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A Scalable Synthesis of the INOS Inhibitor PHA-399733†

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Abstract:

This contribution describes a scalable synthesis of the INOS inhibitor PHA-399733 using the Bucherer−Bergs hydantoin synthesis to introduce the amino acid function.

Introduction

In 1992, *Science* magazine declared nitric oxide the "molecule of the year," because of its ubiquitous evanescence, and has a profound regulatory effect on a host of physiological processes.[1,2] It is also largely responsible for the Los Angeles smog. Its profound effect as a biological messenger led to a medicinal chemistry program to downregulate the induced nitric oxide synthase (iNOS) enzyme complex in order to treat diseases such as osteoarthritis, asthma, and neuropathic pain. This is one of three isoforms of the complex that converts arginine to citrulline with the release of the free radical NO.[3] This program identified PHA-399733 (**28**) as a potential candidate possessing the required potency and selectivity. This report describes enabling work that was done for the large-scale preparation of PHA-399733.

The original synthesis (Scheme 1),[4] when scaled, was found to be capricious with unpredictable yields. The primary issue with this scheme is the alkylation of the iodide **4** with the Schiff base **10** using the Schwesinger[5] base which is rather costly at $2200/L. This reaction was difficult to control with formation of diene **6** as the primary byproduct. Another issue that led us to reevaluate this chemistry was the fact that there was only a single crystalline intermediate in the sequence, mesylate **3**. The heterocycle has some thermal stability issues which are a safety concern. The Finklestein reaction (**3** to **4**) also operates in a narrow safety window since the ARC[6] on the iodide shows an onset of 104 °C given acetone's boiling point of 56.5 °C. The final deprotection of the amidine was cumbersome because of a difficult purification regimen of the desired amino acid **9**. Although not an ideal approach to the desired enantiomer, the chiral chromatography was considered acceptable at this stage of development.

In developing an alternative route, our main goals were to address the safety issues and the robustness problem and to devise a sequence which had the potential for additional crystalline intermediates to facilitate purification. We had hoped to address the robustness problem by constructing the carbon framework using the coupling of propargyl alcohol and methyl vinyl ketone as described in a patent (Scheme 2).[7] Unfortunately, this chemistry could not be reproduced and resulted only in exothermic polymer formation. A variant with the protected nitrogen in place did give partial success (Scheme 3). [8] The coupling product **15** was obtained in low yield but had to be purified by chromatography from what was identified as the MVK dimer **16** and the bis adduct **17**. This chemistry was not pursued because of time constraints and the fact that the ongoing, more parochial approach of Scheme 4was proving to be simple and scalable.

In our modified approach we took advantage of the well-documented alkylation of β-carbonyl derivatives to build the carbon framework. In this approach **18** was alkylated with propargyl chloride to give **19** which was purified by simple distillation.[9,10] Subsequent protection afforded ketal **20** which was readily homologated using well-precedented chemistry to afford alcohol **21**. Although operationally simple, there were some safety concerns with this chemistry. Propargyl chloride is a shock-sensitive reagent,[11] and the other acetylenic intermediates proved to be of high energy. The onset temperatures were fairly high; under ambient conditions this would be acceptable, but during distillation there could be an issue. Table 1 gives the DSC results for the intermediates. Since the onset temperatures were fairly high and the boiling points reasonably low, in the laboratory the distillations were done using a water bath to limit the pot temperature. After developing this

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(11) These were run at 10 °C/min.

