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Pilot-Plant Preparation of an $\alpha_v\beta_3$ Integrin Antagonist. Part 2. Synthesis of *N*-[2-(5-Hydroxy-4,6-tetrahydropyrimidine)]-3-amino-5-hydroxybenzoic Acid

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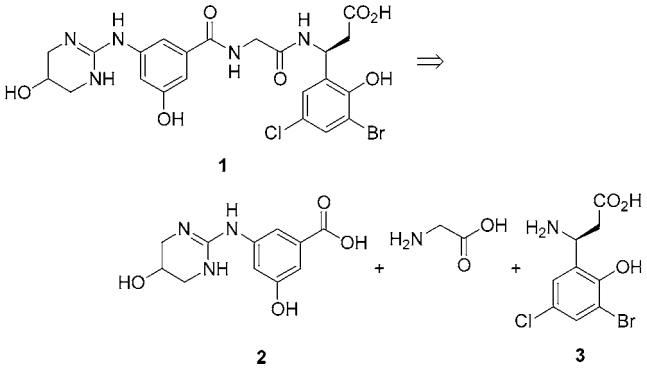
Abstract:

Studies directed toward the process research and development of a scalable method for preparing tetrahydropyrimidine 2, a key intermediate to the $\alpha_v\beta_3$ integrin antagonist 1, are described. A linear approach employing 3-amino-5-hydroxybenzoic acid, methyl isothiocyanate, and 1,3-diaminopropan-2-ol as key reagents is detailed. The results of process development research, a successful pilot run, and a production campaign are explained.

Introduction

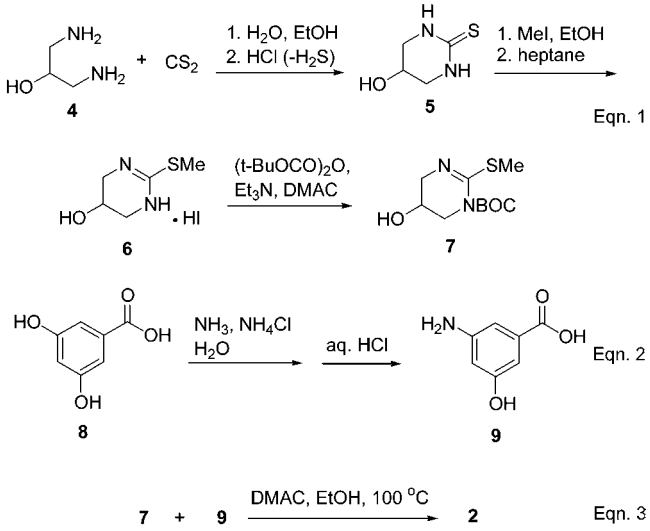
The $\alpha_v\beta_3$ integrin plays an essential role in angiogenesis, the process by which new blood vessels form from preexisting blood vessels.[1] Angiogenesis is required for tumor growth, and therefore, antagonists of $\alpha_v\beta_3$ are being studied for the treatment of cancer. In a program directed toward the discovery of such antagonists, an improved synthesis of **1** (Scheme 1) was required.[2] Accordingly, a process research and development team was formed to evaluate strategies by which **1** could be prepared on-scale. Envisioned was a convergent synthesis of **1** in which tetrahydropyrimidine **2** would be linked via glycine to the β-amino acid ester **3**. With regard to the preparation of **2**, a research program was completed wherein 3-amino-5-hydroxybenzoic acid, methyl isothiocyanate, and 1,3-diaminopropan-2-ol were employed as key reagents. As part two of a three-part report[3] describing the successful pilot-plant preparation of **1**, herein we detail the chemical process research and development of a scalable method for preparing **2**.

Scheme 1. **Key intermediates toward preparation of 1**



1 ⇒ **2** + **3**

Scheme 2. **Enabling route to 2**



4 + CS_2 → **5** → **6** → **7**; **8** → **9**; **7** + **9** → **2**

Enabling Technology

The technology developed to supply interim amounts of **2** is shown in Scheme 2.[2] This convergent approach entailed coupling the methyl thiourea **7** with 3-amino-5-hydroxyben-

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(3) Clark, J. D.; Weisenburger, G. A.; Anderson, D. A.; Colson, P.-J.; Edney, A. D.; Gallagher, D. J.; Kleine, H. P.; Knable, C. M.; Lantz, M. K.; Moore, C. M. V.; Murphy, J. B.; Rogers, T. E.; Ruminski, P. G.; Shah, A. S.; Storer, N.; Wise, B. E. *Org. Process Res. Dev.* **2004**, *8*, 51−61.

