Organic Process Research & Development **2006**, *10*, 78–93

A Practical Enantioselective Synthesis of a Novel Peptide Deformylase Inhibitor

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Abstract:

A practical synthesis of the peptide deformylase inhibitor LBM415, (2*S*)-*N*-(5-fluoro-1-oxido-2-pyridinyl)-1-[(2*R*)-2-[(formylhydroxyamino)methyl]-1-oxohexyl]-2-pyrrolidinecarboxamide, magnesium salt 11, is described. The key chiral intermediate, (2*S*)-*N*-(5-fluoro-2-pyridinyl)-1-[(2*R*)-2-[[formyl(phenylmethoxy)amino]methyl]-1-oxohexyl]-2-pyrrolidinecarboxamide 8, was made by coupling the corresponding amino acid 7 with the carboxamide 25 prepared from L-proline and 2-amino-5-fluoropyridine. Following oxidation of the pyridine nitrogen, selective hydrogenolysis of the benzyl group afforded the free acid of the drug substance, which was converted to the magnesium salt in situ with magnesium chloride. The product was obtained in an overall yield of 16% with an ee > 99%.

Introduction

LBM415-NX (Figure 1) has been shown to be an extremely potent inhibitor of peptide deformylase (PDF)[1] in vitro while at the same time showing good antibacterial activity in vivo. Importantly, LBM415 was found to be active against pathogenic bacterial strains that have acquired resistance to other antibacterial agents. This PDF inhibitor has the potential to be developed into an antibacterial agent with the ability to eradicate many bacterial infections, including those that are refractory to treatment with existing agents. We have developed a practical synthesis to produce multikilogram quantities of this potential medicinal agent as a single enantiomer.

The Discovery synthesis of the free acid form **10** [2] was designed in a modular fashion and was obtained in 9 linear (12 total) steps from mostly readily available starting materials (see Scheme 1).

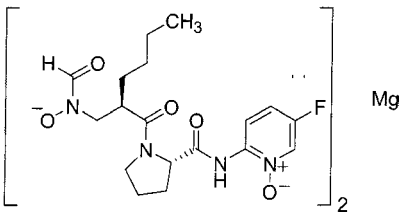

Figure 1. **LBM415.**

Due to the straightforward nature of this route, it was decided to retain the overall strategy for the construction of the molecule for the first Pilot Plant campaign, while employing the necessary modifications to allow for an acceptable fit in the pilot plant. Mainly, we were concerned with the stability of the drug substance along with the large number of chromatographic purifications required and the use of expensive, environmentally unfriendly or undesirable solvents and reagents [e.g., *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), *m*-chloroperoxybenzoic acid (MCPBA), and methylene chloride]. The observed instability of the free acid form **10** was particularly important since no solid form of the compound had as yet been prepared. Thus, in addition to the development of a viable process for the production of this material, we also sought to identify a solid, stable, crystalline derivative. In this paper we provide a full description of the synthesis of LBM415, obtained in 16% overall yield in 13 steps.

Results and Discussion

Following the route elucidated by the Drug Discovery group, our synthesis of LBM415 is illustrated in Scheme 2. *n*-Butylacrylic acid **2** was prepared from *n*-butylmalonic acid **1** and aqueous formaldehyde via a Knoevenagel condensation[3,4] using piperidine as the base. The oily product **2** was obtained in sufficient quality so that no further purification was necessary. In the original procedure developed in Drug Discovery, oxazolidinone chemistry[5] was used to prepare **3** which was purified by preparative chromatography followed by recrystallization from ether/hexane.

In addition to the obvious problems associated with preparative chromatography, there were several other aspects

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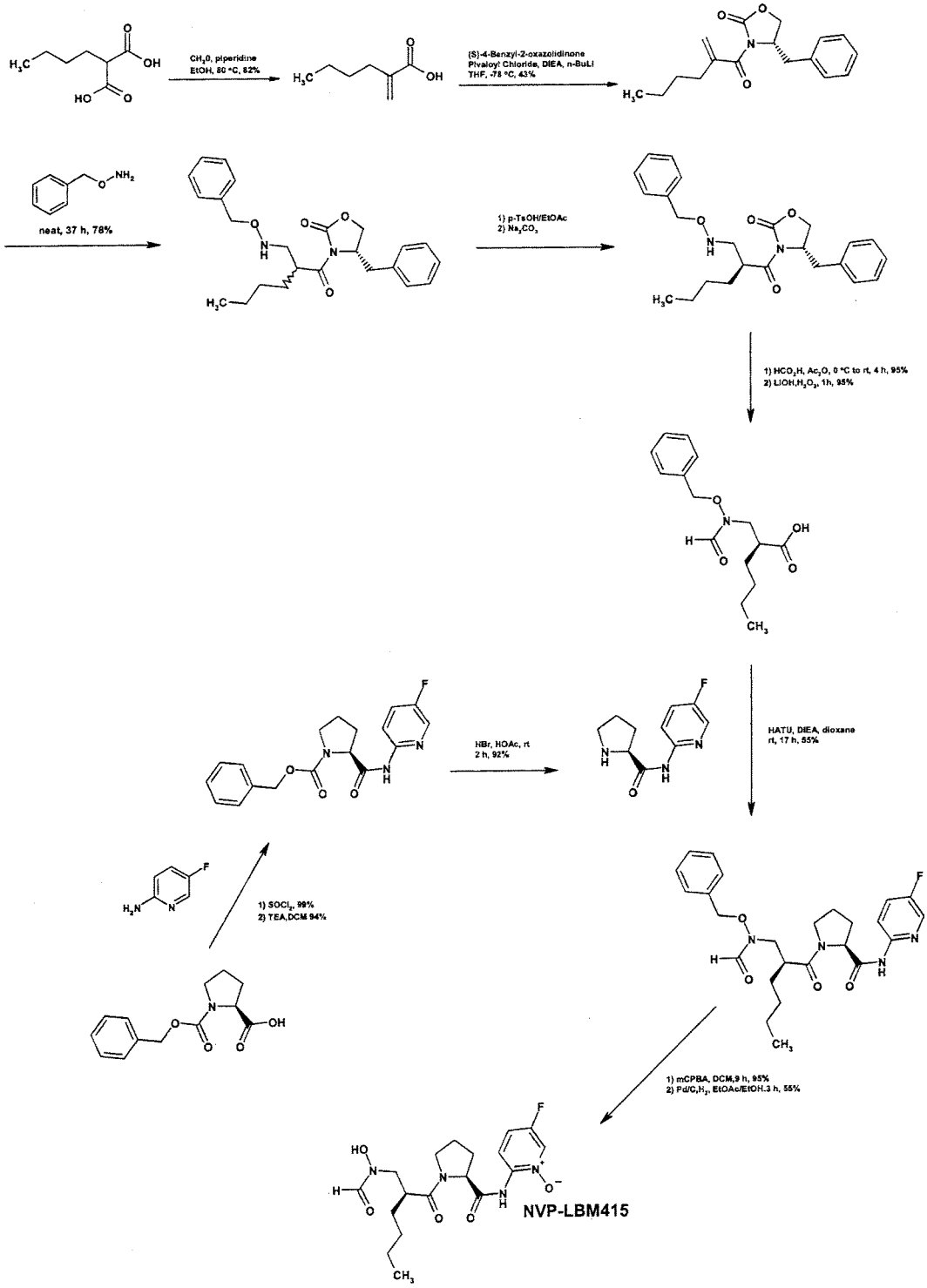
(4) Wilk, B. K. *Tetrahedron* **1997**, *53*, 7097.

(5) (a) Gage, J. R.; Evans, D. A. *Org. Synth.* **1989**, *68*, 77. (b) Evans, D. A. *Aldrichchimica Acta* **1982**, *15*, 23.

Published on Web 12/16/2005

10.1021/op050165y CCC: $33.50

Scheme 1. **Discovery synthesis of LBM415**



of this procedure which needed to be addressed. These were as follows:

(i) the use of low temperature (this would result in lower throughput due to the size of the low-temperature reactor in the pilot plant);

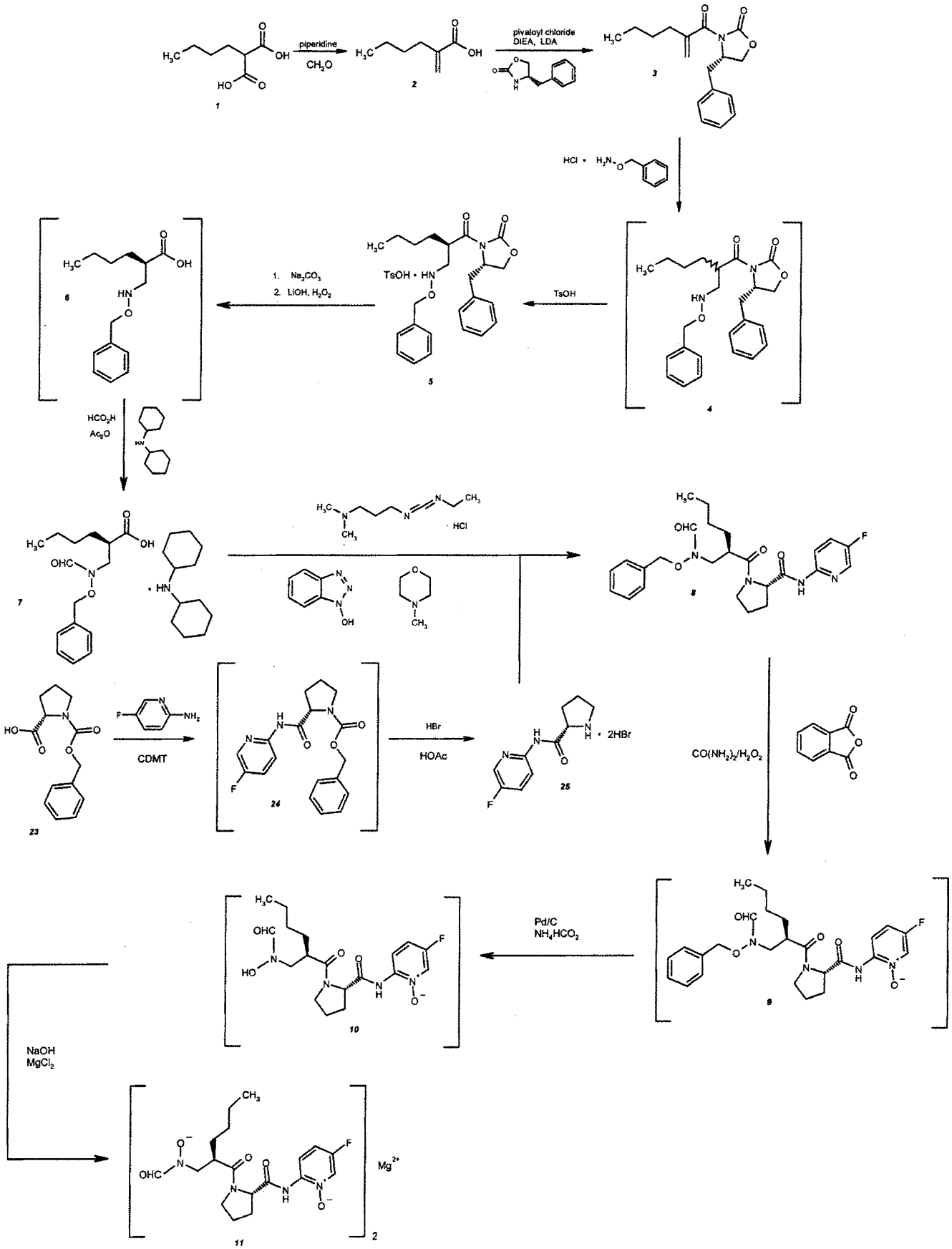
(ii) the need to recrystallize from diethyl ether/hexane; and

(iii) the use of *n*-butyllithium which would generate large quantities of butane gas in the pilot plant.

