

Case Studies in Process Screening and Optimization Utilizing a New Automated Reactor—Analysis System

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Abstract:

The application of a new automated workstation for conducting solution-phase reactions in parallel during chemical process development is described. The fully automated reactor—analysis system is capable of reagent/reactant addition, temperature and agitation control, and sample extraction/quench/injection for online HPLC analysis. Both precision and accuracy of the fluidic delivery and sampling systems were measured through the use of standard ultraviolet chromophore solutions in conjunction with HPLC analysis. Agitation efficiency was evaluated using a phase-transfer catalysis reaction known to be rate-dependent on stirring frequency. Chemistry cases examined included several single and multistep reactions studied to identify the best reagent/solvent combinations (screening) and optimal levels of continuous variables such as temperature, concentration, and stoichiometry (optimization). A multistep route to a chiral substituted imidazolidin-2-one was developed from initial route scouting through reaction condition screening, optimization, and scale-up. Reactions performed included alkylation, reduction, oxidation, reductive amination, displacement, and cyclization.

Introduction

Chemical development in the pharmaceutical industry can be defined to include everything between initial compound discovery and routine batch or continuous production at full manufacturing scale. As a part of chemical development, a synthetic route used by discovery chemists may be developed and optimized, or completely new routes, taken from literature precedent (or new invention), may be investigated for applicability. As there is usually immediate demand for material, both of these activities often occur simultaneously. Historically, the race to discover the most facile, economic, and environmentally acceptable method before regulatory deadlines expire has placed extreme pressure on the development chemist. This has become particularly acute in the pharmaceutical arena where combinatorial chemistry, parallel synthesis, and high-throughput screening have accelerated the promotion of compounds into clinical development.

Automation has played a key role in streamlining many routine laboratory operations. Chemists have benefited from tools such as autosamplers coupled with analytical instrumentation, robotic liquid handlers, and automated reactor devices for synthesis.¹ Combinatorial chemistry has been the main driving force for the development of high-throughput synthesis technologies in drug discovery. As a result,

medicinal chemists now have multiple choices for parallel synthesis equipment ranging from simple manual reactor blocks to fully automated platforms.² More recently, synthesis instruments designed specifically for the activities of process chemists have become commercially available.³

The development of new chemical processes can be divided into four phases: route scouting, screening, optimization, and validation. In *Route Scouting* completely different pathways leading to a target structure are investigated. In *Process Screening* the general route has been established and reaction parameters, that is, temperature, reagent, solvent, and so forth, are examined over broad ranges to determine the most important variables and most effective reagents. Once the reactions are completely defined from these studies, *Process Optimization* is initiated to thoroughly study all parameters, including reproducibility, workup technique, and product isolation. Last, in *Process Validation* the reactions are tested at larger scale to mimic plant conditions and further define process ranges and limits. We have developed an automated workstation (Surveyor) specifically designed for conducting process screening and optimization during chemical process development. The fully automated reactor—analysis system is capable of reagent/reactant addition, temperature and agitation control, and sample extraction/quench/injection for online HPLC analysis. Table 1 summarizes the main instrument features and capabilities. This paper describes the system tests and chemistry case studies in process screening and optimization conducted during the course of instrument development and validation.

Results and Discussion

Reagent Delivery and Sampling. Precise and accurate delivery of starting materials and reagents is of obvious importance in any reaction, automated or otherwise. During the course of instrument development, delivery and sampling were tested and validated prior to conducting chemical reactions. Both precision and accuracy of the fluidic delivery and reactor sampling systems were measured through the use of standard solutions of biphenyl and anthracene in conjunction with HPLC analysis. To test the delivery system in isolation, a standard biphenyl solution was placed in a Surveyor reagent position, and 100 μL aliquots were

(2) Hird, N. W. *Drug Discovery Today* 1999, 4, 265–274.

(3) (a) Wagner, R. W.; Li, F.; Du, H.; Lindsey, J. S. *Org. Process Res. Dev.* 1999, 3, 28–37. (b) Armitage, M. A.; Smith, G. E.; Veal, K. T. *Org. Process Res. Dev.* 1999, 3, 189–195. (c) Emiabata-Smith, D. F.; Crookes, D. L.; Owen, M. R. *Org. Process Res. Dev.* 1999, 3, 281–288. (d) Gooding, O. W. Presented in part at the 3rd International Symposium, The Evolution of a Revolution: Laboratory Automation in Process Research & Development, Boston, 2000.

(1) For a very recent review, see: Harre, M.; Tilstam, U.; Weinmann, H. *Org. Process Res. Dev.* 1999, 3, 304–318.

Table 1. Surveyor features and capabilities

number of reactors	10 independent vessels
reactor volume	57 mL, 15–40 mL (working volume), inertness is maintained by a nitrogen blanket delivered to the headspace
agitation	vertical oscillation, adjustable, two zones
reactor temperature settings	range: –99 to +150 °C; ramping rates are controllable
heating/cooling rates	control accuracy: ± 2 °C between –40 and 150 °C
reagent/solvent additions	rate: ambient to +150 °C (30 min) ambient to –40 °C (30 min)
liquid delivery (fluidics)	automated additions of up to nine different bulk liquids from reservoirs via the fluidic system or up to 176 ^a solutions or slurries from sample vials via robotic syringe; metering rates are controllable
sampling/quench/analysis	manual additions of solutions, slurries, and solids min: 100 μ L, max: 25 mL (per aliquot), increment: 10 μ L automated sampling: up to 176 ^a total samples per run adjustable volume: 100 μ L to 1 mL automated quench, mix, and dilute automated HPLC injection adjustable sampling phase selection
computer interface	drag and drop programming real-time data tracking complete log file generation import/export (Excel, text, etc.)

^a The total number of vial positions available for both reagents and samples.

Table 2. Sampling precision test

sample volume (μ L)	area average ^a	SD ^b	RSD ^c (%)	accuracy ^d (%)
100	506	21	4.23	1.34
300	1470	22	1.49	1.05
500	2367	74	3.15	3.69

^a Average peak area for 15 samples in mAU. ^b Standard deviation. ^c Relative SD. ^d Percent error determined from a calibration curve.

automatically delivered to each of five HPLC vials containing a fixed volume of acetonitrile. Offline HPLC analysis showed that an average of 99.98 ± 3.27 μ L had been delivered as determined by comparison with a standard calibration curve. This was well within the goal of ± 15 μ L at 200 μ L volume. Likewise, the sampling system was tested by a manual addition of a solution containing biphenyl to one of the reactors which was subsequently sampled at three different volume settings (100, 300, and 500 μ L) 15 times each via the robotic needle and deposited into collection vials. The aliquots were then automatically diluted and analyzed by HPLC (Table 2). The precision of sampling was determined directly from the HPLC area count data to have relative standard deviations of 4.23, 1.49, and 3.69% for the 100, 300, and 500 μ L samples, respectively. The accuracy of these sample volumes was determined by comparison with a calibration curve and ranged from 1.05 to 3.69% error. It is important to note that the error associated with the dilution and HPLC analysis is also included in the figure. Precise (reproducible volumes) sampling is of crucial importance because this allows direct comparison of HPLC area counts among different samples without need of an internal standard or the use of peak ratios. For example, in a 10-reaction screening experiment employing 10 different solvents, the sample giving the most area for the product peak in the HPLC would also be the reaction with the most product present.

