Organic Process Research & Development

Article

pubs.acs.org/OPRD

PAT Application in the Expedited Development of a Three-Step, One-Stage Synthesis of the Dipeptide Intermediate of HCV Protease Inhibitor Faldaprevir

Nizar Haddad,* Bo Qu, Heewon Lee, Jon Lorenz, Rich Varsolona, Suresh Kapadia, Max Sarvestani, XuWu Feng, Carl A. Busacca, Dominique Hebrault,‡ Simon Rea,‡ Leen Schellekens,‡ and Chris H. Senanayake

Chemical Development, Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, Connecticut 06877, United States

‡Mettler-Toledo AutoChem, Inc., 7075 Samuel Morse Drive, Columbia, Maryland 21046, United States

Supporting Information

ABSTRACT: A concise scalable synthesis of a chiral dipeptide acid, key substructure of the HCV protease inhibitor faldaprevir, has been developed. A green process with an E-factor of 9.2 was achieved utilizing process analytical technology (PAT) to allow effective processing of multiple-steps in a one-stage operation. Mixed anhydride/oxazolone formation, peptide coupling, saponification, and then crystallization of the desired dipeptide acid were completed within 10 h. MultiMaxIR was used to detect the formation and consumption rates of key intermediates and to provide initial safety data which was subsequently confirmed by more comprehensive process safety testing. Further kinetic analysis was performed to determine the range of operability space to ensure conditions for a robust process.

INTRODUCTION

Our interest in developing small-molecule inhibitors of HCV NS3 protease was recently described in the discovery[1] and process development for the preparation of macrocycle BILN2061.[2] Subsequently, faldaprevir, a nonmacrocyclic inhibitor of HCV protease, has been identified and found to possess the key features of in vivo potency, safety, and bioavailability needed for a new HCV therapy.[3] A highly convergent retrosynthetic route was devised as described in Scheme 1 which was based on the assembly of three advanced intermediates:[4] dipeptide acid **1**, thiazole-quinoline **2**, and the aminoester cyclopropane **3**. The early stage application of PAT in expediting the development of a scalable three-step, one-stage synthesis of dipeptide acid **1** is the highlight of this study.

The Discovery route for the synthesis of dipeptide **1** consisted of a multistep sequence which involved EDC/HOBT procedure for the peptide coupling of capped-*L-tert*-leucine **4** with hydroxyproline methylester (Scheme 2) followed by saponification. In addition to our primary goal of replacing HOBT, which is classified as shock sensitive in Europe,[5] further process considerations had to be addressed in developing a scalable, safe, and economical process. Ultimately, a one-stage process which consists of using a single organic solvent with inexpensive reagents was developed. This method allows for a fast, yet safe, formation of the dipeptide acid without racemization of the *L-tert*-leucine, and culminates in a robust crystallization of **1**. Development of this process, which could serve as the primary means for the preparation and isolation of key intermediate **1** became our goal.

While acid chlorides are the most common acylating agents, the use of a mixed anhydride would be preferred for substrates that are prone to epimerization. Carbonyl diimidazole (CDI) resulted in a poorly reactive imidazolide intermediate which is known to require activation by addition of catalysts such as 1-hydroxybenzotriazole (HOBt) when applied to hindered carboxylic acids and/or weakly nucleophilic amines.[6] Coupling based on carboxylic-sulfonyl-mixed anhydride intermediate using TsCl/DMAP[7] or TsCl/*N*-methylimidazole[8] protocols were considered as an alternative to provide a scalable process.

RESULTS AND DISCUSSION

The bulkiness of the *tert*-butyl substituent in capped-*L-tert*-leucine **4**, accompanied by the carbamate protection of the aminoacid was expected to limit the possibility of epimerization during the peptide coupling under mixed anhydride conditions. Therefore, we first examined the coupling via the mixed-anhydrides of isobutylchloroformate[9] and pivaloyl chloride. In both cases, incomplete regioselectivity was observed[9c] with more than 15% of the undesired products **6** and **7** formed, which accounted in part for the moderate yield (70%) of the desired product under these conditions (Scheme 3). ReactIR monitoring indicated fast formation of the mixed anhydride with pivaloyl chloride in THF or acetonitrile (1811 and 1745 cm^{-1}).[10] However, the slow reaction of the mixed anhydride with hydroxyproline at 5 °C, required 16 h to reach 95% conversion.

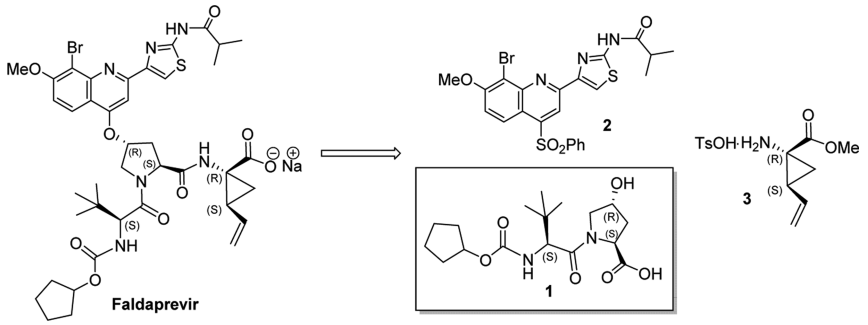
In an attempt to address the low reactivity and regioselectivity of the pivaloyl mixed anhydride, sulfonyl mixed anhydrides of methanesulfonyl chloride (MsCl) and *p*-

