2010

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SYNTHESIS OF MALE SEA LAMPREY PHEROMONES
VIA ALLOMERIZATIONS

presented by

SOONG-HYUN KIM

has been accepted towards fulfillment
of the requirements for the

Ph. D. degree in Chemistry

Robert E Maleczka Jr.
Major Professor's Signature

July 2, 2010
Date

SYNTHESIS OF MALE SEA LAMPREY PHEROMONES VIA ALLOMERIZATIONS

By

Soong-Hyun Kim

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Chemistry

2010

Professor Robert E. Maleczka Jr.

ABSTRACT

SYNTHESIS OF MALE SEA LAMPREY PHEROMONES
VIA ALLOMERIZATIONS

By

Soong-Hyun Kim

3-Keto petromyzonol sulfate (3kPZS) and 3-keto allocholic acid (3kACA) are potent components of the sex pheromone cocktail emitted by male sea lamprey, *petromyzon marinus*. Such pheromones have been successfully tested as population control agents against lamprey residing in the Great Lakes. Owing to these results, a straightforward and practical synthetic route to 3kPZS and 3kACA is needed. For the synthesis of 3kPZS and 3kACA, cholic acid was selected as the common starting material, because cholic acid has the carbon skeleton and functionality suitable for 3kPZS and 3kACA. Allomerization to convert the stereochemistry from 5β (A/B *cis*) to 5α (A/B *trans*) was the crucial step in this synthesis. Several allomerizations were conducted, including a single step allomerization with Raney Ni and a variety of stepwise allomerizations. Crucial intermediate was successfully derived from a single step allomerization with Raney Ni or stepwise allomerization from modified Birch reduction protocol. A plausible synthetic route to male sea lamprey pheromones, 3kPZS and 3kACA was established from the crucial intermediate.

To Jungyoon and Taeho Kim

ACKNOWLEDGMENTS

Wrapping up the very important moment in my career, I really appreciate my life here in East Lansing. I finally achieved and closed another chapter of my life as a doctoral researcher. I need to acknowledge many people for my successful finale.

First of all, I would like to thank Professor Robert E. Maleczka, Jr. for being my advisor. Without his guidance, I could not have made it. I have learned a lot of things, attitude as a scientist, development of critical insight about science, way to make excellent chemistry class, way to make an approach to solve out scientific problems, and especially presentation skills to finish and display my work to other people.

I would also like to thank Professor William Wulff, James Jackson, and Aaron Odom for being my committee members and helping me finish within time through all those rush. Helpful discussion and guidance to be a real chemist gave me such an incredible experience for my achievement.

I wish to thank my colleagues from the Maleczka group for their friendship and help. Spending their countless time and discussion on my research/literature/practice talk made my presentation perfect.

All this achievement would not have been made without support and encouragement of my family. I would like to thank my family members in Korea for giving me a life, teaching me way to be good person, and guiding me to make a reasonable thinking. Lastly, I wish to thank my wife and my son for giving me another life as husband and father. Without Jungyoon's belief in me and her endless love, I could not have made it. I really appreciate it and I always love you, Jungyoon and Taeho.

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CHAPTER 1. INTRODUCTION

1.1. The Sea Lamprey in the Great Lakes

Sea lamprey are among the most primitive vertebrates alive today.[1] They natively inhabit the Atlantic coast of North America and Europe, but can live in both fresh and salt water.[2] The scientific name for sea lamprey is *petromyzon marinus* and it is in the family *petromyzontidae*, the lamprey family.[3] Its genus *petromyzon* is made up of the Latin word '*petra*' meaning 'stone' and the Greek word '*myzon*' meaning 'to suckle'.[3] The "stone sucker" is also their common name, which may have arisen from the habit of males during spawning, when they create a depression in the river bed by wriggling and removing stones with their mouth.[4] Alternatively, sea lamprey are often called cyclostome, which means "round mouth," which refers to the fact that the lamprey have a jawless and round-sucker type mouth.[4]

Figure 1. Sea lamprey, *Petromyzon Marinus*[5]

Sea lamprey resemble eel, which can cause confusion during identification. Unlike eel, however, sea lamprey have a dorsal fin with a notch, and no pectoral or pelvic fins, as depicted in Figure 1.[5] Lamprey have a cartilaginous skeleton, which is another

characteristic of primitive species.[3] Lamprey have gill openings consisting of 7 small lateral clefts on the sides of the head and a single nostril on the upper surface of the head.[4] Lamprey commonly exhibit black or brown mottling on their backs, a metallic violet color on the sides and a whitish gray on the belly.[1] Average lamprey size is 12-20 inches in length and with a weight of 8-13 ounces.[1] A distinguishing characteristic is its inferior, sucker type mouth filled with sharp teeth in concentric circles that surround a file-like tongue (Figure 2).[4] With this unique anatomy, sea lamprey are natively equipped to attack and parasitize other fish. A lamprey uses its mouth to attach itself to a fish, cut through the scales and skin, and feed on the host's body fluids, often scarring or killing its prey.[3] An anticoagulant in the lamprey's saliva keeps the wound open for hours or even weeks so that the lamprey can continue to feed for prolonged periods.[6] Though the less than 1 pound itself, the lamprey is so destructive that it can potentially kill six out of seven fish that it attacks, destroying as much as 40 pounds of fish or more over its lifetime.[7]

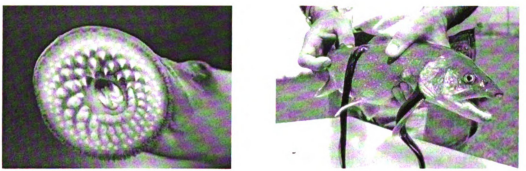


Figure 2. Sea lamprey jawless mouth[8] and sea lampreys feeding on trout[9]

As mentioned above, the native range of sea lamprey in the North American

continent is the east coastal area in the Atlantic Ocean.[2] However, lamprey have expanded their range to include the Great Lakes, arriving through manmade locks and shipping canals (Figure 3).[10] The Welland Canal, which joins Lake Ontario to Lake Erie bypassing Niagara Falls, was in place for nearly nine decades before sea lamprey invaded Lake Erie.[10] Upgrades to the Welland Canal in 1919 appear to have provided an improved avenue for sea lamprey to invade Lake Erie as they were found just two years after improvements were made to the canal.[10] Once lamprey were first discovered in Lake Erie in 1921, it took just 25 years for them to spread to the remaining Great Lakes: Lake Huron in 1932, Lake Michigan in 1936, and Lake Superior in 1946.[10]

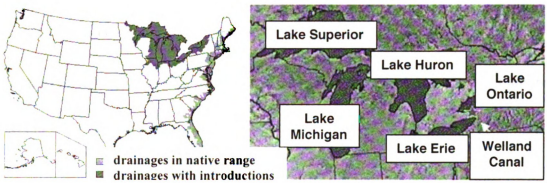


Figure 3. Sea lamprey population in the United States[11] and in the Great Lakes[12]

Due to their intrinsic parasitic habits, sea lamprey are a serious problem for the Great Lakes, devastating fisheries, valued at about 1.6 billion dollars per year.[2] Indeed, the decline of several large native Great Lakes fish is mainly attributed to the introduction of the sea lamprey. Lamprey have had the most devastating effect on a variety of cisco species, lake trout, and walleye.[10] At the height of the sea lamprey population, various sport fish populations were suppressed which resulted in reduced recreational and

commercial fishing activities.[10] Within ten years following the discovery of sea lamprey in Lake Huron, the commercial lake trout fishery fell from 3.4 million pounds to a fishery not worth targeting.[10] Furthermore, a large amount of money continues to be spent in restoring the sport fish populations that have been damaged by sea lampreys.

1.2. Sea Lamprey Population Control in the Great Lakes

1.2.1. Current methods to control sea lamprey

In order to control the sea lamprey population in the Great Lakes, the federal governments of the United States and Canada co-founded the Great Lakes Fisheries Commission (GLFC) in 1956.[2] Since its inception, the GLFC has actively sought methods to eradicate or control the lamprey in the Great Lakes.[2]

Early methods included mechanical wires and electrical barriers.[13] Lamprey barriers have been installed in a number of locations to block the migration of spawning sea lampreys while allowing other fish to pass.

Another technique being used to reduce the sea lamprey population is the sterilization of males.[14] During spawning runs, male sea lampreys are collected, sterilized with a dose of bisazir (*P*,*P*-bis(1-aziridinyl)-*N*-methylphosphinothioic amide,[15] Figure 4) and released back into the tributary. These sterile males compete with fertile males for spawning females. This results in reduced fertilization of the eggs.

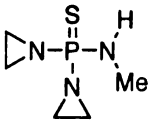


Figure 4. Chemical structure of bisazir

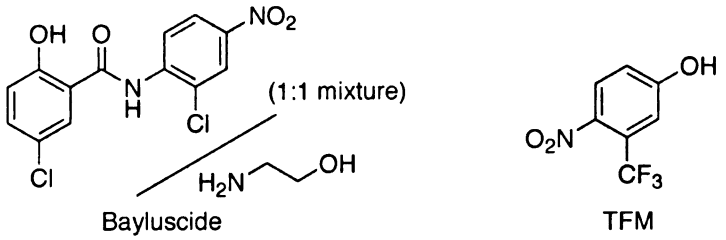


Figure 5. Chemical structures of lampricides

The application of selective lampricides is the primary method used at this time to kill larval sea lamprey in the nursery streams. Chemical structures in Figure 5 represent the two most common lampricides, Bayluscide (mixture of 5-chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide and 2-aminoethanol) and TFM (3-trifluoro methyl-4-nitrophenol).[16]

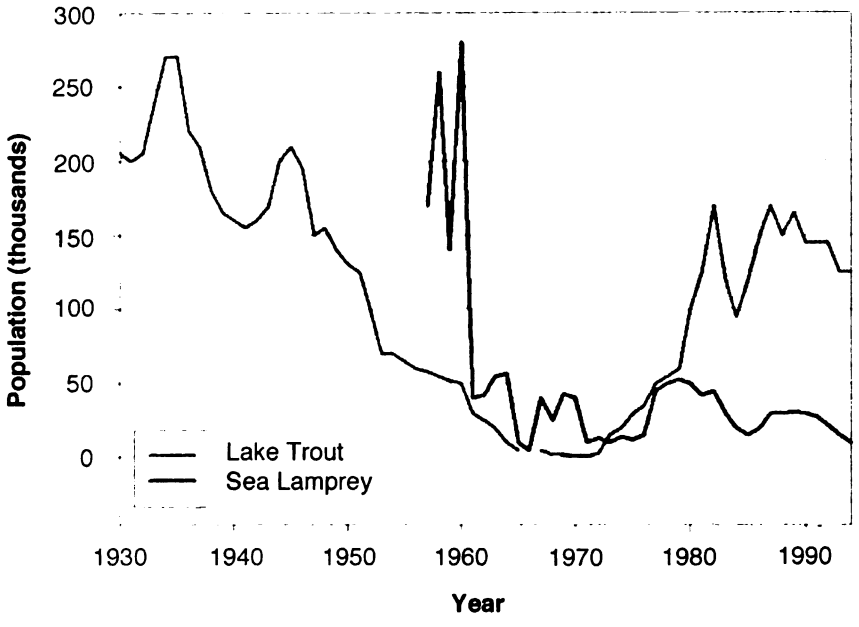


Figure 6. Lake trout populations and sea lamprey populations in Lake Superior[17]

While sea lampreys will never be extirpated from the Great Lakes even with the most aggressive combination of management approaches, the lamprey population can be

suppressed to a low level that will lessen the impact to the native fish. Figure 6 demonstrated the successful control of sea lamprey population in Lake Superior.[18] The population of lake trout seemed to be regulated by natural variation in early days. After the first emergence of sea lamprey in Lake Superior, the trout population dropped down to almost nonexistent levels. Following the founding of the GLFC and application of selective lampricides, mainly TFM, to eradicate sea lamprey, the lamprey population was suppressed by as much as 90% since its population peak. This lowering of the lamprey population was followed by a recovery of the trout population.

Although selective lampricide treatments have been successful at reducing lamprey populations in the presence of native species of fish, this method has brought about public apprehension. There were some reports about acute toxicity associated with TFM, and its price has tripled over the past years.[16] Moreover, the lampricides did not show the same effectiveness in all the Great Lakes. For example, while they have worked effectively in Lake Superior, lampricide use in Lake Huron, especially around the St. Marys River, has been met with only partial success.[19] Owing to these issues, the GLFC decided in 2001 to reduce TFM usage by half and to pursue other management strategies.

1.2.2. Life cycle of sea lamprey and related pheromones

After extensive sea lamprey studies, the GLFC developed several control methods that are closely related to the life cycle of the sea lamprey.[20] Each method mainly targets lamprey in a specific stage: Barrier construction to block the spawning migration of adult

lampreys, sterilizing spermiating male sea lamprey, and the aforementioned application of lampricides to decimate larval sea lamprey in the nursery streams

A closer look at the life cycle of the sea lamprey[7,21] reveals that they hatch from eggs located in tributaries during the spring and early summer. As larvae, the sea lamprey lives in freshwater rivers and feed on microorganisms and detritus. The larvae, which lack their oral disk, are swept downstream and burrow into the sand and silt, where they remain for 3-15 years. During this time, the larval sea lamprey grows to a length of approximately six inches.

At the end of the larval stage, the sea lamprey undergoes metamorphosis into an adult lamprey, developing its oral disc and eyes. As an adult, the lamprey moves out into the Atlantic Ocean, or in the case of the landlocked lamprey, it moves into the open water of the Great Lakes, where it begins feeding on native fish for 12-20 months. It is only during this stage that the sea lamprey poses a threat to other fish.

Once the parasitic stage ends, the sea lamprey becomes sexually mature, virtually blind, and ceases feeding. They leave the Lakes and return to the tributaries where they build nests and spawn. During spawning, there will be a large congregation of lampreys all intertwined in a large ball. An average female spawns 60-70 thousand eggs. After spawning the lamprey dies, thus competing the life cycle.

All these stages of sea lamprey life cycle are coordinated by several key pheromones. Larval lampreys excrete migratory pheromones from gall bladders to attract adult lampreys to their spawning region.[22] Cued by these pheromones, adult lampreys undertake nocturnal migrations into streams to spawn in the spring of their last year of life. Migrating males typically arrive on the spawning grounds before females and

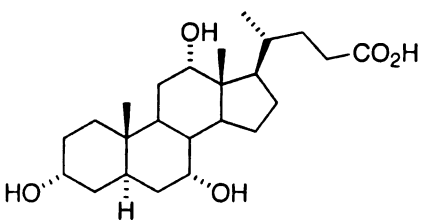
establish nests. During the final stages of maturation (spermiation), male lampreys begin to release sex pheromones that are highly attractive to ovulating females, signaling both reproductive readiness and nest location to potential mates.[23]

Recent investigations aimed at understanding the behavioral responses of lampreys to these pheromones suggest that the pheromones could be used for potential methods to control sea lamprey population in the Great Lakes.

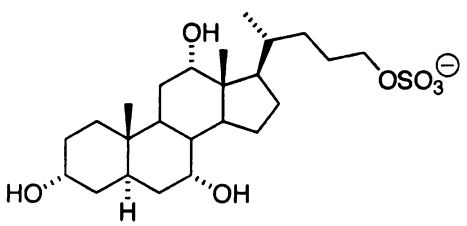
1.2.3. Application of pheromones to sea lamprey control

As part of efforts to seek alternative ways to control sea lamprey in the Great Lakes, pheromones as controlling agents have been investigated and are considered as the most promising substitutes for lampricides.

In pursuit of such use, the Sorensen group at the University of Minnesota studied migratory larval lamprey pheromones.[22] They isolated and characterized several components of the larval pheromone cocktail including allocholic acid ACA, and petromyzonol sulfate PZS (Figure 7). Additionally, they have revealed that adult sea lampreys show active migratory behavior in flowing water treated with as low as 10^{-10}M concentration of ACA and PZS mixture.



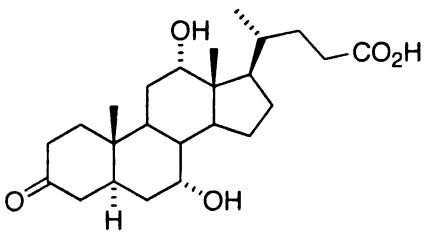
allocholic acid, **ACA**
(3α,7α,12α-trihydroxy-
5α-cholan-24-oic acid)



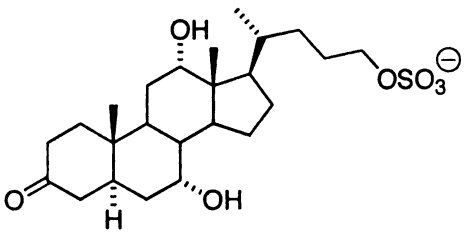
petromyzonol sulfate, **PZS**
(3α,7α,12α,24-tetrahydroxy-
5α-cholan-24-sulfate)

Figure 7. Chemical structures of migratory pheromones from larval sea lamprey

In 2002 and 2003, Weiming Li and co-workers at Michigan State University identified two major components of male sea lamprey pheromones, 3-keto-allocholic acid 3kACA and 3-keto-petromyzonol sulfate 3kPZS (Figure 8).[23] The Li group also reported that the ovulating female lamprey responds to 3kACA and 3kPZS even at the highly dilute concentrations of 10^{-10}M and 10^{-12}M, respectively.



3-keto-allocholic acid, **3kACA**
(7α,12α-dihydroxy-
5α-cholan-3-one-24-oic acid)



3-keto-petromyzonol sulfate, **3kPZS**
(7α,12α,24-trihydroxy-
5α-cholan-3-one-24-sulfate)

Figure 8. Chemical structures of sex pheromones from male sea lamprey

Pheromones that can induce spatial orientation are well known for insects, but not for vertebrates.[23] Typically, sex pheromones in vertebrates tend to have a small range of effectiveness, yet the sea lamprey's pheromones can travel great distances. Data indicates that 3kPZS from male sea lamprey, for instance, is 10^5 times stronger than sex pheromone from female goldfish in terms of effectiveness.[23] In such a highly diluted water stream, the virtually blind adult sea lampreys are guided to the specific spawning area by their sensitive olfactory system. The Sorenson group has revealed that if the nostrils of migratory adults are blocked, they fail to find spawning streams. Studies on the sense of smell of the sea lamprey have demonstrated that its reliance on this sensory system may be greatest among fish.[22]

The lamprey's reliance on chemical communication may be exploited to the future advantage. For example, these naturally occurring cues could be used to lure lamprey into traps or into streams that are not suitable for reproduction. Thus, careful application of migratory and sex pheromones may allow reduced use of lampricides while still maintaining effective sea lamprey control. Indeed, the pheromone application may become an environmentally friendly tool in the integrated management of sea lamprey.

1.2.4. Isolation of sex pheromones for lamprey control agents

For the potential application of pheromones to sea lamprey control, the Li group (Michigan State University) has mainly focused on sex pheromone components released from male sea lamprey, especially 3kPZS which is one of the most potent components showing 100 times stronger activity than other pheromones produced by sea lamprey. The Li group isolated and identified sex pheromones from the following process.[23] During the migration season of sea lamprey from May to July, adult sea lampreys were collected from the field. A spermiating male lamprey was held for 4 hours in 10 L of lake water. Washing water containing sex pheromones was passed through filtration cartridges packed with C18 and then the C18 cartridges were extracted with methanol. About 30 spermiating lamprey were treated by the same procedure. Finally, the mixture of combined sex pheromones was subjected to prep HPLC to isolate two major fractions of 30 mg and 2 mg. After spectroscopic data analysis and electro-olfactogram (EOG) experiments, 3kPZS and 3kACA were structurally identified, respectively, as key signaling components of male lamprey sex pheromone. These isolated pheromone components can be potentially used as species-specific and environmentally benign

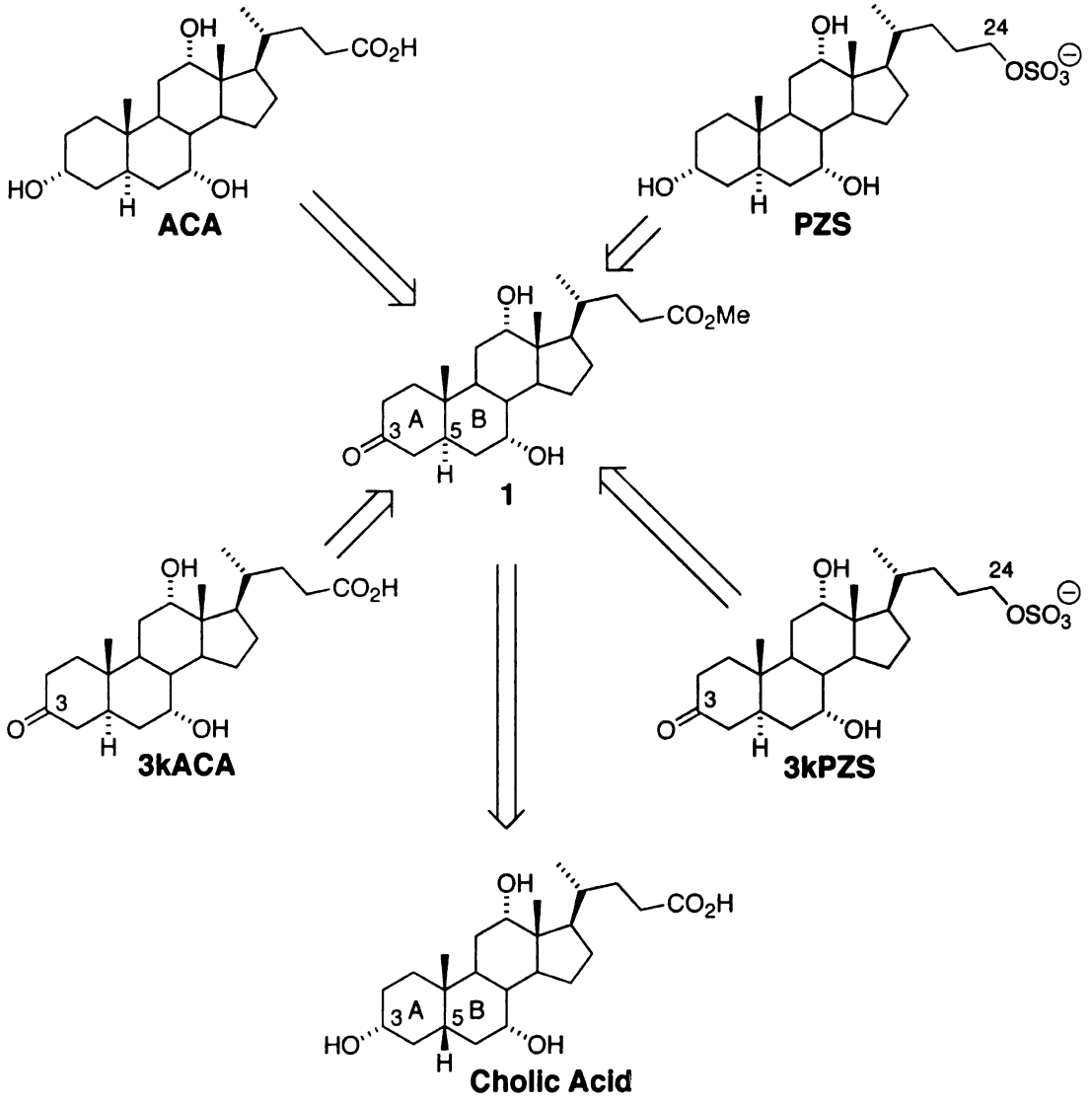
control agents. More intriguingly, a report on capturing ovulating female sea lamprey in pheromone-baited traps shed a promising light upon taking advantage of pheromones as a method to control sea lamprey in the Great Lakes.[24]

For the purpose of utilizing pheromones as selective lamprey control agents, a continuous and economical supply of pheromones is required. The GLFC and its agents have provided funding in search for environmentally friendly methods to control the lamprey population and many research groups have been exploring efficient ways of isolation and synthesis of the pheromones. Due to laborious extraction and isolation efforts with only small acquisitions of pheromones *in vivo*, various types of research to synthesize these pheromones *in vitro* have been conducted by exploiting enzymology and biosynthesis.[25] Nevertheless, the only chemical approaches have been Dr. Mclean's synthesis of petromyzonol (PZ) and Dr. Iida's synthesis of allocholic acid (ACA), *vide infra*. For these reasons, chemical production of sea lamprey pheromones on large scale would be valuable.

1.3. Retrosynthetic Analysis

Given the proposed use, the synthesis of pheromone control agents should be inexpensive, practical and amenable to scale-up route. Furthermore, since the synthetic products might be utilized in the Great Lakes, it is important for the synthetic approach to be environmentally acceptable. A goal of this research is to help the environment, and as such, great care must be taken to avoid introduction of toxic or harmful reagents, even at low concentrations, into the lakes.

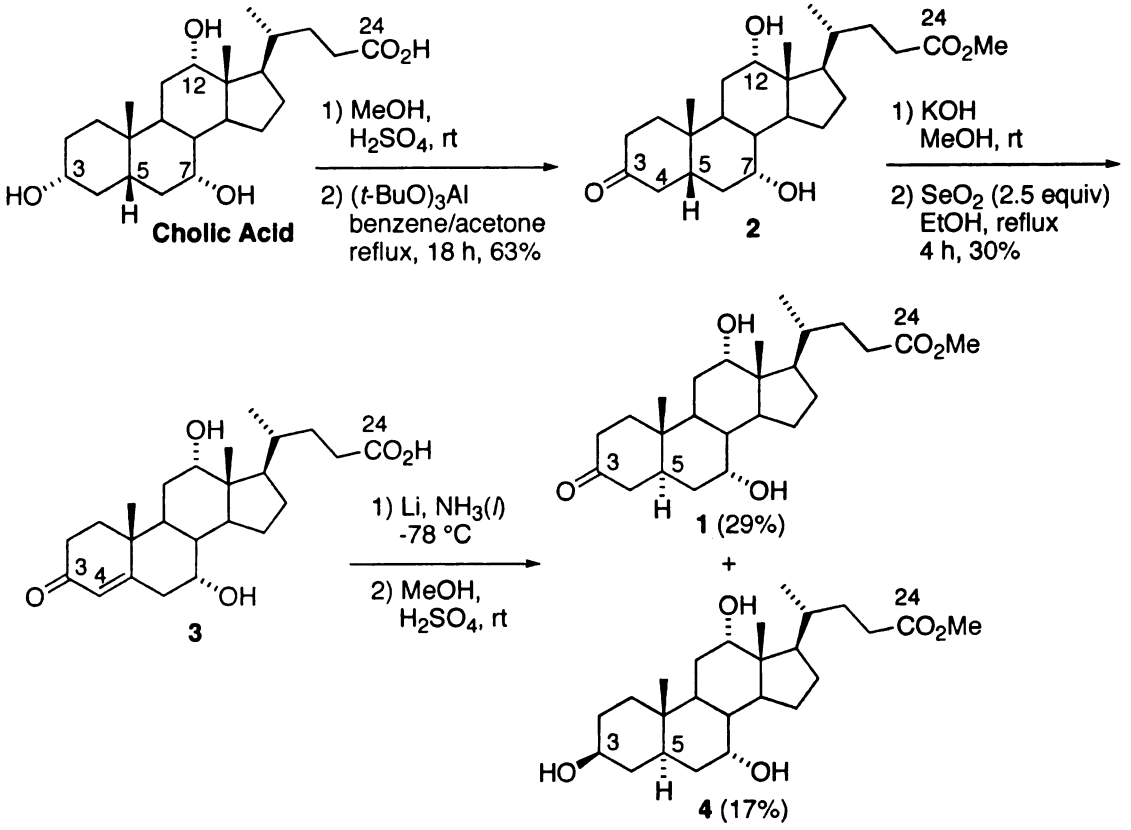
Our synthetic approach to sea lamprey pheromones is presented in Scheme 1. From the retrosynthetic point of view, all four major sea lamprey pheromones could be transformed via relatively straightforward functional group manipulations from common intermediate **1**. For the practical preparation of the intermediate **1**, cholic acid was viewed as a plausible starting material because it is an inexpensive bile acid, bearing the carbon skeleton and functional group precursors of the sea lamprey pheromones. In order to achieve the successful conversion from cholic acid to intermediate **1**, a crucial step of the synthesis would be conversion of stereochemistry from 5β (A/B *cis*) to 5α (A/B *trans*). In steroidal compounds, this type of epimerization to convert stereochemistry from β to α specifically at the C5 position is called allomerization.[26] Thus, the main strategy and key step of the synthesis will be allomerization of this stereogenic center.



Scheme 1. Retrosynthetic analysis of sea lamprey pheromones

1.4. Prior Synthesis

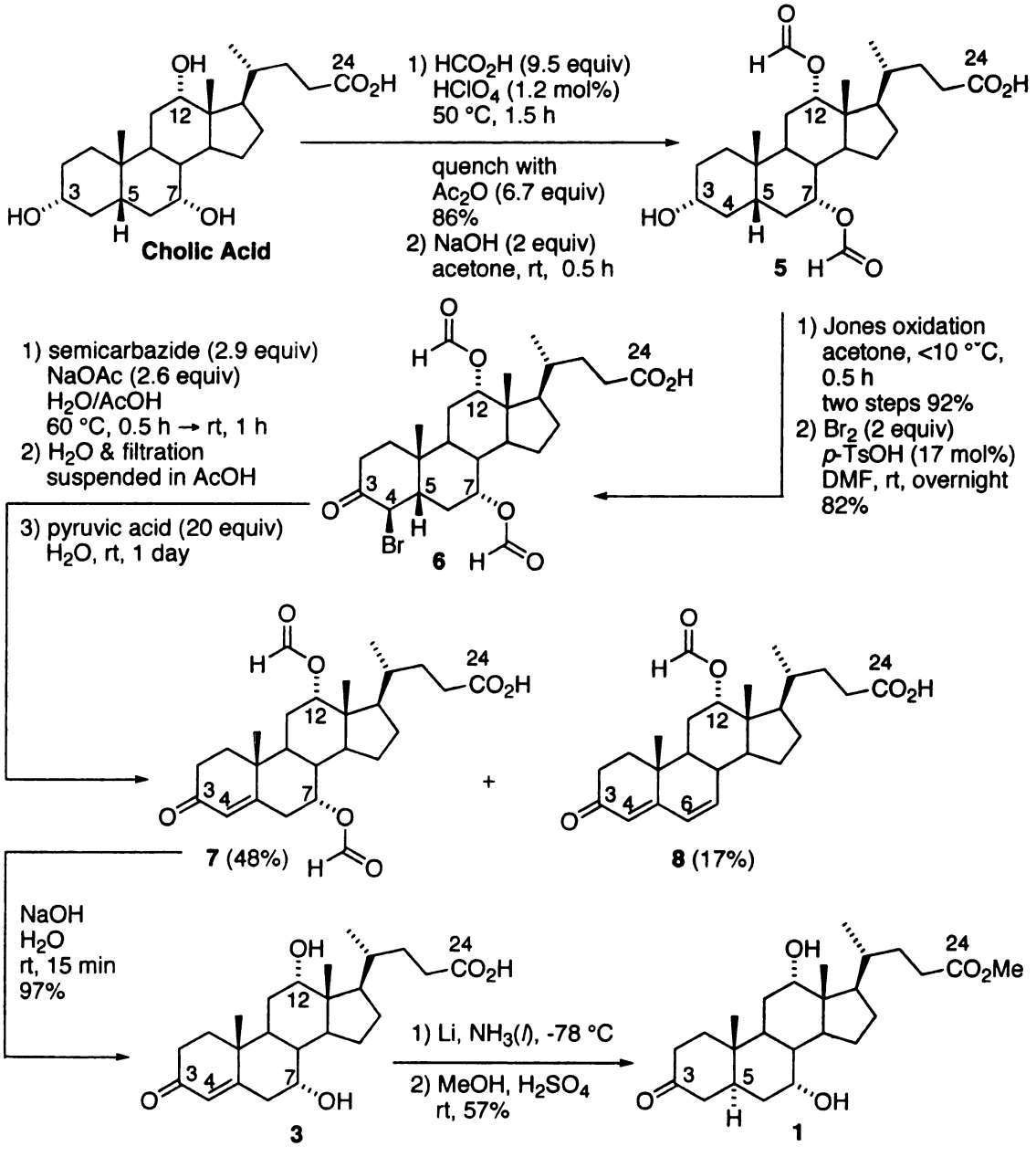
As mentioned from retrosynthesis, the ring conversion of 5β stereochemistry of cholic acid into 5α of common intermediate **1** is crucial for proper synthesis of all sea lamprey pheromones. There are some literature reports of similar target syntheses derived from cholic acid via allomerization.



