Abstract

FETTEROLF, BRANDON JOHN. Synthesis and Analysis of Mechanism Based Inhibitors of Juvenile Hormone Epoxide Hydrolase from Insect *Trichoplusia ni*. (Under the direction of Russell J. Linderman)

The research presented here entails a multi-disciplinary advance at understanding the role of juvenile hormone epoxide hydrolase in the metabolism of juvenile hormone in last stadium *Trichoplusia ni*. The work combines synthetic organic chemistry and biochemistry.

A series of juvenile hormone analogs have been synthesized and their effectiveness to inhibit insect JHEH has been ascertained. Some of the key elements in the synthetic sequence for the fluorinated compounds include chemoselective oxidation of a primary alcohol in the presence of an alpha-fluorinated alcohol, introduction of a geminal difluoro group via a Reformatsky reaction or use of the DAST reagent, and selective nucleophilic and electrophilic epoxidation reactions. The synthesis of some of the non-fluorinated compounds employed regio-selective opening of an epoxide, Sonogashira palladium catalyzed cross coupling, nucleophilic epoxidation, and a variety of protecting group manipulations. Of all the inhibitors tested, the alkene **10** was the most effective alternative substrate with an I_{50} of 4.29 μM, slightly more potent than the benchmark MEMD. We also found that the introduction of fluorine increased the potency of the inhibitors compared to the non-fluorinated analogs. Lastly, inhibitor **5** provided insight to support a novel mechanism based inhibition of juvenile hormone epoxide hydrolase by formation of a tetrahydrofuran intermediate.

Synthesis and Analysis of Mechanism Based Inhibitors of Juvenile Hormone Epoxide Hydrolase from Insect *Trichoplusia ni*

by

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Dedication

To my parents John and Lynn Fetterolf

Thank you

Biography

Brandon J. Fetterolf was born on April 12, 1975 in Pittsburgh, Pennsylvania. He lived in Hatfield, Pennsylvania for eighteen years, graduating from North Penn High School in 1993. Brandon graduated in 1997 from East Stroudsburg University in East Stroudsburg, Pennsylvania where he obtained a Bachelor's of Science degree in Biology. He began his graduate education in organic chemistry at North Carolina State University in the fall of 1998 under the supervision of Dr. Russell J. Linderman. Brandon enjoys the outdoors, especially, backpacking and camping.

Acknowledgements

I would like to first start by thanking my parents, John and Lynn Fetterolf, for supporting and believing in me throughout the years. To my sisters, Denise and Meg, I have always looked up to both of you and thank you for continuing to cheer me on. I know my grandparents, other family members and friends are especially proud of me and I want to thank them for their encouragement.

I would like to thank the past and present members of the Linderman and Roe groups, especially Stacy O'Neill, Sophie Binet, Matt Isherwood, Emmeline Igboekwe, Shengyou Long, Joe Zhang, Dr. Wang, Matt Vanderherchen, Deborah Thompsom, and Doug Anspaugh. Thank you also to my roommate, Wyatt McConnell, for those intense talks about life and Jason Nolan for the Nolan Workout Plan (patent pending) and -- most of all -- for being a good friend.

I would like to acknowledge and thank Dr. Russell J. Linderman and Dr. R. Michael Roe for their leadership and guidance and for the opportunity they provided me to work on such a multi-disciplinary project. A very special thank you goes to Dr. Tal Nahir, formerly of East Stroudsburg University, for believing and encouraging me to pursue chemistry over biology. I hope some day to impact a life as you have mine.

Lastly, I would like to thank Amanda Sramek for her love, support, patience and encouragement throughout the last four years.

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DMF………………………………………………………………. N,N-dimethylformamide
DPT…………………………………………………………... 2-amino-4,5-diphenylthiazole
EH………………………………………………………………….... epoxide hydrolase
JH…………………………………………………………………….. juvenile hormone
JHE………………………………………………………….. juvenile hormone esterase
JHEH.. ……………………………………………... juvenile hormone epoxide hydrolase
*m*CPBA……………………………………………………… 3-chloroperoxybenzoic acid
mEH…………………………………………………… microsomal epoxide hydrolase
MEMD…………………………………..… methyl 10,11-epoxy-11-methyl dodecanoate
MsCl……………………………………………………… methanesulfonyl chloride
NMO………………………………………………………….... morpholine-N-oxide
OTFP…………………………………………. 3-octylthio-1,1,1-trifluoro-2-propanone
PCC……………….. ……………………………………… pyridinium chlorochromate
PDC……………….. …………………………………..… pyridinium dichlorochromate
p-TSA…………………………………………………………... *p*-toluenesulfonic acid
sEH………………………………………………………… soluble epoxide hydrolase
T. ni…………………………………………………………………. *Trichoplusia ni*
TBAF………………………………………………….... tetrabutylammonium fluoride
TBS…………………………………………………………….. *tert*-butyldimethylsilyl
TBSOTf……………………………….. *tert*-butyldimethylsilyl trifluoromethanesulfonate
THP……………………………………………………………………. tetrahydropyran
TLC…………………………………………………… thin layer chromatography

Chapter 1

Introduction

There is a need to develop a method for the control of economically destructive agricultural pests, such as lepidopteran insects. By tradition, pest management has involved the use of non-specific chemical pesticides and this practice has enabled insects to build up a resistance to low level concentrations. This has led to the use of chemical pesticides at a high concentration, generating adverse environmental effects. The introduction of a highly selective pest management system may allow for a decrease in the negative effects on non-specific targets, and also reduce environmental contamination. Insect juvenile hormone (JH) is unique to only arthropods and as far as we know there is no human complement. A more complete understanding of JHs basic physiological function in insects may provide a means of insect control that is more specific for target organisms.

Insect juvenile hormone controls and regulates a variety of necessary biological processes within the insect life cycle. JH functions in metamorphosis, reproduction, and diapause to name a few.[1-5] JH is synthesized by the corpora allata and released into the hemolymph where it is deposited within the tissues.[6] There are a number of different JHs (see Figure 1.1) consisting of a structural core of a methyl ester of farnesoic acid C10, C11 epoxide. Fluctuation in JH concentration at specific times in the life cycle allows the insect to develop. Hammock has shown that a drop in JH concentration in the hemolymph allows for metamorphosis.[3] How are these concentrations decreasing? The first answer is due to decreased synthesis and release of JH by the corpora allata. The second is due to metabolic degradation. There are two primary pathways of juvenile hormone metabolism.[3-5] The first involves juvenile hormone esterase (JHE), which hydrolyzes the methyl ester providing the corresponding acid. The second utilizes juvenile hormone epoxide hydrolase (JHEH) in hydrolysis of the epoxide resulting in the diol. It is important to note that either pathway of degradation renders the metabolite physiologically inactive.

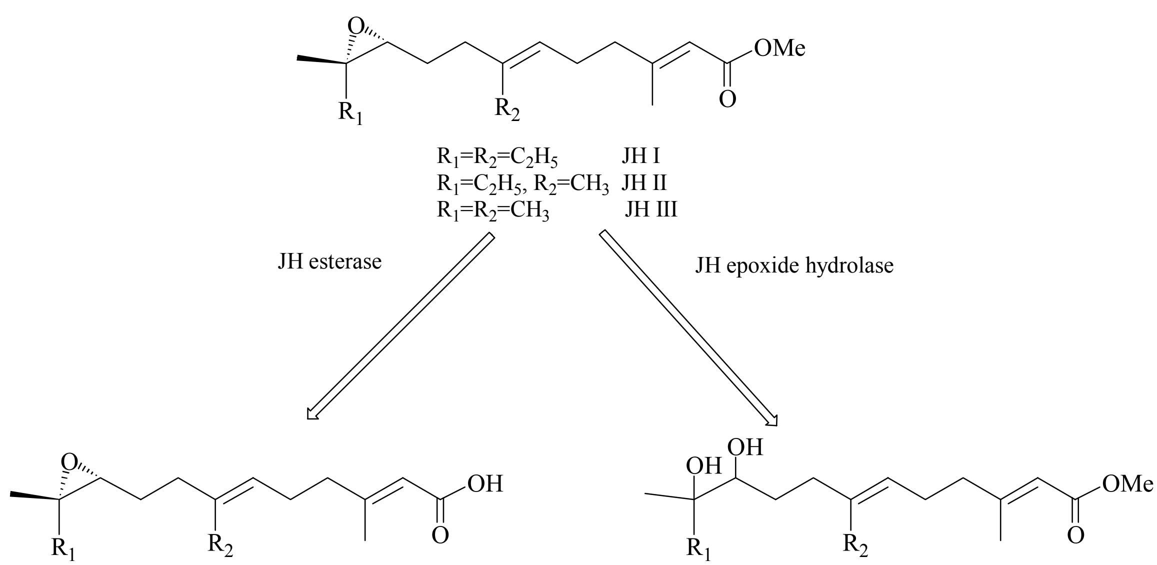


Figure 1.1. Degradation of JHs by the two different pathways

The majority of research on the effects of JH metabolism in development thus far has involved the study of the hydrolysis of JH to the acid by JH esterase. Generally, in Lepidoptera, many researchers have viewed that the primary pathway of insect juvenile hormone degradation involves JHE to form first the acid and in a second step, the epoxide is hydrolyzed generating the acid diol.[7] The inhibition of juvenile hormone esterase has also been associated with morphological changes. For example, in the last stadium of *Trichoplusia ni* a delayed wandering response, deferred pupation, and the development of malformed intermediates were all observed when the larvae were treated with organophosphate or polarized alcohol compounds.[8-10] There's also evidence of two major increases in JHE activity during the last larval instar of Lepidoptera.[4,8,9] The first upsurge triggers metamorphosis and occurs during the prewandering stage when low levels of JH titer are present. The second peak transpires during the prepupal stage and is again associated with decreased levels of JH titer. Recently, evidence indicating inconsistencies between species implies that JH metabolism by JHE may not be the primary pathway after all.[3,4]

An abundant amount of research into juvenile hormone esterase has enabled a better understanding of its relevance and has also brought about the development of JHE specific inhibitors.[11-16] Pauling in the 1940's introduced the idea of compounds being able to inhibit an enzyme by forming a stabilized transition state mimic.[17,18] These

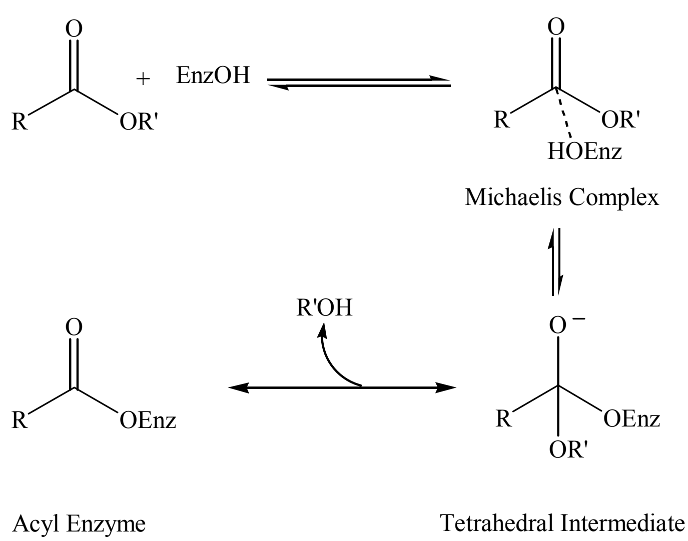


Figure 1.2. Proposed mechanism for ester hydrolysis by serine hydroxyl esterase

compounds were later given the name "transition state" analogs.[12,19-22] Some have estimated that the enzyme actually binds better to the transition state than the corresponding substrate.[19] Thus, it would be possible to create a potent inhibitor by resembling the transition state. Wolfenden characterized these transition state analogs as one that contained a mechanistic relationship from substrate to product.[22] This mechanism involves a Michaelis complex that is transformed to a tetrahedral intermediate before going to the acyl intermediate seen in Figure 1.2.[12] Hammock found a transition state analog 3-octylthio-1,1,1-trifluoro-2-propanone, OTFP, to be a potent inhibitor of JHE, I_{50} of 2.8 x 10^{-9}M.[11-16] The presence of a strong electron withdrawing trifluoromethyl group allows the ketone to remain largely as the hydrate within an aqueous solution (Figure 1.3).[23,24] Importantly, this hemi ketal resembles the normal

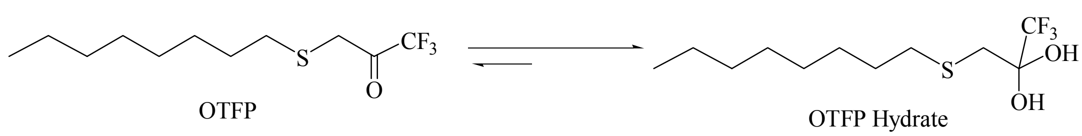


Figure 1.3. Fluoroketones exist predominately as the hydrate in an aqueous solution.

tetrahedral intermediate prior to the formation of the acyl-enzyme intermediate (Figure 1.4).[12] Interestingly, Linderman found that a decrease in inhibition was observed when the ketone of OTFP was reduced to the alcohol.[15,16] This again reinforces the idea of a potent inhibitor being one which mimics the transition state.

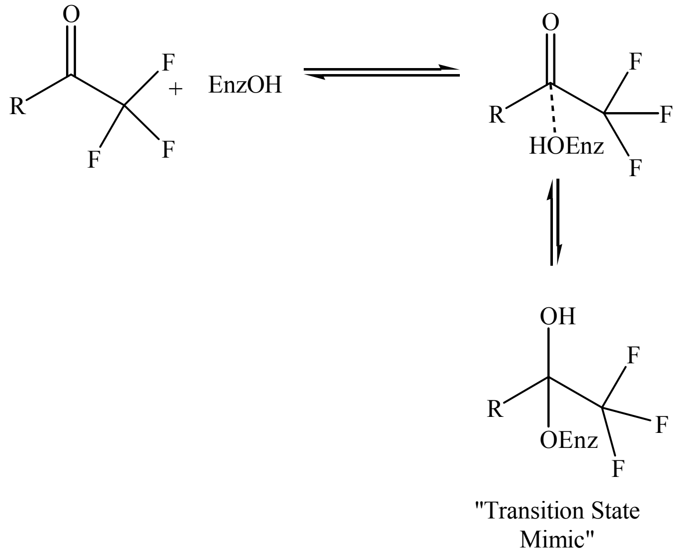


Figure 1.4. Possible mechanism of inhibition by fluoro-ketones by stabilizing the tetrahedral intermediate.

Unlike JH esterase, the contribution of JHEH in the metabolism of JH is less understood and has also been found to vary between species. JHEH was ascertained not to participate in the metabolism of JH in egg homogenates of the house cricket, *Acheta domesticus.*[25,26] Conversely in the tobacco hornworm, *Manduca sexta*, JHEH plays a major role in the degradation within egg homogenates.[27] There has also been research to support a relationship in species and tissues between the activity of JHEH and that of JHE. Jesudason showed in prewandering, last stadium *Manduca sexta,* that increased levels of JH epoxide hydrolase in the integument, fat body, and brain were also associated with high JHE activity in the integument, fat body, brain, midgut, and hemolymph.[28] In contrast, Kallapur et al. established in *Trichoplusia ni* that juvenile hormone esterase levels peaked within the integument, fat body, and midgut in the last stadium (day 2) and also in the fat body of the prepupae (day 4), whereas on day 2 the midgut contained high JHEH activity and the fat body and integument peaked on day 3.[7]

Evidence to support a major role of JHEH in the metabolism of JH in higher order insects has been noted. During the last stadium of *C. quinquefasciatus*, JHEH activity doubles compared to no difference in esterase activity.[29-31] In fact, to refute the idea that JHEH plays a secondary role after the formation of JH acid, Kallapur discovered that at physiological concentrations [^{3}H] JH II is preferred by JHEH over the JH II acid.[7] In addition, the formation of another major metabolite, a polar phosphate conjugate, arises from the diol/ester and not the acid/epoxide.[7] Solely looking at the activity of JHEH in specific tissues and species, it is clear that further investigation needs to be assessed.

Unfortunately, insect juvenile hormone epoxide hydrolase has been understudied compared to its mammalian EH counterpart. The important aspects are to apply the knowledge gained from the mammalian epoxide hydrolase and relate it to insect EH. In mammals, five different classes of epoxide hydrolase can be identified based on the type of substrate metabolized and the location of the EH in the cell; 1) cholesterol epoxide hydrolase, 2) leukotriene A_4 epoxide hydrolase, 3) hepoxilin epoxide hydrolase, 4) microsomal epoxide hydrolase (mEH), and 5) soluble epoxide hydrolase (sEH). The last two, mEH and sEH, are the most consistently studied within mammals. Microsomal EH breaks down a variety of exogenous epoxides such as polycyclic aromatic hydrocarbons (PAH's).[32-35] Whereas, sEH metabolizes a variety of fatty acid derived epoxides.[36,37] Genetic copies of both human and rat mEH and sEH have been successfully created.[38-40] The soluble epoxide hydrolase has also been cloned from mouse [41-43] and plants, ex. potato (*Solanum tuberosum*).[44] The development of these sequences has allowed for comparison between known assemblies. By doing this, regions in both the mEH and sEH that were similar to bacterial haloalkane dehalogenase (HLD1) were found.[45] Interestingly, these regions all contain a similar Glu/His/Asp catalytic triad responsible for metabolism of substrates.

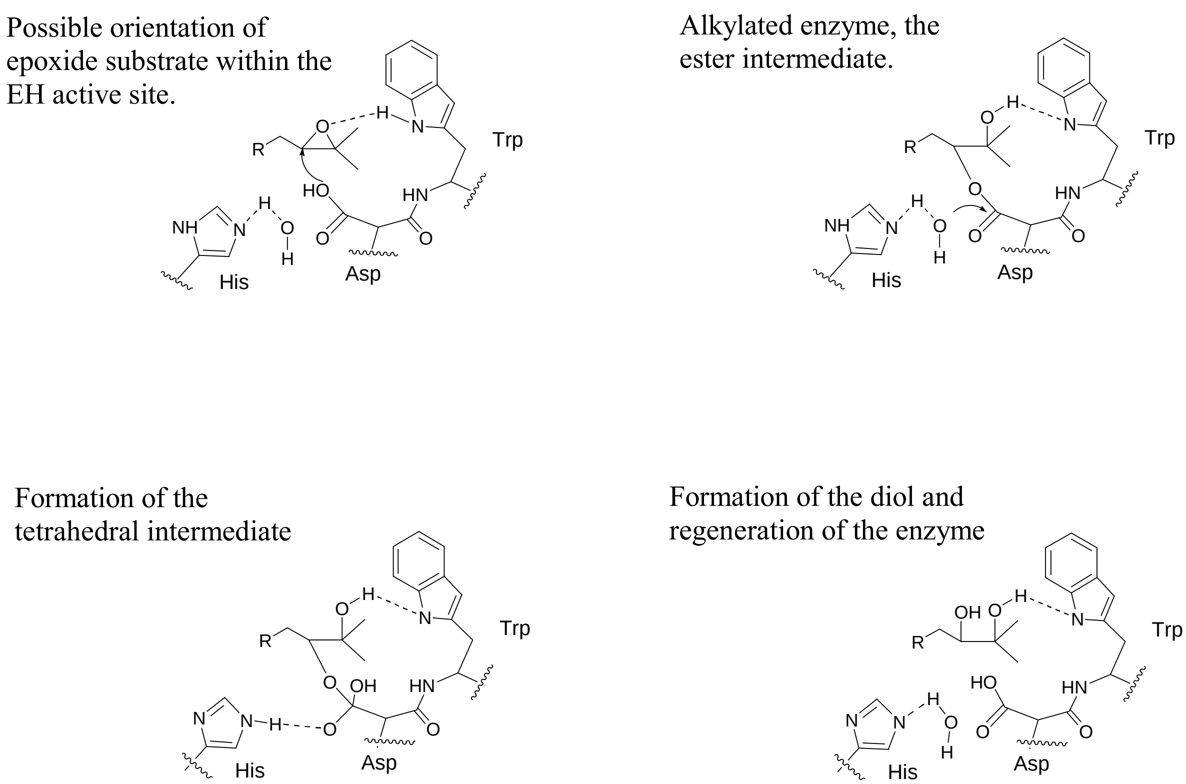


Figure 1.5. Proposed mechanism of mammalian epoxide hydrolase catalyzed epoxide hydration

An X-ray crystal structure of an acyl-enzyme intermediate of haloalkane dehalogenase categorized the enzyme within the α,β-fold family of enzymes.[46,47] Recently, a crystal structure of recombinant mouse sEH[48]and EH from *Agrobacterium radiobacter* AD1[49] have also been published. Interestingly, all of the X-ray crystal structures were shown to contain a highly conserved active site made up of the same acid/base/acid.[48,49]

A proposed mechanism for epoxide hydrolysis shown in Figure 1.5 occurs in two steps. The first step involves nucleophilic attack by an aspartate residue at the least substituted position of the epoxide. This in turn creates a covalently bound ester intermediate. In a second rate and limiting step,[50] a water molecule is activated by a His residue, which hydrolyzes the acyl-enzyme providing the diol and regenerating the active enzyme. Lacourciere and Armstrong were the first to show this proposed mechanism to be true for mEH.[51] By using ^{18}O labeled water, they found no incorporation of the ^{18}O indicator in the resultant diol. Hammock also provided evidence of a two-step mechanism in murine sEH by precipitation of an intermediate [^{3}H] JH III-enzyme adduct in acidic acetone and using an ^{18}O marker.[52,53]

Analogous to the relationship that researchers have found to exist with the mammalian EH and bacterial dehalogenase, scientists have also established a connection with insect JHEH. Wojtasek and Prestwich were able to clone the first JH specific EH from the eggs of *Manduca sexta*.[54] The deduced protein sequence showed 44% identity and 64% similarity to human microsomal EH. Again, conservation of the Glu/His/Asp catalytic triad was observed. Harris used a BLAST simulation to predict similarity of amino acid sequences from *Trichoplusia ni* mEH to human microsomal EH (41% identical) and *Manduca sexta* mEH (46% identical).[55] On the other hand, *T. ni* mEH was divergent with sequences from soluble epoxide hydrolases (18% to 19% identical). Significantly, a high degree of homology with *T. ni* mEH was found between all of the epoxide hydrolases and dehalogenase surrounding the Glu/His/Asp amino acid residues (active site).[55] Linderman found that nucleophilic addition of ^{18}O labeled water occurred exclusively at the C10 position of the epoxide and that the S_N2 mechanism of metabolism was consistent with data from previous studies of the mammalian enzyme.[56] These data imply that the mechanism of epoxide hydrolysis noted in Figure 1.5 for mammalian species should also be operative for insect JHEH.

The two main mammalian epoxide hydrolases differ in substrate specificity and also vary greatly in what type of compounds inhibit the enzyme. Hammock found that cyclohexene oxides and trichloropropene oxides can retard mEH activity, while sEH activity is not hampered.[57,58] Only at 100 times the concentration that inhibits mEH will there be any sEH inhibition.[59] The reverse was also found to be true. Substituted chalcone oxides stall mouse sEH activity at low concentrations and only slightly impede mEH activity at higher concentrations.[59] There has also been an "opposite" effect where a compound may inhibit sEH activity but cause an increase in mEH activity. For example, some analogs of hydroxylated 2-amino-4, 5-diphenylthiazole (DPT) were shown to inhibit sEH, yet amplify levels of mEH activity.[60,61] The majority of these inhibitors contain epoxides and are designed to act as competitive (alternate) substrates. Recently though, Hammock has introduced the idea of epoxides not being important, by observing a reduction in sEH activity using amides and ureas.[62] In general, inhibitors of mammalian mEH and sEH are not as successful in inhibiting insect epoxide hydrolase.

Earlier work in our group has focused on the inhibition of microsomal JHEH in *Trichoplusia ni*, the cabbage looper.[55,63,64] The most potent inhibitors were alternative substrates, which mimicked JH III. The length of the carbon backbone as well as the methyl ester and C10, C11 epoxide are important in substrate binding. In addition, tri-

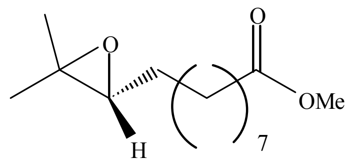
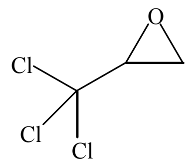
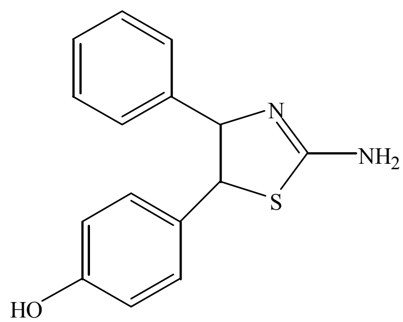
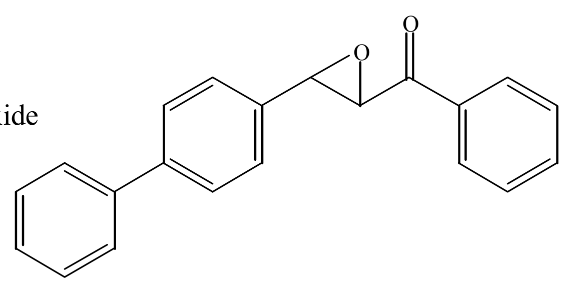
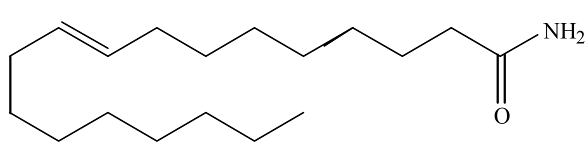
Inhibitor	Molecular Structure	Enzyme/ I_{50}
C10-*R* MEMD		*T. ni* JHEH/ 94 nM
Trichloropropene oxide		mouse mEH/ 2 μM
4OH-DPT		mouse sEH/ 0.313 mM
4 Phenylchalcone oxide		mouse sEH/ 64 nM
Elaidamide		rat mEH/ 1.3 μM

Figure 1.6. Molecular structure, enzyme inhibited, and I_{50} data for different inhibitors

substituted epoxides were found to be better inhibitors than the corresponding di-substituted derivatives. Interestingly, the correct stereochemistry at the C10 position can greatly enhance the potency of the inhibitor. Earlier results concluded that racemic MEMD was the most potent inhibitor of *T. ni* mEH to date with an I_{50} equal to 80 μM. Later work revealed that the C10-*R* enantiomer of MEMD was more potent than the *S*, I_{50} of 9.4 x 10^{-8}M vs. 2.2 x 10^{-6}M respectfully.

The interaction of juvenile hormone epoxide hydrolase has been understudied compared to its counterpart JHE. Many believe that hydrolysis of the epoxide may be the primary route of metabolism of JH. By designing inhibitors of insect EH, we are better able to understand the interaction between hormone and enzyme. This allows for the expansion of our understanding and the possibility of designing inhibitors that mimic the transition state of the enzyme. We herein describe the rationale, synthesis, and *invitro* assay of eleven novel inhibitors of juvenile hormone epoxide hydrolase in larval *Trichoplusia ni*. Additionally, we will show a mechanistic approach to inhibition by interacting with either the alkylated ester intermediate or with the His residue (Figure 1.5) after nucleophilic attack of the aspartate residue.

Chapter 2

Discussion and Synthesis of JH Analogs

We have learned from previous structure activity relationship studies with MEMD that it is important to mimic the 12 carbon backbone of juvenile hormone.[64] Also, conservation of the C10, C11 epoxide and the methyl ester are important in substrate binding. What is noticeably different with the potential inhibitors shown in Figure 2.1 is that we have introduced functionality along the carbon backbone in hope of creating a mechanism based approach to inhibition. Although we have found that the C10 *R*-enantiomer of MEMD enhances the inhibition of JHEH,[64] the analogs presented here are

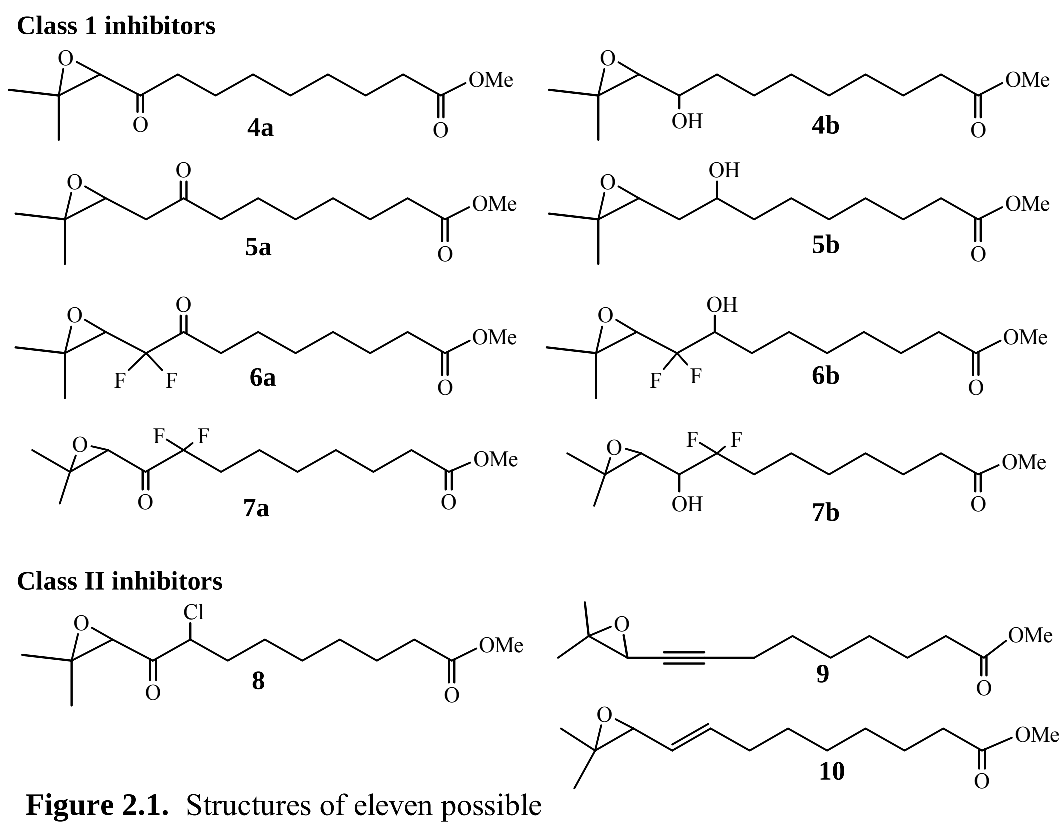


Figure 2.1. Structures of eleven possible inhibitors of JHEH

racemic. The compounds are also divided into two separate classes. The Class I compounds (**4a**-**7b**) are designed to be reversible inhibitors by helping to stabilize the ester intermediate or the tetrahedral intermediate prior to hydrolysis thereby slowing down the release of the enzyme. While the Class II analogs may act in an irreversible mode of inhibition by interacting with the His residue and not allow for regeneration of the enzyme.

Preliminary mechanistic and molecular biology studies done within our group has allowed us to expand our knowledge into the identity of the three amino acid residues believed to be found in the active site of insect JHEH.[55,63,64] Similarities in the sequence homologies between *T. ni* JHEH with that of *M. sexta* JHEH and other EHs have allowed us to presume that an Asp-226, His-430, and Glu-403 make up the catalytic triad.[55] Combining the mechanism of epoxide hydration discussed earlier (Figure 1.5) with the data obtained from the work done with mammalian EH topology, we feel that the analogs may arrange in the active site of JHEH with the long aliphatic chain extending toward the

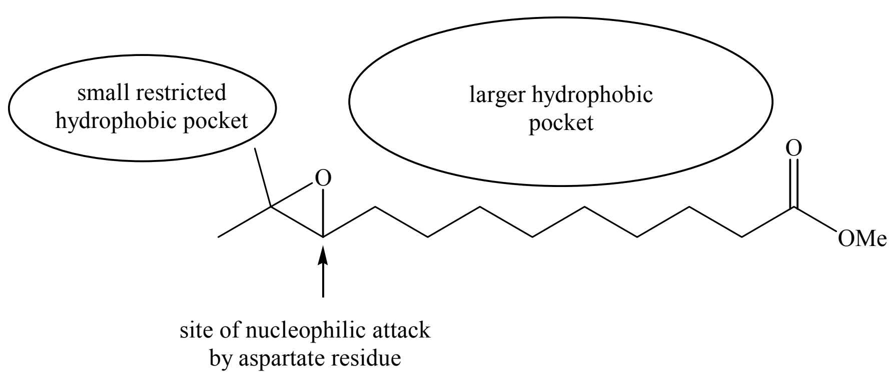


Figure 2.2. Proposed topology and orientation of inhibitor within JHEH active site

His and Glu residues noted in Figure 2.2. In this orientation, it is possible that the aliphatic chain may interfere with the delivery of the water molecule needed to hydrolyze the ester intermediate.

2.1: Class I Analogs

All of the potential inhibitors represented in Class I are transition state analogs and are designed to interact with the catalytic triad, in hope of potentially decreasing the rate of hydrolysis of the ester intermediate. This mode of inhibition first requires nucleophilic attack of the Asp residue on the epoxide generating the acyl-enzyme complex. Effectively, at this point we believe that by incorporating functionality; i.e. ketone, alcohol, or fluorine, we could potentially either interfere or help stabilize the ester intermediate before hydrolysis by the activated water. The orientation of the catalytic triad is not fully known in the insect species and to account for this we have placed the functionality at different positions in hope of increasing the effectiveness of inhibition. We felt that the introduction of a ketone one to two atoms away from the epoxide may act as a site for hydrogen bonding by the nucleophilic water within the active site or by the His residue. One could also envision nucleophilic attack at the

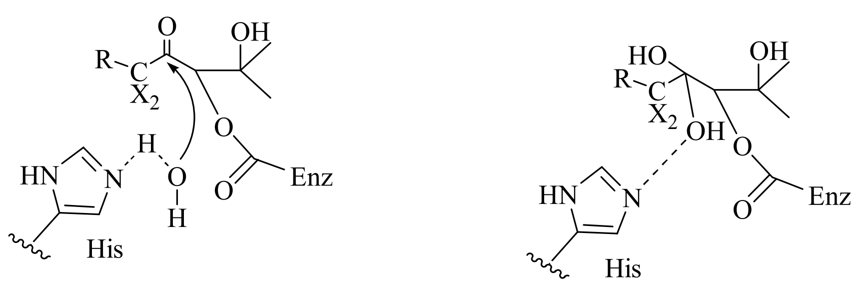
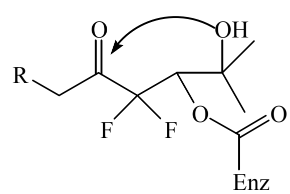


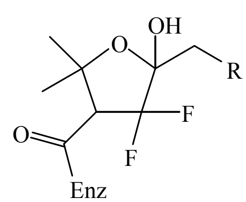
Figure 2.3. Possible mode of inhibition for **4a** (X-H) or **7a** (X-F) via gem diol formation

carbonyl by the activated water normally intended to hydrolyze the ester intermediate on the ketone (Figure 2.3). This in turn would generate a hemi-ketal and possibly decrease the rate of enzyme reactivation (Figure 2.3). We also believe that we could possibly increase the probability of hemi-ketal formation by generating an electron deficient ketone. Introduction of a strong electron-withdrawing group such as a fluorine alpha to a ketone enabled Hammock to successfully inhibit juvenile hormone esterase by mimicking the tetrahedral intermediate of the enzyme.[11-16] Remembering that the ketone is predominately the hydrate in an aqueous solution (Figure 1.3)[23,24] and also that a fluorine is similar to a proton in atomic radius, we could then derive a few new promising mechanisms of inhibition for a fluorinated ketone. We would expect nucleophilic attack on a fluoro-ketone by the activated water to occur more readily than the non-fluorinated ketone (Figure 2.3) due to the more electron deficient carbonyl. In addition, after the

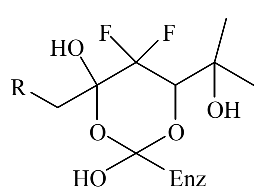
Figure 2.4. Possible mechanism of inhibition for **6a** and **7a**



Ester intermediate formed by alkylation of the enzyme by inhibitor **6a**



THF intermediate result of intramolecular attack



Formation of the hemi ketal by nucleophilic attack of the gem diol formed during hydrolysis of the ester intermediate ester intermediate has been formed, the new C11 hydroxyl group could potentially undergo intramolecular attack on the ketone to form a five membered tetrahydrofuran ring as shown in Figure 2.4. This could induce a conformational change of the protein, potentially reducing the rate of hydrolysis of the ester intermediate. If this were to occur, the mode of action would be mechanism based inhibition. Also, the regiochemistry of the ketone could dictate this route of inhibition. We could expect analog **7a** not to participate in this mode of action due to formation of a four membered heterocycle intermediate that may be too unstable. Lastly, hydrolysis of the ester intermediate by the activated water may allow for nucleophilic attack of the newly formed gem diol at the electron poor ketone (Figure 2.4). This would form a five or six membered heterocycle (Figure 2.4) dependent on the placement of the ketone.