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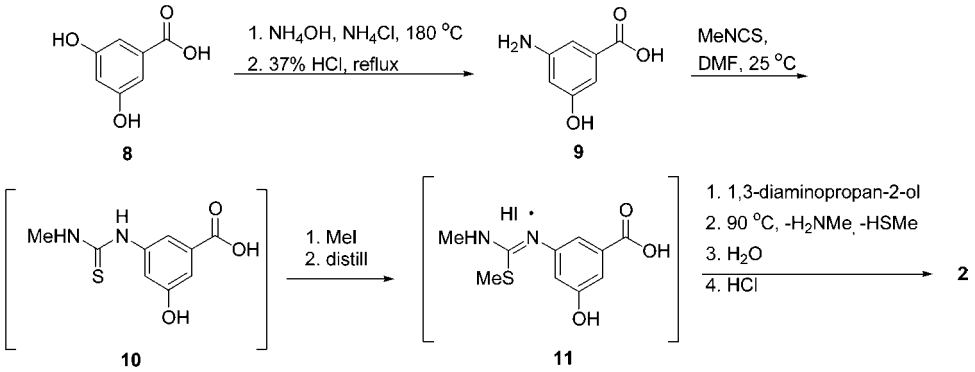
Scheme 3. **Linear approach to 2**



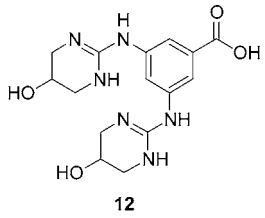
zoic acid (**9**) affording **2** in 18% overall yield. Although this methodology was employed on-scale, the following safety, technical, and operational issues were identified during the course of practicing the chemistry. The process (1) required too many steps to product, (2) gave rise to low yields and high waste loads, (3) entailed relatively low payload and long cycle times, (4) required the use of carbon disulfide, (5) required the use of a protecting group, (6) involved vacuum distillations, and (7) revealed issues associated with the handling and transfer of some intermediates.

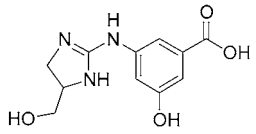
Process Research

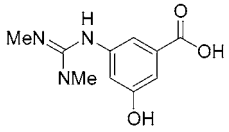
The aforementioned enabling technology used to prepare **2** suffered from a number of drawbacks. Thus, a linear approach to **2** was investigated. Envisioned was the reaction of **9**, prepared from **8**,[4] with an isothiocyanate derivative (Scheme 3). On the basis of in-house experience, we expected that an isothiocyanate should react selectively with the amino substituent. Alkylation of the resulting thiourea derivative followed by reaction with 1,3-diaminopropan-2-ol would then give rise to **2**. Accordingly, a review of the price and availability of isothiocyanates indicated that the methyl analogue should be the most cost-effective. Indeed, when **9** was subjected to a slight excess of methyl isothiocyanate in DMF at ambient temperature, the corresponding thiourea **10** (Scheme 3) was prepared in near quantitative yield as determined by HPLC analysis.

The crude solution of **10** was then subjected to methyl iodide, giving rise to **11**. Treatment of this mixture with excess 1,3-diaminopropan-2-ol was followed by careful heating with venting to a scrubber. Gas evolution was observed at approximately 70 °C. Analysis of the product mixture revealed, however, that the reaction was incomplete. Total conversion was realized upon heating to 90 °C. Isolation of the desired product **2** as either its HCl salt or as the zwitterion proved troublesome at first, but conditions were developed from which zwitterion **2** was prepared in 55−60% overall yield from **8**. The product assay was 85 wt %, with a moisture content of approximately 10 wt %.

Analysis of the impurity profile of **2** by HPLC/MS and HPLC/NMR suggested the presence of the bis-tetrahydropyrimidine **12** at a level of approximately 0.5%, the imidazole-derivative **13** at approximately 0.5%, and the dimethylguanidine-analogue **14** at levels approaching 3%.







Additionally it was found that product mass balance was in the mother liquor. Taking this into account, an outstanding overall chemical yield of 94% of **2** from **9** was realized. Thus, an efficient, three-step, one-pot procedure for the preparation of **2** had been developed.

