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# The Chemical Development of CI-972 and CI-1000: A Continuous Nitration, A $MgCl_2/Et_3N$-Mediated C-Alkylation of a Chloronitropyrimidine, A Catalytic Protodediazotization of a Diazonium Salt, and an Air Oxidation of an Amine

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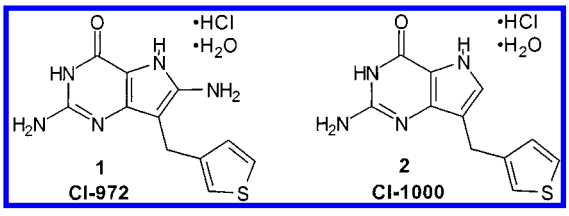
**Abstract:**

**Efficient, large-scale processes were developed for the preparation of the potent PNP inhibitors 2,6-diamino-3,5-dihydro-7-(3-thienylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one hydrochloride, monohydrate (1) and 2-amino-3,5-dihydro-7-(3-thienylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one hydrochloride, monohydrate (2). We report (1) a safe, continuous nitration process for the preparation of 2-amino-6-chloro-5-nitro-4-pyrimidinol (8a) and its stable diisopropylamine salt (8b), (2) the first $MgCl_2/Et_3N$-mediated C-alkylation of a chloronitropyrimidine, (3) a rare catalytic protodediazotization of the diazonium salt 2-amino-4-oxo-7-thiophen-3-ylmethyl-4,5-dihydro-3H-pyrrolo[3,2-*d*]pyrimidine-6-diazonium chloride (14), (4) a single-step process to prepare 2 directly from 2-amino-6-hydroxy-5-nitro-α-(3-thienylmethyl)-4-pyrimidineacetonitrile (12) using a sponge nickel-catalyzed reduction, and (5) a method to convert the over-reduction by-product 2,5-diamino-6-(1-aminomethyl-2-thiophen-3-yl-ethyl)-pyrimidin-4-ol (16) into 2 using air oxidation.**

## Introduction

PNP inhibitors have been under investigation in our Discovery Research program[1] and in those of other companies[2] for the treatment of rheumatoid arthritis and other autoimmune diseases. In a recent effort toward introducing the first PNP inhibitors into man the need for significant amounts of material was required for toxicological, formulation, and phase I clinical studies of **1** (CI-972) and its back-up compound **2** (CI-1000). The similarities between these compounds lent themselves nicely to a convergent synthesis for their production.




## Results and Discussion

**Synthesis of 1.** The general route to **1** was established by medicinal chemists; however, significant modifications to reagents and conditions were required to allow consistent preparation of pure drug substance safely and in high yield on a multikilogram scale.

The synthesis of **1** from thiophene-3-carboxaldehyde (**4**) was completed in six synthetic steps. Knovenagel condensation of methyl cyanoacetate (**3**) and aldehyde **4** in isopropyl alcohol (IPA) with catalytic diisopropylamine (DIPA) afforded the acrylate (**5**) in 93% yield (Scheme 1). The condensation was originally run in toluene or dioxane with piperidine; however, the acrylate **5** could only be obtained in 86% yield if a second crop was recovered. Substituting DIPA for piperidine improved the yield to 92%, but isolation in two crops was still necessary. By using IPA and DIPA, the acrylate **5** could be isolated in the same yield but in a single crop (Table 1).

The acrylate **5** was converted to cyanoester **6** by catalytic hydrogenation over 5% Pd on carbon in 89% yield. This reduction was originally carried out using sodium cyanoborohydride in methanol. A more economical and safe catalytic method in IPA was developed to give crystalline **6** of ≥99.7% purity (Table 2). IPA-wet acrylate **5** was successfully reduced to **6** on 100 kg scale (batch 4), but we found that the isolation of **5** could be avoided completely by subjecting the Knovenagel reaction mixture directly to the hydrogenation conditions, affording **6** in 79% overall yield. This modification reduced the amount of solvent waste by 50%.

Of particular concern from a safety and scale-up viewpoint was the nitration of 2-amino-6-chloro-4-pyrimidinol (**7**). The exothermic nitration ($\Delta H = -100.4$ kJ/mol) of **7** required the use of 90% nitric acid in sulfuric acid[3] (Scheme 2). RSST (reactive system screening tool) evaluation of the reaction mixture showed an exothermic decomposition beginning at 70 °C, which reached nearly 2500 deg/min rate at 91 °C and which peaked at 3260 deg/min rate at 245 °C. A large amount of gas is evolved during this decomposition, resulting in a maximum pressure rate of 515 psig/min at 98 °C. Clearly

(1) Sircar, J. C.; Kostlan, C. R.; Gilbertsen, R. B.; Bennett, M. K.; Dong, M. K.; Cetenko, W. J. *J. Med. Chem.* **1992**, *35*, 1605.

(2) (a) Montgomery, J. A.; Niwas, S.; Rose, J. D.; Secrist, J. A., III; Babu, S.; Bugg, C. E.; Erion, M. D.; Guida, W. C.; Ealick, S. E. *J. Med. Chem.* **1993**, *36*, 55. (b) Guida, W. C.; Elliot, R. D.; Thomas, H. J.; Secrist, J. A., III; Babu, S.; Bugg, C. E.; Erion, M. D.; Ealick, S. E.; J. A.; Montgomery, J. A. *J. Med. Chem.* **1994**, *37*, 1109. (c) Halazy, S.; Eggenspiller, A.; Ehrhard, A.; Danzin, C. *Bioorg. Med. Chem. Lett* **1992**, *2*, 407. (d) Miles, R. W.; Tyler, P. C.; Furneaux, R. H.; Bagdassarian, C. K.; Schramm, V. L. *Biochemistry* **1998**, *37*, 8615. (e) Yokomatsu, T.; Hayakawa, Y.; Suemune, K.; Kihara, T.; Soeda, S.; Shimeno, H.; Shibuya, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2833.

(3) (a) Temple, C., Jr.; Smith, B. H.; Montgomery, J. A. *J. Org. Chem.* **1975**, *40*, 3141. (b) Stuart, A.; West, D. W.; Wood, H. C. S. *J. Chem. Soc.* **1964**, 4769.

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**Scheme 1**

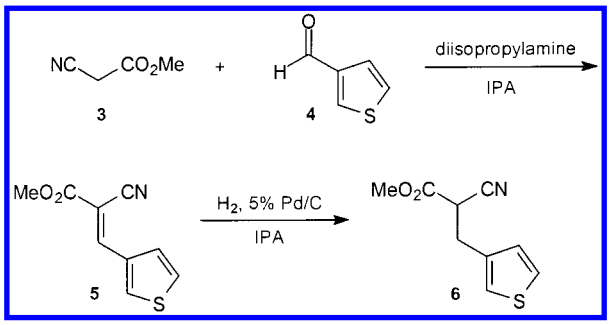



***Table 1.*** **Pilot plant lots of acrylate 5**

| batch | solvent | base | amount produced (kg) | yield[a] (%) | purity (VPC, area %) |
|---|---|---|---|---|---|
| 1 | toluene | piperidine | 21.4 | 83.0 | 100/99.4[b] |
| 2 | toluene | piperidine | 29.5 | 85.5 | 98.9/98.4[b] |
| 3 | toluene | DIPA | 44.1 | 91.7 | 98.0/99.6[b] |
| 4 | IPA | DIPA | 118.6 | 92.6 | 99.8 |
| 5 | IPA | DIPA | 116.5[c] | – | 99.4 |

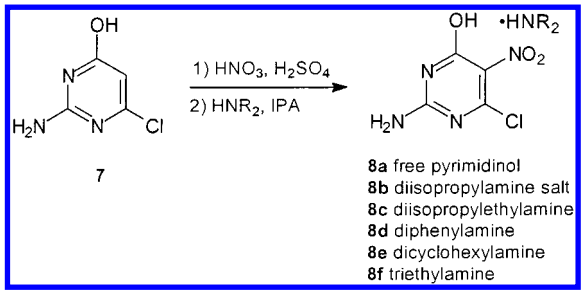
[a] Not corrected for purity. [b] First crop/second crop. [c] Wet cake, carried on directly into the next step.

***Table 2.*** **Pilot plant lots of cyanoester 6**

| batch | amount produced (kg) | yield[a] (%) | purity (VPC, area %) |
|---|---|---|---|
| 1 | 26.5 | 87.2 | 99.7 |
| 2 | 37.8 | 84.6 | 99.7 |
| 3 | 106.3 | 88.8 | 99.9 |
| 4[b] | 95.9 | 81.6[c] | 99.9 |

[a] Not corrected for purity. [b] Acrylate **5** wet cake carried on directly into reduction. [c] Overall yield for Knovenagel condensation and catalytic reduction.