Alternatively, positioning of an alcohol one to two carbon atoms away from the oxirane may compete with the activated water for hydrolysis of the ester intermediate. If this would happen, the configuration of the covalently bound ester intermediate would change by an equilibrium acyl transfer reaction, generating a new alkylated enzyme intermediate (Figure 2.5). This new acyl enzyme derivative may be enough to decrease the extent of regeneration of the enzyme active site residue. The efficiency of the mechanism presented here may be dependent on the regiochemistry of the alcohol. Alcohol **4b** would proceed through a five membered transition state versus a six membered case for **5b**. We would expect **4b** to be favored due to the involvement of a five membered transition state. Proton NMR studies of fluorinated alcohols have shown a greater amount of hydrogen bonding to ethers compared to non-fluorinated alcohols.[65]

In addition, we believe that the fluoro-alcohols **6b** and **7b** will be more acidic compared to **4b** and **5b**.[66] Ballinger showed that for substituted methanol's (RCH_2OH) when

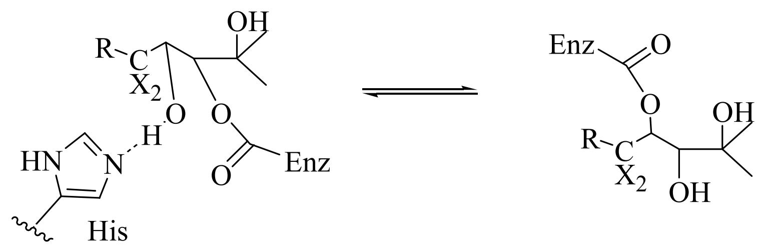


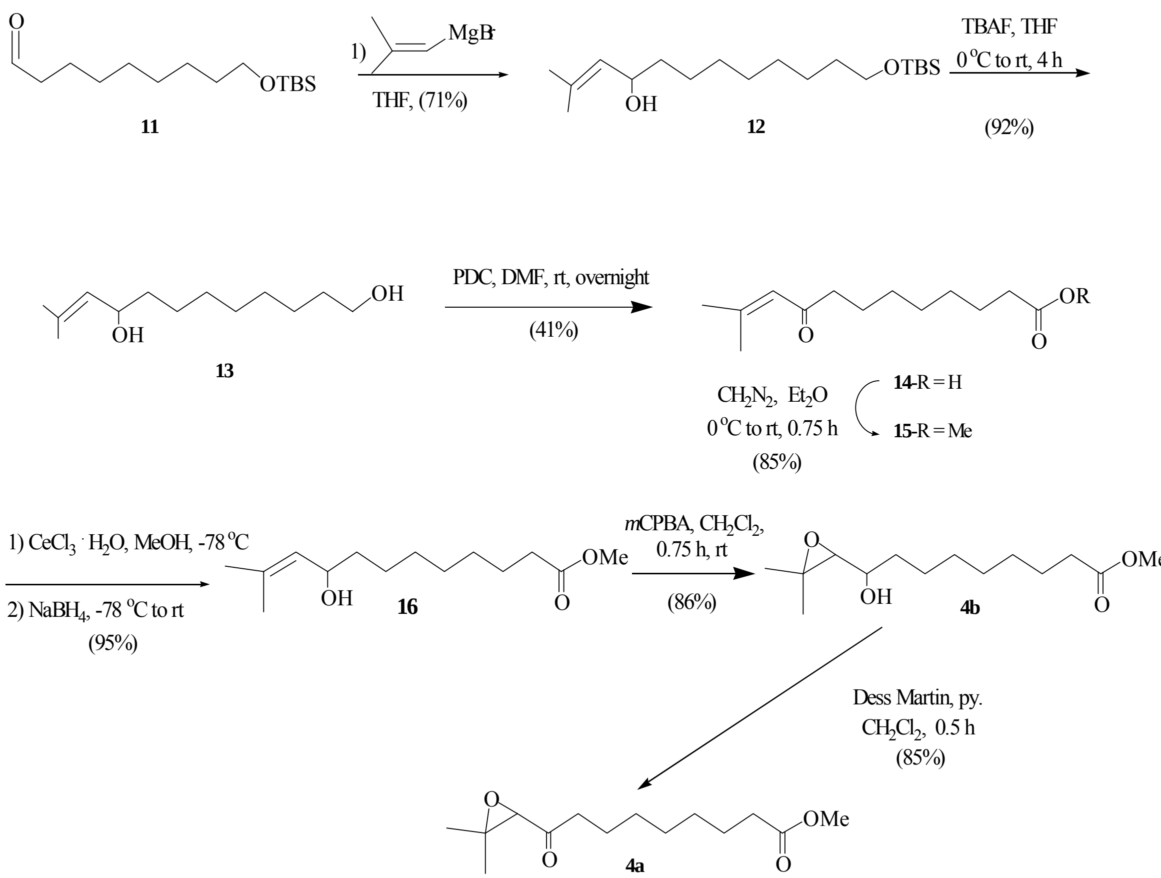
Figure 2.5. Possible mode of inhibition for **4b** (X-H) or **7b** (X-F) by establishment of an equilibrium acyl transfer process

R = CF_3 the pK_{HA} was equal to 12.37 versus that for ethanol at pK_{HA} 15.9.[66] Because of these two factors, there may be an increase in the fluorinated alcohol activation by the His residue to carryout the rearrangement shown in Figure 2.5.

2.1.A. Synthesis of Analogs 4a and 4b

The synthesis of **4a** and **4b** are shown in Scheme 2.1 and are relatively straightforward. Starting aldehyde **11** was not commercially available but was made in 65% yield by mono TBS protection of 1,9-octanediol followed by PCC oxidation. Grignard addition of 1-methyl-2-propenylmagnesium bromide to aldehyde **11** generates allylic alcohol **12** in 71% yield. Deprotection of the primary alcohol using TBAF, followed by oxidation of both alcohols via PDC in DMF[67] provides the keto acid **14** in moderate yield. Esterification using diazomethane followed by chemoselective reduction using Luche[68.69] conditions generated allylic alcohol ester **16** in 90% yield for the two steps. Electrophilic epoxidation via *m*CPBA provided alcohol epoxide **4b**, while subsequent Dess-Martin[70] oxidation produced inhibitor **4a**.

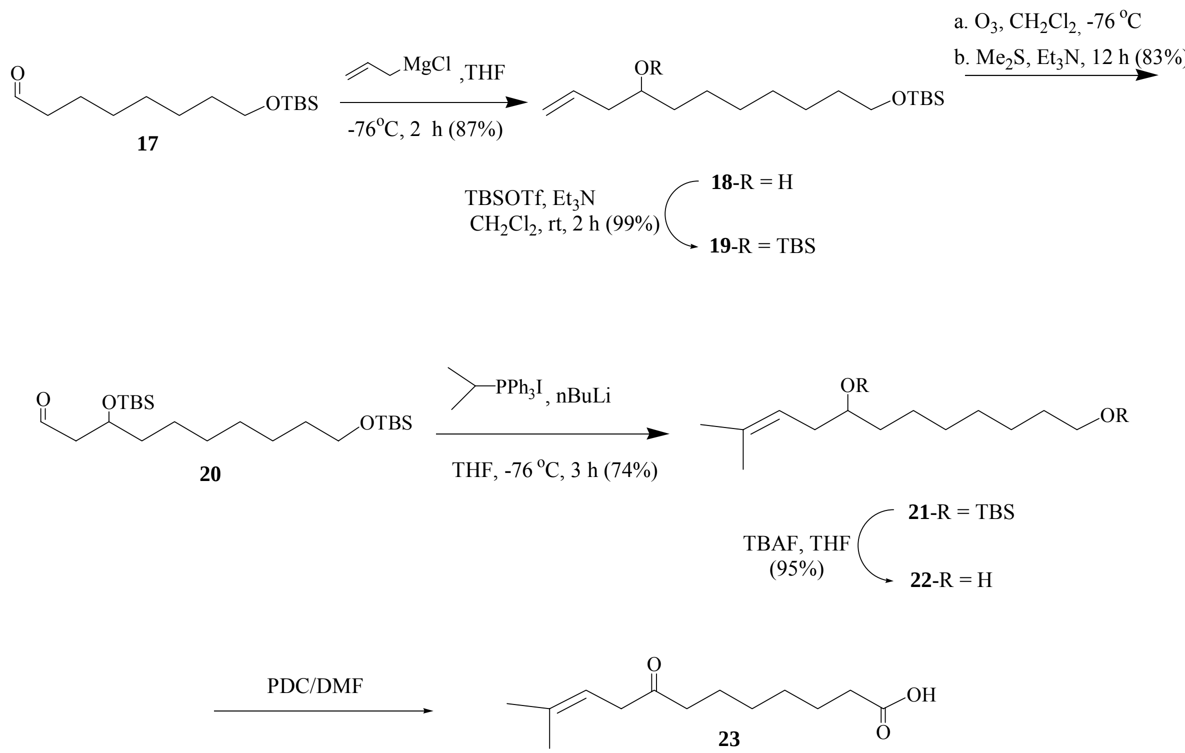
Scheme 2.1. Synthesis of Inhibitors **4a** and **4b**



2.1.B. Synthesis of Analogs 5a and 5b

There have been a variety of different routes attempted for the complete synthesis of inhibitors **5a** and **5b.** As shown in Scheme 2.2, we felt the product could be achieved by reaction between aldehyde disilylether **20** and a Wittig reagent. Aldehyde **17,** obtained by mono-TBS protection of 1,9-nonanediol followed by PCC oxidation, was reacted with allyl Grignard to provide **18** in 87% yield. Next, the homo allylic alcohol was reacted with TBSOTf and triethylamine to give **19** the disilyl ether in excellent yield, 99%.[71] Compound **19** was next subjected to ozonolysis which provided aldehyde **20**. Wittig reaction of **20** with the ylide derived from isopropyltriphenylphosphonium iodide

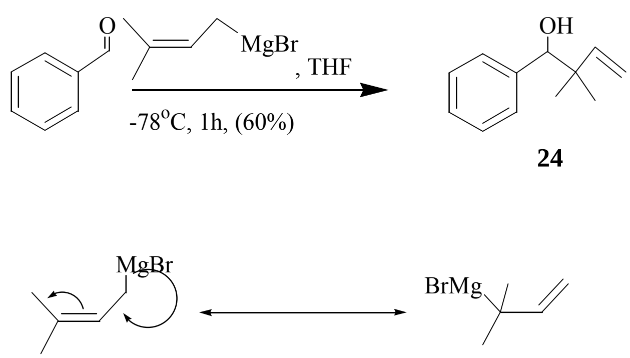
Scheme 2.2. Wittig reaction to generate alkene intermediate **25**



resulted in a good yield (74%) of **21.** Disappointingly, this reaction only worked successfully once and was not reproducible. Careful titration of the *n*-BuLi and varying the amount of base added during Wittig formation did not help. It was observed by TLC that a side reaction was taking place. Low conversion could have been due to deprotonation of the aldehyde followed by condensation. Nevertheless, the product **21** obtained was deprotected with TBAF followed by oxidation of the diol with PDC in DMF[67] to yield crude keto acid **23** (purification was not attempted). Esterification was not tried on this material.

With the previous failed attempt at homologation, we next focused on Grignard addition to an aldehyde.[72] Due to its availability, benzaldehyde was used as the model study for the addition of prenylmagnesium bromide noted in Scheme 2.3. This reaction produced no desired product but the compound formed, **24**, may have come from a S_N2' attack of the rearranged Grignard reagent.

Scheme 2.3: Grignard addition to benzaldehyde

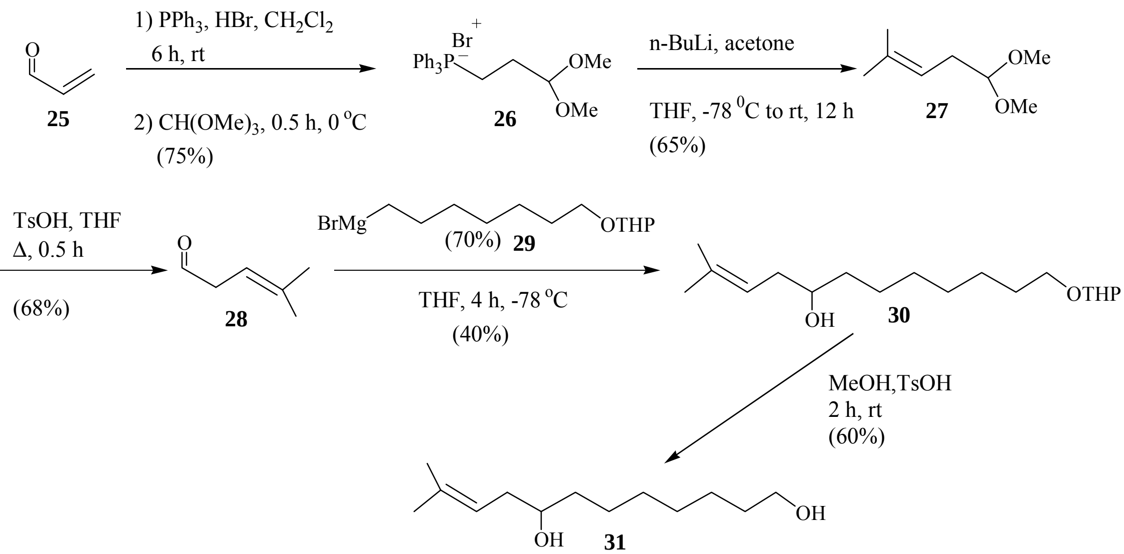


24

An alternative route mirrors the Grignard addition previously discussed. The difference being that we have reversed which side of the product that is derived from the Grignard reagent. We anticipated making aldehyde **28** followed by Grignard addition as shown in Scheme 2.4. Aldehyde **28** is not commercially available but was made following a literature procedure.[73] Acrolein **25** reacted with triphenylphosphine and hydrobromic acid followed by protection with trimethyl orthoformate in a one-pot reaction to provide **26**. Acetone was then treated with the ylide formed from the Wittig salt to provide the masked aldehyde **27**. Removal of the acetal gave the desired aldehyde **28**. Grignard addition of the THP protected ether **29** (prepared by THP protection of 7-bromo-1-heptanol occurring in 70% followed by refluxing with Mg turnings) to aldehyde

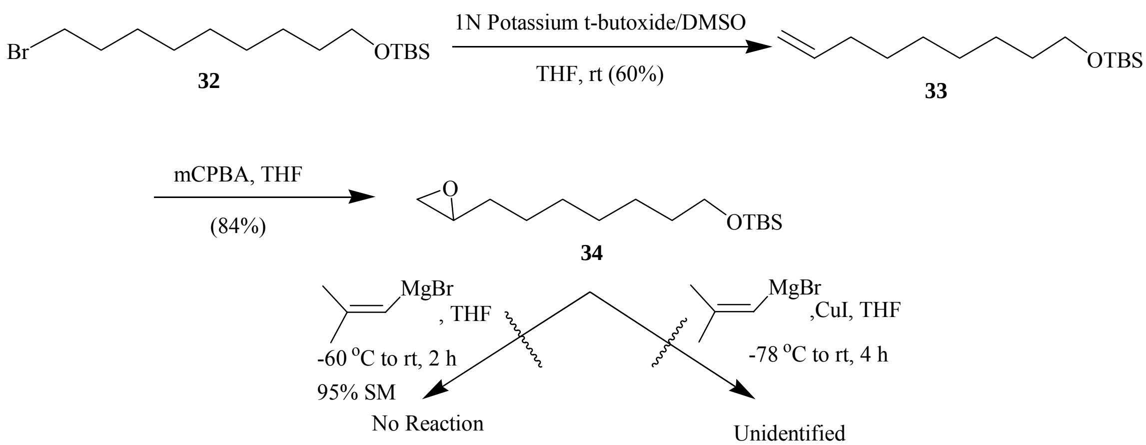
29 provided **30** in low yields. The yield of this reaction was not optimized. NMR spectral data of the higher R_f side product identified the hydrolyzed Grignard product, which may also account for the mass recovery. Deprotection of **30** with *p*-toluene sulfonic acid (*p*-TSA) in methanol resulted in diol, **31**, in moderate yield. Due to the presence of the side product in the Grignard addition, this Scheme was also abandoned.

Scheme 2.4. Grignard addition to aldehyde **31**



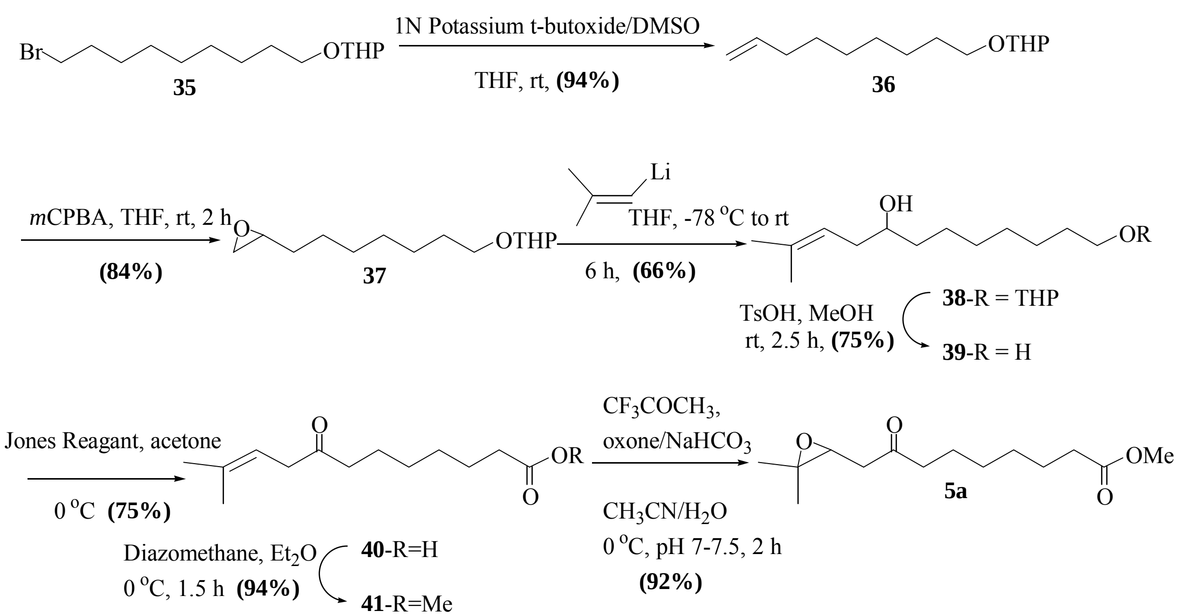
As an alternative, we envisioned that the alkene could be introduced via a regioselective epoxide opening by nucleophilic attack of a Grignard or copper-mediated Grignard reagent (Scheme 2.5). TBS protection of 9-bromo-1-nonanol using TBSOTf and triethylamine provided the ether bromo ether **32**. Elimination of the HBr from **32** with potassium t-butoxide (1N in DMSO)[74] was followed by epoxidation with *m*CPBA to give **34** in 84% yield. Grignard addition resulted in nothing more than recovered starting material, while copper mediated vinyl Grignard addition generated a side product that was not characterized.

Scheme 2.5. Attempted Grignard addition to epoxide **19**

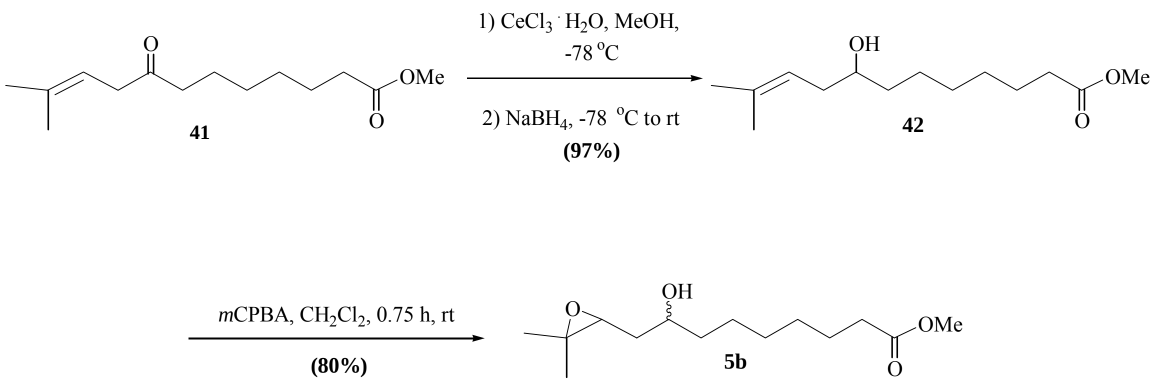


We next changed the protecting group to a THP ether to allow for the use of a lithium-mediated addition as shown in Scheme 2.6a and 2.6b to complete the synthesis. Dihydropyran and *p*-TSA was reacted with 9-bromo-1-nonanol generating the THP ether

Scheme 2.6a. Synthesis of analogs **5a** and **5b** by lithium addition to generate **38**



in good yield. Elimination of HBr from **35**, using potassium t-butoxide (1N in DMSO)[74], provided alkene **36** in 94% yield. Epoxidation by *m*CPBA generated epoxide **37**, the intermediate that would be regioselectively opened. We reacted epoxide **37** with the vinyl lithium reagent obtained from 1-bromo-2-methylpropene and *t*-BuLi to successfully generate the desired regio-isomer **38** as the major product in 66% yield. A small amount of the regioisomeric addition product was obtained but was easily separated via column chromatography. With the homoallylic alcohol in hand, deprotection of the THP ether



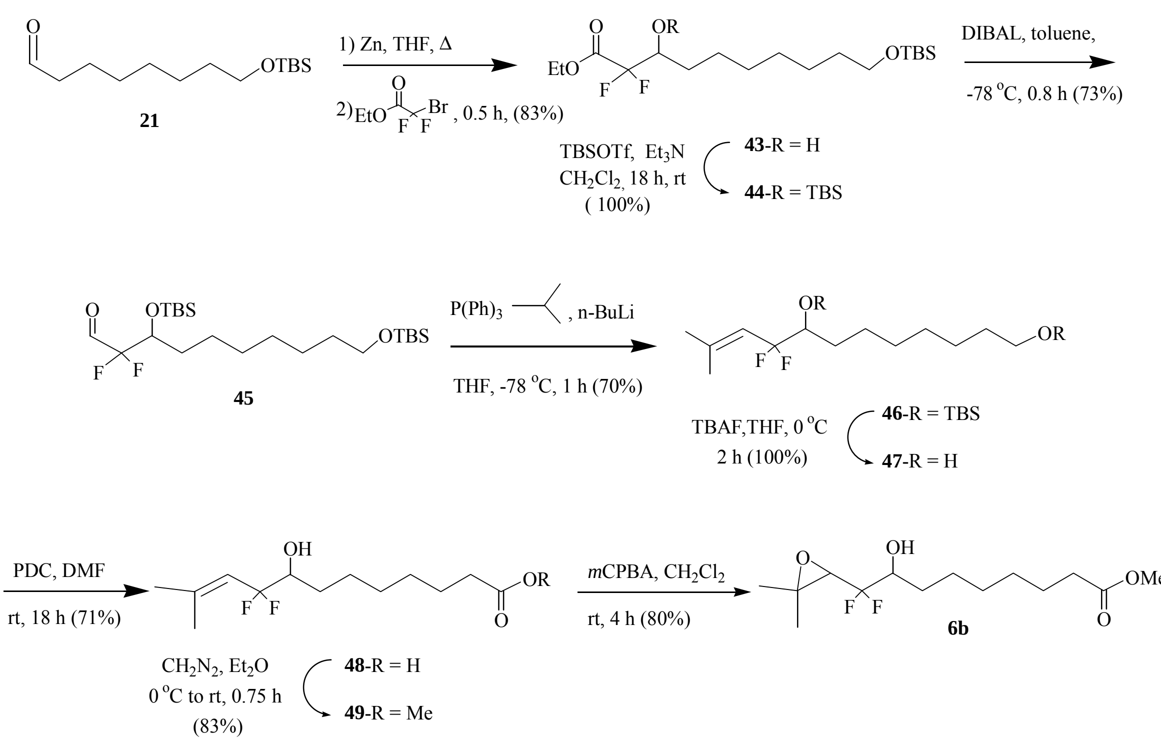
Scheme 2.6b. Completed synthesis of inhibitor **5b**

using *p*-TSA in methanol provided diol **39**. Jones oxidation[75] of the diol produced the keto-acid **40** that was then treated with diazomethane to provide **41** in 94% yield. We generated in-situ methyl (trifluoromethyl) dioxirane[76] in a mixed solvent system of acetonitrile/water at a neutral pH, which formed the epoxide, desired inhibitor **5a,** in 92% yield. Lastly, chemoselective reduction of the ketone in the presence of the ester provided alcohol **42**.[68] The alkene of **42** was then epoxidized using *m*CPBA to form the alcohol epoxide **5b** completing the synthesis.

2.1.C. Synthesis of Analogs 6a and 6b

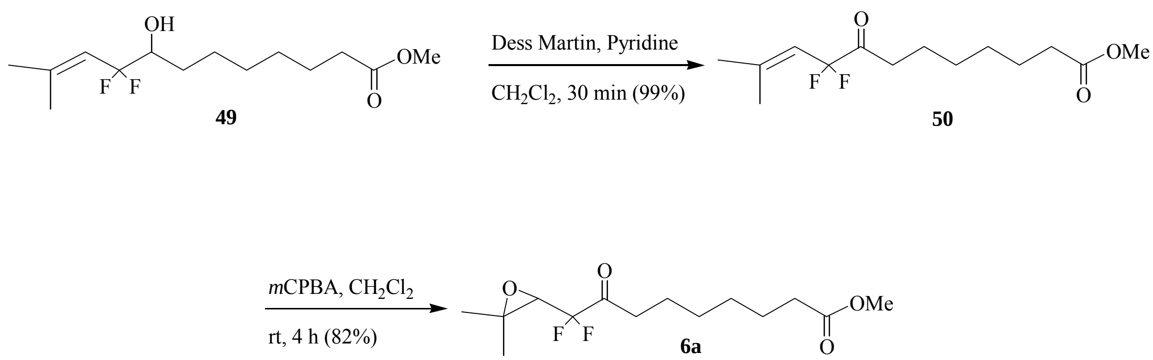
The design of **6a** and **6b** incorporates a Reformatsky reaction to introduce the fluorines and contains a chemoselective oxidation. The synthesis of **6a** and **6b** (Schemes 2.7a and 2.7b) begins with the same aldehyde silyl ether precursor as the nonfluorinated compounds **4a** and **4b**. Introduction of the difluoro ester via Reformatsky reaction[77-80] of aldehyde **21** with ethyl bromodifluoroacetate provides **43** in 83% yield. Next, quantitative protection of the secondary alcohol using TBSOTf[71] resulted in the bis-silyl

Scheme 2.7a. Synthesis of analogs **6a** and **6b**



ether difluoro ester **44**. Using 1 equiv of DIBAL reagent and running the reaction at low temperatures enabled the reduction of the ester to the aldehyde **45** without forming the alcohol. Interestingly, we found that aldehyde **45** was quickly hydrated during column chromatography and the hydrate was carried on to the next step. Large excess

Scheme 2.7b. Completed synthesis of analog **6a**



(2.5 equivalents) of Wittig reagent was used to generate difluoro alkene **46** in 70% yield. Deprotection of both TBS protecting groups with TBAF gave diol **47**. We next took advantage of the difficulty in the oxidation of fluorinated alcohols[80] to chemoselectively oxidize the primary alcohol to the acid **48** via PDC in DMF.[67] Finally, esterification of the carboxylic acid generated the methyl ester precursor for **6a** and **6b**. Direct epoxidation of the alkene afforded inhibitor **6b**, while Dess-Martin oxidation[70] followed by subsequent epoxidation with *m*CPBA provided epoxide keto ester **6a**.

2.1.D. Synthesis of Analogs 7a and 7b

Initially, we wanted to directly and efficiently obtain an alpha-keto ester, the key intermediate, before introducing the fluorines via the DAST reagent. Page and Rosenthal[81] found a direct method of generating α-keto esters efficiently. Use of a catalytic amount of osmium tetroxide and excess *t*-butyl hydrogen peroxide (TBHP) in the presence of the correct alcohol solvent could successfully oxidize trimethylsilyl acetylenes (Figure 2.4). Our attempts (Scheme 2.8) began with commercially available 6-bromo-1-hexanol that was reacted with *p*-TSA and dihydropyran resulting in the bromo

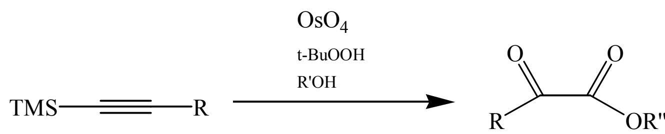
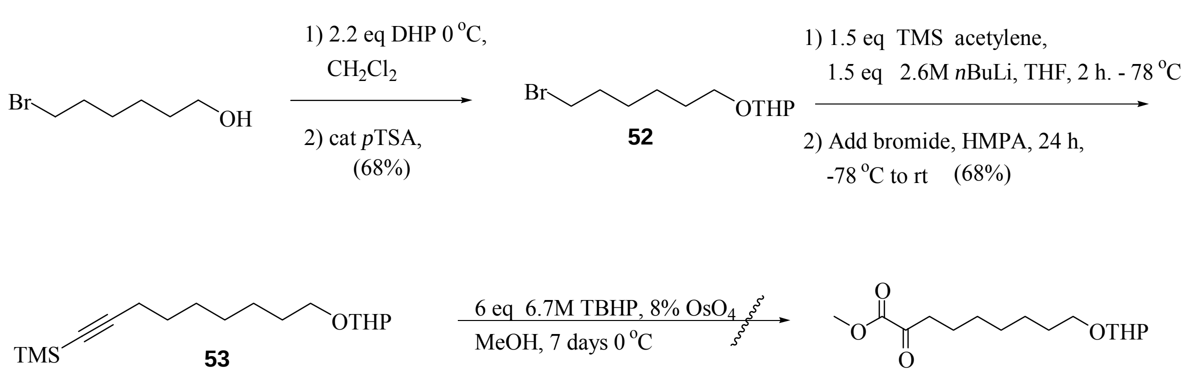


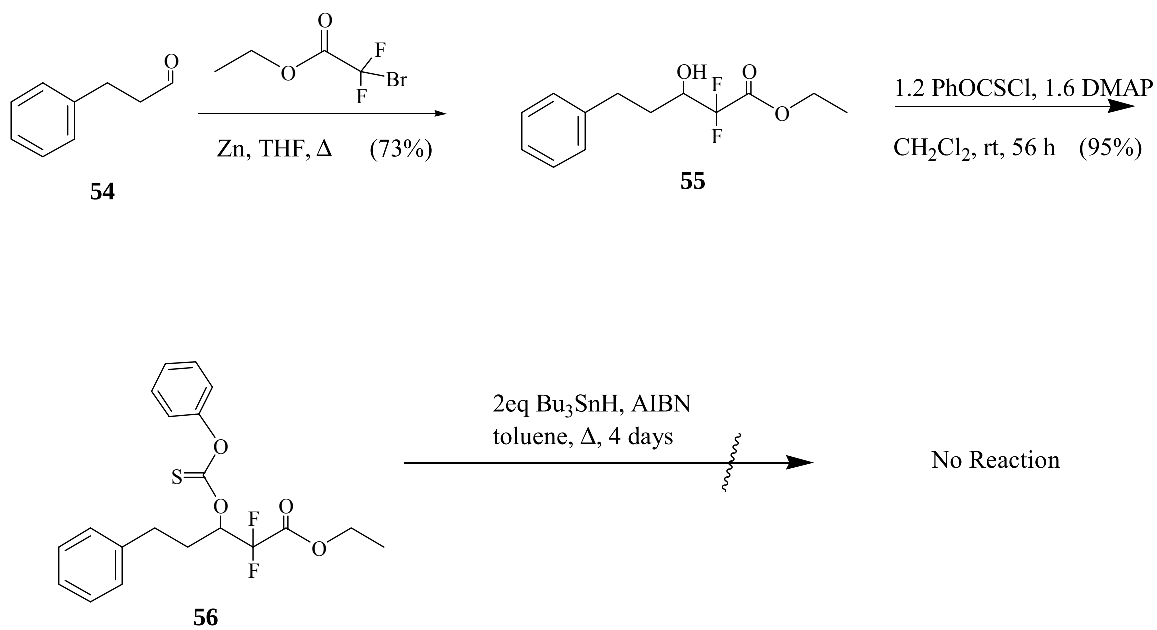
Figure 2.6. Page and Rosenthal's direct formation of an α-keto ester

THP ether **52**. The bromide was then added to a solution of *n*-BuLi and TMS-acetylene, in the presence of HMPA, providing the alkyne intermediate **53** in 68%. Following literature precedent,[81] 8% OsO_4 and excess TBHP was allowed to stir with the alkyne for 7 days at 0 °C; however, no product formation was observed. Closer examination of the literature reaction conditions revealed that the majority of the reactions were run until complete consumption of the starting material occurred. Interestingly, the small print indicated the majority of the yields were based on recovered starting material.

Scheme 2.8. Attempted OsO_4 reaction to form the α-keto ester



We next focused on Scheme 2.9, where we introduced the difluoro group by a Reformatsky reaction.[77-80] This would provide an α-difluoro β-hydroxy ester intermediate that could be deoxygenated by Barton's procedure.[82] Dihydro cinnamaldehyde (due to availability) was used as a model system. The aldehyde **54** was

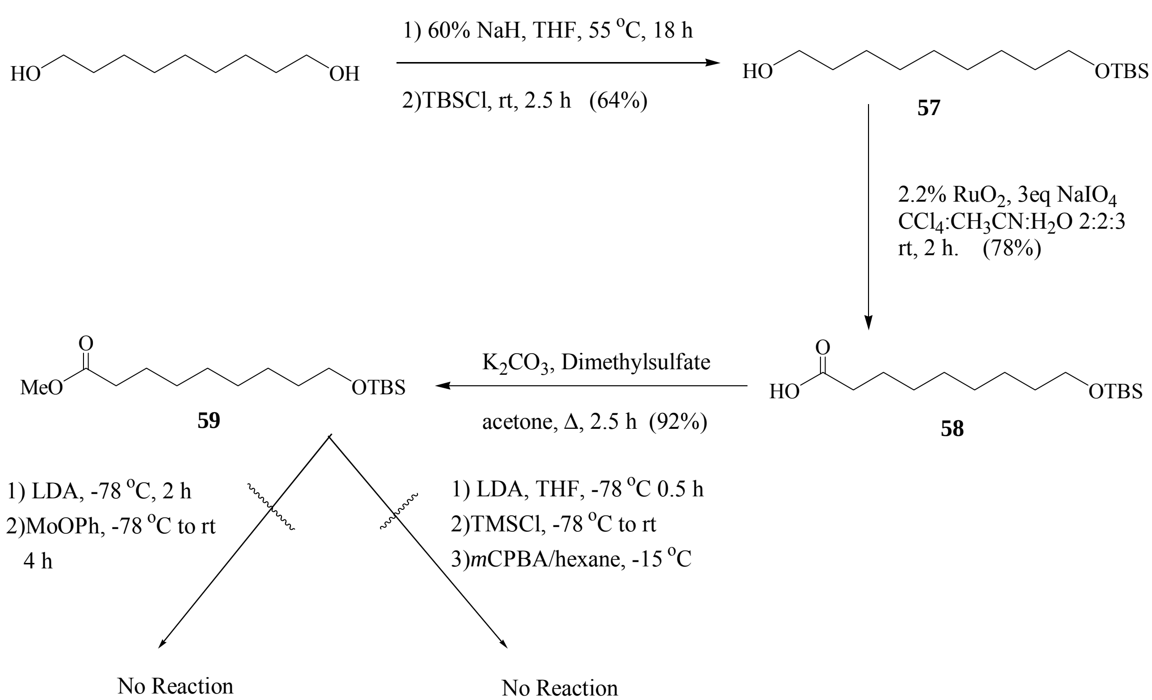


Scheme 2.9. Barton's deoxygentation and the Reformatsky reaction

subjected to Reformatsky conditions[77-80] to provide the α-difluoro β-hydroxy ester **55** in moderate yield. Next, we formed the xanthate ester **56** and attempted AIBN promoted tributyltin hydride deoxygenation.[82] Unfortunately, this reaction did not proceed resulting in primarily decomposed product. Interestingly, Ding et al.[83] also found the same to be true in their attempt at the synthesis of L-4,4-difluoroglutamic acid.