Special Issue: Process Analytical Technologies (PAT) 14

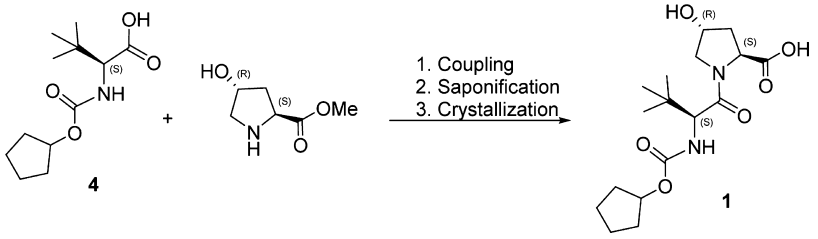
Received: October 11, 2013

dx.doi.org/10.1021/op400285y | Org. Process Res. Dev. XXXX, XXX, XXX–XXX

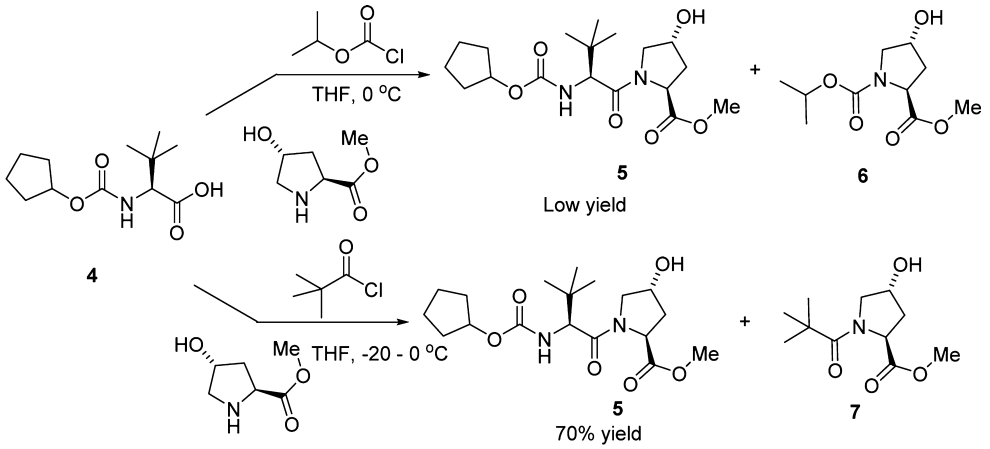
Scheme 1. Retrosynthesis of faldaprevir



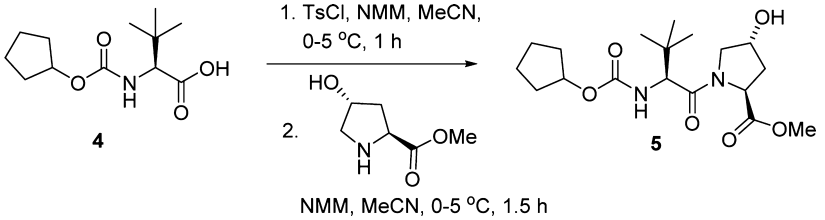
Scheme 2. Three-step, one-stage synthesis of dipeptide acid 1



Scheme 3. Unsatisfactory couplings using isobutylchloroformate or pivaloyl chloride conditions



Scheme 4. Preparation of dipeptide 5 under optimized conditions



toluenesulfonyl chloride (TsCl) became an attractive option for faster reaction[11a] of a less electrophilic[11b] leaving group. Screening of different organic bases and solvents identified *N*-methylmorpholine (NMM) as a suitable base in acetonitrile, resulting in a fast and selective conversion to the desired dipeptide **5** in >90% isolated yield without detectable isomerization[12] (Scheme 4). Acetonitrile was selected due to the rapid and clean reaction profile and its compatibility with the subsequent saponification step.

It should be noted that these reactions were selective and rapid in the absence of catalytic DMAP[7] or *N*-methylimidazole.[8] With these results in hand, TsCl was selected for further development to eliminate potential complications with MsCl on scale-up via possible sulfene formation.[13] For timely development of a robust and safe process, the reaction profile was followed by online IR data recording. The IR data in Figure 1 describe the reaction progress through the different steps. It first indicates a shift of the carboxylic acid frequency (1744 cm^{-1}) after addition of NMM to capped-*L*-*tert*-leucine **4**, followed by fast reaction with TsCl as evidenced by the disappearance of the carboxylic acid absorption at 1711 cm^{-1} and increase in the intermediate frequencies at 1825 cm^{-1} and

