermediate, exothermic decomposition was observed at 170 °C using differential scanning calorimetry. We concluded that 5-chloro-2-nitorobenzoyl chloride could be safely concentrated in toluene solution by performing the concentration process at a minimum of 100 °C below the exothermic temperature and setting the concentration end point to 2 vol. Finally, in the reduction of the nitro moiety using hydrogen and Raney nickel, 1,4-dioxane was replaced with *N*,*N*-dimethylformamide (DMF), and aniline **5** was obtained in 87% yield.

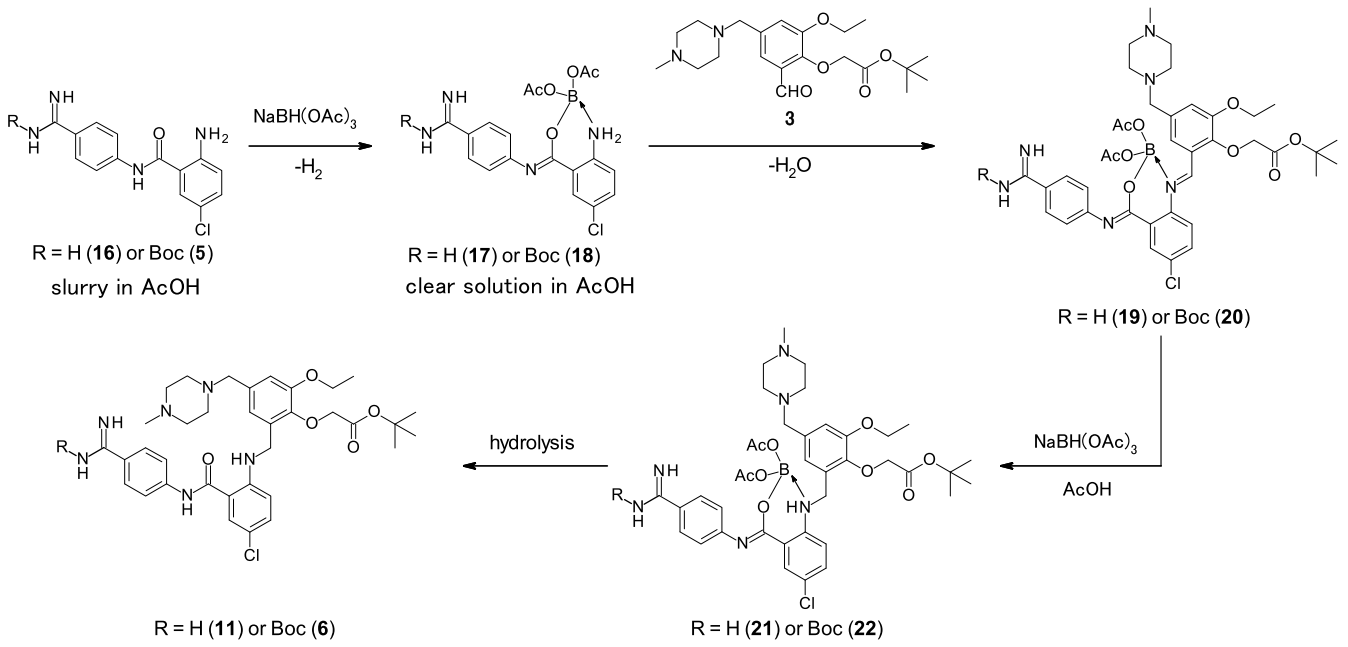
Reductive Amination. In reductive amination of aniline **5** and aldehyde **3**, the discovery method had poor reproducibility because of the uncontrolled formation of impurities, Boc-deprotected compound **11** and the possible cyclic aminal **14**/**15**.[12] Column chromatography purification was required to remove the impurities.

https://dx.doi.org/10.1021/acs.oprd.0c00291
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

Scheme 6. Formation of 14/15



Scheme 7. Potential Reaction Mechanism of Reductive Amination



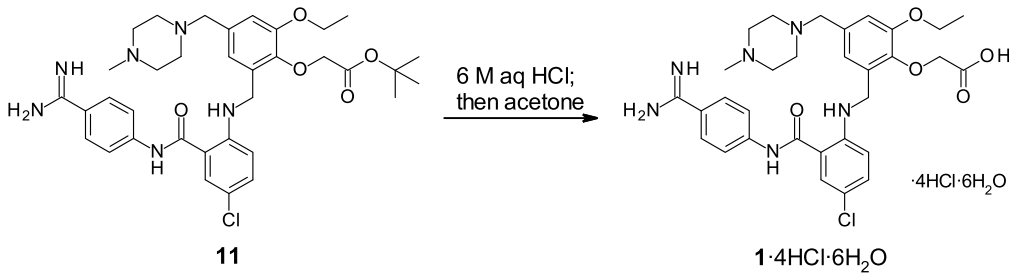
Water caused Boc deprotection, and impurity **11** gradually increased under the reaction conditions. Because water is a byproduct of imine formation, it is impossible to inhibit the water formation and Boc deprotection. Hence, we attempted to converge to Boc-deprotected compound **11** without isolating **6**. After the reductive amination reaction, addition of water to the mixture and stirring at 60 °C led to successful conversion of **6** to **11** (Scheme 5).

In the discovery procedure, the initial mixing of aniline **5** and aldehyde **3** caused the formation of **14/15** by intramolecular cyclization of the imine intermediate **12/13**, and subsequent addition of a reductant did not lead to the conversion of **14/15** to **11/6** (Scheme 6). We predicted that addition of aldehyde **3** to the mixture of aniline **5** and reductant would induce immediate reduction of the corresponding imine intermediate **12/13** to yield the target **11/6** with suppression of formation of **14/15**. Thus, we attempted this method to change the order of the reagents added to the reaction.

As a result, when 3 equiv of sodium triacetoxyborohydride was added to a slurry of aniline **5** in acetic acid, the slurry turned into a clear solution with the generation of hydrogen gas. Thereafter, addition of the aldehyde **3** and subsequent Boc deprotection yielded the target **11** as the main product (75.7 A %), while successfully suppressing the production of **14** (0.4 A %). When the reaction involved reducing the amount of reductant from 3 equiv to 1.5 equiv, the target **11** and **14** were obtained at 29.3 A % and 52.9 A %, respectively. Based on the above results, we suggest the following potential reaction mechanism in Scheme 7: (1) at the mixing of aniline **16/5** and sodium triacetoxyborohydride, the boron complex **17/18** was formed with the generation of hydrogen, which has a much higher solubility than aniline **16/5**, leading the reaction mixture to gradually change from a slurry to a clear solution; (2) aldehyde **3** was added, and boron-complexed imine **19/20** was generated from aldehyde **3** and boron-coordinated aniline **17/18**;[13] (3) because the imine was activated by boron, it was immediately reduced by another sodium triacetoxyborohydride

https://dx.doi.org/10.1021/acs.oprd.0c00291
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

Scheme 8. *tert*-Butyl Ester Deprotection of 11



11 1·4HCl·6H$_2$O

to give **21**/**22**; and (4) the water byproduct of imine formation induced hydrolysis to give the target **11**/**6**. The theoretical need for 2 equiv of sodium triacetoxyborohydride suggested by the potential mechanism indicates that the above result of obtaining **14** at 52.9 A % in the presence of 1.5 equiv of sodium triacetoxyborohydride is reasonable.