Not being able to form the keto-ester early in the synthesis and unable to deoxygenate the xanthate left us with few options. Two reactions have been successful for the oxidation of an enolate generating an α-hydroxy ester, the Rubottom[84] procedure

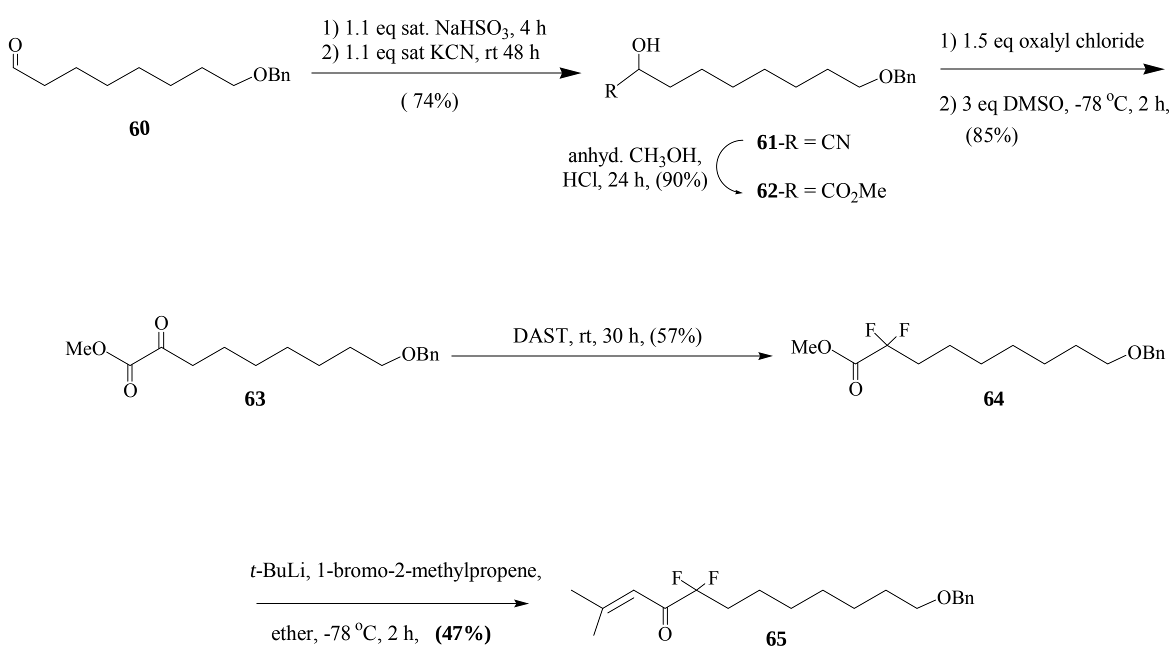
Scheme 2.10. Rubottom procedure and MoOPh reaction



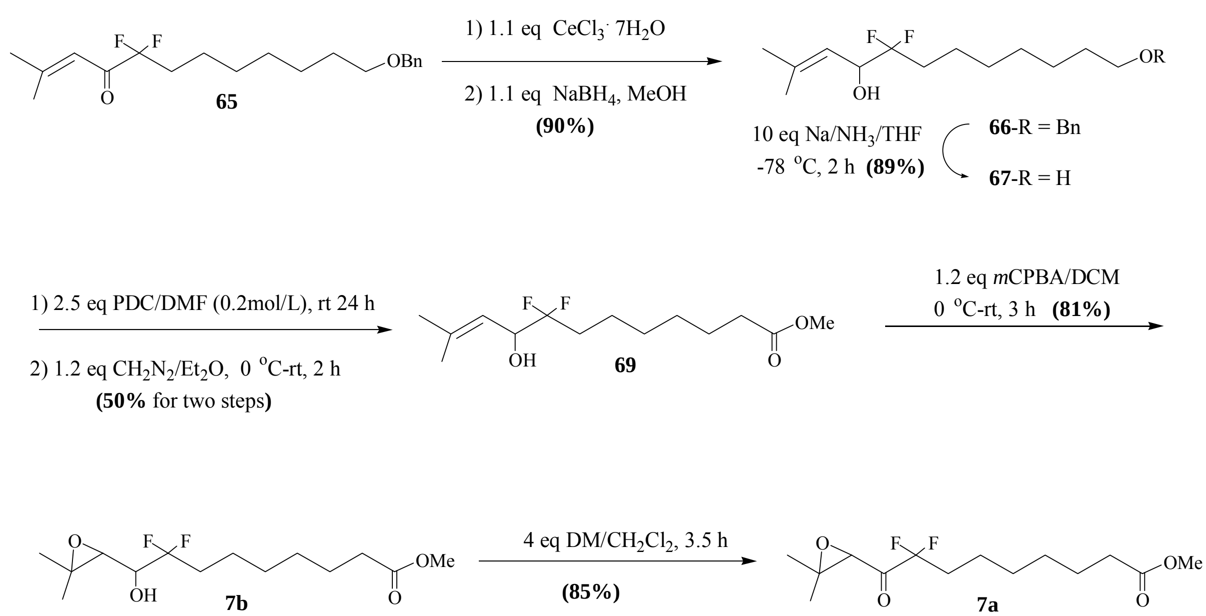
and MoOPh[85] reagent. Oxidation of the alcohol in a second step would provide the desired key intermediate. The synthesis shown in Scheme 2.10 begins with the mono TBS protection of 1,9-nonane diol resulting in the mono silyl ether **57**. By using a catalytic amount of ruthenium oxide in a mixed solvent system, the primary alcohol was oxidized to the acid, followed by esterification providing **59** in 92% yield. A number of attempts were made using both oxidation conditions,[84,85] yet neither provided the desired product.

The completed syntheses of **7a** and **7b** are shown in Scheme 2.11a and 2.11b, and involved generating the alpha hydroxy nitrile as a masked ester. This was than followed by an oxidation to give the desired key intermediate. Addition of KCN to aldehyde **60** resulted in the cyanohydrin **61**. The nitrile was then converted to the ester **62** with hydrochloric acid in methanol followed by Swern Oxidation[86] to give the desired keto-ester **63** in 85% yield. Introduction of the difluoro functional group at the ketone using DAST[87-89] provided the difluoro ester **64,** which was then reacted with an organo lithium reagent producing the α,β-unsaturated ketone **65**. Luche reduction[68,69] of **65** to generate the allylic alcohol was followed by deprotection of the benzyl protecting group using dissolving metal reduction to provide the diol **67** in high yield for the two steps.

Scheme 2.11a. Formation of key intermediate **63**



Linderman et al[80] found that alcohols alpha to a difluoro functional group are often difficult to oxidize. We attempted to take advantage of this selectivity to achieve oxidation of the primary alcohol to the corresponding acid **68** using PDC in DMF[67]. Interestingly, we found that we indeed did get a degree of selectivity but also observed a minor amount, 17%, of over-oxidized product. Esterification of intermediate **68** using diazomethane provided the methyl ester **69** in moderate yield for the two steps. Epoxidation of the alkene generated the desired inhibitor **7b**. Finally, oxidation of the alcohol using Dess-Martin[70] reagent resulted in the ketone analog **7a** in 85% yield.



Scheme 2.11b. Completed synthesis for inhibitors **7a** and **7b**

2.2 Class II Analogs

The class II inhibitors are designed to interact with the His residue within the active site in hope of effecting an irreversible inhibition. Again, in order for this mechanism to occur there must first be nucleophilic attack of the Asp residue opening the epoxide. In a second step, the His residue that normally activates the water for hydrolysis of the ester/enzyme intermediate could possibly be alkylated by an adjacent electrophile.

Alpha-chloroketones have been shown to be irreversible inhibitors of serine esterase.[90] Nucleophilic attack of the ketone by the serine esterase generates a tetrahedral intermediate. In a second step, displacement of the chloride by the His residue provides an irreversible alkylated complex. We believe inhibitor **8** will have the same potential for inhibition of the ester intermediate presumed for compounds **4a** and **5a**. Also, the alpha-chloroketone could potentially inhibit JHEH irreversibly as illustrated in Figure 2.7. Initial nucleophilic attack by the Asp residue opening the followed by addition of the

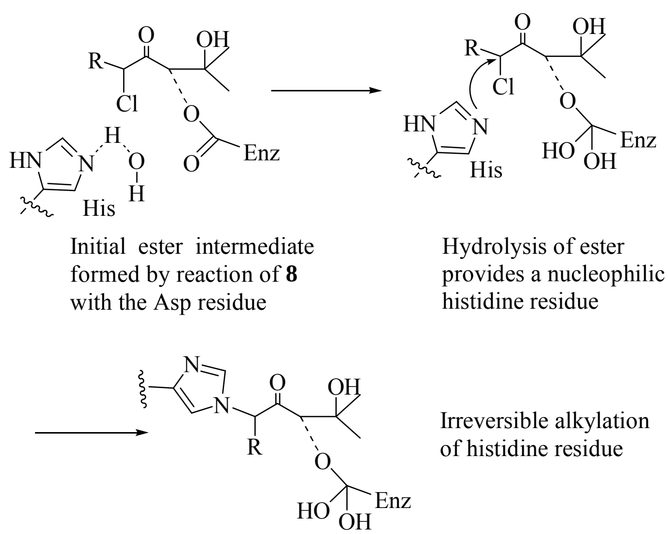


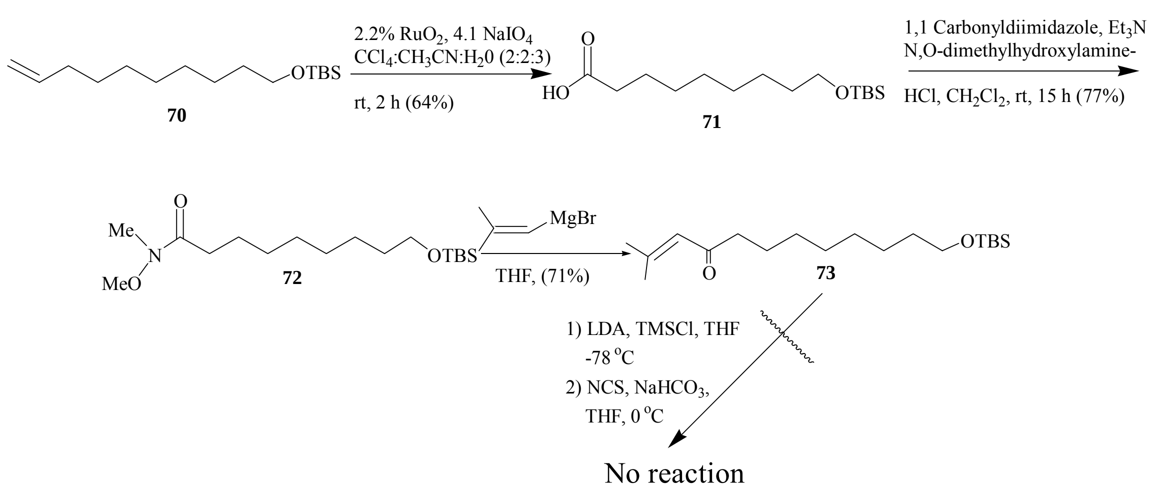
Figure 2.7. Probable mechanism of inhibition for the alpha chloro analog **8**.

activated water to the acyl enzyme epoxide would form a tetrahedral intermediate similar to the one formed for serine esterases. Subsequent alkylation of the His residue by the alpha-chloroketone could result in irreversible inhibition of the enzyme. Interestingly, Debernard used non-selective inhibition of JHEH with 2-bromomethyl acetophenone to implicate a His residue in the catalytic triad of JHEH from *Manduca sexta*.[91]

Irreversible inhibition of juvenile hormone epoxide hydrolase by alkylation of the His residue could also be achieved by introduction of an alkyne **10** or alkene **9** next to the oxirane. Marshal found that both propargylic and allylic acetates can act as efficient electrophiles.[92] Epoxide opening by the Asp residue could provide an intermediate where the His residue can be directly alkylated by S_N2' displacement of the ester group. This would result in irreversible inhibition of the enzyme.

2.2.A. Synthesis of Analog 8

The initial attempts at the synthesis of **8** (Scheme 2.12) began with alkene silyl ether **70,** which was made by TBSCl protection of 9-decen-1-ol**.** Ruthenium catalyzed oxidation[93] of the terminal alkene provides acid **71**. Compound **71** was then transformed to the corresponding Weinreb amide[94] **72** followed by addition of 2-methyl-1-propenyl

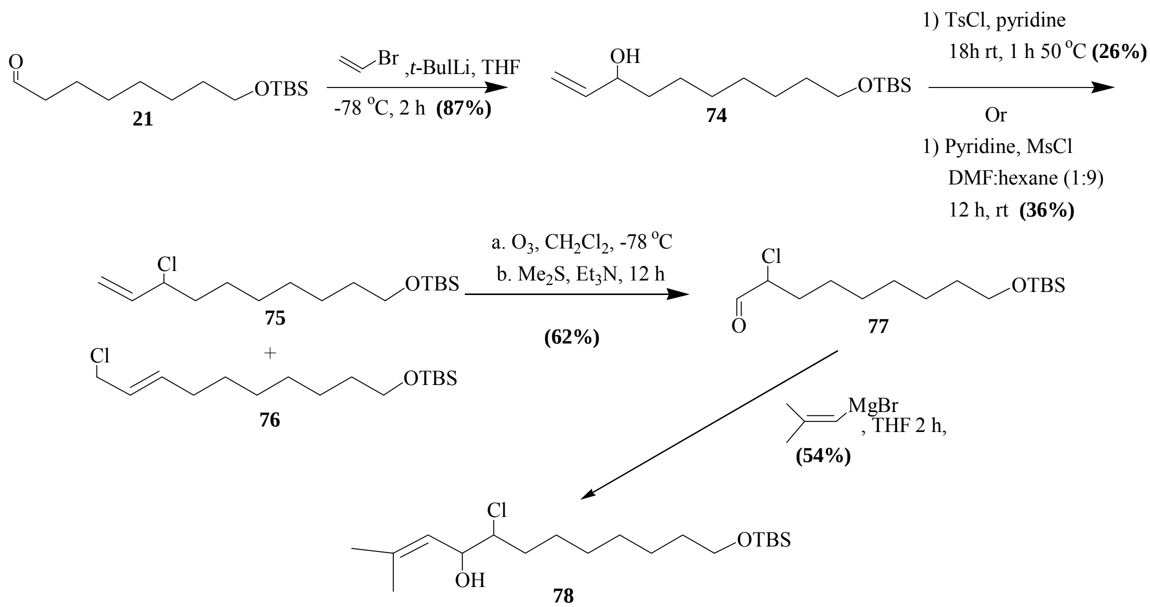


Scheme 2.12. Introduction of the chloride via formation of enolate

magnesium bromide to produce alpha-beta ketone **73**. At this point we decided to attempt to introduce the chlorine at the alpha position. Our first attempt used LDA to form the kinetic enolate of **73** followed by treatment with NCS in one pot. Regrettably, this procedure did not work. We then felt that it may be possible to first trap the enolate with TMSCl and then react the enol ether formed with NCS. Crude NMR analysis of the trapped TMS-enolate of **73** showed that we indeed had formed an enol ether, but it was not the desired kinetic enolate. Instead, the thermodynamic enolate of **73** had been formed.

Abandoning that route, we next began to focus our attention on trying to incorporate the chlorine earlier into the sequence as an allylic alcohol as shown in Scheme 2.13. Vinyllithium addition to aldehyde **21** resulted in allylic alcohol **74** in moderate yield. Our first attempt at chlorinating encompassed the use of TsCl and pyridine in a S_N2-type reaction previously reported by Nardo and Varela.[95] The desired allyl chloride **75** was obtained albeit in a disappointing yield. Tanaka et al[96] showed, in the synthesis of (*S*)-MA20565, that the use of MsCl and pyridine in a DMF/hexane solvent system could provide chlorine substitution products in good yields. The desired product **75** was again produced in low yields, as well as regioisomer **76**. It is interesting to note that all references were using the allylic alcohol with an internal double bond. In 1971, Collington and Meyers[97] reported that when pyridine was used as the base, low yields were also obtained and speculated that the product was lost in the workup as a N-allylpyridinium salt. Nevertheless, the allyl chloride obtained was carried on to the next step. Ozonolysis of alkene followed by Grignard addition generated **78**. The yields of the reactions were low possibly due to displacement of the chlorine by the alkoxide

Scheme 2.13. Displacement of an allylic alcohol with a chloride

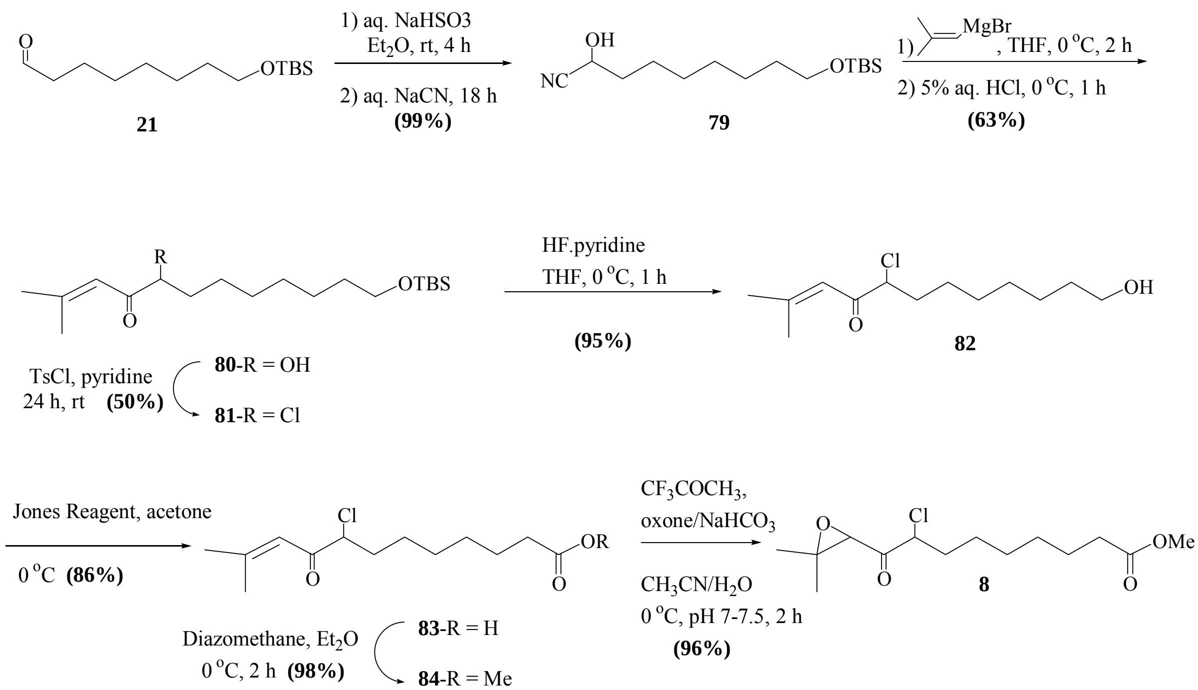


formed, possibly resulting in epoxide formation. As a result of this myriad of problems, this route was also abandoned.

As these experiments depict, we needed to devise a route that would enable us to incorporate the chlorine later in the reaction sequence, after the Grignard addition. The route should also allow us to do a nucleophilic substitution of a tosylate. Reaction Scheme 2.14 shows such a plausible sequence. Starting from aldehyde **21,** reaction with aqueous $NaSO_3$ and aqueous NaCN provided nitrile **79** in excellent yield. Direct Grignard addition using 2-methyl-1-propenyl magnesium bromide with an acidic workup provided the α-hydroxy ketone **80** in one step in 63% yield. Displacement of the alcohol by chloride using tosyl chloride in pyridine[95] provided the alpha chloro ketone **81**. HF·pyridine deprotection of the TBS ether gave the primary alcohol **82** that was oxidized to the acid **83** by titrating with 8N Jones Reagent[75] in 91% yield for the two steps.

Diazomethane esterification of acid **83** resulted in the ester in 98% yield, followed by nucleophilic epoxidation of the unsaturated alkene to provide the desired inhibitor **8**.[76]

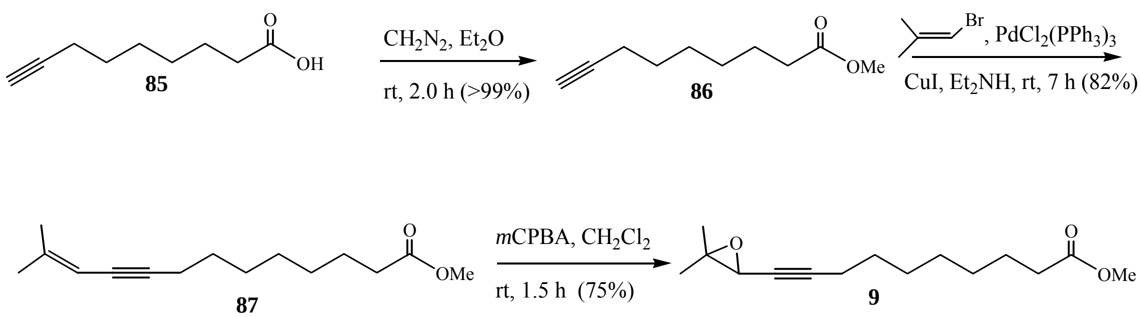
Scheme 2.14. Completed synthesis of analog **8**



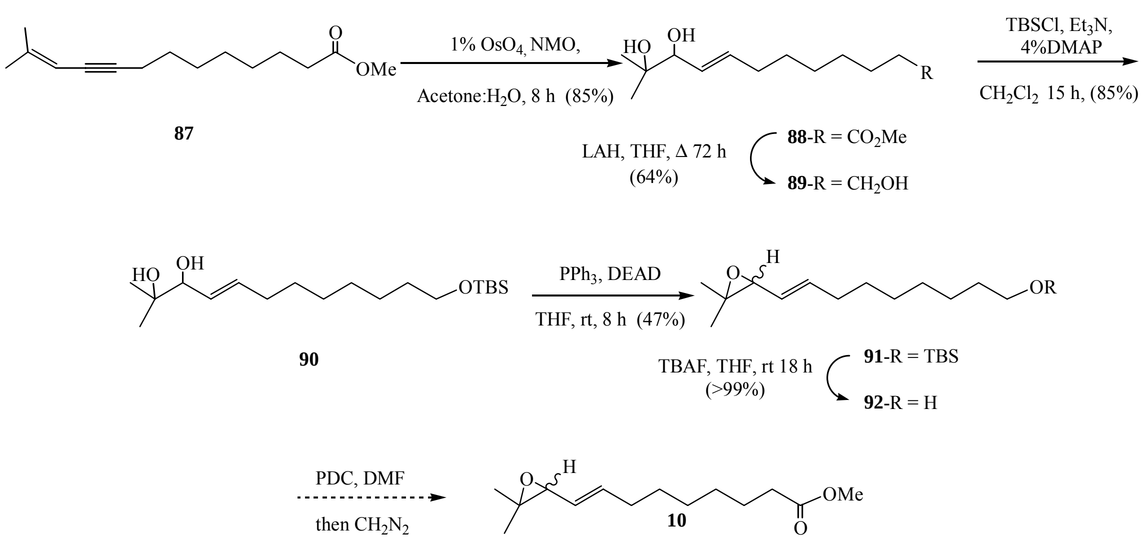
2.2.B. Synthesis of Inhibitors 9 and 10

Synthesis of inhibitors **9** and **10** are shown in Schemes 2.15 and 2.16, respectfully. Esterification of commercially available 8-nonyoic acid **85** using diazomethane gave **86** in near quantitative yield. The ester was then subjected to a Sonogashira[98-100] palladium catalyzed cross coupling between the ester **86** and 1-bromo-2-methylpropene to give **87** in 82% yield, a precursor to both inhibitor **9** and **10**. Lastly, selective epoxidation of **87** with *m*CPBA furnished inhibitor **9**.

Scheme 2.15. Sythesis of inhibitor **9**



Dihydroxylation of **87** using OsO_4/NMO[101] gave **88**. The alkyne was then reduced to the trans alkene **89** with LAH followed by chemoselective TBS protection of the primary alcohol following a literature precedent[102] to give **90** in moderate overall yield for the two steps. Epoxidation via intramolecular Mitzunobu[103] conditions gave **91**. At this point the oxidation of the primary alcohol to the corresponding acid presented new complications. Use of the Jones oxidation reagent was not possible because the epoxide is acid sensitive. Instead PDC in DMF[67] was attempted. Initial trials resulted in no desired product as

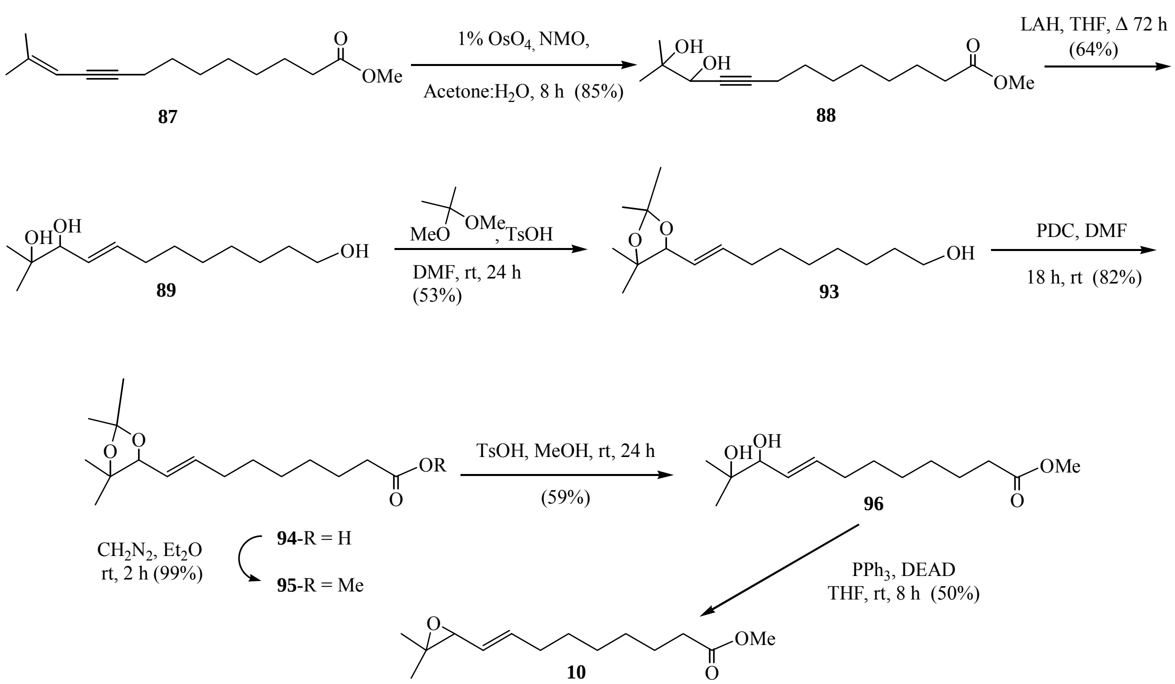


Scheme 2.16. Synthesis of **10** with the early formation of the epoxide **91**

evidence by NMR analysis of crude reaction product, showing what appeared to be loss of the epoxy proton. Oxidation was tried a second time, without purification, and the crude product was reacted with diazomethane in hope that we would be able to simplify the purification. Again this failed as NMR data showed no methyl ester and again loss of the epoxy proton.

If the acid-lability of the epoxide is the issue, introduction of the epoxide later in the sequence may alleviate these problems. Scheme 2.17 shows the desired synthesis of compound **10**. The alkene was oxidized as previously reported with OsO_4/NMO[101] to

Scheme 2.17. Completed synthesis of Inhibitor **10**



give the diol **89**. This time protection of the diol as an acetonide with 2,2-dimethoxy propane provided **93** in moderate yield. PDC oxidation in DMF[67] followed by diazomethane gave ester ketone **95** in 91% yield for the two steps. Deprotection of the acetonide with *p*-TSA in methanol gave again the diol ester **96**. Intramolecular Mitzunobu[103] reaction conditions afforded alkene epoxide, inhibitor **10**.

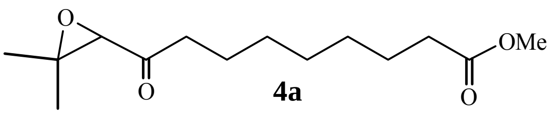
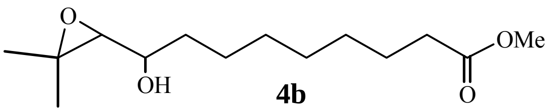
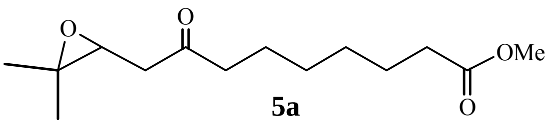
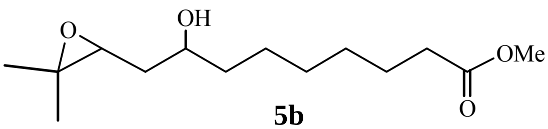
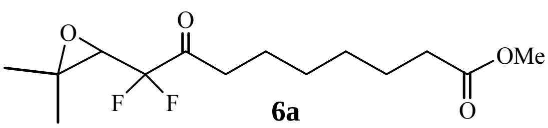
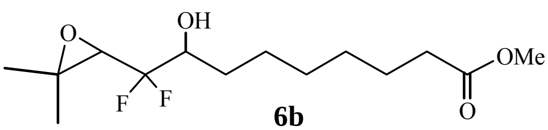
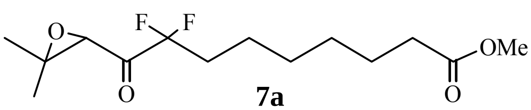
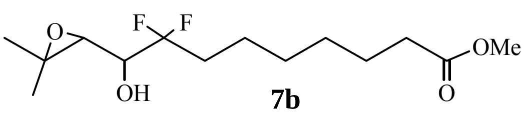
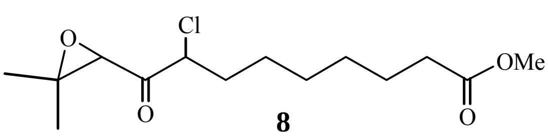
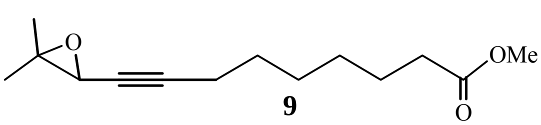
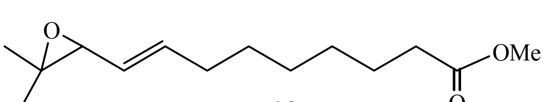
Chapter 3

In-vitro Analysis of Analogs

The eleven analogs synthesized were tested for JHEH inhibition, using an in-vitro partition assay developed by Share and Roe[104] involving [^{3}H]-JH III as the substrate and the use of OTFP for JHE inhibition. The experiments were performed using insect mEH obtained from wandering L_5D_0 *Trichoplusia ni* larvae. All of the following studies were completed within the linear range of enzyme activity with respect to protein concentration and time. Thin layer chromatography was also used as reassurance that complete inhibition of the esterase had occurred. The results are shown in Table 3.1 (see Appendix A for I_{50} Plots).

For the Class I inhibitors, it appeared that in most cases, **4a**, **6a**, and **7a,** the presence of the ketone increased the inhibition over the alcohol derivatives **4b**, **6b**, and **7b**, with one exception, inhibitor **5b** was more potent than **5a**. The inhibitors containing the difluoro functional group, **6a**, **6b**, **7a**, and **7b,** generally showed an order of magnitude increase in the degree of inhibition relative to the non-fluorinated analogs. Determining the regiochemical importance of the functional group on the inhibitory potency was not so straightforward. In some cases, the alcohol was more effective at inhibiting JHEH being two carbons away from the oxirane as in **5b** versus one carbon away as in **4b**. On the other hand, the position of the ketone in **4a** enhanced inhibitory potency 2 orders of magnitude when compared to **5a**. When evaluating the reactivity of these analogs to our benchmark, MEMD (I_{50}=4.8μM), we found that none were as potent, except for **6a** (I_{50}=8.54 μM), which was slightly less effective as an inhibitor.

Table 3.1. I_{50} Data for the Class I and Class II potential inhibitors of insect JHEH

Inhibitor	I_{50} in M
 **4a**	5.1×10^{-5} M
 **4b**	4.2×10^{-3} M
 **5a**	5.2×10^{-3} M
 **5b**	4.3×10^{-4} M
 **6a**	8.5×10^{-6} M
 **6b**	2.7×10^{-4} M
 **7a**	3.6×10^{-5} M
 **7b**	2.6×10^{-4} M
 **8**	1.3×10^{-5} M
 **9**	7.4×10^{-5} M
 **10**	4.3×10^{-6} M

In contrast to the Class I inhibitors, the Class II analogs showed a higher degree of inhibition. The alpha-chloro compound **8** had a greater degree of reactivity compared to all (Class I inhibitors) but one, **6a**. The alkyne **9** that we initially believed may be more potent than the alkene did not perform as expected being an order of magnitude less potent. Surprisingly, the alkene epoxide **10** was the most reactive compound tested of the new series with an I_{50}=4.29 μM and showed a slightly higher degree of inhibition than MEMD.

Linderman et al[64] has previously showed that the "correct" absolute stereochemistry at the C-10 position of MEMD dramatically increased the inhibition of JHEH in insects. Logically, including the absolute stereochemistry in our derivative **10** may shed some insight in reactivity patterns, but we instead wanted to concentrate on the mechanism of these inhibitors. We have shown that these compounds do inhibit JHEH but by just studying the I_{50}s does not fully answer the question of how they are acting.

After the initial studies we decided to take the most potent analog from each class and ascertain if there was any time dependency for the degree of inhibition. Initially, all I_{50}s were run with a 30 second incubation time for the epoxide at 30 ^{o}C prior to the addition of the substrate (JH III). OTFP inhibition of JHE has been shown to be time dependent and inhibition can be increased if the incubation time is longer prior to the addition of the substrate.[48] Table 3.2 represents three different times of incubation at a single concentration. Noteworthy is that there seems to be no observable time dependence for any of our derivatives within 30 minutes. The potency for both MEMD and alkene **10** begins to dramatically drop after 30 minutes. MEMD was designed to act as an alternative substrate, so we expect to see a drop in its efficiency after some amount of time. Analog **10** was predicted to serve as an irreversible inhibitor and unfortunately the decreased inhibition with time suggests that the mechanism previously proposed in

	% Inhibition by Inhibitor		
Time (min)	MEMD	**10**	**6a**
0.5	68.89	74.17	56.76
30	67.22	54.72	60.15
240	14.27	9.92	63.69

Table 3.2. Incubation time dependency of MEMD, **10**, and **6a** at a concentration of 10^{-5} before addition of [^{3}H] JH III.

Figure 2.4 does not apply. Therefore, we define the alkene as an alternative substrate. Since we do not notice a drop in inhibition for **6a** we cannot automatically assume it is an alternative substrate or a reversible/irreversible inhibitor.