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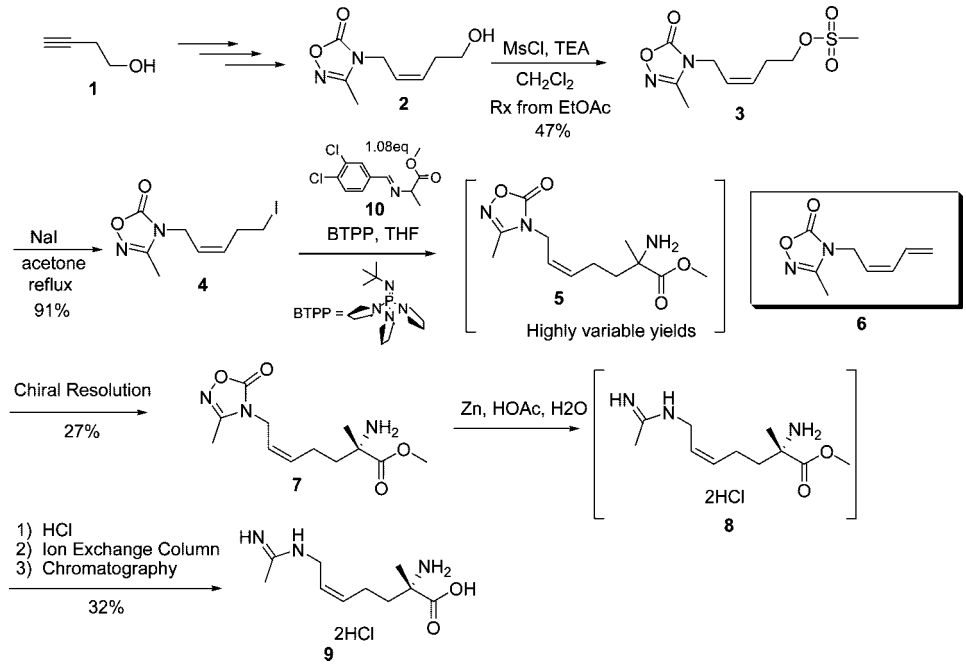
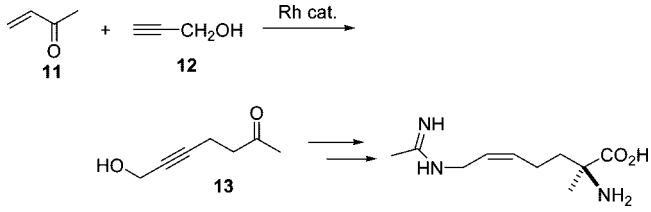
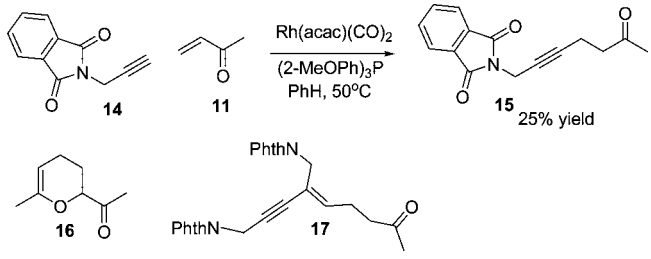
Scheme 1



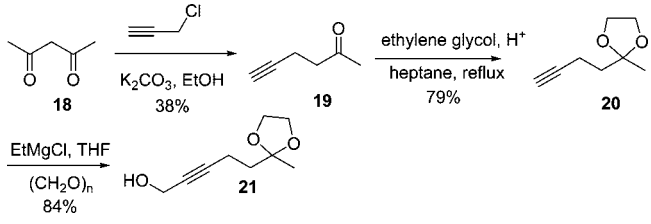
Scheme 2



Scheme 3



Scheme 4



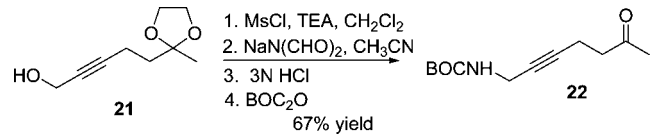
chemistry, a 40 kg batch of alcohol **21** was prepared by an outside vendor that had the ability to safely handle propargyl chloride on scale.

With the key carbon framework in hand, the remainder of the synthesis was pursued by replacing the alcohol in **21**with a protected nitrogen (Scheme 5). Displacement of the derived mesylate with $NaN(CHO)_2$ proceeded efficiently, but the subsequent ketal deprotection could not be performed without also hydrolyzing both formyl groups, giving an amino ketone which had to be reprotected on the nitrogen with $(BOC)_2O$ to give **22** as an oil in 67% yield after chromatography.

Table 1. **DSC results for acetylenes**[12]

compound	onset/energy	boiling point
propargyl chloride	182°, −1290 J/g	56−59 °C
19	182°, −1024 J/g (Arc onset = 165°)	149 °C
20	215°, −635 J/g	76 °C, 20 Torr
21	250°, −400 J/g	274 ± 20 °C, 760 Torr[13]

