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Dihalomethyl carbinol intermediates in natural product synthesis

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Dihalomethyl carbinol intermediates in natural product synthesis

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This is to certify that the Doctoral dissertation of

Xuemei Wang

has met the dissertation requirement of Iowa State University

Signature was redacted for privacy.

Major Professor

Signature was redacted for privacy.

For the Major Program

Signature was redacted for privacy.

For the Graduate College

DEDICATION

To my parents

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ABBREVIATIONS

DMAP	*N,N*-dimethylpiperidine
LDA	Lithium diisopropylamide
LiTMP	Lithium 2,2,6,6-tetramethylpiperidine
MsCl	Methanesulfonic chloride
PTSA	*p*-Toluenesulfonic acid
TBDMSCl	*tert*-Butyldimethylsilyl chloride
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMSCl	Trimethylsilyl chloride

GENERAL INTRODUCTION

Introduction

Organic synthesis is a highly developed, versatile and interdisciplinary branch of the natural sciences. Modern synthetic methods have allowed us to synthesize all kinds of natural and artificial compounds. In the past two decades, with the advances of modern molecular biology and molecular medicine, organic synthesis has been largely employed to synthesize natural compounds and their analogs that have biological activities. A large number of such synthetic compounds have already contributed on the advent of modern medicines. With the discoveries of more and more natural products with potent medicinal applications, organic synthesis will participate more widely and deeply in the revolution of molecular medicine. In order to make organic synthesis more broadly used in large quantity production, new synthetic routes have to be developed constantly for efficiency, economical, and environmental concerns.

Hydroxy hemiacetal structures are widely present in naturally occurring compounds. The conventional and general synthetic route to synthesize hydroxy hemiacetals involves the use of sulfur compounds and mercury salts, which are environmentally inconvenient. This dissertation is to achieve new basic methods involving dihalomethyl carbinol compounds as synthetic intermediates for hydroxy hemiacetal syntheses. By using this method, several natural products have been synthesized, which include sesquiterpene-based endothelin receptor antagonists, phytuberin, aflatoxin M_2 and a tricyclic hydroxy hemiacetal subunit of azadirachtin.

Dissertation organization

This dissertation is composed of five publishable manuscripts. The numbering system adopted for the compounds, schemes and references is independent in each chapter. Although I am the second author in these manuscripts, I am the person principally involved in the data collection, the data analysis, and the writing of the papers.

The first chapter includes some fundamental aspects of the addition of dihalomethyl lithium to carbonyl compounds and the hydrolysis of dihalomethyl carbinol intermediates. The second chapter of the dissertation presents the synthesis of new endothelin receptor antagonists via dibromomethyl carbinol intermediates. The third chapter of the dissertation demonstrates an enantioselective total synthesis of phytuberin, which is a stress metabolite from tomato and tobacco. The fourth chapter of the dissertation illustrates the synthesis of aflatoxin M_2, which is a mycotoxin from milk. The fifth chapter describes the synthesis of the tricyclic hydroxy acetal unit of azadirachtin, a potential pesticide from the Indian neem tree. There is a general conclusion at the end of this dissertation.

CHAPTER I

DIHALOMETHYL CARBINOL INTERMEDIATES IN ORGANIC SYNTHESIS

A paper to be submitted to the *Journal of Organic Chemistry*

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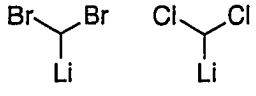
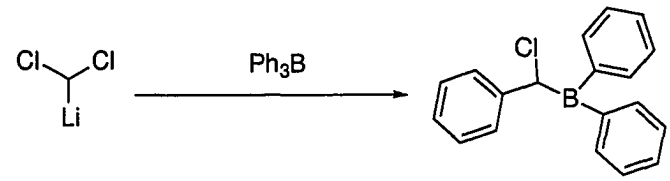
Dihalomethyl carbinol intermediates

Dihalomethyl carbinol compounds are not commonly used as organic intermediates, because of the instability of dihalomethyl lithium reagent and the alkoxides resulting from the addition to carbonyl compounds. It is a challenging task to prepare dihalomethyl lithium reagents.



In 1967, Köbrich[1] first reported the generation of dichloromethyl lithium. Dichloromethyl lithium was prepared from methylene chloride and *n*-butyl lithium in a Trapp mixture (THF: Hexane: Ether 4:1:1) at –115°C. Although the anion could be generated in a minute, dichloromethyl lithium is not stable and easily decomposed when the temperature rose. One purpose of generating dichloromethyl lithium was to produce α-chlorobenzyl diphenylboron[1] (Scheme 1). Since then, making boron reagents has

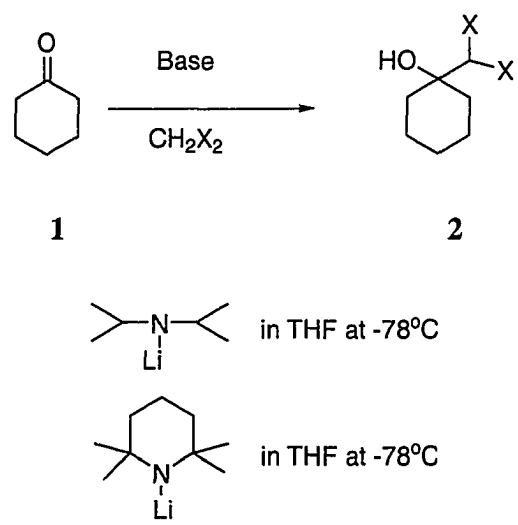
Scheme 1



become the most common application of dichloromethyl lithium over the past thirty years.[2-4]

A more practical procedure was developed for the homologation of boronic esters. Brown and coworkers[5] utilized *in situ* formation of dichloromethyl lithium at–78°C from methylene chloride and base, followed by *in situ* reduction of the α-chloroboronic ester intermediates with potassium triisopropoxyborohydride. This procedure was more practical for large-scale applications and avoided both the low temperature (-100°C) and the use of exactly one equivalent of alkyllithium required by

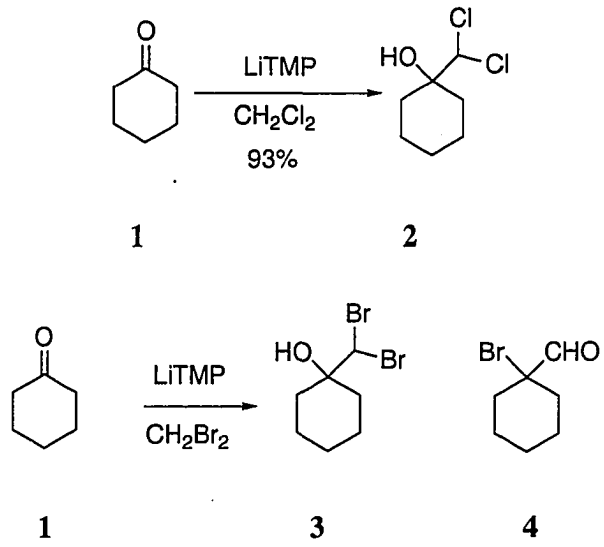
Scheme 2



the earlier procedures.[2-4]

Dichloromethyl lithium addition to carbonyl groups has been rarely reported. Taguchi[6] tried to generate dichloromethyl lithium by altering reaction conditions. In his protocols, dichloromethyl lithium was generated in the presence of different bases. By

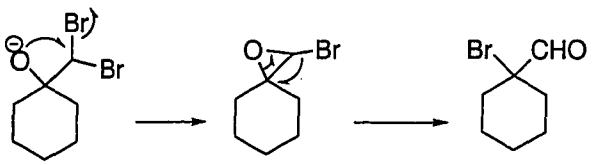
Scheme 3



adding the bases into the mixture of the carbonyl compounds and dihalomethane, the yield of the addition reaction was improved. However, the application of the addition of dihalomethyl lithium to carbonyl compounds in synthesis is still limited.

In our model study with cyclohexanone, lithium diisopropylamide (LDA) and lithium tetramethylpiperidine (LiTMP) were employed in the reaction (Scheme 2). Compared with LDA, LiTMP gave a better yield. When the mixture of cyclohexanone and dihalomethane in dry tetrahydrofuran was treated with LDA or LiTMP at –78°C, followed by working up the reaction at the low temperature, dihalomethyl carbinols can be isolated. Both methylene chloride and methylene bromide gave a high yield of the

Scheme 4

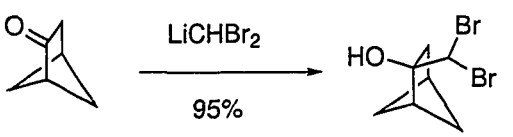


5 **6** **4**

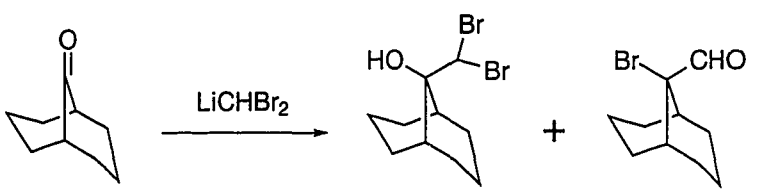
addition products. When the temperature of the reaction mixture rose, especially during the work-up period, a by-product could always be isolated. This by-product was identified to be an α-haloaldehyde.

A possible pathway from the lithium alkoxide to the α-haloaldehyde is described in Scheme 4. The mechanism of the reaction appears to be an intramolecular displacement to give a bromoepoxide, which in turn rearranges to an α-haloaldehyde.

Scheme 5



7 **8**



52% 36%

9 **10** **11**

Murray and coworkers[7] also indicated that the reaction of various bicyclic or tricyclic ketones with dibromomethyl lithium provides a dibromomethyl carbinol and/or α-haloaldehyde (Scheme 5). The reaction of dibromomethyl lithium with bicyclo[2,1,1] hexan-2-one (**7**) gave only 2-(dibromomethyl)bicyclo[2,1,1] hexan-2-ol (**8**) in 95% yield. The reaction of dibromomethyl lithium with ketone **9** gave both 9-(dibromomethyl)bicyclo[3,3,1]nonane-9-ol (**10**) and 9-bromobicyclo[3,3,1]nonane-9-carboxaldehyde (**11**) in yields of 52 and 36% respectively. The product ratios seemed to be determined by the steric interactions.

As these steric interactions increase, the proportion of α-haloaldehyde in the product mixture increases accordingly. In our studies, using methylene chloride often can avoid (or at least minimize) the yield of the α-haloaldehyde, because of the lower reactivity of the dichloroalkoxide. Therefore, in the most of the cases, dichloromethyl lithium was chosen in our synthetic studies.

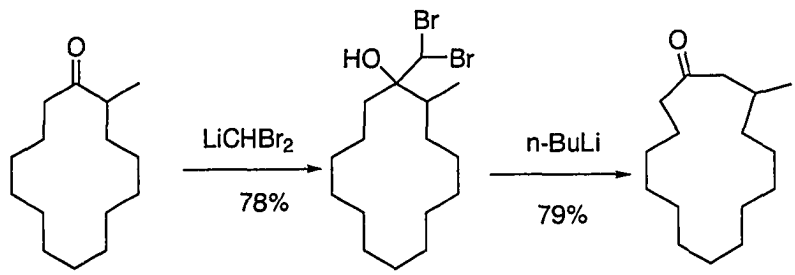
Hydrolysis of the Dihalomethyl carbinols

One of the known transformations from the dihalomethyl carbinol to other products includes one-carbon ring expansion of cyclic ketones.[8-10] When dibromomethyl carbinol **13** was transformed to the halogen-free ketone **14** upon treatment with *n*-butyllithium, α–methyl substituted ketone **12** was exclusively converted to the β-methyl substituted homologue. Therefore, dl-muscone was synthesized from cyclotetradecanone according to this new method (Scheme 6).[11]

Glycinoeclepin A, a natural hatching stimulus for the soybean cyst nematode, was also synthesized through the ring expansion method described above.[12]

Another transformation of dihalomethyl carbinols was for the preparation of haloolefins.[13] Aliphatic, alicyclic, and aromatic 1-bromoalkenes were prepared by the addition of dibromomethyl lithium to aldehydes or ketones, followed by reductive elimination with zinc and acetic acid (Scheme 7).[14]

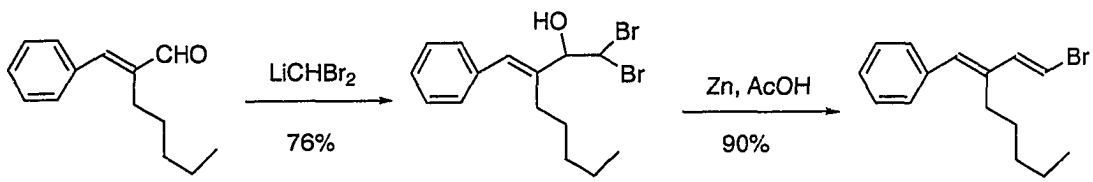
Scheme 6



12 **13** **14**

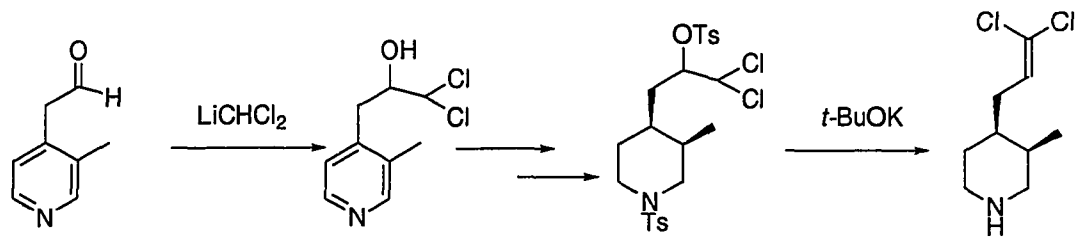
A similar example is shown in Scheme 8. Pyridine aldehyde **18** was reacted with dichloromethyl lithium to afford dichloropiperidinylpropanol **19**. Compound **19** was then converted into the 1,1-dichloro olefin **20** via hydrogenation, tosylation and elimination.[15]

Scheme 7



15 **16** **17**

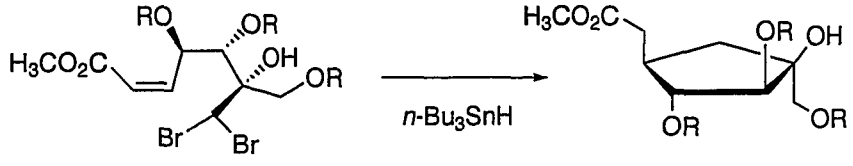
Scheme 8



18 **19** **20**

The third application of dihalomethyl carbinols is in radical reactions to synthesize carbocyclic analogs of D-fructofuranose and D-fructofuranose-6-phosphate (Scheme 9). The synthetic process features a stereospecific addition of dibromomethyl lithium to an unsaturated ketose and cyclization of an intermediate **22** via a radical generated from the unsaturated geminal dibromide.[16]

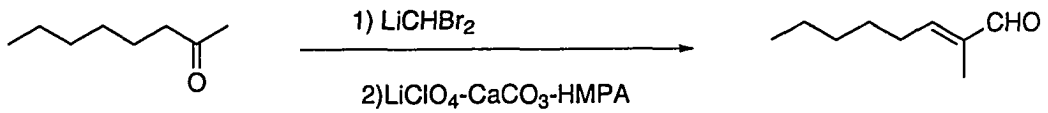
Scheme 9



22 **23**

One additional application of dihalomethyl carbinols is to generate α, β – unsaturated aldehydes or ketones.[17] Reaction of cycloalkanones with dichloromethyl lithium and dehydrochlorination of the α-chloro aldehyde gave 1-cycloalkene-carboxaldehydes (Scheme 10). This method can also be used in acyclic systems. A similar reaction of 2-octanone (**24**) gave α,β–unsaturated aldehyde **25** regio- and stereo-

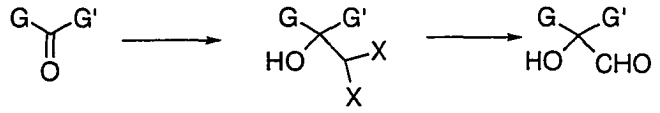
Scheme 10



24 **25**

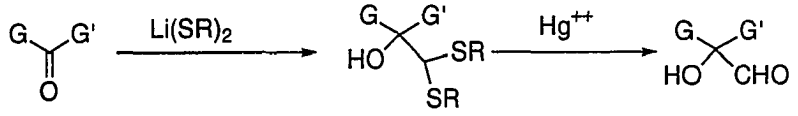
selectively.[18]

Our synthetic goal is to make α-hydroxy aldehydes via dihalomethyl carbinols. Although there were a few examples of the generation of dihalomethyl carbinols in the literature, the hydrolysis of dihalomethyl carbinols to α-hydroxy aldehydes is almost unknown.



The current method from carbonyl compounds to α-hydroxy aldehydes involves the addition of the anion of 1,3-dithianes to carbonyl compounds,[19] followed by hydrolysis with mercury salts (Scheme 11).[20] The disadvantages of this method are that it is environmentally unattractive and that some dithianes cannot be hydrolyzed.

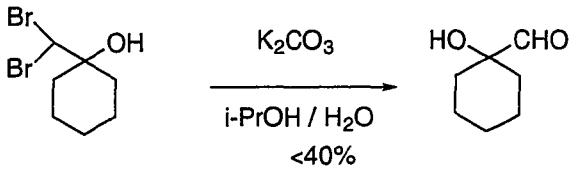
Scheme 11



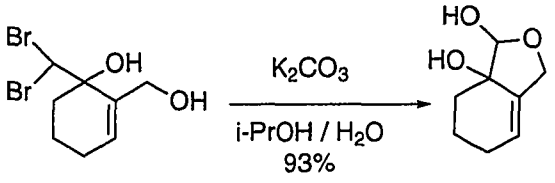
26 **27** **28**

Our initial idea for the hydrolysis of the dichloromethyl carbinols involved silver salts and bases. Silver triflate, silver nitrate and silver borontetraflouride were used to attempt the conversion of both dichloromethyl and dibromomethyl carbinols. Unfortunately, all of these attempts failed.

Scheme 12



3 **29**



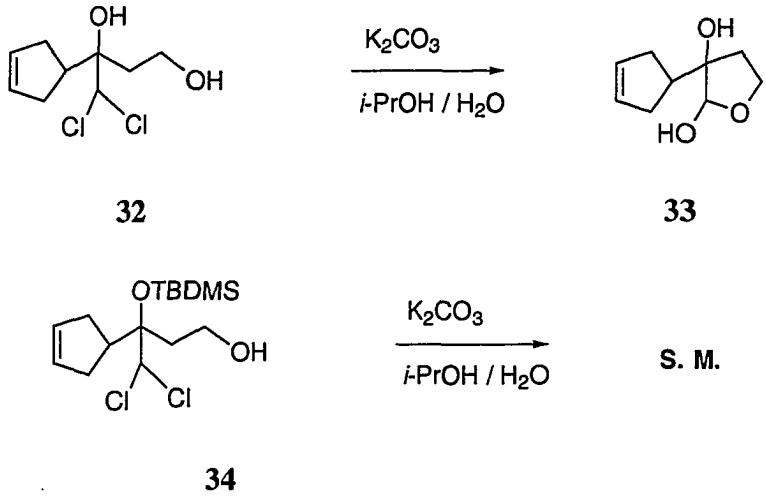
30 **31**

Only one paper reported the generation of α-hydroxy aldehyde from basic hydrolysis of a dichloromethyl carbinol in a modest yield (approximately 33%).[21] By-products, such as α-chloroaldehydes and α,β-unsaturated aldehydes, were produced in variable yields. Even though the α-hydroxy aldehyde was produced under basic conditions, the resulting hydroxy aldehyde easily dimerized. The hydroxy aldehyde dimer required a complicated procedure to convert it back to the monomer.

Since the proximate primary alcohol in **30** might, through neighboring group participation, enhance the hydrolysis and also protect the resulting aldehyde as a hemiacetal, we examined the base-mediated hydrolysis of compound **30** (Scheme 12).

Compared with the simple dibromomethyl carbinol **3**, the basic hydrolysis of δ-hydroxy compound **30** can be completed in a shorter time and higher yield. Under our conditions, the resulting hydroxy hemiacetals are very stable. The corresponding dichloromethyl carbinols gave similar results under the basic hydrolysis conditions.