dx.doi.org/10.1021/op400285y | *Org. Process Res. Dev.* XXXX, XXX, XXX–XXX

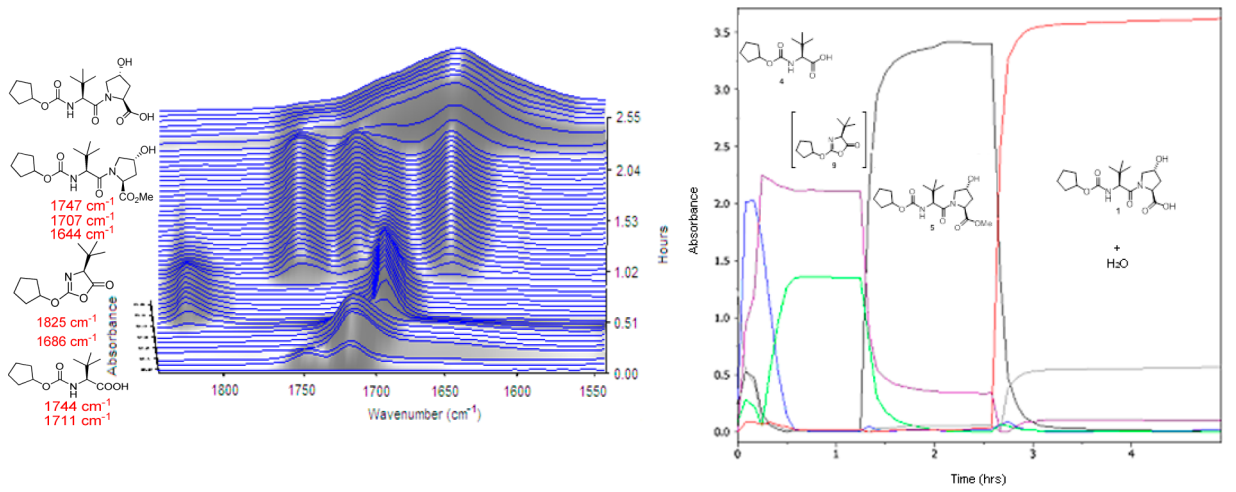
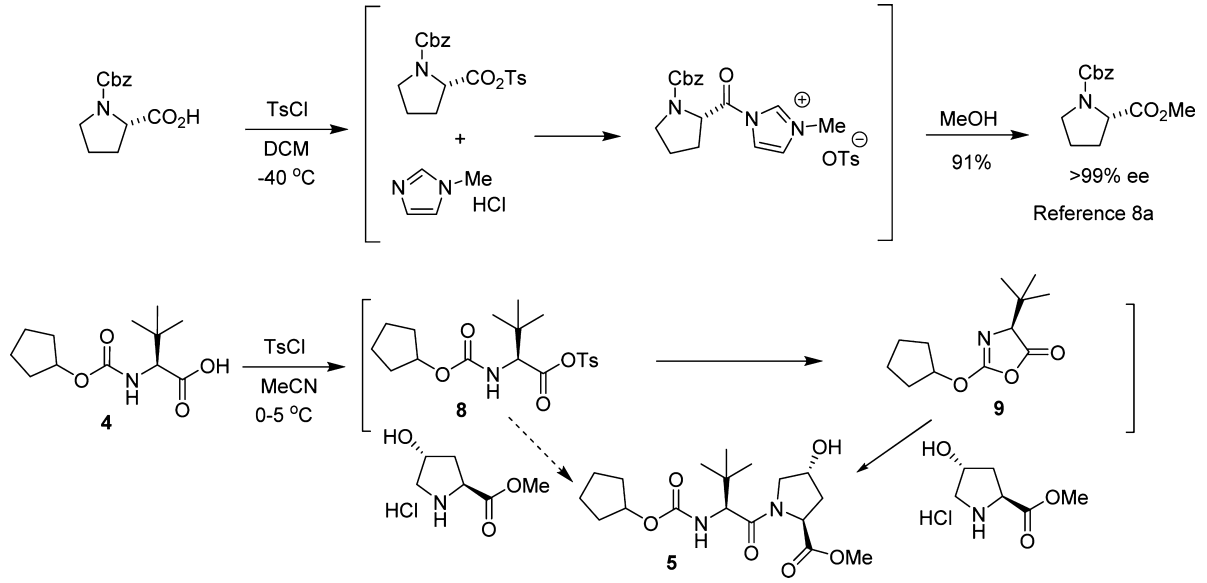


Figure 1. IR data from in situ monitoring throughout the sequence of preparing dipeptide **1**; blue: capped Leu **4**, green and purple: oxazolone **9**, black: dipeptide ester **5**; red: dipeptide acid **1** + H_2O.

Scheme 5. Possible effect of *N*-methylimidazole in activation of the tosyl-mixed anhydride intermediate; oxazolone 9 formed as major intermediate in a similar rate, in presence or absence of NMI



1686 cm^{-1}. The IR data is missing the typical carbamate carbonyl absorption (1745 cm^{-1})[10] that was observed with pivaloyl chloride reaction and expected in intermediate **8** (Scheme 5). However, the IR data was found consistent with the structure of cyclic intermediate 2-alkoxy-5(4*H*)-oxazolone (**9**).[10b,c] A sample of **9** was isolated and fully characterized by NMR, IR, and MS analyses. Addition of hydroxyproline HCl salt to the solution of **9** at 0−5 °C resulted once again in a fast reaction which provided the desired dipeptide ester **5** as evidenced by the intensity decrease of the oxazolone frequencies and formation of the amide signals at 1747, 1707, and 1644 cm^{-1}. The end point of this step was confirmed by HPLC and NMR analysis which indicated <1% of remaining starting material. Finally, the saponification step with LiOH was followed by the decay rate of the methyl ester signal at 1747 cm^{-1}. The desired dipeptide **1** was obtained in >90% yield with less than 0.05 A% of the product derived from epimerization of the *tert*-leucine fragment.

Unlike the reported slow esterification of carboxylic acids in the absence of *N*-methylimidazole (NMI),[8a] our studies indicate fast amide formation with hydroxyproline in the absence of NMI (Scheme 5). While this observation benefits the chemoselective preference for amide vs ester formation upon reaction with hydroxyproline, further evaluation of the addition of NMI to form the corresponding acyl-imidazolidinium intermediate was tested. IR data indicated fast formation of oxazolone **9** as the sole intermediate. Complete overlap in the reaction rates was obtained in the presence and absence of NMI.[8c]

While online IR monitoring has effectively provided valuable kinetic data along with structural confirmation of the key intermediates, the fast reaction rates emphasized the need for early calorimetric evaluation of the process to ensure process safety on multikilogram scale. The T_r and T_r-T_j data obtained from the MultiMax system provided a preliminary indication on the heat of reaction as described in Figure 2. In the MultiMax system, the temperature profile of the reaction in step 1

