Selective DIBAL-H Monoreduction of a Diester

Using Continuous Flow Chemistry: From Benchtop

to Kilo Lab

Nick Uhlig*, Andrew Martins, and Detian Gao

Gilead Alberta ULC, 1021 Hayter Rd. NW, Edmonton, Alberta, T6S1A1 Canada.

KEYWORDS: Flow chemistry, reduction, metal hydrides

Contents

1. Pro	cess Descriptions
1.1.	Batch Reduction of 1 to 2 on 18-kilogram Scale
1.2.	Representative Lab-Scale Continuous Flow Reduction of 1 to 2 4
1.3.	Multikilogram-scale Continuous Flow Reduction of 1 to 27
1.3.	1. Freebasing of 1 •HCl to 1 7
1.3.	2. Continuous flow reactor assembly, components, and setup7
1.3.	.3. Reaction sample collection and analysis
1.3.	.4. Manufacturing procedure
1.3.	.5. Color removal procedure
2. Cal	orimetry Experiments
2.1.	Dosing of DIBAL-H into THF (Figure S1) 14
2.2.	Reduction of 1 to 2 using DIBAL-H (Figure S2)15
2.3.	Quenching of Reaction Mixture with EtOAc (Figure S3)16
3. DIE	3AL-H Aliquot Experiments17
4. Des	sign of Experiments (DoE)
5. Ran	nging studies
6. Sca	le-up equipment selection
6.1.	Heat exchange
6.2.	Mass flow measurement
6.3.	Pumping

7. Ma	anufacturing Observations	32
7.1.	In-process pre-workup sample analyses	32
7.2.	Reaction Sample Analyses	33
7.3.	Final Stock Solution Sample Analyses	34
8. De	termination of precipitated triethylamine hydrochloride from 1 stock solution	35
Chroma	tographic Methods	38
8.1.	HPLC (Method 1)	38
8.2.	UPLC (Method 2)	38
9. Co	mpound Spectra	39
9.1.	Diethyl pyridine-2,4-dicarboxylate (1)	40
9.2.	Ethyl 2-formylpyridine-4-carboxylate (2)	41
9.3.	Ethyl 2-(hydroxymethyl)pyridine-4-carboxylic acid (3)	42
9.4.	Pyridine-2,4-dicarbaldehyde (4)	43
9.5.	4-(hydroxymethyl)pyridine-2-carbaldehyde (5)	44
10. F	References	45

1. **Process Descriptions**

1.1. Batch Reduction of 1 to 2 on 18-kilogram Scale

1 (18.0 kg, 80.6 mol) was charged to the reactor as a stock solution in ethyl acetate. A solvent swap was performed by repeated put-and-take vacuum distillations with 90 kg THF charged on each iteration (up to 4 iterations, using a jacket temperature of ≤ 65 °C). Once the ethyl acetate content was reduced to below 0.5 wt% as measured by q¹H NMR, the pot contents were cooled to -20 °C and DIBAL-H (25 wt% in toluene, 70.2 kg, 1.50 equiv.) was charged slowly, keeping the pot temperature at -20 ± 3 °C. Once the addition was finished, an in-process sample was taken. The reaction was deemed complete if 1 content was below 1.0 %AN as measured by LC.

Once the reaction was complete, 95.4 kg (13.4 equiv.) EtOAc was charged slowly, keeping the pot temperature \leq -15 °C. The reactor contents were agitated for at least 1 h.

In a second reactor, a solution was prepared by mixing 72 kg of sodium potassium tartrate tetrahydrate (Rochelle salt) and 90 kg of water. The quenched organic reaction mixture was slowly transferred onto this aqueous solution (over ca. 1 h) with vigorous agitation, keeping pot temperature \leq 30 °C. This mixture was then agitated at 22 °C for at least 6 h. A layer separation was then performed, discarding the lower aqueous layer. The organic layer was then washed with water (72 kg) and brine (18 kg NaCl in 108 kg water), to yield a stock solution of **2**.

This stock solution was circulated via a sparkler filter containing 9.0 kg of silica gel for 1 h. The solution was then concentrated to a volume of approximately 29.0 L, and the pot temperature adjusted to 50 °C. 142 kg of heptanes was charged, and the contents agitated at 50 °C for 60 minutes prior to ramp cooling to -20 °C over 4 h. After aging for 6 h at -20 °C, the resulting slurry was filtered and rinsed with 14.4 kg heptane to yield pale yellow solids. LC analysis of these solids showed 15.2 %AN **5** and repurification was necessary prior to isolation.

These solids were then purified by silica column chromatography, eluting with 20% EtOAc/heptanes to yield a purified stock solution in EtOAc/heptanes (29.1 kg at 10.9 wt% by quantitative ¹H NMR, 3.16 kg, 22% yield, 99.8 %AN by LC at 257 nm).

Scale performed: 18 kg input of compound 1.

1.2. Representative Lab-Scale Continuous Flow Reduction of 1 to 2

This representative lab-scale experiment was performed using a Vapourtec R-Series flow reactor consisting of four piston pumps with a maximum flow rate of 9.99 mL/min each. Cooling was performed using a pail filled with a dry ice-isopropanol bath kept at -40 ± 3 °C, in which all precooling loops, as well as reaction and quench loops were constantly submerged. Pre-cooling loops consisted of 1.0 mm bore PFA tubing, while reactor loops were 1.59 mm bore PFA tubing. Mixing between the feeds of DIBAL-H and **1** was accomplished using an IMM Caterpillar split-and-recombine micromixer (300 µm internal channel width).^{1–4} Collection and waste diversion were automated by the instrument using a computer-controlled three-way valve.

A stock solution of approximately 14.3 wt% **1** in THF (0.58 M, d = 0.911 g/mL) was prepared by mixing 500 g of solid **1** with 3.00 kg of THF. The assay of this solution was determined using q¹H NMR, and its density measured using a volumetric flask, to allow calculation of volumetric flow rates. This solution was connected to the inlets of pumps A and B of the Vapourtec instrument.

A solution of 25 wt% DIBAL-H in toluene (0.846 g/mL, ca. 1.5 M concentration) was connected to the inlet of pump C of the instrument.