Shannon Harris[105] found that the order of addition (substrate or inhibitor first) had no significance for the MEMD assay results. We observed different results for inhibitor **6a** (Table 3.3). Addition of the substrate first (30 second incubation at 30 ^{o}C) followed by adding the epoxide **6a** resulted in decreased potency. After which, we noted a drop in the inhibition or an increase in the amount of metabolism. This information reflects that the epoxide seems to be interacting with the same active site as the substrate JH III, and therefore is a competitive inhibitor.

	% Inhibition	
Log [I]	Substrate First	Inhibitor **6a** First
-4.5	54.5	68.8
-5.5	24.0	31.8

Table 3.3. Competition Assay- assay run with EtOH as control; 30sec incubation of EI prior to addition of S and vice versa.

At this point we have discovered that inhibitor **6a** competes with JH for the enzyme and over time (up to 4 hours) the inhibitory potency slightly increases. Next, we needed to Figure out how long this increase lasts and whether or not there is ever a time dependent decrease in potency. Figure 3.1 represents a time course (up to 24 hrs) at two concentrations for inhibitor **6a** using MEMD as a control. First, looking at MEMD we notice that the inhibition sharply drops over time and we predict this could be a model for an alternative substrate. However, **6a** does not act the same way as MEMD, but instead the inhibition slightly increases over the first 200 minutes and then slowly drops over 1400 minutes (Note: Incubation of the enzyme over 24 h resulted in a linear line with no drop off, Appendix A). The slight increase from the 0.5 minute to the 200 minute data point may be the result of a temperature dependency of the enzyme activity. If this was occurring you would expect to see the same results in MEMD but this was not observed. Secondly, it may take some time for the inhibitor to enter the active site and this delay may provide an explanation to this phenomena. It is feasible that other enzymes found in the crude microsomal preparation may be metabolizing the inhibitor, and this may account for the decreased inhibition at 24 hours. Indiscriminate protein binding of the inhibitor may also effect the actual concentration of the inhibitor available for binding to the JHEH present.

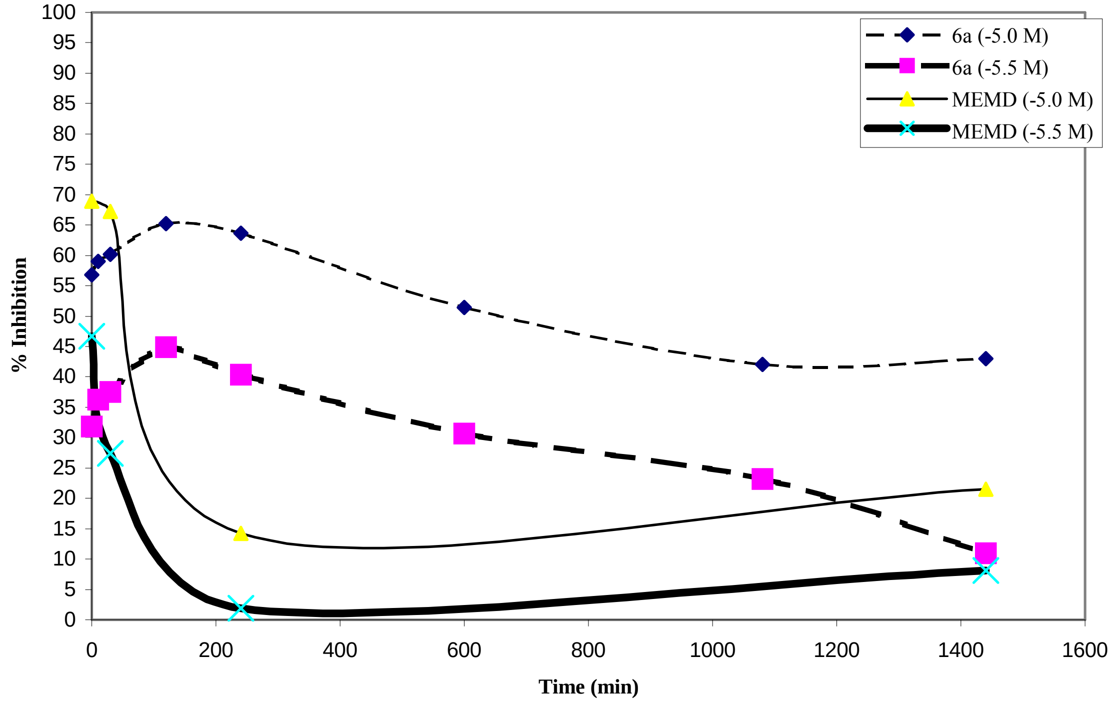
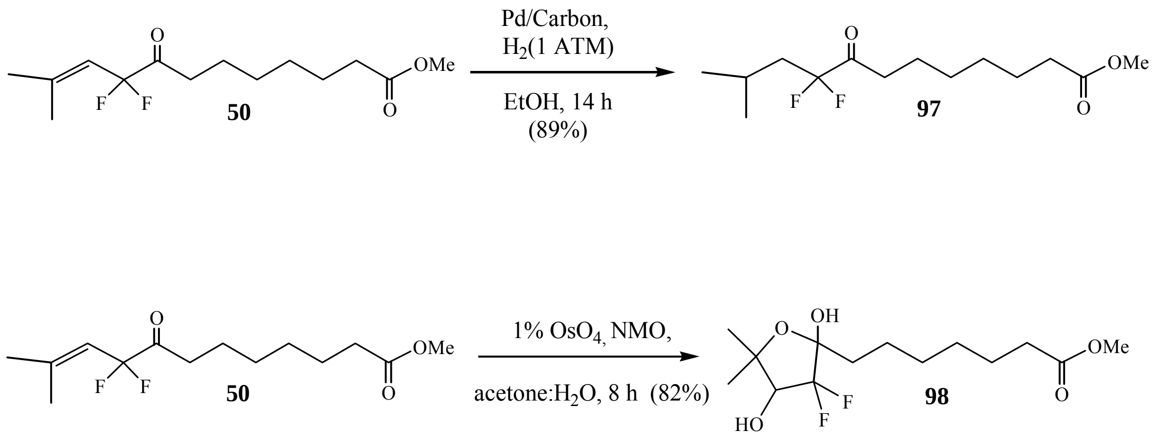


Figure 3.1. Time dependency of **6a** and MEMD

Hammock recently published a paper on urea and amide compounds containing no epoxide that successfully inhibited rat sEH.[62] These results suggest another possible form of inhibition by **6a**. The presence of the electronegative fluorines creates an electron deficient ketone, which may be a competitive site for nucleophilic attack by the Aspartate residue instead of the epoxide. If true, inhibitor **6a** would be a transition state analog similar to that seen with OTFP noted in Figure 1.4. A number of other compounds were made to either rule out or solidify this theory, Table 3.4 and Scheme 3.1. Intermediate **50** (Scheme 6) contains just the alkene ketone. The alkene of **50** was



Scheme 3.1. Synthesis of **6a** analogs

hydrogenated to provide **97 (**Scheme 3.1). Lastly, the alkene of **50** was treated with osmium tetroxide to provide the diol, but unexpectedly formed a tetrahydrofuran ring **98** (Scheme 3.1). Proton NMR data revealed that there was loss of the vinyl proton triplet at 5.38 ppm and also loss of the methylene protons alpha to the ketone at 2.62 ppm.

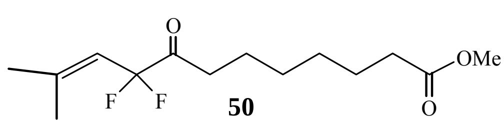
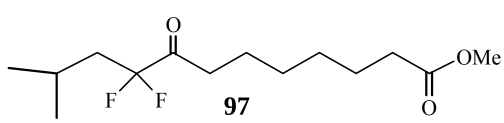
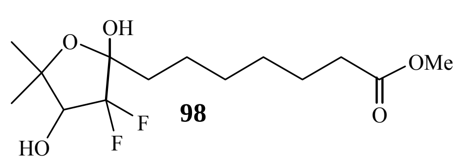
Analog	I_{50} in M
 **50**	5.3×10^{-3}
 **97**	2.2×10^{-2}
 **98**	1.1×10^{-2}

Table 3.4. I_{50} Data for the Analogs of **6a**

These three compounds were all assayed as inhibitors of JHEH as previously described and all were found to not inhibit the enzyme to any degree. More important than the degree of inhibition, the formation of the tetrahydrofuran ring **98** provides a chemical argument that the inhibitor **6a** may be performing as we proposed (Fig 2.3) and provide the reason for slow regeneration of the enzyme past 4 hours seen in Figure 3.1.

In conclusion, we have shown the effectiveness of the eleven compounds to inhibit JHEH in *T. ni*. All of the inhibitors decreased the enzyme activity to an extent with alkene **10** being the most effective with an I_{50} of 4.29 μM. Although **10** exhibited the highest potency for inhibition of the enzyme, we found that **10** was acting solely as an alternative substrate and not interfering with the His residue as we designed (Figure 2.5). In general, the introduction of the fluorine functionality did indeed increase the inhibitory potency compared to the non-fluorinated analogs. Comparisons made between the fluorinated inhibitors led us to believe that the regiochemistry of the ketone for analog **6a** and **7a** may have influenced the inhibitory potency. Chemically, we found that hydrolysis of **6a** generated a tetrahydrofuran structure identical to the mechanism for inhibition proposed in Figure 2.3. On the other hand, **7a** during hydrolysis would form a four membered heterocycle, which may not be as facile as the formation of **98**. Also, we found that for inhibition of JHEH it appears that the presence of an epoxide for substrate binding is essential. Lastly, we have provided insight to support a novel mechanism based inhibition of juvenile hormone epoxide hydrolase by formation of a tetrahydrofuran intermediate.

Conclusion

The work presented here entails a multi-disciplinary advance at understanding the role of juvenile hormone epoxide hydrolase in the metabolism of juvenile hormone in last stadium *Trichoplusia ni*. The work combines synthetic organic chemistry and biochemistry.

A series of juvenile hormone analogs have been synthesized and their effectiveness to inhibit JHEH has been ascertained. Some of the key elements in the synthetic sequence for the fluorinated compounds include chemoselective oxidation of a primary alcohol in the presence of an alpha-fluorinated alcohol, introduction of a geminal difluoro group via the Reformatsky reaction or the DAST reagent, and selective nucleophilic and electrophilic epoxidation reactions. The synthesis of some of the non-fluorinated compounds employed regio-selective opening of an epoxide, Sonogashira palladium catalyzed cross coupling, nucleophilic epoxidation, and a variety of protecting group manipulations. Of all the inhibitors tested, the alkene **10** was the most effective alternative substrate with an I_{50} of 4.29 μM, slightly more potent than the benchmark MEMD. We also found that the introduction of fluorine increased the potency of the inhibitors compared to the non-fluorinated analogs. Lastly, we have provided insight to support a novel mechanism based inhibition of juvenile hormone epoxide hydrolase by formation of a tetrahydrofuran intermediate.

Experimental

<u>General</u>

All reactions were run under an inert argon atmosphere in either flame dried or oven dried glassware, unless otherwise stated. All reagents were purchased from Sigma-Aldrich or Fisher Scientific and purified prior to use unless stated differently. Tetrahydrofuran (THF), ether, and toluene were all freshly distilled from sodium-benzophenone. Methylene chloride and amines were distilled from CaH_2 prior to being used. ^{1}H, ^{13}C and ^{19}F NMR Spectra were recorded on a GE or Varian 300MHz spectrometer using $CDCl_3$ as solvent and standard. Infrared spectral data was obtained using a Fourier Transform Perkin-Elmer1600 series spectrometer. Chromatography was performed on silica gel 60, 230-400 mesh ASTM, obtained from EM Science. Whatman aluminum backed TLC plates containing a coating of fluorescent silica gel were used for TLC analyses. Either UV light or a 5% solution of phosphomolybdic acid in ethanol was used for visualization of TLC plates. Elemental analyses were carried out by Atlantic Microlab Inc., Atlanta, Georgia. A Brinkman polytron, Beckman J2-21 M/E centrifuge, and Beckman L8-70 M ultracentrifuge were used to prepare the crude microsomes. The scintillation counter used for radiometric assays was a Beckman LS 1801.

<u>Microsomal</u> <u>Preparation</u>: 2 g of insect per 5 mL buffer (I = 0.2, pH = 7.4, 0.01 % PTU) were homogenized using a Brickman polytron until relatively smooth. The suspension was than centrifuged at 10,916 x G for 30 min at 4 ^{o}C. This generated two distinct layers in which the top liquid layer or supernatant was taken and the pellet containing the exoskeleton was discarded. The supernatant was then passed though a small pipette column containing glass wool remove solids and placed into pre chilled ultra centrifuge

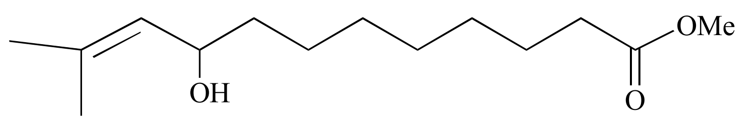
tubes in an ice bath. The microsomes were then removed after spinning at 100,000 x G for 1 h at 4 ^{o}C. The pellet was resuspended in buffer and washed 2 x followed by a 400:1 dilution with buffer.

<u>Partition Assay by Share and Roe</u>: Epoxide analogs were routinely assayed by the addition of 1 µL of ^{3}H-labled JH III substrate (5.0 x 10^{-4} M, approx. 9000 cpm) to 100 µL of *T. ni* L_5D_0 diluted crude microsome in 10 x 75 mm test tubes in ice water. Microsomes were preincubated with 1 µL of 10^{-2} M OTFP (final concentration 10^{-4}) for inhibition of JH esterase for 10 min at 30 ^{o}C and returned to the ice bath before the addition of either 1 µL of epoxide inhibitor or 1 µL 100% ethanol as a control for total epoxide hydrolase activity. The microsomal solution was then incubated for 30 sec. at 30 ^{o}C and returned to the ice bath. After shaking for 30 sec at 0 ^{o}C, 1 µL of [^{3}H] JH III substrate was added, incubated for 20 min at 30 ^{o}C, and returned to ice bath for the addition of 300 µL 100% methanol and 250 µL isooctane. (Note: A 0 time incubation was carried out in order to calculate the partition coefficient). After the addition of the stop bath, each test tube was vigorously shaken (vortex) to ensure complete mixing of the bi-phasic mixture. Each tube was then centrifuged for 4 min at 1000 g. Aliquots of 100 µL from the top and bottom phases were analyzed by liquid scintillation and the resulting cpm corrected for instrument background.

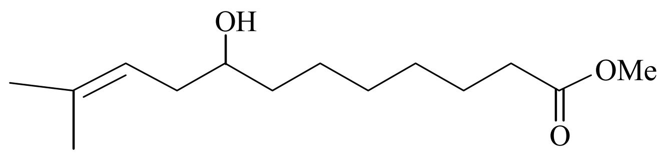
Chemical Syntheses:

General Procedures:

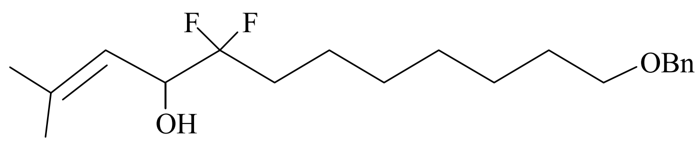
General Procedure of Luche Reduction: A solution of ketone (0.17 mmol) and $CeCl_3 \cdot 7 H_2O$ (0.172 mmol) in 5.7 mL of dry methanol was cooled to -78 °C prior to the addition of sodium borohydride (0.172 mmol). The cooling bath was removed and the reaction mixture was allowed to warm to room temperature. The mixture was then diluted with 25 mL ether and quenched by the addition of 10 mL saturated aqueous NH_4Cl. After stirring for 2 h the two phases were separated and the aqueous phase was extracted with ether (2 x 25 mL). The combined organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The product was obtained in good yield as either the allylic or homoallylic alcohol.



Methyl-9-Hydroxy-11-methyl-dodec-10-enoate 16 (93%). Flash chromatography (30% EtOAc:Hexane); ^1H (300 MHz, $CDCl_3$) δ δ 5.15 (d, J = 8.7 Hz, 1H), 4.34-4.32 (m, 1H), 3.66 (s, 3H), 2.38 (t, J = 7.5 Hz, 2H), 1.72 (s, 3H), 1.68 (s, 3H), 1.52-1.26 (complex, 12H); ^{13}C (75 MHz, $CDCl_3$) δ 174.0, 133.2, 125.8, 68.1, 50.4, 38.2, 33.3, 30.7, 30.0, 29.7, 25.6, 25.4, 24.2, 19.6; IR (neat) υ = 3417.4, 2928.0, 2854.9, 1738.5, 1435.2, 1172.7, 1015.1 cm^{-1}; Anal. Calcd. for $C_{14}H_{26}O_3$: C, 69.38; H, 10.81; Found: C, 69.08; H, 10.76.

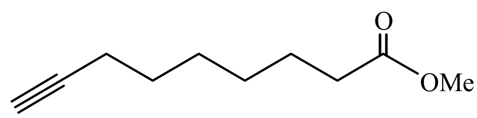


Methyl-8-Hydroxy-11-methyl-dodec-10-enoate 42 (97%). Flash Chromatography (40%EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 3.91-3.85 (comp, 1H), 3.65 (s, 3H), 2.95-2.91 (m, 1H), 2.29 (t, J = 7.6Hz, 2H), 1.86-127 (comp, 19H); ^{13}C (75 MHz, $CDCl_3$) δ 174.3, 71.0, 62.8, 57.9, 51.6, 51.6, 37.5, 35.8, 34.2, 29.4, 29.4, 29.2, 25.5, 25.0, 25.0, 24.9, 19.1; IR (neat) υ = 3447, 2931, 2858, 1737, 1439, 1375, 1251, 1201, 1171, 1109, 1014, 849 cm^{-1}; Anal. Calcd. for $C^{14}H^{26}O^{4}$: C, 65.09; H, 10.14; Found: C, 65.34; H, 10.29.

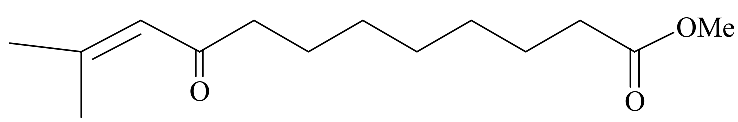


12-Benzyloxy-5,5-difluoro-2-methyl-dodec-2en-4-ol 66 (90%). Flash chromatography (10% EtOAc:Hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36–1.40(m, 8H), 1.55-2.02(m, 4H), 1.74 (s, 3H), 1.80(s, 3H), 2.44-2.46 (d, J = 6.2Hz, 1H), 3.98-4.51(m,1H), 4.52(s, 2H), 5.22-5.31(d, J = 6.2Hz, 1H), 7.30-7.37(m, 5H); ^{13}C (75 MHz, $CDCl_3$) δ 140.2, 138.6, 127.4, 127.7, 127.6, 120.1, 72.9, 70.4, 70.0, 32.7, 29.7, 29.4, 29.3, 26.1, 21.3, 18.6; ^{19}F NMR (282 MHz, $CDCl_3$) δ 112.1; IR (neat) υ =3401, 2931, 2849, 1441 cm^{-1}; Anal. Calcd. for $C_{20}H_{30}F_2O_2$: C, 70.56; H, 8.88; Found: C, 70.38; H 8.99.

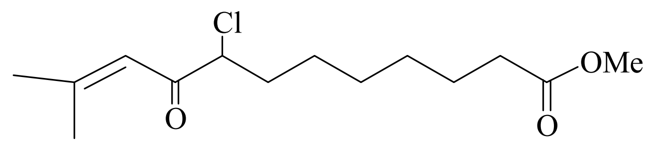
General Procedure for Esterification via Diazomethane: A flame dried round bottom flask was charged with a solution of acid (6.16 mmol) in 7 mL dry ether. The solution was then cooled to $0^{o}C$ and diazomethane (24 mmol, 0.3M in dry ether) was added dropwise. After stirring for approximately 2h at $0^{o}C$, the yellow solvent was removed under reduced pressure. Purification by flash chromatography provided the ester.



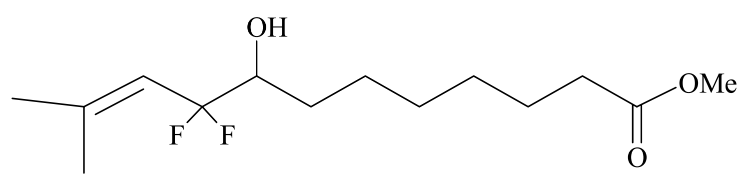
Methyl Non-8-ynoate 86 (99%). Flash chromatography (30% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 3.66 (s, 3H), 2.31 (t, J = 7.2 Hz, 2H), 2.18 (dt, J = 2.4, 4.2 Hz, 1H), 1.93 (t, J = 2.7Hz, 2H), 1.68-1.29 (m, 8H); ^{13}C (75 MHz, $CDCl_3$) δ 172.0, 83.0, 50.4, 33.3, 28.8, 28.6, 28.5, 25.4, 20.0; IR (neat) υ = 3295, 1739, 1436, 1200 , 1172 cm^{-1}; Anal. Calcd. for $C_{10}H_{16}O_2$: C, 71.39; H, 9.59; Found: C, 70.99; H, 9.68.



Methyl 11-Methyl-9-oxo-dodec-10-enoate 15 (85%) : Flash chromatography (15% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 6.06 (s, 1H), 3.66 (s, 3H), 2.38 (t, J = 7.5 Hz, 2H), 2.29 (t, J = 7.5 Hz, 2H), 2.13 (s, 3H), 1.87 (s, 3H), 1.63-1.54 (m, 4H), 1.30 (bs, 6H); ^{13}C (75 MHz, $CDCl_3$) δ 201.5, 174.5, 155.0, 124.0, 51.6, 44.4, 34.2, 29.2, 29.1, 27.9, 25.0, 24.3, 20.8; IR (neat) υ = 2932, 2856, 1739, 1682, 1620, 1437, 1378, 1362, 1197, 1171, 1114, 1044 cm^{-1}; Anal. Calcd. for $C_{14}H_{24}O_3$: C, 69.96; H, 10.07; Found: C, 69.76; H, 9.94.

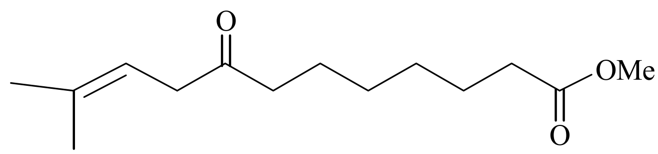


Methyl-8-Chloro-11-methyl-9-oxo-dodec-10-enoate 84 (98%). Flash chromatography (15% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 6.30 (s, 1H), 4.13 (dd, J = 5.7, 8.1Hz, 1H), 3.66 (s, 3H), 2.29 (t, J = 7.5Hz, 2H), 2.19 (s, 3H), 1.96-1.83 (comp, 6H), 1.63-1.31 (comp, 7H); ^{13}C (75 MHz, $CDCl_3$) δ 194.4, 174.3, 160.5, 120.0, 64.6, 51.6, 34.1, 29.0, 28.8, 26.1, 24.9, 21.4; IR (neat) υ = 2936, 2858, 1737, 1689, 1619, 1440, 1377, 1200, 1171, 1024, 841, 732, 686 cm^{-1}; Anal. Calcd. for $C_{14}H_{23}ClO_3$: C, 61.19; H, 8.44; Found: C, 61.45; 8.49.

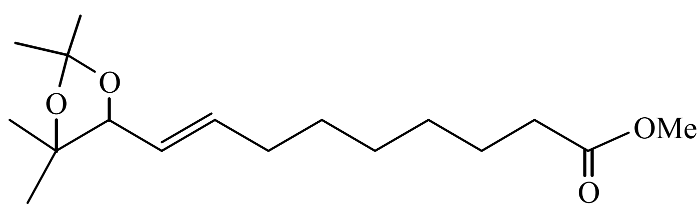


Methyl-9,9-Difluoro-8-hydroxy-11-methyl-dodec-10-enoate 49 (83%). Flash chromatography (20% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 3.98-3.85 (m, 1H), 2.30 (t, J = 7.5Hz, 2H), 3.17-3.05 (m, 1H), 2.33-2.27 (m, 2H) 1.65-1.20 (18H); ^{13}C (75 MHz, $CDCl_3$) δ 172.0, 133.2 (t), 125.8 (t), 115.8 (t), 81.5, 50.4, 33.3, 30.6, 29.9, 25.4, 25.2, 24.8, 24.4, 19.3; ^{19}F (282 MHz, $CDCl_3$) δ –102.6 (t, J = 469.5, 1F), -103.4 (t, J =

263.106, 1F), IR (neat) υ = 3458, 2933, 2859, 1738, 1438, 1379, 1030, 737 cm^{-1}; Anal. Calcd. for $C_{14}H_{24}F_2O_3$: C, 60.41; H, 8.69; Found: C, 60.41; H, 8.69.

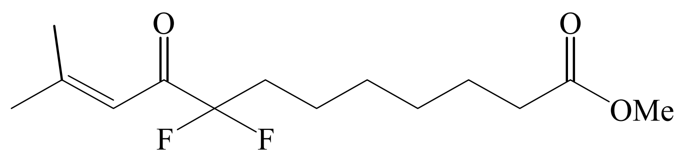


Methyl-11-Methyl-8-oxo-dodec-10-enoate 41 (94%). Flash chromatography (15% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 5.28 (t, J = 3.6Hz, 1H), 3.65 (s, 3H), 3.08 (d, J = 7.2Hz, 2H), 2.39 (t, J = 7.6Hz, 2H), 2.29 (t, J = 7.6Hz, 2H), 1.74 (s, 3H), 1.61-1.46 (m, 7H), 1.30-1.28 (comp, 5H); ^{13}C (75 MHz, $CDCl_3$) δ 209.7, 174.3, 135.8, 116.2, 51.6, 42.8, 42.2, 34.1, 29.0, 29.0, 25.9, 24.9, 23.7, 18.2; IR (neat) υ = 2933, 2859, 1737, 1439, 1368, 1172, 1109, 1012, 834 cm^{-1}; Anal. Calcd. for $C_{14}H_{24}O_3$: C, 69.96, H, 10.0; Found: C, 69.73; H, 9.98.



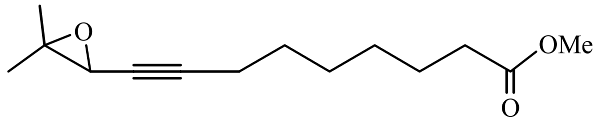
Methyl-9-(2,2,5,5-Tetramethyl-[1,3]dioxolan-4yl)dioxolan-4yl)-non-8-enoate 95 (99%). Flash Chromatography (20% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 5.80 (dt, J = 6.3, 15.6Hz, 1H), 5.4 (dd, J = 9.0, 14.4Hz, 1H), 4.12 (d, J = 8.1Hz, 1H), 3.66 (s, 3H), 2.29 (t, J = 7.8Hz, 2H), 2.01 (q, J = 6.9Hz, 2H), 1.58-1.51 (multiplet, 2H), 1.44-1.22

(complex, 14H), 1.09 (s, 3H); ^{13}C (75 MHz, $CDCl_3$) δ 172.0, 136.8, 124.7, 107.2, 85.2, 50.4, 34.0, 32.4, 28.9, 28.8, 28.6, 27.2, 25.9, 24.7, 23.7; IR (neat) υ = 2979, 2931, 2857, 1741, 1436, 1368, 1216, 1199, 1120, 1036, 972, 914, 860.2 cm^{-1}; Anal. Calcd. for $C_{17}H_{30}O_4$: C, 68.42; H, 10.13; Found: C, 68.33; H, 10.22.

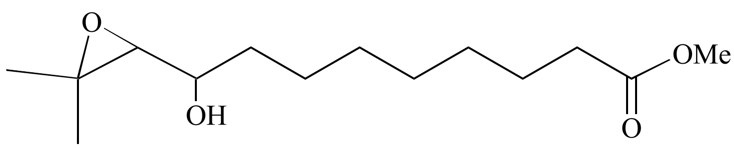


Methyl-8,8-Difluoro -11-methyl-9-oxo -dodec-10-enoate 69 (50%). ^{1}H (300 MHz, $CDCl_3$) δ 1.33–1.45(m, 6H), 1.59-1.64(m, 2H), 1.94-2.01(m, 2H), 1.99-2.01(s, 3H), 2.23(s, 3H), 2.27-2.32(m, 2H), 6.42(s, 1H). ^{13}C (300 MHz, $CDCl_3$) δ 21.4, 21.5, 21.5, 21.9, 24.9, 28.7, 29.0, 29.2, 33.0, 33.3, 33.5, 34.2, 51.7, 116.4, 117.2, 118.9, 121.4, 164.3, 174.4, 189.8. ^{19}F (282 MHz, $CDCl_3$) δ 108.2. IR (neat) υ = 2940, 2861, 1739, 1616(allyl) cm^{-1}; Anal. Calcd. for $C_{14}H_{22}F_2O_3$: C, 60.85; H, 8.03; Found: C, 65.70; H, 8.77.

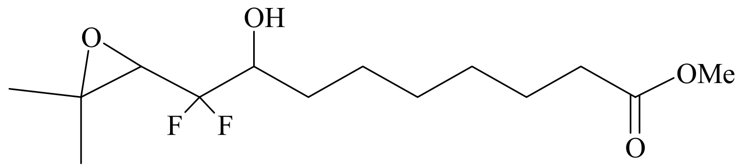
General procedure for mCPBA epoxidation: To an ice cooled solution of alkene (0.22 mmol) in dry dichloromethane (2.4 mL) was slowly added *m*CPBA (0.34 mmol). The ice bath was removed and the mixture allowed to warm to room temperature with hourly monitoring via TLC to access disappearance of starting material. Normally after 2.0 h, the reaction mixture was diluted with 100 mL Et_2O, washed with sat. aqueous sodium bicarbonate (3 x 50 mL), followed by 50 mL sat. aq. sodium chloride. The organic layer was then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography.



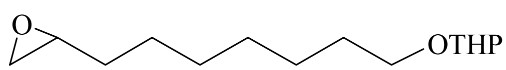
Methyl-9-(3,3-Dimethyl-oxiranyl)-non-8-ynoate 9 (75%). Flash chromatography (15% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 3.66 (s, 3H), 3.20 (t, J = 1.5 Hz, 1H), 2.30 (t, J = 7.2 Hz, 2H), 2.22 (dt, J = 1.8, 5.1 Hz, 2H), 1.62 (quintet, J = 7.8 Hz, 2H), 1.56-1.44 (m, 2H), 1.42-1.28 (complex, 11H); ^{13}C (75 MHz, $CDCl_3$) δ 172.5, 79.8, 78.1, 55.1, 54.6, 50.4, 34.6, 29.0, 28.7, 28.6, 26.1, 22.3, 17.5 ; IR (neat) υ = 2935, 2859, 1738, 1435, 1378, 1306, 1249, 1200, 1170, 882 cm^{-1}; Anal. Calcd. for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59; Found: C, 71.56; H, 9.67.



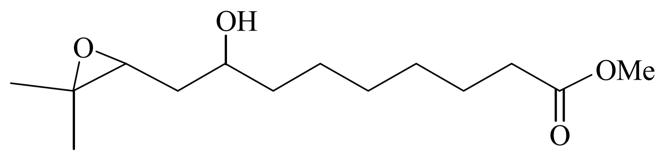
Methyl-9-(3,3-Dimethyl-oxiranyl)-9-hydroxy-nonanoate 4b (86%) : Flash chromatography (20% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 3.66 (s, 3H), 3.50-3.43 (m, 1H), 2.69 (d, J = 8.4 Hz, 1H), 2.30 (t, J = 7.5 Hz, 2H), 1.61 (bt, J = 7.5 Hz, 2H), 1.55-1.30 (complex, 16H); ^{13}C (75 MHz, $CDCl_3$) 174.4, 70.6, 68.0 51.7, 34.2, 29.6, 29.3, 29.2, 25.1, 19.6; ; IR (neat) υ = 3425, 2931, 2857, 1757, 1439, 1247, 1172, 1115, 1032, 898, 821, 729, 694 cm^{-1}; Anal. Calcd. for $C_{14}H_{26}O_4$: C, 65.09; H, 10.14; Found: C, 64.95; H, 10.08.



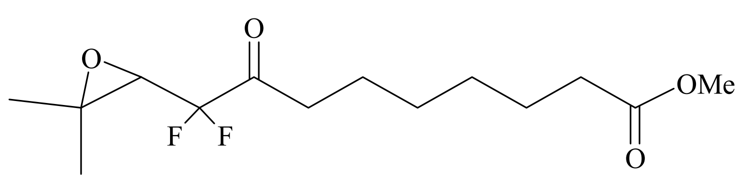
Methyl-9-(3,3-Dimethyl-oxiranyl)-9,9-difluor-8-8-hydroxy-nonanoate 6b (80%) : Flash Chromatography (20% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 5.34 (bt, J = 16.2Hz, 1H), 3.76-3.67 (m, J = 7.2Hz, 1H), 2.35 (t, J = 6.6 Hz, 2H), 1.87 (bs, 3H), 1.82 (bs, 3H), 1.62-1.50 (complex, 10H); ^{13}C (75 MHz, $CDCl_3$) δ 174.5, 120.3 (t), 73.3 (m), 60.4 (m), 51.6, 34.2, 30.4, 29.7, 25.4, 24.1, 24.0, 23.5; ^{19}F (282 MHz, $CDCl_3$) δ -113.6 (dd, J = 51.3, 195.7 Hz, 1F), -118.1 (dd, J = 195.7, 604.3Hz, 1F); IR (neat) υ = 2859, 1712, 1453, 1410, 1379, 1216, 1029 cm^{-1}; Anal. Calcd. for $C_{14}H_{24}F_2O_4$: C, 57.13; H, 8.22; Found: C, 57.34; H, 8.28.



2-(7-Oxiranyl-heptyloxy)-tetrahydro-pyran 37 (84%). Flash chromatography (10% Et_2O:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 5.85-5.75 (m, 1H), 4.98 (d, J = 17.2Hz, 1H), 4.92 (d, J = 10Hz, 1Hz), 4.58-4.56 (m, 1H), 3.89-3.84 (m, 1H), 3.72 (q, J = 9.6Hz, 1H), 3.52-3.47 (m, 1H), 3.37 (q, J = 9.6Hz, 1H), 2.03 (q, J = 7.7Hz, 2H), 1.86-1.78 (m, 1H), 1.74-1.68 (m, 1H), 1.59-1.51 (comp, 6H), 1.39-1.32 (comp, 8H); ^{13}C (75 MHz, $CDCl_3$) δ 139.4, 114.3, 99.0, 67.8, 62.5, 34.0, 31.0, 29.9, 29.5, 29.2, 29.0, 26.4, 25.7, 19.9; IR (neat) υ = 3075, 2927, 2854, 1640, 1351, 1259, 1200, 1136, 1120, 1078, 1034, 991, 906, 869, 815, 724 cm^{-1}; Anal. Calcd. for $C_{14}H_{26}O_3$: C, 69.38; H, 10.81; Found: C, 69.36; H, 10.49.

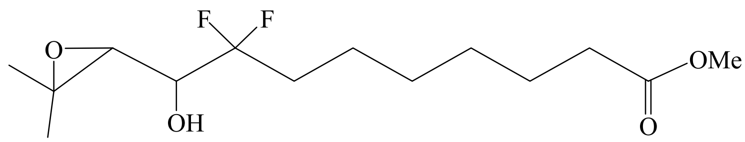


Methyl-9-(3,3-Dimethyl-oxiranyl)-8-hydroxy-nonanoate 5b (80%). Flash Chromatography (20% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 5.14 (t, J = 7.2Hz, 1H), 3.64 (s, 3H), 3.58-3.54 (m, 1H), 2.28 (t, J = 7.6Hz, 2H), 2.15-2.10 (m, 2H), 1.72 (s, 3H), 1.62-1.56 (comp, 5H), 1.43-1.31 (comp. 8H) ^{13}C (75 MHz, $CDCl_3$) δ 174.5, 135.4, 120.3, 71.8, 51.6, 36.9, 36.4, 34.2, 29.5, 25.8, 25.0, 18.2; IR (neat) υ = 3440, 2928, 2855, 1738, 1434, 1375, 1172, 1104, 876, 726 cm^{-1}; Anal. Calcd. for $C_{14}H_{26}O_3$: C, 69.38; H, 10.81; Found: C, 68.98; H, 10.79.



