Electronic Supplementary Information

Laboratory of the future: a modular flow platform with multiple integrated PAT for monitoring multistep reactions

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1. General Information

1.1 Materials and Methods

Solvents and chemicals were obtained from commercial suppliers and were used without any further purification unless otherwise noted. Tetrahydrofuran was purchased from Acros in 99.5 % purity, extra dry over molecular sieves and stabilized. The solvent was filtered with syringe filters (25 mm membrane, 0.45 µm pore size, PTFE) prior to usage to avoid particles in the solutions.

1.2 Flow Equipment

In the flow setup, standard PFA tubing (0.8 mm or 1.6 mm i.d.), fittings, T-pieces manufactured from PTFE or PEEK were used as connectors. The back pressure regulator was obtained from Upchurch Scientific.

1.3 High Field NMR

NMR spectra were recorded on a Bruker 300 MHz instrument. ^{1}H, ^{13}C and ^{19}F spectra were recorded at 300 MHz, 75 MHz and 282 MHz, respectively, with a chemical shift relative to TMS expressed in parts per million. Chemical shifts (δ) are reported in ppm downfield from TMS as the internal standard. The letters s, d, dd, t, tt, q, and m are used to indicate singlet, doublet, doublet of doublets, triplet, triplet of triplets, quadruplet, and multiplet respectively.

1.4 LC-MS

LC-MS analysis were performed on a Shimadzu HPLC system comprised of a degassing unit (DGU-20A), solvent delivering unit (LC-20AD), an autosampler (SIL-20A), thermostated column oven (CTO-20A). The separation was carried out on a Macherey-Nagel Nucleodur C18 HTec column (150 mm × 4.6 mm, particle size 5 µm) at 37 °C using mobile phase A (H_2O/acetonitrile (9+1 v/v) + 0.1% TFA) and B (acetonitrile + 0.1% TFA) at a flow rate of 0.6 mL·min^{-1}). The following gradient was applied: hold 5% of B for 2 minutes, then linear increase from 5% B to 20% B in 6 min, followed by a linear increase from 20% B to 100% B in 8 min, then hold 100% B for 6 min, followed by column equilibration time at 5% B for 5 min. The detection of compounds was accomplished by diode array detector (SPD-M20A) prior electrospray ionization (ESI) using a Shimadzu LCMS-QP2020 instrument. The ESI-MS was operating either in positive or negative mode with in a scan range of 100- 400 m/z or 300- 500 m/z. The interface voltage was either 4.5 kV (positive mode) or –4.5 kV (negative mode) and the detector voltage typically 0.7 kV. The interface temperature was 350 °C, the DL temperature 250 °C and the heat block temperature was 250 °C. Nitrogen was used as carrier gas and the dry gas flow rate was 18 L·min^{-1} and the nebulizer gas flow rate was 1.5 L·min^{-1}.

1.5 HRMS

High resolution mass spectra of pure substances were recorded either in positive or negative mode on a Agilent 6230 TOF LC/MS (G6230B) by flow injections (1 µL) on an Agilent 1260 Infinity Series (HiP Degasser G4225A, Binary Pump G1312B, ALS Autosampler G1329B, TCC Column thermostat G1316A, DAD Detector G4212B). The solvent was 50 % H_2O + 0.1 % of a 5 M ammonium formate solution and 50 % of a MeOH + 0.1 % of a 5 M ammonium formate solution and a flow rate of 0.3 mL·min^{-1}. A Dual AJS ESI source was used with the following settings: (A) negative mode: Gas temperature (N_2) 325 °C, drying gas (N_2): 10 L·min^{-1}; nebulizer: 40 psig; fragmentor voltage: 175 V; skimmer voltage: 65 V, OCT 1 RF Vpp: 750 V; Vcap: 5000 V; nozzle voltage: 2000 V; reference mass: 966.0007; (B) positive mode: Gas temperature (N_2) 350 °C, drying gas (N_2): 10 L·min^{-1}; nebulizer: 40 psig; fragmentor voltage: 200 V; skimmer voltage: 65 V, OCT 1 RF Vpp: 750 V; Vcap: 3500 V; nozzle voltage: 1100 V; reference mass: 121.050873 and 922.009798. The scan range was 100-1100 m/z and 1 spectra per second was recorded.

1.6 Preparative Chromatography

Column chromatography purifications were carried out on an automated flash chromatography system (Biotage SP1) using ethyl acetate/40-60 petroleum ether (HPLC grade) mixtures as eluent.

2 General Flow Configuration

A detailed overview of the flow configuration is given in Fig. S1. Each part of the flow configuration will be discussed below.

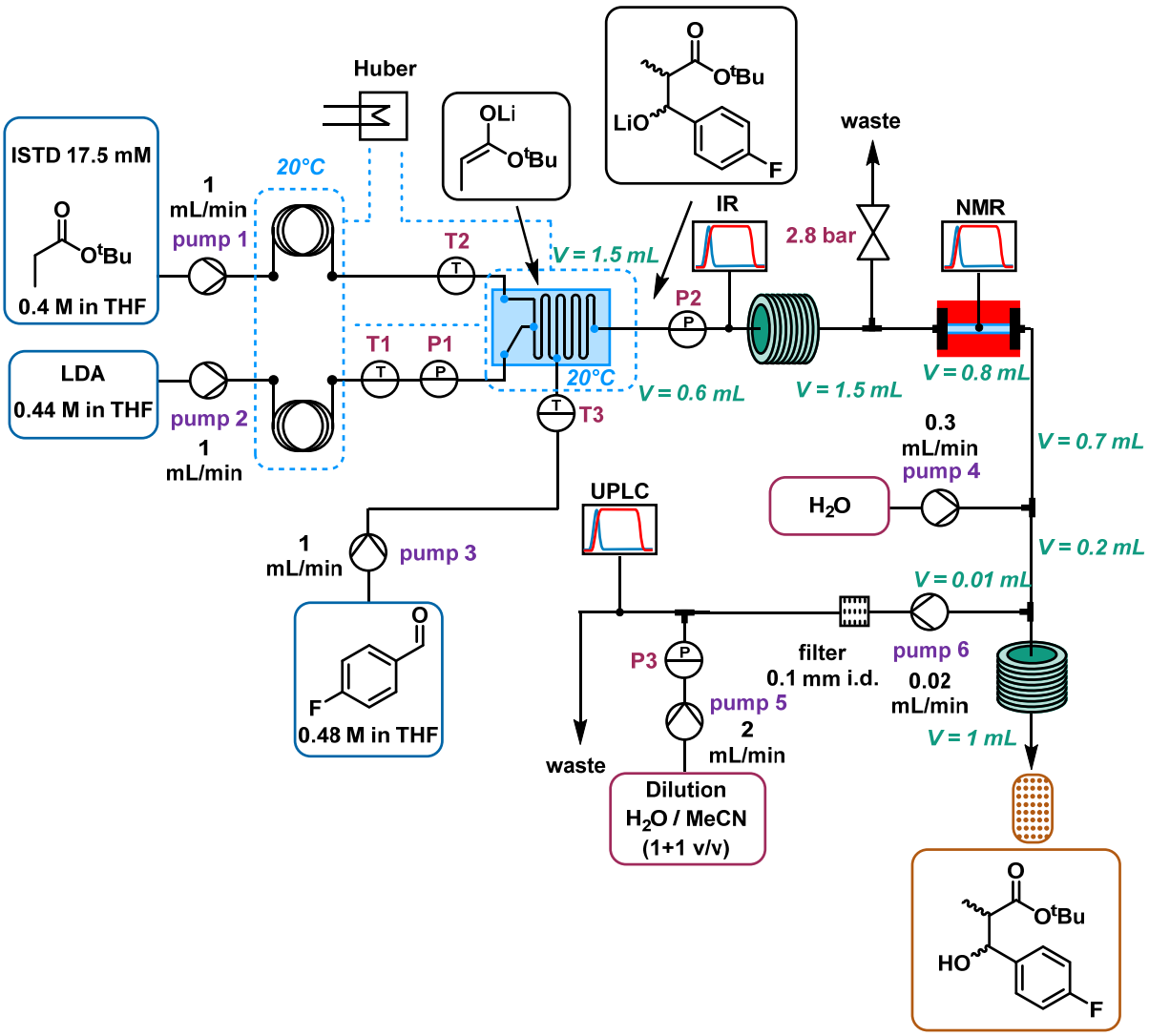


Fig. S1 Schematic view of the final reaction setup used in this study, showing the placement of pumps, sensors (T = temperature probe; P = pressure probe) and PAT instruments.

2.1 HiTec Zang Control Unit and LabVision Software

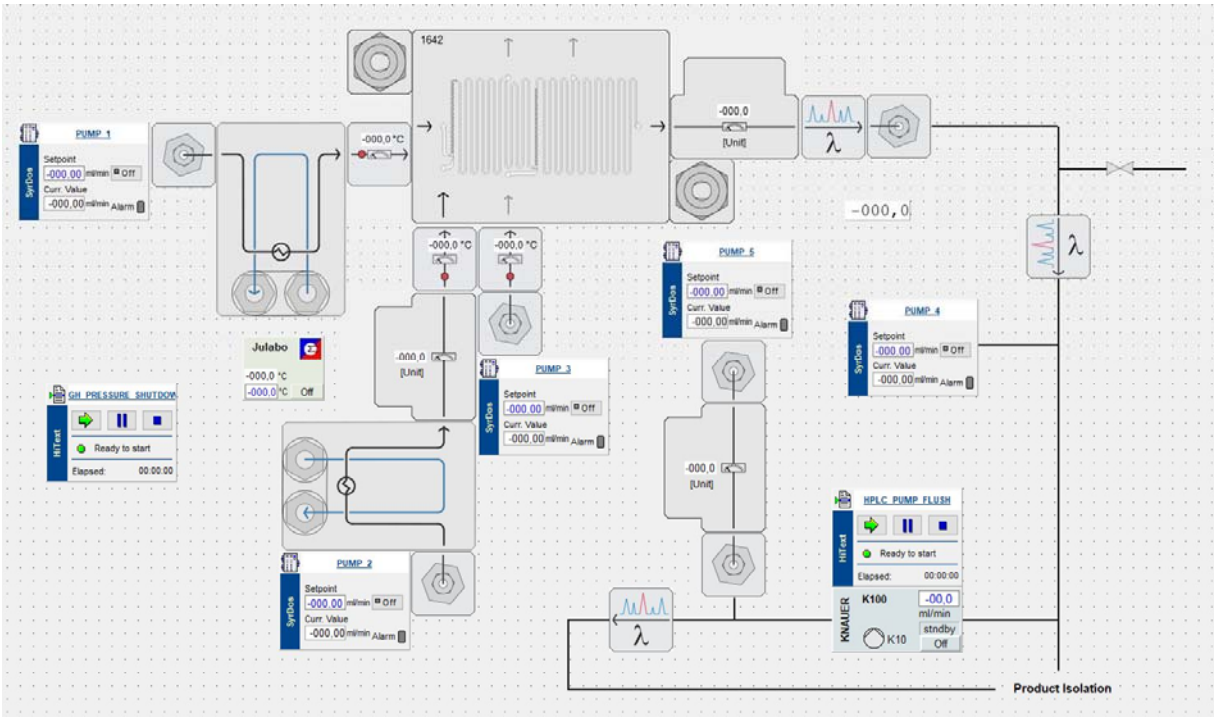


Fig. S2 Schematic of the process in LabVision.

2.2 Pumps

The system consisted of five SyrDos2 pumps (pumps 1 to 5) and a Knauer Azura HPLC pump (pump 6). Each pump was controlled via RS232 interface by a control module (HiTec Zang LabManager) and its associated software (HiTec Zang LabVision). Prior to each experiment the pumps were calibrated using a mass flow controller (MFC) (**Fig. S3**). In the case of a pump not delivering the correct flow rate, the pump was adjusted either changing the prestep value or through initialization of the syringe positions. Of the SyrDos2 pumps, two (pumps 1 and 2) were equipped with a 90 bar valve and three (pumps 3, 4 and 5) were equipped with a 30 bar valve. The pressure in the system was tracked with pressure sensors, if the pressure increased above 25 bar the LabVision Software automatically turned off all pumps for safety reasons.

HiText script for high-pressure shutdown

```
begin
if !P_1 > 25 then
       !PUMP_1.ON=0
       !PUMP_2.ON=0
       !PUMP_3.ON=0
       !PUMP_4.ON=0
       !PUMP_5.ON=0
end if
if !P_1 > 25 then
       !PUMP_1.ON=0
       !PUMP_2.ON=0
       !PUMP_3.ON=0
       !PUMP_4.ON=0
       !PUMP_5.ON=0
end if
goto begin
```

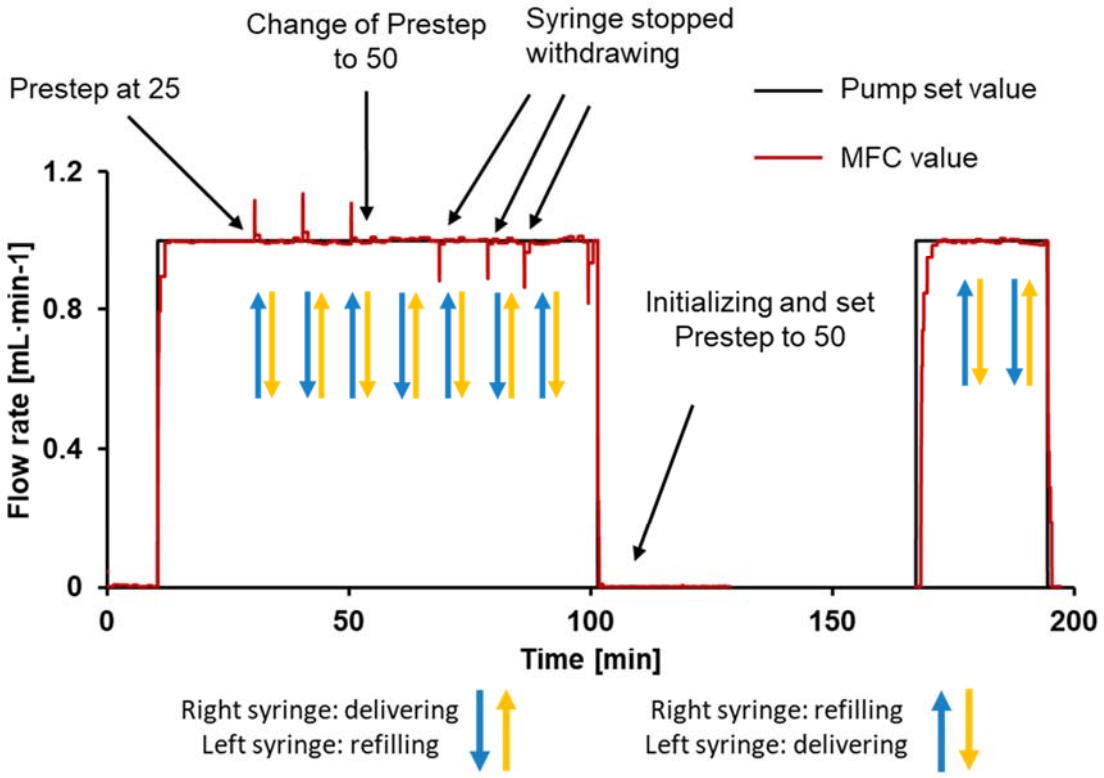


Fig. S3 A graph showing pump calibration using a mass flow controller (MFC).

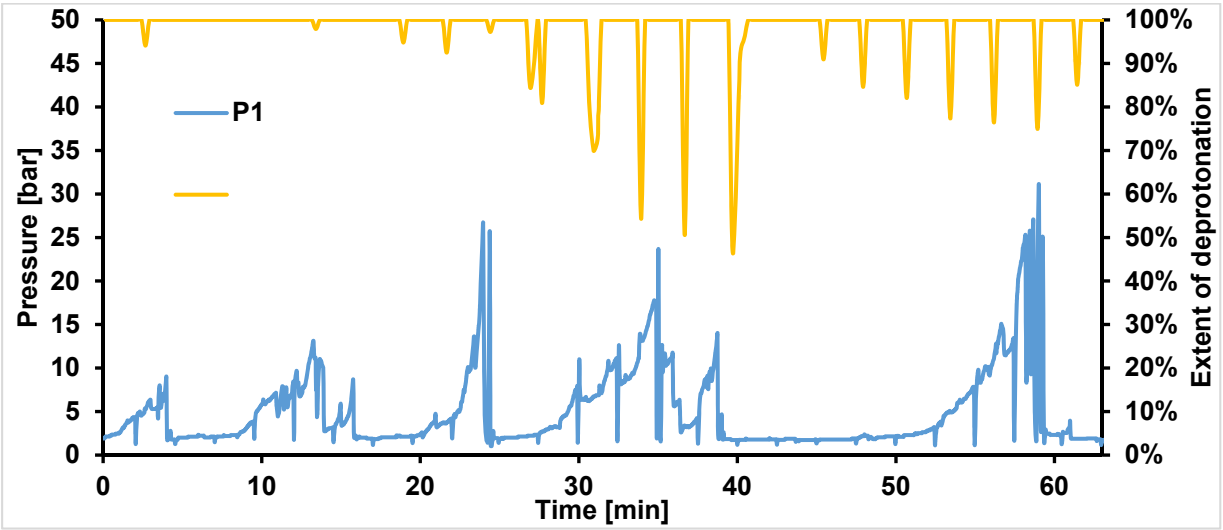
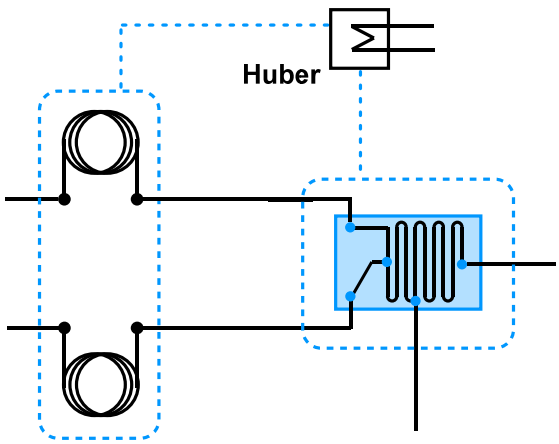
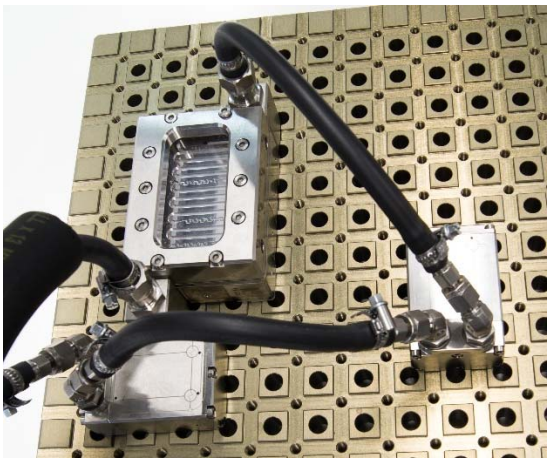


Fig. S4 A graph showing the extent of deprotonation over operation time and the influence of changes in pressure

2.3 Heat Exchanger and Lonza FlowPlate Lab





	Coax heat exchanger	Lonza FlowPlate Lab	
Part	0309-4-0004-F	1701-3-0004-F	
Wetted material	Hastelloy® C 276 (2.4819) FFPM	Hastelloy® C 22 / C 276, Sapphire, FFPM	
Internal volume	1010 µL	long side, near center long side, edge short side	95 µL* 103 µL* 74 µL*
Max. particle size	30 µm	5 µm	
Max. pressure	100 bar	35 bar	
Max. temperature	–20-200 °C	–20-200 °C	
Temperature control	Yes	Yes	

Fig. S5 The heat exchangers and the Lonza FlowPlate Lab were connected to a thermostat (Huber, Ministat 240). The temperature and pumping speed of the cooling/heating liquid was set on the LabVision software and communicated via an RS232 interface with the thermostat.
Note: * this describes internal volume of the Lonza FlowPlate Lab without a process plate fitted.