Table 3. Experimental parameters for the chromophore test

RV#	biphenyl (μ L)	anthracene (μ L)
1	500	2500
2	1000	0
3	1500	2500
4	2000	0
5	2500	2500
6	3000	0
7	3500	2500
8	4000	0
9	4500	2500
10	5000	0

With verification that the delivery and sampling components of the system were performing within specification, a self-test of the entire system working together (chromophore test) was developed. In this test, two Surveyor reservoirs were charged with standard solutions of biphenyl (50 mg/mL) and anthracene (25 mg/mL). Automatic deliveries of anthracene, biphenyl, or both were then made to all 10 reaction vessels (RVs) as defined in Table 3. The volume of biphenyl was progressively increased across RVs 1–10 while the volume of anthracene was held constant and added only to alternating (odd) vessels. In this way accuracy, precision, and cross contamination of all system components were checked simultaneously. Each RV was sampled three times. A plot of the HPLC area counts derived from the biphenyl peaks gave a straight line showing that it had been accurately and precisely delivered and that the sampling volumes were uniform. The anthracene points gave a horizontal line showing that reagent delivery and sampling were uniform with an average relative standard deviation of 3.1%. No cross contamination of anthracene into even RVs was detected (Figure 1).

Agitation. Agitation efficiency is an important reaction variable that must be considered during chemical develop-

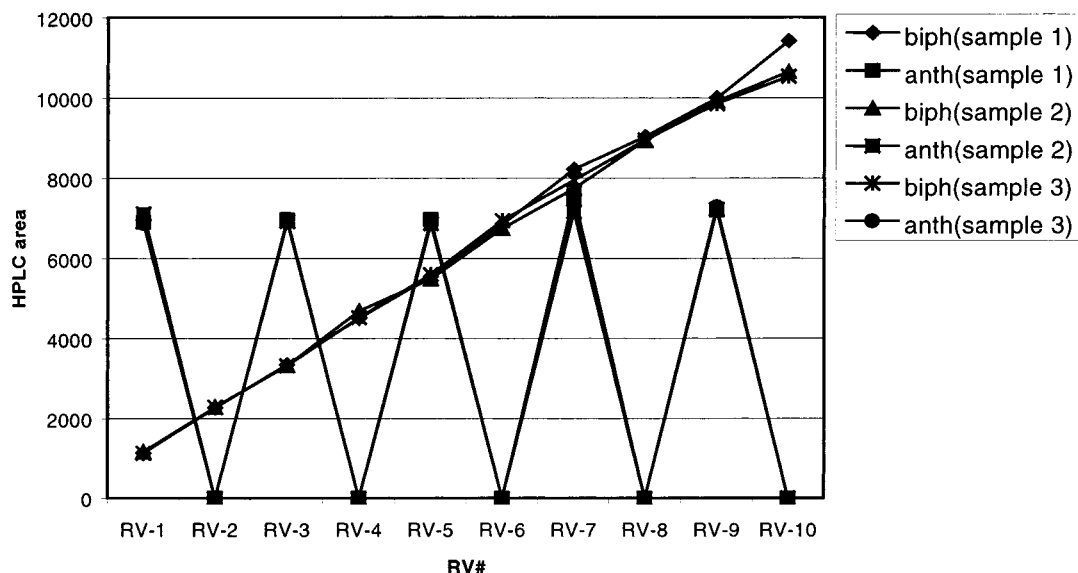
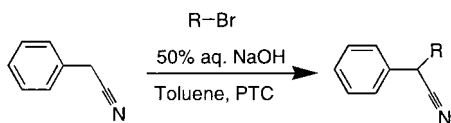


Figure 1. Plot of reaction vessel number (RV#) vs HPLC area for anthracene and biphenyl in the chromophore test.

Scheme 1. Alkylation of phenylacetonitrile (PAN)



ment and scale-up. During progression from discovery through the process development cycle, agitation methodology is often changed from magnetic to mechanical paddles and ultimately to reactor impellers. These graduations can be accompanied by inconsistent results with some reaction types being more sensitive than others. It is therefore desirable to have very efficient (though realistic) agitation in any reactor system used for chemical development, particularly for optimization and validation. After considering the alternatives of magnetic spinning bars and mechanical paddle stirrers, we settled on the technique of magnetically coupled vertical oscillation that had proven effective in other automated reactors.⁴ This technique was more easily implemented than other commonly used forms; however, validation of its performance relative to standard methods was required.

A phase-transfer catalysis (PTC) reaction known to be rate-dependent almost exclusively on stirring frequency⁵ was used to compare agitation by vertical oscillation with the other standard laboratory method of mechanical stirring paddles. The reaction chosen was the alkylation of phenylacetonitrile (PAN) with ethyl bromide in the presence of tetrabutylammonium bromide (TBAB) as shown in Scheme 1.

This dependence may be explained by a mechanism where deprotonation of PAN occurs only at the aqueous/organic interface while the catalyst functions only to transport the anion from the interface into the bulk organic phase where alkylation rapidly occurs. The rate-determining step is the

deprotonation; therefore, the better the mixing, the larger the surface area of biphasic contact, the faster the rate. By measuring the rate of disappearance of PAN (V_0) in a round-bottomed flask at five different stirring speeds (RPM), a series of lines with slope equal to V_0 was obtained (Figure 2). Plotting the log of V_0 vs the log of the RPM (Figure 3) then produced a calibration curve. This curve was a polynomial described by eq 1. Solving eq 1 for RPM provided eq 2. With eq 2 in hand, the rate of this reaction was then measured under various instrument agitation settings, and a corresponding "effective RPM" was calculated.

$$\log V_0 = 3.9694(\log \text{RPM})^2 - 18.731(\log \text{RPM}) + 18.088 \quad (1)$$

$$\text{RPM} = \text{antilog} \left\{ \frac{(18.731 + [(-18.731)^2 - 4(3.9694)(18.088 - \log V_0)]^{0.5})}{2(3.9694)} \right\} \quad (2)$$

Thus, a quantitative method for evaluating the efficiency of vertical oscillation (or any other type of agitation) relative to a standard mechanical paddle in a round-bottomed flask was developed. The instrument's performance at two different vertical oscillation settings and three different volumes is shown in Table 4. At a rate of 1 stroke/second, the effective RPM stirring rate of 1011, 796, and 591 was observed at a volume of 20, 30, and 40 mL, respectively. Cycle times of as little as 0.5 strokes per second are possible so that even more vigorous agitation may be achieved on Surveyor. For comparison purposes the reaction rate was also measured when magnetic stirring was employed. A stir plate with a commonly used setting of 3.5 (provides a good vortex) gave a calculated effective RPM of 464. A setting of 7.0 (maximum setting before decoupling of the magnet occurred) corresponded to 879 rpm. These results suggest that vertical oscillation provides more effective mixing than a standard magnetic stirring plate is capable of. It is important to note that this is only a comparison of agitating a biphasic mixture and will not necessarily be valid for all types of mixtures.

(4) This technique is used in the Quest 210, Quest 205, and First Mate Synthesizers (Argonaut Technologies).

(5) Solaro, R.; D'Antone, S.; Chiellini, E. *J. Org. Chem.* **1980**, *45*, 4179-4183.

