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TETRAHEDRON

Synthesis of 3-Aminopyridine-2-carboxaldehyde Thiosemicarbazone (3-AP)

Chuansheng Niu*,†, Jun Li*,#, Terrence W. Doyle and Shu-Hui Chen

Vion Pharmaceuticals, Inc., 4 Science Park, New Haven, CT 06511

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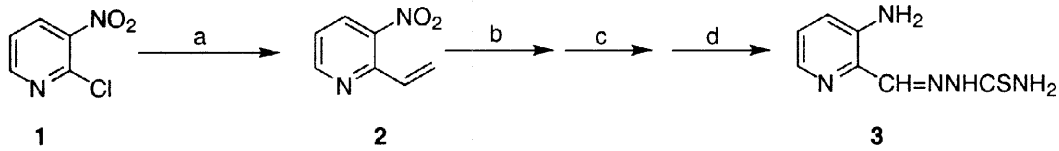
Abstract: Palladium-catalyzed cross-coupling of methylboronic acid with 2-chloro-3-nitropyridine produced 2-methyl-3-nitropyridine **4** in one step in high yield. Oxidation of **4** with selenium dioxide gave aldehyde **5**. Alternatively, condensation of **4** with DMFDMA followed by oxidation gave **5** in a two step higher yielding conversion. Subsequent direct coupling of **5** with thiosemicarbazide followed by reduction of the nitro group using stannous chloride or sodium sulfide provided 3-AP (**3**). Reduction with sodium hydrosulfite gave 3-HAP (**8**). Finally a route which avoids the reduction of a nitro function was devised. Thus direct coupling of styrene with 2-chloro-3-aminopyridine **9** under Heck reaction conditions gave **16** which was converted to **17**, oxidized to the aldehyde **18** and converted to 3-AP (**3**) with *in situ* deblocking of the *t*-Boc functionality.

INTRODUCTION

Ribonucleotide reductases exist in all living cells and catalyze the rate-limiting step in the synthesis of deoxyribonucleoside triphosphates. Inhibitors of ribonucleotide diphosphate reductases are extremely effective in blocking the biosynthesis of DNA because of the low intracellular levels of deoxyribonucleoside triphosphates.[1] An excellent correlation between tumor growth rate and the specific activity of ribonucleotide reductase has been demonstrated.[1b] Thus, strong inhibitors of ribonucleotide diphosphate reductases could be useful as therapeutic agents against cancer.[2] Recently, Sartorelli and co-workers have described several new N-heterocyclic carboxaldehyde thiosemicarbazones (HCTs) as potent inhibitors of ribonucleotide diphosphate reductases.[3] The most active compound of this series, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) showed significant antitumor activity in mice bearing L1210 leukemia, M-109 lung carcinoma, and A2780 human ovarian carcinoma. This activity has led to the selection of 3-AP for clinical development providing the impetus for the development of an efficient synthesis of this agent.

Previously, we reported a synthesis of 3-AP based on palladium-mediated cross-coupling reactions of vinyltin reagents with 2-chloro-3-nitropyridines as shown in Scheme 1.[4] In this paper we report our efforts to further simplify the synthesis of 3-AP.



Scheme 1 *Reagents and conditions:* a) vinyl tributyltin, $Pd(PPh_3)_4$, PPh_3, toluene, reflux, 86%; b) O_3, MeOH, Me_2S; c) $NH_2NHCSNH_2$, EtOH, HCl, 90%; d) Na_2S, 82%.

RESULTS AND DISCUSSION

The original synthesis of 3-AP involved the conversion of 2-methyl-3-nitropyridine **4** to **3** in several steps. Several known methods of preparing **4** suffer from difficult handling,long synthetic sequences and low yields.[5-7] More recently Sartorelli and co-workers[8] described an improved method of preparing **4** involving a two-step sequence in 63% overall yield. Since 2-methyl-3-nitropyridine **4** is a useful starting material for the further synthesis of HCTs, developing a simple and highly efficient methodology for the synthesis of **4** was desirable.