2.4 Lonza FlowPlate Process Plates

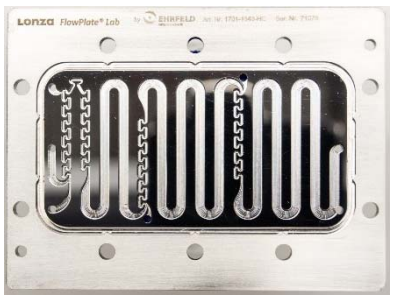
		
Flow plates	1701-1642-HC	1701-1343-HC
Material	Hastelloy® C 22	Hastelloy® C 22
Mixing Structure	SZ-Mixer	SZ-Mixer
Minimum Channel Width	0.2 mm	0.5 mm
Maximum Channel Width	0.6 mm	2.5 mm
Reaction Zone: Deprotonation	150 µL	361 µL
Reaction Zone: Addition of Electrophile	197 µL	1140 µL

Fig. S6 The two different process plates used in this study with specifications.

2.4.1 Pressure Profile

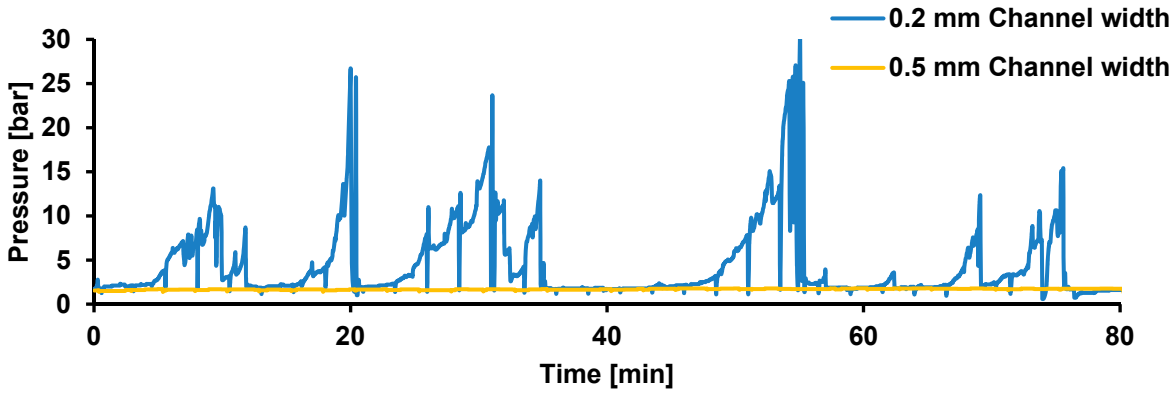
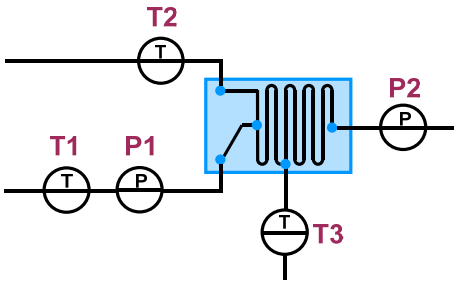


Fig. S7 A typical pressure profile during a scale-out experiment using either the process plate with a channel width of 0.2 mm or with a channel width of 0.5 mm. In the case of 0.2 mm, blockages occurred during the scale-out experiment and pressure increases were observed.

2.5 Pressure and Temperature Probes

Temperature and pressure probes were connected to the HiTec Zang LabManager and the data were recorded in the LabVision software. Data points were collected every 1 second. The temperature probes were placed in front of the process plate to observe the inlet temperature in the reactor. Pressure probes were placed in front (P1) and after (P2) the process plate. This configuration enabled us to identify blockages in or after the process plate. For example in **Fig. S9** a blockage in the process plate was observed around 25-30 min in the process plate and a blockage in the IR flow cell was observed at 45 min. The third pressure sensor (P3) was placed within the dilution and automated injection of the UPLC and will be discussed in more detail in **ESI Section 2.9**.



	Pressure Probe	Temperature Probe
Part	0518-1-6034-F or 0518-1-6044-F	0501-2-1004-X
Wetted material	Hastelloy® C 276 (2.4819) FFPM	Hastelloy® C 276 (2.4819) PTFE, Xyfluor
Measuring range	0-60 bar (6034-F) 0-100 bar [6044-F)	–60-200 °C
Internal volume	560 µL	165 µL
Max. particle size	50 µm	100 µm
Connection	Electrical, analogue (Tuchel plug / DIN 5-pol)	Electrical, analogue (socket M8)

Fig. S8 Placement and specifications of temperature and pressure probes.

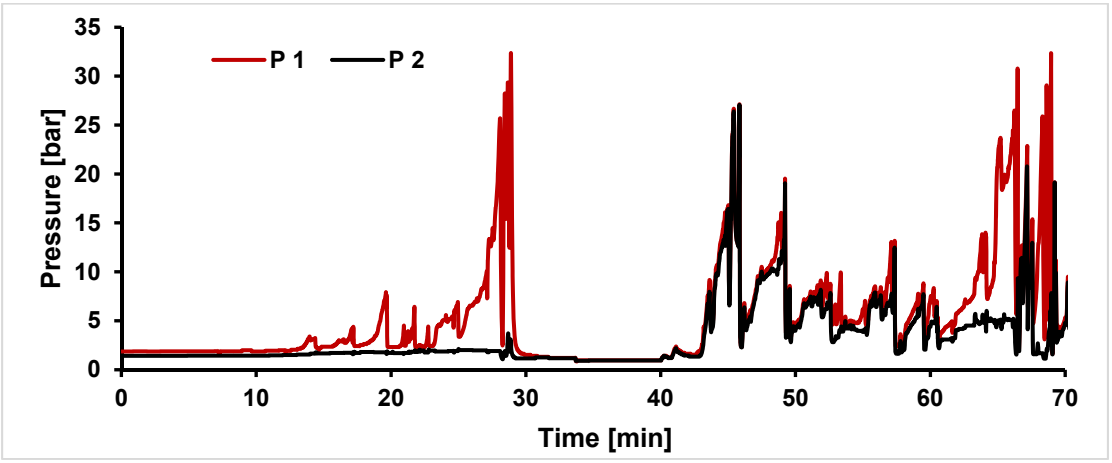


Fig. S9 Pressure profile recorded by pressure probes P1 and P2 during a scale-out experiment.

2.6 IR

2.6.1 Equipment

Inline ATR-FT-IR spectra were recorded on a ReactIR 15 instrument (ReactIR 15 DiComp probe, Mettler Toledo) equipped with a DiComp (Diamond) probe. The acquisition time for a data point was 15 sec and spectra were recorded between 2000 and 650 cm^{-1} using the maximum resolution of 4 cm^{-1}. Prior to starting the experiments it was ensured that the MCT detector was cooled with liquid nitrogen, the signal to noise ratio was above 5000 and the peak height was between 18000 and 24000. A flow cell (Ehrfeld Mikrotechnik, Part 0554-1-0004-F) with an internal volume of 82 µL manufactured from Hastelloy® C 276 (2.4819) and FFPM (wetted materials) allowed inline monitoring of the process stream (**Fig. S10**).

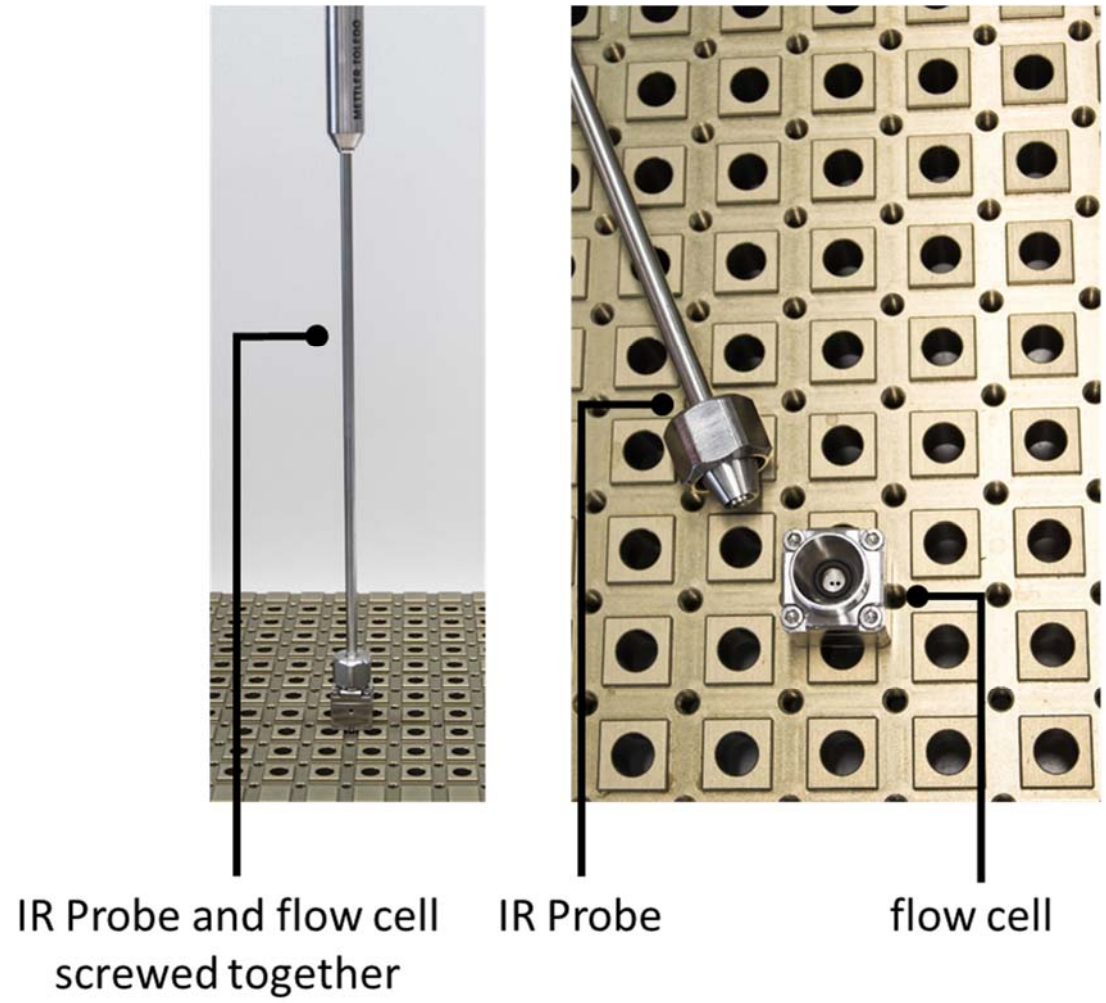


Fig. S10 A labeled image of the ReactIR probe and flow cell.

2.6.2 Deprotonation Experiments

Input solutions were made up with dry THF, in oven-dried round bottom flasks, under an argon atmosphere as follows:
0.4 M **1**: *tert*-butylpropionate (6.0 mL, 40.0 mmol) with THF (93 mL)
0.44 M LDA: diisopropylamine (1.4 mL, 10 mmol), *n*-butyllithium (2.5 M, 3.2 mL, 8.0 mmol) with THF (5.4 mL). The solution of amine was cooled to around –80 °C during the gradual addition of *n*-butyllithium.

The reaction setup shown in **Fig. S11** was used for this experiment, using a 6-port 2-position valve (Upchurch, part# V-450) equipped with a 10 mL PFA sample loop. The thermostat was set to –10 °C and allowed to stabilize. The pumps were set to the desired flow rate (see **Table S1**). After the IR signal stabilized, the sample loop was used to inject 10 mL of the LDA stream. The extent of deprotonation was determined by the remaining peak height of the propionate **1** C=O stretch at 1730 cm^{-1}.

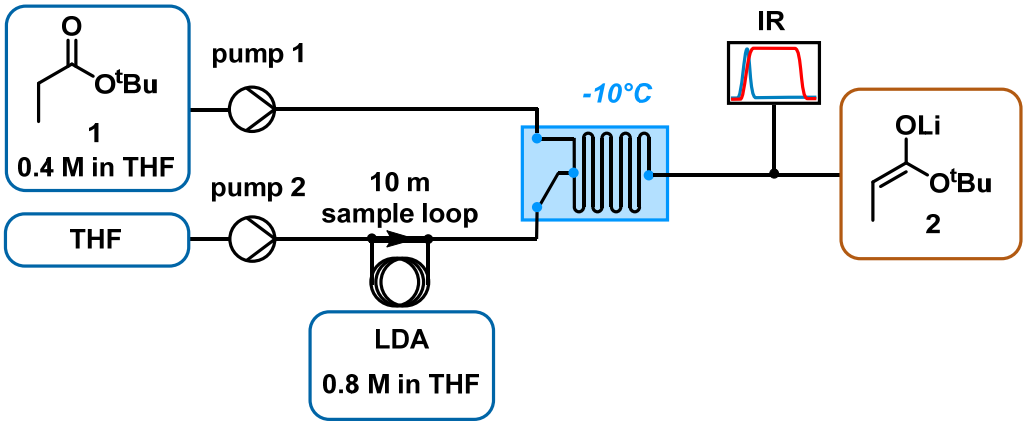


Fig. S11 Schematic representation of the flow setup used to measure deprotonation rate.

Entry	Pump 1 flow rate [mL·min^{-1}]	Pump 2 flow rate [mL·min^{-1}]	Residence time [sec]	Extent of deprotonation
1	0.5	0.1	23	> 95 %
2	1.0	1.0	12	> 95 %
3	3.0	3.0	3.9	> 95 %

Table S1 Flow rates used and results obtained for deprotonation study.

2.6.3 IR Spectra Overlay

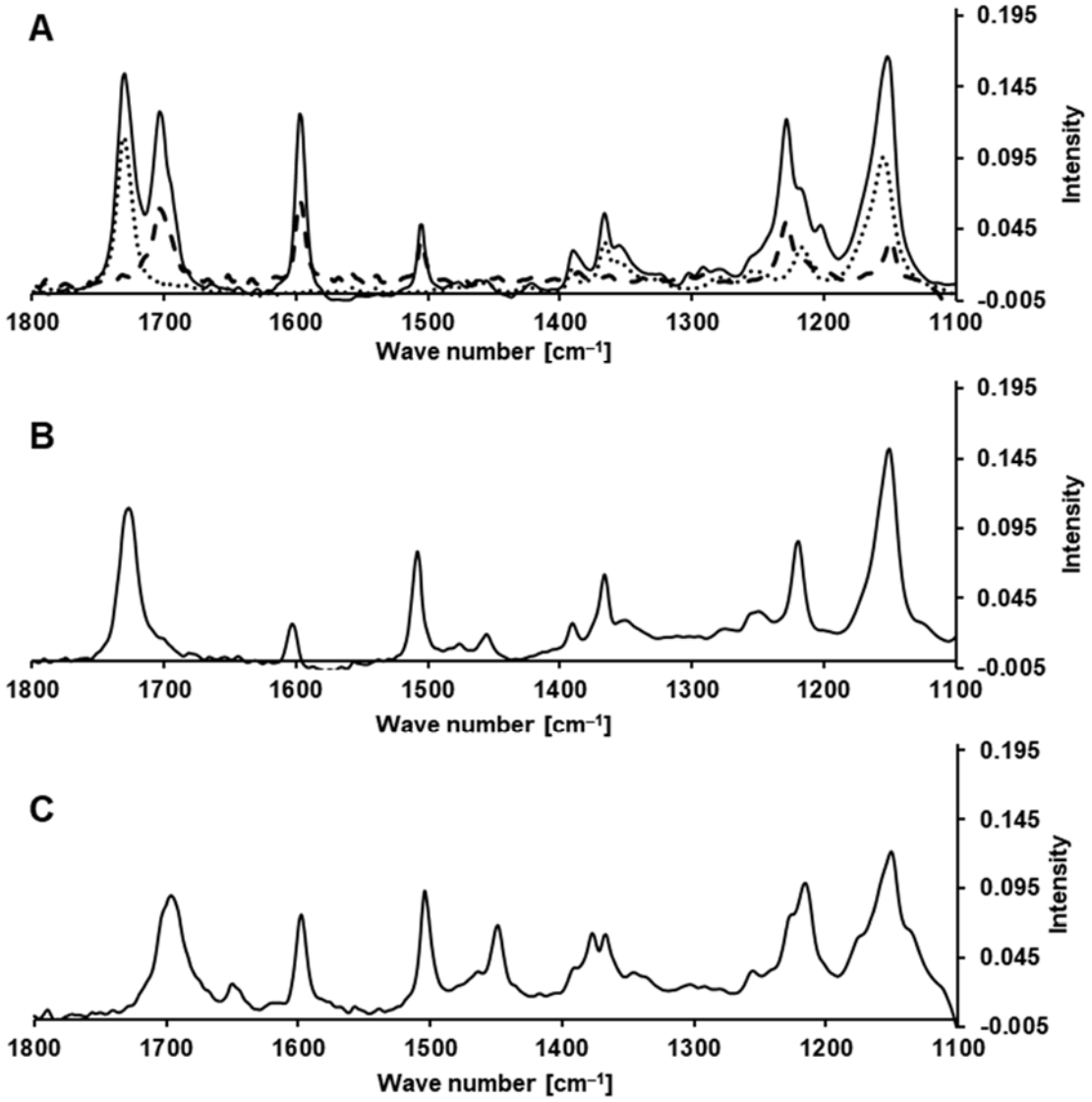


Fig. S12 In the first spectrum (A) the black solid line represents a mixture of the two substrates (*tert*-butyl propionate **1** and 4-fluorobenzaldehyde **3**), the dotted line is the spectrum for substrate **1** and the dashed line is the spectrum for substrate **3**. In (B) the spectrum for the quenched product **5** is given and in (C) a spectrum during reaction monitoring.

2.6.4 IR Calibration

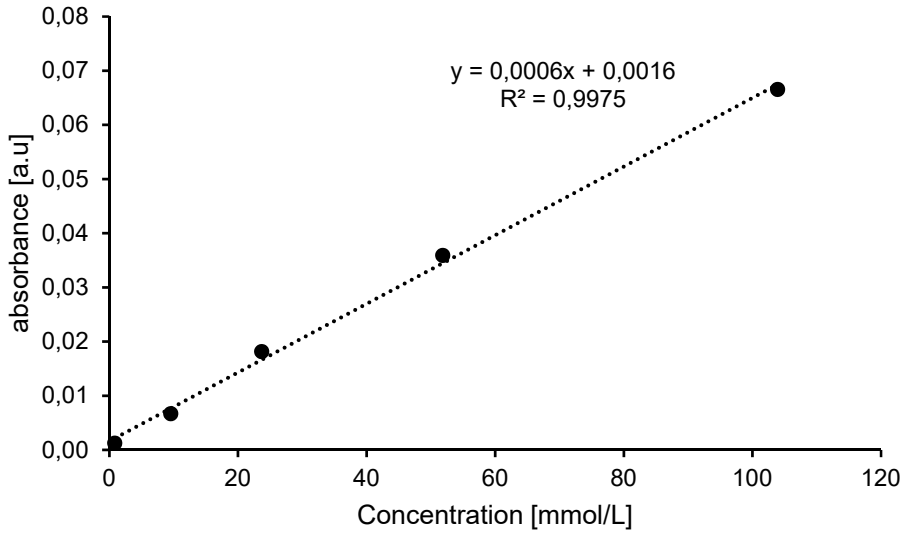


Fig. S13 IR calibration curve for *tert*-butyl propionate **1** in THF. The peak location was between 1750-1716 cm^{-1} and a two point baseline (1750 and 1716 cm^{-1}) was used.

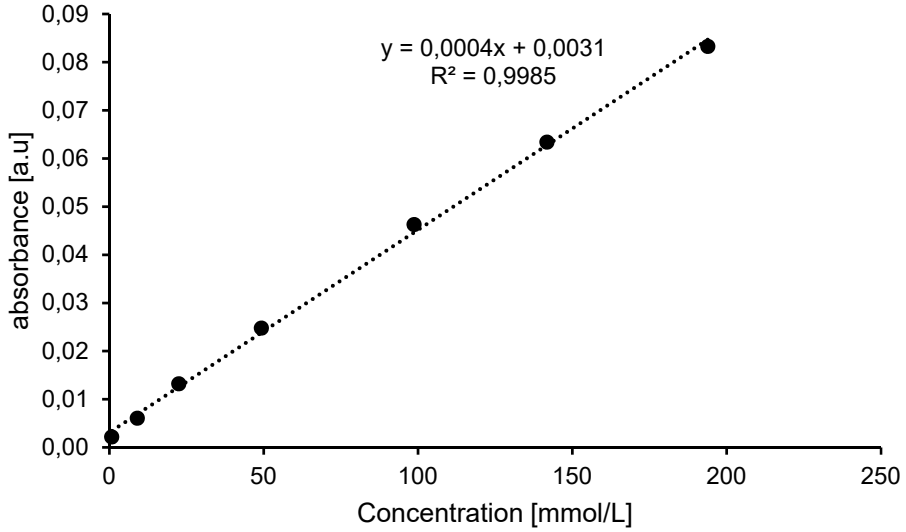


Fig. S14 IR calibration curve for 4-fluorobenzaldehyde **3** in THF. The peak location was between 1715-1687 cm^{-1} and a two point baseline (1715 and 1687 cm^{-1}) was used.

