Organic Process Research & Development **2009**, *13*, 928–932

Development of a Practical Synthesis of a Purine Nucleoside Phosphorylase Inhibitor: BCX-4208

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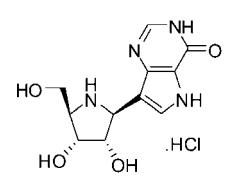
Abstract:

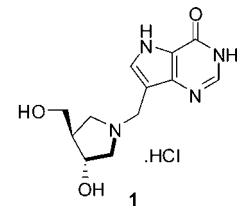
A practical synthesis of the purine nucleoside phosphorylase (PNP) inhibitor BCX-4208 (1) was accomplished in three telescoped steps. Mannich condensation of the 4-benzyloxy-9-deazahypoxanthine with (3*R*,4*R*)-3-hydroxy-4-(hydroxymethyl)pyrrolidine and formaldehyde followed by removal of the protecting group and crystallization furnished the desired product as a hydrochloride salt in 85% overall yield and 99.8% purity. A scalable synthesis of 9-deazahypoxanthine is also reported.

Introduction

The enzyme purine nucleoside phosphorylase (PNP) catalyzes the cleavage of deoxyribonucleosides to the corresponding purine base and sugar-1-phosphate. Children who are deficient in PNP suffer from T-cell immunodeficiency due to the accumulation of dGTP which prevents the proliferation of T-lymphocytes.[1−3] Hence, inhibitors against PNP are immunosuppressive in nature and are active against T-cell malignancies and T-cell disorders.[4] The discovery of potent PNP inhibitors was based on studies by Schramm and co-workers on transition state analysis of the human PNP.[5] The first generation of PNP inhibitors includes forodesine HCl, a compound currently in clinical trials at BioCryst Pharmaceuticals Inc. for relapsed/resistant T-cell leukemia.[6] A second-generation transition state analogue has been identified as BCX-4208 (**1**) whose primary advantage is that it binds more tightly to human PNP.[7a,b] This molecule was prepared by Schramm, Tyler, and co-workers from whom BioCryst acquired the rights. While this was an excellent synthesis for small-scale work, multikilogram quantities required a new process. Herein is described our development of a practical synthesis of **1** and 9-deazahypoxanthine (**2**).



Forodesine HCl



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Results and Discussion

The retrosynthetic analysis of **1** suggested that the molecule could be assembled by the formal condensation of formaldehyde, (3*R*,4*R*)-3-hydroxy-4-(hydroxymethyl)pyrrolidine HCl (**3**) and 9-deazahypoxanthine (**2**) (see Scheme 1). This approach was successful.[7c]

Synthesis of 9-Deazahypoxanthine (2). There have been relatively few syntheses of the pyrrolo[3,2-*d*]pyrimidine ring system. Two early procedures suffered from either low yields or purification challenges and were unlikely to provide scalable processes.[8−10] A recent report[11] describing a direct synthetic route by the condensation of isoxazole and diethyl aminomalonate, appeared initially promising. However, attempts to repeat the process on a large scale led to problems for sourcing isoxazole, monitoring the reaction, workup, stability of intermediates, and reproducibility. This prompted the search for an alternate route.

A report published[12] in 1996 led directly to our multikilogram process as shown in Scheme 2. The pyrrole **5** could be synthesized by refluxing ethyl (ethoxymethylene)cyanoacetate, diethyl aminomalonate, and sodium methoxide in methanol for 24 h. Neutralization of the reaction to ~pH 7 was carried out by addition of acetic acid. This was followed by evaporation of the solvent (~65% of the original volume) to furnish a slurry. Addition of water followed by rapid agitation of the slurry produced **5** in 79% yield and in sufficient purity to be carried directly to the next step. Condensation of **5** with formamidine acetate in ethanol under reflux conditions for 20 h, followed by addition of water to the hot solution resulted in the precipitation of **6** in 72% yield.

