

PROGRESS TOWARD THE TOTAL SYNTHESIS OF (+)-MYRICERIC ACID A

by

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AN ABSTRACT OF A DISSERTATION

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Department of Chemistry  
College of Arts and Sciences

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

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## Abstract

(+)-Myriceric acid A, [(+)-**1.1**], is a natural product isolated in 0.01% yield from the southern bayberry, *myrica cerifera* twigs. It is a specific ET<sub>A</sub> receptor antagonist because it selectively inhibits the endothelin-1 (ET-1) induced increase in [Ca<sup>2+</sup>] (IC<sub>50</sub> = 11 ± 2 nM) and antagonizes the binding of ET-1 (K<sub>i</sub> = 66 ± 15 nM), in rat aortic smooth muscle cells. ET-1 is a potent vasoconstrictor peptide released by the vascular endothelial cell. Over production of this peptide causes vasospasm, which may lead to heart attack, stroke, pulmonary hypertension, and congestive heart failure.

My research involved the development of a total synthesis of (+)-myriceric acid A. This is a triterpenoid compound that has five six-member rings, seven stereo-centers, a carboxylic acid group, and a *trans*-caffeoyl ester side chain. The synthesis was planned to be accomplished by adding the D and E rings to the known ABC ring compound (4a'*S*,4b'*R*,8a'*R*)-1',1',4a',8a'-tetramethyldecahydro-1'*H*-spiro[[1,3]dioxolane-2,2'-phenanthren]-8'(3'*H*)-one [(**-**)-**2.1**].

Many model studies, both convergent and linear syntheses, were conducted to determine the best approach to construct the D and E rings. From these studies it was determined that a linear synthesis was best.

After the ABCD ring compound (4a*R*,4b*R*,6a*R*,10b*R*)-1,1,4a,10b-tetramethyl-4,4a,4b,5,6,6a,7,8,10b,11,12,12a-dodecahydro-1*H*-spiro[chrysene-2,2'-[1,3]dioxolan]-9(3*H*)-one [(**-**)-**3.41a**] was synthesized, several approaches were investigated for the functionalization of the D ring. The best method turned out to be one in which the C14 position of **3.41a** was functionalized by a Michael addition of a nitrile group. Conversion of the nitrile to the aldehyde proved to be problematic, but was overcome by the formation of an interesting cyclic hemiiminal which hydrolyzed cleanly to the aldehyde (4a*R*,4b*R*,6a*R*,10a*S*,10b*R*,12a*R*)-1,1,4a,10b-tetramethyl-9-oxohexadeca-hydro-1*H*-spiro[chrysene-2,2'-[1,3]dioxolane]-10a-carbaldehyde (**4.22**) when treated with acid.

Herein, the studies that led to the tetra-cyclic aldehyde **4.22**, a key intermediate for the synthesis of (+)-myriceric acid A, will be discussed.

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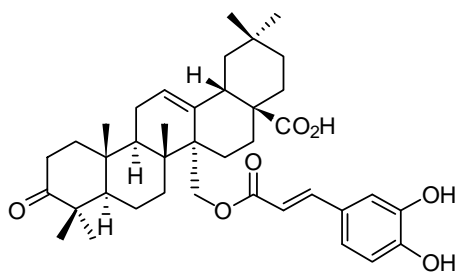
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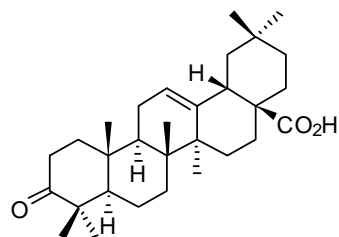
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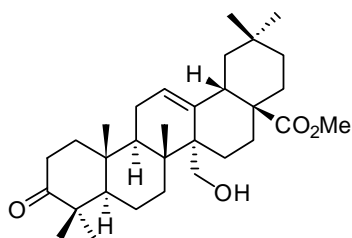
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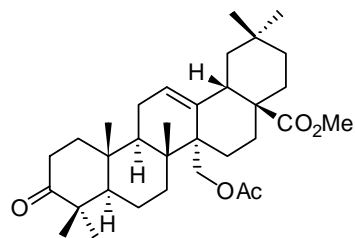
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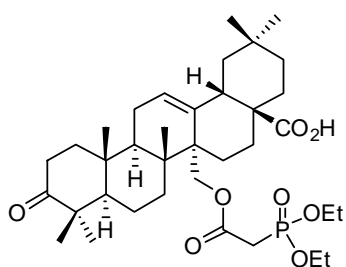
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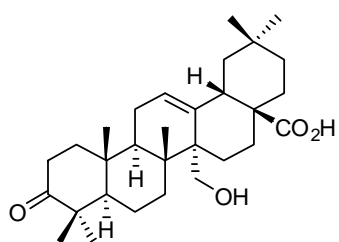
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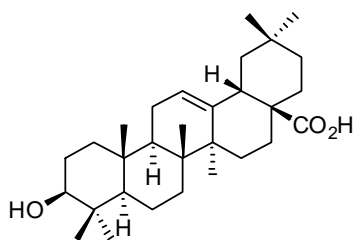
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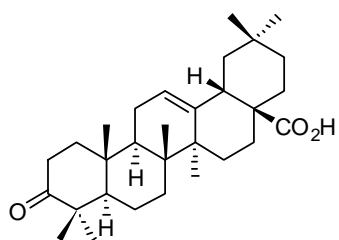
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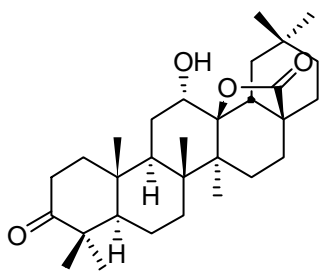
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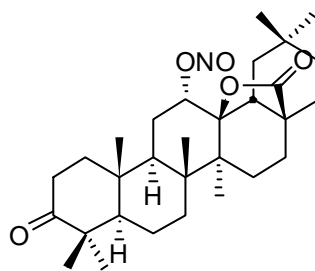
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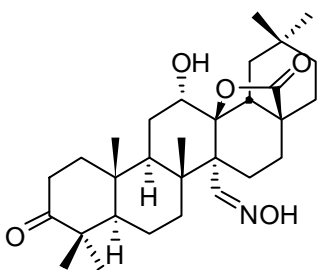
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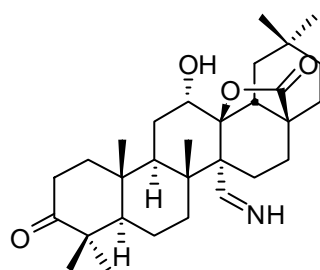
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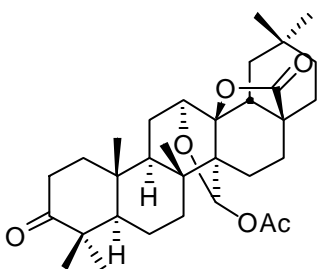
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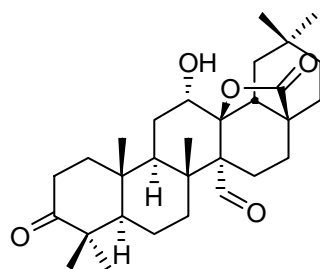
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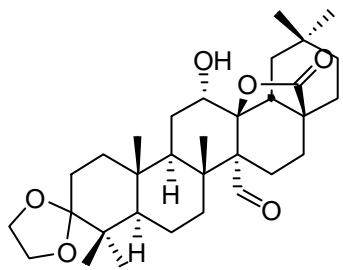
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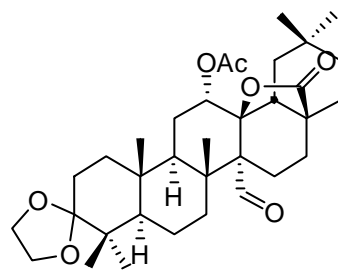
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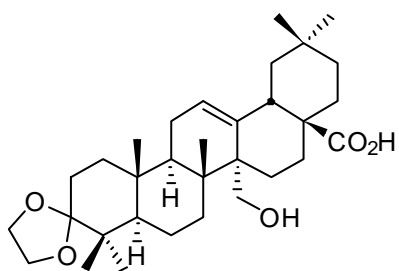


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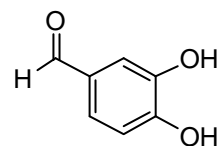


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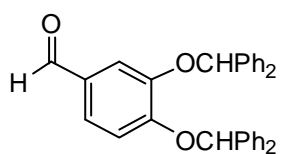




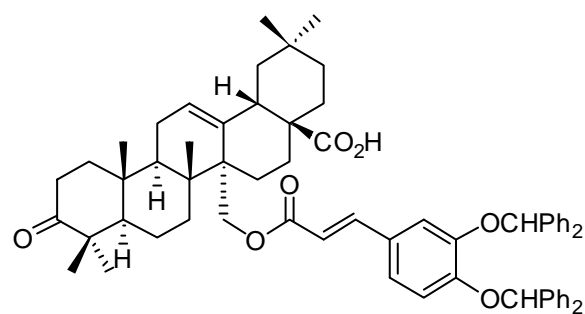
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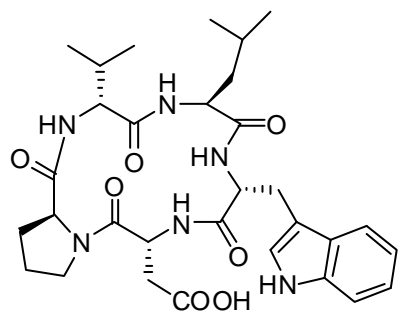
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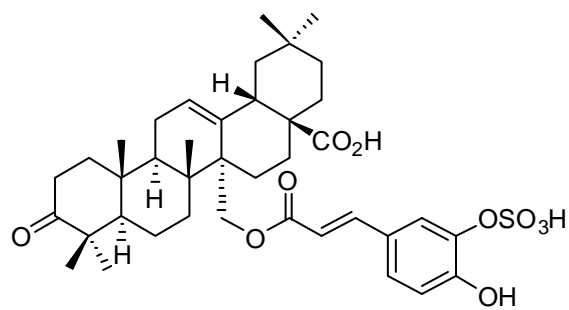
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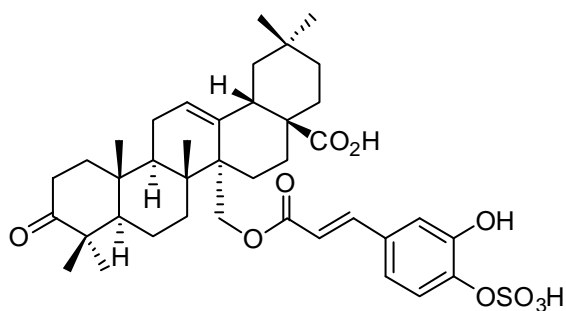
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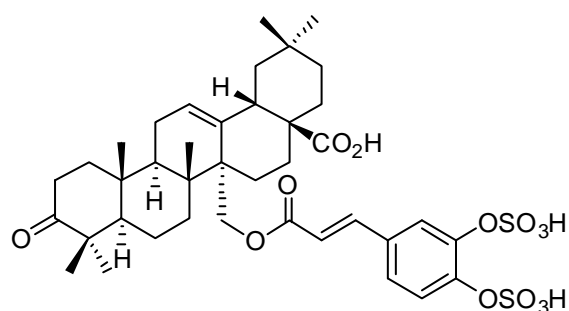
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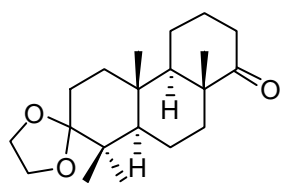
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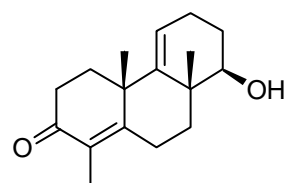
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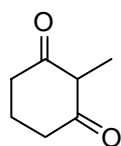
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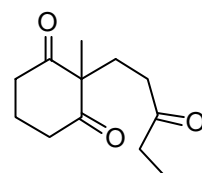
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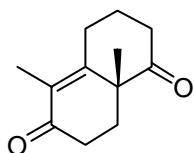
**(+)-2.2**



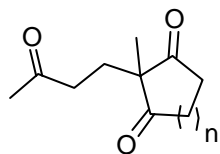
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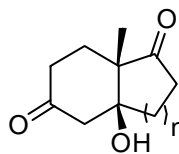
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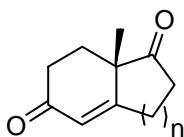
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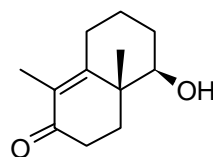
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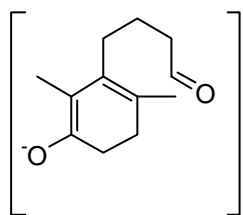
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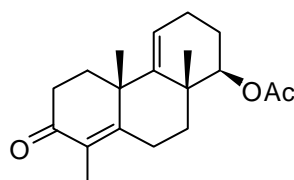
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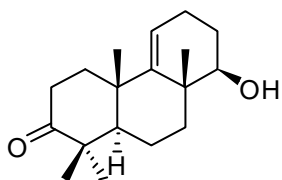
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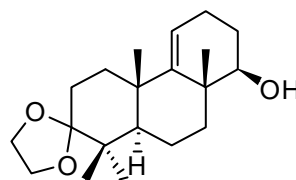
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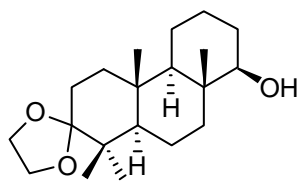
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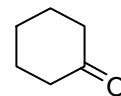
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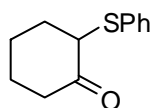
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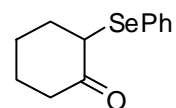
**(-)-2.14**



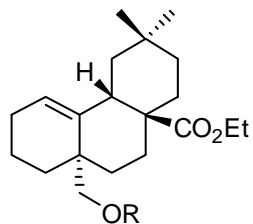
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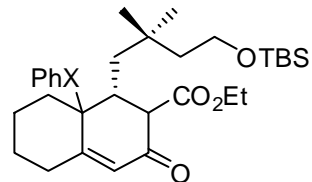
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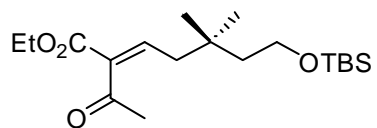
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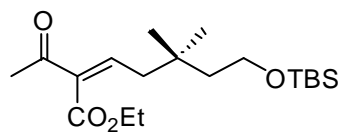
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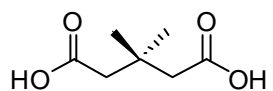
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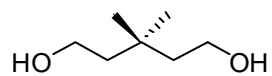
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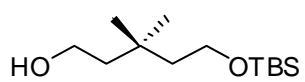
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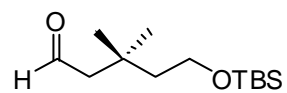
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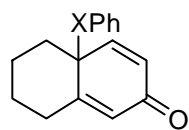
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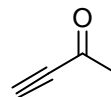
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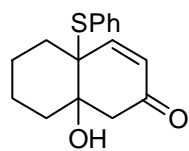
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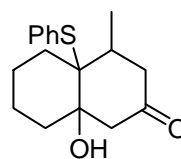
2.25  
X = S or Se



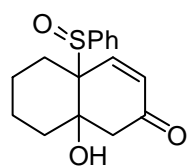
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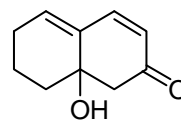
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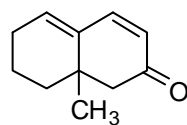
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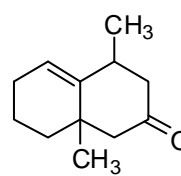
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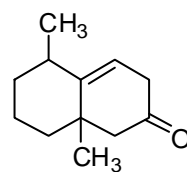
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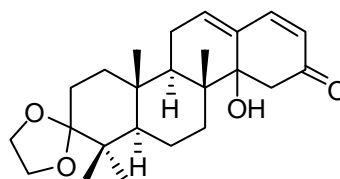
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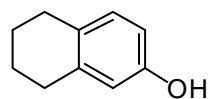
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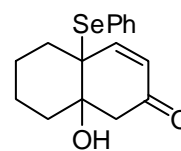
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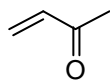
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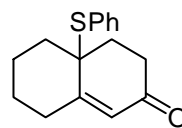
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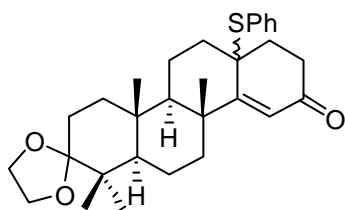
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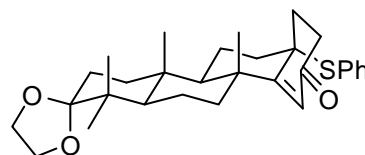
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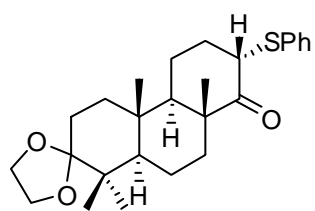
**2.38**



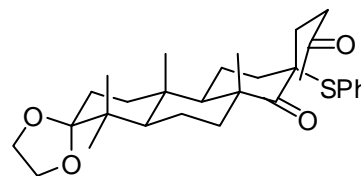
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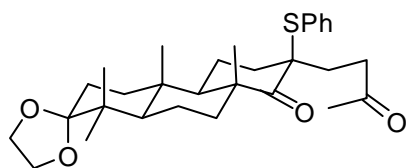
**3.1a**



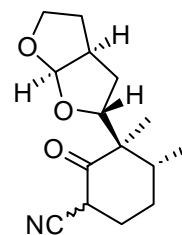
**(+)-3.2**



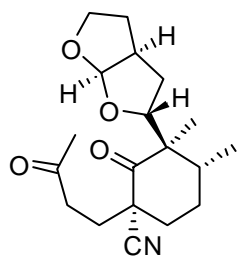
**(-)-3.3a**



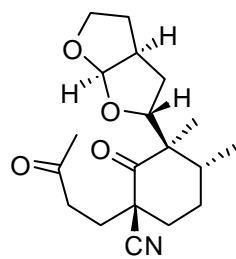
**(-)-3.3b**



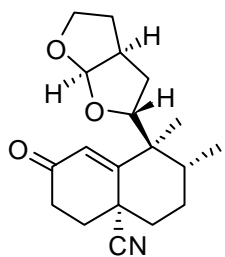
**3.4**



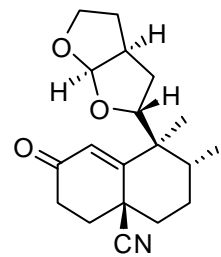
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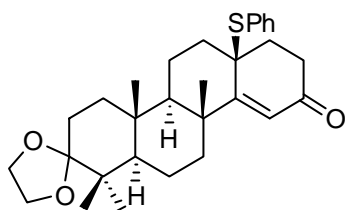
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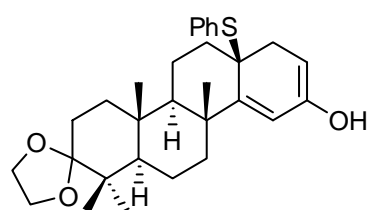
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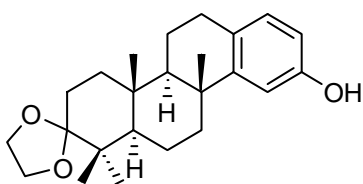
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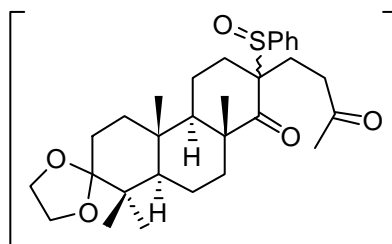
3.9



3.10

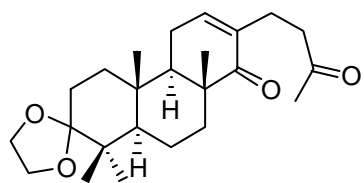


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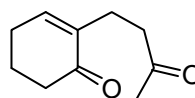


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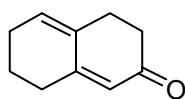




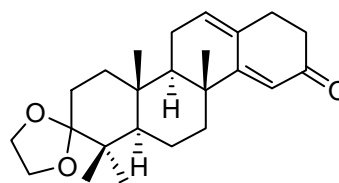
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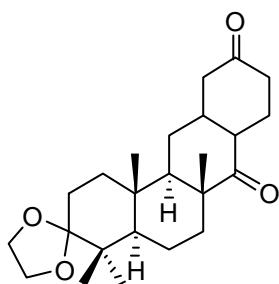
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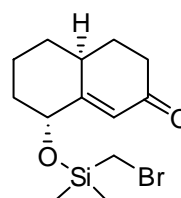
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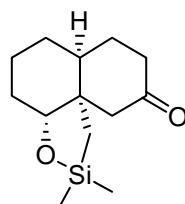
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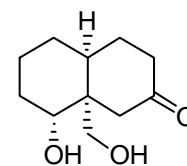
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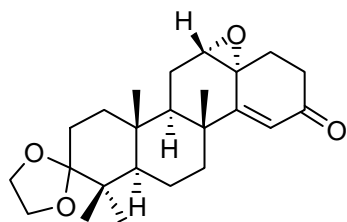
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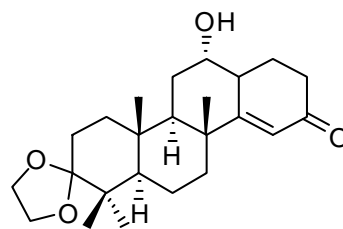
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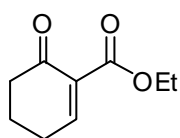
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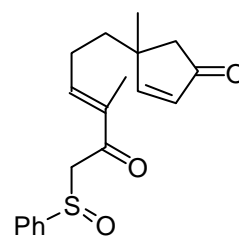
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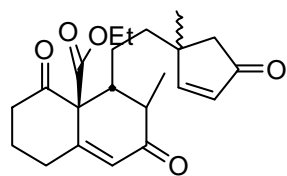
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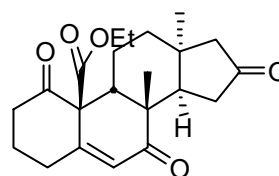
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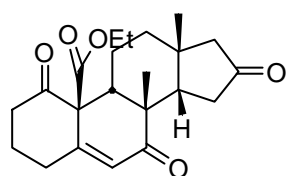
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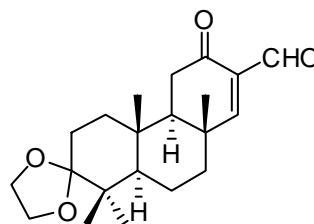
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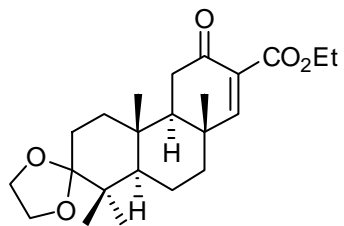
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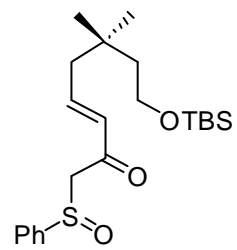
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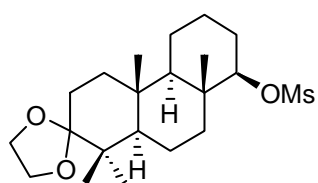
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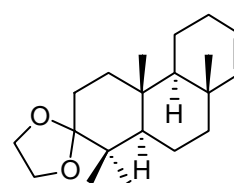
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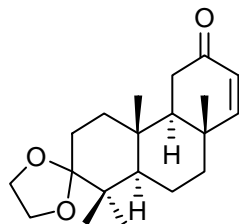
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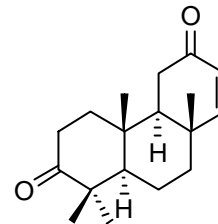
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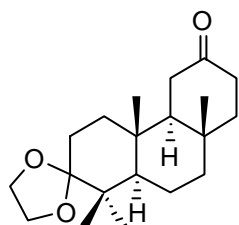
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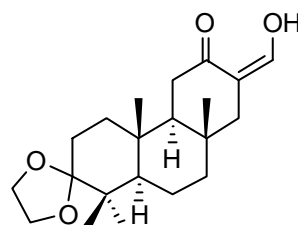
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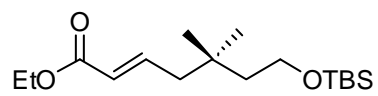
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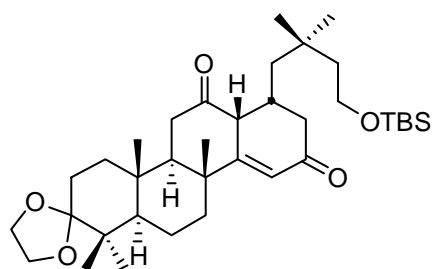
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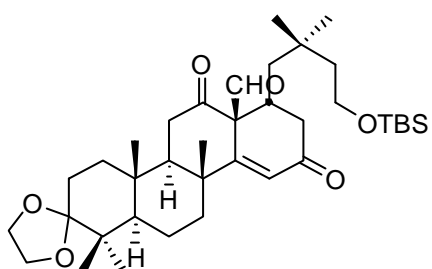
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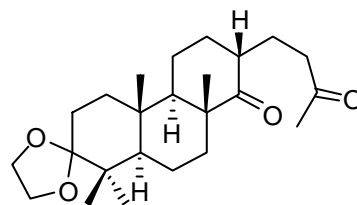
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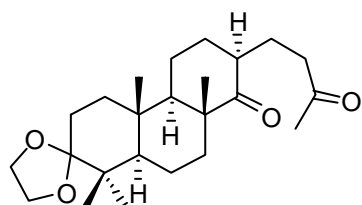
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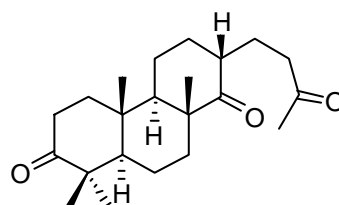
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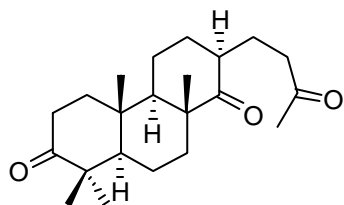
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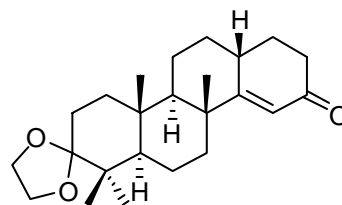
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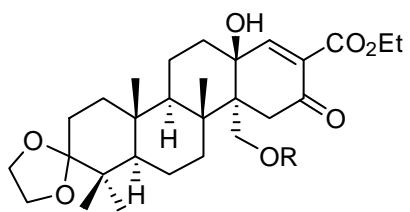
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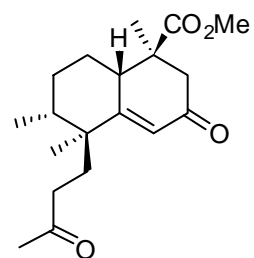
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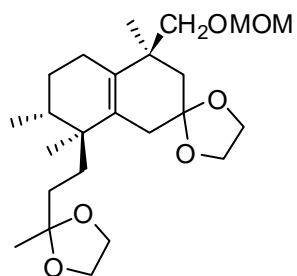
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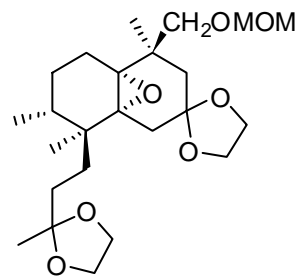
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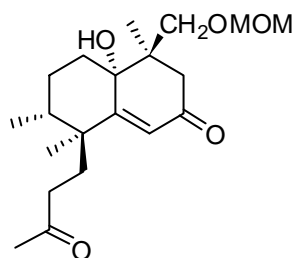
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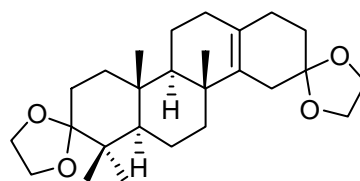
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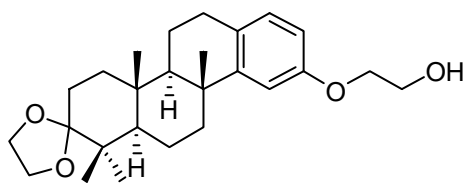
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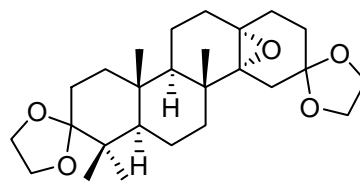
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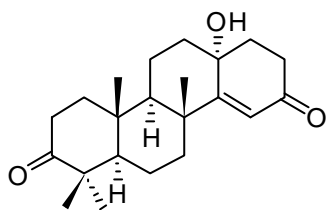
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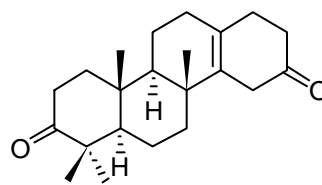
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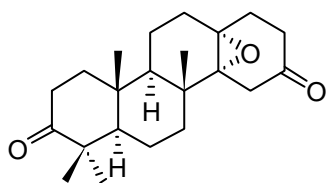
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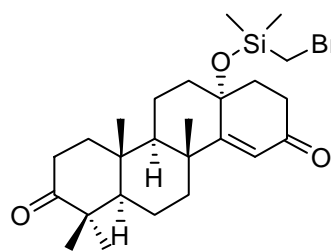
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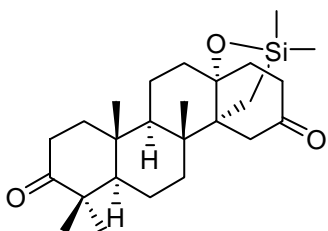
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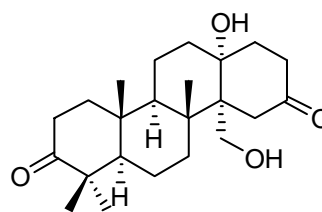
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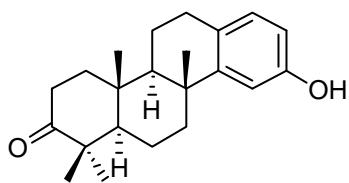
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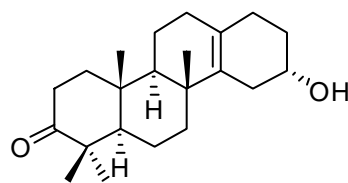
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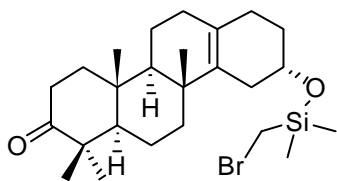
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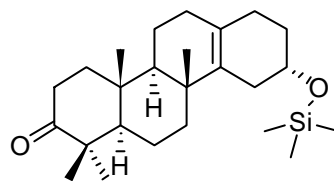
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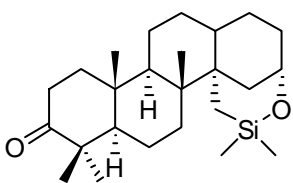
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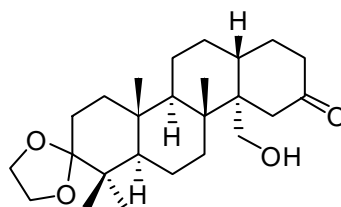
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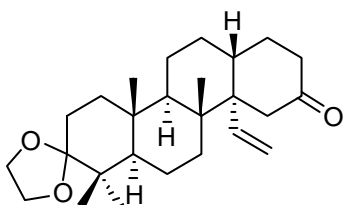
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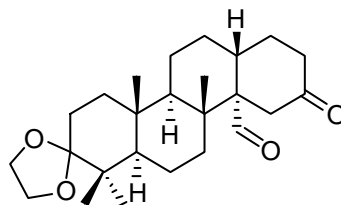
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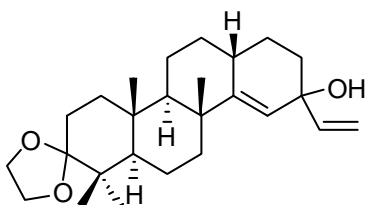
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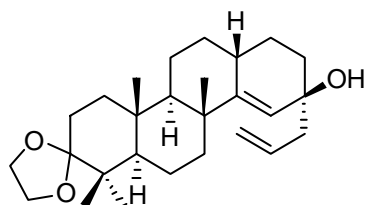
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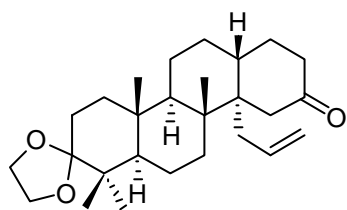
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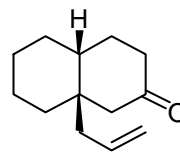
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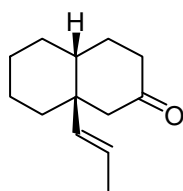
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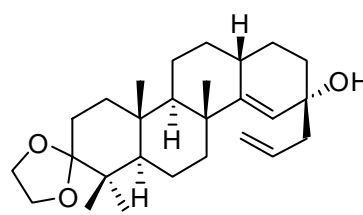
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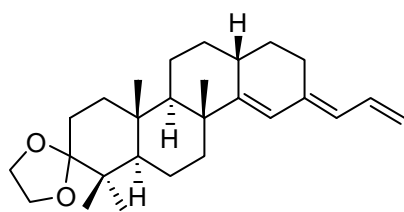
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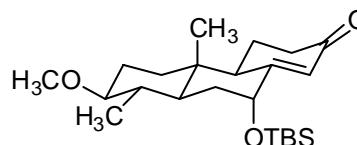
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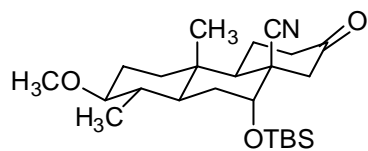
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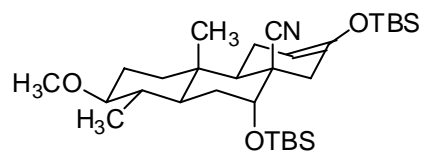
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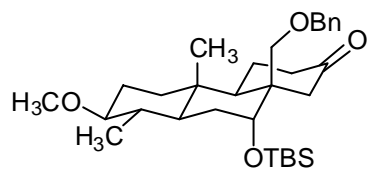


