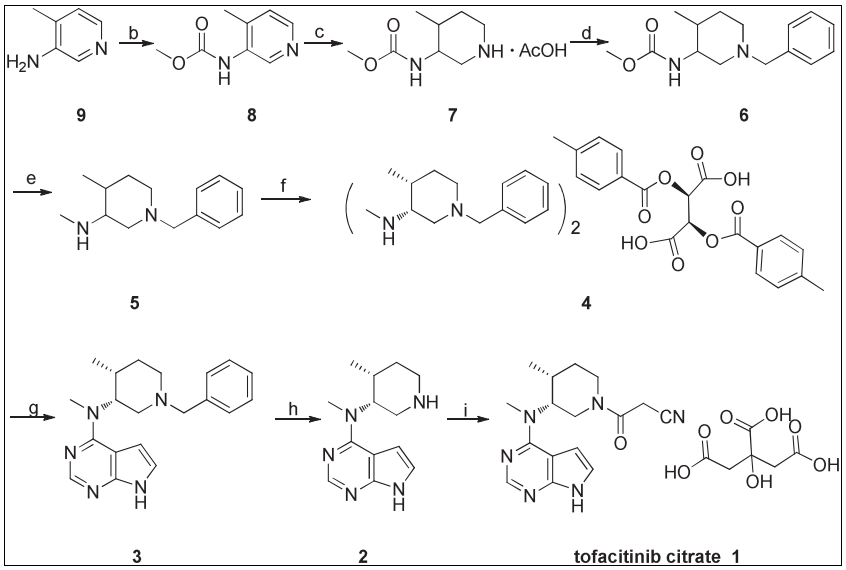
An Efficient Method for Synthesis of Tofacitinib Citrate

Shuang Zhi,[a] Dengke Liu,[b] Ying Liu,[b] Bingni Liu,[b] Donghua Wang,[a] and Ligong Chen[a]*

[a]School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China
[b]Tianjin Key Laboratory of Molecular Design and Drug Discovery, Tianjin Institute of Pharmaceutical Research, Tianjin 300193, China
*E-mail: lgchen@tju.edu.cn

Received August 31, 2014
DOI 10.1002/jhet.2384
Published online 00 Month 2015 in Wiley Online Library (wileyonlinelibrary.com).



An efficient and mild synthetic method was developed for tofacitinib citrate from 3-amino-4-methylpyridine and 4-chloro-7*H*-pyrrolo[2,3-d]pyrimidine. The related reactions were systematically optimized. Sodium hydride instead of potassium *tert*-butoxide employed in the methoxycarbonylation reaction of compound **9** made the reaction proceed effectively to present compound **8** in a better yield. The replacement of benzaldehyde with benzyl bromide simplified the protection process of amino group. Red-Al provided a cost-effective method for the reduction of amides. The introduction of tosyl group into compound **10** enhanced the nucleophilic substitution of **10** with compound **4** dramatically. Thus, under the optimized conditions, tofacitinib citrate was obtained in 13.3% yield (based on compound **9**) with a purity of 99.9%, much better than the reported yield 8.6%. This cost-effective and environmental friendly process is more suitable for scale-up production.

J. Heterocyclic Chem., **00,** 00 (2015).

INTRODUCTION

Tofacitinib (CP-690,550) citrate, chemically named as (3R,4R)-3-[4-methyl-3-[*N*-methyl-*N*-(7*H*-pyrrolo[2,3-*d*] pyrimidin-4-yl)amino]piperidin-1-yl]-3-oxopropionitrile citrate (Fig. 1), is a novel JAK3 inhibitor [1] discovered and developed by Pfizer. It was approved for oral treatment of rheumatoid arthritis in the US, Japan, and Russia. Today, this agent is further being evaluated for treatment of immune-mediated diseases, including psoriasis, Crohn's disease, and inflammatory bowel disease, as well as the prevention of organ transplant rejection [2–5].

Based on its pharmaceutical importance, the synthesis of tofacitinib citrate was extensively studied. As reported, its key intermediate, (3R,4R)-1-benzyl-N,4-dimethylpiperidin-3-amine 20, was synthesized from isoprene 16 [6] or 4-methylpyridine 17 [7,8] (Scheme 1). However, some drawbacks limited their application in scale-up production: (i) an aqueous Diels-Alder reaction required 70 h at 35°C to give compound 18 only in 59% yield [6]. (ii) Boron fluoride ethyl ether hydrolyzed rapidly in damp air to release toxic hydrogen fluoride. (iii) The sulfur-based oxidations left the product contaminated with dimethyl sulfide leading to handling problems.

