

**Cannogenol and Related Cardiotonic Steroids: Concise Synthesis and their Anticancer Activity
Evaluation**

by

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Dedication

To my mom, dad, and grandmother

(You have sacrificed your happiness for the sake of mine. You have compromised so much to make sure we were well educated and more importantly, developed as good human beings. You have encouraged and inspired me to love science. Thank you for everything!)

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List of Abbreviations

AIDS	acquired immune deficiency syndrome
ATP	adenosine triphosphate
BINOL	1, 1'-bi-2-naphthol
BOX	bis(oxazoline)
Bz	benzoyl
<i>c</i>	concentration
CS	cardiotonic steroid
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL-H	diisobutylaluminum hydride
DMF	dimethylformamide
DMP	Dess-Martin periodinane
DNA	deoxyribonucleic acid
dr	diastereomeric ratio
ee	enantiomeric excess
EtOAc	ethyl acetate
HBD	hydrogen bond donor
H-bond	hydrogen bond

HF	hydrogen fluoride
HPLC	high performance liquid chromatography
IC ₅₀	half-maximal inhibitory concentration
Imz	imidazole
KHMDS	potassium hexamethyldisilazide
LiHMDS	lithium hexamethyldisilazide
μM	micromolar
NaHMDS	sodium hexamethyldisilazide
nM	nanomolar
OTf	triflate
<i>p</i> -TSA	<i>para</i> -toluenesulfonic acid
Py	pyridine
SAR	structure-activity relationship
STAB	sodium triacetoxyborohydride
TADDOL	$\alpha,\alpha,\alpha,\alpha$ -tetraaryl-1,3-dioxolane-4,5- dimethanol
TBAF	tetrabutylammonium fluoride
TBS	<i>tert</i> -butyldimethylsilyl
<i>t</i> BuOH	<i>tert</i> -butanol
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TMS	trimethylsilyl

Abstract

Small molecules that are approved as drugs are largely the result of extensive synthetic efforts. A robust synthetic method is required to access the natural products and their analogs to study their biological activities. This thesis largely focuses on the synthesis of cardiogenic steroids, the steroids that are known for the treatment of heart conditions and more recently has shown promising prospect in anticancer studies against various human cancer cell lines. Furthermore, the anti-cancer activities of the molecules of this class were studied and substantial data on structure-activity relationship (SAR) were obtained. In addition, this thesis also presents an anion binding catalysis, the process by which anions are selectively transported between membranes in human body, of a new class of catalyst thiophosphoramidate that showed promising ability to bind with anions.

The first chapter describes the utility of three hydrogen-bond donor, thiophosphoramidate-based catalyst anion binding catalysis. The study illustrates that the thiophosphoramidate-based HBDs could significantly accelerate Cu(II)-catalyzed reactions of potassium carboxylates with diaryliodonium salts. The scope of counterion on both the diaryliodonium salt and the copper salt is explored, followed by the application of this method in a wide-variety of carboxylic acid including some naturally available carboxylic acid.

The second chapter introduces cardiogenic steroids and the discusses the relevant prior synthesis. Impressive synthesis of digitoxigenin by Yoshii and Nakada followed by the synthesis of strophanthidin by Yoshii and Kočovský are discussed in detail. The total synthesis of cannogenol-3-*O*- α -L-rhamnoside that will be discussed in following chapter is inspired by the

work previously published by Nagorny and coworkers on enantioselective synthesis of oxygenated steroids by copper-catalyzed Michael reaction followed by double aldol reaction and the utilization of this method in the total synthesis of 19-hydroxysermentogenin and trewianin aglycone are discussed in this chapter.

The third chapter describes the development of a robust and divergent synthetic pathway to access natural product of the class called cardiotonic steroids. The chapter highlights the first enantioselective total synthesis of two cardiotonic steroids that have been recently of interest because of their anti-cancer activities: 1) cannogenol 2) cannogenol-3-*O*- α -L-rhamnoside. The challenges during the development of this method, that could provide analogs with different heterocycles and sugar moieties with minimum deviations, are described in detail.

Finally, the fourth chapter focuses on the generation of natural products and analogs using the method developed in chapter 3. Another natural product cannogenol-3-*O*-glucopyranoside and an analog of cannogenol-3-*O*- α -L-rhamnoside with alkyne incorporation in the sugar moiety for the target identification studies were synthesized. In addition to the molecules synthesized in our group, other commercial steroids of diverse functional group at different part of the molecule were collected. The collaboration with Dr. Yimon Aye's group at Cornell University presented us with significant structure-activity relationship (SAR) data. In addition, an analog of strophanthidin with amine incorporation instead of alcohol at C19 was synthesized by reductive amination of aldehyde.

Chapter 1

Thiophosphoramides as Cooperative Catalysts for Copper-catalyzed Arylation of Carboxylates with Diaryliodonium Salts

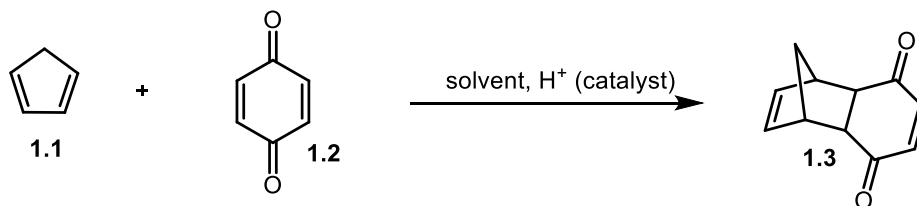
(A part of this work has been published in Bhattarai, B.; Tay, J.H.; Nagorny, P. Thiophosphoramides as cooperative catalysts for copper-catalyzed arylation of carboxylates with diaryliodonium salts. *Chem. Commun.* **2015**, 51, 5398-5401)

1.1. Introduction

Over the past two decades, the use of organocatalysts in asymmetric organic reactions has emerged as a powerful tool for stereoselective organic transformations. This popularity of organocatalysts, in recent years, is due to their robustness, inexpensiveness, ease of availability, and non-toxic nature.¹⁻³ When compared to other methods using metal-based catalysts, organocatalysis does not require demanding reaction conditions and is usually inert towards moisture and oxygen.² In addition, the absence of transition metals makes this field more attractive for pharmaceuticals industry since it avoids the task of controlling and removing trace metal impurities in the final product. Despite the rapid developments in the field in the recent years, there is still much room for advancement with regard to catalytic activity and substrate scope. Due to the historical reasons proline,^{4,5} cinchona alkaloids^{6,7} and other secondary amine derivatives⁸ are among the most frequently used organocatalysts; however, new classes of organic catalysts are emerging. Thus, the studies of H-bond donor-catalysts and their chiral analogs have also become a rapidly growing and important area of organocatalysis and numerous reports have shown these catalysts to be an effective alternative to metal-based catalysts and other organocatalysts.¹⁻³

Although organocatalysis is popularized since 2000s,^{9,10} the reports of organic molecules catalyzing organic reactions date back to 1860, when Liebig performed the reaction of cyanogen in presence of water and acetaldehyde to obtain oxamide.¹¹ Later in 1896, Knoevenagel discovered the condensation reaction of aromatic aldehydes with malonic acid catalyzed by amines as an organocatalyst.¹² The first asymmetric organocatalytic reaction was attained by Bredig and Fiske in 1912 where they reported the ability of cinchona alkaloids to catalyze a reaction of hydrogen cyanide with aldehydes to provide cyanohydrins in ~10% ee.¹³ Since then, protic organic compounds have been used to catalyze various organic reactions. For an instance, Wassermann reported the first example of catalytic Diels-Alder cycloaddition between cyclopentadiene **1.1** and benzoquinone **1.2** in 1942,¹⁴ where he used several organic molecules (Phenol, CH₂ClCO₂H, CH₃CO₂H, CCl₃CO₂H) as single proton donor to catalyze the cycloaddition reaction (**Scheme 1.1**).

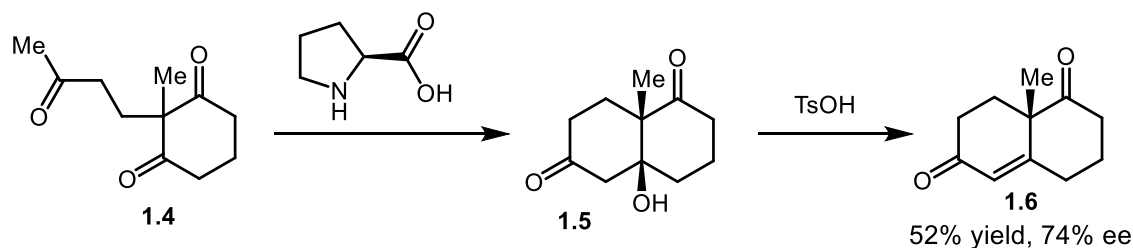
Scheme 1.1 First Example of Catalytic Diels- Alder Cycloaddition Catalyzed by Organic Molecule



H⁺ Catalyst = Phenol, CH₂ClCO₂H, CCl₃CO₂H, CH₃CO₂H

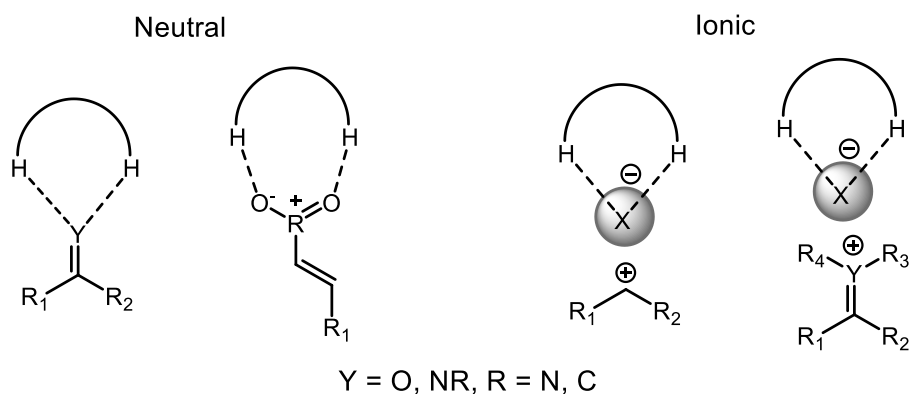
A pioneering work in this field was the discovery of proline in the catalysis of intramolecular aldol reaction in Hajos-Parrish-Eder-Sauer-Wiechert reaction in 1971 (**Scheme 1.2**).^{15,16} This chemistry has since been widely elaborated in the enantioselective synthesis of steroids and various other natural products.

Scheme 1.2 Proline Catalysis in Hajos-Parris-Eder-Sauer-Wiechert Reaction



Organocatalysis based on catalyst's H-bond donating abilities has been explored in a variety of organic reactions. They depend on either the activation or deactivation of a nucleophile or an electrophile to facilitate the reactions (**Figure 1.1**). There are two different modes of activation 1) a neutral substrate can be activated by the enhancement of its electrophilicity, and then undergo a subsequent nucleophilic attack, or 2) binding to an anionic counterion of an electrophile results in a significant enhancement of its electrophilicity and results in a subsequent reaction with a nucleophile.

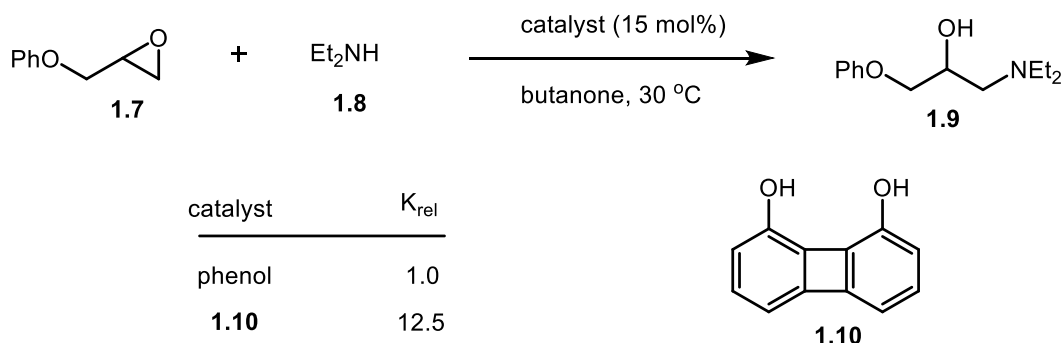
Figure 1.1 Different Modes of Activation by H-Bond Catalysis



Among the early examples of organic hydrogen bond donors (HBDs) is 1,8-Biphenylenediol **1.10** that based on previously reported X-ray crystallographic data, is known to form two H-bonds with a single oxygen atom.¹⁷ In one of a pioneering work by Hine et al., the authors were able to take advantage of this particular characteristic of 1,8-biphenylenediol (**1.10**) to catalyze the reaction of an epoxide **1.7** with diethyl amine **1.8** (**Scheme 1.3**). This catalyst was found to be

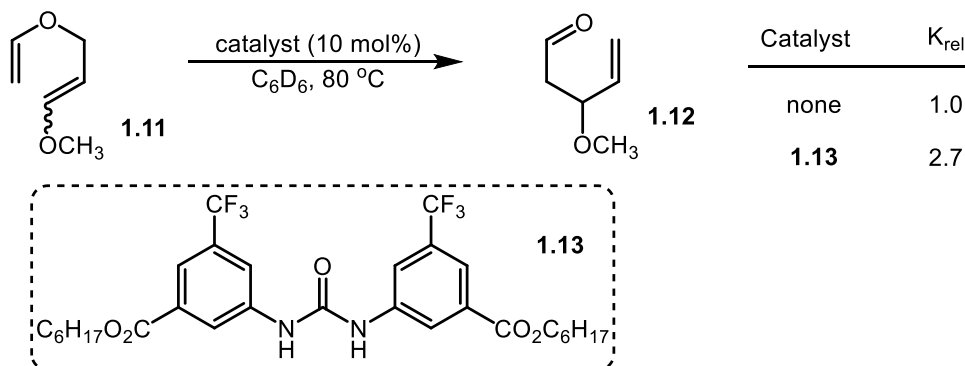
superior to several phenols tested with either electron-donating or electron-withdrawing groups that were explored in this study and the reaction was observed to proceed 12.5 times faster than with a phenol as a catalyst. This effect was attributed to superior substrate activation by **1.10**, which is able to form two hydrogen bonds simultaneously.¹⁸

Scheme 1.3 1,8-biphenylenediol as Two H-bond Donor in Catalysis



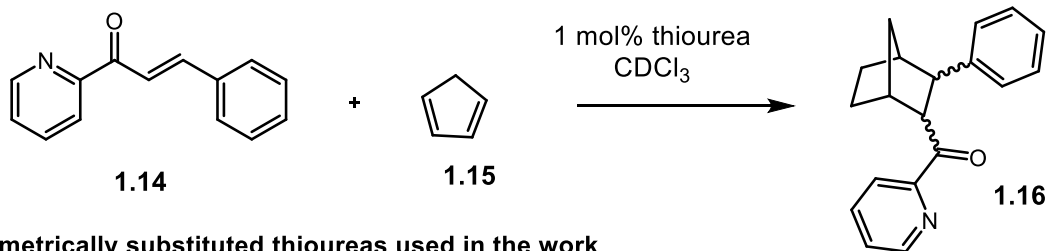
In 1994, Curran and Kuo investigated the diarylureas as the catalyst for the allylation of cyclic α -sulfinyl radicals.¹⁹ For the first time, the authors demonstrated that these H-bond donors could catalyze complex organic transformations of this type. They further studied Claisen rearrangement of allyl vinyl ether catalyzed by thiourea catalyst **1.13** (**Scheme 1.4**).²⁰ The acceleration of the Claisen rearrangement by 2.7 times was observed, as compared to the reaction in the absence of catalyst.

Scheme 1.4. Claisen Rearrangement of Allyl Vinyl Ether Catalyzed by **1.13**

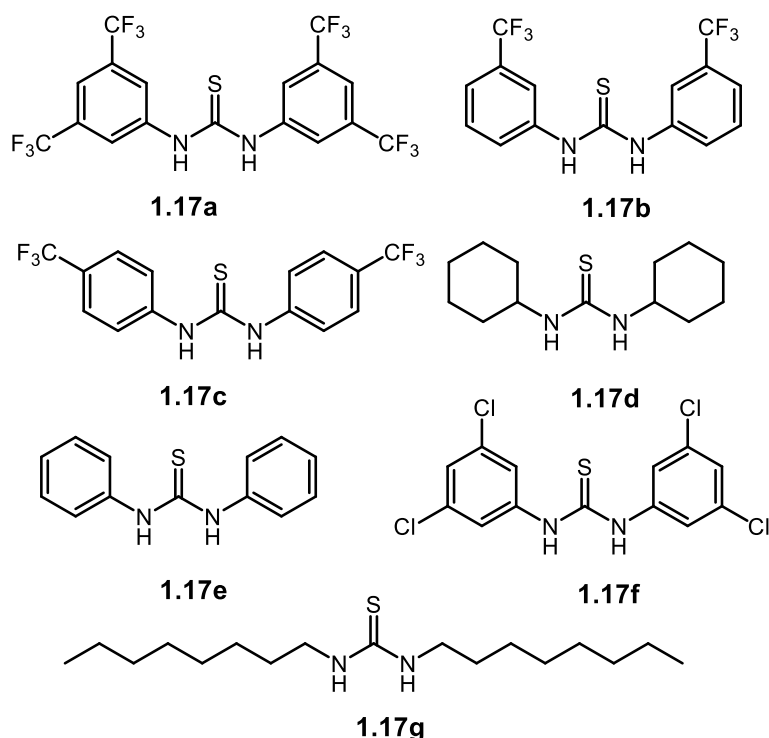


A variant of thiourea **1.13** was later used by Schreiner in 2002, where they showed capability of this class of catalyst towards Diels-Alder reactions.²¹ Furthermore, in 2003, Schreiner et al. screened catalyst efficiencies (**Scheme 1.5**) by carrying out a series of Diels-Alder reactions of cyclopentadiene with α, β -unsaturated carbonyl compounds (**Scheme 1.5**). Most thiourea catalysts demonstrated a catalytic behavior and the introduction of disubstituted non-coordinating electron-withdrawing groups (i.e. CF₃) in the meta positions of the ring enhanced the hydrogen bonding ability of the N–H bonds. These thiourea derivatives, with rigid electron-withdrawing aromatic substituents, were found to be the most effective H-bonding catalysts for the Diels-Alder reactions. Relative rate constant (K_{rel}) (**Table 1.5b**) and conversion of the reaction catalyzed by **1.17a**, however, was significantly higher than for the majority of the other thiourea derivatives.²²

Scheme 1.5. Diels-Alder Reaction of Cyclopentadiene with Dienophile Catalyzed by Thiourea Derivatives



a) Symmetrically substituted thioureas used in the work



b) Relative rate constant of thiourea derivatives

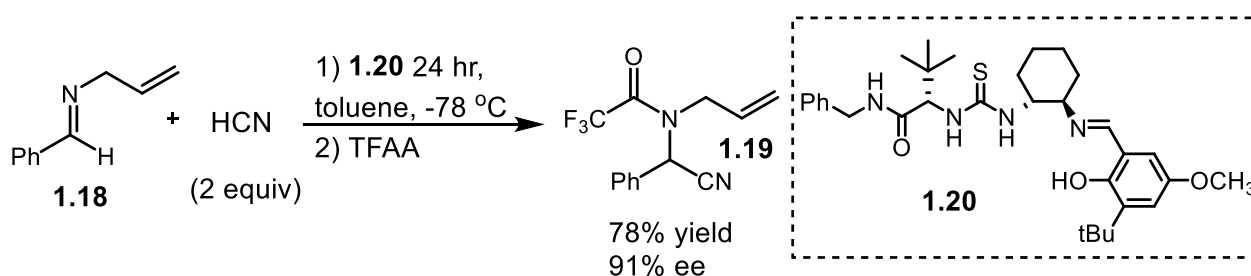
Thiourea	Relative rate constant K_{rel}
1.17a	8.2
1.17b	5.9
1.17c	1.0
1.17d	1.2
1.17e	1.5
1.17f	3.5
1.17g	1.0

Utilization of H-bonding as an activation force is widespread in asymmetric organocatalysis. Chiral ureas and thioureas, squaramides, diols, and phosphoric acids have dominated the field of H-bonding catalysis for the past decade and great advances have been attained.^{2,3,23} During this period, urea and thiourea based organocatalysts have been extensively studied due to their ability of strong activation of carbonyl and nitro-groups through efficient H-bonding interactions. Chiral, bifunctional thiourea-based organocatalysts, that can catalyze the reaction while transferring chiral information, are widely used in enantioselective reactions like acyl-Pictet-Spengler,²⁴ Michael-addition,^{25,26} Baylis-Hillman,²⁷ aza-Henry,⁶ and Mannich^{6,7} reactions. Though most often utilized to activate electrophiles by binding to a carbonyl, chiral thioureas have also been demonstrated to

promote anion abstraction from neutral organic electrophiles to generate highly reactive cationic intermediates.²⁸

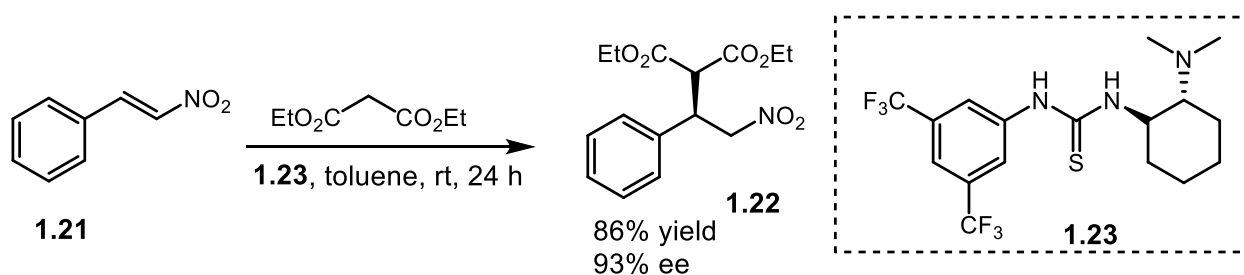
In 1998, one of the first thiourea catalysts was synthesized by the Jacobsen group, which was applied in an asymmetric Strecker reaction. From their parallel synthetic libraries, they identified that **1.20** was an effective catalyst for the asymmetric Strecker reaction as depicted in **Scheme 1.6** (hydrocyanation of **1.18**). Notably, it exhibited promising enantioselectivity both on solid phase and in solution and was easily prepared from inexpensive components.²⁹

Scheme 1.6. Asymmetric Strecker Reaction Catalyzed by **1.20**



In addition to the aforementioned HBD-based catalysts, a variety of hybrid organic catalysts that contain other catalytic sites in addition to the hydrogen bonds have been developed. Thus, Takemoto catalyst (**1.23**) is the first bifunctional thiourea catalyst with rigid electron-withdrawing aromatic substituents (-CF₃ group). This catalyst activates both the nucleophile, by general base catalysis, and the electrophile, by H-bonding to the nitro group. Thiourea catalyst **1.23** worked well to promote the Michael reactions of malonates and various nitroolefins (**1.21**), with high enantioselectivities (**Scheme 1.7**).²⁵

Scheme 1.7. Enantioselective Michael Reaction of Malonate and Nitroolefin Catalyzed by **1.23**

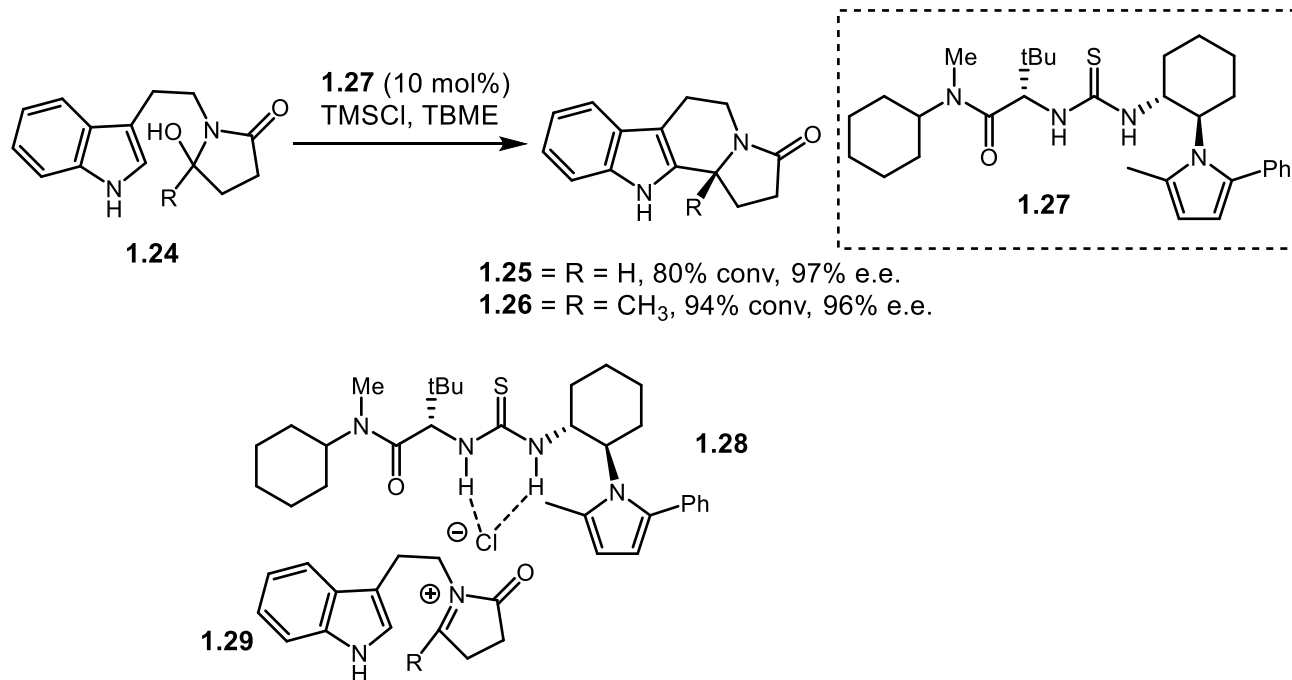


In 2007, Jacobsen et al. reported the successful application of thiourea catalysis to the Pictet-Spengler-type cyclization of hydroxylactams, affording highly enantioenriched indolizidinones and quinolizidinones (**Scheme 1.8**). Furthermore, it was also discovered that thiourea catalyst **1.27** promoted enantioselective cyclization by inducing dissociation of the chloride counterion and formation of a chiral *N*-acyliminium chloride-thiourea complex (**1.28-1.29**).²⁴

Scheme 1.8. Asymmetric Cyclization of Hydroxylactams Catalyzed by **1.27** and the Proposed

Anion

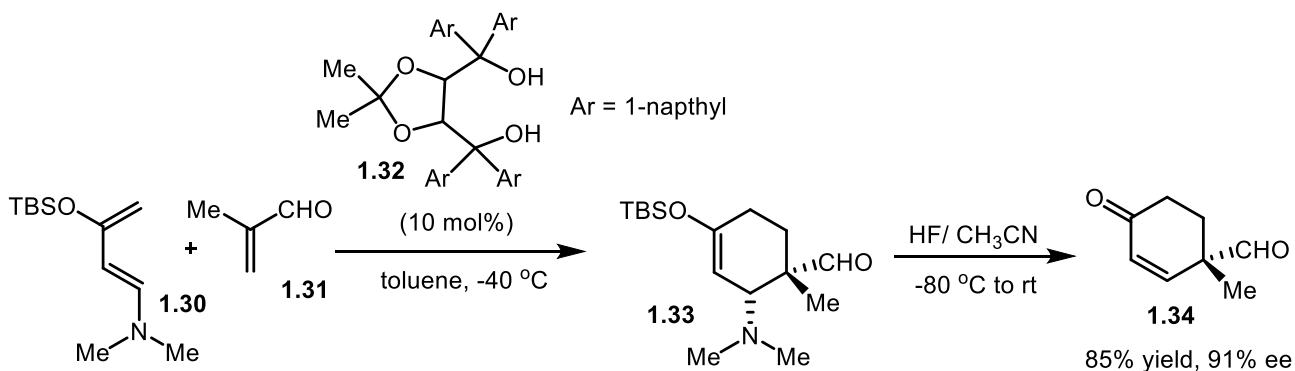
Binding



Interestingly, the scope of HBD-based catalysts is not limited to conventionally strong HBDs such as ureas or thioureas, and even alcohols may serve as the catalysts in certain transformations.

Thus, Rawal's TADDOL **1.32** is considered to be one of first successful application of chiral diols in asymmetric H-bond donating catalysis (ref). In a Diels-Alder reaction of aminosiloxydiene **1.30** and methacrolein **1.31**, aldehyde **1.34** was obtained in 91% ee after desilylation and amine elimination (Scheme 1.9).³⁰ TADDOL **1.32** is a simple and easily available and promoted this reaction as efficiently as a previously known Lewis acid catalyst would. Since the publication of this work, research on various organic transformations with TADDOL, BINOL, BINOL based Chiral Phosphoric Acids and others have followed.³¹⁻³³

Scheme 1.9. Rawal's TADDOL **1.32** in Asymmetric Catalysis

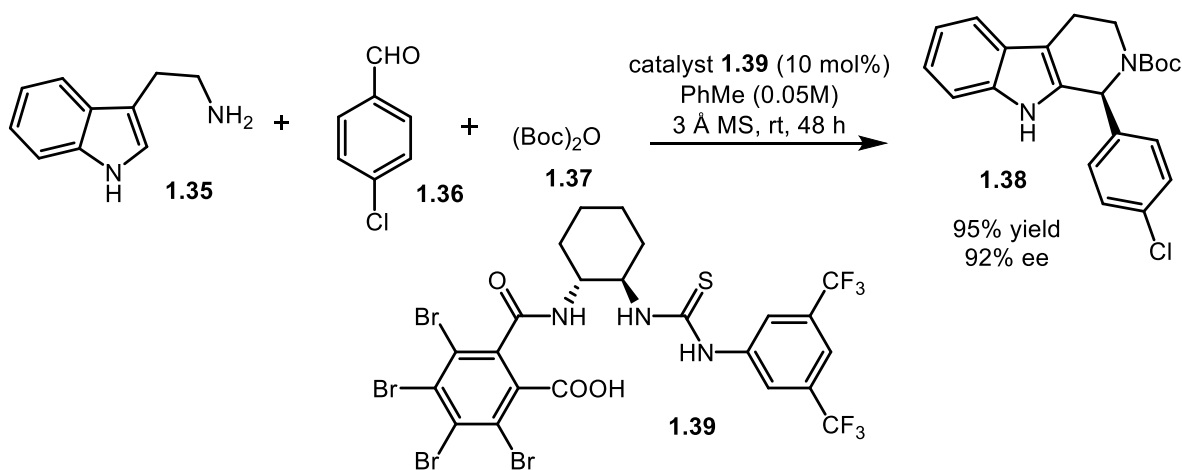


1.2. Three Hydrogen Bond Donors

In addition to the catalysts that may serve as a single or double HBDs, catalysts that may potentially form three HBDs with a substrate have been developed and studied. Seidel and co-workers further explored of the thiourea-based dual catalysis and reported several examples of carboxylic acid-based thiourea that could act as a dual catalyst in a series of organic transformations like Pictet-Spengler reaction,³⁴ Povarov reaction,³⁵ and Intramolecular Aza-Diels-Alder Reactions.³⁶ In the following example, internal anion-binding concept was used using chiral anion/Bronsted acid catalysis. Catalyst **1.39** functions as a dual catalyst, wherein, it contains both anion-recognition site, specifically, thiourea moiety and covalently connected carboxylic acid

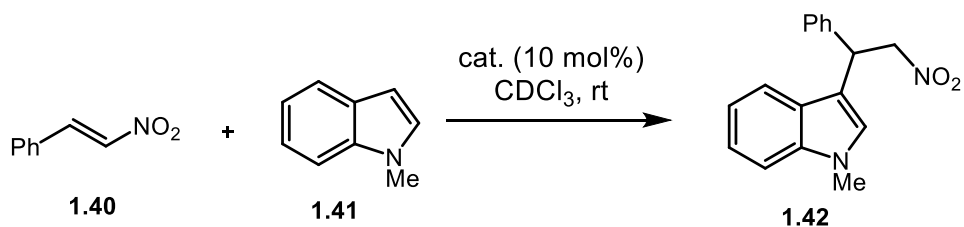
functional group.³⁴ Upon the exposure to this catalyst, the substrate gets protonated and a substrate/catalyst ion pair is formed by anion recognition, which leads to the enhanced electrophilicity of the substrate. The authors screened multitude of catalysts with a variety of carboxylic acid and they found catalyst **1.39** to be the most efficient at promoting Pictet-Spengler reaction of tryptamine **1.35** with aldehyde **1.36**. Following a protection of the amine with Boc generated β -carbolines **1.38** in very good yield and % ee.

Scheme 1.10. Pictet-Spengler Reaction Catalyzed by Dual Catalyst **1.39**

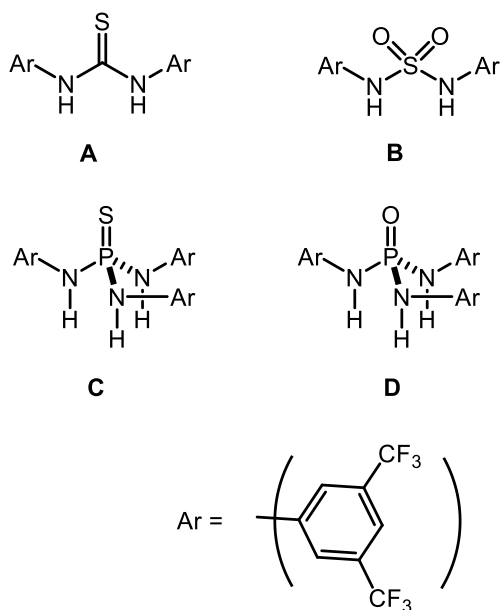


A more direct use of multiple hydrogen bond donors for the substrate activation was reported by the Shea group. The Friedel-Crafts reaction between β -nitrostyrene **1.40** and *N*-methyl indole **1.41** (Scheme 1.11) was executed by Rodriguez et al. in 2009 to compare the catalytic activity of thiourea **A**, sulfamide **B**, thiophosphoric triamide **C**, and phosphoric triamide **D** (Scheme 1.11a). Thiophosphoric triamide **C** displayed a 2.6-fold increase in activity compared to the most active and versatile thiourea catalyst **A** that had been designed by Schreiner. This finding led to the recategorization of the thiophosphoric triamide as an HBD catalyst, and its superior catalytic activity was attributed to its ability to form up the three hydrogen bonds at the same time.²⁷

Scheme 1.11. Friedel-Crafts Reaction of *N*-methyl Indole with β -nitrostyrene



a) HBD catalysts used in the work

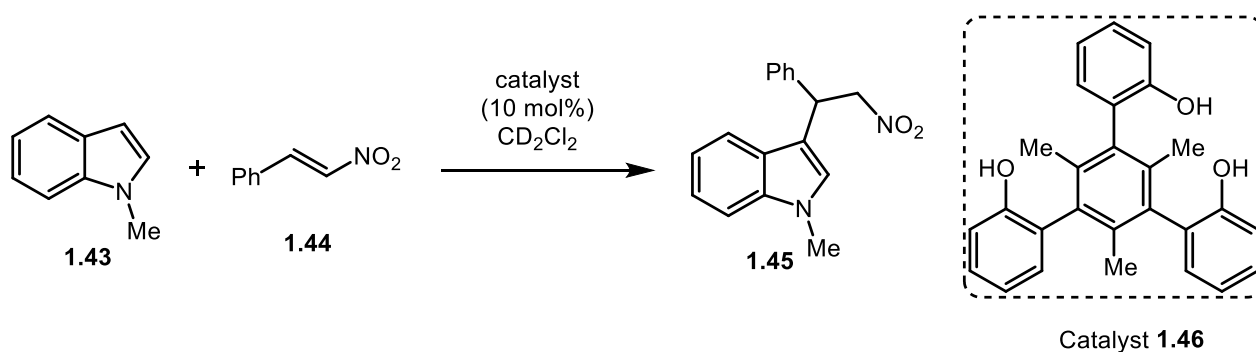


b) Catalyst comparison

Compounds	K_{rel}	Conversion
Thiourea (A)	1	49
Sulfamide (B)	0.3	20
Thiophosphoric triamide (C)	2.6	80
Phosphoric triamide (D)	0.1	8

Another case describing improved substrate activation with additional hydrogen bonds was reported by the Kass group. Thus, in their 2012 publication, a new class of H-bond catalyst **1.46** was presented which could efficiently catalyze a Friedel-Craft reaction of N-methyl indole **1.43** with β -nitrosyrene **1.44** (**Scheme 1.12**). This catalyst was found to use all three of its H-bond donors to bind with a single functional group and consequently, catalyze the reaction 100 times faster. It bound anions more tightly than the other, two H-bond donating counterparts.³⁷

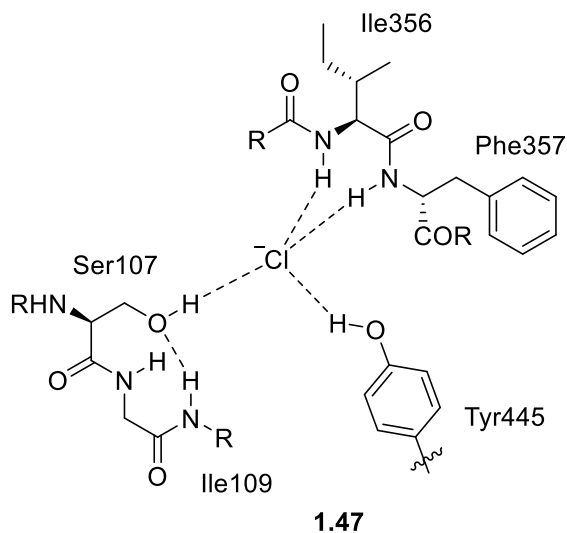
Scheme 1.12. New Class of Three H-bond Donors **1.46**



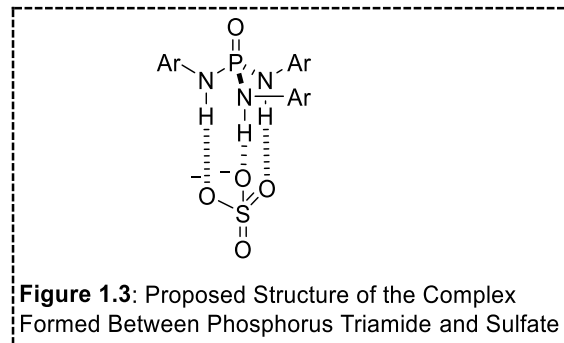
1.3. Anion Recognition

It has been long known that the anion recognition is essential for biological function especially, for the transfer of anions between membranes selectively. Chloride channel is no exception (**Figure 1.2**). Chloride anion is essential for numbers of cellular and physiological tasks that control the membrane potentials. The three amino acids that are highly aligned act as a selective filter for anion channel and enable the selective flow of chloride ions across the cell membranes.³⁸ Such interaction **1.47** is very common in biological systems and the study of the interaction between proteins and anions like nitrate, sulfate, phosphate, chloride and their recognition, binding, and transportation is of interest to the scientific community.³⁸

Figure 1.2. Anion Recognition in Chloride Channel



In that regard, Cranwell et al. demonstrated in 2013 that new H-bonding motifs based on phosphoric triamide and thiophosphoric triamide could be effective anion receptors, which would be capable of mediating the exchange of chloride and nitrate anions across the lipid bilayer membrane. Interestingly, phosphoric triamide was also found to have high selectivity for sulfate ions in DMSO-d₆/0.5% water solution (**Figure 1.3**).³⁹



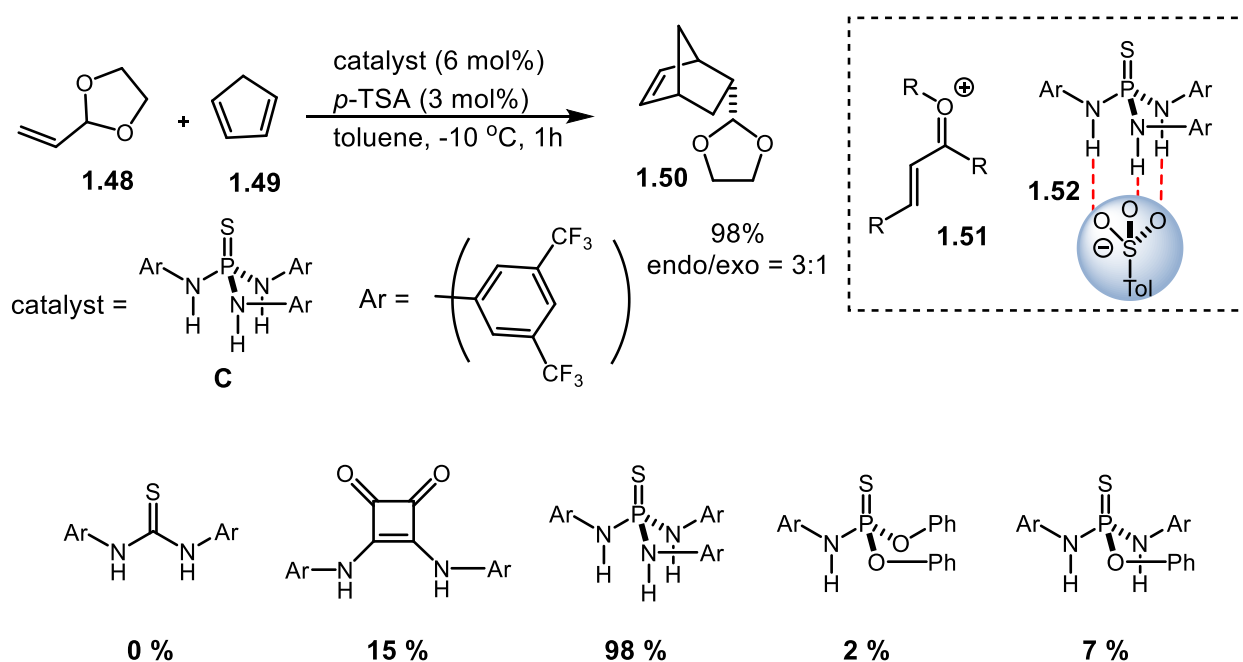
In addition to chloride, nitrate, and sulfate anions, they found **C** to be a good receptor of other halogen, phosphate, carboxylate, and acetate anions as well.

In recent years, ureas and thioureas have gained a great deal of attention for their ability to operate as dual HBD catalysts. However, the discussed above cases imply that additional hydrogen bonds could enhance the substrate activation. Therefore, HBD catalysts capable of forming more than two hydrogen bonds, like thiophosphoramides or their bifunctional modifications represent a newer area of study. Our research group was among the pioneers in exploring thiosphosphoramides as HBD catalysts as these compounds could form three hydrogen bonds with an acceptor, unlike two H-bond donors, but at the same time are significantly more stable to hydrolysis than squaramides and thioureas.

Parallel to Cranwell's finding, our group identified the thiophosphoramide **C** as powerful HBDs which significantly enhanced the reactivity of oxocarbenium ion **1.51** via anion binding **1.52** by forming a more separated ion pair (**Scheme 1.13**).⁴⁰ During the study, Bronsted acid was used as a co-catalyst in the formation of oxocarbenium ion, reactivity of which was enhanced by the separation of counterion, sulfonate, by a binding of thiophosphoramide with the anion. This is the first example of thiophosphoramide being used for anion-binding organocatalysis.

Furthermore, ^1H NMR supported a strong binding affinity of sulfonate anions with thiophosphoramidate. Computational studies indicated the binding of all three of the N–H bond of the catalyst to the oxygen atom of the sulfonate anion (**1.52**). Thiophosphoramidate was also found to be superior to traditionally used two H-bond donor catalysts.

Scheme 1.13. HBD-based Cocatalysts for the Ionic [2 + 4] Cycloaddition and the Proposed Activation of Oxocarbenium Ion by Anion Binding

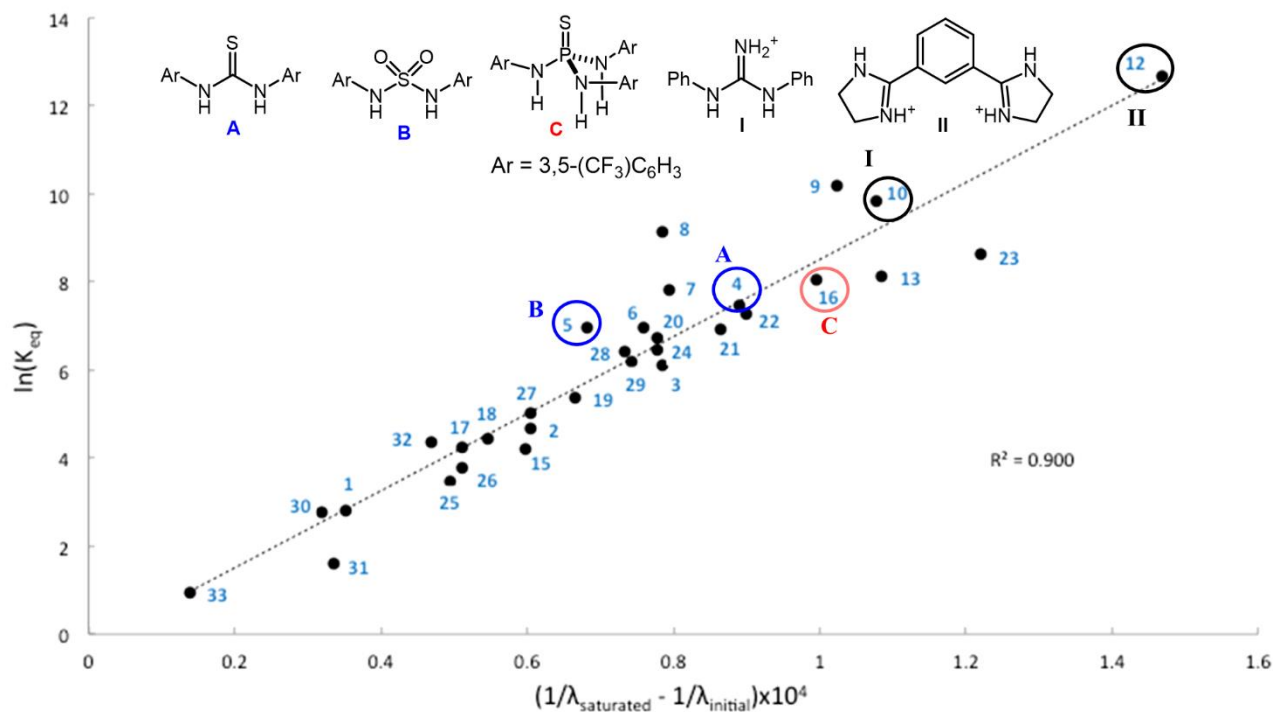


Subsequently, in an effort to quantify the electrophilic activation (LUMO-lowering), Kozlowski and co-workers were able to use spectrophotometric sensor to assess the reactivity of an array of commonly used H-bonding organocatalysts with different structures.⁴¹ Imidazopyrazinone was used as a sensor or chromophore that underwent electronic excitation after binding to H-bond donor and this interaction was detected by UV-vis absorption. Significant visible blue shift was observed even in presence of weaker donors like diphenylthiourea upon interaction with the sensor. The measured shift in sensor wavelength and binding equilibrium

constant of sensor-catalyst complex in a broad range of widely used organocatalysts were plotted (Figure 1.4). In addition, the correlation in wavelength shift and the rate of enhancement in presence of the catalyst in a Diels-Alder reaction between cyclopentadiene and methyl vinyl ketone was also plotted. Similar correlation data was obtained for a Friedel-Crafts reaction. They concluded that the measurement from the sensor provided good assessment of inherent reactivity of these organocatalysts.

Figure 1.4. Correlation between Sensor Wavelength Shift and Sensor-catalyst Binding

Equilibrium



(Figure 1.4 is used directly from <https://pubs.acs.org/doi/abs/10.1021/ja5086244> with permission from American Chemical Society (ACS) and further permissions related to the material excerpted should be directed to the ACS)

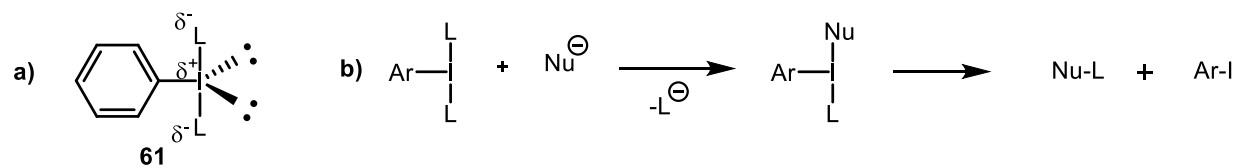
From these studies, they found thiophosphoramidate catalyst C to be one of the best performers and the strongest non-cationic binder because of its ability to act as three N–H donors. It was

1.4. Thiophosphoramides as Cooperative Catalysts for Copper-catalyzed Arylation of Carboxylates with Diaryliodonium Salts

1.4.1. Diaryliodonium Salts

Hypervalent iodine derivatives have been of great importance to organic synthesis. In addition to the standard oxidation state, iodine can form a variety of other organic compounds including the ones with (III) and (V)-valent iodine. Iodine(V) reagents like 2-Iodoxybenzoic acid (IBX) and Dess-Martin Periodinane (DMP) are widely used in natural products synthesis as a mild oxidant of alcohols.^{44,45} Iodine(III) compounds like iodosylbenzene and diacetoxyiodobenzene (PIDA) are similarly used for oxidation of alcohols and have additional applications like α -functionalization of carbonyl groups and oxidation of alkenes.^{46,47} Hypervalent iodine compounds reacts with nucleophile to form I-Nu bond by substituting a ligand. Formal reductive elimination or its mechanistic equivalents follows to form the product Nu-L with the release of ArI (**Figure 1.5b**).⁴⁸

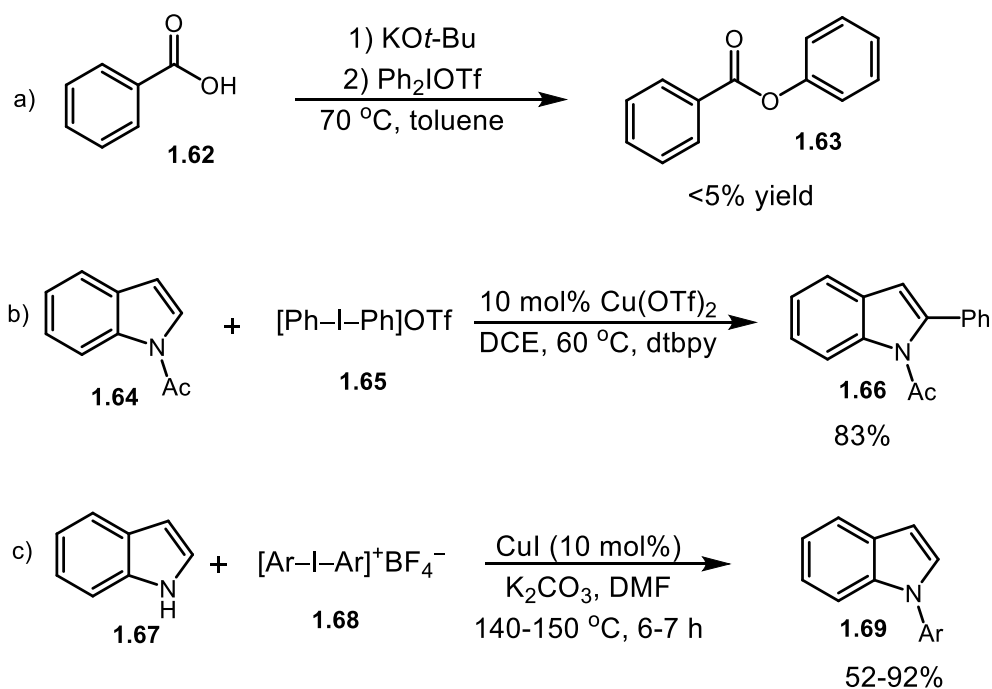
Figure 1.5 Shape of Hypervalent Iodine and General Reactivity



Diaryliodonium salts have gained popularity as inexpensive and readily available electrophilic arylation agents.^{49,49,50} While X-ray studies indicate that diaryliodonium salts have significant secondary bonding with counterions in the solid state, the dissociation of the counterion in solution is often proposed.^{49,51,52} In some instances the observed reactivity trends could be attributed to ion-pairing effects, and certain parallels could be drawn between the reactivity of diaryliodonium salts and classical electrophiles such as iminium or carbenium ions. These salts have received great attention in the recent years due to their exceptional ability to transfer sp^2 - and sp -based carbon electrophiles in a reaction with a variety of nucleophiles. Reactions with nucleophiles could

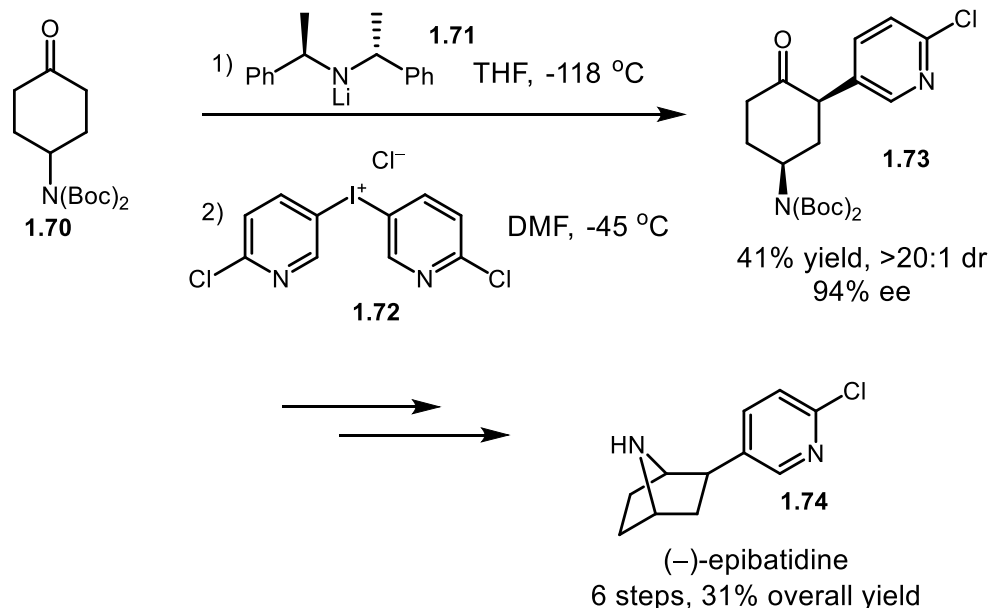
proceed under both metal-catalyzed⁵¹⁻⁵⁵ and metal-free conditions,⁵⁶⁻⁵⁸ and parameters such as the polarity of the solvent and the coordinating ability of the diaryliodonium counterion are often important for the outcome of these reactions.^{49-51,59-61} Diaryliodonium salt has been widely used in organic synthesis to transfer aryl groups to the nucleophile in presence or absence of metal-catalyzed reactions where it is found to be more reactive than traditionally used aryl halides. These reactions could happen with thermal activation or upon addition of an external catalyst such as Cu(I) or Pd(II). Traditionally, strong nucleophiles could directly react with diaryliodonium salts although some of these reactions require heating. Thus, as it has been demonstrated by the Olofsson and group, the carboxylates could be arylated with diaryliodonium salts to form aryl esters, and these reactions are very general to variety of carboxylates and diaryliodonium salts albeit require elevated temperatures.⁶² The arylation of benzoic acid **1.62** with diphenyliodonium triflate occurred at 130 °C to provide phenyl benzoate **1.63** in excellent yield but only trace of the product was observed in upto 70 °C (**Scheme 1.15a**). In a separate example by Gaunt and group, the *N*-acetylated indoles were selectively arylated at C2 or C3 position in presence of Cu (II) catalyst.⁶³ In the following example (**Scheme 1.15b**), *N*-acetylated indole **1.64** was treated with diphenyliodonium triflate **1.65** in presence of Cu(OTf)₂ and dtbpy at 60 °C to provide 9:1 selectivity of C2:C3 in 83% yield. In the work by Zhou and Chen towards the synthesis of *N*-arylidole **1.67**, diaryliodonium tetrafluoroborate **1.68** salts readily arylates the indoles in presence of copper, however, require very high temperature (140-150 °C) as shown in **Scheme 1.15c**.⁶⁴

Scheme 1.15 Reactions of Diaryliodonium Salts with Nucleophiles



Diaryliodonium salts have found abundant applications in the field of organic synthesis and been widely used in total synthesis of natural products.⁶⁵⁻⁶⁸ One of such examples is the work by Olofsson and group in the total synthesis of (-)-epibatidine.⁶⁵ They developed a direct asymmetric α -arylation of cyclohexanones **1.70** where Simpkin's base **1.71** resulted in the asymmetric enolization to desymmetrize the *para*-substituted cyclohexanones and the reaction of diaryliodonium salts yielded 2-aryl ketones **1.73** in excellent enantioselectivities and moderate yields (**Scheme 1.16**). The method was further elaborated to the total synthesis of (-)-epibatidine.