dx.doi.org/10.1021/op400285y | *Org. Process Res. Dev.* XXXX, XXX, XXX−XXX

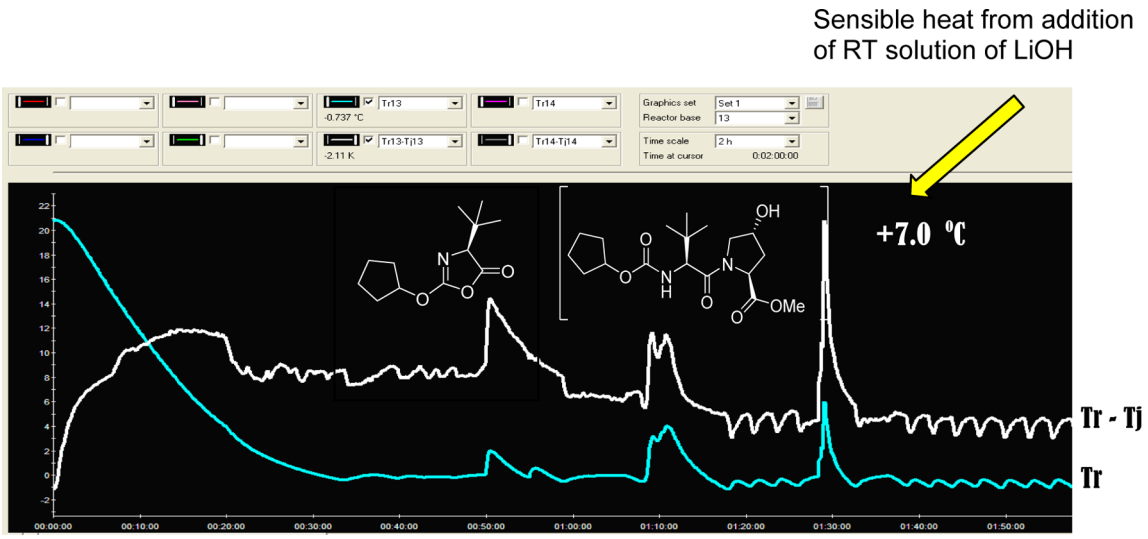
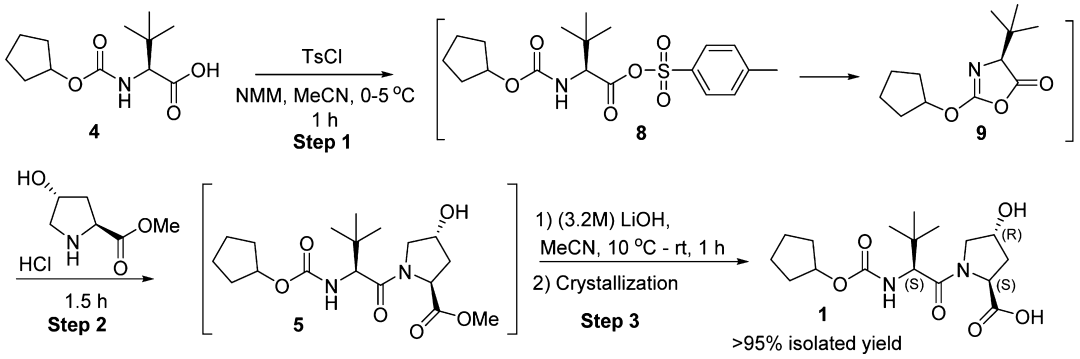


Figure 2. Heat flow data recorded by the MultiMax system throughout the experiment.

Scheme 6. Scale-up process



indicated a low exotherm ($T_r - T_j < 6$ °C) after addition of solid TsCl to the reaction mixture at 0 °C. Similarly, the IR data along with the observed $T_r - T_j < 6$ °C in step 2 after addition of hydroxyproline-HCl solid in one portion indicated a fast, yet safe, step. The saponification step was also completed in about 30 min following the addition of aqueous LiOH solution. At this step the value of $T_r - T_j$ =14 °C was attributed to sensible heat from addition of a higher temperature (RT) solution of the base. These results were subsequently confirmed by conducting more comprehensive process safety testing including differential scanning calorimetry (DSC), adiabatic calorimetry in the Advanced Reactive Systems Screening Tool (ARSST) and in reaction calorimetry (RC1*e*). RC1*e* experiments indicated an adiabatic temperature rise of 61 °C, 38 °C, and 17 °C for the three steps in the process, respectively.

With this information in hand, we obtained initial confidence in the safety and efficiency of this three-step, one-stage process for the formation of the dipeptide acid **1** and its isolation as described in Scheme 6.

The following procedure was thus applied on multikilogram scale: *N*-methylmorpholine was added to the precooled solution of capped-*tert*-leucine **4** in MeCN at 0−5 °C, followed by addition of TsCl solution while keeping the reaction temperature below 5 °C (20 min). The mixture was then stirred for 1 h at 5 °C. A suspension of *L*-4-hydroxyproline methylester HCl in MeCN was then added to the reaction mixture to result in complete conversion to **5** after 1.5 h at 0 °C. Aqueous LiOH solution (3.2 N) was added within 40 min. Stirring was continued at 25 °C for one hour to reach complete conversion to **1**. MeCN was then distilled under vacuum at 35 °C until GC analysis indicated less than 1% w/w of MeCN in the reactor. The pH was first adjusted to about pH = 7 by addition of 6 N HCl followed by heating the mixture to 60 °C. This operation was then followed by a second addition of 6 N HCl to reach a pH of 3.5−4. The batch was then seeded, and a final pH adjustment to about pH = 2 was made with 6 N HCl. Cooling to 22 °C and filtration then provided the desired product in 95% yield and purity of >98 wt %.

With the project advancing in development, our efforts turned to firming up the robustness of the coupling process for our manufacturing facility. The following topics were addressed: sensitivity of reaction rate to reactants concentration (e.g., hydroxyproline concentration) and impact of temperature on the coupling reaction rate. Blackmond's approach to kinetic analysis, Reaction Progress Kinetic Analysis (RPKA),[14] and iC Kinetics, was applied by running a set of experiments geared at evaluating process robustness. The reaction was investigated using EasyMax, a synthetic workstation which provides heat flow information, and ReactIR, an ATR-FTIR instrument that provides mid-IR spectroscopy information in real time.

