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Pilot Plant Preparation of an $\alpha_v\beta_3$ Integrin Antagonist. Part 1. Process Research and Development of a (*S*)-β-Amino Acid Ester Intermediate: Synthesis via a Scalable, Diastereoselective Imino-Reformatsky Reaction

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Abstract:

Described are four process research investigations directed toward discerning a scalable, enantioselective method for preparing (*S*)-β-amino acid ester 3, a key intermediate to the $\alpha_v\beta_3$ integrin antagonist 1. Reported are an asymmetric Michael reaction approach, attempts to enantioselectively hydrogenate an enamine, resolution of (±)-3 via diastereomeric salt formation, and a synthetic route employing a novel, diastereoselective imino-Reformatsky reaction. This last research initiative proved successful and was employed as the enabling route to initial API supply. Process development of this enabling chemistry is reported. The technical issues researched and optimized were (1) the necessity of employing MEM-protection for high yield and diastereoselectivity in the imino-Reformatsky reaction, (2) the reaction kinetics of MEM chloride hydrolysis and the application of these data to an on-scale quench procedure, (3) the efficient formation of the (*S*)-phenylglycinol imine 15 in NMP and a dehydration of this product solution on-scale, employing molecular sieves, (4) a calorimetric study of the Reformatsky reaction and the application of these data, (5) the replacement of $Pb(OAc)_4$ with $NaIO_4$ and the use of methylamine to sequester competing oxazolidine formation, and (6) further development of the isolation and purification protocol for the ethyl ester, *p*-TsOH salt of (*S*)-3. The results and challenges associated with two campaigns in which the potential commercial process was practiced are discussed.

Introduction

The $\alpha_v\beta_3$ integrin plays an essential role in angiogenesis, the process by which new blood vessels form from preexisting blood vessels.[1] Angiogenesis is required for tumor growth, and therefore, antagonists of $\alpha_v\beta_3$ are being studied for the treatment of cancer. In a program directed toward the discovery of such antagonists, an improved synthesis of **1** was required.[2] As part one of a three part report describing the successful pilot-plant preparation of **1**, herein we wish to detail the chemical process research and development of a scalable and enantioselective method of preparing (*S*)-β-amino ester **3**, a key intermediate in the convergent synthesis of **1** (Scheme 1).

A process research and development team was formed to evaluate strategies by which **1** could be prepared on-scale. Envisioned was a convergent synthesis in which tetrahydropyrimidine **2** would be linked via glycine to the β-amino acid ester **3**. With regard to the preparation of **3**, four research programs[3] were completed: (1) an asymmetric Michael addition reaction to an appropriately substituted cinnamic acid ester, (2) the enantioselective hydrogenation of a corresponding enamine, (3) the resolution of (±)-**3** via diastereomeric salt formation and (4) evaluation of a synthetic route developed in-house employing a novel, diastereoselective imino-Reformatsky reaction as the key reaction.[4]

Process Research

Asymmetric Michael Addition Route to 3. Studies directed toward the synthesis of **3** began by exploring the feasibility of employing an asymmetric Michael reaction

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(1) (a) Eliceri, B. P.; Cheresh, D. A. *Cancer* **2000**, *6S3*, 245. (b) Roberto, R.; Angelo, V.; Domenico, R.; Francesco, D. R.; Francesca, M.; Franco, D. *Haematologica* **2002**, **87**, 836. (c) Ruminski, P. G.; Rogers, T. E.; Rico, J. E.; Nickols, G. A.; Westlin, W. F.; Engleman, V. W.; Settle, S. L.; Keene, J. L.; Freeman, S. K.; Carron, C. P.; Meyer, D. M. "Book of Abstracts", 218th National Meeting of the American Chemical Society, New Orleans, 1999; American Chemical Society: Washington, D. C., 1999. (d) Ruminski, P. G.; Rogers, T. E.; Rico, J. E.; Nickols, G. A.; Engleman, V. W.; Settle, S. L.; Keene, J. L.; Freeman, S. K.; Carron, C. P.; Meyer, D. M. "Book of Abstracts", 217th National Meeting of the American Chemical Society, Anaheim, Calif., 1999; American Chemical Society: Washington, D. C., 1999. (e) Flynn, D. L.; Abood, N.; Bovy, P.; Garland, R.; Hockerman, S.; Lindmark, R. J.; Schretzman, L.; Rico, J.; Rogers, T. *Book of Abstracts*, 210th National Meeting of the American Chemical Society, Chicago, IL, 1995; American Chemical Society: Washington, D. C., 1995.

(2) Collins, J. T.; Devadas, B.; Lu, H.-F.; Malecha, J. W.; Miyashiro, J. M.; Nagarajan, S.; Rico, J. G.; Rogers, T. E. U.S. Patent 6100423, 2000.

(3) The results of a bio-resolution route to (*S*)-**3** will be the subject of a separate report.

(4) (a) Colson, P.-J., Awashti, A. K.; Nagarajan, S. R PCT Int. Appl. 1999, WO 9944985. (b) Colson, P-. J.; Boys, M. L.; Farid, P. N.; Awasthi, A. K.; Ainsworth, O., unpublished results. (c) Colson, P-. J.; Boys, M. L.; Cain-Janicki, K. J.; Doubleday: W. W.; Farid, P. N.; Duran, J. E. unpublished results.

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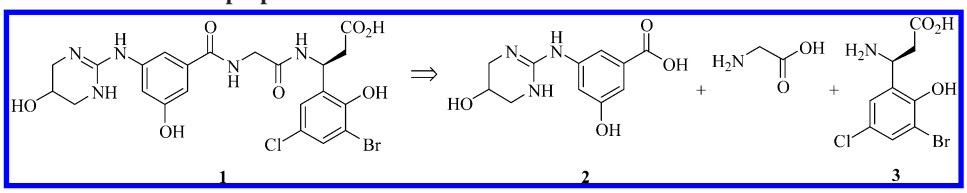
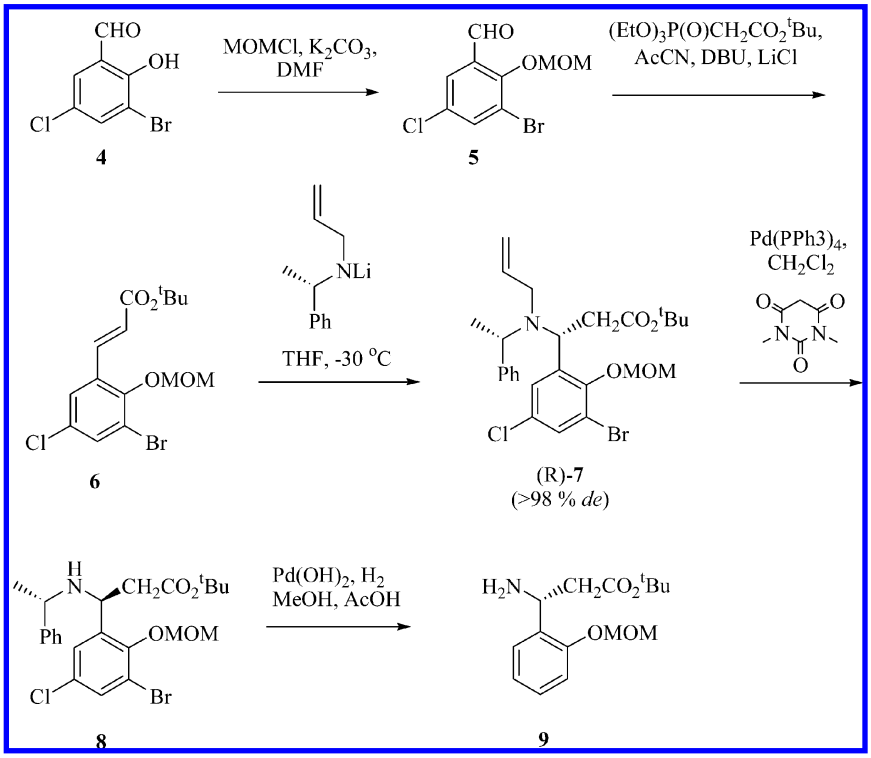
Scheme 1. **Key intermediates toward preparation of 1**