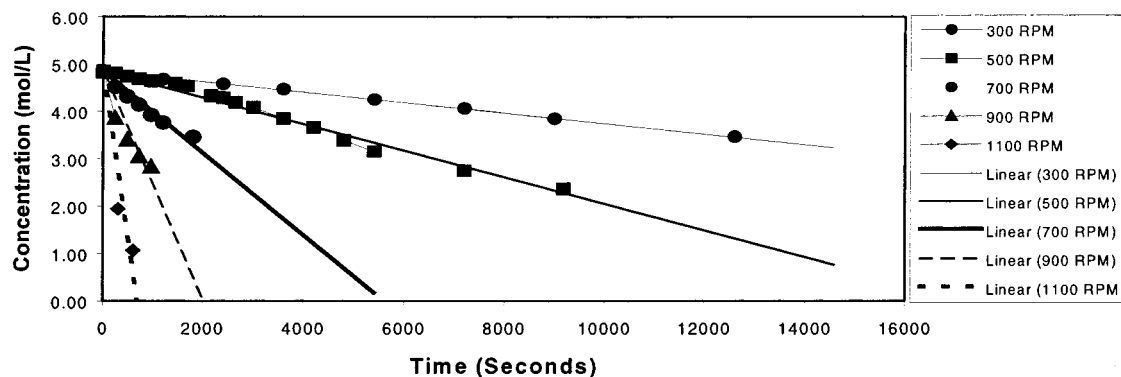


Figure 2. Plot of the reaction time vs concentration of PAN at various stirring rates in a standard round-bottomed flask.

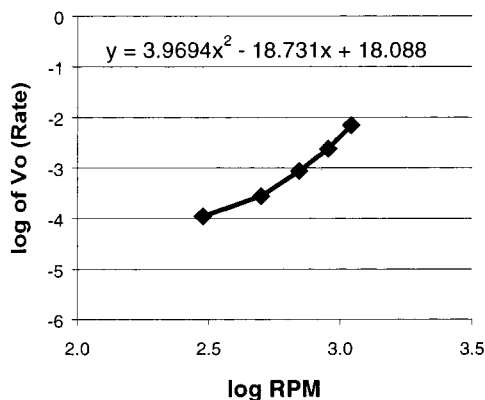


Figure 3. Plot of the log of stirring RPM vs log of the initial rate of disappearance of PAN in a standard round-bottomed flask.

Table 4. Calculated effective RPM rates for Surveyor, magnetic stir plate, and shaker

agitation type, setting	log V_0	calcd log RPM	calcd RPM
Surveyor, 30 mL 1 s/stroke	-2.8451	2.901	796
Surveyor, 30 mL 3 s/stroke	-3.3754	2.759	574
Surveyor, 40 mL 1 s/stroke	-3.3325	2.772	591
Surveyor, 20 mL 1 s/stroke	-2.3778	3.001	1011
Corning stir plate, 3.5	-3.6345	2.666	464
Corning stir plate, 7.0	-2.6535	2.944	879
Gyrotory shaker, 200 rpm	-2.957	2.784	749

Temperature Control. Temperature control and monitoring are of paramount importance during reaction development. The Surveyor is equipped with resistive heaters on each RV that are controlled by thermocouples *inside* each RV. Cooling is achieved by passing liquid nitrogen through a manifold in which there is a separate cryo-valve for each reactor that regulates flow in a jacket surrounding each reactor. Liquid nitrogen and heaters work in concert to control subambient temperature settings. Actual internal temperature is continuously measured, plotted in real time, and written to a log file for later reference. To demonstrate the effectiveness of a heating ramp, each of 10 RVs containing THF was programmed to heat to reflux (70 °C set point) over 30, 60, 90, 120, ..., 270 min, and the real-time plot generated by the Surveyor software is shown in Figure 4. A very linear response was observed, even over the longest ramp time. Refluxing is accomplished through the use of a heat exchanger through which a coolant is

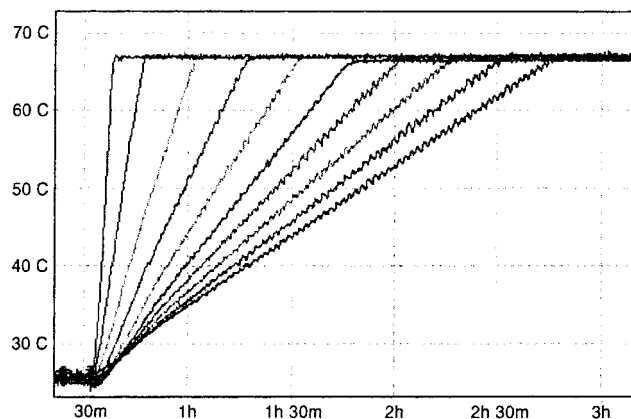


Figure 4. Plot of internal temperature vs time for 10 different heating ramp rates.

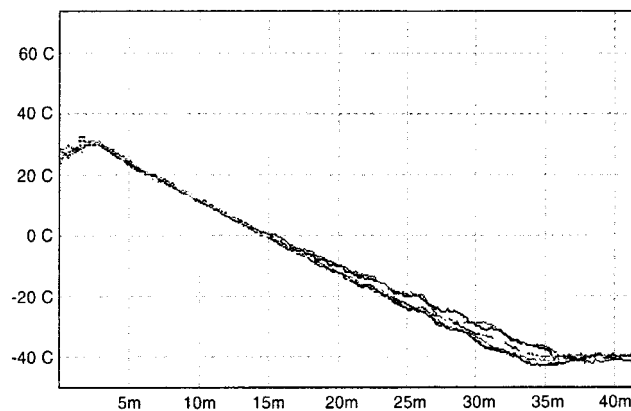


Figure 5. Plot of internal temperature vs time for 10 vessels with a fixed cooling ramp rate of 30 min.

circulated. Likewise, to test the effectiveness of cooling, all 10 RVs containing THF were cooled to -40 °C at a constant rate (Figure 5). The observed internal temperatures were linear and reasonably consistent among all 10 vessels. As a final test of cooling, the RVs were cooled to 10 different temperatures ranging from 20 to -70 °C and held for a period of 1 h (Figure 6). Again, ramps were linear, and the desired internal temperatures were held to within 2 °C of the set point even at -70 °C. It should be noted that these plots were created directly by the control software and are accessible in real time during an experiment.

Chemistry. Case 1. Our targets for chemistry validation centered on materials generally useful for combinatorial chemistry, that is, resins and linkers, or parallel synthesis,

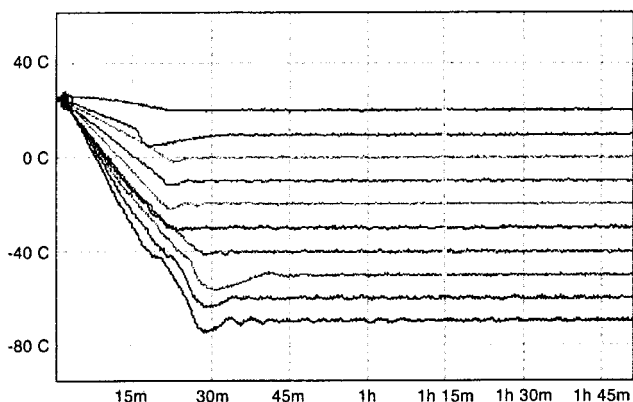
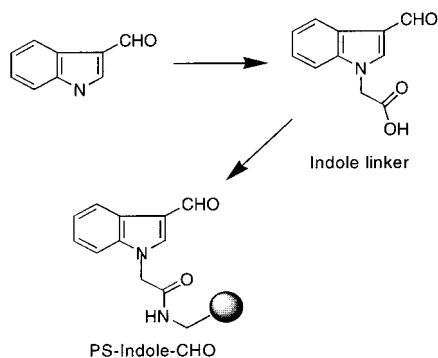


Figure 6. Plot of internal temperature vs time for cooling 10 vessels to different set points.