2.7 NMR

2.7.1 General Reaction Monitoring NMR

Online NMR reaction monitoring was accomplished by recording ^{1}H or ^{19}F spectra, using a low field benchtop 43 MHz NMR (Magritek, Spinsolve Ultra).

2.7.2 Process Integration

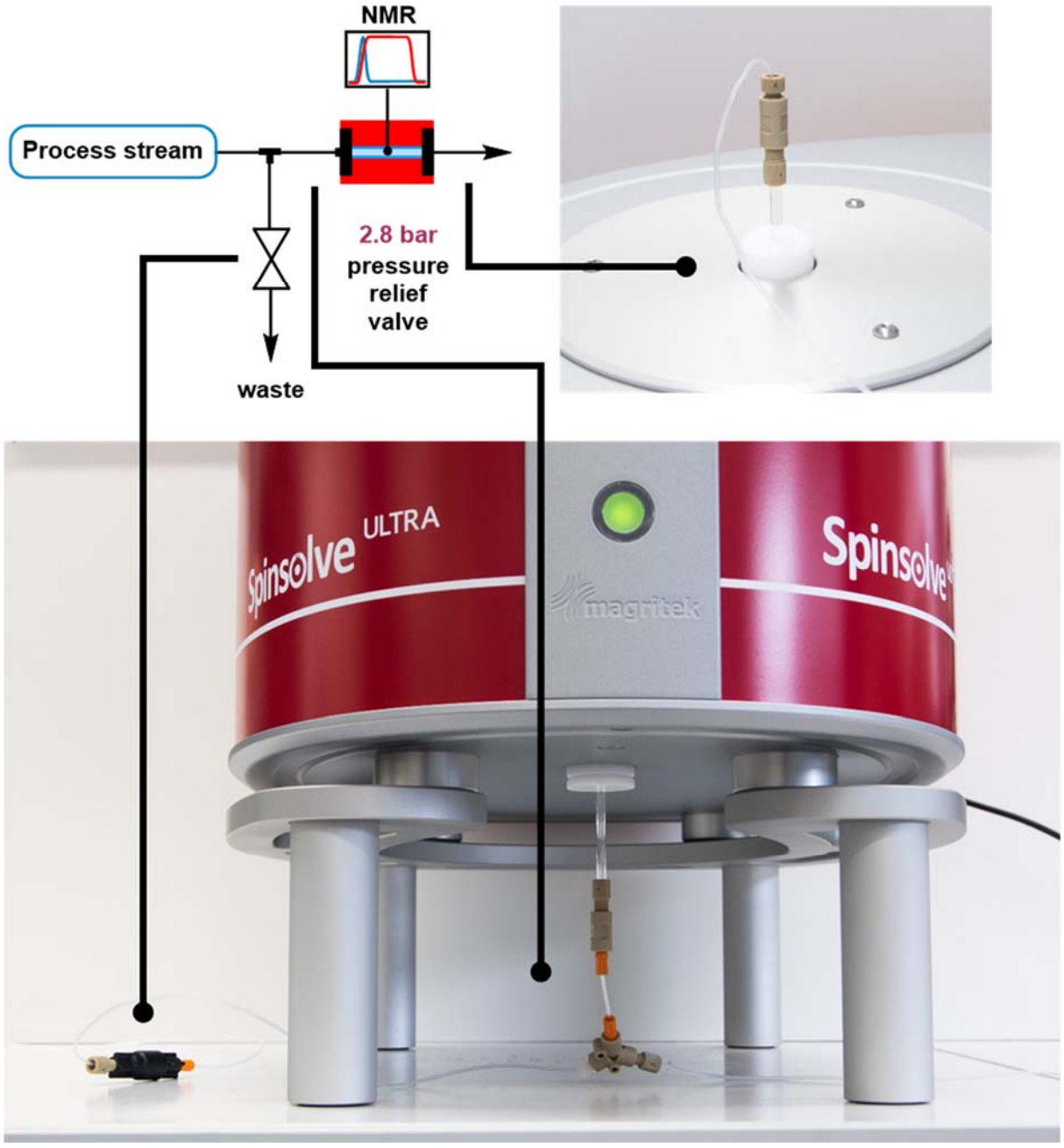


Fig. S15 A detailed overview of the integration of the NMR into the process. The glass flow through cell had an internal volume of 800 µL and a length of 550 mm.

2.7.3 General Overview, Pulse Sequence and Code

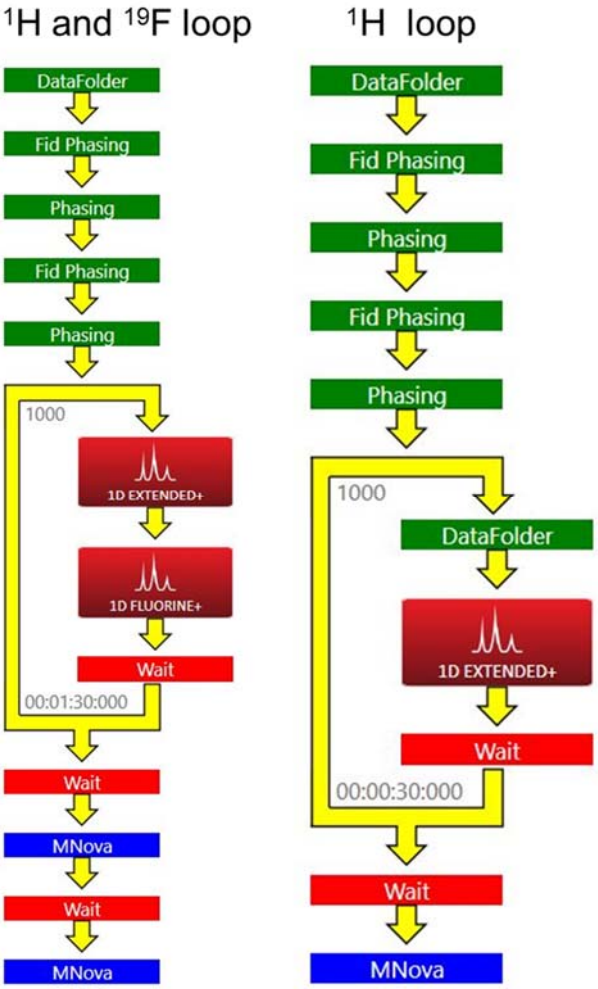


Fig. S16 Flow diagram of scripts used for inline NMR monitoring.

Code for ^{1}H and ^{19}F loop

```
TimeStampFolder           = "c:/ReactionMonitor/1H + 19F"
ProtonPhase               = "FirstScan"
FluorinePhase             = "FirstScan"

# Loop
loop(1000, 00:01:30:000)
  RunProtocol("1D EXTENDED+",   ["Number=4", "RepetitionTime=10",
"PulseAngle=90", "AcquisitionTime=6.4"])
  RunProtocol("1D FLUORINE+",   ["Number=16", "RepetitionTime=4",
"PulseAngle=90", "AcquisitionTime=1.64"])
  wait(00:00:10:000)
endloop

wait(00:00:10:000)
RunMnovaFile("ScriptUtilities/ReactionMonitor.qs", "process", ["1H"])
wait(00:00:10:000)
RunMnovaFile("ScriptUtilities/ReactionMonitor.qs", "process", ["19F"])
```

Code for ^{1}H loop

```
TimeStampFolder           = "c:/ReactionMonitor/PS006"
ProtonPhase               = "FirstScan"


# Loop
loop(1000, 00:00:30:000)
  TimeStampFolder           = "c:/ReactionMonitor/PS006/1H"
  RunProtocol("1D EXTENDED+",   ["Number=4", "RepetitionTime=10",
"PulseAngle=90", "AcquisitionTime=6.4"])
  wait(00:00:00:000)
endloop

wait(00:00:10:000)
RunMnovaFile("ScriptUtilities/ReactionMonitor.qs", "process", ["1H"])
```

2.7.4 ^{19}F NMR

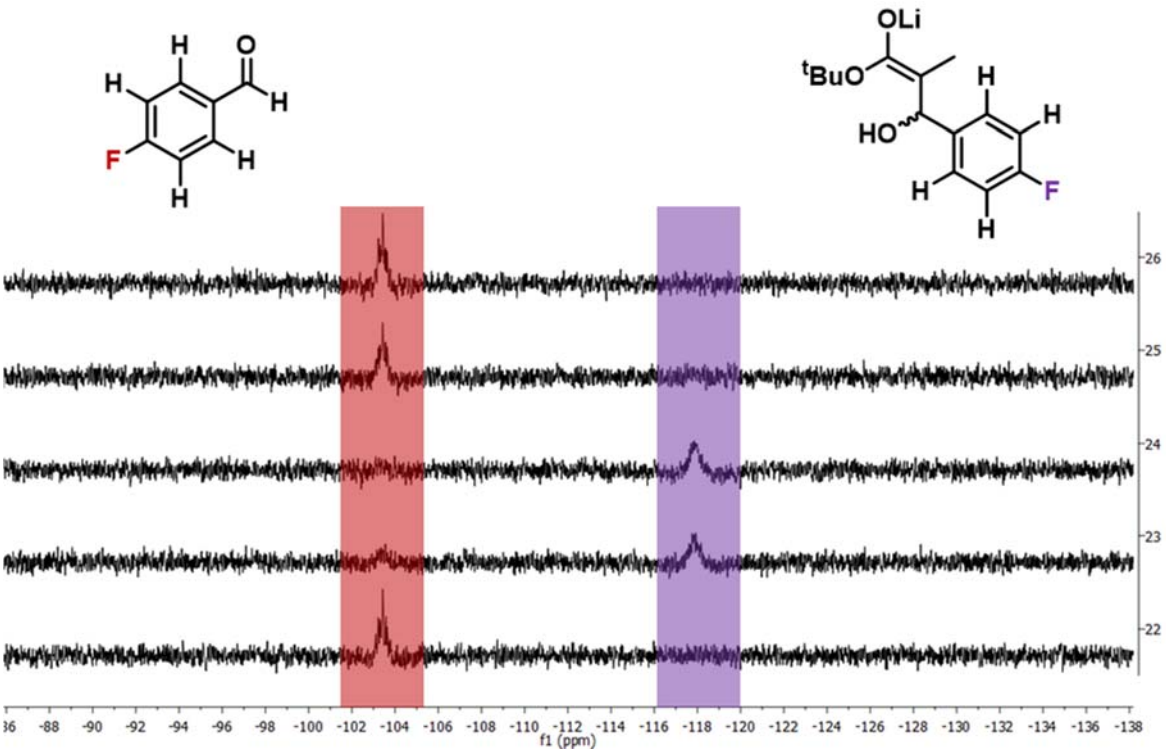


Fig. S17 Example spectra from reaction monitoring with ^{19}F-NMR. A poor signal to noise ratio was observed, therefore, ^{19}F-NMR was not used for the quantification of 4-fluorobenzaldehyde **3** or intermediate **4**.

2.7.5 ^{1}H NMR

The concentration of intermediate **4** could be calculated by taking the integral area 3 (containing signals from both **3** and **4**), and subtracting the area of integral 2 (aryl signals from aldehyde **3**). Example calculations are shown below (**Table S2**).

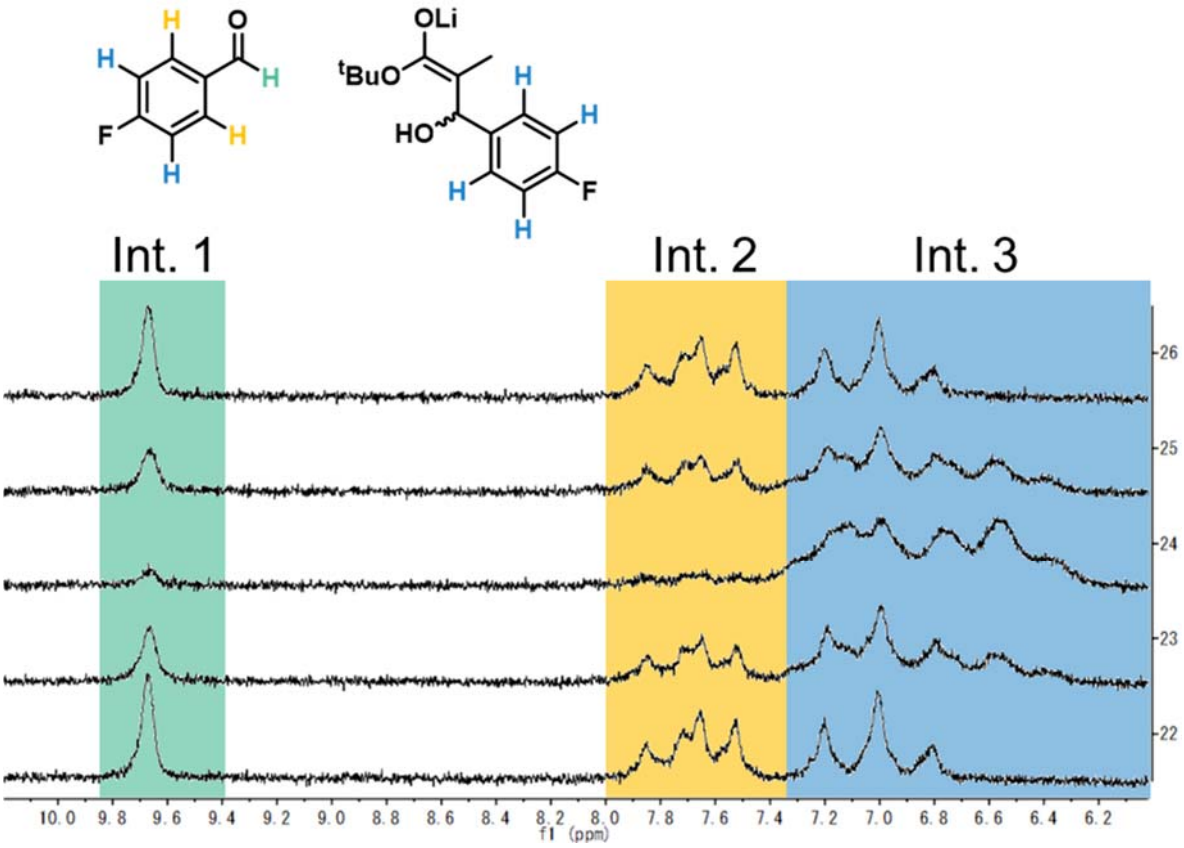


Fig. S18 Example ^{1}H NMR spectra from reaction monitoring, showing aldehyde **3** and intermediate **4**.

	Int. 1	Int. 2	Int. 3	Int. 3 – 2	Int.1 per one ^{1}H	Int. 2 per one ^{1}H	Int. 3 – 2 per one ^{1}H
22	2122	4549	4677	127	2122	2274	31
23	1399	3369	8544	5175	1399	1684	1293
24	385	1367	14946	13579	385	683	3395
25	1130	2978	8513	5535	1130	1489	1383
26	1797	4196	4393	197	1797	2098	49

Table S2 Example values for ^{1}H NMR integration.

2.7.6 NMR Calibration

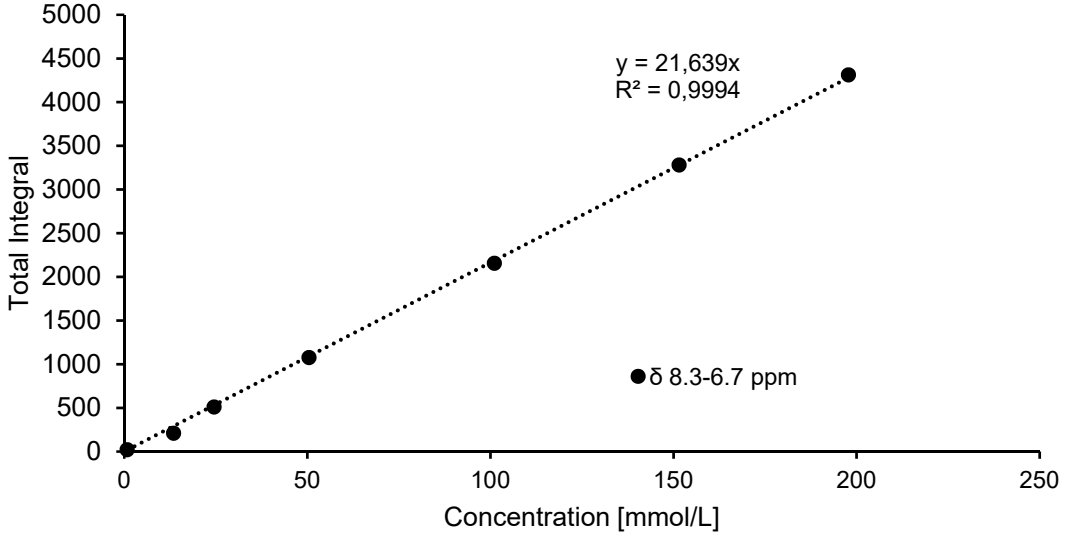


Fig. S19 Calibration curve for ^{1}H-NMR per ^{1}H using the four aryl protons of 4-fluorobenzaldehyde **3**. The integration area was between 8.3 – 6.7 ppm.

Since a linear response was observed for concentrations 25 mM and above (but not for 10 mM), it can be proposed that the limit of quantification is between 10 mM and 25 mM.

2.8 Development of Reaction Quench Setup

A 25 mL round bottom flask equipped with a magnetic stirring bar, was charged with diisopropylamine (1.5 mmol, 0.21 mL) and THF (9.1 mL) under an argon atmosphere. The solution was chilled to –70 °C (acetone + liquid nitrogen) and a 2.3 M solution of *n*-butyllithium (1.5 mmol, 0.64 mL) was added slowly. The reaction mixture was stirred for 5 minutes and propionate **1** (1.3 mmol, 0.20 mL) was added and stirred for 20 minutes at –70 °C. Then the aldehyde **3** (1.5 mmol, 0.21 mL) was added and stirred for 40 minutes at –70°C. The reaction mixture was then allowed to warm to room temperature. Glass vials with the desired quench solution were prepared and 1 mL of the reaction mixture was added in each vial (**Table S3**). For the online UPLC analysis, 3.0 mL/min of reaction mixture and 0.3 mL/min of the quench solution were used which gave a homogeneous solution prior to the UPLC.

Quench	Reaction Solution [mL]	Quench Solution [mL]	Ratio	Observation
H_2O	1	0.1	0.1	Cloudy Solution
H_2O	1	0.2	0.2	2 Phases
H_2O	1	0.3	0.3	2 Phases
H_2O	1	0.5	0.5	2 Phases
0.1 M aq. citric acid	1	0.3	0.3	2 Phases
0.1 M citric acid in MeOH	1	0.3	0.3	Precipitate
sat. NH_4Cl	1	0.3	0.3	2 Phases
sat. NH_4Cl + H_2O (1+9 v/v)	1	0.1	0.1	Cloudy Solution
sat. NH_4Cl + H_2O (1+9 v/v)	1	0.3	0.3	2 Phases

Table S3 Observed results of quench experiments.