Meanwhile, the boron complex **17**/**18**, which was observed as **16**/**5** on HPLC, was unstable under the reaction conditions and gradually decomposed. Because the melting point of acetic acid is 16.7 °C, the reaction temperature cannot be lowered. However, the use of acetic acid/isopropyl acetate as the solvent enables the temperature to be lowered to <0 °C, and the decomposition of **17**/**18** can be suppressed. Given that isopropyl acetate was the extraction solvent for the purification of **3**, the isopropyl acetate solution of **3** can be utilized without solvent switching.

After Boc deprotection for conversion to amidine **11**, crystallization was performed in 2-butanol/*tert*-butyl methyl ether (MTBE). This highly reproducible method solved the problems associated with the discovery method and gave **11** in 88% yield at a 100 g scale.

Cleavage of the *tert*-Butyl Ester and Properties of 1·4HCl. Although *tert*-butyl ester deprotection of **11** under hydrochloric acid/alcohol solvent conditions yielded **1**·3HCl, many impurities—such as amide, in which an amidine moiety was hydrolyzed, and ester, in which *tert*-butyl ester was substituted with an alcohol solvent—were observed. In addition, because **1**·3HCl was obtained as an amorphous solid, purification of **1** was difficult without using column chromatography. We attempted to use an excess amount of hydrochloric acid without an alcohol solvent to suppress the solvent-mediated substitution reaction in the deprotection reaction. As expected, the side reaction was inhibited, and the subsequent addition of acetone as an antisolvent led to the formation of a crystalline rather than an amorphous solid. Elemental analysis of the obtained crystal indicated that it was **1**·4HCl·6H$_2$O, not **1**·3HCl (Scheme 8). Following the observation that the weight of **1**·4HCl changed after drying, we investigated the adsorption and desorption of hydrated water at different levels of relative humidity (RH). We observed a unique hygroscopic profile in which **1**·4HCl adsorbed and desorbed up to 13 equiv of water (Figure S1). Given that the amount of hydrate water in this compound could easily be altered depending on the humidity of its environment, **1**·4HCl·*x*H$_2$O (x = 0−13) was immediately used for the next reaction after drying.

Formation of ASP5069 (1·HCl·2H$_2$O) and Drying Process for the Dihydrate form. Because **1** contains a carboxylic acid moiety and several basic nitrogen atoms, the solubility of ASP5069 (**1**·HCl·2H$_2$O) was largely dependent on pH. The solubility of ASP5069 increased significantly in aqueous acetone at pH < 5.0 (Figure 2). Adjusting the pH to >6.0 led to contamination with an undesired polymorph or

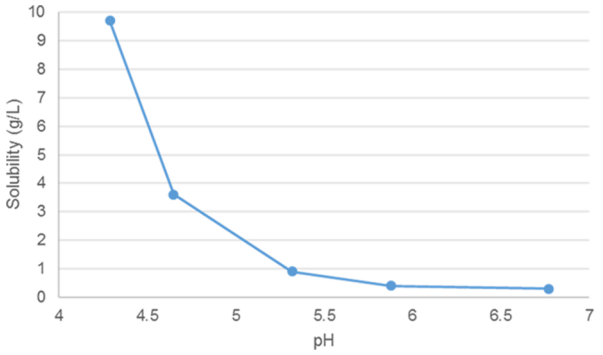
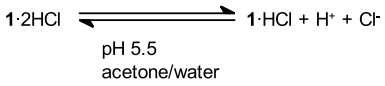


Figure 2. Solubility of ASP5069 in aqueous acetone. Conditions: ASP5069 (100 mg), acetone (10 vol), water (40 vol), NaCl (2 eq), 1 M aqueous HCl for pH adjustment, rt, >5 h.

pseudopolymorph, as observed by X-ray powder diffraction (XRPD) (Figure S2). Given that the pKa values were 11.2, 7.7, 3.7, and 0.9 for base and 2.8 for acid, as calculated using ACD/Percepta,[14] compound **1** may exist mostly as a monohydrochloride in an approximately pH 5.5 aqueous solution. Based on these findings, the target pH was set to 5.5.

After polish filtration of the aqueous **1**·4HCl solution, pH was adjusted with aqueous sodium hydroxide, and acetone was added as an antisolvent for crystallization of **1**·HCl·2H$_2$O. Interestingly, the pH decreased with the precipitation of crystals, leading to a corresponding increase in the loss of **1** to the mother liquor. This phenomenon suggested that the compound **1** may be in equilibrium with dihydrochloride and monohydrochloride in pH 5.5 aqueous acetone, as shown in Scheme 9. Precipitation of **1**·HCl would move the reaction

Scheme 9. Possible Equilibrium of Compound 1 in pH 5.5 Aqueous Acetone



toward the formation of more of **1**·HCl and hydrogen chloride, causing the pH to decrease. pH adjustment was repeated every few hours until the pH stabilized at the target value, which was achieved after several adjustments, and the monohydrochloride form was obtained with decreased loss (4.3%) to the mother liquor.

Meanwhile, there was concern about losing the hydrated water under drying conditions. Figures 3 and 4 show the hygroscopic profiles at 25 °C and 50 °C. At 25 °C, according

https://dx.doi.org/10.1021/acs.oprd.0c00291
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

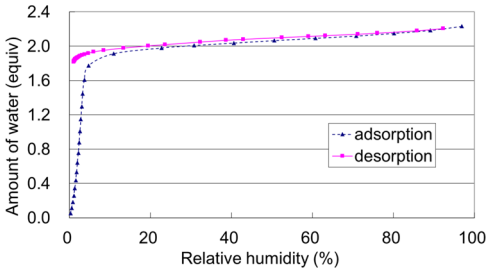


Figure 3. Hygroscopic properties of **1**·HCl at 25 °C.