Scheme 5



Although the chemistry in Scheme 5 was workable, we felt that a more robust protecting group for nitrogen was warranted. Thus, the phthalimide was examined (Scheme 6) in place of the bisformylamide. In this case, all the chemistry proceeded uneventfully to give crystalline intermediates. The displacement of potassium phthalimide on the derived mesylate of alcohol **21** was performed without isolating the mesylate by running the reaction in methyl *tert*-butyl ether, where insoluble TEA/HCl was filtered off and the solution was added to potassium phthalimide followed by the addition of acetonitrile and heating to 45 °C. The crystalline imide **23a** was isolated in 81% yield on a 54 kg scale. Addition of acetic acid during the isolation was required to prevent phthalimide ring-opening. The hydrolysis of the ketal was performed in aqueous acetone/HCl to give the crystalline ketone **23b** in 97.4% yield on a 44.9 kg scale. The partial hydrogenation of the acetylene required some optimization because, during the course of the reduction,

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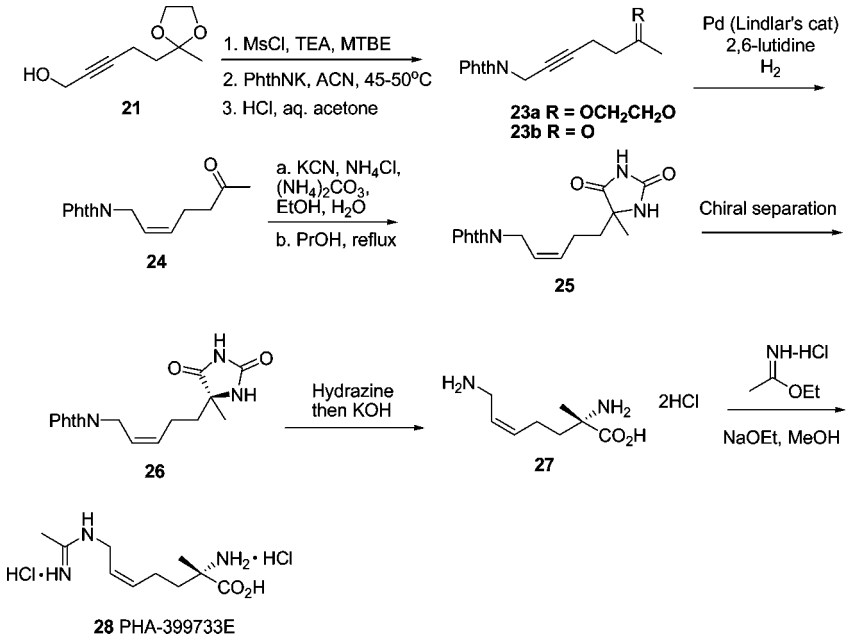
Scheme 6



considerable quantities of the *trans*-alkene formed upon scaling the reaction to 100 g. The reaction was optimized to run in THF with 5 psig of H_2 at 18−22 °C with 2,6-lutidine to modify the Lindlar catalyst. Hydrogenation had to be stopped when 97% of the theoretical amount of H_2 was absorbed to minimize olefin isomerization. Allowing the reaction to progress any further resulted in extensive isomerization of **24**. The product was not isolated but was used as in the Bucherer−Bergs[13] reaction to produce the hydantoin **25** after a solvent displacement with ethanol.

The main issue with the Bucherer−Bergs hydantoin synthesis[13] is handling and disposal of cyanide. The disposal problem was solved by obtaining a stoichiometric amount of the needed cyanide, thus obviating the need to dispose of a partial container. Otherwise, standard safety precautions were adhered to, and the vents were scrubbed with caustic bleach solution. During the course of the hydantoin synthesis the phthalimide opened up to the acid under the basic reaction conditions. Reclosure to the imide was smoothly carried out by refluxing in *n*-propanol. Isolation gave 39.9 kg (75% yield) of hydantoin **25** as a racemate.

A simulated moving bed (SMB) chiral separation (conditions shown in Table 2) was developed to isolate the desired enantiomer **26**. Since SMB chromatography can readily be performed on large scale, no attempts were made to examine more classical crystallization methods to carry out the enantiomer separation. This is also a fairly rapid method to achieve enantiomer separation which is well suited to the timelines of drug development. A 2.3 g sample of the early eluting isomer was processed to the end to identify the desired enantiomer. This processing showed that the late-eluting isomer was the desired isomer based on chiral HPLC. The separation and the remaining steps were not carried forward in the pilot plant due to project reprioritizations. Laboratory work on this sequence was largely finished to show that the final product could be obtained by this route. The deprotection of **26** was done in two transformations. Direct hydrolysis of the hydantoin and the phthalimide with refluxing aqueous KOH failed. Thus, the imide was removed with aqueous hydrazine, and the crude amine was hydrolyzed with KOH at 120 °C. The amino acid **27** was isolated as the bis-HCl salt and then converted to the amidine **28** with ethyl acetamidate-HCl and NaOEt in MeOH.

