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# Synthesis of 2,4-Di-1-pyrrolidinyl-9H-pyrimido[4,5-b]indoles, Including Antiasthma Clinical Candidate PNU-142731A

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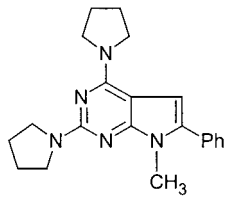
**Abstract:**

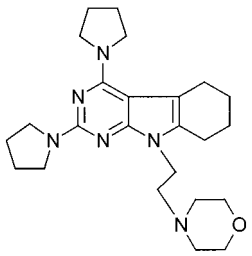
**Two syntheses are described for 1-[(2,4-di-1-pyrrolidinyl-9H-pyrimido[4,5-b]indol-9-yl)-acetyl]pyrrolidine hydrochloride (PNU-142731A), a clinical candidate in the asthma area. The route involving the initial regioselective addition of glycine ethyl ester to commercially available 2,4,6-trichloropyrimidine is particularly well-suited for large-scale operation, as it is short, proceeds in good yield, is operationally straightforward, and requires no chromatographic purification of intermediates.**

## Introduction

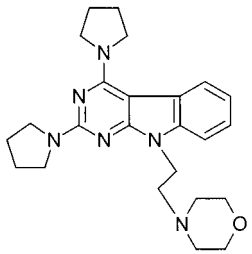
We recently reported[1] the synthesis and preliminary pharmacological evaluation of a series of 2,4-diaminopyrrolo[2,3-d]pyrimidine derivatives, of which structures **1** and **2** are prototypical.



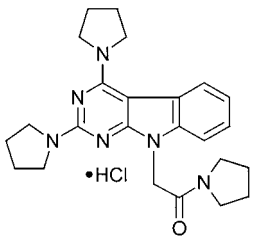
**1** (PNU-87663)



**2** (PNU-101033)



**3** (PNU-104067)



**4** (PNU-142731A)

These heterocycles were synthesized via a novel (and mild) Bischler-type condensation/cyclization between 2,4-di-1-pyrrolidinyl-6-methyl(or 2-morpholinylethyl)amino-pyrimidine[1,2] and the appropriate α-bromoketone (phenacyl bromide for **1** and 2-bromocyclohexanone for **2**). Compounds **1** and **2** are both good one-electron reducing agents, and both have shown good activity as inhibitors of lipid peroxidation. Compound **2** in particular has exhibited neuroprotective effects after oral administration in rat models of cerebral ischemia,[3] and due to its good brain penetration may find utility for the treatment of chronic neurodegenerative disorders (e.g., ALS, Alzheimer's disease, Parkinson's disease) which are thought to be mediated at least in part by oxidative stress.[3,4] As might be anticipated on the basis of their structure, pyrimido[4,5-b]indoles **3** and **4** were much less effective as reducing agents/radical scavengers, but both surprisingly exhibited good activity in several animal models of eosinophilic lung inflammation[1,5] and hence appeared to have potential for the treatment of the inflammatory component of asthma.[6] The development of an efficient process for the large-scale preparation of morpholinoethyl derivative **3** has recently been reported.[7] However, gallbladder/bile duct toxicity precluded the clinical evaluation of **3**. In anticipation of clinical trials in the asthma area with the nontoxic pyrrolidine amide analogue **4**,[5] several alternative routes have been evaluated for its preparation, routes which would be able to provide the needed multikilogram amounts of clinical quality final product.

## Results and Discussion

Our initial approaches to PNU-142731A (**4**), outlined in Schemes 1 and 2, were heavily biased in favor of utilizing primary alcohol **8** as the key intermediate, since this material

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(1) Bundy, G. L.; Ayer, D. E.; Banitt, L. S.; Belonga, K. L.; Mizsak, S. A.; Palmer, J. R.; Tustin, J. M.; Chin, J. E.; Hall, E. D.; Linseman, K. L.; Richards, I. M.; Scherch, H. M.; Sun, F. F.; Yonkers, P. A.; Larson, P. G.; Lin, J. M.; Padbury, G. E.; Aaron, C. S.; Mayo, J. K. *J. Med. Chem.* **1995,** *38*, 4161.

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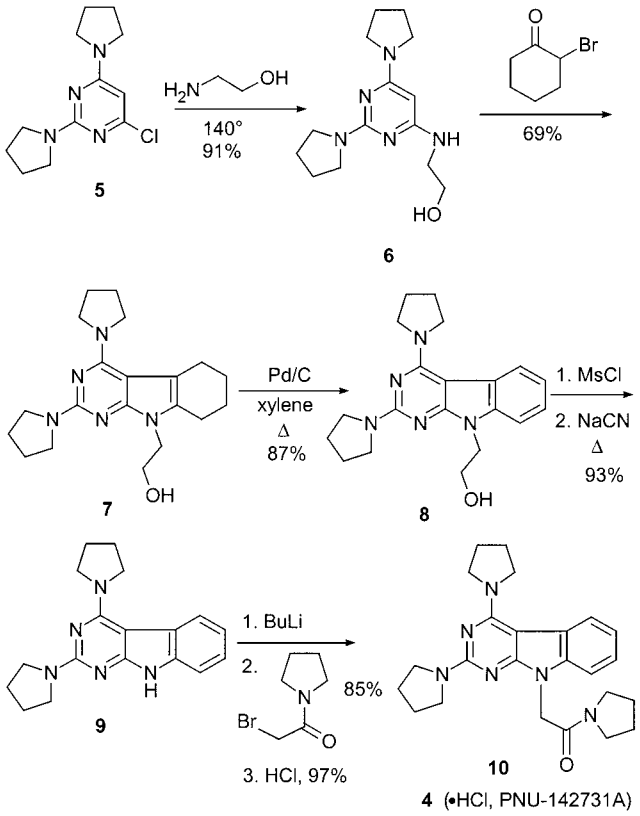
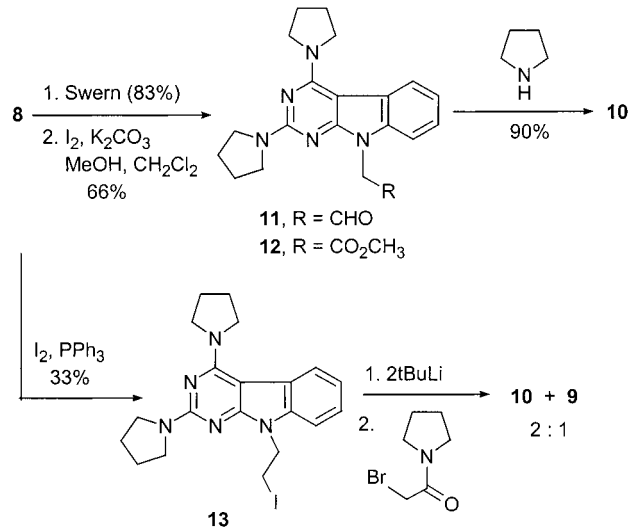
***Scheme 1***




***Scheme 2***




was available in >20 kg quantities from our fairly extensive analogue program which culminated in the preparation of **2** and **3**.[1] (The ethanol side chain had provided a versatile handle for optimization of the indole N-substituent.) Primary alcohol **8** was assembled via the direct, high-yield protocol depicted in Scheme 1, a sequence very similar to that reported earlier for the synthesis of **3**. The operationally simple displacement of the chloro group of diaminopyrimidine intermediate **5**[7] with excess ethanolamine proceeded in excellent yield on up to a 300 g scale in laboratory-sized equipment. The key to this step was the maintenance of careful temperature control (140 °C) especially at the beginning of the reaction. Although no problems were ever encountered in many runs on this scale, calorimetry experiments indicated that this reaction became vigorously exothermic at temperatures near 200 °C. Hence, safety concerns precluded the use of this displacement on a very large scale and provided the impetus to investigate other alternatives. Efforts to effect the displacement under milder conditions, for example via utilization of the dianion of ethanolamine as the nucleophile, proved unsuccessful, and selective O-protection of the ethanolamine was considered not worth the several steps it would have required.

