Title

Modular synthesis, biological evaluation, and structural characterization of trichothecenes

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Modular synthesis, biological evaluation, and structural characterization of trichothecenes

by
Minh Tran

DISSERTATION
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in the

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of the
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

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It's not necessarily a bad sign if work is a struggle,

any more than it's a bad sign to be out of breath while running

-Paul Graham

This thesis is dedicated to Lam Phi Yen

Acknowledgements

First and foremost, I must express my heartfelt gratitude to Ian Seiple. It's hard to believe it's been around 4.5 years since I made the decision to start on my graduate studies at UCSF under his mentorship. Looking back, it's clear that choosing Ian as my mentor was one of the best decisions I've ever made. As I started my thesis work amidst the unprecedented challenges of the COVID-19 era, Ian's unwavering support and boundless optimism were my guiding lights. His belief in my abilities, coupled with his relentless dedication to excellence, not only propelled me forward even though his expectation can feel unachievable sometimes. Much like his portrayal of Phil in his own thesis, Ian's mentorship has been simultaneously humbling and inspiring, shaping me both personally and professionally.

I also want to extend my deepest gratitude to my friend and drinking buddy, Yoshito. During the darkest moments of my PhD journey, when nothing seemed to work, Yoshito's presence and unparalleled expertise in synthesis were invaluable. Despite the absence of formal synthesis training at UCSF, Yoshito's insights and guidance were instrumental in navigating the complexities of our research. Both Ian and Yoshito were the reason I can figure out the key Claisen rearrangement, without whom this thesis would not exist.

To my colleagues in the lab, I am immensely grateful for your support and camaraderie throughout this journey Isabel and Jesus, thanks for putting up with my weird sense of humor and all the random questions. Quinn, it's been real bouncing ideas off you and shooting the breeze. Arthur, thanks for talking me into coming to UCSF and always having my back. Seulki, you're a rock star! It’s been amazing to have someone who always has my best interest at heart, and tolerate my silliness and annoyance. Part of me is thankful for Covid since it brought us closer during our shift. Thank you so much for always being there for me Seulki, and I hope we can be friend for a long

time. And Andrew, you're a straight-up inspiration with your smarts and work ethic. You made me realized what it felt like to work with an exceptional person.

And to my crew outside of the lab: Zach, you're a genius, man. Our chats have opened my eyes to so much, and you've helped me figure out where I wanna go in life. Sophie, thanks for keeping me grounded during grad school madness. You got me into tennis, listened to my rants, and helped me remember there's life beyond the lab. Lauren, you've always had my back, especially when times got tough.

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Last but never least, Mom, you're the real MVP. Thanks for checking in on me every day, for loving me no matter what, and for teaching me that being a good person comes before being a smart scientist. Couldn't have done it without you.

Contributions

The chapters in this dissertation were performed under the guidance of Dr. Ian Seiple, with the collaboration and feedback of many Seiple lab members. My committee members Dr. Adam Renslo and Dr. William Degrado also provided key scientific guidance that helped make this work successful. Other collaborators that were instrumental in moving this work forward include James Fraser and Tushar B. Raskar for cryo-EM structure and Ethel Tackie-Yarboi for help in mammalian cell works.

Chapter 1.1 contains the majority of my results, which center around the total synthesis platform of trichothecene family and fail attempts.

Chapter 1.2 is a structure of diacetyl verrucarol and its biological activity .

Minh Tran provided the experimentation and manuscript preparation for much of the following dissertation, which is a substantive contribution comparable to other dissertations in Chemistry & Chemical Biology. Ian Seiple directed and supervised the research and provided guidance and feedback throughout.

Modular synthesis, biological evaluation, and structural characterization of trichothecenes

Minh Tran

Abstract

Trichothecenes such as T2-toxin are toxic natural products produced by several species of fungi that grow on grain crops. This class of sesquiterpenes comprises over 200 family members that feature a tricyclic core with varying degrees of oxidation and further cyclization, and has received attention for their toxicity as well as their anticancer, antifungal, and immunomodulatory effects. Cellular and animal model studies support the hypothesis that their manifold biological effects arise at least in part due to inhibition of protein synthesis. Here we report a modular synthesis of the minimal trichothecene pharmacophore verrucarol, enabling straightforward structural modifications to investigate toxicity and molecular mechanisms of action. We found that while verrucarol inhibits human cytosolic protein synthesis in vitro, functionalization of the C4 and C15 alcohols is required for potent cellular toxicity in cancer cell lines and fibroblasts. We characterized the binding of verrucarol and diacetylverrucarol to the 50S subunit of the human cytosolic ribosome at 2.7 A resolution, revealing binding determinants that could not be resolved from the previous structures in yeast ribosomes. This work will enable new studies of trichothecenes required for food safety and for exploration of their use as therapeutics.

In the chapter **1.1**, I describe the modular synthetic route for verrucarol as well as all the fail attempts. The route features a selective Claisen rearrangement as its key step to set up two consecutive quaternary centers. Additionally, using the same route, I was able to access other natural product within the same trichothecene family.

In the chapter **1.2**, I show the structure of diacetyl verrucarol bound to 60S subunit of human ribosome. Due to its high resolution, we were able to detect interaction that was not able to resolve with previous structure in yeast ribosome. This work was done by Tushar Raskar in James Fraser lab. Additionaly, using MTT assay, I was able to demonstrate cytotoxicity activity of these minimal pharmacophore in 4 different cell lines. With help of others, we showed diacetyl verrucarol might inhibit both cytosolic and mitochondrial protein synthesis without affecting bacterial ribosome up to 100 uM.

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Chapter 1.1: Modular synthesis platform of trichothecene

1.1.1 Introduction

Trichothecenes are sesquiterpene mycotoxins produced by fungi in the *Hypcreales* order that grow on a wide variety of plants including several food crops and forages. They are structurally characterized by a 12,13-epoxytrichothec-9-ene (**1**, Figure 1) core with varying degrees of oxygenation, cyclization, and additional functionalization.[1] The class is subdivided into four structural subtypes: type A are characterized by monooxygenation at C3 and C15 accompanied by monooxygenation or no oxygenation at C8, type B contain a ketone at C8, type C harbor a C7,C8 epoxide, and type D have alcohols at C4 and C15 that are often connected in an 18-membered macrocycle.[2] Within these subclasses, further functionalization of the core and the bridging macrocycle gives rise to an enormous amount of structural diversity, resulting in a class that contains >200 characterized natural products.[3]

Since the fungi that produce trichothecenes grow on several grain and cereal crops, in forages, and in damp buildings,[4] they have been the subject of extensive research and regulation.[5] The type A trichothecene T2-toxin (**2**) was responsible for mass deaths in the USSR in the 1940s, bringing international attention to the class.[6,7] The type B deoxynivalenol (**3**) is less toxic than T2-toxin, but is the most common trichothecene food contaminant.[8] Type D trichothecenes such as verrucarin A (**4**) are less abundant in nature but are among the most toxic family members, and vary greatly in both their core structure and the bridging macrocycle.[9] In addition to being ingested, some trichothecenes can be absorbed through the skin and aerosolized with LD_{50} in mice in the low mg/kg and sub-ppm ranges, respectively.[2] Due to their toxicity and prevalence, trichothecenes are the subject of extensive attention and regulation from global governments, which often include guidelines for their maximum allowable content in food and for responses to their potential uses as chemical weapons.[10,11]

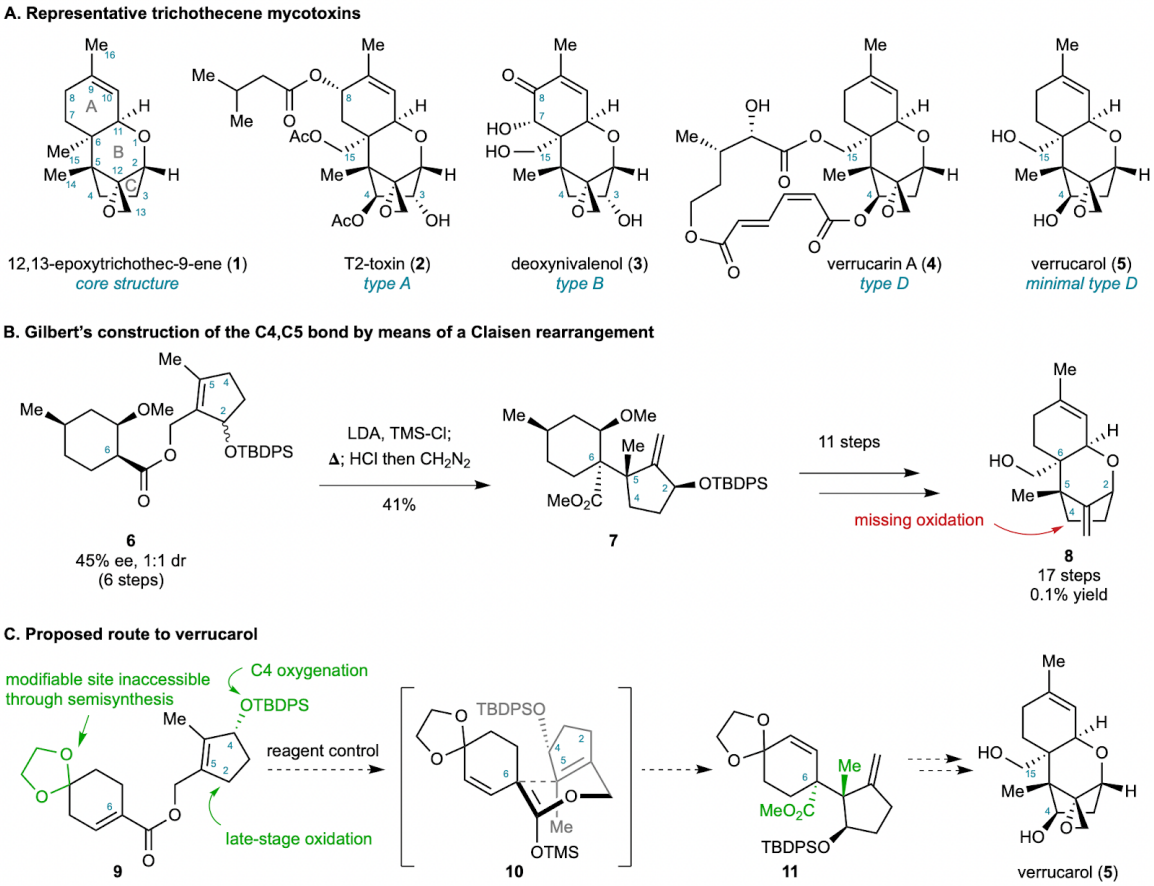


Figure 1: (a) Representative trichothecene mycotoxins from various subgroups. (b) Progress towards the verrucarol core by the Gilbert group featuring a Claisen rearrangement, (c) Proposed route to verrucarol with required functional groups for the complete construction of the natural product.

In addition to research pertaining to trichothecenes' toxicity, there has been much interest in their potential for therapeutic development. The anticancer, antifungal, and immunomodulatory activity of trichothecenes have been active areas of research for decades, and have been recently reviewed.[9,12] The type B trichothecene trichothecin induces apoptosis in several cancer cell lines at sub-micromolar concentrations with no effect on non-cancer cell lines.[13,14] Antibody-drug conjugates with T2-toxin (type A) as the warhead have been shown to be effective at suppressing tumor growth in a mouse model of human colon cancer.[15] Diacetoxyscirpenol (also called anguidine, type A) was evaluated in several phase-II clinical trials against various types of cancer

but was not progressed due to side effects and low response rates,[16–19] although recent research on diacetoxyscirpinol's HIF-1 inhibitory activity has suggested these results be re-visited.[20] The macrocyclic type D trichothecenes have received significant attention for their anticancer, antifungal, and immunomodulatory effects.[9] Most of the studies have been conducted on verrucarin A,[12] but structure activity relationships have been established for many other type D trichothecenes through comparison of natural products and semisynthetic analogs.[9] These studies indicate that small structural changes to the core and the C4,C15 macrocyclic tether can have profound effects on the various biological activities of the type D subclass. Notably, type D trichothecenes can be accessed from the natural product verrucarol (**5**, Figure 1), a minimal type D scaffold that contains the requisite C4, C15 alcohols for macrocycle formation.[21–26]

Trichothecenes have multiple effects on multiple cellular pathways, confounding determination of a single mechanism of action.[27,28] Studies dating back to 1968 have identified their ability to inhibit the eukaryotic cytosolic ribosome during initiation, elongation, or termination, depending on the specific trichothecene.[29,30] It was later shown that trichothecenes also inhibit the mitochondrial ribosome.[31] These two mechanisms of action cause manifold downstream effects and could explain many of the class's toxic effects, although protein synthesis inhibition in cells has been shown to not always correlate with toxicity in mice.[32] The mitochondrial protein synthesis inhibition is also interesting, since the mitoribosome has high homology to the bacterial ribosome,[33] yet trichothecenes generally have poor antibiotic activity,[34] although this could be an effect of differing accumulation in bacteria vs mitochondria.

X-Ray crystallography data characterizing the binding of T-2 toxin, deoxyvalenol, and verrucarin A (types A, A, and D respectively) to the *S. cerevisiae* ribosome was reported in 2014, revealing that trichothecenes bind to the peptidyltransferase center at the A site.[35] This binding site is shared

with homoharringtonine, a chemotherapeutic approved for the treatment of chronic myeloid leukemia. The authors proposed an explanation for the selective inhibition of eukaryotic vs bacterial ribosomes caused by the positioning of U2504 (*E. coli* numbering), but this hypothesis is not in agreement with trichothecenes' ability to inhibit the mitoribosome, in which U2504 is positioned similarly to the bacterial ribosome.[33] A further detailed analysis of this structural data was published in 2021, expounding on the structure–activity relationships that could be gleaned from the differential binding of the three trichothecenes, but also pointing out that the resolution (3.1-3.3 Å) of the structures was limiting as metal ions, hydrogen bonds, and solvent atoms could not be modeled.[36] Higher resolution structural data would be enabling for structure-guided medicinal chemistry on trichothecenes and could reveal the mechanistic underpinnings of selectivity between different types of ribosomes.

Methods to systematically modify trichothecenes by synthesis would enable thorough evaluation of their structure activity relationships, which could lead to improvements in therapeutic effects and reduction of toxicity. Additionally, modification of the trichothecene scaffold coupled with high resolution structural data would provide insight into their disparate effects on bacterial, cytosolic, and mitochondrial ribosomes. Chemical synthesis of trichothecenes, however, presents a formidable challenge due to the congested core containing up to nine contiguous stereocenters surrounding two hindered vicinal tetrasubstituted carbons. Syntheses of several members of this family have been published and are summarized in a recent review.[37] Although some fully synthetic routes have been developed towards the minimal type D pharmacophore verrucarol , these routes are often long (≥17 steps) and non-ideal for analog development.[38–42] Among them, the first total synthesis was reported by Nugent and Schlessinger, with an overall yield of 3.4% achieved over 17 linear steps from the precursor bicycle 1.[22] Trost and Roush concurrently reported

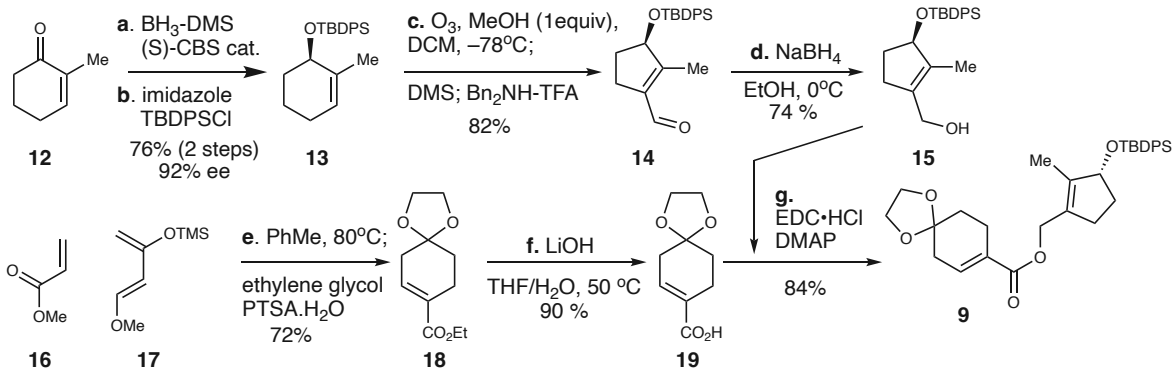
syntheses of (±)-verrucarol in the same year. [23,24] Koreeda later published the synthesis of an advanced intermediate via aluminum-catalyzed intramolecular Diels–Alder reaction that intercepts the Trost and McDougal route.[25] Notably, in 1998, Tadano achieved the enantioselective total synthesis of (−)-verrucarol from d-glucose.[38–42]

The synthesis of compact, highly functionalized terpenes such as trichothecenes, has long been a formidable challenge. We draw inspiration from the approach towards verrucarol reported by the Gilbert lab,[43] which featured the strategic use of Claisen rearrangement to construct vicinal C5,C15 quaternary centers in ~8 steps from commercially available materials.[43, 44] Their approach used a methoxy group at C11 for chelation control during the formation of the Claisen precursor and lacked the required oxygenation at C4, a key determinant of biological activity and an anchor point for the macrocyclic tether in type D trichothecenes.[45] We hypothesized that the C4 alcohol could be incorporated from the start and that the selectivity of the formation of the Claisen precursor could arise from reagent control.[46,47] This would simplify and shorten the approach and allow access to verrucarol, which is itself a precursor to the majority of the macrocyclic type-D trichothecenes

1.1.2 Complete synthetic route toward verrucarol and trichodermin

Total synthesis of verrucarol, using commercially available building blocks **12**, **16**, and **17**, is outlined in Figure **2**. The synthesis of the left half commenced with Corey-Itsuno reduction of **12**, followed by TBDPS protection, yielding R-alcohol **13** with 76% yield and 92% ee. Ozonolysis of **13**, followed by a Robinson annulation in the presence of dibenzylammonium trifluoroacetate, provided an aldehyde in a single step. While addition of methanol was important to accelerate reduction of ozonolide, using methanol as a solvent led to unknown major side product. This

aldehyde was then reduced to yield intermediate **15** (left half). I have spent around 1 month optimizing this reduction using Luche condition ($NaBH_4$ and $CeCl_3.H_2O$) or different batch of $NaBH_4$ as well as vary in temperature which yielded unfruitful results. Switching solvent to ethanol led to reproducible way to reduce enal **14** at large scale.



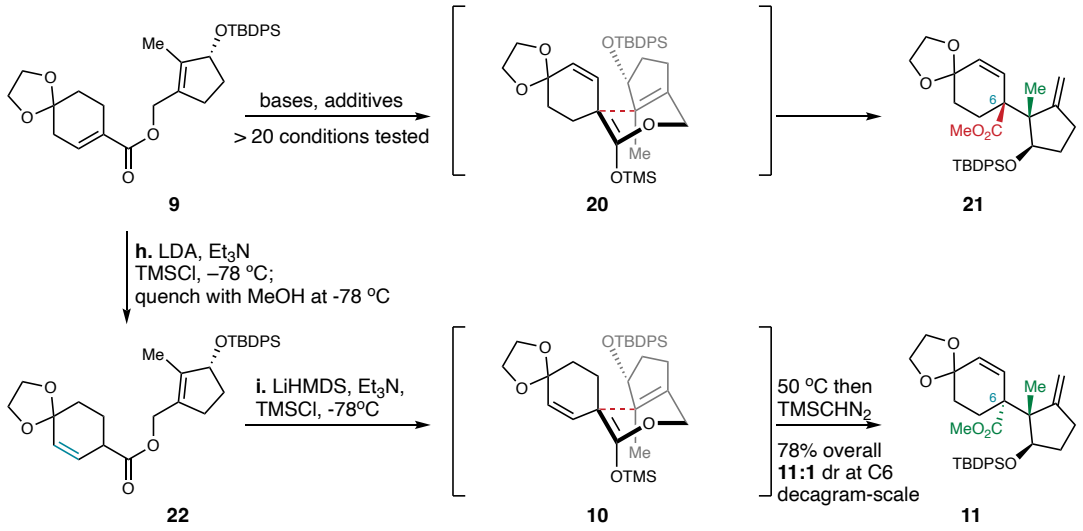
Reagents and conditions: (**a**) BH_3. DMS, (S) - CBS catalyst, THF, 0 °C, 30 mins; (**b**) imidazole, TBDPS-Cl, DMF, 0 °C to rt, 48 hrs, 76% (2 steps), 92% ee; (**c**) O_3, MeOH (1 equiv), DCM, -78 °C, 1 h to 2 hrs, then DMS for 24 hrs, then Bn_2NH - TFA, reflux, 12 hrs, 82 %; (**d**) $NaBH_4$, EtOH, 0 °C, 1 hr, 74%; (**e**) benzene, 80 °C, 48 hrs, then PTSA. H_2O, rt, 30 mins, then ethylene glycol, 8 hrs, dean stark, 72 %; (**f**) LiOH, THF/ H_2O, 60 °C, 12 hrs, 72 % (**g**) EDC. HCl, DMAP, rt, 48 hrs, 72 %

Figure 2: Synthesis of key Claisen precursor **9**.

On the other hand, the right half synthesis began with a Diels–Alder reaction between Danishefsky diene and methyl acrylate. With the assistance of PTSA and ethylene glycol treatment, this reaction generated enone **18** with a high 72% yield. Saponification led to the formation of the right half acid **19**. The coupling of the left half **15** with the right half **19** was achieved through EDC in the presence of DMAP.

A crucial step in our process involved the generation of silyl ketene acetal, followed by heating to initiate an Ireland-Claisen rearrangement, resulting in the formation of intermediates **11** or **21**. The OTBDPS group played a pivotal role in shielding one face of the chair transition state, ensuring the correct stereocenter at C14. Conversely, the formation of the E/Z isomers of silyl ketene acetal **10** and **20** dictated the stereocenter of C6 ester. Drawing from prior research conducted by the Gilbert lab, the desired product could be reliably obtained through the Z isomer. It became evident

that the E/Z isomer ratio of silyl ketene acetal could be influenced by the combination of bases, additives, and solvent, as demonstrated by the Column group.[46,47] Consequently, we embarked on a systematic exploration of various conditions during the optimization of this transformation (refer to the Supplementary Information). Ultimately, only the combination of LDA (2.2 equiv), alkyl amine (TEA 7 equiv), and TMS-Cl (7 equiv) yielded the rearrangement products, which were inseparable, in a 50% yield with a 2.2:1 diastereomeric ratio at C6.

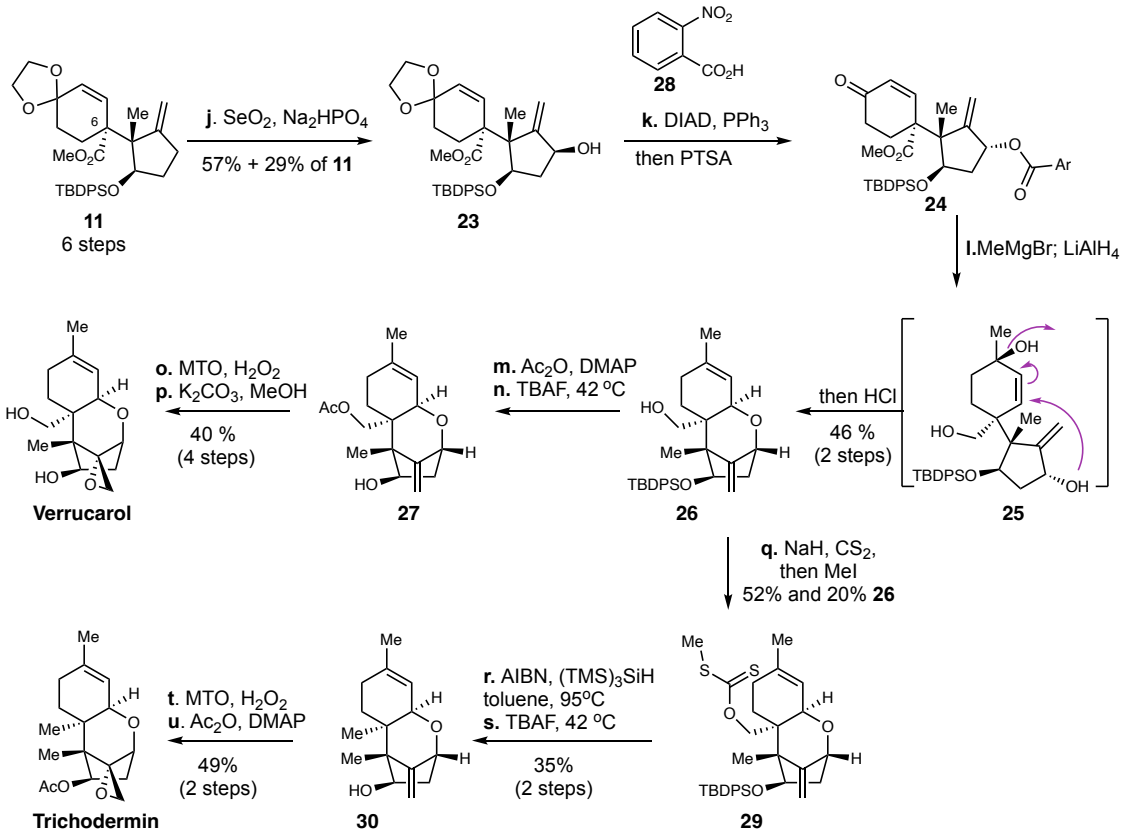


Reagents and conditions: (**a**) LDA, Et_3N, TMS-Cl, THF, -78 °C, 1 hr, then MeOH, 30 mins, then slowly add NH_4Cl; (**i**) LiHMDS, Et_3N, TMS-Cl, THF, -78 °C, 1 hr, then 50 °C, 3 hrs quench NH_4Cl at rt, work-up. then $TMSCHN_2$, MeOH, 0 °C to rt, 5 hrs, 78 % overall, 6:1 dr at C6

Figure 3: Optimization of the Claisen rearrangement.