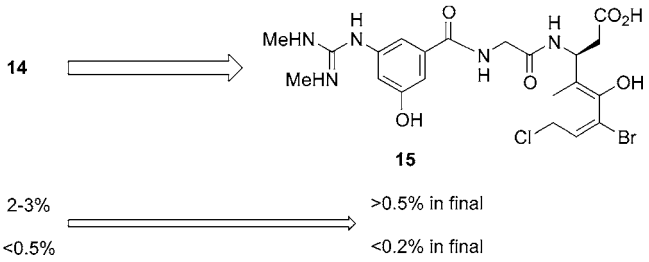
Process Development

The success of the laboratory trial to make **2** via this methyl isothiocyanate route prompted us to further develop this chemistry. Two noteworthy results from the aforementioned laboratory trial were (1) the impurity profile and (2) the high level of water in the isolated solid. To address these issues and prepare the data for transfer to the pilot plant, three significant studies were performed: (1) development of rationale approaches to minimize the impurities, (2) a study directed toward the drying habits of **2**, and (3) a Mettler RC1 reaction calorimetry study of the chemical route to more effectively understand and safely transfer the technology.

It was quickly realized that **12** and **13** were derived from impurities present in the starting materials. In the case of **12**, it was verified that 3,5-diaminobenzoic acid was present at a level of approximately 1% in **9**. Reaction of the diamine with the reagents in excess formed **12**. This was verified through independent preparation of **12** and spiking studies thereof. It was found, however, that **12** was eliminated in the downstream process and did not manifest itself as the corresponding impurity in API. Therefore, investigating ways to diminish the level of 3,5-diaminobenzoic acid in **9** was not necessary.

(4) For the literature preparation of **9** see: (a) Becker, A. M.; Rickards, R. W.; Brown, R. F. C. *Tetrahedron* **1983**, *39*, 4189−4192. (b) Drake, N. L. *Org. React.* **1942**, *5*, 105−128.

Scheme 4. **Impact of 12 on API impurity profile**



It was proposed that **13** might be derived from 2,3-diaminopropan-1-ol present in the 1,3-diaminopropan-2-ol. The presence of this homologue at levels as high as 7 area % was verified by careful GC analysis of the starting material, but directed studies toward the preparation of **13** were not completed. Like **12**, **13** did not significantly impact the impurity profile of **1**.

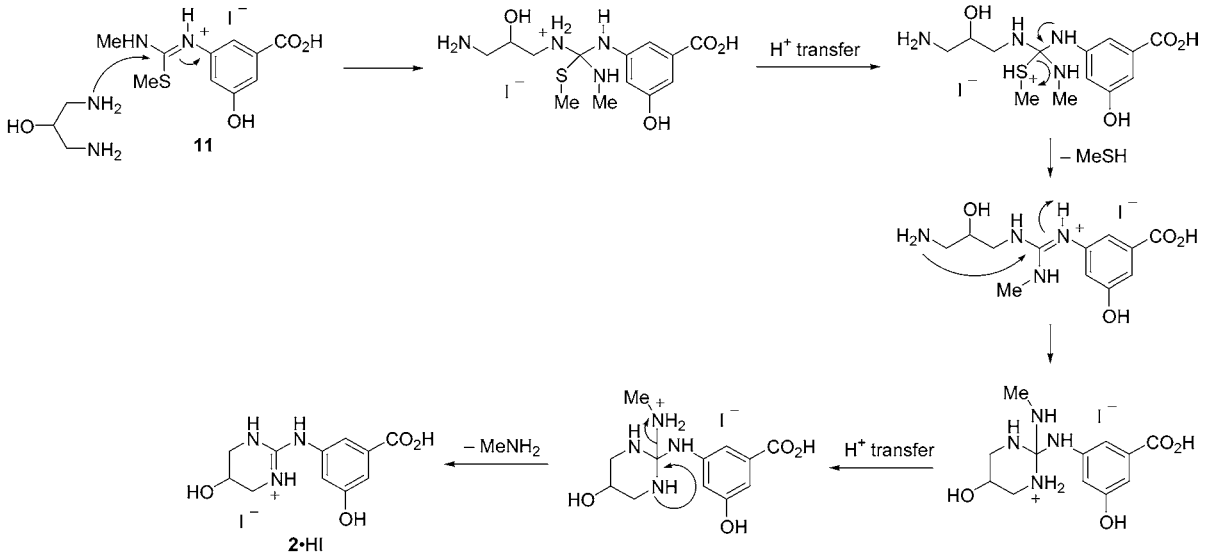
The dimethylguanidine impurity **14** presented a most interesting challenge as it was generated by the chemistry itself. Also, unlike the other impurities, **14** carried downstream and negatively impacted the API purity. For example, when 2−3% of **14** was present in **2**, **1** contained >0.5% of the corresponding impurity **15** (Scheme 4). However, when the level of **14** was <0.5% in **2**, then <0.2% of **15** was observed. Thus, understanding the origin of **14** and further development of the process to minimize it was an important goal.