Conversion of intermediate **6** to the cyclohexyl-fused pyrrolopyrimidine **7** was accomplished in 69% yield via the general alkylation/cyclization protocol with bromocyclohexanone[7] reported earlier.[1,7] (A 50% yield of analytically pure material could be obtained by simple filtration of the reaction mixture; recovery of the additional 19% required chromatographic purification of the mother liquors.) Dehydrogenation with 5% Pd/C in xylene at 142 °C (24 h) then afforded the key pyrimido[4,5-b]indole intermediate **8** in 87% yield. Interestingly, the use of either 10% Pd/C or higher temperatures (decalin, 190 °C) for this reaction led to significant amounts of dehydrogenation of the 2- and 4-pyrrolidine substituents to the corresponding pyrroles. Alternative protocols utilizing either chloranil or DDQ also afforded the desired intermediate **8**, but in yields of only 70% or less, and the material obtained in this manner required extensive chromatographic purification to remove numerous highly colored impurities.

The unusual strategy for the remaining steps in this sequence—that is, removal of the two-carbon ethanol appendage, followed by replacement with a two-carbon side chain at the acid oxidation level—was dictated by our inability to cleanly oxidize **8** or the corresponding aldehyde **11** to the requisite acid or ester **12** without damaging the oxidation-prone heterocyclic ring system (see Scheme 2). With the exception of the Swern oxidation,[8] which afforded aldehyde **11** cleanly in 83% yield, other attempted oxidations of **8** yielded either recovered starting alcohol ($K_2S_2O_8/RuCl_3$/KOH; $KMnO_4$, *t*-BuOH, pH 7; $Pt/O_2$) or complex mixtures including products resulting from destruction of the heterocyclic ring system (Jones reagent; PDC; $NaBrO_3$; $Ca(OCl)_2$, HOAc, MeOH; *t*-BuOCl, pyr, MeOH; TEMPO). The use of 10-I-4 iodinane oxide (IBX),[9] which has been recommended for substrates containing oxidatively sensitive heterocycles, led to oxidative cleavage of the desired aldehyde **11** affording **9**, even under conditions where utilization of the starting alcohol was incomplete. Further oxidation of the Swern-derived aldehyde **11** with $I_2/K_2CO_3/MeOH/CH_2Cl_2$ afforded ester **12** (66% on a 2 g scale), and oxidation with buffered $KMnO_4$/aqueous THF gave the corresponding acid (71%) on small scale, but in both cases, yields declined precipitously as the scale of operation increased. Alternative oxidations of aldehyde **11** led to unusable mixtures ($NaClO_2$; Jones reagent; NaOCl; TMSOMe, AIBN, NBS; $Br_2$, aqueous MeOH; cyanuric chloride, pyr; TEMPO).

(8) Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165.
(9) Frigerio, M.; Santagostino, M.; Sputore, S.; Palmisano, G. *J. Org. Chem.* **1995**, *60*, 7272.

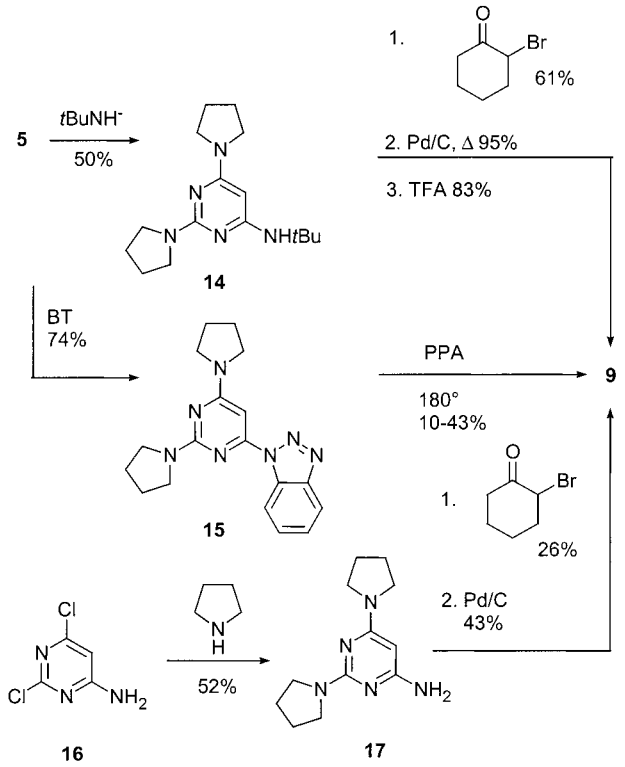
Removal of the N-1 ethanol side chain from **8** (Scheme 1) was accomplished by conversion to the mesylate, displacement with excess cyanide, and subsequent elimination of acrylonitrile in 93% overall yield. (No reaction of the liberated acrylonitrile with product **9** was ever observed.) Depending on the procedure used for the isolation of the mesylate, traces of $Et_3N$ remaining from its formation were sufficient to catalyze the elimination step; alternatively 1−5% of $Et_3N$ could be added to the mixture. Following completion of our work, another example of an analogous cyanide-mediated N-dealkylation of hydroxyethyl intermediates was reported;[10] in this case, however, the authors claimed success only for those cases in which the ethanol side chain bore a phenyl substituent.

The synthesis of **4** was completed by formation of the indole anion with *n*-BuLi, followed by alkylation with the easily prepared pyrrolidine amide of bromoacetic acid[11] (85% yield). Despite the apparent inefficiency of removing and reintroducing the indole side chain, the seven-step sequence in Scheme 1 proceeded in 42% overall yield from **5**, and the whole process was operationally simple enough to allow the facile preparation of a total of ∼6 kg of clinical candidate **4** in laboratory-scale and small pilot plant equipment.

Also summarized in Scheme 2 is an alternative protocol for the removal of the ethanol side chain from key intermediate **8**. Conversion to iodide **13**, followed by reductive cleavage with *t*-BuLi (2.0 equiv, 0 °C)[12] and quenching of the incipient indole anion with *N*-(bromoacetyl)-pyrrolidine provided a 2:1 mixture of the desired final product **10** and N−H indole intermediate **9**. Optimization of this promising transformation was not undertaken, since the Scheme 1 process was working well for the preparation of intermediate amounts (1−2 kg) of PNU-142731A (**4**). Furthermore, it seemed likely that once our stockpile of intermediate **8** was consumed, the subsequent larger-scale synthesis of **4** would probably not proceed via this route.