In the process of trying to understand the mechanism of this reaction, we divided it into two steps, carefully scrutinizing each intermediate. The silyl ketene acetal intermediate was isolated via trituration and analyzed it using HNMR spectroscopy. At 23°C, we observed peaks suggesting one Claisen product, E/Z isomers, but lowering the trituration temperature enabled isolation of a pure E/Z mixture. This revealed one isomer's faster rearrangement, yet we couldn't identify which. Thus, after forming the silyl ketene acetal from **9**, reaction was warmed to room temperature and ran for 30 minutes before quenching with NH_4Cl. This yielded 22% of Claisen product with 7:1 dr, which later confirmed to be an undesired product **21**, indicating the E isomer's faster

rearrangement, and 50% of **22**. A fortuitous discovery occurred when switching from LDA to LiHMDS for compound **22**, providing the desired product **11** with a 11:1 diastereomeric ratio (see Supplementary Information).Treatment of **9** with LDA, Et_3N, and TMS-Cl, quenched with methanol at -78°C to cleanly convert **9** to **22**, avoiding proton sources like HCl, NH_4Cl, or water, which led to mixtures. Sequence from **9** to **11** was done on decagram scale with 78% yield overall and consistent diastereomeric ratios.



Reagents and conditions: (**j**) SeO_2, Na_2HPO_4, dioxane, 50°C, 1.5 hrs, 57% + 29% **11**; (**k**), **28**, DIAD, PPh_3, toluene, 0 °C to rt, 10 hrs, work up, then PTSA, Acetone/ H_2O; (**l**) MeMgBr, Et_2O, 23°C, 8 hrs, then $LiAlH_4$, 0°C -> 23°C, 5 hrs, then quench 6M HCl, 3 hrs, 46% (2 steps); (**m**), Ac_2O, DMAP, DCM, rt, 3 hrs; (**n**), TBAF, THF, 42 °C (**o**) MTO, H_2O_2, pyridine, DCM, rt, 12 hrs; (**p**), K_2CO_3, MeOH, rt, 2 hrs, 40 % (4 steps); (**q**), NaH, CS_2, THF rt, 2hrs, then MeI, 30 mins, 52% + 20% of **26**; (**r**), AIBN, $(TMS)_3SiH$, toluene 95 °C, 1hr; (**s**), TBAF, THF, 42 °C, 15 hours, 35% (2 steps) (**t**) MTO, H_2O_2, pyridine, DCM, rt, 12 hrs; (**u**), Ac_2O, DMAP, DCM, rt, 2 hrs, 49%

Figure 4: Completion of the synthesis of verrucarol and trichodermin

Having obtained compound **11**, allylic oxidation was done using SeO_2 and Na_2HPO4, resulting in a single diastereomer at C2 alcohol. Initially, we hypothesized that the OTBDPS group might bias

the formation of the new alcohol with the correct S stereocenter. However, after a meticulous analysis using NOESY, it was indeed R-alcohol **23**, obtained with a 57% yield and 29% recovery of starting material. Attempts to improve yield by adding more SeO_2 or extending reaction time proved counterproductive. A Mitsunobu reaction on compound **23**, employing DIAD, PPh_3, and **28**, followed by acidic treatment, led to the formation of enone **24**. I was unable to obtain pure **24** as it overlapped with undetermined side product. Thus column chromatography was used to get the product as pure as I could before the next step. Subsequent carbonyl addition with MeMgBr, ester reduction with $LiAlH_4$ in ether, and quenching with 6N HCl induced SN2' cyclization, affording the desired product with the correct stereocenter at C6, achieved in two steps with a 46% yield. Further steps involved the acetylation of the primary alcohol and TBDPS group removal, yielding intermediate **27**. Various attempts to epoxidize the diol analog of **27** proved unsuccessful in achieving regioselectivity, consistent with prior studies on similar substrates. Additionally, while epoxidation of **27** with DMDO demonstrated regioselectivity, but poor stereoselectivity. mCPBA provided the correct stereochemistry on the epoxide at -20°C but lacked regioselectivity if the reaction duration was extended. Fortunately, MTO and H_2O_2 furnished a single diastereomer of the desired product, which, after saponification, yielded verrucarol with a 40% yield over four steps.

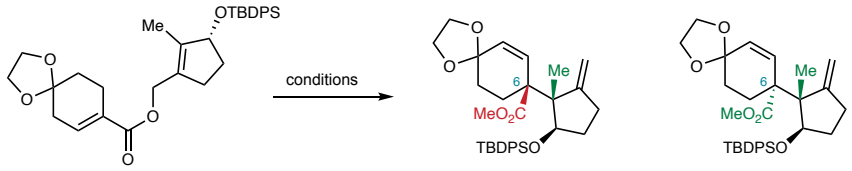
In the course of our investigations, we realized our synthetic route not only allowed for the efficient synthesis of verrucarol but also for the diversification towards other trichothecene natural products like trichodermin, contingent upon the successful deoxygenation of C15 alcohol. Commencing with compound **26**, a strategic sequence involving treatment with NaH, carbon disulfide, and methyl iodide facilitated the formation of xanthate **29**, moderately yielded 27%, and 25% recovery of the starting material initially. Intermediate before methyl iodide addition had similar rf as

starting material; thus, it was challenging to monitor reaction progressed. Despite extend reaction durations or elevated temperature, reaction yield remained low. Fortunately, it was later discovered that reaction yield improved to 52% with 20% recover starting material when using similar equivalent of NaH and CS_2 instead of too much excess of CS_2.

In pursuit of introducing the pivotal O-methyl O-phenyl thiocarbonate functionality as an alternative to methyl xanthate **29**, reaction of **26** with O-phenyl chlorothionoformate and DMAP resulted solely in the recovery of the starting material. Initial attempts employing tributyl tin hydride as the hydride source and AIBN were met with limited success, attributed to the presence of BHT as a radical stabilizer. Subsequent endeavors to purify the tin hydride via distillation proved futile, prompting the exploration of alternative strategies such as silane sources. Fortunately, treatment of intermediate **29** with AIBN and super silane at elevated temperature (95 °C) , followed by treatment with TBAF at 42 °C yielded the desired product **30** at 35% yield**.**

Moreover, while the conversion of intermediate **26** to methyl oxalate proceeded smoothly, attempts to replicate the deoxygenation step under identical conditions resulted in the formation of a complex mixture of products. Nonetheless, through further manipulations encompassing epoxidation, and acetylation, the target trichodermin was ultimately isolated.

1.1.3 Fail attempt to optimize selectivity of Claisen rearrangement



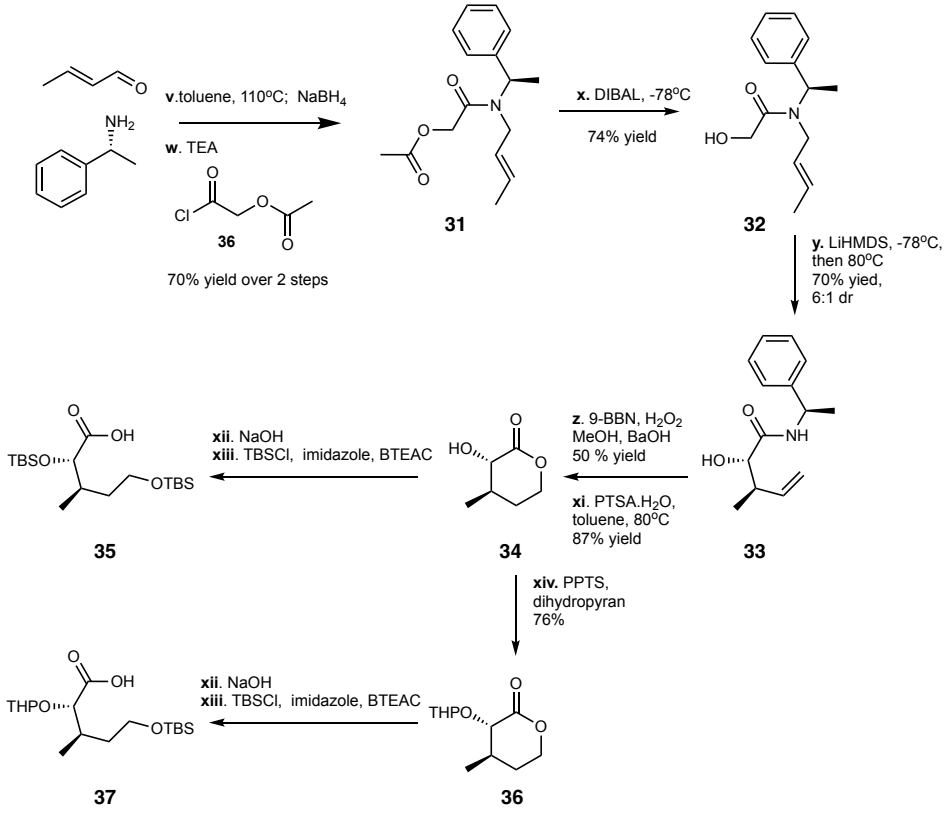
9 **21** **11**

entry	Solvent	Base and additive	Silyl source	Condition	Result (21 : 11)
1	THF	DBU	N/A	50 °C , 24 hrs	recover SM
2	THF	LiHMDS	TMS-Cl	-78 °C, 1 hr then 60 °C, 3 hrs	recover SM
3	CCl_4	LiHMDS	TMS-Cl	-78 °C, 1 hr then 60 °C for 3 hrs	recover SM
4	DCE	LiHMDS	TMS-OTf	23 °C, 24 hrs	recover SM
5	DCE	Et_3N	TMS-OTf	23 °C, 48 hrs	recover SM
6	DCE	Et_3N	TMS-OTf	50 °C, 24 hrs	recover SM
7	THF	LiHMDS, Et_3N	TMS-Cl	-78 °C, 3 hrs then 60 °C, 3 hrs	recover SM
8	THF	LDA	TMS-Cl	23 °C, 5 hrs	recover SM
9	THF	LDA, Et_3N	TMS-Cl	-78 °C, 1 hr then 60 °C, 3 hrs	2 : 1
10	THF	LDA, Et_3N	TMS-Cl	0 °C, 1 hr then 60 °C, 3 hrs	2 : 1
11	THF	LDA, DMPU	TMS-Cl	-78 °C, 1 hr then 60 °C, 3 hrs	recover SM
12	THF	LDA, DMPU	TMS-Cl	0 °C, 1 hr then 60 °C, 3 hrs	complex mixture
13	THF	LDA, HMPA	TMS-Cl	-78 °C, 1 hr then 60 °C, 3 hrs	recover SM
14	THF	LDA, HMPA	TMS-Cl	0 °C, 1 hr then 60 °C, 3 hrs	complex mixture
15	Toluene	LDA, Et_3N	TMS-Cl	-78 °C, 1 hr then 60 °C, 3 hrs	complex mixture
16	THF	LDA, Me_3N	TMS-Cl	-78 °C, 1 hr then 60 °C, 3 hrs	2.2 : 1
17	THF	LDA, nBu_3N	TMS-Cl	-78 °C, 1 hr then 60 °C, 3 hrs	1.5 : 1
18	THF	MgHMDS, Et_3N	TMS-Cl	-78 °C, 1 hr then 60 °C, 3 hrs	recover SM
19	THF	NaHMDS, Et_3N	TMS-Cl	-78 °C, 1 hr then 60 °C, 3 hrs	recover SM
20	THF	nBuLi, Me_2NH, Et_2N	TMS-Cl	-78 °C, 1 hr then 60 °C, 3 hrs	complex mixture
21	THF	nBuLi, Me_2NH	TMS-Cl	-78 °C, 1 hr then 60 °C, 3 hrs	recover SM
22	THF	LDA, Et_3N, LiCl	TMS-Cl	-78 °C, 1 hr then 60 °C, 3 hrs	2.3 : 1
23	THF	LDA, Et_3N	TBS-Cl	-78 °C, 1 hr then 60 °C, 3 hrs	recover SM
24	THF	LDA, Et_3N	TBS-Cl	0 °C, 1 hr then 60 °C, 3 hrs	recover SM
25	THF	LDA, Et_3N	TBS-Cl	23 °C, 1 hr then 60 °C, 3 hrs	recover SM
26	THF	LDA, Et_3N	Tes-Cl	-78 °C, 1 hr then 60 °C, 3 hrs	recover SM
27	THF	LDA, Et_3N	Tes-Cl	0 °C, 1 hr then 60 °C, 3 hrs	recover SM

Figure 5: Representative conditions tested to optimize Claisen rearrangement.

Numerous bases were systematically evaluated for their efficacy in deprotonating the gamma proton of substrate **9** to generate the desired silyl ketene acetyl moiety. Remarkably, a precise combination of LDA, alkyl amine, and TMSCl proved requisite for this transformation. Alternative bases explore failed to elicit the desired reactivity. Furthermore, it was observed that trimethyl silyl ketene acetal had the optimal steric dimensions for the intended application. Attempts employing larger silyl groups were met with limited success, highlighting the unique efficacy of TMS-Cl in this context. Despite tremendous effort, I was unable to control E/Z isomer of silyl ketene acetal generated from **9**. Thus, my best selectivity for nearly 1.5 years was 2:1 favor undesired product showed in figure **5**.

1.1.4 Progress toward total synthesis of Verrucarin A

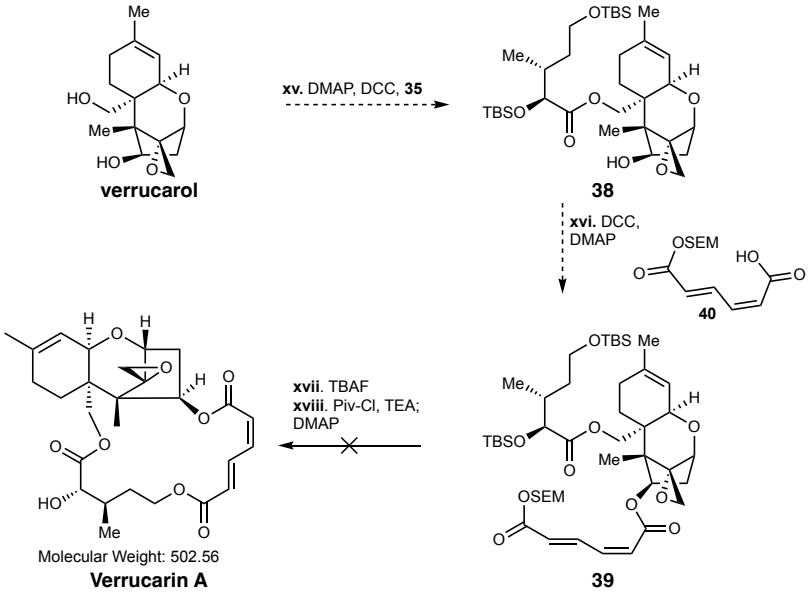


Reagents and conditions: (**v**) reflux, 45 mins, then $NaBH_4$, MeOH, -78 °C -> rt, 15 hours; (**w**), TEA, **36**, DCM, rt, 15 hrs, 70% (2 steps) ; (**x**) DIBAL, THF, -78 °C, 74% yield; (**y**), LiHMDS, toluene, - 78 °C, 1 hr then 80 °C, 15 hrs, 70% yield, 6:1 dr; (**z**), 9-BBN, THF, rt, 2 hrs, then MeOH, BaOH, 0 °C -> rt ,30 mins then H_2O_2 0 °C -> rt ,30 mins, 50% yield; (**xi**), PTSA.H_2O, toluene, 80 °C, 1 hr, 87%; (**xii),** NaOH, MeOH:H_2O, rt, 17 hrs; (**xiii**), TBSCl, imidazole, BTEAC, THF, rt, 15 hrs. (**xiv**) PPTS, dihydropyran, DCM, rt, 15 hrs, 76%

Figure 6: Modular and scalable route to access left half of verrucarin A strap

Inspired by the work of Tsunoda and colleagues, who utilized α-hydroxy amino acids to synthesize glycoamide,[53] which was similar the left moiety of verrucarin A, we started the synthesis of this natural product following the successful synthesis of verrucarol. In the begining, no scalable route for the synthesis of the pivotal aza-Claisen precursor **32** was available. Given the necessity for a trans-alkene to establish the correct stereochemistry of the methyl group, reductive amination of R-(+)-α-phenylethylamine to predominantly afford trans-crotoaldehyde emerged as the optimal strategy. Attempts to alkylate phenylethylamine with E-crotylbromide proved unfruitful. Amidation of the resultant crotylamine to glycolic acid utilizing conventional methods

such as DCC, EDC, or HOBt yielded suboptimal conversions, with the majority of the starting material recovered. Consequently, (chlorocarbonyl)methyl acetate was employed, albeit introducing additional synthetic steps, to enable a scalable route. DIBAL reduction of **31** afforded the desired α-hydroxy compound **32**. Employing milder saponification conditions such as K_2CO_3/MeOH or NaOH, successful hydrolysis was not achieved, presumably due to enolate formation leading to reaction quenching.



Reagents and conditions: (**xv**) DCC, DMAP, **35**, DCM, rt, 15 hours; (**xvii**), EDC.HCl, DMAP, **40**, DCM, rt, 15 hrs, ; (**xvii**) TBAF, THF, rt , 2 hrs; (**xviii**), Piv-Cl, TEA, DCM, rt, 2 hrs, then concentrate, DMAP, toluene, rt, 15 hrs

Figure 7: Initial attempt to synthesize Verrucarin A

Treatment of **32** with LiHMDS to generate a stable enolate, which upon heating underwent an aza-Claisen rearrangement, furnished the desired product **33** in 70% yield, with a diastereomeric ratio of 6:1, comparable to the seminal study. Subsequent hydroboration followed by oxidation delivered the dihydroxy product in 50% yield. Initially envisaging a double silyl protection strategy followed by auxiliary removal, attempts utilizing various acid/base combinations and different protecting groups (TES, TBS, TBDPS) proved futile. High-temperature acidic conditions

were ultimately required for amide cleavage, resulting in the removal of all protecting groups and furnishing verrucarin lactone 34 as the final product. We abandoned our previous approach and resorted to lactone formation utilizing PTSA and toluene. Treatment of the lactone with 1 equivalent of NaOH, followed by TBS-Cl and BTEAC, furnished the desired intermediate for macrocyclization. Notably, this product exhibited instability on TLC, consistently displaying two distinct spots upon staining with CAM. To ascertain **35** was an acid the compound was subjected to treatment with TMS-diazomethane, resulting in the clean conversion to the methyl ester.

Concurrently, the synthesis of the complementary right-half acid was conducted by Dr. Ian Seiple utilizing a known protocol. With both acid components, we embarked on the synthesis of verrucarin A. However, attempts at esterification of verrucarol and the left-half acid **35** using DCC and DMAP proved sluggish, with verrucarol starting material remained even after prolonged reaction times and excess reagents. Following workup, inadvertent loss of verrucarol starting material occurred during the use of DCM as the solvent. Nonetheless, isolation of some ester product selectively at the C15 alcohol position was achieved. Subsequent esterification at the C4 position utilizing EDC·HCl and DMAP proceeded more smoothly, possibly attributed to a more activated acid **38**. Upon assembly of the full linear compound 39, global silyl deprotection with TBAF was done.

Initial attempts at macrocyclization employing DCC and DMAP in DCM proved unfruitful, yielding only starting material. Subsequently, efforts were directed towards generating a Piv-anhydride intermediate by treatment with Piv-Cl and TEA, followed by DMAP. Despite observing the product mass on LCMS, NMR spectra of our crude reaction didn't match verrucarin A spectrae. Preparative TLC analysis revealed the absence of verrucarin A, with only unknown side products

detected. Limited scale of the reaction hindered thorough characterization by 2-D NMR techniques.

After careful analysis of **38** and **39**, we came to realize that I did not make both of them. Various esterification conditions tested to make **38** like EDC, Shiina condition, PivCl, DMAP were unfruitful. Therefore, we decided to THP protected lactone **34** to yield **36**. Following the same sequences shown in Figure **6**, I was able to make acid **37** which was a known starting material to make verrucarin A.[52] Interesting, silyl ester of **37** was quite stable in 1M HCl, and only converted to **37** upon extended acidic treatment.

Chapter 1.2: Biological Activity of Verrucarol and its analog

1.1.1 Cryo-EM structure of diacetyl verrucarol bound to 70S subunit of human ribosome (worked was done by Tushar Raskar

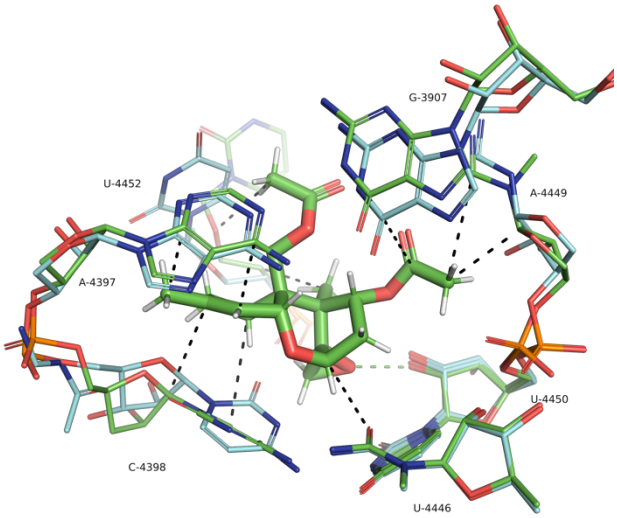


Figure 8: Superposition of human 70S ribosome – diacetyl verrucarol complex with apo human ribosome peptidyl transferase center (apo structure : 4UG0)

Tushar successfully elucidated the cryoEM structure of diacetyl verrucarol bound to the human 70S subunit, achieving an impressive resolution of 2.7 Å. Our initial analysis revealed that the majority of binding interactions predominantly comprised Van der Waals forces, with the exception of a single hydrogen bond formed with base U4450. Overlaying this complex with the structure of the apo human ribosome (see Figure 6) highlighted notable conformational changes induced by diacetyl verrucarol, particularly impacting residues C4398 and U4452. Intriguingly, a similar conformational shift was observed in response to homoharringtonine binding to the 70S subunit. It is important to note, however, that this comparative analysis was somewhat limited by the resolution of the apo structure, which was determined at 3.4 Å, thereby posing challenges in definitively attributing the observed conformational changes solely to inhibitor binding. Notably, leveraging our high-resolution data, we identified a water molecule mediating a bridging hydrogen bond between the carbonyl group of the C4 acetyl moiety and G4451 within the peptidyl

transferase center (PTC). This observation unveils a novel aspect of the molecular interactions underlying diacetyl verrucarol's mode of action within the ribosomal complex. Full characterization and method will be written by Fraser lab and published in our joint paper.

