

Drug-Oriented Synthesis of Cardiotonic Steroids

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Medicinal Chemistry)
in the University of Michigan
2017

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Dedication

I dedicate this dissertation to: my parents, Tom and Debbie; my sister, Claire; and my wife and
soulmate, Alex.

Scones.

Acknowledgments

I would like to acknowledge my advisor, Dr. Pavel Nagorny, whose guidance has helped drive this dissertation project and shape my skills as a chemist. He has always been willing to give helpful advice and support throughout my graduate school career. Dr. Nagorny has spent a lot of time and effort to help me apply for fellowships and scholarships, as well as attend conferences. He has always guided me in how best to present scientific research and was always willing to proofread and edit my writing. His encyclopedic knowledge of synthetic organic chemistry and willingness to help has been a tremendous asset. Perhaps most valuable has been the pep-talks and friendship offered during times of great stress.

I would also like to thank my lab mates Dr. Zhankui Sun, Dr. Nathan Cichowicz, Dr. Enoch Mensah, Bijay Bhattarai, and especially Dr. Hem Raj Khatri and Tay Rosenthal for their friendship and assistance throughout my tenure at Michigan. Talking about chemistry, sports, and life with them has been fun and rewarding. I would like to acknowledge my friends in the medicinal chemistry program, Joe Madak, David Cech, Nathan Scharf, and Eric Lashacz for his help with the biological studies I performed. I would also like to thank Dr. Dan Michele for his assistance and hospitality during my CBI sabbatical and assay development. Finally, I wish to thank my committee members for their service and guidance at each stage of this project.

Table of Contents

Dedication.....	ii
Acknowledgements.....	iii
List of Schemes.....	viii
List of Figures.....	xi
List of Tables.....	xii
List of Abbreviations.....	xiii
Abstract.....	xix
Chapter 1: Cardiotonic Steroids.....	1
1.1. Introduction.....	1
1.2. Cardiotonic Steroid Structural Features and Nomenclature.....	2
1.3. Brief History of Cardenolides.....	4
1.4. Heart Failure and Cardenolide Mechanism of Action.....	5
1.5. Binding Mode of Cardenolides.....	7
1.6. Cardenolide Toxicity and Therapeutic Window.....	10
1.7. Anticancer Activity of Cardenolides.....	12
1.8. Conclusion.....	14

Chapter 2: Previous Approaches to Cardenolides	18
2.1. Introduction.....	18
2.2. Semi-synthesis Vs Total Synthesis.....	18
2.3. First Total Synthesis of a Steroid.....	19
2.4. First Synthesis of Cardenolide Core.....	21
2.5. First Total Synthesis of a Natural Cardenolide.....	22
2.6. Overman's Intramolecular Heck Reaction Approach to Steroidal Cores.....	25
2.7. Deslongchamp's Synthesis of Ouabain.....	27
2.8. Baran's Semi-synthesis of Ouabagenin.....	32
2.9. Inoue's Synthesis of 19-Hydroxysarmentogenin.....	35
2.10. Conclusion.....	39
Chapter 3: Concise Enantioselective Synthesis of Oxygenated Steroids via Cu(II)-BOX-Catalyzed Michael Addition and Intramolecular Aldol Cyclization Reactions	41
3.1. Rationale for New Synthetic Approach.....	41
3.2. Methodological Design Overview.....	42
3.3. Michael Donor Fragment Design.....	43
3.4. Michael Acceptor Fragment Design.....	44

3.5. Investigation of Racemic Michael Reactions.....	45
3.6. Catalyst Screen for Enantioselective Michael Additions.....	48
3.7. Substrate Scope for Enantioselective Michael Reactions and Proposed Mechanism.....	54
3.8. Cyclization of Michael Adducts into Steroidal Cores.....	56
3.9. Origin of Stereodivergent Cyclization Results.....	59
3.10. Stereodivergent Cyclization of Adducts to a Library of Steroidal Cores.....	62
3.11. Experimental.....	66
Chapter 4: Concise Enantioselective Total Synthesis and Biological Evaluation of Cardiotonic Steroids 19-Hydroxysarmentogenin, Trewianin Aglycone, Panogenin, and <i>cis</i>- Panogenin.....	83
4.1. Introduction.....	83
4.2. Selected Targets and Approach Toward Total Synthesis.....	84
4.3. Installing C11 Oxygenation.....	86
4.4. Installing C3 Oxygenation.....	90
4.5. Synthesis of Key Intermediate 175	98
4.6. Elaboration of Intermediate 175 into 19-Hydroxysarmentogenin.....	102
4.7. Elaboration of Intermediate 175 into Trewianin Aglycone.....	106
4.8. Elaboration of Intermediate 175 into Panogenin.....	107

4.9. Elaboration of Intermediate 175 into <i>cis</i> -Panogenin.....	110
4.10. Evaluation via Biochemical Assay.....	113
4.11. Conclusion.....	119
4.12. Experimental.....	121
Chapter 5: Conclusions and Future Directions	175
5.1. Introduction.....	175
5.2. Conclusions.....	176
5.3. Future Directions.....	182

List of Schemes

Scheme 1.1. E. J. Corey's Semi-synthesis of 11- <i>epi</i> -ouabain.....	12
Scheme 2.1. Bachmann's Synthesis of Equilenin.....	20
Scheme 2.2. Daniewski's Synthesis of Cardenolide Core.....	22
Scheme 2.3. Stork's Total Synthesis of Digitoxigenin.....	24
Scheme 2.4. Overman's Intramolecular Heck Approach to Cardenolide Cores.....	26
Scheme 2.5. Deslongchamp's Preliminary Studies on a Michael/Aldol Cascade.....	28
Scheme 2.6. Deslongchamp's Total Synthesis of Ouabagenin.....	30
Scheme 2.7. Deslongchamp's Glycosylation of Ouabagenin to Ouabain.....	32
Scheme 2.8. Baran's Semi-synthesis of Ouabagenin from Cortisone Acetate.....	34
Scheme 2.9. Inoue's Synthesis of Key Fragments Toward 19-Hydroxysarmentogenin.....	36
Scheme 2.10. Inoue's Completion of 19-Hydroxysarmentogenin.....	38
Scheme 3.1. General Overview of Two-step Approach to Steroidal Core.....	43
Scheme 3.2. Synthesis and Structure of Michael Acceptor Fragments.....	44
Scheme 3.3. Copper(II)-Triflate Catalyzed Michael Addition of Fragments 94 and 95	46
Scheme 3.4. Substrate Scope for Cu(OTf) ₂ -Catalyzed Michael Reactions.....	47

Scheme 3.5. Known Asymmetric Michael Additions of Hindered Substrates.....	48
Scheme 3.6. Copper(II)-Based Catalyst Screen for Asymmetric Michael Reactions.....	50
Scheme 3.7. Optimization of Asymmetric Michael Addition with Donor 98	52
Scheme 3.8. Attempt at Mukaiyama-Michael Approach.....	53
Scheme 3.9. Substrate Scope for Enantioselective Michael Addition.....	54
Scheme 3.10. Proposed Mechanism of Cu(II)-BOX-catalyzed Michael Additions.....	56
Scheme 3.11. Approach to Cyclization of Michael Adducts.....	57
Scheme 3.12. Suggested Pathways for Diastereoselective Michael Adduct Cyclization.....	60
Scheme 3.13. Formation of <i>cis</i> - α -C/D Steroidal Scaffolds via DBU Promoted Cyclization.....	63
Scheme 3.14. Two-step Cyclization Approach to <i>cis</i> - β -C/D Steroidal Cores.....	64
Scheme 3.15. Synthesis of <i>cis</i> - β -C/D Steroidal Cores Via Two-step Protocol.....	65
Scheme 4.1. Retrosynthesis of Selected Targets via Key Intermediate 175	86
Scheme 4.2. Plan for Pre-installation of C11 Oxygenation.....	87
Scheme 4.3. Asymmetric Organocatalytic α -Oxidation of Aldehyde 100	88
Scheme 4.4. Michael Addition and Cyclization Tests with Fragment 182	89
Scheme 4.5. Plan for Pre-installation of C3 Oxygenation.....	90
Scheme 4.6 Initial Attempt at Michael Addition with Fragment 188	92
Scheme 4.7. Attempts at Mukaiyama Michael Addition and Subsequent Cyclization.....	93

Scheme 4.8. Synthesis and Michael Addition of Chloroketoester Fragment 192	95
Scheme 4.9. Attempts and Conditions for Cyclization of Adduct 194 to Steroidal Core.....	97
Scheme 4.10. Epimerization of C/D Ring Junction, Affording Steroid 197	99
Scheme 4.11. Global Reduction/Transposition Sequence for the Synthesis of Intermediate 175	100
Scheme 4.12. Total Synthesis of 19-Hydroxysarmentogenin.....	103
Scheme 4.13. Summary of Trewianin Aglycone Total Synthesis.....	107
Scheme 4.14. Total Synthesis of Panogenin.....	108
Scheme 4.15. Total Synthesis of <i>cis</i> -Panogenin.....	111
Scheme 4.16. Proposed Route to <i>cis</i> -Panogenin.....	112
Scheme 4.17. Schematic for Mechanism of Na ⁺ /K ⁺ ATPase Biochemical Assay.....	114
Scheme 5.1. Synthetic Methodology Design for Accessing Steroidal Cores.....	176
Scheme 5.2. Summary of Asymmetric Michael Addition Results.....	177
Scheme 5.3. Summary of Stereodivergent Cyclization Results.....	178
Scheme 5.4. Summary of Route to 175 and Stereodivergent Elaboration to Targets.....	180

List of Figures

Figure 1.1. Selected Steroidal Drug Examples.....	2
Figure 1.2. Cardiotonic Steroid Nomenclature.....	3
Figure 1.3. Selected Cardenolide Examples.....	5
Figure 1.4. Cardenolide Mechanism of Action Schematic.....	7
Figure 1.5. Ouabain- Na ⁺ /K ⁺ -ATPase Crystal Structure Image Created By Fedosova <i>et al.</i>	8
Figure 3.1. Michael Donors 94 and 98	43
Figure 4.1. Required Modifications to Previous Methodology and Selected Targets.....	84
Figure 4.2. Biochemical Assay and Concentration-response Curves.....	116
Figure 5.1. Targets and Required Modifications.....	179
Figure 5.2. Summary of Observed Structure-activity Relationships.....	182

List of Tables

Table 1.1. Reported Activity of Cardiotonic Steroids in Various Cancer Cell Lines – Information Compiled by Prassas, I. and Diamandis, E.....	13
Table 3.1. Major Products from Cyclization of Michael Adduct 96	58
Table 4.1. Summary of IC ₅₀ Values for Tested Compounds.....	118
Table 4.2. Comparison to the reported 19-hydroxysarmentogenin ¹ H NMR (700 MHz, D ₃ COD).....	144
Table 4.3. Comparison to the reported 19-hydroxysarmentogenin ¹³ C NMR (175 MHz, CD ₃ OD).....	146
Table 4.4. ¹³ C NMR Evidence for Configuration at C5.....	170

List of Abbreviations

Ac	acetyl
AIBN	azobisisobutyronitrile
Ar	aromatic (general)
Bn	benzyl
BOX	bis(oxazoline)
br	broad
Bu	butyl
Bz	benzoyl
<i>c</i>	concentration (g/100 mL)
°C	degrees Celsius
COSY	correlation spectroscopy
CSA	camphorsulfonic acid
Cy	cyclohexyl
<i>d</i>	deutero
d	doublet

DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethylazodicarboxylate
DIBALH	diisobutylaluminum hydride
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMP	Dess-Martin periodinane
DMS	dimethyl sulfide
DMSO	dimethylsulfoxide
dr	diastereomeric ratio
<i>E</i>	entgegen (Cahn-Ingold-Prelog system)
ee	enantiomeric excess
eq	equivalent
ESI	electrospray ionization
Et	ethyl
h	hour
HPLC	high performance liquid chromatography
HQ	hydroquinone

HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum correlation
$h\nu$	electromagnetic radiation
Hz	hertz
<i>i</i>	iso
iPr	isopropyl
<i>J</i>	coupling constant
KHMDS	potassium hexamethyldisilazide
LDA	lithium diisopropylamide
LiHMDS	lithium hexamethyldisilazide
<i>m</i>	meta
m	multiplet
M	molar; metal
m/z	mass to charge ratio
<i>m</i> -CPBA	<i>meta</i> -chloroperbenzoic acid
Me	methyl
min	minute(s)
mNBSF	<i>meta</i> -nitrobenzenesulfonic acid pyridinium

mol	mole(s)
MS	mass spectroscopy
NaHMDS	sodium hexamethyldisilazides
NBS	N-bromosuccinimide
ng	nanogram
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser enhancement
OTf	trifluoromethanesulfonate
<i>p</i>	para
Pd/C	palladium on carbon
Pd/SrCO ₃	palladium on strontium carbonate
Ph	phenyl
PMB	4-methoxybenzyl
PMP	4-methoxyphenyl
ppm	parts per million
PPTS	pyridinium <i>para</i> -toluenesulfonate
Pr	propyl
<i>p</i> -TSA	<i>para</i> -toluenesulfonic acid

Py	pyridine
q	quartet
R	rectus (Cahn-Ingold-Prelog system)
R	alkyl group (generic)
rt	room temperature
S	sinister (Cahn-Ingold-Prelog system)
s	singlet
SR	sarcoplasmic reticulum
t	triplet
<i>t</i>	tertiary
T	temperature
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
TBHP	<i>tert</i> -butyl hydroperoxide
TBS	<i>tert</i> -butyldimethylsilyl
<i>t</i> Bu	<i>tert</i> -butyl
TC	thiophene carboxylate
TES	triethylsilyl

TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
TPAP	tetrapropylammonium perruthenate
Z	zusammen (Cahn-Ingold-Prelog system)
δ	chemical shift (parts per million)

Abstract

Steroids are a structurally privileged class of bioactive natural products whose architecture has served as the bedrock for many valuable therapeutics throughout human history. In particular, the cardenolide sub-class of steroids, typified by ouabain and digoxin, has been used clinically since the late 18th century for the treatment of congestive heart failure; a widespread and detrimental affliction. In addition, the recently discovered anticancer activity of cardenolides has brought them back into focus as highly valuable therapeutic agents, although the mechanism of their anticancer activity is not fully understood. Moreover, cardenolides are well known for their low therapeutic windows. The design and construction of novel cardenolide analogues for biological evaluation is highly sought after. However, the chemical complexity of cardenolides makes the synthesis of new analogues a long process which is economically unjustifiable in an industrial setting. Herein we describe our efforts toward the development of a drug-oriented, concise, and stereodivergent synthetic approach to the cardenolide class of steroids culminating in the total synthesis of several natural cardenolides and analogues.

Chapter 1 delves into the relevant background and history of cardenolides as well as their structure and bioactivity. The mechanism of action leading to a positive inotropic response is explained and the mode of binding to the Na⁺/K⁺ATPase is discussed. Some previously reported structure-activity relationship studies are described and evaluated.

Chapter 2 describes several landmark efforts towards the total synthesis and semi-synthesis of cardenolides. A comparison of these two approaches is discussed and various semi-synthetic

and totally synthetic routes aimed at cardenolides are described. Special attention is paid toward butenolide installation, stereochemical control, and introduction of key sites of oxygenation.

Chapter 3 provides a detailed overview of the design and development of a conceptually new two-step synthetic approach to oxygenated steroidal cores. Chiral Cu(II)-BOX catalysts are successfully investigated for their ability to promote the enantio- and diastereoselective Michael addition of cyclic β -ketoesters and functionalized enones. A library of Michael adducts with unique A and D ring sizes, as well as C13 substitution is produced with high selectivities and yields (82–95% yield, 90–96% ee, and 4:1 to >20:1 dr). The Michael adducts then undergo stereodivergent intramolecular double aldol cyclization to afford a library of natural and unnatural steroidal cores. The factors controlling the diastereoselective cyclization are discussed.

Chapter 4 describes the elaboration of the aforementioned methodology to allow the total synthesis of four targets: 19-hydroxysarmentogenin, trewianin aglycone, panogenin, and *cis*-panogenin. Modifications allow the pre-installation of oxygenation at the key positions C3 and C11. These functional handles are carried through the Michael addition/cyclization sequence to afford a heavily functionalized steroid intermediate with 7 stereocenters in a fused tetracyclic array with oxygenation installed at C3, C11, C14, C17, and C19 in only 7 linear steps. This intermediate is then elaborated in a stereodivergent fashion into the four target cardenolides in only 9-11 linear steps. Finally, a biochemical Na^+/K^+ ATPase inhibition assay was developed and concentration-response curves were generated for the synthesized steroids. The resulting data corroborates the importance of the orientation at C17 and C11, while suggesting that the configuration at C5 is of little importance for aglycones.

Chapter 1

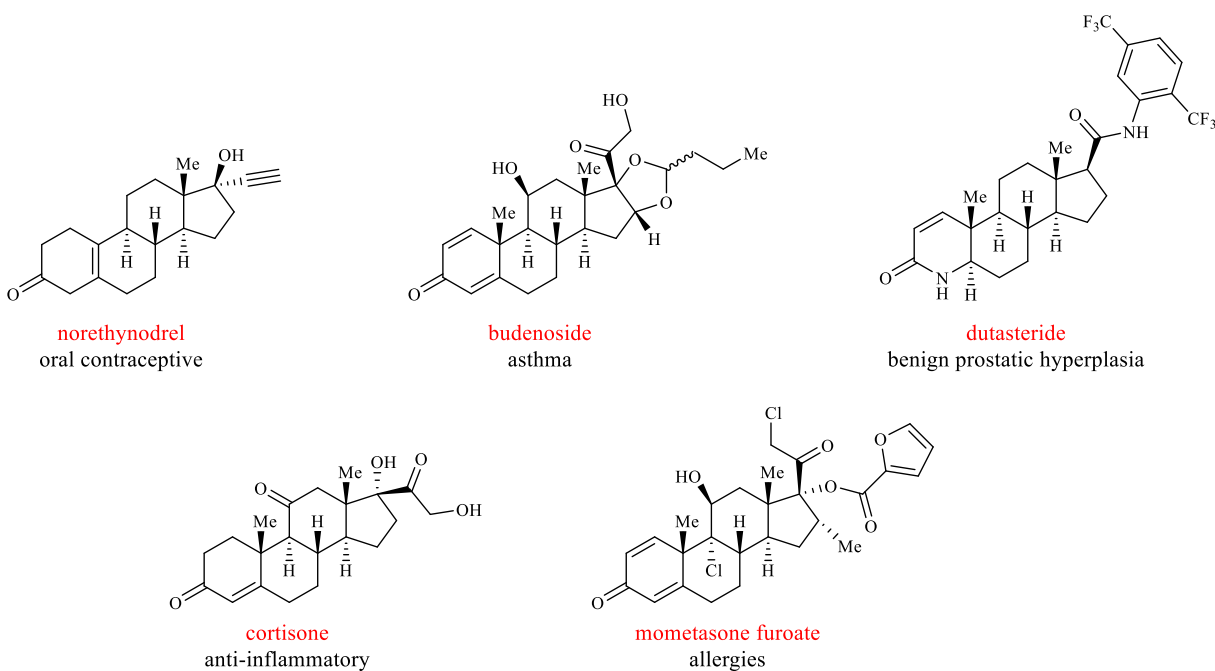
Cardiotonic Steroids

1.1. Introduction

Steroids are a structurally privileged class of bioactive natural products found ubiquitously in nature. These compounds are remarkably prevalent and are used naturally for a wide array of purposes including hormonal/cell signaling, lipid membrane stability, and defense mechanisms.¹ The abundance of steroids as endogenous compounds makes them valuable from a therapeutic standpoint because the human biochemical toolkit is well adapted to recognizing and responding to the steroidal scaffold.

Despite having a common tetracyclic core, steroids with unique stereochemical patterns and peripheral substitutions can be tuned to elicit drastically different physiological responses. For this reason, the steroidal core has served as the bedrock for many valuable FDA-approved drugs which have successfully treated human ailments such as cancer, heart failure, inflammation, allergies, metabolic diseases, and other health related areas like contraception and physical fitness **(Figure 1.1).**²

Figure 1.1. Selected Steroidal Drug Examples.



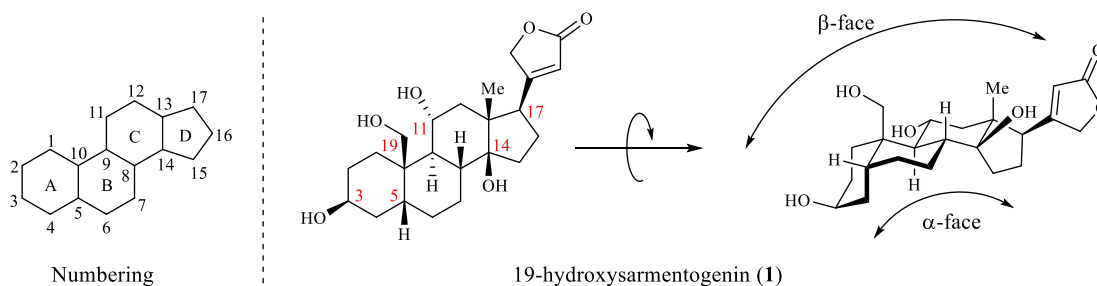
1.2. Cardiotoxic Steroid Structural Features and Nomenclature

The standard convention for steroid numbering and facial reference, which is used throughout this dissertation, is shown in **Figure 1.2**. The three 6-membered rings are labeled A, B, and C proceeding from left to right, and the 5-membered ring is labeled D. The carbon numbering begins at the northern apex of the A-ring and proceeds counter-clockwise around the A and B rings, and then weaves around the C and D rings with carbon units appended at the C13 and C10 position being labeled C18 and C19 respectively. Lastly, the top face of the steroid is denoted the β -face, with the underside being the α -face.

Cardiotoxic steroids represent an important steroidal subclass and are specifically characterized by a unique *cis*- β -A/B ring junction, *trans*-B/C ring junction, and *cis*- β -C/D ring junction which impose a clear U-shape to the molecule where the β -face is convex and the α -face

is concave.³ It should be noted there are exceptions to the *cis*- β -A/B ring junction fusion, particularly in cardiotonic steroids produced by milkweed (*Asclepias*), where the A/B ring is trans fused.⁴ Cardiotonic steroids also contain a β -C14 hydroxy and often have additional β -oriented hydroxy groups at various positions around the core. One of these key hydroxylated positions is found at C3, where the hydroxy group is often glycosylated. Non-glycosylated congeners are typically denoted with the suffix “-genin”. Finally, an unsaturated lactone ring is appended at the C17 position in a β orientation. Cardiotonic steroids can be further divided into cardenolides and bufadienolides, with the former being predominantly plant derived and the latter generally produced by animals.⁵ The key structural determinant which differentiates these subclasses is the heterocycle at C17; cardenolides possess a butenolide ring, while the bufadienolides have a 2-pyrone ring. An example of a cardenolide, 19-hydroxysarmentogenin (**1**), is shown in **Figure 1.2**. These structural characteristics impart an interesting amphipathic nature to many cardiotonic steroids with highly polar β -faces and relatively non-polar α -faces. As a result, cardiotonic steroids are well matched to the Na⁺/K⁺-ATPase binding pocket. The binding mode of these steroids is further discussed in section 1.5.

Figure 1.2. Cardiotonic Steroid Nomenclature.

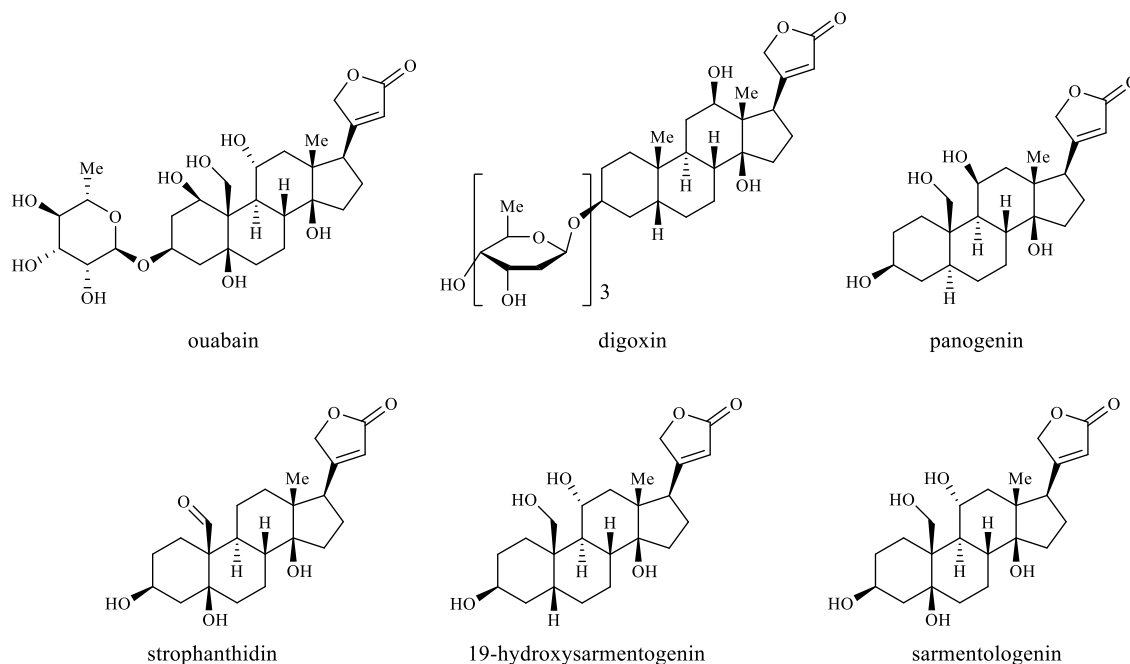


1.3. Brief History of Cardenolides

Cardenolides have been used for hundreds of years for a myriad of purposes, and there is no doubt they have played an important part in human history. In fact, plant extracts containing cardenolides were used by the ancient Egyptians, Romans, Greeks, Chinese, and African Tribes for purposes ranging from poison arrows and rat poison to diuretics and anesthetics.⁶⁻⁸ Cardenolides have even been cited as “the most ingested drugs in history” and the World Health Organization lists Digoxin (a cardenolide) on its Model List of Essential Medicines.^{9,10}

It wasn't until the latter half of the 18th century when an English scientist named William Withering studied and documented how extracts of the foxglove plant, now known to contain the digitalis cardenolides, could be effectively prepared and used to treat congestive heart failure; a condition referred to as dropsy at the time.¹¹ Withering used the scientific method to systematically study and understand how particular chemical components of the foxglove extracts can be properly dosed to treat human disease. His efforts relating to cardenolides are often cited as the “the beginning of modern therapeutics.”¹² Withering successfully described optimized extraction and dosing procedures and he found great success in using the foxglove extract to treat patients suffering from dropsy. Cardenolides have been used to treat heart failure ever since. Some examples of cardenolides are shown in **Figure 1.3**.

Figure 1.3. Selected Cardenolide Examples.

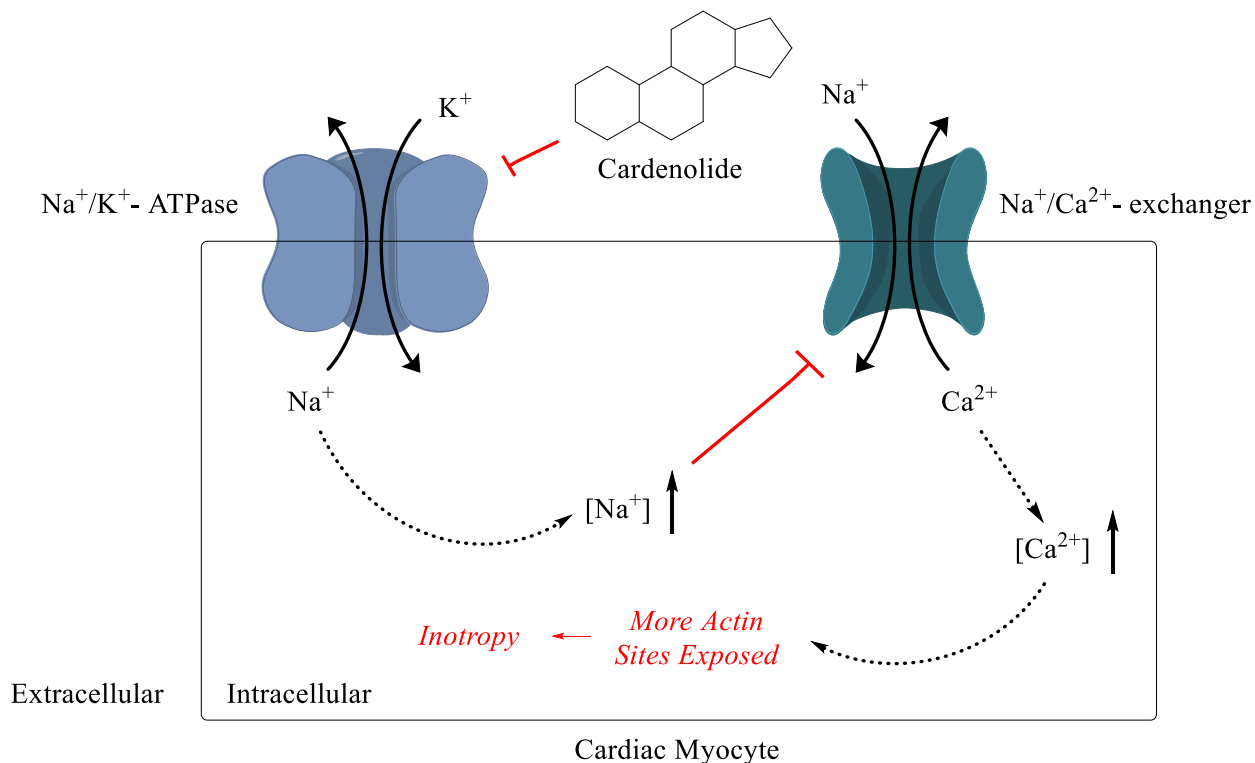


1.4. Heart Failure and Cardenolide Mechanism of Action

Heart failure is a widespread and detrimental disease which currently afflicts 5.8 million people in the United States alone.¹³ It is characterized by an enlargement or weakening of the heart's ventricular muscles which dramatically impairs the heart's ability to effectively pump and circulate blood throughout the body. Approximately 1 in 5 people will develop heart failure and about half of those who develop it will die within 5 years.¹⁴ The economic burden of heart failure in the United States is estimated to be \$39 billion each year.¹³ Due to the financial strain, prevalence, and danger posed by heart failure, new therapies for the effective treatment of this cardiovascular disease are strongly sought. While significant progress in medicine in the last century and the advent of diuretics and angiotensin-converting-enzyme (ACE) inhibitors has undoubtedly curbed these numbers, there remains a large potential for improvement.

Cardenolides act on the heart by binding and inhibiting the catalytic α -subunit (isoforms $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 4$) of the Na^+/K^+ -ATPase pumps found on the surface of cardiac myocytes (**Figure 1.4.**)¹⁵ The Na^+/K^+ -ATPase functions to maintain a cell's resting potential, regulate volume, participate in signal transduction of the MAPK pathway, and modulate intracellular calcium.¹⁶ Its mechanism of action involves the sequential transport of three Na^+ ions out of the cell and two K^+ into the cell, against their respective concentration gradients. The coupled hydrolysis of ATP provides the necessary energy. When the pump is bound by a cardenolide, its function is halted. Consequently, Na^+ efflux is hindered and intracellular Na^+ concentrations are increased. Elsewhere on the cell surface exists the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. This antiporter uses the energy stored in the electrochemical gradient of Na^+ across the cell membrane in order to efflux Ca^{2+} ions from the cell while allowing Na^+ to influx. The increased intracellular Na^+ concentrations resulting from Na^+/K^+ -ATPase inhibition will slow down or stop the function of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. This decreases Ca^{2+} efflux, and as a result, intracellular Ca^{2+} accumulates and is shuttled to the sarcoplasmic reticulum (SR). When the cardiac myocyte receives an action potential to contract and undergoes calcium-induced calcium release from the SR, a surplus of Ca^{2+} is released and these ions bind to troponin to expose myosin binding sites on the actin filament. More calcium leads to more myosin binding sites being exposed which leads to an increased contractile strength. The end result is a positive inotropic response on the heart muscle, wherein the heart contractile force is increased.¹⁶ This physiological response directly counteracts the effects of congestive heart failure by improving the hearts ability to pump and circulate blood.

Figure 1.4. Cardenolide Mechanism of Action Schematic.



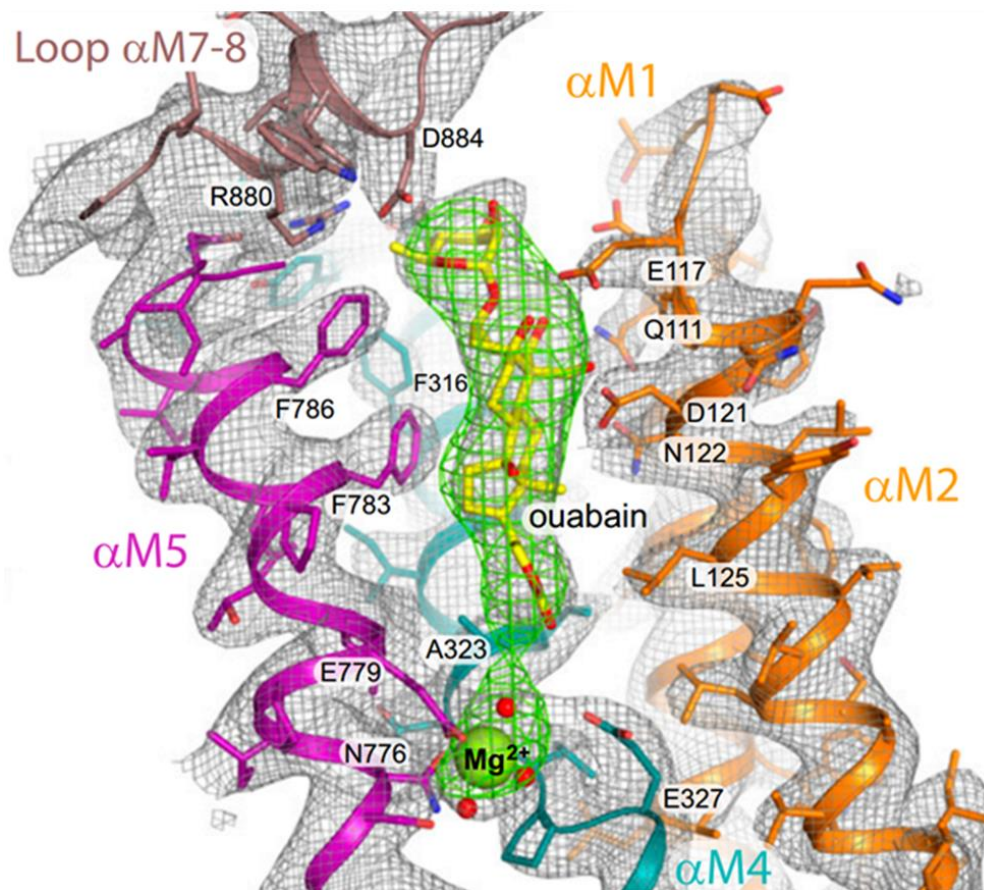
In fact, it was discovered that cardiotonic steroids are even present in small quantities as endogenous human hormones.¹⁷ These steroids serve as natural inhibitors of the Na⁺/K⁺-ATPase and have been shown to have several natural regulatory functions including arterial tension, cell proliferation, insulin release, and blood pressure.¹⁸

1.5. Binding Mode of Cardenolides

X-ray crystallographic analysis of Na⁺/K⁺-ATPase–cardenolide binding has been achieved and revealed valuable insights into the nature of the binding interactions involved. In one case, pig kidney Na⁺/K⁺-ATPase ($\alpha 1$ isoform) was complexed with ouabain and the x-ray was resolved to 3.4 Å resolution.¹⁹ Ouabain is bound in the pocket vertically with the C17 butenolide ring pointing directly into the enzyme while the sugar moiety is nearly solvent exposed at the extracellular

surface of the binding pocket. It is evident that the cardenolide binding pocket on the α -subunit is lined with polar residues on one side and non-polar residues on other (**Figure 1.5**).

Figure 1.5. Ouabain- Na^+/K^+ -ATPase Crystal Structure Image Created By Fedosova *et al.*¹⁹



The pocket is well suited for cardenolides which have polar β -faces and non-polar α -faces. The helices αM2 and αM1 line the polar side of the pocket and protrude glutamate 117, glutamine 111, aspartate 121, asparagine 122, and leucine 125 residues toward the bound cardenolide. These residues form key H-bonding and electrostatic interactions with the multiple β -oriented hydroxy groups on ouabain. At the same time, helices αM4 and αM5 line the opposite side of ouabain with non-polar phenylalanine 316/786/783, isoleucine 315, glycine 319, and leucine 793 residues

forming key hydrophobic van der Waals contacts. In addition, the polar interactions mediated by residues R880 and D884 of loop α M7-8 help bind the sugar component of the steroid.

Some structure-activity relationships have been explored through semi-synthetic modifications of cardenolides.²⁰ It has been shown that the sugar moiety at C3 influences the pharmacokinetic and pharmacodynamic properties. Appended rhamnose was shown to increase potency by a factor of 6-35, while mannose had no effect.²⁰ In addition, glycosylated cardenolides tend to absorb more poorly but exhibit greater metabolic stability. The role of the lactone ring at C17 has also been explored. One study determined that the interaction energy afforded by the butenolide amounts to -4.9 kcal/mol. This is nearly equal to the -5.4 kcal/mol of interaction energy supplied by the rest of the steroid as a whole.²¹ This beneficial interactive force is eliminated if the orientation of the butenolide at C17 is inverted to an α -orientation. Further evidence implicates the likelihood of an induced fit binding mode. The process of steroid binding likely promotes conformational changes within the Na⁺/K⁺-ATPase pocket which then allows deeper binding and optimal orientation of the butenolide ring. Since the butenolide is one of the most defining structural features of cardiotonic steroids, its importance for bioactivity is understandable. Some work has also been done to probe the role of butenolide (cardenolide) vs 2-pyrone (bufadienolides) rings at the C17 position. One study examined deaths resulting from cardiotonic steroids in a non-clinical setting. From 1982 to 2003, it was apparent that bufadienolides were significantly more toxic than cardenolides.²² This implicates the 2-pyrone moiety as a stronger toxicophore than the butenolide substituent. Another important aspect to note is variance of the internal ring junction stereocenters. Changing the orientation of either the A/B or C/D ring junction from *cis* to *trans* appears to decrease interaction energy for some glycosylated cardenolides.²³ However, this effect is not as apparent in the aglycone congeners and suggests that the *cis* orientation of the A/B

junction is necessary for proper spatial arrangement of the sugar group. Yet, there are known examples where aglycones with *trans*-C/D junctions exhibit stronger interactions with the Na⁺/K⁺-ATPase than their *cis*-β-C/D counterparts.²⁰ In addition, there are documented cases of glycosylated *trans*-A/B architectures showing strong Na⁺/K⁺-ATPase inhibition.²⁴ Our understanding of cardenolide structure-activity relationships remains incomplete and additional work is needed in this area.

1.6. Cardenolide Toxicity and Therapeutic Window

Despite the historical success and widespread use of the cardenolides, there remain some important limitations. Even in 1775, William Withering noted the disappointingly narrow therapeutic window of digitalis. Cardenolides have since been well known for their poor toxicity profiles resulting in therapeutic indices as low as two. Cardenolides are produced naturally by plants, likely as a mechanism for deterring herbivores, thus the compounds are naturally designed and well optimized to kill animals via arrhythmia and heart-arrest.²⁵ When cardenolides are used clinically, careful patient dosing is essential. For example, the target plasma concentration of digoxin is 0.8 – 2.0 ng/mL and toxic effects begins to occur at or above 2.0 ng/mL.²⁶ As a result, patients often receive 60% of the toxic dose.

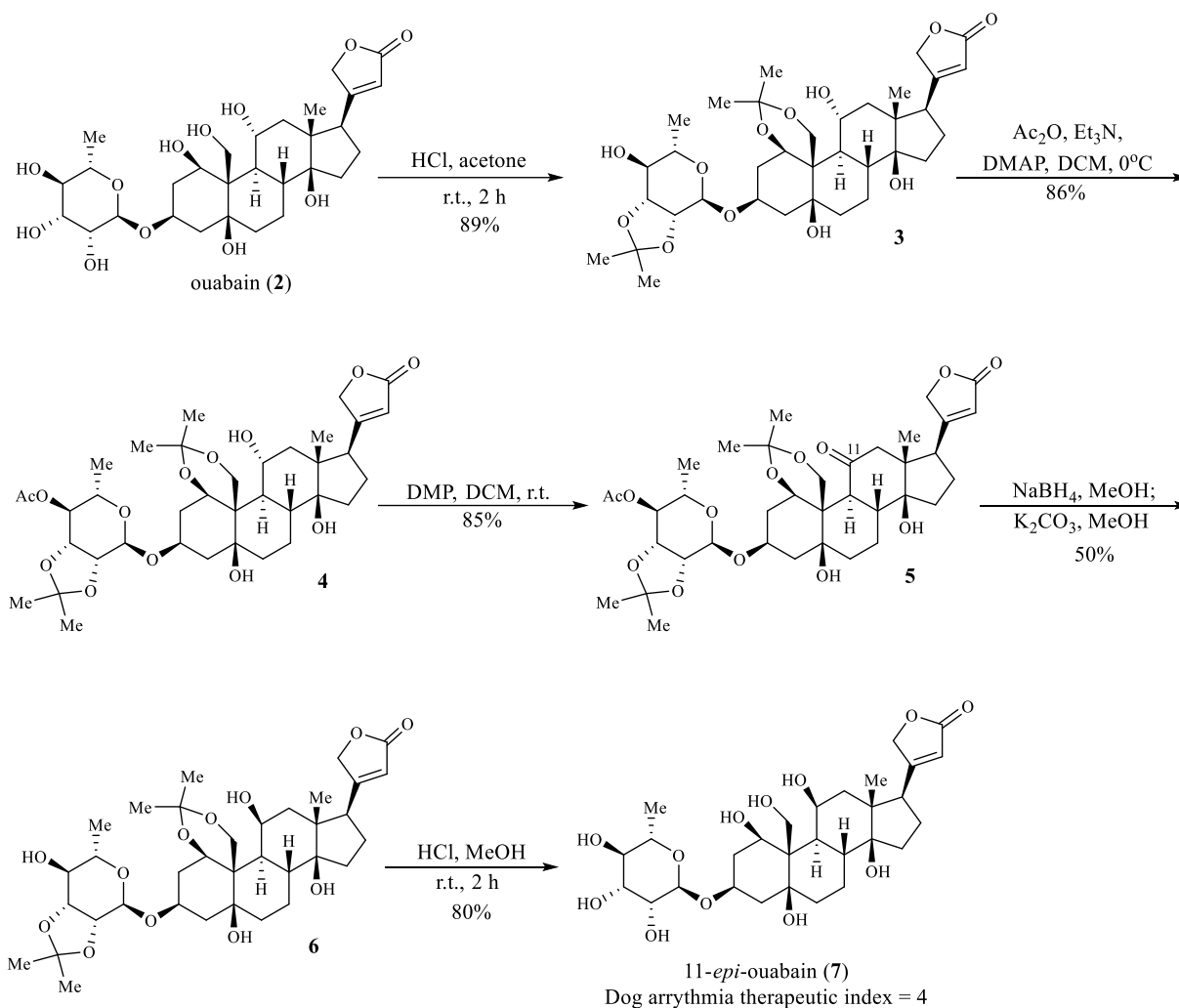
It is documented that two discrete receptor isoforms appear largely responsible for the therapeutic inotropic response and the toxicity. There is some lack of consensus in the literature on this topic. One report tentatively suggests the α1 isoform as the desired target for therapy with the α2 or α3 isoforms being responsible for toxic effects,²⁷ while a more recent report theorizes that selective α2 inhibitors would best expand the therapeutic window.²⁸ It is worth noting that the clinically used digitalis cardenolides show a modest preference for α2 over α1, while the less used ouabain shows a preference for α1 over α2; yet these differing usage levels may be a result of

bioavailability parameters. Furthermore, some studies report that the sugar moiety of glycosylated cardenolides is responsible for isoform differentiation,²⁸ while another study found that the sugar actually reduced isoform selectivity in some cardenolides and went on to strongly implicate the butenolide region in selectivity.²⁹ More research on this topic is needed, however, it is clear that isoform selective inhibitors are highly desired. At this time, the author is unaware of any $\alpha 2$ or $\alpha 3$ co-crystal structures with a cardenolide and so direct x-ray guided structure design for generating isoform selectivity is unavailable.

One study undertaken by E.J. Corey in 2006 at Harvard University demonstrated the capacity for cardenolide analogues to exhibit an improved therapeutic index.³⁰ Corey and his co-workers explored the semi-synthesis of a C11 epimer of ouabain (**2**) and its biological activity (**Scheme 1.1**). They were able to bis-acetalize ouabain (**2**) with HCl/acetone to produce compound **3**. Selective monoacylation of the remaining sugar hydroxy in the presence of the C11 hydroxy generated compound **4**, which was subsequently oxidized with the Dess-Martin periodinane. Steroid **5** was then reduced with NaBH₄, wherein hydride was added to the C11 ketone exclusively from the α -face. After saponification of the acylated alcohols, intermediate **6** was obtained. The final step was hydrolysis of the acetal moieties with HCl/MeOH to furnish 11-*epi*-ouabain (**7**). It was discovered that this C11 epimer of ouabain retained potency as an inotropic agent and displayed a two-fold improvement of the therapeutic index in a dog arrhythmia test (TI = 4). Unfortunately, no mention or study of isoform selectivity was apparent.

There is a clear benefit and desire to improve the toxicity profile associated with the cardenolides and one aspect of the work described in this dissertation aims at improving access to new regions of chemical space associated with these steroids in order to explore ways of increasing the therapeutic window and generating safer drug candidates.

Scheme 1.1. E. J. Corey's Semi-synthesis of 11-*epi*-ouabain.



1.7. Anticancer Activity of Cardenolides

In the 1980s, Stenkvist and colleagues were studying women with breast cancer and some of the women being studied were also taking digitalis.³¹ It was discovered that the cancer cells of the women taking digitalis showed more benign features compared to those not taking digitalis. In addition, Stenkvist noted that the odds of reacquiring breast cancer five years after a mastectomy was 9.6 times lower for the women taking digitalis. Moreover, Stenkvist followed up with the same women twenty years later and reported that those taking digitalis had a 6% mortality rate

(6/32), while those not taking digitalis had a 34% mortality rate (48/143).³² Despite the small sample size, the apparent correlation sparked much interest. Many groups have since independently investigated and confirmed the anticancer activity of cardenolides in various cell lines (**Table 1.1.**)³³

Table 1.1. Reported Activity of Cardiotonic Steroids in Various Cancer Cell Lines – Information Compiled by Prassas, I. and Diamandis, E.³³

Cancer Type	Compounds	Cell Lines
Breast	digitoxin, digoxin, proscillaridin A, ouabain, digoxigenin, gitoxin, gitoxigenin	MCF-7, MDA-MD-435
Prostate	oleandrin, ouabain, digoxin, bufalin, cinobufagenin	PC-3, LNCaP, DU145
Melanoma	digoxin, oleandrin, digitoxin, proscillaridin A, ouabain, digitonin	UACC-62, BRO
Lung	digitoxin, digoxin, ouabain, UNBS1450, oleandrin	A549, NCI-H-358, Calu1, Sklu1, NCI-H6, H69AR
Leukemia	bufalin, oleandrin, digitoxin, proscillaridin A, ouabain	HL60, U937, CCRF-CEM, CEM-VM-1
Neuroblastoma	digoxin, ouabain	SH-SY5Y, Neuro-2a
Renal	digitoxin, digoxin, digitoxigenin, proscillaridin A, ouabain	TK-10, ACHN
Myeloma	digitoxin, digoxin, proscillaridin A, digitoxigenin, ouabain, digitonin, lanatocide C	8226-S, 8226-LR5, 8226-DOX-40
Pancreatic	oleandrin	PANC-1

Since the discovery of cardenolide antiproliferative activity, many research projects have investigated the biological origin. However, the specific mechanism of action by which cardiotonic steroids exert their anticancer activity has not been definitively determined. Many reports have proffered different theories as to the origin of this anticancer activity. Some proposals include alteration of the Na⁺/K⁺-ATPase signalosome which mediates intracellular signaling pathways, inhibition of topoisomerase II, changes in gene expression profiles, increased production of

reactive oxygen species (ROS), inhibition of glycolysis, disruption of ion concentrations, and more.³³ It is important to note that there is little ambiguity about the increased activity of Na⁺/K⁺-ATPase associated with cancerous cells.³⁴ Most, but not all, suggestions for the anticancer activity are associated with binding to the Na⁺/K⁺-ATPase on tumor cells and a recent report implicates the $\alpha 1$ isoform as being a prime target for combating non-small cell lung cancers.²⁴

The investigation of cardiotonic steroids as potential antiproliferative agents is highly sought after. It is the partial aim of the work described here to improve access to novel regions of chemical space surrounding the cardenolides. Access to unique chemical analogues of these steroids could allow a more comprehensive understanding of the structure-activity relationship associated with the anticancer activity and possibly generate promising drug candidates.

1.8. Conclusion

Cardiotonic steroids are an historically important and therapeutically valuable class of natural products. Their use dates back to the ancient Egyptians and has continued through modern times. Since the late 1800's, cardenolides have been used primarily for the treatment of heart failure, a widespread and detrimental affliction. However, these steroids are plagued by high toxicity issues which have limited their therapeutic use. Safer versions of these drugs are highly sought after. In addition, the recently discovered anticancer activity of cardenolides has brought them back into focus as highly valuable therapeutic agents. Nonetheless, the chemical complexity of the cardiotonic steroids make it difficult to synthesize important new analogues and access regions of chemical space which may provide us with safer, yet effective drugs. There is a strong desire within the synthetic and medicinal communities to develop new, more concise and modular syntheses of the cardenolide class of steroids to allow researchers improved access to novel analogues for biological evaluation.

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Chapter 2

Previous Approaches to Cardenolides

2.1. Introduction

The primary difficulty in studying the chemistry and biology of the cardiotonic steroids is the inherent chemical complexity of the molecules involved. The inability to acquire sufficient quantities of specific analogues makes the process slow and economically unjustified in an industrial setting. It is highly desirable to develop a concise and modular synthetic route to the cardenolide class of steroids to enhance our access to unique cardenolide like compounds for biological evaluation. Throughout the last century, there have been many landmark efforts and advancements in the field of steroidal synthesis. Some are described in sections 2.3 through 2.9.

2.2. Semi-Synthesis vs. Total Synthesis

The two most common approaches to acquiring steroids outside of natural production are semi-synthesis and total synthesis. In semi-synthesis, a common and inexpensive natural steroid is acquired through isolation from an organism. The steroid is then chemically transformed through a series of reactions into a different, target steroid. This approach has some benefits and limitations. The primary benefit is that most of the structural features of the targeted steroid, such as the steroidal core, peripheral substituents, and stereochemical patterns, are already present from the outset. Semi-synthesis is thus most useful when one can isolate, from natural sources, a steroidal precursor which is structurally similar to the particular analogue being targeted. This minimizes

the number of chemical transformations needed to convert the isolated steroid into the desired analogue. However, in most cases, isolating certain steroids from natural sources can itself be a long and expensive process with low yield. The semi-synthetic approach also has limitations. The steroidal precursors have preset stereochemical patterns, sites of oxidation, and peripheral substitutions. It can be difficult, sometimes prohibitively so, to chemically modify these functionalities. For example, expanding or contracting ring sizes, inverting internal stereocenters, and functionalizing or oxidizing inert positions are some transformations which may be difficult to achieve in a concise manner. In addition, enantiomers are inaccessible through semisynthetic approaches. The predefined chemical state of an isolated steroid can therefore significantly hinder access to new and important analogues.

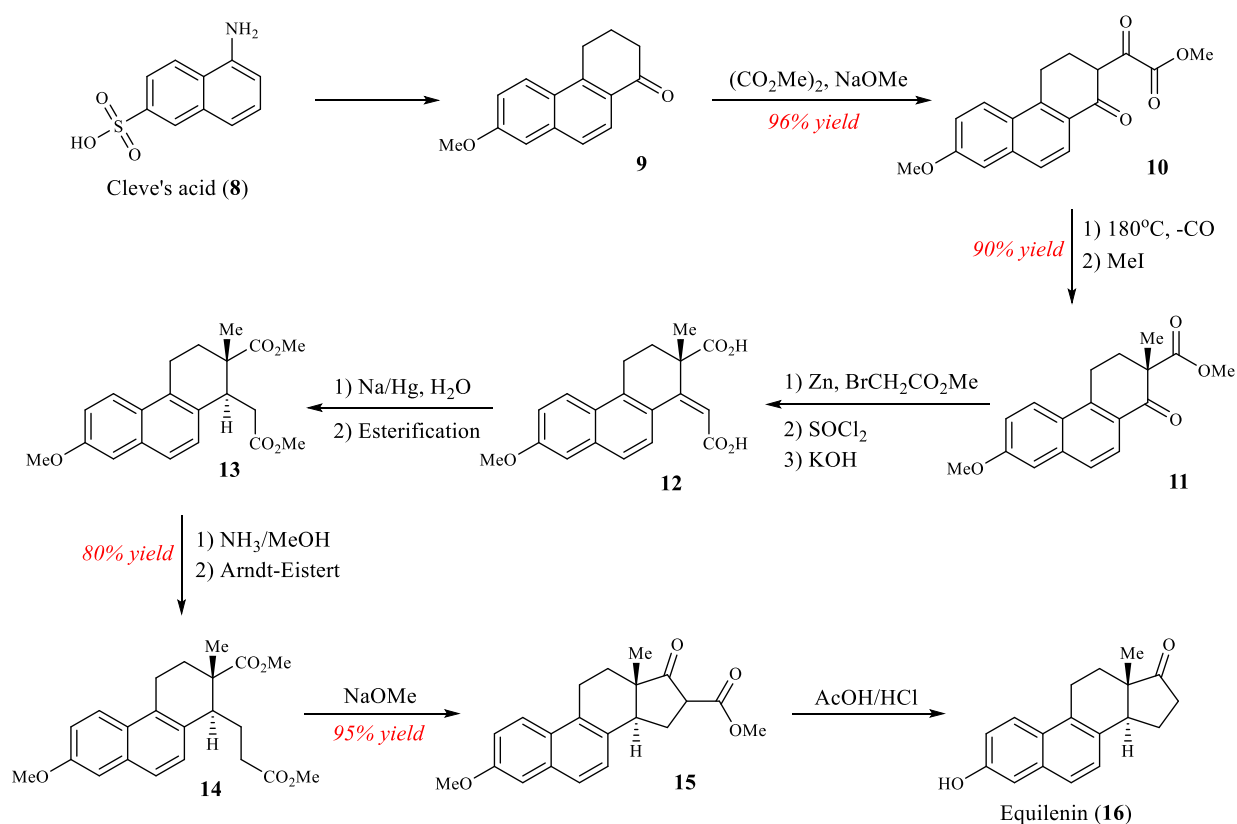
In a totally synthetic approach, cheap and simple starting materials (often no more than 6-8 carbons) are used by the chemist to construct a target steroid. Total synthesis has the benefit of accessing virtually any chemically viable steroid analogue. This allows researchers to explore unique analogues and have the best chance to find the most therapeutically valuable structures. The primary limitation to total synthesis is the time and number of steps required to acquire the target steroid. Total syntheses can often be so long (30 – 40 steps) and unwieldy that the cost does not justify the means and the production of analogues is too slow to maintain a viable drug development program. An optimal solution to the problem of synthesizing complex cardenolides could be a short and modular totally synthetic approach which could serve as a guiding manual for accessing new cardenolide cores in few steps.

2.3. First Total Synthesis of a Steroid

In 1939, Bachmann and colleagues at the University of Michigan developed the first total synthesis of a steroid.¹ While it is not a cardenolide, it represented the first successful approach to

synthesizing the steroidal core. Equilenin (**16**), a sex hormone, was synthesized in 12 steps (Scheme 2.1). Tricycle **9** was first prepared from Cleve's acid (**8**). Acylation with methyl glyoxalate using NaOMe produced triketone **10**. Subsequent decarbonylative pyrolysis and alkylation afforded β -ketoester **11** in 86% yield over two steps. A Reformatsky reaction was then followed by a two-step procedure for dehydration involving the conversion of a tertiary alcohol to a tertiary chloride and elimination of HCl by treatment with KOH.

Scheme 2.1. Bachmann's Synthesis of Equilenin.



Compound **12** was then reduced with sodium amalgam and esterified to the methyl ester. After this reduction, the *trans*-C/D ring junction product could be easily obtained because the *cis* isomer would spontaneously cyclize to the anhydride. Selective saponification with methanolic ammonia and subsequent Arndt-Eistert homologation generated intermediate **14** in 80% yield.

Dieckmann condensation promoted by NaOMe successfully closed the D ring and subsequent decarboxylation and demethylation was accomplished by treatment with AcOH/HCl to afford racemic Equilenin (**16**).

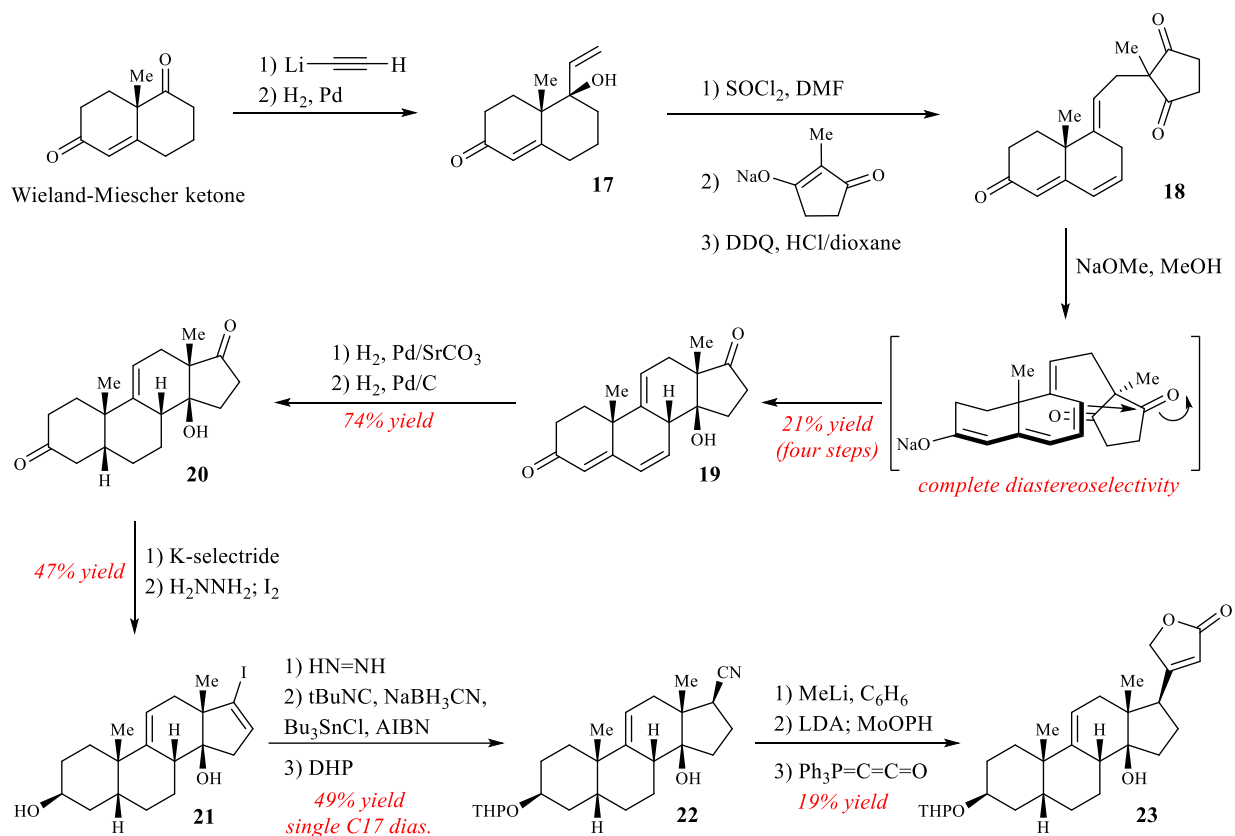
2.4. First Synthesis of Cardenolide Core

In 1979, Daniewski and co-workers reported the first synthesis toward a cardenolide core and completed it in 1988 (**Scheme 2.2.**).^{2,3} The approach taken includes a key aldol closure of the C-ring reminiscent of the strategy seen later by Deslongchamps as well as the methodology described in chapters 3 and 4. Another important note is the method for butenolide installation, which is a 7-step sequence starting from a ketone and proceeding by way of a clever Wittig reaction of the Bestmann ylide with an acyloin at C17. This is one of the two most common approaches to butenolide installation.

The synthesis starts with the well-known Wieland-Miescher ketone. Addition of lithium acetylide followed by hydrogenation produced allylic alcohol **17**. Chlorination of **17** with SOCl₂ and subsequent S_N2 displacement with the sodium salt of 2-methyl-cyclopentane-1,3-dione was performed. Oxidation of the resulting enone with DDQ produced intermediate **18**. The key cyclization was then initiated with NaOMe in dry methanol. The α -face of the extended enolate underwent an aldol reaction at the D-ring ketone with complete diastereoselectivity to produce **19** with the desired *cis*- β -C/D ring junction in 21% overall yield over four steps. The desired *cis*- β -A/B ring junction was then installed by successive hydrogenation using Pd/SrCO₃ and Pd/C. With diketone **20** in hand, the C3 ketone was selectively reduced from the α -face with K-selectride. The Barton conditions for vinyl iodide formation were then utilized to produce vinyl iodide **21** in 47% yield over two steps. Transfer hydrogenation using diimide was employed for the reduction of the C16-C17 olefin to furnish the α -oriented iodide and radical substitution of the iodide with cyanide

followed by protection of the C3 hydroxy by DHP produced steroid **22** in 49% yield over 3 steps. The final goal was installation of the butenolide. To this end, nitrile **22** was treated with MeLi to produce a C17 β -oriented methyl ketone. Using a modified Vedej's oxidation, the α position of the methyl ketone was successfully oxidized to produce an acyloin. Cyclization to the final product rac-9,11-dehydrodigitoxigenin 3-tetrahydropyranyl ether (**23**) was achieved by treatment with the Bestmann ylide.