Scheme 2. Kallner's allomerization of cholic acid to intermediate **1**

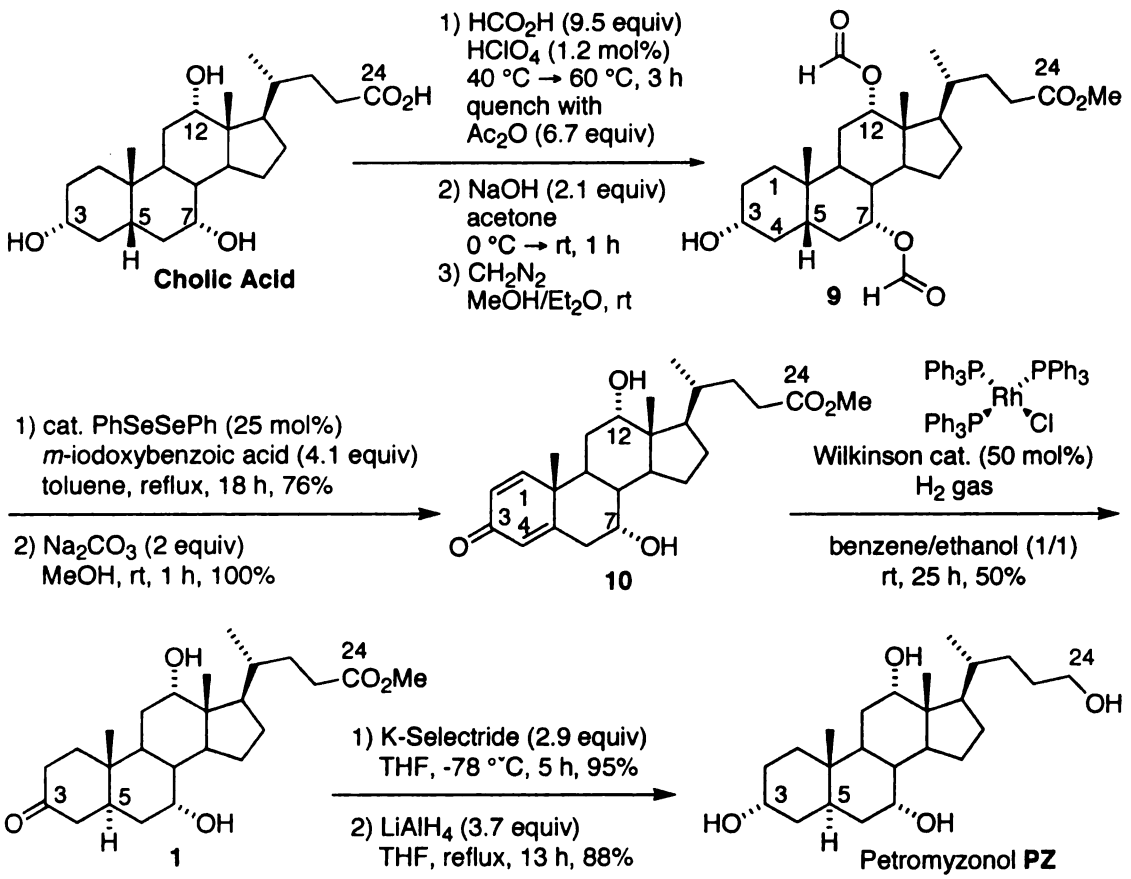
In 1967, Kallner reported the first allomerization of cholic acid to intermediate **1** (Scheme 2).[27] Methylated cholic acid was selectively oxidized only at C3 position to afford methyl 3-ketocholate **2** with Oppenauer's reagent.[28] To achieve allomerization, they destroyed the 5β (A/B *cis*) stereochemistry initially to prepare α,β-unsaturated enone compound **3** by the action of SeO_2 in ethanol. Then, under the conditions of Birch reduction, the stereochemistry of 5α (A/B *trans*) was exclusively installed on both products **1** and **4**. Although this protocol demonstrated the first successful allomerization of cholic acid, yields of the key steps were low, accompanied with significant amount of by-products, which have to be separated by column chromatography. In the

dehydrogenation step, they used stoichiometric amounts of SeO_2, which is highly toxic and expensive. Additionally, Birch reduction for stereoselective conjugate reduction normally requires cryogenic and dry conditions, which along with the handling of liquid ammonia can be problematic on kilo scale synthesis.



Scheme 3. Iida's allomerization of cholic acid via bromoketone **6**

In 1986, Iida group modified procedures for the enone formation without the usage of selenium reagent (Scheme 3).[29] Transformation to the α,β-unsaturated enone was carried out by the sequence of oxidation at C3 to the ketone, then α-bromination, followed by β-elimination. Tserng and Klein's procedure for selective oxidation at C3 position was first applied.[30] Cholic acid was fully formylated on its three hydroxyl groups, and then, mono deformylation at C3 position occurred with. The resulting free hydroxyl compound **5** was oxidized with Jones' reagent to the 3-keto compound in high yield. In order to avoid the usage of selenium, the ketone precursor was activated by α-bromination and then, the resulting bromo compound **6** was transformed into 4-en-3-one **7** via hydrolysis of semicarbazone intermediate. However, once compound **7** is formed, the hydroxyl group at C7 position becomes extremely labile to both acidic and basic conditions.[31] For example, the presence of either a protected or free hydroxyl group at C7 causes complications, mainly facile γ,δ-elimination to deliver 4,6-dien-3-one. Indeed, Iida observed 17% of the eliminated material (**8**) in their allomerization. Finally, selective 5α (A/B *trans*) stereochemistry of the crucial intermediate was installed by Birch reduction due to the benefits of ideal stereocontrol of cholic acid derivatives, even with the aforementioned disadvantages of practicality of Birch reduction.

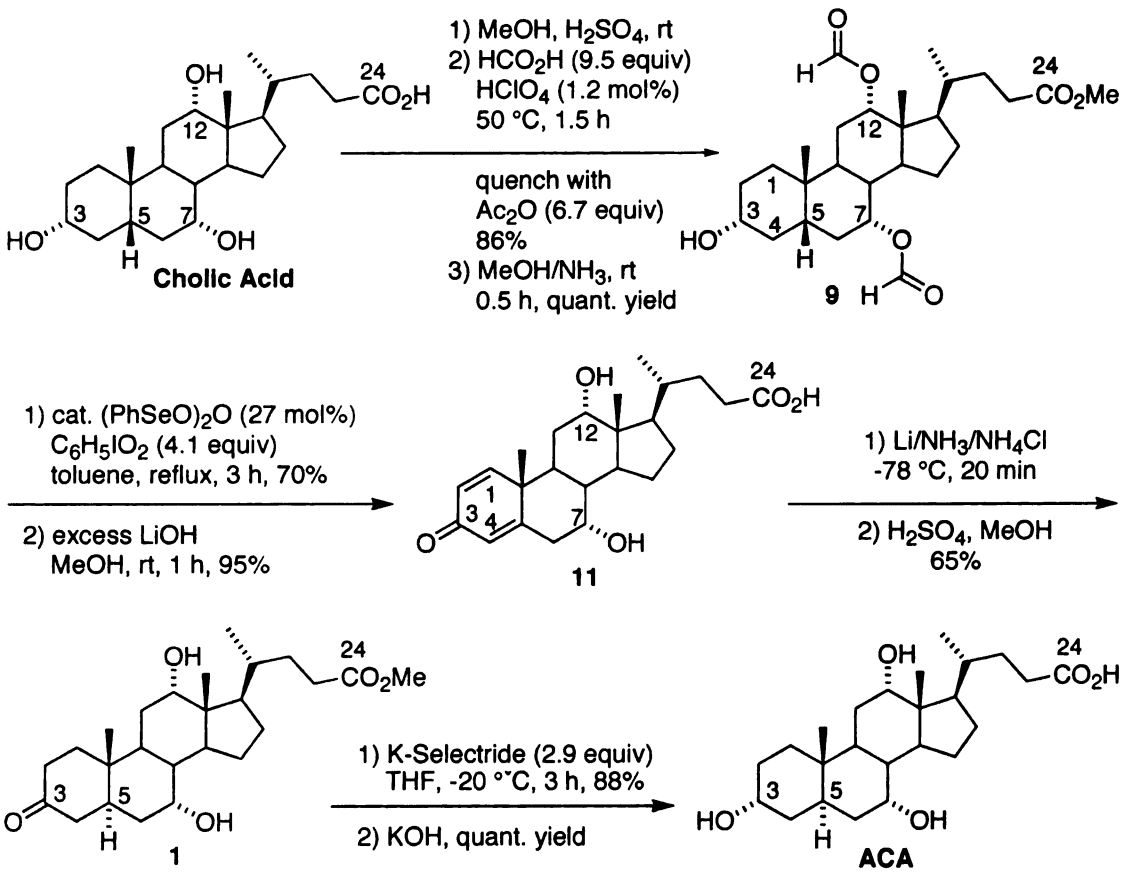


Scheme 4. McLean's allomerization towards the synthesis of petromyzonol **PZ**

In 1987, the Mclean group investigated their own allomerization approach towards the synthesis of petromyzonol (PZ), as illustrated in Scheme 4.[32] Functionalized cholic acid **9** was subjected to oxidation with catalytic amounts of selenium as developed by the Barton group.[33] The subsequent 1,4-dien-3-one **10** was reduced in the presence of Wilkinson's catalyst to afford complete stereoselection of 5α (A/B *trans*), presumably from hydride delivery directed by catalyst complexation with the free 7α-hydroxyl group. The ketone functionality at C3 of the desired 5α compound **1** was then reduced by a bulky hydride source, potassium tri-sec-butyl-borohydride (K-selectride) to provide a single diastereomer of 3α-hydroxyl compound in excellent yield. The last reduction of

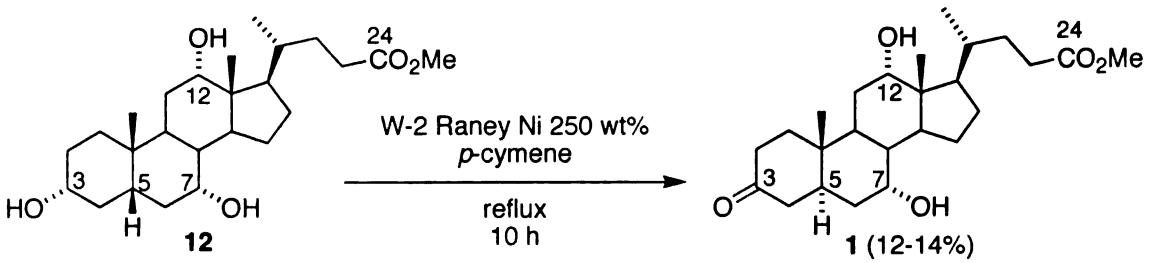
the terminal ester completed the synthesis of petromyzonol PZ. This allomerization procedure, however, is not without unattractive features. For the preparation of mono-deformylated precursor **9**, the methylation was carried out with diazomethane in order to keep acid/base-labile formylated functional groups in tact. Diazomethane is problematic at large-scale due to its hazardous and toxic nature. Secondly, while only catalytic amounts of diphenyl diselenide were needed, to accomplish the one-step sequence of oxidation and dehydrogenation to afford 1,4-dien-3-one **10**, the selenium was relatively high (25 mol%). Furthermore, the authors acknowledged that the reduction with Wilkinson's catalyst proved inconsistent and catalyst-batch-dependent. Moreover, the reduction required 50 mol% loading of Wilkinson's catalyst and provided only 50% yield of product **1**, along with observation of over-reduced alcohol at C3 position.

After 6 years, Iida group combined most of the advantageous methods in the allomerization reports of cholic acid and established the most feasible route to the synthesis of allocholic acid **ACA** to date (Scheme 5).[34] A one-step oxidation and dehydration procedure was chosen to afford a key intermediate 1,4-diene-3-one that was ready for allomerization.[35] Precursor **9** was prepared by the sequence of methylation of cholic acid, formylation, and selective deformylation at C3 position by methanol/ammonia method. With this sequence, step of diazomethane usage was eliminated. Alcohol compound **9** was converted to the 1,4-diene-3-one **11**, catalyzed by selenium reagent (27 mol%). Complete stereoselective reduction to 5α (A/B *trans*) compound **1** was accomplished under Birch reduction conditions, which were found to be preferable to hydrogenation by Wilkinson's catalyst. Again though, the selenium loading was still high and the same concerns over the Birch reduction on large scale remain.



Scheme 5. Iida's allomerization towards the synthesis of allocholic acid **ACA**

As discussed above, most allomerizations of cholic acid derivatives were carried out via the stepwise sequence of oxidation at C3 and α,β-unsaturated ketone formation, followed by stereoselective reduction to convert stereochemistry from 5β to 5α. While new pathways for allomerization have developed and great achievement has been made, the preparation of precursors for allomerization still requires several steps of functional group manipulation. Also, due to various safety and cost issues, none of the allomerization procedures are ideal for an environmentally friendly pest management program in economical way.



Scheme 6. Mitra and Elliott's single-step allomerization with Raney Ni

Interestingly, back in 1968, Mitra and Elliott demonstrated transformation of methyl cholate **12** to desired intermediate **1** via single-step allomerization (Scheme 6).[35] By the action of Raney Ni in boiling *p*-cymene discovered by Chakravarti group,[37] 5β (A/B *cis*) stereochemistry of methyl cholate **12** was smoothly converted into 5α (A/B *trans*), along with concomitant selective oxidation at C3 position in one step. This method is attractive superficially but there are some drawbacks. Although it is single step allomerization, yield of desired product **1** was low (12-14%) and crude reaction mixture included series of by-products in considerable amounts of each. Moreover, all compounds in the mixture are accompanied by their corresponding 5β isomers and the purification or separation of the 5α compounds from the 5β compounds was not practical. Indeed, the Iida group noted that Raney Ni reactions were erratic and resulted in complex mixtures, which required extensive laborious chromatographic separation.[38]

Despite such results and Iida's comment, the Raney Ni reaction is still a single step allomerization, cutting out many steps and the need for expensive and/or toxic reagents. We hypothesized that with the development of practical methods for separation and improved reaction conditions, this single step allomerization would be potent method and thus worth further investigating. Therefore, our first approach to 3kPZS and analogs

commenced with an investigation of the single step allomerization mediated by Raney Ni.

CHAPTER 2. SINGLE STEP ALLOMERIZATION WITH RANEY NICKEL

2.1. Mitra and Elliot's Allomerization with Raney Ni

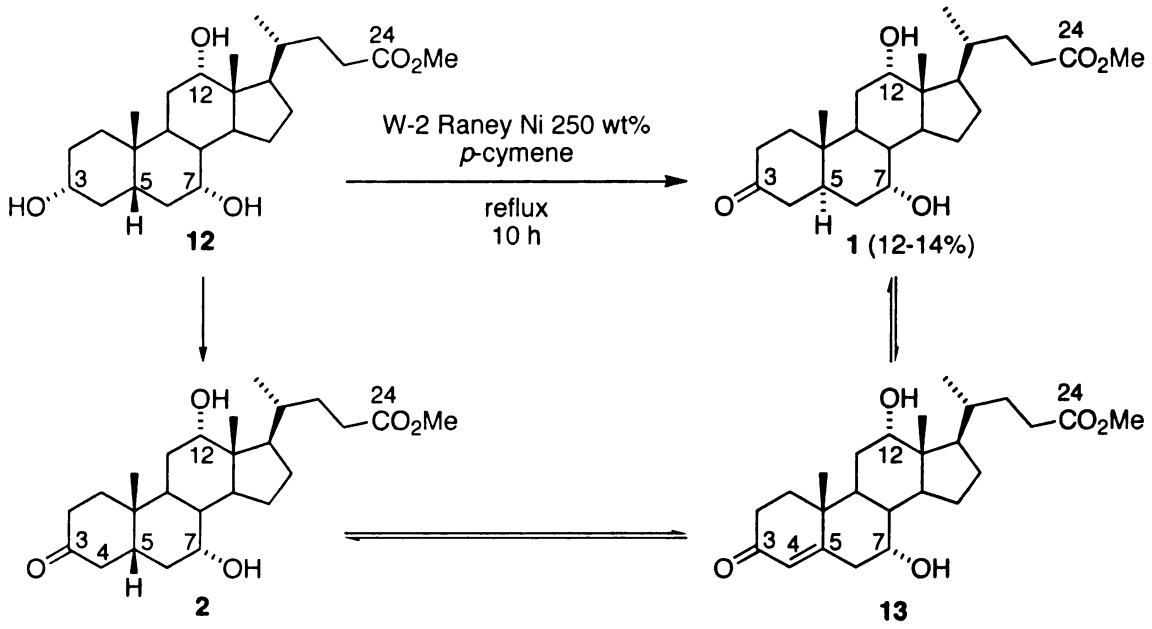
2.1.1. Single step allomerization of methyl cholate

In order to be utilized as sea lamprey population control agents in the Great Lakes, chemical synthesis of pheromone components needs to be practical and environmentally benign. Among the literature reports that seemed to suit these requirements, the allomerization with Raney Ni reported by Mitra and Elliot stands out.[36] Action of Raney Ni in boiling *p*-cymene smoothly transformed methyl cholate **12** into product **1** (Scheme 6). Other reports about allomerization of cholic acid incorporated the stepwise sequence of oxidation at C3 position, dehydrogenation to α,β-unsaturated ketone, and selective reduction, in order to achieve conversion of stereochemistry from 5β (A/B *cis*) to 5α (A/B *trans*). Since cholic acid bears three hydroxyl groups at C3, C7, and C12 positions, these multi step approaches needed to be accompanied by selective protecting group manipulation. In contrast, Mitra and Elliot's allomerization converted stereochemistry from 5β (A/B *cis*) of methyl cholate **12** to 5α (A/B *trans*) of desired product **1** in a single step along with selective oxidation at C3 position without any need of functional group protection. Empirically, this procedure is a one-step allomerization, but reaction actually proceeds via a series of stepwise allomerizations.

2.1.2. Mechanism of allomerization with Raney Ni

In their early reports, Mitra and Elliot presented their mechanism of the allomerization with Raney Ni (Scheme 7).[39] Raney Ni was said to oxidatively add into

the O-H bond of the sterically more accessible 3α-hydroxyl group, followed by β-hydride elimination to afford 3-keto compound **2**. Selectively oxidized keto compound **2** enolizes toward at C4 and Raney Ni promotes β-hydride elimination affords α,β-unsaturated ketone **13**. Lastly, the resulting enone compound **13** is reduced to provide desired product **1** with 5α (A/B *trans*) stereochemistry.



Scheme 7. Proposed mechanism of allomerization with Raney Ni

After chromatographic separation of the reaction mixture on alumina, Mitra and Elliot isolated three major fractions, desired product **1** (12-14%), over-oxidized product **14** (18-20%), and dehydroxylated product **15** (20-22%) as shown in Figure 9.[36b] While all these products have 5α (A/B *trans*) stereochemistry, each product was accompanied with the corresponding 5β (A/B *cis*) isomer prior to another purification by preparative layer chromatography (plc), followed by recrystallization.

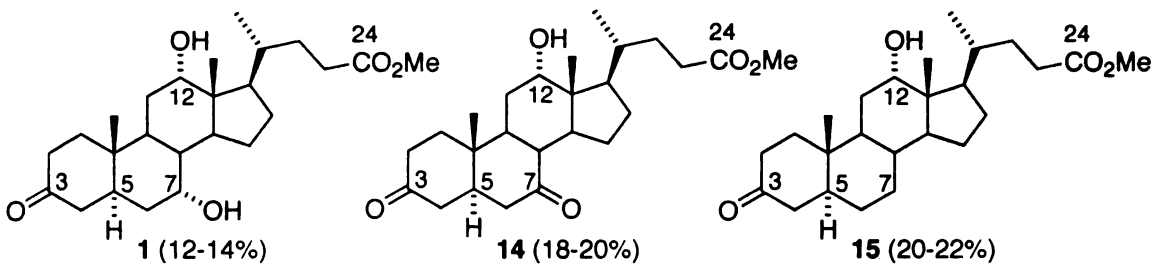
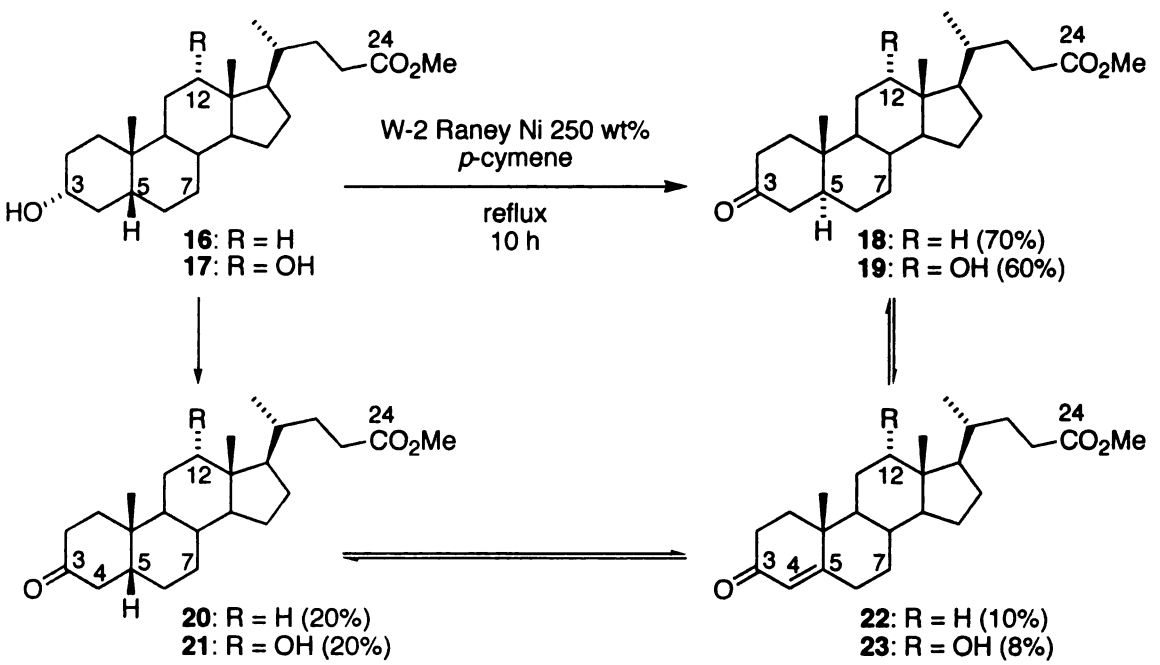


Figure 9. Identified products from allomerization of methyl cholate by Raney Ni

Compound **14** was possibly the result of over-oxidation of the desired product **1** at C7 by Raney Ni. Dehydroxylated compound **15** is also produced via proposed intermediate **13**. Here, once the α,β-unsaturated ketone is set up, the hydroxyl group at C7 becomes easily eliminated to provide 4,6-dien-3-one compound, which is then probably reduced by Raney Ni to afford dehydroxylated product **15**.



Scheme 8. Single step allomerizations with dehydroxylated substrates at C7 position

Later, Mitra and Elliot investigated the mechanism of allomerization of substrates without 7α-hydroxyl group under the same condition of Raney Ni (Scheme 8).[39]

Allomerization of substrates **16** and **17** provided three isolated products, of which the formations were mechanistically examined. Isolation of enone compounds **22** and **23** clearly demonstrated that allomerization by Raney Ni proceeds via the sequence of oxidation, dehydrogenation, and reduction. Moreover, when each isolated product was subjected to the Raney Ni conditions, very similar results were obtained. For instance, the subjection of **18** under the condition of Raney Ni gave three compounds, **18** (70%), **20** (20%), and **22** (10%) probably after reaching equilibrium. The same equilibrium was established with either compound **20** or **22**, affording the same ratios of three products. These data supported the hypothesis that the three products are in equilibrium during the process.

Mitra and Elliot also looked into the role of solvent in these allomerizations.[39] The net reaction is one of epimerization at C5 from 5β (A/B *trans*) to 5α (A/B *cis*) along with selective oxidation at C3. Therefore, the overall reaction is an oxidation, where the starting material loses two hydrogens in the course of the reaction. From labeling studies, Mitra and Elliot found that tritium at C4 of the steroidal compounds was transferred to solvent by way of hydrogenolysis or reduction through the action of Raney Ni. Thus, *p*-cymene, is working as solvent as well as hydrogen acceptor.

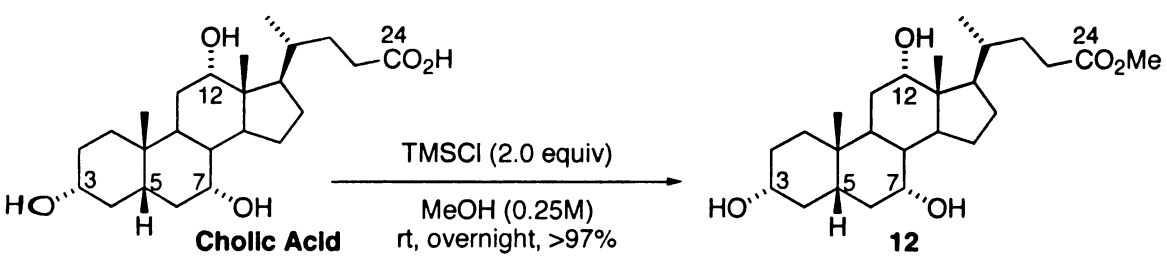
This reaction has advantages as a one-pot allomerization, but there are some drawbacks. Allomerization of methyl cholate **12** provided mixtures of desired product **1**, over-oxidized product **14**, and dehydroxylated product **15**. Compound **15** is especially problematic as it would be a dead-end because its transformation back into product **1** would not be straightforward. Furthermore, each product contained the corresponding 5β (A/B *cis*) isomer prior to additional purification. On the plus side, compound **2**, one of

the 5β isomers, could be recyclable into product **1** through the equilibrium process. Perhaps the biggest operational issue is that purification or separation of 5β/5α mixture requires undue effort. Despite of these issues, this reaction is a single step allomerization, cutting out many steps. Thus, it is still attractive and worth further investigation.

2.2. Optimization of Single Step Allomerization with Raney Ni

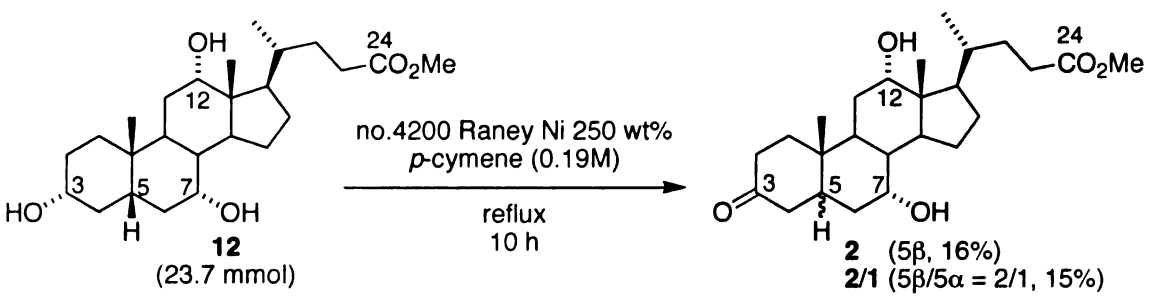
As discussed above, we need to resolve those several negative issues surrounding the single step allomerization with Raney Ni. Obviously, the yield of desired product **1** needs to be improved. Thus, production of dead-end by-products **14** and **15** should be minimized. In tandem with this aim, we need to improve the ease with which the 5β and 5α mixture is purified.

We began by exploring the allomerization of methyl cholate **12** with Raney Ni. Substrate **12** was prepared from commercially available cholic acid. Cholic acid was methylated first in methanol, mediated by 2 equivalents of trimethylsilyl chloride, TMSCl (Scheme 9). This methylation seemed suitable for large-scale synthesis because the reaction proceeded smoothly at room temperature and open-to-air. Concentration of the resulting crude reaction mixture induced the slow formation of colorless needles of methyl cholate **12** in excellent yield.



Scheme 9. Preparation of substrate **12** for single step allomerization

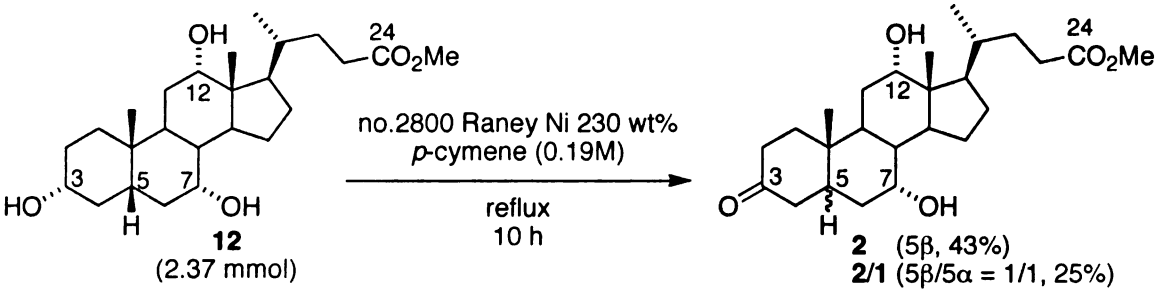
Mitra and Elliot performed the allomerization of methyl cholate with freshly prepared W-2 Raney Ni. This Raney Ni was prepared by the treatment of sodium hydroxide on Raney catalyst powder (no. 2813) according to Mozingo's method.[40] However, the same Raney catalyst powder is no longer available, so we used commercially available Raney Ni powder that was washed right before the reaction. Allomerization of methyl cholate **12** was thus initiated with Raney Ni (no. 4200) in *p*-cymene (Scheme 10).



Scheme 10. Allomerization of methyl cholate **12** with no. 4200 Raney Ni

The reaction mixture was mechanically stirred at reflux for 10 hours, yielding complicated crude mixtures. After separation on 12% deactivated neutral alumina, 10% of starting material **12** remained, and oxidized product **2** (5β, A/B *cis*) was obtained in 16% yield. More importantly, the desired product **1** (5α, A/B *trans*) was obtained as a mixture of **2** (5β, A/B *cis*) and **1** (5α, A/B *trans*) with a ratio of about 2 to 1 favoring compound **2**. The combined yield was 15%. Additionally, there were several unidentified compounds including UV active compounds that were present in significant amounts. Initial attempts at a single step allomerization with no. 4200 Raney Ni did not give us any more promising results.

We worked on optimizing this reaction by screening different Raney Ni catalyst and changing catalyst loading, solvents, concentrations, and reaction times. Under lower catalyst loading and higher concentrations, formation of oxidized product **2** (5β, A/B *cis*) increased up to 50% yield, but there was no considerable change in the calculated yield of desired product **1** (5α, A/B *trans*). One hour of reaction gave us complicated mixtures including desired product **1**. As the reaction was run for longer time, the complicated reaction mixtures got simplified on TLC but there was no change in amount of product **1**. The type of Raney Ni emerged as a key factor in the efficiency in which desired product **1** was formed. Scheme 11 represents our optimized results of the allomerization of methyl cholate **12**.



Scheme 11. Optimized conditions of allomerization with no. 2800 Raney Ni

Starting material **12** was recovered in 5-10% yield, so the reaction did not go to completion. Yields of the by-products were greatly reduced and the only two major products were **2** (5β, A/B *cis*) and **1** (5α, A/B *trans*). We isolated 43% of oxidized product **2** (5β, A/B *cis*), which could be recyclable to desired product **1** (5α, A/B *trans*) through an equilibrium process. A 1 to 1 ratio of 5β/5α mixture was obtained in 25% yield. Finally, separation of the mixture on neutral alumina provided us a small amount (1% yield) of the desired product **1** (5α, A/B *trans*). Stereochemistry of compounds **2** and **1** was confirmed by X-ray crystallography, which was obtained by Dr. Feng Shi

(Figure 10). Additionally, we isolated a trace amount of enone **13**, which indicates the presence of equilibrium in this reaction.