A proposed mechanism for the preparation of **2** is shown in Scheme 5. This suggests that **14** is derived from the interaction of unreacted starting material, or a reactive intermediate thereof, with the methylamine byproduct of the reaction.[5] Indeed, it was found that the level of **14** could be diminished from 3% to less than 0.5% by either adjusting the mode of reagent interaction or by physically facilitating gaseous methylamine expulsion. For example, the mode of reagent addition was changed by either adding the crude solution of **11** to a hot solution of 1,3-diaminopropan-2-ol in DMF (inverse addition) or by increasing the rate of 1,3-diaminopropan-2-ol addition to the crude solution of **11**. Physical facilitation of methylamine expulsion was accomplished by either the installation of a nitrogen sparger or an increase of the reaction temperature during reagent interaction. Independent preparation of **14** and spiking studies thereof verified these results. Ultimately, the approach employed in the pilot plant was to increase the pot temperature containing the solution of **11**, maintain a vigorous nitrogen sweep through the reactor headspace, and add the 1,3-diaminopropan-2-ol as quickly as possible.

The investigations undertaken to discern the drying habits of **2** led to the realization that this zwitterion was hygroscopic. For example, original samples contained ≥13% moisture. However, when samples of **2** were dried at 100 °C under a vacuum of 27.5 in Hg, a weight decrease of 4.4% was measured within 1 h. A loss of 11.3% was observed after 21 h, and eventually, a total of 12% of the sample's original weight was lost after 8 days. On the other hand, if these dried samples were allowed to stand under ambient conditions, the moisture level returned to a level of 13% within 36 h. The water in this key intermediate would also be a parameter of interest in the chemistry used to prepare **1**.[7]

The Mettler RC1 reaction calorimeter study of the conversion of **9** into **10** revealed a moderate heat of reaction of −57.3 kJ/mol of **9** (−63.7 kJ/kg of solution) and an adiabatic temperature rise (ATR) of 33 °C for this process. The data also indicated that the reaction was essentially complete within 6.25 h after dosing. Similar trends were observed in the reaction of **10** with methyl iodide. A heat of reaction of −52 kJ/kg of solution was measured, resulting in an ATR of 27 °C for the process. The reaction was complete within 2 h, including the 60-min addition of methyl iodide. For the conversion of **11** to the desired product **2**, only a small endothermic response was measured when the room temperature 1,3-diaminopropan-2-ol was added to the hot solution of **11**. Thus, the reaction calorimetry experiments did not reveal any significant thermal risk associated with the chemistry.

Scheme 5. **Proposed mechanism for the conversion of 11 to 2**



With these data, the chemistry was scaled to 22-L equipment. No noteworthy issues were observed. Employing 2 kg of **9**, 2.27 kg of **2** was prepared in 72% overall yield with an assay of 85 wt % and 10 wt % water. Therefore, a new linear, three-step, methyl isothiocyanate-based synthesis of **2** from **9** was demonstrated.

Pilot-Plant Campaigns

The hazardous reagents and potential materials of construction issues associated with the new chemistry led us to choose a specific third party manufacturer (TPM). This facility had the high temperature and pressure reactors necessary to prepare **9**, had previous experience in handling methyl isothiocyanate, and manufactured methyl iodide. Thus, a strategy was implemented in which a pilot run would be completed first, and if successful, a production campaign composed of two batches would be completed.

Preparation of 3-Amino-5-hydroxybenzoic Acid (9). Laboratory studies directed toward the preparation of **9** followed literature precedent.[4] However, upon scale-up, mixing the ammonium hydroxide, ammonium chloride, and 3,5-dihydroxybenzoic acid (**8**) as described gave rise to a solid, cement-like mass that could not be agitated. We were able to repeat this phenomenon in the laboratory and found that the solution to the problem was the order of mixing. It was discovered that addition of a premixed solution of ammonium hydroxide and ammonium chloride to **8** avoided the formation of the solid mass. Once this was ascertained, 51.1 kg of **8** was converted to 25.3 kg of **9** in 51% overall yield with an assay of >96%. This procedure was successfully repeated. The major equipment employed was an Inconel 600 clad reactor with a working volume of 300 gal and a maximum pressure tolerance of 1000 psi. The balance of the work required the use of one 100-gal glass-lined reactor, two 200-gal glass-lined reactors, a ceramic-coated filter, and a stainless steel filter.