Scheme 2.2. Daniewski's Synthesis of Cardenolide Core.



2.5. First Total Synthesis of a Natural Cardenolide

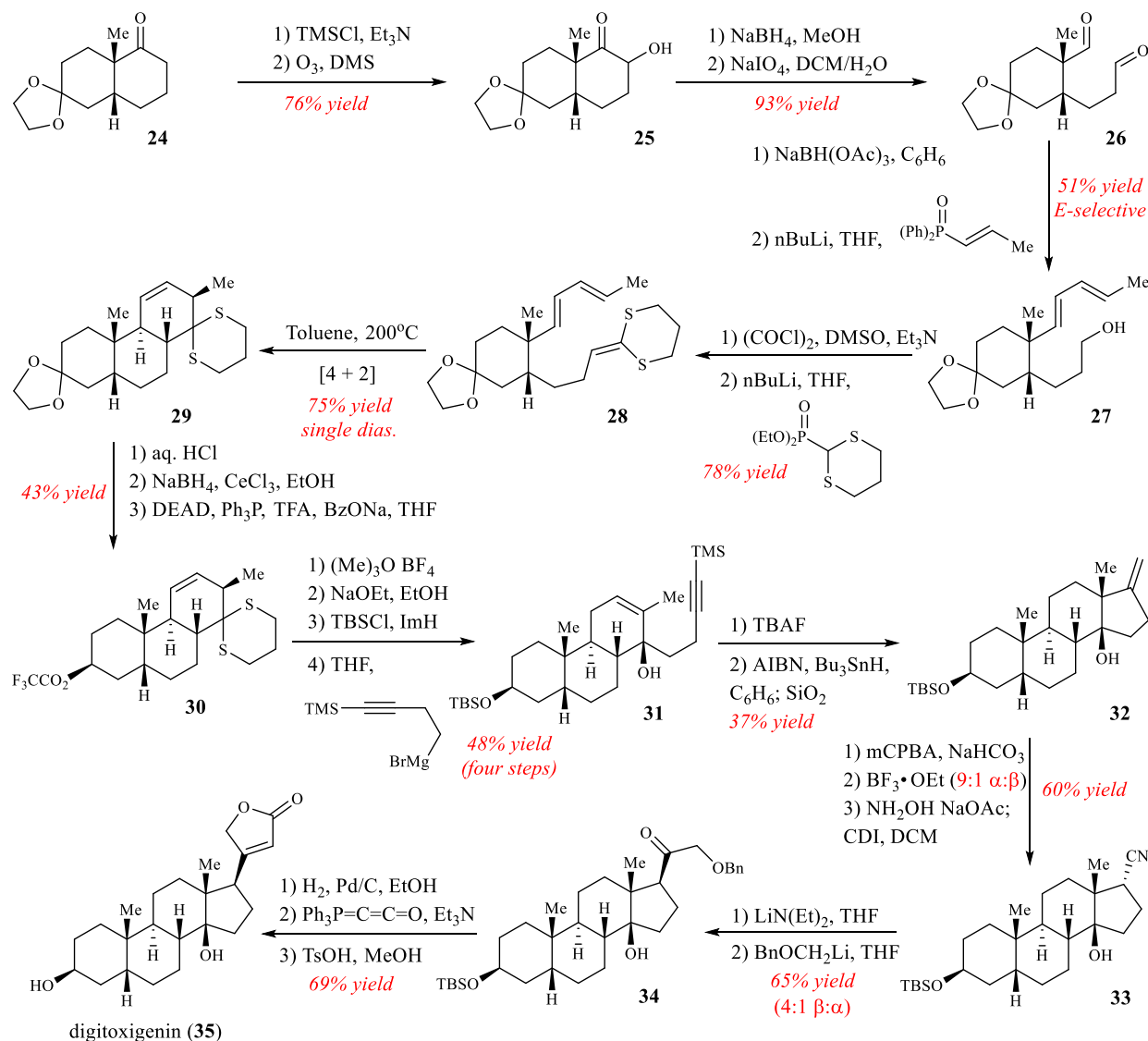
In 1996, Stork and co-workers reported the first total synthesis of a natural cardenolide, digitoxigenin (Scheme 2.3.), proceeding in 29 steps from the Wieland-Miescher ketone.⁴ The key

features of Stork's approach include a diastereoselective intramolecular type 1 Diels-Alder reaction to close the B and C rings, radical cyclization to set the D ring, and the same acyloin/Bestmann ylide combination used by Daniewski to furnish the butenolide at C17.

The synthesis begins by ozonolysis of the trimethylsilyl enol ether of **24** to yield acyloin **25**. Reduction with NaBH₄ produced the diol which was oxidatively cleaved with NaIO₄ to the dialdehyde **26**. Selective reduction of the primary aldehyde in presence of the more sterically hindered tertiary aldehyde was accomplished with NaBH(OAc)₃. Subsequent olefination by the method of Yamamoto furnished diene **27** in 77% yield forming the *E* olefin exclusively. Next, the dienophile component for the Diels-Alder reaction was installed. Swern oxidation of the primary alcohol was followed by a dithiane Hornor-Wadsworth-Emmons reaction. Upon heating to 200°C in toluene, intermediate **28** underwent a type I Diels-Alder reaction to close the B and C rings in the desired *trans* orientation (compound **29**) in 75% yield. Hydrolysis of the acetal at C3 with aqueous HCl produced a C3 ketone which was subjected to Luche reduction conditions to selectively generate the α -oriented hydroxy at C3 in 11:1 diastereomeric ratio. Natural steroids have a β -oriented alcohol at this position so inversion had to be carried out. This was accomplished with a Mitsunobu reaction using trifluoroacetate as the nucleophile to obtain intermediate **30**. Cleavage of the dithiane at C14 was accomplished with trimethyloxonium tetrafluoroborate. With concomitant hydrolysis of the C3 trifluoromethyl ester, the C11-C12 olefin could be isomerized into conjugation with the C14 ketone by treatment with NaOEt to generate an extended enolate which could be protonated at the γ -position. The C3 hydroxy was then protected with TBSCl and a Grignard reagent was added to the α -face of C14 thus generating intermediate **31** in 48% yield over 4 steps. This Grignard addition is noteworthy because it occurs selectively to set the desired C14-OH in a β -orientation. Next, the D ring needed to be closed in a *cis* orientation. Stork and co-

workers decided to accomplish this transformation by a radical cyclization. To do so, the terminal alkyne was first deprotected with TBAF and the product was then subjected to AIBN and Bu_3SnH . The tributyltin radical adds to the terminal alkyne and the resulting vinyl radical at C17 undergoes an allowed 5-exo-trig cyclization with the C13-C12 olefin and the resulting C12 radical abstracts a proton to continue the chain propagation. Destannylation is then accomplished by treatment with silica gel to furnish steroid **32**.

Scheme 2.3. Stork's Total Synthesis of Digitoxigenin.



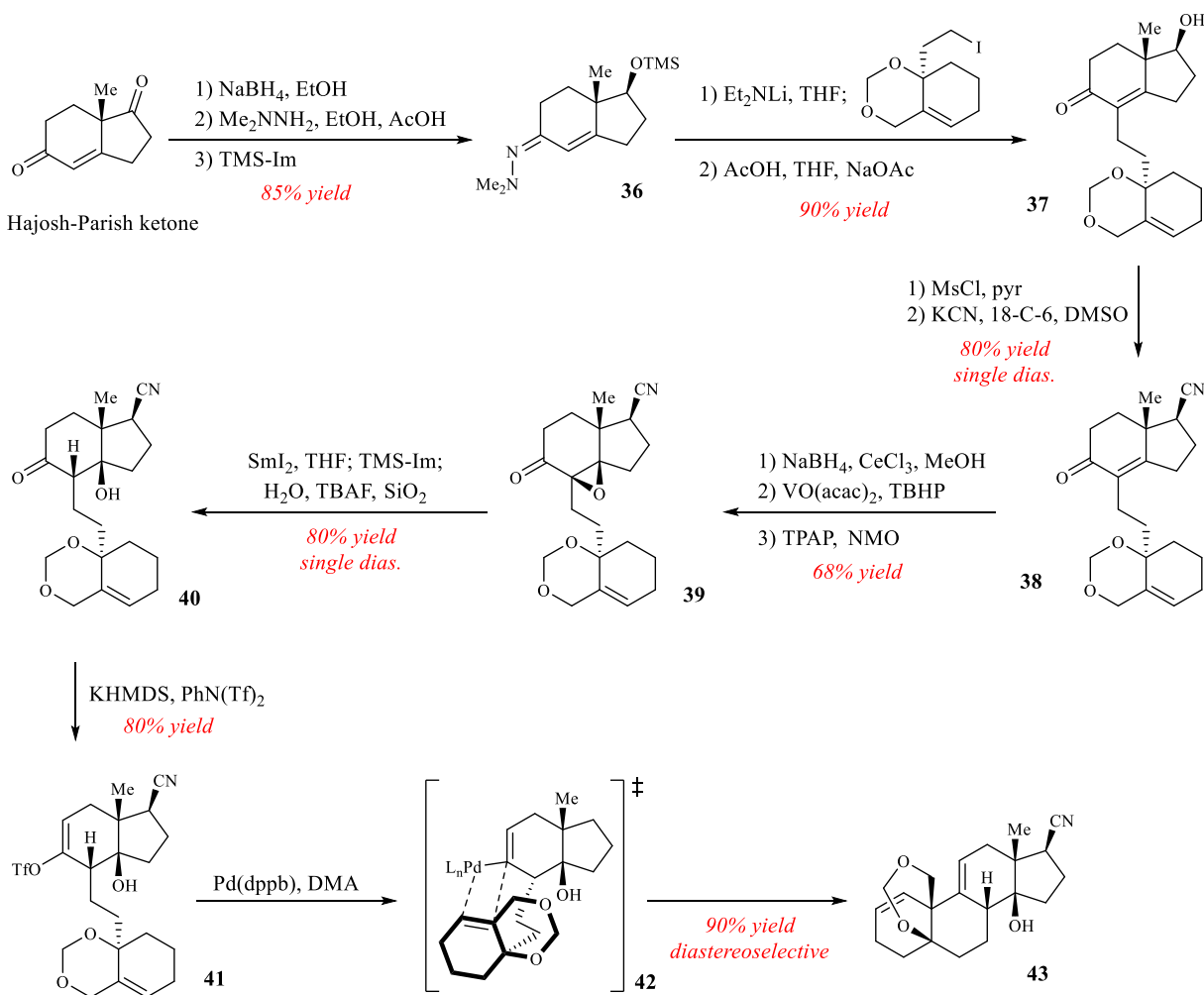
The final consideration was installation of the butenolide ring at C17. Epoxidation of the exocyclic olefin at C17 was achieved with mCPBA. Treatment of the epoxide with the Lewis acid BF_3 etherate promoted 1,2-hydride shift epoxide opening which furnished the undesired α -oriented aldehyde. This is an indication that the epoxidation proceeded selectively from the α -face and subsequent hydride shift occurred on the β -face. To invert the configuration at C17, Stork and co-workers first converted the C17 aldehyde to a nitrile by way of dehydrating an oxime and thus obtaining intermediate **33** in 60% yield over 3 steps from **32**. Exposure of the nitrile to $\text{LiN}(\text{Et})_2$ generated a dianion which would undergo selective protonation by di-tert-butyl-4-methylphenol from the α -face of the steroid thus producing a β -oriented nitrile ($\beta:\alpha > 4:1$) and completing the desired inversion at C17. It is noteworthy that forming the dianion by means of deprotonating both C17 and the C14 hydroxy was a key aspect of this inversion as the effective size of the β -face is increased by formation of the oxyanion at C14 and the possibility of intramolecular protonation of the C17 anion by the C14 hydroxy is eliminated (this process would have produced no inversion at C17). The same result could be achieved by TMS protecting the C14 hydroxy before the deprotonation-reprotonation sequence. The β -oriented nitrile was then treated with BnOCH_2Li to furnish the benzylated acyloin at C17, intermediate **34**. Hydrogenolysis of the benzylated hydroxy was followed by treatment with the Bestmann ylide to promote cyclization to the desired butenolide. The final step was acidic deprotection of the C3 silyl ether with tosic acid to produce digitoxigenin (**35**) in 59% yield over 3 steps from intermediate **34**.

2.6. Overman's Intramolecular Heck Reaction Approach to Steroidal Cores

In 1996, Overman and co-workers reported a unique approach to cardenolide cores.⁵ Their strategy utilized an intramolecular Heck reaction to cyclize the B ring and set the steroid scaffold. In 1998, the methodology was expanded upon to include some important sites of oxidation.⁶

Importantly, their method allows the pre-installation of oxygenation at C19 and C5 while the implementation of the Heck reaction promotes stereospecific formation of a desired *cis*- β A/B ring junction (**Scheme 2.4**). In addition, the C11 and C17 positions are functionalized and amenable to further derivatization toward natural cardenolides.

Scheme 2.4. Overman's Intramolecular Heck Approach to Cardenolide Cores.



The route starts with the Hajosh-Parish ketone. The C17 ketone was selectively reduced with NaBH_4 from the α -face. Next, Me_2NNH_2 was condensed with the C9 enone and the alcohol at C17 was protected by TMS to furnish intermediate **36** in 85% yield over 3 steps. An extended

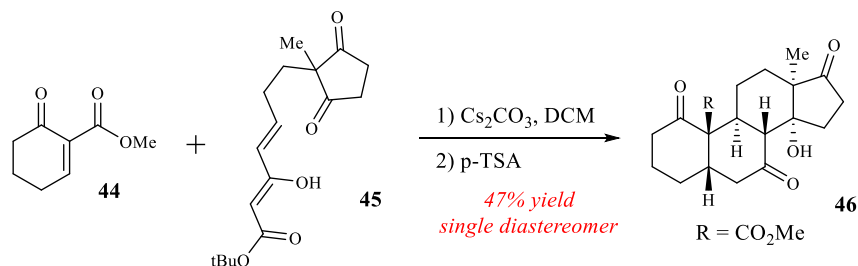
metalloenamine was generated and alkylated at the α -carbon with a primary iodide. The resulting C14-C15 olefin was isomerized into conjugation with concomitant hydrolysis of the C9 hydrazone by treatment with AcOH to form tetracycle **37**. The β -oriented C17 alcohol was then mesylated and displaced with cyanide to produce intermediate **38**. This transformation occurs with net retention of configuration. Next, Luche reduction of the enone at C9 produces the β -oriented alcohol. This alcohol serves as a directing group for subsequent epoxidation with VO(acac)₂/TBHP. Upon reoxidation of the C9 alcohol, epoxide **39** was obtained. Reductive opening of the epoxide was accomplished with SmI₂. The resulting enolate was trapped with TMS-imidazole and then selectively protonated at C8 from the β -face after treatment with TBAF/SiO₂. This sequence generated compound **40** in 80% yield and completed the installation of both the desired β -oriented alcohol at C14 and the β -oriented hydrogen at C8. Triflation of the C9 ketone produces intermediate **41** which is now ready to undergo the key transformation to set the steroidal core. Reaction with a palladium catalyst triggered a diastereoselective intramolecular Heck reaction, suggested to proceed through a transition state resembling **42**, which closes the A/B ring junction in a *cis*- β orientation to furnish steroid **43** in 90% yield. Importantly, **43** contains oxidation at C5, C14, and C19 as well as a functional handle at C17 which could be used for butenolide installation as demonstrated in Daniewski's synthesis (section 2.4.).

2.7. Deslongchamp's Synthesis of Ouabain

Ouabain (**2**) is one of the better known cardenolides due to its historic use and chemical complexity. It contains β -oriented hydroxy groups at C1, C3, C5, C14, C19, and a single α -oriented hydroxy at C11. Its high level of oxygenation makes the steroid difficult to synthesize and work with. As a result, it has been the target of several synthetic chemists. In 2008, the group of Pierre Deslongchamps reported the first total synthesis of ouabagenin and ouabain (**2**) (**Scheme 2.6.** and

Scheme 2.7. respectively).⁷ Their route includes a clever Michael addition cascade to set the A and B rings from functionalized fragments, a diastereoselective aldol reaction to close the *cis*- β -C/D ring junction, and the usual C17 acyloin/Bestmann ylide combination for installing the butenolide. It is important to note some of the results that Deslongchamps and co-workers recorded when investigating the base promoted anionic polycyclization cascade to set the steroidal core. Some preliminary work was done on a model system using fragments **44** and **45** in order to study the diastereoselectivity of the reaction (**Scheme 2.5.**).⁸ In these experiments, it was noted that the C/D ring junction of the product steroid **46** was formed in an undesired *cis*- α orientation. However, in Daniewski's synthesis of a cardenolide core (section 2.4.), a similar aldol reaction was used to close the C ring but the desired *cis*- β junction was obtained. The difference in diastereoselectivity between these two cases is attributed to the unsaturation present at C9-C11 in the case of Daniewski which is not present in the model system of Deslongchamps. To overcome this issue, Deslongchamps and co-workers desymmetrized the D ring prior to cyclization so that only one ketone is available for addition and aldol closure of the C ring will proceed with complete stereocontrol.

Scheme 2.5. Deslongchamp's Preliminary Studies on a Michael/Aldol Cascade.

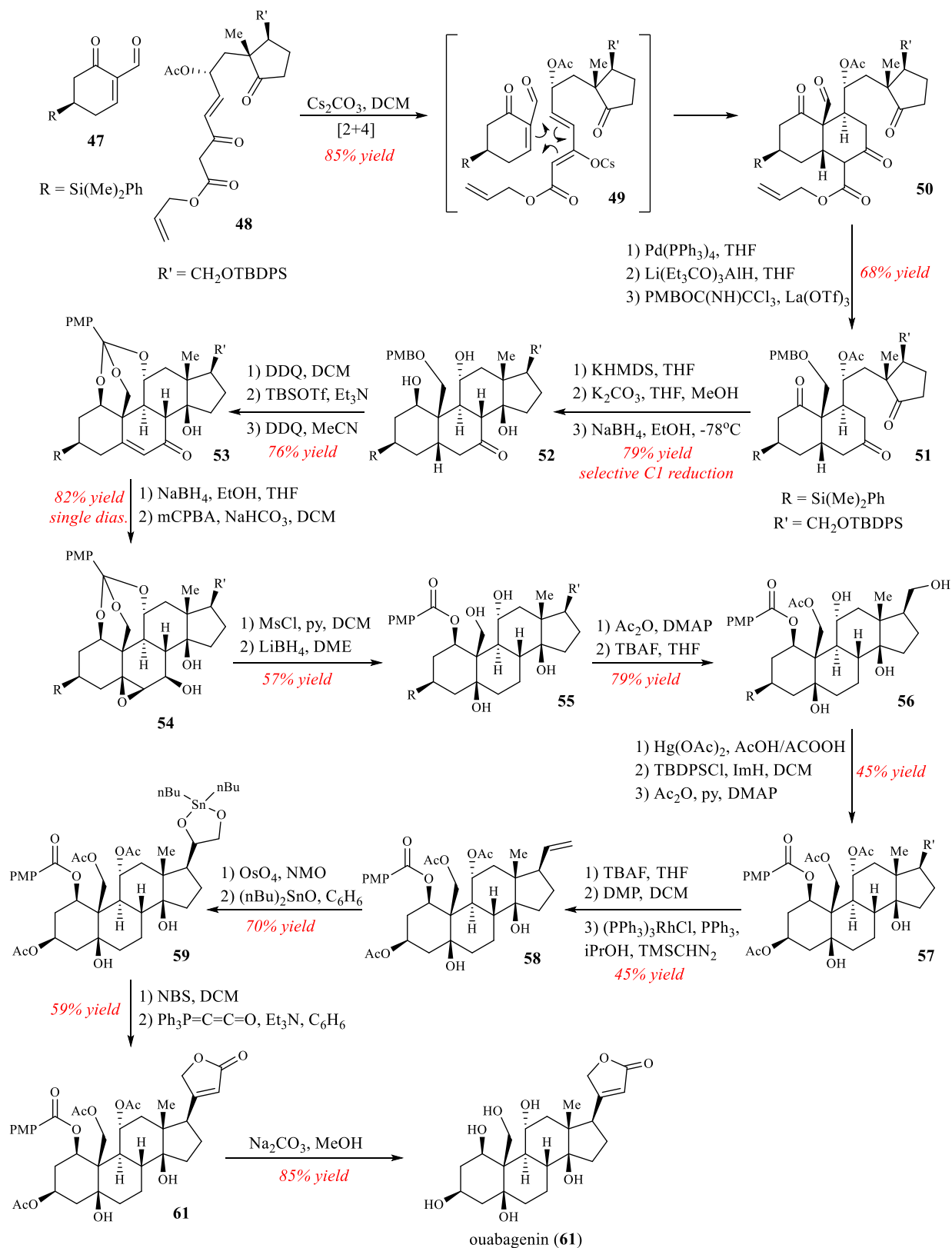


The synthesis begins with the heavily functionalized fragments **47** and **48** (**Scheme 2.6.**). Importantly, these fragments contain preinstalled functionality at key positions including C1, C3,

C7, C11, C17, and C19. A-ring fragment **47** was prepared in seven steps from cyclohexenone, and fragment **48** was generated in fourteen steps from the Hajosh-Perish ketone. Deprotonation of **48** with Cs₂CO₃ promoted its conversion to a diene which is now susceptible to undergo a Diels-Alder cycloaddition with dienophile **47**, depicted as transition state **49**. Notably, this [2+4] cycloaddition proceeds in 85% yield and produces a *cis*-β-A/B ring junction. The product β-ketoester **50** was then decarboxylated upon exposure to palladium tetrakis. Next, the aldehyde at C19 was reduced and protected as its PMB ether. Triketone **51** was then exposed to KHMDS to promote the addition of C8 to C14 and close the C/D ring junction in a *cis*-β orientation selectively. Hydrolysis of the C11 acetate with K₂CO₃ in MeOH was followed by reduction of the C1 ketone using NaBH₄ to obtain compound **52** in 79% yield over 3 steps. Importantly, the selective reduction of the C1 ketone to the desired β-oriented hydroxy was only possible after saponification of the C11 acetate and if performed at low temperature.

The next step was to oxidize the C5 position. To accomplish this, intermediate **52** was first treated with DDQ to form an orthoester and protect the alcohols at C1 and C11. Subsequent treatment with TBSOTf and Et₃N produced the C7 silyl enol ether which was oxidized to the enone **53** upon exposure to DDQ. The enone would then undergo selective 1,2-reduction from the α-face by treatment with NaBH₄ to generate the β-allylic alcohol at C7. It is important to note that selective production of the β-oriented hydroxy at this stage is crucial for the downstream installation of the desired β-oriented hydroxy at C5. Directed epoxidation of the C5-C6 olefin could now be carried out using mCPBA to produce intermediate **54** in 82% yield over 2 steps. Then, mesylation of the C7 alcohol was followed by treatment with LiBH₄ to nucleophilically displace the mesylate and open the epoxide.

Scheme 2.6. Deslongchamp's Total Synthesis of Ouabagenin.

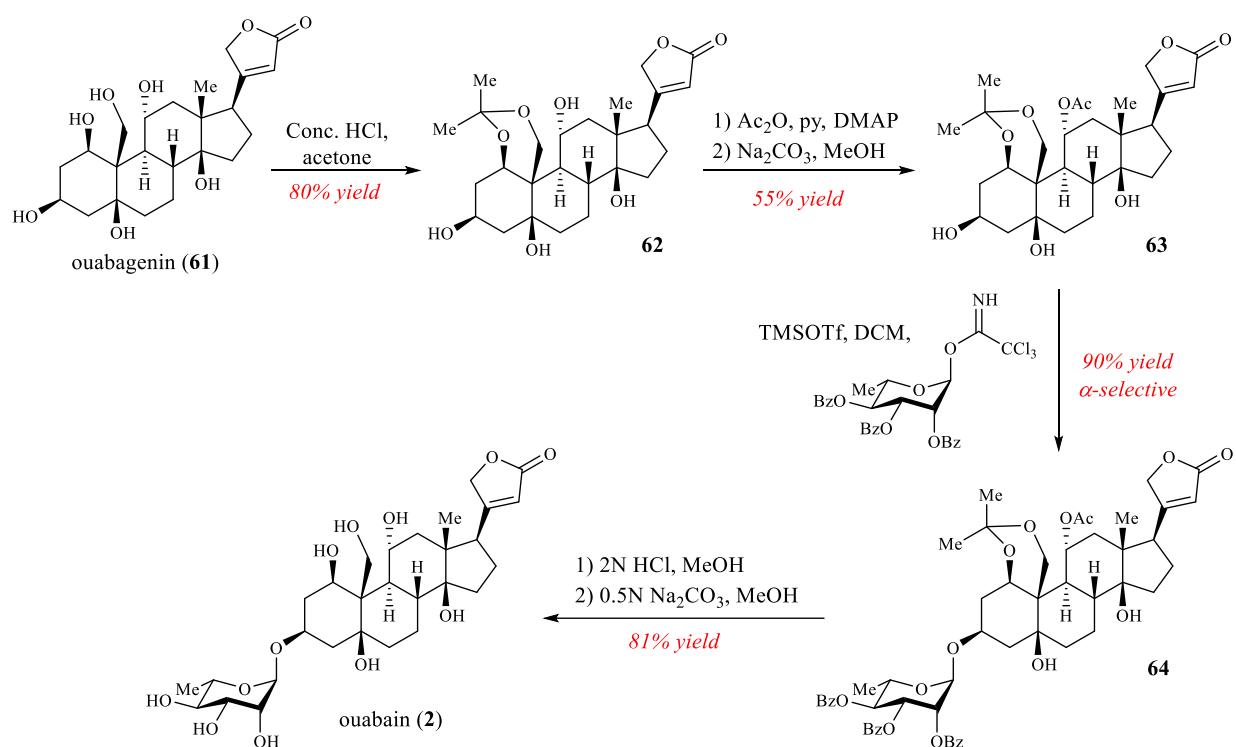


Subsequent exposure to mildly acidic silica gel promoted cleavage of the orthoester. This completed the installation of the C5 oxygenation (intermediate **55**). Selective monoacylation of the primary alcohol at C19 was followed by desilylation with TBAF. With tetraol **56** in hand, the researchers used a Tamao oxidation to install oxygenation at C3 with retention of configuration to obtain the desired β -oriented hydroxy. Silylation of the primary alcohol followed acylation of the C3/C11 alcohols furnished steroid **57** in 45% yield over 3 steps. Deslongchamps and co-workers then focused on installation of the C17 butenolide. First, the D ring alcohol was exposed upon desilylation with TBAF and oxidized to the aldehyde with DMP. Rhodium (I)-catalyzed methylenation with TMS diazomethane afforded olefin **58**. Dihydroxylation of the exocyclic olefin was accomplished with OsO₄/NMO. The resulting diol was then reacted with (nBu)₂SnO to produce the cyclic tin ether **59**. Treatment with NBS then promoted destannylation with selective oxidation of the secondary alcohol to generate a C17 acyloin. Exposure to the Bestmann ylide triggered the desired cyclization to the β -oriented C17 butenolide intermediate **60**. Final global saponification of the acylated alcohols at C1, C3, C11, and C19 proceeded in 85% yield to produce ouabagenin (**61**).

Deslongchamps and co-workers then focused on the glycosylation of ouabagenin (**61**) to complete the total synthesis of the natural cardiac glycoside ouabain (**2**) (**Scheme 2.7.**). The major challenge impeding this conversion is the presence of multiple hydroxy groups which make selective glycosylation at C3 difficult. To circumvent this, the primary alcohol at C19 was first protected as an acetal with the C1 hydroxy. Acylation of the remaining reactive alcohols at C3 and C11 was accomplished by exposing **62** to acetic anhydride. At this point, the C3 acetate could be selectively cleaved by careful saponification with Na₂CO₃ to furnish steroid **63** in 55% yield over 2 steps. With the C3 position having the only reactive alcohol exposed, glycosylation with a

rhamnose derived sugar donor was performed in the presence of TMSOTf to produce glycosylated steroid **64** in excellent yield. Importantly, anchimeric assistance from the neighboring benzoylated alcohols on the sugar donor allow for complete α -selective glycosylation. Finally, deacetylation of the C1/C19 alcohols was performed and subsequent sugar debenzoylation/C11 acetate cleavage with Na_2CO_3 afforded the desired final product ouabain (**2**).

Scheme 2.7. Deslongchamp's Glycosylation of Ouabagenin to Ouabain.



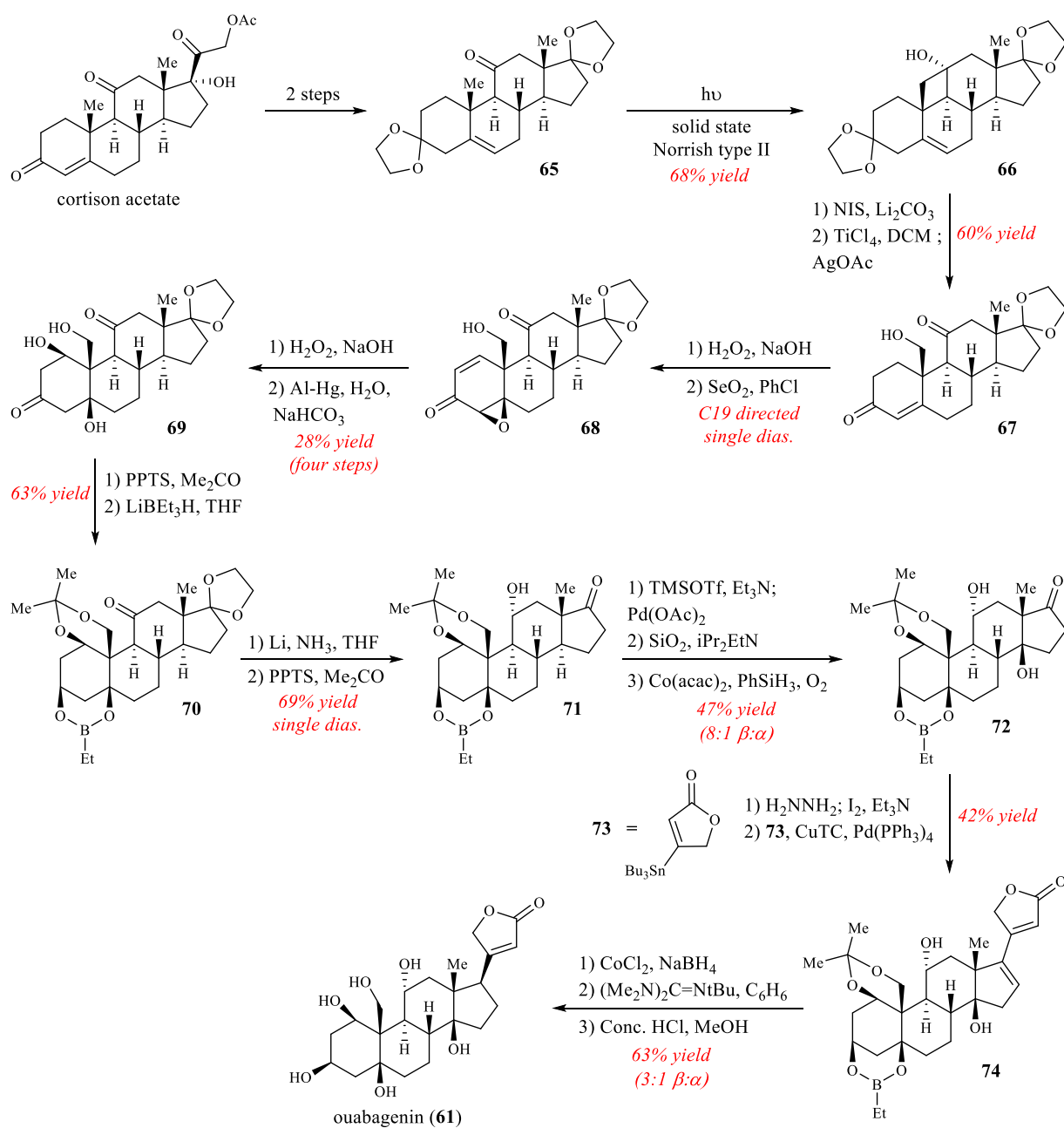
2.8. Baran's Semi-synthesis of Ouabagenin

In 2013, Phil Baran and co-workers reported a 21-step semi-synthesis of ouabagenin (**61**) starting from the readily available steroid cortisone acetate (**Scheme 2.8**).⁹ This preparation of ouabagenin is considerably shorter than any other synthesis reported to date and its key features

include selective C-H oxidation at C19 using a redox relay strategy, Suzuki coupling for installation of the butenolide moiety, and oxidation at C14 to install the β -oriented alcohol.

Starting from cortisone acetate, intermediate **65** was prepared in two steps including an oxidative cleavage and acetalization of the C3 and C17 ketones. Using a redox-relay strategy, the oxidative information at C11 is “donated” to the C19 position through a creative two step sequence. First, a solid-state Norrish type II photochemical reaction is utilized to form a C19-C11 bond to furnish cyclobutane **66** in 68% yield. Next, treatment with *N*-iodosuccinimide triggers iodination at C19 with concomitant cleavage of the C19-C11 bond and restoration of the C11 ketone. Baran and co-workers speculate the presence of a transient hypoiodite species which promotes chemoselective homolysis of the C19-C11 bond followed by radical recombination with Iodine. Subsequent exposure to TiCl₄ and AgOAc promotes hydrolysis of the C19 primary iodide with concomitant deprotection of the C3 ketone to produce diketone **67**. The C19 hydroxy was then used to direct epoxidation to the β -face of the A ring enone with H₂O₂ as the oxidant. Introduction of unsaturation at the C1-C2 bond was accomplished by SeO₂ oxidation to generate enone **68**. Another directed epoxidation with H₂O₂ gave the desired bis-epoxide and completed the necessary oxidation of the C1 and C3 positions. Extensive investigation was then required to find the optimal conditions for opening both epoxides. Eventually, Baran and co-workers determined that reduction with aluminum amalgam in water gave the best results. Thus, the bis-epoxide could be reductively opened to triol **69** in 28% yield over 4 steps. Next, The C1/C19 alcohols were protected as an acetonide and the C3 ketone was selectively reduced to the β -oriented hydroxy using super hydride. During the C3 reduction, the alcohols at C3 and C5 were tethered as their ethyl boronic ester, producing steroid **70**.

Scheme 2.8. Baran's Semi-synthesis of Ouabagenin from Cortisone Acetate.



Next, thermodynamic Birch reduction of the C11 ketone of intermediate **70** furnished the desired α -oriented hydroxy exclusively. This reduction is important to note because it is a commonly used technique for stereoselective reduction of ketones at C11 to produce the thermodynamic α -oriented alcohol. Deacetalization of the C17 ketone with PPTS afforded

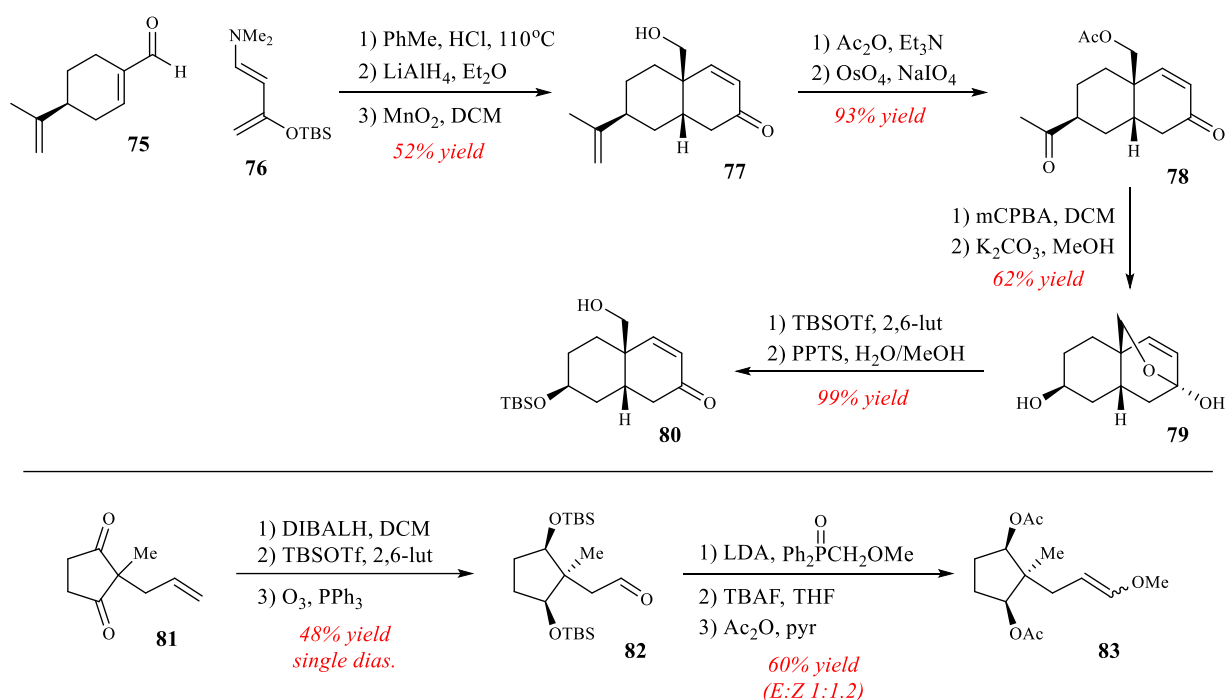
intermediate **71** in 69% yield over 2 steps. The next goal was stereoselective installation of the C14 oxygenation. First, Saegusa-Ito oxidation of the D ring ketone generated an enone. The olefin could then be deconjugated to the C14-C15 position upon treatment with SiO₂ and perfluorinated toluene. Importantly, the perfluorinated solvent proved to be an essential component for the success of this deconjugation. The application of Mukaiyama hydration conditions using cobalt and oxygen successfully oxygenated the olefin at the C14 position in an 8:1 diastereomeric ratio favoring the desired β -oriented hydroxy steroid **72**. The final task was to append the butenolide ring at C17. To accomplish this, the D ring ketone was first converted to a vinyl iodide using the Barton procedure. Next, Stille coupling using known tributylstannyl butenolide **73** was employed for installing the heterocycle and producing intermediate **74** in 42% yield over two steps. Attempts to directly hydrogenate the C16-C17 olefin at this stage occur predominantly from the β -face to yield the undesired α -oriented butenolide at C17. To circumvent this, the olefins are reductively isomerized using CoCl₂ and NaBH₄ to introduce unsaturation between the C17 position and the butenolide ring. Next, Barton's base was used to isomerize the olefin back into conjugation with a 3:1 preference for the desired β -oriented butenolide ring. The final transformation was acid promoted deprotection of the A ring alcohols to furnish ouabagenin (**61**).

2.9. Inoue's Synthesis of 19-Hydroxysarmentogenin

In 2013, Inoue and co-workers reported the first total synthesis of the cardenolide 19-hydroxysarmentogenin (**1**) proceeding in 34 steps.¹⁰ The key steps include a bromine promoted fragment coupling and a radical cyclization to close the B ring. The first task was to synthesize the necessary fragments **80** and **83** in preparation for their coupling (**Scheme 2.9**). The A/B ring fragment was prepared from (*S*)-perillaldehyde (**75**). Diels-Alder reaction with Rawal's diene **76** proceeded with the expected regio- and facial selectivity to provide the *cis*- β -A/B ring junction.

After hydrolysis of the β -amino silyl enol ether, the bicyclic enone was obtained. Reduction with LiAlH_4 produced a diol and the allylic alcohol at C7 was selectively reoxidized with MnO_2 to obtain enone **77**. Next, the C19 alcohol was acylated and the exocyclic olefin at C3 was dihydroxylated and then oxidatively cleaved to furnish intermediate **78** in 48% yield over 5 steps. Baeyer-Villiger oxidation of the C3 acyl group was achieved upon treatment with mCPBA and global saponification yielded acetal **79**. The diol was then bis-TBS protected. Treatment with PPTS promoted the regioselective desilylation of the C7 acetal with concomitant reversion to the ketone in excellent yield, thus affording key fragment **80** in nine total steps.

Scheme 2.9. Inoue's Synthesis of Key Fragments Toward 19-Hydroxysarmentogenin.



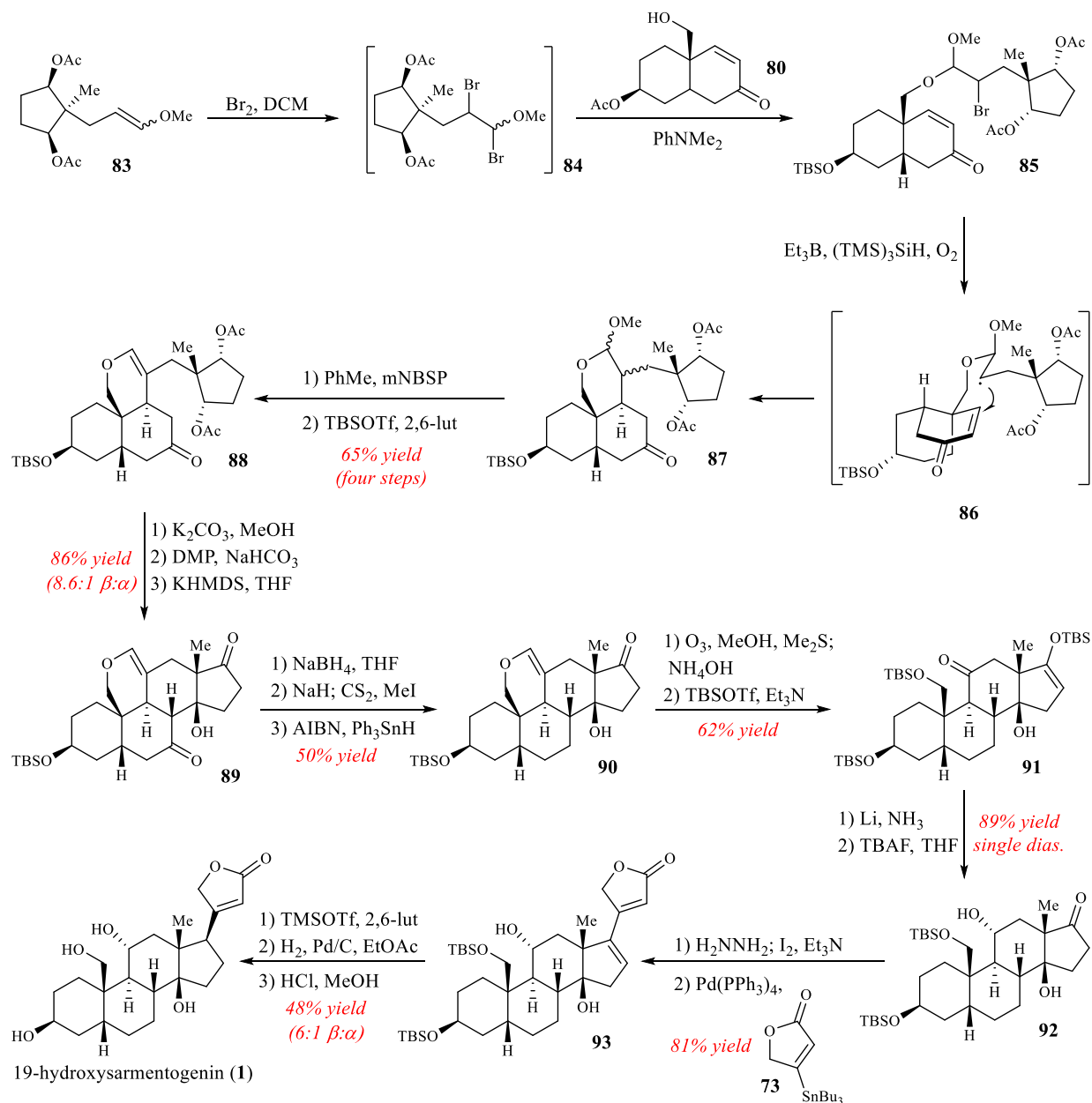
The D ring fragment was prepared from diketone **81**. Exposure to DIBALH caused both ketones to be reduced selectively from the face opposite the terminal olefin. The resulting diol was bis-TBS protected and the olefin was ozonolyzed to furnish aldehyde **82**. Horner-Wittig olefination

was utilized to convert the aldehyde to a methyl enol ether. Finally, the alcohols were deprotected with TBAF and subsequently acylated to complete the synthesis of D ring fragment **83** in six total steps and 29% yield over 6 steps.

With the two key fragments in hand, Inoue and co-workers focused on completing the target 19-hydroxysarmentogenin (**Scheme 2.10**). The first goal was coupling of the fragments and cyclizing the steroidal core. To achieve this, the D ring fragment was reacted with molecular bromine to generate the dibrominated intermediate **84**. The A/B ring fragment **80** was then introduced and the C19 alcohol underwent displacement of the activated bromide to furnish acetal **85** and successfully couple the two fragments.

Next, Inoue and co-workers planned a radical cyclization to form the C9-C11 bond, closing the C ring. By treating tricycle **85** with Et₃B, O₂, and (TMS)₃SiH, the remaining bromide is abstracted and the alkyl radical adds to the B ring enone thus forming the desired connection in a stereoselective manner, favoring the α -oriented hydrogen at C9 and furnishing tetracycle **87**. A proposed stereochemical model for this radical addition is depicted as intermediate **86**. Next, the acetal was eliminated under acidic conditions using *meta*-nitrobenzenesulfonic acid pyridinium salt (mNBSP) to generate a cyclic enol ether and merge the mixture of diastereomers into a single compound. During this elimination, some material underwent desilylation at C3, so the compound was fully resilylated using TBSOTf to give intermediate **88** in 65% yield over 4 steps from **80**. Next, the D ring acetates were cleaved and oxidized to ketones. This sets the stage for an important aldol reaction to close the C ring and set several stereocenters. Treatment with KHMDS generates the B ring enolate which then adds to the C14 ketone of the D ring to furnish steroid **89**. It is important to note that this aldol reaction occurs stereoselectively to yield a *trans*-B/C ring junction and *cis*- β -C/D ring junction which is necessary for synthesizing natural cardenolides.

Scheme 2.10. Inoue's Completion of 19-Hydroxysarmentogenin.



Next, the C7 position is deoxygenated using the Barton-McCombie radical protocol over a three-step sequence to yield compound **90** in 50% yield over 3 steps. The cyclic enol ether is then ozonolyzed, producing a C11 ketone and C19 formylated alcohol. Addition of ammonium hydroxide then promoted saponification at the C19 alcohol. The D ring ketone is then protected as

its silyl enol ether using TBSOTf, this produces steroid **91**. Protecting the D ring ketone was a necessary step for the ensuing Birch reduction which provides exclusively the thermodynamically preferred α -oriented alcohol at C11. The D ring silyl enol ether is quickly and selectively deprotected by exposure to TBAF.

Intermediate **92** is then subjected to the Barton conditions for vinyl iodide formation at C17 followed by Stille cross coupling with stannylated butenolide **73** to generate the coupled product **93** in 81% yield over 2 steps. The final goal was to install the necessary chirality at C17. Inoue and co-workers noted that simple hydrogenation of **93** produces primarily the undesired α -oriented butenolide ring. To overcome this, the C14 alcohol is protected as its TMS ether; this provides steric occlusion of the β -face. Now, hydrogenation proceed to deliver hydrogen predominantly from the α -face, affording an 8:1 mixture of β : α – oriented butenolides. Lastly, the molecule is subject to HCl/MeOH to promote global desilylation and furnish the target 19-hydroxysarmentogenin (**1**) in 48% yield over 3 steps. After completing this synthesis, Inoue and co-workers went on to apply the same methodology toward the successful total synthesis of ouabagenin (**61**) in 2015.¹¹

2.10. Conclusion

There have been many landmark efforts toward synthesizing cardiotonic steroids. The primary challenges include setting the steroidal core, introducing oxygenation, controlling stereochemistry, and installing the C17 butenolide ring. Different approaches described in this chapter accomplish these tasks in various ways, sometimes using similar techniques. However, semi-synthetic techniques remain hindered by the narrow landscape of attainable chemical analogues while total syntheses are often unfavorably long. There continues to be a demand for improving the synthetic approach we use to access the cardenolide class of steroids.

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Chapter 3

Concise Enantioselective Synthesis of Oxygenated Steroids via Cu(II)-BOX-Catalyzed Michael Addition and Intramolecular Aldol Cyclization Reactions¹

(A portion of this work has been published in: Cichowicz, N. R.; Kaplan, W.; Khomutnyk, Y.; Bhattarai, B.; Sun, Z.; Nagorny, P. “Concise Enantioselective Synthesis of Oxygenated Steroids via Sequential Copper(II)-Catalyzed Michael Addition/Intramolecular Aldol Cyclization Reactions” *J. Am. Chem. Soc.* **2015**, *137*, 14341.)

3.1. Rationale for New Synthetic Approach

As discussed in chapter 2, various research groups have successfully developed unique approaches for the total synthesis of cardenolides and other steroids.² One of the main hurdles associated with these approaches is modularity; and the design of more drug-oriented approaches remains a formidable challenge. When a synthetic route is conceived specifically to access a single complex target, it is often not amenable to diversification and the accessible chemical space surrounding the target is limited. For the purpose of exploring new therapeutic agents, it is highly desirable that a methodological approach features access to an entire class of natural products. In this regard, modularity and plasticity should be ingrained in the synthetic foundation of the approach. Designing such a method requires a convergent technique. Ideally, unique fragments containing pre-installed functional handles and diversity elements can be coupled into a steroidal core in an assembly-line like practice. Importantly, the process of coupling the fragments needs to be robust enough to accommodate a wide range of structurally diverse fragments. By inputting the

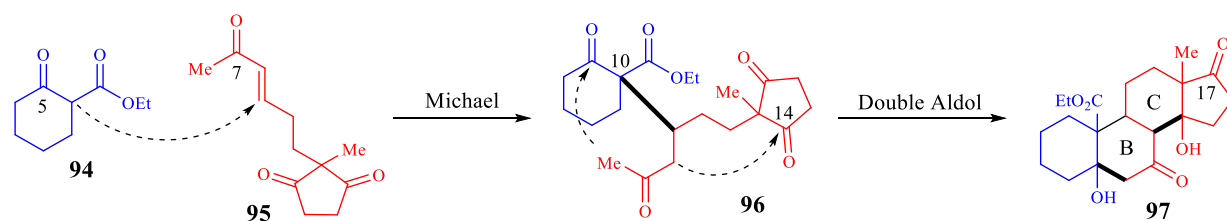
oxidative complexity and functional handles on the chemically simpler fragments, one can circumvent the need for difficult, sometimes prohibitively so, late-stage functionalizations and redox manipulations on more complicated intermediates; especially when those late-stage intermediates are scarce.

In addition to controlling the peripheral sites of substitution and levels of oxidation, stereochemical modulation is a key consideration. Steroidal systems are rigid by nature as a result of their fused tetracyclic array and their internal stereocenters have a profound impact on the overall 3D shape of the molecule. As such, changing stereochemical patterns of the ring system or peripheral moieties can significantly alter the binding contacts within the Na⁺/K⁺ATPase pocket.³ A useful approach to synthesizing cardenolides and their analogues must incorporate access to chemical space which encompasses a diverse pattern of substitution, oxidation, and stereochemistry.

3.2. Methodological Design Overview

The method designed and delineated in this chapter originated from a key conceptual disconnection of the steroidal framework. A two-step approach to the steroidal core was envisioned as illustrated in **Scheme 3.1**. A nucleophilic β -ketoester (**94**, the A ring) is added conjugatively to an electrophilic enone (**95**) to produce a Michael adduct (**96**), which can subsequently undergo two intramolecular aldol reactions to close the B and C rings and set the steroidal frame (**97**). The key features of this approach are the capacity to pre-install functionality on the starting fragments, specifically at C3, C5, C7, C11, C14, C17, and C19, controlling internal stereocenters with asymmetric catalysis, and setting the steroidal core in only two steps.

Scheme 3.1. General Overview of Two-step Approach to Steroidal Core.



3.3. Michael Donor Fragment Design

The Michael donor fragment houses the eventual A ring as well as carbons 1-5, 10, and 19. The β -ketoester moiety, which grants the fragment its nucleophilicity at C10, affords pre-installed oxygenation at the key C5 and C19 positions. In order to make steroidal cores viably accessible through this methodology, the Michael donor fragments must be readily accessible, either through commercial vendors or a small number of synthetic steps. One of the key advantages of the approach outlined in section 3.2. is the ability to access steroids with expanded or contracted A/D ring sizes, a type of architecture which may be difficult to access semi-synthetically. As such, efforts were focused on the Michael reaction using two donor fragments **94** and **98**, **Figure 3.1**. Fragment **94** is a six-membered A ring Michael donor which would provide the natural series of steroids, while fragment **98** is a five-membered Michael donor which would generate steroid-like scaffolds with contracted A rings, allowing access to a unique class of steroidal chemical space. Both are inexpensive and commercially available.

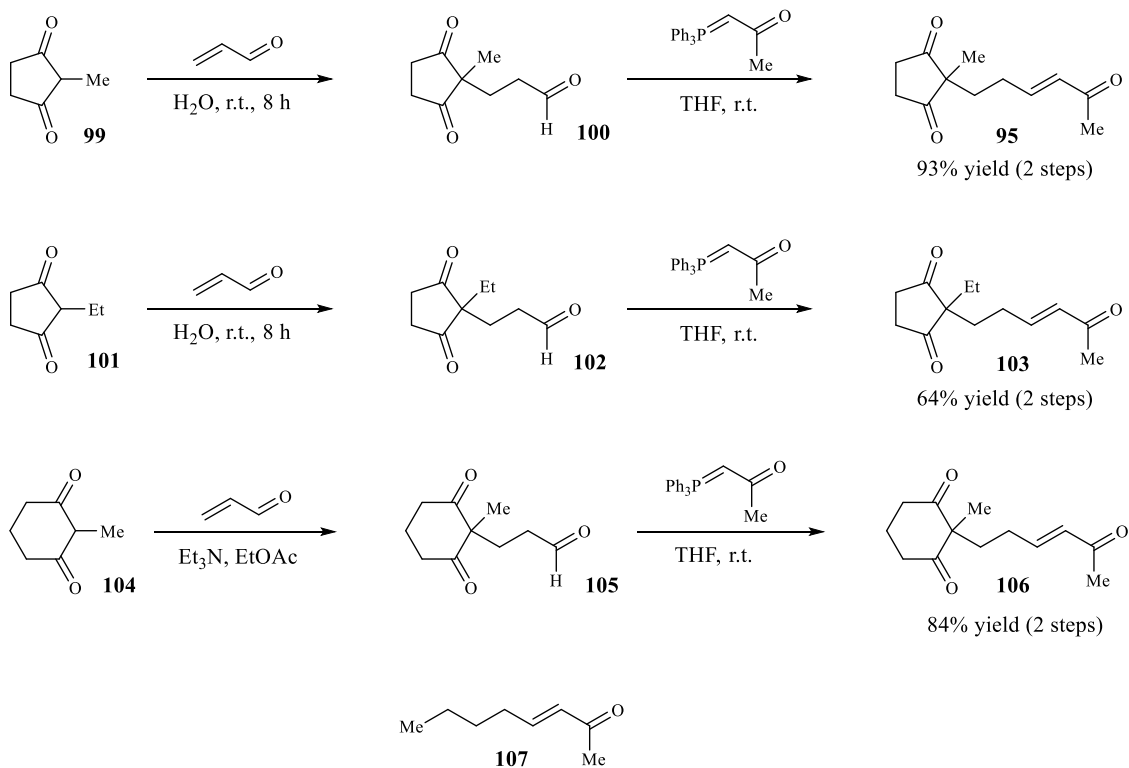
Figure 3.1. Michael Donors **94** and **98**.



3.4. Michael Acceptor Fragment Design

The Michael acceptor fragment contains the eventual D ring and the carbon chain which forms the eventual B and C ring architecture. Oxygenation is preset at key positions including C7, C14, and C17. As with the Michael donors, the acceptor fragments must be amenable to modification and readily accessible. In order to explore the tolerance of the desired approach to variations in the size of the D ring and substitution at C13, various enones were designed and synthesized to serve as coupling partners. These Michael acceptors are shown in **Scheme 3.2**.

Scheme 3.2. Synthesis and Structure of Michael Acceptor Fragments.



Importantly, each acceptor could be synthesized in multi-gram quantities in just two steps. Michael acceptors **95** and **103** represent precursors to the natural steroid series with a 5-membered D ring; the only difference being the presence of a -Me group as opposed to an -Et group at C13,

respectively. Importantly, this simple distinction exemplifies the types of difficult modifications attainable through a modular totally synthetic approach. It would be very difficult to chemically transform a C13 methyl group into an ethyl group through semi-synthetic techniques; whereas this modality is readily achievable through the approach described in this chapter. Fragment **106** would result in a steroid-like derivative containing a 6-membered D ring. Finally, a linear enone (**107**), containing no D ring was also used in these studies. Fragments **103** and **106** were prepared by Dr. Nathan Cichowicz.

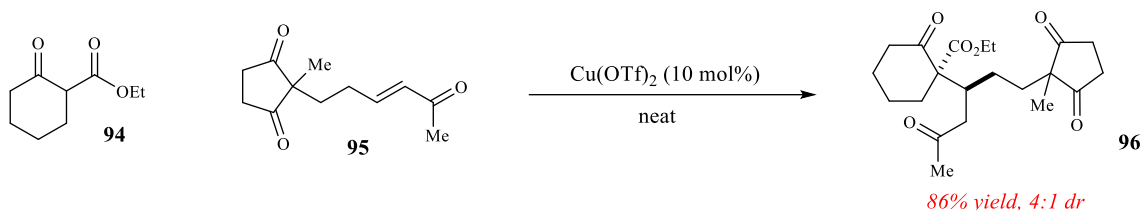
The synthesis of enones **95** and **103** are similar and proceed from the commercially available diketones **99** and **101**, respectively. Stirring the diketones with acrolein in water facilitates a Michael reaction which produces the corresponding aldehydes **100** and **102**. These aldehydes can undergo Wittig olefination with 1-(triphenylphosphoranylidene)-2-propanone in THF to furnish the desired fragments. The route to fragment **106** is different from the previous fragments because the starting diketone has a smaller population of the enol tautomer in solution than the 5-membered diketones. As such, the process was modified by Dr. Cichowicz by adding base and using an aprotic solvent. This proved effective for promoting the conjugate addition to acrolein and generating aldehyde **105**. The same Wittig olefination procedure can now be used to convert the aldehyde to the target enone **106**. Finally, the linear Michael acceptor **107** is commercially available.

3.5. Investigation of Racemic Michael Reactions

The preliminary investigative work described in this section was performed by Dr. Nathan Cichowicz. The construction of vicinal quaternary and tertiary stereocenters via conjugate addition is challenging and not well preceded due to the steric resistance to forming such a moiety.^{4,5} As such, the first step in developing this methodology was to investigate the racemic Michael

additions of the aforementioned fragments in order to determine the optimal conditions for promoting such a difficult transformation. After screening several acids and bases for their ability to promote the Michael addition of **94** to enone **95**, Dr. Cichowicz determined that $\text{Cu}(\text{OTf})_2$ was the most reliable catalyst for promoting conversion to Michael adduct **96** and controlling diastereoselectivity (**Scheme 3.3**).

Scheme 3.3. Copper(II)-Triflate Catalyzed Michael Addition of Fragments **94** and **95**.

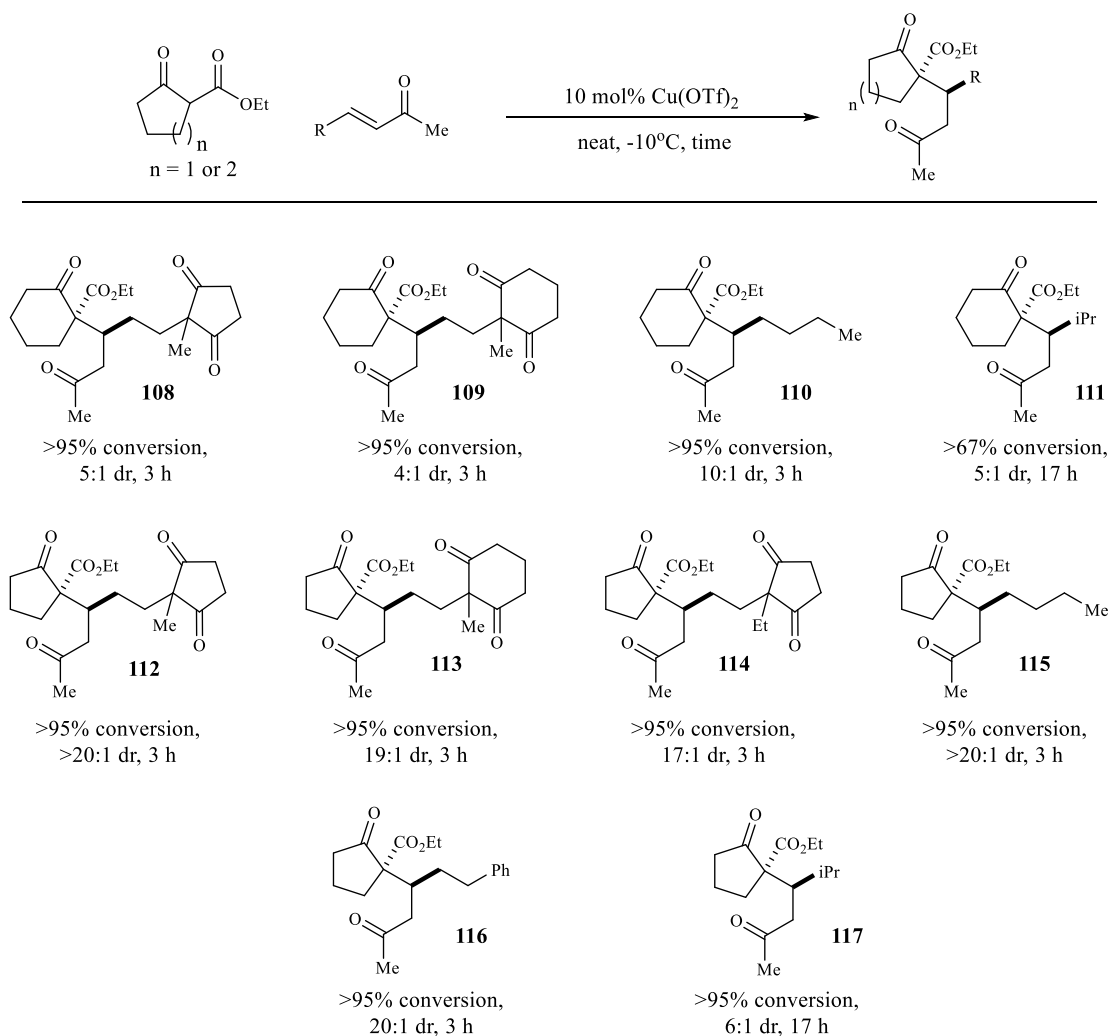


The reaction proceeded with 86% yield and 4:1 diastereomeric ratio (dr) favoring the desired natural isomer. With the optimal catalyst in hand, the substrate scope was investigated. To this effect, each Michael donor and acceptor were coupled using the $\text{Cu}(\text{OTf})_2$ conditions elucidated from the catalyst screen. The results are shown in **Scheme 3.4**. Fortunately, each donor **94** and **98** could be successfully coupled to enone fragments **95**, **103**, **106**, and **107**, to produce Michael adducts **108-117** in good conversion and diastereomeric ratio.