Table 2. **Conditions for chiral separation**

stationary phase	ChiralPak AS (20 μm particles)
columns	six @ 10 mm × 100 mm (1/2/2/1 configuration)
mobile phase	acetonitrile/methanol, 95/5
feed makeup	10 mg/ml PHA-399, 733 in methanol (1 L)
feed rate	0.6 mL/min
eluent rate (mobile phase)	9.54 mL/min
extract rate (more retained, enantiomer 2)	6.16 mL/min
raffinate rate (less retained, enantiomer 1)	3.98 mL/min
recycling rate (between eluent and extract)	15.42 mL/min
valve switching period	0.87 min

In conclusion we have developed a simple, robust, and scalable process to prepare the INOS inhibitor PHA-399733 using the Bucherer−Bergs reaction to prepare the α-substituted amino acid. The synthesis produced racemic material because, for early development, the more difficult chiral synthesis was not warranted, given that enantiomer separations are readily carried out on large scale using the SMB technology.

Experimental Section

1-Hexyne-5-one, 19. A mixture of propargyl chloride (53.2 g, 0.5 mol), acetylacetone (55.1 g, 0.55 mol), K_2CO_3 (77.4 g, 0.56 mol), and 300 mL of EtOH was refluxed overnight. Most of the EtOH was removed by atmospheric distillation. The mixture was taken up in MTBE and washed with water. The

extracts were dried with $MgSO_4$ and concentrated by simple distillation. House vacuum distillation gave the desired product (20.0 g, 38% yield). ^{13}C NMR ($CDCl_3$): 206.3, 82.9, 68.7, 41.97, 29.7, 12.8 ppm.

2-Methyl-2-but-3-yn-1-yl-1,3-dioxolane, 20.[14] The ketone **19** (18.0 g, 0.187 mol), ethylene glycol (11.7 g, 0.189 mol), and TsOH (723 mg) in 180 mL of benzene was refluxed with a Dean−Stark trap for 4 h to remove water. The mixture was washed with $NaHCO_3$ and concentrated to afford the ketal **20** (20.8 g) as a clear oil in 79% yield. ^{13}C NMR ($CDCl_3$): 128.2 (benzene), 108.9, 84.2, 68.0, 64.3, 37.9, 23.7, 13.2 ppm.

4-(2-Methyl-1,3-dioxolan-2-yl)but-2-yn-1-ol, 21.[15] EtMgCl (107 mL) was added to the ketal **20** (20.0 g, 0.143 mol) in 110 mL of THF, keeping the temperature <20 °C. This was heated to 50 °C for 2 h after the addition was complete. The mixture was added to the paraformaldehyde and heated to 45 °C. The reaction temperature rose to reflux. The reaction was held at 45 °C for 2 h and then at rt over the weekend. The mixture was cooled to −5 °C and treated with 55 mL of sat. NH_4Cl very slowly, keeping the temperature at <3 °C. The exotherm stopped after 25 mL of NH_4Cl had been added. This gave a nicely granular solid which was filtered off and washed with EtOAc. The solution was filtered, washed with water, dried over $MgSO_4$, and concentrated to a clear oil to give 20.4 g (84% yield) of the alcohol **21**. ^{13}C NMR ($CDCl_3$): 109, 85.3, 78.4, 64.6, 50.7, 37.8, 23.7, 13.4 ppm.