We first evaluated the consistency between the information provided by EasyMax heat flow and changes in mid-IR signal. As shown in Figure 3, good correlation was obtained between the two plots following the formation of amide **5**.

dx.doi.org/10.1021/op400285y | *Org. Process Res. Dev.* XXXX, XXX, XXX−XXX

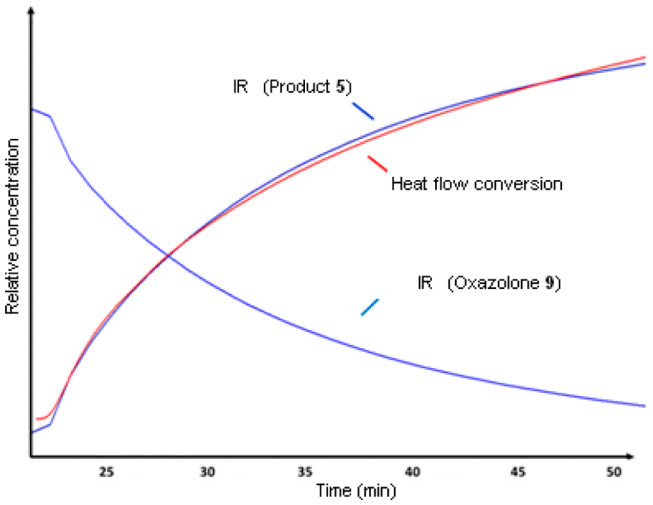


Figure 3. Correlation between conversion of oxazolone **9** to amide **5** vs time, data obtained by heat flow and IR.

Using the Different Excess strategy (DE), as described by Blackmond,[14] we investigated the coupling power law rate equation (Figure 4).

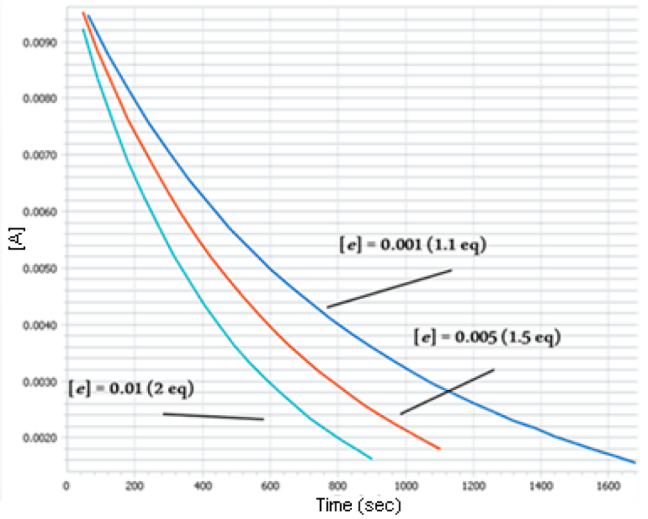


Figure 4. Oxazolone **9** concentration vs time in Different Excess experiments.

The data shown in Figure 4 was then used to build an empirical model using the iC Kinetics algorithm,[15] and determine reaction time to 90% conversion for 400 simulated experimental conditions (Figure 5).

A good linear regression correlation value (0.99) was obtained, which indicates that the amide formation reaction lends itself well to preliminary kinetic investigation and modeling. The model that best fits the experimental data gave partial orders in oxazolone **9** and hydroxyproline (0.78 and 0.69 respectively, rate constant 0.0115 M^{-1} s^{-1}) which likely indicates several elementary steps, or the presence of chemical equilibrium, involved in the formation of the oxazolone intermediate **9**. It should be noted that the initial rate of a fast reaction like this may not provide a full picture for reaction rate compared to a full data set of up to 90% conversion as calculated by the iC Kinetics. Reaction Progress Kinetic Analysis (RPKA) and iC Kinetics allow for going a step

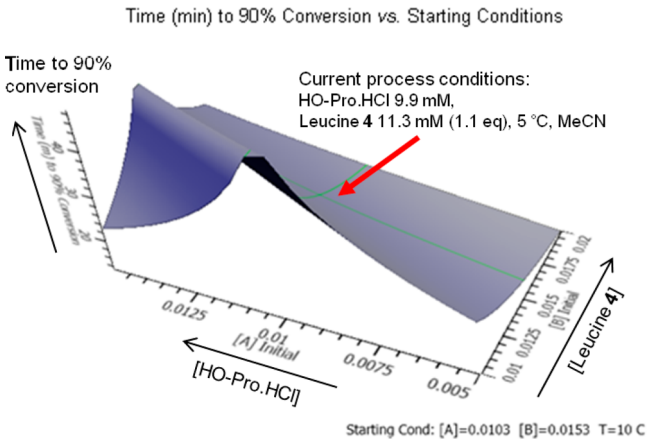


Figure 5. Empirical model for oxazolone 9 reaction with hydroxyproline, built using iC Kinetics

further by using the large number of data points made available by real time reaction monitoring while applying a more complex fit equation software.

We tested the developed model (rate constant and reaction orders) using the Different Excess strategy on a central point and obtained good levels of prediction for time-course concentration change. Furthermore, the calculated rate equation that best fits the model remains the same across the conversion range, confirming a stable reaction and a consistent mechanism throughout the step. This observation increased the level of confidence in the robustness of the process. The reaction was then tested following the consumption of oxazolone **9** at different temperatures across the −10 °C to +30 °C range. Faster conversion was obtained as the temperature increased with good fit (R^2 = 0.998) of the data points into an Arrhenius model.[16] Interestingly, when the reaction sequence was tested at 30 °C, 1.3% epimerization on the *tert*-butyl substituent was detected by HPLC analysis. Considering that the reaction investigated is unlikely an elementary step, the extension of the model outside the −10 to 30 °C temperature range might lead to erroneous conclusions.

In summary, a concise, safe, and scalable multistep synthesis of chiral dipeptide acid **1** was effectively achieved by early-stage utilization of PAT to allow processing of three steps in one operation which is completed within 10 h. MultiMaxIR was used to identify the formation and consumption rates of oxazolone **9** as a key intermediate and temperature profile of the reaction which indicated safe scale-up as subsequently confirmed by reaction safety calorimetry. Oxazolone **9** was effectively formed in the absence of *N*-methylimidazole, and provided dipeptide **1** without detectable epimerization when the reaction temperature was kept below 10 °C.