Several trends became apparent. Reactions with the 5-membered A ring fragment **98** generally proceeded faster and with greater diastereoselectivity than its 6-membered counterpart **94**. Specifically, adducts generated from ketoester **98** had diastereomeric ratios (dr) generally better than 17:1, whereas adducts produced from **94** showed dr around 5:1. It is proposed that enolization of the 5-membered ketoester is more facile compared to the 6-membered ketoester due to an enhanced favorability of unsaturation in 5-member rings resulting from decreased torsional strains; thus, a larger population of the nucleophilic tautomer might be present at a given time,

causing faster reaction. Additionally, slower kinetics are observed upon increasing steric hindrance of the enone, and adducts **111** and **117** require extended reaction times. Overall, a good catalytic system was discovered for promoting the diastereoselective Michael addition of various β -ketoesters to 2-substituted enones in order to produce interesting Michael adducts with unique steroidal features including expanded and contracted ring sizes and peripheral substitutions. The next goal of further developing this approach was incorporating an asymmetric component for production of enantioenriched steroids and steroid-like precursors.

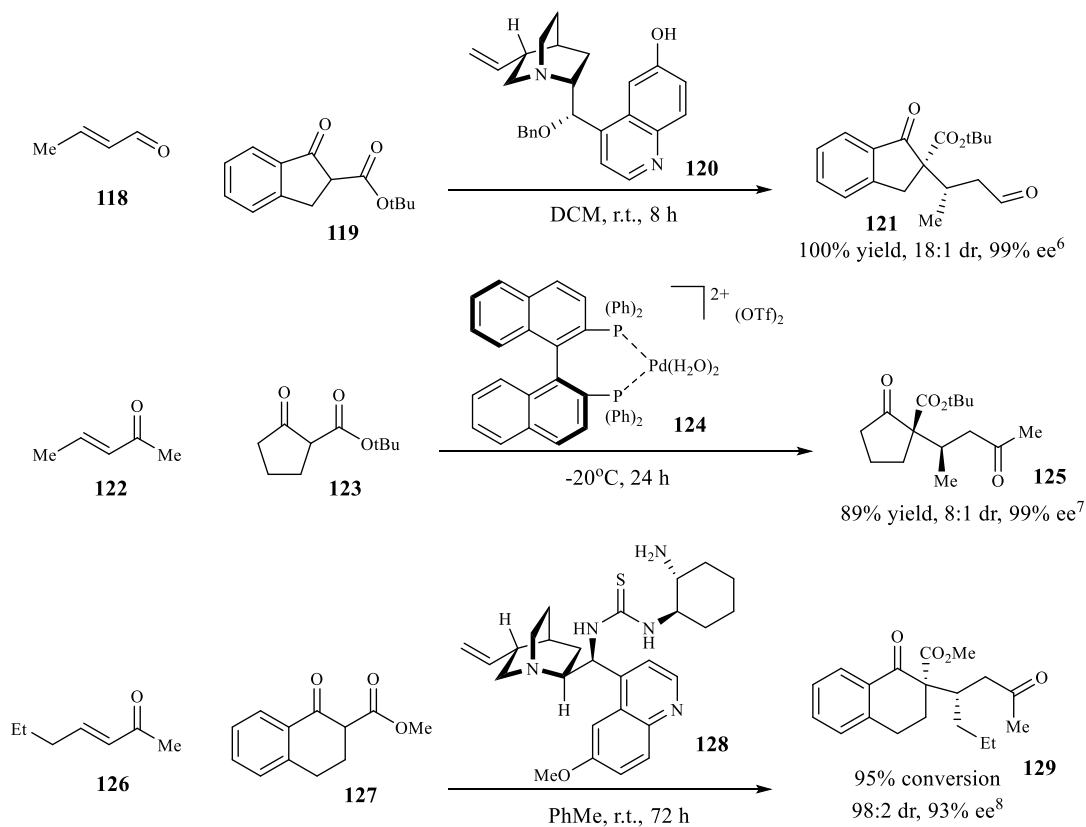
Scheme 3.4. Substrate Scope for $\text{Cu}(\text{OTf})_2$ -Catalyzed Michael Reactions.



3.6. Catalyst Screen for Enantioselective Michael Additions

The process of developing a novel method for producing cardenolide cores requires stereocontrol. Due to the chiral nature of biological targets and variable bioactivity of enantiomeric compounds, it is strongly desirable that synthetic approaches toward natural products allow access to highly enantioenriched compounds. To incorporate this aspect, an enantioselective variant of the Cu(II)-catalyzed Michael additions was investigated. The first attempts utilized literature reported chiral catalysts for promoting the asymmetric Michael reactions. There exist only few reported conditions for asymmetric synthesis of vicinal tertiary and quaternary stereocenters via conjugate addition. Three of the studies most applicable to ours are illustrated in **Scheme 3.5**.⁶⁻⁸

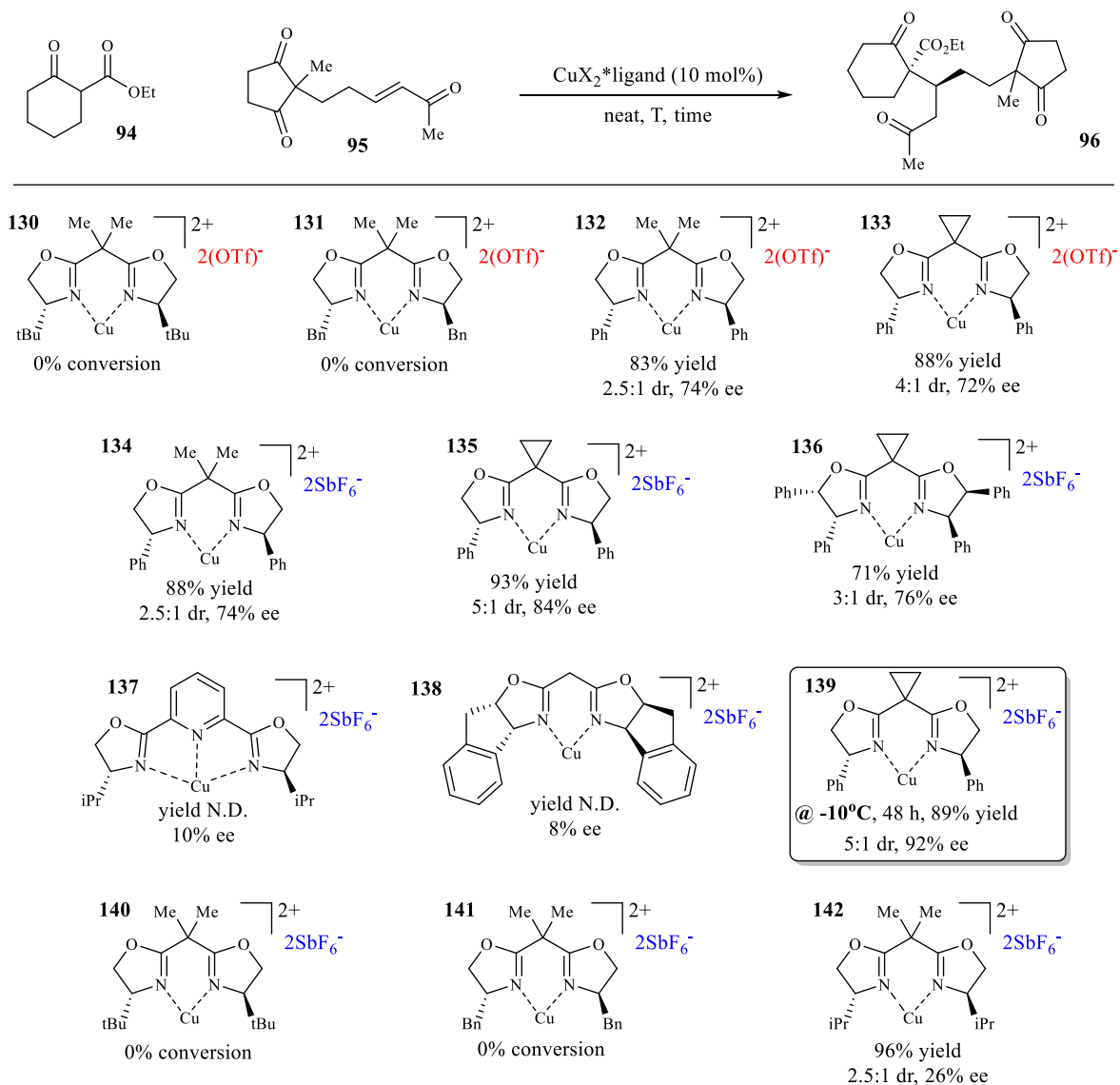
Scheme 3.5. Known Asymmetric Michael Additions of Hindered Substrates.



However, attempts to use the reported chiral catalysts **120**, **124**, or **128** for promoting the asymmetric Michael addition of **94** and **95** failed, and other methods were sought. The first task toward developing an asymmetric protocol for the Michael reaction was a catalyst screen. With copper(II) serving as the Lewis acid, the bisoxazoline class of ligands were investigated. There is considerable literature review on the use and effectiveness of these ligands, particularly when complexed with copper(II) salts, for the purpose of catalyzing Mukaiyama Michael additions.⁹⁻¹² However, some previous attempts to use Cu(II)-BOX catalytic systems toward the Michael addition of 1,3-dicarbonyls and enones were unable to produce enantioselectivity.^{13,14} In order to determine the best method and ligand for promoting the enantioselective Michael additions of our substrates, various Cu(II)-BOX/PyBOX catalysts were synthesized and screened alongside Dr. Nathan Cichowicz and Dr. Zhankui Sun.

Early on in the catalyst screening stage we discovered the chiral enrichment of our Michael adducts via column chromatography with an achiral stationary phase. This phenomenon occurs as a result of diastereomeric interactions between the enantiomers of the substrate. If a sample contains some enantiomeric excess and is prone to forming diastereomeric dimeric complexes, it is possible for the minor enantiomer to be dragged (via complexation) either ahead of or behind the pure portion of the major enantiomer during chromatography. The result of this effect is a gradual change in enantiomeric excess of the material eluting from the column. Over the course of our studies, we discovered that our chiral Michael adducts were prone to this self-promoted enantioenrichment. As a result, we had to adjust our method of determining enantiomeric excess to ensure accurate measurement. This was accomplished by assaying the diastereomeric mixtures of Michael adducts. At this stage, we chose to reevaluate the catalyst screen. The results of the revised screen are summarized in **Scheme 3.6**.

Scheme 3.6. Copper(II)-Based Catalyst Screen for Asymmetric Michael Reactions.

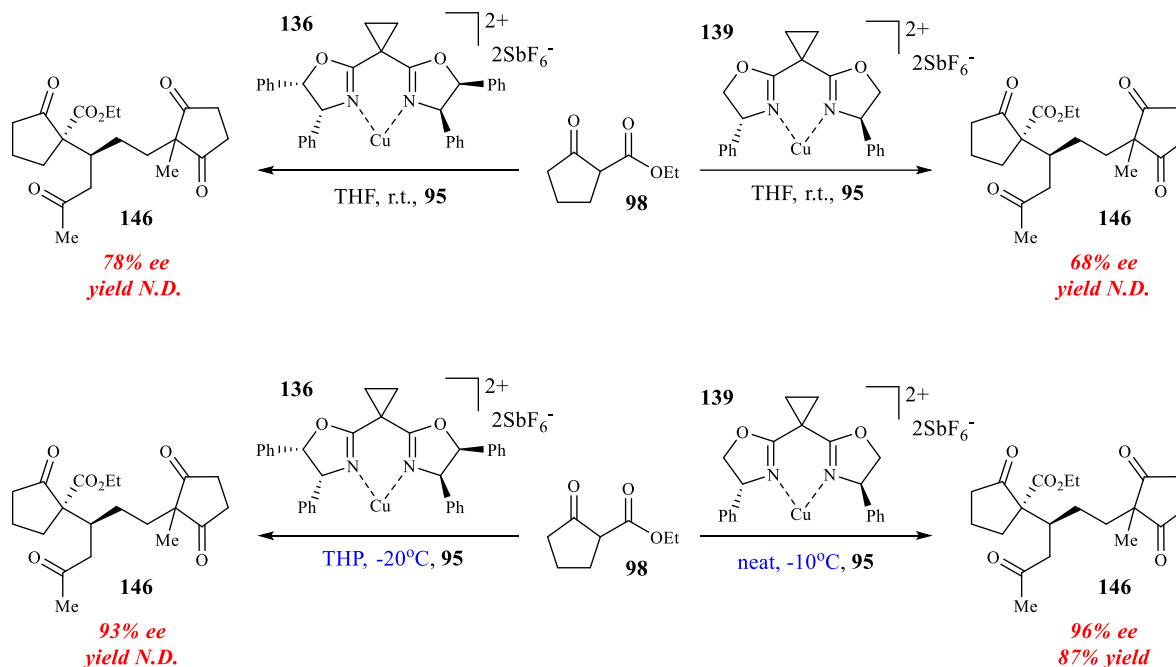


After extensive screening, the first important discovery was the improved reactivity of $\text{Cu}(\text{SbF}_6)_2$ over the original $\text{Cu}(\text{OTf})_2$ system in the presence of BOX ligands. It is suggested that the non-coordinating hexafluorantimonate anion effectively boosts the reactivity of the copper center and allows lower reaction temperatures to be employed without a large setback to reaction times. This capacity to lower the reaction temperatures resulted in a significant improvement in

the enantio- and diastereoselectivity. Ligands with *tert*-butyl or benzyl dimethyl-bisoxazoline substituents (catalysts **130**, **131**, **140**, and **141**) did not promote the desired reaction. Moreover, PyBOX ligands and scandium proved disappointingly ineffective. It is also worth noting that the use of a cyclopropyl bridge (**135**) as opposed to a gem-dimethyl bridge (**134**) on the ligand often afforded improved yields and diastereoselectivity. Eventually, it was determined that the best combination of yield and selectivity could be obtained with catalyst **139** under neat conditions at -10°C for 48 hours.

When comparing the results from the catalyst screen (performed with Michael donor **94**) to earlier studies with Michael donor **98**, we observed some interesting results. We found that while donor **94** would provide the best results with diphenyl catalyst **139** at -10°C under neat conditions, the 5-membered β -ketoester **98** had originally been optimized with tetraphenyl catalyst **136** at -20°C in THP as a solvent, providing 93% ee upon Michael reaction with substrate **95**. This resulted from the observation that donor **98** provided improved enantioselectivities with catalyst **136** as compared to catalyst **139** when run at room temperature and in THF (**Scheme 3.7**). We considered the possibility of describing two unique substrate specific protocols, one for each donor **94** and **98** using catalysts **139** and **136**, respectively. However, in light of our discovery that donor **94** was exhibiting improved selectivities under neat conditions, we fortunately revisited the optimization of donor **98** and found that at -10°C, and under neat conditions, the use of catalyst **139** in conjunction with acceptor **95** provided highly satisfactory results of 96% ee, >20:1 dr, and 87% yield. This aspect of improved selectivities under neat conditions is noteworthy and may result from fewer competing coordinative interactions from solvent molecules. This allowed a more consistent and unified methodology which utilizes the same catalyst and protocol for each substrate.

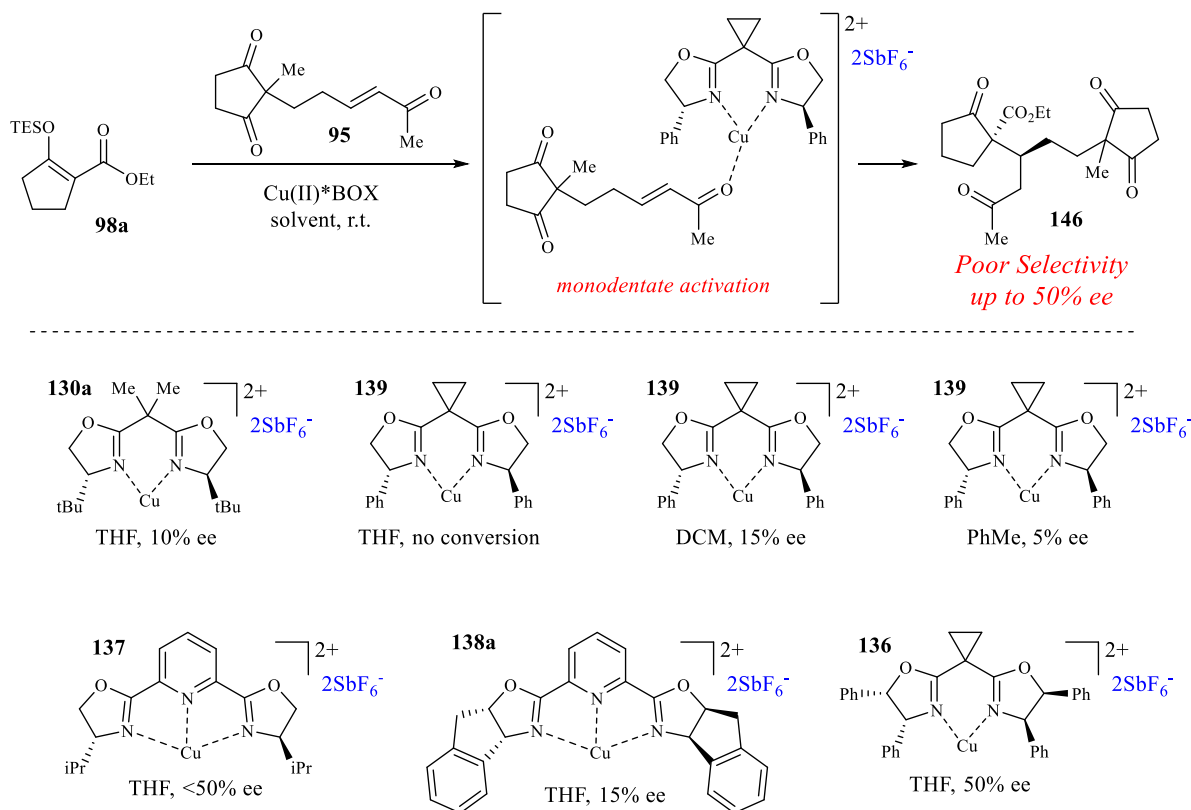
Scheme 3.7. Optimization of Asymmetric Michael Addition with Donor **98**.



Another course which we investigated was to perform the asymmetric Michael reactions via a Mukaiyama-Michael approach. Given the large steric demands of the Michael additions we were studying, we postulated that the silyl enol ethers of the Michael donors **94** and **98** may display enhanced reactivity. This method essentially locks the Michael donors in their nucleophilic tautomer, increasing the chance of productive collision with an activated Michael acceptor. To investigate this possibility, I generated the TES enol ether of Michael donor **98** and treated it with enone **95** in the presence of various Cu(II)-BOX/PyBOX catalysts (**Scheme 3.8.**).

As anticipated, the Michael reaction would readily proceed. However, the use of various Cu(II)-BOX catalysts, and attempts to optimize the reaction were unsuccessful and the enantioselectivities achievable with this approach were low, reaching 50% ee at best with catalyst **136**.

Scheme 3.8. Attempt at Mukaiyama-Michael Approach.



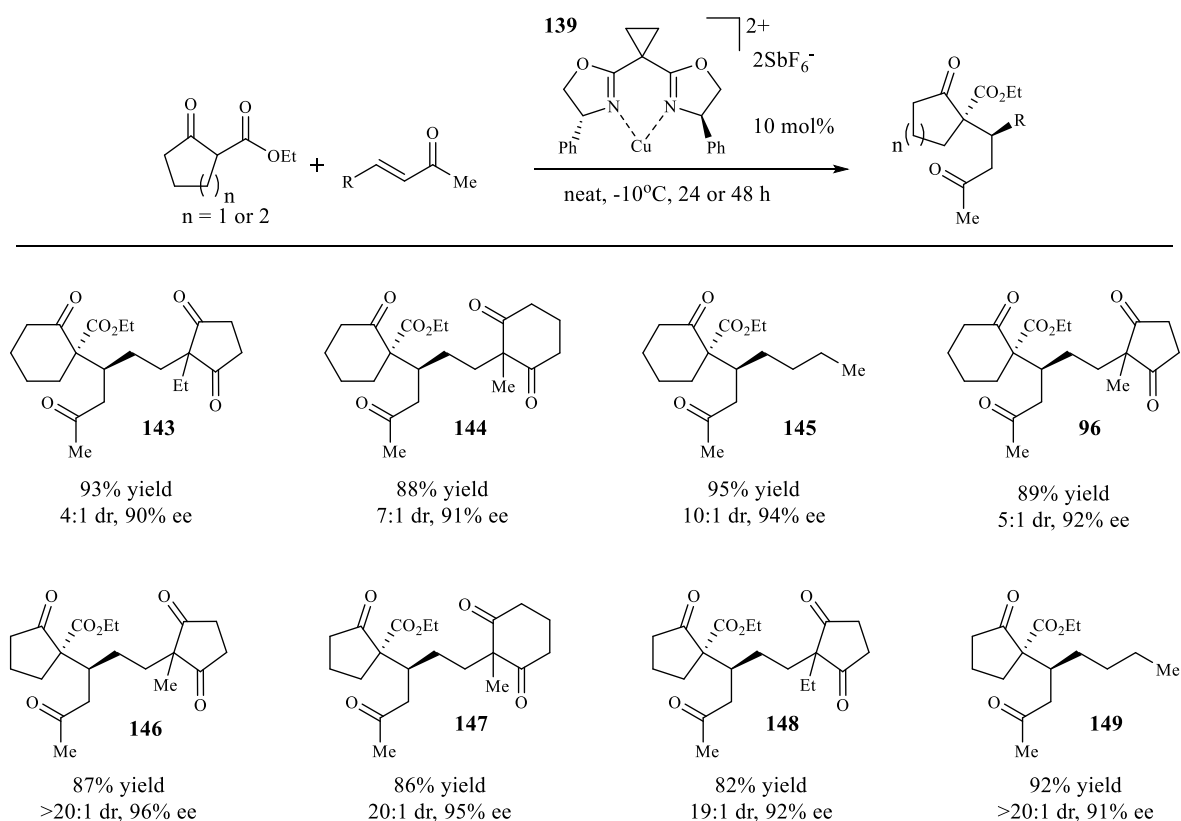
We reasoned that silylation of **98** inhibits its ability to effectively coordinate with the Cu(II) center. In order to obtain high selectivities, it is essential that the coordinative interactions involved in the transition state are compact and well defined. This type of coordination is best attainable with a bidentate substrate, which is a feature of Michael donor **98** that is destroyed upon silylation. Generally, selective Mukaiyama-Michael reactions are done with bidentate Michael acceptors containing 1,3-dicarbonyl motifs, allowing tight bidentate complexation within the chiral pocket.⁹⁻

¹² This feature is absent in the Michael acceptors we are using and is likely the reason that the attempts at Mukaiyama-Michael reactions within our system were unsuccessful. In light of these disappointing enantioselectivities, we decided to abandon this Mukaiyama-Michael approach in favor of the more reliable and selective Michael additions using the unsilylated donors **94** and **98**.

3.7. Substrate Scope for Enantioselective Michael Reactions and Proposed Mechanism

After determining the optimized catalyst and conditions for the enantioselective Michael reaction, the substrate scope was again explored to investigate selectivities in unnatural steroid-like systems. With the assistance of Dr. Cichowicz, I treated Michael donors **94** and **98** with Michael acceptor fragments **95**, **103**, **106**, and **107** under the optimized Cu(SbF₆)₂–**139** system at -10°C for either 24 or 48 hours to generate a library of enantioenriched adducts **96**, **143-149** (Scheme 3.9).

Scheme 3.9. Substrate Scope for Enantioselective Michael Addition.

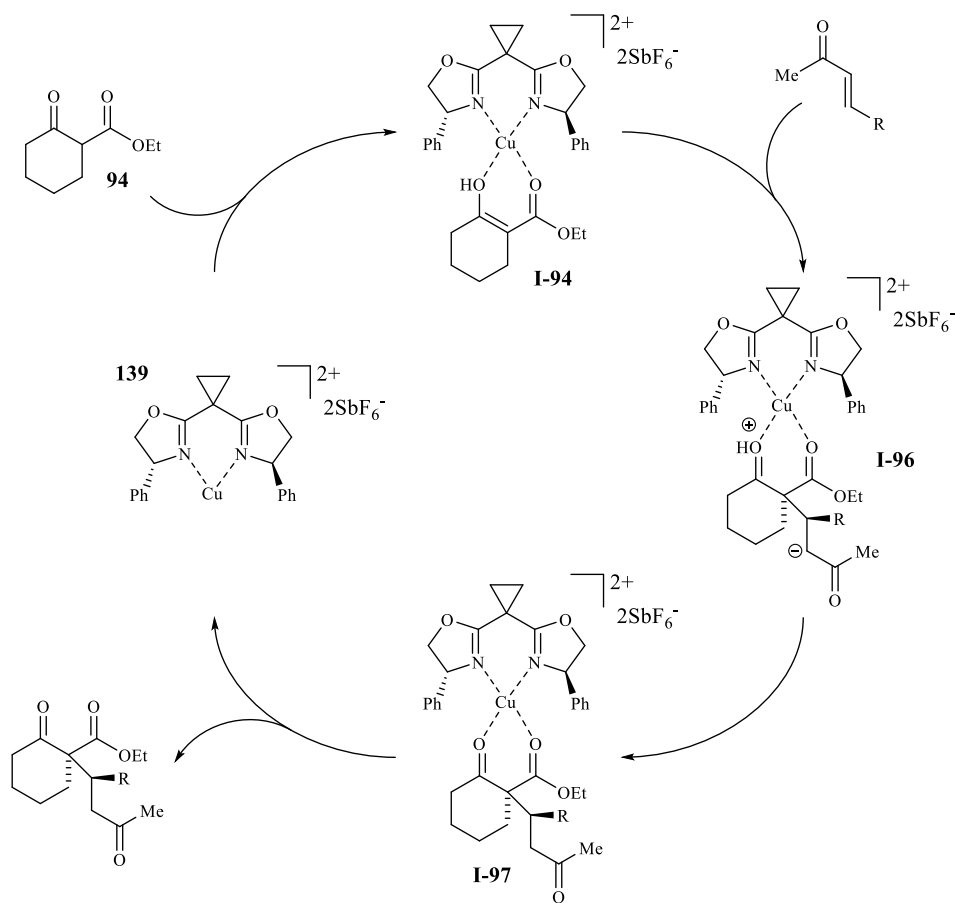


Due to the quicker reaction time of the 5-membered fragment **98**, those reactions were complete within 24 hours; whereas the Michael additions with donor **94** required 48 hours to reach

completion. Yields were excellent and ranged from 82% to 95%. The diastereoselectivity was also good and ranged from 4:1 to 10:1 for donor **94**, and from 19:1 to >20:1 for donor **98**. This follows the same trend noted earlier of better diastereoselectivity with the 5-membered β -ketoester. Lastly, the enantiomeric excess was greater than 90% for each adduct and up to 96% at the highest. Overall, a library of steroid and steroid-like precursors was constructed in a single enantioselective step with excellent yield, enantioselectivity, and diastereoselectivity for each case. Importantly, the method developed allows construction of the Michael adducts containing vicinal quaternary and tertiary stereocenters and with the desired stereochemical configuration at both C9 and C10.

Based on our work, we propose a mechanism for the Cu(II)-BOX-catalyzed Michael additions depicted in **Scheme 3.10**. The Cu(II)-BOX complex **139** undergoes chelation with the enol tautomer of Michael donor **94**. This provides planar complex **I-94**. The coordination of a 1,3-dicarbonyl Michael donor to the Lewis acidic copper center promotes electron withdrawal of the carbonyls, resulting in increased acidity of the Michael donor. Subsequent coordination of the copper center with the Michael acceptor promotes activation of the enone LUMO and the resulting conjugate addition of donor **94** within the chiral pocket of the BOX ligand, generating the zwitterionic complex **I-96**. A rapid proton transfer can occur from the C5 carbonyl to the resulting enolate, affording the catalyst-adduct complex **I-97**. Finally, the Michael adduct is decomplexed and released, refreshing the catalytic cycle.

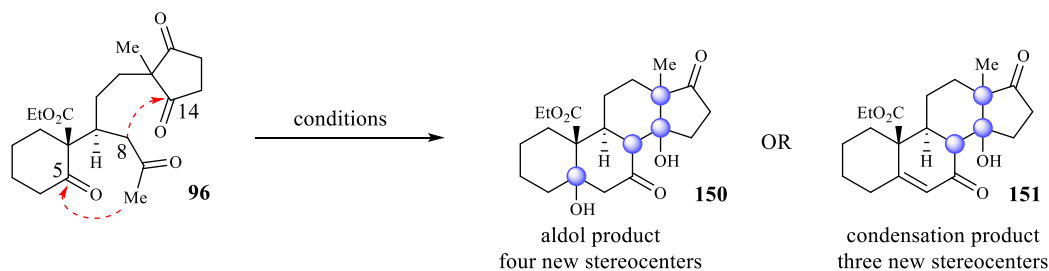
Scheme 3.10. Proposed Mechanism of Cu(II)-BOX-catalyzed Michael Additions.



3.8. Cyclization of Michael Adducts into Steroidal Cores

After successfully developing an asymmetric variant of the key Michael reaction, conditions for the cyclization step were explored. In order to completely set the steroidal scaffold, the Michael adducts must undergo a double aldol cyclization. The first aldol must occur between C6 and the C5 carbonyl of adduct **96**, thus closing the B ring, while the second aldol must form a bond between C8 and the C14 carbonyl, closing the C ring and furnishing a full steroid core **150** or **151** (Scheme 3.11). This process also sets three to four new stereocenters (C5, C8, C13, and C14) and the best conditions must provide adequate diastereocontrol.

Scheme 3.11. Approach to Cyclization of Michael Adducts.

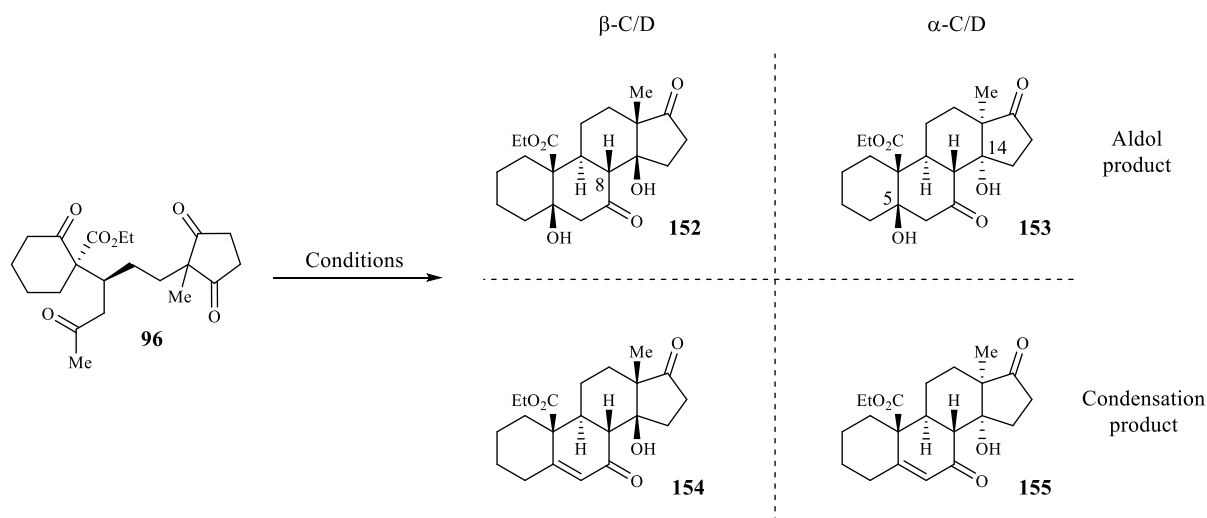


In the interest of pursuing biological evaluation of both natural and unnatural cardenolides, the goal in these studies was to delineate stereodivergent cyclization conditions to allow for the production of unique steroidal isomers. Controlling the internal ring junction stereocenters is a key tenant of our approach. The configuration at the ring junction carbons has a profound effect on the overall shape of the steroid and can readily modulate binding contacts in such rigid ring systems. We are interested in exploring how this region of chemical space can affect isoform selectivity and antiproliferative activity. In order to accomplish this, multiple cyclization procedures must be developed to provide access to unique ring junctions. Initial screening studies performed by Dr. Cichowicz were aimed at understanding how different conditions favor discrete steroidal products. His studies revealed the production of four major steroidal scaffolds depending on the conditions used. A summary of the conditions and results are shown in **Table 3.1**.

Many conditions provided insufficient conversion or selectivity (entries 1, 2, 6-8). However, the use of DBU in refluxing THF delivered steroid **153** exclusively and in high isolated yield (94%, entry 4). Steroid **153** is the result of an aldol addition at C5 which forms a natural *cis*- β -A/B ring junction and an aldol addition at C14 which provides an unnatural *cis*- α -C/D ring junction. In addition, the newly formed stereocenter at C8 has an absolute configuration of *R*,

forming the desired *trans*-B/C ring junction. The origin of this selectivity is discussed in section 3.9.

Table 3.1. Major Products from Cyclization of Michael Adduct **96**.



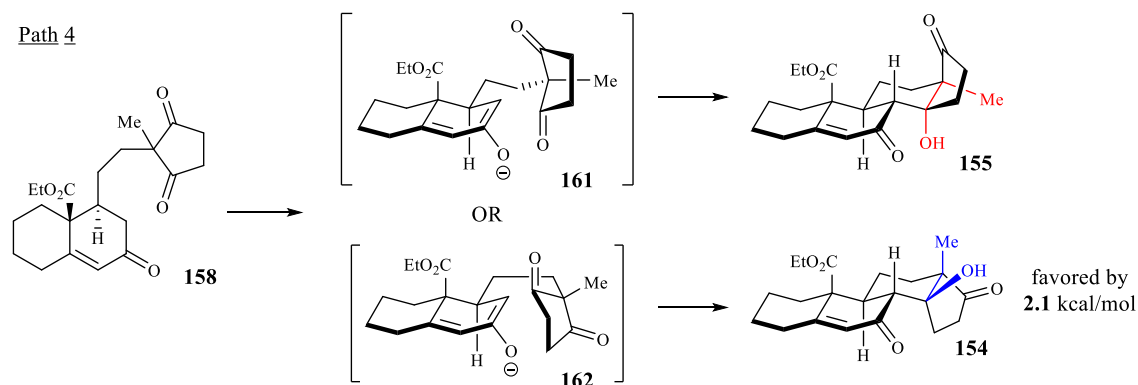
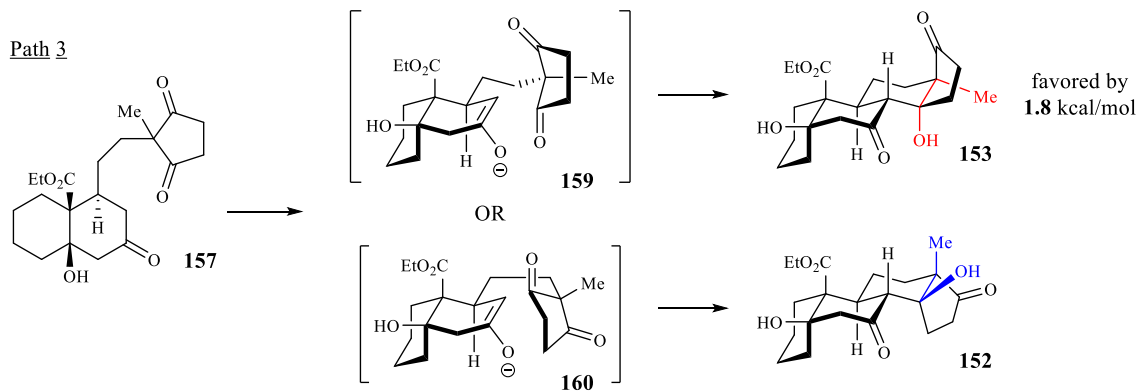
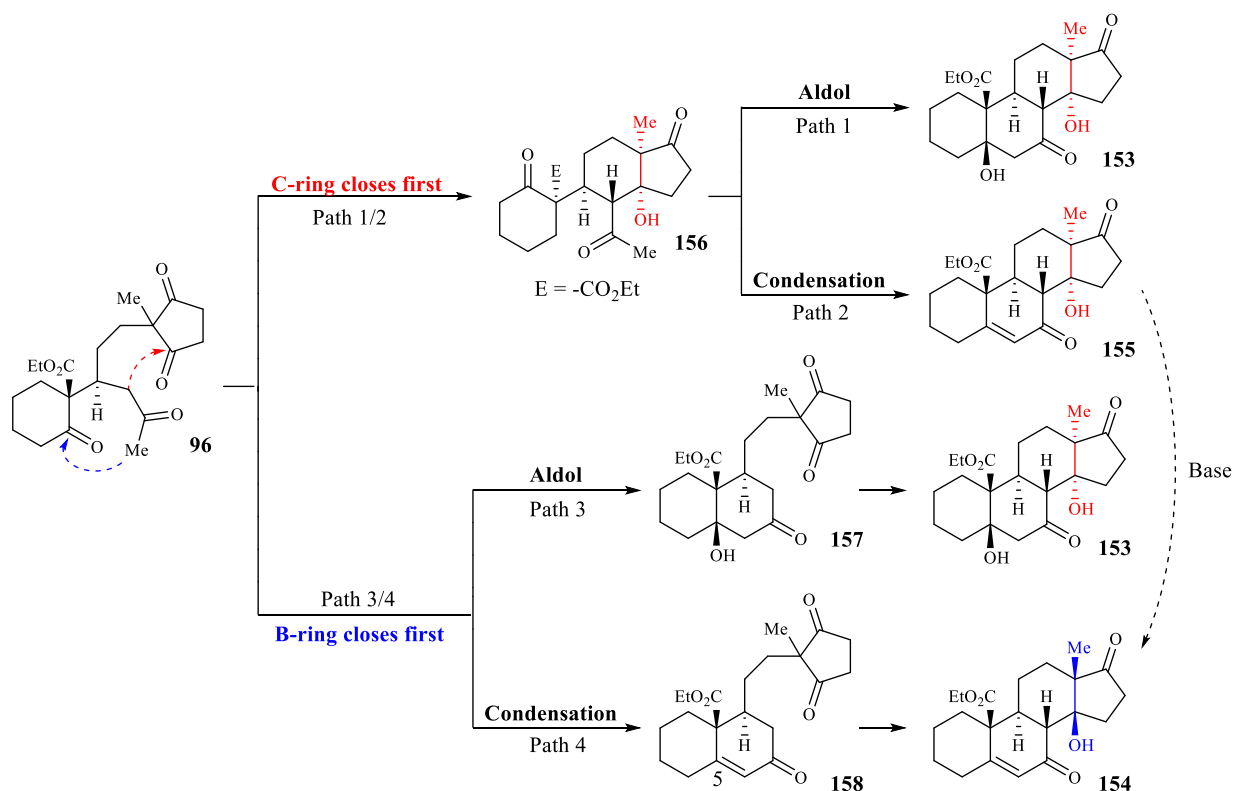
Entry	Conditions	Conversion (yield)	Products	Selectivity
1	D- or L-proline, DMF, r.t., 24 h	0		
2	TiCl ₄ , Et ₃ N, THF, -78 to 0°C	decomposition		
3	p-TSA, toluene, reflux, 18 h	>98	155	only
4	DBU, THF, reflux, 18 h	>98 (94)	153	only
5	piperidine, THF, reflux, 18 h	>98	153	only
6	piperidine, LiCl, THF, reflux, 18 h	>98	153,152,155,154	10:8:43:39
7	KHMDS (1 eq.), THF, r.t., 24 h	>98	152,155,154	35:50:15
8	Cs ₂ CO ₃ , DMF, 140°C, 1 h	>98 (89)	154	only

With good conditions for the exclusive production of steroidal core **153**, additional conditions were explored to investigate the possibility of selecting for other steroidal scaffolds starting from the same Michael adduct **96**. After exploring the use of stronger bases and fine tuning the reaction conditions, Dr. Cichowicz discovered that Cs_2CO_3 in DMF at 140°C would effectively promote the cyclization of adduct **96** into steroid **154** exclusively (entry 9). Importantly, scaffold **154** results from aldol condensation across C5-C6, producing an enone, with closure of the C/D ring occurring with a *cis*- β orientation. This replicates the stereochemical pattern of natural cardenolides at all positions with stereogenic carbons. In short, we were able to discover stereodivergent conditions for the cyclization of Michael adduct **96** into unique steroidal cores with high yield and selectivity.

3.9. Origin of Stereodivergent Cyclization Results

In the interest of understanding the physical origin of the stereodivergence observed during the cyclization studies, some observations, calculations, and previously reported results were considered. Over the course of our studies, we have observed some trends in factors controlling the diastereoselectivity of the cyclization step. These observations are summarized in **Scheme 3.12**. Importantly, the trends described here mirror the results noted by Deslongchamps during his studies on polyanionic cyclizations of steroidal cores.^{2g,2h} There are four likely reaction pathways for completing the cyclization of a Michael adduct into a steroidal core. These are labeled paths 1-4. In paths 1 and 2, the C ring is closed prior to the B ring. In these cases, we observe exclusive formation of a monocyclized compound with a *cis*- α -C/D ring junction, intermediate **156**. At this point the reaction pathway can follow one of two options.

Scheme 3.12. Suggested Pathways for Diastereoselective Michael Adduct Cyclization.



In path 1, the B ring is closed via an aldol reaction which halts prior to dehydration of the resulting C5 hydroxy, producing steroid **153**. In path 2, the B ring is closed via an aldol condensation reaction which generates an olefin at C5-C6 (**155**). In either case, the C/D ring junction remains in a *cis- α* orientation. In contrast, paths 3 and 4 begin with closure of the B ring prior to closure of the C ring. This can proceed via aldol reaction, path 3, producing monocyclized intermediate **157**, or by means of an aldol condensation, path 4, producing enone **158**. Subsequent closure of the C ring proceeds in a diastereoselective fashion depending on the absence (path 3) or presence (path 4) of unsaturation at C5-C6. In the case of a path 3, containing a C5 hydroxy group, closure of the C ring proceeds stereoselectively from **157** to furnish an unnatural *cis- α* -C/D ring junction **153**, the same product which results from path 1. However, when the B ring has closed via aldol condensation path 4, producing enone **158**, subsequent C ring closure proceeds selectively to yield the natural *cis- β* oriented C/D ring junction steroid **154**.

The cyclization step was investigated computationally (DFT, geometry optimization, B3LYP, 6-31+G*). Based on these DFT calculations, the *cis- α* -C/D junction product **153** resulting from intermediate **157** is thermodynamically preferred by 1.8 kcal/mol over its *cis- β* -C/D junction congener **152** (path 3). On the other hand, the natural *cis- β* -C/D product **154** produced from enone **158** is thermodynamically more stable than its *cis- α* -C/D isomer **155** by 2.1 kcal/mol. We suggest this outcome is a result of torsional or angle strains introduced by the C5-C6 unsaturation which dictate a unique confirmation of the A and B rings. This strain bias may be effectively “transmitted” through the ethylene tether at C9 and could impose a conformational preference on the D ring, orienting the *pro-cis- β* -C14 carbonyl for aldol addition. Such a conformational bias would be absent in the case of path 3, intermediate **157**, wherein the C5-C6 bond is saturated. It is likely, though not necessary, that the factors which cause a thermodynamic preference for the

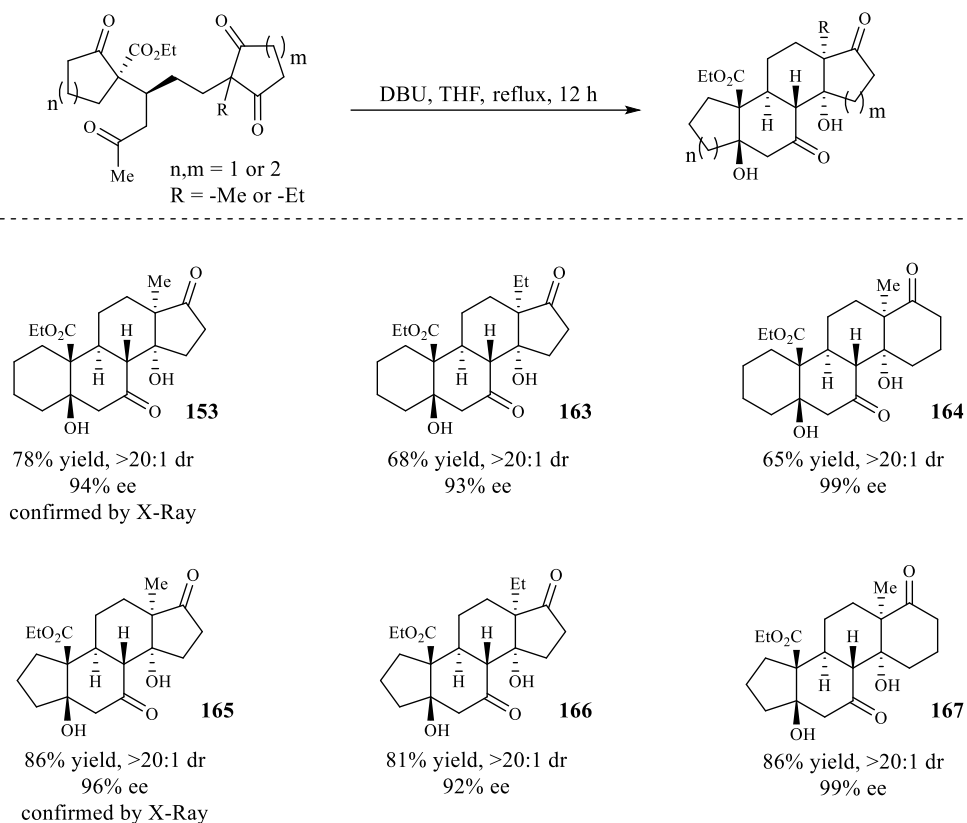
formation of *cis*- β -C/D junction product **154** from enone **158** are also present in the transition state (**162**) leading to that product.

Lastly, it was found that steroids containing a *cis*- α -C/D ring junction with C5-C6 unsaturation (**155**) could be epimerized to the thermodynamically preferred *cis*- β -C/D junction by means of reversible aldol reaction. By treating steroids of structure **155** with base, the C14 hydroxy can be deprotonated and the resulting alkoxide is able to undergo a retro-aldol reaction. This puts the opening and closing of the C ring in flux and promotes thermodynamic equilibration between the two possible C/D junction stereoisomers. In this case, the presence of unsaturation at the C5-C6 bond imposes a preference for the *cis*- β -C/D junction. As a result, we can observe the base promoted epimerization of the C/D ring junction.

3.10. Stereodivergent Cyclization of Adducts to a Library of Steroidal Cores

With effective conditions in hand for promoting the diastereoselective cyclization of Michael adducts, a library of natural and unnatural steroidal cores was synthesized. With the help of co-worker Bijay Bhattarai, Dr. Cichowicz applied the optimized cyclization procedures to the previously prepared Michael adducts. DBU in refluxing THF was used to promote the formation of *cis*- α -C/D steroidal scaffolds (**Scheme 3.13**). Steroidal cores **153**, **163-167** containing an unnatural *cis*- α -C/D junction could be obtained in good yield and excellent diastereomeric ratio with no erosion of enantiomeric excess. Steroid-like substrates containing expanded 6-membered D rings (**164**, **167**) and contracted 5-membered A rings (**165-167**) could be readily accessed. In addition, the formation of C13 ethyl substituted products (**163**, **166**) could be achieved; such an architecture would be very difficult to generate semi-synthetically. The stereochemistry of products **153** and **165** were determined unambiguously by X-ray crystallographic analysis.

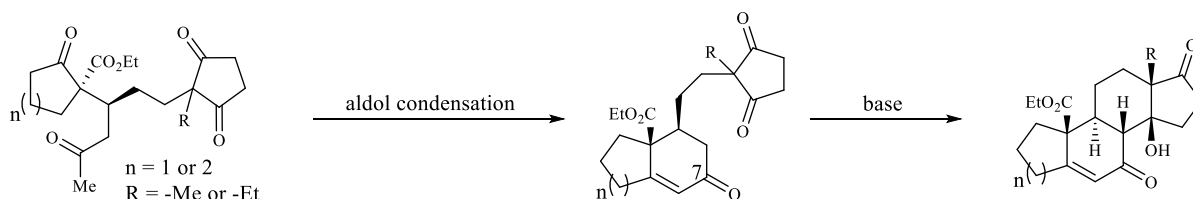
Scheme 3.13. Formation of *cis*- α -C/D Steroidal Scaffolds via DBU Promoted Cyclization.



After successfully synthesizing a library of steroid and steroid-like cores with unnatural configuration at the C/D ring junction, attention was turned toward the selective production of steroidal scaffolds with a natural *cis*- β -C/D junction (**Scheme 3.14.**). Unfortunately, the initially discovered conditions of Cs_2CO_3 in DMF at 140°C proved ineffective. Only the natural series steroidal scaffold with a 6-membered A ring and 5-membered D ring could be reliably cyclized with these conditions; other substrates would provide a complex mixture of degraded products. As such, a new approach was investigated. With an improved knowledge of the factors which govern the configuration of the C/D ring junction, we envisioned a two-step cyclization procedure. First, the B ring would be closed via an aldol condensation followed by subsequent base-promoted aldol

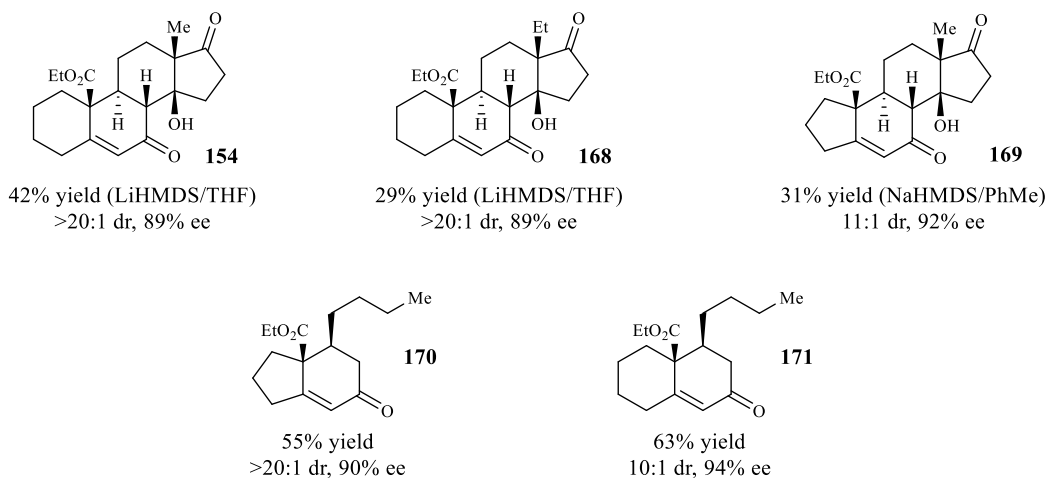
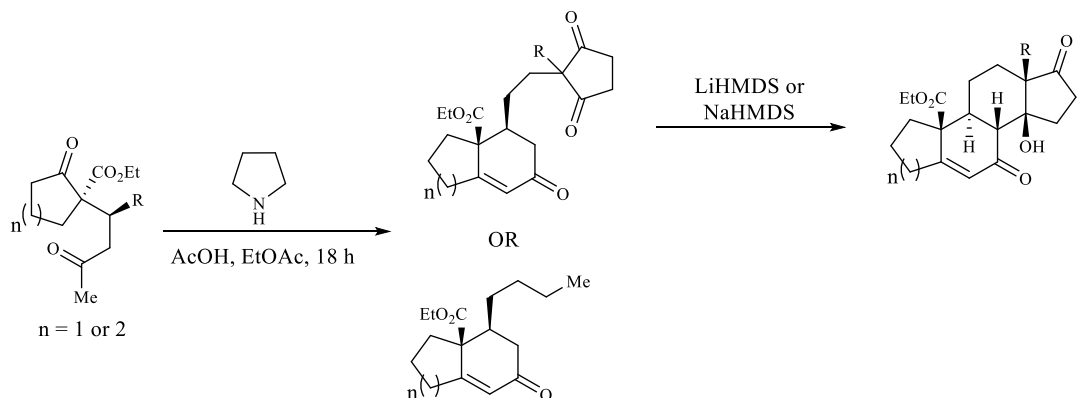
reaction to close the C ring. This approach would allow complete control over the introduction of unsaturation at the C5-C6 bond prior to the key C ring closure. Based on our previous research, we anticipated that this mode of reaction would preferentially deliver the natural *cis*- β -C/D junction.

Scheme 3.14. Two-step Cyclization Approach to *cis*- β -C/D Steroidal Cores.



The aldol condensation closure of the B ring could be effectively produced by treatment with pyrrolidine and acetic acid in ethyl acetate, presumably via an enamine formed at C7. Subsequent closure of the C ring was then accomplished upon treatment with metal hexamethyldisilazides in THF. As expected, this C8-C14 bond forming reaction to set the steroid core would furnish the desired *cis*- β -C/D junction orientation. Accordingly, steroids **154**, **168**, and **169** could be synthesized using this two-step approach (**Scheme 3.15**). In addition, modified Hajos-Parish and Wieland-Miescher ketones **170** and **171**, respectively, could be produced upon cyclization with pyrrolidine and acetic, but cannot be further cyclized due to the absence of available D ring carbonyls. This two-step procedure was successful in delivering the desired stereocontrol in good yields and without erosion of the enantiomeric excess. In the case of producing steroid **169**, it was found that cyclization with LiHMDS in THF provided insufficient diastereoselectivity. After some investigation, it was found that the thermodynamic product could be biased by refluxing with NaHMDS in toluene.

Scheme 3.15. Synthesis of *cis*- β -C/D Steroidal Cores Via Two-step Protocol.



Overall, a library of structurally diverse steroid and steroid-like compounds were synthesized. The key aspects of this concise methodology include the enantio- and diastereoselective Cu(II)-BOX catalyzed Michael reaction which forms sterically congested vicinal quaternary and tertiary stereocenters, stereodivergent cyclization conditions used to control for natural *cis*- β or unnatural *cis*- α -C/D ring junctions, and pre-installed functional handles at C5, C7, C14, C17, and C19. Future work is aimed at increasing the complexity of the substrates and applying the method toward the total synthesis of natural cardenolides.

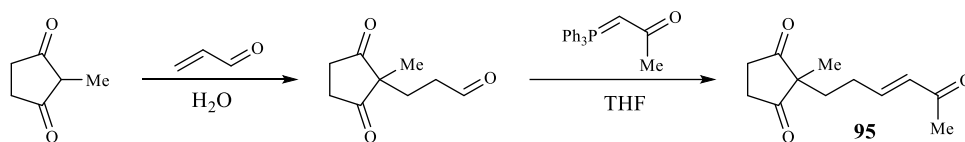
3.11. Experimental (as appears in the corresponding publication)¹

General

All reagents and solvents were purchased from commercial sources and were used as received without further purification unless specified. THF and DMF were purified by Innovative Technology's Pure-Solve System. All reactions were carried out under a positive pressure of nitrogen in flame- or oven-dried glassware with magnetic stirring. Reactions were cooled using cryocool or external cooling baths (sodium chloride/ice water (-10 °C) or dry ice/acetone (-78 °C)). Heating was achieved by use of a silicone bath with heating controlled by electronic contact thermometer. Deionized water was used in the preparation of all aqueous solutions and for all aqueous extractions. Solvents used for extraction and chromatography were ACS or HPLC grade. Purification of reactions mixtures was performed by flash chromatography using SiliCycle SiliaFlash P60 (230-400 mesh). Yields indicate the isolated yield of the title compound $\geq 95\%$ pure as determined by ^1H NMR analysis. Whereas the yields in the article are the average yields of two or more experiments, the yields in the supporting information describe the result of a single experiment. Diastereomeric ratios were determined by ^1H NMR analysis. Enantiomeric excess was determined by HPLC analysis using a Waters e2695 Separations Module with a Waters 2998 photodiode array detector. ^1H NMR spectra were recorded on Varian vnmrs 700 (700 MHz) spectrometer and chemical shifts (δ) are reported in parts per million (ppm) with solvent resonance as the internal standard (CDCl_3 at δ 7.26). Data are reported as (s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, sext = sextet, m = multiplet; coupling constant(s) in Hz; integration). Proton-decoupled ^{13}C NMR spectra were recorded on Varian vnmrs 700 (700 MHz) spectrometer and chemical shifts (δ) are reported in ppm with solvent resonance as the internal standard (CDCl_3 at δ 77.0). High resolution mass spectra (HRMS) were performed and recorded on Micromass

AutoSpec Ultima or VG (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}).

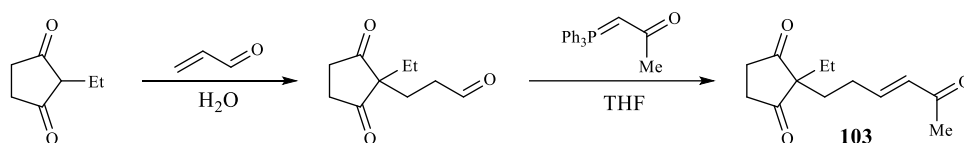
Reaction Protocols and Compound Characterization



(*E*)-2-methyl-2-(5-oxohex-3-en-1-yl)cyclopentane-1,3-dione (95)

2-Methylcyclopentane-1,3-dione (5.37 g, 47.9 mmol, 1.0 equiv.) was taken in H_2O (120 ml, 0.4 M). Acrolein (4.8 mL, 71.9 mmol, 1.5 equiv.) was added. The reaction mixture was allowed to stir overnight and then concentrated in vacuo. The reaction mixture was diluted with EtOAc, dried over MgSO_4 , and concentrated in vacuo to afford 3-(1-methyl-2,5-dioxocyclopentyl)propanal (8.05 g, 47.9 mmol, quantitative) as a colorless oil. Unpurified 3-(1-methyl-2,5-dioxocyclopentyl)propanal (8.05 g, 47.9 mmol, 1 equiv.) was then taken in THF (145 mL, 0.33 M). Added 1-(triphenylphosphoranylidene)-2-propanone (21.3 g, 67.1 mmol, 1.4 equiv.) and the reaction mixture was allowed to stir overnight. The reaction mixture was then concentrated in vacuo and purified directly by column chromatography (grad. 20%→33% EtOAc in hexanes) to afford (*E*)-2-methyl-2-(5-oxohex-3-en-1-yl)cyclopentane-1,3-dione (**95**) (9.268 g, 44.6 mmol, 93% yield) as a colorless oil. ^1H NMR (700MHz, CDCl_3) δ 6.61 (dt, $J = 6.8, 16.0$ Hz, 1H), 5.98

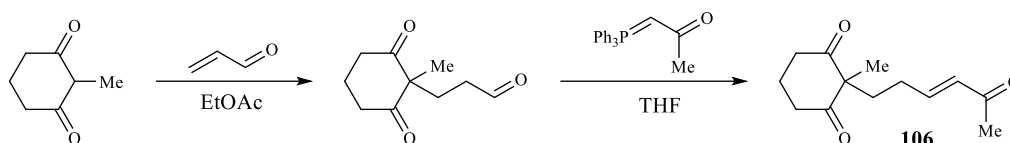
(d, $J = 16.0$ Hz, 1H), 2.86-2.79 (m, 2H), 2.73-2.66 (m, 2H), 2.22 (s, 3H), 2.14-2.11 (m, 2H), 1.86-1.83 (m, 2H), 1.15 (s, 3H); ^{13}C NMR (175 MHz, CDCl_3) δ 215.9, 198.2, 146.2, 131.9, 56.1, 35.0, 32.6, 27.7, 26.8, 20.4; HRMS (ESI): m/z calcd for $\text{C}_{13}\text{H}_{18}\text{O}_3\text{Na}^+$ $[\text{M}+\text{Na}]^+$ 231.0992, found 231.0998; IR (thin film, cm^{-1}): 2922, 1717, 1671, 1626, 1362, 1255, 980.



