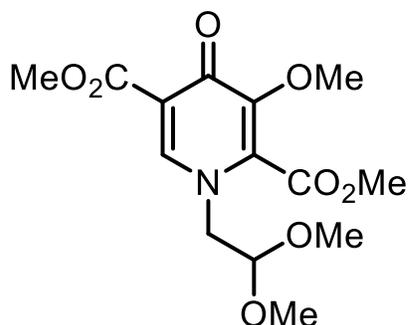


Process Development Report

The Medicines for All Institute

Continuous Synthesis of the Pyridone In Route to Dolutegravir

(dimethyl 1-(2,2-dimethoxyethyl)-
3-methoxy-4-oxo-1,4-dihydropyridine-
2,5-dicarboxylate)



Report Prepared by M4ALL Team
6/12/2019

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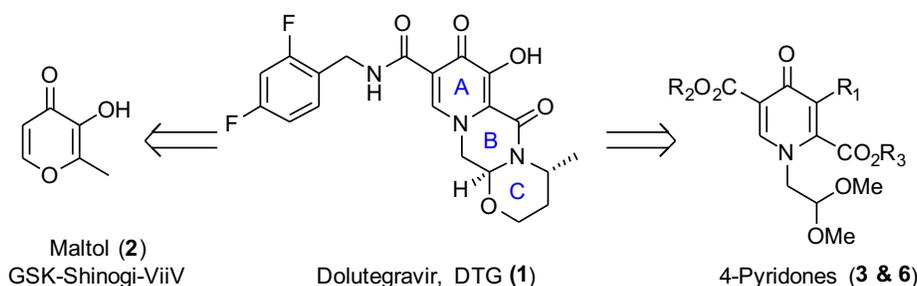
1. Executive Summary

Two continuous routes to produce the pyridone intermediate commonly used to prepare Dolutegravir (DTG) are described. The first consists of a two-stage process whereby impurities are purged before the final step to the pyridone is conducted. The process provides the desired product in an overall yield of 86% and a 69-fold enhancement in space-time-yield relative to state of practice processes. The second process is conducted as a through process, continuously from start to finish. The pyridone is common to dolutegravir, cabotegravir and bictegravir and the following report should be of interest to those who manufacture intermediates or active ingredients

2. Introduction

A number of routes describing the synthesis of dolutegravir (DTG) are known. These approaches are described in patents from the innovator, GSK-Shionogi-ViiV, as well as several generic routes from Mylan, Microlabs, and Lek Pharma, for example. All routes to DTG **1** begin with the construction of the “A” ring. GSK-Shionogi-ViiV derived the “A ring” from maltol **2** early-on and more recently (10.1021/acs.oprd.8b00409). Subsequent approaches have leveraged a pyridone fragment **3** as an early intermediate or starting material (Figure 1).

Figure 1. Literature survey of pyridone fragments



3a GSK (DTG, Cabotegravir): R₁ = OCH₃, R₂ = CH₃ or H, R₃ = CH₃

3b Lek Pharma (DTG): R₁ = H, R₂, R₃ = H, CH₃

6 Mylan (DTG): R₁ = OCH₃; R₂, R₃ = CH₃

3d Micro Labos (DTG): R₁ = OCH₂Ph, R₂, R₃ = H or CH₃

The 4-pyridones shown in Figure 1 are made by similar chemistries using different starting materials or reaction conditions and serve as the starting materials for other integrase inhibitors such as cabotegravir and bictegravir. The prevalence and similar bond-forming paths used to produce this family of starting materials inspired the Medicines for All Institute (M4ALL) team to devise a continuous synthesis that could be adapted to the needs of multiple suppliers. An earlier and less optimized version of the continuous synthesis work presented here was published in *Angewandte Chemie* and presents the entire synthesis of DTG (DOI: 10.1002/anie.201802256).

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3. Prior Art

3.1. Mylan Synthesis of Pyridone 6 (Figure 2)

Although several patents and publications proceed through 4-pyridones, the Mylan patent provided sufficient details enabling meaningful comparisons with the M4ALL process. Several processes are outlined in two patents from the Mylan team (WO 2015/019310 A1, WO 2016/125192 A2). The yields and reaction times are shown in Table 1.

Figure 2. Mylan synthesis of pyridine intermediate **6**

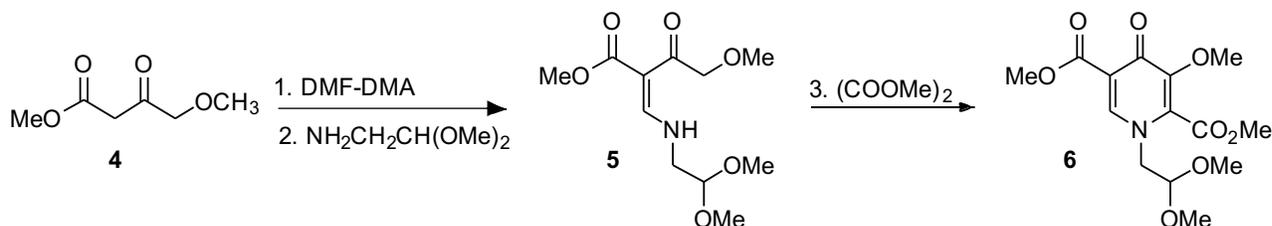


Table 1. Yields and reaction times for Mylan processes

Synthesis Process	Step1 rxn time	Step 2 rxn time	Yield	Step 3 rxn time	Yield	Overall Yield
Example 1	8h	2h	N/A	26h	28.2%	28.2%
Example 2	12h	n.d.	85%	1.5h	19.8%	17%

3.2. GSK Synthesis of Pyridone 6 (Figure 3)

The GSK synthesis of pyridone **6** proceeds through enamine **5** (Figure 3), followed by a condensation to form pyridone **6**, which is not isolated but carried directly on to the mono-carboxylic acid **7**. While the GSK process does not report a yield for the conversion of **5** to **6**, it can be inferred from the overall yield of 64%. From this figure and the described yield for the formation of **5**, a 73% yield is estimated for the Step 3 conversion.

Figure 3. GSK Synthesis of Mono-Carboxylic Acid **7**

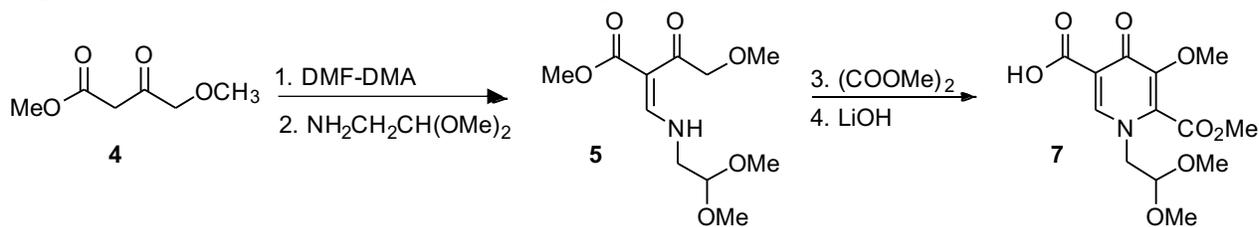


Table 2. Yields and reaction times for the GSK process

Synthesis Process	Step1 rxn time	Step 2 rxn time	Step 1-2 Yield	Step 3 rxn time	Step 3 Yield	Overall Yield to Acid 7
GSK published process	12h	2h	88%	20.5h	73%	64%

4. M4ALL Objectives and Approach

Integrase inhibitors are an important aspect of combination therapies for the treatment of HIV. The emergence of the pyridone as a common intermediate in the manufacture of these active ingredients prompted the M4ALL team to seek a more cost and material efficient route. Our expectation is that such a route will enable manufacturers to produce these active ingredients at a price that can meet global health needs while facilitating sustainable business practices. The synthesis of the pyridone and the total synthesis of DTG was previously described (DOI: 10.1002/anie.201802256) by our team. Herein, we provide more details to aid those wishing to implement the process.