2-[4-(2-Methyl-1,3-dioxolan-2-yl)but-2-yn-1-yl]-1H-isoindole-1,3(2H)-dione, 23a. Alcohol **21** (40 kg, 236 mol) was dissolved in 814 L of MTBE, and 39.3 L, 282 mol of triethylamine was added; the solution was cooled to ∼0 °C. Methanesulfonyl chloride (20 L, 259 mol) was added slowly, maintaining the reaction temperature at ∼20 °C. The triethylamine hydrochloride that formed in the reaction was removed by filtration, and the cake was washed with MTBE. The mother liquor containing the mesylate was then added to dry potassium phthalimide (30.8 kg, 270 mol), and 680 L of acetonitrile was added. The reaction was heated to 45 °C. When the reaction was determined to be complete by TLC analysis, the slurry was cooled to less than 10 °C and the volume reduced by vacuum distillation to remove MTBE. The distillation was chased once with acetonitrile to remove residual MTBE. Water and 13.5 L of acetic acid (for pH) were added to the slurry and heated to 55 to 65 °C to dissolve all solids. The salt-laden aqueous phase was separated, and water was added to the organic layer while the temperature was maintained at 50−55 °C. The product was held at this temperature for 1 h before cooling slowly to ∼0 °C. The product was recovered by filtration, washed twice with a 1:1 solution of acetonitrile/water, and dried using heated nitrogen. The slightly yellow solids of ketal were isolated in 81% chemical yield (54.8 kg) uncorrected for starting material or product quality. The quality was good, at 98.5 wt %. MS calculated for $C_{17}H_{17}NO_4$: M + 1 = 300.12359, Found: M + 1 = 300.1247. ^1H NMR (400 MHz, DMSO): 7.87 (m, 4H), 4.31 (s, 2H), 3.33 (s, 4H), 2.15 (t, J = 7.6 Hz, 2H), 1.71 (t, J = 7.6 Hz, 2H), 1.18 (s, 3H) ppm. ^{13}C NMR (DMSO): 166.8, 134.7, 131.5, 123.3, 108.2, 82.9, 74.2, 64.1, 37.4, 27.0, 23.5 13.0 ppm.

2-(6-Oxohep-2-yn-1-yl]-1H-isoindole-1,3(2H)-dione, 23b. Hydrochloric acid, 1.922 kg, was diluted in 328 L of water and added to a slurry of the ketal in 177.8 L of acetone at 25 °C. The reaction was heated to 50 °C and monitored by HPLC. When the reaction was determined to be complete, the solution was slowly cooled to 40 °C to promote crystallization. After crystals formed, the slurry was gradually cooled to 0 °C and held for 1 h. The product was recovered by filtration, and the product cake was washed with a 20% acetone/water solution followed by a water wash. The product cake was dried with nitrogen to afford 44.9 kg, 97.4% yield of the ketone **23.** ^1H NMR (400 MHz, $CDCl_3$): 7.87, (m, 2H), 7.74 (m, 2H), 4.40 (t, J = 8.3 Hz), 2.62 (m, 2H), 2.40 (m, 2H), 2.15 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$): 206.5, 167.1, 134.1, 132.0, 123.4, 82.1, 73.9, 42.1, 29.8, 27.3, 13.1 ppm.

2-[(2*Z*)-6-Oxohep-2-en-1-yl]-1H-isoindole-1,3(2H)-dione, 24. Lindlar's catalyst, 58.5 g, 3.9 kg of alkyne **23**, 60 kg of THF, and 32.7 g of 2,6-lutidine were charged to a pressure reactor. The vessel was purged with nitrogen and pressure checked at 50 psig. The reactor was adjusted to 18−22 °C, purged with hydrogen, and pressure checked. The hydrogen pressure was adjusted to 5 psig. PF-02377156 was hydrogenated at 18−28 °C and 5 psig ± 2 psig until the hydrogen uptake reached 97% of theory or ceased, and then agitation was stopped immediately. The reaction was assayed by HPLC, and when the reaction was determined to be complete (NMT 3 area % starting material), the palladium was removed by filtration. Eleven lots were run in the 10-gal autoclave. The processing went smoothly. The reductions were performed at about 5 psig hydrogen pressure and took 30−45 min to complete. The uptake stopped abruptly and was fairly easy to recognize. The combined THF solutions of olefin **24** were used as is in the next step. MS calculated for $C_{15}H_{15}NO_3$: M + 1 = 258.11320. Found: M + 1 = 258.1131. ^1H NMR (400Mz, $CDCl_3$): 7.83 (m, 2H), 7.70 (m, 2H), 5.57 (m, 1H), 5.45 (m, 1H), 4.32 (d, J = 6.92 Hz, 2 H), 2.57 (m, 2H), 2.17 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$): 208.0, 167.9, 133.9, 132.4, 132.1, 124.0, 123.1, 43.4, 34.6, 29.9, 21.6 ppm.