As the unreacted (*S*)-4-benzyl-2-oxazolidinone could not be removed by recrystallization, we reasoned that if all of the oxazolidinone could be consumed in the reaction, then purification would be simplified. Based on this assumption, a series of experiments was carried out in which the **2**/oxazolidinone ratio was increased until all of the latter reagent was consumed. By using a 10% excess of acid **2**, complete consumption of the oxazolidinone occurred. The excess acid present in the product could be easily removed using a basic workup procedure.

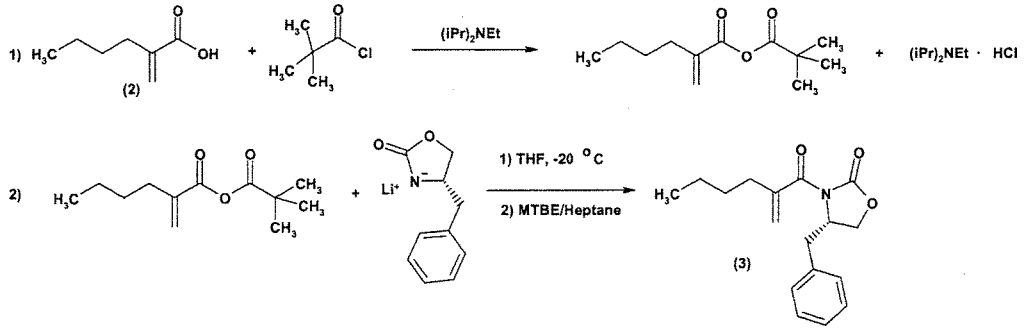
To make the isolation of **3** safe, the original crystallization solvents (ether/hexane) were replaced by methyl *tert*-butyl ether/heptane (1/9). This mixture afforded a white crystalline

Scheme 2. **Process R & D synthesis of LBM415**



solid in 75% overall yield with high purity (>98.5% by HPLC area normalization). To prevent the buildup of static charge during the centrifugation of the batch, 5% ethanol in heptane was used as the final cake wash.

Scheme 3. **Preparation of compound 3**



Another important issue to be addressed was that of throughput. Since *n*-butyllithium was used as the base to deprotonate the oxazolidinone, a reaction temperature of −78 °C was required to prevent side reactions, the main one being nucleophilic attack at the carbonyl of the oxazolidinone ring. This necessitated the use of the low temperature reactor in the pilot plant which only had limited capacity. Another drawback to *n*-BuLi was the fact that butane was produced as a byproduct. This was not desirable in the pilot plant due to the flammability of this gas. To address this problem lithium diisopropylamide (LDA) was investigated. The use of LDA had two advantages: no flammable gas was released on deprotonation, and the selectivity was preserved even at −20 °C with the formation of little or no side products. In our initial experiments, the LDA was used as a 2.0 M solution in THF/heptane/ethylbenzene. However, at the outset, we encountered some difficulties in reproducing both the yield and purity of this reaction. Sometimes the oxazolidinone was consumed, and in some cases, as much as 4% still remained. Even this amount was found to cause problems with the isolation (vide supra).

In conjunction with this, we observed that during the addition of the LDA to the oxazolidinone, the color of the reaction at the end point was variable. Sometimes it was a clear yellow solution, and sometimes it was a dark red-brown solution. Further experimentation revealed that when the end point was yellow, not all of the oxazolidinone was consumed, but when the color changed to red-brown, no oxazolidinone could be detected. Furthermore we knew from previous experience that if less than 1 equiv of LDA was used, the residual oxazolidinone could not be removed easily by recrystallization and that if excess LDA was employed, the yield and purity of **3** would decrease.

Although the concentration of each lot of LDA could be ascertained, we felt that use of the color change as a guide to the completeness of the deprotonation would be more reliable. Thus, the following protocol was used for the development of the process. The dark LDA solution was added slowly to the oxazolidinone in THF at −20 °C. The addition was continued until the color of the solution changed from yellow to dark red-brown.[6] At this point, the addition was stopped and the mixture was held at −20 °C for 15 min. If the color became yellow again, additional quantities of LDA were added until the red-brown color persisted. The anion mixture was then transferred to the solution containing the mixed anhydride (Scheme 3). This procedure was found to work well in the pilot plant as the color change was easily observed through the sight glass.

For the preparation of intermediate **5**, the original Drug Discovery protocol was modified. Thus, 2 equiv of *O*-benzylhydroxylamine were reacted at 50 °C with **3** in the absence of solvent. Ethyl acetate and 4 equiv of *p*-toluenesulfonic were added to precipitate the toluenesulfonic salt of *O*-benzylhydroxylamine. The solvent was then replaced with MTBE, and the desired diastereomer crystallized in 69% yield. A sample of the undesired diastereoisomer was isolated by chromatography from the mother liquors (NMR as well as HPLC indicated that the desired diastereoisomer **5** was obtained in greater than 99% diastereomeric purity).

One other problem which we addressed was the exothermicity of the reaction. Calorimetric studies indicated that an exotherm was occurring at 33 °C (ΔH > 29 J/g) as the reaction was being heated. Since the reaction mixture was a thick oil, it was feared that heat might accumulate in the reactor due to the poor heat transfer to the cooling medium. To eliminate this possibility, the reaction was heated in stages over a 3-h period until the desired temperature (50 °C) was reached.

The diastereoselective conjugate addition of an achiral amine nucleophile to a chiral α,β-unsaturated carbonyl component is well-known in the literature.[7] Specifically for the case of the conjugate addition of *O*-benzylhydroxylamine or its analogues, several examples have been reported.[8−13]

In the present case, we feel that the ratio of the diastereomers is set based on the protonation of the *si* face which is favored for steric reasons. Fortuitously, the desired diastereoisomer is a solid and crystallizes from the reaction mixture. The selective enolate protonation argument has been

(6) A similar color change has been noted for the *N*-acylation of an oxazolidinone using *n*-BuLi; see: Hoekstra, M. S.; Sobieray, D. M.; Schwindt, M. A.; Mulhern, T. A.; Todd, M. G.; Huckabee, B. K.; Hendrickson, V. S.; Franklin, L. C.; Granger, E. J.; Karrick, G. L. *Org. Process Res. Dev.* **1997**, *1*, 26.

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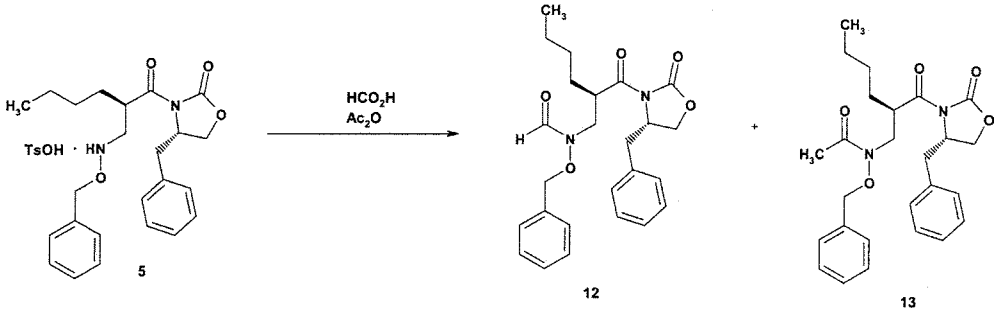
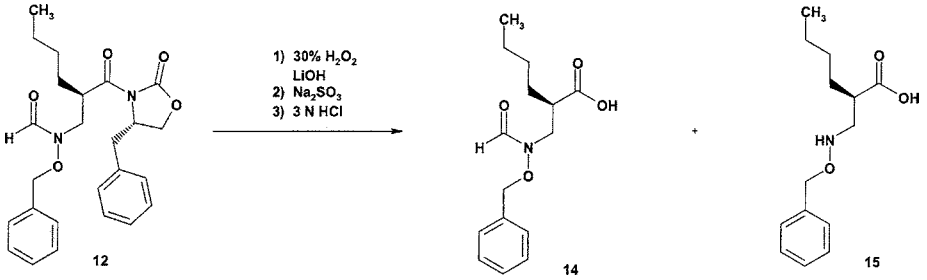
Scheme 4. **Discovery route to amino acid 7 free base: formylation of compound 5**



Scheme 5. **Removal of the chiral auxiliary from 12**



put forth to explain the stereochemistry of the nucleophilic conjugate addition of carbon nucleophiles.[14]

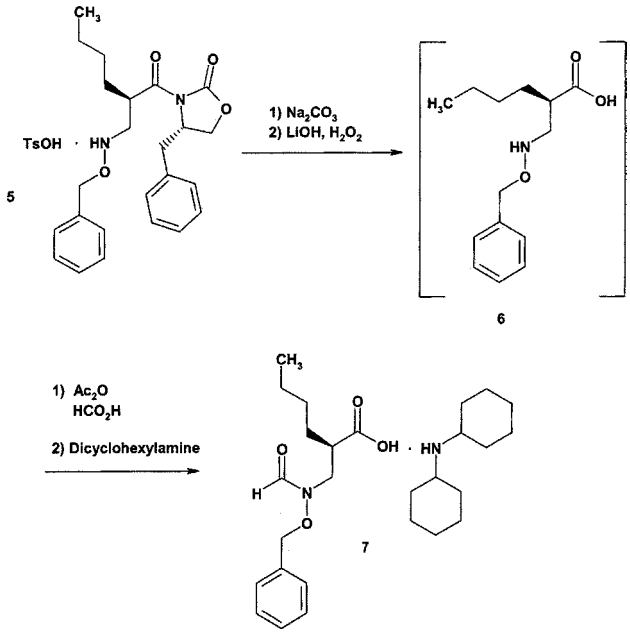
In the original Discovery synthesis, the formylation step was executed prior to the removal of the chiral auxiliary (Scheme 4). Thus, a formic acid solution of **5** was treated with a mixture of formic acid/acetic anhydride (15 equiv/2 equiv) at 0 °C. After an aqueous workup and preparative chromatography, product **12** was isolated in 40% overall yield. The major impurity in the crude product (10%) was identified as the *N*-acetyl derivative **13**.

This procedure was modified by carrying out the formylation at −15 °C. When the reaction was conducted in THF using 8 equiv of formic acid and 2 equiv of acetic anhydride,[15] **12** was obtained in 75% yield with only 1.5% of the *N*-acetyl impurity **13** present.

However, problems were encountered in the following step, i.e., removal of the chiral auxiliary (Scheme 5). Under the standard reaction conditions (2.3 equiv of 30% hydrogen peroxide and 1.2 equiv of lithium hydroxide)[16] **14** was obtained in low yield and purity. Along with the chiral auxiliary, the product was contaminated with intractable polar materials and as much as 10% of the deformylated side product **15**. The latter was identified by comparison with an authentic sample and may have resulted from the reaction between the product **14** and the lithium hydroperoxide.

