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# Pilot-Plant Preparation of an $\alpha_v\beta_3$ Integrin Antagonist. Part 3. Process Research and Development of a Diisopropylcarbodiimide and Catalytic 1-Hydroxybenzotriazole Peptide Coupling†

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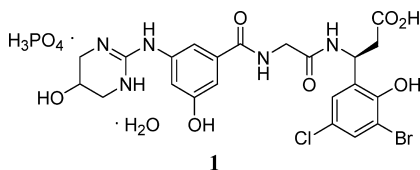
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## Abstract:

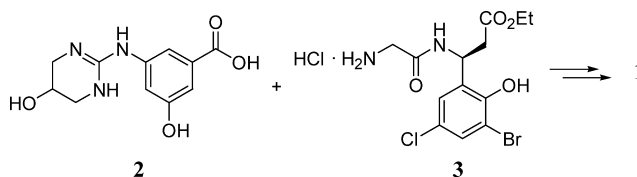
Studies directed toward the process research, development, and scale-up preparation of the potential  $\alpha_v\beta_3$  integrin antagonist **1** are described. A convergent approach is detailed wherein tetrahydropyrimidine hydroxybenzoic acid **2** is linked to the  $\beta$ -amino acid ester **3** via a diisopropylcarbodiimide, catalytic HOBt coupling reaction. Saponification of the resulting ethyl ester, isolation of the corresponding zwitterion,  $\text{H}_3\text{PO}_4$  salt formation, crystallization, and acetonitrile-to-acetone exchange give rise to **1** as a crystalline monohydrate in 67% overall yield from **4**.

## Introduction

The integrin  $\alpha_v\beta_3$  plays an essential role in angiogenesis, the process by which new blood vessels form from pre-existing blood vessels.<sup>1</sup> 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 antagonist, an improved synthesis of the monohydrate, monophosphoric acid salt **1** was required.<sup>2</sup>

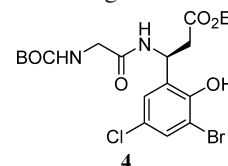


Envisioned was a convergent synthesis wherein hydroxybenzoic acid **2** would be linked to the  $\beta$ -amino acid amide **3**. As part of a series of reports,<sup>3</sup> herein we describe the chemical process research, development, and large-scale preparation of **1**.



## Results and Discussion

Preparation of **1** began by converting **4**<sup>4</sup> to **3**. Initial laboratory-scale experiments facilitated the desired deprotection through the use of 4 N HCl in dioxane, but upon further investigation, use of this reagent on scale gave rise to **3** that



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† Manuscript dedicated to Professor Paul A. Grieco on the occasion of his 65th birthday.

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**Table 1.** Summary of the thermokinetic data for the conversion of **4** to **3**

unit operation	total heat [kJ]	intrinsic heat [kJ/mol x]	adiabatic temp. rise [°C]
hydrogen chloride preparation	-128	136/mol x = AcCl	+79
preparation of <b>3</b>	-0.91	9.2/mol x = <b>4</b>	+0.46

was hygroscopic and contained several percent of the corresponding, undesired carboxylic acid. These findings in conjunction with the potential safety issues associated with the use of 4 N HCl in dioxane on scale prompted development of another procedure.

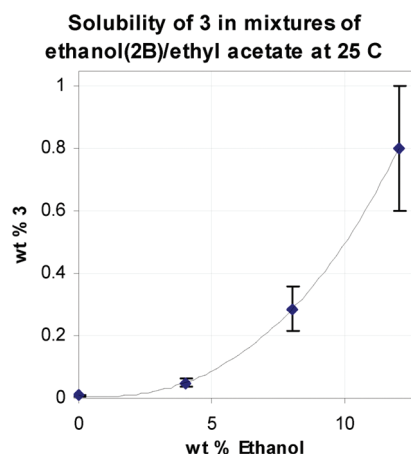
After some experimentation it was determined that deprotection and isolation of **3** could be successfully obtained by generating anhydrous HCl *in situ* from the reaction of 10 equiv of acetyl chloride and ethanol at  $\leq 10$  °C. Following workup, **3** was isolated by filtration as a white solid in >95% yield and >96 HPLC % purity.