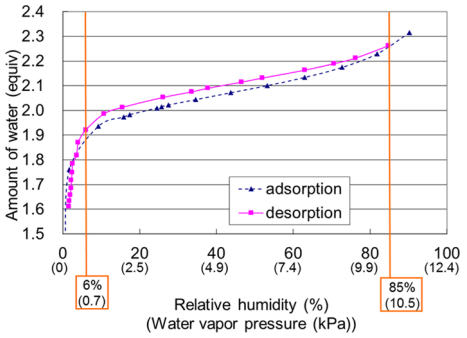


Figure 4. Hygroscopic properties of **1**·HCl at 50 °C.

to the adsorption curve (blue), approximately 2 equiv of water were adsorbed at 10% RH. Thereafter, with increasing RH, the water content gradually increased to 2.3 equiv. The target range of the water content of **1**·HCl was set to 2.1 ± 0.2H_2O, which is the water content at normal humidity (approximately 50% RH) at 25 °C. At 50 °C, the drying temperature, according to the desorption curve (pink), loss of the hydrated water would occur below 6% RH (0.7 kPa of water vapor pressure) (see Figure 4). Previous studies have reported drying methods for preventing anhydride contamination.[15] In our case, the hygroscopic profile at 50 °C suggested that the target 2.1 ± 0.2 hydrate could be obtained by drying the wet **1**·HCl product at 50 °C under a vacuum at 0.7−10.5 kPa. In the >100 g scale experiment, drying was performed under a vacuum at 7−9 kPa until loss on drying (LOD) was ≤1.0%, to give the 2.1 hydrate form of ASP5069 (KF titration: 5.6%) with 98.5 A % purity[16] and ≤0.5 A % individual impurities.

CONCLUSIONS

This study describes the development of a practical, scalable, and reproducible process for manufacturing ASP5069. The process comprised a convergent synthetic approach, eliminated the need for column chromatography, and solved safety and environmental concerns associated with the discovery method. In the Mannich reaction and alkylation sequence, the use of a more sterically hindered solvent suppressed the impurity formation, and *N*-overalkylation was markedly inhibited by using a harder electrophile to give **3** in good yield. We also developed an effective purification method for amine **3** by using an acidic aqueous solution for extraction. For the preparation of **5**, we replaced the solvent with an environmentally friendly alternative and developed the safe process for isolating 5-chloro-2-nitrobenzoyl chloride. For the reductive amination reaction, establishing an appropriate order for the addition of reagents and appropriate reaction temperature suppressed the production of **14**/**15** and improved process reproducibility, and subsequent Boc deprotection yielded **11** in good yield. The final steps were cleavage of the *tert*-butyl ester and hydrochloride salt formation. *tert*-Butyl ester deprotection was performed in the presence of excess amounts of hydrochloric acid to yield **1**·4HCl as a crystal, which had unique hygroscopic properties. Wet ASP5069 was isolated at pH 5.5 under pH-controlled crystallization in an aqueous acid solution. Finally, drying the dihydrate form under controlled vacuum pressure gave ASP5069. This process was successfully used to yield >100 g of ASP5069 with a purity of 98.5 A %.

EXPERIMENTAL SECTION

General Methods. Unless otherwise noted, all reactions were performed under a nitrogen atmosphere. All reagents and solvents purchased from suppliers were used as received unless otherwise noted. NMR spectra were recorded on a Bruker ADVANCE III HD500. Chemical shifts (δ) are reported in ppm in reference to the residual solvent signal (δ 2.50 for ^{1}H NMR in DMSO-d_6 and δ 39.52 for $^{13}C\{^1H\}$ NMR in DMSO-d_6). High-resolution mass spectra were obtained on a Thermo Scientific EXACTIVE Plus. IR spectra were recorded on a SHIMADZU IRAffinity-1S.

HPLC Methods. Method A: YMC-Pack Pro C18 (5 μm), 150 × 4.6 mm I.D.; eluent: 0.1 M aqueous $NaClO_4$ (pH 2.0)/MeCN (6/4); run time: 25 min; flow rate: 1.0 mL/min; temperature: 40 °C; detection: UV at 220 nm. Retention time: 6.5 min for **2**, 2.2 min for **9**, 6.2 min for **3**, 1.9 min for **4**, 3.5 min for **8**, 2.1 min for 5-chloro-2-nitorobenzoic acid, and 10.1 min for *N*-butyl-5-chloro-2-nitrobenzamide.

Method B: YMC-Pack Pro C18 (5 μm), 150 × 4.6 mm I.D.; eluent A: 0.1 M aqueous $NaClO_4$ (pH 2.0); eluent B: MeCN; flow rate: 1.5 mL/min; temperature: 40 °C; detection: UV at 220 nm. Gradient: 0 → 15 min, A/B = 75/25; 15 → 40 min, A/B = 75/25 → 20/80; 40 → 50 min, A/B = 20/80. Retention time: 19.6 min for **3**, 4.7 min for **8**, 22.0 min for **10**, 23.4 min for **5**, 6.2 min for **16**, 29.0 min for **6**, 26.6 min for **11**, and 14.7 min for **1**.

Method C: YMC-Pack ODS-A (5 μm), 150 × 4.6 mm I.D.; eluent: 0.03 M aqueous KH_2PO_4/THF (6/4); run time: 25 min; flow rate: 1.0 mL/min; temperature: 40 °C; detection: UV at 220 nm. Retention time: 12.3 min for **10** and 13.7 min for **5**.

*tert-Butyl {2-Ethoxy-6-formyl-4-[(4-methylpiperazin-1-yl)-methyl]phenoxy}acetate (**3**).* To a slurry of 3-ethoxysalicylaldehyde (**2**) (100 g, 602 mmol) in 2-propanol (800 mL) at 15−25 °C, 1-methylpiperazine (86.8 mL, 1.3 equiv) and formaldehyde (37% in water, 67.2 mL, 1.5 equiv) at 15−25 °C were added. After stirring for 10 min at 15−25 °C, the reaction mixture was warmed to 80 °C and stirred for 67 h at 80 °C until HPLC indicated that aldehyde **2** had been consumed (0.3 A % by HPLC method A). The reaction mixture was cooled to 60 °C and concentrated under a vacuum to 200 mL at 60 °C. The brown solution was diluted with toluene (800 mL) and concentrated under a vacuum at 60 °C to 200 mL. This process was repeated three times to give **9** in a toluene solution.

