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A Catalyzed and Highly Selective Ester Reduction in the Synthesis of an *N*-Acylpyrrolidine: Safe Design through Reaction Calorimetry and Modeling

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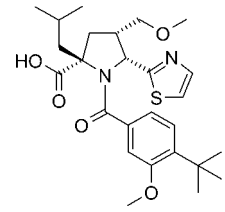
Abstract:

The asymmetric synthesis of an *N*-acylpyrrolidine for HCV inhibition features a unique and highly selective reduction of an ester to an alcohol with $NaBH_4$−MeOH catalyzed by $NaB(OAc)_3H$. This reagent combination provides excellent chemoselectivity while avoiding formation of the thermodynamically favored but undesired epimer. Significant process safety issues including delayed onset of reaction initiation and latent, abrupt release of heat and hydrogen gas are encountered. The pyridine impurity responsible for the reaction inhibition is identified in the reaction calorimetry investigation. A series of reaction calorimetry and modeling studies have led to the safe design of a process which has been scaled up to 300 gallons for production of multikilogram quantities of the *N*-acylpyrrolidine target.

Introduction

Hepatitis C virus (HCV) is a major global public health problem. HCV infection is one of the main causes of cirrhosis and hepatocellular carcinoma (HCC), and HCV-related end stage liver disease is the leading reason for liver transplantation in the United States and other developed nations.[1] The current standard treatment for HCV infection is a combination therapy with interferon-α, particularly the pegylated form, and ribavirin. However only ∼50% of the patients infected with HCV of genotype 1, the prevalent type of HCV infection in the United States, achieve a sustained virological response with the standard treatment as measured by a reduction in serum HCV RNA levels and normalization of liver enzymes.[2] There is a strong need for new therapies to address the issues of safety, broad antiviral response, and viral resistance mutations.

One of the approaches in the discovery of new treatment for HCV infections is to inhibit the HCV polymerase which synthesizes the new viral RNA strands.[3] Compound **1**, a highly substituted *N*-acylpyrrolidine, is a non-nucleoside small molecule which acts as a potent inhibitor of RNA-dependent RNA polymerase (NS5B) in enzymatic assays and inhibits viral RNA replication in cell based replicon assays.[4]



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We recently reported the synthesis of **1** as shown in Figure 1.[5] An important step and significant challenge in the synthetic sequence is the preparation of alcohol **6** from ester **5**, the three stereocenters of which are constructed via an asymmetric [3 + 2] cycloaddition of imino ester **2** and methyl acrylate **3** catalyzed by a complex of silver acetate and hydroquinine. Acylation of the cycloadduct with benzoic acid **4** leads to the reduction precursor **5**. Both the amide bond and the *tert*-butyl ester of **5**, along with the target methyl ester, are susceptible to reduction by a variety of reducing agents. In addition to the chemoselectivity, the C-4 position of **5** is prone to epimerization to the thermodynamically more stable β-epimer prior to being reduced. As a result, common reagents for reduction of an ester to an alcohol such as $LiAlH_4$, *i*-Bu_2AlH, $LiBH_4$, Superhydride, and $NaBH_4$ in combination with various alcohols led to a large amount of over-reduction and C-4 epimerization.

The combination of $NaBH_4$−MeOH (1:2) in the presence of a catalytic amount of $NaB(OAc)_3H$ (2.5 mol % relative to $NaBH_4$) as shown in Scheme 1 turned out to be the only set of conditions we were able to find that did not lead to a significant amount of C-4 epimerization and over-reduction. We postulated that conversion of $NaBH_4$ to $NaB(OMe)_{4-n}H_n$ ($n = 1-3$) was catalyzed by $NaB(OAc)_3H$. To our surprise, the commercially

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(1) Willems, M.; Metselaar, H. J.; Tilanus, H. W.; Schalm, S. W.; de Man, R. A. *Transplant Int.* **2002**, *15*, 61.

(2) (a) Fried, M. W. *Hepatology* **2002**, *36*, 237. (b) Cornberg, M.; Wedemeyer, H.; Manns, M. P. *Curr. Gastroenterol. Rep.* **2002**, *4*, 23. (c) Hugle, T.; Cerny, A. *Rev. Med. Virol.* **2003**, *13*, 361.