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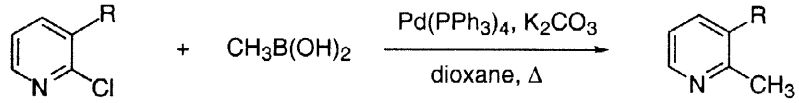
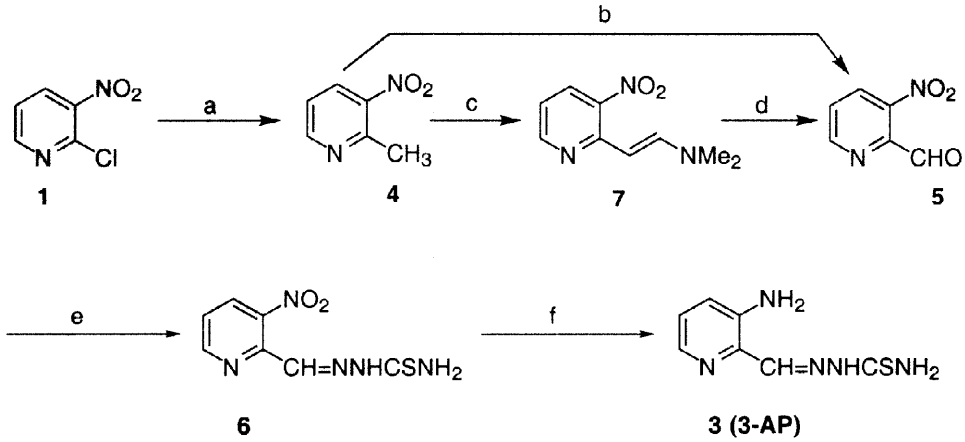
Suzuki coupling approach. The palladium-mediated coupling reaction of arylboronic acids with aryl halides in the presence of a base was first reported by Suzuki in 1981.[9] The scope of the Suzuki reaction has been extended to coupling of aryl- and alkylboronic acids with heteroaryl halides,[10] aryl or vinyl triflates,[11] vinyl halides,[12] allyl bromides[13] and so on.[14] The Suzuki reaction usually gives high yields under very mild conditions and is tolerant of a wide variety of functional groups in either component. We first investigated the Suzuki cross-coupling reaction of substituted 2-chloropyridine derivatives with methylboronic acid in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium(0) (Scheme 2). Interestingly, treatment of 2-chloro-3-nitropyridine with methylboronic acid produced the desired product **4** in 90% yield; but reaction of 2-chloro-3-aminopyridine with methylboronic acid required a longer time to be completed and gave 2-methyl-3-aminopyridine in only 35% yield. In the case of 2-chloropyridine itself, none of the desired product could be detected. Instead, two unknown products as yellow solids were isolated. Neither of them showed a methyl group upon examination of their proton NMR spectra. This study revealed that an electron-withdrawing group at the C-3 position of 2-chloropyridine activated the C-2 position and greatly facilitated the Suzuki reaction.



1 4

Scheme 2 *Substituents and yields:* R = NO_2, 90%; R = NH_2, 35%; R = H, 0%

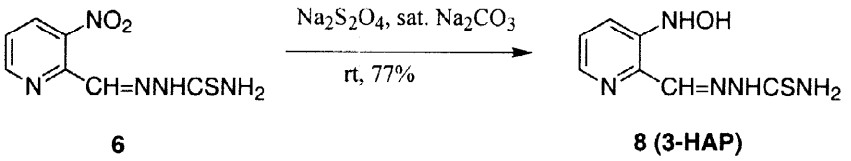
This reaction proved to be a facile entry into the 2-methyl-3-nitropyridine **4**. Oxidation of compound **4** with selenium dioxide followed by direct coupling of aldehyde **5** with thiosemicarbazide gave **6** which was reduced to give 3-AP **3**. By performing the methylation under Suzuki conditions, the total synthesis of 3-AP can be accomplished in a four-step sequence in 46% overall yield. Alternatively, reaction of **4** with dimethylformamide dimethylacetal (DMFDMA)[15] to form enamine **7**, followed by oxidation with sodium periodate or ozone provided the intermediate aldehyde **5** in 84% and 82% yields, respectively. This sequence was a significant improvement over the oxidation procedure of **4** with selenium dioxide. The new methodology, in addition to reducing the number of steps required and improving overall yield, also made handling easier and eliminated the use of selenium dioxide. Clean conversion of the nitro group to the amine could be acheived using either stannous chloride[16] or sodium sulfide (Zinin reduction)[17] to provide **3** in 81% and 91% yields, respectively (Scheme 3).



Scheme 3 *Reagents and conditions:* a) $CH_3B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, dioxane, Δ,90%; b) SeO_2, dioxane, 63-70%; c) DMFDMA; d) $NaIO_4$, 84% or O_3, 82%; e) $H_2NNHCSNH_2$, 80%; f) $SnCl_2$, 81% or Na_2S, 91%.