The Pilot Run. The pilot run was uneventful. The major equipment utilized were two 30-gal Pfaudler reactors, three 200-gal Pfaudler reactors, a 20-in. stainless steel-clad Tolhurst centrifuge, a vacuum tray dryer, and a 50-gal portable, glass-lined vacuum tank.

A slurry of **9** (11.1 kg, 72.7 mol) in DMF was treated with a warm solution of methyl isothiocyanate (5.4 kg, 74.1 mol) in DMF. The reaction was allowed to go to completion by overnight agitation at ambient temperature. In the next step, crude **10** was treated directly with methyl iodide (14.4 kg, 102 mol) and allowed to agitate overnight at ambient temperature. Most of the DMF was removed under vacuum, providing a mobile concentrate.

In the final step, a warm solution of 1,3-diaminopropan-2-ol (19.7 kg, 218 mol) and DMF was added to the concentrate of **11**. This mixture was gradually heated, resulting in off-gassing of methyl mercaptan and methylamine. The gases were scrubbed with solutions of caustic and sulfuric acid. The reaction solution was then diluted with water and the pH adjusted to 6 with concentrated HCl. The zwitterion **2** was collected by centrifugation, the cake was washed with water and acetonitrile and was then transferred to a tray dryer and dried at 60 ± 5 °C until the LOD was measured at less than 1%. This gave rise to 13.0 kg of **2** as a light-tan powder in 71.5% overall yield from **9** with an assay of 97.4%.

Production Campaign. The major equipment used in the production of **2** included two 100-gal Pfaudler reactors, four 500-gal Pfaudler reactors, a 40-in. stainless steel-clad Tolhurst centrifuge, a vacuum tray dryer, and a 50-gal portable, glass-lined vacuum tank. Again the chemistry performed as expected. The one significant operational change implemented was increasing the tray dryer temperature to 70 ± 5 °C. Two batches of **2** were prepared, affording 35.4 kg (73.1% yield) and 37.0 kg (76.2% yield) of product with assays of 96.3 and 96.4 wt %, respectively. Thus, the average yield for all lots was 74.1%, providing a total of 85.6 kg of **2** with an average assay of 96.6 wt %.

Conclusions

Studies directed toward the process research and development of a scalable method for preparing tetrahydropyrimidine **2**, a key intermediate to the $\alpha_v\beta_3$ integrin antagonist **1**, were described. A linear approach employing 3-amino-5-hydroxybenzoic acid, methyl isothiocyanate, and 1,3-diaminopropan-2-ol as key reagents was detailed. Development of this chemistry led to a better understanding of the origin of impurities and how to diminish them. A successful pilot run and production campaign were completed in which a total of 85.6 kg of **2** was prepared with an average assay of 96.6 wt % in an overall yield of 74%.

Experimental Section

General. The actual charges of substrates and reagents are given below. The molar amounts are calculated on the basis of the assays of the materials. Similarly, yields are calculated on the basis of assay-corrected moles of substrates and products. Proton (^{1}H) nuclear magnetic resonance (NMR) spectra were recorded on either a Unity Inova Varian 300 MHz or Unity Inova Varian 400 MHz spectrometer. ^{1}H NMR descriptions are reported as: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad).

Melting points were determined using a Laboratory Devices Mel-Temp instrument equipped with a Fluke 51 thermocouple. Thin-layer chromatography was performed on EM Science 0.25 nm silica gel 60, glass-backed plates with F_{254} indicator. UV light was employed for visualization.

3-Amino-5-hydroxybenzoic Acid (9).[4] To a high-pressure Inconel clad reactor was added **8** (51.1 kg, 332 mol). In a separate reactor, ammonium chloride (68.0 kg) was dissolved into ammonium hydroxide (215.5 kg). A clear solution was obtained. The ammonium hydroxide/ammonium chloride solution was then drawn into the reactor containing **8**. The loading sequence is important because altering the procedure could create a mass that cannot be stirred. The contents of the reactor were agitated, heated to 180−190 °C, and held within this temperature range for 40−42 h.

(5) No impurity derived from the expulsion of methyl mercaptan was observed.
(6) Intermediate **2** was a proposed registered starting material.
(7) The effect of the water associated with **2** on the downstream chemistry to prepare **1** will be a subject of discussion in part three of this series.