(*E*)-2-ethyl-2-(5-oxohex-3-en-1-yl)cyclopentane-1,3-dione (103)

2-Ethylcyclopentane-1,3-dione (3.498 g, 27.8 mmol, 1.0 equiv.) was taken in H_2O (70 mL ml, 0.4 M). Acrolein (2.8 mL, 41.6 mmol, 1.5 equiv.) was added. The reaction mixture was allowed to stir overnight and then concentrated in vacuo. The reaction mixture was diluted with EtOAc, dried over MgSO_4 , and concentrated in vacuo to afford 3-(1-ethyl-2,5-dioxocyclopentyl)propanal (5.07 g, 27.8 mmol, quantitative) as a colorless oil. Unpurified 3-(1-ethyl-2,5-dioxocyclopentyl)propanal (5.07 g, 27.8 mmol, 1 equiv.) was then taken in THF (84 mL, 0.33 M). Added was 1-(triphenylphosphoranylidene)-2-propanone (13.3 g, 41.7 mmol, 1.5 equiv.) and the reaction mixture was allowed to stir overnight. The reaction mixture was then concentrated in vacuo and purified directly by column chromatography (grad. 20%→33% EtOAc in hexanes) to afford (*E*)-2-ethyl-2-(5-oxohex-3-en-1-yl)cyclopentane-1,3-dione (**103**) (3.948 g, 17.8 mmol, 64% yield) as a colorless oil. ^1H NMR (700MHz, CDCl_3) δ 6.60 (dt, $J = 7.0, 15.8$ Hz, 1H), 5.97 (d, $J = 16.0$ Hz, 1H), 2.79-2.73 (m, 2H), 2.70-2.61 (m, 2H), 2.21 (s, 3H), 2.08 (d, $J = 6.8$ Hz, 2H), 1.85-1.82 (m, 2H), 1.67 (q, $J = 7.5$ Hz, 2H), 0.82 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (175 MHz, CDCl_3) δ

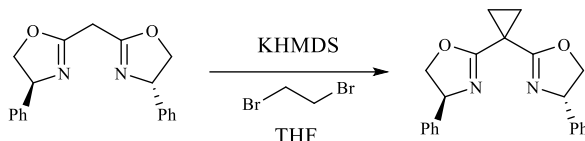
216.5, 198.3, 146.4, 131.9, 60.9, 36.0, 31.3, 29.2, 27.8, 26.8, 8.7; HRMS (ESI): m/z calcd for $C_{13}H_{18}O_3Na^+$ $[M+Na]^+$ 245.1148, found 245.1147; IR (thin film, cm^{-1}): 2922, 1716, 1671, 1626, 1254, 980.



(*E*)-2-methyl-2-(5-oxohex-3-en-1-yl)cyclohexane-1,3-dione (106)

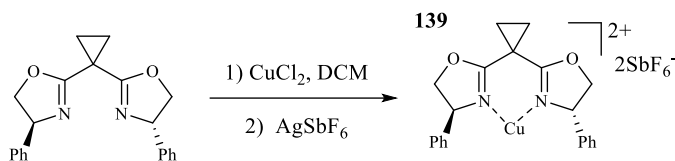
2-Methylcyclohexane-1,3-dione was taken (2.06 g, 16.4 mmol, 1.0 equiv.) was taken in EtOAc (41 mL, 0.4M). TEA (3.4 mL, 24.6 mmol, 1.5 equiv.) and acrolein (1.7 mL, 24.6 mmol, 1.5 equiv.) was added. The reaction mixture was allowed to stir overnight and then concentrated in vacuo to afford 3-(1-methyl-2,6-dioxocyclohexyl)propanal (2.06 g, 16.4 mmol, quantitative) as a colorless oil. Unpurified 3-(1-methyl-2,6-dioxocyclohexyl)propanal (3.0 g, 16.4 mmol, quantitative) was then taken in THF (40 mL, 0.33 M). Added was 1-(triphenylphosphoranylidene)-2-propanone (7.3 g, 22.9 mmol, 1.5 equiv.) and the reaction mixture was allowed to stir overnight. The reaction mixture was then concentrated in vacuo and purified directly by column chromatography (grad. 20%→33% EtOAc in hexanes) to afford (*E*)-2-methyl-2-(5-oxohex-3-en-1-yl)cyclohexane-1,3-dione (**106**) (3.1 g, 13.9 mmol, 84% yield) as a colorless oil. 1H NMR (700MHz, $CDCl_3$) δ 6.67 (dt, $J = 6.8, 16.0$ Hz, 1H), 5.99 (d, $J = 16.0$ Hz, 1H), 2.77-2.66 (m, 2H), 2.62-2.58 (m, 2H), 2.20 (s, 3H), 2.05-2.02 (m, 2H), 1.95-1.89 (m, 4H), 1.27 (s, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 209.9, 198.5, 146.9, 131.5, 64.7, 37.9, 33.7, 27.9, 26.8, 22.0, 17.4; HRMS (ESI): m/z calcd for $C_{13}H_{18}O_3Na^+$ $[M+Na]^+$ 245.1148, found 245.1150; IR (thin film, cm^{-1}): 2956, 1724, 1692, 1672, 1626, 1364, 1246, 980.

Catalysts were prepared based on known procedures¹⁵:



(4*S*,4'*S*)-2,2'-(cyclopropane-1,1-diyl)bis(4-phenyl-4,5-dihydrooxazole)

Bis-(*S*)-4-phenyl-4,5-dihydrooxazol-2-yl)methane (1.0 g, 3.26 mmol, 1.0 equiv.) was dissolved in THF (30 mL, 0.1 M). 1,2-dibromoethane (562 μ l, 6.5 mmol, 2.0 equiv.) and LiHMDS (1.48 g, 8.86 mmol, 2.7 equiv.) were added sequentially. The reaction mixture was allowed to stir for 6 hours. Additional 1,2-dibromoethane (281 μ L, 3.26 mmol, 1.0 equiv.) and LiHMDS (1.08 g, 6.47 mmol, 2.0 equiv.) was added. The reaction mixture was allowed to stir for an additional 16 hours. Then, the reaction mixture was quenched with saturated NH_4Cl solution and then saturated NaHCO_3 solution was added. The aqueous solution was extracted with EtOAc (3 times). The combined organic layers were dried over MgSO_4 , filtered, and concentrated in vacuo. The crude mixture was purified by column chromatography (silica gel was pretreated with TEA, grad. 50% \rightarrow 100% EtOAc in hexanes) to afford (*4S*,*4'S*)-2,2'-(cyclopropane-1,1-diyl)bis(4-phenyl-4,5-dihydrooxazole) (0.95 g, 2.86 mmol, 88% yield) as an orange oil.



(4*S*,4'*S*)-2,2'-(cyclopropane-1,1-diyl)bis(4-phenyl-4,5-dihydrooxazole)copper(II) hexafluoroantimonate (139)

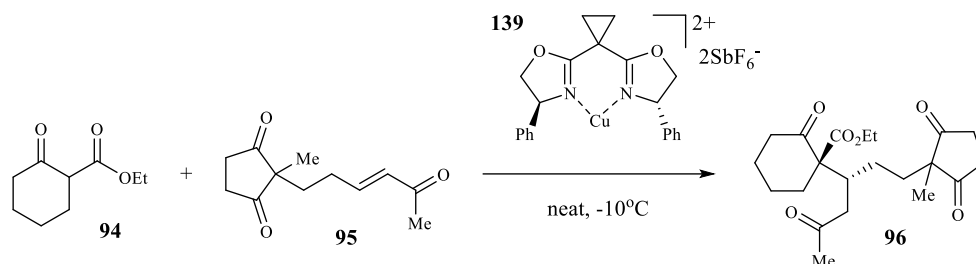
(4*S*,4'*S*)-2,2'-(cyclopropane-1,1-diyl)bis(4-phenyl-4,5-dihydrooxazole) (950 mg, 2.86 mmol, 1.0 equiv.) and copper(II) chloride (380 mg, 2.86 mmol, 1.0 equiv.) were taken in DCM (14 mL, 0.2 M) and stirred for 3 hours. Silver hexafluoroantimonate(V) (1.97 g, 5.72 mmol, 2.0 equiv.) was added as a solution in DCM (4 mL). The resulting reaction mixture was allowed to stir for 2 hours. The reaction mixture was diluted with THP (25 mL) and filtered through a plug of celite. The catalyst solution was concentrated in vacuo and then azeotroped with toluene (3 times). The catalyst was further dried by diluting with DCM (30 mL) and stirred with 4 Å MS (2.0 g) overnight. The green solution was decanted via cannula and the DCM was removed by flow of nitrogen to afford (4*S*,4'*S*)-2,2'-(cyclopropane-1,1-diyl)bis(4-phenyl-4,5-dihydrooxazole) copper(II) hexafluoroantimonate (**139**) (1.9 g, 2.2 mmol, 85% yield) as a dark green solid.

General procedure for the synthesis of racemic Michael adducts 108 - 117:

Michael acceptor (1.0 equiv.), Michael donor (1.0-1.1 equiv.), and copper(II) triflate (0.1 equiv.) were stirred neat at room temperature. After 3 hours, the reaction mixture was purified directly by column chromatography.

General procedure for the synthesis of chiral Michael adducts **96**, **143** - **149**:

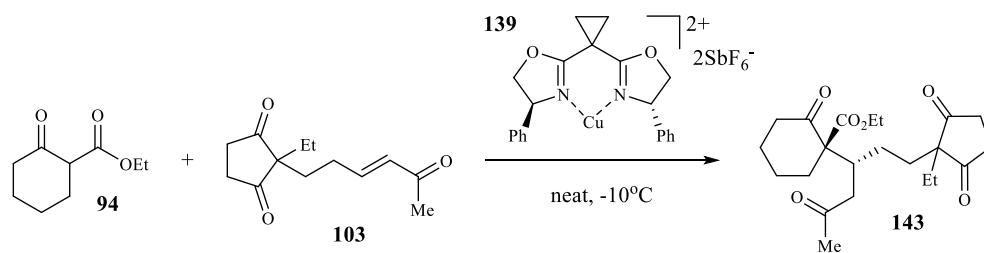
Michael acceptor (1.0 equiv.) and bis(oxazoline)copper(II) complex (0.1 equiv.) were cooled to -10°C. Michael donor (1.1-2.0 equiv.) was then added and the reaction mixture was stirred neat at -10°C until completion (24-48 h). Copper catalyst can be removed by dissolving the reaction mixture in EtOAc and filtering through a plug of silica gel and eluting with Et₂O. Alternatively, the reaction mixture can be purified directly by column chromatography.



Ethyl (*R*)-1-((*R*)-1-(1-methyl-2,5-dioxocyclopentyl)-5-oxohexan-3-yl)-2-oxocyclohexane-1-carboxylate (**96**)

Michael acceptor **95** (238 mg, 1.14 mmol, 1.0 equiv.) and bis(oxazoline)copper(II) complex (95 mg, 0.11 mmol, 0.1 equiv.) were cooled to -10 °C. Michael donor **94** (0.36 mL, 2.29 mmol, 2 equiv.) was then added and the reaction mixture was stirred neat at -10 °C for 48 hours. The reaction mixture was purified directly by column chromatography (grad. 20%→33% EtOAc in hexanes) to afford ethyl (*R*)-1-((*R*)-1-(1-methyl-2,5-dioxocyclopentyl)-5-oxohexan-3-yl)-2-oxocyclohexane-1-carboxylate (**96**) (404 mg, 1.07 mmol, 93% yield, 5:1 dr, 92% ee) as a colorless oil. **Major Diastereomer:** $[\alpha]_D^{26} = +15.7$ (c 1.0, CHCl₃); ¹H NMR (700 MHz, CDCl₃) δ 4.14-4.12 (m, 1H), 4.07-4.05 (m, 1H), 2.73-2.69 (m, 4H), 2.55(dd, J = 4.9, 18.2 Hz, 1H), 2.49-2.47 (m, 1H), 2.37-2.31 (m, 3H), 2.18(dd, J = 4.9, 18.2 Hz, 1H), 2.09 (s, 3H), 1.91-1.89 (m, 1H), 1.74-1.71

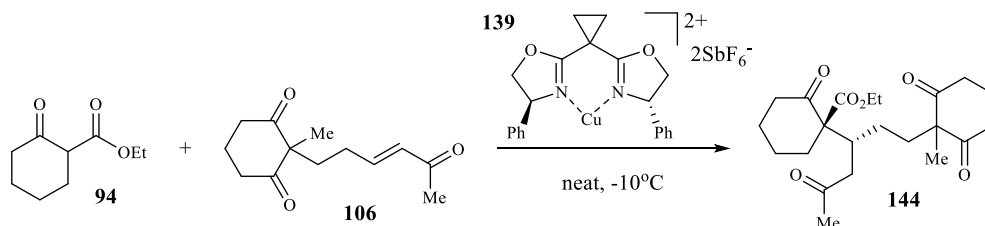
(m, 1H), 1.63-1.39 (m, 5H), 1.21 (t, J = 7.0 Hz, 3H), 1.20-1.18 (m, 1H), 1.01 (s, 3H), 0.93-0.91 (m, 1H); ¹³C NMR (175 MHz, CDCl₃) δ 216.2, 216.0, 207.4, 207.1, 171.4, 64.2, 61.5, 56.8, 45.1, 41.2, 36.1, 35.0, 35.0, 33.7, 32.9, 30.1, 26.9, 26.9, 22.2, 18.8, 14.0; HRMS (ESI): m/z calcd for C₂₁H₃₂O₆⁺ [M+H]⁺ 379.2115, found 379.2117; IR (thin film, cm⁻¹): 2937, 1706, 1363, 1206, 1093. Enantiopurity was determined to be 93% ee by chiral HPLC (DAICEL CHIRALPAK AD-H, 25 cm x 4.6 mm, hexanes/2-propanol = 85/15, flow rate = 1 mL/min, λ = 282.0 nm, RT(minor) = 9.5 min, RT(major) = 12.8 min). **Minor Diastereomer:** ¹H NMR (700 MHz, CDCl₃) δ 4.19-4.14 (m, 2H), 2.76-2.71 (m, 4H), 2.59 (dd, J = 4.2, 18.2 Hz, 1H), 2.52-2.48 (m, 1H), 2.43-2.26 (m, 4H), 2.14 (s, 3H), 1.96-1.93 (m, 1H), 1.77-1.75 (m, 1H), 1.62-1.53 (m, 5H), 1.50-1.46 (m, 1H), 1.28-1.22 (m, 4H), 1.05 (s, 3H), 0.91-0.85 (m, 1H); ¹³C NMR (175 MHz, CDCl₃) δ 216.2, 216.1, 207.2, 207.2, 171.3, 64.5, 61.5, 56.8, 45.7, 41.4, 36.4, 35.1, 35.0, 33.7, 33.3, 30.1, 27.1, 26.7, 22.4, 19.0, 14.1; HRMS (ESI): m/z calcd for C₂₁H₃₁O₆Na⁺ [M+Na]⁺ 401.1935, found 401.1933.



Ethyl (R)-1-((R)-1-(1-ethyl-2,5-dioxocyclopentyl)-5-oxohexan-3-yl)-2-oxocyclohexane-1-carboxylate (143)

Michael acceptor **103** (241 mg, 1.17 mmol, 1.0 equiv.) and bis(oxazoline)copper(II) complex (**104**, 104 mg, 0.12 mmol, 0.1 equiv.) were cooled to -10 °C. Michael donor **94** (0.37 mL, 2.33 mmol, 2 equiv.) was then added and the reaction mixture was stirred neat at -10 °C for 48 hours. The

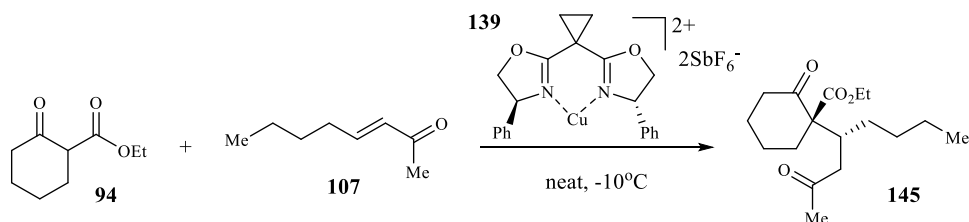
reaction mixture was purified directly by column chromatography (grad. 20%→33% EtOAc in hexanes) to afford ethyl (*R*)-1-((*R*)-1-(1-ethyl-2,5-dioxocyclopentyl)-5-oxohexan-3-yl)-2-oxocyclohexane-1-carboxylate (**143**) (384 mg, 0.98 mmol, 90% yield, 4:1 dr, 90% ee) as a colorless oil. $[\alpha]_D^{25} = +17.9$ (c 1.0, CHCl₃). ¹H NMR (700MHz, CDCl₃) δ 4.19-4.07 (m, 2H), 2.74-2.64 (m, 4H), 2.58 (dd, J = 4.9, 18.6 Hz, 1H), 2.51-2.48 (m, 1H), 2.43-2.32 (m, 3H), 2.23 (dd, J = 5.5, 18.6 Hz, 1H), 2.12 (s, 3H), 1.94-1.91 (m, 1H), 1.76-1.73 (m, 1H), 1.65-1.40 (m, 7H), 1.27-1.18 (m, 4H), 0.94-0.89 (m, 1H), 0.74 (t, J = 7.5 Hz, 3H); ¹³C NMR (175 MHz, CDCl₃) δ 217.0, 216.7, 207.4, 207.1, 171.5, 66.2, 61.5, 61.5, 45.1, 41.2, 36.3, 36.1, 36.1, 36.0, 33.0, 32.5, 30.1, 29.1, 26.9, 22.2, 14.0, 9.0; HRMS (ESI): m/z calcd for C₂₂H₃₂O₆Na⁺ [M+Na]⁺ 415.2091, found 415.2098; IR (thin film, cm⁻¹): 2937, 1713, 1234, 1208, 1046. Enantiopurity was determined to be 90% ee by chiral HPLC (DAICEL CHIRALPAK AD-H, 25 cm x 4.6 mm, hexanes/2-propanol = 85/15, flow rate = 1 mL/min, λ = 282.0 nm, RT(minor) = 8.7 min, RT(major) = 12.4 min).



Ethyl (*R*)-1-((*R*)-1-(1-methyl-2,6-dioxocyclohexyl)-5-oxohexan-3-yl)-2-oxocyclohexane-1-carboxylate (144**)**

Michael acceptor **106** (200 mg, 0.90 mmol, 1.0 equiv.) and bis(oxazoline)copper(II) complex (78 mg, 0.09 mmol, 0.1 equiv.) were cooled to -10 °C. Michael donor **94** (0.29 mL, 1.80 mmol, 2 equiv.) was then added and the reaction mixture was stirred neat at -10 °C for 48 hours. The reaction mixture was purified directly by column chromatography (grad. 20%→33% EtOAc in

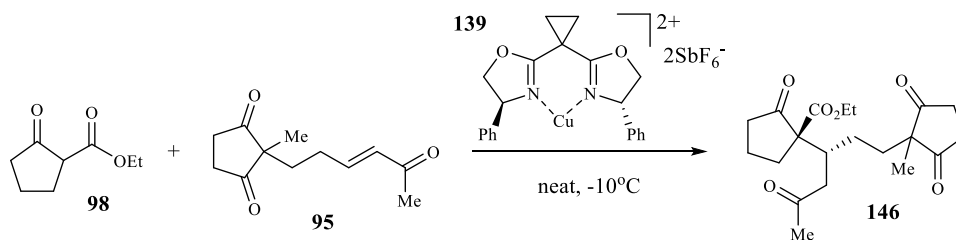
hexanes) to afford ethyl (*R*)-1-((*R*)-1-(1-methyl-2,6-dioxocyclohexyl)-5-oxohexan-3-yl)-2-oxocyclohexane-1-carboxylate (**144**) (310 mg, 0.79 mmol, 88% yield, 7:1 dr, 92% ee) as a colorless oil. $[\alpha]_D^{26} = +17.8$ (c 1.2, CHCl₃); ¹H NMR (700 MHz, CDCl₃) δ 4.14-4.11 (m, 1H), 4.07-4.04 (m, 1H), 2.73-2.69 (m, 2H), 2.61-2.55 (m, 4H), 2.37-2.33 (m, 3H), 2.20(dd, J = 4.9, 17.5 Hz, 1H), 2.10 (s, 3H), 2.00-1.97 (m, 1H), 1.92-1.85 (m, 2H), 1.80-1.71 (m, 2H), 1.63 (td, J = 4.2, 13.3 Hz, 1H), 1.57-1.53 (m, 2H), 1.44-1.42 (m, 1H), 1.22 (t, J = 7.0 Hz, 3H), 1.17-1.13 (m, 1H), 1.13 (s, 3H), 0.89-0.83 (m, 1H); ¹³C NMR (175 MHz, CDCl₃) δ 210.1, 210.0, 207.3, 207.2, 171.4, 65.9, 64.2, 61.5, 45.1, 41.2, 37.7, 37.6, 36.1, 36.1, 32.3, 30.1, 27.3, 26.8, 22.2, 18.5, 17.8, 14.0. HRMS (ESI): m/z calcd for C₂₂H₃₄O₆⁺ [M+H]⁺ 393.2272, found 393.2274; IR (thin film, cm⁻¹): 2940, 1692, 1363, 1206. Enantiopurity was determined to be 91% ee by chiral HPLC (DAICEL CHIRALPAK AD-H, 25 cm x 4.6 mm, hexanes/2-propanol = 85/15, flow rate = 1 mL/min, λ = 290.0 nm, RT(minor) = 8.7 min, RT(major) = 11.4 min).



Ethyl(*R*)-1-((*R*)-4-methyl-2-oxooctan-4-yl)-2-oxocyclohexane-1-carboxylate (**145**)

Michael acceptor **107** (141 mg, 1.12 mmol, 1.0 equiv.) and bis(oxazoline)copper(II) complex (95 mg, 0.11 mmol, 0.1 equiv.) were cooled to -10 °C. Michael donor **94** (0.20 mL, 1.23 mmol, 1.1 equiv.) was then added and the reaction mixture was stirred neat at -10 °C for 48 hours. The reaction mixture was purified directly by column chromatography (grad. 0%→10% EtOAc in hexanes) to afford ethyl (*R*)-1-((*R*)-4-methyl-2-oxooctan-4-yl)-2-oxocyclohexane-1-carboxylate

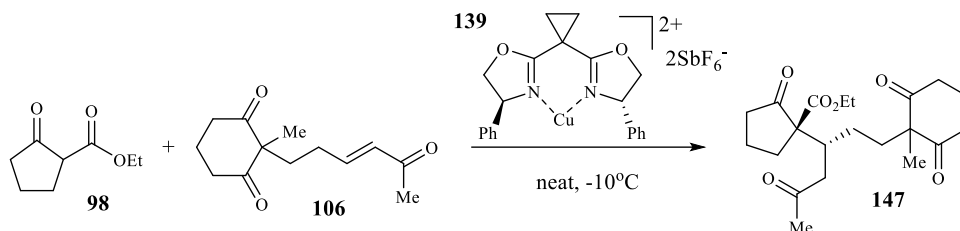
(145) (318 mg, 1.07 mmol, 96% yield, 10:1 dr, 92% ee) as a colorless oil. $[\alpha]_D^{26} = +14.7$ (c 1.0, CHCl_3); $^1\text{H NMR}$ (700 MHz, CDCl_3) δ 4.17-4.12 (m, 1H), 4.10-4.05 (m, 1H), 2.70-2.67 (m, 1H), 2.55 (dd, $J = 4.9, 17.5$ Hz, 1H), 2.42-2.35 (m, 3H), 2.28 (dd, $J = 5.6, 18.2$ Hz, 1H), 2.09 (s, 3H), 1.92-1.89 (m, 1H), 1.77-1.75 (m, 1H), 1.62-1.58 (m, 3H), 1.34-1.08 (m, 9H), 0.82 (t, $J = 7.0$ Hz, 3H); $^{13}\text{C NMR}$ (175 MHz, CDCl_3) δ 207.7, 207.4, 171.7, 64.5, 61.2, 45.6, 41.2, 36.1, 32.7, 31.8, 30.3, 29.9, 26.9, 22.8, 22.2, 14.0, 14.0; HRMS (ESI): m/z calcd for $\text{C}_{17}\text{H}_{30}\text{O}_4^+$ $[\text{M}+\text{H}]^+$ 297.2060, found 297.2063; IR (thin film, cm^{-1}): 2936, 1708, 1363, 1205, 1134. Enantiopurity was determined to be 94% ee by chiral HPLC (DAICEL CHIRALPAK AD-H, 25 cm x 4.6 mm, hexanes/2-propanol = 99.5/0.5, flow rate = 1 mL/min, $\lambda = 285.0$ nm, $\text{RT}(\text{minor}) = 17.4$ min, $\text{RT}(\text{major}) = 18.6$ min).



Ethyl (R)-1-((R)-1-(1-methyl-2,5-dioxocyclopentyl)-5-oxohexan-3-yl)-2-oxocyclopentane-1-carboxylate (146**)**

Michael acceptor **95** (265 mg, 1.27 mmol, 1.0 equiv.) and bis(oxazoline)copper(II) complex (113 mg, 0.13 mmol, 0.1 equiv.) were cooled to -10°C . Michael donor **98** (0.38 mL, 2.29 mmol, 2 equiv.) was then added and the reaction mixture was stirred neat at -10°C for 24 hours. The reaction mixture was purified directly by column chromatography (grad. 20%→33% EtOAc in hexanes) to afford ethyl (R)-1-((R)-1-(1-methyl-2,5-dioxocyclopentyl)-5-oxohexan-3-yl)-2-oxocyclopentane-1-carboxylate (**146**) (396 mg, 1.09 mmol, 86% yield, 96% ee) as a colorless oil.

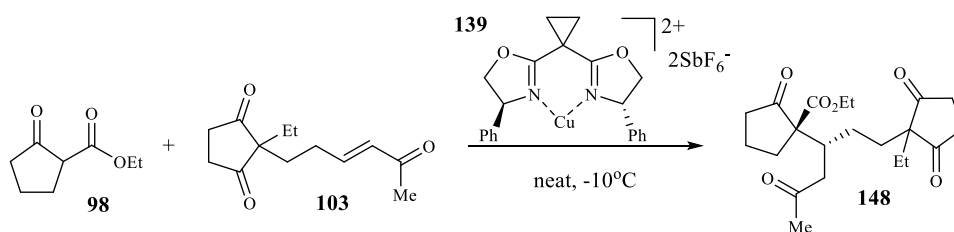
$[\alpha]_D^{26} = -32.1$ (c 1.1, CHCl_3); $^1\text{H NMR}$ (700 MHz, CDCl_3) 4.01 (q, $J = 7.0$ Hz, 2H), 2.90 (dd, $J = 4.9, 18.2$ Hz, 1H), 2.73-2.63 (m, 4H), 2.40-2.37 (m, 1H), 2.31-2.30 (m, 1H), 2.24-2.12 (m, 3H), 2.04 (s, 3H), 1.93-1.89 (m, 1H), 1.85-1.77 (m, 2H), 1.48 (t, $J = 8.4$ Hz, 2H), 1.14 (t, $J = 7.0$ Hz, 3H), 1.11-1.08 (m, 1H), 1.05-1.01 (m, 1H), 0.97 (s, 3H); $^{13}\text{C NMR}$ (175 MHz, CDCl_3) δ 216.0, 216.0, 214.2, 207.6, 170.2, 63.4, 61.3, 56.6, 44.7, 38.3, 36.6, 35.0, 34.9, 33.4, 31.9, 29.9, 26.6, 19.2, 19.0, 13.9; HRMS (ESI): m/z calcd for $\text{C}_{20}\text{H}_{30}\text{O}_6^+$ $[\text{M}+\text{H}]^+$ 365.1959, found 365.1960; IR (thin film, cm^{-1}): 2966, 1714, 1363, 1223, 1157. Enantiopurity was determined to be 96% ee by chiral HPLC (DAICEL CHIRALPAK AD-H, 25 cm x 4.6 mm, hexanes/2-propanol = 88/12, flow rate = 1 mL/min, $\lambda = 290.0$ nm, $\text{RT}(\text{minor}) = 12.5$ min, $\text{RT}(\text{major}) = 15.4$ min).



Ethyl (R)-1-((R)-1-(1-methyl-2,6-dioxocyclohexyl)-5-oxohexan-3-yl)-2-oxocyclopentane-1-carboxylate (147**)**

Michael acceptor **106** (200 mg, 0.90 mmol, 1.0 equiv.) and bis(oxazoline)copper(II) complex (**78** mg, 0.11 mmol, 0.1 equiv.) were cooled to -10°C . Michael donor **98** (0.27 mL, 1.80 mmol, 2 equiv.) was then added and the reaction mixture was stirred neat at -10°C for 24 hours. The reaction mixture was purified directly by column chromatography (grad. 20%→33% EtOAc in hexanes) to afford ethyl (R)-1-((R)-1-(1-methyl-2,6-dioxocyclohexyl)-5-oxohexan-3-yl)-2-oxocyclopentane-1-carboxylate (**147**) (295 mg, 0.78 mmol, 86% yield, 20:1 dr, 95% ee) as a colorless oil. $[\alpha]_D^{26} = -30.4$ (c 1.0, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) 4.07 (q, $J = 7.2$ Hz, 2H),

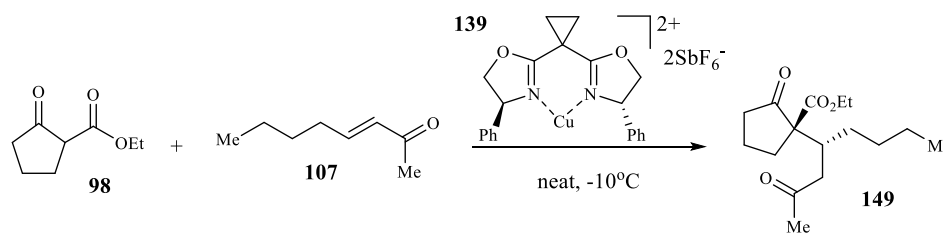
2.90 (dd, $J = 5.6, 18.4$ Hz, 1H), 2.64-2.58 (m, 4H), 2.46-2.42 (m, 2H), 2.31-2.17 (m, 3H), 2.10 (s, 3H), 1.97-1.82 (m, 5H), 1.74-1.69 (m, 2H), 1.19 (t, $J = 7.2$ Hz, 3H), 1.15 (s, 3H), 1.09-1.02 (m, 2H); ^{13}C NMR (175 MHz, CDCl_3) δ 214.4, 210.1, 210.1, 207.9, 170.2, 65.5, 63.8, 61.4, 45.0, 38.4, 37.8, 37.8, 36.9, 35.3, 31.7, 30.1, 27.2, 20.1, 19.3, 17.6, 14.0; HRMS (ESI): m/z calcd for $\text{C}_{21}\text{H}_{32}\text{O}_6^+$ $[\text{M}+\text{H}]^+$ 379.2115, found 379.2116; IR (thin film, cm^{-1}): 2963, 1713, 1692, 1364, 1224, 1160. Enantiopurity was determined to be 95% ee by chiral HPLC (DAICEL CHIRALPAK AD-H, 25 cm x 4.6 mm, hexanes/2-propanol = 90/10, flow rate = 1 mL/min, $\lambda = 291.0$ nm, $\text{RT}(\text{minor}) = 13.0$ min, $\text{RT}(\text{major}) = 15.9$ min). minor) = 12.5 min, $\text{RT}(\text{major}) = 15.4$ min).



Ethyl (R)-1-((R)-1-(1-ethyl-2,5-dioxocyclopentyl)-5-oxohexan-3-yl)-2-oxocyclopentane-1-carboxylate (148)

Michael acceptor **103** (181 mg, 0.82 mmol, 1.0 equiv.) and bis(oxazoline)copper(II) complex (71 mg, 0.08 mmol, 0.1 equiv.) were cooled to -10 °C. Michael donor **98** (0.24 mL, 1.63 mmol, 2 equiv.) was then added and the reaction mixture was stirred neat at -10 °C for 24 hours. The reaction mixture was purified directly by column chromatography (grad. 20%→33% EtOAc in hexanes) to afford ethyl (R)-1-((R)-1-(1-ethyl-2,5-dioxocyclopentyl)-5-oxohexan-3-yl)-2-oxocyclopentane-1-carboxylate (**148**) (254 mg, 0.67 mmol, 82% yield, 19:1 dr, 92% ee) as a colorless oil. $[\alpha]_{\text{D}}^{26} = -23.1$ (c 1.0, CHCl_3); ^1H NMR (700 MHz, CDCl_3) δ 4.10 (q, $J = 7.2$ Hz, 2H), 2.04 (dd, $J = 6.1, 18.4$ Hz, 1H), 2.72-2.65 (m, 4H), 2.47 (qn, $J = 6.0$ Hz, 1H) 2.37-2.21 (m, 4H),

2.12 (s, 3H), 2.01-1.97(m, 1H), 1.93-1.85 (m, 2H), 1.64-1.54 (m, 4H), 1.22 (t, J = 7.2 Hz, 3H), 1.19- 1.15 (m, 1H), 1.21-1.06 (m, 1H), 0.76 (t, J = 7.5 Hz, 3H); ^{13}C NMR (175 MHz, CDCl_3) δ 216.8, 216.7, 214.4, 207.8, 170.3, 63.4, 61.5, 61.4, 44.6, 38.4, 36.9, 36.1, 36.0, 32.3, 30.1, 28.4, 26.8, 19.3, 14.0, 8.9; HRMS (ESI): m/z calcd for $\text{C}_{21}\text{H}_{30}\text{O}_6\text{Na}^+$ $[\text{M}+\text{Na}]^+$ 401.1935, found 401.1939; IR (thin film, cm^{-1}): 2968, 1713, 1222, 1157, 1023. Enantiopurity was determined to be 92% ee by S9 chiral HPLC (DAICEL CHIRALPAK AD-H, 25 cm x 4.6 mm, hexanes/2-propanol = 90/10, flow rate = 1 mL/min, λ = 286.0 nm, RT(minor) = 12.0 min, RT(major) = 15.6 min).



Ethyl (*R*)-1-((*R*)-4-methyl-2-oxooctan-4-yl)-2-oxocyclopentane-1-carboxylate (**149**)

Michael acceptor **107** (150 mg, 1.19 mmol, 1.0 equiv.) and bis(oxazoline)copper(II) complex (100 mg, 0.12 mmol, 0.1 equiv.) were cooled to $-10\text{ }^\circ\text{C}$. Michael donor **98** (0.19 mL, 1.31 mmol, 1.1 equiv.) was then added and the reaction mixture was stirred neat at $-10\text{ }^\circ\text{C}$ for 48 hours. The reaction mixture was purified directly by column chromatography (grad. 0% \rightarrow 10% EtOAc in hexanes) to afford ethyl (*R*)-1-((*R*)-4-methyl-2-oxooctan-4-yl)-2-oxocyclopentane-1-carboxylate (**149**) (295 mg, 1.05 mmol, 88% yield, 91% ee) as a colorless oil. $[\alpha]_D^{26} = -58.5$ (c 1.0, CHCl_3); ^1H NMR (700 MHz, CDCl_3) δ 4.10 (q, J = 7.0 Hz, 2H), 2.95 (dd, J = 6.3, 18.2 Hz, 1H), 2.62-2.58 (m, 1H), 2.53-2.50 (m, 1H), 2.33-2.22 (m, 3H), 2.11 (s, 3H), 2.02-1.87 (m, 3H), 1.30-1.11(m, 9H), 0.84 (t, J = 7.0 Hz, 3H); ^{13}C NMR (175 MHz, CDCl_3) δ 214.6, 208.0, 170.4, 64.0, 61.3, 45.2, 38.6, 37.1, 31.7, 31.5, 30.2, 29.9, 22.7, 19.3, 14.0, 13.9; HRMS (ESI): m/z calcd for $\text{C}_{16}\text{H}_{28}\text{O}_4^+$ $[\text{M}+\text{H}]^+$

283.1904, found 283.1902; IR (thin film, cm^{-1}): 2957, 1714, 1222, 1156. Enantiopurity was determined to be 91% ee by chiral HPLC (DAICEL CHIRALPAK OD-H, 25 cm x 4.6 mm, hexanes/2-propanol = 99/1, flow rate = 1 mL/min, λ = 295.0 nm, RT(major) = 7.5 min, RT(minor) = 9.4 min).

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Chapter 4

Concise Enantioselective Total Synthesis and Biological Evaluation of Cardiotonic Steroids

19-Hydroxysarmentogenin, Trewianin Aglycone, Panogenin, and *cis*-Panogenin¹

(A portion of this work has been published in: Kaplan, W.; Khatri, H. R.; Nagorny, P. “Concise Enantioselective Total Synthesis of the Cardiotonic Steroids 19-Hydroxysarmentogenin and Trewianin Aglycone” *J. Am. Chem. Soc.* **2016**, *138*, 7194.)

4.1. Introduction

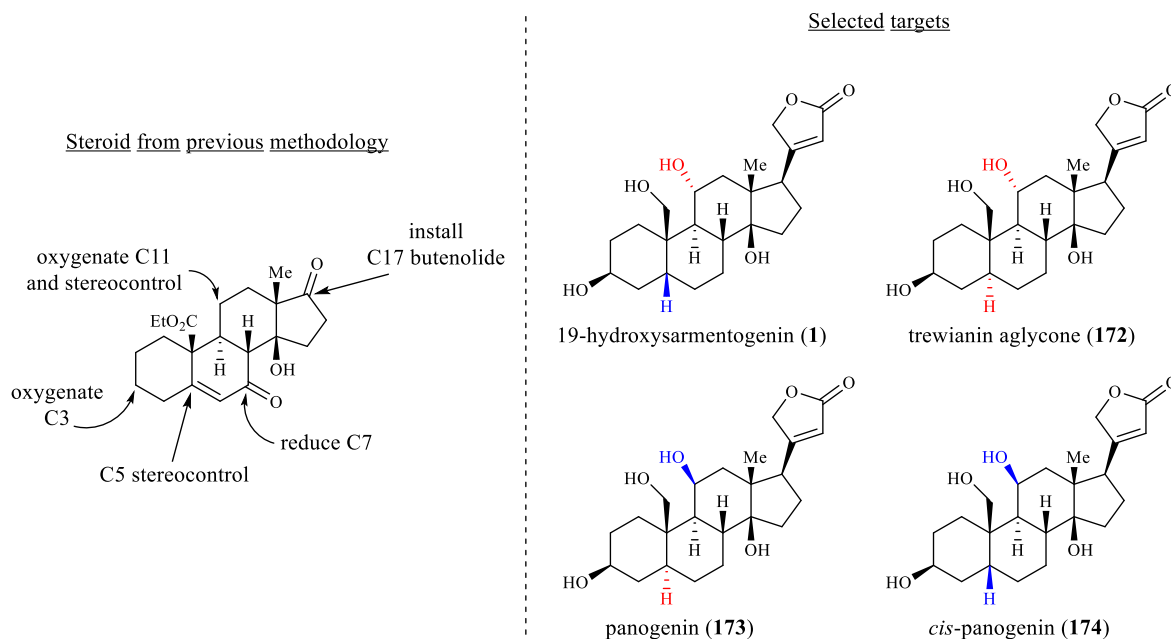
Steroids are some of the most pervasive, naturally occurring small molecules. Despite having a common tetracyclic core motif, steroids with differing stereochemical patterns and peripheral substitutions can elicit a remarkable range of therapeutic effects.²⁻⁶ One particularly useful class of steroids are the cardenolides. These plant-derived steroids are potent inhibitors of the Na⁺/K⁺-ATPase pump on cardiac myocytes; an activity which causes a positive inotropic response in humans and helps combat the effects of congestive heart failure.⁷ Additionally, recent work has demonstrated the anticancer activity of cardenolides.⁸⁻¹⁰ However, a narrow therapeutic window and lack of understanding regarding the antiproliferative effects of cardenolides necessitates the synthesis of new steroid analogues in pursuit of therapeutically optimized drug candidates. The purpose of the work described here is to delineate a new and more effective approach for the synthesis of cardenolide steroids; one which allows unprecedented access to a number of cardenolide and cardenolide-like compound. By developing a concise route to these polyoxygenated steroids, we strive to improve the synthetic access to the region of chemical space

surrounding the cardenolides. This may eventually allow for the development of safer and more effective cardenolide-based therapeutics with improved pharmacokinetic, pharmacodynamic, metabolic, and toxicity profiles for the treatment of congestive heart failure and cancer.

4.2. Selected Targets and Approach Toward Total Synthesis

The initial targets of interest chosen were 19-hydroxysarmentogenin (**1**), trewianin aglycone (**172**), panogenin (**173**), and *cis*-panogenin (**174**). 19-hydroxysarmentogenin was chosen because it was synthesized previously by Inoue and co-workers and would serve as a valuable benchmark for comparison.¹¹ Trewianin aglycone, panogenin, and *cis*-panogenin represent the remaining three possible stereoisomers of the C5 and C11 positions. The goal of this decision is to eventually explore how the configuration at these positions affect the binding and biological activity of these cardenolides. The primary vehicle for synthesizing these targets was predicated upon the methodology outlined in chapter 3.¹² By optimizing and expanding on the tandem Michael addition/intramolecular double aldol approach to steroidal cores developed previously, we envisioned concise access to a number of natural and unnatural cardenolides. This approach, however, necessitates certain modifications. The targets chosen contain oxygenation at both C3 and C11. The functionality of these sites is absent in the methodology described previously, wherein both C3 and C11 are unoxidized. Additionally, our developed method (Chapter 3) results in oxygenation of the C7 position of the steroidal products, while natural cardenolides do not contain oxygenation at C7 and so this position must be reduced to a methylene. Finally, stereodivergent routes for constructing the differential chirality at C5 and C11 must be devised and the characteristic β -oriented butenolide ring at C17 must be installed. These required modifications and the selected targets are illustrated in **Figure 4.1**.

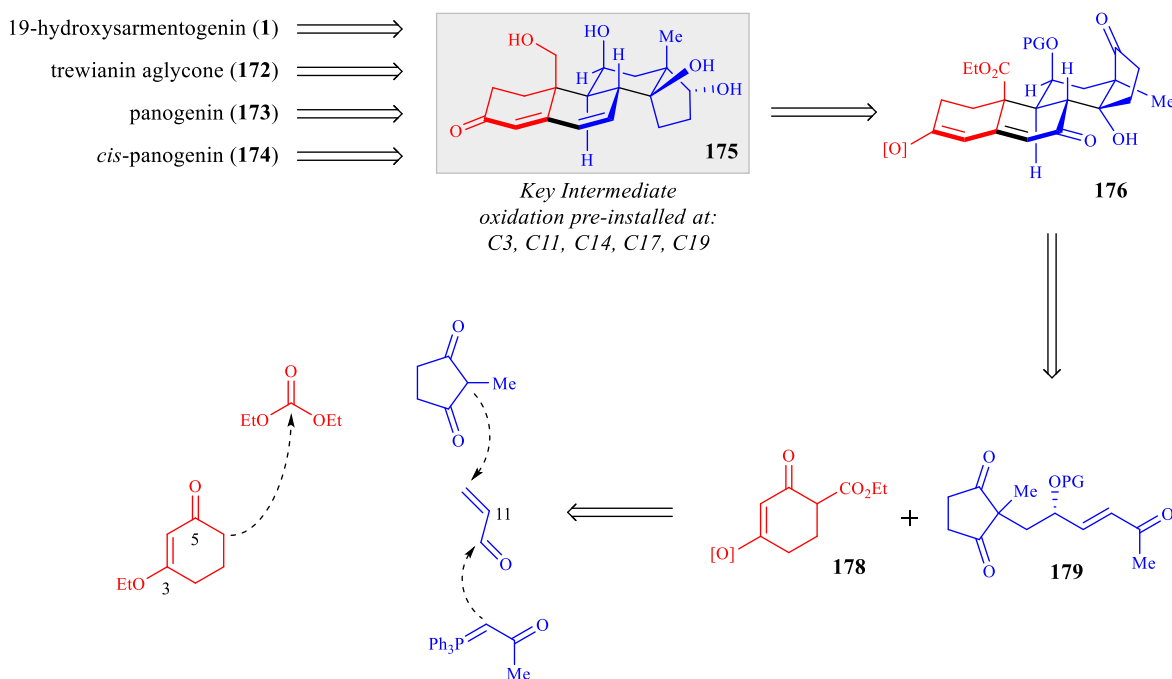
Figure 4.1. Required Modifications to Previous Methodology and Selected Targets.



In order to accomplish the stereodivergent total synthesis of all the targets, we envisioned a retrosynthetic design wherein all four cardenolides could be elaborated from a common, highly functionalized key intermediate. It is desirable to develop a synthetic plan wherein the point of divergence to each individual target occurs as a late as possible – allowing scalable synthesis of the key intermediate which can then be diverted to a desired target in as few steps as possible. To this effect, and utilizing the same disconnection approach described in chapter 3, we conceived of a retrosynthetic design as depicted in **Scheme 4.1**. Divergence occurs from the key intermediate **175**. Importantly, this steroidal core contains pre-installed oxidative information at all positions required for concise, stereodivergent elaboration to the natural cardenolides. Additionally, the construction of **175** is readily attainable from steroid **176**. Fragments of architecture **178** and **179** would be coupled via Michael addition/cyclization to produce intermediate **176**. By pre-installing the oxidative information and functional handles on the chemically simpler starting fragments

prior to coupling and cyclization, we can avoid more difficult late stage redox manipulations and the need to functionalize inert positions – allowing more concise access to a chemically complex and heavily functionalized key intermediate.

Scheme 4.1. Retrosynthesis of Selected Targets via Key Intermediate **175**.

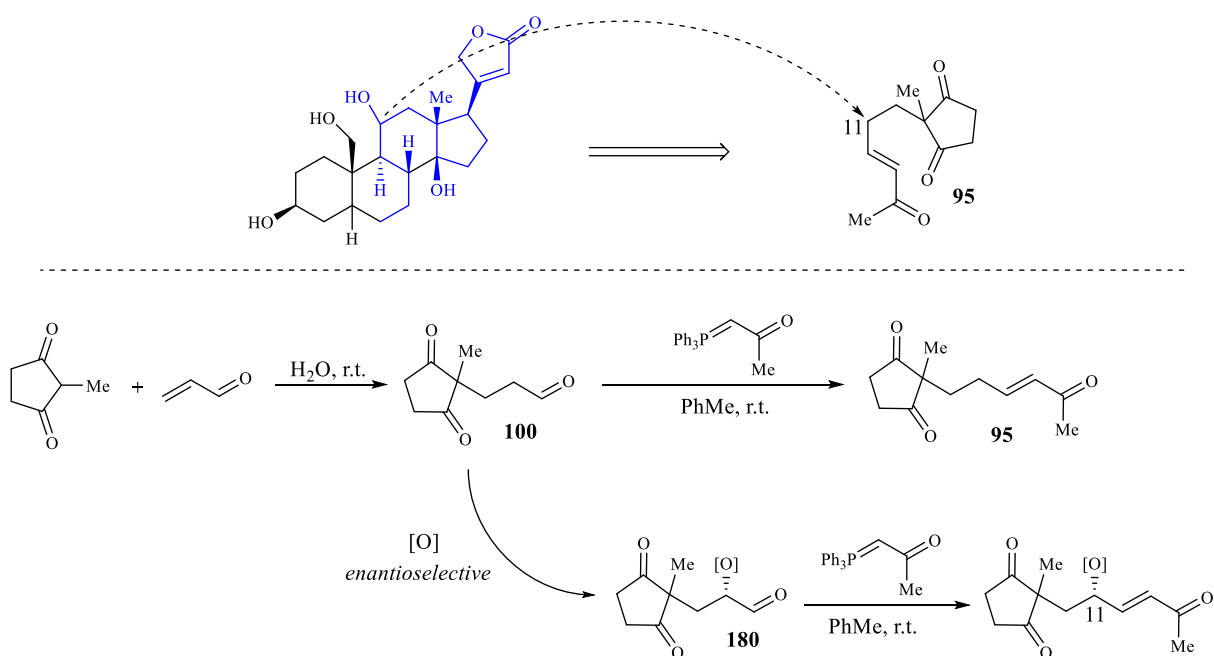


4.3. Installing C11 Oxygenation

The first functionality we examined was the stereoselective pre-installation of C11 oxygenation on the enone fragment. Back-tracing the carbon atoms through the synthetic design reveals that the eventual C11 position is the γ -carbon of the enone fragment. With a well-established and scalable route to the bare enone already established, we examined possibilities for modifying the known route to incorporate oxidation of the γ -carbon with minimal added steps. To achieve this, we quickly focused on the intermediate aldehyde **100** (**Scheme 4.2.**). At this stage, the C11 carbon is α to an aldehyde and most amenable to derivatization due to its high reactivity.

We envisioned here an asymmetric organocatalytic α -oxidation of the aldehyde to install oxygenation in a stereoselective manner, producing structure **180**. Importantly, this early incorporation of oxygenation with a high level of stereocontrol ensures the enantiopurity of all remaining intermediates of the synthesis – requiring only diastereocontrol to set relative configurations at downstream stages in the route.

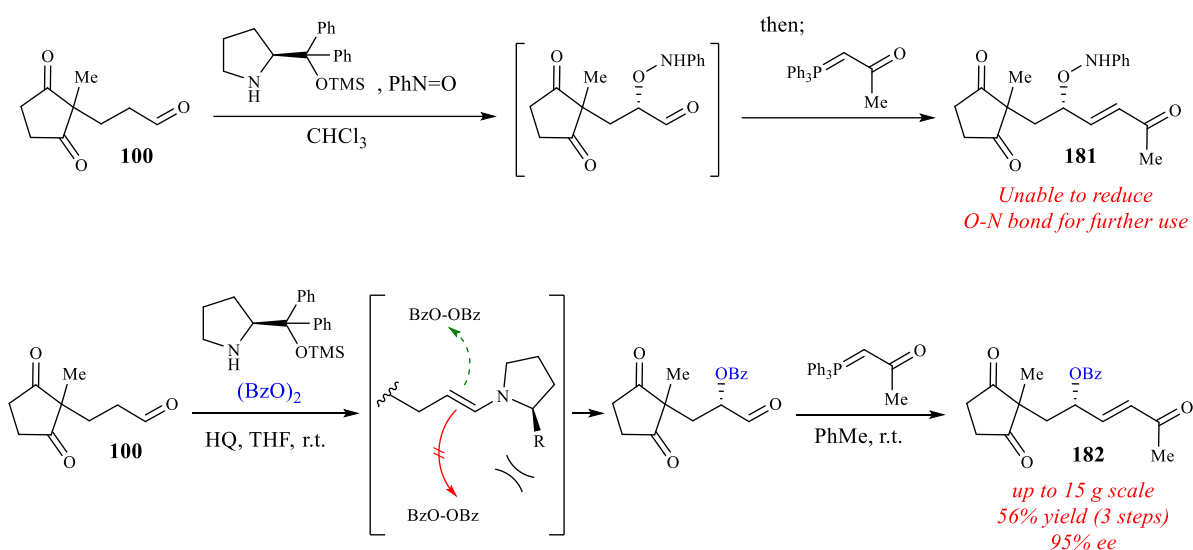
Scheme 4.2. Plan for Pre-installation of C11 Oxygenation.



The first conditions we examined for the oxidation utilizes a proline-derived catalyst for the induction of asymmetry via formation of a chiral enamine in conjunction with nitrosobenzene as the electrophilic oxygen source (**Scheme 4.3**).¹³ The proline-derived catalyst is readily synthesized.¹⁴ We suspected that the oxidation procedure could be coupled with the subsequent Wittig olefination in a single pot procedure to afford the desired oxidized enone fragment in one step. Initial attempts at this one-pot oxidation/Wittig olefination were promising and successfully delivered the desired C11 oxidized enone **181** in moderate yield. However, subsequent attempts to

reduce the O-N bond under various conditions were unsuccessful, leading to a complex mixture of products, or failing to produce the desired allylic alcohol. Attention was turned toward a similar report of an asymmetric α -oxidation of aldehydes which utilized dibenzoyl peroxide (BPO) as the stoichiometric oxidant.^{15,16} This procedure has the added benefit of delivering a benzoyl protected alcohol directly.

Scheme 4.3. Asymmetric Organocatalytic α -Oxidation of Aldehyde **100**.

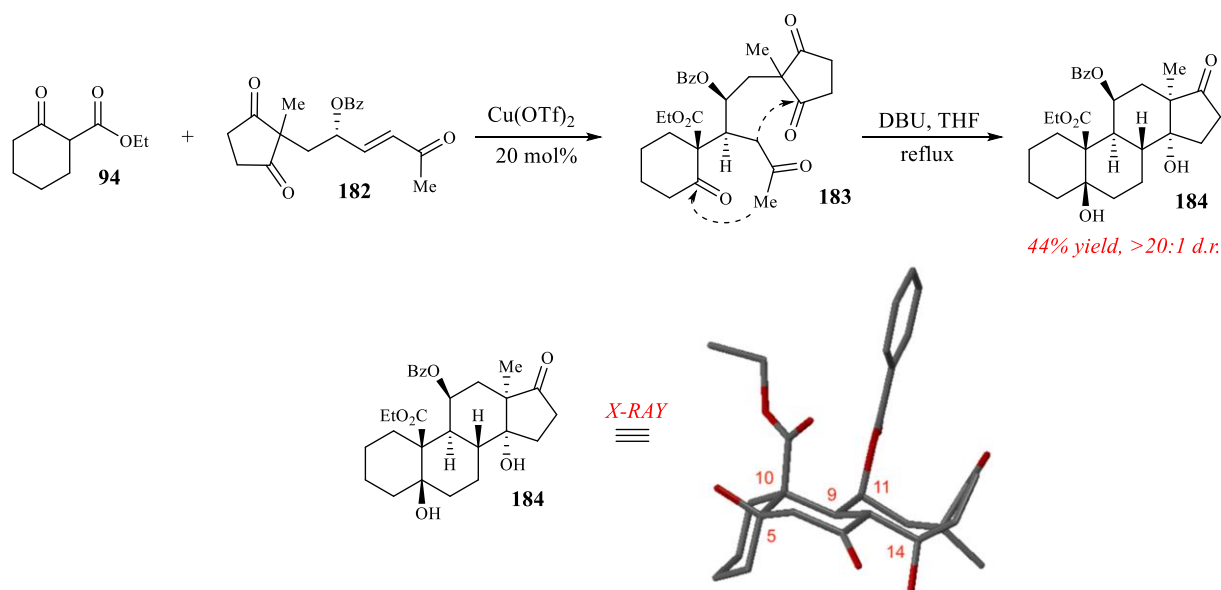


Initial attempts at a one-pot oxidation/olefination procedure were disappointingly unfruitful due to the production of a complex mixture of side products, including triphenyl phosphine oxide (TPPO), which greatly complicated the isolation procedure. However, since the mechanism of oxidation is ionic, and BPO is a known radical generating reagent, the addition of catalytic hydroquinone as a radical scavenger was able to diminish side-product formation. Unfortunately, this modification alone was insufficient to allow high isolated yields from a single-pot oxidation/olefination procedure. Rather, we found that isolation of the α -oxidized aldehyde by column chromatography prior to Wittig olefination afforded much better overall isolated yields.

Importantly, the silica gel purification procedure results in little to no epimerization of the chiral C11 center. After Wittig olefination, the desired fragment **182** with pre-installed oxygenation at C11 could be isolated in 56% yield (over three steps) on a 15 gram scale and in 95% enantiomeric excess as determined by HPLC. The primary concern regarding the development of such a C11 oxidized fragment is steric crowding of the electrophilic β -carbon of the enone potentially leading to an incompatibility in the key Cu(II)-catalyzed Michael addition or unexpected diastereoselectivity in the ensuing cyclization step.

To investigate the viability of fragment **182**, we tested its reactivity with the unfunctionalized A ring β -ketoester fragment **94** (Scheme 4.4.). Fortunately, the Michael addition proceeded to afford the desired adduct **183**. Based on the results of our previously developed methodology, we attempted the cyclization of adduct **183** with DBU in refluxing THF.

Scheme 4.4. Michael Addition and Cyclization Tests with Fragment **182**.



As anticipated, these cyclization conditions afforded a steroidal core of architecture **184**, containing a *cis*- β -A/B ring junction, the desired natural configuration at C8, C9, and C10, as well

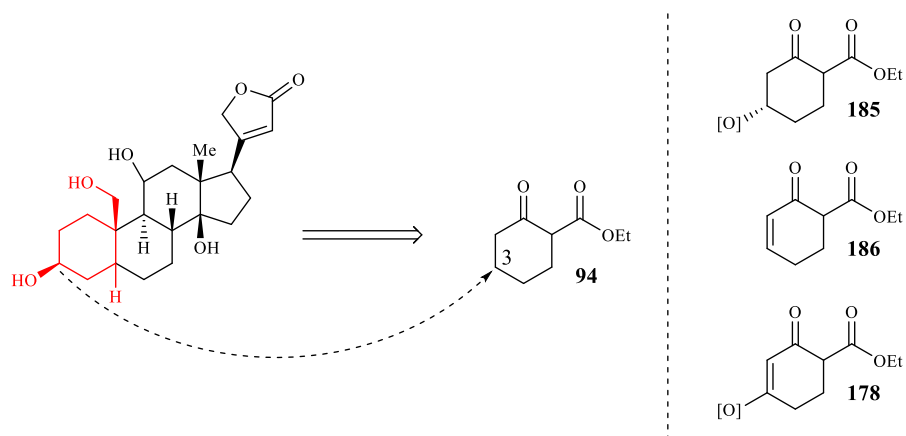
as the expected *cis*- α -C/D ring junction. X-ray crystallographic analysis confirmed the relative stereochemistry of the steroidal core. With sufficient evidence that the current C11 oxidized fragment **182** is able to participate in the designed approach without affecting the mode of diastereoselective cyclization, we turned our attention toward the design of a compatible C3 oxidized A ring fragment.

4.4. Installing C3 Oxygenation

The ability to oxygenate cardenolides at C3 is crucial because all natural cardenolides contain oxygenation at this position. In addition, the C3 hydroxy group serves as the point of sugar attachment for glycosylated cardenolides. Any future efforts at installing glycosides and investigating their effect on biological activity would rely on the presence of C3 oxygenation. Lastly, all cardenolides have a β -oriented hydroxy group at C3, so the ability to control this stereogenic carbon is another downstream task we had to consider.

Back-tracing the carbon positions through the retrosynthetic design reveals that the β ' carbon of the β -ketoester fragment is the eventual C3 position and so we focused on a method for pre-installing the necessary oxidative information at this position (**Scheme 4.5**).

Scheme 4.5. Plan for Pre-installation of C3 Oxygenation.

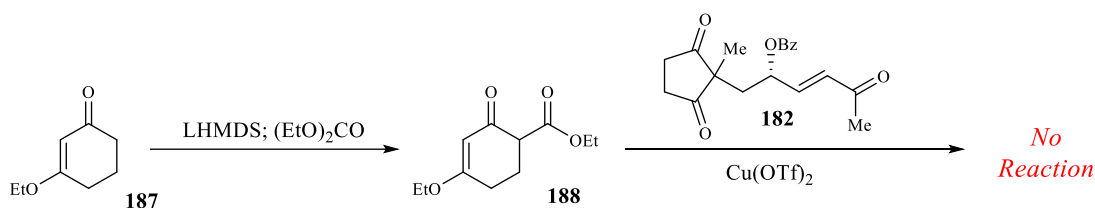


We first devised of three different structural approaches to accomplishing this task, these β -ketoester architectures are shown as **185**, **186**, and **178**. In the case of **185**, we envisioned a stereoselective oxidation of the C3 carbon so that the desired hydroxy group would be installed with the desired configuration. However, the primary concerns with this approach were twofold: 1) Too much complexity was being added at too early a stage. That is, we wanted to avoid the need for another catalytic chiral controller and rather let substrate control determine the configuration at C3 during a later stage, to allow for a more stereodivergent approach. And 2) β -hydroxyketone motifs of the type present in **185** are prone to E₁cb-type elimination and may generate an enone during either the copper(II)-catalyzed Michael addition or cyclization steps, destroying the chirality. Architecture of type **186** was also considered and seemed more viable. However, the presence of an olefin, while providing a useful functional handle, still requires additional downstream steps for installing oxygenation at the desired C3 position. To avoid this, we looked at β -ketoesters of structure **178**. This somewhat combines the desirable aspects of both **185** and **186** and allows for the direct incorporation of oxygenation at C3 (so we won't have to install it later), while avoiding the issues of elimination and enantioselectivity. The primary issue we anticipated with β -ketoesters like **178** was diminished reactivity. The enol ether moiety is a strong electron donor by resonance and is expected to reduce the acidity of the β -ketoester, perhaps by 100-fold or more if a rough comparison is made to the pK_a's of ethyl acetoacetate (pK_a \approx 11, H₂O) and dimethyl malonate (pK_a \approx 13, H₂O). This could lead to a lower concentration of the nucleophilic enol tautomer, and thus slow or halt the Michael addition with fragment **182**.

Initial attempts at making a C3 oxygenated fragment were successful and straightforward. The readily available enol ether **187** could be kinetically enolized with LHMDS and then acylated with diethyl carbonate to afford β -ketoester **188** in a single step and good yield. However, attempts

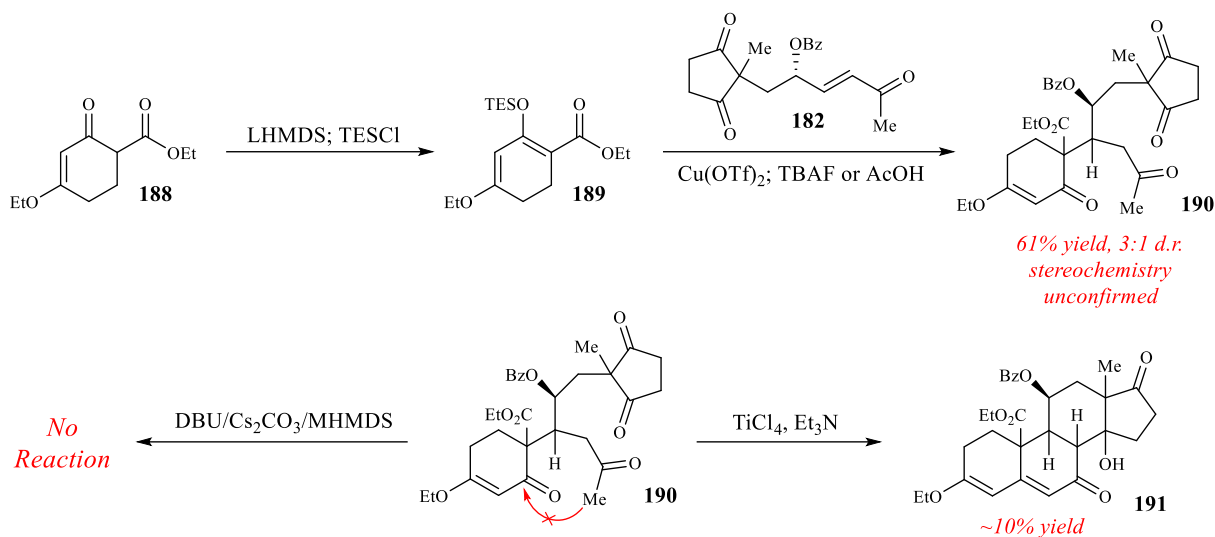
to couple fragment **188** with enone **182** via Cu(II)-catalyzed Michael addition were unsuccessful (**Scheme 4.6.**). As we feared, the added steric occlusion of the enone in conjunction with the deactivation of the β -ketoester resulted in a complete lack of reactivity despite attempts at optimization.