Scheme 13



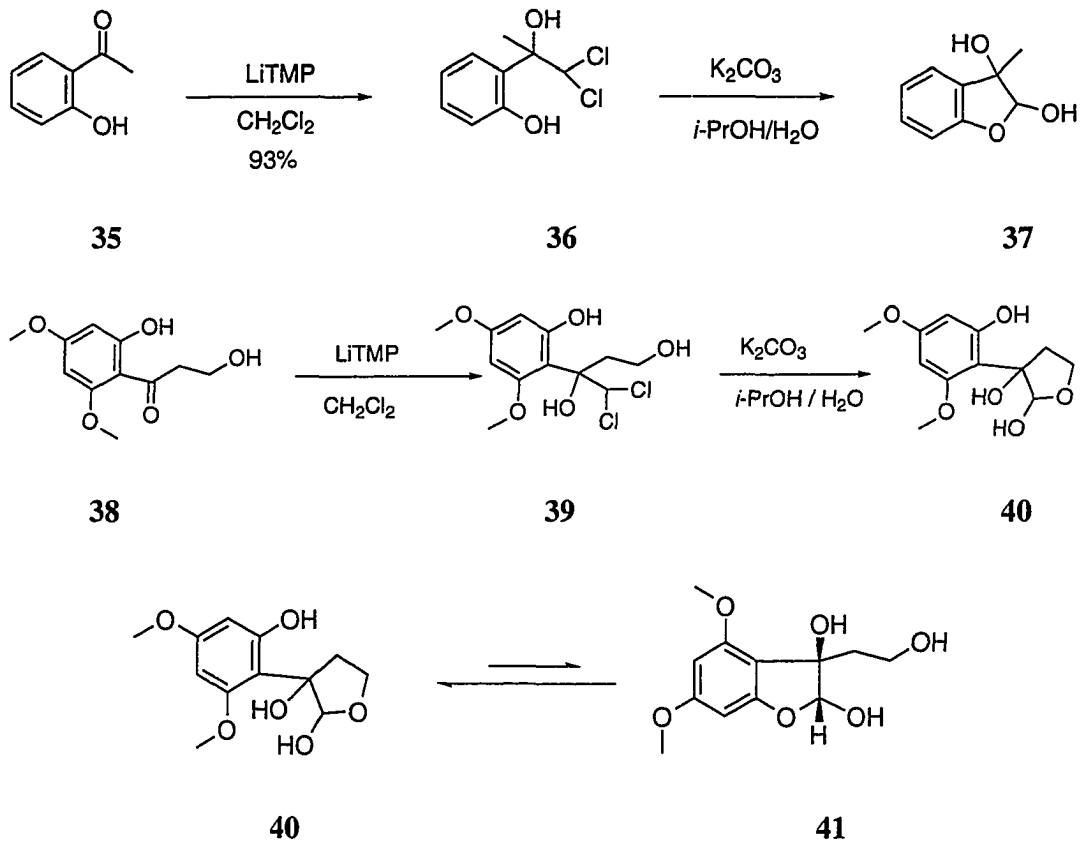
32 **33**

34

The mechanism of the hydrolysis appears to be via a chloroepoxide intermediate. One reaction carried out in the synthesis of an azadirachtin intermediate supports this proposed mechanism (Scheme 13). When compound **32**, which has a free tertiary alcohol in the molecule, was treated with potassium carbonate in aqueous isopropanol, hydroxy hemiacetal **33** was produced in 78% yield. However, when the tertiary alcohol was protected with a *tert*-butyldimethylsilyl group, compound **34** was stable in the basic solution, even when the reaction mixture was heated to 60°C. This experiment supports our belief that the tertiary hydroxyl group is involved in the hydrolysis step.

Besides primary alcohols, a neighboring aromatic hydroxyl group also

Scheme 14



35 **36** **37**

38 **39** **40**

40 **41**

participates in the hydrolysis. As shown in Scheme 14, the dichloromethyl carbinol **36** was hydrolyzed under the basic conditions to afford the hydroxy hemiacetal **37** in 70% yield. When both primary and aromatic hydroxyl groups are present in the molecule, compound **40** was the only hydroxy hemiacetal isolated from dichloromethyl carbinol **39**. One possible reason is that the primary alcohol assists the hydrolysis in compound **39**. Another reason could be that the product **40** is more thermodynamically stable compared to the other possible product **41**.

In summary, the transformation of carbonyl compounds to dihalomethyl carbinol intermediates, followed by δ-hydroxyl assisted hydrolysis of these dihalomethyl

carbinols to hydroxy hemiacetals, offers an extremely direct and efficient way for one-carbon elongation. It also offers a new application of dihalomethyl lithium compounds in organic synthesis. We envisioned that this method could be used to synthesize highly functionalized natural products.

As part of our study of the synthetic potential of dihalomethyl carbinol intermediates in organic synthesis, we have explored several synthetic routes to naturally occurring compounds with interesting biological activity. In this dissertation, we will concentrate on the direct synthesis of endothelin inhibitors, phytuberin, aflatoxin M_2 and azadirachtin.

Experimental

Unless otherwise noted, materials were obtained from commercial suppliers and were used without purification. THF and diethyl ether were distilled from sodium benzophenone ketyl. Dichloromethane was distilled from calcium hydride. Unless otherwise noted, all of the reactions were conducted under an argon atmosphere. All organic extracts were dried over anhydrous magnesium sulfate. Infrared spectra were obtained on a Perkin-Elmer model 1320 spectrophotometer. Nuclear magnetic resonance spectra were determined on either a Varian 300 or a Bruker 400 spectrometer. All chemical shifts are reported relative to tetramethylsilane as an internal standard. Coupling constants are reported in Hz. High-resolution mass spectra were recorded on a Kratos model MS-50 spectrometer. Low-resolution mass spectra were obtained from a Finnegan 4023 mass spectrometer. Flash chromatography was performed on silica gel Kieseldel 60 (mesh 230-400).

A typical procedure for the addition of dihalomethyl lithium to carbonyl compounds is described as followed:

To a solution of carbonyl compound (10 mmol) and dihalomethane (50 mmol) in tetrahydrofuran (50 mL), three to five equivalents of LiTMP [30 mmol, prepared from 2,2,6,6-tetramethylpiperidine (4.23 g, 30 mmol) and *n*-butyllithium (12 mL of 2.5 M solution in hexanes)], were added slowly at –78°C. The reaction mixture was stirred at –78°C for three hours and monitored by thin layer chromatography (TLC). After the reaction was complete, saturated ammonium chloride solution (15 mL) was added at –78°C. The reaction mixture was then extracted with diethyl ether, washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified with flash column chromatography, eluting with a mixture of hexanes and ethyl acetate, to give the pure dihalomethyl carbinol.

1-(Dichloromethyl)cyclohexanol (2): ^{1}H NMR ($CDCl_3$, δ): 5.65 (s, 1H), 4.65 (s, 1H), 1.2-2.5 (m, 10H). ^{13}C NMR ($CDCl_3$, δ): 82.5, 75.5, 33.5, 26.0, 22.5. IR (neat) cm^{-1}: 3318, 1050.

1-(Dibromomethyl)cyclohexanol (3): ^{1}H NMR ($CDCl_3$, δ): 5.65 (s, 1H), 4.65 (s, 1H), 1.2-2.5 (m, 10H). ^{13}C NMR ($CDCl_3$, δ): 75.5, 61.8, 34.0, 26.3, 22.9. IR (neat) cm^{-1}: 3350, 1050.

1-Bromocyclohexanecarboxaldehyde (4): ^{1}H NMR ($CDCl_3$, δ): 9.40 (s, 1H), 1.2-2.5 (m, 10H). ^{13}C NMR ($CDCl_3$, δ): 202.4, 68.4, 50.1, 25.0, 20.6. IR (neat) cm^{-1}: 1627. MS m/z (CI-NH_3): 190.

1-(Dibromomethyl)-2-(hydroxymethyl) cyclohex-2-enol (30): ^{1}H NMR ($CDCl_3$, δ): 6.10 (s, 1H), 6.03 (d, 1H), 4.55(br s, 1H), 4.50 (d, *J* = 3 Hz, 1H), 4.15 (d, *J* = 10.2 Hz, 1H), 1.5-2.3 (m, 6H). ^{13}C NMR ($CDCl_3$, δ): 134.0, 133.3, 75.6, 67.4, 55.6, 31.7, 25.8, 22.7.

1-(Dichloromethyl)-1-(cyclopent-3-enyl) propane-1,3-diol (32): ^{1}H NMR ($CDCl_3$, δ): *5.80* (s, *1H*), *5.69* (s, *2H*), *4.15* (m, *1H*), *3.97* (*s, 1H*), *3.77* (*br s, 1H*), *2.85* (*p, J = 6.4* Hz, 1H), 2.49 (m, 4H), 2.3 (m, 2H), 2.0 (m, 2H). ^{13}C NMR ($CDCl_3$, δ): 129.7, 129.5, 79.7, 78.8, 59.7, 45.3, 41.6, 34.7, 32.8.

1-(Dichloromethyl)-1-(hydroxyphenyl) ethanol (36): ^{1}H NMR ($CDCl_3$, δ): 8.19 (br s, 1H), 7.1-7.2 (m, 2H), 6.8-6.9 (m, 2H), 6.13 (s, 1H), 3.54 (br s, 1H), 1.87 (s, 3H). ^{13}C NMR ($CDCl_3$, δ): 154.6, 130.3, 128.4, 125.6, 120.6, 117.7, 81.0, 78.8, 23.1. IR (neat) cm^{-1}: 3318, 3201, 1584, 1492, 1456, 1231, 789, 751. MS m/z (CI-NH_3): 220, 167, 137, 119. HRMS m/z M+: 220.00557, calcd. for $C_9H_{10}O_2Cl_2$: 220.00579.

1-(Dichloromethyl)-1-(2-hydroxy-4, 6-dimethoxyphenyl) propane-1,3-diol (39): ^{1}H NMR($CDCl_3$, δ): 6.35 (s, 1H), 6.06 (d, *J* = 2.4 Hz, 1H), 6.98 (d, *J* = 2.4 Hz, 1H), 4.24 (m, 2H), 3.75 (s, 3H), 3.75 (s, 3H), 2.2-2.8 (m, 2H). ^{13}C NMR ($CDCl_3$, δ): 161.1, 156.9, 103.2, 95.3, 91.3, 86.0, 78.3, 60.7, 55.6, 37.7.

A typical procedure for the hydrolysis of dihalomethyl carbinols is described as followed:

To the dihalomethyl carbinol (100 mg) in a mixture of isopropanol (5 mL) and

water (5 mL), potassium carbonate (100 mg) was added and then stirred at room temperature from 1 to 12 hours. The reaction was monitored by TLC. After the reaction was complete, isopropanol was removed in vacuo. The aqueous solution was saturated with sodium chloride and extracted with ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with a mixture of hexanes and ethyl acetate) to give a pure compound.

1-Hydroxycyclohexanecarboxaldehyde (29): ^{1}H NMR ($CDCl_3$, δ): 9.49 (s, 1H), 1.2-2.5 (m, 10H). ^{13}C NMR ($CDCl_3$, δ): 204.0, 76.0, 41.8, 31.3, 26.9, 24.9, 20.4. IR (neat) cm^{-1}: 3450, 1715. MS m/z (CI-NH_3): 128.

1,7a-Dihydroxy-1, 3, 5, 6, 7, 7a-hexahydroisobenzofuran (31): ^{1}H NMR ($CDCl_3$, δ): 5.84 and 5.78 (s, 1H), 5.27 (d, J = 5.4 Hz) and 4.90 (d, J = 7.8 Hz) 1H, 4.61 and 4.59 (m, 1H), 4.50 and 4.23 (m, 1H), 3.95 (d, J = 7.8 Hz) and 2.51 (d, J = 3.6 Hz) 1H, 1.2-2.3 (m, 6H). ^{13}C NMR ($CDCl_3$, δ): 137.3 and 137.0, 124.9 and 124.2, 103.1 and 102.8, 76.4 and 71.8, 68.2 and 67.4, 30.0 and 27.9, 25.1 and 24.8, 17.5 and 17.4. (It is a mixture of two isomers with ratio 1.7 to 1.)

3-(Cyclopent-3-enyl)tetrahydrofuran-2, 3-diol (33): ^{1}H NMR ($CDCl_3$, δ): 5.66 (s, 2H), 5.03 (s, 1H), 4.60 (m, 1H), 3.47-3.95 (m, 2H), 1.5-3.0 (m, 7H). ^{13}C NMR ($CDCl_3$, δ): 130.1, 129.9, 99.7, 81.7, 65.6, 43.5, 36.9, 34.1, 33.7.

3-Methyl-2, 3-dihydrobenzofuran-2, 3-diol (37): ^{1}H NMR ($CDCl_3$, δ): 6.7-7.5 (m, 4H), 6.22 (s, 1H), 5.25 (br s, 1H), 3.26 (br s, 1H), 1.39 (s, 3H). ^{13}C NMR ($CDCl_3$, δ):

129.5, 129.3, 126.8, 124.7, 122.0, 117.3, 116.7, 97.8, 42.8.

3-(2-Hydroxy-4, 6-dimethoxyphenyl)-tetrahydrofuran-2, 3-diol (40): ^{1}H NMR ($CDCl_3$, δ): 6.05 (d, J = 2.4 Hz, 1H), 6.05 (d, J = 2.4 Hz, 1H), 5.22 (s, 1H), 4.2 (m, 2H), 3.82 (s, 3H), 3.77 (s, 3H), 2.5-2.8 (m, 2H). ^{13}C NMR ($CDCl_3$, δ): 216.2, 146.9, 111.0, 68.4, 50.1, 44.5, 43.5, 33.2, 25.0, 21.2, 20.6.

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CHAPTER II

SYNTHESIS OF SESQUITERPENE-BASED ENDOTHELIN RECEPTOR ANTAGONISTS

A paper to be submitted to the *Journal of Organic Chemistry*

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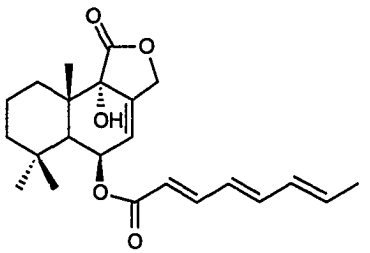
Introduction

Endothelins are a family of potent vasoactive peptides released by endothelial cells.[1] Endothelins exert their biological effects via activation of specific receptors.[2] Endothelin receptors are classified into two subtypes in mammals: ET_A and ET_B.[3] Both receptors are present in vascular smooth muscle to mediate vasoconstriction. ET_B receptors also locate on endothelial cells to mediate endothelium-dependent relaxation.[4]

It has been suggested that many human diseases were related to endothelins, such as hypertension, congestive heart failure, renal failure, ischemia and pulmonary hypertension.[5] Particularly, in many forms of the cadiovascular diseases, effective therapy is not yet available. The discovery of new endothelin receptor antagonists can lead to the development of the new drugs against heart diseases.

Most of the known endothelin receptor antagonists are peptide-like compounds.[6] One of the disadvantages of peptides is degradation after oral intake. Therefore, despite their potential value as pharmacological tools, the peptide-based endothelin receptor

Scheme 1



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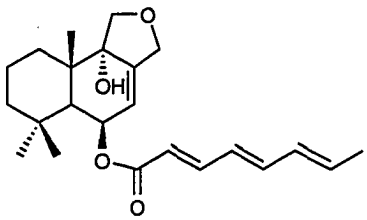

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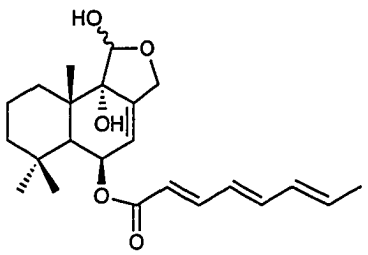

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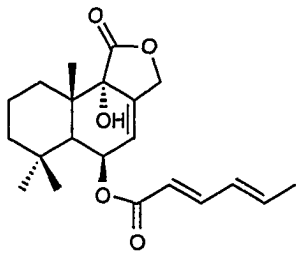


4

antagonists have limited pharmaceutical application. Hence, an important goal in the research new medication against heart disease is to discover non-peptide endothelin receptor antagonists.[7]

Recently, significant progress has been achieved in this area.[8-10] In 1996, novel non-peptide endothelin antagonists RES- 1149-1 and –2 were isolated from the culture broth of a fungus, *Aspergillus sp.*[11-13] RES-1149-1 and RES-1149-2 selectively prevented ET-1 from binding to ET_B receptor with IC_{50} values of 1.5 and 20 μM, respectively. RES-1149-2 was also isolated as a major component with six other novel drimane sesquiterpene esters from fermentation of *A. ustus pseudodeflectus*.[14]

Compounds **1** to **4** shown in Scheme 1 exhibited endothelin receptor binding inhibitory activity against rabbit endothelin A and rabbit endothelin B receptors with

IC_{50} values ranging from 20 to 150 μM. The IC_{50} value of the compounds **1** to **4** against endothelin receptors is listed in Table 1. These compounds had similar levels of binding activity with human endothelin A and endothelin B receptors.[14]

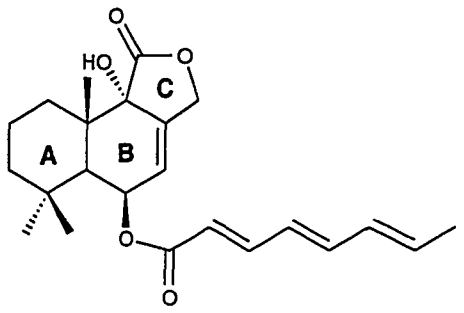
Although the significant biological activity of these endothelin receptor antagonists and their highly functionalized structures are interesting, the synthesis of these compounds hasn't been reported. However, these sesquiterpene-based endothelin receptor antagonists might be efficiently prepared using our synthetic method involving dihalomethyl carbinol intermediates.

Table 1: The IC_{50} value of the compound 1 to 4 against ET

Compound	Rabbit ET_A (μM)	Rabbit ET_B (μM)	Human ET_B (μM)	Human ET_B (μM)
1	155	50	135	73
2	80	55	112	61
3	65	21	62	41
4	50	70	>250	148

Results and discussion

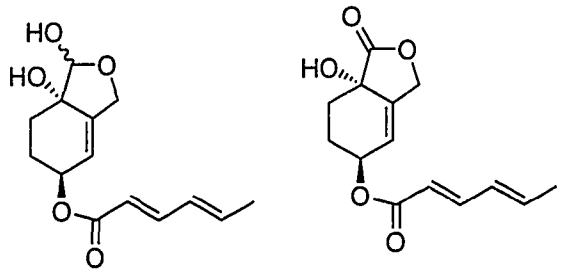
The significant biological activity of these endothelin receptor antagonists and their unique structure are of interest to us. Development of a direct synthetic route to this class of compounds would benefit the study of structure and function and may help lead to new potential chemtheropeutic agents. Since all the compounds in this series



have a simple A ring without any functionality, our expectation was that the A ring is not significant to their biological activity. Therefore, we tried to synthesize analogs of these endothelin receptor antagonists without ring A.

Two compounds (**5** and **6**) were chosen as our initial synthetic targets. Compound **5** contains the tertiary hydroxyl group and hemiacetal subunits of compound 1. Compound **6** is a hydroxy lactone analog.

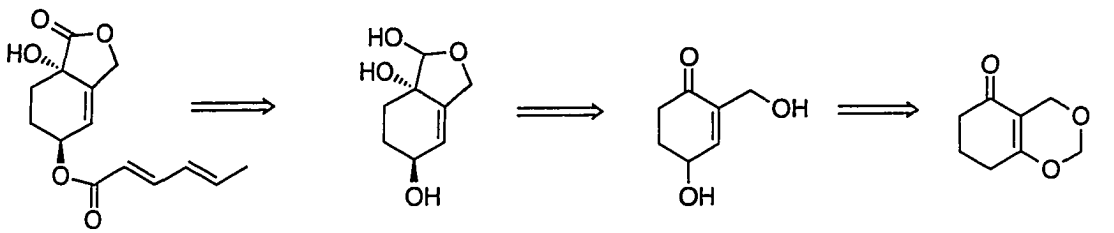
Scheme 2



5 **6**

Compounds **5** and **6** both have the five- membered ring, because the C-ring appears to be important for endothelin receptor binding activity.[14] Compared with the natural products, compounds **5** and **6** have lower molecular weights, which may make them better drug candidates. Our synthesis of these potential endothelin receptor

Scheme 3

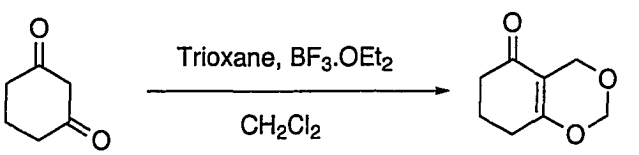


6 **19** **11** **7**

antagonists features the dibromomethyl lithium addition to an enone, followed by δ-alcohol assisted cyclization as the key step. In this retrosynthetic analysis (Scheme 3), compound **19** is a key intermediate, which can be derived from a dibromomethyl carbinol intermediate.

