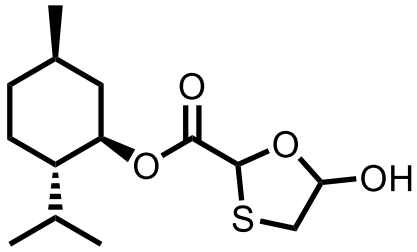
PROCESS DEVELOPMENT REPORT
3/2020

The Medicines for All Institute

Synthesis of (1R,2S,5R)-2-isopropyl-5-methylcyclohexyl 5-hydroxy-1,3-oxathiolane-2-carboxylate



March 20th, 2020

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Executive Summary

We report a synthesis of (1R,2S,5R)-2-isopropyl-5-methylcyclohexyl 5-hydroxy-1,3-oxathiolane-2-carboxylate **1** from low cost commodity feedstocks including thioglycolic acid, sulfuryl chloride and vinyl acetate. This product is a key raw material used in the production of lamivudine (3TC) and emtricitabine (FTC). The current market prices of **1** and 3TC are $34/kg and $140/kg, respectively. With the new route, the cost of goods for **1** is estimated to be $17-21/kg. Incorporating a conservative $10/kg conversion cost brings the total estimated price for compound **1** to $27, a projected 20% decline over market price. This is projected to enable a 14% decline in the cost of 3TC.

Introduction and Prior Art

Lamivudine also known as 3TC is a nucleoside reverse transcriptase inhibitor (NRTI) for treatment of HIV. The fixed dose combination therapy of tenofovir, lamivudine and dolutegravir is becoming the first-line therapy for HIV patients in more than 50 low- to middle-income countries. Lamivudine is also a component of most other treatment regimens as well. The current market price is ~$140/kg. Reducing the associated production costs would enable the maximum number of people to receive treatment and that the market for these medicines remains robust. A 10% reduction in cost is estimated to save $28 MM/yr.

Lamivudine was discovered in 1988 by McGill University and BioChem International and brought to market in 1995.[1] The current manufacturing process (a 5 step process) was developed by Andrew Whitehead and colleagues at GSK and first described in a patent application in 1995 and subsequently in the academic literature in 2005 (Figure 1).[2] Though a number of synthetic approaches have been disclosed,[3] the original synthesis appears to still be practiced today with only minor modifications. Each intermediate in the Whitehead route is observed in high-volume market transaction records, bearing witness to this claim.

M4ALL seeks to improve global healthcare by lowering manufacturing costs and environmental impact of key medicine manufacturing processes. Our approach ranges from creating novel end-to-end routes to focusing on key starting materials or intermediates to improve supply chain conditions. Our approach to cost reduction for lamivudine began with an understanding of the current manufacturing route cost structure, presented in Figure 1. Price for intermediates and reagents were obtained from import/export records of India (Datamyne). From this analysis, it was observed that no one single reagent dominates the cost-structure. Impressively, the highest cost reagents are all relatively inexpensive (menthol ($11/kg), cytosine ($27/kg), and $NaBH_4$ ($18.6/kg)). By mole, the reagents are ranked from highest to lowest price as follows: cytosine ($3.00/mol), menthol ($1.71/mol), dithianediol ($1.53/mol), and sodium borohydride ($0.99/mol).

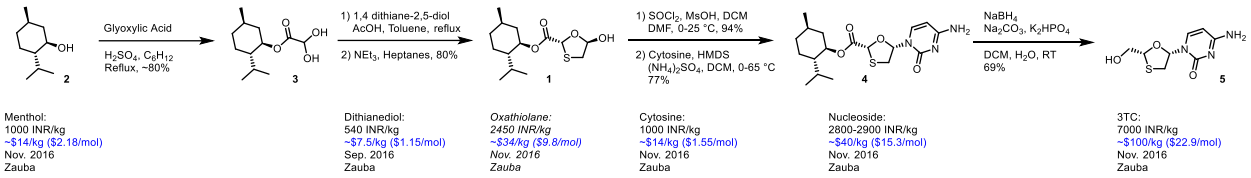


Figure 1: Current manufacturing route for lamivudine.

Once the route and cost drivers were identified, the following opportunities were conceptualized (Figure 2): 1) Replace menthol with a less expensive chiral auxiliary, 2) Insert menthol later in the synthetic scheme rather than in the first step, 3) Use a commodity feedstock, as a raw material in place of dithianediol, and 4) Develop catalytic transformations to set the stereochemistry of the oxathiolane ring.

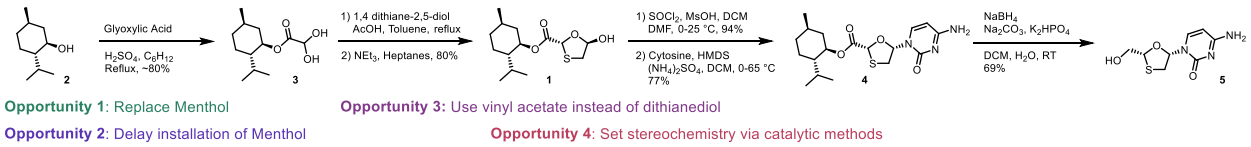


Figure 2: Opportunities selected for investigation to decrease cost of lamivudine.

In this report, we describe a new route to hydroxy-oxathiolane compound **1** (Figure 3). The route uses vinyl acetate and a thioglycolate to construct the oxathiolane ring thereby replacing the glyoxalate hydrate and dithianediol with easy and cheaper to source materials. Our process includes a regioselective 1,2-addition of a sulfenyl chloride formed *in situ* to vinyl acetate. The resulting sulfide-ester is then chlorinated in the *alpha*-position of the ester to form **7**. Unmasking the aldehyde by transesterification in water leads to the ring-closed product. The product is isolated by crystallization from 1% NEt_3 in hexanes. Stereochemical ratios may be measured but recent findings from Lupin indicate that the diastereomeric ratio is not a critical parameter in transformation to lamivudine.[4] We have converted product 1 produced by the described chemistry to lamivudine and obtained chiral purity comparable to commercial material. Measured isomeric ratios of the oxathiolane change in solution due to epimerization of the substrate when dissolved. These ratios also change slowly in the solid state. The approach provides isolated yields ranging from 50-53% over the two steps.

Vinyl acetate is a high-volume commodity used in manufacture of adhesives, the improvement relates to a known intermediate and is thus a drop-in-replacement, and the route change does not occur within most regulatory defined routes. We expect that these factors will facilitate ability to adopt the technology and lead to improved pricing of 3TC and FTC.

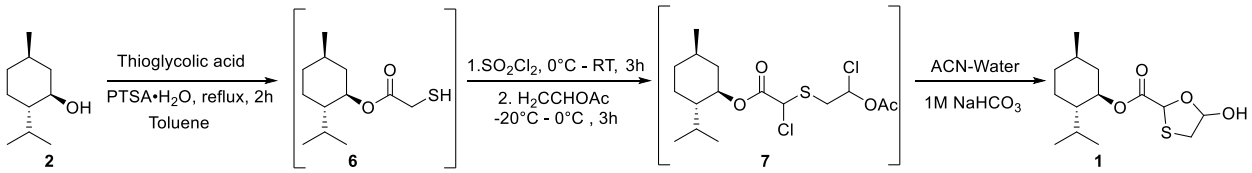


Figure 3: New route to hydroxyoxathiolane**1** from vinyl acetate.

Experimental Procedure

Chemicals	Density (g/mL)	Purity (%)	QTY (g)	QTY (Vol)	M.W	mmole	Molar Ratio	Source	Specs/Grade
L-Menthol		>99%	10.0		156.27	64.0	1.00	Sigma Aldrich	>99%
Thioglycolic acid	1.326	98%	6.19	4.7 mL	92.12	67.2	1.05	Sigma Aldrich	>98%
p-Toluenesulfonic acid monohydrate		>98.5%	0.243		190.22	1.3	0.02	Sigma Aldrich	>98%
Sulfuryl Chloride	1.67	97%	19.0g	11.4 mL	134.97	141	2.2	Sigma Aldrich	97%
Vinyl Acetate	0.934	>99%	11.02g	11.8mL	86.09	128	2.0	Sigma Aldrich	>99%
Toluene				50 mL				Merck	DriSolv Anhydrous
Acetonitrile				400mL				J.T Baker	>99.9%
water				400mL					
1M $NaHCO_3$ solution				100mL 128mL			2.0		

Table 1: Reagents and quantities for the synthesis of compound 1

Safety Precautions

1. The reaction generates HCl vapors (acid scrubber recommended).
2. The reaction generates SO_2 (acid scrubber recommended).
3. Pressure generated by gaseous reagents.

(1R,2S,5R)-2-isopropyl-5-methylcyclohexyl5-hydroxy-1,3-oxathiolane-2-carboxylate (1):

1. Setup a 250 mL three-neck round-bottom flask with a stir bar, Dean Stark distillation receiver, condenser, and probe for internal temperature monitoring. Inspect integrity of seals/O-rings for any defects. Place the setup on a hot plate with heating block.
2. Charge Toluene (50 mL).
3. Charge L- menthol (10.00 g).
4. Charge Thioglycolic acid (4.66 mL).
5. Charge PTSA.H_2O (0.24 g).
6. Stir between 400-500 rpm.
7. Stir at 120 °C (Internal) for 2h. 1.1 mL of water expected to be produced over course of reaction. Record volume of water collected in Dean-Stark trap. Would be useful to advise what quantity is expected. Since next step is used for completion is it even relevant?
8. Sample reaction for completion. Dissolve a 10 µL sample in 1 mL of toluene and analyze using GC-MS. If menthol peak area percent is >3% compared to compound 1, stir for an additional hour and sample again for GC-MS analysis.

9. After consumption of Menthol (<3% by peak area) turn off the heat. Replace the stopper with rubber septum. Remove the Dean Stark distillation receiver. Visually inspect reactor to ensure no water contaminates system. Place system under static positive pressure <5 psig (Accomplished via nitrogen balloon).
10. Cool the reaction mixture to 0 °C ± 2 °C (internal) using ice bath.
11. Charge sulfuryl chloride (11.4 mL) at a constant rate of 0.76 mL/min over 15 min via syringe pump. Record external and internal temperature.
12. Record maximum internal temperature.
13. Record internal temperature at end of addition. We found best results when the rate of sulfuryl chloride addition was performed at the rate provided such that the temperature change was minimized.
14. Stir the reaction at 0 °C to 10 °C (internal) for 1h. Remove the ice bath and stir for an additional 2h at 20 °C ± 5 °C (internal). Reaction mixture becomes yellow in color.
15. Cool the reaction mixture to temperature -20 °C ± 2°C (internal).
16. Charge 11.8 mL vinyl acetate at a constant rate of 0.79 mL/min over 15 min via syringe pump. Record external and internal temperature.
17. Record maximum internal temperature.
18. Record internal temperature at end of addition. We found that when the vinyl acetate was added at the stated rate the temperature change was minimal. We encourage a vinyl acetate rate that does not result in temperature rises.
19. Stir the reaction for 2h keeping internal temperatures between -15 °C and -10 °C and then 1h between -10 °C and 5 °C (internal).
20. Quench the reaction mixture with 1M $NaHCO_3$ (100 mL) over the period of 10-15 min using an addition funnel. Temperature at start of addition should be 0 °C ± 5 °C.
21. Separate the toluene layer. Extract the aqueous layer with 30 mL of toluene.
22. Combine organic layers, dry with sodium sulfate (10 g), and filter into a tared round bottom flask. Rinse filter with 20 mL of toluene. Although we did not azetropically dry the toluene at this point, we suggest that those scaling this process consider doing so.
23. Concentrate the sample using a rotary evaporator. Keep temperature below 35 °C. No more than 20 wt% toluene should be present after evaporation as judged by reaction mass. If sample stored, keep below 0 °C.

24. Setup a 2 L three-neck round-bottom flask with stir bar, condenser and a probe for internal temperature control, place on hot plate with heating block. Place system under static positive pressure <5 psig (accomplished via nitrogen balloon).
25. Transfer material from step 23 using acetonitrile (100 mL).
26. Charge acetonitrile (300 mL).
27. Charge water (400 mL).
28. Stir at 500 rpm.
29. Heat reaction mixture to 70 °C (internal) over period of 30-40 minutes.
30. Add 1M $NaHCO_3$ (128 mL) over 2.5 h using syringe pump with 0.85 mL/min flow rate.
31. Stir for 1 hr after end of $NaHCO_3$ addition.
32. Add toluene (100 mL).
33. Allow the reaction mixture to cool.
34. When temperature is less than 40 °C, separate the organic layer and dry it over sodium sulfate (10 g). Alternatively, dry azeotropically with toluene during concentration during step 35. (Note that analysis shows no loss of product to the aqueous layer)
35. Transfer to a 500 mL single neck RB Flask and concentrate. Again for those choosing to scale this process, azetropic distillation is encouraged.
36. Cool the reaction mixture to 0 °C (external).
37. Add 1% NEt_3 in hexanes (60 mL) dropwise over a period of 30 min using an addition funnel. Continue stirring for 2h at 0 °C ± 5 °C.
38. Store the reaction mixture in freezer overnight at -10 ± 5 °C.
39. Collect the white solid on a Buchner funnel, and wash with chilled hexanes (-10 °C, 40mL). Allow the residue to dry for 1h by passing air through Buchner Funnel.
40. Record final sample weight and submit a sample for LC-DAD analysis.

Results of Reproducibility Assessment

The reproducibility of the route was assessed through three runs beginning with 10 g of menthol. Compound **1** was obtained as a white solid in yields from 50-53% with assay results ranging from 97.8% to 99.3%. Assay values were determined against commercial material using the HPLC method described in the analytical section. Corrected yields were consistently between 50 and 53%.