Scheme 4.6. Initial Attempt at Michael Addition with Fragment **188**.



To overcome this, we considered the possibility of activating fragment **188** as its silyl enol ether. This effectively locks the entire population of molecules in an enol-like configuration and may thus promote Mukaiyama Michael addition with enone **182** in the presence of Lewis acidic Cu(II). We were encouraged by various reports of other Cu(II)-catalyzed Mukaiyama Michael additions.^{17,18} Fortunately, **188** is readily enolized with LHMDS and trapped by the addition of TESC1 to provide silyl enol ether **189** (**Scheme 4.7.**). Initial attempts at the $\text{Cu}(\text{OTf})_2$ -catalyzed Mukaiyama Michael addition of **189** and **182** were very promising and the reaction would readily go to completion, producing Michael adduct **190**. However, the reaction resulted in a disappointing diastereoselectivity of 3:1 at best and would produce a complex mixture of silylated and desilylated Michael adducts. We could optimize the procedure slightly to include the addition of TBAF or AcOH after the reaction was complete to convert any silylated Michael adduct to its desilylated congener, making the mixture easier to interpret and purify. Despite the low diastereomeric ratio, we could isolate useful quantities of the desired major diastereomer **190**.

Scheme 4.7. Attempts at Mukaiyama Michael Addition and Subsequent Cyclization.



We surmise that the low diastereoselectivity is a result of the silylation of the β -ketoester, effectively hindering its ability to undergo coordination with copper which may have otherwise produced a tighter transition state and better selectivity. A significant amount of time and effort was then expended, unfruitfully, on the cyclization of Michael adduct **190**. While the conversion to a silyl enol ether was sufficient for affecting the desired Michael addition, the problem of the electron rich enol ether rose again and attempts to cyclize adduct **190** were made difficult by the attenuated electrophilicity of the C5 carbonyl. The use of the well-established DBU conditions failed to promote the cyclization and we turned instead to stronger bases. Unfortunately, the use of metal hexamethyldisilazides resulted in a complex mixture of degradation with significant amounts of retro-Michael products. Next, we explored the use of soft enolization conditions. At last, using a combination of TiCl_4 and Et_3N , we could observe the formation of some fully cyclized product **191**. However, the yields were very low and several attempts at optimization ultimately failed to produce useful quantities. Unfortunately, the determination of stereochemistry via NOE spectroscopy is only easily accomplished on the rigid steroidal systems following successful

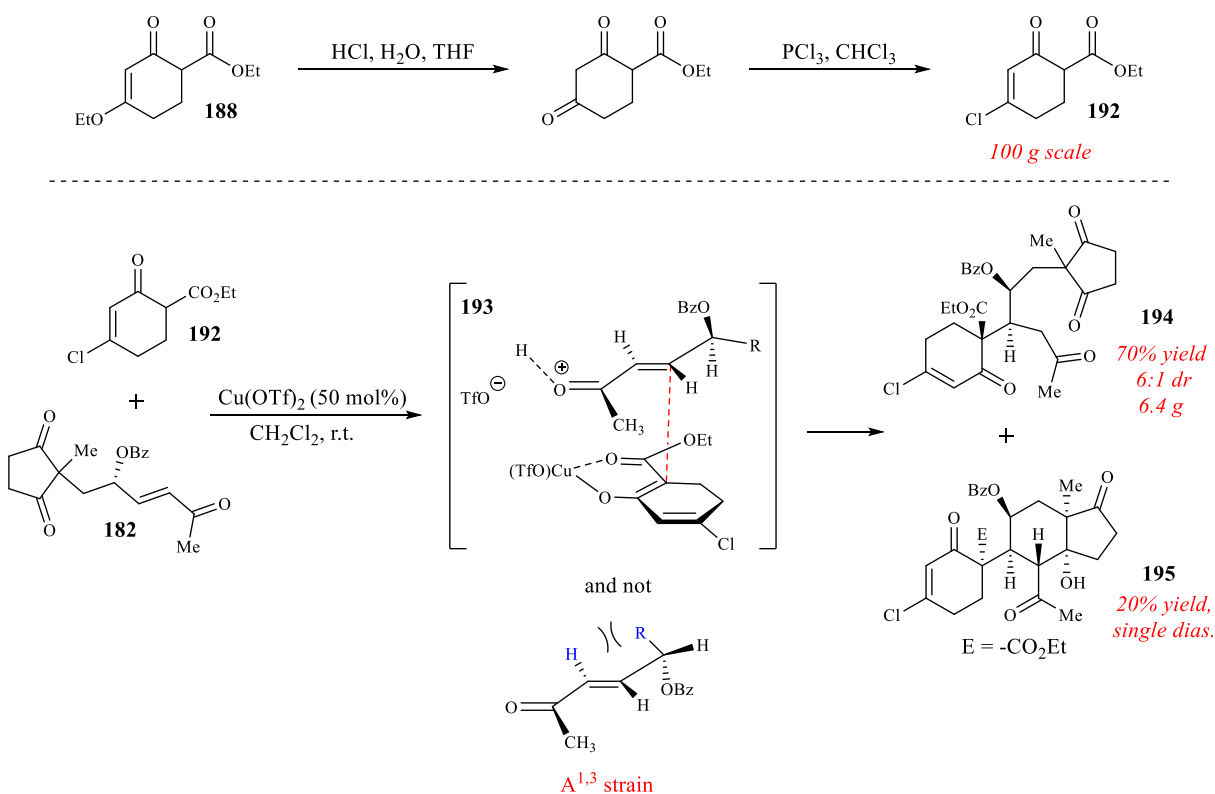
cyclization. Adducts such as **190** have too much rotational freedom and conformational flexibility for reliable NOE analysis under standard conditions. As such, the stereochemical pattern of **190** and **191** have not been elucidated.

It was clear that the electron-rich enol ether moiety was causing too many downstream issues so we focused on a redesign of the A ring fragment. Keeping in theme with the same overall architecture of type **178**, we imagined replacing the enol ether motif with a vinyl chloride, making fragment **192**.¹² This modification still permits straightforward conversion to oxygenation at a later stage without any additional steps and has the benefit of being an inductive activator of the C5 carbonyl, and a much weaker resonance donor; potentially enough to overcome the reactivity issues faced with fragment **188**. The vinyl chloride fragment can be synthesized in two steps from β -ketoester **188** (**Scheme 4.8**). First, the enol ether is hydrolyzed with HCl and water in THF. Subsequent chlorination with PCl_3 in CHCl_3 provides the desired chloroketoester **192** in good yield and high regioselectivity. We found that attempts at chlorination via Vilsmeier-Haack type reaction with oxalyl chloride and DMF produced only 1:1 regioselectivity. The synthesis of **192** was later optimized to allow for its preparation on a 100 gram scale.

With **192** in hand, we explored its ability to react conjugatively with enone **182** (**Scheme 4.8**). Fortunately, chloroketoester **192** was sufficiently reactive to participate in the desired Michael addition without the need for conversion to its silyl enol ether, and Michael adduct **194** could be isolated in 70% yield and 6:1 diastereoselectivity favoring the desired, natural configurations at C10 and C9. However, as we would expect, the reaction proceeds slowly with the typical 10 mol% loading of $\text{Cu}(\text{OTf})_2$. We discovered that by increasing the loading to 50 mol% and running the reaction at high concentration, it is typically complete in less than nine hours. A transition state model **193** is proposed to explain the observed diastereoselectivity. Three

major steric and stereoelectronic interactions are invoked. First, the potential for a vinylogous polar Felkin-Ahn interaction between the σ bonding electrons of the C11-OBz bond and the π -system of the enone may orient the -OBz group to block one face of the enone. Second, the avoidance of $A^{1,3}$ strain between C12 and the olefinic hydrogen would preferentially orient the -OBz group over the *re* face of the enone, exposing the *se* face for the Michael addition. Third, the chiral center at C11, due its saturation, will protrude radially out of the plane of the π -system and the chloroketoester may prefer an approach which places the rotatable -OEt group of the ester, rather than the flat and rigid ring system, closest to the chiral center at C11.

Scheme 4.8. Synthesis and Michael Addition of Chloroketoester Fragment **192**.



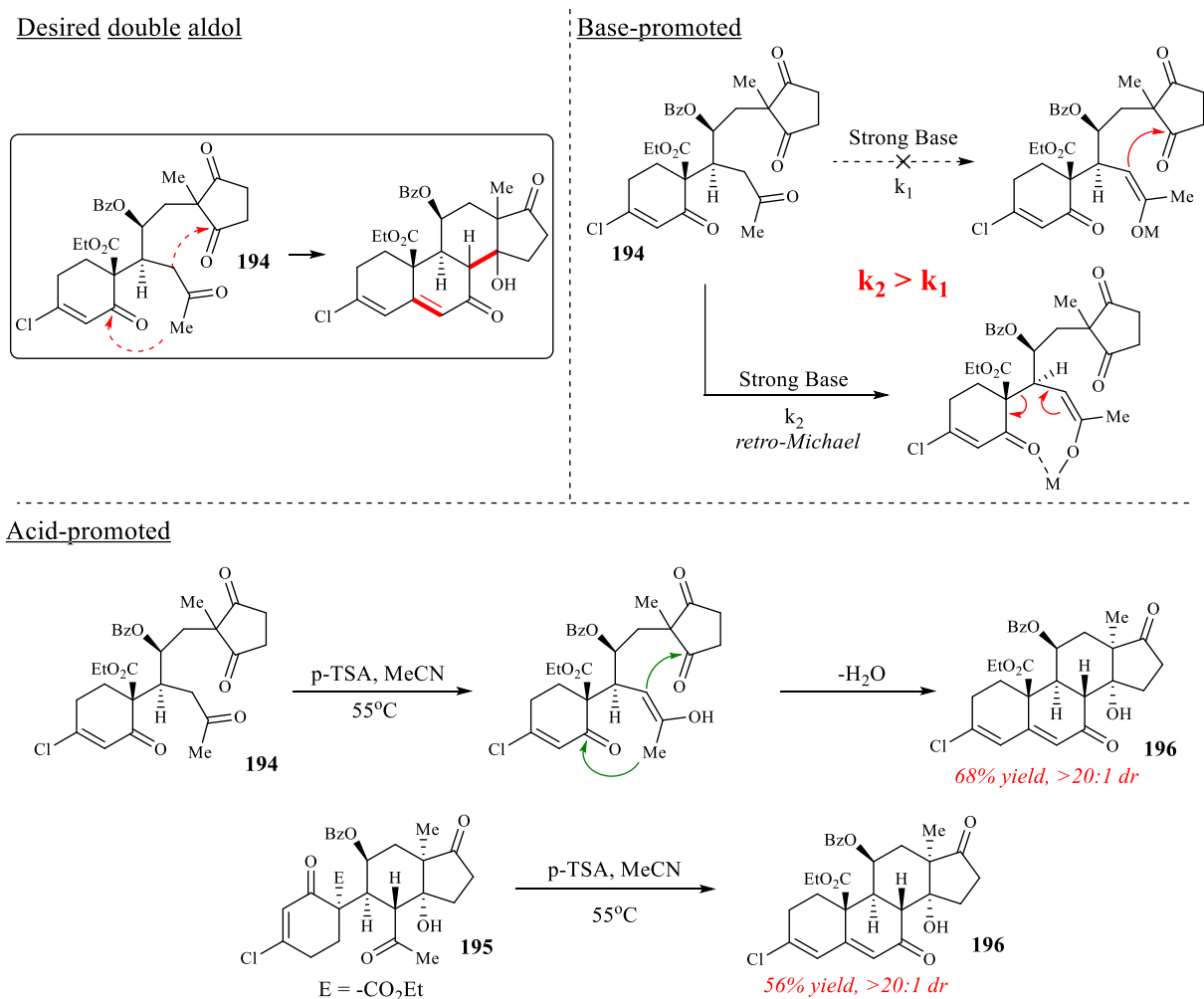
Finally, the last important result of the Michael addition to note is the production of side-product **195**. This compound results from the Cu(OTf)₂-catalyzed C ring closure aldol reaction

between C8 and a C14 carbonyl of the Michael adduct **194** and affords approximately 20% yield of the half-cyclized material which is readily isolated from the desired Michael adduct via column chromatography. We found that the Michael addition is best if monitored closely and halted quickly upon complete consumption of the enone **182**, extended reaction times often allow side-products to accumulate.

With Michael adduct **194** in hand, we focused on the key cyclization step. Keeping in mind the factors which we believe to influence the diastereoselectivity of the cyclizations (Chapter 3, scheme 3.12.), we anticipated the production of a *cis*- α -C/D ring junction no matter which conditions we used. This expectation is explained by the following two observations: 1) The C5 carbonyl of adduct **194** is mildly deactivated as an electrophile due to its conjugation as an enone and so we expect the aldol addition between C8 and C14 to occur faster, this means the reaction would proceed either through path 1 or 2 (Chapter 3, scheme 3.12.) and afford a *cis*- α -C/D ring junction. And 2) Our studies on the Michael addition of chloroketoester **192** and enone **182** revealed the production of half-cyclized side product **195** which already contains a *cis*- α -C/D junction; again, consistent with our previous observations. Nonetheless, and to our dismay, the usual DBU conditions were unsuccessful to promote cyclization of adduct **194**. It appeared the C5 carbonyl was still sufficiently deactivated to serve as an electrophile. Moreover, the use of stronger metal hexamethyldisilazides bases again failed due to the retro-Michael pathway. We reasoned that the generation of an enolate via strong bases is sufficient for creating significant negative charge at C8. In conjunction with the sterically congested C10-C9 bond, the enolized Michael adduct succumbs to rapid degradation and retro-Michael fragmentation before undergoing the desired cyclization. To combat this, we considered an acid-promoted cyclization. Such an approach would allow the double aldol cyclization to proceed by means of enols, not enolates

(Scheme 4.9.). This could translate to a weaker build-up of negative charge at C8 and would hinder the retro-Michael pathway, allowing cyclization to proceed. In addition, the production of half-cyclized side product **195** from the Cu(OTf)₂-catalyzed Michael addition provided some evidence that acidic conditions may allow for the desired aldol reaction to outcompete the retro-Michael pathway.

Scheme 4.9. Attempts and Conditions for Cyclization of Adduct **194** to Steroidal Core.



The use of p-TSA to promote adduct cyclization was preceded in our methodology development described in Chapter 3 (table 3.1.). As such, we investigated its application toward

the cyclization of adduct **194**. Fortunately, the p-TSA promoted cyclization proceeded smoothly. After some optimization, the use of excess p-TSA in MeCN at 55°C for 48 hours proved highly effective for the cyclization of adduct **194** into the steroidal core **196**, in 68% yield on a 5 gram scale and with complete diastereoselectivity. Importantly, **196** results from the dehydration product at C5, delivering an extended enone, and contains the expected but undesired *cis*- α -C/D ring junction stereochemistry.

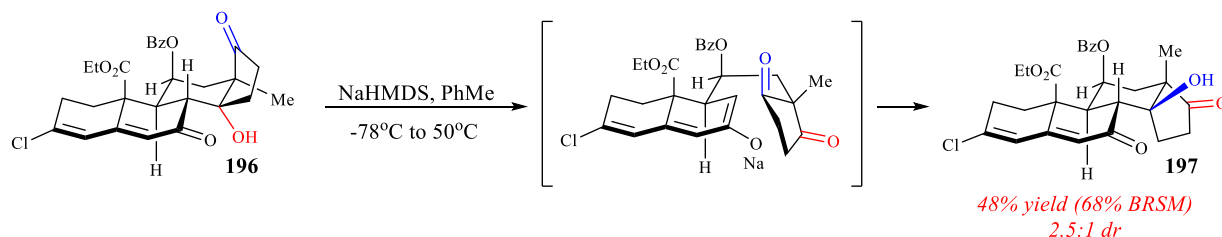
Additionally, the half-cyclized side product **195** from the Michael reaction could be subjected to the same p-TSA cyclization protocol to affect its conversion to the same steroidal core **196**, albeit in a lower 56% yield. With **196** in hand, we focused on its conversion to the key functionalized intermediate **175**. To accomplish this task, several transformations are required. First, the C/D ring junction must be converted to the natural *cis*- β orientation. Second, oxygenation must be removed from C7 and installed at C3. Third, the C19 and C11 hydroxy groups must be reduced/deprotected.

4.5. Synthesis of Key Intermediate **175**

With the successful modification of our developed methodology to allow for the pre-installation of both C11 oxygenation on the enone fragment and C3 oxygenation on the A ring fragment, we focused on the concise synthesis of our idealized key intermediate **175** containing all the necessary functionality for divergence to our target cardenolides. The first objective was to epimerize the *cis*- α -C/D ring junction to a natural *cis*- β -C/D ring junction. We knew from our previous investigations into cyclization protocols that the presence of unsaturation at C5 would thermodynamically favor a *cis*- β -C/D junction. Thus, we imagined that the *cis*- α -C/D junction of steroid **196** could be epimerized under equilibrating conditions which puts the opening and closing of the C ring in flux. Both acidic and basic conditions were extensively tested for their ability to

promote this key epimerization. Unfortunately, acidic conditions would often result in elimination of the C14 hydroxy group prior to, or competing with, any retro-aldol process. Focusing on the use of base, we discovered that weak alkyl amine bases were insufficient to promote any desired reaction. However, the more basic metal hexamethyldisilazides were able to generate the necessary C14 alkoxide, and upon heating, a diastereomeric mixture of steroids was produced. Interestingly, the sodium cation provided the best results in terms of isolated yield and the use of a non-coordinating solvent, toluene in this case, was required. More polar solvents such as THF or DMF would cause formation of an unidentified side-product (perhaps resulting from epimerization at C9) which was very difficult to isolate from the desired product. Using NaHMDS in toluene, steroid **196** could be epimerized to the *cis*- β -C/D junction intermediate **197** in a 2.5:1 diastereomeric ratio (**Scheme 4.10**). The desired product **197** could be isolated in 48% yield (68% BRSM) and the starting material could be recovered in 30% yield, allowing it to be effectively recycled.

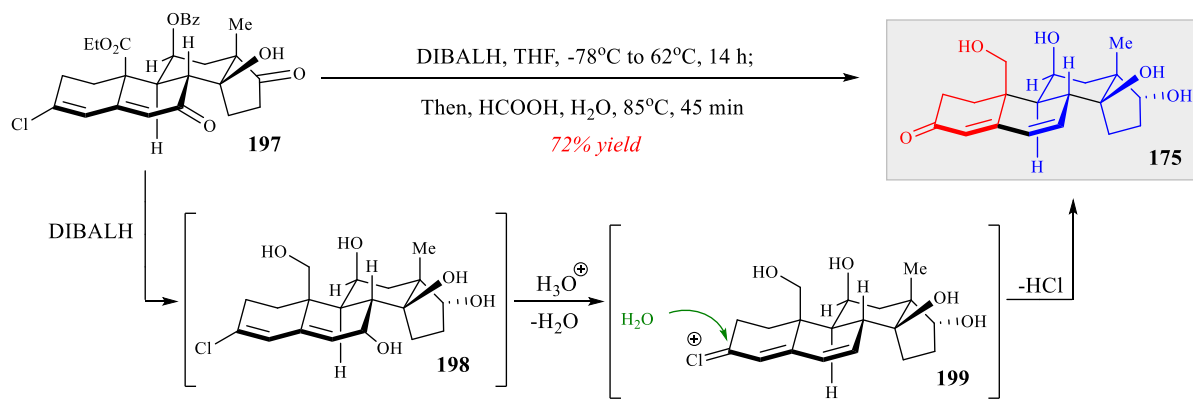
Scheme 4.10. Epimerization of C/D Ring Junction, Affording Steroid **197**.



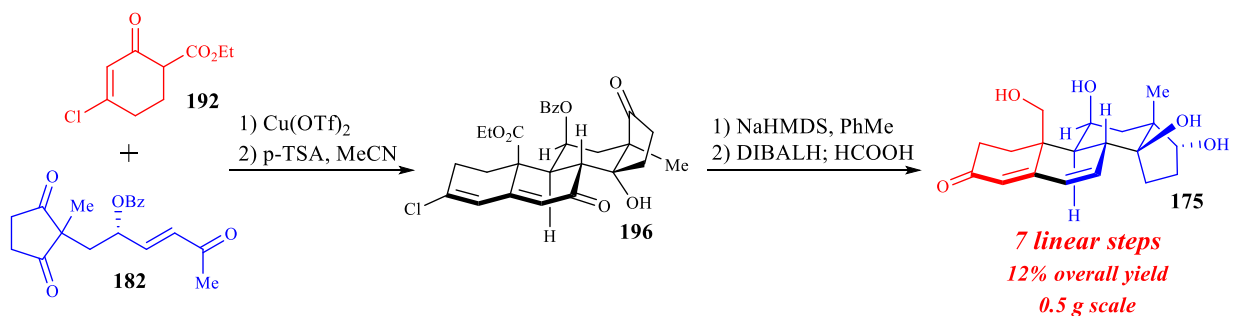
The remaining transformations included reduction of C7 and C19, oxygenation at C3, and deprotection of the C11-OBz group. To accomplish this, we envisioned an imaginative global reduction/transposition sequence. First, steroid **197** would be reduced globally, generating a primary alcohol at C19, secondary alcohols at C7 and C17, and deprotection of the C11 hydroxy

group (intermediate **198**, **Scheme 4.11**). Importantly, the C7 allylic alcohol is then susceptible to acid-promoted allylic transposition. Ionization could occur under acidic conditions to generate a doubly allylic carbocation. Thus, positions C3, C5, and C7 are activated as electrophiles and can undergo hydration in the presence of water. However, the presence of the chlorine at C3 provides a key thermodynamic sink for hydration to eventually occur at that position. When C3 is hydrated, the chlorine can be rapidly eliminated, affording a carbonyl which no longer undergoes reversible ionization. If hydration occurs at C5 or C7, the resulting allylic alcohol can be re-ionized under the acidic reaction conditions to regenerate the doubly allylic carbocation intermediate **199**. Only hydration at C3 leads to irreversible formation of a product and over time, all rehydration pathways converge to produce key intermediate **175** (**Scheme 4.11**).

Scheme 4.11. Global Reduction/Transposition Sequence for the Synthesis of Intermediate **175**.



Overall route to **175**.



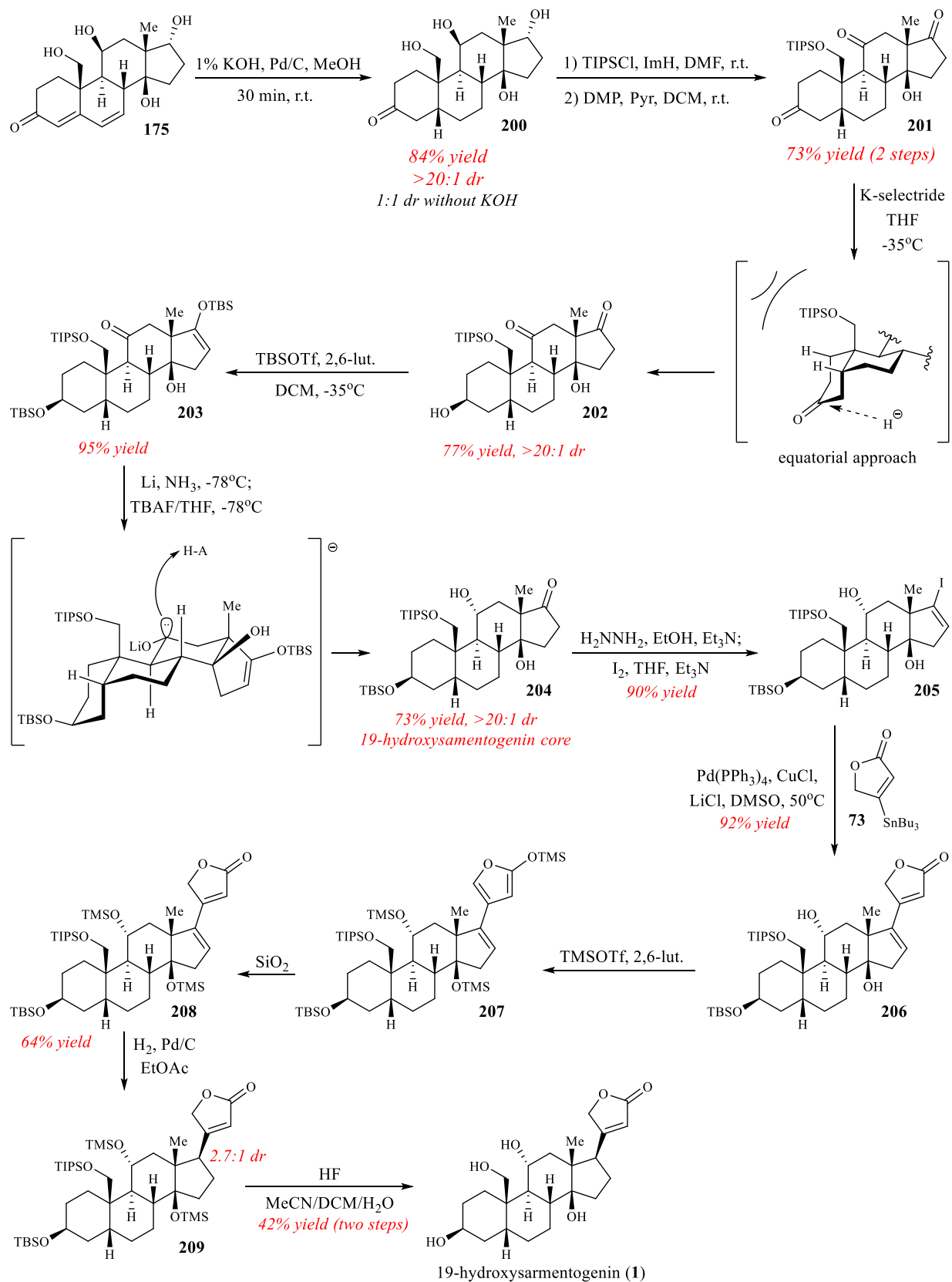
Various reducing agents were evaluated for their ability to promote the global reduction. LiAlH_4 failed because it would reduce the vinyl chloride moiety presumably via single electron transfer. While $\text{LiBH}_4/\text{B}(\text{OMe})_3$ provided the desired reactivity, the reaction was too operationally cumbersome. Fortunately, the use of excess DIBALH at elevated temperature in THF affected the desired reduction of all carbonyls. By starting the reduction at -78°C and allowing the reaction to warm slowly to ambient temperature, the configuration at C17 could be biased and an 8:1 diastereomeric ratio could be achieved favoring the α -orientation; this simplifies the isolation procedure and subsequent transformations.

Upon complete global reduction, the reaction mixture could be quenched with water and acidified with formic acid without the need to isolate intermediate **198**. By heating the acidic solution to 85°C , ionization of C7 is initiated and the transposition proceeds to generate the key intermediate **175** in 72% yield in a single step from **197**. Initially, we explored the use of the weaker acid AcOH to promote the transposition, however, these conditions appeared to produce unwanted side products resulting from elimination and so the switch to the stronger formic acid was sufficient to avoid this issue. This transposition process achieves both the removal of oxygenation from C7 and the installation of oxygenation at C3. Overall, key intermediate **175** could be synthesized in 7 linear steps from the commercially available building blocks. Remarkably, **175** contains all the preset oxygenation for synthesizing the target cardenolides, as well as seven contiguous stereocenters in a fused tetracyclic array. Up to this point, an incredible amount of chemical complexity is installed in very few steps. This foundation was a key tenant of our overall approach; that is, rapidly accessing a heavily functionalized and structurally complex intermediate.

4.6. Elaboration of Intermediate **175** into 19-Hydroxysarmentogenin

The only previous total synthesis of 19-hydroxysarmentogenin was accomplished by Inoue and co-workers in 2013.¹¹ Their synthesis proceeded in 34 total steps and is described in Chapter 2, section 2.9. Our goal is to utilize a modular, drug-oriented approach to develop a more concise route to 19-hydroxysarmentogenin. In order to elaborate key intermediate **175** into the target, several important structural transformations must be made. The C3 carbonyl must be reduced stereoselectively to a β -oriented hydroxy, the C5 position must be reduced to a *cis*- β -A/B ring junction, the C11 hydroxy group must be epimerized to a β -orientation, and finally the butenolide ring must be installed stereoselectively at C17. The first issue we examined was the hydrogenation of the extended enone moiety to set the A/B ring junction. Unfortunately, simple hydrogenation of **175** with activated Pd/C in methanol generated a 1:1 diastereomeric ratio of *cis* and *trans* A/B ring junctions (**Scheme 4.12.**). However, we reasoned that we could utilize the inherent structural features of the substrate to help bias hydrogenation to occur predominantly from the desired β -face, specifically the primary hydroxy group at C19. Heterogeneous hydrogenations over Pd/C are typically not as susceptible to substrate coordination control from polar moieties such as alcohols, amines, amides, or acids as are homogenous hydrogenations. However, exposed hydroxy groups are known to allow directed heterogeneous hydrogenation under certain circumstances.¹⁹ In this vein, we imagined running the hydrogenation under basic conditions in order to generate some population of the C19 alkoxide which may be sufficient for adsorbing to the palladium and directing hydrogenation to occur from the same face. Gratifyingly, running the hydrogenation with 1% KOH in methanol had the desired effect and the *cis*- β -A/B ring junction steroid **200** could be acquired exclusively in good yield.

Scheme 4.12. Total Synthesis of 19-Hydroxysarmentogenin.



Next, the C19 primary hydroxy group had to be protected. This would protect it from downstream oxidations. Surprisingly, attempts to selectively protect C19 as a TBS ether in the presence of other hindered secondary alcohols failed due to rapid bis-silylation of the substrate. Only TIPS was sufficiently bulky to allow for the selective silylation of C19 and **200** could be readily protected with TIPSCl and imidazole in DMF. At this point we focused our attention to the reduction of the C3 carbonyl. We realized that the hydroxy groups at C11 and C17 had to be oxidized at a later stage, and that this may cause complications if the C3 position is already set as a β -oriented hydroxy. As such, we opted to first oxidize the C11 and C17 position to carbonyls prior to attempting a regio- and stereoselective reduction of the C3 ketone. To this effect, the C19 protected steroid was readily oxidized upon exposure to the Dess-Martin periodinane in DCM to afford triketone **201**. We rationalized that a sterically hindered nucleophilic hydride source would allow the selective reduction at C3 for two reasons: 1) the C11 and C17 carbonyls are flanked by substituted positions, sterically encumbering the approach of a nucleophile, and 2) bulky hydride sources are known to prefer an equatorial approach to conformationally biased cyclohexanones.²⁰ Due to the presence of a *cis*- β -A/B ring junction, a hydride delivered equatorially to the C3 carbonyl will approach from the desired α -face. Additionally, the C19-OTIPS group provides additional steric occlusion of the β -face, further biasing a bulky hydride source to react on the desired *re* face at C3. For these reasons, we used K-selectride. At -35°C in THF, the reduction proceeded smoothly to produce intermediate **202** and the desired β -oriented C3 hydroxy exclusively. The next task was to set an α -oriented hydroxy group at C11. To accomplish this, we mimicked the approach used by Inoue and co-workers in 2013.¹¹ A thermodynamic Birch reduction of the C11 position is known to provide the α -oriented equatorial hydroxy as opposed to the use of nucleophilic hydride sources which appear to reduce C11 kinetically to afford a β -

oriented hydroxy. First, the C17 carbonyl is protected as its TBS enol ether upon exposure to TBSOTf and Et₃N; the C3 hydroxy undergoes concomitant silylation under these conditions affording **203**. Subsequent Birch reduction with lithium in ammonia generates a C11 carbanionic intermediate which undergoes exclusive protonation from the β -face. Rapid exposure to TBAF in THF at -78°C will quickly desilylate the C17 TBS enol ether, re-exposing the carbonyl and delivering intermediate **204**. Steroid **204** represents the fully complete core of 19-hydroxysarmentogenin. All oxygenation is properly installed at this stage and with the desired configuration.

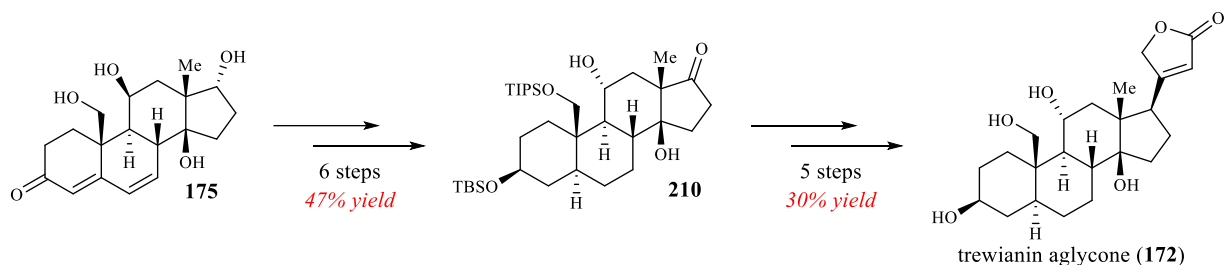
The final task is installation of the β -oriented C17 butenolide ring. Here, we employed a known 5-step sequence.¹¹ In fact, this 5-step approach is rather general and is used for butenolide installation on all four of our original targets. First, the Barton conditions for vinyl iodide formation can be utilized to convert **204** into intermediate **205**. Next, vinyl iodide **205** is subjected to a Stille coupling with known stannyl butenolide **73** in the presence of Pd⁰ and Cu(I). The coupling proceeds smoothly and in high yield to afford coupled product **206**. At this stage, the chiral center at C17 must be set. However, the *cis-trans-cis* ring junction pattern imposes convexity to the β -face and concavity to the α -face, rendering the β -face more sterically accessible. As such, direct heterogeneous hydrogenation of **206** results in hydrogen being delivered to the convex β -face and delivers primarily the undesired α -oriented butenolide. To overcome this, Inoue and co-workers devised and successfully employed the solution of silylating the C14 hydroxy group. This sterically occludes the β -face and helps promote hydrogenation from the α -face. Global silylation of **206** with TMSOTf and 2,6-lutidine initially produces intermediate **207**, wherein the butenolide ring has aromatized as a silyl enol ether. The silylated furan is very labile and undergoes rapid desilylation upon exposure to SiO₂. Subsequent purification affords the desired C14-OTMS

intermediate **208**. In our hands, the silylation procedure would often provide variable and mediocre yields despite the appearance of clean conversion to the desired product based on TLC and NMR analysis. With the C14 hydroxy silylated, hydrogenation over 10% Pd/C could proceed. Whereas Inoue and co-workers reports 8:1 dr favoring the β -oriented butenolide, our hydrogenation provided only 2.7:1 dr favoring **209**. Nonetheless, with the desired configuration set at C17, only a global desilylation step remained. To accomplish this, we used HF in a ternary solvent system comprised of MeCN, DCM, and water. Over the course of 3 days, the compound was fully desilylated and pure 19-hydroxysarmentogenin (**1**) could be isolated. Our synthetic sample of 19-hydroxysarmentogenin matched all available spectroscopic data reported by Inoue and co-workers. Additionally, the measured optical rotation ($[\alpha]_D^{25} = +13.4$ (c = 0.1, MeOH) indicates that the desired natural enantiomer was synthesized. In summary, the complete total synthesis of 19-hydroxysarmentogenin was accomplished in 21 total steps (compared to the previous route which required 34 steps).

4.7. Elaboration of Intermediate **175** into Trewianin Aglycone

The synthesis of Trewianin aglycone was accomplished by a post-doctoral researcher in our lab Dr. Hem Raj Khatri. Trewianin is a naturally occurring cardenolide, similar to 19-hydroxysarmentogenin except that it is epimeric at C5, containing a *trans*-A/B ring junction.²¹ Intermediate **175** was successfully converted to the core of trewianin aglycone, intermediate **210**, in 6 steps and 47% overall yield. Importantly, **210** contains the desired *trans*-A/B ring junction, and configuration at C11. From there, the same 5-step sequence for butenolide installation was employed in 30% overall yield, affording Trewianin aglycone (**172**) in 21 total steps (**Scheme 4.13.**)

Scheme 4.13. Summary of Trewianin Aglycone Total Synthesis.

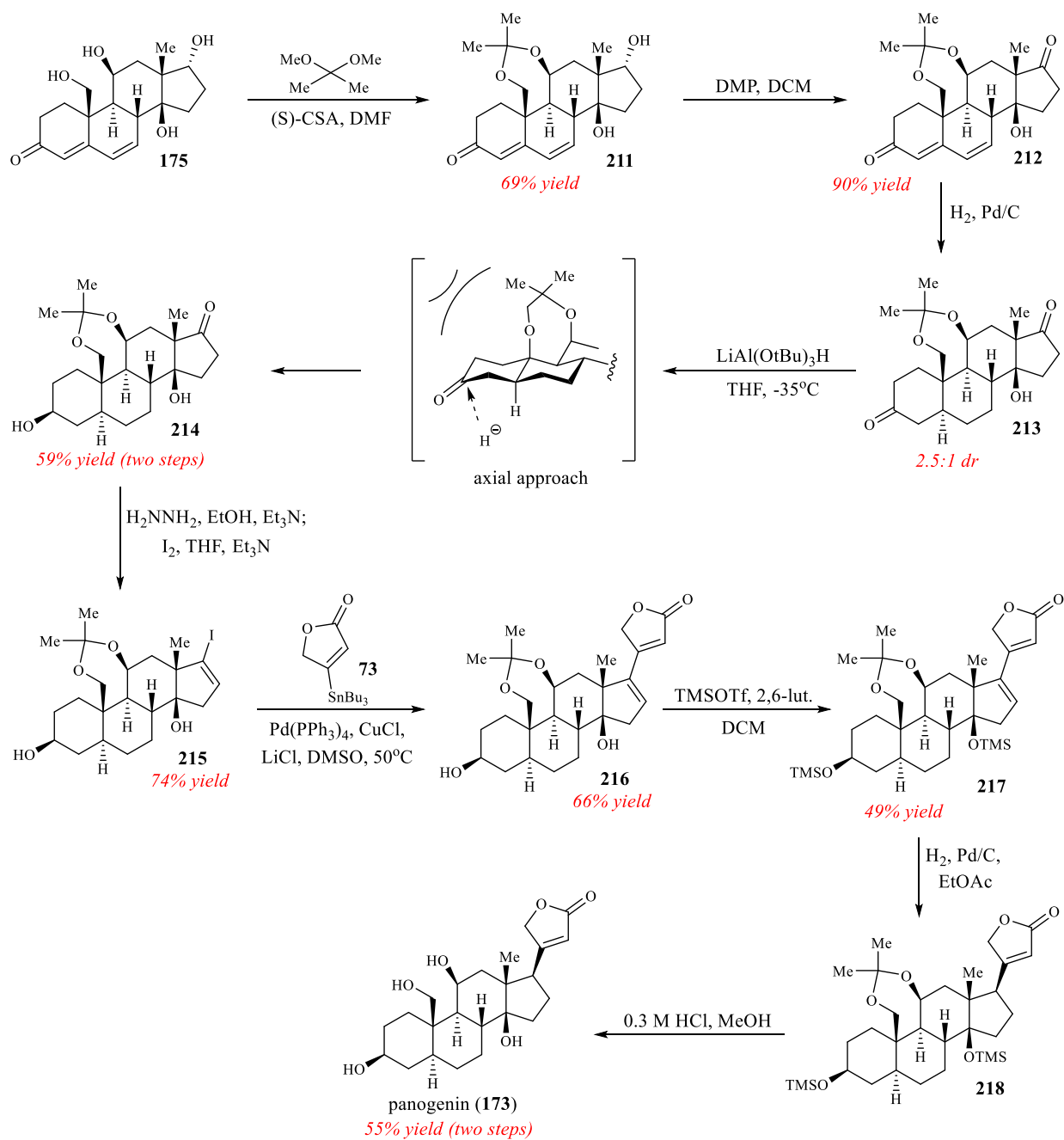


4.8. Elaboration of Intermediate 175 into Panogenin

Panogenin (**173**) is the aglycone of the naturally occurring cardenolide panoside.²² The important features of panogenin include a β -oriented hydroxy at C11, and a *trans*-A/B ring junction. The primary concern for synthesizing panogenin was controlling these stereocenters. Previously completed targets (19-hydroxysarmentogenin and trewianin aglycone) both incorporate α -oriented hydroxy groups at C11. A new approach for the stereocontrol of the key C5 and C11 centers had to be devised. Conveniently, the C11-OH of intermediate **175** is already in the same configuration as panogenin. Rather than allowing the C11 position to be oxidized and then reduced at a later stage as was done previously, we opted to preserve the stereochemical information at C11 from the outset – we imagined this approach would reduce the total number of transformations required to complete the synthesis. To accomplish this, we envisioned the formation of an acetal between the C19 and C11 hydroxy groups. Importantly, such a transformation effectively controls stereochemistry at both C11 and C5 because C11 is protected from downstream oxidation, and the presence of an acetal on the β -face of the steroid provides a steric bias for the hydrogenation of C5 to occur from the desired α -face. We first investigated formation of a cyclic silyl acetal via $\text{Si}(\text{tBu})_2\text{Cl}_2$. However, these attempts produced a complex mixture of products. Instead, acetalization was attempted with 2,2-dimethoxypropane in DMF with catalytic *S*-camphorsulfonic

acid. This acetalization proceeded smoothly to afford cyclic acetal intermediate **211** (Scheme 4.14.). Next, the C17 hydroxy group was oxidized with the Dess-Martin periodinane to cleanly afford compound **212**.

Scheme 4.14. Total Synthesis of Panogenin.



At this stage, the C5 position could be hydrogenated. The C19-C11 acetal successfully provides some steric occlusion of the *se* face at C5, allowing hydrogenation over Pd/C to proceed in 2.5:1 dr favoring the desired *trans*-A/B ring junction **213**. The next goal was the regio- and stereoselective reduction of the C3 carbonyl. At this stage, there is an important conformational distinction to be made between **213** and the corresponding triketone of 19-hydroxysarmentogenin, compound **201**. In the case of **201**, the desired hydride reduction at C3 would provide an axial C3 hydroxy group, therefore requiring an equatorial approach of the hydride source. However, with intermediate **213**, the desired reduction at C3 will provide an equatorial β -oriented C3 hydroxy and so an axial approach of the hydride source is necessary. For this, we opted to use LiAl(OtBu)₃H (LATBH). Though it may seem like a sterically bulky hydride source, similar to K-selectride, LATBH often behaves as a small hydride source and is known to reduce conformationally biased cyclohexanones via axial approach.²³ Indeed, the use of LATBH at -35°C to reduce triketone **213** provided the desired mode of reactivity, and intermediate **214** could be isolated with an equatorially β -oriented hydroxy at C3 with complete diastereoselectivity. Here, the complete core of panogenin, emulating all natural oxygenation and stereochemistry, is completed and only the butenolide installation remains.

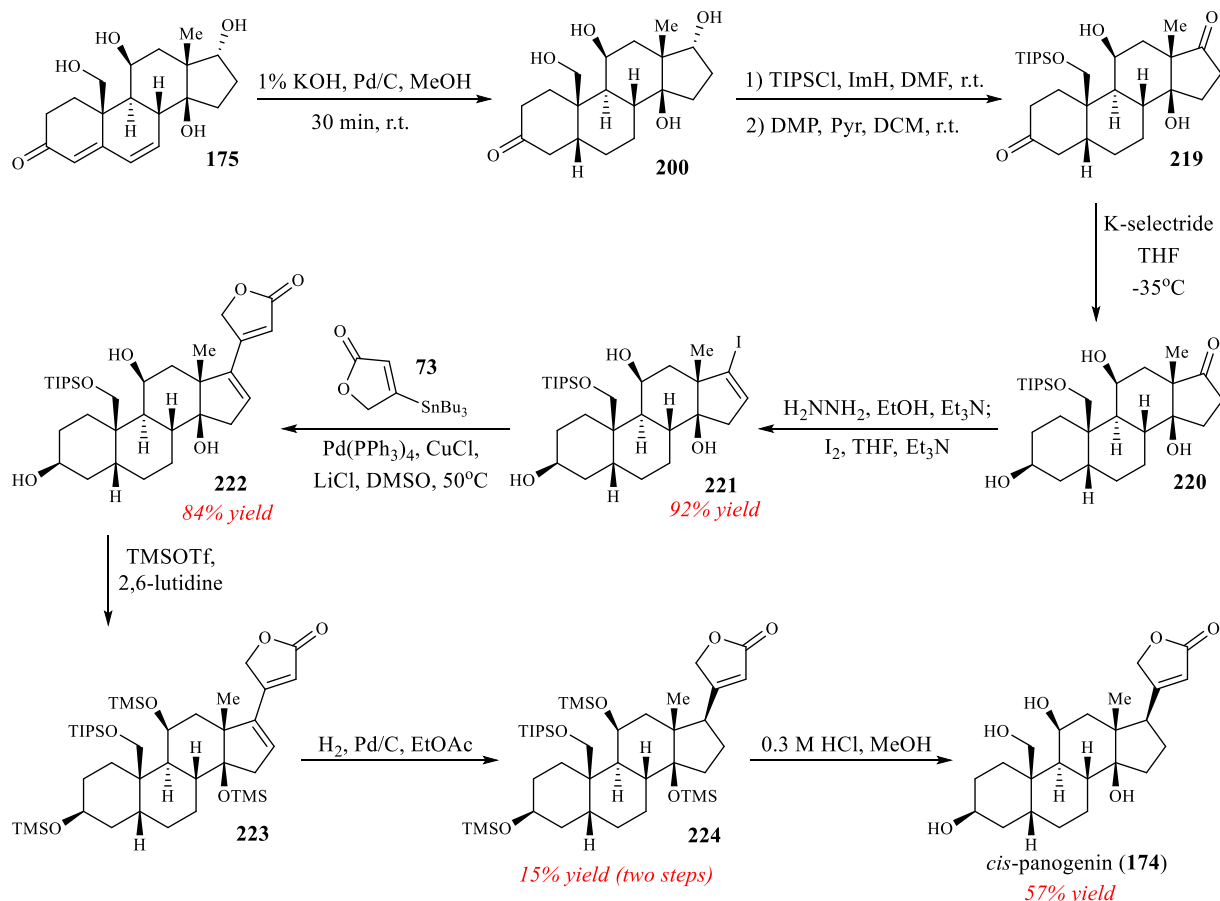
The known 5-step sequence could be employed again for successful butenolide installation. In this regard, conversion of panogenin core **214** to vinyl iodide **215** was accomplished via the Barton vinyl iodide protocol. Subsequent Stille coupling of iodide **215** with the stannyl butenolide **73** afforded coupled product **216**. Global silylation with TMSOTf and 2,6-lutidine set the stage for diastereoselective hydrogenation. Interestingly, hydrogenation of **217** over Pd/C proceeded exclusively from the α -face and the crude material could be immediately subjected for global deprotection. This completely diastereoselective hydrogenation is also observed during the

synthesis of *cis*-panogenin (**174**) described in the next section – perhaps a result of the β -oriented hydroxy group at C11 in these substrates. The presence of the C19-C11 acetal caused us to modify the method for deprotection. Rather than using the usual HF method, we employed the same 0.3 M HCl in MeOH conditions utilized by Inoue and co-workers for deprotection of **218**. In just 30 minutes, all hydroxy groups are deprotected and desired target panogenin (**173**) is produced in 19 total steps. The observed optical rotation ($[\alpha]_D^{27} = +22.4$ ($c = 0.08$, CH₃OH)) closely matches the reported value of +25.8, indicating the natural enantiomer was prepared.²⁴

4.9. Elaboration of Intermediate 175 into *cis*-Panogenin

cis-Panogenin (**174**) has not yet been discovered from natural sources. Its key architectural difference from panogenin is the presence of a *cis*- α -A/B ring junction and it represents the last of four possible diastereomers among the C5 and C11 stereocenters. In an effort to acquire *cis*-panogenin (**174**), intermediate **175** was hydrogenated with KOH to produce a *cis*- β -A/B ring junction intermediate **200** (Scheme 4.15.). The C19 hydroxy was then silylated with TIPS. Next, the material was subjected to the Dess-Martin periodinane with the expectation of acquiring triketone **201** on route to 19-hydroxysarmentogenin. However, only monoxidation of C17 was observed, affording **219** with the C11 hydroxy group unoxidized and retaining a β -orientation. This was a good opportunity to take advantage of the unexpected result and divert the synthesis toward the final remaining C5/C11 diastereomer, *cis*-panogenin (**174**). With a *cis*- β -A/B ring junction installed, the C3 carbonyl could be reduced with K-selectride in a regio- and stereoselective manner to generate intermediate **220**. At this stage, the full core of *cis*-panogenin had been completed, containing all the necessary sites of oxygenation, a *cis*- β -A/B ring junction, and a β -oriented hydroxy at C11.

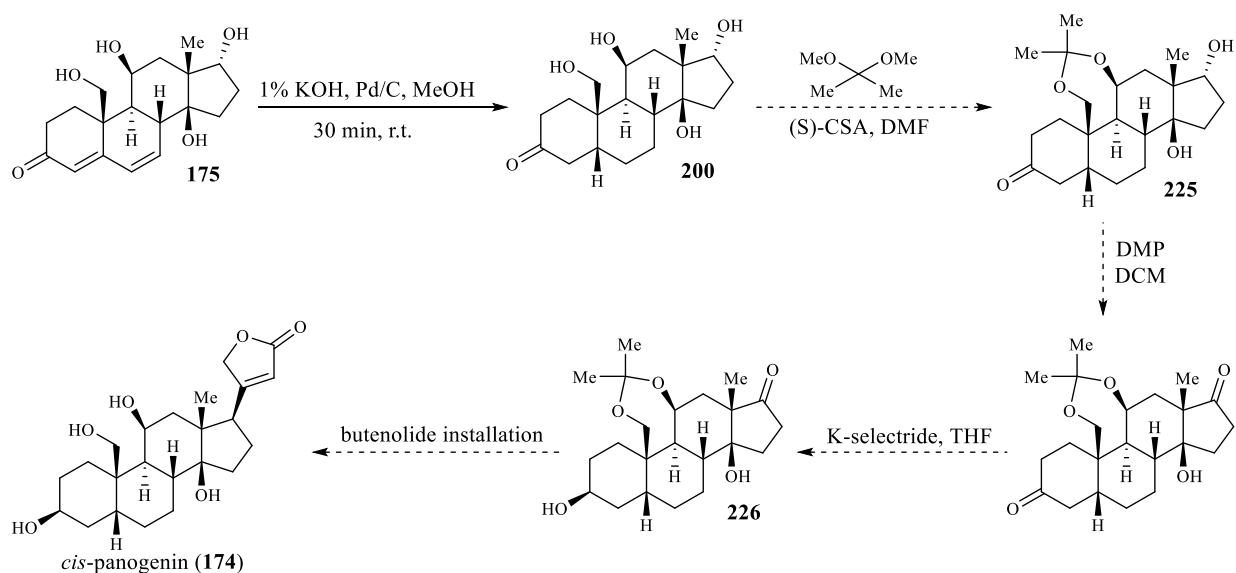
Scheme 4.15. Total Synthesis of *cis*-Panogenin.



The final sequence commenced with vinyl iodide formation using the usual Barton protocol, followed by Stille coupling with the stannyl butenolide to produce the coupled product **222**. Unfortunately, subsequent silylation of the C14 hydroxy provided poor yield of the desired product **223** for unknown reasons. Nonetheless, the ensuing hydrogenation proceeded with complete diastereoselectivity. Generating the β -oriented butenolide **224** exclusively. Global deprotection occurred completely in just 45 minutes upon exposure to 0.3 M HCl in MeOH to afford the target *cis*-panogenin (**174**) in 19 total steps. Despite the presence of a TIPS group on the C19 hydroxy, the final deprotection procedure was changed from the previously described approach with HF in MeCN, DCM, and water because the method using HF should not be

performed in borosilicate glassware and so was found to be operationally more difficult than simple acid-promoted deprotection. Despite the successful synthesis of *cis*-panogenin, it is likely that we would use a revised route for any future attempts at synthesizing this target. This proposed route is outlined in **Scheme 4.16**. The *cis*- β -A/B ring junction is installed by the same C19 directed hydrogenation under basic conditions. However, a more reliable method for setting the stereochemistry at C11 is likely via acetalization of the C19 and C11 hydroxy groups, producing acetal **225**. Subsequent oxidation with the Dess-Martin periodinane and C3 carbonyl reduction by K-selectride could provide *cis*-panogenin core **226**. The same 5-step butenolide installation procedure could be employed to acquire the final target. This proposed route would avoid the need to reproduce the unusual monoxidation leading to **219** and may provide more straightforward access *cis*-panogenin (**174**).

Scheme 4.16. Proposed Route to *cis*-Panogenin.



4.10. Evaluation via Biochemical Assay

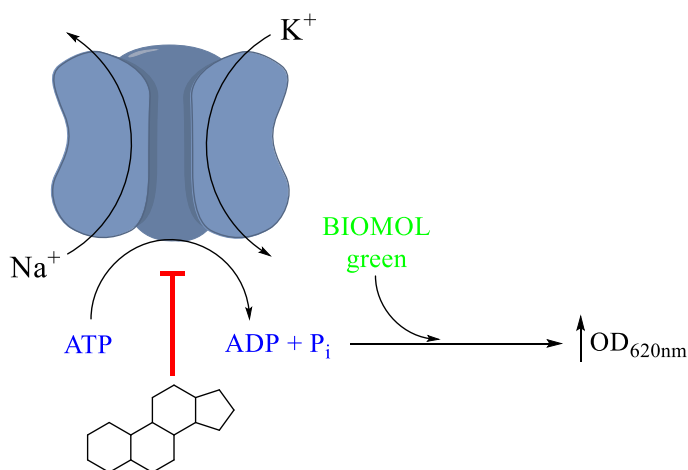
The biological evaluation of cardenolides has much precedence. Some studies have focused on the ability of cardenolides to act as anticancer agents, while others have sought to explore their ability to specifically inhibit the $\text{Na}^+/\text{K}^+\text{ATPase}$.²⁵ The nature of $\text{Na}^+/\text{K}^+\text{ATPase}$ inhibition is better understood due to structure-activity relationship studies and X-ray co-crystallographic analysis.^{26,27} However, only the common cardenolides such as ouabain (**2**) and digitalis tend to be used in these studies. Moreover, the connection between $\text{Na}^+/\text{K}^+\text{ATPase}$ inhibition, anticancer activity, isoform selectivity, and therapeutic window is not well understood. Access to new cardenolides and their analogues for further exploration of these connections is highly desirable. It is the goal of our group to synthesize new cardenolides for the purpose of biological evaluation. Specifically, we hope to explore the connection, if any, between $\text{Na}^+/\text{K}^+\text{ATPase}$ isoform inhibition and anticancer activity and to better understand the structural features which modulate the antiproliferative and toxic effects of cardenolides.

As part of a collaborative effort funded by the Chemistry-Biology Interface training program at the University of Michigan, I spent a 10-week sabbatical in the lab of Dr. Dan Michele (Department of Molecular and Integrative Physiology, University of Michigan) developing a biochemical assay for the evaluation of the cardenolides we've synthesized (**Scheme 4.17.**). In a non-inhibited state, the $\text{Na}^+/\text{K}^+\text{ATPase}$ will bind ATP, followed by three Na^+ ions.²⁸ The bound ATP is then hydrolyzed, leading to phosphorylation of an aspartate residue. This phosphorylation promotes a conformational change and subsequent release of the Na^+ ions on the extracellular surface of the pump. Two K^+ ions will then bind the extracellular surface and dephosphorylation ensues – releasing one equivalent of inorganic phosphate per cycle. Upon release of phosphate,

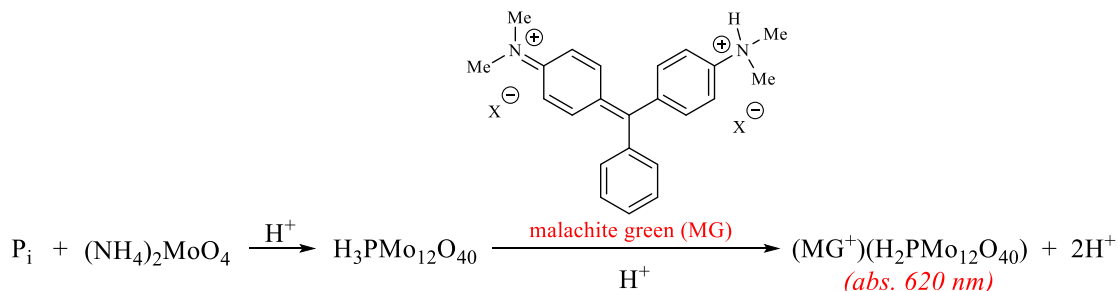
the enzyme reverts to its original confirmation and the bound K^+ is released from the intracellular surface of the pump, thus refreshing the catalytic cycle.

Scheme 4.17. Schematic for Mechanism of Na^+/K^+ ATPase Biochemical Assay.

Assay schematic



Molybdate/Malachite green mechanism



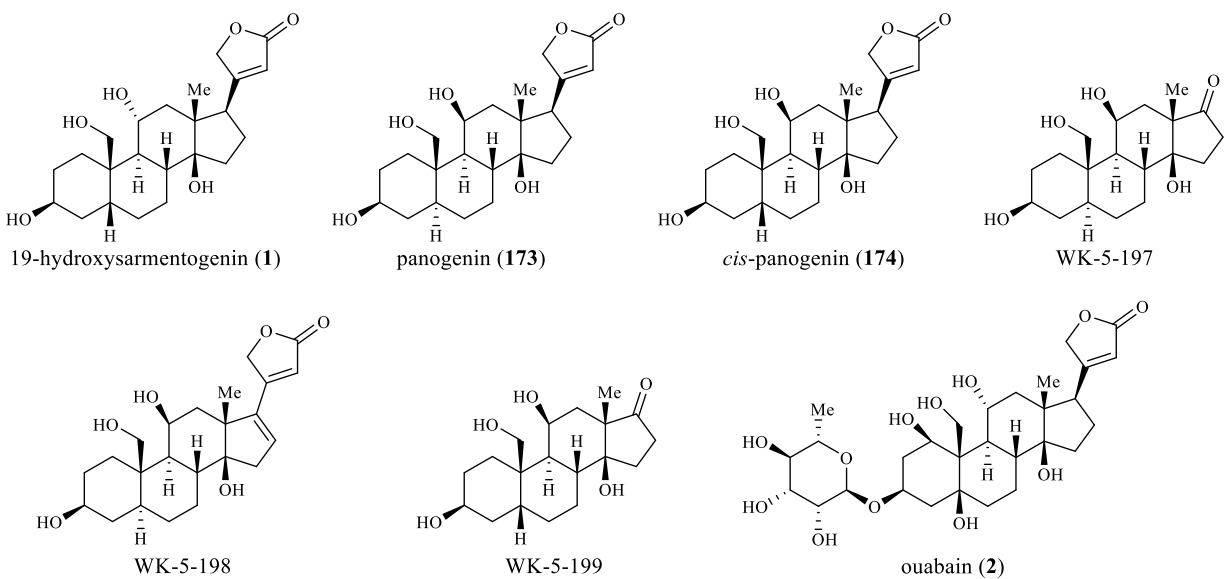
In the presence of an inhibitor, the enzyme's function is slowed or halted and the rate of hydrolysis of ATP is reduced. Therefore, the amount of phosphate produced is directly correlated with the enzyme's activity level. In the assay we developed, the production of phosphate is measured by use of a commercial colorimetric reagent, BIOMOL green (Enzo Life Sciences). This reagent is a preparation of molybdate/malachite green-based phosphate detection. In a manner dependent on the phosphate concentration, a solution of BIOMOL green will display increased

optical density at 620 nm. A non-inhibited Na⁺/K⁺ATPase control will freely produce large quantities of phosphate, resulting in high optical density at 620 nm (OD₆₂₀) following addition of BIOMOL green. In the presence of a strong inhibitor, the production of phosphate will be slowed and the OD₆₂₀ will remain flat after analysis with BIOMOL green. A concentration-response curve can be generated by measuring the OD₆₂₀ which results after the enzymatic reaction is performed in the presence of an inhibitor at serial dilutions. The percent activity of the Na⁺/K⁺ATPase in each test well was determined by subtracting the background signal of the blank wells of the same plate, and then dividing by the signal of the uninhibited controls containing only vehicle.

The Na⁺/K⁺ATPase used in our assay was isolated from the porcine cerebral cortex. All three pig isoforms show >99% amino acid identity with the human isoforms.²⁹ A concentration-response curve of commercially acquired ouabain (**2**) was generated to compare with the measured IC₅₀ values of our test compounds (**Figure 4.2.**). The IC₅₀ value of ouabain was determined to be 660 nM. Neither of the steroids **WK-5-197** or **WK-5-199** showed any activity at concentrations below 399 μM or 150 μM, respectively. This is likely due to the absence of a C17 butenolide ring, which has previously been implicated as a key moiety necessary for binding. Steroid **WK-5-198** is an interesting case. This compound contains an installed butenolide but has a C16-C17 olefin; meaning there is no stereochemical information at C17. Steroid **WK-5-198** showed no obvious ability to inhibit the Na⁺/K⁺ATPase in our assay at concentrations lower than 100 μM. Yet in contrast to the other analogues **WK-5-197** and **WK-5-199**, **WK-5-198** appeared to show the onset of activity in concentrations greater than 120 μM, perhaps due to the butenolide ring.

Figure 4.2. Biochemical Assay and Concentration-response Curves.