4.1. General Experimental Considerations

The following commercial equipment was used to pump/control the described process: Vapourtec R-Series, Syrris ASIA syringe pump, Harvard Apparatus PHD Ultra syringe pumps, and the Eldex 3HI Optos Series HPLC pumps. Fluidic components and backpressure regulators were supplied from IDEX Health & Science Technologies. Reactor modules utilized in the flow synthesis were constructed from high-purity perfluoroalkoxy (PFA) tubing with a 1/16" outside diameter and or 0.04" inside diameter. T-mixers were IDEX Tee Assy 1/8" PEEK 0.050 through, and ferrules were SF, SS Ring, 1/16", TZ.

4.2. Two-Stage Process

The M4ALL Institute has developed a two-stage process leading to the pyridone. The process begins with a telescoped two-bond-forming unit operation terminating in a chromatographic purification yielding 94% of intermediate **5**. The pyridone synthesis is completed in a continuous annulation that provides the desired material in 91% yield after flash chromatography. This two-stage semi-continuous process provides the pyridone in 86% overall yield with a purity of 99.7%.

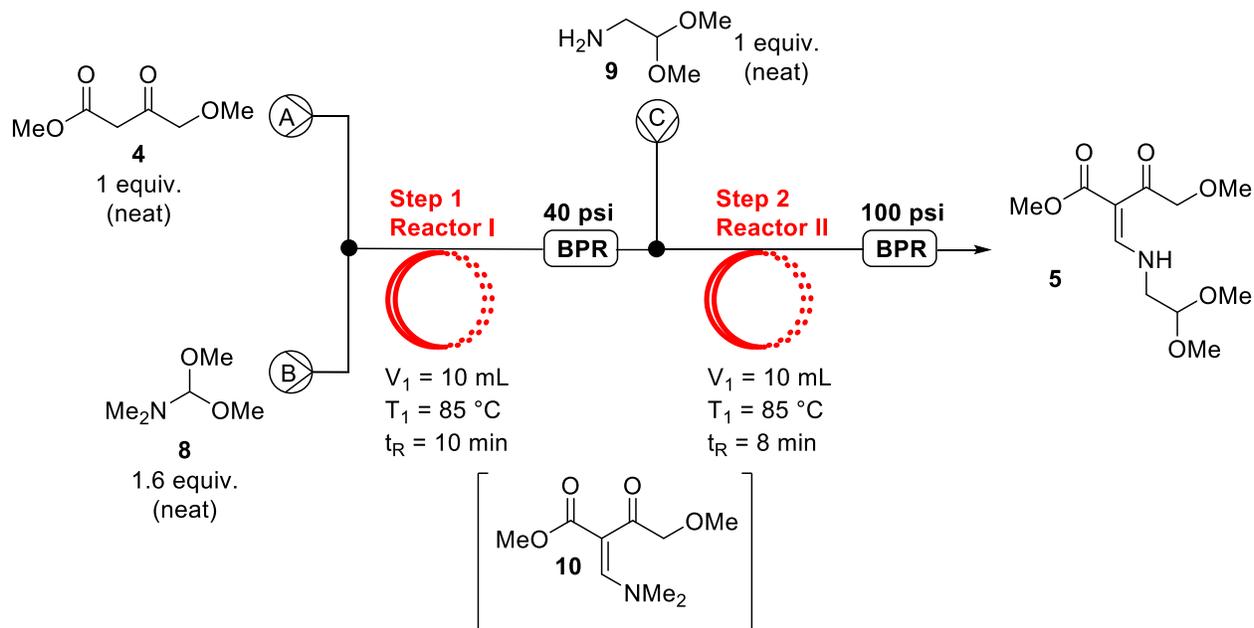
4.2.1. First Stage of the Semi-continuous approach

The fluidic configuration for the first two bond-forming steps is illustrated as a simplified P&ID diagram in Figure 4. Two HPLC pumps were used to deliver solutions (Pump A: methyl-4-methoxyacetate **1**; Pump B: N,N-dimethylformamide dimethyl acetal **8**) to the first of two T-mixers and residence-time loops. The product from this first reaction is then mixed with aminoacetaldehyde dimethylacetal **9** via the second T-mixer delivered via

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another pump. Two back-pressure regulators were used to ensure even flow, eliminate pump cavitation and eliminate back-flow.

Figure 4. Fluidic configuration for continuous flow synthesis of Steps 1 & 2 of DTG Intermediates



MATERIALS SUMMARY

Table 3. Material Amounts for the First Stage of the Semi-Continuous Approach

Material	MW	Density(g/mL)	Amount (g)	Amount (mL)	mmol	Equiv
4	146.14	1.129	4.27	3.78	29.20	1
8	119.16	0.897	5.58	6.22	46.82	1.6
9	105.14	0.965	4.63	4.8	44.06	1

PROCEDURE

1. The system was purged with three volumes of HPLC grade methanol before use.
2. The flow rates of pumps 1 and 2 were set to 0.378 mL/min and 0.622 mL/min respectively, and pump 3 was set at 0.48 mL/min.
3. Each residence-time loop was set to the noted temperature and allowed to equilibrate before the reaction was initiated.
4. Once the reactions began, the system was allowed to equilibrate with the set flow rates and set reaction temperature for approximately three total system residence times.

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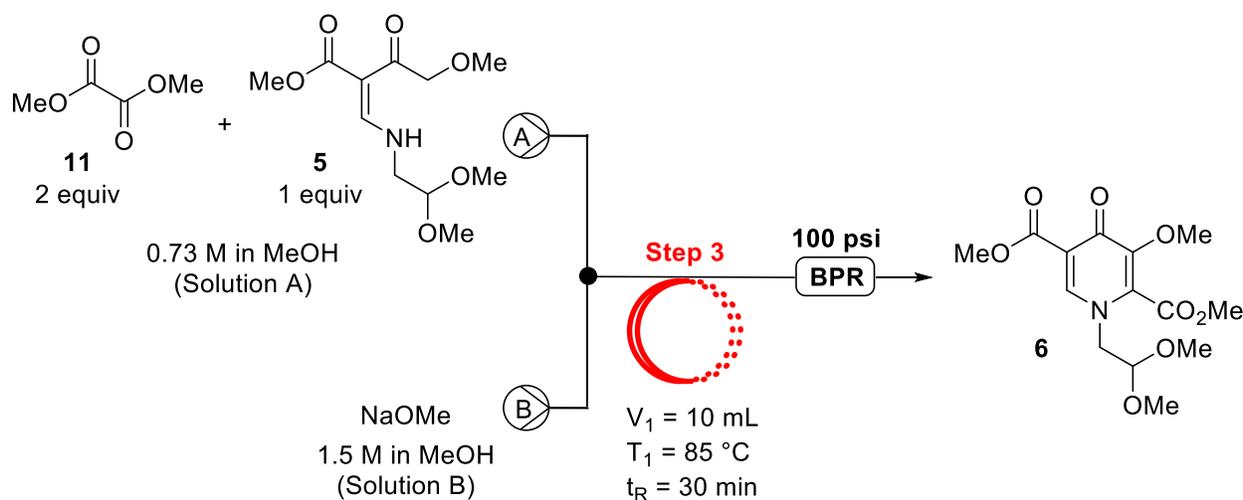
5. The output flow was collected for 10 mins (14.8 mL). The crude product was collected and purified by flash column chromatography yielding compound **5** (7.16 g, 27.4 mmol, 94 %).