**Scheme 2**




this reaction was not suitable for batch scale-up and was run in quantities no larger than 22 L to prepare early lots of material.

Due to the rapid rate of nitration and the desire for a small reaction volume, continuous nitration seemed to be an ideal alternative. A continuous method was developed wherein a solution of the pyrimidine **7** in sulfuric acid was combined with 90% nitric acid in a translucent Teflon tube.[4] The flow of each solution was controlled by means of a dual-head metering pump while the temperature of the reaction was moderated by immersion of the tube in a constant-temperature bath. The tube outlet was connected to a quench tank containing cold water. Typical residence time of the reaction mixture in the tube was approximately 2.5 min. This system offered several advantages: (1) the design was simple and easily scaled as demand grew, (2) the process was much safer than a batch process since only a small amount of nitration reaction mixture was present at any given time, and (3) the open-ended tube alleviated concerns of pressure build-up in the event of gas evolution.

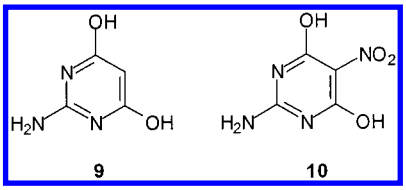
(4) A manuscript detailing the development of this continuous nitration is under preparation.

***Table 3.*** **Pilot plant lots of 8b**

| batch | % nitric acid | amount produced (kg) | yield[a] (%) | purity (HPLC, area %) |
|---|---|---|---|---|
| 1 | 90 | 25.7 | 78.2 | 91.1 |
| 2 | 98 | 44.4 | 44.2 | 99.7 |
| 3 | 98 | 49.1 | 45.9 | 93.6 |
| 4 | 90 | 76.8 | 82.2 | 96.6 |

[a] Corrected for purity

Several parameters including residence time, reactant ratio, and reaction temperature had to be controlled to avoid side reactions. If the residence time was too short, pyrimidine **7** remained. If the residence time was too long or if the concentration of nitric acid too high, a dinitro impurity was formed. It was also necessary to control the dissolution temperature of pyrimidine **7** in sulfuric acid to avoid hydrolysis of **7** to 2-amino-4,6-dihydroxypyrimidine (**9**). This compound would then be nitrated, giving rise to nitrodiol **10** in the isolated product. After some optimization,[5] pyrimidine **7** could be prepared in 82% yield and 97% purity (Table 3).



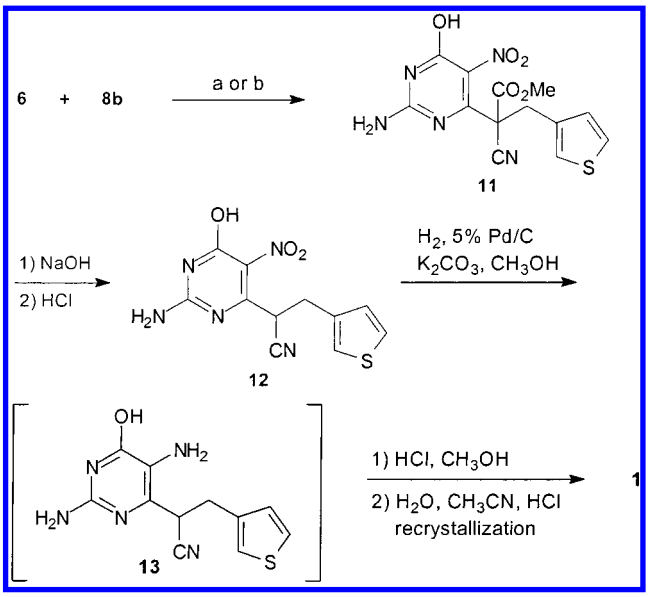

For stability reasons 2-amino-6-chloro-5-nitro-4-pyrimidinol (**8a**) was isolated as its diisopropylamine salt (**8b**).[6] While the pyrimidinol **8a** was difficult to dry and store without some hydrolysis to the nitro diol **10**, we found the diisopropylamine salt to be stable for at least 4 years at ambient temperature.

The coupling of cyanoester **6** with chloronitropyrimidinol **8a** was originally carried out using NaH in DMF at 70 °C overnight.[1] This procedure was initially modified to use $K_2CO_3$ in DMSO due to safety concerns associated with NaH, and to improve the yield from 52 to 75−80%. Workup involved filtering the reaction mixture (to remove excess $K_2CO_3$ and $KHCO_3$) into water followed by acidification with hydrochloric acid to afford the crude cyanoester **11** as a yellow solid. Foaming was a problem due to the clay-like

(5) Over the course of our scale-up work, two batches gave unexpectedly low yields of 45% and 49% (batches 2 and 3). In those runs, 98% rather than 90% nitric acid was used. We postulate that the higher strength acid and possibly higher reaction temperatures resulted in oxidative destruction of the product—no UV absorbing impurities were found by HPLC.

(6) Several persons developed skin rashes and became sensitized to this material over the course of this project. One must exercise care to minimize skin contact when handling this compound.

***Scheme 3***[a]




[a] a) $K_2CO_3$, DMSO, 70 °C. b) $MgCl_2$, $Et_3N$, $CH_3CN$, 22 °C.

nature of the solid. Switching to **8b** in the alkylation (Scheme 3) allowed reduction of the amount of $K_2CO_3$ from 2.0 to 1.05 mol equiv and eliminated the foaming problem. After drying, the crude cyanoester **11** was purified by dissolving in DMF and precipitating with water. Although the purification achieved using this method was slight, it did reduce the amount of residual DMSO in the product which, if not removed, poisoned the catalyst in the next step.

The cyanoester **11** was hydrolyzed in about 90 min at 15−25 °C with aqueous NaOH. The addition of hydrochloric acid resulted in decarboxylation to give the nitrile **12** in 93−95% yield. It was important that **12** be isolated in a timely fashion due to its instability at low pH for extended periods of time. We also found that some degradation of **12** occurred (up to 2−3%) as the material was dried.

Although this process was workable for pilot-scale synthesis, it still had several drawbacks which needed to be addressed: (1) The coupling of **6** and **8** consistently failed to go to completion. HPLC analysis (area %) of a typical $K_2CO_3$/DMSO coupling reaction mixture after 10 h at 70 °C showed 74% cyanoester **11**, 4% nitrile **12**, 11% nitropyrimidinol **8**, and 6% cyanoester **6**. Unfortunately, further heating did not result in the formation of more **11** but only hydrolysis of **11** to **12**. (2) Large amounts of aqueous waste were generated in the work-up and reprecipitation of **11**. (3) DMSO was a safety concern because it (and whatever may be dissolved in it) is readily absorbed through the skin. In addition, traces of DMSO in **12** had been shown to be a catalyst poison in the subsequent hydrogenation. (4) The reprecipitation of **11** added extra isolation and drying steps and removed the approximately 4% of usable nitrile **12** from the cyanoester product **11**.

We were encouraged to discover that **6** and **8** could be cleanly coupled using Rathke-type conditions[7] of $MgCl_2$ (1.5−1.7 mol) and $Et_3N$ (2.0−2.2 mol) in acetonitrile at ambient temperature (Table 4). As far as we know, this is the only example of using Lewis acid conditions to effect

***Table 4.*** **Pilot plant lots of 11 and 12**

| batch | amount produced (kg) | yield[a] (%) | purity (HPLC, area %) |
|---|---|---|---|
| | Coupling Only Batches (**11**) | | |
| 1[b,c] | 8.0 | 79.0 | 98.7 |
| 2[b,c] | 10.6 | 73.8 | 98.9 |
| 3[b,c] | 11.8 | 81.3 | 97.9[d] |
| 4[b,c] | 10.4 | 72.3 | 98.4[d] |
| 5[b,c] | 12.8 | 75.5 | 99.7[d] |
| 6[b,c] | 11.7 | 79.8 | 100.3[d] |
| 7[b,e] | 12.0 | 74.2 | 100.2[d] |
| 8[b,e] | 10.0 | 66.7 | 97.4[d,f] |
| | Hydrolysis Only Batches (**12**) | | |
| 9 | 7.9 | 92.9 | 97.9 |
| 10 | 8.2 | 93.0 | 98.2 |
| 11 | 17.5 | 93.2 | 98.6 |
| 12 | 19.4 | 94.2 | 98.8 |
| 13 | 17.7 | 95.4 | 98.9 |
| | Combined Coupling and Hydrolysis Batches (**12**) | | |
| 14[b,e,g] | 46.7 | 83.9[h] | 94.2 |
| 15[i] | 29.7 | 93.2[h] | 96.0 |
| 16[i] | 59.7 | 89.3[h] | 95.4 |

ND = not determined. [a] Corrected for purity. [b] $K_2CO_3$/DMSO coupling. [c] **8a** used in coupling. [d] w/w %. [e] **8b** used in coupling. [f] 2.1% **12** in product. [g] No DMF purification of crude **11**. [h] Overall yield for coupling and hydrolysis steps. [i] $MgCl_2/Et_3N/CH_3CN$ coupling.