Scheme 2. Synthesis of PS–Indole-CHO resin



that is, scavengers and polymeric reagents. Several single and multistep reactions were studied to identify the best route (scouting), reagent/solvent combinations (screening), or optimal levels of continuous variables such as temperature, concentration, and stoichiometry (optimization). The indole linker⁶ is a key intermediate in the synthesis of PS–Indole-CHO,⁷ a polystyrene resin used in combinatorial synthesis when employing reductive amination/acylation methodology (Scheme 2). Literature conditions for the alkylation of indole-3-carboxaldehyde with ethyl bromoacetate (K_2CO_3 , DMF, 60 °C) gave 50% conversion at best in our hands at the bench. We chose to screen three solvents (DMF, THF, EtOH) and three bases, K_2CO_3 (2.5 equiv) KOH (2.5 equiv), and KOt-Bu (1.5 equiv) in this reaction for a total of nine reactions leaving one RV for a replicate. Operations were conducted as described in Table 5. All 10 RVs were manually charged with solid indole-3-carboxaldehyde followed by the solid bases: K_2CO_3 (RVs 1, 4, 7) or KOH (RVs 2, 5, 8). Solvents were then added automatically followed by the KOt-Bu solution in THF automatically. Agitation was initiated, and ethyl bromoacetate (1.0 equiv) was added neat over a 10-min period. The reactions were sampled, and a second charge of bromide (1.0 equiv) was added. The reactions were sampled again at 30 and 240 min. For ease of visualization graphs were constructed of HPLC area versus sample time for each RV.⁸ A representative plot for observed

peaks is shown for RV1 (Figure 7) and the complete data set is summarized in Table 6.

On the basis of considering this process step by itself, it appeared prudent to select KOH in either DMF or THF for highest conversion in shortest time. However, when the second step in the process (ester hydrolysis) was considered, a different conclusion was reached. From a throughput standpoint it was desirable to omit isolation of the intermediate ester by combining the two reactions into a “one-pot” operation. The second step involving ester hydrolysis (saponification) is normally carried out in polar protic solvents such as water or alcohol. Both DMF and THF were expected to be inferior to EtOH for ester hydrolysis. An additional consideration was that DMF and THF are more expensive than EtOH. Therefore, EtOH appeared to be a good solvent for attempting a one-pot synthesis that would avoid a solvent exchange. Examination of the data set in Table 6 showed that the best base for use in ethanol was KOt-Bu that gave a respectable 91% conversion. To test this hypothesis a scale-up run (1 kg) was then conducted using KOt-Bu in EtOH followed by in situ hydrolysis affording the acid in 84% isolated yield. The improved process was discovered using a combination of automated screening and general process development knowledge. Improvements included: (1) eliminating one isolation/drying step; (2) conversion improvement by selecting a different solvent–base combination; (3) raw materials cost savings and waste minimization; (4) cycle time improvement for both steps; (5) yield increase from 66 to 84%.

Case 2. A novel multistep route to a chiral imidazolidin-2-one was developed from initial route scouting through reaction condition screening, optimization, and scale-up. Our retrosynthetic approach is shown in Scheme 3. Imidazolidin-2-ones have been used as chiral auxiliaries in asymmetric aldol condensations,⁹ alkylations,¹⁰ Michael additions,¹¹ and Diels–Alder reactions.¹² They are also intermediates in the synthesis of a class of HIV protease inhibitors.¹³ The enantioselectivities achieved in alkylation reactions are similar to their analogue oxazolidinones; however, imidazolidin-2-one adducts are usually crystalline and are less susceptible to nucleophilic ring-opening, offering advantages in downstream purification (diastereomeric upgrade) and auxiliary recycling. Substituted imidazolidin-2-ones have been prepared by fusion of 1,2-amino alcohols with urea¹⁴ and by reaction of diamines with phosgene.¹⁵ In this new and general route, chirality is derived from readily available and relatively inexpensive amino acids **1** (Scheme 4). Carboxyl reduction followed by protection at nitrogen would

(6) Estep, K. E.; Neipp, C. E.; Stephens Stramiello, L. M.; Adam, M. D.; Allen, M. P.; Robinson, S.; Roskampet, E. J. *J. Org. Chem.* **1998**, 63, 5300.

(7) PS–Indole-CHO is commercially available from Argonaut Technologies.

(8) This feature is now a part of Surveyor software and is done automatically.

(9) Roder, H.; Helmchen, G.; Peters, K.; von Schnering, H.-G. *Angew. Chem., Int. Ed. Engl.* **1984**, 23, 895–896.

(10) (a) Cardillo, G.; D’Amico, A.; Orena, M.; Sandri, S. *J. Org. Chem.* **1988**, 53, 2354–2356. (b) Konigsberger, K.; Prasad, K.; Repic, O.; Blacklock, T. *J. Tetrahedron: Asymmetry* **1997**, 8, 2347–2354.

(11) Melnyk, O.; Stephan, E.; Pourcelot, P.; Cresson, P. *Tetrahedron* **1992**, 48, 841–850.

(12) Roos, G. H. P.; Jensen, K. N. *Tetrahedron: Asymmetry* **1992**, 3, 1553–1554.

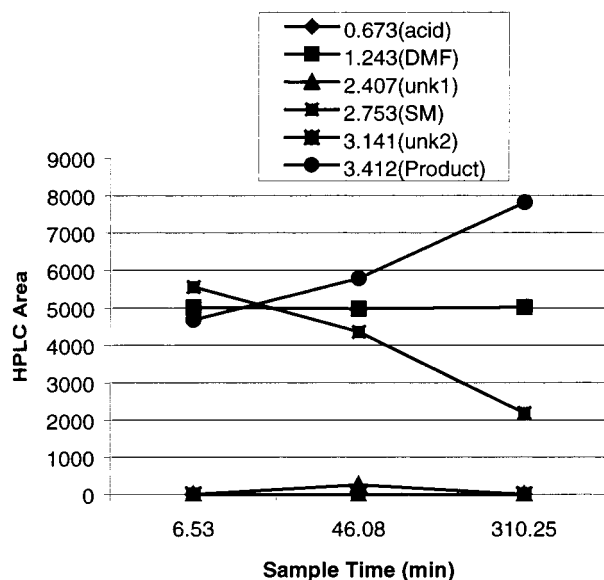
(13) Tung, R. D.; Salituro, F. G.; Deininger, D. D. International Patent WO 97/27180, 1997.

(14) Close, J. W. *J. Org. Chem.* **1950**, 15, 1131–1134.

(15) See ref 10b.

Table 5. Operations used in the indole alkylation solvent–base screening experiment

action	RV-1	RV-2	RV-3	RV-4	RV-5	RV-6	RV-7	RV-8	RV-9	RV-10
add quench to sample vials	×3	×3	×3	×3	×3	×3	×3	×3	×3	×3
manual add, indole (g)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
manual add, KOH (g)		1.94			1.94			1.94		
manual add, K ₂ CO ₃ (g)	4.79			4.79			4.79			
add THF (mL)							20	20	20	20
add EtOH (mL)				20	20	20				
add DMF (mL)	20	20	20							
add KOt-Bu (mL)			12.61			12.61			12.61	12.61
add bromide, 1 equiv (mL)	1.54	1.54	1.54	1.54	1.54	1.54	1.54	1.54	1.54	1.54
wait 5 min, sample all										
add bromide, 1 equiv (mL)	1.54	1.54	1.54	1.54	1.54	1.54	1.54	1.54	1.54	1.54
wait 30 min, sample all										
wait 240 min, sample all										

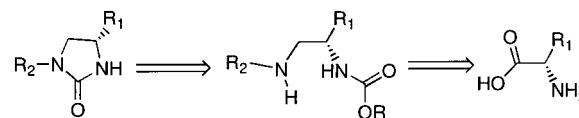

Figure 7. Plot of sample time vs HPLC area for RV-1 in the alkylation of indole-3-carboxaldehyde.
Table 6. Screening of bases and solvents in the alkylation of indole-3-carboxaldehyde