¹H NMR (600 MHz, CDCl₃): δ 10.98 (br. s, 1H), 7.96 (d, *J* = 13.8 Hz, 1H), 4.59 (s, 2H), 4.43 (m, 1H), 3.73 (s, 3H), 3.48 (s, 3H), 3.44 (o, 8H); **¹³C NMR (125 MHz, CDCl₃):** δ 196.9, 166.9, 160.9, 102.8, 98.5, 76.5, 59.1, 54.8, 51.9, 50.8 **HPLC-MS** Consistent with structure.

4.2.2. Second Stage of the Semi-continuous approach

The second stage of this semi-continuous approach was conducted by combining the output of the first stage (compound **5**, 1 equiv., 730mM in MeOH, flow rate = 165μL/min) and dimethyl oxalate **11** (2 equiv.) into a single solution that was combined via a T-mixer with a solution of sodium methoxide (1.5 equiv., 1.5M in MeOH, flow rate = 165μL/min) and subsequently into a residence-time loop.

Figure 5. Fluidic Diagram for Continuous Flow Synthesis of DTG Step 3



MATERIALS SUMMARY

Table 4. Material Amounts for the Second Stage of the Semi-Continuous Approach

Material	MW	Concentration M	Amount (g)	Amount (mL)	mmol	Equiv
5	261.27	0.73	1.89	9.9	7.23	1
Dimethyl oxalate	118.09		1.71		14.45	2
NaOMe (25wt% in MeOH)	54.02	0.945		19.8	68.80	1.5
MeOH for system washing				~500		

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PROCEDURE

1. HPLC Grade MeOH was used as the solvent and the flow rates of each pump set at 165 μ L/min.
2. The residence-time loop was equilibrated at 85°C and the reaction initiated by flowing the solutions into the system.
3. The flow chemistry system was allowed to equilibrate for three system residence times.
4. Product solution was collected once the system equilibrated (19.8 mL, 7.23 mmol of rate limiting material) and purified by flash column chromatography yielding pyridone **6** (2.17 g, 6.58 mmol, 91%, white solid with an HPLC area% of 99.7%). The product can also be isolated with similar purity without chromatography – see the through process (vide infra).

¹H NMR (600 MHz, CDCl₃): δ 8.13 (s, 1H), 4.50 (t, J = 4.9 Hz, 1H), 3.98 (o, 6H), 3.92 (d, J = 4.86 Hz, 2H), 3.91 (s, 3H), 3.40 (s, 6H); **¹³C (125 MHz, CDCl₃):** δ 171.3, 165.8, 162.7, 150.7, 146.3, 133.9, 118.6, 103.0, 60.8, 56.9, 56.0, 53.6, 52.6. **HPLC-MS** – Consistent with structure

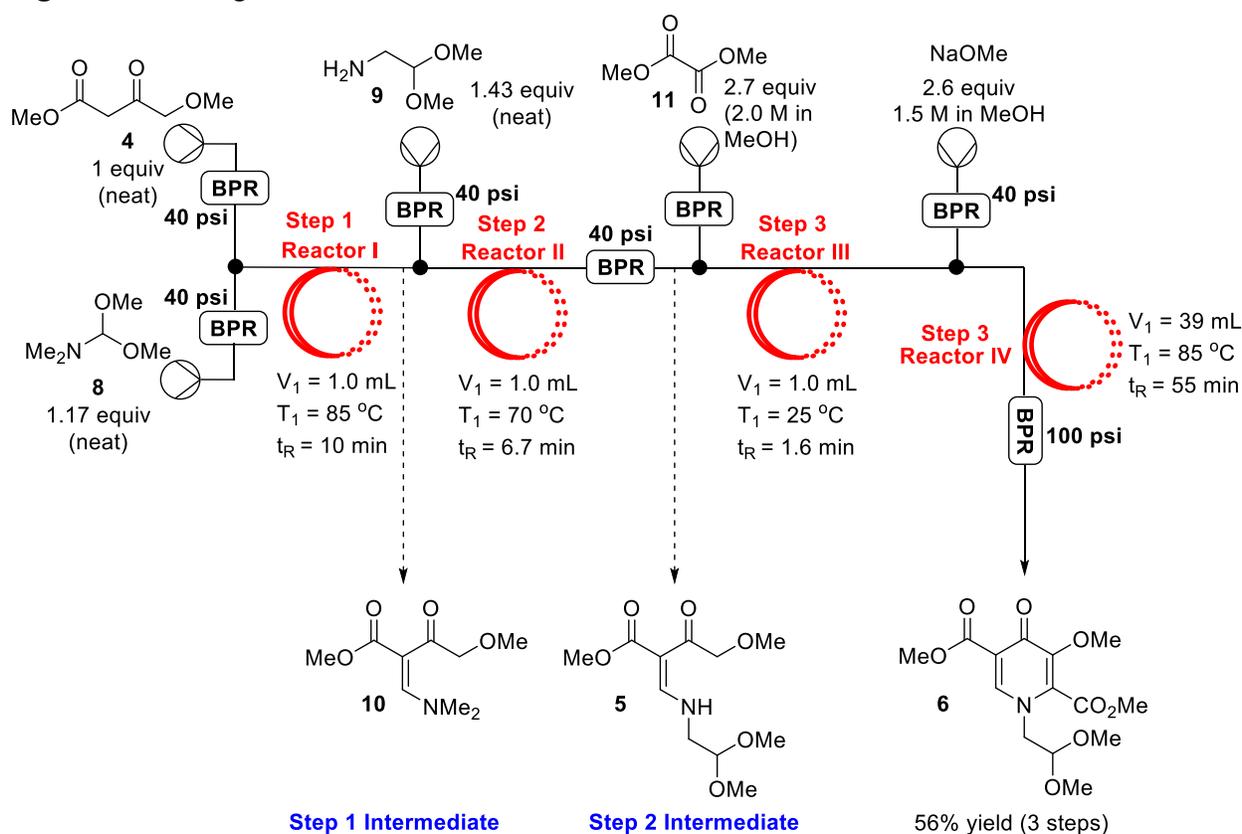
4.3. Through Process

DTG-pyridone is prepared by a three bond-forming through process that mirrors the two-stage example described above but telescopes the last step with the first two. The pyridone was isolated by crystallization from IPAc/Hexanes in 50-57% yield overall.

Neat **4** is combined with neat **8** at 80°C and reacted within a residence time-loop for 15 min. The output is then combined with **9** (10 min residence time at 80°C), the effluent contains >95A% of the step 2 intermediate (**5**). The resulting stream is diluted with MeOH to dissolve solids, and the solution is concentrated to half its original volume to remove dimethylamine. The process is repeated, and the ensuing stock solution is diluted with MeOH. Dimethyl oxalate (**11**) is dissolved in the methanolic solution of the step 2 intermediate and volume adjusted with MeOH (~0.75M). This solution was then mixed with a ~1.8M solution of NaOMe in MeOH at 70°C with 50min residence time. The process stream was worked up by stripping the volatiles to ~1/4 of total volume and dissolved in DCM and pH adjusted with 1M aq. citric acid. Organics were isolated and concentrated to about 1/4 of total volume; then IPAc (3V) added and solution concentrated again. This is repeated with another 3V of IPAc. The mixture was stirred at RT whereupon crystallization occurs forming a light brown slurry. Hexanes (2V) was then added at RT to complete crystallization. After incubation for 2h, the batch was cooled to 0-5°C and aged for 2h. Solids were filtered off, washed with IPAc/hexanes and dried in vacuo at 40°C.