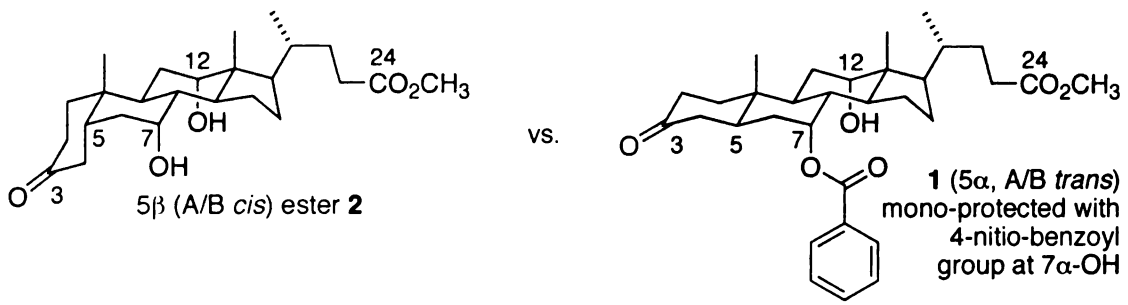


Figure 10. Three-dimensional structures of 5β (A/B *cis*) ester **2** and derivatized 5α (A/B *trans*) ester **1** based on their X-ray crystal structures

We considered this single step allomerization processes to be slightly improved over the original protocol. By utilizing commercially available Raney Ni, the amount of by-products was considerably reduced while the overall yield of usable products increased. By usable we mean, products that are desired or can be converted into the desired compound significantly were able to effect the conversion of **2** (5β, A/B *cis*) into **1** (5α, A/B *trans*). Therefore, we next looked into the reversibility of this reaction.

After subjection of compound **2** (5β, A/B *cis*) to the same Raney Ni conditions, a 1 to 1 ratio of 5β/5α mixture was obtained. Also, we subjected the mixtures of **2/1** (5β/5α=1/1) to the same conditions, but this gave no change in the mixture ratio. On the other hand, subjection of compound **1** (5α, A/B *trans*) in a small scale (10 mg, 0.0237 mmol) to the same conditions provided us degradation of compound **1**, not equilibration due to the stirring issue of paramagnetic nature of Raney Ni with magnetic bar, which should be performed in a dilute concentration with mechanical stirrer or large scale of reaction. From these equilibrium experiments, we knew it was possible to increase the overall yield of desired product **1** (5α, A/B *trans*). Nevertheless, we still had an

abundance of the 5β/5α mixture, so we needed to work on the development of a practical separation protocol.

2.3. Separation of Mixture of 5β (A/B *cis*) 2 and 5α (A/B *trans*) 1

2.3.1. Separation by liquid chromatography on neutral alumina

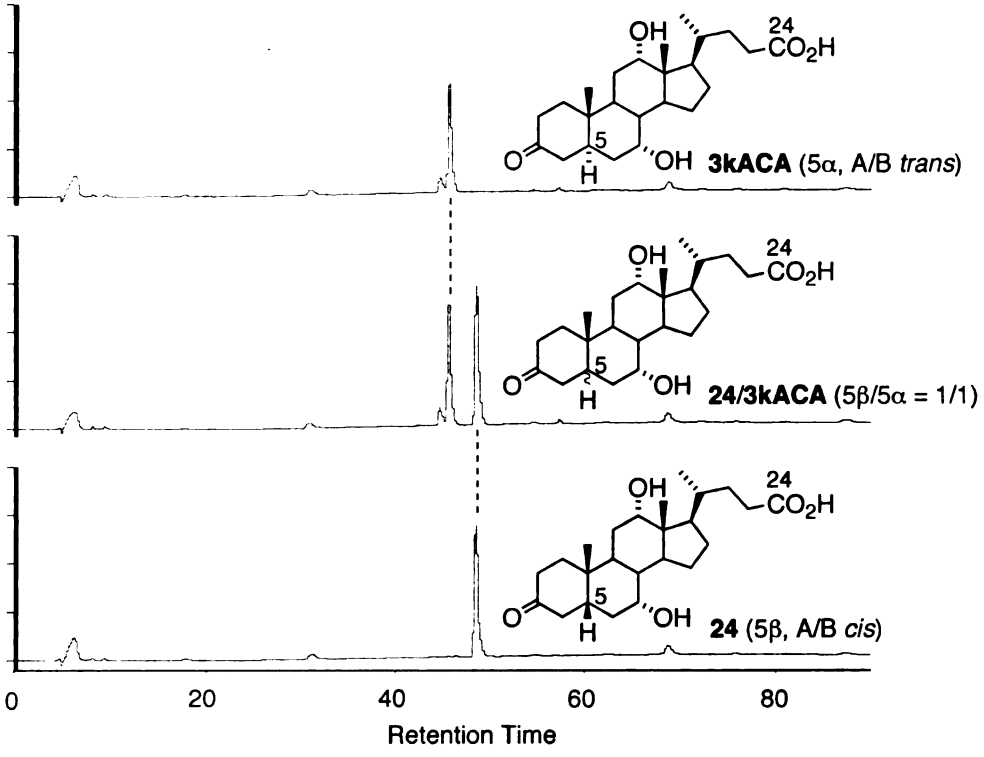
Initially, we followed Mitra and Elliot's separation method.[36] They separated their crude mixture on 12% deactivated neutral alumina to afford three major fractions. The corresponding 5β (A/B *cis*) isomer of each fraction was removed by preparative liquid chromatography (plc) and separated 5α (A/B *trans*) isomers were purified by crystallization.

In our work, we examined several deactivation levels (0%, 3%, 8%, and 12%) of neutral alumina. Contrary to Mitra and Elliot's results, in our hands, liquid chromatography on 3% deactivated neutral alumina gave us pure 5α (A/B *trans*) product **1** without need for a further purification step, however only small amounts of **1** were isolated. As a result, separation by liquid chromatography required multiple runs and it would be impractical for large-scale synthesis.

2.3.2. HPLC separation of mixture of 5β/5α carboxylic acids at C24

While we were working on analysis and separation of 5β/5α mixtures, interesting results were observed. Under the reverse phase of HPLC condition, we could separate 5β (A/B *cis*) and 5α (A/B *trans*) mixtures, but those compounds were carboxylic acids after hydrolysis of methyl ester mixtures at C24 (Figure 11). On the other hand, with 5β/5α methyl ester mixtures, only 5β (A/B *cis*) ester **2** eluted. The 5α (A/B *trans*) ester **1** was never detected under various HPLC conditions. We hypothesize that there might be a selective interaction between 5α (A/B *trans*) ester **1** and the solid support of the normal

phase of HPLC columns. The selectivity might originate from the structural difference between 5β (A/B *cis*) ester **2** and 5α (A/B *trans*) ester **1**.



HPLC condition: prep Nova-Pak HR, C18 3.9X300mm, waters, A/B = 72/28, eluent A =0.01%TFA, eluent B = 70%ACN/0.01%TFA, rate = 0.5 mL/min.

Figure 11. Separation of mixture of 5β/5α carboxylic acids at C24 on HPLC

Based on three-dimensional structures of each compound shown in Figure 12, the 7α-hydroxyl group seems to be the most sterically differentiated functional group between 5β (A/B *cis*) ester **2** and 5α (A/B *trans*) ester **1**. In an intermolecular interaction, the more sterically hindered 7α-hydroxyl group on 5β (A/B *cis*) ester **2** would likely react slower than the 7α-hydroxyl group on 5α (A/B *trans*) ester **1**. For this reason, the 7α-hydroxyl group on 5α (A/B *trans*) ester **1** might interact selectively with the polar functional groups of the solid support in a normal phase of HPLC columns. In contrast, the selective intramolecular reaction of 5β (A/B *cis*) ester **2** could be induced from the

close proximity between the 3-keto functionality and the 7α-hydroxyl group. If we could utilize the reactivity difference between 5β (A/B *cis*) ester **2** and 5α (A/B *trans*) ester **1**, an easy and practical way of separation might be achieved.

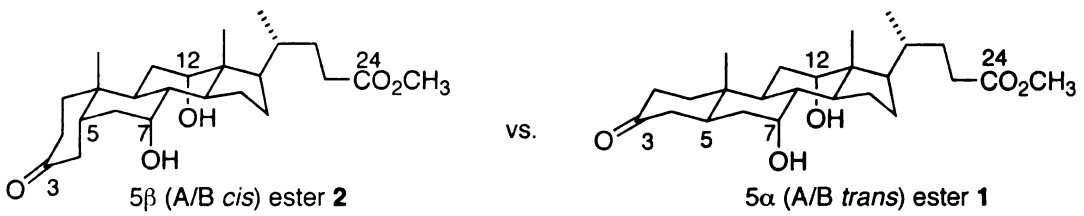
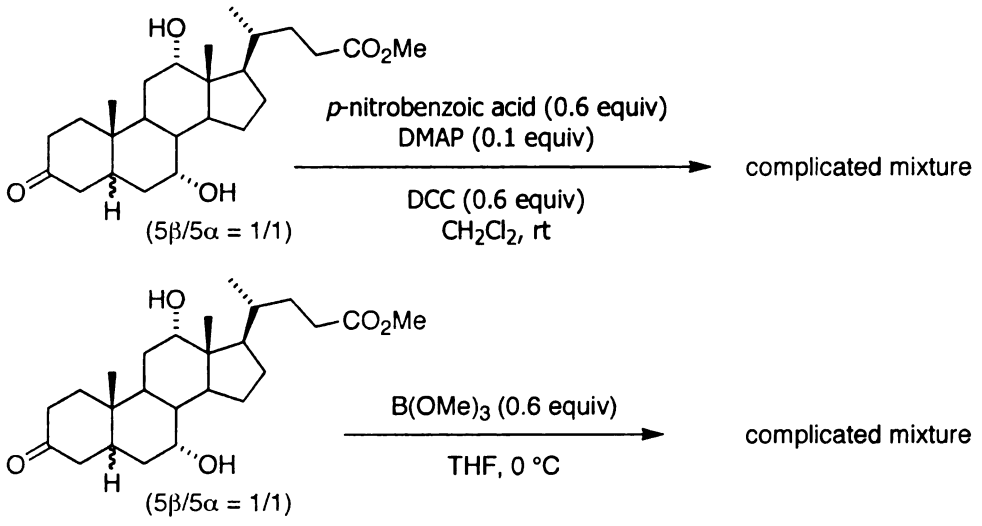


Figure 12. Three-dimensional structures of 5β ester **2** and 5α ester **1**

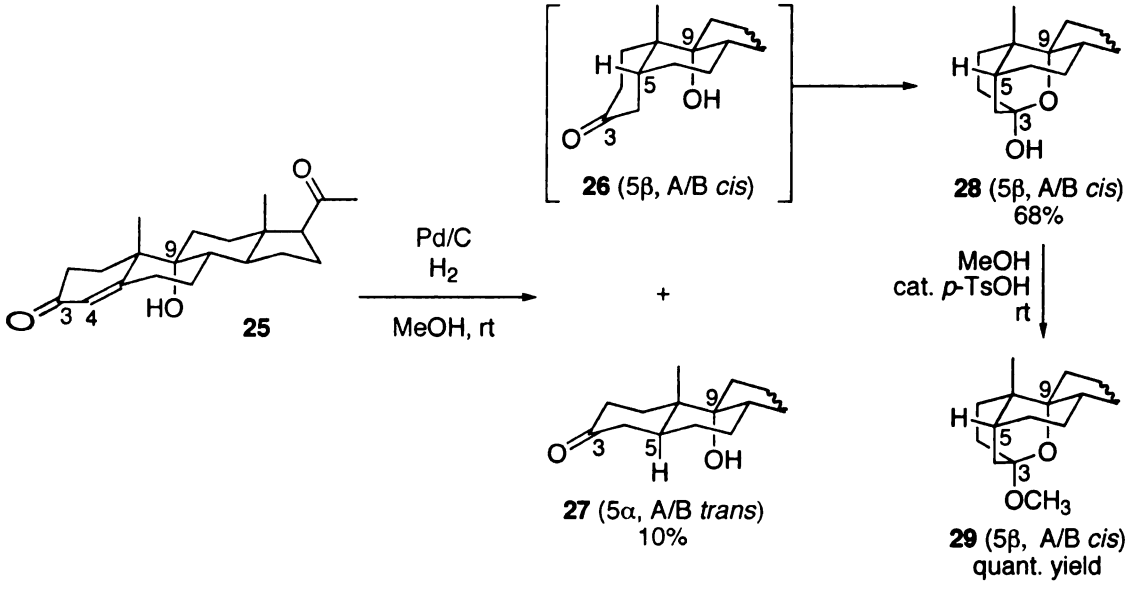
2.3.3. Kinetic resolution of 2/1 (5β/5α) mixtures

In order to induce selective reactivity between 5β (A/B *cis*) ester **2** and 5α (A/B *trans*) ester **1**, we probed their intermolecular reactivity. If the hydroxyl group at C7 could be mono-protected or two hydroxyl groups at C7 and C12 could be tethered with a proper linker, the sterically more accessible 7α-hydroxyl group of 5α (A/B *trans*) ester **1** might react faster than that of 5β (A/B *cis*) ester **2**. Such a kinetic resolution was tried with benzoyl mono-protection of the hydroxyl group at C7 and with boronic ester tethering of the two hydroxyl groups at C7 and C12[41] (Scheme 12).



Scheme 12. Kinetic resolution of 5β/5α mixture

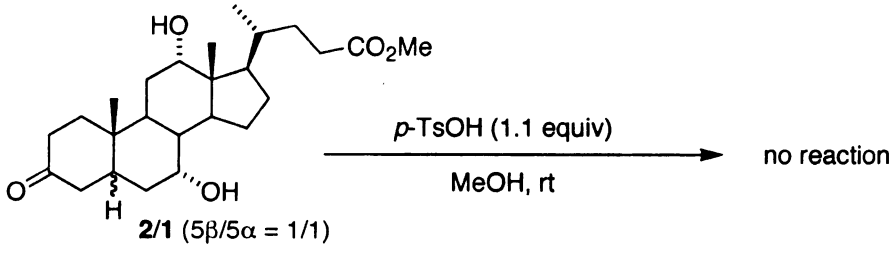
Unfortunately, both of these reactions provided us complicated mixtures. No separation of the 5β/5α mixtures via either mono-protection, bis-protection, or oligomerization, etc were achieved.



Scheme 13. Dodson's separation of 5β/5α mixtures

Then, we turned our attention to selective intramolecular interactions. From the literature, we found excellent separation of 5β/5α mixtures reported by Dodson group

(Scheme 13).[42] Under hydrogenation conditions, 4-en-3-one **25** was reduced to mixtures of their 5β/5α isomers. From the mixture, 5β (A/B *cis*) compound **26** was selectively transformed into hemiacetal **28** as a result of the close interaction between the 3-keto functionality and the 9α-hydroxyl group, while the reduced 5α (A/B *trans*) compound **27** remained unchanged. After reaction was done, several subsequent crystallizations of the residue provided the isolation of **28** (5β) and **27** (5α) easily and in a practical way. Following the lead provided by this example, we tried to selectively form the acetal of a **2/1** (5β/5α) mixture, realizing though that the reacting hydroxyl group of **2** (5β, A/B *cis*) was positioned at C7, not C9 (Scheme 14) and would this make the desired reaction more formidable.



Scheme 14. Resolution of 5β/5α mixtures via intramolecular acetal formation

Under acidic conditions in methanol, the intramolecular acetal formation was attempted as a means to resolve the 5β/5α mixture. Unfortunately, the reaction did not occur and all of the 5β/5α material was recovered. As suggested, this result might be caused by the distance between the 3-keto functionality and the 7α-hydroxyl group of 5β (A/B *cis*) compound **2**. Comparison of Newman projections of the two 5β (A/B *cis*) substrates, **26** and **2** (Figure 13) shows that the 9α-hydroxyl group of **26** is close enough to form hemiacetal **28** or acetal **29** with 3-keto functionality, but the 7α-hydroxyl group of **2** is sufficiently far from the 3-keto functionality to impede acetal formation.

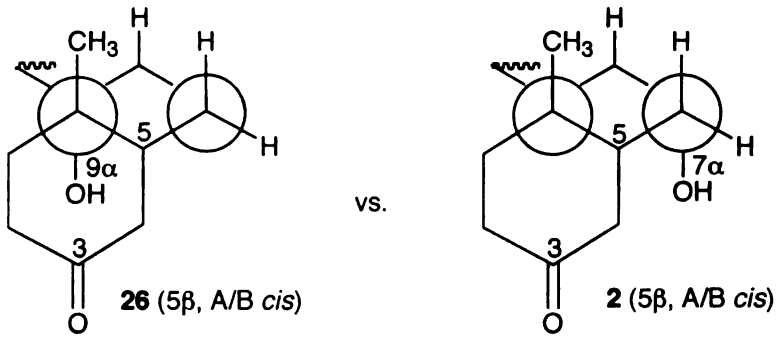
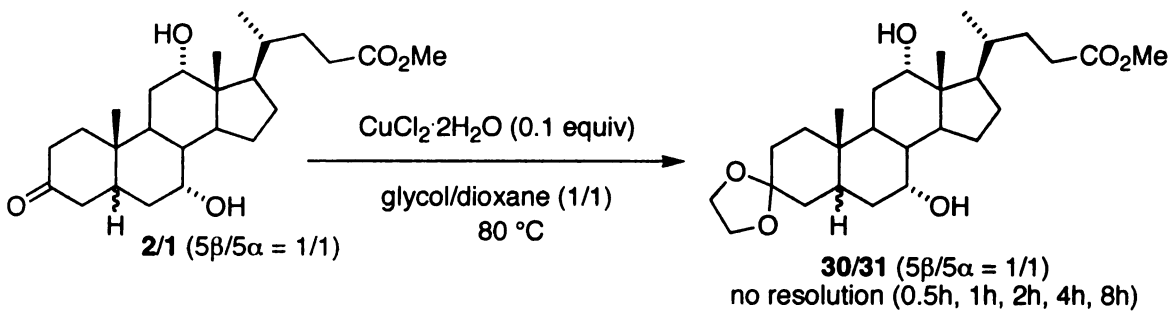


Figure 13. Newman projections of two 5β (A/B *cis*) compounds, **26** and **2**

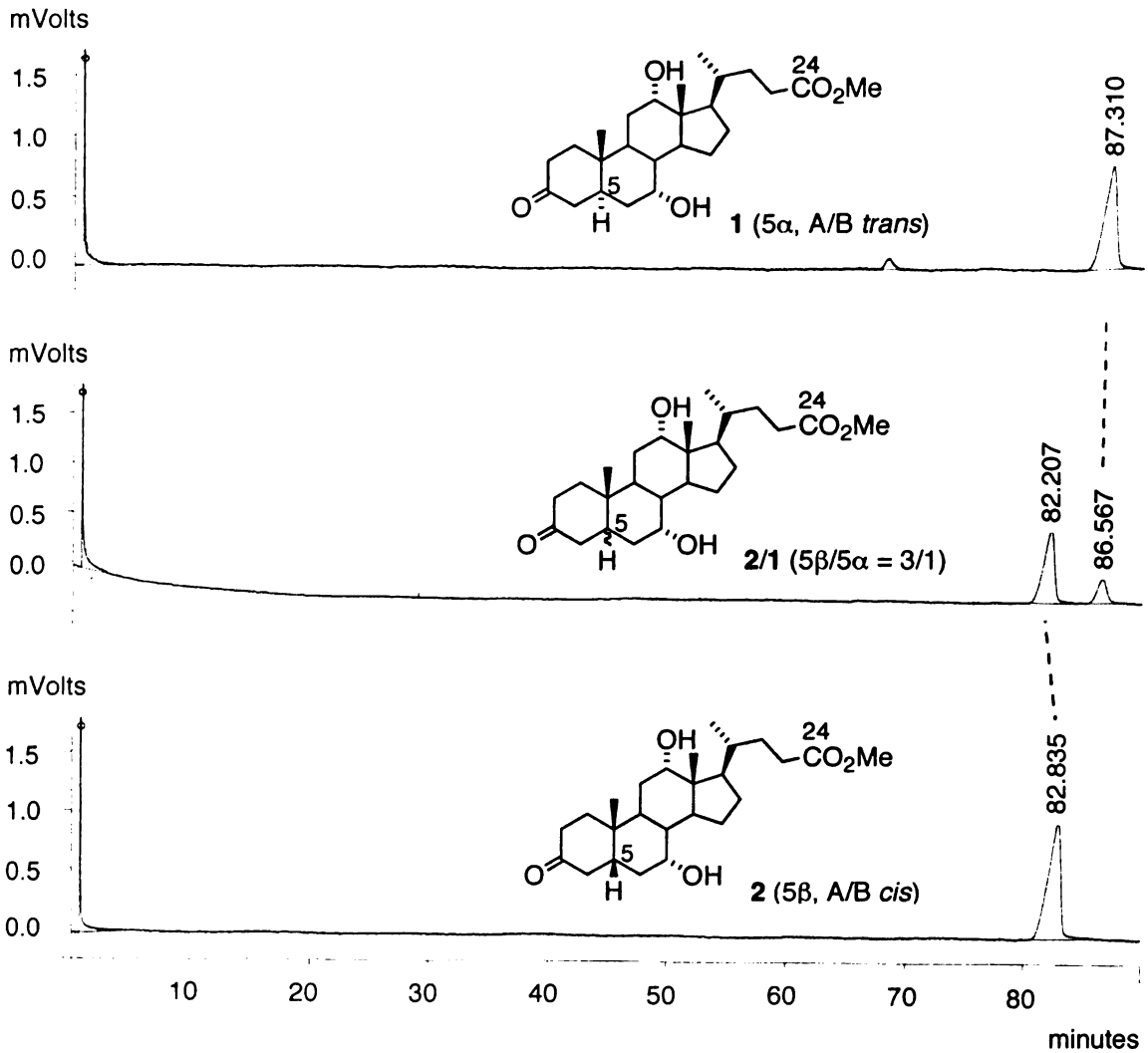
Additionally, we attempted to utilize the 3-keto functionality for the resolution of the **2/1** (5β/5α) mixtures. With acetal formation at the 3-ketone (Scheme 15), we expected faster reaction from the 5α (A/B *trans*) compound **1** over the 5β (A/B *trans*) compound **2** because the 3-keto functionality of the 5α (A/B *trans*) compound **1** is less sterically hindered. In monitoring the reaction by NMR, several aliquots were taken out of the mixture. Each aliquot was treated with $NaBH_4$ to remove remaining ketone. After separation of acetals and reduced alcohols, ratios of the acetal were checked by ^{1}H-NMR. As a result, forming acetals of all aliquots demonstrated the same ratios of 1 to 1 (5β/5α). With these unsatisfactory resolution results, we looked into other separation conditions.



Scheme 15. Kinetic resolution of **2/1** (5β/5α) mixtures via selective acetal formation

2.3.4. Separation of 2/1 (5β/5α) mixtures by gas chromatography

Figure 14 is gas chromatography (GC) traces of mixtures of **2** (5β) and **1** (5α). The molecular weight of these esters is 420.58, so it needed the harsh conditions of GC as indicated in the legend of Figure 14. While we finally observed the resolution of the **2/1** (5β/5α) ester mixtures, prep GC would not be practical during large-scale synthesis.



GC conditions: Factor FourTM: Capillary Column VF-1ms, 15m 0.25mm 0.25μm, Varian flow rate = 65.5 mL/min, oven temp 230 °C, injector temp 250 °C, FID temp 250 °C

Figure 14. Separation of **2/1** (5β/5α) mixtures by gas chromatography

2.3.5. Resolution attempt of 5β/5α mixtures via sublimation

While preparative GC would not suit our needs, discussions with Professor Adam Matzger of the University of Michigan suggested that sublimation under high vacuum might be viable. As such we tested a couple of derivatives of our 5β/5α mixtures (Figure 15) under such conditions. The Matzger group attempted sublimation with 5β/5α mixture of acetyl derivatives **32/33**, and we independently tried sublimation of both ester 5β/5α mixtures under the high vacuum of a silicon oil pump. However, none of these attempts provided us practical separations of the 5β/5α mixtures.

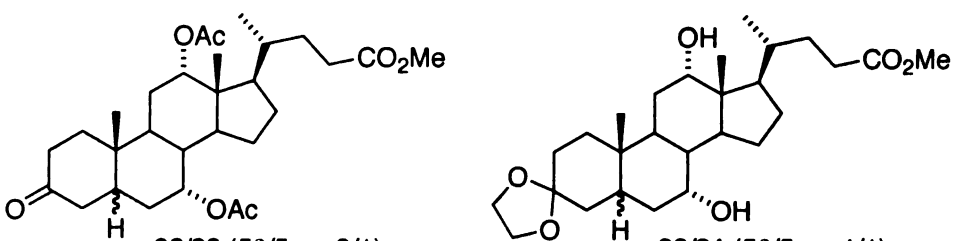
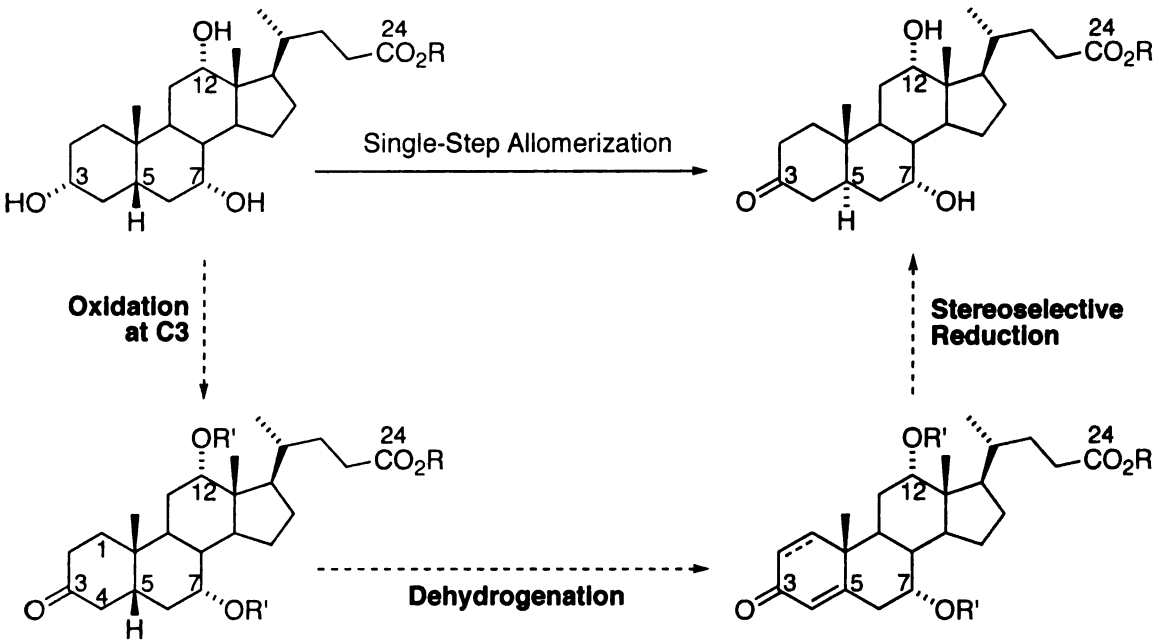


Figure 15. Sublimation attempt to separate 5β/5α mixtures

In the end, while we improved some conditions of a single step allomerization with Raney Ni to increase overall yield of potential materials and minimize dead-end by-products. Recently, from our extensive search for separation of 5β/5α mixtures, scrupulous and multiple column chromatography on silica gel (hexane:acetone=2/1) gave us clear separation of 5β/5α (1/1) mixtures.[43] However, there was still the issue of impractical separations of the 5β/5α mixtures. This unresolved issue of separation led us to a full investigation of stepwise allomerizations.

CHAPTER 3. STEPWISE ALLOMERIZATION

As discussed earlier in Chapter 1, stepwise allomerization was generally achieved via the following sequences: Oxidation at C3, dehydrogenation and stereoselective conjugate reduction (Scheme 16). We explored oxidation at C3 of cholic acid derivatives first.

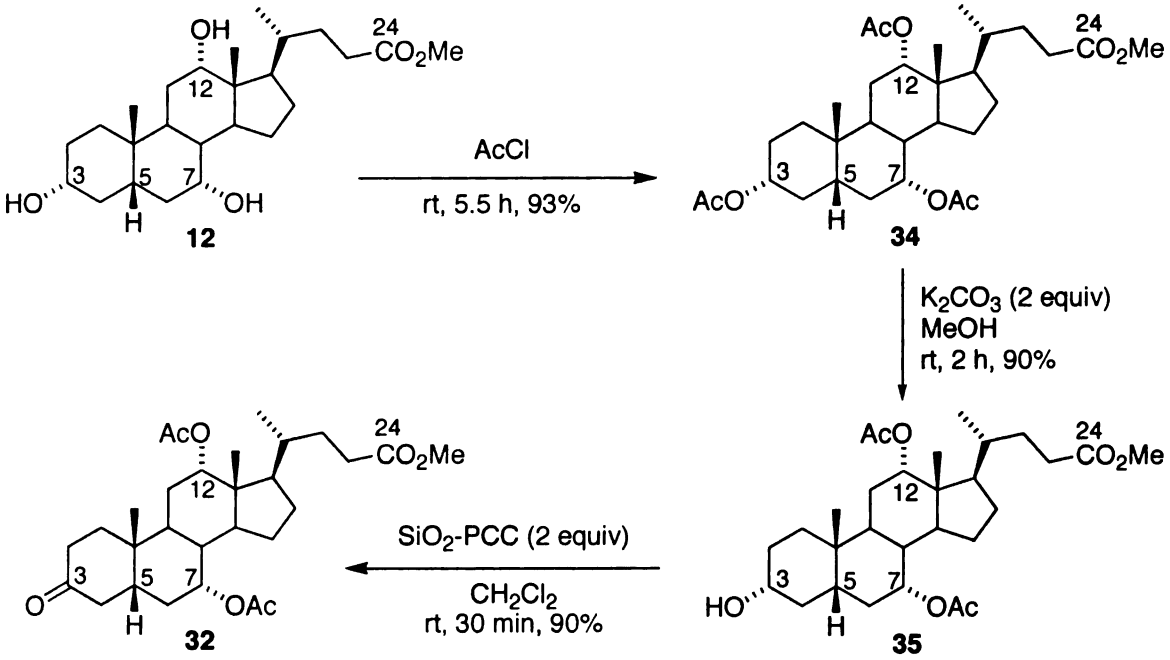


Scheme 16. Stepwise allomerization sequences of cholic acid derivatives: Oxidation at C3, dehydrogenation, and stereoselective reduction

3.1. Oxidation at C3 Position of Cholic Acid Derivatives

Since cholic acid has three hydroxyl groups at C3, C7, and C12, the challenge is to oxidize only the 3α-hydroxyl group with the other hydroxyl groups at C7 and C12 remaining intact. For this selective oxidation at C3, all three hydroxyl groups can be protected first, and then the hydroxyl group at C3 can be selectively deprotected,

followed by oxidation of the resulting 3α-hydroxyl group (see section 1.4).[29,30,34,35] Following this sequence, the 3α-hydroxyl group of methyl cholate **12** was selectively oxidized (Scheme 17).[44]

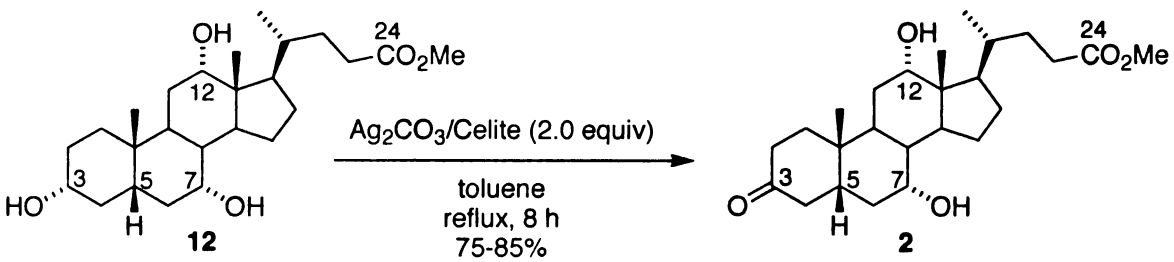


Scheme 17. Oxidation sequence of methyl cholate **12** at C3 position

Neat reaction of methyl cholate **12** in acetyl chloride proceeded at room temperature to provide tri-acetylated product **34** in 93% yield after recrystallization. Selective deacetylation at C3 of compound **34** was efficiently performed with treatment of potassium carbonate in methanol. After recrystallization, selectively deprotected **35** was obtained in good yield. Lastly, a 1:1 mixture of SiO_2 and PCC rapidly oxidized the free hydroxyl group of compound **35** to ketone **32** in high yield. We embraced this protection/deprotection sequence for the oxidation of the 3α-OH because product purification does not require chromatographic separation as recrystallization of the crude reaction mixture provides pure material in excellent yields. Furthermore, the toxicity of chromium reagent is manageable in its solid supported form. Indeed, oxidation of

compound **35** with this PCC reagent has a potential for scalability, given its ease of work-up.