The toluene solution of **9** was diluted with DMF (500 mL) at rt. The resulting deep brown solution was cooled to −10 to 0 °C. K_2CO_3 (91.3 g, 1.1 equiv) was added at −10 to 0 °C. The resulting slurry was aged for 40 min at −10 to 0 °C. *tert*-Butyl chloroacetate (94.7 mL, 1.1 equiv) was added at −10 to 0 °C. The resulting slurry was warmed gradually to 40 °C over 8 h. Another portion of K_2CO_3 (74.9 g, 0.9 equiv) was added at 40 °C. The resulting slurry was stirred for 1 h at 40 °C.

https://dx.doi.org/10.1021/acs.oprd.0c00291
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

Another portion of *tert*-butyl chloroacetate (8.61 mL, 0.1 equiv) was added, and the resulting slurry was stirred for 1.5 h at 40 °C until HPLC indicated that **9** had been consumed (0.7 A % by HPLC method A). The reaction mixture was transferred to H_2O (150 mL) with the seed **3** (100 mg) in another flask over 30 min at 10−20 °C. The resulting slurry was aged for 1 h at 40 °C, cooled to rt, aged for 108 h at 20−25 °C, and then filtered. The wet cake was washed with DMF/H_2O (1/4) (500 mL) and H_2O (1000 mL) and dried under a vacuum at 50 °C for 21 h to give crude **3** (187 g, 476 mmol, 79%; 97.8 A % by HPLC method A; 88.3 A % by HPLC method B) as a light brown solid.

*Purification of Aldehyde **3***. To a solution of crude **3** (136 g, 347 mmol) in *i*-PrOAc/H_2O (680 mL/1000 mL) at rt was added 6 M aqueous HCl (57.8 mL, 1.0 equiv). After vigorous stirring at rt, the biphasic mixture was separated. The organic layer was extracted with H_2O (136 mL) at rt. The combined aqueous layer was washed with *i*-PrOAc (136 mL), diluted with *i*-PrOAc (1020 mL) and then basified with 5 M aqueous NaOH (76.3 mL, 1.1 equiv) at rt. After vigorous stirring, the biphasic mixture was separated. The organic layer was washed with 10% brine (272 mL) and 20% brine (136 mL). The organic layer was concentrated under a vacuum to 272 mL, and *i*-PrOAc (680 mL) was added. This process was repeated. The precipitated inorganic salts were removed by filtration to give aldehyde **3** in *i*-PrOAc solution (0.304 M, 1088 mL, 331 mmol, 95%, 98.7 A % by HPLC method B). The authentic sample was obtained by crystallization from *i*-PrOAc. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.37 (t, 3H, J = 7.0 Hz), 1.38 (s, 9H), 2.14 (s, 3H), 2.16−2.53 (m, 8H), 3.42 (s, 2H), 4.10 (q, 2H, J = 7.0 Hz), 4.76 (s, 2H), 7.19 (d, 1H, J = 1.9 Hz), 7.25 (d, 1H, J = 1.9 Hz), 10.50 (s, 1H). ^{13}C{^{1}H} NMR (DMSO-d_6, 125 MHz): δ 14.5, 27.6, 45.7, 52.4, 54.7, 61.2, 64.4, 69.4, 81.5, 117.7, 119.7, 128.6, 134.6, 148.9, 150.7, 168.3, 190.5. HRMS−ESI (m/z): $[M + H]^+$ calcd for $C_{21}H_{33}O_5N_2$, 393.2384; found, 393.2383. IR (ATR, cm^{-1}): 2936, 2787, 1748, 1144, 812, 725. Mp: 88−90 °C. Assay (QNMR): 100 wt %.

*tert-Butyl [(4-Aminophenyl) (imino)methyl]carbamate (**8**)*. To a slurry of 4-aminobenzamidine dihydrochloride (**4**) (51.1 g, 246 mmol) in THF (128 mL) was added 5 M aqueous NaOH (221 mL, 4.5 equiv) at 0−10 °C. The resulting biphasic solution was stirred for 30 min at 0−10 °C. To the mixture was added Boc_2O (57.5 mL, 1.0 equiv) at 0−10 °C. The resulting slurry was stirred for 3 h at 0−10 °C until HPLC indicated that amidine **4** had been consumed (0.3 A % by HPLC method A). The slurry was added dropwise to H_2O (1022 mL) in another flask at 0−10 °C over 1 h. The reaction flask was rinsed with H_2O (153 mL), and the rinse solution was added to the crystallization flask. The resulting slurry was aged for 3 h at 0−10 °C and filtered. The wet cake was washed with H_2O (511 mL) and dried under a vacuum at 50 °C for 22 h to give **8** (56.3 g, 239 mmol, 97%; 98.5 A % by HPLC method A) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.43 (s, 9H), 5.77 (s, 2H), 6.55 (d, 2H, J = 8.8 Hz), 7.72 (d, 2H, J = 8.8 Hz), 8.03−9.69 (m, 2H). ^{13}C{^{1}H} NMR (DMSO-d_6, 125 MHz): δ 28.1, 77.0, 112.6, 120.4, 129.2, 152.5, 164.0, 166.2. HRMS−ESI (m/z): $[M + H]^+$ calcd for $C_{12}H_{18}O_2N_3$, 236.1394; found, 236.1392. IR (ATR, cm^{-1}): 3146, 1609, 1267, 1146, 1125, 862, 797. Mp:195 °C dec. Assay (QNMR): 99 wt %.

*tert-Butyl {[4-(5-Chloro-2-nitrobenzamido)phenyl](imino)methyl}carbamate (**10**)*. To a slurry of 5-chloro-2-nitorobenzoic acid (120 g, 1.2 equiv) in toluene (816 mL) was added $SOCl_2$ (72.5 mL, 2.0 equiv) at rt. The resulting slurry was warmed to 100 °C and stirred for 6 h at 100 °C until HPLC indicated that 5-chloro-2-nitorobenzoic acid had been consumed (0.3 A % by HPLC method A: the reaction was monitored by mixing the reaction aliquot with *n*-butylamine in a HPLC vial). The reaction mixture was cooled to 60 °C and concentrated under a vacuum to 233 mL at 60 °C. Toluene (936 mL) was added and concentrated under a vacuum to 233 mL at 60 °C. This process was repeated to give 5-chloro-2-nitrobenzoyl chloride in a toluene solution (98.1 A % by HPLC method A: an aliquot of the solution was mixed with *n*-butylamine in a HPLC vial, and checked using HPLC).