Compounds tested:



Dose-response curves:

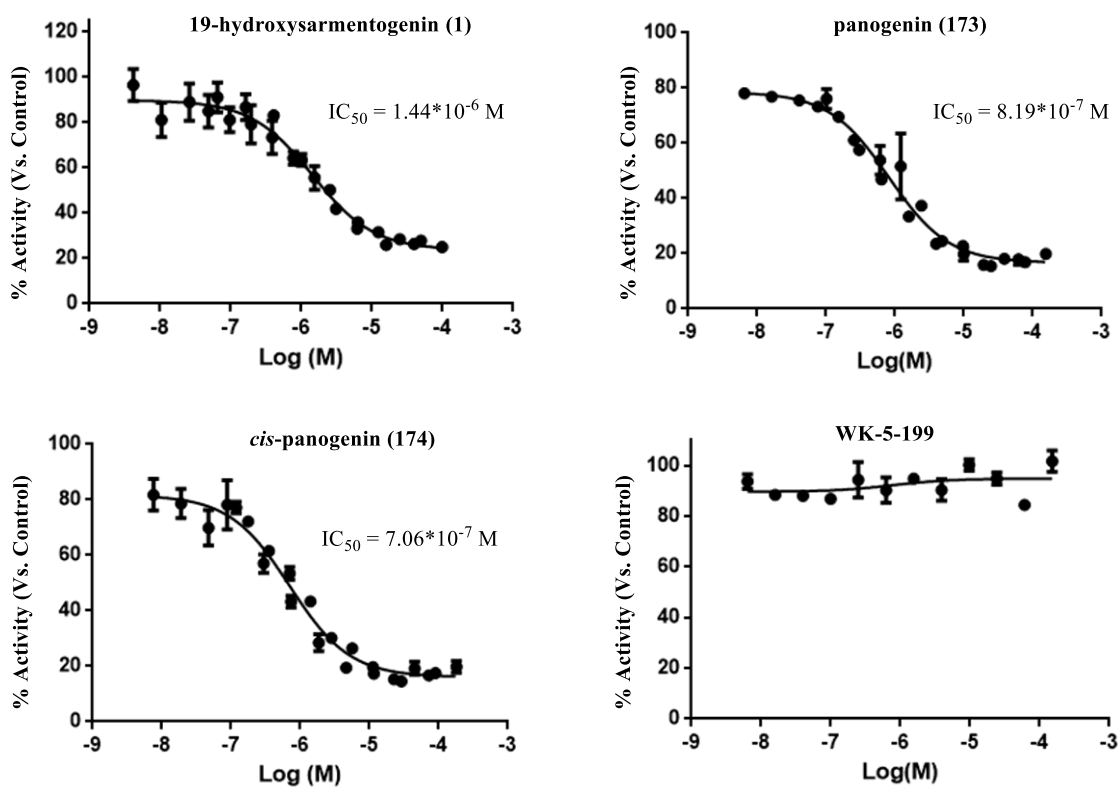
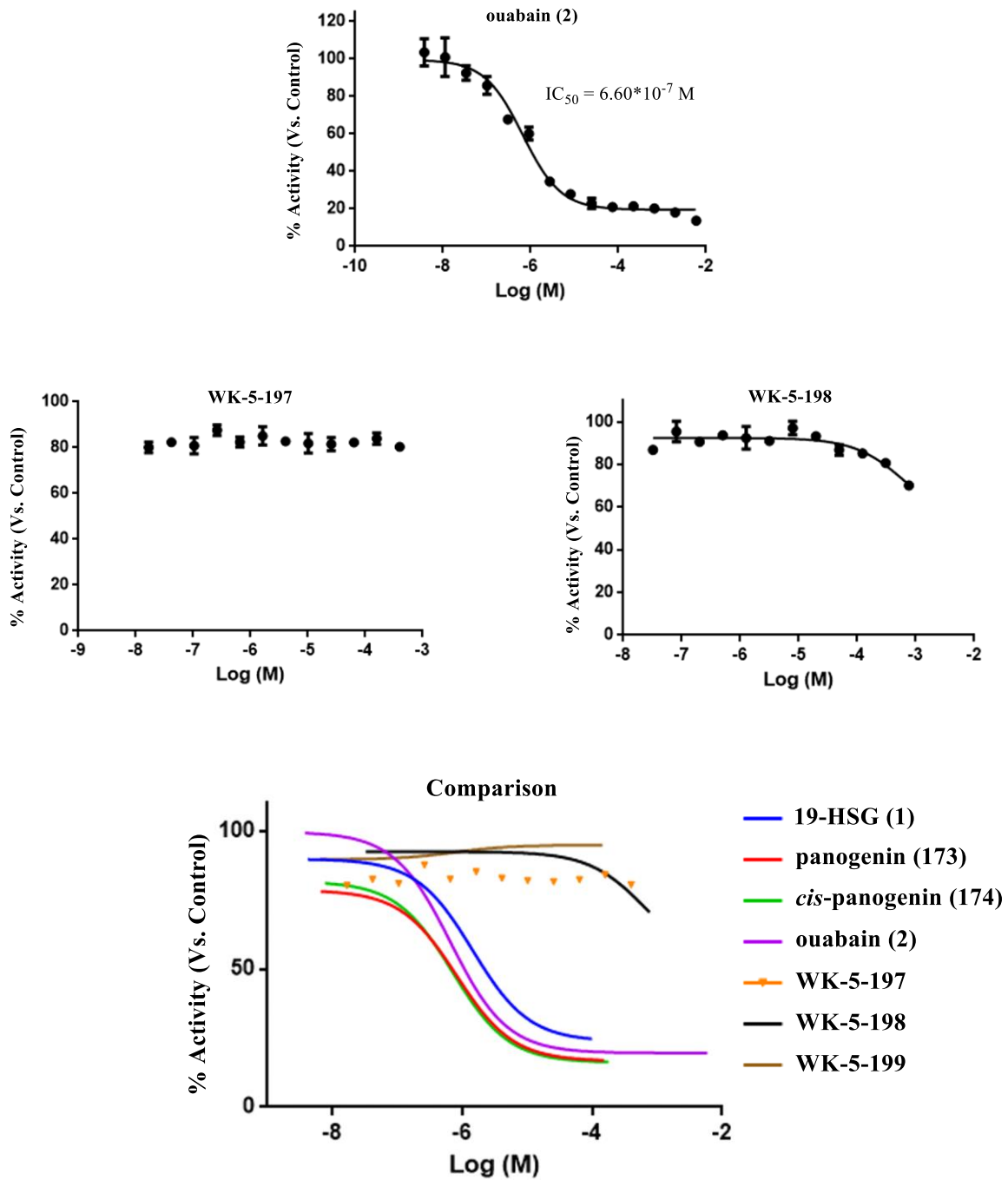


Figure 4.2. Biochemical Assay and Concentration-response Curves (continued).



However, simple reduction of the C16-C17 olefin of compound **WK-5-198** generates panogenin (**173**), which showed much stronger inhibition of the Na⁺/K⁺ATPase with an IC₅₀ of 819 nM. This corroborates the notion that the correct orientation of the C17 butenolide is crucial for proper binding. Additionally, both 19-hydroxysarmentogenin (**1**) and *cis*-panogenin (**174**) showed reliable concentration response curves, with IC₅₀ values of 1.44 μM and 706 nM, respectively. We suspected that the *trans*-A/B ring junction compound panogenin would exhibit poorer binding than its *cis*-β-A/B counterparts, *cis*-panogenin and 19-hydroxysarmentogenin, due its unique 3D shape, but this was not observed to a significant degree and both panogenin and *cis*-panogenin showed similar concentration-response curves. Moreover, the synthetic steroids panogenin and *cis*-panogenin displayed IC₅₀ values approximately half of that for 19-hydroxysarmentogenin (**1**). This result may suggest that a β-oriented C11 hydroxy group affords lower IC₅₀ values, while the orientation at C5 is of little consequence. However, a previous study has implicated the importance of a *trans*-A/B ring junction in α1 isoform selectivity and anticancer activity.³⁰ Our measured IC₅₀ values are compiled in **Table 4.1**.

Table 4.1. Summary of IC₅₀ Values for Tested Compounds.

Compound	IC ₅₀
19-hydroxysarmentogenin (1)	1.44 μM (error range 1.27 – 1.64 μM)
panogenin (173)	819 nM (error range 729 – 918 nM)
<i>cis</i> -panogenin (174)	706 nM (error range 625 – 797 nM)
ouabain (2)	660 nM (error range 571 – 763 nM)
WK-5-197	>1 mM
WK-5-198	>1 mM
WK-5-199	>1 mM

Unfortunately, the preparation of Na⁺/K⁺ATPase we acquired from Sigma Aldrich was a mixture of all three α isoforms from the porcine cerebral cortex, and so no isoform selectivity information could be extracted. We hope to acquire purified Na⁺/K⁺ATPase isoforms for use in the developed assay to allow for comparisons of isoform selectivity. Furthermore, we aim to investigate concentration-response curves of additional cardenolides, including some C3 glycosylated derivatives for which we are currently developing synthetic access.

In addition to the biochemical assay developed for Na⁺/K⁺ATPase inhibition, we have recently begun a collaboration with Dr. Yimon Aye at Cornell University. Her group is set to assist with the evaluation of the antiproliferative effects of the steroids we have synthesized. In conjunction with the results from Aye group, we aim to explore Na⁺/K⁺ATPase inhibition and isoform selectivity to understand how certain structural features modulate binding and bioactivity, and whether there are significant correlations between Na⁺/K⁺ATPase isoform selectivity and anticancer effects.

4.11. Conclusion

We have successfully expanded on the methodology described in chapter 3 to gain expedient access to the cardenolides 19-hydroxysarmentogenin (**1**), panogenin (**173**), trewianin aglycone (**172**), and *cis*-panogenin (**174**). By discovering reliable means of pre-installing key oxygenation on the simple starting substrates in an asymmetric fashion, we circumvented the need for late stage redox manipulations at otherwise inert positions. In doing so, we completed the synthesis of heavily functionalized key intermediate **175** in only 7 linear steps from simple and commercially available building blocks. From there, **175** could be readily diverted to a number of natural and unnatural cardenolides, allowing the concise total synthesis of all four C5/C11 isomers of the general 19-hydroxysarmentogenin architecture (in 19 to 21 total steps). Upon completing

the synthesis of the four major targets, we subjected them to a biochemical assay to determine IC_{50} values of Na^+/K^+ ATPase inhibition and discovered some interesting potential structure-activity relationships. The β -orientation of the C17 butenolide ring appeared to be crucial for effective inhibition while the stereochemistry at C5 had little to no effect. Additionally, a β configuration at C11 may have promoted a slight lowering of the IC_{50} value.

We hope to continue the methodology expansion to access even more heavily oxidized cardenolides such as ouabain. Further, we aim to establish a lasting collaboration for testing the antiproliferative activity of the steroids we synthesize – to further understand the structural features which promote this anticancer effect and determine if there is a relationship with isoform selectivity.

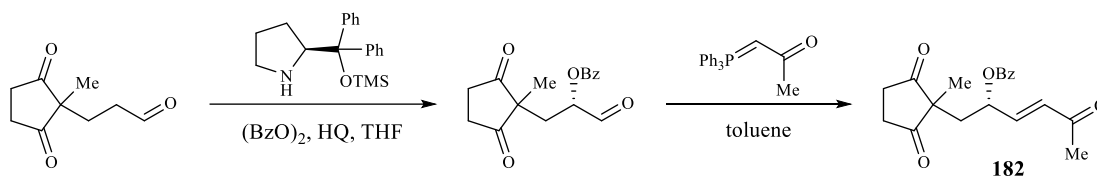
4.12. Experimental

General

All reagents and solvents were purchased from commercial sources and were used as received without further purification unless otherwise specified. THF and DMF were purified by Innovative Technology's Pure-Solve System using basic alumina. All reactions were carried out under a positive pressure of nitrogen in flame- or oven-dried glassware with magnetic stirring. Reactions were cooled using a cryocool or external cooling baths (sodium chloride/ice water (-10°C) or dry ice/acetone (-78°C)). Heating was achieved by use of a silicone oil bath with heating controlled by electronic contact thermometer. Deionized water was used in the preparation of all aqueous solutions and for all aqueous extractions. Solvents used for extraction and chromatography were ACS or HPLC grade. Purification of reactions mixtures was performed by flash chromatography using SiliCycle SiliaFlash P60 (230-400 mesh). Alternatively, purifications were achieved using CombiFlash Rf+ Lumen Automated Flash Chromatography System with UV and ELSD detector using either SiliCycle SiliaFlash P60 (230-400 mesh) or Teledyne RediSep Rf Gold Silica (20-40 μm spherical). Yields indicate the isolated yield of the title compound with $\geq 95\%$ purity as determined by ^1H NMR analysis. Whereas the yields in the article are the average yields of two or more experiments, the yields in the supporting information describe the result of a single experiment. Diastereomeric ratios were determined by ^1H NMR analysis. Enantiomeric excess was determined by HPLC analysis using a Waters e2695 Separations Module with a Waters 2998 photodiode array detector on a Chiralcel AD-H column. ^1H NMR spectra were recorded on a Varian vnmrs 700 (700 MHz), Varian vnmrs 500 (500 MHz), Varian vnmrs 400 (400 MHz), or a Varian Inova 500 (500 MHz) spectrometer and chemical shifts (δ) are reported in parts per million (ppm) with solvent resonance as the internal standard (CDCl_3 at δ 7.26, D_3COD at δ 3.31).

Tabulated ^1H NMR Data are reported as s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, sext = sextet, m = multiplet, ovrlp = overlap, and coupling constants in Hz. Proton-decoupled ^{13}C NMR spectra were recorded on Varian vnmrs 700 (700 MHz) spectrometer and chemical shifts (δ) are reported in ppm with solvent resonance as the internal standard (CDCl_3 at δ 77.0, D_3COD at δ 49.0). High resolution mass spectra (HRMS) were performed and recorded on Micromass AutoSpec Ultima or VG (Micromass) 70-250-S Magnetic sector mass spectrometers in the University of Michigan mass spectrometry laboratory. Infrared (IR) spectra were recorded as thin films a Perkin Elmer Spectrum BX FT-IR spectrometer. Absorption peaks are reported in wavenumbers (cm^{-1}). Optical rotations were measured at room temperature in CHCl_3 or H_3COH on a Jasco P-2000 polarimeter.

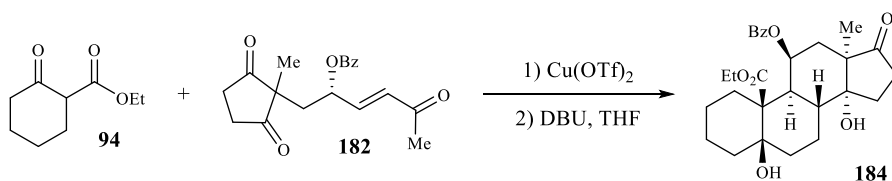
Reaction Protocols and Compound Characterization



(*S,E*)-1-(1-methyl-2,5-dioxocyclopentyl)-5-oxohex-3-en-2-yl benzoate (182)

Benzoyl peroxide (18.7 g, 77.4 mmol) and hydroquinone (990 mg, 9 mmol) were solvated in THF (300 mL) and H_2O (0.5 mL). Then, (*S*)-2-(diphenyl((trimethylsilyl)oxy)methyl)pyrrolidine (1.95 g, 6.0 mmol) was added followed by 3-(1-methyl-2,5-dioxocyclopentyl)propanal (10.0 g, 59.5 mmol). The reaction mixture was then stirred at room temperature under N_2 until the disappearance of starting material by ^1H NMR. After 1.5 h, the reaction mixture was concentrated in vacuo and directly purified using flash column chromatography with 30% EtOAc in hexanes to afford (*S*)-1-(1-methyl-2,5-dioxocyclopentyl)-3-oxopropan-2-yl benzoate (13.5 g, 46.8 mmol) as viscous oil in

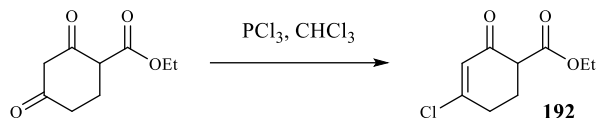
79% yield. The material was immediately used for the next step without further purification. (*S*)-1-(1-methyl-2,5-dioxocyclopentyl)-3-oxopropan-2-yl benzoate (13.5 g, 46.8 mmol) was solvated in toluene (300 mL) and Wittig reagent 1-(tripheylphosphoranylidene)-2-propanone (22.2 g, 69.7 mmol) was added. The reaction was allowed to stir at room temperature for 12 h. The solution was then concentrated in vacuo and directly purified by flash column chromatography with 20% → 30% EtOAc in hexanes to afford title compound (*S,E*)-1-(1-methyl-2,5-dioxocyclopentyl)-5-oxohex-3-en-2-yl benzoate (**182**) (11.5 g, 35.0 mmol) as a white solid in 75% yield, 95% ee (Chiralpak AD-H, 12% iPrOH in hexanes, R-enantiomer 13.2 minutes, S-enantiomer 28 minutes), R_f = 0.3 (33% EtOAc in Hexanes). ¹H NMR (700 MHz, CDCl₃): δ 7.61 (t, J = 7.4 Hz, 1H), 7.48 (t, J = 7.8 Hz, 2H), 6.64 (dd, J = 16.1, 5.3 Hz, 1H), 6.19 (dd, J = 16.1, 1.2 Hz, 1H), 5.63 - 5.49 (m, 1H), 2.82 - 2.69 (m, 2H), 2.57 - 2.49 (m, 1H), 2.49 - 2.36 (m, 2H), 2.25 (s, 3H), 2.15 (dd, J = 14.6, 3.3 Hz, 1H), 1.19 (s, 3H). ¹³C NMR (175 MHz, CDCl₃): δ 215.7, 214.8, 197.6, 165.4, 142.7, 133.8, 130.6, 129.7, 128.7, 128.7, 70.1, 54.3, 37.9, 34.7, 34.6, 27.4, 23.1. HRMS (ESI): m/z calcd for C₁₉H₂₀O₅Na⁺ [M+Na]⁺ 351.1203, found 351.1206. IR (film, cm⁻¹): 1722, 1678, 1364, 1252, 1100, 1070, 982, 715. [α]_D²⁵ = -29 (c = 1.0, CHCl₃).



Ethyl (*5R,8R,9S,10S,11S,13R,14R*)-11-(benzoyloxy)-5,14-dihydroxy-13-methyl-17 oxohexa decahydro-10H-cyclopenta[a]phenanthrene-10-carboxylate (184**)**

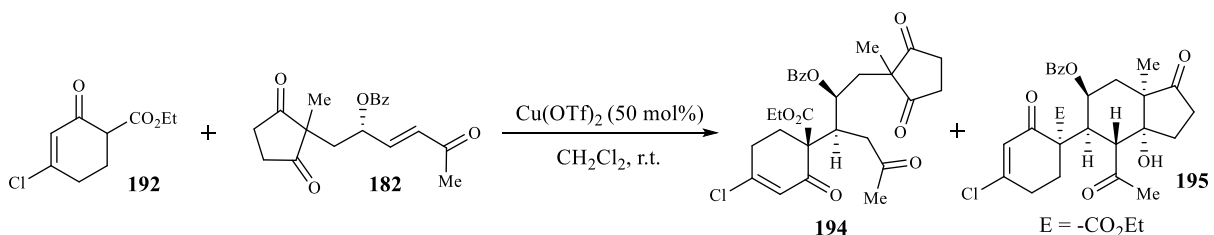
(*S,E*)-1-(1-methyl-2,5-dioxocyclopentyl)-5-oxohex-3-en-2-yl benzoate **182** (328.0 mg, 1.0 mmol), and ethyl 2-oxocyclohexane-1-carboxylate **94** (500 mg, 2.94 mmol) were successively added to a

flask with a stir bar and the vessel was purged with nitrogen. Then, Cu(OTf)₂ (54 mg, 0.015 mmol) was added and the reaction was allowed to stir at room temperature overnight. The next day, the residue was filtered through a plug of silica with diethyl ether as eluent. The crude mixture was then purified by flash column chromatography with 10% → 50% EtOAc in hexanes. The resultant adduct was used for cyclization without further purification. It was solvated in THF (2.5 mL), and 1,8-diazabicyclo[5.4.0]undec-7-ene (50.4 mg, 49 μL, 0.33 mmol) was added via syringe. The solution was allowed to reflux for 18 h. Afterwards, the solution was cooled and concentrated in vacuo. The resulting residue was directly purified by flash column chromatography with 30% EtOAc in hexanes to afford title compound ethyl (5*R*,8*R*,9*S*,10*S*,11*S*,13*R*,14*R*)-11-(benzoyloxy)-5,14-dihydroxy-13-methyl-17-oxohexadecahydro-10H-cyclopenta[*a*]phenanthrene-10-carboxylate (**184**) (219.1 mg, 0.44) in 44% unoptimized yield, R_f = 0.2 (30% EtOAc in hexanes). The product was then crystallized from hexanes/CH₂Cl₂ and submitted for X-ray analysis. ¹H NMR (500 MHz, CDCl₃): δ 7.86 (d, J = 7.2 Hz, 2H), 7.54 (t, J = 7.4 Hz, 1H), 7.42 (t, J = 7.7 Hz, 2H), 5.41 (d, J = 1.1 Hz, 1H), 4.41 (s, 1H), 3.85 - 3.73 (m, 2H), 3.71 (d, J = 12.6 Hz, 1H), 3.31 (s, 1H), 2.58 (dd, J = 15.1, 3.3 Hz, 1H), 2.55 - 2.38 (m, ovrlp, 5H), 2.34 - 2.21 (m, 1H), 2.08 - 2.21 (m, 2H), 1.98 (d, J = 14.5 Hz, 1H), 1.63 - 1.45 (m, ovrlp, 6H), 1.41 - 1.28 (m, 1H), 0.98 (s, 3H), 0.92 (t, J = 7.1 Hz, 3H). ¹³C NMR (125 MHz, CDCl₃): δ 216.1, 212.8, 174.1, 165.0, 133.2, 129.7, 129.6, 128.3, 77.5, 74.9, 67.4, 61.0, 54.1, 51.8, 51.6, 49.0, 40.2, 35.8, 34.8, 33.4, 31.7, 21.1, 20.4, 20.1, 13.5. IR (film, cm⁻¹): 3504 (br), 2932, 1718 (ovrlp), 1451, 1270, 1177, 1071, 1026, 708.



Ethyl 4-chloro-2-oxocyclohex-3-ene-1-carboxylate (**192**)

Ethyl 2,4-dioxocyclohexanecarboxylate (11.0 g, 59.8 mmol, 1.0 equiv.) was taken in chloroform (110 mL, 0.54 M) and cooled to 0°C. PCl₃ (10.5 mL, 119.6 mmol, 2.0 equiv.) was added. After 1 hour, the reaction mixture was warmed to room temperature and additional PCl₃ (7.0 mL, 79.7 mmol, 1.3 equiv.) was added. The reaction mixture was allowed to stir at room temperature and monitored by TLC analysis. After 7 hours, the reaction mixture was concentrated in vacuo and purified by column chromatography (grad. 5%→10% EtOAc in hexanes) to afford ethyl 4-chloro-2-oxocyclohex-3-ene-1-carboxylate (**192**) (8.1 g, 40.1 mmol, 67% yield) as a colorless oil. ¹H NMR (700 MHz, CDCl₃) δ 6.27 (t, J = 1.4 Hz, 1H), 4.25-4.18 (m, 2H), 3.36 (dd, J = 4.9, 9.0 Hz, 1H), 2.83 (dt, J = 5.8, 18.9 Hz, 1H), 2.70 (dt, J = 6.5, 18.9 Hz, 1H), 2.48-2.43 (m, 1H), 2.25 (dq, J = 5.1, 16.7 Hz, 1H); ¹³C NMR (175 MHz, CDCl₃) δ 191.3, 169.2, 158.6, 127.7, 61.6, 51.8, 32.3, 25.1, 14.1; HRMS (ESI): m/z calcd for C₉H₁₁ClO₃ [M+Na]⁺ 225.0289, found 225.0291.



(2*S*,3*S*)-3-((*S*)-4-chloro-1-(ethoxycarbonyl)-2-oxocyclohex-3-en-1-yl)-1-(1-methyl-2,5-dioxocyclopentyl)-5-oxohexan-2-yl benzoate (**194**).

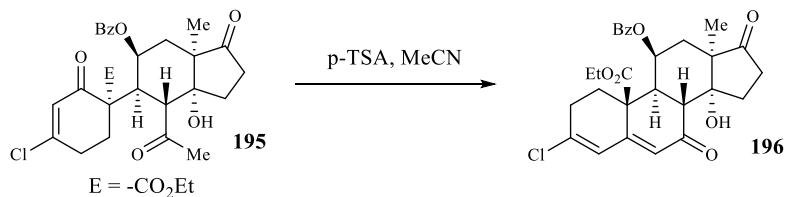
(*S,E*)-1-(1-methyl-2,5-dioxocyclopentyl)-5-oxohex-3-en-2-yl benzoate **182** (5.7 g, 17.3 mmol), ethyl 4-chloro-2-oxocyclohex-3-ene-1-carboxylate **192** (7.0 g, 34.5 mmol), and copper(II) triflate

(3.1 g, 8.6 mmol) were added successively to a flame dried flask. The vessel was purged with N₂ and dry CH₂Cl₂ (10 mL) was added. The reaction mixture was allowed to stir at room temperature until starting material was completely consumed as determined by TLC. After 9 h, the reaction mixture was filtered through a pad of silica gel and eluted with diethyl ether. The filtrate was concentrated and directly purified by flash column chromatography with 30% → 50% EtOAc in hexanes to afford title compound (2*S*,3*S*)-3-((*S*)-4-chloro-1-(ethoxycarbonyl)-2-oxocyclohex-3-en-1-yl)-1-(1-methyl-2,5-dioxocyclopentyl)-5-oxohexan-2-yl benzoate (**194**) (6.4 g, 12.1 mmol) as a white foam in 70% yield, R_f = 0.2 (33% EtOAc in Hexanes), and 6:1 d.r. as well as crude fractions of half cyclized aldol product (3*aR*,5*S*,6*S*,7*R*,7*aR*)-7-acetyl-6-((*S*)-4-chloro-1-(ethoxycarbonyl)-2-oxocyclohex-3-en-1-yl)-7*a*-hydroxy-3*a*-methyl-3-oxooctahydro-1*H*-inden-5-yl benzoate (**195**) (1.8 g, 3.45 mmol) in 20% yield.

(2*S*,3*S*)-3-((*S*)-4-chloro-1-(ethoxycarbonyl)-2-oxocyclohex-3-en-1-yl)-1-(1-methyl-2,5-dioxocyclopentyl)-5-oxohexan-2-yl benzoate (**194**): ¹H NMR (500 MHz, CDCl₃): δ 7.89 (d, J = 7.2 Hz, 2H), 7.59 (t, J = 7.4 Hz, 1H), 7.45 (t, J = 7.7 Hz, 2H), 6.18 (d, J = 1.2 Hz, 1H), 5.20 (d, J = 8.6 Hz, 1H), 3.90 - 3.79 (m, 2H), 3.21 (t, J = 4.8 Hz, 1H), 3.04 (dd, J = 19.1, 5.7 Hz, 1H), 2.84 (dd, J = 19.1, 4.2 Hz, 1H), 2.81 - 2.71 (m, ovrlp, 3H), 2.70 - 2.60 (m, 1H), 2.55 (dt, J = 19.5, 4.6 Hz, 1H), 2.48 - 2.36 (m, ovrlp, 2H), 2.23 (s, 3H), 2.20 (d, J = 10.1 Hz, 1H), 2.08 (dd, J = 14.9, 2.7 Hz, 1H), 1.94 (ddd, J = 14.2, 9.2, 5.3 Hz, 1H), 1.11 (s, 3H), 1.08 (t, J = 7.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 215.0, 214.8, 206.0, 191.9, 169.0, 166.4, 156.8, 133.5, 129.5, 128.6, 127.8, 70.6, 61.9, 58.1, 55.6, 39.5, 39.23, 37.4, 34.19, 34.15, 31.6, 30.0, 27.3, 24.3, 13.7. HRMS (ESI): m/z calcd for C₂₈H₃₁ClO₈Na⁺ [M+Na]⁺ 553.1600, found 553.1603. IR (film, cm⁻¹): 2934, 1722, 1685, 1618, 1419, 1276, 1184, 1070, 715. [α]_D²⁵ = -1.7 (c = 0.5, CHCl₃).

(3*aR*,5*S*,6*S*,7*R*,7*aR*)-7-acetyl-6-((*S*)-4-chloro-1-(ethoxycarbonyl)-2-oxocyclohex-3-en-1-yl)-7*a*-hydroxy-3*a*-methyl-3-oxooctahydro-1*H*-inden-5-yl benzoate (**195**): ¹H NMR (500 MHz, CDCl₃): δ 7.87 (d, J = 7.4 Hz, 2H), 7.57 (t, J = 7.4 Hz, 1H), 7.46 (t, J = 7.7 Hz, 2H), 6.07 (s, 1H), 5.45 (d, J = 1.6 Hz, 1H), 4.31 (s, 1H), 4.11 (d, J = 11.6 Hz, 1H), 3.78 (dq, J = 14.3, 7.1 Hz, 1H), 3.44 (dq, J = 14.3, 7.2 Hz, 1H), 2.92 (d, J = 11.6 Hz, 1H), 2.78 - 2.30 (m, 10H), 2.22 - 2.00 (m, 1H), 1.90 (dd, J = 13.5, 8.8 Hz, 1H), 1.74 (dd, J = 15.4, 3.3 Hz, 1H), 1.02 (s, 3H), 0.87 (t, J = 7.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 217.3, 214.5, 193.2, 168.5, 165.3, 154.1, 133.3, 129.7, 129.5, 128.4, 126.3, 78.0, 68.2, 61.4, 57.3, 51.5, 49.1, 38.6, 36.1, 34.6, 32.5, 31.0, 30.8, 28.5, 19.8, 13.5. HRMS (ESI): m/z calcd for C₂₈H₃₂ClO₈⁺ 531.1786 [M+H]⁺, found 531.1773. IR (film, cm⁻¹): 3457(br), 2916, 1743, 1722, 1682, 1620, 1359, 1271, 1185, 1072, 1043, 756, 713. [α]_D²⁵ = +91.9 (c = 0.5, CHCl₃).

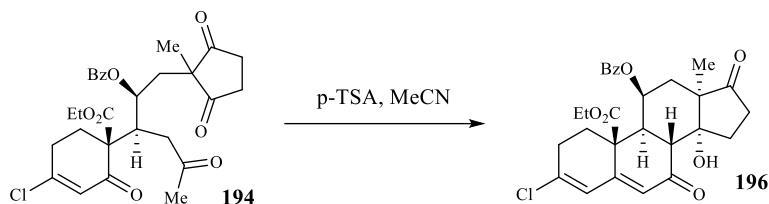
Minor diastereomer of adduct **194** characteristic ¹H NMR peaks (400 MHz, CDCl₃): δ 7.88 (d, J = 7.4 Hz, 2H), 7.59 (t, J = 7.4 Hz, 1H), 7.45 (t, J = 7.7 Hz, 2H), 6.09 (s, 1H), 5.32 (d, J = 10.2 Hz, 1H), 4.16 - 3.95 (m, 2H), 3.24 (t, 1H), 2.99 (dd, J = 16.3, 5.7 Hz, 1H), 2.23 (s, 3H), 2.00 - 1.88 (m, 1H), 1.13 - 1.04 (ovrlp, 6H).



Ethyl (8*R*,9*S*,10*S*,11*S*,13*R*,14*R*)-11-(benzoyloxy)-3-chloro-14-hydroxy-13-methyl-7,17-dioxo-1,2,7,8,9,11,12,13,14,15,16,17-dodecahydro-10*H*-cyclopenta[*a*]phenanthrene-10-carboxylate (196)

(3*aR*,5*S*,6*S*,7*R*,7*aR*)-7-acetyl-6-((*S*)-4-chloro-1-(ethoxycarbonyl)-2-oxocyclohex-3-en-1-yl)-7*a*-hydroxy-3*a*-methyl-3-oxooctahydro-1*H*-inden-5-yl benzoate **195** (7.0 g, 13.2 mmol) and *p*-toluenesulfonic acid monohydrate (26.0 g, 136.8 mmol) were solvated in acetonitrile (350 mL) and the solution was heated to 55°C for 48 h. The reaction mixture was then concentrated and CH₂Cl₂ (400 mL) was added. The *p*-TSA precipitated and was filtered off. The filtrate was then concentrated and directly purified by flash column chromatography with 10% → 30% EtOAc in hexanes to afford title compound ethyl (8*R*,9*S*,10*S*,11*S*,13*R*,14*R*)-11-(benzoyloxy)-3-chloro-14-hydroxy-13-methyl-7,17-dioxo-1,2,7,8,9,11,12,13,14,15,16,17-dodecahydro-10*H*-cyclopenta[*a*]phenanthrene-10-carboxylate (**196**) (3.8 g, 7.4 mmol) as a white solid in 56% yield, *R_f* = 0.4 (50% EtOAc in Hexanes). ¹H NMR (700 MHz, CDCl₃): δ 7.80 (d, *J* = 8.3 Hz, 2H), 7.54 (t, *J* = 7.4 Hz, 1H), 7.41 (t, *J* = 7.8 Hz, 2H), 6.31 (d, *J* = 1.9 Hz, 1H), 5.95 (s, 1H), 5.54 (s, 1H), 3.68 (dq, *J* = 10.9, 7.1 Hz, 1H), 3.41 (d, *J* = 13.2 Hz, 1H), 3.32 (dq, *J* = 10.9, 7.1 Hz, 1H), 3.19 (dd, *J* = 13.0, 9.2 Hz, 1H), 3.08 (s, 1H), 2.87 - 2.74 (ovrlp, 2H), 2.64 (dd, *J* = 15.1, 3.6 Hz, 1H), 2.56 - 2.44 (ovrlp, 3H), 2.39 - 2.29 (m, 1H), 2.12 (td, *J* = 12.5, 9.7 Hz, 1H), 1.63 (ddd, *J* = 13.0, 11.9, 6.1 Hz, 1H), 1.56 (dd, *J* = 15.1, 2.4 Hz, 1H), 1.00 (s, 3H), 0.89 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (175 MHz, CDCl₃): δ 216.5, 200.8, 169.6, 164.8, 152.0, 144.2, 133.3, 129.8, 129.4, 128.3, 126.8, 125.3, 78.5, 66.8, 62.03, 52.1, 46.8, 46.4, 45.7, 34.8, 33.5, 32.1, 31.8, 30.3, 20.6, 13.6. HRMS (ESI): *m/z*

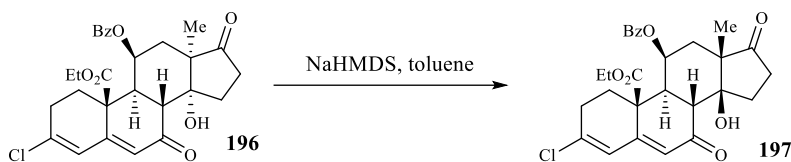
calcd for $C_{28}H_{30}ClO_7^+ [M+H]^+$ 513.1675, found 513.1680. IR (film, cm^{-1}): 3507(br), 2911, 1724, 1697, 1658, 1625, 1451, 1350, 1277, 1177, 1074, 1027, 900, 717. $[\alpha]_D^{25} = -176$ ($c = 1.1$, $CHCl_3$).



Ethyl (8*R*,9*S*,10*S*,11*S*,13*R*,14*R*)-11-(benzoyloxy)-3-chloro-14-hydroxy-13-methyl-7,17-dioxo-1,2,7,8,9,11,12,13,14,15,16,17-dodecahydro-10*H*-cyclopenta[*a*]phenanthrene-10-carboxylate (196)

(2*S*,3*S*)-3-((*S*)-4-chloro-1-(ethoxycarbonyl)-2-oxocyclohex-3-en-1-yl)-1-(1-methyl-2,5-dioxocyclopentyl)-5-oxohexan-2-yl benzoate **194** (8.0 g, 15.1 mmol) and *p*-toluenesulfonic acid monohydrate (28.7 g, 150.9 mmol) were solvated in acetonitrile (400 mL) and the solution was heated to 55°C for 48 h. The reaction mixture was then concentrated and CH_2Cl_2 (500 mL) was added. The *p*-TSA precipitated and was filtered off. The filtrate was then concentrated and directly purified by flash column chromatography with 10% → 30% EtOAc in hexanes to afford title compound ethyl (8*R*,9*S*,10*S*,11*S*,13*R*,14*R*)-11-(benzoyloxy)-3-chloro-14-hydroxy-13-methyl-7,17-dioxo-1,2,7,8,9,11,12,13,14,15,16,17-dodecahydro-10*H*-cyclopenta[*a*]phenanthrene-10-carboxylate (**196**) (5.3 g, 10.4 mmol) as a white solid in 68% yield, $R_f = 0.4$ (50% EtOAc in Hexanes). 1H NMR (700 MHz, $CDCl_3$): δ 7.80 (d, $J = 8.3$ Hz, 2H), 7.54 (t, $J = 7.4$ Hz, 1H), 7.41 (t, $J = 7.8$ Hz, 2H), 6.31 (d, $J = 1.9$ Hz, 1H), 5.95 (s, 1H), 5.54 (s, 1H), 3.68 (dq, $J = 10.9, 7.1$ Hz, 1H), 3.41 (d, $J = 13.2$ Hz, 1H), 3.32 (dq, $J = 10.9, 7.1$ Hz, 1H), 3.19 (dd, $J = 13.0, 9.2$ Hz, 1H), 3.08 (s, 1H), 2.87 - 2.74 (ovrlp, 2H), 2.64 (dd, $J = 15.1, 3.6$ Hz, 1H), 2.56 - 2.44 (ovrlp, 3H), 2.39 - 2.29 (m, 1H), 2.12 (td, $J = 12.5, 9.7$ Hz, 1H), 1.63 (ddd, $J = 13.0, 11.9, 6.1$ Hz, 1H), 1.56 (dd, J

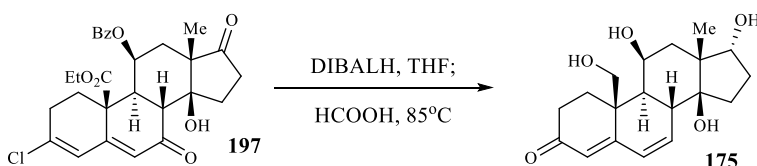
= 15.1, 2.4 Hz, 1H), 1.00 (s, 3H), 0.89 (t, J = 7.1 Hz, 3H). ^{13}C NMR (175 MHz, CDCl_3): δ 216.5, 200.8, 169.6, 164.8, 152.0, 144.2, 133.3, 129.8, 129.4, 128.3, 126.8, 125.3, 78.5, 66.8, 62.03, 52.1, 46.8, 46.4, 45.7, 34.8, 33.5, 32.1, 31.8, 30.3, 20.6, 13.6. HRMS (ESI): m/z calcd for $\text{C}_{28}\text{H}_{30}\text{ClO}_7^+$ $[\text{M}+\text{H}]^+$ 513.1675, found 513.1680. IR (film, cm^{-1}): 3507(br), 2911, 1724, 1697, 1658, 1625, 1451, 1350, 1277, 1177, 1074, 1027, 900, 717. $[\alpha]_{\text{D}}^{25} = -176$ (c = 1.1, CHCl_3).



Ethyl (8*R*,9*S*,10*S*,11*S*,13*S*,14*S*)-11-(benzyloxy)-3-chloro-14-hydroxy-13-methyl-7,17-dioxo-1,2,7,8,9,11,12,13,14,15,16,17-dodecahydro-10H-cyclopenta[a]phenanthrene-10-carboxylate (197)

Ethyl (8*R*,9*S*,10*S*,11*S*,13*R*,14*R*)-11-(benzyloxy)-3-chloro-14-hydroxy-13-methyl-7,17-dioxo-1,2,7,8,9,11,12,13,14,15,16,17-dodecahydro-10H-cyclopenta[a]phenanthrene-10-carboxylate **196** (2.0 g, 3.9 mmol) was solvated in dry toluene (30 mL) and cooled to -78°C under N_2 . Then, a solution of sodium bis(trimethylsilyl)amide (860 mg, 4.7 mmol) in toluene (10 mL) was added dropwise to the reaction vessel. The solution was allowed to stir at -78°C for 1 hour before it was slowly warmed to room temperature and subsequently placed in a 42°C oil bath where it was stirred for an additional 45 minutes. The reaction was cooled and quenched with a saturated NH_4Cl solution (5 mL), concentrated in vacuo, and directly adsorbed onto silica gel and purified by flash column chromatography with 25% EtOAc in hexanes to afford title compound ethyl (8*R*,9*S*,10*S*,11*S*,13*S*,14*S*)-11-(benzyloxy)-3-chloro-14-hydroxy-13-methyl-7,17-dioxo-1,2,7,8,9,11,12,13,14,15,16,17-dodecahydro-10H-cyclopenta[a]phenanthrene-10-carboxylate

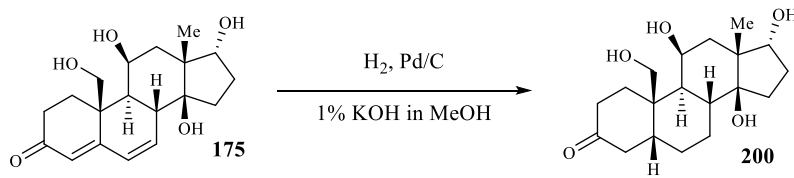
(**197**) (960 mg, 1.9 mmol) as an off white solid in 48% yield and 68% BRSM, $R_f = 0.5$ (50% EtOAc in Hexanes). $^1\text{H NMR}$ (700 MHz, CDCl_3): δ 8.01 (d, $J = 7.2$ Hz, 2H), 7.61 (t, $J = 7.4$ Hz, 1H), 7.47 (t, $J = 7.8$ Hz, 2H), 6.35 (d, $J = 2.1$ Hz, 1H), 5.96 (s, 1H), 5.74 (d, $J = 2.5$ Hz, 1H), 5.24 (s, 1H), 3.79 (dq, $J = 10.9, 7.1$ Hz, 1H), 3.66 (d, $J = 13.6$ Hz, 1H), 3.46 (dq, $J = 10.9, 7.1$ Hz, 1H), 2.96 - 2.83 (m, 1H), 2.79 (dd, $J = 13.1, 5.3$ Hz, 1H), 2.64 - 2.56 (m, 1H), 2.53 (dd, $J = 19.6, 5.7$ Hz, 1H), 2.41 (ddd, $J = 19.3, 6.5, 4.0$ Hz, 1H), 2.28 (dd, $J = 13.6, 2.2$ Hz, 1H), 2.06 - 2.00 (m, 2H), 1.90 (dd, $J = 15.6, 3.1$ Hz, 1H), 1.65 (ddd, $J = 13.3, 11.3, 6.1$ Hz, 1H), 1.45 (dd, $J = 15.6, 2.8$ Hz, 1H), 1.21 (s, 3H), 0.93 (t, $J = 7.1$ Hz, 3H). $^{13}\text{C NMR}$ (175 MHz, CDCl_3): δ 218.9, 202.7, 168.9, 164.8, 153.8, 145.3, 133.7, 129.8, 129.2, 128.6, 126.0, 125.1, 80.9, 66.1, 62.2, 51.7, 49.1, 45.5, 45.2, 34.9, 33.1, 31.6, 30.0, 27.8, 16.9, 13.6. HRMS (ESI): m/z calcd for $\text{C}_{28}\text{H}_{30}\text{ClO}_7^+$ $[\text{M}+\text{H}]^+$ 513.1675, found 513.1680. IR (film, cm^{-1}): 3485 (br), 2926, 1724, 1646, 1615, 1353, 1273, 1076, 1026, 760. $[\alpha]_D^{25} = -235$ ($c = 0.23, \text{CHCl}_3$).



(8*R*,9*S*,10*S*,11*S*,13*R*,14*S*,17*R*)-11,14,17-trihydroxy-10-(hydroxymethyl)-13-methyl-1,2,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[*a*]phenanthren-3-one (175)

To a flame dried flask containing ethyl (8*R*,9*S*,10*S*,11*S*,13*S*,14*S*)-11-(benzoyloxy)-3-chloro-14-hydroxy-13-methyl-7,17-dioxo-1,2,7,8,9,11,12,13,14,15,16,17-dodecahydro-10*H*-cyclopenta[*a*]phenanthrene-10-carboxylate **197** (600.0 mg, 1.17 mmol) in THF (35 mL) under N_2 and cooled to -78°C was added diisobutyl aluminum hydride (3.3 g, 4.2 mL, 23.4 mmol) dropwise. The reaction was allowed to stir at -78°C for 1 h before being heated to 60°C for 12 h. After

completion, the reaction flask was cooled to 0°C and quenched cautiously by the addition of water (~10 mL) until the formation of a viscous emulsion. The solution was gradually acidified with formic acid (~8 mL total) until the emulsions dissipated and the solution became homogeneous (pH < 4). The mixture was then heated to 85°C for 1 h and then quenched by the addition of a saturated NaHCO₃ solution (10 mL) and solid Na₂CO₃ (2 g). The suspension was then filtered through celite with excess MeOH and concentrated. The crude mixture was then adsorbed onto silica gel (with MeOH) and purified directly by flash column chromatography with 5% → 10% MeOH in CH₂Cl₂ to afford title compound (*8R,9S,10S,11S,13R,14S,17R*)-11,14,17-trihydroxy-10-(hydroxymethyl)-13-methyl-1,2,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[*a*]phenanthren-3-one (**175**) (281 mg, 0.842 mmol) in 72% yield and 8:1 d.r., R_f = 0.2 (10% MeOH in CH₂Cl₂). ¹H NMR (700 MHz, D₃COD): δ 6.49 (dd, J = 9.9, 1.8 Hz, 1H), 6.25 (dd, J = 9.9, 2.6 Hz, 1H), 5.69 (s, 1H), 4.18 (d, J = 1.6 Hz, 1H), 4.04 (t, J = 8.9 Hz, 1H), 3.75 (d, J = 12.2 Hz, 1H), 3.43 (d, J = 12.2 Hz, 1H), 2.93 (d, J = 11.4 Hz, 1H), 2.65 - 2.51 (ovrlp, 2H), 2.37 (dd, J = 16.8, 4.8 Hz, 1H), 2.01 (ddd, J = 13.5, 9.3, 4.2 Hz, 1H), 1.84 (ddd, J = 14.6, 12.8, 4.8 Hz, 1H), 1.76 - 1.64 (m, 2H), 1.61 (dd, J = 11.5, 1.2 Hz, 1H), 1.52 - 1.42 (m, 1H), 1.41 - 1.32 (ovrlp, 2H), 1.30 (s, 3H). ¹³C NMR (175 MHz, D₃COD): δ 202.0, 162.9, 141.6, 129.7, 124.6, 84.1, 82.9, 64.9, 61.8, 51.1, 48.4, 43.3, 39.6, 36.4, 34.4, 30.3, 29.0, 28.9, 21.6. HRMS (ESI): m/z calcd for C₁₉H₂₇O₅⁺ [M+H]⁺ 335.1853, found 335.1855. IR (film, cm⁻¹): 3320(br), 2930, 1642, 1610, 1350, 1280, 1228, 1181, 1055, 1021, 971, 874. [α]_D²⁵ = +61 (c = 1.0, MeOH).

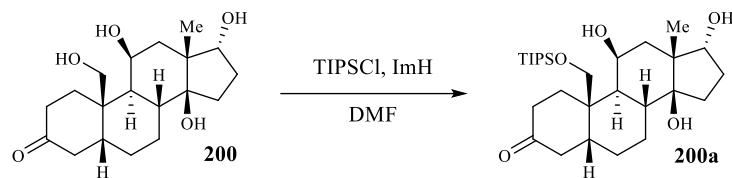


(5*R*,8*R*,9*S*,10*R*,11*S*,13*R*,14*S*,17*R*)-11,14,17-trihydroxy-10-(hydroxymethyl)-13-methylhexadecahydro-3*H*-cyclopenta[*a*]phenanthren-3-one (200)

(8*R*,9*S*,10*S*,11*S*,13*R*,14*S*,17*R*)-11,14,17-trihydroxy-10-(hydroxymethyl)-13-methyl-

1,2,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[*a*]phenanthren-3-one **175** (60 mg, 0.180 mmol) and 10% Pd on activated carbon (15 mg, 25% w/w loading) was solvated in 5.2 mL of MeOH containing 1% KOH (w/v) and 1% quinoline (v/v). The reaction vessel was purged with N₂, and then H₂, and allowed to stir vigorously at room temperature under an atmosphere of H₂. After 30 minutes, TLC showed complete conversion. The solution was quenched with 600 μL of pH = 7 phosphate buffer. The crude mixture was directly adsorbed onto silica gel in vacuo and purified by flash column chromatography with 0% → 5% → 10% MeOH in DCM to afford compound (5*R*,8*R*,9*S*,10*R*,11*S*,13*R*,14*S*,17*R*)-11,14,17-trihydroxy-10-(hydroxymethyl)-13-

methylhexadecahydro-3*H*-cyclopenta[*a*]phenanthren-3-one (**200**) (51 mg, 0.151 mmol) exclusively in 84% yield as a white solid, R_f = 0.2 (10% MeOH in CH₂Cl₂). ¹H NMR (400 MHz, D₃COD): δ 4.29 (d, J = 11.6 Hz, 1H), 4.21 (d, J = 2.3 Hz, 1H), 4.09 (t, J = 8.8 Hz, 1H), 3.23 (d, J = 11.6 Hz, 1H), 2.79 (, J = 15.4 Hz, 1H,), 2.48 (td, J = 14.7, 5.7 Hz, 1H), 2.41 - 2.31 (m, 1H), 2.29 - 2.13 (m, ovrlp, 3H), 2.13 - 2.01 (m, ovrlp, 2H), 2.00 - 1.84 (m, ovrlp, 3H), 1.84 - 1.68 (m, ovrlp, 2H), 1.63 - 1.32 (m, ovrlp, 5H), 1.32 - 1.22 (ovrlp, 4H). ¹³C NMR (100 MHz, D₃COD): δ 215.5, 86.0, 83.4, 68.5, 66.1, 47.8, 44.1, 43.0, 40.6, 40.5, 37.6, 36.6, 36.2, 32.2, 30.4, 28.7, 26.2, 21.1, 20.3. HRMS (ESI): m/z calcd for C₁₉H₃₁O₅⁺ [M+H]⁺ 339.2166, found 339.2158. IR (film, cm⁻¹): 3392 (br), 2940, 1536, 1512, 1466, 1251, 1177, 1033, 814. [α]_D²⁵ = +5.7 (c = 0.7, MeOH).



(5*R*,8*R*,9*S*,10*R*,11*S*,13*R*,14*S*,17*R*)-11,14,17-trihydroxy-13-methyl-10-

(((triisopropylsilyl)oxy)methyl)hexadecahydro-3*H*-cyclopenta[*a*]phenanthren-3-one (200a)

(5*R*,8*R*,9*S*,10*R*,11*S*,13*R*,14*S*,17*R*)-11,14,17-trihydroxy-10-(hydroxymethyl)-13-

methylhexadecahydro-3*H*-cyclopenta[*a*]phenanthren-3-one **200 (40 mg, 0.118 mmol) and**

imidazole (130 mg, 1.912 mmol) were solvated in dry DMF (5 mL) at room temperature.

Triisopropylsilyl chloride (155 μ L, 140 mg, 0.724 mmol) was added by syringe. The reaction

mixture was allowed to stir at ambient temperature for 6 h, until all starting material was consumed.

The solution was then quenched with a saturated NaHCO₃ solution (2 mL) and diluted with EtOAc

(5 mL). The organic layer was washed 3x with 1:1 H₂O:brine, dried over Na₂SO₄, filtered,

concentrated, and directly purified by flash column chromatography with 0% \rightarrow 30% \rightarrow 50% \rightarrow

65% EtOAc in hexanes to afford title compound (5*R*,8*R*,9*S*,10*R*,11*S*,13*R*,14*S*,17*R*)-11,14,17-

trihydroxy-13-methyl-10-(((triisopropylsilyl)oxy)methyl)hexadecahydro-3*H*-

cyclopenta[*a*]phenanthren-3-one (200a**) (48 mg, 0.097 mmol) in 82% yield as a white solid, R_f =**

0.7 (10% MeOH in CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃): δ 4.70 (s, 1H), 4.44 (d, J = 10.4 Hz,

1H), 4.24 - 4.13 (m, ovrlp, 2H), 3.35 (d, J = 10.4 Hz, 1H), 2.64 (t, J = 14.4 Hz, 1H), 2.48 - 2.39

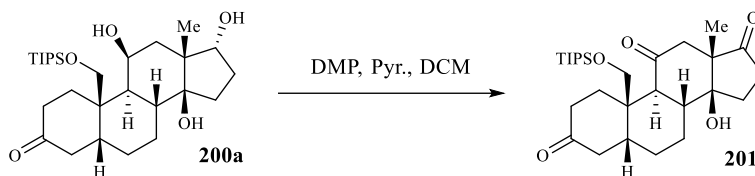
(m, 1H), 2.34 - 2.26 (m, ovrlp, 2H), 2.26 - 2.08 (m, ovrlp, 2H), 2.09 - 1.98 (m, ovrlp, 2H), 1.98 -

1.80 (m, ovrlp, 4H), 1.68 - 1.45 (m, ovrlp, 4H), 1.41 - 1.23 (m, ovrlp, 6H), 1.19 - 1.00 (m, ovrlp,

21H). ¹³C NMR (100 MHz, CDCl₃): δ 211.8, 85.0, 82.6, 69.4, 64.3, 46.7, 43.5, 42.0, 40.2, 39.3,

36.8, 35.7, 34.8, 30.9, 30.0, 28.0, 25.0, 19.8, 18.8, 18.0, 17.9, 11.8. HRMS (ESI): m/z calcd for

$C_{28}H_{51}O_5Si^+$ $[M+H]^+$ 495.3500, found 495.3509. IR (film, cm^{-1}): 3408 (br), 2941, 2866, 1709, 1462, 1264, 1058, 1016, 882, 734. $[\alpha]_D^{25} = +1.8$ ($c = 0.7$, $CHCl_3$)



(5*R*,8*R*,9*S*,10*R*,13*S*,14*S*)-14-hydroxy-13-methyl-10 (((triisopropylsilyl)oxy)methyl)dodecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,11,17(2*H*)-trione (201)

(5*R*,8*R*,9*S*,10*R*,11*S*,13*R*,14*S*,17*R*)-11,14,17-trihydroxy-13-methyl-10-

(((triisopropylsilyl)oxy)methyl)hexadecahydro-3*H*-cyclopenta[*a*]phenanthren-3-one **200a** (90

mg, 0.182 mmol) and the Dess-Martin periodinane (400 mg, 0.943 mmol) was solvated in dry

CH_2Cl_2 (4.0 mL) and pyridine (75 μ L). The reaction mixture was allowed to stir at room

temperature for 2 h until the starting material was consumed. The reaction was quenched with a

saturated $Na_2S_2O_3$ solution (2 mL) and a saturated $NaHCO_3$ solution (2 mL) and allowed to stir

vigorously for 1 hour. The resulting mixture was extracted 3x with CH_2Cl_2 (4 mL each), dried over

Na_2SO_4 , filtered, adsorbed onto silica gel, and directly purified by flash column chromatography

with 30% \rightarrow 40% \rightarrow 50% EtOAc in hexanes to afford title compound (5*R*,8*R*,9*S*,10*R*,13*S*,14*S*)-

14-hydroxy-13-methyl-10 (((triisopropylsilyl)oxy)methyl)dodecahydro-1*H*-

cyclopenta[*a*]phenanthrene-3,11,17(2*H*)-trione (**201**) (80 mg, 0.163 mmol) in 89% yield as a white

solid, $R_f = 0.3$ (50% EtOAc in hexanes). 1H NMR (400 MHz, $CDCl_3$): δ 4.28 (d, $J = 9.5$ Hz, 1H),

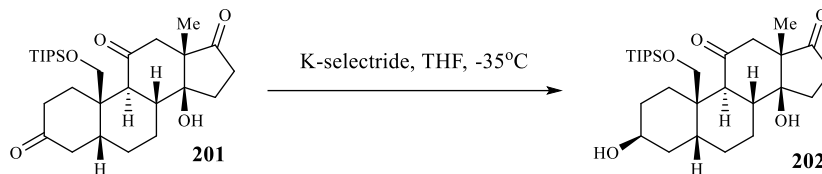
3.95 (d, $J = 9.6$ Hz, 1H), 2.79 (d, $J = 12.7$ Hz, 1H), 2.66 - 2.30 (m, ovrlp, 7H), 2.26 - 2.05 (m,

ovrlp, 6H), 2.00 (d, $J = 13.3$ Hz, 1H), 1.97 - 1.87 (m, ovrlp, 2H), 1.68 - 1.54 (m, 1H), 1.51 - 1.40

(m, 1H), 1.18 - 0.99 (m, ovrlp, 24H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 216.1, 212.3, 205.9, 80.4,

63.4, 56.5, 48.9, 47.1, 43.6, 41.6, 39.7, 37.4, 34.5, 32.9, 30.8, 27.5, 25.5, 20.2, 18.1, 18.1, 13.4,

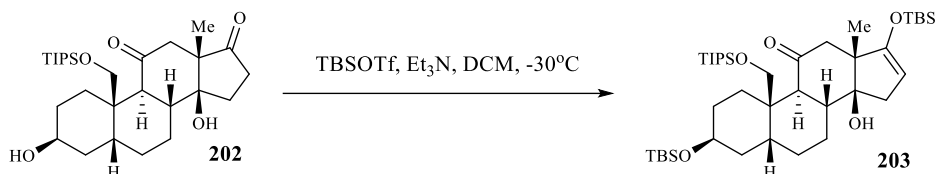
12.0. HRMS (ESI): m/z calcd for $C_{28}H_{46}O_5SiNa^+$ $[M+Na]^+$ 513.3007, found 513.2996. IR (film, cm^{-1}): 3457 (br), 2939, 2866, 1734, 1708, 1462, 1210, 1094, 882. $[\alpha]_D^{25} = +68.7$ ($c = 0.45$, $CHCl_3$).



(3*S*,5*R*,8*R*,9*S*,10*R*,13*S*,14*S*)-3,14-dihydroxy-13-methyl-10-(((triisopropylsilyl)oxy)methyl) tetradecahydro-1*H*-cyclopenta[*a*]phenanthrene-11,17-dione (202**)**

(5*R*,8*R*,9*S*,10*R*,13*S*,14*S*)-14-hydroxy-13-methyl-10(((triisopropylsilyl)oxy)methyl)dodecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,11,17(2*H*)-trione **201** (39 mg, 0.080 mmol) was solvated in dry THF (5 mL) and cooled to $-78^\circ C$ under a N_2 atmosphere. Then, 1M K-Selectride (0.170 mL, 0.170 mmol) was added slowly via syringe. The reaction was allowed to warm to $-35^\circ C$ where it was stirred for 1.5 h until the reaction was complete. The reaction was then quenched with a saturated NH_4Cl solution (2 mL) and allowed to warm to room temperature. The mixture was then extracted 3x with CH_2Cl_2 (4 mL), dried over Na_2SO_4 , filtered, adsorbed onto silica gel, and directly purified by flash column chromatography with 30% \rightarrow 40% \rightarrow 50% EtOAc in hexanes to afford title compound (3*S*,5*R*,8*R*,9*S*,10*R*,13*S*,14*S*)-3,14-dihydroxy-13-methyl-10-(((triisopropylsilyl)oxy)methyl) tetradecahydro-1*H*-cyclopenta[*a*]phenanthrene-11,17-dione (**202**) (30 mg, 0.061 mmol) in 77% yield as a white solid, $R_f = 0.3$ (50% EtOAc in hexanes). 1H NMR (700 MHz, $CDCl_3$): δ 4.14 (d, $J = 9.7$ Hz, 1H), 4.07 (s, 1H), 3.97 (d, $J = 9.7$ Hz, 1H), 2.76 (d, $J = 12.8$ Hz, 1H), 2.57 - 2.53 (m, 2H), 2.48 - 2.41 (m, 2H), 2.35 (d, $J = 13.0$ Hz, 1H), 2.26 (td, $J = 12.6, 3.9$ Hz, 1H), 2.09 (ddd, $J = 13.6, 5.8, 5.3$ Hz, 1H), 2.00 - 1.91 (m, 3H), 1.84 - 1.77 (m, overl, 3H), 1.64 - 1.55 (m, 2H), 1.51 - 1.43 (m, 2H), 1.37 - 1.29 (m, 2H), 1.25 (dd, $J = 8.9, 3.5$ Hz, 1H), 1.17 - 1.05 (m, overl, 21H),

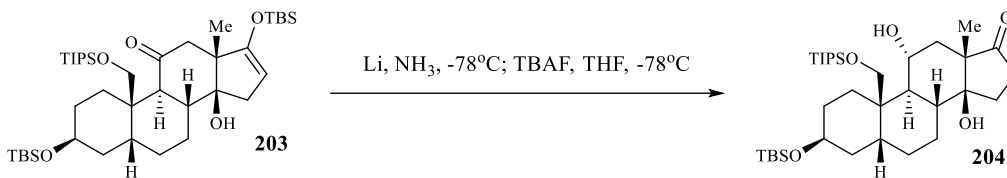
1.02 (s, 3H). ^{13}C NMR (175 MHz, CDCl_3): δ 216.7, 206.9, 80.8, 66.1, 64.6, 56.6, 47.4, 46.5, 43.5, 40.4, 33.3, 33.0, 28.3, 28.0, 27.5, 25.6, 22.7, 20.2, 18.2, 18.1, 13.6, 12.0. HRMS (ESI): m/z calcd for $\text{C}_{28}\text{H}_{49}\text{O}_5\text{Si}^+ [\text{M}+\text{H}]^+$ 493.3344, found 493.3341. IR (film, cm^{-1}): 3473 (br), 2928, 2862, 1737, 1718, 1460, 1378, 1097. $[\alpha]_{\text{D}}^{25} = +68.7$ ($c = 1.13$, CHCl_3).