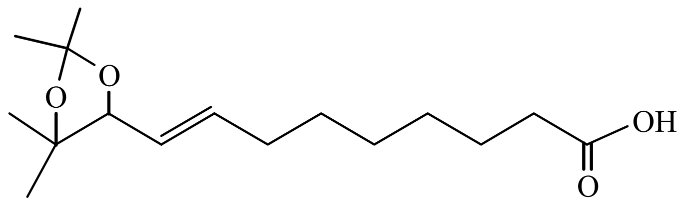
Methyl-9-(3,3-Dimethyl-oxiranyl)9,9-difluoro-8-oxo-nonanoate 6a (82%) : Flash chromatography (35% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 5.30 (s, 1H), 3.66 (s, 3H), 3.13 (t, J = 11.4Hz, 1H), 2.69 (t, 6.9Hz, 2H), 2.30 (t, J = 7.5Hz, 2H), 1.66-1.54 (m, 4H), 1.41-1.31 (complex, 12H); ^{13}C (75 MHz, $CDCl_3$) δ 199.9 (t), 174.2, 114.4 (t), 60.39 (m), 51.6, 36.8, 34.1, 28.9, 28.7, 25.1, 24.9, 22.5, 18.8; ^{19}F (282 MHz, $CDCl_3$) δ -111.1

(dq, J = 8.46, 78.7, 212.1, 2F); IR (neat) υ = 2953, 2860, 1771, 1737, 1436, 1252 cm^{-1}; Anal. Calcd. for $C_{14}H_{22}F_2O_4$: C, 57.52; H, 7.59; Found: C, 57.75; H, 7.65.

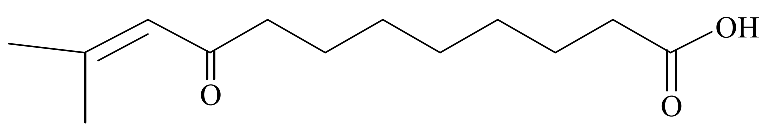


Methyl-9-(3,3-Dimethyl-oxiranyl)-8,8-difluror-9-hydroxy-nonanoate 7b (81%). Flash chromatography (20% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 1.34–1.36(m, 2H), 1.35–1.36(s, 6H), 1.51-1.55(m, 2H), 1.61-1.64(m, 4H), 1.94-1.98(m, 2H), 2.28-2.31(t, J = 9Hz, 2H), 2.65-2.67 (d, J = 6Hz, 1H), 3.03-3.05(d, 1H), 3.66(s, 3H), 3.66(m, 1H). ^{13}C (300 MHz, $CDCl_3$) δ 193.4, 174.4, 126.1, 123.7, 121.3, 70.3, 70.0, 69.7, 61.8, 61.7, 59.8, 51.7, 33.2, 33.0, 32.7, 29.1, 24.9, 21.4, 21.3; ^{19}F (282 MHz, $CDCl_3$) δ 109.1-110.2, 113.2-114.2; IR (neat) υ = 3453, 2939, 2862, 1735, 1639, 1439 cm^{-1}; Anal. Calcd. for $C_{14}H_{24}F_2O_4$: C, 57.13; H, 8.22; Found: C, 56.99; H, 8.21.

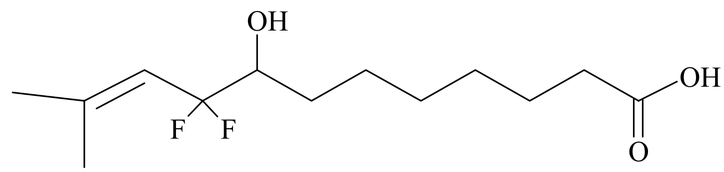
General procedure for PDC oxidation: To a solution of alcohol (0.12mmol) in DMF (1.2mL) at room temperature was added PDC (0.36mmol). The brown suspension was stirred for 18 hours at room temperature before the addition of 12mL of water. The aqueous layer was then extracted with Et_2O (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give a yellow/brown oil. In most cases the acid or acid ketone was purified by column chromatography as the major product.



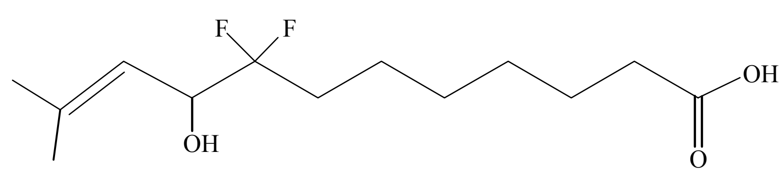
9-(2,2,5,5-Tetramethyl-[1,3]dioxolan-4-yl)-non-8-enoic acid 94 (82%). Flash chromatography (30% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 5.80 (dt, J = 6.3, 15.6Hz, 1H), 5.43 (dd, J = 9.0, 14.4Hz, 1H), 4.12 (d, J = 8.1Hz, 1H), 2.34 (t, J = 7.5Hz, 2H), 2.07 (q, J = 6.9Hz, 2H), 1.58-1.51 (multiplet, 2H), 1.44-1.22 (complex, 14H), 1.09 (s, 3H); ^{13}C (75 MHz, $CDCl_3$) δ 177.0, 136.6, 124.7, 107.2, 85.2, 34.0, 32.4, 28.9, 28.8, 28.6, 27.2, 25.9, 24.7, 23.7; IR (neat) υ = 2979, 2930, 2857, 1710, 1369, 1217, 1198, 1119, 1035, 972, 913, 734 cm^{-1}; Anal. Calcd. for $C_{16}H_{28}O_4$: C, 67.57; H, 9.92; Found: C, 67.33; H, 9.88.



11-Methyl-9-oxo-dodec-10-enoic acid 14 (41%) : Flash chromatography (35% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 6.05 (s, 1H), 2.38 (t, J = 7.5 Hz, 2H), 2.33 (t, J = 7.5 Hz, 2H), 2.13 (s, 3H), 1.87 (s, 3H), 1.63-1.54 (m, 4H), 1.30 (bs, 8H); ^{13}C (75 MHz, $CDCl_3$) δ 201.5, 180.1, 155.2, 124.0, 44.4, 34.2, 29.2, 29.0, 27.8, 24.7, 24.3, 20.8; IR (KBr Pellet) υ = 2930, 1709, 1619, 1445, 1229, 1116 cm^{-1}; Anal. Calcd. for $C_{13}H_{22}O_3$: C, 68.99; H, 9.80; Found: C, 69.23; H, 9.98.

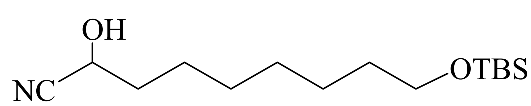


9,9-Difluoro-8-hydroxy-11-methyl-dodec-10-enoic acid 48 (71%) : Flash Chromatography (30% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 5.38 (tt, J = 1.5, 15.6 Hz, 1H), 3.76-3.64 (complex, 4H), 2.30 (t, J = 7.5 Hz, 2H), 1.99-1.80 (m, 6H), 1.65-1.25 (complex, 10H); ^{13}C (75 MHz, $CDCl_3$) δ 177.0, 133.2, 125.6 (t), 116.2 (t), 81.5 (t), 35.8, 30.3, 29.7, 25.3, 25.1, 24.4, 24.2, 19.7; ^{19}F (282 MHz, $CDCl_3$) δ -101.70, -102.61, -103.37 -104.27 -104.31; IR (neat) υ = 3467, 2936, 1731, 1679, 1438, 1379, 1202, 1030, 737 cm^{-1}; Anal. Calcd. for $C_{13}H_{22}F_2O_3$: C, 59.07; H, 8.39; Found: C, 59.30; H, 8.44.

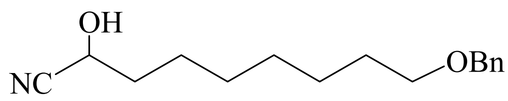


Methyl-8,8-Difluoro-9-hydroxy-11-methyl-dodec-10-enoate 68 (50%). Flash chromatography (35% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 1.25−1.64(m, 8H), 1.73(s, 3H), 1.79(s, 3H), 1.73-1.92(m, 2H), 2.0 (s, 1H), 2.28-2.32 (t, J = 7.2Hz, 2H), 4.42-4.45(m, 1H), 5.24-5.26 (d, J = 6.0 Hz, 1H); ^{13}C (75 MHz, $CDCl_3$) δ 174.4, 127.4, 124.2, 120.0, 70.5, 70.1, 69.8, 51.6, 34.1, 33.0, 32.7, 32.4, 29.2, 29.0, 26.2, 24.9, 21.3, 18.7; ^{19}F NMR (282 MHz, $CDCl_3$) δ 112.2; IR (neat) υ = 3458, 2938, 2863, 1735, 1443 cm^{-1}; Anal. Calcd. for $C_{14}H_{24}F_20_3$: C, 60.41; H, 8.69

General Procedure for the Synthesis of Cyanohydrins: To a solution of aldehyde (0.77 mmol) in ether (3.27 mL) at room temperature was added dropwise a solution of sodium bisulfite (0.85 mmol, 1M in H_2O). After 4 hrs, an aqueous solution of sodium cyanide (0.85 mmol, 1M in H_2O) was slowly added to the mixture forming a white precipitate. The biphasic solution was stirred rapidly for 20 h before being diluted with 20 mL ether. The two phases were then separated and the aqueous layer extracted with Et_2O (2 x 50 mL). The combined organic layers were washed with 50 mL H_2O, dried over magnesium sulfate, filtered and concentrated to an oil. Column chromatography provided the pure cyanohydrin as a clear oil.

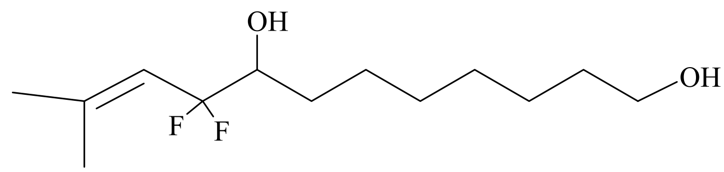


9-(tert-Butyl-dimethyl-silanyloxy)-2-hydroxy-nonanenitrile 79 (99%). Flash Chromatography (20% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 4.48 (q, J = 8.4Hz, 1H), 3.59 (t, J = 8.4Hz, 2H), 1.85 (bq, J = 10.8, 2H), 1.52-1.43 (comp, 3H), 1.32 (bs, 8H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C (75 MHz, $CDCl_3$) δ 120.1, 63.4, 61.5, 35.4, 32.9, 29.3, 29.0, 26.2, 25.8, 24.6, 18.6, -5.0; IR (neat) υ = 3444.3, 2932.6, 2858.8, 2738.3, 1465.4, 1389.7, 1253.8, 1098.1, 938.6, 837.5, 777.5, 714.6, 662.2 cm^{-1}; Anal. Calcd. for $C_{15}H_{31}NO_2Si$: C, 63.10; H, 10.94; Found: C, 62.78; H, 10.98.

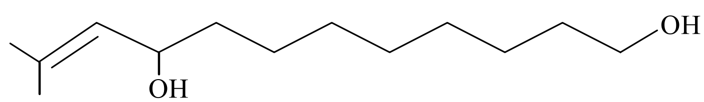


9-Benzyloxy-2-hydroxy-nonanenitrile 61 (74%). Flash Chromatography (25% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 1.35(m, 6H), 1.48-1.62(m, 4H), 1.81-1.86(m, 2H), 2.23(d, J = 6.2 Hz, 1H), 3.45-3.49(t, J = 7.3 Hz, 2H), 4.42-4.49(m, 1H), 4.50(s, 2H), 7.33-7.35(m, 5H); ^{13}C (75 MHz, $CDCl_3$) δ 138.2, 128.5, 128.0, 127.8, 120.4, 73.0, 70.6, 60.8, 35.1, 29.5, 29.1, 28.9, 26.0, 24.5; IR (neat) υ = 3427, 2931, 2856, 1724, 1103 cm $^{-1}$; Anal. Calcd. for $C_{16}H_{23}NO_2$: C, 73.53; H, 8.87; Found: C, 73.33; H, 8.78.

General Procedure for TBAF Deprotection: Tetrabutylammonium fluoride (TBAF) (3.6 mmol, 1M in THF) was added dropwise to a cooled (0 ^{o}C) solution of silyl ether (0.71 mmol) in 3.6 mL of THF. Once the addition was complete the reaction mixture was allowed to warm to room temperature and stirred until complete consumption of starting material had occurred (TLC). The reaction mixture was then quenched by the addition of 5 mL saturated aq. ammonium chloride. The aqueous layer was extracted with ether (3x 50 mL), the organic layers were combined and washed with 50 mL sat. ammonium chloride and 50 mL brine. Drying over anhydrous magnesium sulfate, filtering and concentrating provided the crude diol, which was purified by column chromatography.



9,9-Difluoro-11-methyl-dodec-10-ene-1,8-diol 47 (100%) : Flash chromatography **(**35% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 3.34 (t, J = 13.5Hz, 1H), 3.76-3.70 (m, 1H), 3.64 (t, J = 6.6Hz, 2H), 2.04-1.81 (complex, 7H), 1.59-1.22 (complex, 12H); ^{13}C (75 MHz, $CDCl_3$) δ 134.5, 125.8 (t), 115.8 (t), 81.2 (t), 63.0, 33.9, 30.5, 30.1, 26.5, 25.4, 25.3, 20.7, 19.3; ^{19}F (282 MHz, $CDCl_3$) δ -102.6 (t, J = 219.7, 1F), -103.5 (t, J = 219.7, 1F); IR (neat) υ = 3361, 2931, 2859, 1450, 1380, 1086, 1049 cm^{-1}; Anal. Calcd. for $C_{13}H_{24}F_2O_2$: C, 62.37; H, 9.66; Found: C, 62.83; H, 9.81.



11-Methyl-dodec-10-ene-1,9-diol 13 (92%) : Flash chromatography **(**35% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 5.15 (d, J = 8.7 Hz, 1H), 4.34-4.32 (m, 1H), 3.59 (t, J = 6.6 Hz, 2H), 1.72 (s, 3H), 1.68 (s, 3H), 1.52-1.26 (m, 14H); ^{13}C (75 MHz, $CDCl_3$) δ 134.8, 128.5, 68.8, 62.9, 37.8, 32.8, 29.7, 29.5, 25.8, 25.5, 18.3, 15.3, 14.3; IR (neat) υ = 3330, 2927, 2854, 1446, 1375, 1301, 1057, 1005, 845, 723 cm^{-1}; Anal. Calcd. for $C_{13}H_{26}O_2$: C, 72.84; H, 12.23; Found: C, 72.15; H, 12.25

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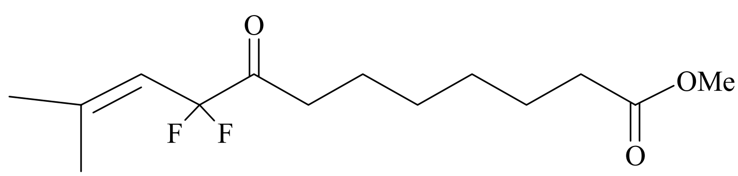
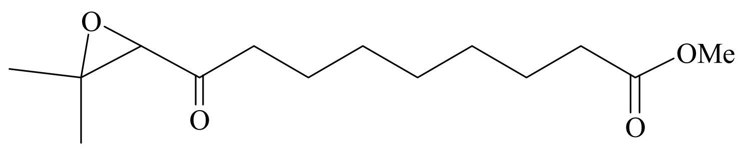
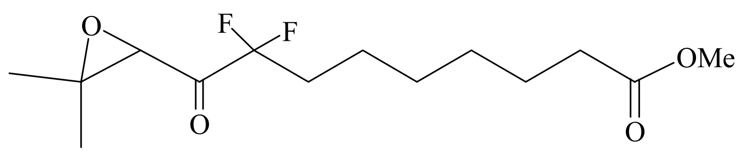
General Procedure for Dess-Martin Oxidation: The carbinol (0.11mmol) in 1.0 mL dichloromethane was added slowly via canula to a solution of Dess-Martin reagent (0.407 mmol) in 11.0 mL CH_2Cl_2 and pyridine (0.407mmol). The mixture was stirred for 2 h and then diluted with Et_2O (100 mL) and poured into a solution of sat. aq. sodium thiosulfate (50 mL) and sat. aq. sodium bicarbonate (10 mL). The layers were separated and the organic phase was washed with saturated sodium bicarbonate (2 x 25 mL) and water (2 x 25 mL). The aqueous solution was back extracted with 100 mL ether. After drying the combined organic phases over $MgSO_4$, solvent removed was under reduced pressure. The resultant oil purified by flash chromatography (15% EtOAc:Hexane) providing the purified keto-ester.



Methyl-9,9-Difluoro-11-8-oxo-dodec-10-enoate 50 (99%). Flash chromatography **(**10% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 5.38 (tt, J = 1.5, 15.6 Hz, 1H), 3.66 (s, 3H), 2.62 (t, J = 6.9Hz, 2H), 2.30 (t, J = 7.5Hz, 2H), 1.84-1.79 (m, 6H), 1.67-1.54 (m, 4H), 1.36-1.29 (m, 5H); ^{13}C (75 MHz, $CDCl_3$) δ 207.1 (t), 172.0, 134.7, 126.5 (t), 117.9 (t), 50.7, 33.3, 29.5, 27.6, 25.0, 24.0, 19.0; ^{19}F (282 MHz, $CDCl_3$) δ –100.6 (d, J = 14.1, 2F); IR (neat) υ = 2940, 2861, 1740, 1669, 1437, 1377, 1199, 1058, 971 cm^{-1}; Anal. Calcd. for $C_{14}H_{22}F_2O_3$: C, 60.85; H, 8.03; Found: C, 61.45; H, 8.17.

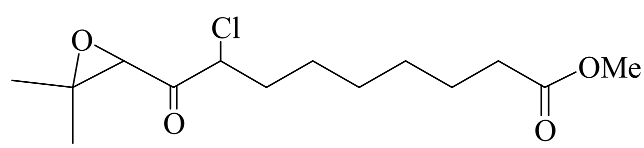


Methyl-9-(3,3-dimethyl-oxiranyl)-9-oxo-nonanoate 4a (84%). Flash chromatography **(**10% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 3.66 (s, 3H), 3.39 (s, 1H), 2.50 (t, J = 7.5 Hz, 2H), 2.29 (t, J = 7.5 Hz, 2H), 1.62-1.55 (m, 4H), 1.43 (s, 3H), 1.30 (s, 3H), 1.25 (s, 3H); ^{13}C (75 MHz, $CDCl_3$) δ 206.8, 174.4, 65.6, 61.1, 51.6, 41.1, 34.2, 29.2, 25.0, 23.3, 18.6; IR 2931, 2859, 1734, 1402, 1173, 821 cm^{-1}; Anal. Calcd. for $C_{14}H_{24}O_4$: C, 65.60; H, 9.44; Found: C, 65.60; H, 9.44.

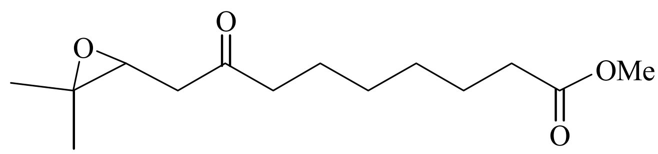


Methyl-9-(3,3-dimethyl-oxiranyl)-8,8-difluoro-9-oxo-nonanoic methyl ester 7a (85%). ^{1}H (300 MHz, $CDCl_3$) δ 1.25 (s, 3H), 1.34-1.6 (m, 2H), 1.50 (s, 3H), 1.48-1.51 (m, 2H), 1.60-1.62 (m, 2H), 1.95-2.08 (m, 2H), 2.28-2.32 (t, J = 7.2Hz, 2H), 3.66(s, 3H), 3.89(s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 18.0, 21.0, 21.1, 21.1, 24.6, 24.7, 24.9, 28.9, 29.1, 29.6, 29.9, 32.3, 32.4, 32.6, 32.8, 33.9, 34.1, 51.7, 62.1, 63.4, 116.1, 118.6, 121.0, 174.3, 195.5. ^{19}F NMR (282 MHz, $CDCl_3$) δ 107.3-107.9. IR (neat) 3467, 2932, 2859, 1741 cm^{-1}; Anal. Calcd. for $C_{14}H_{22}F_2O_4$: C, 57.52; H, 7.59; Found: C, 60.27; H, 8.19.

General Procedure for Epoxidation using an Oxirane generated In-situ: To an acetonitrile solution (7.5 mL) of alkene (5mmol) was added an aqueous solution of Na_2EDTA (5 mL, 4.0 x 10-4 M). The resulting biphasic solution was cooled to 0 ^oC, followed by addition of trifluoroacetone (10.0 mmol) via a precooled syringe. A mixture of sodium bicarbonate (7.75 mmol) and Oxone (5.0 mmol) was then added to the reaction mixture over a period of 30 minutes. Once the addition was complete, the mixture was further stirred with monitoring by TLC for starting material consumption. After all starting material had reacted, the reaction mixture was diluted with 20 mL of water. The aqueous layer was extracted with ether (3 x 50 mL) and the combined organic layers dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. Purification by silica gel gave the desired product.

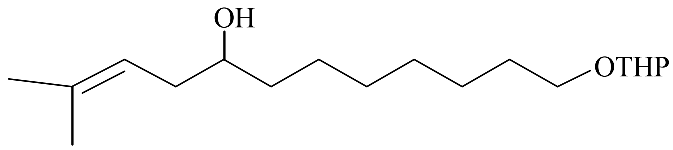


Methyl-8-Chloro-9-(3,3-dimethyl-oxiranyl)-9-oxo-nonanoate 8 (96%). Flash chromatography (12% EtOAC: Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 4.40 (dd, J = 5.1, 8.4 Hz, 1H), 3.70 (s, 1H), 3.66 (s, 3H), 2.30 (t, J = 7.2, 2H), 2.05-1.776(comp, 2H), 1.64-1.24 (comp, 14H); ^{13}C (75 MHz, $CDCl_3$) δ 199.4, 174.1, 64.1, 62.7, 51.5, 34.2, 32.1, 28.9, 28.7, 25.9, 24.8, 24.4; IR (neat) υ = 2934, 2859, 1732, 1436, 1379, 1251, 1198, 1173, 1113, 1025, 921, 818, 733, 678 cm^{-1}**;** Anal. Calcd. for $C_{14}H_{23}ClO_4$: C, 57.83; H, 7.97; Found: C, 58.11; H, 8.12.

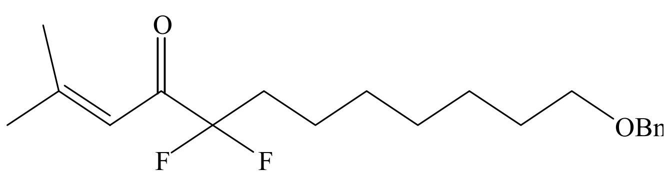


Methyl-9-(3,3-Dimethyl-oxiranyl)-8-oxo-nonanoate 5a (92%). Flash chromatography (20%EtOAc: Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 3.65 (s, 3H), 3.08 (d, J = 8.0Hz, 1H), 2.67 (dq, J = 8.4, 22.4Hz, 2H), 2.46 (t, J = 7.6Hz, 2H), 2.24 (t, J = 7.6Hz, 2H), 1.63-1.55 (m, 4H), 1.34-1.25 (m, 10H); ^{13}C (75 MHz, $CDCl_3$) δ 208.5, 174.3, 59.4, 58.2, 51.6, 43.3, 42.9, 34.1, 29.0, 28.9, 24.9, 24.7, 23.5, 19.0; IR (neat) υ = 2933, 2859, 1738, 1713, 1461, 1434, 1379, 1251, 1198, 1172, 1122, 1012, 854 cm^{-1}; Anal. Calcd. for $C_{14}H_{24}O_4$: C, 65.60; H, 9.44; Found: C, 63.37; H, 9.48.

General Procedure for Organo Lithium Addition: 2-Methyl-1-bromopropene (0.62 mmol) was dissolved in dry THF (0.8 mL) and the solution cooled to –78 °C via dry ice/ acetone. Slow addition of *t*-butyllithium (1.24 mmol, 1.7 M in pentane) into the reaction mixture generated a yellow solution with a small amount of precipitate. After 45 min at –78 °C, the mixture was warmed to room temperature and stirred for 10 min before cooling to –78 °C. Once at –78 °C, a solution of epoxide or aldehyde (0.41 mmol) in 0.5 mL THF was slowly added via canulation. Soon after the addition, the ice bath was removed and the reaction mixture warmed to room temperature. The reaction was stirred for 5 h, followed by slow addition of 2 mL water. The aqueous layer was extracted with ether (3 x 50 mL). The combined organic layers were dried over anhydrous magnesium sulfate, filtered and concentrated. Purification via column chromatography gave the desired intermediate.

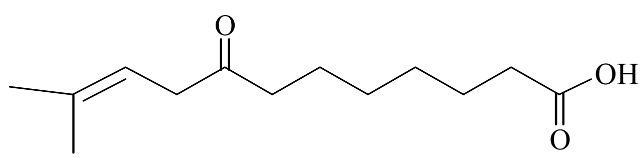


2-Methyl-12-(tetrahydro-pyran-2-yloxy)-dodec-2-en-5-ol 38 (66%). Flash chromatography (10% EtOAc: Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 5.14 (t, J = 7.6Hz, 1H), 4.58-4.56 (m, 1H), 3.89-3.84 (m, 1H), 3.72 (q, J = 9.6Hz, 1H), 3.52-3.47 (m, 1H), 3.37 (q, J = 9.6Hz, 1H), 2.15-2.12 (comp, 2H), 1.86-1.31 (comp, 26H); ^{13}C (75 MHz, $CDCl_3$) δ 135.3, 120.4, 99.0, 71.8, 67.8, 62.5, 36.9, 36.4, 30.9, 29.9, 29.8, 29.6, 26.1, 25.9, 25.6, 19.8, 18.1; IR (neat) υ = 3450, 2929, 1452, 1441, 1381, 1351, 1260, 1200, 1120, 1120, 1077, 1033, 986, 904, 868, 815 cm^{-1}; Anal. Calcd. for $C_{18}H_{34}O_3$: C, 72.44; H, 11.48; Found: C, 72.39; H, 11.51.

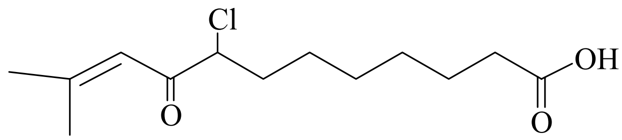


12-Benzyloxy-5,5-difluoro-2-methyl-dodec-2-en-4-one 65 (47%). Flash chromatography (2% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 1.27–1.48 (m, 8H), 1.58-1.67 (m, 2H), 1.89-2.02 (m, 2H), 2.01 (s, 3H), 2.25 (s, 3H), 3.45-3.49 (t, J = 7.2Hz, 2H), 4.51 (s, 2H), 6.44 (s, 1H), 7.30-7.36 (m, 5H); ^{13}C (75 MHz, $CDCl_3$) δ 189.9, 164.1, 138.9, 122.3, 118.9, 117.2, 115.6, 73.1, 70.6, 33.6, 33.3, 33.0, 29.9, 29.4, 29.3, 28.7, 26.2, 21.8, 21.6, 21.5. ^{19}F NMR (282 MHz, $CDCl_3$) δ −108.1. IR (neat) υ = 2933, 2857, 1704(ketone), 1616(allyl) cm^{-1}; Anal. Calcd. for $C_{20}H_{28}F_2O_2$: C, 70.98; H, 8.34.

General Procedure for Oxidation of an alcohol to an acid by Jones Reagent: To a solution of diol (0.18 mmol) in 1.8 mL of acetone at 0 ^{o}C was added dropwise 8N Jones reagent until the color of the solution remained a greenish/blue color. The reaction mixture was then diluted with 50 mL Et_2O, and washed with water (3x 25 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated to provide the crude oil. Purification via column chromatography gave the keto acid as a clear oil.

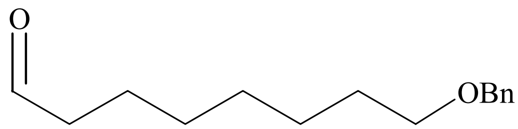


11-Methyl-8-oxo-dodec-10-enoic acid 40 (75%). Flash chromatography (50% EtOAc: Hexane) ^{1}H (300 MHz, $CDCl_3$) δ 5.28 (t, J = 3.6Hz, 1H), 3.08 (d, J = 7.2Hz, 2H), 2.40 (t, J = 7.6Hz, 2H), 2.33 (t, J = 7.6Hz, 2H), 1.74 (s, 3H), 1.62 (comp, 7H), 1.32-1.25 (comp, 5H); ^{13}C (75 MHz, $CDCl_3$) δ 210.0, 180.0, 135.8, 116.1, 42.8, 42.2, 34.1, 28.9, 25.8, 24.6, 23.6; IR (neat) υ = 3342, 2929, 2855, 1737, 1451, 1376, 1112, 1056, 872, 835, 776, 723 cm^{-1}; Anal. Calcd. for $C_{13}H_{22}O_3$: C, 68.99; H, 9.80; Found: C, 69.05; H, 9.82.

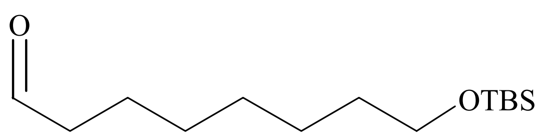


8-Chloro-11-methyl-9-oxo-dodec-10-enoic acid 83 (86%). Flash Chromatography (30% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 6.31 (s, 1H), 4.16 (dd, J = 8, 11.2Hz, 1H), 2.34 (t, J = 10Hz, 2H), 2.19 (s, 3H), 1.96-1.77 (comp, 6H), 1.65-1.25 (comp, 8H); ^{13}C (75 MHz, $CDCl_3$) δ 194.4, 180.2, 160.6, 120.0, 64.5, 34.1, 28.9, 28.8, 28.3, 26.1, 24.6, 21.4; IR (neat) υ = 2934, 2859, 1707, 1618, 1442, 1382, 1231, 947, 840, 732, 686 cm^{-1}; Anal. Calcd. for $C_{13}H_{21}ClO_3$: C, 59.88; H, 8.12; Found: C, 59.93; H, 8.13.

General Procedure for PCC Oxidation: A 250 mL flask was charged with mono protected alcohol (10.1 mmol) and 101 mL methylene chloride followed by the addition of 5.4g Celite and (0.0254mol) pyridinium chlorochromate (PCC). The mixture was stirred at room temperature with reaction progress monitored by TLC analysis. Once complete disappearance of the alcohol was noted, the reaction mixture was then filtered through a short silica gel column. The column was flushed two times with CH_2Cl_2 (100 mL). The organic layers were combined and concentrated to an oil. Purification by flash chromatography provided the aldehyde.

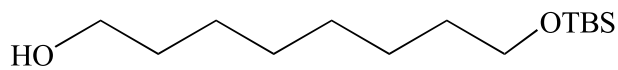


8-Benzyloxy-octaldehyde 60 (75%). Flash Chromatography (4% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 1.34 (m, 6H), 1.59-1.61 (m, 4H), 2.38-2.42 (t, J = 7.2Hz, 2H), 3.44-3.48 (t, J = 6Hz, 2H), 4.50 (s, 2H), 7.33-7.35 (m, 5H), 9.76 (s, 1H); ^{13}C (75 MHz, $CDCl_3$) δ 203.1, 138.9, 128.6, 127.8, 127.7, 73.1, 70.6, 44.1, 29.9, 29.5, 29.4, 29.3, 26.2, 22.2; IR (neat) υ = 3427, 2931, 2856, 1724, 1103 cm^{-1}.

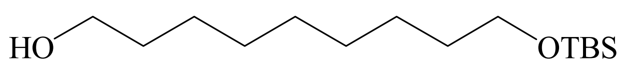


8-(*tert*-Butyl-dimethyl-silanyloxy)-octanal (21). Flash Chromatography (10% EtOAc:Hexane); ^{1}H (300 MHz, $CDCl_3$) δ 9.76 (s, 1H), 3.59 (t, J = 6.3Hz, 2H), 2.41 (t, J = 1.5Hz, 2H), 1.65-1.60 (m, 2H), 1.52-1.47 (m, 2H), 1.31 (bs, 6H), 0.88 (s, 9H), 0.04 (s, 6H). ^{13}C NMR (75 MHz, CDCl3) δ 203.0, 63.4, 44.1, 34.16, 33.00, 29.5, 26.2, 24.88, 22.25, 18.55, -5.07; IR (neat) υ = 2930, 2856, 2710, 1708, 1463, 1411, 1253, 1907 cm^{-1}.

General Procedure for the monoprotection of a diol: A flame dried round bottom flask equipped with a condenser and a magnetic stir bar was charged with sodium hydride (14.1 mmol, 1.1 eq.), diol (13.4 mmol), and 61mL of THF. The reaction was then heated to 58 ^{o}C via an oil bath for 18hr and cooled to room temperature before the addition of tbutyl dimethylsilyl chloride (14.1mmol, 1.1eq). After stirring for 2h, the white solution was then diluted with ether (100 mL), washed with 50 mL 10% aq. K_2CO_3, 50 mL H_2O, and 50 mL saturated aq. NaCl. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification of the crude oil by flash chromatography (15% EtOAc:Hexane) gave the mono TBS protected alcohol.

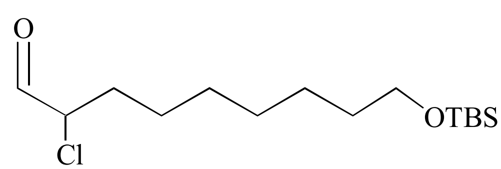


8-(*tert*-Butyl-dimethyl-silanyloxy)-octan-1-ol: ^{1}H (300 MHz, $CDCl_3$) δ 3.60 (m, 4H), 1.57 (m, 5H), 1.30 (s, 9H), 0.921-0.890 m, 9H), 0.05 (s, 6H); ^{13}C NMR (75 MHz, CDCl3) δ 63.46, 62.90, 60.59, 32.9, 26.9, 18.4, 14.3, -3.4, -5.13; IR (neat) υ = 3348, 2923, 2856, 1745, 1472, 1255, 1099 cm^{-1}.

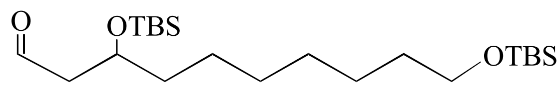


9-(tert-Butyl-dimethyl-silanyloxy)-nonan-1-ol 57: ^{1}H (300 MHz, $CDCl_3$) δ 3.60 (m, 4H), 1.57 (m, 5H), 1.30 (s, 11H), 0.921-0.890 (m, 9H), 0.05 (s, 6H); ^{13}C NMR (75 MHz, CDCl3) δ 63.46, 62.90, 60.59, 32.9, 26.9, 18.4, 14.3, -3.4, -5.13; IR (neat) υ = 3347, 2922, 2856, 1744, 1471, 1255, 1099 cm^{-1}.

General Procedure for Ozonolysis: In a 250 mL tube equipped with a stir bar, alkene (0.26 mmol) was dissolved in anhydrous CH_2Cl_2 (15 mL). The reaction mixture was cooled to –78 ^{o}C under a positive pressure of oxygen followed by introduction of ozone via a glass gas dispersion tube (15% O_3 output and 15 PSI pressure). As soon as the appearance of a blue color the introduction of ozone was immediately terminated and the solution was flushed with nitrogen at –78 ^{o}C for 15 minutes. The gas dispersion tube was removed and replaced with a septum and dimethyl sulfide (2.6 mmol) and Et_3N (1.3 mmol) was added dropwise. The reaction mixture was warmed to room temperate and stirred overnight before removing the volatile solvents under reduced pressure. The thick residue was transferred into a separatory funnel and diluted with ether (150 mL) and washed with water (3 x 20 mL). The aqueous phases were combined and extracted with ether (3 x 25 mL). The combined organic extracts were washed with sat. aq. sodium chloride (30 mL), dried over magnesium sulfate, filtered and the solvents removed under reduced pressure. Flash chromatography gave the desired aldehyde.

