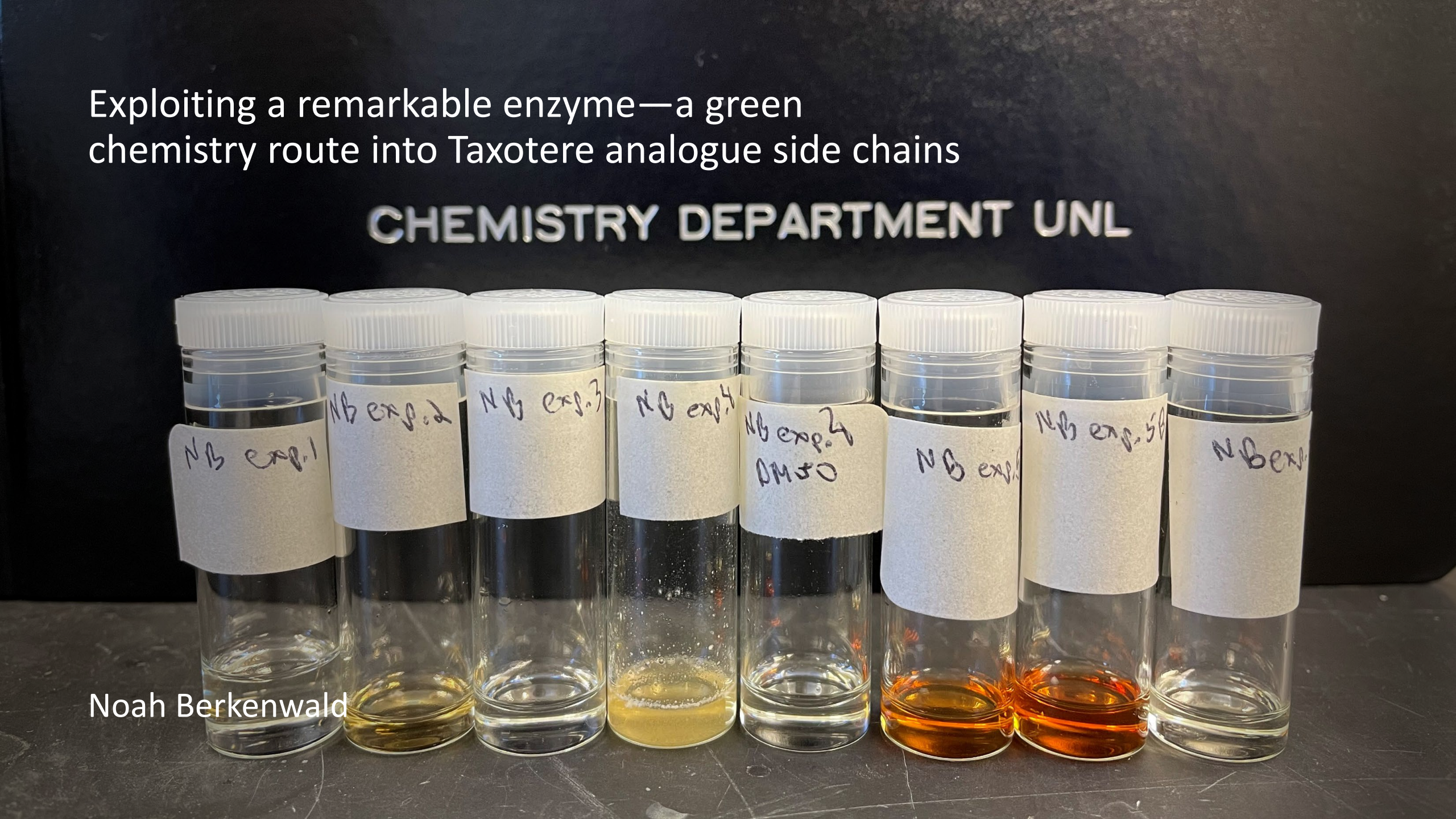
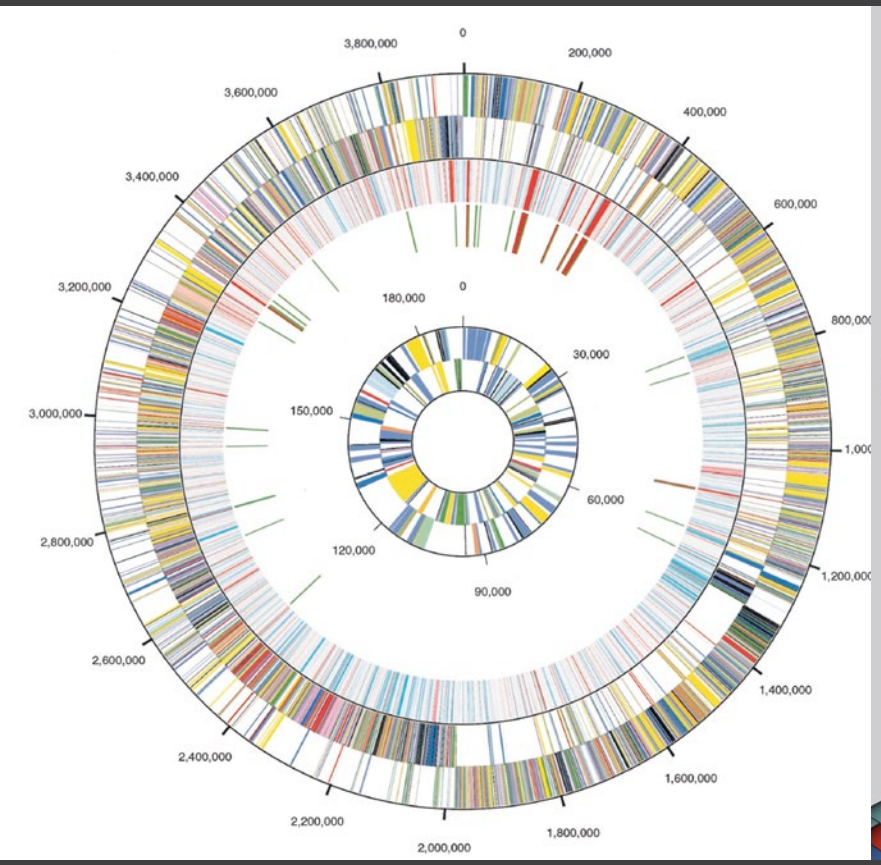
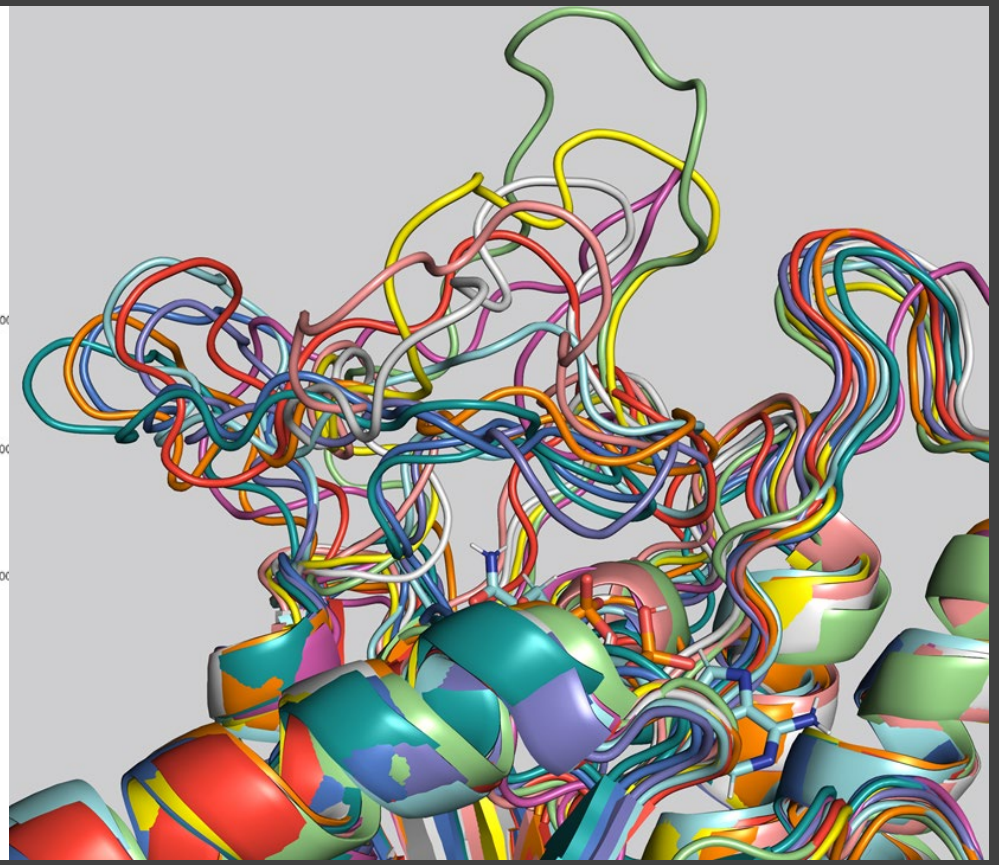
Exploiting a remarkable enzyme—a green chemistry route into Taxotere analogue side chains