Operator	Step 1: Menthol (%)	Step 2: Mass (g)	Step 2: Assay (%)	Step 2: Yield (%)	Step 3: Mass(g)	Step 3: Assay (%)	Step 3: Yield (%)
1	**2.65**	**28.74**	**72.2**	**84.3**	**9.4**	**97.8**	**50**
2	**2.64**	**25.4**	**84.0**	**86.6**	**9.65**	**98.2**	**51**
3	**1.5**	**29.32**	**73.2**[1]	**87.2**	**9.85**	**99.3**	**53**

Analytical Test Results for Compound **1**

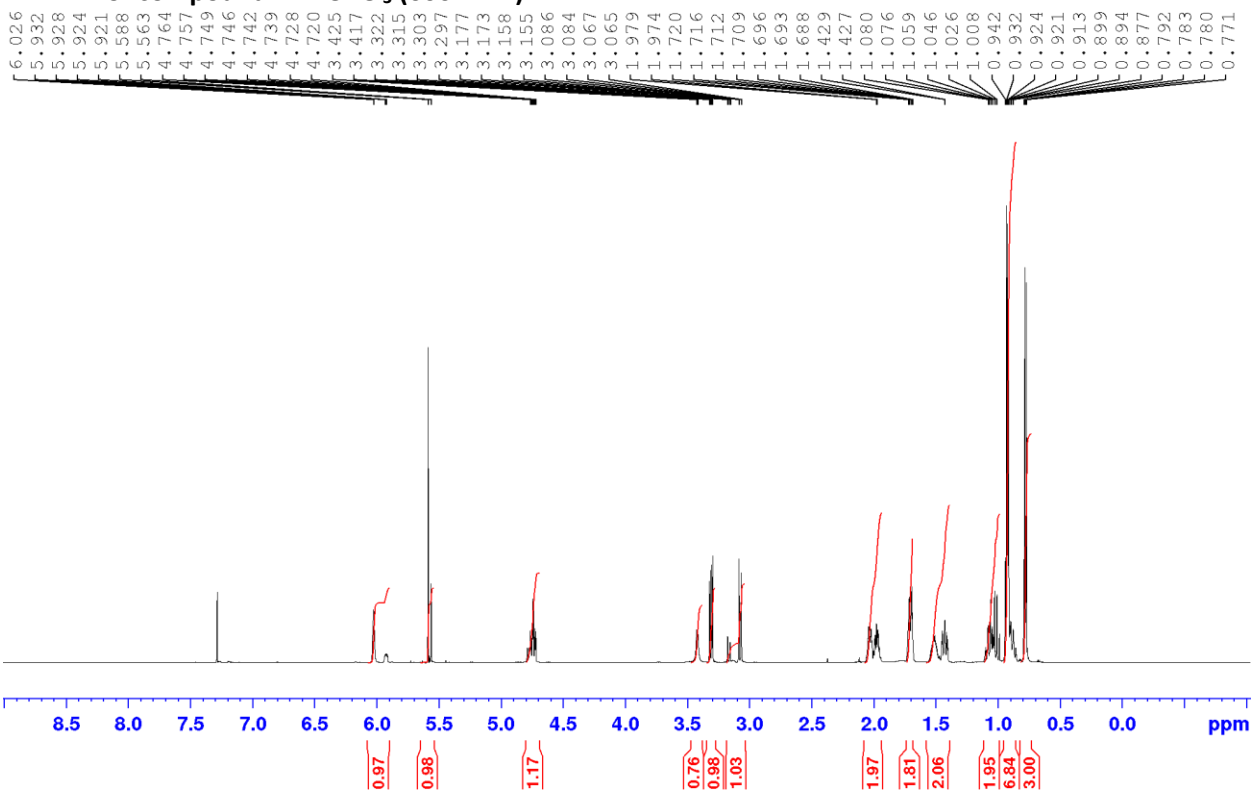
Batch	Weight%	Residual Toluene	Residual ACN	Area% (210 nm)	Impurity 1 (0.7 min)	Impurity 2 (1.7 min)
1	97.8	0.12%	ND	98.6%	0.44%	0.97%
2	98.2	0.14%	ND	98.5%	0.49%	0.96%
3	99.3	0.21%	ND	99.5%	ND	0.51%

Two small impurities were observed which were not identified. Considering the low UV absorptivity of Compound **1** it is expected that these impurities are present at lower concentration than the peaks would suggest. There are no established specifications for this intermediate. Compound 1 produced by this synthesis was subjected to a use-test where it was converted to 3TC using established chemistry. The results of this experiment demonstrated that neither of these impurities carried forward from Compound 1 to the API. At this point, we cannot rule out other by-products being formed.

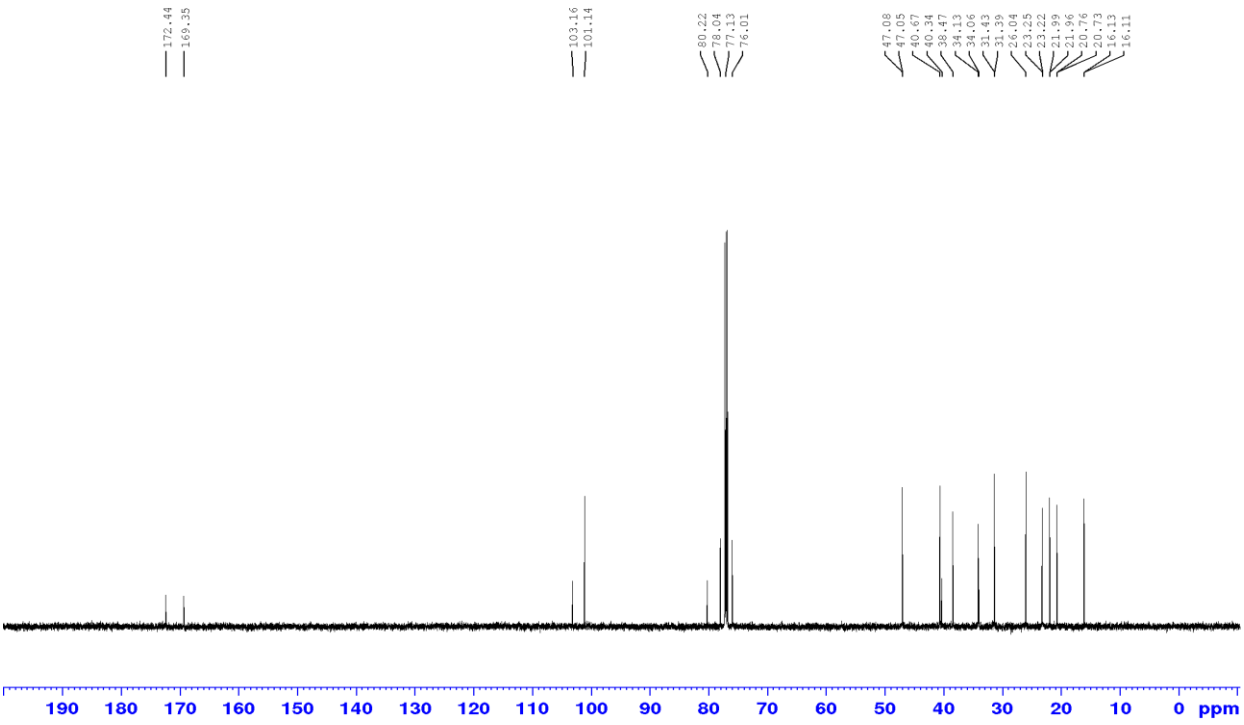
[1] Assay by QNMR. HPLC showed high assay yield 148.5. (erroneous sample prep)

Product Characterization

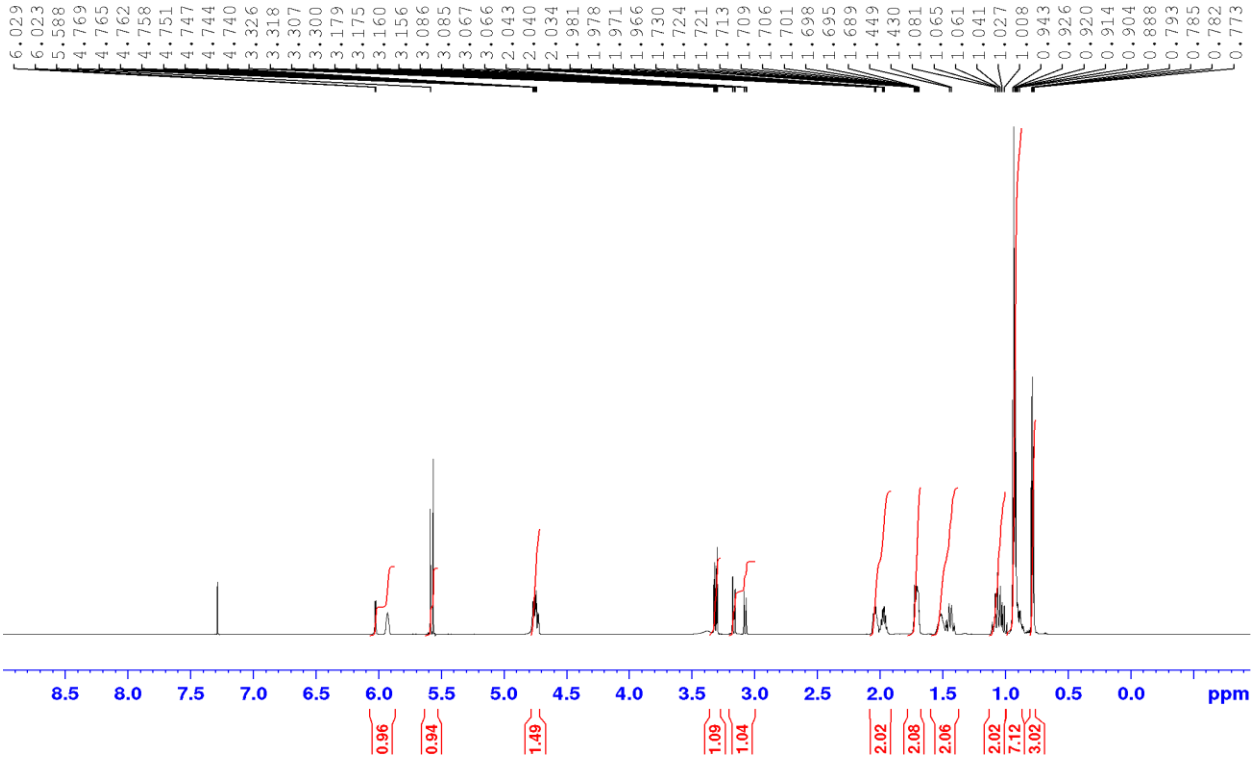
^{1}H NMR of compound 1 in $CDCl_3$ (600 MHz):



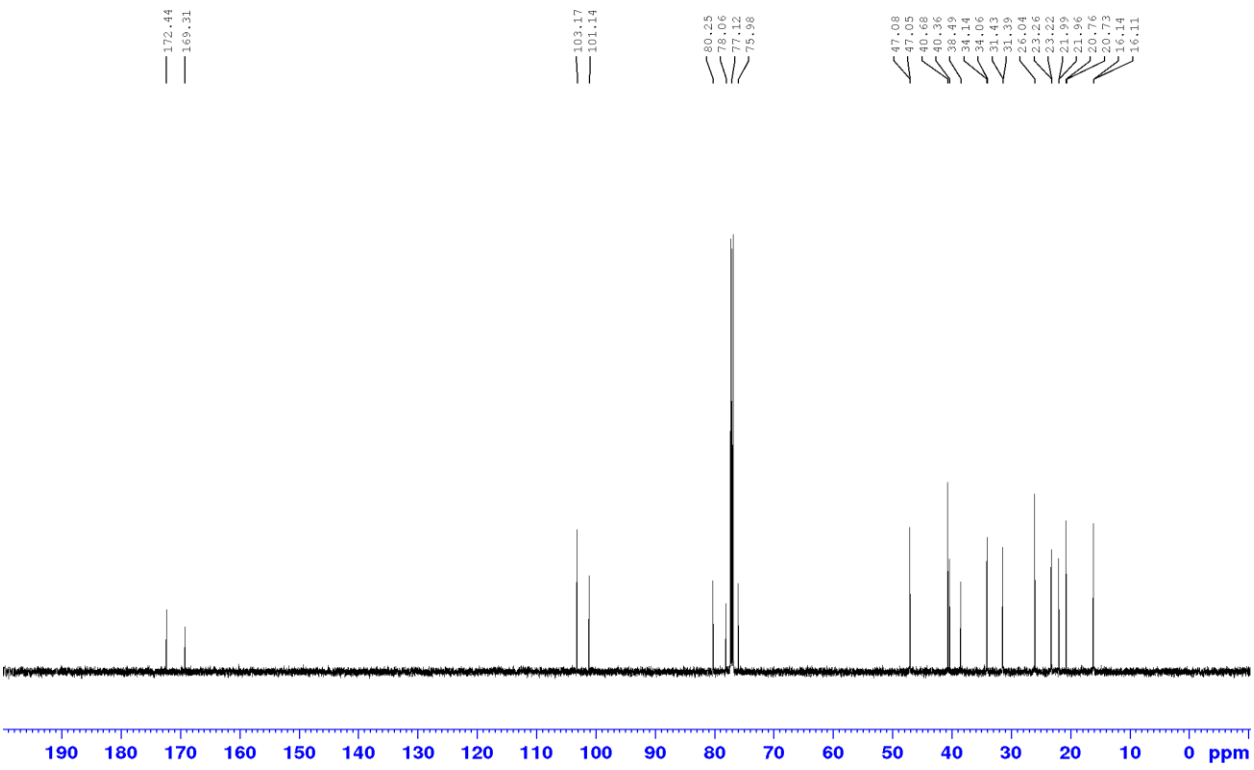
^{13}C NMR of compound 1 in $CDCl_3$ (150 MHz):



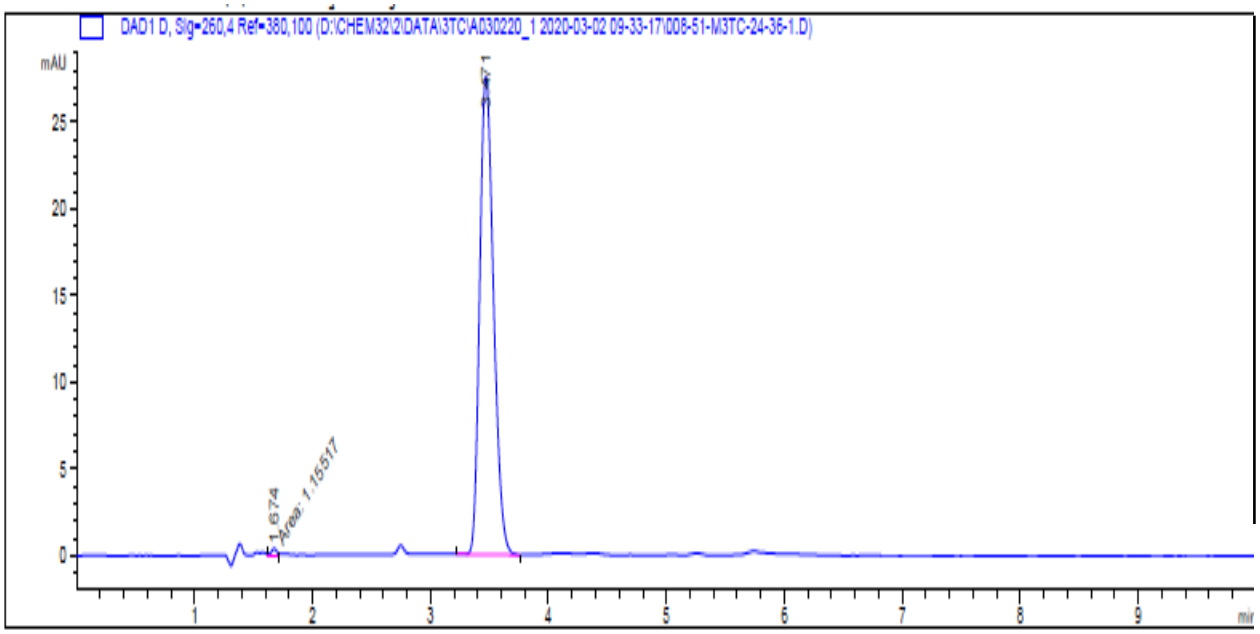
^{1}H NMR of compound 1 (Ambeed, Cat. No. A238462, Lot. No. A238462-001) in $CDCl_3$ (600 MHz):



