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Production Scale Synthesis of the Non-Nucleoside Reverse Transcriptase Inhibitor Atevirdine Mesylate (U-87,201E)

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Abstract:

A practical synthesis of atevirdine mesylate, Pharmacia & Upjohn's first-generation non-nucleoside reverse transcriptase (RT) inhibitor for treatment of AIDS, is described. The route consists of three steps. In the first step, the starting material, 3-amino-2-chloropyridine, is *N*-ethylated by conversion into the acetimidate (1.25 equiv of trimethyl orthoacetate, 0.003 equiv of HOTs·H_2O, neat; then distill off the MeOH to drive the amine/imidate equilibrium to imidate) followed by reduction with DIBAL (2.27 equiv, toluene, <-10 °C). In the second step, the *N*-ethyl derivative is heated in 5.13 equiv of piperazine at ~170 °C in a closed system under moderate pressure (~10 psig) to give 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine, which is purified by crystallization from water. An X-ray crystallographic study revealed that the crystal contains five molecules of water per molecule of 3-(*N*-ethylamino)-2-(1-piperazinyl)-pyridine. The molecules pack in an interesting way, with two layers of piperazinylpyridine molecules sandwiched between layers of water molecules, as in the lipid bilayer structure of the biological cell membrane. The yield for the first two steps is 79.2% (overall, average of six plant runs). In the third step, the pentahydrate is coupled with 5-methoxyindole-2-carboxylic acid (MICA; 1.07 equiv of CDI, CH_2Cl_2, 30 °C, 2−3 h; then add 1.06 equiv of 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine, CH_2Cl_2, 30 °C) to give atevirdine free base, which is converted into the mesylate salt (1.01 equiv of $MeSO_3H$, methanol, 25 °C) and crystallized. The yield of the third step is 83.3% (overall from MICA; average of eight plant runs). The bulk drug typically contains <0.1% total impurities (by HPLC). This process was used to produce multiton quantities of bulk drug used in phase II clinical trials.

Introduction

Over 5 million people have died from AIDS, and another 20 million are infected by HIV, the virus that causes AIDS.[2] HIV replicates by transcribing DNA from an RNA template, the reverse of the process by which human cells replicate. This reverse transcription is also one of the steps in the process by which the retrovirus infects the human immune system. Therefore inhibiting the enzyme reverse transcriptase (RT) is an attractive strategy for treatment of AIDS. The first drug approved by the U.S. FDA for treatment of AIDS was an RT inhibitor, zidovudine (3′-azido-3′-deoxythymidine; AZT; Retrovir). However, HIV rapidly develops resistance to zidovudine.

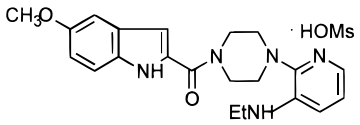

Figure 1. **Atevirdine mesylate (U-87,201E).**

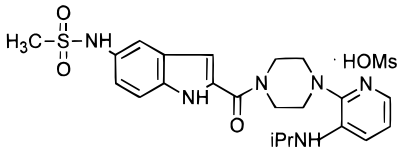

Figure 2. **Delavirdine mesylate (U-90,152S).**

Several years ago scientists in Upjohn Laboratories initiated a program directed toward development of a non-nucleoside RT inhibitor. They envisioned that such a drug when coadministered with zidovudine or another nucleoside RT inhibitor would have a potent antiviral effect, based on the premise that it would be difficult for the virus to develop resistance to different drugs simultaneously. Out of this program the first-generation candidate to emerge was atevirdine mesylate (U-87,201E)[3] (Figure 1). *In vitro* studies confirmed that atevirdine mesylate and zidovudine inhibit zidovudine-resistant clinical isolates of HIV-1 synergistically.[3b] Clinical development progressed into phase II, where development was discontinued in favor of a more active second-generation candidate, delavirdine mesylate (U-90,152S; Rescriptor)[4] (Figure 2). Pharmacia & Upjohn recently filed an NDA asking the U.S. FDA for approval to market delavirdine mesylate for treatment of AIDS.

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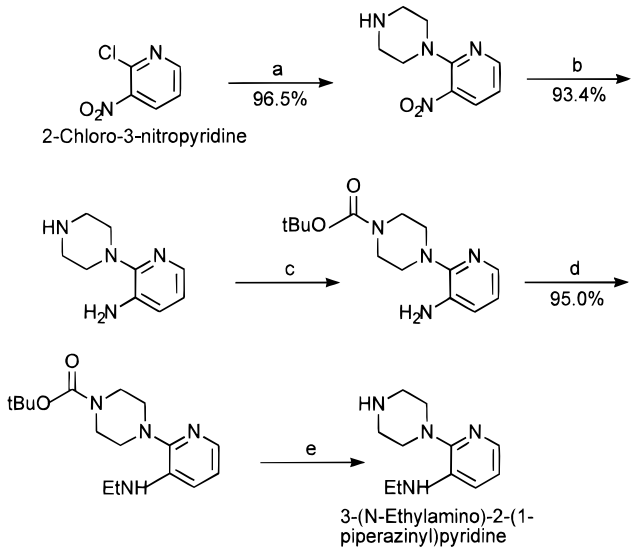
Scheme 1. **Original route to piperazinylpyridine subunit**[a]

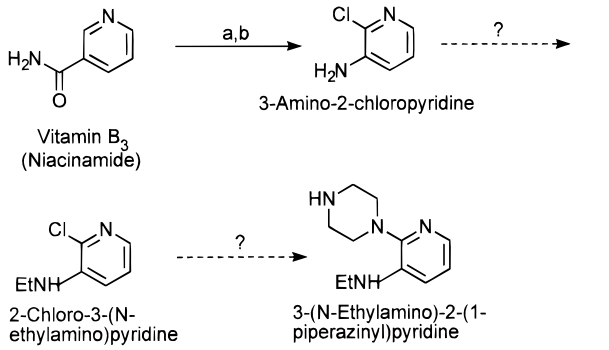


[a] Key: (a) 5.0 equiv of piperazine, iPrOH, rt, 30 min; (b) catalytic Pd/C, H_2, aqueous HCl, EtOH, rt (c) 1.02 equiv of $(tBuO_2C)_2O$, 1.12 equiv of Et_3N, CH_2Cl_2, 0 °C → rt, 30 min; (d) 4.43 equiv of MeCHO, 1.10 equiv of $NaCNBH_3$, MeOH, rt, overnight; (e) aqueous HCl, EtOAc, rt, 2 h.

If clinical development of atevirdine mesylate had continued, Pharmacia & Upjohn would have needed a synthetic route capable of delivering multiton quantities of bulk drug economically. Therefore shortly before clinical development entered phase I, a process research program was initiated. This program led to the development of efficient processes for synthesis of both atevirdine mesylate and delavirdine mesylate. The purpose of this paper is to describe the process to make the former; the process to make the latter is described in a patent application.[5]

Retrosynthetic Analysis. Disconnection of the amide bond generates two subunits, 5-methoxyindole-2-carboxylic acid and 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine. The indole subunit is commercially available in bulk from multiple vendors. Two short, high-yielding synthetic routes are described in the literature.[6,7] However, the piperazinylpyridine subunit is not readily accessible. A five-step route (outlined in Scheme 1),[3d,8−10] which was developed originally for synthesis of lipid peroxidation inhibitor drug candidates, was used to generate the small amounts of material needed for manufacture of bulk drug for phase I clinical studies. However this route is not suitable for generating multiton quantities of material economically. One problem is that the starting material, 2-chloro-3-nitropyridine, is itself not readily accessible. It can be synthesized in one step, by oxidation of 3-amino-2-chloropyridine with hydrogen peroxide and oleum in concd sulfuric acid, but the yield is low (65−84%).[11] Alternatively, it can be synthesized by multistep routes involving nitration of either 2-aminopyridine[12] or 2-hydroxypyridine,[13] but these routes are also low-yielding due to non-regiospecificity of the nitrations. It was felt that the price of this starting material would inevitably reflect the difficulty of synthesizing it. A more fundamental problem is that the route is too long: five steps is too many for synthesis of a simple 2,3-diamino-substituted pyridine. Therefore we decided to investigate alternative routes.