The synthesis started with cyclohexanedione using a method reported by Smith.[15] 1,3-Cyclohexanedione was treated with trioxane and boron triflouride etherate

Scheme 4

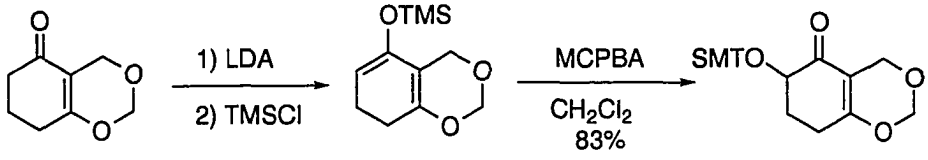


7

in methylene chloride solution. Vinylogous ether **7** was prepared in 84% yield under these conditions (Scheme 4).

The conversion from **7** to **9** is shown in Scheme 5. Compound **7** was treated with LDA at –78°C, but this anion could not be generated completely until the temperature was raised to –20°C. After quenching with trimethylsilyl chloride, enol silyl ether **8** was

Scheme 5

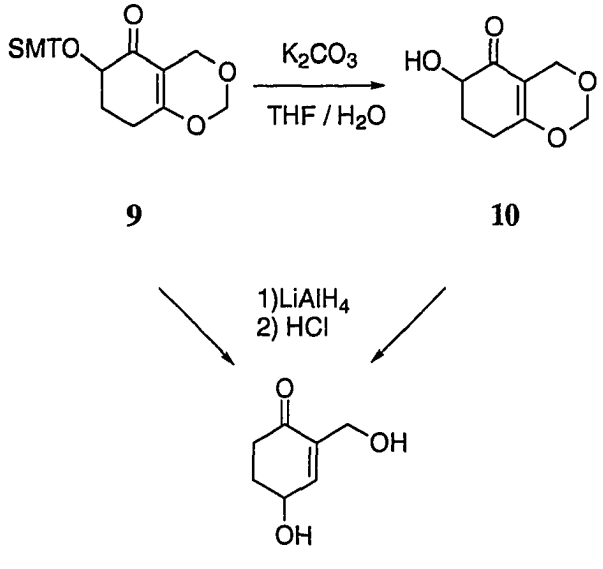


7 **8** **9**

prepared in 98% yield. Epoxidation of **8** with *m*-chloroperbenzoic acid gave compound **9** in 83% yield.

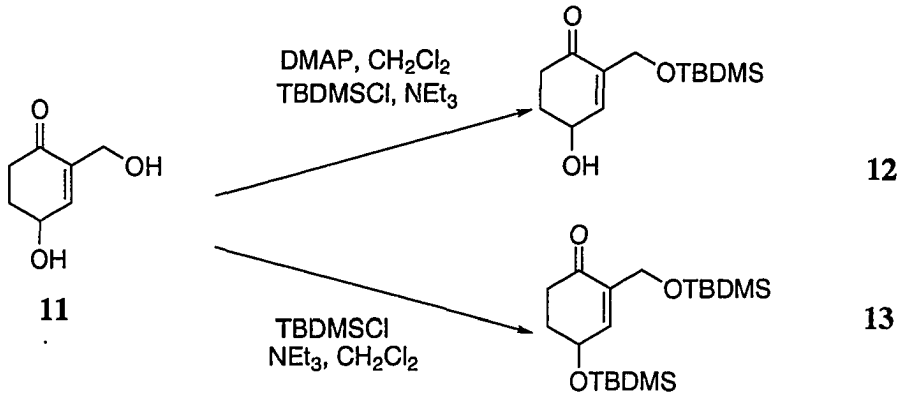
Under basic conditions, compound **9** can be deprotected to afford compound **10** (Scheme 6). Fortunately, both compound **9** and compound **10** can be hydrolyzed under acidic conditions.[15] In this process, α-hydroxy ketone was rearranged to compound **11**. Compound **11** was treated with different equivalents of *tert*-butyldimethylsilyl chloride to give two different addition precursors (Scheme 7). Diol **11** was treated with one

Scheme 6



equivalent of *tert*-butyldimethylsilyl chloride, triethylamine and a catalytic amount of DMAP in methylene chloride, and was selectively converted into protected secondary alcohol **12**. When diol **11** was treated with two equivalents of *tert*-butyldimethylsilyl chloride and triethylamine, compound **13** was achieved.

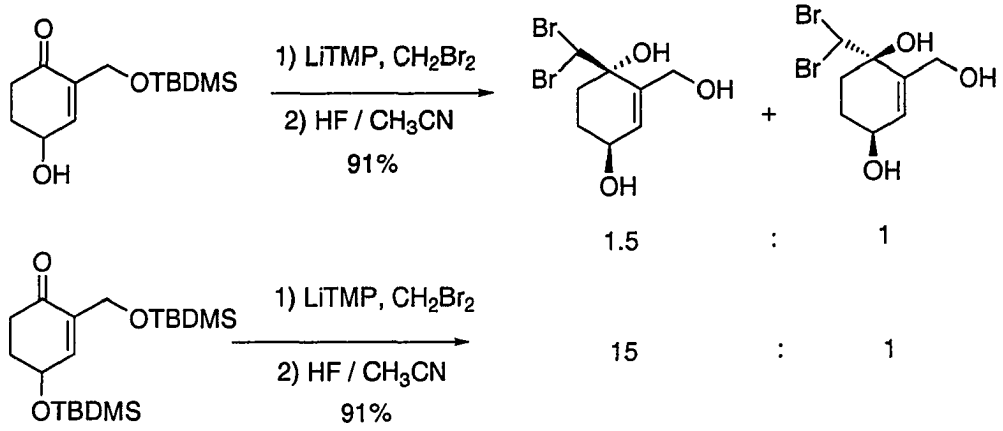
Scheme 7



The addition of dibromomethyl lithium[16] to compounds **12** and **13** gave different ratios of addition products (Scheme 8). When enone **12** was treated with dibromomethyl lithium followed by deprotection, the ratio between compounds **14a** and **14b** is 1.5 to 1. But when enone **13** was used as an addition substrate, the ratio between compounds **14a** and **14b** was increased to 15 to 1. The dramatic improvement in stereoselectivity is due to the bulky *tert*-butyldimethylsilyl group.

Hydrolysis of simple dibromomethyl carbinols in aqueous isopropanol gave only a modest yield of α-hydroxyaldehydes.[17] By-products, such as α-chloroaldehydes and α,β-unsaturated aldehydes, are often produced in comparable yields. In our model study to endothelin receptor antagonists, dibromomethyl carbinol **17** was chosen to investigate the effect of neighboring alcohol participation in the hydrolysis (Scheme 9).

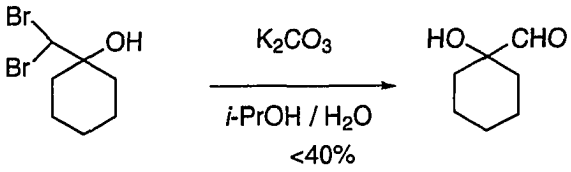
Scheme 8



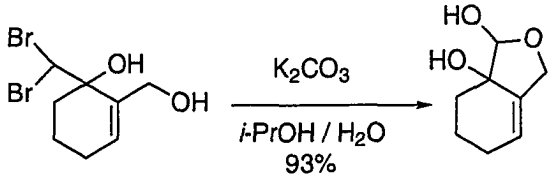
13 **14a** **14b**

Compared with the simple dibromomethyl carbinol **15**, the basic hydrolysis of δ-hydroxy dibromomethyl carbinol **17** can be completed in shorter time and higher yield. Under our conditions, the resulting hydroxy hemiacetals are very stable in aqueous basic conditions.

Scheme 9



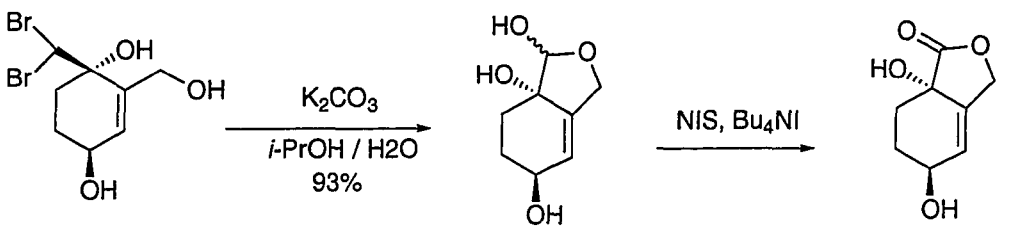
15 **16**



4 **18**

The primary alcohol in **17** enhanced the hydrolysis and also protected the resulting aldehyde as a hemiacetal. We have used this method in our synthesis of endothelin receptor antagonists. The key transformation from dibromomethyl carbinol to hemiacetal **19** is shown in Scheme 10. With the allylic hydroxymethyl group, this cyclization proceeds smoothly. The hemiacetal **20** can be selectively oxidized to a lactone under mild conditions.[18]

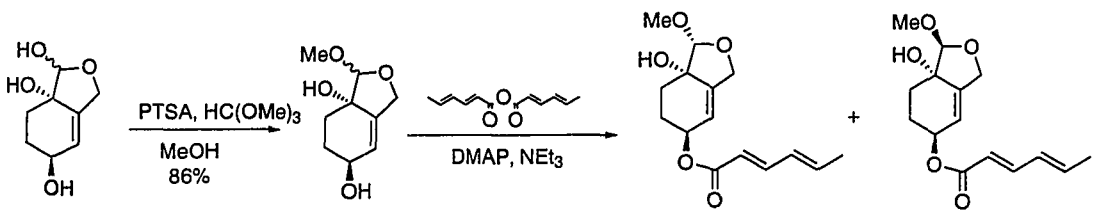
Scheme 10



14 **19** **20**

A direct esterification of compound **19** to compound **5** with the acid chloride of sorbic acid failed. The hemiacetal group in the compound **19** was protected with trimethyl orthoformate under the catalysis of PTSA in methanol (Scheme 11).[19] The protected compound **21** was treated with the anhydride of sorbic acid, which was made

Scheme 11

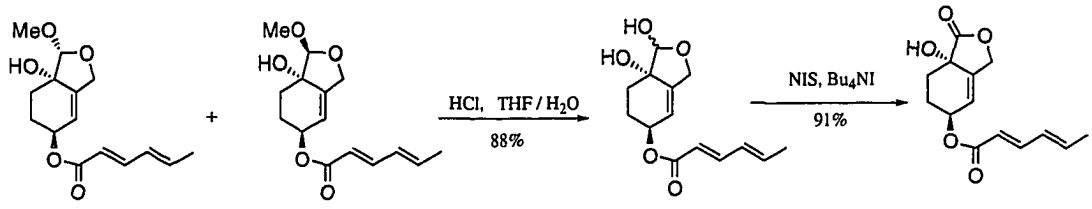


19 **21** **22a** **22b**

from sorbic acid and the corresponding acid chloride. Two diastereomers **22a** and **22b** were isolated from the esterification reaction by column chromatography. The ratio between these two isomers is 3 to 1.

Both diastereomers **22a** and **22b** hydrolyzed under acidic conditions to give hemiacetal **5** in a combined 88% yield (Scheme 12). The derived hemiacetal **5** was selectively oxidized to the lactone by treating **5** with *N*-iodosuccinimide and tetra-*n*-butyl ammonium iodide to afford lactone **6** in 91% yield.[18]

Scheme 12



22a **22b** **5** **6**

The synthesis of the target compound **6** was completed in 29% overall yield in 9 steps. It involved addition of dibromomethyl lithium, followed by hydroxyl-assisted hydrolysis of dibromomethyl carbinol, to a five-membered hemiacetal intermediate as a key transformation step. This transformation extends the application of dibromomethyl lithium in organic synthesis. This direct and efficient synthetic approach can be used to synthesize other highly functionalized terpenes. The biological activities of these potent endothelin receptor antagonists will be reported later.

Experimental

Unless otherwise noted, materials were obtained from commercial suppliers and were used without purification. THF and diethyl ether were distilled from sodium benzophenone ketyl. Dichloromethane was distilled from calcium hydride. Unless otherwise noted, all the reactions were conducted under an argon atmosphere. All organic extracts were dried over anhydrous magnesium sulfate. Infrared spectra were obtained on a Perkin-Elmer model 1320 spectrophotometer. Nuclear magnetic resonance spectra were determined on either a Varian 300 or a Bruker 400 spectrometer. All chemical shifts are reported relative to tetramethylsilane as an internal standard. Coupling constants are reported in Hz. High-resolution mass spectra were recorded on a Kratos model MS-50 spectrometer. Low-resolution mass spectra were obtained from a Finnegan 4023 mass spectrometer. Flash chromatography was performed on silica gel Kieseldel 60 (mesh 230-400).

4, 6, 7, 8-Tetrahydro-5*H*-1, 3-benzodioxin-5-one (7)

To a solution of trioxane (12.8 g, 12 mmol) and boron triflouride etherate (7.38 mL, 60 mmol) in methylene chloride (1 L) at 0°C, a solution of 1,3-cyclohexanedione (2.3 g, 20 mmol) in methylene chloride (20 mL) was added at room temperature over two hours. After addition, the reaction mixture was stirred at room temperature for an additional 16 hours. The reaction mixture was quenched with saturated sodium bicarbonate solution (50 mL) and the aqueous phase was extracted with methylene chloride. The combined organic phases were washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes /ethyl acetate) to give a colorless oil.

Compound **7**: ^{1}H NMR ($CDCl_3$, δ): 4.80 (s, 1H), 4.65 (s, 1H), 3.64 (d, J = 11.2 Hz, 1H), 3.47 (d, J = 10.8 Hz, 1H), 2.50 (m, 2H), 1.6-1.8 (m, 2H), 1.70 (s, 3H), 1.2-1.5 (m, 2H), 1.08 (s, 3H). ^{13}C NMR ($CDCl_3$, δ): 216.2, 146.9, 111.0, 68.4, 50.1, 44.5, 43.5, 33.2, 25.0, 21.2, 20.6. IR (neat) cm^{-1}: 3000, 2950, 1627. MS m/z (CI - NH_3): 156.

5-Trimethylsilyloxy-2, 4, 7, 8-tetrahydrobenzo-1, 3-dioxine (8)

To a stirred solution of diisopropylamine (1.8 mL, 12 mmol) in tetrahydrofuran (15 mL), a solution of butyl lithium (4.4 mL of 2.5 M solution in hexane) was added at 0°C. After 1 hour, the reaction mixture was cooled to –78°C, and a solution of compound **7** (1.40 g, 10 mmol) in tetrahydrofuran (5 mL) was added over a 10 min period. The reaction mixture was stirred at –78°C for 1 hour, and then stirred at –20°C for 2 hours. Chlorotrimethylsilane (1.2 g, 11 mmol) was added to the reaction mixture. After 1 hour, the reaction mixture was diluted with hexanes, filtered through Celite, and then concentrated in vacuo. Compound **8**: ^{1}H NMR ($CDCl_3$, δ): 5.04 (s, 2H), 4.53 (t, J = 5.7 Hz, 1H), 4.28 (d, J = 5.7 Hz, 1H), 2.20-2.25 (m, 4H), 0.18 (s, 9H). ^{13}C NMR ($CDCl_3$, δ): 151.4, 147.2, 105.7, 94.4, 90.7, 63.5, 25.7, 20.9, 0.14.

4, 6, 7, 8-Tetrahydro-6-trimethylsilyloxy-5*H*-1, 3-benzodioxin-5-one (9)

To a solution of compound **8** (2.14 g, 10 mmol) in methylene chloride (50 mL) at 0°C, *m*-chloroperbenzoic acid (2.3 g, 11 mmol) was added and then stirred at room temperature for 2 hours. After filtration, the reaction mixture was concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes /ethyl acetate) to give a white solid. Compound **9**: ^{1}H NMR ($CDCl_3$, δ): 5.12 (d, J = 6 Hz, 1H), 5.02 (d, J = 6 Hz, 2H), 4.36 (dd, J = 2.4, 11.2 Hz, 1H), 4.04 (dd, J = 2.4, 11.2 Hz, 1H), 2.45-2.49 (m, 2H), 1.8-2.2 (m, 2H), 0.10 (s, 9H). ^{13}C NMR ($CDCl_3$, δ): 194.9,

169.2, 110.0, 91.5, 72.1, 62.7, 30.2, 26.2, 0.3. IR (neat) cm^{-1}: 3000, 2950, 1630, 1604.

4, 6, 7, 8-Tetrahydro-6-hydroxy-5*H*-1, 3-benzodioxin-5-one (10)

To an aqueous methanol solution of compound **9** (214 mg, 1 mmol), potassium carbonate (100 mg) was added and then stirred at room temperature for 12 hours. The reaction mixture was extracted with ethyl acetate and the organic layer was dried and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 2:1 hexanes /ethyl acetate) to give a white solid. Compound **10**: ^{1}H NMR ($CDCl_3$, δ): 5.24 (d, J = 6 Hz, 1H), 5.05 (d, J = 6 Hz, 1H), 4.55 (dd, J = 1.9, 14.5 Hz, 1H), 4.36 (dd, J = 2.0, 14.5 Hz, 1H), 4.07 (dd, J = 5.4, 13.1 Hz, 1H), 3.70 (br s, 1H), 2.2-2.8 (m, 3H), 1.86 (m, 1H). ^{13}C NMR ($CDCl_3$, δ): 196.2, 170.8, 109.0, 91.8, 71.0, 62.5, 29.0, 26.8. IR (neat) cm^{-1}: 3310, 2990, 1635, 1150. MS m/z (CI - NH_3): 170.

4-Hydroxy-2-(hydroxymethyl)-2-cyclohexenone (11)

To a solution of compound **9** (280 mg, 12 mmol) or **10** (210 mg, 12 mmol) in dry tetrahydrofuran (25 mL) under argon, lithium aluminum hydride (90 mg, 18 mmol) was added at 0°C and then stirred at room temperature for 2 hours. The reaction mixture was cooled to 0°C and then quenched by the addition of saturated sodium sulfate solution (0.5 mL). After 15 minutes, magnesium sulfate was added to the reaction mixture. The insoluble salts were removed by filtration, and the filtrate was concentrated in vacuo. The resultant oil was then dissolved in tetrahydrofuran (2 mL) and then 3N HCl (0.2 mL) was added. After 1 hour, the reaction mixture was neutralized with aqueous potassium carbonate solution. The reaction mixture was extracted with ethyl acetate and the organic layer was dried and concentrated in vacuo. The residue was purified by flash column chromatography (eluting with ethyl acetate) to give a white solid. Compound

11: ^{1}H NMR ($CDCl_3$, δ): 6.92 (dd, J = 1.2, 1.3 Hz, 1H), 4.58 (br s, 2H), 4.16 (br s, 2H), 4.06 (d, J = 6 Hz, 1H), 2.2-2.5 (m, 3H), 1.65-1.98 (m, 1H). ^{13}C NMR ($CDCl_3$, δ): 198.0, 147.9, 137.8, 66.0, 58.6, 35.8, 32.7. IR (neat) cm^{-1}: 3500, 2950, 1635, 1150. MS m/z (CI - NH_3): 126.