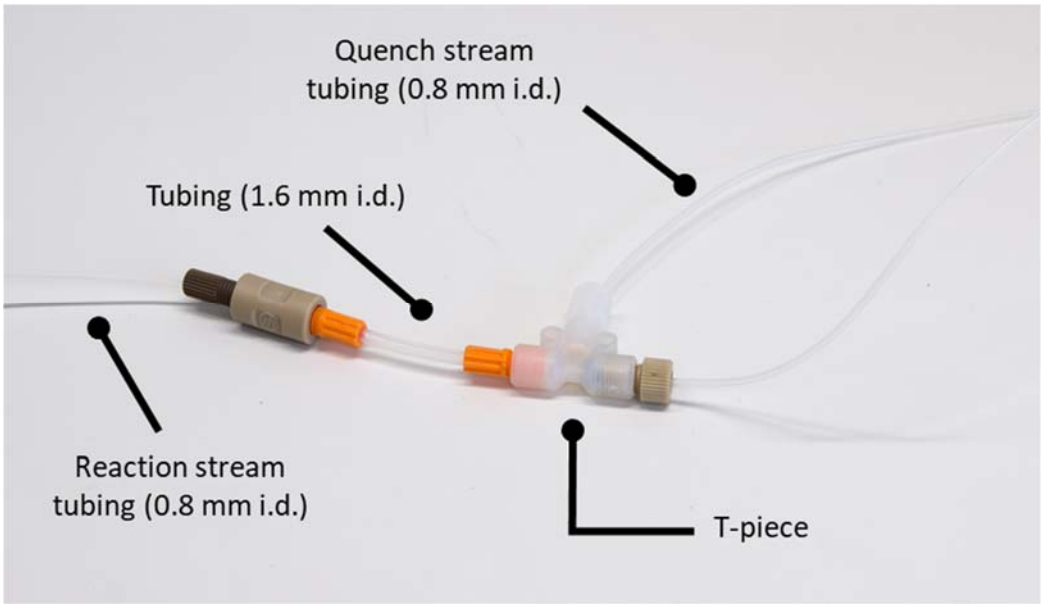


Fig. S20 To avoid blockages the T-piece (i.d. ~2 mm) used for the quench was drilled through to widen the internal diameter. Additionally, a short piece of larger diameter tubing was used in front of the T-piece to avoid blockages.

2.9 Dilution Stream Prior to UPLC Injection

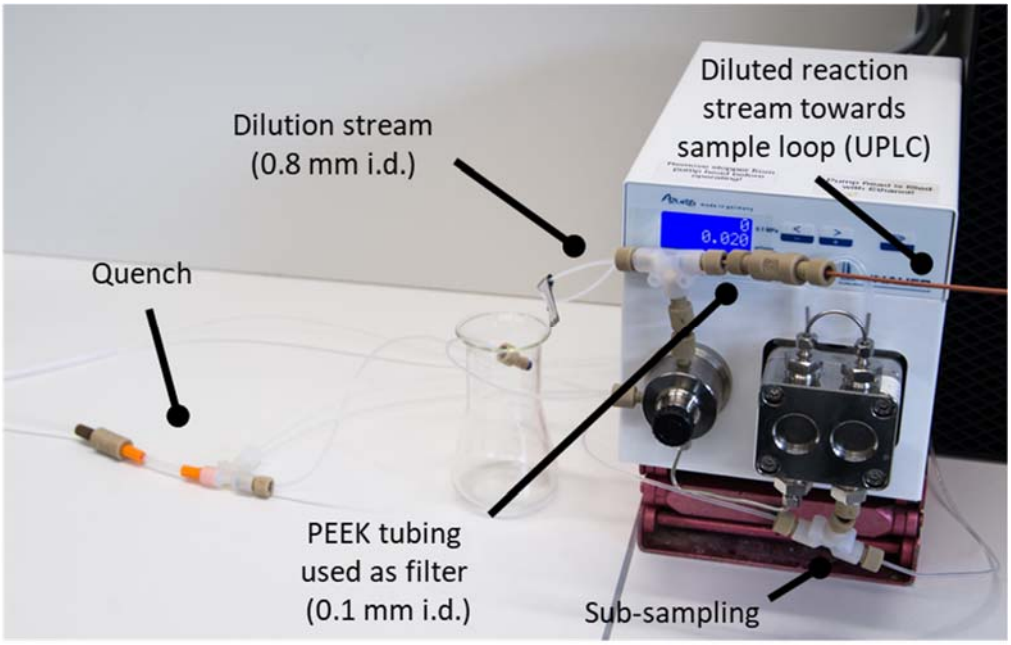


Fig. S21 An overview of the experimental setup for quenching the process stream, sub-sampling and dilution prior to UPLC analysis.

2.9.1 Subsampling with HPLC Pump

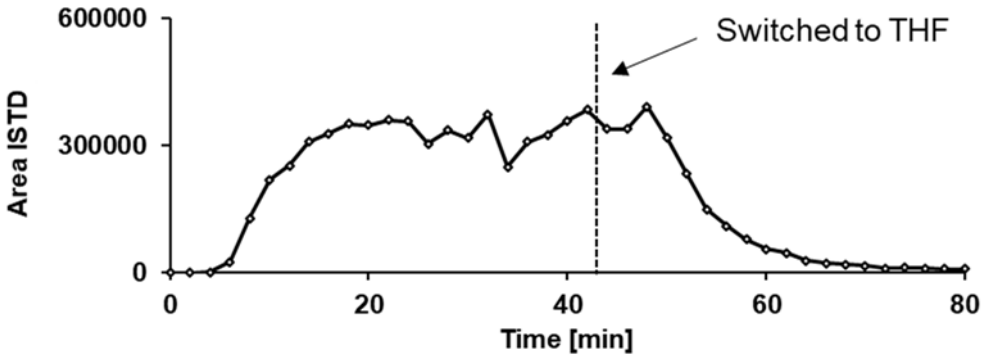


Fig. S22 Sub-sampling from the process stream (internal standard in THF) with the HPLC pump. The system required > 20 minutes to equilibrate after switching to pure THF (42 minute time point) without the "HPLC Sub-sampling "flush" script". When operating the system with the script, the equilibration time was greatly reduced.

HiText Code for the HPLC Sub-sampling "flush" script

```
begin
if !P_3<5 then
wait 5 sec
!HPLC_PUMP.F_W=0.5
wait 20 sec
!HPLC_PUMP.F_W=0.02
end if
goto begin
```

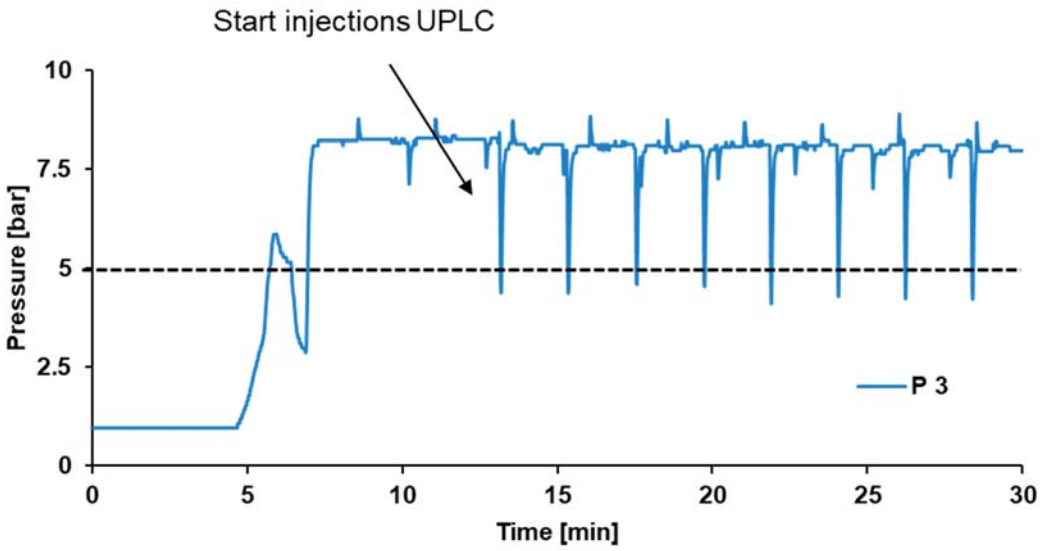


Fig. S23 The HPLC Sub-sampling "flush" script observed the pressure in the dilution stream (P3). The 6-port valve for the injection into the UPLC created a pressure drop in the dilution stream during injection. When the pressure dropped below 5 bar the subsampling pump flushed additional volume through for 20 seconds, to equilibrate the diluted process stream for the next injection.

2.9.2 Analytical Splitter (not used)

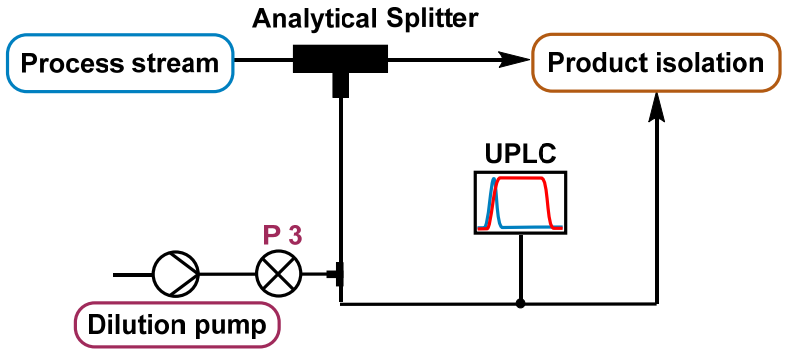


Fig S24 Possible setup for dilution prior to UPLC injection with an analytical splitter (QuickSplit™ Adjustable Flow Splitter, Analytical Scientific Instruments, Part Number 600-PO10-04).

Note: the splitter creates a pressure upstream and is not compatible with THF. For this reason, it was not implemented in the flow setup.

2.9.3 Mass Flow Controller (not used)

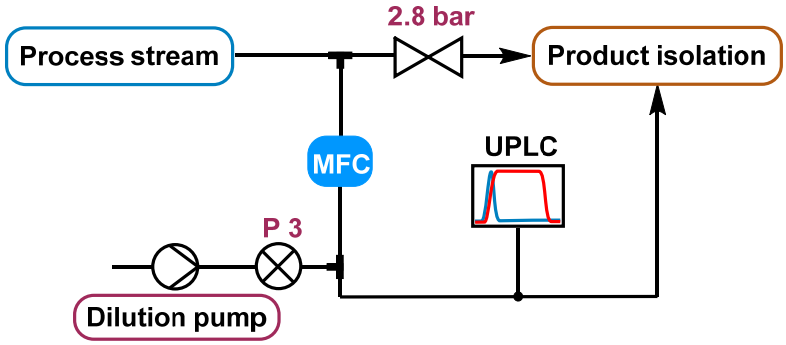
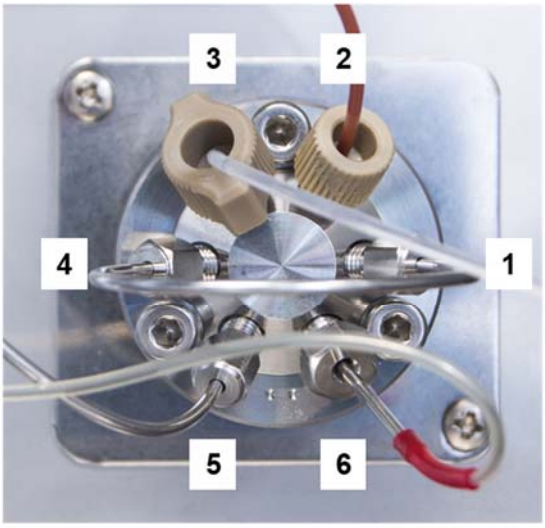


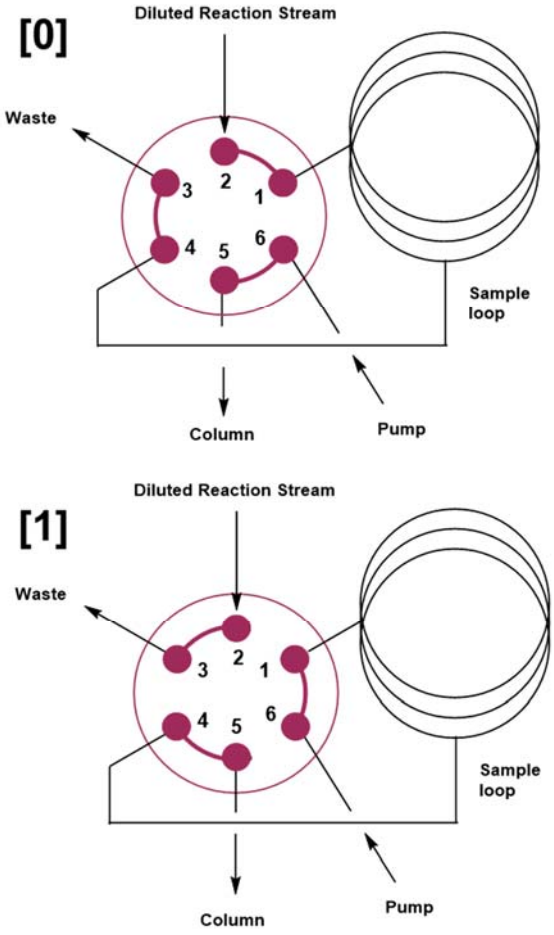
Fig. S25 Possible setup for implementing dilution prior to UPLC with a mass flow controller (Brooks Instrument, Coriolis Quantim Series, product code: QMBC2L1B3A1A2A1YY1C7A1AA).

Note: this specific MFC did not have the right volume range for our application. For this reason, it was not implemented in the flow setup.

2.10 Connecting Diluted Reaction Stream with UPLC

Injection onto the column was carried out via a high pressure six-port valve (Shimadzu FCV-32AH), which was controlled by Shimadzu LabSolutions software. A metal pipe (i.d. 0.1 mm) was cut to a length of 128.74 mm, sanded and connected in position 1 and 4 on the valve. The inner volume of the tube theoretically corresponds to a volume of 1 µL, however, initial testing versus samples injected by the autosampler revealed an injection volume of approximately 2.6 µL. The diluted reaction stream was connected in position 2 and tubing towards the waste in position 3. The UPLC stream from pump was connected in position 6 and the stream towards the column was connected in position 5. Across 10 injections of a 1mM standard solution of 4-fluorobenzaldehyde **3** a relative standard deviation (RSD) of 3 % was observed with this configuration.





Time	Module	Command	Value
0.00	Column Oven	CTO.RVR	[0]
0.01	Column Oven	CTO.RVR	[1]
0.05	Column Oven	CTO.RVR	[0]
2.00	Controller	STOP	

Fig. S26 Detailed overview of the connections and positions of the high pressure six-port valve and the control program in the Shimadzu LabSolutions software.

2.11 UPLC-DAD

2.11.1 Chromatographic Conditions

The UPLC-DAD (Shimadzu Nexera X2) was comprised of a degassing unit (DGU-20A), two solvent delivery units (LC-30AD), a thermostated autosampler (SIL-30AD), thermostated column oven (CTO-20AC) with an integrated high pressure 6-port valve (FCV-32AH), diode array detector (SPD-M30A) and a control unit (CBM-20A). The analysis was carried out on a Phenomenex Luna Omega C18 column (50 mm × 2.1 mm; 1.6 µm particle size; pore size 100 Å) at 45 °C using mobile phase A (H_2O/acetonitrile (9+1 v/v) + 0.1% TFA) and B (acetonitrile + 0.1% TFA) at a flow rate of 0.7 mL·min^{-1}. Compounds were eluted with an isocratic elution mode using 50% of A and B within 2 min. The autosampler was used with the UPLC in offline mode and the high pressure 6-port valve for online mode. UPLC-DAD calibration curves were measured for **3**, **5** and **6** versus internal standard (biphenyl) for quantitative calculations. For quantification, area responses were normalized with respect to biphenyl as the internal standard and corrected for molar relative response factors.

2.11.2 UPLC Method Development

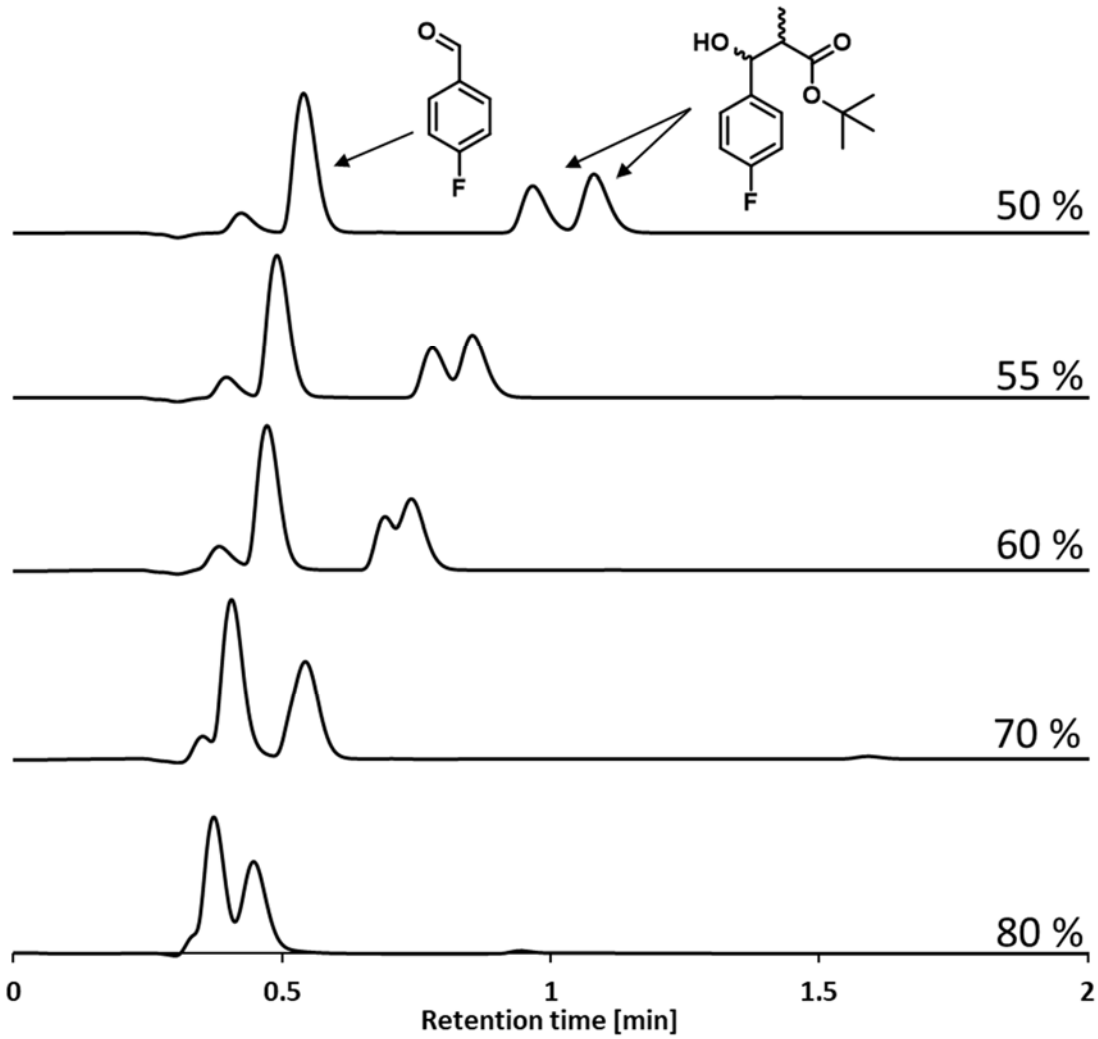


Fig. S27 Representative UPLC–DAD chromatograms of the analysis of a mixture consisting of 2 µM 4-fluorobenzaldehye **3** and product **5** for the investigation of different mobile phase compositions. Column: Luna Omega C18 50 mm × 2.1 mm, 1.6 µm; mobile phase: A: H_2O + MeCN (9+1 v/v) + 0.1 % CF_3COOH, B: MeCN + 0.1 % CF_3COOH; isocratic elution: X % B (X depicted in the figure); flow rate: 0.5 mL·min^{-1}; column oven: 40°C; injection volume: 1 µL; wavelength: 206 nm

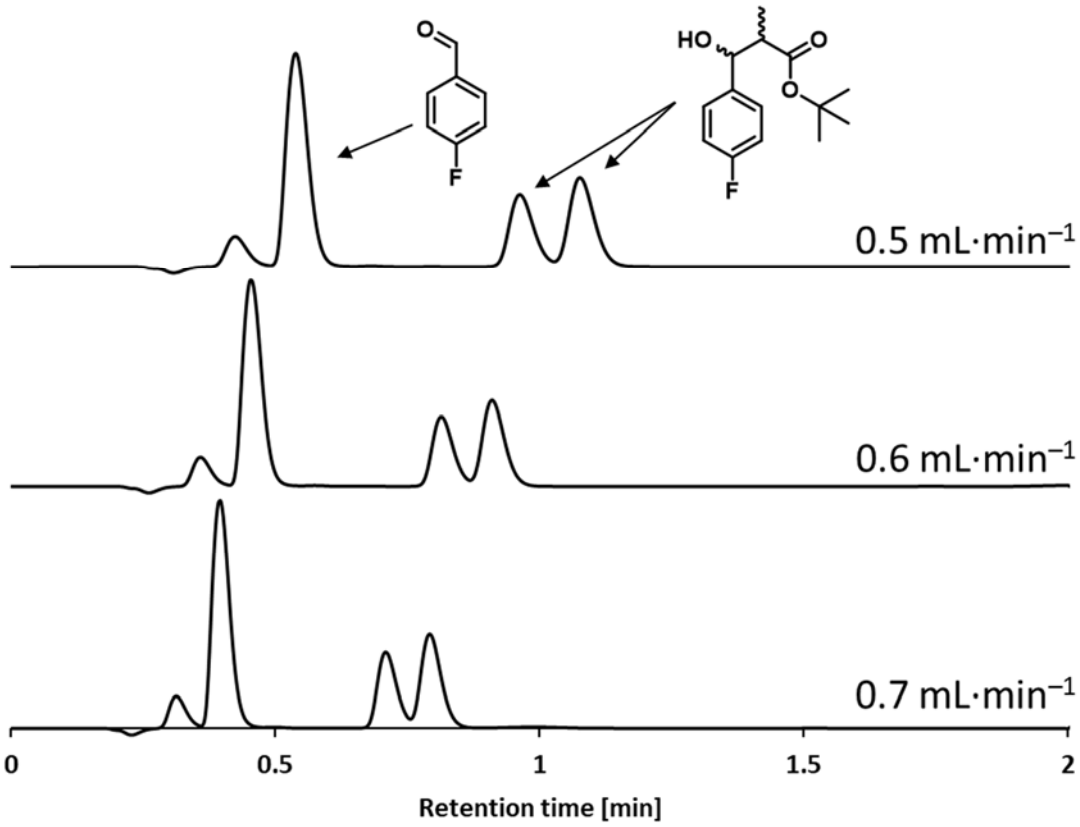


Fig. S28 Representative UPLC–DAD chromatograms of the analysis of a mixture consisting of 2 µM of 4-fluorobenzaldehye **3** and product **5** for the investigation of different flow rates on the separation. Column: Luna Omega C18 50 mm x 2.1 mm, 1.6 µm; mobile phase: A: H_2O + MeCN (9+1 v/v) + 0.1% CF_3COOH, B: MeCN + 0.1% CF_3COOH; isocratic elution: 50% A, 50% B; flow rate: X mL·min^{-1} (X depicted in the figure); column oven: 40 °C; injection volume: 1 µL; wavelength: 206 nm.