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Figure 6. Through Process



MATERIAL SUMMARY

Table 5. Material Amounts for Through Process

Material	MW	Density(g/mL)	Amount (g)	Amount (mL)	mmol	Equiv
4	146.14	1.129	56.45	50	386	1
8	119.16	0.897	53.82	60	451.6	1.17
9	105.14	0.965	57.9	60	550.7	1.42

Note: Material from steps 1 & 2 was reserved for additional experiments and only 40mL of the output was taken through the third step.

5 (40 mL)					77	1
Dimethyl oxylate	118.09		24.6		208	2.7
NaOMe (25wt% in MeOH)	54.02	0.945		46	201	2.6
MeOH for system washing				~500		

PROCEDURE

- 50ml of 4 was charged into reagent bottle A.
- 60ml of 8 was charged into reagent bottle B.

3. 60ml of **9** was charged into reagent bottle C.
4. Two 10mL PFA reactors in series were primed with HPLC grade methanol and a 40 psi Back Pressure Regulator (BPR) was affixed at the effluent of the second reactor. Both reactors were heated to 80 °C while continuing priming with methanol. This step was performed to rinse the reactors – potentially removing any plasticizers or other coatings that might be used in their production. (2 column volumes, 20 mL).
5. Reagent A was pumped at 0.305 mL/min and reagent B at 0.365 mL/min through a T-mixer into reactor 1 at 80 °C while reagent C was pumped at 0.34 mL/min into reactor 2. The effluent of reactor one was connected to reagent C stream through a T-mixer.
6. The first ~20mL of process stream was discarded to eliminate residual methanol used to wash the system.
7. The process stream was collected in 10 mL aliquots and analyzed by HPLC to determine steady state – we observed that the system equilibrated in the first aliquot. Restated, the HPLC areas were consistent from first to second aliquot.
8. Once steady state was reached the process stream was collected – 140-150 mL.
9. After the reagent bottles emptied the system was flushed with MeOH (2 column volumes to push the dead volume from the system).
10. The solution was concentrated to ~1/4 of total volume. MeOH was charged (~3/4 of original volume) and the distillation repeated. When the distillation was complete the concentrate was diluted with MeOH to 200 mL total volume. The above stock solution was used as such without any further processing.
11. Dimethyl oxalate (24.6g) was dissolved in 40 mL of the stock solution produced in step 10 and diluted further with 15-20 mL of MeOH.
12. The solids were dissolved with agitation, and heating to 30-40 °C (if necessary). Once solids dissolved, dilute with MeOH to a volume of 96 mL (0.8M wrt step 2 intermediate). This is solution A.
13. NaOMe (46 mL 25 wt% in MeOH) was charged into a reagent bottle.
14. MeOH (50mL) was added to the NaOMe solution ~2.0 M solution B. This brought the total volume to 96 mL to normalize the volume to that described in step 12. This dilution ensures that flows A and B occur at the same rate.
15. Two 10mL PFA reactors in series were primed with HPLC grade MeOH (5 column volumes, ~50 mL) and a 100 psi BPR was affixed at the exit of the second reactor. Both reactors were heated to 70 °C while priming with MeOH (2 column volumes, ~20 mL). Solutions A & B were pumped through the two reactors at 0.2 mL/min and the first 30 mL were discarded – to eliminate the methanol.
16. Aliquots of 10 mL were collected for HPLC analysis to determine steady state – again steady state was reached within the first two aliquots.

17. When a steady state was reached, process streams were collected until solutions were consumed. Reactors were flushed with MeOH (no less than 20 mL). Volatiles were evaporated on a rotovap to ~1/4 original volume and the concentrate extended in DCM (100 mL) and washed with 1M citric acid (50mL). Then aqueous stream was then back extracted with DCM (50mL).
18. **Note:** By HPLC assay at this point, the desired product peak represents 85% of the area and the hydrolyzed product represents 8% of the area and miscellaneous peaks represent the remaining.
19. The organic phase was washed with 0.5 M NaHCO₃ and concentrated to ~1/4 volume. 100 mL IPAc was charged to this concentrated organic layer and distilled to ~1/4 volume. This step was repeated to achieve a complete solvent exchange to IPAc.
20. The IPAc solution was treated with 2x volume of hexanes and the resultant slurry aged initially at room temperature, and then at 0-5 °C.
21. Solids were filtered off, washed with 1:2 IPAc/Hexanes, and dried at RT in vacuo – 14.59g. This process gave off-white solids with an HPLC area% of 99.5% and 100% assay against chromatographed material.
22. Table 5 features the yield for two runs. The HPLC assay before work-up was coincident with what is described above (85% desired product). The majority of the loss in both cases appears to be the precipitation step and assay of the mother liquor does contain desired product. A more efficient purification might be needed to improve the yield to align the outcome relative to the two-stage example described above.

Table 6. Data for Through Process Runs

Batch	Scale (g)	Yield (wt%) uncorrected	Output Purity (A %)
Run 1	10	57.6	98.3
Run 2	11	50.2	97.4

4.4. Economics (Cost of Goods) and Materials Balance

Prior art for the manufacture of the pyridine DTG intermediate suggests two different viable routes. GSK published an Org Lett. Paper (2015, 17, 564-567) and Mylan has filed two different patents (WO2015/019310 and WO2016/125192). Cost of Goods analysis (chemical inputs only, no conversion costs) suggests \$380/kg and \$425/kg, respectively, for these routes.

Subjecting the M4All continuous process to similar analysis revealed a significant savings from either the two-step or through process.

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Two-Step Process

Material	M.Wt.	Amount	equivalent	density	RMCost-	RM cost	Rmcost
		kg			\$/Kg	\$/batch	\$/Kg Product
Step -1 -2 (Telescoped)							
Methyl-4-methoxyacetoacetate (4)	146.16	146.16	1	1.129	106	15,492.96	
Dimethylformamide dimethylacetal (8)	119.16	190.656	1.6	0.897	10	1,906.56	
Step -2							
Aminoacetaldehyde dimethylacetal (9)	105.14	105.14	1	0.965	182	19,135.48	
Product (Step 2)	261.27						
95%		248.2065				36,535.00	147.20
Step 3 (Independent)							
Step 3 Starter (is Step 2 Product)	261.27	261.27	1		147.20	38457.89	
Dimethyloxalate	118.09	236.18	2		5.00	1180.9	
Sodium methoxide	54.02	81.03	1.5	0.945	1.50	121.545	
Methanol	32.04	431.0955		0.792	0.69	297.4559	
Step 3 Product	329.2						
91%		299.572				40,057.80	133.72
							133.72
							\$/kg

Recycle solvent

Assuming 85% solvent recycle and reported yields the pyridine cost of goods is estimated to be \$134/kg, significantly lower than either the GSK or Mylan process. This analysis does not include the cost of flash chromatography since it is expected that a crystallization process will be adopted at scale. A rough estimate is that solvents, chromatography media and processing time could add as much as \$50/kg to the above COG analysis. Even with that conservative estimate the process is competitive.