(3) For recent reviews on progress and development of small molecule HCV antivirals, see: Nie, Z.-J.; Wagman, A. S. *Curr. Opin. Drug Discovery Dev.* **2004**, *7*, 446–459. (b) Beaulieu, P. L.; Tsantrizos, Y. S. *Curr. Opin. Invest. Drugs* **2004**, *5*, 838. (c) Wu, J. Z.; Yao, N.; Walker, M.; Hong, Z. *Mini-Rev. Med. Chem.* **2005**, *5*, 1103.

(4) (a) Slater, M. J.; Amphlett, E. M.; Andrews, D. M.; Bravi, G.; Burton, G.; Cheasty, A. G.; Corfield, J. A.; Ellis, M. R.; Fenwick, R. H.; Fernandes, S.; Guidetti, R.; Haigh, D.; Hartley, C. D.; Howes, P. D.; Jackson, D. L.; Jarvest, R. L.; Lovegrove, V. L. H.; Medhurst, K. J.; Parry, N. R.; Price, H.; Shah, P.; Singh, O. M. P.; Stocker, R.; Thommes, P.; Wilkinson, C.; Wonacott, A. *J. Med. Chem.* **2007**, *50*, 897. (b) Burton, G.; Ku, T. W.; Carr, T. J.; Kiesow, T.; Sarisky, R. T.; Lin-Goerke, J.; Baker, A.; Earnshaw, D. L.; Hofmann, G. A.; Keenan, R. M.; Dhanak, D. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1553. (c) Burton, G.; Ku, T. W.; Carr, T. J.; Kiesow, T.; Sarisky, R. T.; Lin-Goerke, J.; Hofmann, G. A.; Slater, M. J.; Haigh, D.; Dhanak, D.; Johnson, V. K.; Parry, N. R.; Thommes, P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1930.

(5) Agbodjan, A. A.; Cooley, B. E.; Copley, R. C. B.; Corfield, J. A.; Flanagan, R. C.; Glover, B. N.; Guidetti, R.; Haigh, D.; Howes, P. D.; Jackson, M. M.; Matsuoka, R. T.; Medhurst, K. J.; Millar, A.; Sharp, M. J.; Slater, M. J.; Toczko, J. F.; Xie, S. *J. Org. Chem.* **2008**, *73*, 3094.

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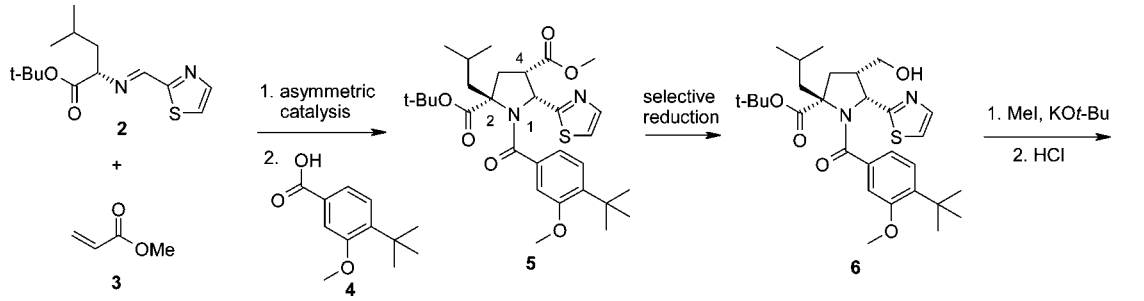
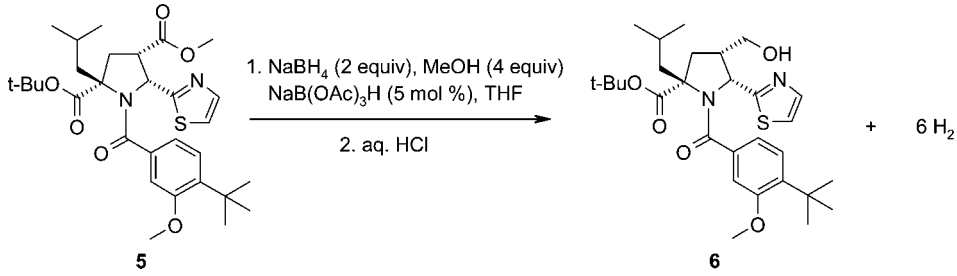


Figure 1. **Synthesis of 1 via asymmetric [3 + 2] cycloaddition and selective ester reduction.**