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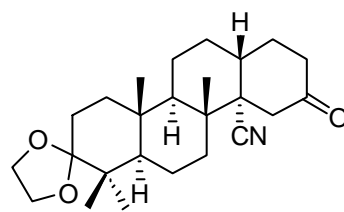


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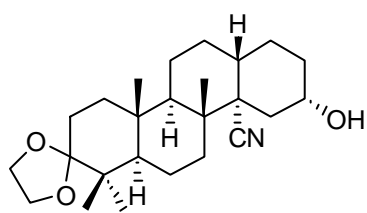




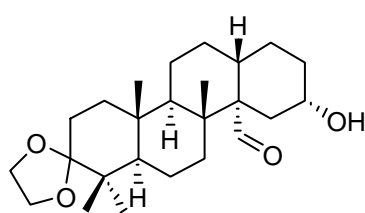
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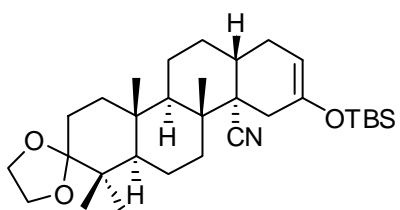
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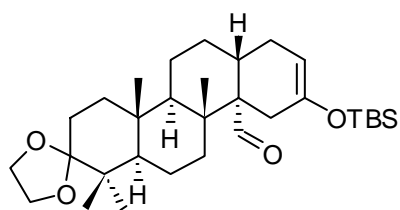
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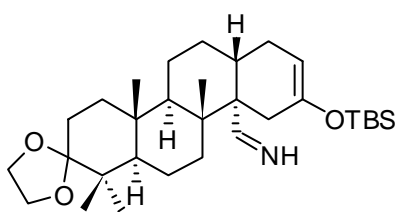
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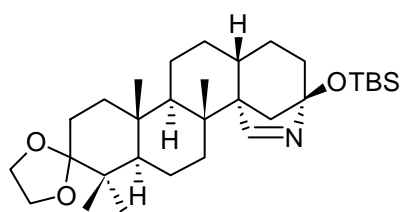
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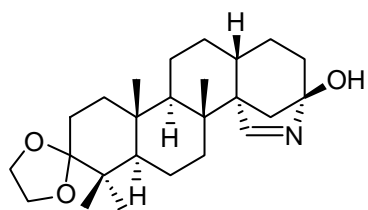
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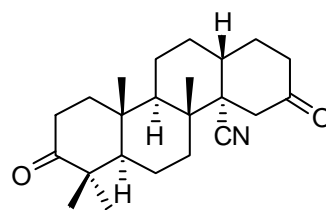
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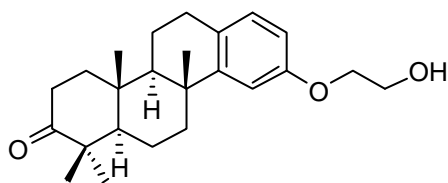
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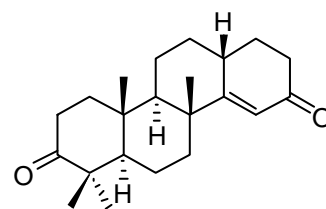
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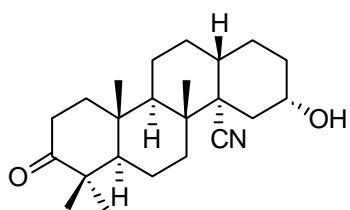
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## List of Abbreviations

Ac	acetyl
Ac <sub>2</sub> O	acetic anhydride
AcOH	acetic acid
AIBN	2,2'-azobisisobutyronitrile
Bn	benzyl
BnBr	benzyl bromide
BuLi	butyl lithium
cat.	Catalytic
<sup>13</sup> C-NMR	carbon nuclear magnetic resonance
CSA	10-camphor sulfonic acid
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DIBAL-H	diisobutylaluminum hydride
DMAP	4-( <i>N,N</i> -dimethylamino)pyridine
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethyl sulfoxide
<i>ee</i>	enantiomeric excess
Et	ethyl
Et <sub>2</sub> O	diethyl ether
Et <sub>3</sub> N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol
<sup>1</sup> H-NMR	proton nuclear magnetic resonance
HPLC	high performance liquid chromatography
HRMS	high-resolution mass spectrometry
IBX	<i>o</i> -iodoxybenzoic acid
LAH	lithium aluminum hydride
LDA	lithium diisopropylamine

<i>m</i> -CPBA	<i>meta</i> -chloroperbenzoic acid
Me	methyl
MeCN	acetonitrile
MeOH	methanol
MOM	methoxymethyl
MsCl	methanesulfonyl chloride
OAc	acetate
OMs	mesylate
OTf	triflate
Ph	phenyl
PhH	benzene
PhMe	toluene
PPh <sub>3</sub>	triphenylphosphate
PPTS	pyridinium <i>p</i> -toluenesulfonate
PTSA	<i>p</i> -toluenesulfonic acid
pyr	pyridine
rt	room temperature
TBAF	tetra- <i>n</i> -butylammonium fluoride
TBS	<i>tert</i> -butyldimethylsilyl
<i>t</i> -Bu	<i>tert</i> -butyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSCl	trimethylsilyl chloride
Ts	<i>p</i> -toluenesulfonyl
TsCl	<i>p</i> -toluenesulfonyl chloride

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I would like to thank my mom, Ester Pech, and my brother, Apolonio, for always believing in me and encouraging me to pursue my dreams. Most importantly, I would like to thank my wife Lizeth, my son Dimitri and my daughter Nailah for always being there for me. They are my inspiration and their love and support are what keeps me going.

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## **Dedication**

I would like to dedicate my thesis to my entire family, both here and in Belize.

To my grandparents, Guadalupe and Oraliah Pech, you made my childhood very pleasant and enjoyable.

My mother Esther, and brother Apolonio. You made me believe in myself and convinced me to pursue my Bachelor degree and now my Ph. D. Without your support and encouragement, I would not have made it this far.

I would especially like to dedicate this thesis to my wife Lizeth, my son Dimitri, and my daughter Nialah. You have shared all my pains and frustrations and your love is what kept me focused. This accomplishment is as much yours as it is mine. Thank you for your love and patience. I love you very much.

# CHAPTER 1 - Introduction: isolation, partial synthesis and biological importance of (+)-myriceric acid A

## 1.1 Isolation and characterization of (+)-myriceric acid A

In 1996 Fujimoto et al.<sup>1</sup>, at Shionogi Research Laboratories in Japan, reported the isolation of (+)-myriceric acid A [(+)-**1.1**] from the plant *Myrica cerifera*. This triterpene was the first non-peptide endothelin A receptor antagonist from a plant.<sup>1</sup>

Endothelin-1-induced increase of cytosolic free Ca<sup>2+</sup> was inhibited by the methanol extract from the fresh twigs of *Myrica cerifera*.<sup>1</sup> Further extraction from chloroform and water showed increased activity of the chloroform fraction. Silica gel chromatography gave an active fraction that showed one major peak on reverse phase HPLC. Semi-preparative reverse phase HPLC produced (+)-myriceric acid A [(+)-**1.1**].<sup>1</sup>

The molecular formula, C<sub>39</sub>H<sub>52</sub>O<sub>7</sub>, was deduced from mass spectrometry analysis of the crystalline (+)-**1.1**.<sup>1</sup> <sup>1</sup>H-NMR and <sup>13</sup>C-NMR of (+)-**1.1** suggested the presence of a *trans*-caffeoyl group, six tertiary methyl groups, a CH<sub>2</sub>OR group, a double bond, a carbonyl group, and a carboxyl group.<sup>1</sup>

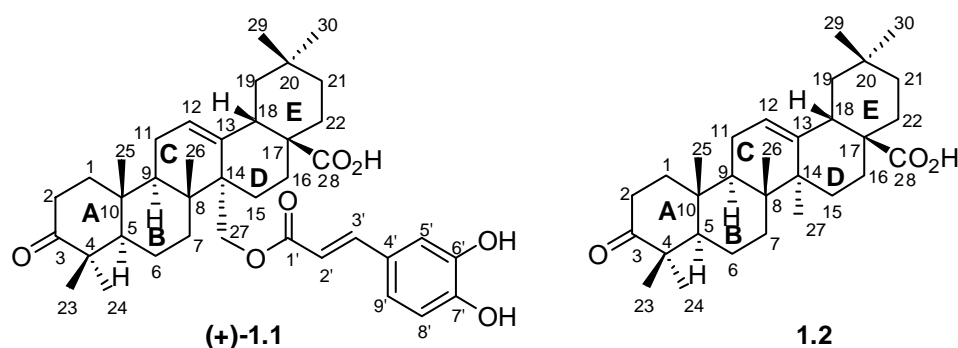
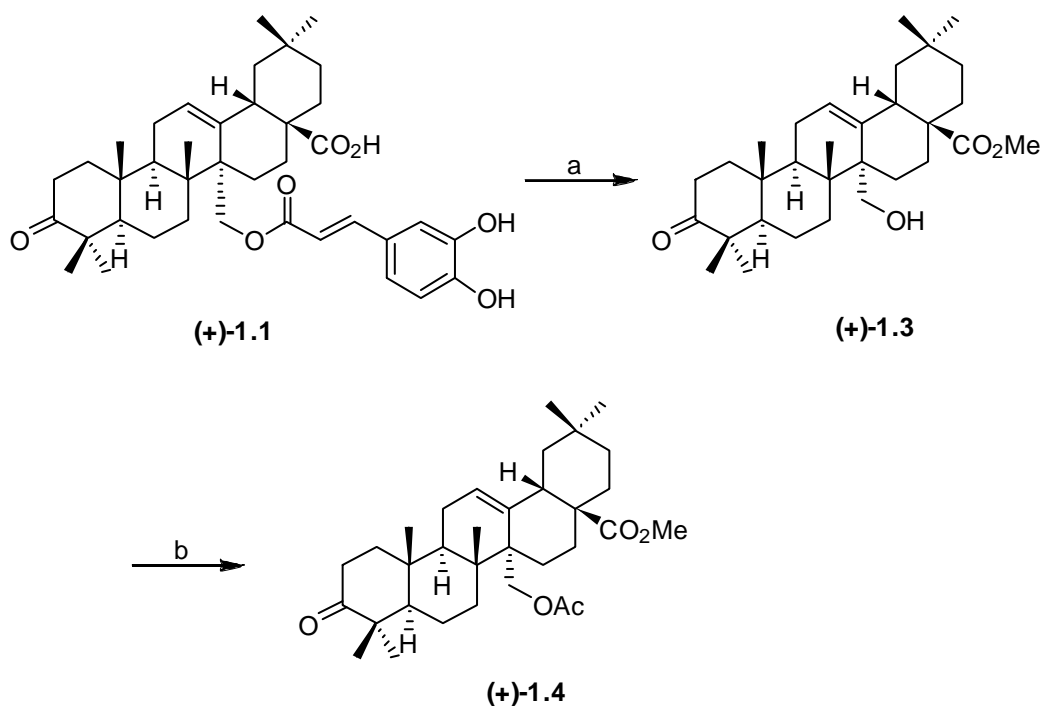


Figure 1.1: Myriceric acid A and oleanoic acid<sup>1</sup>

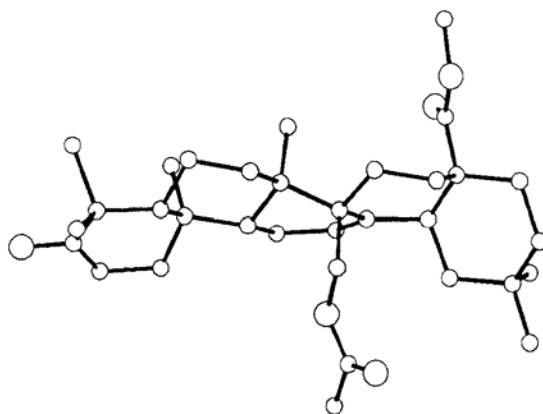
These NMR spectral data indicated that it was a derivative of 3-oxoolean-12-en-28-oic acid (**1.2**) with one of the methyl groups substituted by a *trans*-caffeoyloxy group (Figure 1.1).<sup>1</sup>

**Scheme 1.1: Synthesis of acetate (+)-1.4 from (+)-myiceric acid A<sup>1</sup>**



a.) i.  $\text{CH}_2\text{N}_2$ /ether, MeOH ii. NaOH, MeOH, 50°C 1.5 h b.)  $\text{Ac}_2\text{O}$ , pyridine overnight rt.

Methylation followed by basic hydrolysis of (+)-1.1 produced hydroxy methyl ester (+)-1.3.<sup>1</sup> Acetylation of the hydroxymethyl group in (+)-1.3 produced acetate (+)-1.4 (Scheme 1.1), from which its crystal structure was obtained (Figure 1.2).<sup>1</sup>



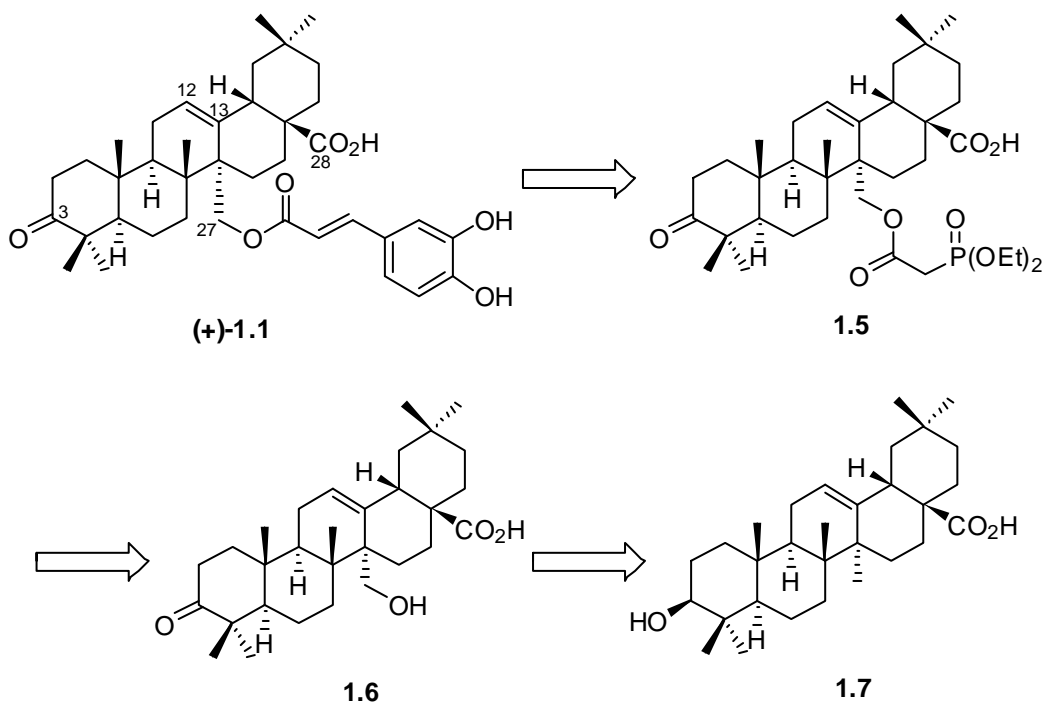
**Figure 1.2: Crystal structure of (+)-1.4<sup>1</sup>**

The crystal structure of (+)-**1.4** confirmed the proposed structure of (+)-myriceric acid A, (+)-**1.1**.

## 1.2 Partial synthesis of myriceric acid A from oleanolic acid<sup>2</sup>

After their isolation of myriceric acid A, Konoike et al.<sup>1,2</sup>, wanted to do further pharmacological evaluations and study the structure-activity relationships of myriceric acid A and its derivatives.<sup>2</sup> Because the content of (+)-**1.1** in the plant was only 0.01%, in 1997 they reported the development of an efficient 14 steps synthesis of (+)-**1.1** from the natural compound oleanolic acid **1.7**.<sup>2</sup>

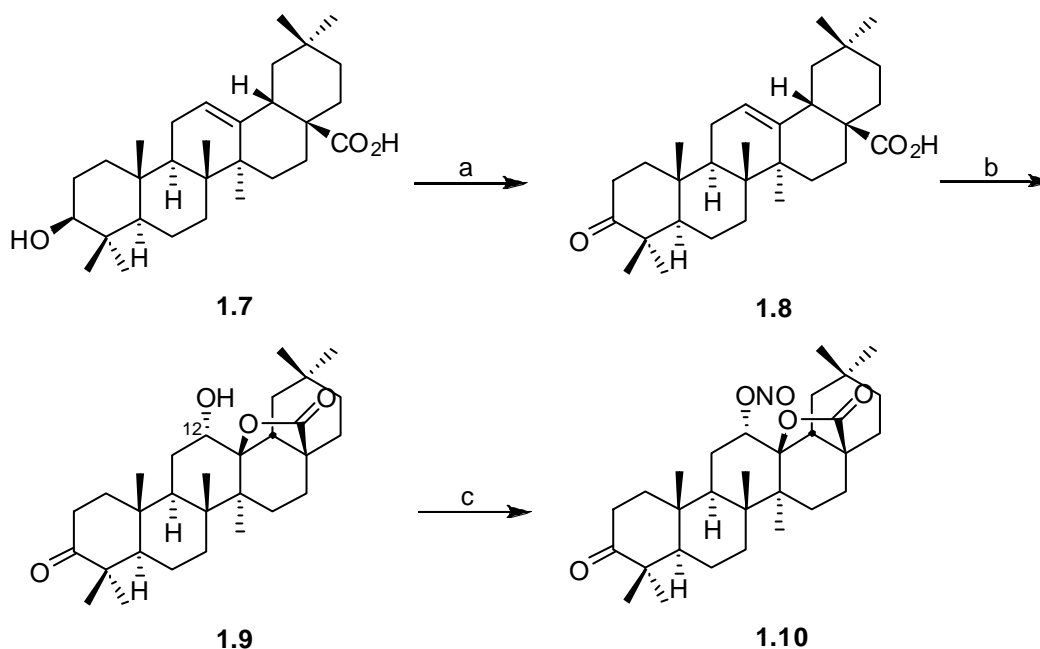
**Scheme 1.2: Retro-synthesis of myriceric acid A [(+)-1.1] from oleanolic acid (1.7)<sup>2</sup>**



As illustrated in their retrosynthetic analysis of (+)-**1.1** (Scheme 1.2), two key intermediates were needed to accomplish the synthesis of (+)-**1.1** from oleanolic acid (**1.7**).<sup>2</sup> Oleanolic acid (**1.7**) already has all the stereocenters required in (+)-**1.1**, the major differences are that **1.7** has a hydroxy group at C3 which should be a ketone in (+)-**1.1** and the C14 methyl

group in **1.7** is replaced by a *trans*-caffeoyloxymethyl group in (+)-**1.1**. Intermediate, myricerone (**1.6**) was obviously a key compound necessary for obtaining these required functionalities.<sup>2</sup> Because direct acylation of the C27 hydroxy group was not efficient and failed when attempted with 3,4-di-*O*-acetylcaffeic acid, Konoike et al.<sup>2</sup> developed a new procedure that required the key intermediate phosphonate **1.5**. This would be used in a Horner-Wadsworth-Emmons (HWE) olefination<sup>3</sup> with a dihydroxy-benzaldehyde derivative. Phosphonate **1.5** was very versatile and could be used with other aldehydes so other derivatives were possible from this intermediate.<sup>2</sup>

**Scheme 1.3: Synthesis of nitrite 1.10 from oleanolic acid (1.7)**<sup>2</sup>



a.) Jones oxidation; b.) O<sub>3</sub>, MeOH, -50°C, 79% yield; c.) NOCl, pyridine, -40°C, 30 min., 94% yield.<sup>2</sup>

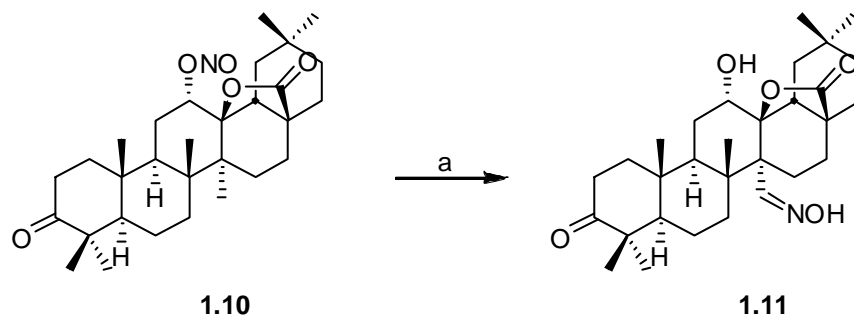
A key step in the conversion of **1.7** to the intermediate **1.6** was introduction of the C27 hydroxy group using the Barton reaction<sup>4</sup>. Three steps, from oleanolic acid (**1.7**), were required to make the starting compound **1.10** for the Barton reaction. Oxidation of the C3 hydroxy group was accomplished by Jones oxidation of **1.7** to produce oleanoic acid (**1.8**).<sup>2</sup> Introduction of ozone to a crude solution of **1.8** produced the 12 $\alpha$ -hydroxy lactone **1.9** in which the 12 $\alpha$ -hydroxy group was the scaffold for the Barton reaction. Nitrite **1.10** was formed by treatment of hydroxy lactone **1.9** with nitrosyl chloride in pyridine.<sup>2</sup> To prevent decomposition of nitrite



**1.10**, it was necessary for the work up and crystallization to be done in the presence of pyridine (Scheme 1.3).<sup>2</sup>

Several reaction conditions were investigated for the Barton reaction of nitrite **1.10** to produce oxime **1.11**.<sup>2</sup> From these investigations they determined that acetone was the best solvent and lower concentrations increased the yield. Also, irradiation should be done between 329-379 nm in less than one hour and exclusion of oxygen was essential. Pyridine was not necessary but prevented decomposition of nitrite **1.10**, and temperature did not affect the reaction.<sup>2</sup> The photochemical reaction of **1.10** was done following the requirements stated above to produce oxime **1.11** (Scheme 1.4).<sup>2</sup>