2.11.3 Offline Mode Calibration (autosampler injection)

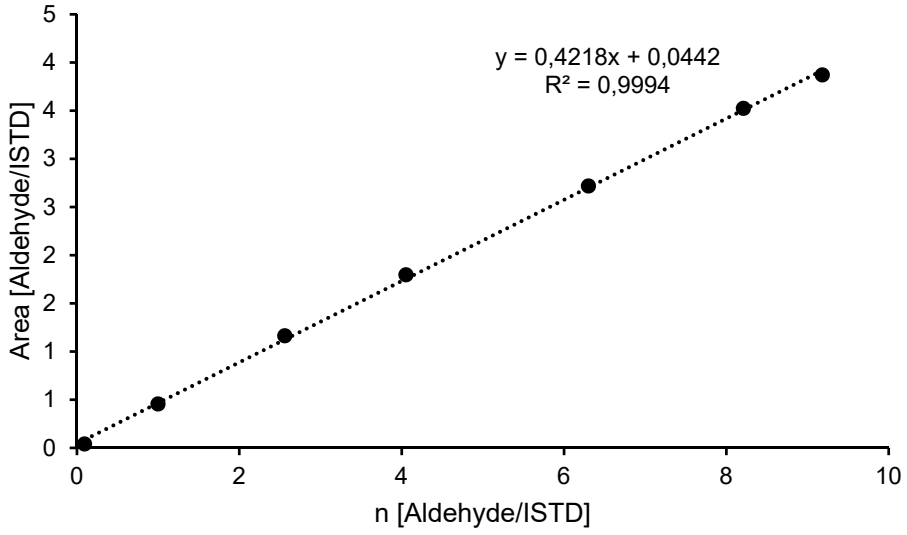


Fig. S29 Calibration curve for offline UPLC-DAD analysis of 4-fluoroaldehyde **3** at a wavelength of 206 nm.

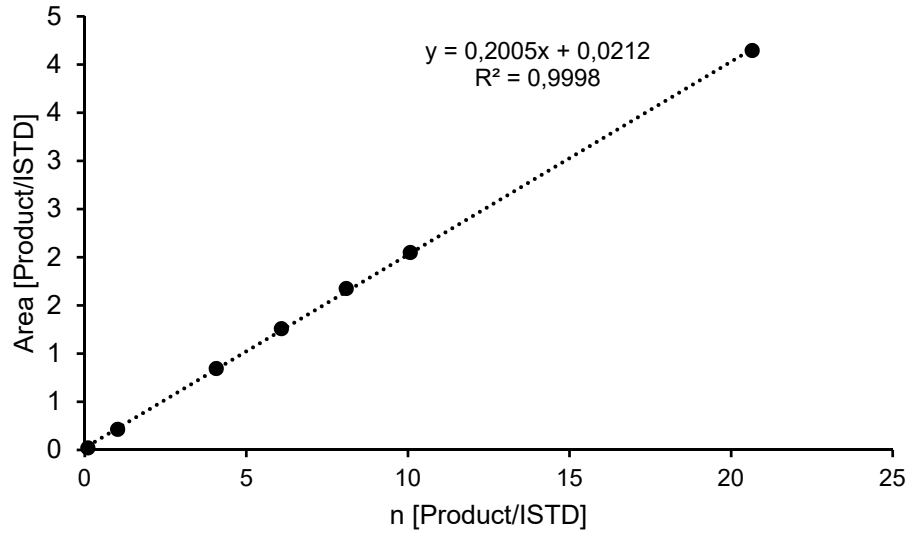


Fig. S30 Calibration curve for offline UPLC-DAD analysis of product **5** at a wavelength of 206 nm.

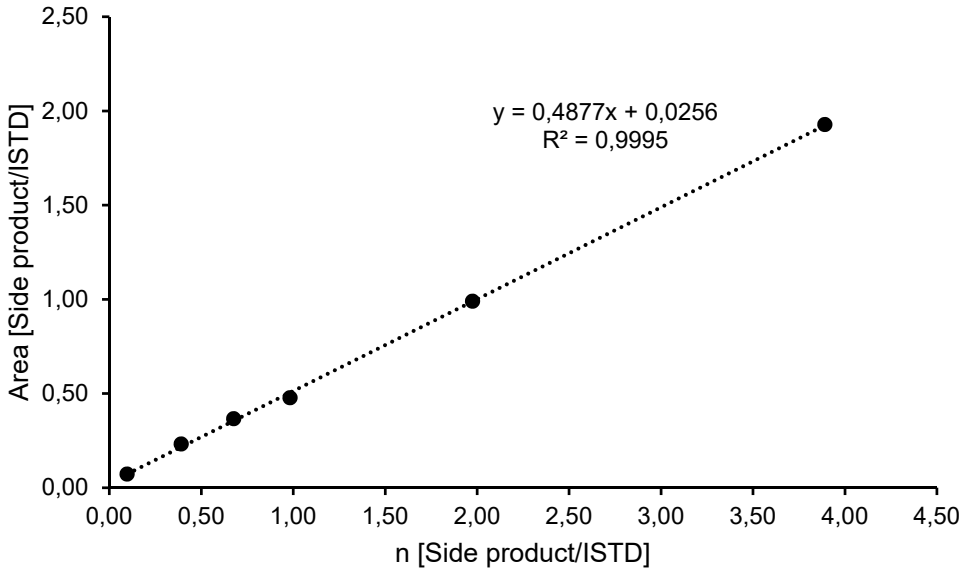


Fig. S31 Calibration curve for offline UPLC-DAD analysis of side product **6** at a wavelength of 206 nm.

2.11.4 Online Mode Calibration (sample loop injection)

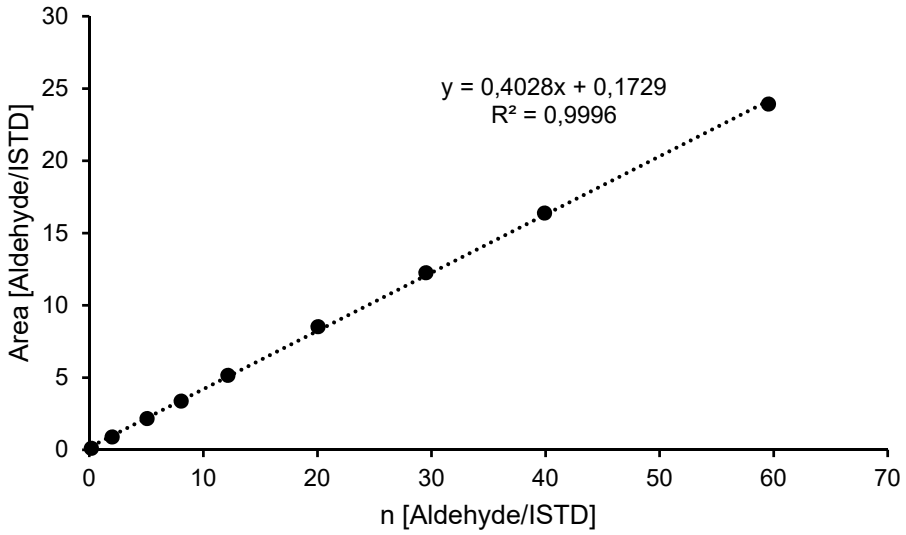


Fig. S32 Calibration curve for online UPLC-DAD analysis of aldehyde **3** at a wavelength of 269 nm.

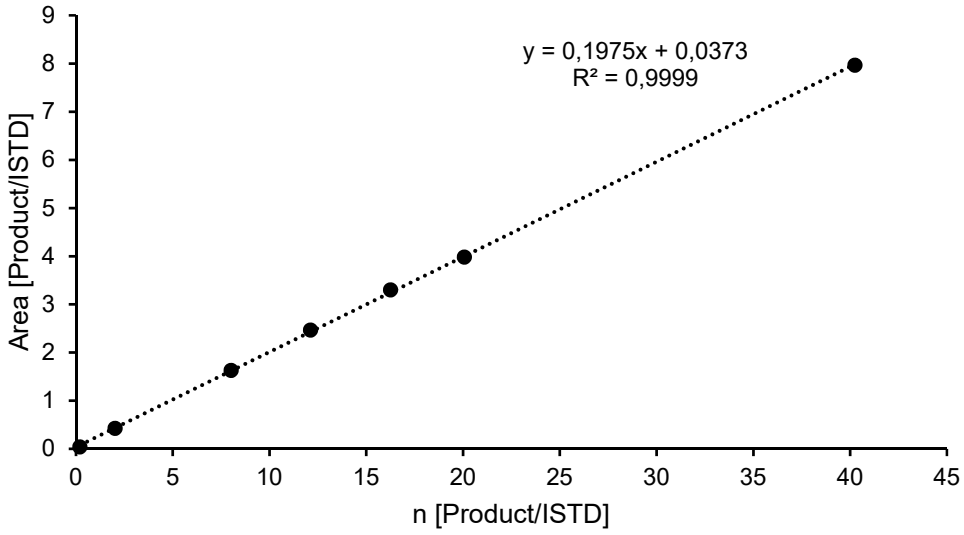


Fig. S33 Calibration curve for online UPLC-DAD analysis of product **5** at a wavelength of 206 nm.

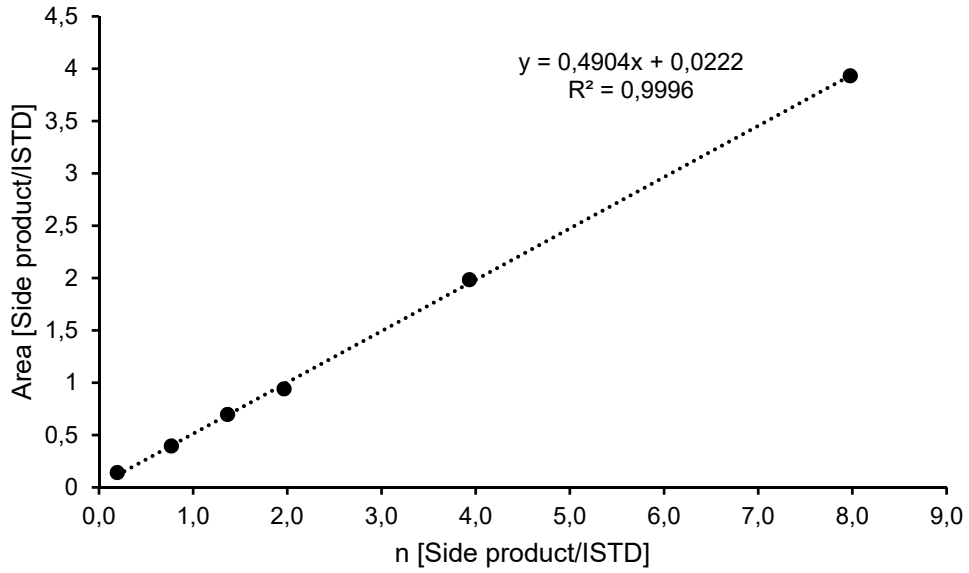


Fig. S34. Calibration curve for online UPLC-DAD analysis of side product **6** at a wavelength of 206 nm.

2.11.5 Comparison Online vs Offline Mode

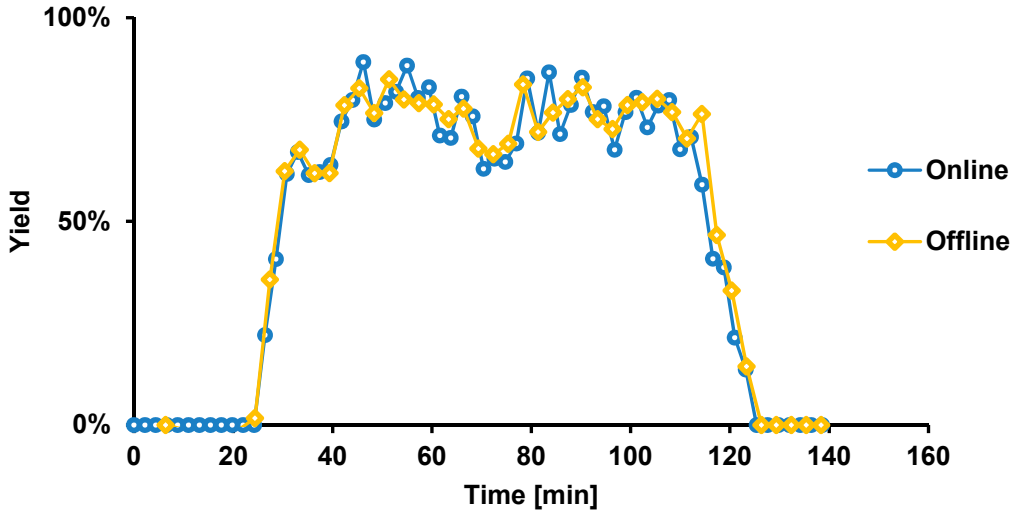


Fig. S35 Comparison of the obtained UPLC-DAD data for product yield in online and offline mode.

3 Reaction Optimization

Input solutions were made up with dry THF, in oven-dried round bottom flasks, under an argon atmosphere as follows:
0.4 M **1**: *tert*-butylpropionate (6.0 mL, 40.0 mmol), biphenyl (0.265 g, 1.72 mmol) with THF (93 mL)
0.44 M **3**: 4-fluorobenzaldehyde (4.7 mL, 44.0 mmol) with 95 mL THF
0.44 M LDA: diisopropylamine (7.7 mL, 54.6 mmol), *n*-butyllithium (2.3 M, 19.1 mL, 44.0 mmol) with THF (73.2 mL). The solution of amine was cooled to around –80 °C during the gradual addition of *n*-butyllithium.

The reaction setup shown in **Fig. S1** was used for this experiment, with a batch quench into aqueous NH_4Cl. The thermostat was set to the desired temperature, and the reactor was flushed with THF (pumps 1-3 set to 1 $mL \cdot min^{-1}$) until a stable IR signal was achieved (this often required increased flow rates in order to clear the IR flow-through cell of any trapped gas). Monitoring by NMR was then initiated. Each feed was swapped from solvent to its respective input solution.

The required flow rates for each experiment were set as shown below (**Table S4**). A period of 3 mins was allowed for the reactor to reach steady state, then 3 × 3 mL fractions were collected (with NH_4Cl quench) for offline UPLC analysis. All flow rate combinations were examined in this manner at 0 °C, 20 °C and 40 °C and when changing between temperatures, an extended period was allowed for the reactor to reach the correct temperature.

Ratio (Propionate:LDA:Aldehyde)	Pump 1 (Propionate) ($mL\ min^{-1}$)	Pump 2 (LDA) ($mL\ min^{-1}$)	Pump 3 (Aldehyde) ($mL\ min^{-1}$)
1.0 : 1.0 : 1.1	1.00	0.91	1.00
1.0 : 1.1 : 1.1	1.00	1.00	1.00
1.0 : 1.2 : 1.1	1.00	1.09	1.00
1.0 : 1.1 : 1.0	1.00	1.00	0.91
1.0 : 1.1 : 1.1	1.00	1.00	1.00
1.0 : 1.1 : 1.2	1.00	1.00	1.09

Table S4 Pump flow rates used for varying reagent stoichiometries.

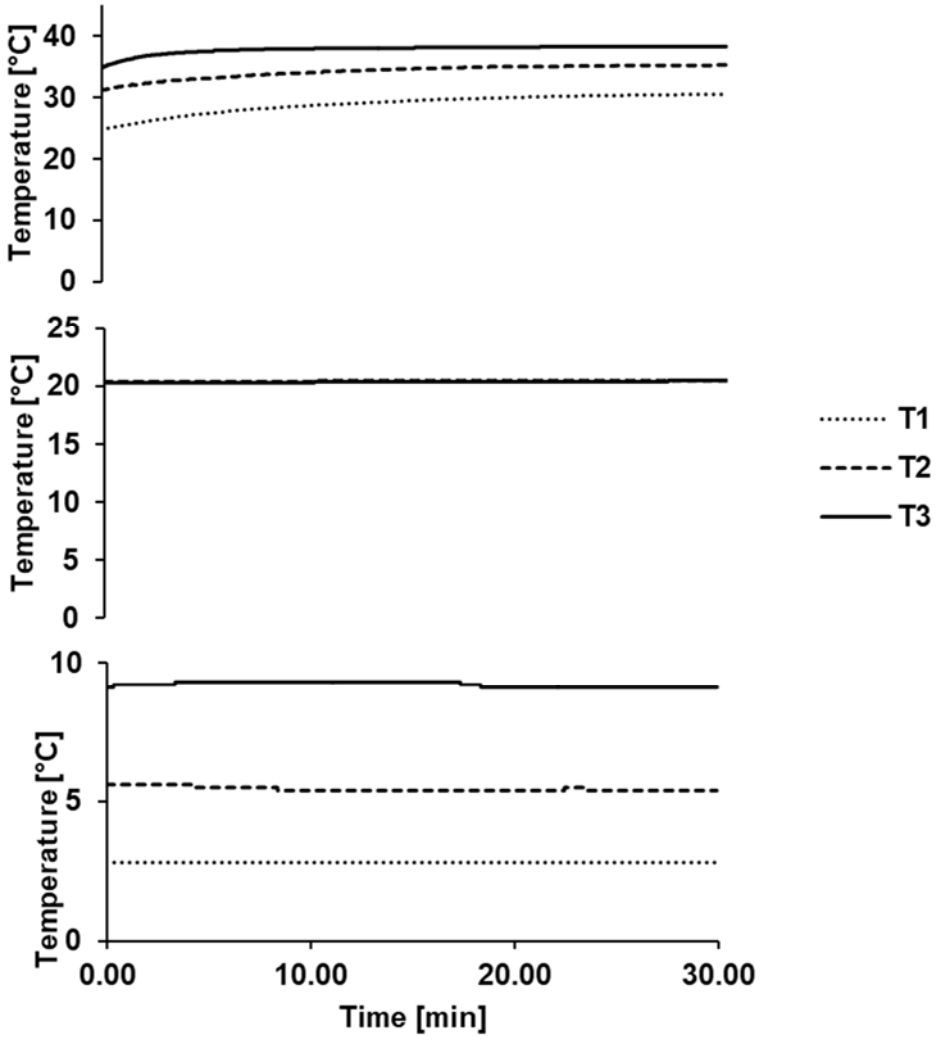


Fig. S36 Temperature traces recorded during the optimization experiments.