Boc amidine **8** (117 g, 497 mmol) was added to a mixed solution of THF (468 mL) and *i*-Pr_2NEt (130 mL, 1.5 equiv) at rt. The resulting slurry was cooled to −15 to −10 °C. The toluene solution of 5-chloro-2-nitrobenzoyl chloride (233 mL, 1.2 equiv) was added dropwise at −15 to −5 °C. The residual acid chloride in the flask was rinsed with THF (58.5 mL). The resulting orange solution was stirred for 2 h at −15 to −5 °C until HPLC indicated that amidine **8** had been consumed (0.2 A % by HPLC method B). The reaction mixture was quenched with 5% aqueous Na_2CO_3 (585 mL) at below 10 °C. The biphasic mixture was stirred vigorously for 20 min and then separated at 10−30 °C. The organic layer was washed with 10% brine (293 mL × 2) and 20% brine (147 mL) and then added dropwise to toluene (2630 mL) with the seed **10** (58.5 mg, 0.05 wt %) in another flask at 48−52 °C over 0.5 h. The residual organic layer in the flask was rinsed with THF (58.5 mL), and the rinse solution was added to the slurry. The resulting yellow slurry was aged for 1 h at 48−52 °C, cooled gradually to 15−25 °C, aged for 68 h at 15−25 °C, and then filtered. The wet cake was washed with toluene (1170 mL × 2) and dried under a vacuum at rt for 1 h and then at 50 °C for 14 h to give **10** (180 g, 430 mmol, 87%, 94.0 A % by HPLC method B) as a colorless solid. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.45 (s, 9H), 7.74 (d, 2H, J = 8.7 Hz), 7.86 (dd, 1H, J = 8.8, 2.3 Hz), 7.99 (d, 2H, J = 8.7 Hz), 7.99 (d, 1H, J = 2.3 Hz), 8.20 (d, 1H, J = 8.7 Hz), 9.00 (br s, 2H), 10.96 (br s, 1H). ^{13}C{^{1}H} NMR (DMSO-d_6, 125 MHz): δ 28.0, 77.6, 118.9, 126.4, 128.6, 129.2, 129.7, 130.9, 134.0, 138.9, 141.8, 144.9, 162.9, 163.7, 165.4. HRMS−ESI (m/z): $[M + H]^+$ calcd for $C_{19}H_{20}O_5N_4Cl$, 419.1117; found, 419.1118. IR (ATR, cm^{-1}): 1618, 1607, 1533, 1497, 1277, 1148, 853, 839. Mp: 187 °C dec. Assay (QNMR): 90 wt %.

*tert-Butyl {[4-(2-Amino-5-chlorobenzamido)phenyl](imino)methyl}carbamate (**5**)*. Nitro-amide **10** (60.0 g, 143 mmol) was dissolved in a mixed solvent of H_2O (43.8 mL) and DMF (300 mL) at rt in a 1000 mL autoclave. To the solution was added pre-rinsed Raney nickel [washed with H_2O (300 mL × 3)] (60.0 g, 100 wt %) in DMF (136 mL) at rt. The resulting mixture was stirred for 16 h at 30 °C under a H_2 atmosphere (44 psi) until HPLC indicated that amide **10** had been consumed (ND by HPLC method C). Then, the reaction mixture was cooled to rt and filtered. Filtration was performed using a pressure filter under a N_2 atmosphere to avoid exposing flammable Raney Ni to air. The filtered solid and vessels were rinsed with DMF/H_2O (10/1) (240 mL). The combined light brown filtrate was warmed to 50 °C. Water (240 mL) was added, and then the mixture was seeded with **5** (30.0 mg, 0.05 wt %). The resulting slurry was aged for 1 h at 50 °C. Another portion of H_2O (360 mL) was added dropwise over 0.5 h. The resulting slurry was aged for 1 h at 50 °C, cooled to rt, aged for 20 h at rt, and then filtered. The wet cake was washed with

https://dx.doi.org/10.1021/acs.oprd.0c00291
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

DMF/H_2O (1/2) (300 mL) and then H_2O (600 mL) and dried under a vacuum at 50 °C for 139 h to give **5** (48.5 g, 125 mmol, 87%, 94.4 A % by HPLC method B) as a gray-brown solid. ^{1}H NMR (DMSO-d_6, 500 MHz): δ1.45 (s, 9H), 6.51 (s, 2H), 6.80 (d, 1H, J = 8.9 Hz), 7.24 (dd, 1H, J = 8.9, 2.5 Hz), 7.71 (d, 1H, J = 2.6 Hz), 7.82 (d, 2H, J = 8.9 Hz), 7.97 (d, 2H, J = 8.9 Hz), 8.49−9.55 (m, 2H), 10.30 (s, 1H). ^{13}C{^{1}H} NMR (DMSO-d_6, 125 MHz): δ 28.0, 77.6, 115.5, 117.8, 118.2, 119.6, 128.0, 128.2, 129.1, 132.1, 142.3, 148.8, 163.7, 165.6, 166.8. HRMS−ESI (m/z): [M + H]$^+$ calcd for $C_{19}H_{22}O_3N_4Cl$, 389.1375; found, 389.1377. IR (ATR, cm^{-1}): 3420, 3312, 2976, 1609, 1508, 1489, 1308, 1292, 1236, 1140, 845, 831. Mp: 195 °C dec. Assay (QNMR): 95 wt %.