Scheme 2. **Asymmetric Michael reaction approach to 3**



(Scheme 2). Envisaged was the preparation of **7**, a precursor to **3**, via the 1,4-conjugate addition of a homochiral lithium α-methylbenzyl alkyl amide to cinnamic acid ester **6**.[5] Indeed, after extensive work, **7** was prepared with high diastereoselectivity. Starting from commercially available salicylaldehyde **4**, the phenol was protected as the MOM ether **5** in 74% isolated yield. The *tert*-butyl cinnamate **6** was prepared by Wadsworth−Emmons[6] chemistry to afford exclusively the *trans*-olefin in 82% isolated yield. Michael addition at −30 °C employing lithium α-methylbenzyl allyl amide[7] gave diastereoselectively **7** in 75% yield and with >98% de. Noteworthy aspects of this chemistry were the use of a MOM protecting group and the *tert*-butyl ester. Other esters and protecting groups were explored, but only this combination diminished competing 1,2-nucleophilic addition and gave rise to high diastereoselectivity. Also noteworthy was completing the desired asymmetric Michael reaction at −30 °C, a temperature much higher than generally reported.[5]

Removal of the allyl group was accomplished successfully on small scale, employing $Pd(PPh_3)_4$ and *N*,*N*-dimethylbarbituric acid in dichloromethane.[8] This afforded **8** as an orange oil in 91% yield. However, removal of the α-methylbenzyl auxiliary also gave rise to dehalogenation of the aromatic ring. For example, hydrogenation using Pearlman's catalyst in MeOH and acetic acid under 1 atm hydrogen resulted in deprotection as well as dehalogenation to afford **9** in quantitative yield. Multiple conditions were surveyed in an attempt to produce the desired *β*-amino acid ester, but competing dehalogenation always interfered. Therefore, despite the high diastereoselectivity of this asymmetric Michael reaction, this research effort was terminated.

Asymmetric Enamine Reduction Approach to 3. Chiral reduction of an appropriately functionalized prochiral enamine[9] was also investigated as a logical method to access a single enantiomer of **3**. The chemistry employed to ascertain the feasibility of such an approach is outlined in Scheme 3. Preparation of the desired prochiral enamine began by brominating commercially available acetophenone **10** employing NBS in DMF. Exposure of this to excess sodium hydride and diethyl carbonate in refluxing toluene afforded **11**. Reaction of **11** with excess ammonium acetate in DMF

(5) See for example, (1) Davies, S. G.; Ichihara, O.; Lenior, I.; Walters, I. A.. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1411, (2) Davies, S. G.; Ichihara, O. *Tetrahedron Asymmetry* **1991**, 183.

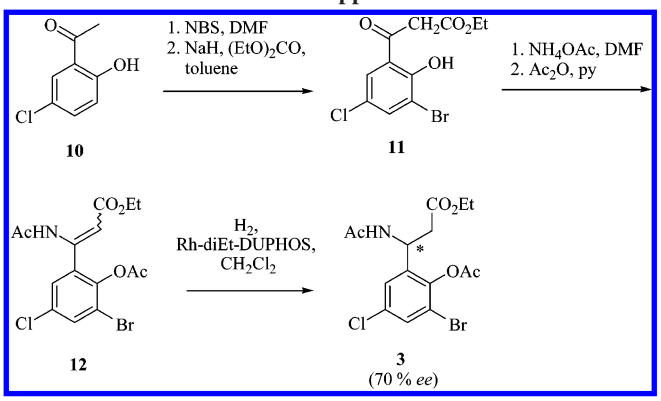
(6) For reviews see: (a) Izod, K. *Coordination Chemistry Reviews* **2002**, *227*(2), 173. (b) Bruckner, R. *Current Organic Chemistry* **2001**, *5* (6), 679. (c) Nicolaou, K. C.; Harter, M. W.; Gunzner, J. L.; Nadin, A. *Liebigs Annalen/Recueil* **1997**, 1283.

(7) (*S*)-α-methylbenzyl allylamine was available and therefore employed to expedite ascertaining the feasibility of this synthetic route.

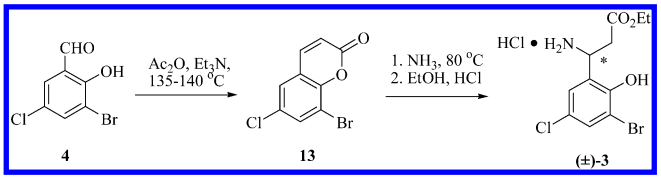
(8) Garro-Helion, F.; Merzouk, A.; Guibe, F. *J. Org. Chem.* **1993**, *58*, 6109.

(9) Abdel-Magid A. F.; Cohen J. H.; Maryanoff C. A. *Current Medicinal Chemistry* **1999**, *6*, 955.

Scheme 3. **Chiral reduction approach to 3**



Scheme 4. **Preparation of (±)-3**



at room temperature followed by acetylation with acetic anhydride and pyridine provided the requisite enamine **12** as a white solid in 70% overall yield from **10**.

Unfortunately asymmetric reduction of **12** was only moderately successful. Six commercially available catalysts and multiple conditions were investigated. The best result obtained was 70% ee using catalytic (+)-1,2-bis-((2S,5S)-2,5-diethylphospholano)benzene(cyclooctadiene) rhodium (I) trifluoromethanesulfonate in dichloromethane under 5 atm of H_2. Also investigated were other analogues of **12** wherein the ester and hydroxyl-protecting groups were either removed and/or changed, but no improvement was observed. Therefore, on the basis of the moderate success of this approach, this research effort was also discontinued.

Resolution via Diastereomeric Salt Formation. Resolution of (±)-**3** via diastereomeric salt formation was investigated. This would represent a viable method of obtaining a single enantiomer of **3** if preparation of the racemate could be implemented on-scale. This was accomplished by employing methodology developed in-house[11] as shown in Scheme 4. Starting with the aforementioned salicylaldehyde **4**, Perkin reaction[12] in refluxing acetic anhydride in the presence of triethylamine provided coumarin **13** in 70% yield. Exposure of **13** to ammonia at 80 °C in a pressure reactor for 24 h accomplished the desired Michael reaction, giving rise to the β-amino acid amide analogue of **3**. After evaporation of the excess ammonia, acidic ethanolysis afforded (±)-**3** as the HCl salt in approximately 60% yield.