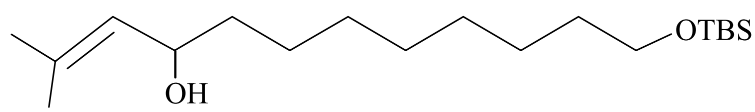


9-(*tert*-Butyl-dimethyl-silanyloxy)-2-chloro-nonanal 77 (62%). Flash Chromatography (20% EtOAc: Hexane); ^{1}H (300 MHz, $CDCl_3$) δ δ 9.48 (s, 1H), 4.17-4.12 (m, 1H), 3.59 (t, J = 6.6Hz, 2H), 2.01-1.91 (m, 1H), 1.86-1.75 (m, 1H), 1.55-1.31 (complex, 9H), 0.88 (s, 9H), 0.01 (s, 6H); IR (neat) υ = 2929, 2855, 1735, 1462, 1254, 1097, 834, 775 cm^{-1}.



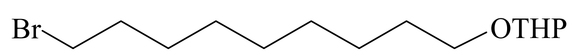
3,10-Bis-(*tert*-Butyl-dimethyl-silanyloxy)-decanal 24 (83%) : Flash Chromatography (15% EtOAc: Hexane); ^{1}H (300 MHz, $CDCl_3$) δ δ 9.76 (s, 1H), 4.35 (quartet, J = 6.6 Hz, 1H), 3.57 (t, J = 6.6 Hz, 2H), 2.50 (dd, J = 11.2, 6.5 Hz, 2H), 1.56-1.43 (m, 4H), 0.12 (bs, 8H), 0.89 (s, 9H), 0.89 (s, 9H), 0.03 (s, 6H), 0.03 (s, 6H); IR (neat) υ = 2930, 2857, 1727, 1471, 1255, 1099, 836, 775 cm^{-1}.

*Syntheses of Inhibitors **4a** and **4b***

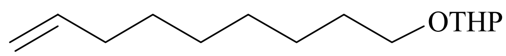


12-(tert-butyl-dimethyl-silanyloxy)-2-methyl-dodec-2-en-4-ol 12 (71%). To a round bottom flask flame dried and flushed with argon was added 2-methyl-1-propenylmagnesium bromide (0.92 mmol, 0.5M in THF) and the solution was cooled to 0 ^{o}C via an ice bath. After stirring for 20 min, a solution of aldehyde **11** (0.38 mmol) in THF (0.5 mL) was slowly added via cannula. The yellow solution was warmed to room temperature and stirred for 2 h before being quenched by the addition of 5 mL sat. aq. NH_4Cl. The resultant biphasic solution was then diluted with 25 mL ether and separated. The aqueous layer was extracted with Et_2O (2 x 25 mL). The combined organic layers were washed with sat. NH_4Cl (50 mL), then brine (50 mL) and dried over $MgSO_4$. Filtration and removal of solvent under reduced pressure provided the crude allylic alcohol as a yellow oil. Purification via flash chromatography (20%EtAOc:Hexane) gave the allylic alcohol as a transparent oil,. ^{1}H (300 MHz, $CDCl_3$) δ 5.158 (d, J = 8.7 Hz, 1H), 4.342-4.324 (m, 1H), 3.592 (t, J = 6.6 Hz, 2H), 1.728 (s, 3H), 1.683 (s, 3H), 1.524-1.264 (m, 14H), 0.882 (s, 9H), 0.044 (s, 6H); ^{13}C (75 MHz, $CDCl_3$) δ 134.8, 128.5, 68.8, 62.9, 37.8, 32.8, 29.7, 29.5, 25.8, 25.5, 20.7, 18.3, 15.3, 14.3, -6.5; IR (neat) υ = 3330, 2927, 2854, 1446, 1375, 1301, 1057, 1005, 845, 723 cm^{-1}; Anal. Calcd. For$C_{19}H_{40}O_2Si$: C, 69.45; H, 12.27; Found: C, 69.35; H, 12.34.

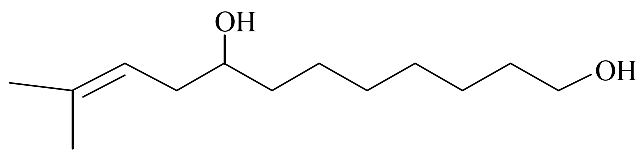
Syntheses of Analogs ***5a*** *and* ***5b***



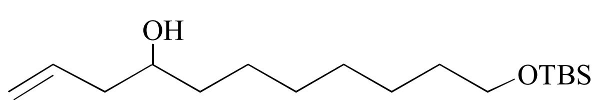
2-(9-Bromo-nonyloxy)-tetrahydro-pyran 35 (80%). To an ice cooled solution of 9-bromo-1-nonanol (4.26 mmol) and freshly distilled dihydropyran (10.64 mmol) in 21.3 mL of dry methylene chloride was added *p*-toluenesulfonic acid monohydrate (8.52 mmol). The reaction was stirred at 0 ^{o}C for 10 minutes and then allowed to warm to room temperature. After 18 h, the reaction mixture was diluted with 100 mL ether and washed with 50 mL sat. aq. $NaHCO_3$, 50 mL water, and brine (2 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated to a dark oil. Purification via flash chromatography (10% Et_2O:Hexane) yielded the OTHP ether as a clear oil. ^{1}H (300 MHz, $CDCl_3$) δ 4.58-4.56 (m, 1H), 3.39-3.84 (m, 1H), 3.72 (q, J = 9.6Hz, 1H), 3.52-3.47 (m, 3H), 1.88-1.80 (m, 2H), 1.74-1.68 (m, 1H), 1.59-1.53 (comp, 7H), 1.42-1.30 (m, 11H); ^{13}C (75 MHz, $CDCl_3$) δ 99.0, 77.3, 67.7, 62.5, 34.1, 32.9, 30.9, 29.8, 29.4, 28.8, 28.2, 26.3, 25.6, 19.9; IR (neat) υ = 2930, 2854, 1464, 1439, 1259, 1200, 1119, 1077, 1033, 981, 905, 868, 814, 729 cm^{-1}; Anal. Calcd. for $C_{14}H_{27}BrO_2$: C; 54.72; H, 8.86; Found: C, 54.87; H, 8.91.



2-Non-8-enyloxy-tetrahydro-pyran 36 (94%) : To a solution of bromine **33** (3.28 mmol) in 16.4 mL dry THF at room temperature was added a 1N solution of potassium *t*-butoxide in DMSO (6.56 mmol). The reaction immediately became a cloudy white. The slurry was stirred for 2 h before adding 25 mL ice-cold water. The aqueous layer was extracted with ether (3x 50 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated to a yellow oil. Flash Chromatography (5% Et_2O:Hexane) provided the alkene as a clear oil. ^1H (300 MHz, $CDCl_3$) δ 4.58-4.56 (m, 1H), 3.39-3.84 (m, 1H), 3.72 (q, J = 9.6Hz, 1H), 3.52-3.47 (m, 3H), 1.88-1.80 (m, 2H), 1.74-1.68 (m, 1H), 1.59-1.53 (comp, 7H), 1.42-1.30 (comp, 11H); ^{13}C (75 MHz, $CDCl_3$) δ 99.0, 77.3, 67.7, 62.5, 34.1, 32.9, 30.9, 29.8, 29.4, 28.8, 28.2, 26.3, 25.6, 19.9; IR (neat) υ = 2930, 2854, 1464, 1439, 1259, 1200, 1119, 1077, 1033, 981, 905, 868, 814, 729 cm^{-1}; Anal. Calcd. for $C_{14}H_{26}O_2$: C, 74.29; H, 11.58; Found: C, 74.05; H, 11.72.

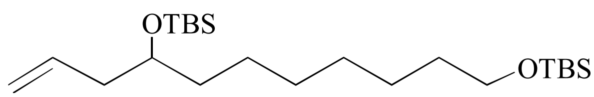


11-Methyl-dodec-10-ene-1,8-diol 39 (75%). To a solution of homoallylic alcohol **38** (0.24 mmol) in 1.2 mL MeOH at 0 ^{o}C was added *p*-toluenesulfonic acid (0.36 mmol). After stirring at 0 ^{o}C for 10 min, the reaction mixture was warmed to room temperature with progress monitored via TLC for absence of starting material. A slight red tint developed after 2h at room temperature and once all of the starting material was consumed the reaction was diluted with 25 mL ether. Afterward, the organic layer was then washed with saturated aq. sodium bicarbonate (15 mL), water (15 mL) and finally, brine (25 mL). The organic layer was dried over magnesium sulfate, filtered, and solvent removed under reduced pressure. The crude oil was then purified by column chromatography (37% EtOAc: Hexane) to a clear oil. ^{1}H (300 MHz, $CDCl_3$) δ 5.14 (t, J = 10.4Hz, 1H), 3.63 -3.54 (m, 3H), 2.13 (t, J = 9.6Hz, 2H), 1.72-1.30 (comp, 21H); ^{13}C (75 MHz, $CDCl_3$) δ 135.3, 120.4, 71.9, 63.1, 36.9, 36.4, 32.9, 29.8, 29.5, 26.1, 25.8, 18.2; IR (neat) υ = 3342, 2929, 2855, 1451, 1376, 1112, 1056, 872, 835, 776, 723 cm^{-1}; Anal. Calcd. for $C_{13}H_{26}O_2$: C, 72.60; H, 12.23: Found; C, 72.32; H, 12.36.

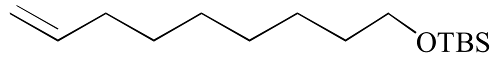


11-(*tert*-Butyl-dimethyl-silanyloxy)-undec-1-en-4-ol 18 (87%) : To a round bottom flask containing a solution of allyl magnesium chloride (6.98 mmol, 2.0 M in THF) in

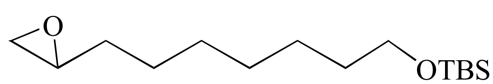
THF (14.1 mL) cooled to -78^{o}C via dry ice/ acetone was added aldehyde **21** (2.32 mmol) via canula. After 2 h the mixture was allowed to warm to room temperature and stir for 30 min before quenching by the addition of 10 mL sat. aq. NH_4Cl. The aqueous layer was extracted with ether (3 x 10 mL). The combined organic layers were washed with 15 mL sat. aq. NH_4Cl and 15 mL brine. The organic phase was dried over $MgSO_4$, filtered and concentrated to an oil. Flash Chromatography (15% EtOAc : Hexane) gave a pure transparent oil. ^{1}H (300 MHz, $CDCl_3$) δ 5.84-5.73 (m, 1H), 5.32 (bd, J = 12.6 Hz, 2H), 3.65-3.57 (complex, 3H), 2.31-2.26 (m, 1H), 2.21-2.09 (m, 1H), 1.54-1.25 (complex, 10H), 0.89 (s, 9H), 0.04 (s, 6H).



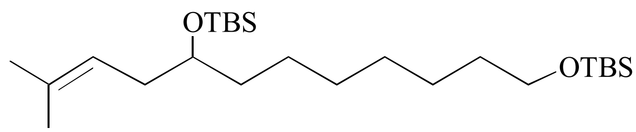
4,11-Bis-(*tert*-Butyl-dimethyl-silanyloxy)-undec-1-ene 19 (99%). A 50 mL round bottom flask flushed with Ar was charged with alcohol **22** (2.0 mmol), triethyl amine (5.0 mmol) and dichloromethane (6.7 mL). After stirring the reaction mixture for 5 min., *t*-butyldimethylsilyl triflate (5.0 mmol) was added dropwise via syringe and stirring was continued overnight. The yellow mixture was diluted with 30 mL ether and washed with chilled sat. aq. $NaHCO_3$ (2 x 15 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. Purification via flash chromatography (10 % CH_2Cl_2 : Hexane) gave a disilyl ether as a yellow oil. ^{1}H (300 MHz, $CDCl_3$) δ δ 5.84-5.73 (m, 1H), 5.00 (d, J = 7.5 Hz, 1H), 4.98 (s, 1H), 3.65 (quin, J = 6.6 Hz, 1H), 3.59 (t, J = 6.6 Hz, 2H), 2.19 (bt, J = 6.8 Hz, 2H), 1.56-1.25 (complex, 12H), 0.89 (s, 18H), 0.02 (s, 12H).



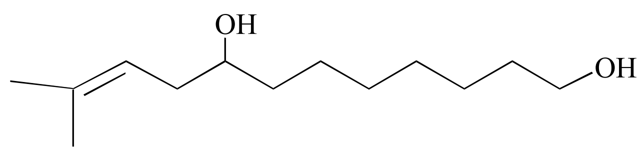
***tert*-Butyl-dimethyl-non-8-enyloxy-silane 33 (60%) :** To a flame dried 50 mL round bottom flask equipped with a stir bar and flushed with Ar was added allyl bromide **17** (1.48 mmol) and 7.4 mL THF. A separate 10 mL round bottom flask was charged with potassium *tert* butoxide (2.96 mmol) in 2.96 mL DMSO and stirred for 15 minutes prior to addition of the solution into the reaction flask. After the addition the mixture was stirred at room temperature overnight, and then quenched by the addition of 10 mL chilled water. The aqueous layer was extracted with CH_2Cl_2 (3 x 25 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Flash Chromatography (20% CH_2Cl_2:Hexane) gave and oil. ^1H (300 MHz, $CDCl_3$) δ δ 5.84-5.78 (m, 1H), 4.98 (dd, J = 1.5, 16.8 Hz, 1H), 4.93 (dd, J = 1.5, 10.2Hz, 1H), 3.59 (t, J = 6.6Hz, 2H), 2.03 (bq, J = 7.2, 2H), 1.54-1.29 (complex, 8H), 0.89 (s, 9H), 0.04 (s, 6H).



***tert*-Butyl-dimethyl-(7-oxiranyl-heptyloxy)-silane 34 (84%).** To an ice cooled solution of alkene **18** (0.44 mmol) in 4.8 mL dry dichloromethane was slowly added *m*CPBA (0.68 mmol). The ice bath was removed and the mixture allowed to warm to room temperature with hourly monitoring via TLC for disappearance of starting material. Normally after 2.0 h , the reaction mixture was diluted with 100 mL Et_2O, washed with sat. aq. sodium bicarbonate (3 x 50 mL), followed by 50 mL sat. aq. sodium chloride. The organic layer was dried over $MgSO_4$, filtered and concentrated. The resultant crude oil was purified by flash chromatography (20% EtOAc:Hexane) providing the epoxide. ^{1}H (300 MHz, $CDCl_3$) δ 3.59 (t, J = 6.6Hz, 2H), 2.93-2.86 (m, 1H), 2.74 (t, J = 3.6Hz, 1H), 2.47-2.44 (m, 1H), 1.53-1.31 (complex, 12H), 0.88 (s, 9H), 0.04 (s, 6H).

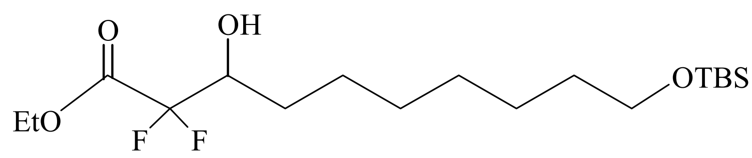


5,12-Bis-(*tert*-Butyl-dimethyl-silanyloxy)-2-methyl-dodec-2-ene 21 (74%) : To a stirred suspension of isopropyltriphenylphosphonium iodide (0.47mmol) in 1.6 mL of THF at –78 ^{o}C via dry ice/acetone was added *n*BuLi (0.47 mmol, 2.5 M in Hexane). After 1 h, a solution of aldehyde **24** (0.16 mmol) in THF was added dropwise via canula to the red/orange suspension. Once the addition was complete the reaction was warmed to room temperature overnight. The once cherry red solution was now yellow. 10 mL water was then added, and extracted with Et_2O (3 x 20 mL). The combined organic layers were dried over $MgSO_4$, concentrated and the crude product purified via flash chromatography (15% CH_2Cl_2:Hexane). ^{1}H (300 MHz, $CDCl_3$) δ 5.16 (t, J = 8.1 Hz, 1H), 3.66-3.56 (m, 3H), 2.17-2.12 (m, 2H), 1.73 (s, 3H), 1.64 (s, 3H), 1.58-1.21 (complex, 12H) 0.89 (s, 9H), 0.89 (s, 9H), 0.03 (s, 6H), 0.03 (s, 6H); IR (neat) υ = 3343, 2928, 2856, 1452, 1376, 1052. 733 cm^{-1}.

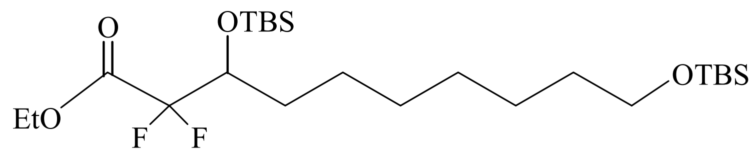


11-Methyl-dodec-10-ene-1,8-diol 22 (95%). Tetrabutylammonium fluoride (3.6 mmol, 1 M in THF) was added dropwise to a cooled (0 ^{o}C) solution of silyl ether **26** (0.71 mmol) in 3.6 mL of THF. Once the addition was complete the reaction mixture was warmed to room temperature and stirred for 12 h before quenching by the addition of 5 mL saturated aq. ammonium chloride. The aqueous layer was than extracted with ether (3 x 10 mL). The organic phases were combined and washed with 15 mL sat. aq. NH_4Cl and 15 mL brine. The organic phase was dried over $MgSO_4$ and concentrated. The crude oil was purified via flash chromatography (35% EtOAc : Hexane) to give an oil. ^{1}H (300 MHz, $CDCl_3$) δ 5.14 (t, J = 10.4Hz, 1H), 3.63 -3.54 (m, 3H), 2.13 (t, J = 9.6Hz, 2H), 1.72-1.30 (comp, 21H); ^{13}C (75 MHz, $CDCl_3$) δ 135.3, 120.4, 71.9, 63.1, 36.9, 36.4, 32.9, 29.8, 29.5, 26.1, 25.8, 18.2; IR (neat) υ = 3342, 2929, 2855, 1451, 1376, 1112, 1056, 872, 835, 776, 723 cm^{-1}.

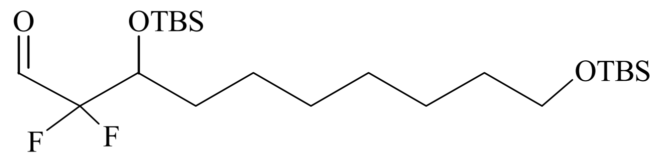
*Syntheses of Inhibitors **6a** and **6b***



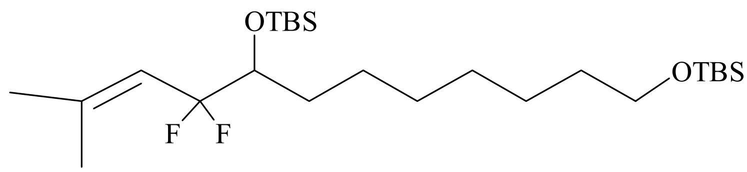
Ethyl-10-(tert-butyl-dimethyl-silanyloxy)-2,2-difluor-3-hydroxy-decanoate 43 (83%). Zinc dust [(182.65 mg, 2.79mmol) activated by stirring in 10% HCl for 2 h, washed with DI water, acetone, ether and dried under vacuum] was suspended in 11.6 mL THF and heated to reflux. Ethyl bromodifluoroacetate (2.79 mmol) was then added to the refluxing suspension (neat) via syringe, immediately generating bubbles. A solution of aldehyde **21** (2.32 mmol) in 2.0 mL THF was canulated into the refluxing suspension and the mixture allowed to stir for 15 minutes before cooling to room temperature. The reaction mixture was then transferred to a solution of EtOAc (100 mL), 1M aq. sodium hydrogen sulfate (25 mL) and saturated aq. sodium bicarbonate (25 mL) and stirred for 15 minutes. The layers were separated, and the aqueous layer extracted with EtOAC (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, concentrated to an oil and purified by flash chromatography (10%EtOAc:Hexane). ^1H (300 MHz, $CDCl_3$) δ 4.36 (q, J = 7.5 Hz, 2H), 4.08-3.94 (m, 1H), 3.59 (t, J = 6.6Hz, 2H), 1.93 (bd, J = 7.7Hz, 1H), 1.58-1.25 (complex, 12H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C (75 MHz, $CDCl_3$) δ 164.0 (t), 115.0, 71.8 (t), 63.4 (d), 33.0, 29.4, 26.1, 25.9, 25.4, 18.5, 14.1, -5.1; ^{19}F (282 MHz, $CDCl_3$) δ -115.3 (dd, J = 7.6, 262.5 Hz, 1F), -122.8 (dd, J = 15.2, 262.5, 1F); IR (neat) υ = 3425, 2933, 2858, 1764, 1465, 1380, 1099, 837, 777 cm^{-1}; Anal. Calcd. for $C_{18}H_{36}F_2O_4Si$: C, 56.51; H, 9.49; Found: C, 56.29; H, 9.55.



Ethyl-3,10-Bis-(*tert*-butyl-dimethyl-silanyloxy)-2,2-difluor-3-hydroxy-decanoate 44 (100 %) : To a cooled (0 ^{o}C) solution of alcohol **43** (2.0 mmol) in 6.7 mL of CH_2Cl_2 and triethylamine (5.0 mmol) was added *tert*-butyldimethylsilyl trifluoromethanesulfonate (5.0 mmol) and the mixture was then allowed to warm to room temperature. After stirring overnight, the reaction mixture was diluted with 100 mL of Et_2O, and washed with chilled aq. $NaHCO_3$ (2 x 50 mL), and saturated aq. NaCl (50 mL). The organic layer was dried over magnesium sulfate, filtered and the solvent removed to give the crude disilylether. The product was purified by column chromatography (10% CH_2Cl_2: Hexane) to a transparent oil. ^{1}H (300 MHz, $CDCl_3$) δ 4.36 (q, J = 7.5 Hz, 2H), 4.08-3.94 (m, 1H), 3.59 (t, J = 6.6Hz, 2H), 1.93 (bd, J = 7.7Hz, 1H), 1.58-1.25 (complex, 12H), 0.88 (s, 18H), 0.04 (s, 12 H); ^{13}C (75 MHz, $CDCl_3$) δ 163.9 (t), 115.5 (t), 73.2 (t), 63.5, 33.0, 31.4, 29.8, 29.5, 26.2, 25.9, 25.5, 18.6, 14.2, -4.3, -5.1; ^{19}F (282 MHz, $CDCl_3$) δ -113.4 (dd, J = 11.0, 255.2 Hz, 1F), -116.3 (dd, J = 9.0, 255.2, 1F); IR (neat) υ = 2934, 2858, 1767, 1466, 1254, 1108, 838, 777 cm^{-1}; Anal. Calcd. for $C_{24}H_{50}F_2O_4Si_2$: C, 58.02; H, 10.14; Found: C, 58.42; H, 10.29.

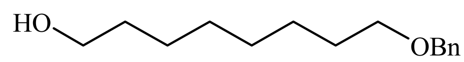


3,10-Bis-(*tert*-butyl-dimethyl-silanyloxy)-2,2-difluoro-decanal 45 (73%) : To a flame dried 25 mL round bottom flask was added the ester **44** (1.41 mmol) and 7.04 mL freshly distilled toluene. The solution was cooled to –78 ^{o}C via dry ice/acetone and a 1M solution of DIBAL (1.48 mmol) in toluene, in a separate flask cooled to –78 ^{o}C, was slowly canulated into the solution. After 1.5 h, TLC showed no presence of starting material, and the reaction mixture was quenched at –78 ^{o}C by the addition of 15 mL of sat. aq. sodium potassium tartrate. The ice bath was removed and the suspension was allowed to warm to room temperature. After stirring for 40 minutes the phases were separated. The aqueous phase was extracted with Et_2O (3 x 50 mL). The combined organic layers were washed with 50 mL water and 50 mL sat. aq. NaCl. The organic layer was dried over $MgSO_4$ and concentrated via under reduced pressure. Purification via flash chromatography (15% EtOAC: Hexane) gave the aldehyde as a clear oil. ^{1}H (300 MHz, $CDCl_3$) δ 9.60 (dd, J = 7.8, 15.6 Hz, 1H), 4.13-3.93 (m, 1H), 3.59 (t, J = 6.6Hz, 2H), 1.52- 1.22 (complex, 12H), 0.88 (s, 18H), 0.04 (s, 12H); ^{13}C (75 MHz, $CDCl_3$) δ 200.6, 142.2, 72.5, 65.8, 34.2, 30.6, 30.3, 26.3, 24.8, 23.8, 20.7, 15.0, 14.7, -6.5; ^{19}F (282 MHz, $CDCl_3$) δ –116.8-127.8 (2F); IR ; Anal. Calcd. for $C_{22}H_{46}F_2O_3Si$: C, 58.36; H, 10.24

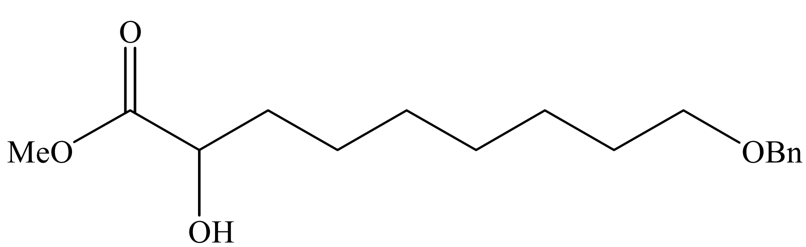


5,12-Bis-(*tert*-butyl-dimethyl-silanyloxy)-4,4-difluoro-2-methyl-dodec-2-ene 46 (70%). To a stirred suspension of isopropyltriphenylphosphonium iodide (0.47 mmol) in 1.6 mL of THF was added *n*BuLi (0.47 mmol, 2.5 M in hexane). After 1 h, a solution of aldehyde **45** (0.16 mmol) in 1 mL THF was added dropwise via a canula and the mixture was allowed to warm to room temperature generating a red/orange suspension. After stirring for 18 h, the once cherry red solution was now yellow. Water (10 mL) was then added, and the aqueous layer extracted with Et_2O (3 x 50 mL). The combined organic phases were dried over anhydrous $MgSO_4$. Filtration and the removal of solvent under reduced pressure gave the crude alkene that was purified by flash chromatography (15% CH_2Cl_2:Hexane). ^{1}H (300 MHz, $CDCl_3$) δ 5.38 (tt, J = 1.5, 15.6 Hz, 1H), 3.77, 3.68 (m, 1H), 3.59 (t, J = 6.6Hz, 2H), 1.82 (d, J = 12.6 Hz, 6H), 1.53-1.25 (complex, 12 H), 0.88 (s, 18H), 0.52 (s); ^{13}C (75 MHz, $CDCl_3$) δ 133.2, 125.8 (t), 116.8 (t), 80.6, 65.8, 34.2, 30.6, 30.3, 26.3, 25.4, 25.3, 20.7, 19.3, 15.0, 14.7, -4.3, -5.1; ^{19}F (282 MHz, $CDCl_3$) δ –95.8 (d, J = 255.2, 1F), -101.5 (dd, J = 19.2, 258.3 Hz, 1F); IR (neat) υ = 3346, 2930, 2857, 1675, 1472, 1255, 1104, 836, 776 cm^{-1}; Anal. Calcd. for $C_{25}H_{52}F_2O_2Si_2$: C, 62.71; H, 10.95; Found: C, 63.11; H, 11.04.

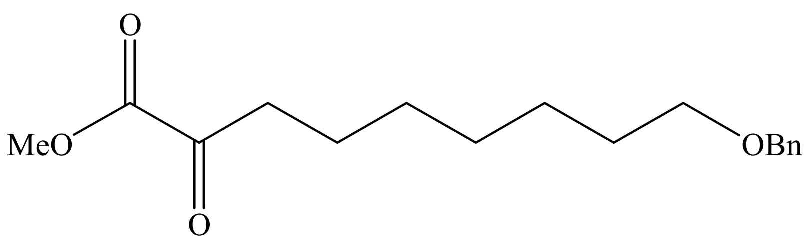
*Syntheses of Analogs **7a** and **7b***



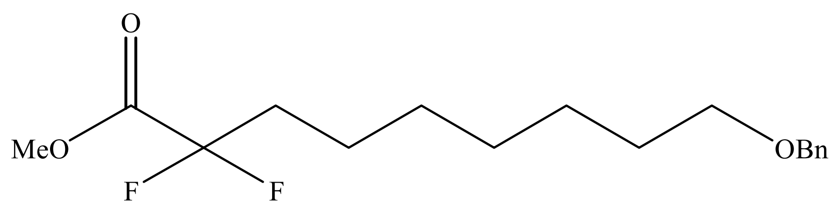
8-Benzyloxy-octan-1-ol 60 (93%). To a suspension of 1,8-octanediol (0.0615 mol) in 100 mL xylene was slowly added thinly sliced pieces of sodium metal (0.031 mol). The mixture was heated via an oil bath to 110 °C neat benzylchloride (0.032 mol) was then added, and the reaction was refluxed for 20 h. After cooling to room temperature, the suspension was carefully quenched by the addition of 50 mL cold water and extracted with 100 mL ether. The organic layer was dried over anhydrous magnesium sulfate, filtered and the solvent removed under reduced pressure. Vacuum distillation ((188-192 °C/ca.10mmHg) provided the pure compound**.** ^{1}H (300 MHz, $CDCl_3$) δ 1.33 (m, 8H), 1.57-1.62 (m, 4H), 3.44-3.49 (t, J = 6Hz, 2H), 3.63-3.65 (t, J = 6Hz 2H), 4.50 (s,2H), 7.33-7.3 (m,5H); ^{13}C (75 MHz, $CDCl_3$) δ 138.5, 128.3, 127.6, 127.4, 72.8, 70.4, 62.4, 32.6, 29.6, 29.4, 26.0, 25.7; IR (neat) υ = 3423, 2921, 2857, 1076 cm^{-1}.



Methyl-9-Benzyloxy-2-hydroxy-nonanoate 62 (91%). HCl gas was bubbled into a cooled solution of nitrile **61** (11.5 mmol) in 50 mL anhydrous methanol for 2 h. After stirring for 24 h at room temperature the solvent was removed in vacuo and the residue taken up in 100 mL ether. The ether was washed with 50 mL brine and dried over magnesium sulfate. Filtration and concentration left a crude oil, which was purified by flash chromatography (15% EtOAc:Hexane) to provide the α-hydroxy ester. ^{1}H (300 MHz, $CDCl_3$) δ 1.31-1.44 (m, 8H), 1.54-1.1.81 (m, 4H), 2.62-2.67 (d, J = 15Hz, 1H), 3.43-3.48 (t, J = 7.2 Hz, 2H), 3.79 (s, 3H), 4.16-4.20 (m, 1H), 4.50 (s, 2H), 7.33-7.35 (m, 5H). ^{13}C (75 MHz, $CDCl_3$) δ 175.9, 138.8, 128.4, 127.7, 127.6, 73.0, 70.6, 70.5, 52.5, 34.5, 29.8, 29.4, 29.3, 26.2, 24.8; IR (neat) υ = 3449, 2931, 2857, 1739 cm^{-1}; Anal. Calcd. for $C_{17}H_{26}O_4$: C, 69.36; H, 8.90; Found: C, 69.35; H, 8.98.



Methyl-9-Benzyloxyl-2-oxo-methylnonanoate 63 (85%). DMSO (1.02 mmol) was slowly added dropwise to a stirred solution of oxalyl chloride (0.51 mmol) in 2mL dichloromethane at -78 °C and stirred for 15 minutes. The reaction mixture was then canulated to a separate 25 mL round bottom flask containing a solution of α-keto ester (0.34 mmol) in 3 mL dichloromethane at -78 °C. After 2 h at –78°C, 0.5 mL triethylamine was added and the mixture allowed to slowly warm to room temperature. The mixture was then diluted with 100 mL ether and washed with 50 mL water (destroyed later by bleach). The organic layer was dried over $MgSO_4$, filtered and the solvent was removed in vacuo. Flash chromatography (10% EtOAc:Hexane) gave 0.085g of the pure oil. ^{1}H (300 MHz, $CDCl_3$) δ 1.26-1.38 (m, 6H), 1.52-1.65 (m, 4H), 3.79-2.81 (t, J = 6Hz, 2H), 3.43-3.48 (t, J = 7.2Hz, 2H), 3.79 (s, 3H), 4.50 (s, 2H), 7.33-7.35 (m, 5H). ^{13}C (75 MHz, $CDCl_3$) δ 194.5, 161.8, 138.9, 128.5, 127.8, 127.7, 73.1, 70.6, 53.1, 39.5, 29.9, 29.3, 29.1, 26.2, 23.1. IR (neat) υ = 2932, 2846, 1728(ketone) cm^{-1}; Anal. Calcd. for $C_{17}H_{24}O_4$: C, 69.84; H, 8.27; Found: C, 65.24; H, 8.26.



Methyl-9-Benzyloxy-2,2-difluoro-nonanoate 64 (57%). To a solution of alpha keto ester **63** (3.15 mmol) in 25 mL dichloromethane at 0°C was added (Diethylamino)sulfur trifluoride (3.47 mmol). The reaction mixture was then allowed to warm to room temperature. After 30 h, 10 mL water and 5 mL saturated aq. sodium bicarbonate were added to the reaction flask. The aqueous layer was then extracted with 100 mL ether and the layers separated. The ether layer was washed with 50 mL sat. aq. NaCl and dried over magnesium sulfate. After filtering and concentrating the organic layer, the solvent was removed under reduced pressure. The crude oil was purified by column chromatography (2% EtOAc:Hexane) to give 0.56 g of the difluoro ester. ^{1}H (282 MHz, $CDCl_3$) δ 1.34-1.46 (m, 8H), 1.50-1.65 (m, 2H), 1.92-2.18 (m, 2H), 3.43-3.49 (t, J = 7.2Hz, 2H), 3.79 (s, 3H), 4.50 (s, 2H), 7.33-7.35 (m, 5H). ^{13}C (75 MHz, $CDCl_3$) δ 165.5, 165.1, 164.7, 138.8, 128.5, 127.8, 127.7, 119.9, 116.6, 113.3, 73.1, 70.5, 53.4, 35.0, 34.7, 34.4, 29.9, 29.2, 26.7, 26.2, 21.6. ^{19}F (282 MHz, $CDCl_3$) δ 106.1. IR (neat) υ = 2931, 2856, 1774 cm^{-1}; Anal. Calcd. for $C_{17}H_{24}F_2O_3$: C, 64.95; H, 7.69; Found: C, 65.04; H, 7.89.

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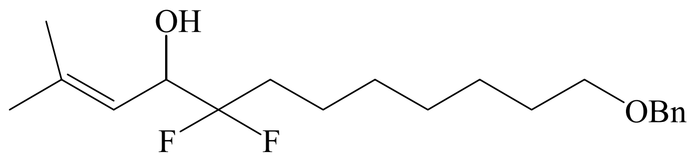
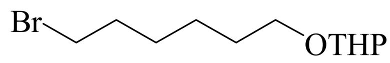
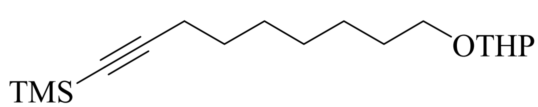


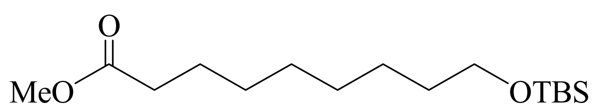
8,8-Difluoro-11-methyl-dodec-10-ene-1,9-diol 67 (89%). A solution of **66** (0.103 mmol) in 3ml THF was added drop wise to a pre-stirred (2 h) suspension of sodium metal (1.03 mmol) in 3ml liquid ammonia[12] and 2ml THF at -78 °C. The cooling bath was removed and the mixture allowed to stir at room temperature for 0.5 h before being quenched by the addition of 10 mL sat. aq. NH_4Cl. After evaporation of the ammonia, the solution was extracted with ether (3 × 20 mL), and the combined organic layers were washed with 50 mL brine, dried over magnesium sulfate, filtered, and concentrated to an oil. Column chromatography (30% EtOAc:Hexane) gave 23 mg of diol. ^1H (300 MHz, $CDCl_3$) δ 1.45-1.59 (m, 2H), 1.74 (s, 3H), 1.80 (s, 3H), 1.74-1.94 (m, 2H), 3.61-3.66 (t, J = 7.2Hz, 2H), 4.45 (m, 1H), 5.24-5.26 (d, J = 6Hz, 1H); ^{13}C (300 MHz, $CDCl_3$) δ 18.7, 21.4, 21.4, 25.7, 26.2, 29.3, 29.6, 32.5, 32.8, 63.1, 70.2, 70.6, 120.0, 124.3, 140.8; ^{19}F (282 MHz, $CDCl_3$) δ -112.2; IR (neat) υ = 3366, 2931, 2860, 1449(allyl) cm^{-1}; Anal. Calcd. for $C_{13}H_{24}F_2O_2$: C, 62.37; H, 9.66; Found: C, 63.48; H, 9.76.