4-Hydroxy-2-(*tert*-butyldimethylsilyloxymethyl)-2-cyclohexenone (12)

To a solution of **11** (1.42 g, 10 mmol) in dry methylene chloride (50 mL) under argon, triethylamine (3 g, 30 mmol), *tert*-butyldimethylsilyl chloride (1.6 g, 11 mmol) and DMAP (500 mg, 3 mmol) were added at 0°C. The solution was stirred at room temperature for 12 hours. The reaction mixture was concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 10:1 hexanes / ethyl acetate) to give a colorless oil. Compound **12**: ^{1}H NMR ($CDCl_3$, δ): 6.94 (t, J = 1.3 Hz, 1H), 4.62 (br s, 1H), 4.34 (t, J = 1.3 Hz, 2H), 2.55-2.64 (m, 1H), 2.31-2.39 (m, 2H), 1.93-2.04 (m, 2H), 1.60 (br s, 1H). ^{13}C NMR ($CDCl_3$, δ): 198.1, 145.6, 138.6, 66.8, 59.6, 35.8, 32.7, 26.0, 18.4, -5.38.

4-(tert-Butyldimethylsilyloxy)-2-(tert-butyldimethylsilyloxymethyl)-2-cyclohexenone (13)

To a solution of compound **11** (1.42 g, 10 mmol) in dry methylene chloride under argon, triethylamine (3 g, 30 mmol) and *tert*-butyldimethylsilyl chloride (3.2 g, 22 mmol) were added at 0°C and stirred at room temperature for 12 hours. The reaction mixture was concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 15:1 hexanes / ethyl acetate) to give a colorless oil. Compound **13**: ^{1}H NMR ($CDCl_3$, δ): 6.87 (s, 1H), 4.55 (m, 1H), 4.32 (s, 2H), 2.55-2.64 (m, 1H), 2.1-2.39 (m, 2H), 1.80-2.04 (m, 1H), 0.92 (s, 9H), 0.07 (s, 6H). ^{13}C NMR

($CDCl_3$, δ): 198.4, 147.3, 137.6, 67.3, 59.5, 36.0, 33.1, 25.9, 18.3, 18.2, -4.6, -4.7, -5.3, -5.4.

A typical procedure for the addition of dihalomethyl lithium to carbonyl compounds is described as followed:

To a solution of carbonyl compound (10 mmol) and dihalomethane (50 mmol) in tetrahydrofuran (50 mL), three to five equivalents of LiTMP [30mmol, prepared from 2,2, 6,6-tetramethylpiperidine (4.23 g, 30 mmol) and *n*-butyllithium (12 mL of 2.5 M solution in hexanes)], were added slowly at –78°C. The reaction mixture was stirred at –78°C for three hours and monitored by thin layer chromatography (TLC). After the reaction was complete, saturated ammonium chloride solution (15 mL) was added at –78°C. The reaction mixture was then extracted with diethyl ether, washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified with flash column chromatography, eluting with a mixture of hexanes and ethyl acetate, to give a pure dihalomethyl carbinol.

1-(Dibromomethyl)-2-hydroxymethylcyclohex-2-ene-1, 4-diol (14): ^1H NMR ($CDCl_3$, δ): 6.23 (s, 1H), 5.93 (s, 1H), 4.74 (s, 1H), 4.3 (m, 2H), 1.5-2.5 (m, 2H). ^{13}C NMR ($CDCl_3$, δ): 136.4, 135.4, 75.3, 66.9, 63.4, 56.8, 29.2, 27.5. IR (neat) cm^{-1}: 3540, 1600, 1050. MS m/z (CI- NH_3): 316, 334 (M+18). HRMS m/z (M-3H_2O): 262.89053, calcd. for $C_8H_7{}^{79}Br^{81}Br$: 262.88953. CHN: anal. calcd. for C 30.41%, found C 30.74%. anal. calcd. for H 3.83%, found H 3.98%.

1-(Dibromomethyl)cyclohexanol (15): ^1H NMR($CDCl_3$, δ): 5.65 (s, 1H), 4.65 (s, 1H), 1.2-2.5 (m, 2H). ^{13}C NMR ($CDCl_3$, δ): 75.5, 61.8, 34.0, 26.3, 22.9. IR (neat) cm^{-1}: 3350,

1050.

1-(Dibromomethyl)-2-(hydroxymethyl)cyclohex-2-enol (17): ^{1}H NMR ($CDCl_3$, δ): 6.10 (s, 1H), 5.95 (d, 1H), 4.30 (dd, J = 1, 11.2 Hz, 2H), 4.17 (s, 1H), 1.5-2.53 (m, 4H). ^{13}C NMR ($CDCl_3$, δ): 216.2, 146.9, 111.0, 68.4, 50.1, 44.5, 43.5, 33.2, 25.0, 21.2, 20.6.

A typical procedure for the hydrolysis of dihalomethyl carbinols is described as followed:

To the dihalomethyl carbinol (100 mg) in a mixture of isopropanol (5 mL) and water (5 mL), potassium carbonate (100 mg) was added and then stirred at room temperature for 1 to 12 hours. The reaction was monitored by TLC. After the reaction was complete, the isopropanol was removed in vacuo. The aqueous solution was saturated with sodium chloride and extracted with ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with a mixture of hexanes and ethyl acetate) to give a pure compound.

1-Hydroxycyclohexanecarboxaldehyde (16): ^{1}H NMR ($CDCl_3$,δ): 9.49 (s, 1H), 1.2-2.5 (m, 10H). ^{13}C NMR ($CDCl_3$,δ): 204.0, 76.0, 41.8, 31.3, 26.9, 24.9, 20.4.

1, 7a-Dihydroxy-1, 3, 5, 6, 7, 7a-hexahydroisobenzofuran (18): ^{1}H NMR ($CDCl_3$, δ): 5.84 and 5.78 (s, 1H), 5.27 (d, J = 5.4 Hz) and 4.90 (d, J = 7.8 Hz) 1H, 4.61 and 4.59 (m, 1H), 4.50 and 4.23 (m, 1H), 3.95 (d, J = 7.8 Hz) and 2.51 (d, J = 3.6 Hz) 1H, 1.2-2.3 (m, 6H). ^{13}C NMR ($CDCl_3$, δ): 137.3 and 137.0, 124.9 and 124.2, 103.1 and 102.8, 76.4 and 71.8, 68.2 and 67.4, 30.0 and 27.9, 25.1 and 24.8, 17.5 and 17.4. (It is a mixture of

two isomers with ratio 1.7 to 1.)

1, 5, 7a-Trihydroxy-1, 3, 5, 6, 7, 7a-hexahydroisobenzofuran (19): ^{1}H NMR (acetone-d_6, δ): 5.64 (s, 1H), 5.10 (m, 1H), 4.2-4.5 (m, 3H), 3.5-3.9 (m, 3H), 2.0 (m, 2H). ^{13}CNMR(acetone-d_6, δ): 140.8, 128.3, 123.2, 102.9, 75.9, 67.6, 27.0, 23.9. IR (neat) cm^{-1}: 3540, 1627, 1604, 1450, 1050. MS m/z (CI): 172, 154. HRMS m/z (M-H_2O): 154.06306, calcd. for $C_8H_{10}O_3$: 154.06299.

5, 8-Dihydroxy-1-oxo-2-oxabicyclo[4, 3, 0]non-4(9)-ene (20)

To a solution of hydroxy hemiacetal **19** (156 mg, 1 mmol) in dry methylene chloride (25 mL) under argon, *N*-iodosuccinimide (1.13 g, 5 mmol) and tetra-*n*-butyl ammonium iodide (370 mg, 1 mmol) were added at 0°C and stirred at room temperature for 12 hours. The reaction mixture was diluted with ethyl acetate, washed with saturated sodium thiosulfate solution and brine, dried and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes / ethyl acetate) to give a white solid. Compound **20:** ^{1}H NMR ($CDCl_3$, δ): 4.80 (s, 1H), 4.65 (s, 1H), 3.64 (d, J = 11.2 Hz, 1H), 3.47 (d, J = 10.8 Hz, 1H), 2.50 (m, 2H), 1.6-1.8 (m, 2H), 1.70 (s, 3H), 1.2-1.5 (m, 2H), 1.08 (s, 3H). ^{13}C NMR ($CDCl_3$, δ): 177.7, 147.9, 109.5, 77.3, 76.4, 42.3, 41.6, 34.6, 26.2, 21.0, 14.1. IR (neat) cm^{-1}: 3368, 2959, 2921, 1770, 1018.

5, 7a-Dihydroxy-1-methoxy-1, 3, 5, 6, 7, 7a-hexahydroisobenzofuran (21):

To a solution of hydroxy hemiacetal **19** (172 mg, 1 mmol) in methanol (25 mL) under argon, trimethyl orthoformate (106 mg, 1 mol) and catalytic amount of *p*-toluenesulfonic acid (10 mg) were added at 0°C. The reaction mixture was stirred at room temperature for 1 hour, and then diluted with ethyl ether. The organic solution was

washed with washed with saturated sodium bicarbonate solution, brine, dried and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes / ethyl acetate) to give a white solid. Compound **21:** ^{1}H NMR ($CDCl_3$, δ): 5.58 (s, 1H), 4.67 (s, 1H), 4.5 (m, 1H), 1.8-2.0 (m, 2H). ^{13}C NMR ($CDCl_3$, δ): 141.4, 125.8, 108.8, 76.1, 68.0, 67.1, 54.8, 28.2, 27.4.

2*E*, 4*E*-Hexadienoic acid, 1-methoxy-7a-hydroxy-1, 3, 5, 6, 7, 7a-hexahydro-isobenzofuran-5-ylester (22)

To a solution of compound **21** (36 mg, 0.2 mmol) in methylene chloride (10 mL) under argon, triethylamine (30 mg, 0.6 mmol) and the anhydride of sorbic acid (40 mg, 0.2 mmol) were added at 0°C, and then stirred at room temperature for 1 hour. The reaction was concentrated in vacuo, the residue was purified with flash column chromatography (eluting with 4:1 hexanes / ethyl acetate) to give a white solid. Compound **22a**: ^{1}H NMR ($CDCl_3$, δ): 7.21-7.27 (m, 1H), 6.13-6.17 (m, 2H), 5.76 (d, J = 16 Hz, 1H), 5.70 (d, J = 16 Hz, 1H), 5.4-5.6 (m, 1H), 4.75 (s, 1H), 4.58 (dt, J = 16, 2.4 Hz, 1H), 4.34 (dt, J = 16, 2.4 Hz, 1H), 3.34 (s, 3H), 2.1-2.2 (m, 2H), 1.8-2.0 (m, 6H). ^{13}C NMR ($CDCl_3$, δ): 167.1, 145.5, 143.0, 139.8, 129.8, 122.2, 118.9, 108.5, 76.0, 70.3, 67.1, 54.8, 27.1, 24.0. IR (neat) cm^{-1}: 3442, 2958, 2924, 2854, 1716, 1616, 1558, 1003. MS m/z (CI-NH_3): 266, 110; 267 (M+1), 284 (M+18).

2E, 4E-Hexadienoic acid, 1, 7a-dihydroxy-1, 3, 5, 6, 7, 7a -hexahydroisobenzofuran-5-yl ester (5):

To an aqueous THF solution of compound **23**, five drops of 10% HCl solution was added at room temperature. The reaction mixture was heated to 60°C for 2 hours. The reaction mixture was extracted with ether, dried and concentrated in vacuo. The

residue was purified with flash column chromatography (eluting with 4:1 hexanes / ethyl acetate) to give a white solid. Compound **5**: ^{1}H NMR ($CDCl_3$, δ): 7.12-7.29 (m, 1H), 6.13-6.19 (m, 2H), 5.75 (dd, *J* = 15, 1.5 Hz, 2H), 5.49 (d, *J* = 16 Hz, 1H), 5.26 (s, 1/2H), 4.93 (s, 1/2H), 4.59 (dt, *J* = 16, 2.4 Hz, 1H), 4.34 (dt, *J* = 16, 2.4 Hz, 1H), 1.5-2.0 (m, 4H). ^{13}C NMR ($CDCl_3$, δ): 167.1, 145.8, 142.5, 140.1, 129.8, 123.4, 118.8, 102.4, 76.4, 70.3, 67.5, 29.7, 24.0, 18.8. IR (neat) cm^{-1}: 3450, 3368, 2959, 2921, 1716, 1616, 1558, 1539, 1050, 1018. MS m/z (CI-NH_3): 252, 110.

2E, 4E-Hexadienoic acid, 1, 3, 5, 6, 7, 7a-hexahydro-7a-hydroxy-1-oxoisobenzofuranon-5-yl ester (6)

To a solution of hydroxy hemiacetal **5** (14.7 mg, 0.05 mmol) in dry methylene chloride (15 mL) under argon, *N*-iodosuccinimide (62 mg, 0.25 mmol) and tetra-*n*-butyl ammonium iodide (20.5 mg, 0.05 mmol) were added at 0°C and stirred at room temperature for 12 hours. The reaction mixture was diluted with ethyl acetate, washed with saturated sodium thiosulfate solution and brine, dried and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes / ethyl acetate) to give a white solid. Compound **6:** ^{1}H NMR ($CDCl_3$, δ): 7.21-7.27 (m, 1H), 6.13-6.22 (m, 2H), 5.98 (d, *J* = 0.9 Hz, 1H), 5.75 (d, *J* = 15.3 Hz, 1H), 5.4-5.6 (m, 1H), 5.04 (dt, *J* = 13, 3.3 Hz, 1H), 4.73 (dt, *J* = 13, 3.3 Hz, 1H), 2.51 (br s, 1H), 2.1-2.3 (m, 3H), 1.8-2.0 (m, 6H). ^{13}C NMR ($CDCl_3$, δ): 175.4, 166.9, 146.0, 140.3, 137.2, 129.7, 126.5, 118.3, 69.9, 69.2, 67.9, 29.8, 23.5, 18.8. IR (neat) cm^{-1}: 3368, 2959, 2921, 1772, 1716, 1616, 1558, 1539, 1018.

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CHAPTER III

AN ENANTIOSELECTIVE FORMAL TOTAL SYNTHESIS OF PHYTUBERIN

A paper to be submitted to the *Journal of Organic Chemistry*

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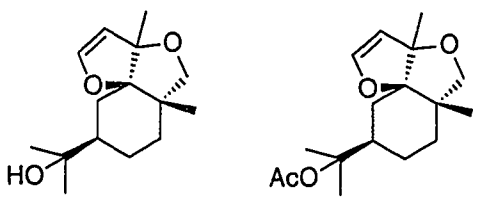
Introduction

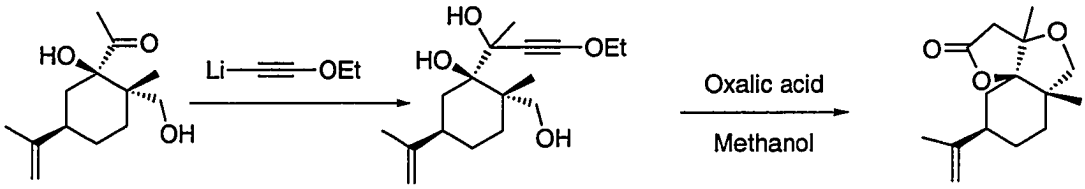
Sesquiterpenoid phytoalexins phytuberol (R = H) and phytuberin (R = Ac) are stress metabolites isolated form tobacco leaves. They were induced by smearing or spraying the leaves of *Nicotinia tabacum* with $ClCH_2CH_2SO_3H$, followed by extraction.[1] These sesquiterpenoids of stress metabolites were also isolated from potato stems and roots.[2-4] The structures of phytuberol and phytuberin are shown in Scheme 1.

Scheme 1



phytuberol **phytuberin**

Scheme 2

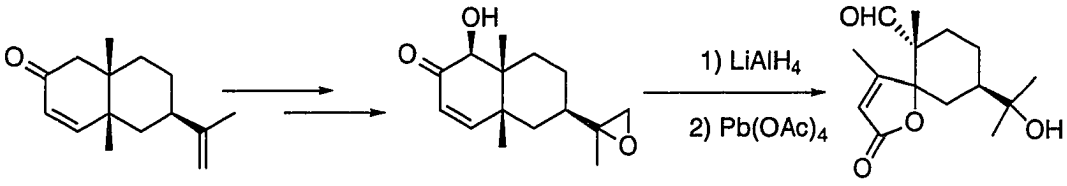


1 **2** **3**

These compounds have been demonstrated to have weak antifungal activity by Sato[5] and Stoessl.[6] They are of interest from both a synthetic and a biological perspective, in that stress metabolites are a key part of signal transduction pathways.[7]

Three total syntheses of phytuberin have been reported in the literature. One strategy is exemplified by a creative synthesis by Findlay (shown in Scheme 2).[8] It features addition of the anion of ethoxyacetylene to ketone **1**, followed by cyclization under mild acidic conditions. This process is direct; however, the production of

Scheme 3



4 **5** **6**

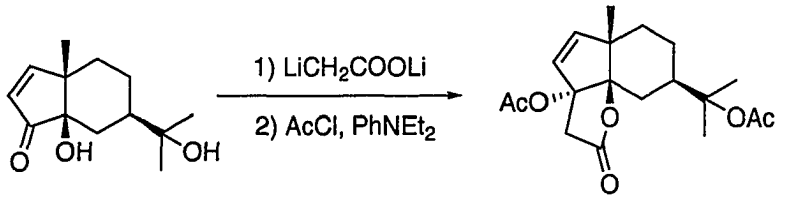
diastereometric mixtures is a drawback.

The second strategy was developed by Murai and coworkers[9] (Shown in Scheme 3). (+) β-Rutunol was chosen as an intermediate. β-Rutunol undergoes α-hydroxylation and epoxidation of the isopropenyl group. Compound **6**, which was the precursor of

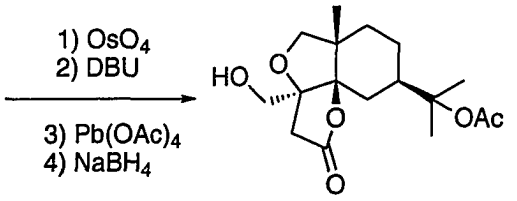
phytuberin, was produced by ring cleavage of compound **5**. This strategy gave high stereoselectivity, but the synthetic route is lengthy.

Another strategy was developed by Kido and coworkers (Scheme 4).[10] It started from elemol. Then *cis*-hydrindenone reacted with the dianion of acetic acid to afford a lactone after acidification of the reaction mixture. The spirocyclic lactone obtained by the double bond cleavage regenerates a tricyclic compound **8** upon reduction. This

Scheme 4



7 **8**



9

strategy features an interesting key step and high stereoselectivity. However, the synthetic route is lengthy.

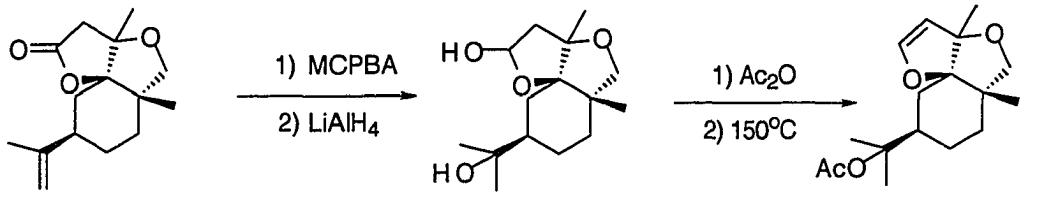
The strategies discussed above have their unique features. However, the skeleton of phytuberin with oxygen-containing substituents could be easily obtained though the method described in the first part of the dissertation.

Results and discussion

The unique structure of phytuberin prompted us to synthesize the skeleton of this compound. Since the transformation of the furanolactone **10** has been reported by Findlay,[8] it was chosen as our target molecule for synthetic studies.

The retrosynthetic analysis is shown in Scheme 6. It requires the preparation of a

Scheme 5

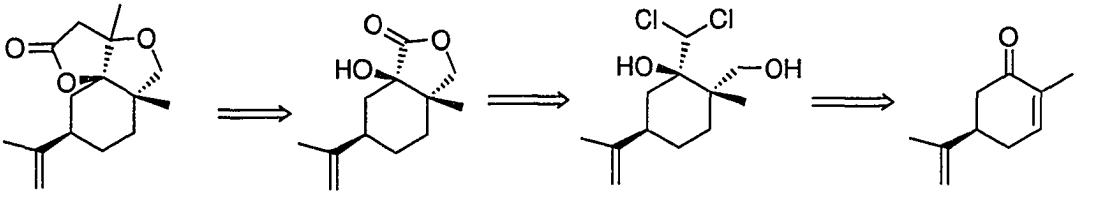


10 **11** **12**

key intermediate **16**, which would be derived from hydrolysis of dichloromethyl carbinol **14**. Compound **14** can be derived from R- (-)-carvone.