Three alternative options for the preparation of late stage N−H intermediate **9**, without the intermediacy of hydroxyethyl derivative **8**, are outlined in Scheme 3. Displacement of the chlorine atom from diaminopyrimidine intermediate **5** with the anion of *tert*-butylamine gave triaminopyrimidine **14** in 50% yield (*tert*-butylamine itself was unreactive with **5** at reflux without added solvent.) The conversion of **14** to **9** via three straightforward steps (alkylation/cyclization/dehydration, dehydrogenation, and removal of the *tert*-butyl-protecting group) proceeded in 48% overall yield. However, the low yield on the **5** → **14** displacement rendered this modification unattractive. Commercially available 4-amino-2,6-dichloropyrimidine, **16**, could also be converted to intermediate **9** without protection of the primary amine. However, unacceptably low yields were encountered for each of the three steps indicated in Scheme 3 (**16** → **17** → **9**), and the intermediate **17** also seemed unusually prone to both air oxidation and reaction with methylene chloride,[13] possibly at the open pyrimidine carbon. The final and most direct

***Scheme 3***




option investigated for the synthesis of **9**, based on literature precedent in simpler systems,[14] involved the displacement of chloride **5** with benzotriazole (120 °C, 18 h, 74%) followed by decomposition of the triazole intermediate **15** in polyphosphoric acid (180 °C, 0.75 h). On a small scale, yields for the **15** → **9** step (43% maximum) were in the same range as those reported for simple indoloquinolines,[14] but unfortunately the yields went down precipitously as the reaction was scaled up. Hence, none of these alternatives for the preparation of **9** offered any advantage over the route presented in Scheme 1.

Although the process in Scheme 1 was operationally straightforward on laboratory scale and indeed was used for the synthesis of kilogram amounts of pure PNU-142731A for preclinical studies, this route was considered to be unsatisfactory for the preparation of the much larger quantities of material needed for clinical development. Negative aspects of this pathway included the need for large quantities of cyanide for the **8** → **9** conversion, the fact that intermediate **9** exhibited some genotoxicity (Ames positive),[15] the need to prepare the final alkylating agent [*N*-bromoacetyl)-pyrrolidine],[11] and the safety concern of the initial ethanolamine displacement.

The concise, high-yield synthetic route to **10** outlined in Scheme 4 provides an attractive alternative which circumvents all of the aforementioned concerns. Treatment of commercially available 2,4,6-trichloropyrimidine **18** with 1.1 equiv of glycine ethyl ester afforded the desired unsym-

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(13) Mills, J. E.; Maryanoff, C. A.; Cosgrove, R. M.; Scott, L.; McComsey, D. F. *Org. Prep. Proced. Int.* **1984**, *16*, 97.
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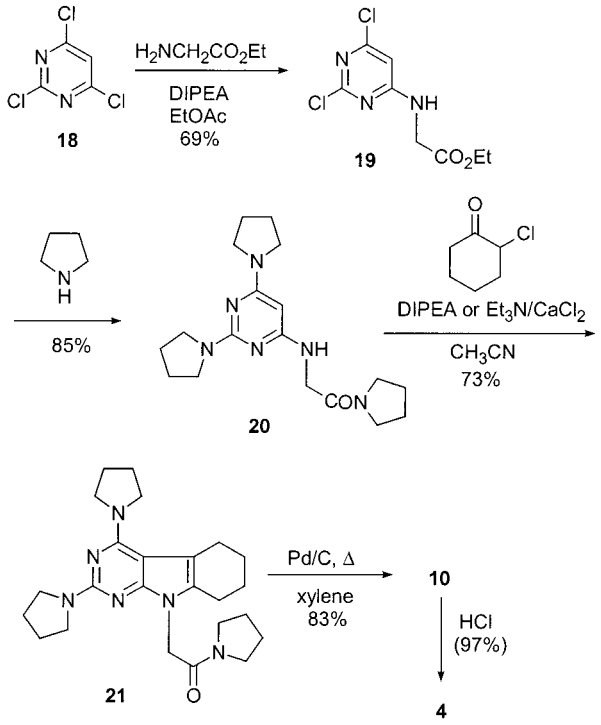
***Scheme 4***




metrical regioisomer **19** in 69% yield following a heptane knock-out which removed most of the C-2 regioisomeric product. Although the adduct **19** isolated in this manner contained <5% of the C-2 regioisomer, it was easily rejected later during the crystallization of **21**, as it does not react with chlorocyclohexanone. The 4:1 ratio of the 4-/2-isomers obtained in the displacement step was both temperature- and solvent-dependent, but it also reflects both the statistical and the modest reactivity advantages[16] of the C-4 chlorine. (Although an exhaustive assessment was not undertaken, the following C-4/C-2 isomer ratios were noted: cyclohexane, reflux, 68/32; THF, −10 °C, 60/40; dioxane, reflux 65/35; MeOH, 25 °C, 80/20. The EtOAc conditions reported in the Experimental Section also afforded an 80/20 mixture but obviated the need for a solvent swap during work-up.) Earlier efforts to avoid the regiochemistry question altogether by displacing the monochloro substituent of intermediate **5** with glycine, its dianion, glycine ethyl ester, or the corresponding pyrrolidine amide were uniformly unsuccessful, presumably due to the deactivation of the pyrimidine ring by the pyrrolidine substituents.

The treatment of intermediate **19** with excess pyrrolidine was initially moderately exothermic (displacement of the first chlorine) and required external cooling, but the second displacement and the ester → amide conversion required atmospheric concentration and heating at reflux for 20 h (85% yield). The synthesis of **10** was then completed by utilizing the usual one-pot alkylation/cyclization/dehydration reaction,[1] mediated by either DIPEA or more recently $Et_3N/CaCl_2$, followed by the Pd/C-mediated dehydrogenation of the fused cyclohexenyl intermediate **21**, both steps proceeding in very good yield.

In summary, we have described two syntheses (Schemes 1 and 4) of the diamino-substituted pyrimido[4,5-b]indole **4**, a clinical candidate in the asthma area. The Scheme 4 pathway is particularly attractive, as it is short (five steps), operationally simple, readily scalable, proceeds in high overall yield (35%), avoids genotoxic intermediate **9**, and requires no chromatographic purifications. This route has been used for the preparation of multikilogram quantities of PNU-142731A (**4**), with lot sizes in the 50 kg range, thus demonstrating the robustness of the chemistry.

## Experimental Section

**General.** All reagents were obtained from Aldrich Chemical Co. and were used as received. Distilled, purified solvents were obtained from Burdick and Jackson, Inc., and were used without further purification. Except as noted, all reactions were carried out with magnetic stirring in flame-dried glassware in an atmosphere of nitrogen. Column chromatographic purifications were performed on E. Merck 230−400 mesh silica gel 60. $^1$H and $^{13}$C NMR spectra were recorded at 300 and 75 MHz, respectively, on $CDCl_3$ solutions. Melting points are uncorrected.