Ultimately, Pfizer Inc established a new four-step method for compound **20** from 3-amino-4-methylpyridine, an appropriately functionalized and commercially available pyridine. It only requires condensation with dimethyl carbonate, hydrogenation of pyridine ring, protection of *sec*-amino group in piperidine ring, and finally reduction of the amides to yield the target compound **20** as shown in Scheme 2 [9]. Although this route looks reasonable, it still suffers from the following troublesome problems: (i) the cream solid is easily formed in the condensation step.

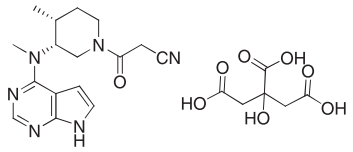
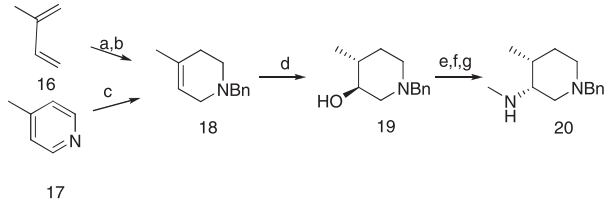


Figure 1. The chemical structure of tofacitinib citrate.

Scheme 1. Two routes to synthesize compound **20** reaction conditions: (a) $BnNH_2$, HCHO, H_2O, 59%; (b) BnCl, acetone, 55°C, 73%; (c) $NaBH_4$, EtOH, 15°C, 73%; (d) (i) $BF_3 \cdot OEt_2$, $BH_3 \cdot THF$, THF; (ii) HCl, H_2O_2; (iii) TsOH, 88%; (e) $SO_3 \cdot$ pyridine, DMSO, Et_3N; (f) $MeNH_2$, $NaHB(OAc)_3$, toluene, EtOH, THF, HOAc; (g) HCl, EtOH, EtOAc, 53%, three steps.



(ii) the cost of reducing agent and quenching agent in the protection of amino group. In addition, a thick emulsion layer was easily formed when the medium pH was controlled improperly during the quenching step. (iii) flammable and explosive lithium aluminum hydride (LAH) with poor solubility in aprotic solvents.

Changelian *et al*. [10] reported that the nucleophilic substitution of compound **10** with compound **20** is so inefficient that only poor yield compound **3** was obtained after 90 h reaction. This is possibly attributed to the low reaction activity of compound **10**.

Thus, in order to overcome the aforementioned problems, we attempted to optimize the synthetic route of tofacitinib citrate. The obtained results were summarized and reported here.

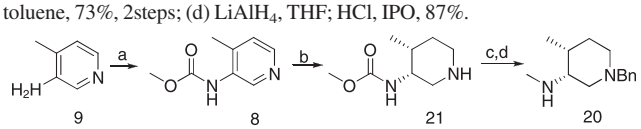
RESULTS AND DISCUSSION

First, the condensation reaction of 3-amino-4-methylpyridine with dimethyl carbonate was examined, as shown in Scheme 3. The carbalkoxylation of 3-amino group, not only protected the amino group, but also provided a convenient way to install methyl group in the final product by reduction of the obtained amide. In which, carbomethoxy is the best one in accordance with the "atom economy" principle. Cai *et al*. [9] reported that the carbalkoxylation of 3-amino-4-methylpyridine with dimethyl carbonate to carbamate was realized with t-BuOK as a catalyst in 2-methyltetrahydrofuran (2-MeTHF). However, when we repeated this experiment, the cream solid was formed when dimethyl carbonate was added to the mixture of compound **9** and t-BuOK in 2-MeTHF due to poor solubility of compound **9** in 2-MeTHF, so a great volume of solvent was required. Thus, methyl chloroformate, instead of dimethyl carbonate, was used as the carbalkoxylation reagent. Unfortunately, its high activity resulted in more impurities. Alternatively, several bases were chosen and examined for this reaction. It was found that sodium hydride (NaH) exhibited excellent catalytic performance in the condensation reaction and made the reaction proceed effectively to present better yield of compound **8**. Furthermore, the employment of NaH resulted in more convenient work-up procedure. One interesting phenomenon was that the addition of NaH to the compound **9** in THF did not cause the obvious release of hydrogen, even prolonging the reaction time or increasing the reaction temperature. Instead, during the addition of dimethyl carbonate, hydrogen was released rapidly. It is possibly attributed to the weaker acid strength of N–H at C3 than that of amino group at C2 and C4 positions (Fig. 2). Hence, without stable resonance structures, amino anion at C3 failed to form quantitatively, dimethyl carbonate reacted with the trace amino anion and promoted the reaction to move forward.