*tert-Butyl {2-({2-[(4-Carbamimidoylphenyl)carbamoyl]-4-chloroanilino}methyl)-6-ethoxy-4-[(4-methylpiperazin-1-yl)-methyl]phenoxy}acetate (**11**).* To a slurry of aniline **5** (100 g, 257 mmol) in *i*-PrOAc (700 mL) was added $NaBH(OAc)_3$ in AcOH [prepared by adding $NaBH_4$ (29.2 g, 3.0 equiv) to AcOH (800 mL) at 16−30 °C. Caution: It is important to add $NaBH_4$ slowly while controlling the reaction temperature and hydrogen production] at −10 to 0 °C over 45 min. Caution: It is equally important to add $NaBH(OAc)_3$ slowly while controlling the reaction temperature and hydrogen production. Residual $NaBH(OAc)_3$ in the flask was rinsed with AcOH (200 mL) and added to the reaction flask. The resulting thin, light brown slurry was stirred for 70 min at −10 to 0 °C. Then, aldehyde **3** in *i*-PrOAc (931 mL, 0.304 M, 1.1 equiv) was added over 80 min at −10 to 0 °C. The resulting yellow slurry was stirred for 4 h at −10 to 0 °C. Another portion of aldehyde **3** in *i*-PrOAc (84.5 mL, 0.304 M, 0.1 equiv) was added over 5 min at −10 to 0 °C. The resulting brown solution was stirred for 2.5 h at −10 to 0 °C until HPLC indicated that aniline **5** and Boc-deprotected aniline **16** had been consumed (ND and 0.4 A %, respectively, by HPLC method B). Water (4.63 mL, 1.0 equiv) was added at −10 to 0 °C. The resulting mixture was warmed to 60 °C and stirred for 20 h at 60 °C until HPLC indicated that the intermediate **6** had been consumed (0.2 A % by HPLC method B). The mixture was cooled to 0 °C and stirred for 9.5 h at 0 °C. To the mixture were added 7.5 M aqueous NaOH (600 mL) and 2-butanol (400 mL) at 0−10 °C. The resulting mixture was warmed to 30 °C and stirred for 1 h at 30 °C and then transferred to a separation funnel via a glass filter to remove insoluble precipitates. The reaction flask was rinsed with *i*-PrOAc/2-butanol/H_2O (3/2/1) (90 mL), and the rinsed solution was transferred to the funnel via the filter. The biphasic mixture was separated. The organic layer was washed with H_2O (250 mL × 2) and 20% brine (200 mL). The solution was concentrated under a vacuum at 50 °C to 500 mL, and 2-butanol (500 mL) was added. This process was repeated three times. Precipitated inorganic salts were removed by filtration, and the filtrate was concentrated to 500 mL. The solution was added dropwise to MTBE (2300 mL) in another flask at 50 °C over 1 h. The residual solution in the flask was rinsed with 2-butanol (280 mL), and the rinsed solution was added to the crystallization flask at 50 °C. The resulting yellow slurry was stirred for 1 h at 50 °C, cooled to 20 °C, stirred for 4 h at 20 °C, and then filtered. The wet cake was washed with MTBE (2000 mL) and dried under a vacuum at 40 °C for 13 h to give **11** (181 g, 226 mmol, 88%; 92.8 A % by HPLC method B). KF, NMR, and elemental analysis indicated that **11** contained 1.3 equiv of AcOH, 0.7 equiv of HCl, and 1.7 equiv of water. Yield and assay are calculated as **11**·1.3AcOH·0.7HCl·1.7H_2O. Yellow solid. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.34 (t, 3H, J = 7.0 Hz), 1.39 (s, 9H), 1.86 (s, CH_3COOH, 3.9H), 2.10 (s, 3H), 2.13−2.45 (m, 8H), 3.31 (s, 2H), 4.02 (q, 2H, J = 7.0 Hz), 4.50 (d, 2H, J = 5.4 Hz), 4.61 (s, 2H), 6.74 (d, 1H, J = 9.2 Hz), 6.79 (d, 1H, J = 1.5 Hz), 6.83 (d, 1H, J = 1.6 Hz), 7.29 (dd, 1H, J = 9.0, 2.5 Hz), 7.82 (d, 1H, J = 2.5 Hz), 7.86 (d, 2H, J = 9.0 Hz), 7.84−7.89 (m, 1H), 7.95 (d, 2H, J = 9.0 Hz), 8.41−10.43 (m, 3H), 10.60 (br s, 1H). ^{13}C{^{1}H} NMR (DMSO-d_6, 125 MHz): δ 14.7, 22.1 (CH_3COOH), 27.7, 41.5, 45.5, 52.2, 54.5, 61.6, 63.9, 69.5, 81.1, 113.0, 113.7, 115.9, 117.9, 120.0, 122.5, 128.5, 128.8, 131.2, 132.6, 133.8, 143.7, 143.9, 148.2, 150.2, 165.0, 167.3, 168.4, 173.2 (CH$_3$COOH). HRMS−ESI (m/z): [M + H]$^+$ calcd for $C_{35}H_{46}O_5N_6Cl$, 665.3213; found, 665.3218. IR (ATR, cm^{-1}): 2970, 1508, 1497, 1489, 1207, 1146, 851, 745, 648. Mp: 140 °C dec. Anal. Calcd for $C_{35}H_{45}ClN_6O_5\cdot 1.3C_2H_4O_2.0.7HCl.1.7H_2O$: C, 56.5; H, 6.9; N, 10.5; Cl, 7.5. Found: C, 56.8; H, 6.8; N, 10.1; Cl, 7.7. KF titration: 3.8%. Assay (QNMR): 95 wt %.

*{2-({2-[(4-Carbamimidoylphenyl)carbamoyl]-4-chloroanilino}methyl)-6-ethoxy-4-[(4-methylpiperazin-1-yl)-methyl]phenoxy}acetic Acid Monohydrochloride Dihydrate (ASP5069, **1**·HCl·2H_2O).* Compound **11** (170 g, 213 mmol) was suspended in 6 M aqueous HCl (850 mL, 24 equiv) at rt. The suspension was stirred for 2 h at 40 °C until HPLC indicated that **11** had been consumed (1.0 A % by HPLC method B). Acetone (4.25 L) was added at 40 °C over 1 h. The resulting slurry was stirred for 3 h at 40 °C, cooled to 20 °C (20 °C/h), stirred for 2 h, and then filtered. The wet cake was washed with acetone (1.7 L) and dried under a vacuum at 50 °C for 10 h to give **1**·4HCl·$x$$H_2O$ (x = 0−13, 155 g, 97.4 A % by HPLC method B). Compound **1**·4HCl·$x$$H_2O$ (x = 0−13, 145 g) was dissolved in water (668 mL) at rt. The solution was filtered through a 0.5 μm filter, and residual **1**·4HCl·$x$$H_2O$ (x = 0−13) in the flask and on the filter was rinsed with water (158 mL). The filtrate was stirred at rt, and acetone (826 mL) was added at rt. The pH of the mixture was adjusted from 1.0 to 5.6 by adding 5 M aqueous NaOH (108 mL). The solution was warmed to 50 °C and stirred for 1 h at 50 °C. Acetone (2878 mL) was added to the slurry over 1 h. The slurry was stirred for 1 h at 50 °C, cooled to 20 °C (10 °C/h), and stirred at 20 °C for 11 h. The pH of the mixture was adjusted to approximately 5.5 by adding 5 M aqueous NaOH and 6 M aqueous HCl, and then the slurry was stirred for 2−14 h. This pH adjustment was repeated until the pH stabilized at approximately 5.5. The slurry was filtered. The wet cake was washed with acetone/H_2O (607 mL/121 mL) and acetone (1.46 L) and dried at 50 °C under a vacuum at 7−9 kPa for 3 days until LOD was ≤1.0% to give ASP5069 (117 g, 172 mmol, 86%; 98.5 A % by HPLC method B) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.34 (t, 3H, J = 6.9 Hz), 2.30 (s, 3H), 2.32−2.72 (m, 8H), 3.36 (s, 2H), 4.02 (q, 2H, J = 6.9 Hz), 4.53 (s, 2H), 4.59 (s, 2H), 6.76 (s, 1H), 6.76 (d, 1H, J = 9.1 Hz), 6.84 (s, 1H), 7.28 (dd, 1H, J = 9.0, 2.4 Hz), 7.81 (d, 1H, J = 2.4 Hz), 7.86 (d, 2H, J = 8.9 Hz), 7.84−7.88 (m, 1H), 7.95 (d, 2H, J = 8.8 Hz), 9.18 (br s, 2H), 9.96 (br s, 2H), 10.63 (s, 1H). ^{13}C{^{1}H} NMR (DMSO-d_6, 125 MHz): δ 14.7, 41.5, 44.2, 50.9, 53.6, 61.2, 63.8, 69.9, 113.0, 113.9, 115.9, 117.8, 119.9, 120.0, 122.5, 128.5, 128.8, 131.7, 132.58, 132.61, 143.9, 144.3, 148.3, 150.5, 165.0, 167.3, 172.4. HRMS−ESI (m/z): [M + H]$^+$ calcd for $C_{31}H_{38}O_5N_6Cl$, 609.2587; found, 609.2589. IR (ATR, cm^{-1}): 1489, 1204, 851, 716, 638. Mp: 209 °C dec. KF titration: 5.6%. Assay (QNMR): 99 wt %.