(3*S*,5*R*,8*R*,9*S*,10*R*,13*S*,14*S*)-3,17-bis((tert-butyldimethylsilyl)oxy)-14-hydroxy-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-1,2,3,4,5,6,7,8,9,10,12,13,14,15-tetradecahydro-11H-cyclopenta[*a*]phenanthren-11-one (203)

(3*S*,5*R*,8*R*,9*S*,10*R*,13*S*,14*S*)-3,14-dihydroxy-13-methyl-10-(((triisopropylsilyl)oxy) methyl) tetradecahydro-1H-cyclopenta[*a*]phenanthrene-11,17-dione **202** (29 mg, 0.059 mmol) was solvated in dry CH_2Cl_2 (2.5 mL) and triethylamine (225 μL) and cooled to -78°C under a N_2 atmosphere. Then, Tert-butyldimethylsilyl trifluoromethanesulfonate (135 μL , 110 mg, 0.495 mmol) was added via syringe. The reaction was allowed to warm to -30°C where it was stirred for 1.5 h until the reaction was complete. The solution was then quenched with a saturated NaHCO_3 solution (1 mL) and extracted 3x with CH_2Cl_2 (2 mL), dried over Na_2SO_4 , filtered, concentrated, and directly purified by flash column chromatography with 0% \rightarrow 5% EtOAc in hexanes to afford title compound (3*S*,5*R*,8*R*,9*S*,10*R*,13*S*,14*S*)-3,17-bis((tert-butyldimethylsilyl)oxy)-14-hydroxy-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-1,2,3,4,5,6,7,8,9,10,12,13,14,15-tetradecahydro-11H-cyclopenta[*a*]phenanthren-11-one (**203**) (40 mg, 0.056 mmol) in 95% yield as a white solid, $R_f = 0.7$ (10% EtOAc in hexanes). ^1H NMR (700 MHz, CDCl_3): δ 4.41 (s, 1H), 4.11 (d, $J = 9.8$ Hz, 1H), 4.02 (s, 1H), 3.56 (d, $J = 9.8$ Hz, 1H), 2.69 (dd, $J = 16.5, 1.8$ Hz, 1H), 2.63 (d, $J = 12.7$

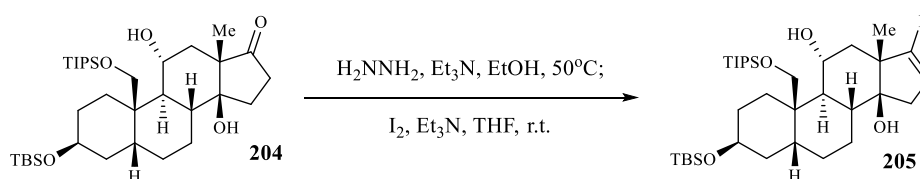
Hz, 1H), 2.44 (td, $J = 12.4, 3.1$ Hz, 1H), 2.27 (ovrlp, 2H), 2.08 (d, $J = 12.8$ Hz, 1H), 2.03 (dd, $J = 16.5, 2.5$ Hz, 1H), 1.93 (d, $J = 13.3$ Hz, 1H), 1.86 (m, ovrlp, 2H), 1.74 - 1.68 (m, ovrlp, 2H), 1.65 (t, $J = 13.7$ Hz, 1H), 1.51 (m, 1H), 1.36 - 1.22 (m, ovrlp, 3H), 1.11 - 1.02 (m, 24H), 0.92 (s, 9H), 0.87 (s, 9H), 0.16 (s, 3H), 0.15 (s, 3H), 0.010 (s, 3H), 0.007 (s, 3H). ^{13}C NMR (175 MHz, CDCl_3): δ 212.0, 156.0, 95.7, 80.2, 68.4, 66.8, 53.6, 49.3, 46.6, 43.7, 41.7, 38.0, 34.9, 30.6, 28.8, 26.8, 25.8, 25.64, 25.61, 24.8, 20.3, 19.3, 18.1, 18.1, 18.07, 18.05, 12.0, -4.7, -4.8, -4.9, -5.1. HRMS (ESI): m/z calcd for $\text{C}_{40}\text{H}_{77}\text{O}_5\text{Si}_3^+$ $[\text{M}+\text{H}]^+$ 721.5073, found 721.5078.



(3*S*,5*R*,8*R*,9*S*,10*R*,11*R*,13*S*,14*S*)-3-((tert-butyldimethylsilyl)oxy)-11,14-dihydroxy-13-methyl-10-(((triisopropylsilyl)oxy)methyl)hexadecahydro-17H-cyclopenta[*a*]phenanthren-17-one (204)

Gaseous ammonia (~1.5 mL) was condensed at -78°C under N_2 and a small chip of lithium metal (~2 mg) was added and allowed to dissolve. After 30 minutes, (3*S*,5*R*,8*R*,9*S*,10*R*,13*S*,14*S*)-3,17-bis((tert-butyldimethylsilyl)oxy)-14-hydroxy-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-1,2,3,4,5,6,7,8,9,10,12,13,14,15-tetradecahydro-11H-cyclopenta[*a*]phenanthren-11-one **203** (9.1 mg, 0.0125 mmol) dissolved in dry THF (0.5 mL) was slowly added to the deep blue reaction ammonia solution via syringe and allowed to stir at -78°C for 30 minutes. Then, the reaction was quenched dropwise by the addition of a saturated NH_4Cl solution until the blue color had completely dissipated. The solution was allowed to warm to room temperature as the ammonia boiled off. The remaining mixture was extracted 2x with CH_2Cl_2 (2 mL), dried over Na_2SO_4 , filtered, and concentrated. The crude mixture was then solvated in dry THF (1 mL) and cooled to

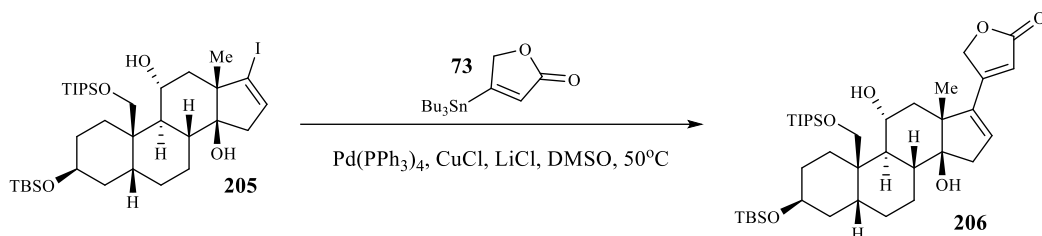
-78°C under N₂. 1M Tetrabutylammonium fluoride in THF (15 μL, 0.015 mmol) was added via syringe and the reaction was stirred for precisely 5 minutes before being quenched with a saturated NH₄Cl solution (1 mL) and warmed to room temperature. The crude mixture was then extracted 3x with CH₂Cl₂ (2 mL), dried over Na₂SO₄, filtered, adsorbed onto silica gel, and directly purified by flash column chromatography with 10% → 20% → 35% EtOAc in hexanes to afford title compound (3*S*,5*R*,8*R*,9*S*,10*R*,11*R*,13*S*,14*S*)-3-((tert-butyldimethylsilyl)oxy)-11,14-dihydroxy-13-methyl-10-(((triisopropylsilyl)oxy)methyl)hexadecahydro-17H-cyclopenta[*a*]phenanthren-17-one (**204**) (5.5 mg, 0.0091 mmol) in 73% yield as a white solid, R_f = 0.7 (50% EtOAc in hexanes). ¹H NMR (500 MHz, CDCl₃): δ 4.27 (d, J = 9.8 Hz, 1H), 4.06 (s, 1H), 3.96 (t, J = 10.6 Hz, 1H), 3.43 (d, J = 9.8 Hz, 1H), 3.15 (d, J = 2.8 Hz, 1H), 2.53 - 2.21 (m, ovrlp, 4H), 1.94 - 1.48 (m, ovrlp, 11H), 1.47 - 1.35 (m, ovrlp, 2H), 1.31 - 1.05 (m, ovrlp, 26H), 0.88 (s, 9H), 0.03 (s, 3H), 0.02 (s, 3H). ¹³C NMR (175 MHz, CDCl₃): δ 219.9, 82.3, 70.3, 69.7, 66.5, 53.8, 41.7, 41.5, 40.6, 40.3, 35.2, 34.4, 33.0, 29.7, 28.8, 27.6, 27.2, 26.7, 25.8, 18.8, 18.1, 18.0, 14.3, 11.9, -4.9. HRMS (ESI): m/z calcd for C₃₄H₆₅O₅Si₂⁺ [M+H]⁺ 609.4371, found 609.4379. IR (film, cm⁻¹): 3483 (br), 2928, 2865, 1731, 1460, 1252, 1065, 830. [α]_D²⁵ = -1.7 (c = 0.57, CHCl₃).



(3*S*,5*R*,8*R*,9*S*,10*R*,11*R*,13*S*,14*S*)-3-((tert-butyldimethylsilyl)oxy)-17-iodo-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-1,2,3,4,5,6,7,8,9,10,11,12,13,15-tetradecahydro-14H-cyclopenta[*a*]phenanthrene-11,14-diol (205**)**

(3*S*,5*R*,8*R*,9*S*,10*R*,11*R*,13*S*,14*S*)-3-((tert-butyldimethylsilyl)oxy)-11,14-dihydroxy-13-methyl-10-(((triisopropylsilyl)oxy)methyl)hexadecahydro-17H-cyclopenta[*a*]phenanthren-17-one **204** (5.5

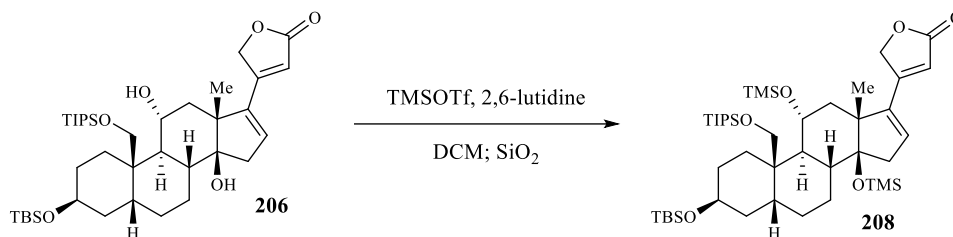
mg, 0.0091 mmol) was solvated in EtOH (1.0 mL) and triethylamine (25 μ L). Hydrazine monohydrate (9 mg, 0.18 mmol) was then added and the solution was heated to 50°C and stirred for 6 h until all starting material was consumed. The resulting mixture was cooled to room temperature and concentrated in vacuo. The crude oil was then dissolved in THF (1.0 mL) and triethylamine (25 μ L). A solution of I₂ (25 mg, 0.0985 mmol) in THF (1 mL) was prepared and 0.25 mL of this solution was added to the reaction vessel. After 10 minutes, additional I₂ solution (0.30 mL) was added until a brown color persisted. The mixture was stirred at room temperature for 1 hour until it was complete. The solution was then quenched with a saturated Na₂S₂O₃ solution (1 mL) and a saturated NaHCO₃ solution (1 mL) and stirred vigorously for 20 minutes. The resulting mixture was then extracted 3x with EtOAc (2 mL each), dried over Na₂SO₄, filtered, adsorbed onto silica gel, and directly purified by flash column chromatography with 0% → 5% → 10% → 20% EtOAc in hexanes to afford title compound (3*S*,5*R*,8*R*,9*S*,10*R*,11*R*,13*S*,14*S*)-3-((tert-butylidimethylsilyl)oxy)-17-iodo-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-1,2,3,4,5,6,7,8,9,10,11,12,13,15-tetradecahydro-14*H*-cyclopenta[*a*]phenanthrene-11,14-diol (**205**) (6.0 mg, 0.0084 mmol) in 90% yield, R_f = 0.9 (50% EtOAc in hexanes). ¹H NMR (700 MHz, CDCl₃): δ 6.13 (s, 1H), 4.30 (d, J = 9.8 Hz, 1H), 4.04 (s, 1H), 3.83 (td, J = 12.4, 3.3 Hz, 1H), 3.43 (d, J = 3.9 Hz, 1H), 3.39 (d, J = 9.8 Hz, 1H), 2.66 (dd, J = 16.5, 1.0 Hz, 1H), 2.31 - 2.24 (m, ovrlp, 2H), 1.91 (dd, J = 13.2, 2.9 Hz, 1H), 1.88 - 1.75 (m, ovrlp, 3H), 1.75 - 1.67 (m, ovrlp, 2H), 1.68 - 1.47 (m, ovrlp, 4H), 1.23 - 1.07 (m, ovrlp, 28H), 0.87 (s, 9H), 0.02 (s, 3H), 0.01 (s, 3H). ¹³C NMR (175 MHz, CDCl₃): δ 133.7, 110.2, 82.1, 70.5, 69.1, 66.6, 55.3, 46.1, 43.1, 41.8, 41.0, 40.5, 35.2, 34.5, 28.8, 27.1, 26.7, 25.8, 20.1, 19.6, 18.1, 18.0, 11.9, -4.9. HRMS (ESI): m/z calcd for C₃₄H₆₄IO₄Si₂⁺ [M+H]⁺ 719.3382, found 719.3381. IR (film, cm⁻¹): 3450 (br), 2926, 2856, 1462, 1380, 1253, 1053, 828. [α]_D²⁵ = -8.1 (c = 0.53, CHCl₃).



4-((3*S*,5*R*,8*R*,9*S*,10*R*,11*R*,13*R*,14*S*)-3-((*tert*-butyldimethylsilyl)oxy)-11,14-dihydroxy-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-2,3,4,5,6,7,8,9,10,11,12,13,14,15-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)furan-2(5*H*)-one (206)

(3*S*,5*R*,8*R*,9*S*,10*R*,11*R*,13*S*,14*S*)-3-((*tert*-butyldimethylsilyl)oxy)-17-iodo-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-1,2,3,4,5,6,7,8,9,10,11,12,13,15-tetradecahydro-14*H*-cyclopenta[*a*]phenanthrene-11,14-diol **205** (15.0 mg, 0.021 mmol) along with Pd(PPh₃)₄ (5 mg, 0.0043 mmol), CuCl (31 mg, 0.313 mmol), and LiCl (18 mg, 0.425 mmol) was solvated in dry DMSO (1.1 mL) under a N₂ atmosphere and stannyl butenolide **73** (30 mg, 0.0804 mmol) was added via syringe. The reaction solution was heated to 50°C for 1 hour until the starting material was consumed. The solution was then quenched with pH = 7 phosphate buffer (1 mL) and extracted 4x with EtOAc (1.5 mL each). The organic layers were combined, dried over Na₂SO₄, filtered, adsorbed onto silica gel, and directly purified by flash column chromatography with 0% → 10% → 20% → 30% EtOAc in hexanes to afford title compound 4-((3*S*,5*R*,8*R*,9*S*,10*R*,11*R*,13*R*,14*S*)-3-((*tert*-butyldimethylsilyl)oxy)-11,14-dihydroxy-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-2,3,4,5,6,7,8,9,10,11,12,13,14,15-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)furan-2(5*H*)-one (**206**) (13.0 mg, 0.0193 mmol) in 92% yield, R_f = 0.3 (30% EtOAc in hexanes). ¹H NMR (700 MHz, CDCl₃): δ 6.11 (s, 1H), 6.00 (s, 1H), 5.00 (dd, J = 16.2, 1.0 Hz, 1H), 4.92 (dd, J = 16.2, 1.0 Hz, 1H), 4.34 (d, J = 9.8 Hz, 1H), 4.05 (s, 1H), 3.93 - 3.85 (m, 1H), 3.52 (d, J = 3.8 Hz, 1H), 3.40 (d, J = 9.8 Hz, 1H), 2.81 (d, J = 18.2 Hz, 1H), 2.41 (dd, J = 18.5, 3.3 Hz, 1H), 2.30 (d, J

= 13.9 Hz, 1H), 2.13 (dd, $J = 13.2, 3.0$ Hz, 1H), 1.91 - 1.66 (m, 6H), 1.66 - 1.48 (m, 3H), 1.36 - 1.32 (m, overlap, 4H), 1.31 - 1.15 (m, overlap, 6H), 1.14 - 1.08 (m, 18H), 0.088 (s, 9H), 0.025 (s, 3H), 0.018 (s, 3H). ^{13}C NMR (175 MHz, CDCl_3): δ 174.1, 157.9, 143.4, 131.8, 112.9, 85.3, 71.6, 70.6, 69.0, 66.5, 52.7, 47.1, 41.8, 40.9, 40.5, 40.5, 35.3, 34.6, 29.7, 28.8, 27.2, 26.7, 25.8, 20.2, 18.1, 18.0, 18.0, 12.0, -4.9. HRMS (ESI): m/z calcd for $\text{C}_{38}\text{H}_{67}\text{O}_6\text{Si}_2^+$ $[\text{M}+\text{H}]^+$ 675.4471, found 675.4466. IR (film, cm^{-1}): 3473 (br), 2925, 2856, 1747, 1622, 1462, 1255, 1045, 828. $[\alpha]_{\text{D}}^{25} = +19.5$ ($c = 0.3$, CHCl_3).

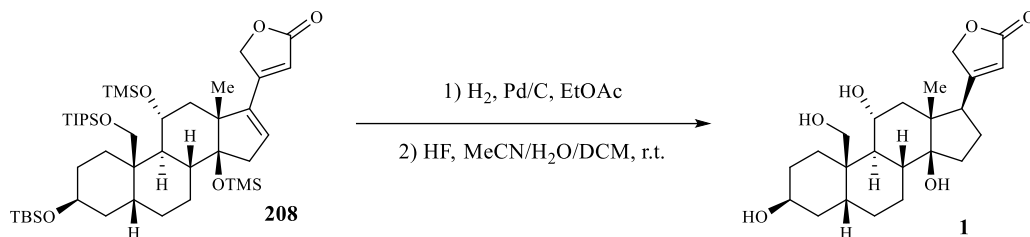


4-((3*S*,5*R*,8*R*,9*S*,10*R*,11*R*,13*R*,14*S*)-3-((tert-butyl dimethylsilyl)oxy)-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-11,14-bis((trimethylsilyl)oxy)-2,3,4,5,6,7,8,9,10,

11,12,13,14,15-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)furan-2(5*H*)-one (208)

4-((3*S*,5*R*,8*R*,9*S*,10*R*,11*R*,13*R*,14*S*)-3-((tert-butyl dimethylsilyl)oxy)-11,14-dihydroxy-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-2,3,4,5,6,7,8,9,10,11,12,13,14,15-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)furan-2(5*H*)-one **206** (13.0 mg, 0.0193 mmol) and 2,6-lutidine (28 μL , 25.9 mg, 0.242 mmol) were solvated in dry CH_2Cl_2 (4 mL) and cooled to -78°C under a N_2 atmosphere. Then, trimethylsilyl trifluoromethanesulfonate (22 μL , 27.0 mg, 0.122 mmol) was added via syringe and the reaction was allowed to warm to room temperature. After 2 h, the reaction was quenched with a saturated NaHCO_3 solution (1.5 mL). The crude mixture was then extracted 3x with CH_2Cl_2 (2 mL each), dried over Na_2SO_4 , filtered, and azeotroped 4x with toluene (2 mL each) to remove remaining 2,6-lutidine. The crude material was then solvated in CH_2Cl_2 (5

mL) and adsorbed onto silica gel (5 g). The silica gel was allowed to sit at room temperature overnight. Then, the crude material was washed off the silica gel by filtering and eluting with 100% EtOAc. The filtrate was concentrated and directly purified by flash column chromatography with 0% → 5% EtOAc in hexanes to afford title compound 4-((3*S*,5*R*,8*R*,9*S*,10*R*,11*R*,13*R*,14*S*)-3-((tert-butyldimethylsilyl)oxy)-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-11,14-bis((trimethylsilyl)oxy)-2,3,4,5,6,7,8,9,10,11,12,13,14,15-tetradecahydro-1H-cyclopenta[*a*]phenanthren-17-yl)furan-2(5H)-one (**208**) (10.0 mg, 0.0122 mmol) in 64% yield, R_f = 0.4 (10% EtOAc in hexanes). ¹H NMR (500 MHz, CDCl₃): δ 6.06 (t, J = 2.5 Hz, 1H), 5.95 (s, 1H), 5.01 (dd, J = 16.2, 1.5 Hz, 1H), 4.92 (dd, J = 16.2, 1.5 Hz, 1H), 4.02 (s, 1H), 3.82 – 3.93 (m, ovrlp, 2H), 3.65 (d, J = 9.5 Hz, 1H), 2.87 (d, J = 17.9 Hz, 1H), 2.44 (dd, J = 18.9, 3.3 Hz, 1H), 2.25 (d, J = 11.9 Hz, 1H), 1.93 - 1.70 (m, 8H), 1.63 - 1.52 (m, 1H), 1.44 - 1.35 (m, 2H), 1.24 - 1.05 (m, 27H), 0.88 (s, 9H), 0.023 (s, 9H), 0.014 (s, 6H), 0.007 (s, 9H). ¹³C NMR (175 MHz, CDCl₃): δ 174.5, 158.4, 143.3, 133.3, 111.8, 88.7, 71.6, 67.6, 67.4, 67.2, 52.8, 46.1, 42.7, 40.8, 40.3, 39.8, 34.9, 30.6, 29.3, 26.9, 25.9, 25.3, 21.6, 19.7, 18.3, 18.2, 18.1, 12.0, 2.6, 0.8, -4.9, -4.9.



19-hydroxysarmentogenin (**1**)

4-((3*S*,5*R*,8*R*,9*S*,10*R*,11*R*,13*R*,14*S*)-3-((tert-butyldimethylsilyl)oxy)-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-11,14-bis((trimethylsilyl)oxy)-2,3,4,5,6,7,8,9,10,11,12,13,14,15-tetradecahydro-1H-cyclopenta[*a*]phenanthren-17-yl)furan-2(5H)-one **208** (10.0 mg, 0.0122 mmol) and 10% Palladium on activated carbon (10 mg) were solvated in EtOAc (1.5 mL) and the

reaction vessel was purged with N₂, and then with H₂, and the solution was allowed to stir vigorously under an atmosphere of H₂. After 30 minutes, all starting material was consumed. The reaction solution was then filtered through a pad of celite and silica gel with 100% EtOAc. The resulting crude filtrate was a 2.5:1 diastereomeric mixture of C17 epimers. The minor diastereomer was then removed by flash column chromatography with 0% → 5% → 10% EtOAc in hexanes. The material was then immediately subjected to HF deprotection without further purification. The residue was solvated in 450 μL of 1:1 CH₂Cl₂:MeCN. Then, a stock solution of 18:3:2 MeCN:H₂O:HF was prepared and 700 μL of this solution was added to the reaction vessel. The resulting solution was allowed to stir at room temperature for 3 days. Once all the TIPS groups had been removed, methoxytrimethylsilane (300 μL) was added and the crude mixture was stirred for 3 h. The resulting solution was then concentrated in vacuo, adsorbed onto silica gel, and directly purified by flash column chromatography with 0% → 10% MeOH in CH₂Cl₂ to afford 19-hydroxysarmentogenin (**1**) (2.0 mg, 0.0049 mmol) in 42% yield over two steps, R_f = 0.3 (15% MeOH in CH₂Cl₂). ¹H NMR (700 MHz, D₃COD): δ 5.91 (s, 1H, H22), 5.02 (dd, J = 18.4, 1.3 Hz, 1H, H21b), 4.92 (dd, J = 18.4, 0.9 Hz, 1H, H21a), 4.03 (m, 1H, H3), 3.85 (ddd, J = 10.6, 4.2 Hz, 1H, H11), 3.77 (d, J = 11.1 Hz, 1H, H19b), 3.74 (d, J = 11.1 Hz, 1H, H19a), 2.90 (dd, J = 9.0, 5.7 Hz, 1H, H17), 2.26 - 2.15 (m, 3H, H1b, 15b, 16b), 2.07 (m, 1H, H5), 1.96 - 1.70 (m, 9H, H1a, 2b, 4b, 6b, 7b, 8, 9, 15a, 16a), 1.67 (dd, J = 13.2, 4.2 Hz, 1H, H12b), 1.53 (dd, J = 13.2, 10.5 Hz, 1H, H12a), 1.57 - 1.50 (m, 1H, H2a), 1.39 - 1.20 (m, 3H, H4a, 6a, 7a), 0.91 (s, 3H, H18). ¹³C NMR (175 MHz, D₃COD): δ 177.7 (C23), 177.1 (20), 118.0 (C22), 85.9 (C14), 75.3 (C21), 69.1 (C11), 67.6 (C3), 66.9 (C19), 51.9 (C17), 51.1 (C13), 50.6 (C12), 42.8, 41.6 (x2) (C10), 35.0, 33.5 (C15), 32.3 (C5), 29.2, 27.9 (C16), 27.7, 27.1, 22.3, 17.6 (C18). HRMS (ESI): Calculated for C₂₃H₃₅O₆⁺ [M+H]⁺ 407.2428, found 407.2435. Calculated for C₂₃H₃₄O₆Na⁺ [M+Na]⁺ 429.2248, found

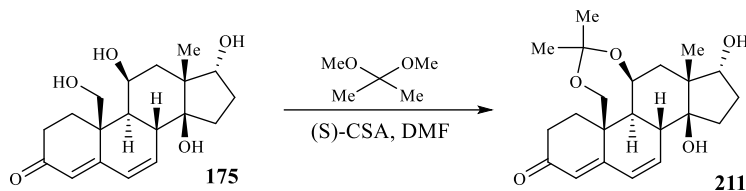
429.2255. IR (film, cm^{-1}): 3315 (br), 2920, 1735, 1632, 1449, 1022. $[\alpha]_{\text{D}}^{25} = +13.4$ ($c = 0.1$, MeOH).

Table 4.2. Comparison to the reported 19-hydroxysarmentogenin ^1H NMR (700 MHz, D_3COD)¹¹:

Position	This work δ_{H} (Multiplicity, J in Hz, No. of H)	Inoue et al. δ_{H} (Multiplicity, J in Hz, No. of H)
22	5.91 (s, 1H)	5.91 (br s, 1H)
21b	5.02 (dd, $J = 18.4, 1.3$, 1H)	5.02 (dd, $J = 18.3, 1.8$, 1H)
21a	4.92 (dd, $J = 18.4, 0.9$, 1H)	4.91 (dd, $J = 18.3, 1.2$, 1H)
3	4.03 (m, 1H)	4.03 (m, 1H)
11	3.85 (ddd, $J = 10.6, 4.2$, 1H)	3.85 (ddd, $J = 10.9, 3.5$, 1H)
19b	3.77 (d, $J = 11.1$, 1H)	3.77 (d, $J = 12.0$, 1H)
19a	3.74 (d, $J = 11.1$, 1H)	3.74 (d, $J = 12.0$, 1H)
17	2.90 (dd, $J = 9.0, 5.7$, 1H)	2.90 (dd, $J = 9.2, 5.2$, 1H)
1b, 15b, 16b	2.26 - 2.15 (m, 3H)	2.26-2.15 (m, 3H)
5	2.07 (m, 1H)	2.07 (m, 1H)
H1a, 2b, 4b, 6b, 7b, 8, 9, 15a, 16a	1.96 - 1.70 (m, 9H)	1.96-1.69 (m, 9H)
12b	1.67 (dd, $J = 13.2, 4.2$, 1H)	1.67 (dd, $J = 13.2, 4.0$, 1H)
12a	1.53 (dd, $J = 13.2, 10.5$, 1H)	1.53 (dd, $J = 13.2, 10.5$, 1H)
2a	1.57 - 1.50 (m, 1H)	1.58-1.50 (m, 1H)
4a, 6a, 7a	1.39 - 1.20 (m, 3H)	1.39-1.20 (m, 3H)
18	0.91 (s, 3H)	0.92 (s, 3H)

Table 4.3. Comparison to the reported 19-hydroxysarmentogenin ¹³C NMR (CD₃OD)¹¹:

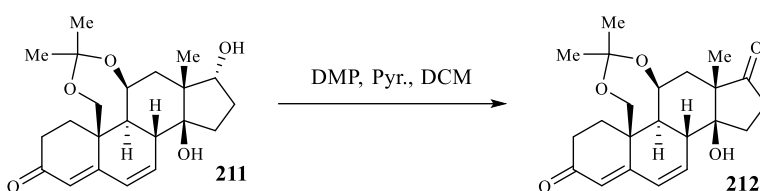
Carbon #	This work	Inoue et al.	Δ (δ)
23	177.7	177.7	0.0
20	177.1	177.1	0.0
22	118.0	118.0	0.0
14	85.9	85.9	0.0
21	75.3	75.3	0.0
11	69.1	69.1	0.0
3	67.6	67.6	0.0
19	66.9	66.9	0.0
17	51.9	51.9	0.0
13	51.1	51.1	0.0
12	50.6	50.5	0.1
	42.8	42.8	0.0
10	41.6	41.6	0.0
	35.0	35.0	0.0
15	33.5	33.5	0.0
5	32.3	32.3	0.0
	29.2	29.2	0.0
16	27.9	27.9	0.0
	27.7	27.7	0.0
	27.1	27.1	0.0
	22.3	22.3	0.0
18	17.6	17.6	0.0



(4*aS*,4*a1S*,8*aS*,9*aR*,10*R*,12*aS*,12*bR*)-10,12*a*-dihydroxy-7,7,9*a*-trimethyl-3,4,8*a*,9,9*a*,10,11,12,12*a*,12*b*-decahydro-5H-cyclopenta[1,2]phenanthro[4,4*b*-de][1,3]dioxepin-2(4*a1H*)-one (211)

To a flame dried flask was added (8*R*,9*S*,10*S*,11*S*,13*R*,14*S*,17*R*)-11,14,17-trihydroxy-10-(hydroxymethyl)-13-methyl-1,2,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[*a*]phenanthren-3-one **175** (50.0 mg, 0.15 mmol) and S-camphorsulfonic acid (CSA) (20.0 mg, 0.086 mmol). The solids were solvated in 4.0 mL of dry dimethylformamide and 2,2-dimethoxypropane (550 μ L) was added via syringe. The solution was allowed to stir at ambient temperature for 20 minutes when TLC indicated all starting material was consumed. The crude mixture was then diluted with EtOAc (4.0 mL) and quenched with a saturated solution of NaHCO₃ (2.0 mL) and Brine (2.0 mL). The organic layer was washed 3x with a 1:1 mixture of water:brine (3.0 mL each). The organic layer was then dried with Na₂SO₄, filtered, and concentrated. The crude residue was then purified by flash column chromatography with 5% \rightarrow 10% MeOH in CH₂Cl₂ to afford title compound (4*aS*,4*a1S*,8*aS*,9*aR*,10*R*,12*aS*,12*bR*)-10,12*a*-dihydroxy-7,7,9*a*-trimethyl-3,4,8*a*,9,9*a*,10,11,12,12*a*,12*b*-decahydro-5H-cyclopenta[1,2]phenanthro[4,4*b*-de][1,3]dioxepin-2(4*a1H*)-one (**211**) (38.6 mg, 0.10 mmol) in 69% yield as a white solid, R_f = 0.5 (10% MeOH in CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃): δ 6.51 (dd, J = 9.9, 2.1 Hz, 1H), 6.22 (dd, J = 10.0, 2.9 Hz, 1H), 5.78 (s, 1H), 4.27 (s, 1H), 4.16 (t, J = 8.8 Hz, 1H), 3.85 (d, J = 12.7 Hz, 1H), 3.32 (dd, J = 12.7, 1.6 Hz, 1H), 3.03 (d, J = 11.0 Hz, 1H), 2.71 - 2.58 (m, ovrlp, 2H), 2.52 - 2.43

(m, 1H), 2.23 - 2.10 (m, 1H), 1.95 - 1.83 (m, 1H), 1.68 (dd, $J = 14.6, 3.4$ Hz, 1H), 1.59 - 1.41 (m, overl, 4H), 1.36 (s, overl, 4H), 1.33 (s, 3H), 1.31 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3): δ 199.7, 157.0, 137.9, 128.8, 126.0, 100.8, 83.1, 82.1, 65.4, 61.8, 48.1, 40.7, 38.8, 35.0, 34.0, 30.8, 29.6, 29.0, 25.4, 24.4, 21.2. IR (film, cm^{-1}): 3346 (br), 2920, 1644, 1613, 1363, 1281, 1220, 1066, 1022, 730. HRMS (ESI): Calculated for $\text{C}_{22}\text{H}_{31}\text{O}_5^+$ $[\text{M}+\text{H}]^+$ 375.2166, found 375.2164. $[\alpha]_{\text{D}}^{25} = +60.2$ ($c = 0.33$, CH_2Cl_2).



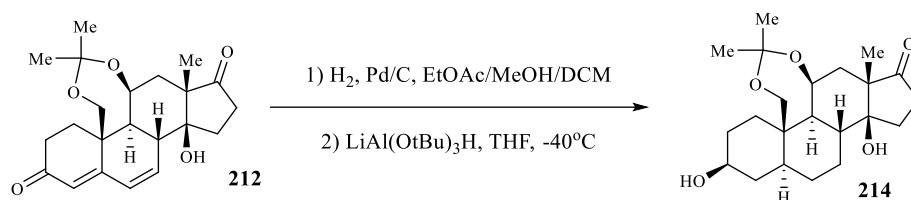
(4*aS*,4*a*1*S*,8*aS*,9*aS*,12*aS*,12*bR*)-12*a*-hydroxy-7,7,9*a*-trimethyl-3,4,4*a*1,8*a*,9,9*a*,11,12,12*a*,12*b*-decahydro-5*H*-cyclopenta[1,2]phenanthro[4,4*b*-de][1,3]dioxepine-2,10-dione (218)

(4*aS*,4*a*1*S*,8*aS*,9*aR*,10*R*,12*aS*,12*bR*)-10,12*a*-dihydroxy-7,7,9*a*-trimethyl-

3,4,8*a*,9,9*a*,10,11,12,12*a*,12*b*-decahydro-5*H*-cyclopenta[1,2]phenanthro[4,4*b*-de][1,3]dioxepin-2(4*a*1*H*)-one **211** (28.0 mg, 0.075 mmol) and Dess-Martin periodinane (140.0 mg, 0.330 mmol) were added successively to a dry flask and solvated in 3.0 mL of CH_2Cl_2 and 30.0 μL of pyridine.

The solution was allowed to stir at room temperature for 1.5 hours until all SM was consumed. If the reaction seems to stall by TLC, 1 drop of H_2O can be added to promote further conversion to product. The solution was then quenched with saturated solutions of $\text{Na}_2\text{S}_2\text{O}_3$ (2.0 mL) and NaHCO_3 (1.5 mL) and stirred at room temperature for 45 minutes. The organic layer was then extracted, dried with Na_2SO_4 , filtered, and concentrated. The crude residue was then purified by flash column chromatography with 5% \rightarrow 10% MeOH in CH_2Cl_2 to afford title compound (4*aS*,4*a*1*S*,8*aS*,9*aS*,12*aS*,12*bR*)-12*a*-hydroxy-7,7,9*a*-trimethyl-3,4,4*a*1,8*a*,9,9*a*,11,12,12*a*,12*b*-

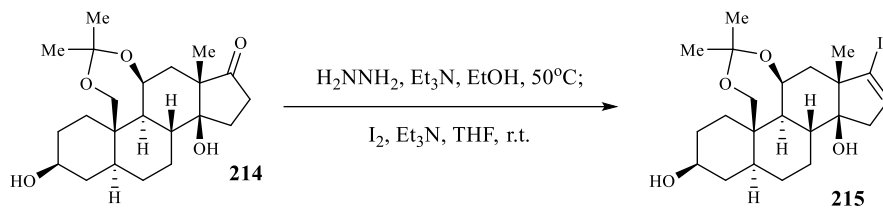
decahydro-5H-cyclopenta[1,2]phenanthro[4,4b-de][1,3]dioxepine-2,10-dione (**218**) (25.0 mg, 0.067 mmol) in 90% yield as a white solid, R_f = 0.7 (10% MeOH in CH₂Cl₂). ¹H NMR (700 MHz, CDCl₃): δ 6.59 (dd, J = 9.9, 2.1 Hz, 1H), 6.28 (dd, J = 10.0, 3.0 Hz, 1H), 5.80 (s, 1H), 4.22 (s, 1H), 3.85 (d, J = 12.8 Hz, 1H), 3.32 (dd, J = 12.8, 1.4 Hz, 1H), 3.17 (d, J = 11.2 Hz, 1H), 2.67 - 2.59 (m, ovrlp, 2H), 2.54 (m, ovrlp, 3H), 2.03 - 1.96 (m, 1H), 1.90 - 1.79 (m, ovrlp, 2H), 1.69 (dd, J = 14.5, 3.3 Hz, 1H), 1.54 - 1.44 (m, ovrlp, 2H), 1.37 (dd, J = 14.5, 2.7 Hz, 1H), 1.35 (s, 3H), 1.33 (s, 3H), 1.29 (s, 3H). ¹³C NMR (176 MHz, CDCl₃): δ 220.4, 199.5, 156.5, 136.8, 129.1, 126.3, 101.0, 81.6, 65.1, 61.6, 53.1, 47.7, 40.7, 38.1, 37.5, 33.9, 33.9, 30.7, 26.8, 25.3, 24.4, 16.9. IR (film, cm⁻¹): S17 3448 (br), 2935, 1729, 1650, 1615, 1376, 1220, 1072, 881. HRMS(ESI): m/z calcd for C₂₂H₂₉O₅⁺ [M+H]⁺ 373.2010, found 373.2006. [α]_D²⁵ = +85.1 (c = 0.9, CH₂Cl₂).



(2*S*,4*aR*,4*a1S*,8*aS*,9*aS*,12*aS*,12*bR*,14*aS*)-2,12a-dihydroxy-7,7,9a-trimethyltetradecahydro-5H-cyclopenta[1,2]phenanthro[4,4b-de][1,3]dioxepin-10(2H)-one (214)

(4*aS*,4*a1S*,8*aS*,9*aS*,12*aS*,12*bR*)-12a-hydroxy-7,7,9a-trimethyl-3,4,4a1,8a,9,9a,11,12,12a,12b-decahydro-5H-cyclopenta[1,2]phenanthro[4,4b-de][1,3]dioxepine-2,10-dione **212** (14.0 mg, 0.0376 mmol) and 10% Palladium on activated carbon (5.0 mg) were added to a vial and solvated in 1.0 mL of a 3:1:1 mixture of EtOAc:MeOH:CH₂Cl₂. The vial was purged with N₂ and then with H₂ and allowed to stir vigorously at room temperature under an atmosphere of H₂. After 20 minutes, TLC showed complete conversion of starting material. The crude mixture was filtered

through a plug of celite and the filtrate was concentrated. By NMR, the crude mixture was a 2.3:1 diastereomeric mixture of *trans:cis* A/B ring junctions. Without further purification, the residue was solvated in dry THF (1.0 mL) and cooled to -78°C under a N₂ atmosphere. Then, a solution of LiAl(OtBu)₃H (22.0 mg, 0.087 mmol) in THF (0.50 mL) was added slowly to the reaction vessel via syringe. The solution was then allowed to warm to -40°C and was stirred at that temperature for 2.5 hours. Then, the reaction was quenched with a saturated solution of NH₄Cl (0.50 mL) and brine (1.50 mL), and diluted with CH₂Cl₂ (2.0 mL) as it was brought to room temperature. The aqueous layer was extracted 2x with CH₂Cl₂ (2.0 mL each) and the combined organic layers were dried with Na₂SO₄, filtered, and concentrated. Crude NMR indicated a retention of 2.3:1 d.r., indicating completely stereoselective reduction of the C3 ketone. The crude residue was then purified by flash column chromatography with 66% EtOAc in hexanes to afford title compound (*2S,4aR,4a1S,8aS,9aS,12aS,12bR,14aS*)-2,12a-dihydroxy-7,7,9a-trimethyltetradecahydro-5H-cyclopenta[1,2]phenanthro[4,4b-de][1,3]dioxepin-10(2H)-one (**214**) (8.4 mg, 0.022 mmol) in 59% yield over 2 steps as a white solid, R_f = 0.2 (66% EtOAc in hexanes). ¹H NMR (500 MHz, CDCl₃): δ 4.11 (s, 1H), 3.77 (d, J = 12.5 Hz, 1H), 3.72 - 3.64 (m, 1H), 3.61 (d, J = 12.5 Hz, 1H), 2.46 - 2.35 (m, ovrlp, 3H), 2.30 - 2.19 (m, 1H), 2.17 - 2.10 (m, 1H), 2.10 - 1.99 (m, 1H), 1.92 - 1.82 (m, 2H), 1.74 - 1.67 (m, 1H), 1.61 - 1.48 (m, 2H), 1.44 - 1.35 (m, 2H), 1.34 - 1.27 (m, ovrlp, 8H), 1.24 (s, 3H), 1.22 - 1.16 (m, ovrlp, 2H), 0.91 (d, J = 11.7 Hz, 1H), 0.70 (td, J = 13.3, 3.4 Hz, 1H). ¹³C NMR (176 MHz, CDCl₃): δ 221.5, 100.5, 82.9, 71.1, 65.1, 57.8, 53.1, 51.1, 43.0, 39.1, 37.8, 37.5, 36.9, 33.6, 33.0, 31.0, 27.6, 26.8, 25.5, 25.2, 24.2, 16.6. IR (film, cm⁻¹): 3461 (br), 2928, 1730, 1457, 1379, 1278, 1218, 1134, 1080. HRMS(ESI): m/z calcd for C₂₂H₃₅O₅⁺ [M+H]⁺ 379.2485, found 379.2487. [α]_D²⁵ = +18.9 (c = 0.4, CHCl₃).

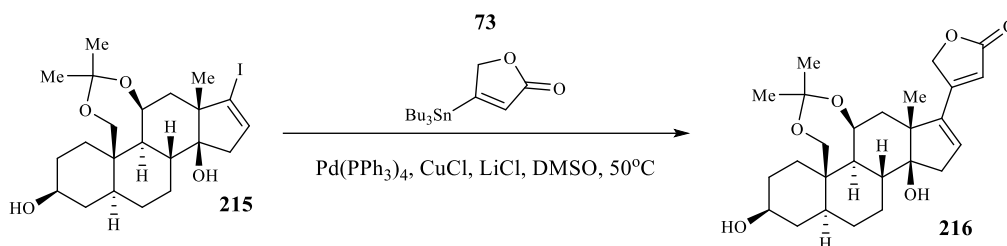


(2*S*,4*aR*,4*a1S*,8*aS*,9*aS*,12*aS*,12*bR*,14*aS*)-10-iodo-7,7,9*a*-trimethyl-

**1,3,4,4*a*1,8*a*,9,9*a*,12,12*b*,13,14,14*a*-dodecahydro-5H-cyclopenta[1,2]phenanthro[4,4*b*-
de][1,3]dioxepine-2,12*a*(2H)-diol (**215**)**

(2*S*,4*aR*,4*a1S*,8*aS*,9*aS*,12*aS*,12*bR*,14*aS*)-2,12*a*-dihydroxy-7,7,9*a*-trimethyltetradecahydro-5H-cyclopenta[1,2]phenanthro[4,4*b*-de][1,3]dioxepin-10(2H)-one **214** (20 mg, 0.0528mmol) was solvated in EtOH (4 mL) and triethylamine (150 μ L). Hydrazine monohydrate (53 mg, 1.058 mmol) was then added and the solution was heated to 50°C and stirred for 6 h until all starting material was consumed. The resulting mixture was cooled to room temperature and concentrated in vacuo. The crude oil was then re-dissolved in THF (4 mL) and triethylamine (150 μ L). A solution of I₂ (25 mg) in THF (1 mL) was prepared and 0.8 mL of this solution was added to the reaction vessel. After 10 minutes, additional I₂ solution (0.30 mL) was added until a brown color persisted. The mixture was stirred at room temperature for 1 hour until it was complete. The solution was then quenched with a saturated Na₂S₂O₃ solution (2 mL) and a saturated NaHCO₃ solution (2 mL) and stirred vigorously for 20 minutes. The resulting mixture was then extracted 3x with EtOAc (5 mL each), dried over Na₂SO₄, filtered, adsorbed onto silica gel, and directly purified by flash column chromatography with 0% → 5% → 10% → 20% EtOAc in hexanes to afford title compound (2*S*,4*aR*,4*a1S*,8*aS*,9*aS*,12*aS*,12*bR*,14*aS*)-10-iodo-7,7,9*a*-trimethyl-1,3,4,4*a*1,8*a*,9,9*a*,12,12*b*,13,14,14*a*-dodecahydro-5H-cyclopenta[1,2]phenanthro[4,4*b*-de][1,3]dioxepine-2,12*a*(2H)-diol (**215**) (19 mg, 0.0389 mmol) in 74% yield as a white solid. ¹H

NMR (700 MHz, CDCl₃) δ 6.07 (dd, *J* = 3.1, 2.0 Hz, 1H), 4.08 (s, 1H), 3.78 (d, *J* = 12.5 Hz, 1H), 3.67 (tt, *J* = 10.6, 4.9 Hz, 1H), 3.60 (dd, *J* = 12.8, 1.4 Hz, 1H), 2.51 (dd, *J* = 16.6, 2.0 Hz, 1H), 2.40 (dt, *J* = 13.0, 3.6 Hz, 1H), 2.33 (td, *J* = 12.2, 4.3 Hz, 1H), 2.26 (dd, *J* = 16.6, 3.1 Hz, 1H), 2.21 (dq, *J* = 12.9, 3.5 Hz, 1H), 1.92 (dd, *J* = 14.1, 3.7 Hz, 1H), 1.88 – 1.82 (m, 1H), 1.69 (ddt, *J* = 12.6, 5.1, 2.6 Hz, 1H), 1.57 – 1.49 (m, 1H), 1.38 (td, *J* = 12.7, 10.8 Hz, 1H), 1.33 (m, 1H), 1.31 (s, 3H), 1.30 (s, 3H), 1.27 (dt, *J* = 13.5, 3.2 Hz, 1H), 1.23 (s, 3H), 1.22 – 1.15 (m, 1H), 1.07 – 1.04 (m, 2H), 0.78 (d, *J* = 11.8 Hz, 1H), 0.70 (td, *J* = 13.4, 3.6 Hz, 1H). ¹³C NMR (176 MHz, CDCl₃) δ 133.3, 111.7, 100.6, 82.4, 71.2, 64.2, 57.8, 54.4, 51.0, 43.6, 43.0, 42.5, 38.9, 37.9, 36.4, 32.9, 31.0, 27.7, 26.7, 25.5, 24.3, 22.0. IR (film, cm⁻¹): 3389 (br), 2926, 1456, 1374, 1154, 1065, 1036, 734. [α]_D²⁸ = -0.7 (c = 0.25, CHCl₃).



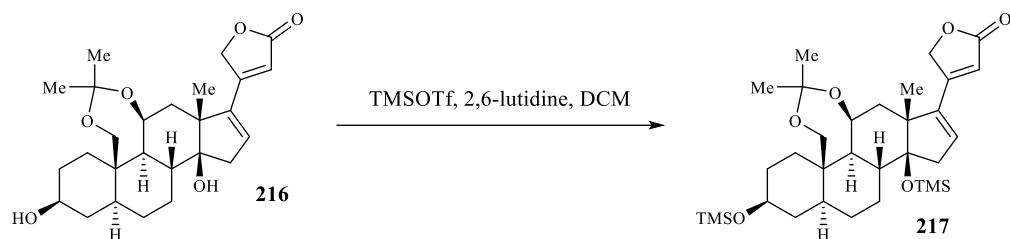
4-((2*S*,4*aR*,4*a1S*,8*aS*,9*aR*,12*aS*,12*bR*,14*aS*)-2,12*a*-dihydroxy-7,7,9*a*-trimethyl-1,2,3,4,4*a1*,8*a*,9,9*a*,12,12*a*,12*b*,13,14,14*a*-tetradecahydro-5H-cyclopenta[1,2]phenanthro[4,4*b-de*][1,3]dioxepin-10-yl)furan-2(5H)-one(216)

(2*S*,4*aR*,4*a1S*,8*aS*,9*aS*,12*aS*,12*bR*,14*aS*)-10-iodo-7,7,9*a*-trimethyl-

1,3,4,4*a1*,8*a*,9,9*a*,12,12*b*,13,14,14*a*-dodecahydro-5H-cyclopenta[1,2]phenanthro[4,4*b-*

de][1,3]dioxepine-2,12*a*(2H)-diol **215** (15.0 mg, 0.031 mmol) along with Pd(PPh₃)₄ (8.5 mg, 0.007 mmol), CuCl (55 mg, 0.555 mmol), and LiCl (33 mg, 0.7783 mmol) was solvated in dry DMSO (2.0 mL) under a N₂ atmosphere and stannyl butenolide **73** (55 mg, 0.147 mmol) was added via syringe. The reaction solution was heated to 50°C for 1 hour until the starting material was

consumed. The solution was then quenched with pH = 7 phosphate buffer (1 mL) and extracted 4x with EtOAc (1.5 mL each). The organic layers were combined, dried over Na₂SO₄, filtered, adsorbed onto silica gel, and directly purified by flash column chromatography with 0% → 10% → 20% → 30% EtOAc in hexanes to afford 4-((2*S*,4*aR*,4*a1S*,8*aS*,9*aR*,12*aS*,12*bR*,14*aS*)-2,12*a*-dihydroxy-7,7,9*a*-trimethyl-1,2,3,4,4*a1*,8*a*,9,9*a*,12,12*a*,12*b*,13,14,14*a*-tetradecahydro-5H-cyclopenta[1,2]phenanthro[4,4*b*-de][1,3]dioxepin-10-yl)furan-2(5H)-one **216** (10.0 mg, 0.0225 mmol) in 66% yield. ¹H NMR (500 MHz, CDCl₃) δ 6.07 (t, *J* = 2.8 Hz, 1H), 5.99 (s, 1H), 5.01 – 4.88 (m, 2H), 4.08 (s, 1H), 3.78 (d, *J* = 12.5 Hz, 1H), 3.68 (dq, *J* = 11.0, 5.3 Hz, 1H), 3.62 (dd, *J* = 12.5, 1.5 Hz, 1H), 2.66 (dt, *J* = 18.5, 1.8 Hz, 1H), 2.44 – 2.37 (m, 2H), 2.33 (td, *J* = 12.1, 4.1 Hz, 1H), 2.22 (dd, *J* = 12.8, 3.7 Hz, 1H), 2.13 (dd, *J* = 14.1, 3.7 Hz, 1H), 1.86 (ddd, *J* = 13.3, 5.3, 2.4 Hz, 1H), 1.70 (ddt, *J* = 12.5, 4.9, 2.4 Hz, 1H), 1.60 – 1.48 (m, 1H), 1.43 (s, 3H), 1.41 – 1.34 (m, 2H), 1.32 (s, 6H), 1.30 – 1.16 (m, 3H), 1.10 (qd, *J* = 12.5, 3.7 Hz, 1H), 0.82 (d, *J* = 11.7 Hz, 1H), 0.70 (td, *J* = 13.4, 3.6 Hz, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 174.3, 158.3, 144.2, 131.9, 112.7, 100.7, 85.6, 71.7, 71.1, 64.4, 57.8, 52.1, 51.1, 44.3, 43.0, 40.2, 39.0, 37.9, 36.2, 32.9, 31.0, 27.7, 26.8, 25.4, 24.5, 20.7. HRMS (ESI): *m/z* calcd for C₂₆H₃₇O₆⁺ [M+H]⁺ = 445.2585, found 445.2587. IR (film, cm⁻¹): 3413 (br), 2933, 2863, 17386, 1621, 1376, 1218, 1038, 731. [α]_D²⁷ = +26.7 (c = 0.45, CH₂Cl₂).

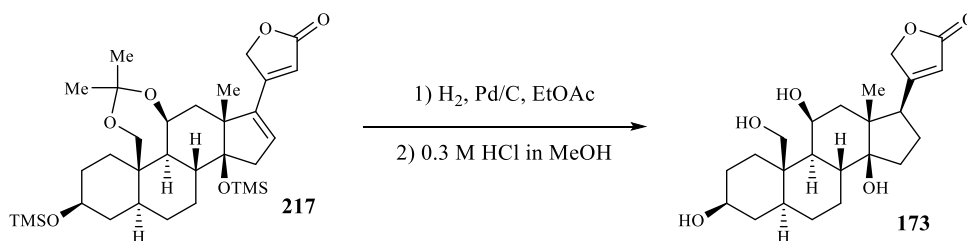


4-((2*S*,4*aR*,4*a1S*,8*aS*,9*aR*,12*aS*,12*bR*,14*aS*)-7,7,9*a*-trimethyl-2,12*a*-bis((trimethylsilyl)oxy)-1,2,3,4,4*a1*,8*a*,9,9*a*,12,12*a*,12*b*,13,14,14*a*-tetradecahydro-5*H*-cyclopenta[1,2]phenanthro[4,4*b*-de][1,3]dioxepin-10-yl)furan-2(5*H*)-one(217)

4-((2*S*,4*aR*,4*a1S*,8*aS*,9*aR*,12*aS*,12*bR*,14*aS*)-2,12*a*-dihydroxy-7,7,9*a*-trimethyl-1,2,3,4,4*a1*,8*a*,9,9*a*,12,12*a*,12*b*,13,14,14*a*-tetradecahydro-5*H*-cyclopenta[1,2]phenanthro[4,4*b*-de][1,3]dioxepin-10-yl)furan-2(5*H*)-one **216** (13.0 mg, 0.0293 mmol) and 2,6 – lutidine (68 μ L, 0.587 mmol) were solvated in dry CH₂Cl₂ (1.5mL) and cooled to -78°C under a N₂ atmosphere. Then, trimethylsilyl trifluoromethanesulfonate (53 μ L, 65.2 mg, 0.294 mmol) was added via syringe and the reaction was allowed to warm to room temperature. After 2 h, the reaction was quenched with a saturated NaHCO₃ solution (1.5 mL). The crude mixture was then extracted 3x with EtOAc (2.5 mL each), dried over Na₂SO₄, filtered, and azeotroped 4x with toluene (5 mL each) to remove remaining 2,6-lutidine. The crude material was then adsorbed onto silica gel and directly purified by flash column chromatography with 0% → 5% EtOAc in hexanes to afford title compound

4-((2*S*,4*aR*,4*a1S*,8*aS*,9*aR*,12*aS*,12*bR*,14*aS*)-7,7,9*a*-trimethyl-2,12*a*-bis((trimethylsilyl)oxy)-1,2,3,4,4*a1*,8*a*,9,9*a*,12,12*a*,12*b*,13,14,14*a*-tetradecahydro-5*H*-cyclopenta[1,2]phenanthro[4,4*b*-de][1,3]dioxepin-10-yl)furan-2(5*H*)-one (**217**) (8.5 mg, 0.0143 mmol) in 49% yield. ¹H NMR (700 MHz, CDCl₃) δ 6.00 (s, 1H), 5.99 (s, 1H), 4.98 (dt, *J* = 16.2, 1.1 Hz, 1H), 4.88 (dt, *J* = 16.2, 1.2 Hz, 1H), 4.02 (s, 1H), 3.76 (d, *J* = 12.4 Hz, 1H), 3.62 (m, 2H,

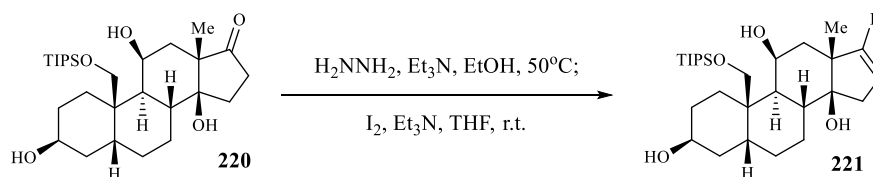
overlap), 2.60 – 2.54 (m, 1H), 2.44 (dd, $J = 18.7, 3.4$ Hz, 1H), 2.36 (dt, $J = 12.9, 3.6$ Hz, 1H), 2.30 (td, $J = 12.3, 4.1$ Hz, 1H), 2.08 (m, 2H, overlap), 1.71 (dd, $J = 13.7, 4.1$ Hz, 1H), 1.62 – 1.53 (m, 2H), 1.44 (td, $J = 12.7, 10.6$ Hz, 1H), 1.37 – 1.31 (m, 1H), 1.32 – 1.31 (s, 6H), 1.30 (s, 3H), 1.27 – 1.11 (m, 3H), 1.00 (qd, $J = 12.6, 3.9$ Hz, 1H), 0.73 (d, $J = 12.0$ Hz, 1H), 0.64 (td, $J = 13.4, 3.6$ Hz, 1H), 0.10 (s, 9H), 0.02 (s, 9H). ^{13}C NMR (176 MHz, CDCl_3) δ 174.4, 158.4, 145.3, 131.7, 112.6, 100.5, 89.9, 71.8, 71.5, 64.3, 57.9, 53.0, 51.1, 44.6, 43.2, 39.3, 39.0, 38.4, 36.7, 33.2, 31.3, 27.9, 27.2, 25.5, 24.4, 21.4, 2.7, 0.4. HRMS (ESI): Calculated for $\text{C}_{32}\text{H}_{32}\text{O}_6\text{Si}_2$ $[\text{M}+\text{H}]^+ = 589.3375$, found 589.3369. IR (film, cm^{-1}): 2938 (br), 1782, 1749, 1621, 1248, 1217, 1089, 836. $[\alpha]_{\text{D}}^{27} = +49.3$ ($c = 0.15$, CHCl_3).



Panogenin (173)

4-((2*S*,4*aR*,4*a1S*,8*aS*,9*aR*,12*aS*,12*bR*,14*aS*)-7,7,9*a*-trimethyl-2,12*a*-bis((trimethylsilyl)oxy)-1,2,3,4,4*a1*,8*a*,9,9*a*,12,12*a*,12*b*,13,14,14*a*-tetradecahydro-5*H*-cyclopenta[1,2]phenanthro[4,4*b*-de][1,3]dioxepin-10-yl)furan-2(5*H*)-one **217** (3.0 mg, 0.0051 mmol) was solvated in EtOAc (1 mL) and 10% Pd/C (1 mg) was added. The vessel was purged with N_2 , and then with H_2 . The solution was stirred vigorously under 1 atm of H_2 until all starting material was consumed. The reaction was then filtered through a pad of celite with EtOAc and concentrated in vacuo. The crude oil was then dissolved in 0.3 M HCl in MeOH (1 mL) and stirred at ambient temperature. After 1 hour, all starting material was consumed and the reaction was quenched with saturated NaHCO_3 until $\text{pH} = 7$. The solution was extracted 5x with a 2:1 mixture of CHCl_3 :EtOH (2 mL each). The

organic layers were combined and dried over Na₂SO₄, filtered, and concentrated. The crude material was then purified by flash column chromatography with 0% → 5% → 10% MeOH in DCM to afford panogenin (**173**) (1.14 mg, 0.0028 mmol) in 55% yield. ¹H NMR (500 MHz, D₃COD) δ 5.90 (s, 1H, H22), 5.02 (dd, *J* = 18.4, 1.8 Hz, 1H, H21a), 4.90 (dd, *J* = 18.3, 1.7 Hz, 1H, H21b), 4.12 (d, *J* = 2.9 Hz, 1H, H11), 3.84 (d, *J* = 11.7 Hz, 1H, H19a), 3.64 (d, *J* = 11.8 Hz, 1H, H19b), 3.61 – 3.54 (m, 1H, H3), 2.85 – 2.78 (m, 1H, H17), 2.30 (dt, *J* = 13.2, 3.6 Hz, 1H, H1a), 2.24 – 2.05 (m, 4H, H8, H16a, H14a, H7a), 1.92 – 1.83 (m, 1H, H16b), 1.83 – 1.73 (m, 3H, H12a, H2a, H14b), 1.69 – 1.62 (m, 1H, H4a), 1.58 (dd, *J* = 14.8, 3.0 Hz, 1H, H12b), 1.47 – 1.35 (m, 2H, H2b, H4b), 1.35 – 1.19 (m, 4H, H5, H6a, H6b, H7b), 1.19 – 1.15 (m, 1H, H9), 1.14 (s, 3H, H18), 0.86 (td, *J* = 13.6, 3.7 Hz, 1H, H1b). ¹³C NMR (126 MHz, CD₃OD) δ 176.3 (C23), 175.2 (C20), 116.0 (C22), 85.3 (C14), 73.4 (C21), 69.4 (C3), 64.8 (C11), 56.6 (C19), 52.8 (C9), 50.7 (C17), 48.7 (C13), 45.0 (C12), 44.6 (C5), 38.5 (C10), 37.0 (C4), 35.1 (C8), 31.4 (C15), 29.9 (C2), 29.7 (C1), 26.6 (C7), 26.4 (C6), 25.9 (C16), 17.7 (C18). HRMS (ESI): *m/z* calcd for C₂₃H₃₅O₆ [M+H]⁺ = 407.2428, found 407.2429. IR (film, cm⁻¹): 3379 (br), 2923, 2491, 1733, 1652, 1448, 1185, 1028. [α]_D²⁷ = +22.4 (c = 0.08, CH₃OH).