Experiments to discern the best conditions for resolution of (±) **3** were accomplished by subjecting the free base to a screen of commercially available chiral acids in a number of solvents. The substrate and chiral acid (1 equiv) were added to the appropriate solvent (5 vols) and heated to reflux. If dissolution was incomplete, the solvent volume was increased in 5-volume portions up to a maximum of 50 volumes. If full dissolution occurred, the reaction was allowed to cool to room temperature and any solid formed analyzed by chiral HPLC. Ten chiral acids in 10 different solvents were tested. Resolution of (±)-**3** into (*S*)-**3** by diastereomeric salt formation was completed with (−)-(1*R*)-camphor-10-sulfonic acid in either ethanol or 2-propanol. The resolution efficiency was 91 and 81% ee, respectively. Resolution of (±) **3** into (*R*)-**3** was consummated by (*R*)-mandelic acid in either acetyl acetate or acetonitrile. The resolution efficiency was 90 and 83% ee, respectively.

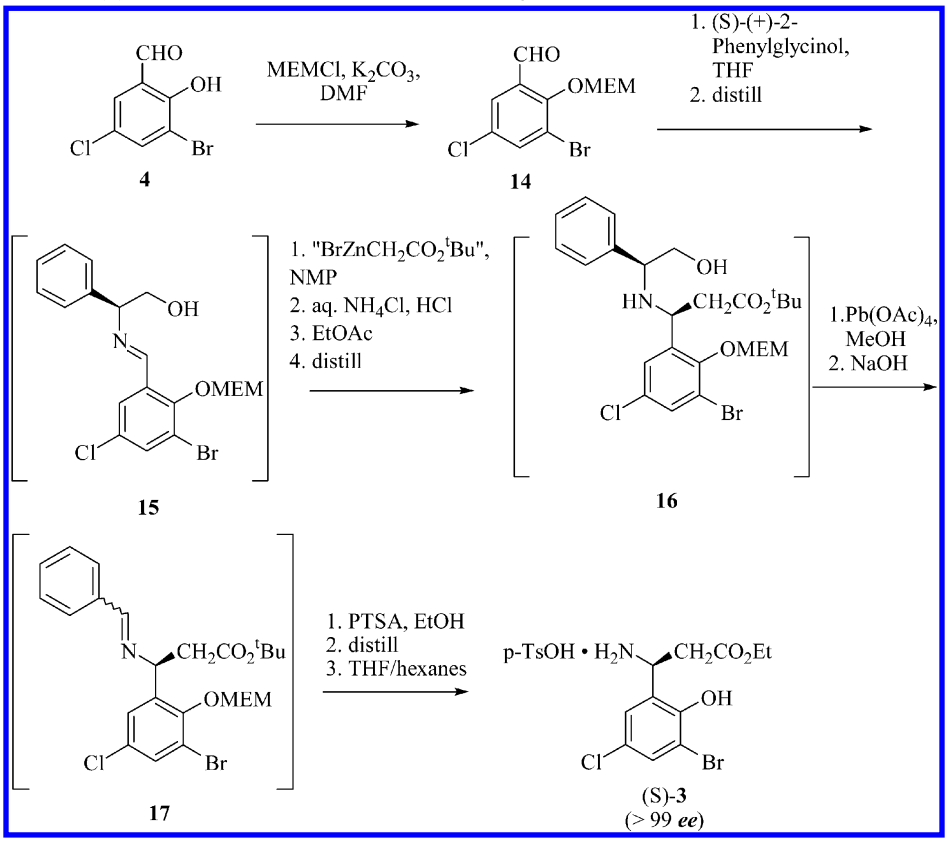
Scale-up of these experiments revealed two major drawbacks. First, the products' ee values on-scale were substantially lower than those acquired during the screening process. For example, the ee obtained for the CSA salt of (*S*)-**3** was 53%, compared to 91%. The ee could be increase to ≥98% with two additional crystallizations, but the overall yield was too low. This was due in part to the second major problem. The product salts were gelatinous solids, making it difficult to manipulate them. Therefore, this approach toward preparing enantiomerically enriched **3** was halted.

Diastereoselective Imino-Reformatsky. Prior to and concomitant with the aforementioned research efforts, work directed toward preparing an analogue of **3** via a novel, diastereoselective imino-Reformatsky reaction was proving to be successful. Drawing upon technology developed in-house,[4] the route shown in Scheme 5 successfully prepared the ethyl ester of **3** as the *p*-TsOH salt. The sequence entailed protecting salicylaldehyde **4** as the methoxyethoxymethyl (MEM) ether. This was accomplished with MEM chloride affording **14** in 92% isolated yield. Reaction of this with (*S*)-(+)-2-phenylglycinol in THF gave imine **15** as a nonisolated intermediate. After removal of the water and in vacuo concentration, dissolution of **15** in NMP and reaction at <−5 °C with an excess of the Reformatsky reagent prepared from *tert*-butyl bromoacetate in THF gave rise to the β-amino acid ester derivative **16**. This intermediate was isolated and characterized in our lab research, but the diastereoselectivity was not determined until **16** was converted to the desired (*S*)-β-amino acid ethyl ester analogue of **3**. In this regard, removal of the phenylglycinol chiral auxiliary was completed in a two-step procedure. Oxidative cleavage of the hydroxymethyl moiety with lead tetraacetate in methanol afforded imine **17** after neutralization with NaOH and concentration. Treatment of this crude oil with a slight excess of *p*-TsOH·H_2O in refluxing ethanol effected imine hydrolysis, deprotection, and transesterification. This provided **3** as the ethyl ester and *p*-TsOH salt in 65% overall from **4**. An ee > 99% was obtained as measured by chiral HPLC analysis.

With the success of this technology and the demand for clinical supplies of **1**, this chemistry was used as the enabling route. Indeed, 7.7 kg of (*S*)-**3** as the ethyl ester, *p*-TsOH salt were prepared from salicylaldehyde **4** via the chemistry shown in Scheme 5 in 57% overall yield with an ee > 99%. This was a remarkable achievement. Nevertheless, practicing this process on-scale accentuated several development opportunities.

(10) See Scheffer, J. R. *Can. J. Chem.* **2001**, *79*, 349 and references therein.
(11) Rogers, T. E.; Ruminski, P. G. US Patent 6013651, 2000.
(12) Rosen, T. *Compr. Org. Synth.* **1991**, *2*, 395.

Scheme 5. **Preparation of (*S*)-3 via a diastereoselective Reformatsky reaction**



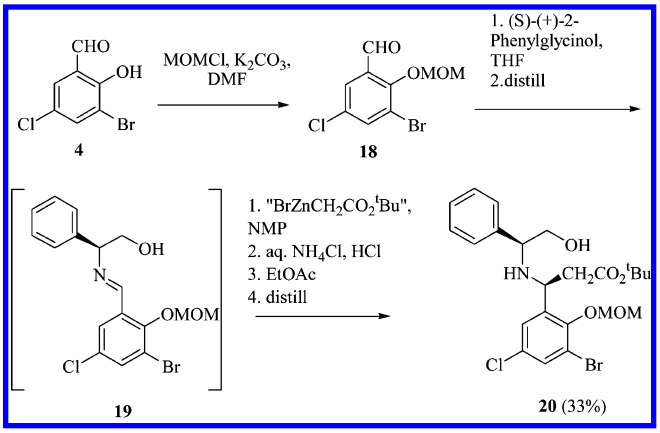
Process Development

Although the aforementioned enabling methodology yielded excellent quality product in very good yield, the following issues were identified. The process (1) employed MEM chloride, a hazardous reagent, (2) involved the isolation of an unstable imine intermediate **15**, (3) engaged a highly energetic imino-Reformatsky reaction, (4) utilized $Pb(OAc)_4$, and (5) displayed inconsistency in the purification and isolation of **3** as the ethyl ester, and *p*-TSA salt. Furthermore the enabling technology involved twelve phase separations, six distillations, three isolations, two $MgSO_4$ dehydrations, one crystallization, and two drying steps. These features tied closely with issues such as safety, engineering, cost of goods, resource allocation, raw materials sourcing as well as the business strategy were the emphasis behind further development of the imino-Reformatsky route to (*S*)-**3**.