In addition to the difficulties mentioned above, we found that **14** was a rather unstable oil which had to be kept from light and stored cold. Because of these problems, it was decided to reverse the order of the steps and remove the chiral auxiliary prior to formylation (see Scheme 6).

Scheme 6. **Development route to intermediate 7**



It was expected that this approach would produce a less deformylated side product and also allow us to separate the oxazolidinone from **6** by acid/base extraction. In addition, it was thought that the properties of **14** could be improved by preparing a stable salt, thus simplifying the isolation of this important intermediate.

This approach was found to be much more suitable for scale-up; however, several problems still existed. In addition to those associated with the use of hydrogen peroxide in the pilot plant (e.g., liberation of oxygen from the reaction mixture due to decomposition of the peroxide[17]), the stability of **6** was also a concern. In one instance in the laboratory, a solution of **6** was concentrated at 50−55 °C on the rotary

(14) Mohrig, J. R.; Fu, S. S.; King, R. W.; Warnet, R.; Gustafson, G. *J. Am. Chem. Soc.* **1990**, *112*, 3665.
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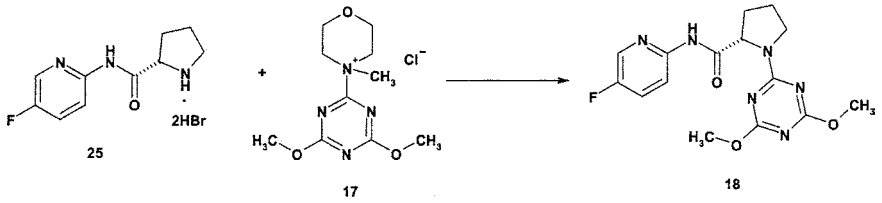
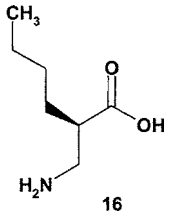
(17) Astbury, G. R. *Org. Process Res. Dev.* **2002**, *6*, 893.

Scheme 7. **Reaction of 25 with morpholinium salt 17**



evaporator. Upon removal, the neat oil was observed to begin offgassing and the internal temperature rose to 150 °C. The sample changed from a colorless oil to a yellow solid. Analysis by HPLC, NMR, and MS indicated that **6** had decomposed to a variety of products. The main constituent was identified as the β-amino acid **16**. Although a definitive explanation is not yet available, we feel that the decomposition may have been brought about by residual amounts of sodium sulfite (used in the work up to decompose the hydrogen peroxide) which could, with heat, cleave the N−O bond of the hydroxylamine.



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With regard to the use of aqueous hydrogen peroxide in the pilot plant, it was felt that the best way to preclude the accumulation of oxygen in the headspace above the reaction mixture was to use a rapid nitrogen sweep across the batch, thus maintaining the level below 8−10% (v/v) (the minimum oxygen level necessary for combustion).[17] To monitor the oxygen level, the reactor to be used was fitted with an oxygen analyzer.

Because of our laboratory experiences with **6**, a significant number of safety tests were carried out to be certain that this intermediate could be handled safely on a large scale. It was found that the neat oil was very unstable with an onset exotherm at 48 °C (both Radex and DSC) with a 612−823 J/g energy release. In addition, it was observed that samples of **6** became less stable over time (lower exotherm detection temperature) with decomposition to the β-amino acid being observed. Based on the stability data, it was decided to store intermediate **6** as a cold ethyl acetate solution and to use it as soon as possible.

Intermediate **6** was *N*-formylated using the conditions mentioned above. However, to improve the yield and reduce the amount of the *N*-acetyl impurity present, the reaction was carried out at lower temperature (−15 °C) and the amount of formic acid was increased from 4 to 8 equiv.

As with **6**, the free acid was unstable to heat and light. Therefore, the compound was isolated as the dicyclohexylamine salt **7**. To further streamline the process, the formylation step was carried out in ethyl acetate so that no solvent exchange or removal was necessary. The overall yield for the two steps was 74%, and the purity was greater than 99% (by HPLC area normalization).

For the preparation of **25**, the protected proline **23** was coupled with 2-amino-5-fluoro-pyridine using CDMT, and the resulting amide **24**, deprotected with HBr/HOAc. The latter conditions were chosen because it was found that the free base obtained by hydrogenolysis of **24** was low melting and difficult to isolate in pure form, whereas the HBr salt was a crystalline solid.

Initially, we envisioned using 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT)[18] as the coupling agent for the synthesis of **8**, since we had employed this reagent successfully on several previous projects. Thus, **7** and **25** were allowed to react in acetonitrile in the presence of a slight excess of CDMT and 4 equiv of *N*-methylmorpholine. Analysis of the crude product indicated that it contained 54% of **8** and 33% of an impurity. The impurity was identified as **18**, the product resulting from the reaction of **25** with the morpholinium salt **17** (Scheme 7).[19] Apparently, the proline amide **25** can compete effectively with the carboxylic acid **7** for the morpholinium salt. Allowing the acid **7** to react with the salt at room temperature prior to the introduction of **25** reduced the level of this impurity to 15%.

Due to the fact that **18** could only be removed from the product by chromatography, alternative conditions for the coupling reaction were investigated. Other coupling agents tried were 1-propanephosphonic acid cyclic anhydride (T3P),[20] isobutyl chloroformate,[21] and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI).[22] Of these, the latter reagent was the only one which afforded appreciable amounts of product.

During the early part of our development work for this step, we observed the formation of a new impurity which was rather difficult to remove. Using NMR and LC/MS, the compound was identified as the acylated urea **20**. This is a rather common type of side product which is observed in carbodiimide-mediated peptide bond formation reactions and comes about by rearrangement of the adduct **19** (Scheme 8).[23] The formation of this compound was suppressed by adding HOBt to the reaction mixture.[24] Thus, the rearrangement of **19** was precluded by the reaction of this intermediate

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(19) Kaminski, Z. J.; Panetti, P.; Rudzinski, J. *J. Org. Chem.* **1998**, *63*, 4248.
(20) Wissman, H.; Kleiner, H. J. *Angew. Chem.* **1980**, *92*, 129.
(21) For a review, see: Ray, T. In *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L., Ed.; John Wiley and Sons: New York; Vol. 4, p 2752. In the DSC, an exotherm was noted beginning at 150 °C [$\Delta H \approx$ 1129 J/g].
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(23) Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Chemical Approaches to the Synthesis of Peptides and Proteins*; CRC Press: Boca Raton, FL, 1997; p 50.
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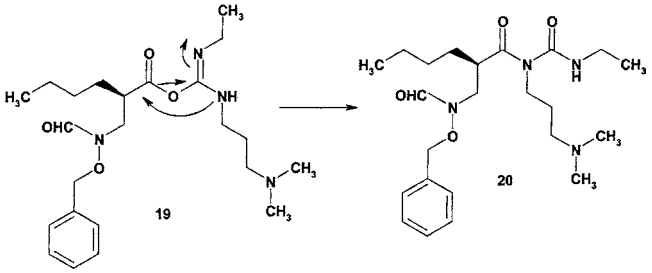
Scheme 8. **Rearrangement of EDCI adduct 19**



with HOBt to form the HOBt ester which then reacted with **25** to give **8**.

As anhydrous HOBt dust is explosive in nature,[21] we conducted the reaction in a two-phase mixture of ethyl acetate and water (2.5/1 v/v). The water served to remove polar impurities and allowed for a simpler workup and purification. The product **8** was then recrystallized to high purity with only minimal losses.

The original procedure used for the oxidation of **8** (MCPBA/methylene chloride) was not considered for reasons of safety and ecology. One major requirement with regard to any new reaction conditions was that complete conversion of **8** to **9** was essential. This was because during the following debenzylation step, any compound **8** remaining would also debenzylate to give the same impurity one would obtain from cleavage of the N−O bond of the pyridine *N*-oxide (see Scheme 9). We had previously observed the latter side reaction during our laboratory work and had found that **21** could only be removed by chromatography (at this point, we had yet to isolate **9** as a solid).

Initially, we investigated the use of Oxone (potassium hydrogen persulfate) for carrying out this oxidation.[25] Based on these early studies we found that the best conditions involved the use of an acetone/water mixture with potassium bicarbonate as a base to reduce the acidity of the reagent. Otherwise, protonation of the pyridine nitrogen would occur rendering it inactive to oxidation. The actual oxidant in this case is dimethyldioxirane **22** (see Scheme 10).[26] Although the reaction worked reasonably well, it could not be driven to completion even when a large excess of oxidant was employed. The best we could accomplish using 4−5 equiv of Oxone and long reaction times (2−4 days) was about 98% conversion with 1−2% **8** still remaining.

Recently, a report appeared[27] in which the authors described the release of singlet oxygen from the reaction of dimethyldioxirane with amine *N*-oxides. In this case it was observed that when dimethyl dioxirane was used to prepare amine *N*-oxides, in some instances it was not possible to achieve complete conversion of the amine to the *N*-oxide despite the use of a large excess of dimethyl dioxirane. According to the experimental data, the authors concluded that the oxidation of tertiary amines and nitrogen heteroarenes by dioxiranes to *N*-oxides may be a reversible process. The in situ generated *N*-oxide may react with the dioxirane at comparable or even higher rates than the amine to afford mixtures of starting material and product. It was concluded that the amine/*N*-oxide ratio could be dependent on the nucleophilicity of the *N*-oxide. Addition of an excess of dioxirane would result in the decomposition of the *N*-oxide to release $^1O_2$. The authors proposed the mechanism shown in Scheme 11. Using this hypothesis, the reaction of Oxone in acetone/water (dimethyldioxirane) with **8** could be represented in the way shown in Scheme 12.

As a final verification that this was indeed occurring, a sample of **9**, free of **8**, was subjected to the oxidation conditions described above. After an overnight hold at room temperature, the reaction mixture was found to contain 1.4% of **8**. Thus, the reversibility of this reaction was proven, and we concluded that it would not be possible under these conditions to effect complete conversion of **8** to **9** due to the apparent reversibility of the reaction brought about by the propensity of the product to react with the excess oxidant.

As an alternative to Oxone, we decided to investigate the use of the urea/hydrogen peroxide/phthalic anhydride (UHP)[28] system for this oxidation since the oxidation of 4-*tert*-butylpyridine to the *N*-oxide had been reported using these conditions.[29]

The advantages to using this reagent mixture were as follows: the mild conditions, the stability of the solid oxidant, the ease of handling, and the fact that the byproducts were water and base-soluble. Since UHP has only limited solubility in nonpolar organic solvents, we chose to investigate acetonitrile and ethyl acetate since the polarity of these two solvents would be sufficient to solubilize the reagent so that the reaction would proceed at a reasonable rate.