Tubing and pumps were set up as shown in Scheme S1. Flow rates were set as follows:

- Pump A (14.3 wt% 1 in THF): 8.10 mL/min
- Pump B (14.3 wt% 1 in THF): 8.10 mL/min

- Pump C (25 wt% DIBAL-H in PhMe): 9.99 mL/min
- Pump D (EtOAc): 9.67 mL/min

A 33 mL loop of 1.59 mm bore PFA tubing (ca. 16.6 m in length) was used for the reactor. Combining the flow rates of DIBAL-H and 1 (26.2 mL/min total) yields a residence time of 1.26 minutes, or 75.6 seconds. A 13.0 mL loop of 1.59 mm bore PFA tubing (ca. 6.6 m in length) served as the quench loop, with a residence time of 22 seconds (total flow rate: 35.9 mL/min).

Scheme S1. Tubing arrangement for lab-scale continuous flow synthesis of 2.



The Vapourtec system was programmed using its proprietary software to divert the first 70.0 mL of eluent to waste via an automated valve. This number was calculated based on the flow rates and volumes used, by the instrument's dispersion modeler, to allow collection only of eluent after the reactor reached steady state.

Pumping was commenced, and the eluent was collected directly into a pre-prepared, stirred solution of Rochelle salt (2.00 kg, in 2.50 kg water) held at 22 °C in a 20 L round-bottom flask, cooled using an ice/water bath. In total, approximately 8.3 L of eluent was collected over a collection time of approximately 4 hours, while keeping the contents of the flask at \leq 30 °C. This biphasic mixture was stirred overnight (16 h) at 22 °C, and then agitation was ceased. The layers were separated, and the organic layer subsequently washed with water (3.5 kg) and brine (0.5 kg sodium chloride in 3.0 kg water) to yield the stock solution of **2**.

A solvent swap was performed by concentrating the stock solution of **2** to approximately 2 volumes (1.0 L) under vacuum using a rotary evaporator (evaporation had to be done in several portions), and then charging 1.0 kg of EtOAc and 2.5 kg of heptanes. This mixture was then filtered through a pad of 700 g silica gel, and the filter cake was rinsed with a mixture of 0.5 kg EtOAc and 1.3 kg heptanes. Upon filtration the previously dark orange to brown solution lightened to a pale yellow solution, which was evaporated by rotary evaporation to dryness to yield **2** as a pale yellow crystalline solid (268.36 g, 1.50 mol, 67%, 98.7 %AN at 257 nm).

1.3.1. Freebasing of 1•HCl to 1

Scheme S2. Freebasing of 1•HCl by triethylamine to produce 1 freebase as a stock solution in THF.



1-HCl (8.00 kg, 30.9 mol) was charged into a reactor with 28.8 kg of THF to form a mobile slurry. The reactor contents were adjusted to ≤ 10 °C, and triethylamine (3.1 kg, 1.0 equiv.) was charged slowly while keeping the pot temperature ≤ 10 °C. The line was rinsed forward with 0.4 kg THF and the reactor contents were adjusted to 22 °C and agitated for at least 60 minutes. The slurry was then filtered, and the filter cake rinsed twice with 1.0 kg THF.

The assay of the resulting filtrate was determined by $q^{1}H$ NMR-corrected total dissolved solids (TDS), and THF was charged if the assay was more than 16.5 wt% **1**, to adjust the assay to 15.0 ± 1.5 wt%. This stock solution was then filtered through a 1.0 µm polypropylene polishing filter.

1.3.2. Continuous flow reactor assembly, components, and setup

All hard-piped tubing and components had wetted parts constructed from 316 stainless steel. Tubing between reactor components (e.g. pumps, heat exchangers, back pressure regulators) was of 6.36 mm (1/4") outer diameter (OD) and 3.18 mm (1/8") internal diameter (ID), with a volume per unit length of ca. 7.94 mL/m, except for the tubing connecting the t-mixer junction between feeds of DIBAL-H and **1** to the heat exchanger serving as the main reactor, which had an OD of 3.18 mm (1/8") and an ID of 1.59 mm (1/16") and a volume per unit length of ca. 1.98 mL/m. Feed

vessels were connected to pump inlets using 6.36 mm OD (1/4") polyethylene tubing. DIBAL-H and 1 feed lines were equipped with in-line sintered metal filters with a 7 micron pore size, to prevent particulate ingress into the pumping mechanisms and reactor tubing.

System back pressure and pump discharge pressure were monitored and controlled using manually adjustable back pressure regulators (BPR) and pressure gauges. Pump discharge pressure was controlled individually for each pump, and was set to 40 psi during manufacturing.

Pre-cooling heat exchangers were oriented horizontally, while the main reactor heat exchanger was angled up at approximately 30° to mitigate the trapping of air bubbles in the reactor. Pre-cooling heat exchangers utilized counter flow of the coolant (refer to Section 4) to ensure maximal reagent feed cooling at the outlet, while the reactor and quench heat exchangers utilized parallel flow to prioritize cooling at the reactor inlet, where the majority of the reaction's exotherm would be localized.

Mixing between the DIBAL-H and **1** solutions was performed using a 1.0 mm inner diameter stainless steel t-mixer. Cooling of the t-mixer junction was accomplished by use of a foam-insulated bath consisting of acetone and dry ice, manually adjusted to a temperature of -45 °C throughout the duration of each manufacturing module by periodic addition of dry ice.

Temperature was measured on the process side and the coolant side using in-line thermoucouples (connected via swaged fittings) and monitored using two paperless data recorders.

1.3.3. Reaction sample collection and analysis

In-process reaction samples were drawn from the reactor outlet, just downstream of the EtOAc quench reactor and upstream of the batch workup vessels. Analysis of eluent samples was performed off-line, using LC. After system startup and 15 minutes of run time, the first sample was taken from the reactor outlet, and on the basis of this result (%AN 2 > 95, integrating only

peaks for **1** and **2** to measure conversion) the run was continued. Samples were then drawn from the eluent every two hours (120 minutes) for information only, to verify that the system remained stable and to glean information regarding impurity content over time.

1.3.4. Manufacturing procedure

The procedure below is scaled to a typical module for the reduction of 3.4 kg of compound 1.