2-[(2*Z*)-6-(6-Methyl-2,5-dioximidazolidin-4-yl)hex-2-en-1-yl]-1H-isoindole-1,3(2H)-dione, 26. The THF solution of ketone **24** was concentrated, and ethanol chases were performed to displace the THF solvent. This ethanolic solution was transferred to a tank in which ammonium carbonate (19.3 kg, 201.0 mol), ammonium chloride (9.7 kg, 181 mol), potassium cyanide (**caution!**) (11.5 kg, 176.6 mol), and 333.0 L of water have been charged. The mixture was heated until the desired hydantoin formation was complete. The reaction mixture was concentrated and the solvent replaced with propanol (920.0 kg used). The mixture was heated to reflux and slowly distilled to complete the phthalimide closure. Once the reaction was complete, the mixture was concentrated and the propanol replaced with propyl acetate (1710.0 kg used). Water, acetic acid (5.0 L), and ethyl acetate (188.0 kg) were added, and the mixture was heated until all of the solids dissolved. The aqueous phase was removed and a second water wash performed. The

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remaining product solution was concentrated and chased with propyl acetate to displace the ethyl acetate and residual water. The slurry was then heated to reflux. The resulting mixture was cooled and filtered. Since the reaction used cyanide, vent streams were scrubbed and waste streams treated with bleach; it was verified that the cyanide has been consumed. Yield 39.9 kg, 75% yield. MS calculated for $C_{17}H_{17}N_3O_4$: M + 1 = 328.12974. Found: M + 1 = 328.1310. ^{1}H NMR (400 Hz, $CDCl_3$): 9.17 (s, NH), 7.84 (m, 2H), 7.71 (m, 2H), 6.76 (s, 1H), 5.56 (m, 1H), 5.49 (m, 1H), 4.35 (m, 1H), 4.22 (m, 1H), 2.50 (m, 1H), 2.21 (m, 1H), 1.95 (m, 1H), 1.79 (m, 1H), 1.50 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$): 177.8, 168.1, 157.0, 134.0, 132.6, 132.0, 63.4, 37.0, 34.5, 23.7, 21.7 ppm.

(2*S*)-(4*Z*)-2,6-Diamino-2-methylhex-4-enoic acid, Dihydrochloride, 27. The phthalimide **26** (40 g, 0.122 mol) in EtOH was treated with hydrazine monohydrate (7.13 mL, 0.15 mol) and heated to 60 °C overnight. LC showed two peaks. The mixture was slowly acidified with HCl in 100 mL of water and filtered. The filtrate was concentrated to remove EtOH, filtered to remove some fines, treated with KOH (39 g, 0.61 mol), and concentrated to about 300 mL. This solution was then heated to 125 °C for 20 h in a Parr vessel. The mixture was cooled to 50 °C and acidified with HCl. LC of the solution prior to acidification showed a single peak. A small amount of precipitate was removed by filtration. The water was exchanged for IPA, and the salts were removed by filtration. The mixture was concentrated to 100 mL and crystallized overnight. Filtration and drying afforded 19.4 g of white solid. The mother liquor was concentrated, and additional product was obtained: 9.7 g (97.1% yield). MS calculated for $C_8H_{16}N_2O_2$: M + 1 = 173.12901. Found: M + 1 = 173.1290. ^{1}H NMR (DMSO, 400 MHz): 8.67 (bs, 3H), 8.27 (bs, 3H), 5.57 (m, 2H), 5.50 (m, 2H), 4.42 (m, 2H), 1.91 (m 4H), 1.45 (s, 3H) ppm. ^{13}C NMR (DMSO): 172.6, 133.3, 123.1, 58.7, 36.0, 35.6, 22.1, 21.5 ppm.

PHA-399733, 28. The diamino acid salt **26** (1.0 g) was dissolved in MeOH and treated with 2.65 M EtONa (3.1 mL) followed by 530 mg of ethyl acetamidate-HCl. The mixture was stirred at rt, and after 1 h HPLC showed the presence of the amidine in 87.4% with 6% sm remaining. When complete, the MeOH was exchanged for IPA, and the salts were removed by filtration through solka floc. Concentration of the solution resulted in the precipitation of the product as a white solid. HPLC of this sample shows it to be 87% pure with 8% starting material and 4.7% of what is probably acetamide. No work was done to see if the product could be recrystallized or if the reaction could be driven to completion. Project cancellation forced us to abandon any further work on this transformation. LC conditions: Shiseido Capsell PAK SCX, type UG 120A, 4.6 mm × 250 mm, 5 μm, 75% A(1% $HClO_4$ in H_2O), 25% B(800 mL H_2O, 200 mL ACN, 10 mL $HClO_4$), 40 °C, 1.0 mL/min. SM R_t = 5.96 min, product = 8.68 min.

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