Scheme 1. **Selective reduction of ester 5 to alcohol 6 with $NaBH_4$ and MeOH catalyzed by $NaB(OAc)_3H$**



available $NaB(OMe)_3H$ led to up to 50% C-4 epimerization.[6] We believe that the $NaB(OMe)_{4-n}H_n$ formed *in situ* was more reactive and less basic, both contributing to the fast and essentially epimerization-free reduction.[7] The reduction was carried out conveniently by mixing the three solids (**5**, $NaBH_4$, and $NaB(OAc)_3H$) in the reactor, followed by sequential addition of THF and methanol. It should be noted that without addition of methanol, the reaction does not proceed in THF. As the process uses a total of 2 equiv of $NaBH_4$, relative to ester **5**, a total of 6 equiv of hydrogen are produced following ester reduction and reaction quenching.

However, concerns with the process were identified during the early laboratory development of the reduction conditions. Early investigations, carried out in a small scale jacketed laboratory reactor (JLR), identified a lack of consistency in the initiation of the reaction during addition of methanol. The delayed onset was often followed by uncontrolled heat output and gas (H_2) evolution. Furthermore, the reaction would occasionally stall and require subsequent additions of $NaB(OAc)_3H$ catalyst and/or $NaBH_4$, a challenging operation on scale. As a result, full hazard evaluation and process safety design were conducted early in the development cycle.

Results and Discussion

Process Safety Investigation. The initial work sought to reproduce the observations that raised concern during the early investigations; specifically, the delayed reaction initiation resulting in significant process safety issues and process irreproducibility. Reaction calorimetry was performed using a Mettler RC1 instrument and materials identical to those used in the problematic JLR preparations.[8] The initial RC1 results successfully reproduced the undesirable delayed onset of reaction, followed by uncontrolled heat and gas evolution, and clearly warranted additional investigation (*vide infra*).

To facilitate the process safety investigation, under a tight timeline with limited starting materials, a screening method was developed to rapidly evaluate the issue around reaction initiation. An Omnical microcalorimeter fitted with a small gas mass flow meter was utilized for this purpose.[9] This enabled a large number of small scale experiments with as little as 5 mL of total reaction volume to be screened rapidly while affording reasonably accurate heat and gas output profiles for both real time and post run evaluation.

Once the screening method to determine reaction initiation was established, a series of Omnical experiments were performed using various batches of starting ester **5**, 1−3 h methanol addition times, and a reaction temperature of 25−45 °C. Of the experiments that failed to initiate or were sluggish in progression, close analytical scrutiny revealed elevated levels of pyridine in the range 1−3 mol %. Pyridine was used as a base as well as an acylation catalyst in the preparation of the reduction precursor **5**.[5] It was determined that ineffective aqueous washing resulted in higher levels of this contamination in some batches. Since the workup conditions for the preparation of ester **5** were slightly acidic with HCl, we suspect that the pyridine might exist as a hydrochloride salt. We postulate that the catalytic conversion of sodium borohydride to various reactive methoxyborohydrides as represented by the formula $NaB(OMe)_nH_{4-n}$ is inhibited by pyridine. It is possible that acetic acid slowly released from $NaB(OAc)_3H$ plays a catalytic

(6) The comparison $NaB(OMe)_3H$ was purchased from Aldrich Chemical Company.

(7) (a) Conditions for HPLC analysis of **5** and its β-epimer were as follows. Column: Phenomenex, Develosil Diol 100A, 4.6 mm × 250 mm, 5 micron; mobile phase: 85:15 heptane/EtOAc; flow rate: 1.5 mL/min; detection: 250 nm; temperature: 25 °C; retention time: 5.95 min for **5** and 5.05 min for its β-epimer. (b) Conditions for HPLC analysis of **6** and its β-epimer were as follows. Column: Phenomenex, Develosil Diol 100A, 4.6 mm × 250 mm, 5 micron; mobile phase: 60:40 heptane/EtOAc; flow rate: 1.0 mL/min; detection: 250 nm; temperature: 25 °C; retention time: 7.59 min for **6** and 5.26 min for its β-epimer.

(8) Mettler RC1e mid-temperature reaction calorimeter was used.

(9) Omnical Super CRC microcalorimeter with Cole-Parmer 10mL/min thermal mass flow meter was used. See the Experimental Section for the screening procedure.