Solutions of 13.9 wt% compound **1** in THF were prepared in three modules as described in Section 1.3.1. The stock solutions from the three freebasing modules were combined, and charged to a 200 L keg, placed on a floor scale to measure its mass (resolution of 1 g). This solution was connected to the feed line of pump 1. This solution and feed vessel were used across all seven flow chemical synthesis modules.

DIBAL-H (25 wt% in toluene) was charged to a 20 L stainless steel keg placed on a floor scale to measure its mass (resolution of 1 g). This solution was connected to the feed line of pump 2. The stainless steel keg was topped up as needed from a cylinder, prior to every manufacturing module.

EtOAc was charged to a 200 L keg. This was connected to the feed line of pump 3. The mass of EtOAc was not measured during manufacturing due to it being used in significant excess. This keg was topped up as needed during manufacturing.

A solution of sodium potassium tartrate tetrahydrate (13.4 kg) and water (16.8 kg) was prepared and split proportionally between two glass-lined reactors of 30 L and 60 L volumes. This solution was adjusted to 22 °C in both reactors, and agitated continuously throughout the manufacturing module. The chiller was turned on and adjusted to a setpoint of -40 °C and allowed to warm up for at least 1 h. The cooling bath for the t-mixer was charged with acetone and dry ice until a temperature of -45 °C was reached.

The pumps were started, and **1**, DIBAL-H, and EtOAc were pumped through the system at target rates of 36 g/min, 19 g/min, and 26 g/min respectively.

Throughout each module, pump flow rates were measured by recording the mass change in the DIBAL-H and **1** feed vessels over time, adjusting pump speed manually as needed, and then measuring the mass change a second time to verify that it was within the desired range.

Workup was performed using the two glass-lined reactors using the previously-established conditions mentioned in Section 1.2. Collection of the eluent was performed directly into the two quench vessels containing the aqueous solution of sodium potassium tartrate. The 30 L vessel was filled first, and then a three-way valve was toggled to begin collecting in the 60 L vessel. Collection was continued until 24.2 kg of the stock solution of **1** (3.4 kg of **1**, 15.1 mol, at 13.9 wt% in THF) had been pumped, as measured by mass difference on the feed vessel. This typically took approximately 11-12 hours of pumping time.

Once collection was complete, the quench mixtures in the two vessels was agitated at 22 °C overnight, and workup was finished the following day by washing the organic layers with water (23.5 kg, split proportionally between the two reactors), and then brine (3.4 kg NaCl dissolved in 20.2 kg water, split proportionally between the two reactors). Combining the resulting stock solutions from manufacturing modules 1-7 yielded a final crude stock solution of **2** (310.2 kg, 3.48 wt% **2** by q¹H NMR, 10.8 kg **2**, 60.3 mol, 68%).

Scale performed (according to input of pure 1):

Module 1: 1.1 kg (4.9 mol)

Modules 2-6: 3.4 kg	(15.1 mol each)
Module 7: 2.0 kg	(9.1 mol)
Total Scale: 19.9 kg	(89.3 mol)

1.3.5. Color removal procedure

To remove solid particulates, color and remaining impurity content, the stock solution of 2 produced according to the procedure in Section 1.3.4 was treated as follows (procedure has been scaled to an input of 5 kg of pure compound 2).

A solvent swap was performed by concentrating the stock solution of **2** (142.9 kg) to 4 volumes (20 L) under vacuum with a jacket temperature of 45 °C, and then charging 19.5 kg of EtOAc and 32.5 kg of heptanes. This mixture was then filtered through a pad of 6.5 kg silica gel using an Aurora filter, and the filter cake was rinsed with a mixture of 10 kg EtOAc and 10 kg heptanes. Upon filtration the previously dark red-brown suspension yielded a pale yellow clear filtrate which was collected in a single portion, and was suitable for use in downstream chemistry as normal. Typical purity of the stock solution after this series of operations was approximately 94 %AN **2** as measured at 242 nm.

2. Calorimetry Experiments

Calorimetry experiments were performed using a Mettler-Toledo EasyMax reactor at a reaction temperature setpoint of -20 °C. Calibrations of the temperature probe were performed prior to every run with a wait time of 10 minutes, and a Δ T of 3 K.

Calorimetry experiments were performed on a 5 g scale relative to input of **1** (22.4 mmol), and all enthalpy values are given in kJ per mole of **1**.

Heat output calculation was done using two different equations:

$$q_{hf} = q_{flow} - q_c + q_{acc} \tag{1}$$

and

$$q_{hf} = q_{flow} - q_c + q_{acc} + q_{dos} \tag{2}$$

Where:

 q_{hf} is the heat flow of the reaction;

 q_{flow} is the heat flow through the jacketed reactor wall;

 q_c is the heat delivered by the calibration probe;

 q_{acc} is the heat accumulation in the reactor; and

 q_{dos} is the correction of heat flow due to the dosing in of a room-temperature reagent.

The latter of these two formulae (2) accounts for the dosing of warm reagent into a cold solution, and is therefore more salient when speaking strictly about the reaction's enthalpy. However, for calculating total cooling requirements for the purposes of scale-up, the uncorrected value derived from (1) is more informative, as feeds must be cooled prior to combination. Table S1 summarizes the results from these experiments for three separate operations, examining the dilution of DIBAL-H into pure THF (entry 1), the reduction of **1** to **2** by DIBAL-H under semi-batch conditions (entry 2), and the quenching of the reaction with EtOAc (entry 3).

Table S1. Summary of reaction calorimetry data obtained from calorimetric experiments for different process steps.

		Uncorrected		Dosing-co	rrected
Entry	Operation	ΔHrxn (kJ/mol 1)	ΔT _{ad} (K)	ΔHrxn (kJ/mol 1)	ΔT _{ad} (K)
1	Charging of DIBAL-H into THF	-102	44	-21	9
2	Reduction of 1 to 2	-319	119	-238	88
3	Quenching reaction with EtOAc	-65	16	-37	9

2.1. Dosing of DIBAL-H into THF (Figure S1)

Into 30.25 g of THF was charged 22 mL (ca. 19 g) of DIBAL-H (25 wt% in toluene) over 60 minutes and the heat output data collected.



Figure S1. Visualization of calorimetry experiment for the dosing of DIBAL-H into THF. Note that the graph does not compensate for the heat of dosing in a room-temperature reagent.