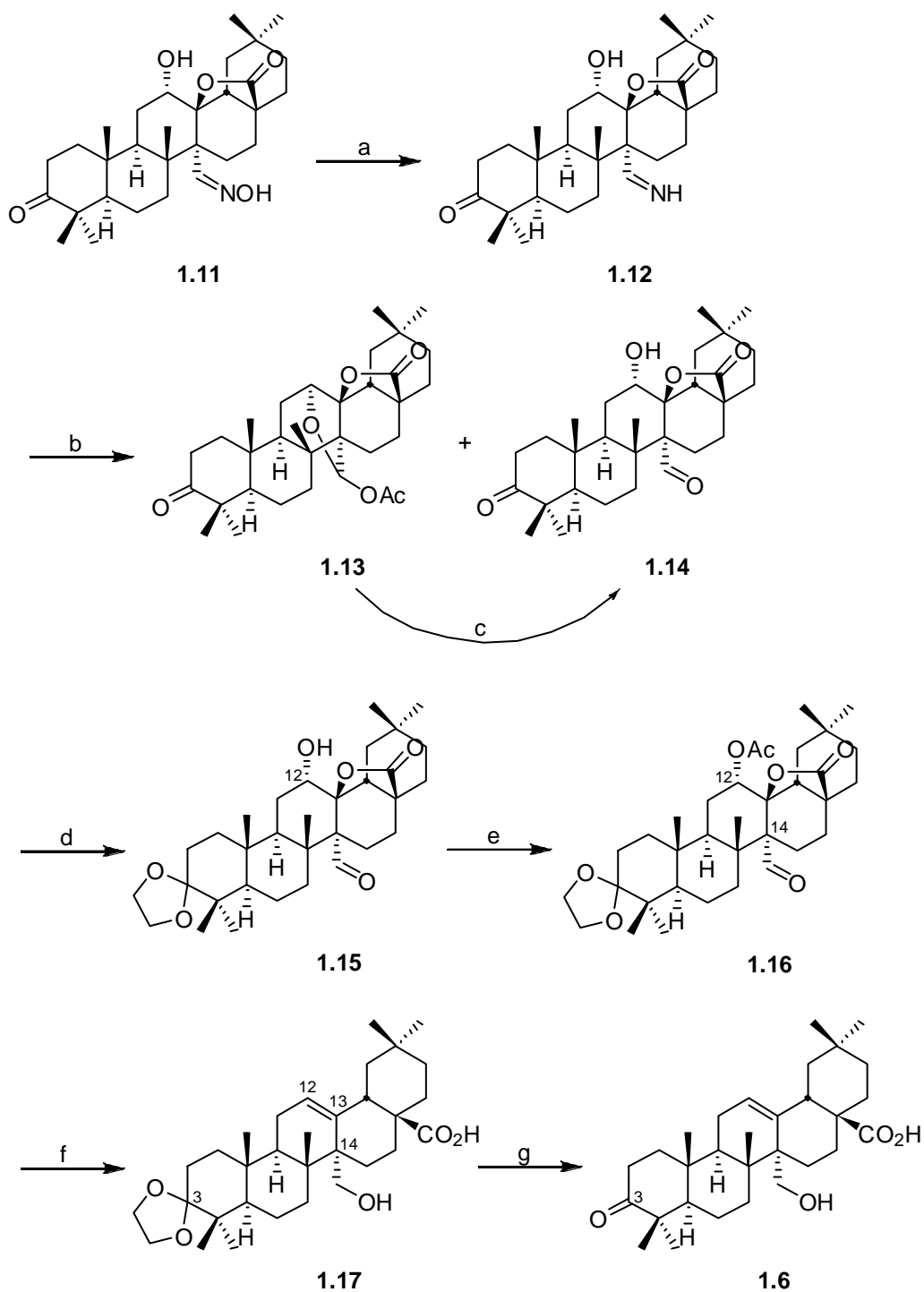
**Scheme 1.4: Barton reaction of 1.10 to produce oxime 1.11<sup>2</sup>**



a.) acetone, pyridine, irradiation 329-379nm, 1 h under nitrogen atmosphere, 74% yield.<sup>2</sup>

Direct formation of its corresponding aldehyde **1.14** from oxime **1.11** was difficult because of steric hindrance around the C27 position, so they generated it through a three-step process starting by formation of imine **1.12** from oxime **1.11**.<sup>2</sup> Treatment of oxime **1.11** with  $\text{TiCl}_3$  produced imine **1.12**, which was stable under normal hydrolysis conditions.<sup>2</sup> Hydrolysis of imine **1.12** was accomplished by treatment with sodium nitrite in aqueous dioxane-acetic acid to produce a mixture of two compounds, hemiacetal acetate **1.13** and hydroxy aldehyde **1.14** in a 2:1 ratio, respectively. Treatment of the mixture of **1.13** and **1.14** with sodium hydroxide in methanol produced aldehyde **1.14** as the sole product (Scheme 1.5).<sup>2</sup>

**Scheme 1.5: Seven step conversion of oxime 1.11 to myricerone (1.6)<sup>2</sup>**

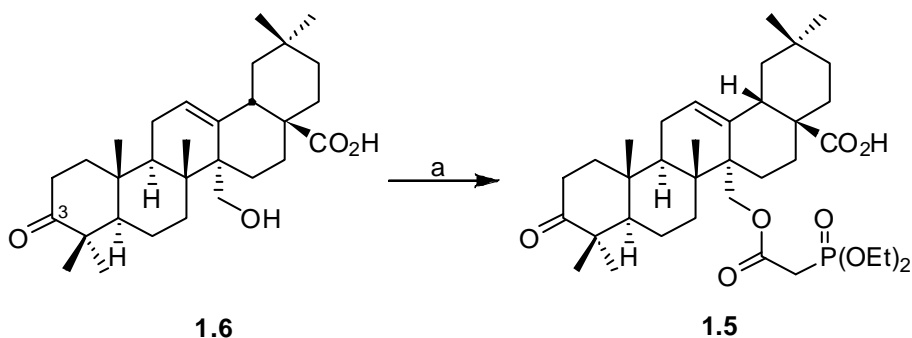


a.) 20%  $\text{TiCl}_3$ , 10:1 dioxane:AcOH, 26°C 4 h, 88% yield. b.) aqueous  $\text{NaNO}_2$ , 10:1 dioxane:AcOH, 40°C 1 h c.)  $\text{NaOH}$ , MeOH, 14-26°C, 2.5 h, 77% yield d.) PPTS, ethylene glycol, Toluene, reflux 4 h e.)  $\text{Ac}_2\text{O}$ , pyridine, DMAP,  $\text{CH}_2\text{Cl}_2$  5 h, 96% yield 2 steps f.) THF, Li,  $\text{NH}_3$  (l), 45 min. g.) HCl, THF, reflux 75 min., 99% yield 2 steps.<sup>2</sup>

From aldehyde **1.14**, four more steps were required to complete the synthesis of the key intermediate, myricerone (**1.6**). The first step was protection of the C3 ketone in aldehyde **1.14**, which was accomplished by treatment with PPTS and ethylene glycol to produce the ethylene ketal **1.15**. Treatment of ketal **1.15** with acetic anhydride in pyridine resulted in the acetylation of the C12 hydroxy group producing acetoxy ketal **1.16**.<sup>2</sup> Formation of the C12-C13 double bond was accomplished by reductive elimination of the acetoxy lactone moiety with lithium in liquid ammonia. Interestingly, the C14 aldehyde was also reduced in the process producing the desired C14-hydroxy methyl group in compound **1.17**.<sup>2</sup> The ethylene-ketal protecting group in **1.17** was removed by treatment with aqueous hydrochloric acid and resulted in the formation of the key intermediate myricerone (**1.6**).<sup>2</sup>

Synthesis of the second key intermediate, phosphonate **1.5**, was accomplished in one step from **1.6**. Commercially available diethoxyphosphonoacetic acid was condensed with myricerone **1.6** by treatment with carbonyl diimidazole to give phosphonate **1.5** (Scheme 1.6) in 39% yield, after 12 steps, from oleanolic acid **1.7**.<sup>2</sup>

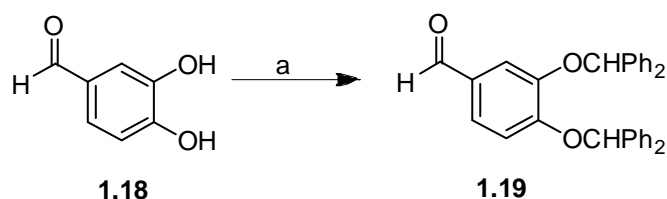
**Scheme 1.6: Synthesis of phosphonate 1.5 from myricerone (1.6)**<sup>2</sup>



a.) diethoxyphosphonoacetic acid, carbonyl diimidazole, CH<sub>2</sub>Cl<sub>2</sub>, rt 6 h, 84% yield.<sup>2</sup>

Since direct HWE olefination of **1.5** with 3,5-dihydroxybenzaldehyde (**1.18**) failed because the phenolic hydroxy groups deactivated the aldehyde. They were protected as diphenylmethyl groups to produce aldehyde **1.19** (Scheme 1.7).<sup>2</sup>

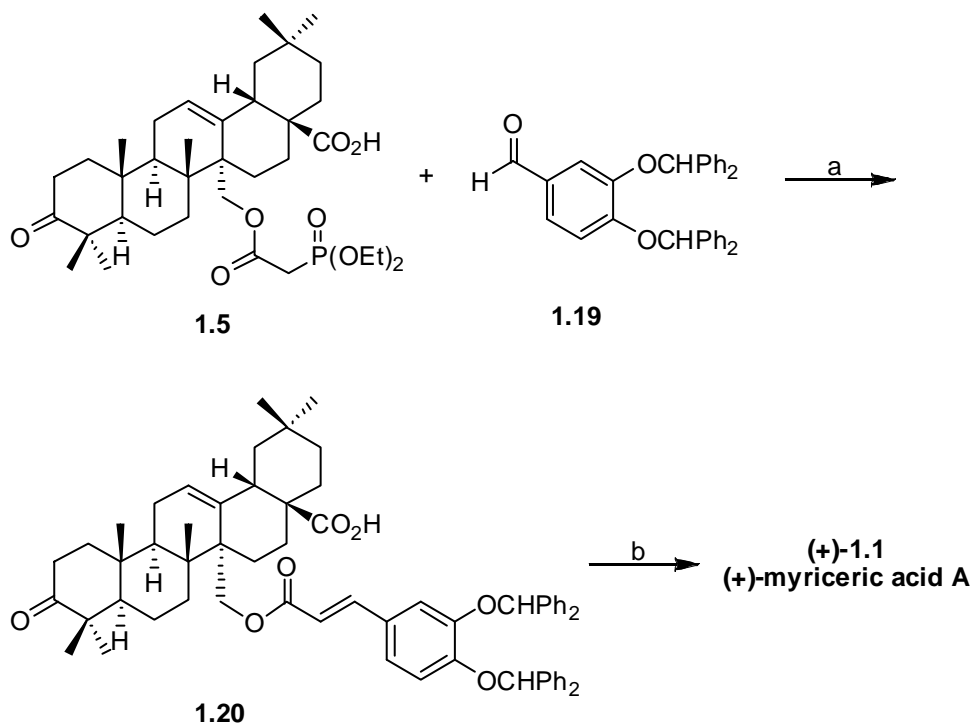
### Scheme 1.7: Synthesis of 1.19 from 1.18<sup>2</sup>



a.) Ph<sub>2</sub>CHBr, K<sub>2</sub>CO<sub>3</sub>, DMF, 80°C 5 h, 23% yield.<sup>2</sup>

The HWE olefination of phosphonate **1.5** and aldehyde **1.19** produced compound **1.20** in 95 % yield. Treatment of **1.20** with trifluoroacetic acid in anisole removed the diphenyl methyl protecting groups and produced (+)-myriceric acid A [(+)-**1.1**] (Scheme 1.8).<sup>2</sup>

### Scheme 1.8: HWE olefination of 1.5 and deprotection of 1.20 to form myriceric acid A [(+)-1.1]<sup>2</sup>



a.) DBU, LiCl, DMF rt 5 h, 95% yield b.) TFA, anisole, rt 1.5 h, 80% yield 2 steps.<sup>2</sup>

Synthesis of (+)-myriceric acid A [(+)-**1.1**] was accomplished in 14 steps and 31% yield starting from the natural product oleanolic acid (**1.7**). No chromatographic purification was necessary until after the reaction of **1.20** to produce the final product (+)-**1.1**.<sup>2</sup>

### 1.3 Importance and biological activity of myriceric acid A.

After its isolation, (+)-myriceric acid A [(+)-1.1] showed inhibition of endothelin-1-induced increase in cytosolic  $\text{Ca}^{2+}$  concentration (median inhibition concentration ( $\text{IC}_{50}$ ) =  $11 \pm 2$  nM) and binding of endothelin-1 (ET-1) in rat aortic smooth muscle cells (binding constant ( $K_i$ ) =  $66 \pm 15$  nM).<sup>1</sup> Since it selectively antagonized binding of ET-1 but not ET-3 it was confirmed that it was a specific  $\text{ET}_A$  receptor antagonist.<sup>1</sup>

#### 1.3.1 Endothelins

In 1988, Yanasigawa et al.<sup>5</sup> reported that from the supernatant of porcine aortic endothelial cells (EC) they isolated and determined the amino acid sequence of a potent 21-residue vasoconstrictor peptide. This new peptide, now known as endothelin-1 (ET-1), has a relative molecular mass ( $M_r$ ) of 2,492 and its four cystein residues form two intra-chain disulfide bonds (Cys1-Cys15 and Cys3-Cys11).<sup>5</sup> Comparing its activity to other vasoconstrictors, ET-1 is the most potent mammalian vasoconstrictor peptide and its contraction is long lasting and difficult to wash out even more than angiotensin II.<sup>6</sup>

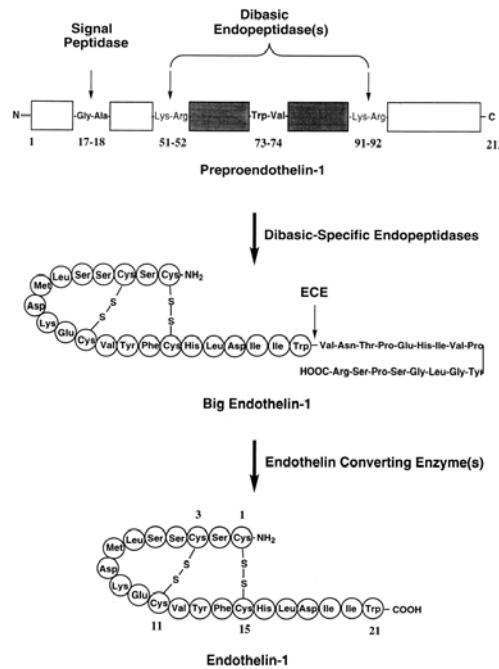
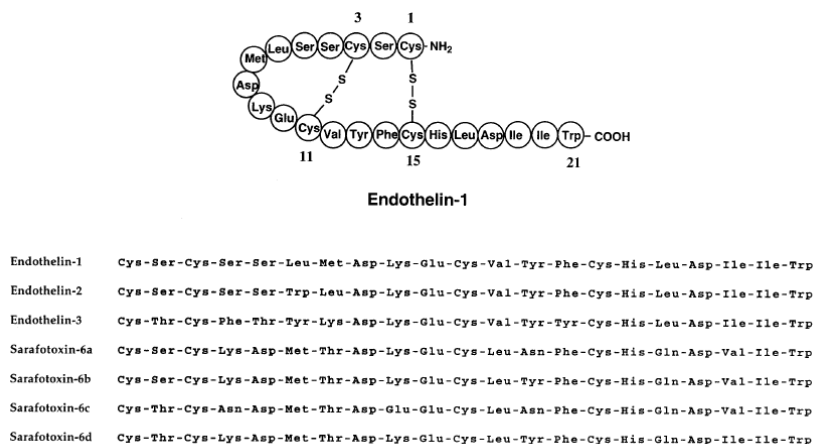


Figure 1.3: Formation of ET-1 from preproET-1<sup>6</sup>

Yanagisawa et al. also molecularly cloned the precursor peptide, which was a 203-residue preproendothelin sequence in pigs<sup>5</sup> and 212 amino acids in humans<sup>6</sup>. This precursor peptide undergoes three proteolytic steps to produce the mature ET-1.<sup>5,6</sup> First it is cleaved between Lys51 and Arg52, then between Arg92 and Arg93 by dibasic endopeptidases to give a 38 amino acid (39 amino acids in humans) intermediate, big endothelin-1 (big ET-1). An unusual specific cleavage between Trp73 and Val74 by an endothelin-converting enzyme (ECE) results in the formation of the mature ET-1 (Figure 1.3).<sup>5,6</sup>

Two other isopeptides, ET-2 and ET-3, were also identified after analysis of the human genomic library.<sup>1</sup> The three isopeptides are very similar in sequence to each other and also to sarafotoxins, which are a family of peptides found in cobra venom.<sup>6</sup> They all consist of 21 amino acids, two disulphide bridges between Cys1-Cys15 and Cys3-Cys11, the charged tripeptide Asp8-Lys-Glu10, and the carboxy-terminal penta-peptide His16-Leu-Asp-Ile-Ile-Trp21 (Figure 1.4).<sup>6</sup> The free carboxylic C-terminal on Trp, and the disulphide bonds have been found to be necessary for biological activity and binding to the ET<sub>A</sub> receptor. It was found that if the internal Cys3-Cys11 was replaced by alanines there was a slight loss of agonist activity on the other hand when Cys1-Cys15 was replaced almost all the activity was lost.<sup>6</sup>



**Figure 1.4: Similarities of ETs and sarafotoxins<sup>6</sup>**

ET-1 is expressed in numerous vascular and non-vascular cells but it is the only one found in vascular endothelial cells, which explains why it is the primary circulating isoform.<sup>6</sup> For the other isoforms, ET-2 is mostly found in the gastrointestinal tract and ET-3 is found in the

neuronal tissues.<sup>6</sup> Because it is one of the most potent and long lasting vasoconstrictors, ET-1 has gotten most attention of the three isopeptides.<sup>6</sup>

### ***1.3.2 Endothelin receptors***

The different potencies of the three isopeptides toward vasoconstrictory and vasodilatory activity:  $ET-1 \geq ET-2 \gg ET-3$  for vasoconstriction and  $ET-1 = ET-2 = ET-3$  for vasodilation indicated that these actions of ETs were mediated by different receptors.<sup>6</sup> Radioligand binding studies revealed that the vasoconstrictory receptor that prefers ET-1 was found in the vascular smooth muscle cells and the vasodilatory receptor that has the same affinity for both ET-1 and ET-3 was found on the endothelium. These two receptors were termed the  $ET_A$  and  $ET_B$  receptors, respectively.<sup>6</sup>

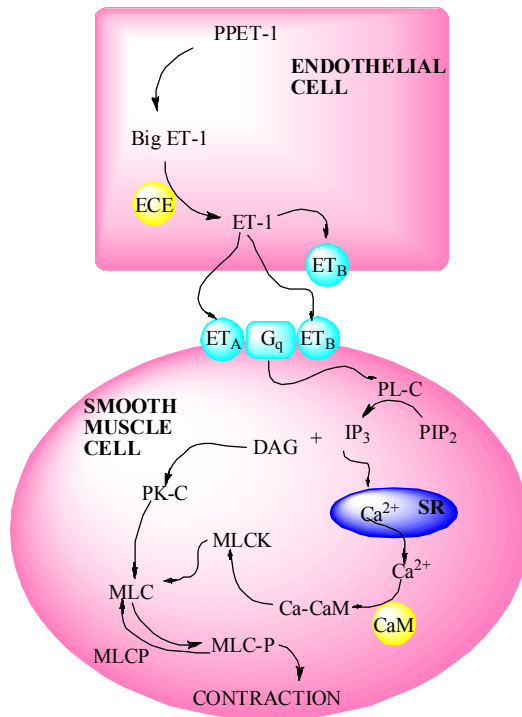
The  $ET_A$  receptor was originally isolated, by Arai et al<sup>7</sup>, from bovine lung and was found to be a protein of 427 amino acids with a molecular mass of 48.5 kilodaltons with seven hydrophobic regions of 22-27 residues.<sup>6</sup> The  $ET_B$  receptor was originally isolated, by Sakuri et al<sup>8</sup>, from rat lung and was found to be a protein of 441 amino acids with a molecular mass of 47 kilodaltons with seven hydrophobic regions.<sup>6</sup> The  $ET_A$  and  $ET_B$  are ~60% homologous to each other and because their trans-membrane regions are highly homologous to that of G-protein coupled receptors (GPCR) they are considered members of the superfamily of GPCRs.<sup>6</sup>

### ***1.3.3 Vascular signal transduction mechanism for contraction***

Two factors determine the contractile status of the vascular smooth muscle cells: the cytosolic  $Ca^{2+}$  concentration and the  $Ca^{2+}$  sensitivity of the myofilaments<sup>13</sup>. The  $ET_A$  receptor is coupled to phospholipase-C (PL-C)-coupled Gq proteins.<sup>10</sup> Binding of ET-1 to the  $ET_A$  receptor activates PL-C which stimulates formation of inositol triphosphate ( $IP_3$ ) and diacylglycerol (DAG) from phosphatidylinositol biphosphate ( $PIP_2$ ).<sup>10</sup>  $IP_3$  then stimulates the sarcoplasmic reticulum to release  $Ca^{2+}$ .<sup>10,11</sup> The free  $Ca^{2+}$  binds to calmodulin (CaM), a calcium binding protein, and the Ca-CaM complex activates myosin light chain kinase (MLCK) that phosphorylates myosin light chain (MLC) in the presence of ATP.<sup>11</sup> Phosphorylation of MLC

to MLC-P leads to cross-bridge formation of the myosin head and actin filaments, which results in contraction.<sup>11</sup> PL-C activation also forms diacylglycerol (DAG), which activates protein kinase C<sup>10</sup>, which can also phosphorylate the MLC, which would result in contraction also (Figure 1.5).

The Ca<sup>2+</sup> sensitivity of the myofilaments depends on the activity ratio of MLCK and myosine light chain phosphatase (MLCP). MLCP dephosphorylates the MLC-P to produce the MLC.



**Figure 1.5: Vascular signal transduction mechanism for contraction<sup>9,10,11</sup>**

Because of the significant presence of endothelin and/or receptors in many mammalian tissues it is believed that they may be involved many diseases like: stroke, migraine, and subarachnoid hemorrhage in the brain; congestive heart failure; pulmonary hypertension, and bronchial asthma in the lung to name a few<sup>6</sup>. To further understand and possibly treat these diseases endothelin receptor antagonists need to be investigated.



## 1.4 Superfused spiral modiolar artery (SMA) of the gerbil as the bioassay for determining ET<sub>A</sub> receptor antagonistic activity of the synthetic intermediates

To evaluate the functional activities of our synthesized intermediates, as specific ET<sub>A</sub> receptor antagonists, we would use the well established bioassay, isolated in vitro superfused spiral modiolar artery (SMA) of the gerbil. In 2001, Wangemann et al.<sup>13</sup> demonstrated that ET-1 induced, via the ET<sub>A</sub> receptors, a transient Ca<sup>2+</sup> concentration [Ca<sup>2+</sup>] increase and a long lasting vasoconstriction of the gerbil spiral modiolar artery (SMA).<sup>13</sup> In the same study they also determined that Ca<sup>2+</sup> mobilization was not necessary for the development of the vasoconstriction, but its maintenance was dependent on influx of extra-cellular calcium.<sup>13</sup> It was concluded that other signaling mechanisms were also present which increased the sensitivity of the myofilaments resulting in lower [Ca<sup>2+</sup>] maintaining the constriction.<sup>13</sup>

### 1.4.1 *Spiral modiolar artery*

The spiral modiolar artery is an arteriole that provides the main blood supply to the cochlea.<sup>13</sup> It has a diameter of 62 μm<sup>14</sup> and loosely surrounds the eighth cranial nerve in the cochlea of the inner ear. The vascular diameter controls the blood flow and the diameter depends on the contractile state of the vascular smooth muscle cells.<sup>13</sup> Access to the ET<sub>A</sub> receptor is ideal, on the SMA, because the wall contains only a single layer of vascular smooth muscle cells and a single layer of endothelial cells.<sup>12</sup> (Figure 1.6)

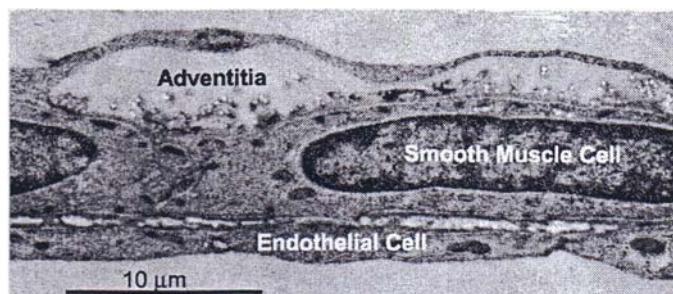
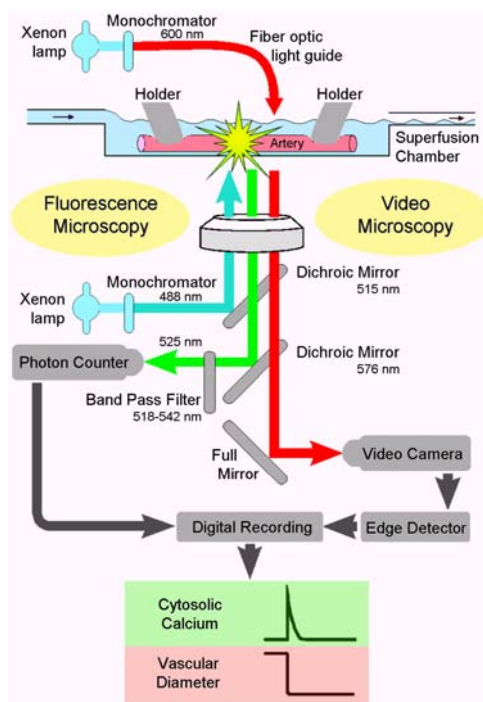


Figure 1.6: Cross section through the wall of the SMA<sup>12</sup>

This makes the isolated spiral modiolar artery (SMA) of the gerbil a suitable bioassay for determining  $ET_A$  receptor antagonists.

#### ***1.4.2 Isolation of the SMA and simultaneous measurement of $[Ca^{2+}]_i$ and vascular diameter***

In this bio-assay the diameter of the SMA and the  $[Ca^{2+}]_i$  were measured simultaneously by video-microscopy and microfluorimetry. Isolation of the SMA was achieved by microdissection, in control solution, of the cochlea obtained from a gerbil.<sup>13</sup>



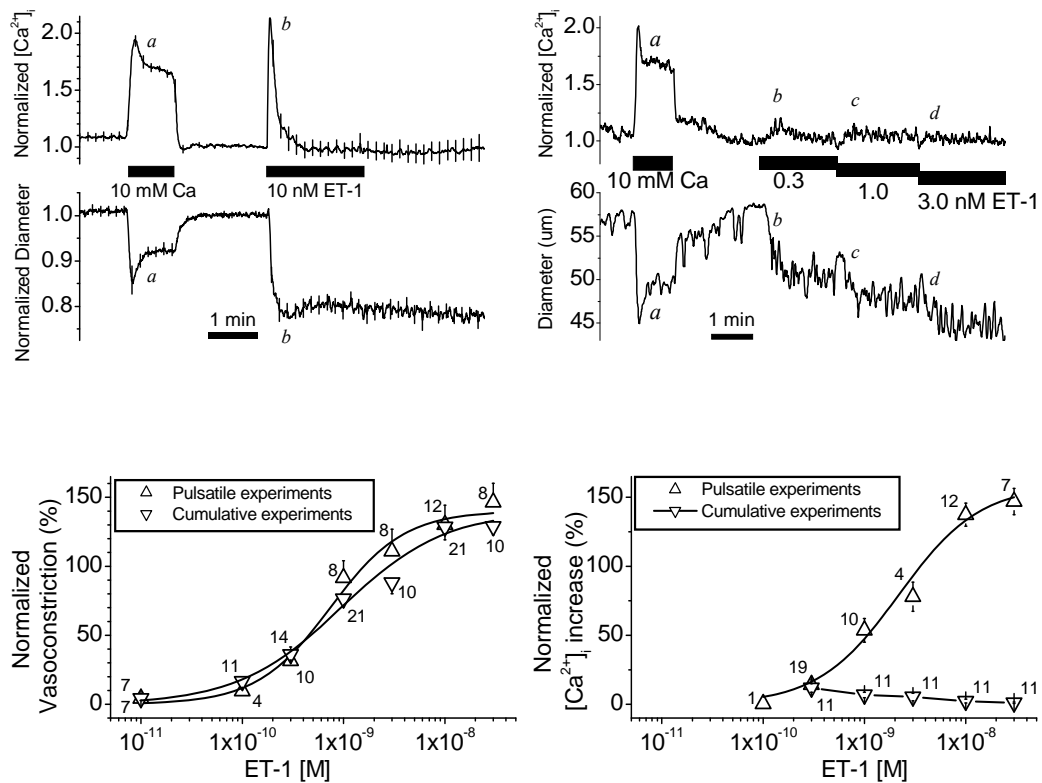
**Figure 1.7: Schematic for simultaneous measurement of  $[Ca^{2+}]_i$  and vascular diameter by microfluorimetry and video microscopy<sup>13</sup>**

A segment of the isolated SMA was treated with molecular probes and transferred to a bath chamber (superfusion chamber), with control solution at 37°C, mounted on the stage of an inverted microscope.<sup>13</sup> After illumination, changes in  $[Ca^{2+}]_i$  were determined by changes in the emission intensity. Simultaneously, the outer diameter of the SMA was monitored and calibrated by video-edge detectors. Changes were monitored on a chart recorder.<sup>13</sup> (Figure 1.7)

### 1.4.3 Endothelin-1 effect on SMA

Two experiments, a pulsatile and a cumulative experiment, were performed to study the effect of ET-1 on the  $[Ca^{2+}]$  and the constriction of the SMA.