The final step was the base-catalyzed decarboxylation of compound **6**. A series of experiments with different bases were attempted, and 10% KOH solution gave the best results.[13−16]

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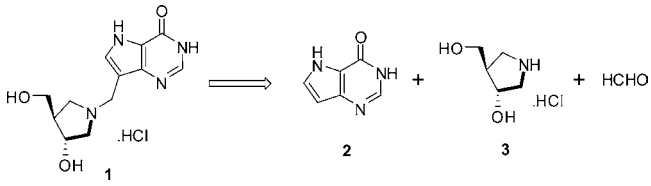
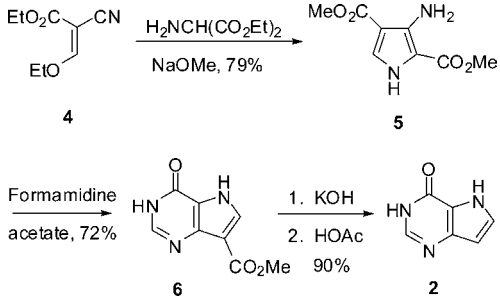
10.1021/op9001142 CCC: $40.75
Published on Web 07/24/2009

Scheme 1. **Retrosynthetic analysis of BCX-4208 (1)**



Scheme 2. **Synthesis of 9-deazahypoxanthine (2)**

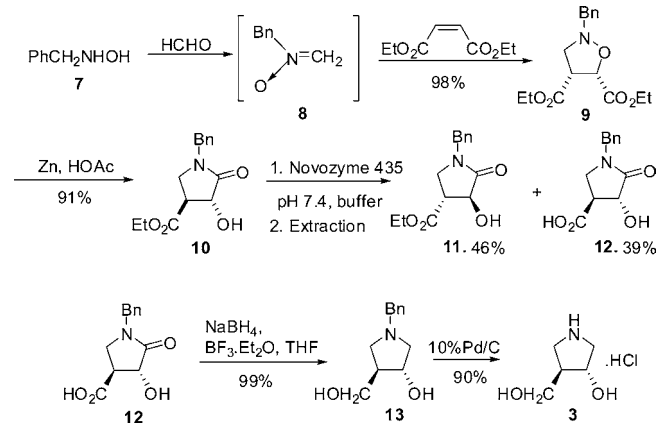


Our optimized process consisted of refluxing **6** in 10% KOH solution for 40 h, followed by neutralization with acid to isolate **2** in 90% yield. The synthesis of 9-deazahypoxanthine (**2**) was thus accomplished in three steps in 51% overall yield and is currently in use for the synthesis of multikilogram quantities.

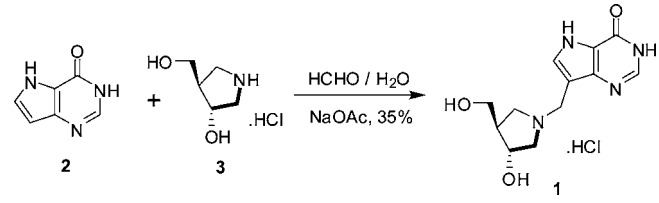
Synthesis of (3*R*,4*R*)-3-Hydroxy-4-(hydroxymethyl)pyrrolidine HCl (3). The synthesis of the western section of **1**, 3-hydroxy-4-(hydroxymethyl)pyrrolidine HCl (**3**) was first prepared by Biel and co-workers[17] from *N*-benzyl glycinate and ethyl acrylate as a mixture of *cis/trans* isomers. The synthesis of pure enantiomer (3*R*,4*R*)-3-hydroxy-4-(hydroxymethyl)pyrrolidine HCl (**3**) was first made from D-xylose over several steps but in low yields by Pedersen and co-workers.[18]