1.2.2 Biological activity of verrucarol and diacetyl verrucarol

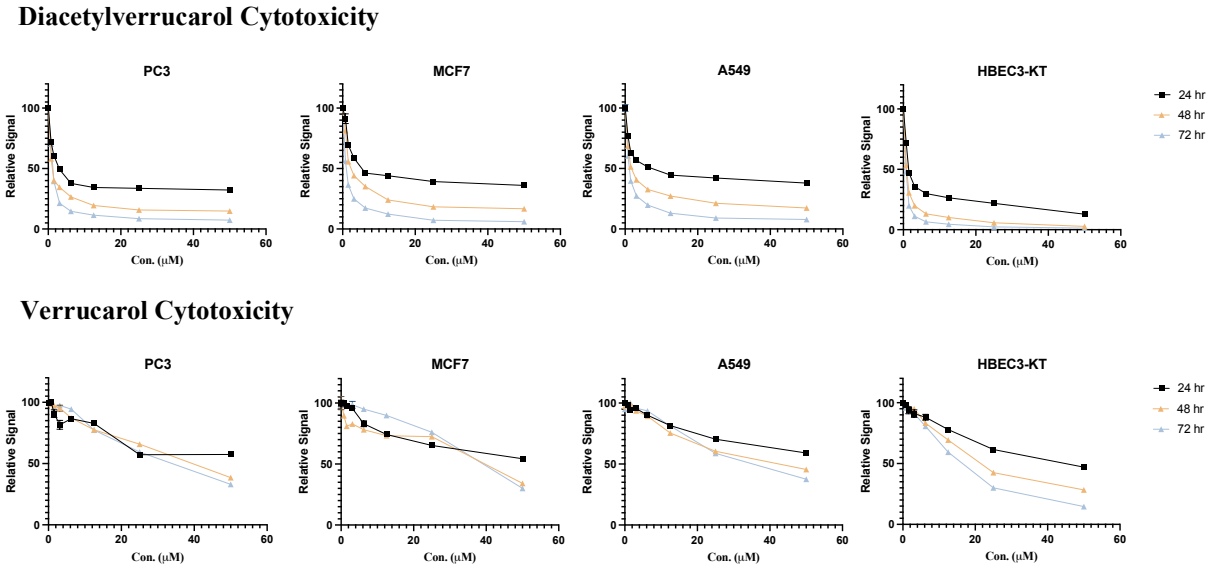


Figure 9: Cytotoxicity of Diacetylverrucarol and Verrucarol. the cytotoxicity of dicaetylverrucarol and verrucarol was evaluated on various cell lines. Cells were seeded in 96-well plates, then treated with compounds for 24 h, 48 h and 72 h, and the cell viability was assayed by MTT

Utilizing the MTT assay, we demonstrated that even with its minimal pharmacophore, verrucarol retains robust cytotoxicity across three distinct cancer cell lines and a mammalian cell line (see Figure 7). Disappointingly, no discernible selective cytotoxicity was observed among the tested cell lines. Huamn bronchial epithelial cells (HBEC3-KT) even exhibited greater sensitivity to verrucarol compared to the cancer cell lines. We postulated that this discrepancy in EC50 values may arise from variations in the growth rates of the respective cell lines.

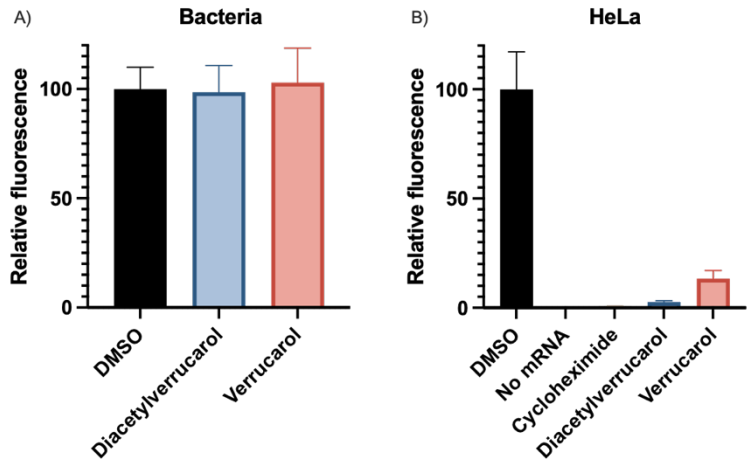


Figure 10: In vitro translation inhibition of diacetylverrucarol and verrucarol at 10 µM. a) bacterial translation inhibition of compounds. Data were acquired using a PURExpress in vitro protein synthesis kit. b) mammalian translation inhibition of compounds measured in vitro coupled transcription / translation Hela cell lysate - based kit

Notably, acetylation of the hydroxyl groups at C4 and C15 resulted in a remarkable enhancement of verrucarol's cytotoxic activity. Drawing insights from the cryoEM structure, we identified that the acetyl moiety at C4 contributes to enhanced compound binding through hydrogen bonding with a water molecule, which interacts with base G4451 within the peptidyl transferase center (PTC). Nevertheless, we reasoned that a single hydrogen bond alone may not account for the pronounced shift in EC50 values observed. To explore this further, we employed an in vitro translation assay in Hela cells, wherein verrucarol exhibited potent inhibition of protein synthesis, albeit marginally less potent than diacetyl verrucarol (see Figure 8). This discrepancy led us to hypothesize that the substantial shift in cytotoxicity EC50 values could potentially be attributed to differences in the cell permeability profiles between verrucarol and diacetyl verrucarol.

Given the inhibitory effect of T2-toxin on mitochondrial protein synthesis, we sought to investigate whether its lack of antibacterial activity could be attributed to a permeability issue within bacterial cells. To address this, we evaluated verrucarol and diacetyl verrucarol, compounds sharing a similar core structure with T2-toxin, in a bacterial in vitro translation assay (see Figure 8).

Remarkably, even at concentrations up to 100 µM, neither compound exhibited any discernible effect on bacterial protein synthesis.

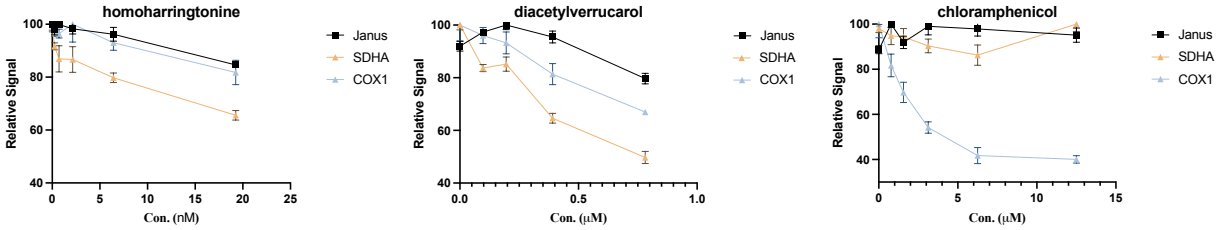


Figure 11: MitoBiogenesis™ In-Cell ELISA Kit to measure mitochondrial protein synthesis inhibition activity of homoharringtonine, diacetyl verrucarol and chloramphenicol at 72 hours in PC3 cells.

To ascertain whether diacetyl verrucarol retained its ability to inhibit mitochondrial protein synthesis, I employed the MitoBiogenesis™ In-Cell ELISA Kit. Notably, diacetyl verrucarol exhibited inhibitory activity against both mitochondrial and cytosolic protein synthesis, albeit displaying a greater potency towards the cytosolic system. The impact of diacetyl verrucarol on mitochondrial ribosomes was most pronounced at concentrations of 0.17 and 0.34 µM, preceding the onset of compound-induced cytotoxic effects. Attempts to conduct this assay at higher concentrations and shorter time frames (6 and 12 hours) proved futile due to the prolonged half-life of the proteins measured in the assay.

In an effort to elucidate the underlying basis for this observed selectivity, we tried to overlay our newly acquired cryo-EM structure with both bacterial and mitochondrial ribosomes. However, despite our efforts, we were unable to discern any notable differences using the current published mitochondrial ribosome structure. Nevertheless, the prospect of elucidating the binding mode of diacetyl verrucarol to mitochondrial ribosomes and delineating its divergence from bacterial

ribosomes presents an intriguing avenue for future investigation, particularly considering the prevailing consensus regarding the structural similarity between the two ribosomal systems.

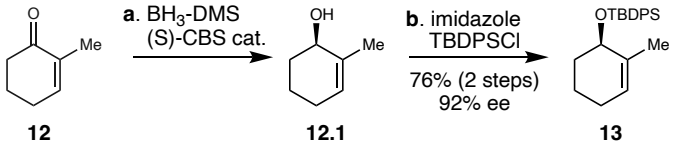
1.3 General Experimental Procedures:

All reactions were conducted in flame- or oven-dried glassware fitted with rubber septa under a positive pressure of nitrogen or argon, unless otherwise noted. All reaction mixtures were stirred throughout the course of each procedure using Teflon-coated magnetic stir bars. Air- and moisture-sensitive liquids were transferred via syringe or stainless steel cannula. Solutions were concentrated by rotary evaporation below 35 °C. Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25-mm, 60-Å pore size, 230−400 mesh, SILICYCLE INC) with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV), and then were stained by submersion in a basic aqueous solution of with an acidic ethanolic solution of anisaldehyde, Cerium Ammonium Molybdate, followed by brief heating.

Materials: DCM, DMF, THF to be used in anhydrous reaction mixtures were dried by passage through activated alumina columns immediately prior to use. Hexanes used were ≥85% *n*-hexane. Reagents were used as received, unless otherwise noted.

Instrumentation: Unless otherwise noted, proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a 400 MHz Bruker Avance III HD 2-channel instrument NMR spectrometer at 23 °C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to residual protium in the NMR solvent ($CHC1_3$: δ 7.26, DMSO-d5: δ 2.50). Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the carbon resonance of the NMR solvent ($CDC1_3$: δ 77.0). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, sxt = sextet, m = multiplet, br = broad, app = apparent), integration, and coupling constant (J) in hertz (Hz).

R- cyclohexanol **13**



A flame-dried 500-L round-bottom flask was charged with a magnetic stir bar, borane–dms complex (1.65 mL, 19.1 mmol, 0.700 equiv) and THF (70.0 mL). A rubber septum with a needle to argon source was affixed, and the solution was cooled to –10 °C (ice–salt bath). A solution of (S)-1-methyl-3,3-diphenyltetrahydro-1H,3H-pyrrolo[1,2-c][1,3,2]oxazaborole in toluene (Sigma, 1.0 M, 5.45 mL, 5.45 mmol, 0.20 equiv) was transferred via cannula over 10 min into the reaction mixture. A solution of enone **12** (3.00 g, 27.2 mmol, 1 equiv) in THF (10 mL) was then added via syringe pump over 45 min. The internal temperature was kept at –10 °C over the course of addition. After 30 min, methanol (10 mL) was added, and the mixture was vigorously stirred for 20 min at –10 °C. Saturated aqueous ammonium chloride (50 mL) was added, and the mixture was allowed to warm to 23 ºC. Stirring was maintained for an additional 30 min. The product mixture was extracted with ethyl acetate (3 x 50 mL). The combined organic layers were dried over magnesium sulfate and concentrated under reduced pressure.

A 250-mL round-bottom flask charged with the crude mixture from the previous reaction was evacuated and flushed with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. A positive pressure of argon was applied with a balloon and dry DMF (24 mL) was added, resulting in a white suspension. The vessel was cooled to 0 ºC in an ice bath. Imidazole (5.56 g, 81.7 mmol, 3 equiv) was added, followed by dropwise TBDPS-Cl (7.00 ml, 27.2 mmol, 1equiv). Reaction was slowly warmed up to 23 ºC and stirring was continued for an

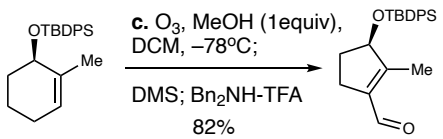
additional 48 h. Water (50 ml) was then added and the mixture was transferred to a separatory funnel and was extracted with EtOAc (4 × 50 mL). The combined organic layers were washed with water (5 × 100 mL) and brine (100 mL) and the washed solution was dried (Na_2SO_4). The dried solution was filtered and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: 100% hexanes) to R- alcohol **13** (7.22g, 76%, 92% ee) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 – 7.66 (m, 4H), 7.46 – 7.32 (m, 6H), 5.46 (s, 1H), 4.05 (s, 1H), 1.99 (s, 1H), 1.71 – 1.61 (m, 4H), 1.58 – 1.47 (m, 2H), 1.38 (s, 1H), 1.14 – 1.02 (m, 9H), 0.86 (d, *J* = 7.9 Hz, 0H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 136.07, 129.48, 129.41, 127.47, 127.36, 124.68, 70.50, 32.46, 27.12, 25.52, 21.33, 19.51, 18.88.

Determination of enantiomeric excess:

To a solution of **12.1** (12 mg, 0.11 mmol, 1 equiv) in DCM (2 mL) at 23 °C was added successively Et_3N (0.12 mL, 0.86 mmol, 8 equiv), DMAP (0.018 g, 0.15 mmol, 1.4 equiv) and (S)- or (R)-Mosher acid chloride (0.08 mL, 0.43 mmol, 4 equiv). After 2 h the mixture was diluted with EtOAc (15 mL). The mixture was transferred to a separatory funnel and washed successively with 1 M aqueous $KHSO_4$ solution (3 x 5 mL), 1 M aqueous NaOH solution (5 mL) and saturated aqueous $NaHCO_3$ solution (3 x 5 mL). The organic phase was dried over $MgSO_4$, the dried solution was filtered, and the filtrate was concentrated. The crude residue was analyzed by ^{1}H-NMR.

Aldehyde **14**



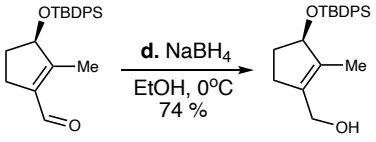
13 **14**

An oven-dried 1-L round-bottom flask equipped with a large stir bar was charged with alcohol **13** (19.16 g, 56.93 mmol, 1 equiv), methanol (2.30 mL, 56.9 mmol, 1 equiv), and DCM (350 mL).

The reaction flask was connected to an ozone generator through plastic tubing and a glass bubbler, and cooled to -78 °C. The system was purged with O2 for 10 min, and ozone generation was initiated. The reaction progress was monitored by TLC (hexane 100 %). Upon complete consumption of starting material (60~120 min), the line from the ozone generator was immediately disconnected. Argon was then bubbled through the reaction mixture for 20 minutes followed by the addition of dimethyl sulfide (8.40 mL, 114 mmol, 2 equiv). The reaction mixture was slowly warmed to 23 ºC and was stirred for 24 h. Dibenzylamine (1.80 mL, 9.36 mmol, 0.164 equiv) and TFA (0.75 mL, 9.85 mmol, 0.173 equiv) was added and the mixture heated at reflux for 12 hours. After complete conversion of the intermediates as indicated by TLC (ADD DETAILS HERE), the reaction mixture was filtered through a short column of silica gel (flushed with DCM).The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography (1% to 3% to 5% acetone in hexanes) to afford enal **14** (16.3 g, 82%) as a clear light yellow oil. NOTE: the crude mixture can be also used directly for next reaction without diminishing yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 10.00 (s, 1H), 7.69 (dddd, *J* = 10.6, 6.4, 2.8, 1.6 Hz, 5H), 7.52 – 7.33 (m, 8H), 4.86 – 4.69 (m, 1H), 2.55 (dd, *J* = 14.6, 10.5 Hz, 1H), 2.22 – 1.87 (m, 6H), 1.77 – 1.59 (m, 1H), 1.17 – 1.02 (m, 12H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 189.24, 137.35, 135.98, 129.89, 129.85, 127.74, 127.64, 82.06, 32.63, 27.02, 26.57, 19.30, 11.85.

Alcohol **15**



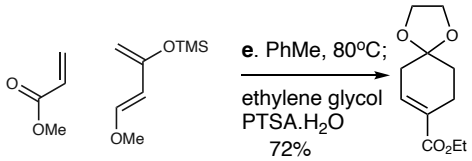
14 **15**

A 250-mL round-bottom flask charged with enal **14** (16.3 g, 46.6 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. Dry ethanol (82 mL) was added, resulting in a clear solution and the vessel was cooled to -78 ºC in a dry ice-acetone bath. $NaBH_4$ (1.76 g, 46.7 mmol, 1 equiv) was slowly added

in 3 different portions, 5 minutes apart. Then, reaction was slowly warmed up to 0 ºC with ice bath. After 1 hour, NH_4Cl was added carefully (Caution: gas evolution!). The vessel was removed from the cooling bath and the system was allowed to warm to 23 ºC while the mixture was rapidly stirred. The biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with DCM (3 × 100 mL). The combined organic layers were washed with water (2 × 100 mL) and brine (100 mL) and the washed solution was dried (Na_2SO_4). The dried solution was filtered and the filtrate was concentrated. The crude residue was purified by flash chromatography (eluent: 5% to 7% to 10% to 12% acetone in hexanes) to afford alcohol **15** (12.6 g, 74%) as a clear oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 – 7.64 (m, 4H), 7.47 – 7.33 (m, 6H), 4.73 (td, *J* = 7.0, 3.6 Hz, 1H), 4.16 (s, 2H), 2.50 – 2.31 (m, 1H), 2.18 – 2.07 (m, 1H), 1.94 (dddd, *J* = 13.2, 8.3, 7.4, 3.0 Hz, 1H), 1.73 – 1.64 (m, 1H), 1.64 – 1.60 (m, 3H), 1.58 (s, 1H), 1.15 (s, 1H), 1.08 (s, 9H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 137.71, 136.30, 136.04, 134.68, 134.32, 129.57, 129.53, 127.52, 127.45, 82.25, 77.35, 77.03, 76.72, 59.64, 32.86, 30.95, 27.10, 19.31, 11.57.

Ester **18**



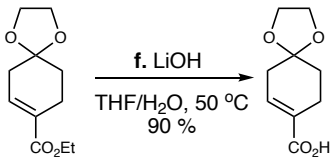
16 **17** **18**

An oven-dried 250 - ml round-bottom flask equipped with a large stir bar was charged with Danishefsky's diene (10.0 g, 58.04 mmol, 1 equiv), ethyl acrylate (12.60 mL, 116.1 mmol, 2 equiv), and toluene (25 mL). Reaction was run at 80 ºC for 48 hrs. Reaction was concentrated under vacuum. Intermediate was diluted with toluene (160 ml) and transferred to oven-dried 250 – ml three-neck flask using cannula. PTSA.H_2O (1.52 g, 8.70 mmol, 0.15 equiv) was added, followed by ethylene glycol (3.88 ml, 69.65 mmol, 1.2 equiv). Dean-stark apparatus as applied, and reaction was heat at 105 ºC for 10 hrs. The vessel was removed from the oil bath and the

system was allowed to cool to 23 ºC. Saturated sodium bicarbonate solution was added, then the biphasic mixture was transferred to a separatory funnel and the layers were separated. The flask was rinsed with ethyl acetate. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated. The crude residue was purified by flash chromatography (eluent: 5% to 10% to 15% ethyl acetate in hexanes) to afford ester **18** (8.84 g, 72%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.87 (tt, *J* = 4.4, 2.4 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.99 (d, *J* = 1.0 Hz, 4H), 2.58 – 2.40 (m, 4H), 1.79 (t, *J* = 6.5 Hz, 2H), 1.27 (td, *J* = 7.1, 1.0 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 166.80, 136.16, 130.09, 107.28, 64.53, 60.38, 36.13, 30.71, 23.58, 14.29.

Acid **19**



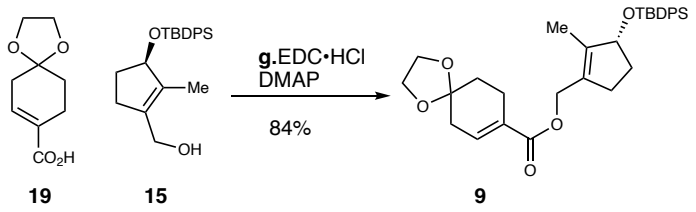
18 **19**

In a 250 – ml round-bottom flask containing solution of ester **18** (8.40 g, 39.6 mmol, 1 equiv) in THF (120 ml) and water (40 ml), lithium hydroxide monohydrate (3.32 g, 79.18 mmol, 2 equiv) was added. The vessel was heated in oil bath at 50 ºC for 8 hrs. The flask was removed from the heat bath and the system was allowed to cool to 23 ºC. Septum was removed and 1M HCl was slowly added until the solution reach pH 1 indicated by pH paper. The mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated to afford acid **19** (6.56 g, 90 %) as yellow solid. The crude was carried forward without further purification.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.04 – 6.95 (m, 1H), 4.00 (s, 4H), 2.58 – 2.44 (m, 4H), 1.86 – 1.76 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.93, 139.11, 129.17, 107.11, 77.34, 77.23, 77.02, 76.71, 64.56, 36.34, 30.63, 23.26.

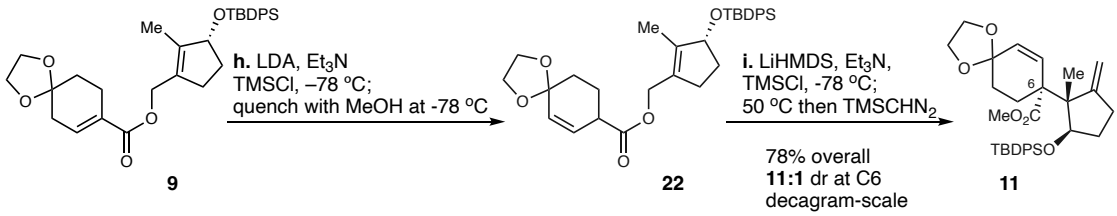
Ester **9**



An oven-dried 250-mL round-bottom flask equipped with a large stir bar was charged with alcohol **15** (4.47 g, 12.2 mmol, 1 equiv), DMAP (150 mg, 1.22 mmol, 0.1 equiv), and DCM (51 mL) under nitrogen. Dry DIPEA (8.50 ml, 48.8 mmol, 4 equiv) was added. Septum was quickly remove to add acid **19** (2.99 g, 16.2 mmol, 1.33 equiv) and EDC. HCl (3.10g, 16.2 mmol, 1.33 equiv). Reaction ran at 23 °C for 48 hrs. Saturated sodium bicarbonate solution was added, then the biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with DCM 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated. The crude residue was purified by flash chromatography (eluent: 5% to 7% to 10% to 15% acetone in hexanes) to afford ester **9** (4.68 g, 72%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 – 7.64 (m, 4H), 7.46 – 7.32 (m, 6H), 6.89 – 6.80 (m, 1H), 4.66 (q, J = 11.9 Hz, 3H), 3.99 (d, J = 0.7 Hz, 4H), 2.56 – 2.47 (m, 2H), 2.43 (s, 2H), 2.32 (d, J = 11.3 Hz, 1H), 2.13 (s, 1H), 1.74 (dt, J = 23.9, 6.4 Hz, 4H), 1.64 (s, 3H), 1.56 (d, J = 0.6 Hz, 5H), 1.27 (d, J = 3.2 Hz, 2H), 1.08 (d, J = 2.5 Hz, 9H), 1.01 – 0.81 (m, 4H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 166.70, 139.79, 136.48, 136.04, 134.23, 132.14, 129.91, 129.58, 129.53, 127.53, 127.46, 107.24, 82.02, 77.35, 77.03, 76.71, 64.53, 61.23, 36.15, 32.83, 31.36, 30.68, 27.08, 23.61, 19.31, 11.71.

Ester **11**



An oven-dried 250-mL round-bottom flask equipped with a large stir bar was charged with dry THF (80 ml) under nitrogen. The solution was cooled to –78 °C in acetone –dry ice bath. Diisopropylamine (4.34 ml, 30.8 mmol, 2 equiv) was added, followed by slowly added n-Buli (Sigma, 2.2 M, 14.0 mL, 30.8 mmol, 2 equiv). Acetone bath was replaced with ice bath and vessel was stirred at 0 °C. After 15 minutes, triethylamine (15 ml, 108.0 mmol, 7 equiv) was added slowly. Reaction was cooled to -78 °C, and stirred for an additional 10 minutes. TMSCl (11.7 ml, 92.4 mmol, 6 equiv) was added quickly (Caution: gas evolution). A solution of ester **9** (8.20 g, 15.4 mmol, 1 equiv) (azeotrope 3 time with toluene) in THF (20 ml) was transferred via cannula dropwise into the reaction mixture. After 1 hour, methanol (12.5 ml, 308.0 mmol, 20 equiv) was slowly added, and reaction was stirred vigorously at -78 °C for additional 30 minutes. NH_4Cl was added carefully (Caution: gas evolution!). The biphasic mixture was transferred to a separatory funnel while reaction mixture still cold and the layers were separated. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated to afford crude ester **22** as yellow oil which was carried forward without further purification.