In order to facilitate the technology transfer of this reaction to the pilot plant and to ascertain the thermal and safety characteristics of the chemistry, reaction calorimetry experiments as well as IR and MS head space gas analysis for the conversion were performed.<sup>5</sup> The results of the calorimetry experiments are provided in Table 1. Both reactions produced moderate to low levels of heat, demonstrating that the chemistry should be easily controlled in a standard reactor setup.

The major gaseous products detected in the reactor headspace were ethanol, CO<sub>2</sub>, HCl, isobutylene, *tert*-butyl chloride, ethyl acetate, and acetyl chloride.<sup>6</sup> Only 43% or 0.04 mol of the theoretical quantity of 0.0938 mol of CO<sub>2</sub> were measured. This was most likely due to the solubility of CO<sub>2</sub> in ethanol<sup>7</sup> and, perhaps, losses in the reactor configuration. Also detected was 0.2 mmol of isobutylene. This translated to only 0.21% of the available moles of substrate vented as isobutylene. It was therefore determined that a standard scrubber configuration would support the disposition of the gaseous products.

The aforementioned procedure was practical for a laboratory-scale reaction, but further work on the concentration and isolation of the product on large scale was required. In this regard, the solubility of **3** in ethyl acetate/ethanol solvent mixtures was measured. The level of ethanol in the solvent mixture was an important parameter for the efficient isolation of **3**. As illustrated in Figure 1, the solubility of **3** in ethyl acetate/ethanol increased significantly as the wt % of ethanol increases versus ethyl acetate. This was verified experimentally. For example, a 30% yield loss of **3** was realized if the ethanol content was 12 wt %. At 5 wt % of the solvent, 12% of **3** was lost.

With this data in hand, **3** was successfully prepared in two pilot plants. A solution of **4** in ethanol was charged with 10



**Figure 1.** Solubility of **3** in ethanol/ethyl acetate.

equiv of acetyl chloride at  $\leq 10$  °C. The solvent was then exchanged to ethyl acetate, being sure to decrease the ethanol content to <1 wt %. The product was collected, washed with cold ethyl acetate, and blown partially dry with warm N<sub>2</sub>. Drying was completed in a tumble dryer affording 125 kg of **3** as a white solid in 93–95% yield.

**Coupling Process Development.** Process development toward the coupling of **2** with **3** to prepare the ethyl ester of **1** began by surveying a number of coupling reagents.<sup>8</sup> 1,3-Diisopropylcarbodiimide (DIC), 1,3-dicyclohexylcarbodiimide (DCC), 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT),<sup>9</sup> and benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate (BOP) were all effective coupling agents. Table 2 summarizes these results.<sup>10</sup>

Of these coupling reagents, DIC was employed in all scale-up work. This reagent proved easy to source, was cost-effective and known to provide greater operator safety.<sup>11</sup> Preliminary research determined that 1.3 equiv of DIC at 20 °C for 23 h provided the best reaction performance. The amount and number of reaction impurities increased when either >1.3 equiv of DIC was employed or the reaction was carried out at >30 °C.

The best solvent system for the desired coupling was found to be a 1:1 (v:v) mixture of DMF/CH<sub>2</sub>Cl<sub>2</sub>.<sup>12</sup> Other aprotic solvents as well as alcohols facilitated the desired coupling reaction. The reaction was faster in alcohols versus DMF/CH<sub>2</sub>Cl<sub>2</sub> but required the addition of 1 equiv of HCl, and more importantly, the workup procedure from alcohols proved more difficult. The rate of the coupling reaction in NMP was greater

(5) The calorimeter used in this study was a RC1 SV01 1L reactor equipped with a pitched blade impeller, addition funnel, a thermocouple, calibration heater, a P6890 MSD GC/MS system used for sampling from the RC1 SV01 reactor head space, and a ASI ReactIR1000 with a diamond ATR probe. IR spectrum analysis was completed using ASI Concert IR analysis software.