This oxidation sequence could also be improved by application of Tserng's method.[45] In 1978, Tserng reported that treatment of methyl cholate with Fetizon's reagent[46] could selectively oxidize 3α-hydroxyl group without the need for protection of the other hydroxyl groups at C7 and C12. In order for oxidation to take place, both the hydroxyl group and the hydrogen on carbon should be attached to the surface of the $AgCO_3$ on celite.[45] Therefore, in this case, the structurally more accessible 3α-hydroxyl group and 3β-hydrogen are selectively absorbed on the surface of the oxidizing reagent to produce the 3-keto compound **2**.[45]



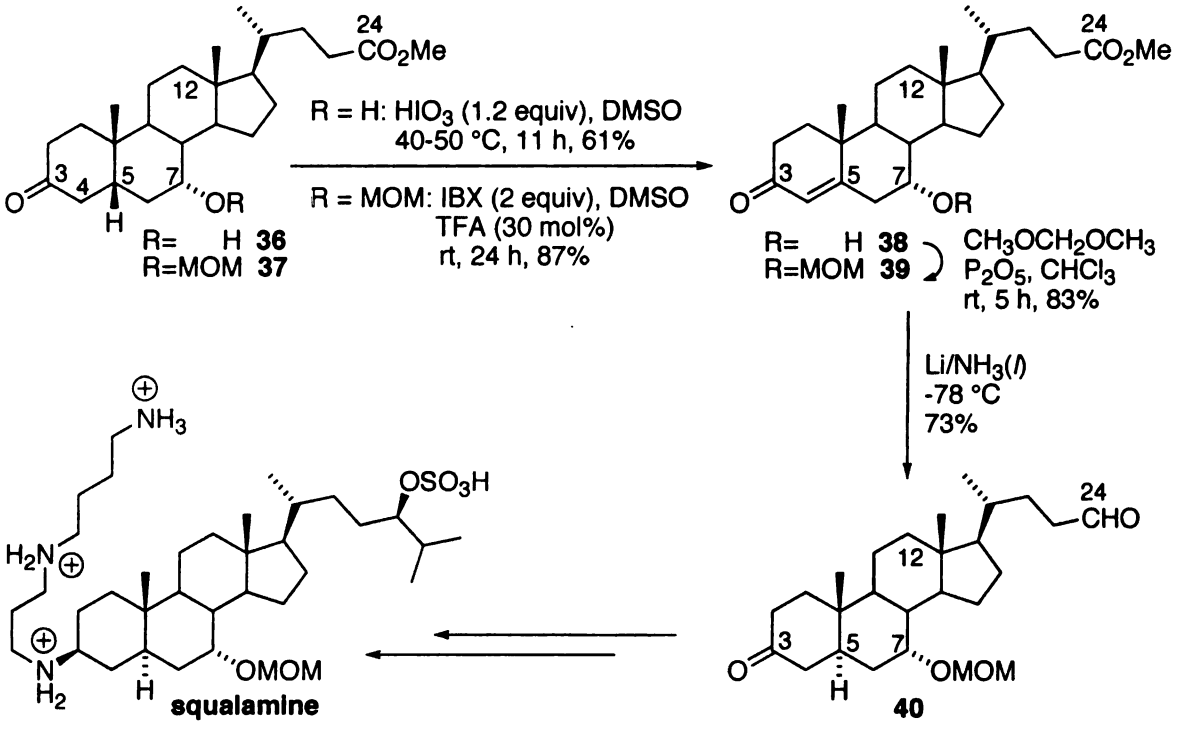
Scheme 18. Selective C3 oxidation of methyl cholate with Fetizon's reagent

We carried out the same oxidation protocol of oxidation with methyl cholate **12** (Scheme 18). Methyl cholate **12** was refluxed in toluene with freshly prepared Fetizon's reagent. In the course of the reaction, water was removed by a Dean-Stark apparatus. Upon completion of the reaction, as monitored by TLC, the oxidizing reagent was filtered off and the filtrate was concentrated to afford crystalline product **2** in good yield. This method proved very efficient and is likely applicable to large-scale synthesis. Moreover, once the oxidation is complete, we would have several choices of alcohol protecting

groups, should the C7 and C12 hydroxyls need protection during later transformation. With the 3-keto compounds in hand, we turned to the next step, dehydrogenation to the enone.

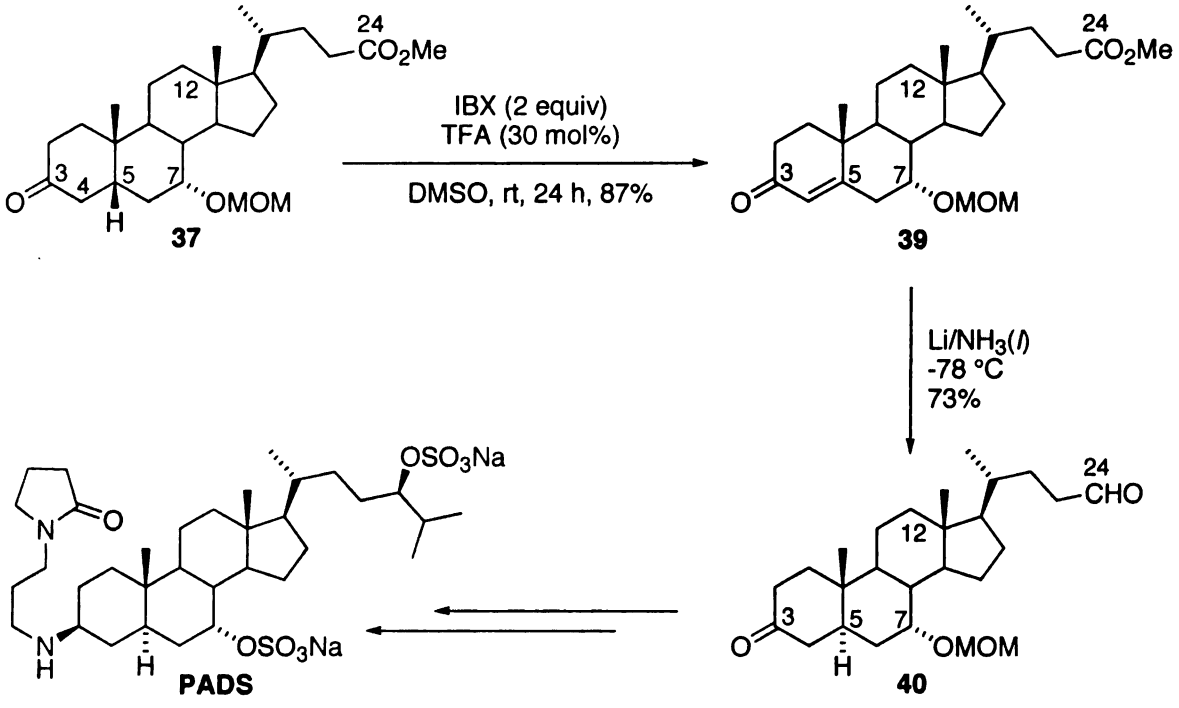
3.2. Dehydrogenation with I(V) Reagents

As shown in Chapter 1 (see section 1.4), the unsaturated 4-en-3-one compounds have been prepared by α-halogenation of the 3-keto compounds, followed by β-elimination[39] or selenium catalyzed direct oxidation/dehydrogenation[27,32,34] of 3α-hydroxyl compounds. However, those methods were not ideal for a large-scale synthesis due to various safety and cost issues of reagents.



Scheme 19. Zhou's dehydrogenation with I(V) chemistry toward the synthesis of squalamine

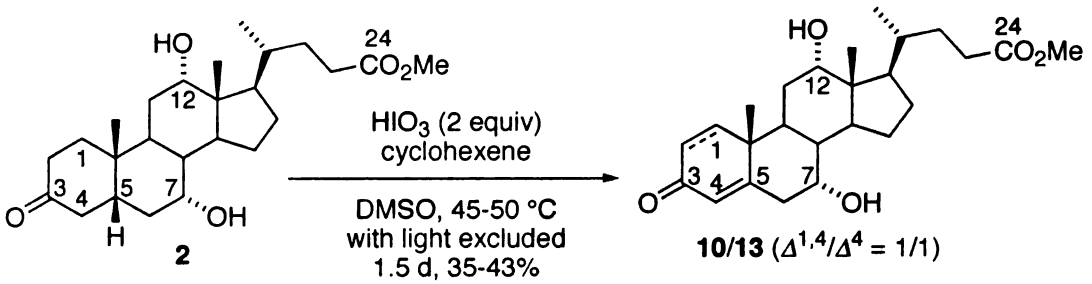
In 2003, the Zhou group reported iodine (V) chemistry dehydrogenations (Scheme 19).[47] Modifying Nicolaou's protocol of iodine chemistry,[48] they utilized iodic acid or acid catalyzed *o*-iodoxybenzoic acid (IBX) in DMSO to convert 5β (A/B *cis*) 3-keto compounds into 4-en-3-one compounds. Furthermore, MOM protected enone compound **39** was allomerized by the Birch reduction conditions to afford 5α (A/B *trans*) compound **40** along with concomitant ester reduction to the aldehyde at C24. Then, they took substrate **40** onto their synthesis of squalamine. Two years later, Hoye and co-workers applied the Zhou's IBX protocol to the synthesis of petromyzonamine disulfate, PADS (Scheme 20).[49]



Scheme 20. Hoye's dehydrogenation with IBX toward the synthesis of PADS

We decided to examine this iodic acid chemistry because it is nontoxic and an inexpensive reagent. Also, alcohols are known to be inert to this oxidant, thus no protection of hydroxyl groups is needed.[47,48] Lastly, substrate **36** is structurally similar

to our methyl 3-ketocholate **2**. Hence, we carried out the dehydrogenation of compound **2** with iodic acid, as illustrated in Scheme 21. A solution of substrate **2** with iodic acid and cyclohexene in DMSO was stirred in a sealed tube at 45-50 °C for 1.5 day with light excluded. After chromatographic separation, we obtained 1 to 1 mixture of 1,4-dien-3-one **10** and 4-en-3-one **13** in combined yield of 35-43%.

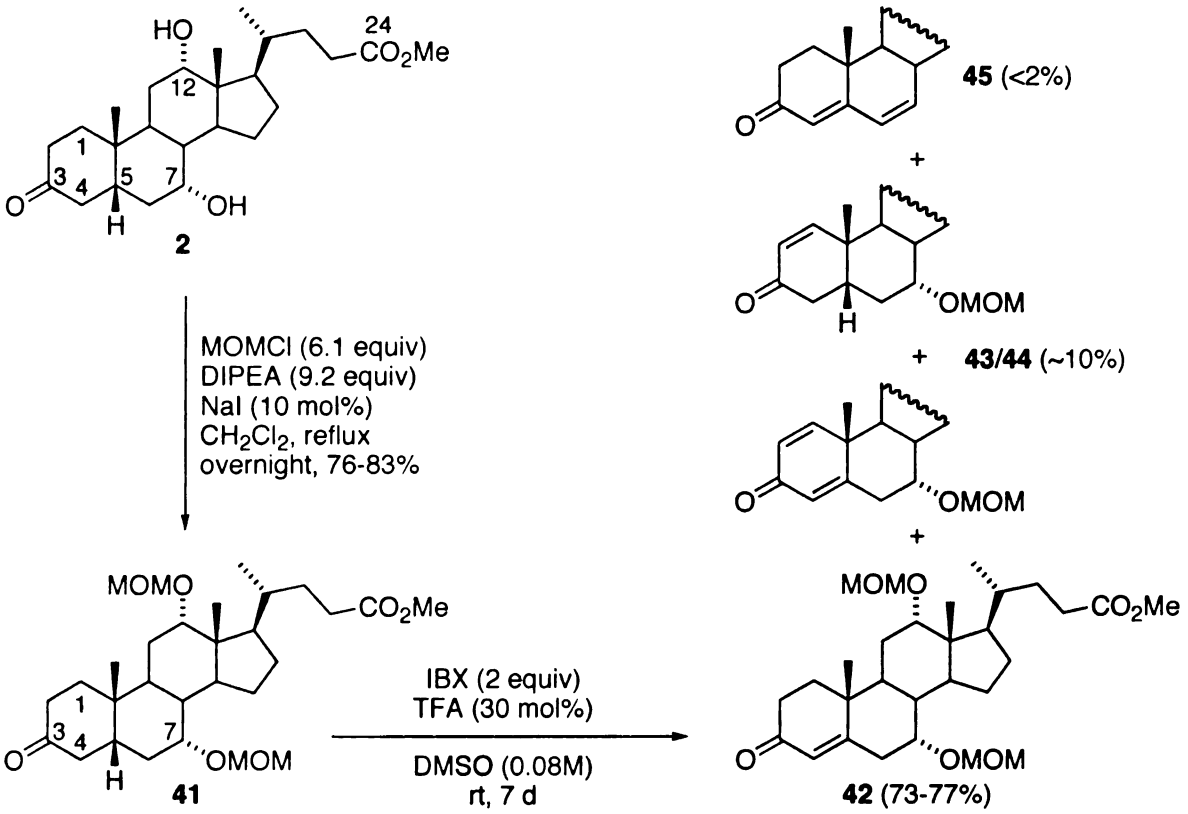


Scheme 21. Dehydrogenation of compound **2** into enones **10/13** with iodic acid

Although a one-pot dehydrogenation was achieved with iodic acid, the reaction proved to be a bit complicated. The reaction provided a complex mixture and the isolated products **10** and **13** were not high yielded. Furthermore, the mixture of **10** and **13** was not separable in our hands. Thus, we turned our attention to other iodine chemistry with IBX.

Scheme 22 shows the dehydrogenation of compound **2** to afford 4-en-3-one compound **42**. Since IBX can oxidize alcohols, the two hydroxyl groups at C7 and C12 need to be protected. Thus, a solution of compound **2** with MOMCl, DIPEA, and NaI in CH_2Cl_2 was stirred at reflux overnight and subsequent chromatographic separation provided us MOM-protected ketone precursor **41** in good yield. Having the compound **41** in hand, we investigated the dehydrogenation with freshly prepared IBX. Under the trifluoroacetic acid (TFA) catalyzed conditions, 5β (A/B *cis*) ketone **41** in DMSO

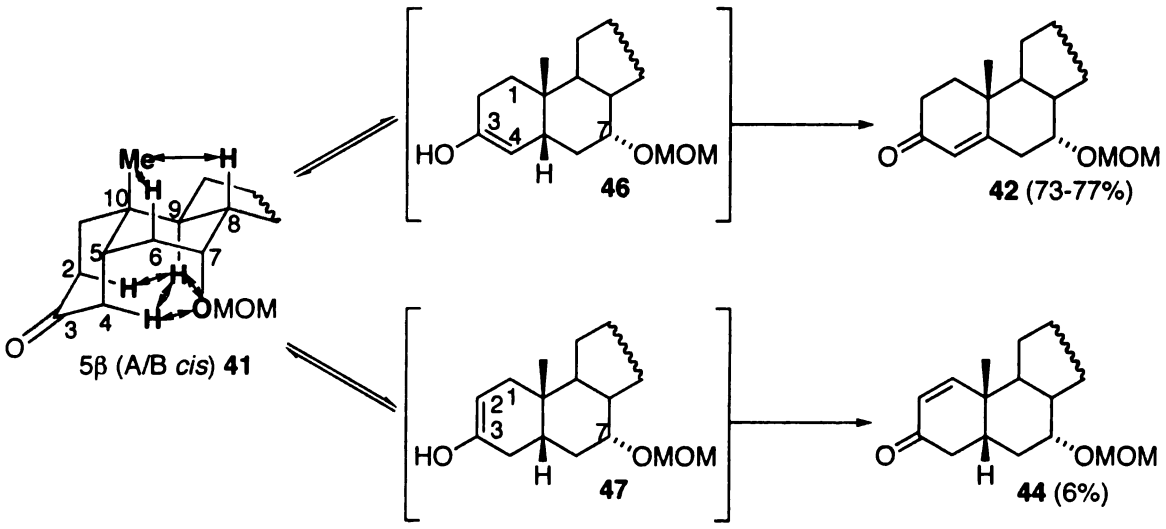
(0.08M) was smoothly transformed at room temperature into dehydrogenated 4-en-3-one compound **42** in 73-77% yield by the action of IBX.



Scheme 22. Dehydrogenation of compound **41** into enone **42** with IBX

Some key features of IBX chemistry are worth noting. IBX was synthesized from oxidation of *o*-iodobenzoic acid by treatment with oxone, following the literature method.[50] IBX prepared by this method is reported to be non-explosive and nontoxic. Although reaction times are long (1 week), the conditions are mild and not sensitive to moisture, so the DMSO solvent does not have to be dry. Another feature is that the formation of double bond is regioselective giving 4-en-3-one **42** as a major product. That said, we did isolate minor amounts of by-products that included mixtures of 1,4-dien-3-one **43** and 1-en-3-one **44** (~10%), and γ,δ-eliminated 4,6-dien-3-one **45** (<2%). This regioselectivity may be derived from the enolization step of compound **41** with IBX. As

illustrated in Scheme 23, 5β (A/B *cis*) compound **41** has six major 1,3 diaxial interactions around A/B ring junction, including interactions of 7α-O↔4α-H, 4α-H↔9α-H, 9α-H↔2α-H, 9α-H↔7α-O, 10β-Me↔6β-H, and 10β-Me↔8β-H. Whereas enolization at C4 (or unsaturation between C3 and C4) removes two 1,3-diaxial interactions relating with 4α-H, enolization at C2 (or unsaturation between C2 and C3) eliminates only one interaction associated with 2α-H. Also, β-hydride elimination of C4-enolized intermediate **46** creates more substituted double bond (**42**) than would be present in C2-enolized intermediate **47**, which generates the less substituted double bond in **44**. Among the isolated enone compounds, compounds **43** and **44** are recyclable with respect to allomerization, but γ,δ-eliminated product **45** is a dead-end compound. When the reaction is run at 0.08M concentration, we observe a precipitation of *o*-iodosobenzoic acid (IBA), which is a by-product formed after oxidation with IBX when the reaction is done. This makes for easy reaction monitoring. Thus an easy filtration, followed by chromatographic separation delivered 4-en-3-one compound **42** as a major product.



Scheme 23. Regioselective enolization of 5β (A/B *cis*) **41** with IBX for enone formation

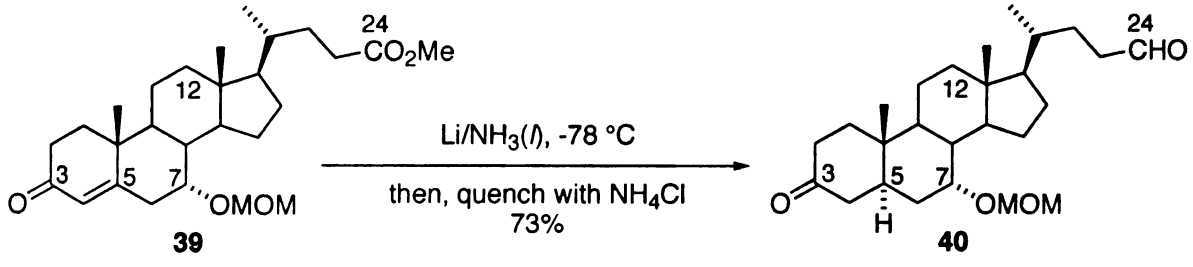
With this isolated pure 4-en-3-one compound **42** in hand, we set out to explore the next step, the stereoseletive reduction of 4-en-3-one to 5α (A/B *trans*) 3-keto product.

3.3. Stereoselective Reduction

3.3.1. Birch reduction

We commenced our stereoselective reduction studies with a Birch reduction of our enones, as this should guarantee full stereoselection to the 5α (A/B *trans*) compounds (see section 1.4).[27,29,34,35]

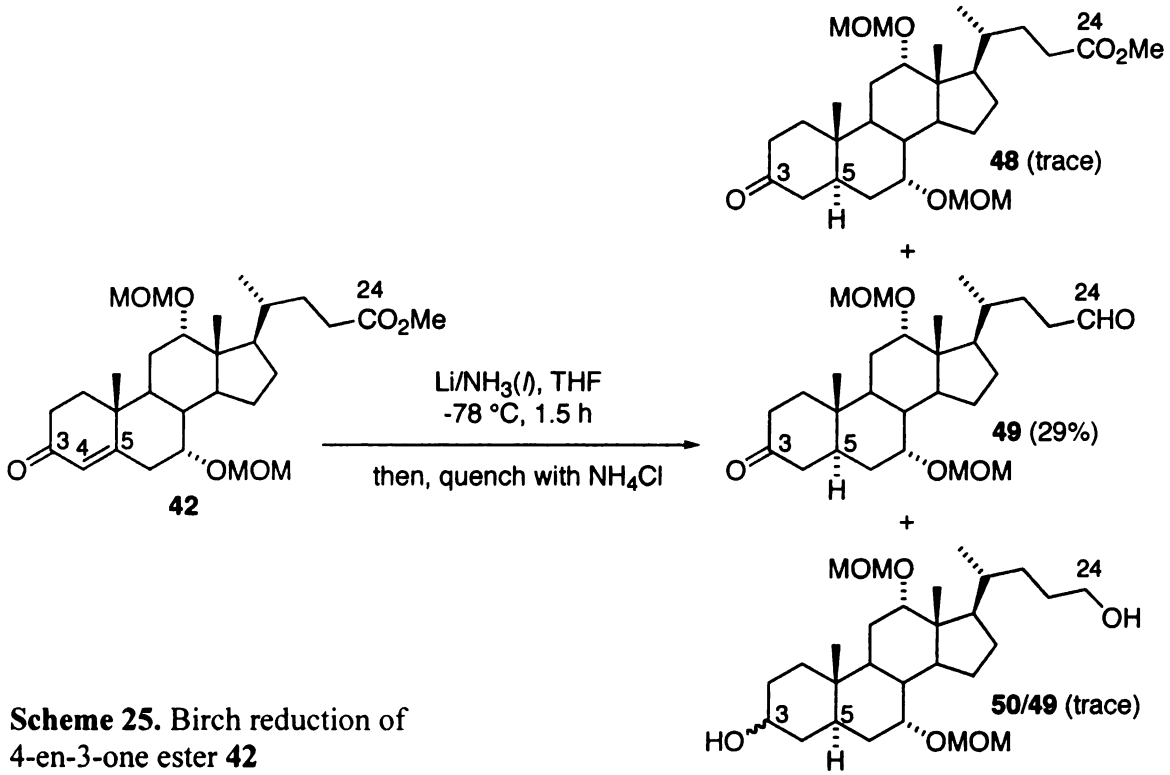
Generally, Birch reductions are carried out on related enone substrates bearing a carboxylic acid at C24. While carboxylic acids stay intact under Birch conditions, esters tend to be reduced to aldehydes or alcohols by the action of the dissolving metal. Such a reaction is often referred to as Bouveault-Blanc type reductions.[51] Utilizing this fact, the Zhou group carried out the Birch reduction on 4-en-3-one substrate **39** having an ester functional group at C24 to afford allomerized 5α (A/B *trans*) compound **40** that bears the reduced aldehyde at C24 in high yield (Scheme 24).[47]



Scheme 24. Zhou's stereoselective conjugate reduction with concomitant reduction of ester to 5α (A/B *trans*) 3-keto aldehyde

Conjugate reduction of the enone with concomitant reduction of the ester is more attractive than conjugate reduction alone, because the resulting product 5α (A/B *trans*) 3-keto aldehyde is on the path to 3kPZS and its analogs. Prior to the last sulfation to 3kPZS, precursor 3kPZ has the hydroxyl functional group at C24. Thus, in order to prepare 3kPZ, the carboxylic acid or aldehyde at C24 should be reduced to alcohol functionality. While reduction of carboxylic acid requires ketone protection at C3, aldehyde at C24 can be reduced selectively over the ketone at C3. Additionally, substrate similarity between 4-en-3-one **39** and 4-en-3-one **42** led us to explore the Birch reduction of enone ester **42**.

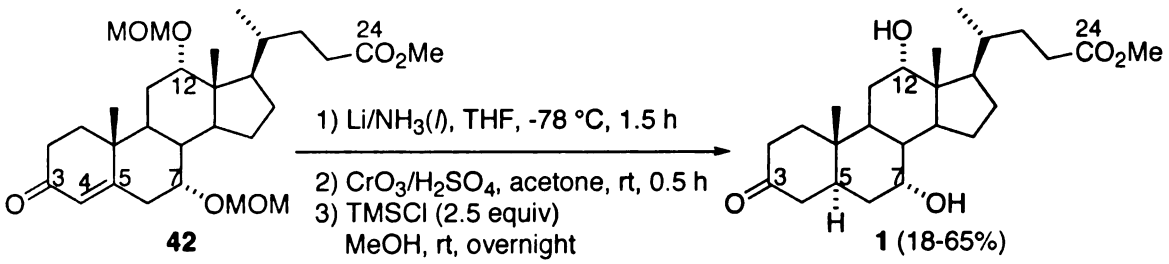
As illustrated in Scheme 25, 4-en-3-one ester **42** was reduced under the dissolving metal conditions. The Birch reduction delivered trace amounts of 3-keto ester **48**, 29% yield of the desired 3-keto aldehyde **49**, and also small amount of a mixture of **49** and overreduced 3-hydroxyl alcohol **50**. As anticipated, the stereochemistry of the A/B ring junction of all products is 5α (A/B *trans*).



Scheme 25. Birch reduction of 4-en-3-one ester **42**

Conjugate reduction along with Bouveault-Blanc type reduction gave us mixtures of compounds bearing ketone/alcohol functionality at C3 and ester/aldehyde/alcohol functionality at C24, as a single A/B *trans* isomer at C5. We then hypothesized that treatment of the crude reaction with Jones' reagent would convert the alcohol at C3 to the ketone and the aldehyde/alcohol at C24 to the carboxlic acid, accompanied with deprotection of the MOM groups under the strong acidic conditions. This would clean up the aforementioned mixtures from the reduction step. Methylation of the resulting 3-keto carboxylic acid would afford 5α (A/B *trans*) 3-keto ester **1**. With this hypothesis in mind, we modified the Birch reduction sequence (Scheme 26). 1) After quenching the Birch reduction of compound **42** with NH_4Cl, the crude reaction in CH_2Cl_2 was washed with water and brine. 2) The concentrated material was dissolved in acetone and stirred at room temperature for 30 minutes with Jones' reagent, CrO_3/H_2SO_4, after which the

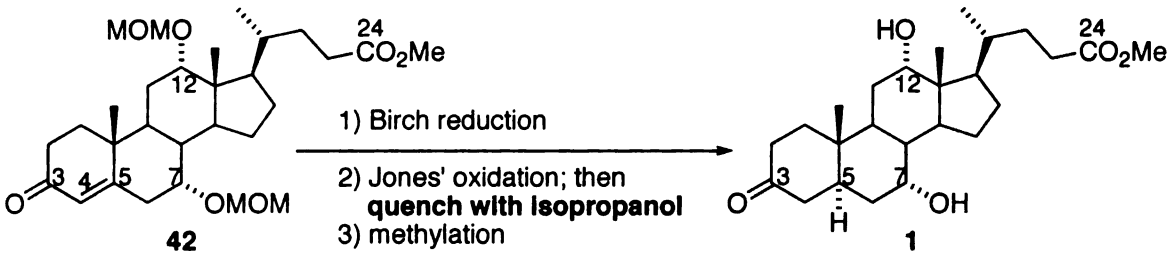
reaction was quenched with excess isopropanol to generate turquoise colored chromium precipitates. The precipitates were filtered and the filtrate was concentrated and redissolved in MeOH. 3) After the TMSCl promoted methylation, final work-up and chromatographic separation provided us single compound of 5α (A/B *trans*) 3-keto ester **1**. Before the last methylation at C24, purification was not necessary but the yield of **1** varied from 18 to 65%.



Scheme 26. Modified sequence of Birch reduction to convert **42** to 5α (A/B *trans*) **1**

During this sequence, we followed the reaction sequence step by step with ^{1}H-NMR and made a few interesting observations. Under the strong acidic conditions of the Jones' oxidation, the MOM groups were still intact but methylation with TMSCl deprotects two MOM groups. Moreover, it turns out that the yield of product **1** critically depends on the quenching time of the Jones' oxidation. After quenching the Jones' oxidation with isopropanol, we saw formation of chromium precipitates, and these precipitates got aggregated to make beads as time went on. Filtration of the precipitates right after the quench requires additional washings with organic solvents and was slower than filtration of beads. However, the yield of product **1** diminished, as the quenching time of the Jones' oxidation got longer (Scheme 27). Perhaps that is because some of product was trapped inside the beads of chromium. Finally, we established that the fast quench of chromium reaction was the best conditions for the Birch modified sequence.

With this protocol, we obtained good yields over three steps without needing any purification of intermediates between steps.



2) quenching time	1 yield (%)
<5 min	**60-65**
3 h	49
9 h	35
18 h	18

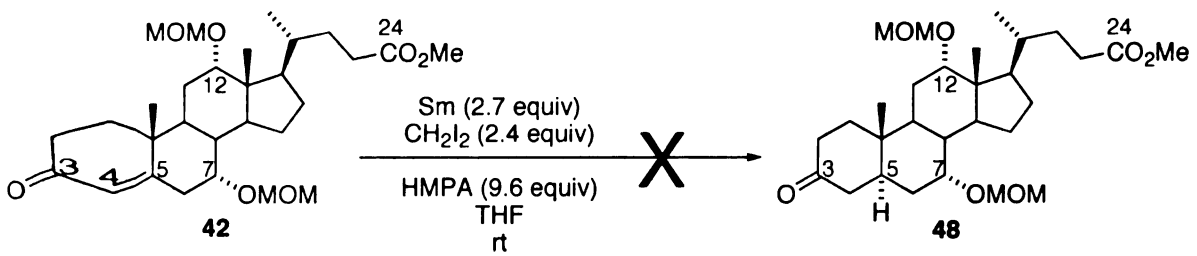
Scheme 27. Yield of product **1** depending on the quenching time of Jones' oxidation

Although the Birch reduction afforded full stereoselection to 5α (A/B trans), the practicality of these reductions is compromised by the need for cryogenic and scrupulously dry conditions. Additionally, handling of liquid ammonia would be an issue for large-scale synthesis. Thus, we gave our attention to other reduction methods.

3.3.2. SET Reduction with SmI_2

The Birch reduction is a single-electron-transfer (SET) process. We observed conjugate reduction along with over-reduction of 4-en-3-one ester **42** at C3 and C24. Normally steroidal esters are known to be tolerant to SmI_2 reductions, which are also single electron transfer reactions.[52] Even though reduction with SmI_2 requires completely dry conditions, the reactions can be run at room temperature. Thus, we applied reduction with SmI_2 to our 4-en-3-one ester **42**, as depicted in Scheme 28. SmI_2 was generated *in-situ* by addition of CH_2I_2 to samarium metal suspended in THF.[53] The

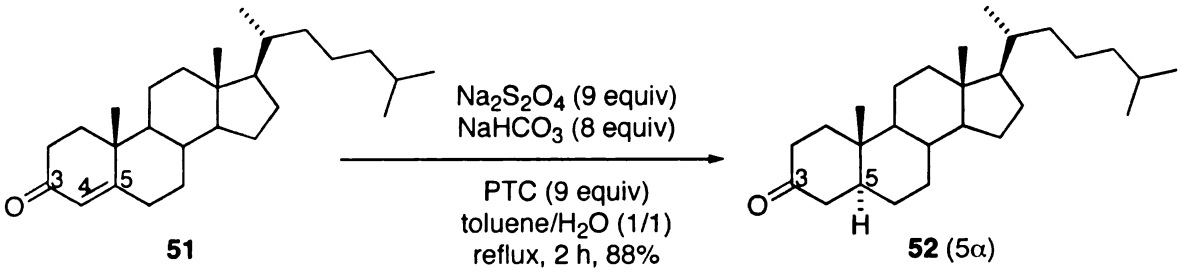
deep blue color of CH_2I_2 solution changed into a deep violet upon the addition of HMPA. Unfortunately, addition of substrate **42** immediately quenched the generated SmI_2 solution turning it into a yellow suspension. As a result, the reaction with substrate **42** did not take place and starting material was recovered quantitatively.