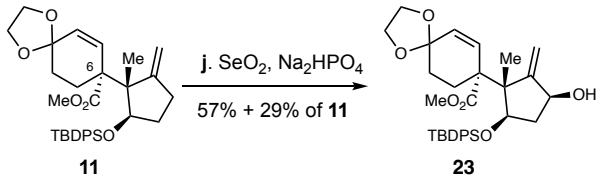
An oven-dried 250-mL round-bottom flask equipped with a large stir bar was charged with dry THF (80 ml) under nitrogen. The solution was cooled to –78 °C in acetone –dry ice bath. HMDS

(6.40 ml, 30.8 mmol, 2 equiv) was added, followed by slowly added n-Buli (Sigma, 2.2 M, 14.0 mL, 30.8 mmol, 2 equiv). Acetone bath was replaced with ice bath and vessel was stirred at 0 °C. After 15 minutes, triethylamine (15 ml, 108.0 mmol, 7 equiv) was added slowly. Reaction was cooled to -78 °C, and stirred for an additional 10 minutes. TMSCl (11.7 ml, 92.4 mmol, 6 equiv) was added quickly (Caution: gas evolution). A solution of crude ester **22** (azeotrope 3 time with toluene) in THF (20 ml) was transferred via cannula dropwise into the reaction mixture. After 1 hour stirring at -78 °C, vessel was transferred to preheat oil bath at 50 °C, and stirred for additional 5 hours. Reaction was then allowed to cool to 23 °C, and NH_4Cl was slowly added to quench reaction. The biphasic mixture was transferred to a separatory funnel, and 1M HCl was added until mixture reached pH 1 indicated by pH paper. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed brine and the washed solution was dried (Na2SO4). The dried solution was filtered, and the filtrate was concentrated to afford foamy oil.

In a oven-dried 250 – ml round-bottom flask containing solution of crude acid above in dry MeOH (50 ml) was cool to 0 °C in ice bath. TMS-diazomethane (Sigma, 2 M, 77.00 ml, 154.0 mmol, 10 equiv) was slowly added. Reaction was slowly warmed to 23 °C over 1 hour, and stirred at that temperature for additional 3 hours. Reaction was then concentrated (Caution: reaction mixture tended to bump during rotatvap). The crude residue was purified by flash chromatography (eluent: 1% to 2.5% to 5% acetone in hexanes) to afford ester **11** (6.58 g, 78%) as a foamy white oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 – 7.66 (m, 4H), 7.39 (q, *J* = 9.7 Hz, 6H), 6.34 (dd, *J* = 10.4, 1.9 Hz, 1H), 5.59 (d, *J* = 10.4 Hz, 1H), 4.94 (d, *J* = 2.5 Hz, 1H), 4.85 (d, *J* = 2.7 Hz, 1H), 4.52 (dd, *J* = 10.3, 6.3 Hz, 1H), 4.09 – 3.82 (m, 4H), 3.61 (s, 3H), 2.27 – 2.14 (m, 1H), 2.02 (dd, *J* = 15.2, 7.1 Hz, 1H), 1.85 – 1.70 (m, 3H), 1.47 – 1.35 (m, 1H), 1.31 – 1.23 (m, 1H), 1.20 (s, 3H), 1.04 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 174.02, 154.55, 136.04, 136.02, 135.01, 134.82, 133.74, 133.45, 129.57, 129.46, 128.10, 127.53, 127.36, 108.88, 104.91, 78.30, 77.36, 77.04, 76.72, 64.70, 64.36, 53.76, 52.82, 51.65, 32.87, 32.48, 31.54, 27.01, 26.49, 20.33, 19.38.

Alcohol **23**

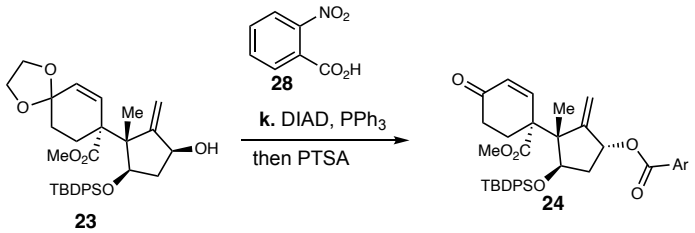


A 100-mL round-bottom flask charged with ester **11** (1.30 g, 2.38 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. Dry 1,4 dioxane (20 mL) was added, resulting in a clear solution. Sodium phosphate dibasic (675 mg, 4.76 mmol, 2 equiv) was added, followed by SeO_2 (528.0 mg, 4.76 mmol, 2 equiv). Reaction was run at 23 °C for 18 hours. DI water (5 ml) was added and mixture was stirred vigorously for 20 minutes. Biphasic layers was filtered through silica plug and washed with ethyl acetate until no yellow streak passed through column. Biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated. The crude residue was purified by flash chromatography (eluent: 5% to 7% to 10% to 12% acetone in hexanes) to afford ester **23** (764 mg, 57%) as a foamy yellow oil, and starting material (377 mg, 29 %).

NOTE: the yield varied depending on SeO_2 batch. Reaction can also be run at 50 °C for 1.5 hour instead of 23 °C for 18 hours to give similar result.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 – 7.61 (m, 4H), 7.40 (dt, *J* = 9.7, 6.9 Hz, 6H), 6.34 (dd, *J* = 10.4, 1.9 Hz, 1H), 5.65 – 5.55 (m, 1H), 4.94 (d, *J* = 2.5 Hz, 1H), 4.85 (d, *J* = 2.7 Hz, 1H), 4.52 (dd, *J* = 10.3, 6.3 Hz, 1H), 4.12 – 3.82 (m, 4H), 3.61 (s, 3H), 2.28 – 2.17 (m, 1H), 2.02 (dd, *J* = 15.2, 7.1 Hz, 1H), 1.78 (t, *J* = 13.3 Hz, 3H), 1.61 (d, *J* = 3.3 Hz, 1H), 1.48 – 1.35 (m, 1H), 1.26 (d, *J* = 7.7 Hz, 1H), 1.20 (s, 3H), 1.04 (s, 9H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 173.95, 157.18, 136.03, 135.98, 133.31, 132.74, 129.72, 129.62, 128.56, 127.61, 127.48, 108.57, 104.74, 77.35, 77.03, 76.71, 72.74, 71.69, 64.74, 64.37, 53.67, 52.90, 51.79, 41.73, 31.45, 30.96, 26.96, 26.81, 20.75, 19.35.

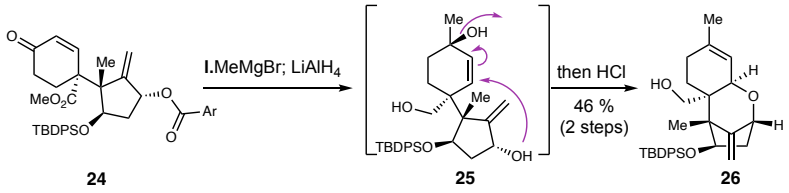
Enone **24**



A flame-dried 100-mL round-bottom flask was charged with a magnetic stir bar, ester **23** (1.28 g, 2.27 mmol, 1 equiv) and toluene (30.0 mL). Vessel was cooled to 0 ºC in an ice-water bath. Septum was quickly removed to add 2-nitrobenzoic acid (403m g, 2.41 mmol, 1.06 equiv) and triphenylphosphine (632 mg, 2.41 mmol, 1.06 equiv). DIAD (0.47 ml, 2.42 mmol, 1.06 equiv) was added dropwise. Reaction was slowly warmed to 23 °C over 1 hour, and stirred at that temperature for additional 12 hours. Saturated sodium bicarbonate was added to quench the reaction. Biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated. The crude residue was purified by flash chromatography (eluent: 7% to 10% to 12% acetone in hexanes).

In a 100-ml round-bottom flask was charged with a magnetic stir bar, product from column above, THF (24.0 mL) and DI water (6 ml). p-Toluenesulfonicacid monohydrate (432.6 mg, 2.27 mmol, 1 equiv) was added, and reaction was run at 23 °C for 24 hours. Saturated sodium bicarbonate was added to quench the reaction. Biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated. The crude product was carried forward without further purification.

Alcohol **26**



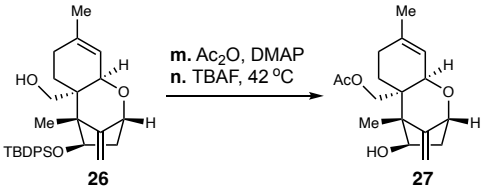
A flame-dried 100-mL round-bottom flask was charged with a magnetic stir bar, crude ester 24 from SI-9 (1.52 g, 2.28 mmol, 1 equiv) and anhydrous diethyl ether (30.0 mL). Vessel was cooled to 0 ºC in an ice-water bath. Methylmagnesium bromide (Sigma, 3 M in hexane, 3.79 ml, 11.4 mmol, 5 equiv) was added dropwise. Reaction was slowly warmed up to 23 ºC and stirred for additional 12 hours. Vessel was then cooled to 0 ºC. LAH (Sigma, 2 M in diethyl ether, 5.69 ml, 11.4 mmol, 5 equiv) was added dropwise. Reaction slowly warmed up to 23 ºC. After 2 hours, reaction was cooled down to 0 ºC, 0.5 ml of 6 M of HCl was added dropwise to quench the reaction. When there was no more gas evolution, removed ice bath and added additional 9.5 ml. After the reaction went to completion indicated by TLC (disappearance of UV spot at rf 0.25 and appearance of spot at rf 0.5 in 1:3 acetone: hexane eluent), the biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated. The crude residue was purified by flash chromatography (eluent: 1% to 2% to 5% acetone in hexanes) to afford alcohol 26 (436 mg, 39% yield over 2 step).

NOTE: alternatively, after 2 hours of LAH treatment, vessel was cooled down to 0 ºC using water bath. 0.4 ml of DI water was added dropwise, followed by 0.4 ml of 2M NaOH, and 1.2 ml of DI water. Reaction was warmed to 23 ºC and stirred for additional 30 minutes. Solution was dried

(Na2SO4). The dried solution was filtered and the filtrate was concentrated. Vessel containing crude mixture was charged with DCM (30.0 ml) and p-Toluenesulfonicacidmonohydrate (433.0 mg, 2.28 mmol, 1 equiv). After 10 minutes stirring at 23 ºC, solution of saturated sodium bicarbonate was added. The biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated. The crude residue was purified by flash chromatography (eluent: 1% to 2% to 5% acetone in hexanes) to afford alcohol 26 with comparable yield as above.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 – 7.62 (m, 4H), 7.51 – 7.33 (m, 6H), 5.26 (dd, *J* = 5.5, 1.6 Hz, 1H), 5.11 (s, 1H), 4.69 (d, *J* = 0.9 Hz, 1H), 4.62 (dd, *J* = 7.2, 3.2 Hz, 1H), 4.31 (d, *J* = 5.2 Hz, 1H), 3.32 (d, *J* = 5.6 Hz, 1H), 3.24 – 3.09 (m, 2H), 2.30 – 2.14 (m, 1H), 1.91 (d, *J* = 8.5 Hz, 2H), 1.82 – 1.66 (m, 3H), 1.54 – 1.35 (m, 4H), 1.25 (s, 3H), 1.05 (d, *J* = 12.3 Hz, 13H), 0.93 – 0.80 (m, 3H), 0.07 (s, 1H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 153.35, 140.64, 136.21, 134.43, 134.24, 129.78, 127.62, 119.01, 104.84, 78.92, 77.35, 77.03, 76.71, 74.59, 66.57, 62.66, 53.00, 44.09, 41.68, 28.28, 27.14, 23.28, 19.82, 19.36, 12.78.

Acetate **27**

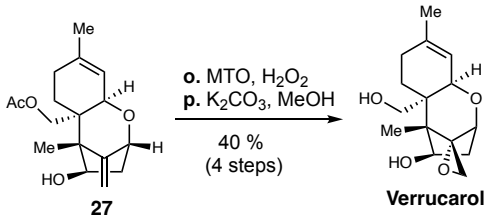


A 5-mL round-bottom flask charged with alcohol 26 (35 mg, 0.072 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. Dry DCM (2 mL) was added, resulting in a clear solution. Septum was quickly removed to add DMAP (8.75 mg, 0.072 mmol, 1 equiv), followed by triethylamine (0.05 ml, 0.358 mmol, 5 equiv). Vessel was cooled to 0 ºC using ice – water bath, and acetic anhydride (20.3 µL, 0.215 mmol, 3 equiv) was added dropwise. Reaction was slowly warmed up to 23 ºC and stirred

for additional 2 hours. Solution of saturated sodium bicarbonate was added. The biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated. The crude was carried forward.

A flame-dried 10-mL round-bottom flask was charged with a magnetic stir bar, crude mixture above, TBAF (1 M in THF, 0.358 mL, 0.358 mmol, 5 equiv) and anhydrous THF (2.0 mL). Reaction was heat to 42 ºC and stirred for 15 hours. Vessel was cooled down to 23 ºC. Saturated sodium bicarbonate was added to quench reaction and the biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated. The crude residue was purified by flash chromatography (eluent: 12% to 15% to17% to 20% acetone in hexanes) to afford alcohol 26.

Verrucarol



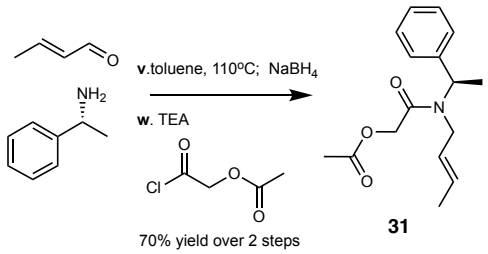
A 5-mL round-bottom flask **(1)** charged with alcohol 27 (20.9 mg, 0.072 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. Dry DCM (1.5 mL) and anhydrous pyridine (40.5 µL, 0.50 mmol, 7 equiv)

were added, resulting in a clear solution. In a separated flame-dried 5-mL round-bottom flask **(2)** was charged with a magnetic stir bar, methyltrioxorhenium (5.34 mg, 0.021 mmol, 0.3 equiv). Solution of H_2O_2 (35% in water, 0.125 ml, 1.43 mmol, 20 equiv) was added to flask **2**, and stirred for 5 minutes resulting in yellow solution. A solution in flask **1** was then transferred to flask **2** using cannula. The mixture ran at 23 ºC for 15 hours. Solution of saturated sodium thiosulfate was added and stirred for 20 minutes until the yellow color disappeared to quench the reaction. The biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated. The crude was carried forward.

In a flame-dried 10-mL round-bottom flask containing crude mixture above and anhydrous methanol (2.0 mL), K_2CO_3 (49.4 mg, 0.357 mmol, 5 equiv) was added. Reaction was run at 23 ºC for 2 hours then filtered through celite and concentrated. The crude residue was purified by flash chromatography (eluent: 25% to 27% to 30% acetone in hexanes) to afford verrucarol (7.7 mg, 40% yield over 4 steps).

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.43 (d, *J* = 4.0 Hz, 1H), 4.61 (d, *J* = 7.5 Hz, 1H), 3.82 (d, *J* = 5.3 Hz, 1H), 3.77 (d, *J* = 11.8 Hz, 1H), 3.61 (d, *J* = 5.7 Hz, 1H), 3.56 (d, *J* = 11.7 Hz, 1H), 3.11 (d, *J* = 3.9 Hz, 1H), 2.81 (d, *J* = 3.8 Hz, 1H), 2.58 (dd, *J* = 15.7, 7.5 Hz, 1H), 2.04 (d, *J* = 5.1 Hz, 1H), 2.00 – 1.84 (m, 1H), 1.72 (s, 2H), 1.27 (d, *J* = 7.6 Hz, 2H), 0.95 (d, *J* = 1.3 Hz, 2H), 0.92 – 0.79 (m, 1H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 141.06, 118.76, 78.66, 77.34, 77.02, 76.71, 74.57, 66.53, 65.69, 62.56, 49.00, 47.63, 43.88, 39.98, 28.25, 23.29, 20.98, 7.31.

Amide **31**



A flame-dried 1- L round-bottom flask was charged with a magnetic stir bar, predominantly trans crotonaldehyde (9.02 ml, 110 mmol, 1 equiv) and toluene (83.0 mL). R-(+)-alpha-Phenylethylamine (14.0 ml, 110 mmol, 1equiv) was added. Rubber septum was quickly replaced with yellow cap, and secured tightly between clamp. Vessel was transferred to preheat oil-bath at 110 ºC. After 30 minutes, flask was removed from oil bath and allowed to cool to room temperature. Sodium sulfate was added and the dried solution was filtered, rinsed 3 times with ethyl acetate and the filtrate was concentrated. The crude was carried forward.

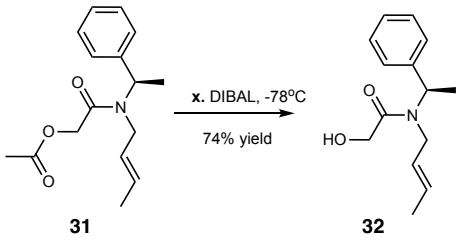
In the same flask, ethanol (83 ml) was added and the vessel was cooled to -78 ºC using dry ice – acetone bath. $NaBH_4$ (4.15 g, 110 mmol, 1 equiv) was slowly added in 1 different portion. Reaction was slowly warmed up to 23 ºC. After 24 hours, 1M NaOH was slowly added and reaction ran for another 30 minutes. The biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated. The crude was carried forward.

A flame-dried 1- L round-bottom flask was charged with a magnetic stir bar, crude secondary amine S13 (19.2 g, 110 mmol, 1 equiv) and and triethylamine (18.4 ml, 132 mmol, 1.2 equiv) in DCM (340.0 mL). (Chlorocarbonyl)methyl acetate (13.0 ml, 121 mmol, 1.1equiv) was added dropwise (Caution: reaction is exothermic and vessel can get very hot if acid chloride was added to quickly). Sodium sulfate was added and the dried solution was filtered, rinsed 3 times with ethyl acetate and the filtrate was concentrated. The crude was carried forward. After 3 hours, solution of saturated sodium bicarbonate was added to quench the reaction. The biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed

solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated. The crude residue was purified by flash chromatography (eluent: 10% to 12% to 15% acetone in hexanes) to afford ester (21.3 g, 70% yield over 2 steps) as yellow oil. Stain dark blue with anisaldehyde

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (p, *J* = 7.1 Hz, 5H), 5.99 (q, *J* = 7.2 Hz, 1H), 5.47 (dq, *J* = 13.5, 6.5 Hz, 1H), 5.20 – 5.05 (m, 1H), 4.68 (s, 2H), 3.65 – 3.41 (m, 2H), 2.17 (s, 3H), 1.65 – 1.58 (m, 3H), 1.49 (d, *J* = 7.2 Hz, 3H), 1.24 (s, 1H), 0.98 – 0.77 (m, 1H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.70, 167.31, 140.20, 128.44, 127.58, 126.90, 77.56, 77.14, 76.71, 61.78, 51.56, 44.51, 31.56, 25.24, 22.63, 20.66, 17.59, 16.47, 14.11.

Alcohol **32**

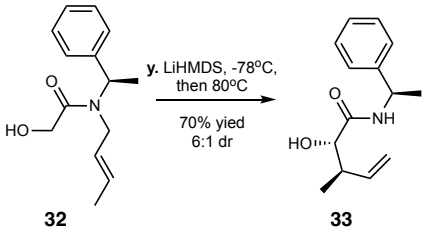


A 1-L round-bottom flask charged with ester **31** (6.30 g, 22.9 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. Dry ethyl ether (180 mL) was added, resulting in a light yellow solution and the vessel was cooled to -78 ºC in a dry ice-acetone bath. DIBAL (1 M in diethyl ether, 45.8 ml, 45.8 mmol, 2 equiv) was added slowly. After running for 12 hours at -78 ºC, MeOH (20 mL) was carefully added, followed saturated aqueous solution of potassium sodium tartrate (200 mL). The vessel was removed from the cooling bath, and the system was allowed to warm to 23 ºC while the mixture was rapidly stirred. After 1 h, the biphasic mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with diethyl ether. The combined organic layers were washed with water (2 × 100 mL) and brine (100 mL), and the washed solution was dried (Na2SO4). The dried solution was filtered, and the filtrate was concentrated. If there was

some water left in the crude after rotavap, DCM was added to the flask followed by sodium sulfate. The dried solution was filtered and concentrated again. The resulting crude residue was purified by flash chromatography (silica gel, eluent: 15% to 17% to 20% acetone in hexanes) to afford alcohol (3.95 g, 74% yield) as yellow oil. Stain dark blue with anisaldehyde

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 – 7.24 (m, 4H), 7.21 (d, *J* = 7.6 Hz, 1H), 5.98 (q, *J* = 7.1 Hz, 1H), 5.57 – 5.33 (m, 2H), 5.07 (dddd, *J* = 15.3, 7.8, 3.9, 1.8 Hz, 1H), 4.16 (s, 2H), 3.47 (dq, *J* = 6.1, 2.0 Hz, 2H), 1.75 – 1.46 (m, 6H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 172.39, 139.98, 128.82, 128.51, 128.39, 127.90, 127.68, 127.56, 126.74, 126.57, 77.53, 77.11, 76.68, 60.45, 52.42, 44.11, 17.57, 16.67

Alcohol **33**

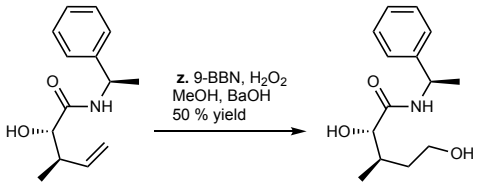


An oven-dried 250-mL round-bottom flask equipped with a large stir bar was charged with dry toluene (15 ml) under nitrogen. The solution was cooled to –78 °C in acetone –dry ice bath. HMDS (1.40 ml, 6.72 mmol, 2.34 equiv) was added, followed by slowly added n-Buli (Sigma, 2.38 M in hexane, 2.82 mL, 6.72 mmol, 2.34 equiv). Acetone bath was replaced with ice bath and vessel was stirred at 0 °C. After 30 minutes, vessel was cooled to -78 °C. A solution of alcohol (azeotrope 3 time with toluene) in toluene (5 ml) was transferred via cannula dropwise into the reaction mixture. After 1 hour stirring at -78 °C, vessel was transferred to preheat oil bath at 80 °C, and stirred for additional 18 hours. Reaction was then allowed to cool to 23 °C, and NH_4Cl was slowly added to quench reaction. The biphasic mixture was transferred to a separatory funnel, and extract with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated.

The crude residue was purified by flash chromatography (eluent: 15% to 17% to 20% acetone in hexanes) to afford amide (470 mg, 70% yield) as yellow oil. Stain dark blue with anisaldehyde

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.20 (m, 5H), 6.78 (s, 1H), 5.99 – 5.82 (m, 2H), 5.24 – 5.09 (m, 5H), 4.13 (d, *J* = 3.2 Hz, 2H), 2.86 (d, *J* = 7.7 Hz, 2H), 2.25 (s, 1H), 1.52 (d, *J* = 6.9 Hz, 5H), 1.04 – 0.83 (m, 5H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 170.96, 143.08, 140.10, 128.68, 127.40, 126.22, 126.15, 116.37, 77.35, 77.23, 77.03, 76.71, 74.08, 48.48, 40.59, 21.87, 11.52.

Alcohol **40**

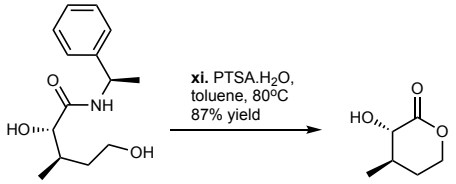


33 **40**

In a flame-dried 100-mL RBF containing (2S,3R)-2-hydroxy-3-methyl-N-((R)-1-phenylethyl)pent-4-enamide (900 mg, 3.86 mmol, 1 equiv) was added slowly 9-BBN (Sigma, 0.5 M in THF, 1.98 g, 16.2 mL, 8.10 mmol, 2.1 equiv) and the solution was then stirred at 23 °C for 2 hours .The solution was then cooled to 0 °C and dry MeOH (5 mL) was added slowly, followed by addition of BaOH (3.30 g, 19.3 mmol, 5 equiv). Reaction was allowed to warm to 23 ºC and stirred vigorously for additional 30 minutes. The flask was cooled to 0 °C , and solution of hydrogen peroxide (35% in water, 1.69 ml, 19.3 mmol, 5 equiv) was added dropwise. Reaction was slowly warmed to 23 ºC and stirred vigorously for 30 minutes. The biphasic mixture was then transferred to a separatory funnel and diluted with Et2O and washed with water. The aqueous layer was extracted with Et2O and the combined organic layers were washed with brine, dried (Na2SO4), filtered, and concentrated . The crude reaction mixture was then purified by flash column chromatography (eluent: 30% to 32% to 35% to 37% acetone in hexanes) to afford amide (480 mg, 50% yield) as white solid. Stain dark pink with anisaldehyde

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (s, 5H), 7.08 (s, 1H), 5.16 – 5.00 (m, 2H), 4.13 (d, *J* = 2.5 Hz, 2H), 3.67 (qt, *J* = 10.6, 5.4 Hz, 4H), 2.29 – 2.16 (m, 2H), 1.63 (q, *J* = 5.8 Hz, 4H), 1.46 (s, 5H), 0.74 (d, *J* = 7.0 Hz, 5H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 172.71, 143.20, 128.63, 127.31, 126.12, 77.36, 77.24, 77.04, 76.72, 74.26, 74.21, 59.72, 48.43, 35.93, 34.50, 34.45, 21.98, 12.57, 12.55.