(6) Except for CO<sub>2</sub> and isobutylene, all gas flows were calculated by assuming that the response factor of gas/Air was equal to 1. Quantitative CO<sub>2</sub> and isobutylene data were calculated by the response factor of CO<sub>2</sub> (*m/z* 44)/Ar (*m/z* 40) = 0.74, isobutylene (*m/z* 56)/Ar (*m/z* 40) = 0.15.

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(10) *N,N'*-Carbonyldiimidazole, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide HCl, bis(2-oxo-3-oxazolidinyl)phosphinic chloride, 2-chloro-1-methylpyridinium iodide, diethyl chlorophosphate, trimethylacetyl chloride, isovaleryl chloride, isobutyl chloride, 1-propanephosphonic acid cyclic anhydride, 2-nitrophenol, 4-nitrophenol, 2,4,5-trichlorophenol, and *O*-benzotriazolyl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate were ineffective coupling agents when using 2 equiv relative to **2**.

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**Table 2.** Comparison of coupling reagents for the reaction of **2** and **3**

reagent	temp. (°C)	time (h)	solvent	equiv	HPLC area % <b>2</b>	HPLC area % <b>3</b>	HPLC area % <b>1</b> ethyl ester
DIC	30	23	DMF CH <sub>2</sub> Cl <sub>2</sub>	1.3	<1	0	64
DCC	30	23	DMF CH <sub>2</sub> Cl <sub>2</sub>	1.3	<1	trace	62
CDMT	0–20	36	DMF	1.2 2 NMM <sup>a</sup>	7	0	48
BOP	18	48	DMF	1.15 2 Et <sub>3</sub> N	10	2	66

<sup>a</sup> NMM = *N*-methylmorpholine.

**Table 3.** Comparison of 0.2 equiv of an auxiliary nucleophile with 1.3 equiv of DIC in DMF at 4 h and 18–20 °C

auxiliary nucleophile	HPLC area % <b>2</b>	HPLC area % <b>3</b>	HPLC area % <b>1</b> ethyl ester
control: no auxiliary	28	26	43
2-hydroxypyridine	20	17	40
2-mercaptopyridine	24	16	35
catechol	25	17	38
HOAt	0.1	0.6	76
HOBt	0.5	0.6	75

than in DMF, which in turn was greater than in 1:1 DMF/CH<sub>2</sub>Cl<sub>2</sub>, but the combination of DMF/CH<sub>2</sub>Cl<sub>2</sub> provided a more efficient workup and isolation of the desired product. The ratios of DMF/CH<sub>2</sub>Cl<sub>2</sub> were also investigated, but again the 1:1 mixture provided the best balance with reaction results versus postreaction isolation. DMAC was also investigated, but the reaction rate was slower than in DMF or the DMF/CH<sub>2</sub>Cl<sub>2</sub> combination.

The use of an auxiliary nucleophile<sup>8</sup> accentuated the rate of formation of the ethyl ester of **1** and gave rise to fewer structurally related impurities. 1-Hydroxybenzotriazole (HOBt) was found to be an excellent auxiliary nucleophile; however, several chemical safety concerns were recognized.<sup>13</sup> The structurally related analogue, 1-hydroxy-7-azabenzotriazole (HOAt), and other nucleophile auxiliaries were also studied, but again there were questions about the safety of HOAt.

HOAt gave similar results when compared to HOBt (Table 3). The reaction was slightly faster, but the thermokinetic data for both reagents revealed no safety advantage for HOAt. The closed pan DSCs for HOBt and HOAt were similar.<sup>15</sup> The other nucleophiles, 2-hydroxypyridine, 2-mercaptopyridine, and catechol, had slower reaction rates versus those of HOBt; thus, it was decided to use the readily available and a safer form of this reagent, a 12 wt % solution of HOBt in DMF,<sup>16</sup> for further scale-up work.

The addition of 0.1–0.4 mol equiv of HOBt<sup>17</sup> to the DIC-based coupling reaction accelerated the rate of reaction, decreasing the reaction time from 23 to 4 h versus no catalytic HOBt. It also gave rise to a higher reaction yield by decreasing the number of impurities as measured by HPLC analysis. However,

HOBt was found in the isolated product when >0.2 equiv was employed. Therefore, 0.2 equiv was used in all further scale-up work.