In both experiments, the  $[Ca^{2+}]$  and vascular diameter were normalized by increasing the  $[Ca^{2+}]$  from 1 mM to 10 mM. In the pulsatile experiment, in which a single dose of ET-1 was added, a transient  $[Ca^{2+}]$  increase was observed along with a significant and long lasting vasoconstriction. The  $[Ca^{2+}]$  quickly returned to normal, however the vasoconstriction remained even after the ET-1 was removed from the perfusate and the SMA was superfused with 1 mM  $Ca^{2+}$  solution. In the cumulative experiment, an increasing dose of ET-1 showed a step-wise decrease of vascular diameter and no transient  $[Ca^{2+}]$  increase was observed.<sup>13</sup> (full procedure is described in chapter 5 and in the experimental section)

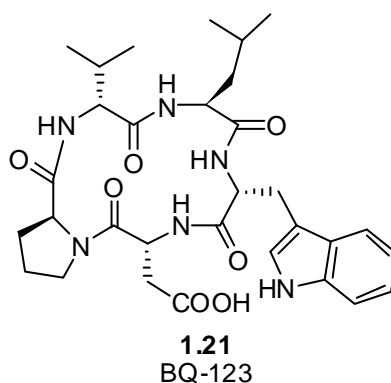


**Figure 1.8: Top – (left) Pulsatile experiment; (right) cumulative experiment; Bottom – (left) Dose response curve for constriction; (right) dose response curve for  $[Ca^{2+}]$ <sup>13</sup>**

Comparison of the dose response curves obtained from both experiments show that the potency of the ET-1 toward constriction is the same in both experiments, however there is a significant difference in  $[Ca^{2+}]$  increase.<sup>13</sup> These data suggested that the transient increase in  $[Ca^{2+}]$  was not necessary for the development of the contraction. However, another experiment (not shown), indicated that influx of  $Ca^{2+}$  was necessary for the maintenance of the constriction.<sup>13</sup> These results were consistent with the view that the sensitivity of the myofilaments toward  $Ca^{2+}$  was increased by ET-1<sup>13</sup>, through a different signaling pathway.

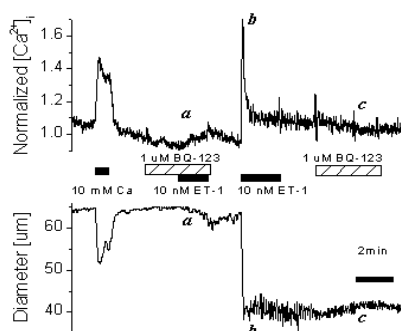
#### 1.4.4 Effect of $ET_A$ receptor antagonists on ET-1 induced constrictions of the SMA

A known selective  $ET_A$  receptor antagonist is the synthetic cyclic peptide BQ-123 (**1.21**) (Figure 1.9). In the SMA bioassay, when BQ-123 was present in the perfusate, no  $[Ca^{2+}]$  increase or vasoconstriction was observed when a single dose of ET-1 was added.<sup>13,14</sup> This indicated that the antagonist prevented both the  $[Ca^{2+}]$  increase and the vasospasm by preventing the binding of the agonist, ET-1, to the  $ET_A$  receptor.



**Figure 1.9: Structure of specific  $ET_A$  receptor antagonist BQ-123 (1.21)**

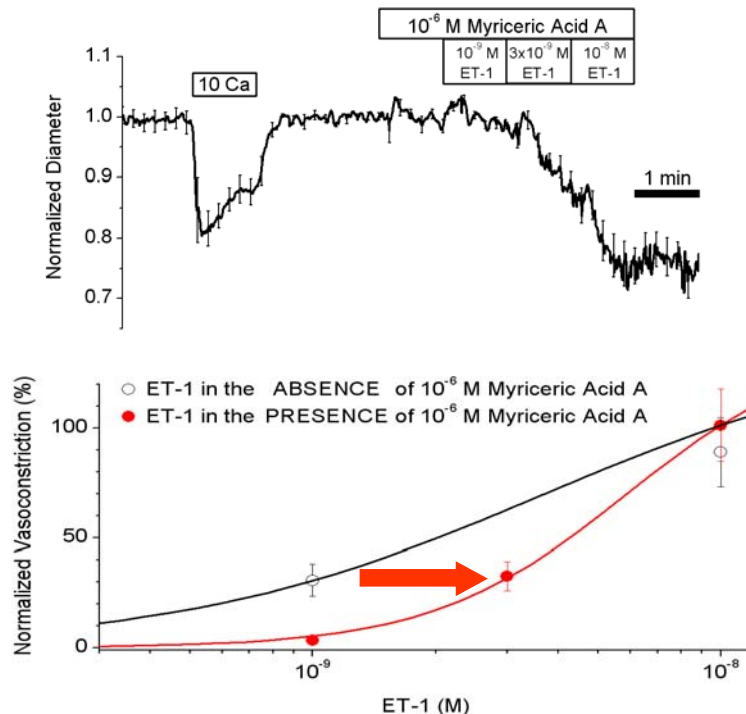
Since it was able to prevent the vasospasm, determination of whether it could reverse it was investigated. After the BQ-123 was washed out, the  $[Ca^{2+}]$  increase and vasospasm were induced by a single dose of ET-1. After the ET-1 was removed from the perfusate and the SMA was superfused with 1 mM  $Ca^{2+}$  solution, as expected, the vasospasm remained. Addition of BQ-123 failed to reverse the vasospasm (Figure 1.10).<sup>14</sup>



**Figure 1.10: Effect of  $ET_A$  receptor antagonist BQ-123 in pulsatile experiment. The antagonist was able to prevent the vasoconstriction but it failed to reverse it.**<sup>14</sup>

It can be concluded from these results that the specific  $ET_A$  receptor antagonists BQ-123 can prevent the vasospasm but it is unable to reverse it. While the SMA is superfused with the antagonist BQ-123, the antagonist binds to the  $ET_A$  receptor and prevents the effect of the agonist, ET-1, and so prevents the vasospasm to occur. After the ET-1 induced the vasospasm the antagonist was unable to reverse it because the maintenance of the constriction is not dependent on the binding of the agonist to the receptor. The initial binding of the ET-1 to activate the receptor stimulates a different signaling mechanism that maintains the constriction. This mechanism is dependent on Rho-kinase inhibition of the myosin light chain phosphatase (MLCP) which results in sensitization of the contractile apparatus. This results in a lower  $Ca^{2+}$  concentration maintaining the constriction. So,  $ET_A$  receptors are necessary for the initiation of the vasospasm but they may not be needed for the maintenance of the constriction.

The affinity constant ( $K_{DB}$ ), which quantitatively indicate the effectiveness of the antagonist at preventing the effect of the agonist, of BQ-123 was determined to be 24 nM in the SMA bioassay. To compare the effectiveness of (+)-myriceric acid A, an experiment in which ET-1 was added cumulatively in the presence of 1  $\mu$ M (+)-myriceric acid A, was performed. From the shifts in the dose response curves, and the assumption that inhibition is competitive, the  $K_{DB}$  of (+)-myriceric acid A was determined to be 500 nM (Figure 1.11).



**Figure 1.11: Top - Effect of  $ET_A$  receptor antagonist myriceric acid A in a cumulative addition of ET-1. Bottom – Dose response curve of myriceric acid A**

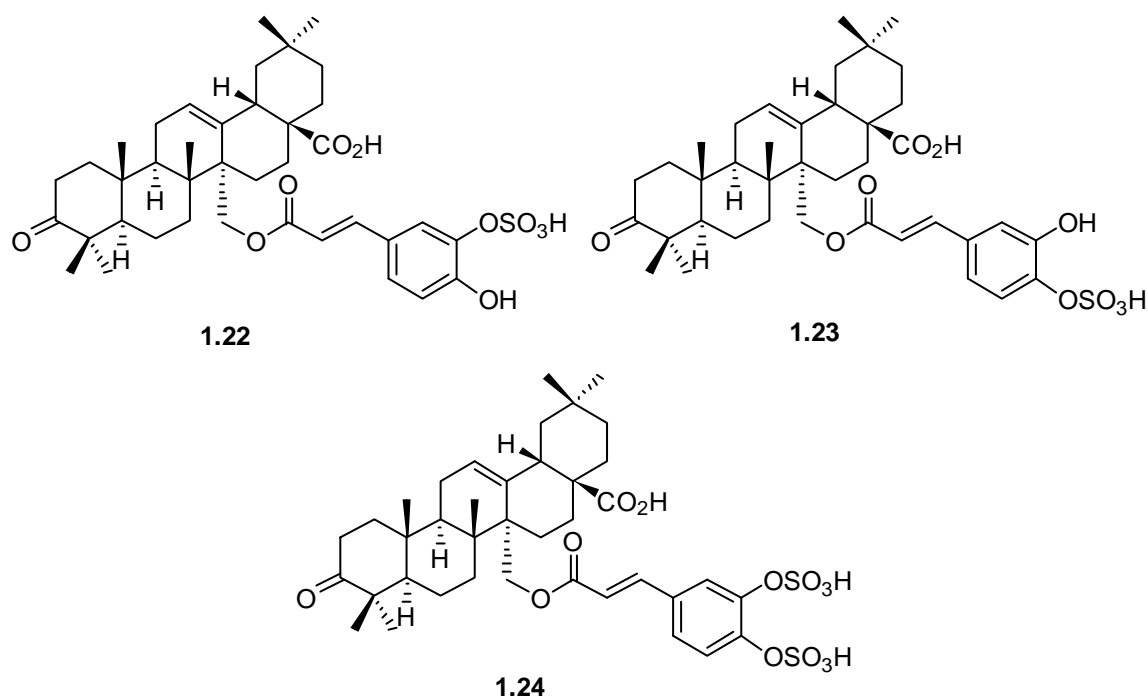
This result show that myriceric acid A is an antagonist which prevents the vasospasm up to 3 nM of ET-1 when the SMA is superfused with 1  $\mu$ M (+)-myriceric acid A,  $K_{DB} = 500$  nM. Because the  $K_{DB}$  of (+)-myriceric acid A is 20 times less than that of BQ-123,  $K_{DB} = 24$  nM, a total synthesis of (+)-myriceric acid A is necessary from which synthetic intermediates can be evaluated and possibly a more active compound can be obtained.

## 1.5 Conclusion:

Because of the significant presence of endothelin and/or receptors in many mammalian tissues it is believed that they may be involved many diseases; like stroke, pulmonary hypertension and congestive heart failure.<sup>6</sup> To treat and prevent these diseases, endothelin receptor antagonists need to be investigated. A non-peptide that is a selective  $ET_A$  receptor antagonist is myriceric acid A.<sup>1</sup> It showed inhibition of endothelin-1-induced increase in

cytosolic  $\text{Ca}^{2+}$  concentration ( $\text{IC}_{50} = 11 \pm 2 \text{ nM}$ ) and binding of endothelin-1 (ET-1) in rat aortic smooth muscle cells ( $\text{K}_i = 66 \pm 15 \text{ nM}$ ).<sup>1</sup>

Although the affinity constant of (+)-myriceric acid A ( $\text{K}_{\text{DB}} = 500 \text{ nM}$ ) is 20 times less than that of the known specific  $\text{ET}_A$  receptor antagonist BQ-123 ( $\text{K}_{\text{DB}} = 24 \text{ nM}$ ), derivatives with higher activities may be obtained from a total synthesis. This is evident in the structure-activity relationship study done by Fujimoto et al.<sup>1</sup>, after the isolation of (+)-myriceric acid A. They synthesized three derivatives of myriceric acid A; **1.22**, **1.23**, and **1.24** which showed higher binding affinities  $\text{K}_i = 15, 3.4, \text{ and } 41 \text{ nM}$ , respectively (Figure 1.12) than (+)-myriceric acid A.<sup>1</sup>



**Figure 1.12: Sulfonic derivatives of myriceric acid A<sup>1</sup>**

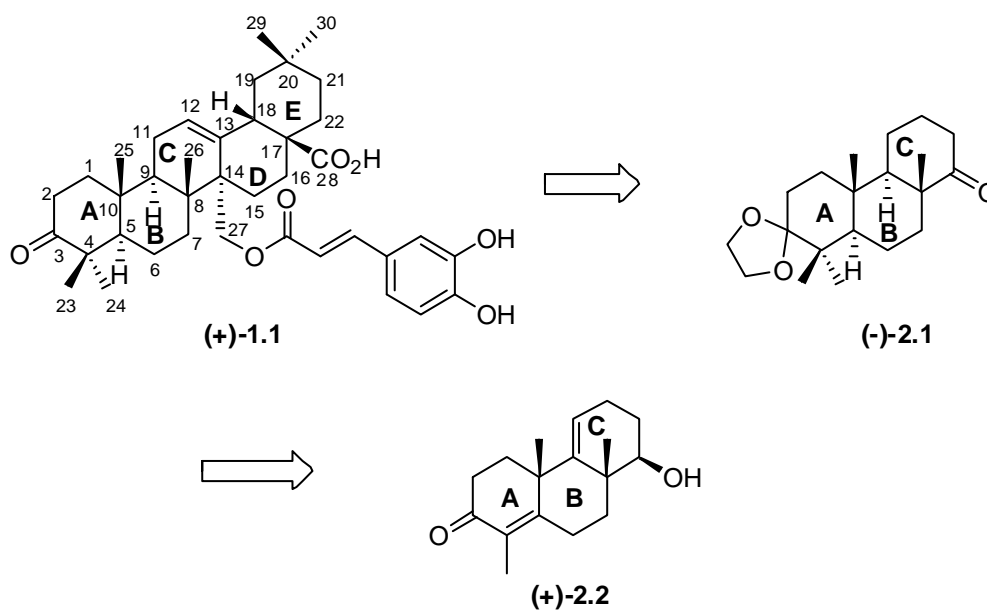
Although the partial synthesis of myriceric acid A could allow for some structure-activity relationship studies, it was limited to derivatization of the existing functional groups on the pentacyclic backbone of the starting natural product oleanolic acid<sup>2</sup>. For a more in depth structure-activity relationship study, a total synthesis of myriceric acid A is necessary, from which synthetic intermediates and novel analogs would be obtained.

## CHAPTER 2 - Synthesis of the ABC rings of (+)-myriceric acid A and model studies for constructing the DE rings

### 2.1 Retrosynthesis of (+)-myriceric acid A

(+)-Myriceric acid A [(+)-1.1] is a pentacyclic triterpenoid isolated from the chloroform soluble fraction of the methanol extract of *Myrica cerifera* twigs. It was the first non-peptide endothelin A receptor antagonist isolated from a plant<sup>1</sup>. It has seven stereo-centers, a double bond between C12 and C13, a caffeic ester side chain connected to a hydroxy methyl group (trans-caffeoyloxymethyl group) at C14 and a carboxylic acid at C17.

#### Scheme 2.1: Retro synthesis of myriceric acid A from tricyclic compound (+)-2.2



We planned to synthesize (+)-1.1 from the known tricyclic compounds (-)-2.1 which could be derived from (+)-2.2<sup>15-19</sup> (Scheme 2.1). Compound (+)-2.2 and (-)-2.1 have been used as key intermediates for the synthesis of several terpenoids<sup>15-19</sup>. Compound (-)-2.1 already has



the ABC rings with the C5, C8, C9, and C10 stereocenters of myriceric acid A. The ketone at C14 in (-)-**2.1** would be used to build the D and E rings.

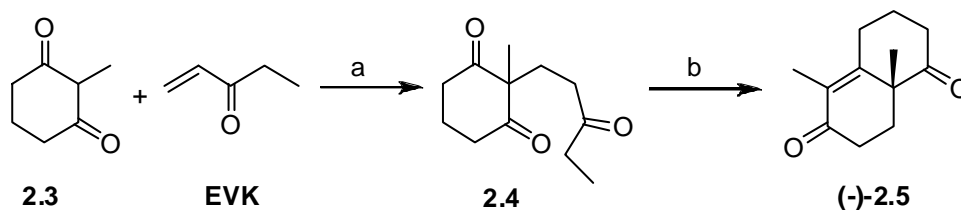
## 2.2 Synthesis of the ABC rings of (+)-myriceric acid A

Compound **2.2** and **2.1** have been used by several research groups for the racemic and asymmetric synthesis of various natural products.<sup>15-19</sup> This is because their synthesis initiates from simple, commercially available, and relatively inexpensive materials. It also contains most of the stereo-centers of the ABC rings of many pentacyclic triterpenes. Because of their proven versatility for the synthesis of several natural products<sup>15-19</sup>, **2.1** and **2.2** will be used as the ABC rings of (+)-myriceric acid A.

### *2.2.1 Assymmetric synthesis of the BC rings using an enantioselective intramolecular aldol condensation as a key step*

The synthesis of (-)-**2.1** was initiated by a Michael addition reaction of 2-methyl-1,2-cyclohexanedione (**2.3**) to ethyl vinyl ketone (EVK), catalyzed by potassium hydroxide in methanol, to give the symmetrical tri-ketone **2.4**. The intramolecular aldol condensation reaction of compound **2.4**, using pyrrolidine in benzene, would result in the formation of racemic **2.5** as described by Heathcock et al.<sup>20</sup>. To accomplish the planned asymmetric synthesis, chiral Wieland-Miescher ketone analogue (-)-**2.5** needed to be obtained. This was accomplished using Hagiwara et al.'s<sup>21</sup> method in which they treated symmetrical triketone **2.4** with an equivalent of the unnatural amino acid D-phenylalanine as an organocatalyst, and 0.5 equivalents of D-camporsulfonic acid in DMF to produce (+)-**2.5** (Scheme 2.2),  $[\alpha]_D -125^\circ$ , 89 % optical purity and after recrystallization  $[\alpha]_D -140^\circ$ <sup>21</sup>, 99.5 % optical purity. The optical purity of this compound was determined by chiral HPLC of its *p*-bromobenzoylated derivative of (-)-**2.9**.<sup>21</sup>

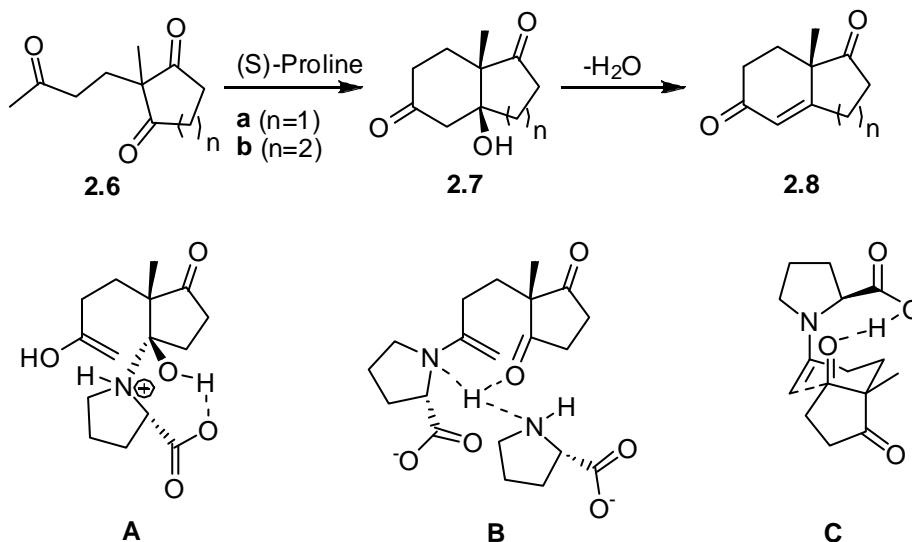
**Scheme 2.2: Hagiwara's synthesis of chiral Wieland-Miescher ketone analogue, (-)-2.5**



(a) KOH, MeOH, reflux, 100%; (b) D-phenylalanine, D-camphorsulfonic acid, DMF 68% yield after 2 steps.

This enantioselective aldol condensation reaction was discovered independently by two groups, the Hajos<sup>22</sup> group and Eder<sup>23</sup> group, in the 1970's. In their reactions, proline catalyzed the enantioselective intramolecular aldol reaction of the symmetric triketone **2.6** to produce aldol compound **2.7** and condensation product **2.8** (Scheme 2.3).<sup>28</sup>

**Scheme 2.3: The Hajos-Parish-Eder-Sauer-Wiechert reaction and proposed transition states.**<sup>22,23,28</sup>

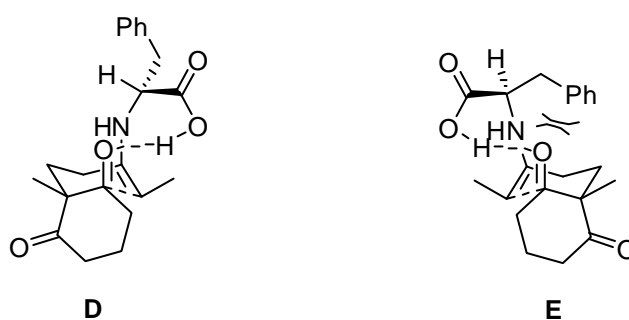


A. Hajos transition state B. Agami transition state C. Houk transition state.

Since then, three mechanisms<sup>28</sup> for this reaction have been proposed: the hemiaminal intermediate **A** by Hajos in 1974<sup>22</sup>; the proline enamine intermediate, that involves two prolines, in the transition state **B** by Agami in 1984<sup>24</sup>; and the proline enamine intermediate **C** proposed by Houk<sup>25-27</sup> in 2001. The Houk intermediate **C** is different from the Agami intermediate **B** because

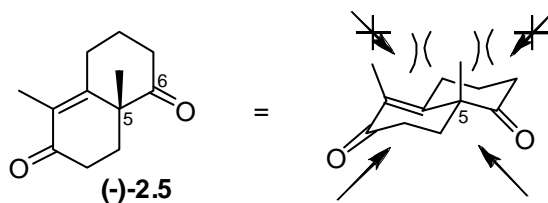
**C** involves a single proline, in which the carboxyl group is hydrogen bonded to one of the enantiotopic ketones in the transition state, and **B** involves two prolines.

Based on kinetic, stereochemical, and dilution experiments List et al. suggest that transition state **C** is the most acceptable mechanism for the asymmetric intramolecular aldol reaction of triketone **2.6**.<sup>27</sup> Following the same mechanism we believe that the Hagiwara et al. synthesis of the Wieland-Miescher ketone analogue **2.5**<sup>21</sup>, which uses D-phenylalanine instead of proline, follows a similar mechanism going through transition state **D** illustrated below (Figure 2.1).



**Figure 2.1: Transition state intermediates for intra-molecular aldol condensation. D – favorable transition state; E – unfavorable transition state because of steric hindrance of the benzyl group**

Furthermore, transition state **D** which is *pro R* is favored over transition state **E** which is *pro S*, because in the latter the benzyl group contributes some steric hindrance. On the other hand, in **D** the benzyl group is away from the enantiotopic carbonyl, so there is less steric hindrance in this transition state. This results in the formation of *R* compound (-)-**2.5**.



**Figure 2.2: Steric hindrance caused by the angular axial methyl group in (-)-2.5**

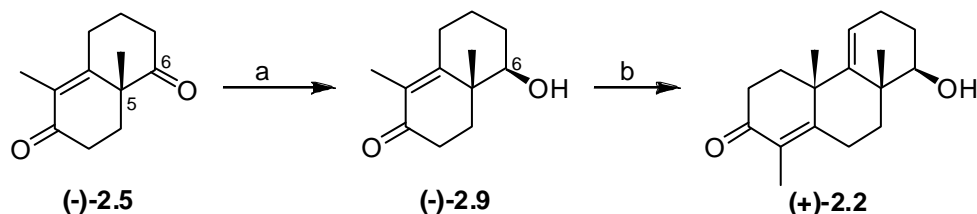
The newly formed stereo-center, with the angular axial methyl group at C5, helps to control the stereo-chemical outcome of the reactions that follow. The axial methyl group blocks

the top face of the compound causing all reactants to come from the bottom face of (-)-2.5 (Figure 2.2).

### 2.2.2 Synthesis of key intermediate (+)-2.2 by addition of the A ring to the BC rings

Continuing the synthesis, the saturated ketone at C6 in (-)-2.5 was regio- and stereo-selectively reduced by treating (-)-2.5 with sodium borohydride in ethanol to produce (-)-2.9 with 96% *ee*. The stereo-selectivity of the reduction was achieved because the addition of hydride occurs from the less hindered bottom face in (-)-2.5 (Scheme 2.4).<sup>16-19</sup>

#### Scheme 2.4: Synthesis of tricyclic compound (+)-2.2



(a) NaBH<sub>4</sub>, ethanol 0°C, 87% yield, (b) NaOMe, MeOH, ethyl vinyl ketone, 69% yield

Treatment of (-)-2.9 with sodium methoxide in methanol formed the thermodynamic dienolate (-)-2.9' (Scheme 2.5). This was reacted with EVK and resulted in a stereo-selective Robinson annulation reaction that produced (+)-2.2. The axial methyl group at C5 controlled the stereo-selectivity of this reaction, again. The structure of (+)-2.2 was confirmed by X-ray analysis of its crystal, which produced its crystal structure (Figure 2.3).

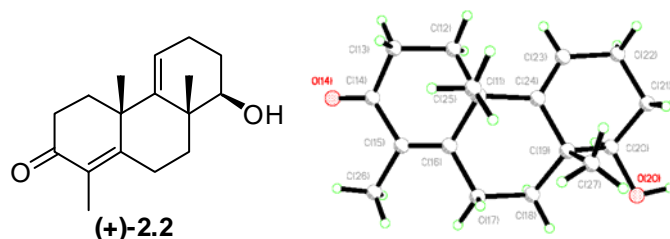
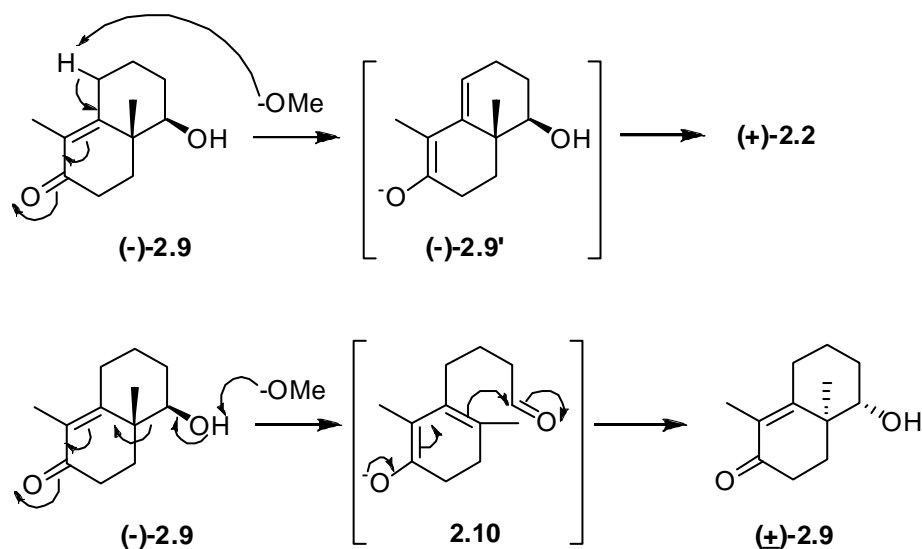


Figure 2.3: Crystal structure of (+)-2.2, by single-crystal X-ray analysis

Interestingly after the reaction of (-)-2.9 to form (+)-2.2 the enantiomeric excess (*ee*) of (+)-2.2 had decreased significantly to 48% *ee*. Honda et al<sup>17</sup> and Takikawa et al<sup>19</sup> report the same result and explain this by a retro-aldol reaction that competes with the formation of the dienolate (Scheme 2.5).<sup>17,19</sup>

**Scheme 2.5: Competing retro-aldol reaction**<sup>17,19</sup>

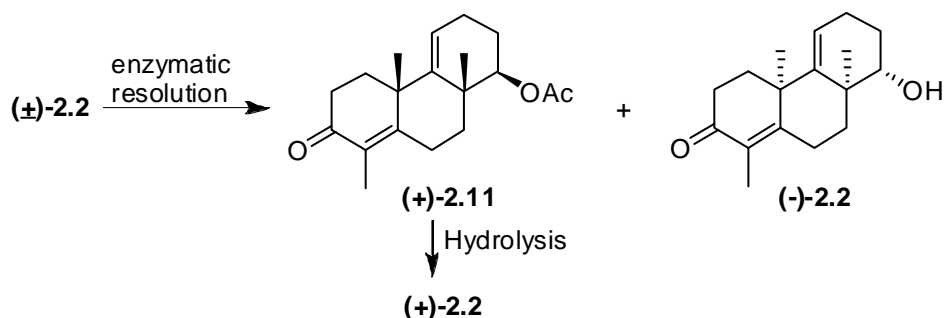


The fact that the product is enriched with (+)-2.2, indicates that the dienolate formation is faster than the racemization. Initially, a significant amount of the dienolate is formed which reacts with EVK to produce the enantiomer (+)-2.2. However, as the reaction progresses, the relative concentration of (-)-2.9 decreases, and results in a slower formation of the dienolate, allowing for the racemization of (-)-2.9, through the retro-aldol, and consequently formation of the racemic ( $\pm$ )-2.2. The initial formation of the high amount of (+)-2.2 is stable to the reaction conditions and results in the optical activity of the product.<sup>17</sup>

To accomplish an asymmetric synthesis, a solution to the racemization was necessary. An obvious solution to this problem would be to protect the alcohol. Honda et al.<sup>17</sup> and Takikawa et al.<sup>19</sup> determined that this was an unsuccessful solution. Honda et al.<sup>17</sup> reported that they obtained racemic benzyl ether under benzylation conditions and the acetyl, pivaloyl, tert-butyl dimethylsilyl groups were removed under the Robinson annulation conditions.<sup>17</sup> Similarly, Takikawa et al.<sup>19</sup> reported that the benzyl and methoxymethyl (MOM) ethers failed to undergo the Robinson annulation reaction.

Takikawa et al.<sup>19</sup> decided to do enzymatic resolution on **(±)-2.2** using Chirazyme L-2 (Roche Diagnostics Co.) to produce 50% of **(-)-2.2** 98.8% ee and 49% of acetate **(+)-2.11** 99.2% ee which was hydrolyzed to **(+)-2.2**  $[\alpha]_D^{22} +114$  (c 0.128, CHCl<sub>3</sub>)<sup>19</sup> (Scheme 2.6).