The synthesis started from R- (-)-carvone. R- (-)-carvone was treated with lithium selectride at –78°C, followed by addition of a THF solution of formaldehyde

Scheme 6

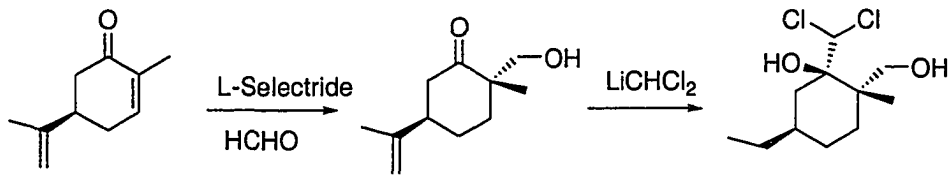


10 **16** **14** **R-(-)-carvone**

under argon, and afforded a mixture of **13a** plus its C-10 (phytuberin numbering system) epimer in 91% yield.[11]

The ratio of these two epimers is 2 to 3. Fortunately, the undesired isomer can be equilibrated to a 5 to 4 mixture of **13a** and its epimer by pyrolysis at 185°C. The addition of dichloromethyl lithium[12] to hydroxy ketone **13a** at –78°C resulted in the

Scheme 7

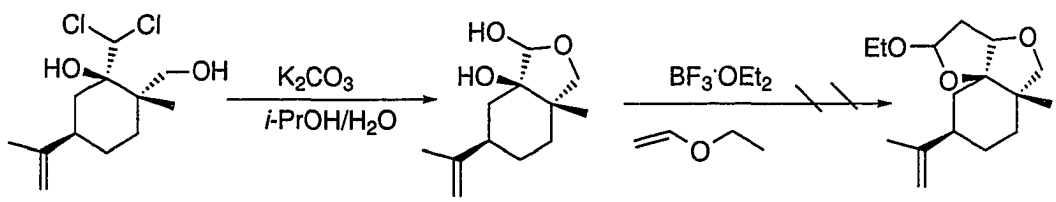


R-(-)-carvone **13a** **14**

formation of a single isomer. The extremely high selectivity might be due to an alkoxide-directed addition of dichloromethyl lithium.

The dichloromethyl carbinol compound **14** can be hydrolyzed with potassium carbonate in a 1:1 mixture of isopropanol and water.[13] Originally, we envisioned that compound **15** would react with ethyl vinyl ether under Lewis acid catalyzed conditions. Unfortunately, it didn't afford the desired product.

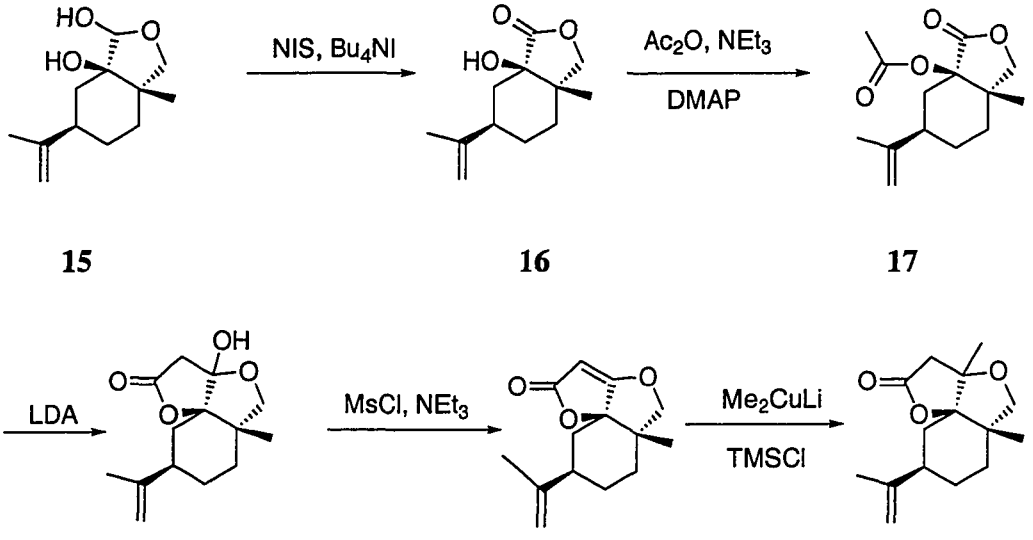
Scheme 8



14 **15**

Instead of using a Lewis acid-catalyzed cyclization, we designed an intramolecular aldol condensation to build the skeleton of phytuberin. Hydroxy hemiacetal **16** was oxidized to a hydroxy lactone under mild conditions with one equivalent of *N*-iodosuccinimide and five equivalents of tetra-*n*-butylammonium iodide.[14] The resulting lactone **16** was treated with acetic anhydride and triethylamine

Scheme 9



and a catalytic amount of DMAP. The acetoxy lactone **17** was reacted with five equivalents of lithium diisopropylamide (LDA) at –78°C to afford tricyclic compound **18** in 93% yield,[15] which contains the main skeleton of phytuberin. The structure of compound **18** was confirmed by x-ray diffraction (Figure 1).

Treating hemiketal **18** with methanesulfonyl chloride and triethylamine at 0°C afforded the elimination compound. Finally, the target molecule was produced by a trimethylsilyl chloride assisted cuprate 1,4-addition to enone **19**.[16]

In summary, the synthesis from R- (-)-carvone to known intermediate **10** was accomplished in eight steps in 16% overall yield. The high stereoselectivity in this synthetic route will provides a convergent method for the construction of phytoalexins and other sesquiterpenes having similar structures.

Experimental

Unless otherwise noted, materials were obtained from commercial suppliers and were used without purification. THF and diethyl ether were distilled from sodium benzophenone ketyl. Dichloromethane was distilled from calcium hydride. Unless otherwise noted, all the reactions were conducted under an argon atmosphere. All organic extracts were dried over anhydrous magnesium sulfate. Infrared spectra were obtained on a Perkin-Elmer model 1320 spectrophotometer. Nuclear magnetic resonance spectra were determined on either a Varian 300 or a Bruker 400 spectrometer. All chemical shifts are reported relative to tetramethylsilane as an internal standard. Coupling constants are reported in Hz. High-resolution mass spectra were recorded on a Kratos model MS-50 spectrometer. Low-resolution mass spectra were obtained from a Finnegan 4023 mass spectrometer. Flash chromatography was performed on silica gel Kieseldel 60 (mesh 230-400).

2*S*-Hydroxymethyl-2-methyl-5*R*-isopropenylcyclohexanone (13a);
2*R*-hydroxymethyl-2-methyl-5*R*-isopropenylcyclohexanone (13b)

To a solution of R- (-)-carvone (4.5 g, 30 mmol) in tetrahydrofuran (350 mL) at –78°C, a solution of lithium selectride (31 mL of 1M solution in hexanes) was added, and then stirred at –78°C for one hour. A freshly prepared anhydrous formaldehyde

solution was added slowly. After addition, the reaction mixture was stirred at –78°C for an hour then warmed to room temperature for 10 hours. The reaction mixture was treated with 5 mL of water. After 15 min, 3N sodium hydroxide solution (100 mL) was added, followed by the addition of 80 mL of 30% hydrogen peroxide. After stirring at room temperature overnight, the reaction mixture was extracted with diethyl ether, washed with sodium thiosulfate solution and brine, dried and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 10:1 hexanes/ ethyl acetate) to give two colorless oils. Compound **13a**: ^{1}H NMR ($CDCl_3$, δ): 4.80 (s, 1H), 4.65 (s, 1H), 3.64 (d, J = 11.2 Hz, 1H), 3.47 (d, J = 10.8 Hz, 1H), 2.50 (m, 2H), 1.6-1.8 (m, 2H), 1.70 (s, 3H), 1.2-1.5 (m, 2H), 1.08 (s, 3H). ^{13}C NMR ($CDCl_3$, δ): 216.2, 146.9, 111.0, 68.4, 50.1, 44.5, 43.5, 33.2, 25.0, 21.2, 20.6. IR (neat) cm^{-1}: 3540, 1627, 1604, 1450, 1050. MS m/z (CI-NH_3): 182, 181, 154. HRMS m/z M+: 182.13078, calcd. for $C_{11}H_{18}O_2$: 182.13070. Compound **13b**: ^{1}H NMR ($CDCl_3$, δ): 4.67 (s, 1H), 4.64 (s, 1H), 3.47 (d, J = 14.4 Hz, 1H), 3.33 (d, J = 14.4 Hz, 1H), 2.1-2.5 (m, 3H), 1.75 (m, 2H), 1.74 (s, 3H), 1.2-1.6 (m, 1H), 1.08 (s, 3H). ^{13}C NMR ($CDCl_3$, δ): 217.2, 1467.3, 109.9, 68.5, 49.4, 46.2, 43.7, 34.1, 26.0, 20.5, 20.2. IR (neat) cm^{-1}: 3540, 1627, 1604, 1450, 1050. MS m/z (CI-NH_3): 182, 181, 154. HRMS m/z M+: 182.13086, calcd. for $C_{11}H_{18}O_2$: 182.13068.

1*R*-(Dichloromethyl)-2*S*-hydroxymethyl-2-methyl-5*R*-isopropenylcyclohexanol (14)

To a solution of carbonyl compound **13** (1.82 g, 10 mmol) and methylene chloride (50 mmol) in tetrahydrofuran (50 mL) at –78°C, excess LiTMP (30mmol), prepared from 2,2,6,6- tetramethylpiperidine (4.23 g, 30 mmol) and *n*-butyllithium (12 mL of 2.5M solution in hexane), was added slowly. The reaction mixture was stirred at –78°C for three hours, and monitored by thin layer chromatography (TLC). After

reaction was completed, saturated ammonium chloride solution was added to the reaction mixture at –78°C. The reaction mixture was then extracted with diethyl ether, washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 1:1 hexanes/ ethyl acetate) to give a colorless oil. Compound **14**: ^{1}H NMR ($CDCl_3$, δ): 5.57 (s, 1H), 4.75 (s, 2H), 4.72 (br s, 1H), 3.27 (d, J = 5.1 Hz, 2H), 1.78 (s, 3H), 1.10 (s, 3H), 0.8-2.0 (m, 6H). ^{13}C NMR ($CDCl_3$, δ): 148.2, 109.3, 74.3, 68.3, 67.8, 43.9, 38.5, 33.1, 25.8, 21.0, 18.0. IR (neat) cm^{-1}: 3445, 1616, 1470, 1090. MS m/z (CI-NH_3): 284 (M+NH_4), 266, 248 (M-H_2O).

3a*S*-Methyl-6*R*-isopropenyl-1, 7a-dihydroxyoctahydroisobenzofuran (15)

To dihalomethyl carbinol **14** (100 mg, 0.8 mmol) in the mixture of isopropanol (5 mL) and water (5 mL), potassium carbonate (100 mg) was added and the mixture was stirred at room temperature for 2 hours. The reaction was monitored by TLC. After removal of isopropanol, the aqueous solution was saturated with sodium chloride and extracted with ethyl acetate. The organic phase was dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 1:4 hexanes/ ethyl acetate) to give a white solid. Compound **15**: ^{1}H NMR ($CDCl_3$, δ): 5.48 (d, J = 9.6 Hz, 1H), 4.73 (d, J = 2.4 Hz, 2H), 3.95 (d, J = 7.8 Hz, 1H), 3.63 (d, J = 9.6 Hz, 1H), 3.49 (d, J = 9.6 Hz, 1H), 2.03 (br s, 1H), 1.8-2.2 (m, 2H), 1.73 (s, 3H), 1.1-1.5 (m, 4H), 1.04 (s, 3H). ^{1}H NMR ($CDCl_3$, D_2O, δ): 5.46 (s, 1H), 4.73 (d, J = 2.4 Hz, 2H), 3.95 (d, J = 7.8 Hz, 1H), 3.49 (d, J = 9.6 Hz, 1H), 1.8-2.2 (m, 2H), 1.73 (s, 3H), 1.1-1.5 (m, 4H), 1.04 (s, 3H). ^{13}C NMR ($CDCl_3$, δ): 148.3, 109.4, 100.0, 79.0, 78.5, 44.1, 42.0, 35.1, 34.9, 26.6, 21.2, 15.2.

3a*S*-Methyl-6*R*-isopropenyl-7a*R*-hydroxyhexahydroisobenzofuran-1-one (16)

To a solution of compound **15** (212 mg, 1 mmol) in dry methylene chloride (25 mL) under argon, *N*-iodosuccinimide (1.13 g, 5 mmol) and tetra-*n*-butylammonium iodide (370 mg, 1 mmol) were added at 0°C and the mixture was stirred at room temperature for 12 hours. The reaction mixture was diluted with ethyl acetate, washed with saturated sodium thiosulfate solution and brine, dried, and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 1:1 hexanes/ ethyl acetate) to give a white solid. Compound **16**: ^{1}H NMR ($CDCl_3$, δ): 4.73 (d, J = 9.6 Hz, 2H), 4.20 (dd, J = 3.6, 8.4 Hz, 1H), 3.81 (dd, J = 3.6, 8.4 Hz, 1H), 2.25 (br s, 1H), 2.17 (m, 1H), 1.8-2.2 (m, 2H), 1.74 (s, 3H), 1.2-1.8 (m, 6H), 1.13 (s, 3H). ^{13}C NMR ($CDCl_3$, δ): 177.7, 147.9, 109.5, 77.3, 76.4, 42.3, 41.6, 34.6, 26.2, 21.0, 14.1.

3a*S*-Methyl-6*R*-isopropenyl-7a*R*-acetoxyhexahydroisobenzofuran-1-one (17)

To a solution of compound **16** (210 mg, 1 mmol) in dry methylene chloride (25mL) under argon, triethylamine (1 g, 10 mmol) and acetic anhydride (106 mg, 1 mmol) and a catalytic amount of DMAP (10 mg) were added at room temperature and the mixture was stirred at 50°C for 12 hours. The reaction was concentrated in vacuo, the residue was purified with flash column chromatography (eluting with 4:1 hexanes / ethyl acetate) to give a white solid. Compound **17**: ^{1}H NMR ($CDCl_3$, δ): 4.72 (d, J = 10 Hz, 2H), 4.10 (d, J = 8.4 Hz, 1H), 3.80 (d, J = 8.4 Hz, 1H), 2.71 (m, 1H), 2.04 (s, 3H), 1.71 (s, 3H), 1.2-1.9 (m, 6H), 1.14 (s, 3H). ^{13}C NMR ($CDCl_3$, δ): 173.1, 168.8, 147.8, 109.6, 82.6, 76.3, 42.7, 41.3, 35.5, 32.1, 25.7, 21.3, 21.0, 15.9.

8*R*-Isopropenyl-5a*S*-methyl-3a*R*-hydroxyoctahydro-1, 4-dioxacyclopenta-indene-2-one (18)

To a stirred solution of diisopropylamine (1.8 mL, 12 mmol) in dry tetrahydrofuran (15 mL), a solution of *n*-butyl lithium (4.4 mL of 2.5M solution in hexanes) was added at 0°C. After 1 hour, the reaction mixture was cooled to –78°C, a solution of compound **17** (252 mg, 1 mmol) in dry ether (5 mL) was added over a 10 min period. The reaction mixture was stirred at –78°C for 3 hours. The reaction mixture was quenched with saturated ammonium chloride, extracted with ether, dried, and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes / ethyl acetate) to give a white solid. Compound **18**: ^{1}H NMR ($CDCl_3$, δ): 4.75 (d, J = 1 Hz, 2H), 3.76 (dd, J = 8.7, 16 Hz, 2H), 3.37 (br s, 1H), 2.95 (dd, J = 12, 16 Hz, 2H), 2.60 (m, 2H), 2.04 (m, 1H), 1.79 (s, 3H), 1.2-1.8 (m, 4H), 1.13 (s, 3H). ^{13}C NMR ($CDCl_3$, δ): 172.2, 148.5, 110.2, 109.4, 95.1, 78.6, 42.8, 41.5, 34.8, 32.6, 25.6, 20.8, 16.3.

8*R*-Isopropenyl-5a*R*-methyl-5, 5a, 6, 7, 8, 9-hexahydro-1, 4-dioxacyclopent-3-enindene-2-one (19)

To a solution of **18** (252 mg, 1 mmol) in dry methylene chloride (25 mL) under argon, triethylamine (5 mL) and methylsulfonyl chloride (0.05 mL) were added at 0°C and the solution was stirred at room temperature for 2 hours. The reaction mixture was quenched with saturated sodium bicarbonate, extracted with ether, dried, and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes / ethyl acetate) to give a white solid. Compound **19**: ^{1}H NMR ($CDCl_3$, δ): 4.93 (s, 3H), 4.78 (d, J = 9 Hz, 1H), 4.25 (d, J = 9.3 Hz, 1H), 2.48 (s, 1H), 1.87 (s, 3H), 1.2-2.2 (m, 6H), 1.02 (s, 3H). ^{13}C NMR ($CDCl_3$, δ): 189.7, 174.9, 145.0,

111.4, 87.3, 86.4, 85.8, 41.7, 36.9, 34.6, 25.4, 23.4, 22.4, 21.0.

8*R*-Isopropenyl-3a*S*, 5a*R*-dimethyloctahydro-1, 4-dioxacyclopentinden-2-one (10)

To a solution of compound **19** (100 mg, 0.5 mmol) in dry ether (10 mL) under argon, trimethylsilyl chloride (100 mg, 1 mmol) was added at 0°C. Then freshly prepared dimethyllithium cuperate was added at 0°C and the mixture was stirred at the same temperature for 2 hours. The reaction mixture was treated with triethylamine (1 g, 10 mmol), and then was diluted with hexanes (150 mL), filtered through Celite, and concentrated in vacuo. The residue was dissolved in tetrahydrofuran (10 mL) and quenched with saturated ammonium chloride solution (10 mL). The mixture was stirred at room temperature for 10 hours and then extracted with ether. The organic phase was dried and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes / ethyl acetate) to give a white solid. Compound **20:** ^{1}H NMR ($CDCl_3$, δ): 4.72 (s, 2H), 3.30 (dd, J = 8.4, 21.6 Hz, 2H), 1.78 (s, 2H), 1.72 (s, 3H), 1.52 (s, 3H), 1.01 (s, 3H), 0.9-2.3 (m, 7H). ^{13}C NMR ($CDCl_3$, δ): 187.3, 131.8, 109.0, 100.0, 77.3, 74.0, 45.4, 42.8, 39.4, 34.7, 29.8, 26.2, 20.8, 17.6, 16.9. IR (neat) cm^{-1}: 1772, 1627, 1604, 1450. MS m/z (CI-NH_3): 250, 227, 181. HRMS m/z: 250.15661, calcd. for $C_{15}H_{22}O_3$: 250.15690.