RV#	base	solvent	SM ^a	product ^a	% conversion ^b
7	K ₂ CO ₃	THF	8225	1112	11.9
4	K ₂ CO ₃	EtOH	7146	2107	22.8
5	KOH	EtOH	5557	4643	45.5
1	K ₂ CO ₃	DMF	2189	7821	78.1
3	KOt-Bu	DMF	813	6932	89.5
6	KOt-Bu	EtOH	632	6126	90.6
10	KOt-Bu	THF	253	6719	96.4
9	KOt-Bu	THF	235	6574	96.5
2	KOH	DMF	216	10768	98.0
8	KOH	THF	0	7735	100.0

^a HPLC area (mAU) for starting material (SM) or product. ^b Calculated using the formula % conversion = product peak area/total peak area.

give the pivotal intermediate **2**. Oxidation to the aldehyde **3** followed by reductive amination would give penultimate intermediate **5**. Alternatively, **5** could be accessible from **2** by a sequence of mesylation, to give **4**, followed by displacement.¹⁶ In either route, the alkoxy carbonyl group (Boc) serves as a protecting group through the early stages of the synthesis

(16) This is similar to the sequence described previously; see ref 13.

Scheme 3


and ultimately provides the urea carbonyl upon cyclization, obviating the need for phosgene (Scheme 6). In this work we employed L-phenylalanine (Phe) as the amino acid and benzylamine¹⁷ as the nitrogen nucleophile (R₁ = R₂ = benzyl) for route-scouting and screening studies. Each route was evaluated and compared from a scale-up standpoint.

In the first stage the synthesis of pivotal intermediate **2** was studied by a one-pot lithium aluminum hydride (LAH) reduction and Boc protection. The in situ N-protection of the polar amino alcohol intermediate facilitated product isolation from the spent aluminum salts. A literature procedure¹⁸ describing the preparation of Boc-phenylglycinol used LAH (2 equiv), THF (25 mL/g phenylglycine), Boc₂O (1.1 equiv), and DMAP (cat) to afford a 53% isolated yield. In our lab, a manual run with Phe in place of phenylglycine gave desired product in 43% isolated yield (average of 2 runs) along with 10–15% of a side product identified as bis-Boc-phenylalaninol. A third run conducted with the omission of DMAP led to a similar yield with no formation of bis-Boc byproduct. Therefore, addition of DMAP was found unnecessary and was not used in the automated run.

An automated run was then conducted in which the LAH charge and the total concentration were varied (Table 7). During the carboxyl reduction step each reaction suspension was sampled at 30, 90, and 270 min. The samples were quenched into aqueous NaOH, diluted with dioxane, allowed to settle, and injected onto an HPLC. This was found to be a clean conversion of Phe to the intermediate amino alcohol that reached a steady state after 90 min. Continuing on to the protection step, each reaction was automatically quenched with aqueous NaOH and treated with a DCM solution of di-*tert*-butyl dicarbonate. The reaction suspensions were again sampled over time, and complete conversion of the intermediate amino alcohol to Boc amine **2** was observed after 120 min. The overall process was found to be very

(17) An ultimate goal of this work is to develop an insoluble, solid-supported version of this chiral auxiliary. In this case, benzylamine serves as a model for aminomethyl polystyrene resin.

(18) Correa, A.; Denis, J.-N.; Greene, A. E. *Synth. Commun.* **1991**, *21*, 1.

Scheme 4. Two routes to the penultimate imidazolidinone intermediate 5

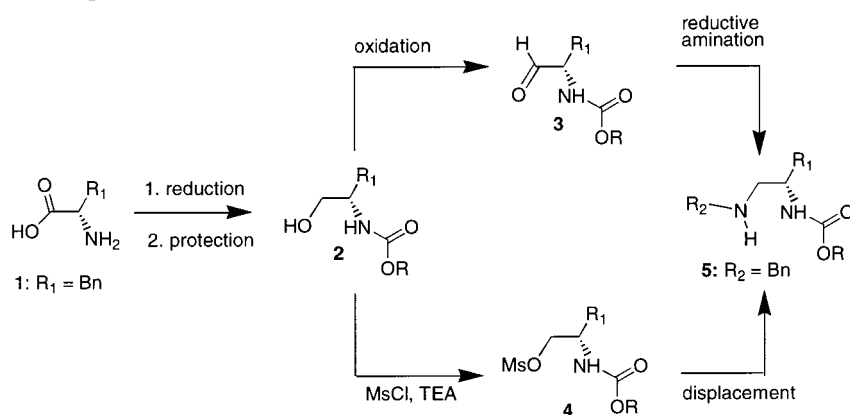


Table 7. Optimization of LAH equivalents and concentration in the conversion of amino acid (1) to *N*-Boc amino alcohol (2)

RV#	LAH (equiv)	concentration (mL/g)	HPLC area ^a
1	2.0	30	7005
2	1.75	30	6360
3	1.50	30	5902
4	1.25	30	3999
5	1.00	30	3893
6	2.00	20	5560
7	1.75	20	5594
8	1.50	20	4376
9	1.25	20	4406
10	1.00	20	3293

^a HPLC area counts in mAU associated with product, no other peaks were observed.

sensitive to concentration as well as stoichiometry. The highest concentration of product, as determined by HPLC area counts, was found in the reaction where 2.0 equiv of LAH was used under the more dilute conditions of 30 mL of THF/g Phe (RV1). Each reaction step was determined to require ~2 h rather than the 6 h for each step reported in the literature. A scale-up run was then conducted at the bench using the optimized conditions affording a 63% isolated yield (vs 43% unoptimized) with the reaction time being cut by a factor of 3 (Table 8). This scale-up run provided material for subsequent route-scouting studies.

Displacement Route. The mesylate **4** was prepared from alcohol **2** in quantitative yield under standard conditions,¹⁹ and the displacement reaction was studied with butylamine and benzylamine. Treatment of mesylate **4** with 20 equiv²⁰ of either amine in dioxane at 60 °C led to clean conversion to the desired amine (90% isolated). Efforts to reduce the amine charge to 3 equiv gave a second product that was isolated and identified as the oxazolidinone **7**. Precedence for this reaction was found in the literature²¹ where it was shown that treatment of analogue tosyl derivatives of **4** with DIEA gave oxazolidinone **7** in excellent yield (Scheme 5). Because it appeared that suppression of this side reaction

Table 8. Comparison of original literature conditions to the optimized conditions for the conversion of amino acid (1) to *N*-Boc amino alcohol (2)

literature conditions			
Step 1		Step 2	
LAH (equiv)	2	Boc ₂ O	1.2
THF (mL/g)	25	DMAP	yes
time	6 h	time	6 h
temp	reflux	temp	reflux
total time		12 h	
isolated yield		43%	
optimized conditions			
Step 1		Step 2	
LAH (equiv)	2	Boc ₂ O	1.0
THF (mL/g)	30	DMAP	No
time	2 hr	time	2 h
temp	60	temp	25
total time		4 h	
isolated yield		63%	

could only be realized through the use of a large excess of amine, this route was ultimately abandoned.

Oxidation/Reductive Amination Route. The second route to penultimate intermediate **5** involved oxidation of alcohol **2** to the aldehyde **3** followed by reductive amination. Preliminary screening of oxidation procedures included the Swern, Moffatt, pyridine-SO₃, and Marko procedures. In summary, Swern oxidation was reliable, affording product in 75–85% conversion based on HPLC. The Moffatt procedure gave clean oxidation, but the product was contaminated with diisopropylurea that presented purification problems. Marko oxidation (CuCl-phenanthroline (cat.) DBAD, K₂CO₃, air²² gave low conversion for this substrate. Pyridine-SO₃-TEA gave mixed results, probably due to the inconsistent purity of commercially available material. Ultimately, the Swern procedure was adopted to produce material needed for downstream studies despite the drawbacks of low-temperature requirements and dimethyl sulfide formation.