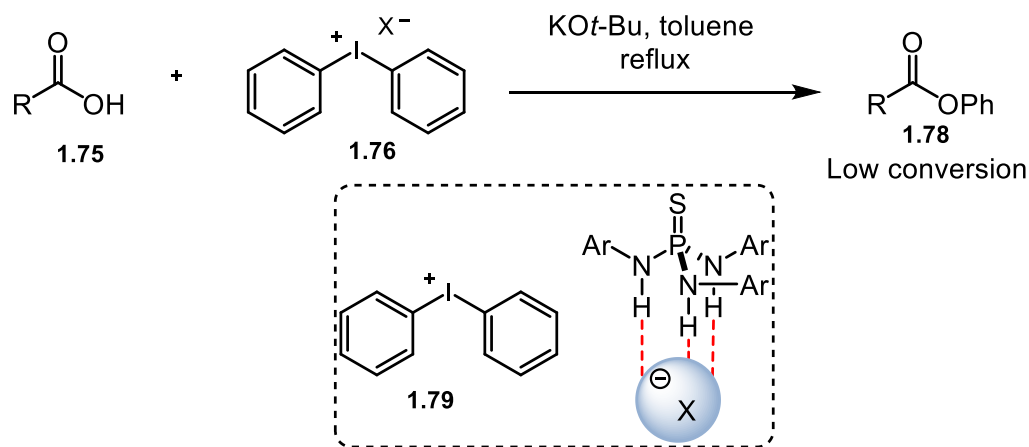
Scheme 1.16 Diaryliodonium Salt in Total Synthesis of (-)-epibatidine



The robustness of thiophosphoramides as well as their unique ability to accomplish counterion activation in comparison to other HBDs^{27,40,41} prompted us to investigate other types of reactions, in which counterion activation has not been accomplished with traditional HBDs.

In most of the examples of the arylation of nucleophiles with diaryliodonium salts, the reactions are general to the wide variety of nucleophile and delivers desired products in excellent yield making diaryliodonium salts as an attractive reagent for arylation, but unfortunately these reactions require very high temperature. Most of the examples require refluxing in solvents like toluene or DMF. We identified the need to further enhance the electrophilicity of diaryliodonium salts and this could potentially be achieved by ion pair separation by using H-bond donors as shown in **Figure 1.6**.

Figure 1.6. Proposed Ion Pair Separation by Three H-bond Donor Catalyst



The use of HBD-based catalysts can potentially enhance the reactions involving diaryliodonium salts and significantly expand the scope of the reaction conditions (i.e. solvents and diaryliodonium counterions) that could be used for the transformations involving these species. This chapter summarizes our studies illustrating that thiophosphoramidate based HBDs could significantly accelerate Cu(II)-catalyzed reactions of potassium carboxylates with diaryliodonium salts.

1.4.2. Initial Optimization

According to studies by Oloffson and coworkers,^{56,62} an uncatalyzed reaction of metal carboxylates and diaryliodonium salts require elevated temperatures and the yield is significantly retarded at 70 °C. To demonstrate that the addition of HBDs as a catalyst or co-catalyst could significantly accelerate this transformation at ambient temperature, we initiated the experimentations summarized in **Table 1.1**. The initial screening to identify the enhancement of rate using thiophosphoramidates was identified by colleague Jia Hui Tay. Thus, arylation of potassium benzoate with diphenyliodonium triflate was investigated. This reaction does not happen at room temperature or at 70 °C after 24 h (entries 1 and 2), and only 18% yield is observed when performed in presence of catalytic quantities of copper(II) trifluoromethanesulfonate (entry 3). The following evaluation of

HBDs **A-C** revealed that this reaction can indeed be accelerated if a HBD-based co-catalyst is used in combination with Cu(OTf)₂. Interestingly, among the evaluated HBDs, thiophosphoramidate **C** was found to outperform traditional two hydrogen bond donors such as Schreiner's thiourea **A**²² or squaramide **1.80**⁴² (Table 1, entries 4-6). In the following control experiments, reactions with **C** were conducted at room temperature and 70 °C in the absence of Cu(OTf)₂ (entries 7-8). These experiments clearly indicated that both the catalysts were important for the activation of diaryliodonium salts. The use of **C** alone resulted in the formation of product **1.82** in 36% yield. Based on this outcome, we postulated that **C** could activate diaryliodonium salts by complexing the counterions; however, this effect was weaker compared to the case where a dual catalytic system was used. Next, the solvent effects were explored (entries 9-13). Surprisingly, no product (**1.82**) was observed in the oxygen-containing polar solvents like DMF, diethyl ether, and THF either in the presence or absence of **C** (entries 9-11). This could be attributed to weaker hydrogen bonding due to the increased polarity and hydrogen bond accepting ability of these solvents. However, reaction yield in non-coordinating solvents such as methylene chloride were found to be comparable to when toluene was used as a solvent (entries 12-13).

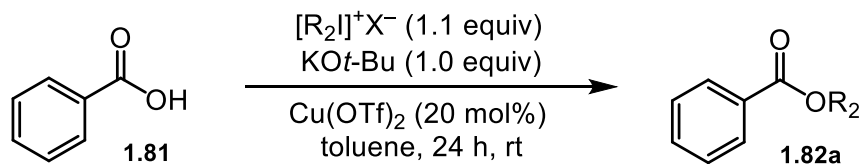
Table 1.1. Optimization of the Reaction Conditions

entry	catalyst	co-catalyst	solvent	T °C	time h	yield (%)
1	none	none	toluene	rt	24	0
2	none	none	toluene	70	24	0
3	Cu(OTf) ₂	none	toluene	rt	21	18
4	Cu(OTf) ₂	A	toluene	rt	24	38
5	Cu(OTf) ₂	1.80	toluene	rt	24	0
6	Cu(OTf) ₂	C	toluene	rt	21	82
7	none	C	toluene	rt	24	0
8	none	C	toluene	70	24	36
9	Cu(OTf) ₂	C or none	THF	rt	24	0
10	Cu(OTf) ₂	C or none	Et ₂ O	rt	24	0
11	Cu(OTf) ₂	C or none	DMF	rt	24	0
12	Cu(OTf) ₂	none	CH ₂ Cl ₂	rt	24	28
13	Cu(OTf) ₂	C	CH ₂ Cl ₂	rt	21	73

1.4.3. Investigation of the Effect of Diaryliodonium Salt Counterions

Having established the optimized conditions (**Table 1.1**, entry 6), we sought out to investigate the scope of the iodonium salts that could be activated in presence of the

combination of **C** and Cu(OTf)₂ (**Table 1.2**). First, the effect of the counterion was evaluated (entries 1–8). In theory, the diphenyliodonium salts containing non-coordinating counterions should have higher reactivities than diphenyliodonium triflate. However, the counterion activation of such salts would be more difficult due to their lower affinity to HBDs. To our knowledge, HBDs are typically not employed for the counterion activation of non-coordinating anions such as hexafluorophosphate and tetrafluoroborate through this mode. However, under the established reaction conditions both [Ph₂I]⁺PF₆⁻ and [Ph₂I]⁺BF₄⁻ provided synthetically useful yields in the presence of **C** and Cu(OTf)₂ (entries 3 and 5). As expected, no reaction was observed with these salts in the absence of the HBD-catalyst (entries 6 and 4). The reactivity of [Ph₂I]⁺OTs⁻ was lower in comparison with the salts containing less coordinating counterions due to the more coordinating nature of tosylate anion. No reaction was observed at room temperature with and without **C**/Cu(OTf)₂. However, when heated to 70° C in the presence of **C** and Cu(OTf)₂, [Ph₂I]⁺OTs⁻ provided the corresponding product in 55% yield. As before, no product was observed at this temperature in the absence of the catalyst even after 24 h (entry 8).

Table 1.2. Investigating Effect of the Diaryliodonium Salt Counterion

entry	$[R_2I]^+X^-$	co-catalyst (20 mol%)	yield of 1.82a (%)
1	$[Ph_2I]^+OTf^-$	C	82
2	$[Ph_2I]^+OTf^-$	–	18
3	$[Ph_2I]^+BF_4^-$	C	76
4	$[Ph_2I]^+BF_4^-$	–	0
5	$[Ph_2I]^+PF_6^-$	C	77
6	$[Ph_2I]^+PF_6^-$	–	0
7	$[Ph_2I]^+OTs^-$	C	55
8	$[Ph_2I]^+OTs^-$	–	0
9		C	84
10		C	67

Finally, in order to demonstrate that the catalytic reaction is not sensitive to the nature of aryl group, arylation with commercially available bis(4-tert-butylphenyl)iodonium triflate (entry 9) and bis(4-bromophenyl)iodonium triflate (entry 10) was investigated. As before, both of these arylations proceeded with good yields in the presence of **C**/ $Cu(OTf)_2$, but no reaction was observed in absence of the catalysts. These studies indicate that the

C/Cu(OTf)₂ based catalytic activation is less sensitive to the nature of diaryliodonium counterion than previously anticipated. However, this result is not entirely surprising considering that in all of the cases described above, trifluoromethanesulfonate anion was also present in the reaction medium as a counterion of Cu(II).

1.4.4. Investigation of the Effect of Copper Salt Counterions

Table 1.3. Investigating the Effect of the Copper Salt Counterion

$\text{Ph-C(=O)OH} \xrightarrow[\text{KOt-Bu (1.0 equiv), toluene, rt, 24h}]{[\text{Ph}_2\text{I}]^+\text{Y}^- (1.1 \text{ equiv}), \text{CuX}_2 (20 \text{ mol}\%)} \text{Ph-C(=O)OPh}$				
entry	[R ₂ I] ⁺ Y ⁻	CuX ₂	co-catalyst (20 mol%)	yield (%)
1	[Ph ₂ I] ⁺ OTf ⁻	Cu(OTf) ₂	C	82
2	[Ph ₂ I] ⁺ OTf ⁻	Cu(OTf) ₂	–	18
3	[Ph ₂ I] ⁺ OTf ⁻	Cu(BF ₄) ₂	C	51
4	[Ph ₂ I] ⁺ OTf ⁻	Cu(BF ₄) ₂	–	0
5	[Ph ₂ I] ⁺ BF ₄ ⁻	Cu(OTf) ₂	C	76
6	[Ph ₂ I] ⁺ BF ₄ ⁻	Cu(OTf) ₂	–	0
7	[Ph ₂ I] ⁺ BF ₄ ⁻	Cu(BF ₄) ₂	C	28
8	[Ph ₂ I] ⁺ BF ₄ ⁻	Cu(BF ₄) ₂	–	0

Another hypothesis for this reaction is that thiophosphoramidate catalyst **C** is likely to complex the trifluoromethanesulfonate anion and consequently activate the arylcopper intermediate bound to this anion.^{59–61} Therefore, the ability of **C** to co-catalyze the reaction should not only depend on the counterion of diaryliodonium salt, but also on the counterion of the Cu(II) catalyst. To validate this proposal, the experiments summarized in **Table 1.3**

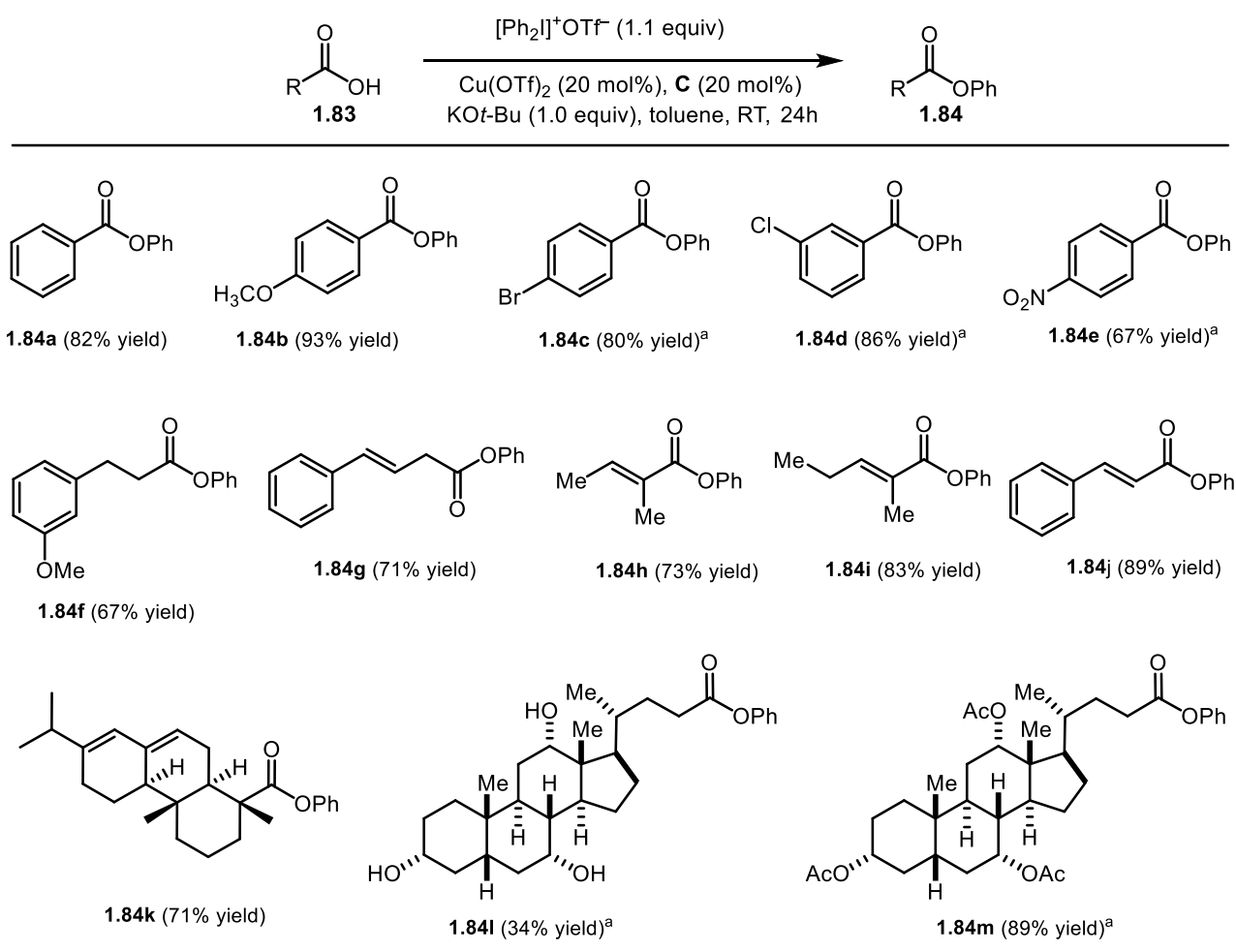
were conducted. In these studies, the counterions on both the Cu(II) catalyst and the diaryliodonium salt was replaced to be tetrafluoroborate instead of trifluoromethanesulfonate. In general, replacement of the more coordinating trifluoromethanesulfonate to a less coordinating tetrafluoroborate counterion resulted in the reduction of the yield. Although such a variation in the diaryliodonium counterion resulted in only a minor reduction in the yield (entry 5), the counterion of Cu(II) seems to play a more significant role. Thus, when Cu(II) tetrafluoroborate was used in combination with $[\text{Ph}_2\text{I}]^+\text{OTf}^-$, a 31% reduction in yield was observed (entry 3) and no reaction happened in the absence of **C** (entry 4). Following this trend, the reaction of $[\text{Ph}_2\text{I}]^+\text{BF}_4^-$ catalyzed by $\text{Cu}(\text{BF}_4)_2$ was significantly less efficient, and only 28% of **1.82** was isolated (entry 7). These results indicated that **C** plays a more important role in activating the organocopper intermediate than it plays in activating the diaryliodonium salt itself.

1.4.5. Substrate Scope

The scope of the nucleophile was investigated next (**Scheme 1.17**). Under the optimized conditions, phenylation of various carboxylates was accomplished in good to excellent yields. Introduction of the substituents onto the aromatic ring (entries **1.84a–1.84e**) was found to affect the reactivity of the carboxylate anion. As expected, reactions with less nucleophilic carboxylates (**1.84c–1.84e**) were significantly slower and required elevated temperatures (40 °C) to proceed to completion. Olofsson's group previously reported that under the catalyst free conditions in refluxing toluene the yields for **1.84d** and **1.84e** were found to be 65% and 59% respectively. However, using the catalytic conditions, we were able to accomplish the formation of **1.84d** and **1.84e** at 40 °C in 67% and 80% yield respectively.

This method was found to be equally effective for the arylation of α , β -unsaturated acids (entries **1.84f** – **1.84h**) and aliphatic acids (entries **1.84i** – **1.84m**). Importantly, the phenylation did not affect electrophile sensitive functionalities such as styrene (**1.84j**) and diene (**1.84k**). However, the presence of competing nucleophiles such as hydroxyl groups of the cholic acid (entry **1.84l**) resulted in a reduced yield. The acetylated cholic acid however, could be esterified in an excellent yield (entry **1.84m**).

Scheme 1.17. Substrate Scope

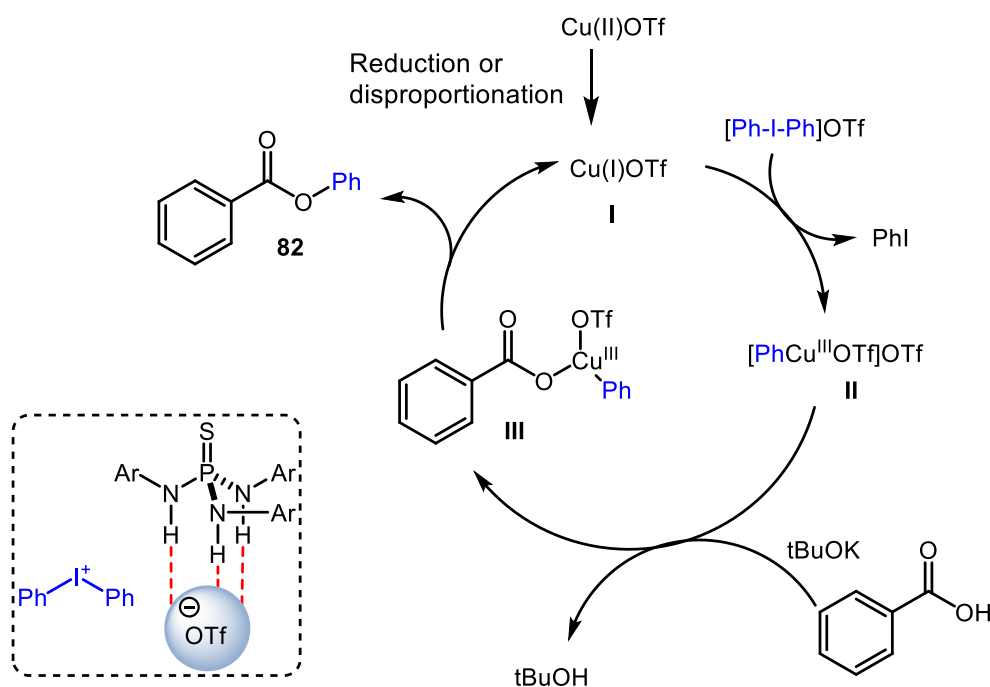


^a The reactions were performed at 40°C.

1.4.6. Proposed Mechanism

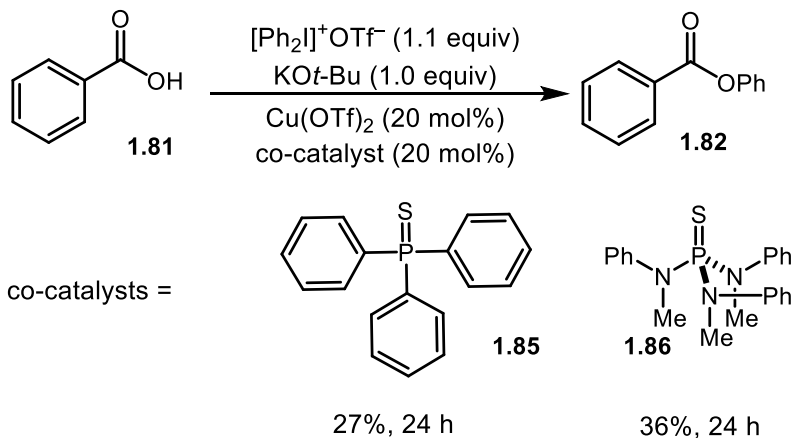
The proposed mechanism of this transformation is shown in **Scheme 1.18**. The addition of copper to the diaryliodonium salt forms an organo-copper intermediate **II**. The carboxylate, deprotonated by the base, would displace the triflate counterion to form organo-copper intermediate **III**, which is followed by reductive elimination to provide ester **1.82**, restoring the Cu catalyst **I**. Thiophosphoramidate could activate the counterion of either diaryliodonium salts directly or from organocopper intermediates.

Scheme 1.18. Proposed Mechanism



The sulfur atom in the thiophosphoramidate could also be involved in a complexation with Cu(I) and Cu(II) salts. While the fact that the observed acceleration effect could arise from such complexations cannot be excluded, our control experiments suggest that the presence of an anion-binding motif in thiophosphoramidate **C** is critical for obtaining **1.82** in high yields. Thus, when **C** is replaced with N,N,N -trimethyl- N',N',N' -triphenylphosphothioic triamide **1.86** lacking the $N\text{-H}$ bonds, **3a** was obtained in only 36% yield after 24 h and with triphenylphosphine sulfide **1.85** as co-catalyst provided 27% yield after 24 h (**Scheme 1.19**).

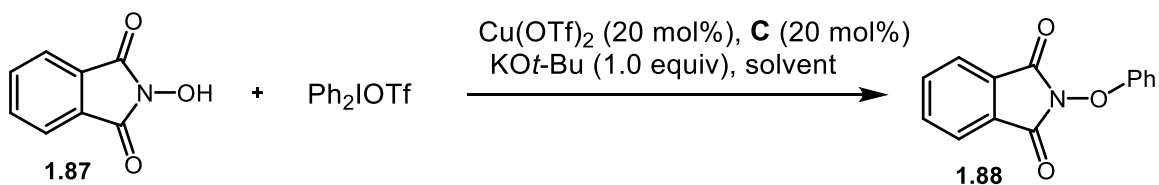
Scheme 1.19. Control Reaction with Co-catalyst **1.85** and **1.86**



1.5. Other Reactions

In theory, this approach can be extended to the arylation of other acidic functionalities. Thus, our attempts focused on the arylation of *N*-hydroxyphthalimide. Using the method that was previously developed for the arylation of carboxylates using diaryliodonium salts discussed above, etherification of *N*-hydroxyphthalimide **1.87** with diphenyliodonium salt was attempted (entry 5, **Scheme 1.20**). Significant acceleration of the reaction in presence of thiophosphoramidate was observed (entry 6 and 7) although the formation of inseparable and unidentifiable side product was formed. No traces of product was observed in absence of the ligand even when $\text{Cu}(\text{OTf})_2$ was present (entry 5 and 8). The further investigation was abandoned after the realization that the use of DMF as a solvent promotes the reaction without the $\text{Cu}(\text{OTf})_2$ or thiophosphoramidate (entry 1 and 2). The poor solubility of starting material in toluene and better solubility in DMF could be credited for the need of ligand when toluene was used as a solvent.

Scheme 1.20. Etherification of *N*-hydroxyphthalimide with Diphenyliodonium Salt



Entry	Solvent	Time	Temperature	Cu(OTf) ₂	Ligand (C)	Yield
1	DMF	2 h	rt	0	0	81%
2	DMF	2h	rt	0	20	79%
3	DMF	2h	0 °C	0	0	nd
4	DMF	2h	0 °C	0	20	slows down reaction
5	Toluene	overnight	rt	20	0	no reaction
6	Toluene	overnight	rt	20	20	57%(71% purity)
7	Toluene	5 h	60 °C	20	20	78%(72% purity)
8	Toluene	5 h	60 °C	20	0	No reaction

1.6. Conclusion

In conclusion, HBDs may act as synergistic co-catalysts of the Cu(II)-catalyzed arylation of potassium carboxylates with diaryliodonium salts. Thiophosphoramides were found to be significantly better HBDs than conventionally used thioureas or squaramides in their ability to accelerate the reaction. The data suggests that the HBD activation is accomplished by the counterion activation of the organocopper intermediate, however, further studies are required to clarify the exact origin of activation. The ability of thiophosphoramide to bind with the organocopper intermediate to increase the solubility and hence increase the reactivity couldn't be denied. This new catalytic variant of this transformation can be used for the arylation of various carboxylic acids including the substrates possessing electrophile-sensitive functionalities. The reaction is limited to the

solvent like toluene and methylene chloride that cannot participate in accepting H-bond donation of the thiophosphoramidate. We envision that these findings can be extended to the other transformations involving diaryliodonium salts.

1.7. Experimental (as it appears in Bhattarai, B.; Tay, J.H.; Nagorny, P. Thiophosphoramides as cooperative catalysts for copper-catalyzed arylation of carboxylates with diaryliodonium salts. *Chem. Commun.* **2015**, 51, 5398-5401)

General Information

Methods and Reagents:

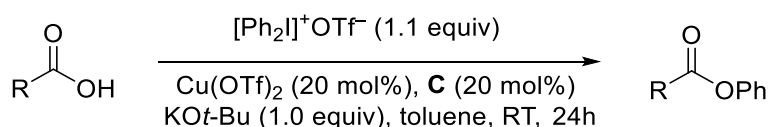
All the reagents were purchased from the commercial sources and used without further purification. All iodonium salts were purchased from TCI America. Abietic acid (technical, ~75% by GC) was purchased from Sigma Aldrich. All reactions were carried out under an atmosphere of nitrogen in flame or oven dried glassware with a magnetic stirrer. 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 purification and extraction were ACS or HPLC grade. toluene, dichloromethane, diethyl ether, tetrahydrofuran, and dimethylformamide were filtered through a column of activated alumina under nitrogen atmosphere (Innovative Technology PS-MD-5). Thin-layer chromatography (TLC) was conducted on precoated glass plates with 230-400 mesh silica gel impregnated with fluorescent indicator (250 nm) for routine monitor of reaction progress and visualized using the combination of UV and ceric ammonium molybdate. All products were purified by flash column chromatography using SiliCycle Silica Flash P60 (230-400 mesh) silica gel.

Instrumentation:

NMR spectra were recorded on Varian vnmrs 700 (700MHz), Varian vnmrs 500 (500 MHz), or Varian Inova 500 (500 MHz) spectrometers and chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane with solvent resonance as the internal standard (CDCl₃ at

δ 7.26, DMSO- d_6 at δ 2.5). Proton coupling patterns are described as singlet (s), doublet (d), triplet (t), quarted (q), and multiplet (m). High resolution mass spectra (HRMS) were obtained from Micromass AutoSpec Ultima or VG (Micromass) 70-250-S Magnetic Sector mass spectrometers at the University of Michigan mass spectrometry facility. Infrared spectra (IR) were recorded as thin films on NaCl plates on a Perkin Elmer Spectrum BX FT-IR spectrophotometer and were reported in wavenumbers (cm^{-1}).

Synthesis and spectra data of compounds **3**



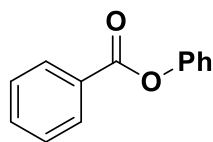
General Procedure:

Potassium *tert*-butoxide (22 mg, 0.2 mmol), Copper(II) trifluoromethanesulfonate (15 mg, 0.04 mmol), and benzoic acid (24 mg, 0.2 mmol) were added to an oven dried and nitrogen flushed 10 mL vial charged with 3 mL dry dichloromethane at rt and then left to stir for 10 minutes. Diaryliodonium salt (95 mg, 0.22 mmol) and **C** (30 mg, 0.04 mmol) were added in one portion and reaction was stirred for 24 h. The reaction was then quenched with H_2O . The product was extracted with diethyl ether and the organic layer was dried over Na_2SO_4 , filtered and concentrated *in vacuo*. Crude material was purified by flash chromatography to give corresponding ester.

Spectroscopic data of compounds **1.84a-m**

Phenyl benzoate (**1.84a**)

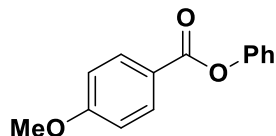
Purification by flash chromatography (99:1→9:1Hexanes: Et₂O) to afford the above compound



1.84a (33 mg, 84%) as white crystal. ¹H NMR (700 MHz, CDCl₃) δ 8.22 (d, *J* = 7.14 Hz, 2H), 7.65 (t, *J* = 7.5 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 2H), 7.44 (t, *J* =

7.9 Hz, 2H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.22 (d, *J* = 7.7 Hz, 2H); ¹³C NMR (175 MHz, CDCl₃) δ 165.34, 151.09, 133.73, 130.32, 129.72, 129.64, 128.71, 126.04, 121.86; IR (film, cm⁻¹): 2918, 1727, 1598, 1589, 1485, 1449, 1256, 1195, 1177, 1062, 1024; HRMS (ESI+) *m/z* calcd for C₁₃H₁₀O₂ [M+H]⁺ 199.0754, found 199.0750;

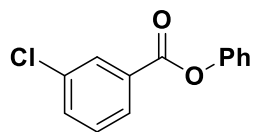
Phenyl 4-methoxybenzoate (**1.84b**)



Purification by flash chromatography (99:1→9:1Hexanes: Et₂O) to afford

the above compound **1.84b** (43 mg, 93%) as white crystal. ¹H NMR (700 MHz, CDCl₃) δ 8.16 (d, *J* = 8.75 Hz, 2H), 7.42 (t, *J* = 7.1 Hz, 2H), 7.26 (t, *J* = 7.46 Hz, 1H), 7.21 (d, *J* = 7.8 Hz, 2H), 6.99 (d, *J* = 8.82 Hz, 2H), 3.90 (s, 3H); ¹³C NMR (175 MHz, CDCl₃) δ 165.06, 164.01, 151.20, 132.43, 129.58, 125.86, 122.02, 121.95, 113.97, 55.67; IR (film, cm⁻¹): 2929, 1724, 1605, 1509, 1485, 1449, 1318, 1255, 1193, 1178, 1162, 1074, 1024; HRMS (ESI+) *m/z* calcd for C₁₄H₁₂O₃ [M+H]⁺ 229.0859, found 229.0868, calcd [M+Na]⁺ 251.0679, found 251.0672;

Phenyl 3-chlorobenzoate (**1.84c**)

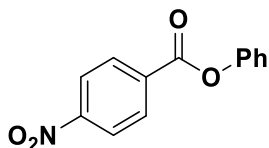


Purification by flash chromatography (95:5→8:2Hexanes: Et₂O) to afford the

above compound **1.84c** (40 mg, 86%) as white solid. ¹H NMR (700 MHz, CDCl₃) δ 8.20 (s, 1H), 8.10 (d, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 7.98 Hz, 1H), 7.49 – 7.42 (m, 3H), 7.30 (t, *J* = 7.5 Hz, 1H),

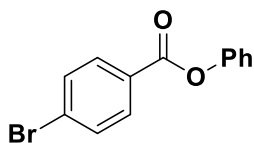
7.22 (d, $J = 8.0$ Hz, 2H); ^{13}C NMR (175 MHz, CDCl_3) δ 164.13, 150.84, 134.89, 133.74, 131.46, 130.32, 130.04, 129.71, 128.42, 126.25, 121.70; IR (film, cm^{-1}): 3068, 2922, 1732, 1590, 1484, 1248, 1195, 1075; HRMS (EI) m/z calcd for $\text{C}_{13}\text{H}_9\text{ClO}_2$ $[\text{M}]^+$ +232.0291, found 232.0292;

Phenyl 4-nitrobenzoate (1.84d)



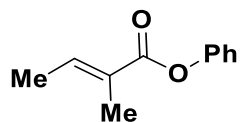
Purification by flash chromatography (9:1 \rightarrow 7:3Hexanes: Et_2O) to afford the above compound **1.84d** (32 mg, 67%) as yellow solid. ^1H NMR (700 MHz, CDCl_3) δ 8.38 (m, 4H), 7.47 (t, $J = 8.0$ Hz, 2H), 7.32 (t, $J = 7.5$ Hz, 1H), 7.24 (d, $J = 7.7$ Hz, 2H); ^{13}C NMR (175 MHz, CDCl_3) δ 163.46, 151.03, 150.63, 135.11, 131.44, 129.83, 126.56, 123.87, 121.55; IR (film, cm^{-1}): 2923, 1738, 1518, 1265, 1182, 1077; HRMS (EI) m/z calcd for $\text{C}_{13}\text{H}_9\text{NO}_4$ $[\text{M}]^+$ +243.0532, found 243.0535;

Phenyl 4-bromobenzoate (1.84e)



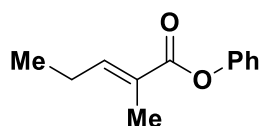
Purification by flash chromatography (95:5 \rightarrow 9:1Hexanes: Et_2O) to afford the above compound **1.84e** (44 mg, 80%) as white crystal. ^1H NMR (700 MHz, CDCl_3) δ 8.07 (d, $J = 8.61$ Hz, 2H), 7.66 (d, $J = 8.61$ Hz, 2H), 7.44 (t, $J = 7.56$ Hz, 2H), 7.29 (t, $J = 7.46$ Hz, 1H), 7.21 (d, $J = 7.56$ Hz, 2H); ^{13}C NMR (175 MHz, CDCl_3) δ 164.64, 150.89, 132.10, 131.80, 129.69, 128.98, 128.62, 126.20, 121.75; IR (film, cm^{-1}): 2922, 2852, 1728, 1583, 1484, 1394, 1265, 1192, 1174, 1155, 1074; HRMS (EI) m/z calcd for $\text{C}_{13}\text{H}_9\text{BrO}_2$ $[\text{M}]^+$ +275.9786, found 275.9789;

Phenyl (E)-2-methylbut-2-enoate (1.84f)



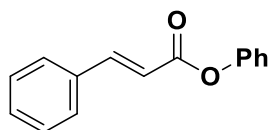
Purification by flash chromatography (99:1→9:1Hexanes: Et₂O) to afford the above compound **1.84f** (27 mg, 73%) as colorless liquid. ¹H NMR (500 MHz, CDCl₃) δ 7.38 (t, *J* = 7.9 Hz, 2H), 7.22 (t, *J* = 7.4 Hz, 1H), 7.15 – 7.08 (m, 3H), 1.96 (s, 3H), 1.89 (d, *J* = 7.1 Hz, 3H); ¹³C NMR (175 MHz, CDCl₃) δ 166.71, 151.28, 139.50, 129.49, 128.32, 125.66, 121.86, 14.78, 12.32; IR (film, cm⁻¹): 2926, 1722, 1650, 1592, 1494, 1485, 1388, 1258, 1242, 1194, 1161, 1110, 1056, 1001; HRMS (ESI) *m/z* calcd for C₁₁H₁₂O₂ [M+H]⁺ 177.0910, found 177.0906;

phenyl (E)-2-methylpent-2-enoate (**1.84g**)



Purification by flash chromatography (99:1→9:1Hexanes: Et₂O) to afford the above compound **1.84g** (31 mg, 83%) as yellow liquid. ¹H NMR (500 MHz, CDCl₃) δ 7.38 (t, *J* = 7.8 Hz, 2H), 7.22 (t, *J* = 7.1 Hz, 1H), 7.11 (d, *J* = 7.7 Hz, 2H), 7.01 (td, *J* = 7.4, 1.1 Hz, 1H), 2.28 (p, *J* = 7.5 Hz, 2H), 1.95 (s, 3H), 1.11 (t, *J* = 7.6 Hz, 3H); ¹³C NMR (175 MHz, CDCl₃) δ 166.87, 151.29, 146.22, 129.47, 126.80, 125.65, 121.86, 22.38, 13.11, 12.48; IR (film, cm⁻¹): 2965, 1725, 1646, 1593, 1487, 1231, 1195, 1162, 1126, 1089, 1066; HRMS (ESI) *m/z* calcd for C₁₂H₁₄O₂ [M+H]⁺ 191.1067, found 191.1067;

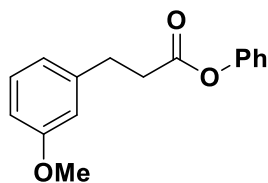
Phenyl cinnamate (**1.84h**)



Purification by flash chromatography (99:1→9:1Hexanes: Et₂O) to afford the above compound **1.84h** (39 mg, 89%) as white crystal. ¹H NMR (700 MHz, CDCl₃) δ 7.88 (d, *J* = 16.0 Hz, 1H), 7.62 – 7.57 (m, 2H), 7.46 – 7.39 (m, 5H), 7.28 – 7.24 (m, 1H), 7.18 (d, *J* = 7.6 Hz, 2H), 6.65 (d, *J* = 16.0 Hz, 1H); ¹³C NMR (175 MHz, CDCl₃) δ 165.54, 150.92, 146.71, 134.30,

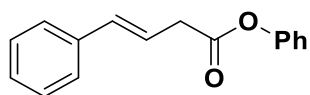
130.84, 129.58, 129.13, 128.44, 125.93, 121.77, 117.44; IR (film, cm^{-1}): 2923, 1724, 1635, 1483, 1305, 1202, 1139; HRMS (APCI+) m/z calcd for $\text{C}_{15}\text{H}_{12}\text{O}_2$ $[\text{M}+\text{H}]^+$ +225.0910, found 225.0899;

Phenyl 3-(3-methoxyphenyl)propanoate (**1.84i**)



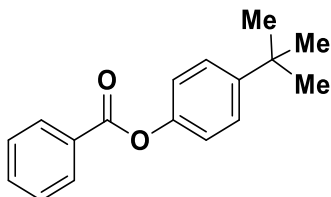
Purification by flash chromatography (95:5→9:1 Hexanes: Et_2O) to afford the above compound **1.84i** (34 mg, 67%) as colorless liquid. ^1H NMR (700 MHz, CDCl_3) δ 7.37 (t, $J = 7.56$ Hz, 2H), 7.27 – 7.21 (m, 2H), 7.04 (d, $J = 7.56$ Hz, 2H), 6.87 (d, $J = 7.56$ Hz, 1H), 6.82 (s, 1H), 6.79 (dd, $J = 8.2, 2.4$ Hz, 1H), 3.81 (s, 3H), 3.06 (t, $J = 7.8$ Hz, 2H), 2.89 (t, $J = 7.8$ Hz, 2H); ^{13}C NMR (175 MHz, CDCl_3) δ 171.52, 159.89, 150.76, 141.86, 129.72, 129.54, 125.94, 121.66, 120.84, 114.26, 111.93, 55.31, 36.06, 31.13; IR (film, cm^{-1}): 2940, 1755, 1593, 1584, 1491, 1454, 1259, 1192, 1161, 1126, 1041; HRMS (APCI+) m/z calcd for $\text{C}_{16}\text{H}_{16}\text{O}_3$ $[\text{M}+\text{H}]^+$ +257.1172, found 257.1172;

Phenyl (E)-4-phenylbut-3-enoate (**1.84j**)



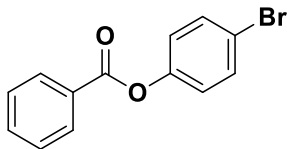
Purification by flash chromatography (99:1→9:1 Hexanes: Et_2O) to afford the above compound **1.84j** (35 mg, 71%) as off-white crystal. ^1H NMR (700 MHz, CDCl_3) δ 7.43 – 7.36 (m, 4H), 7.33 (t, $J = 7.7$ Hz, 2H), 7.27 – 7.22 (m, 2H), 7.11 (d, $J = 7.7$ Hz, 2H), 6.61 (d, $J = 15.9$ Hz, 1H), 6.41 (dt, $J = 15.7, 7.1$ Hz, 1H), 3.50 (d, $J = 7.07$ Hz, 2H); ^{13}C NMR (175 MHz, CDCl_3) δ 170.18, 150.80, 136.84, 134.21, 129.59, 128.74, 127.87, 126.50, 126.06, 121.65, 121.13, 38.60; IR (film, cm^{-1}): 3034, 1742, 1591, 1492, 1364, 1201, 1133; HRMS (APCI+) m/z calcd for $\text{C}_{16}\text{H}_{14}\text{O}_2$ $[\text{M}+\text{H}]^+$ + 239.1067, found 239.1060;

4-(tert-butyl)phenyl benzoate (SI-1)



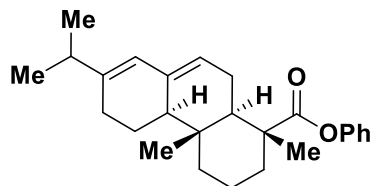
Purification by flash chromatography (99:1→9:1Hexanes: Et₂O) to afford the above compound (42 mg, 84%) as white crystal. ¹H NMR (700 MHz, CDCl₃) δ 8.21 (d, *J* = 7.1 Hz, 2H), 7.64 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.7 Hz, 2H), 7.45 (d, *J* = 8.8 Hz, 2H), 7.15 (d, *J* = 8.8 Hz, 2H), 1.35 (s, 9H); ¹³C NMR (175 MHz, CDCl₃) δ 165.49, 148.83, 148.71, 133.64, 130.29, 129.83, 128.67, 126.53, 121.13, 34.66, 31.58; IR (film, cm⁻¹): 2964, 1730, 1508, 1262, 1203, 1170, 1076, 1060, 1021; HRMS (ESI+) *m/z* calcd for C₁₇H₁₈O₂ [M+H]⁺255.1380, found 255.1378;

4-bromophenyl benzoate (SI-2)



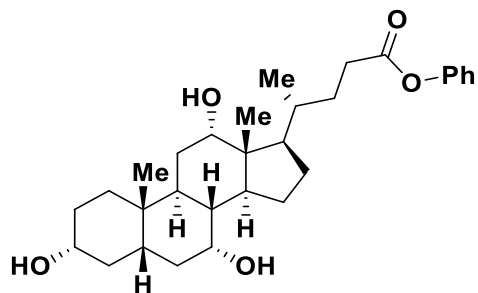
Purification by flash chromatography (99:1→9:1Hexanes: Et₂O) to afford the above compound (38 mg, 70%) as white crystal. ¹H NMR (700 MHz, CDCl₃): δ 8.19 (d, *J* = 7.1 Hz, 2H), 7.65 (t, *J* = 7.5 Hz, 1H), 7.57 – 7.50 (m, 4H), 7.12 (d, *J* = 8.82, 2H); ¹³C NMR (175 MHz, CDCl₃) δ 165.01, 150.10, 133.95, 132.68, 130.35, 129.30, 128.78, 123.69, 119.13; IR (film, cm⁻¹): 2923, 1730, 1482, 1259, 1198, 1160, 1057; HRMS (EI) *m/z* calcd for C₁₃H₉BrO₂ [M]⁺275.9786, found 275.9787;

Phenyl (1R,4aR,4bR,10aR)-7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthrene-1-carboxylate (1.84k)



Potassium *tert*-butoxide (22 mg, 0.2 mmol), copper(II) trifluoromethanesulfonate (15 mg, 0.04 mmol), and abietic acid (60 mg of 75% abietic acid, 0.15 mmol) were added to an oven dried and nitrogen flushed 10 mL vial charged with 3 mL dry dichloromethane at rt and then left to stir for 10 minutes. Diaryliodonium salt (95 mg, 0.22 mmol) and **C** (30 mg, 0.04 mmol) were added in one portion and reaction was stirred for 24 h at 40°C. The reaction was then quenched with H₂O. The product was extracted with diethyl ether and the organic layer was dried over Na₂SO₄, filtered and concentrated. Purification by flash chromatography (95:5→9:1 Hexanes: EtOAc) to afford the above compound **1.84k** (40 mg, 71%) as yellow viscous liquid. Some impurities from the starting material (75% abietic acid) was also observed in the product and was inseparable by accessible purification process. ¹H NMR (700 MHz, CDCl₃) δ 7.36 (t, *J* = 7.8 Hz, 2H), 7.21 (t, *J* = 7.3 Hz, 1H), 7.01 (d, *J* = 7.9 Hz, 2H), 5.81 (s, 1H), 5.43 (s, 1H), 2.31 - 2.27 (m, 1H), 2.26 – 2.21 (m, 1H), 2.11 (d, *J* = 2.7 Hz, 2H), 2.03 – 1.99 (m, 2H), 1.94 (d, *J* = 13.1 Hz, 1H), 1.86 – 1.82 (m, 2H), 1.70 – 1.63 (m, 2H), 1.39 (s, 3H), 1.29 – 1.19 (m, 4H), 1.03 (t, *J* = 6.7 Hz, 6H), 0.89 (s, 3H); ¹³C NMR (175 MHz, CDCl₃) δ 177.26, 151.30, 145.62, 135.71, 129.45, 125.68, 122.46, 121.67, 120.57, 51.03, 46.99, 45.20, 38.42, 37.20, 35.04, 34.74, 27.59, 25.92, 22.62, 21.55, 20.97, 18.29, 17.27, 14.20; IR (film, cm⁻¹): 2928, 1743, 1594, 1492, 1458, 1385, 12766, 1227, 1190, 1162, 1129, 1101, 1025, 1000; HRMS (ESI+) *m/z* calcd for C₂₆H₃₄O₂ [M+H]⁺ 379.2632, found 379.2635;

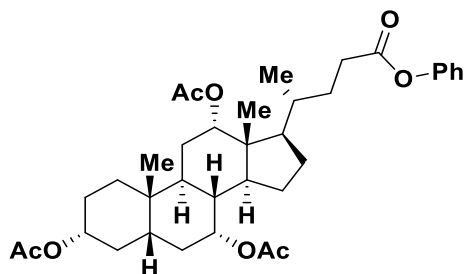
Phenyl (R)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (**1.84l**)



Purification by flash chromatography (1:1→0:1Hexanes:

EtOAc) to afford the above compound **1.84l** (33 mg, 34%) as light-yellow solid. ¹H NMR (700 MHz, CDCl₃) δ 7.37 (t, *J* = 7.9 Hz, 2H), 7.22 (t, *J* = 7.4 Hz, 1H), 7.07 (d, *J* = 7.7 Hz, 2H), 4.00 (s, 1H), 3.85 (s, *J* = 2.5 Hz, 1H), 3.48 (s, 1H), 3.47 – 3.43 (m, 1H), 2.64 – 2.59 (m, 1H), 2.52 – 2.48 (m, 1H), 2.26 – 2.18 (m, 1H), 1.98 – 1.88 (m, 5H), 1.85 – 1.81 (m, 1H), 1.78 (d, *J* = 14.4 Hz, 1H), 1.75 – 1.66 (m, 4H), 1.63 – 1.59 (m, 1H), 1.58 – 1.46 (m, 6H), 1.40 (d, *J* = 12.1 Hz, 2H), 1.37 – 1.32 (m, 1H), 1.14 (dd, *J* = 12.2, 6.1 Hz, 1H), 1.05 (d, *J* = 6.1 Hz, 3H), 1.01 – 0.96 (m, 1H), 0.89 (s, 3H), 0.71 (s, 3H); ¹³C NMR (175 MHz, CDCl₃) δ 172.86, 150.91, 129.53, 125.84, 121.70, 73.15, 72.08, 68.57, 47.28, 46.67, 42.04, 41.58, 39.80, 39.71, 35.36, 35.34, 34.86, 34.77, 31.53, 31.01, 30.64, 28.47, 27.65, 26.73, 23.35, 22.66, 17.53, 12.70; IR (film, cm⁻¹): 3392, 2924, 1755, 595, 1456, 1376, 1259, 1234, 1194, 1076, 1036; HRMS (ESI+) *m/z* calcd for C₃₀H₄₄O₅ [M+NH₄]⁺ 502.3527, found 502.3526;

(3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-10,13-dimethyl-17-((R)-5-oxo-5-phenoxypentan-2-yl)hexadecahydro-1H-cyclopenta[a]phenanthrene-3,7,12-triyl triacetate (1.84m)

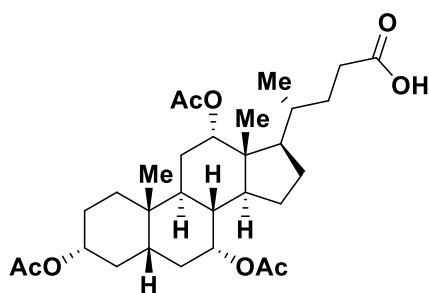


Purification by flash chromatography (9:1→7:3 Hexanes:

EtOAc) to afford the above compound **1.84m** (108 mg, 89%) as colorless solid. ¹H NMR (700

MHz, CDCl₃) δ(t, *J* = 7.98, 2H), 7.22 (t, *J* = 7.4 Hz, 1H), 7.06 (d, *J* = 7.6 Hz, 2H), 5.11 (s, 1H), 4.91 (s, 1H), 4.59 – 4.57 (m, 1H), 2.60 (ddd, *J* = 15.0, 9.6, 5.1 Hz, 1H), 2.47 (ddd, *J* = 15.8, 9.1, 7.0 Hz, 1H), 2.15 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.98 – 1.91 (m, 3H), 1.90 – 1.87 (m, 1H), 1.81 – 1.74 (m, 2H), 1.70 (d, *J* = 5.6 Hz, 1H), 1.68 (d, *J* = 13.1 Hz, 1H), 1.65 – 1.62 (m, 1H), 1.61 – 1.58 (m, 2H), 1.53 – 1.48 (m, 3H), 1.46 – 1.41 (m, 2H), 1.38 – 1.32 (m, 1H), 1.30 – 1.22 (m, 3H), 1.16 – 1.11 (m, 1H), 1.07 (td, *J* = 14.4, 3.3 Hz, 1H), 0.92 (s, 3H), 0.89 (d, *J* = 6.4 Hz, 3H), 0.75 (s, 3H); ¹³C NMR (175 MHz, CDCl₃) δ172.52, 170.48, 170.32, 150.65, 129.38, 125.73, 121.48, 77.25, 77.06, 76.88, 75.35, 74.05, 70.65, 47.36, 45.07, 43.39, 40.90, 37.70, 34.66, 34.61, 34.59, 34.31, 31.21, 31.19, 30.69, 29.66, 28.86, 27.22, 26.86, 25.56, 22.79, 22.54, 21.61, 21.48, 21.43, 17.54, 12.23; IR (film, cm⁻¹): 2922, 1727, 1446, 1379, 1239, 1199, 1136, 1022; HRMS (EI) *m/z* calcd for C₃₆H₅₀O₈ [M+NH₄]⁺ 628.3844, found 628.3848;

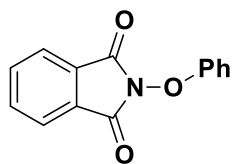
(R)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-triacetoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid (SI-3)



To an ice cooled solution of cholic acid (1 gm; 2.45 mmol) in pyridine (3 mL) and acetic anhydride (2 mL), DMAP (180 mg, 1.47 mmol) was added. The reaction mixture was stirred at rt for 3 h. The solution was concentrated *in vacuo*, dissolved in 50 mL diethyl ether, washed with 0.1 M HCl, NaHCO₃, and brine. The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo* to give the crude product. Purification *via* flash chromatography (8:2→1:1Hexanes: EtOAc) to afford the above compound (831 mg, 63%) as

colorless solid. ^1H NMR (700 MHz, CDCl_3) δ 5.09 (s, 1H), 4.91 (s, 1H), 4.59 – 4.56 (m, 1H), 2.42 – 2.36 (m, 1H), 2.27 – 2.22 (m, 1H), 2.14 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.04 – 1.99 (m, 1H), 1.97 – 1.93 (m, 1H), 1.90 – 1.84 (m, 2H), 1.82 – 1.72 (m, 3H), 1.68 – 1.64 (m, 2H), 1.63 – 1.59 (m, 2H), 1.54 – 1.48 (m, 2H), 1.45 – 1.41 (m, 2H), 1.34 – 1.25 (m, 4H), 1.14 – 1.08 (m, 1H), 1.08 – 1.03 (m, 1H), 0.92 (s, 3H), 0.87 (dt, $J = 14.4, 7.0$ Hz, 2H), 0.83 (d, $J = 6.6$ Hz, 3H), 0.73 (s, 3H); ^{13}C NMR (175 MHz, CDCl_3) δ 179.36, 170.58, 170.54, 170.42, 75.37, 74.08, 70.70, 47.31, 45.04, 43.37, 40.89, 37.71, 34.58, 34.52, 34.30, 31.56, 31.21, 30.75, 30.51, 28.86, 27.14, 26.86, 25.55, 22.63, 22.53, 21.60, 21.47, 17.46, 14.10, 12.21; IR (film, cm^{-1}): 2936, 2013, 1730, 1441, 1374, 1230, 1023; HRMS (EI) m/z calcd for $\text{C}_{30}\text{H}_{46}\text{O}_8$ $[\text{M}+\text{NH}_4]^+$ 552.3531, found 552.3530; Calcd $[\text{M}+\text{Na}]^+$ 557.3085, found 557.3081;

2-phenoxyisoindoline-1,3-dione (1.88)



Potassium *tert*-butoxide (25 mg, 0.2 mmol), copper(II)

trifluoromethanesulfonate (15 mg, 0.04 mmol), and phthalimide (33 mg, 0.15 mmol) were added to an oven dried and nitrogen flushed 10 mL vial charged with 1 mL dry toluene at rt and then left to stir for 10 minutes. The bloody red color of the reaction mixture was observed. Diaryliodonium salt (95 mg, 0.22 mmol) and **C** (30 mg, 0.04 mmol) were added in one portion and reaction was stirred for 24 h at 60 °C. The reaction was then quenched with H_2O . The product was extracted with diethyl ether and the organic layer was dried over Na_2SO_4 , filtered and concentrated. Purification by flash chromatography (95:5→7:3 Hexanes: EtOAc) to afford the above compound **1.88** (29 mg, 78%) as white solid. Some impurities were observed in small quantity and was

inseparable by accessible purification process. ^1H NMR (400 MHz, CDCl_3) δ 7.91 (dd, $J = 5.5$, 3.1 Hz, 2H), 7.80 (dd, $J = 5.5$, 3.1 Hz, 2H), 7.37 – 7.29 (m, 2H), 7.19 – 7.07 (m, 3H).

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Chapter 2

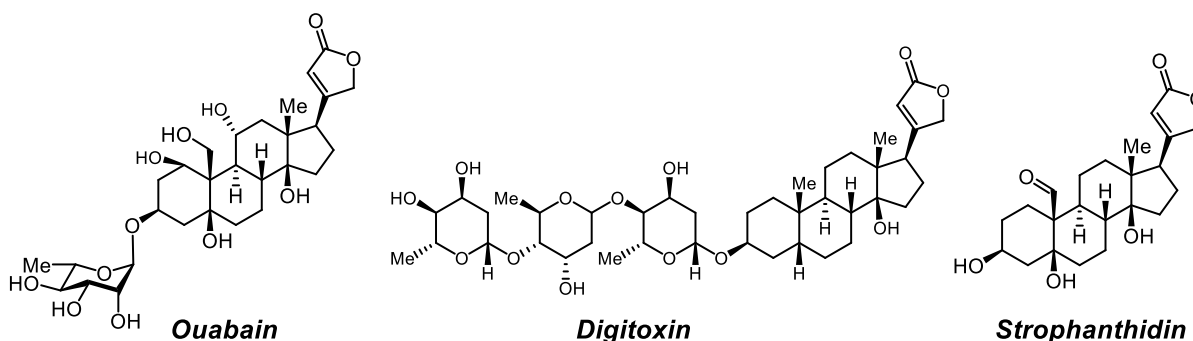
Cardiac Steroids: Introduction and Previous Synthesis

(A part of this work has been published in Bhattarai, B.; Nagorny, P. Enantioselective Total Synthesis of Cannogenol-3-O- α -L-rhamnoside via Sequential Cu(II)-Catalyzed Michael Addition/Intramolecular Aldol Cyclization Reactions. *Org. Lett.* **2018**, 20, 154-157)

2.1. Introduction

Cardiac steroids like *ouabain* and *digitoxin* (**Figure 2.1**) were traditionally used in ancient China and a big part of Africa to treat congestive heart failure.¹ They are also known to be effective antiarrhythmic agents. These steroids inhibit the Na⁺/K⁺-ATPase pump, which elevates the intracellular calcium intake and improves the cardiac contractility to counteract congestive heart failure.² Cardiac glycoside-based compounds are currently in different phases of clinical trials for various ailments such as cancer and hypertension. Some of these steroids include rostafuroxin,³ bufalin,⁴ and UNBS 1450 (**Figure 2.2**).⁵

Figure 2.1. Examples of Common Cardiotonic Steroids.