**2-[[2,6-Di-(1-pyrrolidinyl)pyrimidin-4-yl)amino]ethanol (6).** A solution of chloropyrimidine intermediate **5**[2,7] (300 g, 1.188 mol) in 1000 mL of ethanolamine was heated under nitrogen for 66 h at 140 °C. (Internal reaction temperature was carefully maintained at 140 °C with a J-Kem temperature controller in order to avoid a vigorous exotherm which occurs if the reaction temperature reaches 200 °C.) The mixture was then cooled gradually, and when solids began to precipitate out (approximately 80 °C), 1700 mL of water was added. The thick suspension was stirred vigorously at 25 °C for 2 h and then at 0 °C for 1 h. The solid product was isolated by filtration and washed with four 600 mL portions of ice water. Drying, by pulling nitrogen through the funnel for 1.5 h and then by heating at 40 °C, 0.005 mm for 66 h, afforded pure **6** as a white solid (299.4 g, 91%): mp 150.5−151.5 °C; $^1$H NMR $\delta$ 6.55 (broad s, 1H, exchangeable), 4.83 (m, 1H, exchangeable), 4.75 (s, 1H), 3.75−3.72 (m, 2H), 3.54−3.38 (m, 10H), 1.93−1.85 (m, 8H); $^{13}$C NMR $\delta$ 163.75, 161.56, 159.61, 72.63, 64.35, 46.39, 45.99, 45.16, 25.52, 25.30. Anal. Calcd for $C_{14}H_{23}N_5O$: C, 60.62; H, 8.36; N, 25.25. Found: C, 60.71; H, 8.49; N, 25.45.

**5,6,7,8-Tetrahydro-9-(2-hydroxy-1-ethyl)-2,4-di-1-pyrrolidinyl-5H-pyrimido[4,5-b]indole (7).** A solution of **6** (220 g, 0.79 mol), 2-bromocyclohexanone[7] (280 g, 1.58 mol), and diisopropylethylamine (200 mL, 1.15 mol) in $CH_3CN$ (2 L) was heated at reflux for 48 h, after which approximately 700 mL of $CH_3CN$ was removed by atmospheric distillation. The remaining reaction mixture was cooled to 4 °C (18 h), and pure **7** (142 g, 50%) was isolated by filtration. Chromatographic purification of the mother liquors (10 kg silica gel, 5% EtOAc/$CH_2Cl_2$) provided an additional 52.1 g of clean **7** (total yield 194.1 g, 69%). Further chromatographic purification of a small portion of this material (silica gel, 2.5% acetone/$CHCl_3$) afforded analytical samples of **7** as a white solid: mp 123−125 °C; $^1$H NMR $\delta$ 7.64 (broad

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s, 1H), 4.05 (m, 2H), 3.91 (m, 2H), 3.67 (m, 4H), 3.55 (m, 4H), 2.72 (m, 2H), 2.56 (m, 2H), 2.00−1.70 (m, 12H); HRMS calcd for $C_{20}H_{29}N_5O$ 355.2372, found 355.2368. Anal. Calcd for $C_{20}H_{29}N_5O \cdot 0.5\ H_2O$: C, 65.91; H, 8.29; N, 19.22. Found: C, 65.85; H, 7.89; N, 19.00.

**9-(2-Hydroxy-1-ethyl)-2,4-di-1-pyrrolidinyl-5H-pyrimido[4,5-b]indole (8).** A mixture of hydroxyethyl intermediate **7** (700 g, 1.97 mol) and 5% Pd/C (245 g; 50% $H_2O$) in 7 L of xylene was heated at 142 °C for 24 h (fairly vigorous and steady evolution of $H_2$, which was carried away through a trap with a gentle stream of $N_2$.) The mixture was then cooled to 20 °C, and the catalyst was removed by filtration through Celite. The filter cake was washed with $CH_2Cl_2$, and the combined filtrate was evaporated to a small volume in vacuo. The residue was diluted with heptane, cooled to −10 °C, and filtered, and the solids were washed with additional heptane, thereby affording **8** (604 g, 87%) as a white solid, sufficiently pure for subsequent transformations. Recrystallization of a small portion of this material (ether/hexane) afforded an analytical sample: mp 110−111 °C; $^1$H NMR $\delta$ 7.89 (m, 1H), 7.23 (m, 2H), 7.13 (m, 1H), 6.97 (broad s, 1H), 4.38 (m, 2H), 4.05 (m, 2H), 3.92 (m, 4H), 3.62 (m, 4H), 2.0−1.90 (m, 8H); $^{13}$C NMR $\delta$ 159.6, 158.3, 157.7, 137.3, 122.3, 122.2, 121.1, 120.5, 108.4, 90.2, 63.8, 50.1, 46.6, 26.0, 25.9; MS(EI) 351 ($M^+$), 323, 307, 296, 279. Anal. Calcd for $C_{20}H_{25}N_5O$: C, 68.35; H, 7.17; N, 19.93. Found: C, 68.28; H, 7.31; N, 19.93.

**2,4-Di-1-pyrrolidinyl-5H-pyrimido[4,5-b]indole (9).** A stirred, −10 °C solution of hydroxyethyl intermediate **8** (125 g, 0.36 mol) and triethylamine (36.4 g, 0.36 mol) in 2000 mL of $CH_2Cl_2$ was treated with a solution of methanesulfonyl chloride (40.8 g, 0.36 mol) in 200 mL of $CH_2Cl_2$, added over 30 min. After an additional 30 min, TLC analysis showed a small amount of **8** remaining, and therefore, an additional 5 mL of $Et_3N$ and 1 mL of MsCl were added, and stirring at −10° was continued for 15 min. The reaction mixture was then poured into 1 L of 10% aqueous sodium bicarbonate and extracted thoroughly with $CH_2Cl_2$. The extracts were washed with brine, dried over anhydrous sodium sulfate, and concentrated, thereby affording the crude solid mesylate. This material (containing small amounts of $Et_3N$) was used directly in the cyanide displacement/elimination which follows. (For other purposes, residual triethylamine and trace by-products could easily be removed from the mesylate by trituration with 60/40 EtOAc/hexane.)

A solution of the above mesylate and sodium cyanide (186 g, 3.8 mol) in DMSO (3.3 L) and water (300 mL) was heated at 115 °C for 12 h. Approximately two-thirds of the DMSO was then removed in vacuo (bath temperature 100 °C), and the residue was cooled to 25 °C and partitioned between $CH_2Cl_2$ (2L) and 10% aqueous $NaHCO_3$ (800 mL). The aqueous layer was extracted with additional $CH_2Cl_2$ (3 × 300 mL), and the combined extracts were washed with brine (800 mL) and dried with $Na_2SO_4$. The residue resulting from removal of the solvents was triturated with acetone (400 mL), filtered, washed with cold acetone, and dried (0.05 mm, 40 °C, 48 h) to afford **9** (101.5 g, 93%) as a white solid, completely clean by TLC and NMR: mp 209−211 °C; $^1$H NMR $\delta$ 9.70 (s, 1H), 7.87 (m, 1H), 7.22 (m, 1H), 7.15−7.06 (m, 1H), 3.95 (m, 4H), 3.67 (m, 4H), 2.02−1.95 (m, 8H); $^{13}$C NMR $\delta$ 159.8, 158.2, 158.0, 135.9, 122.1, 121.8, 120.6, 119.8, 110.4, 90.4, 49.6, 46.8, 25.7; MS (FAB) 308 ($M^+$ + H), 307. Anal. Calcd for $C_{18}H_{21}N_5$: C, 70.33; H, 6.89; N, 22.78. Found: C, 70.28; H, 7.20; N, 22.67.