The MEM-Protecting Group: Essential for Success. The first stage in the enabling process to supply (*S*)-*β*-amino acid ethyl ester **3** for preclinical development entailed the conversion of **4** to the corresponding MEM protected aldehyde **14**. An alternative to this protecting group was desired because of the toxicity and expense of MEM chloride. It was recognized, however, that the high diastereoselectivity (>99% de) and yield of the subsequent conversion of imine **15** to (*S*)-**3** might be adversely affected by this change.[4,13]

The first alternate protecting group investigated was methoxymethyl (MOM). This group might provide the aforementioned complexation site and may have the added advantage in that it could be introduced by using dimethoxymethane, avoiding the use of the carcinogenic reagent chloromethyl methyl ether. For these feasibility studies, however, MOM chloride was employed to expedite evaluation of this idea. Accordingly, **4** was converted to the corresponding MOM ether **18** in 74% isolated yield and transformed to imine **19** in near quantitative yield. Reaction of **19** with the Reformatsky reagent derived from *tert*-butyl bromoacetate unfortunately afforded **20** in only 33% overall yield from **4** after column chromatography (Scheme 6). Attempts to optimize the desired reaction were unsuccessful; therefore, results obtained from the MOM protecting analogue **18** could not compete with those obtained from MEM ether **14**.

Scheme 6. **Feasibility studying employing MOM protection**



(13) Two equivalents of the Reformatsky were essential for complete reaction. It is envisaged that at least one of the two oxygen atoms of the MEM moiety assists in the observed diastereoselectivity by complexation with one equivalent of reagent.

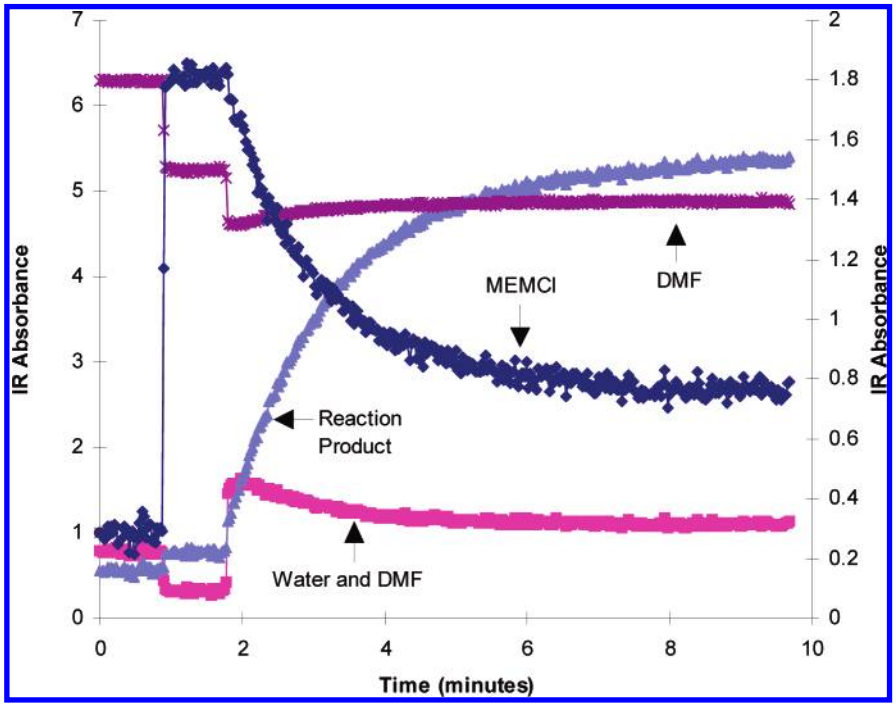


Figure 1. **FT-IR data for the hydrolysis of MEM chloride with DMF/H_2O.**

Other protecting groups were explored and found to fail in comparison. For example, base labile groups such as acetate did not hold up to the downstream chemistry. Base stable groups such as pivaloyl resulted in low yields and poor diastereoselectivity. Groups requiring hydrogenation for removal such as benzyl or benyloxycarbonyl resulted in dehalogenation of the aromatic ring. Thus, it was established that the MEM protecting group was required for successful completion of this chemistry.

Recognition of this fact focused our research toward full development of the safety protocols necessary for use MEM chloride on-scale as well as the destruction of excess reagent postreaction. Accordingly, the rate constant for hydrolysis of MEM chloride in DMF/H_2O was measured by FT-IR and ^{1}H NMR analysis. In the FTIR experiment, water was added to a solution of MEM chloride in DMF and the hydrolysis monitored using the ReactIR.[14] Spectra were recorded every 1.4 s for approximately 10 min. A ConcIRT analysis[15] of the data was performed to extract the individual components. A plot of the data is shown in Figure 1.

The initial MEM chloride and water concentrations are known from the amounts initially charged. The final concentrations and final time were estimated from Figure 1. Essentially all of the MEM chloride was destroyed within 8 min. The data were fit using Scientist,[16] a modeling program, and the second-order rate constant[17] was calculated to be $k = 1 \times 10^{-2}$ M^{-1} s^{-1}. The data and fit are shown in Figure 2.

The rate of MEM chloride hydrolysis as measured by ^{1}H NMR analysis started by adding it to a small vial containing DMF-d_7 and mesitylene as an internal standard. The solution was transferred to an NMR tube and the ^{1}H NMR spectrum acquired. D_2O was added and the tube inverted to allow for mixing. Fifteen spectra (eight acquisitions each) were sequentially recorded. The time from D_2O addition to the first acquisition was approximately 1 min. Twenty-five seconds were required for eight acquisitions. The first spectrum was complete within 85 s, and 3% of the MEM chloride remained. The second-order rate constant was calculated to be $k = 1 \times 10^{-2}$ M^{-1} s^{-1} using the integrated rate equation shown in eq 1.

$$k = \frac{1}{t([\mathrm{MEMCl}_{\mathrm{initial}}] - [\mathrm{H_2O}_{\mathrm{initial}}])} \ln \frac{[\mathrm{H_2O}_{\mathrm{initial}}][\mathrm{MEMCl}_{\mathrm{final}}]}{[\mathrm{MEMCl}_{\mathrm{initial}}] - [\mathrm{H_2O}_{\mathrm{final}}]} \quad (1)$$

Thus, it was shown by both spectroscopic techniques that the second-order rate constant for hydrolysis of MEM chloride is $k = 1 \times 10^{-2}$ M^{-1} s^{-1}. The experimental result, therefore, suggest that essentially all remaining MEM chloride would be quenched by water if the mixture was allowed to stir at ambient temperature for approximately 1 h.

This result was confirmed in the laboratory by direct quantitative GC analysis of the MEM chloride in DMF and water solutions. For example, after 83 min the MEM chloride was not destroyed by DMF alone. However, the addition of water to the DMF greatly increases the rate at which MEM chloride is degraded. In a 1:1 mixture of DMF/water, MEM chloride could not be detected after 21 min.

(14) All in situ infrared spectra were collected by means of an ASI ReactIR 1000 equipped with a 5/8-in. DiComp ATR probe. ASI Applied Systems, Inc., Millersville, MD 21108.