Through Process

Material	M.Wt.	Amount	equivalent	density	RMCost-	RM cost	Rmcost
		kg			\$/Kg	\$/batch	\$/Kg Product
Step -1 -2 (Telescoped)							
Methyl-4-methoxyacetoacetate (4)	146.16	146.16	1	1.129	106	15,492.96	
Dimethylformamide dimethylacetal (8)	119.16	139.4172	1.17	0.897	10	1,394.17	
Step -2							
Aminoacetaldehyde dimethylacetal (9)	105.14	149.2988	1.42	0.965	182	27,172.38	
Product (Step 2)	261.27						
75%		195.9525				44,059.51	224.85
Step 3 (Independent)							
Step 3 Starter (is Step 2 Product)	261.27	261.27	1		224.85	58746.02	
Dimethyloxalate	118.09	318.843	2.7		5.00	1594.215	
Sodium methoxide	54.02	140.452	2.6	0.945	1.50	210.678	
Methanol	32.04	431.0955		0.792	0.69	297.4559	
Step 3 Product	329.2						
75%		246.9				60,848.37	246.45
							246.45
							\$/kg

Recycle solvent

Note: For each step in the above cost model, we assume that each step begins with 1 mole of the limiting starting material.

Even with the markedly lower yields for the through process the estimated cost of goods for the pyridone intermediate is \$246.45/kg, markedly lower than existing processes.

Another measure of process efficiency is defined as “space-time yield” (STY, g L⁻¹ hr⁻¹), which is the time needed to process one reactor volume of fluid. The higher the space-

time yield, the more material you can produce in a given reactor in a given period of time, which corresponds to better plant utilization. Space-time yields have been calculated for the Mylan, GSK, and M4ALL processes and are shown in Table 7, indicating the significant improvement from a process intensity standpoint. Space-time yields calculated for the Mylan (0.83 and 1.02 g/(L*h)), GSK (1.19 g/(L*h)), and M4ALL (69 g/(L*h)) processes indicate that *the M4ALL process produces approximately 58 times the amount of material as the next competitive process (GSK)*. These data only reflect the reaction time and do not factor in the work-up or other post-reaction operations.

Table 7. Comparison of Space-Time Yields

Process	Stage 1 RxnTime	Stage 2 Rxn Time	Stage 3 Rxn Time	Overall Yield of 6	Space-Time Yield
Mylan 1	8 h	2 h	26 h	28%	0.83
Mylan 2	12 h	N/A	1.5 h	17%	1.02
GSK	12 h	2 h	20.5 h	64%	1.19
M4All	10 min	8 min	20 min	86%	69.3

4.5. Characterization Data

Collected from the Two Stage Process

The conversion of Compound **5** to Compound **6** can be monitored by HPLC.

Column: Agilent Eclipse Plus-C18 (4.6 mm ID X 150 mm L, 3.5 μ m particles)

Mobile phase: 35% Methanol vs 0.1% H_3PO_4 at 1.0 mL/min

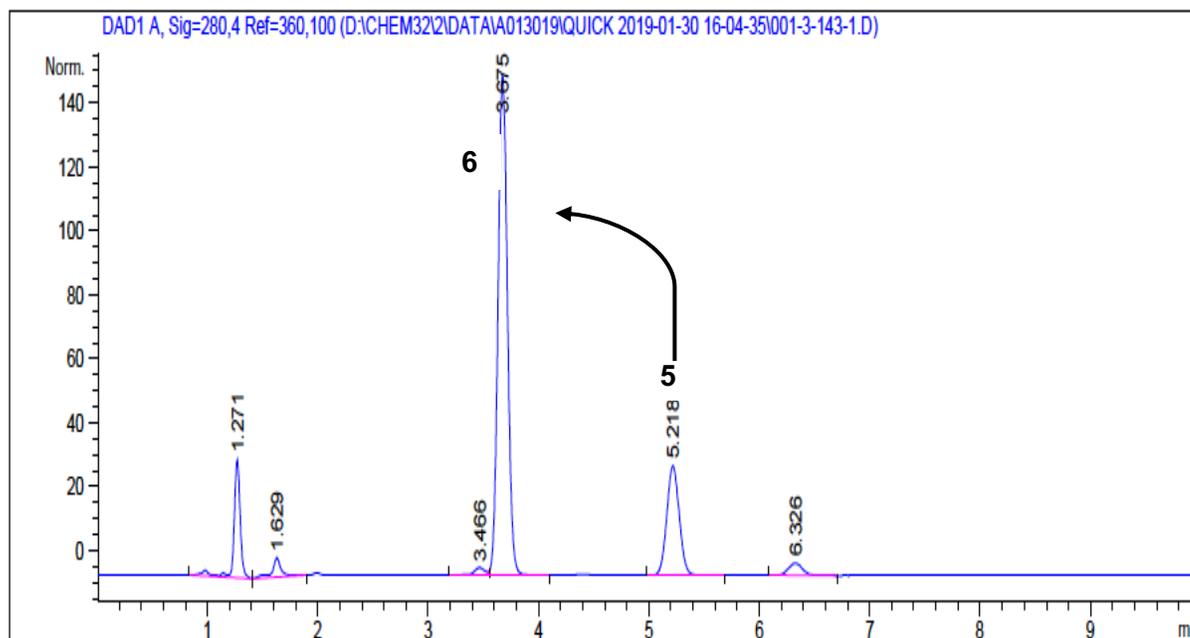
Column oven: 25°C

Injection: 1 μ L

Detection: 280 nm – note that the molar absorptivity of **5** is 1.13X that of **6**