https://dx.doi.org/10.1021/acs.oprd.0c00291
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.0c00291.

^{1}H and ^{13}C{^{1}H} NMR spectra of compounds **3**, **8**, **10**, **5**, **11**, and ASP5069 (**1**·HCl·2H$_2$O) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Shun Hirasawa − *Pharmaceutical Science & Technology Labs., Pharmaceutical Technology, Astellas Pharma Inc., Tsukuba-shi, Ibaraki 305-8585, Japan;* orcid.org/0000-0002-1659-3424; Email: shun.hirasawa@astellas.com

Author

Yoshinori Kohmura − *Pharmaceutical Science & Technology Labs., Pharmaceutical Technology, Astellas Pharma Inc., Tsukuba-shi, Ibaraki 305-8585, Japan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.oprd.0c00291

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank M. Fujimoto and Y. Hashimoto for analytical support and T. Tsunoda, S. Ieda, T. Takahashi, and M. Okada for project management advice.

■ REFERENCES

(1) (a) Ueno, A.; Oh-ishi, S. Roles for the Kallikrein-Kinin System in Inflammatory Exudation and Pain: Lessons From Studies on Kininogen-Deficient Rats. *J. Pharmacol. Sci.* **2003**, *93*, 1−20. (b) Moreau, M. E.; Garbacki, N.; Molinaro, G.; Brown, N. J.; Marceau, F.; Adam, A. The Kallikrein-Kinin System: Current and Future Pharmacological Targets. *J. Pharmacol. Sci.* **2005**, *99*, 6−38. (c) Bryant, J.; Shariat-Madar, Z. Human plasma kallikrein-kinin system: Physiological and biochemical parameters. *Cardiovasc. Hematol. Agents Med. Chem.* **2009**, *7*, 234−250. (d) Campbell, D. J. The Kallikrein-Kinin System In Humans. *Clin. Exp. Pharmacol. Physiol.* **2001**, *28*, 1060−1065. (e) Schmaier, A. H.; Mccrae, K. R. The plasma kallikrein-kinin system: its evolution from contact activation. *J. Thromb. Haemostasis* **2007**, *5*, 2323−2329.

(2) Levy, J. H.; O'Donnell, P. S. The therapeutic potential of a kallikrein inhibitor for treating hereditary angioedema. *Expert Opin. Invest. Drugs* **2006**, *15*, 1077−1090.

(3) Fujisawa, H.; Ito, H.; Kashiwagi, S.; Nomura, S.; Toyosawa, M. Kallikrein-kinin system in chronic subdural haematomas: its roles in vascular permeability and regulation of fibrinolysis and coagulation. *J. Neurol. Neurosurg. Psychiatr.* **1995**, *59*, 388−394.

(4) Liu, J.; Gao, B.-B.; Clermont, A. C.; Blair, P.; Chilcote, T. J.; Sinha, S.; Flaumenhaft, R.; Feener, E. P. Hyperglycemia-induced cerebral hematoma expansion is mediated by plasma kallikrein. *Nat. Med.* **2011**, *17*, 206−210.

(5) Wanaka, K.; Okamoto, S.; Bohgaki, M.; Hijikata-Okunomiya, A.; Naito, T.; Okada, Y. Effect of a highly selective plasma-kallikrein synthetic inhibitor on contact activation relating to kinin generation, coagulation and fibrinolysis. *Thromb. Res.* **1990**, *57*, 889−895.

(6) (a) Ishihara, T.; Miura, M.; Ohne, K.; Takuwa, T.; Shirakami, S.; Ibuka, R.; Ohnuki, K.; Seki, N.; Shigenaga, T.; Hirayama, F.; Hirabayashi, A.; Kai, Y.; Kobayashi, J.; Hirasawa, H.; Kondou, A.; Yamada, K. Carboxylic acid derivative or salt thereof. WO 2006070878 A1, December 28, 2005. (b) Shigenaga, T.; Imamura, Y.; Suzuki, D.; Takuwa, T.; Honma, T.; Hirabayashi, A.; Hirasawa, H.; Kondou, A. Plasma kallikrein inhibitor. WO 2011118672 A1, March 24, 2011.

(7) (a) Zhou, Y.; Feng, Q.; Di, F.; Liu, Q.; Wang, D.; Chen, Y.; Xiong, L.; Song, H.; Li, Y.; Li, Z. Synthesis and insecticidal activities of 2,3-dihydroquinazolin-4(1*H*)-one derivatives targeting calcium channel. *Bioorg. Med. Chem.* **2013**, *21*, 4968−4975. (b) Mizutani, T.; Nagase, T.; Ito, S.; Miyamoto, Y.; Tanaka, T.; Takenaga, N.; Tokita, S.; Sato, N. Development of novel 2-[4-(aminoalkoxy)-phenyl]-4(3H)-quinazolinone derivatives as potent and selective histamine H3 receptor inverse agonists. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6041−6045. (c) Kim, D.; Wang, L.; Hale, J. J.; Lynch, C. L.; Budhu, R. J.; MacCoss, M.; Mills, S. G.; Malkowitz, L.; Gould, S. L.; DeMartino, J. A.; Springer, M. S.; Hazuda, D.; Miller, M.; Kessler, J.; Hrin, R. C.; Carver, G.; Carella, A.; Henry, K.; Lineberger, J.; Schleif, W. A.; Emini, E. A. Potent 1,3,4-trisubstituted pyrrolidine CCR5 receptor antagonists: effects of fused heterocycles on antiviral activity and pharmacokinetic properties. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2129−2134. (d) Wright, W. B., Jr.; Tomcufcik, A. S.; Chan, P. S.; Marsico, J. W.; Press, J. B. Thromboxane synthetase inhibitors and antihypertensive agents. 4. N-[(1H-imidazol-1-yl)alkyl] derivatives of quinazoline-2,4(1H,3H)-diones, quinazolin-4(3H)-ones, and 1,2,3-benzotriazin-4(3H)-ones. *J. Med. Chem.* **1987**, *30*, 2277−2283. (e) Tani, J.; Yamada, Y.; Oine, T.; Ochiai, T.; Ishida, R.; Inoue, I. Studies on biologically active halogenated compounds. 1. Synthesis and central nervous system depressant activity of 2-(fluoromethyl)-3-aryl-4(3*H*)-quinazolinone derivatives. *J. Med. Chem.* **1979**, *22*, 95−99.