(15) ConcIRT is an algorithm used by ASI ReactIR software to pull individual components out of a complex mixture.

(16) Scientist for Windows, Version 2.01, MicroMath Scientific Software, P.O. Box 21550, Salt Lake City, UT 84121, (801) 943−0290.

(17) α-Chloroethers have previously been shown to hydrolyze by an SN1 mechanism.[18] Since the mechanism has not been rigorously established under these conditions, we chose to use a second-order model which led to a more conservative estimate of the MEM chloride concentration.

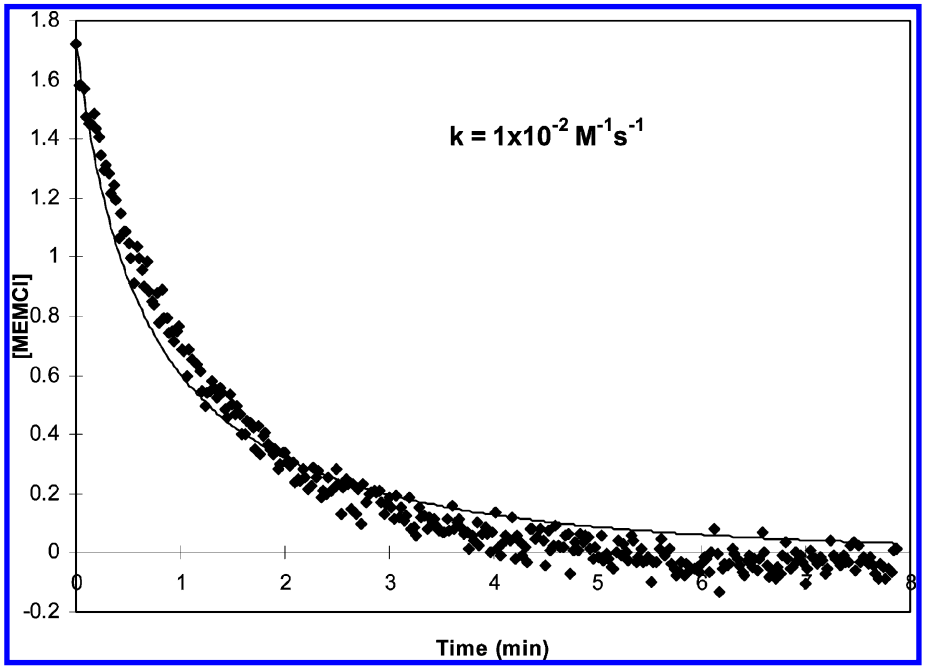


Figure 2. **Mathematical fit of the MEM chloride hydrolysis FT-IR data.**

However, additional evidence was obtained by analyzing isolated samples of **14** for MEM chloride. When the reaction mixture was stirred with water for 1 h or more, no MEM chloride was detected by gas chromatographic analysis. On the other hand, traces for MEM chloride in **14** were detected if the reaction mixture quench was allowed to stir for only 10 min.

The mode of quench addition also affected the MEM chloride levels entrapped in **14**. No MEM chloride was detected if the reaction mixture was charged to an excess of water; however, if water was added to the reaction mixture, MEM chloride could be detected in isolated **14** even after stirring for 1 h. It was found that the addition of water to the reaction immediately precipitated small amounts of **14** enriched in MEM chloride. Therefore, the practiced pilot-plant procedure for the conversion of salicylaldehyde **4** to its MEM protected analogue **14** was to add powered potassium carbonate (1.06 equiv) to a solution of **4** in DMF. MEM chloride (1.05 equiv) was added via pressure canister, while maintaining a temperature of 20−25 °C. After complete addition, the reaction was stirred for 3 h at ambient temperature. The product mixture was charged to a reactor containing water (4.6 L/L DMF). The slurry was stirred for 30−60 min and the white solid (mp 32.5−33 °C) isolated by either centrifugation or by pressure filter. The wet cake was washed with water and dried in vacuo at ambient temperature to afford **14** in 92% yield.

Imine 15, an Unstable Intermediate. In the enabling technology imine **15** was prepared by reaction of **14** with (*S*)-phenylglycinol in THF. After the desired conversion was complete, the reaction mixture was concentrated to dryness under reduced pressure. The distillation served mainly to the remove the water generated during imine formation to levels of less than 0.1 wt. %. The product concentrate was then diluted with NMP and reacted with the Reformatsky reagent prepared form *tert*-butyl bromoacetate to provide **16**. This procedure seemed to perform effectively in all laboratory runs, but during the first piloting run the total batch was lost due to decomposition. Accordingly, a study was initiated to solve this heretofore unknown problem.

The laboratory work began with attempts to repeat the decomposition observed in the pilot plant. Employing supplies of (*S*)-phenylglycinol prepared from two third-party manufacturers (TPM), we found that one source did not give rise to decomposition for a period of up to 6 days. In fact, accelerated decomposition studies on neat **15** revealed no change at temperatures as high as 90 °C for periods of 1−15 h as determined by ^{1}H NMR spectrum analysis. It was not until **15** was exposed to 90 °C for 65 h that signs of decomposition began to emerge.

On the other hand, the decomposition phenomenon could be repeated in the lab when employing the same phenylglycinol employed in the pilot run, reagent sourced from a second TPM. Surprisingly, in contrast to the aforementioned phenylglycinol, this lot gave rise to decomposition in the THF solution itself, before concentration! Therefore, on the basis of these preliminary laboratory findings, it was evident that the sources of phenylglycinol were behaving differently.

Our focus turned toward finding the cause of the decomposition. A ^{1}H NMR study was completed in which the two different sources of phenylglycinol were employed and compared directly. An empirical observation was made. The different sources of phenylglycinol gave rise to vastly different rates of imine formation. For example, the reaction was complete within 30 min using the TPM's phenylglycinol. Conversely, it took the same reaction approximately 6 h to go to completion when employing the lab lot of phenylglycinol.

This prompted us to hypothesize that an agent in the TPM's phenylglycinol, perhaps an acid or a metal, caused the differences in the observed rates of reaction and decomposition. Unfortunately, no definitive cause could be

found within the accelerated timeline of the project. In fact, on the basis of all analytical information received, the two lots of phenylglycinol appeared compositionally identical. Titration, trace metals analysis, elemental analysis, and extensive liquid chromatography/mass spectral analysis of the two lots could not resolve the difference. Thinking that the method by which the phenylglycinol was manufactured may shed some light on a possible difference, a request was made of both manufacturers to provide a process description. Unfortunately, the manufacturer of the phenylglycinol that did not promote decomposition would not release their preparative method because it was considered a trade secret.

Nevertheless, a robust method to effect the desired reaction and avoid the aforementioned decomposition was found. The answer was NMP, the solvent used in the forthcoming Reformatsky reaction. In this solvent, the rate of formationof **15** was slower (20−30 h), but acceptable, and the aforementioned decomposition was eliminated. For example, the lot of phenylglycinol that caused decomposition in THF was applied using NMP. No decomposition of imine **15** was observed for 8 days, even at elevated temperatures. NMP had an additional advantage in that its use eliminated one distillation used in the enabling process.