Noah Berkenwald

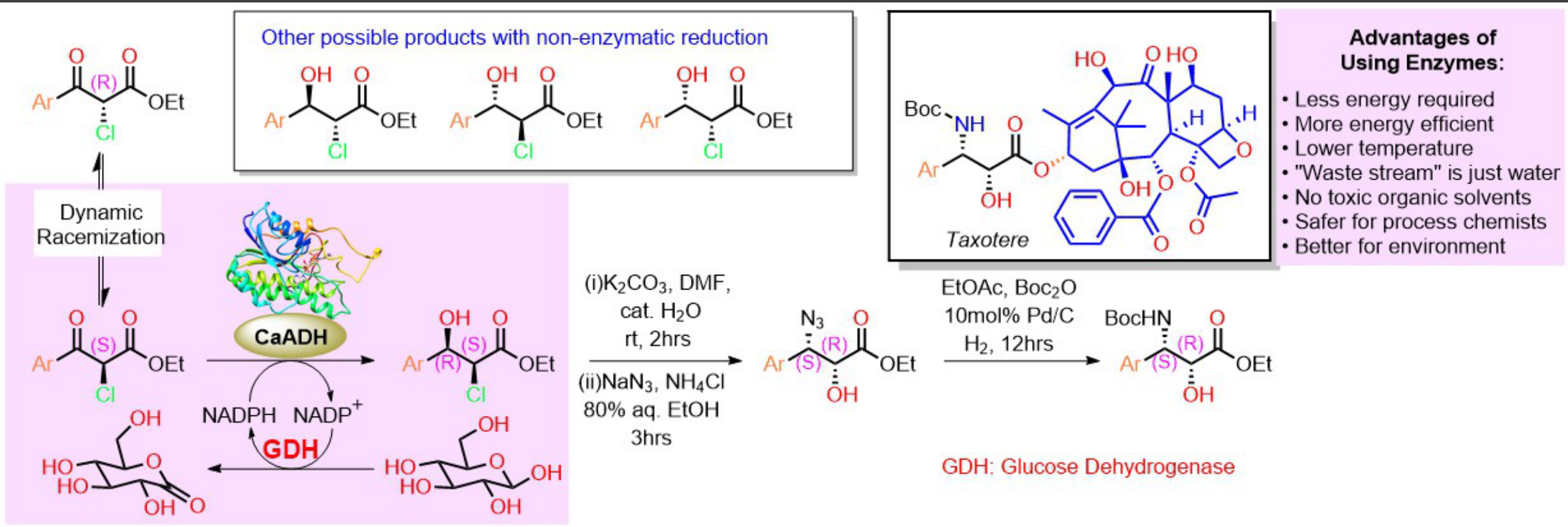
CaADH: Clostridium acetobutylicum alcohol dehydrogenase
-A short chain dehydrogenase (SDR) enzyme.



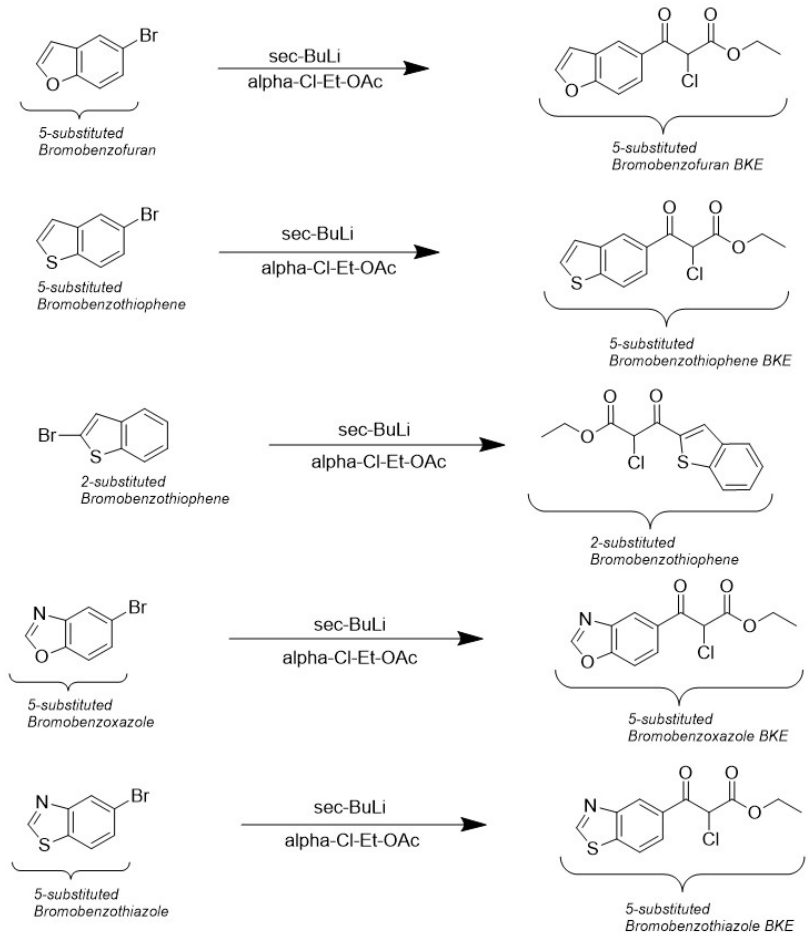


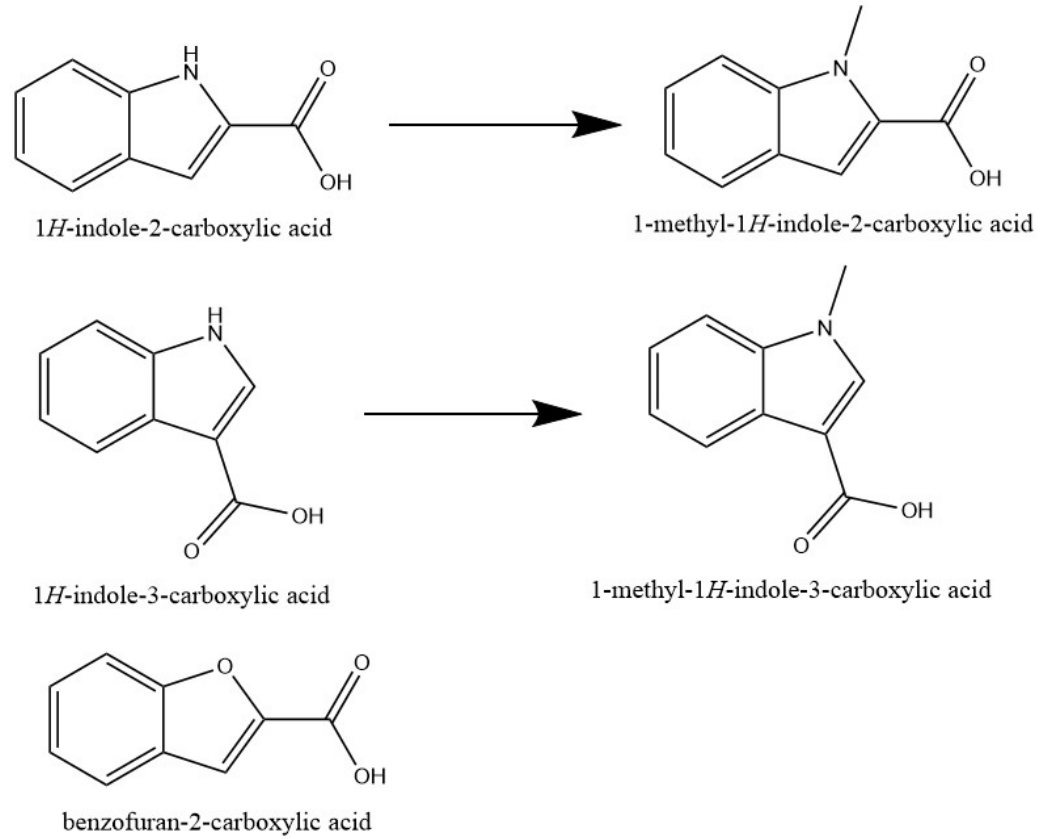
- This enzyme reduces carbonyl compounds such as aldehydes, esters, and ketones.
- CaADH displays substrate promiscuity yet stereochemical fidelity.
- DYRKR: Dynamic Reductive Kinetic Resolution