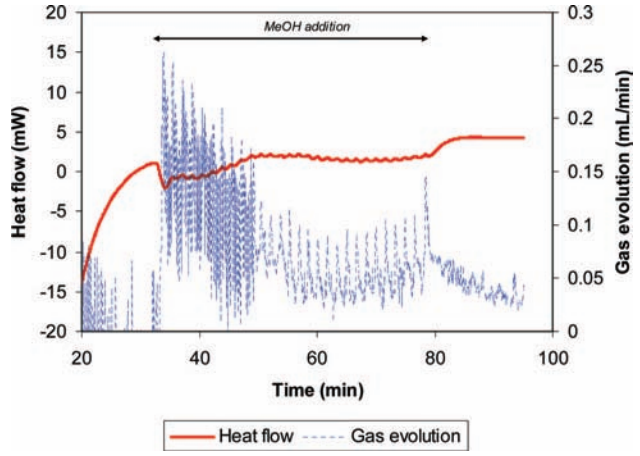


Figure 2. **Omnical heat output, gas evolution, and methanol addition vs time showing failed reaction initiation for reduction of ester 5 with $NaB(OMe)_nH_{4-n}$/$NaB(OAc)_3H$.[9]**

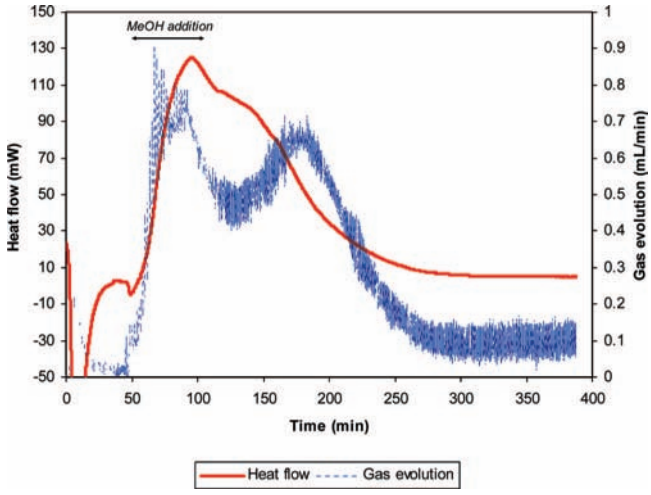


Figure 3. **Omnical heat output, gas evolution, and methanol addition vs time showing successful reaction initiation for reduction of purified ester 5 with $NaB(OMe)_nH_{4-n}$/$NaB(OAc)_3H$.[9]**

role in the formation of the reactive methoxyborohydrides. If so, the inhibition of the catalysis by pyridine is not too surprising.

In Figure 2, an example of failed reaction initiation is observed, as evidenced in the negligible amount of observed heat output and gas evolution in the Omnical profile data. This was confirmed by HPLC analysis which revealed ~98% unreacted starting ester **5** and essentially no product formation. A subsequent experiment with twice the catalyst ($NaB(OAc)_3H$) loading also did not initiate immediately. There was essentially no product formed by HPLC monitoring following the methanol addition, and the reaction only progressed to ~50% completion after aging at 35 °C for 16 h.

To demonstrate the link between pyridine contamination and failed reaction initiation/progression, starting ester **5** from the experiment profiled in Figure 2 was reworked to reduce levels of pyridine to below 1 mol %. The purified material was then subjected to the standard screening experiment. The reaction initiated immediately, followed by progression with desired results (Figure 3). Approximately 20% of **5**, as measured by HPLC, remained shortly after completion of methanol addition and less than 2% was seen after 12 h hold.

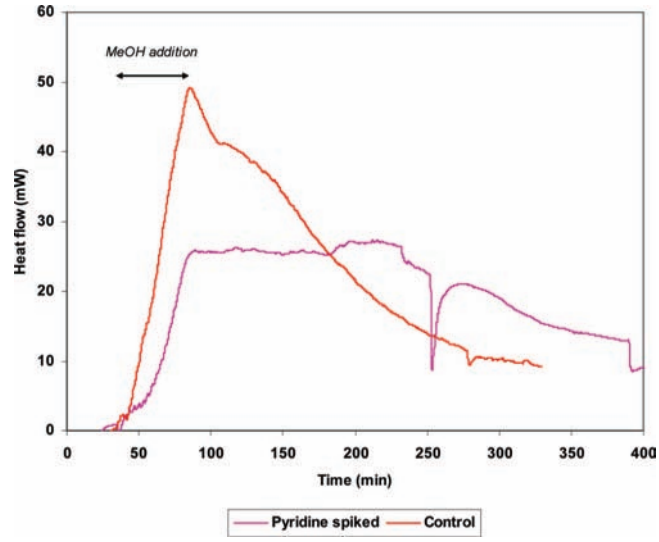


Figure 4. **Omnical heat output and methanol addition vs time for reduction of ester 5 showing effect of pyridine contamination.[9]**