Having determined that NMP would resolve the decomposition problem and simplify the process, our studies turned toward dehydrating the product solution. Spiking studies on the effect of water in the forthcoming Reformatsky reaction revealed that the presence of 1 mol equiv of water, the theoretical amount, led to a 75% decrease in product yield, whereas, only 5% loss was observed when the equivalent of water present was ≤0.4 with respect to **15**. These investigations also revealed that the type of dehydrating agent was important. The use of an inorganic salt or other solid dehydrating agent in NMP always led to filtration problems. However, a column of 4 Å molecular sieves proved successful. Studies found that the water level could be decreased to less than 0.3 wt % by simply passing the NMP solution through a column of untreated sieves.

As it turns out, this method of dehydrating the reaction mixture was very robust. The technology was employed successfully on 12 different occasions and at three different pilot plants. Dehydration was effected either by employing a column of sieves or a Sparkler filter filled with sieves. In a properly designed column system the level of water in the reaction mixture was diminished to well below the effective level by passing the solution through only one time. In the Sparkler filter system, the solution was recirculated through the unit until the desired level of dehydration was achieved. Thus, the general procedure employed for preparation of an NMP solution of **15** entailed dissolution of aldehyde **14** into NMP. Phenylglycinol (1.005 equiv) was charged in one portion and the reaction mixture agitated for 20−30 h. The product solution was then pumped through a 15-cm column of untreated 8−12 mesh, 4 Å molecular sieves and used without further purification.

The Diastereoselective Imino-Reformatsky Reaction. The preparation of chiral *β*-amino acid esters via an imino-Reformatsky reaction has been employed successfully by Pfizer on a number of occasions.[4] This experience led us to appreciate the energy of this conversion, and therefore, studies directed toward understanding the thermo- and reaction kinetics of the conversion of **15** to **16** were completed. Such data would help in designing a safe and effective protocol for practicing this chemistry on-scale.

Table 1. **Summary of thermokinetic data for the conversion of 14 to 15 and 15 to 16**

step	Cp initial [J/kg/K]	Cp final [J/kg/K]	total heat [kJ]	molar heat [kJ/mol]	adiabatic temp [°C]
14 → 15	1806	1713	11	37	20
"$BrZnCH_2CO_2{}^tBu$"	1601	1728	149	205	173
15 → 16	950	1600	17	56	13
quench	1600	2300	29	183	1R

The study was performed employing a Mettler RC1 SV01 1L reaction Calorimeter equipped with a ReactIR[13] to obtain FTIR-derived kinetic information. Spectra were recorded every 5 min for approximately 500 min. A ConcIRT analysis[14] of the data was performed in order to extract the individual components. A probe for GC−MS analysis of the reactor headspace was also installed so that the rate of ethylene generation during zinc activation with 1,2-dibromoethane could be monitored and quantified.

Ethylene gas was detected during reflux only. The maximum rate of ethylene formed was measured at 3% of the ethylene volume per minute, assuming complete conversion. Extrapolation of this information indicated that a pilot plant's scrubber or flume system or both would adequately handle this rate of ethylene release.

A summary of the thermokinetic data obtained for the conversion of **14** to **15** and then **15** to **16** is given in Table 1. The information indicates that the formation of the reagent itself is the most energetic component of the reaction sequence, giving rise to a calculated $\Delta H_{rxn} = -205$ kJ/mol *tert*-butyl bromoacetate and an adiabatic temperature rise of 173 °C.

The data were acquired by the addition of *tert*-butyl bromoacetate over 2 h to a suspension of activated zinc in THF at 50 °C. The heat generation profile for this experiment is shown in Figure 3. Note a significant, but not surprising, observation. A 15-min induction period was observed; thus, important information for controlling the rate of *tert*-butyl bromoacetate addition and predicting reaction behavior, particularly on-scale, was obtained.

After the reaction was complete, the THF used to generate $BrZnCH_2CO_2{}^tBu$ was partially distilled off. It was important to monitor the amount of THF removed because eliminating too much led to precipitation of the THF solvate of the Reformatsky reagent. On-scale this could result in the agitator freezing up. Studies in this regard found that optimum results were obtained when 40 wt % of the original THF charge was removed. In cases where 45−50% of the THF was removed, the agitator became sluggish and started to stall.

The thermal data obtained for the desired Reformatsky reaction is shown in Figure 4. In this experiment, 88% of imine **15** (1.0 equiv) in NMP was added via pump over a 1.5-h period to a −10 °C NMP/THF solution. The remaining

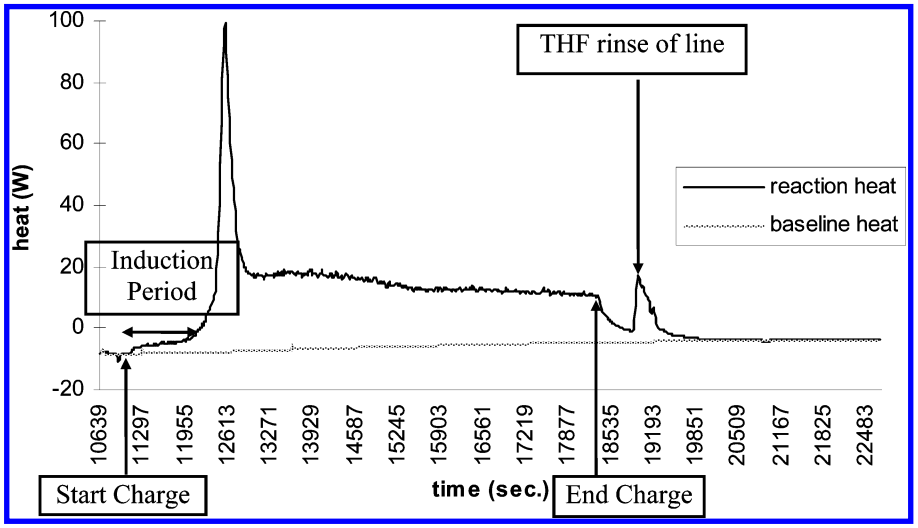


Figure 3. **Preparation of "BrZnCH$_2$CO$_2$^{t}Bu". Heat released and accumulation data, watts vs time.**

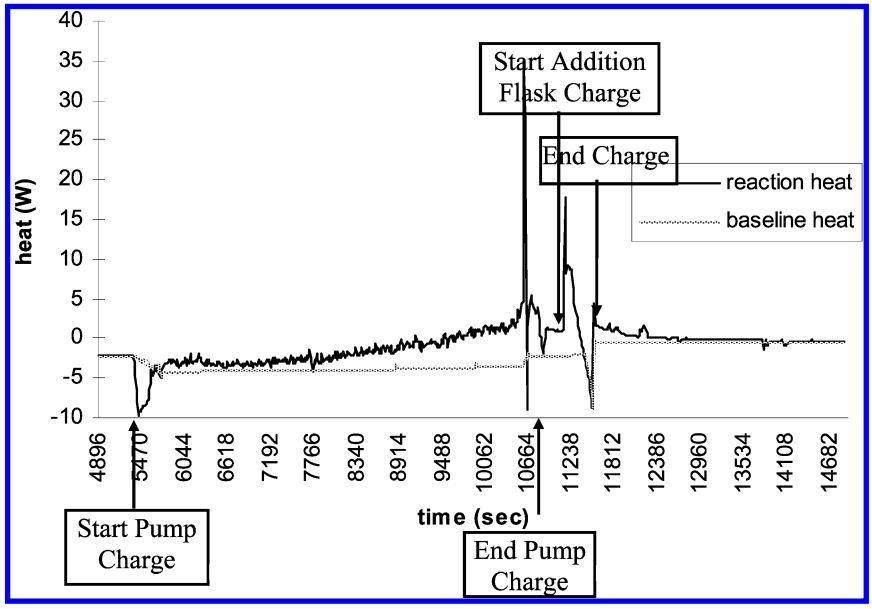


Figure 4. **Conversion of 15 to 16. Heat released and accumulation data, watts vs time.**

12% was added over a 6-min period using an addition funnel. The overall adiabatic temperature rise of the Reformatsky reaction was calculated to be 13 °C, with a molar heat of reaction of approximately −56 kJ/mol of **14**, assuming 100% conversion to **16**. The heat flow data indicated that the conversion of **15** to **16** is dose controlled. Reaction time based on the FTIR data indicated that the reaction was nearly dosed controlled and complete once all of **15** was added.