^{13}C NMR of compound 1 (Ambeed, Cat. No. A238462, Lot. No. A238462-001) in $CDCl_3$ (150 MHz):

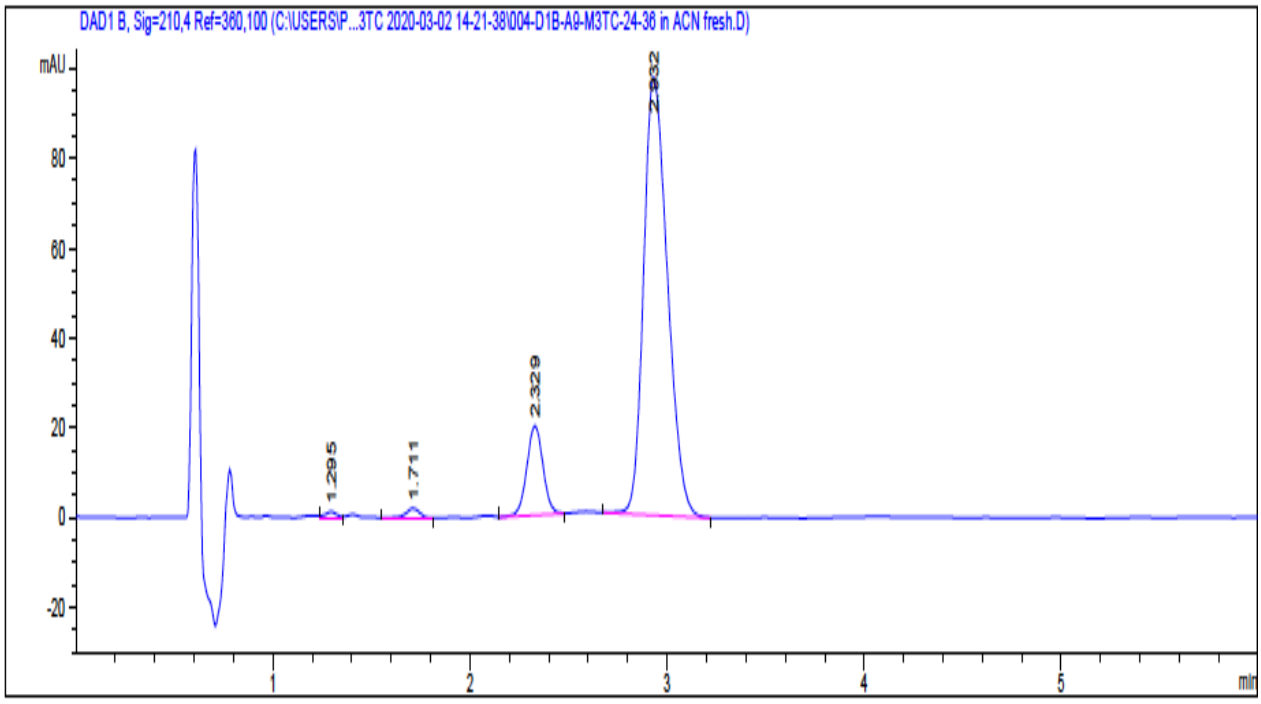

172.44
169.31
103.17
101.14
80.25
78.06
77.12
75.98
47.08
47.05
40.68
40.36
38.49
34.14
34.06
31.43
31.39
26.04
23.26
23.22
21.99
21.96
20.76
20.73
16.14
16.11
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

HPLC of Compound 1 (see Analytical section for method description)



The peak at 2.7 minutes corresponds to toluene. This method may be used to monitor drying and assay toluene level in the isolated material.

Chiral SFC of Compound 1 (see Analytical section for method description)



The peak at 2.9 minutes corresponds to the target isomer. The other peaks correspond to stereoisomers of the oxathiolane ring. Compound **1** epimerizes both in solution and in the solid state so results of this

analysis can be confounded. The subsequent synthetic step pulls other stereoisomers to the correct form so this result is for information only.

Conclusions

Oxathiolane **1** was made from low cost commodity feedstocks including thioglycolic acid, sulfuryl chloride and vinyl acetate. This product is a key raw material used in the production of lamivudine (3TC) and emtricitabine (FTC). The current market prices of **1** and 3TC are $34/kg and $140/kg, respectively. With the new route, the cost of goods for **1** is estimated to be $17-21/kg. Incorporating a conservative $10/kg conversion cost brings the total estimated price for compound **1** to $27, a projected 20% decline over market price. This is projected to enable a 14% decline in the cost of 3TC.

References

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Analytical Methods

GC-MS Method to Monitor Step 1 Conversion

GC-MS analysis was performed on the reaction mixture at the completion of step 1 to ensure conversion of menthol to intermediate **6**. Prior to GC-MS analysis 10 µL of reaction mixture was dissolved in 1 mL of toluene. The peak area for any observed menthol was compared to intermediate **6** peak area to ensure that < 3% menthol remained.

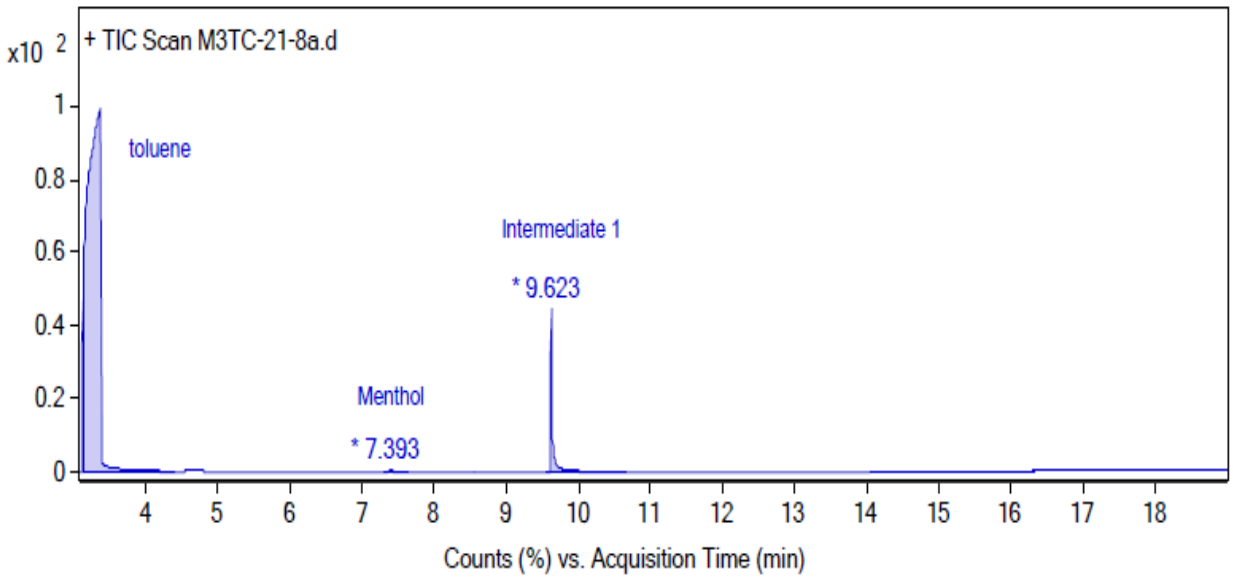
Table 2. GC-MS method for monitoring the conversion of Menthol to Intermediate **6**

GC Parameters	
Oven Temperature 1	50 ˚C
Hold Time 1	3 min
Ramp Rate 1	25 ˚C/min
Oven Temperature 2	250 ˚C
Hold Time 2	3 min
Ramp Rate 2	25 ˚C/min
Oven Temperature 3	300 ˚C
Hold Time 3	3 min
Injection Port Temperature	325 ˚C
Solvent Delay	0 min
Split Ratio	50:1
Split Flow	60 mL/min
Column	HP-5MS 30 m X 0.25 mm, 0.25 µm
Runtime	19 min
Injection Volume	0.5 µL
MS Parameters	
Transfer Line Temperature	250 ˚C
Source Temp	230 ˚C
Quad Temp	150 ˚C
Electron Energy	70 eV

The method separates solvent (acetonitrile and toluene), menthol and intermediate **6** (Figure 4).

Intermediate **6**

Figure 4. GC-MS Chromatogram for Batch 21, Step 1, conversion of menthol to Intermediate **6**



HPLC Method

Conversions of intermediate **6** to intermediate **7** and intermediate **7** to compound **1** were monitored using LC-DAD. Intermediate **7** was received as a clear oil while compound **1** was received as a white powder. Samples were dissolved in LC-UV grade or higher acetonitrile prior to analysis. Weight assays were performed on the oils/solids for intermediate **7** and compound **1**. Residual toluene in compound **1** was also determined using this method. Figures 5-7 show example chromatograms from batch 21 for intermediate **7** and compound **1**. The acquisition method is described in Table 3. The use of a subambient column temperature is required to minimize on-column equilibration and resulting peak distortion of Compound **1**.

Table 3. HPLC-DAD isocratic method for monitoring the conversion of Intermediate **6** to Intermediate **7** and Intermediate **7** to Compound **1**.

Mobile Phase A	15%: 2.5 mM Ammonium Formate in 18 MΩ Water, pH 4.12
Mobile Phase B	85%: Acetonitrile
Flowrate	1.5 mL/min
Column Compartment Temperature	10 ◦C
Column	Zorbax Eclipse XDB-C18 4.6 x 250 mm, 5 µm
Runtime	10 min
Injection Volume	10 µL
Monitored Wavelengths (nm)	210, 260

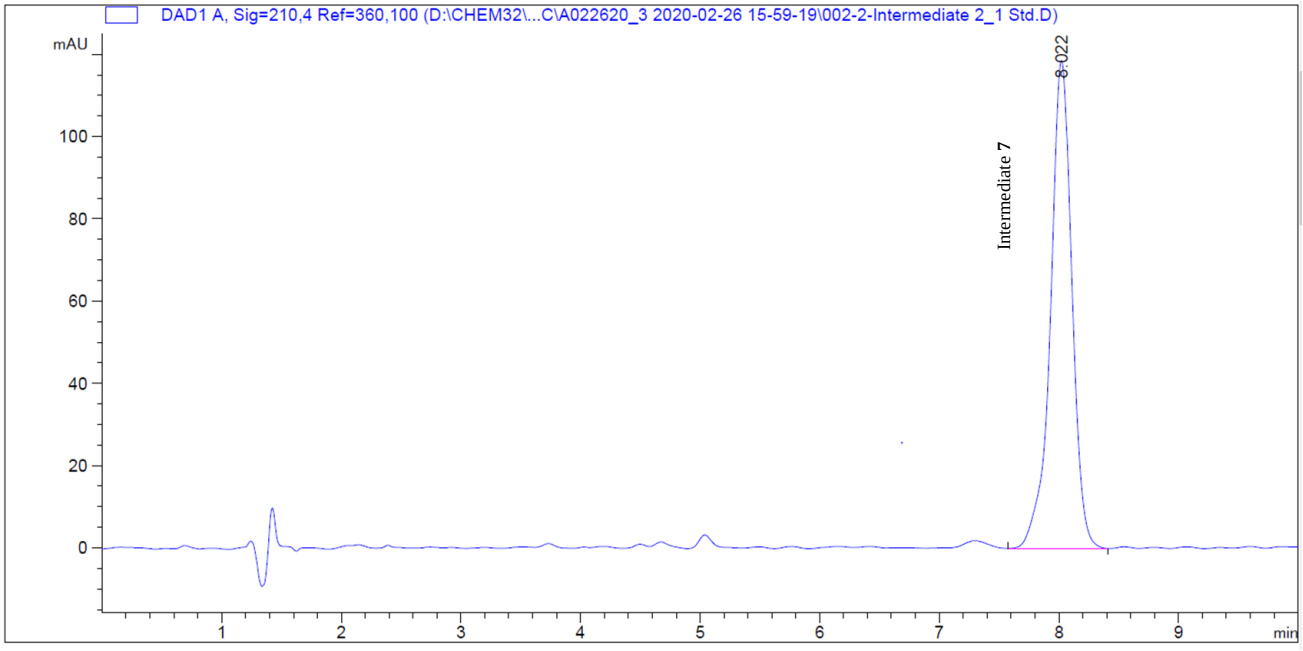
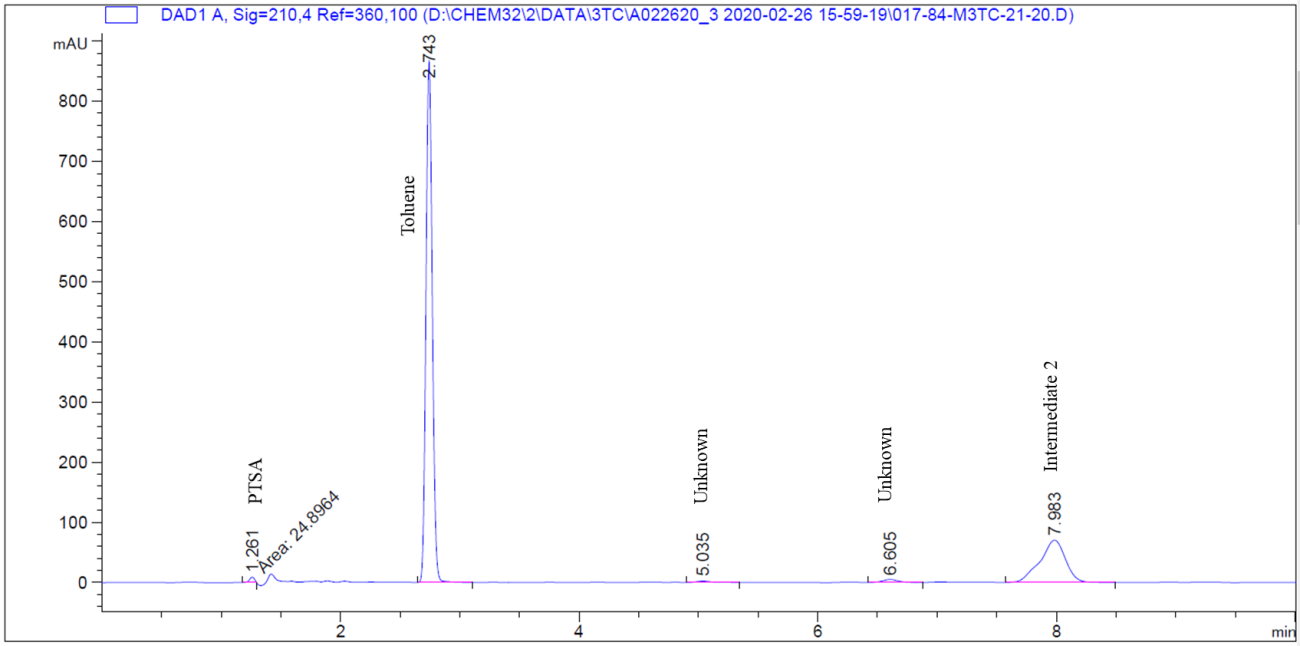


Figure 5. HPLC-DAD Chromatogram for Intermediate **7** standard

Intermediate 7

Figure 6. LC-DAD Chromatogram for Batch 21, Step 2 – Formation of Intermediate **7**.