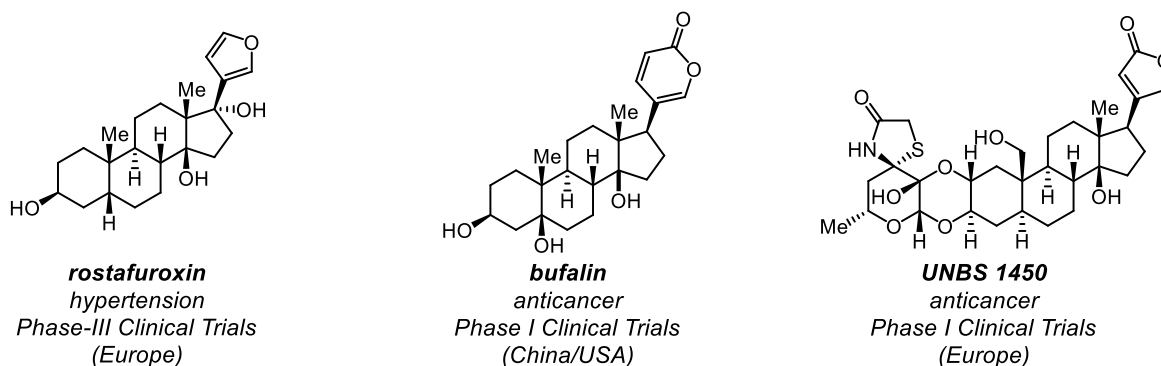


In addition, *strophanthidin* and *ouabain* (**Figure 2.1**) have recently been identified as potent inhibitors of DNA double-strand break repair in a study investigating homologous recombination (HR) and non-homologous end joining (NHEJ) by Herzon and co-workers.⁶ This new development

has prompted several in vivo and in vitro studies. As a result, these cardiac glycoside-based compounds are now on clinical trials for several other human diseases.

The therapeutic index, the comparison of amount of reagents to show therapeutic effect to the amount that leads to toxicity, of these cardiac steroids are narrow and sometimes there is an overlap of a dose required to treat the failing heart with one that can show toxic symptoms.⁷ This has been one of the limiting factor in the development of these compounds as drugs. One way to tackle this unfortunate limitation would be to test activity of chemical entities with similar scaffolds. Hence, there is an urgent need to utilize the tools of organic synthesis to develop methods to access the derivatives of these steroids in quest of less toxic clinical candidates with retained or improved activity.

Figure 2.2. Representative Cardiac Steroids and Analogs in Different Phases of Clinical Trials.



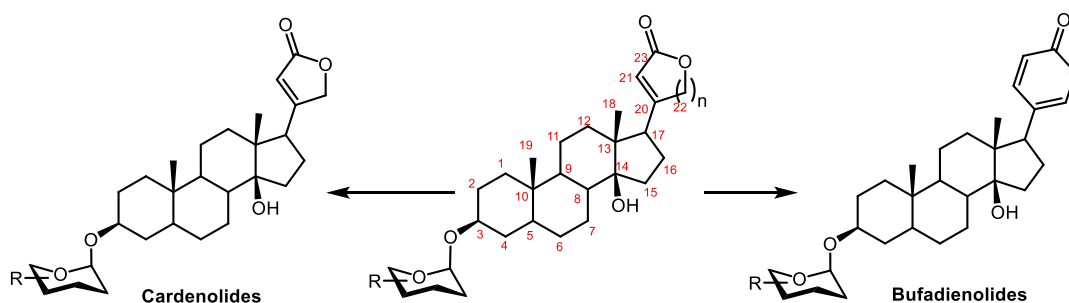
Unfortunately, most of the compounds of this class are found in indigenous plants and animals in very low concentration, and are not easily accessible for extraction.^{8,9} In recent years, the interest in developing the synthetic strategies to cardiotonic steroids has been rising, with more focus on the members with higher degree of oxygenation.¹⁰⁻¹² These approaches either require multiple steps to access the chiral building blocks^{11,12} prior to the construction of steroid framework or rely on semisynthetic approaches^{10,13} which require downstream redox manipulations at key positions and often are limited to the access of diverse stereochemistry at ring junctions. Therefore, a

modular approach, which utilizes simple achiral building blocks and quickly constructs the oxygenated core in a divergent manner, would highly improve the accessibility of various natural and unnatural cardiotonic steroids and significantly increases the scope of the subsequent biological activity evaluation studies.

2.2. Class of Cardiotonic Steroids Based on Size of Lactone Ring

Cardiotonic steroids can be broadly divided into two subclasses based on the heterocycle moiety at C17 (**Figure 2.3**). Cardenolides are mostly extracted from plant extracts and contain 5-membered unsaturated gamma-butyrolactone, also called butenolide at C-17. Bufadienolides, on the other hand, are extracted from animal sources, commonly from toads of genus *Bufo* and contain 6-membered, double unsaturated, delta valerolactone or 2-pyrone. This difference in substituent at the C17 site results in distinct biological activities. Natural products and their analogs with different heterocycles in place of butenolide and 2-pyrone have been studied and have shown stimulating results in variety of biological activities.

Figure 2.3. General Structural Characteristics and Numbering of Cardiac Steroids



Glucose, rhamnose, digitoxose, and mannose are common natural sugars that are attached with the steroid aglycone, although a wide variety of other monosaccharides, disaccharides, and trisaccharide sugar moiety are prevalent. Despite no known distinct role of these sugar moieties towards biological activity, the attachment of these groups in the cardiac steroids has shown a significant difference in their pharmacokinetic and pharmacodynamic profile. In several cases,

changing the identity of the sugar moiety changes the potency of the compound. For instance, mannoside has no significant effect on the potency, however, introduction of a rhamnoside is shown to increase the potency of these steroids by several folds.¹⁴

2.3. Previous Synthesis of Cardiotonic Steroids

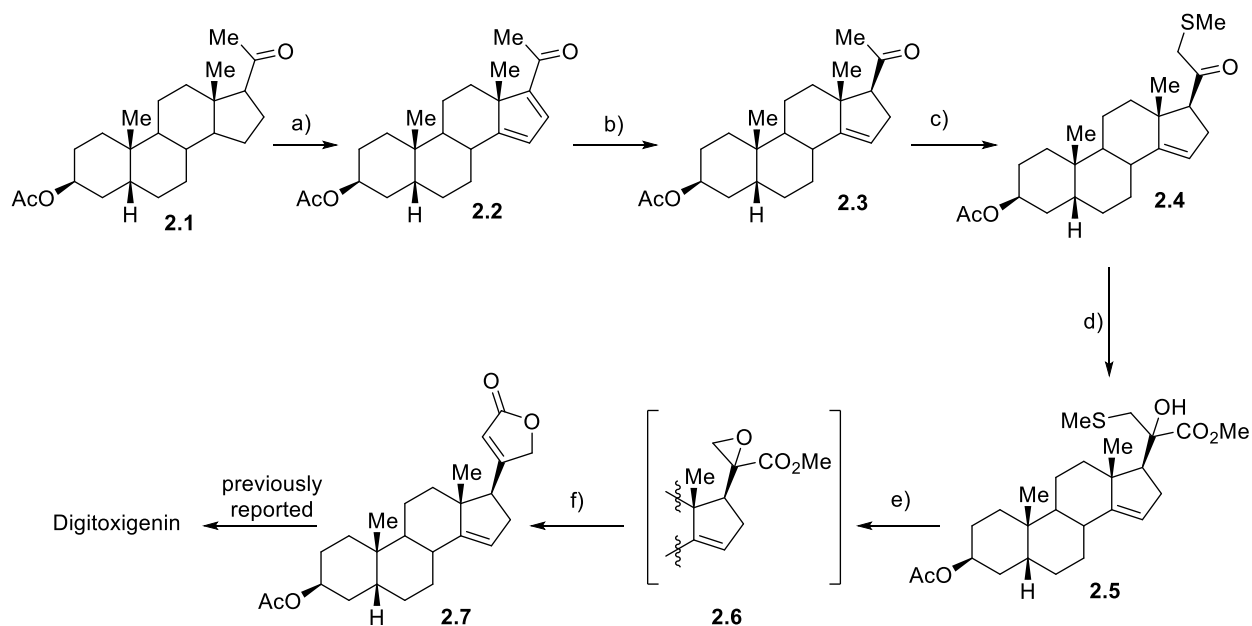
The biological activities of cardiotonic steroids are well studied and thus, several attempts have been made towards the synthesis of these molecules. Due to its biological activity and relatively simple structure, digitoxigenin has been one of the most common target, the synthesis of which was achieved by Sondheimer,¹⁵ Yoshii,¹³ and Wiesner¹⁶ through semi-synthetic efforts. Later, Stork¹⁷ (first enantioselective) and Nakada¹⁸ completed the total synthesis of digitoxigenin. One of the most challenging molecules to synthesize in this class is ouabain; it contains numerous stereocenters and is highly oxygenated. The first total synthesis of this molecule was completed by Deslongchamps using polyanionic cyclization strategy.^{12,19} In 2013, the Baran group used a strategic redox- and stereochemical-relay approach to complete a more practical synthesis of ouabagenin, the aglycone of ouabain, albeit through a semi-synthetic route from the readily available cortisone acetate.^{10,20} Other notable total syntheses in this class of natural product include the synthesis of rhodexin based on an inverse-electron-demand-Diels-Alder reaction by Jung and Yoo,²¹ and an acetal formation/intramolecular radical coupling strategy to 19-hydroxysermentogenin by Inoue¹¹ and more recently by Nagorny.²²

2.3.1. Semi-synthesis of Digitoxigenin by Yoshii and Coworkers¹³

The early synthesis of digitoxigenin marks as an important precedence on the synthesis of cardiotonic steroids. Pregnan-3 β -ol-20-one acetate **2.1** is used as a commercial starting material in several synthesis of cardiotonic steroids^{13,23,24} including Yoshii's semi-synthesis of digitoxigenin.¹³ Compound **2.1** was provided by the allylic bromination of starting material with

NBS or bromine to obtain dibrominated compound which is then dehydrobrominated to obtain extended enone **2.2**. Selective reduction of Δ^{16-17} olefin in presence of triethylsilane or triphenylstannane provided **2.3** which underwent subsequent α -methylsulfenylation of **2.3** to obtain **2.4**. Refluxing **2.4** with activated zinc dust and methyl bromo-acetate in benzene gave Reformatsky product **2.5** that was methylated with trimethyloxonium tetrafluoroborate and exposed to aqueous NaOH to provide epoxy ester **2.6** which lactonized upon passing through alumina chromatography to give compound **2.7**. The method to obtain digitoxigenin from **2.7** via formation of bromohydrin at C14-C15 was previously reported²⁵ and hence this completed the formal synthesis of digitoxigenin.

Scheme 2.1. Semi-synthesis of Digitoxigenin by Yoshii

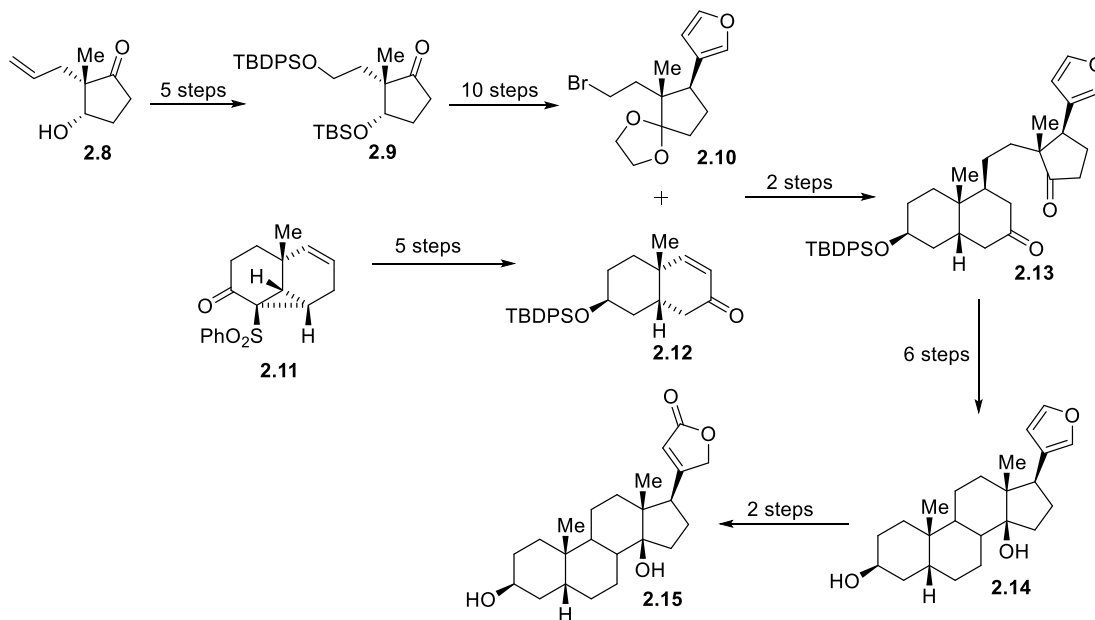


- a) AcOH, Br₂, rt, then, LiBr, DMF, 95 °C, 3h; b) Triphenylstannane, toluene, 140 °C, 4h; c) Diethyl oxalate, NaOMe, benzene, rt then, CH₃COOK, Methyl thiosylate, 50 °C, 3.5h; d) Methylthiomethyl ketone, Zn dust, methyl bromoacetate, benzene, rt; e) Nitromethane, Me₃⁺OBF₄⁻, 0.5h, rt; f) 0.5 N NaOH, DCM, 1h

2.3.2. Enantioselective Total Synthesis of (+)-digitoxigenin by Nakada and Coworkers¹⁸

The enantioselective total synthesis of (+)-digitoxigenin completed by Nakada and coworkers is summarized below. Compound **2.8** was obtained from reduction mediated by baker's yeast of the 1,3-cyclopentanedione derivative of **2.8** and used as the starting material. Compound **2.12** was obtained by Pd-catalyzed Suzuki-Miyaura cross coupling reaction followed by diastereoselective hydrogenation directed by the hydroxyl group. Compound **2.12** was obtained from tricyclo[4.4.0.0]decene **2.11**, the enantioselective synthesis of which was previously reported using CuOTf -BOX ligand.²⁶ Coupling of **2.10** and **2.12** provided diketone **2.13**. Intramolecular aldol reaction followed by deoxygenation at C7 provided advanced furan intermediate **2.14** that was then oxygenated to obtain digitoxigenin as a single enantiomer.

Scheme 2.2. Enantioselective Total Synthesis of (+)-digitoxigenin by Nakada



As discussed earlier, currently available synthetic methods are designed mostly to synthesize cardiotonic steroids containing the C19 and C11 oxygenation, with multiple semi and total synthesis of ouabain,^{19,20} 19-hydroxysermentogenin,^{11,22} and trewianin aglycone appeared in the recent decade.²² However, efforts towards the synthesis of the steroids of this class that contains oxygenation at the C19 but lacks oxygenation at the C11 are rare. In fact, strophanthidin **2.34** is

the only such molecule that has been synthesized despite these compounds showing equal or better potential towards a variety of biological activities.

2.3.3. *Semi-synthesis of Strophanthidin by Yoshii and Coworkers*

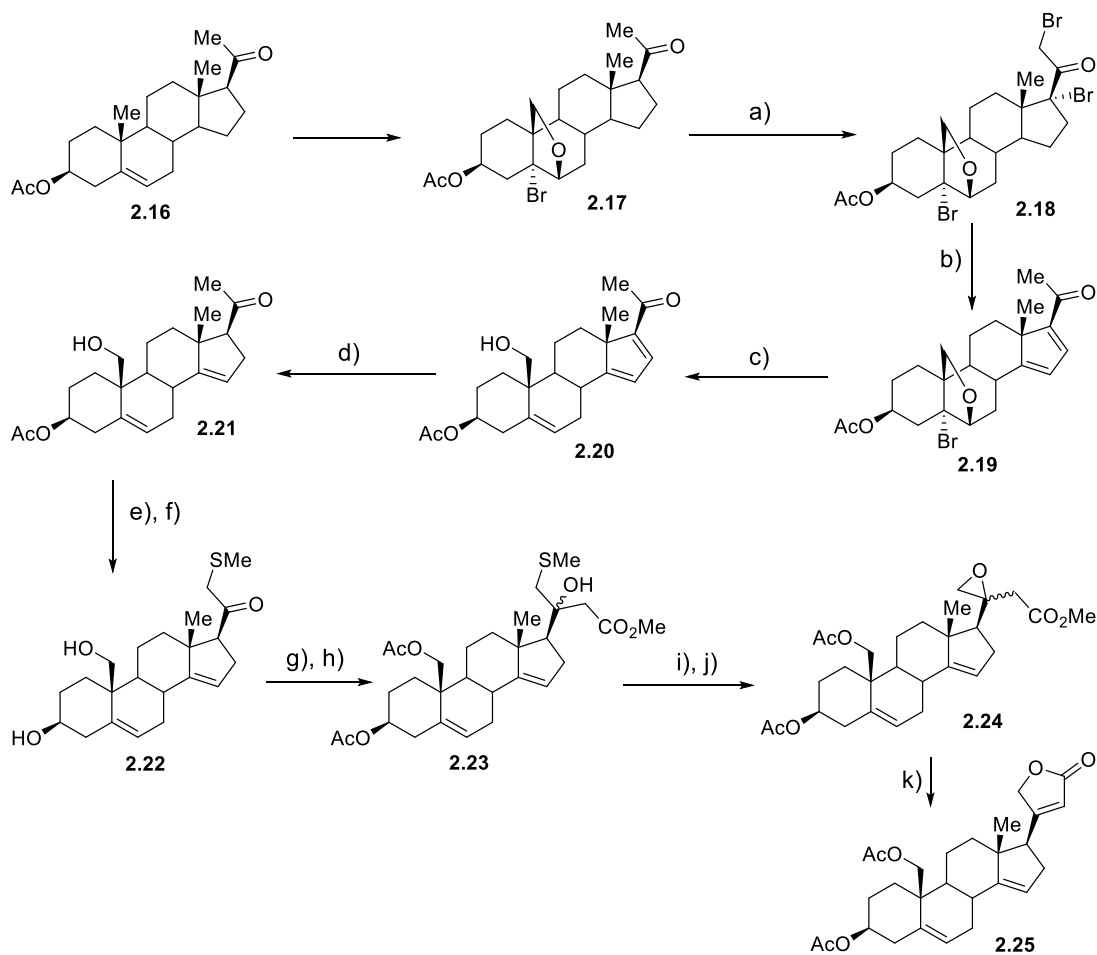
The synthesis of strophanthidin was first completed by Yoshii in 1978²³ and is described below. The semi-synthesis commenced with the readily available steroid pregnenolone acetate **2.16** and it was based on the following stages: 1) installation of C19-hydroxide and formation of double bond at Δ^{14-15} , 2) installation of butenolide without affecting the functionality in the steroidal core, 3) formation of β - hydroxides at C5 and C14, and finally 4) selective oxidation to form C19 aldehyde.

Pregnenolone acetate **2.16** was exposed to the previously developed method²⁷ to obtain 5-bromo-6,19-oxidopregnenolone acetate **2.17** which was then brominated to obtain dibromo compound **2.18** in 63% yield (**Scheme 2.3**). This was followed by dehydrobromination in presence of LiBr in DMF to obtain extended enone **2.19**, which was refluxed in presence of zinc dust and acidic isopropanol to obtain olefin at Δ^{5-6} to get trienone **2.20**. Hydrogenation of conjugated dienone **2.20** in presence of triphenylstannane reduced the olefin Δ^{16-17} selectively to get compound **2.21** in 82% yield. This completed the first phase of their synthesis and the next challenge was the construction of butenolide ring from the intermediate **2.21**.

Base-catalyzed reaction of **2.21** with diethyl oxalate followed by exposure of the intermediate oxalyl derivate to methyl thiosylate and potassium acetate in ethanol provided thiol **2.22** in 44% yield. Protection of C3 and C19 alcohol with acetate protecting group and followed by reflux of the diacetate in benzene in presence of zinc dust and methyl bromoacetate provided the Reformatsky product **2.23**. Reaction of **2.23** with the previously developed sequence during the synthesis of ditoxigenin¹³ was followed: a) reaction with trimethyloxonium tetrafluoroborate in

nitromethane, b) stirring with 0.5 N sodium hydroxide in methylene chloride, and c) absorption on alumina, which finally yielded in anhydropachygenol diacetate **2.25**.

Scheme 2.3. Synthesis of Key Intermediate **2.25** Towards the Synthesis of Strophanthidin

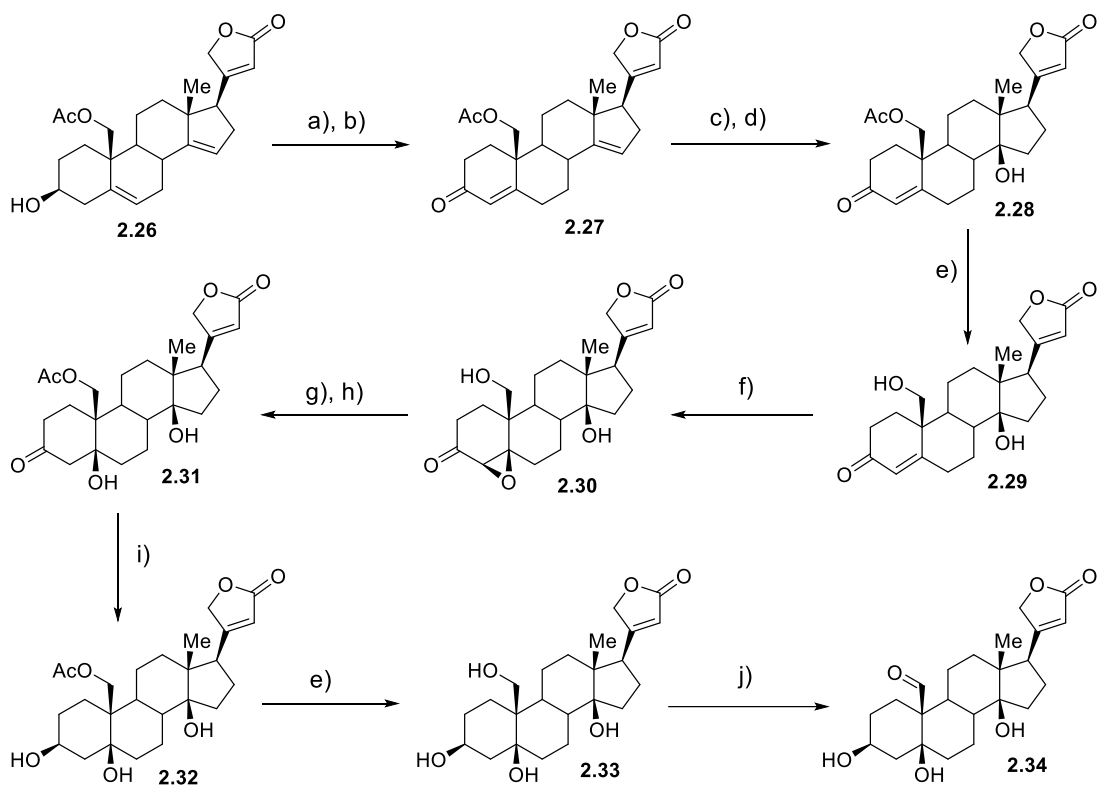


a) Br₂, AcOH; b) LiBr, DMF; c) Zn, i-PrOH-AcOH; d) Ph₃SnH, PhMe; e) (CO₂Et)₂, MeOK;
 f) TsSMe, AcOK; g) Ac₂O, Pyr.; h) BrCH₂CO₂Me, Zn; i) Me₃⁺OBF₄⁻; j) 0.5 N NaOH, CH₂Cl₂;
 k) Alumina

With **2.25** in hand, they focused on the installation of two tertial hydroxy group with β-orientation and the selective oxidation of the C19 alcohol to get an aldehyde (**Scheme 2.4**). Exposure of diacetate **2.25** to weakly basic condition hydrolyzed the C3 acetate group selectively in the presence of the primary acetate and gave **2.26**. This, upon oxidation of the C3 alcohol, in the presence of chromic acid, followed by immediate reaction with oxalic acid, provided enone

2.27 in 60% yield. To install C14 hydroxy group, previously reported procedure that involved subjecting **2.27** to hypobromous acid with *N*-bromoacetamide, followed by hydrogenolysis of the bromohydrin intermediate with Raney Ni to give the C14 hydroxylated steroid **2.28**, was employed. Hydrolysis of C19 acetate in presence of potassium bicarbonate followed by the treatment of **2.29** with basic hydrogen peroxide yielded epoxide **2.30** in 87% yield. Epoxide ring-opening was performed after the C19 protection with acetate through a reductive cleavage reaction using chromium acetate in ethanol to obtain 1:1 mixture of desired product **2.31** and elimination of the C5 alcohol to retrieve **2.28**. Reduction of the C3 ketone with Urushibara Nickel A²⁸ followed by saponification of **2.32** with potassium bicarbonate provided strophanthidol **2.33**. The final oxidation step was challenging as commonly used oxidants like PCC and SO₃•Py in DMSO oxidized the C3 hydroxyl group instead of C19, however, chromic trioxide in presence of hexamethylphosphoric triamide provided strophanthidin **2.34** selectively with no ketonic product observed.

Scheme 2.4. Completion of the Synthesis of Strophanthidin



a) Cr(VI); b) (COOH)₂; c) AcNHBr, H₂O; d) Raney Ni.; e) KHCO₃; f) H₂O₂, NaOH; g) Ac₂O, pyr.; h) Cr(OAc)₂; i) Urushi-bara Ni A.; j) CrO₃, (Me₂N)₃PO;

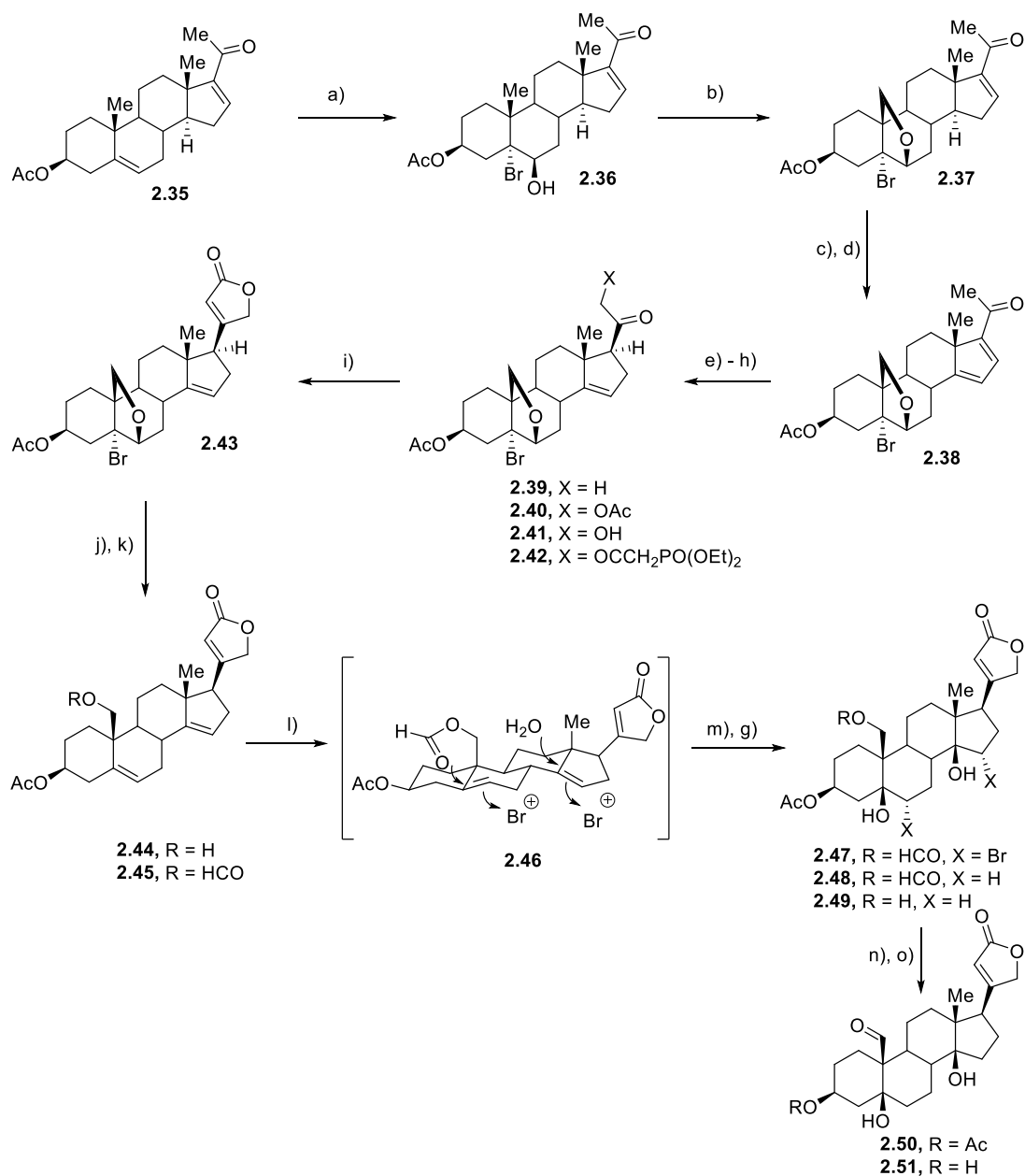
Following this synthesis of Strophanthidin and strophanthidol by Yoshii,²³ Kočovský et al. reported another synthesis of these compounds in 1989.²⁴

2.3.4. Semi-synthesis of Strophanthidin by Kočovský and Coworkers²⁴

Commercially available 5,16-pregnadien-3 β -yl-20-one acetate **2.35** was used as a starting material. The synthesis involves similar strategy as Yoshii's synthesis to form bromohydrin compound **2.36**, followed by installation of cyclic bromoether **2.37**. This was followed by the reduction to form extended enone **2.37** and selective hydrosilylation in presence of palladium metal to obtain **2.39**. The formation of butanolide involves the following sequence: 1) oxygenation of C21 by lead tetraacetate by formation of kinetic enol-ether formed from methyl-ketone and MeOH/BF₃ 2) saponification of **2.40** with KHCO₃ 3) esterification by diethylphosphonoacetic acid to obtain ester **2.42** and finally, 4) cyclization of **2.42** in presence of *t*-BuOK to obtain lactone **2.43**.

The synthesis utilizes a creative way towards the installation of C14 and C5 alcohol through the formation of double bromohydrin **2.47**. The installation of these hydroxy groups is performed by the formation of unstable double bromohydrin **2.47** followed by the radical reduction with tri-n-butyltin hydride, hydrolysis of the formate group with KHCO_3 to obtain strophanthidol-3-acetate **2.49**. Jones oxidation of the C19 alcohol produced **2.50**, the saponification of which was previously published²⁹ to obtain strophanthidin **2.51**. This completed the formal synthesis of strophanthidin in 16 steps.

Scheme 2.5. Synthesis of Strophanthidin by Kočovský and Coworkers



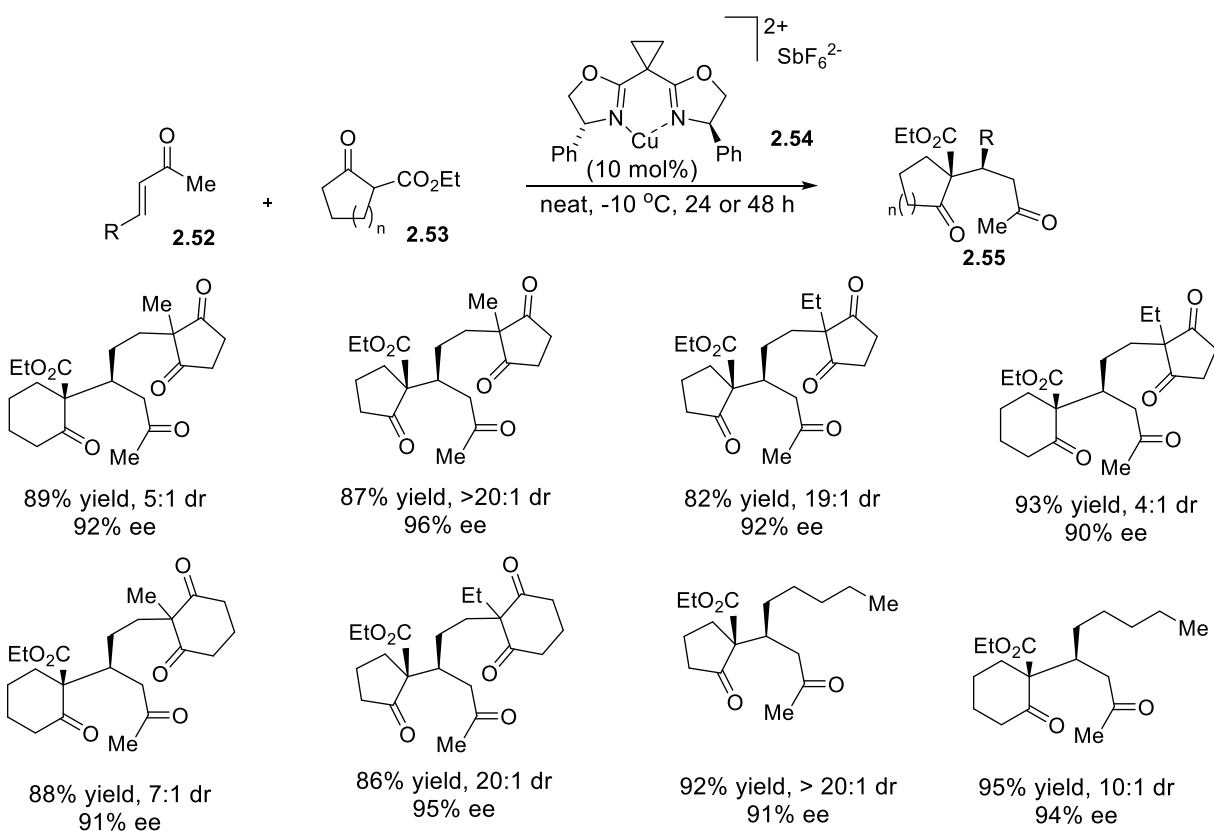
a) CH₃CONHBr, HClO₄, Et₂O, H₂O, rt; b) (AcO)₄Pb, I₂, CaCO₃, C₆H₆, reflux; c) NBS, AIBN, CCl₄, reflux; d) LiI, Li₂CO₃, DMF, 100 °C; e) Ph₂SiH₂, (dppe)₂Pd, ZnCl₂, CHCl₃; f) (AcO)₄Pb, MeOH, BF₃Et₂O, rt; g) KHCO₃, C₆H₆, MeOH, H₂O; h) (EtO)₂P(O)CH₂CO₂H, DCC, DMAP, Et₂O, C₅H₅N, rt; i) t-BuOK, THF, rt; j) Zn, AcOH, MeOH, 80 °C; k) 85% HCO₂H, 70 °C; l) CH₃CONHBr, HClO₄, H₂O, dioxane, rt; m) Bu₃SnH, AIBN, C₆H₆, reflux; n) CrO₃, Me₂CO, H₂O, H₂SO₄, 0 °C; o) Et₃N

2.3.5. Enantioselective Synthesis of Oxygenated Steroids by Nagorny and Coworkers

In 2015, Nathan Cichowicz, Will Kaplan, and Zhankui Sun from the Nagorny group developed an enantioselective synthesis of oxygenated steroids by utilizing copper-catalyzed Michael

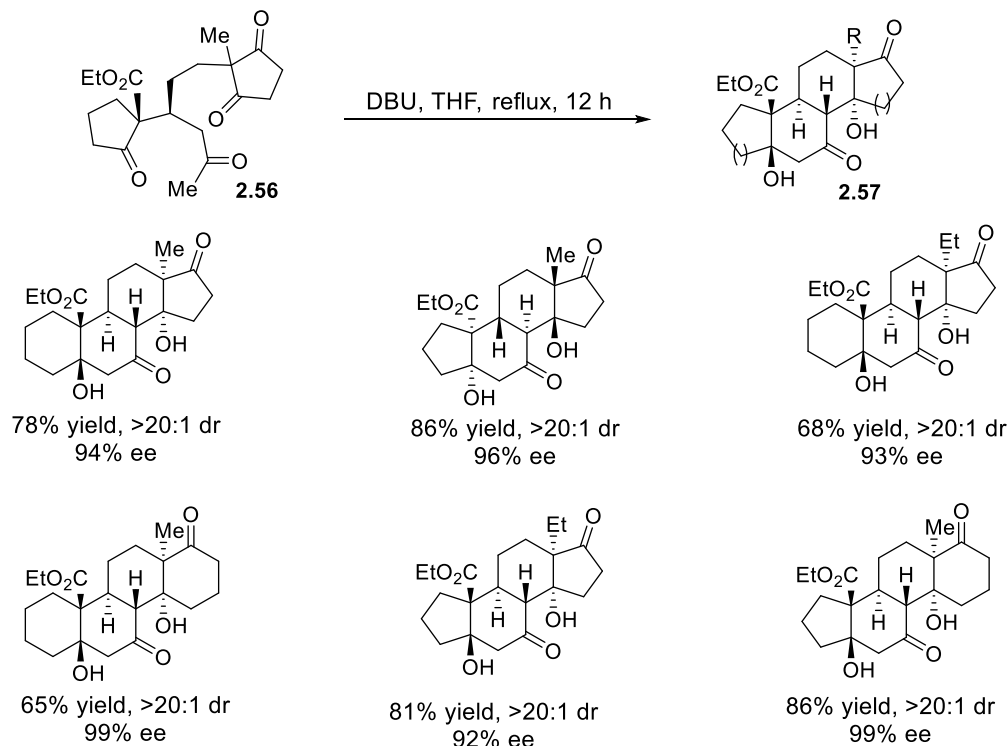
reaction followed by a double aldol reaction to generate steroidal scaffolds of various ring sizes and functionalities in only a few steps.³⁰ The substrate scope (**Scheme 2.6**) was investigated with varying A/D ring sizes and substituents at C13. Five-membered β -ketoesters were found to be more reactive than the six-membered counterparts and proceeded with higher diastereoselectivity. Changes in the sizes of ring and alteration of substituents at C13 was well tolerated and corresponding Michael adducts were obtained in good yields and good selectivities.

Scheme 2.6. Substrate Scope for Enantioselective Michael Reaction



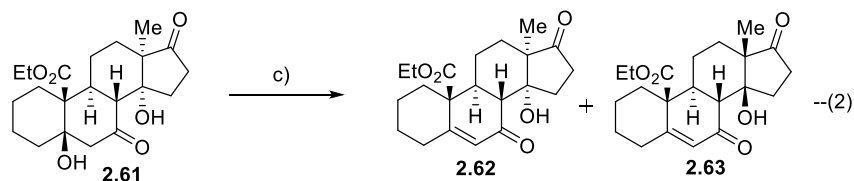
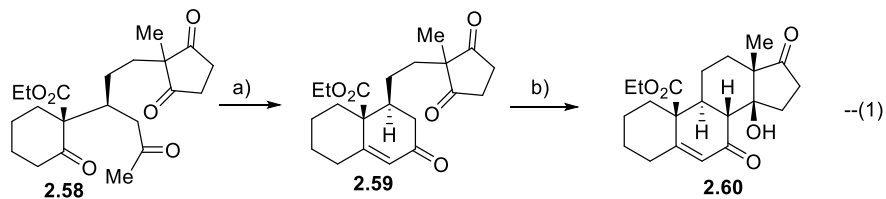
The Michael adducts thus generated were subjected to a double aldol cyclization, which was promoted by DBU to generate epimeric steroids with unnatural configuration i.e. α -CD-ring junction. This reactivity was general for all the Michael adducts generated in **Scheme 2.6** and proceeded with excellent yields and selectivities (**Scheme 2.7**).

Scheme 2.7. Diastereoselective Steroid Formation with Unnatural Configuration



In order to make this reaction more applicable towards the synthesis of natural cardenolides, the method to generate natural β -CD ring junction in a very efficient manner was established from the unnatural steroids (obtained from **Scheme 2.7**) or directly from the corresponding Michael adducts (**Scheme 2.6**). Cyclization of Michael adduct **2.7** promoted by pyrrolidine acetate to form enamine activates the first aldol condensation to get monocyclized enone **2.59** followed by the base promoted aldol addition to obtain steroidal core **2.60** (**Scheme 2.8.1**). Alternately, the steroids with unnatural C13/C14 stereocenter can be epimerized in presence of Cs_2CO_3 to get **2.63** as major product (**Scheme 2.8.2**).

Scheme 2.8. Selected Examples for the Diastereoselective Formation of Steroids with Natural Configuration



a) Pyrrolidine, AcOH, EtOAc; b) LiHMDS, THF, -78 °C to 60 °C; c) Cs₂CO₃, DMF, dr = 1:3

2.3.6. Total Synthesis of 19-hydroxysarmentogenin and Trewianin Aglycone by Nagorny Group

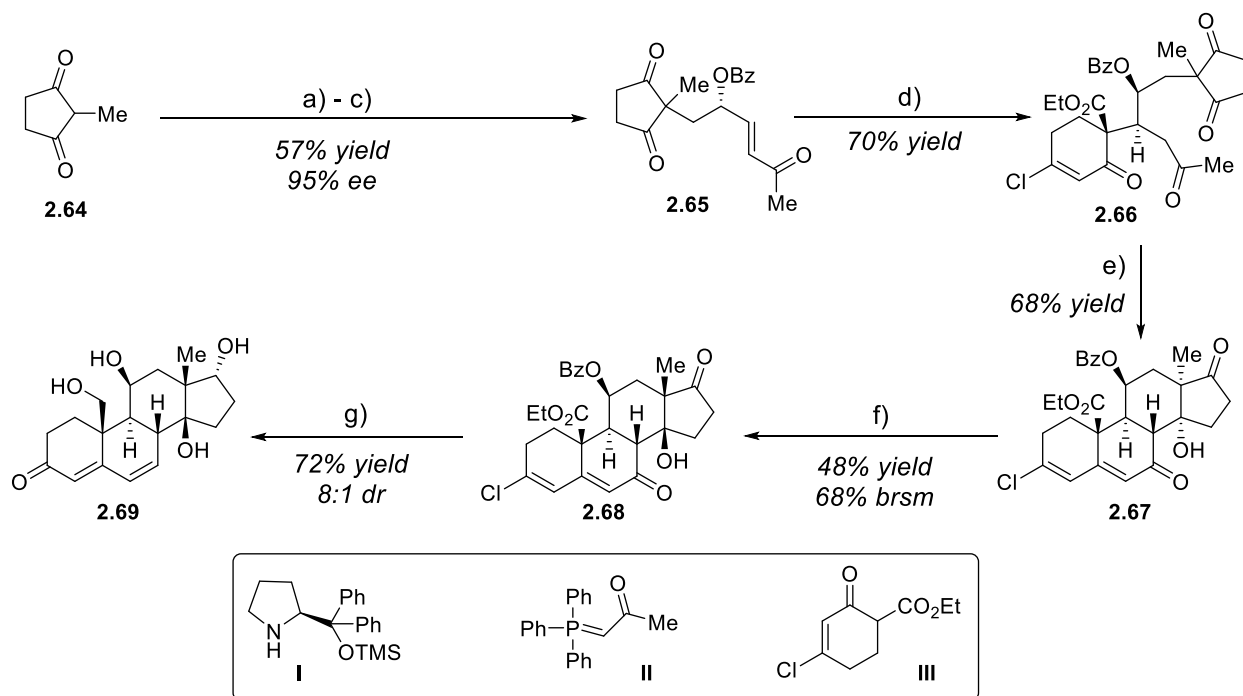
After successful development of the method to rapidly construct the steroid core based on the enantioselective Michael-double aldol approach, Will Kaplan and Dr. Hem Raj Khatri reported a concise strategy to synthesize oxygenated cardiotonic steroids.²⁷ While there are several semi-synthetic and total synthetic approaches to the oxygenated cardiotonic steroids,^{10–12,19,20,35} the divergent synthesis of two epimeric cardenolides, 19-hydroxysarmentogenin and trewianin aglycone, reported in 2016 is the most concise one.

The approach for these total syntheses involved designing the Michael reaction fragments with preinstalled oxygenations. After a diastereoselective Michael-double aldol cyclization sequence, the A/B ring junction would be constructed through a C19-OH controlled regiodivergent hydrogenation. The butenolide at C17 would be installed at a late stage of the synthesis using Stille coupling reaction.

The forward synthesis was begun with the synthesis of chiral enone **2.65**. The diketone **2.64** was reacted with acrolein to achieve a Michael addition, which was followed by an organocatalytic α -oxidation^{36,37} using prolinol-derived catalyst **I** to get the chiral aldehyde. After the Wittig reaction with 1-(triphenylphosphoranylidene)-2-propanone **II**, chiral enone **2.65** was furnished in high yields and high enantiomeric excess (95% ee). Enone **2.65** was reacted under Cu(OTf)₂-

catalysed Michael reaction with ketoester **III**, with preset latent oxidation at C3 in the form of vinyl chloride, to give Michael-adduct **2.66** in high diastereoselectivity. This Michael adduct was subjected to an acid-mediated cyclization with an excess *p*-TSA to produce the cyclized product **2.67** in good yield, albeit with an unnatural configuration at C/D ring junction. Thus, in the next step, the C/D ring junction in tetracyclic steroid **2.67** was epimerized under basic conditions via retro-Aldol-re-Aldol sequence to generate compound **2.68** with the natural configuration at C/D ring junction. Steroid **2.68** was then subjected to global reduction with DIBAL-H followed by hydrolysis and an unprecedented transposition of vinyl chloride to generate ketone at C3 in an efficient, one-pot transformation. At the end of this sequence of reactions, this intermediate **2.69** contains oxygenations at C3, C11, C14, C17, and C19 as well as an unsaturation at C5 which is required for translation to various natural products.

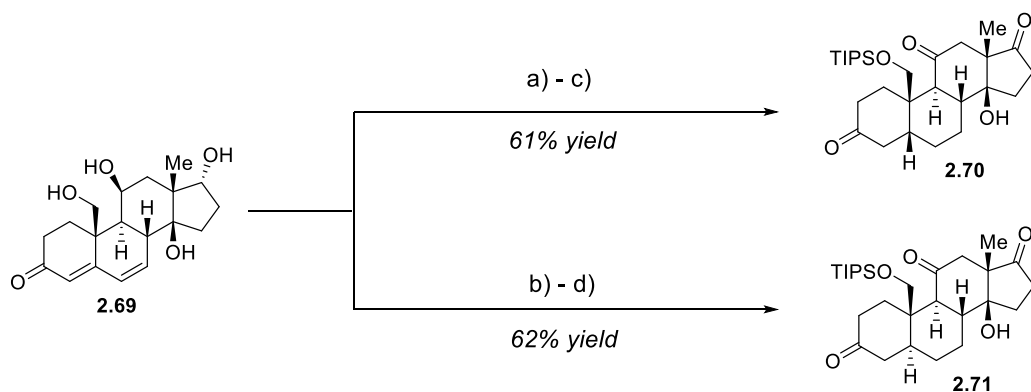
Scheme 2.9. Synthesis of Key Intermediate with Pre-set Oxygenation



a) Acrolein, H₂O, 12 h; b) **I**, (BzO)₂, hydroquinone, THF, H₂O, 1.5 h; c) **II**, toluene; d) **III**, Cu(OTf)₂, DCM, 9 h; e) *p*-TSA, CH₃CN, 55 °C; f) NaHMDS, toluene, -78 °C to 42 °C; g) DIBAL-H, THF, -78 °C to 60 °C, 12 h then, HCOOH, H₂O, 85 °C.

The key intermediate **2.69** was then transformed into two cardiotonic steroids 19-hydroxysarmentogenin and trewianin aglycone, which are epimeric at C5. The enone in **2.69** was hydrogenated under slightly alkaline condition,³⁸ directed by C19-OH, to cleanly produce cis A/B ring junction *en-route* to 19-hydroxysarmentogenin. Following a TIPS protection of C19 and DMP oxidation of C11 and C17 alcohols afforded triketone **2.70**.

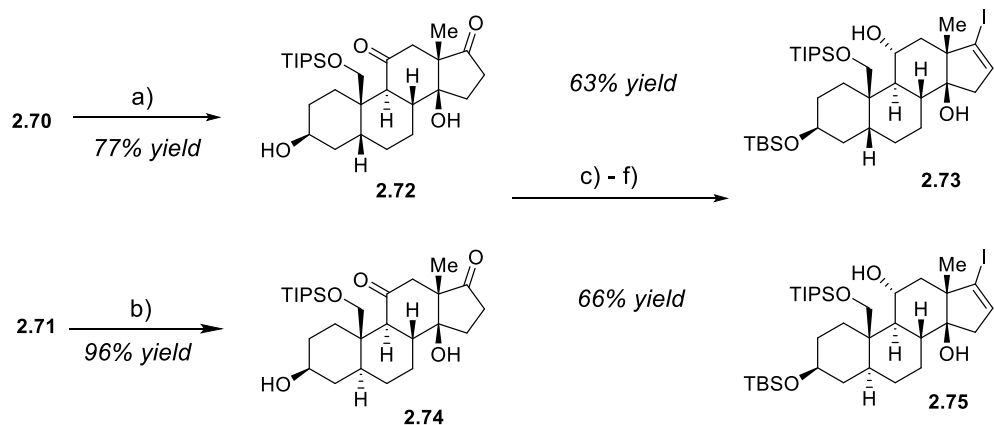
Scheme 2.10. Divergent Synthesis Towards Functionalized Core from Key Intermediate



a) H₂, 10% Pd/C, KOH, quinoline, MeOH; b) TIPSCI, ImH, DMF, 6 h; c) DMP, Py, DCM 2 h; d) H₂, Pd/C, MeOH, Py

Next series of transformations included: 1) a chemo- and stereoselective reduction of C3 ketone with K-selectride, 2) a subsequent protection of resulting C3 alcohol with TBS-ether and the masking of C17 ketone as an enol-TBS ether, 3) a Birch reduction of C11 ketone to get an equatorial alcohol, 4) an unmasking of C17 ketone using TBAF, and 5) the Bartons's condition to convert C17 ketone to a vinyl iodide **2.73**.³⁹ This resulted in a fully functionalized 19-hydroxysarmentogenin core.

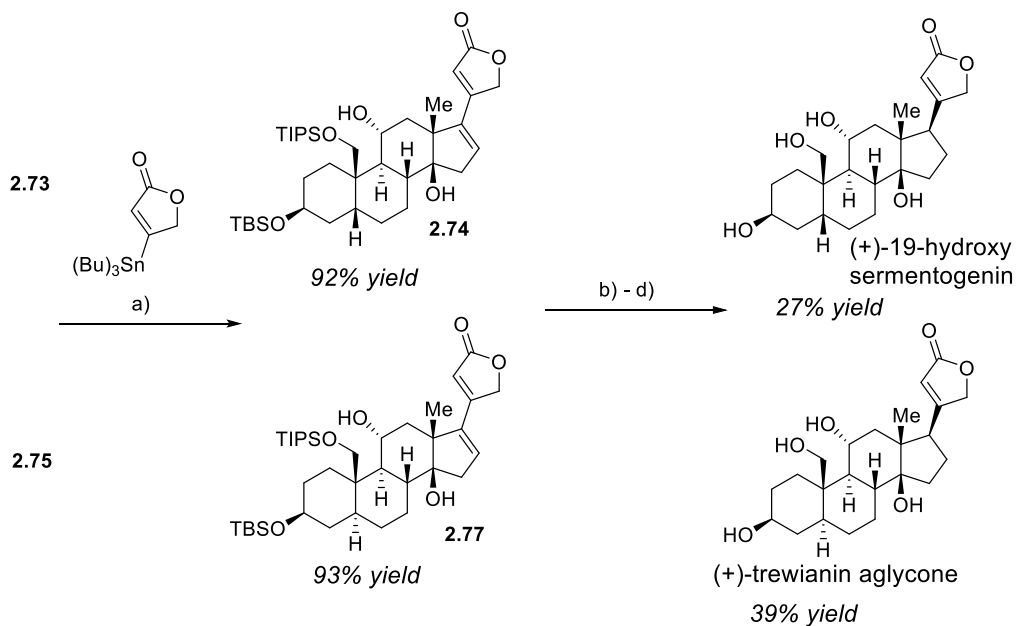
Scheme 2.11. Synthesis of Fully Oxygenated Vinyl Iodides Epimeric at C5



a) K-selectride, THF, -78 °C to -30 °C, 1.5 h; b) LiAlH(OtBu)₃, THF, -78 °C to -40 °C, 4 h; c) TBSOTf, Et₃N, DCM, -78 °C to -30 °C, 1.5 h; d) Li, NH₃, THF -78 °C, 30 min; e) TBAF, THF, -78 °C, 5 min; f) N₂H₄·H₂O, Et₃N, EtOH, 50 °C, 6 h then, I₂, Et₃N, THF, rt, 1 h

Finally, butenolide was installed at C17, thus completing the synthesis of 19-hydroxysermentogenin via a 4-step sequence involving 1) Stille coupling of vinyl iodide **2.73** with stannane of the butenolide, 2) TMS protection of C11 and C14 alcohols, 3) hydrogenation of C16-C17 double bond, and 4) global deprotection of silyl-protecting groups with aqueous HF.

Scheme 2.12. Total Synthesis of (+)-19-hydroxysermentogenin, and (+)-Trewianin Aglycone



a) Pd(PPh₃)₄, CuCl, LiCl, DMSO, DMSO, 50 °C, 1 h; b) TMSOTf, 2,6-lutidine, DCM, -78 °C to rt, 2 h, then SiO₂(dry), 10 h; c) H₂, Pd/C, EtOAc, 30 min; d) HF in CH₃CN/H₂O/DCM, 3 days

In an analogous sequence, the total synthesis of trewianin aglycone was completed from intermediate **2.69**. The TIPS protection of C19 alcohol imparted enough steric bias to construct a trans-A/B ring junction during hydrogenation and thus forming triketone **2.71** following a DMP oxidation. With A/B trans junction assembled, subsequent diastereoselective reduction of C3 ketone was achieved using $\text{Li}(\text{tBuO})_3\text{AlH}$ to obtain β -oriented C3 alcohol. The rest of the synthesis followed same sequence of reactions to that of 19-hydroxysarmentogenin.

The continued interest of our group towards these interesting cardiotonic steroids provoked us to develop a concise and divergent synthetic pathway towards numerous natural products and their analogs of this class of steroids and subsequent biological activity studies. The total synthesis of two natural products cannogenol and cannogenol-3-O- α -L-rhamnoside, with C19 oxygenation but lacking C11 oxygenation, would be discussed in **Chapter 3** and the follow-up study on the anticancer studies of cannogenol based natural products and analogs as well as several cardiotonic steroids will be discussed in **Chapter 4**.