Project Scheme

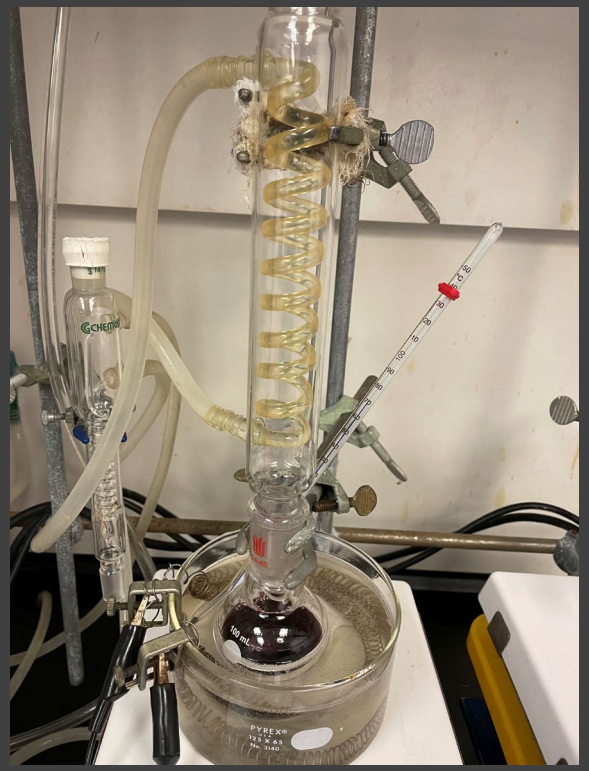


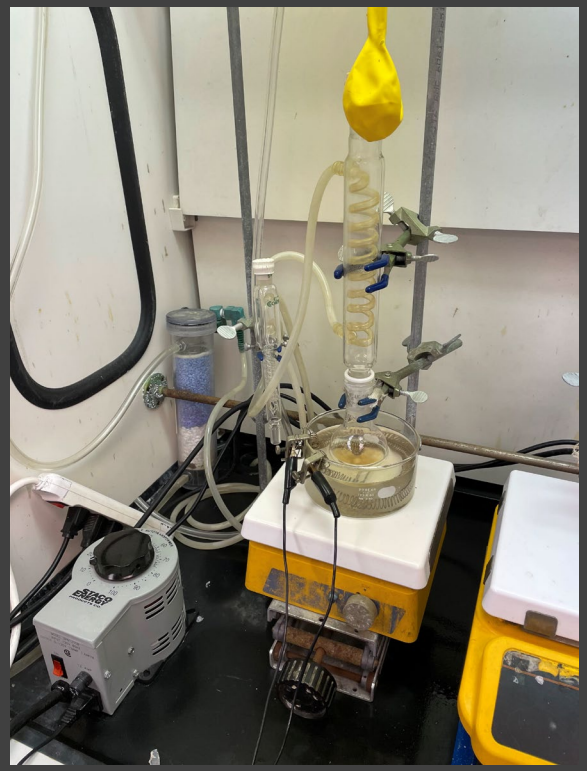
Substrates/Aryl Groups





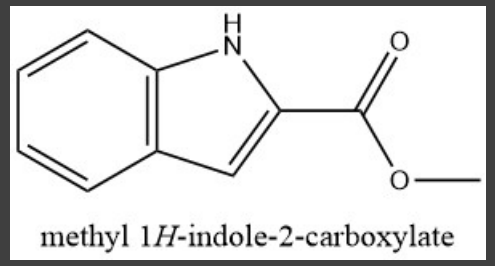
Esterification

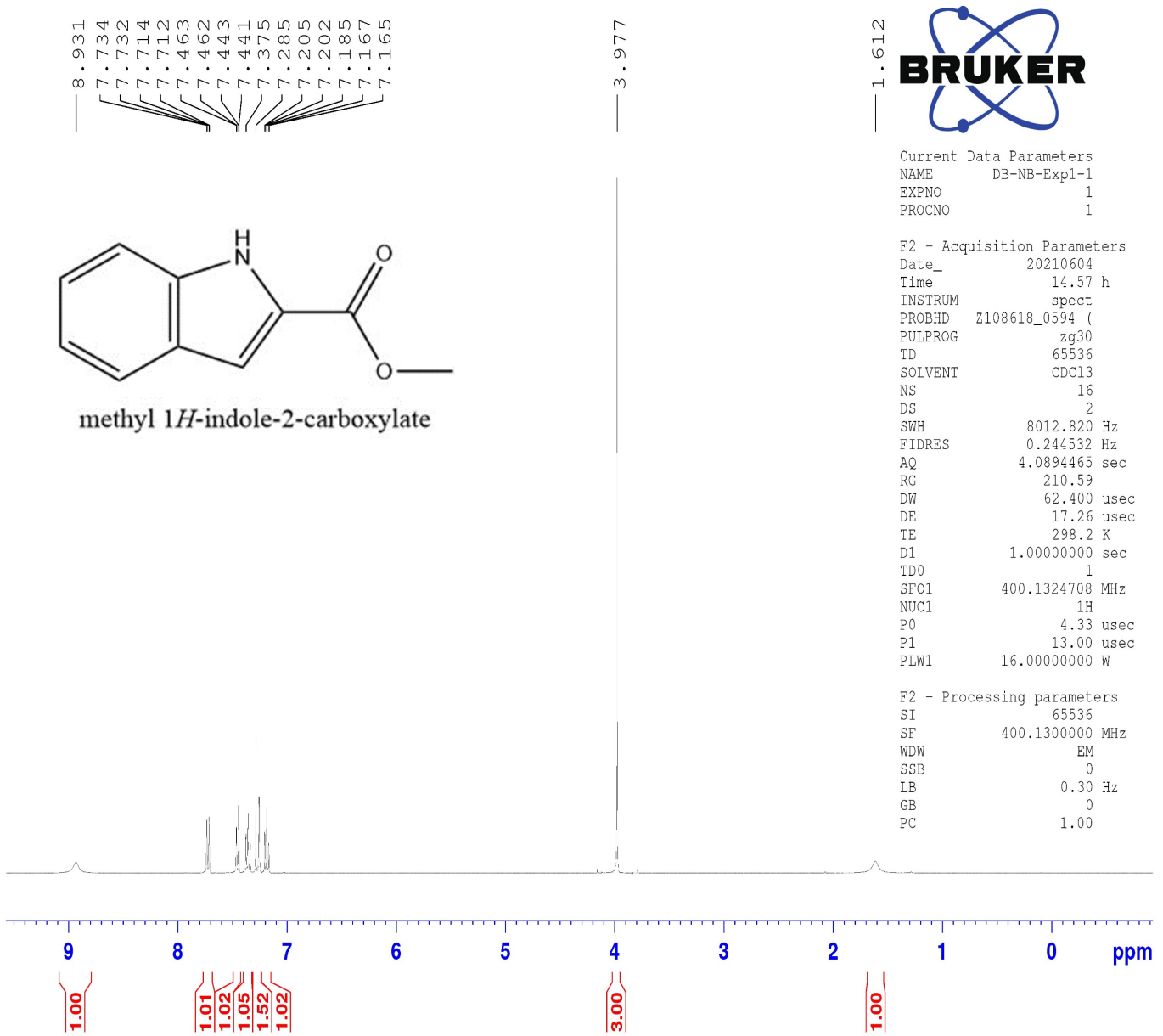




Indole-2 methyl ester

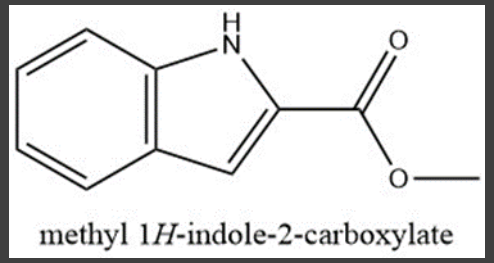
- 70 degrees Celsius, MeOH solvent, catalytic H2SO4
- First reaction I did
- 1mmol (~.162g) scale
- 82.2% yield

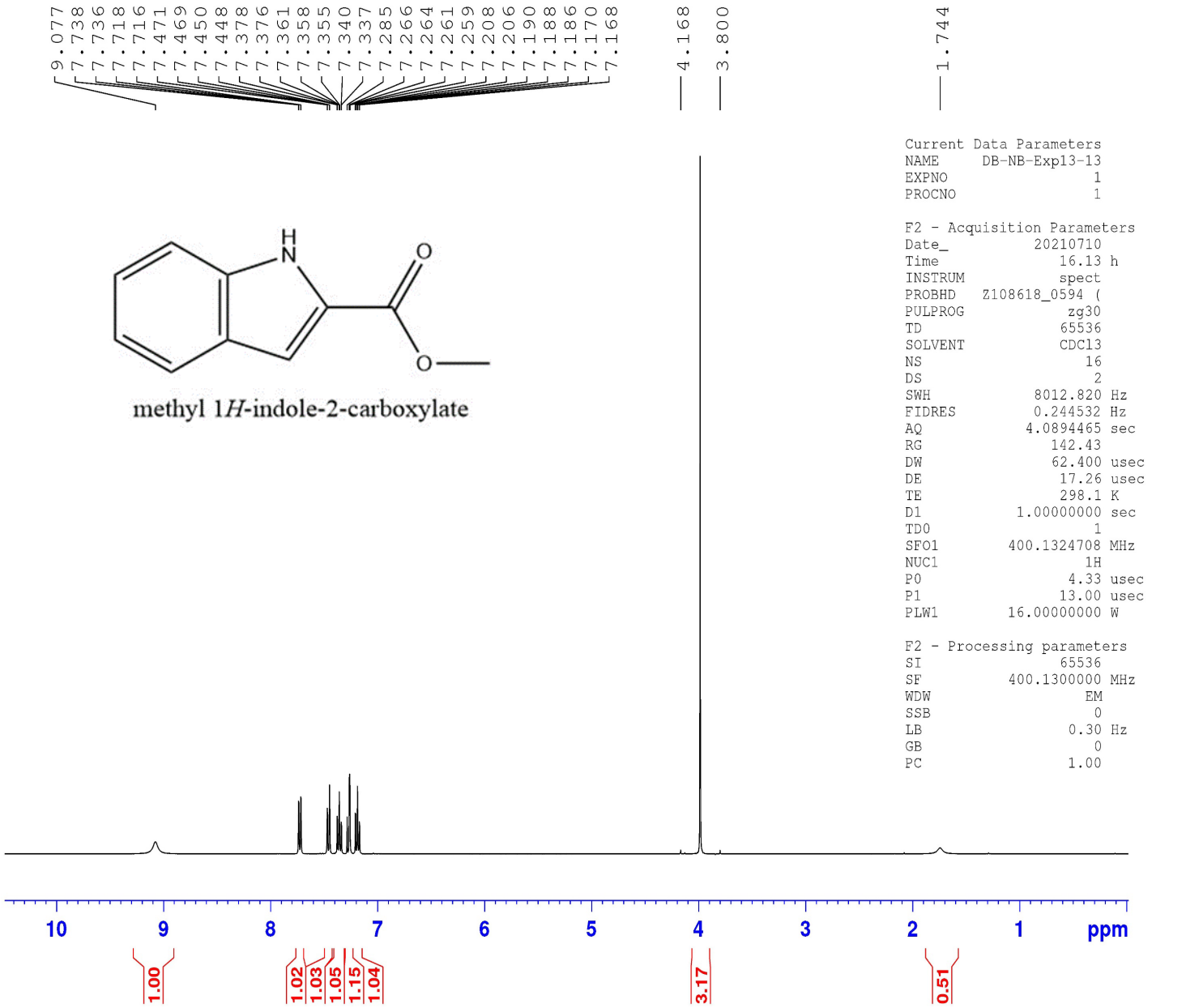




More indole-2 esterification

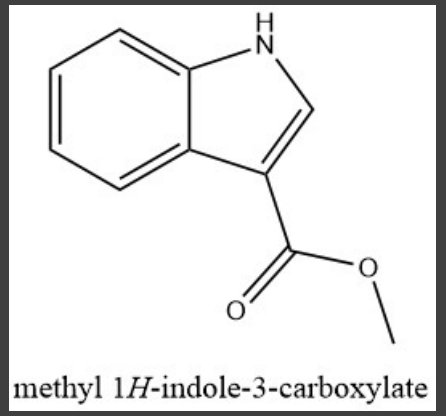
- Successful reaction with 91.1% yield.
- 2g scale