**Scheme 2.6: Enzymatic resolution of **(±)-2.2****<sup>19</sup>

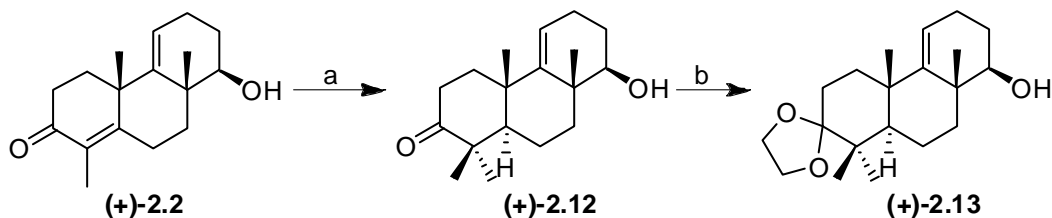


On the other hand Honda et al.<sup>17</sup> continued the synthesis and they were able to increase the enantiomeric purity at a later stage in the synthesis<sup>17</sup>. We decided to follow Honda et al.'s procedure<sup>17</sup> as described below.

### 2.2.3 Synthesis of the ABC ring ketone compound **(-)-2.1**

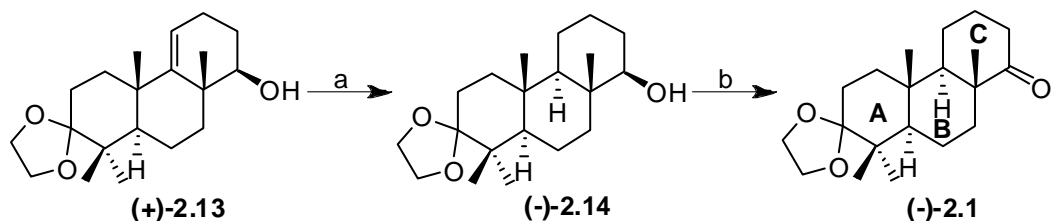
Using **(+)-2.2** with low *ee* we continued the synthesis by treating **(+)-2.2** with lithium in liquid ammonia to do a Birch reduction of the enone moiety. The resulting enolate was treated with iodomethane to give **(+)-2.12** in 45-50% yield. It was important in this reaction that after the iodomethane was added and the reaction was allowed to react for two hours and quenched with methanol to prevent reduction of the ketone. Ketalization of **(+)-2.12** was done by treating **(+)-2.12** with ethylene glycol, catalytic amount of pyridinium *p*-toluenesulfonate (PPTS) in refluxing toluene with azeotropic distillation to give ketal **(+)-2.13** (Scheme 2.7). After column chromatography, **(+)-2.13** was obtained with an optical activity of  $[\alpha]_D^{27} +18$  (c = 0.40, CHCl<sub>3</sub>). Honda et al.<sup>17</sup> recrystallized the ketal to get an improved optical activity of  $[\alpha]_D^{27} +22$  (c 0.26, CHCl<sub>3</sub>) which after removal of the ketal formed **(+)-2.12**  $[\alpha]_D^{27} +47$  (c 0.38, CHCl<sub>3</sub>). The % *ee* of **(+)-2.12** was determined to be 90% by <sup>1</sup>H and <sup>19</sup>F NMR, of the (-)-R-MTPA ester derivative of **(+)-2.12**.<sup>17</sup>

### Scheme 2.7: Synthesis of ketal (+)-2.13



Having the optically enriched ketal (+)-2.13 the synthesis was continued by reducing the double bond in (+)-2.13. Treatment of (+)-2.13 with a catalytic amount of palladium on carbon in ethanol and one atmosphere of hydrogen gas produced the alcohol (-)-2.14. Oxidation of the hydroxyl group of (-)-2.14 was accomplished by treatment with *o*-iodoxybenzoic acid (IBX) in dimethyl sulfoxide to give the ABC ring ketone compound (-)-2.1 (Scheme 2.8).<sup>18,19</sup>

### Scheme 2.8: Synthesis of the ABC ring ketone compound (-)-2.1



Having ketone (-)-2.1 a plan for the construction of the D and E rings of (+)-myriceric acid A was needed. Several plans that were investigated will be discussed in the next section.

## 2.3 Model studies for the construction of the D and E rings of (+)-myriceric acid A

### 2.3.1 C ring model studies

With the ABC rings of (+)-myriceric acid A constructed, we decided to use a model to study the construction of the D and E rings. As a model we decided to use the simplest form of the C ring, cyclohexanone **2.15** (Figure 2.4).

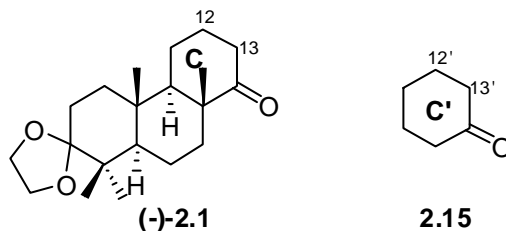


Figure 2.4: Cyclohexanone as the model of the C ring

Two requirements were needed to continue our synthesis 1) the position  $\alpha$  to the ketone at C13 needed to be functionalized with something that would activate this position and, 2) this functionality should allow for the formation of the double bond between C12 and C13 needed in the final product. To accomplish these requirements we decided to functionalize the C13' position of **2.15** with either a phenyl sulfide or phenyl selenide group. Either of these would increase the acidity of the C13' hydrogen and stabilize the resulting anion. Another advantage of these groups was that when they are oxidized they form sulfoxides or selenoxides, respectively, which undergo syn- $\beta$ -elimination to form the necessary double bond between C12 and C13.

#### Scheme 2.9: Synthesis of $\beta$ -keto-sulfide **2.16** and $\beta$ -keto-selenide **2.17**



(a) LDA, THF, phenyl disulfide,  $-78^{\circ}\text{C}$ , 62% yield; (b) LDA, THF, phenyl selenyl bromide,  $-78^{\circ}\text{C}$ , 86% yield

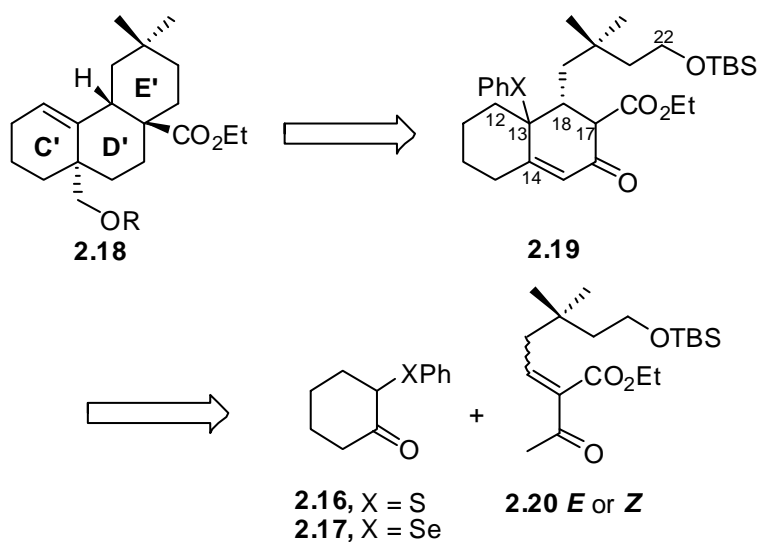


Cyclohexanone **2.15** was treated with freshly prepared lithium diisopropyl amine (LDA) to form the enolate. The enolate was then reacted with phenyl disulfide to produce the  $\alpha$ -phenyl sulfide **2.16** or phenyl selenyl bromide to give the  $\alpha$ -phenyl selenide **2.17**. (Scheme 2.9)

### 2.3.2 Convergent approach for the formation of the D and E rings

In our original plan we intended to do a convergent synthesis for the construction of the D' and E' rings. Our plan is summarized in the retro-synthetic scheme below (Scheme 2.10).

**Scheme 2.10: Retro-synthesis of the model convergent synthesis plan**

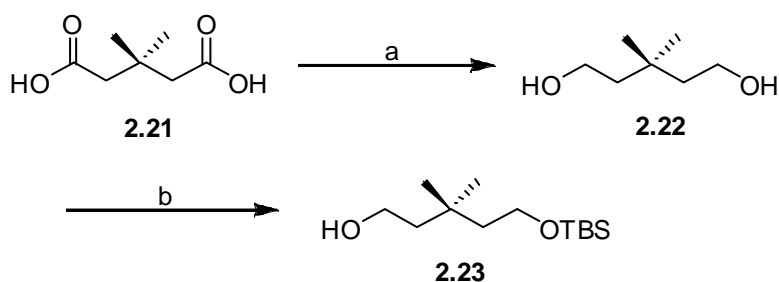


Robinson annulation of **2.16** or **2.17** to **2.20 E** or **Z** would result in the formation of the D ring enone **2.19**. This compound has all the functional groups necessary in the D ring and the side chain at C18 could be used to form the E ring. The ethyl ester at C17 makes the hydrogen at C17 more acidic and also it can easily be hydrolyzed to the required carboxylic acid in the final product. The silyl protected hydroxyl group on C22 can be converted to a leaving group which would allow for the stabilized anion formed at C17 to do a substitution reaction to form the E ring. The phenyl sulfide or phenyl selenide on C13 could then be oxidized to undergo a syn- $\beta$ -elimination to form the necessary double bond between C12 and C13. Finally, C14 is the  $\beta$  position of a  $\alpha,\beta$ -unsaturated ketone to which a Michael addition reaction can be used to

functionalize it. The group that was added should be one that can be converted to the hydroxyl methyl group to which the caffeic ester chain is attached in (+)-myricic acid A.

To investigate this approach we needed to synthesize the **2.20 E** and **Z** compounds. This was initiated by the reduction of 3,3-dimethylglutaric acid (**2.21**) with lithium aluminum hydride ( $\text{LiAlH}_4$ ) in THF to give 3,3-dimethylpentane-1,5-diol (**2.22**) quantitatively.<sup>29</sup> Treatment of diol **2.22** with DMAP, imidazole, TBSCl in DMF resulted in a mixture of three compounds – diol **2.22**, alcohol **2.23**, and di-silylated compound. To avoid this mixture we used McDougal et al.'s<sup>30</sup> method by treating diol **2.22** with one equivalent of sodium hydride to deprotonate one alcohol and reacted this with TBSCl to obtain monoalcohol **2.23** in 71% yield along with some recovered diol **2.22** (Scheme 2.11).

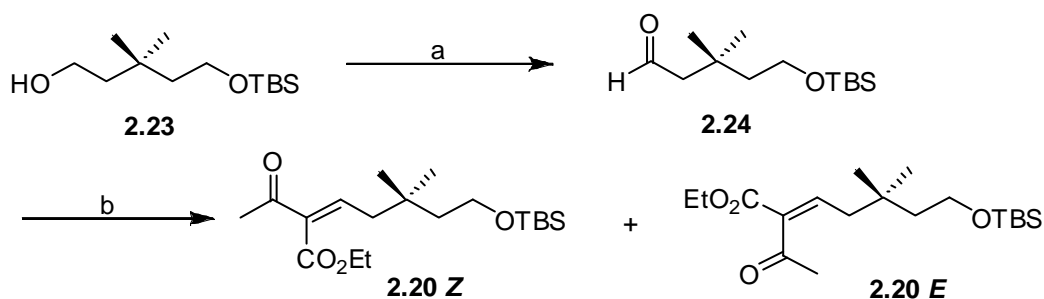
#### Scheme 2.11: Synthesis of monoalcohol **2.23**



(a)  $\text{LiAlH}_4$ , THF quantitative yield; (b)  $\text{NaH}$ , THF 1 h; TBSCl 1 h, 71% yield.

Monoalcohol **2.23** was oxidized to its corresponding aldehyde **2.24** by treatment with IBX in dimethyl sulfoxide (DMSO) overnight. A Knoevenagel condensation, following Tietze et al.'s procedure<sup>31</sup>, of aldehyde **2.24** with ethyl acetoacetate was accomplished by treatment with acetic acid and piperidine to give an almost quantitative yield of a 50/50 mixture of the  $\beta$ -ketoesters **2.20E** and **2.20Z** which were easily separated by column chromatography (Scheme 2.12).

### Scheme 2.12: Synthesis of $\beta$ -ketoesters **2.20 E** and **2.20 Z**

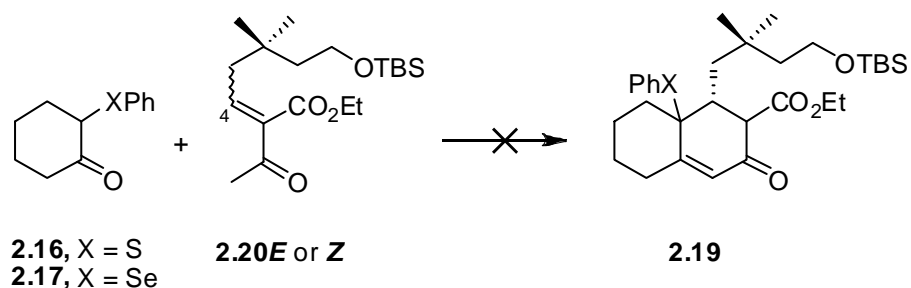


(a) IBX, DMSO overnight 81% yield; (b) ethyl acetoacetate, piperidine, AcOH,  $\text{CH}_2\text{Cl}_2$   $0^\circ\text{C}$ , quantitative yield.

These isomers, we believed, would help us to investigate the stereochemical outcome of the initial Michael addition of the Robinson annulation to form the D ring.

Having the conjugated  $\beta$ -ketoesters **2.20E** and **2.20Z** we attempted the Robinson annulation reaction of both phenyl sulfide **2.16** and phenyl selenide **2.17** with either **2.20E** or **2.20Z**. Unexpectedly and unfortunately using various bases under various reaction conditions we were unable to accomplish the Robinson annulation reaction. (Scheme 2.13)

### Scheme 2.13: Failed Robinson annulation of **2.16** or **2.17** with **2.20E** or **Z**

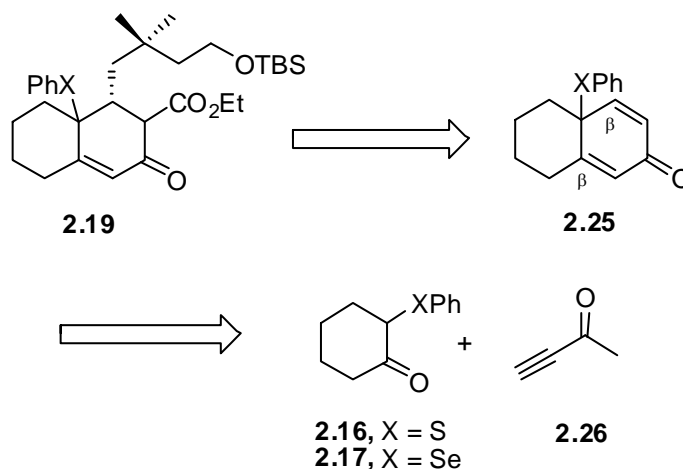


It is suspected that the failure of the Robinson annulation reaction is due to the great steric hindrance at the 4-position caused by 3,3-dimethylbutanosilyl ether group. This prevents the initial Michael addition needed for the Robinson annulation reaction. Another reason may be, as discovered later; that the conjugated  $\beta$ -ketoester **2.20E** or **Z** decomposes very easily under basic conditions. Since the Michael addition reaction is carried out under basic conditions, **2.20E** or **Z** may have decomposed before any reaction occurred. These results convinced us that a different approach for the construction of the D and E rings needed to be investigated.

### 2.3.3 Linear approach for the construction of the D ring

As described in the previous section, it was suspected that the side chain in **2.20E** or **Z** created significant steric hindrance that it prevented the initial Michael addition needed to accomplish the Robinson annulation reaction. An obvious solution to this problem would be to eliminate the side chain from the Michael acceptor; however, we still needed a way to add the side chain so we could construct the E ring. To fulfill these requirements we expected the conjugated triple bond compound 3-butyn-2-one (**2.26**) to be a good candidate.

#### Scheme 2.14: Retro-synthesis of the linear approach synthetic plan

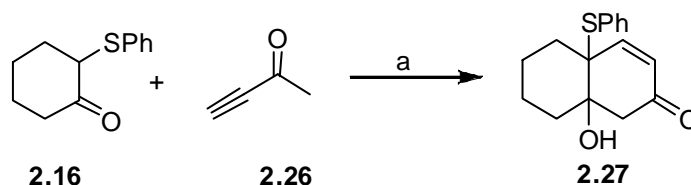


Robinson annulation of **2.16** or **2.17** with **2.26** was expected to result in the formation of the D ring as the dienone **2.25**. In **2.25** the less substituted β-position of the ketone would be expected to be more reactive toward a nucleophile. This would allow for the regio-selective Michael addition of the side chain and the resulting enolate may be trapped with ethyl cyanofornate to give the desired compound **2.19** (Scheme 2.14).

Our investigation of this approach was started by treating the α-phenyl sulfide **2.16** with sodium hydride in THF to form its enolate that was then reacted with 3-butyn-2-one (**2.26**). This reaction did not go through the full Robinson annulation reaction sequence; it did the Michael addition followed by the intramolecular aldol addition where it stopped and produced ketol **2.27** (Scheme 2.15). The elimination reaction did not occur because it was carried out under aprotic

conditions so there was no proton available to protonate the tertiary alkoxide formed by the intramolecular aldol addition so no  $\beta$ -elimination could occur.

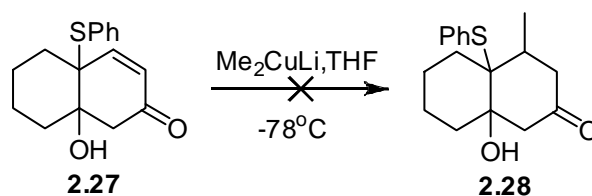
### Scheme 2.15 Synthesis of bicyclic compound 2.27



(a) NaH, THF, **2.26**, 30% yield.

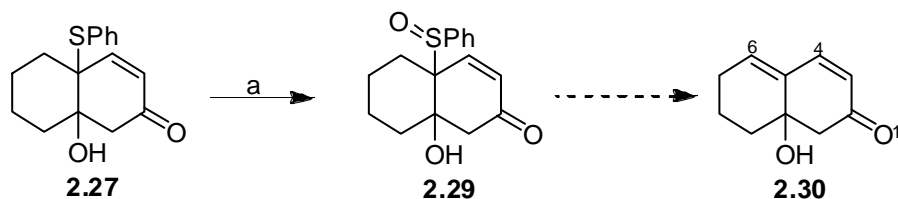
Ketol **2.27** was still useful; in fact, it improved the regio-selectivity of the Michael addition reaction that was planned to add the side chain. After the addition of the side chain, elimination of the  $\beta$ -hydroxyl group would produce the substituted  $\alpha,\beta$ -unsaturation. To determine whether this amendment to the plan was possible, we decided to test the Michael addition to enone **2.27** by treating it with the simple methyl cuprate in THF at  $-78^\circ\text{C}$ . This reaction unexpectedly resulted in the decomposition of ketol **2.27**, to unidentifiable materials (Scheme 2.16).

### Scheme 2.16: Michael addition of methylcuprate to enone 2.27



This may be because of the phenyl sulfide in the  $\gamma$ -position possibly causing steric hindrance for the Michael addition. It may also be that the phenyl sulfide was eliminated since it can be a leaving group. It was decided that the phenyl sulfide group would be removed by oxidation to the sulfoxide **2.29**. Heating of the sulfoxide would result in a syn- $\beta$ -elimination to form the conjugated dienone **2.30** (Scheme 2.17).

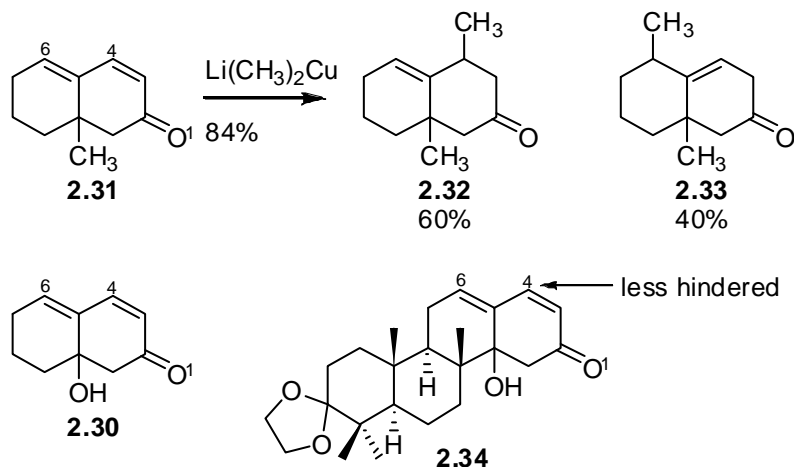
**Scheme 2.17: Plan for the synthesis of conjugated dienone 2.30**



(a) oxone, 4:1 dioxane:H<sub>2</sub>O, 39% yield.

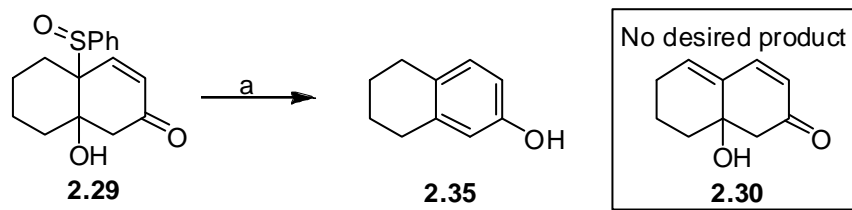
Formation of the conjugated dienone **2.30** would raise the question whether the Michael addition would occur at the 4-position or the 6-position. From Marshall et al.'s<sup>32</sup> studies it has been determined that the regioselectivity of the Michael addition to conjugated dienones is primarily directed by sterics.<sup>32</sup> In his first example, which best resembles our model compound **2.30**, there is a slight preference for addition to the 4-position.<sup>32</sup> Because of this, along with the fact that in our proposed compound **2.34** the 4-position is less hindered than the 6-position we expect that the organocuprate addition would result in the desired 1,4-addition (Scheme 2.18).

**Scheme 2.18: Marshall's study of regioselective Michael addition to conjugated dienones<sup>32</sup>**



Oxidation of the phenyl sulfide **2.27** was accomplished by treating it with oxone in a solution of 4:1 dioxane:water to give the phenyl sulfoxide **2.29**. The syn-β-elimination to produce **2.30** was attempted by refluxing **2.29** in toluene.

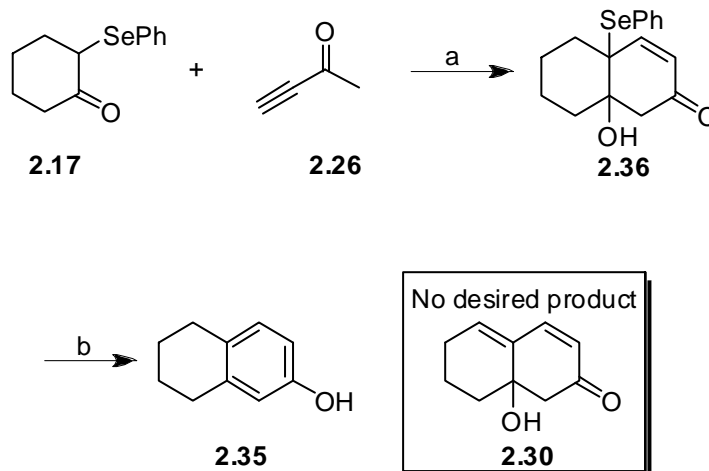
### Scheme 2.19: Aromatization of the model D ring



(a) Toluene, reflux, 28% yield.

Refluxing **2.29** in toluene produced a new product. After isolation it was determined that it was not the desired conjugated dienone **2.30**, instead the model D ring had aromatized to form the phenol **2.35** (Scheme 2.19). It was suspected that this resulted from the high temperature required to accomplish the syn- $\beta$ -elimination reaction of the sulfoxide. To avoid the high temperature reaction, we believed that we could use the phenyl selenide analogue **2.36**. Its oxidation to the selenoxide would result in syn- $\beta$ -elimination even at very low temperatures to produce the dienone **2.30** directly.

### Scheme 2.20: Aromatization of the model D ring at low temperature

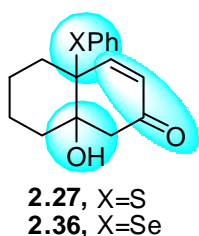


(a) NaH, THF, **2.26**, 10% yield; (b) oxidation,  $0^\circ\text{C}$ .

Following the same procedure as with the  $\alpha$ -phenyl sulfide **2.16**,  $\alpha$ -phenyl selenide **2.17** was treated with sodium hydride in THF. The resulting enolate was reacted with 3-butyn-2-one to form the phenyl selenide ketol analogue **2.36**. Unfortunately, the oxidation of selenide **2.36** using different oxidants and conditions at low temperatures still resulted in the aromatization of

the model D ring to form phenol **2.35** (Scheme 2.20). These results forced us to rethink our approach and try to design a new one.

From analysis of intermediates **2.27** and **2.36** it can be seen that the model D ring is highly functionalized. It has a phenyl sulfide or phenyl selenide group, a tertiary hydroxyl group, which are reasonable leaving groups and an  $\alpha,\beta$ -unsaturated ketone group (Figure 2.5).

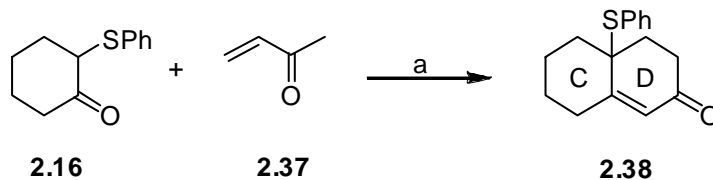


**Figure 2.5: Highly functionalized model D ring**

The high substitution on the model D ring may be contributing to its facile aromatization. To see whether aromatization could be prevented we decided to reduce the amount of functional groups on the model D ring. The unsaturation of the  $\alpha,\beta$ -unsaturated ketone was removed because it seems to be the simplest functionality to remove and also to regenerate.

Synthesis of the new compound was accomplished by treating the  $\alpha$ -phenyl sulfide **2.16** with sodium methoxide in methanol to form the enolate. This was then reacted with methyl vinyl ketone **2.37** to undergo a Robinson annulation reaction to give the new model CD rings as enone **2.38** in high yield and no aromatization (Scheme 2.21).

**Scheme 2.21: Robinson annulation reaction to form the D ring without aromatization**



(a) NaOMe, MeOH, reflux, 92 % yield.

After obtaining this high yielding Robinson annulation reaction we believed this was a promising route for the synthesis of the D and E rings of (+)-myriceric Acid A [(+)-**1.1**]. So, it



was decided that the study of this linear approach would be continued with the actual ABC ring compound (-)-**2.1**.

## 2.4 Conclusion

The ABC rings (-)-**2.1** of (+)-myriceric acid A were successfully synthesized. The first asymmetric center was formed using an asymmetric aldol condensation reaction employing the unnatural amino acid D-phenylalanine as the organo-catalyst to control the enantioselectivity. This new center having an axial angular methyl group helped to control the formation of the other stereocenters in the molecule. A major problem encountered was that some of the enantiomeric purity was lost in the second Robinson annulation reaction from (-)-**2.9** to (+)-**2.2** as a result of a competing retro-aldol reaction in the started material. The enantiomeric purity was improved at a later stage by ketalization and purification of the ketal (+)-**2.13** by column chromatography and recrystallization.

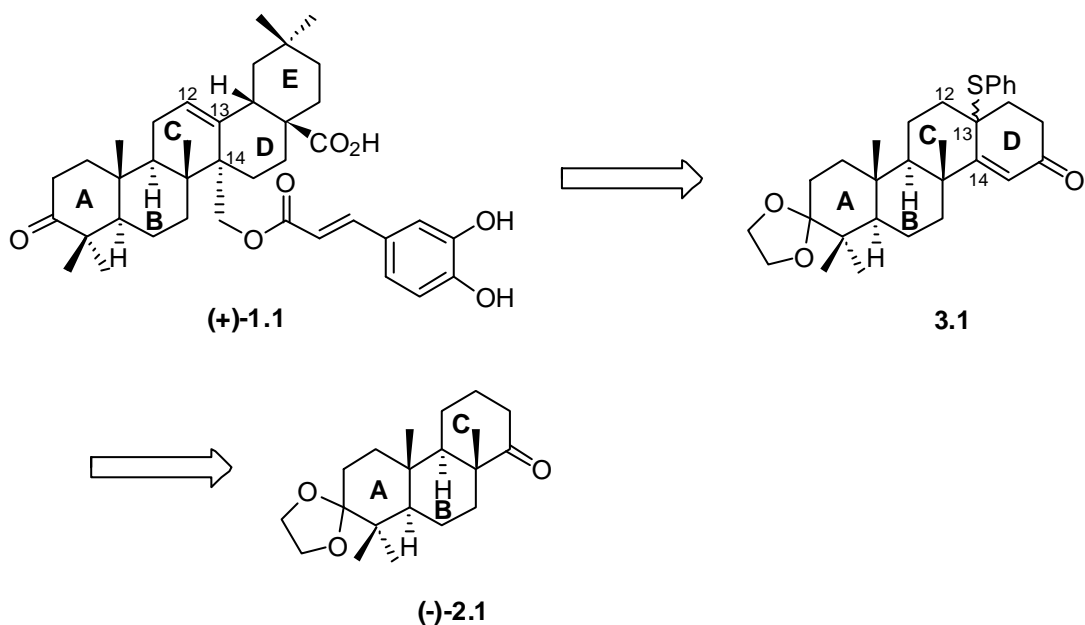
Model studies were done to study the construction of the DE rings using cyclohexanone (**2.15**) as a simple model of the C ring. The initial convergent synthesis plan was unable to be accomplished because the  $\beta$ -position of the enone **2.20E** or **Z** was hindered and the enone easily decomposed under basic conditions. After this discovery, two linear approaches were investigated: the first was using 3-buten-2-one (**2.26**) as the Michael acceptor and D ring precursor; and the second using methyl vinyl ketone (**2.37**) as the Michael acceptor and D ring precursor. The initial approach resulted in aromatization of the D ring to phenol **2.35** because of the high functionalization on the D ring. The second approach provided the D ring compound **2.38** through a high yielding Robinson annulation. The model D ring **2.38** had two of the essential functional groups that could be used to further functionalize the D ring and the final product. Having these results we decided to continue our studies of this approach with our ABC ring compound (-)-**2.1**.