Verrucarin lactone **34**

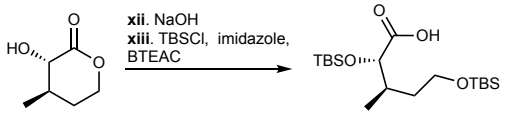


40 **34**

A 250-mL round-bottom flask charged with amide (1.60 g, 6.4 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. Dry toluene (120 mL) was added, resulting in a clear solution. Rubber septum was briefly removed to added PTSA. H_2O (3.0 g, 16 mmol, 2.5 equiv). Flask was transferred to oil bath and heat to 90 ºC for 1 hour. Reaction was then allowed to cool to 23 ºC. Solution of saturated bicarbonate was added to quench the reaction. The biphasic mixture was then transferred to a separatory funnel and extracted with ethyl acetate three times. The combined organic layers were washed with brine, dried (Na2SO4), filtered, and concentrated . The crude reaction mixture was then purified by flash column chromatography (eluent: 20% to 22% to 25% acetone in hexanes) to afford verrucarin lactone (720 mg, 87% yield) as white solid. Stain with CAM

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.47 – 4.24 (m, 2H), 3.84 (d, *J* = 10.5 Hz, 1H), 2.20 – 1.91 (m, 2H), 1.78 – 1.58 (m, 2H), 1.24 (d, *J* = 6.3 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 175.06, 77.34, 77.23, 77.03, 76.71, 72.71, 67.68, 33.71, 30.05, 19.23.

Acid **35**



34 **35**

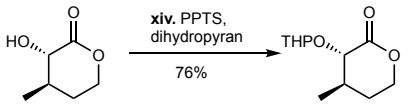
A 25-mL round-bottom flask charged with verrucarin lactone (97.0 mg, 0.745 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. Methanol/ H_2O (6 ml, v/v, 5:1) was added, followed by addition of NaOH

(29.8 mg, 0.745 mmol, 1 equiv) (NOTE: stock solution of NaOH was prepared in MeOH/ H_2O, v/v, 5:1 to ensure exact 1 equiv of NaOH added). The reaction mixture was stirred for 17 hours at 23 ºC. The solvent was removed, azeotrope with toluene three time, and the residue was further dried under high vacuum for 5 h to removed excess water and afford the corresponding sodium carboxylate as a clear crystal.

The suspension of the sodium carboxylate in anhydrous THF (6 mL) was treated with BnNEt3Cl (170 mg, 0.745 mmol, 1equiv) resulting in a clear solution. Rubber septum was briefly removed to added imidazole (304 mg, 4.47 mmol, 6 equiv) and TBS-Cl (449 mg, 2.98 mmol, 4 equiv) leading to a cloudy solution. Reaction was stirred vigorously for 24 hours at 23 ºC. Water (2ml) was added, followed by addition of 2M HCl (2 ml), and stirred for 30 minutes. The biphasic mixture was then transferred to a separatory funnel and extracted with ethyl acetate three times. The combined organic layers were washed with brine, dried (Na2SO4), filtered, and concentrated resulting in a clear oil. The crude was carried forward to next reaction without purification. Stain with CAM

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.07 (d, *J* = 3.2 Hz, 1H), 3.79 – 3.55 (m, 2H), 2.22 – 2.06 (m, 1H), 1.68 (dq, *J* = 13.0, 6.4 Hz, 1H), 1.55 (s, 4H), 1.49 – 1.33 (m, 1H), 0.97 – 0.82 (m, 16H), 0.27 (d, *J* = 2.0 Hz, 3H), 0.07 (s, 5H).

Lactone **36**



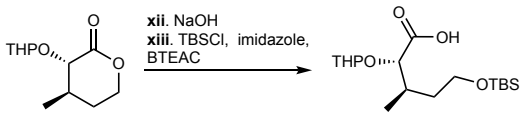
34 **36**

A 25-mL round-bottom flask charged with verrucarin lactone (160.0 mg, 1.23 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. DCM (5 ml) was added, followed by addition of dihydropyran (0.15 ml, 1.60 mmol, 1.3 equiv) and PPTS (30.9 mg, 0.12 mmol, 0.1 equiv). The reaction mixture was stirred

for 17 hours at 23 ºC. Solution of saturated sodium chloride was added. The biphasic mixture was then transferred to a separatory funnel and extracted with DCM three times. The combined organic layers were washed with brine, dried (Na2SO4), filtered, and concentrated . The crude reaction mixture was then purified by flash column chromatography (eluent: 5% to 7% to 10% acetone in hexanes) to afford verrucarin lactone **36** (201 mg, 76% yield) as clear oil. Stain with CAM.

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.03 (s, 1H), 4.38 – 4.19 (m, 2H), 4.03 (d, *J* = 9.5 Hz, 1H), 3.97 – 3.83 (m, 1H), 3.52 (dd, *J* = 11.0, 4.7 Hz, 1H), 2.22 – 2.05 (m, 2H), 1.88 – 1.79 (m, 2H), 1.79 – 1.59 (m, 3H), 1.56 (s, 3H), 1.23 (d, *J* = 6.2 Hz, 3H).

Acid **37**



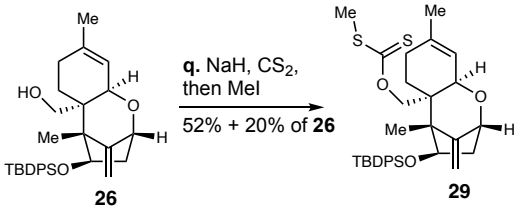
36 **37**

***follow the same procedure from 34 to 35**

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68 (dd, *J* = 4.1, 2.9 Hz, 1H), 4.31 (d, *J* = 4.2 Hz, 1H), 3.85 (ddd, *J* = 11.3, 8.1, 3.3 Hz, 1H), 3.80 – 3.64 (m, 2H), 3.57 – 3.47 (m, 1H), 2.17 (s, 1H), 1.89 – 1.65 (m, 4H), 1.65 – 1.50 (m, 4H), 1.01 (d, *J* = 6.9 Hz, 3H), 0.90 (d, *J* = 6.2 Hz, 10H), 0.13 – 0.03 (m, 6H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 176.52, 97.60, 77.34, 77.02, 76.70, 62.69, 60.72, 35.88, 32.92, 30.40, 25.93, 25.34, 19.24, 18.31, 14.74, -5.36, -5.38.

Xanthate **29**



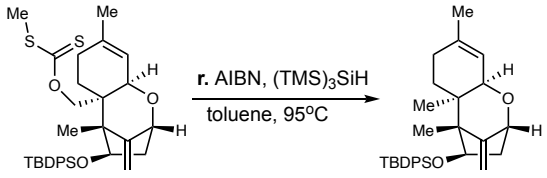
26 **29**

A oven-dried 10-mL round-bottom flask charged with alcohol (40 mg, 0.082 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. Dry THF (2 mL) was added, followed by CS_2 (50 uL, 0.82 mmol, 10 equiv) resulting in a slight yellow solution. Reaction was then cooled to 0 ºC using water-ice bath. Rubber septum was briefly removed to add NaH (60% in mineral oil, 9.82 mg, 0.246 mmol, 3 equiv). Ice

bath was removed, reaction was allowed to warm to 23 ºC and stirred for additional 1 hour. MeI (100 uL, 1.55 mmol, 19 equiv) was then added and reaction was run for another 30 minutes. Solution of saturated ammonium chloride was added to quench the reaction. The biphasic mixture was then transferred to a separatory funnel and extracted with ethyl acetate three times. The combined organic layers were washed with brine, dried (Na2SO4), filtered, and concentrated . The crude reaction mixture was then purified by flash column chromatography (eluent: 1% to 2% acetone in hexanes) to afford xanthate (13 mg, 27% yield) as yellow oil and recover SM (10 mg, 25%)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 – 7.58 (m, 4H), 7.48 – 7.29 (m, 6H), 5.27 (d, *J* = 5.5 Hz, 1H), 5.14 (s, 1H), 4.76 (s, 1H), 4.53 (dd, *J* = 6.8, 3.3 Hz, 1H), 4.35 (d, *J* = 12.5 Hz, 1H), 4.31 (d, *J* = 4.6 Hz, 1H), 4.09 (d, *J* = 12.1 Hz, 1H), 3.40 (d, *J* = 5.5 Hz, 1H), 2.51 (s, 3H), 2.17 – 2.04 (m, 1H), 2.02 – 1.52 (m, 8H), 1.27 (bd, 1H), 1.20 – 0.79 (m, 13H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 214.61, 152.63, 140.74, 136.01, 135.88, 134.30, 133.71, 129.85, 129.80, 127.76, 127.66, 118.49, 105.49, 78.90, 77.37, 77.05, 76.73, 74.62, 73.02, 66.17, 53.06, 43.57, 41.62, 28.02, 27.08, 23.24, 20.16, 19.31, 18.82, 12.68.

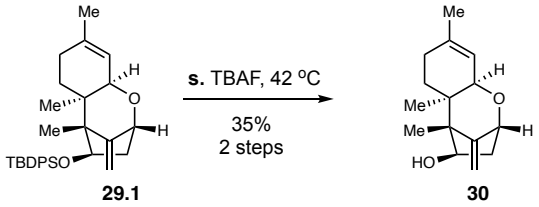
Alcohol **29.1**



29 **29.1**

A flame-dried 1- L round-bottom flask was charged with a magnetic stir bar, xanthate (13 mg, 0.023 mmol, 1 equiv) and AIBN (2.7 mg, 0.016 mmol, 0.73 equiv) in toluene (340.0 mL). Tris(trimethylsilyl)silane (30 uL, 0.097 mmol, 4.3 equiv) was added, and argon was then bubbled through the reaction mixture for 2 minutes. The flask was transferred to oil bath and heat to 95 ºC for 2 hours. Reaction was then cooled to 23 ºC, concentrated and purified by flash column chromatography (eluent: 0.5% to 0.7% to 1% acetone in hexanes) to afford alcohol (5.4 mg, 51% yield) as clear oil.

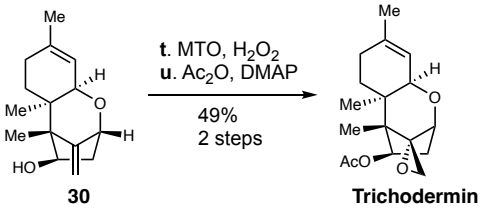
Alcohol **30**



A flame-dried 5-mL round-bottom flask was charged with a magnetic stir bar, alcohol **29.1** (5.4 mg, 0.011 mmol, equiv) , TBAF (1 M in THF, 0.11 mL, 0.11 mmol, 10 equiv) and anhydrous THF (0.3 mL). Reaction was heat to 42 ºC and stirred for 15 hours. Vessel was cooled down to 23 ºC. Saturated sodium bicarbonate was added to quench reaction and the biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated. The crude reaction mixture was then purified by flash column chromatography (eluent: 5% to 7% to 10% acetone in hexanes) to afford alcohol **30.**

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.35 (dq, *J* = 5.6, 1.5 Hz, 1H), 5.13 (d, *J* = 0.8 Hz, 1H), 4.71 (d, *J* = 0.8 Hz, 1H), 4.39 (d, *J* = 5.3 Hz, 2H), 3.56 (d, *J* = 5.6 Hz, 1H), 2.59 (dd, *J* = 15.4, 7.4 Hz, 1H), 2.01 – 1.75 (m, 3H), 1.68 (s, 1H), 1.67 (s, 4H), 1.36 – 1.22 (m, 2H), 1.02 (s, 3H), 0.85 (d, *J* = 0.6 Hz, 3H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 152.63, 139.73, 118.98, 105.49, 78.65, 77.34, 77.03, 76.71, 73.13, 70.27, 40.69, 28.03, 23.68, 23.30, 16.11, 10.27.

Trichodermin



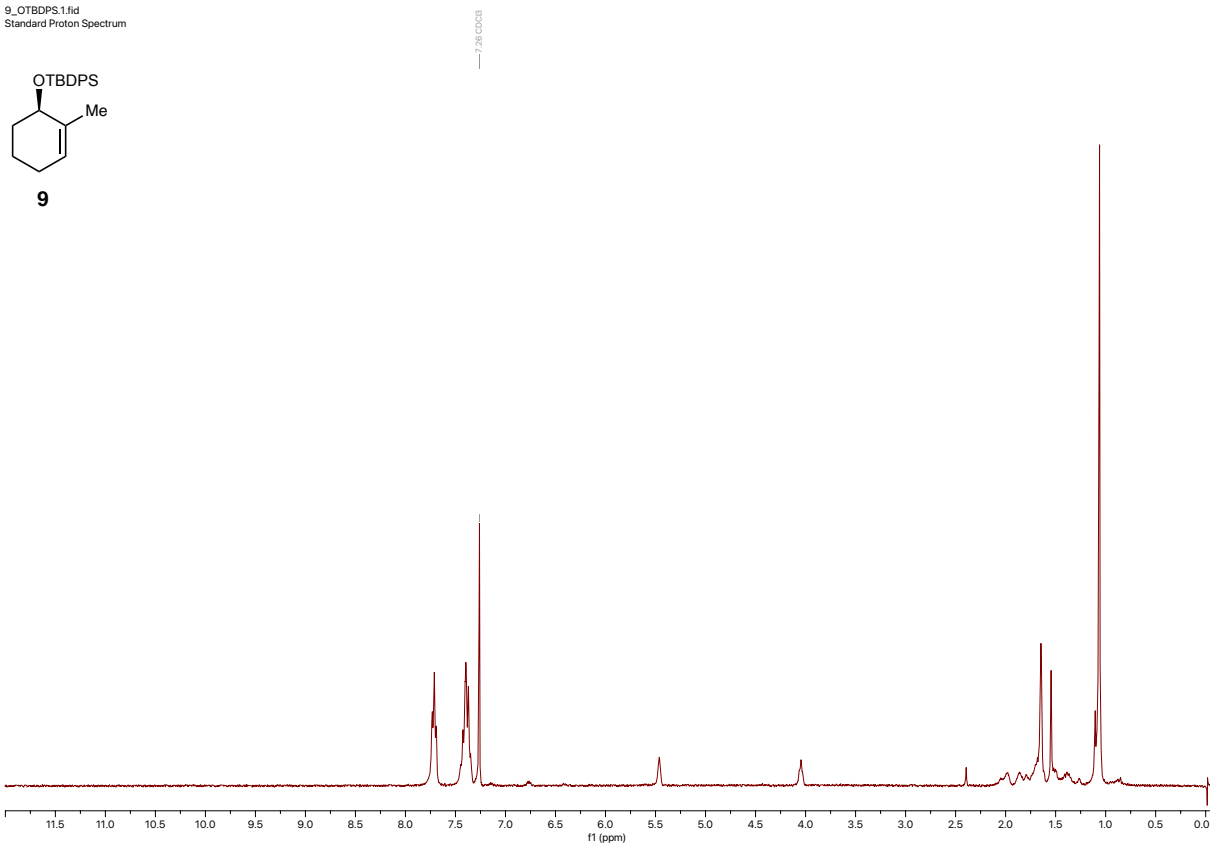
A 5-mL round-bottom flask **(1)** charged with crude product above was evacuated and flushed with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. Dry DCM (0.3 mL) and anhydrous pyridine (10 μL, 0.12 mmol, 11 equiv) were added, resulting in a

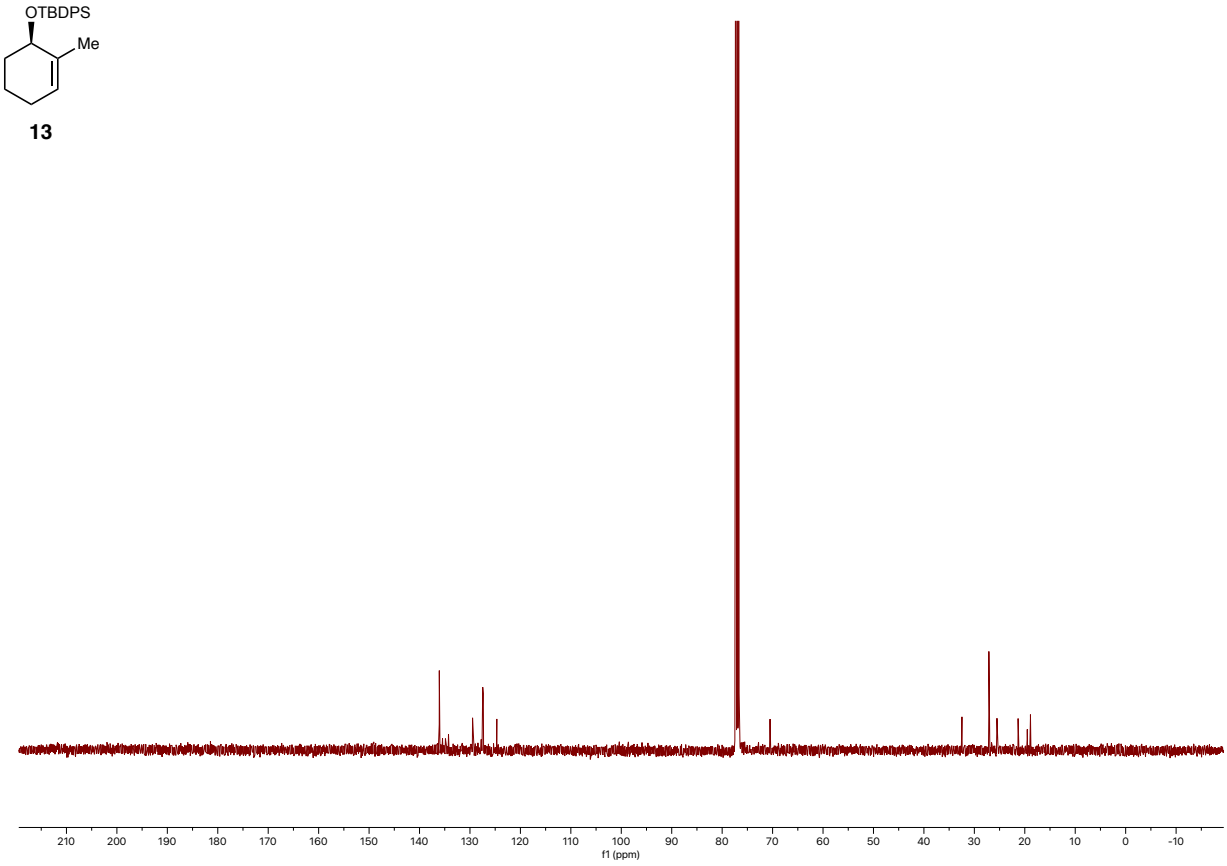
clear solution. In a separated flame-dried 5-mL round-bottom flask **(2)** was charged with a magnetic stir bar, methyltrioxorhenium (2.87 mg, 0.011 mmol, 1 equiv). Solution of H_2O_2 (35% in water, 30 μL, 0.34 mmol, 30 equiv) was added to flask **2**, and stirred for 5 minutes resulting in yellow solution. A solution in flask **1** was then transferred to flask **2** using cannula. The mixture ran at 23 ºC for 15 hours. Solution of saturated sodium thiosulfate was added and stirred for 20 minutes until the yellow color disappeared to quench the reaction. The biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated. The crude was carried forward.

A 5-mL round-bottom flask charged with crude epoxide and DMAP (14.1 mg, 0.11 mmol, 10 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. Dry DCM (0.5 mL) was added, resulting in a clear solution. Acetic anhydride (20 μL, 0.21 mmol, 18 equiv) was added dropwise. Reaction was run at 23 ºC for 2 hours. Solution of saturated sodium bicarbonate was added. The biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with ethyl acetate 3 times. The combined organic layers were washed with brine and the washed solution was dried (Na2SO4). The dried solution was filtered and the filtrate was concentrated. The crude reaction mixture was then purified by flash column chromatography (eluent: 2% to 5% to 7% acetone in hexanes) to afford trichodermin.

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.56 (dd, *J* = 7.9, 3.7 Hz, 1H), 5.41 (s, 1H), 3.82 (d, *J* = 5.2 Hz, 1H), 3.60 (d, *J* = 5.6 Hz, 1H), 3.12 (d, *J* = 4.0 Hz, 1H), 2.83 (d, *J* = 4.0 Hz, 1H), 2.53 (dd, *J* = 15.4, 7.9 Hz, 1H), 2.03 (d, *J* = 31.9 Hz, 7H), 1.56 (s, 1H), 0.93 (s, 3H), 0.71 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.01, 140.26, 118.63, 79.16, 77.34, 77.02, 76.71, 75.12, 70.54, 65.56, 48.95, 47.90, 40.45, 36.70, 28.01, 24.49, 23.28, 21.18, 16.02, 5.84.