Scheme 2. **Alternative route to piperazinylpyridine subunit**[a]



[a] Key: (a) 1.2 equiv of Br_2, 3.8 equiv of NaOH, H_2O, 75 °C; (b) 1.3 equiv of H_2O_2, 11.5 equiv of concd HCl, 80 °C.

We were particularly interested in the possibility of using 3-amino-2-chloropyridine as the starting material since it is inexpensive and readily available in bulk. Presumably it is synthesized by Hofmann degradation[14,15] of vitamin B_3 (niacinamide) followed by regioselective chlorination at C-2,[14,16] based on the fact that commercial material contains minor amounts of 3-aminopyridine and/or 3-amino-2,6-dichloropyridine.[17] Moreover, only two steps (*N*-ethylation and piperazine displacement) are required in principle to convert this starting material into the desired piperazinylpyridine subunit. Therefore we undertook process research on this route (Scheme 2).

Results and Discussion

Step 1: *N*-Ethylation. In the original route to 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine,[3d,8−10] the Borch pro-

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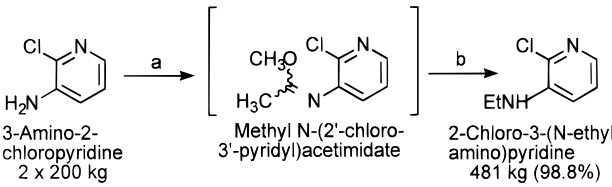
cedure (sodium cyanoborohydride/acetaldehyde)[18] was used to ethylate 3-amino-2-[4-(*tert*-butoxycarbonyl)-1-piperazinyl]-pyridine. The desired *N*-ethyl derivative was formed in excellent yield (95.0%), but it was contaminated with minor amounts of unethylated and diethylated impurities, which caused purification problems downstream. Removal of the former impurity was particularly troublesome, requiring silica gel chromatography (delayed to the stage of the crude bulk drug). Therefore, we ruled out the use of the Borch procedure as well as any other procedure that involves reductive amination with acetaldehyde.

To eliminate the possibilities of under-reaction and over-reaction, we initially chose to study reduction of the acetamide. The acetamide can be produced cleanly by stirring the amine in neat Ac_2O at 75 °C (without any base), following a published procedure.[16a,19] Clean reduction (81.6% yield isolated) could be effected by borane in THF, but excess reagent was required (2 molar equiv in theory,[20] 4.75 equiv in practice) because the product amine complexes 1 equiv of borane and the amine−borane is not a sufficiently strong reducing agent to reduce the acetamide. DIBAL (3.5 equiv; toluene, −40 °C to rt, 45 min) reduced the acetamide but also gave a significant amount of 3-(*N*-ethylamino)-pyridine by reduction of the 2-chlorine.

As it was clear that the acetamide was too stable to be easily reduced to the amine, we chose to make the more easily reducible[21] acetimidate. The literature contains conflicting recommendations for making acetimidates of aromatic amines. One paper recommends simply heating the aromatic amine together with a trialkyl orthoacetate neat without an acid catalyst.[22] Acid is claimed to be detrimental because it catalyzes amidine formation. Another paper states that amidine formation is not a problem, particularly with aromatic amines that bear electron-withdrawing groups, and recommends heating the aromatic amine and triethyl orthoformate together with an acid catalyst.[23] In our initial experiment we heated 3-amino-2-chloropyridine with 1.23 equiv of trimethyl orthoacetate at 108−118 °C in the absence of acid. After 20 h the reaction mixture had turned black and acetimidate formation was only about half complete by TLC. When we repeated the reaction in the presence of a catalytic amount of HOTs·H_2O, the acetimidate formed rapidly and cleanly with no evidence of amidine.

This procedure was then developed into a workable process, which goes as follows (Scheme 3). To a mixture of 3-amino-2-chloropyridine and HOTs·H_2O (0.005 equiv) is added neat trimethyl orthoacetate (TMOA, 1.24 equiv) at 24 °C. The 3-amino-2-chloropyridine slowly dissolves with an endotherm of about 10 °C. The mixture is warmed to 30 °C, and methanol is removed by vacuum distillation. This is an equilibrium reaction[24] and will only go ∼94% to completion without methanol distillation, which drives the reaction to 98.9−99.9% acetimidate. At the end of the methanol distillation the reactor contains a neat liquid consisting of methyl *N*-(2′-chloro-3′-pyridyl)acetimidate contaminated only with excess TMOA, HOTs catalyst, and 0.1−1.1% unreacted 3-amino-2-chloropyridine.

Scheme 3. **Step 1: two representative production runs**[a]



[a] Key: (a) 1.24 equiv of $(MeO)_3CCH_3$, 0.005 equiv of HOTs·H_2O, neat, 24 °C, endotherm to 16 °C; then distill off MeOH, add toluene; (b) 2.21−2.49 equiv of DIBAL, toluene, −10 °C; then quench with 1.59 equiv of MeOH, add to aqueous HCl (3.08 equiv) at 45−60 °C, discard aqueous layer, concentrate.

On the basis of a paper that recommends use of $NaBH_4$ for reduction of formimidates to *N*-methylamines,[21] we initially tried to reduce the acetimidate to the desired *N*-ethyl secondary amine using $NaBH_4$ (1.67 equiv, MeOH, Δ, 3.5 h). However, at most, ∼20% conversion to 2-chloro-3-(*N*-ethylamino)pyridine occurred.

We next investigated stronger reducing agents and found that DIBAL reduces the acetimidate to 2-chloro-3-(*N*-ethylamino)pyridine cleanly at <0 °C. DIBAL is inexpensive in bulk and available on site at Pharmacia & Upjohn's Kalamazoo plant. Generally DIBAL is not used for reductions that give amines because the aluminum salt byproducts cannot be separated by extraction with aqueous acid without losing significant amounts of the amine product.[25] However, 2-chloro-3-(*N*-ethylamino)pyridine is a weak enough base due to the inductive effect of the chlorine that one can wash a solution of it in toluene with pH 3 water to remove the aluminum salts without incurring significant product loss.[26]

The reduction is conducted by the following procedure. The acetimidate is diluted with toluene, then DIBAL (2.3−2.5 equiv; either neat or 25% in toluene) is added while the temperature is kept at <−10 °C. A minor amount (<0.5%) of dechlorination to 3-(*N*-ethylamino)pyridine occurs under these conditions. However, this is not a serious concern, since this impurity is cleanly removed in the pH 3 extraction.

Next the reaction mixture is prequenched at <−10 °C with methanol (0.45 equiv, enough to quench the excess hydride but not the isobutyl groups). This must be done slowly because hydrogen gas is evolved. The purpose of the prequench is to make it possible to hold the reaction mixture indefinitely without dechlorination while the quench is carried out.

Next the reaction mixture is further quenched into aqueous HCl (1.2 equiv/mol of DIBAL); the final pH will be 2.5−

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(26) The effect of pH on the partition coefficient of 2-chloro-3-(*N*-ethylamino)-pyridine between toluene and water is given below.

pH	% in PhMe/% in aqueous phase
4.0	99.3/0.7
3.0	98.8/1.2
2.2	94.9/5.1
1.7	83.7/16.3
1.3	44.3/55.7
1.0	23.9/76.1
0.6	6.0/94.0
0.3	0.6/99.4

3.5. **CAUTION:** It is **critical** that the aqueous HCl be kept at >40 °C throughout the quench so that hydrolysis of the isobutyl groups be instantaneous. The danger is that, if the isobutyl−aluminum species are allowed to accumulate, they might suddenly exothermically hydrolyze with evolution of a large volume of isobutane gas, resulting in an eruption.

The amount of HCl is also critical. If too much is used, a significant amount of product will be lost in the aqueous phase;[26] if too little is used, the aluminum salts will not dissolve, causing an emulsion.

Since the quench is done hot (40−55 °C) and since aqueous HCl has a significant vapor pressure at that temperature, a small amount of HCl can become entrained in the evolved isobutane vapors, making scrubbing necessary. Scrubbing would not be necessary if a nonvolatile acid such as tartaric acid, citric acid, or a mixture of acetic acid and propionic acid were used. However, the workup volumes would be larger because the aluminum salts of these organic acids have lower water solubility than the chloride salt. Since the costs associated with scrubbing are insignificant relative to the cost of decreasing the batch size, the decision was made to use the HCl workup.