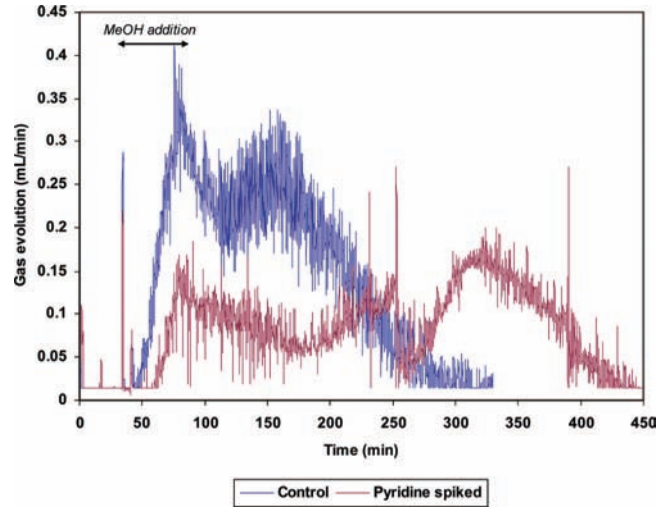


Figure 5. **Omnical gas evolution and methanol addition vs time for reduction of ester 5 showing effect of pyridine contamination.[9]**

To verify the pyridine contamination hypothesis, a spiking experiment was performed. For the control, a purified sample of starting ester **5** was subjected to the standard Omnical screening conditions at half the scale described in the general procedure. As expected, this resulted in immediate reaction initiation and typical progression, similar to what is depicted in Figure 3. The starting ester **5** was then subjected to the same conditions, but with 5 mol % (relative to starting ester **5**) of pyridine added prior to addition of methanol. This resulted in a significant delay in reaction initiation and progression, as evidenced by the heat and gas output profiles. Comparisons of the heat and gas output profiles for the reduction involving pure **5** (control) and the pyridine spiked **5** are shown in Figures 4 and 5.

We suspect that pyridine suppresses the ester reduction by inhibiting the formation of reactive $NaB(OMe)_{4-n}H_n$ from $NaBH_4$ and methanol. Therefore, the impact of pyridine can be observed in the absence of ester **5**. To support this notion, a second series of spiking experiments were performed using

Table 1. **Effect of pyridine addition on total heat output during formation of $NaB(OMe)_{4-n}H_n$[a]**

		total heat output (joules)		
entry	time	control	10 mol % pyridine	20 mol % pyridine
1	end of MeOH addition	81	22	12
2	30 min post-addition	128	50	29
3	60 min post-addition	128	68	39
4	6 h post-addition	–	129	57

[a] General procedure for Omnical screening as described in the Experimental Section was used in the absence of ester **5**.

Table 2. **Effect of pyridine addition on maximum rate of gas evolution during formation of $NaB(OMe)_{4-n}H_n$[a]**

entry	experiment	maximum gas evolution (mL/min)
1	control	2.3
2	10 mol % pyridine	0.68
3	20 mol % pyridine	0.24

[a] General procedure for Omnical screening as described in the Experimental Section was used in the absence of ester **5**.

the general Omnical screening procedure *sans* starting ester **5**. For the control, $NaBH_4$, $NaB(OAc)_3H$, and THF were charged followed by addition of methanol. This was followed by two spiking experiments, run under the same conditions, except that pyridine (10 and 20 mol %, respectively) was added prior to methanol addition. The addition of pyridine resulted in a dramatic, negative effect on heat and gas output, indicative of incomplete formation of $NaB(OMe)_{4-n}H_n$ (see Tables 1 and 2).

Development of Scale-Up Conditions. Once the solution to ensuring immediate reaction initiation had been determined, other process safety issues had to be addressed prior to scale-up. Specifically, a control strategy for the addition-limited heat output and gas evolution was required. Parsed addition of the sodium borohydride was considered but determined to be undesirable given the intended scale of operation, reactive nature of the solid, and available equipment in-house. Following the Omnical screening work, several RC1 experiments were performed to establish a safe methanol dosing regime at an elevated reaction temperature of up to 45 °C. An in-process check was introduced to ensure that incoming starting ester **5** was essentially free (<1 mol %) of pyridine contamination. The final conditions employed eight separate additions of methanol over a period of ~3 h to afford adequate process control over the heat output. The process also limited accumulation and enabled safe dilution and venting of the liberated hydrogen gas. A comparison of reaction calorimetry data for the initial uncontrolled process and final scale-up conditions is illustrated in Figures 6 and 7.