EXPERIMENTAL SECTION

General. Capped-*tert*-leucine **4** was prepared according to our reported procedure.[4] Data collection of adiabatic calorimetry was performed using ARSST manufactured by Fauske and Associates. Kinetic experiments were performed using METTLER TOLEDO EasyMax synthesis workstation equipped with 100 mL vessels in conjunction with iControl software. The accuracy of temperature control of this system is 0.01 °C. A METTLER TOLEDO ReactIR 15, based on attenuated total reflectance-Fourier transform IR spectroscopy

dx.doi.org/10.1021/op400285y | Org. Process Res. Dev. XXXX, XXX, XXX−XXX

(ATR-FTIR) and equipped with diamond-composite insertion fiber probe was used for real-time in situ process monitoring. HPLC analysis was carried out on an Agilent 1100 system equipped with a Waters YMC ODS-AQ, S-5, 120A, 250 mm × 4.6 mm column and detected at 210 nm. Mobile phase: **A**: mixture of 0.1% H_3PO_4 and 0.05% $HClO_4$ in HPLC grade H_2O with **B**: HPLC grade MeCN, was run in a gradient of 90%**A**/5% **A**, flow rate = 1.0 mL/min. HPLC retention times: **4** (15.73 min); **5** (15.6 min); (1*S*,3*R*,5*S*)-**1** (11.72 min), (1*S*,3*R*,5*R*)-diastereomer **1** (11.26 min); (1*R*,3*R*,5*S*)-diastereomer **1** (11.45 min); (1*S*,3*S*,5*S*)-diastereomer **1** (12.28 min); All NMR spectra were collected on Bruker spectrometers equipped with a 5 mm BBI probe (^1H,^{13}C). ^1H and ^{13}C chemical shifts were calibrated vs the deuterated solvent used.

Methyl (*S*)-2-(Cyclopentyloxycarbonylamino)-3,3-dimethylbutanoate (5). *Pivaloyl Chloride Procedure.* To a 50 mL vessel, equipped with nitrogen inlet, mechanical stirrer, IR probe recording every 30 s, and thermocouple, was added NMM (5.5 mL, 49.0 mmol) to a cold (5 °C) solution of capped-*tert*-leucine **4** (2.38 g, 9.8 mmol) in THF or MeCN (10 mL) while stirring at 300 rpm. A solution of pivaloyl chloride (1.3 mL, 10.76 mmol) in MeCN (1.5 mL) was added in over 2 min. The reaction mixture stirred for an additional 1 h at 5 °C. *L*-4-Hydroxyproline methylester HCl (1.95 g, 1.1 equiv) was added in one portion. The reaction stirring was continued at 5 °C for 16 h, at which point IR indicated the end of the reaction. Water (10 mL) was added, the organic solvent was removed under reduced pressure, and then the product was extracted with EtOAc (30 mL), washed with water (5 mL), dried over $MgSO_4$, and then concentrated under reduced pressure (30 mmHg). Purification by silica gel column, using 25% EtOAc in hexanes (R_f = 0.3), provided 2.5 g of the product in 70% yield.

Tosyl Chloride Procedure. To a 50 mL reaction vessel, equipped with a nitrogen inlet, mechanical stirrer, IR probe recording every 30 s, and thermocouple, was added NMM (5.5 mL, 49.0 mmol) to a cold (5 °C) solution of capped-*tert*-leucine **4** (2.38 g, 9.8 mmol) in THF or MeCN (10 mL) while stirring at 300 rpm. Tosyl chloride (2.05 g, 10.76 mmol) was added in one portion. The resultant mixture was stirred for 1 h at 5 °C until IR data indicated complete consumption of **4**. *L*-4-Hydroxyproline methylester HCl (1.95 g, 1.1 equiv) was then added in one portion. The reaction stirring was continued at 5 °C and was followed by recording IR for 1.5 h. Water (10 mL) was then added, the organic solvent was removed under reduced pressure, and the product was then extracted with EtOAc, washed with water (5 mL), dried, and then concentrated under reduced pressure. Purification by SGC (25% EtOAc in hexanes, R_f = 0.3) provided 3.2 g (89%) of the desired product. ^1H NMR (400 MHz, $CDCl_3$, major rotamer reported): 5.44 (d, *J* = 9.4 Hz, 1H), 5.02 (br s, 1H), 4.65 (t, *J* = 8.5 Hz, 1H), 4.50 (s, 1H), 4.25 (d, *J* = 9.5 Hz, 1H), 3.97 (d, *J* = 11.1 Hz, 1H), 3.73 (m, 1H), 3.71 (s, 3H), 2.45 (m, 1H), 2.0 (m, 1H), 1.9−1.5 (m, 8H), 1.02 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$, both rotamers): 172.6, 170.9, 156.8, 78.1, 70.1, 59.0, 57.8, 56.5, 52.2, 37.5, 35.7, 32.9, 32.6, 26.2, 23.7. Exact Mass: Calculated, [$C_{18}H_{30}N_2O_6$ + H^+] 371.2165; Experimental, 371.2177.

Kinetics Experiments. To a 200 mL reaction vessel, equipped with nitrogen inlet, mechanical stirrer, IR probe and thermocouple, was added NMM (2.8 mL, 25.5 mmol) to a cold (0 °C) solution of capped-*tert*-leucine **4** (1.2 g, 5.19 mmol) in MeCN (20 mL) while stirring at 250 rpm. A solution of tosyl chloride (1.0 g, 5.2 mmol) in MeCN (2.5 mL) was added in over 20−30 s; then the resulted mixture was stirred for 1 h at 10 °C. *L*-4-Hydroxyproline methylester HCl (2.04 g, 5.71 mmol) was added in one portion. The reaction was followed by recording IR (256 scans/min, 1 spectrum/min acquisition) for 1.5 h.