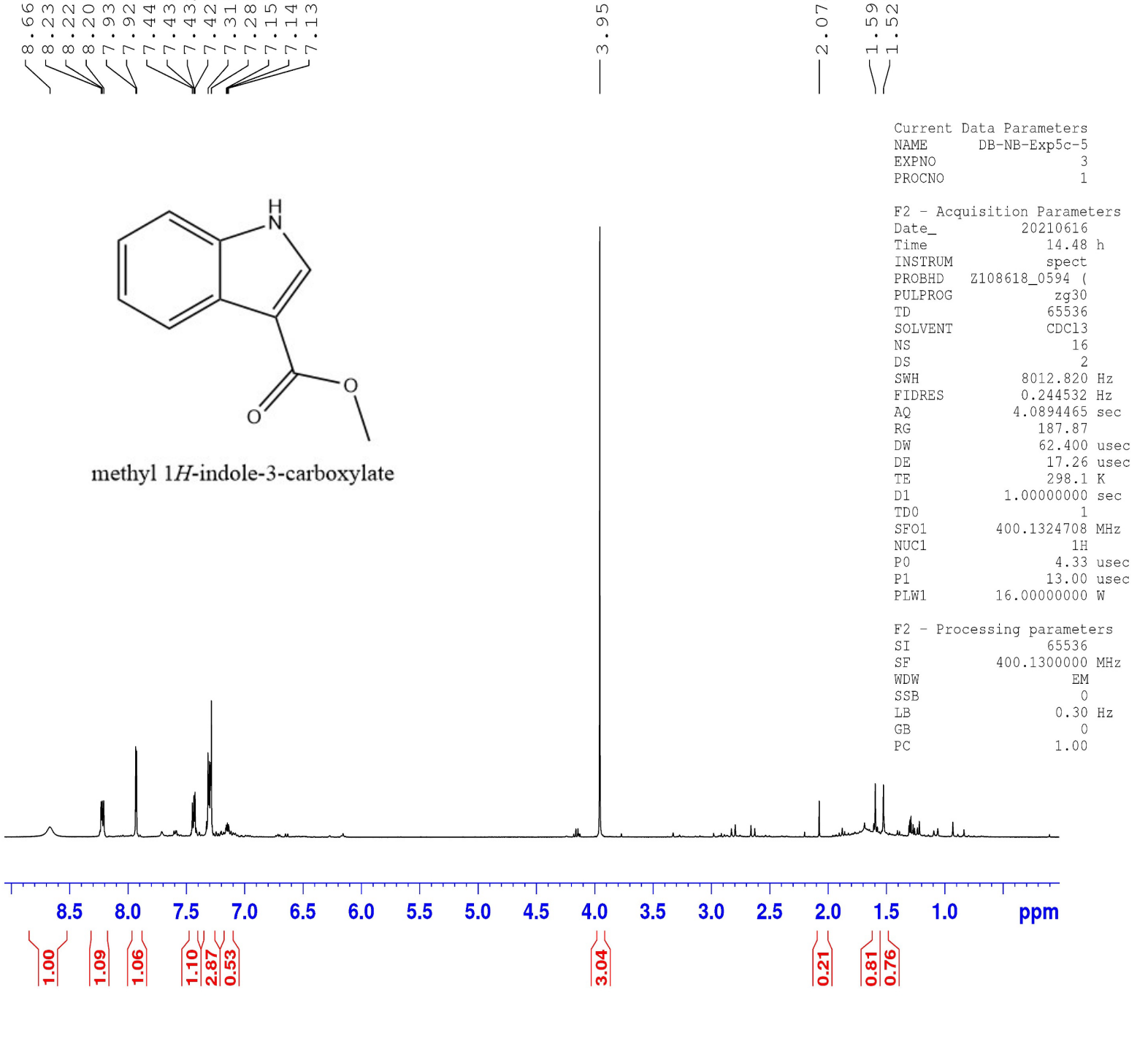




Indole-3 methyl ester

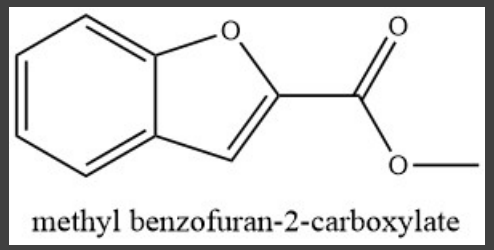
- 2g scale
- 99% yield



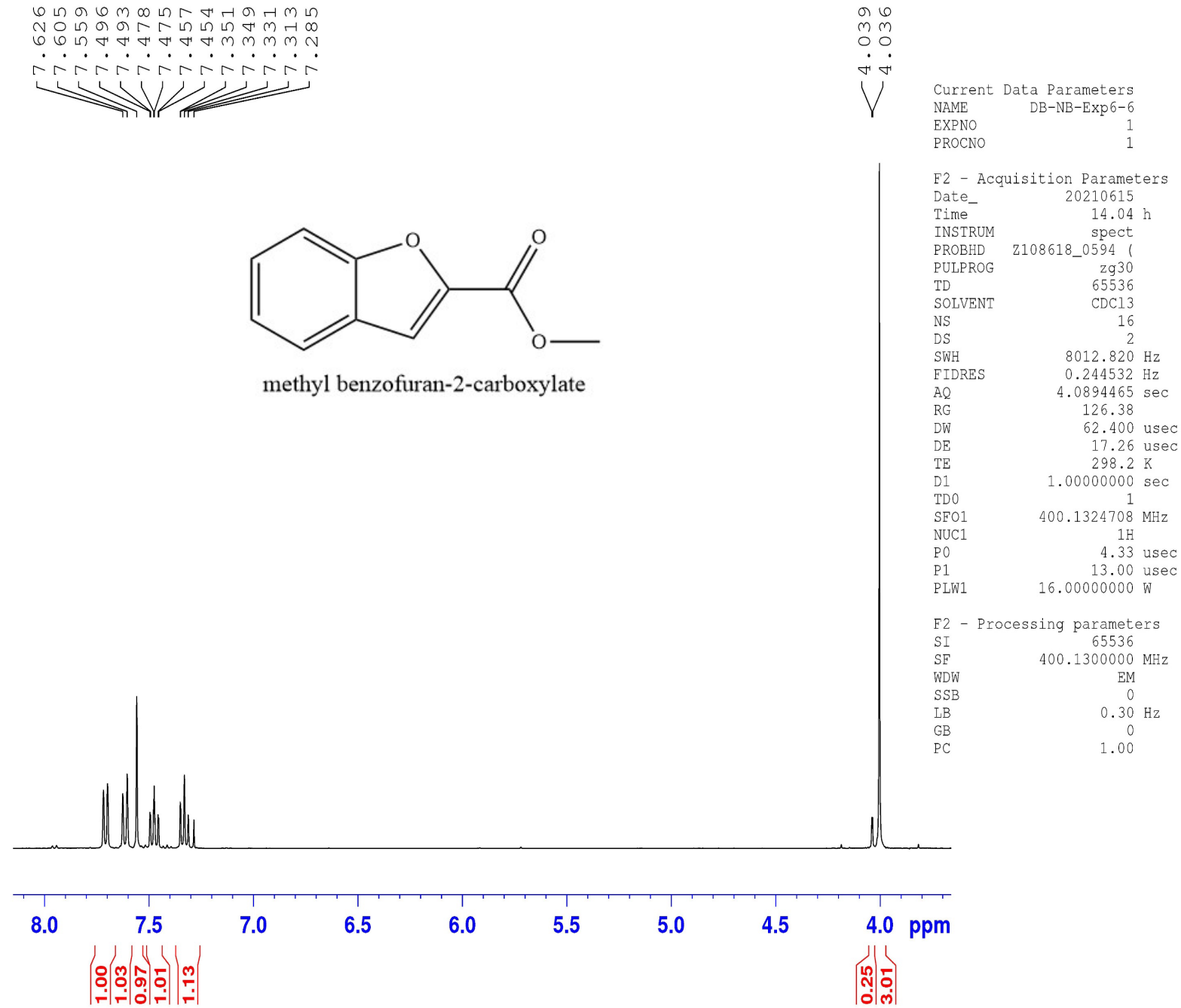


Benzofuran-2 methyl ester

- Yield: 95.7%
- 2g scale



2-Benzo

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RG                126.38
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DE                17.26 usec
TE                298.2 K