Isolation of the ethyl ester or the corresponding ethyl ester HCl salt of **1** could not be demonstrated in the laboratory. It could be separated from the diisopropylurea byproduct and HOBt via precipitation or extraction, but attempted isolation gave rise to either an oil or a gummy solid with >10% impurities. Chromatographic purification of the ethyl ester was also studied, but again the results of this investigation were not practical due to the insolubility of the product and competing hydrolysis to the carboxylic acid. Conversion of the ethyl ester to the corresponding sodium carboxylate and isolation of the zwitterion species was therefore investigated.

Preparation of the sodium carboxylate analogue of **1** was easily accomplished. Addition of 7.5 equiv of 2.5 N NaOH directly to the coupling reaction completed the saponification within 2 h. The product mixture was then extracted with methylene chloride to remove some DMF, the isopropylurea byproduct, and the HOBt before further manipulation.

Extensive experimentation was completed to define the protocol for isolation of the zwitterion of **1**. It was determined that pH-controlled precipitation was required at or near the zwitterion's isoelectric point of 5.7. Laboratory studies found that exceeding a pH of 7.5 or going below pH 3.8 caused a large portion of the solids to dissolve, resulting in the product falling out of the reaction mixture as a sticky, oily material. However, when the pH was controlled within the range of 5.0–7.2, the desired zwitterion was precipitated as a white to light-gray amorphous solid. Experimentally, this was accomplished by separate and simultaneous addition of the sodium carboxylate product phase and 1.2 N HCl to a pH 7, aq 0.1 N KH<sub>2</sub>PO<sub>4</sub>/NaOH buffer solution. A pH of approximately 7 was maintained throughout the additions by controlling the rate at which the streams were added. Once all of the carboxylate solution was introduced, more 1.2 N HCl was added adjusting the pH to 5.5–6.0. The desired zwitterion was collected by filtration and dried, providing the product in 86–89% yield.

Two engineering concerns were recognized during the course of this laboratory work. First, zwitterion prepared in this manner coated the reactor walls, making isolation difficult. Fortunately this issue was easily resolved by subsurface addition of both the HCl and sodium carboxylate product streams. The second concern observed was the physical properties and manipulation of the wet cake. Cracks formed in the filter cake, preventing the water level from decreasing to <50 wt %. Polyethylene bags or rubber dams on the filter cakes helped slightly, but the drying time to <15% LOD required days to complete. Confounding this, the water would separate and the solids would turn into a

(13) 1-Hydroxybenzotriazole: MSDS; Aldrich Chemical Company, 1999. For a current MSDS see, for example: [http://www.reagentworld.com/products/msds2.asp?proid\\_2=18491](http://www.reagentworld.com/products/msds2.asp?proid_2=18491).

(14) 1-Hydroxy-7-azabenzotriazole: MSDS; Aldrich Chemical Company, 1999. For a current MSDS see, for example: <http://www.mctony.com/hoat%20msds.htm>.

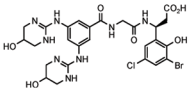
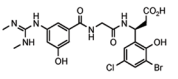
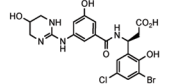
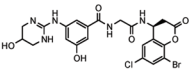
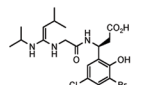
(15) HOBt had an endotherm @ 155–158 °C and an exotherm @ 208 °C (1772 J/g). HOAt had an exotherm at 212 °C (1789 J/g).

(16) Wehrstedt, K. D.; Wandrey, P. A.; Heitkamp, D. J. *Hazard. Mater.* **2007**, *A126*, 1.

(17) HOBt is available as both a wet or dry reagent. It was found that up to 36% water based on HOBt had no effect on the coupling reaction.