Extent of deprotonation of propionate 1 (IR)						
LDA	0 °C		20 °C		40 °C	
	Mean	STD	Mean	STD	Mean	STD
1	96%	± 3%	100%	± 1%	100%	± 1%
1.1	100%	± 1%	100%	± 1%	100%	± 1%
1.2	100%	± 1%	100%	± 1%	99%	± 1%
Ald						
1	98%	± 3%	100%	± 1%	99%	± 1%
1.1	93%	± 3%	100%	± 1%	100%	± 1%
1.2	95%	± 2%	100%	± 1%	99%	± 1%

Table S5 Results of reaction optimization experiments, measuring the extent of deprotonation of starting material **1**. Each percentage value is the mean of 12 measurements.

Remaining aldehyde 3 (NMR)						
LDA	0 °C		20 °C		40 °C	
	Mean	STD	Mean	STD	Mean	STD
1	24%	± 1%	24%	± 1%	29%	± 1%
1.1	11%	± 4%	12%	± 2%	10%	± 4%
1.2	15%	± 3%	9%	± 3%	7%	± 1%
Ald						
1	17%	± 3%	3%	± 2%	6%	± 3%
1.1	22%	± 3%	12%	± 4%	10%	± 4%
1.2	22%	± 7%	14%	± 3%	19%	± 1%
Remaining aldehyde 3 (UPLC)						
LDA	0 °C		20 °C		40 °C	
	Mean	STD	Mean	STD	Mean	STD
1	33%	± 1%	37%	± 1%	42%	± 1%
1.1	18%	± 1%	19%	± 1%	15%	± 3%
1.2	14%	± 1%	12%	± 1%	11%	± 1%
Ald						
1	18%	± 1%	6%	± 1%	11%	± 1%
1.1	25%	± 1%	18%	± 1%	15%	± 3%
1.2	30%	± 1%	19%	± 1%	24%	± 1%

Table S6 Results of reaction optimization experiments, measuring the remaining quantity of 4-fluorobenzaldehyde **3**. Each percentage value is the mean of 3 measurements for UPLC, and 4 measurements for NMR.

Intermediate 4 (NMR)						
LDA	0 °C		20 °C		40 °C	
	Mean	STD	Mean	STD	Mean	STD
1	73%	± 1%	69%	± 1%	63%	± 1%
1.1	87%	± 2%	90%	± 2%	93%	± 2%
1.2	93%	± 1%	100%	± 2%	100%	± 1%
Ald						
1	82%	± 1%	96%	± 1%	89%	± 1%
1.1	80%	± 2%	92%	± 3%	93%	± 2%
1.2	81%	± 2%	97%	± 3%	89%	± 2%
Product 5 (UPLC)						
LDA	0 °C		20 °C		40 °C	
	Mean	STD	Mean	STD	Mean	STD
1	60%	± 2%	65%	± 1%	58%	± 1%
1.1	75%	± 1%	81%	± 1%	84%	± 4%
1.2	74%	± 1%	82%	± 1%	83%	± 1%
Ald						
1	67%	± 2%	87%	± 1%	80%	± 1%
1.1	63%	± 2%	81%	± 2%	84%	± 4%
1.2	63%	± 1%	89%	± 1%	78%	± 3%

Table S7 Results of reaction optimization experiments, measuring the yield of intermediate **4** (NMR) or product **5** (UPLC). Each percentage value is the mean of 3 measurements for UPLC, and 4 measurements for NMR.

3.1 Reaction Optimization Data Model Fitting

The data for the optimization were fitted in MODDE (version 11, Umetrics). The data were imported from Excel. Models were fitted for the NMR and UPLC data for 4-fluorobenzaldehyde **3**, and for the NMR and UPLC data for desired product **5** by using multiple linear regression (MLR), including main, square and interaction terms, and then removing any where their potential contribution to the overall response was zero. It was necessary to remove one anomalous experiment with high residual error in order to improve the overall fit (R^2).

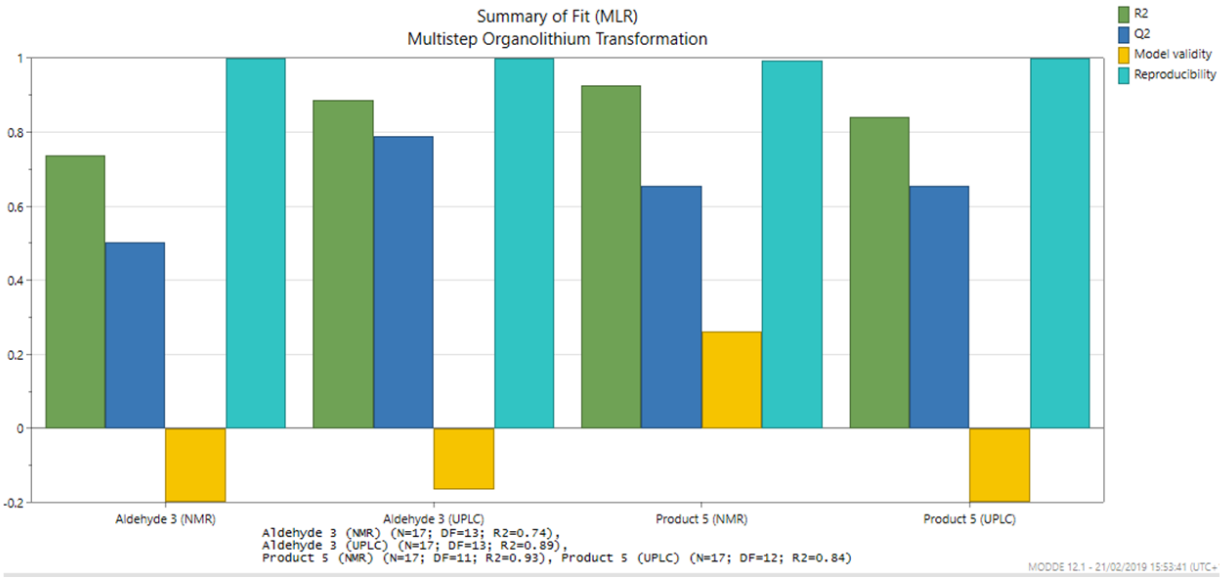


Fig S37 Summary of fit for all models. R^2 is a measure of how well the model fits the experimental data points. Q^2 measures how well the model predicts future data (should be greater than 0.1 for a significant model and greater than 0.5 for a good model). Reproducibility is a measure of experimental error. Model validity can be low (negative) in very good models due to very good replicates.

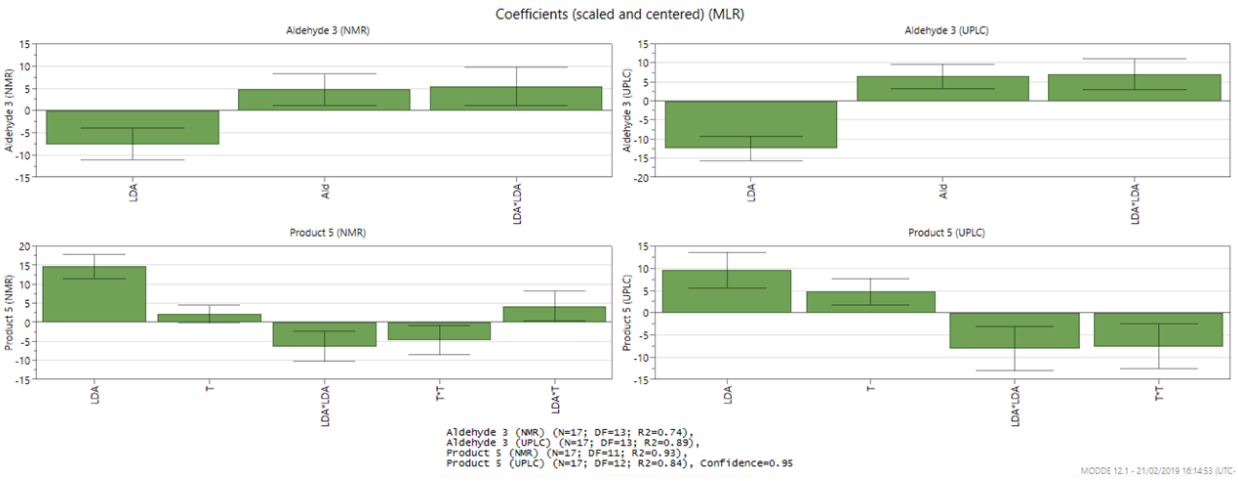


Fig. S38 Coefficients and terms for all models after non-significant terms removed.

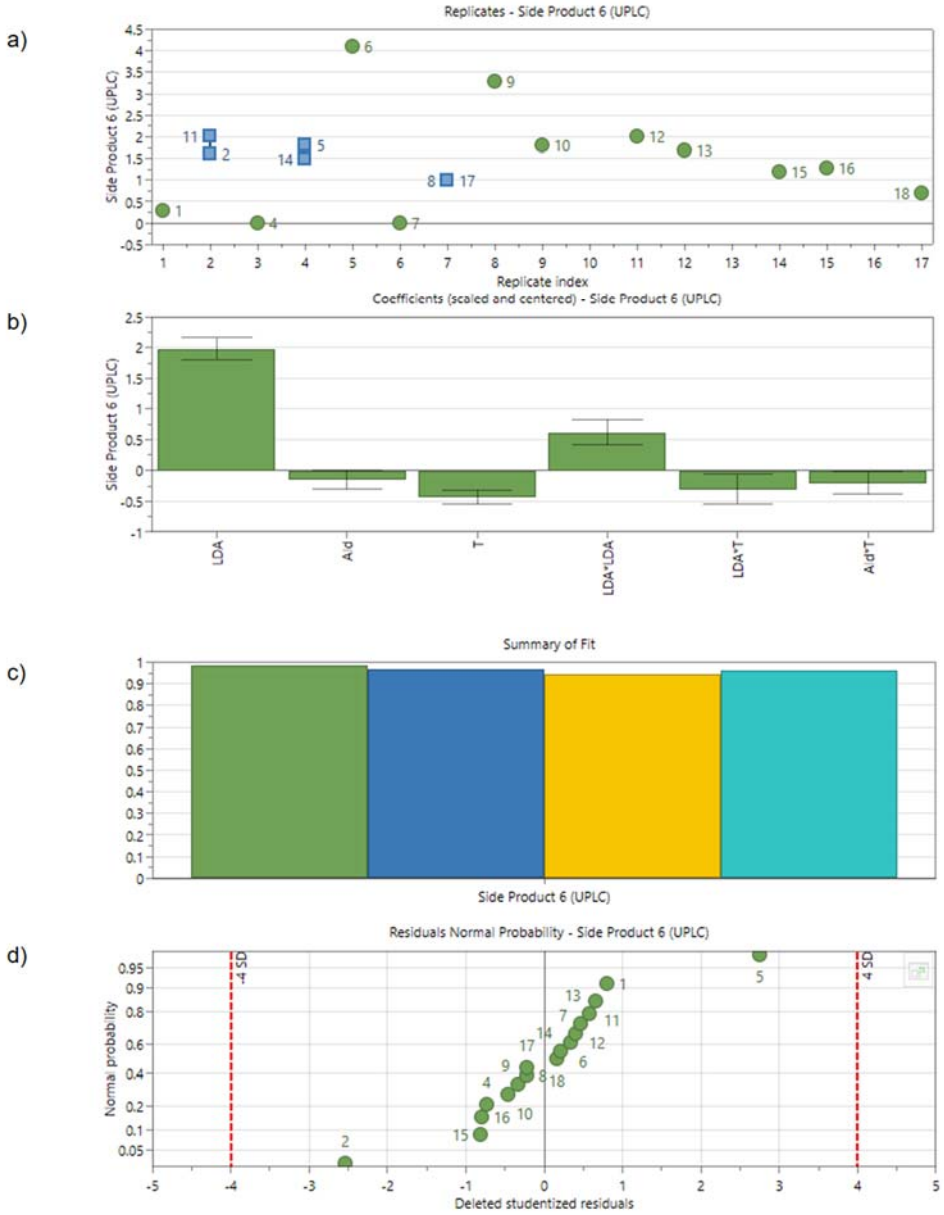


Fig. S39 Model fitting for side product 6 formation based on UPLC data: a) Replicates plot; b) Coefficients and terms for model after non-significant terms removed; c) Summary of fit for all models; d) Residuals normal probability plot.

4 Scale-out Reactions

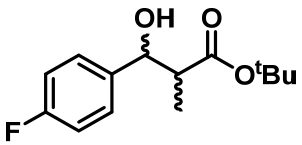
4.1 Experimental Procedure

Input solutions were made up with dry THF, in oven-dried round bottom flasks, under an argon atmosphere as follows:
0.4 M **1**: *tert*-butylpropionate (6.6 mL, 43.9 mmol), biphenyl (0.265 g, 1.72 mmol) with THF (103 mL)
0.48 M **3**: 4-fluorobenzaldehyde (5.15 mL, 48.0 mmol) with 95 mL THF
0.44 M LDA: diisopropylamine (7.7 mL, 54.6 mmol), *n*-butyllithium (2.3 M, 19.1 mL, 44.0 mmol) with THF (73.2 mL). The solution of amine was cooled to around –80 °C during the gradual addition of *n*-butyllithium.

The reaction setup shown in **Fig. S1** was used for this experiment. The thermostat was set to 20 °C, and the reactor was flushed with THF (pumps 1-3 set to 1 mL·min^{-1}) until a stable IR signal was achieved (this often required increased flow rates in order to clear the IR flow-through cell of any trapped gas). Pump 4 (quench) was set to 0.3 mL·min^{-1}, pump 5 (dilution) was set to 2 mL·min^{-1} and pump 6 (subsampling) was set to 0.02 mL·min^{-1}. Monitoring by NMR and UPLC was then initiated, as well as pump 6 "flush" script. Each feed was swapped from solvent to its respective input solution and these solutions were each delivered at a rate of 1 mL·min^{-1} (total flowrate of 3 mL·min^{-1}). The reactor was allowed to equilibrate until a stable IR trace was observed. The reactor effluent was then collected for 69 min, in 9 mL fractions (3 min per fraction). After the reaction, each fraction was analyzed by offline UPLC.

To the combined reaction eluent was added saturated brine solution (30 mL) and ethyl acetate (100 mL). The layers were separated, and the organics were extracted with ethyl acetate (2 × 50 mL). The combined organics were dried over Na_2SO_4, filtered, then solvent was removed *in vauco*. The resulting residue was purified by flash column chromatography (elution gradient 0-20% ethyl acetate in 40-60 petroleum ether). The appropriate fractions were combined and solvent removed *in vacuo* to afford the desired product **5** (4.89 g, 70%) as a pale yellow oil (mixture of diastereomers) and side product **6** as a white crystalline solid.

***tert*-Butyl 3-(4-fluorophenyl)-3-hydroxy-2-methylpropanoate (5)**



5

Diastereomer 1

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 – 7.26 (m, 2H), 7.10 – 6.95 (m, 2H), 4.68 (d, *J* = 7.9 Hz, 1H), 3.20 (s, 1H), 2.72 – 2.57 (m, 1H), 1.43 (s, 9H), 1.01 (d, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 175.4, 162.5 (d, *J* = 245.7 Hz), 137.8 (d, *J* = 3.1 Hz), 128.4 (d, *J* = 8.1 Hz), 115.3 (d, *J* = 21.4 Hz), 81.5, 75.8, 47.9, 28.2, 14.8.

^{19}F NMR (282 MHz, $CDCl_3$) δ -114.74 (tt, *J* = 8.7, 5.4 Hz).

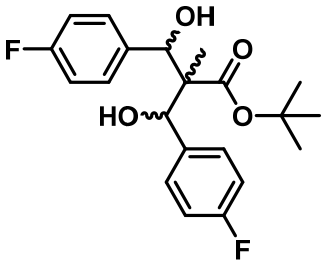
Diastereomer 2

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 – 7.26 (m, 2H), 7.10 – 6.95 (m, 2H), 5.00 (d, *J* = 4.0 Hz, 1H), 3.20 (s, 1H), 2.72 – 2.57 (m, 1H), 1.40 (s, 9H), 1.08 (d, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 175.4, 162.2 (d, *J* = 245.2 Hz), 137.4 (d, *J* = 3.1 Hz), 127.9 (d, *J* = 8.0 Hz), 115.1 (d, *J* = 21.4 Hz), 81.4, 75.8, 73.3, 47.2, 11.2.

^{19}F NMR (282 MHz, $CDCl_3$) δ -115.35 (tt, *J* = 8.7, 5.4 Hz).
^{1}H and ^{13}C NMR spectra are in agreement with those reported previously.[S1]

4.2 Side Product Identification



6

The side product **6** (*tert*-butyl 3-(4-fluorophenyl)-2-((4-fluorophenyl)(hydroxy)methyl)-3-hydroxy-2-methylpropanoate) was identified after preparative column chromatography using LC-MS (**Fig. S39**, **Fig. S40**) and high field NMR (see below).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.29 (m, 2H), 7.24 – 7.16 (m, 2H), 7.10 – 6.89 (m, 4H), 5.48 (s, 1H), 5.04 (d, *J* = 9.2 Hz, 1H), 4.82 (d, *J* = 8.7 Hz, 1H), 3.39 (s, *J* = 3.0 Hz, 1H), 1.21 (s, 9H), 0.80 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 175.1, 162.5 (d, *J* = 246.1 Hz), 162.4 (d, *J* = 246.0 Hz), 136.4 (d, *J* = 3.2 Hz), 135.8 (d, *J* = 3.2 Hz), 129.5 (d, *J* = 8.0 Hz), 129.4 (d, *J* = 8.0 Hz), 114.9 (d, *J* = 21.3 Hz), 114.8 (d, *J* = 21.3 Hz), 82.9, 76.9, 75.4, 56.1, 27.9.

^{19}F NMR (282 MHz, $CDCl_3$) δ -114.52 (tt, *J* = 8.6, 5.4 Hz), -114.84 (tt, *J* = 8.6, 5.4 Hz).

HRMS (TOF ESI): m/z: calc. for $C_{22}H_{25}F_2O_6$ $[M+COOH]^-$: 423.16247 found: 423.16371 Δm= 2.6 ppm

[S1] S. Iwasa, N. Sasaki, Y. Kawasaki, I. Fujisawa, K. Kitahara and K. Shibatomi, *Nat. Commun.*, 2017, **8**, 15600.

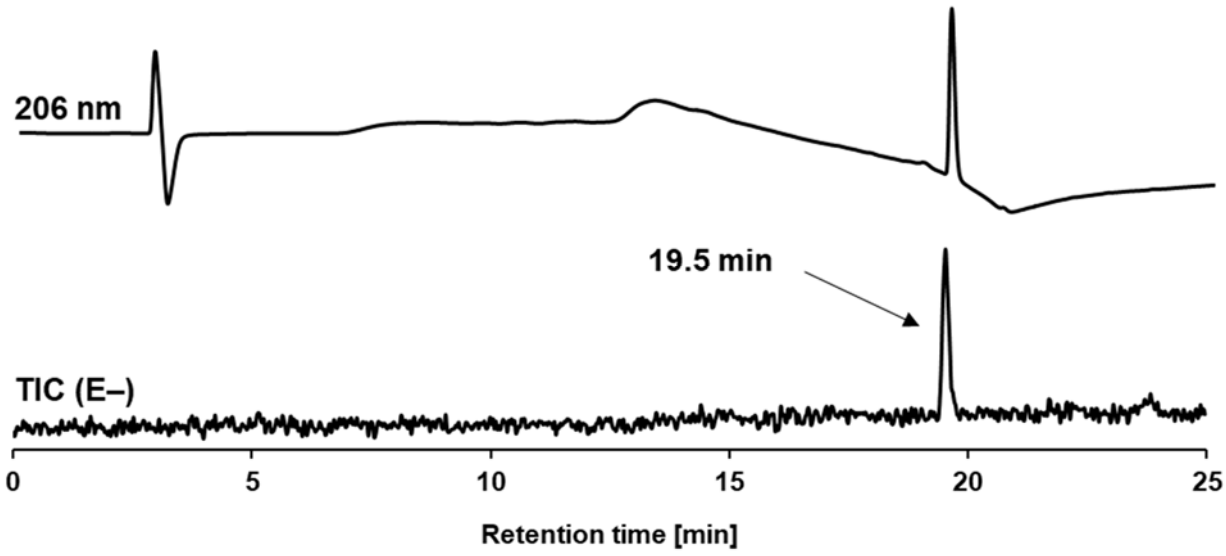


Fig. S40 LC-MS trace of the side product **6**.