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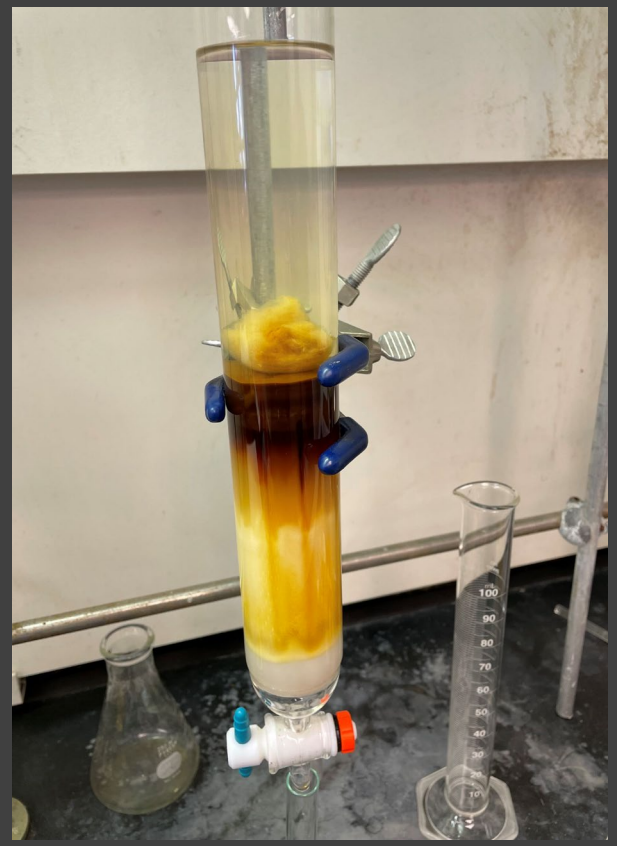
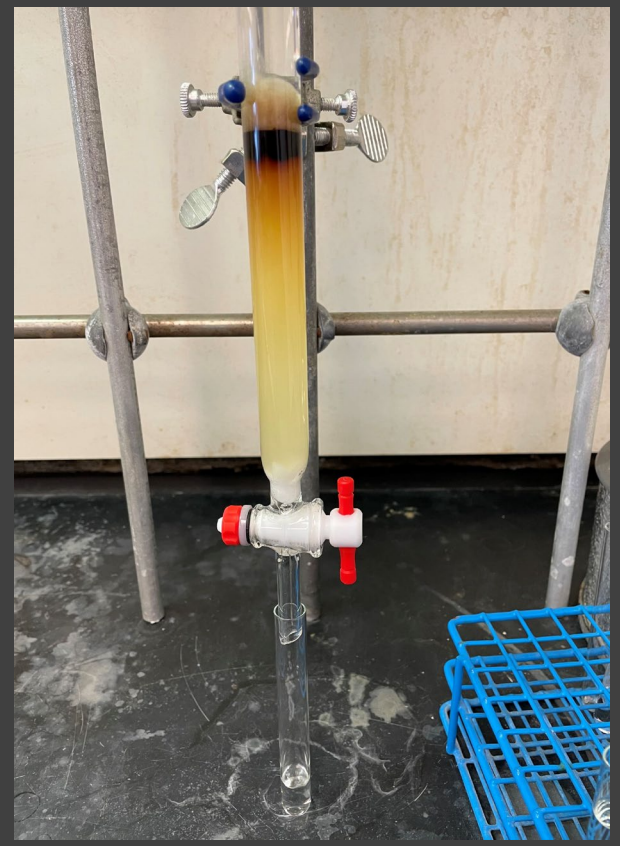
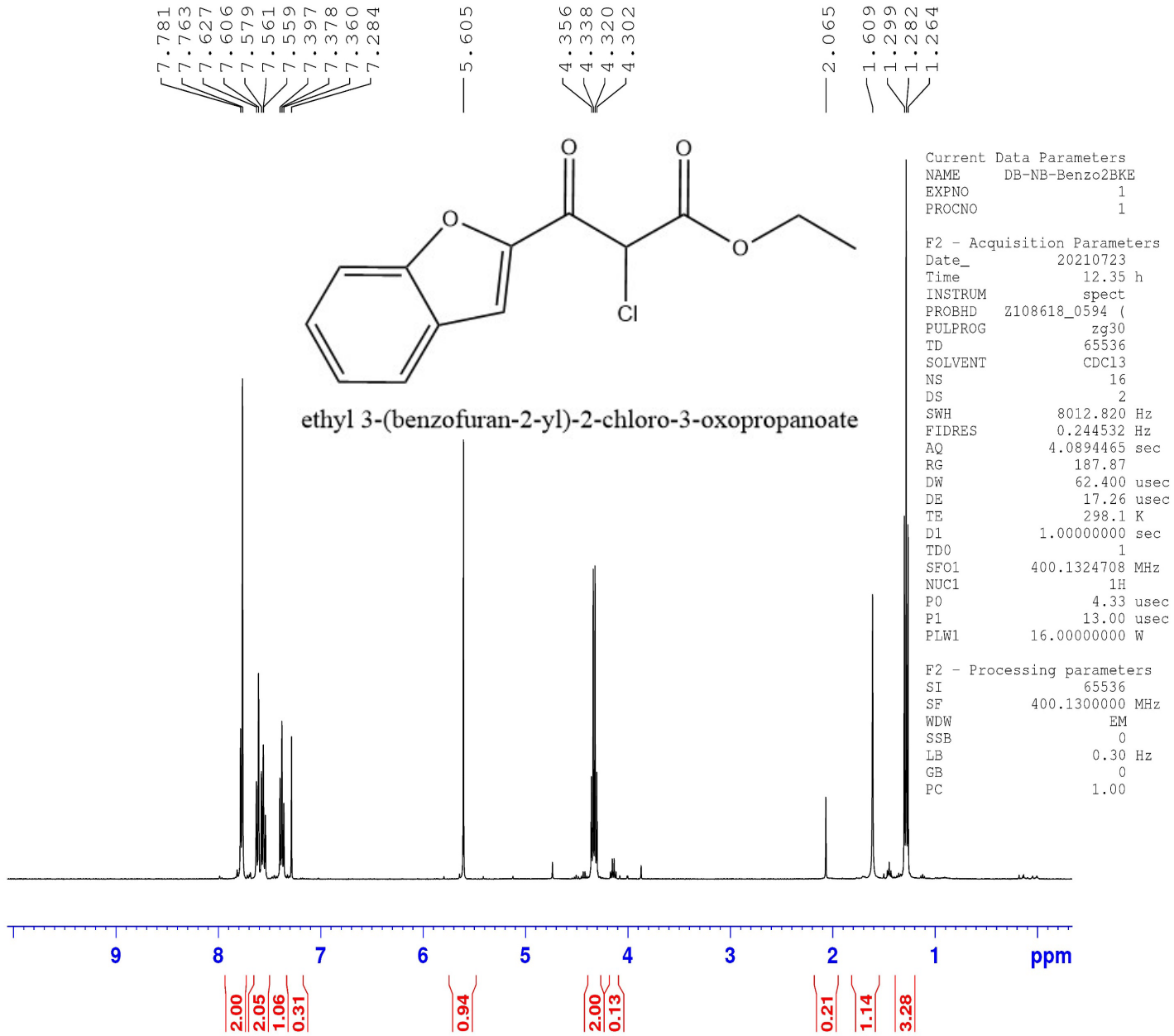




Claisen Condensation

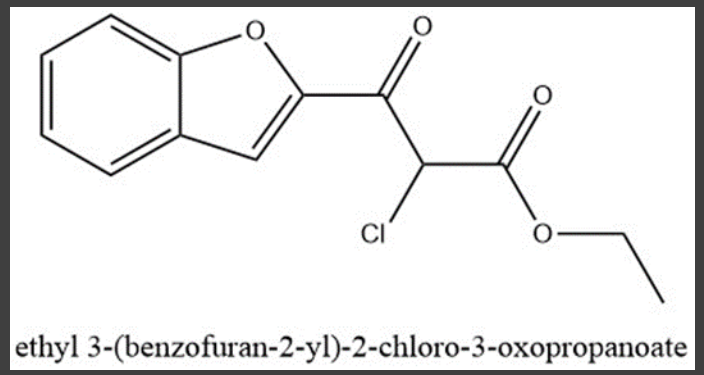
Gaurav's CC of benzofuran-2

- My mentor Gaurav succeeded in a Claisen condensation reaction of my benzofuran-2 methyl ester, and we successfully reacted it with the enzyme, but I haven't obtained the data from that reaction yet.