The workup is completed by separating the phases and concentrating the organic layer. Because the product is slightly volatile (bp 80−84 °C at 0.5−1.2 mmHg), a small amount is lost in the toluene distillate [0.2−0.3% yield in typical lab and plant runs; 4.4% in worst case (a lab run)]. If desired, this can be recycled into the DIBAL work-up of the next run, thus increasing the yield of that run. Even without the recycle, the desired product 2-chloro-3-(*N*-ethylamino)pyridine is isolated in near-quantitative yield.

Step 2: Piperazine Displacement. It is known that it is difficult to displace the chlorine of 3-amino-2-chloropyridine by amines. The literature teaches that it is necessary to run the reaction at high temperature and/or use copper sulfate catalysis. Even so, yields are low. For example, reaction of 3-amino-2-chloropyridine with aniline requires 190 °C (20 h; 54% yield).[16a,27] Reaction with cyclohexylamine requires 200−210 °C (20 h; 67%).[28] Reactions of 3-amino-2-chloropyridine with ammonia, methylamine, *n*-propylamine, and dimethylamine in the presence of copper sulfate require 130 °C (20 h; 58.9%),[16a,27] 150 °C (17 h; 48.2% yield),[16a,27] 180 °C (18 h, 55% yield[29]), and 170 °C (18 h, 46% yield[30]), respectively. Also in the presence of copper sulfate, reaction of 3-(*N*-methylamino)-2-chloropyridine with ammonia requires 130 °C (30 h, 54% yield[15a,27]) and reaction with methylamine requires 160 °C (20 h, 55% yield[31]).

In our first experiment we refluxed 2-chloro-3-(*N*-ethylamino)pyridine in 6 equiv of neat piperazine (137−142 °C) containing 10 vol % toluene (to dissolve the piperazine that sublimes in the condenser) in the presence of sodium carbonate (to scavenge the 1 equiv of HCl that is evolved), following a procedure that we had previously developed for displacement of a chloropyrimidine by piperazine.[32] The desired adduct was formed cleanly and in high yield. However, the displacement was very slow, requiring 24 h to reach 95% conversion. Repetition in the presence of copper sulfate (and absence of sodium carbonate) gave a satisfactory rate acceleration (3−4 times faster), but reductive dechlorination competed to give ∼5% 3-(*N*-ethylamino)-pyridine along with several unidentified byproducts.

Attributing the low reactivity of 3-(*N*-ethylamino)-2-chloropyridine to the π electron donating effect of the ethylamino substituent, we next studied 3-acetamido-2-chloropyridine as the substrate. A competition experiment revealed that 3-acetamido-2-chloropyridine reacts 21 times faster than 3-(*N*-ethylamino)-2−chloropyridine (5.83 equiv of neat piperazine, 1.31 equiv of Na_2CO_3, 144 °C).[33] However, the adduct could not be reduced to the desired product 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine using the previously developed DIBAL reduction procedure because the product is too basic for the aluminum salts to be removed by low pH extraction. Therefore this strategy was not pursued further.

The most practical solution to the problem turned out to be to run in a sealed reactor under pressure at 170 °C. Such a high temperature is generally difficult to reach in general-purpose equipment with pressure steam as the heat source. However, the piperazine displacement reaction, despite being extremely slow, is moderately exothermic, due to the heat of neutralization of the evolved anhydrous HCl by piperazine.[34] Thus the required temperature can be conveniently reached by heating the reaction mixture to ∼140 °C with pressure steam and then releasing the pressure on the jacket and allowing the reaction to freely exotherm. In a typical run carried out in a 2000 gal reactor starting with 509 kg of 3-(ethylamino)-2-chloropyridine, the temperature exothermed from ∼140 °C to 173 °C over 3.5 h. The pressure rose from −12.8 psig to +10 psig, which is comfortably under the pressure ceiling of the reactor. This heating technique works only on production scale. On lab scale and pilot scale [9.0 kg of 2-chloro-3-(*N*-ethylamino)pyridine], the heat of reaction dissipates to the surroundings sufficiently rapidly that no exotherm is seen and thus it is necessary to reflux the reaction mixture for over 24 h to drive the reaction to completion.

Prior to running this exothermic reaction in a sealed reactor near the boiling point of the reaction mixture on production scale, we considered the danger of overpressurization. We reached the conclusion that the reaction could be run safely under these conditions based primarily on previous experience at Pharmacia & Upjohn with scale-up of a similar piperazine displacement reaction involving a chloropyrimidine.[32] In a typical run of that reaction, in which the reactor was charged with 528 kg of piperazine, ∼260 kg of 6-chloro-2,4-di-1-pyrrolidinylpyrimidine, and 325 kg

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(31) Oyama, K.; Stewart, R. *J. Chem. Soc., Perkin Trans. 1* **1973**, 673.
(32) Pearlman, B. A.; Padilla, A. G. US 5,225,555, July 6, 1993.

(33) The relative rate of displacement (K_a/K_b) was determined by monitoring the degree of conversion by HPLC, calibrated with authentic samples of both substrates and products, using the Ingold−Shaw equation (eq i, where a_f and a_i are the final and initial concentrations of species a, respectively); see: Ingold, C. K.; Shaw, F. R. *J. Chem. Soc.* **1927**, 2918. In both reactions, the only byproducts observed by LC or TLC were the bisadducts.

$$K_a/K_b = (\ln a_f - \ln a_i)/(\ln b_f - \ln b_i) \quad \text{(i)}$$

It was also determined that 2-chloro-3-(*N*-ethylamino)pyridine reacts 1.39 times faster than 3-amino-2-chloropyridine.

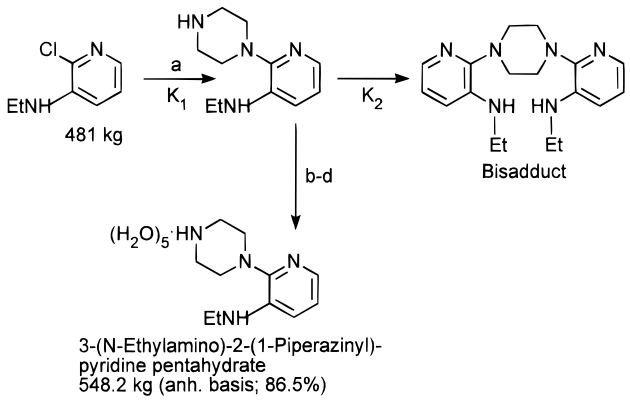
(34) ARC (accelerating rate calorimetry) testing revealed an exotherm with an adiabatic temperature rise of 59 °C and a true onset temperature of 113 °C.

of Isopar H, an ~11 °C exotherm (from ~134 °C to 145 °C) over 1.5 h was seen. Even though the chloropyridine reaction is run more concentrated (due to omission of the co-solvent Isopar H), this experience ruled out the possibility of an exotherm large enough to cause overpressurization, particularly since piperazine is known to have a low vapor pressure above its boiling point (e.g., 4.6 psig at 160 °C).

It is preferable to run the reaction without the acid scavenger (sodium carbonate). One benefit is that the reaction goes faster (by a factor of ~1.8). Presumably the displacement is autocatalyzed by the HCl byproduct. Hydrochloric acid has been used to accelerate displacements of chloroheterocycles by aniline (pK_a 4.6) and substituted anilines,[35] but not by any amine as basic as piperazine (pK_a 9.8) to our knowledge. Another benefit of omitting the sodium carbonate is that less pressure develops due to a boiling point elevating effect of piperazine hydrochloride, the byproduct of the displacement. When the reaction is run in the presence of sodium carbonate, the boiling point of the mixture is 152 °C whereas, when the reaction is run with the sodium carbonate omitted, the boiling point is 158 °C (at the end of the reaction). The option of including extra piperazine hydrochloride to further elevate the boiling point was investigated. Including 1 equiv (so that ~2 equiv is present at the end of the reaction) raised the boiling point to 168 °C, ~2 equiv raised it to 170 °C, and ~3 equiv raised it to 181 °C. A reaction carried out under the latter conditions was run in an open system at reflux and was complete in 3−5 h. However, dilution with only a small amount of xylene caused the piperazine hydrochloride to precipitate all at once to form an unstirrably thick slurry. By contrast, in experiments with sodium carbonate omitted, most of the toluene can be added before much of the piperazine hydrochloride precipitates, so that at no point does the slurry become too thick to stir. Thus in deciding whether to include extra piperazine hydrochloride, the benefit of its boiling point elevating effect must be weighed against the increased risk of problems in the piperazine/piperazine hydrochloride crystallization. The decision was made to omit the sodium carbonate but not to add extra piperazine hydrochloride.