2.2. Reduction of 1 to 2 using DIBAL-H (Figure S2)

Into 34.36 g of a 14.57 wt% solution of **1** in THF was charged 22 mL (19 g) of DIBAL-H over 60 minutes and the heat output data collected.



Figure S2. Visualization of calorimetry experiment for the reduction of **1** to **2** using 1.5 equivalents of DIBAL-H in semi-batch mode.

2.3. Quenching of Reaction Mixture with EtOAc (Figure S3)

Into the resulting reaction mixture from the procedure in Section 2.3 was charged 26.5 g (ca. 30 mL) EtOAc over 40 minutes, and the heat output data was collected (Figure S3).



Figure S3. Visualization of calorimetry experiment for the quenching of the reduction of 1 to 2 using EtOAc.

3. DIBAL-H Aliquot Experiments

The reaction was performed on a 15 g scale (input of **1**) according to the standard lab scale procedure in Section 1.2. Aliquots of 0.1 equiv. (3.8 g, 4.6 mL) of 25 wt% DIBAL-H in toluene were added, and after a 5 minute agitation period an in-process sample was taken for analysis by LC. Due to differing absorption of the impurities, the analyses were performed at 257 and 277 nm. The results of these analyses can be seen in Figure S4. All integrations shown in each plot were normalized to a total of 100 %AN to yield relative proportions of each analyte.



Figure S4. LC analysis (corrected %AN at 257 nm, top, and 277 nm, bottom) of the reaction mixture after addition of 0.1 equivalent aliquot additions of DIBAL-H revealed significant impurity formation even before full conversion was reached. Impurities **4** and **5**, visible at 257 nm (top), had extremely low absorbance and so were not visible at 277 nm (bottom). Conversely, impurity **3** showed poor absorbance at 257 nm and was instead measured at 277 nm.

4. **Design of Experiments (DoE)**

DoE studies were planned and analyzed, and all figures and charts generated, using Fusion QbD software version 9.9.0, by S-Matrix. The study was performed using a 3-level face-centered central composite design (CCF), which included 15 initial runs followed by 5 model refinement and verification runs. The factors chosen for study were temperature (A), residence time (B), and DIBAL-H equivalents (C). The responses analyzed were %AN values of compounds 1, 2, and 3, and the combined %AN of compounds 4 and 5.

For all experiments, LC analysis was performed on pre-workup reaction eluent (after dilution with EtOAc, but prior to aqueous workup) which was collected directly from the reactor outlet, after the reactor reached steady state (as predicted by the dispersion calculator included with proprietary software). LC analysis was performed using the method described in Section 8.2.

Pre-cooling and reactor tubing were submerged in a dry ice and isopropanol bath to reach the desired temperatures. Reactor setup was similar to the one presented above in Scheme S1, with the exception of the mixing at the junction between the DIBAL-H and compound **1** feeds, which was achieved via a 0.254 mm (1/100") inner-diameter t-mixer. Residence times were altered by adding or removing tubing to change the reactor volume (11 mL volume for 40 seconds, 22 mL volume for 80 seconds, and 33 mL volume for 120 seconds). In all experiments the combined flow rate of DIBAL-H (25 wt% in toluene) and **1** (ca. 14.3wt% in THF) was kept the same (16.5 mL/min, refer to Table S2) to eliminate variation in mixing and dispersion in the reactor volume.

Table S2. Compound 1 and DIBAL-H solution flow rates used for DoE studies at various loadings of DIBAL-H.

DIBAL-H loading (equiv.)	Compound 1 Flow Rate (mL/min)	DIBAL-H Flow Rate (mL/min)	Combined Flow Rate (mL/min)
1.0	11.6	4.9	16.5
1.5	10.1	6.4	16.5
2.0	8.9	7.6	16.5

Factors and responses for all DoE experiments can be found in Table S3, with supplementary model refinement and verification experiments also displayed in Table S4. From these sets of data, the models used for predicting reaction outcomes were generated. The contributions of different terms to the generated models for %AN 2 and %AN 4+5 responses can be seen in Figure S5 and Figure S6 respectively. Analysis settings, regression statistics, and analysis-of-variance (ANOVA) statistics can be found in Table S5, Table S6, and Table S7 (for modeling of the %AN 2 response) and Table S8, Table S9, and Table S10 (for modeling of the %AN 4+5 response).

In the model for predicting %AN **2**, temperature (A) and DIBAL H loading (C) proved highly important (Figure S5). A second-level interaction with temperature (A*C) and a quadratic effect (C^2) represent the two most significant model terms.

The model for %AN **4**+**5** also showed DIBAL H loading (C) and temperature (A) as having the most significant effects, including a second-level interaction (A*C) (Figure S6).

In the initial run of 15 experiments, the center point—representing the standard conditions identified through previous screening—provided the best results (Table S3, run 10), with high levels of conversion (0.4 %AN 1 remaining) and a low level of impurity formation (1.1 %AN 3, 7.0 %AN combined 4+5).

While the model predicted the best results at lower DIBAL-H loadings and higher temperatures (e.g. 1.0 equiv. DIBAL-H at -20 °C), verification and model refinement runs shown in Table S4 demonstrated that such conditions led to significant under-reaction with ca. 8 %AN of 1 remaining (run 20).

Table S3. Factors (temperature, residence time, and DIBAL-H loading) and responses (%AN of **1**, **2**, **3**, and [**4**+**5**]) for the 15 original DoE experiments performed in the study. Scheme below displays reaction setup at left, and three-dimensional representation of CCF study design at right.



			DIRAL-H _		Pre-workup Compo	^a LC %AN, ound #	
Run	Temperature (°C)	τ (s)	Loading (equiv.)	1	2	3	4+5 ^b
1	-40	120	1.5	0.1	89.4	1.3	8.3
2	-40	80	2.0	0.1	80.9	1.2	17.0
3	-20	120	2.0	0.0	60.1	1.6	37.3
4	-60	40	2.0	8.9	81.3	1.3	7.3°
5	-60	80	1.5	17.5	72.9	0.8	8.0
6	-20	40	1.0	9.9	84.2	1.4	3.3
7	-40	80	1.0	10.2	84.8	0.9	2.9
8	-40	40	1.5	3.7	89.3	1.1	4.8
9	-60	120	2.0	3.2	83.9	1.1	10.9
10	-40	80	1.5	0.6	89.9	1.1	7.0
11	-20	40	2.0	0.0	71.0	1.9	26.0
12	-60	120	1.0	17.1	78.8	0.8	2.2
13	-20	80	1.5	0.1	87.4	1.9	9.2
14	-20	120	1.0	7.8	85.5	1.6	3.6
15	-60	40	1.0	26.4	69.2	0.9	2.1

^a Samples taken directly from reactor outlet (after mixing with EtOAc, but prior to aqueous workup). %AN measured at 242 nm. <0.05 = ND, $\le 0.1 =$ trace.