The addition strategy was devised such that a plant cooling failure, realized at any point during the addition sequence, would result in a temperature excursion incapable of boiling the reaction mixture with a total heat output of 727 kJ/kg **5** and a predicted adiabatic temperature rise (PATR) of 84 °C. This would avoid the dangerous situation of flooding the overhead

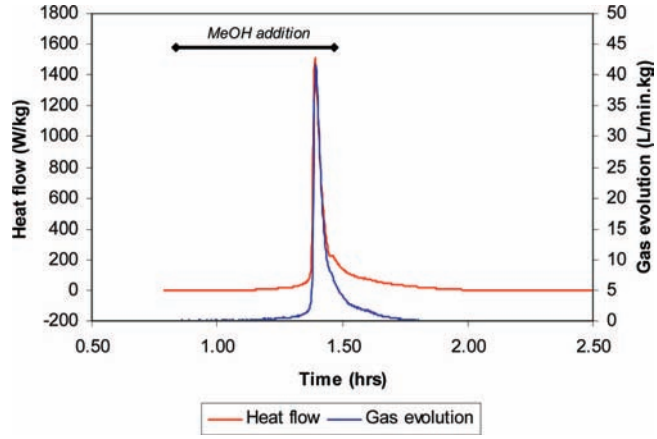


Figure 6. **RC1 heat output, gas evolution, and methanol addition vs time for reduction of ester 5 with $NaB(OMe)_nH_{4-n}$/$NaB(OAc)_3H$ using initial process conditions: delayed onset accompanied with sharp release of heat and hydrogen.**[8]

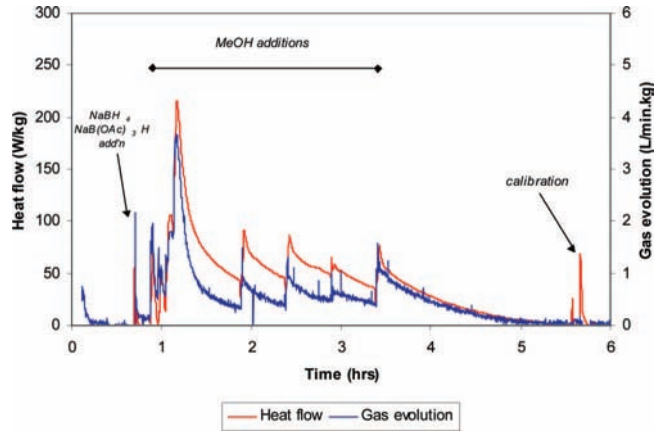


Figure 7. **RC1 heat output, gas evolution, and methanol addition vs time for reduction of ester 5 with $NaB(OMe)_nH_{4-n}$/$NaB(OAc)_3H$ using final process conditions: quick onset accompanied with pulsed release of heat and hydrogen.**[8]

piping with solvent during the period of significant gas evolution, thereby creating the potential for reactor overpressurization.

Prior to plant scale-up, this methodology was modeled using DynoChem simulation software and was then demonstrated on a 400 g scale in a 2 L JLR, which had been characterized to determine the heat transfer coefficient. Using the determined parameters, the process temperature was well controlled and consistent with the predicted profile (Figure 8).

Following successful demonstration in the laboratory, a model for the anticipated scale-up in a pilot plant vessel was developed (Figure 9). An average jacket temperature set point of ~28 °C was determined for maintaining the desired batch temperature of <45 °C.