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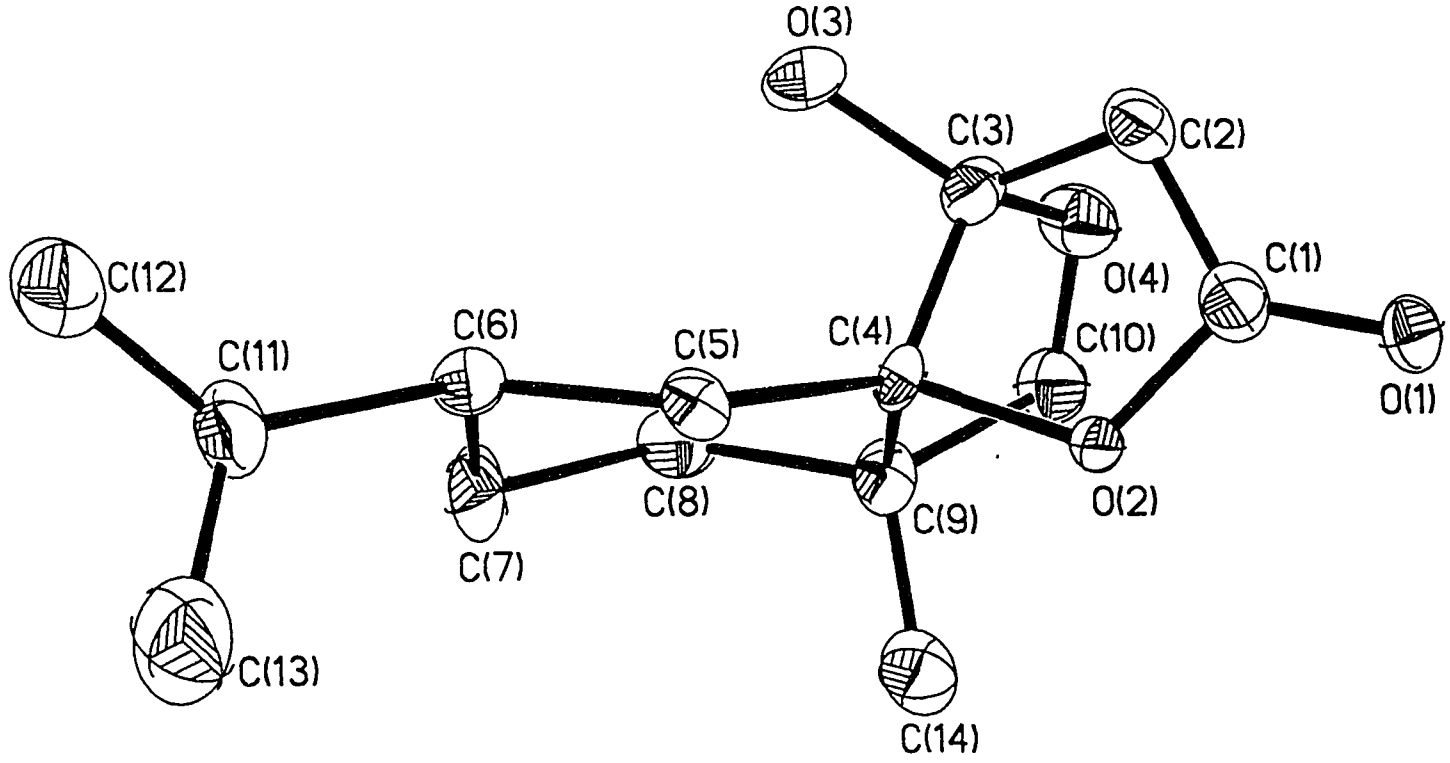

Figure 1: X-ray Structure of Compound 18

CHAPTER IV

A DIRECT FORMAL TOTAL SYNTHESIS OF AFLATOXIN M_2

A paper to be submitted to the *Journal of Organic Chemistry*

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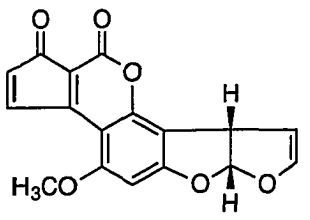
Introduction

Aflatoxins and their relatives belong to a family of mycotoxins.[1] Most of these toxins are produced during the spoiling of foods through the action of fungi. Aflatoxins are present in a variety of foods, including peanuts, rice, wheat, corn, cottonseed, soybean, dairy and wines. These aflatoxins are extremely toxic and most of them are carcinogens.[2,3]

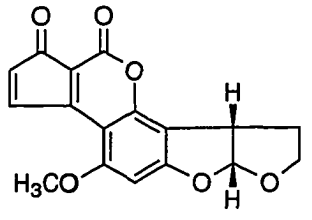
Aflatoxins B_1, B_2, and G_1 are the most widely spread and major toxins contained in the foods. Aflatoxins M_1, M_2, and GM_1 possibly are intermediates in the breakdown of aflatoxins B_1, B_2, and G_1, respectively.[4] Because aflatoxins M_1, M_2, and GM_1 were isolated from the extracts of milk, they are also called "milk toxins".[1] The structures of these aflatoxins are shown in Scheme 1.

The contents of these harmful toxins in milk and dairy products are closely related to human daily life. The concentration of aflatoxins M_1, M_2, and GM_1 in milk and some other dairy products is very important in quality control.[5] In order to better

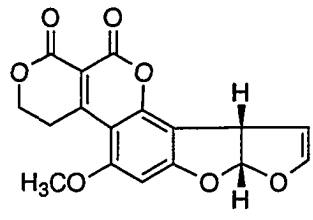
Scheme 1



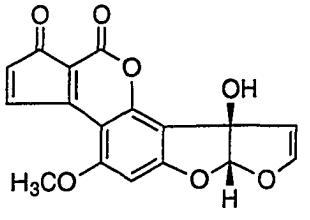
aflatoxin B_1



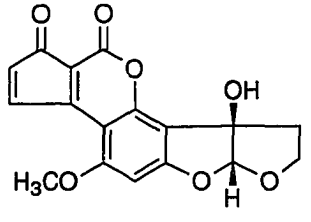
aflatoxin B_2



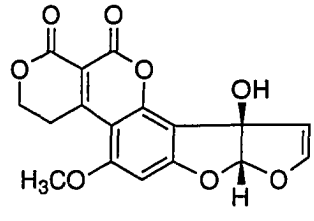
aflatoxin G_1



aflatoxin M_1



aflatoxin M_2



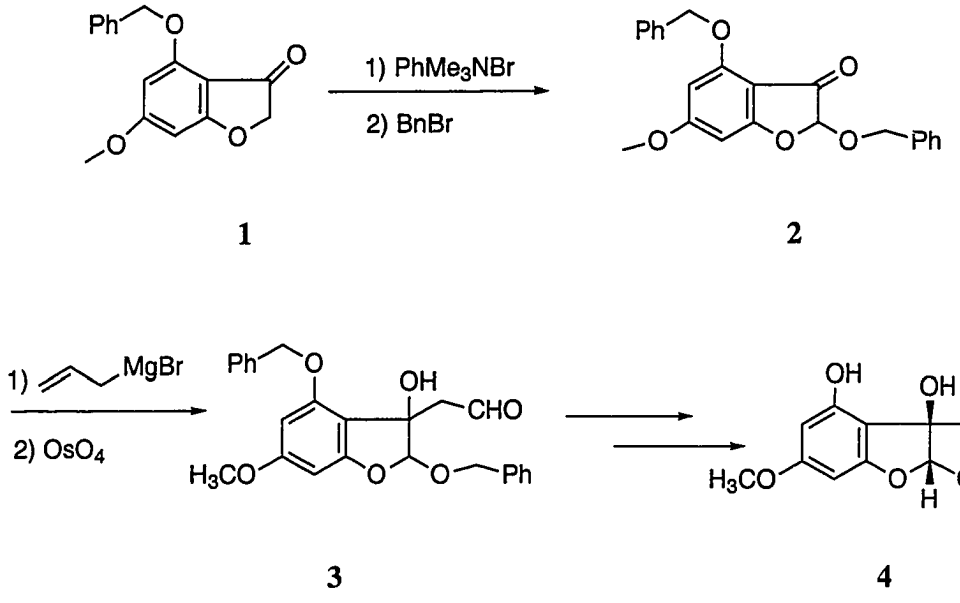
aflatoxin GM_1

understand the mechanism of their toxicity and carcinogenic properties, authentic samples of aflatoxins M_1, M_2, and GM_1 are required.

There have been several reports on the total synthesis of aflatoxins. Most are syntheses of aflatoxin B_1. Fewer are on aflatoxin M, due to its tertiary alcohol, which makes their total synthesis more difficult.

The initial synthetic study of aflatoxin M_1, M_2, and GM_1 was reported by Büchi (Scheme 2).[6] Aflatoxin M_1 was prepared via tricyclic phenol **4**. Starting with benzofuranone **1,** furo[2,*3-b*]benzofuran **4** was prepared in 11 steps. This new coumarin synthesis is generally applicable and useful whenever acid-sensitive phenols interfere with the practice of the classical Pechmann synthesis. This method can also be applied in the total synthesis of aflatoxin B_1, G_1.[7] The synthetic route toward tricyclic intermediate **4** was lengthy, and also involved several different protecting groups.

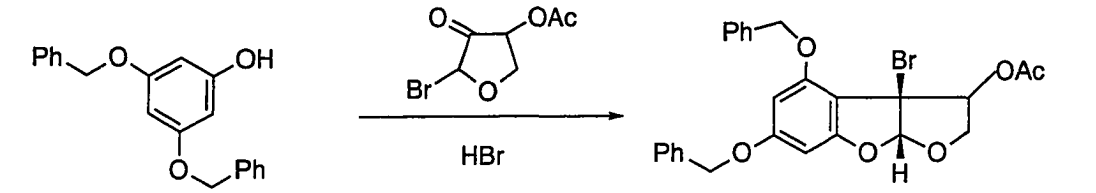
Scheme 2



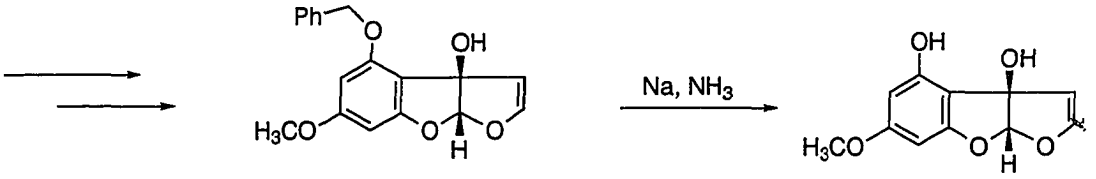
Ten years later, Büchi improved his own synthesis of aflatoxin M_1 (Scheme 3).[8] Starting with substituted phloroglucinol and 1,4-anhydroerythritol, the tricyclic phenol unit of aflatoxin M_1 was prepared in an overall yield of 5%. The key step involved a condensation between substituted phloroglucinol **5** and 4-acetoxy-2-bromotetrahydrofuran-3-one to afford tricyclic compound **6**. Regioselective hydrogenolysis of the benzyl protecting group at C-6 in **6**, and removal of the second benzyl group in compound **7** by Birch reduction gave tricyclic precursor **4**. This new synthesis improved the yield, but the synthesis still involved five different protecting groups.

All of the syntheses reported by Büchi shared the same pathway from tricyclic benzofuranoacetal to final products (Scheme 4).[6] Condensation of compound **4** with 2-carbethoxy-3-bromocyclopentenone in methylene chloride in the presence of zinc

Scheme 3



5 **6**

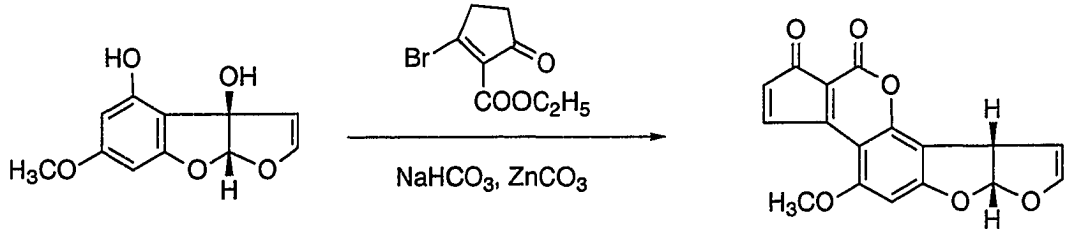


7 **4**

carbonate produced racemic aflatoxin M_1. This method can also be applied in the total synthesis of aflatoxin B_1, G_1, M_1 and GM_1.

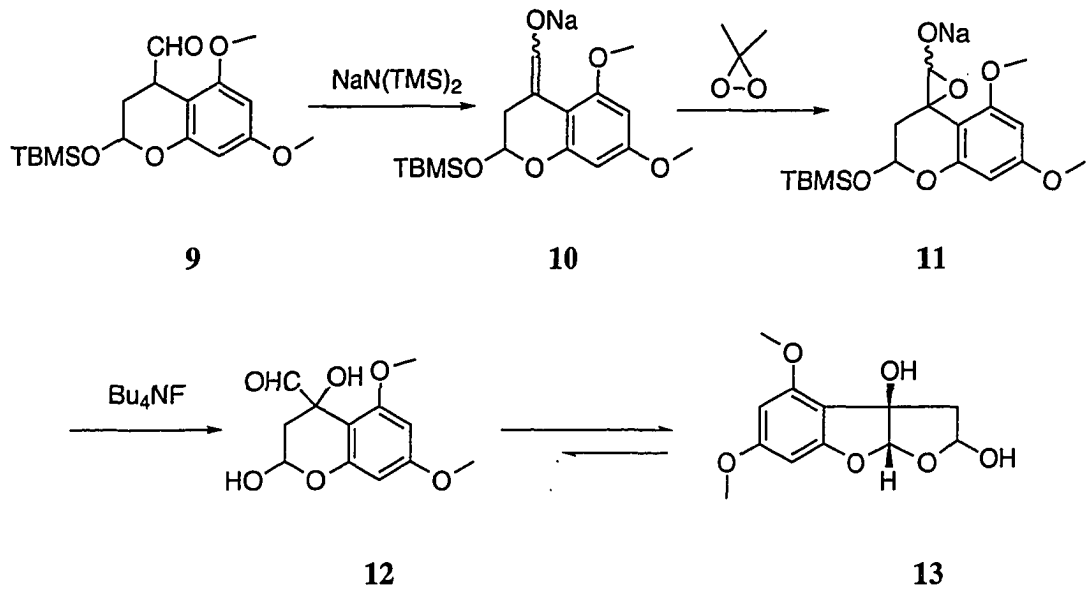
A one-pot procedure[9] from compound **9** involving four steps (enolate formation, epoxidation, epoxide opening, and deprotection) constitutes the key process for

Scheme 4



4 **8**

Scheme 5

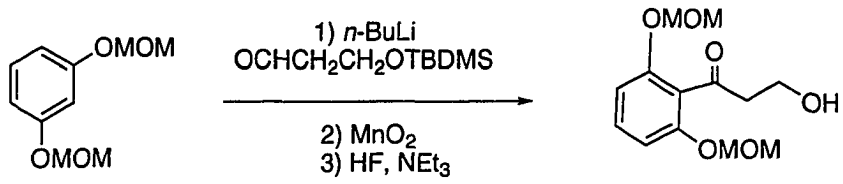


obtaining the hydroxyfuro[2,3-*b*]benzofuran moiety **13** present in aflatoxin M_1 (Scheme 5). The above sequence led to the generation of the benzopyran aldehyde **12**. As expected, this aldehyde underwent equilibration in solution to benzofuran **13**. This epimeric mixture can be converted to aflatoxin M_1.

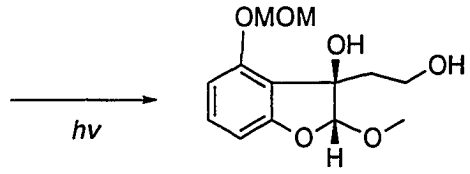
New synthesis of Aflatoxin M_2 are needed, Kraus and Schwinden[10] synthesized aflatoxin precursor **16** by utilizing the type II photocyclization as a key step (Scheme 6). Photocyclization of 2,6-disubstituted acetophenones **15** provides a direct synthetic route to benzofuranol **16**, which can be a precursor to aflatoxin M_2.

The strategies discussed above have their unique features. However, we believe that our method discussed in chapter one will lead to an improved synthetic route towards aflatoxin M.

Scheme 6



14 **15**

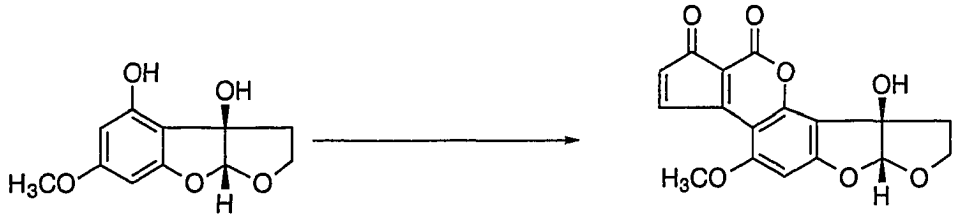


16

Results and discussion

The unique structure of aflatoxin M_2 prompted us to apply our method to this compound. Since the one-step transformation from tricyclic benzofuran acetal **4** to aflatoxins has been reported by Büchi and Weinreb,[6] compound **4** was chosen as our

Scheme 7

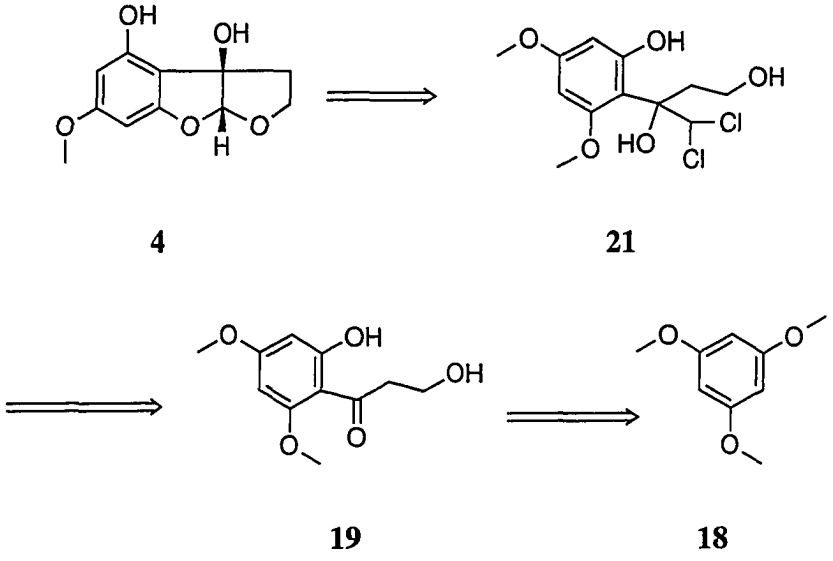


4 **aflatoxin M_2**

target molecule for synthetic studies toward aflatoxin M_2 (Scheme 7).

A retrosynthetic analysis is shown in Scheme 8. It requires the preparation of key intermediate **21**, which in turn would be derived from addition of dichloromethyl lithium to ketone **19**. Compound **19** can be derived from 1,3,5-trimethoxybenzene.

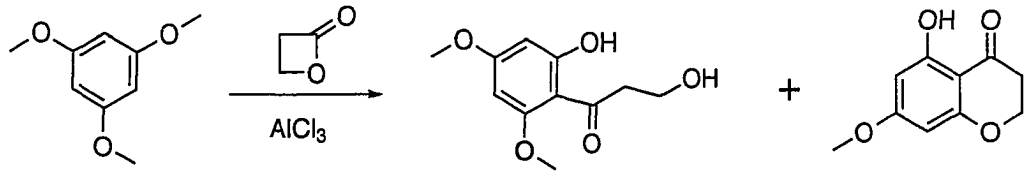
Scheme 8



4 **21** **19** **18**

The synthesis started from 1,3,5-trimethoxybenzene (Scheme 9). It undergoes a Friedel-Crafts reaction with β-propiolactone and excess aluminum trichloride.[11] Depending on the reaction conditions, compounds **19** and **20** were isolated in varying amounts. The best yield of **19** was achieved at 0°C. After addition, the mixture was

Scheme 9

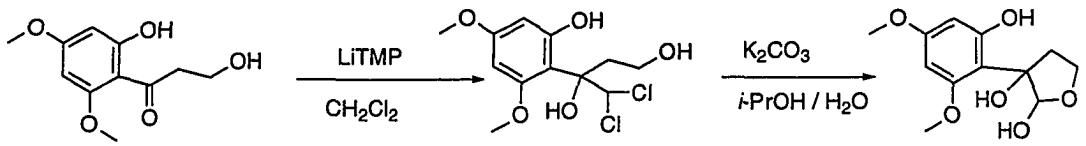


18 **19** **20**

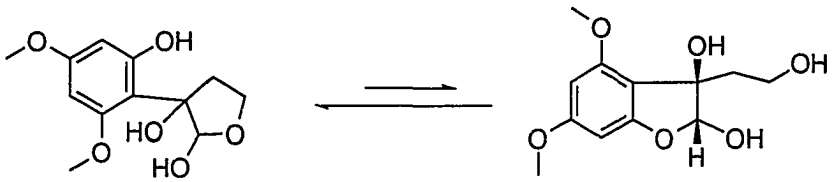
heated to 40°C for 1 hour and then stirred at room temperature for 12 hours.