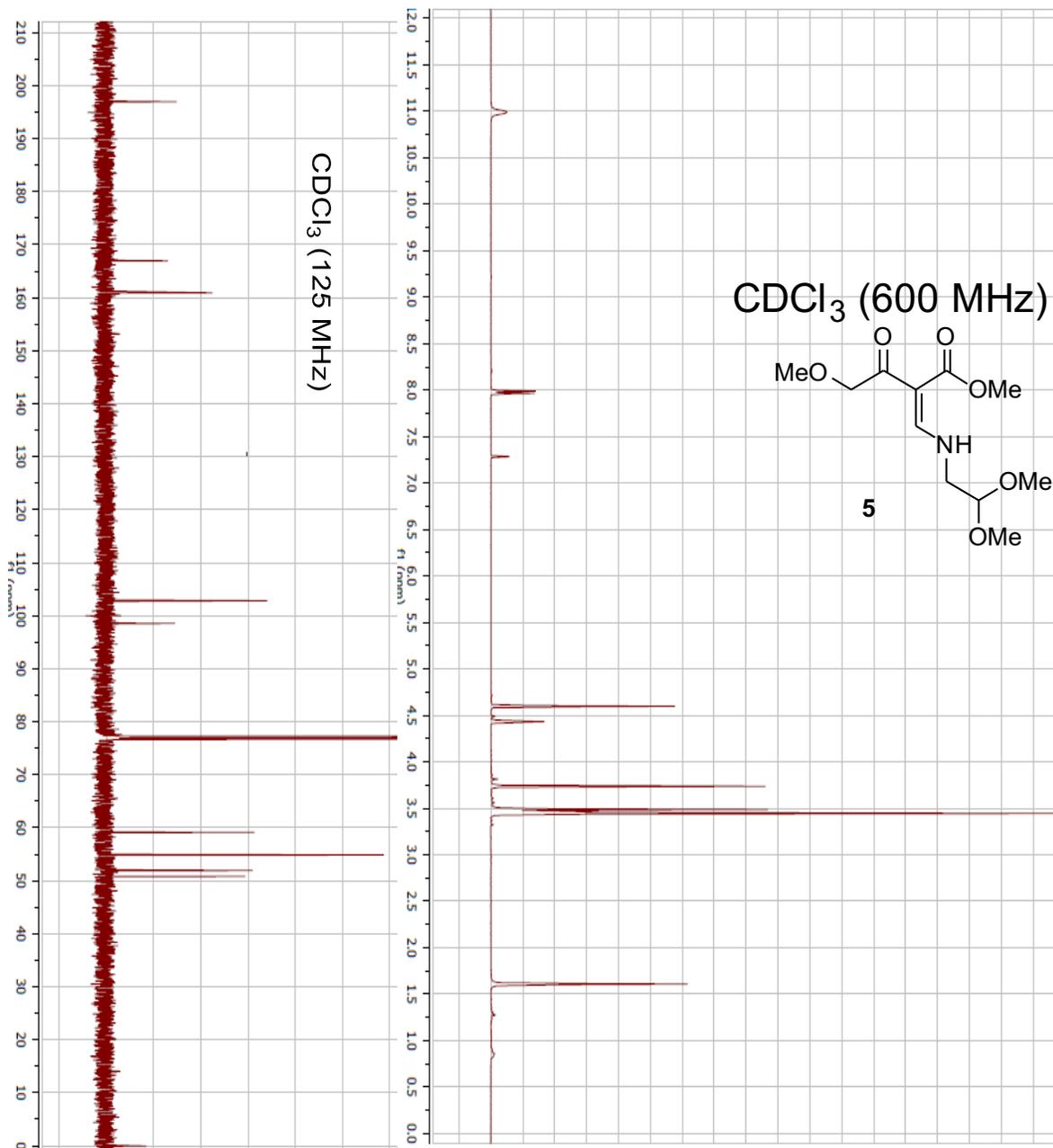
Sample Prep: target 1 mg/mL in methanol

Elution times: Compound **5** = 5.2 minutes; Compound **6** = 3.7 minutes



Compound 5 Characterization

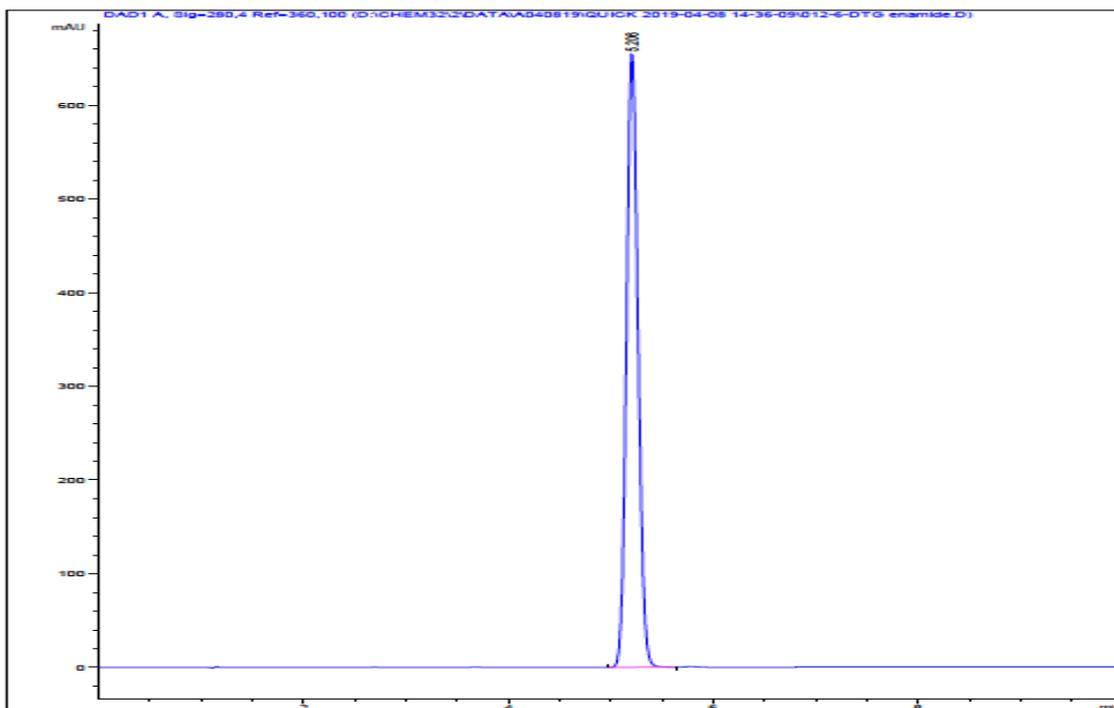
¹H and ¹³C NMR



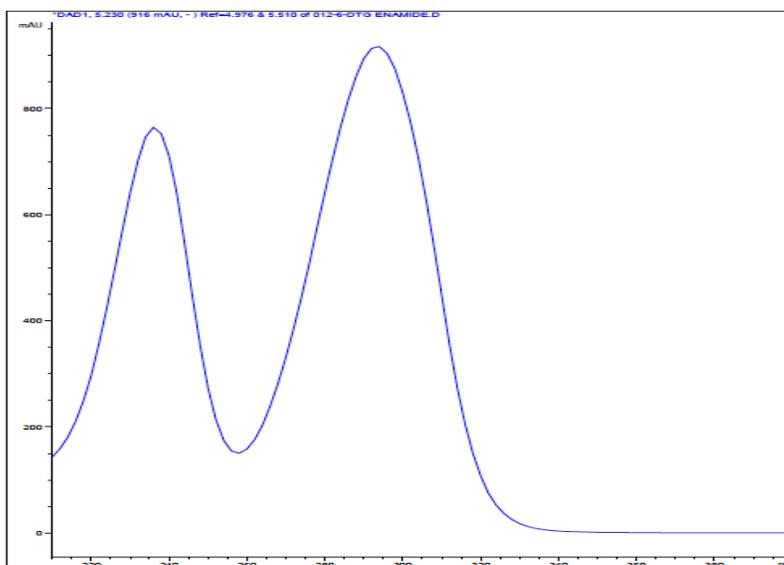
¹H NMR (600 MHz, CDCl₃) δ 10.98 (br. s, 1H), 7.96 (d, *J* = 13.8 Hz, 1H), 4.59 (s, 2H), 4.43 (m, 1H), 3.73 (s, 3H), 3.48 (s, 3H), 3.44 (o, 8H);

¹³C NMR (125 MHz, CDCl₃) δ 196.9, 166.9, 160.9, 102.8, 98.5, 76.5, 59.1, 54.8, 51.9, 50.8.