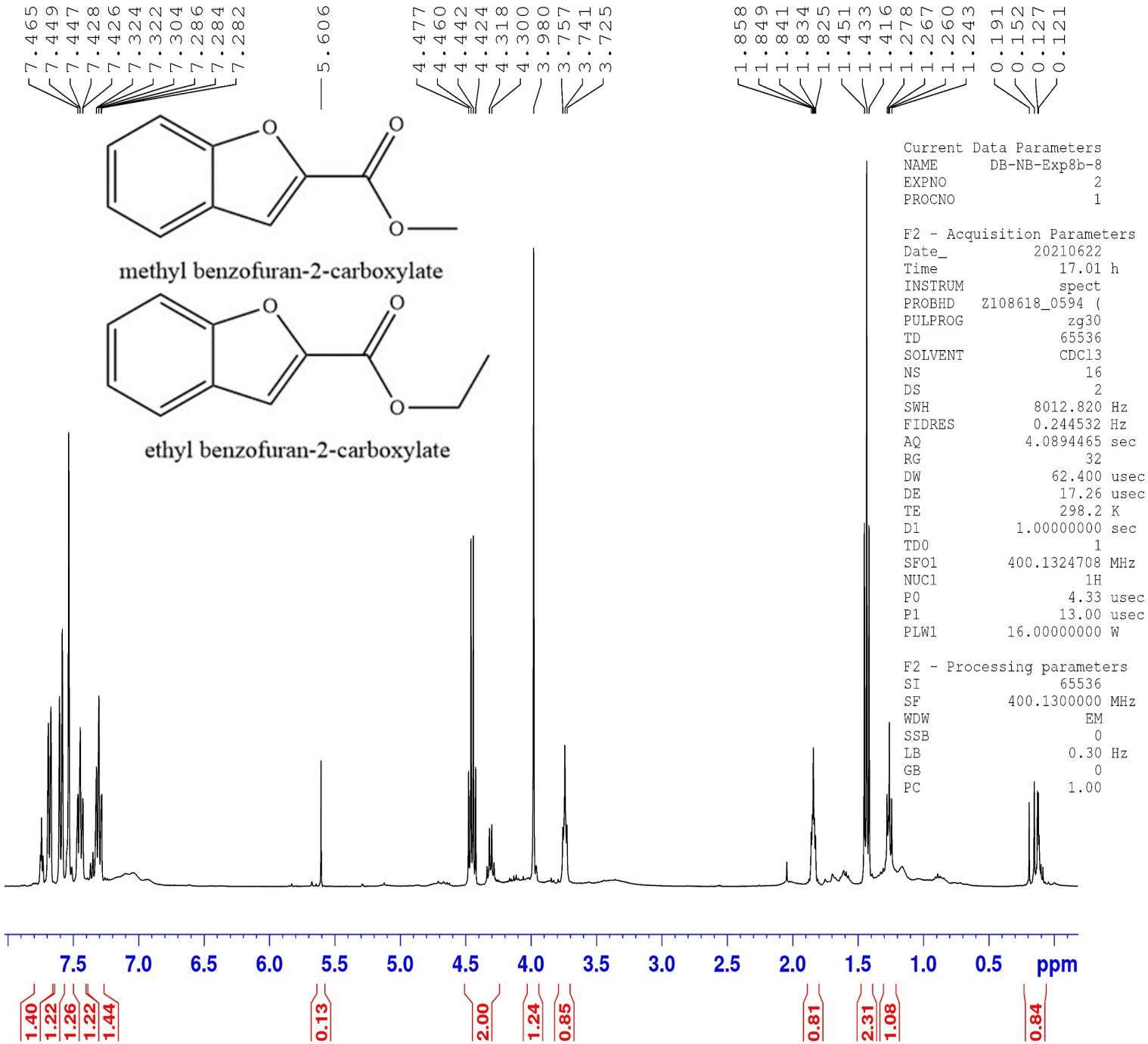


2-benzofuran beta-keto-ester

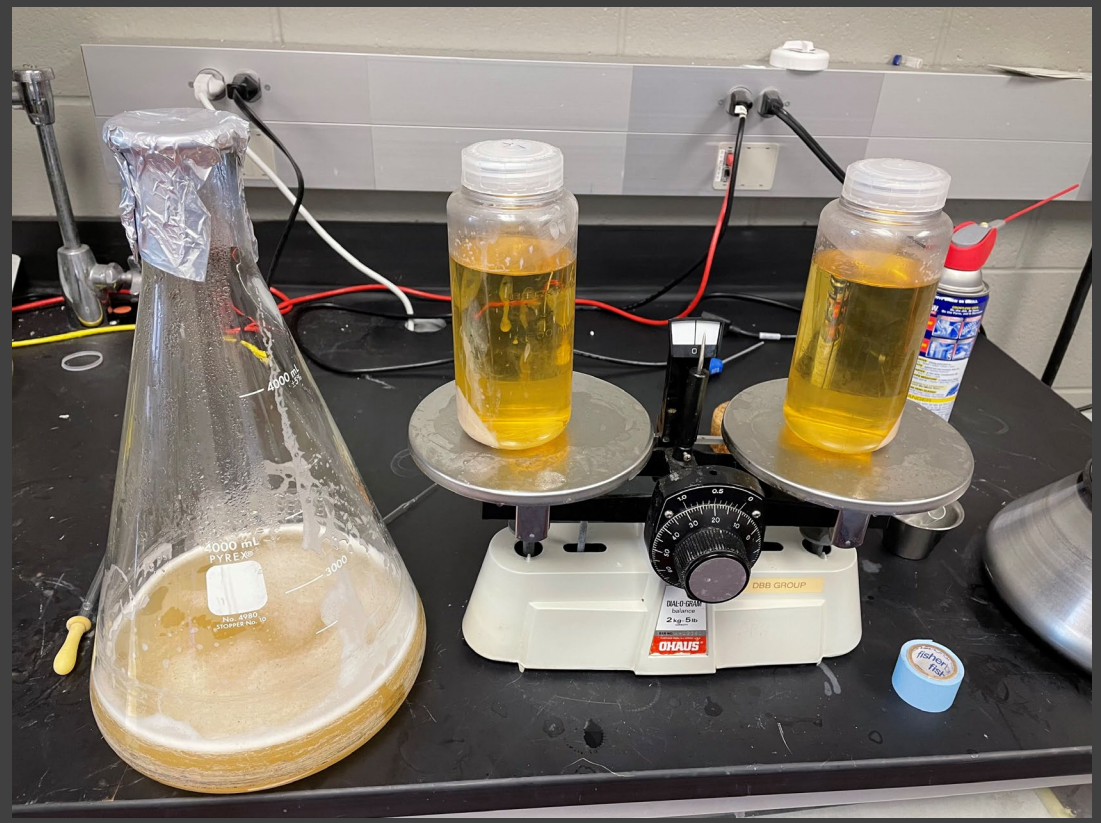
- 2 eqv. LiHMDS, 1.1 eqv. Alpha-chloro-ethyl-acetate, in dry THF for 0.2M.
- Ended up with starting material (methyl ester) and ethyl ester, no BKE.

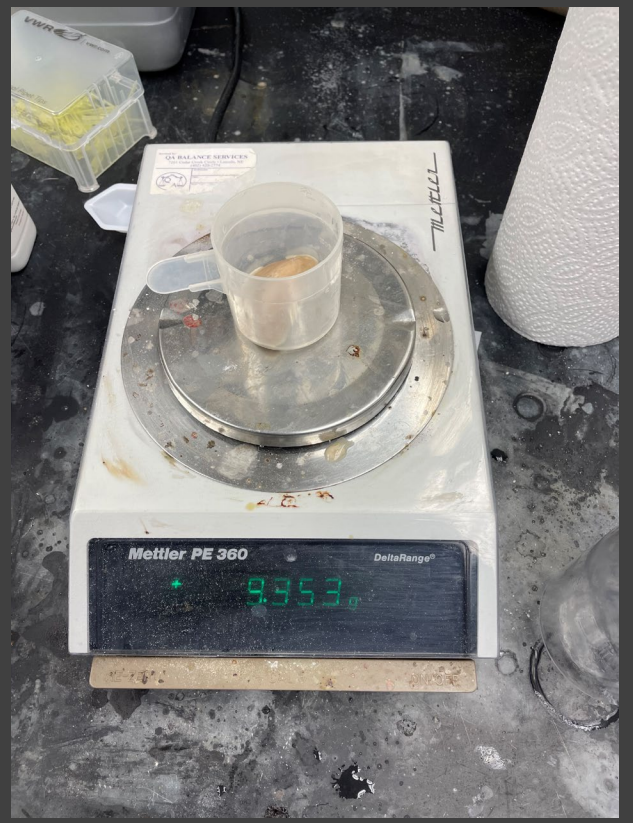


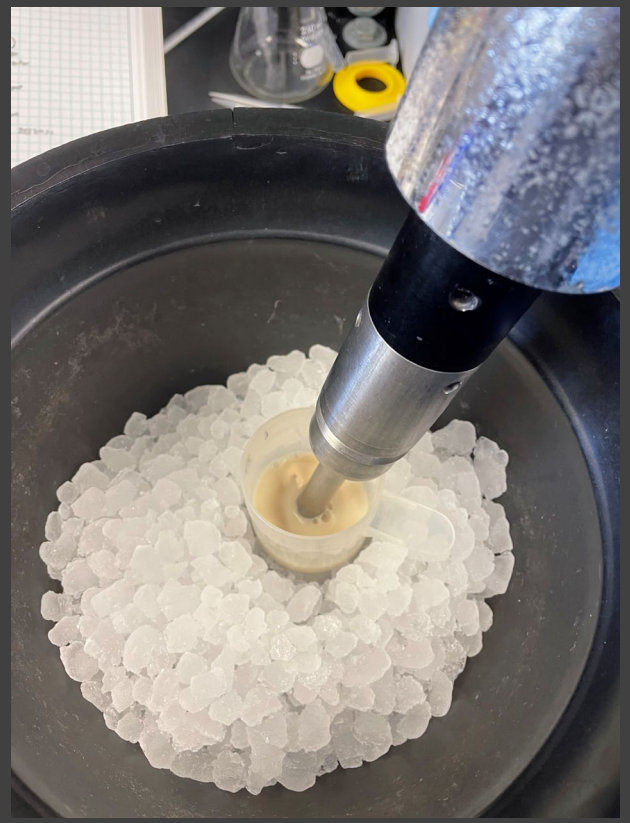
ethyl 3-(benzofuran-2-yl)-2-chloro-3-oxopropanoate