Running in neat piperazine causes technical problems due to its peculiar physical properties. Piperazine melts at 108−110 °C and boils at 144−148.5 °C, depending on its exact water content. Thus piperazine tends to crystallize on the cool upper surfaces of the reactor. The simplest solution to this problem is to maintain all surfaces of the reactor above 110 °C, which insures that all the piperazine is in the liquid state. This is best achieved in production equipment by sealing the reactor at the condenser and tracing the riser with steam tubing. In the lab, it is more convenient to include toluene (approximately 5−10 vol %) in the reaction mixture and run in reflux mode. With 1 atm of steam through the condenser, the piperazine/toluene refluxes nicely without crystallizing.

Scheme 4. **Step 2: representative production run**[a]



[a] Key: (a) 6.01 equiv of piperazine, toluene; then seal under vacuum, heat to 145 °C; exotherm to 167 °C (9 psig) over 3 h; then cool to 150 °C over 9 h; (b) add toluene, cool to ~16 °C, filter off piperazine; (c) extract filtrate with 1.90 equiv of aqueous HCl (final pH of aqueous layer: 5.7); (d) basify with 50% aqueous NaOH (2.99 equiv) at <40 °C, then cool to 20 °C, filter.

The displacement is quite clean. The only significant byproduct is the bisadduct. The yield of bisadduct is a function of the number of equivalents of piperazine. Five to six equivalents is enough to reduce the yield of bisadduct to an acceptable level (2.8−3.1% on a mole basis) while still permitting a reasonable batch size. The excess piperazine does not add significantly to the cost since piperazine is inexpensive ($2.50/lb in bulk[36]). The bisadduct is easily separated from the desired monoadduct by partitioning between toluene and pH 5−6 water. Thus the bisadduct is of little concern.

Analysis of the yields of monoadduct and bisadduct over numerous runs revealed that the rate constant for the desired reaction (K_1, defined in Scheme 4) was consistently slightly greater than the rate constant for over-reaction to bisadduct (K_2). We calculate that $K_1/K_2 = 1.72$ (in the presence of Na_2CO_3; average of three runs) or $K_1/K_2 = 1.66$ (in the absence of sodium carbonate; average of six runs).[37] Thus, 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine is a significantly weaker nucleophile than piperazine. This explains why so little bisadduct is formed.

Initially we attempted an aqueous workup. The crude reaction mixture was diluted with water, treated with 1.24 equiv of sodium hydroxide (to convert the sodium bicarbonate into the more soluble sodium carbonate), and then

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(36) *Chem. Mark. Rep.* **1996**, *250* (11), 29 (Sept 9).

(37) The rate constant ratio $K_1/K_2 = K$ can be calculated using eqs ii, where K_1 and K_2 are the rate constants for reaction and over-reaction, respectively, X is the number of equivalents of piperazine, and S is the number of equivalents of piperazine remaining after addition of T equiv of 2-chloro-3-(*N*-ethylamino)pyridine. For a derivation, see Appendix I (available as Supporting Information). To solve this equation for K given values for S and X, it is necessary to use a numerical approximation method. We suggest using Newton's method (described by Uspensky: Uspensky, J. V. *Theory of Equations* McGraw-Hill Book Co.: New York, 1948; pp 174−180). It generates approximations accurate to >11 decimal places after only a few iterations provided the first approximation is not grossly inaccurate. For a listing of a short computer program (in Microsoft BASIC-80) that performs the required calculations, see Appendix II (available as Supporting Information).

$$0 = (2K-2)\left(\frac{S}{X}\right) + 2K\left(\frac{S}{X}\right)^{\left(\frac{1}{2K}\right)} + \frac{T(2K-1)}{X} + 2 - 4K \qquad (K \neq {}^1/_2)$$

$$0 = S + \frac{T-2X}{2-\ln\left(\frac{S}{X}\right)} \qquad (K = {}^1/_2) \qquad \text{(ii)}$$

extracted at 50 °C with *o*-dichlorobenzene/toluene. However, four extractions were required to recover 98.8% of the product due to significant partitioning of the product in the aqueous phase. A nonaqueous workup was therefore developed. The procedure goes as follows. First the reaction mixture is cooled to 140−145 °C, and then toluene is added. Cooling is necessary to prevent the toluene from flashing off; however, overcooling must be avoided as the reaction mixture will solidify at ∼100 °C. The mixture is then further cooled; when the temperature reaches about 50 °C the piperazine crystallizes. The slurry is further cooled to 0−5 °C and filtered. The filtrate contains the product 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine as well as bisadduct and ∼0.5 equiv of piperazine. The temperature of the filtration is important. In one run in which the slurry was filtered at room temperature, the filtrate contained a larger amount (1.15 equiv) of piperazine and the final product 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine pentahydrate was contaminated with a detectable amount of piperazine by TLC (not quantified). The piperazine cake contains only a negligible amount of product (typically 1−2% yield), regardless of whether sodium carbonate is included or omitted. Thus, essentially all the HCl in the system selectively crystallizes as piperazine hydrochloride, rather than as the hydrochloride of the product, perhaps because piperazine is the stronger base. The filtration is very fast; filtration through a small screen mounted in the bottom valve would be feasible and indeed is recommended if the decision is made to recycle the piperazine.

With the nonaqueous workup the piperazine is recovered in anhydrous form and can be recycled. Recycling the piperazine saves piperazine cost, eliminates a waste stream, and results in 1−2% higher yield since that much product typically cocrystallizes with the piperazine. If sodium carbonate is included in the reaction mixture, the cake can presumably be recycled several times at least. If sodium carbonate is omitted, then the piperazine cake can only be recycled once; after two recycles, the accumulated piperazine hydrochloride precipitates in large chunks on addition of toluene. The possibility of adding an extra equivalent of sodium carbonate to each run to prevent the buildup of HCl in the cake was not investigated.

It is critical that water be scrupulously excluded from the system. Piperazine hydrochloride is very hygroscopic and absorbs water to give a sticky hydrate that is difficult to filter. In one run in which the piperazine was contaminated with 8.4% water, the piperazine/piperazine hydrochloride crystallized as solid chunks which could not be removed from the flask during workup. Also the boiling point was depressed to 137−149 °C from the normal 152−157 °C. Thus, the piperazine should be assayed by KF prior to use to insure that it has <1% water.

After the piperazine/piperazine hydrochloride has been filtered off, water is added to the filtrate and the pH adjusted to ∼5 with aqueous HCl. The product and a small amount of piperazine partition in the aqueous phase (as their monohydrochloride salts), and the bisadduct along with any unreacted 2-chloro-3-(*N*-ethylamino)pyridine partition in the toluene phase. The toluene phase is separated and discarded.

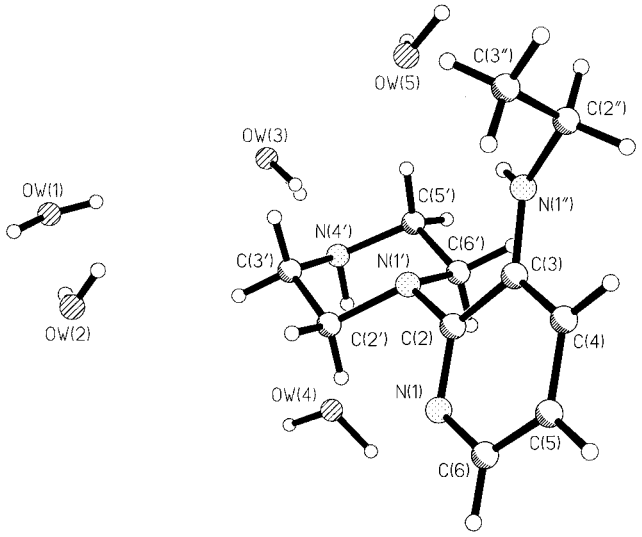

Figure 3. **ORTEP drawing of asymmetric unit.**

The pH 5 aqueous phase is then basified to ∼pH 9 with aqueous NaOH. The free base separates as an oil, which crystallizes (with seeding if necessary) to give a nice white slurry. The pH is then raised to >12.5 to insure complete formation of free base, and then the slurry is cooled to +16 °C and filtered. The temperature of the filtration is important. In one run in which the product was filtered at −2 °C, the product was contaminated with a detectable amount of piperazine (by TLC, not quantified). The cake is then washed with water and dried with room temperature nitrogen until the (off-white) solids appear dry (approximately 35−45 wt % H_2O by Karl Fischer). The mp is 44−46 °C. Overdrying should be avoided since the product will begin to oil out at 30.2 wt % H_2O, which is the theoretical water content of the pentahydrate. The yield is 80.0−85.4% (overall from 3-amino-2-chloropyridine).