Scheme 28. Conjugate reduction of 4-en-3-one ester **42** by *in-situ* generated SmI_2

3.3.3. SET Reduction with sodium dithionite ($Na_2S_2O_4$)

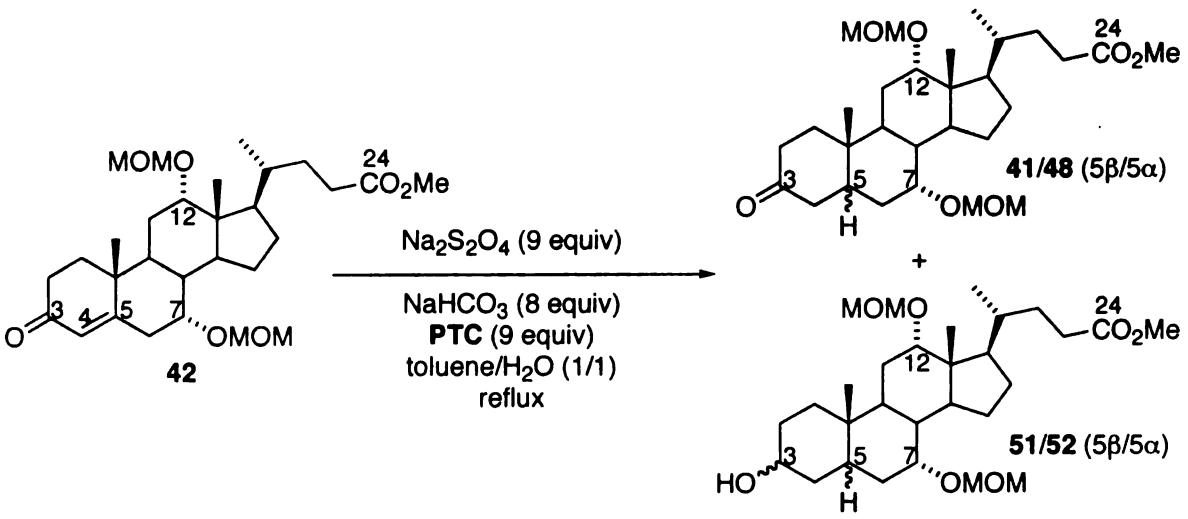
We turned our attention to other SET reductant, namely sodium dithionite ($Na_2S_2O_4$). This reagent is nontoxic, inexpensive, and industrially attractive. Literature examples showed that $Na_2S_2O_4$ is a very efficient reagent for reducing steroidal 4-en-3-one compounds to their corresponding 5α (A/B *trans*) stereoisomers (Scheme 29).[54] Furthemore, $Na_2S_2O_4$ tolerates ketones at C3 and steroidal esters.[54] $Na_2S_2O_4$ is an inorganic reductant, and thus the reaction is typically run in a biphasic solution in the presence of a phase transfer catalyst (PTC). Normal reaction conditions may need high temperature but this is preferred over the cryogenic conditions at large scale. More importantly, the reaction is not sensitive to moisture or air, so open-to-air conditions would be acceptable.



Scheme 29. Stereoselective conjugate reduction of 4-en-3-one **51** with $Na_2S_2O_4$

When the reaction was attempted on 4-en-3-one **42** (Scheme 30), the enone was dissolved in toluene and mixed with a solution of sodium bicarbonate and $Na_2S_2O_4$ in water. PTC was added to the solution and the reaction mixture was allowed to reflux prior to work-up. We tested two different PTCs, *n*-$C_{15}H_{31}NMe_3Br$ and Aliquat 336®. The first PTC in entry 1 provided us 17% recovery of starting material **42** and 71% combined yield of inseparable mixtures of **41/48** (5β/5α) but selectivity was low (5β/5α=1/1.5). The second PTC, Aliquat 336® (entry 2) gave interesting results. The initial conjugate reduction products **41/48** (5β/5α) were formed in only 9% yield with similar selectivity (5β/5α=1/1.8) but we also isolated over-reduced products **53/54** in 84% yield. Indeed, both **53/54** mixtures contained four isomers. Hydroxyl groups of both **53** and **54** isomers are epimeric at C3. Although there were possible complications in determining ratios of 5β/5α, ^{1}H-NMR spectrum showed clear separation of two sets of 7β-H and 12β-H signals, revealing a selectivity of 1 to 5.5 favoring the 5α (A/B *trans*) stereoisomer. Since we noticed that Jones' oxidation could oxidize both epimers of the C3 alcohols to a single ketone and without loss of the MOM groups, we reoxidized the **53/54** mixtures to ketones **41/48** under the Jones' oxidation conditions. The overall yield

of the reoxidized **41/48** was still good at 81% and the 5β/5α selectivity showed the same as 1 to 5.5 ratios favoring the 5α (A/B *trans*) stereoisomer.



Entry	PTC	Yield **41/48** (5β/5α)	**51/52** (5β/5α)
1	*n*-$C_{15}H_{31}NMe_3Br$ (0.5 h)	71% (1/1.5)[a]	
2	Aliquat 336® (2 h)	9% (1/1.8) 81% (1/5.5)[b]	84% (1/5.5)

a) 17% of starting material **42** was recovered.
b) reoxidized from **51/52** mixture by Jones [O]

Scheme 30. Stereoselective conjugate reduction of 4-en-3-one **42** with $Na_2S_2O_4$

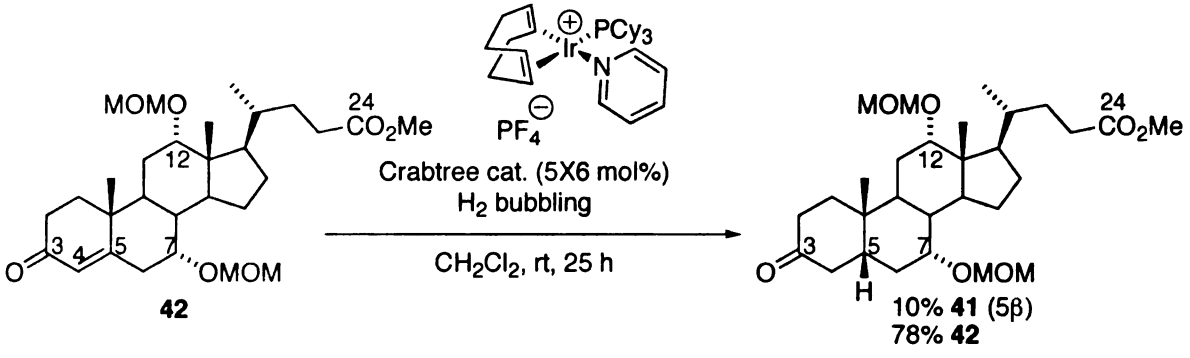
This result was quite interesting. The selectivity of initially isolated **41/48** (5β/5α=1/1.8) mixtures was different from the selectivity of reoxidized **41/48** (5β/5α=1/5.5) mixtures. After the first conjugate reduction of enone to ketone mixtures, the second reduction may induce rate difference in conversion of **41/48** (5β/5α) mixtures to alcohols, favoring 5α compound. Thus we asked if another way to separate 5β (A/B *cis*) and 5α (A/B *trans*) mixtures would be to use kinetic resolution by selective reduction of the 5β/5α 3-keto mixtures with $Na_2S_2O_4$.

Although the reductions with $Na_2S_2O_4$ were promising, the reactions were not without their shortcomings. The reactions were not completely stereoselective for the desired 5α (A/B *trans*) stereochemistry. Additionally, the **41/48** (5β/5α) mixtures after conjugate reduction were not separable even with chromatographic separation.

Having explored single electron transfer processes for the stereoselective reduction of enone compounds to 5α (A/B *trans*) isomers, our attention next turned to other reduction methods.

3.3.4. Directed hydrogenation with Crabtree's catalyst

Selective hydrogenation could be achieved with metal hydride delivery directed by metal complexation with proper functional groups on the substrate. Crabtree's catalyst has been utilized for selective hydrogenation directed by functional groups.[55] Ketones, especially, are among the functional groups that can direct hydrogenation with Crabtree's catalyst.



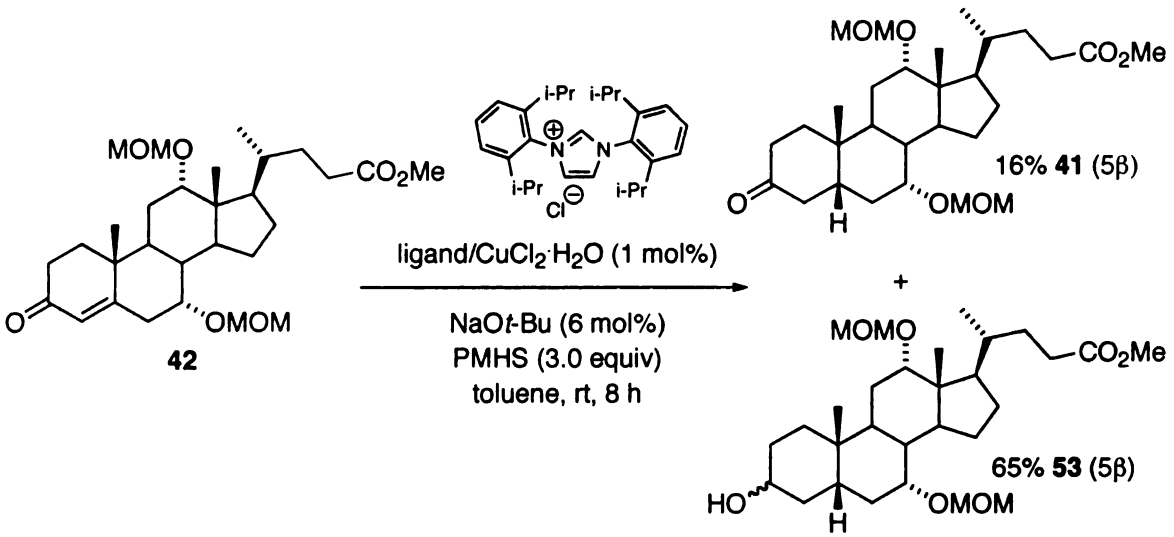
Scheme 31. Directed hydrogenation of 4-en-3-one **42** with Crabtree's catalyst

Having a ketone functional group in enone **42**, we looked to hydrogenate this compound with Crabtree's catalyst (Scheme 31). H_2 gas was bubbled into a solution of enone **42** in CH_2Cl_2 that was stirred at room temperature under the Crabtree catalyst

conditions. The hydrogenation was slow and the additional Crabtree's catalyst needs to be added portion-wise over the course of the reaction. Most of the starting material **42** was recovered (78%) and only 10% conversion to reduced ketone product **41** was observed with complete stereoselectivity favoring 5β (A/B *cis*) isomer, and not the desired 5α (A/B *trans*) isomer.

3.3.5. Stereoselective reduction with *in-situ* Cu-H

Several Cu mediated conjugate reductions with selective hydride delivery have been reported. Recently, Buchwald and co-workers demonstrated a *in-situ* generated Cu-H catalyst for stereoselective conjugate reductions.[56] Following this literature report, we carried out the conjugate reduction of enone **42** catalyzed by *in-situ* Cu-H as illustrated in Scheme 32. A solution of enone **42** in toluene was added dropwise via cannula into the *in-situ* generated Cu-H solution resulting from a mixture of carbene ligand and $CuCl_2$ that was treated with base and polymethylhydrosiloxane (PMHS) in toluene. The reaction mixture was stirred at room temperature for 8 hours prior to work-up to deliver 3-keto compound **41** in 16% yield and 65% yield of the over-reduced alcohol **53**. However, both products were determined to have exclusively the 5β (A/B *cis*) stereochemistry.

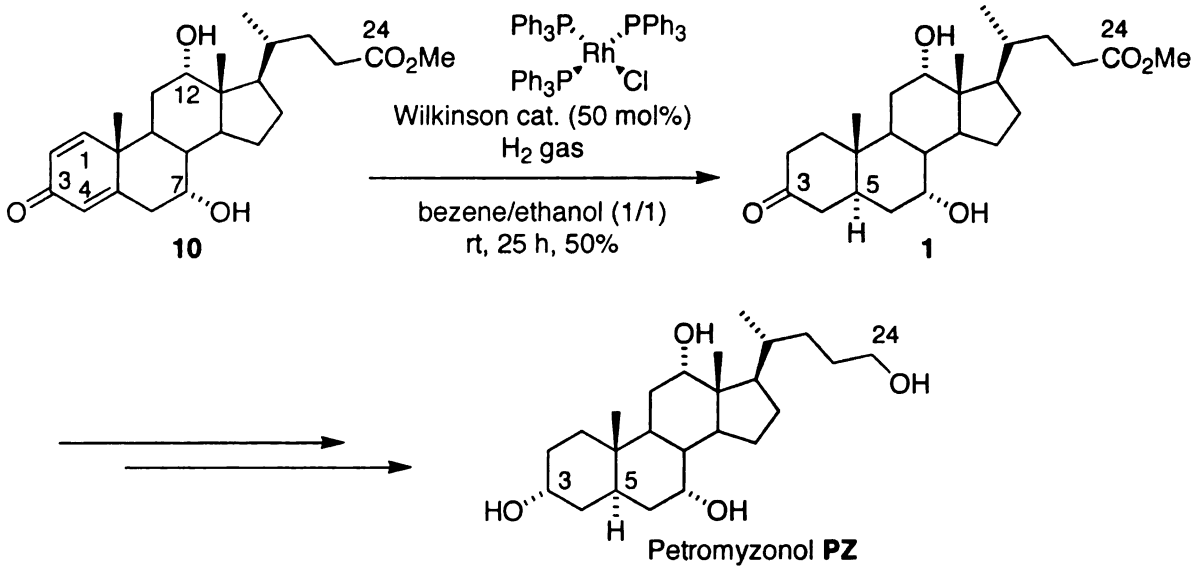


Scheme 32. Stereoselective conjugate reduction of enone **42** with *in-situ* Cu-H

As metal-hydride reduction of enone substrate **42** with MOM protecting groups only afforded the 5β (A/B *cis*) stereochemistry, we decided to focus on the free 7α-hydroxyl group as a directing group.

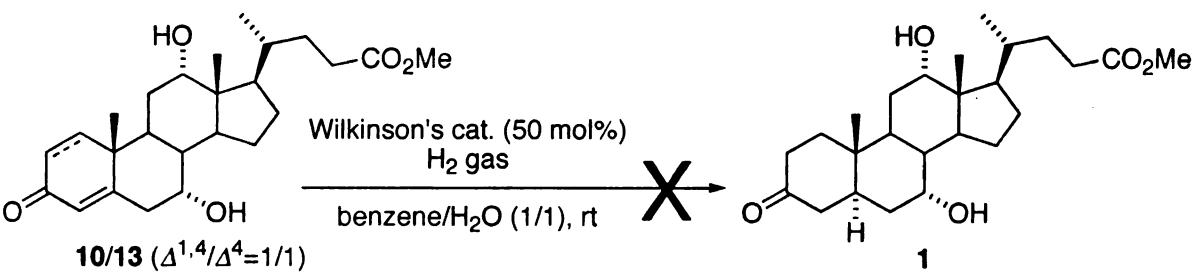
3.3.6. Directed hydrogenation with Wilkinson's catalyst

As discussed in Chapter 1 (see section 1.4), the Mclean group previously carried out reductions with Wilkinson's catalyst to set up the 5α (A/B *trans*) stereochemistry of compound **1** (Scheme 33).[32] Here the selectivity might come from hydride delivery directed by catalyst complexation with the free 7α-hydroxyl group.



Scheme 33. McLean's stereoselective reduction of dienone **10** with Wilkinson's catalyst

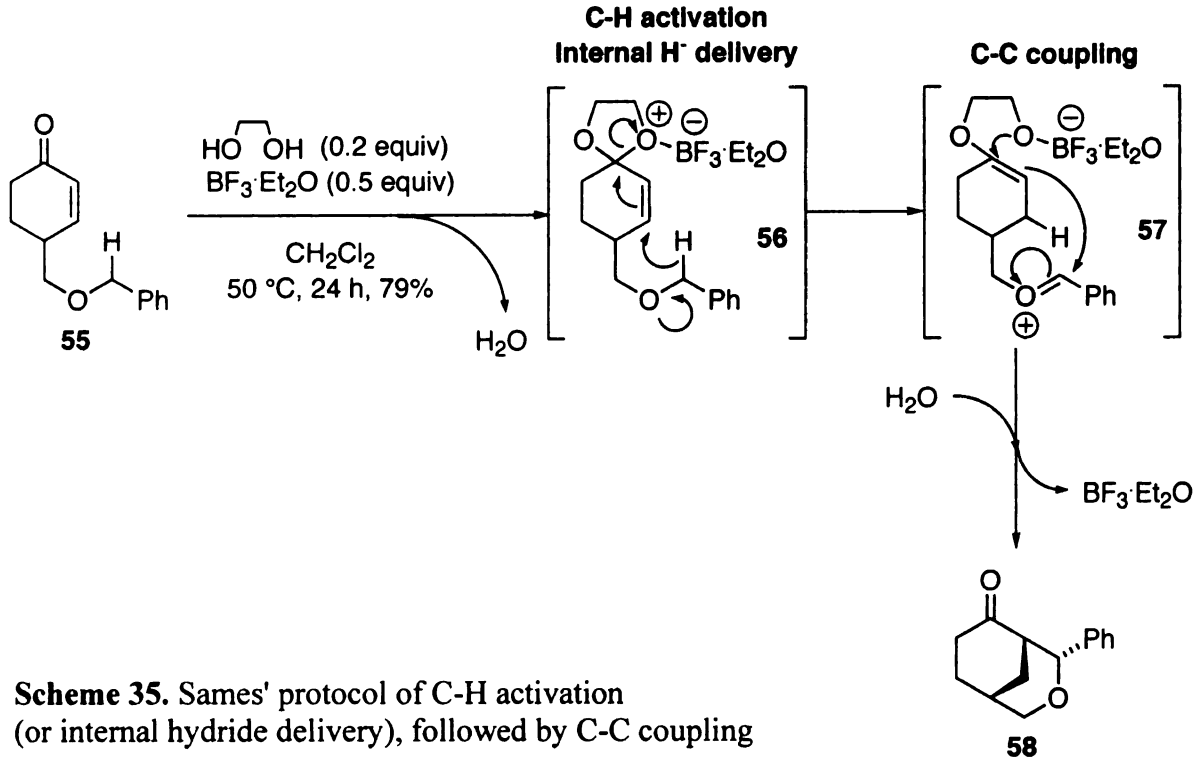
Since we prepared 1 to 1 mixtures of 1,4-dien-3-one **10** and 4-en-3-one **13** (see section 3.2) and both components of the mixture contain a free 7α-hydroxyl group, selective hydrogenation with Wilkinson's catalyst might be possible through hydroxyl direction. A solution of enone mixtures **10** and **13** with Wilkinson's catalyst in benzene/ethanol was charged with H_2 gas and allowed to stir at room temperature as depicted in Scheme 34. Unfortunately in our hands, the reaction did not take place even with three different batches of catalyst. This was not entirely surprising as the authors of the original paper acknowledged that the reaction proved inconsistent and was catalyst-batch dependent.



Scheme 34. Directed hydrogenation of enones **10/13** with Wilkinson's catalyst

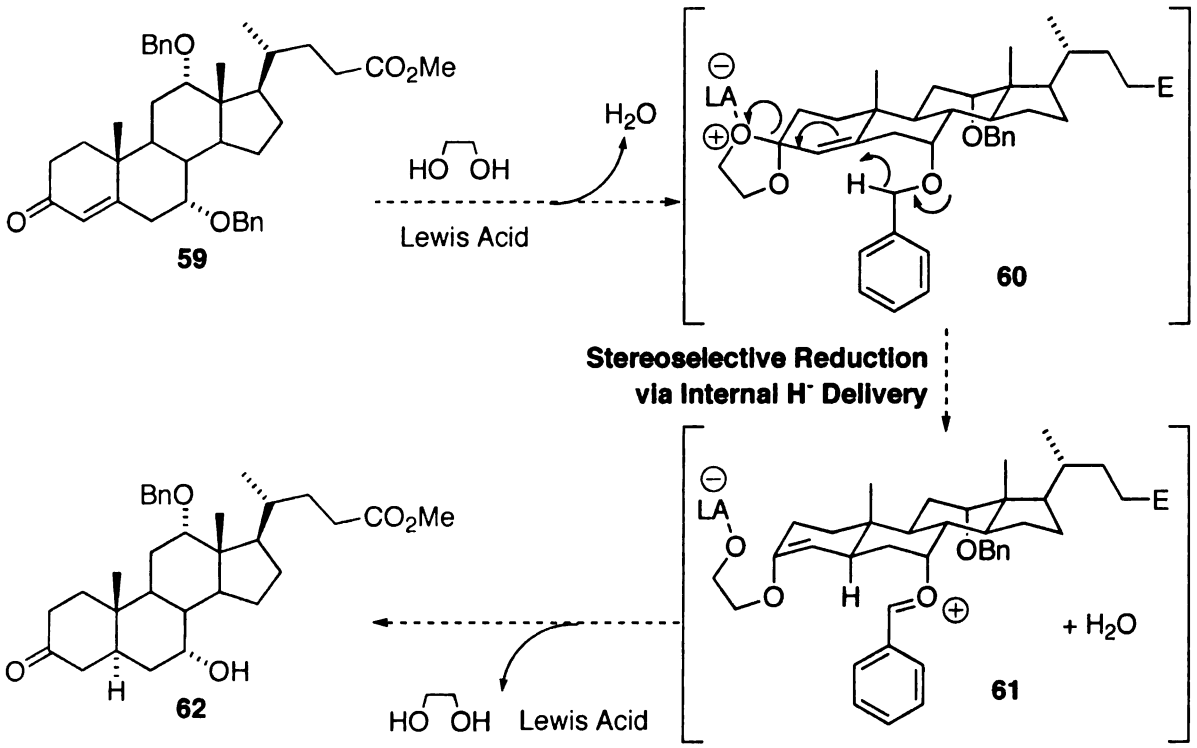
3.3.7. Selective reduction via internal hydride delivery

With unsuccessful results of hydride delivery via metal complexation with a directing functional group, we turned our attention to the internal hydride delivery.



Scheme 35. Sames' protocol of C-H activation (or internal hydride delivery), followed by C-C coupling

The Sames group has focused on C-H activation or internal hydride delivery, followed by C-C coupling as shown in Scheme 35.[57] Benzyloxy enone **55** undergoes *in-situ* acetal formation, and then it is activated by Lewis acid to become intermediate **56**. The lone pair electrons on oxygen serve to deliver a hydride to an activated enone acetal. The resulting enol intermediate **57** undergoes C-C coupling with an aldehyde electrophile to relieve the resultant oxonium species. Then, hydrolysis of the acetal selectively affords product **58** in high yield. We have substrate similarity here. Our enone substrate **42** bears 7α-hydroxyl group, which is two carbon units away from enone.

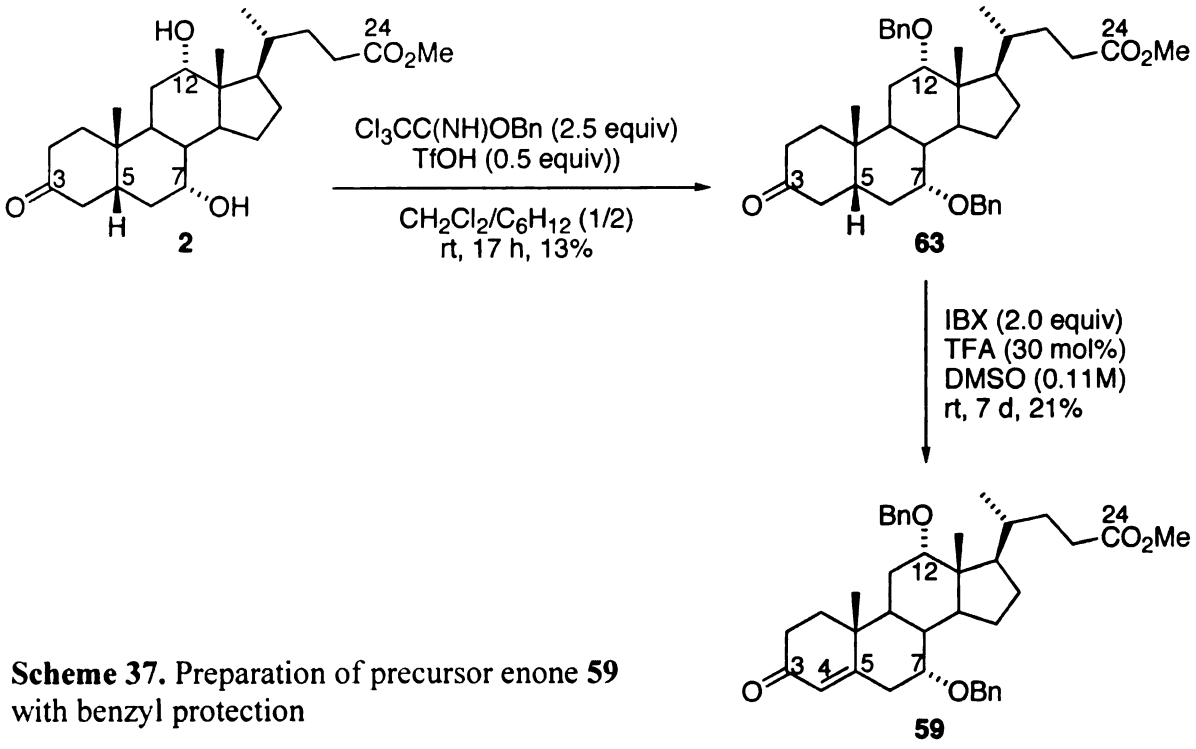


Scheme 36. Stereoselective reduction of enone **59** via internal hydride delivery

If the same chemistry could be applied to our substrate **59**, with benzyl protection at 7α-hydroxyl group as shown in Scheme 36, acetal formation with activation by a Lewis acid may lead to the similar complex intermediate **60**. Then, the benzyl oxygen may deliver the hydride to reduce the activated enone with stereoselectivity for the 5α (A/B *trans*) isomer to afford intermediate **61**. Our hypothesis was that once the hydride is delivered, intermediate **61** would have a rigid A/B *trans* fused ring and thus cannot flip. Consequently, instead of C-C coupling, *in-situ* generated water would hydrolyze either the benzyl oxonium or the acetal to afford mono-deprotected 3-keto compound **62** with 5α (A/B *trans*) stereochemistry.

With this concept in mind, we prepared the substrate **59** (Scheme 37). Acid-catalyzed benzyl protection was performed at the C7 and C12 hydroxyl groups.[58] Dehydrogenation was carried out with IBX chemistry under the acid-catalyzed conditions

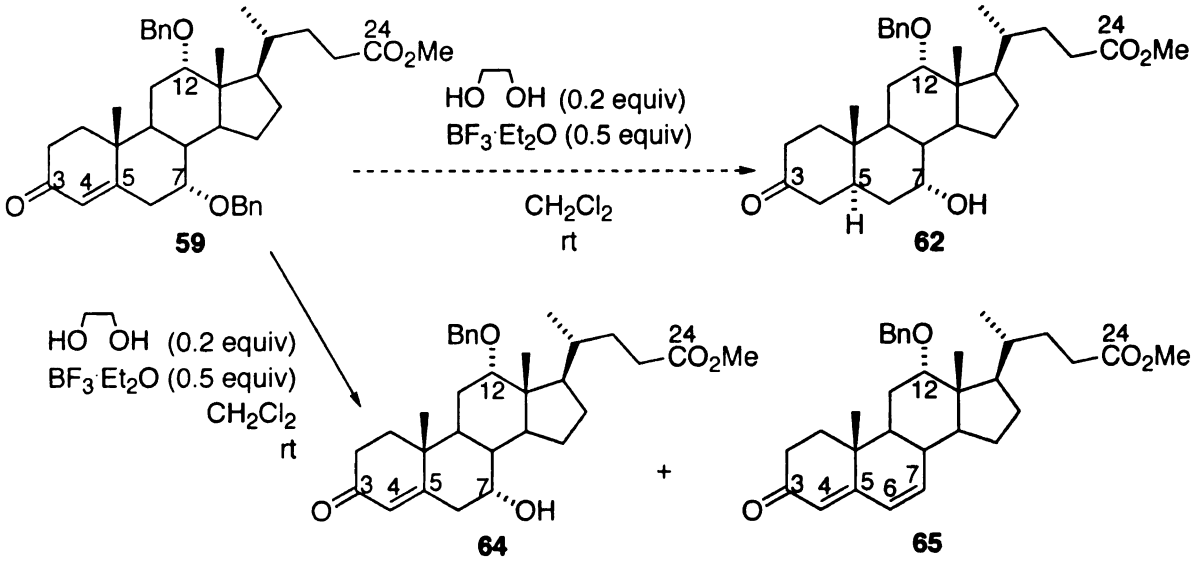
to afford corresponding precursor enone **59**.[47] Both sets of reaction conditions would still need to be optimized for future applications.



Scheme 37. Preparation of precursor enone **59** with benzyl protection

Having this substrate enone **59** in hand, initial tests of the Sames' protocol were attempted (Scheme 38). A solution of benzyl protected enone **59**, ethylene glycol, and Lewis acid in CH_2Cl_2 was allowed to stir at room temperature. The reaction was monitored by TLC, but we did not see formation of any of the desired product **62** with the 5α (A/B *trans*) chemistry. Instead, we observed mono-deprotected compound **64** and trace γ,δ-eliminated 4,6-dien-3-one compound **65** by TLC. Intriguingly enough, aldehyde formation was suggested by a leak at δ = 10.01 ppm in the ^{1}H-NMR spectrum of the crude material. Presumably this is the result of reduction of ester at C24 or hydrolysis of the oxonium intermediate. The possibility of internal hydride delivery in

this system should not be ruled out. Additional experimentations are required to investigate this reaction further.



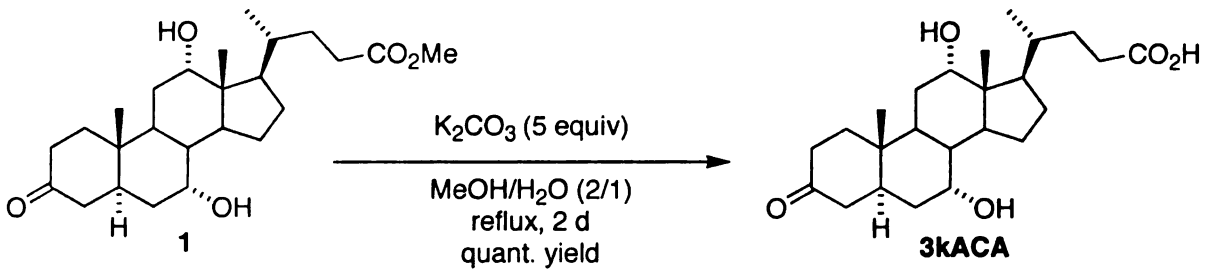
Scheme 38. Stereoselective reduction of enone **59** via internal hydride delivery

To sum up all the allomerization steps, we tried single step allomerization and stepwise allomerizations. Up until now, the desired methyl 3-keto-allocholate **1** was available from either single step allomerization with Raney Ni or Birch reduction sequence of stepwise allomerization. Having crucial compound **1** in hand, we carried on to the final synthesis of male sea lamprey pheromones.

CHAPTER 4. SYNTHESIS OF MALE SEA LAMPREY PHEROMONES AND FUTURE STUDIES

4.1. Synthesis of ACA

With the crucial compound **1** in hand, 3-keto allocholic acid (3kACA), one of the male sea lamprey pheromones, could be easily prepared (Scheme 39), by saponification of the ester to carboxylic acid. A solution of compound **1** with K_2CO_3 in $MeOH/H_2O$ was stirred at reflux for 2 days to afford a quantitative yield of 3kACA. The spectroscopic data of synthetic 3kACA were matched with those of the natural product.