C-alkylation of chloronitropyrimidines. In general, chloronitropyrimidines are readily coupled with nitrogen nucleophiles,[8] but carbon-based nucleophiles are much more resistant to this reaction. This chemistry should prove useful in preparing other pyrrolo[3,2-*d*]pyrimidines.[9]

This method offered several advantages over the $K_2CO_3$/DMSO procedure: (1) the $MgCl_2/Et_3N/CH_3CN$ reaction is complete in only 3 h at 25 °C versus 10 h at 70 °C, (2) the lower coupling temperature is safer for handling **8** due to decomposition concerns,[10] (3) the purification of **11** is no longer necessary, and the material can be used as a wet cake in the hydrolysis step, (4) the problematic solvent DMSO is eliminated, (5) the product **12** was obtained as a more crystalline, better filtering solid due to the elimination of traces of DMSO, (6) the overall yield from **8** was 93 versus 84% for the combined step 4/5 DMSO process and 73% for the original NaH/DMSO process.

One drawback of using $MgCl_2/Et_3N$ for the coupling of **6** and **8b** was the formation of a new impurity (**17**) in approximately 3% yield, which resulted from addition of

(7) Rathke and Cowan report the use of these conditions to acylate diethyl malonate or ethyl acetoacetate with acid chlorides. Rathke, M. W.; Cowan, P. J. *J. Org. Chem.* **1985**, *50*, 2622.

(8) For some recent examples of amine additions to **8a**, see: (a) Bailey, S. W.; Chandrasekaran, R. Y.; Ayling, J. E. *J. Org. Chem.* **1992**, *57*, 4470. (b) Ogawa, K.; Nishii, M.; Inagaki, J.; Nohara, F.; Saito, T.; Itaya, T.; Fujii, T. *Chem, Pharm. Bull.* **1992**, *40*, 1315. (c) Baxter, A. D.; Penn, C. R.; Storer, R.; Weir, N. G.; Woods, J. M.; *Nucleosides Nucleotides* **1991**, *10*, 393. (d) Haddow, J.; Suckling, C. J.; Wood, Hamish C. S. *J. Chem Soc., Perkin Trans 1* **1989**, 1297. (e) Nair, M. G.; Murthy, B. R.; Patil, S. D.; Kisliuk, R. L.; Thorndike, J.; Gaumont, Y.; Ferone, R.; Duch, D. S.; Edelstein, M. P.; *J. Med. Chem.* **1989**, *32*, 1277. (f) Boyle, P. H.; Hughes, E. M.; Khattab, H. A.; Lockhart, R. J. *Tetrahedron Lett.* **1987**, *28*, 5331. (g) Lever, O. W., Jr.; Vestal, B. R. *J. Heterocycl. Chem.* **1986**, *23*, 901.

(9) Amarnath, V.; Madhav, R. *Synthesis*, **1974**, 837.

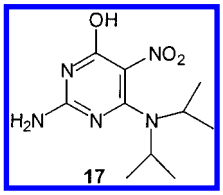
(10) Accelerated rate calorimetry (ARC) analysis of the $K_2CO_3$/DMSO reaction mixture showed an exotherm beginning at 91 °C along with a rapid exothermic decomposition at 166 °C.

***Table 5.*** **Pilot plant lots of 1**

| batch | amount produced (kg) | yield[a] (%) | purity (HPLC, w/w %) |
|---|---|---|---|
| 1 | 4.6 | 53.7 | 101.8 |
| 2 | 3.3 | 37.6 | 99.6 |
| 3 | 13.9 | 64.7 | 100.1 |
| 4 | 12.6 | 60.9 | 99.4 |
| 5 | 11.9 | 62.0 | 100.6 |
| 6 | 23.6 | 62.1 | 98.9 |

[a] Overall yield for reduction, cyclization, and recrystallization.

diisopropylamine to **8**. Fortunately, the formation of **17** did not affect the isolation or purity of **1** or **2**. We prepared bulkier amine salts (diisopropylethylamine **8c**, diphenylamine **8d**, dicyclohexylamine **8e**, and triethylamine **8f**[11]) of **8** and found the dicyclohexylamine salt to be the most promising in light of cost, preparation yield, stability toward amine addition, and overall yield to **11**. Interestingly, we also found that using **8e** in the coupling with **6** required warmer temperatures (45−55 °C) to give complete reaction in a reasonable time (2−5 h).

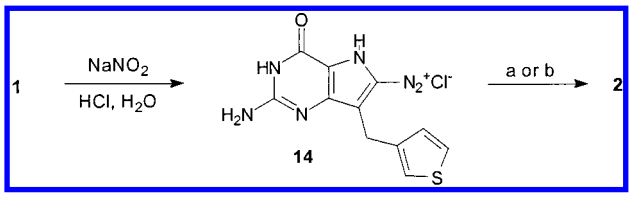



The final conversion of nitrile **12** to **1** was accomplished in two steps (Scheme 3). The nitro group was hydrogenated over 5% palladium on carbon, giving intermediate aminonitrile **13,** which when refluxed with methanolic HCl cyclized to **1**. Crude **1** was isolated and recystallized from either aqueous acetonitrile or aqueous methanol to afford **1** as the monohydrate, monohydrochloride salt in 61−65% overall yield from **12** after some optimization (Table 5). Since **1** is weakly basic, the addition of hydrochloric acid in the recrystallization was necessary to obtain the full 1:1 hydrogen chloride salt.

**Synthesis of 2.** Compound **1** (CI-972) was the first PNP inhibitor tested in man, but poor efficacy prompted the decision to develop the back-up compound **2** (CI-1000), which exhibited better solubility and PNP binding. Because we had a suitable synthesis of **1** as well as sizable quantities of **1** on hand, we initially looked for a method to prepare **2** from **1**. We opted to investigate a diazotization/reductive cleavage sequence to prepare the initial lots of **2** (Scheme 4).

The diazonium chloride, tetrafluoroborate, hexafluorophosphate, and hydrogen sulfate salts of **1** were prepared using standard literature procedures.[12] The hexafluorophosphate salt was cost-prohibitive, while the hydrogen sulfate salt was not stable enough for long-term use. The tetrafluoroborate salt was the most stable of the salts prepared. The chloride salt was the most reactive and the most attractive due to cost. DSC analysis of the chloride and tetrafluoroborate salts showed the onset of exothermic decomposition at 130 and 150 °C, respectively. As an added precaution the diazonium salts were handled and stored as wet cakes for safety reasons.[13]

***Scheme 4***[a]




[a] a) 1. $H_3PO_2$, $CuSO_4$, IPA, $H_2O$ 2. NaOH; 3. chromatography and recrystallization. b) 1. $H_2$, 5% Pd/C, IPA, $H_2O$, HCl; 2. $NaNO_2$; 3. aqueous HCl recrystallization.

Many known protodediazotization methods including $H_3PO_2$,[14,15] $PPh_3$/MeOH,[16] isoamyl nitrite/DMF,[17] $NaBH_4$,[18] $Et_3SiH$,[19] Zn/EtOH,[20] Al(O-*i*Pr)$_3$/IPA, alkaline formaldehyde,[21] PhSH,[22] and $SmI_2$ were evaluated to reduce diazonium salt **14** to **2**. Hypophosphorus acid ($H_3PO_2$) reduction, although not especially clean, showed the most promise initially; therefore, we directed our development efforts on this method for making the first small batches of **2**. We found that addition of 5−10 mol % of $CuSO_4$[14b] improved the rate of reduction and that addition of IPA to the reaction mixture helped to control foaming. Since the reaction was exothermic, $H_3PO_2$ was generally added to a cold slurry (0 °C) of the diazonium salt, which was then slowly warmed to 15 °C and held there until the reduction was complete.