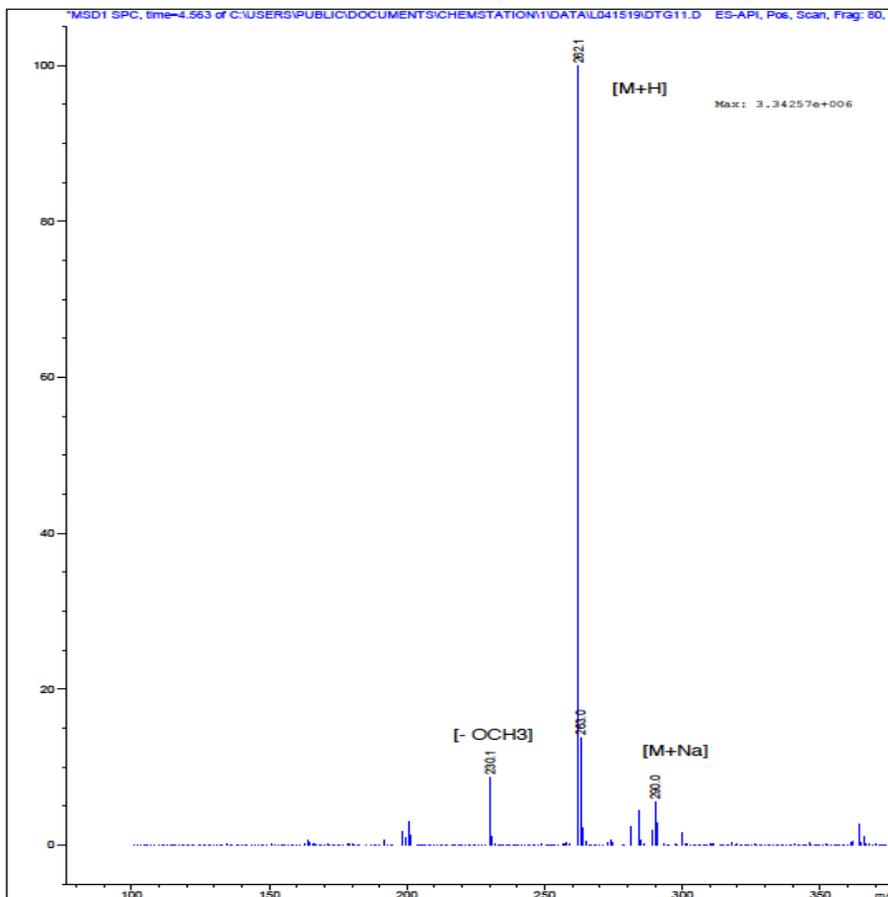
HPLC – method described above



UV Spectrum – UV maxima at 236 and 294 nm

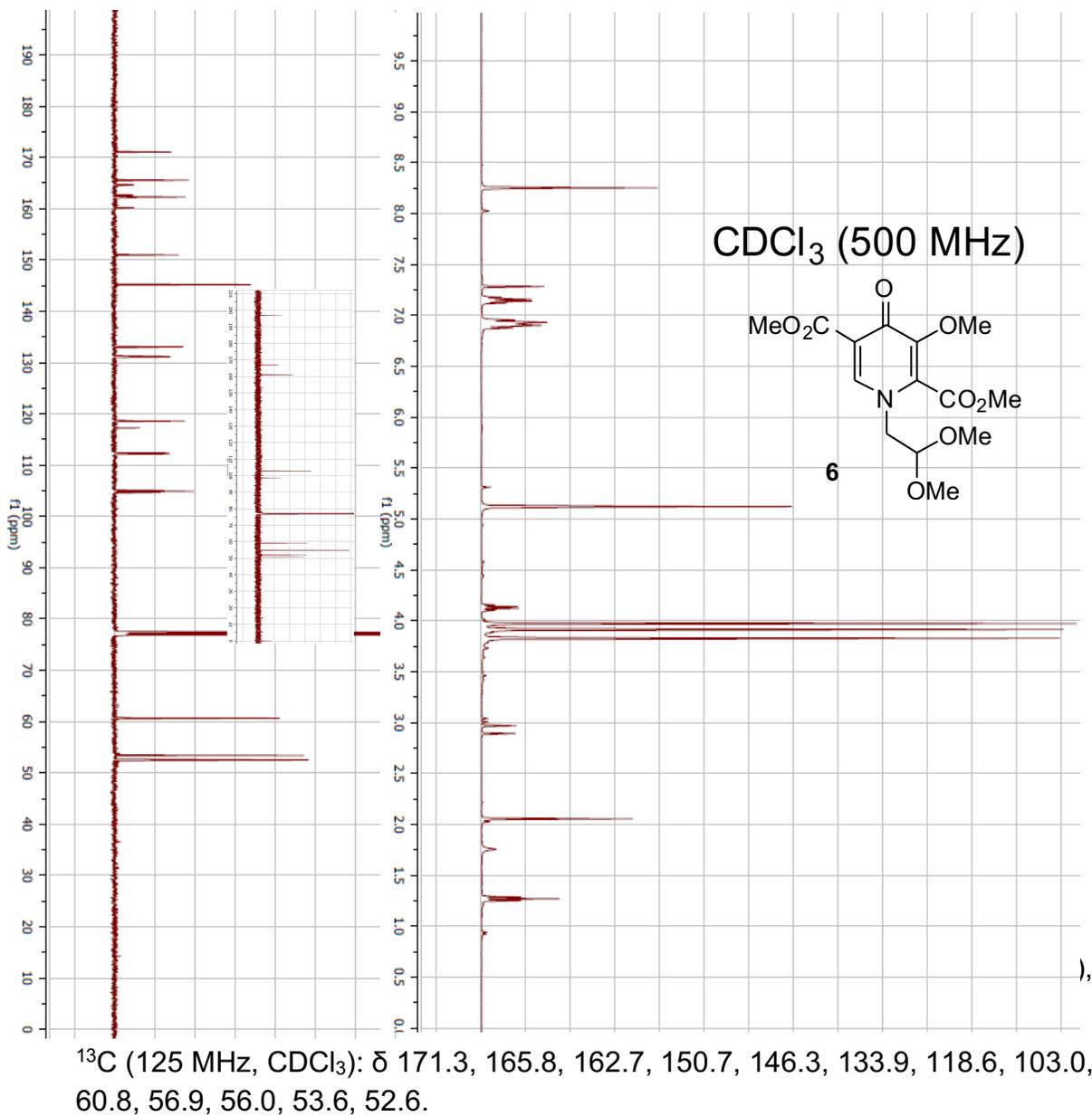


HPLC Mass Spectrum – ions observed at 262.1 $[M+H]^+$, 290.0 $[M+Na]^+$ and 230.1 corresponding to the loss of OCH_3 .