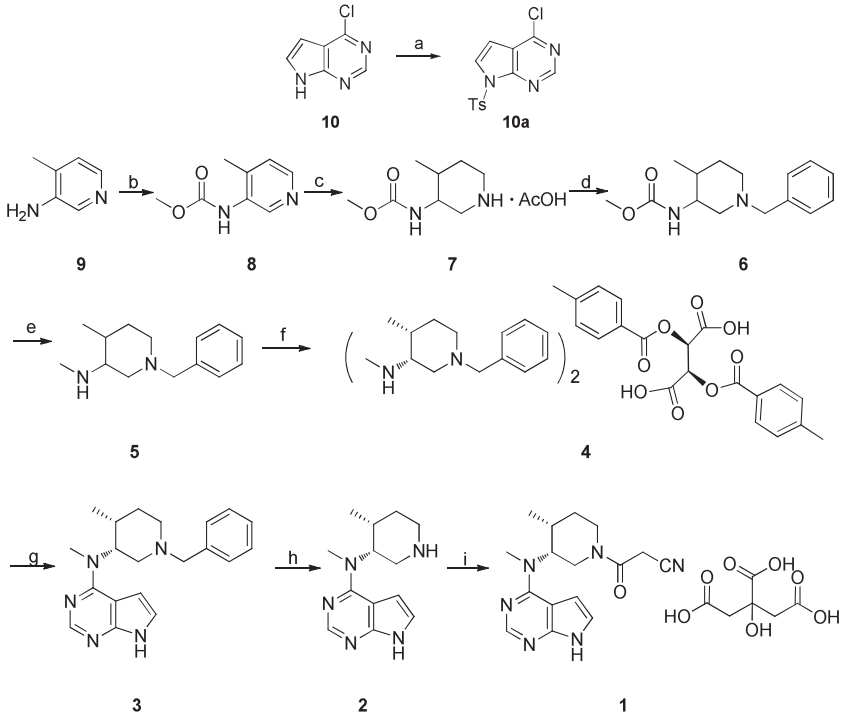
In the benzyl protection of *sec*-amino group in piperidine ring, benzaldehyde and sodium triacetoborohydride were utilized by Pfizer. Except for the cost of the agent, a thick emulsion layer was easily formed during the quenching process. Here, we chose the cheap and commercially available benzyl bromide as benzylation reagent and anhydrous potassium carbonate as catalyst. The reaction parameters, including molar ratio, reaction temperature and catalyst amount were optimized to improve the chemoselectivity of nucleophilic substitution reaction. Thus, under the optimized conditions, the reaction proceeded effectively to afford compound **6** in a satisfied yield.

The obtained methyl carbamate was reduced to the methylamino compound by LAH. However, LAH was characterized with poor solubility in aprotic solvents and high activity in atmosphere. Red-Al, as a solution of sodium dihydro-bis-(2-methoxyethoxy) aluminate in toluene, is always an ideal alternative for the reduction of amide [11,12] with advantages of good solubility in aromatic solvents and low cost. Thus, Red-Al was employed in our experiments, as expected, it mildly and effectively promoted

Scheme 2. The synthesis of compound **20** from **9** reaction condition: (a) t-BuOK, $(MeO)_2CO$, 2-MeTHF; toluene, 87%; (b) 5% Rh/C (JM type C101023-5), AcOH, H_2; (c) PhCHO, $NaHB(OAc)_3$, toluene, 73%, 2steps; (d) $LiAlH_4$, THF; HCl, IPO, 87%.



