Development of Novel Anticancer Therapuetics: Total Synthesis of Lactimidomycin and its Analogs and Synthetic Strategies Towards Diterpene Natural Products

by

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Dedication

I'd like to dedicate this dissertation to my parents, Scott and Martha Larsen, and my big brother, Erik. Your influences have pushed me to work harder and strive higher than I ever thought myself capable. I will always be grateful for love and support you have given me, which has helped me through all these years.

Furthermore, I'd like to dedicate this dissertation to my lovely and wonderful wife, Laura, who has stood by and supported me throughout my entire graduate career. I am eternally grateful that I met you during our first few weeks at Michigan. I'd also like to thank Jimmy, Sharon, Stephen, and Katie Pfund, who have become like second family to me.

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Table of Contents

List of Figures

List of Schemes

List of Tables

List of Abbreviations

Ac	acetyl
acac	acetylacetonate
Ar	aromatic (generic)
Boc	*tert*-butoxycarbonyl
BOX	bis(oxazoline)
Bn	benzyl
br	broad
br s	broad singlet
Bu	butyl
Bz	benzoyl
c	concentration (g/100 mL)
ºC	degrees Celsius
CAN	ceric ammonium nitrate
CAM	ceric ammonium molybdate
(*S*)-CBS	(*S*)-(−)-2-Methyl-CBS-oxazaborolidine
CCM	cancer cell migration
CD	circular dichroism
COD	cycloocta-1,5-diene
COSY	correlation spectroscopy
Cp*	pentamethylcyclopentadienyl

CSA	camphorsulfonic acid
Cy	cyclohexyl
d	deutero
d	doublet
dd	doublet of doublets
dt	doublet of triplets
dq	doublet of quartets
ddd	doublet of doublet of doublets
dddd	doublet of doublet of doublet of doublets,
dqd	doublet of quartet of doublets,
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DBU	1,8-diazabicyclo[5.4.0]undec-5-ene
DEAD	diethylazodicarboxylate
DIBALH	diisobutylaluminum hydride
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMB	3,4-dimethoxybenzyl
DME	1,2-dimethoxyethane
DMF	dimethylformamide
DMP	Dess-Martin Periodinane
DMPU	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
DMSO	dimethylsulfoxide
dr	diastereomeric ratio
DTS	diverted total synthesis

E	entgegen
ECA	extended chain analog
ee	enantiomeric excess
equiv	equivalent
ES	electrospray
ESI	electrospray ionization
Et	ethyl
FAB	fast atom bombardment
h	hour
HBTU	O-(benzotriazol-1-yl)-N,N,N’,N’-tetramethyluroniumhexafluorophosphate
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum correlation
hv	quantum of light (electromagnetic radiation)
HWE	Horner-Wadsworth-Emmons
Hz	hertz
i	iso
ImH	imidazole
J	coupling constant
KHMDS	potassium hexamethyldisilazide
LAH	lithum aluminun hydroxide
LDA	lithium diisopropylamide
LiHMDS	lithium hexamethyldisilazide

m	meta
m	multiplet
M	i) molar (moles/liter); ii) metal (in tables and schemes)
m/z	mass to charge ratio
m-CPBA	*meta*-chloroperbenzoic acid
Me	methyl
MIC	maximum inhibitory concentration
min	minute(s)
mol	mole(s)
MS	mass spectroscopy
MTPA	*a*-methoxy-*a*-(trifluoromethyl)phenylacetate
NaHMDS	sodium hexamethyldisilazide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser enhancement
OTf	trifluoromethanesulfonate
Oxz	4,5-diphenyloxazole
p	pentet
p	*para*
Pd/C	palladium on carbon
$Pd/CaCO_3$	palladium on calcium carbonate
$Pd(OH)_2/C$	palladium (II) hydroxide on carbon
Ph	phenyl
PMB	4-methoxybenzyl

PMP	4-methoxyphenyl
ppm	parts per million
PPTS	pyridinium *para*-toluenesulfonate
Pr	propyl
PT	5-phenyltetrazole
Py	pyridine
Q	quartet
qd	quartet of doublets
RCAM	ring-closing alkyne metathesis
RCM	ring-closing metastasis
R	rectus (Cahn-Ingold-Prelog system)
R	alkyl group (generic)
rbf	round bottom flask
rt	rt
S	sinister (Cahn-Ingold-Prelog system)
s	singlet
SWH	scratch wound healing [assay]
t	triplet
t	tertiary
tert	tertiary
T	temperature
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide

TBDPS	*tert*-butyldiphenylsilyl
TBHP	*tert*-butyl hydroperoxide
TBS	*tert*-butyldimethylsilyl
TBSCl	*tert*-butyldimethylsilylchloride
TES	triethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TlOEt	thallium(I) ethoxide
TMEDA	tetramethylethylenediamine
TMS	trimethylsilyl
Ts	*p*-toluenesulfonyl
Z	zusammen
d	chemical shift (parts per million)

Abstract

In this dissertation, various glutarimide- and macrolactone-containing natural products are described in relation to their isolation, biological studies, and synthetic preparations. In particular, synthetic and biological studies on lactimidomycin (and related analogs) in regards to its previously reported activity as a cancer cell migration inhibitor compared to recently reporterd activity as a highly antiproliferic (cytotoxic) agent are discussed.

Furthermore, the studies culminating in the total synthesis of the glutarimide-containing eukaryote translation elongation inhibitor lactimidomycin are described. The optimized synthetic route featured a Zn(II)-mediated intramolecular Horner-Wadsworth-Emmons (HWE) reaction resulting in a highly stereoselective formation of the strained 12-membered macrolactone of lactimidomycin on nearly a half gram scale. The synthetic route featured a late-stage installation of the glutarimide functionality via an asymmetric catalytic Mukaiyama aldol reaction, which allowed for a quick generation of an extended chain lactimidomycin homolog, which was tested in various biological assays alongside lactimidomycin and its truncated precursors.

Finally, the biosynthetic origin, previous C-H modification, and synthetic studies towards isopimerane- and pimerane-containing natural diterpenes are discussed. The assymetric strategy towards a key chiral intermediate was achieved, establishing a highly scalable and convergent. Furthermore, previous studies along with preliminary model studies we have conducted demonstrated the feasibility of achieving a proposed key Michael-double reaction sequence to the core of isopimerane-containing natural products such as sphaeropsidin A and C, compactone, aspewentin A and B, and their derivitized analogs.

Overall, the development of enantioselective routes to lactimidomycin, its structural analogs, and diterpene-type intermediates has successfully been accomplished.

Chapter 1: Background and previous syntheses of select anticancer macrolactone- and glutarimide-containing natural products and analogs

1.1 Introduction: natural products as a source of novel organic scaffolds

The chemical synthesis of natural products is one of the important driving forces in the development of molecular medicine. However, the gaps in synthetic methodology represents a serious impediment to the discovery of new drugs.[1-3] Natural products and their derivatives play an essential role in the drug discovery process, accounting for approximately 50% of the approved small-molecule-based drugs developed between 1981 and 2010.[4]

There are still significant synthetic challenges in the production of large quantities of molecules with complex organic scaffolds in a rapid and scalable fashion. As complex and important natural products continue to be isolated and identified, the development and application of novel synthetic methodology to conduct shorter, simpler and more scalable routes is vital in accelerating the development and future production of new therapeutics.[5]

1.2 Applications of total synthesis towards future anti-cancer therapeutics

Despite current advancements in prevention and treatment, cancer remains one of the most dangerous and lethal diseases.[6] One of the major factors responsible for the high mortality rate of various cancers is tumor metastasis, the spread of cancer from one organ or part of the body to another which is not directly connected. Therefore, developing new strategies to combat the spread of cancer could play a major role in reducing the number of deaths caused by it.[7-10] More recently, natural product-based selective cancer cell migration (CCM) inhibitors have attracted significant attention from the scientific community due to their abilities to combat the progression of metastasis, either by themselves or in combination with other therapeutic agents.[9-24]

While it is true that semi-synthetic methods and biological approaches to these compounds and other anti-cancer therapeutics do exist, the best and sometimes only way to quickly access many natural product-based analogs to assess their biogical usefulness is through synthetic methods. Organic synthesis allows for a near infinite degree of compound derivatization in forming structural analogs, while semi-synthetic methology is currently limited in its capacity to which analogs can be accessed. It is vital for researchers to establish methods that are capable of rapidly synthesizing and accessing these types of compounds in order to aid in the development of novel pharmaceuticals, as demonstrated by previous successes of natural products in drug discovery.[25]

1.3 Bioactive glutarimide-containing natural products

The glutarimide-containing natural polyketides **1**–**8** (Figure 1.3.1 and Figure 1.3.2) have attracted considerable attention in biomedical research due to their potential application as anti-cancer therapuetics.[14, 20, 26-41] These natural compounds are typically isolated from bacteria,[14, 26, 27, 33, 42] and feature a glutarimide functionality attached to a linear (**1**-**5**) or a macrolactone (**6**-**8**) side-chain.

Figure 1.3.1: Linear glutarimide-containing natural products

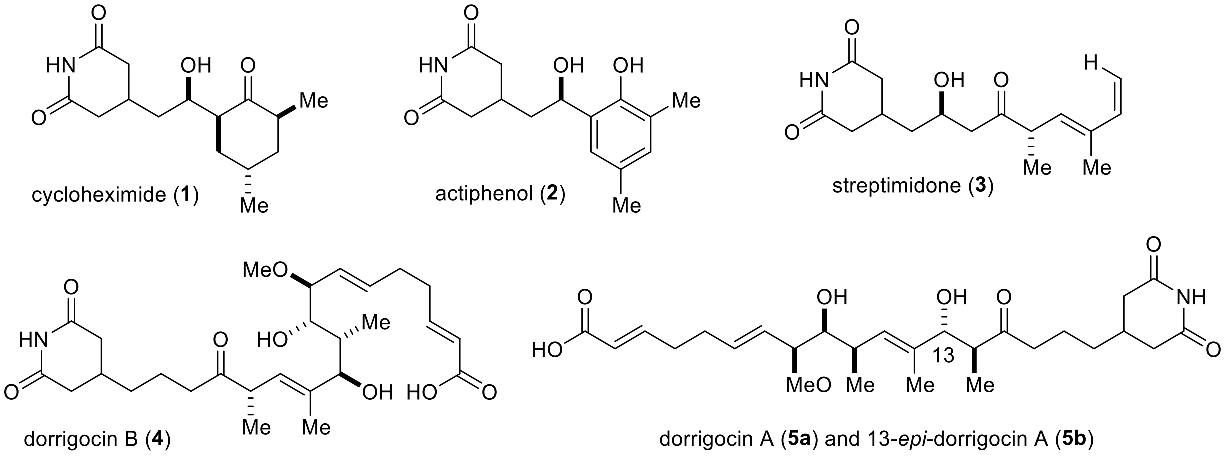
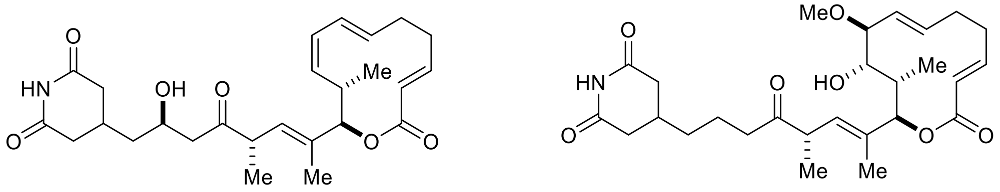
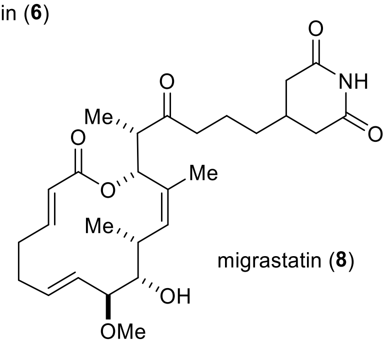


Figure 1.3.2: Macrolactone- and glutarimide-containing natural products



lactimidomycin (**6**)

isomigrastatin (**7**)

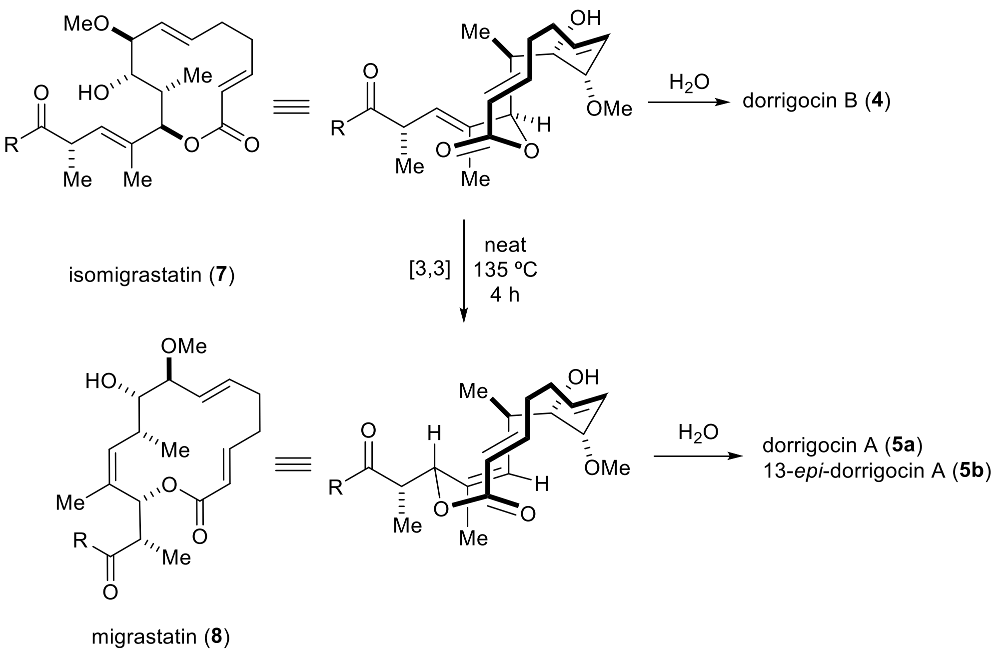


migrastatin (**8**)

One of the most well-studied members of this family, cycloheximide (**1**), is routinely used a potent inhibitor of eukaryotic translation elongation; however, its dehydrated/aromatized counterpart, actiphenol (**2**) lacks this activity.[33] While the glutarimide moiety generally represents the key biological element of these natural products, the macrolactone portion of these molecules has also found to be an important factor in the biological properties of some of these compounds.[14, 26-35]

1.4 Biosynthetic relationship of migrastatin, isomigrastatin, and dorrigocins A and B

The presence of an unsaturated macrolactone in **6–8** significantly affects their stabilities. The Shen group has demonstrated that isomigrastatin **7** can be derived into shunt metabolites **8**, **4**, and **5a**, as well as 13-*epi*-dorrigocin A (**5b**) via an H_2O-mediated rearrangement (Scheme 1.4.1).[43] It was observed that the thermolysis of **7** in the presence of water resulted in an unprecedented [3,3] sigmatropic rearrangement/ring expansion that produced migrastatin (**8**). The macrolactone moieties of **7** and **8** are prone to hydrolysis, and the formation of **4**, **5a**, and **5b** were also observed. Interestingly, the presence of an 8*E*-olefin in lactimidomycin (**6**) prevents this thermal rearrangement, with no ring-expansion products detected for **6**. However, the 12-membered macrolactone of lactimidomycin possesses significant ring strain and was found to undergo polymerization and decomposition upon storage in the neat state or when exposed to acids.[44, 45]

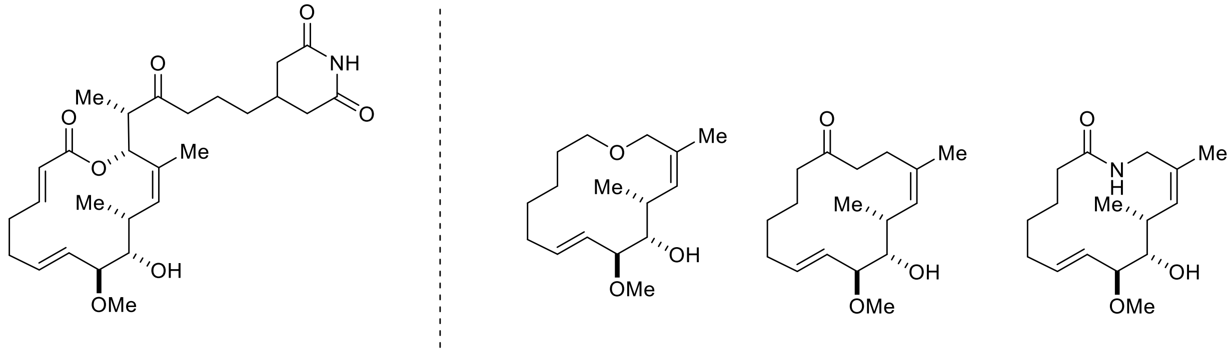


Scheme 1.4.1: Derivatization of 7 into shunt metabolite 8 and the dorrigocins (4, 5a-b)

1.5 Diverted total synthesis of migrastatin and its analogs

Migrastatin (**8**) was first isolated from *Streptomyces sp.* MK929-43F1 and was found to possess modest tumor cell migration inhibitory activity.[14] Many recent studies have focused on investigating the anticancer activity of **8**, as this compound's analogs were found to selectively and significantly inhibit cancer cell migration during the seminal work by Danishefsky and his group.[20-23] Through their pioneering studies, they were able to accomplish the first total synthesis of migrastatin (**8**) and utilize a diverted total synthesis (DTS) to discover several analogs that contained impressive cell-migration inhibition activity. In particular, their DTS route allowed them to produce the simple and stable analogs **8a**–**8c**, which display potent activity against breast cancer metastasis (Scheme 1.5.1).

Interestingly, these studies demonstrated that the glutarimide side-chain of **8** could be removed without affecting the antimetastatic activity of migrastatin. Subsequent *in vivo* work demonstrated that some of these analogs, in particular, **8a**, could serve as potential selective and nontoxic inhibitors of various types of breast cancer metastasis.[46-48]

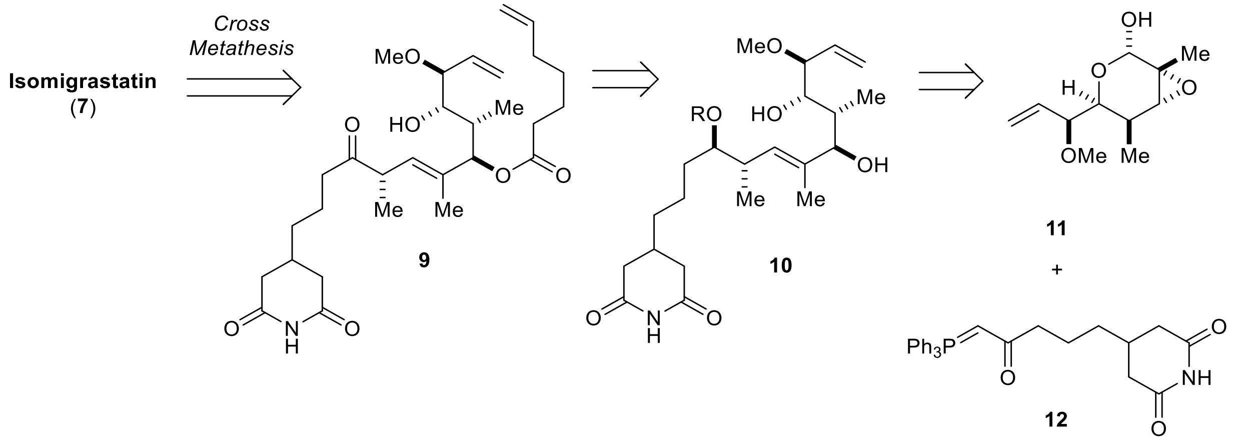


migrastatin (**8**) macroether (**8a**) macroketone (**8b**) macrolactam (**8c**)

Scheme 1.5.1: Migrastatin and related macrocyclic anticancer analogs 8a-8c

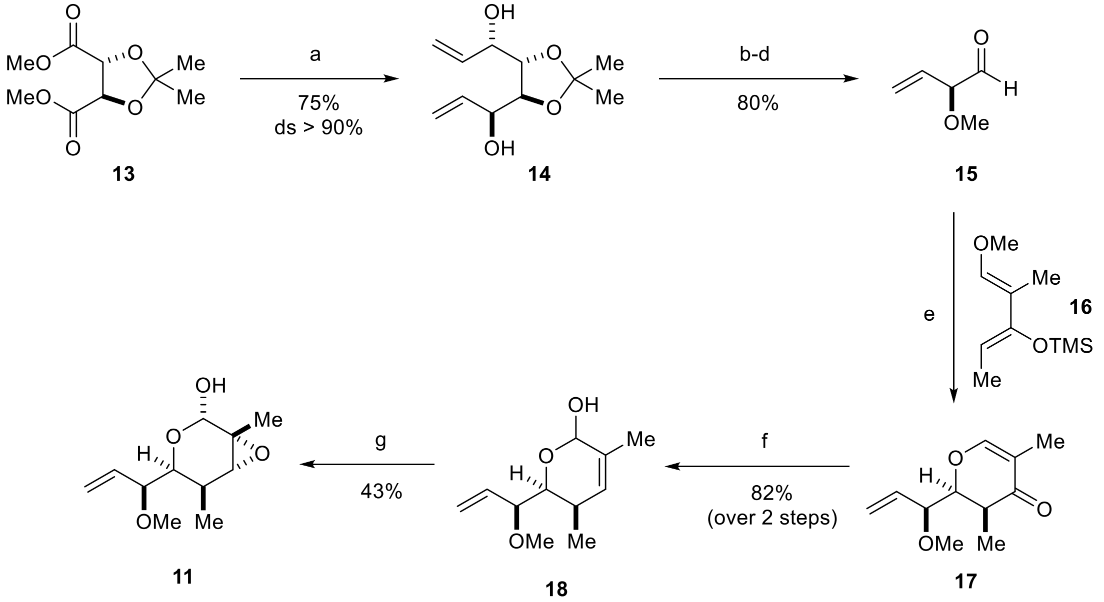
1.6 Danishefsky's total synthesis of isomigrastatin

Isomigrastatin (**7**) was first isolated in 2002 as the main product from *Streptomyces platensis* strain NRRL18993.[49] With promising reported biological activity, Danishefsky's group quickly moved to develop a synthetic route.[50] Their retrosynthetic plan involved a ring-closing metathesis of **9** to form the 12-membered macrolactone of **7**, utilizing intermediates similar to their previous work on migrastatin (**11**-**12**) to lead to **10** and **9** (Scheme 1.6.1).



9 **10** **11** **12**

Scheme 1.6.1: Retrosynthetic analysis of isomigrastatin (7)

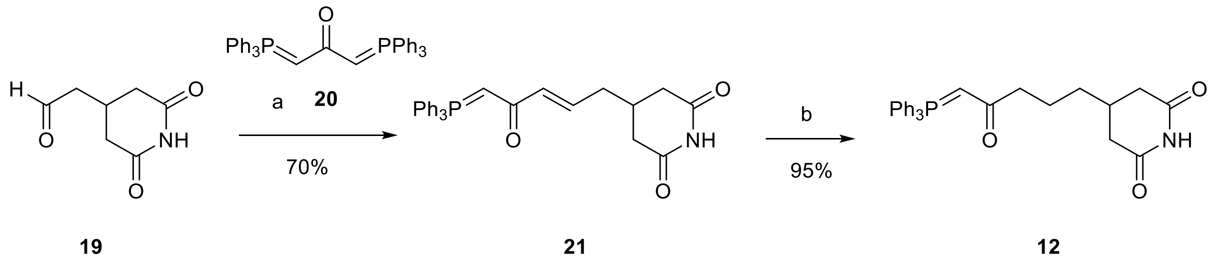


Conditions and Yields: a) DIBAL; then $ZnCl_2$, $H_2C{=}CHMgBr$; b) NaH, MeI; c) HCl, THF, reflux; d) $Pb(OAc)_4$, Na_2CO_3; e) i. **16**, $TiCl_4$, ii. TFA; f) i. $NaBH_4$, $CeCl_3{:}H_2O$, ii. CSA, THF/H_2O (9:1); g) *m*-CPBA, K_2CO_3, DCM, 0 °C.

Scheme 1.6.2: Synthesis of epoxy lactol intermediate 11

The hetero-Diels Alder of known aldehyde **15** with known diene **16** proceeded using a catalytic amount of titanium tetrachloride, and removal of the TMS group with TFA gave the desired pyrone **17**. Reduction with sodium borohydride and treatment with acid (Ferrier rearrangement) formed **18**. Finally, the olefin was treated with *m*-CPBA to form the epoxy lactol **11**.

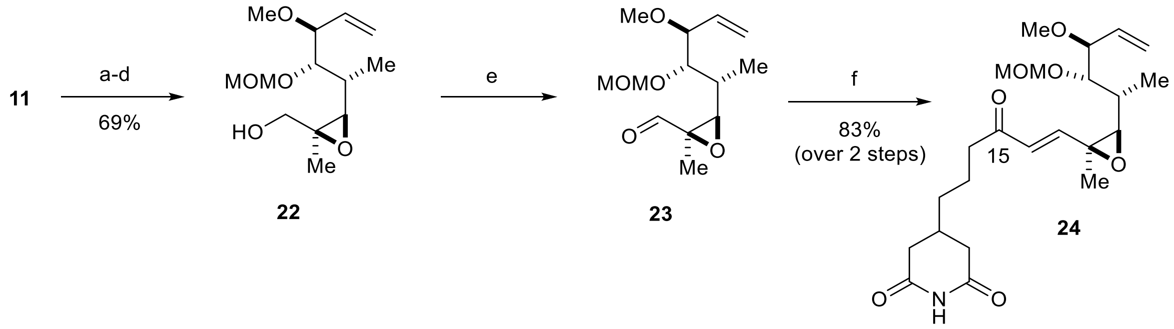
Separately, the known glutarimide-containing aldehyde **19** was olefinated utilizing the known phosphanylidene **17** (Scheme 1.6.3). Compound **21** was hydrogenated using palladium on carbon to generate the functionalized Wittig reagent **12**.



a) **20**, DMSO, rt; b) H_2, Pd/C, THF/MeOH

Scheme 1.6.3: Synthesis of the glutarimide-containing Wittig reagent 12

A series of reduction, protection, and oxidation reactions were conducted to form the alcohol 23 from 11. After oxidation to aldehyde 23, this compound could undergo the desired Wittig reaction with **12** to produce **24** (Scheme 1.6.4).

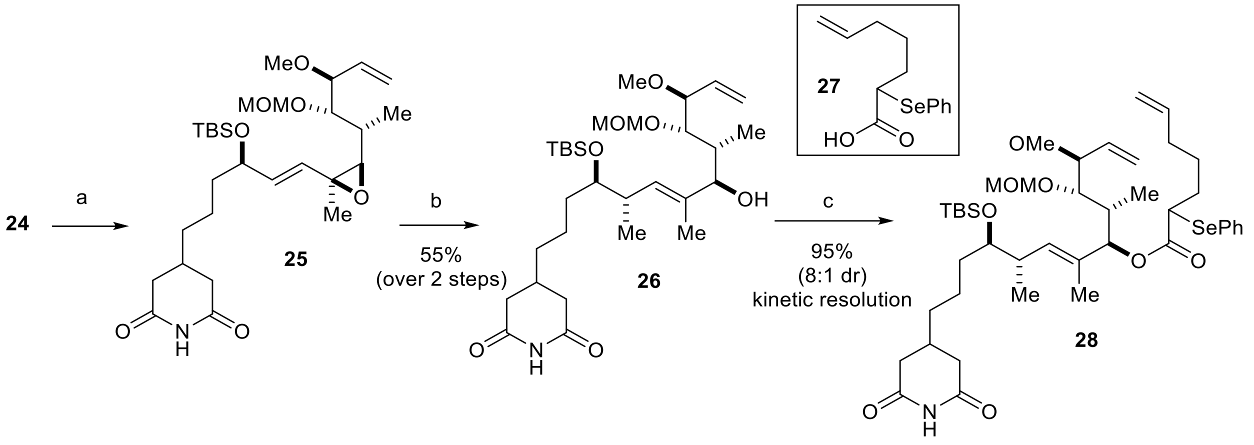


a) $LiBH_4$, THF/H_2O; b) Ac_2O, pyridine; c) MOMCl, iPr_2NEt; d) K_2CO_3, MeOH; e) $(COCl_2)$/DMSO, -78 °C; then iPr_2NEt_2; f) **12**, $DMSO/CHCl_3$, rt.

Scheme 1.6.4: Synthesis of glutarimide-containing intermediate 23

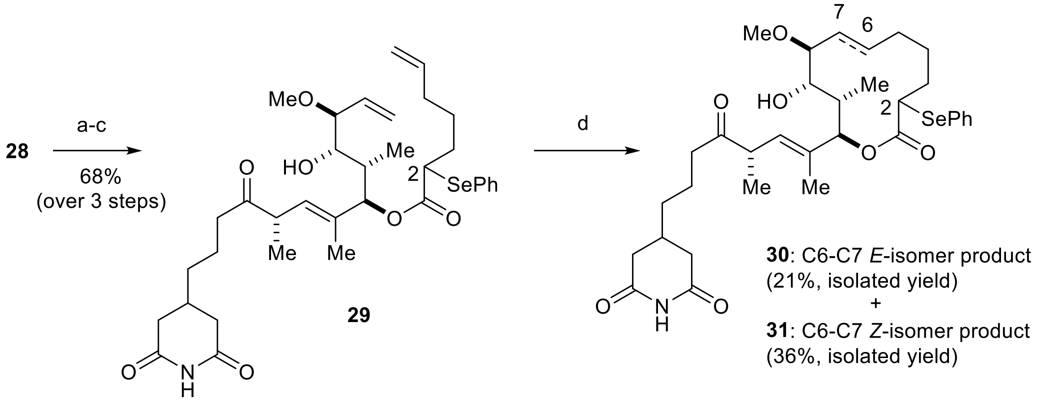
A diastereoselective reduction of the C-15 ketone (**24**) with the (*S*)-CBS catalyst, followed by TBS protection yielded **25** (Scheme 1.6.5). This TBS-protected alcohol functionality in **24** acted as a control element for the stereodirected ring-opening reaction with methylcuperate to proceed with good diastereoselectivity (13:1 dr, 83% yield) to form **26**. As the macrocycle of **7** is known to be notoriously unstable due to the C2-C3 *E*-olefin (Scheme 1.4.1),[43] it was seen best to hide this functionality to avoid issues of decomposition. A selenidoxide elimination was envisioned to do this, allowing introduction this olefin at a later stage. Furthermore, the absence of this olefin was predicted to help the RCM reaction proceed, rather than the direct formation of the highly-strained

12-membered macrolactone of **7**. Accordingly, esterification of **26** was done with **27** to provide the desired α-phenylselenoester **28**. A routine series of deprotection/oxidation reactions were conducted on **28** to form RCM precursor **29** (Scheme 1.6.6).



a) (*S*)-Me-CBS, BH_3;Me_2S, TBSCl, DMAP, Et_3N; b) MeCuCNLi, Et_2O; c) (±)-**27** (excess), EDCI, DMAP, (3 equiv each)

Scheme 1.6.5: Diastereoselective reduction, stereodirected addition with methylcuperate, and esterification reaction to transform 24 into 28



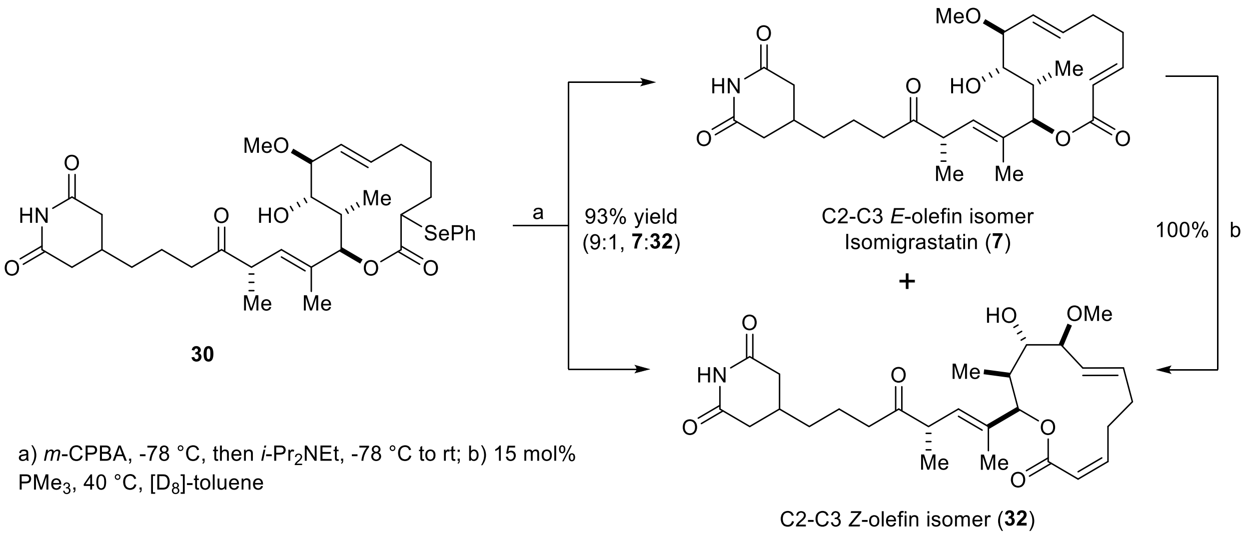
30: C6-C7 *E*-isomer product (21%, isolated yield)
+
31: C6-C7 *Z*-isomer product (36%, isolated yield)

a) HF•pyridine; b) $(COCl)_2$, DMSO, -78 °C, then *i*-Pr_2NEt; c) Me_2BBr, *i*-Pr_2NEt, - 78 °C, then THF/$NaHCO_3$;
d) Grubb's II (20 mol %), toluene, 110°C, 2 min

Scheme 1.6.6: Synthesis of 29 and RCM reaction to form key macrocycle 30 and stereoisomer 31

The 2^{nd} generation Grubbs' catalyst was used to effect this ring-closing metastasis (RCM) to form **30** in only 2 minutes, but resulted in a lower than desired yield of the C6-C7 *trans*-olefin product. In fact, a greater amount of the starting material was transformed into the undesired C6-C7 *cis*-isomeric product, **31**.

The second *E*-olefin was introduced by subjecting **30** to *m*-CPBA, causing the predicted oxidative elimination of -SePh to install the C2-C3 *trans*-olefin of **7** (), finishing the first total synthesis of isomigrastatin (**7**). The spectroscopic data of the synthetic sample of **7** matched to the previously described data of its corresponding biologically isolated sample.[49]



a) *m*-CPBA, -78 °C, then *i*-Pr_2NEt, -78 °C to rt; b) 15 mol% PMe_3, 40 °C, [D_8]-toluene

Scheme 1.6.7: Oxidative elimination of 29 to isomigrastatin (7) and isomerization of 7 to 32

Interestingly, while the oxidative elimination resulted in the desired C2-C3 *E*-olefin isomer, it was also observed that the compound was also forming the C2-C3 *Z*-olefin isomer, **32**. This major decomposition route was determined to be driven by the thermodynamically favorable removal of ring strain via isomerization. This was demonstrated through the treatment of **7** to PMe_3-catalyzed *E-Z* equilibrating conditions, which resulted in complete conversion of the material to the more stable isomer, **32**.

1.7 Lactimidomycin: A highly bioactive 12-membered macrolactone

Lactimidomycin (**6**) is a glutarimide-containing natural product isolated by Sugawara *et al.*

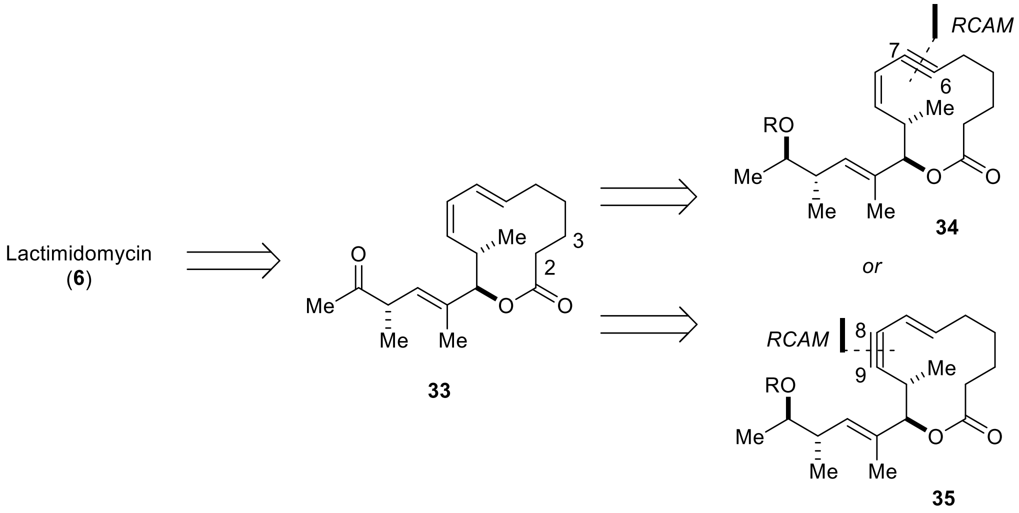
in 1992 from *Streptomyces amphibiosporus* strain ATCC 53964 and reported to contain a variety of biological activities (antifungal, antibiotic, antiproliferative).[26] Furthermore, Sugawara *et al.* demonstrated that **6** exhibits inhibitory activity against P388 leukemia tumors implanted in mice through observing the extended survival time of mice treated with the compound.[26]

Initially, *in vitro* studies by the Shen group indicated that **6** strongly inhibits the migration of human MDA-MB-231 (IC_{50} = 0.6 nM) and mouse 4T1 (IC_{50} = 5.03 nM) mammary adenocarcinoma cells.[51] In 2010, studies by the Liu group showed that lactimidomycin (**6**) is a strong inhibitor of eukaryotic translation elongation (IC_{50} = 38 nM);[52] in other words, the antitumor activity from **6** may occur through blocking the translocation of the E-site of the 60S ribosomal subunit. Furthermore, they demonstrated that **6** had an appreciable effect on tumor growth of MDA-MB-231 breast cancer cells *in vivo*. In 2013,[53] the Fürstner group found, in contrast to previous studies, lactimidomycin's antiproliferic activity against certain cancer cell lines was even higher than previous reported, although his results are in conflict to the previous findings that **6** can act as a CCM inhibitor in subtoxic doses.[51]

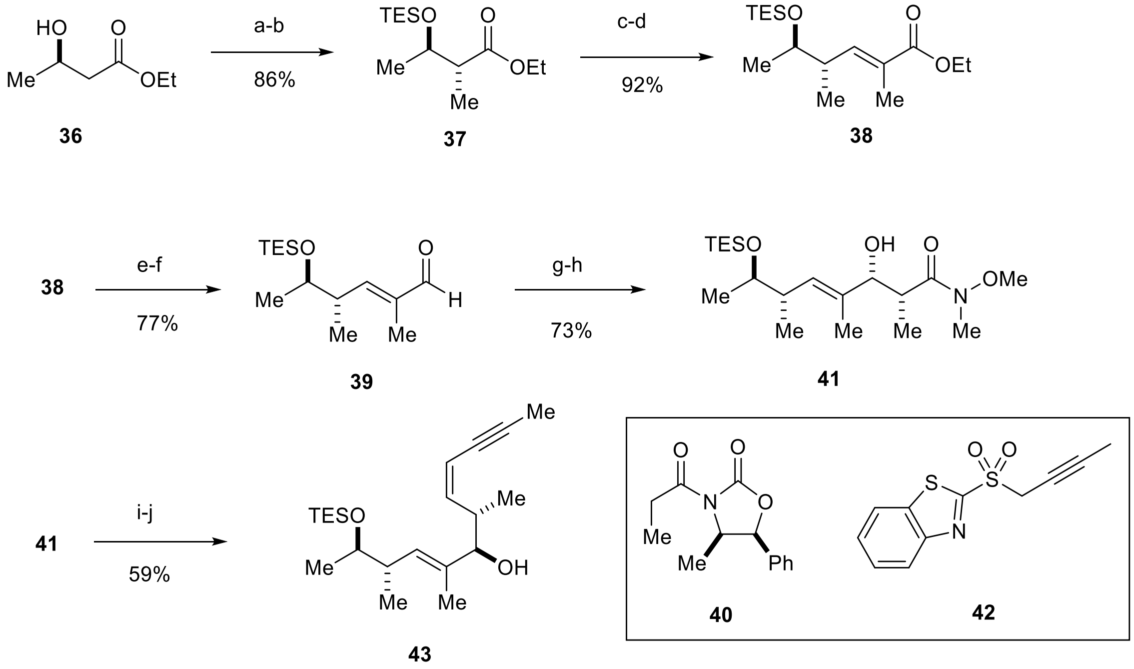
This data suggests that lactimidomycin (**6**) is a potent anticancer agent, but its relatively high *in vivo* toxicity (lethal dose 32 mg/kg in mice),[26] instability in solution,[51] and conflicting reports of CCM inhibition activity[51, 53] have significantly complicated the use of this natural product as an anticancer therapeutic.

1.8 Fürstner's first total synthesis of lactimidomycin

In 2010, the Fürstner group published the first total synthesis of lactimidomycin.[54] The route is notable in that it utilizes a unique ring-closing alkyne metathesis (RCAM) reaction to form the precursor to the strained 12-membered macrolactone ring.



Scheme 1.8.1: Fürstner's retrosynthetic analysis of lactimidomycin (6)

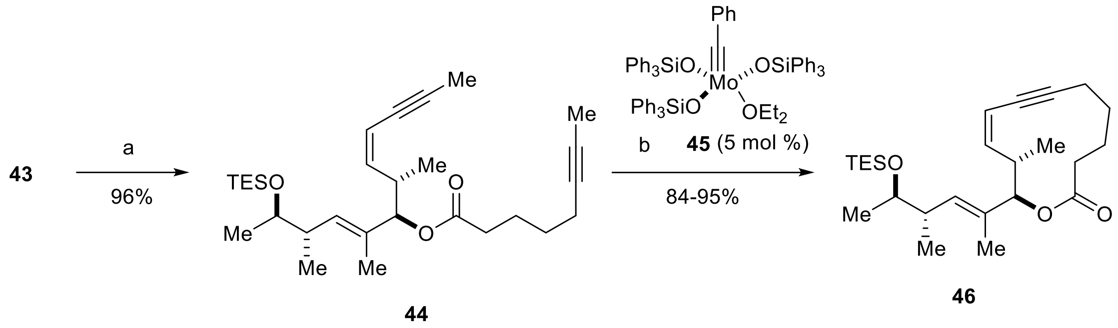


a) LDA (2 equiv), THF/HMPA, MeI, -78 °C to 0 °C; b) TESCl, Py, DCM; c) DIBAL-H, CH_2Cl_2, -78 °C; d) $Ph_3P{=}C(Me)COOEt$, THF, Δ; e) DIBAL-H, CH_2Cl_2, -78 °C to 0 °C; f) PCC, DCM; g) **40**, Bu_2BOTf, Et_3N, CH_2Cl_2, -78 °C to rt, h) MeNH(OMe)·HCl, Me_3Al, THF, -10 °C,; i) $LiAlH_4$, THF, -78 °C to 0 °C; j) **42**, KHMDS, THF, -55 °C

Scheme 1.8.2: Synthetic route to the alkyne intermediate 43

Their initial proposal involved a retrosynthetic breakdown of the molecule into one of two RCAM precursors, **34** and **35** (Scheme 1.8.1). Both of these intermediates could be selectively reduced into ketone **33**, which they planned to attach the glutarimide functionality.

First, a low-temperature mediated stereoselective alkylation of commercially available **36** with MeI was performed, followed by a TES protection to form **37** (Scheme 1.8.2). Intermediate **37** was reduced with DIBAL-H into its corresponding aldehyde, which was treated with a Wittig reagent to form **38**. The material was submitted to another DIBAL-H reduction and was transformed into the aldehyde **39** via a PCC oxidation reaction. With the desired aldehyde in hand, **39** was treated with the chiral auxiliary **40** to establish two new stereocenters, which was transformed into Weinreb's amide **41**. Compound **41** was reduced to its aldehyde form which could undergo a selective Julia coupling with **42** to form isomerically pure *Z*-enyne **43**.



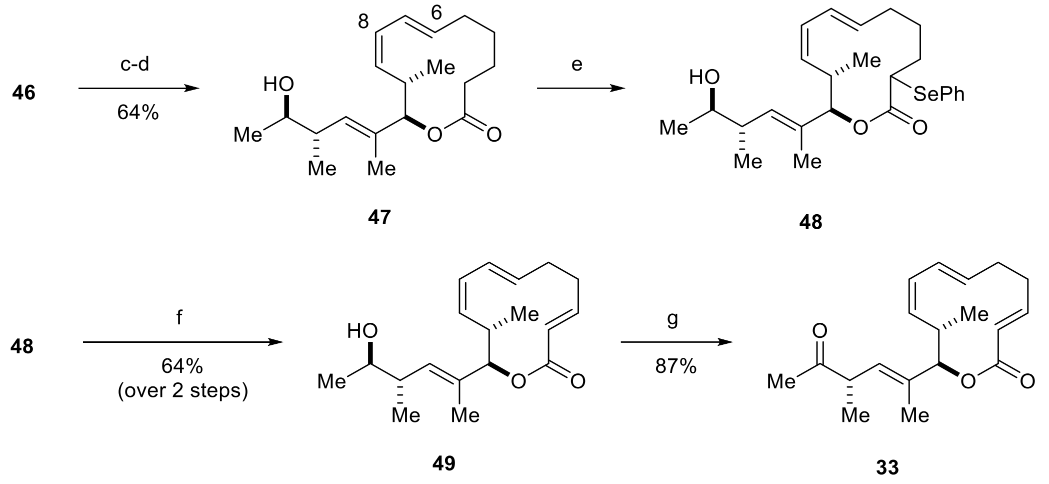
a) 6-octynoic acid, EDC, DMAP, DCM, 0 °C to rt; b) **45** (5 mol %) toluene, MS 5 Å, 80 °C

Scheme 1.8.3: Key RCAM to the macrolactone 46

With intermediate alcohol **43** in hand, they converted it to **44** via a Yamaguchi esterification reaction with 6-octynoic acid (Scheme 1.8.3). Next, they were able to successfully close the ring using catalyst **45** under high dilution conditions via a RCAM, resulting in yields of 84% and 95% on scales of 240 mg and 1.2 g, respectively, of the desired 12-membered enyne **46**.