Purification of **2** obtained from this reduction proved to be difficult. Crude **2** was dark in color and contained salts and tarry materials (typical yield of material was 115% with a purity of 60−70% by w/w HPLC). In addition, a small amount of **1** that was formed proved to be very difficult to remove. We explored recrystallization (in conjunction with several different carbon treatments), extraction, Sohlet extraction, and carbon absorption/deabsorption (**2** is tightly bound to carbon, especially Calgon ADP carbon), but column chromatography was ultimately needed to prepare the first two lots of acceptable **2** (Table 6).

In our search for a cleaner method of reduction we discovered that catalytic hydrogenation over Pd on carbon was a viable method for preparing **2** from **14**. Church[23] et al. reported that the diazonium salt of 9-amino-7-nitro-6-

(11) Brown, R.; Joseph, M.; Leigh, T.; Swain, M. *J. Chem. Soc., Perkin Trans. 1* **1977**, *9*, 1003.
(12) The salts were generally prepared at 0−5 °C in water using 2−3 mol equiv of acid and 1.2−1.4 mol equiv of sodium nitrite. The resulting slurry was then warmed to ambient temperature and the diazonium salt isolated by filtration.
(13) On one occasion, removal of some **dry** diazonium chloride salt from a filter funnel—scraping with a metal spatula—resulted in rapid decomposition, giving a black foam.
(14) (a) Kornblum, N. *Organic Reactions;* John Wiley and Sons Inc.: New York, 1944; Vol. II, pp 277−282. (b) Kornblum, N.; Cooper, G. D.; Taylor, J. E. *J. Am. Chem. Soc.* **1950**, *72*, 3013.
(15) Patai, S. *Chemistry of Diazonium and Diazo Groups Pt 2*; Wiley: New York, 1978; pg 564.
(16) Yasui, S.; Fujii, M.; Kawano, C.; Nishimura, Y.; Ohno, A. *Tetrahedron Lett.* **1991**, *32*, 5601.
(17) Doyle, M. P.; Dellaria, J. F.; Siegfried, B.; Bishop, S. W. *J. Org. Chem.* **1977**, *42*, 3494.
(18) Hendrickson, J. B. *J. Am. Chem. Soc.* **1961**, *83*, 1251.
(19) Nakayama, J.; Yoshida, M.; Simamura, O. *Tetrahedron* **1970**, *26*, 4609.
(20) Roe, A.; Graham, J. R. *J. Am. Chem. Soc.* **1952**, *74*, 6297.
(21) Brewster, R. Q.; Poje, J. A. *J. Am. Chem. Soc.* **1939**, *61*, 2418.
(22) Shono, T.; Matsumura, Y.; Tsubata, K. *Chem. Lett.* **1979**, 1051.

***Table 6.*** **Lab and pilot plant lots of 2**

| batch | reduction method | amount produced (kg) | yield (%) | purity (HPLC, w/w %) |
|---|---|---|---|---|
| 1[a] | $H_3PO_2$ | 19 g | 17.0[b] | 99.8[c] |
| 2[a] | $H_3PO_2$ | 250 g | 37.1[b] | 99.8[c] |
| 3[a] | $H_2$, Pd/C | 212 g | 53.1[b] | 99.4 |
| 4[a] | $H_2$, Pd/C | 11.4 kg | 51.1[b] | 100.5 |
| 5[d] | sponge Ni | 2.3 kg | 50.0[e] | 99.3 |
| 6[d] | sponge Ni | 30.8 kg | 59.7[e] | 99.7 |

[a] Using diazotization/reduction of **1**. [b] Overall yield from **1**. [c] Area %. [d] Using sponge nickel reduction of **12**. [e] Overall yield from **12**.

demethyl-6-deoxytetracycline could be reduced to the corresponding hydrocarbon using 10% Pd on carbon in the presence of formaldehyde and hydrogen. We are not aware of any other reported examples of protodediazonation using palladium on carbon and hydrogen alone.

The catalytic reduction of **14** to **2** was typically run in mixtures of water and alcoholic solvents under 50 psig hydrogen pressure[24] at 20−30 °C in the presence of hydrochloric acid.[25] Work-up consisted of filtering the reaction mixture and diluting with water. Most of the alcohol was removed by distillation and the product isolated by filtration. Aqueous isopropyl alcohol was superior to aqueous methanol with a 3:1 ratio of isopropyl alcohol to water being optimal.[26] Palladium was found to be a better catalyst than Pt, Rh, Ru, or sponge nickel.

Again, preparing colorless **2** free of **1** was difficult. The problem of removing **1** was finally accomplished by treating the reduction filtrate with a small amount of $NaNO_2$ to cleanly diazotize any **1** present into the water-soluble derivative **14**, which was then left behind in the aqueous filtrate when **2** was isolated. Unfortunately, this diazotization added to our already difficult color problem. This problem was partially solved (at the expense of significant yield loss) by resorting to multiple recrystallizations from dilute HCl and Calgon pulverized ADP carbon.[27] Nevertheless, even after two recrystallizations, the product was off-white to pale yellow in color. Two lots of **2** were prepared from **1** using this chemistry in 51−53% yield (Table 6).

Although the diazotization/reduction route provided a necessary short-term solution, we knew that this process was not attractive for long-term production for a number of reasons: (1) unstable intermediates, (2) a low-yielding final step, (3) high cost, and (4) large waste volumes. We reasoned that an attractive alternate route would convert nitrile **12** to **2** directly, thereby eliminating two synthetic steps (Scheme 5). However, due to the fact that several competing reaction pathways are possible, the key to our success required being able to selectively reduce the nitrile functionality of **13** to the imine **15** faster than rearrangement to **1**, *and* to cyclize **15** with loss of ammonia to give **2** faster than further reduction to give triamine **16**.

We were encouraged that initial results using hydrogen and a sponge nickel catalyst showed some desired product,[28] but high levels of **1** and **16** were also present.[29] Stepwise reduction using Pd/C to first reduce the nitro moiety followed by sponge nickel reduction to reduce the nitrile was only slightly more successful. We found that the relative product ratio of **2**, **1**, and **16** was dependent on pH, temperature, catalyst type, hydrogen pressure, and solvents used (including water). The best ratio of products was obtained using one molar equivalent of potassium carbonate and Activated Metals sponge nickel catalyst A-5000 in methanol at 50 psig hydrogen pressure. The reaction was cleaner if the batch was kept below 30 °C for the initial nitro group reduction and then warmed to 40−50 °C for the nitrile reduction and cyclization. Analysis of the reaction mixture after the reduction was complete typically showed (HPLC area %): 65−70% **2**, 9−11% **1**, and 18−20% **16**. Unfortunately, purifying **2** of this quality was tedious and low-yielding.

However, a key observation was made that the HPLC area % of **2** seemed to increase slightly in reaction mixtures from which catalyst had been filtered and left standing overnight. We confirmed that **16** was being air oxidized to the desired product **2** and exploited this transformation by bubbling air through the reaction mixture (after catalyst filtration) at 20−30 °C for several hours (Scheme 6).[30] Charcoal proved to be the best additive for increasing the oxidation rate, but longer times were still required as the reaction scale increased. Since crude **2** from the sponge nickel reduction is typically dark-colored, the addition of carbon to the oxidation step eliminated a second recrystallization which was usually needed to remove color.

Another advantage of the air oxidation was that the troublesome impurity **1** in the product stream was degraded during the oxidation period. Compound **2** is quite stable to strongly acidic and basic conditions; however, **1** readily decomposes under basic conditions to by-products which are easily removed in the final recrystallization. As a result, the

(23) Church, R. F. R.; Schaub, R. E.; Weiss, M. J. *J. Org. Chem.* **1971**, *36*, 723. Church notes that this reduction is unusual.

(24) The pressure increases over the course of the reaction presumably due to the formation of a mole of $N_2$ and HCl.

(25) The addition of HCl is not required, but it does appear to increase the rate of reaction. We added excess acid primarily to obtain the full 1:1 HCl salt of the weakly basic **2**.

(26) A higher percentage of water resulted in a faster reduction rate, but more impurities were formed.