1.4 NMR spectra

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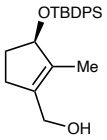
OTBDPS
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OH

15

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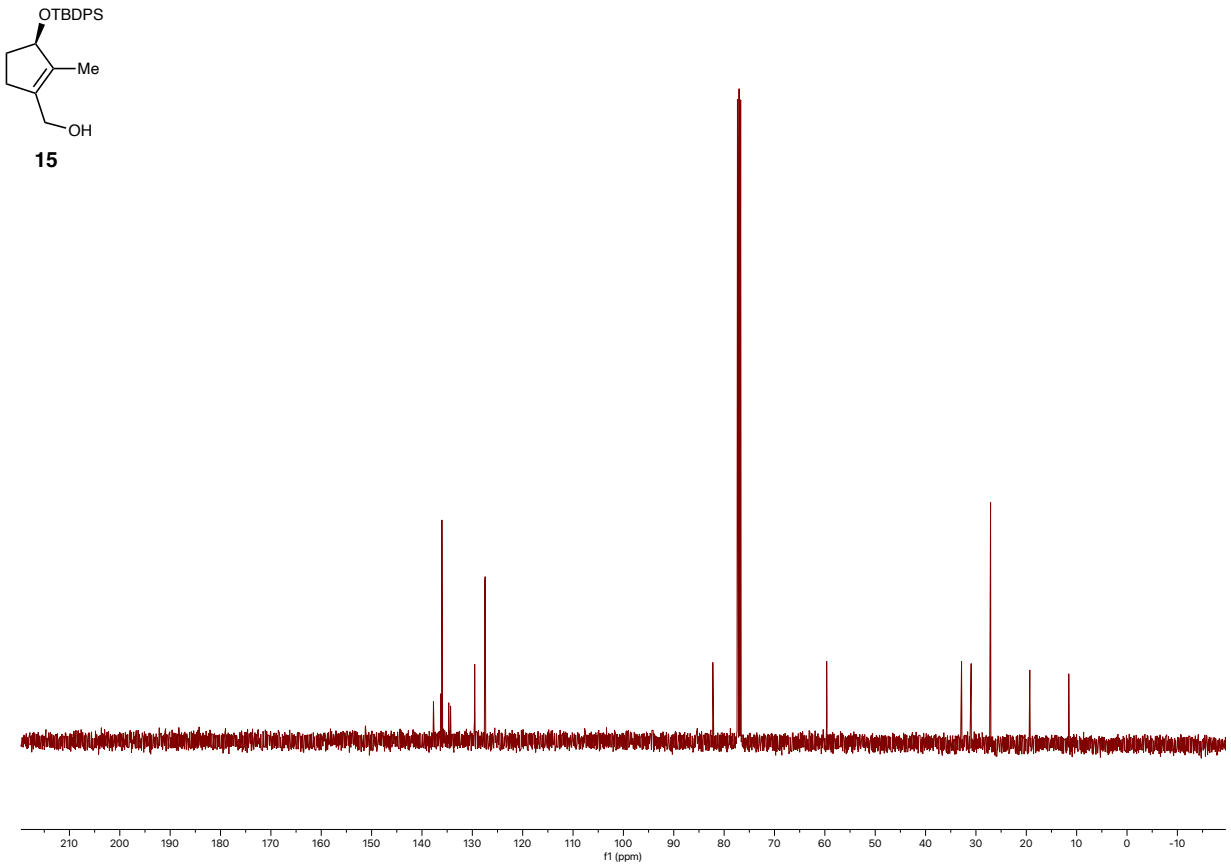
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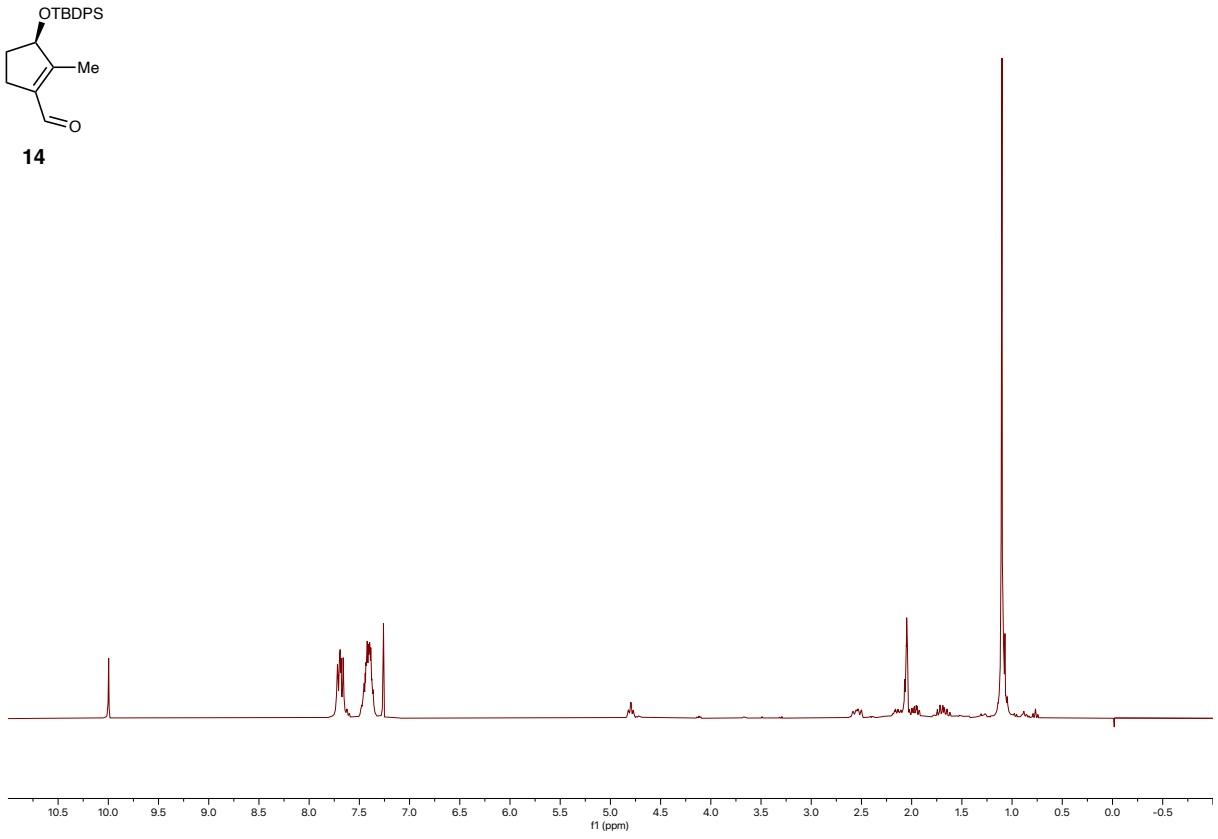
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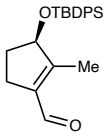
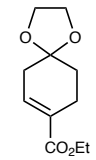
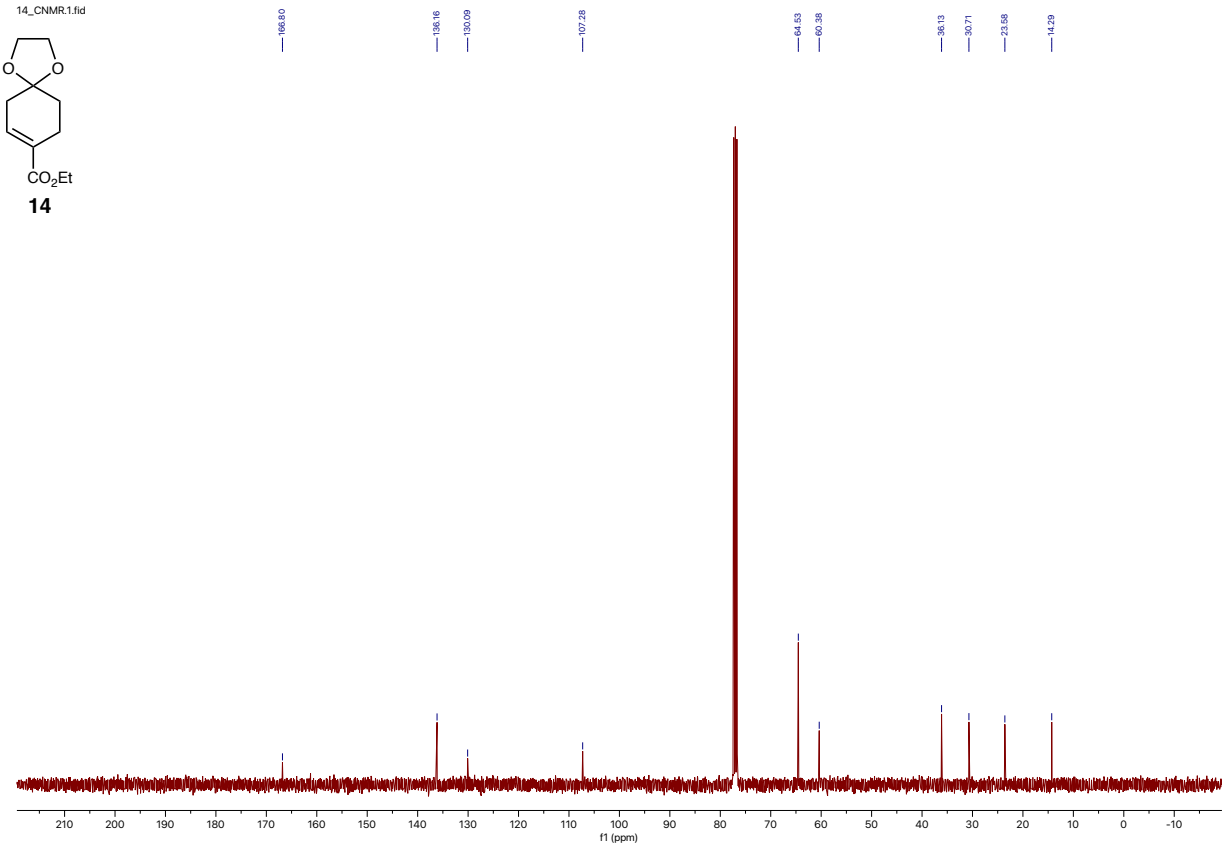


14

14_ethyl ester before acid.1.fid
Standard Proton Spectrum



14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180
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150
140
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120
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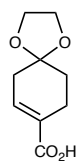
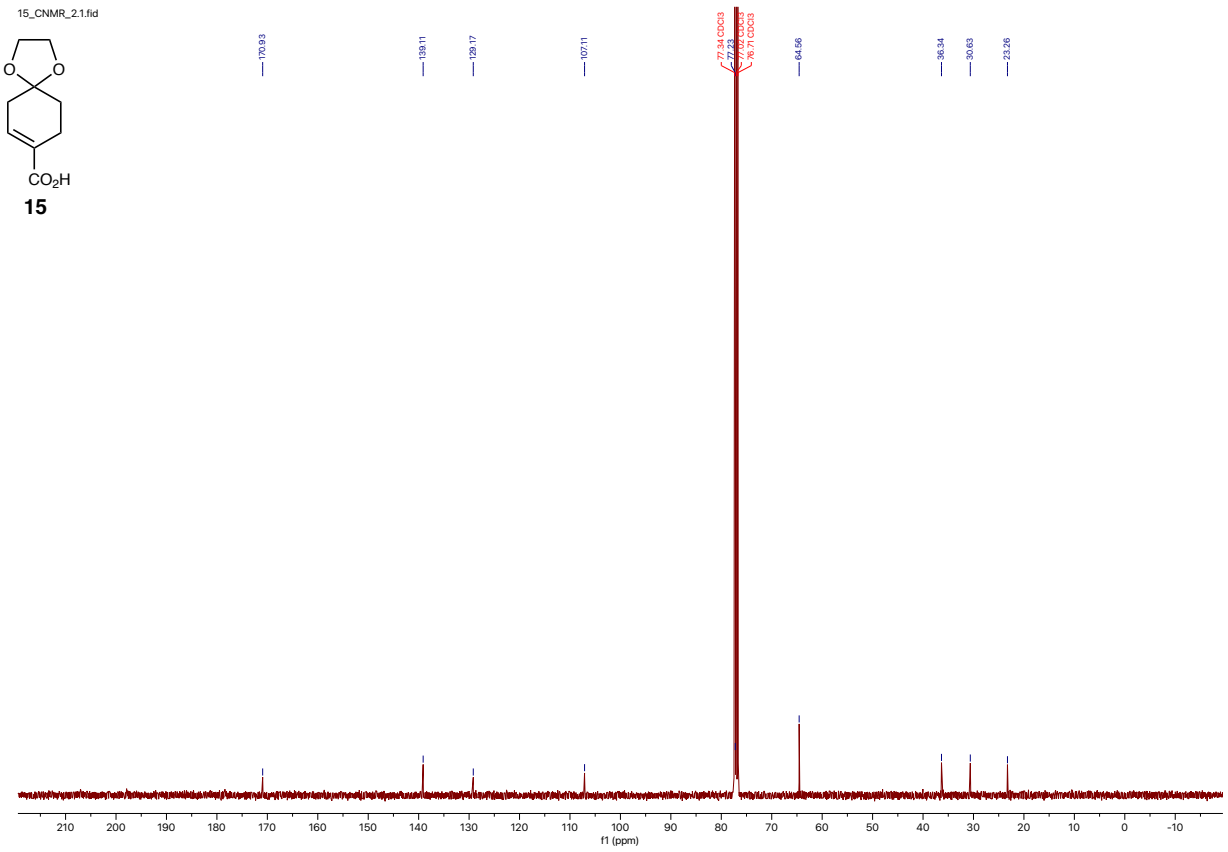
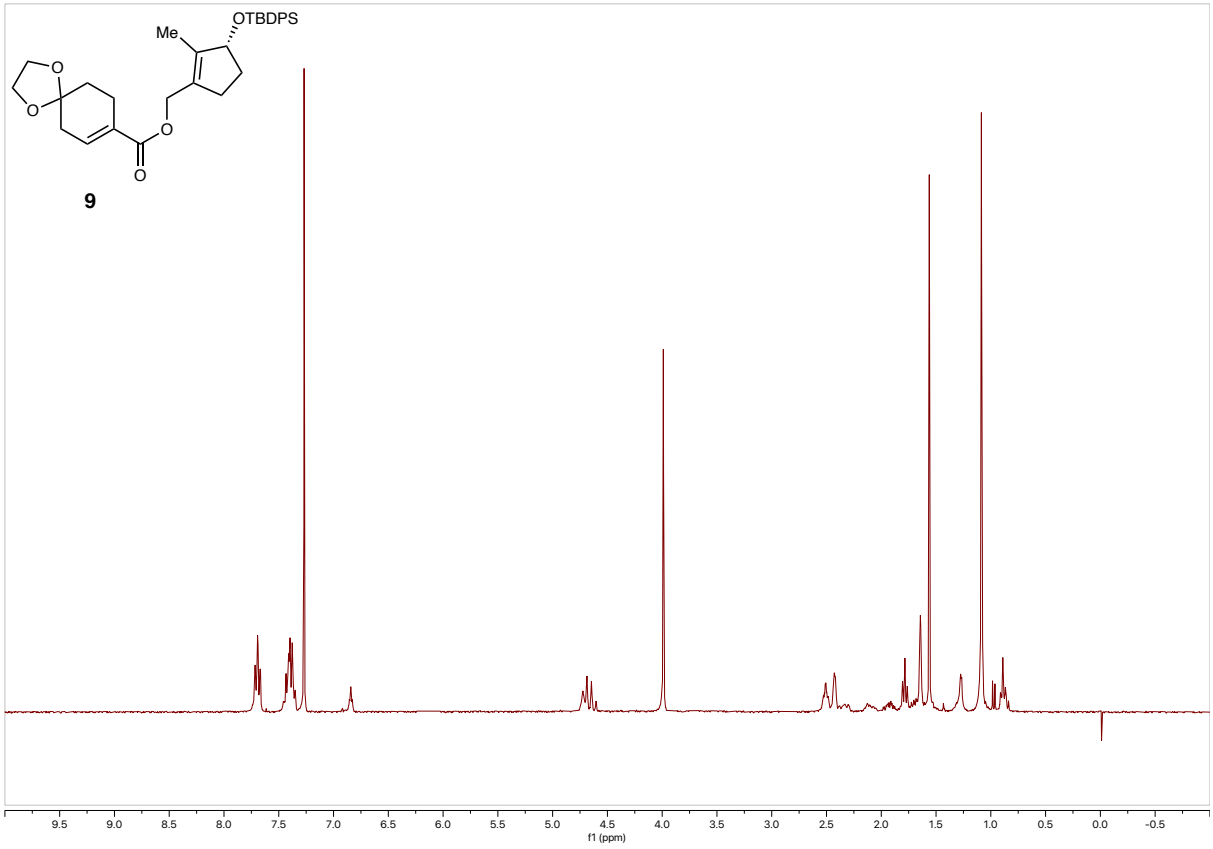
f1 (ppm)

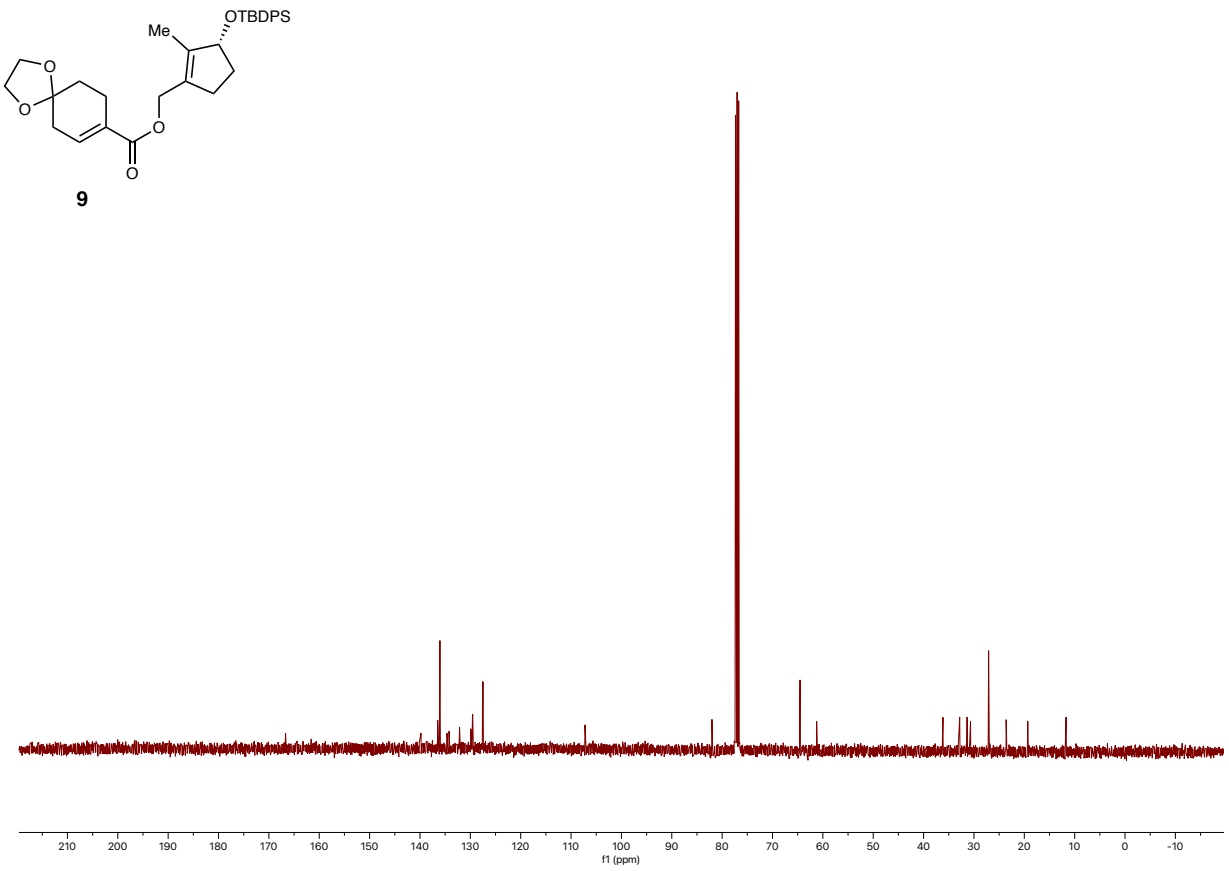
15_acid right half.1.fid
Standard Proton Spectrum



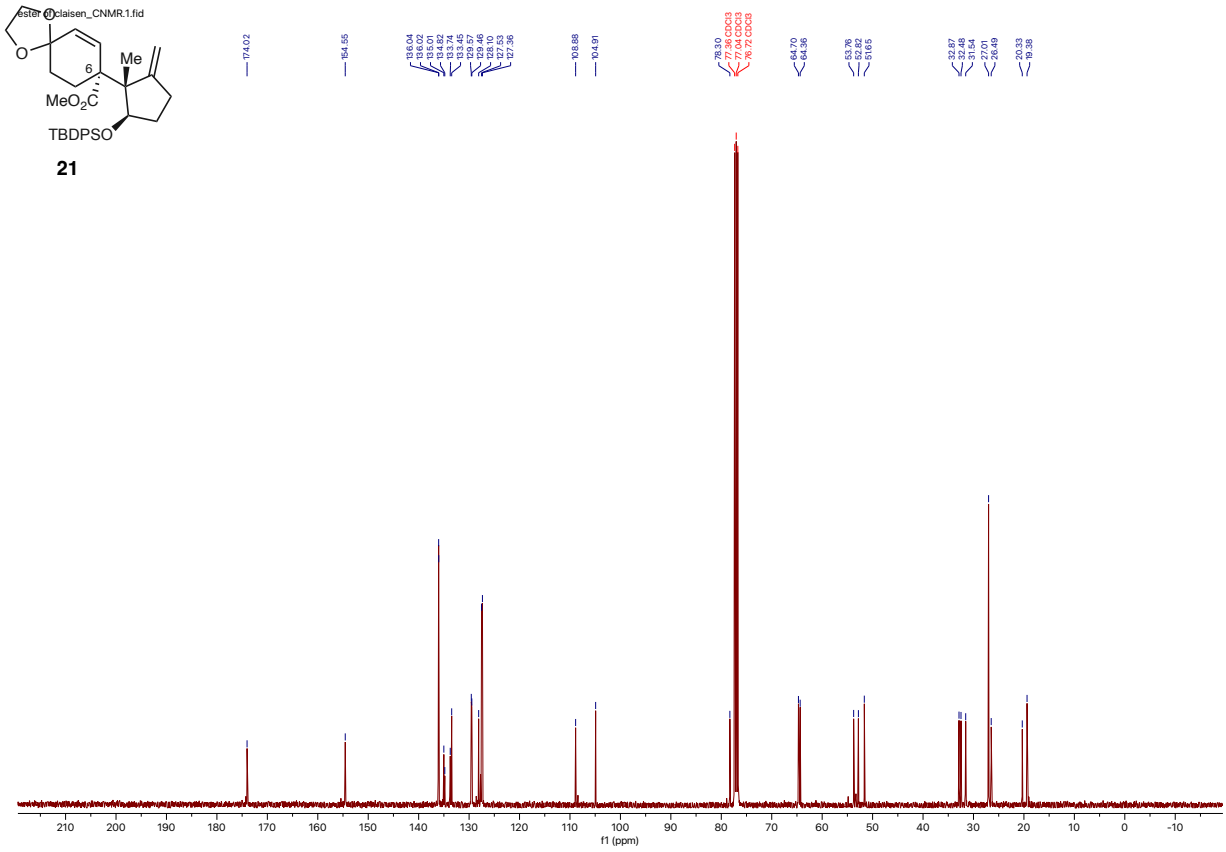
15


15_CNMR_2.1.fid
O
O
CO2H
15
170.93
139.11
129.17
107.11
77.34 CDCl3
77.03
76.71 CDCl3
64.56
36.34
30.63
23.26
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

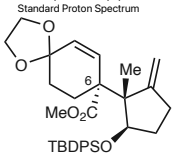

OTBDPS
Me
O
O
O
O
9
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


OTBDPS
Me
O
O
O
O
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

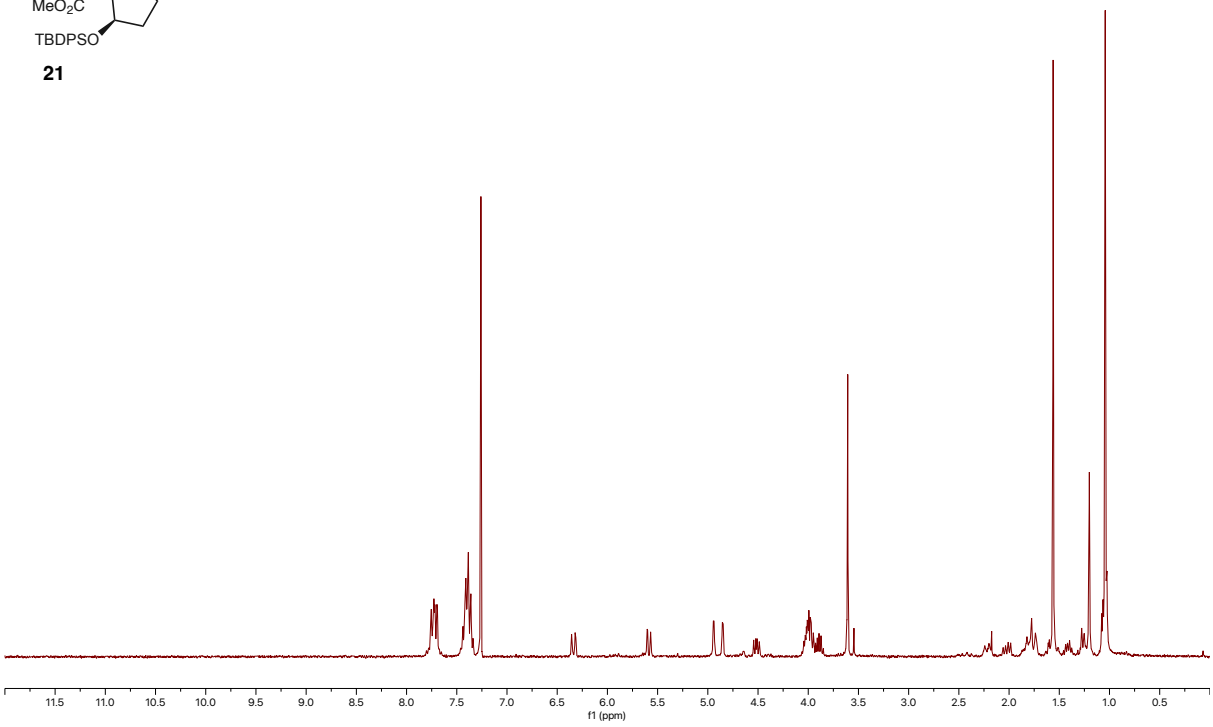
9

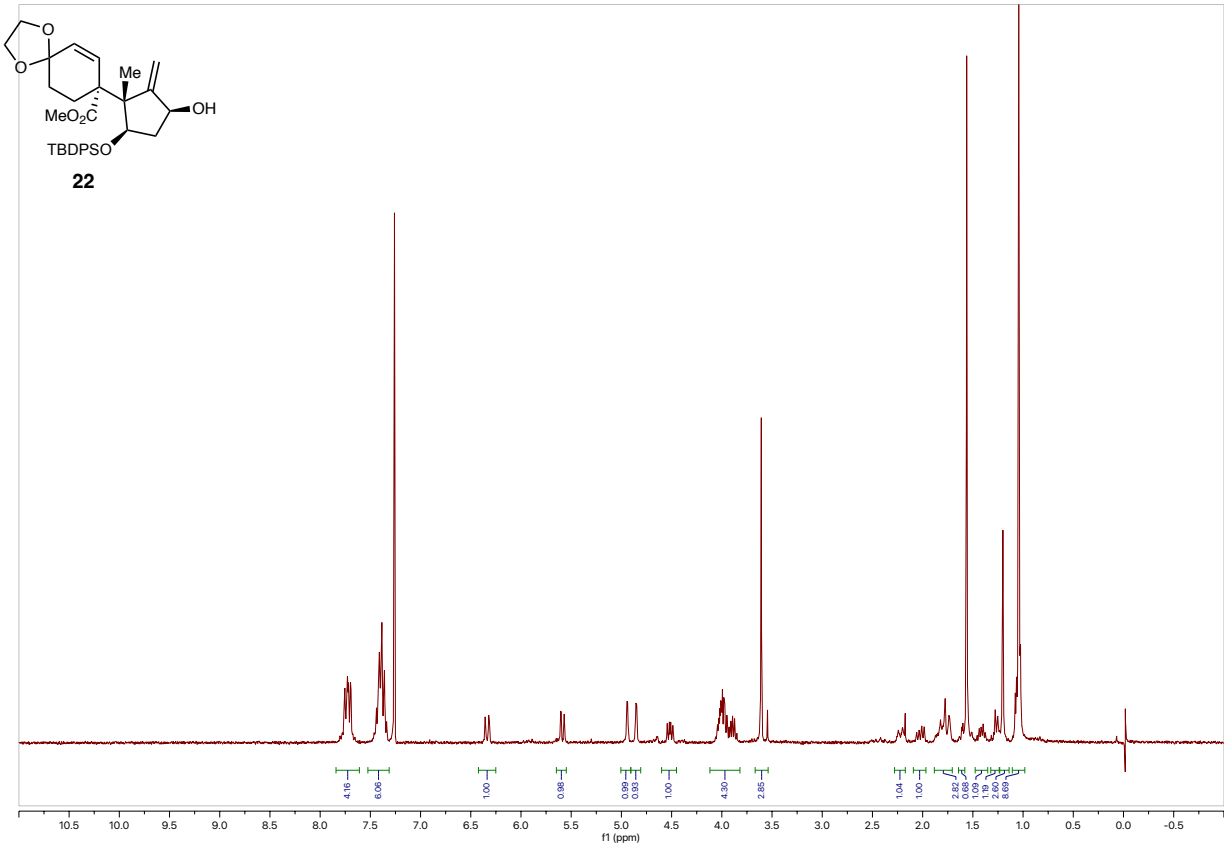

ester Claisen_CNMR.1.fid
O
6
Me
MeO2C
TBDPSO
21
174.02
164.55
136.04
135.92
135.01
134.82
133.74
133.45
129.57
129.46
128.10
127.53
127.36
110.88
104.91
78.30
77.25 CDCl3
77.04 CDCl3
76.72 CDCl3
64.70
64.36
53.76
52.82
51.65
32.87
32.48
31.54
27.01
26.49
20.33
19.38
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