This compound was treated with $BnMe_2SiH$ and $[Cp^*Ru(MeCN)_3]PF_6$ followed by TBAF to effect a *trans*-hydrosilylation/protodesilyation, converting intermediate **46** into the alcohol **47** (), a key intermediate in every published formal and total synthesis of lactimidomycin (**6**). This compound was transformed to its lithium enolate form with LDA, which was quenched with PhSeBr to form

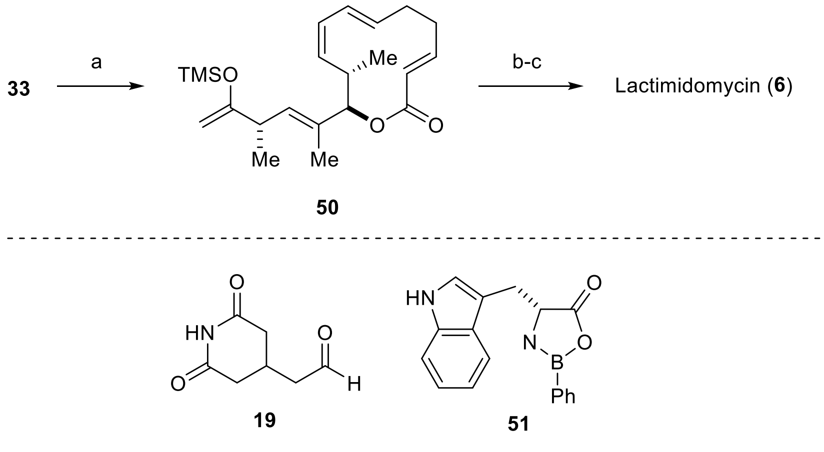
48. An oxidative elimination with *m*-CPBA yielded the desired C2-C3 unsaturated alcohol **49**, another key intermediate. Oxidation of this alcohol **49** with DMP generated the ketone form, **33**.



c) $BnMe_2SiH$, $[Cp^*Ru(MeCN)_3]PF_6$ (10 mol %), CH_2Cl_2, 0 °C to rt; d) TBAF, THF, 0 °C to rt; e) i. LDA, THF, -78 °C to 0 °C; ii. PhSeBr, -78 °C to 0 °C; f) i. *m*-CPBA, DCM, -78 °C; ii. *i*-$PrNEt_2$, -78 °C to rt; g) DMP, CH_2Cl_2.

Scheme 1.8.4: Late stage introduction of the C6-C7 and C2-C3 olefins in the formation of alcohol 49 and ketone 33

Utilizing the ketone intermediate (**33**), the crude silyl enol ether form (**50**) was prepared (Scheme 1.8.5).



a) LiHMDS, TMSCl, Et_3N, THF, -78 °C; b) **19,** EtCN, then **51,** -78 °C; c) HF·pyridine, THF/pyridine, 0 °C; 60% (over 3 steps)

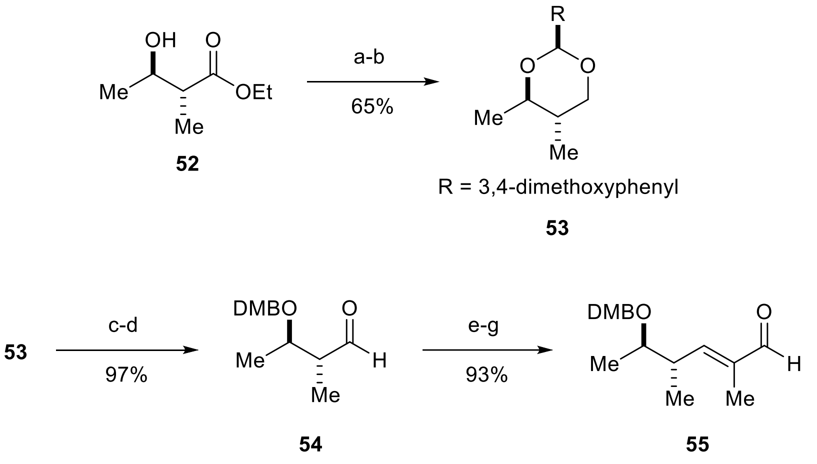
Scheme 1.8.5: Completion of the total synthesis of 6 via a key chiral-catalyzed Mukiyama aldol reaction

A chiral Mukiyama aldol reaction of **50** with aldehyde **19**, in presence of chiral catalyst **51**, stereoselectively formed TMS-protected form of **6**. This crude material was treated with buffered HF•pyridine complex and isolated via preparatory TLC to finish the first total synthesis of lactimidomycin (**6**). The synthetic **6** was formed in 60% yield over these 3 final steps, and its spectroscopic data matched with that of the previous literature.[26, 51]

1.9 Fürstner's studies towards lactimidomycin via RCM reactions

Following this work, the Fürstner group published another paper demonstrating a formal total synthesis of **6**. Following a similar approach to Danishefsky's route to **7**,[50] they proposed to access the 12-membered macrolactone of **6** via a silyl-directed ring closing metathesis (RCM) reaction. Through the use of trisubstitued vinyl silane into the typical tetraene macrocyclization precursor, they hoped to increase the yields of the RCM reaction. The main focus of their work was to showcase the additional benefits of the vinyl silicon substituent in the field of metathesis in that it can act as both a protecting and stereochemical directing group for the formation of conjugated dienes; the bulky silyl group renders its olefin unreactive and also directs the newly formed olefin to the less hindered geometry.

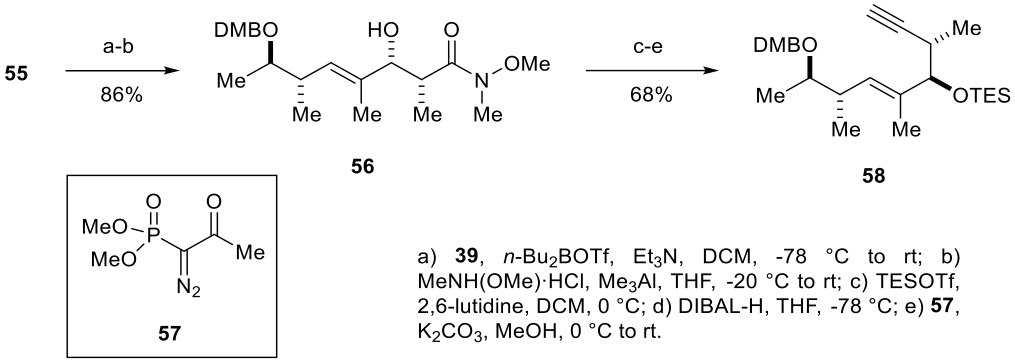
In a route similar to their previous one, but featuring a DMB protecting group this time,[54] aldol **52** was converted to aldehyde **55** through a known series of reactions (Scheme 1.9.1).[55]



a) $LiAlH_4$, Et_2O, 0 °C; b) $(MeO)_2C_6H_3CHO$, *p*-TsOH, benzene, 80 °C; c) DIBAL-H, DCM, -50 °C to 0 °C; d) $(COCl)_2$, DMSO, Et_3N, DCM, -78 °C to 0 °C; e) Ph_3P=(Me)COOEt, DCM; f) DIBAL-H, DCM, -78 °C; g) SO_3•Py, DMSO, Et_3N, DCM

Scheme 1.9.1: Transformation of aldol 52 into aldehyde 55

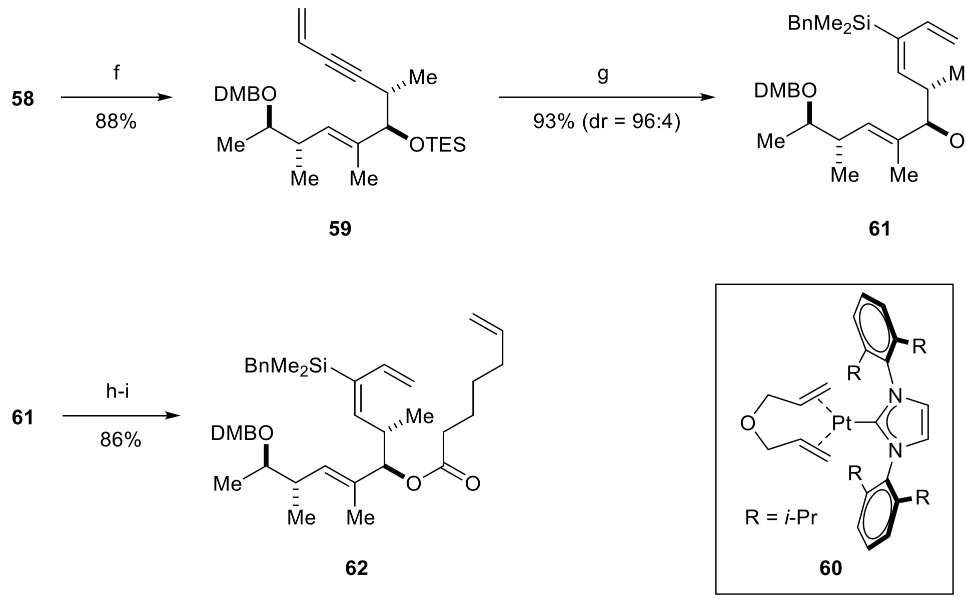
With DMB-protected **55**, they utilized the same series of reactions as before to reach **58**. Two stereocenters with auxiliary **39**¸ and then removed this auxiliary to form **56** (Scheme 1.9.2).



a) **39**, *n*-Bu_2BOTf, Et_3N, DCM, -78 °C to rt; b) MeNH(OMe)·HCl, Me_3Al, THF, -20 °C to rt; c) TESOTf, 2,6-lutidine, DCM, 0 °C; d) DIBAL-H, THF, -78 °C; e) **57**, K_2CO_3, MeOH, 0 °C to rt.

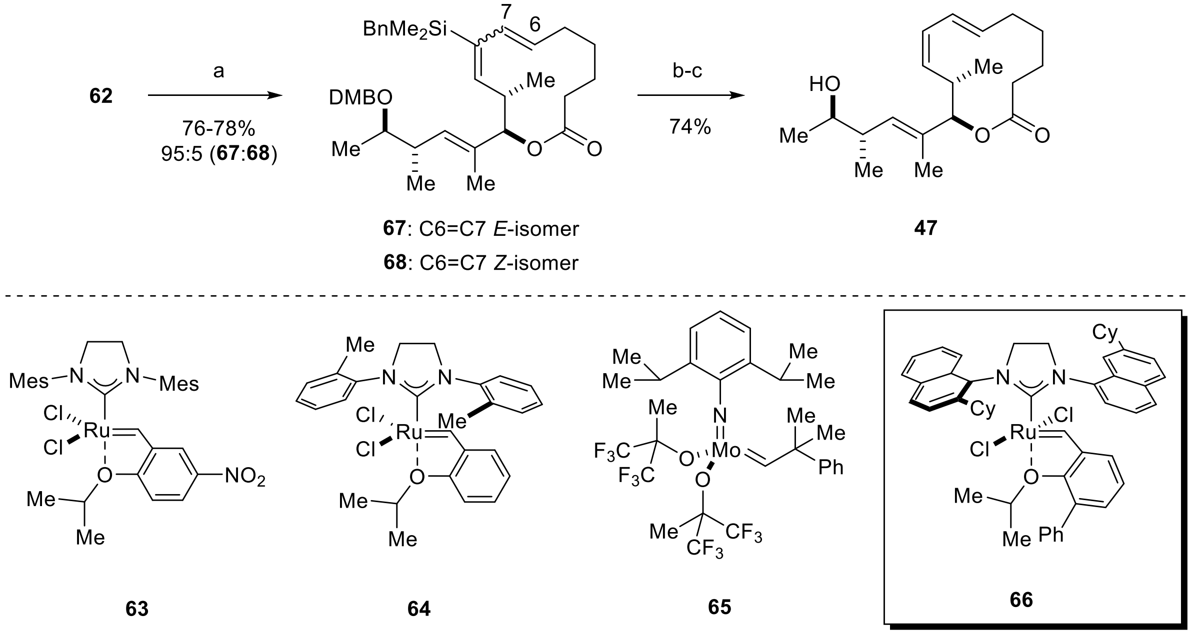
Scheme 1.9.2: Synthesis of alkyne 58

After protection with TES and DIBAL reduction to the aldehyde, they diverged significantly from their first generation approach, utilizing Ohira-Bestmann reagent **57** to form alkyne **58**.[56, 57] The double protected diol **58** was submitted to a Sonagashira reaction to form **59** (Scheme 1.9.3).[58]



f) $H_2C{=}CHBr$, CuI, $(PPh_3)_2PdCl_2$ (5 mol %), *i*-Pr_2NH, THF; g) **60** (1 mol %), $BnMe_2SiH$ (3 eq); h) PPTS (cat.), EtOH;i) 6-heptenoic acid, EDC·HCl, DMAP, DCM, 0 °C to rt

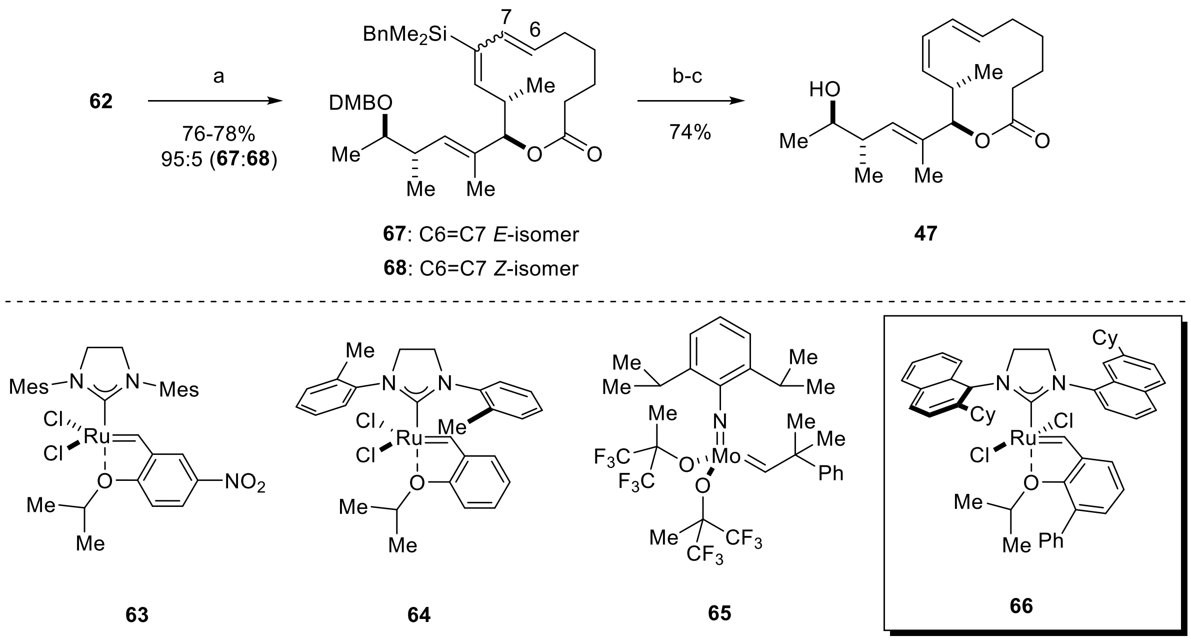
Scheme 1.9.3: Preparation of RCM precursor 62 with silyl directing group



a) Catalyst **66** (10 mol %), 120 °C, b) DDQ, CH_2Cl_2, pH 7 buffer, 0 °C; c) TBAF, THF, 60 °C

Scheme 1.9.4: Completion of the formal total synthesis via a vinyl-silane directed intramolecular *E*-selective RCM

A series of steps were then used, including a regioselective hydrosilyation of **59** with platinum complex **60** to form **61** (dr = 96:4, 93% yield), removal of the TES group, and esterification with 6-heptenoic acid to form the RCM precursor, **62**. With intermediate **62** in hand, a variety of conditions were screened to optimize the intramolecular RCM reaction to preferentially promote formation of the C6-C7 *E*-olefin containing macrolactone **67** over its *Z*-isomeric form **68** (Scheme 1.9.5).



a) Catalyst **66** (10 mol %), 120 °C, b) DDQ, CH_2Cl_2, pH 7 buffer, 0 °C; c) TBAF, THF, 60 °C

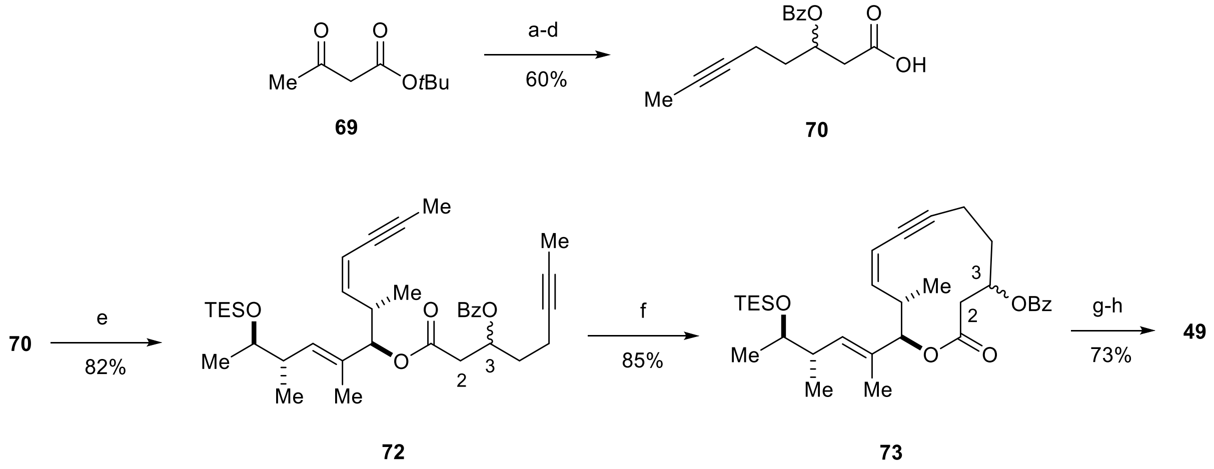
Scheme 1.9.5: Completion of the formal total synthesis via a vinyl-silane directed intramolecular *E*-selective RCM

The hindered Dorta catalyst **66** was identified as superior to the other screened catalysts (**63**,[59] **64**, and **65**) in selectively bringing together the terminal olefins to form the C6-C7 *E*-olefin containing **67**. This compound was then submitted to two deprotection steps, removing the DMB and silyl protecting groups, capturing intermediate **47** (Scheme 1.8.3) featured in their previous publication.[54]

1.10 Scaled up synthesis of lactimidomycin (6)

As previously detailed, two routes to lactimidomycin (**6**) were demonstrated by the Fürstner group in 2010 and 2011, utilizing novel, but commercially unavailable catalysts (**45**, **66**), and through late stage establishment of the α,β-unsaturated lactone functionality.[54, 60] Following their previous work, an optimized protocol detailing the syntheses of **6** and **7** was published by Fürstner group in 2013.[53]

First, acid **70** was prepared from β-ketoester **69** in 4 steps (Scheme 1.10.1). This acid was used to esterify intermediate **42** under Yamaguchi conditions[61, 62] and form **72**. The racemic form of **72** was produced with the reasoning that in eventually forming **49**, this stereocenter would be lost upon elimination anyways.



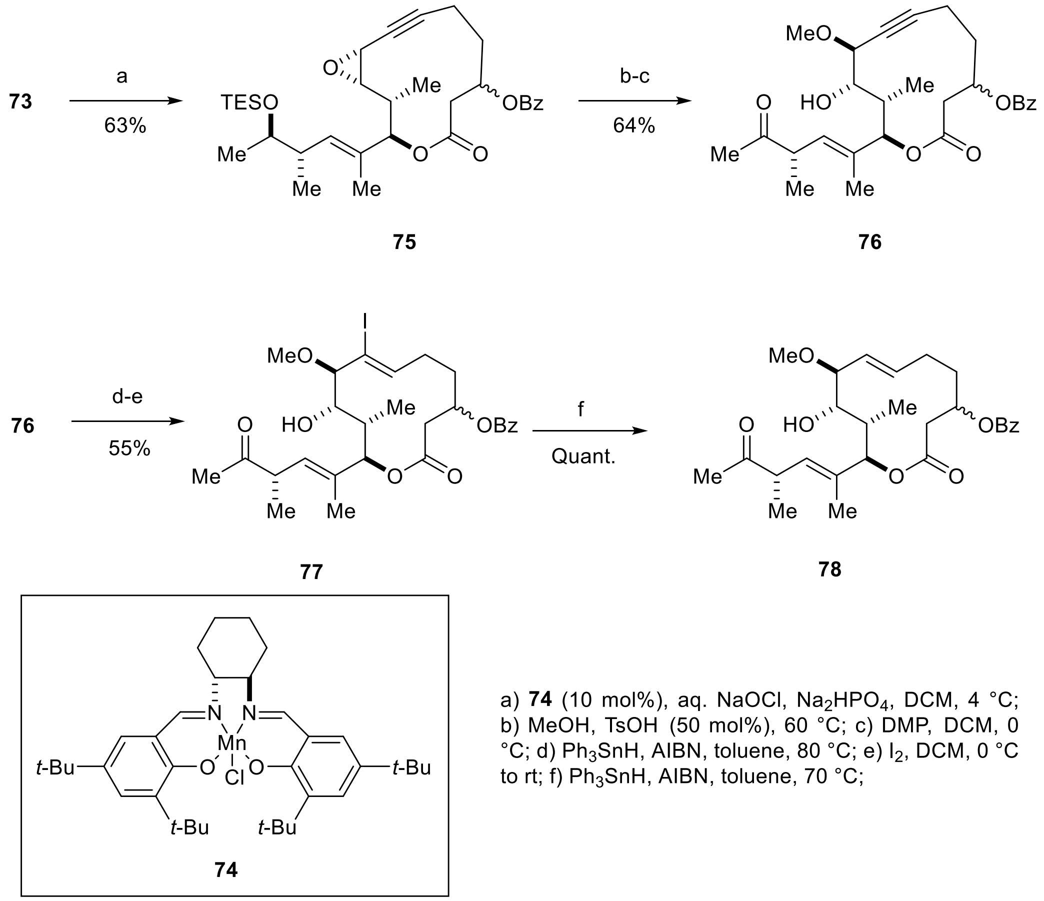
a) i. NaH, THF, 0 °C; ii. *n*-BuLi, 1-bromo-2-butyne, 0 °C; b) $NaBH_4$, THF; c) BzCl, Et_3N, DMAP, CH_2Cl_2; d) TFA, CH_2Cl_2, quant.; e) 2,4,6-trichlorobenzoyl chloride, Et_3N, toluene, 0 °C, then **42**, DMAP; f) **44** (5 mol%), 5Å MS, toluene, 80 °C; g) $[Cp^*Ru(MeCN)_3]PF_6$, (10 mol%), $BnMe_2SiH$, DCM, 0 °C to rt; h) TBAF, THF, 0 °C to rt

Scheme 1.10.1: Second generation RCAM-based methodology in the total synthesis of lactimidomycin

Once again, the RCM precursor **72** lacked the C2-C3 olefin, in an effort to reduce ring strain and promote stability in the desired macrocycle **73**. The alkyne functionality of **73** was transformed into the *trans*-olefin via the previously established 2-step hydrosilylation/desilylation protocol, which, in tandem, eliminated the C3-OBz functionality to form of the C2-C3 olefin. The resulting ketone intermediate **49** was formed and was confirmed to contain the fully functionalized lactimidomycin macrolactone. This material was further transformed following the same methodology as before (Scheme 1.8.5),[54] but on a much larger scale (138 mg, largest batch).[63]

1.11 Diverted total synthesis: The second total synthesis of isomigrastatin (7)

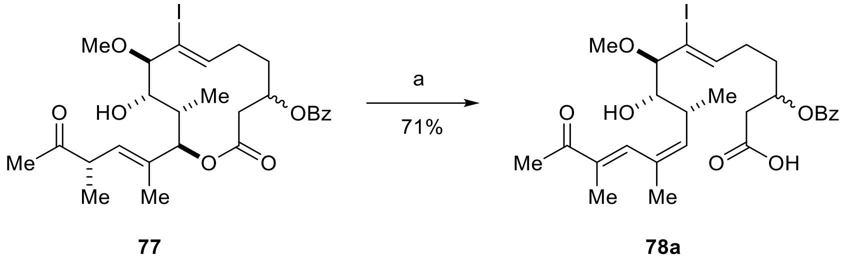
The key intermediate in the scaled up synthesis of lactimidomycin (Scheme 1.10.1), compound **73**, could also be stereoselectively epoxidized into **75**, which was converted into **76** via a methanolysis/deprotection sequence (Scheme 1.11.1).



a) **74** (10 mol%), aq. NaOCl, Na_2HPO_4, DCM, 4 °C; b) MeOH, TsOH (50 mol%), 60 °C; c) DMP, DCM, 0 °C; d) Ph_3SnH, AIBN, toluene, 80 °C; e) I_2, DCM, 0 °C to rt; f) Ph_3SnH, AIBN, toluene, 70 °C;

Scheme 1.11.1: Diverted total synthesis of 73 to isomigrastatin intermediate 77

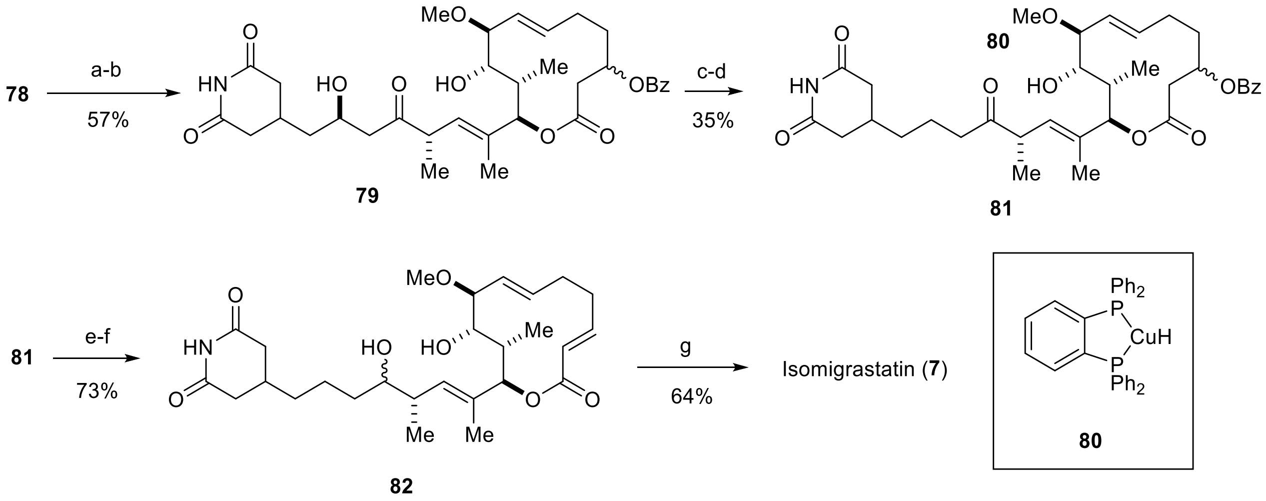
Intermediate **76** was subjected to a reduction/iodination sequence to form iodide **77**, which was dehalogenated to form **78**. Interestingly, the a DBU promoted elimination of the OBz group was initially tested on **77** and was found to inadvertently promote a ring-opening decomposition pathway into **78a**, further demonstrating the inherent instability of these advanced macrocycle intermediates (Scheme 1.11.2).



a) DBU, THF, 4Å MS, then aq. HCl

Scheme 1.11.2: Base-catalyzed decomposition of the macrolactone-ring to acid 82

Compound **78** was subjected previously employed conditions (Scheme 1.8.5) to attach the glutarimide side chain to form **79** (Scheme 1.11.3).



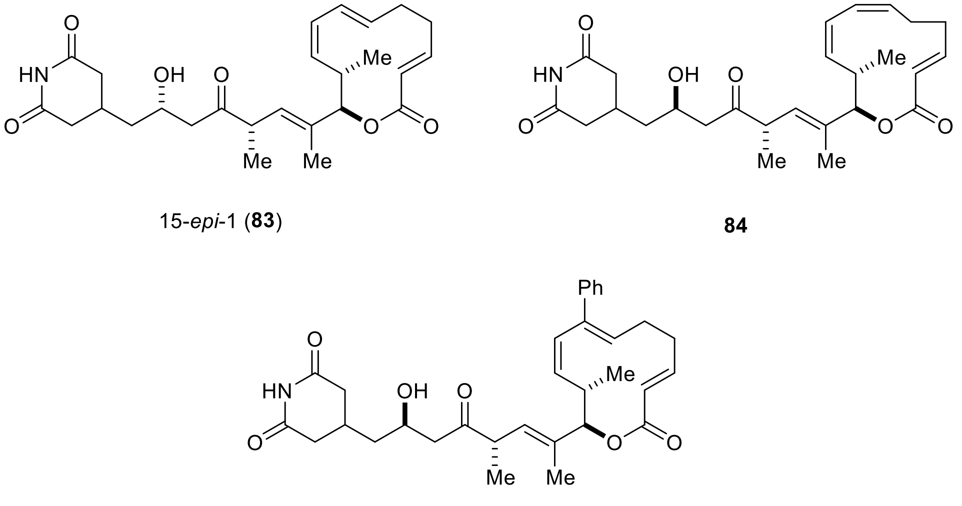
a) TMSCl, Et_3N, THF, -78 °C, then LiHMDS; b) **19**, 4Å MS, propionitrile, then **51**, -78 °C; c) I_2, ImH, PS-PPh_3, DCM, 0 °C to rt; d) **80** (excess), toluene; e) $NaBH_4$, THF, 0 °C to rt; f) DBU, THF; g) DMP, DCM, 0 °C

Scheme 1.11.3: Completion of the diverted total synthesis to form isomigrastatin (7)

Intermediate **79** was treated to a condensation reaction, eliminating the alcohol functionality, followed by a selective hydrogenation with excess **80** to form **81**. The ketone functionality of **81** was unselectively reduced with sodium borohydride, and careful treatment with DBU eliminated the OBz group to form **82**. Impressively, these DBU conditions did not promote the previously seen decomposition (Scheme 1.11.2), and a DMP oxidation of alcohol **82** completed the second reported total synthesis of isomigrastatin (**7**).

1.12 Biological results observed by the Fürstner group on 6, 7, and their analogs

Furthermore, the Fürstner group synthesized a variety of macrolactone-based analogs, including **83-85** (Scheme 1.12.1).[53]



15-*epi*-1 (**83**)

84

85

Scheme 1.12.1: Selected lactimidomycin (6) analogs with minor CCM inhibitory activity

Unfortunately, both the analogs (**83-85**) and the natural products (**6**, **7**) showed only minimal inhibition of migration at concentrations just below their one day cytotoxicity (IC_{50}) values in the SWH Assay (Table 1.12.1).[53]

Table 1.12.1: Minimal inhibition of migration of 4T1 cells in SWH assay observed

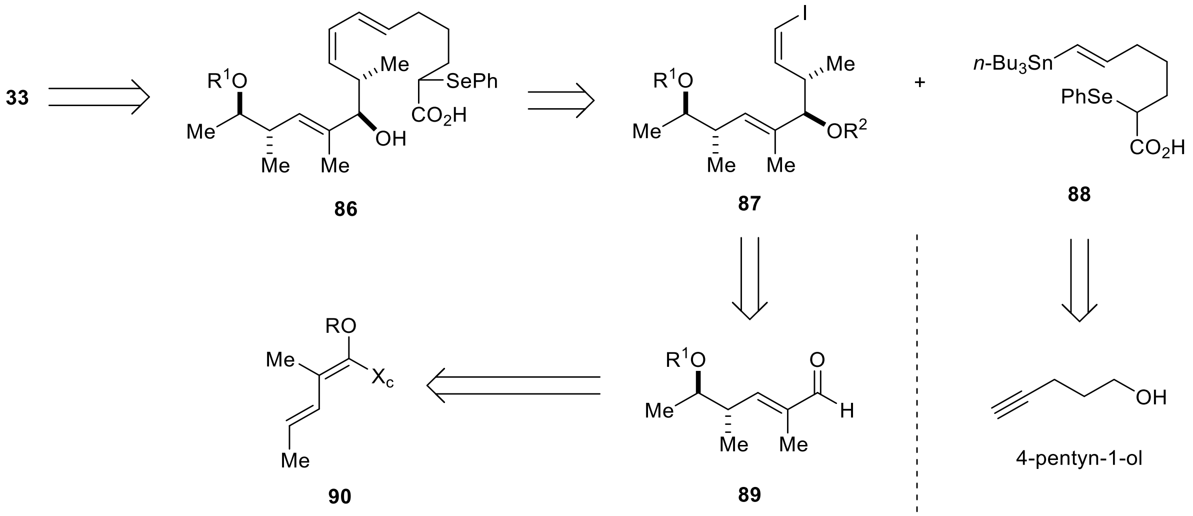
Compound	Concentration[a]	% Inhibition
6	1.6 nM	10%
7	100 nM	20%
83	25 nM	14%
84	6.2 nM	35%
85	12.5 nM	3%

[a]Just below their one day cytotoxicity IC_{50}

Interestingly, their complete biological studies indicated that lactimidomycin (**6**) and isomigrastatin (**7**) are much more potent in their antiproliferative (cytotoxic) activity against 4T1 cells after one day and do not inhibit cancer cell migration in subtoxic doses for LoVo, MDA-MB-231, and 4T1 cancer cell lines, unlike the results previously reported by the Shen group.[51]

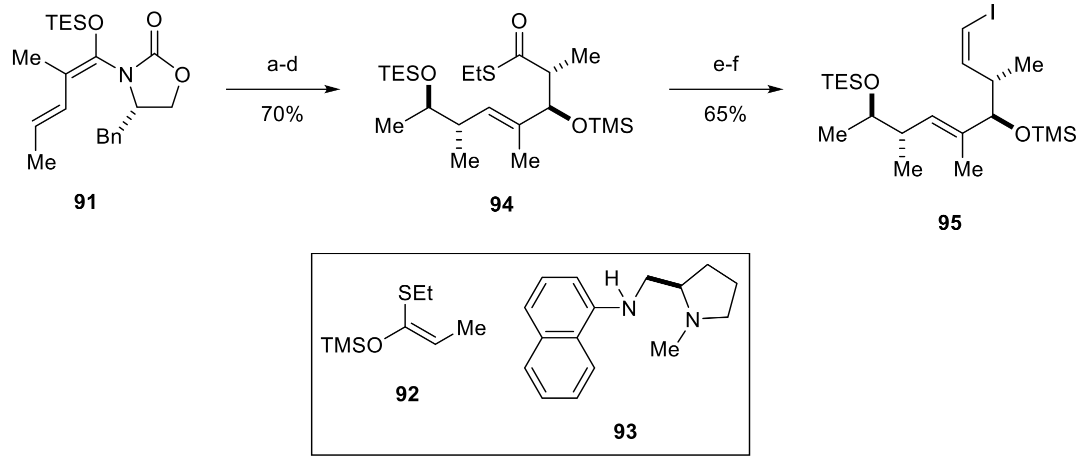
1.13 Kuwahara's formal total synthesis of lactimidomycin

Concurrently with our own report (Chapter 2),[64] the Kuwahara group disclosed the formal total synthesis of lactimidomycin's core alcohol via macrolactonization.[65] In a similar fashion to the Danishefsky and Fürstner approaches, the macrocyclization required the protection of the Δ^2-olefin in the macrolactone via a C2-phenylselenide. This synthesis is important in that it demonstrates a highly optimized and convergent route using traditional synthetic methods. Retrosynthetically, Kuwahara's group planned out their approach to **6** by breaking down the molecule, planning a key Yamaguchi macrolactonization of **86** followed by an oxidative elimination to form the key alcohol close the 12-membered macrolactone of **6** (Scheme 1.13.1).



Scheme 1.13.1: Kuwahara's retrosynthetic analysis towards a formal total synthesis of lactimidomycin (6)

The *E,Z*-diene containing macrolactonization precursor **86** was planned to be formed from a Stille coupling of *cis*-vinyl iodide **87** and *E*-stannyl alcohol **88**. Compound **87** could be formed from Fürstner's aldehyde **89** which could arise from known intermediate **90**; meanwhile, compound **88** could be formed from commercially available 4-pentyn-1-ol.

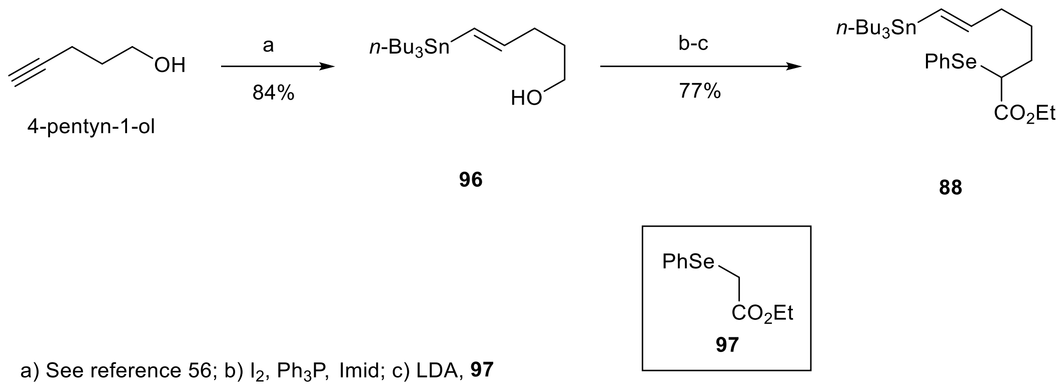


a) MeCHO, $TiCl_4$; b) TESCl, Imidazole; c) DIBAL-H; d) **92**, **93**, $Sn(OTf)_2$, EtCN, e) Pd/C, Et_3SiH; f) NaHMDS, (Iodomethyl)triphenylphosphonium iodide

Scheme 1.13.2: Synthesis of intermediate *cis*-vinyl iodide fragment 95

Starting from the known ketene silyl *N*,*O*-acetal **91**,[66-71] a series of reactions was performed, including a vinylogous Lewis-acid promoted aldol reaction with acetaldehyde, TES protection, cleavage of the auxiliary to the aldehyde form, and treatment with ketene silyl *S*,*O*- acetal **92** in the presence of catalytic $Sn(OTf)_2$ and chiral amine **93** in propionitrile to obtain thioester **94** (Scheme 1.13.2).[72]

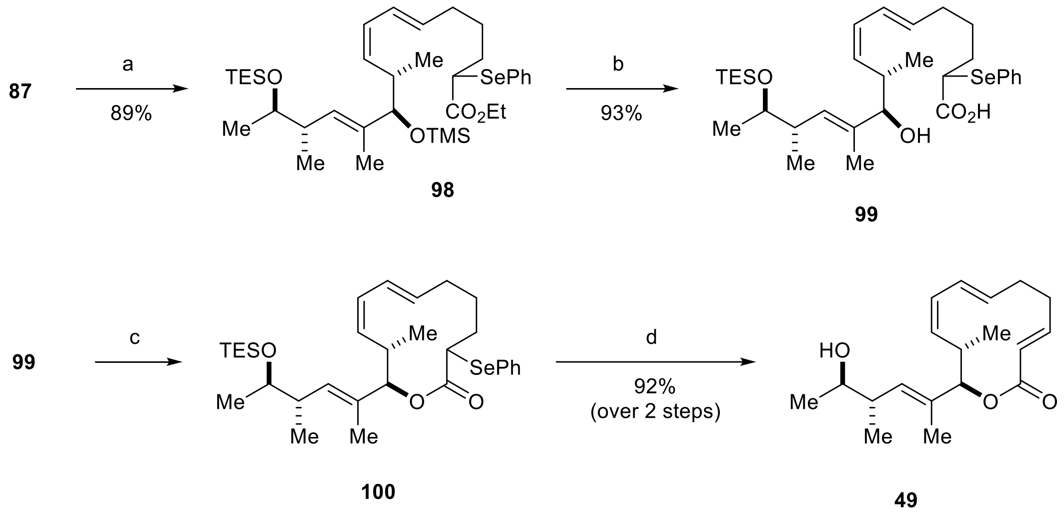
Fukuyama reduction of **94** proceeded in almost quantitative yields to the aldehyde form, which could undergo a *Z*-selective Stork-Zhao olefination reaction (*Z*/*E* > 20:1) with the Wittig reagent to obtain **95**.[73] Next, they pursued the synthesis of the *E*-stannyl alcohol fragment **88** (Scheme 1.13.3). Starting with 4-pentyn-1-ol, a palladium catalyzed syn-hydrostannation was used to form the known *E*-stannyl alcohol **96**.[74] Alcohol **96** was converted to its corresponding iodide via an Appel reaction, which was used to alkylate ethyl 2-(phenylseleno)acetate **97** to successfully form intermediate **88**.



a) See reference 56; b) I_2, Ph_3P, Imid; c) LDA, **97**

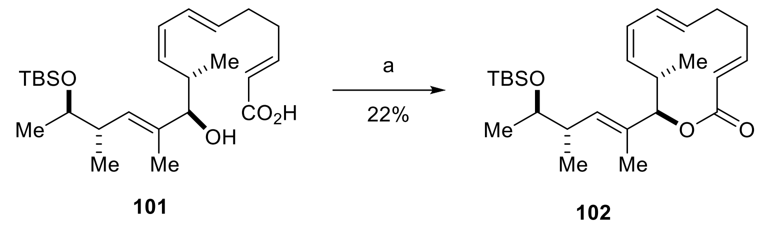
Scheme 1.13.3: Synthesis of *E*-stannyl alcohol fragment 88

With fragments **87** and **88** in hand, a Stille coupling under Corey's modified conditions was employeed to form the *E*,*Z*-diene containing **98** in great yield and selectivity[75, 76] (Scheme 1.13.4). A selective deprotection of the TMS group was achieved under LiOH conditions to form seco-acid **99**. Finally, treatment with Yamaguchi lactonization conditions transformed **99** into intermediate **100**, followed by unmasking of the Δ^2-olefin via an oxidative elimination, yielding the key intermediate alcohol **49**.



a) **88**, $Pd_2(dba)_3$-$CHCl_3$, LiCl, CuCl; b) LiOH, aq THF; c) $Cl_3C_6H_2COCl$, Et_3N, DMAP; d) $NaIO_4$, aq THF

Scheme 1.13.4: Key Suzuki and Yamaguchi lactonization steps to complete formal total synthesis



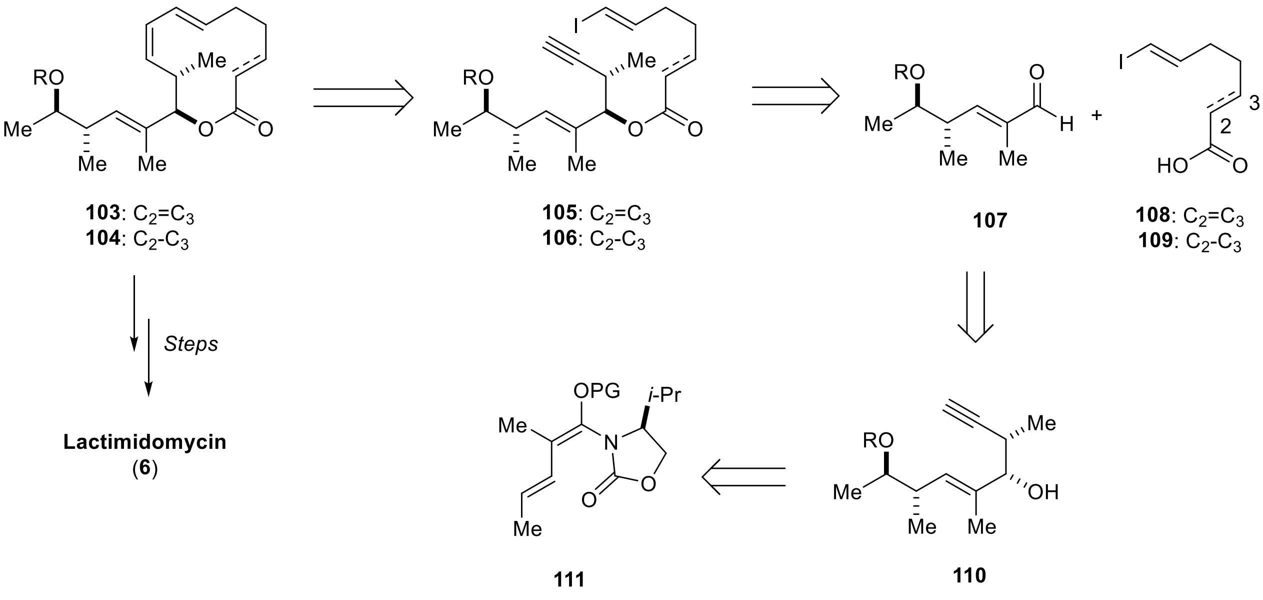
a) $Cl_3C_6H_2COCl$, Et_3N, DMAP

Scheme 1.13.5: Poor-yielding direct cyclization into the C2-C3 unsaturated macrolactone

Interestingly, they observed that cyclization of the TBS-protected, Δ^2-olefin containing, seco-acid **101** provided only 22% of the macrolactone **102** (Scheme 1.13.5); in contrast, the previously described cyclization was significantly more efficient, giving 92% yield over the final two steps.