**Table 4. Representative reaction or product mixture profile as measured by RP-HPLC analysis<sup>18</sup>**

Entry	Impurity	Relative retention time (min)	Reaction mixture Area %	Post-zwitterion ppt Area %	Final Product 1 Area %
1	?	0.11	0.53	0	0
2	<b>2</b>	0.16	2.51	0.02	0.01
3	DMF	0.20	3.59	0	0
4	HOBt	0.34	9.86	0	0
5		0.52	2.42	0.21	0.18
6	DIU	0.56	5.63	0	0
7	?	0.74	0.81	0	0
8	<b>1</b>	1	68.61	96.35	98.36
9	Conformational isomer	1.04	1.83	0.63	0.44
10	Dibromo analog	1.10	0.16	0.27	0.28
11		1.14	1.58	0.32	0.28
12		1.24	0.24	0.30	0.28
13		1.28	0.26	0.25	0.12
14		1.72	1.65	1.33	0
15	Trihalophenyl analogs	1.92	0.32	0.32	0.05

glassy, caramelized mass. The annealed solids were very difficult to work with, but HPLC analysis revealed no apparent degradation. Nevertheless a procedure had to be developed that would avoid these phenomena. In this regard, laboratory pressure filtration and drying test were completed. After extensive experimentation, it was found that the materials could not be pressure filtered to dryness. A two step process had to be followed. The zwitterion was first pressure filtered to a 45–48% moisture content, then immediately dried on a rotoevaporator *in vacuo*. In this manner an LOD of <4% could be obtained providing gray, amorphous solids. This experience suggested that the correct units of operation for drying on scale would be pressure filtration followed by tumble drying. In conjugation with the development of the methodology to isolate the zwitterion of **1**, the impurities were analyzed by HPLC throughout the process in order to ascertain the effectiveness of the procedure. A representative RP-HPLC profile of the reaction mixture before and after precipitation of the zwitterion as well as the final product **1** is provided in Table 4.

Most impurities were removed or diminished to <0.5 area %, but none of the nonpolar impurities were significantly decreased by the precipitation procedure. Thus, further API purification would be required either before or during isolation.

Conversion of the zwitterion to **1** was extensively studied.<sup>19</sup> Three predominate monophosphoric acid salt crystal forms were

discovered: anhydrous, a nonstoichiometric hydrate, and the monohydrate **1**. These pseudomorphic forms were found to arise via a solution-mediated phase transformation with the desired product **1** determined to be the thermodynamically most stable form.<sup>19</sup> Employing zwitterion obtained directly from the aforementioned precipitation procedure, it was realized that impurities did adversely effect the kinetics of various events during crystallization, giving rise to **1** with purities ranging of 92.8 to 98.2 HPLC area %. However, with an API purity target set at a minimum of 97.9% (93 wt %), it was decided that the zwitterion would have to be chromatographed before conversion to **1**.

To facilitate the technology transfer of the coupling reaction and zwitterion precipitation process to the pilot plant as well as ascertain the thermal and safety characteristics of the chemistry, again reaction calorimetry experiments were completed.<sup>20</sup> Table 5 lists a summary of the results. No concerns were revealed. No gases or foaming were observed.

**Pilot-Plant Preparation of 1.** The aforementioned procedure was completed on scale. In a typical batch, 35 kg of zwitterion with a purity of 90% before chromatography was produced from 14.8 kg of **2** and 24.5 kg of **3**. Drying of one batch was attempted at 60 °C and 350 Torr using a 30 gallon glass-lined tumble dryer, but the product adhered to the walls of the tumble dryer and caramelized, contrary to the rotovap laboratory studies. To avoid this, the wet cake was directly dissolved and purified by column chromatography.

The zwitterion was purified on large scale. RP-HPLC analysis was performed on each fraction to determine the area % of product. Those fractions containing ≥96 area % were combined and concentrated. After pH adjustment, the zwitterion was collected by filtration, and the solids were dried.

Large-scale conversion of the zwitterion to **1** entailed mixing the chromatographed, dried zwitterion with water and acetonitrile. Without agitation, H<sub>3</sub>PO<sub>4</sub> was added to the mixture and heated to 60 °C. Heating without agitation was an important operation, because mixing the slurry at ambient temperature resulted in a cement-like material that literally stopped mechanical agitation! Heating without agitation afforded a flowable mixture which quickly became a clear solution, making agitation possible.