Synthesis of 3-HAP 8. Given that Sartorelli had previously reported that hydroxylamino compounds in this series retained biological activity it was decided to attempt selective reduction of **6**. A variety of reductants and solvents such as $Na_2S_2O_4$/$NaHCO_3$, $Na_2S_2O_4$/THF-H_2O, $SnCl_2$/C_2H_5OH or THF, H_2/Pd(OH)$_2$-C/C_2H_5OH or Na_2S/S/H_2O-C_2H_5OH were used and generally, an unseparable mixture of 3-HAP and 3-AP was

obtained. Finally, selective reduction was acheived by treatment of nitro compound **6** with $Na_2S_2O_4$ in saturated Na_2CO_3 solution to produce 3-HAP **8** as a single product in 77% yield (Scheme 4). Biological evaluation of 3-HAP and 3-AP conducted in house showed that 3-AP exhibited significant anticancer activity, but 3-HAP was found inactive against M109 murine lung carcinoma.



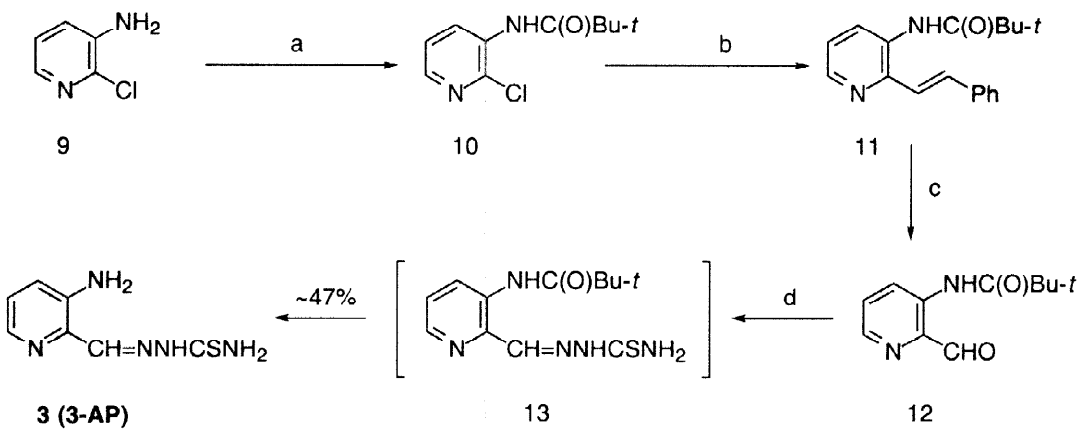
6 **8 (3-HAP)**

Scheme 4 Synthesis of 3-HAP **8**

2-Chloro-3-aminopyridine Route. Although the synthesis of 3-AP was very efficient and high yielding, rather tedious work-up and purification procedures were required for the final $SnCl_2$-mediated nitro-reduction step, which limited large scale production. More critically, we recently encountered problems associated with the complete removal of tin residues from the final product. Since recent *in vivo* experiments appeared to show that metal-chelated 3-AP is much more toxic than its non-chelated counterpart.[4b] Development of a new procedure to synthesize pure 3-AP became very important for our further preclinical studies.

With the aim of developing a practical synthesis which would be suitable for scale-up production of 3-AP free of any metal contamination, we began looking into the possibility of employing 2-chloro-3-amino-pyridine **9**, instead of the 2-chloro-3-nitropyridine **1** used in our previous syntheses, as the starting material for the current synthesis. By doing so, we could eliminate the problems stemming from the use of $SnCl_2$ or Na_2S-mediated nitro-reduction, thereby avoiding residual contamination in the final product.

Towards this end, our first endeavor, as shown in Scheme 5, was to test the feasibility of attaching a vinyl group at the C-2 position of **9**. Initially, reaction of **9** with vinyltributyltin under Stille vinylation conditions[18] failed to yield the expected 2-vinyl-aminopyridine. We then decided to try a Heck reaction[19] with 2-chloro-3-pivaloylamido-pyridine **10**, which was prepared *via* N-pivaloylation of **9**. To our satisfaction, reacting **10** with excess styrene in the presence of palladium(II) acetate, triphenylphosphine and sodium acetate afforded the desired adduct **11** (98%) and thereafter the aldehyde **12** (94%), upon ozonolysis.[20] Further coupling of **12** with thiosemicarbazide in the presence concentrated HCl led to intermediate **13** in good yield. However, the N-pivaloyl group was found to be quite stable even at elevated temperature (80°C). Thus, the anticipated *in situ* N-deprotection[21] of **13** was rather inefficient, and a mixture of the desired 3-AP and **13** was obtained.