## CHAPTER 3 - Synthesis of the D ring of (+)-myriceric acid A

### 3.1 Robinson annulation of $\alpha$ -phenyl sulfide approach

In the previous chapter the synthesis of tricyclic ketone (-)-**2.1** was described. This compound contains the ABC rings with four of the stereocenters of our target compound (+)-myriceric acid A [(+)-**1.1**]. Our original plan, as described in Chapter 1, was a convergent synthesis. However, after several model studies we determined that our best approach would be a linear synthesis in which we first build the D ring, functionalize it and then add the E ring.

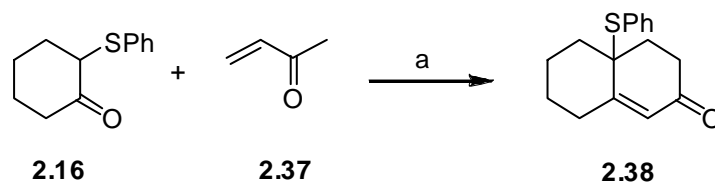
Scheme 3.1: Linear retro-synthetic plan toward (+)-myriceric acid A



From the model studies described in Chapter 2 it was determined that the  $\alpha$ -phenyl sulfide on compound **2.16** would be used as a model for the C ring. Its  $\alpha$ -phenyl sulfide serves two purposes; 1.) It stabilizes the anion formed from removal of the  $\alpha$ -hydrogen and 2.) It can be oxidized and eliminated to form the double bond between C13-C14 in the target compound. The linear synthetic plan summarized in the retro-synthesis above (Scheme 3.1) was based on the results obtained in the model study. In the model studies we accomplished a Robinson

Annulation reaction with compound **2.16** and methyl vinyl ketone (**2.37**) to give the model D ring compound **2.38** (Scheme 3.2).

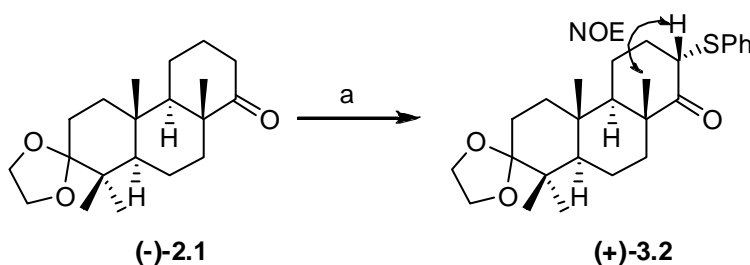
**Scheme 3.2: Robinson annulation reaction accomplished in the model studies**



a.) NaOMe, MeOH, rt overnight, 92% yield.

Continuing our total synthesis using this approach, our first task was to synthesize the tricyclic form of **2.16** from our tricyclic ketone (-)-**2.1**. It was expected that the phenyl sulfide addition would be regio- and stereo-selective because only one  $\alpha$ -position of the ketone is available for deprotonation and the other  $\alpha$ -position had a quaternary center with an axial methyl group that blocks the top face.

**Scheme 3.3: Synthesis of tricyclic  $\beta$ -ketosulfide (+)-3.2**



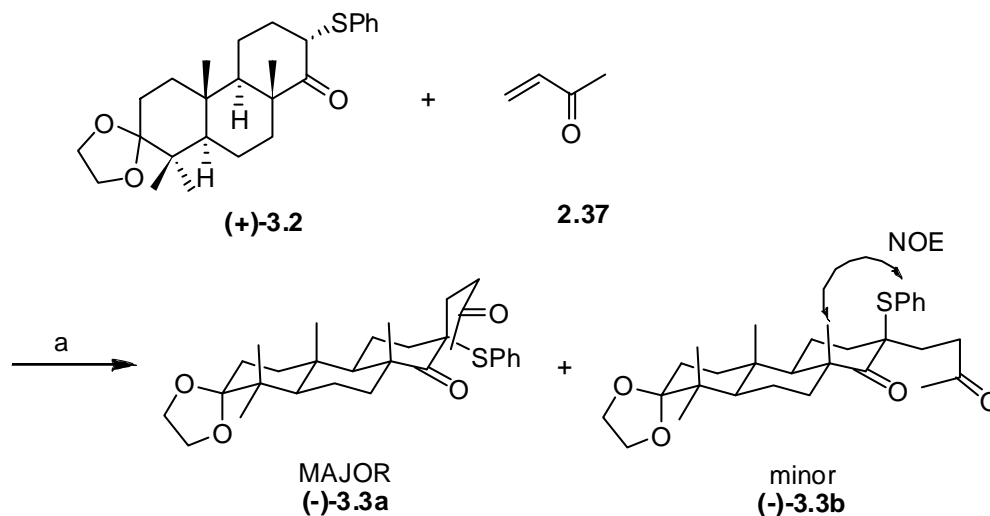
a.) LDA, THF rt, PhSSPh (phenyl disulfide), 71% yield.

As expected, the enolate of (-)-**2.1** was easily formed using LDA and it was then reacted with phenyl disulfide to give the expected regio- and stereo-selective  $\alpha$ -phenyl sulfide compound (+)-**3.2**. The phenyl sulfide group was on the opposite face from the axial methyl group, which was confirmed by NOE observed between the axial methyl group and the  $\alpha$ -hydrogen (Scheme 3.3).

Following the model, our next step was to do a Robinson annulation reaction with (+)-**3.2** and methyl vinyl ketone (MVK) (**2.37**). Using the same conditions as in the model, compound

(+)-**3.2** was treated with sodium methoxide followed by addition of the methyl vinyl ketone at room temperature, but this resulted in no reaction. Because (+)-**3.2** was a more complex molecule with more steric hindrance than the model compound **2.16** we believed that the reaction could occur at elevated temperatures. Upon increasing the temperature to 55-60°C the reaction resulted in the formation of two products as observed by TLC. Unexpectedly, after isolation it was determined that these products were not Robinson Annulation products. Instead they were the two diastereomers of the intermediate Michael addition reaction that produced (-)-**3.3a** and (-)-**3.3b** in quantitative yield (Scheme 3.4).

**Scheme 3.4: Michael addition of tricyclic  $\beta$ -ketosulfide (+)-3.2 with MVK**



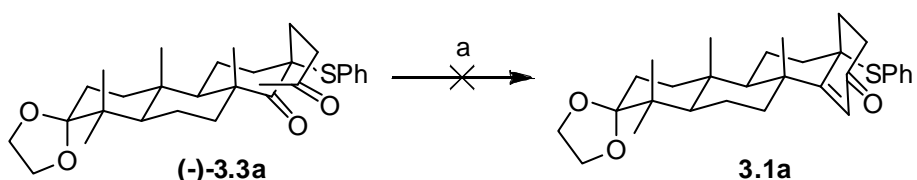
a.) NaOMe, MeOH/THF, 55-60°C 2h, 100% yield, 3:1 **3.3a**:**3.3b**.

The mixture of diastereomers was easily separated to give a 3:1 product mixture with diastereomer (-)-**3.3a**, which has the phenyl sulfide equatorial as the major product, and diastereomer (-)-**3.3b**, which has the phenyl sulfide axial as the minor product, respectively. Based on steric hindrance the opposite ratio was expected, however, this product ratio can be rationalized based on transition state energy of the enolate in the carbanion resonance structure. The carbanion, just before attack, would start approximating  $sp^3$  hybridization before forming the Michael addition product. If the carbanion attacks from the top then the phenyl sulfide is in a more stable equatorial position and lower energy of transition state giving (-)-**3.3a** as the major product. Attack from the bottom face places the phenyl sulfide in an axial position leading to

steric interaction with the adjacent axial methyl group thereby increasing the energy of that transition state and giving (-)-**3.3b** as the minor product. This stereochemical outcome was not a problem because in the later stages of the synthesis it would be destroyed.

At first glance the Michael addition products (-)-**3.3a** and (-)-**3.3b** were expected to undergo the intramolecular aldol condensation very easily to give **3.1a** or **3.1b**, respectively. To investigate this, the intramolecular aldol condensation was attempted with the major diastereomer (-)-**3.3a**.

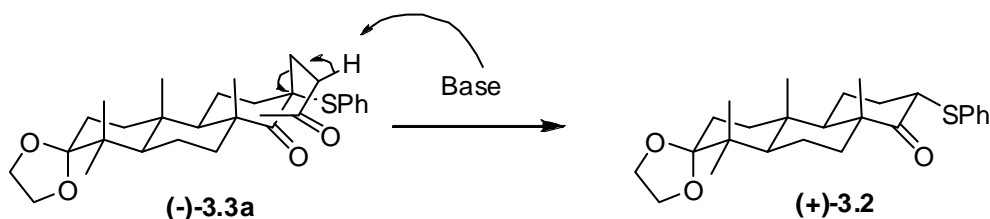
**Scheme 3.5: Failed intramolecular aldol condensation of (-)-3.3a**



a.) several basic conditions

The aldol condensation of (-)-**3.3a** was attempted with many different bases, in different solvents and at different temperatures (Scheme 3.5). Most of the conditions resulted in no reaction. Some, however, resulted in reverse Michael addition in which (+)-**3.2** was isolated (Scheme 3.6).

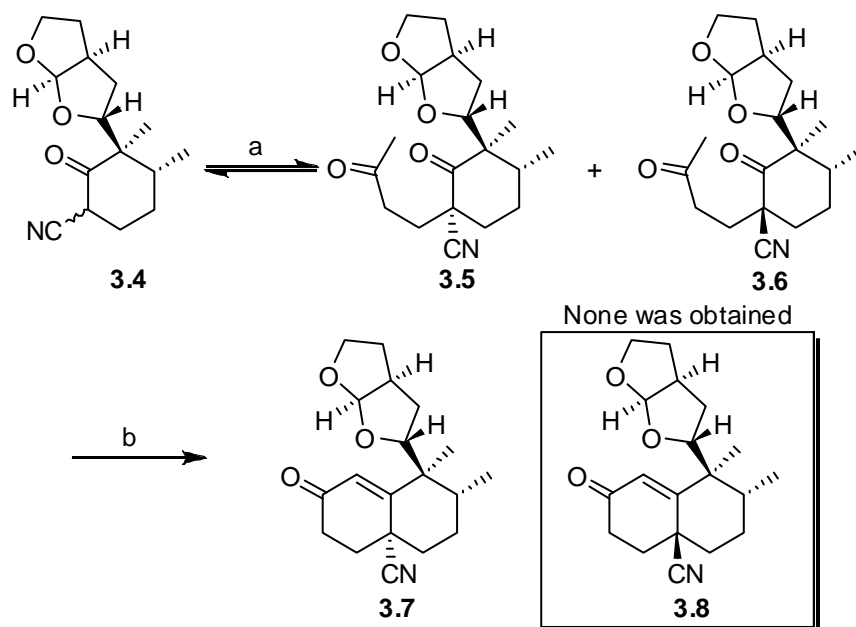
**Scheme 3.6: Reverse Michael addition reaction in (-)-3.3a**



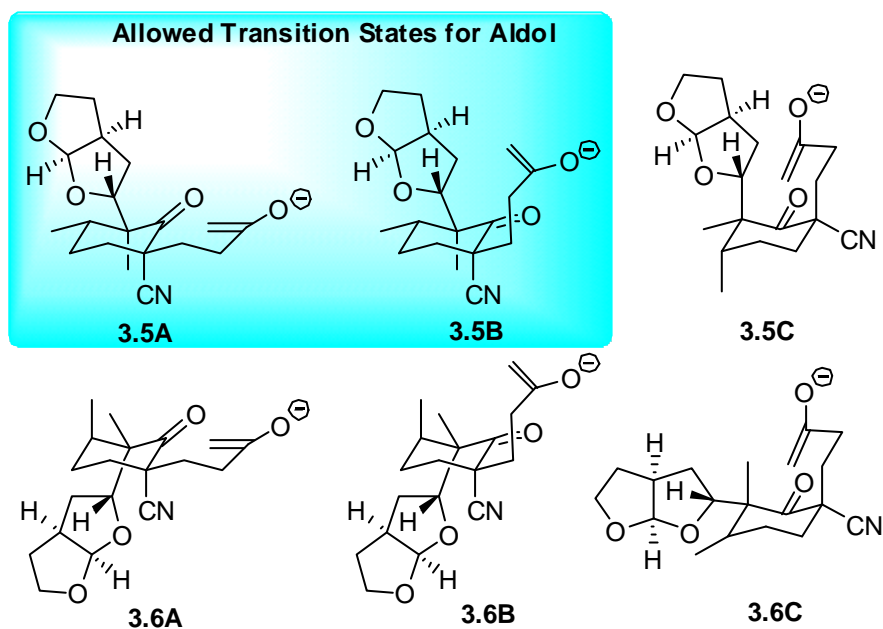
The stability of the anion of the  $\alpha$ -phenyl sulfide compound (+)-**3.2** makes it a reasonable leaving group. The axial methyl group  $\alpha$  to the ketone blocks the top face of the carbonyl in (-)-**3.3a** and makes the retro Michael reaction more favorable than the intramolecular aldol in (-)-**3.3a**. De Groot et al.<sup>33</sup> report a similar behavior in their study of the synthesis of “functionalized decalones by Robinson annulation of substituted cyclohexanones”<sup>33</sup> using the sterically congested cyano ketone **3.4**. The initial Michael addition of **3.4** with methyl vinyl ketone occurs

smoothly to give a mixture of diastereomers **3.5** and **3.6**. They reported that under basic conditions cyclization was not achieved, instead a retro Michael addition reaction was observed. However, under Knoevenagel conditions they were able to accomplish the cyclization. For the cyclization reaction of the two diastereomers **3.5** and **3.6** only one product **3.7** was obtained<sup>33</sup> (Scheme 3.7).

**Scheme 3.7: De Groot's Robinson annulation of substituted cyclohexanones<sup>33</sup>**



a.) NaOMe, MVK, benzene. b.) Pyrrolidine, AcOH, toluene.



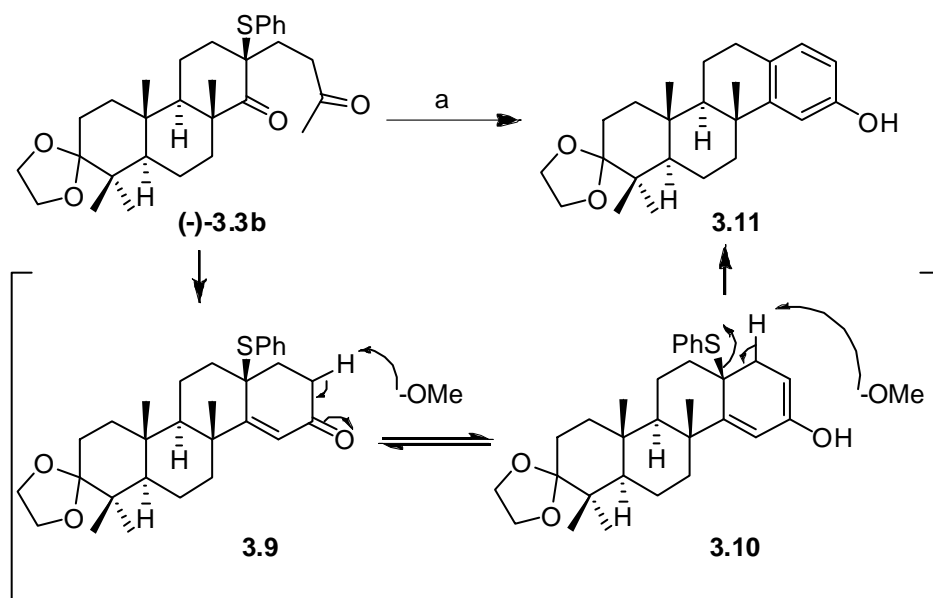
**Figure 3.1: Transition States for aldol condensation of compound 3.5 and 3.6.<sup>33</sup>**

The outcome of the intramolecular aldol condensation was rationalized based on the transition states of the cyclization of **3.5** and **3.6**.<sup>33</sup> Transition states **3.5A** and **3.5B** show that the cyclization of **3.5** can go smoothly to **3.7**. On the other hand, all the transition states of **3.6** (**3.6A**, **3.6B**, or **3.6C**) were both conformationally and geometrically unfavorable for the cyclization (Figure 3.1). Molecule **3.6** instead underwent a retro Michael addition followed by re-addition of the MVK to **3.5** and in that way cyclization to **3.7** occurred.<sup>33</sup>

Comparing the C-ring of (-)-**3.3a** with **3.5** and **3.6** it can be seen that it would have a transition state similar to **3.5C** and **3.6C** in which both the enolate group and methyl group are axial and on the same face which are the most unfavorable transition states for the cyclization. De Groot et al could accomplish the cyclization using Knoevenagel conditions because the one ring system in **3.5** can flip to adapt the more favorable conformations, **3.5A** and **3.5B**. We attempted these conditions without any success. This is because in our case, the tricyclic ring system is very rigid and the transition state is locked as the unfavorable conformation. The retro Michael addition is therefore more favorable in (-)-**3.3a**.

Based on these results, we decided to investigate whether our minor isomer (-)-**3.3b**, which has the favorable conformation as in **3.5A** and **3.5B** in which the enolate group is equatorial and on the opposite face of the axial methyl group, would result in cyclized product.

**Scheme 3.8: Aromatization of the Robinson annulation product of (-)-3.3b**



a.) NaOMe, MeOH reflux, 31% yield.

As suspected, treating (-)-**3.3b** with sodium methoxide in methanol and refluxing resulted in cyclization, however the cyclized product turned out to be the aromatized D ring compound **3.11**. From this result it can be speculated that the intramolecular aldol reaction took place, forming enone **3.9**. However, under the thermodynamic conditions the enol **3.10** was formed from **3.9** and finally under the basic conditions the phenyl sulfide acted as a leaving group and was eliminated to give the phenol **3.11** (Scheme 3.8).

The studies of de Groot et al.<sup>33</sup> and our results indicated that for the intramolecular aldol condensation reaction to occur, to form our D ring, the enolate group must be in an equatorial position or a conformation that would allow the enolate to react with the ketone from the opposite face of the axial methyl group. It was also indicated that the gamma phenyl sulfide group needed to be removed or replaced to prevent aromatization of the D ring. This however posed the problem on how to reform the necessary double bond at that position.

## 3.2 Dienone Approach

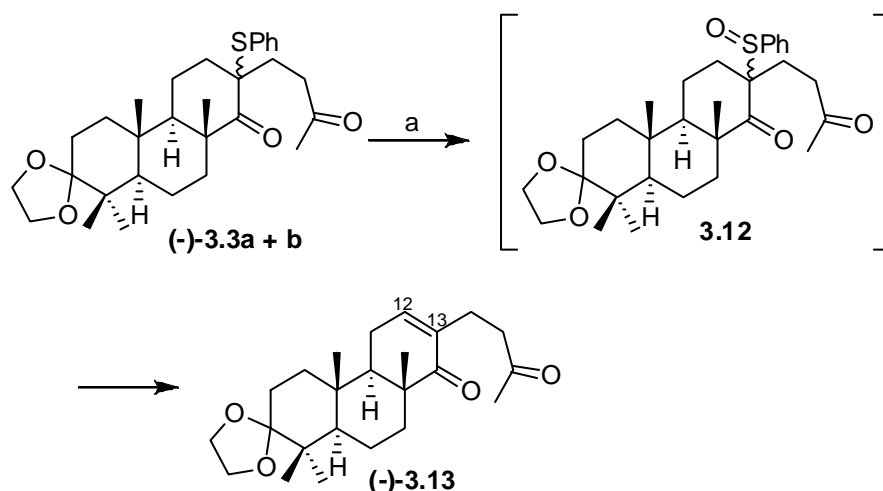
To overcome the steric limitations and elimination of the phenyl sulfide encountered in the previous section with compounds (-)-**3.3a** and (-)-**3.3b**, respectively, we decided to modify our approach. By oxidizing the mixture of sulfide diastereomers to their corresponding sulfoxide **3.12**, followed by heating to accomplish a  $\beta$ -syn-elimination, the C12-C13 double bond in (-)-**3.13** and in the final product would be formed. This double bond alleviates three problems; 1.) It eliminates the steric limitations by making the C13  $sp^2$  hybridized, and therefore flat, allowing for the enolate on the side chain to react from the opposite face of the axial methyl group. 2.) Because the phenyl sulfide leaving group is no longer at C13, no elimination is possible for aromatization of the D ring. 3.) In this route both diastereomers are useful because the diastereocenter has been destroyed so the overall yield of the synthesis is increased.

The diastereomeric mixture of (-)-**3.3** was oxidized by treatment with *m*-chloroperbenzoic acid in methylene chloride at 0°C for 15 minutes. Interestingly, the isolated product was not the sulfoxide **3.12**; instead the deprotected (-)-**3.13** was obtained. Removal of the ketal protecting group is believed to be caused by the sulfinic acid that is produced by the  $\beta$ -syn-elimination of the sulfoxide in **3.12**. To prevent the deprotection, the acid was neutralized



by the addition of sodium bicarbonate to the reaction, which then resulted in quantitative yield of enone (-)-**3.13**. The sulfoxide **3.12** was not isolated because of the rigidity in compound **3.12** facilitated the  $\beta$ -syn-elimination reaction so no high temperature was needed to form (-)-**3.13** (Scheme 3.9).

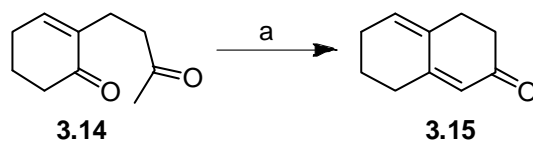
**Scheme 3.9: Removal of the phenyl sulfide group by oxidation**



a.)  $\text{CH}_2\text{Cl}_2$ , *m*CPBA,  $\text{NaHCO}_3$ ,  $0^\circ\text{C}$  15 min, 100% yield.

Precedence for the formation of conjugated dienones by aldol condensation of a similar  $\beta$ -enone-ketone **3.14** was shown by El Gaied et al.<sup>34</sup>. They showed that when **3.14** was treated with potassium carbonate in ethanol it produced the conjugated dienone **3.15** in 67% yield<sup>34</sup> (Scheme 3.10).

**Scheme 3.10: Intramolecular aldol condensation of  $\beta$ -enone-ketone **3.14**<sup>34</sup>**

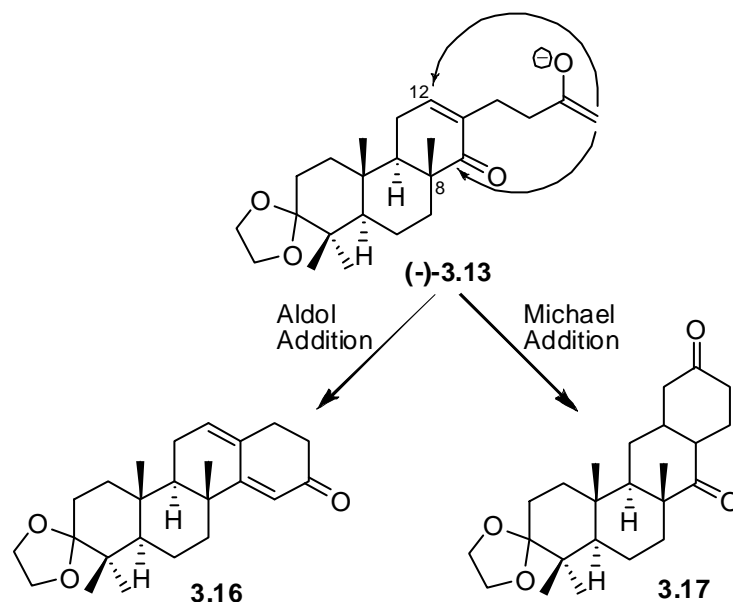


a.)  $\text{K}_2\text{CO}_3$ , EtOH, 67% yield.

Although El Gaied et al.<sup>34</sup> report their reaction gives **3.15** as the major product, it is obvious that there are two possible reactions that can occur in both compound **3.14** and compound (-)-**3.13**. The side chain enolate could either undergo an intra-molecular 1,2-addition

reaction (aldol condensation) or an intra-molecular 1,4-addition (Michael addition), each resulting in a different product (Scheme 3.11).

### Scheme 3.11: Aldol condensation vs. Michael addition



Many different bases were tried.

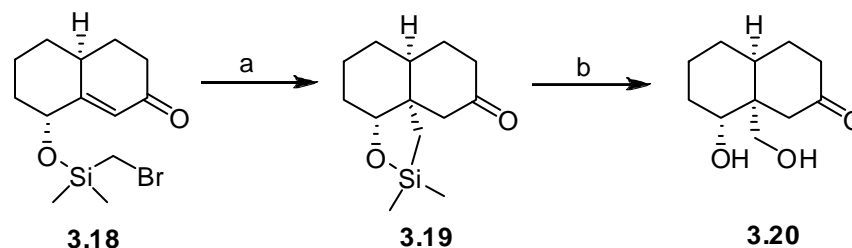
As speculated, treatment of (-)-3.13 under the similar reaction conditions (potassium carbonate in ethanol) resulted in both reactions; the aldol addition forming compound 3.16 and the Michael addition forming compound 3.17. To continue our synthesis we required compound 3.16. However, the major product in this reaction turned out to be the Michael addition adduct 3.17 with a trace amount of conjugated dienone 3.16. In an attempt to change the outcome of the reaction, toward preferential formation of the desired conjugated dienone 3.16, different reaction conditions (different bases, solvents and temperatures) were investigated. Unfortunately, most of the reaction conditions produced the undesired compound 3.17 as the major product and a trace amount  $\leq 10\%$  of the desired dienone 3.16.

This preference for the Michael addition in compound (-)-3.13 is caused by the C8 quaternary center sterically hindring the ketone resulting in a sluggish aldol condensation. On the other hand the C12 position is less hindered favoring the Michael addition and consequently formation of adduct 3.17.

While trying to improve the aldol condensation reaction, studies to continue the synthesis with conjugated dienone **3.16** were initialized. Conjugated dienone **3.16** already possessed the C12-C13 double bond of the final product. The next goal was to functionalize the C14 position with a hydroxy methyl group. As discovered in the previous studies, nucleophilic addition to C14 would be sluggish because of the adjacent quaternary center. Also, if another less hindered electrophilic position was available the reaction would prefer to occur at that position. This was encountered again with the conjugated dienone **3.16**, which has two possible electrophilic positions. Since the C12 is the less hindered electrophilic position, the nucleophile would prefer to add at C12 instead of the C14 position.

To overcome these problems it was decided that the double bond would be masked as an alcohol. This alcohol would be used as an anchor to stereoselectively direct a Stork silyl methyl radical cyclization reaction followed by a Fleming-Tamao oxidation to produce the desired C14 hydroxy methyl group.<sup>35</sup> Lallemand et al.<sup>35</sup> employed this method to form the cyclic silyl ether **3.19** and consequently diol **3.20** with a similar angular hydroxy methyl group that we require (Scheme 3.12).