2-(6-Bromo-hexyloxy)-tetrahydro-pyran 52 (68%). To an ice cooled solution of 6-bromo-1-hexanol (7.64 mmol) and freshly distilled dihydropyran (22.93 mmol) in 50 mL of dry methylene chloride was added *p*-toluenesulfonic acid monohydrate (0.764 mmol). The reaction was stirred at 0 ^{o}C for 10 minutes before warming to room temperature. After 20 h, the reaction mixture was diluted with 100 mL ether and washed with 50 mL sat. aq. $NaHCO_3$, 50 mL water, and brine (2 x 50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated to a dark oil. Purification via flash chromatography (10% Et2O: Hexane) gave a clear oil; ^{1}H (300 MHz, $CDCl_3$) δ 4.58-4.56 (m, 1H), 3.39-3.84 (m, 1H), 3.72 (q, J = 9.6Hz, 1H), 3.52-3.47 (m, 3H), 1.88-1.80 (m, 2H), 1.74-1.68 (m, 1H), 1.59-1.53 (comp, 3H), 1.42-1.30 (comp, 3H); IR (neat) υ = 2930, 2854, 1464, 1439, 1259, 1200, 1119, 1077, 1033, 981, 905, 868, 814, 729 cm^{-1}.

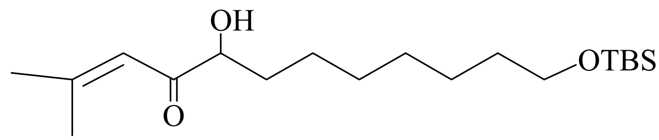


Trimethyl-[9-(tetrahydro-pyran-2-yloxy)-non-1-ynyl]-silane 53 (68%). *n*-BuLi (5.27 mmol, 1.9 M in hexane) was added to a solution of trimethylsilyl acetylene (5.24 mmol) in 8.43 mL of THF at –78 ^{o}C. After stirring for 2 h, 2.11 mL HMPA was added to the solution and the mixture stirred for another 5 minutes prior to the addition of **52** (5.312 mmol) via canula. The yellow solution was then allowed to warm to room temperature and monitored by TLC for starting material consumption. The reaction was then quenched by the addition of 20 mL sat. aq. ammonium chloride and extracted with ether (2 x 50 mL). The organic layers were combined and washed with 15 mL water and brine (15 mL) dried over MgSO4, filtered, and concentrated to provide a crude oil. The oil was then purified by chromatography (5% EtOAc:Hexane). ^{1}H (300 MHz, $CDCl_3$) δ 4.58-4.56 (m, 1H), 3.39-3.84 (m, 1H), 3.72 (q, J = 9.6Hz, 1H), 3.52-3.47 (m, 3H), 1.95 (t, J = 2.7Hz, 2H), 1.77-1.66 (m, 2H), 1.63-1.55 (m, 2H), 1.58-1.37 (m, 10H), 0.08 (s, 6H).

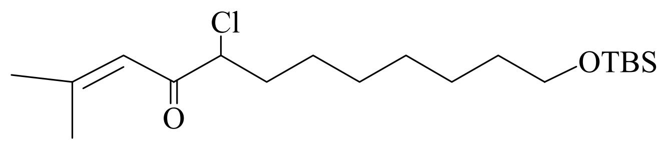


Methyl 9-(*tert*-Butyl-dimethyl-silanyloxy)-nonanoate 59 (92%). To a 25 mL round bottom flask equipped with a magnetic stir bar, reflux condenser, and heating mantle was added **58** (1.064 mmol), 2.5 mL acetone and K_2CO_3 (1.170 mmol). Dimethyl sulfate was then added and the mixture refluxed for 2.5 h. The solution was filtered through a celite pad and washed with acetone. The solvent was removed and 293.8 mg of the ester was obtained by Kuegelror distillation (100-110^{o}C). ^{1}H (300 MHz, $CDCl_3$) δ 3.66 (s, 3H), 3.59 (t, J = 6.6 Hz, 2H), 2.30 (t, J = 7.2 Hz, 2H), 1.65-1.56 (m, 2H), 1.52-1.47 (m, 2H), 1.30 (bs, 8H), 0.89 (s, 9H), 0.04 (s, 6H).

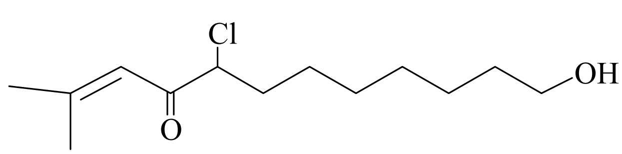
*Syntheses of Inhibitor **8***



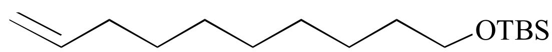
12-(*tert*-Butyl-dimethyl-silanyloxy)-5-hydroxy-2-methyl-dodec-2-en-4-one 80 (63%). To a solution of 2-methyl-1-propenyl magnesium bromide (0.84 mmol, 1.0 M in THF) in 1.68 mL THF at $0^{o}C$ was added by canulation a solution of nitrile **81** (0.35 mmol) in 1.0 mL THF. The reaction mixture immediately turned yellow. After 0.5 h, a mixture of 5% HCl and EtOAc (approximately 1mL) was added and the reaction mixture stirred for 1 hour at $0^{o}C$. The aqueous layer was then extracted with Et_2O (3 x 50 mL). The combined organic layers were then dried over $MgSO_4$, filtered and concentrated to a yellow oil. Purification via flash chromatography (8% EtOAc:Hexane) gave the α-hydroxy-ketone; ^{1}H (300 MHz, $CDCl_3$) δ 6.08 (s, 1H), 4.17-4.12 (m, 1H), 3.58 (t, J = 8.4Hz, 2H), 2.22 (s, 3H), 1.96 (s, 3H), 1.83-1.21 (comp, 13H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C (75 MHz, $CDCl_3$) δ 194.2, 146.8, 122.8, 86.7, 65.8, 34.3, 32.1, 30.6, 30.4, 26.2, 24.4, 23.8, 20.7, 18.8, -6.4; IR (neat) υ = 3461, 2928, 2867, 1682, 1622, 1462, 1386, 1252, 1098, 1034, 937, 838, 776, 661 cm^{-1}; Anal. Calcd. for $C_{19}H_{39}O_3Si$: C, 66.61; H, 11.18; Found: C, 66.86; H, 11.24.



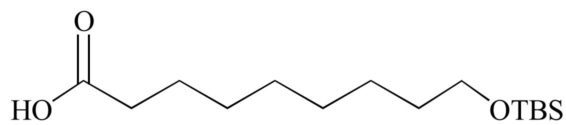
12-(tert-Butyl-dimethyl-silanyloxy)-5-chloro-2-methyl-dodec-2-en-4-one 81 (50%). To a cooled solution of α-hydroxy ketone **80** (0.70 mmol) in dry pyridine (1.74 mL) was added tosyl chloride (2.10 mmol). The mixture was allowed to warm to room temperature and stir for 18 h before being diluted with 50 mL ether. The organic layer was subsequently washed with 25 mL water, 25 mL sat. aq. sodium bicarbonate, and 25 mL brine. Drying over $MgSO_4$, filtering and concentrating generated a yellow slurry. The crude product was then purified by column chromatography (5%EtOAc:Hexane) to give the α-chloro-ketone. ^{1}H (300 MHz, $CDCl_3$) δ 6.31 (s, 1H), 4.16 (dd, J = 8, 11.2Hz, 1H), 3.58 (t, J = 8.4Hz, 2H), 2.19 (s, 3H), 1.96-1.78 (comp, 5H), 1.57-1.30 (comp, 10H), 0.88 (s, 9H), 0.03 (s, 6H); ^{13}C (75 MHz, $CDCl_3$) δ 194.4, 160.3, 120.0, 64.6, 63.3, 34.2, 32.9, 29.3, 29.1, 28.2, 26.2, 26.1, 25.8, 21.3, 18.5, -5.0; IR (neat) υ = 2929, 2855, 1690, 1620, 1462, 1445, 1385, 1359, 1253, 1099, 1006, 938, 835, 775, 661, 530 cm^{-1}; Anal. Calcd. for $C_{19}H_{37}ClO_2Si$: C, 63.21; H, 10.33; Found: C, 63.58; H, 10.39.



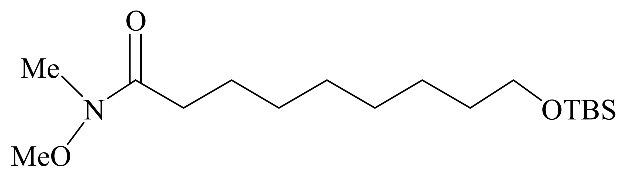
5-Chloro-12-hydroxy-2-methyl-dodec-2-en-4-one 82 (95%). A test tube equipped with a magnetic stir bar was cooled to 0 °C before being charged with the α-chloro ketone **81** (0.03 mmol) and 0.30 mL THF. HF·Pyridine (0.12mmol) was then added dropwise via syringe. After 1 h, TLC showed no starting material. The reaction mixture was cautiously transferred by pipette to a separate test tube cooled to 0 ^{o}C containing 10 mL saturated sodium bicarbonate. The mixture was further diluted with 20 mL ether and the layers were separated. The aqueous layers were then extracted with ether (3 x 20 mL). The combined organic layer was dried over $MgSO_4$, filtered and concentrated. Flash chromatography (20 % EtOAC: Hexane) provided the deprotected alcohol; ^{1}H (300 MHz, $CDCl_3$) δ 6.31 (s, 1H), 4.16 (dd, J = 8, 11.2Hz, 1H), 3.63 (t, J = 8.4Hz, 2H), 2.19 (s, 3H), 1.96-1.77 (comp, 5H), 1.55-1.18 (comp, 11H); ^{13}C (75 MHz, $CDCl_3$) δ 194.5, 160.5, 119.9, 64.5, 62.9, 34.1, 32.7, 29.2, 29.0, 26.1, 25.7, 21.3; IR cm-1 3364, 2931, 2856, 1686, 1618, 1444, 1381, 1356, 1233, 1150, 1056, 842, 726, 687 cm^{-1}; Anal. Calcd. for $C_{13}H_{23}ClO_2$: C, 63.27; H, 9.39; Found: C, 62.98; H, 9.27.



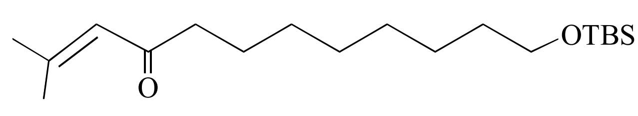
***tert*-Butyl-dec-9-enyloxy-dimethyl silane 70 (76%).** To a solution of sodium hydride (3.76 mmol) in dry THF (15.65 mL) was added first 9-decen-1-ol (3.13 mmol) followed by addition of *tert*-butyldimethylsilyl chloride (3.76 mmol). The reaction was stirred at room temperature for 18 h and then diluted with 20 mL ether. The organic layer was washed sequentially with 10 mL sat. aq. potassium bicarbonate, 10 mL water, and 10 mL brine, dried over $MgSO_4$, filtered and concentrated to an oil. Flash Chromatography (10% CH_2Cl_2:Hexane) yielded the silyl ether as a clear oil**.** ^{1}H (300 MHz, $CDCl_3$) δ 5.84-5.78 (m, 1H), 4.98 (dd, J = 1.5, 16.8 Hz, 1H), 4.93 (dd, J = 1.5, 10.2Hz, 1H), 3.59 (t, J = 6.6Hz, 2H), 2.03 (bquartet, J = 7.2, 2H), 1.54-1.29 (complex, 8H), 0.89 (s, 9H), 0.04 (s, 6H)



Methyl 9-(*tert*-Butyl-dimethyl-silanyloxy)-nonanoate 58,71 (64%). To a solution of alkene (2.33 mmol) in CCl_4:CH_3CN:H_20 (4.66 mL : 4.66 mL : 6.99 mL) was added solid sodium periodate (9.5 mmol). The suspension was allowed to stir at room temperature until two distinct phases appeared. At this time RuO_2 (0.05 mmol) was slowly added and the suspension turned a deep purple color. The reaction was allowed to stir at room temperature for 6 h before adding 5 mL water. The aqueous layer was washed with CH_2Cl_2 (3 x 20 mL). The combined organic layers were filtered though a pad of Celite to remove the dark color, dried over $MgSO_4$, filtered and concentrated to an oil. Purification via Flash Chromatography (25% EtOAc:Hexane) gave the acid as a clear oil. ^{1}H (300 MHz, $CDCl_3$) δ 3.59 (t, J = 6.6Hz, 2H), 2.39 (t, J = 7.8Hz, 2H), 1.65-1.56 (m,2H), 1.52-1.47 (m, 2H), 1.30 (bs, 8H), 0.88 (s, 9H), 0.04 (s, 6H); IR (neat) υ = 2930, 2857, 1711, 1471, 1255, 1098, 836, 775 cm^{-1}.

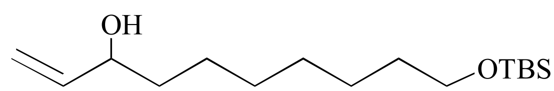


9-(*tert*-Butyl-dimethyl-silanyloxy)-nonanoic acid dimethyl amide 72 (77%) : A round bottom flask equipped with a magnetic stir bar and flushed with Ar was charged with acid **71** (0.35 mmol) in 1.8mL of CH_2Cl_2. To this solution was added 1,1-carbonyl imidazole (0.38 mmol) and the mixture was allowed to stir at room temperature for 10 minutes prior to the addition of N,O-dimethylhydroxylamine hydrochloride (0.38 mmol) and triethyl amine (0.38 mmol). The mixture was stirred over night and then diluted with 20 mL ether. The organic layer was washed with 10 mL 3% aq. HCl, 10 mL sat. aq. sodium bicarbonate, and finally 10 mL brine, dried over $MgSO_4$, filtered and concentrated to an oil. Flash Chromatography (20% EtOAc:Hexane) gave the amide as an oil. ^{1}H (300 MHz, $CDCl_3$) δ 3.67 (s, 3H), 3.59 (t, J = 6.6Hz, 2H), 3.17 (s, 3H), 2.40 (t, J = 7.8Hz, 2H), 1.65-1.50 (m, 2H), 1.52-1.47 (m, 2H), 1.30 (bs, 8H), 0.88 (s, 9H), 0.04 (s, 6H).

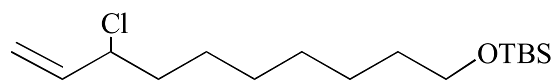


12-(*tert*-Butyl-dimethyl-silanyloxy)-2-methyl-dodec-2-en-4-ol (73) 71% : To a round bottom flask flushed with Ar containing a chilled (0 ^{o}C) solution of amide **72** (0.24 mmol) in 1.2 mL of THF was added 2-methyl-1-propenyl magnesium bromide (0.60 mmol, 0.5M in THF) and the mixture was then allowed to warm to room temperature.

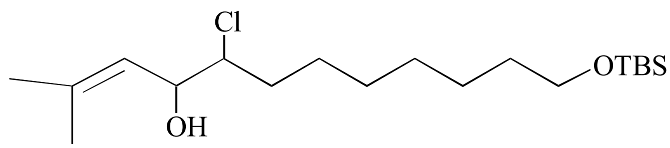
After 3 h, the reaction was quenched by the addition of 5 mL saturated aqueous ammonium chloride followed by separation of the layers. The aqueous layer was extracted with ether (3 x 10 mL). The combined organic layers were washed with 15 mL of brine, dried over $MgSO_4$, filtered and concentrated to a yellow oil. Purification by Flash Chromatography (20% CH_2Cl_2:Hexane) produced ketone as a clear oil. ^1H (300 MHz, $CDCl_3$) δ 6.05 (s, 1H), 2.38 (t, J = 7.5 Hz, 2H), 2.13 (s, 3H), 1.87 (s, 3H), 1.63-1.54 (m, 4H), 1.30 (bs, 8H), 0.88 (s, 9H), 0.04 (s, 6H).



10-(*tert*-Butyl-dimethyl-silanyloxy)-dec-1-en-3-ol 74 (87%) : Vinyl bromide (1.5 mmol) was dissolved in dry ether (2.0 mL) and the solution cooled to –78 ^oC via dry ice/ acetone. *t*-Butyllithium (3.0 mmol, 1.35 M in Pentane) was added to the solution slowly over 10 minutes. The mixture was kept at –78 ^oC for 1. 5h then allowed to warm slowly to room temperature and stirred for 10 minutes. The mixture was cooled to –78 ^oC and the aldehyde **21** (1.3 mmol, in 1 mL ether) solution was slowly added via canula. The once cloudy solution immediately went clear. After 1.5 h the reaction mixture was slowly warmed to 0 ^oC quenched by the addition of 10 mL sat. aq. NH_4Cl and allowed to warm to room temperature. The mixture was diluted with 10 mL ether and the aqueous layer extracted with ether (3 x 10 mL). The combined organic layers were washed with 10 mL sat. aq. $NaHCO_3$, 10 mL sat. aq. NaCl, dried over $MgSO_4$, filtered and concentrated to a clear oil. Flash Chromatography (15 % EtOAc:Hexane) gave the allyl alcohol as a clear oil. ^1H (300 MHz, $CDCl_3$) δ 5.92-5.80 (m, 1H), 5.27 (d, J = 18Hz, 1H), 5.14 (d, J = 11.1Hz, 1H), 4.13-4.05 (m, 1H), 3.59 (t, J = 6.3Hz, 2H), 1.54-1.30 (m, 10H), 0.88 (s, 9H), 0.03 (s, 6H); IR (neat) υ = 3357, 2930, 2856, 1471, 1463. 1255, 1101, 774 cm^{-1}.

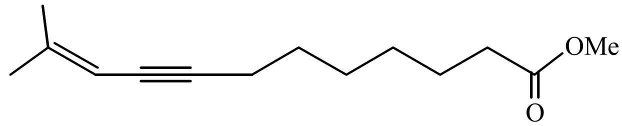


***tert*-Butyl-(8-chloro-undec-9-enyloxy)-dimethyl silane 75 (26%) :** To a cooled solution (0 ^oC) of allylic alcohol **74** (0.70 mmol) in dry pyridine (1.74 mL) was added tosyl chloride (2.10 mmol) and the mixture was allowed to warm to room temperature. Stirring for 18 h indicated no starting material (via TLC). The reaction mixture was warmed to 50^{o}C via oil bath for 1 h, and then cooled to room temperature. The mixture was diluted with 25 mL ether and washed with 15 mL water, 15 mL sat. aq. sodium bicarbonate, and 15 mL brine. The organic phase was dried over $MgSO_4$, filtered and concentrated to a yellowish slurry. Purification by flash chromatography (5% Et_2O:Hexane) gave allyl chloride as a clear oil. ^1H (300 MHz, $CDCl_3$) δ 5.93-5.81 (m, 1H), 5.27 (d, J = 18.0Hz, 1H), 5.14 (d, J = 11.1Hz, 1H), 4.32 (quartet, J = 7.2 Hz, 1H), 3.59 (t, J = 6.3 Hz, 2H), 1.84-1.75 (m, 2H), 1.55-1.25 (m, 10H), 0.89 (s, 9H), 0.04 (s, 6H); IR (neat) υ = 2928, 2855, 1462, 1253, 1099, 835, 774 cm^{-1}.

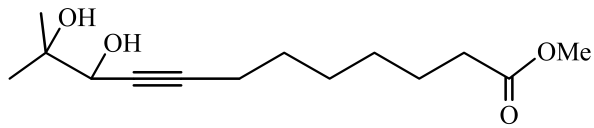


11-(*tert*-Butyl-dimethyl-silanyloxy)-4-chloro-undec-1-en-3-ol 78 (54%) : To a 10 mL round bottom flask flame dried and flushed with argon was added 2-methyl-1-propenylmagnesium bromide (0.92 mmol, 0.5 M in THF) and cooled to 0 ^{o}C via ice bath. After stirring for 20 min, aldehyde **77** (0.38 mmol) in THF (0.5 mL) was slowly added via a cannula. The yellow solution was warmed to room temperature and stirred for 2 h before being quenched by the addition of 1 mL sat. aq. NH_4Cl and stirred for an additional 0.5 h. The mixture was then diluted with 5 mL ether and the aqueous layer extracted with Et_2O (3x 5 mL). The combined organic layers were washed with 5 mL sat. aq. NH_4Cl, and 5 mL brine. The clear solution was then filtered and concentrated leaving a yellow oil. Purification via flash chromatography (20%EtAOc : Hexane) gave as a transparent oil. ^{1}H (300 MHz, $CDCl_3$) δ 5.35-5.31 (m, 1H), 4.49-4.32 (m, 1H), 4.08-4.00 (m, 1H), 3.59 (t, J = 6.3Hz, 2H), 1.76 (s, 3H), 1.72 (s, 3H), 1.68-1.49 (complex, 4H), 1.36-1.28 (complex, 8H), 0.88 (s, 9H), 0.04 (s, 6H); IR (neat) υ = 3417, 2929, 2856, 1462, 1098, 835, 775 cm^{-1}.

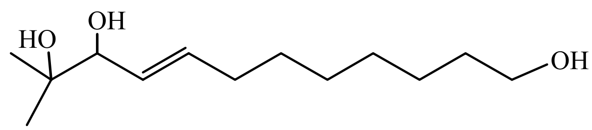
*Synthesis of Analogs **9** and **10***



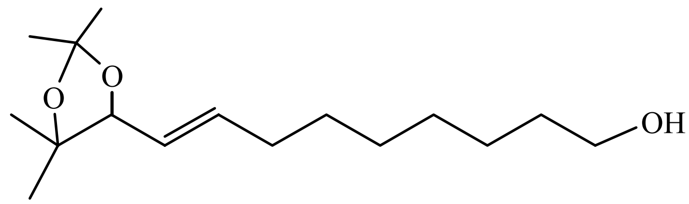
Methyl-11-Methyl-dodec-10-en-8-ynoate 87 (82%). To a solution of 1-bromo-2-methylpropene (2.95 mmol) in 20 mL diethylamine was added bis(triphenyl phosphine) palladium (II) chloride (0.15 mmol) and copper (II) iodide which provided a yellow/green solution. Soon after the appearance of this color, a solution of 8-nonyoic ester **86** (3.24 mmol) in 1 mL of diethyl amine was slowly canulated drop wise into the mixture. The solution immediately turned a bluish/green and within 15 minutes became yellow with a small amount of precipitate. After 8 h, the reaction mixture was diluted with 20 mL Et_2O followed by quenching by the addition of 20 mL saturated ammonium chloride and stirred for 30 min. The aqueous layer was extracted with Et_2O (2 x 50 mL). The combined yellow organic layer was then washed with 50 mL sat. NH_4Cl and dried over $MgSO_4$. Filtration and evaporation of the solvents provided a crude yellow oil. Flash chromatography (10% EtOAC : Hexane) gave the alkene-alkyne ester in **82%** yield. Product UV active. ^{1}H (300 MHz, $CDCl_3$) δ 5.22 (s, 1H), 3.66 (s, 3H), 2.33-2.28 (m, 4H), 1.86 (s, 3H), 1.77 (s, 3H), 1.67-1.33 (complex, 8H); ^{13}C (75 MHz, $CDCl_3$) δ 173.0, 148.0, 107.5, 91.1, 76.9, 50.4, 33.3, 29.1, 28.8, 25.4, 24.1, 18.1; IR (neat) υ = 2933, 1739, 1435, 1200, 1171 cm^{-1}; Anal. Calcd. for $C_{15}H_{24}0_2$: C, 76.23; H, 10.24; Found: C, 76.53; H, 10.30.



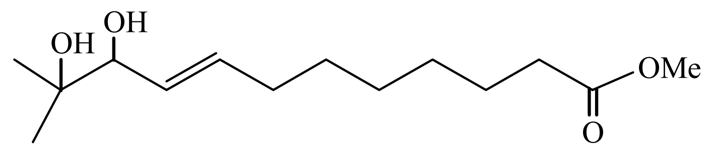
Methyl-10,11-Dihydroxy-11-methyl-dodec-8-ynoate 88 (85%). A catalytic amount of osmium tetroxide (14 μL, 4 wt. % in H_2O) was added to a biphasic solution of water (0.26 mL) and acetone (0.85 mL) containing the alkene-alkyne **87** (0.22 mmol) and N-methylmorpholine *N*-oxide (0.27 mmol). After 4.5 h, the once clear solution was now yellow and after 8 h at room temperature the reaction was quenched by the addition of sodium hydrosulfite (176 mg). The mixture was stirred for 0.5h before being filtered through a short celite pad and flushed with 60 mL Et_20. The aqueous layer was separated and extracted with EtOAc (3 x 50 mL). The organic layers were combined and washed with 50 mL brine, dried over $MgSO_4$, filtered, and concentrated to a yellow oil. Flash chromatography (40% EtOAc : Hexane) gave the diol. ^{1}H (300 MHz, $CDCl_3$) δ 4.12 (s, 1H), 3.65 (s, 3H), 2.29 (t, J = 6.9Hz, 2H), 2.21 (dt, J = 2.1, 4.8 Hz, 2H), 1.61 (quin, J = 1.8 Hz, 2H), 1.53-1.47 (m, 2H), 1.39-1.31 (complex, 5H), 1.27 (d, J = 5.1 Hz, 6H); ^{13}C (75 MHz, $CDCl_3$) δ 172.0, 79.8, 78.5, 78.1, 75.7, 51.2, 34.1, 29.7, 28.6, 22.6, 18.0; IR (neat) υ = 3423, 2974, 2935, 2859, 1739, 1437, 1202, 1171, 1141 cm^{-1}; Anal. Calcd. for $C_{15}H_{26}O_4$: C, 66.64; H, 9.69; Found: C, 66.45: H, 9.75.



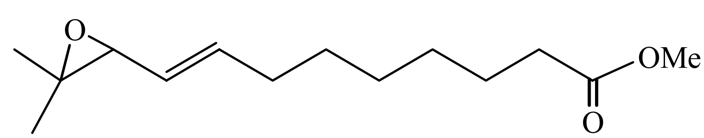
(E)-11-Methyl-dodec-8-ene-1,10,11-triol 89 (64%) : A suspension of $LiAlH_4$ (0.8 9mmol) in dry THF (7 mL) was cooled to 0°C prior to the slow addition of diol **88** (0.22 mmol) in 1.0 mL THF via canulation. (Bubbling occurred and mixture became extremely fluffy). The gray suspension was than heated to reflux by a heating mantle for 3 days before being quenched slowly by the addition of approximately 10 mL water. The two phases were separated. The aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic layer was washed with 50 mL sat. aq. NaCl, dried over $MgSO_4$, filtered and concentrated. Purification by a silica gel column (80% EtOAc : Hexane) provided a clear oil. ^{1}H (300 MHz, $CDCl_3$) δ 5.78-5.68 (m, 1H), 5.52-5.44 (m, 1H), 3.85 (dd, J = 2.4, 7.5 Hz, 1H), 3.63 (bt, J = 6.3 Hz, 2H), 2.88-2.01 (m, 3H), 1.55 (t, J = 7.5 Hz, 2H), 1.40-1.22 (m, 8H), 1.20 (s, 3H), 1.17 (s, 3H); ^{13}C (75 MHz, $CDCl_3$) δ 131.8, 128.9, 90.8, 76.8, 63.2, 33.6, 33.1, 30.7, 30.4, 30.2, 26.4, 23.8; IR (neat) υ = 3426, 2980, 2928, 2856, 1462, 1368, 1265, 1119, 1034, 971 cm^{-1}; Anal. Calcd. for $C_{12}H_{24}O_3$: C, 66.23; H, 11.18; Found: C, 61.95; H, 11.10.



9-(2,2,5,5-Tetramethyl-[1,3]dioxolan-4-yl)-non-8-en-1-ol 93 (53%). To a solution of triol (0.2 mmol) in 1 mL methylene chloride at room temperature was added p-toluene sulfonic acid (0.1 mmol), followed by the addition of dimethoxy propane. The suspension was stirred for 20 h with reaction progress monitored via TLC. The mixture was then diluted with 5 mL of water and the phases separated. The aqueous layer was then extracted with Et_2O (3 x 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated to an oil. The crude product was purified by flash chromatography (80% EtOAc:Hexane) providing the acetonide as a clear oil. ^1H (300 MHz, $CDCl_3$) δ 5.80 (dt, J = 6.3Hz, J = 15.6Hz, 1H), 4.43 (dd, J = 9, 14.4Hz, 1H), 4.11 (d, J = 8.1Hz, 1H), 3.63 (t, J = 6.6Hz, 2H), 2.07 (J = 6.9Hz, 2H), 1.58-1.51 (multiplet, 2H), 1.44-1.22 (complex, 17H), 1.09 (s, 3H); ^{13}C (75 MHz, $CDCl_3$) δ 136.8, 124.7, 107.2, 85.2, 81.0, 63.2, 32.9, 32.5, 29.4, 29.2, 29.0, 28.6, 27.2, 25.9, 25.8, 23.7; IR (neat) υ = 3426, 2980, 2928, 2856, 1462, 1368, 1265, 1217, 1199, 1119, 1034, 971, 913, 860 cm^{-1}; Anal. Calcd. for $C_{16}H_{30}O_3$: C, 68.42; H, 10.13; Found: C, 68.56; H, 10.22.

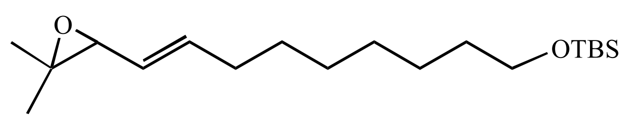


Methyl-10,11-Dihydroxy-11-methyl-dodec-8-enoate 96 (59%). Acetonide-ester **95** (0.035 mmol) was dissolved in 0.35 mL of methanol at room temperature prior to the addition of a small amount of *p*-toluene sulfonic acid (0.007 mmol). The reaction was stirred at room temperature for 24 h and then diluted with 1mL of water. The aqueous layer was then extracted with EtOAc (3 x 10 mL). The organic layers were combined and dried over MgSO4. Filtration, concentration, and purification by flash chromatography (35% EtOAc:Hexane) gave the diol as a yellow oil. ^{1}H (300 MHz, $CDCl_3$) δ 5.72 (dt, J = 7.5, 15.3Hz, 1H), 5.48 (dd, J = 7.8, 15.3Hz, 1H), 3.84 (dd, J = 2.4, 4.5Hz, 1H), 3.66 (s, 3H), 2.30 (t, J = 7.5Hz, 2H), 2.08-2.01 (comp, 2H), 1.66-1.56 (comp, 2H), 1.42-1.24 (comp, 6H), 1.20 (s, 3H), 1.14 (s, 3H); ^{13}C (75 MHz, $CDCl_3$) δ 174.5, 135.3, 128.6, 80.04, 72.9, 51.7, 34.2, 32.4, 29.0, 29.0, 28.9, 26.5, 25.0, 23.9; IR (neat) υ = 3424, 2972, 2929, 2855, 1739, 1438, 1371, 1170, 1097, 1026, 971 cm^{-1}; Anal. Calcd. for $C_{14}H_{26}O_4$: C, 65.09; H, 10.14; Found: C, 64.92; H, 10.20.

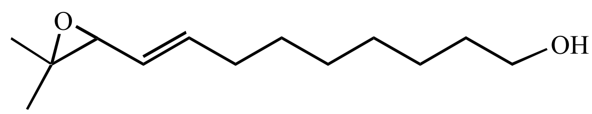


Methyl-9-(3,3-Dimethyl-oxiranyl)-non-8-enoate 10 (50%). A round bottom flask was charged with a solution of diol **96** (0.082 mmol) in 0.82mL THF and triphenylphosphine (0.09 mmol) and cooled to 0 ^{o}C via ice/water. Once at 0^{o}C, diethyl azodicarboxylate

(0.09mmol) was added slowly (neat) by syringe. The resultant solution was stirred for 2 h at 0 °C, followed by warming to room temperature for 4 h. The yellow solution was then concentrated under reduced pressure to give a slushy crude yellow oil. In an attempt to remove triphenylphosphine oxide, the crude oil was suspended in a small amount of ether (1 mL) followed by the addition of hexane (10 mL) and filtered. Removal of the solvent in vacuo resulted again in a yellow slurry. Purification via flash chromatography (10%EtOAc:Hexane) afforded the desired inhibitor, the epoxide alkene ester. ^{1}H (300 MHz, $CDCl_3$) δ 5.88, (dt, J = 6.9, 15.3Hz, 1H), 5.31 (dd, J = 7.8, 15.3Hz, 1H), 3.66 (s, 3H), 3.16 (d, J = 8.1Hz, 1H), 2.12-2.04 (comp, 2H), 1.64-1.56 (comp, 2H), 1.46-1.27 (comp, 12H); ^{13}C (75 MHz, $CDCl_3$) δ 174.4, 137.8, 125.4, 64.5, 60.2, 51.6, 34.2, 32.6, 29.1, 28.9, 28.9, 25.0, 24.8, 19.1; IR (neat) υ = 2972, 2929, 2855, 1739, 1438, 1371, 1170, 1097, 1026, 971 cm^{-1}; Anal. Calcd. for $C_{14}H_{24}O_3$: C, 69.96; H, 10.07; Found: C, 70.10; H, 10.15.

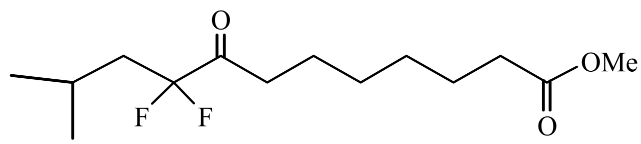


***tert*-Butyl-[(E)-9-(3,3-dimethyl-oxiranyl)-non-8-enyloxy]-dimethyl-silane 91 (47%) :** diethyl azodicarboxylate (0.27 mmol) was added neat via syringe to a cooled (0 °C) solution of diol **90** (0.25 mmol), PPh_3 (0.27 mmol), and THF (1.25 mL). The ice bath was removed and the mixture warmed to room temperature developing a yellow color. After 4 h stirring at room temperature, the solvent was evaporated leaving a yellow oil, which was diluted with a minimal amount of Et_2O then hexane. The white precipitate was filtered and the filtrate reconcentrated to a yellow slurry. Purification via flash chromatography (10% Et_2O : Hexane) provided a clear oil. ^1H (300 MHz, $CDCl_3$) δ 5.89 (dt, J = 6.9, 15.3Hz, 1H), 5.29 (dd, J = 7.8, 15.6Hz, 2H), 3.63 (bq, J = 5.7Hz, 2H), 3.16 (d, 8.1Hz, 1H), 2.08 (bq, J = 6.6Hz, 2H), 1.58-1.51 (m, 2H), 1.41-1.24 (complex, 16H), 0.89 (s, 9H), 0.04 (s, 6H).