(19) Gooding, O. W.; Bansel, R. P. *Synth. Commun.* **1995**, 25, 1155.

(20) Twenty equivalents of amine was used for a similar substrate in the Vertex patent, see ref 13.

(21) Curran, T. P.; Pollastri, M. P.; Abelleira, S. M.; Messier, R. J.; McCollum, T. A.; Rowe, C. G. *Tetrahedron Lett.* **1994**, 35, 5409.

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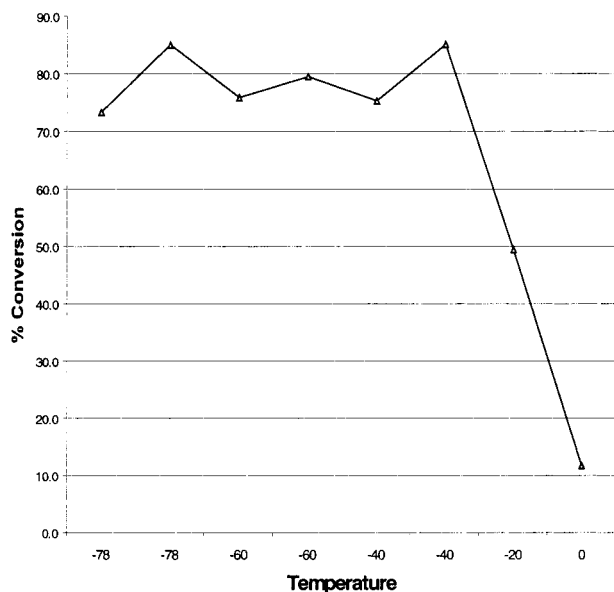
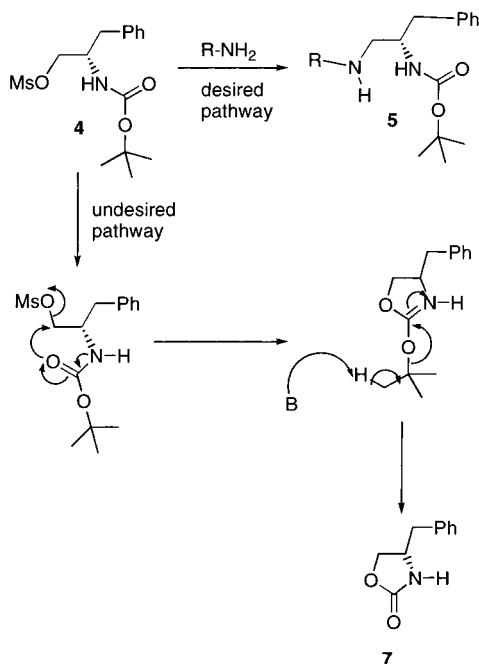


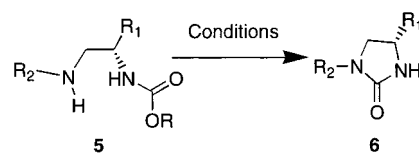
Figure 8. Plot of temperature vs % conversion for the automated Swern oxidation experiment.

Scheme 5. Decomposition pathway leading from mesylate **4** to oxazolidinone **7**



An initial screening experiment on the Surveyor examined the effect of varying temperature and stoichiometry on the Swern oxidation conversion when using DCM as a solvent. Not unexpectedly, this revealed that temperature was the most important variable. As for stoichiometry, conversions did not suffer until oxalyl chloride, DMSO, and DIEA charges dropped below 1.5, 3.0, and 6.0 equiv, respectively. A second experiment held solvent and stoichiometry constant at these levels and looked at five different temperatures, -78 , -60 , -40 , -20 , and 0 °C for the reaction. The oxidations were done at the prescribed temperatures. After warming to 25 °C samples were taken at 60 and 240 min. As shown in Figure 8, conversions remained between 73 and 85% until the reaction temperature was above -60 °C. Note that there

Scheme 6



are two points plotted for each reaction temperature; the first was from the 60-min sample and the second from the 240-min sample. It is interesting that the reaction continued to proceed after warming to 25 °C as Swern oxidations are normally worked up immediately after warming.

The reductive amination reaction (**3** to **5**) was studied using sodium triacetoxyborohydride reagent in a procedure known to be very general and reliable.²³ An automated run was conducted in which four solvents: DCM, THF, DCE, and ACN were screened. The charges of benzylamine (1.2 equiv) and reducing agent (1.9 equiv) were held constant, and the reaction was sampled over time. All reactions went to completion within 1 h with no differences detected. Because this reaction was found not to be sensitive to solvent, we standardized on DCM for further studies.

Because Boc-aminoaldehydes are known to be unstable to storage and can be difficult to isolate in pure form, it was desirable to seek a one-pot procedure for converting **2** to **5** to avoid isolating this material.

A scale-up run was conducted using the optimal conditions for each step. Thus, alcohol (1.0 equiv), oxalyl chloride (1.5 equiv), DMSO (3 equiv), and DIEA (6 equiv) in DCM at -60 °C followed by warming to 25 °C for 1 h gave clean oxidation by HPLC. Direct in situ treatment with benzylamine (1.2 equiv) and $\text{NaBH}(\text{OAc})_3$ (1.9 equiv) provided a 62% isolated yield of pure material at the 10-g scale. This provided sufficient material to study the cyclization reaction while further reaction screening/optimization experiments on this step were continued.

Cyclization. Although the literature precedent¹³ for the conversion of **5** to **6** used a deprotection/phosgene treatment to conduct cyclization, we envisioned that the final cyclization reaction (Scheme 6) could be facilitated either thermally, under base catalysis, or Lewis acid catalysis. An initial broad screen showed that bases were most effective. A Surveyor run was conducted in which two solvents (THF and toluene), three bases (potassium *t*-butoxide, NaHMDS, DIEA), at two different stoichiometries (2.5 and 5 equiv) were examined as shown in Table 9. Samples were taken at 20 and 60 min for HPLC analysis. It was found that K-*Ot*Bu and NaHMDS were effective at both charge levels in THF but not toluene. DIEA was not effective in either solvent. A control experiment with no base in toluene at 110 °C gave partial conversion (thermal conditions). From a manufacturing standpoint the case of 2.5 equiv of K-*Ot*Bu in THF for 1 h (complete conversion) was the method of choice for scale-up. These conditions were subsequently applied on a 6-g scale, affording the imidazolidin-2-one in 90% isolated yield.

(23) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849.

Table 9. Screening study of the cyclization of 5 to 6

RV#	solvent	base	equiv	% conversion ^a	
				20 min	60 min
1	THF	KOt-Bu	2.5	88.6	97.1
2	toluene	KOt-Bu	5	0.0	21.1
3	THF	KOt-Bu	2.5	95.5	100.0
4	toluene	KOt-Bu	5	0.7	28.6
5	THF	NaHMDS	2.5	82.8	100.0
6	THF	DIEA	2.5	3.9	2.8
7	THF	NaHMDS	5	88.5	97.9
8	THF	DIEA	5	6.8	5.7
9	THF	KOt-Bu	5	54.9	94.7
10	toluene	none	0	36.5	38.6

^a Calculated using the formula % conversion = product peak area/total peak area.