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Chapter 3

Enantioselective Total Synthesis of Cannogenol-3-O- α -L-rhamnoside via Sequential Cu(II)-Catalyzed Michael Addition/Intramolecular Aldol Cyclization Reactions

(A part of this work has been published in Bhattarai, B.; Nagorny, P. Enantioselective Total Synthesis of Cannogenol-3-O- α -L-rhamnoside via Sequential Cu(II)-Catalyzed Michael Addition/Intramolecular Aldol Cyclization Reactions. *Org. Lett.* **2018**, 20, 154-157)

3.1. Introduction of Cannogenol-3-O- α -L-rhamnoside and its Anticancer Activities

Cannogenol and cannogenol-3-O- α -L-rhamnoside is commonly isolated from plant source of genus *Convallaria*, mostly from *Convallaria majalis* (popularly known as Lily of the Valley).¹ This plant holds cultural and biblical popularity in native to northern Hemisphere in Asia and Europe where it grows and although sweetly scented, is highly poisonous.²

There has not been as much report as other members of this class of cardiac steroid on the Na⁺/K⁺-ATPase activity of these two natural products. Instead, there have been a few studies on their anticancer activities against a variety of cancer lines as reported in 2007 and 2008 (**Table 3.1 and 3.2**) which will be discussed in detail in the following chapter. These reports show nanomolar half maximal inhibitory concentration (IC₅₀) of cannogenol-3-O- α -L-rhamnoside against several cancer cell lines that were studied (**Table 3.2**).^{3,4} Although it exhibited an impressive activity against these cells, there have been no follow up reports on the elaboration of the medicinal chemistry studies and diversification of cannogenol to generate its different analogs. We believe this to be largely due to their low concentration in natural sources and the difficulty associated in the extraction of these toxic molecules from these sources. Organic synthesis may significantly improve the accessibility of cannogenol and provide otherwise challenging-to-access derivatives.

Table 3.1. Reported Anticancer activities of Cannogenol (μM)⁵

KB	KB-VIN	A549	MCF-7	U-87-MG	PC-3	1A9	CaKI-1	HCT-9	S-KMEL-2
0.45	0.5	0.050	0.21	0.50	0.15	0.12	0.11	0.46	0.70

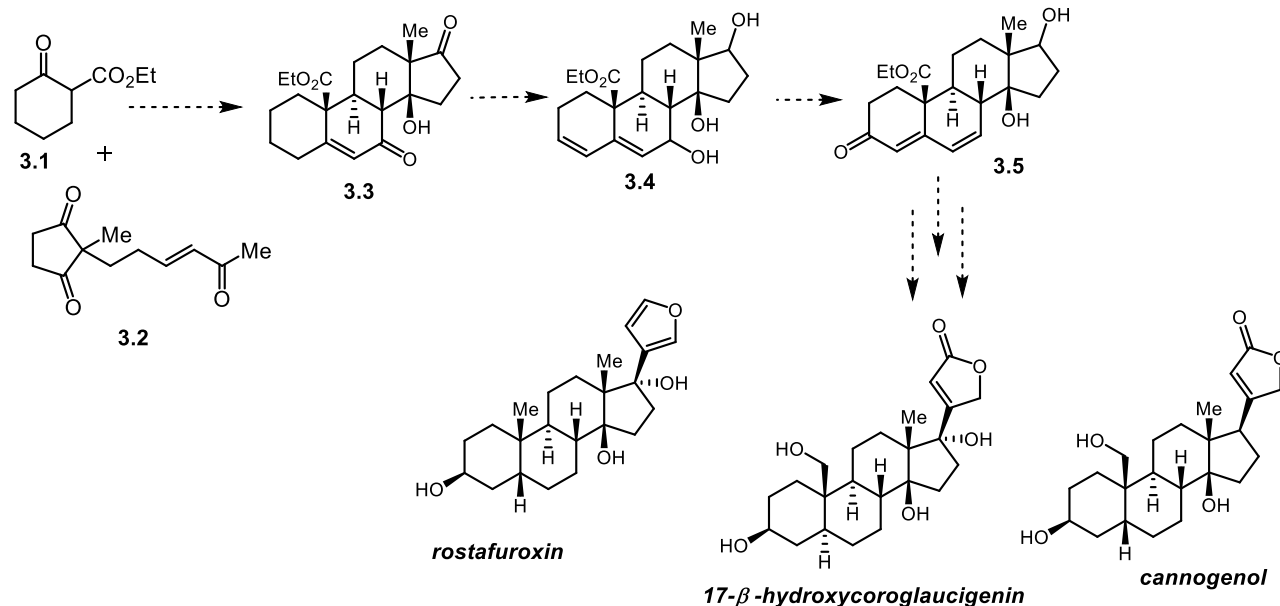
Table 3.2. Reported Anticancer activities of cannogenol-3-*O*- α -L-rhamnoside^{3,4} (nM)

BGC-823	Bel-7402	H460	LNCaP	MCF-7	SW-480	Hela	A549	Calu-6
77	28	10.8 \pm 5	43 \pm 17	37 \pm 2	66 \pm 47	28 \pm 10	18 \pm 6	163 \pm 48

3.2. Initial Objective and Proposed Synthetic Route

The overarching objective of this project was to build up on the previously established methodology from our group and elaborate it to achieve the total synthesis of cannogenol, coroglaucinogenin, and several other related steroids and their derivatives like rostafuroxin, an analog of this family that is in phase III clinical trial against hypertension. Enantioselectivity in the steroid could be introduced early in the total synthesis by utilizing the previously developed enantioselective Michael reaction of keto-ester **3.1** and enone **3.2** followed by rapid steroid assembly using double aldol condensation (**Figure 3.1**). This intermediate **3.4** can be transformed into the steroidal core with proper oxygenation by a reduction followed by acid promoted transposition. The functional group interconversion of this molecule **3.5** followed by a Pd-catalyzed cross-coupling reaction would allow us to easily install various heterocycles and generate multitude of analogs.

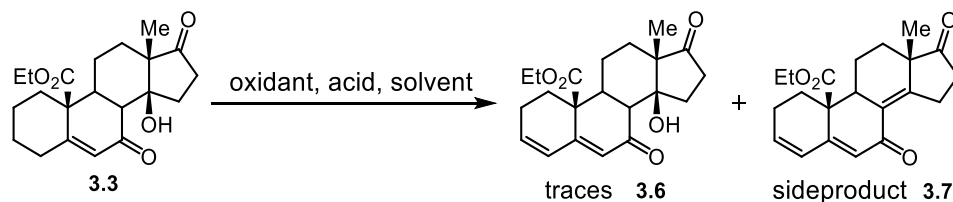
Figure 3.1. Initial Synthetic Route Towards Cannogenol and Other Relevant Cardiotonic Steroids



As mentioned above, intermediate **3.3** can be easily synthesized through a reaction previously developed in our group.⁶ However, the generation of the diene **3.4** was necessary for the subsequent key reaction, the transposition. We planned to install oxygen at C3 and remove unwanted oxidation at C7 in a single step by S_N2 -type mechanism using H_2O , a transformation with limited literature precedence (**Scheme 3.4a**). This key intermediate **3.5** could then be further elaborated to several natural products and biologically relevant derivatives as shown in **Figure 3.1**.

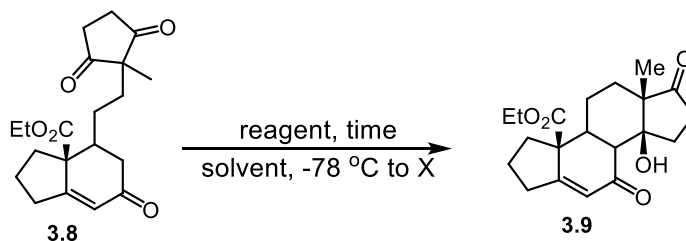
Several attempts to achieve oxidation of **3.3** to an extended enone **3.6** were made. Many reaction conditions were tried, but all of them in vain (**Scheme 3.1**). Organic oxidants like chloranil and DDQ were used in several solvents (toluene, *t*BuOH, 1,4 dioxane, benzene, xylene) either in presence or absence of various acids (acetic acid, *p*-toluenesulfonic acid, trifluoroacetic acid). However, it resulted in either no reaction, a very slow reaction, or formation of eliminated side product **3.7** (observed by MS) when subjected to acidic reflux conditions (**Scheme 3.1**).

Scheme 3.1. Oxidation Attempts to Synthesize Extended Enone **3.6**



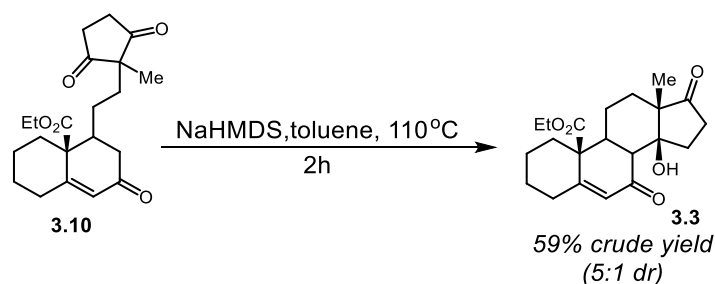
During a collaborative effort with Dr. Nathan Cichowicz towards the development of a concise enantioselective synthesis of oxygenated steroids via sequential copper (II)-catalyzed Michael Addition/intramolecular aldol cyclization reaction, an alternative strategy was developed to achieve similar scaffold that we were interested in (**Table 3.3**). The cardenolide core was synthesized through cyclization of monocyclized enone with LiHMDS or NaHMDS. This resulted in a clean diastereoselective formation of the corresponding cardenolide core **3.9** with natural configuration (**Table 3.3**). Moreover, this sequential approach allowed us to cleanly extend unsaturation in A ring (found to be troublesome to execute in fully cyclized tetracycle previously) prior to the closure of B/C ring and C/D ring.

Table 3.3. Optimization of Aldol Addition to Generate Natural Stereocenters at C13 and C14



Reagent	Temperature (X)	Solvent	Time	yield (isolated)	dr	Comments
KHMDS	60 °C	Toluene	1.5 h	traces		
LiHMDS	60 °C	Toluene	20 min	45%	1:1	
NaHMDS	60 °C	THF	1.5 h	57% (40%)	1.5:1	
NaHMDS	110 °C	Toluene	2h	67% (42%)		
NaHMDS	60 °C	THF	8h			didn't go to completion
NaHMDS	140 °C	DMF	1h	traces		too many side products
NaHMDS	110 °C	Toluene	50 min	(72%)	11:1	clean reaction
NaHMDS	110 °C	Toluene	5h	(46%)		side products
LiHMDS	rt	Toluene	2d	traces		very slow
DBU	60 °C	Toluene	2d	traces		very slow
PTSA	60 °C	Toluene	2d	traces		C-14 eliminated product

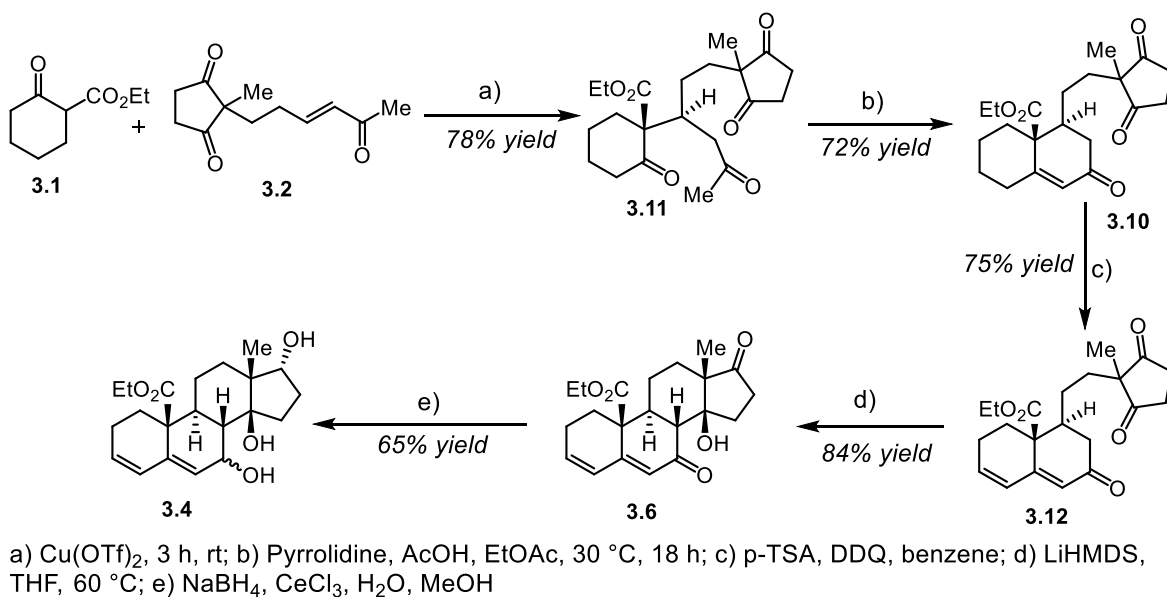
Scheme 3.2. Formation of Steroid 3.3 with Natural Configuration



Following the promising result from this investigation, elaboration of the described work toward the synthesis of cannogenol was attempted. Our synthesis commenced with Cu (II)-catalyzed intermolecular Michael reaction of 2-substituted beta-ketoesters **3.1** and beta-substituted enones **3.2** resulting in vicinal quaternary and tertiary stereocenters (**Scheme 3.3**). The enantioselective variant of this reaction can be achieved using Cu-BOX ligand.⁶ The Michael adduct **3.11** was then treated with pyrrolidine acetate to form an enamine that undergoes first aldol condensation reaction to afford efficient formation of monocyclized enone **3.10**. This intermediate presented us the opportunity to extend the unsaturation to A ring as there is no potentially prone

to elimination tertiary alcohol which impeded our previous attempts. Consequently, this enone **3.10** was further oxidized under acidic condition using DDQ as an oxidant, which cleanly resulted in the extended enone **3.12**. Base-promoted cyclization developed during our previous efforts (**Scheme 3.2**) to generate natural C13/C14 stereocenter was performed next to achieve the desired intermediate **3.6**. LiHMDS instead of NaHMDS provided us with diastereoselective formation of the steroidal core with natural stereocenters through an aldol addition reaction. Diketone **3.6** was then reduced under Luche condition to afford the triol **3.4**. This Intermediate, however, has an unwanted oxidation at C7 and lacks a crucial oxidation at C3.

Scheme 3.3. Initial Route Towards the Synthesis of Cannogenol

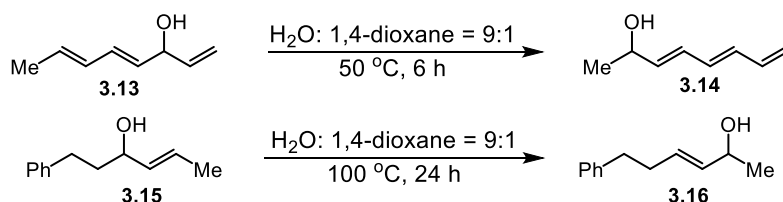


Next, we needed to synthesize the key intermediate **3.5** (**Figure 3.1**) through a crucial transposition reaction. The transposition of similar alcohol system had only been reported in an acyclic system⁷ (**Scheme 3.4a**) and the translation of the reported method in our cyclic and conformationally locked substrate was not trivial. An exhaustive screen of the solvent systems (H_2O : dioxane or benzene) either in presence or absence of acids (acetic acid) was run in attempts of getting transposition going. Our initial hypothesis was that the triol **3.4** would transpose in

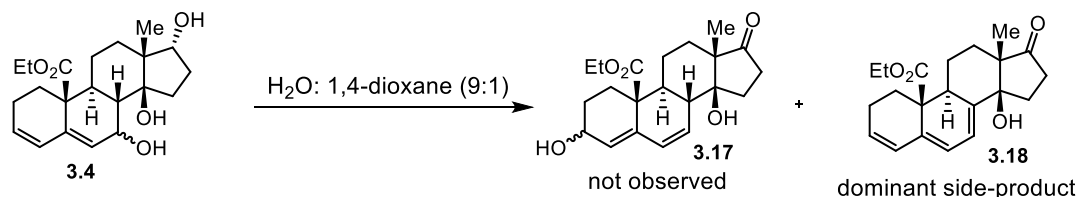
presence of H₂O through an S_N2-like mechanism and might be promoted by acid. However, due to the high temperature required for this transformation to occur, elimination of C14 hydroxyl was frequently observed to form the side product, possibly triene **3.18** as observed by MS (**Scheme 3.4b**). Use of a mild oxidant like manganese oxide to trap any possible carbocation formed at C3 was not successful in formation of desired product either.

Scheme 3.4. Literature Precedence and Optimization of Transposition Reaction

a) *Transposition: Ref 7.*



b) *Transposition Attempt*



3.3 New Retrosynthetic Analysis

After various unsuccessful attempts to synthesize a key intermediate in our original strategy, we re-designed the synthetic pathway to access molecules of this class. In our new strategy, we wanted to design fragments in such a way that necessary oxygenations to be installed early if possible and build a more general common intermediate that could be easily functionalized and at the very least, was a robust method that would allow us to explore various analogs with different sugar and heterocycle moiety appended to the core.

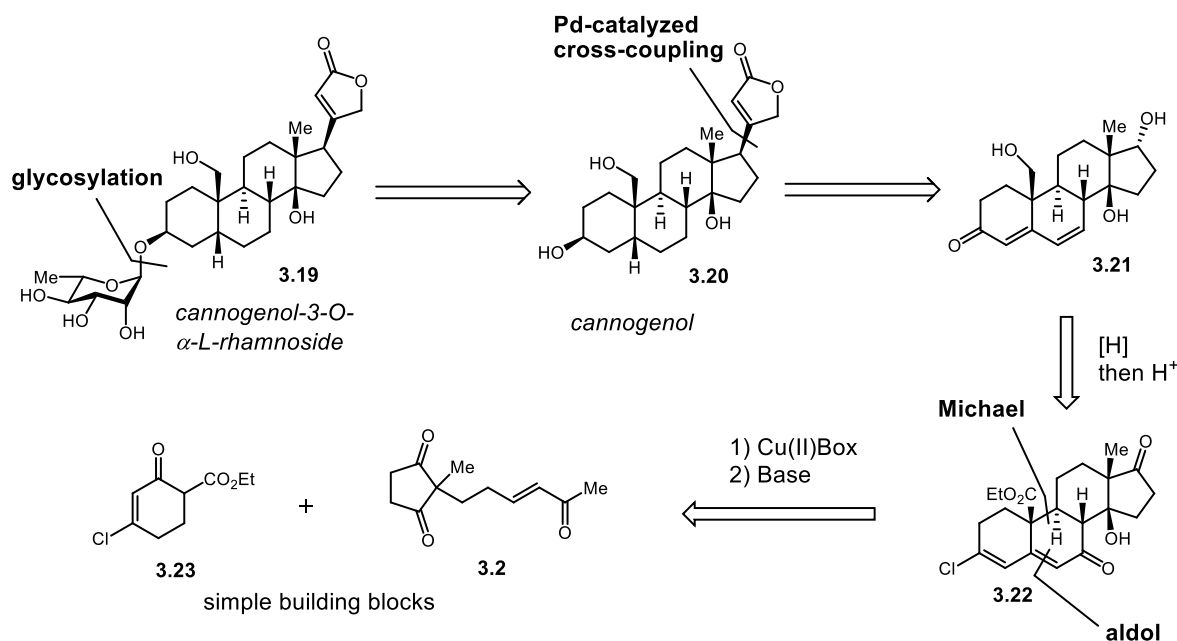
As it was nicely defined by K. C. Nicolaou in his synthesis of Taxol “the route should be short and flexible to allow for the eventuality of producing the natural product and a variety of its analogs in a practical way and to deliver the target molecule in its enantiomerically pure and correct form.”⁸

The new retrosynthetic analysis was designed considering all the reaction and challenges described above. The literature analysis indicated that the functionalization of the A-ring, C3-glycoside and C17-heterocycle play the most important roles in defining the biological activity of cardiotonic steroids as they are found to interact with the enzyme during formation of the cardiotonic steroid-enzyme complex.⁹ Hence, our approach to cannogenol and related steroids required flexibility in adjusting the functionalities of the A-ring and heterocyclic moiety at C17 position. We wanted to design a general synthetic pathway that would give us an access to a large number of members in this class of natural product with minimum, if any, deviation from original pathway to develop analogs with different heterocycles and sugar moieties. The key retrosynthetic disconnection was breaking the C3–C1' bond to facilitate the final stage glycosylation reaction for the sugar installation. Another disconnection that we hypothesized would make late stage functionalization easier is the C17–C20 bond.

To put it all together, we envisioned that we could achieve the synthesis of cannogenol-3-*O*- α -L-rhamnoside **3.19** by selective C3 glycosylation of another bioactive natural product, cannogenol **3.20** (**Figure 3.2**). We hypothesized that a robust method for the heterocycle installation would be a well-studied Pd-catalyzed cross-coupling reaction on steroidal core with proper stereocenters already set. Key intermediate **3.21** would be formed by transposition of intermediate **3.22** with pre-installed functionality that can be removed during the transposition process and circumvents the formation of triene as discussed above (**Scheme 3.4**). This intermediate **3.22** in turn can be achieved by the method discussed above: Michael reaction followed by an aldol addition/aldol cascade reaction.⁶ The proposed starting materials, chloroketoester **3.23** and enone **3.2** lack any stereochemistry, hence, the development of an enantioselective variant of Michael reaction would be required to complete the asymmetric total

synthesis of cannogenol-3-*O*- α -L-rhamnoside. The synthesis of chloroketoester and enone have been previously reported and are known to work in Cu(II)-catalyzed Michael reactions in different setting.⁶

Figure 3.2. Retrosynthetic Analysis of Cannogenol-3-*O*- α -L-rhamnoside



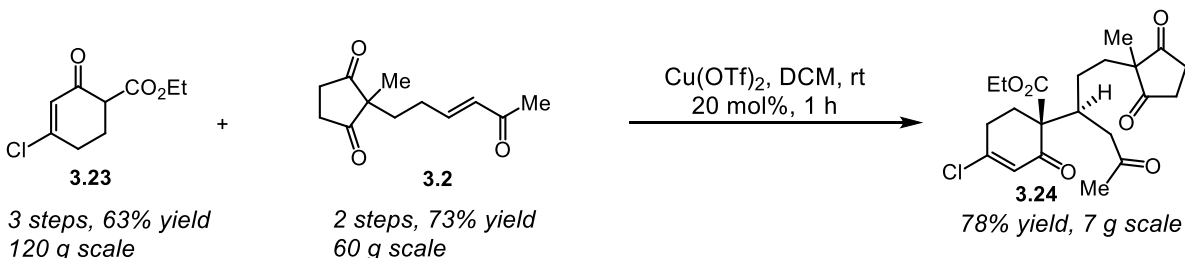
3.4. Enantioselective Total Synthesis of Cannogenol

We began our studies by generating sufficient quantities of known precursors **3.23** and **3.2** (Scheme 3.5). β -ketoester **3.23** was prepared in three steps from commercially available 3-ethoxy-2-cyclohexenone (up to 120 g scale) and enone **3.2** was synthesized in two steps from 2-methyl-1,3-cyclopentanedione (up to 60 g scale). Initially, Cu(OTf)₂, which is a previously reported efficient catalyst for these Michael reactions,^{6,10} was tested for the viability of the reaction. To our delight, we obtained the desired product **3.24** in 78% yield. In order to generate enantiomerically pure cannogenol, these substrates were subjected to an asymmetric variant of Michael reaction⁶ using a 2,2'-(cyclopropane-1,1-diyl)bis(4-phenyl-4,5-dihydrooxazole) ligand pre-complexed with Cu(SbF₆)₂ as the catalyst (10 mol %) under neat conditions.¹¹ As expected, this transformation

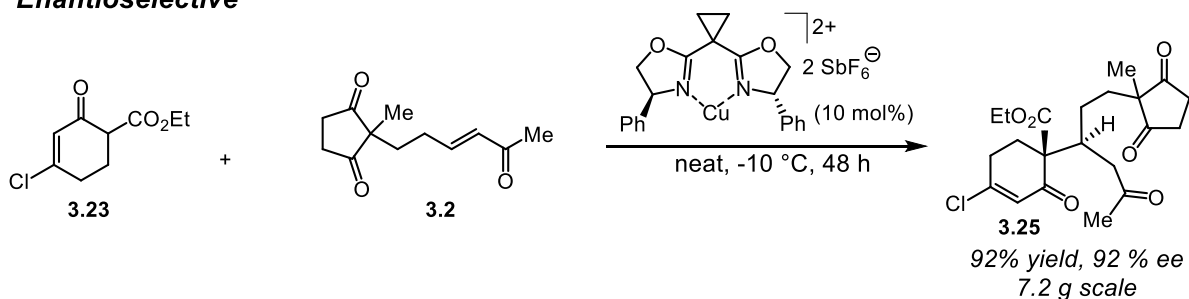
proceeded efficiently, and Michael adduct **3.25** was consistently generated on 7.2 g scale in 92% yield, >20:1 dr, and 92% ee (**Scheme 3.5**).

Scheme 3.5. Development of Enantioselective Michael Reaction

Racemic

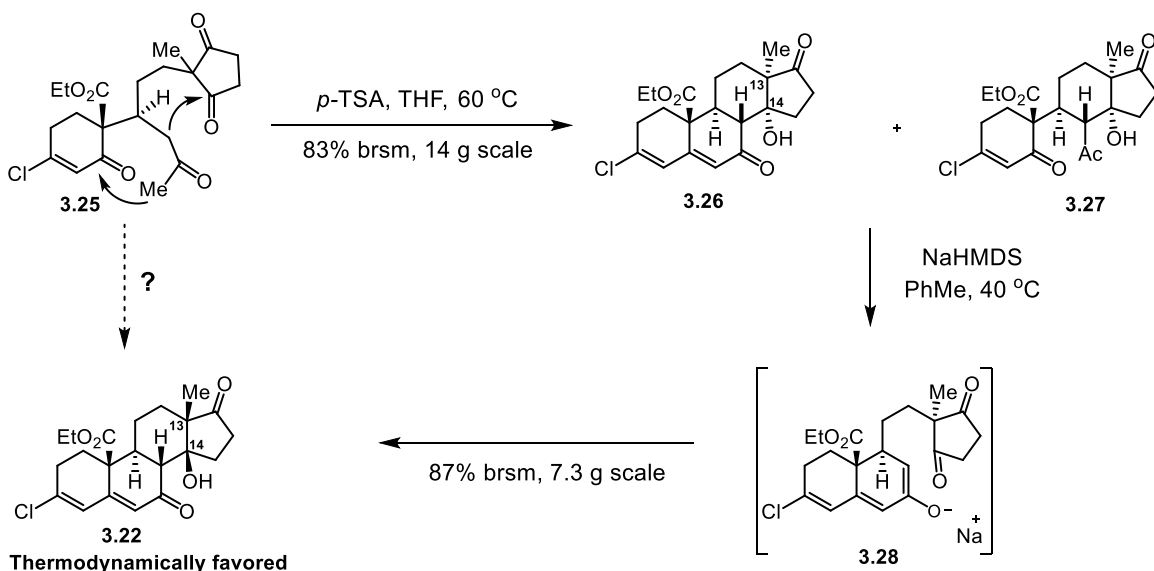


Enantioselective



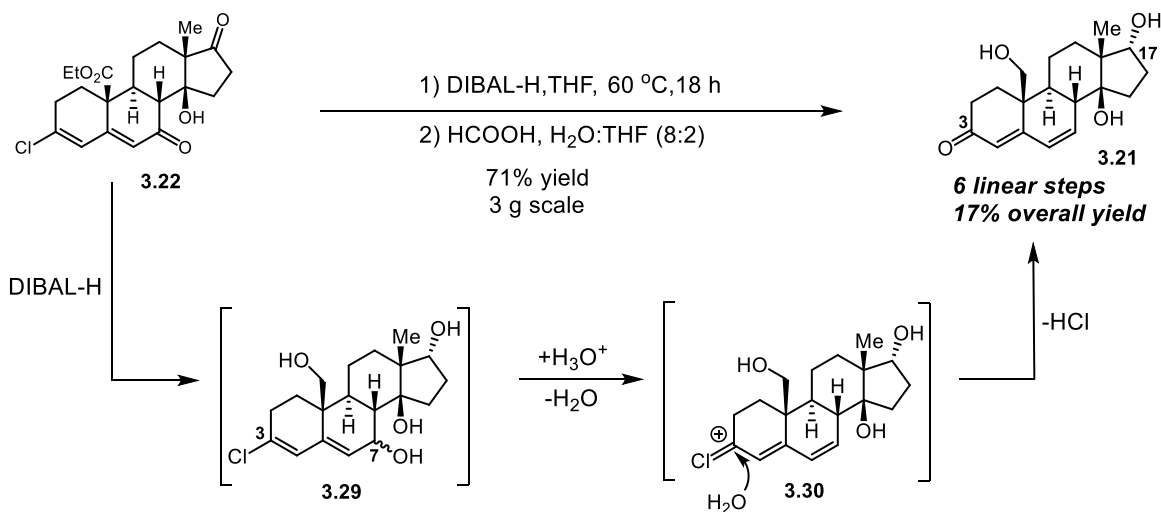
The Michael adduct **3.25** was then subjected to a double aldol cyclization with *p*-TSA, which resulted in the steroid **3.26** with an unnatural α -C13/C14 configuration (57%, >20:1 dr, 14 g scale, **Scheme 3.6**). Based on our previous knowledge on a related system containing C11 oxygenation,¹⁰ we were confident that such an unnatural stereochemistry in these steroids could be epimerized under basic conditions to form the thermodynamically-preferred, natural β -configuration at the C13 and C14 centers. Indeed, subjecting **3.26** to NaHMDS resulted in formation of the desired diastereomer **3.22** in good yield, presumably through the intermediacy of retro-aldol product **3.28** (64%, >20:1 dr; 7.3 g scale). This epimerization is very counterion specific as other counterion (such as K^+ , Li^+) on HMDS did not provide comparable yield.

Scheme 3.6. Generation of Steroidal Framework with Natural Configuration at C/D Ring Junction



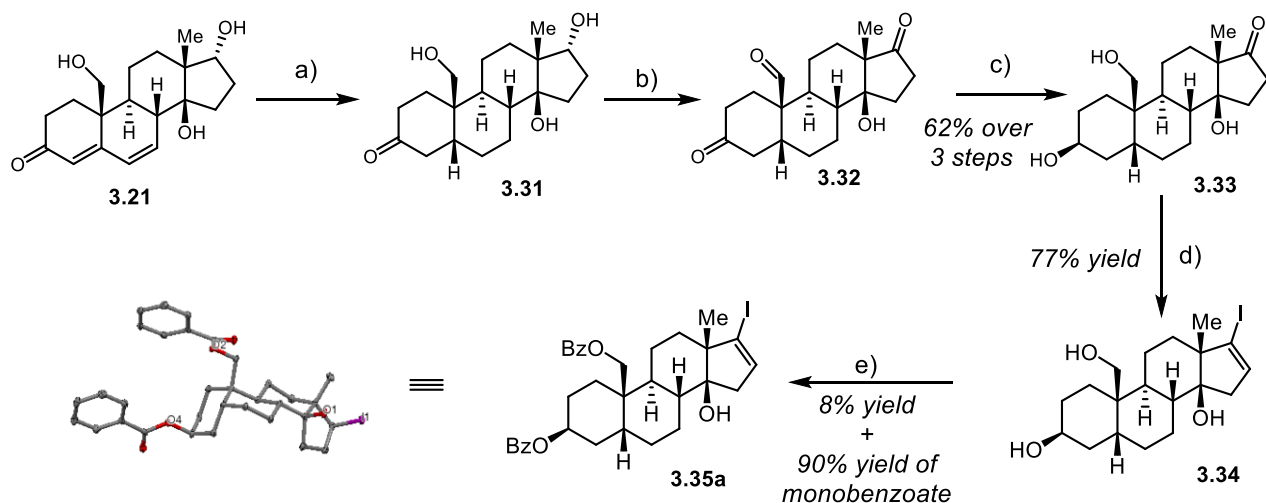
Intermediate **3.22** was then treated with DIBAL-H (3 g scale), which led to **3.29**, which was stable at room temperature for up to 2 h (**Scheme 3.7**). The intermediate **3.29** can be isolated, and the NMR characterization of this intermediate has been performed. However, attempts to store this intermediate in vacuum, bench top, or as a solution of methanol in counter-top or freezer led to decomposition to form black precipitate of unknown identity. In most instances, the triol **3.29** was not isolated, but rather the excess DIBAL-H was quenched with water, and the crude mixture was heated to reflux in formic acid (enough to bring pH to about 3) and 8:2 water/THF to accomplish transposition/vinyl chloride hydrolysis to produce a mixture of C17 epimers in 71% yield, 8:1 dr. This mixture was purified by SiO₂ column chromatography to afford α -C17 diastereomer **3.21** in 63% yield. This transformation allowed us to remove the unwanted oxygenation at C7 and introduce important oxygenation at C3 in a single step to set the proper steroidal framework mimicking the natural product. Thus, the key intermediate **3.21** was synthesized in 6 linear steps (9 overall steps) from commercially available building blocks in 17% overall yield and 92% ee.

Scheme 3.7. Reduction and Transposition Reaction to Afford Key Intermediate **3.21**



With the key intermediate **3.21** in hand, we turned our attention to installation of the stereocenter at C5 and the butenolide ring at C17. Steroid **3.21** was subjected to hydrogenation with Pd/C in presence of 1% potassium hydroxide in methanol (**Scheme 3.8**). This C19 alcohol-directed hydrogenation resulted in the exclusive formation of the β -C5 center. The subsequent global oxidation of the crude material using Dess-Martin periodinane (DMP) followed by the selective reduction of the C19 aldehyde and C3 ketone in the presence of the C17 ketone by a bulky K-selectride yielded **3.33** in 62% yield over three steps after a single purification at the end of the third step. The iodination of **3.33** under Barton's protocol¹² produced vinyl iodide **3.34** in 77% yield. To confirm the stereochemistry of thus formed steroidal core, compound **77** was benzoylated to provide monobenzoate **3.35b** (90% yield) along with the crystalline bis-benzoate **3.35a** in 8% yield, the absolute and the relative configuration of which was confirmed by X-ray crystallographic analysis (**Scheme 3.8**).

Scheme 3.8. Synthesis of Key Vinyl Iodide **3.34**

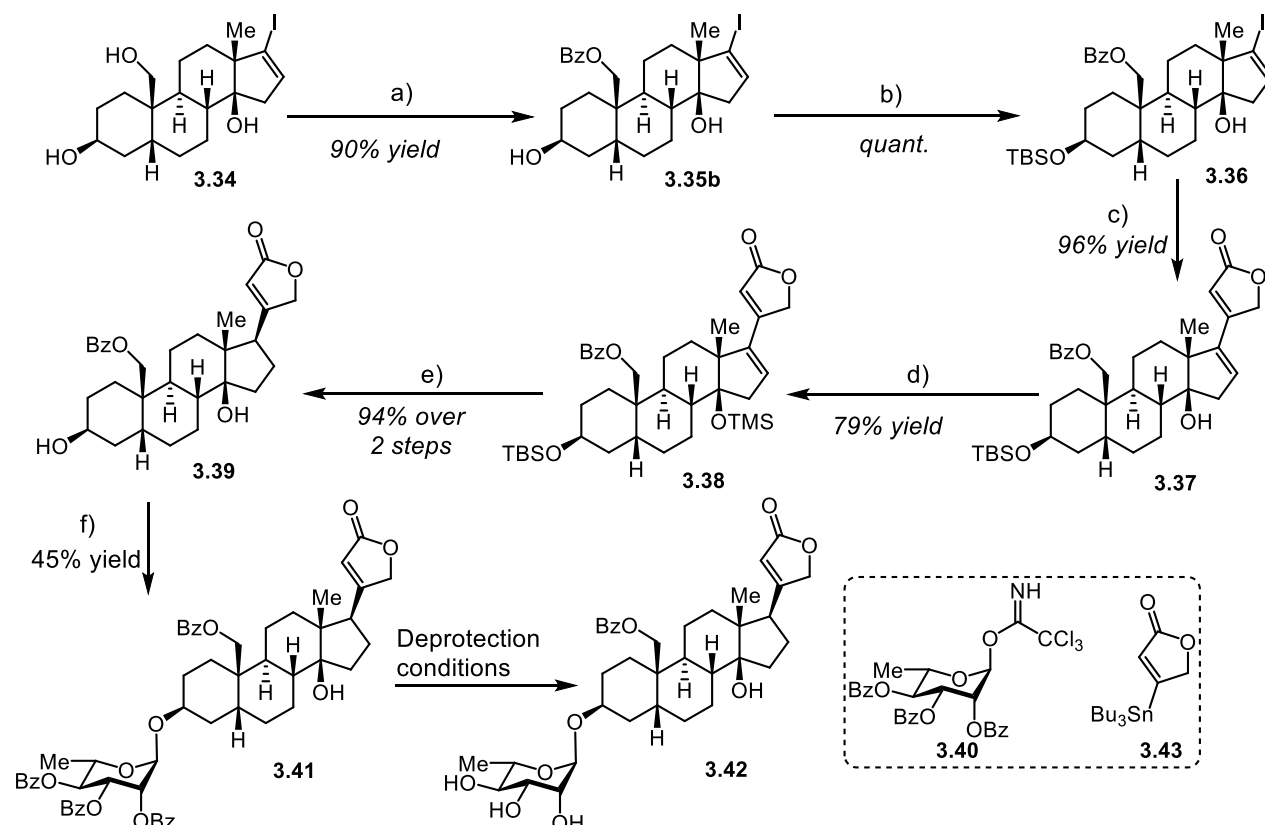


a) 1% KOH in MeOH, H₂, Pd/C (50 mol%), 1h; b) DMP (5 equiv), DCM, 3h; c) K-selectride, THF, -78 °C to -10 °C, 3h; d) H₂NNH₂, THF, Et₃N, 65 °C, 6h, then, I₂, Et₃N, rt, 30 min; e) BzCl, pyridine, DCM, 0 °C, 12h

Initial attempts to differentiate and selectively protect C19 alcohol as deemed necessary to selectively install sugar moieties at C3 at later stage with a variety of different silyl triflates or chlorides met with failure because of the steric bulk pocket around the primary alcohol presented by C1 and C11 methylene backbone. Despite this, we were pleased to find that acyl chlorides can differentiate the two alcohol and we were able to selectively protect the primary alcohol in excellent yield. As shown in **Scheme 3.8** and **Scheme 3.9**, protection of primary alcohol with benzoyl chloride in presence of pyridine afforded **3.35b** in 90% yield and the overreaction resulted in bis benzoyl protected compound **3.35a** in 8% yield. Protection of secondary alcohol with TBSOTf at C3 proceeded smoothly to provide vinyl iodide **3.36** which could then be subjected to Stille coupling reaction. The Stille coupling of **3.36** and stannylated butenolide **3.43** proceeded efficiently and resulted in steroid **3.37** in 96% yield. This product was elaborated to benzoylelled cannogenol **3.39** via a three-step sequence in 74% yield.^{13–15} This sequence involved TMS protection of the C14 tertiary alcohol, hydrogenation of the Δ16-olefin, and global deprotection of the silyl protecting groups. The TMS protection of the C14 tertiary alcohol was required to achieve a steric bias for the subsequent hydrogenation to give exclusive β-C17 center. Late stage

glycosylation for installing sugar proceeded with moderate yield. Under various basic conditions for ester hydrolysis (i.e., Na_2CO_3 , K_2CO_3 , NaOMe , LiOH , or NH_3), deprotection of the benzoyl esters on the rhamnose moiety could be achieved. However, the deprotection of the C19 benzoyl was slow and proceeded with the formation of multiple side products due to concomitant opening and isomerization of butenolide.

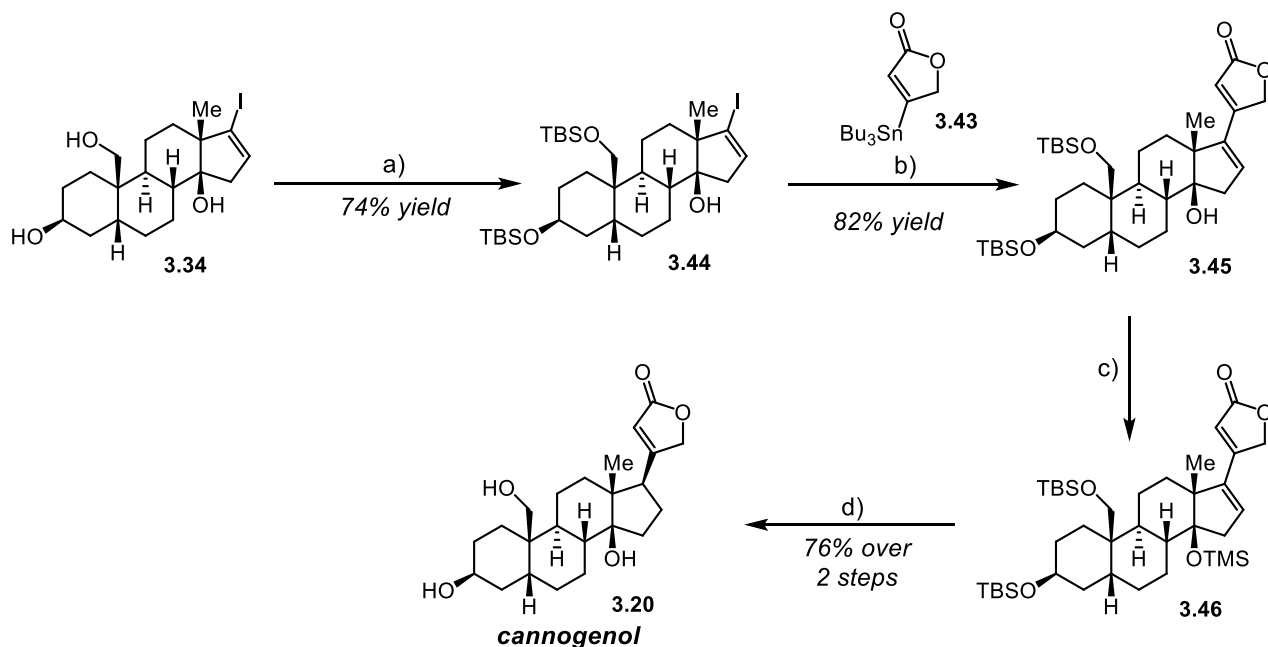
Scheme 3.9. Initial Route Towards the Total Synthesis of Cannogenol-3-*O*- α -L-rhamnoside



As the conditions for the deprotection of the C19 benzoate were not compatible with the butenolide functionality, alternative approach was employed. In this approach, cannogenol was first synthesized, then other protecting groups were explored for C19 alcohol. Hence, vinyl iodide **3.34** was subjected to TBS protection (**Scheme 3.10**) resulting in intermediate **3.44** in 74% yield.

This protection was necessary, as the direct Stille coupling of **3.34** and **3.43** was sluggish and resulted in lower yields. In contrast, the Stille coupling of **3.44** and commercially available stannylated butenolide **3.43** proceeded efficiently and resulted in the steroid **3.45** in 82% yield. This was followed by TMS protection, hydrogenation, and global deprotection of silyl groups as was described for benzoylated cannogenol to obtain the aglycone in 69% yield over 3 steps (**Scheme 3.10**). It should be noted that an attempt to accomplish TMS protection of C14 hydroxyl prior to the Stille coupling reaction, however, resulted in poor yields of the cross-coupled product. Thus, the sequence as depicted in **Scheme 3.10** constitutes the first enantioselective total synthesis of cannogenol.

Scheme 3.10. Total Synthesis of Cannogenol



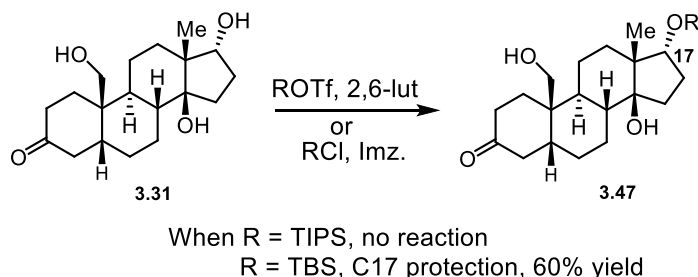
a) TBSOTf, 2,6-lutidine, DCM, -20 °C, 2 h; b) Pd(PPh₃)₄, CuCl, LiCl, DMSO, 70 °C, 2 h; c) TMSCl, imidazole, DMF, rt, 1 h; d) H₂, Pd/C, EA, 1 h then HCl, 3N MeOH, rt, 1 h

3.5. Enantioselective Total Synthesis of cannogenol-3-O- α -L-rhamnoside

3.5.1. Attempts towards differentiation of C3 and C19 alcohol with Silane Protecting Group

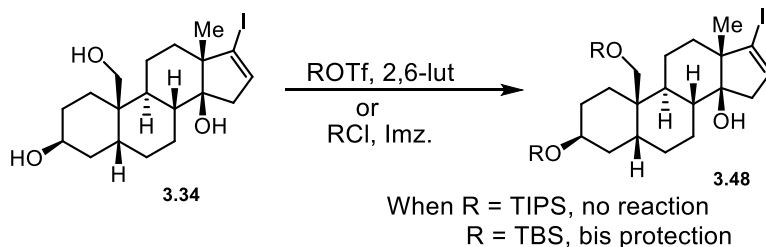
In order to avoid the challenging deprotection of primary esters in presence of base sensitive lactone moiety, we attempted to differentiate the C3 and the C19 alcohol using different silane protecting groups in various intermediates at different stages. We first attempted the selective silane protection of primary alcohol at C19 in presence of secondary alcohol at C17 in intermediate **3.31**, but to our disappointment, after exhaustive optimization of temperature and various reagents, no desired product was observed at all (**Scheme 3.11**). TIPSCl and TIPSOTf were too bulky and did not undergo the protection reaction. Similarly, no reaction was observed when TBSCl was used as a reagent. Unfortunately, heating the reaction with TBSOTf gave the undesired protection of C17 alcohol in 60% yield with no desired product observed.

Scheme 3.11. Attempt to Differentiate C19 and C17 Alcohol of Triol **3.31**



Similar attempt to differentiate the C19 and C3 alcohol of vinyl iodide **3.34** with TIPSOTf or TIPSCl was not successful (**Scheme 3.12**). Use of TBSCl or TBSOTf as the reagent provided no selectivity even after exhaustive screening and bis-TBS protected compound was isolated in each attempted reaction.

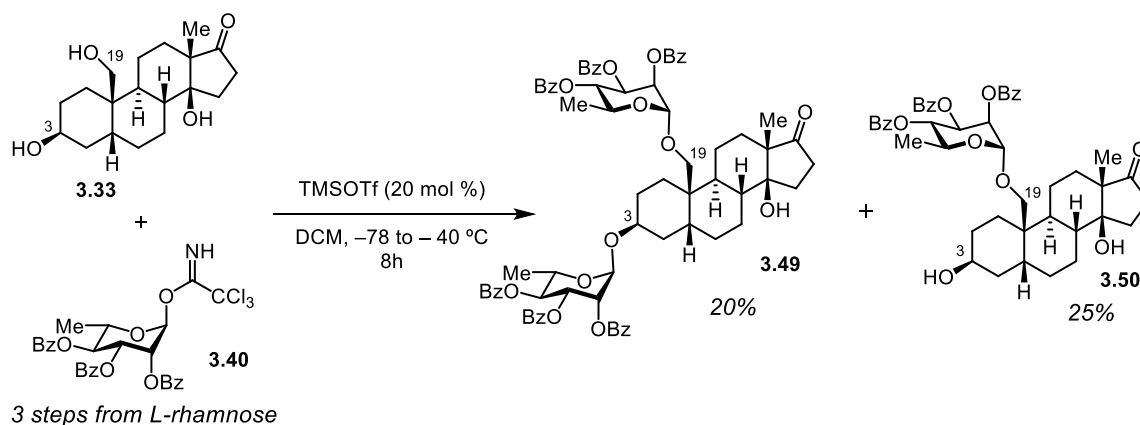
Scheme 3.12. Attempt to Differentiate C19 and C3 Alcohol of Vinyl Iodide **3.34**



3.5.2. Regioselective Glycosylation

After the successful synthesis of cannogenol, we investigated the possibility of adopting this route to the synthesis of cannogenol-3-*O*- α -L-rhamnoside (**3.19**). Our initial studies commenced with attempts to accomplish C3-selective glycosylation of triol **3.33** as a model substrate (**Scheme 3.13**). Attempts to glycosylate **3.33** with trichloroacetimidate **3.40**, which is available in three steps from L-rhamnose,¹⁶⁻¹⁸, however, resulted in the equimolar mixture of products **3.49** and **3.50** formed in ~70% conversion. This result indicated that the C19 hydroxyl is more reactive than the secondary C3 alcohol towards glycosylation. Attempts towards regioselective glycosylation to favor C3 hydroxyl using chiral phosphoric acids provided similar selectivity.

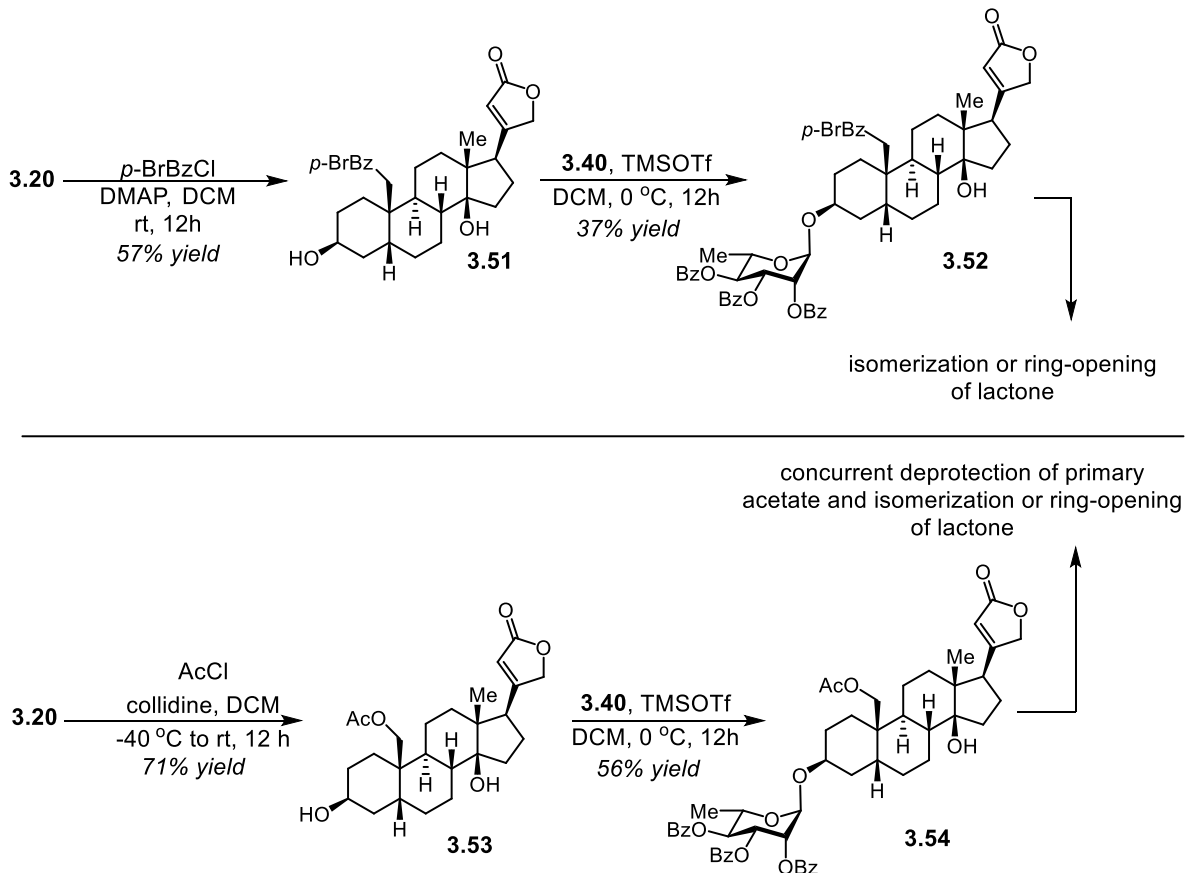
Scheme 3.13. Regioselective Glycosylation Attempt in a Model Substrate



3.5.3. C19 protection group exploration

Upon treating bromobenzoyl-protected C19 primary alcohol under basic conditions resulted in the similar side reactions that was observed in benzoyl-protected primary alcohol (**Scheme 3.14**). When acetate group was used as a protecting group for the C19 primary alcohol, in addition to similar isomerization and ring opening, to our excitement, we also detected concurrent deprotection of the acetate protecting group at C19 (**Scheme 3.14**) unlike C19 benzoyl group which was previously noticed to be difficult to cleave under basic conditions.

Scheme 3.14. Optimization of Protecting Group at C19



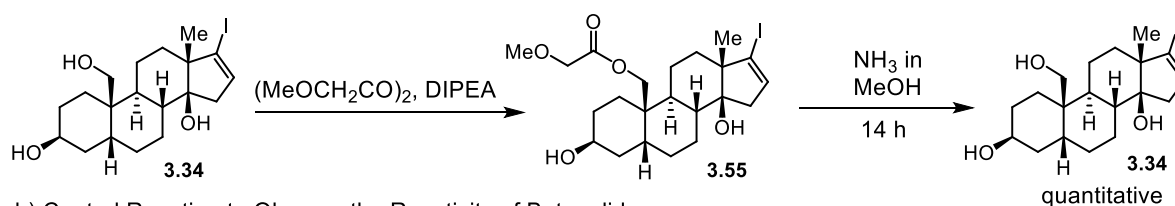
To address the protecting group challenges encountered earlier, a more labile methoxyacetate was investigated. This ester is reported to be almost 20 times easier to hydrolyze than the acetate protecting group.¹⁹ The selective protection of primary alcohol C19 was developed in an intermediate vinyl iodide as a model and highly selective C19 protection was observed (**Scheme 3.15a**). The reaction condition optimized in the model substrate was then replicated in the C19 protection of cannogenol (**3.20**), and not surprisingly the methoxyacetate protected cannogenol **3.56** was prepared in 99% yield (**Scheme 3.16**). This compound was subjected to TfOH catalyzed glycosylation with **3.40**, which resulted in α -rhamnoside **3.57** in 81% yield (>20:1 dr). The use of TMSOTf as promoter for glycosylation, however, resulted in diminished yield.

Two control experiments, as described below, were performed before the investigation of methoxyacetate as a protecting group in cannogenol:

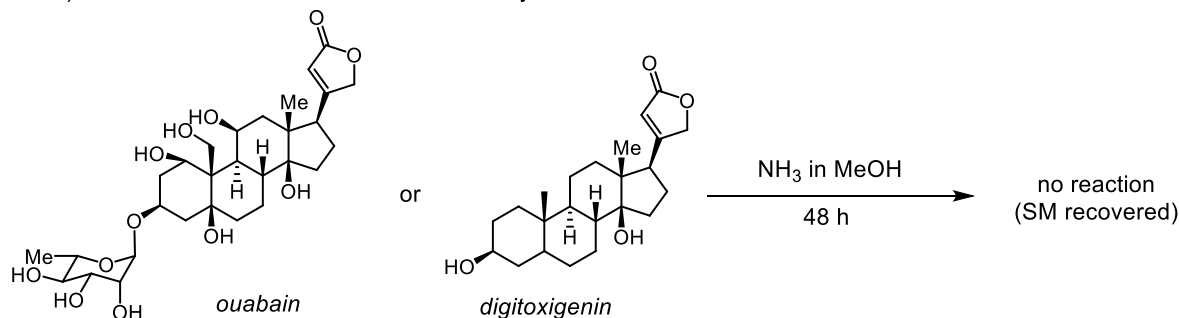
- i) *Determining the total time required for the deprotection of methoxyacetate from the C19 alcohol in model system.* In order to do that, the methoxyacetate protected vinyl iodide was exposed to ammonia solution in methanol and complete deprotection of methoxyacetate was observed after overnight stirring in sealed vial (**Scheme 3.15a**).