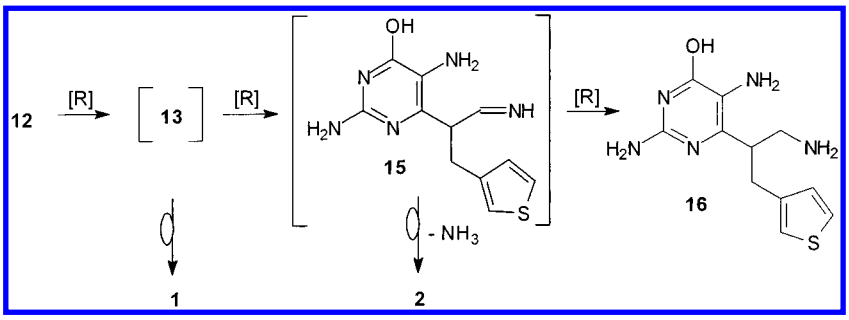
(27) ADP carbon was found to be superior to the 15 or more carbons that were evaluated.

(28) Elliot and co-workers who were also developing similar PNP inhibitors at the time reported that Raney nickel reduction of **12** gave detectable levels of **2**, but due to extensive decomposition products it was not amendable to isolation. Elliott, A. J.; Kotian, P. L.; Montgomery, J. A.; Walsh, D. A. *Tetrahedron Lett.* **1996**, *37*, 5829.

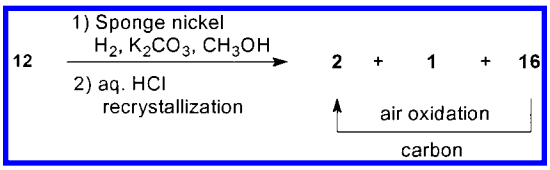
(29) Hicks, J. L. *J. Labelled Cmpds. Radiopharm.* **1995**, *34*, 1029.

(30) The oxidation may be nickel-catalyzed—running the reaction mixture through a column of Duolite C-467 ion-exchange resin, which efficiently removes nickel, completely stopped the oxidation. Concentration of the eluent from the ion-exchange column and analysis of the resulting solid by ICP showed 7.5 times less nickel than solid obtained by concentration of the reduction mixture. All other metals analyzed for by ICP (Fe(II), Mn(I), Mo(I), Cr(III), Zn(III), Al(III), and Cu(I)) were found at similar or greater levels in the Duolite-treated solid. Many metal additives (Fe, $Fe(acac)_3$, $FeSO_4$, $RhCl_3$, $PdCl_2$, $ZnF_2$, CuCl, $CuCl_2$, $Cu(OAc)_2$, $Na_2MoO_4$, $12MoO_3{\cdot}1/3H_3PO_4$, $NiCl_2$, $Ni(OH)_2$, $Ni(acac)_2$, Ni peroxide, $(Ph_3P)_2NiCl_2$, Al−Ni, $Al(OiPr)_3$, $AcAl(OH)_2{\cdot}1/3H_3BO_3$, $AlCl_3$, and Al foil) and oxidizing agents ($Na_2S_2O_8/AgNO_3$, Oxone, $BaMnO_4$, $H_2O_2$, NaClO, 3,5-di-*tert*-butyl-1,2-benzoquinone, MMPP, $NaIO_4$, and CAN) were evaluated in an attempt at improving the oxidation with respect to rate and purity; however, they were either ineffective or resulted in decomposition of **16** without conversion to **2**. The use of Cu(II) salts did increase the rate of air oxidation, but the reaction mixtures became very dark.

**Scheme 5**




**Scheme 6**




addition of $NaNO_2$, which was used in the diazotization/reduction method to remove residual **1** and which gave rise to color in the final product, was no longer needed. Using this process, two lots of **2** were prepared on pilot scale in 50−60% overall yield from **12** (Table 6).

Although sponge nickel catalyst is inexpensive, the biggest drawback in using this reduction methodology was the heavy loading of catalyst required. We typically used a 75% catalyst loading (by weight). If the loading of catalyst was decreased (single or multiple dose) the reduction failed to go to completion; if increased, the level of **16** also increased.

It also should be noted that there are hazards associated with the use of sponge nickel. *On one occasion, a rapid decomposition occurred as sponge nickel was being added to a nitrogen-purged hydrogenation vessel containing nitrile **12** and potassium carbonate. Black carbonaceous material, yellow powder, gas, and smoke were generated.* It was concluded that the sponge nickel became hot on contact with air which in turn ignited **12**. It is noteworthy that this ignition occurred even with standard nitrogen inertion procedures. To circumvent problems in subsequent runs, a slurry of **12** and potassium carbonate in methanol was charged to a sealed, nitrogen-purged vessel containing the sponge nickel catalyst. No further problems were encountered.

The sponge nickel reduction route offers several advantages over the diazotization/reduction route toward preparing **2**: (1) six versus eight steps, (2) a 40% increase in yield, (3) 33−50% reduction in waste, (4) stable intermediates, (5) chemistry not affected by traces of DMSO, (6) 50% decrease in cost, and (7) no changes to the final purification step. The last benefit was especially important in maintaining consistent physical properties of the drug substance over the course of development.

## Conclusions

Efficient, large-scale processes for the preparation of **1** and **2** were developed and proven on pilot scale. In this effort a safe, high-yielding continuous nitration of **7** was developed, a $MgCl_2$-mediated alkylation of a chloronitropyrimidine (**8**) was discovered, a protodediazotization sequence using catalytic hydrogenation was developed, and an air oxidation of by-product **16** to product **2** was discovered and exploited.

## Experimental Section

**General.** All reagents, solvents, and processing aids were from commercially available sources and used as received unless otherwise noted. Proton NMR spectra were obtained from either a Varian Gemini 200 spectrometer operating at 200 MHz for proton ($^1$H) and at 50 MHz for carbon ($^{13}$C), from a Bruker AM250 spectrometer operating at 250 MHz for proton ($^1$H) and at 63 MHz for carbon ($^{13}$C), or from a Varian Unity 400 spectrometer operating at 400 MHz for proton ($^1$H) and at 100 MHz for carbon ($^{13}$C). Chemical shifts are reported in ppm using $Me_4Si$ or residual nondeuterated solvent as reference. IR spectra were measured on an Analect DS-20 or AQS-20 spectrometer with strong absorbances reported in wavenumbers ($cm^{-1}$).

**Methyl 2-Cyano-3-(thienyl)acrylate (5).** To a cold (0−5 °C) solution of thiophene-3-carboxaldehyde (74.3 kg, 663 mol), methyl cyanoacetate (65.7 kg, 663 mol), and isopropyl alcohol (305 L) was slowly added a solution of diisopropylamine (8.3 kg, 82 mol) in isopropyl alcohol (42 L) followed by a 10 L isopropyl alcohol rinse. An exotherm to 40 °C was observed during the diisopropylamine addition, and **5** began to crystallize. The slurry was cooled to 20−25 °C, stirred for 3 h, and then cooled to 0−10 °C for 1 h. The solid was collected on a centrifuge and washed with cold isopropyl alcohol (120 L) and vacuum-dried at 45−50 °C to a constant weight, affording acrylate **5** (118.6 kg, 93% yield) as an off-white crystalline solid. VPC assay: 99.8% (area) (DB-5, 15 m, 50 °C (5 min) to 280 °C (10 min) @ 15 °C/min, injector temp 150 °C, detector temp 300 °C, 10:1 split). $^1$H NMR ($CDCl_3$) $\delta$ 8.23 (m, 1H), 8.19 (ddd, $J$ = 0.6, 1.3, 2.9 Hz, 1H), 7.86 (ddd, $J$ = 0.5, 1.3, 5.2, 1H), 7.47 (ddd, $J$ = 0.6, 3.0, 5.2, 1H), 3.93 (s, 3H); $^{13}$C NMR ($CDCl_3$) $\delta$ 163.5, 148.1, 136.0, 134.6, 127.9, 127.8, 116.1, 100.9, 53.5.