Summary

We have developed a new automated reactor–analysis system for use during chemical process development. The fully automated system is capable of reagent/solvent addition, temperature and agitation control, and sample extraction/quench/injection for online HPLC analysis. Both precision and accuracy of the fluidic delivery and sampling components were measured through the use of standard solutions of biphenyl and anthracene in conjunction with HPLC analysis. Agitation efficiency was evaluated using a phase-transfer catalysis reaction known to be rate-dependent on stirring frequency. The instrument's performance at various agitation settings was determined and compared to mechanical and magnetic stirring. The efficiency of agitation by vertical oscillation compared well with both magnetic and mechanical stirring with effective stirring rates of >1000 rpm being measured. Chemistry cases examined included several single-step and multistep reactions studied to identify the best reagent/solvent combinations (screening) and optimal levels of continuous variables such as temperature, concentration, and stoichiometry (optimization). The system was shown to be a useful tool for performing process screening and process optimization during chemical development.

Experimental Section

Solvents and reagents were used as received from commercial suppliers. HPLC was performed on a HP-1050 or HP-1100 system equipped with a Platinum EPS C18 100A 3- μ m rocket column from Alltech (unless otherwise specified) and UV detector. All NMR experiments were done on a Varian 300 spectrometer and are reported in ppm. Elemental analyses were obtained from Desert Analytics, Tucson, AZ.

General Procedure for Accuracy and Precision Testing. Stock solutions of biphenyl (50 g/L) and anthracene (25 g/L) were prepared and diluted using standard volumetric techniques. Delivery and sampling precision was calculated by taking the standard deviation of the HPLC peak area counts for all replicates. Accuracy was determined by comparison with a calibration curve constructed by plotting concentration versus peak area for a set of serial dilutions centered around the concentration range of the samples. The

chromophore test was programmed as described in Table 3. HPLC analysis: (mobile phase, acetonitrile/water, gradient, 60–80% acetonitrile in 4 min; flow rate, 2 mL/min; UV detector 250 nm); retention time of anthracene = 2.45 min and biphenyl = 1.96 min.

General Methods for the Agitation Efficiency Experiments. The round-bottomed flask experiments were performed in a 100-mL three-necked flask fitted with a standard Teflon stirring paddle and a mechanical stirring motor. Revolutions per minute (RPM) were measured with an optical tachometer. A stock solution (20 mL) containing 4.85 M phenylacetoneitrile (PAN), 5.79 M bromoethane (RBr), and 0.115 M tetrabutylammonium bromide (TBAB) was prepared by combining 11.2 mL of PAN, 8.64 mL of RBr, and 0.0741 g of TBAB. This solution was charged to the flask followed by 40 mL of 50 wt % aqueous NaOH. The flask was heated to 55 °C, and stirring at the specified RPM was initiated. Sampling was accomplished by stopping the agitation, removing 0.200 mL of organic layer via syringe, and quenching into 1.0 mL of 0.1 N aqueous hydrochloric acid using a rinse of 0.80 mL of THF. Samples were further diluted with 0.60 mL of diethyl ether and the upper layer was analyzed by HPLC. Samples were taken at 0, 4, 8, 12, 16, 20, 30, 40, 50, 60, 90, and 120 min. Data from these runs were processed using Excel²⁴ to determine V_0 at each RPM and to plot log RPM versus log V_0 . The automated run was conducted in an analogous manner with the appropriate volume of stock solution delivered automatically and the NaOH solution added manually because of its high viscosity. Samples were automatically withdrawn, quenched, and rinsed in a manner analogous to the manual runs. The total reaction volume and agitation settings were as described in Table 4. Effective RPMs were calculated using eq 2. HPLC analysis: (mobile phase, acetonitrile/water, gradient 40–75% acetonitrile in 3.5 min, flow rate 2 mL/min; UV detection 254 nm); retention time of PAN = 1.57 min, ethylated PAN = 2.20 min.

General Methods for Indole Linker Screening Experiments. The automated run was programmed as described in Table 5. Solids were manually added through a powder funnel inserted into the top port of the vessels. HPLC analysis: (mobile phase, acetonitrile/water, gradient 10–80% acetonitrile in 6 min, flow rate 2 mL/min; UV detection 254 nm); retention time of indole-3-carboxaldehyde = 2.67 min, ethyl 3-formyl-indol-1-yl-acetate = 3.29 min, 3-formyl-indol-1-yl-acetic acid = 1.78 min.

3-Formyl-indole-1-yl-acetic Acid. A dry 22-L flask was charged with indole-3-carboxaldehyde (1.000 kg, 6.889 mol) followed by ethanol (10 L). Agitation was initiated and potassium *tert*-butoxide solution (1.65 M in THF, 4.6 L) was added over 10 min during which time the temperature rose to 37 °C. After 20 min the flask was charged with ethyl bromoacetate (1.265 kg, 7.58 mol, 1.1 equiv) at a rate of 20 g min⁻¹. The reaction was allowed to proceed for a further 20 min, at which time the flask was charged with potassium hydroxide (463 g, 8.26 mmol, 1.2 equiv) portionwise over 20 min. The solution was heated and solvent distilled as water

(24) Excel is a registered trade name of Microsoft Corp.

was added to make up the lost volume (4 L). When the head temperature reached 93 °C, the flask was cooled to 20 °C and washed twice with 60:40 ethyl acetate/hexanes to remove unreacted starting material. The aqueous layer was then heated to 45 °C with agitation and treated with aqueous hydrochloric acid (0.746 L, 12 M, 1.3 equiv) added at a rate of 20 mL per min. The mixture was cooled to 3 °C and aged 30 min; the product was collected by filtration, washing the cake with water (1.5 L). The product was then vacuum-dried to constant weight (50 °C, 0.1 bar) affording 1.183 kg (84.6%) as an orange solid. HPLC purity >98%, physical properties were identical to those previously reported.⁶

General Methods for LAH Reduction Optimization Experiment. The automated run was programmed as described in Table 7. A 1.00 M suspension of lithium aluminum hydride (LAH) was prepared from crushed pellets by agitation in a round-bottomed flask while heating at 60 °C. Sample quench vials were automatically loaded with 3 mL of dioxane. The solid L-Phe (1.00 g, 6.05 mmol) was added manually at the beginning of the experiment followed by the appropriate volume of THF automatically. Agitation was started, and the appropriate volume of LAH suspension was added manually via syringe with a 16-gauge needle. After being held at an internal temperature of 60 °C for 2 h, the vessels were cooled to 20 °C and quenched with 2 M NaOH solution (1.6 mL/g LAH used) followed by water (2.0 mL/g LAH used). DCM was added (12 mL to RVs 1–5 and 8 mL to RVs 6–10) followed by di-*tert*-butyl dicarbonate (1.32 g, 6.05 mmol). Samples were taken (0.300 mL from RVs 1–5 and 0.200 mL from RVs 6–10). Sample size was proportional to total volume so that HPLC peak areas could be compared directly. Final results are given in Table 7. HPLC analysis: (mobile phase, acetonitrile with 0.1% TFA/water, gradient 0–100% acetonitrile with 0.1% TFA in 6 min, flow rate 2 mL/min; UV detection 214 nm); retention time of Phe = 1.00 min, phenylalaninol = 2.39 min, *N*-(*tert*-butoxycarbonyl)phenylalaninol = 4.17 min.