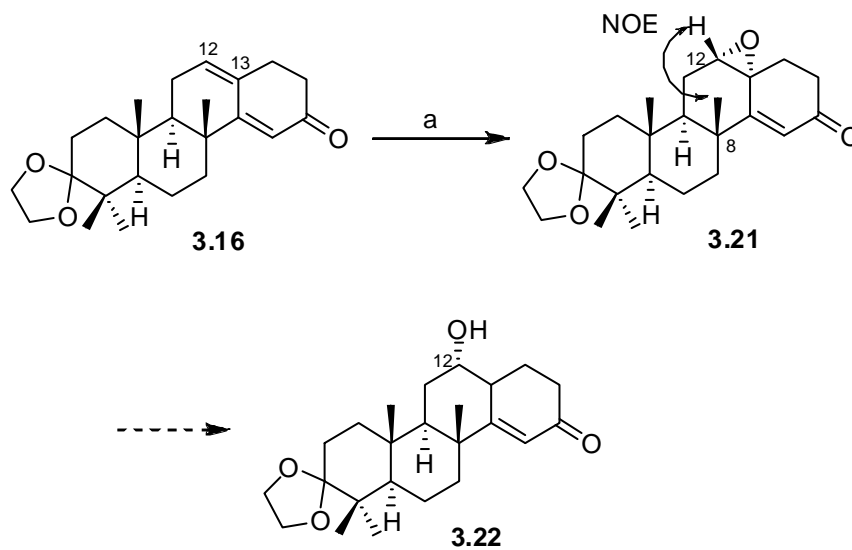
**Scheme 3.12: Lallemand's stereoselective formation of an angular hydroxy methyl group<sup>35</sup>**



a.) (n-Bu)<sub>3</sub>SnH (1.5eq), AIBN (cat.), benzene, reflux 4 h 100% yield; b.) 30% H<sub>2</sub>O<sub>2</sub> (excess), NaHCO<sub>3</sub> (1.3eq), MeOH/THF (1/1), reflux 18 h 65% yield.

To convert the C12-C13 double bond to an alcohol, it was first oxidized to an epoxide. Treatment of compound **3.16** with *m*-CPBA in methylene chloride produced epoxide **3.21** with the epoxide in the β-face of the molecule. This stereochemistry was expected based on steric hindrance of the axial methyl group at C8 and it was confirmed by NOE observed between the C12-hydrogen and the hydrogens on the axial methyl group on C8 (Scheme 3.13).

### Scheme 3.13: Epoxidation of conjugated dienone 3.16



a.) *m*-CPBA, CH<sub>2</sub>Cl<sub>2</sub>, rt, 80% yield.

The next step was to selectively open the epoxide to give the alcohol on C12 as in compound **3.22**. Since the alcohol would be on the bottom face, it could be used as an anchor to direct the functionalization of the  $\beta$ -position with the desired hydroxy methyl group following Lallemand et al.'s<sup>35</sup> procedure. The C12 hydroxy group could then be eliminated to reform the C12-C13 double bond. Because of the small amount of the product, investigations for the conversion of the epoxide **3.21** to the alcohol **3.22** were not possible.

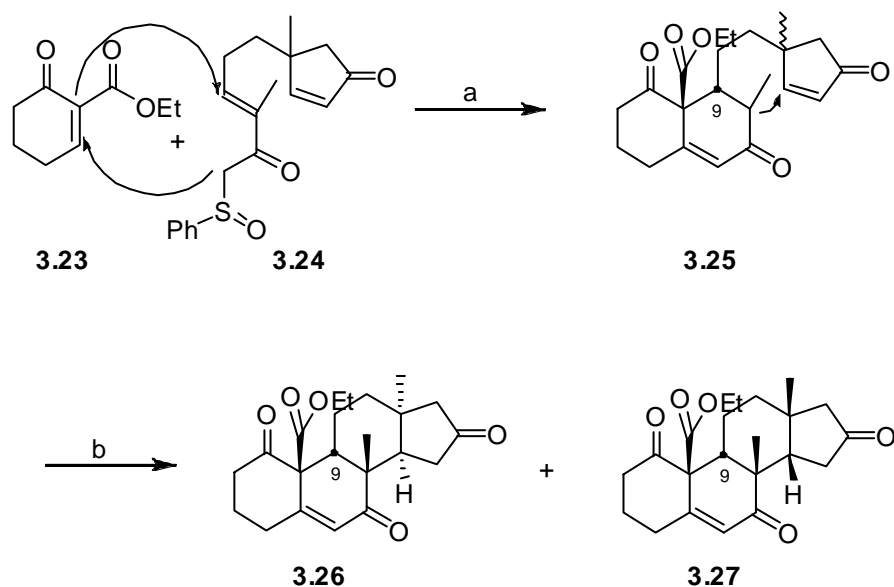
Although the yield of the aldol condensation reaction of **3.16** could not be improved, the studies indicated that the aldol condensation reaction was possible but sluggish compared to the Michael addition reaction. To force the aldol condensation reaction to occur the competing electrophilic position at C12 needed to be protected or eliminated. If this is done, a method for reforming the C12-C13 double bond would be necessary.

### 3.3 Convergent approach: double Michael addition

From a review of recent literature, a new convergent synthetic approach to synthesize the D and E rings was envisioned. Deslongchamps et al. reported a cascade polycyclization<sup>36-39</sup> approach, which they used to synthesize tetracyclic compounds **3.26** and **3.27**.<sup>36</sup> Sulfoxide **3.24**

and activated cyclohexenone **3.23** were treated with cesium carbonate in methylene chloride, which resulted in a double Michael addition reaction and during their purification the sulfoxide was eliminated to produce enone **3.25**. After refluxing **3.25** with p-toluenesulfonic acid in benzene, another Michael addition reaction was accomplished from which they obtained tetracyclic enones **3.26** and **3.27**<sup>36</sup> (Scheme 3.14).

**Scheme 3.14: Cascade polycyclization reaction**<sup>36</sup>

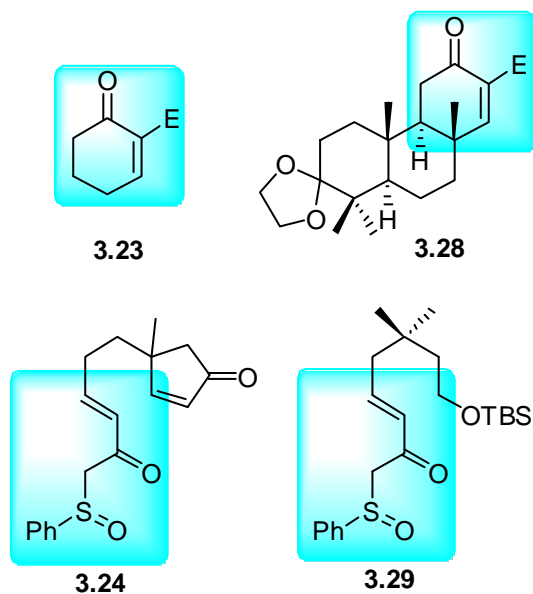


a.) Cs<sub>2</sub>CO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>; b.) p-TsOH, benzene, reflux, 13% of **3.26** and 13% of **3.27**.

The initial double Michael addition and subsequent elimination of sulfoxide to produce **3.25** was envisioned as a method that could be used for the formation of the D ring. If an appropriate side chain was designed the E ring could also be formed. There are two important results that were key to our synthesis: 1.) Deslongchamps et al.<sup>36</sup> discovered that by using  $\beta$ -ketosulfoxide **3.24** they obtained a configuration at C9 in which the hydrogen was cis to the ester as in enone **3.25**. This configuration is key for our synthesis. 2.) The elimination of the sulfoxide produced an  $\alpha,\beta$ -unsaturated ketone. As mentioned before the  $\beta$ -position could be functionalized using a Michael addition with an appropriate nucleophile that could be converted to the required hydroxymethyl group.

To use this method for our synthesis, two compounds were needed. For the equivalent of **3.23**, the C ring of our compound needed to be converted to an activated cyclohexenone like

compound **3.28**. To construct the D and E rings an equivalent of sulfoxide **3.24** needed to be synthesized with an appropriate side chain that could be used to construct the E ring like compound **3.29** (Figure 3.2).

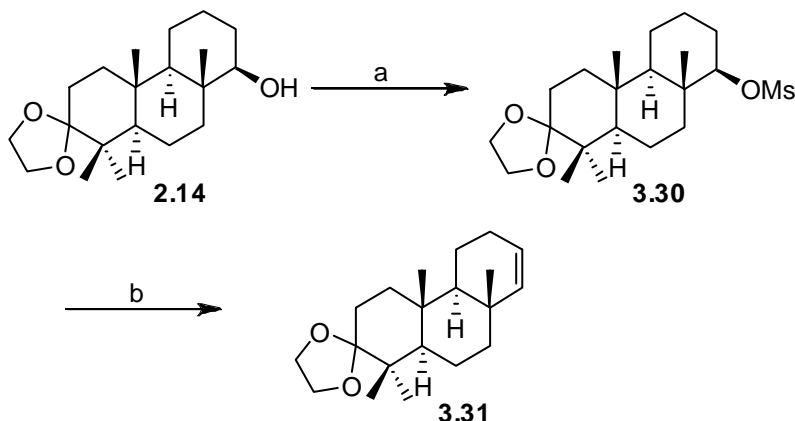


E = CHO(**3.28a**), CO<sub>2</sub>Et(**3.28b**)

**Figure 3.2: Analogues for the double Michael addition reaction**

Synthesis of **3.28** was started from the previously synthesized ABC ring alcohol **2.14**. Treatment of alcohol **2.14** with triethylamine and methanesulfonyl chloride in methylene chloride produced quantitative yield of mesylate **3.30**. Initially, elimination of the mesylate was attempted using DBU as the base, which resulted in no reaction. Using a method employed by Rivera et al.<sup>40</sup> to eliminate a tosylate, mesylate **3.30** was treated with lithium bromide and lithium carbonate in refluxing DMF to produce alkene **3.31** in high yield (Scheme 3.15).

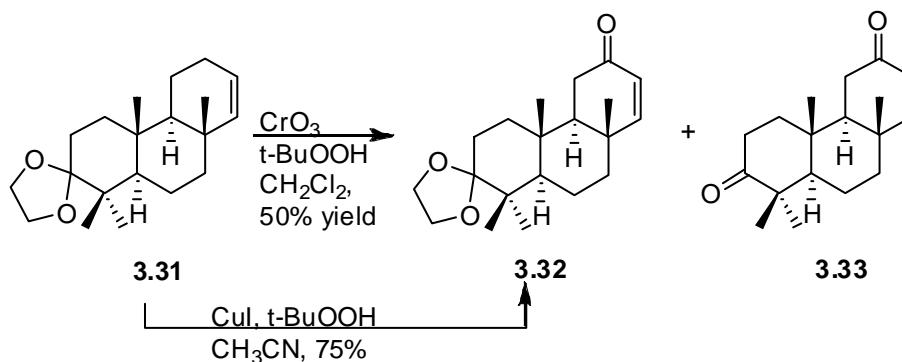
### Scheme 3.15: Synthesis of tricyclic alkene 3.31



a.)  $\text{Et}_3\text{N}$ ,  $\text{MsCl}$ ,  $\text{CH}_2\text{Cl}_2$  100% yield. b.)  $\text{LiBr}$ ,  $\text{Li}_2\text{CO}_3$ ,  $\text{DMF}$  reflux 2 h, 76% yield.

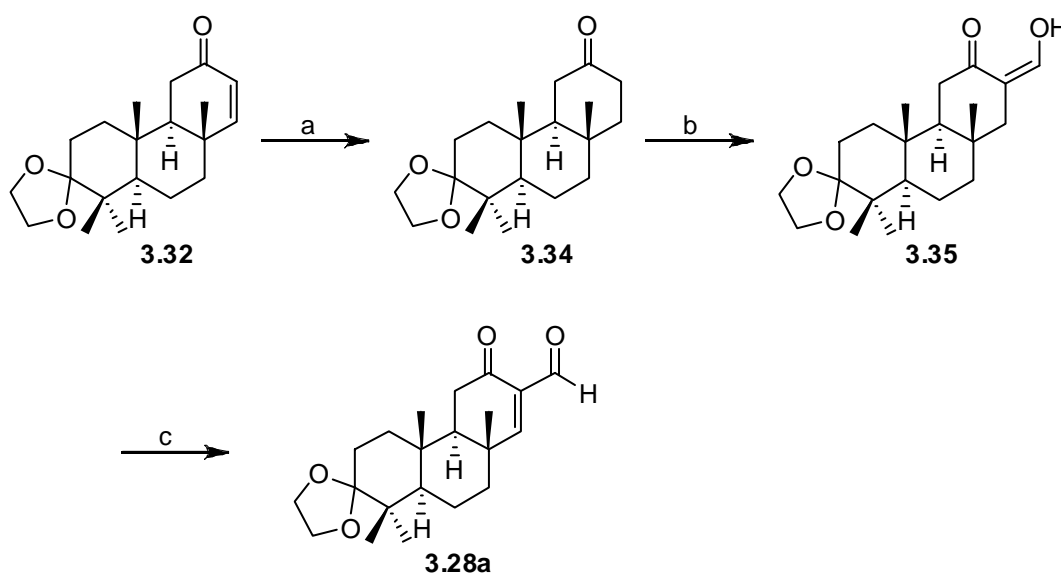
Alkene **3.31** has only one allylic position so the allylic oxidation was expected to occur without any problems. Gribble et al.<sup>16,17</sup> used chromium trioxide and *t*-butylhydrogenperoxide in methylene chloride to accomplish an allylic oxidation to produce a tricyclic enone similar to **3.32**. Applying this procedure to alkene **3.31** produced the desired enone **3.32** but, because the conditions produce chromic acid this also catalyzed the removal of the ketal protecting group. A mixture of **3.32** and **3.33** in less than 50% yield along with other unidentified side products were obtained. These results prompted the investigation of other allylic oxidation procedures from which a mild reaction condition was discovered. Auhmani et al.<sup>41</sup> and Ho et al.<sup>42</sup> produced  $\alpha,\beta$ -unsaturated ketones by allylic oxidation in which they employed *t*-butylhydrogenperoxide, and catalytic copper iodide in acetonitrile at 50°C. When alkene **3.31** was treated with these conditions, enone **3.32** was produced in 75% yield as the only product (Scheme 3.16).

### Scheme 3.16: Allylic oxidation to form tricyclic enone 3.32



It was decided that the proposed double Michael addition reaction would be investigated first using the activated cyclohexenone as the  $\beta$ -ketoaldehyde **3.28a**. To obtain this the unsaturation on **3.32** was reduced by treatment with catalytic amount of palladium on carbon, in ethanol, and under 1 atmosphere of hydrogen gas to produce saturated ketone **3.34**. Using the reaction series employed by Meyer et al.<sup>43</sup> and Grieco et al.<sup>44</sup>, ketone **3.34** was treated with sodium hydride in benzene to produce its kinetic enolate, which was reacted with ethyl formate to produce  $\alpha$ -hydroxymethylene ketone **3.35**. This was then oxidized by treatment with DDQ and acetic acid in dioxane to produce the  $\beta$ -ketoaldehyde **3.28a** (Scheme 3.17).

**Scheme 3.17: Synthesis of  $\beta$ -ketoaldehyde 3.28a**



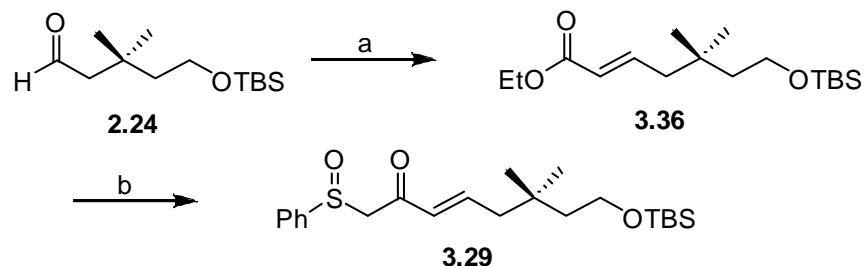
a.) 10% Pd-C (cat.), EtOH, 1atm H<sub>2</sub>, 59% yield; b.) NaH, benzene, ethylformate; c.) DDQ, AcOH, dioxane, 67% yield, 2 steps.

After the synthesis of the activated ketone **3.28a**, the synthesis of the  $\beta$ -ketosulfoxide **3.29** needed to be accomplished before the double Michael addition reaction could be attempted. The synthesis of  $\beta$ -ketosulfoxide **3.29** was initiated from the previously synthesized aldehyde **2.24**. A Wittig reaction, using aldehyde **2.24** and (carbethoxymethylene)triphenylphosphorane in methylene chloride produced the  $\alpha,\beta$ -unsaturated ethyl ester **3.36** in high yield (81% yield).<sup>36</sup> Methylphenylsulfoxide was treated with LDA in THF to form its methyl carbanion, which was



reacted with ethyl ester **3.36** to produce the desired  $\beta$ -ketosulfoxide **3.29** in 62% yield<sup>45</sup> (Scheme 3.18).

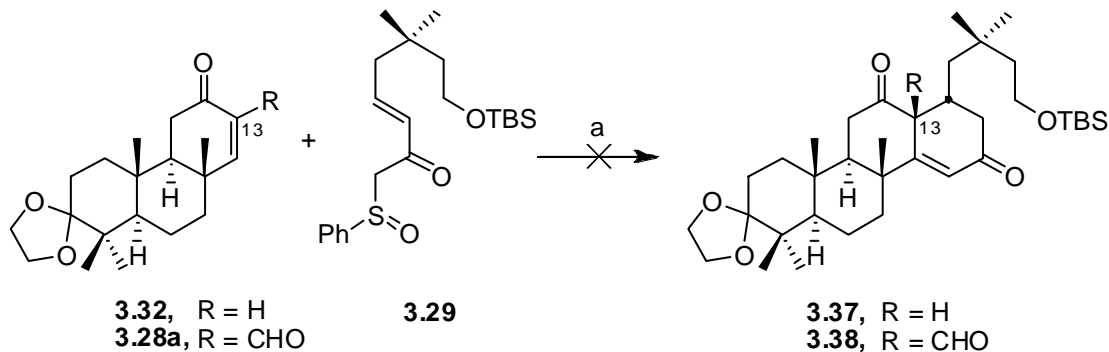
**Scheme 3.18: Synthesis of  $\beta$ -ketosulfoxide 3.29**



a.)  $\text{P}(\text{Ph})_3\text{CHCO}_2\text{Et}$ ,  $\text{CH}_2\text{Cl}_2$ , reflux, 81% yield. b.) LDA,  $\text{CH}_3\text{-S(O)-Ph}$ , THF, 62% yield.

Since the aldehyde at C13 was not necessary for our synthesis, the double Michael addition reaction was first attempted with the unactivated  $\alpha,\beta$ -unsaturated ketone **3.32**. When enone **3.32** and sulfoxide **3.29** were treated with cesium carbonate in methylene chloride no reaction occurred. This indicated that the activated  $\alpha,\beta$ -unsaturated ketone **3.28a** was necessary for the reaction to occur. However, when enone **3.28a** and sulfoxide **3.29** were treated under the similar reaction conditions, only sulfoxide **3.29** was recovered indicating that the enone **3.28a** may have decomposed (Scheme 3.19).

**Scheme 3.19: Attempt of the double Michael addition reaction for the formation of the D ring**



a.)  $\text{Cs}_2\text{CO}_3$ ,  $\text{CH}_2\text{Cl}_2$ .

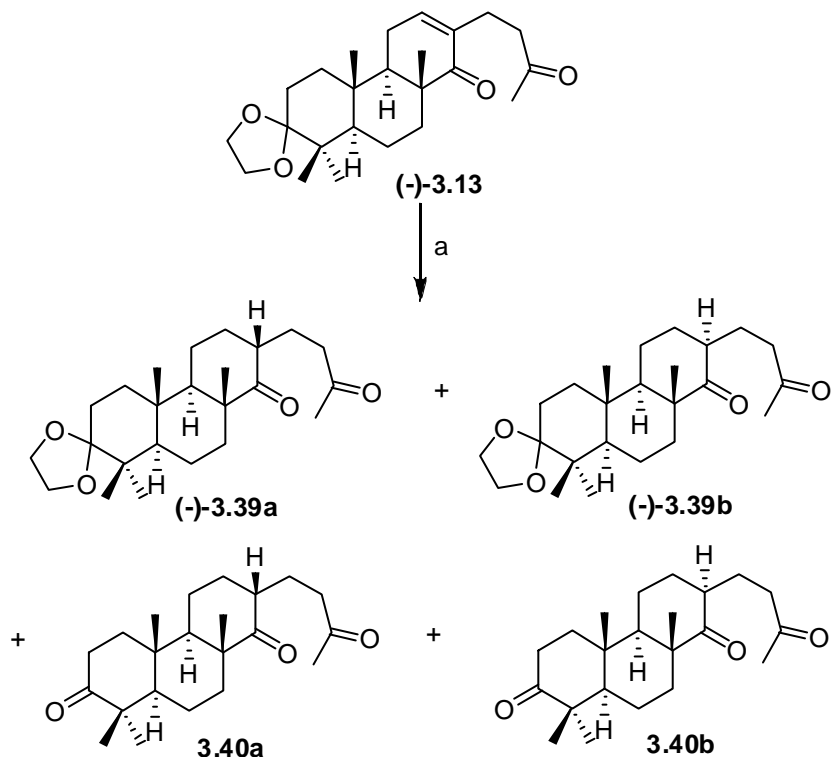
Perhaps changing the electron-withdrawing group at C13 to an ester as in **3.28b**, proposed earlier, may prevent decomposition and result in the double Michael addition reaction.

Because of the lack of material and the prospect of a more feasible alternative route, we did not pursue this approach further. Instead the investigations were focused on another linear approach.

### 3.4 Synthesis of the D ring: aldol condensation of diketone

In section 3.1 it was discovered that the intramolecular aldol condensation reaction cannot occur if the enolate group at C13 was in the axial position, in compound (-)-**3.3a**. For its diastereomer (-)-**3.3b** with the opposite configuration at C13, the condensation reaction occurred, but the phenyl sulfide was eliminated resulting in aromatization of the D ring. In section 3.2, the C13 stereocenter was destroyed by converting it to a double bond between C12-C13, compound (-)-**3.13**. This also formed a competing reaction position at C12, which turned out to be more favorable. To overcome the problems encountered in section 3.1 and 3.2, it was decided that the double bond between C12-C13 would be eliminated completely so only the aldol condensation reaction was possible.

**Scheme 3.20: H<sub>2</sub>/Pd-C reduction of enone (-)-3.13**

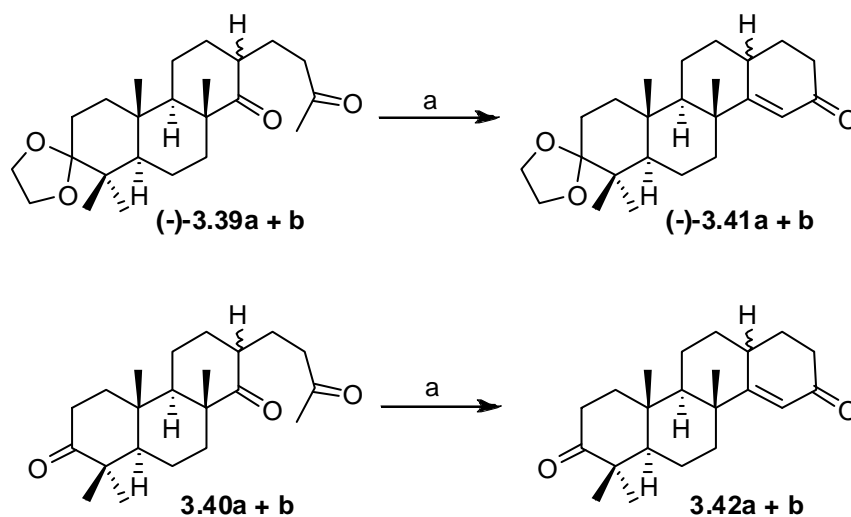


a.) Pd-C, EtOH, 1 atm H<sub>2</sub>, 60% yield **3.39a + b** and 25% yield **3.40a + b**.

The C12-C13 double bond in diketone enone (-)-**3.13** was reduced by treatment with a catalytic amount of palladium on activated carbon, in ethanol, and under one atmosphere of hydrogen gas to produce a mixture of four compounds. These were easily separated by column chromatography to give; diastereomers **3.39a** and **3.39b** and their deprotected analogs **3.40a** and **3.40b**, respectively (Scheme 3.20).

The diastereomeric mixture of **3.39a** and **3.39b** was treated with potassium carbonate in refluxing ethanol to produce the intramolecular aldol condensation adducts as a diastereomeric mixture of **3.41a** and **3.41b**. Similarly the mixture of **3.40a** and **3.40b** was also treated under the same conditions to produce the diastereomeric mixture of **3.42a** and **3.42b** (Scheme 3.21).

**Scheme 3.21: Intramolecular aldol condensation reaction, using potassium carbonate in ethanol**

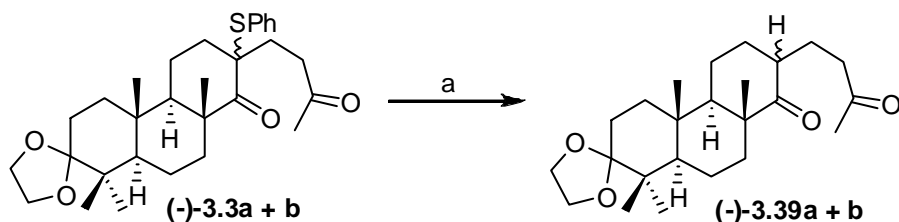


a.)  $K_2CO_3$ , EtOH, reflux, 100 % yield.

Although reduction of diketoenone (-)-**3.13** produced the desired products **3.39a** and **3.39b**, it also produced the unwanted deprotected diastereomers **3.40a** and **3.40b**. To avoid this complicated mixture, a more efficient route to produce the desired products **3.39a** and **3.39b** was necessary. Treatment of the mixture of phenyl sulfide diastereomers (-)-**3.3a** and (-)-**3.3b** with catalytic amount of AIBN and tributyltin hydride in refluxing toluene<sup>46</sup>, proved to be a very

clean and efficient method to produce **3.39a** and **3.39b** directly and in high yield (Scheme 3.22). It made the synthesis of **3.39a** and **3.39b** one step shorter and increased their overall yield.

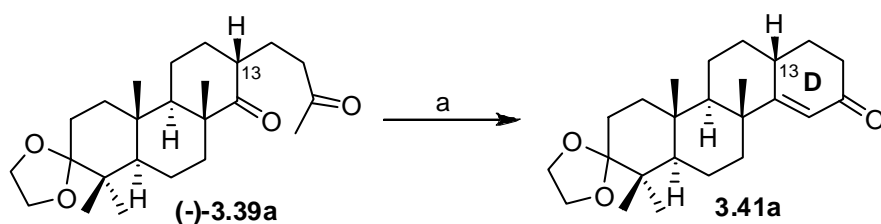
**Scheme 3.22: Radical elimination of phenyl sulfide group to produce diketones 3.39**



a.) AIBN (cat.), (n-Bu)<sub>3</sub>SnH, Toluene, reflux, 89% yield, 1:1 ratio of **3.39a**:**3.39b**

Treating the diastereomers (-)-**3.39a** and (-)-**3.39b** separately with sodium methoxide in methanol at room temperature, followed by refluxing, showed that diastereomer (-)-**3.39a** was converted cleanly to its corresponding cyclization adduct **3.41a** (Scheme 3.23).

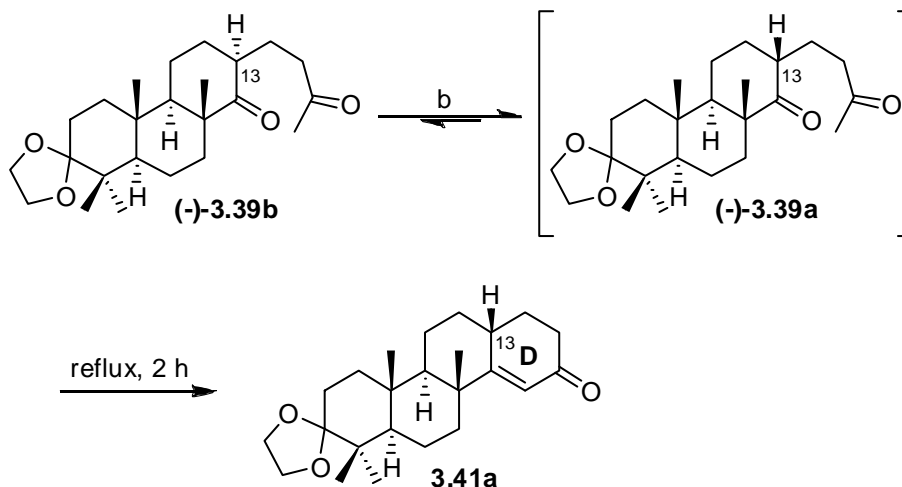
**Scheme 3.23: Intramolecular aldol condensation using sodium methoxide**



a.) NaOMe, MeOH rt. 2 h, reflux 2 h, 92% yield.

On the other hand (-)-**3.39b** produced a mixture of **3.41a** and **3.41b**. This indicated that the C13 hydrogen of (-)-**3.39b** was partially epimerized producing its diastereomer (-)-**3.39a** and consequently **3.41a**. However, it was discovered that by treating (-)-**3.39b** with excess sodium methoxide for three days followed by refluxing for two hours, the reaction outcome was controlled to produce exclusively **3.41a** as the product. This can be rationalized by an equilibration of diastereomer (-)-**3.39b** to (-)-**3.39a** under high concentration of sodium methoxide and consequent aldol condensation of (-)-**3.39a** to form its corresponding annulation adduct **3.41a** (Scheme 3.24).

**Scheme 3.24: Epimerization of C13 hydrogen and formation of 3.41a as the only diastereomer**



b.) NaOMe, MeOH rt. 3 days, reflux 2 h, 81% yield.

Compound **3.41a** now has the ABCD rings of (+)-myriceric acid A, however the D ring needs to be functionalized. The functionalization will be discussed in the following chapter.