The antisolvent acetonitrile was added to the hot, stirred solution over a 1.5 h period, producing a supersaturated solution. However, nucleation did not occur during the charge, but rather 3–6 h after acetonitrile addition. Agitation and temperature were maintained for 16–24 h to allow for the solution-mediated phase transformation<sup>19</sup> to the desired monohydrate polymorph and to allow the system to attain its equilibrium concentration at 60 °C. X-ray diffraction (PXRD) analysis confirmed the formation of **1** (Figure 2). The slurry was cooled and the API collected by filtration. The cake was washed and dried under vacuum to provide **1** in 89–91% yield and 96.9 to 99.1 HPLC area % purity.

The resulting product contained 2.4–2.8 wt % acetonitrile, which could not be reduced further by drying! This level of acetonitrile was a concern, since it was calculated to be too close to ICH guidelines.<sup>21</sup> Fortunately, it was found that the

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(20) Reaction calorimetry was performed using a 2L A10 RC-1 reactor equipped with a pitched blade impeller, ReactIR ATR FTIR probe, a thermocouple, a calibration heater, and two addition funnels.

**Table 5.** Summary of the thermokinetic data for the DIC, catalytic HOBt peptide coupling of **2** and **3**, ethyl ester saponification, and zwitterion precipitation

unit operation	total heat (kJ)	intrinsic heat (kJ/ mol x)	reaction mass (kg)	heat capacity (J/[kg K])	ATR (°C)
dilution DMF and CH <sub>2</sub> Cl <sub>2</sub>	-17.2	-4.9, x = DMF	0.652	1560	+16.9
coupling reaction	-18.3	-127, x = <b>3</b>	0.805	1420	+16.0
saponification	-41.1	-38.0, x = NaOH	1.28	2680	+12.0
precipitation	-30.0	-60.9, x = HCl	1.36	4200	+5.27

acetonitrile could be “exchanged” with acetone. Indeed, stirring in excess acetone produced **1** in 97% yield containing 0.2–0.26% acetonitrile and 3.1–3.4 wt % acetone.<sup>21</sup> A representative impurity profile of the final product **1** is listed in Table 4. A photomicrograph of **1** is shown in Figure 3. Employing the procedures detailed herein, 104 kg of GMP quality **1** was prepared.

## Conclusion

Studies directed toward the process research, development, and scale-up preparation of the potential  $\alpha_v\beta_3$  integrin antagonist **1** were described. A procedure for the large-scale *tert*-butoxycarbonyl (BOC) deprotection of **4** producing the  $\beta$ -amino acid ester **3** was provided. A convergent approach employing the tetrahydropyrimidine hydroxybenzoic acid **2** linked to **3** via a DIC, catalytic HOBt coupling reaction was detailed. Saponification of the resulting ethyl ester, isolation of the corresponding zwitterion, H<sub>3</sub>PO<sub>4</sub> salt formation, crystallization, and acetonitrile-

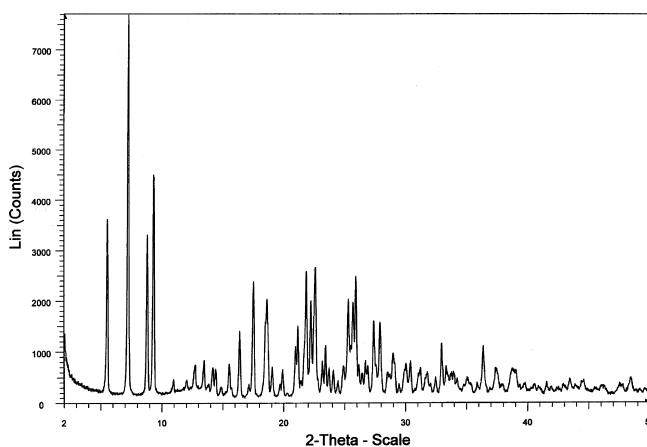
to-acetone exchange provided 104 kg of **1** as a crystalline monohydrate with an average area % purity of 97.4% and in 67% overall yield from **4**.