**1-[(2,4-Di-1-pyrrolidinyl-9H-pyrimido[4,5-b]indol-9-yl)acetyl]pyrrolidine Hydrochloride (4).** To a stirred −40 °C solution of **9** (93.1 g, 0.303 mol) in 2150 mL of dry THF was added, over 30 min under a $N_2$ atmosphere, a solution of *n*-BuLi (246 mL of a 1.6 M hexane solution; 0.394 mol, 1.3 equiv). The resulting homogeneous dark solution was stirred at −40 °C for an additional 30 min and then treated over 15 min with a solution of the pyrrolidine amide of bromoacetic acid[11] (93.1 g; 0.484 mol, 1.6 equiv) in THF (750 mL). The reaction mixture was allowed to warm gradually to 25 °C (over 2 h), then recooled to −78 °C, and filtered through a medium porosity sintered glass funnel. The solids were then washed with additional cold (−78 °C) THF (2 × 200 mL). The solids were partitioned between $CH_2Cl_2$ and $H_2O$, and the organic layer was dried ($Na_2SO_4$) and concentrated, thereby affording **10** (107.3 g, 85%) as a white solid homogeneous by NMR and TLC ($R_f$ 0.46; 10% acetone/$CH_2Cl_2$): mp 201−204 °C; $^1$H NMR $\delta$ 7.87 (m, 1H), 7.38 (m, 1H), 7.21−7.08 (m, 2H), 5.05 (s, 2H), 3.92 (m, 4H), 3.62 (m, 4H), 3.48 (m, 2H), 3.33 (m, 2H), 1.96 (m, 8H), 1.85−1.73 (m, 4H). $^{13}$C NMR $\delta$ 167.3, 158.4, 137.8, 122.5, 122.4, 120.8, 120.6, 109.6, 90.2, 50.0, 47.0, 46.8, 46.6, 45.5, 26.8, 26.1, 26.0, 24.3. HRMS calcd for $C_{24}H_{30}N_6O$ 418.2481, found 418.2476. Anal. Calcd for $C_{24}H_{30}N_6O$: C, 68.87; H, 7.22; N, 20.08. Found: C, 68.79; H, 7.34; N, 19.80.

Conversion of pyrrolidine amide **10** into the hydrochloride salt **4** is described in the final experiment.

**2-(2,4-Di-1-pyrrolidinyl-9H-pyrimido[4,5-b]indol-9-yl)-acetaldehyde (11).** A solution of oxalyl chloride (2.51 mL, 28.76 mmol) in $CH_2Cl_2$ (65 mL) was cooled to −70 to −75 °C under nitrogen and was then treated with a solution of 4.12 mL (58.16 mmol) of DMSO in 10 mL of $CH_2Cl_2$, added over 5 min. After 2 min longer at −75 °C, a solution of 8.0 g of **8** (22.79 mmol) was added and stirred for 15 min at −75 °C, then treated with 8.24 mL (59.1 mmol) of triethylamine, stirred 5 min longer at −75 °C, and finally allowed to warm to room temperature over about 30 min. The mixture was diluted with methylene chloride (200 mL) and water (100 mL) and stirred for 5 min. The mixture was transferred to a separatory funnel, and the product was isolated by extraction with $CH_2Cl_2$. The extracts were washed with water and brine, dried over sodium sulfate, and evaporated. The crude product was triturated with 30 mL of 15% ethyl acetate/hexane (25 °C), filtered, and the solids were washed with 2 × 10 mL of the same mixed solvent and dried (18 h, 40 °C, 0.04 mm). The aldehyde **11**, a white solid, weighed 6.62 g (83%) and exhibited mp 154−155 °C (decomp). (An additional 5−10% of the product could be recovered, if desired, by chromatography of the mother liquors on silica gel with 10% EtOAc/$CHCl_3$.) $^1$H NMR $\delta$ 9.75 (s, 1H), 7.92 (d, $J$ = 8.2 Hz, 1H), 7.23−7.09 (m, 3H), 4.93 (s, 2H), 3.97−3.92 (m, 4H), 3.63−3.58 (m, 4H), 2.05−1.93 ppm (m, 8H); $^{13}$C

NMR $\delta$ 199.4, 159.9, 158.6, 158.3, 137.2, 122.9, 122.3, 121.2, 120.7, 108.2, 90.2, 51.2, 50.0, 46.9, 26.1, 26.0. HRMS calcd for $C_{20}H_{23}N_5O+H$ 350.1981, found 350.2002.

**2-(2,4-Di-1-pyrrolidinyl-9H-pyrimido[4,5-b]indol-9-yl)-acetic Acid, Methyl Ester (12).** The aldehyde **11** (2.5 g, 7.2 mmol) was dissolved in MeOH (20 mL) and $CH_2Cl_2$ (20 mL) and cooled to 0 °C. Solid $K_2CO_3$ (5.0 g, 36.2 mmol) was added. A solution of iodine (4.5 g, 17.7 mmol) was then added over 4 min, and no exotherm was noted. After the reaction mixture stirred at 0 °C for 30 min, TLC analysis (50% EtOAc/hexane) showed only a trace of starting material. The cold reaction mixture was quenched into saturated $Na_2SO_3$ aqueous solution (150 mL) and EtOAc (150 mL). Water (50 mL) was added to dissolve a small amount of solid. The layers were separated, and the organic layer was washed with saturated $NaHCO_3$ and saturated NaCl aqueous solutions (150 mL each). The organic layer was dried by passing through solid $Na_2SO_4$ and concentrated to an oil. Chromatographic purification of the crude product (300 g of silica, $CH_2Cl_2$ followed by 1% acetone/$CH_2Cl_2$) yielded pure ester **12** (1.8 g, 66%) as a white solid: mp 172−175 °C; $^1$H NMR $\delta$ 7.89 (d, $J$ = 8.7 Hz, 1H), 7.20−7.11 (m, 3H), 5.06 (broad s, 2H), 3.93 (m, 4H), 3.72 (s, 3H), 3.62 (m, 4H), 1.96 (m, 8H). $^{13}$C NMR $\delta$ 169.6, 137.0, 121.9, 120.7, 120.3, 108.0, 52.4, 49.6, 46.6, 25.7, 25.6. HRMS calcd for $C_{21}H_{25}N_5O_2$ 379.2008, found 379.2007. Anal. Calcd for $C_{21}H_{25}N_5O_2$: C, 66.47; H, 6.64; N, 18.46. Found: C, 66.26; H, 6.55; N, 18.32.