^b 4 and 5 closely eluted in the chromatographic method used, and so were integrated together.

^c Run #4 was identified as an outlier in the modeling of %AN 4+5 (see Table S8).

			DIBAL-H		Pre-workup Compo	^a LC %AN, ound #	
Run	Temp (°C)	Res. Time (s)	Loading (equiv.)	1	2	3	4+5 ^b
16	-41	120	1.3	1.4	91.1	1.5	5.4
17	-38	120	1.4	Trace	91.1	1.4	6.5
18	-35	107	1.5	Trace	87.9	1.7	9.6
19°	-35	107	1.5	Trace	89.1	1.5	8.5
20	-20	80	1.0	8.3	86.1	1.5	3.3

Table S4. Parameters and responses for additional verification and model refinement runs.

^a Samples taken directly from reactor outlet (after mixing with EtOAc, but prior to aqueous workup). %AN measured at 242 nm. <0.05 = ND, $\le 0.1 =$ trace.

^b 4 and 5 closely eluted in the chromatographic method used, and so were integrated together.

^c Repeat of run 18.



Figure S5. Model term ranking Pareto chart for factors affecting the %AN 2 response in the reduction of 1. All terms displayed show negative effects.

Table S5. Settings for the Regression Analysis of the Model for %AN 2 Responses in the Designof-Experiments Studies of the Reduction of 1 to 2.

Setting	Value
Wizard Mode	Automated
Model Source	Quadratic
Auto Correct	True
Stepwise Regression Option	Combined
F to Add/Remove	4.00
Recommended Transformation	None
Selected Transformation	None
Analysis has an outlier	False

Table S6. Regression Statistics for the Modeling of the %AN 2 Response in the Design-of-Experiments Studies of the Reduction of 1 to 2.

Regression Statistic	Value
R-squared	0.9881
Adjusted R-squared	0.9722
Standard Error (±)	1.45
Observations	15

Table S7. Regression Analysis of Variance (ANOVA) Statistics for the Modeling of the %AN 2 Response in the Design of Experiments Studies on the Reduction of 1 to 2.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	P-value
Regression	1042.2389	8	130.2799	62.1888	<0.0001
Residual	12.5695	6	2.0949		
Total	1054.8084	14			



Figure S6. Model term ranking Pareto chart for factors affecting the (4 + 5) %AN response in the reduction of 1. All terms displayed show positive effects.

Table S8. Settings for the Regression Analysis of the Model for %AN 4+5 Responses in the Design-of-Experiments Studies of the Reduction of 1 to 2.

Setting	Value
Wizard Mode	Automated
Model Source	Quadratic
Auto Correct	True
Stepwise Regression Option	Combined
F to Add/Remove	4.00
Recommended Transformation	Natural Log
Selected Transformation	Natural Log
Analysis has an outlier	False
Outlier run number	4
Outlier dropped from analysis	True

Table S9. Regression Statistics for the Modeling of the %AN 4+5 Response in the Design-of-Experiments Studies of the Reduction of 1 to 2.

Regression Statistic	Value
R-squared	0.9938
Adjusted R-squared	0.9884
Standard Error (±)	0.10
Observations	14

Table S10. Regression Analysis of Variance (ANOVA) Statistics for the Modeling of the %AN **4+5** Response in the Design of Experiments Studies on the Reduction of **1** to **2**.

Source of		Degrees of			
Variation	Sum of Squares	Freedom	Mean Square	F-Ratio	P-value
Regression	10.2738	6	1.7123	185.5567	< 0.0001
Residual	0.0646	7	0.0092		
Total	10.3384	13			

5. Ranging studies

To further probe the robustness of the reaction and workup towards deviations from the chosen conditions, experiments examining the acceptable ranges for stoichiometry and residence times were performed. A 10% undercharge of DIBAL-H was well tolerated (Table S11, entry 1), while a 10% overcharge resulted in significantly lowered pre-workup purity (Table S11, entry 2). In both cases the workup yielded crude solids with >90 %AN purity which was suitable for downstream usage. Shorter residence times were found to have little effect on reaction outcomes (Table S11, entry 3).

			Pre-workup ^b LC %AN, Compound #			Crude solids ^c LC %AN, Compound #					
Entry	Scale	beviation from standard conditions	1	2	3	4+5 ^d	1	2	3	4+5 ^d	vield (%)
1	30.0 g	None ^e	ND	88.0	1.3	8.3	ND	94.4	1.4	0.4	83
2	5.0 g	1.35 equiv. DIBAL-H (-10%)	1.6	86.0	3.3	5.7	1.5	93.7	0.9	1.2	77
3	5.0 g	1.65 equiv. DIBAL-H (+10%)	Trace	78.8	4.0	9.2	ND	93.9	1.3	0.6	75
4	5.0 g	1.13 min residence time (-10%)	0.3	86.8	2.7	7.0	0.4	94.4	1.2	0.7	80

Table S11. Effect of DIBAL-H stoichiometry and residence time on the formation of 2 in flow.^a

 a Unless otherwise noted, all reactions used a reactor volume of 33 mL, a residence time of 75 s, and a bath temperature of -40 $^{\circ}$ C.

^b Samples taken directly from reactor outlet (after mixing with EtOAc, but prior to aqueous workup). %AN measured at 242 nm. <0.05 = ND, $\le 0.1 =$ trace.

^c Purity of crude solids after full aqueous workup and concentration of the resulting organic mixture to dryness. %AN measured at 242 nm. <0.05 = ND, $\le 0.1 =$ trace. Please refer to the Section 1.2 for further details on the workup procedure.

^d Compounds 4 and 5 co-eluted using this method and were integrated together.