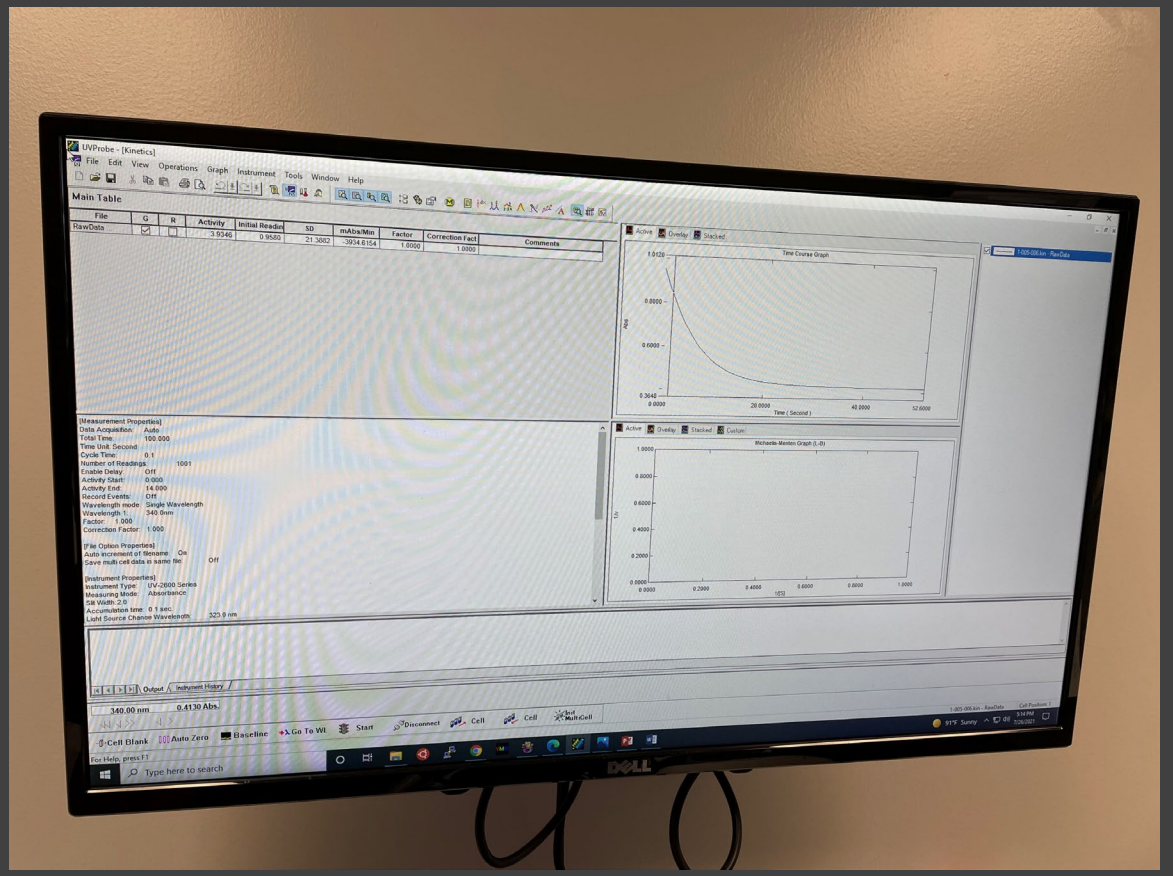


Growing Up Enzymes



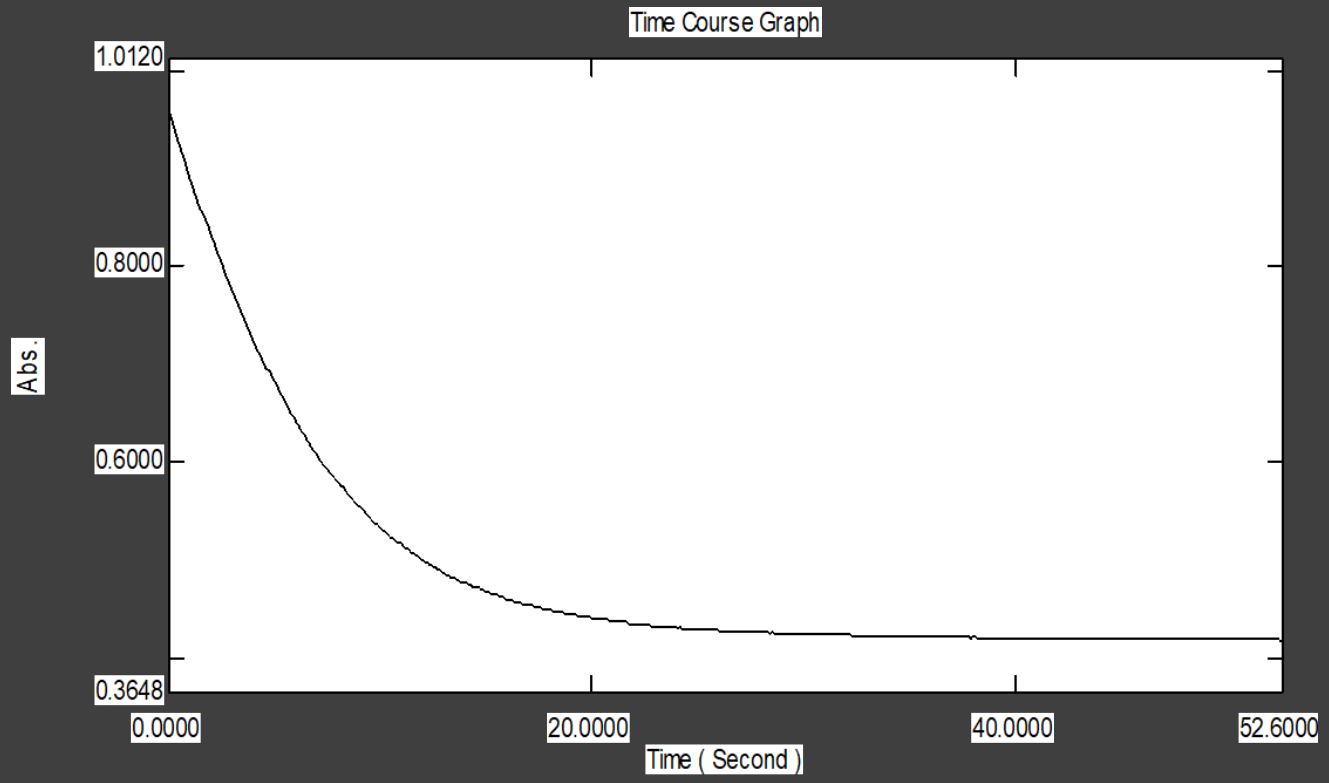






Enzyme Activity

Enzymology- Activity Assay



- Enzymatic reduction of 10mM benzaldehyde with 0.2nM NADPH cofactor in 100mM buffer with 5% DMSO, 1mL volume and 1cm path length, analyzed by UV-vis spectrophotometer.
- 7.6g cell
- -3934.6154mAbs/Min

Enzymology- Activity Assay -Calculations

$$\frac{1umol\ NADPH}{1mL\ vol} = 1mM\ NADPH$$

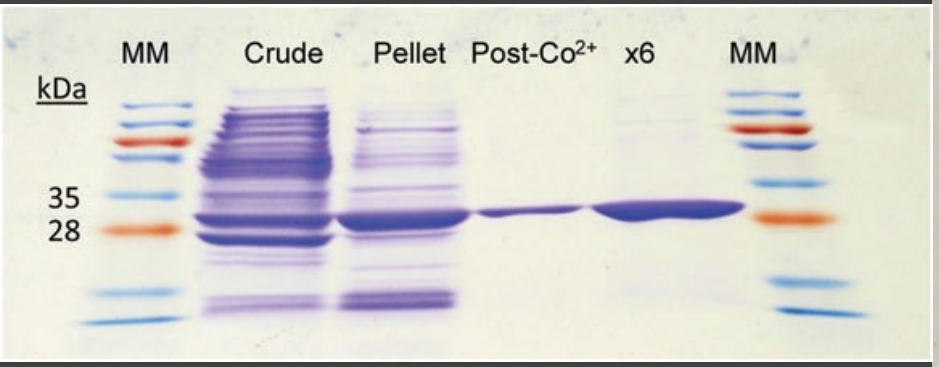
$$\Delta Abs^{340nm} = 6.22mM^{-1}cm^{-1}$$

$$\frac{3934.6\ mAbs}{min} \times \frac{1umol}{6220\ mAbs} = 0.6325\ \frac{umol}{min}$$

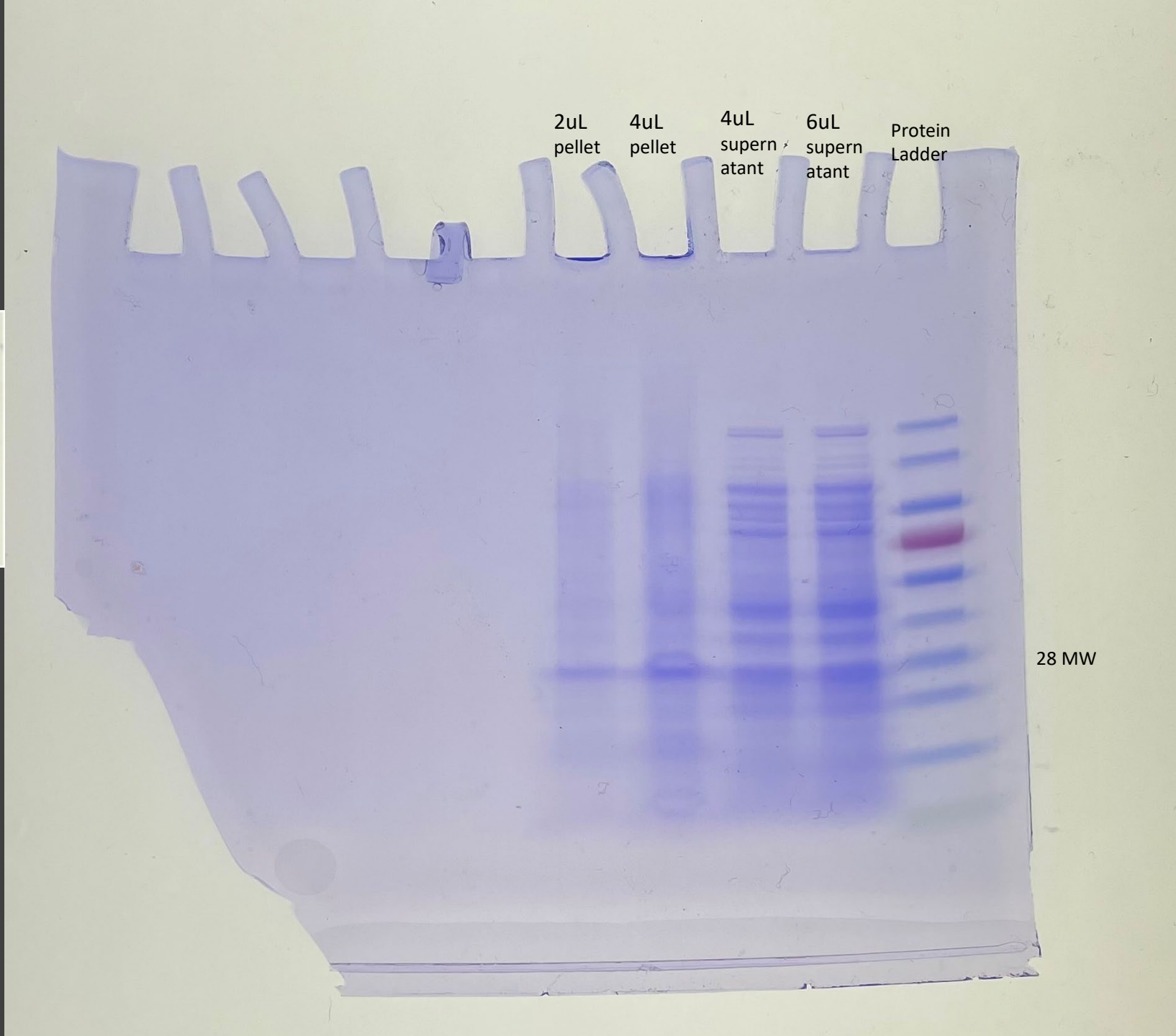
$$0.6325\frac{umol}{min} \times \frac{10{,}000uL\ enzymes}{20uL\ enzymes\ taken} = 316.28\ units\ enzyme$$