**9-(2-Iodo-1-ethyl)-2,4-di-1-pyrrrolidinyl-5H-pyrimido-[4,5-b]indole (13).** To a stirred solution of $Ph_3P$ (341 mg, 1.3 mmol) and imidazole[17] (89 mg, 1.3 mmol) in $CH_2Cl_2$ (4 mL) was added iodine (330 mg, 1.3 mmol) followed by a solution of hydroxyethyl intermediate **8** (351 mg, 1 mmol) in $CH_2Cl_2$ (1 mL). After 30 min at 25 °C, TLC analysis (5% EtOAc/$CHCl_3$) indicated that no starting material remained. The reaction mixture was concentrated and then chromatographed rapidly (30 g of silica gel, 3% EtOAc/$CHCl_3$), thereby affording clean iodide **13** (152 mg, 33%) as a light tan solid, which was used immediately in the next transformation. $^1$H NMR $\delta$ 7.87 (d, $J$ = 7.7 Hz, 1H), 7.30−7.05 (m, 3H), 4.62 (t, $J$ = 7.6 Hz, 2H), 3.92 (m, 4H), 3.64 (m, 4H), 3.48 (t, $J$ = 7.7 Hz, 2H), 1.97 (m, 8H).

**Alternative Routes to 10 via 8-Derived Intermediates.** (a) Via ester **12**: A solution of ester **12** (36.7 g, 96.8 mmol) in 121 mL of pyrrolidine was heated at 60 °C for 1.5 h. The mixture was cooled to 25 °C, diluted with 400 mL of hexane, and filtered. Chromatographic purification of the crude solids (195 g of silica gel, 10% $CH_3CN/CH_2Cl_2$ yielded pure **10** as a white solid (36.3 g, 90%), identical by TLC and NMR to the fully characterized **10** from an earlier experiment. (b) Via iodide **13**: A stirred 0 °C solution of iodide **13** (145 mg, 0.314 mmol) in 5 mL of dry THF was treated with *t*-BuLi (0.37 mL) of 1.7 M solution in pentane, 0.629 mmol) added over 1 min, and the resulting homogeneous light yellow solution was stirred for an additional 15 min at 0 °C. A solution of *N*-(bromoacetyl)pyrrolidine (72 mg, 0.375 mmol) in 1 mL of THF was added in one portion, and stirring was continued at 0 °C for 1 h and then at 25 °C for 2 h. The mixture was poured into cold brine and extracted with $CH_2Cl_2$. The combined extracts were washed with water, dried (anhydrous $Na_2SO_4$), and concentrated, to afford a 2:1 mixture (TLC estimate) of amide **10** and N−H intermediate **9** (140 mg). Purification of this mixture was not undertaken.

**Alternative Syntheses of Intermediate 9.** (a) Via *tert*-butyl-protected intermediate **14**: A three-neck, 500 mL, round-bottom flask was charged with *tert*-butylamine (21.7 g, 0.30 mol) and THF (100 mL) and then cooled to −40 °C. *n*-Butyllithium (170 mL, 0.29 mol, 1.6 M in hexane) was added in 30 mL increments over 15 min. The reaction was warmed to 0 °C for 2 h and then recooled to −40 °C. Chloropyrimidine intermediate **5** (25 g, 99 mmol) in THF (50 mL) was added over a 30 min period to the reaction. The reaction was allowed to warm to room temperature and stirred overnight. The reaction was quenched by addition of $H_2O$ (50 mL) followed by concentration in vacuo. The residue was partitioned between $CH_2Cl_2$ (200 mL) and 10% $NaHCO_3$ (200 mL). The organic layer was removed and the aqueous portion extracted with $CH_2Cl_2$ (2 × 100 mL). The organic extracts were combined, washed with brine (400 mL), dried ($Na_2SO_4$), filtered, and concentrated in vacuo. Chromatographic purification (600 g of silica, 5% EtOAc/hexane) afforded pure **14** (14.3 g, 50%) as an off-white solid: $^1$H NMR $\delta$ 4.77 (s, 1H), 4.25 (s, 1H), 3.51 (M, 4H), 3.40 (m, 4H), 1.89 (m, 8H), 1.41 (s, 9H).

A solution of intermediate **14** (14.0 g, 48.4 mmol) and diisopropylethylamine (9.27 mL, 53.2 mmol) in acetonitrile (200) was treated with 2-bromocyclohexanone (17.3 g, 97.7 mmol). The reaction was stirred at room temperature for 16 h and then heated to reflux for 24 h. Following removal of 50 mL of acetonitrile via distillation the solution was allowed to cool to room temperature with stirring and then further cooled to 0 °C for 18 h. Filtration afforded the pure tetrahydropyrimido[4,5-b]indole (10.8 g, 61%) as a white solid: $^1$H NMR $\delta$ 3.66 (m, 4H), 3.57 (m, 4H), 2.89 (m, 2H), 2.75 (m, 2H), 1.91 (m, 4H), 1.83 (s, 9H), 1.80 (m, 6H), 1.65 (m, 2H).

A portion of the preceding product (5.0 g, 13.6 mmol), decahydronaphthalene (200 mL) and 10% Pd/C (1.25 g) were heated to reflux. After 4 h TLC detected no starting material. The reaction was cooled to room temperature and diluted with $CH_2Cl_2$ (200 mL). The reaction was filtered through a Celite pad, and the pad was washed with $CH_2Cl_2$ (4 × 50 mL). The filtrate was concentrated in vacuo to afford the dehydrogenated product (5.0 g, 95%) as a glassy solid: $^1$H NMR $\delta$ 7.77 (m, 2H), 7.10 (m, 2H), 3.80 (M, 4H), 3.63 (m, 4H), 2.01 (s, 9H), 1.97 (m, 8H).

A portion of the preceding product (4.0 g, 11.0 mmol) and freshly distilled TFA (100 mL) were heated to reflux. After 2 h 50 mL of TFA was removed by distillation. The reaction was cooled to 0 °C and adjusted to pH 10 by the addition of 25% NaOH. The reaction was transferred to a separatory funnel and extracted with $CH_2Cl_2$ (3 × 50 mL). The organic extracts were combined, washed with brine (80 mL), dried ($Na_2SO_4$), filtered and concentrated in vacuo. Chromatographic purification (300 g of silica, 5% EtOAc/

(17) Garegg, P. J.; Samuelsson, B. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2866.

$CH_2Cl_2$) yielded pure N−H intermediate **9** (2.8 g, 83%) as a white solid, identical by TLC and NMR to material prepared in an earlier experiment.