Asymmetric 1,3-dipolar cycloaddition of an azomethine ylide and camphor sultam auxiliary gave **3**, but was unsuitable for large-scale operation as the camphor sultam auxiliary was very expensive.[19] An elegant synthesis by Tyler and co-workers for the synthesis of **3** has been published.[20] Nitrone cycloaddition to diethyl maleate, followed by resolution of the desired isomers by enzymatic transformation, gave the desired product in excellent yields. As shown in Scheme 3, the 1,3-cycloaddition reaction of nitrone **8** (formed *in situ*) to diethyl maleate proceeded to give the diester cyclized compound **9**. Reductive cleavage of the N−O bond with zinc and acetic acid furnished a racemic mixture of β-amino alcohol, **10**. A number of enzymes were screened for effective resolution. Among them, Novozyme 435 gave the best results in terms of yield and enantiomeric purity. The compounds **11** and **12** were separated efficiently by extraction using ethyl acetate. Reduction of the carboxylic acid using borane generated *in situ* from sodium

Scheme 3. **Synthesis of (3*R*,4*R*)-3-hydroxy-4-(hydroxymethyl)pyrrolidine HCl (3)**



Scheme 4. **Synthesis of BCX-4208 (1) via Mannich condensation**



borohydride and $BF_3 \cdot Et_2O$ in THF gave **13** as a crystalline compound. Hydrogenolysis of **13** furnished **3** as a light-orange syrup. The new process for the synthesis of **3** had fewer transformations than previously known methods. The method was scaled up to multikilogram quantities by BioCryst without any further modifications to the process.

Initial Approach to BCX-4208 (1). The Mannich reaction provides a direct route for the formation of carbon−carbon and carbon−nitrogen bonds.[21] Tyler and co-workers have reported the synthesis of several analogues of **1** using the Mannich reaction.[7c] A mixture of (3*R*,4*R*)-3-hydroxy-4-(hydroxymethyl)pyrrolidine HCl (**3**), sodium acetate, water, aqueous formaldehyde, and 9-deazahypoxanthine (**2**) was heated. The resulting solid residue produced after workup was purified by column chromatography and converted to the hydrochloride salt in 35% yield. Although **1** could be synthesized by this one-step operation, the process was not amenable to scale up as it involved column chromatography and the active pharmaceutical ingredient (API) was contaminated with process impurities resulting in only a 35% yield (see Scheme 4).

The low yields for the Mannich reactions could possibly due to the compatibility of the two starting materials. Compound **2** can be dissolved with great difficulty in hot water. Hence, the Mannich reaction had to be carried out under reflux conditions in water, thereby resulting in the formation of process impurities. These have been identified as 9-hydroxyl 9-deazahypoxanthine, C-9 methylene-bridged dimer of **2**, and unreacted **2**.

Process Development of BCX-4208, (1). A number of derivatives of **2** were synthesized by introduction of protecting groups at the C-4 position of **2**. Among those included the widely used methyl and the benzyl groups. The methyl group

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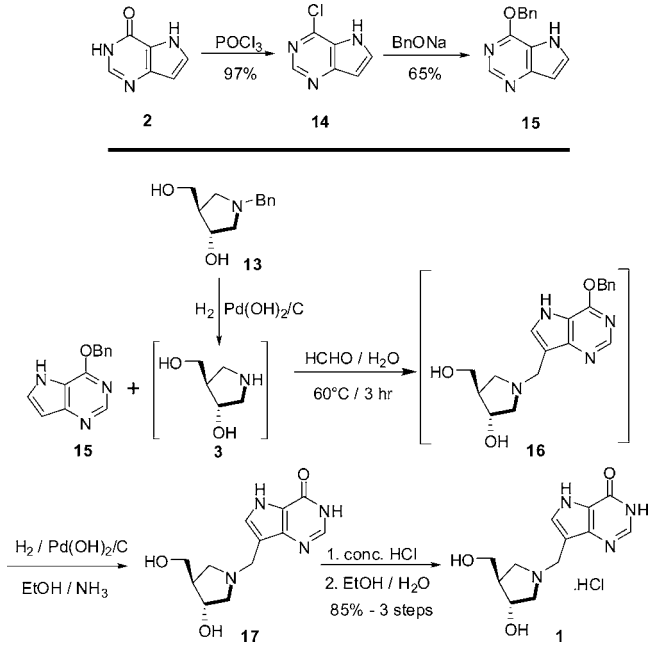
Scheme 5. **Improved synthetic route to make 4-benzyloxy 9-deazahypoxanthine (15) and BCX- 4208 (1)**