The thermokinetic data for the quench reaction (1:1 aqueous NH_4Cl/concentrated HCl) revealed an adiabatic temperature rise of 16 °C and a molar heat of reaction of 183 kJ/mol HCl. On the basis of the recorded heat profile, the reaction time was dosed controlled and nearly complete within the first few percent of the quench solution addition.

To supplement the calorimetry study, temperature experiments were completed to ascertain this effect on the conversion of **15** to **16**. This revealed that **16** was obtained in near quantitative yield when the substrate was combined with the reagent in such a manner that the internal temperature remained between −10−0 °C. If the reaction temperature was allowed to remain within a temperature range of 0−5 °C, a small decrease in yield to approximately 95% was observed. A loss of approximately 40% was observed if the reaction temperature reached 20 °C. Interestingly, no loss in diastereoselectivity[19] was observed within this temperature range, a true testimony to the robustness of this chemistry.

From the aforementioned data, the following standard protocol for large-scale conversion of **15** to **16** was developed and practiced. Zinc (4.0 equiv/mol **15**) was charged to a reactor, followed by THF and 1,2-dibromoethane (0.077 equiv/mol **15**). The contents were heated to reflux (∼66 °C) and held for at least 1 h. The mixture was cooled and *tert*-butyl bromoacetate (3.83 equiv/mol **15**) added in approximately five equal aliquots within a temperature range

(18) (a) Jones, T. C.; Thornton, E. R. *J. Am. Chem. Soc.* **1967**, *89*, 4863. (b) Tou, J. C., Kallos, G. J. *Anal. Chem.* **1974**, *46*, 1866. (c) Richard, J. P.; Amyes, T. L.; Rice, D. J. *J. Am. Chem. Soc.* **1993**, *115*, 2523.

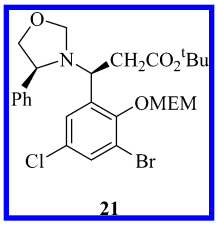
(19) Diastereoselectivity measured by chiral HPLC analysis of the product mixture and by analysis of the product derived from conversion to (±)-**3** ethyl ester, *p*-TsOH salt.

of 48−50 °C. An exotherm of 5−7 °C was generally observed with each aliquot. The contents were then held at 50 ± 5 °C for at least 1 h. The contents of the reactor were cooled to 0 ± 5 °C, and vacuum was applied to the system. Approximately 40% of the THF was removed by vacuum distillation. Following this, NMP was charged, and the contents were cooled to −15 ± 5 °C.

The NMP solution of imine **15** in a second reactor was added to the Reformatsky reagent while maintaining the batch temperature below −5 °C. The contents were held for at least 3 h at ≤0 °C and then filtered to remove the excess zinc. The zinc complex of **16** in NMP was quenched by the addition of an ammonium chloride and hydrochloric acid solution while maintaining the temperature at ≤10 °C. The contents of were warmed to 20 ± 5 °C and MTBE added to extract **16**. Following a series of aqueous washes, the MTBE was removed under reduced pressure until minimum stir volume was reached. Methanol was charged and this solution used without further purification.

Replacing $Pb(OAc)_4$ with $NaIO_4$. The enabling procedure for the conversion of the imino-Reformatsky reaction product **16** to benzyl imine **17** entailed treating a methanol solution of **16** with a molar equivalent of $Pb(OAc)_4$ at 0−5 °C. Sodium hydroxide was then added to the reaction mixture and the methanol removed by vacuum distillation. The product **17** was extracted from the mixture into ethyl acetate, dried, concentrated to an oil, and taken into the next step without further purification. Although this procedure provided quality product in good yield, the waste-handling issues associated with the use of lead gave rise to an effort to replace this oxidant. A number of alternatives to $Pb(OAc)_4$ were therefore examined. Only sodium periodate in methanol gave the desired product. Noteworthy was the fact that methanol was required for successful conversion. A slow reaction rate was observed in ethanol. It is believed that this subtle difference is related to the relative solubility of periodate in ethanol versus that in methanol.

Although reaction of **16** with $NaIO_4$ in methanol gave the desired product **17**, this reaction also afforded oxazolidine **21** as 23% of the product mixture. Evidently the liberated formaldehyde was reacting with substrate.



21

Attempts to minimize the formation of **21** met with limited success at first. Factors such as solvent, periodate counterion, equivalents of periodate, concentration, temperature, and order of addition were investigated, all giving only incremental improvements. The problem of oxazolidine **21** formation was solved, however, when we looked into sequestering the formaldehyde formed. A number of acids and bases were scanned, and it was established that the formation of **21** was terminated with either acetic acid or methylamine.

The conversion of **16** to **17** was monitored carefully by both ^1H NMR and LC analysis to determine which formaldehyde-sequestering agent would be best suited for scale-up. This revealed no **21** at any time with methylamine present, but the oxazolidine content increased to a maximum (approximately 16% by HPLC area) and then decreased to near zero in the presence of acetic acid. Comparative analysis of the two methods further revealed that a higher-quality product and more efficient workup were obtained when using methylamine. Thus, methylamine was used on-scale.

The following general procedure for preparation of benzyl imine **17** was employed in the pilot plant. To a vessel were charged $NaIO_4$ (2.0 equiv), methanol, and methylamine (1.2 equiv). The contents were heated to 30 °C. The methanol solution of **16** was then added to the reactor containing the $NaIO_4$ mixture and held for 12 h. Upon complete reaction, ethyl acetate was charged and this slurry filtered through a precoated filter system. The filter cake was washed with ethyl acetate, and the combined organic layers were distilled in vacuo until the concentrate contained ≤2.5% methanol by GC analysis. This was important to maximize product yield in the following aqueous treatments. The distillation was repeated if necessary. The concentrate was then taken back up in ethyl acetate and washed sequentially with 20% aqueous sodium thiosulfate and brine. Vacuum distillation afforded the crude product, which was taken up into ethanol and used without further purification.

Purification and Isolation of (*S*)-3 Ethyl Ester, *p*-TSA Salt. The enabling route for conversion to (*S*)-**3** entailed exposure of an ethanol solution of **17** to a slight excess of $TsOH \cdot H_2O$ in refluxing ethanol. The ethanol was then removed in vacuo and the residue taken up into tetrahydrofuran. This was reconcentrated and taken up again in THF. After heating to reflux, hexane was added and the mixture cooled to allow crystallization. The product was filtered and the cake washed with acetone. Thus, four solvents and two distillations were employed to carry out this operation. Additionally, it was observed that during vacuum drying of (*S*)-**3** ethyl ester, *p*-TsOH salt, a color change from white to brown took place and the product solid appeared to be nonhomogeneous. Recrystallization from EtOH/EtOAc removed the color and gave rise to a homogeneous white solid. Analysis of the mother liquor revealed trace amounts of benzylaldehyde and *p*-TsOH. Interestingly it was found through spiking studies that neither of these contaminants effected on their own the color change but together created the browning phenomenon. The origin of this effect was not investigated further.