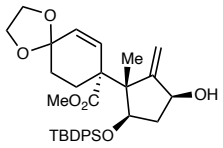
Claisen product for paper.1.fid
Standard Proton Spectrum



21



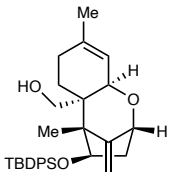

O
O
Me
MeO2C
OH
TBDPSO
22
4.16
6.06
1.00
0.98
0.99
0.93
1.00
4.30
2.85
1.04
1.00
2.82
0.68
1.09
1.19
2.00
8.69
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


O
O
Me
MeO2C
OH
TBDPSO

22

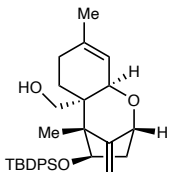
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

cyclized product.1.fid
Standard Proton Spectrum

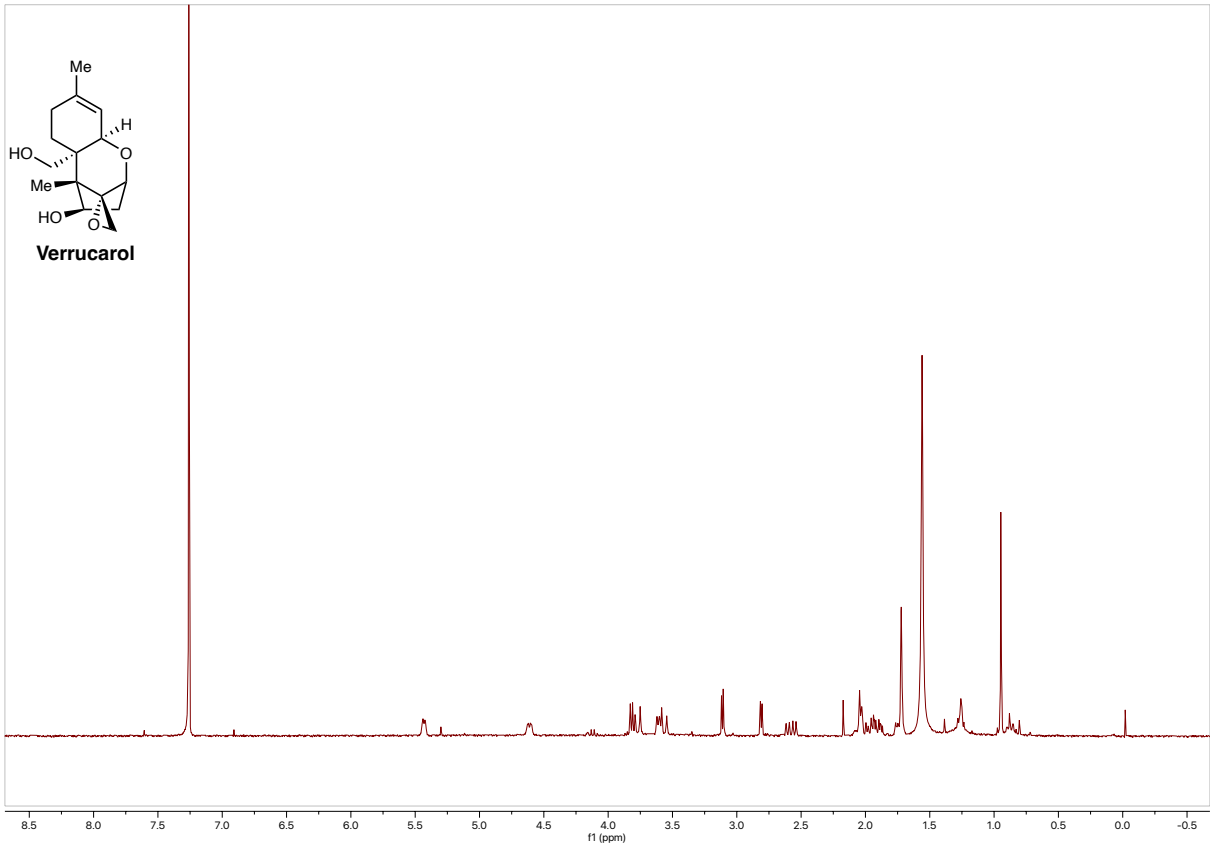


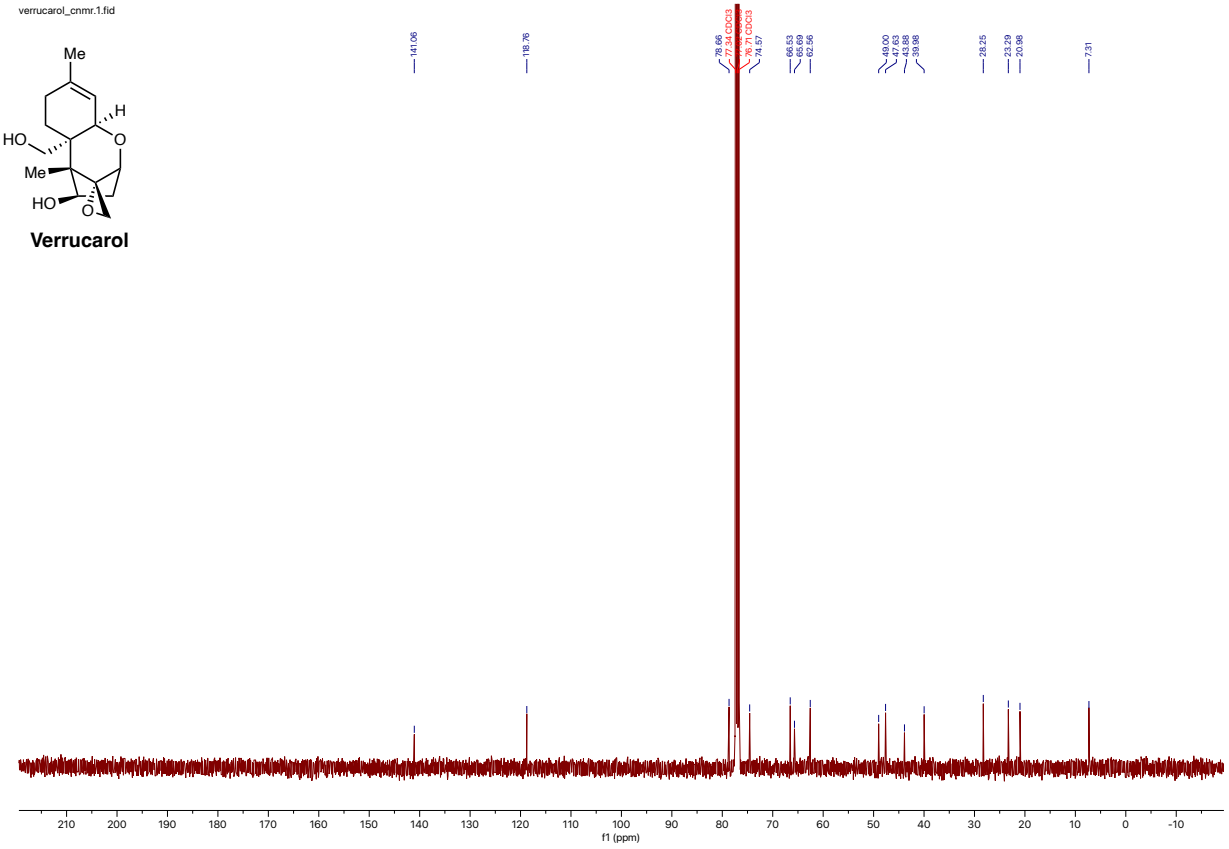
26

cyclized product_CNMR.1.fid

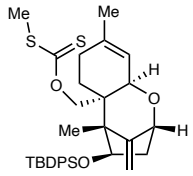


26

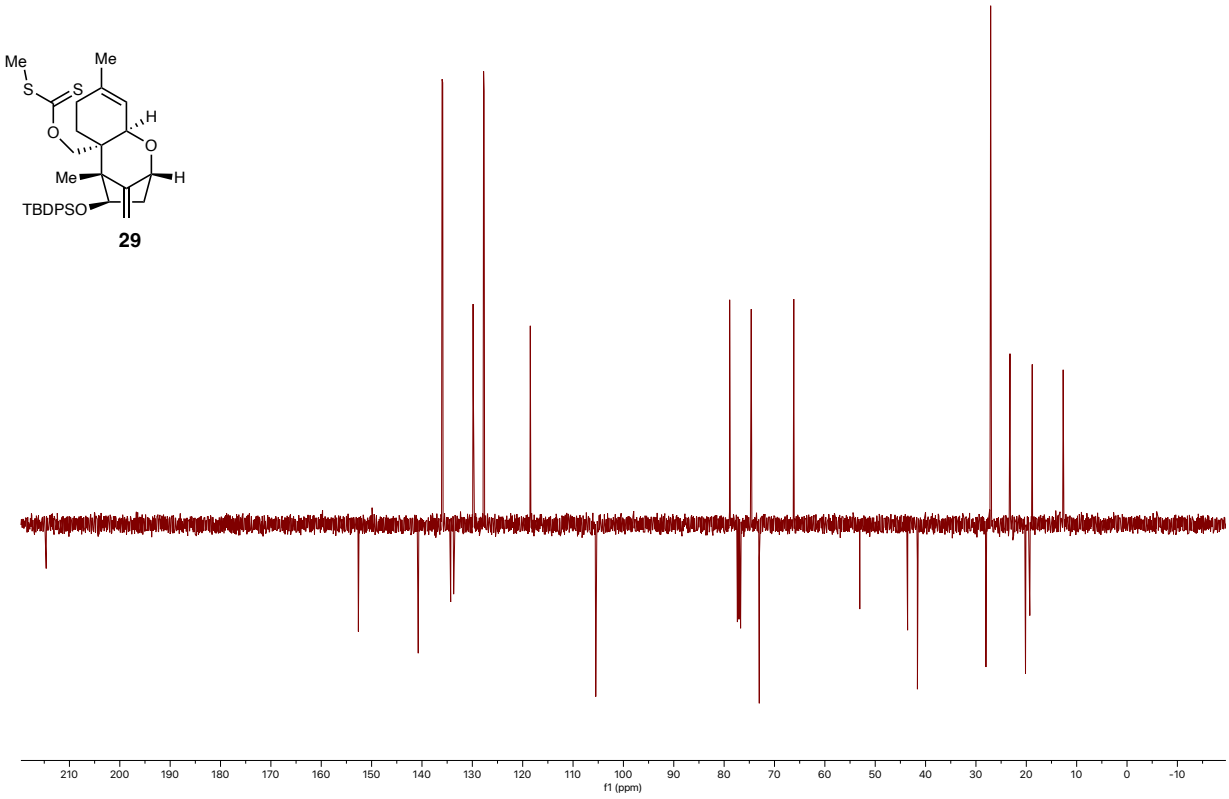

Me
H
HO
O
Me
HO
O
Verrucarol
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

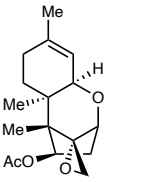

verrucarol_cnmr.1.fid
Me
H
HO
O
Me
HO
O
Verrucarol
141.06
118.76
78.66
77.41 CDCl3
77.16 CDCl3
76.71 CDCl3
74.57
66.53
65.69
62.56
49.10
47.63
43.88
39.98
28.25
23.29
20.98
7.31
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

MTIII-853b.1.fid
Standard Proton Spectrum

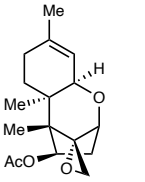


29

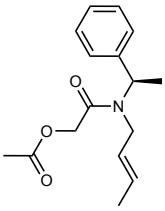

Me
S
Me
S
O
H
O
Me
H
TBDPSO
29
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)



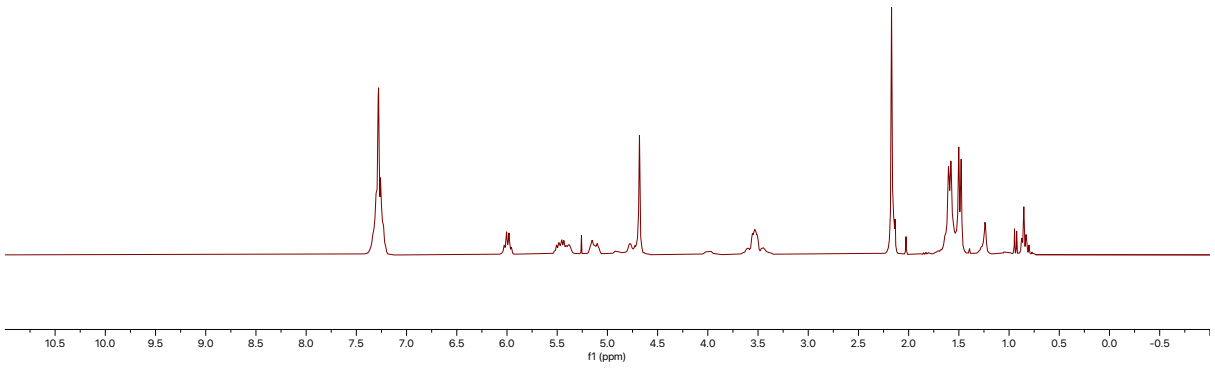
Trichodermin

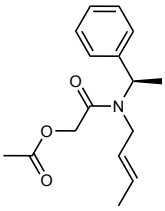


Trichodermin


O
N
O
O

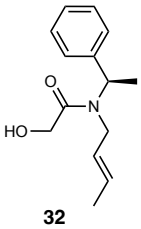
31


10.5
10.0
9.5
9.0
8.5
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
-0.5
f1 (ppm)

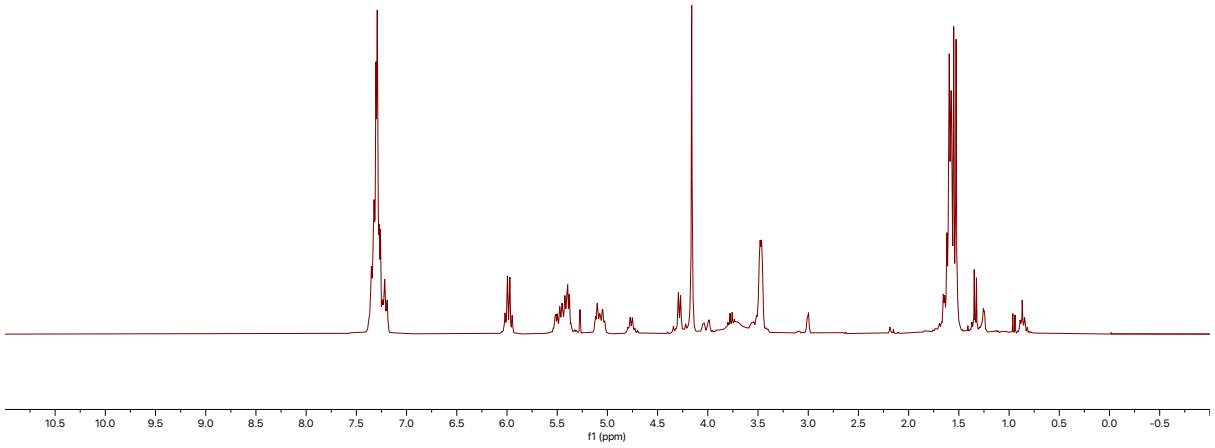

O
N
O
O

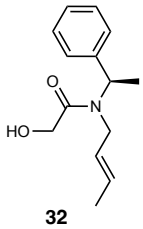
31

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
f1 (ppm)

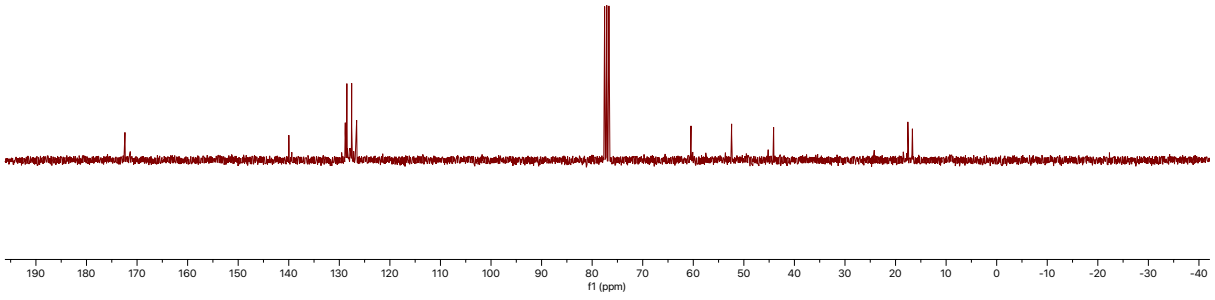

O
N
HO

32

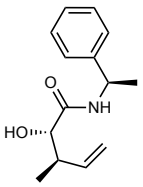

10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


O
N
HO

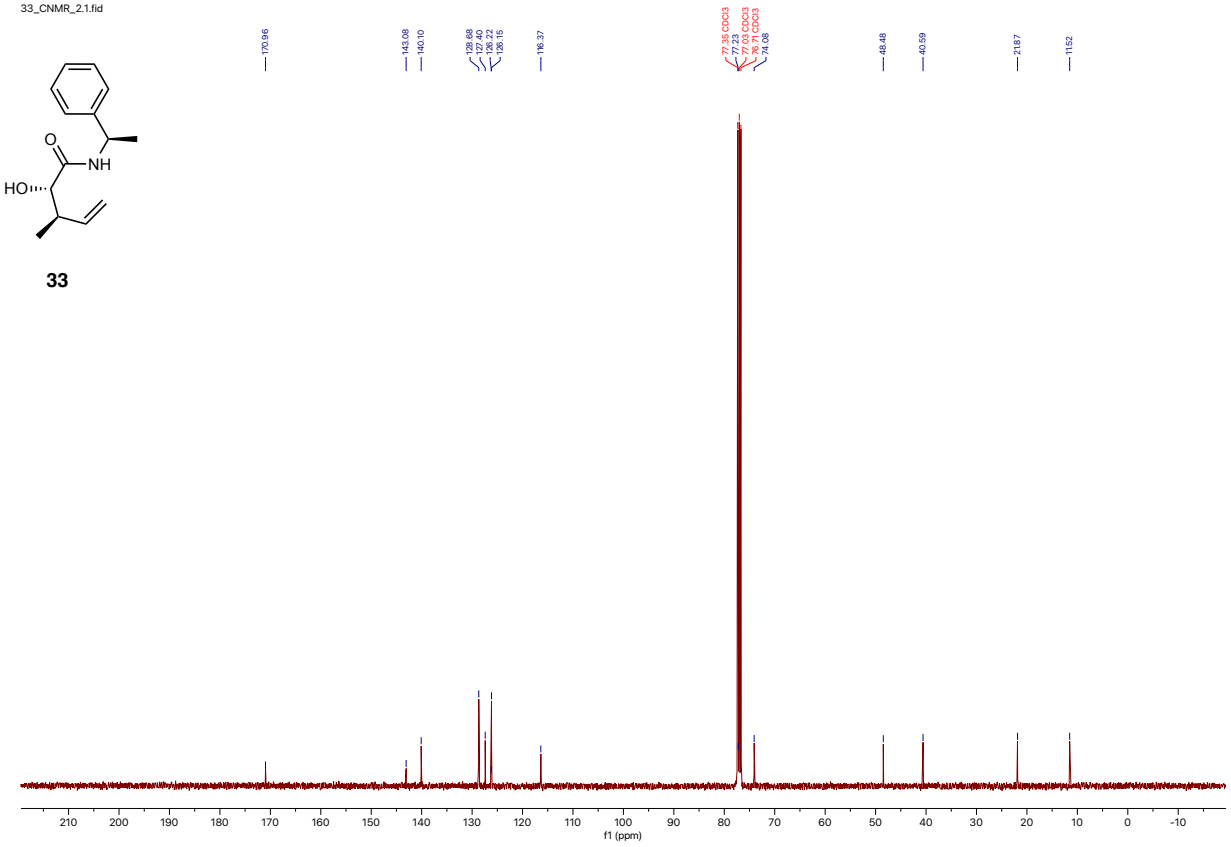
32


190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
f1 (ppm)

33_strap claisen product.1.fid
Standard Proton Spectrum

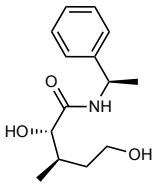


33

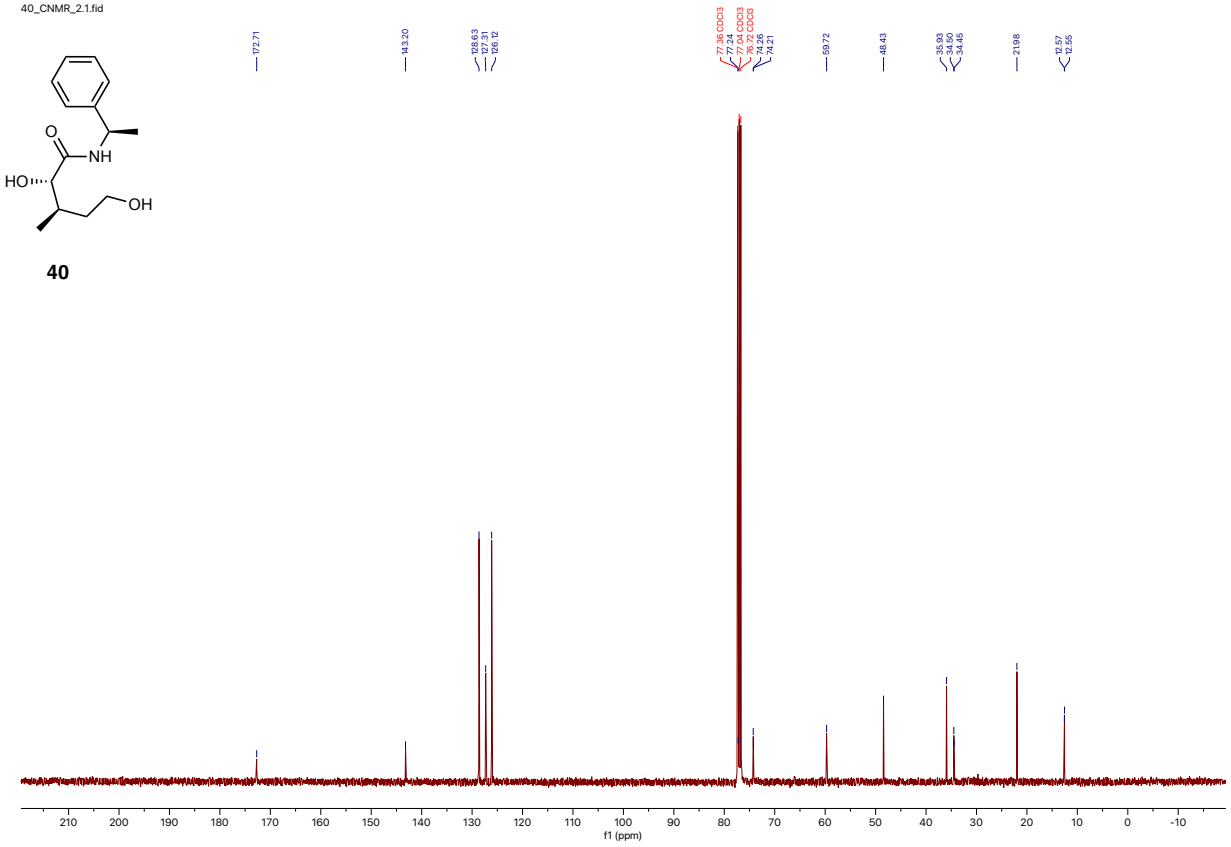

33_CNMR_2.1.fid
170.96
143.08
140.10
128.68
127.60
126.22
126.15
116.37
77.35 CDCl3
77.23
77.00 CDCl3
76.71 CDCl3
74.08
48.48
40.59
21.87
11.52
O
NH
HO
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