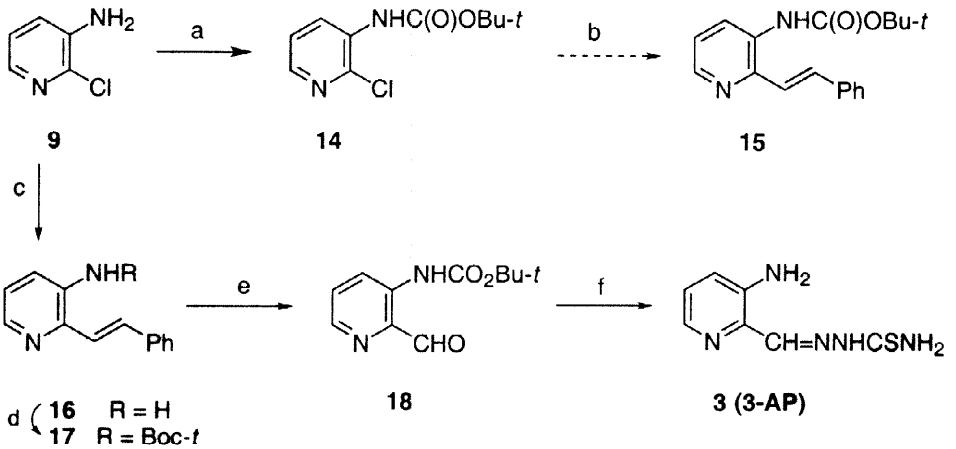


9 **10** **11** **12** **13** **3 (3-AP)**

Scheme 5 *Reagents and conditions:* a) PivCl/Et_3N, 100%; b) Styrene, $Pd(OAc)_2$, PPh_3, NaOAc, DMF, 135°C, 98%; c) O_3, MeOH, 94%; d) $H_2NNHC(S)NH_2$, HCl (conc.), 47% overall.

To circumvent this problem, we decided to use *t*-Boc, a more acid labile group, to protect the C-3 amino function in **9**. Thus, reaction of compound **9** with $(t\text{-Boc})_2O$ in the presence of Et_3N gave the N-Boc pyridine derivative **14** in moderate yield (67%). However, efforts to couple the vinyl group to **14** under Stille vinylation or Heck reaction conditions failed to give any desired products due to the instability of **14** under the reaction conditions (Scheme 6). Alternatively, compound **9** was directly reacted with styrene under the Heck conditions

employed in Scheme 5, affording **16** in 75% yield. Treatment of a warm *t*-BuOH solution of **16** with $(t\text{-Boc})_2O$ led to the desired N-Boc pyridine derivative **17**[22] which was converted to the aldehyde **18** *via* ozonolysis in 92% overall yield. An ethanol solution of aldehyde **18** was treated with thiosemicarbazide in the presence of concentrated HCl to afford the targeted product 3-AP (93%) after the anticipated *in situ* N-deprotection. As shown in Scheme 6, the overall yield for this four-step synthesis of **3** is 64%, which is 6-fold higher than the published method.[3] Furthermore, the chemistry outlined in Scheme 6 requires only one chromatographic separation, rendering it practical for scale-up. More importantly, the 3-AP prepared according to the procedure is pure (>99.5%) and without any metal contamination. This route has been utilized for GMP manufacture of high purity 3-AP. It is also worthwhile to mention that the same reaction sequence described was used for synthesizing radio-labeled 3-AP for metabolism studies.



Scheme 6 *Reagents and conditions:* a) *t*-Boc_2O, Et_3N, 67%; b) Stille or Heck reactions; c) Styrene, $Pd(OAc)_2$, PPh_3, $NaHCO_3$, DMF, 135°C, 75%; d) *t*-Boc_2O; e) O_3, MeOH, 92% for two steps; f) $H_2NNHC(S)NH_2$, HCl (conc.), 93%

In summary, a highly efficient method for the synthesis of 3-HAP and 3-AP has been developed. The new methodologies involving either Suzuki methylation or direct coupling of vinyl groups under Heck conditions to 2-halo pyridines provided a short synthetic protocol, made handling much easier and produced the target compounds in good yield. In addition, these new methodologies can also be applied to the synthesis of α-(*N*)-hetereocyclic carboxaldehyde thiosemicarbazones (HCTs) to produce novel antitumor agents in relatively fewer steps in comparison with the reported synthetic method.