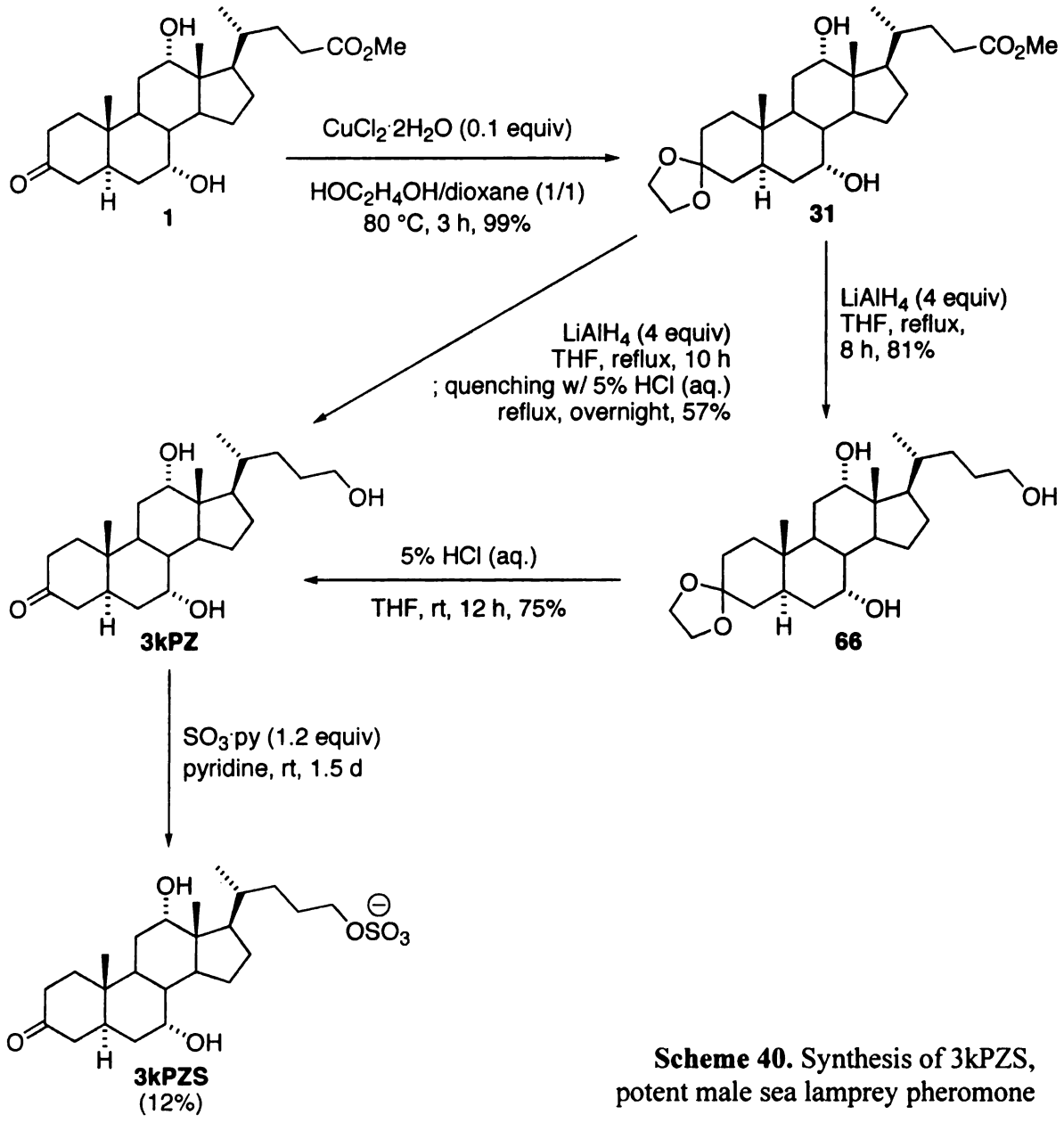


Scheme 39. Synthesis of 3kACA via saponification of 5α (A/B *trans*) compound **1**

4.2. Synthesis of 3kPZS

From the relatively straightforward transformation of compound **1**, the synthesis of 3-keto petromyzonol sulfate (3kPZS), which is the most potent component in male sea lamprey pheromones, could be completed (Scheme 40). The key transformation of compound **1** to 3kPZS would be reduction of ester to alcohol, followed by final sulfation. In order to achieve this sequence successfully, the ketone functionality of compound **1** at C3 needed to be protected as an acetal, and deprotected after reduction of ester. Although several acetal forming conditions were examined, Lewis acid catalyzed acetal

formation turned out to be the most practical method. Compound **1** with $CuCl_2$ in a 1 to 1 mixture of ethylene glycol and dioxane was allowed to stir at reflux for 2 hours. This afforded clean acetal formation.[59] Contrary to typical methods for acetal formation, ethylene glycol and dioxane do not have to be dry and *in-situ* generated water did not hamper acetal formation. Moreover, normal work-up and concentration provided analytically pure acetal compound **31**, which could be carried on to the next step without any further purification. Once the ketone at C3 was protected, the ester at C24 was reduced with lithium aluminum hydride ($LiAlH_4$).[32] A solution of acetal protected ester **31** in THF was added dropwise to the suspension of $LiAlH_4$ in THF. The reaction mixture was stirred at reflux for 8 hours prior to work-up with Rochelle's salt solution to hydrolyze the aluminum salts. The resulting mixture was purified by column chromatography to afford the acetal protected alcohol **66** in 81% yield. Acetal alcohol **66** was then deprotected with HCl aqueous solution in THF at room temperature to deliver 75% yield of 3-keto petromyzonol (3kPZ) after chromatographic separation. Lastly, the primary alcohol at C24 of 3kPZ was sulfated following the literature method.[60] 3kPZ in dry pyridine was treated with SO_3·pyridine at room temperature. The reaction mixture was quenched with MeOH and concentrated, followed by column chromatography to provide us the main target of 3-keto petromyzonol sulfate (3kPZS) in about 12% yield. The spectroscopic data were identical to the natural material, but showed a minor amount of the presumed disulfated product, which was also observed in the natural material by the Li group (Michigan State University).

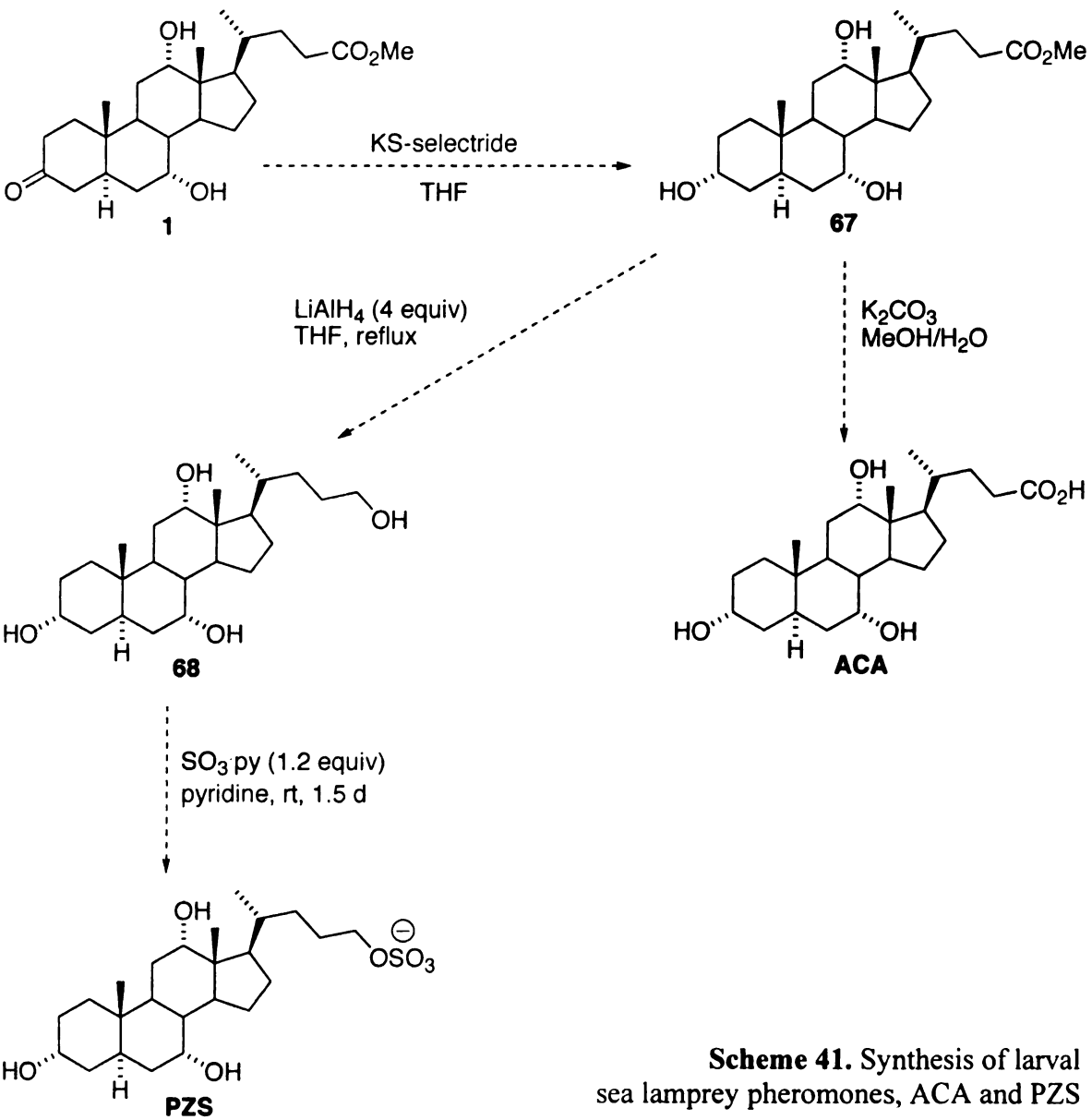


Scheme 40. Synthesis of 3kPZS, potent male sea lamprey pheromone

4.3. Synthesis of Larval Lamprey Pheromones, PZS and ACA

From our retrosynthetic viewpoint (see section 1.3), the 5α (A/B *trans*) compound **1** is the key intermediate for a plausible route to synthesize not only male sea lamprey pheromones (3kPZS and 3kACA), but also larval lamprey pheromones (PZS and ACA). Scheme 41 illustrates that the synthetic pathways to PZS and ACA from a common

intermediate. The compound **1** could be stereoselectively reduced at C3 with a bulky hydride, KS-selectride to provide the 3α-hydroxyl as a single diastereomer.[32] Hydrolysis of the ester of compound **67** would lead to allocholic acid (ACA). On the other hand, compound **67** could be transformed to the corresponding alcohol by $LiAlH_4$ reduction.[32] Then, selective sulfation of the resulting alcohol **68** would similarly afford petromyzonol sulfate (PZS).[60]



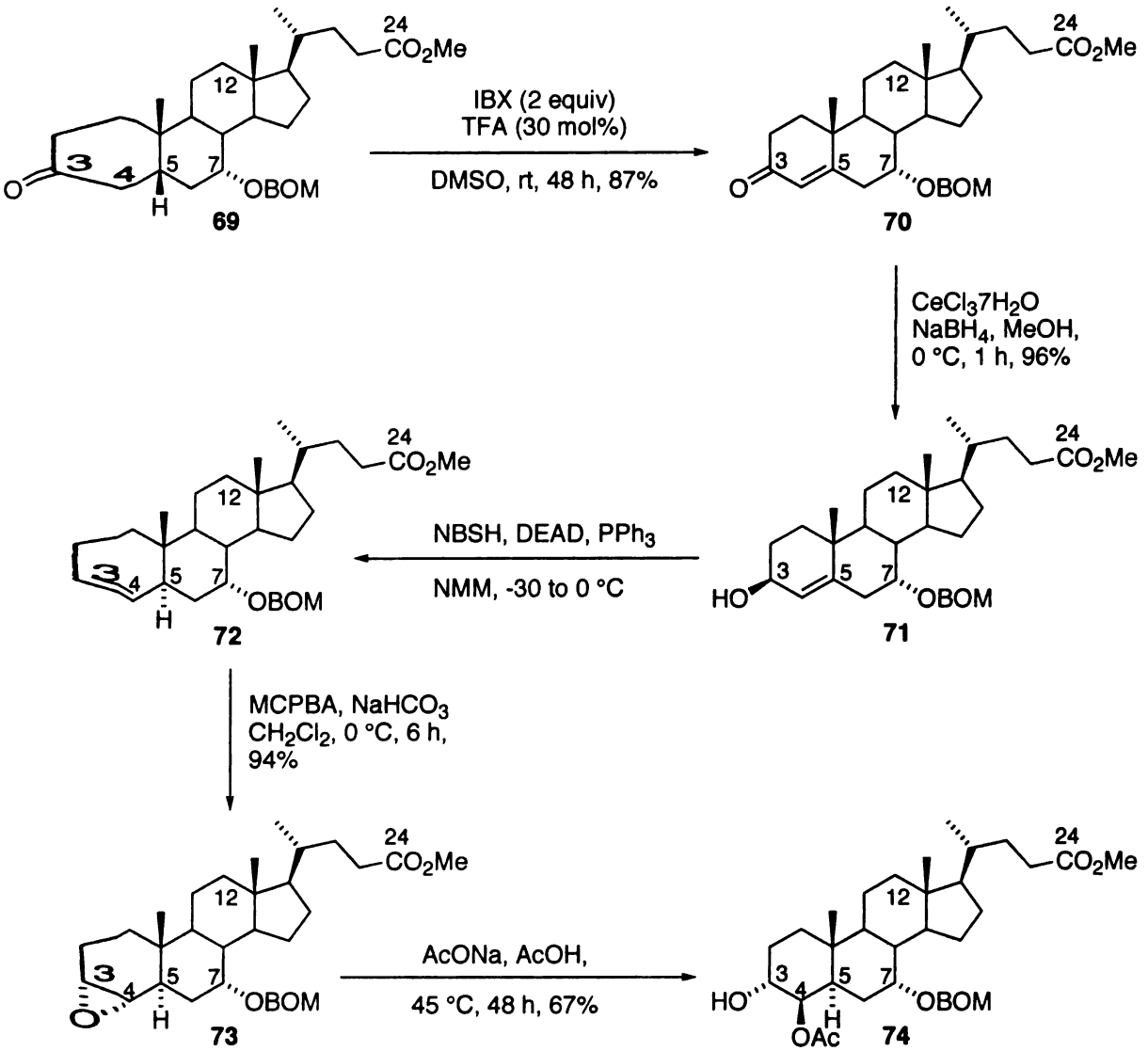
Scheme 41. Synthesis of larval sea lamprey pheromones, ACA and PZS

4.4. Conclusions and Future Studies

3-Keto petromyzonol sulfate, 3kPZS, is a potent component of the sex pheromone cocktail emitted by male sea lamprey, *petromyzon marinus*. Such pheromones have been successfully tested as population control agents against lamprey residing in the Great Lakes.[24] Owing to these results, a straightforward and practical synthetic route to 3kPZS is needed. For the synthesis of 3kPZS, cholic acid was selected as the starting material, because cholic acid has the carbon skeleton and functionality suitable for 3kPZS. Allomerization to convert the stereochemistry from 5β to 5α was the crucial step in this synthesis. Several allomerizations were conducted, including a single steps allomerization with Raney Ni and a variety of stepwise allomerizations. Material derived from allomerizations was used to establish a synthetic route to male sea lamprey pheromones, 3kPZS and 3kACA. The search for a practical and scalable allomerization of cholic acid and its derivatives continues. Even more, recently the Li group tested the single component of synthesized 3kPZS in the field: Synthetic 3kPZS induces upstream movement of females and lures ~50% into traps.[61] Due to this fascinating result, 3kPZS should be continuously supplied via development of practical and environmentally benign synthesis

Our synthesis of 3kPZS was successful, but more needs to be done in order to make this a commercial process. As discussed in section 3.3.7, taking advantage of the directionality of the 7α-hydroxyl groups would be promising way to induce stereoselectivy at C5.[57] Activated by Lewis acid, acetal formation promotes hydride delivery from the benzyl protected 7α-hydroxyl group to establish the 5α

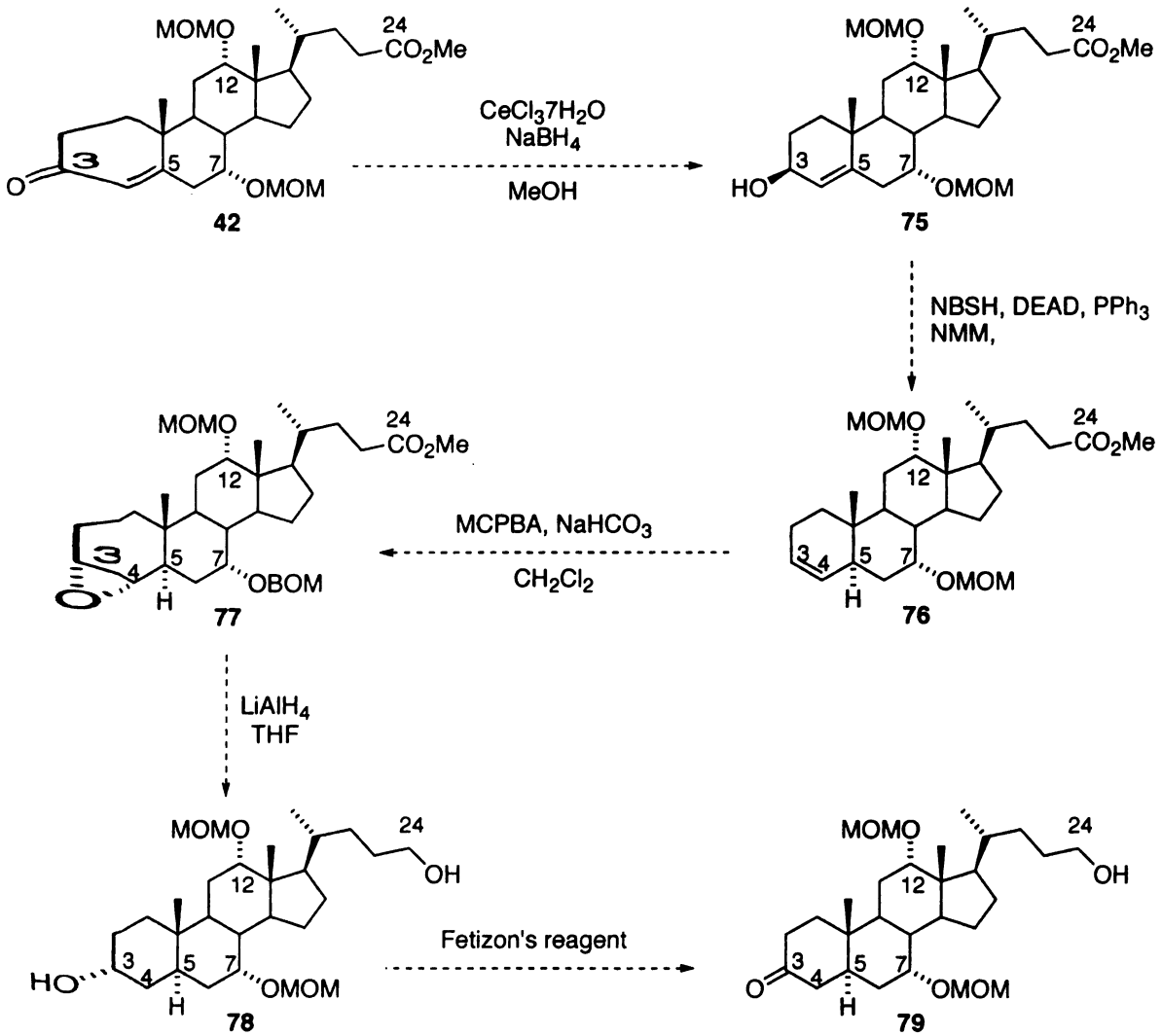
stereochemistry. However, the substrate preparations and reaction conditions are not yet optimized.



Scheme 42. Oishi's allomerization with Myers protocol

The Oishi group reported another allomerization directed by 3β-hydroxyl group instead of 7α-hydroxyl group (Scheme 42).[62] Following Zhou's IBX chemistry, they obtained the enone compound **70**, which was allomerized using Myers protocol.[63] A ketone functional group was reduced by Luche conditions to 3β-hydroxyl group.[64] As a

key step, reductive 1,3-transposition of allylic alcohol was achieved with 5α (A/B *trans*) stereochemistry installation to afford olefin **72**. The hydroxyl group was reinstalled through substrate-controlled stereoselective epoxidation,[65] followed by diaxial opening[66] of the epoxide with acetate attack to obtain compound **74** as a single diastereomer.



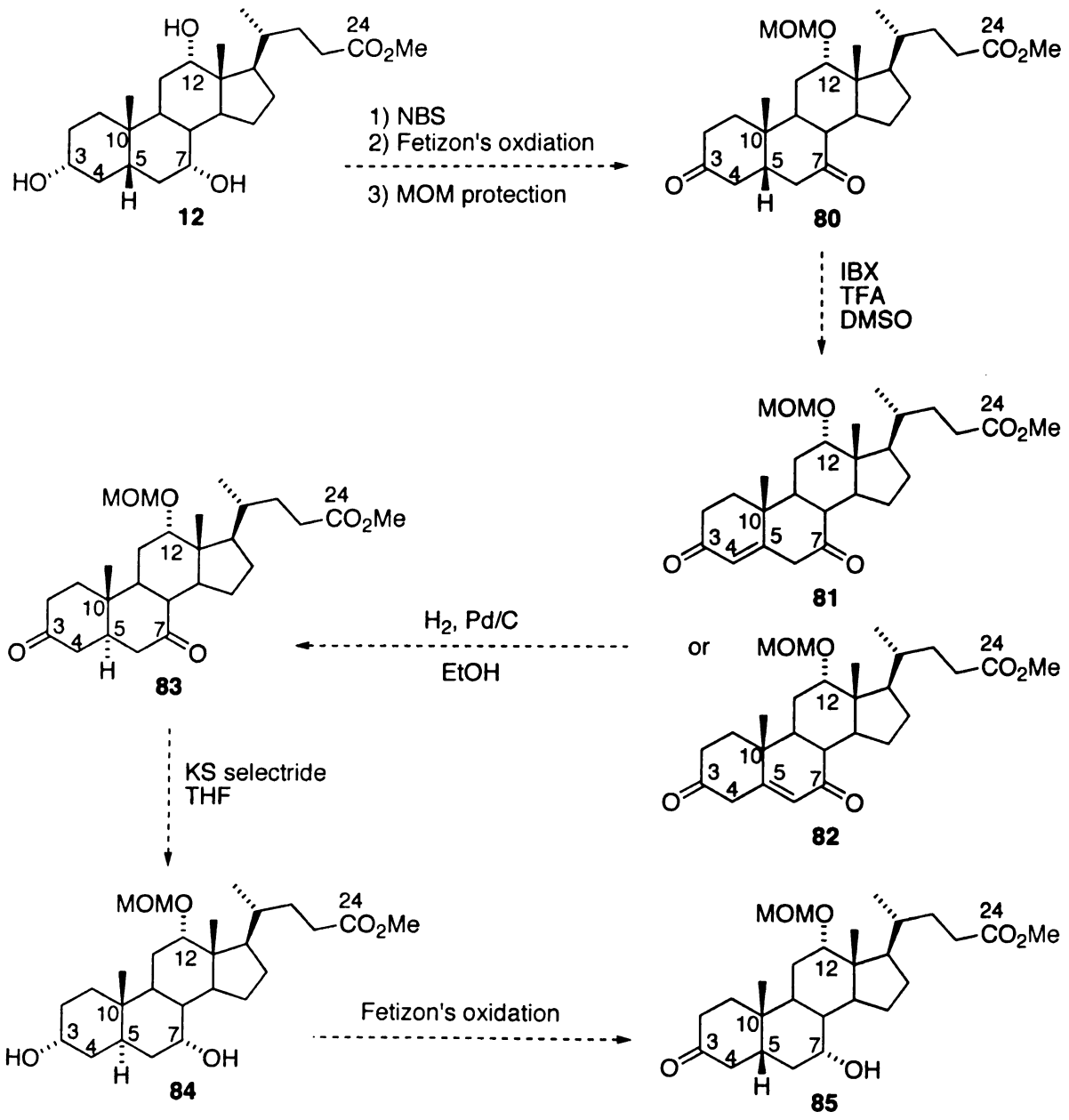
Scheme 43. Allomerization of enone **42** applying Oishi's protocol

This sequence of allomerization could be applied to our system. As illustrated in Scheme 43, an enone compound **42** may be reduced under the Luche conditions[64] to

provide 3β-hydroxyl compound **75**. Applying Myers protocol,[63] 5α (A/B *trans*) stereochemistry could be installed via 1,3-transposition of allylic alcohol to afford olefin **76**. Then, the 3α-hydroxyl group would be reinstalled by stereoselective epoxidation,[65] followed by diaxial attack[66] of hydride alone with the reduction of the ester at C24 to provide compound **78**. According to the Tserng's method,[45] the 3α-hydroxyl group can be selectively oxidized with treatment of Fetizon's reagent.[46]

The net function of the hydroxyl groups especially those bearing the α stereochemistry in an allomerization might be tricky. If the hydroxyl group works as directing functional group for the stereoselective reduction, it would install the 5α (A/B *trans*) stereochemistry. In other cases where the hydroxyl group does not serve as a directing functionality, it may just serve as steric factor. As a result, reduction of enone might give 5β/5α mixtures due to the steric interactions with the 10β-methyl group or 7α-hydroxyl group. Therefore, if one of the competing groups, especially the 7α-hydroxyl group, loses directionality, the other completing group (10β-methyl group) would govern the stereoselectivity of reduction to afford the 5α stereochemistry. With this idea in mind, we propose another allomerization route (Scheme 44). Methyl cholate **12** could be converted to a diketo compound **80** via double oxidation with NBS[67] and Fetizon's reagent,[46] followed by MOM protection.[47] Smooth dehydrogenation with IBX could provide us mixtures of enones **81**/**82**.[47] Since the stereochemistry at C7 is lost, a simple hydrogenation (H_2 gas, Pd/C) could stereoselectively install the 5α (A/B *trans*)

stereochemistry due to the steric interaction between incoming hydrogen and 10β-methyl group. Once 5α (A/B *trans*) stereochemistry is set up, 3α and 7α hydroxyl groups can be installed from reduction with KS-selectride.[32] Lastly, 3α-hydroxyl group could be selectively oxidized with Fetizon's reagent.[46]



Scheme 44. Allomerization of enediketo compound **81/82**

APPENDIX. EXPERIMENTAL DETAILS

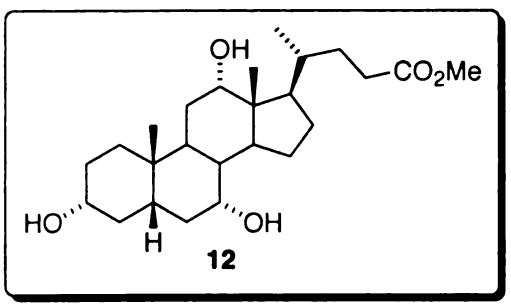
General Materials and Methods

Tetrahydrofuran was freshly distilled from sodium/benzophenone under nitrogen. Benzene, toluene, dichloromethane, triethylamine and chlorotrimethylsilane were freshly distilled from calcium hydride under nitrogen. Benzene-d6, DMSO-d6, and chloroform-d were purchased from the Cambridge Isotope Labs and used without further purification. Deionized water was used unless otherwise noted.

All commercial reagents were used without purification unless otherwise noted. Series of Raney Ni powders suspended in water were purchased from Aldrich. Composition of no.2800 Raney Ni is Ni and Al, and size of the particle is 45-90 microns.[68] Composition of no.4200 Raney Ni is Ni and Al, and size of the particle is 20-50 microns.[68] Flash chromatography was performed with silica gel 60 Å (230–400 mesh) purchased from Silicycle. TLC was performed on aluminum backed TLC plates by Silicycle. All other yields refer to chromatographically and spectroscopically pure compounds. Melting points were determined on a Thomas-Hoover Apparatus, uncorrected. Infrared spectra were recorded on a Nicolet IR/42 spectrometer. ^{1}H, ^{13}C NMR spectra were recorded on 300 and 500 MHz spectrometer with chemical shifts reported relative to the residue peaks of solvent chloroform (δ 7.24 for ^{1}H and δ 77.0 for ^{13}C). High-resolution mass spectra were acquired at the Michigan State University Mass Spectrometry facility using a Waters QTOF Ultima mass spectrometer equipped with an electrospray ionization (ESI) source by Luis Sanchez.

Standard Reaction Method

All reactions were carried out in oven-dried glassware, with magnetic stirring, and monitored by thin-layer chromatography with 0.25-mm precoated silica gel plates, unless otherwise noted. Visualization of reaction progress was achieved by UV lamp, phosphomolybdic acid stain or potassium permanganate stain.



12

Methyl cholate 12:

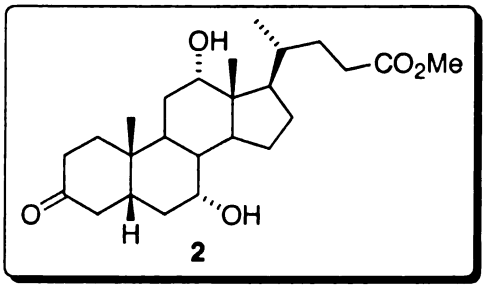
Into a 1 L round bottom flask charged with cholic acid (20.43 g, 50 mmol), which was dissolved into MeOH (360 mL, 0.14M), TMSCl (12.8 mL, 100 mmol) was syringed in and the solution open to air was stirred at room temperature overnight. Upon the full conversion of starting material into product on TLC, reaction mixture was concentrated with nitrogen gas blowing. During concentration, white solid was produced. The solid was collected by suction filtration and washed with deionized water (200 mL×3). The filtrate solution was evaporated to deliver the white precipitate, which was suspended in deionized water (500 mL). Insoluble white precipitate was filtered and the filtered solid was washed with deionized water (200 mL×3). The combined white solid was dried overnight under high vacuum (80 °C) to provide desired product **12** (20.89 g, 49.5 mmol, 99% yield). Spectroscopic data matched literature reports.[69]

mp 154-155 °C (lit.[70] mp 155-156 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 3.96 (m, 1 H), 3.84 (m, 1 H), 3.64 (s, 3 H), 3.43 (m, 1 H), 2.4-0.9 (m, 27 H), 0.96 (d, J = 5.9 Hz, 3 H), 0.87 (s, 3 H), 0.66 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 174.8, 73.0, 71.8,

68.3, 51.4, 50.2, 46.8, 46.3, 41.4, 39.3, 39.2, 35.2, 35.1, 34.7, 34.5, 31.0, 30.8, 30.1, 28.0, 27.4, 26.1, 23.1, 22.3, 17.1, 12.3.

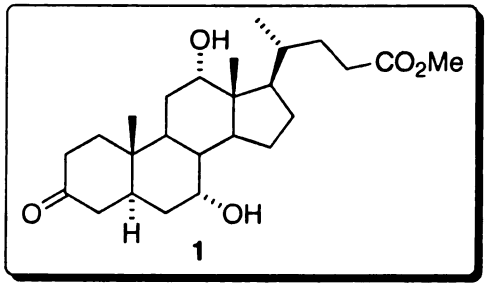
Representative Single Step Allomerization with Raney Ni (Scheme 10 & 11)

To a pre-weighed two-neck round bottom flask was added commercially available Raney Ni in water suspension. The water was pipetted out, and washed with 2×25 mL isopropanol and 2×25 mL *p*-cymene in the same manner. For about 250 wt% of Raney Ni, methyl cholate **12** was added into the flask, followed by *p*-cymene (0.19M). The flask was flushed with N_2, and a mechanical stirrer was set up in the middle neck of the flask and the other neck was connected with an air condenser. Upon stirring, the flask was mechanically stirred at reflux for 10 hours. TLC (EtOAc) showed the starting material was close to be consumed and a series of new spots was observed. The whole mixture was cooled to room temperature and filtered through celite. Filter cake was washed with EtOAc till colorless and the cake was quenched with dilute HCl. The solvent was concentrated with N_2 blowing. The residue was then dissolved into minimum amount of CH_2Cl_2 and purified by flash column chromatography on deactivated neutral alumina (CH_2Cl_2/EtOAc=1/3) to recover the starting material **12** (5-10%), and isolate 5β (A/B *cis*) isomer **2** as white crystalline solid and 5β/5α mixtures (~1/1 by NMR) with a trace amount of 4-en-3-one intermediate **13**. After repeated subjection of 5β/5α mixtures on the 3% deactivated neutral alumina, minor amount of 5α (A/B *trans*) isomer **1** was isolated.