The experiment was repeated while varying the *L*-4-hydroxyproline methylester-HCl amounts as follows: Experiment 1: 2.04 g, 1.1 equiv; Experiment 2: 2.78 g, 1.5 equiv; Experiment 3: 3.71 g, 2.0 equiv.

(*S*)-4-(*tert*-Butyl)-2-cylopentyloxy-5(4*H*)-oxazolone (9). The above tosyl chloride procedure was repeated on 1.2 g of capped-*tert*-leucine **4**. A 2 mL sample was removed from the reaction mixture after 1 h from the addition of tosyl chloride. The sample was passed through a short column containing 10 g silica gel and eluted with 30 mL MeCN. The volatiles were removed under reduced pressure (30 mbar) to give 120 mg of oxazolone **9** in good purity and ∼80% yield: ^1H NMR (400 MHz, $CDCl_3$, 5.3 (m, 1H), 4.0 (s, 1H), 2−1.5 (m, 8H), 1.05 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): 174.8, 156.9, 83.4, 73.7, 31.9, 24.8, 22.9. Exact Mass: Calculated, [$C_{12}H_{19}NO_3$ + H^+] 226.1438; Experimental, 226.1428.

(2*S*,4*R*)-1-((*S*)-2-(Cyclopentyloxycarbonylamino)-3,3-dimethylbutanoyl)-4-hydroxy-pyrrolidine-2-carboxylic Acid (1). *Pilot Plant Procedure.* A 100-gal glass reactor was charged with capped-*tert*-leucine **4** (15.0 kg, 61.65 mol, 1 equiv) and MeCN (30.0 L). The mixture was cooled to 0 °C followed by addition of NMM (31.4 kg, 308.3 mol, 5.0 equiv) to give a colorless solution. The internal temperature was then reduced to −20 °C, and a solution of tosyl chloride (12.15 kg, 63.75 mol, 1.03 equiv) in MeCN (30.0 L) was added over 80−100 min. The internal temperature was maintained below 5 °C. The resulting mixture was stirred for an additional 1 h from the end of the addition at 0 °C, then cooled to below −2 °C. A slurry of *L*-4-hydroxyproline methylester HCl (12.25 kg, 67.50 mol, 1.1 equiv) in MeCN (30.0 L) was then added over 25−30 min, maintaining the temperature below 10 °C during the addition. The mixture was held for 1.5 h at 0−15 °C, and HPLC analysis showed that less than 0.22% of **9** remained (checked as the benzylamide derivative). LiOH (3.2 M, 90.0 L, 12.10 kg in 85.5 L H_2O; 288.0 mol,4.67 equiv) was then added to this mixture of ester **5** over 40 min, while maintaining the internal temperature below 15 °C. The mixture was then warmed to 23 °C and stirred for an additional 1 h; HPLC analysis showed complete consumption of the intermediate ester. The MeCN was removed by distillation under reduced pressure (30−45 °C/30 mbar), then HCl (6 N, 25.5 L, 28.4 kg, 153.0 mol) was added over 25 min while the internal temperature was maintained below 35 °C. The final pH value was 7−8. Residual MeCN was then removed by distillation (45 °C/30 mbar) to below 1% w/w. The mixture was then heated to 60 °C, and HCl (6 N, 28.75 kg, 154 mol) was slowly added to reach pH 3.6; then the batch was seeded with a slurry of 75 g dipeptide acid **1** seeds in 1 L H_2O. The agitation was continued at 60 °C for 30 min to establish a seed bed. An additional charge of HCl (6 N, 11.05 kg) was then added over 1 h to reach pH 0.99 at 60 °C (pH 1.3 at 23 °C). After stirring for an additional 1 h at 60 °C, the mixture was cooled to 23 °C over 1 h and then aged at that temperature for an additional 1 h. The slurry was then filtered (about 50 min), and the filter cake was washed with HCl (0.1 N, 2 × 25.0 L), then with H_2O (25.0 L). The solid was dried at 50 °C/30 mbar with an N_2 bleed to give 20.65 kg of dipeptide acid **1** as a white crystalline solid in 91% overall yield. HPLC purity: 99.91%; wt % purity: 101.14%;

dx.doi.org/10.1021/op400285y | *Org. Process Res. Dev.* XXXX, XXX, XXX−XXX

H_2O = 0.093% (KF); thermogravimetric analysis loss = 0.854%. Mp 117−124 °C; ^{1}H NMR (400 MHz,$CDCl_3$, major rotamer reported): 5.51 (d, J = 9.2 Hz, 1H), 5.02 (br s, 1H), 4.70 (t, J = 8.8 Hz,1H), 4.52 (s, 1H), 4.31 (d, J = 9.2 Hz, 1H), 4.01 (d, J = 11.2 Hz, 1H), 3.68 (dd, J = 3.6, 11.6 Hz, 1H), 3.06 (s, 1H, 0.33 MTBE), 2.08 (m,1H), 1.87−1.48 (m, 9H), 1.09 (s, 3H, 0.33 MTBE), 0.92 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$, both rotamers): 173.5 (s), 172.5 (s), 157.1 (s), 78.5 (d), 69.8 (d), 59.1 (d), 58.2 (d), 56.8 (t), 49.6 (MTBE), 36.7 (t), 35.5 (s), 32.8 (t), 32.7 (t), 26.3 (d), 23.7 ppm (t). Elemental Analysis: Calculated, C: 57.29; H: 7.92; N: 7.86; Experimental, C: 57.16; H: 7.88; N: 7.86.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, IR-spectra, selected ^{1}H NMR spectra for reactions study, ^{1}H and ^{13}C NMR spectra for all new compounds, full structure determination of oxazolone **9** and calorimetric data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: nizar.haddad@boehringer-ingelheim.com

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) (a) Llinas-Brunet, M.; Bailey, M. D.; Bolger, G.; Brochu, C.; Faucher, A. M.; Ferland, J. M. *J. Med. Chem.* **2004**, *47*, 1605−1608. (b) Lamarre, D.; Anderson, P. C.; Bailey, M.; Beaulieu, P.; Bolger, G.; Bonneau, P. *Nature* **2003**, *426*, 186−189. (c) LaPlante, S. R.; Llinas-Brunet, M. *Curr. Med. Chem.: Anti-Infective Agents* **2005**, *4*, 111−132. (d) Tsantrizos, Y. S. *Bioplymers* **2004**, *76*, 309−323.