EXPERIMENTAL SECTION

General Methods. Melting points were determined with a BÜCHI 535 apparatus and are uncorrected. All NMR spectra were measured at 300 MHz for ^1H and at 75 MHz for ^{13}C on QE Plus 300 MHz NMR spectrometer and BRUKER 300 MHz NMR spectrometer. MS spectra were recorded on VG ZAB-SE mass spectrometer and VG 70-SE-4F instrument. All chemical reagents and solvents were purchased from Alderich Chemical Company. TLC was carried out on SiO_2 (silica gel 60 F_{254}, Merck). Chromatography refers to flash chromatography and was carried out on SiO_2 (silica gel 60, SDS, 230-400 mesh ASTM).

2-Methyl-3-nitropyridine 4. Suzuki Reaction. A mixture of 2-chloro-3-nitro-pyridine **1** (793 mg, 5 mmol), methylboronic acid (329 mg, 5.5 mmol), $Pd(PPh_3)_4$ (578 mg, 0.5 mmol) and K_2CO_3 (2.073 g, 15 mmol) in dioxane (25 mL) was refluxed for 2 days, then cooled to room temperature and filtered. The solvent was removed and the residue was isolated by chromatography (hexanes-EtOAc = 1:1) to provide 623 mg (90%) of 2-methyl-3-nitropyridine **4**. Its proton NMR spectrum is identical with that reported in the literature. **4**: m.p. 29-30°C; ^1H NMR ($CDCl_3$) δ 2.88 (s, 3H), 7.37 (dd, 1H, J = 4.8 & 8.4 Hz), 8.28 (dd, 1H, J = 1.2 & 8.1 Hz), 8.74 (dd, 1H, J = 1.2 & 4.5 Hz).

3-Nitropyridine-2-carboxaldehyde 5. *Method 1.* To a solution of 2-methyl-3-nitropyridine **4** (2.07 g, 0.015 mol) in 35 mL of dioxane was added selenium dioxide (1.88 g, 0.017 mol). The reaction mixture was refluxed for 16 h, then cooled to room temperature and filtered. The solvent was removed under reduced pressure and the residue was purified by chromatography (hexanes-EtOAc = 1:1) to give 1.60 g (70%) of the desired aldehyde **5**.
Method 2. A solution of **4** (276 mg, 2 mmol) and DMFDMA (477 mg, 4 mmol) in DMF (1 mL) was heated at 140°C under N_2 for 7 h, then stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was dried in vacuum. The reaction was quite clean and gave only one product, enamine **7**, which was used in the subsequent oxidation without further purification.
Oxidation with $NaIO_4$: A solution of **7** prepared above and $NaIO_4$ (1.283 g, 6 mmol) in 50% aqueous THF (20 mL) was stirred at room temperature for 2 h, filtered and extracted with CH_2Cl_2 several times. The combined extracts were washed with brine and dried over anhydrous Na_2SO_4. Isolation by chromatography (hexanes-EtOAc = 1:1) gave 256 mg (84%) of aldehyde **5**.
Ozonolysis: The crude enamine **7** prepared from **4** (138 mg, 1 mmol) following the procedure described above was dissolved in CH_2Cl_2. The solution was bubbled with ozone at -78°C for 5 min, and then bubbled with N_2 to remove the excess ozone. The solvent was then removed and the residue was isolated by chromatography to give 124 mg (82%) of aldehyde **5**, which is identical with the authentic sample in its reported proton NMR spectrum. ^{1}H NMR ($CDCl_3$) δ 7.71 (dd, 1H, J = 4.8 & 8.2 Hz), 8.29 (dd, 1H, J = 1.1 & 8.0 Hz), 9.01(dd, 1H, J = 1.1 & 4.5 Hz), 10.31 (s, 1H).

3-Nitropyridine-2-carboxaldehyde Thiosemicarbazone 6. A mixture of aldehyde **5** (750 mg, 4.93 mmol) and thiosemicarbazide (540 mg, 5.92 mmol) in 70% ethanol (25 mL) was stirred at room temperature for 6 h and filtered. The crude product was washed with H_2O, C_2H_5OH, ether and dried *in vacuo* to give 893 mg (80%) of the desired hydrazone **6** as a yellow solid: m.p. 225-227°C; ^{1}H NMR (DMSO-d_6) δ 7.09 (br, 1H), 7.67 (dd, 1H, J = 4.9 & 8.2 Hz), 8.27 (s, 1H), 8.38 (d, 1H, J = 7.7 Hz), 8.60 (br, 1H), 8.85 (d, 1H, J = 4.4 Hz), 11.97 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 125.1, 132.7, 138.3, 144.7, 145.6, 152.9, 179.3; CIMS calcd for $C_7H_8N_5O_2S$ 226 (MH^+), found 226; HRMS(FAB) calcd for $C_7H_8N_5O_2S$ 226.0398, found 226.0399.