The crystallization gives remarkable upgrading. Even though the crude 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine contains 0.1−1.1% of the desethyl analog, the crystals contain <0.1% of desethyl and the mother liquor contains only 2−5% yield of product. Crystallization of product intentionally spiked with 1.15% desethyl gave crystals that contained only 0.1% desethyl in good recovery (86.9%).

The pentahydrate has interesting physical properties. The crystals can absorb water up to ∼45% of their weight and remain dry and free-flowing. The molecule is oxygen sensitive like most amines, and the crystals will turn brown and eventually melt if allowed to stand in contact with the atmosphere for a prolonged period of time at room temperature. However, the crystals can be exposed to the atmosphere for short periods of time without showing any tendency to melt or effloresce. The crystals have a reasonably sharp melting point (44−46 °C), close to the melting point of the anhydrous crystals (46−48 °C).[10b]

X-ray Crystallographic Study of 3-(*N*-Ethylamino)-2-(1-piperazinyl)pyridine Pentahydrate. An X-ray crystallographic study of the crystals revealed that the crystal contains five molecules of water per molecule of 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine. An ORTEP drawing of the asymmetric unit is shown in Figure 3. A packing drawing (stereoview) is shown in Figure 4.

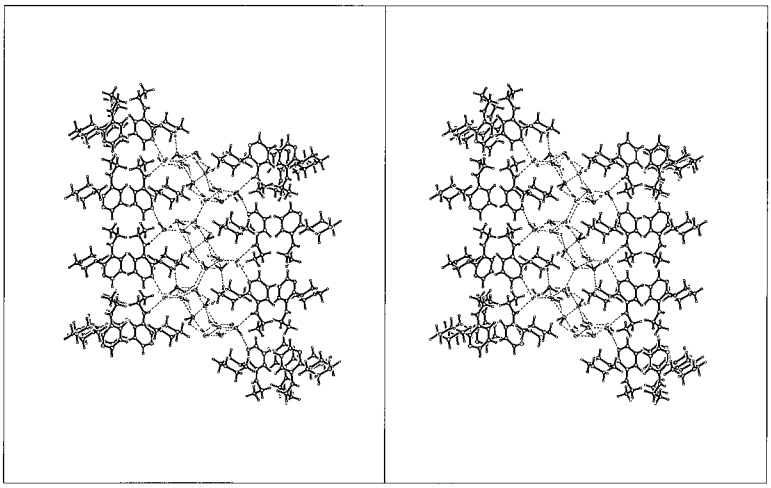

Figure 4. **Packing drawing (stereoview) showing "lipid bilayer" structure; dotted lines are hydrogen bonds.**

The molecules pack in an interesting way, with two layers of piperazinylpyridine molecules sandwiched between layers of water molecules, as in the lipid bilayer structure of the biological cell membrane (see Figure 4). Each piperazinylpyridine layer has a polar piperazine surface that faces a water layer and a nonpolar pyridine surface that faces the pyridine surface of another piperazinylpyridine layer; the pyridine/pyridine contacts are edge-to-face. There is extensive hydrogen bonding at the organic/aqueous interfaces: N(1″)-H is hydrogen bonded to OW(5); N(1) is hydrogen bonded to H-OW(5) of a neighboring asymmetric unit; N(4′)-H is hydrogen bonded to OW(4); and N(4′) is hydrogen bonded to H-OW(3). Piperazine hexahydrate crystallizes in a similar sandwich structure, with alternating layers of water molecules and piperazine molecules.[38]

The conformation of the molecule is also interesting. The pyridine and piperazine rings are non-coplanar. The dihedral angle between the plane of the pyridine ring and the plane defined by the four carbon atoms of the piperazine ring is 52.8°. Therefore the lone pair of electrons on N(1′) of the piperazine ring do not overlap optimally with the pyridine π electrons. Presumably the twisted conformation is adopted to relieve steric replusions in the planar conformation, as has been suggested to rationalize the similar conformation of atevirdine (free base).[39]

Many crystalline polyhydrates of amines have been described previously. Examples include piperazine hexahydrate (mp 44 °C),[38] hexamethylenetetramine hexahydrate (mp 13.5 °C),[40] $(Et_2NH)_3(H_2O)_{26}$ (mp −7 °C),[41] $(tBuNH_2)_4(H_2O)_{39}$ (mp ∼−1 °C),[42] and polyhydrates of dimethylamine (mp −16.9 °C), trimethylamine (mp 5.3−5.9 °C), ethylamine (mp −7.5 °C), *n*-propylamine (mp −13.5 °C), and isopropylamine (mp −4.2 °C).[43] The subject has been reviewed.[44]

Step 3: Coupling. In the original route,[3e] 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine was coupled with 5−methoxyindole-2-carboxylic acid (MICA) using *N*,*N*′-carbonyldiimidazole (CDI) as coupling agent. Since this procedure gave satisfactory results, we did not examine alternative coupling agents, but rather sought to make the original procedure operationally simpler. Our optimized procedure goes as follows. The crystals of 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine pentahydrate are covered with methylene chloride, and the slurry is warmed to 30 °C. The 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine dissolves in the methylene chloride, and the water collects as a separate phase. The water phase is separated and discarded. The methylene chloride phase is suitable for use in the coupling without further drying. Meanwhile, another reactor is dry charged with MICA (1.00 equiv) and CDI (1.07 equiv), and then methylene chloride is added and the mixture stirred at 30 °C until activation of MICA is complete (2−3 h). Then the solution of 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine (1.06 equiv) in methylene chloride is added to the solution of activated MICA and stirring continued at 30 °C. When the reaction is judged complete by HPLC, the reaction mixture is washed with water, and then the methylene chloride is removed by vacuum distillation and replaced with ethyl acetate. The slurry is then cooled to 0 °C and filtered to give atevirdine (free base) in 96.1% yield (based on MICA).

The free base is converted into the mesylate salt by the following procedure. The crystals of free base are slurried in MeOH at 25 °C and then treated with 1.01 equiv of methanesulfonic acid. The free base dissolves to form a solution, and then the mesylate salt crystallizes out. Crystallization is completed by addition of MTBE and cooling to −10 °C. The slurry is filtered to give atevirdine mesylate in 97.7% yield.

The final step was run eight times in production with uniformly satisfactory results. The bulk drug typically contained less than 0.1% total impurities by HPLC. The results of a representative run are given in Scheme 5.

Summary

An efficient three-step process for synthesis of atevirdine mesylate was developed. The process was run successfully in production to generate multiton quantities of bulk drug that was used in phase II clinical studies.

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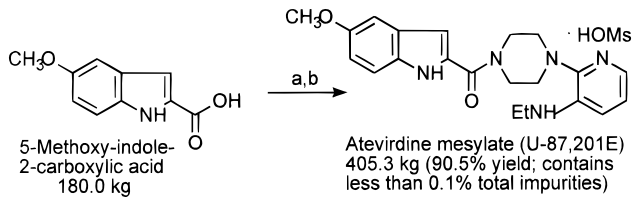
(44) Jeffrey, G. A.; McMullan, R. K. *Prog. Inorg. Chem.* **1967**, *8*, 43.