Compound 1

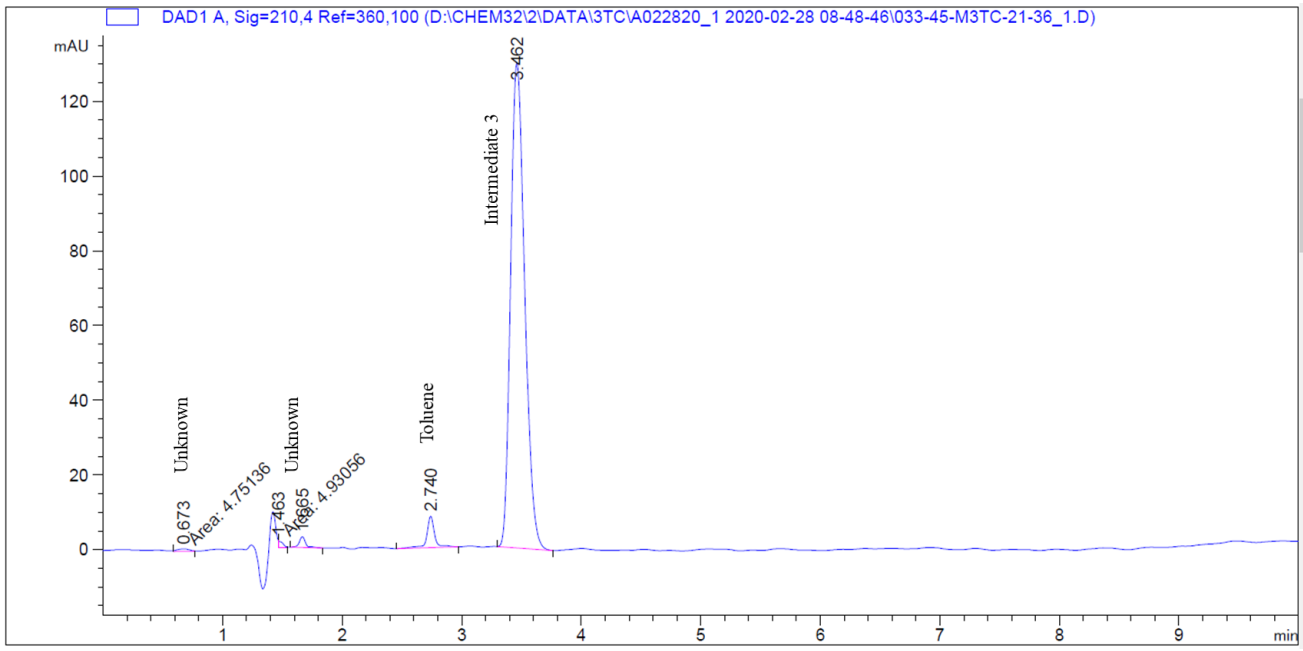


Figure 7. LC-DAD Chromatogram for Batch 21, Step 3.

Chiral Analysis

Chiral analysis was accomplished using the chiral SFC method described below. All four stereoisomers are present in M4All and commercially-prepared samples. The peak at 2.9 minutes corresponds to the target stereoisomer. Compound **1** epimerizes both in solution and in the solid state so results of this analysis should not be considered critical. The subsequent synthetic step pulls other stereoisomers to the correct form so this result is for information only.

Table 4. SFC method for chiral analysis.

Mobile Phase A	80%: CO_2
Mobile Phase B	20%: EtOH
Flowrate	2 mL/min
Column	ChiralPak IG 4.6 x 100 mm, 5 µm
Runtime	15 min
Injection Volume	10 µL
Monitored Wavelengths (nm)	210, 260

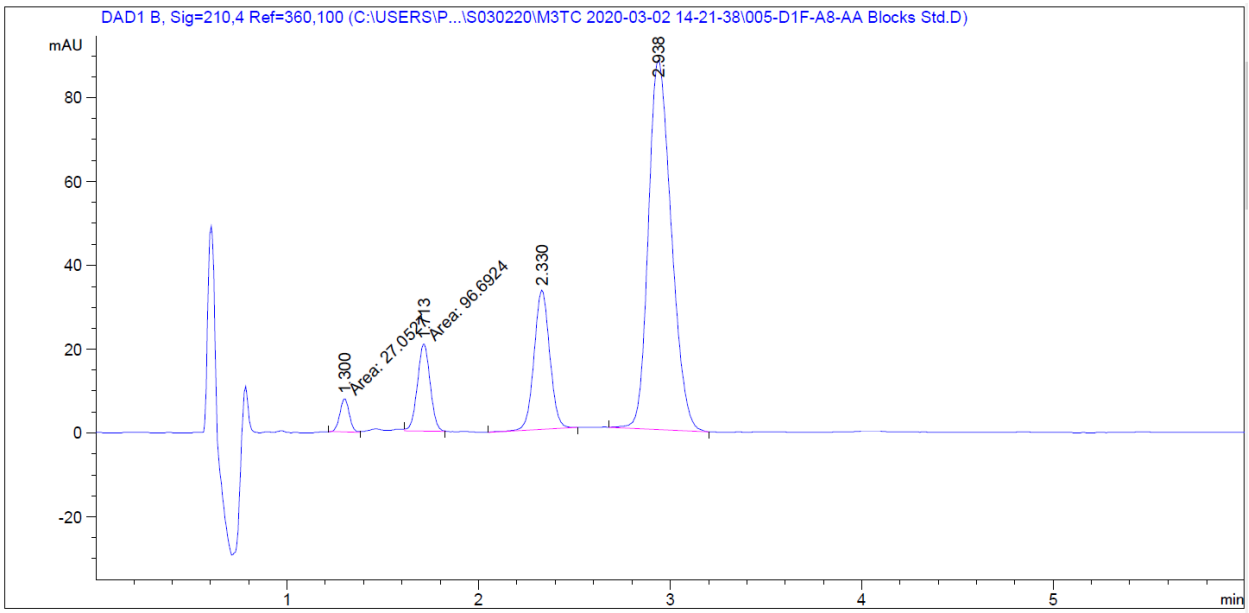


Figure 8. SFC Chromatogram for Compound **1** standard, AA Blocks

Economic Analysis

The economic analysis of (1R,2S,5R)-2-isopropyl-5-methylcyclohexyl 5-hydroxy-1,3-oxathiolane-2-carboxylate produced using the reported process is shown in the table below. Chemical input costs are based on the amount of material required to produce 1 kg of product. The analysis starts with 1 mole of starting material along with the stoichiometric ratios of other materials, factors in the percent yield obtained and the cost per kilogram of materials. This generates an overall raw material cost which is converted back to a per kilogram basis. In the example below quantities are actual amounts used in Batch 3 of the reproducibility study, along with the observed isolated corrected yield. An 85% solvent recycle is assumed along with a 60% recycle of menthol. This is conservative based on private communications. Conversion costs can vary between manufacturing facilities so a rough approximation of $10/kg may be added. This cost is a reasonable assumption for processes run at the expected 50MT/year scale. This brings the total estimate to $27/kg. A variance of $4/kg can be estimated by comparing the range between worst- and best-case scenarios. The current market price for this intermediate is $34/kg, based on one manufacturer. This price may not be consistent across manufacturers and it is further complicated by the fact that this manufacturer recycles menthol. We expect that manufacturers will perform their own cost analysis to assess the benefits of our chemistry in their facility.

Raw materials	Mol. Wt.	Amount	Amount kg	Equivalent	RM Cost	RM Cost	RM Cost	kg RM
		gram	/ kg Product		$ / Kg	$ / batch	$ / kg Product	
Thioglycolic acid	92.11	6.19	0.63	1.05	5.00	30.94	3.16	6.19
L-menthol	156.27	10.00	1.02	1	4.00	40.00	4.09	10.00
p-Toluenesulfonic acid monohydrate	190.213	0.24	0.02	0.02	0.92	0.22	0.02	0.24
Sulfuryl Chloride (SO2Cl2)	134.97	19.00	1.94	2.2	0.91	17.29	1.77	19.00
Vinyl acetate	86.1	11.02	1.13	2.0	1.17	12.89	1.32	11.02
Toluene	92.14	6.50	0.66	5 V	0.60	3.90	0.40	6.50
Acetonitrile(CH3CN)	41.05	47.16	4.82	40 V	0.97	45.75	4.68	47.16
Water	18	400.00	40.89	40 V	0.01	4.00	0.41	400.00
NaHCO3 (1M solution)	84	10.75	1.10	2.0	0.28	3.01	0.31	10.75
Water for NaHCO3 solution	100.2	117.23	11.99		0.01	1.17	0.12	117.23
Triethylamine 1% in hexanes	100.2	0.44	0.04		1.41	0.61	0.06	0.44
hexanes	101.19	6.53	0.67	6 V	0.80	5.23	0.53	6.53
Step 2 product: L-menthyl-5R-hydroxyl-1,3-oxathiolanes-2R-carboxylate								
53%	288.41	9.78	1.00			165.02	16.87	635.07
		NOTE:	These amounts are actually in grams per the Medicines for All batch record				$ / kg	
Overall yield	53.00%							
							16.87	
Solvent Recycle	85%							

			A%
Yield:	Isolated (As is)	9.85	99.3
Yield:	Corrected (with A%)	9.78	

Using a 50% yield in place of 53% increases the cost by $1. The process described in this document has not been optimized and it is reasonable to expect final production costs to actually be lower than predicted by this model. Current solvent volumes are quite high at this scale which should be reduceable in scale-up development. Without solvent recycle the cost model predicts $48/kg which would likely not be competitive with current syntheses.

Appendix

Sensitivity Assessment

Failure modes for each stage of the reaction were examined and critical parameters were identified (Figure 9). Each item will be discussed in more detail below.

Esterification:

- Removal of water
- Reaction time

Chlorination:

- Headspace control
- Operating temperature
- Quantity of sulfuryl chloride
- SO_2Cl_2 and vinyl acetate mode of addition

Ring Closing:

- pH adjustment
- Heat history of reaction mass
- ACN/water ratio

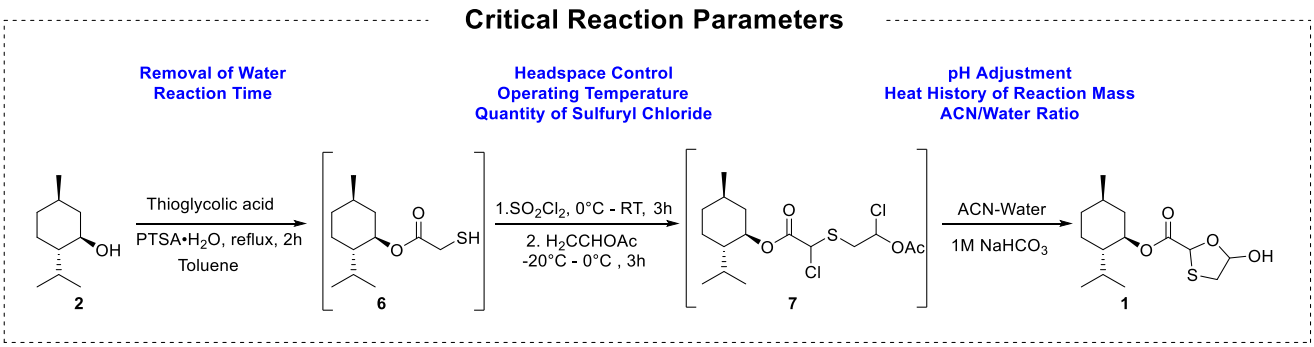


Figure 9. Critical reaction parameters

Esterification:

Esterification was robust with high yields obtained regularly; however, it is important to recognize several points which can impact downstream steps.

1) Full removal of water is essential. Chlorination of the menthyl ester with SO_2Cl_2 will fail if water is not adequately eliminated through reflux/distillation. On occasion, water was captured in cold spots in the reactor rather than the Dean-Stark trap. Subsequently, the water reentered the system. While esterification went to completion, predictably there were problems with chlorination. Presence of water can be difficult to detect by Karl-Fisher due to phase separation with toluene.

Water entering system via reactor cold-spot

2) Time of reaction impacts degree of esterification. Increasing quantity of acid catalyst and thioglycolic acid will accelerate conversion. Use of lesser quantities will increase time

required for reaction to go to completion. We observed that 0.5 volume of solvent is sufficient for reaction completion. We synthesized up to 1kg of compound with quantitative yields using 0.5 vol of solvent; however, 5 volumes of solvent was chosen based on integration with subsequent steps.

Entry	Thioglycolic acid	PTSA.H_2O equiv	Toluene (vol)	Time	Yield (%)
1.	1.01	0.01	5	5h	87
2.	1.01	0.01	5	10h	93
3.	1.03	0.01	5	5h	90
4.	1.05	0.01	5	5h	94
5.	1.05	0.02	5	2h	97-99
6.	1.05	0.08	5	1	96

Table 5: Impact of Thioglycolic acid, PTSA·H_2O, and time on formation of menthyl ester (**6**).

Chlorination:

Controlling degree of chlorination is critical to reaction success, and several key parameters were identified. Lack of control over these factors results in either under or over chlorination of substrate. It is important to maintain control over:

1) Quantity of sulfuryl chloride addition
2) Reaction headspace (static)
3) Operating temperature
4) SO_2Cl_2 and vinyl acetate mode of addition

Data below shows impact of these variables. Other parameters impact the reaction but were less likely to be sources of deviation from ideal results.