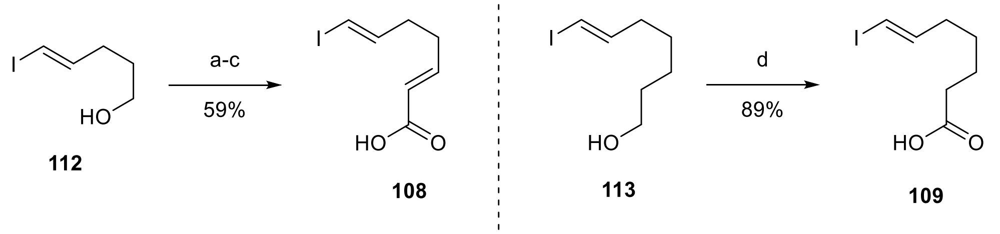
1.14 Georg's formal total synthesis of lactimidomycin (6)

Recently, in 2015, Georg's group published yet another formal total synthesis of lactimidomycin,[77] further demonstrating the interest in the scientific community in forming these bioactive molecular scaffolds (Figure 1.3.2). They envisioned forming the lactimidomycin core using a method based on the Castro-Stephens coupling and an *in situ* alkyne reduction (Scheme 1.14.1).



Scheme 1.14.1: Georg's retrosynthetic analysis of lactimidomycin (6)

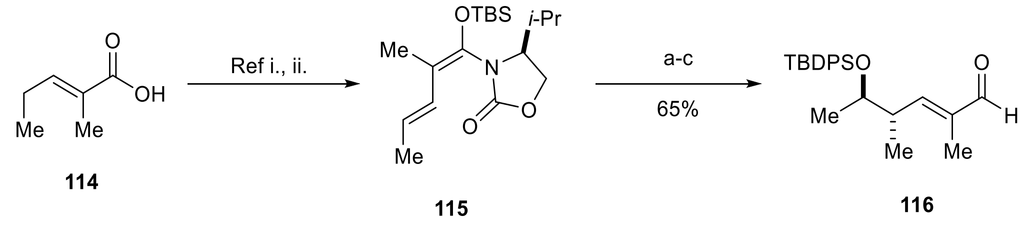
As there was no precedent for this reaction in having the olefin functionality next to the carbonyl preinstalled or later established, they proposed testing both seco acids (**108**, **109**, Scheme 1.14.2) in their key ring-closing step



a) $(COCl)_2$, DMSO, Et_3N, DCM, -78 °C; b) $Ph_3P{=}CH\text{-}CO_2Et$, DCM; c) $(Bu_3Sn)_2O$, PhMe, 105 °C; d) CrO_3, H_2SO_4, acetone, 0 °C

Scheme 1.14.2: Synthesis of unsaturated (108) and saturated (109) seco acids

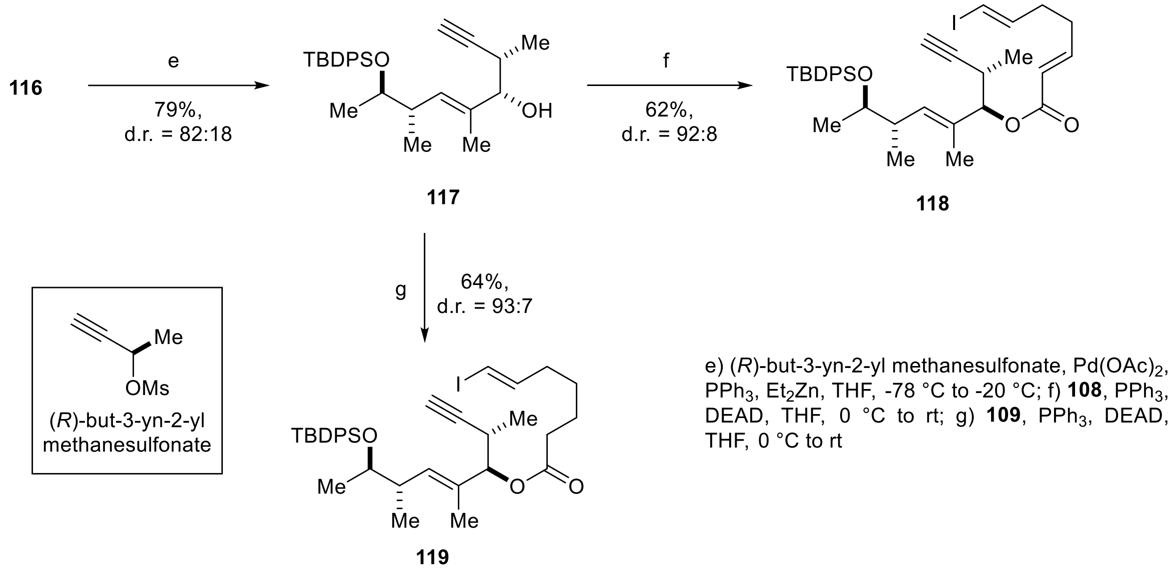
Acid **108** was formed by submitting iodo-alcohol **112** to a sequence of reactions, including a Swern oxidation, Wittig coupling, and tin-promoted ester cleavage (Scheme 1.14.2);[78] acid **109** was formed via Jones oxidation of known iodo-alcohol **113**.[79] With these fragments prepared they pursued the formal total synthesis, following a sequence reminiscent of previous routes transforming acid **114** into **115** (Scheme 1.14.3).[80, 81]



a) CH_3CHO, $TiCl_4$, DCM, -78 °C (d.r. > 30:1); b) TBDPSCl, imidazole, DMAP, DCM; c) DIBAL-H, DCM, -78 °C;

Scheme 1.14.3: Synthesis of aldehyde 116

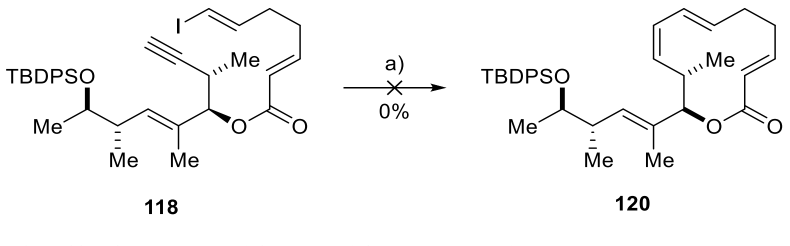
Intermediate **115** underwent similar reactions as before, but was protected with a TBDPS silyl group, and after a DIBAL-H reduction, formed **116**. Next, this intermediate was coupled with a chiral allenylzinc reagent, which was formed in situ from (*R*)-but-3-yn-2-yl methanesulfonate, in a highly selective Marshall's propargylation reaction to form alcohol **117** (Scheme 1.14.4). This alcohol could then undergo a Mitsunobu esterification reaction with acids **108** or **109** to form **118** or **119**, respectively, both with a small amount of diastereomeric material carrying through from the previous reaction.



e) (*R*)-but-3-yn-2-yl methanesulfonate, Pd(OAc)$_2$, PPh$_3$, Et$_2$Zn, THF, -78 °C to -20 °C; f) **108**, PPh$_3$, DEAD, THF, 0 °C to rt; g) **109**, PPh$_3$, DEAD, THF, 0 °C to rt

Scheme 1.14.4: Synthesis of ring-closing step precursors 118 and 119

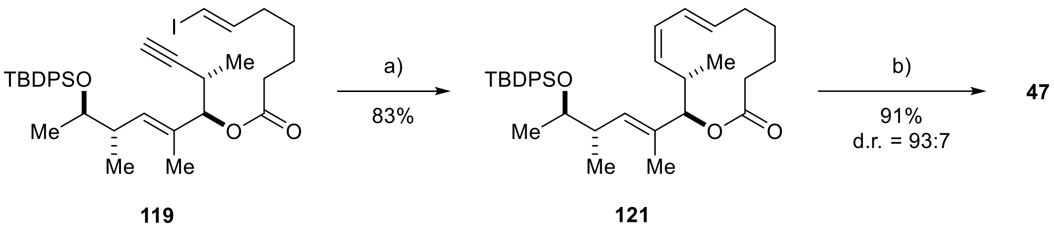
Finally, intermediates **118** and **119** were both submitted to the key ring closing methodology based on the Castro-Stephens coupling and *in situ* alkyne reduction (Scheme 1.14.5 and Scheme 1.14.6, respectively).



a) Cu(OAc)$_2$, BINAP, HCO$_2$Na, K$_2$CO$_3$, DMF 120 °C

Scheme 1.14.5: Unsuccessful ring-closing coupling/*in situ* alkyne reduction of 118 to 120

Surprisingly, **118** failed to produce any detectable amount of **120**, while **119** yielded the desired macrocycle product **121** in an 83% yield, further demonstrating the difficulty in forming this strained C2-C3 *E*-olefin containing 12-membered macrocyclic ring system directly. A final TBAF-mediated deprotection reaction yielded the key Fürstner intermediate **47**, completing the formal total synthesis of **6**.



a) $Cu(OAc)_2$, BINAP, HCO_2Na, K_2CO_3, DMF 120 °C; b) TBAF, THF, 50 °C

Scheme 1.14.6: Completion of the formal total synthesis of 6 by reaching the key saturated C2-C3 alcohol 47

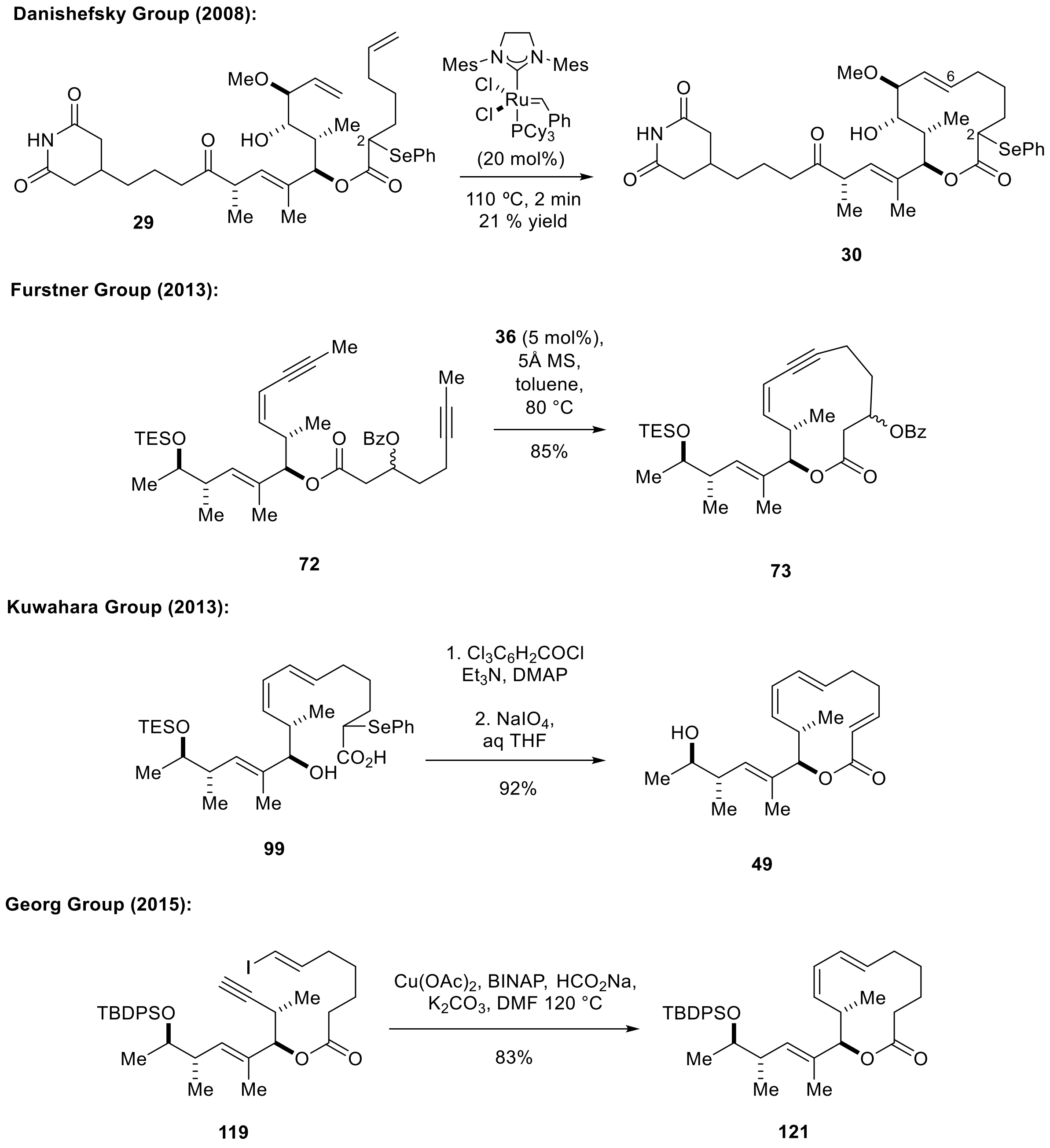
1.15 Summary of synthetic methodologies covered

The following optimized key ring-closing steps in forming the 12-membered macrolactone during formal and total syntheses of **6** or **7** have been described (Scheme 1.15.1). The Danishefsky group utilized a late-stage ring-closing metathesis of **29** to close the 12-membered ring of **7** and to install the C6 *trans*-olefin. In order to facilitate the formation of the macrocycle, the ring strain in the RCM product **30** was reduced by masking the Δ^2-olefin of **29** as the C2 selenide. This *E*-enoate functionality was re-installed later by subjecting **30** to oxidation with *m*-CPBA, thereby converting it into **7**.

Fürstner's initial RCAM approach to **6** encountered oligermerization issues if the alkyne was present at the C8 position rather than the C6 position; therefore, a selective reduction was used to introduce the C6 olefin functionality after the key RCAM. The RCAM sequence was later optimized in 2013, establishing a unified approach to isomigrastatin (**7**) and allowing for a larger scale synthesis of lactimidomycin (**6**, 138 mg) to be achieved. This approach was based on ring-closing alkyne metathesis of substrate **72** leading to **73**. In a similar fashion to Danishefsky's synthesis of isomigrastatin, the RCM precursor **72** lacked the Δ^2-olefin, reducing the ring strain in product **73**. The alkyne functionality of **73** could undergo either a stereoselective epoxidation reaction, followed by a series of steps to convert it into isomigrastatin (**7**), or a 2-step hydrosilylation/desilylation protocol that also resulted in the elimination of the C3-OBz functionality and formation of the Δ^2-olefin containing compound **33**.

Concurrently with our own report,[64] the Kuwahara group described a formal total synthesis of lactimidomycin via the macrolactonization of seco acid **101** followed by oxidative elimination to form key alcohol intermediate **49**; more recently, in 2015, another formal total synthesis has been reported by the Georg group in which **119** is submitted to conditions based on a Castro-Stephens coupling and a tandem *in situ* alkyne reduction to form **121**.

These recent formal total synthesis papers provide excellent examples of the difficulties encountered in synthesizing the 12-membered macrolactone of **6** directly, containing three units of unsaturation. In particular, having the C2-olefin preinstalled during or prior to the ring-closing step appeared to significantly complicate attempted cyclizations: Kuwahara observed a 22% yield when attempting to directly cyclize **101** to **102** (Scheme 1.13.5), and Georg observed no reactivity at all in the cyclization reaction of **118** to **120** (Scheme 1.14.5).


Danishefsky Group (2008):

29 → **30**

Furstner Group (2013):

72 → **73**

Kuwahara Group (2013):

99 → **49**

Georg Group (2015):

119 → **121**

Scheme 1.15.1: Summary of key ring-closing steps utilized in forming 12-membered macrolactones of 6 and 7

1.16 Conclusion

Among the previous synthetic studies towards lactomidomycin (**6**) and isomigrastatin (**7**), one of the most challenging problems appears to be the formation of their similar, strained 12-membered macrocycle moieties. In all of these studies, post-assembly installation of the unit of unsaturation at the C2-C3 position of the macrolactone was either required or would yield significantly more favorable results compared to direct cyclizations into the C2-C3 unsaturated forms. Ultimately, this issue provided an interesting synthetic challenge in designing a scalable route to lactimidomycin (**6**) in the process of developing novel, natural product based analogs of this natural product.

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Chapter 2: Synthesis and biological evaluation of lactimidomycin and its analogs

2.1 Background

Due to its valuable biological and medicinal properties and potential for treatment of various cancers,[1-8] we undertook designing a general and scalable synthetic approach to lactimidomycin (**6**). A synthetic approach to **6** and its analogs could aid in identifying new anticancer agents with improved stability and therapeutic properties; however, the high ring strain energy associated with the unsaturated 12-membered macrolactone of lactimidomycin significantly complicates the preparation of this macrolide. As a consequence, at the time our studies commenced (2010), only Danishefsky's group route to isomigrastatin[9] and Fürstner's group's initial approaches to lactimidomycin[10, 11] could provide access to synthetic **6** and **7**.[12-14] Along with the initial report by the Shen group, which indicated that 12-membered glutarimide-containing macrolides lactimidomycin (**6**) and isomigrastatin (**7**) inhibited cancer cell migration *in vitro*,[15] we were inspired to develop a scalable approach to **6** and its analogs. Although a following study by the Furstner group contradicted the findings of the Shen and coworkers,[12] the initial studies showing that **6** can serve as a very potent antitumor agent, due to its ability to inhibit the translocation step in eukaryotic protein translation initiation, still made this an interesting target for biological studies.[16-18]

While there exists a few methods to generate these glutarimide-containing natural products **6**-**7** via biological isolation,[16, 19] formal[11, 20, 21] and total synthetic means,[10, 12] at the time of starting our research in 2010, none offered scalable and easily conductible route without commercially available catalysts and toxic reagents such as selenium or alkylated tin(IV) compounds;

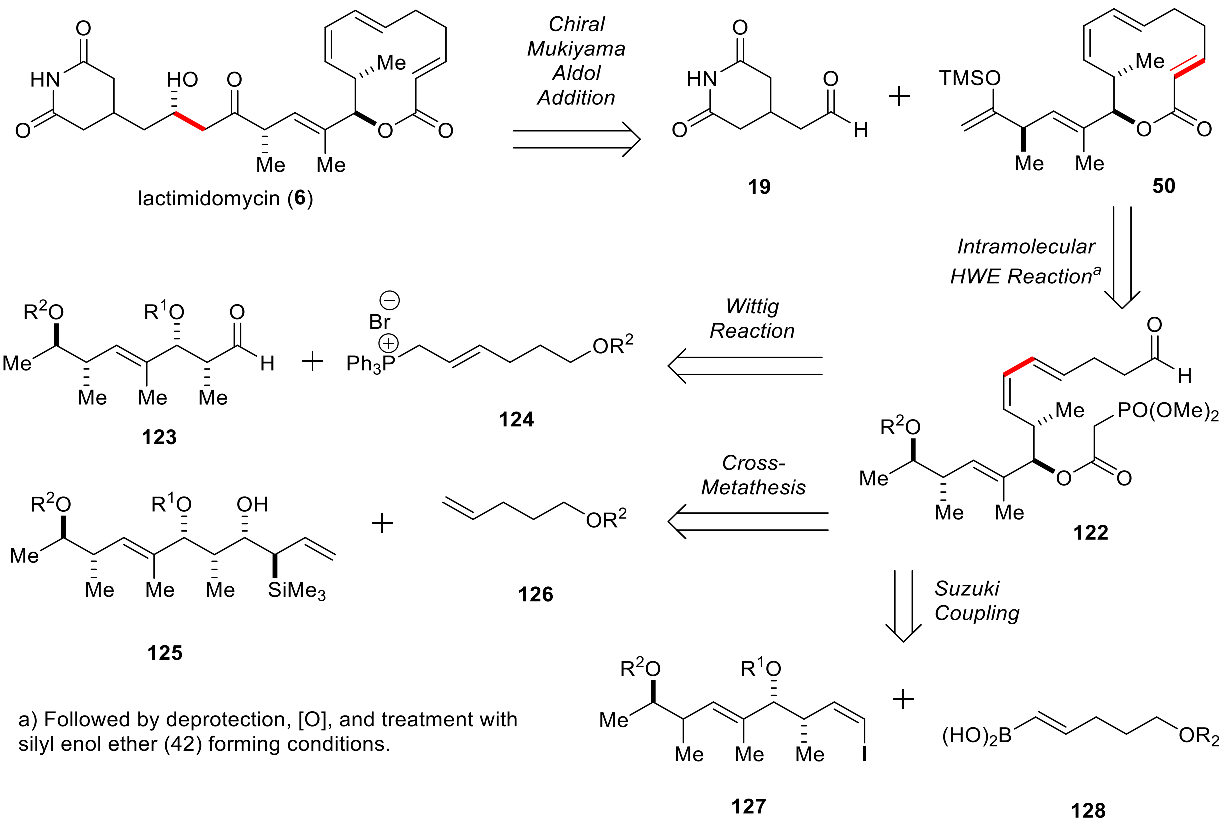
furthermore, we envisioned a diverted total synthetic approach which would allow we to achieve the rapid formation of otherwise unreachable analogs of **6**, and potentially **7**.

2.2 Research plan

In developing a viable, general strategy to lactimidomycin (**6**), it was vital that the number of synthetic steps required after the formation of the macrocyclic ring were minimized, in order to avoid the decomposition and isomerization reactions of the synthetic intermediates.[22-25] To accomplish this, we proposed to establish both the macrocyclization and installation of the Δ^2-enoate double bond in a single step through an intramolecular Horner-Wadsworth-Emmons (HWE) cyclization of protected precursor **122** (Scheme 2.2.1), eventually leading to the silyl enol ether **42** intermediate, after conducting the standard procedures previously described by Fürstner. This would then allow for a rapid, scalable route to the lactimidomycin (**6**) scaffold, allowing for the development of less toxic, more stable, and, more potent cancer cell migration inhibitors.

As the synthesis of the *E,Z*-diene functionality represented one of the key challenges in the construction of precursor **122**, several different approaches were envisioned. Thus, a *Z*-selective Witting reaction of **123** and **124**,[26] a cross metathesis reaction of **125** and **126** followed by a stereoselective Peterson elimination[27] as well as a Suzuki cross-coupling of **139** and **140** were among the possibilities that we considered to investigate.

This chapter summaries our studies on the stereoselective construction of various unsaturated 12-membered macrocycles and application of this strategy to the total synthesis of lactimidomycin (**6**).



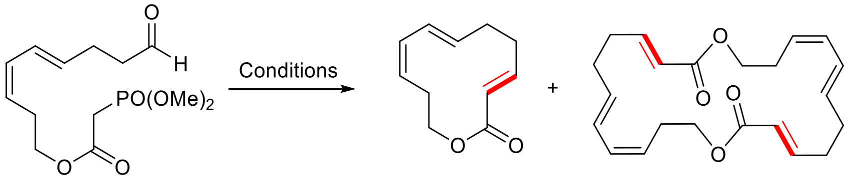
a) Followed by deprotection, [O], and treatment with silyl enol ether (42) forming conditions.

Scheme 2.2.1: Full retrosynthetic analysis[10, 12]

2.3 Model studies on the intramolecular HWE on the 12-membered macrocycle

While intramolecular olefination reactions could be utilized for the construction of larger macrocycles, *E*-selective cyclizations leading to 12-membered macrolactones have little precedence.[28, 29] In addition, the presence of the *(E,Z)*-diene moiety decreases the overall flexibility of the macrocycle, which could affect the relative rates for the intermolecular versus intramolecular pathways. To evaluate the feasibility of the approaches depicted in Scheme 2.2.1, we initiated another series of model studies with the unfunctionalized phosphonate **141** (Table 2.3.1). Intramolecular cyclization of **141** under standard conditions employing potassium carbonate (entry 1)[30] or lithium hexamethyldisilazide (entry 2)[28] as bases did not result in the formation of **130** and only diolide **131** was isolated. Subjecting **141** to the standard Masamune-Roush olefination protocol (entry 3)[31] provided only minor quantities of *E*-enoate **130** (13% yield) and mostly resulted in diolide **131** (60% yield). Encouraged by these results we decided to evaluate other soft enolization conditions,[32-35] surmising that the Lewis acidity of the counterion correlates with its ability to bring together the termini of **141**. Gratifyingly, the combination of zinc (II) trifluoromethanesulfonate, TMEDA and trimethylamine[36, 37] (entry 4) promoted the *E*-selective formation of the macrolactone **130** (78% yield) and only a minor quantity of diolide **131** was observed (9% yield).

Table 2.3.1: Optimization of HWE-Macrocyclization



129 **130** **131**

Entry	Conditions	**130** (yield, %)[a]	**131** (yield, %)[a]
1	K_2CO_3, 18-C-6, 1.3 mM	0	65
2	LiHMDS, 1.3 mM	0	36
3	LiCl, DBU, 1.3 mM	13	60
4	$Zn(OTf)_2$, TMEDA Et_3N, 1.7 mM	78	9

a) Yields are measured over two steps (oxidation of an alcohol precursor to **129** followed by the HWE cyclization to **130** and **131**)

Although Zn(II)-mediated macrocyclizations could be used to construct the lactimidomycin core, it was unclear whether this method could be applied to the preparation of other 12-membered macrocyclic systems such as the one of isomigrastatin (**7**). In order to understand the role of 6E- and 8Z- units of unsaturation, another series of studies were conducted. In addition to **141**, phosphonates **132** with various degrees of unsaturation at the C6- and C8- positions were subjected to Zn(II)-mediated macrocyclizations (Table 2.3.2).[i] The removal of one of the olefins at either the

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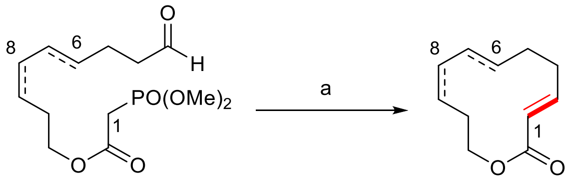
[i] Experiments were conducted by Dr. Zhankui Sun.

C6- (entry 3) or C8- (entry 2) positions did not significantly affect the formation of the 12-membered ring, and the corresponding products, **133-2** and **133-3**, were obtained in 73% and 69% yields, respectively. Furthermore, the successful formation of (*6E*)-**133-2** was remarkable in that it indicates this method could be applied for the preparation of isomigrastatin (**7**) macrolactone. The saturated substrate **132-4** lacking the diene moiety did not cyclize to produce a 12-membered ring **133-4**, and the corresponding diolide **133-4a** (see SI section) was isolated as the only product. It is noteworthy that due to the high ring strain of the *E*-olefin containing macrolactones **133-1** and **133-2**, these compounds were found to be unstable and could be easily polymerized upon storage in neat state.[22]

Table 2.3.2: Investigating the scope of Zn(II)-mediated HWE-macrocyclization

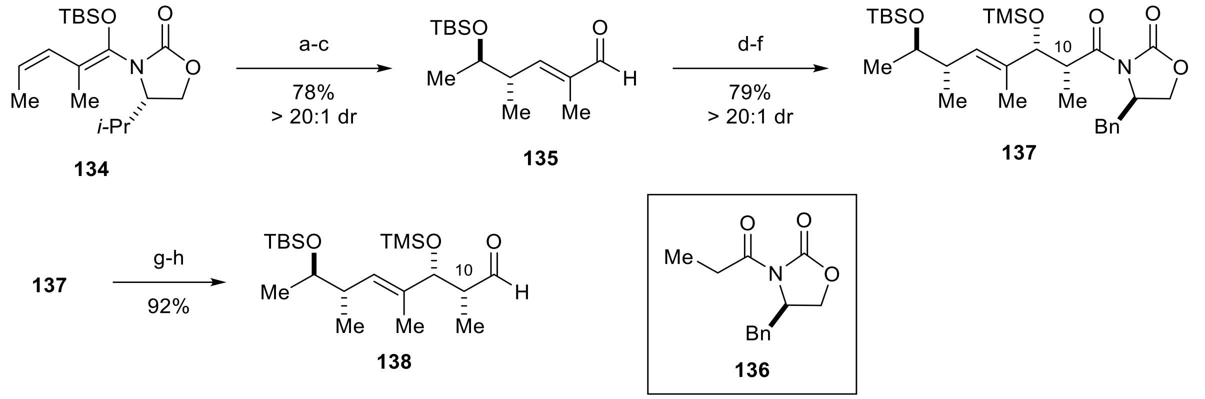


129 or **132** → **130** or **133**

Entry	Starting Material	Unsaturation	Product (yield, %)^b
1	**129**	(6E, 8Z)	**130** (78%)
2	**132-2**	(6E)	**133-2** (69)
3	**132-3**	(8Z)	**133-3** (73)
4	**132-4**	No unsaturation	**133-4** (0%)

a) Zn(OTf)$_2$, TMEDA, Et$_3$N; b) Yields are measured over two steps (oxidation of the alcohol precursor **132a**, followed by the HWE cyclization of **132** into **133**)

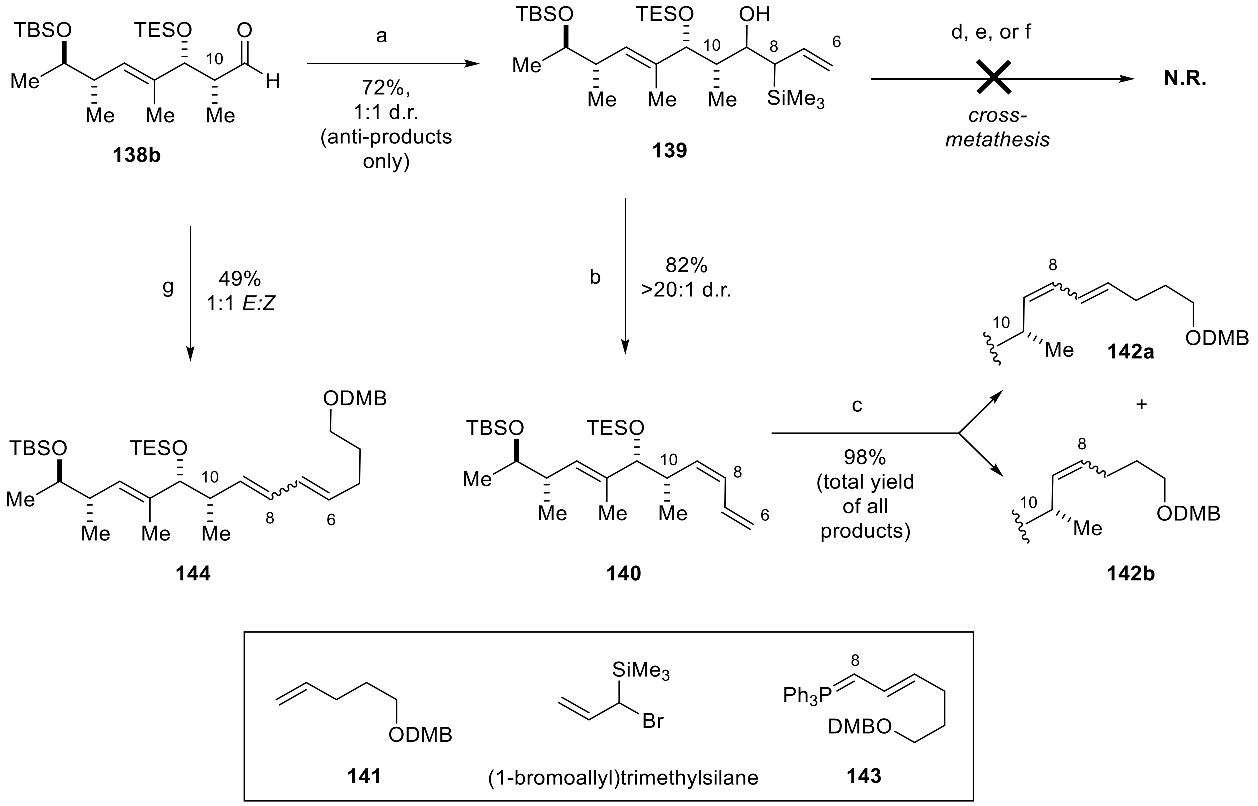
With these encouraging model study results in hand, the synthesis of lactimidomycin (**6**) was pursued next (Scheme 2.3.1). These studies commenced with the known vinylogous Mukaiyama aldol reaction of **134** and acetaldehyde to provide the corresponding aldol adduct (93% yield, >20:1 dr).[38, 39] Protection of this adduct (TBSCl, DMAP, ImH) followed by reductive removal of the auxiliary (NaBH$_4$, 84% yield, 2 steps) and Parikh-Doering oxidation of the resultant alcohol provided aldehyde **135**. Aldehyde **135** was then subjected to the Evans *syn*-selective aldol reaction[40] with a chiral oxazolidinone **136** to provide the corresponding aldol adduct (>20:1 dr) that was converted to silyl ether **137** (79% yield, 2 steps). With compound **137** in hand, the approaches to the construction of the *(E,Z)*-diene outlined in Scheme 2 were investigated next. Thus, the chiral auxiliary of **137** was removed through the formation of a thioester (EtSLi, 83% yield), which was reduced by DIBAL-H to provide the corresponding aldehyde **138**.[41, 42]



a) $TiCl_4$, CH_3CHO, DCM, -78 °C; b) TBSCl, imidazole, DMAP, DCM, 0 °C to rt; c) $NaBH_4$, THF/H_2O, 0 °C to rt; d) SO_3-Py, Et_3N, DMSO, 0 °C to rt; e) *n*-Bu_2BOTf, Et_3N, DCM then **136**, -78 to 0 °C; f) TMSCl, imidazole, DCM, rt; g) *n*-BuLi, EtSH, THF; -20 °C; h) DIBAL-H, DCM, -78 °C

Scheme 2.3.1: Initial steps to form the aldehyde precursor to the desired *E*-*S* diene

When initially conducting the previously described route, the TES form (**138b**) of the aldehyde **138** was generated in a similar reaction sequence; later, the TMS form would be chosen as a selective deprotection of this silyl group was more easily achieved with the TMS derivative. Therefore, all initial attempts to form the *E-Z* diene are described in relation to using the TES-containing aldehyde`**138b**. This compound was first subjected to a known Cr(II)-mediated allylation with (1-bromoallyl)trimethylsilane (Scheme 2.3.2).[43, 44] The resultant *anti*-products **139** were obtained in 72% yield and 1:1 diastereoselectivity. These products (**139**) were then subjected to a base-promoted stereoselective Peterson elimination resulting in the terminal diene **140** (>20:1 d.r.). Diene **140** was subjected to the cross-metathesis with DMB-protected alkene **141** using various Ru-based catalysts; however, despite the large reported yield, a complex mixture of products was observed. The NMR and mass-spectrometric analysis of these products indicated that a significant isomerization of the *Z*-diene took place and a mixture of dienes **132a** was formed; in addition, significant formation of the inseparable truncated products **132b** were also detected.

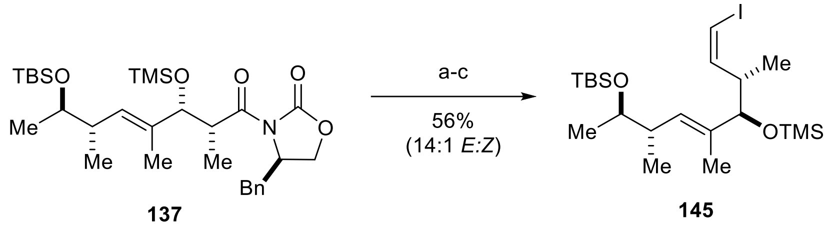


a) (1-bromoallyl)trimethylsilane, $CrCl_2$, THF; b) KH, THF; c) **141**, Grubbs' II (10 mol%), DCM; d) **141**, Grubbs' II; e) **141**, Hoveyda-Grubbs' II; f) **141**, Stewart-Grubbs'; g) **143**, HMPA, THF, -78 °C to rt

Scheme 2.3.2: Early attempts to install the (*6Z,8E*)-diene functionality during the first generation synthesis of lactimidomycin

These results indicate that both olefins of **140** are equally reactive under the cross metathesis conditions,[25] and, to avoid this problem, the mixture of diastereomers (**139**) was directly subjected to the cross-metathesis conditions. However, the attempts to accomplish the cross metathesis of **139** and **141** led only to dimerization of **141**; no reaction of the starting **139** observed by crude

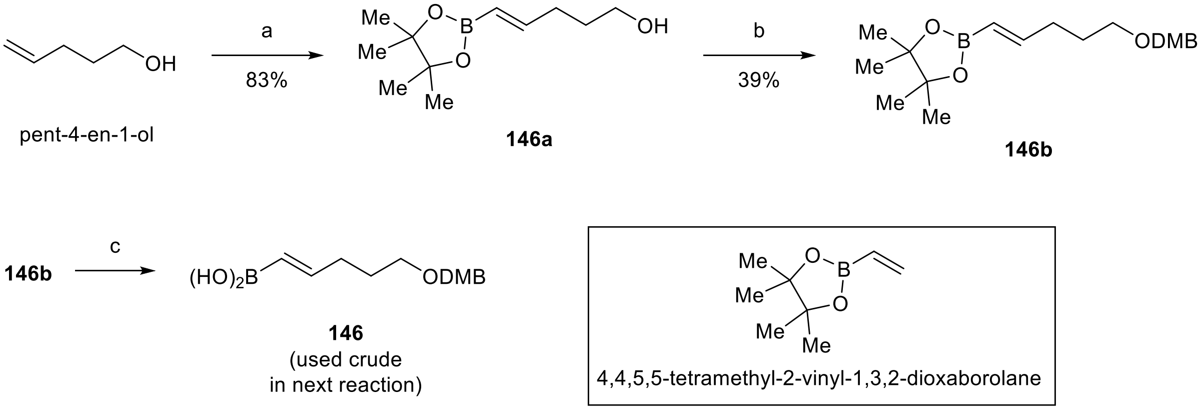
^{1}HNMR. The reduced reactivity of **139** could be attributed to the steric hindrance exhibited by the allylic triethylsilyl group; however, the use of the Grubbs-Stewart catalyst,[45] which is less sensitive to steric effects, did not result in the formation of the desired cross-metathesis products. In addition to the aforementioned studies, the possibility of executing *(Z)*-selective Wittig coupling with allylic phosphorane **143** and aldehyde **138b** was investigated. This coupling was executed under the conditions employed by the Smith group in their synthesis of latrunculin A,[26] and resulted in diene **131** as a 1:1 mixture of *EE* and *ZE* dienes (49% yield).



a) *n*-BuLi, EtSH, THF; -20 °C; b) DIBAL-H, CH_2Cl_2, -78 °C; c) $[Ph_3P^+CHI]\ I^-$, NaHMDS, THF, -78 °C to rt

Scheme 2.3.3: Synthesis of *cis*-vinyl iodide (134)

With little success achieved in the aforementioned studies, we pursued the assembly of the *(E,Z)*-diene moiety through a Suzuki cross-coupling strategy (Scheme 2.3.3). Thus, the TMS-protected aldol adduct **137** was converted to a corresponding aldehyde that was transformed into vinyl iodide **145** via a Stork-Zhao olefination protocol (56% yield over 3 steps, 14:1 *E:Z*).[46]

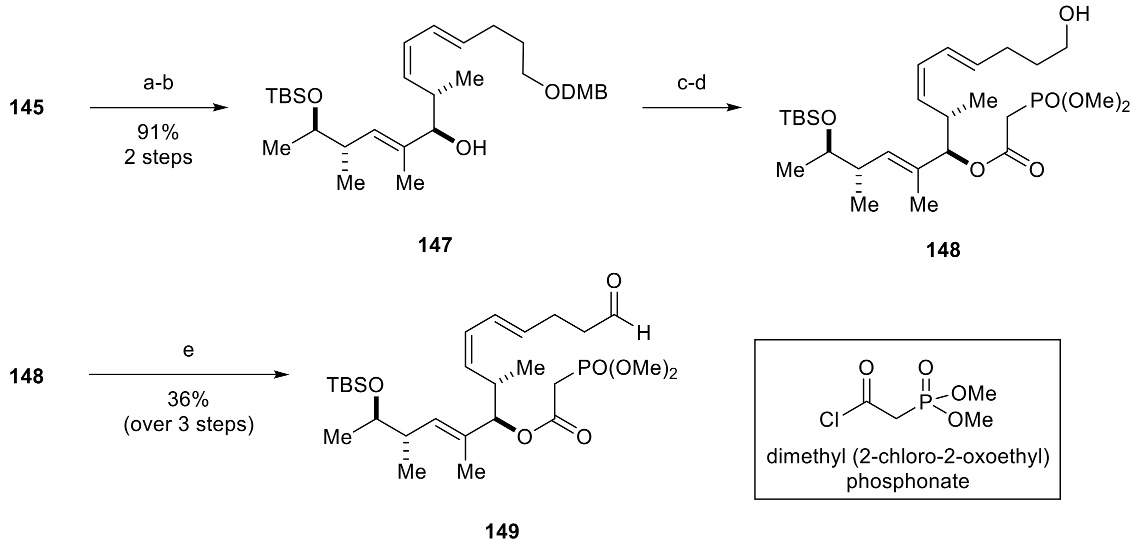


a) 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane, Grubb's II, DCM; b) NaH, DMB-Br, TBAI, THF:DMF (1:2), 0 °C to rt; c) $NaIO_4$, NH_4OAc, Acetone:H_2O (2:1)

Scheme 2.3.4: Synthesis of the DMB-protected alcohol/boronic acid fragment 135

With fragment **145** in hand, a protected cross coupling partner was identified, a DMB-protected alcohol/boronic acid, **146** (Scheme 2.3.4). It was hypothesized that the DMB protecting group would be easy to remove in the presence of the potentially isomerizable *E-Z* diene. The protected boronic acid **146** was synthesized in 3 steps starting from pent-4-en-1-ol which could undergo a cross metathesis reaction with 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane to form **146a**. Next, a DMB protection reaction of this material formed **146b** in acceptable yields, and treatment with sodium periodinate in ammonium acetate produced the crude boronic acid **146**.

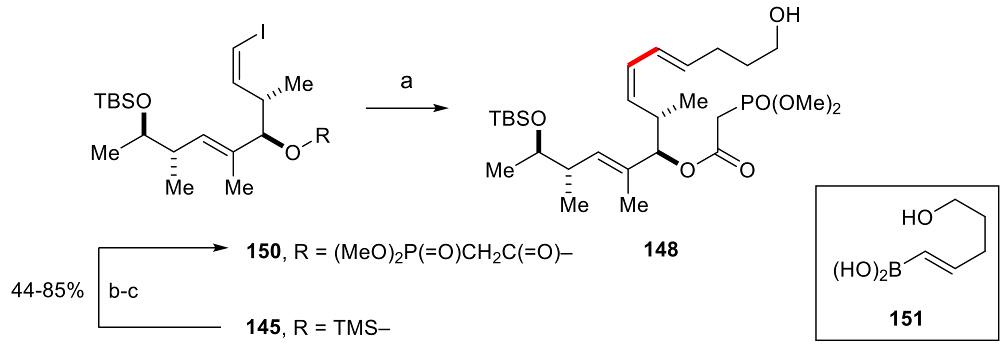
Iodide **145** was then coupled directly with the crude **146**. It is known that vinyl iodides similar to **145** could undergo an intramolecular Heck cyclization, and that this pathway could be suppressed by an addition of thallium(I) ethoxide.[47] Indeed, subjecting the mixture of **145** and **146** to $Pd(PPh_3)_4/TlOEt$[48] resulted in the rapid formation of the desired cross-coupling product containing the desired *(E,Z)*-diene functionality.



a) **146**, $Pd(PPh_3)_4$ (10 mol %), TlOEt, THF/H_2O (3:1); b) *p*-TSA, DCM/MeOH, 0 °C; c) dimethyl (2-chloro-2-oxoethyl)phosphonate, DMAP, pyridine; d) DDQ, $NaHCO_3$(sat), DCM, 0 °C; e) DMP, $NaHCO_3$, DCM

Scheme 2.3.5: First generation cross-metathesis leading to the formation of the HWE precursor

The TMS group of the cross-coupling product was removed under acidic conditions to provide compound **147** in 91% yield over 2 steps from **145**. The alcohol functionality of **147** was acylated with dimethyl(2-chloro-2-oxoethyl)phosphonate, DMAP, and pyridine, and the DMB group was removed with DDQ to form alcohol **148**. Finally alcohol **148** was converted into aldehyde **149** by Dess-Martin oxidation (36%, 3 steps).



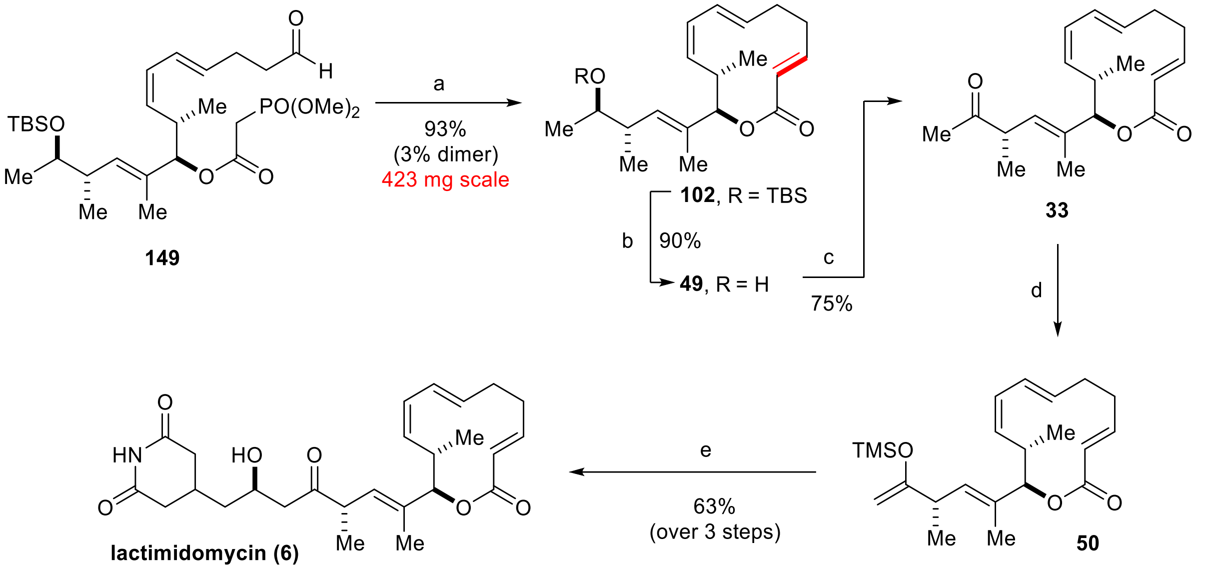
entry	conditions	time	yield (conversion), %
1	$Pd(PPh_3)_4$, TlOEt THF/H_2O (3:1)	40 min	89 (>95)
2	$Pd(PPh_3)_4$, Ag_2O THF/H_2O (3:1)	3 h	n.d. (>90)
3	$Pd(OAc)_2$, X-Phos, Ag_2O, toluene/THF/H_2O	17 h	n.d. (>10)
4	PEPPSI-IPR K_2CO_3, Dioxane	12 h	0 (0)
5	$Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ NaOH (1M) THF/H_2O (3:1)	20 h 18 h	86 (>95)[a] 84 (>95)[b]

a) **137**, **138**, conditions (see table); b) *p*-TSA, DCM/MeOH, 0 ºC; c) dimethyl (2-chloro-2-oxoethyl)phosphonate, DMAP, pyridine

Table 2.3.3: Optimization of the suzuki cross-coupling reaction with DMB-free boronic acid

Considering that the phosphono-aldehyde **149** is the key precursor to the HWE macrocyclization, further optimizations were conducted to reduce the number of steps leading to this compound and, in particular, to deliver sufficient quantities of alcohol intermediate **148** required for the completion of the synthesis and generation of the analogs. The approach to **148** previously depicted (Scheme 2.3.5) suffered from a low-yielding deprotection of the DMB protecting group, highly toxic TlOEt required as the base for the Suzuki coupling leading to **147** as well as the difficulties in the preparation and purification of boronate **146**. In order to avoid the aforementioned problems, the vinyl iodide **145** was directly converted to the phosphonate **150** and the cross-coupling of **150** with unprotected boronate **151** (available in 1 step from pent-4-yn-1-ol) was investigated (Scheme 2.3.3).