Pilot Plant Scale-Up. The process was successfully scaled in the pilot plant, running several times at 50 kg scale, using the final process conditions developed in the reaction calorimeter, simulation software, and laboratory scale demonstration. Analysis of the plant temperature profile data (Figure 10) revealed that these conditions were successful in managing the overall heat output and maintaining the process temperature within the desired range. The small temperature decrease observed prior to reaction heating and methanol addition

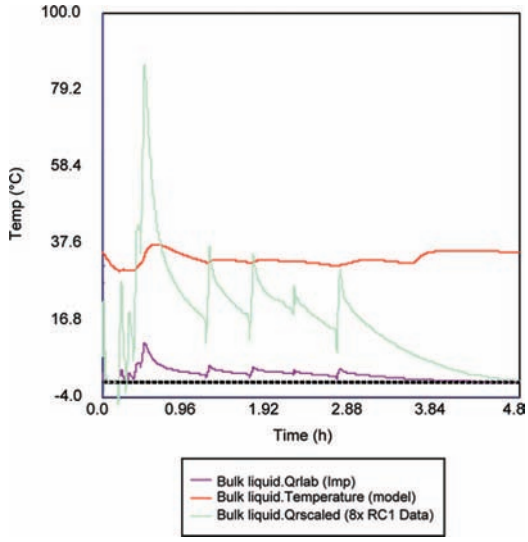


Figure 8. **Dynochem model and 2 L laboratory demonstration data for reduction of ester 5 using final process conditions.**

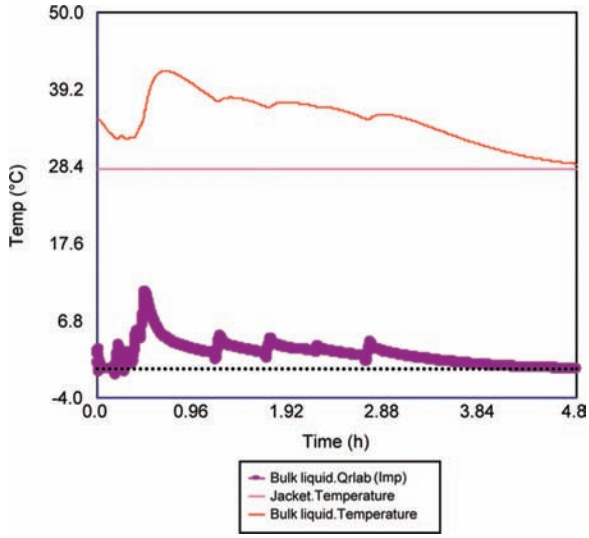


Figure 9. **Dynochem model for 50 kg scale-up reduction of ester 5 using final process conditions.**

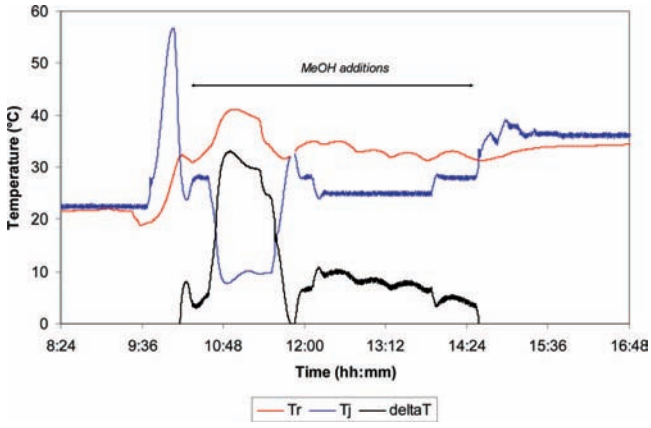


Figure 10. **Pilot plant temperature profile data for 50 kg reduction of ester 5 using final process conditions.**

coincided with dissolution of charged solids. The excellent chemoselectivities were maintained, and there was less than 1%, as measured by HPLC, of epimerized alcohol in the crude reaction mixture.[7] Crystallization provided alcohol **6** of less than 0.2% of the epimer in 80% yield.[10]

The absolute structure of alcohol **6** was confirmed by the X-ray structural analysis.[5] The reduction product was further processed following established conditions to provide multikilograms of drug candidate **1** to support the HCV drug development program.

Conclusion

A basis of safe operation was established for the unique reduction system using $NaBH_4$−MeOH catalyzed by $NaB(OAc)_3H$. Several significant process safety challenges were identified through reaction calorimetry investigation, including discovery of a low-level process impurity responsible for reaction inhibition. Methodology for controlling the pyridine contaminant was developed, and a strict methanol dosing regime was devised with the help of calorimetrical studies and modeling to provide final process conditions suitable for scale-up. The process was safely and successfully run on a multikilogram scale to afford alcohol **6** for subsequent processing, enabling the preparation of clinical trial material under a tight timeline.