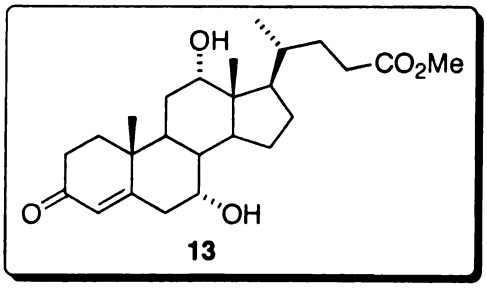
2

5β isomer 2: mp 173-174 °C (lit.[71] 170 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.99 (m, 1 H), 3.89 (m, 1 H), 3.63 (s, 3 H), 3.67 (dd, J = 15.1, 13.7 Hz, 1 H), 2.44-2.08 (m, 7 H), 2.00-1.52 (m, 14 H), 1.41-1.27 (m, 4 H), 1.14-1.11 (m, 1 H), 0.96 (s, 3 H), 0.95 (d, J = 6.8 Hz, 3 H), 0.69 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 213.3, 174.8, 72.8, 68.3, 51.5, 47.2, 46.6, 45.5, 43.0, 41.8, 39.4, 36.7, 36.6, 35.2, 34.9, 33.8, 31.0, 30.8, 28.5, 27.4, 27.1, 23.1, 21.6, 17.3, 12.5.



1

5α isomer 1: mp 149-151 °C (lit.[32] 150-152 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 3.97 (m, 1 H), 3.85-3.84 (m, 1 H), 3.64 (s, 3 H), 2.38-2.02 (m, 8 H), 1.95-1.12 (m, 28 H), 0.97 (s, 3 H), 0.96 (d, J = 6.3 Hz, 3 H), 0.93-0.84 (m, 1 H), 0.69 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 211.7, 174.7, 72.6, 67.3, 51.5, 47.2, 46.5, 44.1, 41.9, 39.6, 39.1, 38.7, 38.0, 37.9, 36.5, 35.3, 35.1, 31.0, 30.8, 28.7, 27.3, 23.0, 17.3, 12.5, 10.3



13

4-en-3-one 13: mp 176-178 °C (lit.[72] mp 178-179 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.78 (s, 1 H), 3.98 (m, 1 H), 3.94 (m, 1 H), 3.63 (s, 3 H), 2.57-0.99 (m, 23 H), 1.14 (s, 3 H), 0.95 (d, J = 6.1 Hz, 3 H), 0.71 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 198.9, 174.7, 167.7, 126.8,

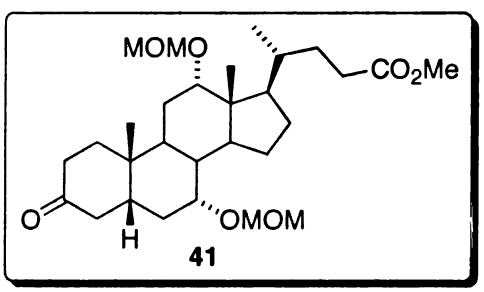
72.3, 68.2, 51.5, 47.1, 46.3, 41.9, 40.8, 39.9, 38.8, 38.0, 35.2, 35.1, 33.8, 31.0, 30.8, 28.4, 27.3, 23.0, 17.3, 16.8, 12.5; HRMS (ESI+) (m/z) calcd for C25H39O5 [M+H]+ 419.2797, found 419.2799.

Separation of 5β/5α mixtures

1 g of 5β/5α (1/1.1) mixtures was dissolved in acetone and EtOAc. 20 mL of silica was added and the slurry was rotavapped dry. This powder was added to the top of the column (d = 3.3 cm, 150 mL silica gel, hexane/acetone = 2:1) and dry. Eluent was used to wash out and down the silica from the flush and funnel. After 200 mL solvent, 8 mL fractions were collected. After 51 fractions, 408 mL of fraction was collected and remaining was rinsed with acetone. An additional 175 mL solvent was collected. Second column was performed with all fractions after 31 from the first column. 10 mL of silica gel was used for dryloading on column (d = 2.5 cm, 75 mL silica gel, hexane/acetone = 2:1). After 100 mL, 8 mL of fractions were collected by the total of 240 mL (30 fractions). 75 mL of acetone was added to top and another 20 fractions were collected. Mixture fractions were collected and resubjected on the third column (d = 2.5 cm, 75 mL silica gel, hexane/acetone = 2:1). The compound mixture was dissolved in eluent and applied directly to the column. After 100 mL, 8 mL of fractions were collected. 0.472 mg of 5α (A/B *trans*) compound **1** was isolated with trace amount of hexane and some impurity at 2.8 ppm but without 5β (A/B *cis*) compound **2**.

Preparation of compound 2 with Fetizon's Reagent (Scheme 18)

Into a 1 L round bottom flask charged with methyl cholate **12** (10.57 g, 25 mmol), which was suspended into toluene (350 mL, 0.07M), Ag_2CO_3 on celite (29.93 g from 1 mmol/0.5986 g batch, 50 mmol) was added. Onto the flask were set up Dean-Stark trap and reflux condenser with nitrogen balloon and the suspended mixture was refluxed in preheated oil bath at 135 °C for 8 hours. Upon full conversion into product on TLC, reaction mixture was cool down to room temperature and precipitate was filtered on celite. Filtered solid was washed with EtOAc (2x300 mL) and combined filtrate solution was concentrated under reduced pressure to provide crude material (10.26 g), which was recrystalized with EtOAc to afford 8.94 g of product **12** in 85% yield.



41

Synthesis of Compound 41:

To an oven-dried 100 mL of round bottom flask was placed 4.206 g (10 mmol) of compound **2** and 150 mg (1 mmol) of NaI. The solid mixture was suspended in 30 mL of CH_2Cl_2 and then, 4.6 mL (61 mmol) of MOMCl was added to the suspension. 16 mL (92 mmol) of diisopropylethylamine was added slowly to the mixture under ice bath. The reaction mixture was refluxed for 8 hours. The reaction was quenched with 30 mL of sat. $NaHCO_3$ and transferred into separatory funnel. Organic layer was separated and aqueous layer was extracted with 20 mL of CH_2Cl_2. Combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue material was purified

by flash chromatography on silica gel (Hexane/EtOAc = 3/1) to afford 4.230 g of white solid in 83 % yield.

mp (softened at 63 °C) 70-71 °C; IR (NaCl) 2984 (s), 2951 (w), 1730 (s), 1708 (s) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 4.65 (d, J = 6.8 Hz, 2 H), 4.62 (dd, J = 95.7, 6.9 Hz, 2 H), 3.79 (m, 1 H), 3.65 (m, 1 H), 3.64 (s, 3 H), 3.37 (s, 3 H), 3.35 (s, 3 H), 3.34 (m, 1 H), 2.47-2.07 (m, 7 H), 1.99-1.23 (m, 15 H), 1.09-1.00 (m, 1 H), 0.98 (s, 3 H), 0.92 (d, J = 6.4 Hz, 3 H), 0.69 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 213.4, 174.7, 96.5, 96.0, 80.8, 74.8, 56.2, 56.0, 51.5, 46.3, 46.1, 45.1, 43.1, 41.9, 39.5, 36.7, 36.6, 35.5, 34.9, 31.1, 31.0, 30.6, 28.2, 27.7, 25.7, 23.4, 21.8, 17.6, 12.5; HRMS (ESI+) (m/z) calcd for C29H48O7Na [M+Na]+ 531.3298, found 531.3284.

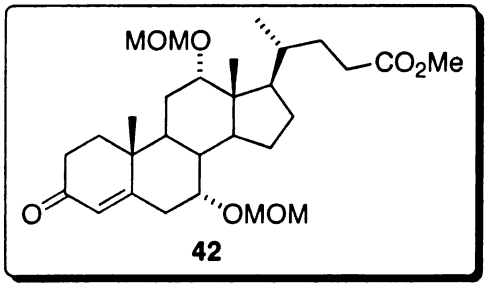
Representative Dehydrogenation with Iodic Acid (Scheme 21):

1.759 g (10 mmol) of iodic acid was heated in 10 mL of DMSO at 80 °C for 1 hour with light excluded from the reaction flask. After cooling down to room temperature, 1 mL (1 mmol) of this readily prepared solution was added to a solution of 0.210 g (0.5 mmol) of compound **2** and 0.13 mL (1.2 mmol) of cyclohexene in 0.5 mL of DMSO. The reaction mixture was heated in the seal tube at 50 °C for 1.5 day, with light excluded from the tube. The reaction mixture was diluted with 50 mL of ethyl acetate and washed with 20 mL of saturated $NaHSO_3$ aqueous solution and 20 mL of brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to provide 0.50 g of crude material, which

was further purified by flash chromatography on silica gel (CH_2Cl_2/EtOAc=1/5) to afford 91 mg (43%) of enone **10** and dienone **13** as a mixture (1/1 ratio by NMR).

Representative Dehydrogenation with IBX (Scheme 22):

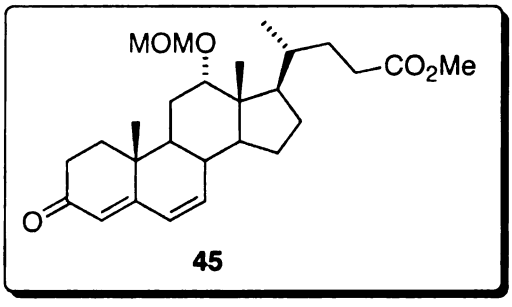
To a 100 mL of 1 neck round bottom flask was placed 1.017 g (2.0 mmol) of compound **41** and dissolved in 25 mL of DMSO. Into the solution was added 1.120 g (4.0 mmol) of IBX and then, 52 mL (35%mol) of TFA was added slowly. The reaction mixture was stirred at room temperature, monitored by TLC. Insoluble solid was formed in the course of reaction. After 7 days, the reaction mixture was quenched with 28 mL of saturated $NaHCO_3$ aqueous solution. To the suspension was added more 80 mL of H_2O and extracted with 4×30 mL of EtOAc. The combined organic layer was washed with 50 mL of brine and dried over Na_2SO_4, followed by filtration through a pad of celite. The solvent was removed in vacuo to afford 1.09 g of crude material, which was further purified by flash chromatography on silica gel (Hexane/EtOAc = 3/2, v'/v) to provide 9 mg of impure starting material, 83 mg of impure 1-en-3-one, 15 mg of 1-en-3-one/4-en-3-one, 735 mg of pure desired 4-en-3-one **10** (73%), and 102 mg of 4-en-3-one/1,4-dien-3-one (1/4 ratio) with accompanied by γ,δ-eliminated product, 4,6-dien-3-one **45**.



42

mp 73-74 °C; IR (PTFE card) 2945 (s), 2821 (w), 1736 (s), 1671 (s), 1618 (w), cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 5.71 (s, 1 H), 4.63 (d, J = 7.1 Hz, 2 H), 4.62 (dd, J = 52.5, 6.9 Hz, 2 H), 3.75 (m, 1 H), 3.72 (m, 1 H), 3.62 (s, 3 H), 3.35 (s, 3 H), 3.31 (s, 3 H), 2.60 (dd, J = 15.1, 2.9

Hz, 1 H), 2.42-2.15 (m, 5 H), 2.04-1.21 (m, 14 H), 1.14 (s, 3 H), 1.13-1.06 (m, 1 H), 0.89 (d, J = 6.3 Hz, 3 H), 0.70 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 198.8, 174.4, 168.6, 126.2, 96.6, 95.9, 80.4, 75.0, 56.0, 55.6, 51.3, 46.0, 46.0, 42.2, 39.9, 39.7, 38.4, 37.8, 35.3, 35.1, 33.8, 30.9, 30.8, 27.4, 25.7, 23.3, 17.4, 17.0, 12.3; HRMS (ESI+) (m/z) calcd for C29H46O7Na [M+Na]+ 329.3141, found 529.3146.



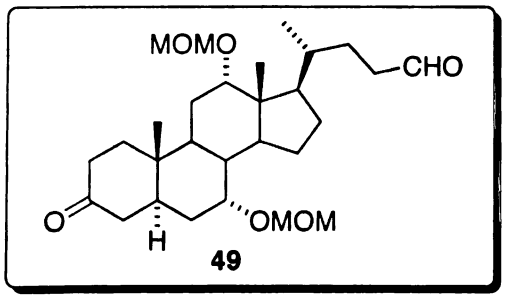
45

mp (softened at 60 °C) 67-68 °C; IR (NaCl) 3054 (s), 2984 (s), 2950 (w), 1733 (s), 1662 (s), 1614 (w), cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.09 (m, 2 H), 5.64 (s, 1 H), 4.65 (m, 2 H), 3.79 (m, 1 H), 3.63 (s, 3 H), 3.33 (s, 3 H), 2.54-1.22 (m, 19 H), 1.07 (s, 3 H), 0.90 (d, J = 6.5 Hz, 3 H), 0.75 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 199.8, 174.6, 164.0, 141.4, 127.8, 123.5, 97.0, 96.8, 80.9, 56.2, 51.5, 47.2, 46.3, 45.9, 44.0, 37.7, 35.7, 35.4, 33.9, 33.6, 30.9, 27.5, 25.7, 23.2, 17.5, 16.2, 12.2; HRMS (ESI+) (m/z) calcd for C27H41O5 $[M+H]^+$ 445.2954, found 445.2959.

Representative Birch Reduction (Scheme 25):

A 100 mL of 3 neck round bottom flask, which was equipped with dry condenser and magnetic stirring bar, was charged with N_2 gas and then flushed with NH_3 gas. Dry condenser was charged with dry ice and the reaction flask was cool down to –78 °C to start collecting about 15 mL of liquid ammonia. 56 mg of Li (8.1 mmol) was inserted rapidly into this solution and stirred for 15 minutes until the color of the solution turned into dark blue. A solution of 0.101 g of compound **42** (0.20 mmol) in 8 mL of dry THF,

was transferred to the ammonia via cannular over 5 minutes and washed/added (2×0.5 mL). The reaction mixture was stirred at the same temperature for 1.5 hour. The reaction was quenched with 1.0 g of NH_4Cl. After the color of the solution turned back to colorless, the Dewar bath was removed to evaporate ammonia gas overnight. Into the suspension was added 20 mL of H_2O, and 1N HCl aqueous solution was added slowly to pH 3. The solution was extracted with 3×30 mL of CH_2Cl_2 and combined organic layer was washed with 20 mL of brine and dried over Na_2SO_4. The solvent was removed in vacuo to provide 0.12 g of crude mixture, which was further purified by flash chromatography on silica gel (25mm×130mm, hexane/EtOAc = 1/1, v'/v) to deliver trace amount of desired 5α (A/B *trans*) product **48**, 29% of aldehyde **49**, and mixtures of **49** and **50** (trace).



49

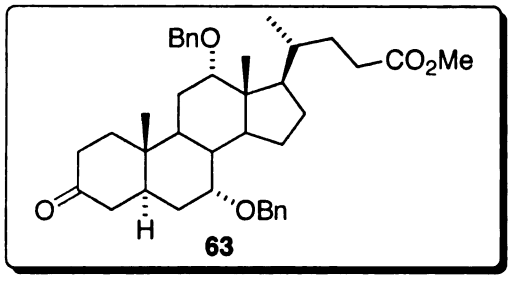
mp 68-70 °C; IR (KBr) 2985 (s), 2952 (w), 1729 (s), 1707 (s) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 9.75 (t, J = 1.8 Hz, 3 H), 4.65 (d, J = 9.5 Hz, 2 H), 4.62 (dd, J = 59.5, 7.0 Hz, 2 H), 3.74 (m, 1 H), 3.59 (m, 1 H), 3.38 (s, 3 H), 3.32 (s, 3 H), 2.37-0.86 (m, 24 H), 0.98 (s, 3 H), 0.91 (d, J = 6.6 Hz, 3 H), 0.69 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 211.7, 203.0, 96.6, 96.2, 80.7, 74.6, 56.0, 55.6, 46.3, 46.3, 44.2, 42.3, 40.9, 39.8, 39.6, 38.1, 38.0, 35.4, 35.2, 33.9, 32.7, 27.9, 27.7, 26.1, 23.3, 17.7, 12.5, 10.5; HRMS (ESI+) (m/z) calcd for C28H50NO6 $[M+NH_4]+$ 496.3638, found 496.3651.

Modified Sequence of Birch Reduction (Scheme 26 & 27):

The exactly same Birch reduction step of enone **42** was carried out. After acidic work-up and concentration, the crude material was checked by ^{1}H-NMR, which indicated that two MOM protecting groups were intact and methyl ester at C24 position was reduced completely incorporating generation of trace amount of aldehyde. The crude was suspended in acetone (3 mL, 0.05M) and the solution was cool down to 0 °C, followed by the addition of Jones' reagent (0.5 mL, 1.33 mmol from 2.67M aqueous solution of CrO_3/H_2SO_4) to provide red solution. After 30 minutes at room temperature, the solution was quenched with isopropanol (3 mL) at 0 °C to produce turquoise precipitate, which was filtered out right away through celite. The filter cake was washed with acetone (20 mL×3) and the filtrate solution was concentrated under reduced pressure to deliver crude material (53 mg), which has MOM protecting groups attached. The crude was dissolved in MeOH (3 mL, 0.05M), followed by the addition of TMSCl (0.1 mL, 0.78 mmol). The mixture was allowed to stir at room temperature overnight. Most of methanol was evaporated and water (15 mL) and saturated $NaHCO_3$ aqueous solution (15 mL) were added, which then mixture was extracted with CH_2Cl_2 (25 mL×3). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give yellow crude, which indicated no presence of MOM protection with trace amount of aldehyde. The crude was further purified by flash chromatography on silica gel (100mm×15mm, CH_2Cl_2/EtOAc = 1/3) to provide up to 65% yield of desired 5α (A/B *trans*) product **1**.

Representative Conjugate Reduction with $Na_2S_2O_4$ (Scheme 30)

To a 50 mL of 1 neck round bottom flask was prepared the solution of 0.101 g (1.20 mmol) of $NaHCO_3$ and 0.546 g (1.35 mmol) of Aliquat® 336 in 6 mL of H_2O. Into the solution was added the solution of 0.076 g (0.15 mmol) of compound **42** in 6 mL of toluene. To the solution mixture was added 0.235 g (1.35 mmol) of $Na_2S_2O_4$ and refluxed for 2 hours. The reaction mixture was cooled down to room temperature and 20 mL of H_2O was added. The organic layer was separated and the aqueous layer was extracted with 3×30 mL of EtOAc. The combined organic layer was dried over Na_2SO_4. The solvent was removed in vacuo to afford 0.081 g of crude material, which was further purified by flash chromatography on silica gel (Hexane/EtOAc = 2/1, v'/v) to provide 7 mg of reduced products **41/48** (ratio of 5β/5α = 1/1.8, 9 %), and 65 mg of overreduced alcohol mixtures of **53/54** (5β/5α = 1/5.5, 84%). The overreduced mixtures were dissolved in 2 mL of acetone, followed by addition of Jones' reagents (1 mL, 2.67 mmol) until the solution was turned into red at 0 °C. The excess of Jones' reagents was quenched with isopropanol at 0 °C and the solution was filtered through celite. The filtrate was concentrated under the reduced pressure to give 66 mg of crude mixture, which was further purified by flash chromatography on silica gel (Hexane/EtOAc = 2/1, v'/v) to obtain 61 mg of oxidized product **41/48** (ratio of 5β/5α = 1/5.5, 81%).



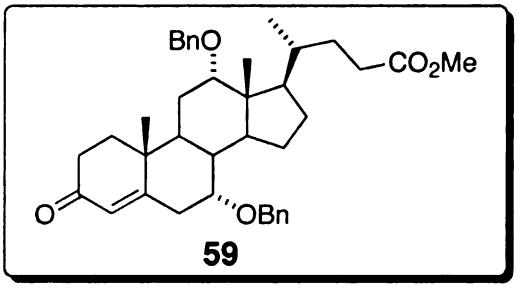
63

Synthesis of Compound 63:

Into a 1 neck of round bottom flask (50 mL) was placed compound **2** (0.2103 g, 0.50 mmol), which was dissolved in cyclohexane/CH_2Cl_2 (2/1, 14.5 mL). Following the addition of benzyl trichloroacetimidate (230 mL, 1.24 mmol), mixture was cool down to 0 °C, at which a 0.5M solution of trifluoromethanesulfonic acid in cyclohexane/CH_2Cl_2 (2/1) (0.5 mL, 0.25 mmol) was added dropwise into the mixture. The resulting mixture was stirred at room temperature overnight. During the course of reaction, the color of the solution was getting brown. After 17 hours, the mixture was diluted with CH_2Cl_2 (10 mL) and then, quenched with H_2O (10 mL). All the mixture was transferred into separatory funnel and washed with saturated $NaHCO_3$ aqueous solution (20 mL). Layers were separated and the aqueous layer was extracted with CH_2Cl_2 (10 mL). The combined yellow organic layer was washed with brine (30 mL) and dried over Na_2SO_4 (3.05 g) and concentrated to deliver red brown crude, which was further purified by flash chromatography on silica gel (100mm×24mm, EtOAc/hexane = 1/6 → EtOAc/hexane = 1/1) to provide the desired product **63** in 13% yield.

^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.33-7.19 (m, 10H), 4.49 (dd, J = 35.5, 12.2 Hz, 2 H), 4.42 (dd, J = 168.7, 11.5 Hz, 2 H), 3.72 (m, 1H), 3.64 (s, 3 H), 3.51 (m, 1 H), 2.24-1.04 (m, 24 H), 0.98 (s, 3H), 0.92 (d, J = 6.3 Hz, 3 H), 0.73 (s, 3 H); ^{13}C NMR (125

MHz, $CDCl_3$) δ (ppm) 213.1, 174.8, 139.7, 138.8, 128.3, 127.4, 127.3, 127.1, 126.7, 80.8, 75.6, 70.7, 70.0, 51.4, 46.5, 46.3, 44.6, 43.5, 42.3, 39.9, 36.7, 36.7, 35.2, 34.9, 31.1, 31.0, 28.5, 27.5, 23.4, 23.4, 21.8, 17.6, 12.5.

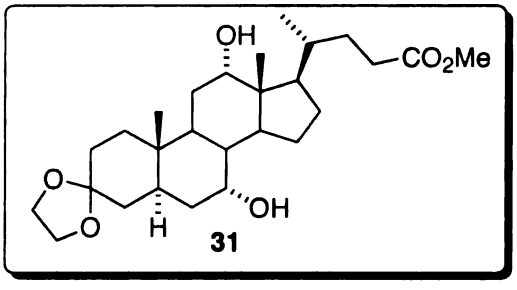


59

Synthesis of Compound 59:

A 20 mL of vial was charged with starting material **63** (0.0378 g, 0.063 mmol), which was dissolved in DMSO (0.57 mL, 0.11M). Into the solution was added IBX (0.0353 g, 0.126 mmol) and then, TFA (1.4 mL, 0.019 mmol) was added. The reaction mixture was stirred at room temperature for 7 days to start forming insoluble solid and then, the reaction mixture was quenched with 2 mL of saturated $NaHCO_3$ aqueous solution. H_2O (20 mL) and CH_2Cl_2 (20 mL) were added into the mixture and then whole mixture was transferred to separatory funnel. The funnel was shaken to extract organic material to bottom layer and settled down. Layer was separated and aqueous layer was extracted with another fresh CH_2Cl_2 (20 mL). The combined organic layer was washed with brine (30 mL), dried over Na_2SO_4 (2.17 g) and concentrated under reduced pressure to deliver crude material (0.0398 g), which was further purified by flash chromatography on silica gel (190mm×24mm, EtOAc/hexane = 1/2) to provide the desired enone compound **59** in 21% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.32-7.21 (m, 10H), 4.46 (dd, J = 104.5, 12.0 Hz, 2 H), 4.44 (dd, J = 79.8, 12.0 Hz, 2 H), 3.69 (m, 1H), 3.63 (s, 3 H), 3.58 (m, 1 H), 2.63-0.95 (m, 21 H), 1.15 (s, 3H), 0.88 (d, J = 6.3 Hz, 3 H), 0.72 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 198.9, 174.7, 168.7, 139.5, 138.9, 128.2, 128.2, 127.3, 127.3, 127.1, 127.1, 126.3, 80.3, 75.1, 70.4, 70.1, 51.4, 46.3, 46.2, 42.3, 40.5, 39.9, 37.8, 36.6, 35.2, 35.1, 33.8, 31.0, 30.9, 29.7, 27.4, 23.3, 23.3, 17.5, 17.2, 12.5.



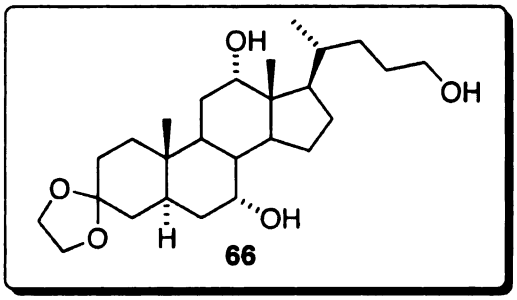
31

Synthesis of Compound 31:

To a solution of 0.10 g (0.238 mmol) of compound **1** in 7.2 mL of ethylene glycol/dioxane (1/1) was added 4.1 mg (0.024 mmol) of cupric chloride. The reaction mixture was placed into the preheated oil bath at 80 °C and heated for 3 hours. The mixture was poured into the 30 mL of H_2O and extracted with 3×20 mL of diethyl ether. The extract was dried over Na_2SO_4 and concentrated to provide 0.11 g of white solid in 99% yield.

mp 155-156 °C; IR (NaCl) 3430 (s, br), 2986 (s), 1729 (s) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 3.93 (m, 1 H), 3.90 (s, 4H), 3.80 (m, 1 H), 3.64 (s, 3 H), 2.38-2.32 (m, 1 H), 2.24-2.18 (m, 1 H), 1.95-1.07 (m, 28 H), 0.95 (d, J = 6.4 Hz, 3 H), 0.87-0.82 (m, 2 H), 0.78 (s, 3 H), 0.67 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 174.7, 109.1, 72.8, 67.7, 64.2, 64.1, 51.5, 47.2, 46.5, 42.2, 39.7, 39.2, 37.5, 36.2, 36.2, 35.6, 35.2, 35.1, 31.2,

31.0, 30.9, 28.7, 27.3, 23.0, 17.3, 12.6, 10.3; HRMS (ESI+) (m/z) calcd for C27H45O6 [M+H]+ 465.3216, found 465.3215.



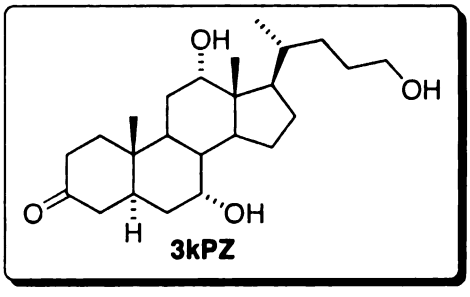
66

Synthesis of Compound 66:

To oven dried 50 mL of 1 neck round bottomed flask was suspended 28 mg (0.72 mmol) $LiAlH_4$ in 4.8 mL of THF. The separately prepared solution of 85 mg (0.18 mmol) of compound **31** in 4.8 mL of THF was added dropwise to the suspension over 5 minutes. All the reaction mixture was set into the preheated oil bath to 70 °C and refluxed for 8 hours. Once the mixture was cooled down to room temperature, 1 mL of Rochellie's salt solution was added slowly and the mixture was reheated to 70 °C to hydrolyze lithium salts for additional 12 hours. After cool down to room temperature, the mixture was transferred to the separatory funnel and washed with 10 mL of H_2O and 10 mL of brine. The organic layer was dried over Na_2SO_4 and concentrated to afford 90 mg of mixture, which was further purified by flash chromatography on silica gel (EtoAc) to provide 65 mg of white solid in 81 % yield.

mp 194-196 °C; IR (NaCl) 3428 (s, br), 2986 (s) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 3.95 (m, 1 H), 3.90 (s, 4H), 3.80 (m, 1 H), 3.59 (td, J = 6.5, 2.0 Hz, 2 H), 1.95-1.80 (m, 3 H), 1.74-1.38 (m, 22 H), 1.36-1.22 (m, 4 H), 1.15-1.06 (m, 2H), 0.97 (d, J = 6.6 Hz, 3 H), 0.78 (s, 3 H), 0.67 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 109.2, 72.8, 67.7, 64.1, 64.1, 63.4, 47.3, 46.4, 42.1, 39.7, 39.0, 37.5, 36.2, 36.1, 35.5, 35.4, 35.2, 31.8,

31.1, 29.4, 28.5, 27.5, 23.1, 17.7, 12.6, 10.2; HRMS (ESI+) (m/z) calcd for C26H45O5 [M+H]+ 437.3267, found 437.3269.



3kPZ

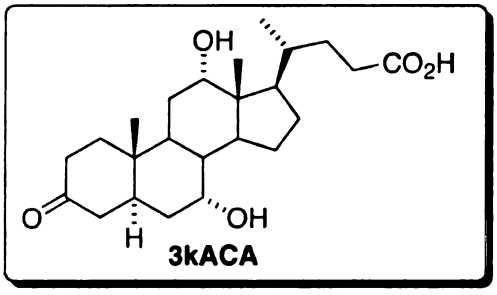
Preparation of 3kPZ:

a) To 10 mL of 1 neck round-bottomed flask was placed 38 mg (0.087 mmol) of compound **66** and dissolved in THF. Then, 0.3 mL of 5% HCl (aq.) was added and the reaction mixture was stirred at room temperature for 12 hours. Solvent was removed under reduced pressure and crude mixture was filtered through silica gel eluenting with CH_2Cl_2/EtOH (7/1). Filtrate was evaporated to afford 35 mg of white solid, which then was purified by flash chromatography on silica gel with EtOAc to provide 25 mg of white solid in 75 % yield.

b) A 50 mL of 1-neck round bottom flask was charged with $LiAlH_4$ (0.066 g, 1.74 mmol), which was suspended into THF (7.7 mL) and was cool down to 0 °C. Into the suspension, a solution of compound **31** (0.202 g, 0.435 mmol) in THF (5 mL) was added dropwise via cannular over 5 minutes and the flask was washed/added with THF (2×0.5 mL). The resulting suspension was refluxed for 10 hours. Upon the complete consumption of starting material on TLC, the mixture was cool down to room temperature and quenched dropwise with 5% HCl aqueous solution (15 mL). The mixture was reheated at 80 °C overnight to hydrolyze lithium salts and cooled down to room temperature. The whole mixture was transferred to separatory funnel to let layers separated and aqueous layer was extracted with EtOAc (50 mL). Combined organic layer was washed with deionized water (50 mL) and brine (50 mL), and then dried over

Na_2SO_4 (5.2 g) and concentrated under reduced pressure to deliver white solid, which then was purified by flash chromatography on silica gel with EtOAc to provide 98 mg of white solid in 57 % yield.

mp 172-173 °C; IR (NaCl) 3427 (s, br), 2986 (s), 1707 (s) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 3.99 (m, 1 H), 3.86-3.84 (m, 1 H), 3.60 (td, *J* = 6.6, 2.0 Hz, 2 H), 2.35-1.82 (m, 8 H), 1.74-1.08 (m, 19 H), 1.36-1.22 (m, 4 H), 1.15-1.06 (m, 2H), 0.98 (d, *J* = 6.6 Hz, 3 H), 0.98 (s, 3 H), 0.71 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 211.9, 72.8, 67.3, 63.4, 47.4, 46.4, 44.1, 41.9, 39.6, 39.2, 38.7, 38.0, 38.0, 36.5, 35.4, 35.3, 31.9, 29.4, 28.6, 27.5, 23.1, 17.7, 12.6, 10.3; HRMS (ESI+) (m/z) calcd for C24H41O4 [M+H]+ 393.3005, found 393.3001.