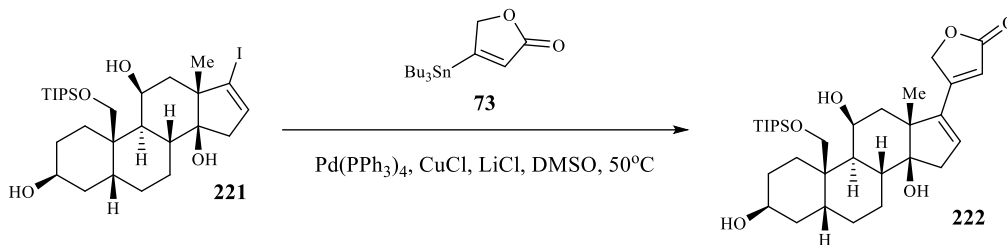


(3*S*,5*R*,8*R*,9*S*,10*R*,11*S*,13*S*,14*S*)-17-iodo-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-1,2,3,4,5,6,7,8,9,10,11,12,13,15-tetradecahydro-14*H*-cyclopenta[*a*]phenanthrene-3,11,14-triol(221**)**

(3*S*,5*R*,8*R*,9*S*,10*R*,11*S*,13*S*,14*S*)-3,11,14-trihydroxy-13-methyl-10-

(((triisopropylsilyl)oxy)methyl)hexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-one **220** (50

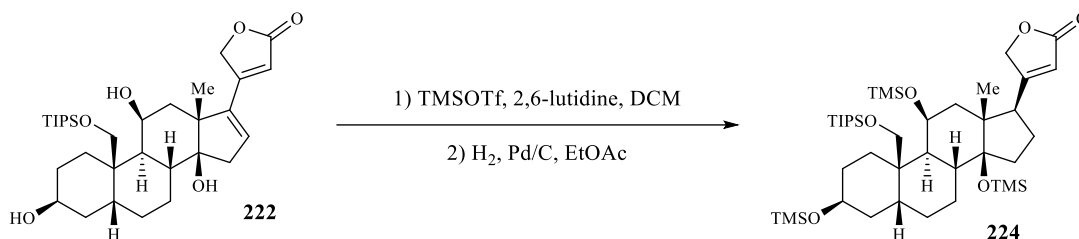
mg, 0.101 mmol) was solvated in EtOH (8 mL) and triethylamine (286 μ L). Hydrazine monohydrate (101.4 mg, 2.024 mmol) was then added and the solution was heated to 50°C and stirred for 6 h until all starting material was consumed. The resulting mixture was cooled to room temperature and concentrated in vacuo. The crude oil was then re-dissolved in THF (8 mL) and triethylamine (286 μ L). A solution of I₂ (84 mg) in THF (1 mL) was prepared and 0.25 mL of this solution was added to the reaction vessel. After 10 minutes, additional I₂ solution (0.30 mL) was added until a brown color persisted. The mixture was stirred at room temperature for 1 hour until it was complete. The solution was then quenched with a saturated Na₂S₂O₃ solution (3 mL) and a saturated NaHCO₃ solution (3 mL) and stirred vigorously for 20 minutes. The resulting mixture was then extracted 3x with EtOAc (5 mL each), dried over Na₂SO₄, filtered, adsorbed onto silica gel, and directly purified by flash column chromatography with 0% → 5% → 10% → 20% EtOAc in hexanes to afford title compound (*3S,5R,8R,9S,10R,11S,13S,14S*)-17-iodo-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-1,2,3,4,5,6,7,8,9,10,11,12,13,15-tetradecahydro-14H-cyclopenta[*a*]phenanthrene-3,11,14-triol (**221**) (56 mg, 0.0926) in 92% yield. ¹H NMR (700 MHz, CDCl₃) δ 6.07 (s, 1H), 4.98 (s, 1H), 4.46 (d, *J* = 10.3 Hz, 1H), 4.13 (s, 1H), 4.05 (s, 1H), 3.34 (d, *J* = 10.3 Hz, 1H), 2.55 (dd, *J* = 16.4, 1.9 Hz, 1H), 2.34 – 2.25 (m, 2H), 2.14 (dd, *J* = 14.4, 3.5 Hz, 1H), 2.00 – 1.83 (m, 3H), 1.70 (d, *J* = 12.4 Hz, 1H), 1.65 – 1.57 (m, 2H), 1.57 – 1.45 (m, 2H), 1.37 – 1.31 (m, 1H), 1.31 (s, 3H), 1.27 – 1.21 (m, 2H), 1.18 (dt, *J* = 15.2, 7.5 Hz, 3H), 1.10 (t, *J* = 6.9 Hz, 21H), 1.07 – 1.02 (m, 1H). ¹³C NMR (176 MHz, CDCl₃) δ 132.9, 112.5, 83.1, 70.2, 66.0, 63.2, 54.0, 43.2, 42.9, 39.7, 39.3, 35.6, 34.9, 33.2, 27.5, 25.4, 23.6, 21.3, 20.6, 18.0, 18.0, 11.9. HRMS (ESI): *m/z* calcd for C₂₈H₅₀IO₄Si⁺ [M+H]⁺ = 605.2523, found 605.2512. [α]_D²⁷ = -4.5 (c = 0.175, CHCl₃).



4-((3*S*,5*R*,8*R*,9*S*,10*R*,11*S*,13*R*,14*S*)-3,11,14-trihydroxy-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-2,3,4,5,6,7,8,9,10,11,12,13,14,15-tetradecahydro-1H-cyclopenta[*a*]phenanthren-17-yl)furan-2(5H)-one(222)

(3*S*,5*R*,8*R*,9*S*,10*R*,11*S*,13*S*,14*S*)-17-iodo-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-1,2,3,4,5,6,7,8,9,10,11,12,13,15-tetradecahydro-14H-cyclopenta[*a*]phenanthrene-3,11,14-triol **221** (50 mg, 0.083 mmol) along with Pd(PPh₃)₄ (20 mg, 0.0165 mmol), CuCl (123 mg, 0.124 mmol), and LiCl (70.3 mg, 1.655 mmol) were solvated in dry DMSO (7.0 mL) under a N₂ atmosphere and stannyl butenolide **73** (105 mg, 0.280 mmol) was added via syringe. The reaction solution was heated to 50°C for 1 hour until the starting material was consumed. The solution was then quenched with pH = 7 phosphate buffer (4 mL) and extracted 4x with EtOAc (7 mL each). The organic layers were combined, dried over Na₂SO₄, filtered, adsorbed onto silica gel, and directly purified by flash column chromatography with 0% → 10% → 20% → 30% EtOAc in hexanes to afford 4-((3*S*,5*R*,8*R*,9*S*,10*R*,11*S*,13*R*,14*S*)-3,11,14-trihydroxy-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-2,3,4,5,6,7,8,9,10,11,12,13,14,15-tetradecahydro-1H-cyclopenta[*a*]phenanthren-17-yl)furan-2(5H)-one (**222**) (39 mg, 0.069 mmol) in 84% yield. ¹H NMR (500 MHz, CDCl₃) δ 6.07 (d, *J* = 2.8 Hz, 1H), 6.04 (s, 1H), 5.03 – 4.86 (m, 2H), 4.46 (d, *J* = 10.3 Hz, 1H), 4.14 (s, 1H), 4.05 (s, 1H), 3.35 (d, *J* = 10.4 Hz, 1H), 2.70 (dd, *J* = 18.4, 2.3 Hz, 1H), 2.45 (dd, *J* = 18.3, 3.5 Hz, 1H), 2.38 – 2.22 (m, 2H), 2.02 – 1.83 (m, 4H), 1.76 – 1.71 (m, 1H), 1.66 – 1.57 (m, 2H), 1.56 – 1.46 (m, 2H), 1.51 (s, 3H), 1.40 – 1.32 (m, 2H), 1.26 (t, *J* = 7.1

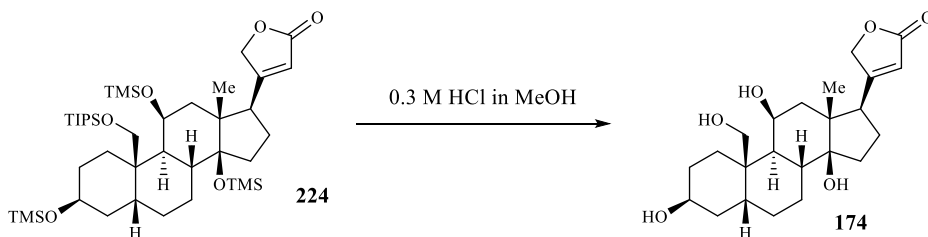
Hz, 1H), 1.24 – 1.13 (m, 2H), 1.14 – 1.04 (m, 21H). ¹³C NMR (126 MHz, CDCl₃) δ 174.3, 158.2, 144.8, 131.4, 112.8, 86.3, 71.6, 70.2, 65.9, 63.3, 51.6, 43.9, 40.6, 39.6, 39.3, 35.5, 34.7, 33.2, 27.5, 25.4, 23.6, 20.7, 19.9, 18.1, 18.0, 11.9. HRMS (ESI): m/z calcd for C₃₂H₅₂O₆SiNa⁺ [M+Na]⁺ = 583.3431, found 583.3424. [α]_D²⁵ = +25.8 (c = 0.525, CHCl₃).



4-((3*S*,5*R*,8*R*,9*S*,10*R*,11*S*,13*R*,14*S*,17*R*)-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-3,11,14-tris((trimethylsilyl)oxy)hexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)furan-2(5*H*)-one(224)

4-((3*S*,5*R*,8*R*,9*S*,10*R*,11*S*,13*R*,14*S*)-3,11,14-trihydroxy-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-2,3,4,5,6,7,8,9,10,11,12,13,14,15-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)furan-2(5*H*)-one **222** (33 mg, 0.0589 mmol) and 2,6 – lutidine (137 μL, 1.18 mmol) were solvated in dry CH₂Cl₂ (5 mL) and cooled to -78°C under a N₂ atmosphere. Then, trimethylsilyl trifluoromethanesulfonate (107 μL, 0.59 mmol) was added via syringe and the reaction was allowed to warm to room temperature. After 2 h, the reaction was quenched with a saturated NaHCO₃ solution (3 mL). The crude mixture was then extracted 3x with EtOAc (2.5 mL each), dried over Na₂SO₄, filtered, and azeotroped 4x with toluene (5 mL each) to remove remaining 2,6-lutidine. The crude material was then adsorbed onto silica gel and directly purified by flash column chromatography with 0% → 5% EtOAc in hexanes. The resulting polysilylated product was immediately redissolved in EtOAc (4 mL) and 10% Pd/C (6 mg) was added. The vessel was flushed with N₂, and then with H₂. The reaction was stirred at ambient temperature under 1 atm of H₂ for

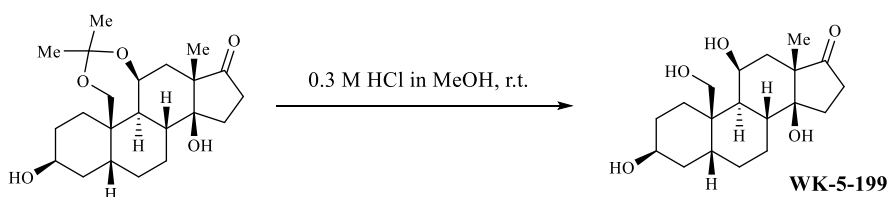
15 minutes. The crude mixture was filtered through celite with EtOAc and concentrated in vacuo. The resulting residue was directly purified by flash column chromatography with 0% → 5% EtOAc in hexanes to afford 4-((3*S*,5*R*,8*R*,9*S*,10*R*,11*S*,13*R*,14*S*,17*R*)-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-3,11,14-tris(trimethylsilyl)oxy)hexadecahydro-1H-cyclopenta[*a*]phenanthren-17-yl)furan-2(5H)-one (**224**) in 15% yield. ¹H NMR (700 MHz, CDCl₃) δ 5.83 (s, 1H), 4.74 (dd, *J* = 3.5, 1.8 Hz, 2H), 4.09 – 3.95 (m, 3H), 3.67 (d, *J* = 10.4 Hz, 1H), 2.70 (d, *J* = 13.3 Hz, 1H), 2.48 (t, *J* = 7.7 Hz, 1H), 2.21 (td, *J* = 14.2, 3.8 Hz, 1H), 2.14 (td, *J* = 12.4, 3.5 Hz, 1H), 2.06 – 1.97 (m, 1H), 1.96 – 1.84 (m, 2H), 1.85 – 1.71 (m, 4H), 1.72 – 1.66 (m, 1H), 1.48 (d, *J* = 13.8 Hz, 1H), 1.40 – 1.33 (m, 3H), 1.32 – 1.24 (m, 2H), 1.20 (d, *J* = 13.9 Hz, 1H), 1.14 (d, *J* = 14.5 Hz, 1H), 1.08 (d, *J* = 3.7 Hz, 21H), 1.04 (s, 3H), 0.13 (s, 9H), 0.13 (s, 9H), 0.07 (s, 9H). ¹³C NMR (176 MHz, CDCl₃) δ 174.3, 173.8, 117.3, 91.9, 73.9, 67.2, 66.9, 65.7, 51.6, 50.4, 47.6, 41.6, 40.0, 35.3, 34.3, 34.1, 28.8, 27.2, 27.0, 25.5, 24.0, 23.1, 21.2, 18.2, 12.2, 2.9, 1.0, 0.2. HRMS (ESI): *m/z* calcd for C₄₁H₈₂NO₆Si₄⁺ [M+NH₄]⁺ = 796.5219, found 796.5212. [α]_D²⁵ = +58.8 (c = 0.1, CHCl₃).



***cis*-panogenin (174)**

4-((3*S*,5*R*,8*R*,9*S*,10*R*,11*S*,13*R*,14*S*,17*R*)-13-methyl-10-(((triisopropylsilyl)oxy)methyl)-3,11,14-tris(trimethylsilyl)oxy)hexadecahydro-1H-cyclopenta[*a*]phenanthren-17-yl)furan-2(5H)-one **224** (7 mg, 0.009 mmol) was solvated in 0.3 M HCl in MeOH (2 mL). The solution was allowed to stir at ambient temperature for 1 hour. The reaction was then quenched with sat. NaHCO₃ until pH =

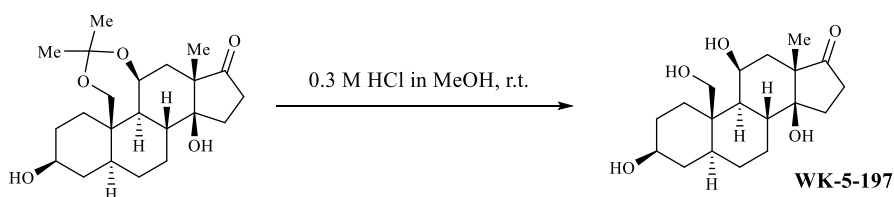
7. The solution was extracted 5x with a 2:1 mixture of CHCl₃:EtOH. The combined organic layers were dried with Na₂SO₄, filtered, and concentrated. The resulting crude material was adsorbed onto silica gel and purified directly by flash column chromatography with 0% → 5% → 10% MeOH in DCM to afford 4-((3*S*,5*R*,8*R*,9*S*,10*R*,11*S*,13*R*,14*S*,17*R*)-3,11,14-trihydroxy-10-(hydroxymethyl)-13-methylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)furan-2(5*H*)-one (**174**) (2.1 mg, 0.0052 mmol) in 57% yield. ¹H NMR (700 MHz, CD₃OD) δ 5.90 (s, 1H), 5.03 (d, *J* = 18.7 Hz, 1H), 4.91 (d, *J* = 18.4 Hz, 1H), 4.25 (d, *J* = 11.4 Hz, 1H), 4.05 (s, 2H), 3.16 (d, *J* = 11.4 Hz, 1H), 2.83 (dd, *J* = 9.2, 5.6 Hz, 1H), 2.22 – 2.13 (m, 3H), 1.99 – 1.92 (m, 2H), 1.93 – 1.85 (m, 3H), 1.84 – 1.78 (m, 3H), 1.72 (tt, *J* = 14.0, 4.6 Hz, 1H), 1.65 – 1.57 (m, 3H), 1.56 – 1.50 (m, 1H), 1.38 (qd, *J* = 13.5, 4.6 Hz, 1H), 1.32 (dd, *J* = 13.8, 2.9 Hz, 1H), 1.24 – 1.19 (m, 1H), 1.14 (s, 3H). ¹³C NMR (176 MHz, CD₃OD) δ 176.3, 175.2, 116.0, 85.5, 73.4, 67.6, 65.0, 64.9, 50.8, 48.8, 45.2, 38.7, 37.8, 34.8, 34.4, 32.0, 31.5, 26.1, 26.0, 24.7, 22.6, 20.4, 17.8. HRMS (ESI): *m/z* calcd for C₂₃H₃₄O₆Na⁺ [*M*+Na]⁺ = 429.2253, found 429.2271. [α]_D²⁵ = +20.7 (*c* = 0.1, MeOH).



(3*S*,5*R*,8*R*,9*S*,10*R*,11*S*,13*S*,14*S*)-3,11,14-trihydroxy-10-(hydroxymethyl)-13-methylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-one (WK-5-199)

(2*S*,4*aR*,4*a1S*,8*aS*,9*aS*,12*aS*,12*bR*,14*aR*)-2,12*a*-dihydroxy-7,7,9*a*-trimethyltetradecahydro-5*H*-cyclopenta[1,2]phenanthro[4,4*b*-de][1,3]dioxepin-10(2*H*)-one (9 mg, 0.0238 mmol) was solvated in 0.3 M HCl in MeOH (2 mL). The solution was allowed to stir at ambient temperature for 1 hour. The reaction was then quenched with sat. NaHCO₃ until pH = 7. The solution was extracted 5x

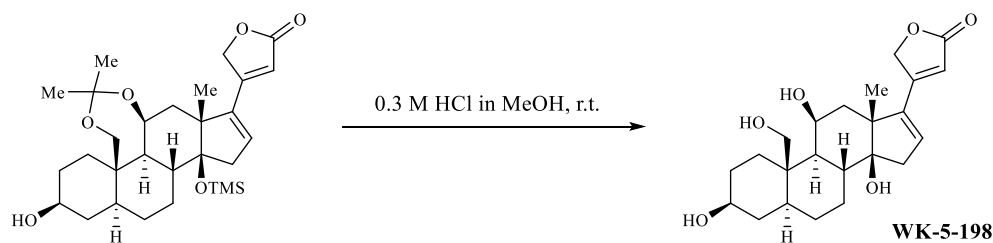
with a 2:1 mixture of CHCl₃:EtOH. The combined organic layers were dried with Na₂SO₄, filtered, and concentrated. The resulting crude material was adsorbed onto silica gel and purified directly by flash column chromatography with 0% → 5% → 10% MeOH in DCM to afford (3*S*,5*R*,8*R*,9*S*,10*R*,11*S*,13*S*,14*S*)-3,11,14-trihydroxy-10-(hydroxymethyl)-13-methylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-one (**WK-5-199**) (4.5 mg, 0.0133 mmol) in 56% yield. ¹H NMR (700 MHz, CD₃OD) δ 4.24 (d, *J* = 11.5 Hz, 1H), 4.10 (d, *J* = 2.7 Hz, 1H), 3.58 (ddt, *J* = 14.0, 7.9, 4.6 Hz, 1H), 3.16 (d, *J* = 11.5 Hz, 1H), 2.47 – 2.40 (m, 1H), 2.36 – 2.22 (m, 3H), 2.10 (dt, *J* = 14.7, 3.5 Hz, 1H), 2.01 – 1.97 (m, 1H), 1.97 – 1.86 (m, 2H), 1.80 – 1.71 (m, 3H), 1.57 (dd, *J* = 14.5, 3.1 Hz, 2H), 1.50 (dd, *J* = 14.8, 3.2 Hz, 3H), 1.37 – 1.25 (m, 3H), 1.23 (s, 3H), 1.18 (td, *J* = 14.5, 3.7 Hz, 1H). ¹³C NMR (176 MHz, CD₃OD) δ 222.9, 82.2, 69.9, 67.3, 63.3, 52.5, 39.6, 38.7, 38.3, 37.2, 34.8, 33.9, 32.0, 28.8, 28.2, 25.8, 24.8, 18.5, 14.9. HRMS (ESI): *m/z* calcd for C₁₉H₃₀NaO₅⁺ [*M*+Na]⁺ = 361.1991, found 362.1988. [*α*]_D²⁵ = +33.8 (*c* = 0.225, MeOH).



(3*S*,5*S*,8*R*,9*S*,10*R*,11*S*,13*S*,14*S*)-3,11,14-trihydroxy-10-(hydroxymethyl)-13-methylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-one (WK-5-197**)**

(2*S*,4*aR*,4*a1S*,8*aS*,9*aS*,12*aS*,12*bR*,14*aS*)-2,12*a*-dihydroxy-7,7,9*a*-trimethyltetradecahydro-5*H*-cyclopenta[1,2]phenanthro[4,4*b*-de][1,3]dioxepin-10(2*H*)-one (20 mg, 0.053 mmol) was solvated in 0.3 M HCl in MeOH (4 mL). The solution was allowed to stir at ambient temperature for 1 hour. The reaction was then quenched with sat. NaHCO₃ until pH = 7. The solution was extracted 5x with a 2:1 mixture of CHCl₃:EtOH. The combined organic layers were dried with Na₂SO₄, filtered,

and concentrated. The resulting crude material was adsorbed onto silica gel and purified directly by flash column chromatography with 0% → 5% → 10% MeOH in DCM to afford (3*S*,5*S*,8*R*,9*S*,10*R*,11*S*,13*S*,14*S*)-3,11,14-trihydroxy-10-(hydroxymethyl)-13-methylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-one (**WK-5-197**) (8 mg, 0.0237 mmol) in 45% yield. ¹H NMR (700 MHz, CD₃OD) δ 4.16 (d, *J* = 2.8 Hz, 1H), 3.87 (d, *J* = 11.8 Hz, 1H), 3.67 (d, *J* = 11.8 Hz, 1H), 3.58 (tt, *J* = 10.5, 4.8 Hz, 1H), 2.42 – 2.35 (m, 1H), 2.34 – 2.26 (m, 3H), 2.20 – 2.10 (m, 2H), 1.91 – 1.84 (m, 1H), 1.80 – 1.76 (m, 1H), 1.67 (dt, *J* = 12.4, 2.6 Hz, 1H), 1.56 (dd, *J* = 14.7, 3.2 Hz, 1H), 1.47 – 1.37 (m, 3H), 1.38 – 1.28 (m, 4H), 1.28 – 1.20 (m, 1H), 1.23 (s, 3H), 1.17 (dd, *J* = 12.4, 2.4 Hz, 1H), 0.86 (td, *J* = 13.5, 3.6 Hz, 1H). ¹³C NMR (176 MHz, CD₃OD) δ 222.9, 82.1, 69.3, 63.5, 56.5, 52.8, 52.5, 44.5, 38.5, 37.0, 36.8, 34.1, 32.0, 29.8, 29.7, 26.2, 25.8, 24.7, 14.9. HRMS (ESI): *m/z* calcd for C₁₉H₃₀NaO₅⁺ [M+Na]⁺ = 361.1991, found 362.1987. [α]_D²⁵ = +30 (c = 0.4, MeOH).



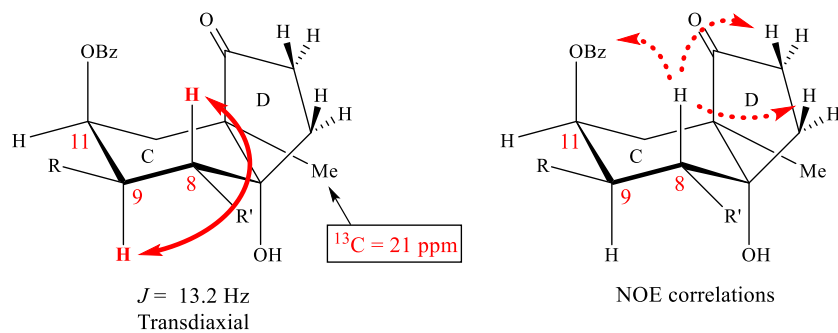
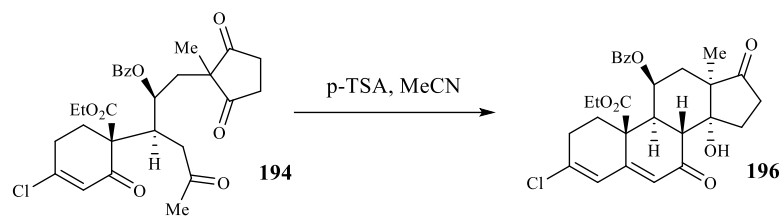
4-((3*S*,5*S*,8*R*,9*S*,10*R*,11*S*,13*R*,14*S*)-3,11,14-trihydroxy-10-(hydroxymethyl)-13-methyl-2,3,4,5,6,7,8,9,10,11,12,13,14,15-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)furan-2(5*H*)-one (WK-5-198)

4-((2*S*,4*aR*,4*a1S*,8*aS*,9*aR*,12*aS*,12*bR*,14*aS*)-2-hydroxy-7,7,9*a*-trimethyl-12*a*-((trimethylsilyl)oxy)-1,2,3,4,4*a1*,8*a*,9,9*a*,12,12*a*,12*b*,13,14,14*a*-tetradecahydro-5*H*-cyclopenta[1,2]phenanthro[4,4*b*-de][1,3]dioxepin-10-yl)furan-2(5*H*)-one (4 mg, 0.0077 mmol) was solvated in 0.3 M HCl in MeOH (4 mL). The solution was allowed to stir at ambient

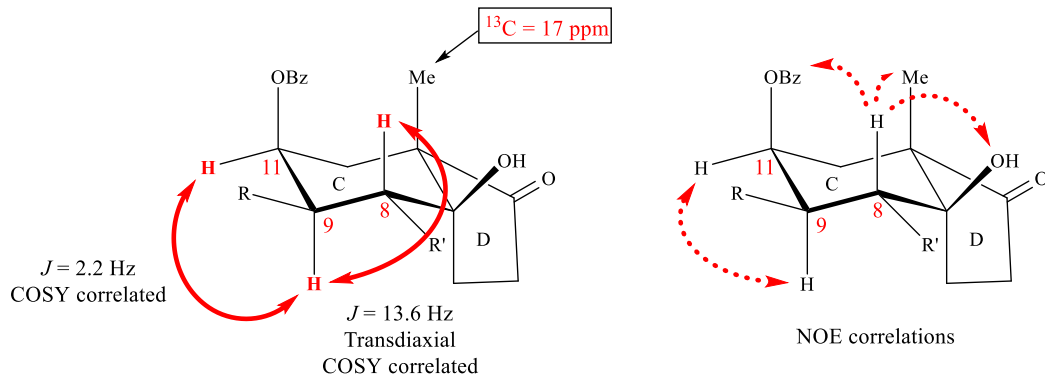
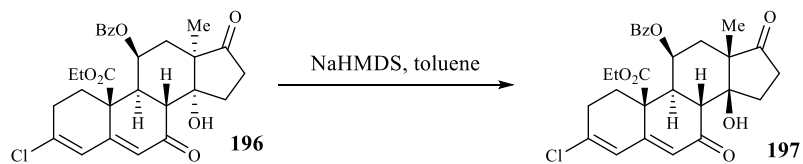
temperature for 1 hour. The reaction was then quenched with sat. NaHCO₃ until pH = 7. The solution was extracted 5x with a 2:1 mixture of CHCl₃:EtOH. The combined organic layers were dried with Na₂SO₄, filtered, and concentrated. The resulting crude material was adsorbed onto silica gel and purified directly by flash column chromatography with 0% → 5% → 10% MeOH in DCM to afford 4-((3*S*,5*S*,8*R*,9*S*,10*R*,11*S*,13*R*,14*S*)-3,11,14-trihydroxy-10-(hydroxymethyl)-13-methyl-2,3,4,5,6,7,8,9,10,11,12,13,14,15-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)furan-2(5*H*)-one (**WK-5-198**) (2 mg, 0.0049 mmol) in 64% yield. ¹H NMR (700 MHz, CD₃OD) δ 6.23 (s, 1H), 6.00 (s, 1H), 5.13 (d, *J* = 16.7 Hz, 1H), 5.00 (d, *J* = 16.8 Hz, 1H), 4.15 (d, *J* = 3.0 Hz, 1H), 3.89 (d, *J* = 11.8 Hz, 1H), 3.68 (d, *J* = 11.8 Hz, 1H), 3.58 (tt, *J* = 10.7, 4.8 Hz, 1H), 2.77 – 2.69 (m, 1H), 2.41 – 2.33 (m, 3H), 2.28 (dd, *J* = 14.7, 3.6 Hz, 1H), 2.21 (d, *J* = 10.6 Hz, 1H), 1.78 (d, *J* = 12.9 Hz, 1H), 1.66 (d, *J* = 13.0 Hz, 1H), 1.46 (s, 3H), 1.41 (dt, *J* = 23.6, 12.6 Hz, 2H), 1.37 – 1.20 (m, 5H), 1.08 (d, *J* = 12.4 Hz, 1H), 0.84 (td, *J* = 13.5, 3.5 Hz, 1H). ¹³C NMR (176 MHz, CD₃OD) δ 175.2, 159.9, 143.4, 132.6, 110.1, 85.3, 71.6, 69.4, 63.2, 56.5, 52.8, 50.6, 44.2, 43.1, 39.4, 38.4, 37.0, 33.8, 29.8, 26.4, 26.3, 18.9. HRMS (ESI): *m/z* calcd for C₂₃H₃₂NaO₆⁺ [M+Na]⁺ = 427.2097, found 427.2092. [α]_D²⁵ = +69 (c = 0.1, MeOH).

Structural Evidence

cis- α -C/D junction of **196**



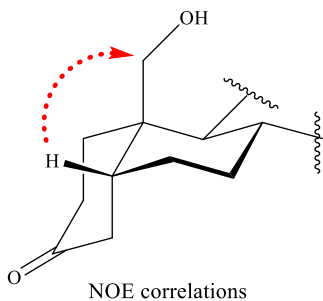
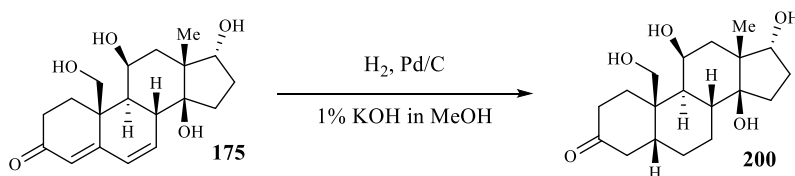
Epimerization of **196** to *cis*- β -C/D junction



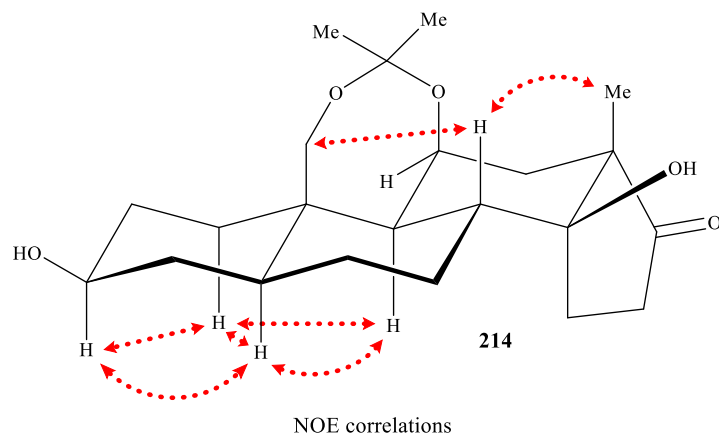
C18 Carbon shift

In addition to the more concrete structural proof, our research into the topic of steroidal C/D ring junctions revealed an interesting and strong correlation between the C18 carbon NMR shift and the ring junction stereochemistry. It became apparent that steroids of similar structure having a *cis*-C/D junction with β -oriented $-Me$ and β -oriented $-OH$ (natural junction) often produce a characteristic C18 shift of 15 – 17 ppm. In contrast, a *cis*-C/D junction with α -oriented $-Me$ and α -oriented $-OH$ produce a C18 shift in the range of 20-21 ppm.³¹ This is a theme we have noticed in the literature and throughout our own work on the synthesis of cardenolides.

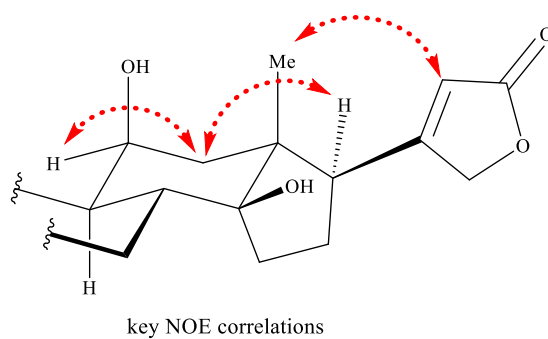
Hydrogenation of **175** to *cis*- β -A/B junction



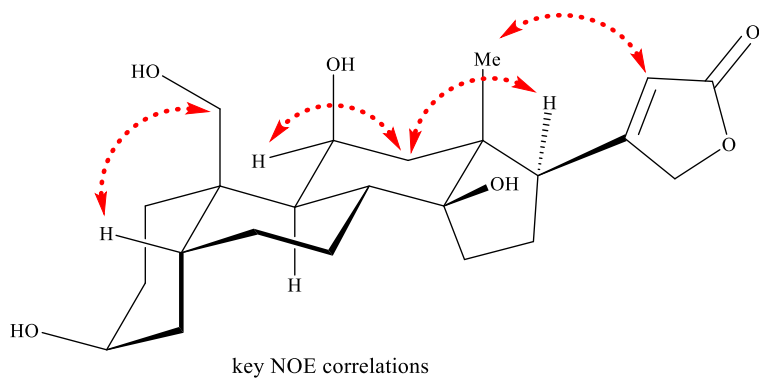
NOE of panogenin core 214

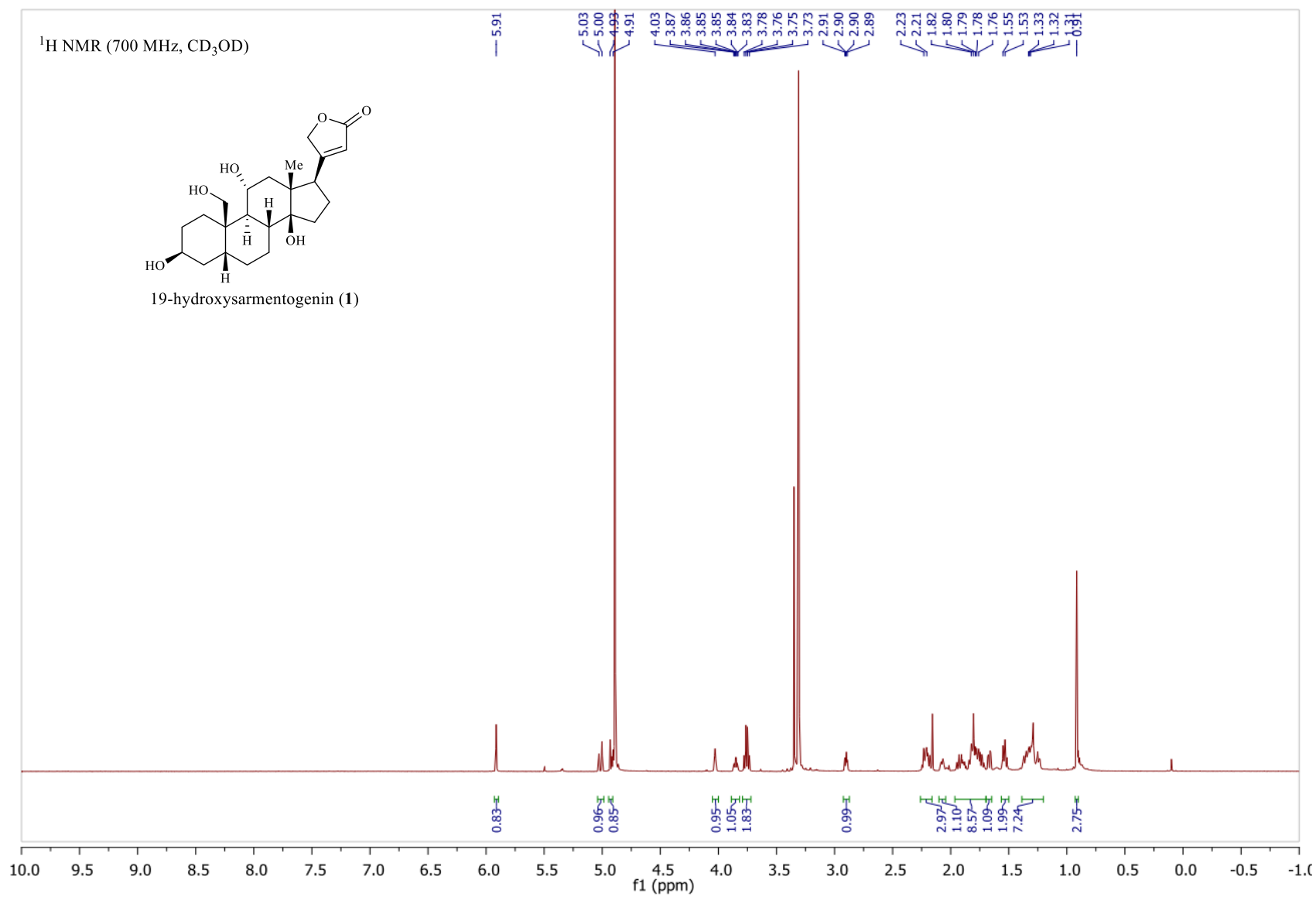


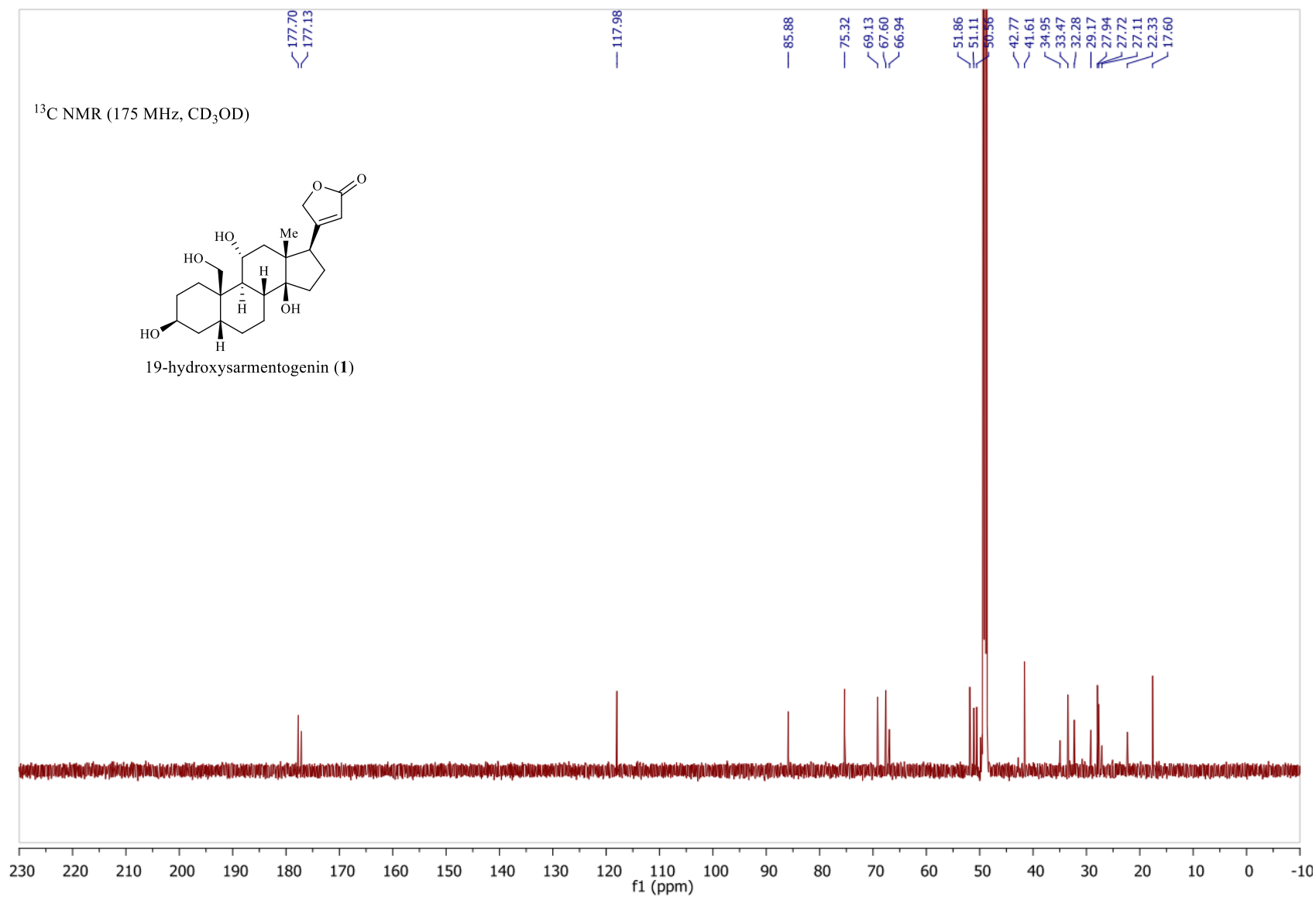
NOE of panogenin (173)



NOE of *cis*-panogenin (174)







C5 stereochemical evidence derived from ^{13}C NMR shift correlations:

A thorough collection and review of cardiac glycoside ^{13}C NMR shifts has been published.³¹ Several strong correlations and patterns relating to specific ^{13}C shifts and stereochemical/structural features were noted. In particular, certain isomers often show characteristic carbon shifts which deviate significantly enough from its other isomers to constitute evidence of a steroids configuration. The authors note the following tell-tale shifts (**Table 4.4.**):

Table 4.4. ^{13}C NMR Evidence for Configuration at C5.

Carbon No.	5 α – derivatives	5 β – derivatives
C5	44.7 \pm 0.4	36.0 \pm 0.6
C9	50.0 \pm 0.3	35.5 \pm 0.4

Accordingly, our synthetic sample of cis-panogenin (174) shows carbon shifts of:

C5: 34.84 C9: 37.79

These shifts are in accord with those noted for 5 β -derivatives.

And our sample of panogenin (173) shows carbon shifts of:

C5: 44.99 C9: 52.78

Again, in agreement with known shifts for 5 α -derivatives.

For 19-hydroxysarmentogenin (1), the shifts are:

C5: 32.28 C9: 41.61

In better alignment with those expected for 5 β -derivatives.

Na⁺/K⁺ATPase Inhibition Assay

Serial dilutions (double digit nanomolar to millimolar range) of the test compounds were prepared in 2:1 DMSO:S3 buffer. The S3 buffer contains 0.01 M NaCl, 0.01 M MgCl₂, 0.1 M Tris + HCl to pH 7.2. The Na⁺/K⁺ATPase purchased from Sigma-Aldrich was isolated from porcine cerebral cortex and contained 0.4 units/mg protein, 14.4% protein content, and 0.0576 units/mg solid. A solution of the enzyme preparation dissolved in the S3 buffer was made at a concentration of 1.7 mg crude enzyme solid per 1.0 mL S3 buffer. 93 μL of the enzyme/S3 solution was pipetted into each well designated to receive the enzyme. Then, 7 μL of test compound solution (or vehicle) was pipetted into the designated wells. Test compounds were allowed to incubate with the enzyme for 10 minutes at 37°C. At the same time, a solution of ATP disodium salt in the S2 buffer was prepared. The S2 buffer contains 0.10 M NaCl, 0.005 M KCl, 0.003 M MgCl₂, 0.001 M EGTA, and 0.08 M Tris + HCl to pH 7.4. The ATP solution was prepared at a concentration of 0.01088 M ATP. After the 10 minute preincubation time of the enzyme/inhibitor mixture, 50.0 μL of the ATP solution is added to the designated wells to begin the enzymatic reaction. The reaction is allowed to proceed for 10 minutes at 37°C. It was determined that the enzymatic reaction and phosphate production is linear over this timeframe by testing the phosphate production at various concentrations of the enzyme. After the allotted time, 100 μL of Biomolgreen (a commercial phosphate detection reagent sold by Enzo Life Sciences) is pipetted into each well to stop the reaction. After 30 minutes, optical density at 620 nm is measured. The measured OD₆₂₀ values are blanked to the control wells (background signal is subtracted), and then divided by the maximum control signal (signal of the uninhibited control) to generate a percent activity value, which is plotted and used to determine IC₅₀ (using the log(inhibitor) vs response profile on PRISM). Values of duplicate experiments are averaged. Errors were calculated by the program PRISM.

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Chapter 5

Conclusions and Future Directions

5.1. Introduction

The development of new synthetic approaches to complicated chemical scaffolds can allow medicinal chemists to access new structures for biological evaluation. The ability to test novel architectures and analogues is crucial for the discovery and development of new or improved therapeutics. Natural products architectures of high chemical complexity are often economically unjustifiable to pursue in an industrial setting. Synthetic routes can be too long and arduous to justify the work required to produce a single target analog. Often, these complicated analogues are instead targeted via a semi-synthetic approach. However, semi-synthetic approaches can dramatically hinder the region of chemical space which is easily accessible because the starting material has such predefined chemical moieties and configurations which are often challenging to overcome. For example, expanding or contracting ring sizes, accessing and controlling internal stereocenters, functionalizing inert positions, and producing enantiomers are some tasks which can be difficult to achieve via semi-synthesis.

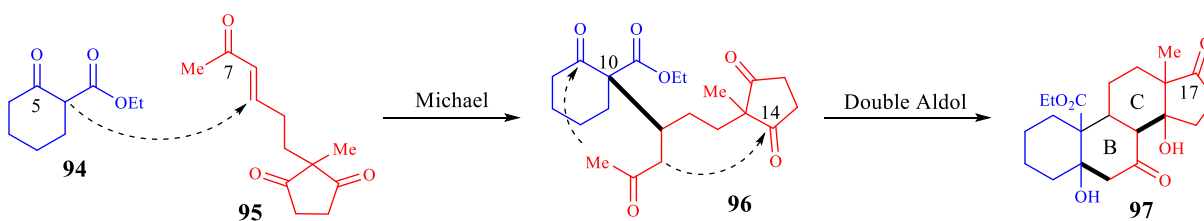
Cardenolides are a class of cardiotonic steroids which have been used clinically for over 200 years to treat congestive heart failure, and recent discoveries have demonstrated highly promising anticancer activity as well (see discussion in Chapter 1, section 1.7.). These complex steroids are unoptimized for the human body and are well known for their poor therapeutic windows. As a result, there has been much interest in developing new synthetic access to this class

of steroids and many groups have successfully reported landmark efforts at synthetically accessing cardenolides (see discussion in Chapter 2). However, total syntheses are often too long (30+ steps) or semi-synthetic approaches are insufficiently modular for a practical drug-oriented approach to these steroids.

5.2. Conclusions

In Chapter 3, we set out to design a drug-oriented approach to the cardenolide class of steroids. This meant we ingrained the aspects of diversification and modularity from the outset of the synthetic design. We devised a disconnection approach illustrated in **Scheme 5.1**.¹

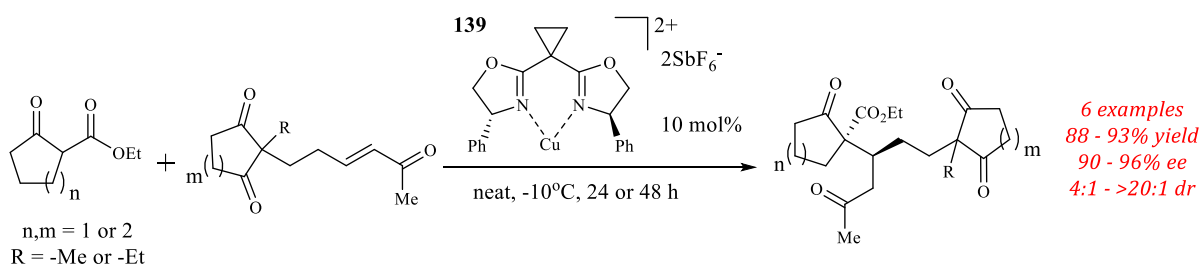
Scheme 5.1. Synthetic Methodology Design for Accessing Steroidal Cores.



This approach was attractive because we imagined it would allow for the pre-installation of functional handles and diversity elements on the starting substrates **94** and **94**. These handles could be carried through the sequence to provide steroidal cores of unique structure. The described method could also allow for great stereocontrol. The ability to control internal ring junction stereocenters and peripheral configurations was paramount to our approach. In these rigid ring systems, stereochemical manipulation can have a profound impact on the overall shape of the molecule and modulate binding contacts in ways which may lead to unique and improved bioactivity. Finally, this approach is highly concise; allowing the steroidal cores (6 stereocenters in a fused tetracyclic array) to be completed in only two steps from chemically simple building blocks.

Our first goal was to describe an asymmetric variant of the initial Michael addition. This reaction is highly hindered and already worth noting since only a handful of examples exist in the literature describing such conjugate additions forming vicinal quaternary and tertiary stereocenters. A catalyst screen revealed the optimal chiral controller as being **139**; a cyclopropyl biphenyl BOX ligand in conjunction with Cu(II) hexafluorantimonate. A variety of uniquely structured β -ketoesters and 2-substituted enones could be successfully coupled under these conditions. This allowed the synthesis of a library of Michael adducts in excellent selectivity. This ability to introduce asymmetry and control these hindered stereocenters is imperative in a drug-development approach. We could achieve 82–95% yield, 90–96% ee, and 4:1 to >20:1 dr across the board for Michael adducts (**Scheme 5.2.**). Some important features to note include uniquely sized ring systems, such as 5-membered A rings and 6-membered D rings, as well variable alkyl substitution at the C13 position – a modification which would be highly difficult to produce semi-synthetically.

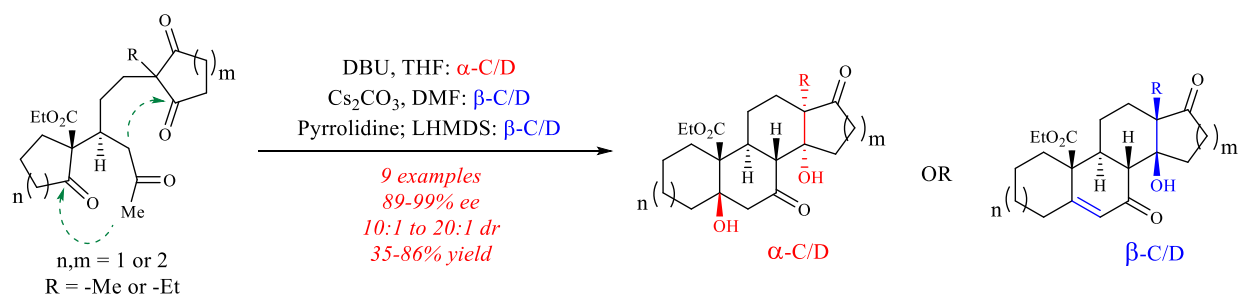
Scheme 5.2. Summary of Asymmetric Michael Addition Results.



At this stage cyclization studies were undertaken to affect a double aldol reaction and complete the steroidal nucleus. We discovered stereodivergent conditions for producing the steroidal cores. Depending on the conditions used, we could produce *cis*- α -C/D or *cis*- β -C/D ring junctions (**Scheme 5.3.**) in high selectivities and yield. Again, this stereocontrol is a primary goal of our overall approach. To this effect, our library of Michael adducts could be converted to a

library of steroidal cores containing the same unique structural features such as unnatural ring sizes, sites of substitution, and stereochemical patterns. In Chapter 3 section 3.9., there is a detailed discussion of the factors which control this diastereoselective C ring closure reaction.

Scheme 5.3. Summary of Stereodivergent Cyclization Results.



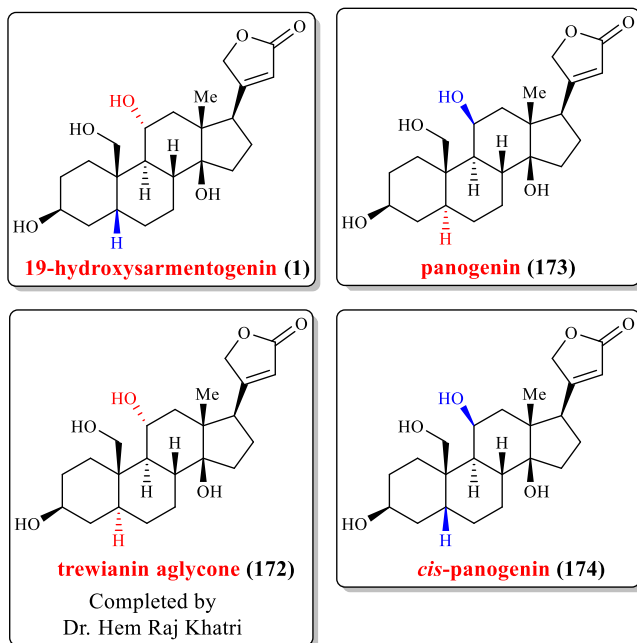
In Chapter 4, we describe our efforts to expand and elaborate upon the developed methodology for the total synthesis of several natural and unnatural cardenolides. We chose four targets: 19-hydroxysarmentogenin, trewianin aglycone, panogenin, and *cis*-panogenin. These targets represent all four isomers of the C5/C11 position of the general 19-hydroxysarmentogenin architecture. To access these targets, several modifications, illustrated in **Figure 5.1**, had to be addressed.

We envisioned making these adjustments in key phases. In line with our primary tenant, we planned to pre-install the oxygenation at C3 and C11 before the key Michael addition/cyclization sequence. This would allow the production of a highly functionalized key intermediate amenable to divergence. This would conclude phase 1. Phase 2 would be the stereodivergent control of the C5 and C11 positions – completing the core structure of each target. While phase three is installation of the butenolide ring. Importantly, we hoped to minimize the steps required to complete phase 1. At this stage, all the important oxygenation is set in the molecule and only stereodivergent elaboration of the cores remains. By constructing a complex

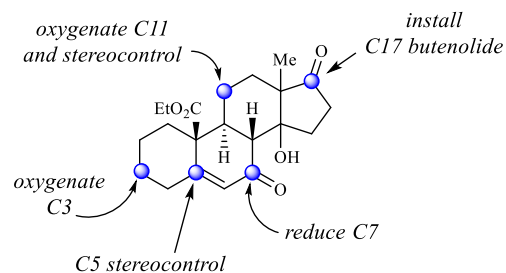
and functionalized intermediate in a concise fashion, we can gain access to a variety of potential derivatives and analogues resulting from subsequent transformations.

Figure 5.1. Targets and Required Modifications.

Targets:



Required modifications:



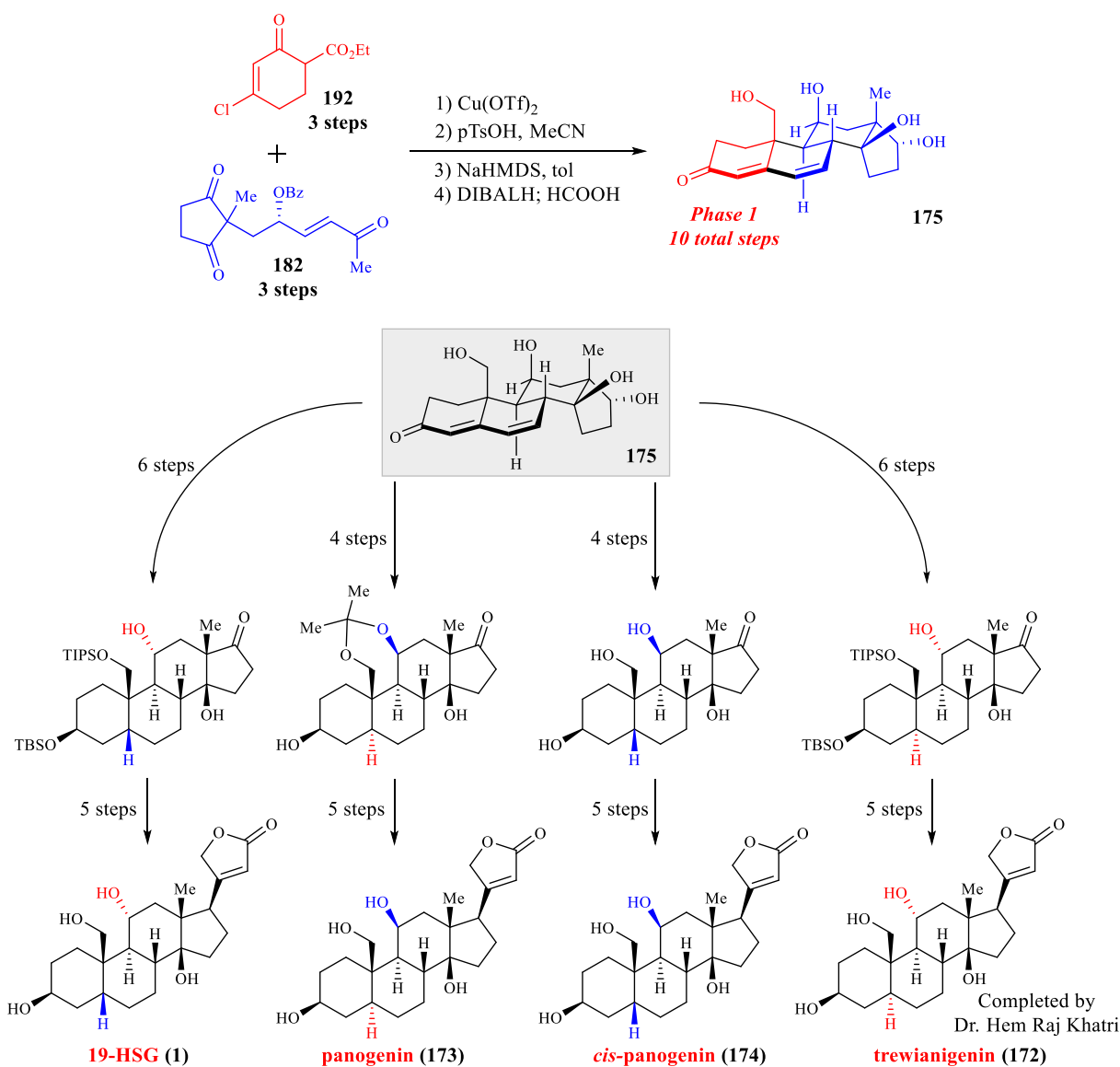
Phases

- 1) Introduce [O] at C3, C11; remove [O] at C7
- 2) Divergent control of configuration at C5, C11
- 3) Install butenolide at C17

We discovered that oxygenation at the eventual C11 position could be achieved via an organocatalytic α -oxidation of an intermediate aldehyde, allowing the synthesis of fragment **182** in 3 steps, 15 g scale, and up to 95% ee. The oxygenation at C3 proved to be more difficult. After much trial and error of unique fragments, we found vinyl chloride **192** to be the most effective fragment for the ensuing sequence. After the Michael addition/cyclization sequence, a *cis*- α -C/D steroidal core is produced. Following epimerization of the *cis*- α -C/D ring junction into a *cis*- β orientation, a global reduction/transposition sequence effectively removes oxygenation at C7 and transfers it to the key C3 position and completes the phase 1 synthesis of key intermediate **175** (Scheme 5.4).² At this stage, comparison to the route described by Inoue *et al.* reveals significant progress. Our key intermediate **175** is produced in 10 total steps, nearly the same steps required to

make only the A/B ring fragment in the previous approach. In addition, one of the key intermediates in the previous approach requires 23 total steps to access, which is more steps than will be required for the completion of the entire target via our method (21 total steps).

Scheme 5.4. Summary of Route to **175** and Stereodivergent Elaboration to Targets.



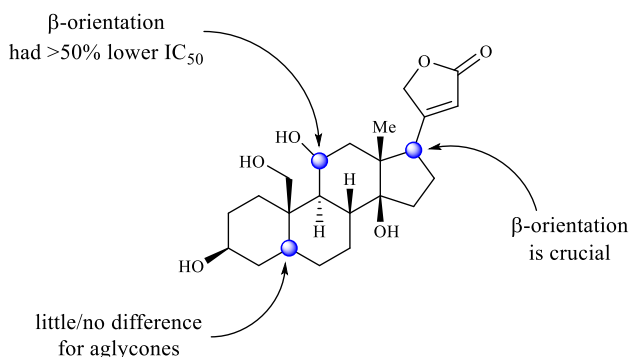
At this point, key intermediate **175** is elaborated in a stereodivergent fashion to the cores of all four targets (**Scheme 5.4.**). Targets with an α -oriented hydroxy at C11 require 6 steps to produce the core, while targets with a β -oriented hydroxy group require 4 steps. Planning of the

synthetic routes and using the inherent structural features of the intermediates allowed strategic stereocontrol at the key positions C5 and C11. With the cores in hand, we completed the synthesis by installing the C17 β -oriented butenolide ring on all four targets via a known 5 step sequence. Overall, we synthesized all four C5/C11 isomers in 19-21 total steps. This represents a significant improvement over previous approaches as well as the capacity to access multiple cardenolide structures from a single, drug-oriented approach.

With the desired compounds in hand, I spent time developing a biochemical inhibition assay to test the ability of our steroids to inhibit the Na^+/K^+ ATPase pump. The assay I developed is based on the colorimetric detection of phosphate production, which is directly correlated to the activity of the Na^+/K^+ ATPase pump. We discovered a few structure-activity relationships which corroborate both the mode of binding and previously suggested results (see discussion in Chapter 1, section 1.5.) (**Figure 5.2.**). First, the orientation at C5 seems to be of little or no importance for our aglycones. It is surmised that the C5 configuration will have a more dramatic effect on IC_{50} with glycosylate congeners. This is because the difference between a *cis*- and *trans*-A/B ring junction has a significant impact on the 3-dimensional orientation of the key C3 hydroxy group which could affect the position of the sugar moiety within the binding pocket. Additionally, the orientation of the C11 alcohol appears to be significant. Our results suggest that a β -oriented alcohol improves the inhibitory capacity. Examination of the binding mode described by co-crystallographic analysis with cardenolides indicates the importance of polar binding contacts on the β -face of these steroids while the primary interactive force on the α -face is nonpolar in nature, corroborating these results. Finally, the removal of the butenolide ring at C17 completely destroyed inhibitory activity up to the millimolar range and when the butenolide ring is present, changing its orientation from β -oriented to planar (by introducing C16-C17 unsaturation) dramatically reduces

its inhibitory capacity. It appears that the raw presence of the butenolide ring is insufficient for inhibiting the pump in this assay, and the β -orientation at C17 is crucial. This supports previous reports that the specific orientation at C17 is paramount to the activity of cardenolides.

Figure 5.2. Summary of Observed Structure-activity Relationships.



Overall, a new synthetic approach was designed from a drug-oriented perspective to allow for the concise, two step synthesis of uniquely functionalized steroidal cores with excellent stereocontrol. This methodology was expanded upon to allow for the expedient synthesis of a heavily functionalized key intermediate with preinstalled functional handles. Here, remarkable chemical complexity is constructed in just 10 total steps from simple substrates. This intermediate is then elaborated in a stereodivergent fashion into four cardenolides epimeric at the C5/C11 positions in just 19-21 total steps. These steroids were then evaluated in a biochemical inhibition assay to derive some interesting structure-activity relationships.

5.3. Future Directions

Currently, our lab continues to explore the capacity of our described approach to access more heavily functionalized cardenolides. We are interested in producing additional structural analogues of these cardenolides including C3 glycosylated derivatives, appending unique heterocycles at C17, exploring steroids of unnatural isomeric configurations at positions other than C5 and C11, and building up additional sites of oxygenation including C1 and C5. We hope to

acquire purified α -isoforms of the Na^+/K^+ ATPase pump to allow for isoform selectivity studies to be performed via biochemical assay. It will be interesting to determine if some unique relationships exist between the isoform selectivity and anticancer activity of these cardenolides.

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