Addition of dichloromethyl lithium[12] to hydroxy ketone **19** at –78°C resulted in a single isomer (Scheme 10). During the addition process, a trianion was generated, so the reaction was conducted in dilute tetrahydrofuran. The dichloromethyl carbinol **21** can be hydrolyzed with potassium carbonate in aqueous isopropanol.[13]

Scheme 10



19 **21** **22**



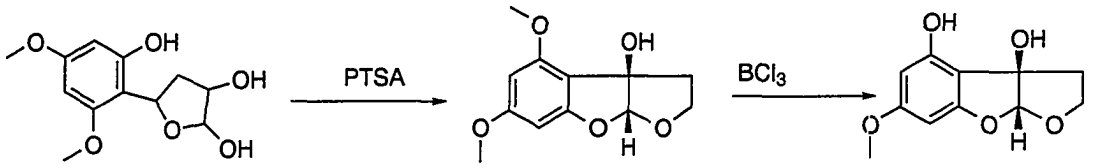
22 **23**

Originally, we predicted two possible products would be isolated from the reaction, because both a primary and a neighboring aromatic hydroxyl in **21** might,through neighboring group participation, enhance the hydrolysis and also protect the resulting aldehyde as a hemiacetal. However, compound **22** was the only hydroxy hemiacetal isolated from dichloromethyl carbinol **21**. This might have occurred because that product **22** is thermodynamically more stable compared with the other possible product **23**.

Hemiacetal **22** was treated with *p*-toluenesulfonic acid to afford **24** (Scheme 11).

Selectively demethylation[14] of the tricyclic benzofuran unit with boron trichloride in methylene chloride solution gave compound **25**. The structures of compounds **24** and **25** were characterized by 2D NOESY NMR experiments. The total synthesis of aflatoxin M_2 from compound **25** can be accomplished in one step by the method reported by Büchi.[6]

Scheme 11



22 **24** **25**

In summary, the synthesis of the precusor of aflatoxin M_2 was accomplished in five steps in 15% overall yield. Compared with other syntheses, this strategy is the most efficient and direct one. It could be used in the synthesis of aflatoxin M_1 and GM_1. Synthetic studies toward these compounds are under investigation.

Experimental

Unless otherwise noted, materials were obtained from commercial suppliers and were used without purification. THF and diethyl ether were distilled from sodium benzophenone ketyl. Dichloromethane was distilled from calcium hydride. Unless otherwise noted, all the reactions were conducted under an argon atmosphere. All organic extracts were dried over anhydrous magnesium sulfate. Infrared spectra were

obtained on a Perkin-Elmer model 1320 spectrophotometer. Nuclear magnetic resonance spectra were determined on either a Varian 300 or a Bruker 400 spectrometer. All chemical shifts are reported relative to tetramethylsilane as an internal standard. Coupling constants are reported in Hz. High-resolution mass spectra were recorded on a Kratos model MS-50 spectrometer. Low-resolution mass spectra were obtained from a Finnegan 4023 mass spectrometer. Flash chromatography was performed on silica gel Kieseldel 60 (mesh 230-400).

3-Hydroxy-1-(2-hydroxy-4, 6-dimethoxyphenyl)propan-1-one (19)

To a suspension of aluminum chloride (2 g, 14 mmol) in methylene chloride (50 mL) at 0°C, a mixture of β -propiolactone (0.216 g, 3 mmol) and 1,3,5-trimethoxybenzene (0.504 g, 3 mmol) in methylene chloride (5 mL) were added slowly. After addition, the reaction mixture was stirred at 40°C for an hour then stirred at room temperature for 12 hours. The reaction mixture was quenched with crushed ice and 15% hydrochloric acid and extracted with diethyl ether. The combined organic layers were washed with saturated sodium bicarbonate solution and then extracted with 1% cold sodium hydroxide solution. The sodium hydroxide solution was acidified to pH 3, and then extracted with ether. The organic phase was dried and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes/ ethyl acetate) to give a white solid. Compound **19**: ^{1}H NMR ($CDCl_3$, δ): 13.79 (s, 1H), 6.04 (d, J = 2.4 Hz, 1H), 5.90 (d, J = 2.4 Hz, 1H), 3.93 (t, J = 5.2 Hz, 1H), 3.83 (s, 3H), 3.80 (s, 3H), 3.23 (t, J = 5.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, δ): 204.9, 167.8, 166.5, 163.1, 105.9, 93.7, 91.0, 58.5, 55.7, 46.2. IR (neat) cm^{-1}: 3540, 1627, 1604, 1450, 1050. MS m/z (CI): 227 (M+1), 181, 154. HRMS m/z M+: 226.08421, calcd. for $C_{11}H_{14}O_5$: 226.08413.

1-(Dichloromethyl)-1-(2-hydroxy-4, 6-dimethoxyphenyl)propan-1, 3-diol (21)

To a solution of carbonyl compound **19** (0.226 g, 1 mmol) and methylene chloride (5 mmol) in tetrahydrofuran (10 mL) at –78°C, LiTMP [3mmol, prepared from 2,2,6,6-tetramethylpiperidine (0.423 g, 3 mmol) and *n*-butyllithium (1.2 mL of 2.5M solution in hexanes)], was added slowly. The reaction mixture was stirred at –78°C for three hours, and monitored by thin layer chromatography (TLC). After reaction was completed, saturated ammonium chloride solution was added to the reaction mixture at –78°C. The reaction mixture was then extracted with diethyl ether, washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes/ ethyl acetate) to give a colorless oil. Compound **21**: ^{1}H NMR ($CDCl_3$, δ): 6.35 (s, 1H), 6.06 (d, J = 2.4 Hz, 1H), 5.98 (d, J = 2.4 Hz, 1H), 4.2-4.3 (m, 2H), 3.75 (s, 3H), 3.75 (s, 3H), 2.2-2.8 (m, 2H). ^{13}C NMR ($CDCl_3$, δ): 161.1, 156.9, 103.2, 95.3, 91.3, 86.0, 78.3, 60.7, 55.6, 37.7. IR (neat) cm^{-1}: 3540, 1627, 1604, 1450, 1050.

3-(2-Hydroxy-4, 6-dimethoxyphenyl)tetrahydrofuran-2, 3-diol (22)

To a dichloromethyl carbinol **21** (100 mg, 0.4 mmol) in the mixture of isopropanol (5 mL) and water (5 mL), potassium carbonate (100 mg) was added and the mixture was stirred at room temperature for 2 hours. The reaction was monitored by TLC. After removal of isopropanol, the aqueous solution was saturated with sodium chloride and extracted with ethyl acetate. The organic phase was dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 1:1 hexanes/ ethyl acetate) to give a white solid. Compound **22**: ^{1}H NMR ($CDCl_3$, δ): 6.05 (d, J = 2.4 Hz, 1H), 5.95 (d, J = 2.4 Hz, 1H), 5.22 (s, 1H), 4.2 (m, 2H), 3.82 (s, 3H), 3.77 (s, 3H), 2.5-2.8 (m, 2H). ^{13}C NMR ($CDCl_3$,

δ): 216.2, 146.9, 111.0, 68.4, 50.1, 44.5, 43.5, 33.2, 25.0, 21.2, 20.6.

3a-Hydroxy-4, 6-dimethoxy-2, 3, 3a, 8a-tetrahydrofuro[2, 3, *b*]benzofuran (24)

To a solution of hydroxy hemiacetal **22** (100 mg, 0.4 mmol) in dry methylene chloride (10 mL) under argon, catalytic amount of the *p*-toluenesulfonic acid (10 mg) was added. The reaction mixture was stirred at 35°C for 4 hours and then diluted with methylene chloride. The organic layer was washed with brine, dried, and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes/ ethyl acetate) to give a white solid. Compound **24**: ^{1}H NMR ($CDCl_3$, δ): 6.35 (s, 1H), 6.09 (d, J = 2.4 Hz, 1H), 5.99 (d, J = 2.4 Hz, 1H), 3.8-4.0 (m, 1H), 3.76 (s, 3H), 3.73 (s, 3H), 2.2-2.8 (m, 2H). IR (neat) cm^{-1}: 1627, 1604, 1450, 1050.

3a, 4-Dihydroxy-6-methoxy-2, 3, 3a, 8a-tetrahydrofuro[2, 3, *b*]benzofuran (25)

To a solution of tricyclic benzofuran unit **24** (10mg, 0.045mmol) in dry methylene chloride (10mL) under argon, 1M boron trichloride solution in methylene chloride (0.045mL, 0.045mmol) was added at 0°C. The reaction mixture was stirred at 0°C for 1 hour and at room temperature for additional 2 hours. After the reaction was complete, the reaction mixture was diluted with methylene chloride, washed with brine, dried, and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes/ ethyl acetate) to give a white solid. Compound **25**: ^{1}H NMR ($CDCl_3$, δ): 6.34 (d, J = 2.4 Hz, 1H), 6.26 (s, 1H), 6.24 (d, J = 2.4 Hz, 1H), 3.8-4.0 (m, 1H), 3.75 (s, 3H), 2.2-2.8 (m, 2H). IR (neat) cm^{-1}: 3350, 1627, 1580, 1450, 1050.

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CHAPTER V

SYNTHESIS OF THE TRICYCLIC HYDROXY HEMIACETAL OF AZADIRACHTIN

A paper to be submitted to the *Journal of Organic Chemistry*

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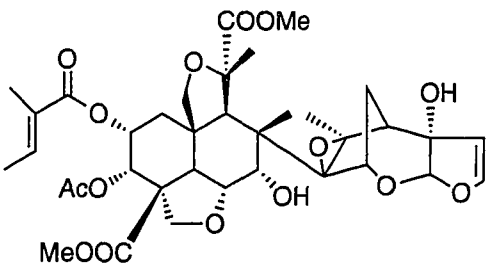
Department of Chemistry, Iowa State University, Ames, IA 50011

Introduction

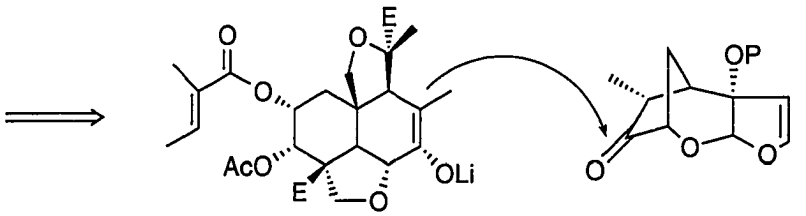
The natural product azadirachtin is a powerful antifeedant and insect growth-disrupting compound. It was isolated from Indian Neem Tree *Azadirachta indica A.* Azadirachtin is extremely active toward a wide range of insect species while exhibiting very low toxicity against mammals.[1] It is difficult to obtain large amounts of azadirachtin for toxicological studies, because the content of azadirachtin in nature is very low. It was also difficult to extract azadirachtin, due to its hydrophilicity.[2]

Scheme 1



Azadirachtin

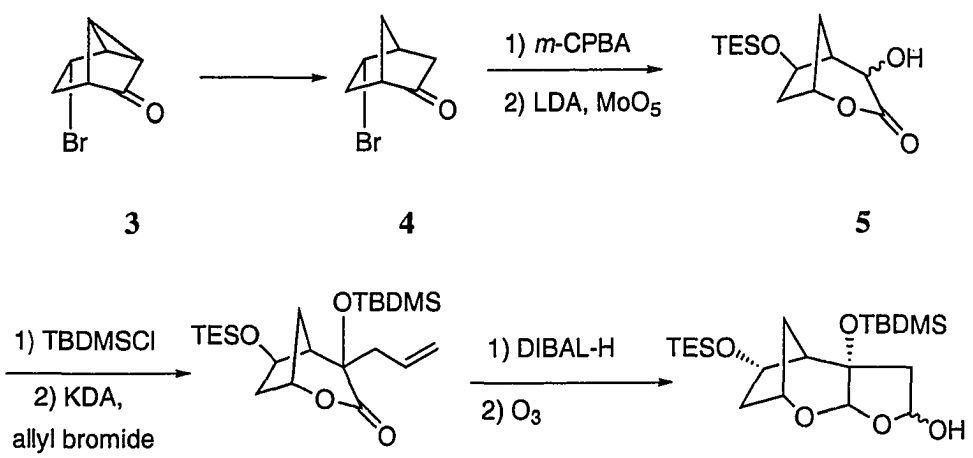
Scheme 2



1 **2**

The structure of azadirachtin is shown in Scheme 1. Azadirachtin contains sixteen stereogenic centers and is highly oxygenated. Although azadirachtin has been isolated twenty years ago,[3] no total synthesis of azadirachtin has been reported in the literature. Ley reported that decalin **1** and tricyclic hemiacetal **2** can be useful intermediates for the total synthesis of azadirachtin (Scheme 2).[4] The complicated structure of azadirachtin and its biological activities has resulted in many synthetic approaches. Both highly oxygenated decalin and the tricyclic hemiacetal units have

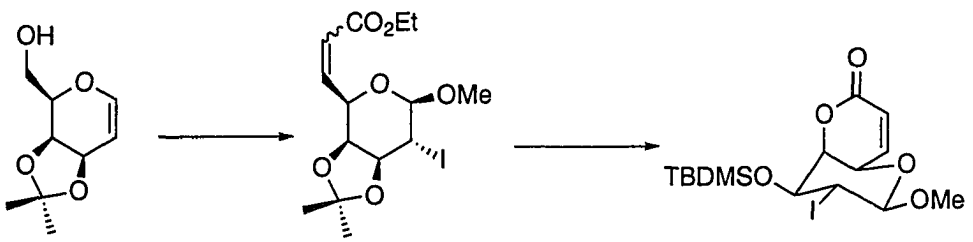
Scheme 3



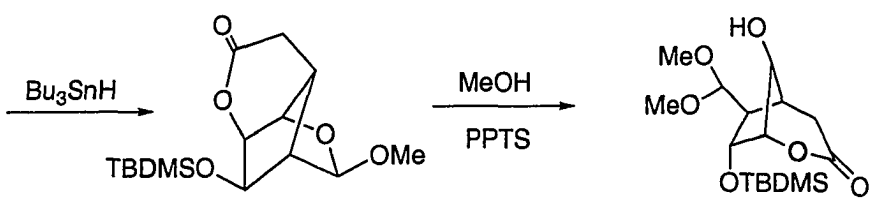
been synthesized. The synthesis of decalin **1** and its analogs was reported independently by Ley,[5-7] Murai,[8] and Blaney.[9,10] Several approaches have used efficient intramolecular Diels-Alder cycloadditions as the key transformation.

In the biological evaluation of azadirachtin and its fragments, both azadirachtin and its tricyclic acetal fragments show significant antifeedant activity against insects. The tricyclic acetal fragment has more promise as a simple analog having good biological activity.[2]

Scheme 3



8 **9** **10**



5 **12**

The most advanced synthetic effort toward the tricyclic hydroxy hemiacetal of azadirachtin has been reported by Ley and coworkers.[11,12] The general strategy toward the tricyclic acetal included a Bayer-Villager reaction as a key step. As shown in Scheme 3, a key intermediate **7** was prepared optically pure from the known bromotricycloheptanone **3**.

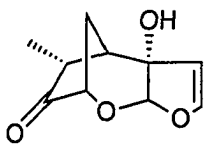
A different strategy was developed by Fraser-Reid and Henry (Scheme 4).[13] Iodolactone **10**, derived from D-galactal, undergoes tributyltin hydride mediated transannular radical cyclization to give the tricyclic lactone **11**, which can be easily converted into an advanced precursor to the tricyclic dihydrofuran portion of azadirachtin.

The strategies discussed above have their unique features; however, the skeleton of tricyclic hydroxy hemiacetal unit of azadirachtin could be easily obtained though the method we discovered.

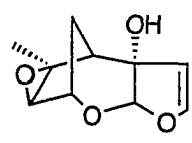
Results and discussion

The unique structure of azadirachtin and its biological activity prompted us to investigate the synthesis of the tricyclic hydroxy acetal unit of azadirachtin. Since the tricyclic furan moiety of azadirachtin, especially the *tert*-hydroxyl and epoxide, is responsible for the antifeeding activity,[2] we chose compounds **13** to **16** as our synthetic targets (Scheme 4). All of these compounds are possible intermediates for a total synthesis of azadirachtin.

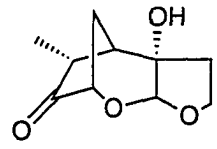
Scheme 4



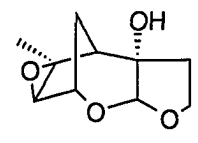
13



14

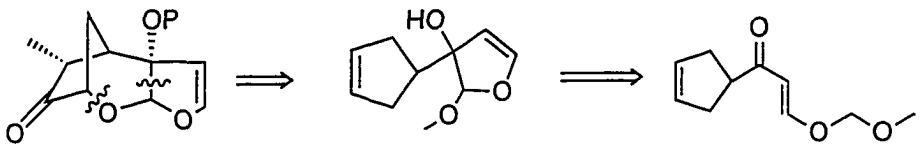


15



16

Scheme 5

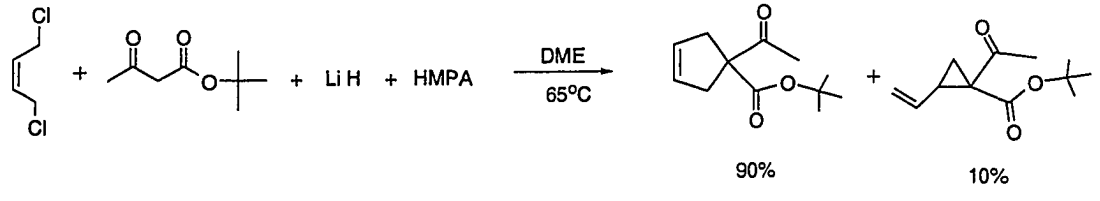


17 **18**

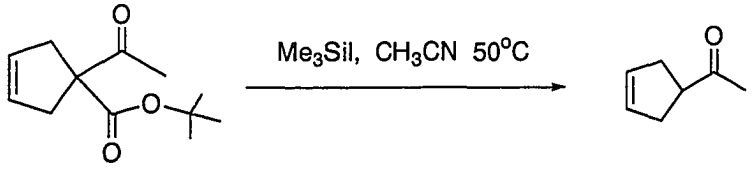
Our initial strategy to prepare compounds **13** and **14** is shown in Scheme 5. It required the preparation of a key intermediate **17**, which would be derived from compound **18** through a δ-hydrogen atom abstraction.