^e Standard conditions: 1.50 equiv. DIBAL-H, 75 s reaction residence time.

6. Scale-up equipment selection

What follows is a brief listing of design choices used in the construction of the flow reactor system reported in the main text, along with criteria that led to their selection.

Parameter	Lab Scale (≤ 500 g)	Scale-up Criteria	Multikilogram-scale (3.4 kg module)
Pumping	HPLC-style piston pumps, computer-controlled interface (Vapourtec)	Low pulsation, ease of control	Magnetic drive gear pump and suction shoe pump head, manual control (Ismatec and Micropump)
Feed lines	Unfiltered, 1.59 mm bore PFA tubing	Avoid particulate ingress to prevent pump damage	6.38 mm (1/4") OD PE tubing equipped with 7 micron sintered metal in-line filters (Swagelok)
Flow measurement and control	Pre-run calibration of pumps, automated control of flow rates	Simple, low-cost, direct mass measurement (non- volumetric)	Direct mass measurement of feed kegs, and manual pump RPM adjustments by operators
Flow rate (sum of DIBAL-H and 1 flow rates, mL/min)	26.2	Pumping time of ≤12 h to synthesize up to 3.4 kg in a single module	62.0
Reactor productivity (kg/h 2 output, theoretical)	0.11	Increase reactor productivity, at least 2-fold	0.24
Mixing apparatus	300 μm caterpillar split-and- recombine micromixer (Institüt für Mikrotechnik Mainz, IMM)	Effective mixing with acceptable pressure drop, evaluated empirically	1.0 mm ID T-mixer (Upchurch Scientific)
Main reactor	1.59 mm ID PFA tubing (IDEX)	Similar diameter, avoid long tubing lengths to reduce back pressure	 37 x 1.88 mm ID (75.5 mL) 316L jacketed stainless steel tubes in parallel (23-series shell & tube heat
			exchanger, Exergy LLC)

Table S12. Comparison of lab-scale equipment used for manufacturing of up to 500 g **2**, and chosen multikilogram-scale equipment., with scale-up selection criteria.

Parameter	Lab Scale (≤ 500 g)	Scale-up Criteria	Multikilogram-scale (3.4 kg module)
Heat removal	Pre-cooling and reaction tubing immersed in dry-ice and isopropanol bath	Ease of control, avoid use of baths as much as possible	T-mixer cooled using insulated dry ice/isopropanol bath
			Pre-cooling, main reactor, and quench reactor use jacketed heat exchangers (see above) with siloxane polymer heat exchange medium (Dow) and chiller (Julabo)
Temperature monitoring	Bath temperature only	Accurate and recordable temperature monitoring of reactor contents	Process side: In-line thermocouples at pre- cooling outlets and reactor/quench outlets, connected to temperature recording unit Coolant side: in-line thermocouples at chiller inlet and outlet
In-process controls	Off-line analysis of reaction eluent by LC	Maintain steady state reactor conditions for longer pumping times of up to 12 hours	Verify conversion using initial off-line LC analysis after 15 minutes; Information-only off-line
			analysis of reaction eluent every 2 h by LC to verify steady state
Quench	Excess EtOAc in-line quench of DIBAL-H, then aqueous Rochelle salt workup	Increase batch reactor space to allow larger modules	Excess EtOAc in-line quench of DIBAL-H, then aqueous Rochelle salt workup
	(max. 20 L round-bottom flask)		(30 + 60 L glass-lined cylindrical reactors)

6.1. *Heat exchange*

On lab scale, the PFA tubing (1.59 mm inner diameter) used for the reaction medium required a length of approximately 26 m. For the flow rate of ca. 62 mL/min planned in manufacturing, the tubing would need to be 49 m long to offer a 75 second residence time. At such a narrow diameter,

the pressure drop would be approximately 8 bar (ca. 115 psi). This is independent of any other reactor components, tubing bends, volumes, or elements.

To shorten path length while maintaining a small tubing diameter, an approach similar to "numbering up" was taken, wherein the number of parallel tubes was increased. Shell and tube heat exchangers—where bundles of narrow tubes with a shared inlet and outlet are surrounded by a jacket for a heat exchange liquid—were selected which contained 37 tubes at 1.88 mm inner diameter, with a total volume of 77.5 mL including fittings (Figure S7).



Figure S7. Simplified schematic of an Exergy 23 series "shell & tube" heat exchanger, with illustrations of coolant and process flow. Process material flows through 37 tubes of 1.88 mm inner diameter, while the heat exchange medium flows around and past the tubes in the shell portion.

These heat exchangers allow the choice of parallel or counter (co-current or counter-current) flow for the heat exchange medium. In our system, counter flow was chosen for pre-cooling the reagents due to its ability to allow the lowest possible outlet temperature for the reagent feeds. Parallel flow was used for the reaction cooling, as in a reaction such as this the vast majority of heat is evolved early in the reaction, and so rapid and efficient cooling is most critical at the reaction inlet.⁵ All heat exchangers and coolant lines were wrapped in insulating thermal tape.

Cooling was accomplished by use of a recirculating chiller with a siloxane polymer heat exchange fluid. Temperature monitoring was performed both on the process side (at the outlet of each pre-cooling heat exchanger, as well as at the outlet of the reaction tube), as well as on the coolant side (at the coolant supply and return ports of the chilling unit), and in the cooling bath containing the t-mixer, using in-line thermocouples, attached via swaged fittings and connected to paperless data recorders (Figure S8).



Figure S8. Paperless data recorders displaying thermocouple-measured process side temperatures (left, channels 1-4), coolant side temperatures (left, channels 5-6), and t-mixer bath temperature (right, channel 1). Blue rectangles are used to hide identifying manufacturer information.

6.2. Mass flow measurement

As described in Section 5, the reduction of 1 proved highly robust to changes in stoichiometry and even residence time when carried out on lab scale. In light of these data, highly precise flow control via methods such as mass flow controllers (MFC, which have been successfully used for scale up of other organometallic processes)^{6,7} was deemed unnecessary, and instead direct

measurement of mass flow was accomplished simply by monitoring feed vessel mass change over time using a floor scale, and adjusting pump speed to maintain the desired flow rates.