Scheme 3.15. Control Experiments to Ensure the Viability of Final Deprotection Step

a) Control Reaction for C19 Protection with Methoxyacetate



b) Control Reaction to Observe the Reactivity of Butenolide



- ii) *Determine the total time required for the reaction condition to react with butenolide.* The reactivity of ammonia in methanol towards the butenolide ring was tested in commercial cardiotonic steroid like digitoxigenin and ouabain and no side product from the reaction was observed when they were exposed to this reaction condition for up to 2 days. (**Scheme 3.15b**)

From these two control reactions, it was safely assumed that the final deprotection step would undergo completion overnight without impacting the lactone ring. And to our delight, when compound **3.57** was subjected to ammonia (50% solution in methanol), the desired cannogenol-3-

O- α -L-rhamnoside (**3.19**) was obtained in 69% yield. The $^1\text{H}/^{13}\text{C}$ NMR data and optical rotation data ($[\alpha]_{\text{D}}^{20} = -10.8$, $c = 0.147$ in MeOH, reported^{1,20} $[\alpha]_{\text{D}}^{20} = -15.5$, $c = 0.55$ in MeOH) of the synthetic **3.19** were in good agreement with the corresponding data obtained for the natural sample of **3.19**.^{1,3,4,20}

Scheme 3.16. Total Synthesis of Cannogenol-3-*O*- α -L-rhamnoside

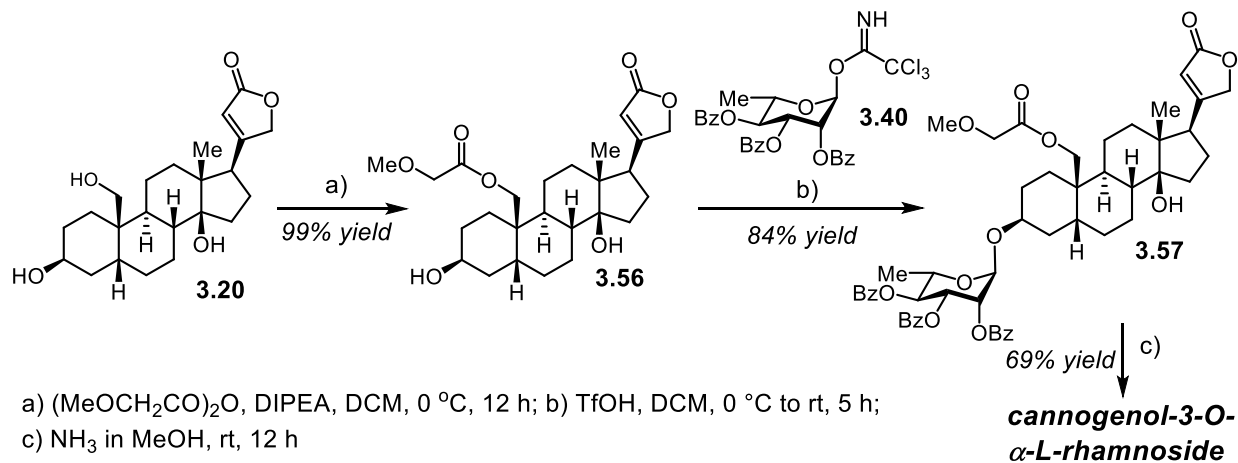
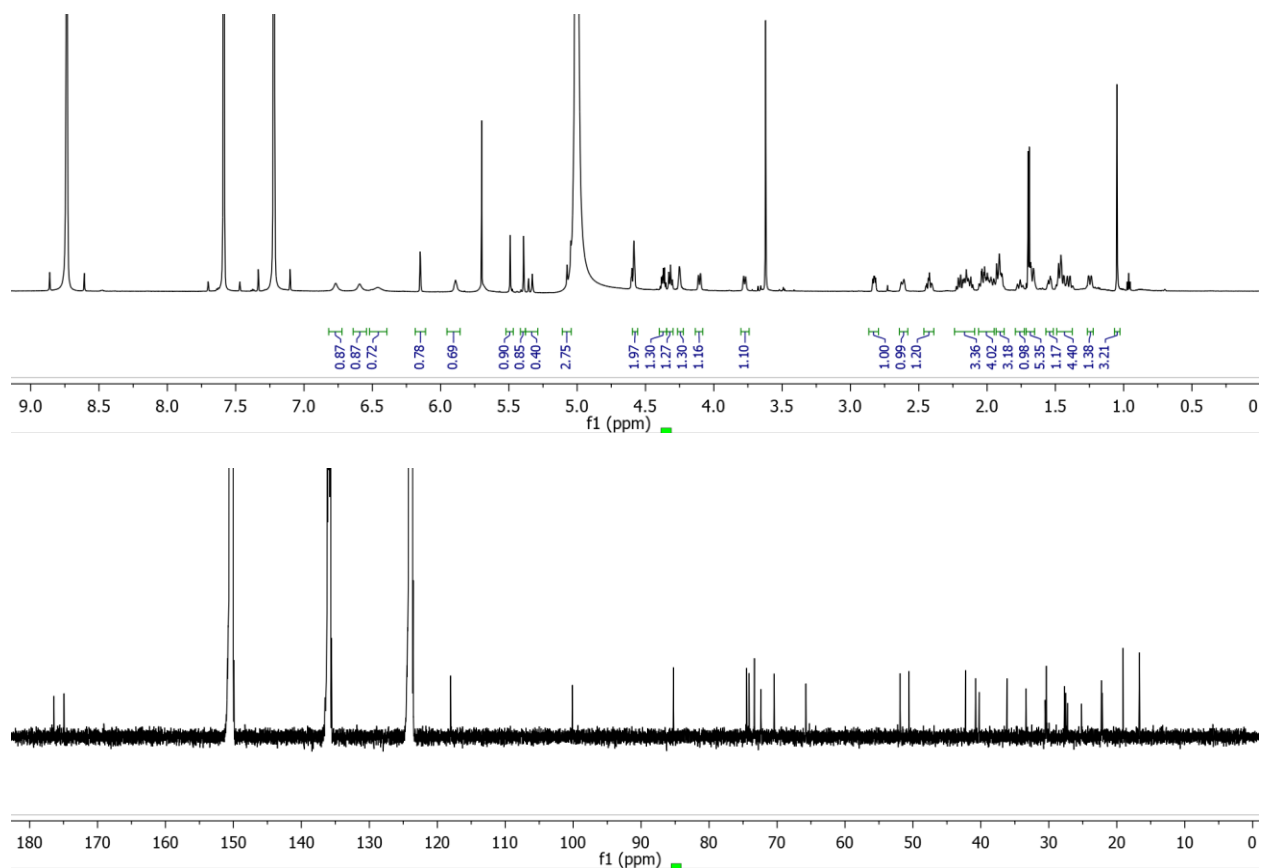


Figure 3.3. ^1H NMR and ^{13}C NMR of Synthetic Cannogenol-3-*O*- α -L-rhamnoside



3.6. Conclusion

In conclusion, a new asymmetric route to cardenolide steroids carrying the C19 oxygenation has been developed and applied to the synthesis of the natural products cannogenol **3.20** and cannogenol-3-*O*- α -L-rhamnoside **3.19**, the biological activity of which will be discussed in the following chapter. This approach features 3 g scale enantioselective synthesis of the functionalized cardenolide core **3.21** in 6 linear steps, 17% yield, and 92% ee involving an enantioselective Michael/tandem Aldol addition sequence established by our group earlier. This key intermediate could be elaborated to cardenolide cannogenol **3.20** in 9 steps, 20% yield. Cannogenol **3.20** contains multiple hydroxyl functionalities, and a strategy for the selective introduction of sugar moieties at the C3 position of **3.20** was developed and successfully applied to the formation of

cannogenol-3-*O*- α -L-rhamnoside **3.19**. The herein described strategies will expedite medicinal chemistry studies on C19- hydroxylated cardenolides related to cannogenol, and the exploration of **3.19**, **3.20**, and related analogs is the subject of ongoing investigation by our group.

3.7. Experimental (as it appears in Bhattarai, B.; Nagorny, P. Enantioselective Total Synthesis of Cannogenol-3-O- α -L-rhamnoside via Sequential Cu(II)-Catalyzed Michael Addition/Intramolecular Aldol Cyclization Reactions. *Org. Lett.* **2018**, 20, 154-157 and a part derived from Cichowicz, N. R.; Kaplan, W.; Khomutnyk, Y.; Bhattarai, B.; Sun, S.; Nagorny, P. Concise Enantioselective Synthesis via Sequential Copper (II)-Catalyzed Michael Addition/Intramolecular Aldol Cyclization Reactions. *J. Am. Chem. Soc.* **2015**, 137, 14341-14348)

I. General Information

i) Reaction equipment, solvents, reagents, and conditions

All the reagents were purchased of highest quality from the commercial sources and used without further purification unless otherwise noted. All reactions were carried out under an atmosphere of nitrogen in flame or oven dried glassware with a magnetic stirrer. 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 purification and extraction were ACS or HPLC grade. Toluene, dichloromethane, diethyl ether, tetrahydrofuran, and dimethylformamide were filtered through a column of activated alumina under nitrogen atmosphere (Innovative Technology PS-MD-5). Dry ice/acetone bath was used to create -78 °C reaction temperature and Ice/water bath was used to create 0 °C. Cryocool was used for extended low temperature experiments and all other low temperature.

ii) Reaction monitoring and purification

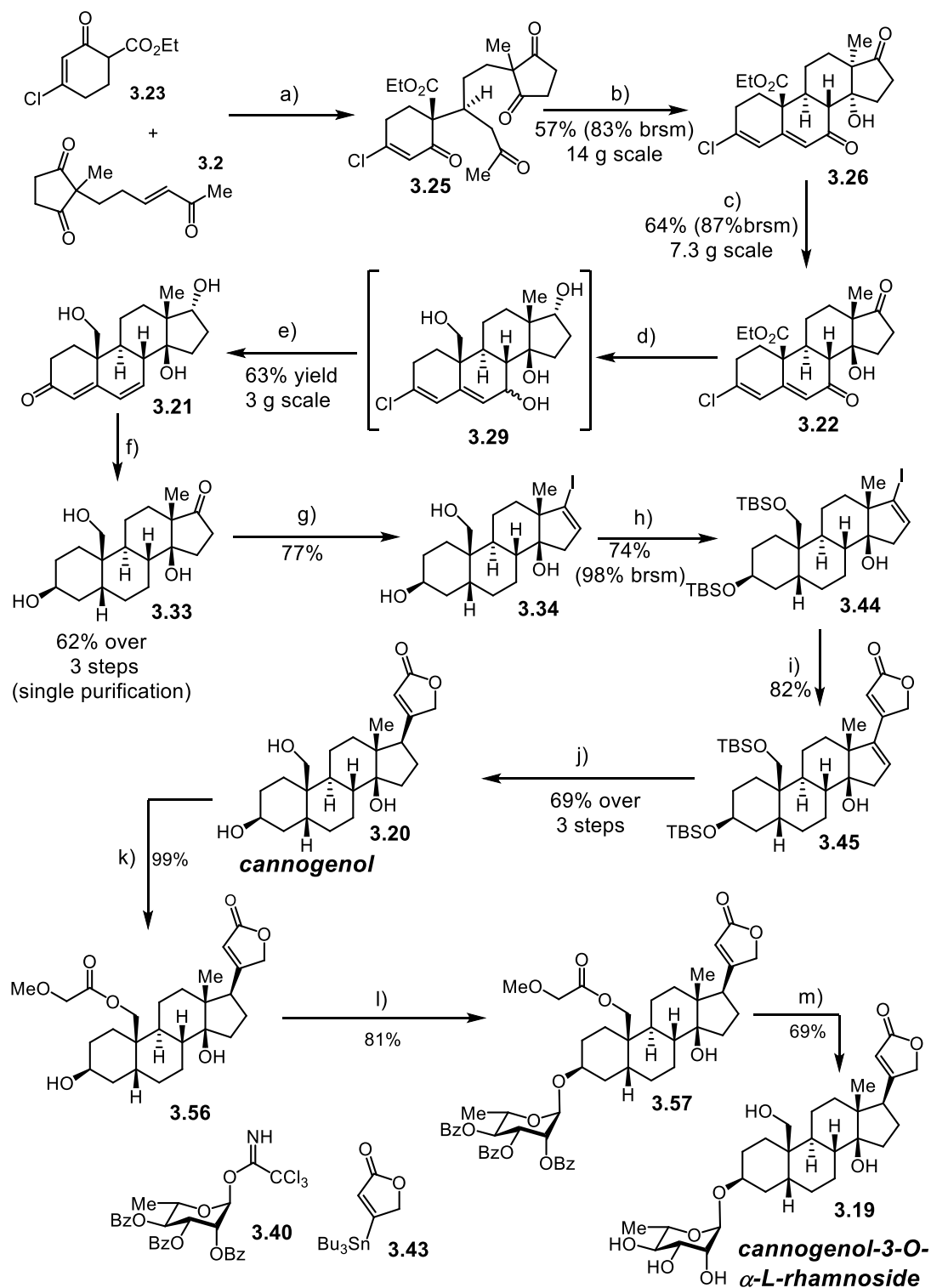
Thin layer chromatography (TLC) was conducted on precoated glass plates with 230-400 mesh silica gel impregnated with fluorescent indicator (250 nm) for routine monitor of reaction progress and visualized using the combination of UV and ceric ammonium

molybdate. All products were purified by flash column chromatography using SiliCycle Silica Flash P60 (230-400 mesh) silica gel. Alternatively, Combiflash Rf+ Lumen Automated Flash Chromatography System with UV and ELSD detector using SiliCycle Silica Flash P60 (230-400 mesh) silica gel was used for purification.

iii) Analysis and characterizations

High-performance liquid chromatography (HPLC) analysis was performed using Waters e2695 Separations Module with a Waters 2998 photodiode array detector on a DAICEL CHIRALPAK AD column to determine the enantiomeric excess. NMR spectra were recorded on Varian vnmrs 700 (700MHz), Varian vnmrs 500 (500 MHz), or Varian Inova 500 (500 MHz) spectrometers and chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane with solvent resonance as the internal standard (CDCl_3 at δ 7.26, CD_3OD at 3.32, or DMSO-d_6 at δ 2.5, $\text{C}_5\text{D}_5\text{N}$ at δ 7.22). Proton coupling patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). High resolution mass spectra (HRMS) were obtained from Agilent 6230 TOF HPLC-MS Magnetic Sector mass spectrometers at the University of Michigan mass spectrometry facility. Infrared spectra (IR) were recorded as thin films on a Perkin Elmer Spectrum BX FT-IR spectrophotometer and were reported in wavenumbers (cm^{-1}). Optical rotation was measured at room temperature in methylene chloride or methanol on a Jasco P-2000 polarimeter.

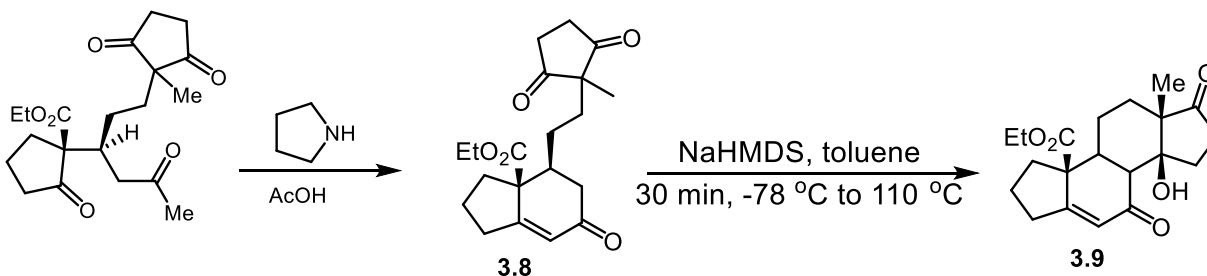
II. Experimental Procedures and Spectroscopic Data



a) Cu(BOX), neat, -10 °C; b) *p*-TSA, THF, 60 °C; c) NaHMDS, PhMe, 40 °C; d) DIBAL-H, THF, 60 °C, 18 h; e) HCOOH, H₂O:THF (8:2); f) H₂, Pd/C in KOH/MeOH, then DMP, DCM, then, K-selectride; g) H₂NNH₂, EtOH, then, I₂, Et₃N; h) TBSOTf, 2,6-lutidine; i) **3.43**,

Pd(PPh₃)₄, CuCl, LiCl; j) TMSOTf, imidazole, then, H₂, Pd/C, then, HCl, MeOH; k) (MeOCH₂CO)₂O, DIPEA, DCM; l) **3.40**, TfOH, DCM, 0 °C to rt; m) NH₃ in MeOH.

Ethyl (3a*S*,5a*S*,8a*S*)-8a-hydroxy-5a-methyl-6,9-dioxo-2,3,3*b*,4,5,5a,6,7,8,8a,8*b*,9-dodecahydrodicyclopenta[*a,f*]naphthalene-3a(1*H*)-carboxylate (3.9**)**



Michael adduct (390 mg, 1.07 mmol, 1.0 equiv.) was dissolved in EtOAc (10 mL, 0.1 M). Pyrrolidine (90 μ L, 1.07 mmol, 1.0 equiv.) and AcOH (66 μ L, 1.07 mmol, 1.0 equiv.) were added and the reaction mixture was stirred overnight. The reaction mixture was concentrated in vacuo and purified directly by column chromatography (grad. 0% \rightarrow 20% acetone in hexanes) to afford **3.8** along with the recovered starting material (78 mg, 0.225 mmol, 21% yield).

HRMS (ESI-MS) calculated for C₂₂H₃₀O₅ [M+H]⁺: m/z 347.1853, found: 347.1857

Compound **3.8** (72 mg, 0.21 mmol, 1.0 equiv.) was dissolved in toluene (2 mL, 0.1 M) and cooled to -78 °C. A solution of NaHMDS (46 mg, 0.25 mmol, 1.2 equiv.) in toluene was added dropwise. The reaction mixture was then stirred for 15 minutes at -78 °C. The reaction mixture was then immediately heated to 110 °C and stirred for 30 minutes. The reaction mixture was then filtered through a plug of silica gel and washed with EtOAc to afford **3.9** (43 mg, 0.34 mmol, 60% yield, 20:1 dr, 92% ee) as a white solid along with the recovered starting material (6.0 mg, 0.048 mmol, ~14%). Enantiopurity was determined to be 92% ee by chiral HPLC (DAICEL CHIRALPAK OJ-H, 25 cm x 4.6 mm, hexanes/2-propanol = 95/5, flow rate = 1 mL/min, λ = 245.0 nm, RT(major) = 19.9 min, RT(minor) = 33.2 min).

¹H NMR (500 MHz, CDCl₃) δ 6.01 (s, 1H), 4.81 (s, 1H), 4.26 (dq, J = 7.2, 10.9 Hz, 1H), 4.19 (dq, J = 7.2, 10.9 Hz, 1H), 2.77-2.73 (m, 2H), 2.71-2.67 (m, 1H), 2.58-2.49 (m, 2H), 2.37 (ddd, J = 3.4, 7.3, 19.1 Hz, 1H), 2.04-2.01 (m, 2H), 1.96-1.86 (m, 2H), 1.77-1.71 (m, 2H), 1.53 (qd, J = 3.8, 13.5 Hz, 1H), 1.45-1.37 (m, 2H), 1.33-1.25 (m, 5H), 1.07 (s, 3H)

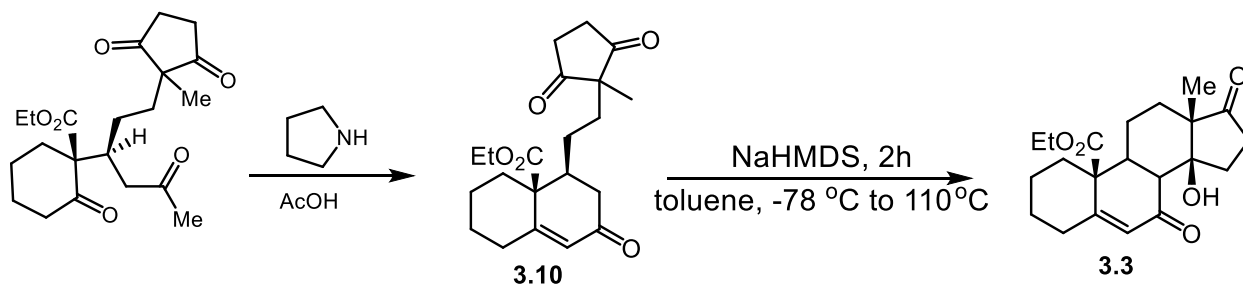
¹³C NMR (126 MHz, CDCl₃) δ 219.9, 202.6, 171.2, 170.4, 124.3, 80.2, 61.8, 58.5, 53.2, 48.7, 46.7, 36.4, 32.9, 30.9, 30.8, 27.7, 23.5, 21.4, 14.3, 13.0

[α]_D²⁵ = +31.8 (c = 0.4, CHCl₃)

HRMS (ESI-MS) calculated for C₂₀H₂₆O₅ [M+H]⁺: *m/z* 347.1853, found: 347.1851

IR = (thin film, cm⁻¹) 3458 (br), 2936, 1731, 1645, 1391, 1209, 1046, 732

Ethyl (10S,13S,14S)-14-hydroxy-13-methyl-7,17-dioxo-1,2,3,4,7,8,9,11,12,13,14,15,16,17-tetradecahydro-10H-cyclopenta[a]phenanthrene-10-carboxylate (3.3)



Michael adduct (518 mg, 1.32 mmol, 1.0 equiv.) was dissolved in EtOAc (13 mL, 0.1 M). Pyrrolidine (110 μL, 1.32 mmol, 1.0 equiv.) and AcOH (82 μL, 1.32 mmol, 1.0 equiv.) were added and the reaction mixture was stirred overnight. The reaction mixture was diluted with EtOAc and washed with aq. NaHCO₃ and brine. The organic layer was then dried over MgSO₄, filtered, and concentrated in vacuo. The reaction mixture was then purified by column chromatography (grad. 20%→40% EtOAc in hexanes) to afford **3.10** (328 mg, 0.60 mmol, 19:1 dr, 56% yield, 88% ee). Enantiopurity was determined to be 88% 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 = 223.0 nm, RT(minor) = 8.5 min, RT(major) = 11.0 min).

¹H NMR (500 MHz, CDCl₃) δ 5.92 (s, 1H), 4.17-4.13 (m, 2H), 2.76-2.59 (m, 5H), 2.42-2.38 (m, 2H), 2.26-2.21 (m, 1H), 2.08-2.03 (m, 1H), 1.84-1.82 (m, 1H), 1.78-1.71 (m, 3H), 1.63 (q, J = 7.0 Hz, 2H), 1.51-1.44 (m, 3H), 1.37 (qt, J = 3.5, 12.6 Hz, 1H), 1.27-1.19 (m, 1H), 1.22 (t, J = 7.0 Hz, 3H), 1.05 (td, J = 4.2, 13.3 Hz, 1H), 0.78 (t, J = 7.7 Hz, 3H), 0.67 (qd, J = 4.6, 11.2 Hz, 1H)

¹³C NMR (126 MHz, CDCl₃) δ 216.9, 216.6, 198.5, 170.7, 163.0, 126.4, 61.3, 61.3, 52.3, 44.5, 38.8, 36.2, 36.1, 34.9, 31.7, 28.9, 26.5, 25.0, 23.1, 14.3, 8.8

$[\alpha]_D^{20}$ = -29.3 (c = 0.095, CH₂Cl₂)

HRMS (ESI-MS) calculated for C₂₂H₃₀O₅ [M+H]⁺: *m/z*. 375.2166, found: 375.2169

Compound **3.10** (33 mg, 0.088 mmol, 1.0 equiv.) was dissolved in toluene (0.88 mL, 0.1 M) and cooled to -78 °C. A solution of LiHMDS (15 mg, 0.88 mmol, 1.0 equiv.) in THF was added slowly. The reaction mixture was then stirred for 15 minutes at -78 °C. The reaction mixture was then immediately heated to 60 °C and stirred for 45 minutes. THF was removed by concentrating in vacuo and the reaction mixture was purified directly by column chromatography (grad. 5%→15% acetone in hexanes) to afford **3.3** (17 mg, 0.045 mmol, 52% yield, 88% ee) as a white solid. Enantiopurity was determined to be 88% ee by chiral HPLC (DAICEL CHIRALPAK AD-H, 25 cm x 4.6 mm, hexanes/2-propanol = 90/10, flow rate = 1 mL/min, λ = 225.0 nm, RT(minor) = 8.1 min, RT(major) = 10.2 min).

¹H NMR (700 MHz, CDCl₃) δ 5.97 (s, 1H), 4.52 (s, 1H), 4.29 (dq, J = 7.0, 10.5 Hz, 1H), 4.22 (dq, J = 7.0, 10.5 Hz, 1H), 2.80 (d, J = 13.3 Hz, 1H), 2.75-2.73 (m, 1H), 2.52-2.43 (m, 2H), 2.35-2.30 (m, 1H), 2.12-2.04 (m, 3H), 1.94-1.88 (m, 2H), 1.85-1.77 (m, 3H), 1.73 (dt, J = 2.8, 14.0 Hz,

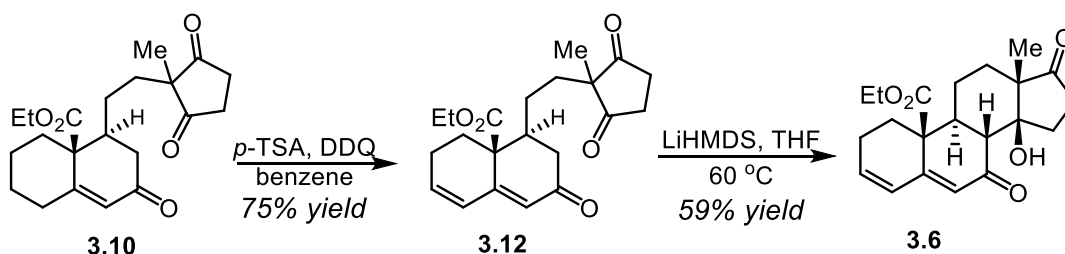
1H), 1.58 (dt, $J = 3.5, 14.0$ Hz, 1H), 1.43 (qt, $J = 3.9, 14.1$ Hz, 1H), 1.35 (sext, $J = 7.4$ Hz, 1H), 1.29 (t, $J = 7.7$ Hz, 3H), 1.21-1.13 (m, 2H), 1.11-1.07 (m, 1H), 1.06 (t, $J = 7.7$ Hz, 3H)

^{13}C NMR (175 MHz, CDCl_3) δ 220.3, 201.9, 170.3, 164.1, 126.8, 81.6, 61.6, 54.3, 52.2, 48.6, 45.3, 36.6, 34.8, 33.3, 28.0, 26.7, 26.5, 23.2, 22.0, 20.3, 14.4, 8.9;

HRMS (ESI-MS) calculated for $\text{C}_{22}\text{H}_{30}\text{O}_5$ $[\text{M}+\text{H}]^+$: m/z 375.2166, found: 375.2165

IR (thin film, cm^{-1}) 3517 (br), 2938, 1730, 1654, 1185

Ethyl (8R,9S,10S,13S,14S)-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 (3.6)



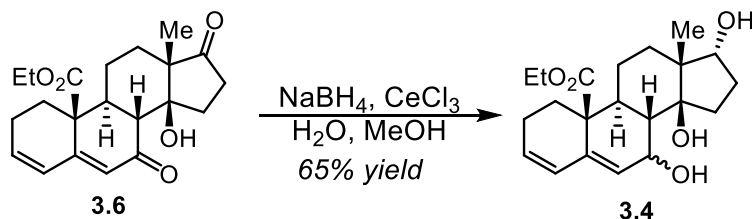
Monocyclized enone **3.10** (3.2 mmol, 1.17 g, 1 equiv.) was dissolved in 100 mL benzene and $p\text{-TsOH}$ (6.4 mmol, 1.1 g, 2 equiv.) was added to the reaction vial. DDQ (3.9 mmol, 0.89 g, 1.2 equiv.) was dissolved in 30 mL benzene and transferred to the reaction mixture and heated to $80\text{ }^\circ\text{C}$. After 2 h, The reaction mixture was dissolved in 50 mL DCM, washed with water, 2% NaOH , and brine then dried with MgSO_4 and concentrated *in vacuo* to obtain slightly yellow residue of **3.12** that appeared pure by crude NMR and was subjected to next step without further purification. To a 10 mL vial with crude extended enone **3.12** (0.279 mmol, 100 mg, 1 equiv.) was added 2 mL THF and cooled to $-78\text{ }^\circ\text{C}$. In a different vial, LiHMDS (0.335 mmol, 56 mg, 1.2 equiv.) was weighed and diluted in 1 mL THF and added dropwise to the solution of enone at $-78\text{ }^\circ\text{C}$. The reaction mixture was stirred at $-78\text{ }^\circ\text{C}$ for 20 minutes then transferred to preheated oil bath at $60\text{ }^\circ\text{C}$. Color change from yellow to dark brown was observed. The reaction was monitored by TLC and stopped after 2 h or upon completion. The reaction mixture was passed through silica plug and

concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (3:2 Hexanes: EtOAc) to give **3.6** (84 g, 84%) as white solid.

¹H NMR (700 MHz, Benzene-*d*₆) δ 5.88 (s, 1H), 5.80 (dd, *J* = 9.8, 2.8 Hz, 1H), 5.74 (dd, *J* = 5.9, 2.6 Hz, 1H), 5.17 (s, 1H), 3.87 (dq, *J* = 10.9, 7.0 Hz, 1H), 3.65 (dq, *J* = 11.1, 7.2 Hz, 1H), 2.87 (d, *J* = 13.2 Hz, 1H), 2.48 (dt, *J* = 19.3, 9.9 Hz, 1H), 2.36 (dd, *J* = 13.0, 5.1 Hz, 1H), 2.27 (dddd, *J* = 16.8, 11.5, 5.2, 2.4 Hz, 1H), 2.12 (ddd, *J* = 18.8, 9.3, 1.4 Hz, 1H), 1.97 – 1.89 (m, 1H), 1.79 (dt, *J* = 19.7, 5.6 Hz, 1H), 1.65 – 1.55 (m, 2H), 1.52 – 1.45 (m, 1H), 1.32 (s, 3H), 1.25 (qd, *J* = 13.3, 3.6 Hz, 1H), 1.19 (dt, *J* = 14.0, 3.4 Hz, 1H), 0.94 (ddd, *J* = 13.1, 11.5, 5.4 Hz, 2H), 0.78 (t, *J* = 7.1 Hz, 3H).

HRMS (ESI-MS) calculated for C₂₁H₂₆O₅ [M+H]⁺: *m/z* 359.1858, found: 359.1855

Ethyl (8*S*,9*S*,10*S*,13*R*,14*S*,17*R*)-7,14,17-trihydroxy-13-methyl-1,2,7,8,9,11,12,13,14,15,16,17-dodecahydro-10*H*-cyclopenta[*a*]phenanthrene-10-carboxylate (3.4)

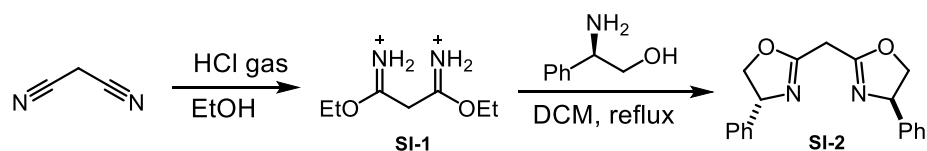


To the vial was added **3.6** (0.025 mmol, 9 mg, 1 equiv.) and dissolved in 1 mL MeOH. CeCl₃·7H₂O (0.028 mmol, 10 mg, 1.1 equiv.) was added to the reaction mixture and cooled to -78 °C. NaBH₄ (0.0375 mmol, 1.5 mg, 1.5 equiv.) was then added slowly and stirred for 4 h. The reaction mixture was quenched with water and the aqueous layer was extracted with EtOAc (3×5 mL), then with 5 mL brine. The combined organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (9:1 DCM: MeOH) to give **3.4** (5.8 mg, 65%) as white solid.

¹H NMR (700 MHz, CDCl₃) δ 6.05 (dd, *J* = 9.7, 2.5 Hz, 1H), 5.74 (t, *J* = 7.9 Hz, 1H), 5.60 (d, *J* = 2.7 Hz, 1H), 4.46 (d, *J* = 8.7 Hz, 1H), 4.34 – 4.23 (m, 1H), 4.15 (ddt, *J* = 10.5, 7.2, 3.6 Hz, 1H), 3.73 (d, *J* = 6.3 Hz, 1H), 2.69 (d, *J* = 9.4 Hz, 1H), 2.51 (dd, *J* = 12.9, 5.6 Hz, 1H), 2.38 – 2.31 (m, 1H), 2.28 – 2.18 (m, 1H), 2.16 – 2.07 (m, 2H), 2.02 (td, *J* = 7.1, 6.2, 3.5 Hz, 2H), 1.96 – 1.86 (m, 1H), 1.80 – 1.68 (m, 1H), 1.42 (td, *J* = 12.5, 4.2 Hz, 1H), 1.37 – 1.31 (m, 3H), 1.29 (td, *J* = 7.2, 0.0 Hz, 3H) 1.09 (s, 3H), 0.88 (t, *J* = 6.8 Hz, 1H).

Synthesis of catalyst

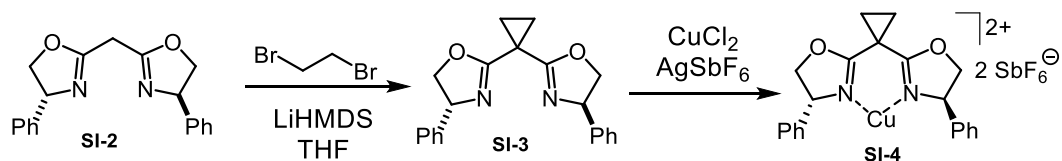
Bis((R)-4-phenyl-4,5-dihydrooxazol-2-yl)methane (SI-2)



To the flask with malonitrile (0.151 mol, 10 g, 1 equiv.) in EtOH (0.31 mol, 18 mL, 2.05 equiv.) and dioxane (230 mL) at 0 °C was bubbled HCl gas (produced by H₂SO₄ and NaCl) upon which white precipitation was observed. The reaction was warmed to rt and HCl gas was bubbled slowly for 2 days. The solution was filtered and the solid was washed with dry Et₂O. The resulting white solid was dried and used in subsequent step. The spectral data obtained matches those reported previously.²¹

The flask with diethylmalonimidate **SI-1** (0.0865 mol, 20 g, 1 equiv.) and R(-)-2-phenylglycinol (0.182 mol, 25 g, 2.1 equiv.) in DCM (290 mL) was refluxed overnight. The solution was quenched with 120 mL water and the aqueous layer was extracted with DCM (3 × 450 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (9:1 DCM: MeOH) to give **SI-2** (21 g, 83%) as brown viscous oil. The spectral data obtained matches those reported previously.²²

Cyclopropanation and complexation (**SI-4**)²³



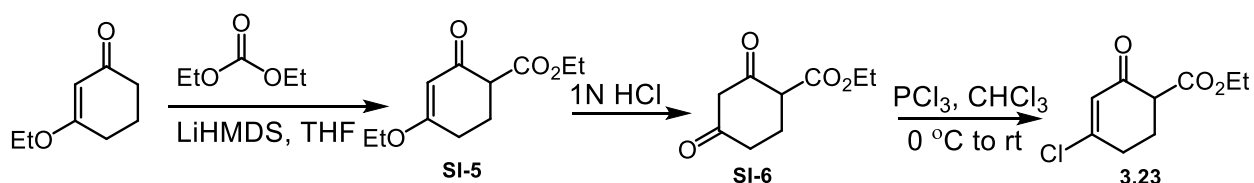
To a flask with bis((R)-4-phenyl-4,5-dihydrooxazol-2-yl)methane **SI-2** (3.3 mmol, 1 g, 1 equiv.) in dry THF was added dibromoethane (6.6 mmol, 560 μ L, 2 equiv.) and LiHMDS (8.9 mmol, 1.5 g, 2.7 equiv.) at rt and stirred under N₂ for 6 h. Additional LiHMDS (3.3 mmol, 550 mg, 1 equiv.) and dibromoethane (3.3 mmol, 280 μ L, 1 equiv.) was added to the reaction mixture and stirred for 16 h. The reaction mixture was quenched with saturated aqueous NH₄Cl and the aqueous layer was extracted with EtOAc (3 \times 100 mL). The combined organic layers were washed with saturated aqueous NaHCO₃, brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on triethylamine pretreated silica gel (2:0.5:7.5 EtOAc: Et₃N: Hexanes to 9.5: 5 EtOAc: Et₃N) to give **SI-3** (806 mg, 74%) as brown viscous oil. The spectral data obtained matches those reported previously.⁶

To the flask with (4R,4'R)-2,2'-(cyclopropane-1,1-diyl)bis(4-phenyl-4,5-dihydrooxazole) **SI-3** (5.87 mmol, 1.95 g, 1 equiv.), azeotroped with toluene, was added CuCl₂ (5.7 mmol, 0.765 g, 0.97 equiv.), dissolved in DCM (29 mL) and stirred for 3 h. AgSbF₆ (11.4 mmol, 3.9 g, 1.94 equiv.) dissolved in 8 mL DCM was added to the reaction mixture and stirred for 2.5 h. Upon the addition of dry THP to this brown reaction mixture, white precipitate was observed while the solution turned green. This mixture was filtered through plug of activated celite into a dry flask and rinsed with THP several times. The resulting green solution was concentrated and azeotroped with toluene. The catalyst was further dried by dissolving it in dry DCM and dried with activated 4 Å molecular sieves overnight. The solution was transferred to a dry flask and dried by blowing

nitrogen and then left it in vacuum before transferring the dark green solid **SI-4** (3 g, 56%) to the glovebox.

Synthesis of Building blocks:

Ethyl 4-chloro-2-oxocyclohex-3-ene-1-carboxylate (**3.23**)

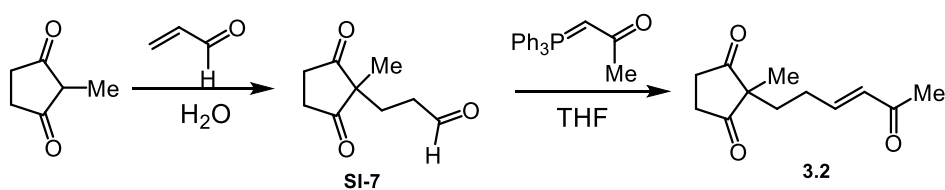


To the solution of LiHMDS (1.712 mol, 286 g, 2 equiv.) in THF (3 L) cooled to -78 °C was added 3-ethoxy-2-cyclohexenone (0.856 mol, 120 g, 1 equiv.) and stirred for 1 h. Diethyl carbonate (1.712 mol, 202 g, 2 equiv.) was added slowly to the reaction mixture and stirred for 10 minutes. The resulting yellow solution was slowly warmed to rt and stirred overnight. The reaction was quenched with saturated aqueous NH₄Cl and the aqueous layer was extracted with DCM (4 × 800 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The crude yellow solid was triturated with hexanes to obtain white solid **SI-5** upon filtration.

The crude residue thus obtained was dissolved in THF (780 mL) and water (70 mL). After slow addition of 1N HCl (560 mL), the reaction mixture was stirred for 6 h at rt. The reaction was quenched with saturated aqueous NaHCO₃ and the aqueous layer was extracted with DCM (3 × 500 mL) and the organic layer was discarded. To the aqueous layer, conc. HCl was added to obtain pH of 2 and extracted with DCM (3×700 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo* to obtain yellow solid **SI-6** that was used directly in the subsequent reaction without further purification.

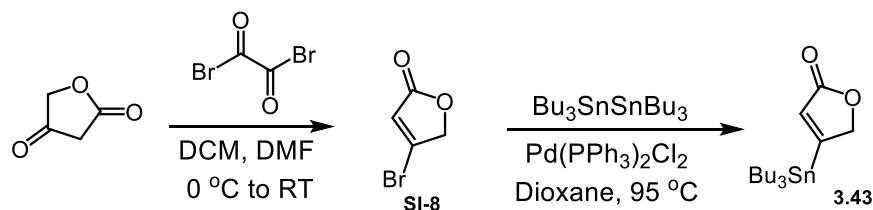
To the solution of crude **SI-6** in CHCl_3 (900 mL) at $0\text{ }^\circ\text{C}$ was added PCl_3 (1.712 mol, 153 mL, 2 equiv.) slowly and stirred for 2 hr. The reaction mixture was warmed to rt and stirred overnight. Resulting yellow solution was concentrated directly to remove excess PCl_3 and redissolved in CHCl_3 . This solution was filtered through cotton and concentrated. The oily residue was then purified by flash chromatography on silica gel (9:1 Hexanes: Ethyl acetate) to give chloroketoester **3.23** (109 g, 63%) as colorless oil. The spectral data obtained matches those reported previously.⁶

(E)-2-methyl-2-(5-oxohex-3-en-1-yl)cyclopentane-1,3-dione (3.2)



To the flask with 2-methyl-1,3-cyclopentanedione (0.535 mol, 60 g, 1 equiv.) in water (1.2 L) at rt was added acrolein (0.749 mol, 50 mL, 1.4 equiv.) and stirred at rt overnight under N_2 . The reaction mixture was extracted with EtOAc ($3 \times 500\text{ mL}$). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , filtered and concentrated *in vacuo* to obtain colorless liquid **SI-7** that was used directly in the subsequent reaction without further purification. To the solution of crude residue in THF (1.2 L) was added 1-(triphenylphosphoranylidene)-2-propanone (y) (0.749 mol, 238 g, 1.4 equiv.) and allowed to stir overnight at rt. This solution was concentrated *in vacuo* and purified directly by flash chromatography on silica gel (7:3 Hexanes: Ethyl acetate) to give enone **3.2** (81 g, 73%) as viscous colorless oil. The spectral data obtained matches those reported previously.⁶

4-(tributylstannyl)furan-2(5H)-one (3.43)

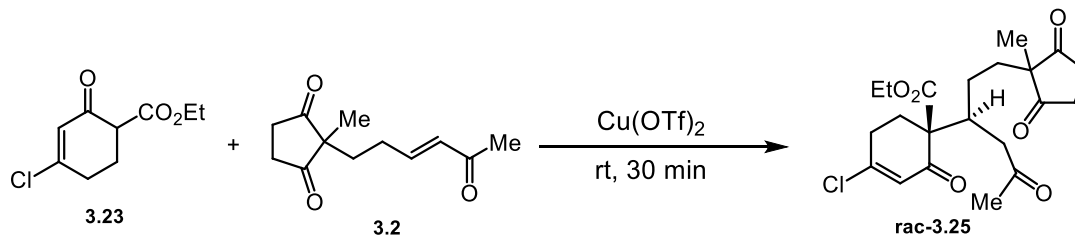


To the solution of tetronic acid (0.05 mol, 5 g, 1.0 equiv.) in DCM (110 mL) and DMF (5 mL) at 0 °C was added oxalyl bromide (0.06 mol, 5.6 mL, 1.2 equiv.) over 1 hr to obtain orange colored solution. The reaction was warmed to rt and stirred for 2 h. The resulting greenish orange solution was quenched with water (100 mL) and organic layer was separated. The aqueous layer was extracted with diethyl ether (3 × 100 mL). The combined organic layers were washed with water, saturated aqueous NaHCO₃, brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The dark brown solid was recrystallized with ether to obtain brown needles shaped solid **SI-8** (6.5 g, 80% yield). The spectral data obtained matches those reported previously.²⁴

To a flask in glovebox was added 4-bromofuran-2(5H)-one **SI-8** (8.3 mmol, 1.35 g, 1 equiv.), hexabutylstannane (8.3 mmol, 4.8 g, 1 equiv.), and Pd(PPh₃)₂Cl₂ (0.83, 580 mg, 10 mol%). This mixture was dissolved in dioxane (80 mL) and the resulting yellow colored solution was stirred at 95 °C for 16 h. The solution was concentrated *in vacuo* and purified directly by florisil (8:2 Hexanes: Ethyl acetate) to give **3.43** (1.9 g, 73%) as viscous colorless oil. The spectral data obtained matches those reported previously.²⁵

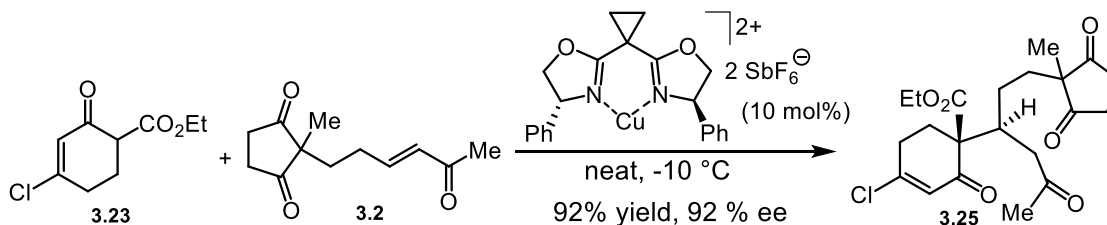
Michael Reaction: Ethyl (S)-4-chloro-1-((S)-1-(1-methyl-2,5-dioxocyclopentyl)-5-oxohexan-3-yl)-2-oxocyclohex-3-ene-1-carboxylate (3.25)

Racemic synthesis:



To the flask with enone (**3.23**) and chloroketoester (**3.2**) was added $\text{Cu}(\text{OTf})_2$ in the glovebox at room temperature. After stirring the reaction mixture neat for 1 h, the mixture was filtered through silica with diethyl ether. The ether layer was concentrated *in vacuo*, then purified by using flash chromatography on silica gel (6:4 Hexanes: Ethyl Acetate) to give light-yellow viscous oil.

Enantioselective Synthesis:



To the flask with enone **3.2** (0.0346 mol, 7.2 g, 1 equiv.) and chloroketoester **3.23** (0.0692 mol, 14 g, 2 equiv.) was added $[\text{Cu}(\text{R,R})\text{-PhBox}](\text{SbF}_6)_2$ **SI-4** (0.00346 mol, 3 g, 0.1 equiv.) in the glovebox at room temperature. The reaction mixture was diluted with DCM (5 mL) and stirred at $-10\text{ }^\circ\text{C}$ for 2 days. After confirming the completion of reaction by crude NMR of aliquote, the mixture was filtered through silica with diethyl ether. The ether layer was concentrated *in vacuo*, then purified by flash chromatography on silica gel (6:4 Hexanes: Ethyl Acetate) to give Michael adduct **3.25** (13 g, 92% yield) as a light-yellow viscous oil. Enantiopurity was determined to be 92% ee by chiral HPLC (DAICEL CHIRALPAK AD, 25 cm \times 4.6 mm, hexanes/2-propanol = 95/5, flow rate = 1 mL/min, $\lambda = 237\text{ nm}$, RT (major) = 27.87 m, RT (minor) = 32.33).

Rf (Hexanes: EtOAc 6:4): 0.3

¹H NMR (500 MHz, CDCl₃) δ 6.17 (d, *J* = 2.1 Hz, 1H), 4.09 (dddd, *J* = 14.2, 10.8, 8.7, 5.3 Hz, 2H), 2.99 – 2.65 (m, 4H), 2.73 – 2.66 (m, 2H), 2.65 – 2.55 (m, 2H), 2.40 – 2.24 (m, 2H), 2.11 (s, 3H), 1.92 (ddd, *J* = 14.5, 10.0, 5.4 Hz, 1H), 1.73 – 1.61 (m, 1H), 1.52 (td, *J* = 13.2, 12.8, 4.4 Hz, 1H), 1.21 (t, *J* = 7.2 Hz, 3H), 1.16 – 1.07 (m, 2H), 1.05 (s, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 216.23, 216.01, 206.81, 192.78, 170.24, 157.23, 128.51, 62.05, 59.62, 56.84, 44.52, 35.61, 35.17, 35.12, 33.61, 31.75, 30.21, 27.56, 27.36, 19.20, 14.05.

[α]_D²⁰ = -29.3 (c = 0.095, CH₂Cl₂)

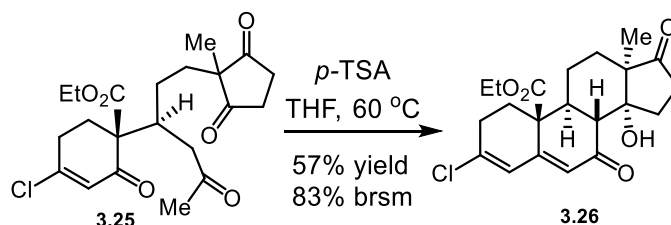
HRMS (ESI-MS) calculated for C₂₁H₂₇ClO₆ [M+NH₄]⁺: *m/z* 428.1834, found: 428.1845

IR = 2936, 2355, 1710, 1680, 1618, 1419, 1364, 1295, 1236

Ethyl (8R,9S,10S,13R,14R)-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

(3.26)



To the solution of Michael adduct **3.25** (0.034 mol, 14 g, 1 equiv.) in THF (420 mL) was added PTSA (0.340 mol, 65 g, 10 equiv.) and heated at 60 °C for 60 h. The reaction was quenched with saturated aqueous NaHCO₃ and the aqueous layer was extracted with DCM (3 × 300 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (7:3 Hexanes: Ethyl acetate) to give unnatural steroid **3.26** (7.6 g, 57%) as yellow solid and unreacted starting material (4.4 g, 83% brsm).

R_f (Hexanes: Ethyl acetate 7:3): 0.6

¹H NMR (700 MHz, CDCl₃) δ 6.30 (d, *J* = 1.8 Hz, 1H), 5.87 (s, 1H), 4.24 (dd, *J* = 10.9, 7.1 Hz, 1H), 4.07 (dd, *J* = 10.9, 7.1 Hz, 1H), 3.49 (s, 1H), 2.81 (d, *J* = 7.6 Hz, 1H), 2.68 (ddd, *J* = 18.7, 12.8, 7.0 Hz, 2H), 2.55 (d, *J* = 12.4 Hz, 1H), 2.54 – 2.51 (m, 1H), 2.47 (dd, *J* = 19.4, 5.6 Hz, 1H), 2.16 – 2.03 (m, 3H), 2.01 (d, *J* = 13.8 Hz, 1H), 1.79 (dd, *J* = 13.5, 3.2 Hz, 1H), 1.58 – 1.50 (m, 1H), 1.34 (td, *J* = 13.7, 3.6 Hz, 1H), 1.20 (t, *J* = 7.1 Hz, 3H), 1.02 (s, 3H), 0.82 (dd, *J* = 13.1, 3.2 Hz, 1H).

¹³C NMR (176 MHz, CDCl₃) δ 218.51, 200.90, 169.66, 152.44, 144.32, 126.54, 125.70, 77.87, 61.90, 54.40, 50.61, 48.71, 43.28, 34.90, 32.02, 31.68, 30.86, 28.28, 24.40, 19.87, 14.49.

[α]_D²⁰ = -391.6 (c 0.08 in CH₂Cl₂)

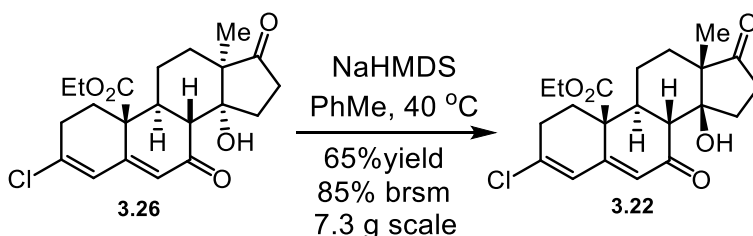
HRMS (ESI) calculated for C₂₁H₂₅ClO₅ [M+H]⁺: 393.1463 *m/z*, found: 393.1466

IR = 3508, 2925, 2028, 1727, 1655, 1649, 1620, 1446, 1372, 1351, 1254, 1217

Ethyl (8R,9S,10S,13S,14S)-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

(3.22)



To a solution of **3.26** (0.0185 mol, 7.3 g, 1 equiv.) in toluene (370 mL) at -78 °C was added the solution of NaHMDS (0.0223 mol, 4 g, 1.2 equiv.) in toluene (30 mL) slowly and let it stir for 25 minutes. The flask was then moved to a pre-heated oil bath at 40 °C and stirred for 1.5 hrs. The resulting brown solution was filtered quickly through silica plug and the eluent was concentrated *in vacuo*. The residue was then purified by flash chromatography on silica gel (7:3 Hexanes: Ethyl

acetate) to give natural steroid **3.22** (4.74 g, 65%) as off-white solid and unreacted starting material (1.7 g, 85% brsm).

Alternately, the reaction was quenched with saturated aqueous NaHCO₃ solution (100 mL) then extracted with EtOAc (3 × 200 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (7:3 Hexanes: Ethyl acetate) to obtain comparable yield.

Rf (Hexanes: Ethyl acetate 7:3): 0.5

¹H NMR (700 MHz, CDCl₃) δ 6.38 (d, *J* = 2.3 Hz, 1H), 5.92 (s, 1H), 4.75 (s, 1H), 4.30 (dd, *J* = 10.9, 7.0 Hz, 1H), 4.19 (dd, *J* = 10.9, 7.1 Hz, 1H), 2.83 – 2.75 (m, 1H), 2.73 (d, *J* = 13.2 Hz, 2H), 2.58 – 2.48 (m, 2H), 2.38 (ddd, *J* = 19.2, 7.7, 3.2 Hz, 1H), 2.10 – 1.95 (m, 3H), 1.87 (d, *J* = 15.5 Hz, 1H), 1.60 (dt, *J* = 11.6, 5.3 Hz, 1H), 1.45 – 1.31 (m, 3H), 1.28 (t, *J* = 7.1 Hz, 3H), 1.06 (s, 3H).

¹³C NMR (175 MHz, CDCl₃) δ 219.79, 202.07, 169.46, 153.95, 144.89, 126.09, 125.96, 80.54, 62.25, 53.10, 49.72, 48.57, 45.24, 32.98, 31.88, 31.27, 30.91, 27.97, 22.21, 14.55, 13.12.

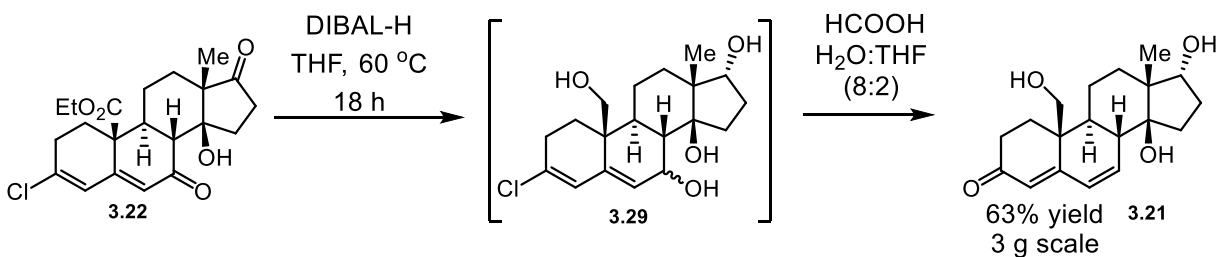
[α]_D²⁰ = -244.9 (c 0.128 CH₂Cl₂)

HRMS (ESI) calculated for C₂₁H₂₅ClO₅ [M+H]⁺: *m/z* 393.1463, found: 393.1463

IR = 3502, 2928, 2862, 2359, 1733, 1644, 1616, 1444, 1373, 1350, 1247, 1206

(8R,9S,10S,13R,14S,17R)-14,17-dihydroxy-10-(hydroxymethyl)-13-methyl-

1,2,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-3-one (3.21)



To the solution of **3.22** (0.0076 mol, 3 g, 1 equiv.) in THF (300 mL) at -78 °C was added DIBAL-H (0.0917 mol, 16 mL, 12 equiv.) and stirred for 30 minutes. The resulting yellow solution was heated to 60 °C and stirred overnight. Quantitative conversion to the reduced intermediate was observed by TLC (9:1 DCM: MeOH). The reaction mixture was cooled to 0 °C and quenched with saturated aqueous solution of sodium potassium tartrate tetrahydroborate very slowly. The resulting cloudy mixture was then extracted with DCM (3 × 150 mL) and the organic layer was removed *in vacuo*. The intermediate **3.29** can be isolated if desired by flash chromatography on silica (8:2 Ethyl acetate: Hexanes) and is stable at room temperature for up to 2 hrs.

The crude mixture obtained after reduction was dissolved in 8:2 H₂O: THF (100 mL) and 50% formic acid (10 mL) and heated to 70 °C for 2 hrs. White precipitate was observed upon the addition of formic acid but slowly disappeared. The resulting colorless solution was then cooled to -10 °C and quenched with saturated aqueous NaHCO₃ solution very slowly. The aqueous layer was extracted with 2:1 mixture of chloroform: ethanol (4 × 150mL). The combined organic phases were dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (9:1 DCM: MeOH) to give key intermediate **3.21** (1.5 g, 63%) as a white solid.