## Experimental Section

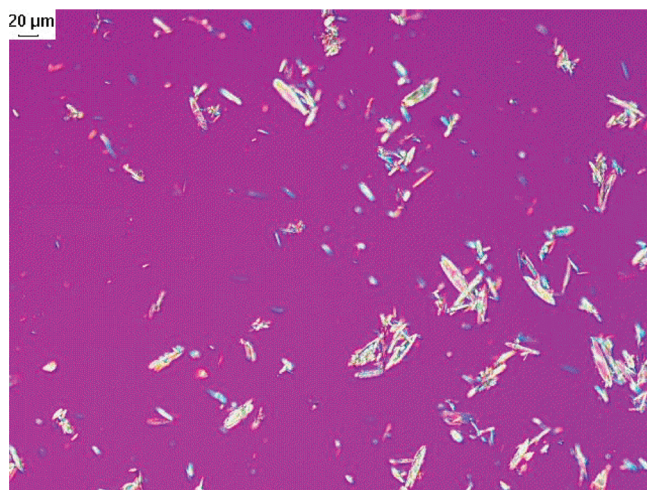
**(3S)-Glycyl-3-(3-bromo-5-chloro-2-hydroxyphenyl)- $\beta$ -alanine Ethyl Ester, Monohydrochloride (**3**).** A reaction vessel was charged with **4** (37.4 kg, 72.3 mol) and ethanol **2B** (322 kg). The mixture was stirred and cooled to 0–5 °C. Acetyl chloride (61.7 kg) was charged while maintaining the temperature below 10 °C. The mixture was heated to 50 °C and held for 3 h. The mixture was cooled to 20 °C and concentrated by vacuum distillation. The concentrate was cooled to 35 °C, ethyl acetate (300 kg) was added and the distillation repeated. Additional ethyl acetate (250 kg) was charged and the distillation repeated again. The concentrate was cooled to 25 °C and ethyl acetate (337 kg) charged. The mixture was cooled to 5 °C and stirred for 3 h. The product was collected by filtration, washed with precooled (5 °C) ethyl acetate (337 kg) and dried at 50 °C until the LOD was measured at <3.0% providing 28 kg (93%) of **3**. The product was shown to be identical to an authentic sample by RP-HPLC, <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>), and <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) analyses.<sup>2</sup>

**(3S)-N-[3-Hydroxy-5-[(1,4,5,6-tetrahydro-5-hydroxy-2-pyrimidinyl)amino]benzoyl]glycyl-3-(3-bromo-5-chloro-2-hydroxyphenyl)- $\beta$ -alanine.** A reaction vessel was charged with **2** (14.8 kg, 58.9 mol), **3** (24.5 kg, 58.9 mol), DMF (89 kg) and 12 wt % 1-hydroxybenzotriazole (13 mol) in DMF (15.0 kg). Methylene chloride (162 kg) was charged to the mixture, which was stirred at 20 °C. *N,N'*-Diisopropylcarbodiimide (9.6 kg, 76 mol) was charged and the mixture stirred for 6 h at 20 °C. A second reaction vessel was charged with water (153 L) and 50% sodium hydroxide (35.3 kg). This solution was transferred to the first reaction vessel and the mixture stirred for 2–2.5 h. Methylene chloride (325 kg) was charged to the reaction vessel and the mixture stirred. The phases were separated and the aqueous phase washed twice with methylene chloride (325 kg for each wash). In a separate vessel a 1.5 wt % aqueous KH<sub>2</sub>PO<sub>4</sub> solution (162 kg water, 2.4 kg KH<sub>2</sub>PO<sub>4</sub>) was prepared, and then the pH of the KH<sub>2</sub>PO<sub>4</sub> solution was adjusted to between 6.5 and 7 with 2.5 N NaOH (~5.27 kg). To a third vessel was charged 4.3 wt % HCl (141 L water, 19.8 kg of 37% HCl).