Refinement of the aforementioned isolation of (*S*)-**3** ethyl ester, *p*-TsOH salt was studied. Precipitations directly from EtOH using an antisolvent were investigated, but scale-up results were not consistent. The use of a combination of MTBE/EtOH was also investigated, and looked promising since precipitation of a white solid took place at reflux, but the product was identified as the *tert*-butyl ester analogue of **3**. Although this was not a bad result, the application of the *tert*-butyl ester of **3** in the synthesis of **1** led to lower yields in the ensuing chemistry.

Therefore, despite extensive investigation, our efforts turned toward simplifying the enabling procedure. In this regard, three significant observations were noted. First, the yield of (*S*)-**3** ethyl ester, *p*-TsOH salt was highly dependent upon the ethanol content of the crystallization solvent system. For example, if the ethanol content was ≥5 wt % before antisolvent addition, a yield loss of >10% resulted. Second, if the product mixture was concentrated too much, an extremely viscous mixture formed that stopped agitation! And third, high temperature (65 °C) addition of the antisolvent afforded better product quality and color.

These procedural modifications led to the following pilot-plant procedure. The ethanol of solution of **17** and *p*-TsOH·H_2O (1.3 mol equiv) were charged to a steam-jacketed vessel. The solution was heated to reflux, maintained at this temperature for 8 h, and then cooled to 10 °C. The contents were distilled under vacuum until either the minimum stir volume of the reactor was reached or the batch temperature reached 40 °C. THF was charged, and the contents were again concentrated under vacuum until the batch temperature reached 40 °C. The contents were diluted again with THF and heated to 65 °C, and heptane was added while maintaining temperature. During heptane addition, (*S*)-**3** ethyl ester, *p*-TsOH salt precipitates out of solution. The contents were cooled to 5 °C and held at this temperature for 2 h. The solids were filtered and washed with cold acetone three times to yield (*S*)-**3** ethyl ester, *p*-TsOH salt in 48−57% overall yield from salicylaldehyde **4**. Analysis of the free base of the product by chiral HPLC measured an enantiomer excess of >99.7%.

Pilot-Plant Campaigns

The technology shown in Scheme 5 was transferred directly to toll manufacturers. The procedure was practiced on three different occasions. Learnings from these campaigns were then incorporated into a potential commercial process. Planned was the preparation of approximately 600 kg of (*S*)-**3** ethyl ester, *p*-TsOH salt. Additionally, two aspects of the process development effort were incorporated into the operating instructions at this juncture—replacing $Pb(OAc)_4$ with $NaIO_4$ and practicing the revised (*S*)-**3** ethyl ester, *p*-TsOH salt crystallization protocol.

In the course of transferring the technology to two potential commercial manufacturing sites, significant engineering limitations were realized. Centrifugation was not available at either facility. Therefore, isolation of product had to be carried out using some sort of pressure filtration system. Second, a bottleneck was recognized when filtering the NMP solution of the Reformatsky product **16** before isolation. Regardless, appropriate strategies were devised, and equipment was installed or modified at each facility.

The equipment employed for Campaign 1 utilized two 200-gal glass-lined reactors, one 200-gal stainless steel reactor, two 100-gal glass-lined reactors, a 36-in. seven-plate Sparkler stainless steel filter (for the molecular sieves) and a 24-in. glass-lined pressure filter. The first three of four batches at this location processed fairly uneventfully. Employing 87 kg of **4** in each run, 241 kg of (*S*)-**3** ethyl ester, *p*-TsOH salt was produced in 60% overall yield (assay-adjusted yield). However, the fourth batch gave the desired product in only 32% overall yield. Additionally troubling was the average assay for all product prepared—only 77%.

The pilot-plant for Campaign 2 was chartered to produce approximately 350 kg of (*S*)-**3** ethyl ester, *p*-TsOH salt. This facility used three 750-gal glass-lined reactors, one 100-gal stainless steel reactor, a 750-gal glass-lined receiver, a molecular sieve column, a bag filter system, and a Nustche filter. The aforementioned throughput restrictions within this equipment and at this scale necessitated a tiered approach to making product. First 240 kg of **14** was converted to the Reformatsky product **16**. This batch was then split in two and processed to product separately. The split lot was held in cold storage until it was ready for use. This pattern was repeated two more times. All of this work was completed within 12 weeks, giving rise to 350 kg of (*S*)-**3** ethyl ester, *p*-TsOH salt with an average assay of 90 wt %. Although this result was superior to that obtained for Campaign 1, the assay was still somewhat lower than anticipated.

Research directed toward ascertaining the reason(s) behind the lower than expected yields and assays were completed. Postprocess analysis of reaction stream samples revealed an accentuated level of an impurity in the Reformatsky product mixture. These studies also concluded that this contaminant carried through into product. This impurity was identified as the amino acid itself, (*S*)-**3**. Further tests revealed that the amount of (*S*)-**3** present was proportional to the time the Reformatsky product solution was held before quench. For example, if the solution of **16** was held for 4.8 h before quench, 17 area % of (*S*)-**3** was present in the product mixture. If this material was held for 8.6 h, the level of this impurity increased to 32 area %. These data were compared to results from laboratory trials and verified. Thus, prior to quench, the *tert*-butyl ester of **16** was converting to the corresponding acid (or precursor thereof) and carried through to the isolation of the ethyl ester, *p*-TsOH salt of (*S*)-**3**, a direct result of the tiered approach to processing this much material.

Fortunately, a simple rework protocol for poor-quality material was devised that entailed reslurry of the product in acetone. This procedure provided product with an average assay of 95 wt %. Approximately 95% of the available product in this material was recovered. The poorer quality batches from Campaigns 1 and 2 were reworked in this manner to provide in total 591 kg of acceptable product.

Conclusions

Four process research investigations directed toward discerning a scalable, enantioselective method for preparing a key intermediate to the $\alpha_v\beta_3$ integrin antagonist **1**, (*S*)-β-amino acid ester **3**, were completed. A synthetic route developed in-house employing a novel, diastereoselective imino-Reformatsky reaction was selected for development.

Process development of this chemistry into a potential commercial process addressed several technical issues. Discovered was the necessity of employing the MEM-protected salicylaldehyde **15** in the Reformatsky reaction. This maintained high yield and diastereoselectivity. The reaction kinetics of MEM chloride hydrolysis were utilized

to determine an amenable quenching procedure for the safe and effective application of this reagent on-scale. The efficient formation of the (*S*)-phenylglycinol imine **15** in NMP and the dehydration of this product solution employing molecular sieves were described. A calorimetry study on the Reformatsky reaction and the application of this thermal and kinetic data to the development of an effective, large-scale procedure were completed. $Pb(OAc)_4$ used in the enabling process was replaced with $NaIO_4$ in methanol. The use of methylamine to sequester competing oxazolidine formation was successful and employed in the commercial process. Finally, an isolation and purification protocol for (*S*)-**3** ethyl ester, *p*-TsOH salt was developed.

The potential commercial process was practiced. Yields and assays were lower than expected. It was found that the Reformatsky product **16** degraded during processing. Nevertheless, after rework, 591 kg of acceptable product was successfully prepared.

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Supporting Information Available

Experimental procedures for the preparation of most compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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