**Methyl 2-Cyano-3-(thienyl)propionate (6). From 5.** The hydrogenation was carried out in two part lots. A mixture of acrylate **5** (60 kg, 310.5 mol), 5% Pd/C (11 kg, Johnson Matthey type 87L, 50% water wet) and isopropyl alcohol (526 L) was hydrogenated under 50 psig hydrogen at 50 °C for 18 h. After cooling the mixture to ambient temperature the catalyst was removed by filtration and washed with isopropyl alcohol (75 L). The combined filtrate was concentrated by vacuum distillation until 300−318 kg of distillate had been collected. The product solution was cooled

to 25 °C and held for the second part lot. A second batch of acrylate **5** (58.5 kg, 302.8 mol) was hydrogenated over 5% Pd/C (10.7 kg) in isopropyl alcohol (513 L) in a similar fashion (50 psig hydrogen, 50 °C, 22 h). After cooling, the mixture was filtered and the catalyst washed with isopropyl alcohol (73 L). The filtrate and wash were combined with the first part lot and concentrated by vacuum distillation until 300−318 kg of distillate has been collected. The solution was cooled to 0−5 °C over 2 h to precipitate the product and held for at least 1 h. The crystalline solid was collected on a centrifuge, washed with isopropyl alcohol (51 L), and vacuum-dried at 25 °C to a constant weight to give cyanoester **6** (106.3 kg, 89% yield) as a white solid. VPC assay: 99.9% (area) (DB-5, 15m, 50 °C (5 min) to 280 °C (10 min) @ 15 °C/min, injector temp 150 °C, detector temp 300 °C, 10:1 split). $^1$H NMR (DMSO-$d_6$) $\delta$ 7.48 (dd, $J$ = 4.9, 3.0 Hz,1H), 7.34 (m, 1H), 7.05 (dd, $J$ = 4.9, 1.1 Hz, 1H), 4.52 (t, $J$ = 6.6 Hz, 1H), 3.73 (s, 3H), 3.21 (d, $J$ = 6.6 Hz, 2H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 166.3, 136.2, 128.2, 126.3, 123.3, 117.0, 53.1, 38.1, 29.2.

**From Thiophene-3-carboxaldehyde.** To a cooled solution (12 °C) of thiophene-3-carboxaldehyde (11.1 g, 0.1 mol), methyl cyanoacetate (9.9 g, 0.1 mol), and isopropyl alcohol (75 mL) was added diisopropylamine (0.2 g, 0.002 mol). After the initial exotherm of 2 °C subsided, the mixture was allowed to stir at ambient temperature for 3 h. Then 5% Pd/C (3.0 g, 50% water wet, Johnson Matthey type 87L) was added and the mixture subjected to hydrogen at 50 psig and 50 °C overnight. The catalyst was removed by filtration and washed with isopropyl alcohol. The combined filtrate was concentrated to approximately a 40 mL volume and seeded. The resulting slurry was cooled in an ice bath for about 1 h. The solid was filtered, washed with cold isopropyl alcohol, and air-dried to afford cyanoester **6** (15.4 g, 79% yield) as a white solid.

**2-Amino-4-chloro-6-hydroxy-5-nitro-pyrimidine, Diisopropylamine Salt (8b).** The continuous nitration was carried out in a Teflon tube immersed in a constant-temperature bath and connected to an aqueous quench tank. The flow and mixing of the pyrimidine/sulfuric acid and nitric acid solutions were controlled by means of a dual-head metering pump.

2-Amino-4-chloro-6-hydroxypyrimidine (45 kg, 309.3 mol) was dissolved in sulfuric acid (290 kg) while maintaining the temperature below 15 °C. After the addition was complete, the solution was held at approximately 15 °C. Water (1000 L) was charged to a 2000 L quench tank and cooled to 0−5 °C. Nitric acid (90%, 60 kg, 958.8 mol) and the pyrimidine/sulfuric acid solution were combined in the tube at a flow rate ratio of 4.4:1 over 7 h. The bath temperature was held at 45 °C. The product mixture in the quench tank was maintained at <6 °C. After the pyrimidine solution was consumed, the pale yellow solid in the quench tank was collected on a centrifuge and washed with water (400 L) and isopropyl alcohol (200 L), affording pyrimidine **8a** as an isopropyl alcohol wet cake. **8a** was combined with isopropyl alcohol (700 L) and agitated to a smooth mixture. Diisopropylamine (47 kg, 473.7 mol) was added and the mixture stirred at 25 °C for about 30 min. After cooling to 0 °C the bright yellow solid was collected on a centrifuge and rinsed with isopropyl alcohol (200 L). The product was dried (21 °C, 2 mmHg, at least 24 h) to a constant weight to afford pyrimidine **8b** (76.8 kg, 81.6% yield corrected for residual IPA 0.7% (w/w) and purity) as a bright yellow powder. HPLC assay 96.6% (area), (Zorbax CN, 5 $\mu$m, 250 × 4.6 mm, 1.0 mL/min, 225 nm, 15% $CH_3CN$:85% 0.036 M $Et_3N$ in $H_2O$ pH = 3 with concentrated $H_3PO_4$). $^1$H NMR (DMSO-$d_6$) $\delta$ 6.6 (br s, 4H), 3.33 (septet, $J$ = 6.6 Hz, 2H), 1.19 (d, $J$ = 6.6 Hz, 12H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 161.2, 161.1, 150.2, 123.4, 46.4, 19.3; IR (1% KBr pellet) 3447, 3318, 3220, 3184, 3176, 3034, 2989, 2873, 1646, 1597, 1556, 1466, 1448, 1394, 1356, 1332, 1245, 1022, 910, 844, 794.

**2-Amino-4-chloro-6-hydroxy-5-nitro-pyrimidine, Diisopropylethylamine Salt (8c).** To a slurry of pyrimidine **8a** (10 g, 0.052 mol) in IPA (100 mL) was added diisopropylethylamine (9.1 mL, 0.052 mol). The yellow mixture was heated to 78 °C for 1 h and then cooled to 15 °C. The solid was filtered, washed with IPA (150 mL), and dried under vacuum to a constant weight to afford pyrimidine **8c** (9.3 g, 56% yield) as a bright yellow powder (HPLC assay 94% (area)). The filtrates were concentrated to afford a second crop of **8c** (4.0 g, 24% yield) as a bright yellow solid (HPLC assay 89% (area), Zorbax SB CN, 5 $\mu$m, 250 × 4.6 mm, 1.0 mL/min, 225 nm, 50% $CH_3OH$:50% 0.05 M $NH_4H_2PO_4$ in $H_2O$ pH = 3 with concentrated $H_3PO_4$).

**2-Amino-4-chloro-6-hydroxy-5-nitro-pyrimidine, Diphenylamine Salt (8d).** Prepared as for **8c** (0.14 mol) but using diphenylamine afforded pyrimidine **8d** (24.2 g, 44% yield) as a pale yellow powder (HPLC assay 87% (area)).

**2-Amino-4-chloro-6-hydroxy-5-nitro-pyrimidine, Dicyclohexylamine Salt (8e).** Prepared as for **8c** (0.63 mol) but using dicyclohexylamine and heating to 50 °C afforded pyrimidine **8e** (219.5 g, 94% yield) as a bright yellow powder (HPLC assay 94% (area)).

**2-Amino-α-cyano-6-hydroxy-5-nitro-α-(3-thienylmethyl)-4-pyrimidineacetic acid, Methyl Ester (11). $K_2CO_3$/DMSO Process.** A mixture of pyrimidine **8a** hydrate (8.8 kg, 53.8 mol), cyanoester **6** (9.5 kg, 48.7 mol), and dry milled $K_2CO_3$ (13.1 kg, 94.8 mol) in DMSO (76 kg) was heated to 70 °C for 10 h. Celite (1.5 kg) was added and the mixture filtered at 60−65 °C into water (270 L) and ice (52 kg), washing the filter cake with DMSO (23 kg). The mixture was slowly acidified to pH 4.0 using 18% HCl (7.5 L). The resulting solid was collected, washed with water (325 L), and dried under vacuum to a constant weight (60 °C, $H_2O$ (KF) 2.1%) to afford crude cyanoester **11** (12.8 kg) as a light yellow solid. The crude cyanoester **11** was dissolved in DMF (23 kg) and treated with carbon (0.5 kg, Darco G-60) and Celite (0.5 kg) for 1 h at ambient temperature. The mixture was filtered and the filter cake rinsed with acetone (55 kg). With efficient agitation, water (347 L) was added to the combined filtrates and the resulting slurry cooled to 10−15 °C for 2 h. The solid was collected on a centrifuge, washed with water (200 L), and dried under vacuum to a constant weight (60 °C, $H_2O$ (KF) 1.2%) to afford cyanoester **11** (11.7 kg, 80% yield) as a light yellow solid. HPLC assay: 100.3%

(w/w), 99.2% (area), (Zorbax CN, 5 $\mu$m, 250 × 4.6 mm, 1.0 mL/min, 225 nm, 30% $CH_3CN$:70% 0.036 M $Et_3N$ in $H_2O$ pH = 3 with concentrated $H_3PO_4$), DMSO 0.05% (w/w). $^1$H NMR (DMSO-$d_6$) $\delta$ 12.2 (br s, 1H), 8.7 (br s, 1H), 7.47 (dd, $J$ = 4.9, 3.0 Hz, 1H), 7.4 (br s, 1H), 7.39 (m, 1H), 7.02 (dd, $J$ = 4.9, 1.3 Hz, 1H), 3.67 (m, 5H).