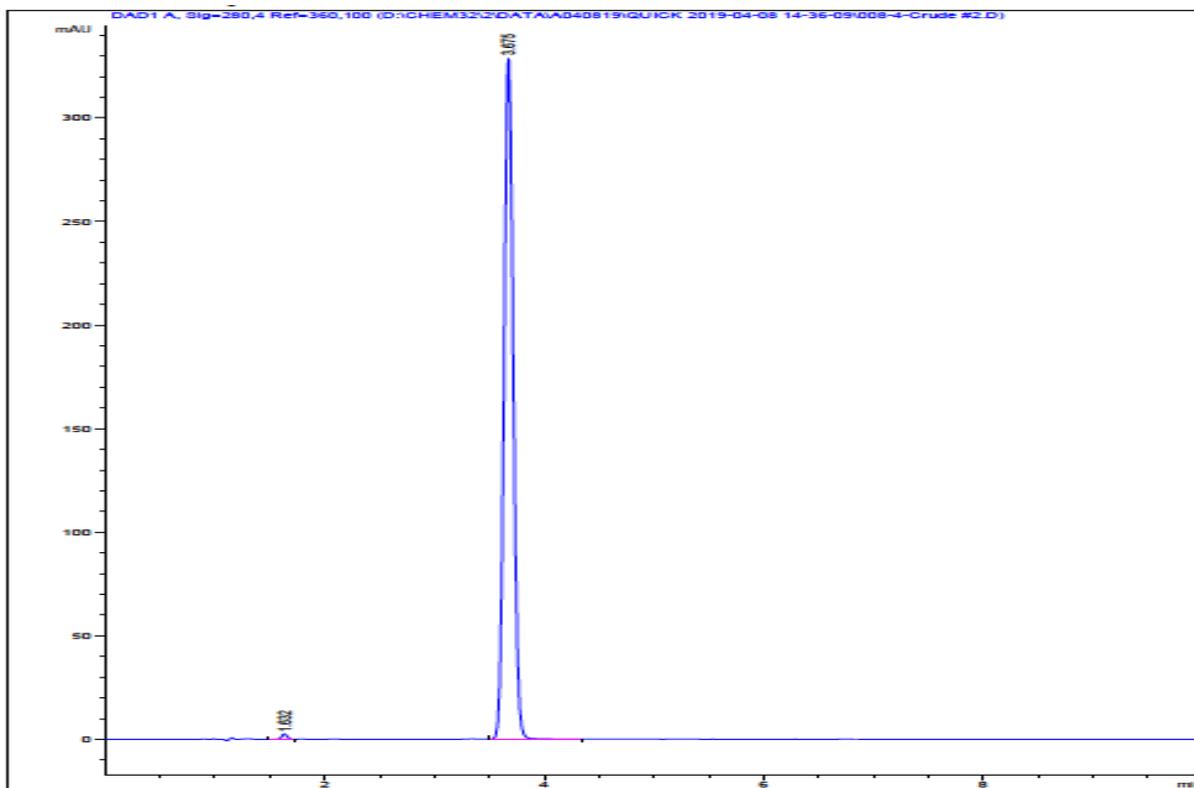


Compound 6 Characterization

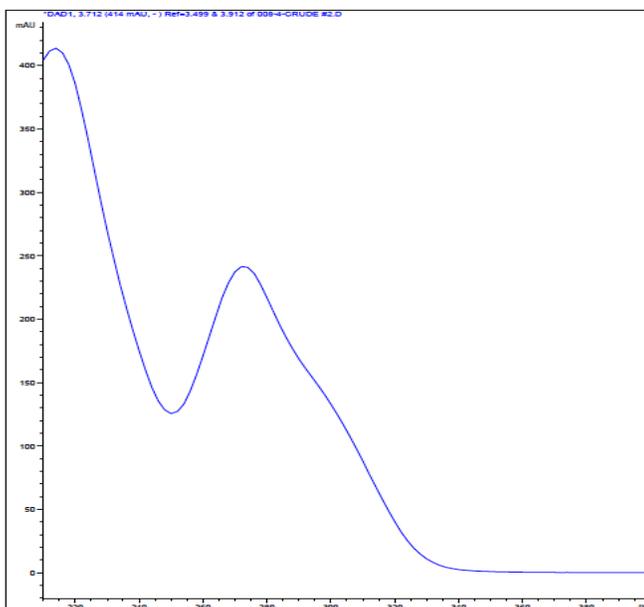
¹H and ¹³C NMR



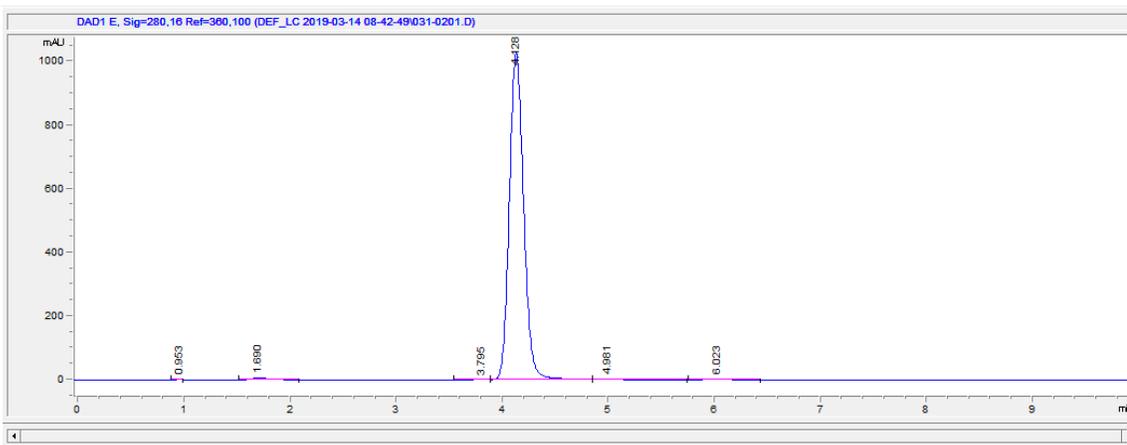
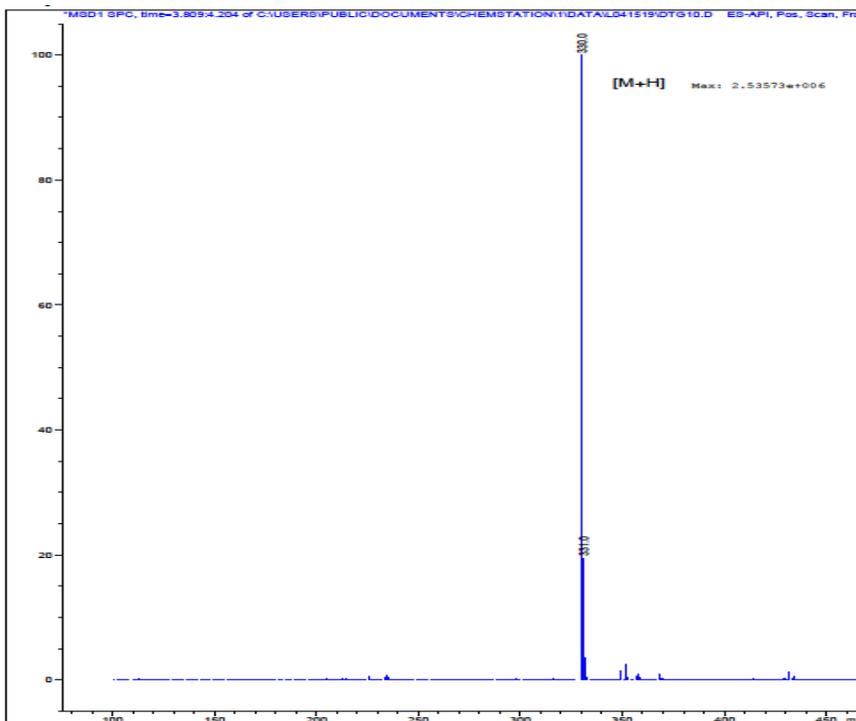
HPLC – method described above



UV Spectrum – UV maximum at 273 nm



HPLC Mass Spectrum – major ion observed at 330.0 [M+H]



File Information		#						
LC-File	031-0201.D	1	0.953	7.7	3	0.0418	0.078	1.014
File Path	C:\CHEM32\2\DATA\DEF_LC 2019-03-14 08-42-49\	2	1.69	54.8	6.3	0.1208	0.555	0.531
Date	14-Mar-19, 08:56:18	3	3.795	15.3	1.6	0.1463	0.155	1.181
Sample	AK-002-15S	4	4.128	9711.3	1027.4	0.1483	98.309	0.829
Sample Info		5	4.981	45.6	2.8	0.2224	0.462	0.561
Barcode		6	6.023	43.6	3.6	0.1844	0.442	0.861
Operator	hari							
Method	DTG-NEW METHOD_LC.M							
Analysis Time	10 min							
Sampling Rate	0.0067 min (0.402 sec), 1501 datapoints							