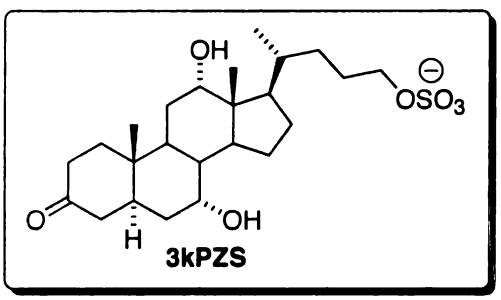


3kACA

Synthesis of 3kACA:

To a 10 mL of 1 neck round-bottomed flask were added 0.100 g (0.238 mmol) of compound **1** and 0.164 g (1.20 mmol) of K_2CO_3. The mixture was dissolved in 3 mL of H_2O/MeOH (1/2) and refluxed overnight. After cooling down to room temperature, 2 mL of 1N HCl aqueous solution was added slowly. The solution was transferred to separatory funnel and 2×20 mL of CH_2Cl_2 was extracted. The organic layer was washed with brine (30 mL) and dried over Na_2SO_4 (3.5 g). The solvent was evaporated under the reduced pressure to provide impure 96 mg of white solid in 99% yield.

mp 189-191 °C; IR (NaCl) 3427 (s, br), 2984 (s), 1707 (s), 1609 (s) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 11.94 (s br, 1H), 3.77 (m, 1 H), 3.60 (m, 1 H), 2.42-2.07 (m, 5 H), 1.98-1.53 (m, 11H), 1.51-1.11 (m, 8 H), 0.92 (s, 3H), 0.91 (d, J = 6.4 Hz, 3 H), 0.61 (s, 3 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm) 210.8, 175.0, 70.9, 65.5, 46.1, 45.8, 44.0, 41.4, 39.5, 38.9, 38.2, 37.9, 37.8, 36.9, 35.0, 35.0, 30.8, 30.8, 28.9, 27.2, 22.8, 16.9, 12.3, 10.0; HRMS (ESI+) (m/z) calcd for C24H41O6 $[M+H_3O]^+$ 425.2903, found 425.2906.



3kPZS

Synthesis of 3kPZS:

To a flame-dried vial was added 7 mg (0.016 mmol) of compound 3kPZ and dissolved in 1 mL of pyridine, dried with KOH. Then, 5.1 mg (0.032 mmol) of SO_3·pyridine was added and the mixture was stirred under N_2 gas atmosphere at room temperature for 8.5 hours. The reaction was quenched with 1 mL of MeOH and stirred for 15 minutes at room temperature. The mixture was concentrated and purified through pipette column with CH_2Cl_2/MeOH/acetic acid (100/10/1) to afford about 12% yield of 3kPZS. The fraction which has low Rf value was gathered and checked by NMR and HPLC, comparing with natural 3kPZS and 3kPZS synthesized by BridgeOrganics.

IR (NaCl) 3433 (s, br), 2944 (s), 2251 (s), 2125 (s), 1662 (s) cm^{-1}; HRMS (ESI+) (m/z) calcd for C26H47N2O7S $[M+ACN+NH_4]+$ 531.3104, found 531.3116.

Table 1. Crystal data and structure refinement for ml112404

Identification code	ml112404
Empirical formula	C32 H42 NO8
Formula weight	568.67
Temperature	173(2) K
Wavelength	0.71073 A
Crystal system	Monoclinic
Space group	P2(1)
Unit cell dimensions	a = 7.3966(15) A
	b = 12.178(2) A
	c = 17.057(3) A
	alpha = 90 deg.
	beta = 101.18(3) deg.
	gamma = 90 deg.
Volume	1507.3(5) A^3
Z	2
Density (calculated)	1.253 Mg/m^3
Absorption coefficient	0.089 mm^{-1}

(Table 1. continued)

F(000)	610
Crystal size	0.4 x 0.2 x 0.2 mm
Theta range for data collection	2.07 to 28.16 deg.
Index ranges	-9<=h<=9, -16<=k<=16, -22<=l<=22
Reflections collected / unique	18038 / 7076 [R(int) = 0.0296]
Completeness to theta = 28.16	98.0%
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	7076 / 1 / 370
Goodness-of-fit on F^2	0.773
Final R indices [I>2sigma(I)]	R1 = 0.0399, wR2 = 0.1044
R indices (all data)	R1 = 0.0623, wR2 = 0.1183
Absolute structure parameter	-0.4(8)
Largest diff. peak and hole	0.229 and -0.199 $e.A^{-3}$

Table 2. Atomic coordinates (x 10^4), equivalent isotropic displacement parameters (A^2 x 10^3), and occupancies for ml112404

	x	y	z	U(eq)	Occ.
O(1)	6692(2)	6320(2)	4275(1)	58(1)	1
C(2)	5466(3)	6824(2)	4506(1)	41(1)	1
C(3)	3546(3)	6371(2)	4441(1)	38(1)	1
C(4)	3044(2)	6331(2)	5280(1)	28(1)	1
C(5)	3192(2)	7470(2)	5697(1)	27(1)	1
C(6)	5191(3)	7882(2)	5747(1)	36(1)	1
C(7)	5794(3)	7918(2)	4929(1)	44(1)	1
C(8)	1848(3)	8325(2)	5232(1)	35(1)	1
C(9)	1158(2)	5793(2)	5246(1)	30(1)	1
C(10)	729(2)	5646(2)	6077(1)	27(1)	1
C(11)	927(2)	6724(2)	6551(1)	25(1)	1
C(12)	2808(2)	7292(2)	6560(1)	26(1)	1
C(13)	2973(3)	8358(2)	7061(1)	33(1)	1
C(14)	2599(2)	8200(2)	7913(1)	31(1)	1
O(15)	4077(2)	7534(1)	8345(1)	38(1)	1
C(16)	697(2)	7664(2)	7887(1)	28(1)	1

(Table 2. continued)

C(17)	648(2)	6573(2)	7414(1)	27(1)	1
C(18)	-1128(3)	6009(2)	7549(1)	34(1)	1
C(19)	-1225(3)	6324(2)	8421(1)	37(1)	1
C(20)	266(3)	7228(2)	8694(1)	31(1)	1
C(21)	-841(3)	8474(2)	7503(1)	34(1)	1
C(22)	-331(3)	8035(2)	9288(1)	35(1)	1
C(23)	1021(3)	8987(2)	9520(1)	47(1)	1
C(24)	-591(3)	7378(2)	10036(1)	48(1)	1
C(25)	-1153(3)	8052(2)	10708(1)	51(1)	1
C(26)	-2981(3)	8608(2)	10467(1)	42(1)	1
O(27)	-3044(2)	9519(2)	10896(1)	64(1)	1
C(28)	-4794(4)	10087(3)	10749(2)	89(1)	1
O(29)	-4271(2)	8272(2)	9986(1)	56(1)	1
O(30)	2085(2)	4853(1)	6500(1)	30(1)	1
C(31)	1485(3)	3912(2)	6770(1)	28(1)	1
O(32)	-124(2)	3654(1)	6709(1)	38(1)	1
C(33)	3050(3)	3200(2)	7162(1)	29(1)	1
C(34)	4892(3)	3507(2)	7231(1)	34(1)	1
C(35)	6295(3)	2801(2)	7575(1)	37(1)	1
C(36)	5808(3)	1801(2)	7864(1)	36(1)	1
C(37)	3989(3)	1478(2)	7808(1)	40(1)	1
C(38)	2614(3)	2176(2)	7447(1)	34(1)	1

(Table 2. continued)

C(39)	7290(3)	1045(2)	8241(1)	44(1)	1
O(40)	8850(2)	1200(1)	8123(1)	51(1)	1
O(41)	6876(3)	298(2)	8653(1)	73(1)	1

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

Table 3. Bond lengths [A] and angles [deg] for ml112404

O(1)-C(2)	1.223(3)
C(2)-C(7)	1.511(3)
C(2)-C(3)	1.508(3)
C(3)-C(4)	1.547(3)
C(4)-C(9)	1.532(3)
C(4)-C(5)	1.553(3)
C(5)-C(8)	1.548(3)
C(5)-C(6)	1.548(2)
C(5)-C(12)	1.568(2)
C(6)-C(7)	1.547(3)
C(9)-C(10)	1.522(3)
C(10)-O(30)	1.477(2)
C(10)-C(11)	1.533(3)

(Table 3. continued)

C(11)-C(17)	1.537(2)
C(11)-C(12)	1.552(2)
C(12)-C(13)	1.546(3)
C(13)-C(14)	1.543(3)
C(14)-O(15)	1.444(2)
C(14)-C(16)	1.544(3)
C(16)-C(21)	1.551(3)
C(16)-C(17)	1.551(3)
C(16)-C(20)	1.564(3)
C(17)-C(18)	1.539(3)
C(18)-C(19)	1.551(3)
C(19)-C(20)	1.564(3)
C(20)-C(22)	1.538(3)
C(22)-C(23)	1.532(3)
C(22)-C(24)	1.548(3)
C(24)-C(25)	1.532(3)
C(25)-C(26)	1.496(3)
C(26)-O(29)	1.203(3)
C(26)-O(27)	1.335(3)
O(27)-C(28)	1.446(3)
O(30)-C(31)	1.342(2)
C(31)-O(32)	1.215(2)

(Table 3. continued)

C(31)-C(33)	1.497(3)
C(33)-C(34)	1.396(3)
C(33)-C(38)	1.398(3)
C(34)-C(35)	1.387(3)
C(35)-C(36)	1.388(3)
C(36)-C(37)	1.387(3)
C(36)-C(39)	1.479(3)
C(37)-C(38)	1.376(3)
C(39)-O(41)	1.223(3)
C(39)-O(40)	1.224(2)
O(1)-C(2)-C(7)	122.4(2)
O(1)-C(2)-C(3)	122.7(2)
C(7)-C(2)-C(3)	114.81(18)
C(2)-C(3)-C(4)	109.59(16)
C(9)-C(4)-C(3)	111.05(15)
C(9)-C(4)-C(5)	112.47(15)
C(3)-C(4)-C(5)	112.77(15)
C(8)-C(5)-C(4)	112.64(15)
C(8)-C(5)-C(6)	108.78(15)
C(4)-C(5)-C(6)	107.29(15)
C(8)-C(5)-C(12)	111.00(15)
C(4)-C(5)-C(12)	107.30(14)

(Table 3. continued)

C(6)-C(5)-C(12)	109.74(14)
C(7)-C(6)-C(5)	113.49(16)
C(2)-C(7)-C(6)	110.99(17)
C(10)-C(9)-C(4)	111.62(15)
O(30)-C(10)-C(9)	106.66(14)
O(30)-C(10)-C(11)	108.45(13)
C(9)-C(10)-C(11)	112.01(15)
C(10)-C(11)-C(17)	112.69(14)
C(10)-C(11)-C(12)	112.39(14)
C(17)-C(11)-C(12)	109.27(14)
C(13)-C(12)-C(11)	110.76(14)
C(13)-C(12)-C(5)	113.19(14)
C(11)-C(12)-C(5)	112.16(14)
C(14)-C(13)-C(12)	113.99(15)
O(15)-C(14)-C(13)	107.14(15)
O(15)-C(14)-C(16)	111.85(15)
C(13)-C(14)-C(16)	110.75(15)
C(14)-C(16)-C(21)	109.43(16)
C(14)-C(16)-C(17)	107.87(14)
C(21)-C(16)-C(17)	112.57(15)
C(14)-C(16)-C(20)	117.30(15)
C(21)-C(16)-C(20)	109.20(14)

(Table 3. continued)

C(17)-C(16)-C(20)	100.27(15)
C(11)-C(17)-C(18)	118.30(15)
C(11)-C(17)-C(16)	113.68(15)
C(18)-C(17)-C(16)	103.84(14)
C(17)-C(18)-C(19)	103.46(16)
C(18)-C(19)-C(20)	107.33(15)
C(22)-C(20)-C(16)	119.81(16)
C(22)-C(20)-C(19)	111.70(15)
C(16)-C(20)-C(19)	103.31(15)
C(23)-C(22)-C(20)	113.40(16)
C(23)-C(22)-C(24)	110.93(18)
C(20)-C(22)-C(24)	108.11(17)
C(25)-C(24)-C(22)	115.8(2)
C(26)-C(25)-C(24)	113.49(19)
O(29)-C(26)-O(27)	123.3(2)
O(29)-C(26)-C(25)	126.0(2)
O(27)-C(26)-C(25)	110.6(2)
C(26)-O(27)-C(28)	115.36(19)
C(31)-O(30)-C(10)	119.19(14)
O(32)-C(31)-O(30)	125.12(17)
O(32)-C(31)-C(33)	123.19(17)
O(30)-C(31)-C(33)	111.69(15)

(Table 3. continued)

C(34)-C(33)-C(38)	119.81(17)
C(34)-C(33)-C(31)	122.66(16)
C(38)-C(33)-C(31)	117.51(16)
C(35)-C(34)-C(33)	120.47(18)
C(34)-C(35)-C(36)	118.05(18)
C(35)-C(36)-C(37)	122.62(19)
C(35)-C(36)-C(39)	118.64(19)
C(37)-C(36)-C(39)	118.74(18)
C(38)-C(37)-C(36)	118.58(19)
C(37)-C(38)-C(33)	120.43(18)
O(41)-C(39)-O(40)	123.9(2)
O(41)-C(39)-C(36)	117.72(19)
O(40)-C(39)-C(36)	118.36(18)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($A^2 \times 10^3$) for ml112404

	U11	U22	U33	U23	U13	U12
O(1)	46(1)	76(1)	58(1)	12(1)	24(1)	16(1)

(Table 4. continued)

C(2)	38(1)	55(1)	30(1)	13(1)	11(1)	5(1)
C(3)	43(1)	42(1)	29(1)	0(1)	10(1)	2(1)
C(4)	28(1)	31(1)	26(1)	3(1)	6(1)	0(1)
C(5)	26(1)	27(1)	26(1)	4(1)	3(1)	-1(1)
C(6)	32(1)	42(1)	32(1)	6(1)	6(1)	-9(1)
C(7)	37(1)	57(2)	40(1)	9(1)	13(1)	-8(1)
C(8)	37(1)	34(1)	33(1)	7(1)	5(1)	3(1)
C(9)	31(1)	31(1)	28(1)	-2(1)	4(1)	-3(1)
C(10)	25(1)	28(1)	29(1)	2(1)	4(1)	-2(1)
C(11)	24(1)	24(1)	28(1)	3(1)	5(1)	1(1)
C(12)	25(1)	27(1)	26(1)	5(1)	4(1)	-1(1)
C(13)	36(1)	30(1)	33(1)	0(1)	8(1)	-7(1)
C(14)	32(1)	30(1)	30(1)	-2(1)	5(1)	-1(1)
O(15)	32(1)	47(1)	32(1)	-3(1)	1(1)	5(1)
C(16)	26(1)	27(1)	29(1)	1(1)	6(1)	4(1)
C(17)	26(1)	26(1)	29(1)	2(1)	7(1)	1(1)
C(18)	35(1)	32(1)	38(1)	-1(1)	13(1)	-3(1)
C(19)	39(1)	35(1)	40(1)	2(1)	17(1)	2(1)
C(20)	30(1)	33(1)	30(1)	2(1)	8(1)	7(1)
C(21)	37(1)	29(1)	35(1)	2(1)	5(1)	7(1)
C(22)	33(1)	42(1)	30(1)	1(1)	9(1)	11(1)
C(23)	43(1)	56(1)	41(1)	-16(1)	8(1)	6(1)

(Table 4. continued)

C(24)	60(1)	54(1)	35(1)	7(1)	19(1)	24(1)
C(25)	57(1)	67(2)	30(1)	2(1)	12(1)	20(1)
C(26)	41(1)	49(1)	39(1)	-6(1)	15(1)	3(1)
O(27)	38(1)	60(1)	91(1)	-35(1)	4(1)	4(1)
C(28)	44(2)	67(2)	150(3)	-57(2)	4(2)	10(1)
O(29)	45(1)	66(1)	56(1)	-27(1)	8(1)	3(1)
O(30)	31(1)	24(1)	36(1)	3(1)	7(1)	-1(1)
C(31)	37(1)	26(1)	22(1)	-3(1)	8(1)	-3(1)
O(32)	36(1)	36(1)	41(1)	3(1)	7(1)	-8(1)
C(33)	36(1)	27(1)	24(1)	-1(1)	9(1)	0(1)
C(34)	38(1)	26(1)	37(1)	3(1)	10(1)	-4(1)
C(35)	36(1)	33(1)	42(1)	0(1)	9(1)	-5(1)
C(36)	40(1)	30(1)	37(1)	2(1)	9(1)	7(1)
C(37)	50(1)	27(1)	47(1)	6(1)	22(1)	0(1)
C(38)	38(1)	28(1)	40(1)	2(1)	15(1)	-3(1)
C(39)	51(1)	31(1)	52(1)	4(1)	13(1)	8(1)
O(40)	41(1)	42(1)	70(1)	1(1)	7(1)	3(1)
O(41)	70(1)	54(1)	100(2)	41(1)	28(1)	21(1)

The anisotropic displacement factor exponent takes the form:

$$-2\,pi^2\,[\,h^2\,a^{*2}\,U11 + \ldots + 2\,h\,k\,a^*\,b^*\,U12\,]$$

Table 5. Hydrogen coordinates (x 10^4),

isotropic displacement parameters (A^2 x 10^3),

and occupancies for ml112404

	x	y	z	U(eq)	Occ.
H(3A)	3482	5639	4214	45	1
H(3B)	2673	6833	4091	45	1
H(4A)	3955	5854	5609	34	1
H(6A)	5299	8613	5977	43	1
H(6B)	6023	7405	6103	43	1
H(7A)	5106	8485	4599	53	1
H(7B)	7093	8102	5007	53	1
H(8A)	2113	8422	4707	52	1
H(8B)	1993	9013	5512	52	1
H(8C)	604	8070	5188	52	1
H(9A)	212	6245	4927	37	1
H(9B)	1141	5082	4989	37	1
H(10A)	-521	5355	6036	33	1
H(11A)	-38	7223	6283	30	1
H(12A)	3762	6790	6834	31	1
H(13A)	4203	8655	7099	39	1

(Table 5. continued)

H(13B)	2108	8894	6784	39	1
H(14A)	2622	8919	8174	37	1
H(15A)	4449	7800	8788	56	1
H(17A)	1680	6126	7689	32	1
H(18A)	-1053	5219	7490	41	1
H(18B)	-2195	6281	7177	41	1
H(19A)	-982	5686	8766	44	1
H(19B)	-2440	6603	8447	44	1
H(20A)	1377	6854	8975	37	1
H(21A)	-751	9135	7815	50	1
H(21B)	-2024	8142	7489	50	1
H(21C)	-700	8646	6969	50	1
H(22A)	-1528	8345	9039	42	1
H(23A)	1156	9384	9049	70	1
H(23B)	2197	8702	9778	70	1
H(23C)	558	9471	9880	70	1
H(24A)	-1522	6821	9869	58	1
H(24B)	555	7003	10250	58	1
H(25A)	-1197	7571	11157	61	1
H(25B)	-218	8605	10885	61	1
H(28A)	-4720	10717	11092	134	1
H(28B)	-5740	9602	10857	134	1

(Table 5. continued)

H(28C)	-5084	10320	10202	134	1
H(34A)	5181	4190	7044	40	1
H(35A)	7525	2993	7611	44	1
H(37A)	3704	804	8010	48	1
H(38A)	1387	1965	7391	41	1

REFERENCES

1. Global Invasive Species Database: *Petromyzon marinus* (fish). http://www.issg.org/database/species/ecology.asp?si=542&fr=1&sts=sss (accessed March 2010).

2. Great Lakes Fishery Commission. *Sea Lamprey Control – Sea Lampreys: A Great Lakes Invader*. http://www.glfc.org/lampcon.php (accessed May2010).

3. http://www.arkive.org/sea-lamprey/petromyzon-marinus/ (accessed May 2010)

4. www.in.gov/dnr/files/SEA_LAMPREY1.pdf (accessed May 2010)

5. Photo courtesy of new York State Department of Environmental Conservation

6. Sea Lamprey. http://www.seagrant.wisc.edu/greatlakesfish/sealamprey.html (accessed June 2005)

7. Great Lakes Fishery Commission. *Fact Sheet 3. Sea Lamprey: A Great Lakes Invader*, Ann Arbor, MI, 2000.

8. http://www.dfo-mpo.gc.ca/science/Publications/article/img/sea_lamprey.gif (accessed April 2010)

9. Photo courtesy of Great Lakes Fishery Commission.

10. Great Lakes Fishery Commission. *Fact Sheet 1. Sea Lamprey: A Great Lakes Invader*, Ann Arbor, MI, 2000.

11. http://www.umesc.usgs.gov/invasive_species/sea_lamprey/images/great_lakes_map_200.gif

12. http://www.iisgcp.org/EXOTICSP/images/pe_marin.gif

13. Great Lakes Fishery Commission. *Fact Sheet 5. Sea Lamprey Barriers: New Technologies Help Solve and Old Problem*, Ann Arbor, MI, 2000.

14. Great Lakes Fishery Commission. *Fact Sheet 6. Sea Lamprey Control – SterileMaleReleaseTechnique: An Innovative Sea Lamprey Control Method*, Ann Arbor, MI, 2000.

15. Siefkes, M. J.; Bergstedt, R. A.; Twohey, M. B.; Li, W. *Can. J. Fish. Aquat. Sci.* **2003**, *60*, 2331.

16. Great Lakes Fishery Commission. *Fact Sheet 4. TFM and Sea Lamprey Control: A Success Story*, Ann Arbor, MI, 2000.

17. http://www.umesc.usgs.gov/invasive_species/sea_lamprey/images/sea_lamprey_lake_trout_620.gif

18. Technical Assistance Provided to the Great Lakes Fishery Commission for Lampricides. http://www.umesc.usgs.gov/invasive_species/sea_lamprey/tech_assistance.html (accessed June 2010).

19. Great Lakes Fishery Commission. *Fact Sheet 9. International Sea Lamprey Management: On the St. Mary's River*; Ann Arbor, MI, 2000.

20. Great Lakes Fishery Commission. *Strategic Vision of the Great Lakes Fishery Commission for the First Decade of the New Millennium*, Ann Arbor, MI, 2001.

21. USGS Great Lakes Science Center, Invasive Fish, Sea Lamprey. http://www.glsc.usgs.gov/main.php?content=research_lamprey (accessed June 2010).

22. Polkinghorne, C. N.; Olson, J. M.; Gallaher, D. G.; Sorensen, P. W. *Fish Physiol. Biochem.* **2001**, *24*, 15-30.

23. (a) Yun, S. -S.; Scott, A. P.; Bayer, J. M.; Seelye, J. G.; Close, D. A.; Li, W. *Steroids* **2003**, *68*, 515-523. (b) Li, W.; Scott, A. P.; Siefkes, M. J.; Yan, H. Liu, Q.; Yun, S. – S.; Gage, D. A. *Science* **2002**, *296*, 138-141.

24. (a) Wagner, C. M.; Jones, M. L.; Twohey, M. B.; Sorensen, P. W. *Can. J. Fish. Aquat. Sci.* **2006**, *63*, 475-479. (b) Johnson, N. S.; Siefkes, M. J.; Li, W. *North Am. J. Fish. Man.* **2005**, *25*, 67-72.

25. (a) Venkatachalam, K. V. *BioEssays* **2005**, *27*, 222-228. (b) Venkatachalam, K. V. **2003** *Project Completion Report*, Great lakes Fishery Commission. (c) Collodi, P. **2000** *Project Completion Report*, Great lakes Fishery Commission.

26. The systematic nomenclature of Fieser and Fieser, Steroids, Rheinhold Publishing Corp., New York, 1959.

27. Kallner, A. *Acta Chem. Scand.* **1967**, *21*, 322-328.

28. Oppenauer, R. V. *Recl. Trav. Chim. Pays-Bas* **1937**, *56*, 137-144.

29. Iida, T.; Momose, T.; Nambara, T.; Chang, F. C.; Nambara, T. *Chem. Pharm. Bull.* **1986**, *34*, 1934-1938.

30. Tserng, K.-Y.; Klein, P. D. *Steroids* **1977**, *29*, 635-647.

31. Leppik, R. A. *Steroids* **1983**, *41*, 475-484.

32. Zhu, X.; Amouzou, E.; Mclean, S. *Can. J. Chem.* **1987**, *65*, 2447-2449.

33. (a) Barton, D. H. R.; Lester, D. J.; Ley, S. V. *J. Chem. Soc Chem. Commun.* **1978**, 130. (b) Barton, D. H. R.; Godfrey, C. R. A.; Morzycki, J. W.; Motherwell, W. B.; Ley, S. V. *J. Chem. Soc. Perkin Trans. 1* **1982**, 1947.

34. Iida, T.; Nishida, S.; Chang, F. C.; Niwa, T.; Goto, J.; Nambara, T. *Chem. Pharm. Bull.* **1993**, *41*, 763-765.

35. Iida, T.; Shinohara, T.; Nishida, S.; Goto, J.; Nambara, T.; Chang, F. C. *J. Lipid Res.* **1988**, *29*, 1097-1101.

36. a) Mitra, M. N.; Elliott, W. H. *J. Org. Chem.* **1968**, *33*, 175-181. b) Mitra, M. N.; Elliott, W. H. *J. Org. Chem.* **1968**, *33*, 2814-2818.

37. Chakravarti, D.; Chakravarti, R. N.; Mitra, M. N. *Nature* **1962**, *193*, 1071.

38. Iida, T.; Tamura, T.; Matsumoto, T.; Chang, F. C. *J. Lipid Res.* **1985**, *26*, 874-881.

39. Mitra, M. N.; Elliott, W. H. *J. Org. Chem.* **1969**, *34*, 2170-2175.

40. Mosingo, R. *Organic Syntheses, Coll. Vol. III*, John Wiley and Sons, Inc., New York, N. Y., 1955, p 181.

41. Personal discussion with Professor William Reusch at Michigan State University.

42. Dodson, R. M.; Muir, R. D. *J. Am. Chem. Soc.* **1961**, *83*, 4631-4635.

43. This procedure was performed by Norberg, Anna Monica, *unpublished results*.

44. This procedure was performed by Dr. Pellerito, Andrea M.

45. Tserng, K.-Y. *J. Lipid Res.* **1978**, *19*, 501-504.

46. Retizon, M.; Golfier, M. *C. R. Acad. Sci. Ser. C.* **1968**, *267*, 900-903.

47. (a) Zhang, D.-H.; Cai, F.; Zhou, X.-D.; Zhou, W.-S. *Org. Lett.* **2003**, *5*, 3257-3259. (b) Zhang, D.-H.; Cai, F.; Zhou, X.-D.; Zhou, W.-S. *Chin. J. Chem.* **2005**, *23*, 176-181.

48. (a) Nicolaou, K. C.; Montagnon, T.; Baran, P. S. *Anew. Chem. Int. Ed.* **2002**, *41*, 1386-1389. (b) Nicolaou, K. C.; Montagnon, T.; Baran, P. S.; Zhong, Y.-L. *J. Am. Chem. Soc.* **2002**, 124, 2245-2258.

49. (a) Sorensen, P. W.; Fine, J. M.; Dvornikovs, V.; Jeffrey, C. S.; Shao, R.; Wang, J.; Vrieze, L. A.; Anderson, K. R.; Hoye, T R. *Nature Chem. Biol.* **2005**, *1*, 324-328. (b) Hoye, T. R.; Dvornikovs, V.; Fine, J. M.; Anderson, K. R.; Jeffrey, C. S.; Muddiman, F. S.; Sorensen, P. W.; Wang, J. *J. Org. Chem.* **2007**, *72*, 7544-7550.

50. Frigerio, M.; Santagostino, M.; Sputore, S. *J. Org. Chem.* **1999**, *64*, 4537-4538.

51. (a) Bouveault, L.; Blanc, G. *Compt. Rend.* **1903**, *136*, 1676. (b) Bouveault, L.; Blanc, G. *Bull. Soc. Chim. France* **1904**, *31*, 666.

52. (a) Watanabe, H.; Takano, M.; Umino, A.; Ito, T.; Ishikawa, H.; Nakada, M. *Org. Lett.* **2007**, *9*, 359-362. (b) Davies, S. G.; Rodriquez-Solla, H.; Tamayo, J. A.; Cowley, A. R.; Concellon, C.; Garner, A. C.; Parkes, A. L.; Smith, A. D. *Org. Biomol. Chem.* **2005**, *3*, 1435-1447.

53. Girard, P.; Namy, J. L.; Kagan, H. B. *J. Am. Chem. Soc.* **1980**, *102*, 2693-2698.

54. (a) Akamanchi, K. G.; Patel, H. C.; Meenakshi, R. *Synth. Commun.* **1992**, *22*, 1655-1660. (b) Lambeth, D. O.; Palmer, G. *J. Biol. Chem.* **1973**, *248*, 6095-6103. (c) Dhillon, R. S.; Singh, R. P.; Kaur, D. *Tetrahedron Lett.* **1995**, *36*, 1107-1108. (d) Camps, F.; Coll, J.; Riba, M. *J. Chem. Soc,. Chem. Commun.* **1979**, 1080-1081. (e) Brosa, C.; Rodriguez-Santamarta, C. *Tetrahedron* **1999**, *55*, 1793-1798.

55. Crabtree, R. H.; Quirk, J. M. *Tetrahedron Lett.* **1981**, *22*, 303-306.

56. Jurkauskas, V.; Sadighi, J. P.; Buchwald, S. L. *Org. Lett.* **2003**, *5*, 2417-2420.

57. McQuaid, K. M.; Sames, D. *J. Am. Chem. Soc.* **2009**, *131*, 402-403.

58. Iversen, T.; Bundle, D. R. *J. Chem. Soc. Chem. Commun.* **1981**, 1240-1241.

59. Satoh, J. Y.; Yokoyama, C. T.; Haruta, A. M.; Nishizawa, K.; Hirose, M,; Hagitani, A. *Chem. Lett.* **1974**, 1521-1522.

60. (a) Zhang, X.; Rao, M. N.; Jones, S. R.; Shao, B.; Feibush, O.; McGuigan, M.; Tzodikov, N.; Feibush, B.; Shrkansky, I.; Snyder, B.; Mallis, L. M.; Sarkahian, A.; Wilder, S.; Turse, J. E.; Kinney, W. A. *J. Org. Chem.* **1998**, *63*, 8599-8603. (b) Kinney, W. A.; Zhang, X.; Williams, J. I.; Johnston, S.; Michalak, R. S.; Deshpande, M.; Dostal, L.; Rosazza, J. P. N. *Org. Lett.* **2000**, *2*, 2921-1922. (c) Liu, Y.; Lien, I. F.; Ruttgaizer, S.; Dove, P.; Taylor, S. D. *Org. Lett.* **2004**, *6*, 209-212.

61. Johnson, N. S.; Yun, S.-S.; Thompson, H. T.; Brant, C. O.; Li, W. *PNAS* **2009**, *106*, 1021-1026.

62. Oishi, T.; Ootou, K.; Shibata, H.; Murata, M. *Tetrahedron Lett* **2010**, *51*, 2600-2602.

63. Myer, A. G.; Zheng, B. *Tetrahedron Lett.* **1996**, *37*, 4841.

64. Gemal, A. L.; Luche, J.-L. *J. Am. Chem. Soc.* **1981**, *103*, 5454.

65. Silva, E. J.; Melo, M. L.; Neves, A. S. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1649.

66. Royals, E. E.; Leffingwell, J. C. *J. Org. Chem* **1966**. *31*, 1937.

67. Fieser, L. F.; Rajagopalan, S. *J. Am. Chem. Soc.* **1949**, *71*, 3935-3938.

68. http://www.graceraney.com/Slurry%20Grades.htm (accessed June 2010).

69. Petrini, M.; Ballini, R.; Marcantoni, E. *Syn. Commun.* **1988**, *18*, 847-853.

70. Afla Aesar

71. Aher, N. G. *Bioorg. & Med. Chem. Lett.* **2009**, *19*, 5411-5414.

72. Fujii, T *J. Biochemistry* **1957**, *44*, 383-387.