33

40_hydroxyl borylation.1.fid
Standard Proton Spectrum

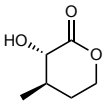


40

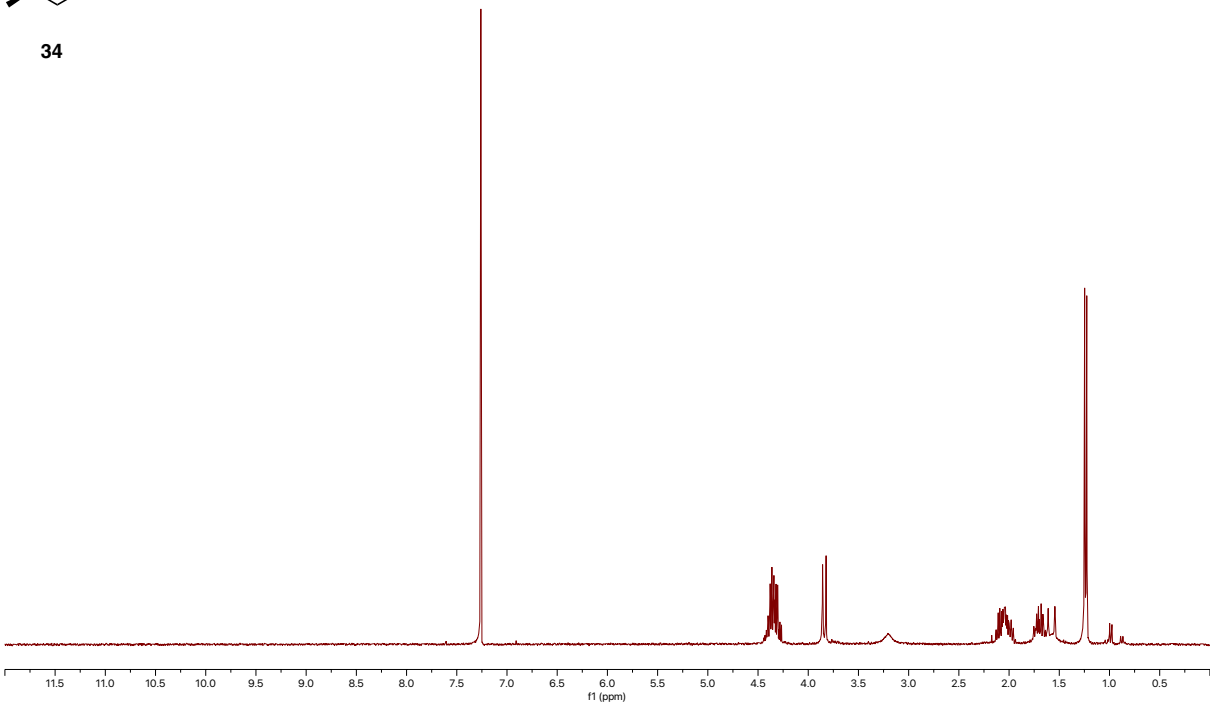

40_CNMR_2.1.fid
172.71
143.20
128.63
127.31
126.12
77.36 CDCl3
77.04
77.04 CDCl3
76.72 CDCl3
74.56
74.21
59.72
48.43
35.93
34.50
34.45
21.98
12.57
12.55
O
NH
HO
OH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

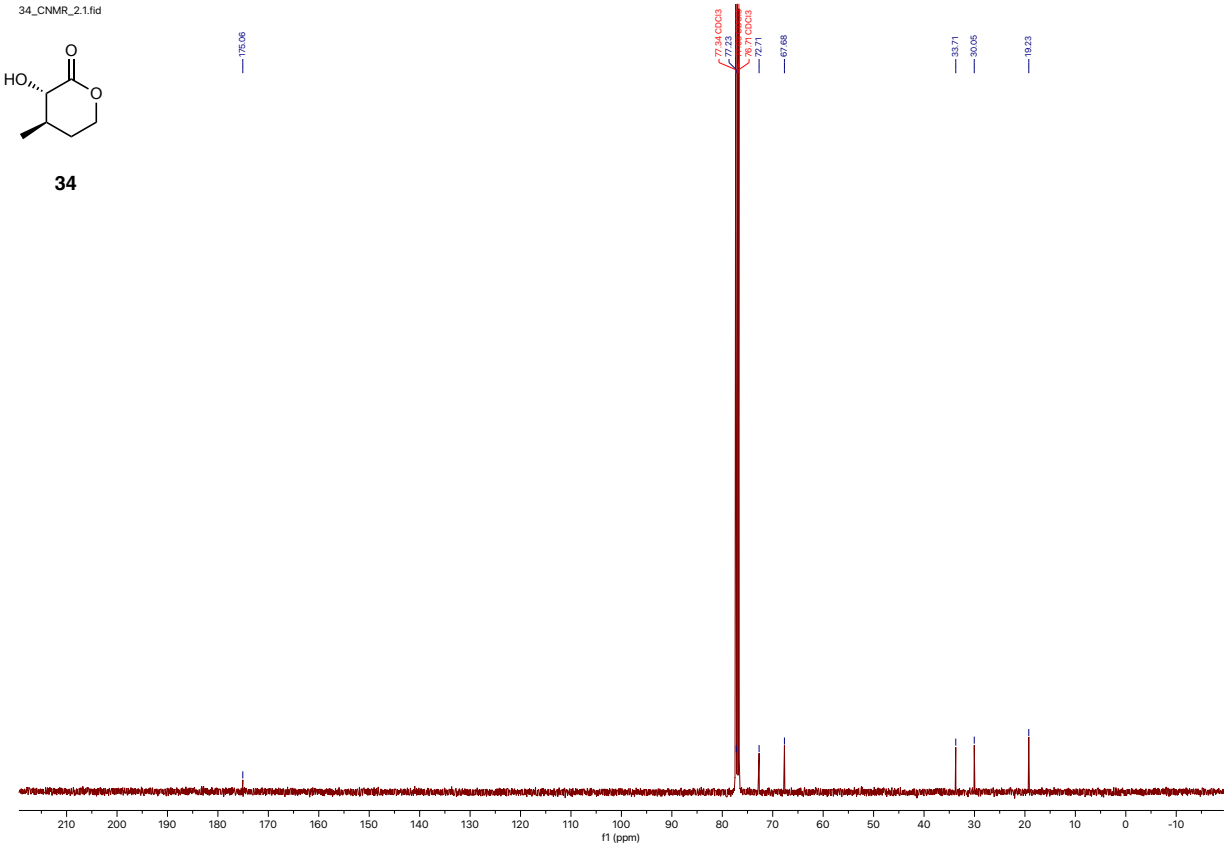
40

34_verrucarin lactone.1.fid
Standard Proton Spectrum

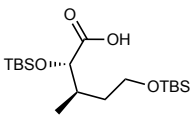


34

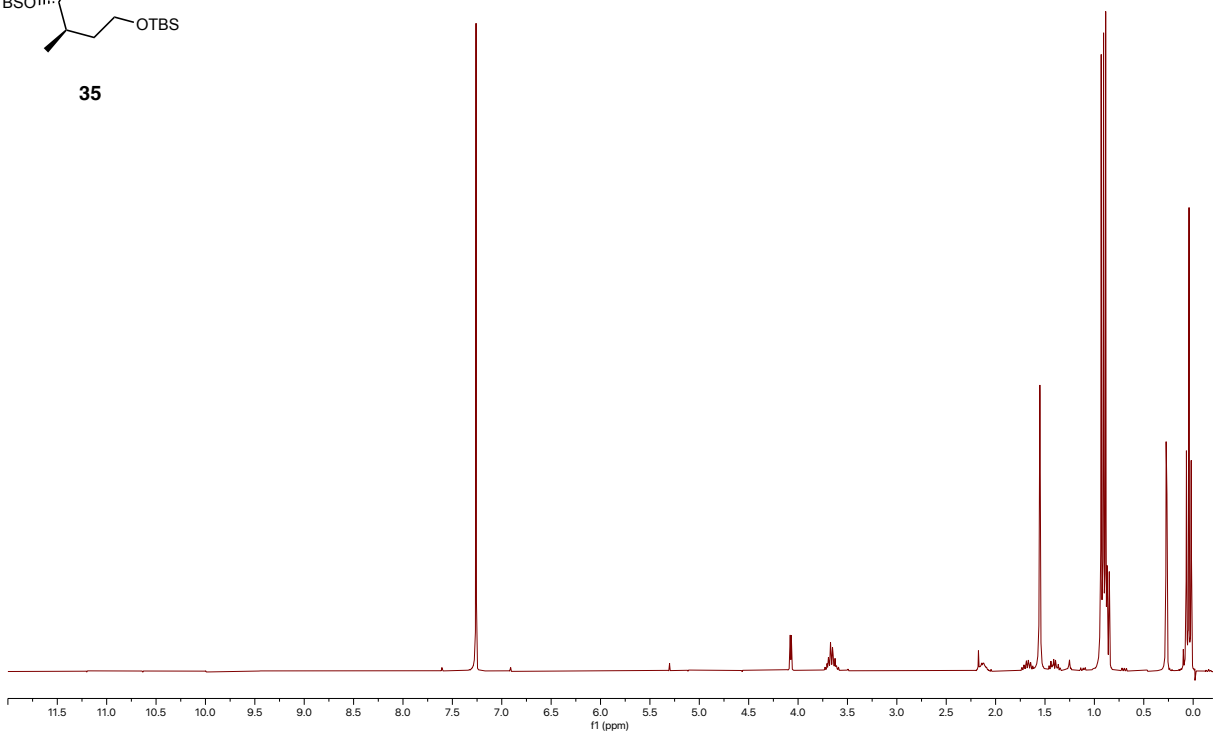


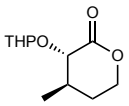

34_CNMR_2.1.fid
HO
O
O
34
175.06
77.34 CDCl3
77.23
77.02
76.71 CDCl3
72.71
67.68
33.71
30.05
19.23
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

MTVIII-49_after 1M HCl treatment.1.fid
Standard Proton Spectrum

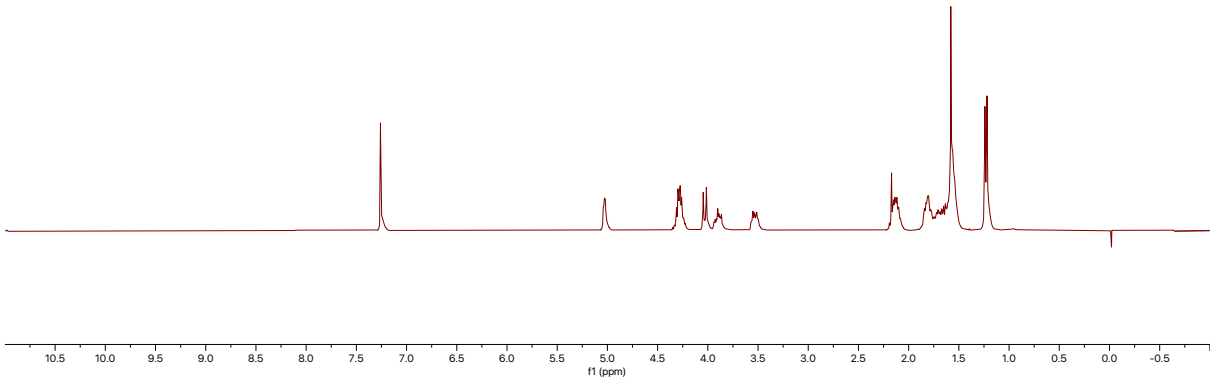


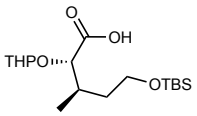
35



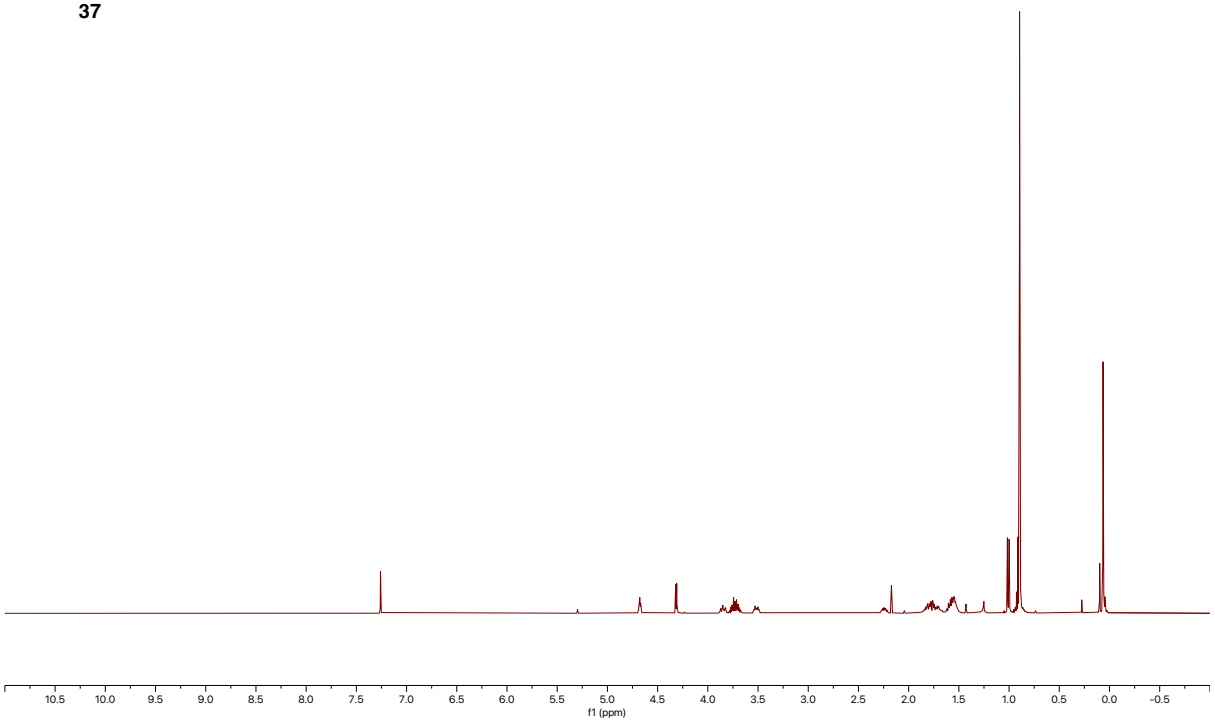

THPO
O
O

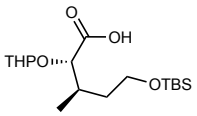
36


10.5
10.0
9.5
9.0
8.5
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
-0.5
f1 (ppm)

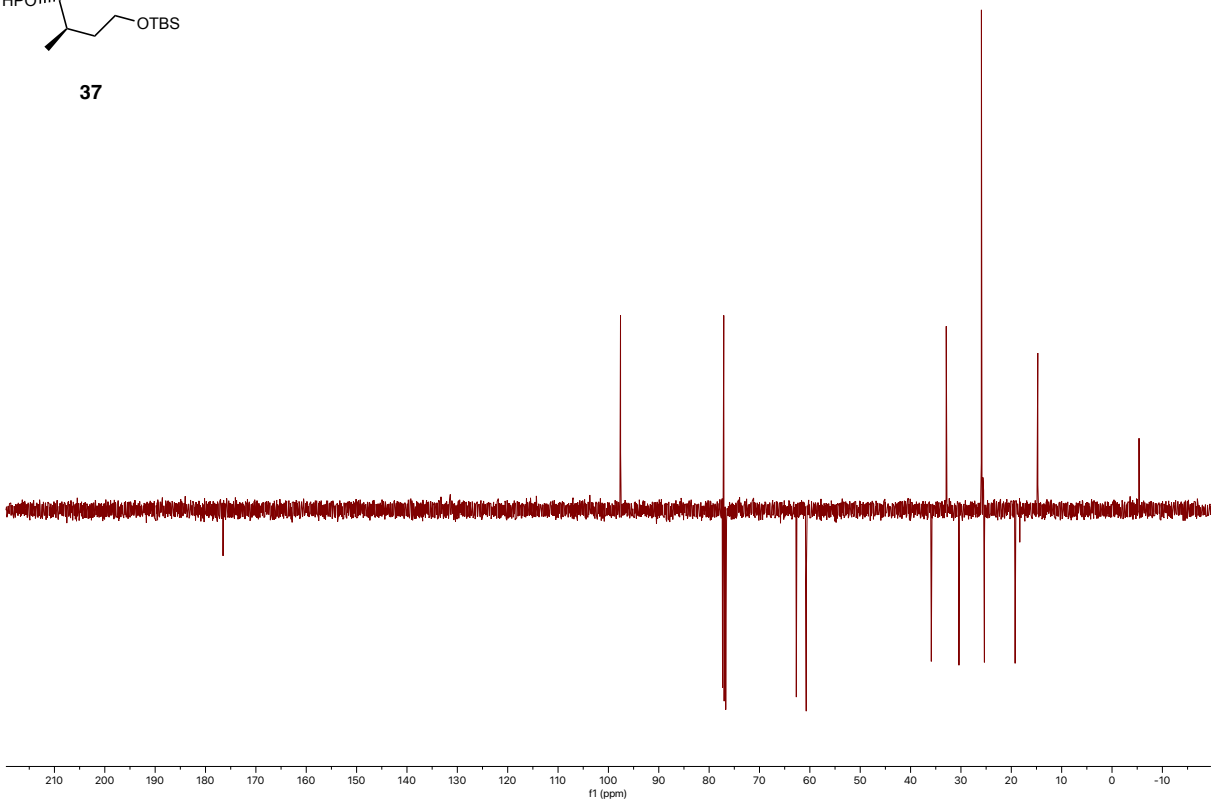

O
OH
THPO
OTBS

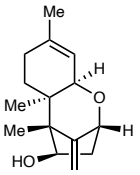
37


10.5
10.0
9.5
9.0
8.5
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
-0.5
f1 (ppm)

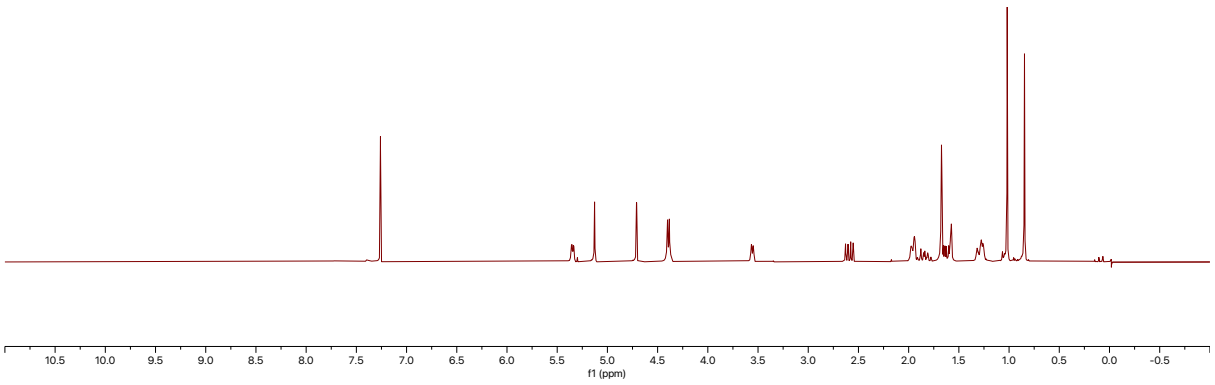

O
OH
THPO
OTBS

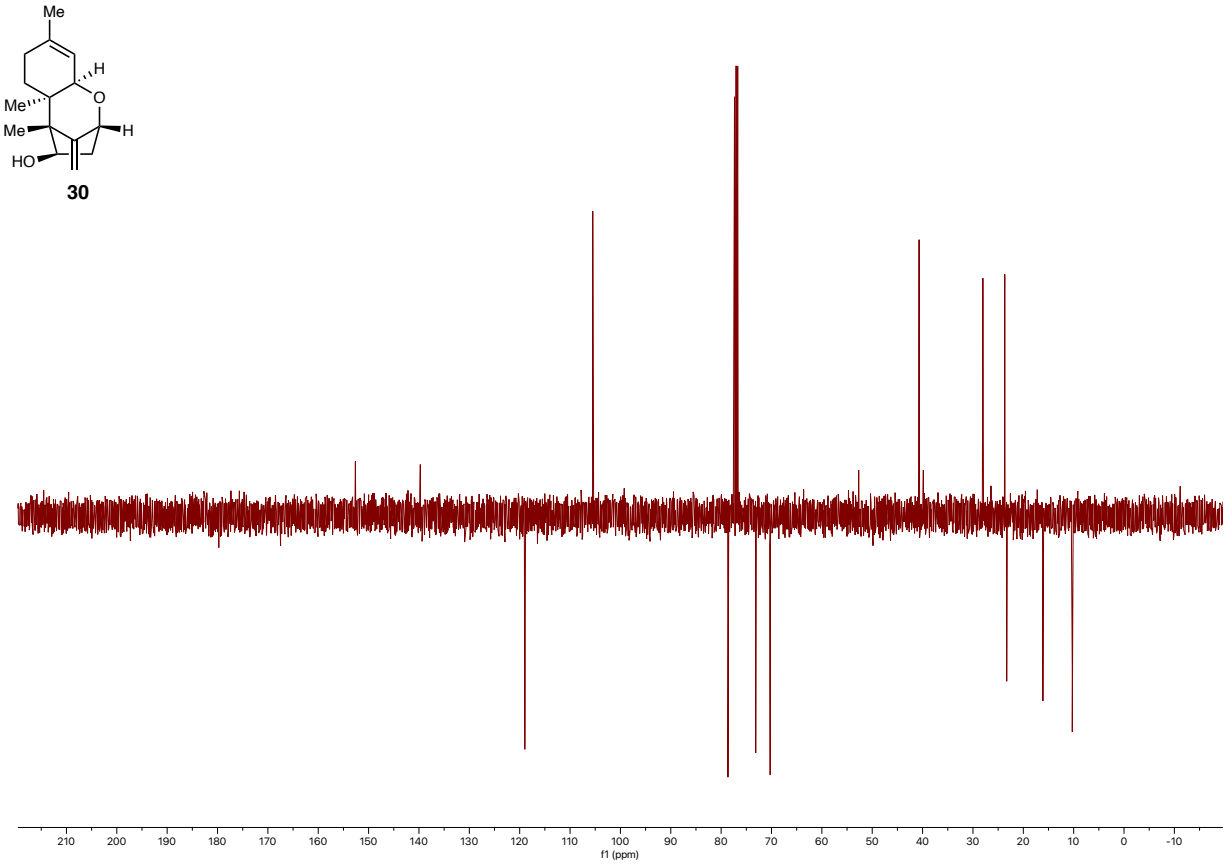
37


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


Me
H
Me
O
Me
H
HO

30


10.5
10.0
9.5
9.0
8.5
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
-0.5
f1 (ppm)


Me
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Me
O
Me
H
HO
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

30

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