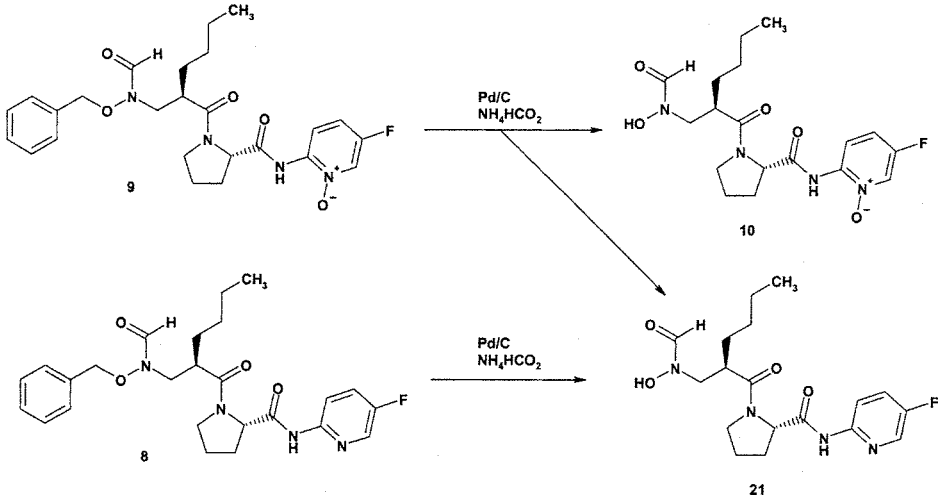
In both solvents the reactions were complete after 7 h at room temperature using 2 equiv of UHP and 3 equiv of phthalic anhydride. Since the results were similar, ethyl acetate was chosen as the reaction solvent, since the workup using this solvent was simpler (aqueous sodium carbonate wash).

Due to the fact that small amounts of water can increase the solubility of UHP in ethyl acetate and that a higher concentration of UHP in solution should accelerate the reaction rate, the reaction conditions were modified to include the addition of 10% water by volume to the mixture. This reduced the reaction time to less than 5 h. As mentioned previously, since a method for the crystallization of **9** was not yet available, an assay method was developed, and the material was carried forward to the next step as an ethyl acetate solution.

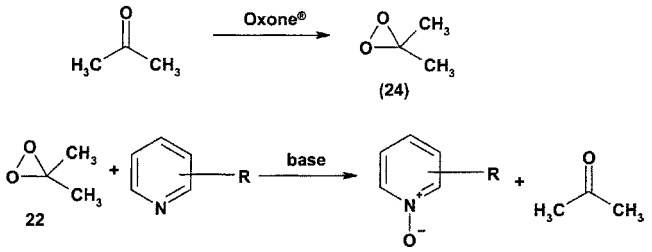
Conversion of 9 to LBM415. In the Discovery synthesis, the debenzylation of **9** was accomplished by catalytic hydrogenation under 1 atm of hydrogen using 10% Pd/C in a 1:1 mixture of ethyl acetate/ethanol. Under these conditions, approximately 10% of **21** was formed resulting from cleavage of the pyridine N−O bond. Since **10** was not a solid, the only method of purification was preparative chromatography. Upon removal of the mobile phase, the drug substance was isolated as a solidified foam in moderate yield.

(25) Bell, T. W.; Young-Moon, C.; Firestone, A.; Healy, K.; Liu, J.; Ludwig, R.; Rothenberger, S. D. *Org. Synth.* **1990**, *69*, 226.
(26) Murray, R. W.; Jeyaraman, R. *J. Org. Chem.* **1985**, *50*, 2847.
(27) Ferrer, M.; Sánchez-Baeza, F.; Messeguer, A.; Adam, W.; Golsch, D.; Görth, F.; Kiefer, W.; Nagel, V. *Eur. J. Org. Chem.* **1998**, 2527.
(28) For a review, see: Heaney, H. *Aldrichchimica Acta* **1993**, *26*, 35.
(29) Kaczmarek, L.; Balicki, R.; Nantka-Namirski, P. *Chem. Ber.* **1992**, *125*, 1965.

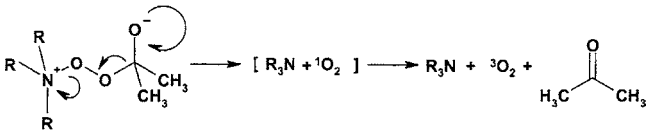
Scheme 9. **Debenzylation of compound 9**



Scheme 10. **Oxidation with dimethyldioxirane in acetone**



Scheme 11. **Reaction of *N*-oxides with excess dimethyldioxirane**

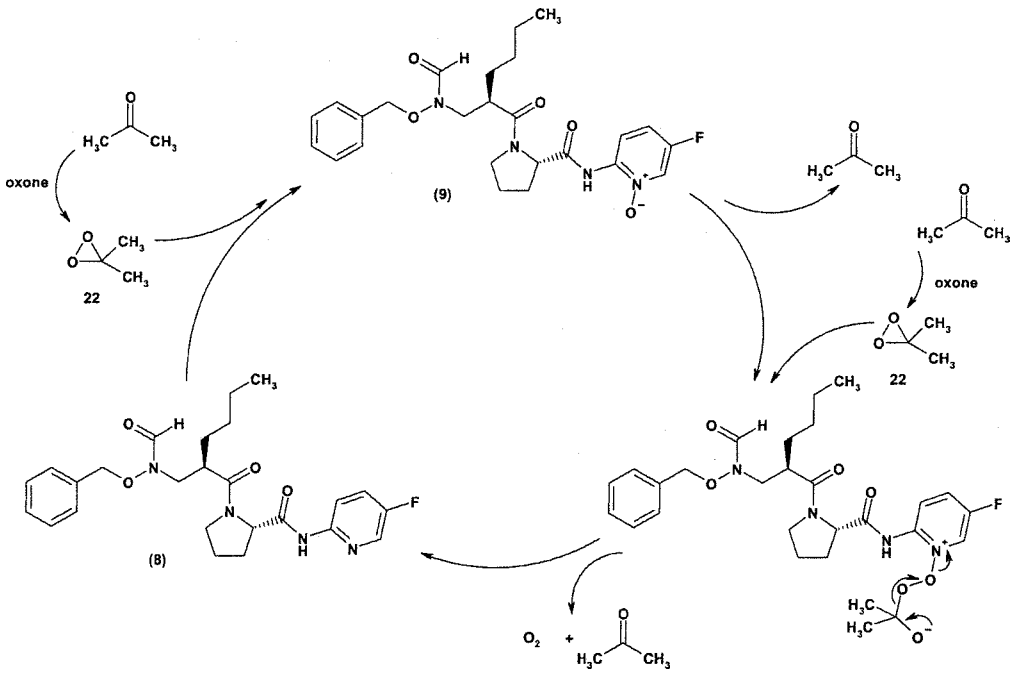


This was acceptable for small-scale preparations; however, we learned that chromatographic purification on a large scale (300 g) was not feasible due to the instability of **10** to prolonged exposure to silica gel. To circumvent this problem, alternative hydrogenolysis conditions were sought which, it was hoped, would minimize the undesired side reaction (reduction of the *N*-oxide) and yet still promote the debenzylation reaction.

At this point, we did not have a scaleable procedure for the final step, nor did we have a method of isolating the drug substance in a solid form. Thus, a parallel investigation was initiated wherein various hydrogenolysis conditions were tried while, at the same time, salt screening was carried out.

Switching the catalyst from Pd/C to 5% $Pd/BaSO_4$ (40 wt % catalyst loading) in ethyl acetate afforded between 2% and 3% of the deoxy impurity **21**. Both commercial and in situ reduced $Pd/BaSO_4$ were tried. With the Lindlar catalyst ($Pd/CaCO_3$ with Pb), the reaction was too slow to be practical. Using a more polar solvent such as ethanol served

Scheme 12. **Reaction of 9 with dimethyldioxirane**



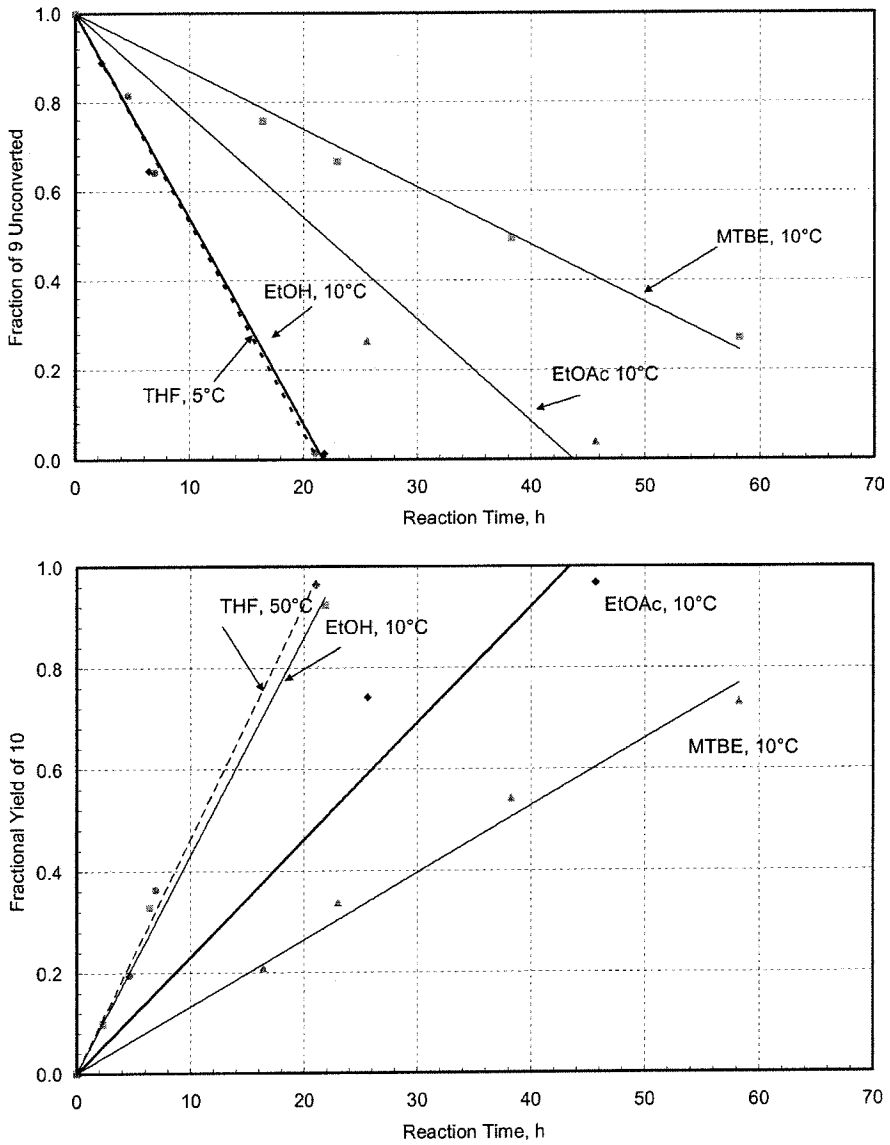


Figure 2. **Kinetics of the debenzylation of 9 and the formation of 10 in various solvents.**

to increase the amount of the deoxy impurity up to as much as 16%.

Thus, the best conditions for this reaction involved the use of unreduced 5% Pd/$BaSO_4$ (40 wt %) in ethyl acetate at 20 °C and 22 psi of hydrogen for 20 h. These conditions afforded complete conversion of **9** while at the same time giving 2.5 wt % of the deoxy impurity **21**.

Based upon the conditions described above, a series of experiments was conducted to quantify the impact of temperature and solvent on the kinetics and selectivity in order to further optimize this reaction. As mentioned previously, minimizing the amount of the deoxy impurity **21** was especially important because of the purification issues.