(b) Via benzotriazole intermediate **15**: A mixture of chloropyrimidine intermediate **5** (20 g, 79.4 mmol) and benzotriazole[14] (18.95 g, 159.1 mol) was heated to 120 °C under nitrogen for 18 h. The reaction mixture was cooled to 25 °C and partitioned between aqueous $NaHCO_3$ and $CH_2Cl_2$. The combined $CH_2Cl_2$ extracts were washed with water, dried ($Na_2SO_4$), and concentrated in vacuo. Chromatographic purification of the crude product (900 g of silica, 50% EtOAc/hexane) yielded clean triazole intermediate **15** 18.1 g, 74%) as a white solid: $^1$H NMR $\delta$ 8.67 (d, $J$ = 8.3 Hz, 1H), 8.08 (d, $J$ = 8.3 Hz, 1H), 7.54 (m, 1H), 7.40 (m, 1H), 6.56 (s, 1H), 3.67−3.56 (m, 8H), 2.01 (m, 8H); $^{13}$C NMR 162.1, 160.0, 157.5, 146.7, 131.9, 128.3, 124.5, 119.6, 115.6, 80.2, 46.6, 46.4, 25.6, 25.3.

A solution of **15** (76 mg, 0.227 mmol) in 1 mL of polyphosphoric acid was heated at 130 °C for 0.5 h and then at 180 °C for 45 min. The mixture was cooled to 25 °C, poured into ice/$NaHCO_3$, and extracted with $CH_2Cl_2$. The extracts were dried ($Na_2SO_4$) and concentrated, and chromatographic purification of the residue (10 g of silica, 50% EtOAc/hexane) yielded 30 mg of pure **9** (43%), identical by TLC and NMR to standard samples.

(c) Via primary amine **17**: Dichloropyrimidine intermediate **16** (from Alfa Aesar) (150 g, 0.91 mol) was added in portions with good stirring to 1 L of pyrrolidine (exothermic; temperature was kept below 50 °C with external cooling.) The resulting solution was then heated at reflux under nitrogen for 18 h. The mixture was cooled to 25 °C, and the excess pyrrolidine was removed in vacuo. The residue was partitioned between $CH_2Cl_2$ (1 L) and 10% aqueous $NaHCO_3$ (1.5 L), and the aqueous layer was extracted with additional $CH_2Cl_2$. The extracts were dried ($Na_2SO_4$) and concentrated, and the solid residue was recrystallized from EtOAc/$CH_2Cl_2$, thereby affording 110 g of pure **17** (52%) as a white solid: mp 155−159 °C; $^1$H NMR $\delta$ 4.85 (s, 1H), 4.61 (broad s, 2H, exchangeable), 3.56−3.48 (m, 4H), 3.46−3.39 (m, 4H), 1.94−1.86 (m, 8H); $^{13}$C NMR $\delta$ 161.3, 161.0, 73.1, 46.4, 45.9, 25.3, 25.0.

A solution of intermediate **17** (2.01 g, 8.62 mmol), 2-bromocyclohexanone (5.34 g, 30.1 mmol), and diisopropylethylamine (2.7 mL, 15.5 mmol) in $CH_3CN$ (44 mL) was heated at reflux under nitrogen for 18 h. The mixture was cooled to 25 °C and concentrated, and the residue was diluted with aqueous $NaHCO_3$ and extracted with $CH_2Cl_2$. The extracts were dried and concentrated, and the residue was purified by chromatography (200 g of silica, 35% methyl *tert*-butyl ether/$CH_2Cl_2$), thereby affording the 5,6,7,8-tetrahydro derivative of **9** (694 mg, 26%) as a pale yellow solid: $^1$H NMR $\delta$ 8.45 (s, 1H, exchangeable), 3.68 (m, 4H), 3.57 (m, 4H), 2.69 (m, 2H), 2.57 (m, 2H), 1.91 (m, 8H), 1.80−1.77 (m, 4H).

A portion of the above product (267 mg) and 10% Pd/C (55 mg) were suspended in Decalin, and the mixture was heated at 180 °C for 48 h. (Additional 100 mg portions of the 10% Pd/C were added at 18 and 26 h.) After cooling and removal of the Pd/C by filtration, chromatography of the residue (10 g of silica, 2% acetone/$CH_2Cl_2$) yielded pure **9** (114 mg, 43%) as a white solid, identical to standard samples by TLC and NMR.

***N*-(2,6-Dichloropyrimidin-4-yl)-glycine Ethyl Ester (19).** A clean, dry 3000 L glass-lined reaction vessel under an inert atmosphere was charged with 181 kg (987 mol) of 2,4,6-trichloropyrimidine and 1179 kg of ethyl acetate, and the mixture was held with agitation at 25 °C. A separate clean, dry 4000 L glass-lined reaction vessel under an inert atmosphere was charged with 150 kg (1075 mol) of glycine ethyl ester hydrochloride and 590 kg of ethyl acetate, and this was cooled with agitation to ∼0 °C. The trichloropyrimidine solution was then added to the glycine ethyl ester hydrochloride mixture over ∼2 h, maintaining the internal temperature below 10 ° C. When the transfer was complete, 299 kg (2313 mol) of diisopropylethylamine was charged over ∼2 h, and after 30 min the reaction mixture was warmed to 25 °C, and the reaction progress was monitored by HPLC analysis.

HPLC conditions: Zorbax C8 (4.6 mm × 150 mm); mobile phase, MeOH 2000, $CH_3CN$ 800, $H_2O$ 800, $Et_3N$ 3.2, HOAc 1.6; 254 nm; injection volume 20 $\mu$L; flow rate 1 mL/min; retention times: **19**, 2.6 min; starting material **18**, 3.1 min; undesired C-2 isomer, 3.5 min.

After 5.5 h of stirring, the reaction was complete, but due to analysis problems, the mixture was inadvertently stirred a total of 15.5 h at 25 °C. At this point ∼153 L of water was added. After 30 min of stirring, the phases were allowed to separate, and the organic phase was washed with 687 kg of 20% aqueous sodium chloride solution, filtered through a bag filter, and concentrated under reduced pressure until a total of 1616 kg of ethyl acetate distillate was collected. *n*-Heptane, 716 kg, was added, and the resulting slurry was cooled to <20 °C and stirred for ∼3 h. The product was isolated by centrifuge, washed with ∼45 kg of *n*-heptane, and dried under nitrogen. A total of 171 kg (69.2% yield) of product of 94 HPLC area % isomeric purity was obtained.

Chromatographic purification of a small sample (silica gel, 25% EtOAc/hexane) provided completely pure material, mp 91−93 °C; $^1$H NMR $\delta$ 6.37 (broad s, 1H), 5.91 (broad s, 1H), 4.25 (q, 2H), 4.17 (m, 2H), 1.29 (t, 3H); $^{13}$C NMR $\delta$ 163.5, 160.1, 62.1, 43.06, 14.16. Anal. Calcd for $C_8H_9Cl_2N_3O_2$: C, 38.42; H, 3.63; N, 16.80; Cl, 28.35. Found: C, 38.37; H, 3.51; N, 3.51; N, 16.67; Cl, 28.73.