Scheme 3. Synthetic route for tofacitinib citrate reaction conditions: (a) *p*-toluenesulfonyl chloride, H_2O, acetone; (b) $(MeO)_2CO$, NaH, THF, 35°C; (c) Rh/C, H_2, AcOH, 1.5 MPa; (d) $PhCH_2Br$, K_2CO_3, 2 M HCl/EtOH; (e) Red-Al, toluene, 40°C; (f) L-DTTA, H_2O, IPA, MeOH; (g) **10a**, K_2CO_3, H_2O, reflux; saturated aqueous solution of NaOH; (h) $Pd(OH)_2/C$, TFA, MeOH; (i) ethyl 2-cyanoacetate, DBU, n-BuOH; citrate acid, H_2O.



the reduction reaction of compound **6** to compound **5** at room temperature for only 1 h.

After the chiral resolution of compound **5** with di-*p*-toluoyltartrate, optical pure compound **4** was successfully obtained by recrystallization. The nucleophilic substitution of compound **10** with compound **4** yield compound **3**. However, this reaction always displayed poor yield due to the low activity of compound **10**. Thus, we introduced a tosyl group [13] into compound **10** to promote its activity (compound **10a**). As a result, shown in Scheme 3, the nucleophilic substitution of compound **10a** with compound **4** underwent smoothly in shorter reaction time and an improved yield. This indicated that tosyl group effectively reduced the electron density of pyrrolopyrimidine ring by its strong electron-withdrawing and conjugative effect.

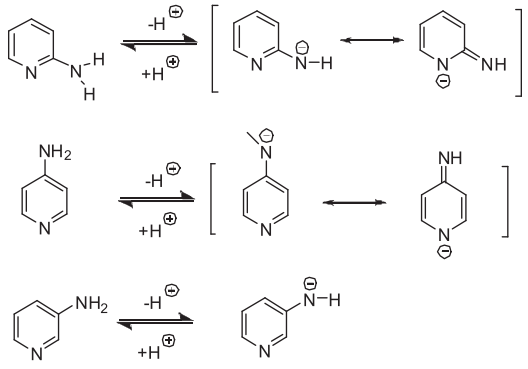


Figure 2. The resonance structures of amino-substituted pyridines.

Subsequently, the tosyl group was hydrolyzed in aqueous solution of sodium hydroxide to afford compound **3** in a nice yield. Thus, a combination of nucleophilic substitution and hydrolysis was successfully carried out in one pot under alkaline condition.

CONCLUSION

To summarize, an environmental friendly, economical and efficient route was successfully developed for the synthesis of tofacitinib citrate. All the related reactions and purification processes were systematically optimized, including the selections of catalyst, benzyl protection agent, and reduction agent, these works obviously improved the yield of tofacitinib citrate. The combination of nucleophilic substitution reaction and hydrolyzation reaction in one pot provided a more acceptable way to compound **3**. The structures of all products and intermediates were confirmed by high-resolution mass spectrometry (HRMS) and ^{1}H-NMR. Thus, with the improved synthetic route, tofacitinib citrate was obtained in a total yield of 13.3% with a purity of 99.9% (based on compound **9**) compared with 8.6% [8,9,14]. This synthetic route is more suitable for large-scale production.

EXPERIMENTAL

Reagents and solvents were obtained from commercial suppliers. All reactions were monitored by thin-layer chromatography. Flash

chromatography was conducted by Reveleris® X2 flash chromatography system (petroleum ether and ethyl acetate, or dichloromethane and methanol, gradient elution). The purity of synthetic compounds was determined with an Agilent 1260 equipped with a Grace C18 column (5 μ, 250 mm × 4.6 mm, Lot No. 55/182). The optical purity was confirmed using an instrument with a Chiralpak ID column (5 μ, 250 mm × 4.6 mm, Lot No.ID00CE-PI029) and a mobile phase of ethanol/hexane or isopropanol/hexane. ^{1}H-NMR was recorded on BRUKER AV400 NMR. HRMS was detected on Bruker microTOF-Q II. Optical rotations were collected at 589 nm on a WXG-4 Disc Polarimeter.