In a similar fashion to the previously developed cross-coupling of **145** and **146** (Scheme 2.3.5), the reaction of **150** and **151** cleanly provided the desired product **148** (89% yield) when TlOEt was employed as the base (entry 1). The possibility of substituting Tl(I) base with Ag(I) oxide was investigated next (entries 2–3). Although we observed that Ag_2O can substitute TlOEt as the base (entries 2–3),[49] the resultant product **148** was contaminated with the inseparable impurities (entry 2) or its formation was sluggish (entry 3). Finally, in the search for the alternative conditions (entries 4 and 5),[50, 51] the conditions previously developed by the Tan group[52] cleanly resulted in the formation of **148** (entry 5) on both a small (100 mg) and large (900 mg) scale. Remarkably, no saponification of the ester functionality of **150** or **148**, intramolecular Heck reaction of **150** or isomerization of the *Z*-vinyl iodide moiety of **150** was observed under these conditions.

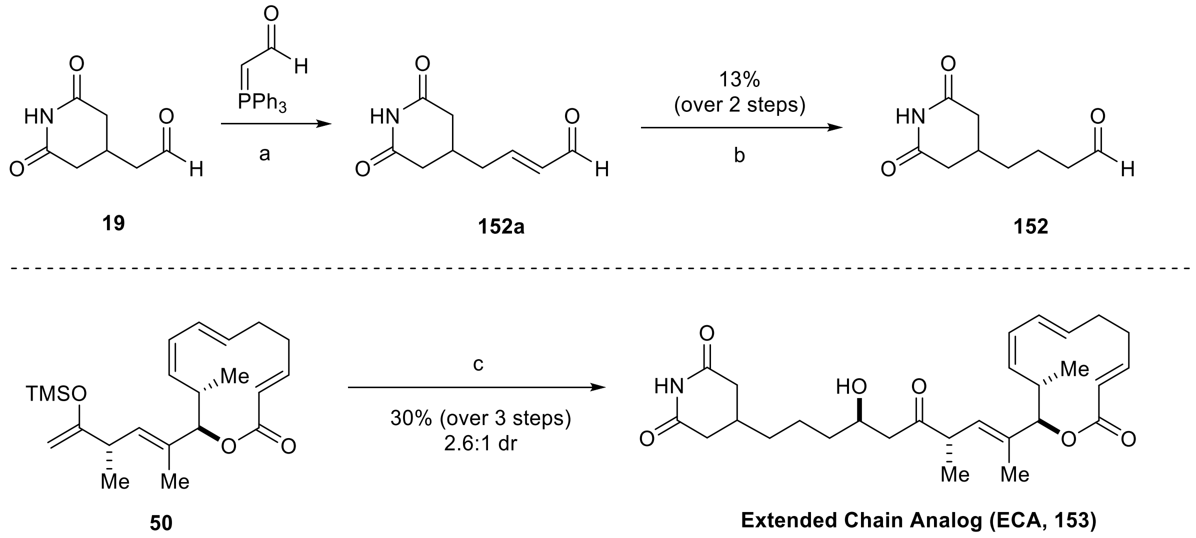


a) Zn(OTf)$_2$, TMEDA, Et$_3$N THF, 18 h; b) HF-Py, THF, 0 ºC to rt; c) DMP, NaHCO$_3$, DCM; d) LiHMDS, TMSCl, Et$_3$N, THF, -78 ºC; e) **19**, EtCN, 4Å MS, **93** (100 mol%), -78 ºC, then HF-Py/THF/Py, 0 ºC to rt

Scheme 2.3.6: Optimized synthesis of lactimidomycin (6)

With a robust synthetic route to macrocyclization precursor **149** developed, the possibility of the formation of lactimidomycin macrolactone using the Zn(II)-mediated intramolecular HWE reaction conditions determined previously (Table 2.3.1) was investigated. Remarkably, this cyclization proceeded with high yields and diastereoslectivity and resulted in the desired *(E)*-**102** (93% yield), with only minor quantities of the corresponding diolide observed (*ca.* 3% yield). This cyclization was executed on a large scale to provide 423 mg of **149** without erosion in the yield or selectivity (*vide infra*). The formal total synthesis of lactimidomycin was completed by TBS-group deprotection that resulted in alcohol **49** (90% yield), a key intermediate in Furstner's syntheses of lactimidomycin.[24, 53] The ^{1}H and ^{13}C NMR spectra as well as the optical rotation of **49** ($[\alpha]_D$ = –234, c = 1.16, $CHCl_3$) matched the previously published physical characteristics ($[\alpha]_D$ = –233, c = 1, $CHCl_3$).

This compound was then elaborated to lactimidomycin following the previously published route.[24, 53] Thus, **49** was oxidized to produce unstable ketone **33**, which was observed to decompose in the neat state upon prolonged storage. Ketone **33** was also found to be unstable to multiple purifications by column chromatography and to traces of DCl in $CDCl_3$. The methyl ketone functionality of **33** was converted into the corresponding silyl enol ether **50** (LiHMDS, Et_3N, Me_3SiCl). Without purification, **50** was subjected to the Mukaiyama aldol addition reaction with aldehyde **19** catalyzed by chiral oxazaborolidinone **93** (100 mol%, EtCN, –78 ºC). The resultant aldol adduct was treated with buffered HF•Py solution in pyridine/THF to remove the trimethylsilyl ether and produce lactimidomycin (**6**). In addition to **6**, the silyl enol ether **50** was reacted with the homologous, 4-carbon chain, glutarimide-containing aldehyde **151** (Scheme 2.3.7), resulting in the formation of the extended chain analog (ECA) of lactimidomycin (**152**), which contained two additional carbons between the glutarimide side-chain and macrolactone moieties.



a) 2-(triphenyl-l^5-phosphanylidene)acetaldehyde, DCM; b) Pd/C, H_2, THF; c) **152**, EtCN, 4Å MS, **93** (100 mol%), -78 ºC, then HF-Py/THF/Py, 0 ºC to rt

Scheme 2.3.7: Synthesis of extended (4-carbon) chain glutarimide aldehyde (55)

2.4 Optical rotation confirmation of the formation of lactimidomycin (6)

The ^1H and ^{13}C NMR data of the synthetic lactimidomycin were in good accordance with those previously reported by the Shen[54] and Fürstner[24, 53] groups. However, the optical rotation value for synthetic **6** ([α]D = –20, c = 0.25, DMSO) was consistent with the corresponding value reported by the Sugawara group ([α]D = –20, c = 0.5, DMSO),[16] but varied from the optical rotation values of Shen ([α]D = +23, c = 0.52, DMSO),[55] and Fürstner ([α]D = +6.9, c = 0.5, DMSO)[24, 53] groups. In contrast, the CD spectrum of synthetic **6** was in good agreement with the corresponding spectrum of the natural sample.[56]

The discrepancies in the optical rotation could be attributed to the presence of optically active impurities that arise from the decomposition of lactimidomycin. Consistent with our observations for macrolactone **29** as well as with our previous studies, **6** is unstable and could Decompose upon storage in the neat state (*vide supra*).

2.5 Biological activity

Table 4. *In vitro* evaluation of the anticancer activity and toxicity of lactimidomycin (**6**) and its analogues **102**, **49**, **33**, and **153**.

compound[a]	MDA-MB-231 (2D) GI_{50} (μM)	MDA-MB-231 (3D) GI_{50} (μM)	HMEC GI_{50} (μM)	Therapeutic Index (TI)
lactimidomycin (**6**)	0.023 ± 0.005	0.047 ± 0.02	< 0.0001	<0.002
102	11 ± 3	20 ± 8	0.64 ± 0.2	0.03
49	9.2 ± 3	1.2 ± 0.4	0.69 ± 0.2	0.58
33	13 ± 4	4.3 ± 0.4	7.0 ± 1	1.6
ECA (**153**)	2.7 ± 0.4	0.98 ± 0.1	0.54 ± 0.2	0.55

[a] Obtained by dilution of the stock solution of the tested compound in DMSO with physiological buffer (*cf.* SI). MDA-MB-231 = human mammary adenocarcinoma cells; HMEC = human mammary epithelial cells; GI_{50} = 50% growth inhibition (*cf.* SI); TI = GI_{50} HMEC / GI_{50} MDA-MB-231 3D.

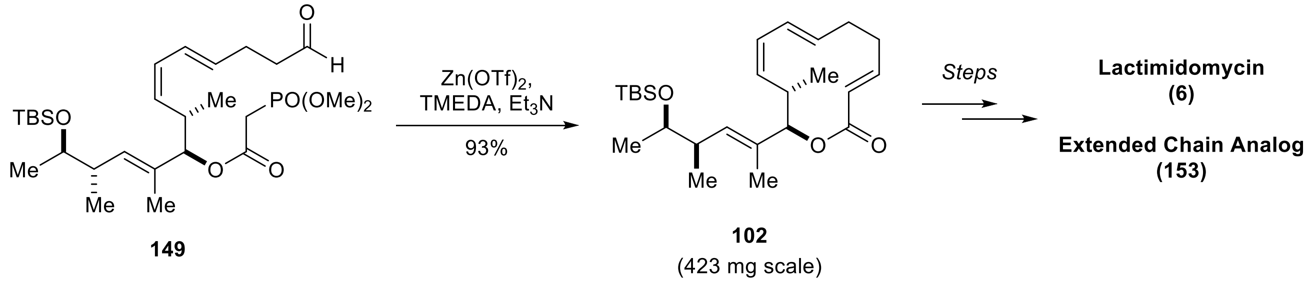
Lactimidomycin (**6**) and its analogs (**102**, **49**, **33**, **153**) were next evaluated for their ability to slow the proliferation of a triple-negative breast cancer cell line (MDA-MB-231) previously demonstrated to be growth inhibited by lactimidomycin (Table 4).[5] In addition to 2-dimensional cell culture on polystyrene, the anti-migration activity of each compound was evaluated in a 3-dimensional cell culture model using basement membrane extract. Consistent with previous reports, lactimidomcyin (**6**) is a potent inhibitor of MDA-MB-231 cell proliferation and migration (Table 4). It is worth noting that lactimidomycin (**6**) does not display enhanced anti-migration activity compared to its anti-proliferative activity, which is in accordance with the observations made by Fürstner and coworkers.[5] The truncated analogs that are devoid of the glutarimide side chain (**102**, **49**, **33**) have significantly weaker anti-proliferative and anti-migration activities compared to lactimidomycin (**6**). Analog **153**, with an increased linker length between the glutarimide side chain and macrolactone, has modest activity in both assays.

To discriminate between selective anti-cancer activity and general cell cytotoxicity, the proliferation of primary, low passage human mammary epithelial cells (HMEC) was evaluated in the presence of each compound. Lactimidomycin (**6**) has highly potent anti-proliferative activity against this non-cancer cell line (Table 4). This result also suggests that the anti-cancer activity observed with lactimidomycin (**6**) is due to general cell cytotoxicity. The analogs (**50–52**, **55**) were also examined for their activities against HME cells and were found to be modestly anti-proliferative (Table 4). The use of a primary non-cancer cell line enables the calculation of a therapeutic index (TI = GI_{50} HMEC / GI_{50} MDA-MB-231 3D) for each compound. Lactimidomycin (**6**) has a very poor therapeutic index (<0.002), demonstrating higher activity in a healthy cell compared to the cancer cell. The analogs (**102**, **49**, **33**, **153**), however, have significantly improved therapeutic indices (Table 4). For example, the extended chain analog (**153**) has a therapeutic index that is 275-fold higher than that of lactimidomycin (**6**). While this impressive improvement is attributed to the addition of two methylene spacers between the glutaramide side chain and the macrolactone, it should be noted that the biological profiles of **153** and **49**, which lacks the glutarimide moiety, are similar.

Together, these results raise questions about the promise of lactimidomycin (**6**) as a potential anti-cancer therapeutic, but indicate that analogs of lactimidomycin have the potential to be selective anti-cancer inhibitors. These results may also suggest a difference in the mechanism of action for lactimidomycin (**6**) and its analogs (**102**, **49**, **33**, and **153**) in analogy to migrastatin case.[1d,2,3] Prior studies highlight the importance of the glutarimide moiety for the inhibition of the eukaryotic ribosome,[1j,1k] and the mechanism, by which the analogs lacking the glutarimide side-chain (**102**, **49**, **33**) is yet to be clarified.

Conclusions

In summary, a scalable enantioselective approach to eukaryotic translation elongation and cancer cell migration inhibitor, lactimidomycin (**6**)[57] as well as its extended chain analog **55**[ii] have been developed. These syntheses feature Zn(II)-mediated *E*-selective Horner-Wadsworth-Emmons reaction to construct the strained 12-membered macrolactone of **6**.



Scheme 2.5.1: Key high-yielding, scalable intramolecular HWE ring-closing step in the route to lactimidomycin (6)

[ii] Manuscript submitted Sept. 3rd, 2015: Brian J. Larsen, Zhankui Sun, Eric Lachacz, Iaroslav Khomutnik, Matthew B. Soellner* and Pavel Nagorny* "Synthesis and biological evaluation of lactimidomycin and its analogs," *Chemistry – A European Journal.*

The macrocyclization reaction was found to be sensitive to the backbone unsaturation, which is important for restricting the conformational flexibility of macrocyclization precursors. The synthetic route features a late-stage installation of the glutarimide functionality via an asymmetric catalytic Mukaiyama aldol reaction, which allows for a quick generation of lactimidomycin homolog with two additional carbons in the glutarimide side chain. Similarly to lactimidomycin, this analog was found to possess cytotoxicity against MDA-MB-231 breast cancer cells ($GI_{50} = 1$–$3\ \mu M$). This activity was found to be similar to the activities of lactimidomycin precursor lacking the glutarimide moiety ($GI_{50} = 1$–$3\ \mu M$). Although lactimidomycin was found to be the most potent in terms of anti-cancer activity, **152** as well as truncated analogs lacking the glutarimide side-chain were found to be significantly less toxic against human mammary epithelial cells.

Chapter 2: Supporting Information

2.6 General reaction methods

All reactions were carried out under an atmosphere of nitrogen in flame– or oven–dried glassware with magnetic stirring, unless otherwise noted. Air–sensitive reagents and solutions were transferred via syringe or cannula and were introduced to the apparatus through rubber septa. Intermediates used in water sensitive reactions were azeotroped in toluene or benzene prior to use. Reactions were cooled via external cooling baths: ice water (0 °C), dry ice–acetone (-78 °C), or Neslab CB 80 immersion cooler (-20 to -78 °C). Heating was achieved by use of a silicone bath with heating controlled by an electronic contact thermometer. Deionized water was used in the preparation of all aqueous solutions and for all aqueous extractions. Solvents used for extraction and flash column chromatography were ACS or HPLC grade. Reagents were purified prior to use following the guidelines of Perrin and Armarego.[58] Tetrahydrofuran (THF), dichloromethane (DCM), toluene and diethyl ether (Et_2O) were filtered through a column (Innovative Technologies) of activated alumina under nitrogen atmosphere. Chloroform ($CHCl_3$) and deuterated chloroform ($CDCl_3$) were filtered through a column of basic alumina and magnesium sulfate prior to use with acid sensitive materials. Purification of the reactions mixtures was performed by flash column chromatography using SiliCycleSiliaFlash P60 (230-400 mesh) silica gel. 4Å molecular sieves were pre-activated before use.

2.7 Spectral data acquisition

^1H NMR spectra were recorded on Varian vnmrs 700 (700 MHz), Varian vnmrs 500 (500 MHz), Varian INOVA 500 (500 MHz) or Varian MR400 (400-401 MHz) spectrometers and chemical shifts (δ) are reported in parts per million (ppm) with solvent resonance as the internal standard ($CDCl_3$ at δ 7.26, C_6D_6 at δ 7.15). Data are reported as follows (s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, dq = doublet of quartets, ddd = doublet of doublet of doublets, dddd = doublet of doublet of doublet of doublets, dqd = doublet of quartet of doublets, t = triplet, q = quartet, qd = quartet of doublets, m = multiplet; coupling constant(s) in Hz; integration). Proton-decoupled ^{13}C NMR spectra were recorded on Varian vnmrs 700 (175 MHz), Varian vnmrs 500 (125-126 MHz), Varian INOVA 500 (125-126 MHz) or Varian MR400 (100 MHz) spectrometers and chemical shifts (δ) are reported in ppm with solvent resonance as the internal standard ($CDCl_3$ at δ 77.000, C_6D_6 at δ 127.683). High resolution mass spectra (HRMS) were recorded on MicromassAutoSpecUltima or VGtd (Micromass) 70-250-S Magnetic sector mass spectrometers in the University of Michigan mass spectrometry laboratory. Infrared (IR) spectra were recorded as thin films on NaCl plates on a Perkin Elmer Spectrum BX FT-IR spectrometer. Absorption peaks were reported in wavenumbers (cm^{-1}). Optical rotations were measured in a solvent of choice on a JASCO P-2000 or Autopol III digital polarimeter at 589 nm (D-line) and reported as follows: $[\alpha]^{TEMP}_{D}$ (*c* = g/100 mL, solvent). Circular Dichroism was measured in spectroscopic grade ethanol on a JASCO J-815 Circular Dichroism Spectrometer.

2.8 General biological methods

Cell growth inhibition assays: WST-1 reagent was obtained from Roche Applied Science. The cell proliferation colorimetric assay using WST-1 was performed according to manufacturer's procedure.[iii]

Cell Culture and Seeding Procedure: Cells were dispersed from flasks and collected by centrifugation (200xg for 5 minutes at rt). An aliquot of the resuspended cells was mixed with trypan blue solution and the cell number was quantified using a hemacytometer. In general, depending on the growth rate of the untreated cells, the cells were plated at 5.0 – 7.5 x 10^3 cells

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[iii] https://cssportal.roche.com/LFR_PublicDocs/ras/11644807001_en_11.pdf

per well. The cells were plated into sterile, clear bottom 96 well plates and cultured under normal growth conditions overnight prior to dosing with compound.

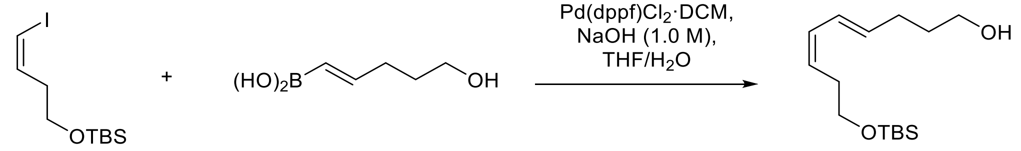
Dosing: 100% DMSO compound stocks were prepared to 100X the final concentration desired in the assay. 3 μL of the DMSO stock solution was then added to 297 μL of the cell growth media to give a DMSO concentration of 1%. The cell media was removed by aspiration for adherent cells and replaced with 100 μL per well of the cell growth media containing the compound. In general, each compound concentration was dosed in triplicate wells.

Assay: After the dosing period (72 hours) was complete, the plates were removed from the incubator and 10 μL per well of WST-1 reagent was added. The plates were returned to the incubator and incubated for 1 h, followed by shaking on a plate shaker for 60 seconds prior to the absorbance read (450 nm) on a BioTek Synergy 4 multimode plate reader.

Data Analysis: The reference absorbance reading was subtracted from the formazan absorbance at 690 nm (background control well no compound added, 1% DMSO) and the data was plotted as a percentage of the vehicle (1% DMSO alone). Data analysis and curve fitting was performed using Graphpad Prism. For each cell line, there were n = 3 data points for each concentration. Each dose response curve was performed at least thrice, providing $n \geq 9$ for each data point.

3D cell culture: Prior to plating cells, 50 μL Cultrex basement membrane extract BME, Trevigen) was added to each well of a 96-well plate incubated on ice and then allowed to gel (cushion formation) over a 30-minute time period in a 37 °C incubator. Cells (MDA-MB-231) were then dispersed from flasks and collected by centrifugation (200xg for 5 minutes at rt). An aliquot of the resuspended cells was mixed with trypan blue solution and the cell number was quantified using a hemacytometer. 100% DMSO compound stocks were prepared to 100X the final concentration that is desired in the assay. 3 μL of the DMSO stock solution was then added to 297 μL of the cell suspension supplemented with 5% BME to give a DMSO concentration of 1%. The cells were plated at about 1.0 x 10^4 cells per well (100 μL/well) in triplicate for each compound concentration.

2.9 Optimization of HWE-macrocyclization (procedures & analytical/spectral data)



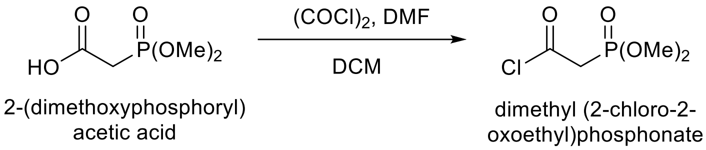
(*Z*)-*tert*-butyl((4-iodobut-3-en-1-yl)oxy)dimethylsilane

(*E*)-(5-hydroxypent-1-en-1-yl)boronic acid

129a

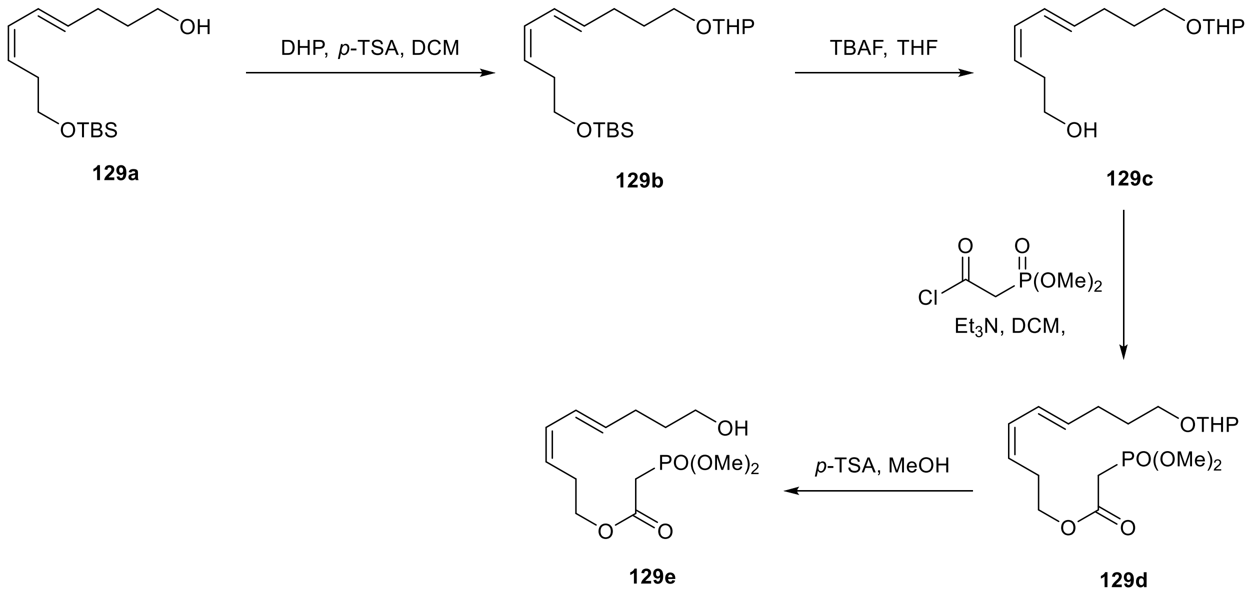
Scheme 2.9.1: Synthesis of (4*E*,6*Z*)-9-((*tert*-butyldimethylsilyl)oxy)nona-4,6-dien-1-ol (129a)

(*Z*)-*tert*-butyl((4-iodobut-3-en-1-yl)oxy)dimethylsilane[59] (312 mg, 1.0 mmol) and (*E*)-(5-hydroxypent-1-en-1-yl)boronic acid[60] (195 mg, 1.5 mmol) were added to THF (5 mL). $Pd(dppf)Cl_2 \cdot DCM$ (33 mg, 0.04 mmol) and NaOH (1.0 M, 3 mL) were added. The mixture was stirred at rt for 15 h and then diluted with water and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The mixture was purified by flash column chromatography (hexanes/EtOAc, 80/20) to provide the diene compound **129a** (242 mg, 89% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 6.36 – 6.29 (m, 1H), 6.00 (t, J = 10.8 Hz, 1H), 5.70 – 5.63 (m, 1H), 5.35 – 5.30 (m, 1H), 3.66 – 3.60 (m, 4H), 2.40 – 2.35 (m, 2H), 2.18 (q, J = 6.8 Hz, 2H), 1.70 – 1.63 (m, 2H), 0.87 (s, 9H), 0.04 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.0, 130.1, 126.3, 126.1, 62.8, 62.5, 32.2, 31.5, 29.1, 25.9, 18.4, -5.3; HRMS (ESI) *m/z* calcd for $C_{15}H_{31}O_2Si^+$ $[M+H]^+$ 271.2088, found 271.2090. IR (thin film, cm^{-1}) 3333, 2929, 1472, 1255, 1097.



Scheme 2.9.2: Synthesis of dimethyl (2-chloro-2-oxoethyl)phosphonate

2-(Dimethoxyphosphoryl) acetic acid (840 mg, 5.0 mmol) and DMF (0.1 mL, 1.3 mmol) were added to DCM (10 mL). Oxalyl chloride (1.27 g, 10.0 mmol) was added slowly. The mixture was stirred at rt for 30 min before the solvent was removed *in vacuo*. Freshly synthesized acyl chloride, dimethyl (2-chloro-2-oxoethyl)phosphonate, was dried under high vacuum for 2 h before it was used in subsequent reactions.



Scheme 2.9.3: Synthesis of (3Z,5E)-9-hydroxynona-3,5-dien-1-yl 2-(dimethoxyphosphoryl)acetate (129e)

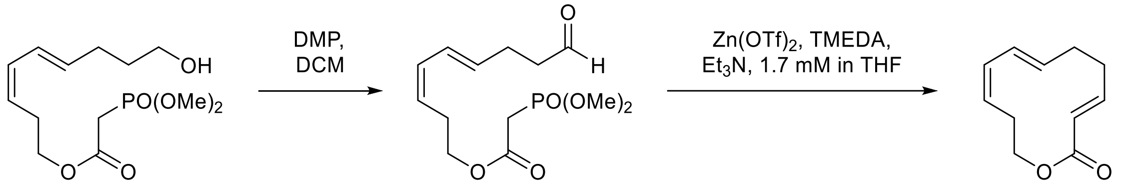
The diene **129a** (242 mg, 0.90 mmol) was added to DCM (10 mL). Next, 3,4-dihydropyran (126 mg, 1.5 mmol) and *p*-toluenesulfonic acid (8.6 mg, 0.05 mmol) were added. The mixture was stirred at rt for 30min. TLC showed complete reaction. DCM (40 mL) was added. The mixture was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude product **129b**.

The crude product **129b** was added to THF (10 mL). TBAF (1.5 mL, 1 M in THF) was added. The mixture was stirred at rt for 3 h. TLC showed complete reaction. The solvent was removed *in vacuo.* DCM (40 mL) was added. The mixture was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo.* The mixture was purified by flash column chromatography (hexanes/EtOAc, 70/30) to provide compound **129c** (188 mg), which was used immediately in the next reaction.

The mono protected diol **129c** (188 mg, 0.78 mmol) was dissolved in DCM (10 mL). Et_3N (1.6 mmol, 162 mg) was added. The freshly prepared dimethyl (2-chloro-2-oxoethyl)phosphonate (296 mg, 1.6 mmol) in DCM (2 mL) was added slowly. The mixture was stirred at rt for 2 h. TLC showed complete reaction. DCM (40 mL) was added to the mixture. The mixture was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude product **129d** which was used directly in the next step.

The crude product **129d** was added to MeOH (5 mL). *p*-Toluenesulfonic acid (17.2 mg, 0.1 mmol) was added. The mixture was stirred at rt for 1 h at which point TLC showed the reaction had gone to completion. The solvent was removed *in vacuo.* DCM (40 mL) was added. The mixture was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography (100% EtOAc) to provide compound **129e** (187 mg, 68% yield over 4 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.35 – 6.28 (m, 1H), 6.06 (t, J = 10.8 Hz, 1H), 5.75 – 5.69 (m, 1H), 5.29 – 5.26 (m, 1H), 4.15 (t, J = 6.8 Hz, 2H), 3.80 (d, J = 11.6 Hz, 6H), 3.65 (t, J = 6.4 Hz, 2H), 2.95 (d, J = 21.6 Hz, 2H), 2.54 – 2.49 (m, 2H), 2.19 (q, J = 7.2 Hz, 2H), 1.73 (br, 1H), 1.70 – 1.63 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.6 (d, J = 5.7 Hz), 135.2, 131.3, 125.7, 123.8, 64.9, 62.1, 53.2(d, J = 6.2 Hz), 33.9, 32.8, 32.0, 29.0, 27.0; ^{31}P NMR (200 MHz, $CDCl_3$) δ 22.5; HRMS (ESI) *m/z* calcd for $C_{13}H_{24}O_6P^+$ $[M+H]^+$ 307.1305, found 307.1309.

IR (thin film, cm^{-1}) 2921, 1734, 1261, 1030.



129e

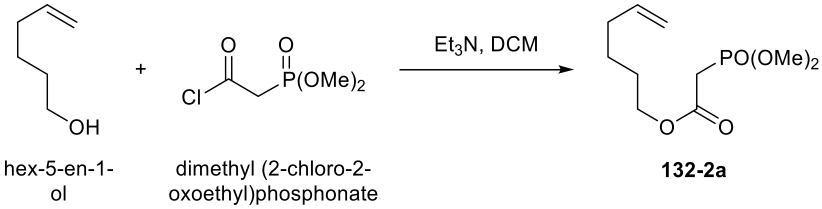
129
(Table 1, entry 4)
(Table 2, entry 1)

130
(Table 1, entry 4)
(Table 2, entry 1)

Scheme 2.9.4: Synthesis of (3*E*,7*E*,9*Z*)-oxacyclododeca-3,7,9-trien-2-one (130)

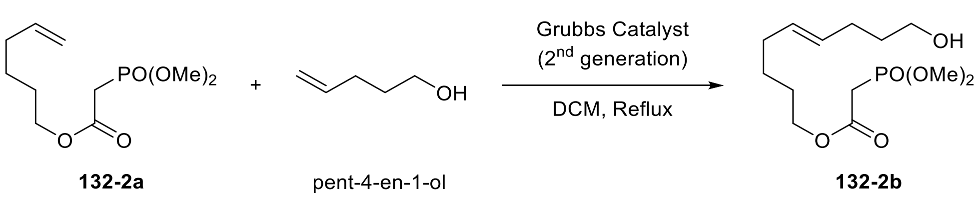
Phosphono-alcohol **129e** (50 mg, 0.16 mmol) was added to DCM (5 mL). To this, Dess-Martin periodinane (80 mg, 0.18 mmol) was added. The mixture was stirred at rt for 30 min before it was diluted with DCM (20 mL) and 1 mL of a 1:1 mixture of saturated $NaHCO_3$ and 2.0% aq. $Na_2S_2O_3$. The mixture was stirred vigorously for 5 min (until the organic phase was clear) and the phases were separated. The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield phosphono-aldehyde **129**, which was used directly in the next step.

Tetramethylethylenediamine (0.036 mL, 0.24 mmol) and triethylamine (0.112 mL, 0.8 mmol) were added to a suspension of $Zn(OTf)_2$ (203.7 mg, 0.440 mmol) in THF (80 mL) at rt. The mixture was stirred for 15 min before phosphono-aldehyde **129** in THF (20 mL) was added drop-wise over 1 h by syringe pump. The resulting mixture was stirred for 15 h at rt. TLC showed complete reaction. The reaction mixture was concentrated to 5 mL. The residue was diluted with ethyl acetate (100 mL) and washed with brine (20 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford a clear oil that was purified by flash column chromatography (hexanes/EtOAc, 90/10) to afford compound **130** (22.7 mg, 78% for two steps). ^{1}H NMR (400 MHz, C_6D_6) δ 6.35 – 6.27 (m, 1H), 6.01 (t, *J* = 10.8 Hz, 1H), 5.65 (dd, *J* = 16.0, 10.8 Hz, 1H), 5.40 (d, *J* = 16.0 Hz, 1H), 5.14 – 5.07 (m, 1H), 5.00 – 4.95 (m, 1H), 4.05 – 4.02 (m, 2H), 2.02 – 2.00 (m, 2H), 1.68 – 1.63 (m, 4H). ^{13}C NMR (125 MHz, C_6D_6) δ 166.4, 147.0, 133.7, 130.9, 128.1, 127.9, 125.8, 63.2, 31.8, 30.8, 27.6; HRMS (ESI) *m/z* calcd for $C_{11}H_{15}O_2^+$ $[M+H]^+$ 179.1067, found 179.1062. IR (thin film, cm^{-1}) 2926, 1722, 1644, 1184, 1148.



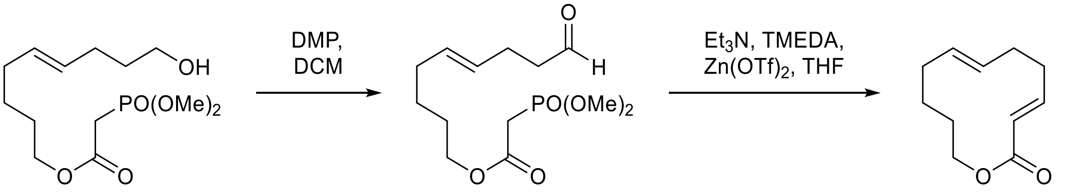
Scheme 2.9.5: Synthesis of hex-5-en-1-yl 2-(dimethoxyphosphoryl)acetate (132-2a)

Hex-5-en-1-ol (250 mg, 2.5 mmol) was dissolved in DCM (20 mL). Et_3N (5.0 mmol, 505 mg) was added. Freshly prepared dimethyl (2-chloro-2-oxoethyl)phosphonate (930 mg, 5.0 mmol) in DCM (5 mL) was added slowly. The mixture was stirred at rt for 2 h. TLC showed complete reaction. The mixture was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The mixture was purified by flash column chromatography (hexanes/EtOAc, 50/50) to provide compound **132-2a** (562 mg, 89% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 5.59 – 5.54 (m, 1H), 4.81 – 4.72 (m, 2H), 3.92 (t, $J = 6.5$ Hz, 2H), 3.58 (d, $J = 11.5$ Hz, 6H), 2.77 (d, $J = 21.5$ Hz, 2H), 1.89 – 1.84 (m, 2H), 1.48 – 1.42 (m, 2H), 1.29 – 1.24 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.4(d, $J = 5.7$ Hz), 137.9, 114.6(t, $J = 25.6$ Hz), 65.2(t, $J = 19.9$ Hz), 52.8(m), 33.6(t, $J = 16.2$ Hz), 32.9(m), 32.5(t, $J = 14.4$ Hz), 27.7, 24.7(m); ^{31}P NMR (200 MHz, $CDCl_3$) δ 22.6; HRMS (ESI) m/z calcd for $C_{10}H_{20}O_5P^+$ $[M+H]^+$ 251.1043, found 251.1044. IR (thin film, cm^{-1}) 2956, 1733, 1274.



Scheme 2.9.6: Synthesis of (*E*)-9-hydroxynon-5-en-1-yl 2-(dimethoxyphosphoryl)acetate (132-2b)

Hex-5-en-1-yl 2-(dimethoxyphosphoryl)acetate **132-2a** (250 mg, 1 mmol), pent-4-en-1-ol (215 mg, 2.5 mmol) and Grubbs II catalyst (42 mg, 0.05 mmol) were added to DCM (20 mL). The mixture was refluxed for 24 h. Then it was loaded on column directly and purified by flash column chromatography (100% EtOAc) to provide compound **132-2b** (215 mg, 70% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.42 – 5.40 (m, 2H), 4.14 (t, J = 6.5 Hz, 2H), 3.79 (d, J = 11.5 Hz, 6H), 3.63 (t, J = 6.5 Hz, 2H), 2.96 (d, J = 22.0 Hz, 2H), 2.12 – 1.99 (m, 4H), 1.82 (br, 1H),1.67 – 1.58 (m, 4H), 1.44 – 1.40 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.6(d, J = 5.7 Hz), 130.3, 129.9, 65.6, 62.0, 53.1(d, J = 6.7 Hz), 33.8, 32.7, 32.3, 31.9, 28.7, 27.7, 25.4; ^{31}P NMR (200 MHz, $CDCl_3$) δ 22.6; HRMS (ESI) *m/z* calcd for $C_{13}H_{26}O_6P^+$ $[M+H]^+$ 309.1462, found 309.1469. IR (thin film, cm^{-1}) 3431, 2932, 1735, 1274, 1059.



132-2b

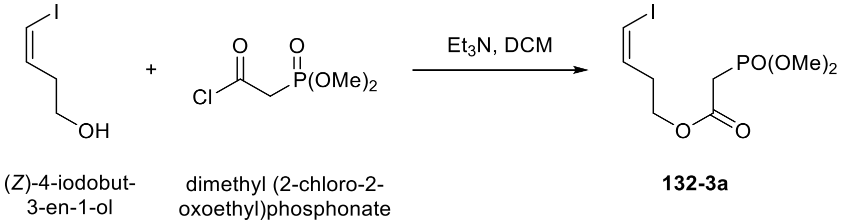
132-2
(Table 2, entry 2)

133-2
Table 2 (entry 2)

Scheme 2.9.7: Synthesis of (3*E*,7*E*)-oxacyclododeca-3,7-dien-2-one (133-2)

Phosphono-alcohol **132-2b** (50 mg, 0.16 mmol) was added to DCM (5 mL). To this, Dess-Martin periodinane (80 mg, 0.18 mmol) was added. The mixture was stirred at rt for 30 min before it was diluted with DCM (20 mL) and 1 mL of a 1:1 mixture of saturated $NaHCO_3$ and 2.0% $Na_2S_2O_3$. The mixture was stirred vigorously for 5 min (until the organic phase was clear) and the phases were separated. The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield aldehyde **132-2** which was used directly in the next step.

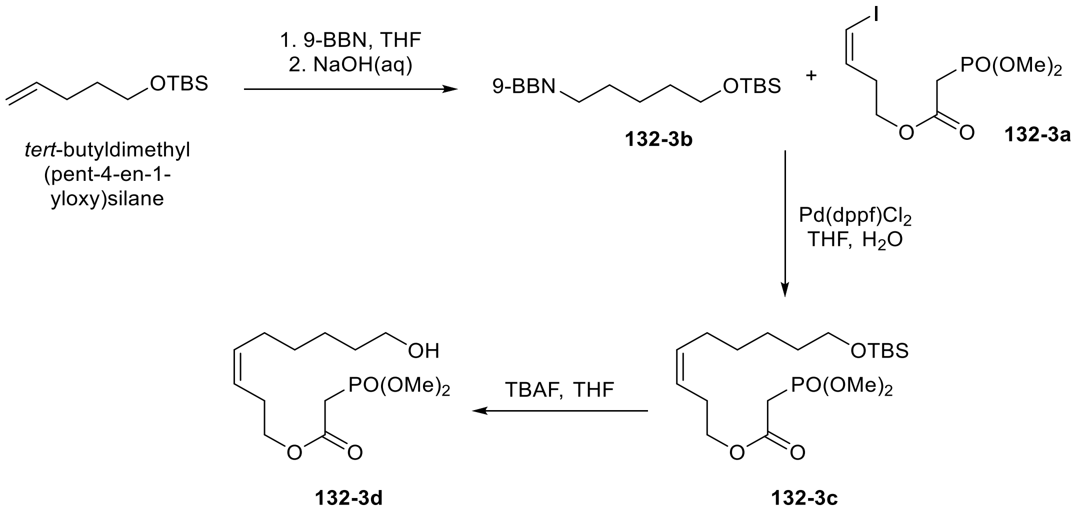
Tetramethylethylenediamine (0.036 mL, 0.24 mmol) and triethylamine (0.112 mL, 0.8 mmol) were added to a suspension of $Zn(OTf)_2$ (203.7 mg, 0.440 mmol) in THF (80 mL) at rt. The mixture was stirred for 15 min before phosphono-aldehyde **132-2** in THF (20 mL) was added drop-wise over 1 h by syringe pump. The resulting mixture was stirred for 15 h at rt. TLC showed complete reaction. The reaction mixture was concentrated to 5 mL. The residue was diluted with ethyl acetate (100 mL) and washed with brine (20 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford a clear oil that was purified by flash column chromatography (hexanes/EtOAc, 90/10) to afford compound **133-2** (20.2 mg, 69% for two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.60 – 6.54 (m, 1H), 5.78 (d, *J* = 16.0 Hz, 1H), 5.38 – 5.32 (m, 1H), 5.25 – 5.22 (m, 1H), 4.17 (t, *J* = 6.4 Hz, 2H), 2.30 – 2.27 (m, 4H), 2.03 – 1.99 (m, 2H), 1.59 – 1.54 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9, 148.7, 133.4, 130.0, 123.6, 66.3, 31.8, 30.6, 30.5, 26.4, 25.5; HRMS (ESI) *m/z* calcd for $C_{11}H_{17}O_2^+$ $[M+H]^+$ 181.1223, found 181.1220. IR (thin film, cm^{-1}) 2930, 1719, 1646, 1453, 1239, 1142.



Scheme 2.9.8: Synthesis of (*Z*)-4-iodobut-3-en-1-yl 2-(dimethoxyphosphoryl)acetate (132-3a)

(*Z*)-4-Iodobut-3-en-1-ol[59] (495 mg, 2.5 mmol) was dissolved in DCM (20 mL). Triethylamine (505 mg, 5.0 mmol) was added. Dimethyl (2-chloro-2-oxoethyl)phosphonate (930 mg, 5.0 mmol) in DCM (5 mL) was added slowly. The mixture was stirred at rt. TLC analysis showed complete reaction after 2 hours. The mixture was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The mixture was purified by flash column chromatography (hexanes/EtOAc, 50/50) to provide compound **132-3a** (760 mg, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.39 – 6.37 (m, 1H), 6.26 – 6.24 (m, 1H), 4.23 (t, *J* = 6.4 Hz, 2H), 3.81 (d, *J* = 11.2 Hz, 6H), 2.99 (d, *J* = 21.2 Hz, 2H), 2.54 – 2.49 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5(d, *J* =

5.7 Hz), 136.5, 85.3, 63.4, 53.2 (d, *J* = 5.7 Hz), 34.2, 33.9, 32.8 ; ^{31}P NMR (200 MHz, CDCl$_3$) δ 22.3; HRMS (ESI) m/z calcd for $C_8H_{15}IO_5P^+$ $[M+H]^+$ 348.9696, found 348.9704; IR (thin film, cm^{-1}) 2956, 1733, 1458, 1272.

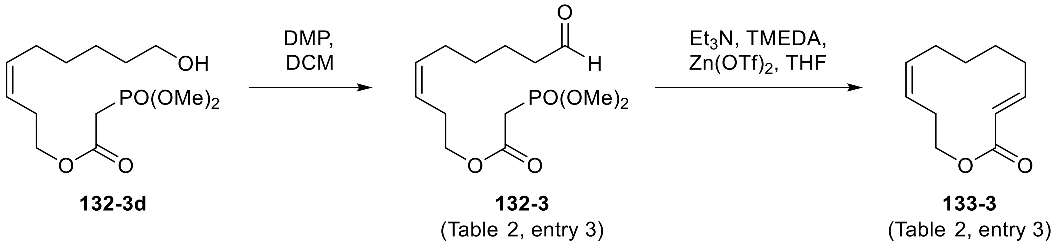


Scheme 2.9.9: Synthesis of (*Z*)-9-hydroxynon-3-en-1-yl 2-(dimethoxyphosphoryl)acetate (132-3d)

tert-Butyldimethyl(pent-4-en-1-yloxy)silane (300 mg, 1.5 mmol) was dissolved in THF (5 mL). To this solution, 9-BBN (6.0 mL, 3.0 mmol, 0.5 M in THF) was added. The mixture was stirred at rt for 3 h. Then, NaOH (1.0 N, 3 mL) was added to the mixture and the reaction was stirred for an additional 30 min to form **132-3b** *in situ*.

To this, phosphate **132-3a** (348 mg, 1 mmol) and Pd(dppf)Cl$_2$·DCM (33 mg, 0.04 mmol) were added. This mixture was stirred for 15 h at rt before being quenched with brine and extracted with EtOAc. The organic layer was washed with brine, dried over MgSO$_4$, filtered, and concentrated *in vacuo* to give **132-3c**.