Sulfuryl chloride: Impact of sulfuryl chloride equivalents was screened (2.0, 2.1, 2.2, 3.0 and 4), and it was found that 2.2 equivalents are required for completion of reaction. At lesser equivalents (2.0 and 2.1) monochlorinated product was observed (Figure 10). At 3 and 4 equivalents trichlorinated compound was observed (Figure 11).

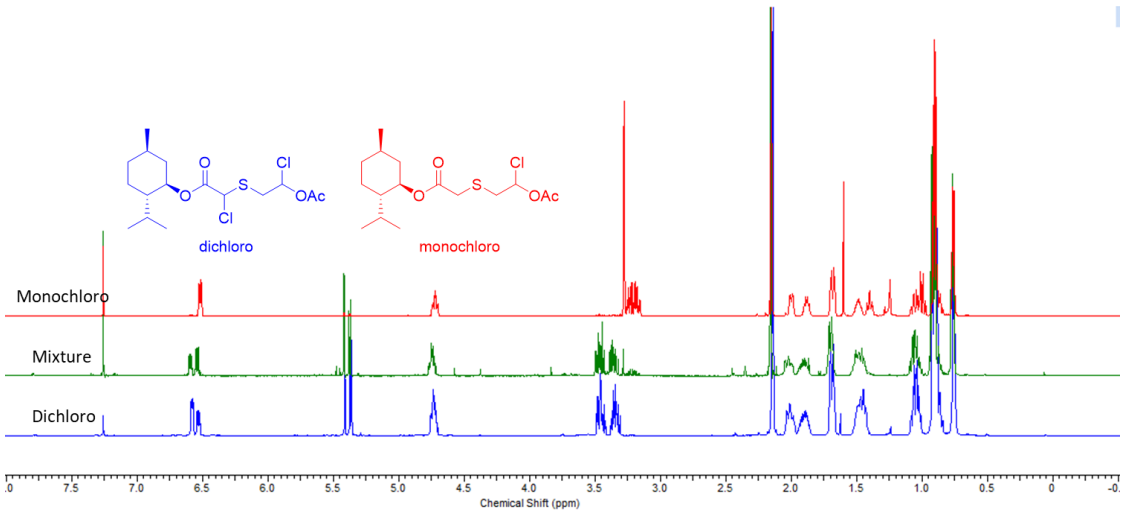


Figure 10: Monochloro compound formation at 2.0 and 2.1 equiv of sulfuryl chloride

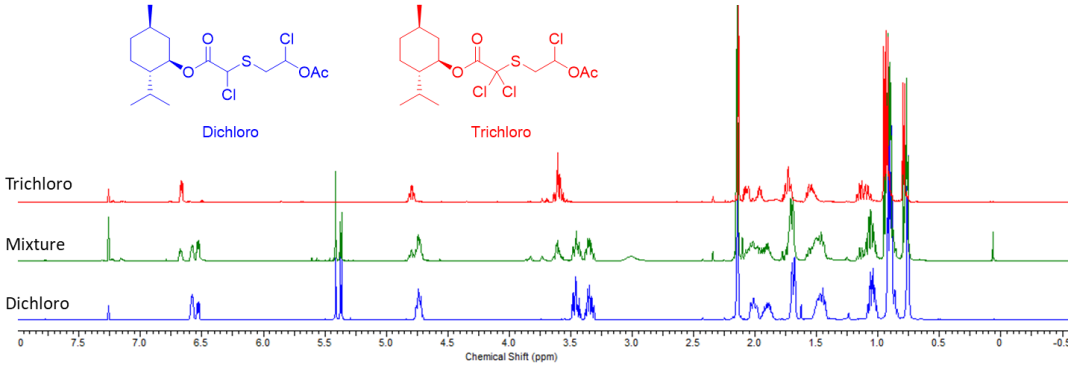
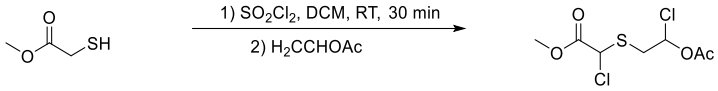


Figure 11: Trichloro compound formation with excess of sulfuryl chloride

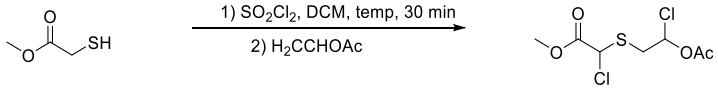
Maintaining a static headspace: Initial scoping with methyl thioglycolate demonstrated the importance of a sealed vessel with contained headspace. Light pressure is generated in the course of the reaction, and keeping the gaseous species in the reaction system

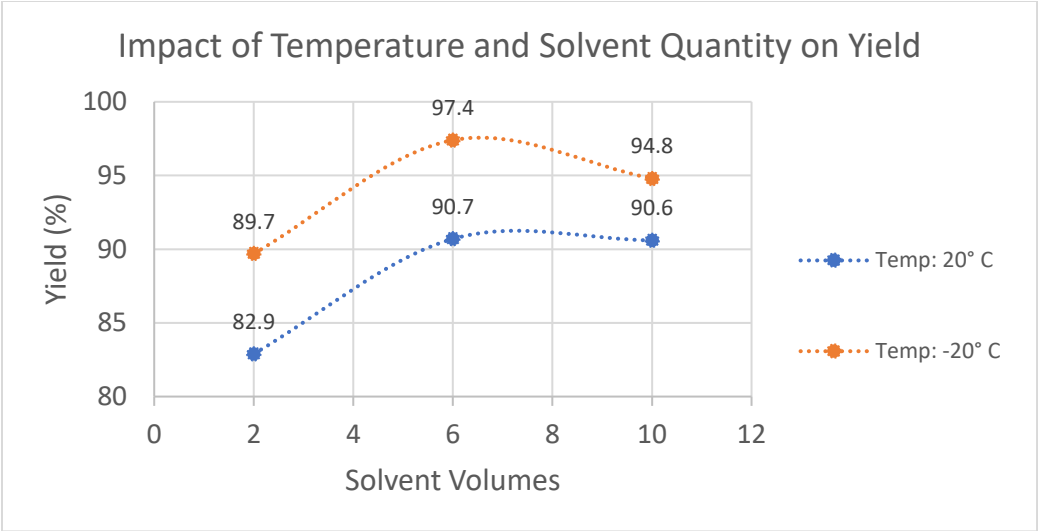
improves yield. Typically this was accomplished by either sealing the vessel or use of a balloon to maintain atmospheric pressure while containing gases in the headspace. Cooling the reaction to -20 °C also increased solvation of gases and increased yield.



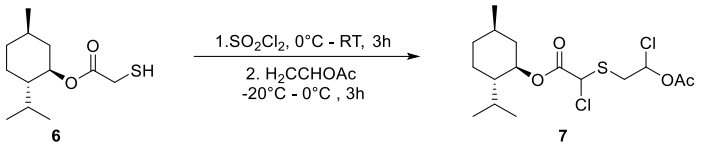
Entry	Reaction Setup	Yield
1	Vial open to atmosphere	76%
2	Sealed tube	91%

Operating Temperature: Initial exploration was again performed with methyl thioglycolate, this time demonstrating impact of reaction temperature and volume on yield. Deviating from set temperature and volume can be expected to decrease yield. Colder temperatures were preferred to warm temperature, and running at too high of a concentration can be expected to decrease performance as well. This can likely be explained in light of need to maintain a static headspace. Here warming will decrease solubility of gases in solution, and low solvent volumes will exacerbate effects of an exothermic reaction.





Vinyl acetate equivalents: The need for an excess of vinyl acetate was articulated in latter screens with the menthyl ester. With 2 equivalents of vinyl acetate, the reaction was most robust. A byproduct of the reaction is chloroethyl acetate, produced in the reaction of HCl with the olefin. For this reason, an excess of vinyl acetate is required.



Entry	Vinyl Acetate (Equiv.)	Yield
1	1.3	84%
2	1.5	88%
3	1.8	94%
4	2.0	96%

Solvent: Toluene was selected for the reaction considering its usage in the first step, menthol esterification. Fortunately, it was compatible with chlorination as well. The chlorination was also performed in acetonitrile, chloroform, dichloromethane, and acetone, but acetonitrile and acetone did not lead to formation of the desired product.

SO_2Cl_2 and vinyl acetate mode of addition: Chlorination results were most consistent when reagents were added at steady rate by syringe pump. Slight changes in impurities were observed if reagents were added manually, and in the next step these batches occasionally suffered from phase separation of the chlorinated intermediate and reaction medium. When the intermediate separated from the acetonitrile/water mix, conversion slowed dramatically. Use of a syringe pump prevented these issues related to starting material agglomeration.

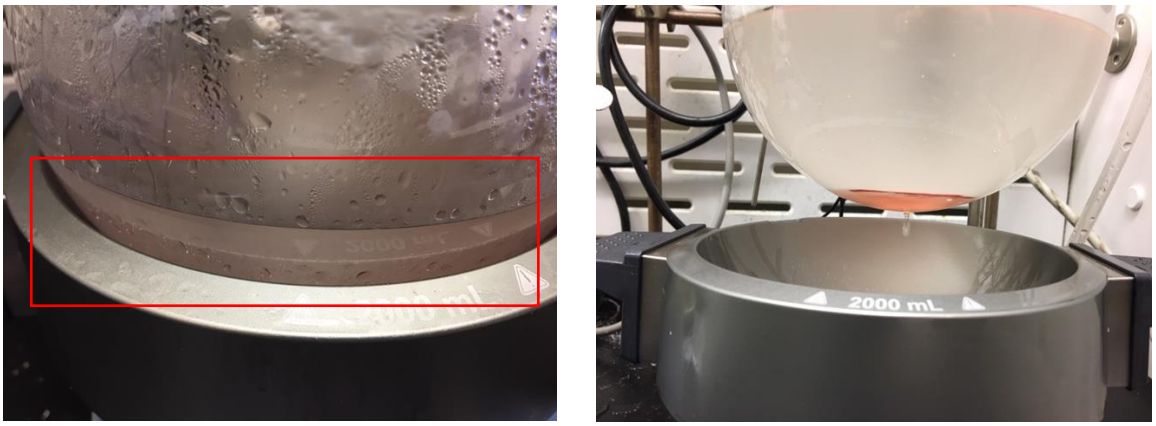


Figure 12: Phase separation during oxathiolane formation.

Figure 13: Dispersion of dichloroacetate in acetonitrile/water solvent system.

Ring-Closing:

Oxathiolane formation was dependent on presence of acid, time of heating, and bulk solvent properties. As such is important to monitor pH and reaction progress and to maintain the desired ratio of acetonitrile to water.

Dependence on acid: Acid accelerates the ring-closing sequence, presumably by initiating trans-esterification. This was observed in reaction of methanol and chlorinated intermediate. Reaction progress is slow to commence when acid is not present in the system; however, by including one equivalent of HCl, product is converted much more quickly. For this reason, acid is not fully neutralized after chlorination. pH is checked before heating the acetonitrile/water mixture and should be between 1.8 and 3.0 when sample is diluted 10-fold.

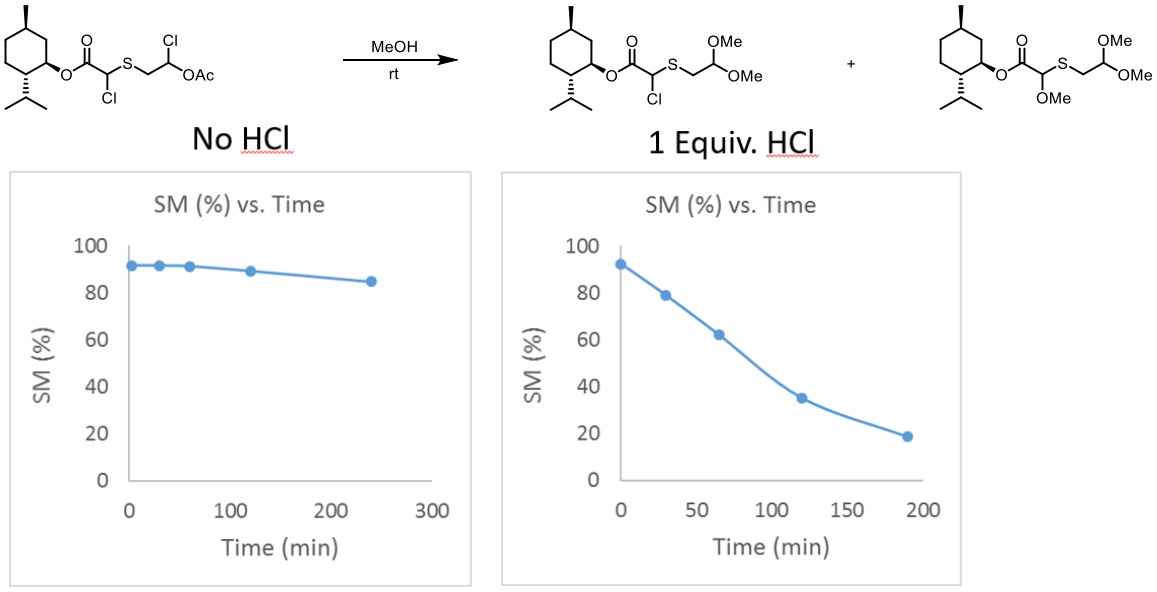


Figure 14: Conversion of SM dependent on presence of HCl.

Time dependence: Oxathiolane yield is dependent on time of reaction. Yield crests and then declines in the absence of base. Addition of base during the reaction slows hydrolysis of the menthol ester, the primary route of yield erosion.