4-Chloro-7-tosyl-7H-pyrrolo[2,3-d]pyrimidine (10a). To a suspension of 4-chloro-7*H*-pyrrolo[2,3-*d*] pyrimidine (15.3 g, 100 mmol), prepared by method of Davoll [15] and Kima [16], in acetone (70 mL) was added *p*-toluenesulfonyl chloride (20.9 g, 110 mmol). After the reaction mixture was cooled below 10°C, sodium hydroxide solution (2 M, 60 mL) was added at a rate to maintain the temperature below 10°C. Then the reaction mixture was warmed to 35°C and stirred for 5 h. After being cooled to room temperature, the resulting solid was isolated by filtration and washed with acetone/water (1:1). After drying under vacuum, 27.4 g (89.4%) of the title compound as white solid was obtained. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.40 (s, 3H), 6.70 (d, J = 4.0 Hz, 1H), 7.33 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 4.0 Hz, 1H), 8.09 (d, J = 8.4 Hz, 2H), 8.77 (s, 1H). ESI-HRMS: Calcd for $C_{13}H_{10}ClN_3O_2S$ (M + H) 308.0255, found 308.0260.

3-Methoxycarbonylamino-4-methylpyridine (8). To a solution of 3-amino-4-methylpyridine (21.6 g, 200 mmol) in absolute tetrahydrofuran (THF, 150 mL) was added the suspension of sodium hydride (5.1 g, 200 mmol) in absolute THF (100 mL) in batches at 0°C. The reaction mixture was stirred at room temperature for 1 h. After being cooled to 0–5°C, dimethyl carbonate was dropwise added to avoid hydrogen rapid release. The reaction mixture was stirred at 40°C for 9 h and then was quenched with water (30 mL). The organic solvent was evaporated to yield a slurry. Petroleum ether was added to it and stirred for 1 h below 5°C. The light brown solid was obtained by filtration and dried in vacuum drying oven at 50°C to afford the crude product **8** (31.0 g, 93.4%), which was directly used in the next step without further purification. Purity: 98.7% (HPLC), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.27 (s, 3H), 3.8 (s, 3H), 6.39 (s, 1H), 7.10 (d, J = 5.2 Hz, 1H), 8.27 (d, J = 4.8 Hz, 1H), 8.86 (s, 1H). ESI-HRMS: Calcd for $C_8H_{10}N_2O_2$ (M + H) 167.0815, found 167.0818.

cis-3-Methoxycarbonylamino-4-methylpiperidine acetate salt (7). A solution of methyl (4-methyl-pyridin-3-yl) carbamate (**8**, 33.2 g, 200 mmol) in acetic acid (200 mL), 5% Rh/C (4.2 g) and acetic acid (100 mL) were added to a autoclave. The reaction mixture was stirred for at least 15 min and then purged sequentially with nitrogen and hydrogen, heated to 55–60°C and pressurized with hydrogen gas at 1.5 MPa until hydrogen uptake ceased. After being cooled to room temperature, the catalyst was filtered through a pad of Celite, and the solvent was removed by azeotropic distillation with toluene. The residue was stirred in ethyl ether (50 mL) for at least 30 min (adding several drops of ethanol to improve the dispersion of precipitate), and the precipitate was filtered to give the desired product **7** (16.1 g, 69.3%) as white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.96 (d, J = 6.4 Hz, 3H), 1.52–1.63 (m, 2H), 1.79–1.80 (m, 1H), 2.04 (s, 3H), 2.72 (t, J = 10.8 Hz, 1H), 2.90 (d, J = 12.0 Hz, 1H), 3.18 (t, J = 5.4 Hz, 2H), 3.67 (s, 3H), 4.01 (d, J = 8.4 Hz, 1H), 7.12–7.15 (m, 3H). ESI-HRMS: Calcd for $C_8H_{16}N_2O_2$ (M + H) 173.1285, found 173.1289.

cis-N-Benzyl-3-methoxycarbonylamino-4-methylpiperidine Hydrochloride (6). To the slurry of **7** (23.2 g, 100 mmol) in dichloromethane (200 mL) was added anhydrous potassium carbonate (34.5 g, 250 mmol) and benzyl bromide (18.8 g, 110 mmol). The reaction mixture was stirred at 40°C for 5 h. The reaction was monitored by GC (90.5% cis). After quenching with water (150 mL), the organic phase was separated and the aqueous phase was extracted by dichloromethane (50 mL × 3). The organic phases were combined, dried over anhydrous $MgSO_4$, and concentrated. The residue was dissolved in the solution of HCl in ethanol (2 M, 60 mL). After the resulting mixture was stirred for at least 1 h, the precipitate was filtered to afford the desired product **6** (27.9 g, 93.7%), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.89 (d, J = 6.8 Hz, 3H), 1.29–1.41 (m, 2H), 1.55–1.60 (m, 1H), 1.92 (t, J = 13.0 Hz, 3H), 2.13 (d, J = 11.6 Hz, 1H), 2.76 (d, J = 11.2 Hz, 2H), 3.425 (s, 2H), 3.67 (s, 3H), 3.64 (s, 3H), 3.77 (d, J = 8.8 Hz, 1H), 5.41 (d, J = 9.2 Hz ,1H), 7.20–7.30 (m, 5H). ESI-HRMS: Calcd for $C_{15}H_{22}N_2O_2$ (M + H) 263.1754, found 263.17763.