was introduced by reacting **14** with 1 *M* sodium methoxide in methanol under reflux conditions. Mannich coupling under standard conditions, followed by deprotection of the methoxy group with 37% hydrochloric acid and methanol (1:1, v/v) under reflux conditions resulted in formation of a unstable dihydrochloride salt. Introduction of a benzyl group at the C-4 position would eliminate this problem, thereby allowing a handle on the outcome of the final product. Accordingly, **2** was treated with neat phosphorous oxychloride to produce **14** as a yellow solid in 97% yield after workup.[22] This was in turn treated with 1 *M* sodium benzyloxide solution at reflux to furnish **15** as a tan solid in 65% yield. While this required the addition of a protection/deprotection sequence to the process, the overall improvements in yield and processability were deemed a good trade-off.

Once **13** was hydrogenated using $Pd(OH)_2/C$ in ethanol to furnish **3**, the catalyst was filtered over a Celite pad, and the filtrate was telescoped to the next step. The reaction mixture was heated followed by addition of aqueous formaldehyde and **15**. Upon completion of the reaction, the mixture was cooled, and the filtrate (containing product **16**) was telescoped to the next step. The filtrate was presaturated with ammonia and hydrogenated over $Pd(OH)_2/C$ to give **17** as a solid following workup. Following the production of the HCl salt **1** in aqueous ethanol by the addition of 37% hydrochloric acid and recrystallization from the same solvent mixture, crystals of the API were isolated in 85% yield and 99.8 wt % purity (see Scheme 5).

Optimized Process Route-Pilot Scale. The kilo-scale process was examined for further improvements prior to scale-up in the pilot plant. The first step of the process required a long reaction time for complete hydrogenation and used inconveniently large volumes of ethanol and water. Experimentation with different catalysts showed that the hydrogenation step was sensitive to catalyst-poisoning agents. This problem was overcome by a charcoal treatment of the solution prior to hydrogenation. The reaction was observed to be complete in a short period of time using absolute ethanol and at higher temperature. Compound **13** was dissolved in absolute ethanol and passed through an activated charcoal filter. Hydrogenation was carried out using 10% Pd/C catalyst. The reaction was complete in 5 h as compared to 24 h on kilo scale. The catalyst was filtered off and the filtrate telescoped to the next step. The Mannich reaction was explored by varying the time and temperature. It was concluded that longer reaction times led to slight improvement in the yield without affecting the formation of any new side products. The reaction proceeded to completion in 6 h (as compared to 3 h on kilo scale). The final hydrogenation step was optimized by adjusting the concentration with ethanol/water, and the reaction was carried out using 10% Pd/C catalyst. The catalyst was filtered at 40−50 °C to facilitate rapid filtration. The filtrate was distilled under vacuum to ∼40% of the original volume to furnish a suspension of the crude product that was filtered and dried. The crude product was suspended in a mixture of ethanol/water and heated to reflux with the addition of 32% hydrochloric acid. The suspension was cooled to ambient temperature and stirred for an additional 3 h to precipitate out the product. Crystallization of the crude API in water/ethanol mixture furnished crystals of BCX-4208 (**1**) in yields comparable to the kilo-scale process.

Conclusion

An improved method for the manufacture of BCX-4208, (**1**) has been developed. The process was streamlined with the help of three telescoped steps without any column chromatography or isolation of the intermediates to furnish the desired product in excellent yields. The process has been successfully scaled up to 25 kg batches for use in toxicology studies and ongoing phase I/II clinical trials.