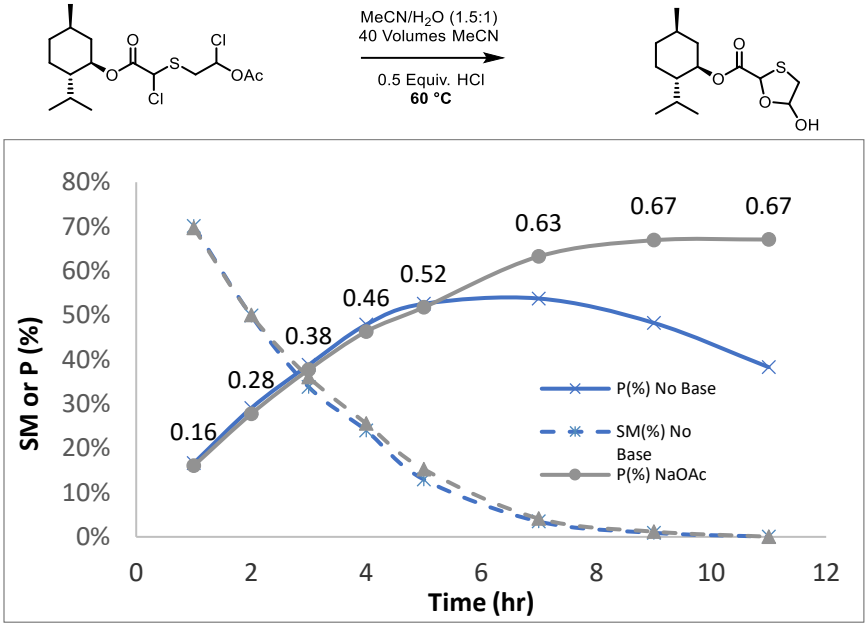
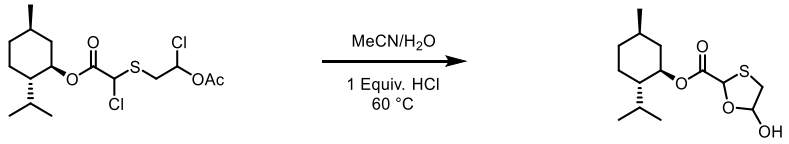


Figure 15: Excess heat history will lead to product decomposition in the absence of base (NaOAc).

Cosolvent ratio: The cosolvent ratio of MeCN to water impacts mass balance of the reaction. A deficiency of acetonitrile will lead to lower selectivity for product. Acetonitrile was observed to boil off the reaction in the case of certain equipment failure (corroded o-ring). As a result of MeCN loss, yield decreased.



Vol	MeCN/H_2O	Acid Equiv.	SM (%)	P (%)	SM+P	P/(Conv.)	P/SM
20	0.5	0.33	71	8.2	79.2	0.28	0.12
20	0.5	1	73.1	8.8	81.9	0.33	0.12
20	1.5	0.33	39.2	33.8	73	0.56	0.86
20	1.5	1	39.7	30.5	70.2	0.51	0.77
40	0.5	0.33	45	14.6	59.6	0.27	0.32
40	0.5	1	56.3	16.1	72.4	0.37	0.29
40	1.5	0.33	20.5	42	62.5	0.53	2.05
40	1.5	1	21.7	40.2	61.9	0.51	1.85

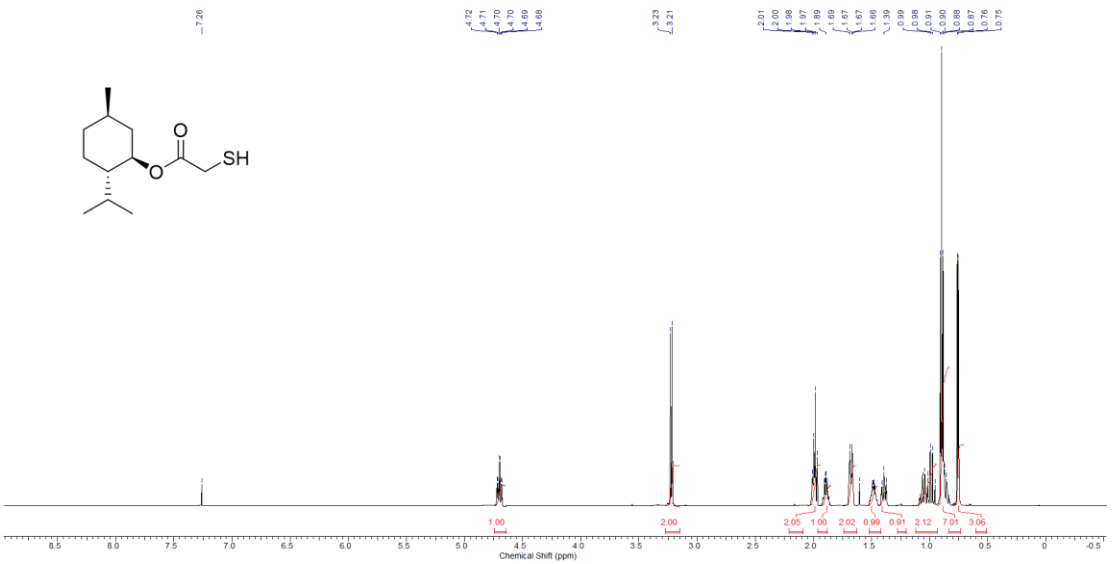
Effect	SM (%)	P (%)	SM+P (%)	P/(Conv.)	P/SM
Vol.	-19.88	7.90	-11.98	0.00	1.13
Acid	3.78	-0.75	3.02	0.02	-0.08
Ratio	-31.08	24.70	-6.37	0.21	1.17

Solvent: Reaction yield was highly impacted by choice of solvent. Various solvent mixtures were explored such as THF-water, acetone-water, dioxane-water, toluene-water, and ACN-water. Best results were obtained in acetonitrile water mixtures.

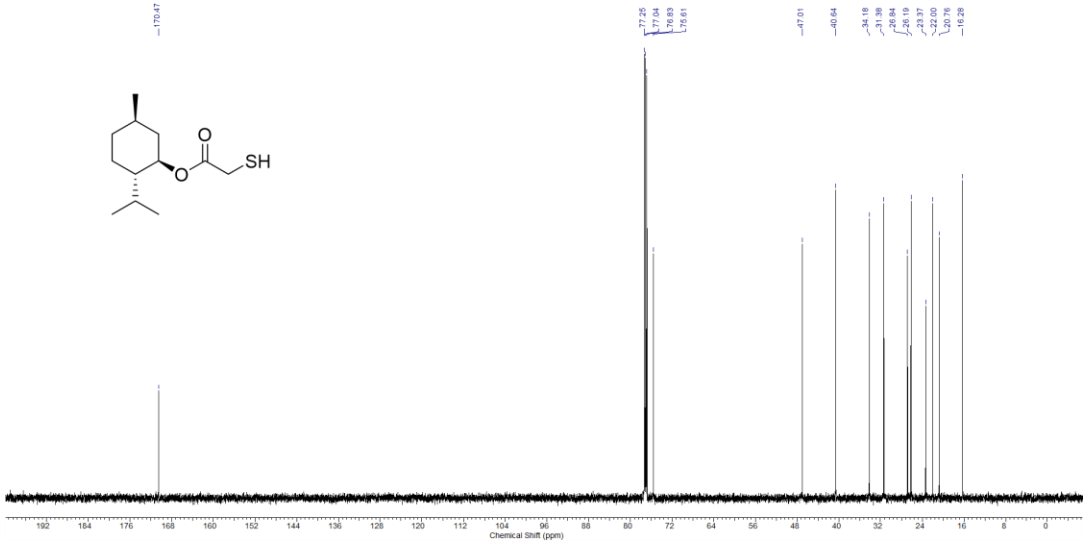
Solvent	Water (vol)	Temp (°C)	Additive	Time	observation
Hexanes 20 vol	20	70	1M $NaHCO_3$	2h	Menthol was observed as major compound. No desired product formation was observed
Dioxane 20 vol	20	70	1M $NaHCO_3$	2h	Menthol was observed as major compound. No desired product formation was observed
THF 20 vol	20	70	1M $NaHCO_3$	2h	Menthol was observed as major compound. No desired product formation was observed
ACN 20 vol	20	70	1M $NaHCO_3$	2.5h	50-55% product

Characterization of Intermediates 6 and 7

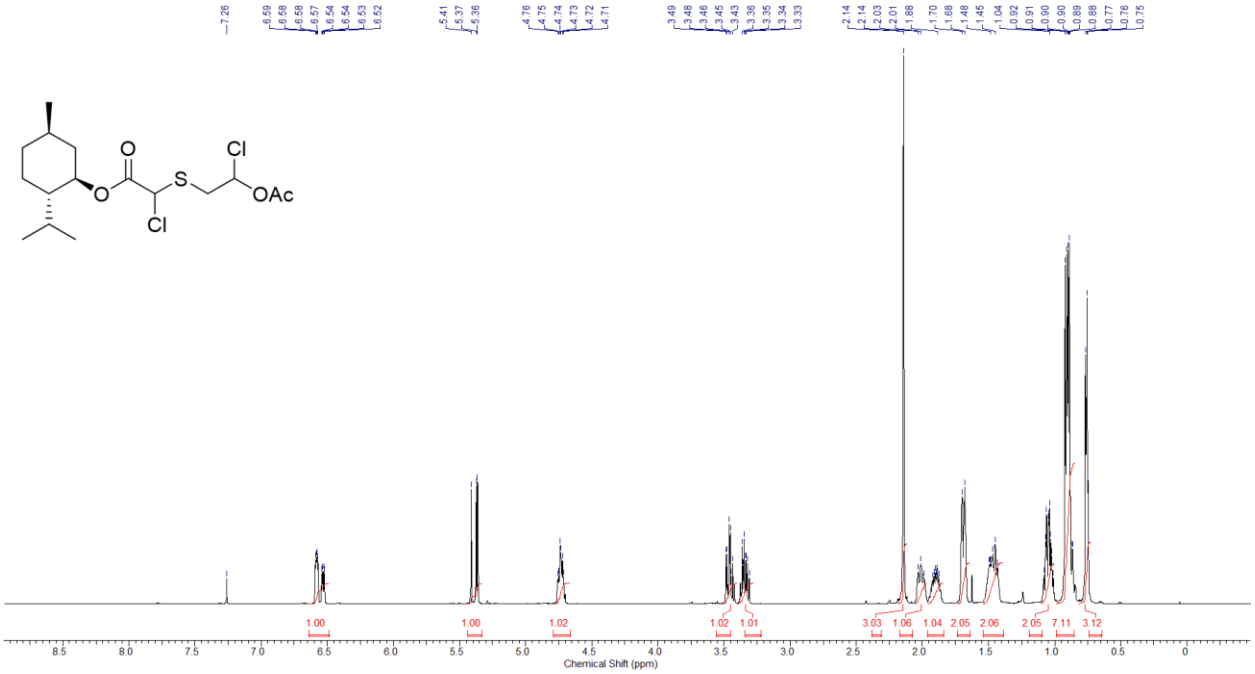
^{1}H NMR of compound 6 in CDCl3 (600 MHz):



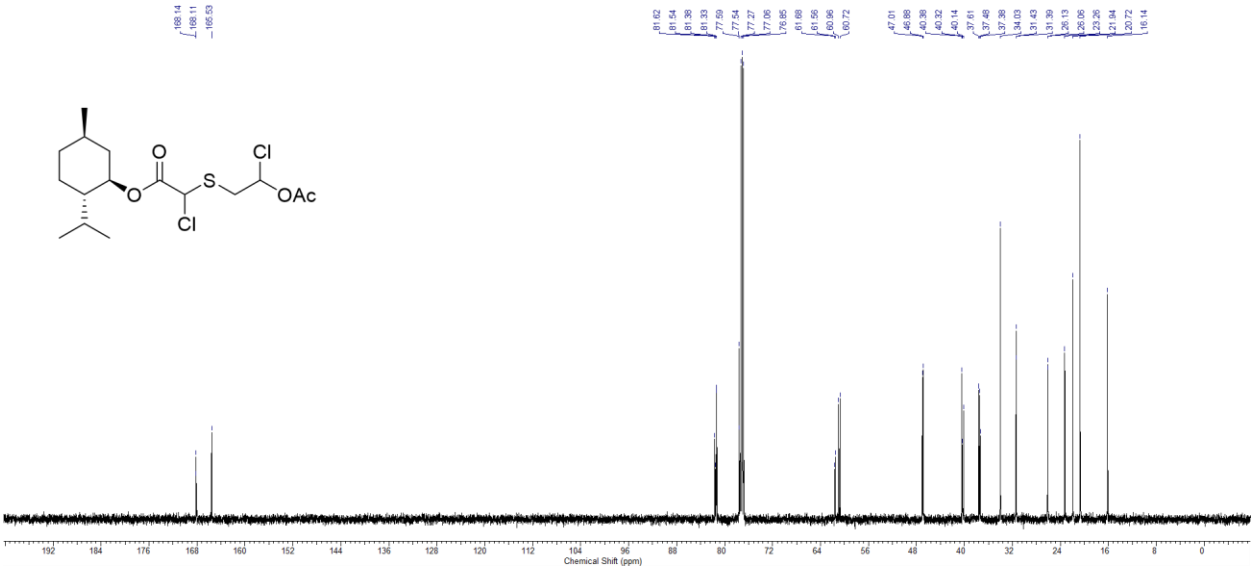
^{13}C NMR of compound 6 in CDCl3 (150 MHz):



^{1}H NMR of compound 7 in CDCl3 (600 MHz):



^{13}C NMR of compound 7 in CDCl3 (150 MHz):



Discussion of Optical Activity:

The product is isolated by crystallization from 1% NEt_3 in hexanes, though recent findings from Lupin indicate that the diastereomeric ratio is not a critical parameter.[4] Further, there is little description in the literature which characterizes optical activity, likely due to rapid epimerization of the substrate when dissolved.

^1H NMR was used to characterize effectiveness of the recrystallization since polarimetry and chiral chromatography did not provide suitable analysis. To further confirm material viability, product was additionally taken forward to produce authentic 3TC.

The thioacetal signal corresponding to the four possible diastereomers of **1** could be well resolved in toluene-d8 (Table 6). Partial resolution of signal was observed in benzene-d6, but $CDCl_3$, DMSO-d6, and MeCN-d3 gave unacceptable resolution.

The four diastereomers are observed prior to recrystallization; however, only two diastereomers are observed afterwards (Figure 16). It is presumed that recrystallization sets the thioacetal stereocenter and that the anomeric hydroxyl group is present in both *R/S* configuration. There appears to be less than 2% of undesired thioacetal epimer (Figure 17). It is worth noting that very rapid epimerization of all stereocenters was observed in DMSO-d6, MeCN-d6, and $CDCl_3$. Degradation of optical purity was even observed in toluene at room temperature over the course of an hour, and was complete overnight. At 40 °C, the epimerization in toluene became quite fast (observed within 10 min, VT-NMR).

Intermediate **1** was carried forward to generate 3TC and thus confirm viability of material. 3TC produced in this method matched commercially available sample (Figures 23 and 24).

Table 6: Analysis of commercial **1** from Combi-Blocks. **1** is a mixture of four diastereomers.