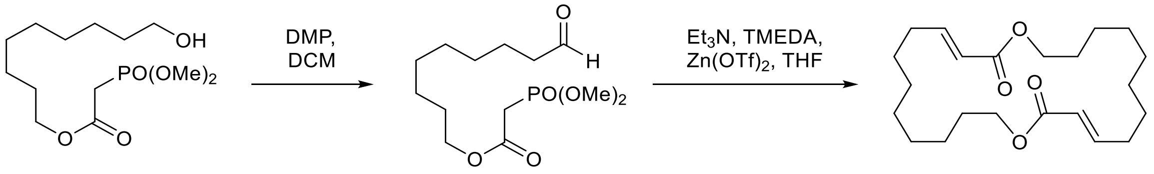
The crude product **132-3c** was dissolved in THF (10 mL) and TBAF (2.0 mL, 2.0 mmol, 1.0 M in THF) was added. The mixture was stirred at rt for 3 h, then diluted with water and extracted with EtOAc. The combined organic layers were washed with brine, dried over MgSO$_4$, filtered, and concentrated *in vacuo*. The mixture was purified by flash column chromatography (100% EtOAc) to provide compound **132-3d** (204 mg, 67% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.51 – 5.48 (m, 1H), 5.35 – 5.32 (m, 1H), 4.13 (t, *J* = 6.8 Hz, 2H), 3.80 (d, *J* = 11.2 Hz, 6H), 3.62 (t, *J* = 6.4 Hz, 2H), 2.97 (d, *J* = 21.6 Hz, 2H), 2.42 – 2.37 (m, 2H), 2.06 – 2.03 (m, 2H), 1.58 – 1.53 (m, 2H), 1.38 – 1.34 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.6 (d, *J* = 5.7 Hz), 132.9, 124.0, 65.2, 62.7, 53.2 (d, *J* = 5.7 Hz), 33.9, 32.8, 32.6, 29.2, 27.2, 26.6, 25.3; ^{31}P NMR (200 MHz, $CDCl_3$) δ 22.5; HRMS (ESI) *m/z* calcd for $C_{13}H_{26}O_6P^+$ $[M+H]^+$ 309.1462, found 309.1470. IR (thin film, cm^{-1}) 3433, 2931, 1737, 1269, 1032.



Scheme 2.9.10: Synthesis of (3*E*,9*Z*)-oxacyclododeca-3,9-dien-2-one (133-3)

Phosphono-alcohol **132-3d** (50 mg, 0.16 mmol) was added to DCM (5 mL). To this, Dess-Martin periodinane (80 mg, 0.18 mmol) was added. The mixture was stirred at rt for 30 min before it was diluted with DCM (20 mL) and 1 mL of a 1:1 mixture of saturated $NaHCO_3$ and 2.0% $Na_2S_2O_3$. The mixture was stirred vigorously for 5 min (until the organic phase was clear) and the phases were separated. The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield aldehyde **132-3** which was used directly in the next step.

Tetramethylethylenediamine (0.036 mL, 0.24 mmol) and triethylamine (0.112 mL, 0.8 mmol) were added to a suspension of $Zn(OTf)_2$ (203.7 mg, 0.440 mmol) in THF (80 mL) at rt. The mixture was stirred for 15 min before phosphono-aldehyde **132-3** in THF (20 mL) was added drop-wise over 1 h by syringe pump. The resulting mixture was stirred for 15 h at rt. TLC showed complete reaction. The reaction mixture was concentrated to 5 mL. The residue was diluted with ethyl acetate (100 mL) and washed with brine (20 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield a clear oil that was purified by flash column chromatography (hexanes/EtOAc, 90/10) to afford compound **133-3** (21.5 mg, 73% for two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.10 – 7.04 (m, 1H), 5.80 (d, J = 16.0 Hz, 1H), 5.38 – 5.31 (m, 2H), 4.33 – 4.30 (m, 2H), 2.28 – 2.17 (m, 4H), 2.01 – 1.97 (m, 2H), 1.65 – 1.60 (m, 2H), 1.50 – 1.49 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.1, 152.5, 133.0, 125.2, 122.4, 64.3, 33.1, 28.8, 27.2, 26.9, 26.8; HRMS (ESI) *m/z* calcd for $C_{11}H_{17}O_2^+$ $[M+H]^+$181.1223, found 181.1218. IR (thin film, cm^{-1}) 2924, 1729, 1272, 1121.



9-hydroxynonyl 2-(dimethoxyphosphoryl acetate)

132-4
(Table 2, entry 4)

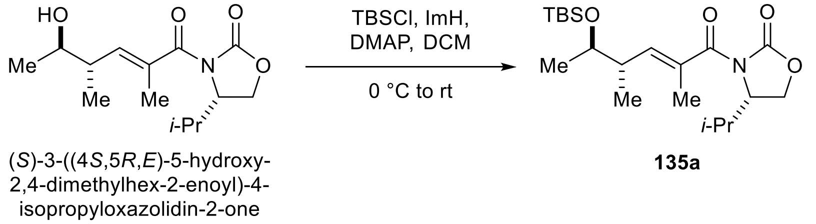
133-4a

Scheme 2.9.11: Synthesis of (3*E*,15*E*)-1,13-dioxacyclotetracosa-3,15-diene-2,14-dione (133-4a)

9-hydroxynonyl 2-(dimethoxyphosphoryl)acetate[61] (50 mg, 0.16 mmol) was added to DCM (5 mL). To this, Dess-Martin periodinane (80 mg, 0.18 mmol) was added. The mixture was stirred at rt for 30 min before it was diluted with DCM (20 mL) and 1 mL of a 1:1 mixture of saturated $NaHCO_3$ and 2.0% $Na_2S_2O_3$. The mixture was stirred vigorously for 5 min (until the organic phase was clear) and the phases were separated. The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield aldehyde **132-4** which was used directly.

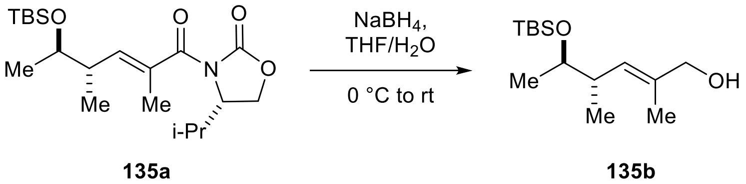
Tetramethylethylenediamine (0.036 mL, 0.24 mmol) and triethylamine (0.112 mL, 0.8 mmol) were added to a suspension of $Zn(OTf)_2$ (203.7 mg, 0.440 mmol) in THF (80 mL) at rt. The mixture was stirred for 15 min before phosphono-aldehyde **132-4** in THF (20 mL) was added drop-wise over 1 h by syringe pump. The resulting mixture was stirred for 15 h at rt. TLC showed complete reaction. The reaction mixture was concentrated to 5 mL. The residue was diluted with ethyl acetate (100 mL) and washed with brine (20 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield a clear oil that was purified by flash column chromatography (hexanes/EtOAc, 90/10) to afford compound **133-4a** (24.5 mg, 83% for two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.96 – 6.93 (m, 2H), 5.83 (d, J = 16.0 Hz, 2H), 4.16 (t, J = 6.0 Hz, 4H), 2.26 – 2.18 (m, 4H), 1.69 – 1.27 (m, 24H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.8, 149.3, 121.6, 64.3, 31.7, 28.9, 28.6, 28.5, 27.8, 27.4, 26.0; HRMS (ESI) *m/z* calcd for $C_{22}H_{37}O_4^+$ $[M+H]^+$ 365.2686, found 365.2689. IR (thin film, cm^{-1}) 2930, 1712, 1654, 1462, 1265.

2.10 Total synthesis of lactimidomycin (procedures & analytical/spectral data)



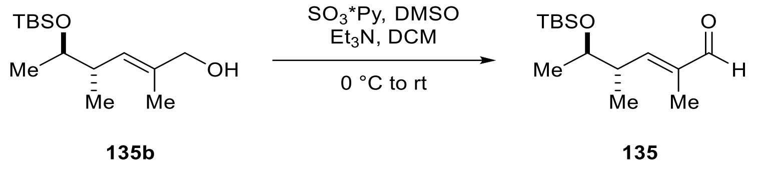
Scheme 2.10.1: Synthesis of (*S*)-3-((4*S*,5*R*,*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethylhex-2-enoyl)-4-isopropyloxaz-olidin-2-one (135a)

Imidazole (5.29 g, 77.6 mmol) and DMAP (948 mg, 7.76 mmol) were added to a stirred solution of (*S*)-3-((4*S*,5*R*,*E*)-5-hydroxy-2,4-dimethylhex-2-enoyl)-4-isopropyloxazolidin-2-one[62, 63] (6.97 g, 25.9 mmol) and DCM (100 mL) at 0 °C. The resulting mixture was stirred for 15 minutes at this temperature before adding TBSCl (7.8 g, 51.8 mmol). The reaction was allowed to warm to rt and stirred for 19 h before quenching with water (150 mL) and stirring for 5 minutes. The aqueous phase was extracted with DCM (3 x 40 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product **135a** (10.2 g) was a yellow oil which was advanced to the next step without purification. An analytical quantity of this product was purified by flash column chromatography (hexanes/EtOAc, 80/20) to assess spectral information. $[\alpha]^{24}_{D}$ = +27.8 (*c* 5.53, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.07 – 6.02 (m, 1H), 4.50 (ddd, *J* = 8.8, 5.4, 4.3 Hz, 1H), 4.29 (t, *J* = 8.9 Hz, 1H), 4.15 (dd, *J* = 8.9, 5.5 Hz, 1H), 3.79 (qd, *J* = 6.2, 3.6 Hz, 1H), 2.52 (dqd, *J* = 10.3, 6.8, 3.5 Hz, 1H), 2.42 – 2.29 (m, 1H), 1.90 (d, *J* = 1.4 Hz, 3H), 1.11 (d, *J* = 6.3 Hz, 3H), 0.99 (d, *J* = 6.9 Hz, 3H), 0.90 (t, *J* = 6.8 Hz, 6H), 0.87 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 153.5, 141.3, 130.6, 71.2, 63.3, 58.2, 40.2, 28.2, 25.8, 21.3, 18.0, 17.8, 15.9, 14.9, 13.6, -4.3, -4.9; HRMS (ESI) *m/z* calcd for $C_{20}H_{38}NO_4Si^+$ $[M+H]^+$: 384.2565, found 384.2565; HRMS (ESI) *m/z* calcd for $C_{20}H_{37}NO_4SiNa^+$ $[M+Na]^+$: 406.2384, found 406.2382; IR (thin film, cm^{-1}) 2961, 2930, 2858, 1788, 1684, 1487, 1472, 1464, 1388, 1365, 1300, 1258, 1208, 1109, 1096, 1062, 1036, 1008, 965, 942, 880, 838, 801, 775, 756, 733, 696, 684, 666.



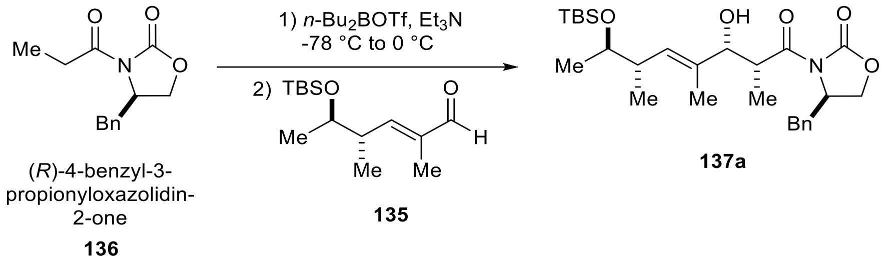
Scheme 2.10.2: Synthesis of (4*S*,5*R*,*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethylhex-2-en-1-ol (135b)

$NaBH_4$ (4.9 g, 129.4 mmol) in water (50 mL) was added to a stirred solution of crude oxazolidinone **135a** (9.93 g) in THF (100 mL) at 0 °C. The reaction was allowed to warm to rt and stirred for 12.5 h before quenching with saturated NH_4Cl (100 mL) slowly. The aqueous phase was extracted with DCM (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography (hexanes/EtOAc, 80/20) to yield **135b** as a clear, colorless oil (5.6 g, 84% yield across two steps). $[\alpha]^{24}_{D}$ = -4.7 (*c* 5.38, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.30 (ddd, *J* = 9.7, 2.4, 1.2 Hz, 1H), 4.01 (d, *J* = 0.8 Hz, 2H), 3.68 (qd, *J* = 6.2, 4.5 Hz, 1H), 2.46 – 2.35 (m, 1H), 1.67 (d, *J* = 1.3 Hz, 3H), 1.35 (s, 1H), 1.04 (d, *J* = 6.2 Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.88 (s, 9H), 0.03 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.5, 129.1, 71.8, 69.2, 39.5, 25.9, 20.8, 18.1, 16.4, 13.9, -4.4, -4.8; HRMS (ESI) *m/z* calcd for $C_{14}H_{30}O_2SiNa^+$ $[M+Na]^+$: 281.1907, found 281.1907; IR (thin film, cm^{-1}) 3316, 2958, 2930, 2858, 1472, 1462, 1374, 1362, 1255, 1167, 1132, 1106, 1066, 1030, 1006, 958, 940, 838, 798, 774, 666.



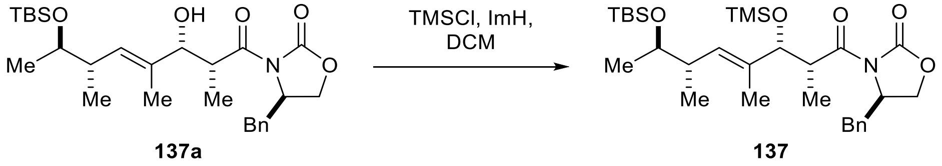
Scheme 2.10.3: Synthesis of (4*S*,5*R*,*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethylhex-2-enal (135)

DMSO (16.09 mL, 226.6 mmol) was added to a solution of alcohol **135b** (3.93 g, 15.2 mmol) in DCM (80 mL). The resulting mixture was cooled to 0 °C. Triethylamine (10.75 mL, 77.1 mmol) and SO_3-Pyridine (9.75 g, 61.3 mmol) were added, and the mixture was stirred for 2 h at 0 °C. Additional SO_3-Pyridine (3.44 g, 21.61 mmol) was added, and the reaction was stirred for another 2 h at 0 °C and then at rt for 1 h. After a total of 5 h, the reaction was diluted with EtOAc (100 mL) and quenched with HCl (2.0 N, 50 mL). The aqueous phase was extracted with EtOAc (2 x 25 mL). The combined organic layers were washed with saturated $NaHCO_3$ (70 mL) and brine/water (1/1, 70 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product **135** (4.1 g) was a yellow oil, which was advanced to the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (s, 1H), 6.46 (d, *J* = 10.0 Hz, 1H), 3.86 – 3.73 (m, 1H), 2.74 – 2.63 (m, 1H), 1.75 (s, 3H), 1.10 (d, *J* = 6.2 Hz, 3H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.88 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H).



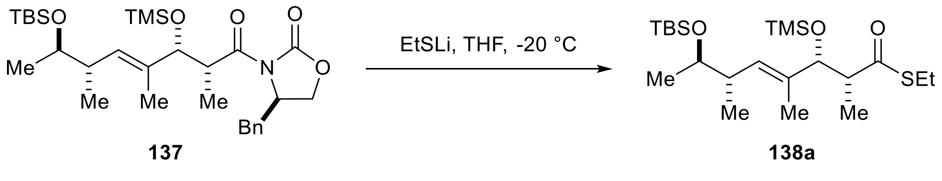
Scheme 2.10.4: Synthesis of (*R*)-4-benzyl-3-((2*R*,3*R*,6*S*,7*R*,*E*)-7-((*tert*-butyldimethylsilyl)oxy)-3-hydroxy-2,4,6-trimethyl-oct-4-enoyl)oxazolidin-2-one (137a)

n-Bu_2BOTf (17.53 mL, 17.53 mmol, 1.0 M in DCM) and triethylamine (2.97 mL, 21.3 mmol) were added to a stirred solution of (*R*)-4-benzyl-3-propionyloxazolidin-2-one (3.91, 16.8 mmol) **(136)** in DCM (50 mL) at -10 °C. This mixture was stirred for 1 h and was cooled to -78 °C before adding crude aldehyde **135** (4.1 g). The reaction was kept at -78 °C for 1 hour, warmed to 0°C, and stirred for an additional 2 h. The reaction was quenched with MeOH/pH 7 buffer (2/1, 39 mL) and MeOH/H_2O_2 (30%) (2/1, 39 mL) and stirred for 1 h at 0°C. The reaction mixture was concentrated *in vacuo*, diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$. The aqueous phase was extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with brine (75 mL), and the aqueous phase was back extracted with EtOAc (40 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. This material was filtered through a short column of silica (hexanes/EtOAc/Et_3N, 81/18/1) to yield the crude product **137a** (7.21 g) as a clear, yellow-colored oil, which was advanced to the next step without further purification.



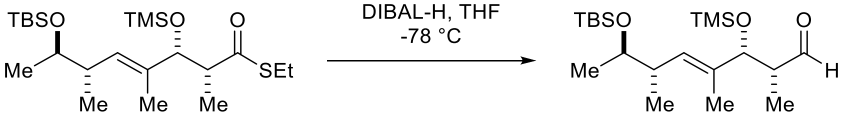
Scheme 2.10.5: Synthesis of (*R*)-4-benzyl-3-((2*R*,3*R*,6*S*,7*R*,*E*)-7-((*tert*-butyldimethylsilyl)oxy)-2,4,6-trimethyl-3-((trimethylsilyl)oxy)oct-4-enoyl)oxazolidin-2-one (137)

Imidazole (4.01 g, 58.9 mmol) and TMSCl (3.74 mL, 29.4 mmol) were added to a solution of compound **137a** (7.21 g) in DCM (55 mL). This mixture was stirred for 20 h at rt, before it was diluted with EtOAc (100 mL). The organic phase was washed with HCl (0.2 N, 45 mL), and the aqueous phase was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography (hexanes/EtOAc/Et_3N, 85/14/1 → 79/20/1) to yield **137** as a clear, colorless oil (6.82 g, 79% yield across three steps). $[\alpha]^{24}_D$ = -21.8 (*c* 5.315, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.16 (m, 5H), 5.35 (d, *J* = 9.8 Hz, 1H), 4.54 (dddd, *J* = 9.7, 7.5, 3.1, 2.2 Hz, 1H), 4.31 (d, *J* = 6.6 Hz, 1H), 4.15 (dd, *J* = 9.1, 2.2 Hz, 1H), 4.12 – 4.04 (m, 2H), 3.69 (qd, *J* = 6.2, 2.8 Hz, 1H), 3.27 (dd, *J* = 13.3, 3.1 Hz, 1H), 2.75 (dd, *J* = 13.3, 9.7 Hz, 1H), 2.42 – 2.32 (m, 1H), 1.58 (d, *J* = 1.2 Hz, 3H), 1.18 (d, *J* = 6.8 Hz, 3H), 0.95 (d, *J* = 2.7 Hz, 3H), 0.94 (d, *J* = 2.1 Hz, 3H), 0.90 (s, 9H), 0.07 (s, 9H), 0.03 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 174.9, 153.0, 135.4, 134.8, 129.4, 129.4, 128.9, 127.3, 78.5, 71.4, 65.8, 55.7, 42.0, 39.1, 37.7, 25.9, 20.8, 18.1, 16.4, 12.8, 11.9, -0.1, -4.4, -4.8; HRMS (ESI) *m/z* calcd for $C_{30}H_{51}NO_5Si_2Na^+$ $[M+Na]^+$: 584.3198, found 584.3202; IR (thin film, cm^{-1}) 2958, 2930, 2858, 1784, 1702, 1472, 1455, 1377, 1362, 1291, 1251, 1209, 1195, 1106, 1073, 1030, 967, 909, 890, 839, 799, 775, 760, 702, 668.



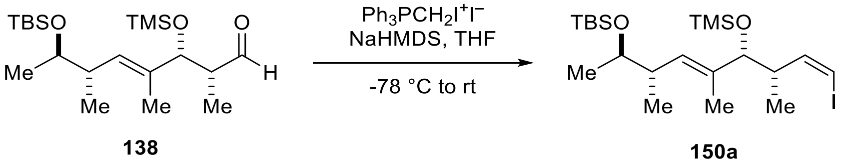
Scheme 2.10.6: Synthesis of (2*R*,3*R*,6*S*,7*R*,*E*)-*S*-ethyl 7-((*tert*-butyldimethylsilyl)oxy)-2,4,6-trimethyl-3-((trimethylsilyl)oxy)oct-4-enethioate (138a)

n-BuLi (9.27 mL, 14.83 mmol, 1.6 M in THF) was added to a solution of ethanethiol (1.69 mL, 22.8 mmol) in THF (60 mL) at –20 °C. In a separate flask, compound **137** (6.60 g, 11.8 mmol) in THF (50 mL + 5 mL rinse) was cooled to -20 °C and then added to the reaction mixture. After 2 h, the reaction was diluted with EtOAc (50 mL), quenched with saturated NH_4Cl (75 mL), and warmed to rt. The aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with saturated $NaHCO_3$ (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by a flash column chromatography (hexanes/EtOAc/Et_3N, 82/17/1) to yield **138a** as a clear, colorless oil (4.35 g, 83% yield). $[\alpha]^{24}_D = -17.6$ (*c* 5.18, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.23 (d, *J* = 9.7 Hz, 1H), 4.13 (d, *J* = 8.2 Hz, 1H), 3.68 (qd, *J* = 6.2, 3.1 Hz, 1H), 2.88 – 2.78 (m, 2H), 2.73 (dq, *J* = 13.3, 7.5 Hz, 1H), 2.42 – 2.35 (m, 1H), 1.60 (d, *J* = 1.3 Hz, 3H), 1.20 (t, *J* = 7.5 Hz, 3H), 1.17 (d, *J* = 6.8 Hz, 3H), 0.92 (dd, *J* = 6.5, 4.3 Hz, 6H), 0.89 (s, 9H), 0.06 (s, 9H), 0.02 (d, *J* = 4.5 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.5, 134.6, 130.5, 80.2, 71.0, 53.1, 39.0, 25.9, 23.0, 20.0, 18.1, 15.3, 14.5, 14.0, 11.4, 0.1, -4.4, -4.9; HRMS (ESI) *m/z* calcd for $C_{22}H_{46}O_3SSi_2Na^+$ $[M+Na]^+$: 469.2598, found 469.2618; IR (thin film, cm^{-1}) 2958, 2931, 2858, 1690, 1472, 1456, 1412, 1375, 1320, 1297, 1251, 1212, 1187, 1166, 1132, 1099, 1072, 1029, 1006, 964, 907, 887, 869, 839, 799, 774, 749, 725, 667, 642, 634, 608, 542.



Scheme 2.10.7: Synthesis of (2*R*,3*R*,6*S*,7*R*,*E*)-7-((*tert*-butyldimethylsilyl)oxy)-2,4,6-trimethyl-3-((trimethylsilyl)oxy)oct-4-enal (138)

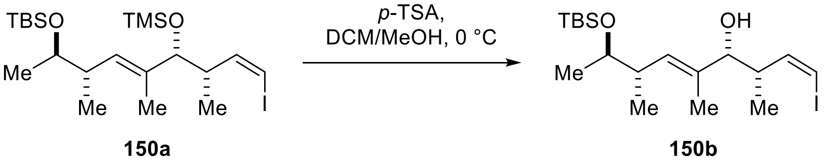
DIBAL-H (12.6 mL, 12.6 mmol, 1.0 M in DCM) was added via syringe pump over 10 minutes to a stirred solution of thioester **138a** (4.31 g, 9.66 mmol), in DCM (100 mL) at -78 °C. After 30 minutes, the reaction was quenched with MeOH (20 mL) cooled to -78 °C. The reaction was warmed to rt and was partially concentrated *in vacuo* to remove about half of the solvent. The reaction mixture was diluted with EtOAc (75 mL) and Rochelle's salt solution (75 mL), and the biphasic mixture was stirred for 30 minutes and then separated. The aqueous phase was extracted with EtOAc (3 x 30 mL), and the combined organic layers were washed with NaOH (1.0 M, 2 x 40 mL), saturated $NaHCO_3$ (50 mL), and brine (75 mL). The combined aqueous layers were further extracted with EtOAc (3 x 50 mL) and washed with brine (100 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. This material was filtered through a short column of silica (hexanes/EtOAc, 90/10) to yield the crude product **138** (3.81 g) as a clear, colorless oil, which was used immediately in the next reaction. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.63 (d, *J* = 2.2 Hz, 1H), 5.37 (d, *J* = 9.7 Hz, 1H), 4.26 (dd, *J* = 6.4, 0.5 Hz, 1H), 3.72 (qd, *J* = 6.2, 2.9 Hz, 1H), 2.51 (pd, *J* = 6.8, 2.2 Hz, 1H), 2.42 – 2.34 (m, 1H), 1.56 (d, *J* = 1.3 Hz, 3H), 1.03 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.2 Hz, 3H), 0.96 (d, *J* = 6.9 Hz, 3H), 0.88 (s, 9H), 0.08 (s, 9H), 0.03 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.5, 134.5, 129.6, 77.9, 71.4, 50.6, 39.2, 25.9, 21.1, 18.1, 16.7, 12.3, 9.2, 0.1, -4.3, -4.9; LCMS (ESI) *m/z* for $C_{20}H_{42}O_3Si_2Na^+$ $[M+Na]^+$: 409.1.



Scheme 2.10.8: Synthesis of (4*R*,7*S*,8*R*,*E*)-4-((*S*,*Z*)-4-iodobut-3-en-2-yl)-2,2,5,7,8,10,10,11,11-nonamethyl-3,9-dioxa-2,10-disiladodec-5-ene (150a)

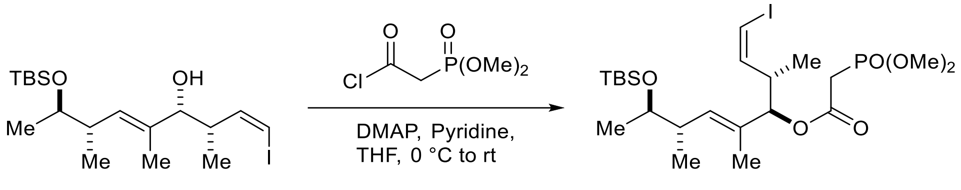
NaHMDS (19.38 mL, 19.38 mmol, 1.0 M in THF) was added to a stirred suspension of iodomethylenetriphenylphosphonium iodide in THF (50 mL). The reaction mixture was stirred for 7 minutes, sonicated for 3 minutes, and cooled to -78 °C. Aldehyde **138** (3.81 g) was added in THF (5 mL, 2 x 3 mL rinse) slowly. The reaction mixture was stirred for 45 minutes at -78 °C, let warm to rt over 30 minutes, and quenched with saturated NH_4Cl (75 mL). The biphasic mixture was extracted with EtOAc (3 x 50 mL), and the combined organic layers were washed with saturated

$NaHCO_3$ (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography (hexanes/EtOAc/Et_3N, 98.5/0.5/1,) to yield **150a** as a yellow colored oil (3.35 g, 68% yield across two steps). $[\alpha]^{24}_{D}$ = +64.5 (*c* 4.76, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.06 (dd, *J* = 7.4, 0.5 Hz, 1H), 5.85 (dd, *J* = 9.2, 7.4 Hz, 1H), 5.18 (d, *J* = 9.7 Hz, 1H), 3.83 (d, *J* = 7.9 Hz, 1H), 3.71 (qd, *J* = 6.2, 2.8 Hz, 1H), 2.73 – 2.59 (m, 1H), 2.43 – 2.30 (m, 1H), 1.57 (d, *J* = 1.3 Hz, 3H), 0.99 (d, *J* = 6.7 Hz, 3H), 0.96 (d, *J* = 6.3 Hz, 3H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.89 (s, 9H), 0.07 (s, 9H), 0.04 (s, 3H), 0.03 ([s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.2, 136.2, 129.1, 81.9, 80.5, 71.6, 43.8, 38.9, 25.9, 21.0, 18.1, 16.5, 15.5, 11.9, 0.1, -4.3, -4.8; HRMS (ESI) *m/z* calcd for $C_{21}H_{43}IO_2Si_2Na^+$ $[M+Na]^+$: 533.1738, found 533.1742; IR (thin film, cm^{-1}) 2957, 2928, 2857, 1472, 1461, 1406, 1374, 1312, 1279, 1250, 1165, 1104, 1094, 1067, 1028, 1006, 962, 885, 838, 798, 774, 748, 706, 669.



Scheme 2.10.9: Synthesis of (1*Z*,3*S*,4*R*,5*E*,7*S*,8*R*)-8-((*tert*-butyldimethylsilyl)oxy)-1-iodo-3,5,7-trimethylnona-1,5-dien-4-ol (150b)

p-TSA in DCM/MeOH (90/10, 2 mL) was added slowly to a stirred solution of compound **150a** (3.35 g, 6.56 mmol) in DCM/MeOH (90/10, 68 mL) at 0 °C. After 40 minutes, the reaction was quenched with saturated $NaHCO_3$/water (1/1, 50 mL) and warmed to rt while stirring. The aqueous layer was extracted with EtOAc (3 x 30 mL), and the combined organics were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product **150b** (2.71 g) as a yellow oil, which was advanced to the next step without purification. $[\alpha]^{24}_{D}$ = +74 (*c* 0.97, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.12 (d, *J* = 7.4 Hz, 1H), 5.94 (dd, *J* = 9.2, 7.4 Hz, 1H), 5.28 (d, *J* = 9.6 Hz, 1H), 3.94 (dd, *J* = 7.7, 2.3 Hz, 1H), 3.70 (qd, *J* = 6.2, 3.4 Hz, 1H), 2.78 – 2.65 (m, 1H), 2.38 (dqd, $J = 10.1, 6.8, 3.4$ Hz, 1H), 1.62 (d, $J = 1.3$ Hz, 3H), 1.47 (s, 1H), 1.06 (d, $J = 6.7$ Hz, 3H), 0.98 (d, $J = 6.2$ Hz, 3H), 0.95 (d, $J = 6.8$ Hz, 3H), 0.88 (s, 9H), 0.03 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.8, 135.9, 130.1, 81.1, 80.9, 71.6, 43.1, 39.2, 25.9, 21.0, 18.1, 16.7, 15.0, 12.2, -4.3, -4.8; HRMS (ESI) *m/z* calcd for $C_{18}H_{35}IO_2SiNa^+$ $[M+Na]^+$: 461.1343, found 461.1342.



150b

150

Scheme 2.10.10: Synthesis of (1Z,3S,5E,7S,8R)-8-((tert-butyldimethylsilyl)oxy)-1-iodo-3,5,7-trimethylnona-1,5-dien-4-yl 2-(dimethoxyphosphoryl)acetate (150)

DMAP (226 mg, 1.85 mmol) was added to a solution of the crude alcohol **150b** (2.71 g) in THF (50 mL) at 0 °C. Pyridine (2.55 mL, 31.7 mmol) and acyl chloride (21.8 mL, 0.8 M in THF) were added slowly, the reaction was stirred at 0 °C for 5 min and then warmed to rt. After 90 minutes, the reaction was diluted with DCM (200 mL), washed with HCl (1.0 N, 100 mL), $CuSO_4$ (0.1 M, 100 mL), water (2 x 100 mL), and saturated $NaHCO_3$ (100 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography (hexanes/EtOAc, 50/50) to yield **150** as a clear, yellow colored oil (1.681 g, 44% yield[iv] across 2 steps). $[\alpha]^{24}_D = +63.9$ (*c* 1.03, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.18 (d, $J = 7.5$ Hz, 1H), 5.91 (dd, $J = 9.2, 7.5$ Hz, 1H), 5.34 (d, $J = 9.6$ Hz, 1H), 5.15 (d, $J = 8.5$ Hz, 1H), 3.80 (dd, $J = 11.2, 1.7$ Hz, 6H), 3.69 (qd, $J = 6.2, 3.2$ Hz, 1H), 3.00 (dd, $J = 21.7, 1.7$ Hz, 2H),

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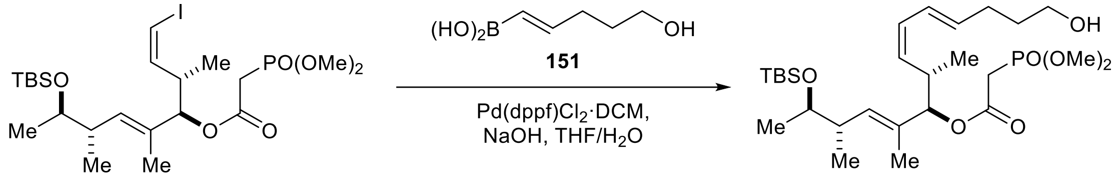
[iv] This is an unoptimized yield. Executing similar procedure on a smaller scale (100 mg) using a 0.06 M concentration of crude compound **47a** in THF and adding a 0.4 M concentration of acyl chloride in THF provided the desired product in significantly higher yield (85% across 2 steps).

2.97 – 2.87 (m, 1H), 2.42 – 2.28 (m, 1H), 1.62 (s, 3H), 1.03 (d, *J* = 6.7 Hz, 3H), 0.95 (d, *J* = 6.3 Hz, 3H), 0.94 (d, *J* = 6.9 Hz, 3H), 0.88 (s, 9H), 0.03 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 164.6 (d, *J* = 6.4 Hz), 142.3, 132.6, 131.2, 83.1, 82.2, 71.5, 53.0 (dd, *J* = 7.5, 6.6 Hz), 41.8, 39.2, 33.9, 33.2, 25.8, 21.0, 18.0, 16.5, 15.2, 12.6, -4.4, -4.8; HRMS (ESI) *m/z* calcd for $C_{22}H_{42}IO_6PSiNa^+$ $[M+Na]^+$: 611.1425, found 611.1423; IR (thin film, cm^{-1}) 2957, 2929, 2856, 1738, 1472, 1462, 1375, 1272, 1258, 1185, 1107, 1059, 1033, 964, 931, 837, 803, 774, 705, 668.

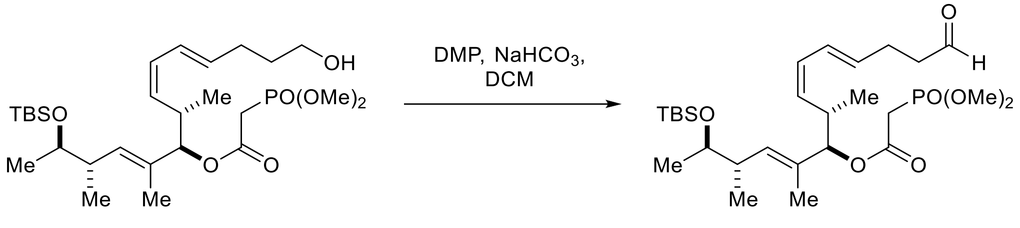


150 **148**

Scheme 2.10.11: Synthesis of (2*R*,3*S*,4*E*,6*R*,7*S*,8*Z*,10*E*)-2-((*tert*-butyldimethylsilyl)oxy)-14-hydroxy-3,5,7-trimethyltetra-deca-4,8,10-trien-6-yl 2-(dimethoxyphosphoryl)acetate (148)

Cis-iodide **150** (907 mg, 1.54 mmol) and boronic acid **151**^{3} (493 mg, 3.85 mmol) were added to THF/H_2O (3:1, 12.5 mL) and stirred until they dissolved. NaOH (1.0 M, 3.1 mL) was added to the solution, which was then stirred for 2 minutes. Next, Pd(dppf)Cl_2·DCM (63 mg, 0.077 mmol) in THF/H_2O (1 mL + 0.5 mL wash) was added. The reaction mixture was stirred for 18 h and quenched with pH 7 buffer (30 mL). The aqueous phase was extracted with EtOAc (3 x 35 mL), and the combined organic layers were washed with brine (2 x 50 mL), back extracted with EtOAc (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (100% EtOAc) to yield **148** as a yellow oil (711 mg, 84% yield). $[\alpha]^{24}_D$ = +56.8 (*c* 3.595, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.34 – 6.22 (m, 1H), 5.89 (t, *J* = 11.0 Hz, 1H), 5.74 – 5.62 (m, 1H), 5.33 (d, *J* = 9.7 Hz, 1H), 5.06 – 4.93 (m, 2H), 3.80 (dd, *J* = 11.2, 4.4 Hz, 6H), 3.66 (dd, *J* = 7.8, 5.2 Hz, 3H), 3.07 – 2.90 (m, 3H), 2.38 – 2.26 (m, 1H), 2.19 (q, *J* = 7.2 Hz, 2H), 1.71 – 1.64 (m, 2H), 1.56 (d, *J* = 1.2 Hz, 3H), 1.02 (d, *J* = 6.6 Hz, 3H), 0.94

(d, $J = 6.2$ Hz, 3H), 0.92 (d, $J = 6.8$ Hz, 3H), 0.87 (d, $J = 2.7$ Hz, 9H), 0.01 (s, 6H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 164.7 (d, $J = 6.2$ Hz), 134.7, 132.7, 131.4, 130.6, 128.5, 125.7, 84.9, 71.4, 62.1, 53.0 (dd, $J = 6.5$, 5.2 Hz), 39.1, 34.6, 33.9, 33.1, 32.1, 29.1, 25.8, 20.6, 18.0, 17.4, 16.2, 12.2, -4.5, -4.9; HRMS (ESI) *m/z* calcd for $C_{27}H_{51}O_7PSiNa^+$ $[M+Na]^+$: 569.3034, found 569.3039; IR (thin film, cm^{-1}) 3440, 2957, 2930, 2857, 1736, 1472, 1462, 1375, 1270, 1257, 1215, 1185, 1106, 1060, 1035, 982, 964, 928, 877, 837, 805, 774, 667.



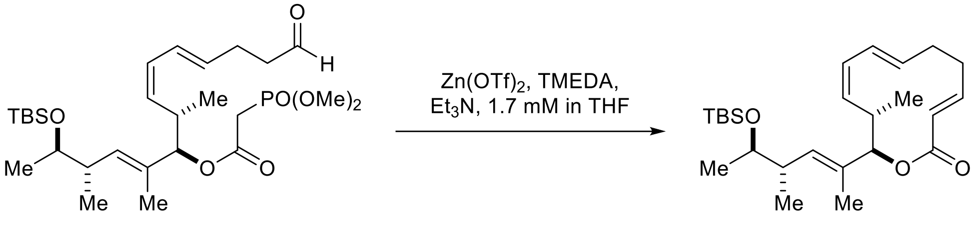
148

149

Scheme 2.10.12: Synthesis of (2*R*,3*S*,4*E*,6*R*,7*S*,8*Z*,10*E*)-2-((*tert*-butyldimethylsilyl)oxy)-3,5,7-trimethyl-14-oxotetradeca-4,8,10-trien-6-yl 2-(dimethoxyphosphoryl)acetate (149)

$NaHCO_3$ (109.2 mg, 1.30 mmol) and DMP (882.4 mg, 2.08 mmol) were added to a solution of phosphono-alcohol **148** (710.9 mg, 1.30 mmol) in DCM (37 mL). This reaction mixture was stirred for 2 h 45 min, quenched with saturated $NaHCO_3$ (30 mL) and $Na_2S_2O_3$ (0.5 M, 30 mL), and then stirred for an additional 10 minutes. The aqueous layer was extracted with DCM (3 x 30 mL) and the combined organic layers were washed with brine (50 mL). The aqueous brine wash was extracted again with DCM (15 mL), and the organic phase was washed with brine (15 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. This material was filtered through a short column of silica gel (hexanes/EtOAc, 92/8) to yield **149** as a yellow oil, which was used immediately in the next reaction (591.6 mg, 84% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.76 (t, $J = 1.4$ Hz, 1H), 6.33 – 6.24 (m, 1H), 5.85 (t, $J = 11.0$ Hz, 1H), 5.69 – 5.56 (m, 1H), 5.32 (d, $J = 9.6$ Hz, 1H), 5.04 (t, $J = 10.6$ Hz, 1H), 4.97 (d, $J = 9.0$ Hz, 1H), 3.78 (dd, $J = 11.2$, 2.9 Hz, 6H), 3.63 (qd, $J = 6.2$, 3.3 Hz, 1H), 2.97 (dd, $J = 21.7$, 0.7 Hz, 3H), 2.53 (t, $J = 7.0$

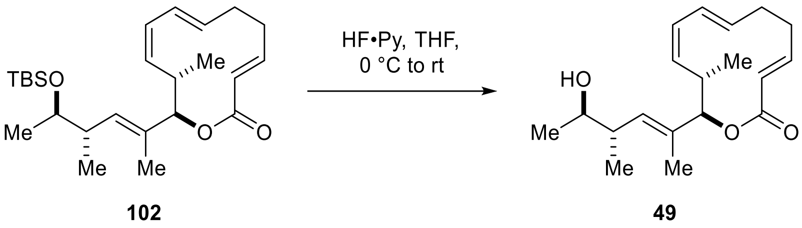
Hz, 2H), 2.42 (q, *J* = 7.1 Hz, 2H), 2.37 – 2.23 (m, 1H), 1.54 (d, *J* = 1.2 Hz, 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.92 (d, *J* = 6.3 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H), 0.86 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.6, 164.7 (d, *J* = 6.4 Hz), 132.8, 132.6, 131.5, 131.4, 128.0, 126.4, 84.8, 71.4, 53.0 (dd, *J* = 6.5, 2.4 Hz), 43.2, 39.1, 34.7, 34.1, 33.0, 25.8, 25.3, 20.7, 18.0, 17.4, 16.3, 12.2, -4.5, -4.9.



149 **102**

Scheme 2.10.13: Synthesis of (3*E*,7*E*,9*Z*,11*S*,12*R*)-12-((4*S*,5*R*,*E*)-5-((*tert*-butyldimethylsilyl)oxy)-4-methylhex-2-en-2-yl)-11-methyloxacyclododeca-3,7,9-trien-2-one (102)

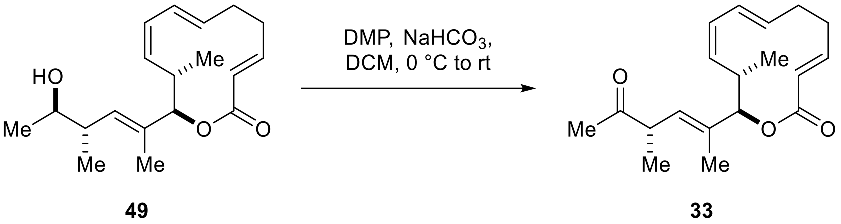
TMEDA (0.194 mL, 1.30 mmol) and Et_3N (0.606 mL, 4.34 mmol) were added to a solution of $Zn(OTf)_2$ (871 mg, 2.40 mmol) in THF (610 mL). This whitish-clear suspension was stirred for 30 minutes, and phosphono-aldehyde **149** (591.6 mg) dissolved in THF (40 mL) was added via syringe pump over 30 minutes. The reaction mixture was stirred for a total of 18 h and concentrated *in vacuo* to remove the majority of the solvent. The remaining crude oil was dissolved in EtOAc (125 mL), washed with brine (150 mL) and back extracted with EtOAc (2 x 30 mL), washed with 1% HCl (100 mL) and back extracted with EtOAc (30 mL), and finally washed with saturated $NaHCO_3$ (100mL) and brine (150 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography (hexanes/EtOAc/Et_3N, 96/3/1 → 91/8/1) to yield **102** as a clear oil product, which solidified under vacuum into a white crystalline solid (423 mg, 93% yield). $[\alpha]^{24}_D$ = -146.4 (*c* 1.28, $CHCl_3$); $[\alpha]^{24}_D$ = -159.1 (*c* 2.46, benzene); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.48 (ddd, *J* = 15.9, 10.3, 5.3 Hz, 1H), 6.03 (t, J = 10.8 Hz, 1H), 5.76 (dd, J = 15.6, 10.7 Hz, 1H), 5.56 (d, J = 16.1 Hz, 1H), 5.47 – 5.37 (m, 2H), 5.34 (d, J = 4.8 Hz, 1H), 5.13 (t, J = 11.0 Hz, 1H), 3.76 (qd, J = 6.2, 3.1 Hz, 1H), 3.15 – 3.02 (m, 1H), 2.61 – 2.48 (m, 2H), 2.47 – 2.39 (m, 1H), 2.05 – 1.80 (m, 2H), 1.68 (d, J = 1.3 Hz, 3H), 1.06 (d, J = 6.2 Hz, 3H), 0.98 (d, J = 6.8 Hz, 3H), 0.95 (d, J = 6.8 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.8, 146.3, 134.6, 133.5, 132.3, 129.5, 128.6, 128.4, 127.5, 83.7, 71.6, 39.4, 35.9, 32.3, 31.2, 25.8, 21.3, 18.0, 17.5, 16.8, 14.6, -4.3, -4.8; HRMS (ESI) *m/z* calcd for $C_{25}H_{42}O_3SiNa^+$ $[M+Na]^+$: 441.2795, found 441.2807; IR (thin film, cm^{-1}) 2959, 2929, 2858, 1724, 1644, 1472, 1462, 1454, 1375, 1337, 1328, 1314, 1287, 1273, 1253, 1189, 1154, 1140, 1100, 1086, 1064, 1034, 1005, 958, 940, 837, 803, 791, 775, 679, 666.