6.3. Pumping

For a reaction in which both residence time and stoichiometry are so important, pumps that offered intrinsically low pulsation were desired. In our case, gear pumps were chosen due to their modularity, compact footprint, moderate cost, and simple operation. Based on the desired scale and the associated flow rates, magnetic-drive gear pump bodies with digital control were selected, and accompanied by suction shoe pump heads, which offered a nominal discharge of 0.092 mL/rev (maximum speed = 5500 RPM) and a maximum differential pressure tolerance of 5.2 bar (75 psi).

Testing was carried out to verify the pressure response of these pumps and pump heads, using process solvents. Measured performance at various pressures and rpm settings confirmed that the pumps would perform as required for manufacturing, but that back pressure must be maintained at a reasonable and stable level to avoid fluctuating discharge rates. To mitigate pump slippage resulting from pressure fluctuations, manually adjustable back pressure regulators were used to maintain a consistent pump discharge pressure of 40 psi (ca. 2.8 bar, 276 kPa). This allowed more consistent performance and reduced the need for adjustment. Pressure relief vales were also used to prevent potential over-pressurization from occurring and causing pump damage.

Because of their pumping mechanism, gear pumps can be highly sensitive to friction and particulate damage. Additionally, with the narrow tubing diameters used, it was necessary to mitigate the risk of clogging. To address this concern, DIBAL-H and **1** feeds were both equipped with micron sintered metal in-line filters to prevent particulates from damaging the wetted parts of the gear pumps.



Figure S9. a) Gear pump equipped with a pressure relief valve (top tight) and b) back pressure regulator used in the flow reactor system displaying downstream system pressure drop (gauge at left) and the set pump discharge pressure (gauge at right).

7. Manufacturing Observations

7.1. In-process pre-workup sample analyses

In-process pre-workup samples were taken after 15 minutes of elapsed pumping time. According to the testing method, only the peaks for 1 and 2 were quantified in order to measure conversion. The module was continued as long as conversion measured \geq 95 %AN 2 at this sample point. Details on the sampling procedure can also be found in Section 1.3.3.

Table S13. Summary of initial in-process sample analysis results from continuous flow manufacturing modules.

	Flanced time	Pre-workup %AN ^a	Pre-workup %AN ^a
Module	(hh:mm)	Compound 1	Compound 2
1	00:15	1.4	98.6
2	00:20	2.1	97.9
3	00:16	1.9	98.1
4	00:15	1.8	98.2
5	00:16	1.2	98.8
6	00:15	1.3	98.3
7	00:18	1.6	98.4

^a Samples taken directly from reactor outlet (after mixing with EtOAc, but prior to workup). %AN measured at 242 nm.

7.2. Reaction Sample Analyses

Information-only pre-workup samples were taken approximately every two hours after the initial in-process sample described in Section 7.1, with all peaks being integrated. Details on the sampling procedure can also be found in Section 1.3.3.

Table	S14 .	Summary	of	in-process	reaction	sample	analysis	results	from	continuous	flow
manufa	acturin	ng modules	1-7	7.							

		Pre-workup ^a LC %AN (242 nm)			
Module	Elapsed Time (hh:mm)	1	2	3	4+5 ^a
1	02:04	1.5	74.1	0.5	7.6
1	03:44	2.3	84.9	0.9	8.3
	01:40	4.4	84.3	1.0	6.6
	03:40	1.6	83.3	0.9	9.2
2	05:40	1.6	84.5	0.8	8.7
	07:40	1.7	83.1	0.8	9.6
	09:40	2.5	84.6	0.8	8.0
	02:18	1.1	85.3	0.6	10.7
	04:18	1.2	85.0	0.8	10.6
3	06:18	1.4	85.0	0.6	9.8
	08:18	1.1	82.6	0.9	10.0
	10:18	0.5	79.8	1.2	12.7
4	02:15	1.2	85.2	0.7	10.2
	04:15	1.2	85.4	0.6	10.0
	06:15	1.3	84.9	0.6	9.9
	08:19	1.5	84.2	0.8	10.3
	10:19	1.4	81.8	0.8	5.7
	02:16	1.1	79.6	1.0	8.4
	04:16	1.3	78.1	1.0	8.3
5	06:16	1.0	83.0	1.1	10.0
	08:15	1.2	83.0	1.0	9.8
	10:23	1.0	80.5	1.1	11.9
	02:23	1.2	84.4	0.7	9.9
6	04:15	1.3	83.8	0.8	9.1
0	06:15	0.9	84.6	0.8	11.3
	08:15	1.0	84.7	0.9	10.2

		Р	re-workup ^a L	C %AN (242	nm)
Module	Elapsed Time (hh:mm)	1	2	3	4+5 ^a
	10:15	1.4	85.2	0.9	9.9
	02:18	1.2	85.3	0.7	10.5
7	04:18	0.9	84.4	0.8	11.1
	06:02	0.8	84.2	0.8	11.9

^a Samples taken directly from reactor outlet (after mixing with EtOAc, but prior to workup). %AN measured at 242 nm. $<0.05 = ND, \le 0.1 = trace$.

^b 4 and 5 co-elute in the method used, and so are quantified together.

7.3. Final Stock Solution Sample Analyses

Table S15. Summary of analysis results for final stock solutions from continuous flow manufacturing modules 1-7.

	LC %AN (242 nm)						
Module	1	2	3	4+5 ^a			
1	2.2	88.7	1.0	0.4			
2	2.6	89.6	1.3	0.5			
3	1.4	90.1	1.5	0.4			
4	1.6	88.3	1.5	0.6			
5	1.4	89.2	1.4	0.7			
6	1.4	89.9	1.1	0.5			
7	1.2	89.0	1.1	0.5			

^a Purity of organic stock solution after full aqueous workup. %AN measured at 242 nm. <0.05 = ND, $\le 0.1 = trace$.

^b 4 and 5 co-elute in the method used, and so are quantified together.

8. Determination of precipitated triethylamine hydrochloride from 1 stock solution

Approximately 1 kg of 1 stock solution in a 1 L Nalgene bottle was stored at 2-8 °C for several days. Upon removing the solution from refrigeration, significant quantities of particulate matter were observed in the stock solution despite its prior polish filtration. The water content of the stock solution was measured by coulometric Karl-Fisher titration, and found to be 0.02 wt%.