Scheme 5. **Step 3: representative production run**[a]



[a] Key: (a) 1.04 equiv of CDI, CH_2Cl_2, 25 °C, 13 h; meanwhile, in another reactor, dissolve 1.16 equiv of 3−(*N*-ethylamino)-2-(1-piperazinyl)pyridine pentahydrate in CH_2Cl_2 at 25 °C; add CH_2Cl_2 phase to activated MICA, stir at 30 °C for 2 h, then wash with water, vacuum concentrate while diluting with EtOAc until CH_2Cl_2 has been removed, cool to 0 °C, filter; (b) 1.03 equiv of $MeSO_3H$, MeOH, 25 °C; then add MTBE, cool to −10 °C, filter.

Experimental Section

General Procedures. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker 300 spectrometer in $CDCl_3$. Melting points were determined in unsealed capillary tubes and are uncorrected. HPLC analyses were carried out with a Varian 5000 liquid chromatograph equipped with a Biotage 4.6 × 250 mm, 8 μm Unisphere CN column, with UV detection at 254 nm, with the following mobile phase: reservoir A = 1000:5 water/30% aqueous ammonia; reservoir B = 1000:5 acetonitrile/30% aqueous ammonia; gradient elution from 95:5 A/B to 50:50 A/B over 30 min. The elution rate was 2.0 mL/min. 3-Amino-2−chloropyridine was purchased from Weyl, GmbH (Mannheim, Germany). Anhydrous *p*-toluenesulfonic acid was purchased from BIT Manufacturing Inc. (Copperhill, TN). Piperazine was purchased from Texaco Chemical Co. (Houston, TX). Dry solvents for laboratory scale reactions were purchased from Baxter Healthcare Corp., Burdick and Jackson Division (Muskegon, MI). All other reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used as received.

Methyl *N*-(2′-Chloro-3′-pyridyl)acetimidate. To a mixture of 3-amino-2-chloropyridine (20.0 g, 0.156 mol) and anhydrous *p*-toluenesulfonic acid (0.080 g, 0.47 mmol, 0.0030 equiv) was added trimethyl orthoacetate (23.36 g, 0.1945 mol, 1.25 equiv). An endotherm from 22 °C to 10 °C was observed. The mixture was warmed to 30 °C with concomitant dissolution of starting material. The solution was maintained at 25−30 °C for 10 min, and then the methanol was distilled by slowly applying vacuum to 70 mmHg, during which time the pot temperature fell to 10 °C. The methanol was further distilled with heating to 30 °C at 70 mmHg to give a crude oil (99.5% pure by HPLC, containing 0.2% 3-amino-2-chloropyridine). An analytical sample (colorless oil) was obtained by flash chromatography on silica gel eluting with 50% ethyl acetate/hexane: weight, 27.4 g (0.148 mol, 95.6%); ^{1}H NMR δ 1.82 (s, 3H), 3.84 (s, 3H), 7.16 (dd, 1H, J = 7.8, 2.0), 7.21 (dd, 1H, J = 7.8, 4.4), 8.08 (dd, 1H, J = 4.4, 2.0); ^{13}C NMR δ 16.33 (q), 53.44 (q), 122.75 (d), 130.03 (d), 142.40 (s), 142.99 (s), 143.40 (d), 163.64 (s); HRMS calcd for $C_8H_9ClN_2O$ *m/e* 184.0403, found *m/e* 184.0406. Anal. Calcd for $C_8H_9ClN_2O$: C, 52.04; H, 4.91; N, 15.17. Found: C, 52.06; H, 5.04; N, 15.28.

2-Chloro-3-(*N*-ethylamino)pyridine. Crude methyl *N*-(2′-chloro-3′-pyridyl)acetimidate (from 20.0 g of 3-amino-2-chloropyridine, 0.156 mol) was diluted with toluene (150 mL) and the mixture cooled to −20 °C. Neat diisobutylaluminum hydride (DIBAL; 50.282 g, 0.3536 mol, 2.272 equiv) was added dropwise with stirring while the temperature was maintained at −17 ± 3 °C. The mixture was stirred for 15 min at −20 °C, at which point HPLC showed <0.2% methyl *N*-(2′-chloro-3′-pyridyl)acetimidate. The mixture, a thin slurry, was then quenched by slow addition of methanol (2.84 mL, 0.0701 mol, 0.451 equiv) at −17 ± 3 °C with concomitant evolution of hydrogen gas. To a second flask were added in sequence water (100 mL) and 32 wt % hydrochloric acid (49.09 g, 0.431 mol, 2.770 equiv). The DIBAL reaction mixture was then cannulated into the aqueous HCl with an exotherm to, and then maintenance of, 45−55 °C via ice bath cooling. This addition was done carefully to allow for distillation of isobutane from the quench mixture. The mixture was stirred at 45 °C for 15 min, at which point a two-phase mixture had formed. The cloudy lower aqueous phase (pH 3.08) was discarded. The organic layer was concentrated under 70 mmHg vacuum at 90 °C to give a crude yellow oil: weight, 23.91 g [94.6% yield corrected for purity: 96.4% 3-(*N*-ethylamino)-2-chloropyridine, 3.0% toluene, 0.2% 3-amino-2-chloropyridine, 0.1% methyl *N*-(2′-chloro-3′-pyridyl)acetimidate]. The crude oil was purified by vacuum distillation (80−84 °C, 0.5−1.2 mmHg) to give an analytical sample: ^{1}H NMR δ 1.32 (t, 3H, J = 7.3), 3.18 (q, 2H, J = 7.3), 4.3 (br s, 1H), 6.87 (dd, 1H, J = 8.1, 1.3), 7.09 (dd, 1H, J = 7.8, 4.6), 7.69 (dd, 1H, J = 4.6, 1.3); ^{13}C NMR δ 14.46 (q), 37.86 (t), 117.21 (d), 123.41 (d), 136.11 (d), 136.95 (s), 140.87 (s); HRMS calcd for $C_7H_9ClN_2$ *m/e* 156.0454, found *m/e* 156.0456. Anal. Calcd for $C_7H_9ClN_2$: C, 53.83; H, 5.81; N, 17.95; Cl, 22.41. Found: C, 53.68; H, 5.79; N, 17.89; Cl, 22.64.

3-(*N*-Ethylamino)-2-(1-piperazinyl)pyridine Pentahydrate. Piperazine (66.948 g of material that assayed at 0.26% KF, 66.774 g on anhydrous basis, 0.7752 mol, 5.13 equiv) and crude 2-chloro-3-(*N*-ethylamino)pyridine [from 19.43 g (0.1511 mol) of 3-amino-2-chloropyridine] were combined and heated to reflux at 152 °C, using 1 atm of steam on the condenser. During the warmup, a large amount of piperazine sublimed into the upper part of the flask, but on reaching reflux this was washed back down. The mixture was stirred at reflux until the reaction was complete [0.58 mol % 3-(*N*-ethylamino)-2-chloropyridine, 93.6 mol % 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine, 3.09 mol % bisadduct, and 0.43 mol % 3-amino-2-(1-piperazinyl)pyridine] by HPLC (14 h), at which point it was a two-phase mixture. The reflux temperature slowly rose to 157.5 °C through the course of the reaction. The reaction mixture was then cooled to 145 °C and toluene (150 mL) added dropwise adiabatically. At the end of the addition the temperature was 90 °C. The slurry was further cooled to 5 °C. The solids were collected by vacuum filtration and washed with 0 °C toluene (2 × 40 mL). To the combined filtrate and washes was added water (89 mL), yielding a thin slurry. The pH was adjusted from 11.6 to 5.0 with 32 wt % hydrochloric acid (28.41 g, 0.249 mmol, 1.65 equiv), to give a two-phase mixture. The organic phase was discarded and the aqueous phase washed with toluene (90 mL). The aqueous phase was adjusted to pH 9.0 with 50 wt % aqueous sodium hydroxide (11.59 g, 0.145 mol, 0.96 equiv). The mixture was cooled to 25 °C and seeded with a prior lot of 3-(*N*-ethylamino)-2-(1-piperazinyl)-

pyridine pentahydrate. A thick but easily stirred slurry formed. The pH was further adjusted to >12.5 with 50 wt % aqueous sodium hydroxide (12.54 g, 0.157 mol, 1.04 equiv) while the temperature was maintained at <30 °C. The resultant slurry was cooled to 12 °C. The product was collected by vacuum filtration and washed with 10 °C water (178 mL). The solids were dried by a stream of 5 psi of nitrogen for 20 min, to give an off-white crystalline solid: weight, 44.60 g [loss on drying (LOD) = 46.9%, 23.683 g on anhydrous basis, 0.1148 mol, 76.0% yield overall from 3-amino-2-chloropyridine, 99.8 area % pure by HPLC]; silica gel TLC R_f = 0.37 (eluant: 89:10:1 dichloromethane/methanol/29% aqueous ammonia); mp 44−46 °C; ^{1}H NMR δ 1.31 (t, 3H, J = 8), 1.79 (br s, 1H), 3.02 (s, 8H), 3.12 (quint, 2H, J = 7), 4.23 (br s, 1H), 6.81 (m, 1H), 6.91 (m, 1H), 7.71 (m, 1H); ^{13}C NMR δ 14.80 (q), 38.13 (t), 46.55 (t), 50.56 (t), 115.94 (s), 119.87 (s), 135.21 (s), 137.57 (s), 151.00 (s); HRMS calcd for $C_{11}H_{18}ClN_4$ *m/e* 206.1531, found *m/e* 206.1530.