Using ethyl acetate as the solvent, a Pd/$BaSO_4$ loading of 3.8 wt %, and 25 psig pressure, the reaction was more rapid at 20 °C but also resulted in good conversion of **9** to **10**; however a significantly greater amount of **21** was also formed (4.3%). The conversion and yield were calculated from component concentrations, the latter based on experimental determination of absolute HPLC response factors for **9**, **10**, and the deoxy impurity **21** using external standards.

The reactivity and selectivity in more polar (EtOH, THF) and less polar solvents (MTBE) were also investigated (keeping reactant and catalyst loadings as well as pressure constant). The debenzylation was significantly faster in EtOH and THF relative to EtOAc (see Figure 2).

With MTBE, the reaction was very slow, with only a 73% conversion of **9** after 58 h. The conversion **9** and the formation of **10** followed zero-order kinetics, based on the almost linear variation of yield with reaction time, consistent with high catalyst surface coverage of reactant and products. The best selectivity for **21** (viz., yield/conversion) of 0.019 was obtained with EtOAc at 10 °C (Table 1). However, subsequent experiments showed that better selectivities could be obtained using transfer hydrogenation. Consequently, catalytic hydrogenation using molecular hydrogen was not considered further.

Hydrogenolysis of **9** by catalytic transfer hydrogenation[30] in ethanol with 5% Pd/C (10 wt %), ammonium formate

(30) For a review, see: Johnstone, R. A. W.; Wilby, A. H. *Chem. Rev.* **1985**, *85*, 129.

Table 1. **Conversion of 9 and product yields at final reaction times**

conditions	reaction time (h)	conversion of **9** (%)	**21** (%)	selectivity
EtOH, 10 °C	22	98.7	8.2	0.083
MTBE, 10 °C	58	73.0	1.65	0.023
THF, 5 °C	21	96.2	6.1	0.061
EtOAc, 10 °C	46	96.2	1.85	0.019

(1.25 equiv), and sodium bicarbonate (3 equiv) was investigated, and the results looked promising. Our experiments indicated that the reaction could be carried out without the sodium bicarbonate, which had been added to neutralize any acid that was present. Under these conditions, hydrogenolysis was complete in 30−60 min for small scale reactions and **10** was obtained in 90−95% purity (HPLC area %) with $0.7 \pm 0.2\%$ of **21**. Prolonged reaction times resulted in lower purity with the formation of more side products. Subsequently it was found that **21**, as well as most of the other impurities, could be removed or significantly reduced through salt formation and recrystallization.

The hydroxylamine **10** is relatively stable in ethanol. After heating at 75 °C for 2 h, the purity of a 10% solution of **10** in ethanol decreased by only 1−2%. However, the decomposition of **10** in the transfer-hydrogenolysis reaction mixture was found to be much faster; about 1−2% for every 15 min at 75 °C. It was assumed that the acceleration of decomposition was due to the presence of ammonia, which is a byproduct of the reaction and which could react with **10** by a number of pathways, including deformylation, and cleavage of the proline amide bond.

Supporting evidence for this assumption came from a calorimetry experiment (see below) in which a slightly positive nitrogen pressure (1.03−1.1 bar) was maintained throughout the reaction. This prevented the ammonia generated in the reaction from escaping. After an extra 30 min of heating at 73 °C (for calibration purposes), the amount of **10** present in the reaction mixture had decreased to 79.2% by HPLC with a concomitant increase in impurity levels. This is about a 10% lower concentration than expected. However, the magnesium salt (vide infra) obtained from this reaction mixture was still of excellent purity (98.2%), although the product was isolated in lower yield (62% vs 75%).

Substituting formic acid or calcium formate for ammonium formate resulted in no product formation. Addition of acetic acid or sodium dihydrogen phosphate did not help reduce the amount of decomposition observed. A nitrogen sweep over the reaction mixture was found to be helpful. For the pilot plant batches, the nitrogen flow was about 1.7 times that of the lab. The purity of **10** in the reaction mixture was 92.6% under these conditions.

Catalyst, concentration, number of equivalents of ammonium formate, and heating profile were some other factors that were found to influence the course of the reaction. Since 5% Pd/C did not always afford complete conversion of **9**, even with extended time and additional ammonium formate, we decided to use 10% Pd/C (50% water wet) instead. This change resulted in an increase in the amount of **21** produced. We found that different lots of catalyst had different activities and that the reaction concentration was related to the activity of the catalyst. For Pd/C (old stock from the pilot plant), the reaction was carried out with ∼14% concentration of **9** in ethanol (w/v) and the amount of **21** produced was 0.7%. However, a fresh batch of catalyst which was ordered for the Pilot Plant campaign gave 1.3% of **21** under the same conditions. This was attributed to the higher activity of the catalyst. By decreasing the concentration to 10%, the amount of **21** produced decreased to below 0.6%. Thus, the concentration for all of the pilot plant batches was lowered accordingly.

With regard to what is reasonable for the pilot plant, a heating and cooling rate of about 1 °C/min is practical. Thus, the reaction was carried out in such a way that the temperature was increased from 20 to 65 °C over ∼40 min and from 65 to 72 °C over 15 min. At this point, there was usually about 5% of **9** remaining. Then, an additional heating period of 10 min was carried out before cooling was started. Subsequently, it was decided to do without this final heating, since the cooling temperature ramp (from 72 to 50 °C over 20 min) was sufficient to allow the reaction to continue, and thus the amount of unreacted **9** was reduced to 0−1%.

In addition, the amount of ammonium formate was increased from 1.25 to 1.3 equiv to help increase the rate. However, we found that a large excess of ammonium formate would result in more **21** being formed. We found that hydrogenolysis did not occur at 20 °C. Analysis by HPLC indicated that no **10** was formed at 20 °C for 20 min. At about 40 °C, gas evolution from the reaction mixture was obvious, and white solids, presumably ammonium carbonate, were observed on the wall of the condenser.

When the catalyst (10% Pd/C, 50% wet) and solid ammonium formate were combined, an exotherm accompanied by gas evolution was observed. Thus it was decided that the Pd/C should be added first and that the ammonium formate would be added after the ethanol had been introduced.

Since **10** was prepared as an ethyl acetate solution, a solvent switch to ethanol was necessary for the salt formation step. This was carried out in the following manner. After concentrating **10** from ∼25% to 45% at ∼200 mbar, ethanol was added and the batch was distilled at reduced pressure (less than 80 mbar). This procedure effectively reduced the residual ethyl acetate to below 1%.

It was found that filtration through Celite was effective in removing the catalyst. The residual Pd content was below 0.5 ppm in the crystallized product using this procedure.[31] The filtrate was found to be stable at low temperature. For example, at −14 °C for 7 days, the decomposition of **10** was about 1% by HPLC. Thus, this solution could be used as a hold point in the pilot plant.

The reaction was carried out in the pilot plant in the following manner. After increasing the temperature from 25 °C to 71 °C over 60 min, the mixture was cooled to 15 °C

(31) For leading methods of removing residual amounts of palladium from organic compounds, see for example: Thayer, A. *Chem. Eng. News* **2005**, *83* (36), 55. Garrett, C. E.; Prasad, K. *Adv. Synth. Catal.* **2004**, *346*, 889.

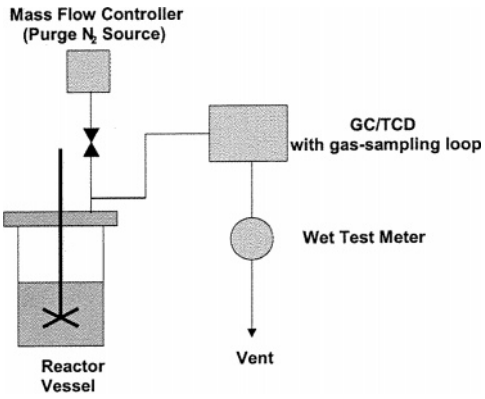


Figure 3. **Schematic diagram of the apparatus used for the determination of the offgas volume, flow rate, and composition.**

over ~55 min. At this point, the in-process analysis indicated virtually complete conversion [less than 1% of **9** remaining].

To ensure a safe scale-up in the pilot plant, an experiment was performed to quantify heat and gas evolution rates. The apparatus comprised a Mettler-Toledo RC1 reaction calorimeter with a wet test meter (Ritter Model TG05) placed at the reactor exit to monitor offgas volume. The offgas composition was also determined on-line using a GC equipped with a thermal conductivity detector and a sampling loop placed upstream of the wet test meter (Figure 3). A flow of nitrogen gas was maintained throughout the experiment. Formation of offgas containing CO_2 and NH_3 was expected based on the in situ decomposition of ammonium formate:[32]

$$NH_4^+HCOO^- \rightarrow NH_3 + CO_2 + H_2$$

The debenzylation was performed by first adding 8.9 g of catalyst (10% Pd/C, 50% wet), 444 g of **9** dissolved in EtOH (containing 69 g of **9**), and 11.6 g of ammonium formate to the reactor at room temperature. The mixture was subsequently heated under agitation to 65 °C over 40 min, heated to 72 °C over 16 min, held at that temperature for 5 min, and finally cooled to below room temperature (ca. 15 °C) over 1 h. The feasibility of these heating and cooling times in the pilot plant was confirmed with the project engineer. The reaction was slightly exothermic initially (Figure 4) but became endothermic as gas was evolved. The maximum gas evolution rate (69 g of **9** basis] of 100 cm^3/min and the maximum heat evolution rate (ca. 10 W/kg) were both acceptable for safe scale-up.

GC analysis indicated that the offgas contained carbon dioxide but no ammonia. However, the presence of some ammonia in the offgas was not ruled out based on the pH paper color change (indicating the presence of a base) when the paper was exposed to the offgas. The capability of the GC method to detect ammonia was checked in a separate experiment in which aqueous NH_4OH was placed in the calorimeter and heated to 45 °C to desorb NH_3 gas; NH_3 was easily detected by GC (Figure 5). We therefore infer that the volume of ammonia evolved in the debenzylation of **9** was very small and that most of the NH_3 produced by ammonium formate decomposition remained in the reaction mixture.

As white solids were found on the reactor fittings after the reaction, it is hypothesized that at least some NH_3 reacted to form ammonium carbonate,

$$2NH_3 + CO_2 + H_2O \rightarrow (NH_4)_2CO_3$$

with water originating from the 50 wt % wet Pd/C catalyst. Ammonium bicarbonate might also form. The high solubility of ammonia in the ethanol solvent and the possibility of salt formation by its reaction with **10** are also consistent with the observed low volume of evolved ammonia gas.

Salt Prescreening and Manufacture. As was mentioned previously, a solid derivative of **10** was needed to facilitate the isolation, to provide needed stability, and to assist with the formulation studies. Specifically, we sought to take advantage of the moderate acidity ($pK_a = 8$) of the proton on the hydroxyl group attached to the *N*-formylated nitrogen. Since we were unsuccessful using organic bases for salt formation, we reasoned that we might have a better chance of success with a metal salt. Initially, formation of the sodium salt was investigated; however, this compound was found to be an amorphous solid. Our salt screening indicated that magnesium and calcium could form salts. This was later extended to zinc. A search of the literature provided little information with regard to the formation of salts of hydroxylamines using any of these metals.[33]

It was found possible to prepare the three salts by treatment of **10** with NaOH in water, followed by the addition of $CaCl_2$, $MgCl_2$, or $ZnSO_4$. The purity of the salts was slightly better than that of the starting material, and two of the salts (Mg and Zn) were crystalline after drying. Crystallization of the Ca and Zn salts was problematic because both gave amorphous precipitates before crystallization. During the production of the Ca salt, gel formation occurred, resulting in poor processability (i.e., a viscous mixture that filtered slowly). The Mg salt was the only one of the three that initially precipitated as a crystalline material. Starting from **10**, about 6 g of each salt were prepared for the salt program and preformulation studies.