Rf (DCM:MeOH 9:1) : 0.2

¹H NMR (500 MHz, CD₃OD) δ 6.48 (dd, *J* = 9.9, 2.1 Hz, 1H), 6.27 (dd, *J* = 10.0, 2.8 Hz, 1H), 5.78 (s, 1H), 4.16 (t, *J* = 8.7 Hz, 1H), 3.84 (d, *J* = 11.6 Hz, 1H), 3.69 (d, *J* = 11.7 Hz, 1H), 2.77 (ddd, *J* = 18.1, 14.3, 5.9 Hz, 1H), 2.58 – 2.48 (m, 2H), 2.43 – 2.31 (m, 1H) 2.09 (dt, *J* = 9.3, 4.5 Hz, 1H), 1.93 (ddd, *J* = 14.4, 12.5, 5.3 Hz, 1H), 1.77 – 1.67 (m, 3H), 1.62 – 1.43 (m, 3H), 1.39 (ddd, *J* = 14.8, 10.1, 5.0 Hz, 1H), 1.22 – 1.13 (m, 1H), 1.08 (s, 3H).

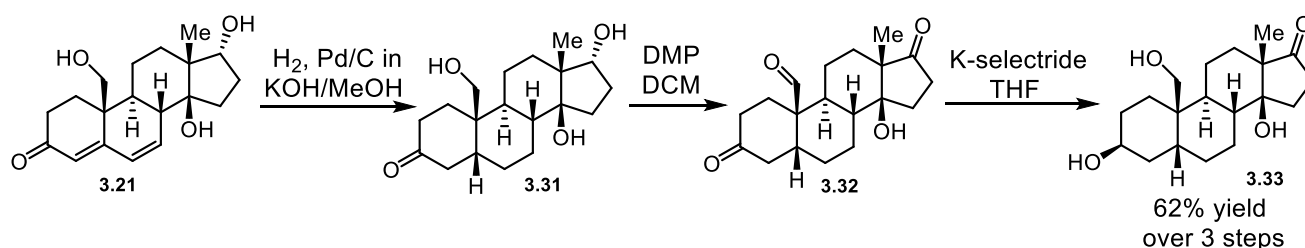
^{13}C NMR (175 MHz, CD_3OD) δ 202.78, 162.58, 141.04, 130.16, 125.82, 83.73, 81.25, 63.84, 48.94, 48.42, 45.15, 42.72, 35.11, 31.24, 30.38, 30.12, 28.77, 21.83, 17.47

$[\alpha]_{\text{D}}^{20} = +9.956$ (c 0.095 in MeOH)

HRMS (ESI) calculated for $\text{C}_{19}\text{H}_{26}\text{O}_4$ $[\text{M}+\text{H}]^+$: m/z 319.1909, found: 319.1903

IR = 3350, 2932, 2030, 1640, 1611, 1578, 1469, 1446, 1418, 1364, 1278, 1223, 1202

(3S,5R,8R,9S,10R,13S,14S)-3,14-dihydroxy-10-(hydroxymethyl)-13-methylhexadecahydro-17H-cyclopenta[a]phenanthren-17-one (3.33)



To a flask with **3.21** (1.099 mmol, 350 mg, 1 equiv.) and 10% Pd on activated carbon (0.550 mmol, 58 mg, 0.5 equiv.) was added a solution of 1% KOH in MeOH (29 mL) and purged with N_2 . The flask was then purged with H_2 and stirred under H_2 atmosphere for 1 h at rt. The completion of the reaction was confirmed by TLC. The reaction mixture was then quenched with pH 7 phosphate buffer, then filtered through celite. The eluent was concentrated *in vacuo* and the crude mixture was used in the next step without further purification. The intermediate **3.31** can easily be purified if desired by flash chromatography on silica gel (9:1 DCM: MeOH).

To the solution of crude **3.31** in dry DCM (73 mL) was added DMP (5.5 mmol, 2.331 g, 5 equiv.) and stirred vigorously at rt for 3 h under N_2 atmosphere. The completion of the reaction was determined by crude NMR. Upon completion, the reaction mixture was quenched with 1:1 mixture of saturated aqueous solutions of $\text{Na}_2\text{S}_2\text{O}_3$: NaHCO_3 (30 mL) and stirred for 1 h. The mixture was extracted with DCM ($3 \times 50\text{mL}$). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , filtered and concentrated *in vacuo*. The aldehyde **3.32** obtained was directly

used in the next step without further purification. The intermediate can easily be purified if desired by flash chromatography on silica gel (7:3 Ethyl Acetate: Hexanes).

To the solution of crude **3.32** in dry THF (57 mL) was added K-selectride (3.3 mmol, 0.805 mL, 3 equiv.) dropwise at -78 °C. The reaction mixture was slowly warmed to -10 °C and stirred for 3 h. The resulting mixture was quenched by addition of saturated aqueous NH₄Cl solution and the aqueous phase was extracted with DCM (3 × 50 mL). The combined organic phases were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (9:1 DCM: MeOH) to give triol **3.33** (220 mg, 62% over 3 steps) as a white solid.

Rf (DCM: MeOH 9:1): 0.2

¹H NMR (700 MHz, CD₃OD) δ 4.05 (t, *J* = 3.0 Hz, 1H), 3.82 (d, *J* = 11.2 Hz, 1H), 3.46 (d, *J* = 11.2 Hz, 1H), 2.45 – 2.37 (m, 1H), 2.34 – 2.25 (m, 2H), 2.21 (d, *J* = 13.8 Hz, 1H), 1.98 (td, *J* = 13.9, 2.9 Hz, 1H), 1.93 – 1.82 (m, 4H), 1.81 – 1.73 (m, 2H), 1.69 – 1.62 (m, 1H), 1.56 (dt, *J* = 13.3, 3.4 Hz, 2H), 1.44 – 1.33 (m, 4H), 1.30 – 1.17 (m, 3H), 1.00 (s, 3H).

¹³C NMR (175 MHz, CD₃OD) δ 224.82, 83.56, 67.43, 66.13, 55.36, 41.95, 40.55, 36.51, 34.12, 33.96, 33.50, 29.97, 28.18, 27.55, 27.15, 24.23, 21.08, 20.27, 13.62.

[α]_D²⁰ = +33.55 (c 0.094 in MeOH)

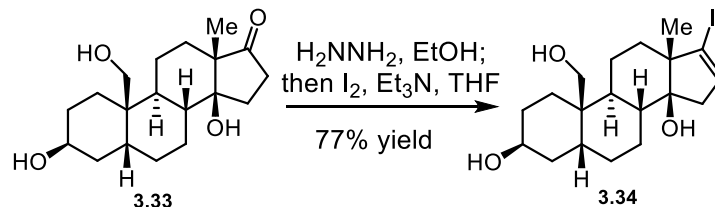
HRMS (ESI) calculated for C₁₉H₃₀O₄ [M+Na]⁺: *m/z* 345.2036, found: 345.2039

IR = 3321, 2849, 2200, 1719, 1648, 1452

(3S,5R,8R,9S,10R,13S,14S)-10-(hydroxymethyl)-17-iodo-13-methyl-

1,2,3,4,5,6,7,8,9,10,11,12,13,15-tetradecahydro-14H-cyclopenta[a]phenanthrene-3,14-diol

(3.34)



To the solution of **3.33** (0.620 mmol, 200 mg, 1 equiv.) in ethanol (60 mL) were added $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$ (12.4 mmol, 600 μL , 20 equiv.) and triethylamine (12.4 mmol, 1.7 mL, 20 equiv.) and heated at 65 °C for 6 h. The resulting colorless solution was concentrated *in vacuo* and dissolved in THF (35 mL). Triethylamine (12.4 mmol, 1.7 mL, 20 equiv.) was added to the reaction mixture followed by dropwise addition of solution of iodine (0.744 mmol, 188 mg, 1.2 equiv.) in THF (2 mL) which resulted in white precipitation. The resulting brown solution was stirred for 30 minutes and quenched with 1:1 mixture of saturated aqueous solution of $\text{Na}_2\text{S}_2\text{O}_3\cdot\text{NaHCO}_3$. It was then extracted with ethyl acetate (3 \times 50mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (9:1 Ethyl acetate: Hexanes) to give vinyl iodide **3.34** (205 mg, 77%) as a white solid.

Rf (EtOAc: hexanes 8:2): 0.2

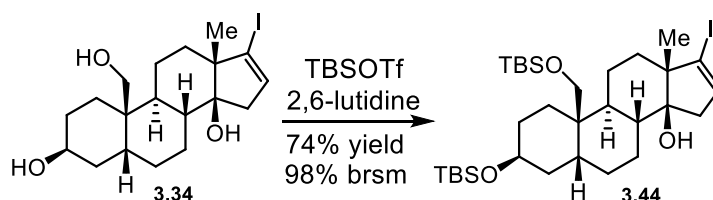
^1H NMR (500 MHz, CD_3OD) δ 6.06 (t, $J = 2.3$ Hz, 1H), 4.03 (s, 1H), 3.83 (d, $J = 11.2$ Hz, 1H), 3.41 (d, $J = 11.2$ Hz, 1H), 2.59 (dd, $J = 16.3, 1.9$ Hz, 1H), 2.27 – 2.09 (m, 2H), 1.97 (td, $J = 14.0, 3.0$ Hz, 1H), 1.93 – 1.79 (m, 3H), 1.74 (ddd, $J = 21.7, 11.3, 3.3$ Hz, 2H), 1.67 (dd, $J = 11.6, 2.7$ Hz, 1H), 1.62 – 1.53 (m, 2H), 1.50 (dd, $J = 13.0, 3.3$ Hz, 1H), 1.43 – 1.35 (m, 2H), 1.29 (dd, $J = 12.5, 4.3$ Hz, 1H), 1.26 – 1.19 (m, 1H), 1.01 (s, 3H), 1.09 – 0.90 (m, 2H).

^{13}C NMR (175 MHz, CD_3OD) δ 134.81, 111.63, 83.54, 67.46, 66.10, 55.75, 42.99, 42.68, 40.51, 39.55, 37.31, 34.11, 29.75, 28.28, 27.22, 24.21, 21.66, 20.89, 18.61.

$[\alpha]_{\text{D}}^{20}$ = +23.26 (c 0.113 in MeOH)

IR = 3365, 2927, 2361, 2337, 1750, 1646, 1540

(3S,5R,8R,9S,10R,13S,14S)-3-(((tert-butyl dimethylsilyl)oxy)-10-(((tert-butyl dimethylsilyl)oxy)methyl)-17-iodo-13-methyl-1,2,3,4,5,6,7,8,9,10,11,12,13,15-tetradecahydro-14H-cyclopenta[a]phenanthren-14-ol (3.44)



To a solution of vinyl iodide **3.34** (0.370 mmol, 160 mg, 1 equiv.) in dry DCM (37 mL) at $-78\text{ }^{\circ}\text{C}$ was added 2,6 lutidine (3.70 mmol, 430 μL , 10 equiv.) followed by dropwise addition of TBSOTf (1.850 mmol, 425 μL , 5 equiv.) and the resulting solution was warmed to $-20\text{ }^{\circ}\text{C}$ and stirred for 2 h. The resulting colorless solution was quenched by addition of saturated aqueous NaHCO_3 solution and the aqueous phase was extracted with DCM ($3 \times 30\text{ mL}$). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (8:2 Hexanes: Ethyl acetate) to give **3.44** (180 mg, 74%) as a white fluffy solid and unreacted starting material (40 mg, 98% brsm).

Rf (Hexanes: EtOAc 9:1): 0.5

$^1\text{H NMR}$ (500 MHz, CDCl_3) δ 6.11 (t, $J = 2.3$ Hz, 1H), 4.01 (s, 1H), 3.74 (d, $J = 9.7$ Hz, 1H), 3.39 (d, $J = 9.7$ Hz, 1H), 2.57 (dd, $J = 16.5, 1.9$ Hz, 1H), 2.26 (d, $J = 14.3$ Hz, 1H), 2.21 (dd, $J = 16.5, 3.2$ Hz, 1H), 1.92 (ddd, $J = 13.8, 11.3, 6.9$ Hz, 1H), 1.82 – 1.67 (m, 5H), 1.53 – 1.38 (m, 4H), 1.33 – 1.14 (m, 5H), 1.07-1.01 (m, 1H), 1.04 (s, 3H), 0.89 (d, $J = 7.6$ Hz, 18H), 0.02 (dd, $J = 9.2, 1.2$ Hz, 12H).

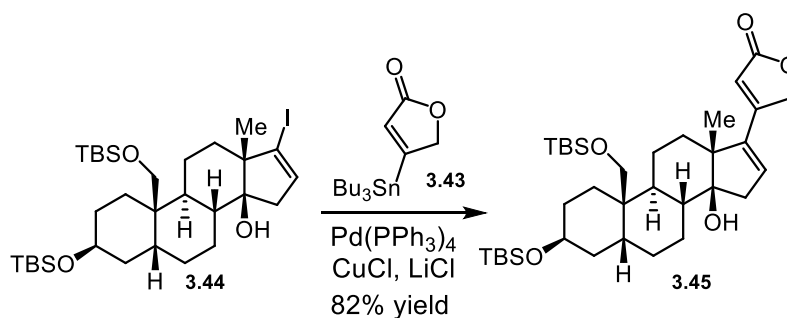
^{13}C NMR (175 MHz, CDCl_3) δ 133.64, 111.56, 82.86, 67.20, 66.27, 54.96, 42.63, 41.48, 39.55, 38.27, 36.20, 34.43, 28.83, 28.67, 26.27, 26.11, 26.03, 23.66, 20.93, 19.98, 18.43, 18.25, 18.17, -4.70, -4.74, -5.36, -5.42.

$[\alpha]_{\text{D}}^{20} = +12.06$ (c 0.0667 in CH_2Cl_2)

HRMS (ESI-MS) calculated for $\text{C}_{31}\text{H}_{57}\text{IO}_3\text{Si}_2$ $[\text{M}+\text{Na}]^+$: m/z 683.2789, found: 683.2781

IR = 2928, 2856, 2183, 1475, 1460, 1359, 1250, 1218

4-((3S,5R,8R,9S,10R,13R,14S)-3-((tert-butyldimethylsilyl)oxy)-10-(((tert-butyldimethylsilyl)oxy)methyl)-14-hydroxy-13-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 (3.45)



To a flask with **3.44** (0.0696 mmol, 46 mg, 1 equiv.) was added $\text{Pd}(\text{PPh}_3)_4$ (0.00348 mmol, 4 mg, 0.05 equiv.), LiCl (1.32 mmol, 56 mg, 20 equiv.) and CuCl (1.044 mmol, 103 mg, 15 equiv.) in a glovebox followed by addition of DMSO (7.5 mL) and stannane precursor **3.43** (0.2088 mmol, 78 mg, 3 equiv.) and heated to 70 °C for 2 h. The resulting brown solution was cooled to rt and quenched with pH 7 phosphate buffer. It was then filtered through glass wool to remove solid residue and the aqueous phase was extracted with ethyl acetate (3×15 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (6:4 Hexanes: Ethyl acetate) to give **3.45** (35 mg, 82%) as a white fluffy solid.

Rf (Hexanes: Ethyl Acetate 6:4): 0.5

¹H NMR (500 MHz, CDCl₃) δ 6.10 (s, 1H), 5.96 (s, 1H), 5.05 – 4.88 (m, 2H), 4.02 (s, 1H), 3.74 (d, *J* = 9.7 Hz, 1H), 3.41 (d, *J* = 9.7 Hz, 1H), 2.72 (dd, *J* = 18.5, 2.2 Hz, 1H), 2.36 (dd, *J* = 18.4, 3.1 Hz, 1H), 2.28 (d, *J* = 12.9 Hz, 1H), 2.04 – 1.96 (m, 1H), 1.91 (td, *J* = 13.3, 5.5 Hz, 1H), 1.82 – 1.76 (m, 4H), 1.65 – 1.52 (m, 1H), 1.51 – 1.37 (m, 3H), 1.28- 1.18 (m, 4H), 1.25 (s, 3H), 1.15 – 1.00 (m, 2H), 0.89 (d, *J* = 7.6 Hz, 18H), 0.02 (dd, *J* = 9.2, 1.2 Hz, 12H).

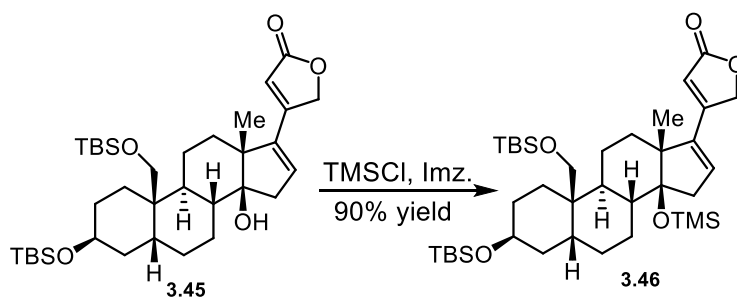
¹³C NMR (175 MHz, CDCl₃) δ 174.41, 158.34, 143.95, 132.09, 112.37, 85.81, 71.67, 66.94, 66.27, 52.18, 41.00, 40.31, 39.33, 39.05, 35.97, 34.28, 28.77, 28.50, 26.14, 25.94, 25.85, 23.50, 20.86, 19.93, 18.27, 18.08, 16.69, -4.87, -4.90, -5.52, -5.58.

[α]_D²⁰ = +38.73 (c 0.225 in CH₂Cl₂)

HRMS (ESI-MS) calculated for C₃₅H₆₀O₅Si₂ [M+H]⁺: *m/z* 617.4058, found: 617.4046

IR = 3420, 2927, 2120, 2010, 1750, 1730, 1620, 1210

4-((3S,5R,8R,9S,10R,13R,14S)-3-((tert-butyldimethylsilyl)oxy)-10-(((tert-butyldimethylsilyl)oxy)methyl)-13-methyl-14-((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 (3.46)



To a solution of **3.45** (0.0486 mmol, 30 mg, 1 equiv.) in DMF (1 mL) at rt was added imidazole (0.486, 33 mg, 10 equiv.) and stirred for 5 minutes. TMSCl (0.243 mmol, 31 μL, 5 equiv.) was added dropwise to the reaction mixture and stirred overnight in a sealed vial. The resulting colorless solution was quenched by addition of saturated aqueous NaHCO₃ solution dropwise and

the aqueous phase was extracted with DCM (3 × 15 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (8:2 Hexanes: Ethyl acetate) to give **3.46** (30 mg, 90%) as a white fluffy solid.

Rf (Hexanes: Ethyl Acetate 9:1): 0.6

¹H NMR (700 MHz, CDCl₃) δ 6.03 (s, 1H), 5.95 (s, 1H), 4.95 (ddd, *J* = 66.0, 16.4, 1.6 Hz, 2H), 4.01 (d, *J* = 3.6 Hz, 1H), 3.73 (d, *J* = 9.7 Hz, 1H), 3.37 (d, *J* = 9.7 Hz, 1H), 2.62 (d, *J* = 19.5 Hz, 1H), 2.41 (dd, *J* = 18.7, 3.4 Hz, 1H), 2.25 (d, *J* = 13.4 Hz, 2H), 1.95 (d, *J* = 13.5 Hz, 1H), 1.87 (d, *J* = 5.8 Hz, 1H), 1.82 – 1.74 (m, 3H), 1.70 (d, *J* = 12.7 Hz, 1H), 1.52 (m, 1H), 1.42 (d, *J* = 11.7 Hz, 3H), 1.29 – 1.22 (m, 2H), 1.20 (s, 3H), 1.21- 1.18 (m, 1H), 1.09 (d, *J* = 13.3 Hz, 1H), 1.04 – 0.94 (m, 1H), 0.90 (dd, *J* = 17.8, 1.1 Hz, 18H), 0.06 (t, *J* = 1.5 Hz, 6H), 0.01 (dd, *J* = 10.1, 1.1 Hz, 15H).

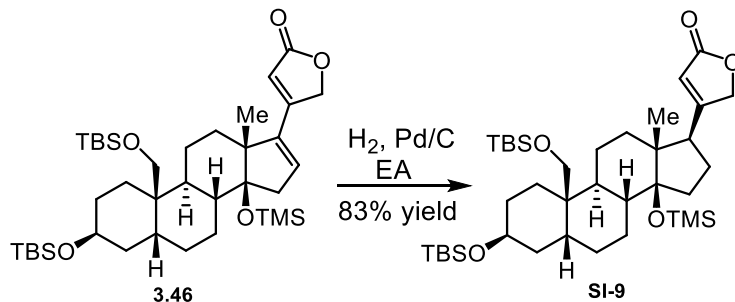
¹³C NMR (175 MHz, CDCl₃) δ 174.65, 158.67, 145.07, 132.16, 112.42, 90.35, 71.72, 67.19, 66.80, 53.15, 42.13, 39.54, 39.45, 39.29, 36.16, 34.49, 29.31, 28.71, 26.53, 26.13, 26.03, 23.72, 21.22, 20.11, 18.40, 18.26, 17.50, 2.84, -4.69, -4.74, -5.37, -5.38.

[α]_D²⁰ = +10.86 (c 0.125 in CH₂Cl₂)

HRMS (ESI-MS) calculated for C₃₈H₆₈O₅Si₃ [M+Na]⁺: *m/z* 711.4267, found: 711.4276

IR = 3745, 2928, 2856, 2359, 2338, 1783, 1751, 1456, 1250

4-((3S,5R,8R,9S,10R,13R,14S,17R)-3-((tert-butyldimethylsilyl)oxy)-10-(((tert-butyldimethylsilyl)oxy)methyl)-13-methyl-14-((trimethylsilyl)oxy)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)furan-2(5H)-one (SI-9)



To a solution of **3.46** (0.0436 mmol, 30 mg, 1 equiv.) in ethyl acetate (3 mL) was added 10% Palladium on activated carbon (0.0109 mmol, 1.3 mg, 0.25 equiv.) and purged with N₂. The flask was then purged with H₂ and stirred under H₂ atmosphere at rt for 1 h. The completion of the reaction was confirmed by TLC. The resulting mixture was then filtered through celite and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (9:1 Hexanes: Ethyl acetate) to give **SI-9** (25 mg, 83%) as a white solid.

Rf (Hexanes: EtOAc 9:1): 0.5

¹H NMR (500 MHz, CDCl₃) δ 5.83 (s, 1H), 4.84 – 4.59 (m, 2H), 4.02 (t, *J* = 2.8 Hz, 1H), 3.67 (d, *J* = 9.6 Hz, 1H), 3.43 (d, *J* = 9.6 Hz, 1H), 2.56 (t, *J* = 7.8 Hz, 1H), 2.24 (d, *J* = 13.4 Hz, 1H), 2.10 – 2.01 (m, 1H), 1.98 – 1.87 (m, 3H), 1.82 – 1.76 (m, 1H), 1.76 – 1.66 (m, 4H), 1.63 – 1.52 (m, 3H), 1.46 – 1.35 (m, 4H), 1.34 – 1.20 (m, 3H), 1.20 – 1.16 (m, 1H), 0.89 (d, *J* = 5.9 Hz, 18H), 0.86 (s, 3H), 0.20 – -0.07 (m, 21H).

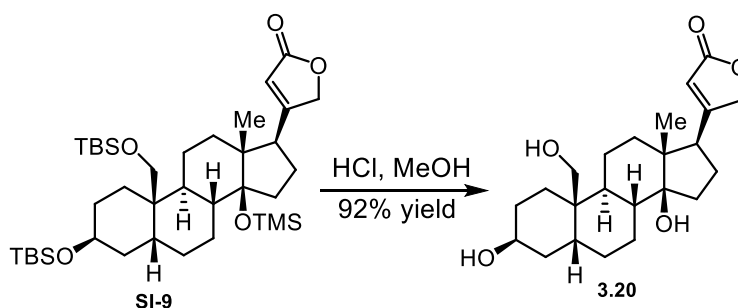
¹³C NMR (126 MHz, CDCl₃) δ 174.49, 174.20, 117.15, 91.70, 74.06, 67.19, 66.34, 51.11, 50.84, 42.33, 40.79, 40.02, 36.50, 34.61, 33.96, 28.84, 28.52, 27.42, 26.51, 26.11, 26.02, 23.76, 23.19, 21.19, 18.46, 18.35, 18.25, 3.07, -4.69, -4.73, -5.39, -5.53.

[α]_D²⁰ = +16.92 (c 0.20 in CH₂Cl₂)

HRMS (ESI-MS) calculated for C₃₈H₇₀O₅Si₃ [M+NH₄]⁺: *m/z* 708.4869, found: 708.4867

IR = 2937, 2928, 2857, 2155, 1781, 1752, 1471, 1250

4-((3S,5R,8R,9S,10R,13R,14S,17R)-3,14-dihydroxy-10-(hydroxymethyl)-13-methylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)furan-2(5H)-one (3.20)



To a solution of **SI-9** (0.065 mmol, 45 mg, 1 equiv.) in MeOH (3 mL) was added 3M HCl in MeOH (600 μ L) and stirred at rt for 1 h. The resulting colorless solution was quenched by addition of saturated aqueous NaHCO₃ solution dropwise and the aqueous phase was extracted with 2:1 chloroform: ethanol (3 \times 15mL). The combined organic layers were dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (9:1 DCM: MeOH) to give **3.20** (25 mg, 92%) as a white solid.

Rf (DCM: MeOH 9:1): 0.2

¹H NMR (500 MHz, CD₃OD) δ 5.90 (s, 1H), 5.09 – 4.90 (m, 2H), 4.04 (t, J = 2.9 Hz, 1H), 3.83 (d, J = 11.2 Hz, 1H), 3.43 (d, J = 11.2 Hz, 1H), 2.83 (dd, J = 9.1, 5.9 Hz, 1H), 2.32 – 2.09 (m, 3H), 1.99 – 1.91 (m, 1H), 1.87 (dt, J = 13.0, 6.9 Hz, 2H), 1.83 – 1.76 (m, 2H), 1.76 – 1.54 (m, 4H), 1.54 – 1.41 (m, 3H), 1.37 (dq, J = 13.4, 3.2 Hz, 2H), 1.33 – 1.18 (m, 4H), 0.88 (s, 3H).

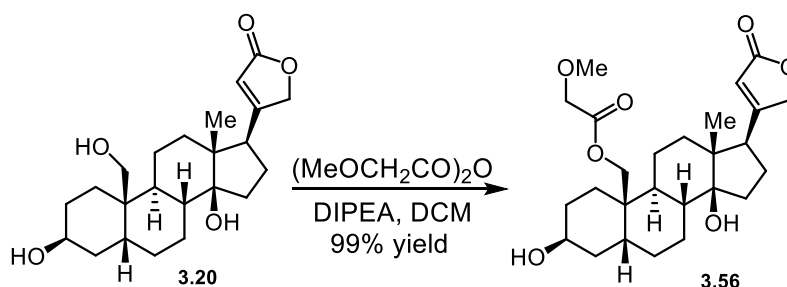
¹³C NMR (175 MHz, CD₃OD) δ 178.41, 177.23, 117.79, 86.56, 75.35, 67.47, 66.04, 52.13, 51.05, 42.53, 41.32, 40.54, 36.36, 34.12, 33.17, 29.81, 28.23, 28.03, 27.44, 24.20, 22.44, 22.17, 16.41.

$[\alpha]_D^{20}$ = +8.48 (c 0.15 in MeOH)

HRMS (ESI-MS) calculated for C₂₃H₃₃DO₅ [M+H]⁺: m/z 392.2547, found: 392.2540

IR = 3367, 2925, 2494, 1727, 1666, 1626, 1448, 1370

((3S,5R,8R,9S,10R,13R,14S,17R)-3,14-dihydroxy-13-methyl-17-(5-oxo-2,5-dihydrofuran-3-yl)hexadecahydro-10H-cyclopenta[a]phenanthren-10-yl)methyl 2-methoxyacetate (3.56)



To a solution of cannogenol **3.20** (0.0589 mmol, 23 mg, 1 equiv.) in DCM (2.5 mL) at 0 °C was added DIPEA (0.141 mmol, 18 mg, 2.4 equiv.) and (MeOCH₂CO)₂O (0.07068 mmol, 11.5 mg, 1.2 equiv.) and stirred overnight. The resulting colorless solution was quenched by addition of saturated aqueous NaHCO₃ solution dropwise and the aqueous phase was extracted with DCM (3 × 8mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (8:2 Ethyl acetate: Hexanes) to give **3.56** (27 mg, 99%) as a brown solid.

R_f (DCM: MeOH 9:1): 0.5

¹H NMR (700 MHz, CDCl₃) δ 5.88 (d, *J* = 2.2 Hz, 1H), 4.97 (dd, *J* = 18.1, 1.8 Hz, 1H), 4.80 (dd, *J* = 18.1, 1.8 Hz, 1H), 4.44 (d, *J* = 11.2 Hz, 1H), 4.12 (s, 1H), 4.09-4.06 (m, 1H), 4.06 (s, 2H), 3.46 (s, 3H), 2.78 (dd, *J* = 9.3, 5.5 Hz, 1H), 2.21 – 2.09 (m, 3H), 1.93 – 1.84 (m, 2H), 1.83 – 1.69 (m, 5H), 1.63 – 1.51 (m, 5H), 1.50 – 1.41 (m, 2H), 1.40 – 1.35 (m, 1H), 1.32 – 1.19 (m, 3H), 0.88 (s, 3H).

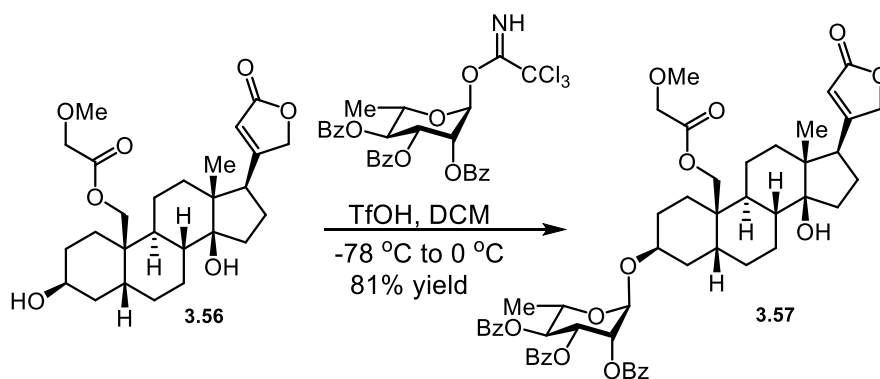
¹³C NMR (175 MHz, CDCl₃) δ 174.30, 174.05, 170.55, 117.86, 85.51, 73.35, 69.84, 67.64, 66.11, 59.43, 50.79, 49.49, 41.77, 40.13, 38.50, 35.02, 33.17, 32.92, 29.16, 27.46, 26.79, 25.99, 23.39, 21.40, 20.80, 15.79.

[α]_D²⁰ = +30.73 (c 0.10 in MeOH)

HRMS (ESI-MS) calculated for C₂₆H₃₈O₇ [M+NH₄]⁺: *m/z* 480.2961, found: 480.2958

IR = 3505, 2946, 1936, 1793, 1747, 1617, 1447, 1263

(2R,3R,4R,5S,6S)-2-(((3S,5R,8R,9S,10R,13R,14S,17R)-14-hydroxy-10-((2-methoxyacetoxy)methyl)-13-methyl-17-(5-oxo-2,5-dihydrofuran-3-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)-6-methyltetrahydro-2H-pyran-3,4,5-triyl tribenzoate
(3.57)



To a solution of acetylated cannogenol **3.56** (0.019 mmol, 8.8 mg, 1.0 equiv.) in dry DCM (800 μ L) was added 4Å MS and cooled to 0 °C. The solution of donor **3.40** (0.0095 mmol, 6.6 mg, 0.5 equiv.) in DCM (100 μ L) was added to the flask followed by dropwise addition of the solution of TfOH (0.0057 mmol, 0.5 μ M, 0.3 equiv.) and the resulting mixture was stirred at 0 °C for 1 h. Additional 0.5 equiv. of donor was added portion wise every hour for the next three hours. After the addition of 2 equiv. of total donor, the reaction mixture was warmed to rt and stirred for 1 h. The resulting colorless solution was quenched by addition of excess triethylamine dropwise and filtered through celite. This eluent was concentrated *in vacuo* and the residue was purified by flash chromatography on silica gel (6:4 Hexanes: Ethyl acetate) to give **3.57** (12.1 mg, 81%) as a colorless solid.

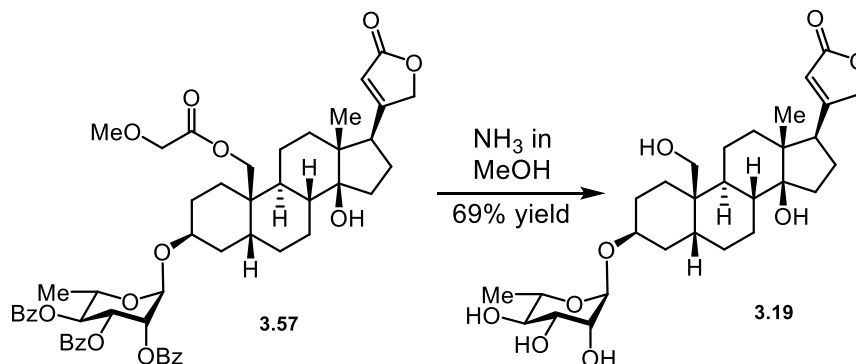
Rf (EtOAc: Hexanes 6:4): 0.4

¹H NMR (700 MHz, CDCl₃) δ 8.10 (d, *J* = 7.4 Hz, 2H), 7.98 (d, *J* = 7.5 Hz, 2H), 7.82 (d, *J* = 7.6 Hz, 2H), 7.62 (t, *J* = 7.5 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.50 (t, *J* = 7.7 Hz, 2H), 7.43 (t, *J* = 7.4 Hz, 1H), 7.39 (t, *J* = 7.7 Hz, 2H), 7.26 (t, *J* = 7.7 Hz, 2H) 5.89 (s, 1H), 5.83 (dd, *J* = 10.2, 3.4 Hz, 1H), 5.69 (t, *J* = 9.9 Hz, 1H), 5.61 (dd, *J* = 3.5, 1.8 Hz, 1H), 5.10 (d, *J* = 1.7 Hz, 1H), 4.99 (dd, *J* = 18.1, 1.8 Hz, 1H), 4.81 (dd, *J* = 18.1, 1.7 Hz, 1H), 4.50 (d, *J* = 11.1 Hz, 1H), 4.26 – 4.19 (m, 3H), 4.09 (d, *J* = 11.2 Hz, 1H), 4.08 (s, 1H), 3.44 (s, 3H), 2.80 (dd, *J* = 9.3, 5.5 Hz, 1H), 2.27 – 2.22 (m, 1H), 2.22 – 2.10 (m, 2H), 1.95 (td, *J* = 14.2, 3.9 Hz, 1H), 1.92 – 1.87 (m, 1H), 1.84 – 1.75 (m, 5H), 1.74 – 1.71 (m, 1H), 1.66 – 1.61 (m, 3H), 1.60 – 1.53 (m, 3H), 1.43 – 1.38 (m, 2H), 1.35 (d, *J* = 6.3 Hz, 3H), 1.31 (d, *J* = 3.4 Hz, 2H), 0.90 (s, 3H).

¹³C NMR (175 MHz, CDCl₃) δ 174.45, 174.19, 171.05, 165.97, 165.89, 165.65, 133.67, 133.51, 133.30, 130.04, 129.85, 129.77, 129.56, 129.44, 129.34, 128.76, 128.60, 128.47, 118.06, 96.00, 85.66, 73.51, 72.94, 71.95, 71.62, 70.28, 69.88, 67.26, 67.07, 59.44, 50.98, 49.66, 42.01, 40.32, 38.59, 35.48, 33.12, 29.86, 29.25, 26.97, 26.30, 26.21, 24.31, 21.58, 21.03, 17.87, 15.98.

[α]_D²⁰ = +42.50 (c 0.133 in CH₂Cl₂)

4-((3S,5R,8R,9S,10R,13R,14S,17R)-14-hydroxy-10-(hydroxymethyl)-13-methyl-3-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)furan-2(5H)-one (3.19)



To a solution of protected glycoside **3.57** (0.00543 mmol, 5.0 mg, 1.0 equiv.) was added half saturated solution of ammonia in methanol (600 μ L) and let it stir at rt overnight in a sealed vial. The resulting solution was concentrated *in vacuo* with silica gel and purified by flash column chromatography on silica gel (9:1 \rightarrow 8:2 DCM: MeOH) to give cannogenol-3-O- α -L-rhamnoside **3.19** (2 mg, 69%) as an off-white solid.

Rf (DCM:MeOH 9:1) : 0.2

$^1\text{H NMR}$ (700 MHz, $\text{C}_5\text{D}_5\text{N}$) δ 6.80 – 6.74 (m, 1H, C3-OH), 6.59 (s, 1H, C2-OH), 6.47 (s, 1H, C4-OH), 6.15 (t, $J = 1.8$ Hz, 1H, H-21), 5.88 (t, $J = 5.6$ Hz, 1H, C19-OH), 5.49 (s, 1H, H-1'), 5.39 (s, 1H, C14-OH), 5.34 (dd, $J = 18.1, 1.8$ Hz, 1H, H-22), 5.06 (dd, $J = 18.1, 1.8$ Hz, 1H, H-22), 4.66 – 4.56 (m, 2H, H-2' and H-4'), 4.40 – 4.34 (m, 1H, H-5'), 4.32 (t, $J = 9.1$ Hz, 1H, H-3'), 4.27 – 4.23 (m, 1H, H-3), 4.11 (dd, $J = 10.9, 4.6$ Hz, 1H, H-19), 3.78 (dd, $J = 10.9, 5.2$ Hz, 1H, H-19), 2.83 (dd, $J = 9.7, 5.5$ Hz, 1H, H-17), 2.62 (d, $J = 13.3$ Hz, 1H, H-5), 2.43 (td, $J = 14.4, 4.1$ Hz, 1H, H-1), 2.23 – 2.10 (m, 3H, H-15, H-7, and H-16), 2.06 – 1.94 (m, 4H, H-6, H-8, H-16, and H-2), 1.94 – 1.87 (m, 3H, H-4, H-9, and H-15), 1.76 (t, $J = 14.0$ Hz, 1H, H-2), 1.70 (d, $J = 6.1$ Hz, 3H, H-6'), 1.71 – 1.63 (m, 2H, H-4 and H-1), 1.57 – 1.52 (m, 1H, H-11), 1.50 – 1.37 (m, 4H, H-11, H12, H-7, and H-12), 1.25 (dt, $J = 13.8, 2.8$ Hz, 1H, H-6), 1.05 (s, 3H, H-18).

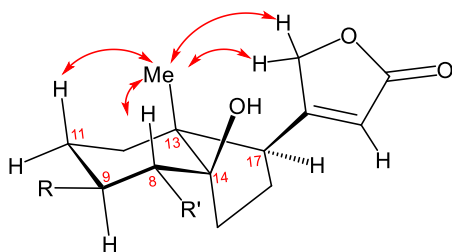
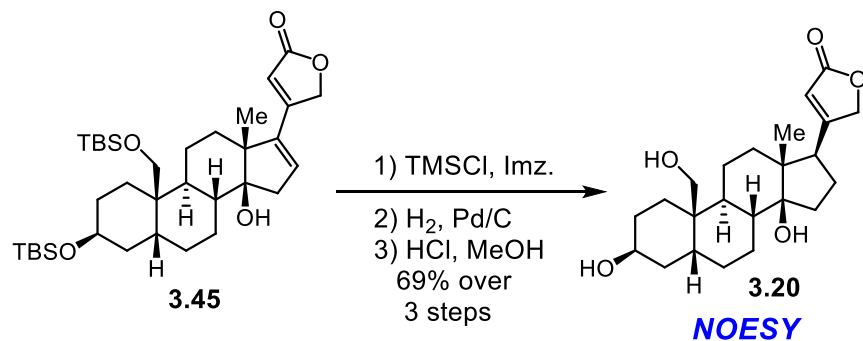
$^{13}\text{C NMR}$ (175 MHz, $\text{C}_5\text{D}_5\text{N}$) δ 176.43 (C23), 174.93 (C20), 118.02 (C21), 100.12 (C1'), 85.26 (C14), 74.49 (C3'), 74.12 (C22), 73.35 (C2'), 73.33 (C4'), 72.38 (C3), 70.42 (C5'), 65.76 (C19), 51.90 (C17), 50.58 (C13), 42.27 (C8), 40.77 (C12), 40.24 (C10), 36.16 (C9), 33.35 (C15), 30.54 (C4), 30.36 (C5), 27.70 (C16), 27.52 (C6), 27.25 (C2), 25.19 (C1), 22.26 (C7), 22.14 (C11), 19.07 (C6), 16.68 (C18).

$[\alpha]_{\text{D}}^{20}$ = -10.8 (c 0.147 in MeOH) reported -15.5 (c 0.55 in MeOH)

HRMS (ESI-MS) calculated for $\text{C}_{29}\text{H}_{44}\text{O}_9$ $[\text{M}+\text{Na}]^+$: m/z 559.2883, found: 559.2869

IR = 3375, 2927, 2905, 1731, 1626, 1450, 1380, 1313

NOE correlations to confirm the stereocenter at C17



NOE correlations

Table S1 Comparison of NMR Data between the Synthetic Cannogenol-3-O- α -L-rhamnoside and the Natural Product Reported by Schenk¹

C #	Natural	Synthetic	$\Delta\delta$
1	25.3	25.2	0.1
2	27.3	27.3	0
3	74.3	74.5	-0.2
4	30.6	30.6	0
5	30.5	30.4	0.1
6	27.5	27.5	0
7	22.3	22.3	0
8	42.2	42.3	-0.1
9	36.1	36.2	-0.1
10	40.2	40.2	0
11	22.3	22.1	0.2
12	40.8	40.8	0
13	50.6	50.6	0
14	85.2	85.3	-0.1
15	33.4	33.4	0
16	27.8	27.8	0
17	51.8	51.9	-0.1
18	16.7	16.7	0
19	65.5	65.8	-0.3
20	176.3	176.4	-0.1
21	73.2	73.3	-0.1
22	117.8	118	-0.2
23	174.8	174.9	-0.1
1'	99.9	100	-0.1
2'	73.2	73.4	-0.2
3'	72.4	72.4	0
4'	74.1	74.1	0
5'	70.3	70.4	-0.1
6'	19.1	19.1	0

Appendix A

X-ray Crystal Structure of Bis-benzoylated Vinyl Iodide 3.35a

Structure Determination.

Colorless needles of **3.35a** were grown from a dichloromethane/hexane solution of the compound at 22 deg. C. A crystal of dimensions 0.11 x 0.08 x 0.08 mm was mounted on a Rigaku AFC10K Saturn 944+ CCD-based X-ray diffractometer equipped with a low temperature device and Micromax-007HF Cu-target micro-focus rotating anode ($\lambda = 1.54184$ Å) operated at 1.2 kW power (40 kV, 30 mA). The X-ray intensities were measured at 85(1) K with the detector placed at a distance 42.00 mm from the crystal. A total of 2028 images were collected with an oscillation width of 1.0° in ω . The exposure times were 1 sec. for the low angle images, 4 sec. for high angle. Rigaku d*trek images were exported to CrysAlisPro for processing and corrected for absorption. The integration of the data yielded a total of 43539 reflections to a maximum 2θ value of 138.48° of which 5235 were independent and 5234 were greater than $2\sigma(I)$. The final cell constants (Table 1) were based on the xyz centroids 38541 reflections above $10\sigma(I)$. Analysis of the data showed negligible decay during data collection; the data were processed with CrystalClear 2.0 and corrected for absorption. The structure was solved and refined with the Bruker SHELXTL (version 2014/6) software package, using the space group $P2(1)2(1)2(1)$ with $Z = 4$ for the formula $C_{33}H_{37}O_5I$. All non-hydrogen atoms were refined anisotropically with the hydrogen atoms placed in a combination of idealized and refined positions. Full matrix least-squares refinement based on F^2 converged at $R1 = 0.0182$ and $wR2 = 0.0477$ [based on $I > 2\sigma(I)$], $R1 = 0.0182$ and $wR2 = 0.0477$ for all data. Additional details are presented in Table 1 and are given as Supporting Information in a CIF file. Acknowledgement is made for funding from NSF grant CHE-0840456 for X-ray instrumentation.

Sheldrick, G.M. SHELXTL, v. 2014/6; Bruker Analytical X-ray, Madison, WI, 2014.

CrystalClear Expert 2.0 r16, Rigaku Americas and Rigaku Corporation (2014), Rigaku Americas, 9009, TX, USA 77381-5209, Rigaku Tokyo, 196-8666, Japan.

CrysAlisPro 1.171.38.41 (Rigaku Oxford Diffraction, 2015).

Table A.1. Crystal data and structure refinement for **3.35a**.

Identification code	bb3136
Empirical formula	C ₃₃ H ₃₇ I O ₅
Formula weight	640.52
Temperature	85(2) K
Wavelength	1.54184 Å
Crystal system, space group	Orthorhombic, P2(1)2(1)2(1)
Unit cell dimensions	a = 7.86976(5) Å alpha = 90 deg. b = 10.63397(5) Å beta = 90 deg. c = 33.8380(2) Å gamma = 90 deg.
Volume	2831.80(3) Å ³
Z, Calculated density	4, 1.502 Mg/m ³
Absorption coefficient	9.211 mm ⁻¹
F(000)	1312

Crystal size	0.110 x 0.080 x 0.080 mm
Theta range for data collection	2.612 to 69.239 deg.
Limiting indices	-9<=h<=9, -12<=k<=12, -37<=l<=40
Reflections collected / unique	43539 / 5235 [R(int) = 0.0405]
Completeness to theta = 67.684	99.80%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.00000 and 0.66548
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	5235 / 0 / 358
Goodness-of-fit on F ²	1.002
Final R indices [I>2sigma(I)]	R1 = 0.0182, wR2 = 0.0477
R indices (all data)	R1 = 0.0182, wR2 = 0.0477
Absolute structure parameter	-0.0113(16)
Extinction coefficient	0.00050(7)
Largest diff. peak and hole	0.551 and -0.499 e.A ⁻³

Table A.2. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for bb3136.

U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
I(1)	5508(1)	6429(1)	3707(1)	17(1)
O(1)	5931(3)	3031(2)	4608(1)	16(1)
O(2)	9188(2)	4645(2)	6303(1)	14(1)
O(3)	9987(3)	2646(2)	6171(1)	18(1)
O(4)	4113(2)	5421(2)	6778(1)	14(1)
O(5)	2242(3)	6882(2)	6985(1)	16(1)
C(1)	4980(4)	5494(3)	4236(1)	15(1)
C(2)	3477(4)	5051(3)	4341(1)	17(1)
C(3)	3584(4)	4440(3)	4745(1)	16(1)
C(4)	5516(4)	4209(2)	4790(1)	13(1)
C(5)	6332(3)	5322(2)	4555(1)	12(1)
C(6)	8100(4)	5021(3)	4398(1)	18(1)
C(7)	6363(3)	6530(3)	4814(1)	14(1)
C(8)	7163(3)	6310(3)	5219(1)	14(1)
C(9)	6154(3)	5311(2)	5450(1)	11(1)
C(10)	6726(3)	5147(2)	5889(1)	12(1)

C(11)	5712(3)	4065(2)	6087(1)	11(1)
C(12)	5829(3)	2838(2)	5853(1)	13(1)
C(13)	5201(3)	3008(2)	5428(1)	13(1)
C(14)	6175(3)	4060(2)	5216(1)	11(1)
C(15)	6438(3)	6359(3)	6133(1)	14(1)
C(16)	4569(4)	6707(2)	6189(1)	15(1)
C(17)	3558(4)	5632(3)	6371(1)	14(1)
C(18)	3837(3)	4405(3)	6153(1)	15(1)
C(19)	8643(4)	4861(3)	5899(1)	14(1)
C(20)	9809(3)	3511(3)	6402(1)	14(1)
C(21)	10266(3)	3434(2)	6826(1)	12(1)
C(22)	11273(4)	2429(3)	6948(1)	16(1)
C(23)	11740(4)	2327(3)	7342(1)	17(1)
C(24)	11180(4)	3203(3)	7615(1)	17(1)
C(25)	10158(4)	4198(3)	7496(1)	17(1)
C(26)	9713(3)	4329(2)	7102(1)	14(1)
C(27)	3314(3)	6104(2)	7058(1)	13(1)
C(28)	3893(3)	5780(2)	7463(1)	13(1)
C(29)	3160(4)	6444(3)	7778(1)	18(1)
C(30)	3618(4)	6156(3)	8162(1)	22(1)
C(31)	4814(4)	5225(3)	8236(1)	23(1)
C(32)	5546(4)	4564(3)	7925(1)	19(1)
C(33)	5077(4)	4836(3)	7538(1)	16(1)

Table A.3. Bond lengths [Å] and angles [deg] for bb3136.

I(1)-C(1)	2.090(3)
O(1)-C(4)	1.436(3)
O(1)-H(1)	0.72(4)
O(2)-C(20)	1.343(3)
O(2)-C(19)	1.452(3)
O(3)-C(20)	1.213(4)
O(4)-C(27)	1.349(3)
O(4)-C(17)	1.462(3)
O(5)-C(27)	1.207(3)
C(1)-C(2)	1.322(4)
C(1)-C(5)	1.526(4)
C(2)-C(3)	1.516(4)
C(2)-H(2)	0.9500
C(3)-C(4)	1.547(4)
C(3)-H(3A)	0.9900
C(3)-H(3B)	0.9900
C(4)-C(14)	1.539(4)
C(4)-C(5)	1.565(4)
C(5)-C(6)	1.523(4)
C(5)-C(7)	1.554(4)
C(6)-H(6A)	0.9800
C(6)-H(6B)	0.9800
C(6)-H(6C)	0.9800
C(7)-C(8)	1.529(4)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-C(9)	1.538(4)
C(8)-H(8A)	0.9900
C(8)-H(8B)	0.9900

C(9)-C(14)	1.548(3)
C(9)-C(10)	1.562(4)
C(9)-H(9)	1.0000
C(10)-C(19)	1.539(4)
C(10)-C(11)	1.553(3)
C(10)-C(15)	1.548(4)
C(11)-C(12)	1.529(3)
C(11)-C(18)	1.535(4)
C(11)-H(11)	1.0000
C(12)-C(13)	1.530(4)
C(12)-H(12A)	0.9900
C(12)-H(12B)	0.9900
C(13)-C(14)	1.534(4)
C(13)-H(13A)	0.9900
C(13)-H(13B)	0.9900
C(14)-H(14)	1.0000
C(15)-C(16)	1.529(4)
C(15)-H(15A)	0.9900
C(15)-H(15B)	0.9900
C(16)-C(17)	1.524(4)
C(16)-H(16A)	0.9900
C(16)-H(16B)	0.9900
C(17)-C(18)	1.516(4)
C(17)-H(17)	1.0000
C(18)-H(18A)	0.9900
C(18)-H(18B)	0.9900
C(19)-H(19A)	0.9900
C(19)-H(19B)	0.9900
C(20)-C(21)	1.483(4)
C(21)-C(22)	1.393(4)
C(21)-C(26)	1.402(4)
C(22)-C(23)	1.388(4)

C(22)-H(22)	0.9500
C(23)-C(24)	1.384(4)
C(23)-H(23)	0.9500
C(24)-C(25)	1.388(4)
C(24)-H(24)	0.9500
C(25)-C(26)	1.388(4)
C(25)-H(25)	0.9500
C(26)-H(26)	0.9500
C(27)-C(28)	1.486(4)
C(28)-C(33)	1.394(4)
C(28)-C(29)	1.401(4)
C(29)-C(30)	1.385(4)
C(29)-H(29)	0.9500
C(30)-C(31)	1.388(4)
C(30)-H(30)	0.9500
C(31)-C(32)	1.390(4)
C(31)-H(31)	0.9500
C(32)-C(33)	1.388(4)
C(32)-H(32)	0.9500
C(33)-H(33)	0.9500
C(4)-O(1)-H(1)	111(3)
C(20)-O(2)-C(19)	118.9(2)
C(27)-O(4)-C(17)	116.0(2)
C(2)-C(1)-C(5)	113.0(2)
C(2)-C(1)-I(1)	125.3(2)
C(5)-C(1)-I(1)	121.61(19)
C(1)-C(2)-C(3)	110.2(2)
C(1)-C(2)-H(2)	124.9
C(3)-C(2)-H(2)	124.9
C(2)-C(3)-C(4)	102.3(2)
C(2)-C(3)-H(3A)	111.3

C(4)-C(3)-H(3A)	111.3
C(2)-C(3)-H(3B)	111.3
C(4)-C(3)-H(3B)	111.3
H(3A)-C(3)-H(3B)	109.2
O(1)-C(4)-C(14)	103.6(2)
O(1)-C(4)-C(3)	108.7(2)
C(14)-C(4)-C(3)	116.1(2)
O(1)-C(4)-C(5)	110.3(2)
C(14)-C(4)-C(5)	114.6(2)
C(3)-C(4)-C(5)	103.5(2)
C(6)-C(5)-C(1)	114.6(2)
C(6)-C(5)-C(7)	110.8(2)
C(1)-C(5)-C(7)	108.1(2)
C(6)-C(5)-C(4)	113.2(2)
C(1)-C(5)-C(4)	99.5(2)
C(7)-C(5)-C(4)	110.1(2)
C(5)-C(6)-H(6A)	109.5
C(5)-C(6)-H(6B)	109.5
H(6A)-C(6)-H(6B)	109.5
C(5)-C(6)-H(6C)	109.5
H(6A)-C(6)-H(6C)	109.5
H(6B)-C(6)-H(6C)	109.5
C(8)-C(7)-C(5)	112.7(2)
C(8)-C(7)-H(7A)	109.0
C(5)-C(7)-H(7A)	109.0
C(8)-C(7)-H(7B)	109.0
C(5)-C(7)-H(7B)	109.0
H(7A)-C(7)-H(7B)	107.8
C(7)-C(8)-C(9)	110.4(2)
C(7)-C(8)-H(8A)	109.6
C(9)-C(8)-H(8A)	109.6
C(7)-C(8)-H(8B)	109.6

C(9)-C(8)-H(8B)	109.6
H(8A)-C(8)-H(8B)	108.1
C(8)-C(9)-C(14)	109.2(2)
C(8)-C(9)-C(10)	114.2(2)
C(14)-C(9)-C(10)	112.7(2)
C(8)-C(9)-H(9)	106.7
C(14)-C(9)-H(9)	106.7
C(10)-C(9)-H(9)	106.7
C(19)-C(10)-C(11)	110.3(2)
C(19)-C(10)-C(15)	107.2(2)
C(11)-C(10)-C(15)	108.1(2)
C(19)-C(10)-C(9)	109.0(2)
C(11)-C(10)-C(9)	110.3(2)
C(15)-C(10)-C(9)	111.9(2)
C(12)-C(11)-C(18)	109.5(2)
C(12)-C(11)-C(10)	112.1(2)
C(18)-C(11)-C(10)	112.4(2)
C(12)-C(11)-H(11)	107.5
C(18)-C(11)-H(11)	107.5
C(10)-C(11)-H(11)	107.5
C(11)-C(12)-C(13)	111.5(2)
C(11)-C(12)-H(12A)	109.3
C(13)-C(12)-H(12A)	109.3
C(11)-C(12)-H(12B)	109.3
C(13)-C(12)-H(12B)	109.3
H(12A)-C(12)-H(12B)	108.0
C(12)-C(13)-C(14)	111.4(2)
C(12)-C(13)-H(13A)	109.4
C(14)-C(13)-H(13A)	109.4
C(12)-C(13)-H(13B)	109.4
C(14)-C(13)-H(13B)	109.4
H(13A)-C(13)-H(13B)	108.0

C(13)-C(14)-C(4)	110.2(2)
C(13)-C(14)-C(9)	112.5(2)
C(4)-C(14)-C(9)	112.7(2)
C(13)-C(14)-H(14)	107.1
C(4)-C(14)-H(14)	107.1
C(9)-C(14)-H(14)	107.1
C(16)-C(15)-C(10)	114.1(2)
C(16)-C(15)-H(15A)	108.7
C(10)-C(15)-H(15A)	108.7
C(16)-C(15)-H(15B)	108.7
C(10)-C(15)-H(15B)	108.7
H(15A)-C(15)-H(15B)	107.6
C(17)-C(16)-C(15)	111.8(2)
C(17)-C(16)-H(16A)	109.3
C(15)-C(16)-H(16A)	109.3
C(17)-C(16)-H(16B)	109.3
C(15)-C(16)-H(16B)	109.3
H(16A)-C(16)-H(16B)	107.9
O(4)-C(17)-C(18)	106.5(2)
O(4)-C(17)-C(16)	109.9(2)
C(18)-C(17)-C(16)	111.8(2)
O(4)-C(17)-H(17)	109.5
C(18)-C(17)-H(17)	109.5
C(16)-C(17)-H(17)	109.5
C(17)-C(18)-C(11)	114.4(2)
C(17)-C(18)-H(18A)	108.7
C(11)-C(18)-H(18A)	108.7
C(17)-C(18)-H(18B)	108.7
C(11)-C(18)-H(18B)	108.7
H(18A)-C(18)-H(18B)	107.6
O(2)-C(19)-C(10)	110.0(2)
O(2)-C(19)-H(19A)	109.7

C(10)-C(19)-H(19A)	109.7
O(2)-C(19)-H(19B)	109.7
C(10)-C(19)-H(19B)	109.7
H(19A)-C(19)-H(19B)	108.2
O(3)-C(20)-O(2)	124.3(2)
O(3)-C(20)-C(21)	123.5(3)
O(2)-C(20)-C(21)	112.2(2)
C(22)-C(21)-C(26)	120.0(2)
C(22)-C(21)-C(20)	117.9(2)
C(26)-C(21)-C(20)	122.1(2)
C(23)-C(22)-C(21)	119.7(3)
C(23)-C(22)-H(22)	120.1
C(21)-C(22)-H(22)	120.1
C(22)-C(23)-C(24)	120.2(3)
C(22)-C(23)-H(23)	119.9
C(24)-C(23)-H(23)	119.9
C(23)-C(24)-C(25)	120.3(3)
C(23)-C(24)-H(24)	119.8
C(25)-C(24)-H(24)	119.8
C(26)-C(25)-C(24)	120.1(3)
C(26)-C(25)-H(25)	120.0
C(24)-C(25)-H(25)	120.0
C(25)-C(26)-C(21)	119.6(3)
C(25)-C(26)-H(26)	120.2
C(21)-C(26)-H(26)	120.2
O(5)-C(27)-O(4)	123.5(2)
O(5)-C(27)-C(28)	124.2(2)
O(4)-C(27)-C(28)	112.3(2)
C(33)-C(28)-C(29)	120.0(3)
C(33)-C(28)-C(27)	122.7(3)
C(29)-C(28)-C(27)	117.2(2)
C(30)-C(29)-C(28)	119.6(3)

C(30)-C(29)-H(29)	120.2
C(28)-C(29)-H(29)	120.2
C(29)-C(30)-C(31)	120.2(3)
C(29)-C(30)-H(30)	119.9
C(31)-C(30)-H(30)	119.9
C(32)-C(31)-C(30)	120.4(3)
C(32)-C(31)-H(31)	119.8
C(30)-C(31)-H(31)	119.8
C(31)-C(32)-C(33)	119.8(3)
C(31)-C(32)-H(32)	120.1
C(33)-C(32)-H(32)	120.1
C(32)-C(33)-C(28)	119.9(3)
C(32)-C(33)-H(33)	120.0
C(28)-C(33)-H(33)	120.0

Symmetry transformations used to generate equivalent atoms:

Table A.4. Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **3.35a**.