Scheme 2.10.14: Synthesis of (3*E*,7*E*,9*Z*,11*S*,12*R*)-12-((4*S*,5*R*,*E*)-5-hydroxy-4-methylhex-2-en-2-yl)-11-methyloxacyclodo-deca-3,7,9-trien-2-one (49)

To a plastic Falcon Tube equipped with a septum, macrolactone **102** (40 mg, 0.096 mmol) and THF (2.73 mL) were added, stirred and cooled to 0 °C. HF-Pyridine complex (0.55 mL, 70% w/w) was added dropwise, and the reaction mixture was stirred 2 h at 0 °C and for 1 h 45 min at rt. Once the reaction was deemed complete by TLC, the reaction was cooled to 0 °C and slowly quenched by the dropwise addition of saturated $NaHCO_3$ (30 mL) over 10 minutes. The resulting solution was diluted with EtOAc (20 mL), and the resulting biphasic mixture was separated. The aqueous layer was extracted with EtOAc (2 x 20 mL), and the combined organic layers were washed with $CuSO_4$ (0.1 M, 15 mL) and water (15 mL). The organic phase was then dried over

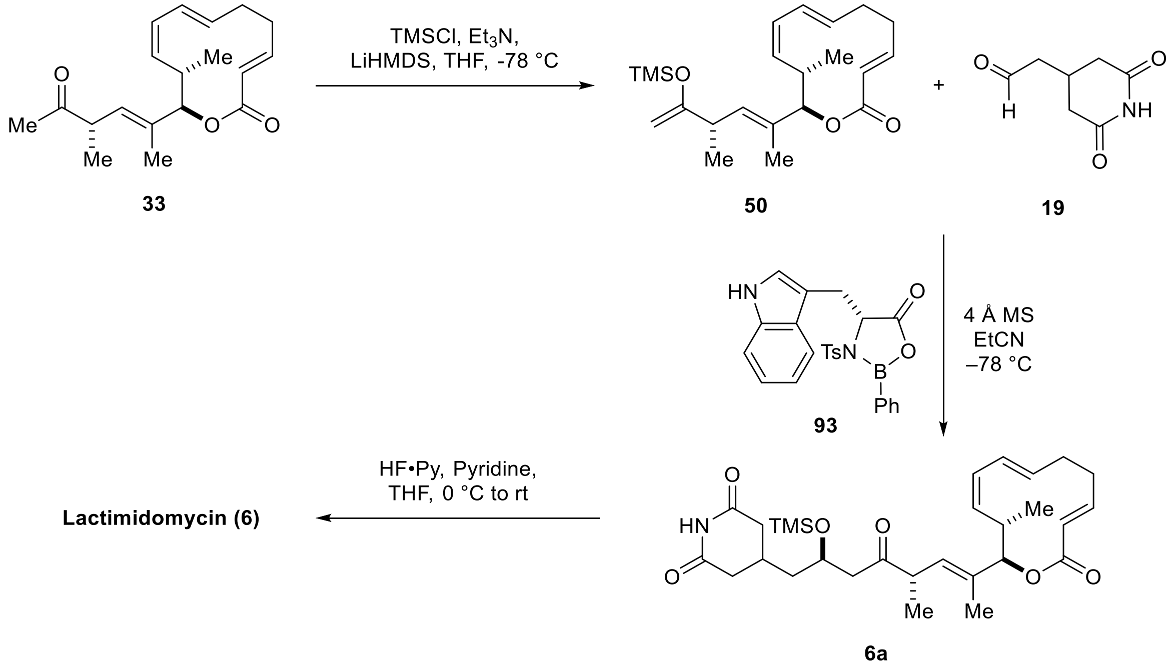
$MgSO_4$, filtered, and concentrated *in vacuo* to yield a crude yellow oil. The crude product was purified by flash column chromatography (hexanes/EtOAc, 80/20) to yield **49** as a clear, colorless oil (26.3 mg, 90% yield). $[\alpha]^{24}_D = -234.4$ (*c* 1.16, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 6.47 (ddd, *J* = 15.9, 10.3, 5.2 Hz, 1H), 6.03 (dd, *J* = 10.8, 10.8 Hz, 1H), 5.73 (dd, *J* = 15.6, 10.7 Hz, 1H), 5.55 (d, *J* = 16.1 Hz, 1H), 5.40 (ddd, *J* = 15.2, 8.7, 6.1 Hz, 1H), 5.34 (d, *J* = 4.4 Hz, 1H, overlap), 5.33 (d, *J* = 8.9 Hz, 1H, overlap), 5.10 (dd, *J* = 10.9, 10.9 Hz, 1H), 3.60 (qd, *J* = 6.2, 6.2 Hz 1H), 3.16 – 3.02 (m, 1H), 2.62 – 2.48 (m, 2H), 2.48 – 2.35 (m, 1H), 2.03 – 1.82 (m, 2H), 1.72 (d, *J* = 1.2 Hz, 3H), 1.56 (br s, 1H), 1.18 (d, *J* = 6.2 Hz, 3H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.7, 146.4, 134.5, 133.1, 132.0, 131.7, 128.9, 128.3, 127.7, 83.3, 71.5, 40.1, 35.6, 32.3, 31.2, 20.3, 17.5, 16.4, 15.0; HRMS (ESI) *m/z* calcd for $C_{19}H_{28}O_3Na^+$ $[M+Na]^+$: 327.1931, found 327.1930; IR (thin film, cm^{-1}) 3448, 2965, 2928, 2873, 1717, 1642, 1452, 1375, 1337, 1328, 1314, 1273, 1190, 1142, 1086, 1002, 957, 927, 848, 830, 801, 735, 679.



Scheme 2.10.15: Synthesis of (3*E*,7*E*,9*Z*,11*S*,12*R*)-11-methyl-12-((*S*,*E*)-4-methyl-5-oxohex-2-en-2-yl)oxacyclododeca-3,7,9-trien-2-one (33)

A suspension of Dess-Martin periodinane (66.7 mg, 0.16 mmol) and $NaHCO_3$ (8.3 mg, 0.098 mmol) in DCM (1.0 mL + 3 x 0.5 mL rinse) was added to a solution of alcohol **49** (26 mg, 0.085 mmol) in DCM (2 mL) at 0 °C. The mixture was stirred at 0 °C for 5 min and stirred at rt for 1 h 30 min before the sample was concentrated *in vacuo*. The residue was purified by flash column chromatography (hexanes/EtOAc, 87/13) to yield **33** as a clear, colorless oil (19.6 mg, 75%).[10]

$[\alpha]^{24}_{D}$ = -26.2 (*c* 1.07, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$) δ 6.48 (ddd, *J* = 15.9, 10.3, 5.3 Hz, 1H), 6.04 (dd, *J* = 10.8, 10.8 Hz, 1H), 5.73 (dd, *J* = 15.6, 10.6 Hz, 1H), 5.55 (d, *J* = 16.1 Hz, 1H), 6.48 (ddd, *J* = 15.9, 10.3, 5.3 Hz, 1H), 5.38 – 5.33 (m, 2H, overlap), 5.08 (dd, *J* = 10.9, 10.9 Hz, 1H), 3.43 (dq, *J* = 9.5, 6.8 Hz, 1H), 3.14 – 3.06 (m, 1H), 2.59-2.48 (m, 2H), 2.13 (s, 3H), 2.00 – 1.87 (m, 2H), 1.78 (d, *J* = 1.2 Hz, 3H), 1.17 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 209.1, 166.5, 146.6, 134.5, 133.3, 131.5, 129.8, 129.1, 128.2, 127.8, 82.6, 46.9, 36.0, 32.2, 31.2, 28.0, 17.4, 16.2, 14.9; HRMS (ESI) *m/z* calcd for $C_{19}H_{26}O_3Na^+$ $[M+Na]^+$: 325.1774, found 325.1775; IR (thin film, cm^{-1}) 2966, 2931, 2873, 1718, 1643, 1453, 1373, 1353, 1337, 1314, 1243, 1189, 1141, 1089, 1002, 958, 873, 848, 829, 800, 766, 733, 719.



Scheme 2.10.16: Synthesis of lactimidomycin (6)[6]

Me_3SiCl (29.2 µL, 0.23 mmol) and triethylamine (32.1 µL, 0.23 mmol) were added to a solution of ketone **33** (6.9 mg, 0.023 mmol) in THF (0.82 mL) at –78 °C. Next, LiHMDS (92 µL, 0.092 mmol, 1.0 M in THF) was slowly introduced and the resulting mixture was stirred at –78 °C for 1 h. The reaction was then quenched with pH 7 phosphate buffer and extracted with DCM (3 x

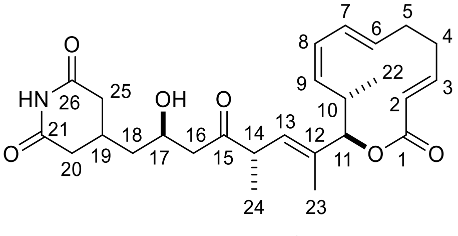
1 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the corresponding silyl enol ether **19**, which was used in the next step without further purification.

Molecular sieves (4Å, ca. 82 mg) and aldehyde **19** (4.6 mg, 0.030 mmol)[64] were added to a solution of the crude silyl enol ether **15** in propionitrile (0.41 mL). The mixture was cooled to –78 °C before a solution of compound **50** [prepared upon stirring of a solution of $PhBCl_2$ (3.0 µL, 0.023 mmol) and N-tosyl-*D*-tryptophan (8.1 mg, 0.023 mmol) in DCM (0.20 mL) for 1 h, followed by removal of the solvent][65] in propionitrile (0.12 mL) was added dropwise. After stirring for 22 h at –78°C, the reaction was quenched with saturated $NaHCO_3$ (1 mL). The aqueous phase was extracted with DCM (3 x 3 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude product **6a**.

This resulting compound **6a** was dissolved in THF (4 mL) at 0 °C and treated with 0.41 mL of a stock solution of buffered HF-pyridine [prepared from THF (3.63 mL), pyridine (1.35 mL) and HF-pyridine complex (0.27 mL, 70% w/w)]. The mixture was stirred at 0 °C for 2 h and at rt 30 min to complete the desilylation. The reaction was diluted with DCM (30 mL), and the organic layer was washed with saturated $NaHCO_3$ (10 mL) and $CuSO_4$ solution (0.1 M, 3 x 15 mL), dried over $MgSO_4$, filtered and concentrated in vacuo to leave a thin oil residue. This crude product was purified by flash column chromatography through a pipette (hexanes/EtOAc, 20/80) to give product **6** as a white solid (6.6 mg, 63%). $[\alpha]^{24}_{D}$ = -29.6 (c 0.66, $CHCl_3$); $[\alpha]^{24}_{D}$ = -20.1 (c 0.25, DMSO); ^1H NMR (700 MHz, $CDCl_3$) δ 7.81 (br s, 1H), 6.49 (ddd, J = 15.9, 10.3, 5.3 Hz, 1H), 6.06 (dd, J = 10.8, 10.8 Hz, 1H), 5.73 (dd, J = 15.6, 10.6 Hz, 1H), 5.55 (d, J = 16.1 Hz, 1H), 5.42 (ddd, J = 15.3, 8.9, 6.1 Hz, 1H), 5.37 – 5.32 (m, 2H, overlap), 5.06 (dd, J = 10.9, 10.9 Hz, 1H), 4.15-4.08 (m, 1H), 3.43 (dq, J = 9.6, 6.8 Hz, 1H), 3.22 (d, J = 1.9 Hz, 1H), 3.14 – 3.08 (m, 1H), 2.83 – 2.72 (m, 2H), 2.60 – 2.55 (m, 3H), 2.55 – 2.47 (m, 2H), 2.37 – 2.29 (m, 2H), 2.02 – 1.86 (m, 2H), 1.78 (d, J = 1.2 Hz, 3H), 1.61 (ddd, J = 14.2, 10.6, 5.0 Hz, 1H), 1.32 (ddd, J = 24.9, 14.0, 10.3 Hz, 1H), 1.19 (d, J = 6.8 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 212.5, 172.1, 172.0, 166.7, 147.1, 134.7, 134.2, 131.3, 129.6, 129.1, 128.3, 128.2, 82.5, 65.0, 47.6, 46.8, 40.9, 38.6, 37.3, 36.1, 32.5, 31.4, 27.3, 17.7, 16.3, 15.5; HRMS (ESI) m/z calcd for $C_{26}H_{35}NO_6Na^+$ $[M+Na]^+$: 480.2357, found 480.2362; IR (thin film, cm^{-1}) 3480, 3226, 2961, 2926, 2856, 1704, 1453, 1376, 1261, 1190, 1142, 1087, 1006, 830, 797, 766, 736, 702.

2.11 Lactimidomycin (6) (natural vs. synthetic NMR spectral data)

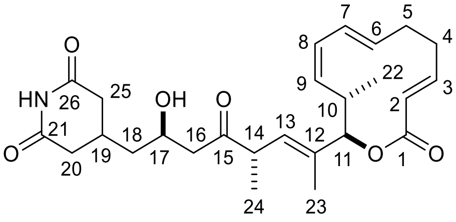
Table 2.11.1: Comparison of the recorded ^{1}H NMR spectral data ($CDCl_3$) of lactimidomycin (6) with reported literature spectral data[54]



Lactimidomycin (**6**)

Position	Literature (500 MHz) δ (in ppm) mult. (*J* in Hz)	Experimental (700 MHz) δ (in ppm) mult. (*J* in Hz)	Δδ
2	5.53 d (16.0)	5.55 d (16.1)	+0.02
3	6.49 ddd (16.0, 10.0, 5.0)	6.49 ddd (15.9, 10.3, 5.3)	0
4	1.96 m/2.56 m	1.94 m/2.56 m	-0.02/0
5	1.96 m/2.54 m	1.94 m/2.53 m	-0.02/-0.01
6	5.42 m	5.42 m	0
7	5.72 dd (15.5, 10.5)	5.73 dd (15.6, 10.6)	+0.01
8	6.06 t (11.0)	6.06 t (10.8)	0
9	5.06 t (11.0)	5.06 t (10.9)	0
10	3.11 m	3.11 m	0
11	5.34 m	5.34 m	0
13	5.34 m	5.34 m	0
14	3.44 m	3.43 m	-0.01
16	2.59 m	2.58 m	-0.01
17	4.12 m	4.12 m	0
18	1.33 ddd (14.0, 9.0, 3.0)	1.32 ddd (13.9, 8.9, 2.9)	-0.01
	1.60 ddd (14.0, 10.5, 4.5)	1.61 (14.2, 10.6, 5.0)	+0.01
19	2.48 m	2.50 m	+0.02
20	2.34 m/2.76 m	2.33 m/2.77 m	-0.01/0
22	0.92 d (6.5)	0.92 d (6.8)	0
23	1.78 d (1.5)	1.78 d (1.2)	0
24	1.19 d (7.0)	1.19 d (6.8)	0
25	2.32 m/2.80 m	2.33 m/2.79 m	+0.01/-0.01
NH	7.98 br s	7.81 br s	-0.17
OH	N/A	3.22 d (1.9)	N/A

Table 2.11.2: Comparison of the recorded ^{13}C NMR spectral data (CDCl$_3$) of lactimidomycin (6) with reported literature spectral data[54]



Lactimidomycin (**6**)

Position	Literature (125 MHz)	Experimental (175 MHz)	Δδ
1	166.7	166.7	0
2	128.4	128.3	-0.1
3	147.0	147.1	+0.1
4	32.4	32.5	+0.1
5	31.4	31.4	0
6	128.2	128.2	0
7	134.6	134.7	+0.1
8	129.6	129.6	0
9	131.1	131.3	+0.2
10	36.1	36.1	0
11	82.5	82.5	0
12	134.1	134.2	+0.1
13	129.1	129.1	0
14	46.8	46.8	0
15	212.5	212.5	0
16	47.6	47.6	0
17	64.9	65.0	+0.1
18	40.9	40.9	0
19	27.3	27.3	0
20	38.6	38.6	0
21	172.2	172.1	-0.1
22	17.7	17.7	0
23	15.4	15.5	+0.1
24	16.3	16.3	0
25	37.3	37.3	0
26	172.1	172.0	-0.1

2.12 Lactimidomycin (6) (comparison of circular dichroism [CD] spectral data)

Figure 2.12.1: CD as reported by Ben Shen *et. al.*

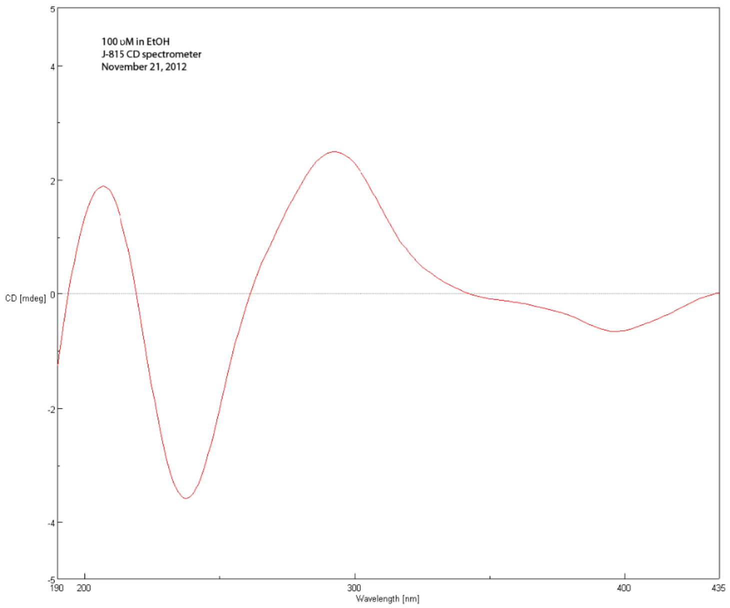
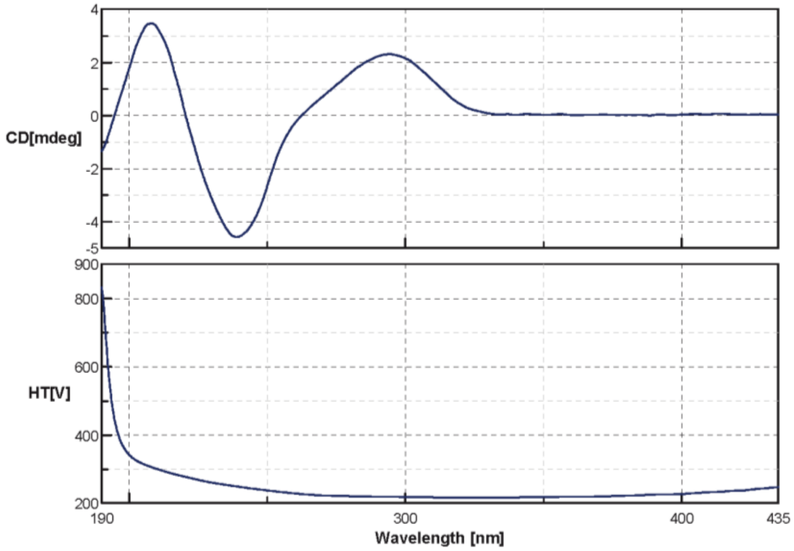
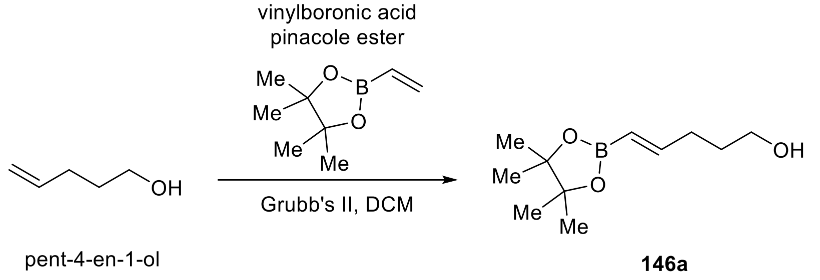


Figure 2.12.2: CD as reported by Nagorny *et. al.*



Date	2/5/2013 3:53PM
File name	LTM (100 μM in Ethanol).jws
Model	J-815
Band width	2 nm
Response	2 sec
Sensitivity	Standard
Measurement range	435 - 190 nm
Data pitch	0.2nm
Scanning speed	50 nm/min
Accumulation	10
Temperature	Room Temperature

2.13 DMB side-route (procedures & analytical/spectral data)



Scheme 2.13.1: Synthesis of (*E*)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pent-4-en-1-ol (146a)[66]

A solution of the Grubb's II catalyst (98.6 mg, 0.116 mmol) in DCM (11.6 mL) were added to a flame-dried rbf equipped with reflux condenser and kept under N_2 pressure. Next, 4-penten-1-ol (200 mg, 2.32 mmol, 0.24 mL) and vinylboronic acid pinacol ester (715 mg, 4.64 mmol, 0.78 mL) were each added via syringe, and the resulting mixture was stirred and refluxed at 45 °C for 20 h. The reaction was allowed to cool to rt and was concentrated in vacuo to remove the majority of the DCM. The crude brown sludge-like mixture was purified via flash column chromatography (hexanes/EtOAc, 2/1) to yield the pure, viscous, brown-colored oil product **146a** (410.9 mg, 83% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.61 (dt, *J* = 17.8, 6.4 Hz, 1H), 5.44 (dd, *J* = 17.9, 1.6 Hz, 1H), 3.62 (t, *J* = 6.5 Hz, 2H), 2.27 – 2.15 (m, 2H), 1.76 – 1.70 (br s, 1H), 1.71 – 1.63 (m, 2H), 1.24 (s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.57, 83.02, 62.22, 31.94, 31.06, 24.70;[v] IR (thin film, cm^{-1}) 2977, 2935, 2857, 1723, 1684, 1639, 1609, 1594, 1517, 1465, 1420, 1398, 1322, 1267, 1238, 1144, 1102, 1031, 1000, 971, 849, 807, 766, 744, 678, 641.

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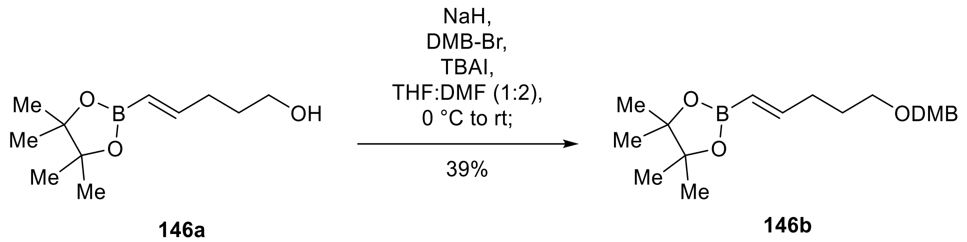
[v] Typical to not see ^{13}CNMR signal of carbon atom next to boron atom, as it is split by the ^{11}B and ^{10}B isotopes into an unobservable, flattened multiplet.



Scheme 2.13.2: Synthesis of (*E*)-2-(5-((3,4-dimethoxybenzyl)oxy)pent-1-en-1-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane) (146b)

To a 15 mL RBF was added **146a** (270 mg, 1.27 mmol) dissolved in THF/DMF (1:2, 4 mL) and TBAI was added (46.8 mg, 0.127 mmol). The reaction mixture was stirred and cooled to 0 °C. After 5 min, NaH (101.4 mg [60% w/w in mineral oil], 2.54 mmol) was added to this stirred mixture rapidly, put back under N_2, and stirred at 0 °C for another 10 min, and then let warm to 0 °C. Next, DMB-Br (587 mg, 2.54 mmol) dissolved in THF/DMF (1:2, 3 mL) was added via syringe, and the reaction was stirred for another 10 min at 0 °C and let warm to rt. After stirring at rt for 20 h, the reaction mixture was diluted with EtOAc (15 mL), washed with brine/water mixture (15 mL, 1:1), and extracted with EtOAc (3 x 25 mL). The combined organic fractions were washed with brine and dried over $MgSO_4$, filtered, and concentrated in vacuo to yield the crude product. This was purified by flash column chromatography (hexanes/EtOAc, 4/1) to yield the product **146b** (180 mg, 39% yield). ^{1}H NMR (401 MHz, $CDCl_3$) δ 6.90 – 6.79 (m, 3H), 6.62 (dt, *J* = 17.9, 6.4 Hz, 1H), 5.44 (dt, *J* = 18.0, 1.6 Hz, 1H), 4.41 (s, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 3.45 (t, *J* = 6.6 Hz, 2H), 2.27 – 2.17 (m, 2H), 1.79 – 1.66 (m, 2H), 1.25 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.64, 148.93, 148.43, 131.08, 120.10, 110.90, 110.82, 82.98, 72.73, 69.47, 55.86, 55.76, 32.30, 28.19, 24.72;[vi] LRMS (ESI) *m/z* calcd for $C_{20}H_{31}BO_5^+$ [M+Na]$^+$: 385.2, found 385.1; IR (thin film, cm^{-1}) 2977, 2935, 2857, 2282, 2000, 1723, 1684, 1639, 1609, 1594, 1517, 1465, 1420, 1398, 1363,

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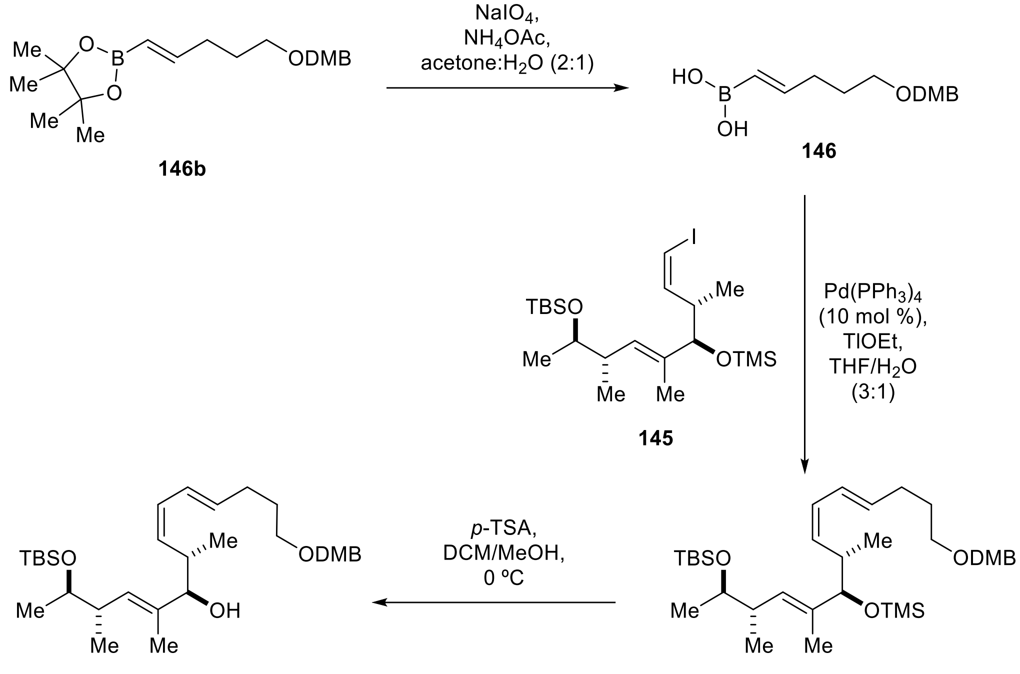
[vi] Typical to not see ^{13}CNMR signal of carbon atom next to boron atom, as it is split by the ^{11}B and ^{10}B isotopes into an unobservable, flattened multiplet.

1267, 1144, 1102, 1031, 1000, 971, 925, 901, 849, 807, 766, 679, 642, 578, 520, 440, 434, 428, 422, 410.



Scheme 2.13.3: Synthesis of (2*R*,3*S*,4*E*,6*R*,7*S*,8*Z*,10*E*)-2-((*tert*-butyldimethylsilyl)oxy)-14-((3,4-dimethoxybenzyl)oxy)-3,5,7-trimethyltetradeca-4,8,10-trien-6-ol (147)

Preparation of crude **146**:[vii] To a 10 mL RBF was added DMB-protected **146b** (85 mg, 0.234 mmol) in acetone/H_2O (5 mL, 2:1), $NaIO_4$ (156 mg, 0.727 mmol), NH_4OAc (54.3 mg, 0.704 mmol).[67] The resulting white-colored suspension was stirred at rt for 42 h. The reaction mixture was concentrated in vacuo to remove most of the organic solvents, and from this crude liquid was

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[vii] 301.3 mg scale: To a RBF was added DMB-protected **146b** (301.3 mg) in acetone/water (9 mL, 2/1), $NaIO_4$ (552 mg, 2.58 mmol), NH_4OAc (192 mg, 2.50 mmol). The resulting white-colored suspension was stirred at rt for 46 h, 15 min. The reaction mixture was concentrated in vacuo to remove most of the organic solvents, and from this crude liquid was extracted with EtOAc (3 x 20 mL). The combined organic fractions were rinsed with brine (50 mL), dried over $MgSO_4$ for 20 min, filtered, and concentrated in vacuo to yield the crude product. The final product (**146**) was frozen in benzene and was utilized without further purification in subsequent reactions.

extracted with EtOAc (3 x 20 mL). The combined organic fractions were rinsed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to yield the crude product **146**. This crude product was frozen in benzene to store and was used directly in the subsequent Suzuki cross-coupling reaction without further purification or characterization.

<u>Step 1 – Suzuki cross coupling</u>:[viii] To a 5 mL RBF (oven dried) was added the vinyl iodide **145** (56.4 mg) and boronic acid **146** (43 mg, crude), and the container was put under argon gas. Next, THF/H_2O (0.7 mL, 3/1, pre-degassed under Ar) was added to the RBF under argon. Next, thallium ethoxide[ix] (2.6 μL, 0.026 mmol) was added, followed by $Pd(PPh_3)_4$ (12.7 mg, 0.11 mmol) dissolved[x] in degassed THF/H_2O (3:1, 1.25 mL). The reaction mixture was stirred for 3 h and formed a solid yellow precipitate in solution. After 3 hours, the reaction appeared incomplete by TLC, so an addition portion of thallium ethoxide (5 μL, 0.071 mmol) and $Pd(Ph_3)_4$ (7 mg, 6.1 μmol) was added, and a small amount of THF/H_2O was carefully added to replace partially evaporated solvent, and the reaction was left to stir overnight. During the course of the reaction it

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[viii] <u>270 mg scale</u>: The boronic acid **146** (207.3 mg, semi-crude) and vinyl iodide **145** (269.8 mg, 0.53 mmol mmol) were dissolved in THF/H_2O (3.70 mL, 3/1). The solution was degassed (freeze-pump-thaw, three cycles), replacing the nitrogen atmosphere with an argon one. $Pd(PPh_3)_4$ (97.7 mg, 0.0846 mmol) was added quickly, and the solution stirred for 3 min under positive Argon pressure. Next, thallium ethoxide[ix] (18.0 μL, 0.254 mmol) was added quickly. A dark yellow precipitate formed immediately, and the solution was stirred overnight. The reaction mixture was quenched with pH 7 phosphate buffer and diluted with EtOAc. The heterogeneous mixture was stirred for 5 min and filtered through celite to remove the thallium salts, and the aqueous and organic phases were seperated. The aqueous fraction was reextracted with EtOAc, and the combined organic fractions were washed with brine, dried over $MgSO_4$, and conc. in vacuo to yield an orange-colored, viscous oil crude product **147a** (468 mg, crude), which was used directly in the larger scale deprotection reaction (footnote xi) without further purification or characterization.

[ix] CAUTION: Thallium is highly toxic! Handle with extreme care.

[x] Sonicated under Argon gas prior to addition via syringe.

turned from yellow to orange to brownish/blackish in color. The reaction was quenched with 3 pipettes full of pH 7 buffer and the result mixture was transferred to a 125 mL separatory funnel. The round bottom flash was washed with EtOAc (3 x 15 mL) and pH 7 buffer (20) and all combined fractions were transferred to a separatory funnel. The organic layer was extracted, and the aqueous phase was back extracted with EtOAc (15 mL). The combined organic fractions were washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to yield an orange-colored, viscous oil crude product (**147a**) which was used directly in the subsequent deprotection reaction without further purification or characterization.

Step 2 – Deprotection of TMS:[xi] To a 5 mL RBF was added **147a** (92.1 mg, crude) in DCM/MeOH (90:10, 1.45 mL). This solution was put under N_2, cooled to 0 °C in an ice bath, and stirred. Next, PTSA (0.273 mL) was quickly added via syringe. After stirring for 5 min, the reaction was checked by TLC and deemed incomplete. Let continue to stir for 45 min, at which point most of the SM had converted to product by TLC, so the reaction was quenched with $NaHCO_3$(sat):H_2O (1:1, 5 mL), let stir for 30 min, and transferred to a separatory funnel. The mixture was extracted with EtOAc (3 x 7 mL), and the combined organic phases were dried over $MgSO_4$, filtered, and

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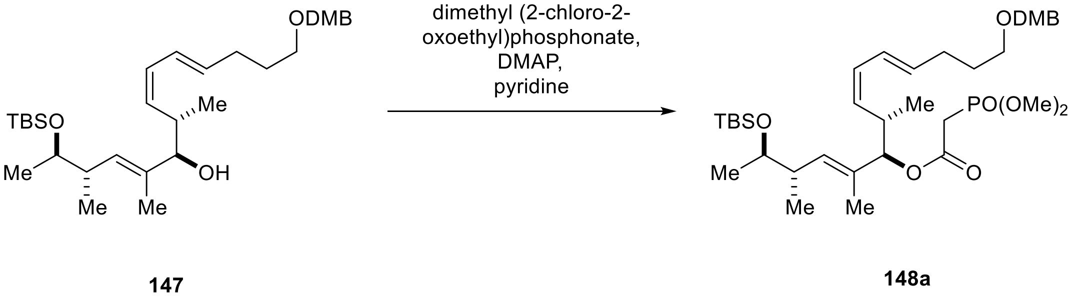
[xi] 468 mg scale: To a 25 mL RBF was added TMS-protected **147a** (468 mg, crude) in DCM/MeOH (90:10, 7.56 mL). This solution was put under N_2, cooled to 0 °C in an ice bath, and stirred. Next, PTSA (1.44 mg, 7.56 μmol) dissolved in DCM/MeOH (9:1, 0.144 mL) was quickly added via syringe. After stirring for 3 hours at 0 °C, the reaction was quenched with sat. $NaHCO_3$:H_2O (1:1, 50 mL), was stirred for 30 min, and was frozen in the freezer to work up the next day. After thawing, the organic phases were extracted using EtOAc (2 x 20 mL), combined, dried over $MgSO_4$, filtered, and conc. in vacuo to yield the crude product. This product was purified via flash column chromatography (hexanes/EtOAc, 5/1) to obtain the pure product **147** (240 mg) as well as recovering unreacted starting material **147a** and mixed fractions. This unreacted material **147a** and mixed fractions were resubjected to reaction conditions following the same general procedure, using PTSA (0.63 mg, 3.31 μmol) and DCM:MeOH (9:1, 3.3 mL). After running for 12 hours, the reaction was quenched same as before, followed by extraction with EtOAc (3 x 20 mL). After flash column chromatography (hexanes/EtOAc, 5/1), additional pure product **147** (31.1 mg) was obtained, raising the total amount of the of pure product **147** (271.1 mg, 67% yield over 2 steps) obtained.

concentrated in vacuo to yield the crude product. This product was purified via flash column chromatography (hexanes/EtOAc, 4/1) to obtain the pure product **147** (55.1 mg, 91% yield across 2 steps from intermediate **145**). $[\alpha]^{24}_D = +61.8$ (*c* 3.14, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 6.93 – 6.79 (m, 3H), 6.36 – 6.24 (m, 1H), 5.86 (t, *J* = 11.0 Hz, 1H), 5.65 (dt, *J* = 14.5, 6.9 Hz, 1H), 5.29 – 5.21 (m, 1H), 5.06 (t, *J* = 10.6 Hz, 1H), 4.43 (s, 2H), 3.89 (s, 3H), 3.88 (s, 3H), 3.78 (dd, *J* = 7.9, 3.6 Hz, 1H), 3.67 (qd, *J* = 6.2, 3.4 Hz, 1H), 3.46 (t, *J* = 6.5 Hz, 2H), 2.83 – 2.74 (m, 1H), 2.35 (dqd, *J* = 10.1, 6.8, 3.4 Hz, 1H), 2.19 (q, *J* = 7.1 Hz, 2H), 1.76 – 1.66 (m, 2H), 1.55 (d, *J* = 1.3 Hz, 3H), 1.47 (d, *J* = 3.7 Hz, 1H), 1.05 (d, *J* = 6.6 Hz, 3H), 0.97 (d, *J* = 6.2 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.88 (s, 9H), 0.02 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.95, 148.47, 136.01, 134.30, 132.34, 131.08, 130.28, 127.82, 125.90, 120.15, 110.92, 110.82, 82.43, 77.00, 72.84, 71.54, 69.56, 55.90, 55.80, 39.10, 36.19, 29.47, 29.39, 25.85, 20.68, 18.06, 17.38, 16.41, 11.81, -4.39, -4.83; HRMS (ESI) *m/z* calcd for $C_{32}H_{54}O_5SiNa^+$ $[M+Na]^+$: 569.3638, found 569.3633; IR (thin film, cm^{-1}) 2956.0, 2929.2, 2856.3, 1593.9, 1516.6, 1463.2, 1373.5, 1257.5, 1157.8, 1137.9, 1096.5, 1030.4, 960.0, 836.5, 774.0, 429.7, 414.8, 407.9.



Scheme 2.13.4: Synthesis of (2*R*,3*S*,4*E*,6*R*,7*S*,8*Z*,10*E*)-2-((*tert*-butyldimethylsilyl)oxy)-14-((3,4-dimethoxybenzyl)oxy)-3,5,7-trimethyltetradeca-4,8,10-trien-6-yl 2-(dimethoxyphosphoryl)acetate (148a)

To a solution of alcohol **147** (164.4 mg, 0.301 mmol), THF (1.5 mL), and DMAP (11 mg). This reaction mixture was put under N_2, cooled to 0 °C, and stirred. Pyridine (0.125 mL, 1.541 mmol,) and dimethyl (2-chloro-2-oxoethyl)phosphonate (3 mL, 0.4 M in THF) were both slowly added, in sequence, and the reaction mixture was allowed to stir for 15 min at 0 °C, let warm to rt and let stir overnight. The reaction mixture was concentrated in vacuo, dissolved in DCM, and washed with HCl (1.0 N), $CuSO_4$ (0.1 M), and H_2O (D.I.), sequentially. After each wash, the aqueous phase was back extracted with DCM, and the combined organic fractions were dried over $MgSO_4$, filtered, and conc. in vacuo to yield a clear, colorless, crude oil product. This crude product was purified by flash column chromatography (hexanes/EtOAc, 1/2) to yield a faintly yellow-colored, viscous oil product **148a**[xii] (191.8 mg, 92% yield). $[\alpha]^{24}_D$ = +58.8 (*c* 2.15, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 6.89 – 6.81 (m, 3H), 6.25 (dd, *J* = 14.9, 11.0 Hz, 1H), 5.86 (t, *J* = 10.9 Hz, 1H), 5.65 (dt, *J* = 14.5, 6.9 Hz, 1H), 5.31 (dd, *J* = 9.7, 1.6 Hz, 1H), 5.03 – 4.95 (m, 2H), 4.42 (s, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 3.79 (d, *J* = 3.0 Hz, 3H), 3.77 (d, *J* = 3.0 Hz, 3H), 3.64 (qd, *J* = 6.2, 3.0 Hz, 1H), 3.45 (t, *J* = 6.5 Hz, 2H), 3.02 – 2.93 (m, 3H), 2.30 (dqd, *J* = 9.9, 6.8, 3.1 Hz, 1H), 2.17 (q, *J* = 7.3 Hz, 2H), 1.69 (p, *J* = 6.8 Hz, 2H), 1.54 (s, 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.92 (d, *J* = 6.2 Hz, 3H), 0.90 (d, *J* = 6.8 Hz, 3H), 0.86 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.69 (d, *J* = 6.2 Hz), 148.90, 148.44, 134.82, 132.75, 131.43, 130.99, 130.52, 128.47, 125.55, 120.11, 110.87, 110.77, 84.88, 72.79, 71.39, 69.51, 55.84, 55.74, 52.99 (d, *J* = 6.2 Hz), 39.08,

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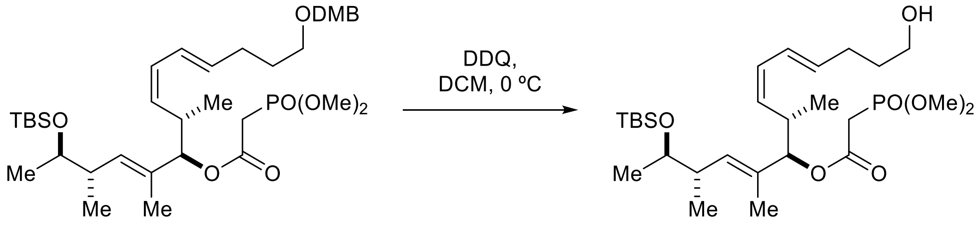
[xii] 55.1 mg scale: To a 5 mL RBF was added alcohol **(147)** (55.1 mg, 0.0967 mmol), THF (1.6 mL), and DMAP (1.0 mg, 8.19 μmol). This reaction mixture was put under N_2, cooled to 0 °C, and stirred. Pyridine (0.0404 mL, 0.5 mmol) was added slowly, followed by dropwise addition of dimethyl (2-chloro-2-oxoethyl)phosphonate (0.74 mL, 0.4 M in THF), stirred 15 min @ 0 °C, warmed to rt and let run 20 h. By TLC, the reaction appeared to be approximately 50% complete. After concentrating the reaction down, and redissolving in THF (1.0 mL), DMAP (3.0 mg, 24.6 μmol) was added. The mixture was stirred, put under N_2 atmosphere, and cooled to 0 °C in an ice bath. Next, pyridine (0.044 mL, 0.55 mmol) and fresh dimethyl (2-chloro-2-oxoethyl)phosphonate (0.65 mL, 0.5 M) were both slowly added, in sequence, and the reaction mixture was allowed to stir for 15 min at 0 °C, let warm to rt and stir overnight. The crude product was purified via flash column chromatography (hexanes/EtOAc, 1/2), but DMAP and pyridine coeluted with the product. To remove these impurities, the product was dissolved in DCM, washed with HCl (1.0 N), $CuSO_4$ (0.1 M), and H_2O (D.I.). After each wash, the aqueous phase was back extracted with DCM, and the combined organic fractions were dried over $MgSO_4$, filtered, and conc. in vacuo to field the clear, colorless, crude oil product **148a** (30.3 mg, 45% yield).

34.58, 34.08, 33.01, 29.46, 29.34, 25.79, 20.63, 17.99, 17.36, 16.19, 12.14, -4.48, -4.89; HRMS (ESI) *m/z* calcd for $C_{36}H_{61}O_9PSiNa^+$ $[M+Na]^+$: 719.3715, found 719.3727; IR (thin film, cm^{-1}) 2955.7, 2930.2, 2855.4, 1735.3, 1593.7, 1516.7, 1463.4, 1419.3, 1374.1, 1266.5, 1185.5, 1157.8, 1103.6, 1031.4, 963.8, 928.3, 837.1, 804.3, 774.4, 668.5, 431.0, 423.1, 415.6, 410.2.

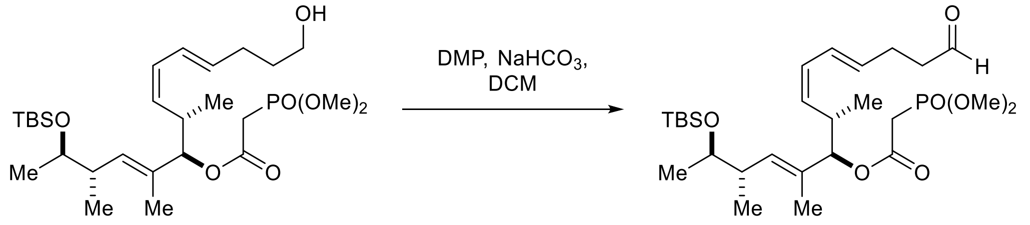
Scheme 2.13.5: Synthesis of (2*R*,3*S*,4*E*,6*R*,7*S*,8*Z*,10*E*)-2-((tert-butyldimethylsilyl)oxy)-14-hydroxy-3,5,7-trimethyltetradeca-4,8,10-trien-6-yl 2-(dimethoxyphosphoryl)acetate (148)



148a

148

To a 5 mL RBF was added **148a** (30.3 mg, 0.0435 mmol) followed by DCM/pH 7 buffer (18:1, 1.0 mL).[68] This solution was stirred and cooled to 0 °C in an ice bath. To this solution was added DDQ (13 mg, 0.057 mmol), and the reaction mixture turned yellow. After 35 min, the reaction was deemed complete by TLC and was quenched with $NaHCO_3$ (saturated, 3 mL) and Na_2SO_3 (1.0 M, 3 mL), and was stirred for an additional 10 minutes before transferring to a separatory funnel. The mixture was extracted with DCM (5 x 15 mL), and the combined organic fractions were dried over $MgSO_4$, filtered, and conc. in vacuo to yield the crude product, which was purified via flash column chromatography (hexanes/EtOAc, 1/2) to yield the pure clear oil product **148**[xiii] (23.6 mg, >99% yield). $[\alpha]^{24}_{D}$ = +56.8 (*c* 3.595, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.34 – 6.22 (m, 1H), 5.89 (t, *J* = 11.0 Hz, 1H), 5.74 – 5.62 (m, 1H), 5.33 (d, *J* = 9.7 Hz, 1H), 5.06 – 4.93 (m, 2H), 3.80 (dd, *J* = 11.2, 4.4 Hz, 6H), 3.66 (dd, *J* = 7.8, 5.2 Hz, 3H), 3.07 – 2.90 (m, 3H), 2.38 – 2.26 (m, 1H), 2.19 (q, *J* = 7.2 Hz, 2H), 1.71 – 1.64 (m, 2H), 1.56 (d, *J* = 1.2 Hz, 3H), 1.02 (d, *J* = 6.6 Hz, 3H), 0.94 (d, *J* = 6.2 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.87 (d, *J* = 2.7 Hz, 9H), 0.01 (s, 6H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 164.7 (d, *J* = 6.2 Hz), 134.7, 132.7, 131.4, 130.6, 128.5, 125.7, 84.9, 71.4, 62.1, 53.0 (dd, *J* = 6.5, 5.2 Hz), 39.1, 34.6, 33.9, 33.1, 32.1, 29.1, 25.8, 20.6, 18.0, 17.4, 16.2, 12.2, -4.5, -4.9; HRMS (ESI) *m/z* calcd for $C_{27}H_{51}O_7PSiNa^+$ $[M+Na]^+$: 569.3034, found 569.3039; IR (thin film, cm^{-1}) 3440, 2957, 2930, 2857, 1736, 1472, 1462, 1375, 1270, 1257, 1215, 1185, 1106, 1060, 1035, 982, 964, 928, 877, 837, 805, 774, 667.