(2) Yee, N. K.; Farina, V.; Houpis, I. N.; Haddad, N.; Frutos, R. P.; Gallou, F.; Wang, X.-J.; Wei, X.; Simpson, R. D.; Feng, X.; Fuchs, V.; Xu, Y.; Tan, J.; Zhang, L.; Xu, J.; Smith-Keenan, L. L.; Vitous, J.; Ridges, M. D.; Spinelli, E. M.; Johnson, M.; Donsbach, K.; Nicola, T.; Brenner, M.; Winter, E.; Kreye, P.; Samstag, W. *J. Org. Chem.* **2006**, *71* (19), 7133−7145.

(3) Highleyman, L. Experimental HCV Protease Inhibitor BI 201335 Shows Robust Antiviral Activity in Genotype I Non-Responders. In Proceedings of the 45th Annual Meeting of the European Association for Study of the Liver (EASL), Vienna, Austria, April 14−18, 2010.

(4) Busacca, C. A.; Wei, X.; Haddad, N.; Kapadia, S.; Lorenz, J. C.; Saha, A. K.; Varsolona, R. J.; Berkenbusch, T.; Campbell, S. C.; Farina, V. *Asian J. Org. Chem.* **2012**, *1*, 80−89.

(5) Weisenburger, G. A.; Vogt, P. F. *Org. Process Res. Dev.* **2006**, *10* (6), 1246−1250.

(6) (a) Dunn, P. J.; Hoffmann, W.; Kang, Y.; Mitchell, J. C.; Snowden, M. J. *Org. Process Res. Dev.* **2005**, *9*, 956. (b) Carey, J. S.; Laffan, D.; Thomson, C.; Williams, M. T. *Org. Biomol. Chem.* **2006**, *4*, 2337−2347.

(7) (a) Wakasugi, K.; Nakamura, A.; Tanabe, Y. *Tetrahedron Lett.* **2001**, *42*, 7427−7430. (b) Wakasugi, K.; Nakamura, A.; Iida, A.; Nishii, Y.; Nakatani, N.; Fukushima, S.; Tanabe, Y. *Tetrahedron* **2003**, *59*, 5337−5345. (c) Hamada, T.; Horikoshi, H.; Ishibashi, K.; Kojima, K.; Kurata; H. U.S. Patent 5,536,714, 1996.

(8) (a) Wakasugi, K.; Iida, A.; Misaki, T.; Nishii, Y.; Tanabe, Y. *Adv. Synth. Catal.* **2003**, *345*, 1209−1214. (b) Angelaud, R.; Davies, I.; Maguire, C.; Lau, S.; O'Shea, P.; Shultz, S.; Patent WO/2010/11584 A2, 2010. (c) Please refer to Supporting Information for a comparison graph of reaction rates in the presence and absence of *N*-methylimidazole.

(9) (a) Beaulieu, P. L.; Lavallee, P.; Abraham, A.; Anderson, P. C.; Boucher, C.; Bousquet, Y.; Duceppe, J. S.; Gillard, J.; Gorys, V.; Grand-Maitre, C.; Grenier, L.; Guindon, Y.; Guse, I.; Plamondon, L.; Soucy, F.; Valois, S.; Wernic, D.; Yoakim, C. *J. Org. Chem.* **1997**, *62*, 3440−3448. (b) Herrmann, M.; Ehrler, J.; Kayser, H.; Rindlisbacher, A.; Hofle, G. *Eur. J. Org. Chem.* **1999**, 3381−3392. (c) Valeur, E.; Bradley, M. *Chem. Soc. Rev.* **2009**, *38*, 606−631.

(10) (a) Wilder, R.; Mobashery, S. *J. Org. Chem.* **1992**, *57*, 2755−2756. (b) Coste, J.; Frerot, E.; Jouin, P. *J. Org. Chem.* **1994**, *59*, 2437−2446. (c) Benoiton, N. L.; Chen, F. M. F. *Can. J. Chem.* **1981**, *59*, 384−389.

(11) (a) Relative rates are consistent with the pK_a values in acetonitrile for sulfonic acids (TsOH = 8.5) and carboxylic acids (acetic acid = 23.5); pK_a values from: Eckert, F.; Leito, I.; Kaljurand, I.; Kutt, A.; Klamt, A.; Diedenhofeni, M. *J. Comput. Chem.* **2008**, *30*, 799−810. (b) Um, I.; Chun, S. M.; Bae, S. K. *Bull. Korean Chem. Soc.* **2005**, *26*, 457−460.

(12) HPLC method that separates dipeptide acid **1** from its corresponding diasteromers (1*S*,3*R*,5*R*), (1*R*,3*R*,5*S*), and (1*S*,3*S*,5*S*) was developed. Please refer to Supporting Information for details.

(13) (a) Opitz, G.; Ehlis, T.; Rieth, K. *Chem. Ber.* **1990**, *123*, 1989−1998. (b) King, J. F.; Lam, J. Y. L.; Skonieczny, S. *J. Am. Chem. Soc.* **1992**, *114*, 1743−1749.

(14) Blackmond, D. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 4302−4320.

(15) iC Kinetics is a graphical method for analyzing how different experimental conditions influence the speed of a chemical reaction. It imports concentration data from in situ spectroscopic measurements or conversion values from reaction calorimetric for kinetic analysis. The kinetic model created by the software can be used to simulate the effect of concentration and temperature parameters on the performance of the reaction. An extensive overview of the Reaction Progress Kinetic Analysis (RPKA) approach, adopted in iC Kinetics, is described in reference 14.

(16) Please refer to Supporting Information for additional details.