Experimental Section

General. The samples were analyzed using HPLC run on a Hewlett-Packard system, model no. HP-1100 with a photodiode array detector. Analytical HPLC conditions were: Zorbax SB-C3 150 mm × 4.6 mm and Zorbax SB-C3 250 mm × 4.6 mm plumbed in series. Mobile phase A: 0.05 M formic acid, B: acetonitrile. Gradient from 0 to 13% B for 12 min; 13−50% B for 6 min; hold 50% B for 4 min; 0% B for 2 min; hold 100% A for 2 min; post time 10 min; flow rate: 1.3 mL/min; 25 °C.

3-Amino-1H-pyrrole-2,4-dicarboxylic Acid Dimethylester (5). Sodium methoxide (9.18 L, 42.5 mol) was added to a solution of methanol (20 L) and diethyl aminomalonate hydrochloride (3.0 kg, 14.1 mol) followed by ethyl (ethoxymethylene)cyanoacetate **4** (2.40 kg, 14.1 mol) added over 1 h at ≤45 °C. The mixture was refluxed for 24 h and then cooled to ambient temperature. The reaction mixture was neutralized by addition of glacial acetic acid (1.62 L, 28.3 mol), and solvent (∼55%) was distilled to furnish a slurry. Water (15 L) was added to the slurry followed by strong agitation. The slurry was filtered, and the solids were washed with water (5 L) to furnish **5** (2.23 kg, 79%) as a tan solid. Mp 165 −166 °C; ^{1}H NMR

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(300 MHz, DMSO-d_6): δ 11.66 (bs, 1 H), 7.26 (s, 1 H), 5.64 (s, 2 H), 3.74−3.72 (2 × s, 6 H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.3, 161.3, 126.7, 103.9, 101.8, 73.7, 50.8, 50.7; IR ν_{max} 3491, 3265, 1676 cm^{-1}. Elem. anal.: calculated for $C_8H_{10}N_2O_4$: C, 48.46; H, 5.08; N, 14.13, found: C, 48.49; H, 5.13; N, 14.15.

4-Oxo-4,5-dihydro-3H-pyrrolo[3,2-*d*]pyrimidine-7-carboxylic Acid Methyl Ester (6). Formamidine acetate (4.70 kg, 45.1 mol) was added to a solution of compound **5** (2.20 kg, 11.2 mol) in ethanol (20 L), and the reaction was refluxed for 20 h. Water (5 L) was added to the hot solution, and the resulting slurry was filtered to furnish **6** (1.57 kg, 72%) as a tan solid. Mp 220−222 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.97−7.96 (2 × s, 2 H), 3.73 (s, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.5, 154.4, 144.8, 143.7, 132.7, 119.6, 112.1, 51.1; IR ν_{max} 3432, 2902, 1676 cm^{-1}. Elem. anal.: calculated for $C_8H_7N_3O_3$: C, 49.72; H, 3.65; N, 21.76, found: C, 50.02; H, 3.59; N, 21.72.

3,5-Dihydropyrrolo[3,2-*d*]pyrimidin-4-one (2). Compound **6** (1.08 kg, 5.67 mol) was refluxed with 10% aqueous KOH (11 L, 22.5 mol) for 40 h. The reaction mixture was cooled to 25−35 °C and then neutralized to pH 6.5−7.5 with addition of glacial acetic acid (1.32 L, 22.5 mol) with gas evolution. The precipitate was filtered and washed with water (5 L). The solid was dried *in vacuo* at 80 °C to furnish **2 (**688.5 g, 90%) as a solid. Mp 260−262 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.05 (bs, 1 H), 11.82 (bs, 1 H), 7.77 (s, 1 H), 7.36 (s, 1 H), 6.35 (s, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 153.8, 144.8, 141.6, 127.5, 117.9, 103.1; IR ν_{max} 3109, 3041, 1674 cm^{-1}. Elem. anal.: calculated for $C_6H_5N_3O$: C, 53.33; H, 3.73; N, 31.10, found: C, 53.35; H, 3.73; N, 31.06.