148

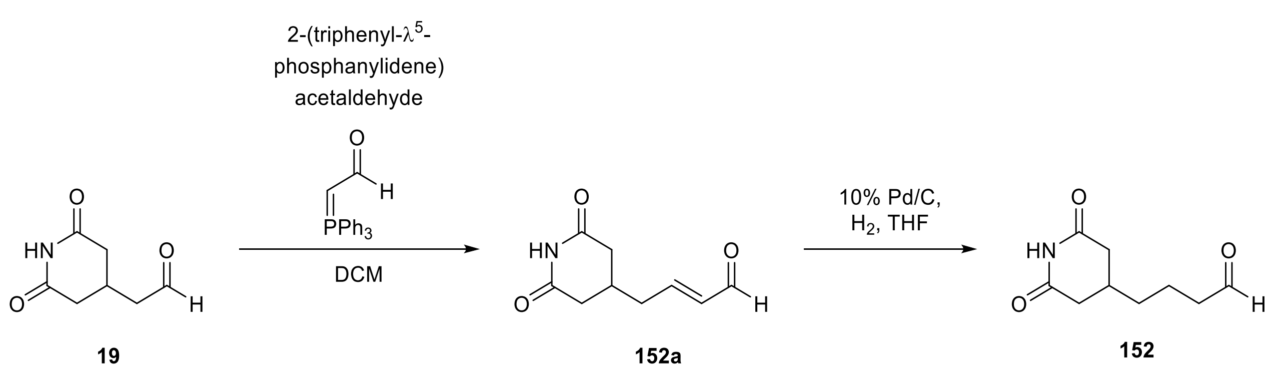
149

[xiii] 163 mg scale: Starting material **148a** (163 mg) was dissolved in DCM/pH 7 buffer (18:1, 2.35 mL). Next, DDQ (125 mg, 0.55 mmol) was added. After 2 h, the reaction didn't appear done by TLC, so an additional portion of DDQ (25 mg, 0.11 mmol) was added, and the mixture was stirred an additional 30 min. As a side note, during the course of this reaction, the solution turned from green to blackish to yellowish-grey. The reaction mixture was quenched with $NaHCO_3$ (saturated, 5 mL) and $Na_2S_2O_3$ (0.5 M, 5 mL), and was stirred for an additional 10 minutes. Excess solvents were utilized, including including Na_2SO_3 (saturated), brine, H_2O (D.I.), and DCM, but suspension of "yellow cloud" in sep. funnel difficult to remove. Therefore, it is important to note that $Na_2S_2O_3$ should be avoided, as it did not prove suitable to induce good seperation. Despite this, addition of EtOAc finally gave a good separation between the layers, allowing isolation of the organic and aqueous fractions. The combined organic fractions were dried over $MgSO_4$, filtered, and concentrated in vacuo to yield the crude product, which could be purified via flash column chromatography (EtOAc) to yield the pure viscous brown oil product **148** (159.8 mg, 80% yield).

Scheme 2.13.6: Synthesis of (2*R*,3*S*,4*E*,6*R*,7*S*,8*Z*,10*E*)-2-((*tert*-butyldimethylsilyl)oxy)-3,5,7-trimethyl-14-oxotetradeca-4,8,10-trien-6-yl 2-(dimethoxyphosphoryl)acetate (149)

In a 5 mL conical RBF was added alcohol **148** (20.0 mg, 0.366 mmol), DCM (0.85 mL), $NaHCO_3$ (3.0 mg, 0.036 mmol), and DMP (25 mg, 0.059 mmol). The mixture bubbled and turned a white-greyish color while stirring. After 2 h, the reaction was complete by TLC and was quenched with $NaHCO_3$ (saturated solution, 10 mL) and $Na_2S_2O_3$ (0.5 M, 10 mL). The aqueous phase was extracted with DCM (3 x 15 mL), and the combined organic fractions were washed with brine (40 mL), dried over $MgSO_4$, and conc. in vacuo to yield the crude product. This crude product was filtered through a short column of silica gel (hexanes/EtOAc, 1/2) to yield **149** as a yellow oil, which was used immediately in the macrocyclization reaction (19.5 mg, 98% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.76 (t, *J* = 1.4 Hz, 1H), 6.33 – 6.24 (m, 1H), 5.85 (t, *J* = 11.0 Hz, 1H), 5.69 – 5.56 (m, 1H), 5.32 (d, *J* = 9.6 Hz, 1H), 5.04 (t, *J* = 10.6 Hz, 1H), 4.97 (d, *J* = 9.0 Hz, 1H), 3.78 (dd, *J* = 11.2, 2.9 Hz, 6H), 3.63 (qd, *J* = 6.2, 3.3 Hz, 1H), 2.97 (dd, *J* = 21.7, 0.7 Hz, 3H), 2.53 (t, *J* = 7.0 Hz, 2H), 2.42 (q, *J* = 7.1 Hz, 2H), 2.37 – 2.23 (m, 1H), 1.54 (d, *J* = 1.2 Hz, 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.92 (d, *J* = 6.3 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H), 0.86 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.6, 164.7 (d, *J* = 6.4 Hz), 132.8, 132.6, 131.5, 131.4, 128.0, 126.4, 84.8, 71.4, 53.0 (dd, *J* = 6.5, 2.4 Hz), 43.2, 39.1, 34.7, 34.1, 33.0, 25.8, 25.3, 20.7, 18.0, 17.4, 16.3, 12.2, -4.5, -4.9.

2.14 Extended-chain analog synthesis (procedures & analytical/spectral data)



Scheme 2.14.1: Synthesis of 4-(2,6-dioxopiperidin-4-yl)butanal (152)

In a 50 mL round-bottomed flask was added aldehyde **19** (150.2 mg, 0.968 mmol)[64] in anhydrous DCM (4.84 mL) to give a white suspension. The mixture was cooled to 0 °C under N_2, and 2-(triphenyl-λ5-phosphanylidene)acetaldehyde (760 mg, 2.50 mmol) suspended in DCM (3 x 1.6 mL) and added slowly to give an orange-colored solution. The obtained solution was warmed to rt and stirred overnight for 25.5 h (under N_2). A small sample of the reaction was checked by NMR, showing the reaction was incomplete,[xiv] so additional 2-(triphenyl-λ5-phosphanylidene)acetaldehyde (176 mg, 0.57 mmol)[xv] dissolved in 1.5 mL anhydrous DCM was added. The reaction was then stirred for an additional 18.5 h. The final reaction mixture was concentrated in vacuo to yield a red residue. This crude product was partially purified via flash column chromatography through a short column (EtOAc), to remove the excess 2-(triphenyl-λ5-phosphanylidene)acetaldehyde, but Ph_3PO co-eluted with the desired product **152a**. This crude material was carried onto the next step without further purification.

In a 50 mL round-bottomed flask was added crude **152a**[xvi] in THF (12.75 mL) to give an orange solution. The flask was purged with argon gas, 10% wt. Pd/C (300 mg) was quickly added, and the flask was purged with H_2. The reaction was stirred under positive H_2 pressure (via balloon) overnight for 18.5 h. The resultant reaction mixture was filtered through celite, and the filter cake was rinsed with EtOAc three times. The resulting solution was concentrated in vacuo to yield a

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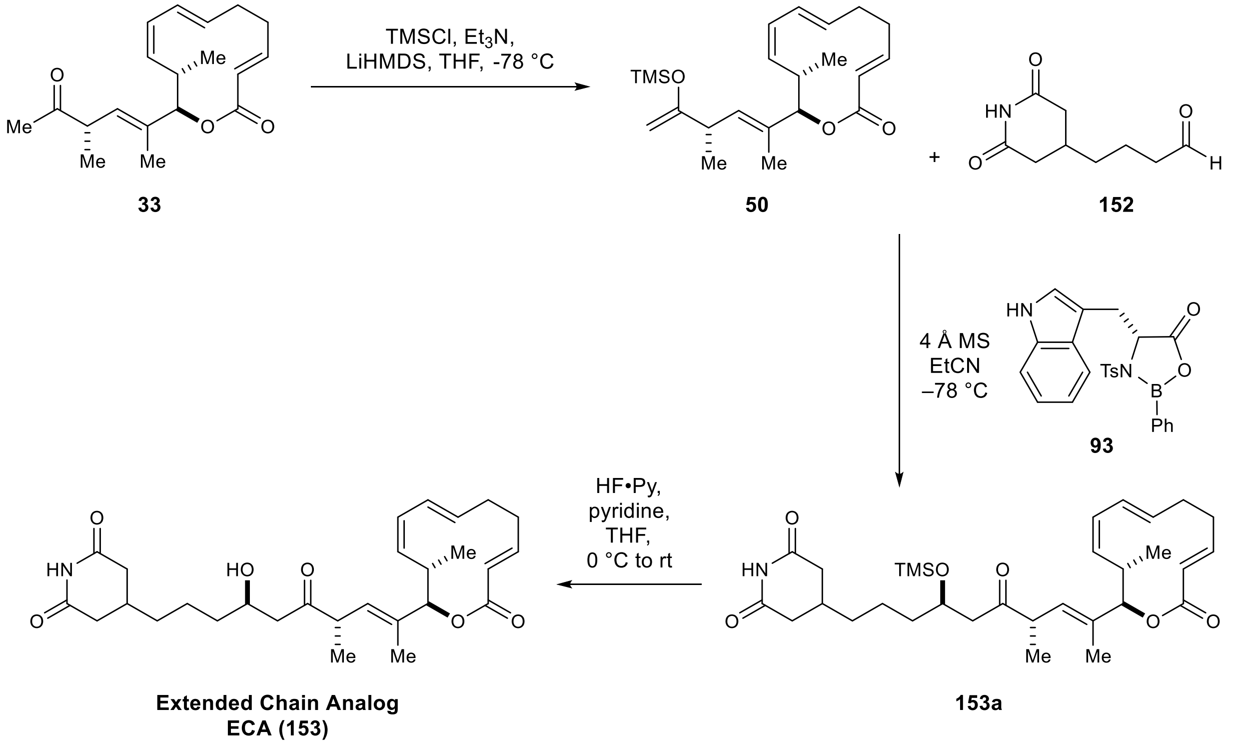
[xiv] By NMR: Approximately 77% converted to product and 23% remaining starting aldehyde.

[xv] Total amount used of 2-(triphenyl-λ5-phosphanylidene)acetaldehyde: 936 mg, 3.07 mmol.

[xvi] Crude weight not recorded, since a significant amount of impurity was present (which may vary).

white crude solid. The crude product was purified by flash column chromatography (DCM/methanol/, 95/5 to 93.5/6.5)[xvii] to yield the pure white solid product **152** (23.1 mg, 13% yield[xviii] across 2 steps). ^{1}H NMR (700 MHz, $CDCl_3$) δ 9.76 (t, *J* = 1.3 Hz, 1H), 8.47 (s, 1H), 2.71 (dd, *J* = 17.1, 4.2 Hz, 2H), 2.48 (td, *J* = 7.1, 1.3 Hz, 2H), 2.31 – 2.22 (m, 2H), 2.19 – 2.09 (m, 1H), 1.70 – 1.62 (m, 2H), 1.44 – 1.38 (m, 2H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 201.39, 172.17, 43.37, 37.61, 34.08, 30.24, 18.84; HRMS (ESI) *m/z* calcd for $C_9H_{13}NO_3^+$ $[M+Na]^+$: 206.0788, found 206.0785; IR (thin film, cm^{-1}) 3200, 3091, 2944, 2869, 2733, 1724, 1684, 1377, 1270, 1146, 875.



Scheme 2.14.2: Synthesis of 4-((4*R*,7*S*,*E*)-4-hydroxy-7-methyl-9-((2*R*,3*S*,4*Z*,6*E*,10*E*)-3-methyl-12-oxooxacyclododeca-4,6,10-trien-2-yl)-6-oxodec-8-en-1-yl)piperidine-2,6-dione (153)

[xvii] Multiple columns were used, trying EtOAc as a solvent at first, with poor separation. The purest fractions were carried forward after each column. The best solvent system was finally identified and is reported to separate the pure product.

[xviii] Unoptimized yield reported.

In a 10 mL round-bottomed flask chlorotrimethylsilane (0.053 mL, 0.417 mmol) and triethylamine (0.058 mL, 0.417 mmol) were added to a solution of **33** (12.6 mg, 0.042 mmol) in THF (1.46 mL) at -78 °C to form a colorless solution. Next, LiHMDS (0.104 mL, 0.104 mmol, 1.0 M in THF) was added dropwise to give a colorless solution. The reaction mixture was stirred at -78 °C for 1.5 hour and was confirmed complete by TLC [R_f of (**33**) = 0.3 and R_f of (**50**) = 0.6, both in D.S. of hexanes/EtOAc (4/1)]. The reaction mixture was quenched with pH 7 buffer (5 mL), and warmed to rt, while stirring, over 10 minutes. The aq layer was back extracted with DCM (3 x 3 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and conc. in vacuo, to yield the resulting crude yellow oil **50**, which was stored frozen in benzene prior to the next steps and used without further purification.

In a 15 mL conical round-bottomed flask was added **50**[xix] (15.7 mg, crude), **152**[xix] (9.6 mg, 0.053 mmol), and molecular sieves (4Å, ca. 150 mg) in dry propionitrile (575 µL) to give a yellow suspension. The reaction was put under N_2, cooled to -78 °C, and stirred for 5 minutes. Next, **93**[xx] (23.3 mg, 53 µmol) dissolved in propionitrile (145 µL) was added dropwise, resulting in a yellow suspension. The reaction was stirred overnight in the cryocool at -78 °C (dry ice used to keep cool). After 23 h, the reaction was checked by TLC and LCMS, which showed the reaction to be incomplete. Fresh catalyst was generated and was dissolved in propionitrile (250 µL). After an additional 14 h (37 h total reaction time), the reaction was quenched with conc. $NaHCO_3$ (3 mL). The mixture was warmed to rt and the aqueous phase was extracted with DCM (3x5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and conc. in vacuo to yield the crude

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[xix] Azeotroped in dry benzene three times prior to reaction.

[xx] Prepared same as before during the synthesis of lactimidomycin (**6**).

oil **153a** (7.5 mg, 13 μmol, 32.0% crude yield), which was submitted to the next reaction without further purification.

In a 15 mL falcon vial[xxi] was added 1**53a** (23 mg, crude) in THF (7.5 mL) to form a suspension. Next, the reaction was put under nitrogen, stirred, and cooled to 0 °C, and buffered HF-pyridine complex solution (0.75 mL, prepared by mixing THF [723.5 μL], pyridine [269 μL], and HF-pyridine [54 μL] in a separate 15 mL falcon vial) was added slowly. The mixture was stirred for 2 h at 0 °C and warmed to rt for 30 min to complete the reaction. The reaction mixture was diluted with DCM (30 mL) and transferred to a plastic separatory funnel. The mixture was washed with sat $NaHCO_3$ (15 mL) and $CuSO_4$ (0.1 M, 3 x 15 mL), dried over $MgSO_4$, filtered, and conc. in vacuo. The resulting residue was purified via a short pipette column (EtOAc/hexanes, 3/1), giving the white-colored product **153** (6.1 mg, 30% yield, 2.6:1 dr at the C-17 position prior to further purification). This material was then further purified via a series of short pipette columns (EtOAc/hexanes, 3/1) to give a sample for characterization and biological testing. ^{1}H NMR (400 MHz, C_6D6) δ 7.50 (br s, 1H), 6.44 (ddd, *J* = 15.5, 10.3, 4.9 Hz, 1H), 6.07 (dd, J = 10.8, 10.8 Hz, 1H), 5.68 (dd, *J* = 15.5, 10.7 Hz, 1H), 5.59 (d, *J* = 4.8 Hz, 1H), 5.49 (d, *J* = 16.1 Hz, 1H), 5.23 (d, *J* = 9.7 Hz, 1H), 5.16 – 5.06 (m, 2H, overlap), 3.90 (dd, *J* = 14.3, 7.1 Hz, 1H), 3.26 – 3.10 (m, 2H, overlap), 2.90 (br s, 1H), 2.36 (dd, *J* = 17.5, 8.9 Hz, 1H), 2.18 – 2.06 (m, 3H), 2.02 – 1.90 (m, 2H), 1.70 (d, *J* = 1.3 Hz, 3H), 1.54 – 1.43 (m, 4H), 1.40 – 1.28 (m, 1H), 1.26 – 1.08 (m, 3H),1.04 (d, *J* = 6.8 Hz, 3H), 1.04 – 0.95 (m, 1H), 0.85 (d, *J* = 6.8 Hz, 3H), 0.78 – 0.63 (m, 2H); ^{13}C NMR (126 MHz, C_6D_6) δ 211.21, 171.13, 166.07, 146.53, 134.78, 134.08, 131.74, 129.86, 129.78, 128.35, 82.74, 67.28, 47.52, 46.85, 37.68, 37.64, 36.57, 36.39, 34.53, 32.27, 31.26, 29.99, 22.59, 17.56,

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[xxi] Reaction and buffered solutions were run and prepared, respectively, in plastic, falcon vial containers to avoid reacting HF-Pyridine with glass containers.

16.02, 15.14; HRMS (ESI) *m/z* calcd for $C_{28}H_{39}NO_6^+$ $[M+Na]^+$: 508.2670, found 508.2664; IR (thin film, cm^{-1}) 3422, 2925, 2854, 1716, 1700, 1684, 1653, 1540, 1456, 1259, 1171, 1031.

Figure 2.14.1: MDA-MB-231 2D

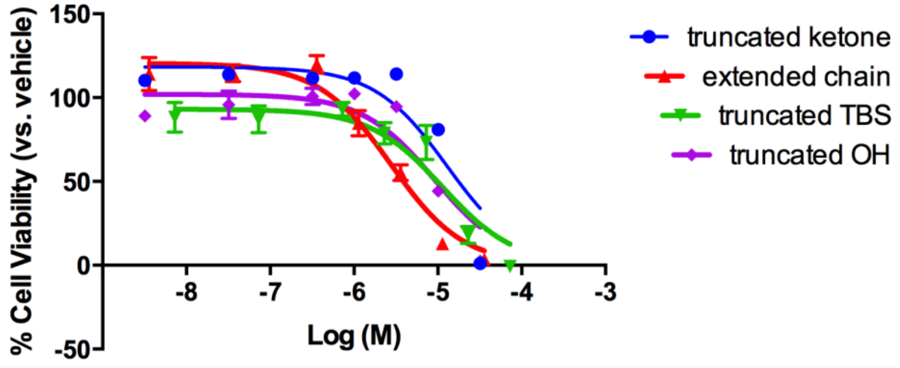


Figure 2.14.2: MDA-MB-231 3D

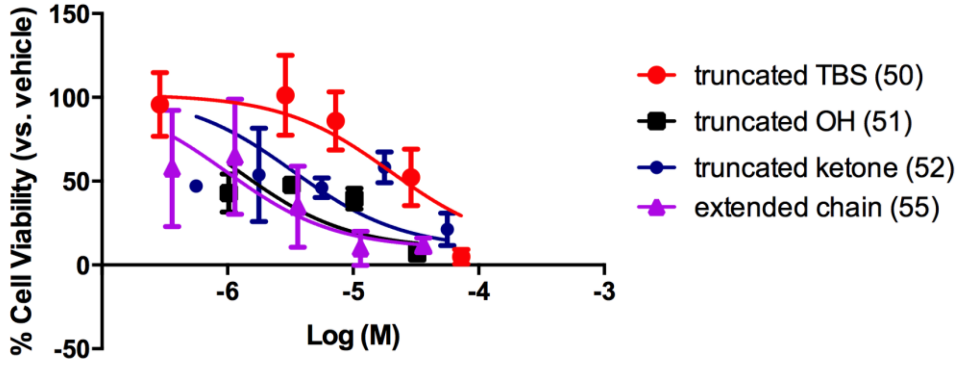


Figure 2.14.3: Human mammary epithelial cells

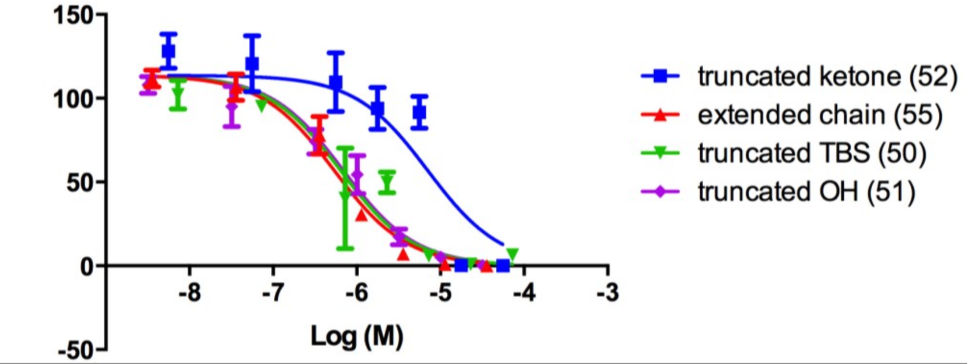


Figure 2.14.4: MDA-MB-231 cell growth 2D with lactimidomycin (6)

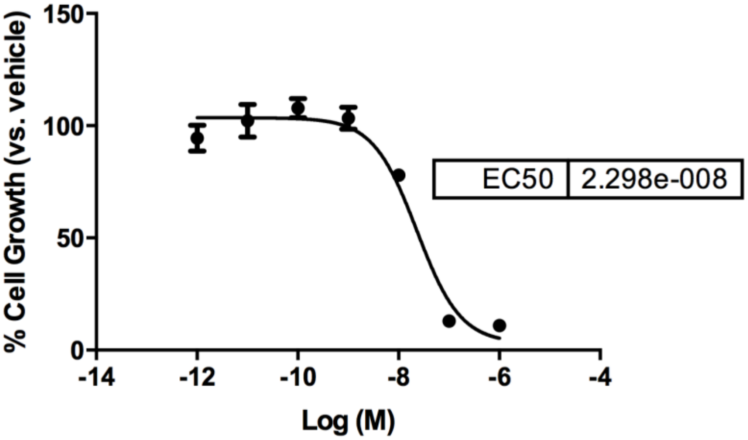


Figure 2.14.5: MDA-MB-231 3D cell growth with lactimidomycin (6)

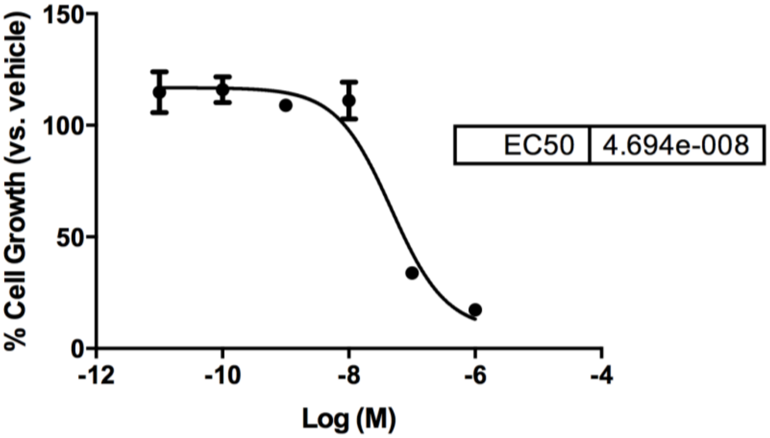
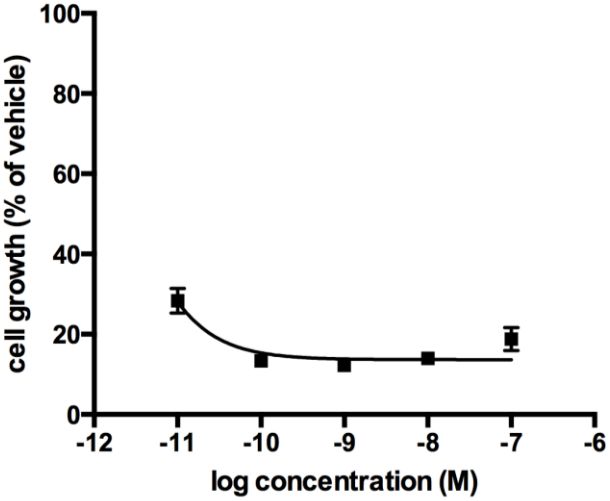


Figure 2.14.6: Human mammary epithelial cells with lactimidomycin (6)



2.15 Chapter 2 References

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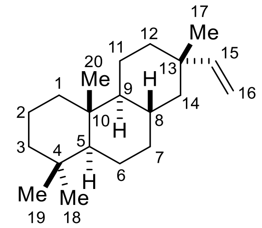
Chapter 3: Synthetic studies towards the convergent, scalable total synthesis of natural tricyclic diterpenoid products and analogs in pursuit of novel anticancer therapuetics

3.1 Diterpenoid natural product scaffolds: pimeranes, isopimeranes, kauranes, and more

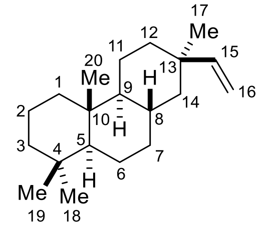
Pimeranes (**154**) and isopimeranes (**155**) are members of an abundant group of plant and fungal diterpenes,[1, 2] some of which are deeply rooted in traditional Asian medicinal treatments.[3, 4] Characteristic features of these compounds include a tricyclic ring system and two quaternary stereocenters at the C10 and C13 positions. The members of pimarane and isopimarane families possess a range of biological activities, and both enantiomeric forms of these types natural products have been isolated and identified to be bioactive. In addition, similar structural motifs are present in a related class of tetracyclic terpenes, kauranes (**156**),[5, 6] a group that exhibits anti-bacterial, anti-tumor, and antimalarial activity. Remarkably, despite these biological activites, relatively few asymmetric total synthetic strategies have been developed in the construction of these scaffolds in their higher oxidation states, as most reported analogs of these compounds arise from biosynthetic or semi-synthetic methodologies; therefore, it is quite difficult to form structural analogs containing varying degrees of oxidation, units of unsaturation, and stereoisomeric forms.[7-14]

Figure 3.1.1: Selected tricyclic diterpene scaffolds: pimerane, isopimerane, and kaurane-type



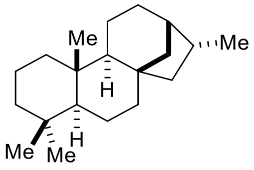
pimarane-type scaffold

154



isopimarane-type scaffold

155



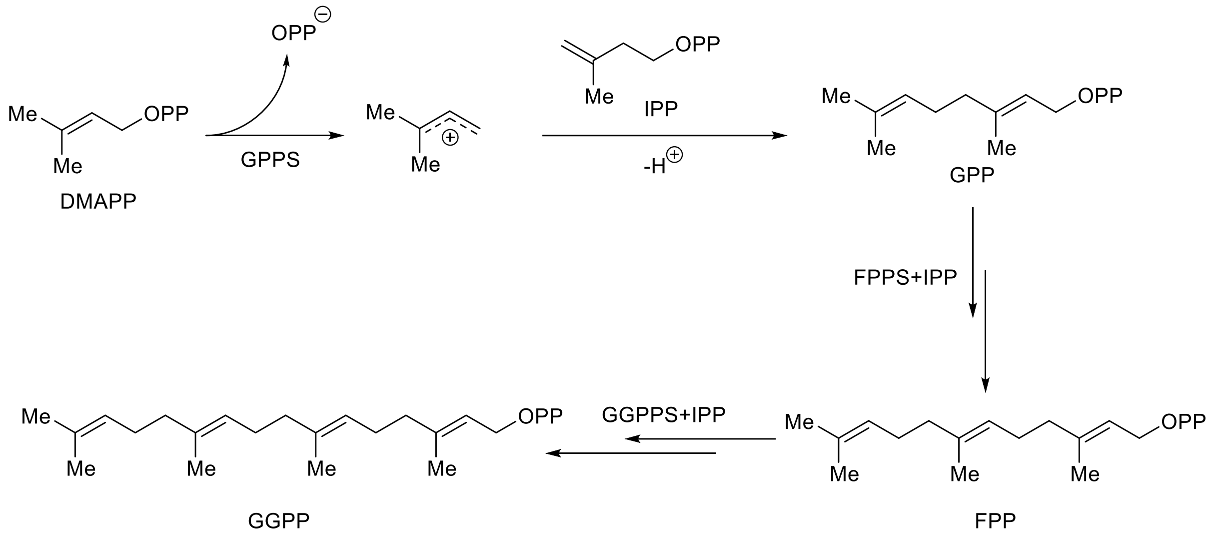
kaurane

156

3.2 Biosynthesis of isopimerane and pimerane-type scaffolds (1-2)

The exact biosynthetic pathways towards specific diterpene natural products are yet to be fully described, as there are a multitude of enzymes that have yet to be isolated and identified.[15] Therefore, a more general review on the biosynthetic pathway leading to the formation of molecules structurally similar to **1** and **2** will be described.

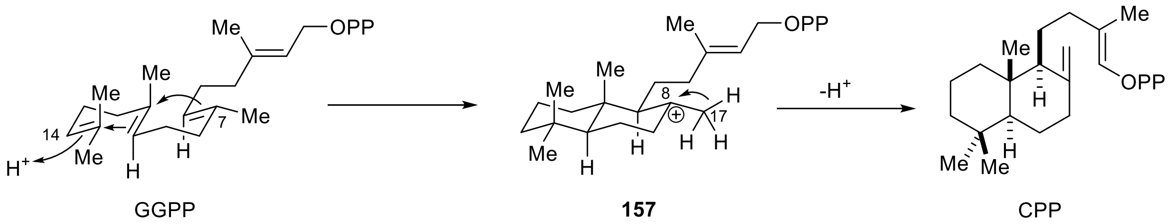
Dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP) are the two substrates used in the biosynthesis of diterpenes (Scheme 3.2.1).[16] DMAPP and IPP condense to form geranyl pyrophosphate (GPP), then GPP and IPP form farnesyl diphosphate (FPP), and finally FPP and IPP form geranylgeranyl diphosphate (GGPP) in a series of reactions catalyzed by their enzymatic partners, geranyl diphosphate synthase (GPPS), farnesyl diphosphate synthase (FPPS), and geranylgeranyl diphosphate synthase (GGPPS), respectively. The compound GGPP is of extreme importance, given that it is the starting point from which all diterpenes are biosynthetically formed.



Scheme 3.2.1: Biosynthesis of GGPP from simple substrates DMAPP and IPP

Derivitization of GGPP into various dirtenes via biosynthetic pathways are induced by one of several enzymes commonly referred to as cyclases (or synthases). As there are many different cyclases still yet to be isolated and identified, it is easier to categorize the transformations by their cyclization pathway, of which two main mechanistic pathways can be observed, Type A and B cyclizations which are defined by how they initiate the cyclization. Type A cyclizations are initiated by the ionization of a diphosphate, and Type B cyclizations are initiated by the protonation at the C14-C15 double bond.

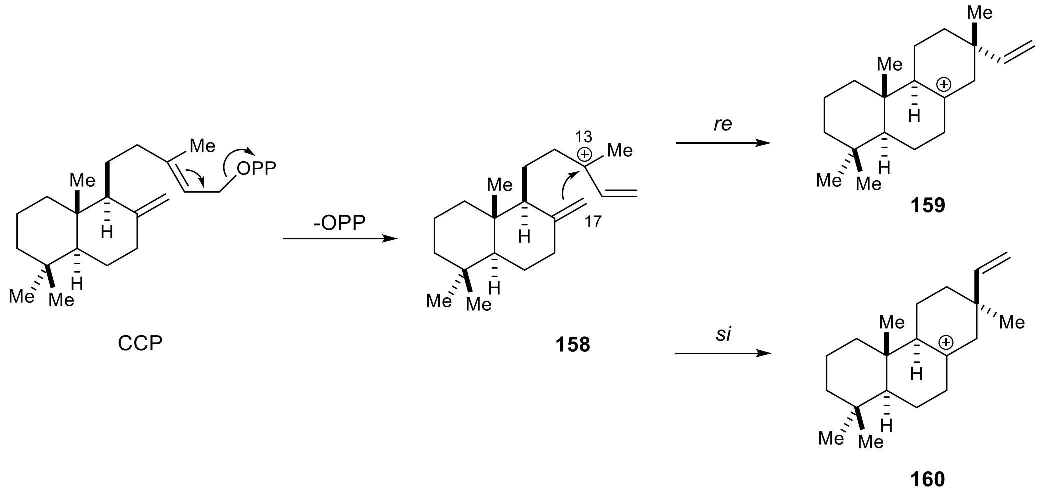
It is important to note, depending on the cyclase, an A and/or B pathway can take place, or a A-B or B-A dicyclization can take place, all enzymatically promoted by a single cyclase. To further complicate things, some dicyclizations are promoted by two different cyclases which are working on only the first or second cyclization. As there are a variety of different possible enzymes present in biological system, the number of diterpenes is accordingly increasingly complexe, considering that the monocyclized, dicyclized products can undergo a variety of different cationic rearrangements, transformations via later stage oxidation via oxidase enzyme(s), and the conditions present in the biosynthetic system (presence of acid/base, etc.).



Scheme 3.2.2: Type B1 cyclization via the chair-chair confirmation to form CPP

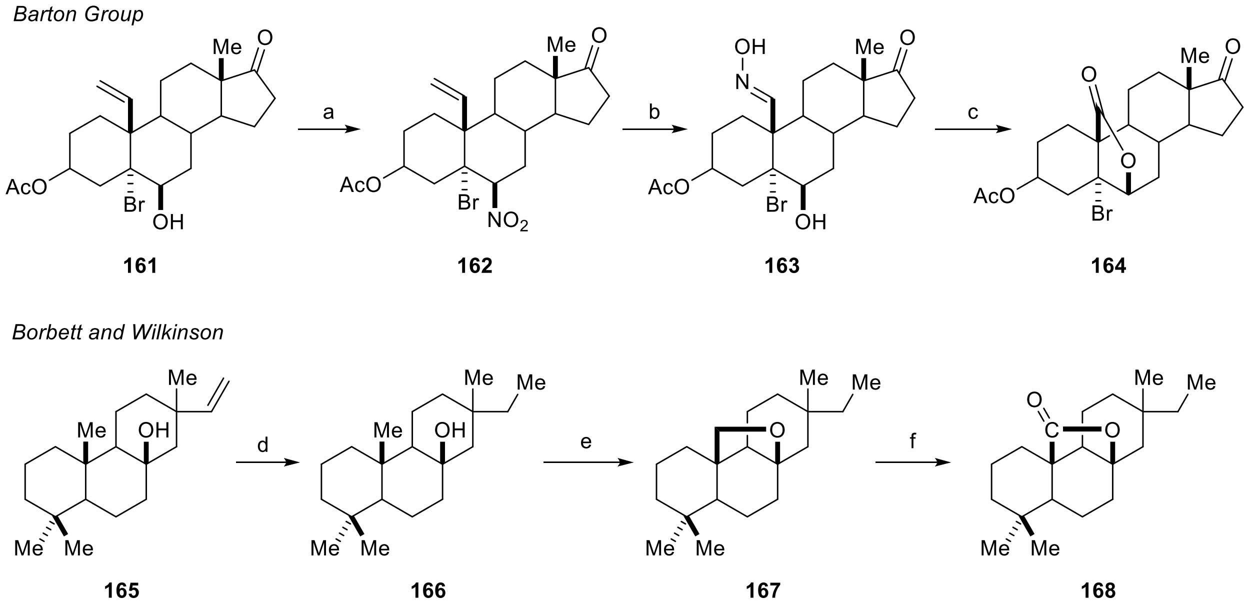
In the production of isopimarane- and pimarane-skeleton-containing natural products, most likely a Type B-Type A series of cyclizations takes place via two distinct mechanistic pathways.

First, GGPP undergoes a cyclase-promoted Type B1 cyclization (chair-chair confirmation) via the protonation of the C14-C15 bond and subsequent cationic rearrangment of **157** to form the normal form of CCP (Scheme 3.2.2). Second, the newly formed CCP undergoes a cyclase-promoted Type A cyclization initiated via ionization of its diphosphate to form the C17 carbocation-containing intermediate **158** (Scheme 3.2.3). This intermediate then undergoes the cyclization as the C13 carbon (double bond) attacks C17 carbocation from either the *re*- or *si*-face, leading to the formation of carboncation intermediates **159** (isopimerane-type) or **160** (pimerane-type), respectively, which can further be further transformed via further enzymatically and/or thermodynamically driven pathways. In particular, oxidases are responsible for installing units of oxydation at various stereocenters; however isolation and identification of these enzymes along with identification of the exact biosynthetic pathway of many diterpenes, a large class of compounds which continues to grow as new derivatives are still being discovered.



Scheme 3.2.3: Type A cyclization via OPP ionization and rearrangement to form 10 and 11

3.3 C-H functionalization at the C20 position of various natural products

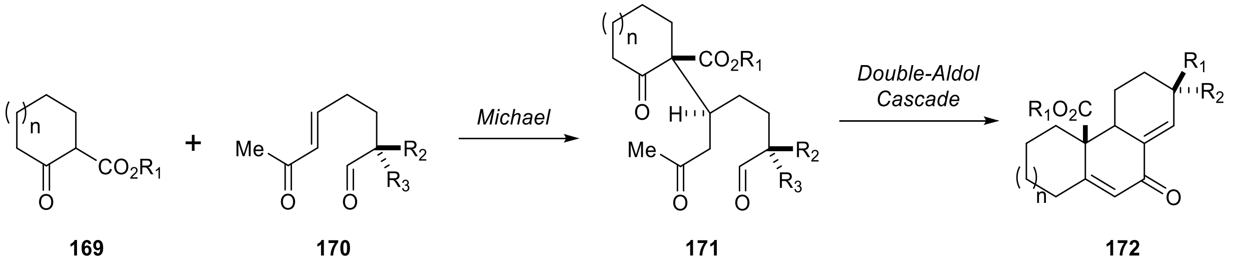


a) ClNO, Pyr; b) *hv*; c) HNO_2, AcOH; d) H_2-Pt; e) Hg-O, *hv*; f) CrO_3, Ac_2O

Scheme 3.3.1: Previous work on promotion of the C-H oxidation at the C20 (or similarly positioned) methyl group of various terpenoids and steroids

To date very few viable chemical transformations exist that can selectively oxidize the desired C20 position of pimerane and isopimerane skeleton directly in forming structural diveritives. Barton and co-workers have discussed a method employing photolysis of a nitrite group to produce the desired oxygenation at a similar position on their norsteroids (Scheme 3.3.1).[17] Later, Borbett and Wilkins found they could functionalize certain methyl groups found in isopimarane and hopanol derivatives. Both of these methods require UV radiation of a nitrite ester to an oxime, which would then form the corresponding hemiacetal;[16] furthermore, they all suffer from the use of toxic and functional group intolerant reagents. Many structural motifs in these compounds are not stable in the presence of oxidative radical species.

3.4 General Michael and double-aldol cascade utilized in the synthesis of steroid and terpene scaffolds

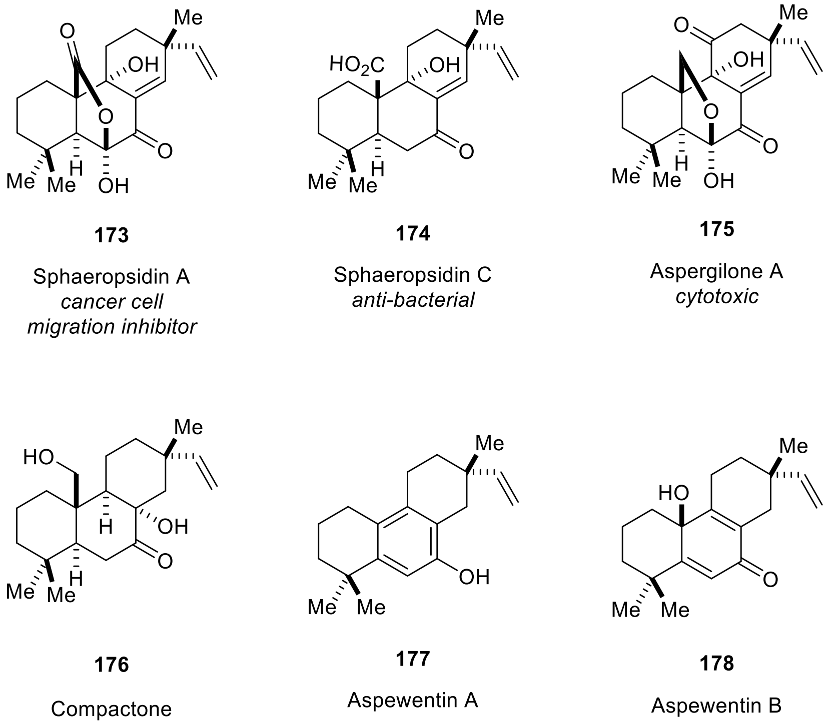


Scheme 3.4.1: Nagorny et al. general approach to forming various terpenoid scaffolds

As previously discussed, the formation of multiple highly substituted stereocenter in a single step is rather limited. One versatile reaction that has been underutilized is that of the asymmetric intramolecular Michael reaction to construct vicinal quaternary and tertiary stereocenters. Previous examples of these are limited and did not provide an efficient enantioselective formation of the desired Michael adduct for the desired substrates.

We has previously shown that cyclic ketoesters **169** and enones **170** can undergo a Lewis acid-catalyzed Michael addition to **171**, followed by a double aldol cascade cyclization can lead to the various terpenoid-type products (**172**) with high diastereoselectivity (and enantioselectivity if the correct chiral ligands are used on copper[II]) and high yields (Scheme 3.4.1).

3.5 Selected diterpene-core containing natural products of interest



173 Sphaeropsidin A *cancer cell migration inhibitor*

174 Sphaeropsidin C *anti-bacterial*

175 Aspergilone A *cytotoxic*

176 Compactone

177 Aspewentin A

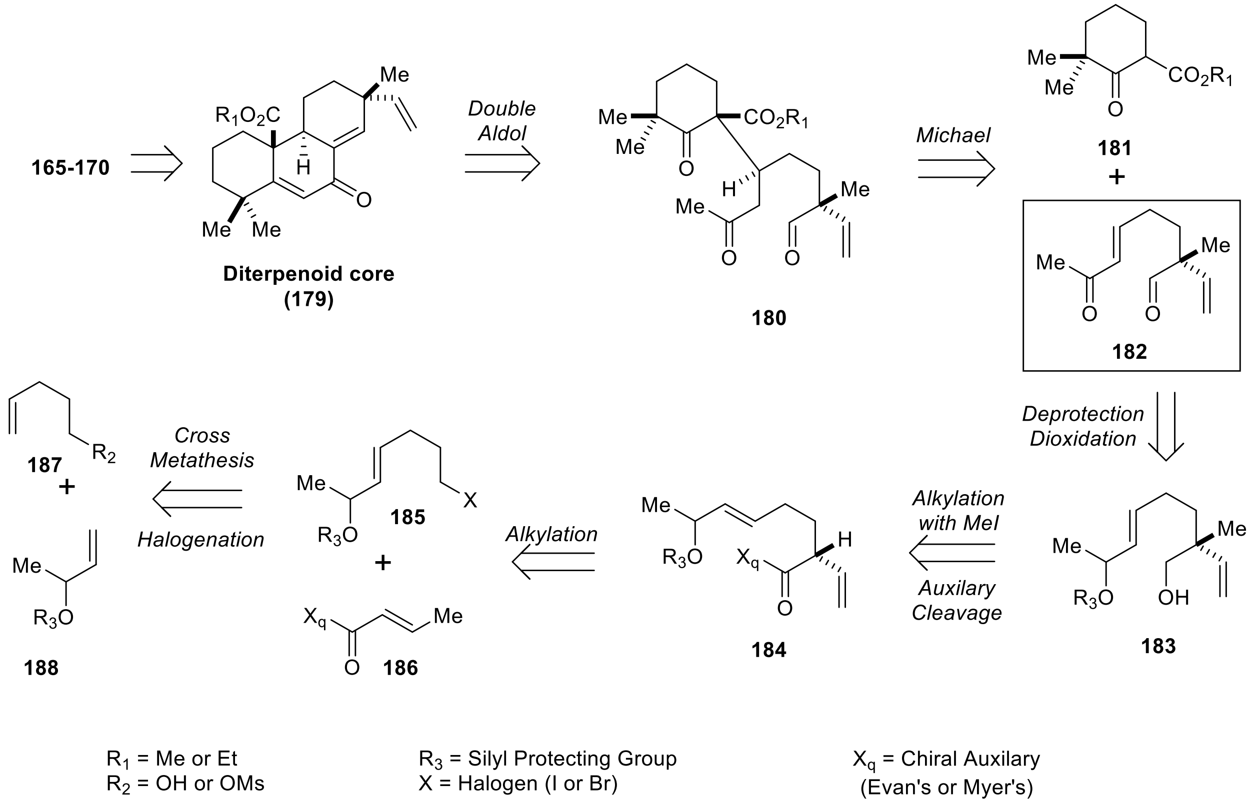
178 Aspewentin B

Figure 3.5.1: Diterpene skeletons and their biologically active derviatives.

Today both mammalian and plant-based steroids make up a significant portion of the top selling pharmaceuticals[18] In order to promote greater selectivity of new therapeutics, researchers have increasingly focused more on chiral molecules over “flat” molecules (which lack such stereochemistry).[19] Inserting stereocenters, especially quaternary ones, has shown marked improvements in improving efficacy and reducing unwanted side effects of various drugs.[20] Synthesis of tricyclic poly-oxygenated diterpenes and related skeletons bearing multiple quaternary stereocenters is a common goal among research groups, due to the difficulty in producing such constrained and complex structures with multiple stereogenic centers.[21] Some of these (iso)pimerane-containing natural products include sphaeropsidins A (**173**) and C (**174**), aspergilone A (**175**), compactone (**176**), and aspewentin A (**177**) and B (**178**).

Sphaeropsidin A (**173**) and C (**174**) are fungal metabolites produced by *Diplodia cupressi* and *Smardaea sp. AZ0432*. In particular, sphaeropsidin A (**173**) has been found to inhibit the migration of triple negative breast cancer cells (MDA-MB-231, IC_{50} = 1.5 μM). In addition, both **173** and **174** have been shown to exhibit antimicrobial activity against *Xanthomonas oryzae*, which is the leading cause of blight, a disease of rice plants which negatively impacts the crop yields, causing major issues in the food supply across the globe.[8, 9, 22] Aspergilone A (**175**) is a cytotoxic molecule structurally similar to **173** (shifted site of oxygenation).[23] Compactone (**176**)[24] may serve as an important intermediate to synthetically accessing kaurane(**156**)-containing or interrelated scaffolds. Aspewentin A (**177**) and B (**178**) have been reported to contain interesting biological activites against different microorganisms. In particular, the importance of the aromatic ring in **177** has been suggested to promote different bioactivity in these norisopimarane diterpenes, in particular against the zooplankton (*Artemia saline*) over the other marine phytoplanktons.[25] Development of a rapid synthetic route to any of these natural products would allow access to various diterepene-based truncated analogs.