The anisotropic displacement factor exponent takes the form:

$$-2 \pi^2 [h^2 a^{*2} U_{11} + \dots + 2 h k a^* b^* U_{12}]$$

	U11	U22	U33	U23	U13	U12
I(1)	21(1)	20(1)	9(1)	4(1)	0(1)	4(1)
O(1)	28(1)	13(1)	8(1)	-3(1)	-3(1)	2(1)
O(2)	19(1)	14(1)	9(1)	0(1)	-4(1)	0(1)
O(3)	24(1)	19(1)	12(1)	-4(1)	0(1)	2(1)
O(4)	18(1)	17(1)	8(1)	-1(1)	1(1)	3(1)
O(5)	20(1)	15(1)	14(1)	1(1)	1(1)	3(1)
C(1)	22(1)	14(1)	9(1)	1(1)	0(1)	4(1)
C(2)	20(1)	18(1)	14(1)	-1(1)	-4(1)	2(1)
C(3)	19(1)	19(1)	12(1)	1(1)	-2(1)	-3(1)
C(4)	18(1)	11(1)	9(1)	-2(1)	0(1)	-1(1)
C(5)	17(1)	14(1)	7(1)	1(1)	0(1)	2(1)
C(6)	19(1)	20(1)	15(1)	4(1)	3(1)	6(1)
C(7)	18(1)	12(1)	14(1)	1(1)	1(1)	-2(1)
C(8)	20(1)	11(1)	11(1)	1(1)	0(1)	-3(1)
C(9)	15(1)	9(1)	9(1)	0(1)	-1(1)	-1(1)
C(10)	14(1)	12(1)	8(1)	-1(1)	-1(1)	-2(1)
C(11)	14(1)	11(1)	8(1)	1(1)	1(1)	-3(1)
C(12)	17(1)	11(1)	10(1)	1(1)	0(1)	-2(1)
C(13)	18(1)	11(1)	10(1)	-1(1)	-1(1)	-2(1)
C(14)	15(1)	11(1)	8(1)	-1(1)	-1(1)	-1(1)
C(15)	18(1)	15(1)	9(1)	-1(1)	0(1)	-5(1)
C(16)	22(1)	14(1)	10(1)	-2(1)	1(1)	1(1)
C(17)	18(1)	18(1)	7(1)	0(1)	-1(1)	-1(1)
C(18)	17(1)	16(1)	11(1)	-1(1)	2(1)	-3(1)
C(19)	17(1)	17(1)	6(1)	2(1)	-2(1)	-1(1)
C(20)	12(1)	16(1)	12(1)	0(1)	2(1)	-2(1)
C(21)	13(1)	14(1)	9(1)	0(1)	1(1)	-5(1)
C(22)	18(1)	16(1)	14(1)	-2(1)	3(1)	-3(1)
C(23)	18(1)	17(1)	17(1)	4(1)	0(1)	2(1)
C(24)	18(1)	22(1)	11(1)	2(1)	-1(1)	-5(1)
C(25)	21(1)	18(1)	11(1)	-4(1)	2(1)	-3(1)
C(26)	16(1)	13(1)	14(1)	-2(1)	1(1)	-1(1)
C(27)	15(1)	12(1)	13(1)	-1(1)	4(1)	-4(1)
C(28)	14(1)	11(1)	14(1)	-1(1)	1(1)	-2(1)

C(29)	20(1)	16(1)	17(1)	-2(1)	0(1)	2(1)
C(30)	32(2)	22(2)	13(1)	-4(1)	-1(1)	3(1)
C(31)	32(2)	24(1)	12(1)	0(1)	-5(1)	0(1)
C(32)	21(1)	15(1)	22(2)	2(1)	-3(1)	1(1)
C(33)	17(1)	13(1)	16(1)	-2(1)	2(1)	-1(1)

Table A.5. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{Å}^2 \times 10^3$) for bb3136.

	x	y	z	U(eq)
H(1)	5700(50)	3030(30)	4401(12)	19(10)
H(2)	2474	5108	4186	20
H(3A)	2941	3641	4753	20
H(3B)	3152	5008	4954	20
H(6A)	8033	4309	4215	27
H(6B)	8849	4804	4619	27
H(6C)	8556	5757	4260	27
H(7A)	7010	7192	4674	17
H(7B)	5185	6836	4849	17
H(8A)	7173	7108	5370	17
H(8B)	8353	6027	5186	17
H(9)	4947	5602	5456	13
H(11)	6230	3909	6353	13
H(12A)	5138	2184	5985	15
H(12B)	7024	2547	5849	15
H(13A)	3974	3214	5431	15
H(13B)	5348	2210	5281	15
H(14)	7388	3785	5197	14
H(15A)	6965	6248	6397	17
H(15B)	7025	7065	6001	17
H(16A)	4487	7456	6361	19
H(16B)	4070	6926	5929	19
H(17)	2321	5847	6369	17
H(18A)	3267	4458	5893	17
H(18B)	3288	3719	6304	17
H(19A)	8886	4107	5737	16
H(19B)	9281	5578	5786	16
H(22)	11640	1817	6762	19
H(23)	12447	1652	7425	21
H(24)	11496	3124	7885	21
H(25)	9763	4790	7686	20
H(26)	9039	5021	7019	17
H(29)	2353	7089	7727	21
H(30)	3112	6596	8376	27
H(31)	5135	5039	8500	27

H(32)	6364	3929	7976	23
H(33)	5563	4377	7326	19

Table A.6. Torsion angles [deg] for bb3136.

C(5)-C(1)-C(2)-C(3)	-2.9(3)
I(1)-C(1)-C(2)-C(3)	-179.66(19)
C(1)-C(2)-C(3)-C(4)	-18.6(3)
C(2)-C(3)-C(4)-O(1)	-85.7(2)
C(2)-C(3)-C(4)-C(14)	158.0(2)
C(2)-C(3)-C(4)-C(5)	31.5(3)
C(2)-C(1)-C(5)-C(6)	143.6(3)
I(1)-C(1)-C(5)-C(6)	-39.5(3)
C(2)-C(1)-C(5)-C(7)	-92.3(3)
I(1)-C(1)-C(5)-C(7)	84.6(2)
C(2)-C(1)-C(5)-C(4)	22.6(3)
I(1)-C(1)-C(5)-C(4)	-160.47(18)
O(1)-C(4)-C(5)-C(6)	-38.1(3)
C(14)-C(4)-C(5)-C(6)	78.4(3)
C(3)-C(4)-C(5)-C(6)	-154.2(2)
O(1)-C(4)-C(5)-C(1)	83.9(2)
C(14)-C(4)-C(5)-C(1)	-159.6(2)
C(3)-C(4)-C(5)-C(1)	-32.2(2)
O(1)-C(4)-C(5)-C(7)	-162.7(2)
C(14)-C(4)-C(5)-C(7)	-46.2(3)
C(3)-C(4)-C(5)-C(7)	81.2(3)
C(6)-C(5)-C(7)-C(8)	-74.3(3)
C(1)-C(5)-C(7)-C(8)	159.4(2)
C(4)-C(5)-C(7)-C(8)	51.7(3)
C(5)-C(7)-C(8)-C(9)	-60.4(3)
C(7)-C(8)-C(9)-C(14)	60.3(3)
C(7)-C(8)-C(9)-C(10)	-172.3(2)
C(8)-C(9)-C(10)-C(19)	-55.2(3)
C(14)-C(9)-C(10)-C(19)	70.3(3)
C(8)-C(9)-C(10)-C(11)	-176.5(2)
C(14)-C(9)-C(10)-C(11)	-51.0(3)
C(8)-C(9)-C(10)-C(15)	63.2(3)
C(14)-C(9)-C(10)-C(15)	-171.3(2)
C(19)-C(10)-C(11)-C(12)	-66.5(3)
C(15)-C(10)-C(11)-C(12)	176.6(2)
C(9)-C(10)-C(11)-C(12)	54.0(3)
C(19)-C(10)-C(11)-C(18)	169.6(2)
C(15)-C(10)-C(11)-C(18)	52.7(3)
C(9)-C(10)-C(11)-C(18)	-69.9(3)
C(18)-C(11)-C(12)-C(13)	68.2(3)

C(10)-C(11)-C(12)-C(13)	-57.3(3)
C(11)-C(12)-C(13)-C(14)	56.2(3)
C(12)-C(13)-C(14)-C(4)	-179.9(2)
C(12)-C(13)-C(14)-C(9)	-53.4(3)
O(1)-C(4)-C(14)-C(13)	-63.8(3)
C(3)-C(4)-C(14)-C(13)	55.2(3)
C(5)-C(4)-C(14)-C(13)	175.9(2)
O(1)-C(4)-C(14)-C(9)	169.7(2)
C(3)-C(4)-C(14)-C(9)	-71.3(3)
C(5)-C(4)-C(14)-C(9)	49.4(3)
C(8)-C(9)-C(14)-C(13)	179.7(2)
C(10)-C(9)-C(14)-C(13)	51.5(3)
C(8)-C(9)-C(14)-C(4)	-55.1(3)
C(10)-C(9)-C(14)-C(4)	176.8(2)
C(19)-C(10)-C(15)-C(16)	-174.2(2)
C(11)-C(10)-C(15)-C(16)	-55.2(3)
C(9)-C(10)-C(15)-C(16)	66.3(3)
C(10)-C(15)-C(16)-C(17)	55.3(3)
C(27)-O(4)-C(17)-C(18)	-150.4(2)
C(27)-O(4)-C(17)-C(16)	88.3(3)
C(15)-C(16)-C(17)-O(4)	67.4(3)
C(15)-C(16)-C(17)-C(18)	-50.7(3)
O(4)-C(17)-C(18)-C(11)	-69.6(3)
C(16)-C(17)-C(18)-C(11)	50.6(3)
C(12)-C(11)-C(18)-C(17)	-178.1(2)
C(10)-C(11)-C(18)-C(17)	-52.7(3)
C(20)-O(2)-C(19)-C(10)	117.0(2)
C(11)-C(10)-C(19)-O(2)	-56.0(3)
C(15)-C(10)-C(19)-O(2)	61.4(3)
C(9)-C(10)-C(19)-O(2)	-177.28(19)
C(19)-O(2)-C(20)-O(3)	1.7(4)
C(19)-O(2)-C(20)-C(21)	-178.5(2)
O(3)-C(20)-C(21)-C(22)	14.2(4)
O(2)-C(20)-C(21)-C(22)	-165.5(2)
O(3)-C(20)-C(21)-C(26)	-165.6(3)
O(2)-C(20)-C(21)-C(26)	14.6(3)
C(26)-C(21)-C(22)-C(23)	-0.6(4)
C(20)-C(21)-C(22)-C(23)	179.5(2)
C(21)-C(22)-C(23)-C(24)	1.3(4)
C(22)-C(23)-C(24)-C(25)	-0.5(4)
C(23)-C(24)-C(25)-C(26)	-1.0(4)
C(24)-C(25)-C(26)-C(21)	1.6(4)
C(22)-C(21)-C(26)-C(25)	-0.8(4)
C(20)-C(21)-C(26)-C(25)	179.0(2)

C(17)-O(4)-C(27)-O(5)	-2.6(4)
C(17)-O(4)-C(27)-C(28)	176.9(2)
O(5)-C(27)-C(28)-C(33)	176.9(3)
O(4)-C(27)-C(28)-C(33)	-2.6(4)
O(5)-C(27)-C(28)-C(29)	-1.2(4)
O(4)-C(27)-C(28)-C(29)	179.3(2)
C(33)-C(28)-C(29)-C(30)	0.0(4)
C(27)-C(28)-C(29)-C(30)	178.2(3)
C(28)-C(29)-C(30)-C(31)	0.9(5)
C(29)-C(30)-C(31)-C(32)	-0.8(5)
C(30)-C(31)-C(32)-C(33)	0.0(5)
C(31)-C(32)-C(33)-C(28)	0.9(4)
C(29)-C(28)-C(33)-C(32)	-0.9(4)
C(27)-C(28)-C(33)-C(32)	-178.9(3)

Symmetry transformations used to generate equivalent atoms:

Table A.7. Hydrogen bonds for bb3136 [A and deg.].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1)...O(3)#1	0.72(4)	2.14(4)	2.832(3)	160(4)

Symmetry transformations used to generate equivalent atoms:

#1 $x-1/2, -y+1/2, -z+1$

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Chapter 4

Synthesis and Structure-Activity Relationships of Cardiotonic Steroids and Their Analogs to Identify Nanomolar Inhibition Against Cancer Cell Lines

(The manuscript for the work in this chapter is in preparation. All the biological data presented in this chapter is performed by Dr. Marcus John Curtis Long from our collaboration with Aye Research Group at Cornell University. Some of the analogs described in this chapter is synthesized by Dr. Hem Raj Khatri and Dr. Jia-Hui Tay and will be acknowledged where needed.)

4.1. Introduction

Cancer has been closely studied for a few decades and despite a major portion of the research and development funding being invested in studying the measures to counteract it, the desired progress in decreasing its effects or preventing it has been unsatisfactory. More recently, cardiotonic steroids (CS) have attracted attention as anticancer agents and interest in advancement of the applicability of these steroids for this purpose has raised progressively. Cardiotonic steroids had been known and used for their beneficial properties in traditional medicine around the world. Frogs^{1,2} and plants^{3,4} extract containing these steroids had been used as arrow poisons in parts of Africa, especially in Maasai plains of Kenya and East African Somalis.⁵ Foxglove plant, one of the natural source of these steroids, was used as juice for sprains and bruises by ancient Romans and Greeks.⁶ Foxglove juice was also found to release excess fluid by stimulating the kidneys and tea made from foxglove leaves were used to treat Dropsy. The publication “On the account of the foxglove” by Sir William Withering⁷ in 1785 opened a frontier of this plant to new medical

applications and physicians then started using it to treat chronic heart failure and irregular heartbeats, one of the more traditional use of cardiotonic steroids where they received their name from. In addition, the reports on the use of oleander, another natural plant source rich in CS, to treat cancer as well as AIDS patients have been documented in different geographical locations since the late Middle ages.^{8,9}

Several of these steroids are found as endogenous human hormones in mammalian body fluids and tissues. Cardenolides like digoxin have been detected in human urine¹⁰ and ouabain has been evidenced in human plasma, adrenal gland, and hypothalamus.^{11,12} In addition to this, 19-norbufalin has been identified in human lenses and marinobufagenin,¹³ and proscillaridin-like bufadienolides observed in human urine and plasma.^{11,14}

4.2. Mode of Action of Cardiotonic Steroids

4.2.1. Cardiovascular Effects

Cardiotonic steroids have widely been used in biological research and lately their interaction with human body has been better understood. Traditionally, these steroids were used in the treatment of congestive heart failure by increasing a positive inotropic response. This response occurs when a cardiotonic steroid binds as a ligand to sodium pump or Na^+/K^+ -ATPase, the ion transport protein that is inserted in the cell membrane. It contains two subunits: α -subunit, which is a trans-membrane protein that has binding sites for Na^+ , K^+ , cardiotonic steroids, and ATP and, β -subunit, which is a trans-membrane protein with site for glycosylation that is needed for enzyme complex activity.

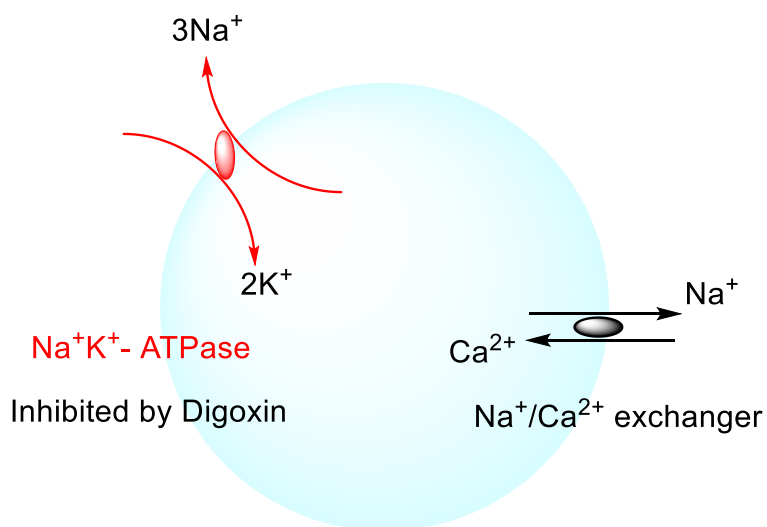
Na^+/K^+ -ATPase is a receptor of cardiotonic steroids and that the steroids are specific inhibitors of sodium pump. The binding of these steroids in Na^+/K^+ -ATPase enzyme increases the level of intracellular sodium ions and reduces the concentration of potassium ions in myocytes. This ion

exchange is facilitated by hydrolysis of the ATP. The concentration of Na^+ ion controls the activity of $\text{Na}^+/\text{Ca}^{2+}$ -exchanger that is involved in exchange of Na^+ and Ca^{2+} in a cell (**Figure 4.1**). An increase in Na^+ inside the cell exchanges for calcium results in the increase in intracellular concentration of Ca^{2+} ions, also called positive inotropic effect. An increase in the concentration of Ca^{2+} activates myocardial contractility due to the availability of more Ca^{2+} for contractile proteins. This way, the use of cardiac steroids like ouabain or digoxin during the congestive heart failure counteracts the condition and minimizes fatality. Regardless of the fact that cardiotonic steroids that are currently used as a drug have very narrow therapeutic doses, they are the best available treatment of congestive heart failure at the moment and about 1.7 million patients receive digoxin for atrial fibrillation or heart failure.¹⁵

Figure 4.1. Mode of Action of Cardiotonic Steroids

4.2.2. Anticancer Effects

In addition to cardiovascular effects, the anticancer properties of steroids of this class have been studied and more recently, sodium pump has been a target in the anticancer therapy. There are several reports of cardiotonic glycosides and



their derivatives, obtained from either direct isolation or semi-synthesis, showing promising results against cancer cells of various types.^{16,17} Although there have been several reports on these anticancer properties, the precise mechanism of action of these cardiac glycosides have not been sufficiently elucidated. There are several theories that describe independent pathways responsible

for these effects.^{18,19} Most of these studies make it clear that there lies a strong correlation of anticancer activities to inhibition of Na⁺/K⁺-ATPase. Different isoforms of α -subunits are found to overexpress in different types of cancer (α -1 in melanoma, glioma, renal carcinoma and α -3 in colon carcinoma).²⁰⁻²² In addition to positive inotropic effects, the Na⁺/K⁺-ATPase can also activate intracellular signaling cascades.^{18,23} Although signaling cascades are very similar, if not the same, the final response of the tumor cells are abnormal and show difference in expression patterns than the normal cells.²⁴ This difference in activity and expression of Na⁺/K⁺-ATPase of cancer cells (usually a significant increase in activity) in comparison to normal cells may be the reason for this difference in response. This difference in response is something that scientists believe could be explored in order to develop a unique anticancer drug of the future.

Lopez-Lazaro et al. validated the anticancer activity of cardiotonic steroids by inhibiting the growth of a variety of cancer cells and presented a different but interesting hypothesis that the anticancer activity might be a result of inhibition of glycolysis that occurs with the inhibition of Na⁺/K⁺-ATPase.²⁵ Growth of cancer cells required initiation of glycolysis and a higher rate of aerobic glycolysis is a known characteristic of cancer cells (Warburg effect).^{26,27} Difference in the activity of tumor cells in comparison to the normal cells and the ability of cardiotonic steroids to selectively kill them can be attributed to the long known ability of cardiotonic steroids to suppress aerobic glycolysis.²⁸ Cardiotonic steroids are also reported to act as immunogenic agents.^{14,29} Inhibition of Na⁺/K⁺-ATPase alters the homeostasis of K⁺, Na⁺, and Ca²⁺ and homeostasis of Ca²⁺, in particular, induces immunogenic cell death.³⁰ This theory is supported by both *in vitro* studies and an *in vivo* study in knockout mice.²⁹

4.3. Cardiotonic Steroids as Anticancer Agent

In 1967, the first antiproliferative activity of cardiotonic steroids against transformed cells was reported.³¹ Moreover, there are data that indicate that cancer patients who were exposed to digitalis therapy had lower mortality rates than those who were not exposed.^{32–34} Similarly, in 1980s, a study on cell-proliferation activity by Stenkvis et al. in breast cancer cells obtained from patients suffering from breast cancers showed that the proliferation capacity of the cells were lower in patients who underwent digitalis therapy. Moreover, a follow up study on this result showed that after 5 years, the recurrence rate of breast cancer of patients on digitalis therapy was 10 times lower than the patients who were not on digitalis therapy.³⁴ After 20-years, it was disclosed that the mortality rate of patients on digitalis from breast carcinoma was 6% (2 of 32) in comparison to 34% (48 of 143) of patients among patients not on digitalis.³⁵

Following the 1967 finding and Stenkvis's reports, several scientists have investigated cardiotonic steroids against many other cancer cells lines including pancreatic,³⁶ lung,^{16,37} breast,³⁸ melanoma,³⁹ renal,³⁸ leukemia,^{40,41} prostate,⁴² myeloma,⁴³ and neuroblastoma⁴⁴ and these studies (**Table 4.1**) show promising activity *in vitro* and *in vivo*.

Table 4.1. *In Vitro* Anticancer Studies of Cardiotonic Steroids in Cancer Cells

Cancer type	Steroids tested	Cancer Cell lines	Refs
Pancreatic	oleandrin	PANC-1	35
Lung	Digitoxin, digoxin, ouabain UNBS1450	A549, NCI-H-358, Calu1, Sklu1, NCI-H6, H69AR	36, 37
Breast	Digitoxin, digoxigenin, digoxin gitoxigenin, gitoxin, proscillaridin A, ouabain	MCF-7, MDA-MD-435	38
Melanoma	Digitonin, digitoxin, digoxin, oleandrin, ouabain, proscillaridin A	UACC-62, BRO	39
Myeloma	digitonin, Digitoxigenin, digitoxin lanatocide C, ouabain, proscillaridin A	8226-S, 8226-LR5, 8226-DOX-40	43
Renal	Digitoxigenin, digitoxin, digoxin ouabain, proscillaridin A	TK-10, ACHN	38
Leukaemia	Bufalin, digitoxin, oleandrin, ouabain proscillaridin A	HL60, U-937, CCRF-CEM, CEM-VM-1	40, 41
Prostate	bufalin, cinobufagenin, digoxin, oleandrin, ouabain	PC-3, LNCaP, DU145	42
Neuroblastoma	digoxin, ouabain	SH-SY5Y, Ueuro-2a	44

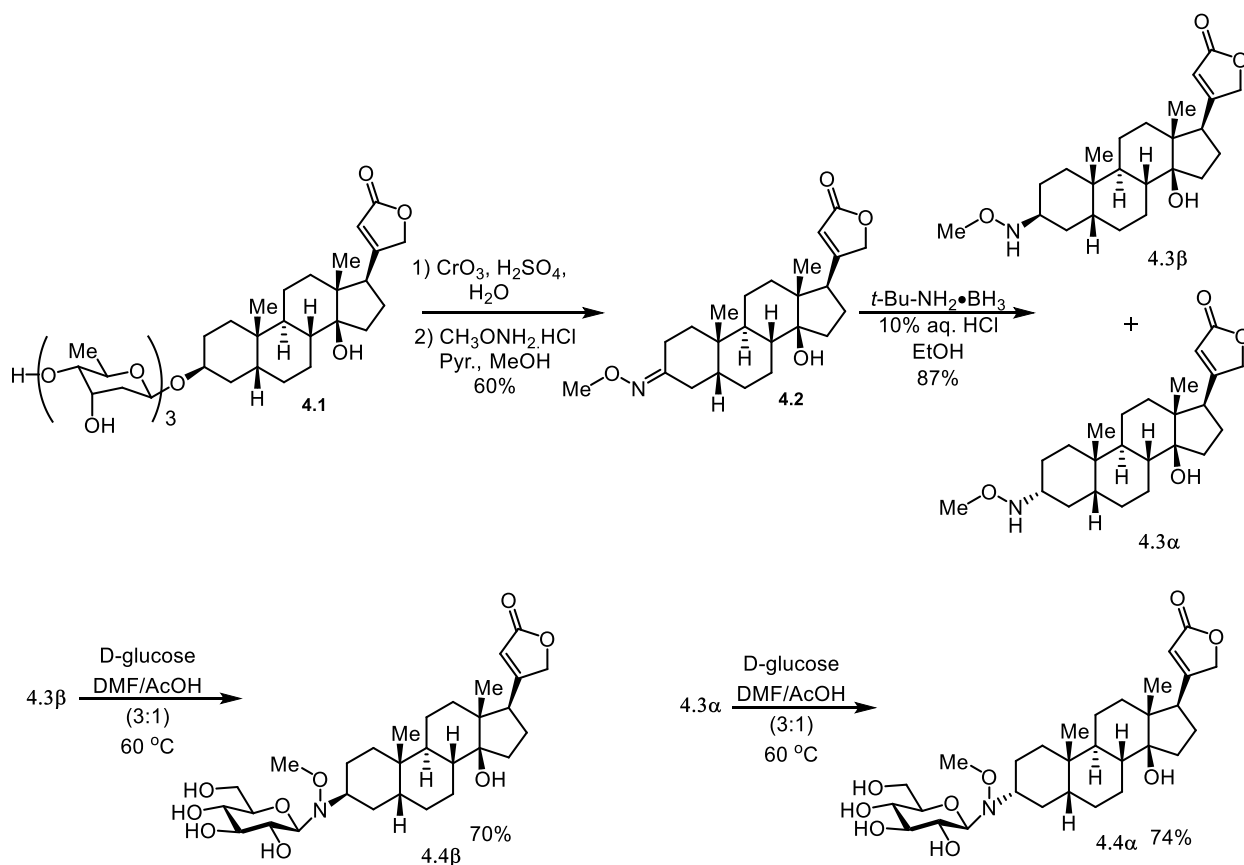
Several *in vitro* studies have suggested an anti-proliferative action of cardiotonic steroids against cancer cell lines (**Table 4.1**). Some of the steroids have been shown to selectively kill cancer cells in the presence of normal cells. In fact, out of 9000 chemicals and known drugs tested in an *in vitro* study by Johnson et al. against human prostate cancer cell lines PC-3, digitoxin and ouabain were the most potent inhibitors.⁴⁵ While many such reports on *in vitro* studies of these steroids for performance against cancer are known, only limited examples on the *in vivo* studies have been reported to date.⁴⁶⁻⁴⁸ These steroids demonstrate inhibition of tumor cell growth in *in vivo* studies but are either highly toxic (like ouabain) or lack practical anticancer activity (like digitoxin) at reasonable dose.⁴⁷ From these aforementioned studies, it is quite evident that a

significant medicinal chemistry effort need to be employed to reduce the toxicity and increase the activity of these cardiotoxic steroids in order for them to be developed as useful anticancer agents.

One of a remarkable work on synthesis of numerous analogs of these steroids in an efficient fashion was done by Thorson and co-workers in 2005 on their glycorandomization studies.⁴⁹ Their method allowed for a direct installation of sugars without the need for protection or for promoter during glycosylation. For this study, they used digitoxin as a model aglycone to test neoglycorandomization reaction. This method would allow for the generation of a library of analogs with a variety of sugar in a single aglycone. The reaction between secondary alkoxyamine of an aglycone with reducing sugars created a new glycosidic linkage to form neoglycosides. As shown in **Scheme 4.1**, **4.3 β** and **4.3 α** was synthesized in three steps from parent glycoside digitoxin. The reaction of **4.3 β** and **4.3 α** separately with the glucose in presence of 3:1 DMF/AcOH provided neoglycosides **4.4 β** and **4.4 α** in >70% yield. Using this method, β and α neoglycosides of 39 reducing sugars were synthesized to generate 78 derivatives of digitoxin. The authors were able to generate neoglycosides with a variety of reducing sugars like D-sugars, L-sugars, disaccharides, dideoxy sugars, and uronic acids and test these analogs against nine human cancer cells including ovary, lung, CNS, liver, breast, colon, and normal epithelial lines using high-throughput assay. During their study, they were able to find neoglycosides with substantially enhanced potency and cancer specificity but with diminished Na⁺/K⁺-ATPase inhibition than the parent glycoside digitoxin. The SAR obtained from this study shows that all 38 β -neoglycosides performed better than the corresponding α neoglycosides, confirming the previous studies. L-riboside and D-mannoside showed a better potency and good selectivity; D-taloside showed good selectivity but modest cytotoxicity; D-lyxoside and L-xyloside showed significant enhancement in cytotoxicity compared to digitoxin, however, lacked selectivity just like the parent glycoside.

One similarity among these five glycosides is the axial C2' stereochemistry (S-configuration) and their epimer neoglycosides at C2' shows relatively diminished potency. Unlike most of the proposed mechanism of cardiotonic steroids that shows strong correlation of anticancer activity with Na⁺/K⁺-ATPase inhibition,^{13,50} these results present a new class of glycosides specific to the tumor cells and cytotoxins without inhibiting Na⁺/K⁺-ATPase.⁴⁹

Scheme 4.1. Method for the Synthesis of Neoglycosides

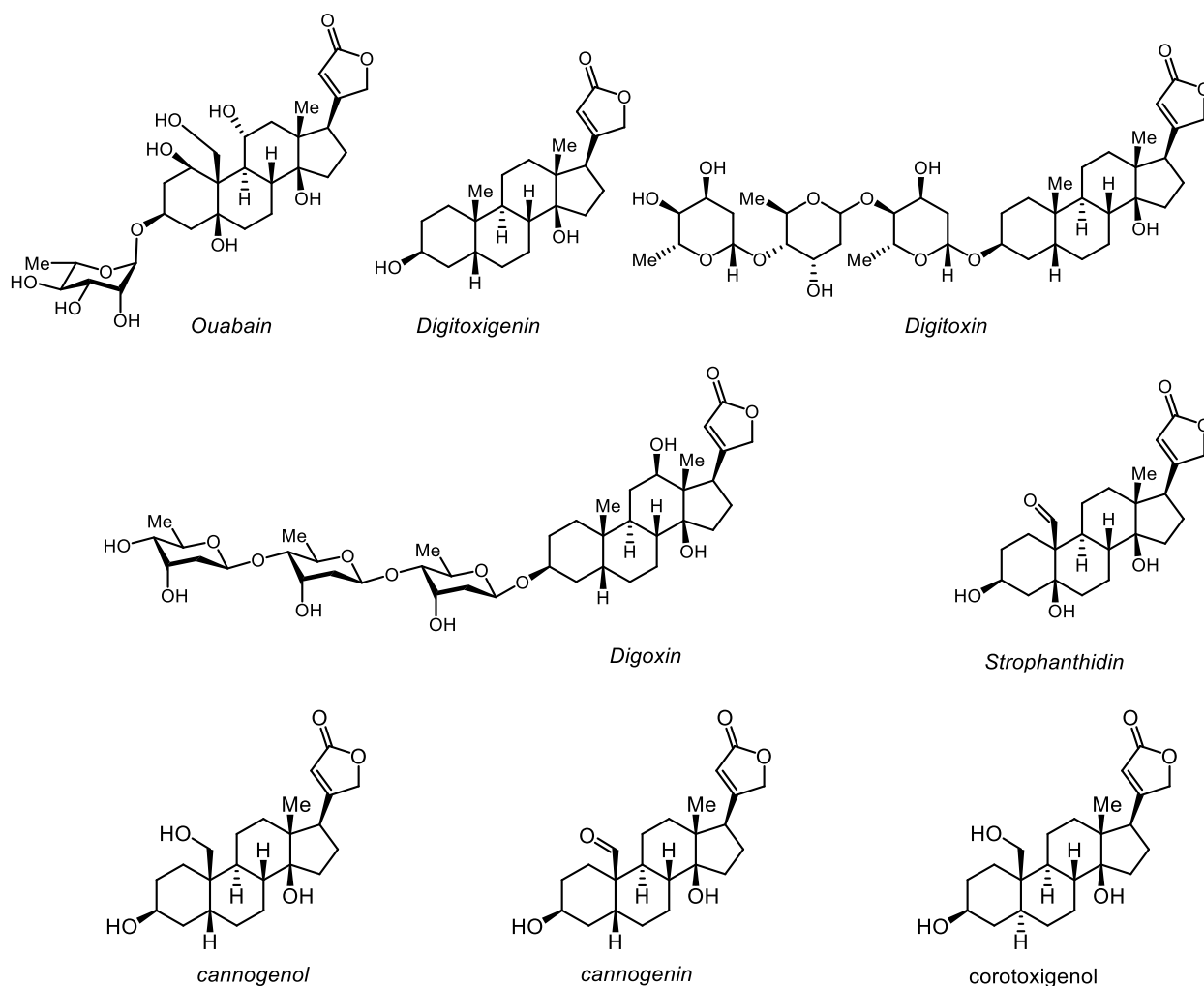


Easily accessible steroids like ouabain, digitoxigenin, digitoxin, digoxin, strophanthidin and their analogs, mostly obtained from their natural source, are subjects of most of these investigation on cardiotonic steroids, however, they present only a limited opportunity for modification and limit the scope of these studies.

4.4. C19 Oxygenated Steroids and Their Scope in Anticancer Activities

There have been few recent studies on steroids with C19 hydroxyl group present, but only on highly oxygenated molecules. Despite the promising anticancer activity shown by molecules like digitoxigenin and strophanthidin against the variety of cancer cell lines,^{51,52} their C-19 hydroxylated counterparts such as corotoxigenol, cannogenol and the respective glycosides (**Figure 4.2**) have not been well studied. This can be attributed to the global supply of these steroids, the complexity in their extraction from poisonous indigenous plants, their low concentration in these plant sources, and most importantly there being no robust method available to synthesize these steroids and their analogs.

Figure 4.2. C10 Methyl and C19 Hydroxy Steroids with Promising Potency



4.5. Isolation of Cannogenol Based Steroids and their Anticancer Activity

Cannogenol-based steroids have been isolated from plant sources for a long time. Cannogenol-3-*O*- α -L-rhamnoside, itself, was first isolated in 1967 by Kislichenko et al. and since then, several other research groups have identified various other cannogenol (with hydroxyl at C19) and cannogenin (with aldehyde at C19) glycosides during the isolation of cardiac glycosides from different plant sources. Abe et al. reported the isolation of twenty-two glycosides from roots of *Apocynum cannabinum* including cannogenin, and cannogenol glycosides **4.20**, **4.21**.⁵³ Compound **4.19**, another cannogenol glycoside was isolated from seeds of *Corchorus olitorius* along with eight other closely related cardiotonic glycosides by Nakamura et al.⁵⁴

There has not been as much report on the Na⁺/K⁺-ATPase activity of cannogenol based natural products as other members of this class. One report on the cardiotonic activity of cannogenin-3-*O*- α -L-rhamnoside (Malayoside) showed similar safety index and potency to ouabain in the atria of guinea pigs.⁵⁵

In a study on antiproliferation of cardiac glycosides, Liu et al. isolated fifteen new and seventeen previously reported cardiac glycosides from the Latex of *Antiaris toxicaria*.⁵⁶ Some of the relevant glycosides (**Table 4.2**, Compound **4.5-4.18**) are listed for discussion. The toxicity of steroids (**4.5-4.18**) were investigated against human lung cancer cells NIH-H460 (**Table 4.3**). Compound **4.5**, **4.9**, **4.10**, **4.12**, and **4.16-4.18** significantly inhibited the viability of NIH-H460 at 50 nM concentration. There is a clear SAR information that can be gathered from this table. Orientation of sugar is very important and steroids with alpha-orientation of the sugar at C3 did not inhibit growth of the cancer cells (**4.6-4.8**). Having sugar at C19 instead of C3 displayed weak activity (**4.13**). The inhibitory rate of rhamnose glycosides were better than other sugar moieties. The functionality at C19 played little role and only small change in inhibitory rate was observed

(4.14-4.18). In conclusion to this study, cannogenol-3-*O*- α -L-rhamnoside was one of the best candidates out of thirty-two steroids isolated.

Table 4.2. Cardiotonic Steroids with C19 Oxygenation Isolated from Various Plants^{53,54,56,57}

Compound	R ₁	R ₂	R ₃	R ₄	Compound	R ₁	R ₂	R ₃	R ₄
4.5	CHO	β-OH	OH	β-O-4,6-dideoxy-β-D allose	4.22	H	H	H	α-L-thevetose
4.6	CHO	β-OH	OH	α-O-α-L-rhamnose	4.23	OH	H	H	α-L-thevetose
4.7	CHO	β-OH	OH	α-O-6-deoxy-β-D-allose	4.24	CH ₃	H	H	α-L-thevetose
4.8	CHO	β-OH	OH	α-O-6-deoxy-β-D-gulose	4.25	CH ₂ OH	H	H	α-L-thevetose
4.9	CHO	α-OH	OH	β-O-α-L-rhamnose	4.26	CHO	H	H	α-L-thevetose
4.10	CHO	β-OH	H	β-O-6-deoxy-β-D-gulose	4.27	COOH	H	H	α-L-thevetose
4.11	CHO	β-OH	H	β-O-6-deoxy-β-D-allose	4.28	CH ₂ OH	H	H	β-D-glc-(1→4)-α-L-thevetose
4.12	CHO	β-OH	H	β-O-α-L-rhamnose	4.29	CH ₂ OH	H	H	β-gentiobiosyl-(1→4)-α-L-thevetose
4.13	COOglc	H	OH	β-OH	4.30	CH ₂ OH	H	H	β-D-glc-(1→4)-α-L-acofriose
4.14	CH ₃	H	OH	rhamnoside	4.31	CH ₃	H	α-H	α-L-thevetose
4.15	CHO	H	OH	A					
4.16	CHO	H	OH	rhamnoside					
4.17	CH ₂ OH	H	OH	rhamnoside					
4.18	CH ₂ OH	H	H	rhamnoside					
4.19	CH ₂ OH	H	H	B					
4.20	CHO	H	H	B					
4.21	CH ₂ OH	H	H	C					

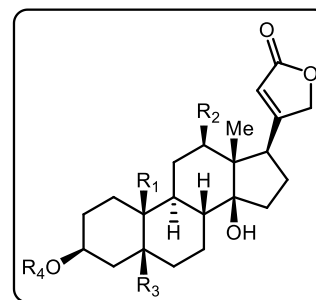
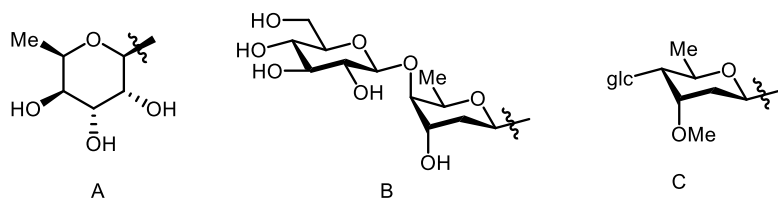
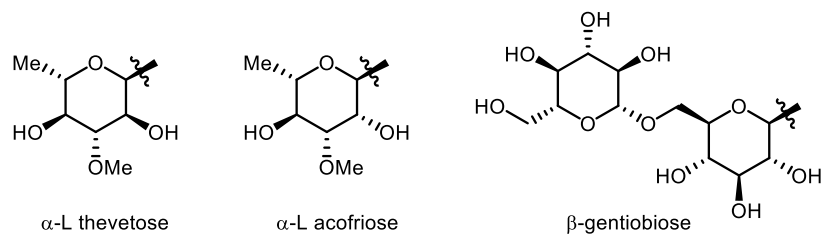


Table 4.3. Inhibitory Rate of Cardiotonic Steroids Isolated by Liu et al.⁵⁶

Compound	Inhibitory rate (%)	Compound	Inhibitory rate (%)
4.5	81.6	4.12	81.9
4.6	6.3	4.13	11.1
4.7	6.9	4.14	75.5
4.8	3.2	4.15	76.9
4.9	82.8	4.16	82.1
4.10	82.3	4.17	80.7
4.11	78.9	4.18	82.4

In a separate study by Tian et al., anticancer activity of thirty-three cardiac glycosides, isolated from the seeds of *Thevetia peruviana*, against three cancer cell lines (human P15 lung cancer cells, human MGC-803 gastric cancer cell, and human SW1990 human pancreatic cancer cells) and a normal LO2 hepatocyte cell line was studied.⁵⁷ Selected relevant steroids (**Table 4.2**, Compound **4.22-4.31**) and their performance in comparison to cannogenol and cannogenin glycosides are shown in **Table 4.4**. Across the board, thevetosides **4.22**, **4.24**, **4.25**, **4.26**, **4.28**, and **4.31** showed promising inhibitory effects against P15, MGC-803, and SW1990 but did not show any cytotoxicity against the normal LO2 cells in up to 10 μM concentration. The study showed a general trend in the effect of the sugar on cytotoxicity and observed a reduced cytotoxicity with increase in number of sugar moieties (trisachharide **4.29**<disachharide **4.28**< monosachharide **4.25**). The carboxylic acid at C10 and 19-nor cardenolides showed significant reduction of toxicity in comparison to hydroxymethyl, formyl, or methyl group. Furthermore, **4.22**, **4.24**, **4.25**, and **4.31** were assessed for apoptosis-inducing abilities against MGC-803 cells and at half maximal inhibitory concentration (IC_{50}) values of 0.53, 0.03, 0.03, 0.02 μM respectively, they showed

massive apoptosis. In summary, cannogenol-3-*O*- α -L-thevetose shows promising anticancer activities against P15, MGC-803, and SW1990 and did not have any effect in normal hepatocyte cell line LO2.

Table 4.4. Cytotoxicity (IC₅₀ values in μ M) of the Steroids Isolated from *Thevetia peruviana* by Tian et al.⁵⁷

Compound	P15	SW1990	MGC-803	LO2
4.22	0.21	0.57	0.53	>10
4.23	1.52	2.14	0.49	>10
4.24	0.06	0.02	0.03	>10
4.25	0.08	0.04	0.02	>10
4.26	0.03	0.02	0.02	>10
4.27	>10	3.93	>10	>10
4.28	0.12	0.32	0.13	>10
4.29	>10	9.52	7.70	>10
4.30	4.91	5.75	2.14	>10
4.31	0.01	0.7	0.03	>10

Shi et al. reported the isolation of cannogenol along with fifty-three other known and new cardiac glycosides and aglycones from trunk bark of *Antiaris toxicaria* and tested them against several human cancer cell lines (discussed in Chapter 2).⁵⁸ In a separate isolation and anticancer study by Wang et al. in 2007, cannogenol-3-*O*- α -L-rhamnoside and nine other cardenolides were isolated from the whole dried plants of *Saussurea stella* and showed very impressive activity against human gastric cancer and human hepatoma (Chapter 2).⁵⁹ Similarly in 2008, in a study by Jiang et al., eleven cardenolides including cannogenol-3-*O*- α -L-rhamnoside were isolated from stem of *Antiaris toxicaria* and their ability to inhibit cancer cell growth was studied (discussed in chapter 2). All these three reports showed nanomolar half maximal inhibitory concentration (IC₅₀)

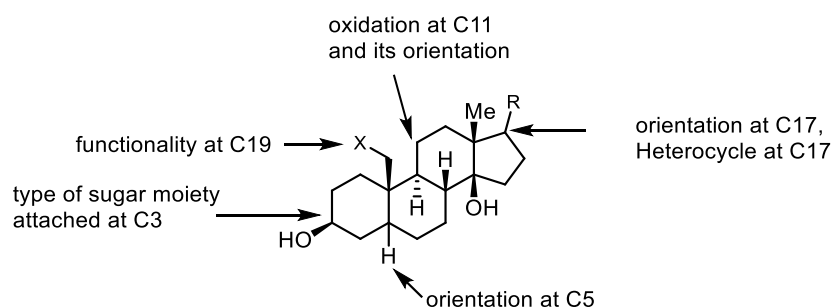
of cannogol-3-*O*- α -L-rhamnoside against several cancer cell lines that were studied and in all these cases, this molecule was one of the best candidates.^{59,60}

Although it exhibited an impressive activity against these cells, there have been no further reports on the elaboration of cannogol based steroids towards medicinal chemistry studies and diversification of the mentioned natural products to generate other analogs for similar testing. As mentioned earlier, we believe this to be largely due to the difficulty in their extraction from natural sources compounded by a very low concentration of these compounds in their natural sources. This is a gap that an organic chemist could fill using their expertise in synthetic organic chemistry.

4.6. Generation of Relevant Biological Analogs

The continuous interest of our group towards cardiotoxic steroids encouraged for us to design a concise synthetic pathway that would allow us an access to the natural products of this class. In addition, we wished to generate relevant analogues with minimum deviation from the synthetic pathway. The developed method would allow us to change functional group and stereochemistry at key position as well as installation of heterocycle and sugar moiety at late stage with minimum modifications.

Figure 4.3 Sites of Modification in the Steroidal Core

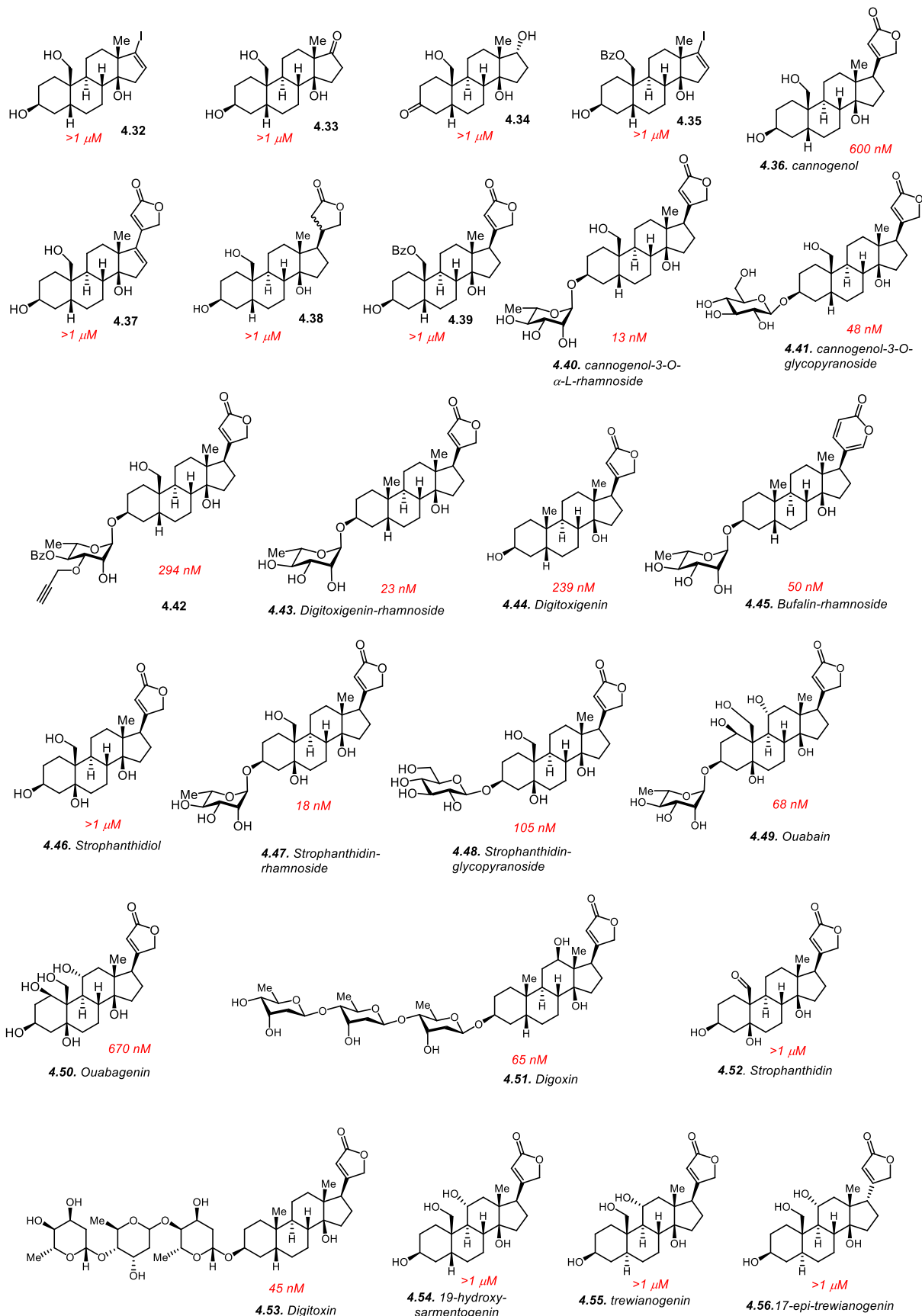


As discussed in the previous chapter, we were able to develop a strategy to synthesize cardiotoxic steroids which could potentially be used to generate their analogs with modification of different functionality of the steroidal core (**Figure 4.4**) to obtain good structure-activity relationship

correlations. While there were a plentiful of studies on anticancer activity of cardiotonic steroids as described earlier, a comprehensive parallel study including natural and synthetic cardenolides and their derivatives along with some of the commercially available cardiotonic steroids was not performed before. Therefore, using our concise and divergent synthetic approach, we were able to generate a reasonable number of natural products, synthetic analogues, and their relevant synthetic intermediates and use them in such a parallel study.

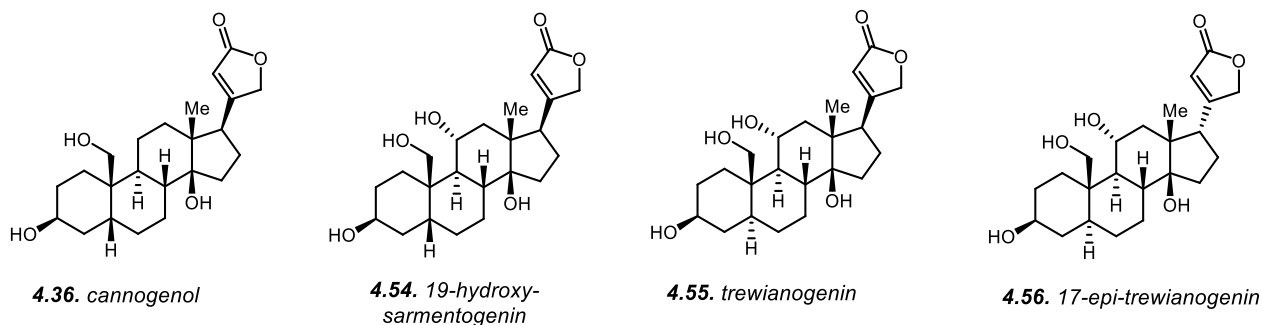
Compounds **4.44**, **4.49**, **4.51- 4.53** were obtained from commercial sources. Compounds **4.43** and **4.45** were obtained by colleague Dr. Hem Raj Khatri and **4.46**, **4.47**, and **4.48** were synthesized by Dr. Jia Hui Tay and Valentin Dorokhov by semi-synthesis from commercially available cardiotonic steroids using the method developed in our lab and discussed in chapter three. Compounds **4.54-4.56** were synthesized by Dr. Hem Raj Khatri and Dr. Will Kaplan and the synthesis of **4.54** and **4.56** are discussed in chapter 2.⁶¹

Figure 4.4. List of all the Cardiotonic Steroids and their Relevant Analogs Included in the Study and their EC₅₀ Values against HEK393T



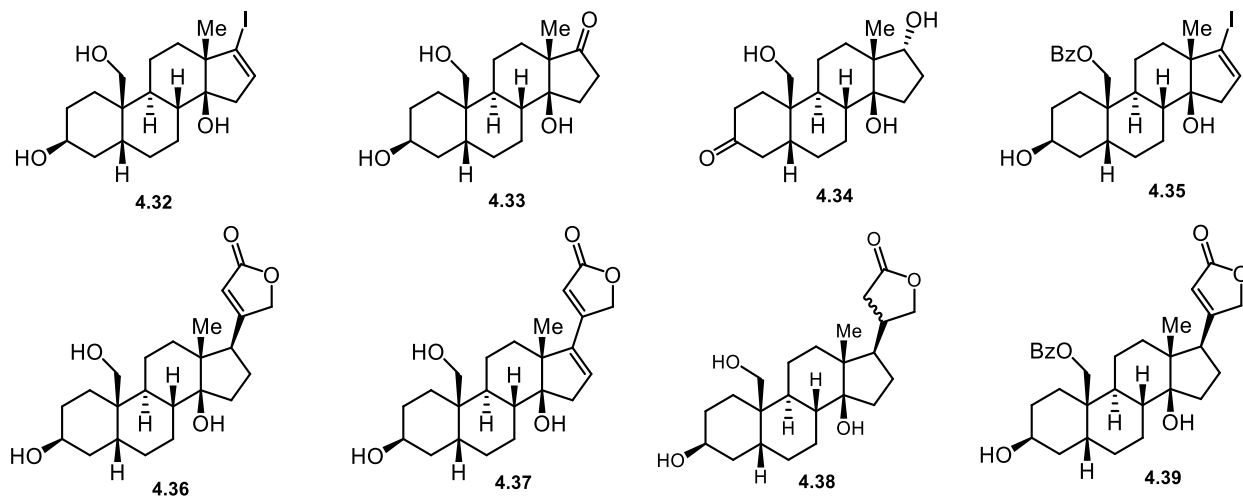
Initial biological activity of cannogenol along with other synthetic cardiotonic steroids were impressive, which encouraged us to construct several other natural products and biologically relevant analogs. The goal of this study was to ultimately design more potent analogs by studying the structure-activity relationships (SARs) of these molecules for their anticancer properties in collaboration with Dr. Yimon Aye's Research Group at the Cornell University. The initial biological activities on the four aglycone of cardiotonic steroids **4.36** and **4.54-4.56** (Figure 4.5) were obtained through NIH CANVAS high-throughput screen against a set of over 30 whole-cell assays designed to sample several areas (rare and neglected diseases, inflammation, cancer, and others). The NCGC ID for cannogenol is NCGC00488733-01, 19-hydroxy-sermentogenin is NCGC00488734-01, trewianogenin is NCGC00488732-01, and 17-epi trewianogenin is NCGC00488735-01 and full results of their assays can be obtained from NIH CANVAS website. High potency of cannogenol was observed during this screen against several assays which included against several cancer cell lines. This encouraged us to further investigate the molecules as described below. Cannogenol **4.36** showed an impressive activity (500 - 900 nM) against several cancer cell lines (HeLa, Cos-7, HEK, A2780cis, KB-3-1) and outperformed the other three, higher-oxygenated steroids such as 19-hydroxy sarmentogenin **4.54**, trewianogenin **4.55**, and 17-epi-trewianogenin **4.56**, that were also synthesized by our group.