Activity	mAbs/Min	umol/Min	Units	Vol supernatant	Mass Cell
1.3880	-3934.6	0.6325	316.28	10mL	7.6g
			250.0		**10g**

Enzymology
-Gel Electrophoresis



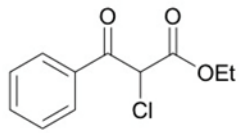
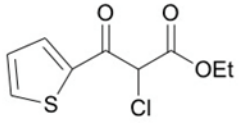
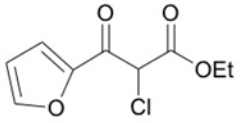
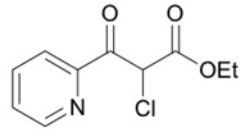
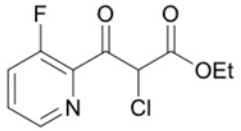
- Gel on left: SDS-PAGE gel illustrating the purification of CaADH: outside lanes=molecular markers (MM); then l to r: (i) crude supernatant; (ii) crude pellet; (iii) eluent from Co2+-column; (iv) 6x loading of (iii).
- Gel on the right: Mini-Protean TGX gel illustrating crude CaADH, and overexpression of the protein at 28 kDa.



CaADH Profile Across Taxoid Side Chains and Future Work/Learning:

- Chiral HPLC
- Growing enzymes
- Finishing with new substrates

e-discr = enantiodiscrimination at the C-Cl stereocenter
fd = facial discrimination at the carbonyl center
bsrm = based on starting material recovered

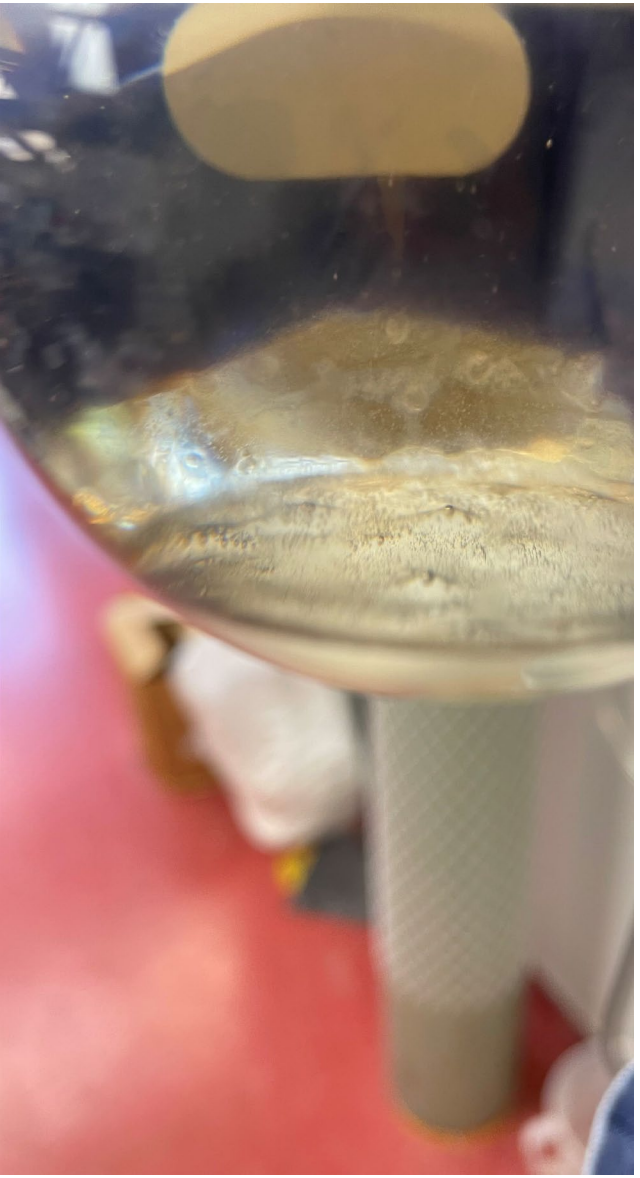
Substrate	%Yield	*e-discr*	fd	D-*syn* / D-*anti**	L-*syn** / L-*anti**
	98%	S 98.5:1.5	D 98.5:1.5	98.5% (98.5%** / <0.1%)	1.5% (1.4% / <0.1%)
	92%	S >97:3	D 97:3	97% (96% / 1%)	3% (2% / 1%)
	83%	S ND	D #	#	#
	96% bsrm	S 62:38	D 95:5 (19:1)	95% (58% / 37%)	5 % (1% / 4%)
	83% bsrm	S 91:9 (11:1)	D 85:15 (11:1)	85% (83% / 2%)	15% (7% / 8%***)

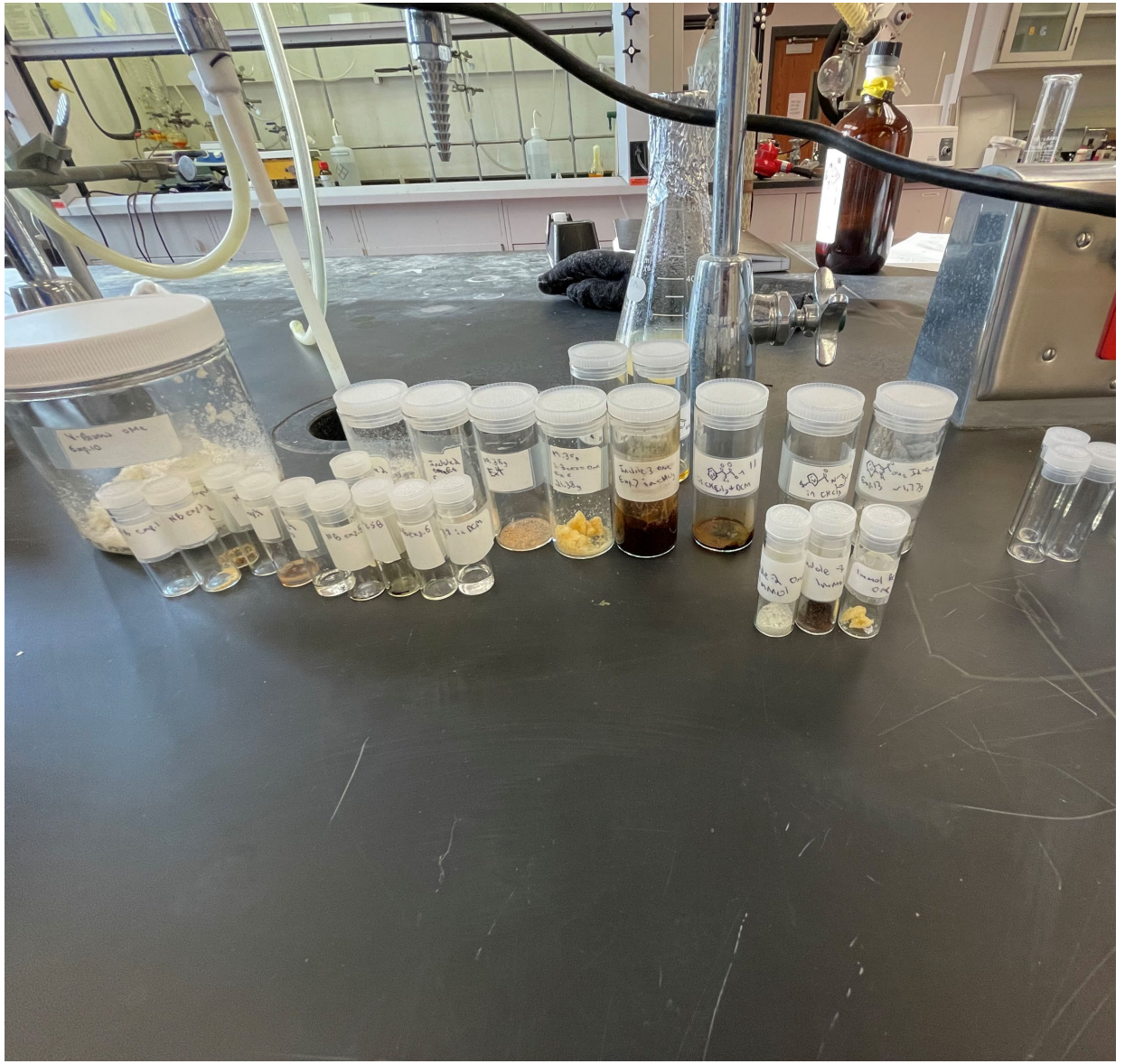
Note: Chiral HPLC not yet completed; NMR shows almost exclusively one diastereomer, presumably the syn-diastereomer

* Note: While the diastereomeric identity of HPLC peaks can be established by correlation with NMR, the absolute stereochemistry of individual peaks, particularly minor ones, if often by analogy and so, where not corroborated by rot'n or x-ray is not definitive.

** Absolute stereochemistry established by optical rotation.

***Absolute stereochemistry established by x-ray structure deterimination (anomolous dispersion) for the L-*anti* isomer





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- Dr. Nivesh Kumar
- Guarav Kudalkar
- Berkowitz lab group

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