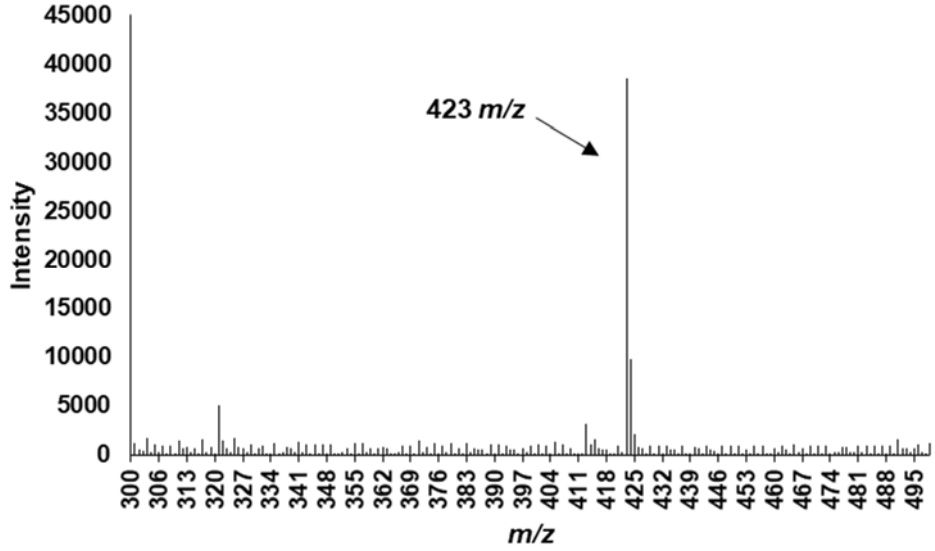


Fig. S41 Obtained mass spectrum from the chromatogram in **Fig. S39** at the retention time of 19.5 min. The mass corresponds to the $[M + COOH]^-$ ion of the side product **6**.

4.3 Reactor Fouling

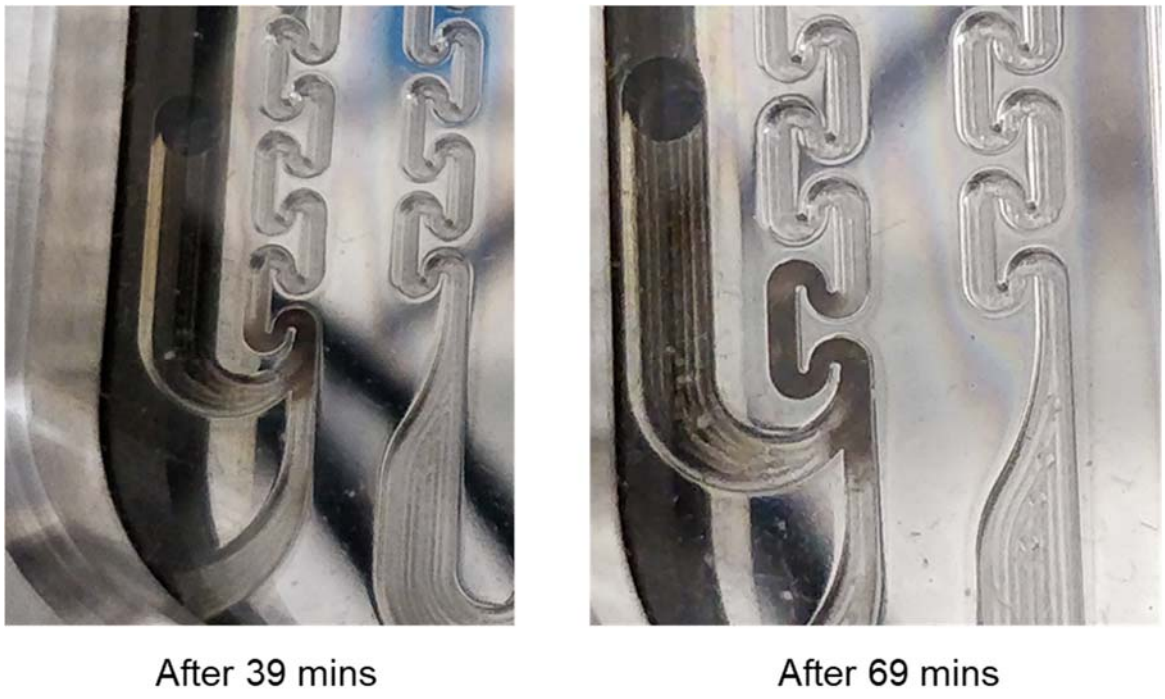
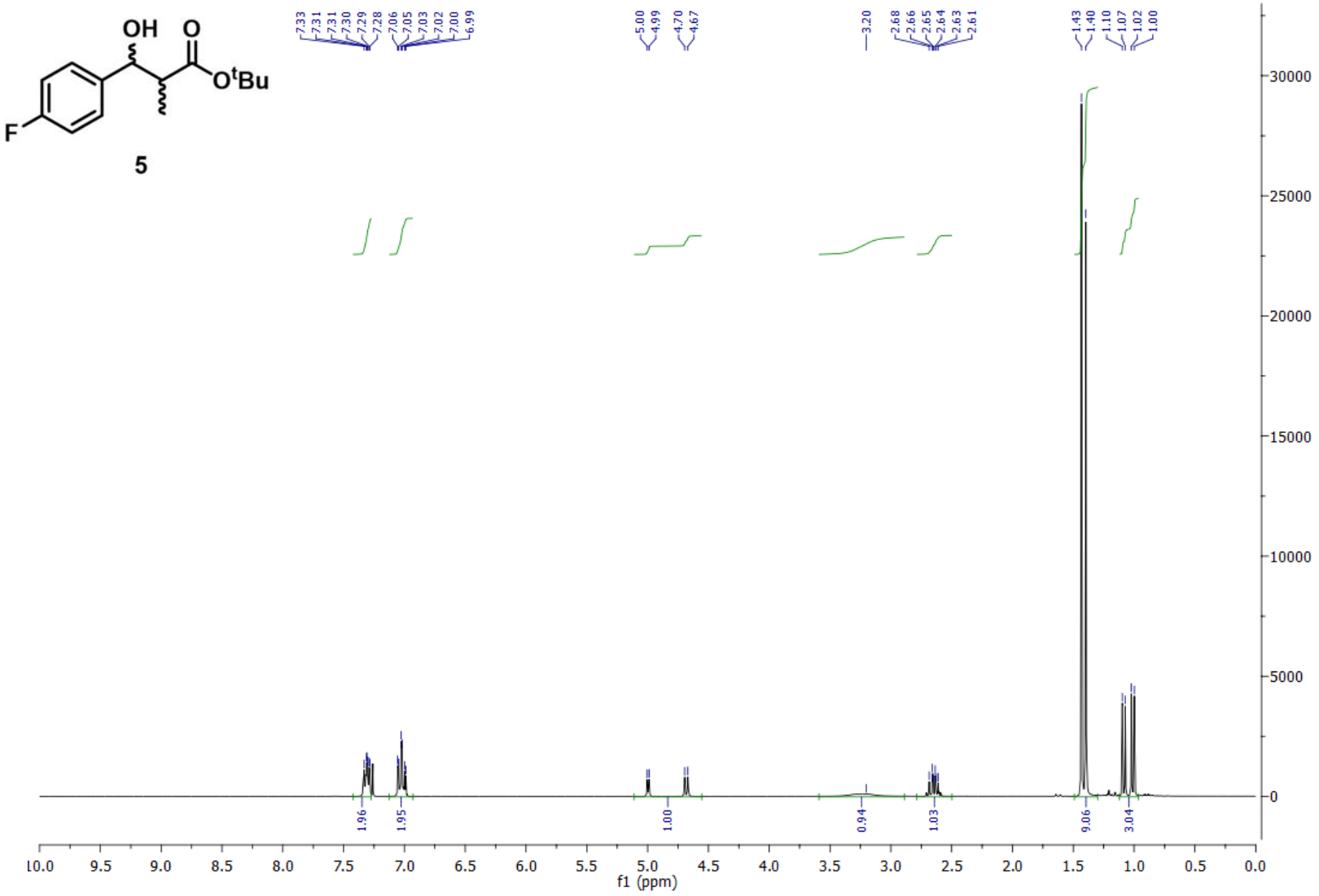
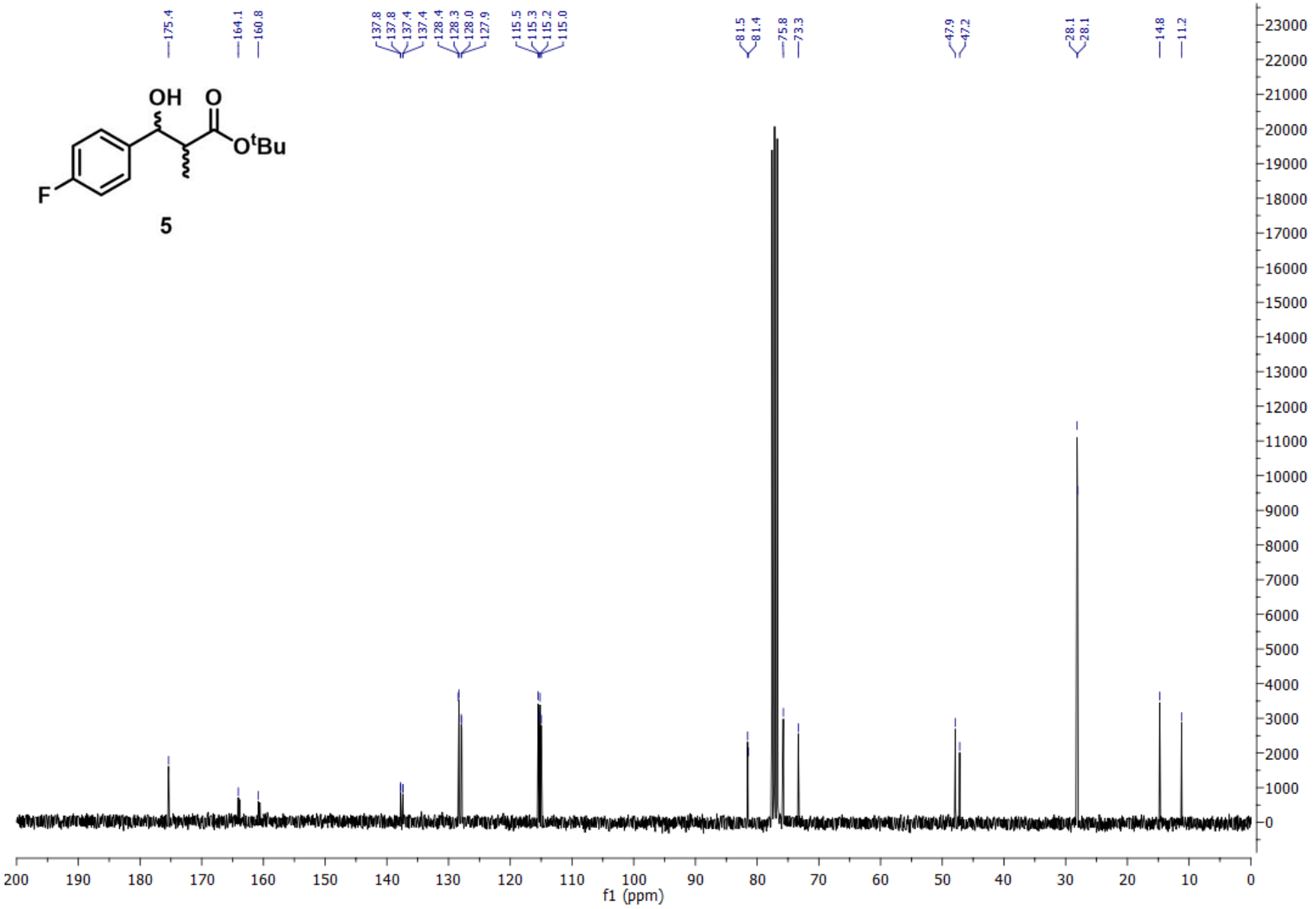
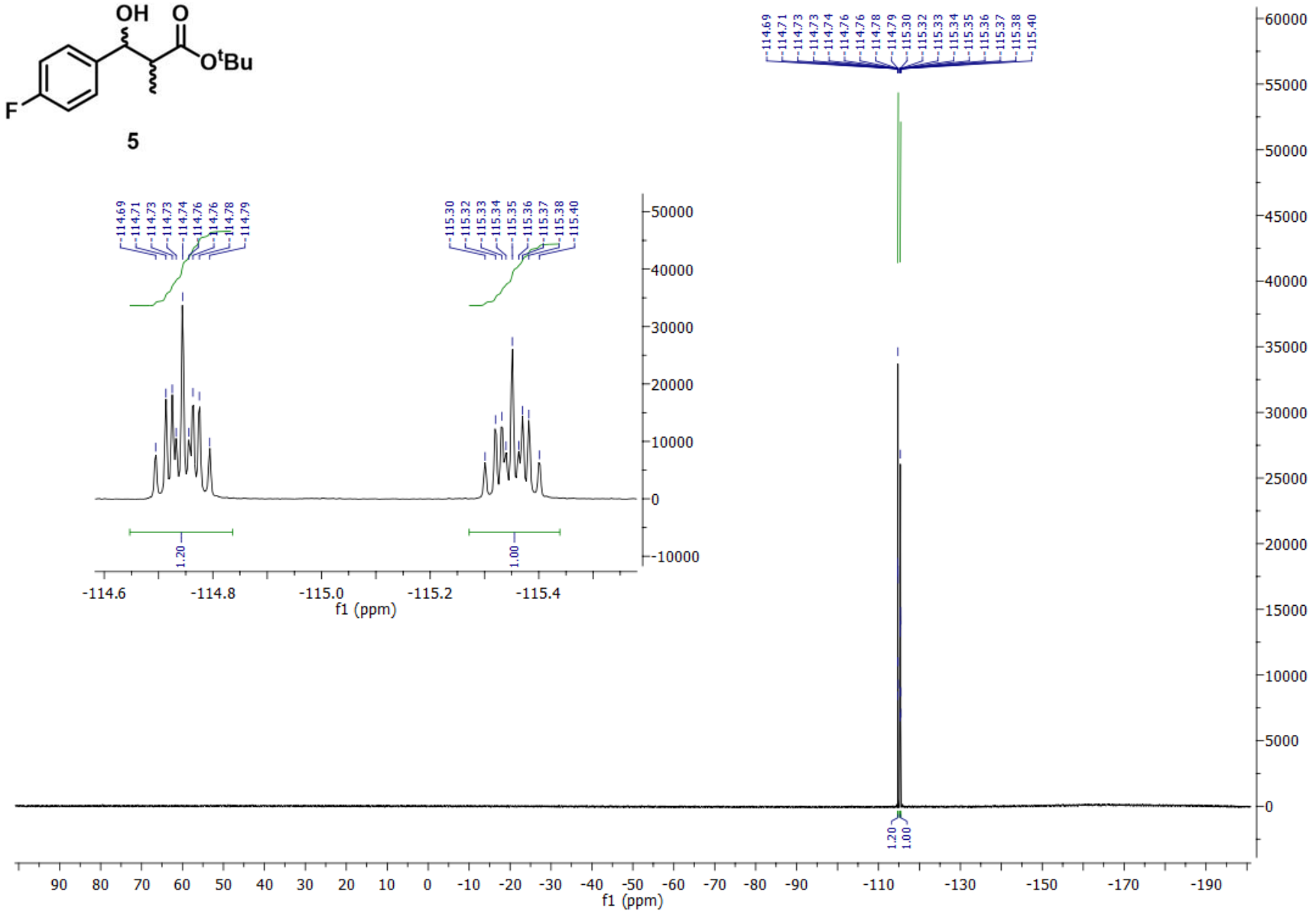


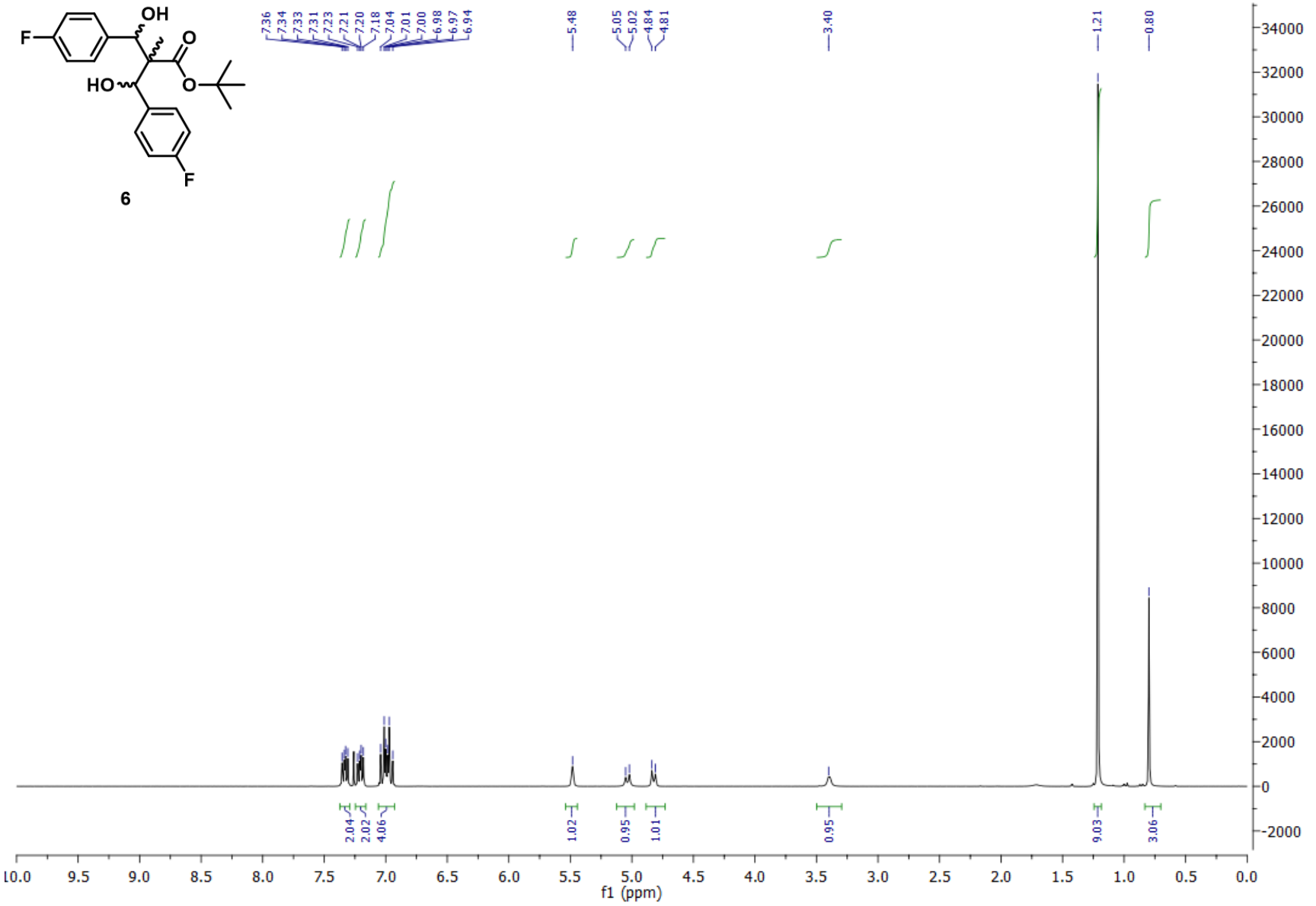
Fig. S42 Process plate during scale-out run, showing the state of "gumming" in the reaction channel (where LDA and ester **1** mix) after 39 and 69 min.