(S)-*N*-(*tert*-Butoxycarbonyl)phenylalaninol (2). A dry 3-L flask was flushed with nitrogen and charged with THF (1.8 L). Agitation was started, and crushed pellets of lithium aluminum hydride (34.2 g, 900 mmol) were added over 5 min. The flask was charged with solid (S)-(-)-phenylalanine (61.0 g, 0.369 mol) portionwise over 1 h. The reaction was heated to 62 °C and held for 2 h prior to cooling to 20 °C in an ice bath. The reaction was quenched by the addition of aqueous NaOH (68.4 mL, 2 M) followed by water (55 mL). **CAUTION: exothermic, hydrogen gas evolution!** When the addition was complete, the flask was allowed to cool to 5 °C, and a solution di-*tert*-butyl dicarbonate (98.2 g, 450 mmol) in DCM (0.300 L) was added at a rate of 20 mL min⁻¹, during which time the temperature rose to 29 °C. The bath was removed, and the suspension was agitated at 25 °C for 1 h. The suspension was treated with diatomaceous earth (100 g) and filtered through a sintered glass funnel. The cake was rinsed twice with THF (0.5 L), and the combined organics were charged to a 3-L flask and concentrated by distillation while adding 500 mL of heptane to make up the volume loss. When the head temperature reached 80

°C, the heat was turned off, and the flask allowed to naturally cool to 20 °C. The thick mixture was further diluted with hexanes (400 mL) and cooled to 5 °C. The product was collected by filtration and vacuum-dried to constant weight at 25 °C, affording 58.0 g (63%) as a white crystalline solid. HPLC purity >99%. Physical properties were identical to authentic commercial material.²⁵

General Methods for Oxidation Experiment. At the beginning of the experiment sample vials were automatically loaded with aqueous sodium bicarbonate (0.30 mL) and acetonitrile (1.40 mL) to quench the samples prior to HPLC analysis. Each of five RVs were dried by heating dry THF under reflux, draining hot, and blowing dry with nitrogen purge. The program added DCM (13.6 mL) followed by oxalyl chloride solution (50% in DCM, 0.69 mL, 2.98 mmol). The vessels were cooled to -78, -60, -40, -20, and 0 °C, and a solution of DMSO (25% in DCM, 2.3 mL, 5.97 mmol) was metered in over 2 min. The program then metered in a solution of alcohol **4** (2.99 mL, 1.0 M, 1.99 mmol). The temperature was held for 1 h, and a solution of DIEA in DCM (50%, 4.17 mL, 11.94 mmol) was metered in over 2 min. The vessels were taken to 0 °C using a linear ramp over 30 min and sampled. The vessels were further warmed to 25 °C and sampled again at 60, and 240 min for on-line analysis. Results for the 60- and 240-min points are shown in Figure 8. HPLC analysis: (column, HP-Zorbax XDA-C8; mobile phase, acetonitrile/water; gradient 40–70% acetonitrile in 5 min; flow rate 1 mL/min; UV detection 214 nm); retention time of **2** = 4.51 min, **3** = 3.21 min.

General Methods for Reductive Amination Experiment. Aldehyde **3** (0.40 g, 1.6 mmol) was charged to each of four RVs followed by one of four solvents, DCM, THF, 1,2-dichloroethane, or acetonitrile (60 mL/g). Agitation was started, and the reactions were treated with benzylamine (0.24 g, 2.2 mmol) and sodium triacetoxyborohydride (0.64 g, 3.0 mmol). The reactions were periodically sampled into vials containing acetonitrile and analyzed by HPLC. All reactions were found complete within 1 h. HPLC analysis: (mobile phase, acetonitrile/water, gradient 40–70% acetonitrile in 5 min, flow rate 1 mL/min; UV detection 214 nm); retention time **3** = 3.21 min, **4** = 4.32 min.

(S)-2-(*tert*-Butoxycarbonylamino)-3-phenyl-1-(*N*-benzyl)propylamine (5). A dry 500-mL round-bottomed flask was charged with DCM (50 mL) and DMSO (4.7 g, 60 mmol, 3.0 equiv). The flask was placed in a dry ice/acetone bath, and neat oxalyl chloride (3.8 g, 30 mmol) was added over 30 s via syringe. After 30 min a solution of alcohol **2** (5.00 g, 20 mmol) in DCM (50 mL) was added dropwise over 15 min, and the solution was stirred for an additional 30 min. A solution of diisopropylethylamine (15.5 g, 120 mmol) in DCM (50 mL) was added dropwise over 15 min, and the solution was stirred for an additional 30 min. A solution of benzylamine (2.4 g, 22 mmol) in DCM (10 mL) was added followed immediately by solid sodium triacetoxyborohydride (6.36 g, 30 mmol). After an additional 30 min the flask was removed from the bath and allowed to warm

(25) (S)-2-(*tert*-Butoxycarbonylamino)-3-phenyl-1-propanol was obtained from Aldrich.

to ambient temperature overnight. The solution was treated with aqueous NaHCO₃ with agitation for 10 min, and the layers were separated. The organic phase was dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified on a column of silica gel, eluting with 20:80 ethyl acetate/DCM. Fractions containing product were concentrated, affording 4.2 g of analytically pure product (62%). ¹H NMR(CDCl₃): 7.35–7.16(m, 10H), 4.75–(br s, 1H), 3.90(br s, 1H), 3.79(d, 1H, *J* = 13.2), 3.75(d, 1H, *J* = 13.2), 3.00–2.61(m, 5H), 1.41(s, 9H). ¹³C NMR(CDCl₃): 155.85, 140.5, 138.3, 129.6, 129.5, 128.7, 128.6, 128.3, 127.2, 126.5, 79.4, 54.0, 51.6, 39.4, 28.6. Anal. Calcd for C₂₁H₂₈N₂O₂: C, 74.08; H, 8.29; N, 8.23. Found: C, 73.80; H, 8.50; N, 8.81.

General Methods for Cyclization Experiment. The automated run was programmed as described in Table 9. At the beginning of the experiment sample vials were loaded with aqueous HCl (0.30 mL) and THF (0.70 mL). Each of 10 RVs were manually charged with solid amine **5** (0.50 g, 1.47 mmol). The program then added the appropriate solvent followed by the base (either 2.5 or 5 equiv). RVs 1–9 were heated to 60 °C, and 10 was heated to 110 °C, and samples were quenched into vials at 20 and 60 min for on-line analysis. HPLC analysis: (mobile phase, acetonitrile/water, gradient 20–70% acetonitrile in 7 min, flow rate 2 mL/min; UV detection 214 nm); retention time of **6** = 5.17 min.

(S)-1-Benzyl-4-benzylimidazolidin-2-one (6). A dry 250-mL round-bottomed flask was charged with amine **5** (5.76 g, 16.9 mmol) and THF (50 mL). The resulting solution was

treated with a solution of potassium-*tert*-butoxide (31.9 mL, 51 mmol, 1.6 M), and the resulting yellow solution was heated to 60 °C for 3 h. The solution was cooled to ambient temperature and acidified with aqueous HCl (35 mL, 1 M) and concentrated under reduced pressure. The aqueous residue was extracted with ethyl acetate, and the organic phase was washed twice with brine, dried over MgSO₄, and concentrated under reduced pressure to a viscous oil that solidified on standing, affording 4.07 g (90%). ¹H NMR(CDCl₃): 7.32–7.07(m, 10H), 4.32(s, 2H), 3.84–3.80(m, 1H), 3.33(t, 1H, *J* = 8 Hz), 2.99(t, 1H, *J* = 8 Hz), 2.73(d, 2H, *J* = 7 Hz). ¹³C NMR(CDCl₃): 161.7, 137.2, 137.1, 129.2, 129.0, 128.8, 128.2, 127.7, 127.1, 51.45, 49.8, 47.7, 42.2. Anal. Calcd for C₁₇H₁₈N₂O: C, 76.66; H, 6.81; N, 10.52. Found: C, 76.40; H, 6.86; N, 10.58.

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