cis-N-Benzyl-3-methylamino-4-methylpiperidine (5). Compound **6** (3.0 g, 10 mmol) was added to toluene (30 mL), followed by the addition of sodium bis(2-methoxyethoxy) aluminum dihydride (Red-Al) in toluene (70% solution in toluene, 8.7 g) at a rate to maintain the temperature below 10°C. The resulting orange solution was stirred for 1 h at 40°C and then cooled to 0°C. Aqueous NaOH (1 M, 60 mL) was added over 30 min. The solid was separated and washed with water (30 mL × 3). The aqueous phase was extracted with toluene (30 mL × 3). The organic phases were combined, dried over anhydrous Mg_2SO_4, and concentrated to afford the compound **5** (2.6 g, 93.2% cis by GC) in 90.1% yield, which was directly used in chiral resolution without further purification.

Bis-(3R,4R)-(1-benzyl-4-methylpiperidin-3-yl)-methylamine di-p-toluoyl-L-tartrate (4). Compound **5** (2.9 g, 10 mmol) was added in the mixture of methanol (4 mL) and isopropanol (16 mL), followed by the addition of water (20 mL) and di-*p*-toluoyl-L-tartrate (2.0 g, 5.1 mmol). The reaction mixture was heated to reflux until homogeneous. After being slowly cooled to room temperature, the reaction mixture stood still until the appearance of compound **4** as white solid, filtered to present target compound (1.7 g, 41.4%). ^{1}H-NMR (400 MHz, CD_3OD) δ: 1.00 (d, J = 7.2 Hz), 1.49–1.62 (m, 4H), 1.88–1.90 (m, 2H), 2.17–2.23 (m, 2H), 2.37 (d, J = 6.8 Hz, 12H), 2.82–2.92 (m, 4H), 3.07 (s, 2H), 3.40 (d, J = 12.8 Hz, 2H), 3.61 (d, J = 12.8 Hz, 2H), 5.84 (s, 2H), 7.22–7.28 (m, 6H), 7.30–7.34 (m, 8H), 8.03 (d, J = 8.0 Hz, 4H). ESI-HRMS: Calcd for $C_{14}H_{22}N_2$ (M + H) 219.1856, found 219.1862.

N-((3R,4R)-1-benzyl-4-methylpiperidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (3). To a three-necked flask were added **4** (8.2 g, 10 mmol), potassium carbonate (4.1 g, 30 mmol), water (50 mL), and 4-chloro-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine **10a** (6.2 g, 20 mmol). The reaction mixture was stirred at reflux for 10 h. At that time, a sample was taken from the reaction mixture, which was cooled to room temperature and the resulting solid was filtered to give *N*-((3R,4R)-1-benzyl-4-methylpiperidin-3-yl)-*N*-methyl-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine. $[\alpha]_D^{25}$ = +23.8 (c 0.84, CH_3Cl), ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.82 (d, 3H), 1.52–1.57 (m, 1H), 1.58–1.65 (m, 1H), 1.97–1.99 (m, 1H), 2.05–2.07 (m, 1H), 2.34 (s, 3H), 2.51(s, 1H), 2.61 (s, 1H), 2.72–2.76 (m, 1H), 3.42–3.46 (m, 5H), 5.04 (brs, 1H), 6.89 (s, 1H), 7.18–7.23 (m, 1H), 7.28–7.31 (m, 4H), 7.41 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 4.0 Hz, 1H), 7.96 (d, J = 8.4 Hz, 2H), 8.18 (s, 1H). ESI-HRMS: Calcd for $C_{23}H_{26}N_6O_3S$ (M + H) 490.2271, found 490.2274.