A basic procedure for the preparation of these salts was developed: an aqueous solution of **10** in water was filtered, and 1 equiv of NaOH was added. Then, 0.5 equiv of an inorganic salt ($ZnSO_4$, $MgCl_2$, $CaCl_2$) was introduced. After stirring for the designated time period, the salt was isolated by filtration, washed, and dried.

An improvement which was made involved adding the 0.5 equiv of NaOH before the first filtration. This served to increase the solubility of **10** in water, and thus the total amount of water could be reduced resulting in an increased yield for the Zn and Mg salts.

The yields were 77%, 70%, and 36% for the zinc, magnesium, and calcium salts, respectively. The amount of the deoxy impurity (now present as the salt) was nearly the same as that in **10** for the Zn and Mg salts (2.0 to 2.3%), or even higher for the calcium salt (from 2.3 to 3.5%).

(32) Rajagopal, S.; Spatola, A. F. *J. Org. Chem.* **1995**, *60*, 1347.

(33) Metal complexes of simple hydroxylamines are known; see for example: Sarukhabnov, M. A.; Val'dman, S. S.; Parpiev, N. A. *Zhurnal Neorganicheskoi Khimii* **1973**, *18*, 838; *Chem. Abs.* **1973**, 131508.

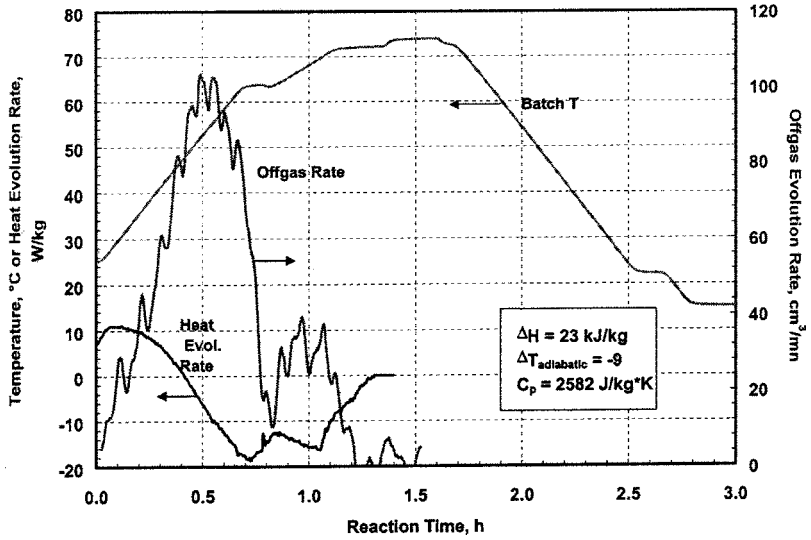


Figure 4. **Heat and offgas evolution rates.**

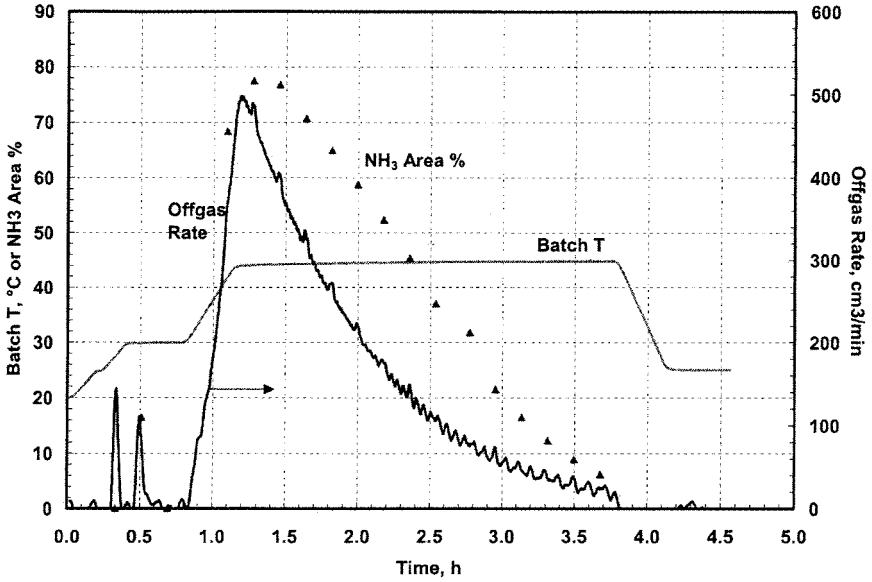


Figure 5. **Offgas rate and composition in which NH_4OH was charged to the RC-1 calorimeter to test the GC method.**

A considerable effort was made to remove this impurity from **11**. In a solubility test, the magnesium salt **11** (made from material which contained 2.2% of **23**) was found to dissolve readily in ethanol (1 g/5 mL) below 40 °C and partially to dissolve in ethanol/water, THF/water, and aqueous $MgCl_2$. However, no significant reduction in the deoxy impurity content was observed after digestion of the Mg salt in any of these solvents at elevated temperatures (40−45 °C) for 10 h. In addition, recrystallization from ethanol/water, ethyl acetate, acetone/water, methanol/water, THF/water, 2-propanol, or acetonitrile proved to be ineffective since the magnesium salt of the deoxy impurity was less soluble than the magnesium salt **11**.

However, when the recrystallization method was applied to the magnesium salt **11** which contained a smaller amount of the deoxymagnesium salt impurity (0.5−0.8%), the level of the deoxy compound present was reduced to 0.3−0.5%. Other impurities, although present at higher levels, could also be removed. Thus, a solution of **10** in acetone (or ethanol or methanol) was treated with aqueous $MgCl_2$ and aqueous NaOH at 40−45 °C followed by the slow addition of water and digestion at this temperature before cooling. The magnesium salt **11** was obtained in 65−70% yield and ≥98.5% purity. Thus we felt that salt formation was a viable method of effecting purification as long as the level of the deoxy impurity **21** was kept below 2% in **10**.

Due to concerns regarding residual acetone in the drug substance, ethanol was chosen as the solvent for the precipitation of the salt. Thus, **10** in ethanol was first concentrated under a vacuum to 1 g/3.3 mL of EtOH. To this solution was added an aqueous $MgCl_2$ solution (0.5 equiv) followed by an aqueous NaOH (∼1 equiv) to pH 9.1. At this stage, the concentration was 1 g of **10** in 3.3 mL of EtOH and 3.3 mL of H_2O, and precipitation of the salt began. After digestion at 45 °C for 1 h, another 3.3 mL of water/g was added over ∼1 h, and the digestion was allowed to proceed for another hour before cooling the suspension to 20 °C and isolation of the solids.

Additionally, the question of the possible decomposition of the Mg salt at 45 °C was addressed. This was based on

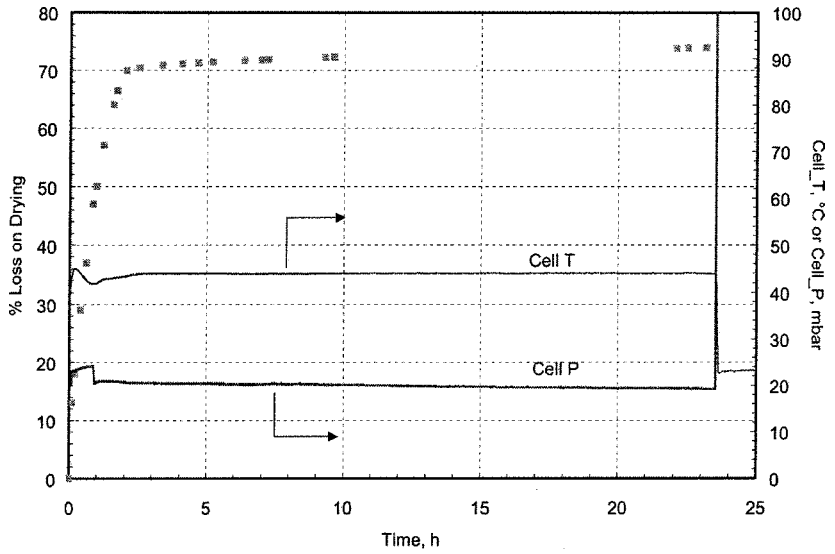


Figure 6. **Percent loss on drying vs time.**

some preliminary data which indicated that ~93% decomposition of a 0.1% aqueous solution of **10** occurred at 50 °C in pH 9 solution after 7 days. Based on our experiences with the Mg salt, we felt that this compound was much more stable than the free acid under the conditions we were using. This is because we had been able to recrystallize the material to high purity using the ethanol/water solvent mixture under conditions similar to the isolation conditions.

To support this assumption, an additional experiment was carried out prior to the Pilot Plant campaign. A solution of **11** (1 wt % in 1:2 ethanol/water, pH 9.6, almost saturated at 45 °C) was heated at 45 °C for 20 h. HPLC analysis indicated 91% purity (i.e., <10% decomposition). Since we have calculated that only about 10% of the salt was actually in solution, this would translate as 1% decomposition of the total amount of Mg salt. It is thus reasonable to believe the decomposition loss during salt formation is merely 1−2%.

We also observed that when the impurity level in **11** was higher, the loss of material in the mother liquor was also higher. The dissolution loss in the mother liquor ranged from 6 to 12 mg/mL for crude **11**.

Since the crystalline form of the magnesium salt was determined to be a tetrahydrate, and since it was known that the anhydrous material was not crystalline, there was some concern regarding overdrying of the product in the pilot plant. Therefore, a drying study was conducted using vacuum thermogravimetry. When the Mg salt was dried by vacuum thermogravimetry at 40−45 °C and 20 mbar, a sharp increase in loss on drying occurred, approaching a nearly constant (plateau) value. However, with longer times, the sample continued to lose mass at a much slower rate (see Figure 6). Thus, overdrying of the product in the pilot plant could occur if a longer drying time is used. To avoid significant overdrying, recommendations were made to closely monitor the loss on drying at periodic intervals. In addition, the residual ethanol values after drying times of 4 and 24 h indicated that small amounts of ethanol were still present, meaning that a small portion of ethanol remained bound in the solid (thermodynamic activity < 1).

In summary, we have developed a practical enantioselective route to LBM415. The conjugate addition of *O*-benzylhydroxylamine to oxazolidinone **3** furnished tosylate **5** (after salt formation) with good selectivity. Thus, chemical resolution was not required. Removal of the chiral auxiliary followed by *N*-formylation gave optically pure **7** in good overall yield. Coupling of acid **7** with proline amide **25** was carried out using the readily available peptide coupling agent EDCI/HOBt and provided key intermediate **8** in good yield and high optical and chemical purity. Oxidation to **9** using relatively stable and nonhazardous urea/hydrogen peroxide was followed by debenzylation and salt formation without the need for isolation of either of the previous intermediates and gave LBM415 in 16% overall yield (ee > 99%) in 12 steps.