4-Chloro-5*H*-pyrrolo[3,2-*d*]pyrimidine (14). Compound **2** (500 g, 3.72 mol) was refluxed for 1 h with phosphorous oxychloride (970 mL, 10.4 mol). The reaction mixture was cooled to 25−35 °C and poured onto chipped ice (14 L) with vigorous stirring. The pH of the solution was adjusted to 7.0−8.5 using 30% ammonium hydroxide (∼1.5 L). The resulting precipitate was collected by vacuum filtration and washed with water (4 L). The resulting solid was dried *in vacuo* at 100 °C to furnish **14** (551 g, 97%) as a yellow solid. Mp 191−193 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.43 (s, 1 H, D_2O exchangeable), 8.61 (s, 1 H), 7.97 (dd, J = 2.8, 2.8 Hz, 1 H), 6.72 (dd, J = 1.7, 3.5 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 151.3, 149.6, 142.1, 134.8, 124.3, 102.7; IR ν_{max} 3128, 3078, 2979, 1621 cm^{-1}. Elem. anal.: calculated for $C_6H_4N_3Cl$: C, 46.93; H, 2.63; N, 27.36; Cl 23.09, found: C, 47.10; H, 2.79; N, 27.15; Cl 22.93.

4-Benzyloxy-5*H*-pyrrolo[3,2-*d*]pyrimidine (15). Compound **14** (540 g, 3.51 mol) was refluxed for 48 h with 1 *M* solution of sodium benzyloxide in benzyl alcohol (3 L). The reaction mixture was cooled to 0−5 °C and neutralized to pH 6.5−7.5 with addition of glacial acetic acid (750 mL). Water (8 L) was added to the reaction mixture and stirred for 2 h followed by addition of ethyl acetate (10 L). The phases were split, the aqueous phase was extracted further with ethyl acetate (3 × 10 L), and the organic layer was evaporated to dryness to furnish a syrup. The crude was crystallized in hot ethyl acetate (6 L). The product was filtered and dried overnight to furnish **15** (515 g, 65%) as a tan-colored powder. Mp 158−160 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.33 (s, 1 H), 7.20−7.70 (m, 6 H), 6.57 (d, J = 3.6 Hz, 1 H), 5.58 (s, 2 H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 154.8, 150.3, 148.3, 136.6, 130.4, 128.4, 128.1, 128.0, 114.1, 101.6, 66.8; IR ν_{max} 3128, 2979, 1621 cm^{-1}. Elem. anal.: calculated for $C_{13}H_{11}N_3O$: C, 69.35; H, 4.92; N, 18.66, found: C, 69.30; H, 4.92; N, 18.36.

(3*R*,4*R*)-3-Hydroxy-4-(hydroxymethyl)pyrrolidine (3). A mixture of **13** (45 kg, 217 mol), activated charcoal (2.25 kg), and ethanol (110 L) was stirred at 20−25 °C for 0.5 h and pressure filtered. The filtrate, 10% Pd/C catalyst (7.6 kg), and ethanol (15.7 kg) were pressurized to 5 bar with hydrogen gas and heated to 55−60 °C. The reaction mixture was stirred for 5 h until there was no more uptake of hydrogen gas. Upon completion of the reaction (<5% of **13** remaining by HPLC) the reactor was cooled to 20−25 °C. The pressure was released from the vessel, which was then flushed several times with nitrogen. The contents of the vessel were filtered under pressure through a lenticular filter, and the filtrate (303 kg, containing **3**) was telescoped to the next step.