The solution was warmed to room temperature, sampled, and the sample evaporated to dryness by rotary evaporation, yielding a mixed residue of **1** and the precipitate. Analysis of these mixed residues by ¹H NMR showed that apart from **1**, there was approximately 5 mol% of what was suspected to be TEA•HCl, based on the integration of the quartet at 3.12 ppm in the sample, relative to **1** (Figure S10). This molar ratio would correspond to a concentration of approximately 4 g/L.

Further confirmation of the identity of this impurity was obtained by chilling a portion of this stock solution to -20 °C for 24 h, which led to the visible formation of needle-like crystalline solids on the bottom of the storage container. The supernatant was decanted, and the crystalline solids analyzed using ¹H NMR in D₂O solvent (Figure S11). When compared with the NMR spectrum for pure TEA•HCl in D₂O (Figure S12) the identity of these solids was conclusively assigned as TEA•HCl.



Figure S10. ¹H NMR spectrum of stock solution residue, showing **1** and the presence of 5 mol% triethylamine hydrochloride, in CDCl₃.



Figure S11. ¹H NMR spectrum of stock solution residue, containing 1 and triethylamine hydrochloride, in D_2O .



Figure S12. ¹H NMR spectrum of triethylamine hydrochloride in D₂O.

Chromatographic Methods

8.1. HPLC (Method 1)

Column: SIELC Primesep P, 5 µm particle size, 4.6 mm x 250 mm, P.N. P-46.250.0510

Mobile Phase A: 0.2 % TFA in water

Mobile Phase B: 0.2 % TFA in acetonitrile

Detection: 257 nm, 277 nm

8.2. UPLC (Method 2)

Column: Waters Acquity BEH C18, 1.7 µm particle size, 2.1 x 150 mm, P.N. 186002353

Mobile Phase A: 0.1 % Ammonium hydroxide in water

Mobile Phase B: 0.1 % Ammonium hydroxide in Acetonitrile

Detection: 242 nm

9. Compound Spectra

All NMR spectra were measured on a Bruker 400 MHz spectrometer, and chemical shifts (δ) are reported in parts per million relative to internal standard TMS (0 ppm). All spectra were recorded at room temperature unless otherwise noted.

9.1. Diethyl pyridine-2,4-dicarboxylate (1)

¹**H NMR** (CDCl₃, 400 MHZ): 8.92 (m, 1H), 8.65 (m, 1H), 8.04 (m, 1H), 4.52 (q, 2H, *J* = 7.2 Hz), 4.46 (q, 2H, *J* = 7.2 Hz), 1.47 (t, 3H, *J* = 7.2 Hz), 1.44 (t, 3H, *J* = 7.2 Hz).



9.2. Ethyl 2-formylpyridine-4-carboxylate (2)

¹**H NMR** (CDCl₃, 400 MHz): 10.16 (s, 1H), 8.96 (m, 1H), 8.49 (m, 1H), 8.11 (m, 1H), 4.46 (q, 2H, *J* = 7.2 Hz), 1.45 (t, 3H, *J* = 7.2 Hz).



9.3. Ethyl 2-(hydroxymethyl)pyridine-4-carboxylic acid (3)

¹**H NMR** (CDCl₃ 400 MHz): 8.10 (m, 1H), 7.16 (m, 1H), 7.79 (m, 1H), 4.86 (s, 2H), 4.44 (q, 2H, *J* = 7.2 Hz), 3.6 (bs, 1H), 1.43 (t, 3H, *J* = 7.2 Hz).

Spectrum shown below is a ca. 60:40 mixture of compounds **3** and **5**. Only peaks corresponding to compound **3** are labeled.



9.4. Pyridine-2,4-dicarbaldehyde (4)

¹H NMR (CDCl₃, 400 MHz): 10.16 (s, 1H), 10.15 (s, 1H), 9.03 (m, 1H), 8.33 (m, 1H), 7.94 (m, 1H).

Spectrum shown below is a ca. 50:50 mixture of compounds 1 and 4, with small quantities of compound 5 also present. Only peaks

corresponding to compound 4 are labeled.



9.5. 4-(hydroxymethyl)pyridine-2-carbaldehyde (5)

¹H NMR (CDCl₃, 400 MHz): 10.10 (s, 1H), 8.76 (m, 1H), 7.97 (m, 1H), 7.58 (m, 1H), 4.86 (s, 2H), 3.6 (bs, 1H).

Spectrum shown below is a ca. 60:40 mixture of compounds 3 and 5. Only peaks corresponding to compound 5 are labeled.



10. References

- 1. Panić, S.; Loebbecke, S.; Tuercke, T.; Antes, J.; Bošković, D. Experimental Approaches to a Better Understanding of Mixing Performance of Microfluidic Devices. *Chem. Eng. J.* **2004**, *101*, 409–419.
- 2. 2. Falk, L.; Commenge, J.-M. Performance Comparison of Micromixers. *Chem. Eng. Sci.* 2010, 65, 405–411.
- 3. 3. Schwolow, S.; Hollmann, J.; Schenkel, B.; Röder, T. Application-Oriented Analysis of Mixing Performance in Microreactors. *Org. Process Res. Dev.* **2012**, *16*, 1513–1522.
- 4. 4. Carrier, O.; Funfschilling, D.; Debas, H.; Poncin, S.; Löb, P.; Li, H.-Z. Pressure Drop in a Split-and-Recombine Caterpillar Micromixer in Case of Newtonian and Non-Newtonian Fluids. *AIChE J.* **2013**, *59*, 2679–2685.
- 5. 5. Çengel, Y.; Turner, R.; Cimbala, J. *Fundamentals of Thermal-Fluid Sciences*, 5th ed.; McGraw Hill: New York, 2017.
- 6. Thaisrivongs, D. A.; Naber, J. R.; McMullen, J. P. Using Flow To Outpace Fast Proton Transfer in an Organometallic Reaction for the Manufacture of Verubecestat (MK-8931). Org. Process Res. Dev. 2016, 20, 1997–2004.
- 7. 7. Thaisrivongs, D. A.; Naber, J. R.; Rogus, N. J.; Spencer, G. Development of an Organometallic Flow Chemistry Reaction at Pilot-Plant Scale for the Manufacture of Verubecestat. *Org. Process Res. Dev.* **2018**, *22*, 403–408.