3-Aminopyridine-2-carboxaldehyde Thiosemicarbazone (3-AP) 3. *Method 1.* To a solution of $SnCl_2{\cdot}2H_2O$ (2.256 g, 10 mmol) in 6 mL of ethanol was added nitro compound **6** (450 mg, 2.0 mmol). The reaction mixture was refluxed overnight under N_2 and filtered. The crude solid was dissolved in 30 mL of hot water and filtered. The filtrate was then adjusted to pH 7.5 with saturated $NaHCO_3$ solution and stirred at room temperature for 30 min, filtered, washed with H_2O, C_2H_5OH and ether. The resulting yellow solid was further extracted with THF several times. The combined THF extracts were evaporated and the residue was dried *in vacuo* to provide 316 mg (81%) of 3-AP as a yellow solid.
Method 2. A mixture of nitro compound **6** (450 mg, 2.0 mg) and Na_2S (468 mg, 6 mmol) in H_2O/C_2H_5OH (1:1, 20 mL) was stirred at room temperature for 18 h and concentrated. The residue was adjusted to pH 7.5 with 1N HCl solution and filtered. The yellow solid was washed with H_2O, C_2H_5OH, CH_2Cl_2 and dried *in vacuo* to give 355 mg (91%) of 3-AP **3**: m.p. 232-234°C; ^{1}H NMR (DMSO-d_6) δ 11.29 (s, 1H), 8.31 (s, 1H), 8.15 (br, 1H), 7.95 (br, 1H), 7.80 (dd, J = 1.2 & 4.2 Hz, 1H), 7.07 (m, 2H), 6.43(br, 2H); ^{13}C NMR (DMSO-d_6) δ 177.0, 149.0, 144.0, 137.1, 132.7, 124.4, 122.3; HRMS(FAB) calcd for $C_7H_{10}N_5S$ (M+1) 196.0657, found 196.0657; Anal. calcd for $C_7H_9N_5S$: C, 43.06; H, 4.65; N, 35.87; S, 16.42. Found, C, 42.93; H, 4.77; N, 35.74; S, 16.58.

3-Hydroxylaminopyridine-2-carboxaldehyde thiosemicarbazone 8. A mixture of **6** (1.125 g, 5 mmol) and $Na_2S_2O_4$ (3.480 g, 20 mmol) in sat. Na_2CO_3 solution (30 mL) was stirred at room temperature for 14 hrs and filtered. The aqueous filtrate was extracted with THF. The combined extracts were evaporated. The combined solid was washed with H_2O, H_2O/C_2H_5OH (1:1), C_2H_5OH, ether and dried in vacuum to give 0.752 g (77%) of 3-HAP **8** as a yellow solid: ^{1}H NMR (DMSO-d_6) δ 7.27 (m, 1H), 7.60 (d, 1H, J = 8.1 Hz), 7.99 (d, 1H, J = 3.3 Hz), 8.21 (br, 1H), 8.27 (s, 1H), 8.33 (br, 1H), 8.76 (s, 1H), 9.17 (br, 1H), 11.37 (br, 1H); ^{13}C NMR (DMSO-d_6) δ 119.4, 124.5, 134.2, 139.1, 146.4, 147.4, 177.6; HRMS(FAB) calcd for $C_7H_{10}N_5SO$ 212.0606, found 212.0606.

2-Methyl-3-aminopyridine. A mixture of 2-chloro-3-aminopyridine (643 mg, 5 mmol), methylboronic acid (329 mg, 5.5 mmol), $Pd(PPh_3)_4$ (578 mg, 0.5 mmol) and K_2CO_3 (2.073 g, 15 mmol) in dioxane (25 mL) was refluxed for 5 days, then cooled to room temperature and filtered. The solvent was

removed and the residue was isolated by chromatography (hexanes-EtOAc = 1:1) to provide 189 mg (35%) of 2-methyl-3-aminopyridine as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3H), 3.68 (br. 2H, D_2O exchangeable), 6.94 (m, 2H), 7.94 (dd, 1H, J = 1.5 & 4.6 Hz); ^{13}C NMR ($CDCl_3$) δ 20.2, 121.0, 122.0, 139.1, 140.4, 143.6; CIMS calcd for $C_6H_9N_2$ 109 (MH^+), found 109.