3-(*N*-Ethylamino)-2-(1-piperazinyl)pyridine Pentahydrate (with Sodium Carbonate). Piperazine (66.98 g, 0.7776 mol, 5.00 equiv), sodium carbonate (21.54 g, 0.203 mol, 1.305 equiv), and crude 2-chloro-3-(*N*-ethylamino)-pyridine (from 20.942 g of 3-amino-2-chloropyridine, 162.90 mmol) were combined and heated to reflux at 144 °C, using 1 atm of steam on the condenser. During the warmup, a large amount of piperazine sublimed into the upper part of the flask, but on reaching reflux this was washed back down. The mixture was stirred at reflux until complete [0.89 mol % 3-(*N*-ethylamino)-2-chloropyridine, 94.1 mol % 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine, 2.79 mol % bisadduct, and 0.08 mol % 3-amino-2-(1-piperazinyl)pyridine] by HPLC (16 h), at which point it was a thin slurry. The reflux temperature slowly rose to 152 °C through the course of the reaction. The reaction mixture was then cooled to 147 °C, and toluene (239 mL) was added dropwise adiabatically; at the end of the addition the temperature was 74 °C. The slurry was further cooled to −3 °C. The solids were collected by vacuum filtration and washed with 0 °C toluene (2 × 48 mL). To the combined filtrate and washes was added water (150 mL), yielding a thin slurry. The pH was adjusted from 10.9 to 5.4 with 37 wt % hydrochloric acid (24.05 g, 0.244 mmol, 1.57 equiv), to give a two-phase mixture. The upper phase was discarded. The aqueous phase was adjusted to pH 8.9 with 50 wt % aqueous sodium hydroxide (9.77 g, 0.122 mol, 0.784 equiv). The mixture was cooled to 20 °C and seeded with a prior lot of 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine pentahydrate. A thick but easily stirred slurry formed. The pH was further adjusted to >12.5 with 50 wt % aqueous sodium hydroxide (14.80 g, 0.1502 mol, 0.9653 equiv) while the temperature was maintained at <30 °C. The resultant slurry was cooled to 16 °C. The product was collected by vacuum filtration and washed with 15 °C water (100 mL). The solids were dried under 5 psi of nitrogen for 20 min, to give 38.71 g of an off-white crystalline solid [LOD = 30.5%, 80.0% yield overall from 3-amino-2-chloropyridine, HPLC 99.5 area % 3-(*N*-ethylamino)-2-(1−piperazinyl)pyridine pentahydrate; mp 46−48 °C].

Crystallization of 3-(*N*-Ethylamino)-2-(1-piperazinyl)-pyridine from Water When Contaminated with 3-Amino-2-(1-piperazinyl)pyridine. To 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine pentahydrate [9.916 g; LOD = 46.4%; 25.765 mmol; 99.8 area % pure containing <0.1 area % 3-amino-2-(1-piperazinyl)pyridine by HPLC] was added 3-amino-2-(1-piperazinyl)pyridine (62 mg, 1.15 wt % on anhydrous basis). Water (25 mL) was added, and the mixture was warmed to 48 °C to give a homogeneous solution. The mixture was cooled to 20 °C and the product collected by vacuum filtration and washed with water (40 mL). The mixture was dried in an air stream for 10 min to afford an off-white solid [7.899 g; LOD = 41.6%; 86.9% recovery; 99.7% 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine and 0.1% 3-amino-2-(1-piperazinyl)pyridine by HPLC]. The filtrate contained a 9.9% yield of product (by HPLC).

1-[(5-Methoxyindol-2-yl)carbonyl]-4-[3-(*N*-ethylamino)-2-pyridyl]piperazine, Monomethanesulfonic Acid Salt (Atevirdine Mesylate; U-87,201E). 5-Methoxyindole-2-carboxylic acid (MICA; 10.00 g, 52.30 mmol) and *N*,*N*′-carbonyldiimidazole (CDI; 9.08 g, 56.00 mmol, 1.07 equiv) were dry loaded into a nitrogen-inerted flask. To this mixture was added 50 mL of methylene chloride. The resulting foamy slurry (CO_2 evolution) was stirred at 30 °C under nitrogen until no MICA remained as determined by HPLC (reaction typically complete in 2−3 h). In another flask, 3-(*N*-ethylamino)-2-(1-piperazinyl)pyridine pentahydrate (11.44 g on anhydrous basis, 55.46 mmol, 1.06 equiv) was stirred with 46 mL of methylene chloride at 25−30 °C to give a two-phase solution. The methylene chloride layer was separated and added to the slurry of activated MICA prepared above over a period of 15−30 min; the aqueous layer was discarded. The reaction mixture was stirred at 30 °C until the reaction was judged to be complete by HPLC (no activated MICA left). The reaction mixture, now a clear yellow solution, was washed with two 50 mL portions of water. The methylene chloride solution was vacuum-concentrated to a volume of 100−150 mL. This mixture was then diluted with 100 mL of ethyl acetate and concentrated again to a slurry volume of 100−150 mL. The dilution with ethyl acetate and concentration were repeated a second and a third time. The final slurry volume was 80−90 mL. The slurry was stirred at 0−5 °C for 1 h and filtered. The cake was washed twice with 10 mL of 0−5 °C ethyl acetate and dried by a nitrogen stream to give atevirdine: yield, 19.07 g (50.25 mmol, 96.1%). To a slurry of atevirdine (15.00 g, 39.53 mmol) in 75 mL of methanol under nitrogen at 25 °C was added neat methanesulfonic acid (2.58 mL, 3.82 g, 39.76 mmol, 1.006 equiv) over 1−2 min; a 5 °C exotherm was noted. The product began to come out of solution within 5 min. This slurry was stirred for ca. 30 min at 25 °C, and then methyl *tert*-butyl ether (110 mL) was added slowly. The resulting slurry was cooled at −10 °C for 30 min and then filtered. The cake was washed with methyl *tert*-butyl ether (2 × 25 mL) and dried overnight in a 55−60 °C vacuum oven, to give atevirdine mesylate, >99% pure by HPLC: yield, 18.374 g (38.64 mmol, 97.7%). The product was identical by HPLC to an authentic sample made by a similar procedure that was fully analyzed and characterized.[3d]

Table 1. **Bond lengths (Å), angles (deg), and torsion angles (deg)**

A. Bond Lengths

N(1) C(2)	1.322(5)	N(1″) C(2″)	1.460(5)
N(1) C(6)	1.363(5)	C(2″) C(3″)	1.526(6)
C(2) C(3)	1.424(5)	N(1′) C(2′)	1.479(5)
C(2) N(1′)	1.408(5)	N(1′) C(6′)	1.481(5)
C(3) C(4)	1.391(5)	C(2′) C(3′)	1.518(6)
C(3) N(1″)	1.384(5)	C(3′) N(4′)	1.468(6)
C(4) C(5)	1.380(5)	N(4′) C(5′)	1.480(5)
C(5) C(6)	1.378(5)	C(5′) C(6′)	1.512(5)