Experimental Section

General Procedure for Omnical Screening. A 17 mL vial was charged with 1.25 g (2.23 mmol) of ester **5**, 0.17 g (4.49 mmol) of $NaBH_4$, 25 mg (0.12 mmol) of $NaB(OAc)_3H$, and 3.1 mL of THF. The vial was placed in the sample holder of the microcalorimeter, and a solvent blank was placed in the reference holder. The contents were heated from 25 to 45 °C and allowed to stabilize. To the reaction was added 0.14 g (4.38 mmol) of methanol via a syringe pump over 1−3 h while monitoring the heat output and gas evolution. Following addition, reaction progression was checked periodically via HPLC analysis.[7] Note that only 2 equiv of methanol relative to ester **5** were used for the screening to determine if reaction initiation had occurred through detection of heat and gas evolution. Although a nearly complete reaction could be achieved at times with 2 equiv of methanol, a complete reaction was more securely obtained with 4 equiv of methanol as illustrated in the conditions for the pilot plant runs.

Procedure for the Pilot Plant Operations. 1,1-Dimethylethyl (4*S*,5*R*)-1-{[4-(1,1-Dimethylethyl)-3-(methyloxy)phenyl]carbonyl}-4-(hydroxymethyl)-2-(2-methylpropyl)-5-(1,3-thiazol-2-yl)-L-prolinate (6). A nitrogen-purged 300 gallon reaction vessel was charged with 49.2 kg (88.1 mol) of ester **5**, 6.89 kg (182 mol) of $NaBH_4$, 0.984 kg (4.64 mol) of $NaB(OAc)_3H$, and 125 L of THF. The slurry was stirred and heated to 35 °C, and 11.3 kg (353 mol) of methanol were added in eight equal portions following a predetermined protocol. The first four portions were added 5−10 min apart, followed by a 45 min hold to ensure reaction initiation had occurred. Following initiation, the last four portions were added 30 min apart. The mixture was stirred for 6 h, and an HPLC sample showed completion of the reduction with less than 2% of ester **5** left and less than 1% of the epimer of alcohol **6** formed.[7] The reaction was cooled to 20 °C and held overnight for ∼12 h.

(10) In 50 L pilot reactions when a second crop was collected, the yield was over 95%.

The reaction mixture was heated to 30 °C and treated with 6.90 kg (176 mol) of methanol in three portions over 15 min. After being stirred for 15 min, the mixture was treated with 17.5 kg (179 mol) of concentrated HCl over 45 min. The mixture was stirred for 20 min, and 150 kg of water were added. The pH of the reaction mixture should be indicative of the acidic aqueous layer (3.0−6.5) to ensure complete quenching of any reactive borohydride. After addition of 50 kg of MTBE, the layers were separated. The aqueous layer was back-extracted with 115 kg of MTBE. The combined organic layers were washed with 125 kg of 15 wt % brine. The organic layer was concentrated in vacuo to ∼130 L. After being diluted with 120 kg of MeCN, the solution was further distilled to 125 L. Crystallization started near the end of the distillation. The mixture was cooled to −5 °C, stirred for 2 h, filtered, washed with 30 kg of MTBE, and dried at 55 °C to give 37.3 kg (80%) of alcohol **6** as a crystalline solid: ^{1}H NMR (300 MHz, MeOH-d_4) δ 7.36 (s, 2 H), 7.05 (d, J = 9.0 Hz, 1 H), 6.62 (d, J = 3.0 Hz, 1 H), 6.34 (s, 1 H), 5.46 (d, J = 9.0 Hz, 1 H), 3.63 (m, 1 H), 3.52 (s, 3 H), 3.27 (m, 1 H), 3.09 (m, 1 H), 2.74 (m, 1 H), 2.27 (m, 1 H), 2.18 (m, 2 H), 1.93 (m, 2 H), 1.50 (s, 9 H), 1.20 (s, 9 H), 1.01 (d, J = 6.0 Hz, 6 H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 170.5, 169.5, 168.1, 156.9, 139.6, 137.8, 134.0, 124.9, 118.9, 115.4, 107.5, 80.4, 69.7, 62.4, 59.5, 52.6, 33.0, 27.3, 25.9, 23.6, 23.2, 22.5. Anal. Calcd for $C_{29}H_{42}N_2O_5S\cdot 0.5H_2O$: C, 64.53; H, 8.02; N, 5.18; S, 5.94, O, 16.3. Found: C, 64.15; H, 7.99; N, 5.14; S, 5.89, O, 16.52.

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