The aqueous phase in the first reaction vessel and 4.3 wt % HCl solution were charged subsurface to the KH<sub>2</sub>PO<sub>4</sub>/NaOH solution simultaneously such that the pH was maintained between 6.0 and 7.2. When the addition was complete, the pH of the resulting solution was further adjusted to 5.8 with the 4.3 wt % HCl solution. The product was collected by filtration, washed with water (294 L), and dried on the filter with a warm



**Figure 2.** PXRD of the final product **1**.



**Figure 3.** Photomicrograph of the final product **1**.

stream of N<sub>2</sub> to an LOD of 45–48% to give the corresponding partially dried zwitterion (35 kg).

A reaction vessel was charged with the zwitterion (35 kg) and sufficient phosphoric acid, acetonitrile, and water to ensure that there are two equivalents of phosphoric acid and a 50% acetonitrile/water solution. The mixture was heated to 60 °C to ensure dissolution and analyzed for the weight % of the zwitterion using HPLC analysis. This value was used to calculate the number of chromatography injections based on the total amount of contained **1**. Water was charged to achieve a concentration of greater than 90% water as determined by Karl-Fisher analysis. The mixture was purified on either a 2 in. × 250 mm or a 4 in. × 1000 mm stainless steel column packed with a YMC ODS-AQ, 50-μm silica reverse phase media using phosphoric acid (H<sub>3</sub>PO<sub>4</sub>), acetonitrile, and water as eluting solvents at a flow rate of 130 mL/min or 1000 mL/min, respectively. After injecting the sample, the column was eluted with 3.4 equivalent volumes of 90:10:0.2 water/acetonitrile/H<sub>3</sub>PO<sub>4</sub> (v:v:v). Those fractions containing ≤96 area % of **1** were combined and heated to 60 °C to ensure complete dissolution of the product. The solution was filtered to remove particulate matter and analyzed by HPLC to determine the wt % of **1**. The mixture was concentrated by vacuum distillation to remove acetonitrile. The product was precipitated at 30 °C using NaOH (7.6 wt %) until a pH of 5.5 ± 0.5 units was obtained. The mixture was cooled to 25 °C and allowed to stir for 2–3 h. The product was collected by filtration, washed with water, and dried on the filter using a warm stream of N<sub>2</sub> for 21 h at 350 Torr until the water content was ≤5.0% as determined by KF analysis to give 22.6 kg (62.4%) of the zwitterion. The product was shown to be identical to an authentic sample by RP-HPLC, <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>), and <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) analyses.<sup>2</sup>

**(3S)-N-[3-Hydroxy-5-[(1,4,5,6-tetrahydro-5-hydroxy-2-pyrimidinyl)amino]benzoyl]glycyl-3-(3-bromo-5-chloro-2-hydroxyphenyl)-β-alanine Monophosphate (1:1) (Salt) Monohydrate (1).** A crystallization vessel was charged with water (86.0 kg) and the zwitterion (21.5 kg). Without agitation, a solution of acetonitrile (67.6 kg) and phosphoric acid (4.25 kg) was charged. The mixture was heated to 65 °C. Once all the solids dissolved, agitation was started and additional acetonitrile (135 kg) charged while

maintaining a temperature of 60 °C. The contents were held at this temperature for at least 16 h. The crystallization vessel was cooled to 5 °C over at least 5 h and the product collected by filtration. The cake was washed with acetonitrile (33.8 kg) and dried under vacuum until an LOD assay of <4% was achieved.

The crude **1** (21.5 kg) was charged to a reaction vessel. Acetone (153 kg) and water (21.5 kg) were added, and agitation was initiated. The contents of the vessel were heated to 40 °C and held for 3 h. The mixture was cooled to 20 °C and the product collected by filtration and dried on the filter with a warm stream of N<sub>2</sub> for 31 h at 350 Torr to give 21.0 kg (84%) of **1** containing <0.26 wt % acetonitrile and 3.6 wt % acetone as determined by GC analysis. The water content was measured as 3% as determined by KF analysis. The product was shown to be identical to an authentic sample by RP-HPLC, <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>), <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>), and PXRD analyses.<sup>2</sup>

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