**$MgCl_2$/$Et_3N$/$CH_3CN$ Process Using 8e.** To a mixture of cyanoester **6** (5.3 g, 0.027 mol) and pyrimidine **8e** (10.0 g, 0.027 mol) in $CH_3CN$ (150 mL) was added $MgCl_2$ (3.8 g, 0.040 mol), resulting in a 5 °C exotherm. $Et_3N$ (7.5 mL, 0.054 mol) was added and the orange mixture heated to 50 °C for 3 h. The batch was poured into aqueous HCl (11.3 mL of 37% HCl and 500 mL of water) and cooled to ambient temperature. The solid was filtered, washed with water (200 mL), and dried under vacuum to a constant weight to afford cyanoester **11** (8.1 g, 86% yield) as a pale yellow powder (HPLC assay 91% (area)).

**2-Amino-6-hydroxy-5-nitro-α-(3-thienylmethyl)-4-pyrimidineacetonitrile (12). $K_2CO_3$/DMSO Process.** To a cooled mixture (10−15 °C) of cyanoester **11** (22.0 kg, 63.0 mol) in water (264 L) was added 50% NaOH (22 kg, mol) over 10 min, keeping the temperature <20 °C. After 3 h at 10−15 °C, the solution was acidified to pH 4 by addition of 37% HCl (29 kg). Without delay the resulting solid was collected on a centrifuge, washed with water (110 L), and dried under vacuum to a constant weight (45−50 °C, $H_2O$ (KF) 1.1%) to afford nitrile **12** (17.7 kg, 97% yield) as a tan solid. HPLC assay: 94.9% (area) (Zorbax CN, 5 $\mu$m, 250 × 4.6 mm, 1.0 mL/min, 225 nm, 30% $CH_3CN$:70% 0.05% $Et_3N$ in $H_2O$ pH = 3 with concentrated $H_3PO_4$). $^1$H NMR (DMSO-$d_6$) $\delta$ 12.1 (br s, 1H), 8.4 (br s, 1H), 7.52 (dd, $J$ = 4.9, 3.0 Hz, 1H), 7.4 (br s, 1H), 7.37 (m, 1H), 7.06 (dd, $J$ = 4.9, 1.3 Hz, 1H), 4.67 (dd, $J$ = 9.3, 5.8 Hz, 1H), 3.26 (dd, $J$ = 14.0, 5.8 Hz, 1H), 3.19 (dd, $J$ = 14.0, 9.4 Hz, 1H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 160.3, 156.31, 156.28, 136.7, 128.2, 127.3, 126.6, 123.3, 118.8, 37.4, 32.3; IR (1.0% KBr pellet) 3429, 3322, 3219, 1641, 1578, 1485, 1329, 1296, 1219, 847, 789, 766.

**$MgCl_2$/$Et_3N$/$CH_3CN$ Process.** To a mixture of cyanoester **6** (20.5 kg, 105.0 mol) and pyrimidine **8b** (34.0 kg, 116.5 mol) in $CH_3CN$ (133 kg) was added $MgCl_2$ (16.6 kg, 174.3 mol) in portions over 45 min, maintaining the temperature below 8 °C (a jacket temperature of −15 °C was used during the addition). $Et_3N$ (23.5 kg, 232.2 mol) was then added, resulting in an exotherm from 8 to 23 °C. The batch was stirred at 23−25 °C for 3 h and then slowly quenched into aqueous HCl (23.1 kg 37% HCl, 551 L water), rinsing the reaction vessel with acetonitrile (20 L). The resulting solid was collected on a centrifuge and washed water (100 L) to give cyanoester **11** (98.8 kg, HPLC assay 96.4% (area)) as a pale yellow, water-wet solid.

The wet cyanoester **11** was combined with water (289 L) and stirred to an even suspension. After cooling to 10−15 °C, sodium hydroxide (50% aqueous, 30.4 kg, 379 mol) was added and the dark brown solution stirred at 15−25 °C for 1.5 h. Hydrochloric acid (37%, 37.6 kg, final pH = 3.5) was added to the batch, while maintaining the temperature at 10−20 °C. Without delay, the resulting solid product was collected on a centrifuge, washed with water (150 L), and dried under vacuum to a constant weight (50−55 °C, 13 mmHg) to afford nitrile **12** (29.7 kg, 93.1% yield corrected for purity) as a yellow solid (HPLC assay 96.0% (area)).

**2,6-Diamino-3,5-dihydro-7-(3-thienylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one Hydrochloride, Monohydrate; CI-972; (1).** To a 400 L stainless steel hydrogenator was charged 5% Pd/C (3.0 kg, 50% water-wet, Johnson Matthey type 21R), potassium carbonate (12.6 kg, mol), nitrile **12** (17.7 kg, mol), and methanol (250 L). The mixture was hydrogenated under 50 psig hydrogen pressure at 20−35 °C for 6.75 h. The reaction mixture was filtered and the catalyst cake washed with methanol (70 L). The combined filtrate was cooled to 15−20 °C, and a 20% HCl in methanol solution was slowly added (35 kg, pH = 3.5 using a 0−6 pH test strip). The precipitated potassium chloride was removed by filtration and the cake rinsed with methanol (35 L). More 20% methanolic HCl solution (18.2 kg) was added to the combined filtrate, which was then heated to reflux for 5 h. The solution was concentrated by vacuum distillation to a batch volume of 175 L to partially crystallize the product. The slurry was cooled to 0−5 °C and held for at least 1 h. The solid was collected on a centrifuge, washed with isopropyl alcohol (80 L) and dried (50 °C) to afford **1** (14.9 kg, 62.1% yield) as a tan solid. The material was recrystallized from aqueous acetonitrile (1:2, 482 L) and carbon (2.6 kg, Calgon ADP pulverized, 12 L water rinse) at 70 °C. After complete dissolution the carbon was removed by filtration and washed with aqueous acetonitrile (1:2, 27 L). Hydrochloric acid (9.0 kg, 37% HCl, 15.3 L water) was added to the combined filtrate and the solution cooled to 5−10 °C for at least 1 h. The product was collected on a centrifuge, washed with water (31 L), dried (50 °C) to a constant weight, and milled to afford **1** (11.9 kg, 60% yield) as a white solid. HPLC assay: 100.6 (w/w), 99.5% (area) (Zorbax CN, 5 $\mu$m, 250 × 4.6 mm, 1.0 mL/min, 225 nm, 15% $CH_3CN$:85% 0.036 M $Et_3N$ in $H_2O$ pH = 3 with concentrated $H_3PO_4$), chloride 11.43% (w/w), water 6.83% (w/w). $^1$H NMR (DMSO-$d_6$) $\delta$ 12.9 (br s, 1H), 11.5 (br s, 1H), 11.1 (s, 1H), 7.7 (s, 2H), 7.4 (m, 1H), 7.3 (m, 1H), 7.1 (m, 1H), 5.9 (br s, 2H), 3.8 (s, 2H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 128.5, 125.8, 120.7, 150.8, 148.7, 147.3, 141.2, 133.7, 101.3, 89.7, 21.7; IR (1.0% KBr pellet) 3454, 3311, 3213, 3167, 3076, 2993, 1686, 1618, 1552, 1433, 1408, 1304, 748.

**2-Amino-3,5-dihydro-7-(3-thienylmethyl)-4*H*-pyrrolo-[3,2-*d*]pyrimidin-4-one Hydrochloride, Monohydrate; CI-1000; (2). From 1 Using Diazotization and Hypophosphorus Acid Reduction.** To a cold (0−5 °C) slurry of **1** (294.7 g, 0.93 mol), water (2.2 L), and hydrochloric acid (37%, 156 mL, 1.86 mol) was slowly added (to control foaming) a solution of sodium nitrite (90.1 g, 1.31 mol) in water (285 mL), keeping the temperature between 5 and 10 °C. The mixture was carefully warmed to 20 °C and held at that temperature for 3 h. The solid was collected on a Büchner funnel, washed with water (2 × 285 mL), and pulled dry using a rubber dam to give the diazonium chloride salt **14** (753 g) as a bright yellow, water-wet solid.