2-Chloro-3-pivaloylamidopyridine 10. To a solution of 2-chloro-3-aminopyridine **9** (10.0 g, 77.8 mmol) and triethylamine (15.74 g, 155.57 mmol) in dichloromethane (50 mL) was added at 0°C pivaloyl chloride (11.25 g, 93.34 mmol) dropwise. The reaction was stirred at 0°C for 2 hr and kept at 5°C overnight. At this point, the reaction was quenched with water (20 mL) and extracted with dichloromethane (3 x 50 mL). The combined organic layers was washed with brine and dried over Na_2SO_4. After filtration and concentration, the resulting residue was chromatographed on silica gel (20% EtOAc/hexanes) to provide 17.1 g (~100%) of **10**: m.p. 35-36°C; ^{1}H NMR ($CDCl_3$) δ 8.80-8.74 (m, 1H), 8.11-8.08 (m, 1H), 8.00 (bs, 1H), 7.25 (dd, J = 4.2 & 8.4 Hz, 1H), 1.35 (s, 9H); HRMS(CI) calcd For $C_{10}H_{14}ClN_2O$ 213.0793, Found 213.0795.

2-Styryl-3-pivaloylamidopyridine 11. A DMF suspension containing **10** (1.06 g, 5.0 mmol), styrene (5.7 mL, 50 mmol), NaOAc (0.82 g, 10 mmol), triphenylphosphine (0.52 g, 2.0 mmol) and $Pd(OAc)_2$ (56 mg, 0.25 mmol) was heated in a sealed tube at 135-140°C for 24 hr. The reaction mixture was cooled to rt and quenched with water (20 mL). The resulting mixture was extracted with EtOAc (3 x 50 mL). The combined organic phase was washed with brine and concentrated. After silica gel column purification (25% EtOAc/hexanes), 1.37 g (98%) of the desired product **11** was obtained as white solids: m.p. 78-83°C; ^{1}H NMR ($CDCl_3$) δ 8.43 (dd, J = 1.2 & 4.5 Hz, 1H), 8.15 (dd, J = 1.2 & 8.7 Hz, 1H), 7.72 (d, J = 15.6 Hz, 1H), 7.57-7.18 (m, 8H), 1.39 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 177.1, 148.1, 146.1, 136.4, 134.8, 132.6, 131.0, 128.6, 128.5, 127.0, 122.1, 121.3, 39.5, 27.4; HRMS(CI) calcd For $C_{18}H_{21}N_2O$ 281.1649, Found 281.1654.

2-Formyl-3-pivaloylamidopyridine 12. A methanol solution (40 mL) of **11** (0.376 g, 1.34 mmol) was subjected to ozonolysis at -78°C for 20 min. The reaction was then quenched with Me_2S (2 mL) and stirred at room temperature overnight. Upon solvent removal and column purification on silica gel (20% EtOAc/hexanes), 260 mg (94%) of desired product **12** was obtained as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 11.26 (bs, 1H), 10.13 (s, 1H), 9.16 (d, J = 8.7 Hz, 1H), 8.48 (d, J = 4.5 Hz, 1H), 7.50 (dd, J = 4.5 & 8.7 Hz, 1H), 1.37 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 197.3, 178.4, 143.8, 138.4, 137.0, 128.4, 127.2, 40.1, 27.1; HRMS(CI) calcd For $C_{11}H_{15}N_2O_2$ 207.1141, Found 207.1134.