NMR Solvent	Observed # of Diastereomers (^1H NMR)	Observed # of Diastereomers (^{13}C NMR)	^1H NMR Resolved? (Thioacetal)	Ratio of Diastereomers
$CDCl_3$	4	4	No	0.14 : 0.47 : 0.38
DMSO-d6	2 (4*)	2	No	0.49 : 0.51 (0.81 : 0.19)
MeCN-d3	2	3-4	No	0.22 : 0.78
Toluene-d8	4	-	**Yes**	0.16 : 0.20 : 0.28 : 0.34
Benzene-d6	4	-	Partial	0.15: 0.24 : 0.25 : 0.36

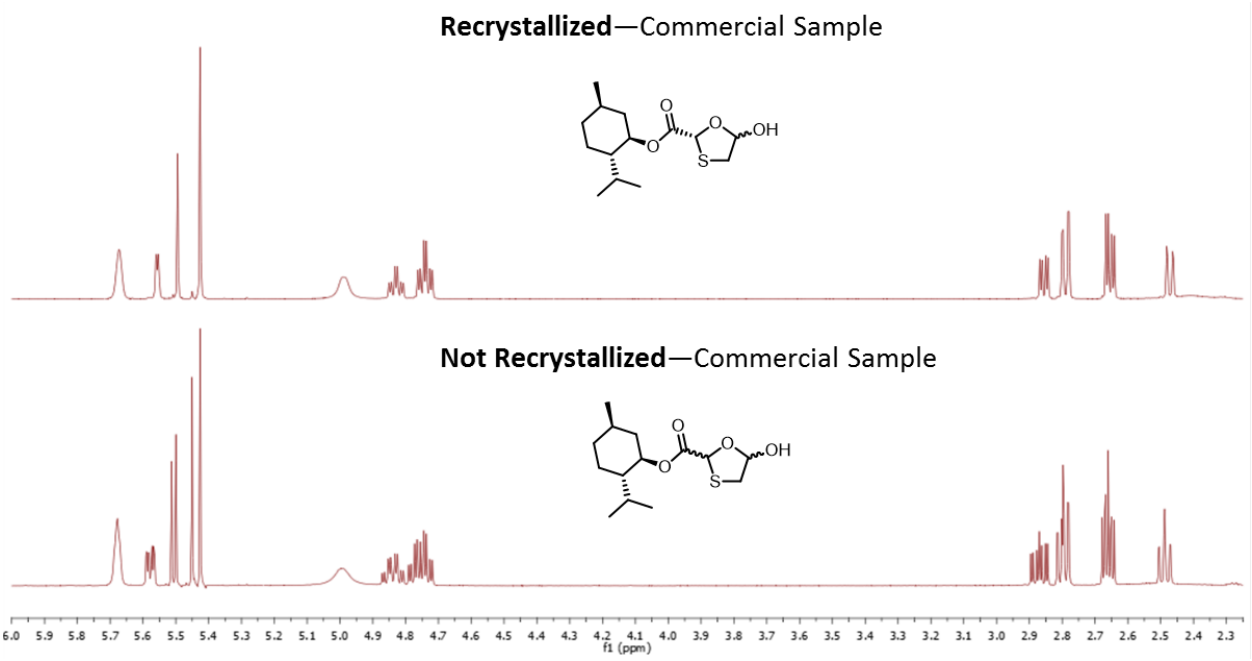


Figure 16: Recrystallization of commercial **1** demonstrating removal of two diastereomers. ^{1}H NMR taken in toluene-d8.

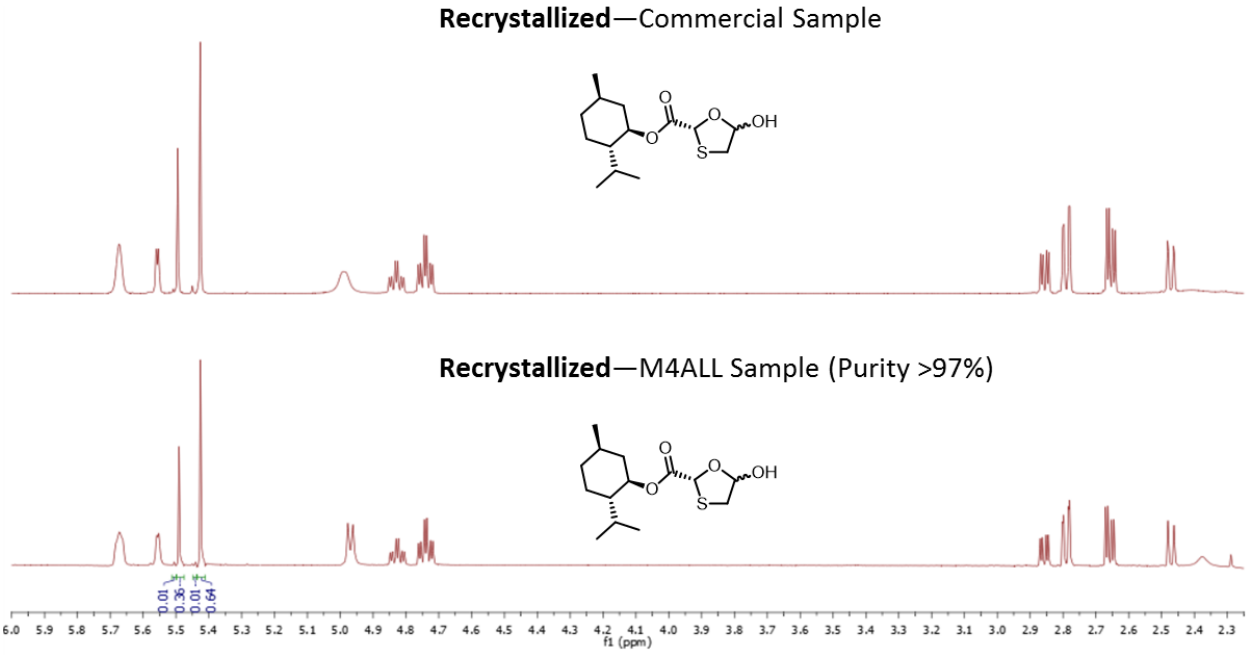


Figure 17: Comparison of **1** from M4ALL process and recrystallized commercial sample. Less than 2% of undesired isomers.

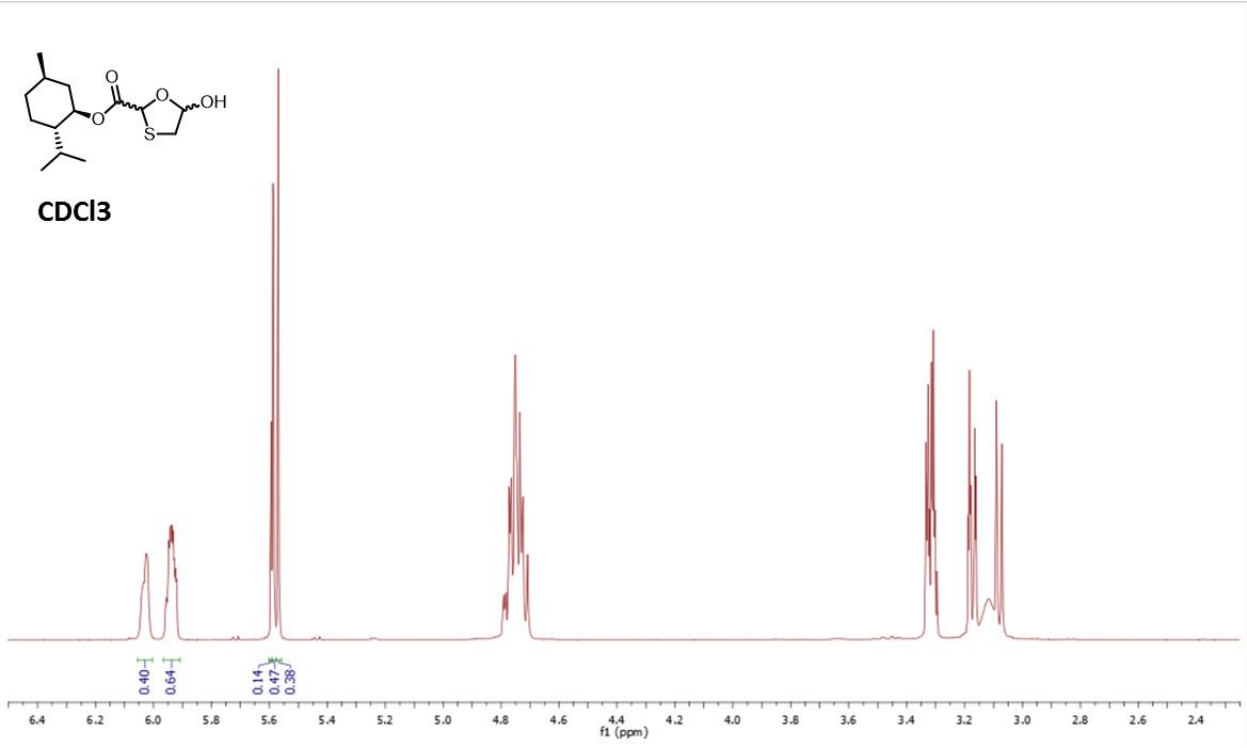


Figure 18: Mixture of four diastereomers in CDCl3.

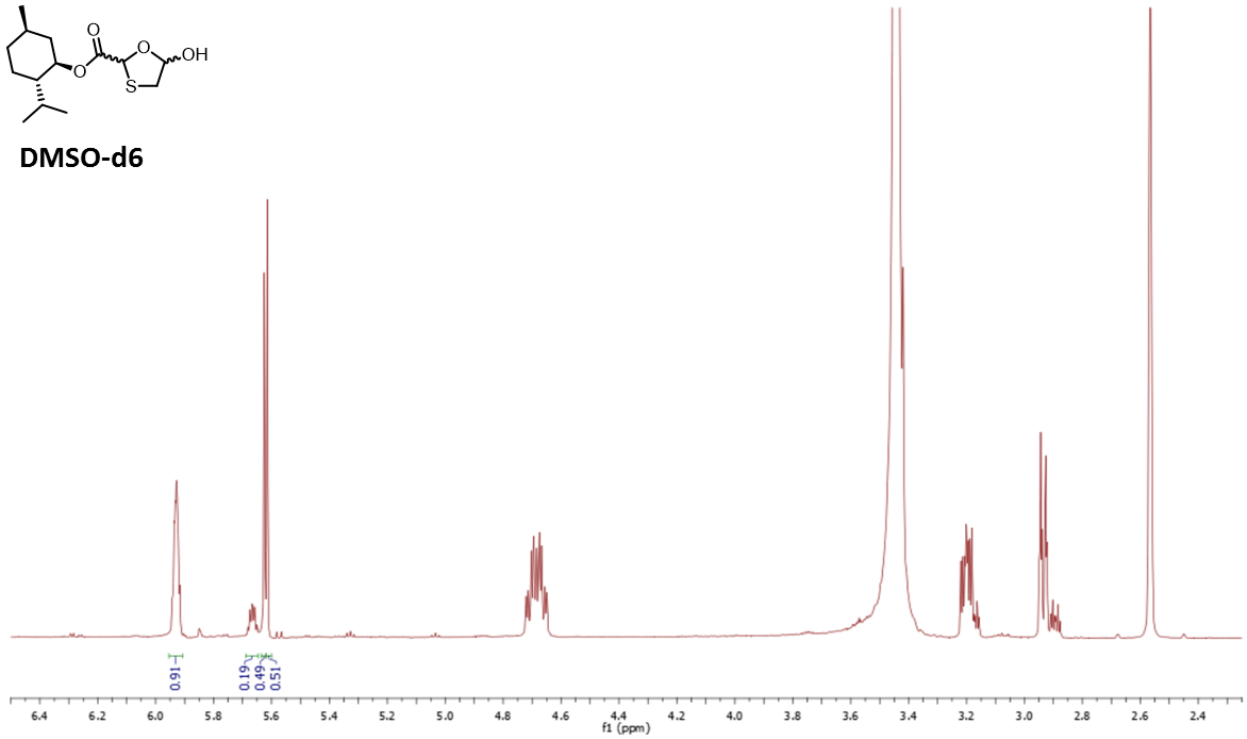


Figure 19: Mixture of four diastereomers in DMSO-d6.

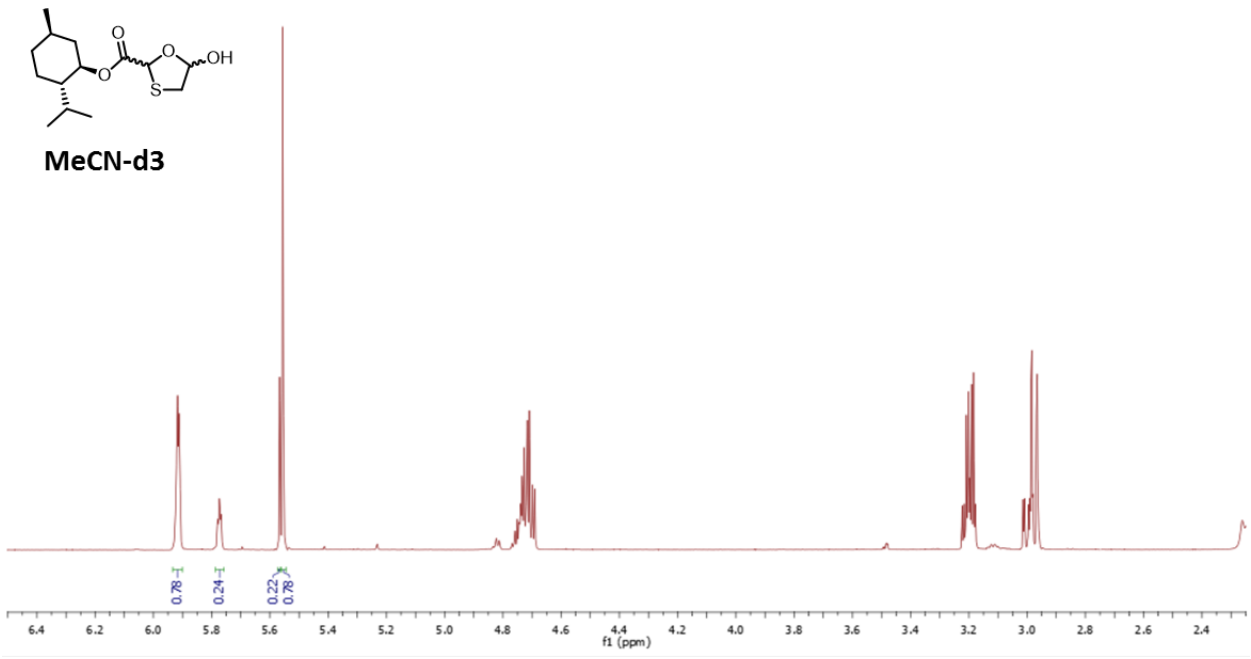


Figure 20: Mixture of four diastereomers in MeCN-d3.