To a mixture of water-wet **14**, deionized water (1.5 L), $CuSO_4$ (13.8 g, 0.086 mol), and isopropyl alcohol (215 mL) at 0−5 °C was added 50% aqueous $H_3PO_2$ (896 mL, 8.65 mol) over 18 min—the temperature increased from 4 to 8 °C over the course of the addition. The mixture was warmed to 15 °C (15 min) and held at that temperature for 1.75 h. The batch was neutralized to a pH of 6.9 by the addition of 50% NaOH (512 mL), while keeping the temperature at 15−20 °C. The solid was filtered, washed with water (500 mL), and dried in vacuo at 50 °C for at least 24 h to afford crude **2** freebase (364 g) as a brown solid. The crude **2** freebase was purified by flash chromatography (6 in. diameter column, 7 kg 60 Å 230−400 mesh silica gel) eluting with $CH_2Cl_2$/$CH_3OH$/$NH_4OH$ (16:1:0.05 to 6:1:0.05) to afford **2** freebase (63.5 g) as a white solid.

Several lots of **2** freebase prepared as described above were recrystallized together: To a solution of **2** freebase (238 g), water (12.3 L), and 37% HCl (595 mL) at reflux was added carbon (23.5 g, Calgon ADP pulverized) in water (100 mL). After refluxing for 30 min the mixture filtered through Celite and washed with hot water (500 mL). The combined filtrate was cooled to 0−5 °C and held for 2 h. The precipitate was filtered, washed with water (500 mL), and dried to a constant weight (50 °C, 24−48 h) to afford **2** (250.3 g, 37.1% overall yield from **1**) as a white solid. HPLC assay 99.8% (w/w) (Zorbax CN, 5 $\mu$m, 250 × 4.6 mm, 1.0 mL/min, 225 nm, 15% $CH_3CN$:85% 0.036 M $Et_3N$ in $H_2O$ pH = 3 with concentrated $H_3PO_4$), chloride 11.77% (w/w), water 7.13% (w/w). $^1$H NMR (DMSO-$d_6$) $\delta$ 12.6 (br s, 1H), 12.33 (d, $J$ = 2.6 Hz, 1H), 7.94 (s, 2H), 7.44 (dd, $J$ = 4.9, 3.0 Hz, 1H), 7.28 (m, 2H), 7.08 (dd, $J$ = 4.9, 1.2 Hz, 1H), 3.9 (s, 2H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 151.9, 151.3, 140.8, 130.6, 128.5, 127.6, 126.1, 121.3, 111.1, 110.2, 21.9; IR (1.0% KBr pellet) 3099, 2932, 2868, 1703, 1667, 1481, 1389, 1129, 1063, 825, 759, 731, 702. Anal. Calcd for $C_{11}H_{10}N_4$-OS?HCl?$H_2O$: C, 43.40; H, 4.44; N, 18.40; S, 10.50. Found: C, 43.22; H, 4.10; N, 18.28; S, 10.79.

**From 1 Using Diazotization and Catalytic Hydrogenation.** To a cold (0−5 °C) slurry of **1** (23.4 kg, 74.1 mol), water (240 L), and hydrochloric acid (37%, 14.9 kg, 151.2 mol) was slowly added (40 min) a solution of sodium nitrite (7.9 kg, 114.5 mol) in water (20 L), keeping the temperature at <4 °C. The mixture was carefully warmed to 20−25 °C over about an hour and held at that temperature for about an hour. The solid was collected on a centrifuge and the cake washed with water (90 L) to give the diazonium chloride salt **14** (63.5 kg) as a bright yellow, water-wet solid.

Hydrogenation of the diazonium salt **14** was carried out in two-part lots: To a 400 L glass-lined hydrogenator was charged approximately one-half of the water-wet **14**, 5% Pd/C (3.5 kg, 50% water-wet, Johnson Matthey type 87L), isopropyl alcohol (225 L), water (75 L), and 37% hydrochloric acid (18.7 kg). The mixture was hydrogenated at 50 psig[31] over 2 h, while maintaining the temperature at 20−30 °C. The mixture was filtered and the catalyst cake washed with isopropyl alcohol (75 L). The filtrate was transferred to a second reactor, followed by an isopropyl alcohol rinse (75 L), and cooled to 0−5 °C. The remaining diazonium chloride salt **14** was reduced in the same manner and combined with the first-part lot. After warming the combined filtrates to 20−25 °C, they were treated with ADP carbon (7.2 kg), sodium nitrite (0.5 kg), and Celite (4.0 kg). The mixture was agitated for about 1 h and filtered, and the carbon cake was rinsed with methanol (400 L). The filtrate was diluted with water (160 L) and the batch concentrated by vacuum distillation to one-quarter of its original volume (approximately 160 L of volume remained). More water (100 L) was charged to the mixture, and the distillation continued until less than 5% isopropyl alcohol and methanol were detected in the distillate (1.7% MeOH and 2.4% IPA were detected in this batch. A volume of about 200 L remained in the still). The resulting mixture was cooled to 0−5 °C for at least 1 h and the crude product collected on a centrifuge and washed with cold (0−5 °C) water (150 L), yielding crude **2** (19.3 kg) as a wet (LOD 23%), light brown solid.

The crude **2** was combined with water (500 L), 37% HCl (19.5 kg), carbon (3.3 kg, Calgon ADP pulverized), and Celite (2.0 kg) and heated to reflux. After about 20 min, the mixture was filtered and the cake rinsed with hot (95 °C) water (50 L). The combined filtrates were cooled to 0−5 °C and held for at least 1 h. The precipitate was collected on a centrifuge and rinsed with cold water (150 L) to afford **2** (18.7 kg) as light gold colored needles (LOD 32%).

The material was recrystallized a second time for color in a similar fashion using water (460 L) 37% hydrochloric acid (15.3 kg), ADP carbon (1.3 kg), and Celite (1.0 kg). The product was collected on a centrifuge, washed with water (150 L), dried to a constant weight (50−55 °C, 28 in. Hg, at least 24 h), and milled to give **2** (11.4 kg, 51% yield from **1**) as a pale yellow solid (HPLC assay 100.5% (w/w), 99.7% (area), chloride 11.86% (w/w), 6.14% water (w/w)).

**From 12 Using Sponge Nickel Reduction.** To an inerted 800 L stainless steel hydrogenator containing sponge nickel catalyst (37.5 kg, Activated Metals type A-5000) was charged a mixture of nitrile **12** (50 kg, 171.6 mol), potassium carbonate (23.7 kg, 171.6 mol), and methanol (378 L), followed by a methanol rinse (50 L). The mixture was cooled to 5−15 °C, and the mixture was hydrogenated under 50 psig hydrogen, while maintaining the temperature at <30 °C. After the initial exotherm subsided (about 1.25 h), the mixture was warmed to 40−50 °C and the hydrogenation continued for an additional 18 h. The mixture was filtered onto carbon (4.4 kg Calgon ADP pulverized) and Celite (4.4 kg) using methanol (171 L) as a catalyst cake wash. Air was gently sparged through the filtrate with agitation at 20−30 °C for 72 h. The mixture was then filtered and the cake washed with methanol (100 L). The orange filtrate was diluted with water (1345 L) and 37% hydrochloric acid (50.7 kg) and the solution concentrated by atmospheric distillation until a batch temperature of 98−100 °C was obtained (6 h). The solution was cooled to 0−5 °C and held for at least 1 h. The precipitate was collected on a centrifuge and washed

(31) Presumably due to the formation of hydrogen chloride and nitrogen during the reduction, the batch pressure actually increases as the hydrogen is consumed. During this hydrogenation the pressure was relieved twice from 60 to 50 psig and once from 60 to 5 psig, followed by repressurizing to 50 psig with hydrogen. The hydrogenation was essentially complete after 1 h.

with water (100 L) to afford crude **2** (41.7 kg, HPLC assay >99% (area)) as dark brown needles.

**Recrystallization for Color.** The crude **2** was dissolved at reflux in water (1470 L) and 37% hydrochloric acid (23.7 kg) and then treated with a slurry of carbon (3.6 kg, Calgon ADP pulverized) and Celite (3.6 kg) in water (20 L). After 15 min the mixture was filtered hot and the carbon cake washed with boiling water (150 L). The combined aqueous filtrate was cooled to 0−5 °C and held for at least 1 h. The precipitate was collected on a centrifuge, washed with water (100 L), and dried to a constant weight (50−55 °C, 29 in. Hg) to afford **2** (30.8 kg, 60% yield) as an off-white solid. HPLC assay: 99.1% (w/w), 99.7% (area) (Lichrosorb RP8, 5 $\mu$m, 250 × 4.6 mm, 1.1 mL/min, 225 nm, 26% $CH_3CN$: 74% 0.02M $NH_4OAc$ in $H_2O$ pH = 6.8 with $NH_4OH$), chloride 11.94% (w/w), water 6.09% (w/w), nickel <10 ppm).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