(4*R*)-1-((4-Benzyloxy)-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-methyl)-4-(hydroxymethyl)pyrrolidin-3-ol (16). The reactor was charged with the ethanolic solution of **3** (303 kg of ethanol containing ∼25.4 kg of **3**). Ethanol (∼220 L) was distilled off at reduced pressure (1.5 bar). Water (80 kg) was charged followed by the addition of **15** (42.5 kg, 189 mol) and then 37% aqueous formaldehyde solution (21.6 kg, 266 mol). The contents were heated to 55−60 °C for 6 h. Upon completion of the reaction (<5% of **15** remaining by HPLC) the reaction mixture was cooled to 20−25 °C followed by the addition of activated charcoal (1.5 kg), and the mixture stirred for 30 min. The contents were filtered under pressure through a lenticular filter, and the filtrate (412 kg) was telescoped to the next step.

7-(((4*R*)-3-Hydroxymethyl)pyrrolidin-1-yl)methyl-3*H*-pyrrolo[3,2-*d*]pyrimidin-4(5*H*)-one (17). The filtrate (∼206 kg) containing **16** was transferred to a pressure vessel, and 25% ammonia solution (6.4 kg) was added followed by the addition of 10% Pd/C (1.0 kg) catalyst suspended in water (10 kg). The vessel was pressurized to 3 bar with hydrogen gas, and the contents were stirred at 20−25 °C until there was no more uptake of hydrogen gas. Upon completion of the reaction (<5% of **16** remaining by HPLC) the pressure was released from the vessel which was then flushed several times with nitrogen. The contents was heated to 45−50 °C and then filtered under pressure through a lenticular filter. The filtrate was concentrated by distilling off ∼40% of the solvent under vacuum (1.5 bar) at 45 °C until **17** precipitated out of the reaction mixture. The solid was isolated by centrifugation and then dried under vacuum at 60 °C to furnish the desired product, **17** (25 kg), as a free base.

7-(((4*R*)-3-Hydroxymethyl)pyrrolidin-1-yl)methyl-3*H*-pyrrolo[3,2-*d*]pyrimidin-4(5*H*)-one Hydrochloride (1). A solution of **17** (25 kg, 94.7 mol), water (32 kg), 32% hydrochloric acid (10.3 kg, 0.95 equiv), and ethanol (224 kg) were stirred for 30 min and then refluxed at 72−75 °C for 1 h. The suspension was cooled to 20−25 °C over 1 h and stirred for an additional 3 h. The product was filtered by centrifugation,

washed with ethanol (100 kg), and dried in a vacuum oven at 70 °C. The crude was taken directly to the crystallization step.

The crude material and activated charcoal (1.5 kg) in water (60 kg) were stirred at 60 °C for 30 min and then transferred via a lenticular filter to another reactor. The crude was further washed with water (15 kg). The filtrate (~50 kg of water) was distilled down at 1.5 bar followed by addition of ethanol (235 kg) to furnish a suspension. Upon complete addition of ethanol, the contents of the reactor were refluxed for 1 h at 72−75 °C. The contents of the reactor was cooled to 20−25 °C over 1 h and stirred for an additional 3 h. The precipitated product was isolated by centrifugation and then dried under vacuum at 70 °C to constant weight to furnish BCX-4208 (**1**) as a white solid (25.3 kg, 85% yield). HPLC analysis: 99.8%. Mp 226 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.46 (bs, 1 H), 12.14 (bs, 1 H), 10.89 (bs, 1 H), 7.91 (d, J = 2.6 Hz, 1 H), 7.65 (d, J = 2.6 Hz, 1 H), 5.49 (s, 1 H), 4.90 (bs, 1 H), 4.33 (m, 2 H), 4.07−4.14 (m, 1 H), 3.04−3.56 (m, 6 H), 2.22 (m, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 153.5, 143.6, 142.6, 129.8, 117.9, 105.9, 70.1, 59.8, 58.6, 53.2, 48.2, 47.1; IR ν_{max} 3339, 3104, 2900, 1706, 1597 cm^{-1}. Elem. anal.: calculated for $C_{12}H_{17}ClN_4O_3$: C, 47.93; H, 5.70; N, 18.63; Cl, 11.79, found: C, 47.70; H, 5.45; N, 18.43; Cl, 11.90.

Received for review April 9, 2009.

OP9001142