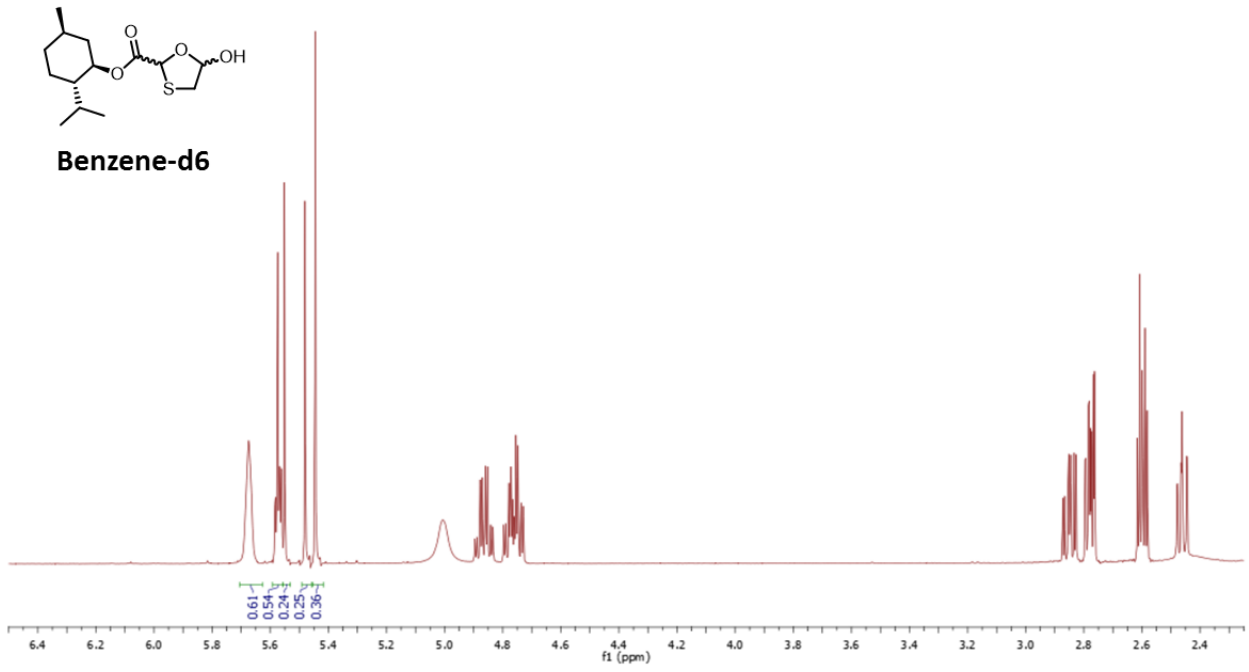


Figure 21: Mixture of four diastereomers in benzene-d6.

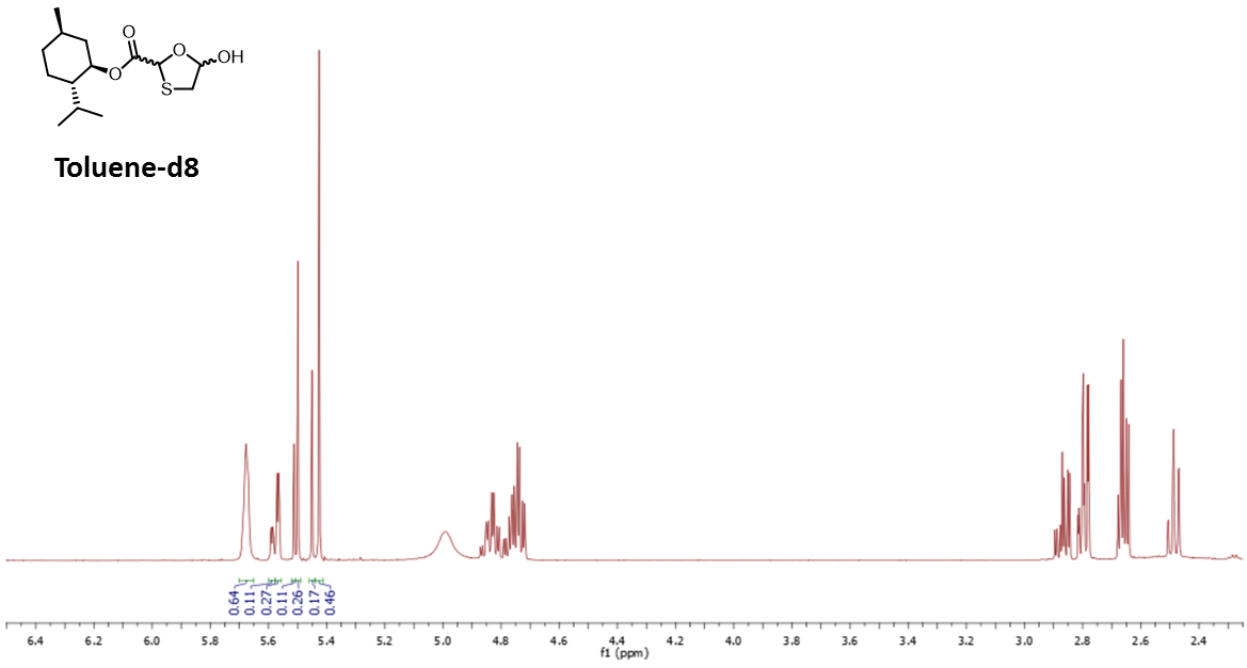
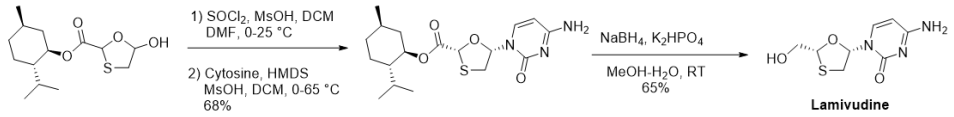
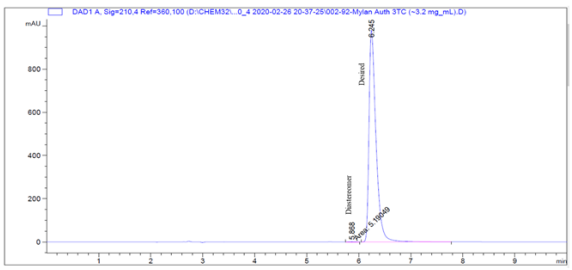


Figure 22: Mixture of four diastereomers in toluene-d8.

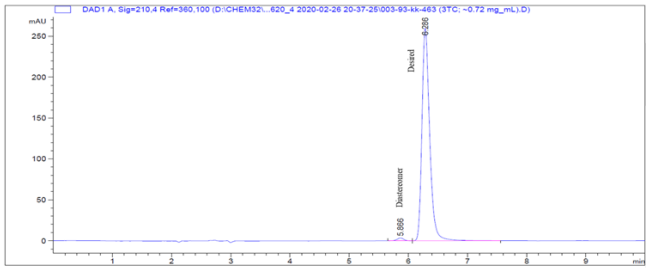
Results of Use-Test of M4ALL Oxathiolane:

A use-test of oxathiolane produced by M4All was performed using established procedures to product 3-TC[4]. The results of this work demonstrated that our Compound 1 does give the desired product, although the obtained material contained more diastereomer than specified (1.1% vs 0.2%) and slightly more enantiomer (0.41% vs 0.30%). This is not surprising since the work was done on a lab scale and no effort was made to optimize the purification. It is reassuring that no other impurities were observed, indicating that the low-level impurities observed in M4All compound **1** do not carry forward.





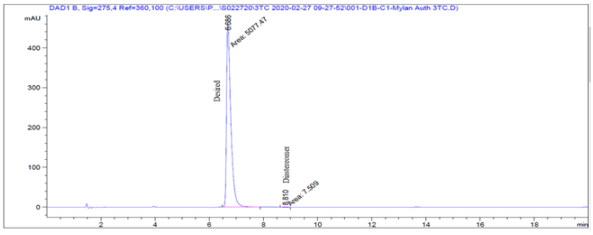
LC-DAD Chromatogram for Standard Lamivudine



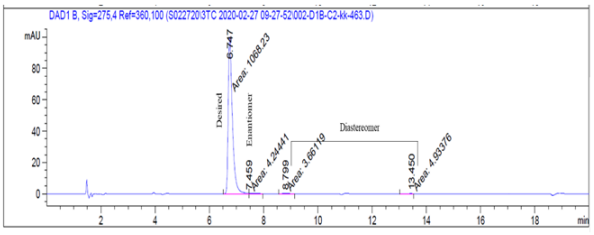
LC-DAD Chromatogram for Synthesized Lamivudine

LC Results

ID	Area % @ 210 nm for desired peak	Area % @ 210 nm for diastereomer peak	Peak Area % for desired @ 210 nn	Peak Area % for desired @ 260 nn
Mylan 3TC std	99.94%	0.06%	99.811%	99.803%
kk-463	98.90%	1.10%	98.688%	98.670%



Chiral Chromatogram for Standard Lamivudine

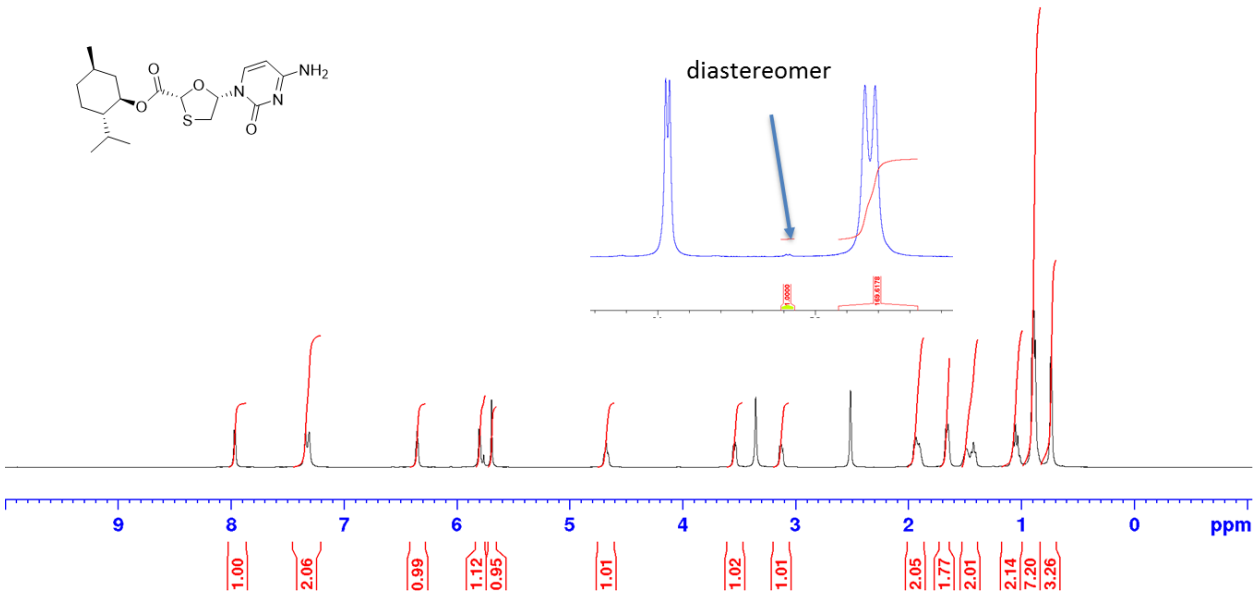


Chiral Chromatogram for Synthesized Lamivudine

SFC Results (Chiral)

ID	Area % @ 210 nm for desired peak	Area % @ 210 nm for enantiomer peak	Area % @ 210 nm for diastereomer peak 1	Area % @ 210 nm for diastereomer peak 2
Mylan 3TC std	99.96%	-	0.04%	-
Synthesized	98.84%	0.41%	0.31% %	0.43%

Figure 23: Production of lamivudine from **1**. **1** made from M4ALL process and matches authentic material.


diastereomer
9
8
7
6
5
4
3
2
1
0
ppm
1.00
2.06
0.99
1.12
0.95
1.01
1.02
1.01
2.05
1.77
2.01
2.14
7.20
3.26

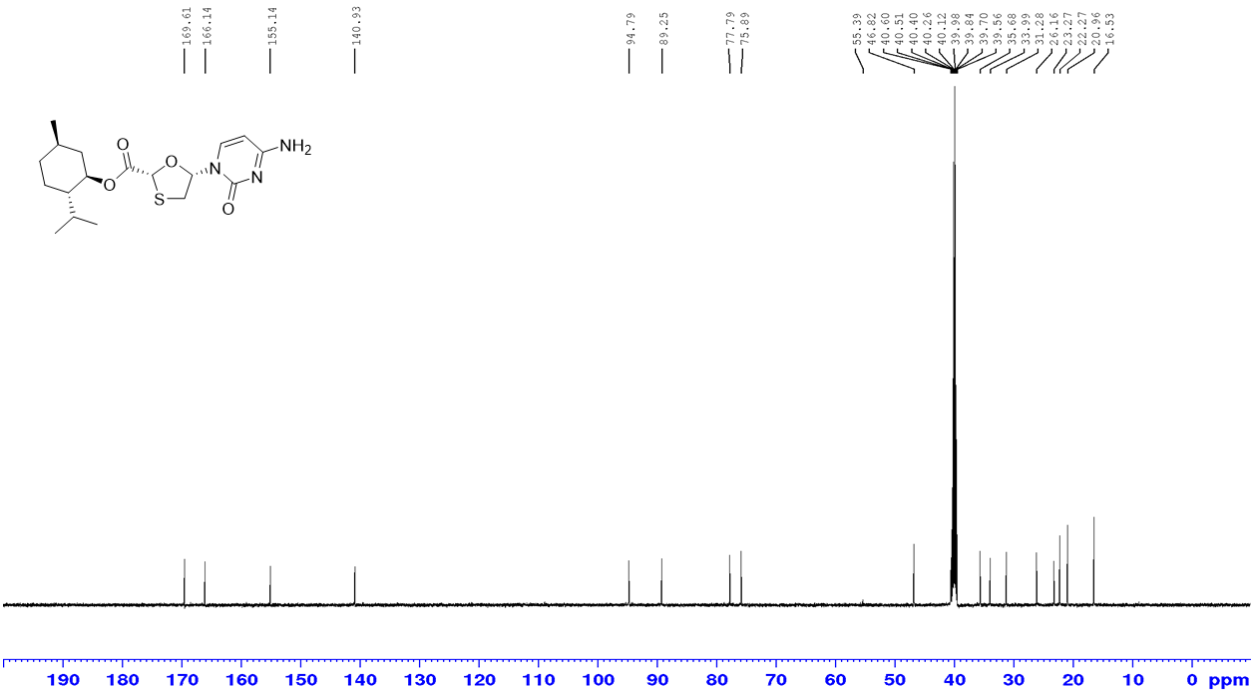


Figure 24: Coupling **1** with cytosine. **1** made from M4ALL process.