Then, the reaction mixture was cooled to room temperature, saturated NaOH solution (100 mL) was added at a rate of controlling the temperature below 30°C. The resulting reaction mixture was refluxed for 6 h. After being cooled to room temperature, the reaction mixture was stirred for 2 h at room temperature. The solid was isolated by filtration to afford off-white solid **3** (5.0 g, 75.0%). $[\alpha]_D^{25} = +29.4$ (c 1.01, MeOH); purity: 99.8% (C18 HPLC); 100% ee (chiralpak ID, 95:5 hexane/ isopropanol, retention time of 16.86 min); ^{1}H-NMR(400 MHz, DMSO-d_6) δ: 0.88 (d, $J = 6.8$ Hz, 3H), 1.59–1.63 (m, 1H), 1.70 (s, 1H), 2.13 (s, 1H), 2.28 (s, 1H), 2.54 (dd, $J_1 = 4.0$ Hz, $J_2 = 11.2$ Hz, 1H), 2.61 (s, 1H), 2.77 (dd, $J_1 = 6.0$ Hz, $J_2 = 11.2$ Hz, 1H), 3.44–3.52 (m, 5H), 5.09 (s, 1H), 6.53 (s, 1H), 7.08 (t, $J = 2.6$ Hz, 1H), 7.20–7.23 (m, 1H), 7.28–7.31 (m, 4H), 8.05 (s, 1H), 11.55 (s, 1H). ESI-HRMS: Calcd for $C_{20}H_{25}N_5$ (M + H) 336.2183, found 336.2189.

N-methyl-N-((3R,4R)-4-methylpiperidin-3-yl)-7H-pyrrolo[2,3-d] pyrimidin-4-amine (2). To the mixture of compound **3** (6.7 g, 20 mmol), methanol (100 mL) and trifluoroacetic acid (3.4 g, 30 mmol) was added 20 wt% $Pd(OH)_2$/C (5.0 g, 54% water wet). The reaction mixture was purged sequentially with nitrogen and hydrogen, and pressurized with hydrogen gas at 70–80 psi for 5 h. After the catalyst was filtered through a pad of Celite, the solvent was removed under reduced pressure, and the residue was dissolved in dichloromethane. The organic phase was washed with 10% aqueous NaOH, dried over anhydrous $MgSO_4$, and concentrated to afford colorless oil **2**, which was directly used in the next step without further purification.

3-((3R,4R)-4-methyl-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl) amino)piperidin-1-yl)-3-oxopropanenitrile citrate (1). To the mixture of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 1.5 g, 10 mmol) and ethyl cyanacetate (3.4 g, 30 mmol) in n-BuOH (20 mL) was added the amine **2** (4.9 g, 20 mmol). The reaction mixture was stirred at 40°C for 18 h. Upon reaction completion, citric acid monohydrate (6.3 g, 30 mmol) was added, followed by the addition of water (1 mL) and 1-butanol (10 mL). The mixture was heated to 80°C and held at that temperature for 30 min. After being cooled slowly to 10–15°C and standing for 12 h, the solid was filtered, washed with 1-butanol (20 mL), and dried in a vacuum oven to afford **1** (9.1, 87.6%) as an off-white solid. $[\alpha]_D^{25}$ (free base) = 10.1 (c 0.99, MeOH); Purity (free base): 99.9% (C18 HPLC); 100% ee (chiralpak ID, 70:30 hexanes/ethanol, retention time of 21.16 min). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.00–1.01 (m, 3H), 1.54–1.79 (m, 2H), 2.35–2.50 (m, 1H), 2.69 (ABq, $J = 15.2$ Hz, 4H), 3.16–3.17 (m, 3H), 3.36–3.45 (m, 1H), 3.61–4.14 (m, 5H), 4.85 (s, 1H), 6.55 (s, 1H), 7.12 (d, $J = 2.8$ Hz, 1H), 8.09 (d, $J = 5.6$ Hz, 1H), 11.63 (s, 1H), 12.24–12.41 (brs, 2H). ESI-HRMS: Calcd for $C_{16}H_{20}N_6O$ (M + H) 313.1771, found 313.1777.