B. Bond Angles

C(2) N(1) C(6)	118.7(3)	C(3) N(1″) C(2″)	121.2(3)
N(1) C(2) C(3)	122.7(3)	N(1″) C(2″) C(3″)	114.0(3)
N(1) C(2) N(1′)	119.2(3)	C(2) N(1′) C(2′)	114.8(3)
C(3) C(2) N(1′)	118.1(3)	C(2) N(1′) C(6′)	112.9(3)
C(2) C(3) C(4)	117.4(3)	C(2′) N(1′) C(6′)	110.0(3)
C(2) C(3) N(1″)	118.7(3)	N(1′) C(2′) C(3′)	108.9(3)
C(4) C(3) N(1″)	123.9(3)	C(2′) C(3′) N(4′)	112.9(3)
C(3) C(4) C(5)	119.8(3)	C(3′) N(4′) C(5′)	109.7(3)
C(4) C(5) C(6)	119.2(3)	N(4′) C(5′) C(6′)	112.5(3)
N(1) C(6) C(5)	122.3(3)	N(1′) C(6′) C(5′)	110.3(3)

C. Torsion Angles

C(6) N(1) C(2) C(3)	0.2(6)	C(2) C(3) N(1″) C(2″)	162.3(3)
C(6) N(1) C(2) N(1′)	−178.5(3)	C(4) C(3) N(1″) C(2″)	−18.7(5)
C(2) N(1) C(6) C(5)	−1.3(6)	C(3) C(4) C(5) C(6)	−1.5(6)
N(1) C(2) C(3) C(4)	0.2(5)	C(4) C(5) C(6) N(1)	2.0(6)
N(1) C(2) C(3) N(1″)	179.3(3)	C(3) N(1″) C(2″) C(3″)	−73.0(4)
N(1′) C(2) C(3) C(4)	179.0(3)	C(2) N(1′) C(2′) C(3′)	172.3(3)
N(1′) C(2) C(3) N(1″)	−1.9(5)	C(6′) N(1′) C(2′) C(3′)	−59.1(4)
N(1) C(2) N(1′) C(2′)	24.6(5)	C(2) N(1′) C(6′) C(5′)	−171.6(3)
N(1) C(2) N(1′) C(6′)	−102.6(4)	C(2′) N(1′) C(6′) C(5′)	58.7(4)
C(3) C(2) N(1′) C(2′)	−154.2(3)	N(1′) C(2′) C(3′) N(4′)	58.3(4)
C(3) C(2) N(1′) C(6′)	78.7(4)	C(2′) C(3′) N(4′) C(5′)	−54.8(4)
C(2) C(3) C(4) C(5)	0.4(5)	C(3′) N(4′) C(5′) C(6′)	53.4(4)
N(1″) C(3) C(4) C(5)	−178.6(3)	N(4′) C(5′) C(6′) N(1′)	−56.2(4)

Accelerating Rate Calorimetry (ARC) Experiment. ARC testing of the reaction mixture [3.105 g of piperazine, 1.179 g of 2-chloro-3-(*N*-ethylamino)pyridine (6.2% by volume toluene)] was carried out using a Hastelloy C sample bomb with a $^1/_4$ in. neck. The start temperature of the run was 100 °C using a heat step of 5 °C, a wait time of 10 min, a search time of 10 min, and a slope sensitivity of 0.02 °C/min. The Φ-factor for the run was 1.6432. Two separate exotherms were detected. The first, corresponding to the desired reaction, yielded a true onset of 113 °C with an adiabatic temperature rise of 59 °C (normalized to Φ-factor = 1) and a time to maximum rate of 254 min. The second exotherm was a high-temperature decomposition with a true onset of 256 °C and an adiabatic temperature rise of 201 °C (normalized to Φ-factor = 1) and a time to maximum rate of 1082 min.

X-ray Crystallographic Study of 3-(*N*-Ethylamino)-2-(1-piperazinyl)pyridine Pentahydrate. Crystal data: $C_{11}H_{18}N_4O_5$, M_r = 296.4, orthorhombic, *Pbcn*, a = 32.848(5) Å, b = 6.615(1) Å, c = 15.414(3) Å, V = 3349.3(7) Å^3, Z = 8, D_c = 1.175 gm/cm^3, graphite-monochromatized Cu $K\alpha$, λ = 1.5418, μ = 6.91 cm^{-1}, T = 135 K, R = 0.106 for 3057 unique reflections.

A clear, thin plate of dimensions 0.07 × 0.13 × 0.32mm was used for intensity measurements on a Siemens $P2_1$ X-ray diffractometer controlled by a Harris computer. Cu $K\alpha$ graphite-monochromatized radiation was used as the source. The step-scan technique was used with a scan speed of 4° min^{-1}, a scan width of 3.4°, and a $2\theta_{max}$ of 138°. Ten reflections periodically monitored showed no loss in intensity during the data collection. Of the 3057 reflections, 1783 had intensities $>3\sigma$. Standard deviations in the intensities were approximated by the equation

$$\sigma^2(I) = \sigma^2(I)_{\text{counting statistics}} + (0.0177I)^2$$

where the coefficient of I was calculated from the variations in intensities of the monitored reflections. Unit-cell parameters were determined accurately by least-squares fit of Cu $K\alpha_1$ 2θ values [λ(Cu $K\alpha_1$) = 1.5402] for 25 high-2θ reflections.[45] Lorentz and polarization corrections appropriate for a monochromator with 50% perfect character were applied, but with no absorption correction. The structure was solved by direct methods using MULTAN80.[46] The structure was refined by least squares with the coordinates and anisotropic thermal parameters for non-hydrogen atoms included in the refinement. The hydrogen atom positions were found in difference maps very close to positions generated using planar and tetrahedral geometry, so generated positions were used. Isotropic thermal parameters for hydrogen atoms were set $^1/_2$ unit higher than the isotropic equivalent of the thermal parameters of the attached heavier

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Table 2. **Hydrogen bonds**[a]

D	A	symmetry	D···A	H···A	D−H···A
N(1″)	N(1′)	x, y, z	2.754(4)	2.42	98
N(1″)	OW(5)	x, y, z	3.018(4)	2.38	120
OW(3)	N(4′)	x, y, z	2.743(4)	1.72	159
N(4′)	OW(4)	x, y, z	3.063(4)	2.10	162
OW(1)	OW(2)	x, y, z	2.831(4)	2.31	114
OW(2)	OW(1)	x, y, z	2.831(4)	2.30	163
OW(2)	OW(3)	$x, y-1, z$	2.788(4)	2.09	146
OW(3)	OW(2)	$x, y+1, z$	2.788(4)	2.30	102
OW(4)	OW(2)	$1-x, y+1, {}^1/_2-z$	2.799(4)	1.88	132
OW(5)	N(1)	$x, -y, z-{}^1/_2$	2.835(4)	1.93	158
OW(4)	OW(5)	$x, 1-y, z+{}^1/_2$	2.860(4)	2.19	120
OW(5)	OW(4)	$x, 1-y, z-{}^1/_2$	2.860(4)	2.04	146
OW(1)	OW(5)	$1-x, -y, -z$	2.836(4)	2.10	135
OW(1)	OW(3)	$1-x, 1-y, -z$	2.759(4)	2.14	132

[a] D represents donor, A acceptor; distances are in angstroms, and angles are in degrees. Standard deviations are in parentheses.

atom. The function minimized in the refinement was $\sum w(F_o^2 - F_c^2)^2$, where weights w were $1/\sigma^2(F_o^2)$. Atomic form factors were from Doyle and Turner,[47] except for hydrogens, which were from Stewart, Davidson, and Simpson.[48] In the final refinement cycle, all shifts were $<0.38\sigma$. The final R was 0.070 (for $F_o^2 > 3\sigma$), and the standard deviation of fit was 2.62. A final difference map showed no peaks >0.48 e Å^{-3}. The CRYM system of computer programs was used.[49] An ORTEP[50] drawing with atom numbering is shown in Figure 3. A packing drawing is shown in Figure 4. The bond lengths, angles, and torsion angles are listed in Table 1. The hydrogen bond lengths and angles are listed in Table 2. The final coordinates are listed in Table 3. The anisotropic thermal parameters are listed in Table 4. Tables 3 and 4 are available as Supporting Information.

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Supporting Information Available

Derivation of eqs ii (Appendix I), listing of a computer program for solving eqs ii (Appendix II), and supporting X-ray crystallographic data including final coordinates and anisotropic thermal parameters (4 pages). See any current masthead page for ordering and Internet access instructions.

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