3.6 Retrosynthetic analysis of the diterpenoid core (179)

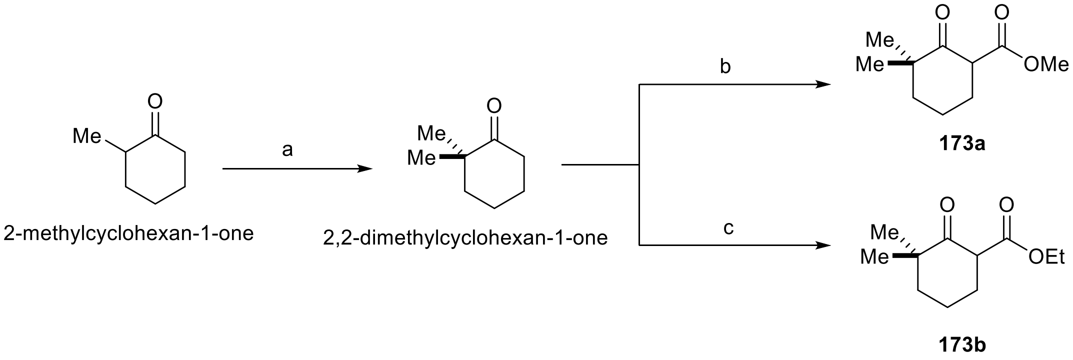


Scheme 3.6.1: Final retrosynthetic analysis in forming core diterpene towards natural products

Retrosynthetically, we envisioned forming the diterpenoid core (**179**) via a key Michael-double aldol sequence, through which ketoester **181** and aldehyde-enone **182** would be first treated with a lewis acid to promote a Michael addition to form aldehyde **180**, followed by treatment with an optimized base to promote the diastereoselective double aldol reaction to produce **179** (Scheme 3.6.1). Upon beginning our studies, the formation of a cyclic *gem*-dimethyl ketoesters **182** was predicted to be simple; however, the assymetric synthesis of **182** proved a more formidable task than was initially envisioned.

After several chiral auxilaries derived from tiglic acid and/or crotonyl chloride starting materials, it was discovered the best was to access this chiral aldehyde-enone **182** was through its precursor alcohol **183** via deprotection of the silyl group to the diol intermediate, followed by a dioxidation. Using **184**, a diastereoselective alkylation was predicted to introduce the final methyl group of the quaternary stereocenter, which, upon removal of the auxilary via reductive conditions was predicted to form **183**. Compound **184** is accessible via a diastereoselective alkylation reaction of **186**, derived from crotonyl chloride and Myer's auxilary, and alkyl halide **185**, derived from a cross metathesis reaction of **187** and **188** followed by subsequent halogenation.

3.7 Synthesis of the ketoesteres 173a and 173b



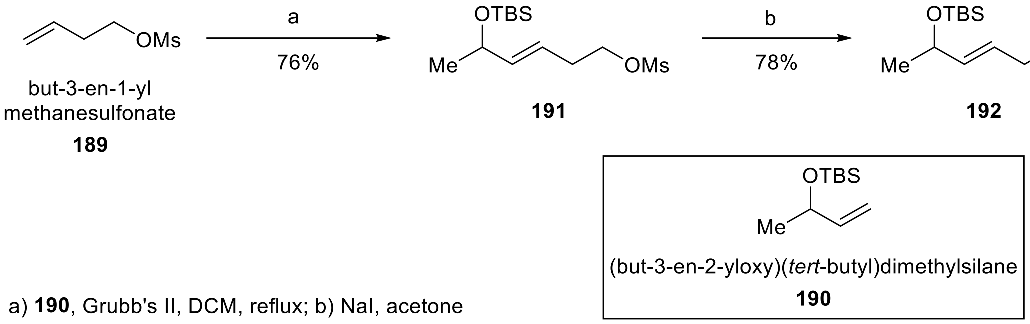
a) NaOEt, MeI, Et_2O; b) NaH, dimethyl carbonate, THF, reflux; c) LiHMDS, diethyl carbonate, THF, -78 °C to rt

Scheme 3.7.1: Synthesis of ketoesters 173a and 173b from 2-methylcyclohexan-1-one

A simple series of known reactions were used to form **173a** and **173b** from 2-methylcyclohexane-1-one over 2 steps, and both compounds were prepared in gram scale quantities.

3.8 Rapid preparation of iodide 192

The synthesis of a key iodide intermediate **192** using a rapid sequence (Scheme 3.8.1) was achieved in only 3 linear steps (4 steps total).



a) **190**, Grubb's II, DCM, reflux; b) NaI, acetone

Scheme 3.8.1: Synthesis of key TBS-protected iodide fragment 192

First, the mesylate **189** and TBS-protected alcohol **190**[26] were prepared via previously detailed procedures. With these two protected components in hand, a Grubb's cross methathesis was utilized to form **191**, which was converted via a Finkelstein SN2 displacement reaction to form iodide **192**.

3.9 Synthesis of the quaternary carbon-containing 182

Despite the push for green chemistry and atom-economic methodology, chiral auxiliaries have become known for being generally reliable and versatile, through enabling the synthesis of a large number of enantiomerically pure compounds in a time-efficient manner. Because of this, they are often the method of choice, especially in the early phases of drug development.[27] [28, 29]

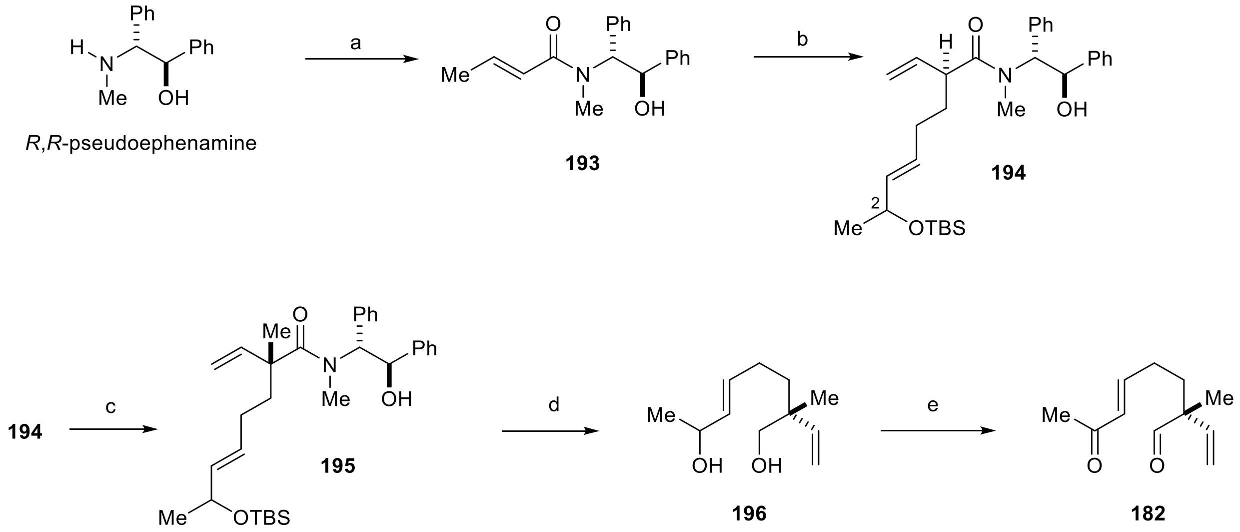
Originally, we tested pseudoephedrine (Figure 3.9.1) as our auxilary in final reaction sequence, demonstrating the reactions with the R=Me containing intermediates proceeded cleanly and that the chemistry was possible; however, based on the prior precedent from Movassaghi's group, a less than desirable (>6:1) or from pseudoephedrine-containing intermediates could be enhance to 29:1 if pseudoephenamine-containing intermediates, are employed.

Figure 3.9.1: Myer's auxilaries - pseudoephedrine vs. pseudoephenamine

R,*R*-pseudophedrine

R,*R*-pseudoephenamine

The simple to prepare and recyclable Myer's auxilary, (*R,R)*-pseudoephenamine (Figure 3.9.1) was selected as the auxilary of choice for the optized route.[30] This novel auxilary was selected as it had previously been shown to promote a highly stereoselective series of alkylations in the formation of typically difficult to form quaternary stereocenters.[30, 31]



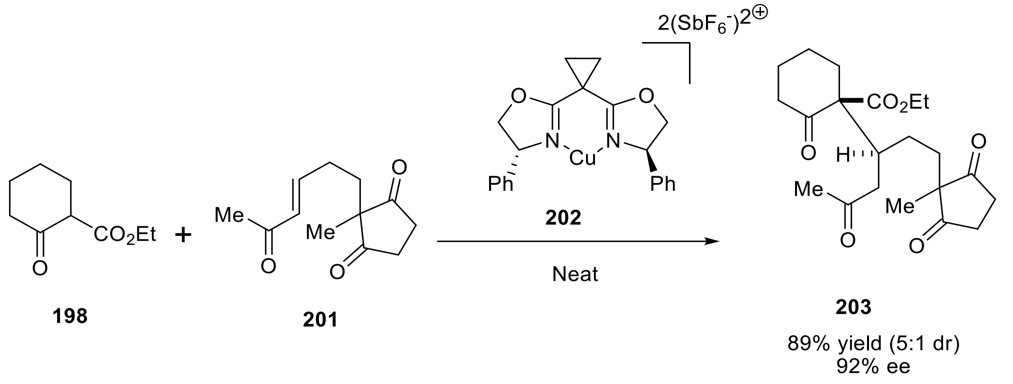
a) (*E*)-but-2-enoyl chloride, Et_3N, 73%; b) **192**, LiCl, *n*-BuLi, TMP, THF; c) MeI, *n*-BuLi, DMPU, DIPA, LiCl, 49% (over 2 steps); d) i. lithium aminoborohydride, ii. HF-Pyridine, 65% (over 2 steps); e) DMP, DCM, yield not calculated (taken immediately to next step)

Scheme 3.9.1: Scalable synthesis of chiral intermediate 182

In this reaction sequence, the formly synthesized iodide **192** was utilized as the electrophile for the addition with the auxiliary-containing **193**, which was available from *R*,*R*-pseudoephenamine following previously published procedures.[32, 33] The product of this first alkylation **194** was then submitted to another alkylation reaction with MeI in the prescence of DMPU, LDA, and LiCl to form **195**. This product was converted into the diol **196** via a two-step reduction/oxidation sequence. Finally, **196** was subjected to oxidation using DMP to form the chiral substrate **182**. Upon completion of optimizing the alkylation sequence to build the chiral quaternary stereocenter, this protocol was repeated on a larger scale by Danielle Singleton.

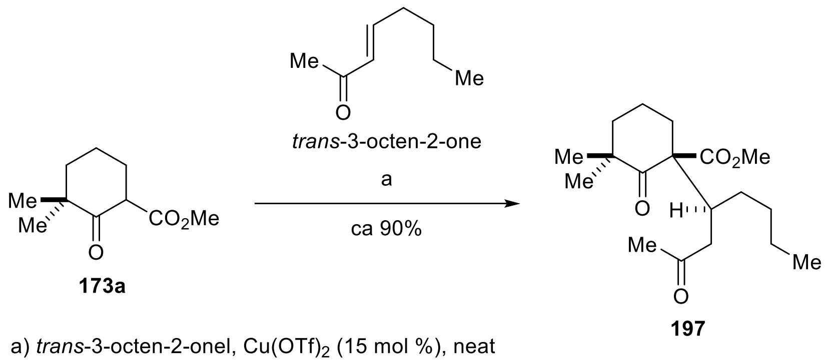
3.10 Model studies towards the synthesis of the isopimarane-type core diterpene

We have also developed a Michael (followed by double aldol condensation; not shown) sequence employing a copper (II) catalyst with a chiral box ligand followed by treatment with a base has been developed and tested (Scheme 3.10.1).



Scheme 3.10.1: Highly enantioselective chiral Cu(II) promoted Michael reaction

In order to demonstrate the feasibility of the Michael reaction leading to the formation of **182**, a series of model studies were conducted. First, ketoester **173a** was reacted with commercially available (*E*)-7-oxooct-5-enal with $Cu(OTf)_2$ under neat conditions. As a result, the corresponding product **197** was isolated in ca. 90% yield. This demonstrates that the reaction is tolerant to the *gem*-dimethyl substitution on the ketoester **173a**.

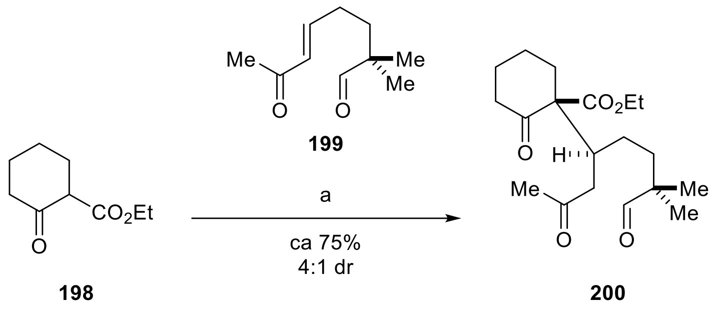


a) *trans*-3-octen-2-onel, $Cu(OTf)_2$ (15 mol %), neat

Scheme 3.10.2: Michael reaction of *gem*-dimethyl ketoester 173a and commercially available unfunctionalized *trans*-3-octen-2-one

Furthermore, readily available ketoester **198** and enone **199** were reactioned with $Cu(OTf)_2$

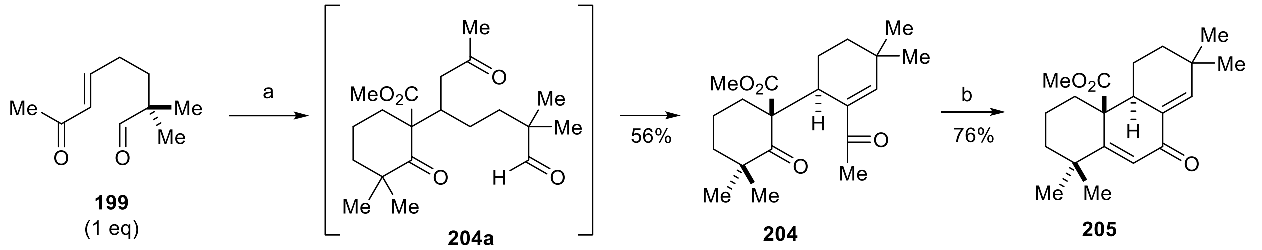
under neat conditions.[i] As a result, the corresponding product **200** was isolated in ca. 75% yield with a d.r. of 4:1. This demonstrates that the reaction is tolerant to the dimethyl substitution next to the aldehyde functionality on the enone **199**.



a) **199**, Cu(OTf)$_2$, (15 mol %), neat

Scheme 3.10.3: Michael reaction of unfunctionalized ketoester 192 and dimethyl aldehyde-enone 199

With these promising studies, further model studies were attempted. In order to optimize the enantioselectivity of the Michael reactions without having to use valuable chiral starting material **182**, model studies were conducted employing a commercially available un-functionalized enone. Accordingly, the racemic reaction was tested[ii] between the of functionalized non-chiral enone **199** and ketoester **173a**.[34, 35] However, instead of sole formation of the Michael adduct, the cyclized product **204** was acquired in 56% yield as a single diastereomer.



a) **173a** (1.1 eq), Cu(OTf)$_2$ (0.5 eq), rt, 4 days; b) LiHMDS, reflux, THF

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[i] Conducted by Dr. Nathan Cichowicz

[ii] Conducted by Danielle Singleton

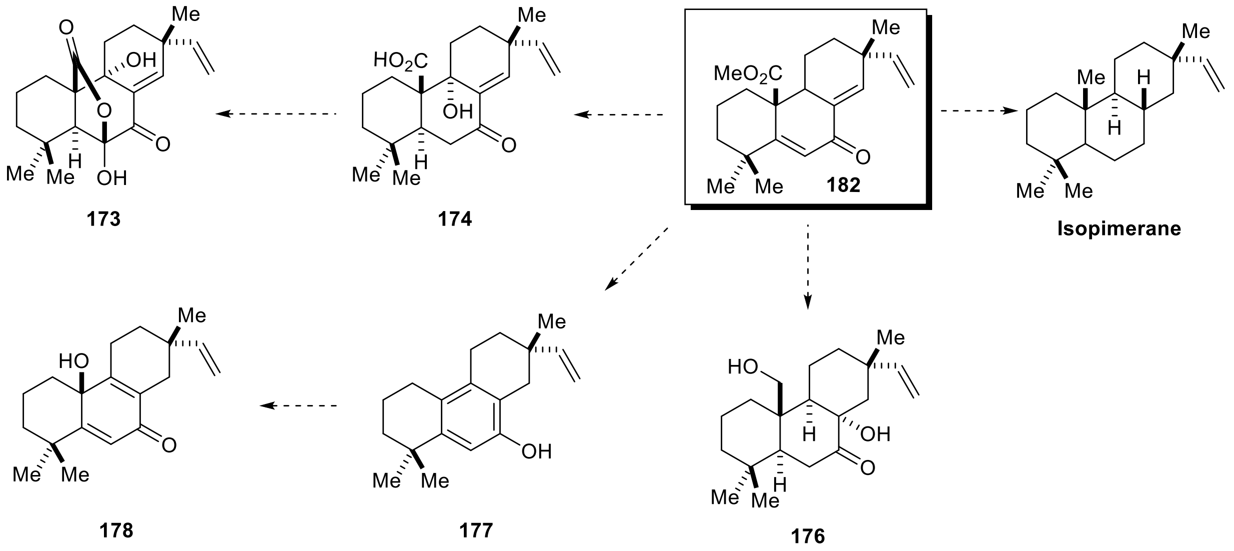
Scheme 3.10.4: Racemic reaction with functionalized enone and closing of cyclized core with LiHMDS

This result was encouraging in that there was concern that the aldehyde in our chiral substrate would interfere with the formation of the Michael adduct, instead it was observed to assist in the formation of the desired core, and spontaneous cyclization was observed to **204a**. At this time, few conditions for the second cyclization had been tested, but the best results were obtained using LiHMDS. Having obtained moderate yields of the desired tricyclic core **205**, we were assured that this method would be appropriate for the chiral substrate.

3.11 Summary

The synthesis of isoterpene-core intermediate **182** is the subject of ongoing studies in the Nagorny group. The synthesis of **182** should allow access to various diterpene-type natural products (**173-178**). It is noteworthy that using *S*,*S*-(-)-pseudoephenamine, the enantiomer of the starting *R*,*R*-(+)-pseudoephenamine (Figure 3.9.1), pimarane-type compounds (**1**), rather than isopimarane-type compounds (**2**), could be also obtained.

Besides the polyoxygenated diterpenes, a number of different alkaloid families of natural products can potentially be accessed including hetidine, hestisine, aza-atisine and atasine sub-classes of diterpene-alkaloids utilizing similar starting materials.



Scheme 3.11.1: Proposed routes for accessing various isopimarane derivatives via the cyclic diterpene intermediate 182

The described synthetic studies have contributed towards the eventual development of a synthetic strategy utilizing both a novel chiral auxilary and an asymmetric strategy in the synthesis of these polycyclic molecules containing multiple quaternary stereocenters. The results of this reasearch are significant in that this privileged tricyclic diterpenoid class of biologically active compounds could be reached in a short, total synthetic manner, potentially broadening the scope of bioactive molecules that can be developed and improving the availability of these compounds and structurally related-analogs for biological evaluation as potential targets in the pursuit of new and novel anticancer therapeutics.

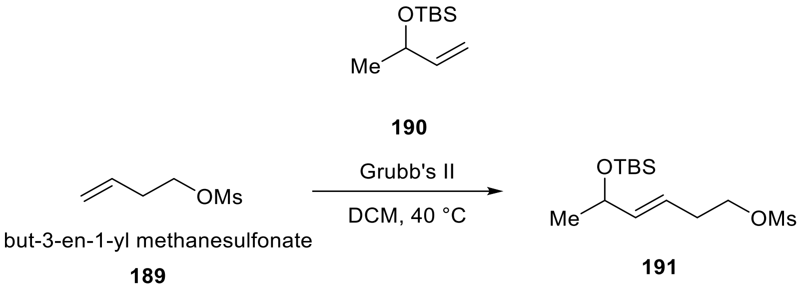
Chapter 3: Supporting Information

3.12 General reaction methods

All reactions were carried out under an atmosphere of nitrogen in flame– or oven–dried glassware with magnetic stirring, unless otherwise noted. Air–sensitive reagents and solutions were transferred via syringe or cannula and were introduced to the apparatus through rubber septa. Intermediates used in water sensitive reactions were azeotroped in toluene or benzene prior to use. Reactions were cooled via external cooling baths: ice water (0 °C), dry ice–acetone (-78 °C), or Neslab CB 80 immersion cooler (-20 to -78 °C). Heating was achieved by use of a silicone bath with heating controlled by an electronic contact thermometer. Deionized water was used in the preparation of all aqueous solutions and for all aqueous extractions. Solvents used for extraction and flash column chromatography were ACS or HPLC grade. Reagents were purified prior to use following the guidelines of Perrin and Armarego.[36] Tetrahydrofuran (THF), dichloromethane (DCM), toluene and diethyl ether (Et_2O) were filtered through a column (Innovative Technologies) of activated alumina under nitrogen atmosphere. Chloroform ($CHCl_3$) and deuterated chloroform ($CDCl_3$) were filtered through a column of basic alumina and magnesium sulfate prior to use with acid sensitive materials. Purification of the reactions mixtures was performed by flash column chromatography using SiliCycleSiliaFlash P60 (230-400 mesh) silica gel. 4Å molecular sieves were pre-activated before use.

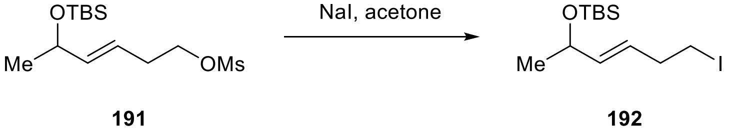
3.13 Spectral data acquisition

^{1}H NMR spectra were recorded on Varian vnmrs 700 (700 MHz), Varian vnmrs 500 (500 MHz), Varian INOVA 500 (500 MHz) or Varian MR400 (400-401 MHz) spectrometers and chemical shifts (δ) are reported in parts per million (ppm) with solvent resonance as the internal standard ($CDCl_3$ at δ 7.26, C_6D_6 at δ 7.15). Data are reported as follows (s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, dq = doublet of quartets, ddd = doublet of doublet of doublets, dddd = doublet of doublet of doublet of doublets, dqd = doublet of quartet of doublets, t = triplet, q = quartet, qd = quartet of doublets, m = multiplet; coupling constant(s) in Hz; integration). Proton-decoupled ^{13}C NMR spectra were recorded on Varian vnmrs 700 (175 MHz), Varian vnmrs 500 (125-126 MHz), Varian INOVA 500 (125-126 MHz) or Varian MR400 (100 MHz) spectrometers and chemical shifts (δ) are reported in ppm with solvent resonance as the internal standard ($CDCl_3$ at δ 77.000, C_6D_6 at δ 127.683). High resolution mass spectra (HRMS) were recorded on MicromassAutoSpecUltima or VGtd (Micromass) 70-250-S Magnetic sector mass spectrometers in the University of Michigan mass spectrometry laboratory. Infrared (IR) spectra were recorded as thin films on NaCl plates on a Perkin Elmer Spectrum BX FT-IR spectrometer. Absorption peaks were reported in wavenumbers (cm^{-1}). Optical rotations were measured in a solvent of choice on a JASCO P-2000 or Autopol III digital polarimeter at 589 nm (D-line) and reported as follows: $[\alpha]^{TEMP}_{D}$ (*c* = g/100 mL, solvent). Circular Dichroism was measured in spectroscopic grade ethanol on a JASCO J-815 Circular Dichroism Spectrometer.



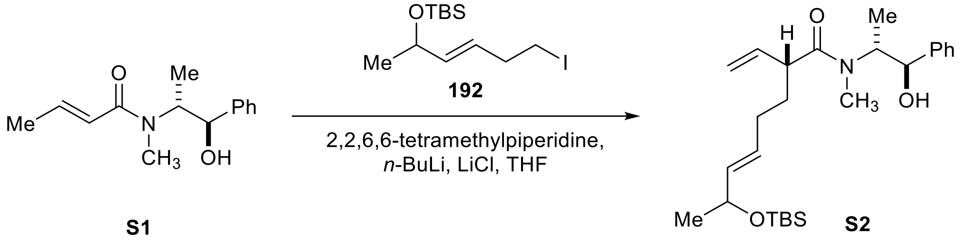
Scheme 3.13.1: Synthesis of (*E*)-5-((*tert*-butyldimethylsilyl)oxy)hex-3-en-1-yl methanesulfonate (191)

In a small oven-dried conical rbf (10 mL) was added mesylate-protected **189**[37] (227 mg, 1.44 mmol) and TBS-protected **190**[26] (545 mg, 2.92 mmol), a reflux condenser was attached, and the mixture was put under N_2. Next, Grubb's II (19.4 mg, 0.023 mmol) dissolved in dry DCM (2.5 mL) was added carefully via syringe. The mixture was refluxed at 40 °C for 12 h, cooled to rt, and carefully concentrated in vacuo to approximately 0.5 mL in volume. The crude mixture was purified via flash column chromatography (hexanes/EtOAc, 90/10 to 85/15) to yield the pure volitile oil product **191** (337 mg, 76 % yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.72 – 5.46 (m, 2H), 4.36 – 4.18 (m, 3H), 3.00 (d, *J* = 1.2 Hz, 3H), 2.51 – 2.43 (m, 2H), 1.19 (d, *J* = 6.3 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H); HRMS (ESI) *m/z* calcd for $C_{13}H_{28}O_4SSiH^+$ $[M+H]^+$ 309.1550, found 309.1548; HRMS (ESI) *m/z* calcd for $C_{13}H_{28}O_4SSiNa^+$ $[M+Na]^+$ 331.1370, found 331.1367.



Scheme 3.13.2: Synthesis of (*E*)-*tert*-butyl((6-iodohex-3-en-2-yl)oxy)dimethylsilane (192)

In an oven-dried RBF, add sodium iodide (0.840 g, 5.60 mmol) and put the reaction under N_2 with a reflux condenser on top. Next, compound **191** (0.150 g, 0.49 mmol) dissolved in acetone (3.03 mL) was added via syringe. The resulting mixture was refluxed at 57 °C for 2 hr and then let cool to rt. The reaction mixture was filtered through a fritted glass funnel, rinsed with acetone (3 times), and conc. in vacuo. The obtained material diluted with Et_2O (50 mL) and washed with Brine (2 x 25 mL). The combined organic fractions were dried over $MgSO_4$, filtered, and carefully conc. in vacuo[iii] to yield the crude transparent, yellow-colored oil (156.6 mg crude). The crude product was purified via flash column chromatography (hexanes/Et_2O, 50/50) to yield the pure product was obtained as a clear, colorless, volitile oil, **192** (129 mg, 78% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 5.65 – 5.42 (m, 2H), 4.27 (p, *J* = 6.1 Hz, 1H), 3.15 (t, *J* = 7.3 Hz, 2H), 2.64 – 2.49 (m, 2H), 1.20 (d, *J* = 6.3 Hz, 3H), 0.90 (s, 9H), 0.06 (s, 3H), 0.06 (s, 3H). LRMS (ESI) *m/z* calcd for $C_{12}H_{25}IOSiNa^+$ $[M+Na]^+$ 363.06.[iv]



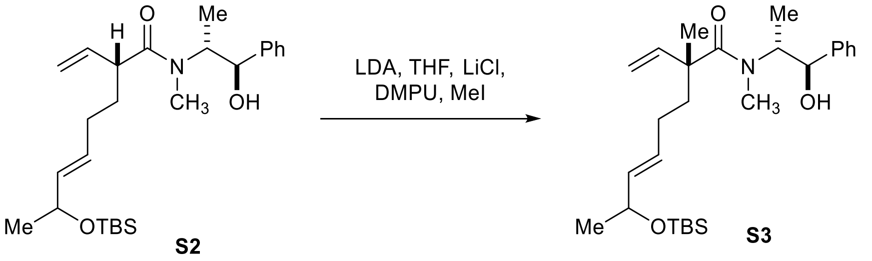
Scheme 3.13.3: Synthesis of (*E*)-7-((*tert*-butyldimethylsilyl)oxy)-*N*-((1*S*,2*S*)-1-hydroxy-1-phenylpropan-2-yl)-*N*-methyl-2-vinyloct-5-enamide (S2)

To a suspension of lithium chloride (118 mg, 2.78 mmol) in THF (0.489 mL) at -78 °C was added 2,2,6,6-tetramethylpiperidine (0.180 mL, 1.06 mmol) dropwise via syringe. A solution of *n*-BuLi (0.59 mL, 0.95 mmol, 1.6 M in THF) was added via syringe, and the resulting mixture was warmed to 0 °C. After 20 min, an ice-cooled solution of **S1**[38] (108 mg, 0.46 mmol) in THF (1.22 mL) was added via cannula. The transfer was rinsed with additional THF (2 x 0.204 mL). After 10 min, the reaction mixture was cooled to -78 °C. After 10 min, iodide **192** (314.3 mg, 0.924 mmol)

[iii] Material was somewhat volatile; cooled rotovap bath down and avoided using high vacuum when concentrating in vacuo.

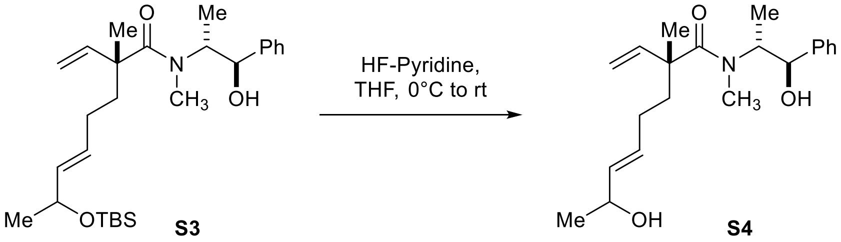
[iv] “after several ionization techniques… have not yet been successful in getting a representative spectrum.” – Paul Lennon.

was added via syringe followed by a rinse with THF (50 uL). After 40 min, the reaction mixture was warmed to 0 °C. After 4.5 h, saturated aqueous ammonium chloride solution was added to quench the lithium alkoxide salts. Brine and ethyl acetate were added, and the layers were seperated. The aqeuous layer was futher extracted with ethyl acetate (2x). the combined organic layers were dried over anhydrous $MgSO_4$, were filtered, and were concentrated under reduced pressure. The resudue was purified by flash column chromatography (hexanes/EtOAc, 65/35 to 60/40) to yield the pure product **S2**. ^1H NMR (700 MHz, $CDCl_3$) δ 7.40 – 7.26 (m, 5H), 5.84 – 5.70 (m, 1H), 5.58 – 5.34 (m, 2H), 5.18 – 5.01 (m, 2H), 4.69 – 4.07 (m, 4H), 3.49 – 3.07 (m, 1H), 2.96 – 2.80 (m, 3H), 2.04 – 1.79 (m, 3H), 1.65 – 1.47 (m, 1H), 1.27 – 1.10 (m, 6H), 0.88 (s, 9H), 0.07 – 0.02 (m, 6H). LRMS (ESI) *m/z* calcd for $C_{26}H_{43}NO_3SiNa^+$ $[M+Na]^+$ 468.3, found 468.1.



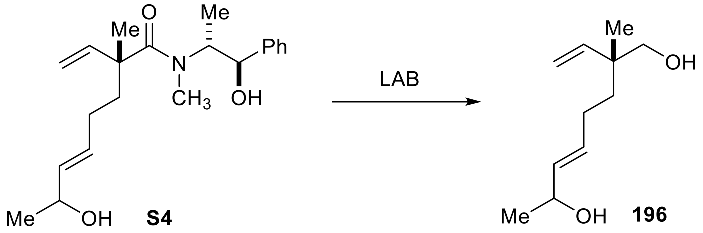
Scheme 3.13.4: Synthesis of (2*R*,*E*)-7-((*tert*-butyldimethylsilyl)oxy)-*N*-((1*R*,2*R*)-1-hydroxy-1-phenylpropan-2-yl)-*N*,2-dimethyl-2-vinyloct-5-enamide (S3)

Diisopropylamine (18.62 µL, 0.133 mmol) was added via syringe to a suspension of LiCl (14.98 mg, 0.353 mmol) in THF (105 µL) at -78 °C. A solution of *n*-BuLi (76 µL, 0.122 mmol, 1.6 M in THF) was added via syringe, and the resulting mixture was warmed to 0 °C. After 5 min, the resulting solution was cooled to -78 °C. An ice-cooled solution of **S2** (26.2 mg, 0.059 mmol) in THF (175 µL) was added via cannula. The transfer was rinsed with additional THF (70 µL), and the resulting mixture was warmed to 0 °C. After 1 h, the reaction mixture was cooled to -40 °C, and DMPU (17.8 µL, 0.148 mmol) was added via syringe. After 10 min, the reaction mixture was cooled to -60 °C, and iodomethane (27.4 µL, 0.441 mmol) was added slowly via syringe. After 5 min, the reaction mixture was warmed to -50 °C. After 42 h, saturated NH_4Cl (1 mL) was added to quench the lithium alkoxide salts, and the resulting biphasic mixture was allowed to warm to rt. Brine (10 mL) and DCM (12.5 mL) were added, and the layers were seperated. The aqueous layer was further extracted with DCM (3 x 12.5 mL). The combined organic layers were dried over anhydrous $MgSO_4$, were filtered, and were conc. in vacuo. The crude product was purified via flash column chromatography to yield the pure product **S3**. ^1H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.27 (m, 5H), 6.11 – 4.18 (m, 7H), 2.98 – 2.67 (m, 3H), 2.04 (s, 2H), 2.00 – 1.59 (m, 3H), 1.38 – 0.93 (m, 10H), 0.88 (s, 9H), 0.21 – -0.10 (m, 6H). LRMS (ESI) *m/z* calcd for $C_{27}H_{45}NO_3SiNa^+$ $[M+Na]^+$ 482.3, found 482.3.



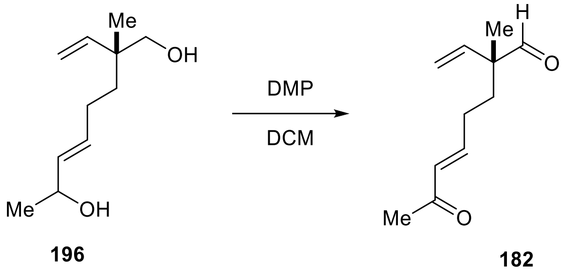
Scheme 3.13.5: Synthesis of (2*R*,*E*)-7-hydroxy-*N*-((1*R*,2*R*)-1-hydroxy-1-phenylpropan-2-yl)-*N*,2-dimethyl-2-vinyloct-5-enamide (S4)

To a 15 mL falcon tube (plastic) under N_2 was added **S4** (47.6 mg, 0.104 mmol) in THF (2.96 mL). The reaction mixture was stirred, vented with N_2, and cooled to 0 °C before adding HF-pyridine (0.597 mL, 0.104 mmol) dropwise via syringe. The reaction was stirred for 2 hours at 0 °C, warmed to rt, and stirred for an additional 2 hours at rt. The reaction was cooled down to 0 °C again, quenched with saturated $NaHCO_3$ (30 mL) added dropwise over 10 min (moved to larger plastic vial halfway through quenching), and diluted with 30 mL EtOAc. The organic layer was seperated, and the aq layer was backextracted with EtOAc (2 x 20 mL). The combined organic layers were washed with $CuSO_4$ (0.1 M, 15 mL) and H_2O (15 mL). The organic layer was dried with $MgSO_4$, filtered and conc. in vacuo to yield a crude, yellow-tinted oil, **S4** (31.1 mg, crude weight). This material was used without any further purification directly in the next reaction step (Scheme 3.13.6). LRMS (ESI) *m/z* calcd for $C_{21}H_{31}NO_3Na^+$ $[M+Na]^+$ 368.2, found 368.3.



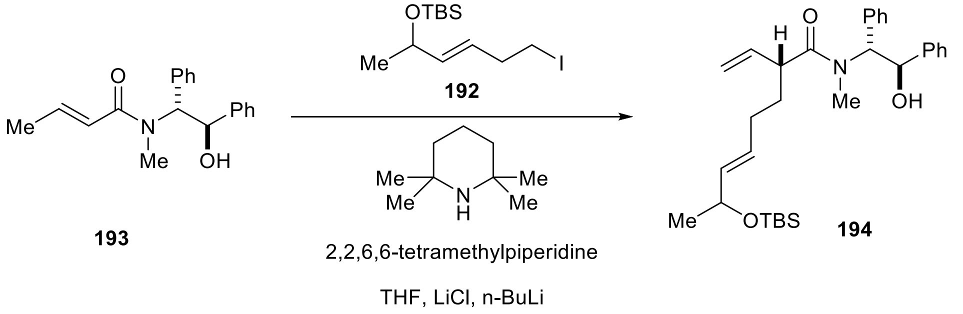
Scheme 3.13.6: Synthesis of (2*R*,*E*)-2-methyl-2-vinyloct-5-ene-1,7-diol (196)

To a small, flame-dried vial under N_2 was added THF (421 µl) via syringe, and the solvent was cooled to -78 °C. Next diisopropylamine (53.1 µl, 0.379 mmol) and *n*-BuLi (219 µl, 0.350 mmol, 1.6 M in THF) were added sequentially via syringe. The resulting yellow solution was stirred at -78 °C for 10 min, then at 0 °C for 5 min (ice-water bath). Borane-ammonium complex (12.4 mg, 0.362 mmol) was added quickly (remove septa and add) and the contained was resealed. The reaction was ran, worked up, and purified following a previously published methodology with LAB,[39] and **196** was isolated. ^{1}H NMR (401 MHz, $CDCl_3$) δ 5.79 – 5.37 (m, 3H), 5.18 (dd, *J* = 10.9, 1.3 Hz, 1H), 5.06 (dd, *J* = 17.6, 1.3 Hz, 1H), 4.25 (p, *J* = 6.4 Hz, 1H), 3.37 (q, *J* = 10.8 Hz, 2H), 1.97 (dq, *J* = 16.6, 5.9 Hz, 2H), 1.51 – 1.34 (m, 4H), 1.24 (d, *J* = 6.3 Hz, 3H), 1.02 (s, 3H); LRMS (ESI) *m/z* calcd for $C_{11}H_{20}O_2Na^+$ $[M+Na]^+$ 207.1, found 207.1.



Scheme 3.13.7: Synthesis of (*R*,*E*)-2-methyl-7-oxo-2-vinyloct-5-enal (182)

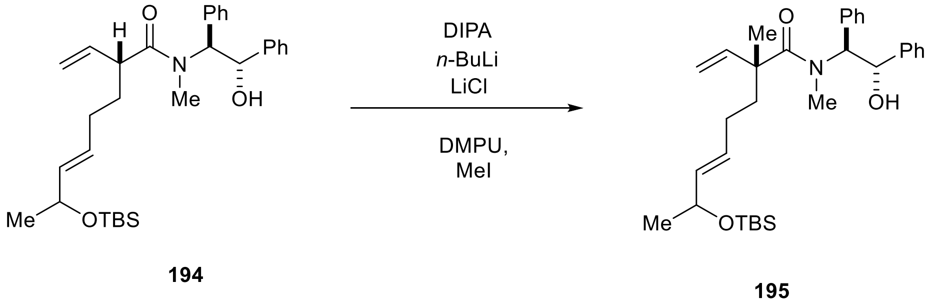
To a small vial (oven-dried) was added (2*R*,*E*)-2-methyl-2-vinyloct-5-ene-1,7-diol (6.2 mg, 0.034 mmol) in DCM (1.35 mL) which was stirred, put under N_2, and cooled to 0 °C with an ice bath. The septa was removed, the starting material was added quickly, and the mixture was resealed and put under N_2 again. The mixture was stirred at 0 °C for 5 min and at room temperature for 1 h 50 min. Additional DMP (7.0 mg) in DCM (0.25 mL) was added, and the reaction was stirred for an additional 30 min, before the sample was concentrated in vacuo. The crude material was purified through a short column of silica gel (hexanes/EtOAc, 75/25) to yield the pure product **182**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.39 (s, 1H), 6.77 (dt, J = 16.0, 6.7 Hz, 1H), 6.08 (dt, J = 15.9, 1.6 Hz, 1H), 5.77 (dd, J = 17.6, 10.8 Hz, 1H), 5.33 (dd, J = 10.8, 0.6 Hz, 1H), 5.17 (dd, J = 17.6, 0.6 Hz, 1H), 2.24 (s, 3H), 2.21 – 2.14 (m, 2H), 1.79 – 1.73 (m, 2H), 1.22 (s, 3H); LRMS (ESI) *m/z* calcd for $C_{11}H_{16}O_2Na^+$ $[M+Na]^+$ 203.1, found 203.1.



Scheme 3.13.8: Synthesis of (2*R*,*E*)-7-((*tert*-butyldimethylsilyl)oxy)-*N*-((1*R*,2*R*)-2-hydroxy-1,2-diphenylethyl)-*N*-methyl-2-vinyloct-5-enamide (194)

2,2,6,6-tetramethylpiperidine (0.398 mL, 2.34 mmol) was added via syringe to a suspension of lithium chloride (258 mg, 6.09 mmol) in THF (1.12 mL) at -78 °C. A solution of *n*-BuLi (1.31 mL, 2.09 mmol, 1.6 M in THF) was added via syringe, and the resulting mixture was warmed to 0 °C. After 20 min, an ice-cooled solution of **193**[33] (300 mg, 1.02 mmol) in THF (2.79 mL) was added via cannula. The rbf and cannula were rinsed with additional THF (2 x 0.47 mL; 1 x 1.00 mL). After 15 min, the reaction mixture was cooled to -78 °C. After 5 min, **192** (691 mg, 2.03 mmol) was added via syringe, followed by a rinse with THF (50 µL). After 40 min, the reaction was warmed to 0 °C. After 4.5 h, saturated NH_4Cl solution was added to quench the lithium alkoxide salts, and the reaction mixture was let warm to rt.

Brine and ethyl acetate were added, and the layers were separated. The aqueous layer was further extracted with ethyl acetate (2x). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under vacuo. The crude product was purified via flash column chromatography (70/30, hexanes/EtOAc) to yield a clear, colorless, highly-viscous liquid product **194** (326 mg, 63% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 7.61 – 7.07 (m, 10H), 5.91 – 4.96 (m, 7H), 4.26 – 4.02 (m, 1H), 3.33 – 3.08 (m, 1H), 2.97 – 2.80 (m, 3H), 2.13 – 1.07 (m, 8H), 1.03 – 0.73 (m, 9H), 0.04 (s, 3H), 0.02 (s, 3H); HRMS (ESI) *m/z* calcd for $C_{31}H_{45}NO_3SiH^+$ $[M+H]^+$ 508.3241, found 508.3240; HRMS (ESI) *m/z* calcd for $C_{31}H_{45}NO_3SiNa^+$ $[M+Na]^+$ 530.3061, found 530.3060.



Scheme 3.13.9: Synthesis of (2*R*,*E*)-7-((*tert*-butyldimethylsilyl)oxy)-*N*-((1*S*,2*S*)-2-hydroxy-1,2-diphenylethyl)-*N*,2-dimethyl-2-vinyloct-5-enamide (195)

Diisopropylamine (0.185 mL, 1.32 mmol) was added via syringe to a suspension of LiCl (149 mg, 3.51 mmol) in THF (1.04 mL) at -78 °C. A solution of *n*-BuLi (0.76 mL, 1.21 mmol, 1.6 M in THF) was added via syringe, and the resulting mixture was warmed to 0 °C. After 5 min, the resulting solution was cooled to -78 °C. An ice-cooled solution of **194** (297 mg, 0.584 mmol) in THF (1.74 mL) was added via cannula. The transfer was quantitated with additional THF (348 μL) and a second portion of THF (348 μL), and the resulting mixture was warmed to 0 °C. After 1 h (at 0 °C), the reaction mixture was cooled to -40 °C, and DMPU (177 µL, 1.47 mmol) was added via syringe. After 10 min, the reaction mixture was cooled to -60 °C (via addition of dry ice to cryocool), and iodomethane (273 µL, 4.38 mmol) was added slowly via syringe. After 5 min, the reaction mixture was allowed to warm to -50 °C (set on cryocool, not an instant change). After 42 h, saturated aqueous ammonium chloride (3.0 mL) was added to quench the lithium alkoxide salts, and the resulting biphasic mixture was allowed to warm to rt. Brine (20 mL) and DCM (25 mL) were added, and the layers were seperated. The aqueous layer was further extracted with DCM (3 x 25 mL). The combined organic layers were washed again with Brine (50 mL), backextracted aqueous Brine with DCM (25 mL), and final combined organic layers were dried over anhydrous $MgSO_4$, filtered, and conc. in vacuo. The crude material (R_f [starting material] = 0.25 [hexanes/EtOAc, 80/20]; R_f [product] = 0.295 [hexanes/EtOAc, 80/20]) was purified via flash column chromatography (hexanes/EtOAc, 85/15 to 80/20) to yield the pure product **195** (223 mg, 73% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 – 7.01 (m, 10H), 6.00 – 5.79 (m, 1H), 5.76 – 5.55 (m, 1H), 5.55 – 5.28 (m, 3H), 5.15 – 4.92 (m, 2H), 4.23 (p, *J* = 6.0 Hz, 1H), 2.85 (s, 3H), 2.04 – 1.54 (m, 5H), 1.24 (s, 3H), 1.20 – 1.14 (m, 3H), 0.89 (s, 9H), 0.19 – -0.11 (m, 6H); LRMS (ESI) *m/z* calcd for $C_{32}H_{47}NO_3SiNa^+$ $[M+Na]^+$ 544.3, found 544.1.

3.14 Chapter 3 References

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