Experimental Section

2-Methylenehexanoic Acid (2). A suspension of *n*-butylmalonic acid **1** (110 kg, 680 mol) in absolute ethanol (633 L) was treated at room temperature with 37% aqueous formaldehyde (258 kg, 3180 mol). The suspension was stirred at 20 °C until all of the solids had dissolved (15 min). Piperidine (70 kg, 810 mol) was added at such a rate that the internal temperature was kept below 45 °C. The time of the addition was 20 min. The reaction mixture was stirred at 45 °C for 1 h, heated to 72 °C, and held at this temperature for 30 min. The batch was then heated to the reflux temperature and held for 2 h. At this point, the reaction mixture was concentrated under vacuum at 60 °C to a predetermined volume (396 L). After the reaction mixture cooled to 20 °C, 2 N HCl (880 kg, 1760 mol) and ethyl acetate (440 L) were charged to the batch while the temperature was maintained below 27 °C. The aqueous layer was separated and back extracted with ethyl acetate (220 L). The organic portions were combined and washed with 2 N HCl (220 kg, 440 mol), saturated sodium chloride (220 kg), and water (220 kg). Most of the ethyl acetate was removed by distillation at atmospheric pressure (final volume 385 L), and after the solution cooled to ambient temperature,

heptane (376 kg) was added. Distillation at atmospheric pressure to a predetermined volume (495 L) was followed by filtration at 55 °C to afford **2** as a solution in heptane (72.1 kg, 82%): ^{1}H NMR ($CDCl_3$) δ 6.30 (s, 1H), 5.66 (s, 1H), 2.32 (t, J = 7.0 Hz, 2H), 1.44 (m, 4H), 0.94 (t, J = 7.0 Hz, 4H). Note: Due to concerns regarding the stability of this compound, it was not purified further but was used "as is" in the following step.

(4*S*)-3-(2-Methylene-1-oxohexyl)-4-(phenylmethyl)-2-oxazolidinone (3). A solution of 2-methylenehexanoic acid (21.6 kg, 170 mol) in THF (233 L) was cooled to −20 °C. To this solution was added *N*,*N*-diisopropylethylamine (27.8 kg, 215 mol) followed by a THF rinse (22 L). After stirring at −20 °C for 30 min, pivaloyl chloride (19.7 kg, 163 mol) was added followed by a THF rinse (22 L). The mixture was stirred for 30 min at −20 °C, allowed to warm to 10−15 °C for 30 min, and then cooled back down to −20 °C. In a separate vessel, a solution consisting of (*S*)-benzyl-2-oxazolidinone (26.4 kg, 149 mol) in THF (136 L) was prepared and cooled to −20 °C. A 2 M solution of lithium diisopropylamide in heptane (68 kg, 166 mol) was added at such a rate that the temperature was maintained below −20 °C. Upon completion of the addition, the solution of the anion was added to the solution of the mixed anhydride while the temperature was maintained below −20 °C. The reaction mixture was held for 30 min at −20 °C, allowed to warm slowly to 15 °C, and held at this temperature for 30 min. At this point, a suspension consisting of 1 M potassium bicarbonate solution (150 kg, 690 mol) and Celite (5.5 kg) was introduced, and the mixture was stirred for 20 min. The suspension was filtered, and the filter cake was washed with ethyl acetate (49 kg, 54 L). The mixture was filtered, and the aqueous portion separated. The organic portion was washed twice with 1 M potassium bicarbonate (150 kg) and concentrated under reduced pressure to a final volume (50 L). To the concentrate was added ethyl acetate (136 L). The ethyl acetate solution was washed twice with water (55 L) and concentrated to a final volume (50 L) under reduced pressure. The concentrate was diluted with ethyl acetate (95 L), and the distillation was repeated until no more distillate was observed. The concentrate was warmed to 50 °C, and heptane (136 L) was added to form a solution. The mixture was concentrated under reduced pressure to a final volume (65 L). The concentrate was dissolved in *tert*-butyl methyl ether (40.8 L) and warmed to 45 °C. The solution was diluted with heptane (368 L), cooled to 0 °C, and held at this temperature for 2 h. The solids were filtered, washed with a cold (0 °C) 95/5 (w/w) heptane/ethanol solution, and dried in vacuo at 30 °C overnight to give **3** [29.4 kg, 63% (corrected)] as a white crystalline solid: mp 46 °C; ^{1}H NMR ($CDCl_3$) δ 7.29 (m, 5H), 5.40 (d, J = 7 Hz, 2H), 4.44 (m, 1H), 4.22 (m, 2H), 3.37 (m, 1H), 2.82 (m, 1H), 2.39 (m, 1H), 1.43 (m, 4H), 0.93 (m, 3H).

(4*S*)-3-[(2*R*)-1-Oxo-2-[[(phenylmethoxy)amino]methyl]-hexyl]-4-(phenylmethyl)-2-oxazolidinone, *p*-Toluenesulfonic Acid Salt (5). *O*-Benzylhydroxylamine hydrochloride (43.7 kg, 274 mol) was dissolved in 2 N potassium hydroxide (300 L) with stirring. The solution was extracted twice with *tert*-butyl methyl ether (191 and 47 L). The combined organic portions were washed with water (47 L), and the *tert*-butyl methyl ether was removed by distillation under reduced pressure to afford *O*-benzylhydroxylamine free base as a clear oil.[34] The oil was added to **3** (33.3 kg, 116 mol), and the mixture was heated to 50 °C for 22 h. At this point, the reaction mixture was cooled to room temperature and diluted with ethyl acetate (139 L). This mixture of the free base **4** was added to a solution of *p*-toluenesulfonic acid monohydrate (88 kg, 463 mol) in ethyl acetate (325 L) followed by an ethyl acetate rinse (118 L). The suspension was stirred for 1.5 h and filtered to remove the *O*-benzylhydroxylamine *p*-toluenesulfonate salt. The salt was washed with ethyl acetate (115 L), and the wash was combined with the filtrate. The filtrate was concentrated under reduced pressure to a final volume (170 L) and cooled to 45 °C at which point *tert*-butyl methyl ether (764 L) was added. The mixture was cooled to 22 °C and stirred for 2 h. The resulting solids were filtered, washed with water-saturated *tert*-butyl methyl ether (231 L), and dried in vacuo at 50 °C for 12 h to give **5** [45.9 kg, 68% (corrected)]: mp 132 °C; ^{1}H NMR (DMSO-d_6) δ 10.7 (s br, 1H), 7.52 (d, J = 6 Hz, 2H), 7.38 (m, 6H), 7.26 (m, 3H), 7.13 (m, 4H), 4.98 (s, 2H), 4.56 (m, 1H), 4.29 (m, 1H), 4.19 (m, 1H), 4.02 (d, J = 8 Hz, 1H), 3.70 (m, 1H), 3.41 (d, J = 12 Hz, 1H), 2.84 (d, J = 12 Hz, 1H), 2.50 (s, 3H), 2.24 (m, 1H), 1.63 (m, 1H), 1.48 (m, 1H), 1.26 (m, 4H), 0.86 (m, 3H).

(2*R*)-2-[[(Phenylmethoxy)amino]methyl]hexanoic Acid (6). A suspension of **5** (27.5 kg, 0.47 mol) in ethyl acetate (95 L) and water (24 L) was treated with a 1 M aqueous solution of sodium carbonate (96 kg). After the mixture was stirred for 15 min, the aqueous layer was separated and discarded. The organic layer was washed with water (2 × 24 L) and concentrated under reduced pressure until the ethyl acetate content was less than 8 wt %. The concentrate was dissolved in THF (129 L) and water (41 L) and cooled to 0 °C. To this was added 30% hydrogen peroxide (12.3 kg, 108 mol), followed by a 4.5% lithium hydroxide solution (50.6 kg, 54 mol), while the temperature was maintained at 0 °C. The reaction mixture was stirred at this temperature for 45 min. The reaction was quenched by the addition of a cold (0 °C) aqueous solution of sodium sulfite (20.5 kg, 163 mol in 403 L of water). The reaction mixture was allowed to warm to 20 °C and stirred at this temperature for 30 min. The solution was concentrated under reduced pressure (maximum jacket temperature = 33 °C) to a final volume of 560 L. The concentrate was extracted with ethyl acetate (6 × 58 L) and then acidified with 3 N HCl (42.1 kg) to a final pH of 4−5. The product was extracted into ethyl acetate (2 × 113 L), and the extracts were combined and washed with water (52 L). Due to the very limited stability of **6**, the compound was held as a solution for use in the next step. The solution was assayed for content of **6** (7.9 kg, 66% yield). A small sample of the solution was removed and carefully concentrated for characterization: ^{1}H NMR ($CDCl_3$) δ 7.37 (s, 5H), 6.86 (s br, 2H), 4.75 (t, J = 12 Hz, 2H), 3.19

(34) *O*-Benzylhydroxylamine free base (oil) was found to be stable at 160 °C for 10 h (RADEX dynamic autoclave) and at 120 °C for 16 h (stainless steel pan) (RADEX isoperibolic autoclave).

(m, 2H), 2.77 (m, 1H), 1.70 (m, 1H), 1.55 (m, 1H), 1.35 (m, 4H), 0.90 (m, 3H).

(2*R*)-2-[Formyl(phenylmethoxy)amino]methylhexanoic Acid Dicyclohexylamine Salt (7). An ethyl acetate solution of **6** (16.2 kg, 64 mol, total weight of solution = 384 kg) was cooled to −10 °C and treated with a cold (5 °C) solution of acetic anhydride (13.2 kg, 129 mol) and 96% formic acid (23.7 kg, 514 mol), which was added over 20 min. The reaction mixture was stirred an additional 20 min at −10 °C. Water (5 L) was added, and the reaction mixture was allowed to slowly warm to 15 °C. The ethyl acetate was removed by distillation in vacuo while the internal temperature was maintained below 40 °C to a final volume (60 L). Toluene (210 L) was added, and the distillation was continued to reach a final volume (60 L). The toluene addition and distillation were carried out a second time. At this point, the mixture was cooled to room temperature, and a sample was taken to determine the acetic acid content of the batch (<2%). A solution of dicyclohexylamine (14 kg, 77 mol) in heptane (206 L) was added over 15 min. Seed crystals of **7** were added, and the solution was stirred for 2 h during which time the precipitation occurred. The suspension was diluted with heptane (149 L) and held for 8 h at room temperature with stirring. The solids were isolated by filtration, washed with a solution of dicyclohexylamine (2.9 kg, 16 mol) in heptane (86 L), and dried at a maximum temperature of 27 °C in vacuo for 8 h to give **7** [20.6 kg, 69% (corrected)]: mp 83−86 °C; ^{1}H NMR (DMSO-d_6) δ 8.04 (d, 1H), 7.40 (m, 5H), 4.91 (s, 2H), 3.50 (m, 2H), 2.77 (m, 2H), 2.51 (m, 2H), 1.49 (m, 27H), 0.84 (s, 3H).