2-Styryl-3-aminopyridine 16. (a) Reaction performed in a sealed tube. A DMF (20 mL) suspension of 2-chloro-3-aminopyridine **9** (1.28 g, 10.0 mmol), styrene (5.72 mL, 50.0 mmol), sodium bicarbonate (1.68 g, 20.0 mmol), triphenylphosphine (1.31 g, 5.0 mmol) and $Pd(OAc)_2$ (0.12 g, 0.50 mmol) was heated at 130°C for 24 hrs in a sealed tube. At this point, the reaction mixture was cooled to room temperature, and quenched with saturated $NaHCO_3$ (10 mL) and water (10 mL). The reaction mixture was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine, dried over sodium sulfate and filtered. The filtrates were concentrated *in vacuo*, the residue was chromatographed on silica gel (25% ethyl acetate in hexanes) to provide 1.47 g (75%) of desired product **16**.
(b) Reaction performed at 1 atm. A DMF (50 mL) suspension of 2-chloro-3-aminopyridine **9** (6.43 g, 50.0 mmol), styrene (28.7 mL, 250.0 mmol), sodium bicarbonate (1.68 g, 20.0 mmol), triphenylphosphine (6.55 g, 25.0 mmol) and $Pd(OAc)_2$ (0.56 g, 2.50 mmol) was heated at 130°C for 48 hrs. At this point, the reaction mixture was cooled to room temperature, and then quenched with brine (50 mL). The resulting dark brown solution was extracted with EtOAc/Et_2O (2:1) four times (100 mL each time). The combined organic layers were washed with water and brine. The resulting organic phase was dried and concentrated *in vacuo*. The residue was chromatographed on silica gel (20-40% ethyl acetate in hexanes) to provide 5.51 g (56%) of the desired product **16** as yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.12 (t, J = 3.0 Hz, 1H), 7.07-7.57 (m, 3H), 7.40-7.18 (m, 4H), 7.01 (d, J = 3.0 Hz, 2H), 3.82 (bs, 2H); ^{13}C NMR ($CDCl_3$) δ 141.5, 140.3, 140.0, 137.0, 132.8, 128.6, 128.0, 126.9, 123.3, 122.9, 121.9; LRMS (CI) calcd. for $C_{13}H_{13}N_2$ (M+1) 197, found 197; HRMS(CI) calcd For $C_{13}H_{13}N_2$ 197.1071, Found 197.1078.

2-Formyl-3-N-Bocpyridine 18. 2-Aminopyridine derivative **16** (5.00 g, 25.51 mmol) was dissolved in warm *tert*-butanol (100 mL). To this warm solution (~40°C) was added $(t\text{-Boc})_2O$ (6.68 g, 30.61 mmol). After stirring this solution at room temperature for a few hours, addition amount of $(t\text{-Boc})_2O$ (2.78 g, 12.76 mmol) was added. The reaction was further stirred at room temperature for 15 hr. At this point, the milky

suspension was subjected to solvent evaporation. After solvent removal, the resulting residue was taken up with (1:1) EtOAc/Et_2O (100 mL). The resulting solution was washed with brine. The organic layer was separated and saved. The aqueous layer was back extracted with same mix-solvent (3 x 50 mL). The combined organic layers were dried and concentrated in vacuo to provide ~10 g crude 2-styryl-3-Boc-aminopyridine **17** as light brown solid. The crude **17** (~25.5 mmol) was dissolved in MeOH (120 mL) and dichloromethane (30 mL). The resulting solution was cooled to -78°C and subjected to ozonolysis for ~45 mins. The reaction was then quenched with Me_2S (8 mL) and stirred at room temperature overnight. Solvents were removed in vacuo, the residue was purified using silica gel chromatography (10-15% ethyl acetate in hexanes) to provide 5.23 g (92% for two-step) of the desired aldehyde **18** as white solids: m.p. 78-80°C; ^1H NMR ($CDCl_3$) δ 10.23 (bs, 1H), 10.08 (s, 1H), 8.84 (d, J = 8.7 Hz, 1H), 8.41 (dd, J = 1.2 & 4.2 Hz, 1H), 7.46 (dd, J = 4.5 & 8.7 Hz, 1H), 1.54 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 197.1, 152.7, 143.2, 139.2, 128.6, 126.2, 81.6, 28.1; HRMS(CI) calcd For $C_{11}H_{15}N_2O_3$ 222.1074, Found 222.1083.

3-Aminopyridine-2-carboxaldehyde Thiosemicarbazone (3-AP) 3. To a mixture of aldehyde **18** (1.468 g, 6.61 mmol) and thiosemicarbazide (662 mg, 7.27 mmol) in EtOH/H_2O (22.5 mL, 67% ethanol content) was added 3 mL of conc. HCl. The resulting solution was heated to reflux for 3 hr. The reaction was cooled to room temperature and filtered. The crude yellownish 3-AP-HCl salt was transferred to a flask. To this flask was added 40 mL hot water and 8 mL 10% NaHCO3. The mixture was stirred at r.t. for 1 hr (at pH ~ 7.5). The solids were filtered and rinsed with water (10 mL), EtOH (3 mL) and Et_2O (10 mL). The solids obtained were dried under high vacuum for a few hours to provide 1.195 g (93%) of the desired 3-AP **3**.

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References and Notes:

† To whom correspondence should be addressed. Current address: Wyeth-Ayerst Research, Chemical Research Laboratory, Bldg. 222/Room 3087, 401 N. Middletown Road, Pearl River, NY 10965.
* Principal investigator of this project.
# Current address: Medicinal Chemistry, Bristol-Myer-Squibb Pharmaceutical Research Institute, P.O. Box 5400, Princeton, NJ 08543-5399.

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