REFERENCES AND NOTES

[1] Flanagan, M. E.; Blumenkopf, T. A.; Brissette, W. H.; Brown, M. F.; Casavant, J. M.; Shang-Poa, C.; Doty, J. L.; Elliott, E. A.; Fisher, M. B.; Hines, M.; Kent, C.; Kudlacz, E. M.; Lillie, B. M.; Magnuson, K. S.; McCurdy, S. P.; Munchhof, M. J.; Perry, B. D.; Sawyer, P. S.; Strelevitz, T. J.; Subramanyam, C.; Sun, J.; Whipple, D. A.; Changelian, P. S. J Med Chem 2010, 53, 8468.

[2] Ghoreschi, K.; Jesson, M. I.; Li, X.; Lee, J. L.; Ghosh, S.; Alsup, J. W.; Warner, J. D.; Tanaka, M.; Steward-Tharp, S. M.; Gadina, M.; Thomas, C. J.; Minnerly, J. C.; Storer, C. E.; LaBranche, T. P.; Radi, Z. A.; Dowty, M. E.; Head, R. D.; Meyer, D. M.; Kishore N.; O'Shea, J. J. J Immunol 2011, 186, 4234.

[3] Williams, N. K.; Bamert, R. S.; Patel, O.; Wang, C.; Walden, P. M.; Wilks, A. F.; Fantino, E.; Rossjohn, J.; Lucet, I. S. J Mol Biol 2009, 387, 219.

[4] Dymock, B. W.; See, C. S. Exp Opin Ther Patents 2013, 23, 449.

[5] Burmester, G.R.; Blanco, R.; Charles-Schoeman, C.; Wollenhaupt, J.; Zerbini, C.; Benda, B.; Gruben, D.; Wallenstein, G.; Krishnaswami, S.; Zwillich, S. H.; Koncz, T.; Soma, K.; Bradley, J.; Mebus, C. Lancet 2013, 381, 451.

[6] Larsen, S. D.; Grieco, P. A. J Am Chem Soc 1985, 107, 1768.

[7] Brown Ripin, D. H.; Abele, S.; Cai, W. L.; Blumenkopf, T.; Casavant, J. M.; Doty, J. L.; Flanagan, M.; Koecher, C.; Laue, K. W.; McCarthy, K.; Meltz, C.; Munchhoff, M.; Pouwer, K.; Shah, B.; Sun, J. M.; Teixeira, J.; Vries, T.; Whipple, D. A.; Wilcox, G. Org Proc Res Dev 2003, 7, 115.

[8] Wu, J. J.; Tang, W. J.; Pettman, A.; Xiao, J. L. Advanced Synthesis & Catalysis 2013, 355, 35.

[9] Cai, W. L.; Colony, J. L.; Frost H.; Hudspeth, J. P.; Kendall, P. M.; Krishnan, A. M.; Makowski, T.; Mazur, D. J.; Phillips, J.; Brown Ripin, D. H.; Ruggeri, S. G.; Stearns, J. F.; White, T. D. Organic Process Research & Development 2005, 9, 51.

[10] Changelian, P. S.; Zwillch, S. H. International Patent, 2,008,029,237; Chem Abstr 2008, 148, 347337.

[11] Hoffmann-Emery, F.; Hilpert, H.; Scalone, M.; Waldmeier, P. J Org Chem 2006, 71, 2000.

[12] Dix, A. V.; Meseck, C. M.; Lowe, A. J.; Mitchell, M. O. Bioorg Med Chem Lett 2006, 16, 2522.

[13] Ruggeri, S.G.; Hawkins, J. M.; Makowski, T. M.; Rutherford, J. L.; Urban, F. J. International patent, 2,007,012,953; Chem Abstr 2007, 146, 206328.

[14] Price, K. E.; Larrivée-Aboussafy, C.; Lillie, B. M.; McLaughlin, R. W.; Mustakis, J.; Hettenbach, K. W.; Hawkins, J. M.; Vaidyanathan, R. Org Lett 2009, 11, 2003.

[15] Davoll, J. J Am Soc 1960, 82, 131.

[16] Kima, J; Wang, L. G.; Li, Y. F.; Becnel, K. D.; Frey, K. M.; Garforth, S. J.; Prasad, V. R.; Schinazi, R. F.; Liotta, D. C.; Anderson, K. S. Bioorg Med Chem Lett 2012, 22, 4064.