Pressures over the course of heating reached 365 psig at the onset and attained a maximum pressure of 431 psig.

After cooling the contents, the reaction mass was transferred to a glass-lined 200-gal reactor. The liquid appeared to be translucent, homogeneous, and approximately dark-maroon in color. The excess ammonia was removed from the system to a sulfuric acid scrubber and the material concentrated to a volume of approximately 60 gal. A dark-maroon to coffee-black colored, homogeneous, and fairly opaque product mixture was produced. The product mass was acidified with 37% HCl (127 kg) and refluxed at 100−112 °C for 16−17 h. After reflux, the material was precipitated by cooling to 20 °C and then collected on a ceramic filter to give crude **9** (127 kg) as a damp, dark-brown to maroon solid.

The crude was then purified by dissolving the material in 2% aqueous sodium hydroxide (359 kg), adding Darco KB activated carbon (6.8 kg), filtering the resulting slurry, and adjusting the pH to 3.5 ± 0.1 with 37% HCl (21.3 kg). The precipitate was collected by centrifugation, affording 33.8 kg of damp **9** with an assay of 91.7% by HPLC analysis.

Further purification was accomplished by redissolving crude **9** into 3.7% caustic (238 kg). This was filtered (without charcoal), the pH was adjusted to 3.5 ± 0.1 with 37% HCl (21.3 kg), and the product was isolated via filtration. The product was dried in a tray dryer with a vacuum of 25 in. Hg at 60−65 °C to provided 25.3 kg of **9** (50%) as a light-gray powder with an assay of 96.5% by HPLC analysis: mp 230.5−234 °C. This material was identical to an authentic sample.[2]

3-Hydroxy-5-[[(methylamino)thioxomethyl]amino]-benzoic Acid (10). Into a 100-gal reactor was charged DMF (57.6 kg) and methyl isothiocyanate (14.7 kg, 202 mol). To a separate 100-gal reactor was added **9** (28.8 kg, 199 mol) and DMF (57.6 kg). With agitation, the methyl isothiocyanate/DMF mixture was charged into the reactor containing **9** and allowed to agitate for 12 h at room temperature. Thin-layer chromatographic analysis indicated that no starting material was present. This solution was used without further purification.

Thiomethyl Adduct 11. To crude **10** was slowly added methyl iodide (38.2 kg, 269 mol). The internal temperature of the mixture was maintained below 40 °C during addition, applying cooling to the jacket when necessary. The reaction was shown complete by thin-layer chromatographic analysis. The methyl iodide adduct was concentrated under reduced pressure (27−29 in. Hg) to remove excess DMF and excess methyl iodide. The internal temperature during the distillation was not allowed to exceed 60 °C. Crude **11** was used without further purification.

***N*-[2-(5-Hydroxy-4,6-tetrahydropyrimidine)]-3-amino-5-hydroxybenzoic Acid (2).** To a separate 100-gal reactor vented to sequential bleach and sulfuric acid scrubbers was charged premelted (mp 40−45 °C) 1,3-diaminopropan-2-ol (50.8 kg, 597 mol) and DMF (41.7 kg). The 1,3-diaminopropan-2-ol/DMF solution was transferred to the reactor containing **11** at such a rate to keep the contents below 50 °C. Upon complete addition, the contents were heated to 90 °C. Vigorous off-gassing began at approximately 60 °C. Once the reactor contents reached 90−95 °C, it was held within this range for 3 h.

The reactor jacket was cooled to 40 °C. Into a separate 500-gal reactor was charged 181 kg of water. With agitation, the solution containing **2** was transferred into the 500-gal reactor. The contents were then cooled to 10 °C, and the pH was carefully adjusted to pH 6 with 37% aqueous HCl (27.7 kg). The resulting precipitate was isolated via a variable-speed 40-in. Tolhurst centrifuge. The damp cake was washed with water (18 kg) and acetonitrile (14 kg) and allowed to spin dry. The product was transferred to a vacuum tray dyer and dried overnight at 70−75 °C at 25−28 in. Hg to give 37 kg (74.9%) of **2** with an HPLC assay of 96.4%: TLC: $R_f = 0.53$ ($CHCl_3$/MeOH/H_2O, 25:25:5); ^{1}H NMR (D_2O, 90 MHz) δ 7.42 (s, 2H), 7.01 (t, 1H), 4.41 (t, 1H), 3.46 (br s, 4H). This product was identical to an authentic sample as determined by ^{1}H NMR, ^{13}C NMR, and HPLC analysis.[2]

Acknowledgment

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