Figure 4.5. Cardiotonic Steroids Aglycones Submitted for NIH CANVAS High-throughput Screening



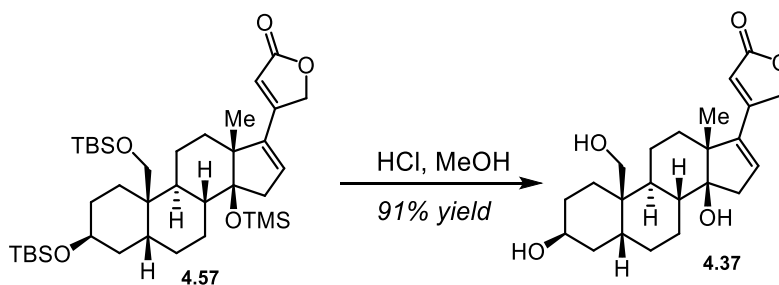
During initial collaboration with Aye's group, the following compounds (**Figure 4.6**) were tested against cancer cell lines to obtain a better idea on effects of different functional group, heterocycles, and stereocenters on their biological activity. The synthesis of **4.32-4.36**, **4.39**, and **4.40** are described in the previous chapter (chapter 2).

Figure 4.6. Steroids **4.32- 4.39** Included in Initial Assay



To understand the importance of stereocenter at C17, compound **4.37** was synthesized (**Scheme 4.2**) by global deprotection of the silanes from compound **4.57** (an intermediate discussed in chapter 2).

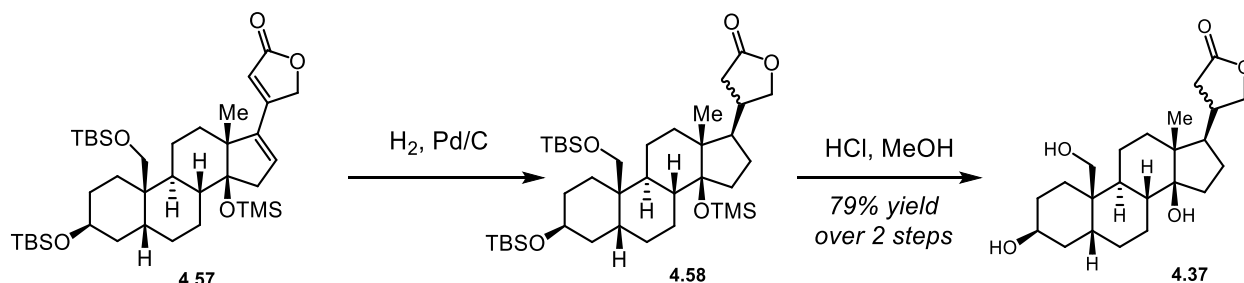
Scheme 4.2. Synthesis of **4.37** with no stereocenter at C17



Reduced lactone **4.37** was synthesized (**Scheme 4.3**) as a variant to recognize the importance of lactone ring. Synthesis of **4.38** was carried out by exhaustive hydrogenation of **4.37** to get

compound **4.58** with ~1:1 dr at C20 followed by global deprotection of the silane groups to obtain the diastereomeric mixture of **4.38** in 79% yield over 2 steps.

Scheme 4.3. Synthesis of **4.39** with reduced lactone



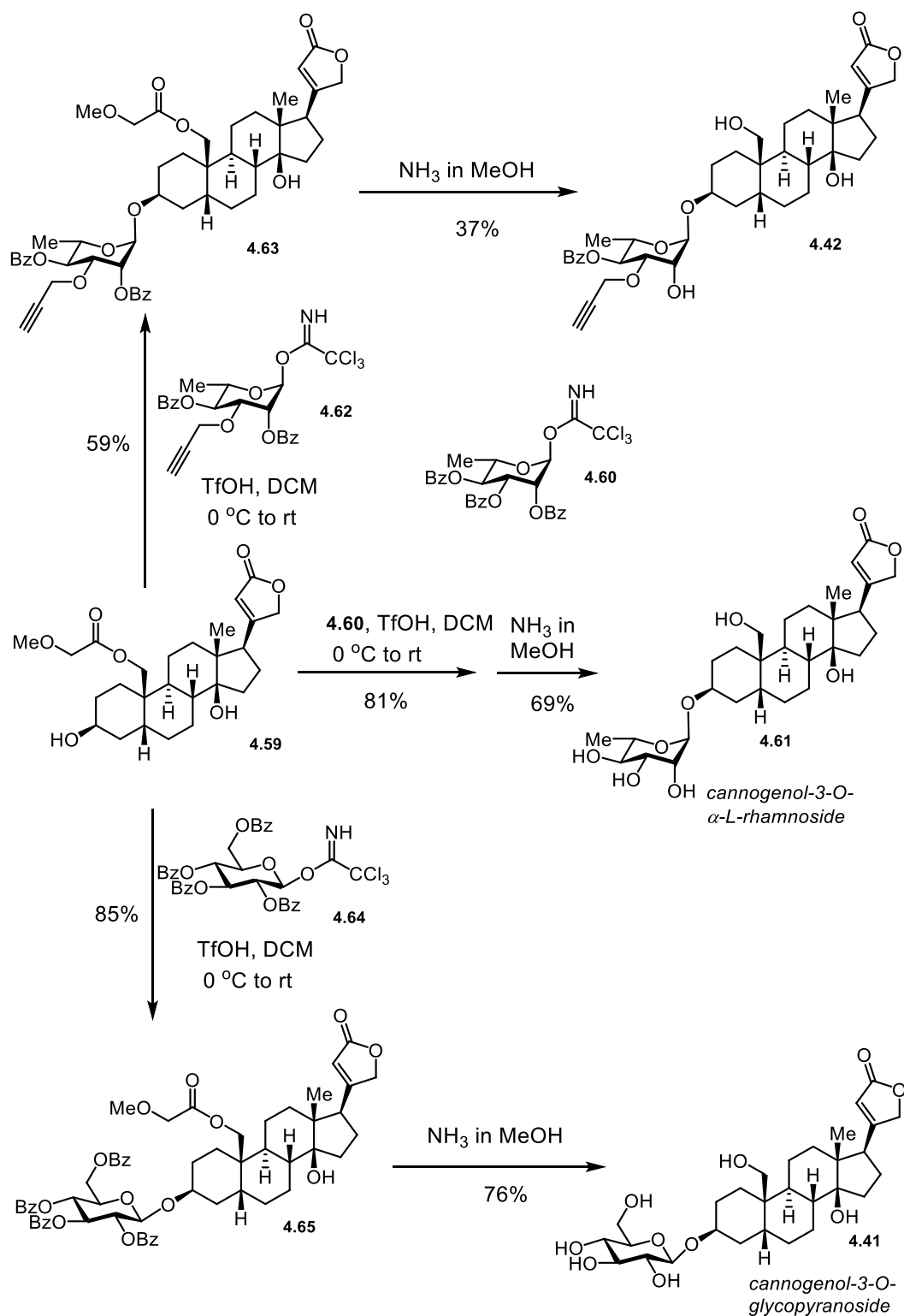
4.7. Total Synthesis of Cannogenol-3-O-gluco-pyranoside and Analog 4.42

The synthetic pathway towards the total synthesis of cannogenol-3-O- α -L-rhamnoside is robust and efficient. Late stage functionalization for generation of natural products or analogs with different sugar moiety or heterocycle was achieved using this method with minimum deviation. Described below is the synthesis of the natural product cannogenol-3-O-glycopyranoside and an analog with incorporated alkyne moiety at C3 of the sugar (**Scheme 4.4**). This was synthesized to identify the target protein of the cardiotoxic steroids. Both molecules were synthesized using the same method developed for cannogenol-3-O- α -L-rhamnoside without any synthetic modification.

The synthesis of cannogenol-3-O-glycopyranoside **4.41** commenced with glycosylation of methoxyacetate-protected cannogenol **4.59** (synthesized in chapter 2) catalyzed by TfOH to provide **4.65** in 85% yield. Compound **4.65** was subjected to ammonia (in methanol) to obtain the desired cannogenol-3-O-glycopyranoside (**4.41**) in 76% yield. Alkyne incorporated steroid **4.42** was obtained using the same method. Methoxyacetate protected cannogenol **4.59** was subjected to TfOH-promoted glycosylation with **4.62** (obtained in 6 steps from L-rhamnose), which resulted in α -rhamnoside **4.63** in 59% yield (>20:1 dr) and the deprotection of esters in presence of ammonia resulted in analog **4.42** in 37% yield. The deprotection of C-4' benzoyl group was very sluggish

and resulted into the serious concomitant side reactions at butenolide at prolonged reaction time which resulted in a lower yield.

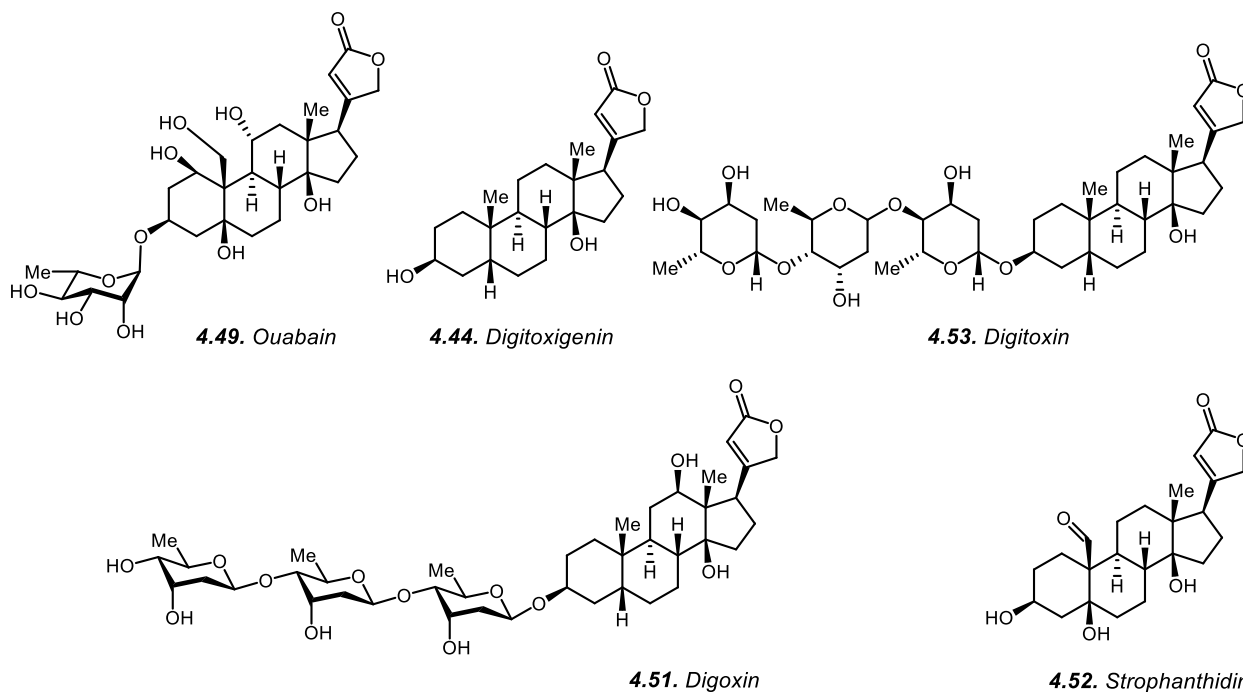
Scheme 4.4. Total Synthesis of Cannogenol-3-O-glucoopyranoside and Analog **4.42**



Commercially available, biologically active natural cardiotonic steroids **4.44**, **4.49**, and **4.51-4.53** (Figure 4.7) were also included in our study to understand the anticancer properties. These

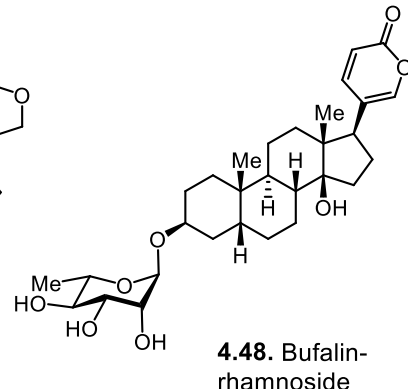
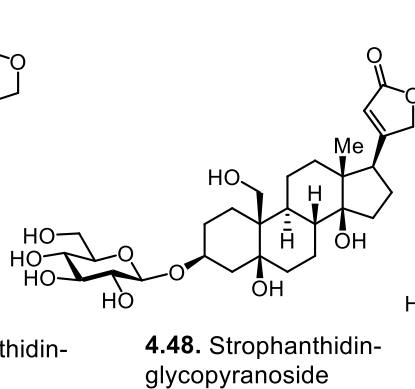
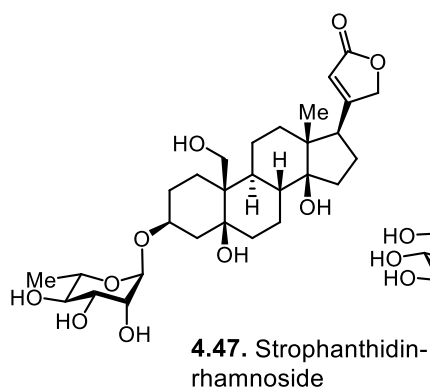
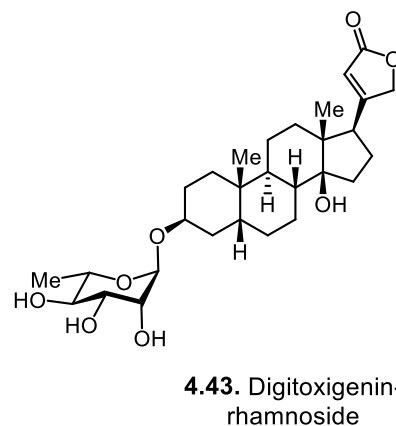
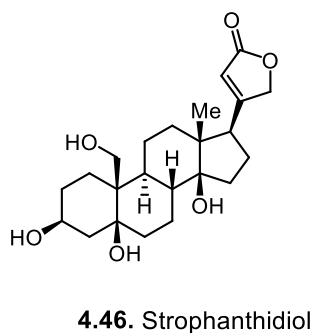
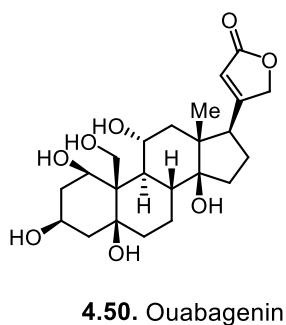
steroids are generally included in several Na^+/K^+ -ATPase studies. But more recently, they have been included to anticancer studies as well and some of them have shown promising inhibitory activities in these studies.

Figure 4.7. Commercially Available Cardiotoxic Steroids Used During this Study



The following steroids **4.43**, **4.45-4.48**, and **4.50** (**Figure 4.8**) were synthesized by semi-synthetic methods by my co-workers Jia-Hui Tay and Dr. Hem Raj Khatri and the respective publication of detailed synthetic methods is underway. Ouabagenin was synthesized by deglycosylation of ouabain by stirring in conc. HCl for 2 days. Strophanthidiol was obtained by NaBH_4 reduction of aldehyde of strophanthidin and the corresponding glycosides were obtained by TfOH-promoted glycosylation as discussed in **Scheme 4.4** and chapter 3. Digitoxigenin-rhamnoside and bufalin-rhamnoside were obtained from Digitoxigenin and bufalin respectively by the glycosylation method described in **Scheme 4.4** as well.

Figure 4.8. Cardiotoxic Steroids Synthesized by Semi-Synthesis from Commercial Steroids



4.8. Structure Activity Relationships (SARs) Results from Figure 4.3.

There has been clear literature reports indicating a correlation of the heterocycle and the sugar with the biological activities of these molecules including their anticancer activities.⁶² This study is no exception. From our collaboration with the Aye's Research Group, the SAR we obtained by comparing the anticancer activities of the above listed molecules show a clear correlation of both the heterocycle and the sugar moiety confirming previously reported results on other cardiotonic steroids like strophanthidin, digitoxigenin, and bufalin.^{50,62} Substantial amount of SAR information were gathered on the importance of heterocycle, different sugar, C19, C11, C5, C1 oxidations, stereochemistry at C17 and C5, among others.

4.8.1. Importance of heterocycle and orientation at C17

From the initial assay against HEK and Hela cell lines, cannogenol was found to have nanomolar half maximal inhibitory concentration (EC_{50}) that encouraged us to further explore this

area. The lactone moiety is known to interact with the active site through electrostatic interaction with the two binding points (one to the carbonyl oxygen by hydrogen bond and second at the electron deficient C20 by electrostatic interaction).⁶³ Hence, it led us to believe that changes in this part of the molecule would likely change the potency of the steroids. By comparing cytotoxic activities of the analogues from the same set of assays, the importance of heterocycle was elucidated (μM activity of **4.32-4.35** vs nm EC₅₀ of cannogenol **4.36**). Steroids **4.32-4.35** shows no activity in inhibition of cancer cell growth. The lactone ring plays an important role in the cytotoxicity of these steroids and hence the lack of stereocenter at C17 in compound **4.37** or reduced lactone ring in compound **4.38** shuts off the activity of these molecules (compare **4.37**, **4.38** and **4.36**). From the initial NIH CANVAS screening, trewianogenin **4.55** was found to have 15-25 mM toxicity against COV-362 (human ovarian epithelial carcinoma), A2780cis (human ovarian carcinoma), KB-3-1 (oral cancer), and PANC-1 (human pancreatic cancer cells) but the C17 epimer of trewianogenin **4.56** showed no activity against human cancer cell at all.

4.8.2. Importance of rhamnose and effect of changing sugar

To our excitement, synthetic cannogenol-3-O- α -L-rhamnoside **4.40** performed the best out of our set of compounds, with EC₅₀ of 13 nM, which is consistent with reports on the increased activity because of the sugar moiety.^{50,62} The difference in activity can be visualized by comparing cannogenol **4.36** and cannogenol-3-O- α -L-rhamnoside **4.40** (600 nM vs 13 nM). The same trend was observed when comparing ouabagenin **4.50** to ouabain **4.49** (670 nM vs 68 nM), strophanthidol **4.46** to strophanthidol rhamnoside **4.47** (<1 μM vs 18 nM) and, digitoxigenin **4.44** to digitoxigenin-rhamnoside **4.43** (239 nM vs 23 nM). Rhamnoside was found to be crucial towards the biological activity of cardiotonic steroids and it has been uncovered that the activity can be tuned by changing the sugar fragment.⁵⁰ Consistent with this result, introduction of glucose

instead of rhamnose in the cannogenol slightly decreased the EC₅₀ value against HEK 293T although it remained highly potent (48 nM of **4.41** and 13 nM of **4.40**). Similar trend was observed with strophanthidol as an aglycone. Glucose significantly reduced the activity (105 nM of **4.48** vs 18 nM of **4.47**). This is also consistent with isolation and anticancer study by Shi et al. where they reported significant reduction of the activity of aglycone and impressive anticancer activity of the rhamnose glycoside against several cancer cell lines.⁵⁸

4.8.3. Role of C19 functionality

Protection of primary alcohol with esters significantly diminished the potency of cannogenol (μ M of **4.39** and nm of **4.36**). Hence, the free alcohol is an important functionality in dictating the biological activity for C19 hydroxy-cardiotonic steroids. Although both **4.37**, and **4.45** are active against the cancer cell lines, having hydroxy group at C19 slightly enhances the potency (compare **4.43**, **4.45**, and **4.40**). Having an aldehyde at C19 as in strophanthidin **4.52** significantly diminishes the inhibition in comparison to strophanthidol.

4.8.4. Other SAR

The comparison of activity of six-membered lactone in bufalin rhamnoside **4.45** to five membered lactone in digitoxigenin-rhamnoside **4.43** showed that both steroids were potent anticancer agents with steroid containing five-membered lactone having slightly better activity. The C11 oxygenated natural products 19-hydroxysermentogenin showed no activity in preliminary assay performed by NIH CANVAS in comparison to cannogenol, which proved to be much better in inhibiting growth of cancer cells. Commercial steroids like digitoxin **4.53** and digoxin **4.51** with trisaccharide sugar, digitoxose showed promising activity as well.

4.8.5. Other Studies

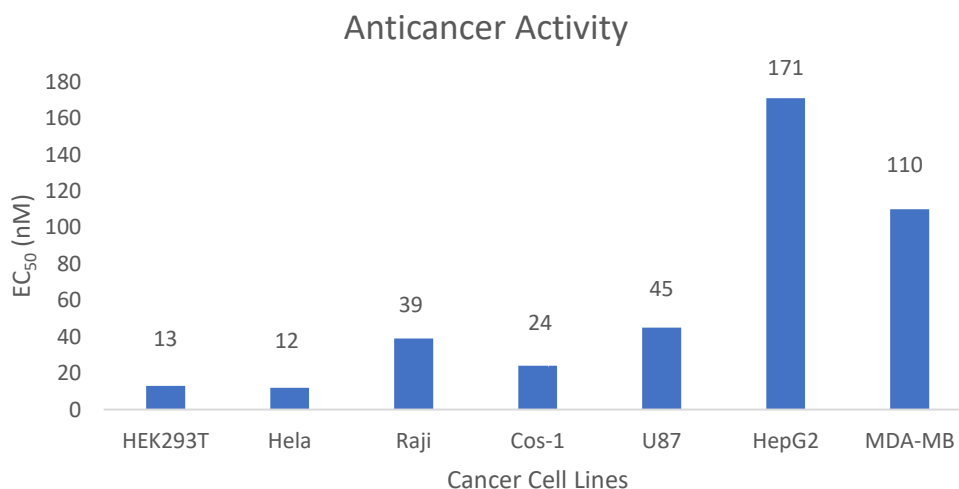
In order to identify the binding target of these class of steroids, a steroid with sufficient potency needed to be synthesized. Therefore, molecule **4.42**, cannogenol-3-O- α -L-rhamnoside with an alkyne incorporated in the molecule, was constructed. To our excitement, compound **4.42** had EC₅₀ of 294 nm and study on target identification is currently underway.

4.9. Cannogenol-3-O- α -L-rhamnoside as Anticancer Agent

4.9.1. Potency of Cannogenol-3-O- α -L-rhamnoside Against Variety of Transformed Cell Lines

Cannogenol-3-O- α -L-rhamnoside, after being proven superior out of all the compound tested in our series, was assayed against a variety of cancer cell lines. It showed remarkable inhibition (**Figure 4.9**) against transformed human embryonic kidney cells (HEK293T), blood cancer cell line (Raji), monkey kidney cell line transformed with simian virus (Cos-1), glioblastoma line (U87), and liver tumor line (HepG2). It also showed promising potency against triple-negative breast cancer cell line (MDA-MB), which is an aggressive form of breast cancer with limited treatment options.⁶⁴

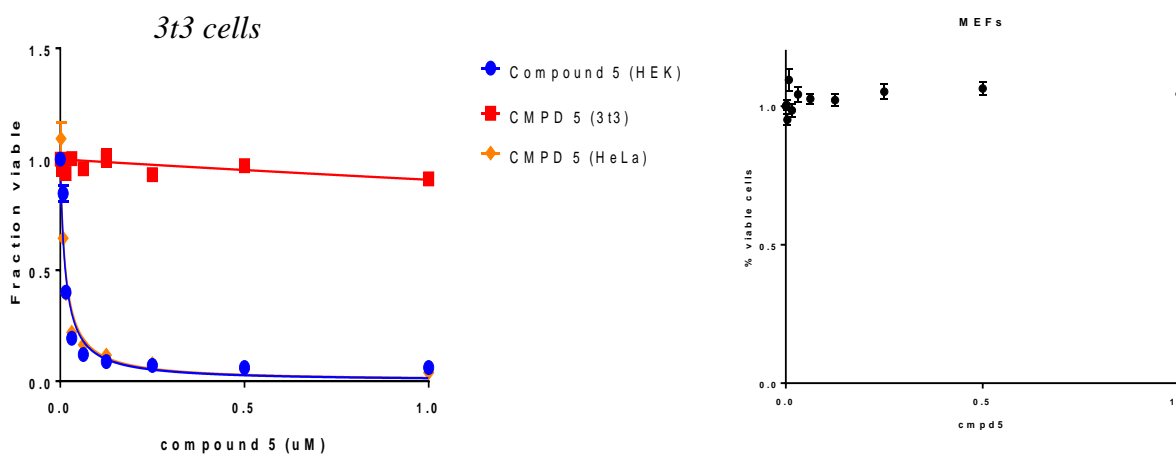
Figure 4.9. Cannogenol-3-O- α -L-rhamnoside as Anticancer Agent



4.9.2. Selectivity Studies Against Normal 3t3 and MEF Cell Lines

Cardiotonic steroids in our study showed promising inhibition against transformed cell lines. However, it is important for them to be selective to these transformed cell lines as well as show no cytotoxicity towards normal cell lines. To our delight, cannogenol-3-O- α -L-rhamnoside was very selective to the transformed cell lines and not too toxic to normal mouse fibroblast (3t3) and mouse embryonic fibroblast (MEF) at up to 1 μ M concentration (**Figure 4.10**).

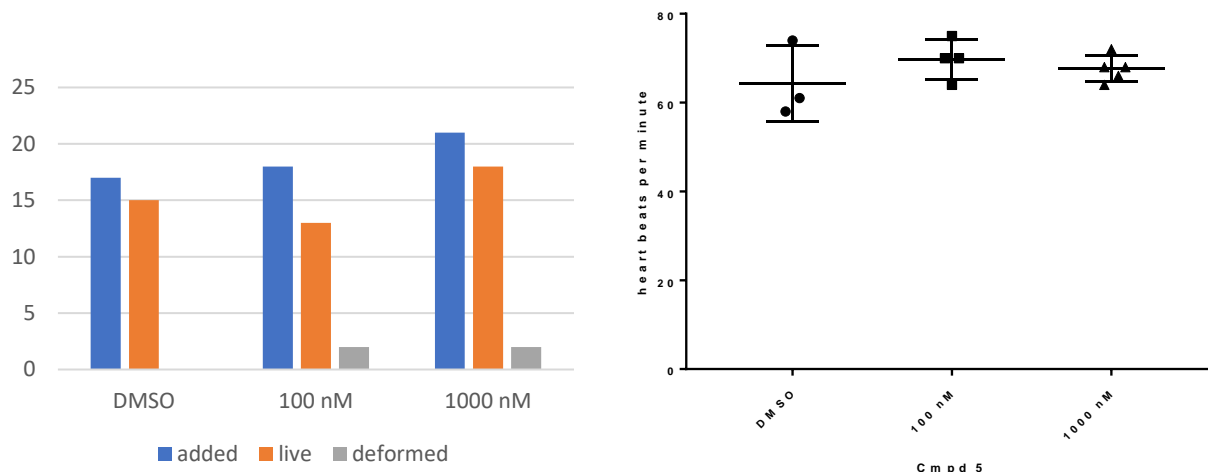
Figure 4.10. Selectivity Studies Against Normal 3t3 and MEF Cell Lines



4.9.3. Selectivity Study Against Zebra Fish Embryo

Furthermore, to confirm the selectivity, zebra fish embryos were exposed to cannogenol-3-O- α -L-rhamnoside for two days and the teratogenicity was studied. **4.40** was well tolerated at up to 1000 nM. Out of twenty-one fish embryos used at 1000 nM of cannogenol, only two were deformed after two days (**Figure 4.11a**). The study also showed that cannogenol-3-O- α -L-rhamnoside did not affect the heart beating in two-day old fish (**Figure 4.11b**).

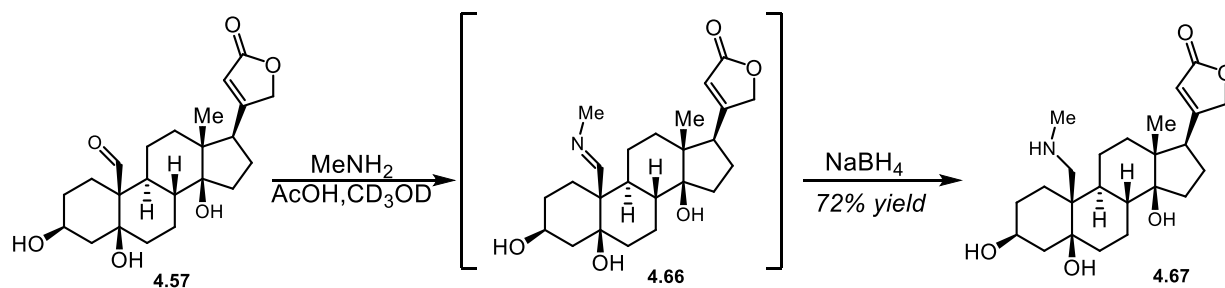
Figure 4.11. Teratogenicity Studies of **4.40** Towards Zebra Fish Embryo



4.10. 2nd Generation Analogs

Amines are known to increase the solubility of organic compounds when compared to alcohol, while still maintaining the H-bonding ability, and in some instances increasing the potency of cardiotoxic steroids.⁵¹ Hence, we desired to introduce the variant of cannogenol to incorporate amines into the molecule. Strophanthidin is commercially available and its C3 rhamnoside was found to be equally potent to cannogenol-3-O- α -L-rhamnoside, against Hela cells, hence initial optimization to convert the C19 alcohol to amines was performed in strophanthidin. The interconversion of C19 aldehyde to amine was performed by reductive amination of the aldehyde using methyl amine and NaBH₄. Strophanthidin **4.52** formed an intermediate imine **4.66**, the reduction of which produced amine analog **4.67**. The formation of imine wasn't observed when t-butyl carbamate was used in an attempt to synthesize primary amine. The reduction of intermediate **4.66** wasn't observed when sodium triacetoxyborohydride (STAB) was used and sodium cyanoborohydride led to a slower reaction.

Scheme 4.5. Synthesis of Amine-incorporated Analog **4.67**

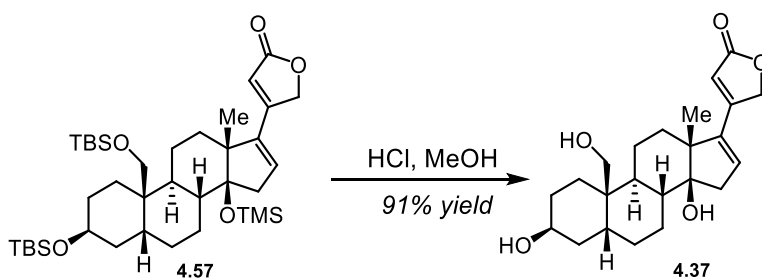


4.11. Current and Future Directions:

The current work is focused on designing more analogs of cannogenol-3-O- α -L-rhamnoside, and the analogs of strophanthidin, the molecules that have proven themselves in our medicinal chemistry studies. With the studies described above, we have been able to identify functionalities that can change the potency of these molecules through the SAR studies and in future, we will attempt to generate relevant analogs to tune the bioactivity. Amines are known to increase the bioactivity of these compounds towards anticancer as well as are better Na^+/K^+ -ATPase inhibitor. To this direction, current work is based on replacing the C19 alcohol with amine to increase the solubility of strophanthidin based steroids and hence, potentially increase their bioactivity.

4.12. Experimental

4-((3S,5R,8R,9S,10R,13R,14S)-3,14-dihydroxy-10-(hydroxymethyl)-13-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



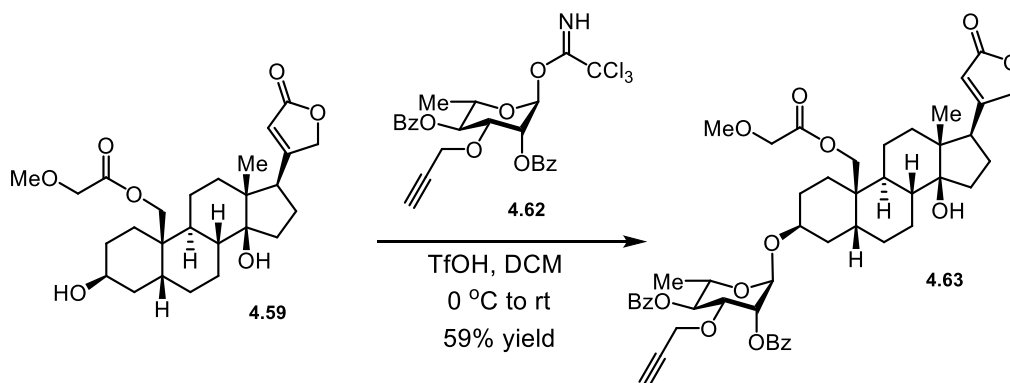
To a solution of **4.57** (0.0090 mmol, 6.0 mg, 1 equiv.) in MeOH (1 mL) was added 3M HCl in MeOH (300 μ L) and stirred at rt for 1 h. The resulting colorless solution was quenched by addition of saturated aqueous NaHCO₃ solution dropwise and the aqueous phase was extracted with 2:1 chloroform: ethanol (3 \times 5mL). The combined organic layers were dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (9:1 DCM: MeOH) to give **4.37** (3 mg, 91%) as a white solid.

¹H NMR (700 MHz, Methanol-*d*₄) δ 6.25 (s, 1H), 5.99 (s, 1H), 5.14 (d, $J = 16.7$ Hz, 1H), 5.01 (d, $J = 16.7$ Hz, 1H), 4.04 (s, 1H), 3.86 (d, $J = 11.2$ Hz, 1H), 3.44 (d, $J = 11.2$ Hz, 1H), 2.77 (d, $J = 18.2$ Hz, 1H), 2.32 (dd, $J = 18.4, 3.1$ Hz, 1H), 2.23 (d, $J = 13.6$ Hz, 1H), 2.09 – 2.05 (m, 1H), 1.99 (t, $J = 13.0$ Hz, 1H), 1.90 (td, $J = 13.9, 4.0$ Hz, 2H), 1.86 – 1.78 (m, 2H), 1.72 (t, $J = 10.0$ Hz, 1H), 1.66 – 1.54 (m, 2H), 1.49 (s, 1H), 1.40 (d, $J = 14.4$ Hz, 2H), 1.36 – 1.30 (m, 1H), 1.27 (s, 3H), 1.24 (s, 1H), 1.15 (t, $J = 8.6$ Hz, 2H).

¹³C NMR (700 MHz, Methanol-*d*₄) δ 177.28, 161.95, 145.02, 134.72, 112.00, 86.82, 73.53, 67.47, 66.13, 53.44, 42.02, 40.92, 40.51, 40.00, 37.09, 34.13, 29.75, 28.28, 27.26, 24.23, 21.83, 21.02, 16.97.

HRMS (ESI-MS) calculated for $C_{23}H_{32}O_5$ $[M+H]^+$: m/z 389.2328, found: 389.2321

(2R,3R,4R,5S,6S)-2-(((3S,5R,8R,9S,10R,13R,14S,17R)-14-hydroxy-10-((2-methoxyacetoxy)methyl)-13-methyl-17-(5-oxo-2,5-dihydrofuran-3-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)-6-methyl-4-(prop-2-yn-1-yloxy)tetrahydro-2H-pyran-3,5-diyl dibenzoate



To a solution of compound **4.59** (5.0 mg, 0.011 mmol) in dry CH_2Cl_2 (800 μ L) was added 4Å MS and cooled to 0 °C. The solution of donor **4.62** (3.0 mg, 0.0055 mmol) in CH_2Cl_2 (100 μ L) was added to the flask followed by dropwise addition of the solution of TfOH (10 μ L, 0.0033 mmol taken from the stock solution prepared by dissolving 3 μ L TfOH in 100 μ L CH_2Cl_2) and the resulting mixture was stirred at 0 °C for 1 h. Additional 0.5 equiv. of donor was added portion wise every hour for the next three hours. After the addition of 2 equiv. of total donor, the reaction mixture was warmed to room temperature and stirred for 1 additional hour. The resulting colorless solution was quenched by addition of excess triethylamine dropwise and filtered through celite. This filtrate was concentrated in *vacuo* and the residue was purified by flash chromatography (6:4 Hexanes: Ethyl acetate) to give 59% (5.5 mg, 0.0064 mmol) of compound **4.63** as a colorless solid, R_f = 0.8 (10% MeOH in CH_2Cl_2).

1H NMR (700 MHz, $CDCl_3$): δ 8.10 (d, J = 7.8 Hz, 4H), 7.59 (td, J = 7.2, 4.1 Hz, 2H), 7.47 (dt, J = 11.5, 7.7 Hz, 4H), 5.89 (d, J = 1.8 Hz, 1H), 5.52 (dd, J = 3.3, 1.8 Hz, 1H), 5.40 (t, J = 9.8 Hz,

1H), 5.03 (d, $J = 1.8$ Hz, 1H), 4.98 (dd, $J = 18.0, 1.7$ Hz, 1H), 4.81 (dd, $J = 17.9, 1.7$ Hz, 1H), 4.48 (d, $J = 11.2$ Hz, 1H), 4.34 (dd, $J = 9.7, 3.3$ Hz, 1H), 4.26 – 4.16 (m, 2H), 4.15 – 4.06 (m, 4H), 4.03 (t, $J = 2.9$ Hz, 1H), 3.45 (s, 3H), 2.80 (dd, $J = 9.3, 5.5$ Hz, 1H), 2.38 (t, $J = 2.4$ Hz, 1H), 2.24 – 2.06 (m, 3H), 1.89 (dtd, $J = 17.5, 8.7, 8.0, 5.2$ Hz, 1H), 1.85 – 1.69 (m, 7H), 1.69 – 1.50 (m, 6H), 1.47 – 1.36 (m, 2H), 1.35 – 1.28 (m, 5H), 0.90 (s, 3H);

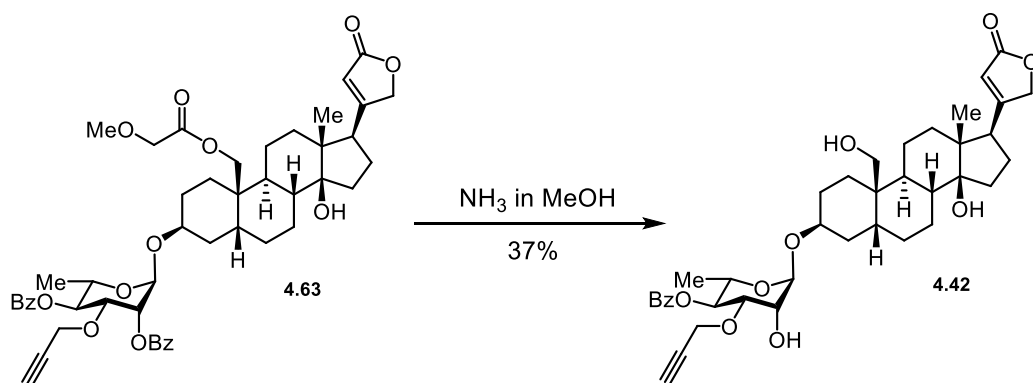
^{13}C NMR (176 MHz, CDCl_3): δ 174.5, 174.2, 170.7, 166.2, 166.0, 133.5, 133.4, 130.1, 130.0, 130.0, 129.7, 128.7, 128.5, 118.1, 96.2, 85.6, 79.6, 75.2, 74.4, 73.5, 73.1, 72.6, 70.2, 69.9, 67.8, 67.3, 59.6, 57.0, 51.0, 49.6, 42.0, 40.3, 38.6, 35.5, 33.2, 30.0, 29.9, 27.0, 26.3, 26.2, 24.4, 21.6, 21.0, 17.9, 16.0;

IR (thin film, cm^{-1}): 711, 913, 1027, 1069, 1110, 1266, 1317, 1451, 1621, 1726, 2935, 3514 (br.);

HRMS (ESI-MS) calculated for $\text{C}_{49}\text{H}_{58}\text{O}_{13}$ $[\text{M}+\text{H}]^+$: 855.3956, found 855.3940;

$[\alpha]_{\text{D}}^{25} = +49.7$ ($c = 0.1$, CHCl_3).

(2S,3S,4S,5R,6R)-5-hydroxy-6-(((3S,5R,8R,9S,10R,13R,14S,17R)-14-hydroxy-10-(hydroxymethyl)-13-methyl-17-(5-oxo-2,5-dihydrofuran-3-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)-2-methyl-4-(prop-2-yn-1-yloxy)tetrahydro-2H-pyran-3-yl benzoate



Compound **4.63** (5.5 mg, 0.0064 mmol) was dissolved in half saturated MeOH (1 mL) and stirred at room temperature for 24 hours. The reaction mixture was then concentrated in *vacuo* and

purified by flash column chromatography (5% to 10% MeOH in CH₂Cl₂) to afford 37% (1.6 mg, 0.0024 mmol) compound **4.42** as a white film, R_f = 0.5 (10% MeOH in CH₂Cl₂).

¹H NMR (700 MHz, CD₃OD): δ 8.05 (d, *J* = 7.7 Hz, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.49 (t, *J* = 7.7 Hz, 2H), 5.90 (d, *J* = 2.0 Hz, 1H), 5.27 (t, *J* = 9.7 Hz, 1H), 5.04 (d, *J* = 17.6 Hz, 1H), 4.94-4.89 (m, 2H), 4.25 – 4.18 (m, 2H), 4.14 (dd, *J* = 9.7, 3.1 Hz, 1H), 4.11 (t, *J* = 2.5 Hz, 1H), 4.08 – 4.02 (m, 1H), 3.99 (t, *J* = 3.0 Hz, 1H), 3.88 (d, *J* = 11.2 Hz, 1H), 3.41 (d, *J* = 11.2 Hz, 1H), 2.84 (t, *J* = 7.2 Hz, 1H), 2.26 – 2.15 (m, 3H), 2.03 (m, 1H), 1.96 (td, *J* = 13.8, 4.5 Hz, 1H), 1.90-1.81 (m, 4H), 1.79 – 1.64 (m, 4H), 1.64 – 1.41 (m, 5H), 1.37 – 1.25 (m, 3H), 1.17 (d, *J* = 6.3 Hz, 3H), 0.89 (s, 3H);

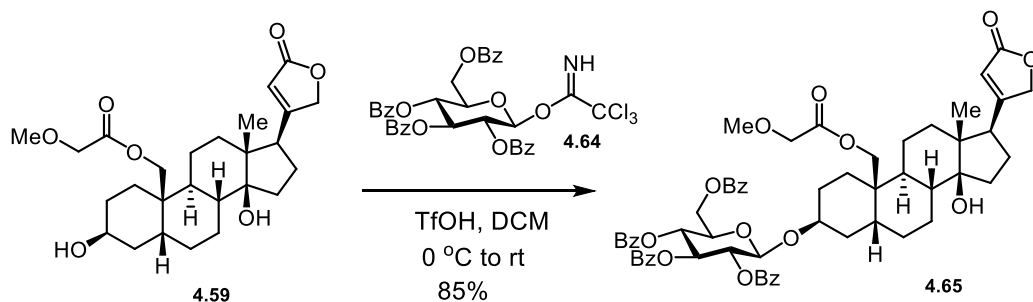
¹³C NMR (176 MHz, CD₃OD): δ 178.4, 177.3, 167.6, 134.4, 131.4, 130.7, 129.6, 117.8, 99.7, 86.6, 80.6, 77.6, 76.2, 75.4, 74.6, 73.8, 69.8, 68.2, 65.6, 57.5, 52.1, 51.1, 42.6, 41.3, 40.5, 36.5, 33.2, 30.7, 30.1, 28.0, 27.5, 27.2, 24.9, 22.4, 22.2, 17.9, 16.4;

IR(thin film, cm⁻¹): 637, 713, 1027, 1070, 1271, 1451, 1621, 1728, 2115, 2937, 3436(br.);

HRMS (ESI-MS) calculated for C₃₉H₅₀O₁₀ [M+H]⁺: 679.3482, found 679.3491;

[α]_D²⁵ = +1.7 (c = 0.08, CH₃OH).

(2R,3R,4S,5R,6R)-2-((benzyloxy)methyl)-6-(((3S,5R,8R,9S,10R,13R,14S,17R)-14-hydroxy-10-((2-methoxyacetoxy)methyl)-13-methyl-17-(5-oxo-2,5-dihydrofuran-3-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl tribenzoate



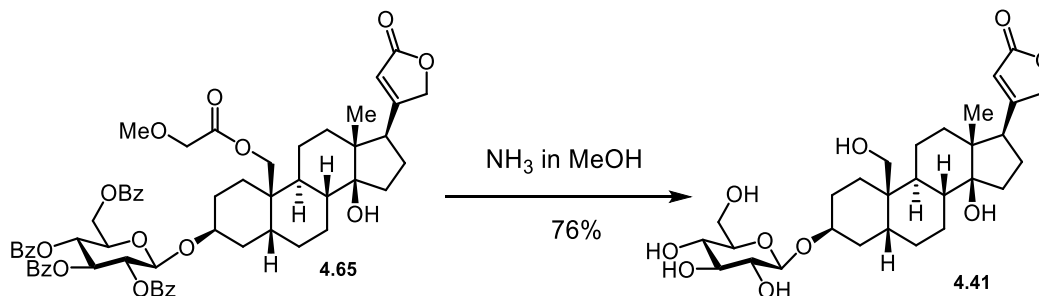
To a solution of compound **4.59** (10.0 mg, 0.021 mmol) in dry CH₂Cl₂ (1.2 mL) was added 4Å MS and cooled to 0 °C. The solution of donor **4.64** (8.0 mg, 0.011 mmol) in CH₂Cl₂ (100 µL) was added to the flask followed by dropwise addition of the solution of TfOH (10 µL, 0.00648 mmol taken from the stock solution prepared by dissolving 5.7 µL TfOH in 100 µL CH₂Cl₂) and the resulting mixture was stirred at 0 °C for 1 h. Additional 0.5 equiv. of donor was added portion wise every hour for the next three hours. After the addition of 2 equiv. of total donor, the reaction mixture was warmed to room temperature and stirred for 1 additional hour. The resulting colorless solution was quenched by addition of excess triethylamine dropwise and filtered through celite. This filtrate was concentrated in *vacuo* and the residue was purified by flash chromatography (6:4 Hexanes: Ethyl acetate) to give 85% (19.0 mg, 0.0183 mmol) of compound **4.65** as an off-white solid, R_f = 0.4 (60% EtOAc in hexanes).

¹H NMR (700 MHz, CDCl₃) δ 7.99 (d, *J* = 7.8 Hz, 2H), 7.90 (dd, *J* = 14.4, 7.9 Hz, 4H), 7.84 (d, *J* = 7.8 Hz, 2H), 7.54 (t, *J* = 7.6 Hz, 2H), 7.50 (dt, *J* = 14.9, 7.6 Hz, 1H), 7.44 (t, *J* = 7.3 Hz, 1H), 7.39 (q, *J* = 7.2 Hz, 4H), 7.34 (t, *J* = 7.7 Hz, 2H), 7.30 (t, *J* = 7.6 Hz, 2H), 5.90 (t, *J* = 9.7 Hz, 1H), 5.85 (s, 1H), 5.67 (t, *J* = 9.7 Hz, 1H), 5.55 – 5.51 (m, 1H), 4.94 (d, *J* = 18.1 Hz, 1H), 4.85 (d, *J* = 7.9 Hz, 1H), 4.77 (d, *J* = 18.1 Hz, 1H), 4.62 (dd, *J* = 12.0, 3.3 Hz, 1H), 4.51 (dd, *J* = 11.9, 5.2 Hz, 1H), 4.04 – 3.92 (m, 5H), 3.46 (s, 3H), 2.74 (dd, *J* = 9.3, 5.6 Hz, 1H), 2.18 – 2.11 (m, 1H), 2.03 (d, *J* = 3.7 Hz, 1H), 1.89 – 1.81 (m, 1H), 1.78 (d, *J* = 13.2 Hz, 1H), 1.65 (dd, *J* = 25.0, 12.4 Hz, 5H), 1.56 (d, *J* = 10.6 Hz, 1H), 1.51 – 1.42 (m, 3H), 1.37 (d, *J* = 14.0 Hz, 1H), 1.33 – 1.28 (m, 4H), 1.11 (q, *J* = 13.1 Hz, 2H), 0.81 (s, 3H).

¹³CNMR (176 MHz, CDCl₃) δ 174.45, 174.22, 170.79, 166.25, 165.98, 165.37, 164.98, 133.59, 133.45, 133.41, 133.24, 129.95, 129.91, 129.86, 129.77, 129.72, 129.47, 128.97, 128.94, 128.62, 128.56, 128.48, 128.46, 117.97, 100.57, 85.59, 75.34, 73.51, 73.06, 72.26, 72.04, 70.14, 69.65,

67.40, 63.48, 60.54, 59.50, 50.89, 49.61, 41.82, 40.23, 38.26, 35.28, 34.28, 32.97, 30.51, 29.85, 29.79, 26.96, 26.24, 25.63, 23.94, 21.49, 20.88, 15.87, 14.35.

4-((3S,5R,8R,9S,10R,13R,14S,17R)-14-hydroxy-10-(hydroxymethyl)-13-methyl-3-(((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)furan-2(5H)-one



Compound **4.65** (9.0 mg, 0.0086 mmol) was dissolved in half saturated MeOH (1 mL) and stirred at room temperature for 24 h. The reaction mixture was then concentrated in *vacuo* and purified by flash column chromatography (5% to 15% MeOH in CH_2Cl_2) to afford 76% (3.6 mg, 0.0065 mmol) compound **4.41** as a white film, $R_f = 0.15$ (10% MeOH in CH_2Cl_2).

^1H NMR (700 MHz, Pyridine-*d*₅) δ 6.14 (s, 1H), 5.37 (s, 1H), 5.33 (d, $J = 18.1$ Hz, 1H), 5.07 (d, $J = 18.1$, 1H), 4.60 (d, $J = 10.6$ Hz, 1H), 4.45 (s, 1H), 4.41 (dd, $J = 11.7, 5.4$ Hz, 1H), 4.33 – 4.19 (m, 1H), 4.11 (d, $J = 10.9$ Hz, 1H), 4.06 (t, $J = 8.1$ Hz, 1H), 4.02 – 3.98 (m, 1H), 3.79 (d, $J = 10.7$ Hz, 1H), 3.62 (s, 1H), 2.89 – 2.74 (m, 2H), 2.59 – 2.50 (m, 1H), 2.22 – 2.08 (m, 5H), 2.07 – 1.95 (m, 2H), 1.95 – 1.83 (m, 4H), 1.80 – 1.73 (m, 1H), 1.67 (d, $J = 14.0$ Hz, 1H), 1.55 – 1.43 (m, 4H), 1.41 – 1.36 (m, 1H), 1.28 (d, $J = 13.4$ Hz, 1H), 1.03 (s, 3H).

^{13}C NMR (176 MHz, pyridine) δ 176.45, 174.93, 117.98, 103.67, 85.28, 79.15, 78.86, 75.63, 74.74, 74.11, 72.23, 65.98, 63.34, 51.88, 50.57, 42.27, 40.76, 40.19, 36.24, 33.33, 30.95, 30.36, 27.69, 27.50, 27.42, 25.17, 22.26, 22.12, 16.67.

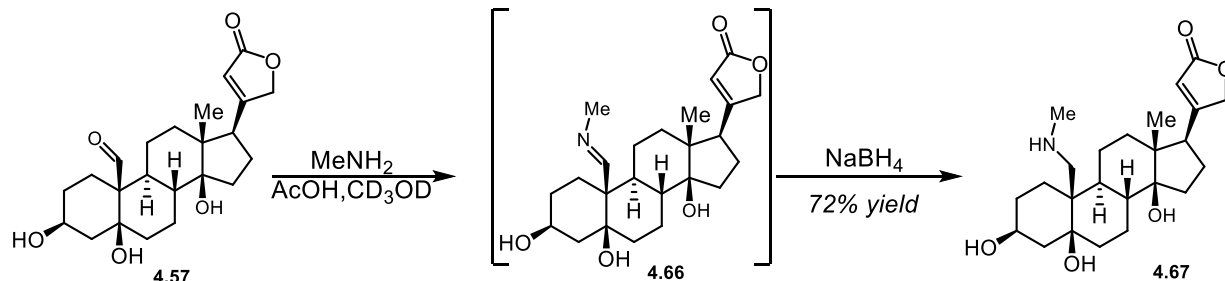
IR (thin film, cm^{-1}): 626, 1024, 1076, 1352, 1381, 1591, 1737, 2927, 3390 (br.);

HRMS (ESI-MS) calculated for C₂₉H₄₄O₁₀ [M+H]⁺: 575.2832, found 575.2826

[α]_D²⁵ = +1.3 (c = 0.2, CH₃OH).

4-((3S,5S,8R,9S,10R,13R,14S,17R)-3,5,14-trihydroxy-13-methyl-10-

**((methylamino)methyl)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)furan-2(5H)-
one**⁶⁵



To a stirred solution of strophanthidin **4.57** (14 mg, 0.034 mmol, 1 equiv.) in CD₃OD (0.5 mL) in an NMR tube at 0 °C, was added AcOH (2 μ L, 2 equiv.). MeNH₂ in EtOH (30%) (18 μ M, 0.136 equiv.) was added to the reaction mixture slowly and stirred for 2 h at that temperature. The proton NMR showed complete conversion of aldehyde into imine by a characteristic peak at ~8.2 ppm. NaBH₄ (4 mg, 3 equiv.) was added to the reaction mixture, and slowly warmed. Let it stir for 2.5 h at room temperature. The crude NMR suggested the consumption of imine. The reaction was then quenched with aqueous saturated NaHCO₃, and the aqueous layer was extracted with EtOAc (5 mL \times 3). The collective organic layer was dried with Na₂SO₄ and concentrated in vacuo followed by purification by flash column chromatography (5-15% MeOH in CH₂Cl₂) to afford (10 mg, 72%) of compound **4.67**.

¹H NMR (700 MHz, CD₃OD) δ 5.91(s, 1H), 5.03 (d, 1H), 4.92(d,1H), 4.11 (s, 1H), 3.85 (d, 1H), 2.91 (d, 1H), 2.85 (dd, 1H), 2.56 (s, 3H), 2.19 (m, 4H), 1.98 (m, 1H), 1.88 (m, 2H), 1.75 (m, 4H), 1.51 (m, 7H), 1.28 (m, 2H), 0.89 (s, 3H)

¹³C NMR (176 MHz, CD₃OD) δ 178.09, 177.16, 118.00, 117.95, 86.09, 78.36, 75.30, 68.44, 54.14, 51.83, 43.06, 41.07, 40.60, 39.79, 37.81, 36.81, 35.74, 33.21, 28.09, 27.80, 25.01, 22.21, 20.57, 16.17

HRMS(ESI): [M+H]⁺ calculated for C₂₄H₃₇N₅O₅ 420.2750, found 420.2760

[α]_D²⁵ = +23.5 (c = 0.4, CH₃OH).

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