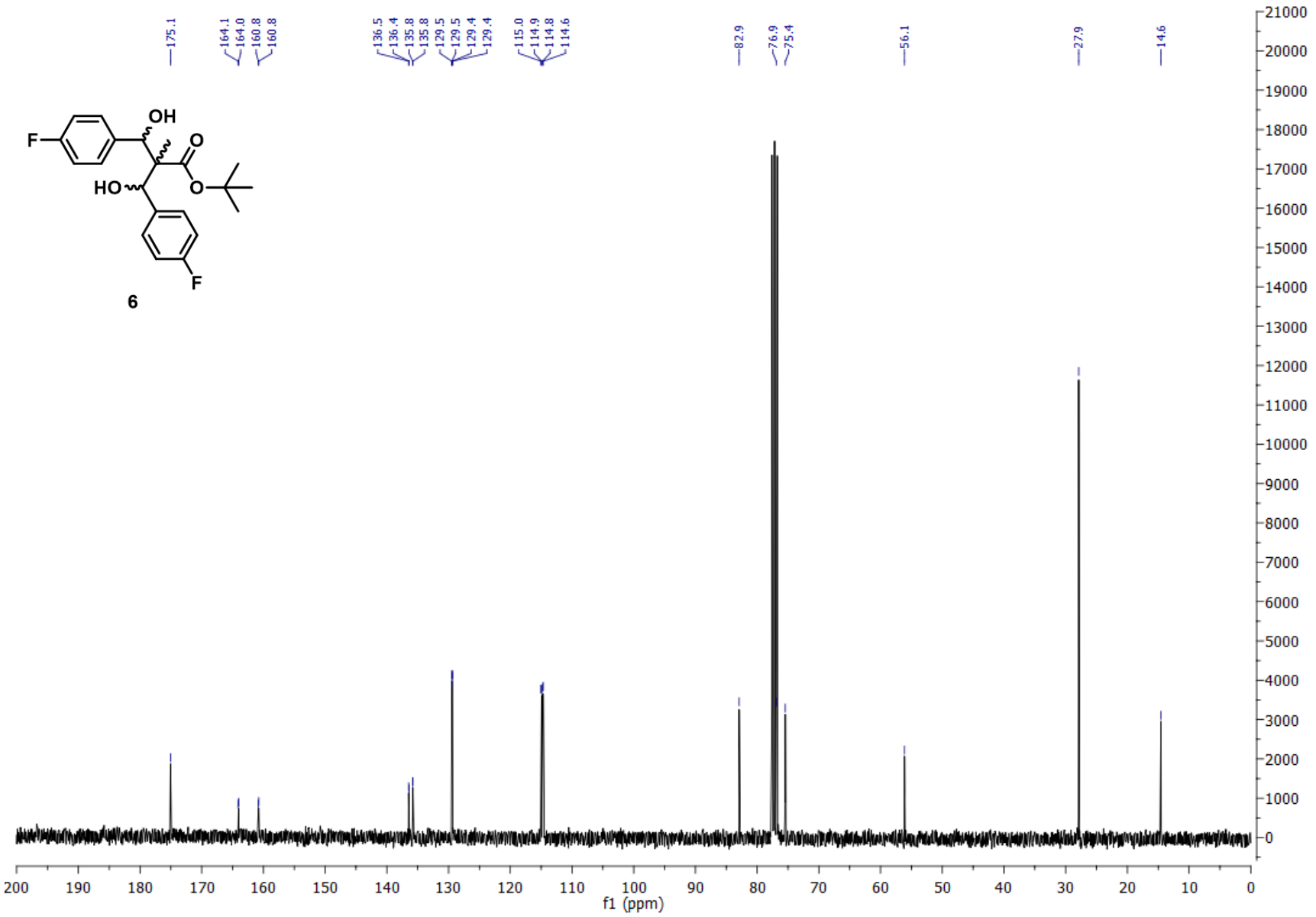
5 NMR Spectra

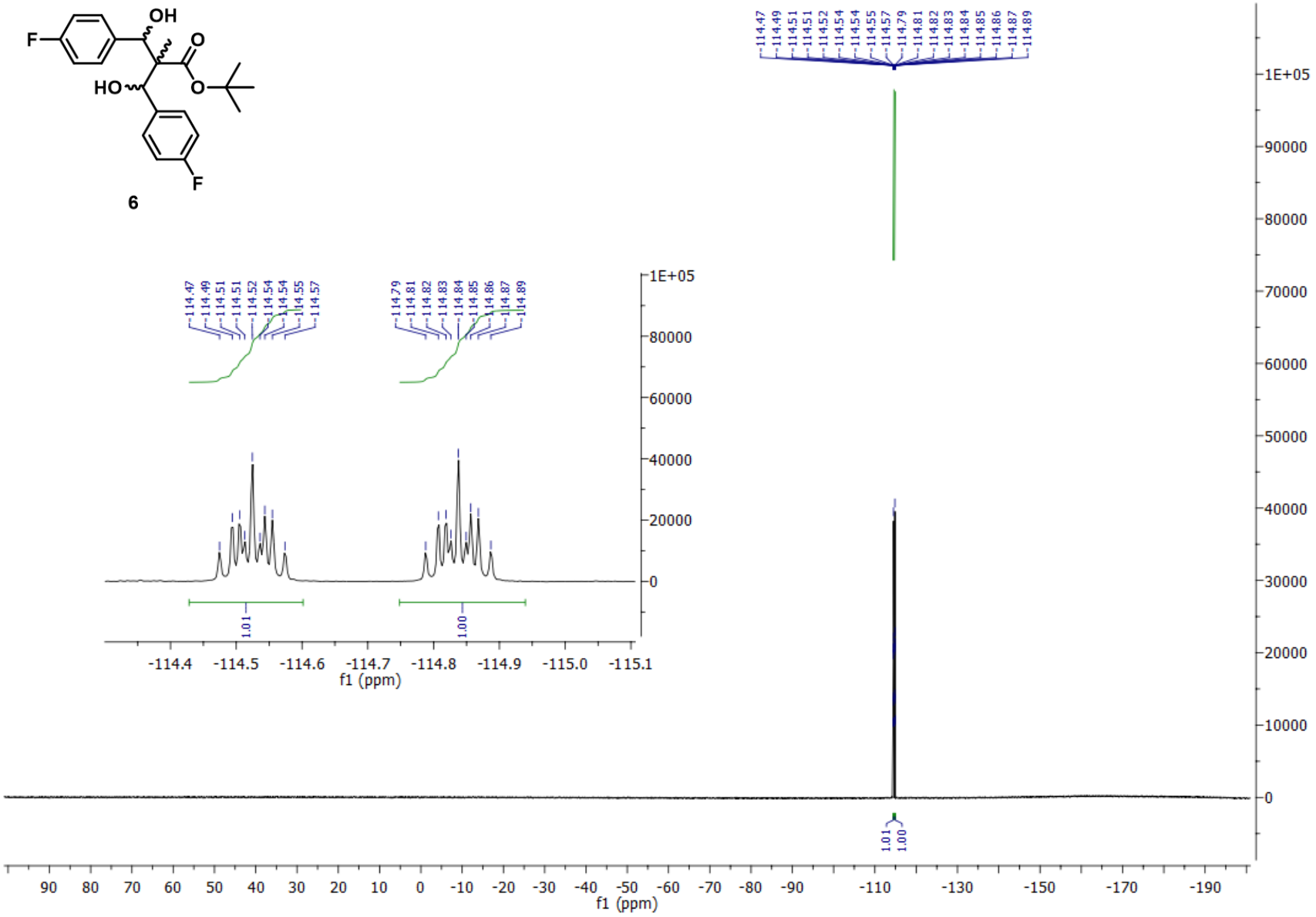


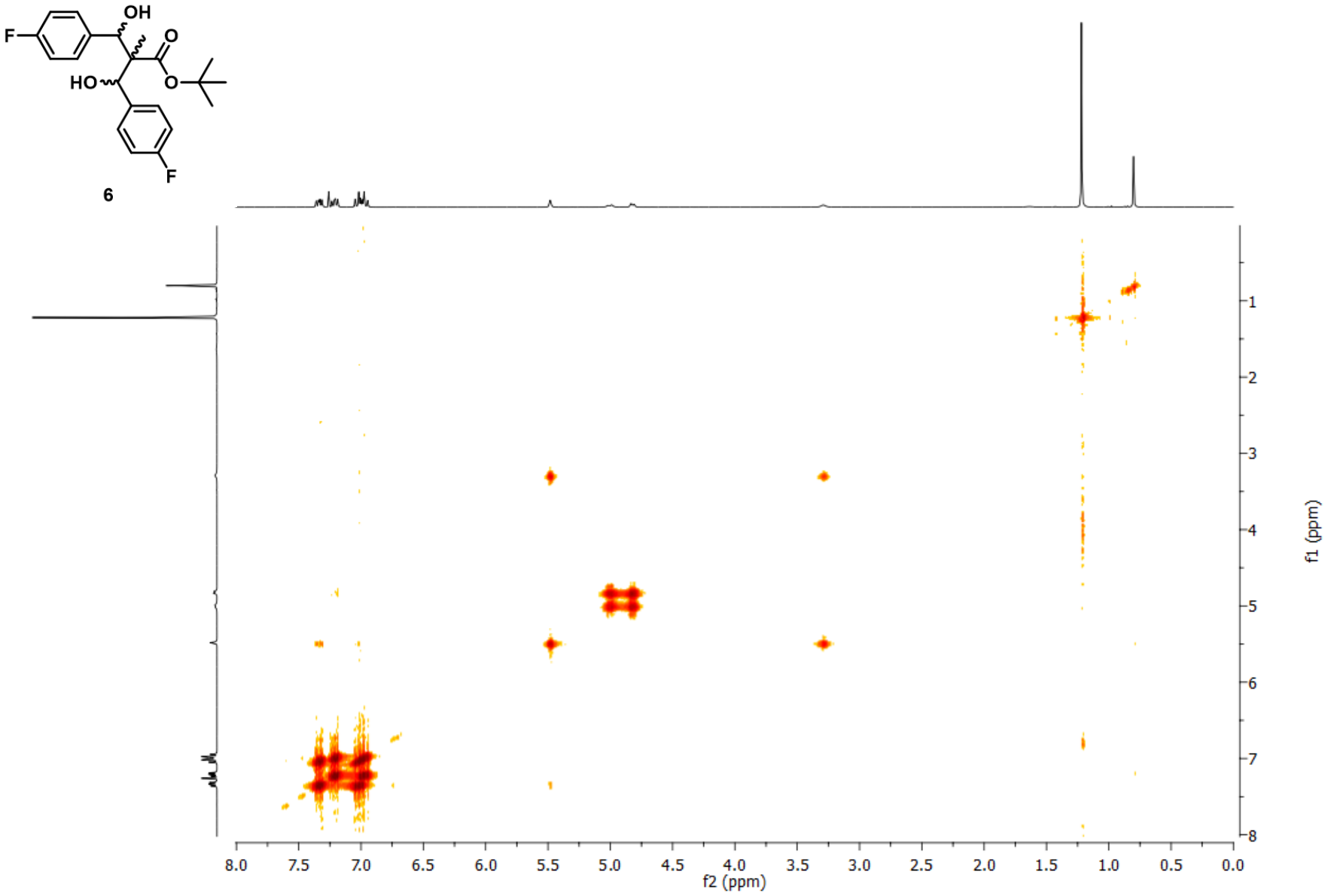

175.4
164.1
160.8
137.8
137.8
137.4
137.4
128.4
128.3
128.0
127.9
115.5
115.3
115.2
115.0
81.5
81.4
75.8
73.3
47.9
47.2
28.1
28.1
14.8
11.2
OH
O
O^{t}Bu
F
5
f1 (ppm)

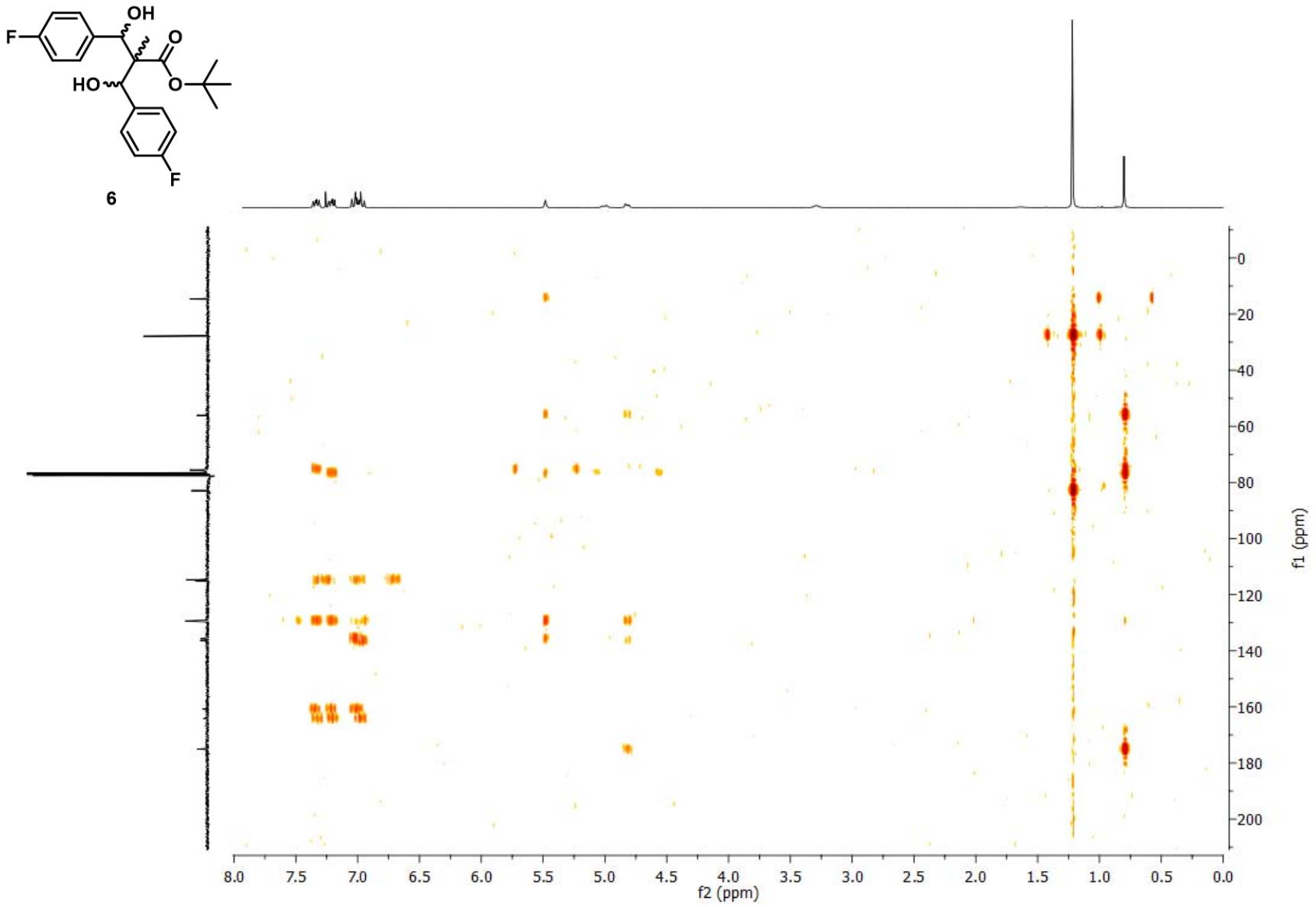

OH
O
O^{t}Bu
F
5
114.69
114.71
114.73
114.73
114.74
114.76
114.76
114.78
114.79
115.30
115.32
115.33
115.34
115.35
115.36
115.37
115.38
115.40
1.20
1.00
f1 (ppm)

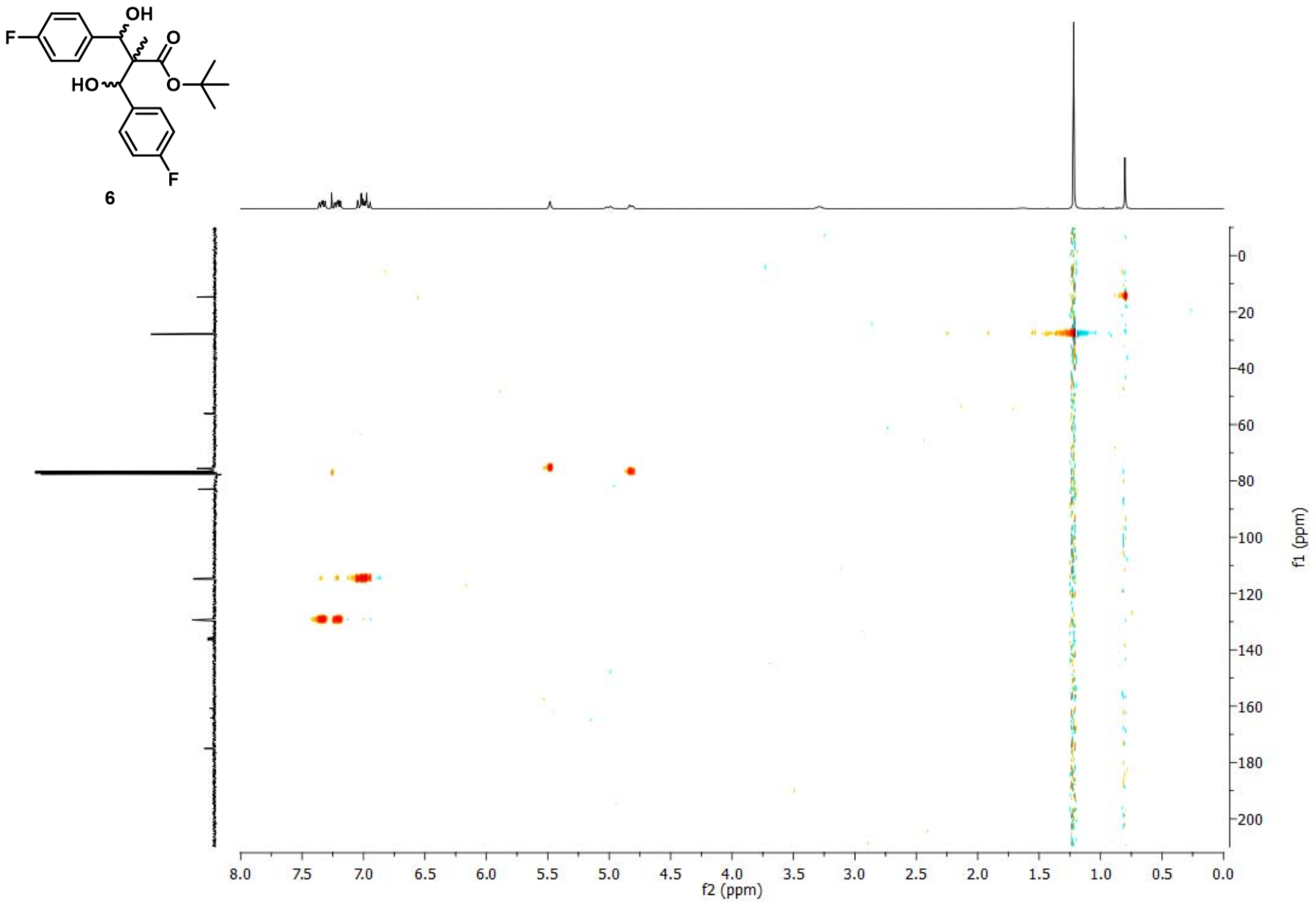

OH
F
O
HO
O
F
6
7.36
7.34
7.33
7.31
7.23
7.21
7.20
7.18
7.04
7.01
7.00
6.98
6.97
6.94
5.48
5.05
5.02
4.84
4.81
3.40
1.21
0.80
2.04
2.02
4.06
1.02
0.95
1.01
0.95
9.03
3.06
f1 (ppm)


175.1
164.1
164.0
160.8
160.8
136.5
136.4
135.8
135.8
129.5
129.5
129.4
129.4
115.0
114.9
114.8
114.6
82.9
76.9
75.4
56.1
27.9
14.6
OH
F
O
HO
O
F
6
f1 (ppm)


OH
F
O
HO
O
F
6
114.47
114.49
114.51
114.51
114.52
114.54
114.54
114.55
114.57
114.79
114.81
114.82
114.83
114.84
114.85
114.86
114.87
114.89
1E+05
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
1.01
1.00
-114.4
-114.5
-114.6
-114.7
-114.8
-114.9
-115.0
-115.1
f1 (ppm)
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-110
-130
-150
-170
-190
f1 (ppm)


OH
F
O
HO
O
F
6
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f2 (ppm)
1
2
3
4
5
6
7
8
f1 (ppm)


OH
F
O
HO
O
F
6
f2 (ppm)
f1 (ppm)


OH
F
O
HO
O
F
6
f2 (ppm)
f1 (ppm)