The synthesis began with the preparation of 4-acetylcyclopentene (Scheme 6). *cis*-1,4-Dichlorobutene and *tert*-butyl acetoacetate were reacted with lithium hydride and HMPA in dimethoxyethane to obtain a mixture of ketoesters **19** and **20**.[14] After

Scheme 6

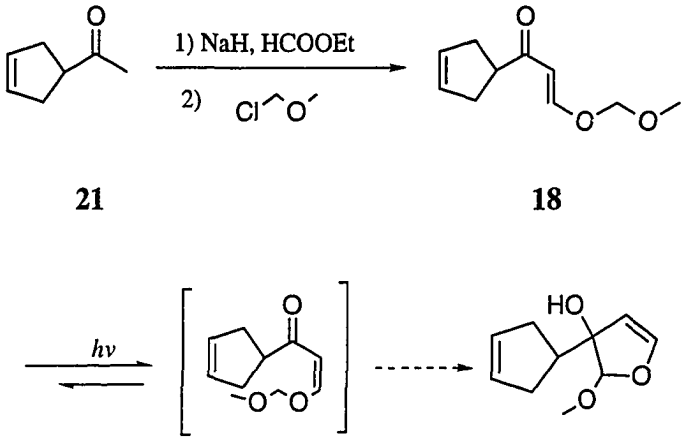


4 **20**



19 **21**

Scheme 7

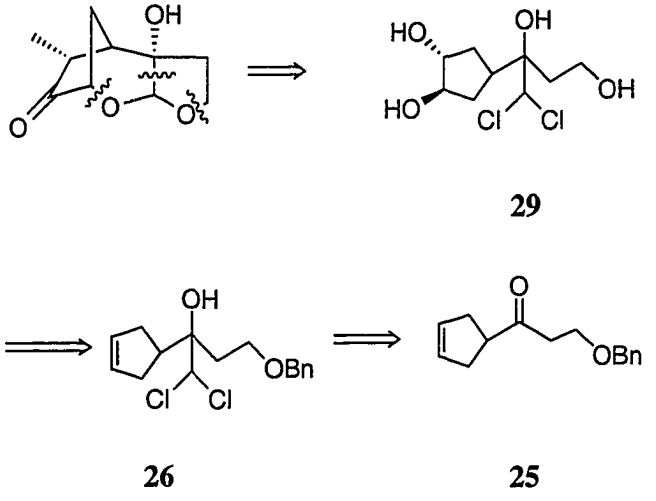


21 **18**

purification, compound **19** was treated with trimethylsilyl iodide to get 1-(cyclopent-3-enyl)ethanone.[15]

1-(cyclopent-3-enyl)ethanone (**21**) was treated with sodium hydride, followed by addition of ethyl formate and methoxymethyl chloride to achieve compound **18** (Scheme 7). Compound **18** was expected to undergo hydrogen atom abstraction and

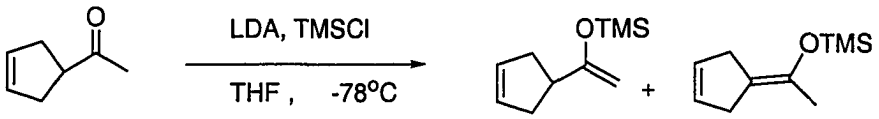
Scheme 8



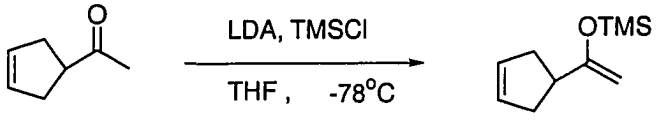
29

26 **25**

Scheme 9



21 **22** **23**

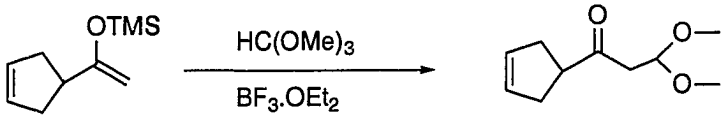


6 **22**

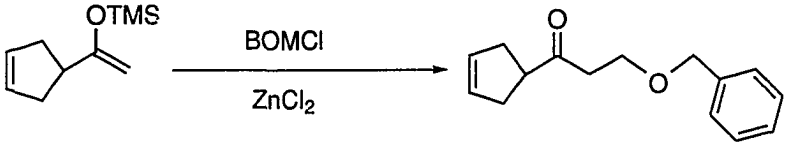
photocyclization to obtain intermediate **17**. During the photolysis, the transformation from *trans*-enone **18** to *cis*-enone was detected, but the cyclization product was not found under these conditions.

While the photocyclization was being studied, we developed a synthetic route involving a dihalomethyl carbinol intermediate. This was used in our second approach.

Scheme 10



22 **24**

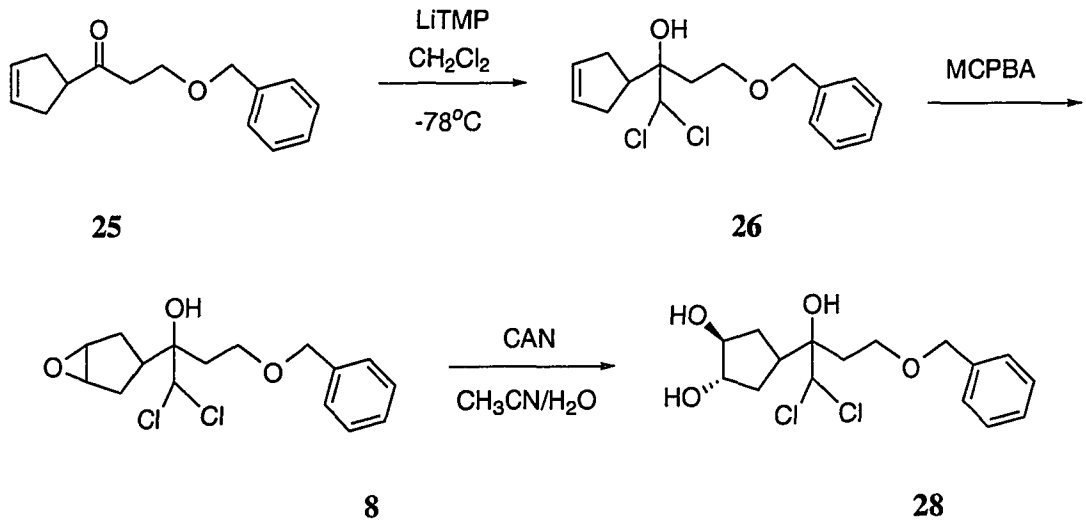


22 **25**

The synthetic analysis is shown in Scheme 8. The tricyclic skeleton of azadirachtin can be derived from key intermediate **29** via hydrolysis. Compound **29** can be prepared from the addition of dichloromethyl lithium to ketone **25**, followed by epoxidation and hydrolysis of epoxide.

The synthesis started from compound **21** (Scheme 9). When compound **21** was treated with LDA and TMSCl, a mixture of two enol silyl ethers was obtained. However, under the LiTMP conditions, compound **22** was the only product.

Scheme 11



25 **26** **8** **28**

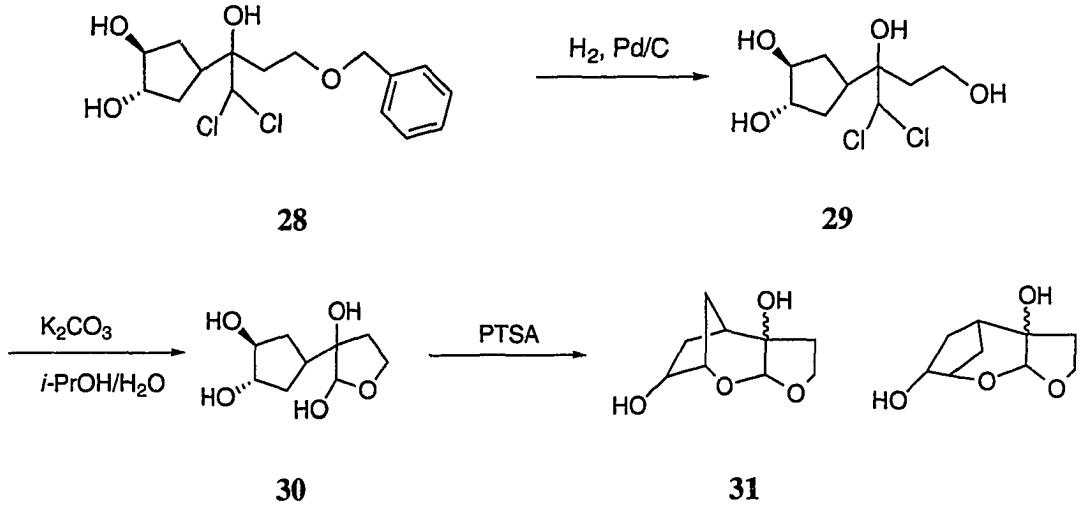
Noyori-type reactions with compound **22** were then investigated (Scheme 10). When enolate **22** was treated with trimethyl orthoformate and boron triflouride etherate,[16,17] compound **24** was obtained; when enolate **22** was treated with benzyl chloromethyl ether and zinc chloride, compound **25** was isolated.[18,19]

Compound **25** can be a precursor in the synthesis of our target molecules **15** and **16** (Scheme 11). The addition of dichloromethyl lithium[20] to compound **25**, followed by epoxidation with MCPBA, provided epoxide **27**. With catalysis by ceric ammonium

nitrate, epoxide **27** was hydrolyzed in a mixture of acetonitrile and water to get trans diol **28**.[21,22]

Hydrogenation of benzyl ether **28**, followed by hydrolysis of the resulting dichloromethyl carbinol with potassium carbonate in isopropanol and water, led to hemiketal **30**.[23] Treating hemiketal **30** with PTSA produced the tricyclic skeleton of

Scheme 12



28 **29**

30 **31**

azadirachtin. Unfortunately, undesired diastereomers were also obtained from this cyclization and could not be separated by column chromatography.

In summary, the synthesis of the tricyclic acetal **31** was achieved using a dichloromethyl carbinol intermediate. The cyclization to the tricyclic structure of azadirachtin needs to be further investigated. The current strategy with modification may be a promising method for the synthesis of the tricyclic acetal unit of azadirachtin. This efficient synthetic route provides a direct entry for the construction of related natural products.

Experimental

Unless otherwise noted, materials were obtained from commercial suppliers and were used without purification. THF and diethyl ether were distilled from sodium benzophenone ketyl. Dichloromethane was distilled from calcium hydride. Unless otherwise noted, all the reactions were conducted under an argon atmosphere. All organic extracts were dried over anhydrous magnesium sulfate. Infrared spectra were obtained on a Perkin-Elmer model 1320 spectrophotometer. Nuclear magnetic resonance spectra were determined on either a Varian 300 or a Bruker 400 spectrometer. All chemical shifts are reported relative to tetramethylsilane as an internal standard. Coupling constants are reported in Hz. High-resolution mass spectra were recorded on a Kratos model MS-50 spectrometer. Low-resolution mass spectra were obtained from a Finnegan 4023 mass spectrometer. Flash chromatography was performed on silica gel Kieseldel 60 (mesh 230-400).

1-Acetyl-cyclopent-3-enecarboxylic acid, *tert*-butyl ester (19)

To a solution of *tert*-butyl acetoacetate (7.9 g, 50 mmol) in dimethoxyethane (450 mL) and HMPA (45 mL) at 0°C, lithium hydride (0.96 g) was added in portions. The reaction mixture was stirred at 0°C for one hour. *cis*-1,4-Dichloro-2-butene (6 mL, 50 mmol) was added slowly. After addition, the reaction mixture was stirred at 65°C for 72 hours. The reaction mixture was diluted with a mixture of ether (200 mL) and hexanes (200 mL), then quenched with water. The reaction mixture was extracted with the 1:1 mixture of hexanes and ether, washed with brine, dried and concentrated in vacuo. The residue was distilled under vacuum. (b.p. 84-86 °C / 20 mm Hg) Compound **19**: ^{1}H NMR ($CDCl_3$, δ): 5.56 (s, 2H), 2.6-2.8 (m, 4H), 2.18 (s, 3H), 1.46 (s, 9H).

1-(Cyclopent-3-enyl)ethanone (21)

To a solution of ketoester **19** (21 g, 0.1 mol) in acetonitrile (350 mL) at 0°C, trimethylsilyl iodide (25 g, 0.12 mol) was added slowly then stirred at 50°C for 8 hours. The reaction mixture was quenched with saturated sodium thiosulfate (25 mL), then was extracted with diethyl ether. The organic phase was washed with brine, dried and concentrated in vacuo. The residue was distilled under the vacuum. (b. p. 64-66°C / 80 mm Hg) Compound **21**: ^{1}H NMR ($CDCl_3$, δ): 5.65 (s, 2H), 3.23 (p, J = 5.6 Hz, 1H), 2.58 (m, 4H), 2.18 (s, 3H).

1-(Cyclopent-3-enyl)vinyloxyltrimethylsilane (22)

To a stirred solution of diisopropylamine (1.8 mL, 12 mmol) in tetrahydrofuran (15 mL), a solution of *n*-butyllithium (4.4 mL of 2.5M solution in hexane) was added at 0°C. After 1 hour, the reaction mixture was cooled to –78°C, and a solution of compound **21** (1.40 g, 10 mmol) in tetrahydrofuran (5 mL) was added over 10 min period. The reaction mixture was stirred at –78°C for 1 hour, and then stirred at –20°C for 2 hours. Chlorotrimethylsilane (1.2 g, 11 mmol) was added to the reaction mixture. After 1 hour, the reaction mixture was diluted with hexanes, filtered through Celite, and then concentrated in vacuo. Compound **22**: ^{1}H NMR ($CDCl_3$, δ): 5.65 (s, 2H), 4.84 (t, J = 6 Hz, 2H), 3.35 (s, 6H), 3.25 (p, J = 6.9 Hz, 1H), 2.77 (d, J = 5.4 Hz, 1H), 2.5 (m, 4H).

1-(Cyclopent-3-enyl)-3, 3-dimethoxypropan-1-ol (24)

To a solution of compound **22** (8.03 g, 50 mmol) in dry ether (500 mL) under argon, methyllithium (32 mL of 1.4M solution in ether) was added at 0°C, and the mixture was stirred for 1 hour. The reaction mixture was cooled to –95°C, trimethyl

orthoformate (4.82 mL, 50 mmol) was added followed by fast addition of boron trifluoride etherate (5.3 g, 50 mmol) in one portion under vigorous stirring, then stirred at –78°C for 1 hour. The reaction mixture was quenched by addition of saturated ammonium chloride solution and shaken vigorous. The mixture was extracted with ether, dried and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes / ethyl acetate) to give a colorless oil. Compound **24**: ^{1}H NMR ($CDCl_3$, δ): 7.31 (m, 5H), 5.61 (s, 2H), 4.49 (s, 2H), 3.73 (t, J = 6.4 Hz, 2H), 3.25 (p, J = 6.9 Hz, 1H), 2.74 (t, J = 6.4 Hz, 2H), 2.5 (m, 4H).

1-(Cyclopent-3-enyl)-3-benzyloxypropan-1-one (25)

To a solution of compound **22** (0.91 g, 5 mmol) in dry ether (50 mL) under argon, zinc chloride (10 mmol, 1 mL of 1M solution in methylene chloride) was added at 0°C followed by addition of an ether solution of benzyl chloromethyl ether (0.94 g, 6 mmol). After addition, the mixture was stirred for 1 hour, then 1,4-dioxane (5 mL) and hexanes (50 mL) was added to the reaction mixture. And then the reaction mixture was filtered to remove zinc salts. The filtrate was concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes / ethyl acetate) to give a colorless oil. Compound **25**: ^{1}H NMR ($CDCl_3$, δ): 7.2-7.4 (m, 5H), 5.80 (s, 1H), 5.67 (s, 2H), 4.53 (d, J = 6.9 Hz, 2H), 4.34 (m, 1H), 4.01 (m, 1H), 3.79 (m, 1H), 2.83 (p, J = 6.9 Hz, 1H), 2.45 (m, 1H), 2.33 (m, 5H), 2.10 (m, 1H).

1-(Cyclopent-3-enyl)-1-dichloromethyl-3-benzyloxypropan-1-ol (26)

To a solution of carbonyl compound **25** (2.2 g, 10 mmol) in tetrahydrofuran (50mL) and methylene chloride (50 mmol) at –78°C, excess LiTMP [30 mmol, prepared from 2,2,6,6-tetramethylpiperidine (4.23 g, 30 mmol) and butyllithium (12 mL of 2.5M

solution in hexanes)], was added slowly. The reaction mixture was stirred at –78°C for three hours, and monitored by TLC. After reaction was complete, saturated ammonium chloride solution was added to the reaction mixture at –78°C. The reaction mixture was then extracted with diethyl ether, washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes/ ethyl acetate) to give a colorless oil. Compound **26**: ^{1}H NMR ($CDCl_3$, δ): 8.19 (br s, 1H), 7.1-7.2 (m, 2H), 6.8-6.9 (m, 2H), 6.13 (s, 1H), 3.54 (br s, 1H), 1.87 (s, 3H).

1-(3, 4-Epoxycyclopentane)-1-dichloromethyl-3-(benzyloxy)propan-1-ol (27)

To a solution of compound **26** (388 mg, 1 mmol) in methylene chloride (25 mL) at 0°C, *m*-chloroperbenzoic acid (200 mg, 1.1 mmol) was added and then stirred at room temperature for 2 hours. After the filtration, the reaction mixture was concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 4:1 hexanes/ ethyl acetate) to give a colorless oil. Compound **27**: ^{1}H NMR ($CDCl_3$, δ): 7.2-7.3 (m, 5H), 5.67 (s, 2H), 4.50 (dd, J = 15, 1.3 Hz, 2H), 4.0 (m, 1H), 3.7 (m, 1H), 3.56 (s, 2H), 1.7-2.2 (m, 7H).

1-(Cyclopentan-*tran*-3, 4 -diol)-1-dichloromethyl-3-(benzyloxy)propan-1-ol (28)

To dihalomethyl carbinol **27** (100 mg, 0.6 mmol) in the mixture of acetonitrile (8 mL) and water (2 mL), ceric ammonium nitrate (20 mg, 0.005 mmol) was added at room temperature. The reaction was monitored by TLC. The reaction mixture was saturated with sodium chloride and then extracted with ethyl acetate. The organic phase was dried and concentrated with vacuo. The residue was purified with flash column chromatography (eluting with 1:1 hexanes / ethyl acetate) to give a colorless oil.

Compound **28**: ^{1}H NMR ($CDCl_3$, δ): 7.2-7.3 (m, 5H), 5.76 (s, 1H), 5.74 (s, 1H), 4.94 (d, *J*=6.4 Hz, 1H), 4.54 (d, *J* = 6.0 Hz, 1H), 3.5-4.0 (m, 4H), 2.70 (p, *J* = 6.9 Hz), 2.0-2.3 (m, 4H), 1.5-1.8 (m, 2H).

1-(Cyclopentan-*tran*-3, 4-diol)-1-dichloromethyl-propane-1, 3-diol (29)

To a solution dichloromethyl carbinol **28** (100mg, 0.4mmol) in methanol (25mL), palladium on carbon catalyst (100mg, 10%) was added. The reaction mixture was stirred under a hydrogen atmosphere at room temperature for 12 hours. After filtration to remove palladium catalyst, the filtrate was concentrated in vacuo. The residue was purified with flash column chromatography (eluting with ethyl acetate) to give a colorless oil. Compound **29**: ^{1}H NMR ($CDCl_3$, δ): 5.76 (s, 1H), 4.81 (s, 1H), 4.36 (m, 1H), 3.96 (m, 1H), 2.8 (m, 1H), 2.0-2.5 (m, 4H).

3-(Cyclopentan-*tran*-3, 4-diol)tetrahydrofuran-2, 3-diol (30)

To a dihalomethyl carbinol **29** (100 mg, 0.6 mmol) in the mixture of isopropanol (5 mL) and water (5 mL), potassium carbonate (100 mg) was added and then stirred at room temperature for 2 hours. Reaction was monitored by TLC. After removal of isopropanol, the aqueous solution was saturated with sodium chloride and extracted with ethyl acetate. The organic phase was dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with 10:1 ethyl acetate /methanol) to give a white solid. Compound **30**: ^{1}H NMR ($CDCl_3$, δ): 4.93 (s, 1H), 4.2-4.5 (m, 2H), 3.8-4.0 (m, 4H), 2.70 (p, *J* = 6.9 Hz, 1H), 2.0-2.3 (m, 4H), 1.5-1.8 (m, 2H).

6-Hydroxy-1, 9-dioxotricyclo [6.2.1.0 ^{2,6}] undercan-2, 9-diol (31)

To a solution of hydroxy hemiacetal **30** (50 mg, 0.3 mmol) in dry methylene chloride (10 mL) under argon, a catalytic amount of *p*-toluenesulfonic acid (5 mg) was added. The reaction mixture was stirred at 35°C for 4 hours and then diluted with methylene chloride. The organic layer was washed with brine, dried, and concentrated in vacuo. The residue was purified with flash column chromatography (eluting with hexanes/ ethyl acetate 4:1) to give a white solid. Compound **31**: ^{1}H NMR ($CDCl_3$, δ): 5.95 (s, 1H), 4.78 (d, J = 9 Hz, 1H), 4.25 (d, J = 9.3 Hz, 1H), 2.48 (s, 1H), 1.87 (s, 3H), 1.2-2.2 (m, 6H), 1.02 (s, 3H).

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GENERAL CONCLUSIONS

The task of building complex natural products is often facilitated by the introduction of new synthetic methodology. In this work, we have developed a general method to generate hydroxy hemiacetals via dihalomethyl carbinol intermediates. This method is direct and versatile. By using this method, several natural products have been synthesized, which include sesquiterpene-based endothelin receptor antagonists, phytuberin, aflatoxin M_2 and the tricyclic hydroxy hemiacetal subunit of azadirachtin.

We hope that this new methodology developed here will broaden the application of methylene chloride and methylene bromide as economical chemical reagents. And we also hope this method will provide an alternative route α-hydroxy aldehydes.

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