(2*S*)-*N*-(5-Fluoro-2-pyridinyl)-2-pyrrolidinecarboxamide Dihydrobromide (25). A mixture of (2*S*)-1,2-pyrrolidinecarboxylic acid 1-(phenylmethyl)ester **23** (50.6 kg, 203 mol) and 2-amino-5-fluoropyridine (17.5 kg, 156 mol) in ethyl acetate (67 L) was treated with a solution of 2-chloro-4,6-dimethoxy-1,3,5-triazine (32.9 kg, 188 mol) in ethyl acetate (67 L) followed by a rinse with ethyl acetate (33 L). This was followed by the addition of *N*-methylmorpholine (23.7 kg, 243 mol) at such a rate that the temperature was maintained below 25 °C. The reaction mixture was stirred at 22 °C for 5 h. At this point, water (156 L) was added and the suspension was filtered. The filter cake was washed with ethyl acetate (46 L), and the wash was combined with the filtrate. The aqueous layer was separated and extracted with ethyl acetate (2 × 46 L). The ethyl acetate layers were combined and washed with 1 N sodium hydroxide (46.8 kg) and saturated sodium chloride solution (2 × 62 kg). The solution was concentrated at atmospheric pressure (95 °C maximum jacket temperature) to a final volume (100 L). At this point, the water content by Karl Fischer titration was <0.5%. The solution of crude (2*S*)-2-[[(5-fluoro-2-pyridinyl)-amino]carbonyl]phenylmethyl ester **24** was diluted with ethyl acetate (37 L) and cooled to 5 °C. Hydrogen bromide solution (30 wt % in acetic acid, 166.1 kg, 616 mol) was added while the temperature was maintained below 10 °C. After stirring for 1 h, the batch was warmed to 23 °C and stirred for an additional 2 h. The solids were collected by centrifugation, washed with ethyl acetate (69 L), and dried at 45 °C in vacuo for 12 h to give **25** [48.7 kg, 84% (corrected for the purity of the limiting reagent)]: mp 180 °C (dec); ^{1}H NMR (DMSO-d_6) δ 11.2 (s, 2H), 9.35 (broad s, 1H), 8.73 (broad s, 1H), 8.40 (s, 1H), 8.09 (m, 1H), 7.84 (m, 1H), 4.43 (m, 1H), 3.28 (s, 1H), 2.42 (m, 1H), 1.96 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 167.33, 157.52, 154.21, 149.83, 147.40, 114.99, 59.36, 45.72, 29.60, 23.46; MS (ES$^+$) 210 (MH^+, free base).

(2*S*)-*N*-(5-Fluoro-2-pyridinyl)-1-[(2*R*)-2-[[formyl(phenylmethoxy)amino]methyl]-1-oxohexyl-2-pyrrolidinecarboxamide (8). A solution of **7** (41.7 kg, 90.1 mol) in ethyl acetate (360 L) was treated at room temperature with a 10% solution of citric acid (360 kg), and the mixture was stirred until all of the solids had dissolved. The aqueous layer was separated, and the organic layer was washed with water (2 × 270 L). Compound **25** (40.1 kg, 108.1 mol) was added, and the mixture was cooled to 5 °C. Water (72 L) was introduced followed by 1-hydroxybenzotriazole hydrate (HOBt) (15.4 kg, 99.1 mol) and 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (98%) (48.4 kg, 252.2 mol). Another water (72 L) addition was followed by the slow introduction of *N*-methylmorpholine (57.5 kg, 567.5 mol) while the temperature was maintained below 20 °C. The reaction mixture was stirred at 21 °C for 10 h or until the amount of **7** remaining was less than or equal to 2% by HPLC area normalization. The aqueous layer was separated and discarded. The organic layer was washed with water (4 × 270 L) to remove the last traces of HOBt. The ethyl acetate solution containing **8** was split into two equal portions (85.7 kg each), and each portion was filtered through a bed of silica gel (50 kg) using ethyl acetate as the eluent. Fractions (approximately 20 kg each) were collected and analyzed by HPLC. The appropriate fractions were combined, and this solution was concentrated by distillation under vacuum (maximum jacket temperature = 45 °C) to a final volume (260 L). The batch was heated to 55 °C, and heptane (815 L) was added. The solution was cooled to 45 °C, seeded, cooled further to 35 °C, held at this temperature for 2 h, and then cooled to 20 °C, and held at this point for a minimum of 8 h. Finally, the suspension was cooled to −10 °C and stirred for another 2 h. The product was collected by centrifugation and washed with a mixture of heptane (94 L), ethyl acetate (48 L), ethanol (2.7 L), and water (0.14 L). The filter cake was dried at 42 °C in vacuo for 8 h, and the crude product was recrystallized from ethyl acetate (173 L)/heptane (695 L) to afford **8** [28.9 kg, 68% (corrected)] as an off-white crystalline solid: mp 98 °C; ^{1}H NMR (DMSO-d_6) δ 10.89, 10.63 (s, 1H), 8.29 (s, 1H), 8.01 (m, 2H), 7.70 (m, 1H), 7.35 (s, 5H), 4.81 (s, 2H), 4.54 (broad s, 1H), 3.52 (m, 4H), 2.92 (m, 1H), 1.85 (m, 4H), 1.20 (m, 6H), 0.81 (s, 3H); Assay by HPLC 98.9%; Purity by DSC 98.2%.

(2*S*)-*N*-(5-Fluoro-1-oxido-2-pyridinyl)-1-[(2*R*)-2-[[formyl-(phenylmethoxy)-amino]methyl]-1-oxohexyl]-2-pyrrolidinecarboxamide (9). A solution of **8** (12.8 kg, 27 mol) and urea-hydrogen peroxide (7.7 kg, 81.9 mol) in ethyl acetate (117 L) was cooled to 10 °C, and solid phthalic anhydride (12.0 kg, 81 mol) was added in portions. The reaction mixture was stirred at 20 °C for 6 h at which point there

was less than 0.4% **8** remaining by HPLC analysis (area normalization). After the reaction mixture was cooled to 5 °C, an 8.6% sodium sulfite solution (159.7 kg) was introduced while the temperature was maintained below 25 °C. At the completion of the addition, the batch was held at 22 °C for 55 min. The top layer was tested for the absence of peroxides, and the aqueous layer was separated and discarded. The organic layer was washed with 0.9 M sodium carbonate (160.4 kg) and a 12.7% sodium chloride solution (109.5 kg). The organic portion was concentrated in vacuo (maximum batch temperature = 50 °C) to a final volume (60 L) (water content by Karl Fischer titration ≤1.0%) which was assayed by HPLC for the content of **9** [12.8 kg, 96% (corrected)]. A sample was removed and concentrated to dryness for characterization purposes: ^{1}H NMR ($CDCl_3$) δ 10.39 (s, 1H), 8.42 (m, 1H), 8.17 (m, 2H), 7.40 (s, 5H), 7.08 (m, 1H), 4.83 (m, 2H), 4.41 (broad d, 1H), 3.81 (m, 2H), 3.58 (m, 2H), 3.02 (m, 1H), 1.68 (m, 10H), 0.85 (s, 3H); MS (ES^+) 487 (MH^+).

(2*S*)-*N*-(5-Fluoro-1-oxido-2-pyridinyl)-1-[(2*R*)-2-[(formylhydroxyamino)methyl]-1-oxohexyl]-2-pyrrolidinecarboxamide Magnesium Salt (2:1) (11). The ethyl acetate solution of **9** from the previous step (53.1 kg, 26 mol) was concentrated under reduced pressure (maximum batch temperature = 50 °C) to a final volume (24 L). The batch was cooled to room temperature, and ethanol (71 L) was added. The distillation was resumed under the same conditions to a final volume (24 L). Ethanol (72 L) was added, and the distillation was carried out once more to the same final volume (ethyl acetate content ≤ 4%). The ethanol solution of **9** was added to 10% Pd/C (1.3 kg, 50% water wet), and to this suspension was added ammonium formate (2.1 kg, 32.3 mol). The batch was heated to an internal temperature of 65 °C over 40 min. The temperature was then raised to 72 °C over 14 min and held at this temperature an additional 6 min. The mixture was cooled to 10 °C rapidly (approximately 1 °C/min cooling rate), and a sample was taken to check for completeness (≤6% of **9** remaining). The reaction was filtered through Celite (5 kg), and the filter cake was washed with ethanol (38 L) which was combined with the batch. The solution containing **10** was concentrated under reduced pressure (maximum jacket temperature = 50 °C) to a final volume (41 L). After cooling to 25 °C, water (14.7 L) was added followed by a solution of magnesium chloride (13.5 kg) which was prepared from magnesium chloride hexahydrate (5.5 kg, 27 mol) and water (23 L). The reaction mixture was heated to 43 °C (internal temperature), and 3 N sodium hydroxide solution (10.1 kg) was added over 15 min to afford a final pH of 8.7. After stirring for 70 min at 43 °C, water (33.1 L) was added over 1 h and the suspension was stirred for an additional 1 h at 43 °C. The batch was cooled to 20 °C and stirred for 1 h. The solids were collected by centrifugation, washed with a cold (5 °C) solution of water (13 L) and ethanol (6.5 L), and dried in vacuo at 42 °C for 4.5 h to afford **11** [9.1 kg, 79% (corrected)] as an off-white crystalline solid. A second batch was prepared, and the combined products (17.8 kg) were recrystallized from ethanol/water using the following procedure. The salt was dissolved in absolute ethanol (86 L) and heated to 40 °C to effect dissolution. Upon cooling to room temperature, the solution was filtered and the filter and lines were rinsed with ethanol (19 L), after which the rinse was combined with the batch. The solution was concentrated under reduced pressure (maximum jacket temperature = 50 °C) to a final volume (65 L). The concentrate was warmed to 44 °C, and water (51.5 L) was added. Stirring was continued at this temperature for 1 h after precipitation started. Water (51.5 L) was added, and stirring was continued for an additional 55 min. The suspension was cooled to 3 °C and stirred for a total of 3 h. The solids were collected by centrifugation, washed with cold (3 °C) ethanol/water (7L/13.8 L), and dried in vacuo for 7.5 h to afford LBM415 (**11**) [16.0 kg, 70% overall (corrected)]: ^{1}H NMR (CD_3OD) δ 8.55 (s, 1H), 8.50 (t, 1H), 7.55 (s, 1H), 7.45 (t, 1H), 4.76 (broad s, 1H), 4.03 (broad s, 1H), 3.70 (broad s, 2H), 3.44 (broad s, 1H), 3.41 (broad s, 1H), 2.30 (broad s, 1H), 2.07 (broad s, 3H), 1.60 (broad s, 1H), 1.46 (broad s, 2H), 1.35 (broad s, 3H), 0.91 (t, 3H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 175.66, 172.49, 156.38, 155.74, 143.03, 128.86, 118.41, 116.45, 62.59, 54.70, 49.28, 42.45, 31.50, 30.43, 30.18, 25.87, 24.00, 14.35. Anal. Calcd for $C_{18}H_{24}FN_4O_5 \cdot 1/2Mg \cdot 2H_2O$: C, 48.72; H, 6.36; N, 12.62. Found: C, 48.64; H, 6.23; N, 12.53.

Acknowledgment

We thank the Analytical Research and Development group for their support and assistance throughout this work. We extend our appreciation to Dr. Hanumantha Marepalli for his help in analyzing the NMR spectra of compound **11** and Robert Duval for the safety tests. We also thank the reviewers for many valuable suggestions.

Note Added after ASAP Publication: This article was published on the web December 16, 2005 with a minor error in Table 1. The version posted December 21, 2005 and the print version are correct.

Received for review September 8, 2005.

OP050165Y