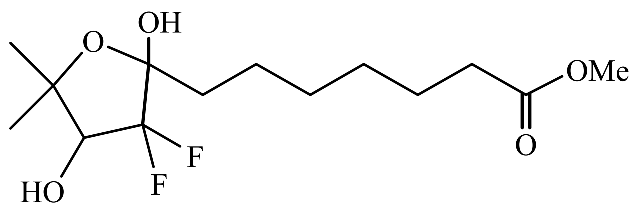


(E)-9-(3,3-Dimethyl-oxiranyl)-non-8-en-1-ol 92 (99%) : A solution of epoxide **91** (0.033 mmol) in THF (0.165 mL) was cooled to 0 °C before the dropwise addition of tetrabutylammonium fluoride (0.039 mmol, 1M solution in THF). Once the addition was complete the mixture (yellow) was allowed to warm to room temperature and remain overnight. After 18 h, the reaction was diluted with 5 mL ether and quenched by the addition of 1 mL water. Organic layer became a white solution. The aqueous layer extracted with 5 mL ether. The organic layers were combined and dried over $MgSO_4$, filtered and concentrated. Flash chromatography (25% EtOAc:Hexane) provided **92**. ^1H (300 MHz, $CDCl_3$) δ 5.89 (dt, J = 6.9, 15.3Hz, 1H), 5.29 (dd, J = 7.8, 15.6Hz, 2H), 3.63 (bq, J = 5.7Hz, 2H), 3.16 (d, 8.1Hz, 1H), 2.08 (bq, J = 6.6Hz, 2H), 1.58-1.51 (m, 2H), 1.41-1.24 (complex, 16H).

*Syntheses of **6a** derivatives*



Methyl-9,9-Difluoro-11-methyl-8-oxo-dodecanoate 97 (89%). To a solution of **50** (0.40 mmol) in EtOH (5 mL) under argon was added 10% Pd/C (40 mg). The flask was flushed with H_2 and the mixture stirred under atmospheric H_2 pressure (balloon) for 24 h. The catalyst was removed by filtration through a short column of celite. After evaporation of the solvent in vacuo, the residue was dissolved in MeOH (5 mL) and the solution filtered through a pipette plugged with celite. This process was repeated before concentration *in vacuo* afforded **97**. ^{1}H (300 MHz, $CDCl_3$) δ 3.66 (s, 3H), 2.65 (t, J = 7.2Hz, 2H), 2.30 (t, J = 7.8Hz, 2H), 1.98-1.80 (m, 3H), 1.65-1.56 (m, 6H), 1.35-1.30 (m, 4H), 0.97 (d, J = 6Hz, 6H); ^{13}C (300 MHz, $CDCl_3$) δ 201.7 (t), 174.4, 118.8 (t), 51.7, 40.6 (t), 36.3, 34.2, 29.0, 28.8, 24.9, 23,6, 22.9, 22.7; ^{19}F (282 MHz, $CDCl_3$) δ -106.0 (t, J = 16.3 Hz, 2F); IR (neat) υ = 2948, 2870, 1740, 1436, 1368, 1172, 1023, 866 cm^{-1} Anal. Calcd. for $C_{14}H_{24}F_2O_3$: C, 60.41; H, 8.69; Found: C, 60.15; H, 8.74.



Methyl-7-(3,3-Difluoro-2,4-dihydroxy-5,5-dimethyl-tetrahydro-furan-2-yl)-heptanoate 98 (82%). A catalytic amount of osmium tetroxide (17 μL, 4 wt. % in H_2O) was added to a biphasic solution of 0. 32 mL water and 1.0 mL acetone containing the alkene **50** (0.27 mmol) and 4-methylmorpholine *N*-oxide (0.33 mmol). After 8 h at room temperature the reaction was quenched by the addition of sodium hydrosulfite (176 mg). The mixture was stirred for 0.5 h before being filtered through a short celite pad and flushed with 30 mL Et_20. The aqueous layer was separated and extracted with EtOAc (3 x 10 mL). The organic layers were combined and washed with 50 mL brine, dried over $MgSO_4$, filtered, and concentrated to a yellow oil. Flash chromatography (40% EtOAc : Hexane) gave the THF compound **98**. ^{1}H (300 MHz, $CDCl_3$) δ 4.33 (quintet, J = 6.3Hz, 1H), 3.64 (s, 3H), 2.28 (t, J = 5.7 Hz, 2H), 1.49-1.28 (complex, 20H); ^{13}C (300 MHz, $CDCl_3$) δ 174.8, 122.9 t, 102.3 t, 82.5, 80.2, 51.8, 34.2, 33.8, 29.5, 29.0, 28.2, 25.0, 24.6, 22.2; ^{19}F (282 MHz, $CDCl_3$) δ 2F –117.4 (d, J = 181.3Hz), -124.7 (d, J = 173.1Hz), -126.7 (d, J = 180.8Hz), -130.8 (d, 173.7Hz); IR (neat) υ = 3438, 2937, 2858, 1720, 1439, 1369, 1190, 1090, 1021 cm^{-1}; Anal. Calcd. for $C_{14}H_{24}F_2O_5$: C, 54.18; H, 7.80; Found: C, 54.36; H, 7.75.

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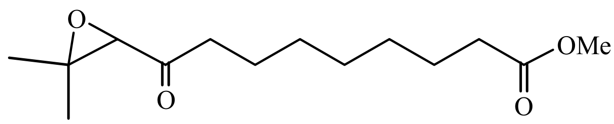
Appendix A

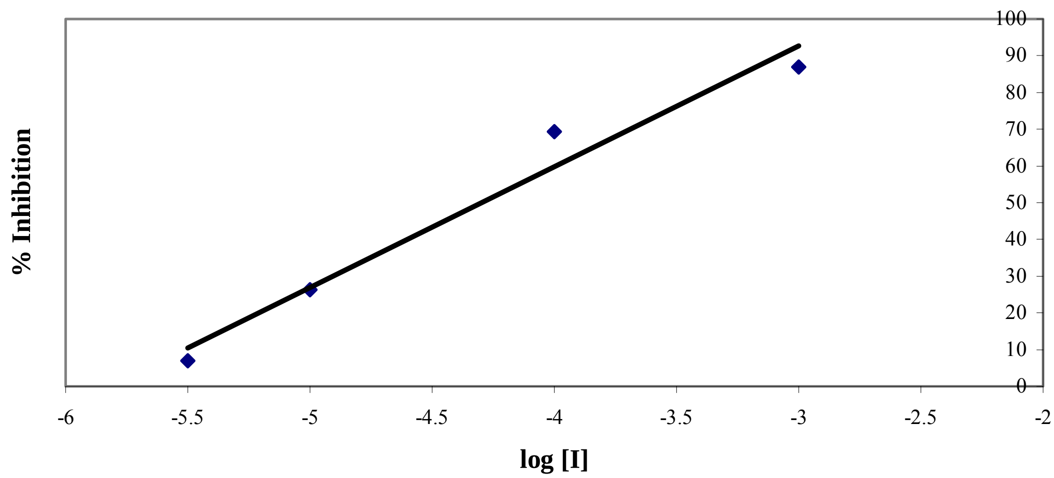
I_{50} Plots

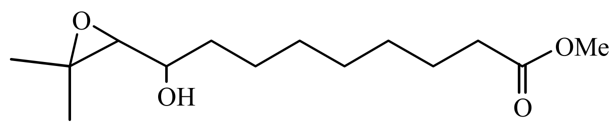


4a

$I_{50} = 50.6\ \mu M$

y= 32.91x + 191.37, R^2= 0.967

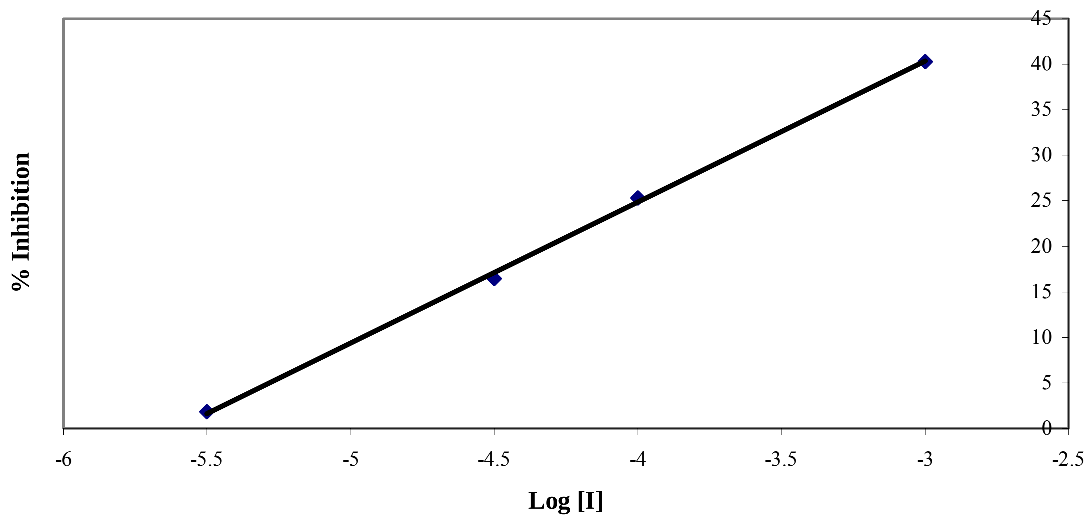


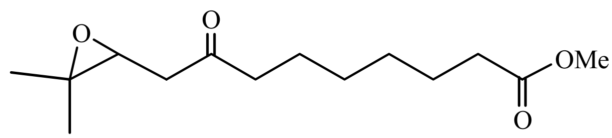


4b

$I_{50} = 4210\ \mu M$

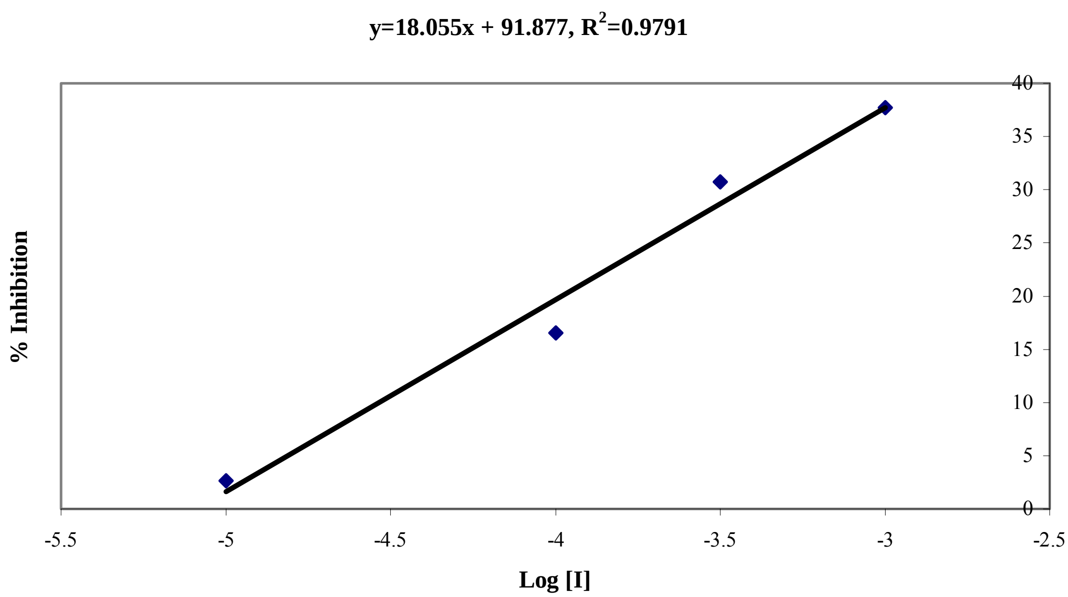
y= 15.476x + 86.763, R^2=0.999

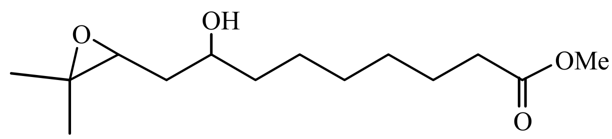




5a

$I_{50} = 4700\ \mu M$

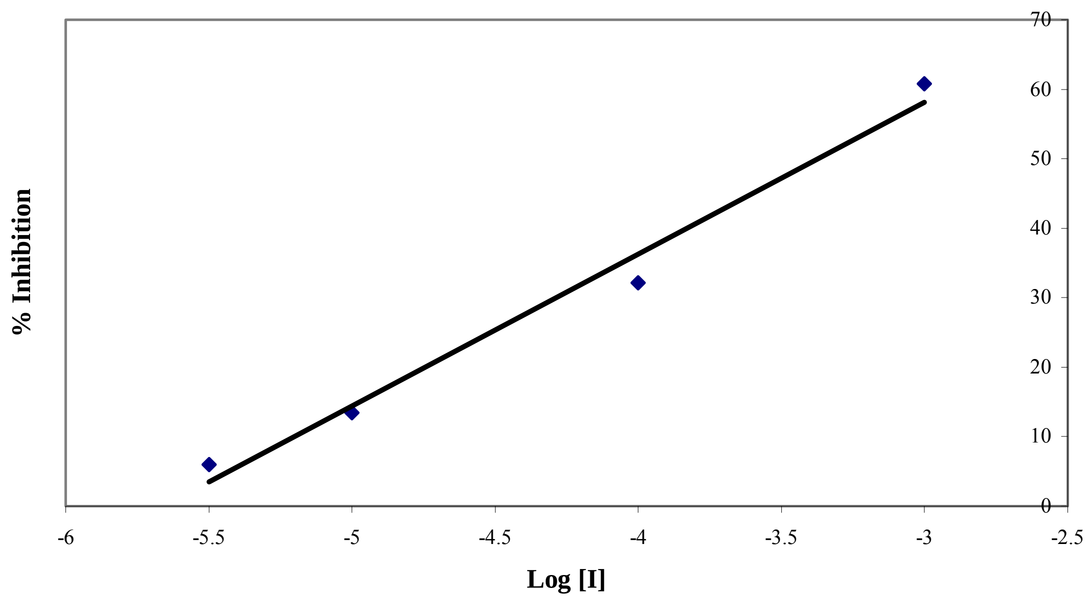


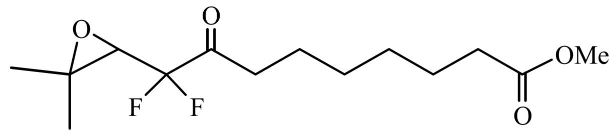


5b

$I_{50} = 424.7\ \mu M$

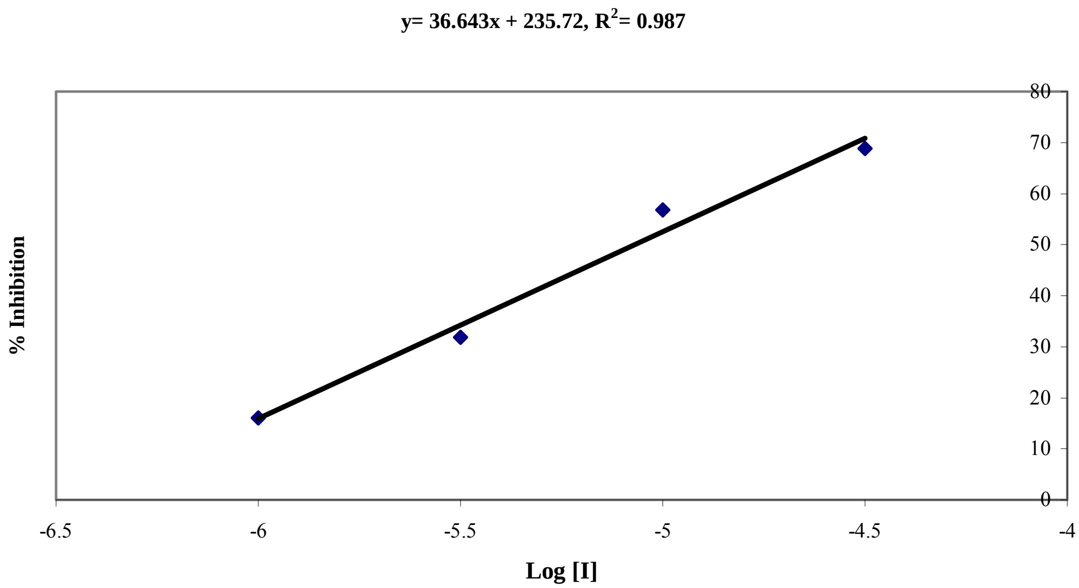
y= 21.842x + 123.65, R^2= 0.983

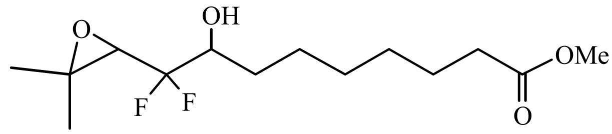




6a

$I_{50} = 8.5\mu M$

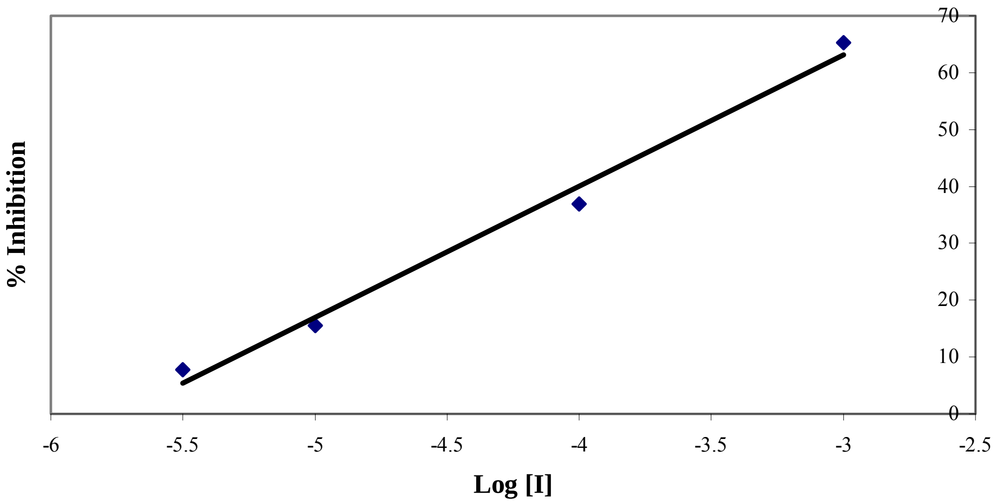

y= 36.643x + 235.72, R2= 0.987
% Inhibition
Log [I]
0
10
20
30
40
50
60
70
80
-6.5
-6
-5.5
-5
-4.5
-4

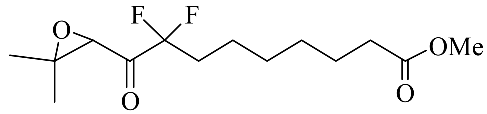


6b

$I_{50} = 244\ \mu M$

y = 23.097x + 132.43, R^2 = 0.9889

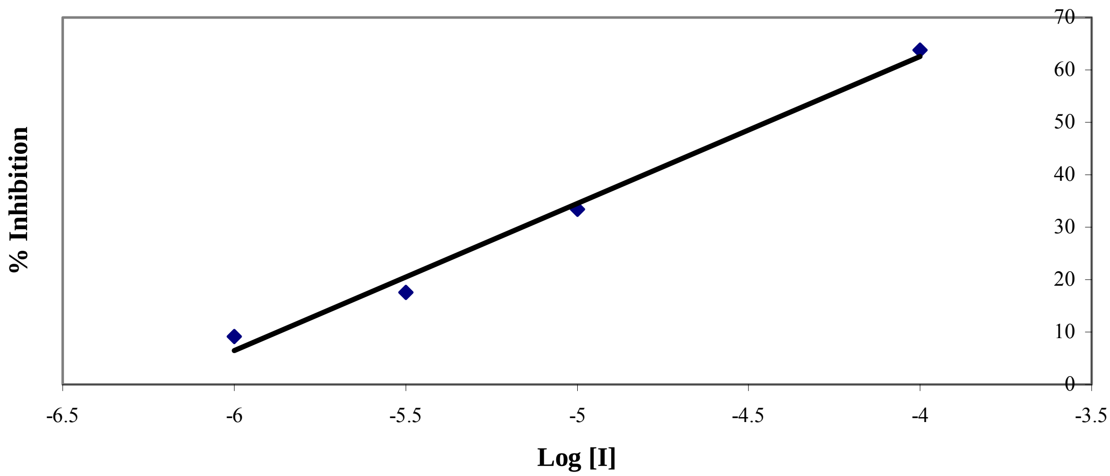


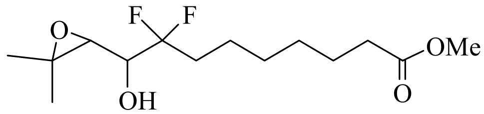


7a

$I_{50} = 35.7\ \mu M$

y= 28.021x + 174.61, R^2=0.9894

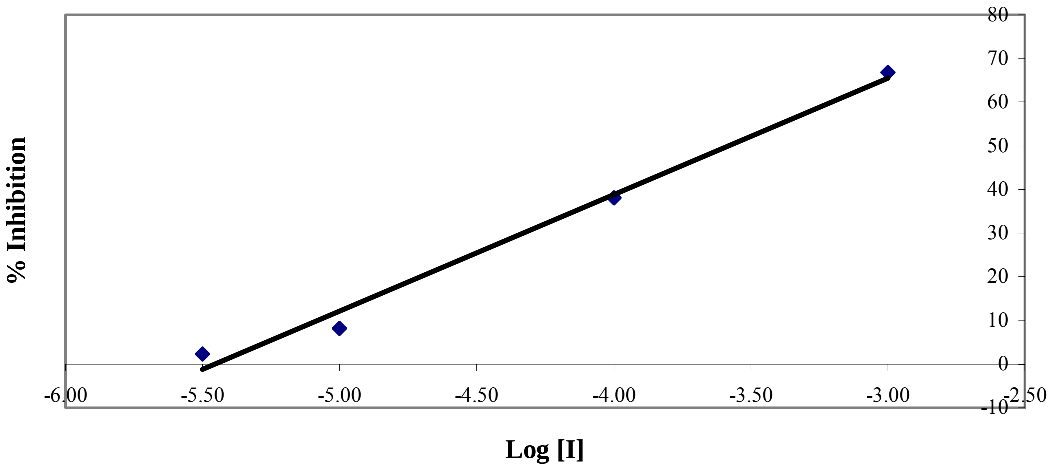


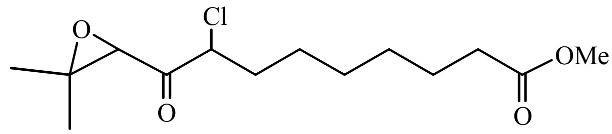


7b

$I_{50} = 262.2\ \mu M$

y=26.683 + 145.56, R^2=0.9885

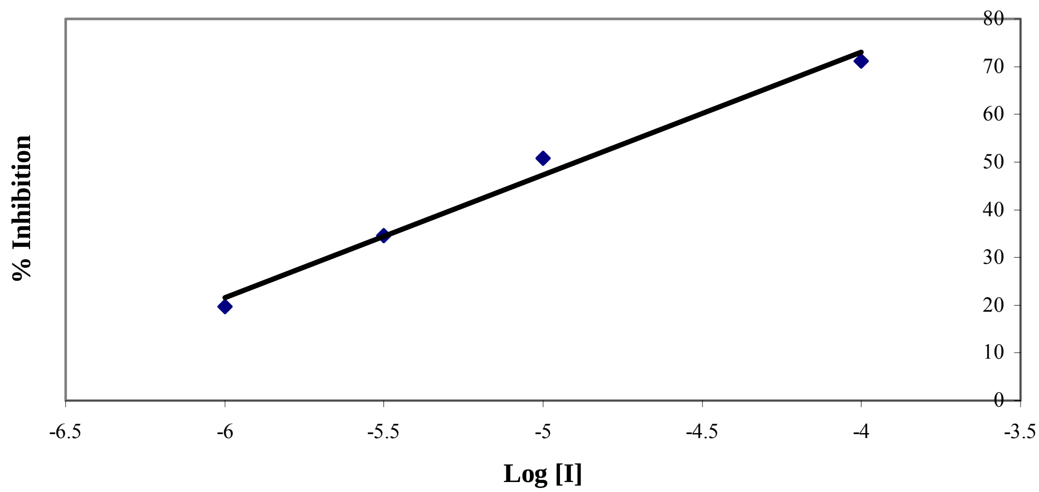


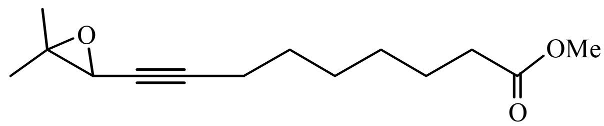


8

$I_{50} = 12.8\ \mu M$

y= 25.7x + 175.78, R^2= 0.987

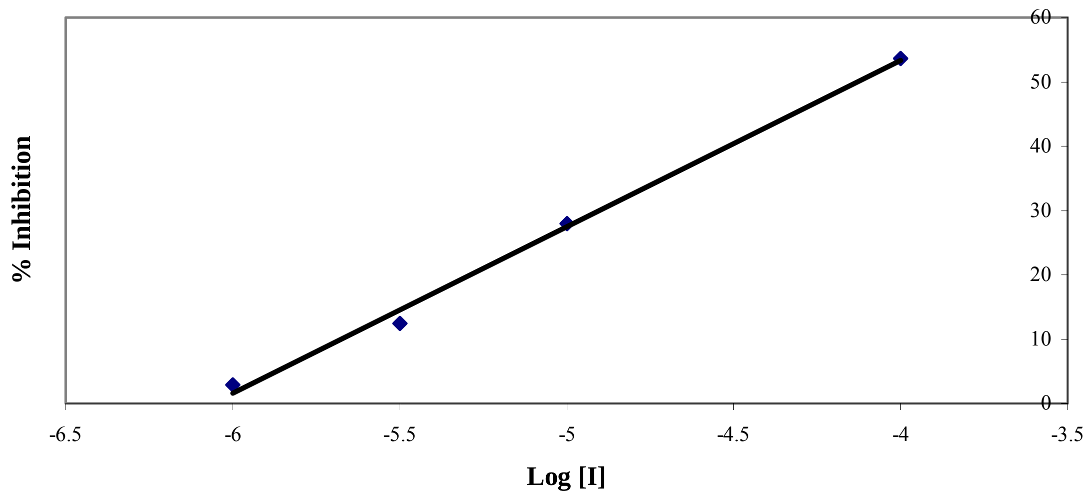


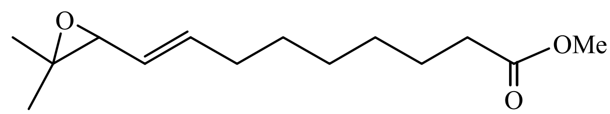


9

$I_{50} = 74.1\ \mu M$

y= 25.913x + 157.03, R^2= 0.996

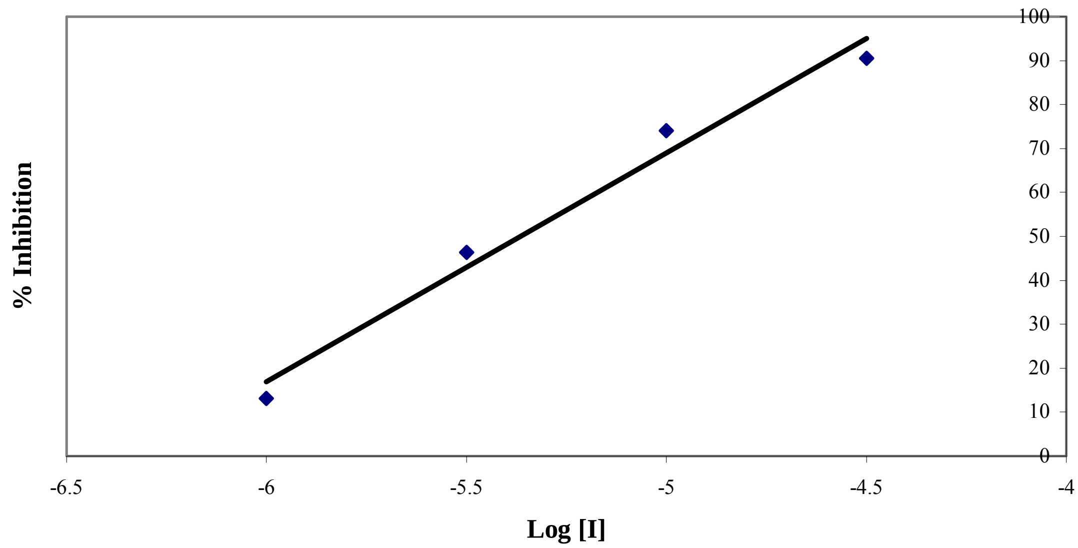


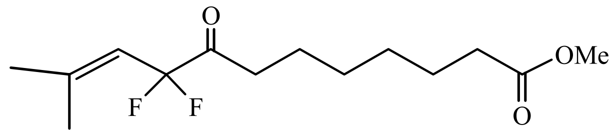


10

$I_{50} = 4.3\ \mu M$

y= 52.015x + 329.07, R^2= 0.979

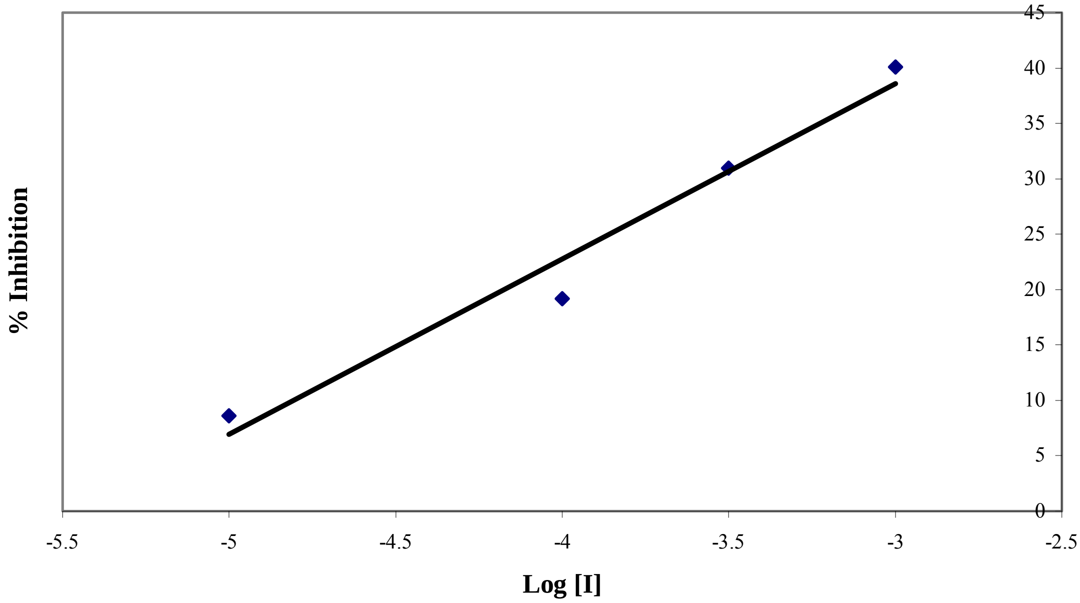


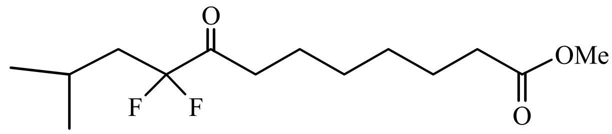


50

I_{50} = 5300 μM

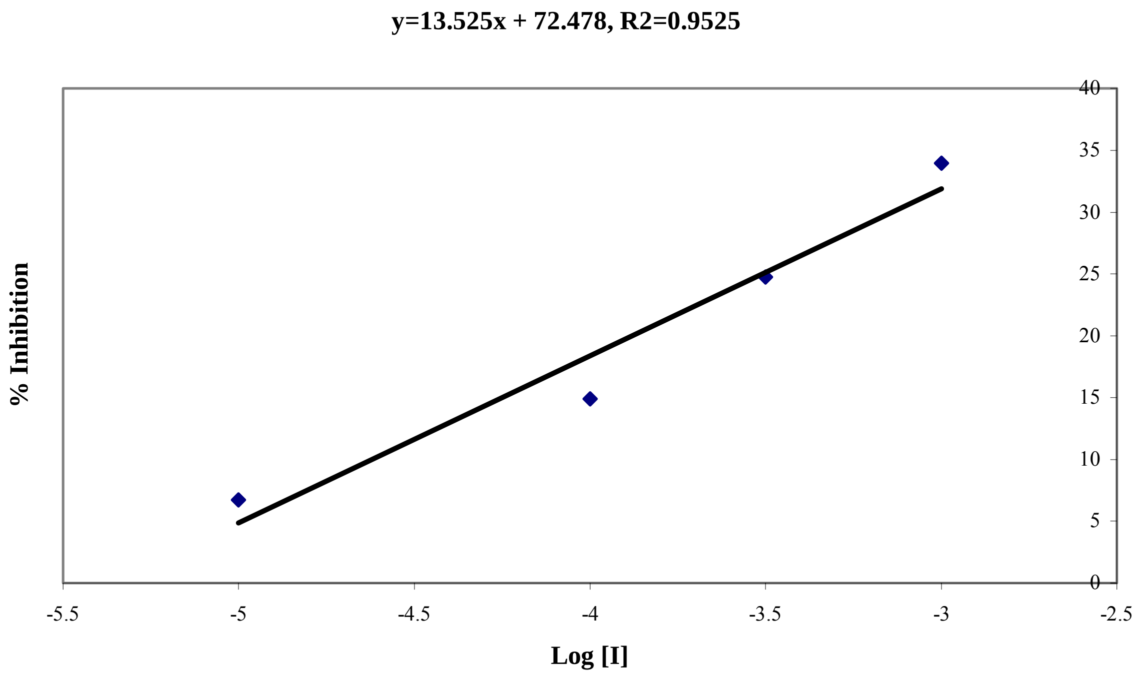
y=15.827x + 86.046, R^2= 0.9682

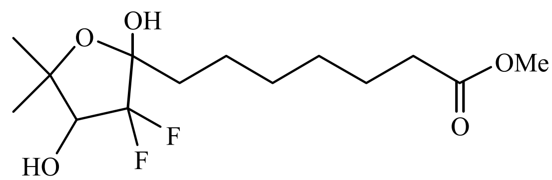




97

I_{50} = 22000 μM

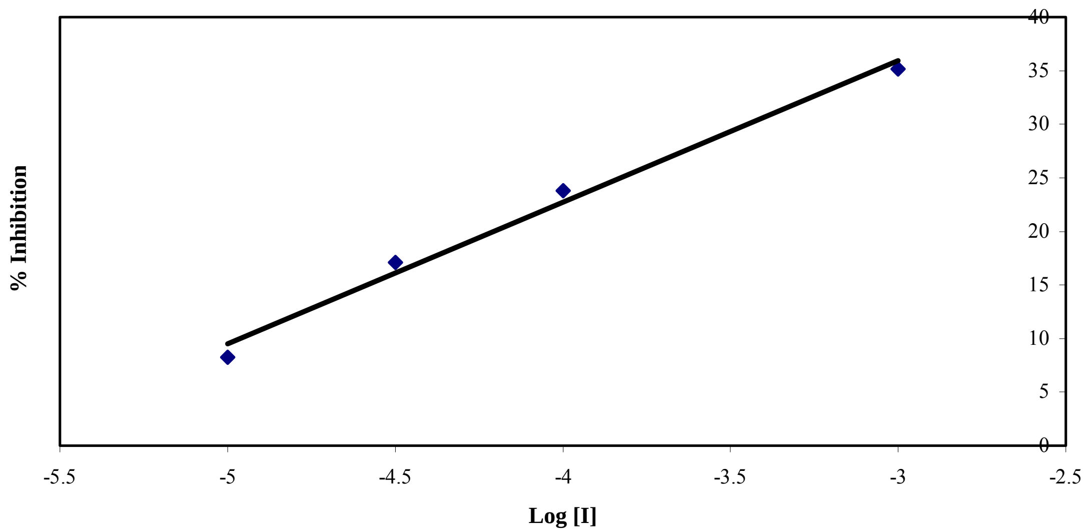


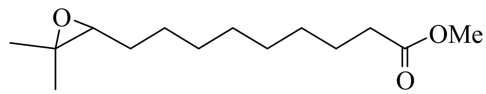


98

$I_{50} = 11000\ \mu M$

y=13.223x + 75.625, R^2=0.9888





MEMD

I_{50}= 4.8 μM

y=62.552x +382.56, R^2=0.9916