***N*-2,6-Di-1-pyrrolidinylpyrimidin-4-yl)-glycine, Pyrrolidine Amide (20).** A clean, dry 3000 L glass-lined reaction vessel under an inert atmosphere was charged with 127 kg (508 mol) of *N*-(2,6-dichloro-4-pyrimidinyl) glycine ethyl ester and 362 kg of methanol. The solution was held with agitation at 25 °C. A clean, dry inert 4000 L glass-lined reaction vessel under an inert atmosphere was charged with 825 kg (11600 mol) of pyrrolidine and cooled to <0 °C with agitation. The substrate in methanol solution was then added to the pyrrolidine over a period of ∼4 h, keeping the internal temperature below 10 °C. When the transfer was complete, the stirred reaction mixture was warmed first to 45 °C and then heated to >80 °C, collecting ∼362 kg of

distillate. The mixture was then refluxed for 22−24 h, at which time HPLC analysis indicates less than 1% of di-addition intermediate remaining. The excess pyrrolidine (407 kg) was distilled off, and 1270 kg of water was added over ∼3 h, keeping the temperature at >75 °C. The reaction mixture was cooled to <20 °C, and the product was isolated by filtration. The wet cake was washed with ∼13 L of water and dried at 50 °C under vacuum. The average yield for a three-lot campaign was 92.9% of material which contained 8−11 HPLC area % of the 2-glycine isomer.

HPLC conditions: Zorbax C8 (4.6 × 150 mm); mobile phase: same as preceding experiment; retention times: starting material **19**, 2.6 min; bis-pyrolidine ester intermediate, 2.8 min; 2-isomer of product, 5.0 min; product **20**, 5.6 min.

Recrystallization of a portion of the product from EtOAc afforded clean amide **20** as a white solid: mp 215−219 °C; $^1$H NMR $\delta$ 5.21 (broad s, 1H), 4.78 (s, 1H), 4.04 (d, 2H), 3.53−3.39 (m, 12H), 2.20−1.82 (m, 12H); $^{13}$C NMR $\delta$ 168.0, 162.9, 161.8, 160.4, 126.3, 73.1, 46.2, 46.0, 45.9, 45.4, 43.8, 26.0, 25.6, 25.3, 24.2; MS (EI) 344 ($M^+$), 246, 218, 211, 121, 70, 55; HRMS calcd for $C_{18}H_{28}N_6O$ + H 345.2403, found 345.2416.

**(5,6,7,8-Tetrahydro-2,4-di-1-pyrrolidinyl-9H-pyrimido-[4,5-b]indolyl)-acetic Acid, Pyrrolidine Amide (21).** A clean, dry inert 4000 L glass-lined reaction vessel under an inert atmosphere was charged with ∼82 kg (238 mol) of intermediate **20** and 6.4 kg of calcium chloride, and after reestablishing the inert atmosphere, 51 kg (505 mol) triethylamine and 817 kg of acetonitrile were added. With agitation, ∼62 kg (468 mol) of 2-chlorocyclohexanone was added, and the reaction mixture was heated to 75 °C for 8 h, during which time reaction progress was monitored by HPLC analysis. Water (40 L) was added to the warm reaction mixture, and the mixture was slowly cooled to −10 °C over 3 h. After 6 h of stirring at −10 °C, the product was isolated by centrifugation and dried under nitrogen at 40 °C. The two-batch campaign provided **21** in an average yield of 73%, corrected for starting material quality.

HPLC conditions: Zorbax SB-C8 (4.6 × 250 mm); mobile phase: 350 $CH_3CN$, 650 0.05 M ammonium dihydrogen phosphate adjusted to pH 2.5 with phosphoric acid; injection volume 10 $\mu$L; flow rate 1 mL/min; retention times: starting amide **20**, 7.8 min; product **21**, 11.7 min.

Chromatographic purification of a portion of the product (10% acetone/$CH_2Cl_2$) gave pure **21** as a white solid: mp 219−225 °C; $^1$H NMR $\delta$ 4.77 (s, 2H), 3.67 (m, 4H), 3.54 (m, 4H), 3.46 (m, 4H), 2.71 (m, 2H), 2.63 (m, 2H), 1.93−1.77 (m, 16H); MS (FAB) 422 ($M^+$), 423, 352, 324, 310.

**Preparation of 10 from Glycine-Based Intermediate 21.** A clean, dry, inert 400 L Hastelloy-C reaction vessel under an inert atmosphere was charged with 27.7 kg (65.6 mol) of intermediate **21** and 20.5 kg of 5% Pd/C (50% water wet). The inert atmosphere was reestablished and 208 kg of xylenes was added. The reaction mixture was heated to ∼95 °C to remove the water by azeotropic distillation and heated to 140 °C with a positive nitrogen sweep. The reaction progress was monitored by HPLC, and after 8 h the starting material was consumed. The mixture was cooled to ∼70 °C, and 166 kg of THF was added. The spent catalyst was then removed by filtration. The catalyst cake was thoroughly washed with two 55 kg portions of THF and was then deactivated for safe recycle by soaking and washing with 40 L of water. The combined reaction filtrate and THF washes were concentrated at 130 °C and then cooled to ∼70 °C. Mixed heptanes, 55 kg, were added over ∼1.5 h. The resulting slurry was cooled to <0 °C, and the product was isolated by filtration. After washing with ∼83 kg of mixed heptanes, the product was dried under vacuum at 40 °C. The seven-batch campaign afforded **10** in an average yield of 83%. This material was identical to standard samples by TLC, NMR, and MS, described earlier in this manuscript.

HPLC conditions: Zorbax SB-C8 (4.6 × 75 mm); mobile phase: A-$CH_3CN$:$H_2O$: TFA = 5:95:0.001, B-$CH_3CN$:$H_2O$: TFA = 75:25:0.001; 50:50 A/B (0−7 min), 0:100 A/B (7.1−11 min), 50:50 A/B (11.1−13 min); retention times:starting material **21**, 5.9 min; product **10**, 3.5 min.

**1-[(2,4-Di-1-pyrrolidinyl-9*H*-pyrimido[4,5-b]indol-9-yl)acetyl]pyrrolidine hydrochloride (4).** A clean, dry inert 4000 L glass-lined reaction vessel under an inert atmosphere was charged with 32.0 kg (76.5 mol) of intermediate **10**. After reestablishing the inert atmosphere, 571 L of 2B3 ethyl alcohol was added, and the mixture was heated to a gentle reflux to dissolve the solids. The hot reaction mixture was polished through a 0.6 $\mu$m polypropylene line filter. A solution of polish-filtered ethyl acetate (550 L) and 37% hydrochloric acid (7.54 kg, 80.4 mol) was added to the alcoholic free base solution over ∼30 min, and the reaction mixture was distilled under vacuum to .∼170 L final volume. The mixture was seeded, diluted with 540 L of ethyl acetate and concentrated again under vacuum to ∼170 L final volume. This dilution/concentration operation was repeated four more times, or until the ethanol content of the supernatant was less than 5%. The slurry was filtered at 20−25 °C, rinsed with 95 L of polish-filtered ethyl acetate, and dried under 40 °C filtered nitrogen. The yield was 33.90 kg (97.4%) of clinical quality bulk drug **4**. The product **4** was a nonhygroscopic white solid which exhibited mp 250−251 °C. Anal. Calcd for $C_{24}H_{30}N_6O$·HCl: C, 63.35; H, 6.87; N, 18.47; Cl, 7.79. Found: C, 63.24; H, 6.96; N, 18.56; Cl, 7.81.


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