(8) (a) Clark, R. H.; Wagner, E. C. Isatoic Anhydride. I. Reactions with Primary and Secondary Amines and with Some Amides1. *J. Org. Chem.* **1944**, *09*, 55−67. (b) Coppola, G. M. The Chemistry of Isatoic Anhydride. *Synthesis* **1980**, *1980*, 505−536.

(9) Pearson, R. G. Hard and Soft Acids and Bases. *J. Am. Chem. Soc.* **1963**, *85*, 3533−3539.

(10) Anderson, N. G. *Practical Process Research & Development*, 2nd ed.; Academic Press: Oxford, U.K.. 2012; p 129.

(11) *Bretherick's Handbook of Reactive Chemical Hazards*, 5th ed.; Urben, P., Ed.; Butterworth-Heinemann: Oxford, 1995; Vol. *1*, p 856.

(12) Several cyclic aminal syntheses under acidic or basic conditions with benzaldehyde analogs and 2-amino-*N*-phenylbenzamide analogs have been recently reported. For selected references, see; (a) Mostafavi, H.; Islami, M. R.; Khabazzadeh, H.; Khaleghi, M. Synthesis of New Quinazolin-4-(3H)-one Derivatives and Evaluation of Their Biological Activities. *ChemistrySelect* **2019**, *4*, 3169−3174. (b) Khachatryan, D. S.; Belus, S. K.; Misyurin, V. A.; Baryshnikova, M. A.; Kolotaev, A. V.; Matevosyan, K. R. Synthesis and properties of 1,2-dihydro-4(3*H*)-quinazolinones. *Russ. Chem. Bull.* **2017**, *66*, 1044−1058. (c) Mahdavi, M.; Pedrood, K.; Safavi, M.; Saeedi, M.; Pordeli, M.; Ardestani, S. K.; Emami, S.; Adib, M.; Foroumadi, A.; Shafiee, A. Synthesis and anticancer activity of N-substituted 2-arylquinazolinones bearing trans-stilbene scaffold. *Eur. J. Med. Chem.* **2015**, *95*, 492−499. (d) Rajaka, L.; Penumati, N. R.; Nagaiah, K.; Poornachandra, Y.; Kumar, C. G. Convenient and Scalable Synthesis of 2,3-Dihydroquinazolin-4(1*H*)-one Derivatives and Their Anticancer Activities. *Synth. Commun.* **2015**, *45*, 1893−1901. (e) Dindulkar, S. D.; Oh, J.; Arole, V. M.; Jeong, Y. T. Supported ceric ammonium nitrate: A highly efficient catalytic system for the synthesis of diversified 2,3-substituted 2,3-dihydroquinazolin-4(1H)-ones. *Compt. Rendus Chem.* **2014**, *17*, 971−979. (f) Noel, R.; Gupta, N.; Pons, V.; Goudet, A.; Garcia-Castillo, M. D.; Michau, A.; Martinez, J.; Buisson, D.-A.; Johannes, L.; Gillet, D.; Barbier, J.; Cintrat, J.-C. *N*-Methyldihydroquinazolinone Derivatives of Retro-2 with Enhanced Efficacy against Shiga Toxin. *J. Med. Chem.* **2013**, *56*, 3404−3413. (g) Prakash, S.; Jayakumar, S.; Kesavan, V. Investigation of the Enantioselective Synthesis of 2,3-Dihydroquinazolinones Using Sc-(III)−*inda*-pybox. *Synthesis* **2013**, *45*, 2265−2272. (h) Prakash, M.; Kesavan, V. Highly Enantioselective Synthesis of 2,3-Dihydroquinazolinones through Intramolecular Amidation of Imines. *Org. Lett.* **2012**, *14*, 1896−1899. (i) Sharma, M.; Pandey, S.; Chauhan, K.; Sharma, D.; Kumar, B.; Chauhan, P. M. S. Cyanuric Chloride Catalyzed Mild Protocol for Synthesis of Biologically Active Dihydro/

Spiro Quinazolinones and Quinazolinone-glycoconjugates. *J. Org. Chem.* **2012**, *77*, 929−937.
(13) For imine formation by a nitrogen coordinated to borane and a carbonyl group, see: Nefkens, G. H. L.; Zwanenburg, B. Reactions of Boroxazolidones with Aromatic Aldehydes. *Tetrahedron* **1985**, *41*, 6063−6066.
(14) pK_a values were calculated using GALAS Algorithm. Software name and version: ACD/Percepta 14.3.0 (Build 3063).
(15) (a) Cypes, S. H.; Wenslow, R. M.; Thomas, S. M.; Chen, A. M.; Dorwart, J. G.; Corte, J. R.; Kaba, M. Drying an Organic Monohydrate: Crystal Form Instabilities and a Factory-Scale Drying Scheme to Ensure Monohydrate Preservation. *Org. Process Res. Dev.* **2004**, *8*, 576−582. (b) Variankaval, N.; Lee, C.; Xu, J.; Calabria, R.; Tsou, N.; Ball, R. Water Activity-Mediated Control of Crystalline Phases of an Active Pharmaceutical Ingredient. *Org. Process Res. Dev.* **2007**, *11*, 229−236. (c) Savage, S. A.; Jones, G. S.; Kolotuchin, S.; Ramrattan, S. A.; Vu, T.; Waltermire, R. E. Preparation of Saxagliptin, a Novel DPP-IV Inhibitor. *Org. Process Res. Dev.* **2009**, *13*, 1169−1176.
(16) The target quality of the active pharmaceutical ingredient is ≥97 A %.

https://dx.doi.org/10.1021/acs.oprd.0c00291
Org. Process Res. Dev. XXXX, XXX, XXX−XXX