### 3.5 Conclusion

Many approaches were investigated to construct the D ring of (+)-myriceric acid A from which it was discovered that the axial methyl group at C8 plays a significant role in the reactivity of the C14 position. In the first approach, the axial methyl group blocked the enolate, which was on the same face in compound (-)-3.3a, preventing the aldol condensation reaction and a retro Michael addition occurred instead. Cyclization did occur with its diastereomer (-)-3.3b in which the enolate group was trans and in the equatorial position, but the C13 phenyl sulfide was eliminated resulting in aromatization of the D ring. In the second approach the sulfide was oxidized and eliminated to form a C12-C13 double bond in compound (-)-3.13. This destroyed the C13 stereocenter and flattened the position allowing the enolate to come from the opposite face of the axial methyl group. It also formed a less hindered electrophilic position at C12, which was more favorable and resulted in the undesired Michael addition adduct as the major product. The third approach was a convergent approach, which was not investigated thoroughly

due to the lack of material and discovery of the fourth approach. Finally in the fourth approach the C12-C13 double bond was removed. This eliminated the possibility of the competing Michael addition reaction and resulted in the synthesis of the D ring compound **3.41a** of (+)-myriceric acid A.

Functionalization of the D ring and construction of the E ring will be discussed in the following chapter.

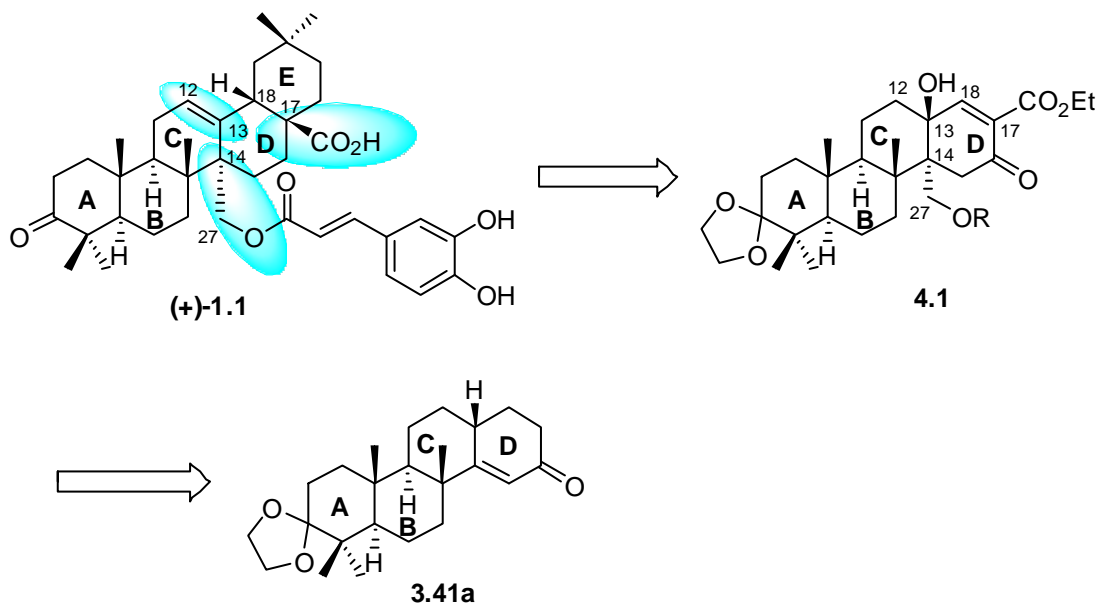
## CHAPTER 4 - Functionalization of the D ring of (+)-myriceric acid

### A

#### 4.1 Retro Synthesis

In chapter 2 the ABC rings with all the stereocenters of (+)-myriceric acid A [(+)-1.1] were constructed. In chapter 3, after many approaches, the construction of the D ring was accomplished as tetracyclic enone **3.41a**. The D ring of (+)-myriceric acid A has three key functional groups: 1) the C12-C13 double bond, 2) the angular hydroxy methyl group at C14 to which the caffeic ester side chain is attached, and 3) the carboxylic group on C17.

**Scheme 4.1: Retrosynthesis of (+)-myriceric acid A from the ABCD compound 3.41a**



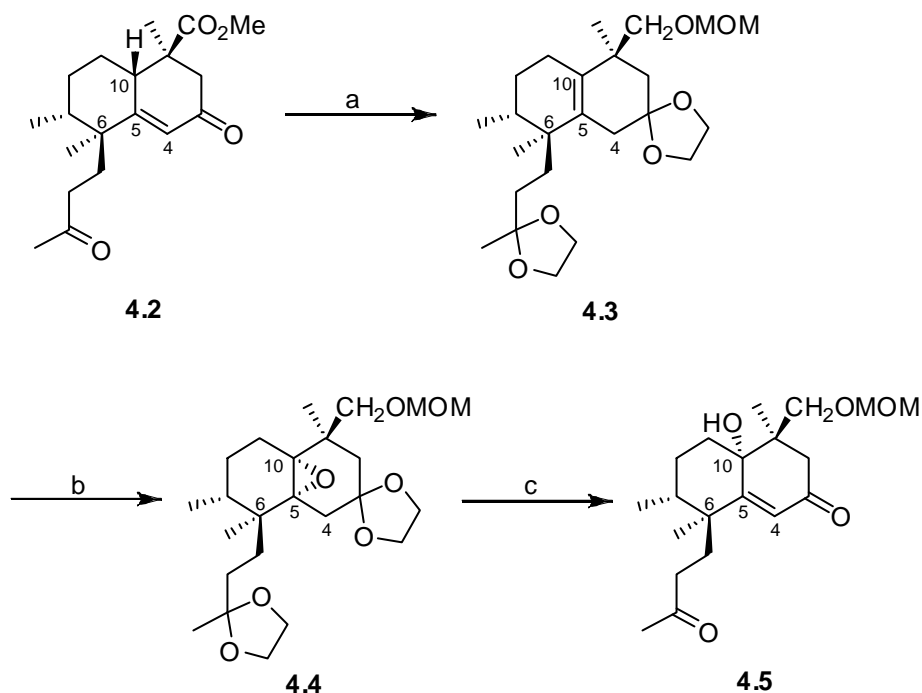
The ABCD ring compound **3.41a** has none of the functional groups required in the D ring of (+)-myriceric acid A. To accomplish the synthesis of (+)-1.1 from **3.41a**, it was envisioned that **3.41a** had to first be converted to **4.1** which has the D ring fully functionalized. In compound **4.1**, the hydroxy group at C13 would allow for the formation of C12-C13 double bond, the hydroxy methyl group at C14 would allow for the addition of the caffeic ester group,

the ethyl ester group at C17 could be hydrolyzed to the carboxylic acid group, and the C17-C18 double bond would be used to construct the E ring. This chapter will discuss the approaches investigated for the synthesis of **4.1**.

## 4.2 Radical cyclization approach 1

The challenge of using the ABCD ring compound **3.41a**, to continue the synthesis of (+)-myriceric acid A, was the formation of the C12-C13 double bond. Our first task was to figure out a way to form this double bond. In their synthesis of (-)-chettaphanin I and II, Marcos et al.<sup>47,48</sup>, used a reaction series that could be useful for the formation of the C12-C13 double bond in enone **3.41a**. By ketalizing the ketones in enone **4.2**, they moved the C4-C5 double bond to the C5-C10 position forming compound **4.3**. Epoxidation of the double bond in **4.3** followed by removal of the ketal protecting groups in **4.4** produced the  $\gamma$ -hydroxy enone **4.5**.

**Scheme 4.2: Marcos's synthesis of  $\gamma$ -hydroxy enone **4.5**<sup>47,48</sup>**



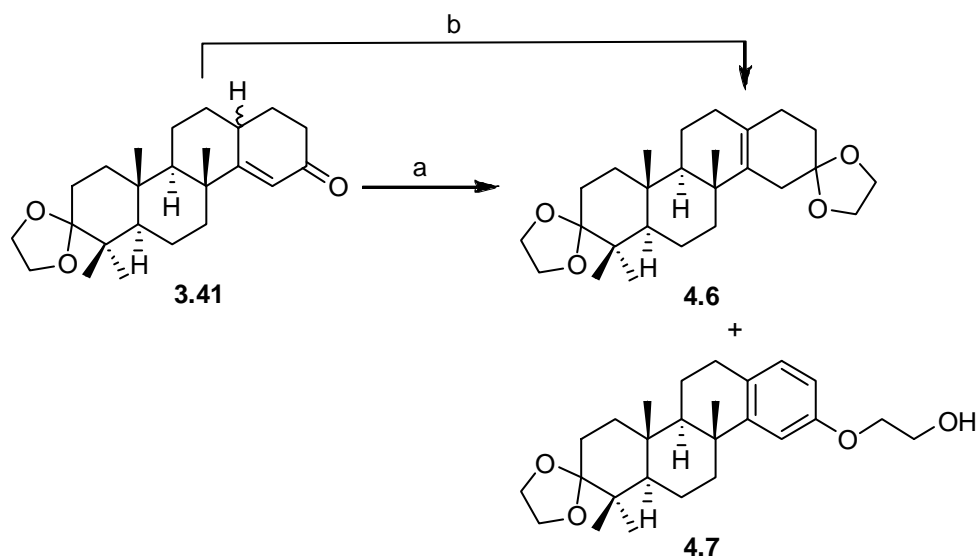
a.) 1.) Ethylene glycol, *p*-TsOH, benzene, 8 h; 2.) LAH, Et<sub>2</sub>O, 1.5 h; 3.) DMM, LiBr, *p*-TsOH, 12 h, rt. b.) *m*-CPBA, CH<sub>2</sub>Cl<sub>2</sub>, 30 min, rt. c.) *p*-TsOH, acetone, 2 h, rt.



The key results from this reaction series were the formation of the  $\gamma$ -hydroxy group which, in our case, could be eliminated to form the C12-C13 double bond, and the preservation of the  $\alpha,\beta$ -unsaturated ketone, which could be used for functionalization of the C14 position.

Following Marcos et al.'s procedure<sup>47,48</sup>, **3.41a** was treated with catalytic *p*-toluenesulfonic acid (PTSA) and excess ethylene glycol in refluxing toluene under azeotropic distillation. This produced two compounds; the epimerized double bond diketal compound **4.6** and the undesired aromatic compound **4.7**. It was discovered that by replacing *p*-toluenesulfonic acid with pyridinium *p*-toluenesulfonic acid (PPTS), the reaction produced the desired diketal compound **4.6** as the only product.

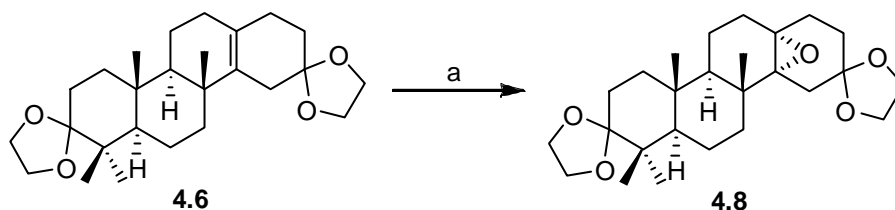
#### Scheme 4.3: Synthesis of diketal alkene compound **4.6**



a.) *p*-TsOH, ethylene glycol, toluene, 2 h, 41% yield of **4.6** and 12% yield of **4.7**. b.) PPTS, ethylene glycol, toluene, 2 h, 90% yield of **4.6**.

Epoxidation of the double bond in **4.6** was accomplished by treatment with mCPBA in methylene chloride to produce epoxide **4.8**. Based on the steric effect of the axial methyl group on C8, it was postulated that the epoxidation occurred from the opposite face producing epoxide compound **4.8** with the epoxide on the bottom face.

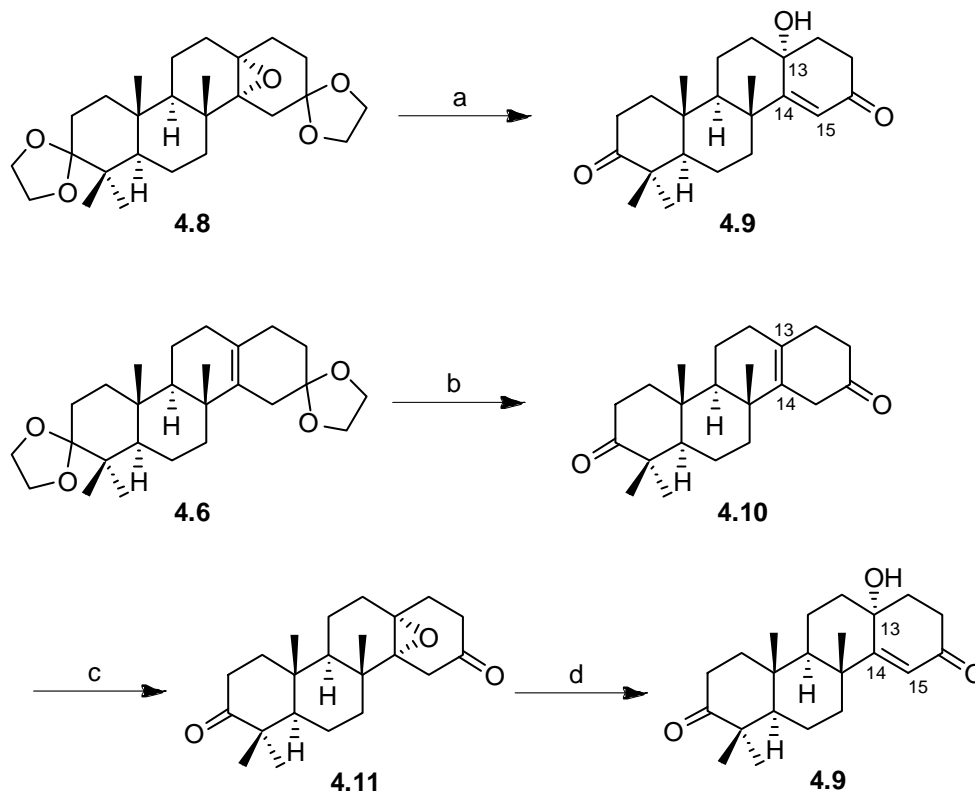
#### Scheme 4.4: Epoxidation of compound 4.6 to form epoxide compound 4.8



a.) *m*-CPBA, CH<sub>2</sub>Cl<sub>2</sub>, rt, 100% yield.

Treatment of diketal epoxide **4.8** with PTSA in acetone produced  $\gamma$ -hydroxy enone **4.9** along with a mixture of other compounds. To avoid the complex mixture, another route to **4.9** was developed. Treatment of **4.6** with 1:1 THF:1M HCl at 40°C produced dione **4.10**. Dione **4.10** was then treated with *m*CPBA in methylene chloride to form dione epoxide **4.11**, which was treated with potassium carbonate to produce the  $\gamma$ -hydroxy enone **4.9** cleanly.

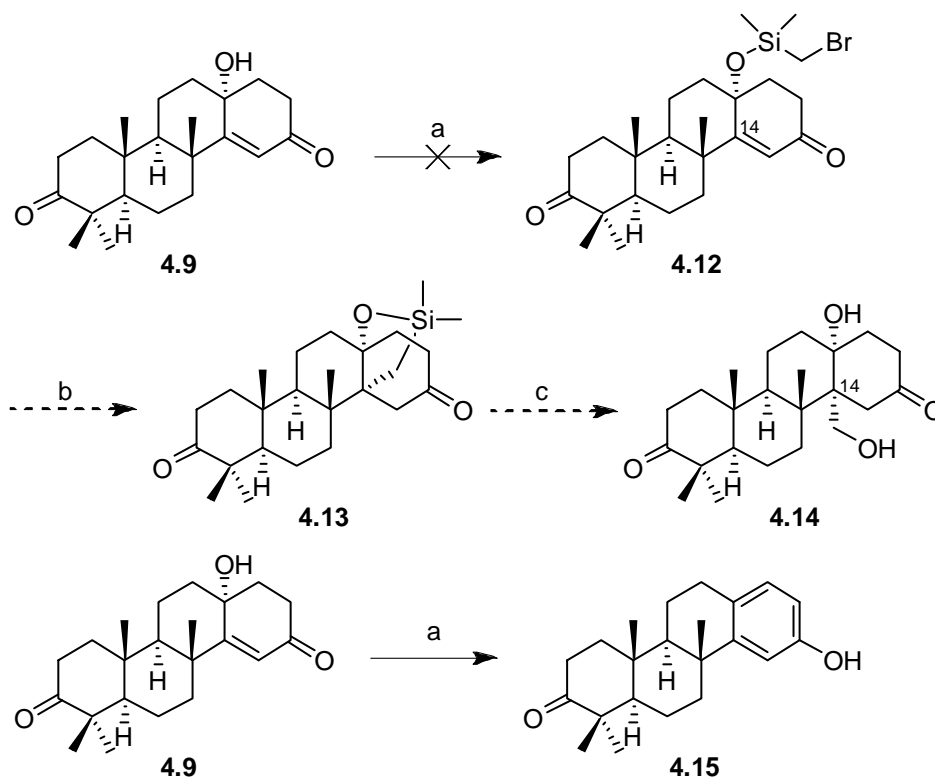
#### Scheme 4.5: Synthesis of $\gamma$ -hydroxy enone 4.9



a.) PTSA, acetone, 44% yield of **4.9**. b.) 1:1 THF: 1 M HCl, 40°C, 74% yield; c.) *m*CPBA, CH<sub>2</sub>Cl<sub>2</sub>, rt, 72% yield. d.) K<sub>2</sub>CO<sub>3</sub>, ETOH, rt, 100% yield.

Formation of the hydroxy group at C13 in compound **4.9** had two purposes: 1.) it could now be used for the formation of the C12-C13 double bond, and 2.) because it is on the bottom face, it could be used as an anchor to direct the functionalization of the C14 carbon. By using Lallemand et al.'s reaction sequence<sup>35</sup>, described in the previous chapter, the hydroxy group could be converted to the bromomethyl dimethylsilyl ether **4.12**. Since the silyl ether would be on the bottom face, the Stork-silyl-methyl radical cyclization would occur forming the cyclic silyl ether on the bottom face, as in compound **4.13**. Fleming-Tamao oxidation, using hydrogen peroxide and sodium bicarbonate in a mixture of refluxing methanol and THF, would produce compound **4.14** having the C13 hydroxy group, the desired hydroxymethyl group at C14, and the desired configuration at C14.

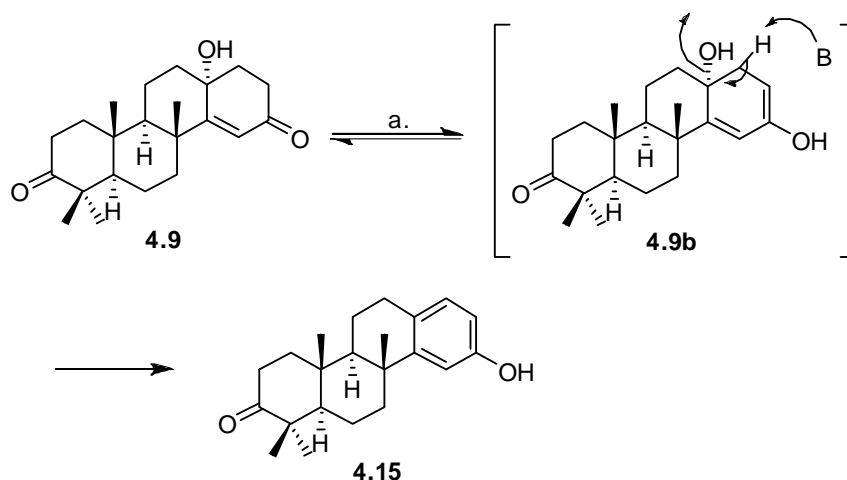
**Scheme 4.6: Proposed radical cyclization approach for the formation of the C14 hydroxymethyl group**



a.) Imidazole, DMAP, DMF,  $\text{BrCH}_2(\text{CH}_3)_2\text{SiCl}$  or  $\text{NaH}$ , THF,  $\text{BrCH}_2(\text{CH}_3)_2\text{SiCl}$ . b.) cat. AIBN,  $\text{Bu}_3\text{SnH}$ , Toluene, reflux. c.) 30%  $\text{H}_2\text{O}_2$ ,  $\text{NaHCO}_3$ ,  $\text{MeOH/THF}$  (1/1), reflux.

Treatment of **4.9** using two different conditions: imidazole, DMAP, bromomethyldimethylsilylchloride in DMF, or; sodium hydride, bromomethyldimethyl silylchloride, in THF; did not result in the silyl ether **4.12**. Instead, they formed the phenol compound **4.15**. Formation of the silyl ether did not occur because the alcohol is tertiary and very hindered. The aromatization possibly resulted from first, the enolization of the ketone to transition state **4.9b**, followed by elimination of the C13 hydroxy group catalyzed by basic conditions.

**Scheme 4.7: Proposed mechanism for aromatization of compound 4.9 to phenol 4.15**



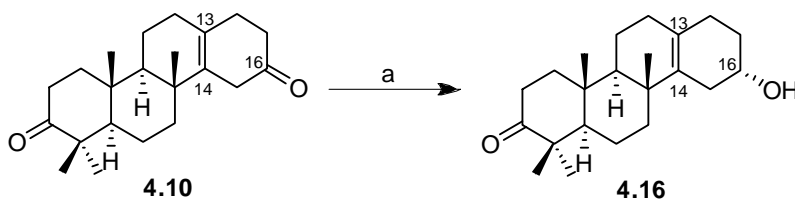
a.) Imidazole, DMAP, DMF,  $\text{BrCH}_2(\text{CH}_3)_2\text{SiCl}$  or  $\text{NaH}$ , THF,  $\text{BrCH}_2(\text{CH}_3)_2\text{SiCl}$ .

Because of the low reactivity of the tertiary alcohol at C13 and the aromatization of **4.9** under strong basic conditions, the approach needed to be modified. For the radical cyclization to be accomplished, two requirements were needed: 1.) the silyl ether had to be formed and 2.) a double bond must be present, at the C14 position, for reaction with the radical. It was decided that dione **4.10** would be used to fulfill these requirements. It had a double bond between C13-C14 and the ketone at C16 could be reduced to a secondary alcohol, which would serve as the anchor for the radical cyclization reaction.

Compound **4.10** has two ketones, so a regio- and stereo selective reduction of the ketone on C16 was needed. The regio-selective reduction could be accomplished by using a bulky hydride donor because the C16 ketone is the less hindered of the two ketones. Similarly, the “steric approach control”<sup>49</sup> would result in the bulky hydride donor to approach the ketone on its

equatorial face and consequently produce the desired axial alcohol, as in compound **4.16**. As expected, treatment of dione **4.10** with the bulky hydride, K-selectride, regioselectively reduced the alcohol at C16 and only one diastereomer was formed. This diastereomer was presumed to be the axial alcohol **4.16**, based on the “steric approach control”<sup>49</sup>.

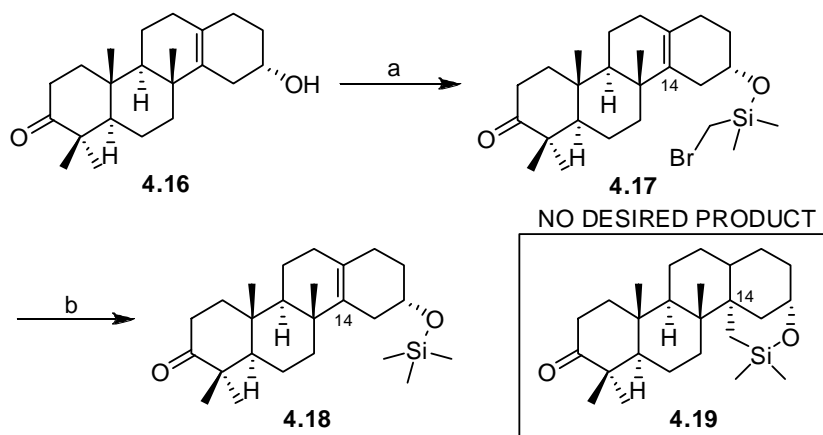
**Scheme 4.8: Regio- and stereo-selective reduction of the C16 ketone in 4.10 to produce alcohol 4.16**



a.) K-selectride, THF, -78°C, 91% yield.

Secondary alcohol **4.16** was treated with imidazole, DMAP, bromomethyldimethyl silylchloride in methylene chloride at room temperature to produce quantitative yield of silyl ether **4.17**. This was immediately purified by column chromatography in which the silica was neutralized by washing with 1 % triethylamine. The product was used immediately for the next reaction. Treatment of **4.17** with catalytic AIBN, tributyltin hydride in refluxing benzene produced one single product. After isolation, it was determined that, the product was not the desired cyclic silyl ether **4.19**, instead quantitative yield of the trimethylsilylether **4.18** was produced.

**Scheme 4.9: Failed Stork-silyl-methyl radical cyclization reaction**



a.) Imidazole, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, BrCH<sub>2</sub>(CH<sub>3</sub>)<sub>2</sub>SiCl, 60% yield. b.) cat. AIBN, Bu<sub>3</sub>SnH, benzene, reflux, 50% yield.

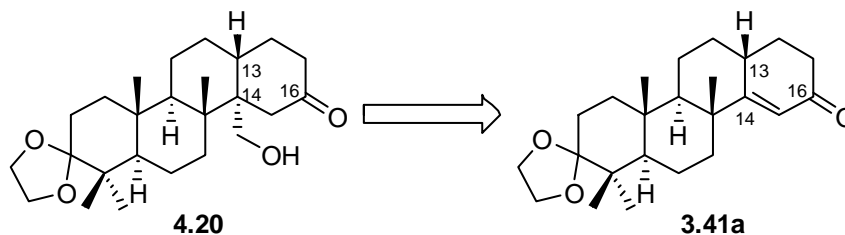
Failure of the radical cyclization in **4.17** may be rationalized based on the low reactivity of the C13-C14 double bond. The degree of substitution at the C14 is high compared to Lallemand et al.'s compound<sup>35</sup>. Additionally, the quaternary center adjacent to C14 adds steric hindrance that reduces the reactivity of this position. The C14 position could possibly be activated if it were the  $\beta$ -position of an  $\alpha,\beta$ -unsaturated ketone.

From the investigation of this approach it was determined that the C12-C13 double bond may be formed by formation of a hydroxyl group at the C13 position. This however may cause aromatization so functionalization of the C14 position needs to be done before the formation of the C12-C13 double bond.

### 4.3 Michael addition of vinyl cuprate or anionic oxy-Cope rearrangement

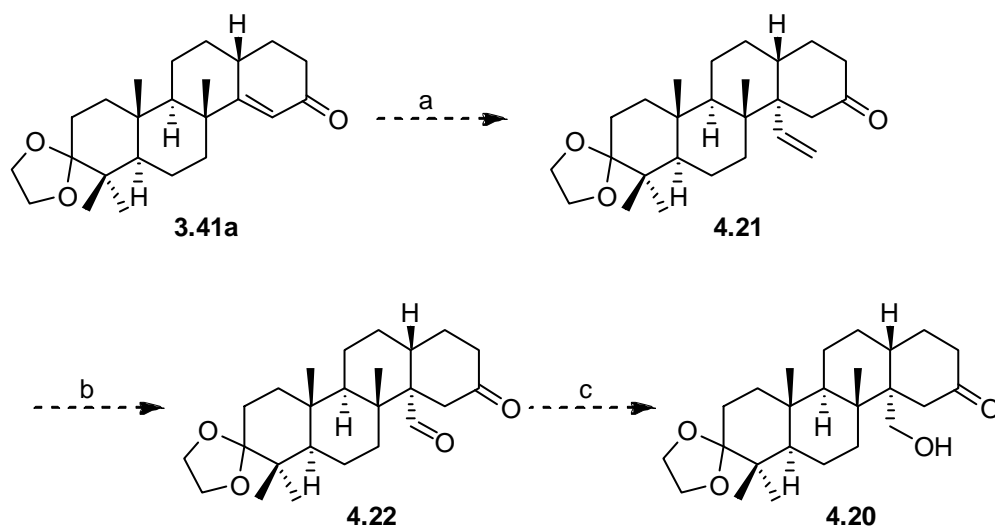
In the previous section it was determined that functionalization of the C14 position was necessary to prevent aromatization of the D ring. The functional group required at the C14 position is a hydroxymethyl group to which the caffeic ester side chain is attached in (+)-myriceric acid A. The initial ABCD ring compound **3.41a** is an  $\alpha,\beta$ -unsaturated ketone. This should allow for a conjugate addition at the C14 position. Unfortunately, no nucleophile exists that would form the angular hydroxymethyl functional group directly. To overcome this, a nucleophile that can be converted to the hydroxymethyl functionality needs to be used for the Michael addition reaction.

**Scheme 4.10: Proposed hydroxy methyl intermediate 4.20**



Vinyl Grignard reagents mixed with catalytic copper iodide have been used as Michael addition nucleophiles to  $\alpha,\beta$ -unsaturated ketones<sup>50-53</sup>. The axial methyl group at C8 should block the top face and control the Michael addition of the vinyl nucleophile to come from the bottom face to give **4.21**. Ozonolysis of the vinyl group<sup>50,51</sup> should produce an aldehyde **4.22**. This could then be reduced to the desired hydroxymethyl group at C14 in compound **4.20**.

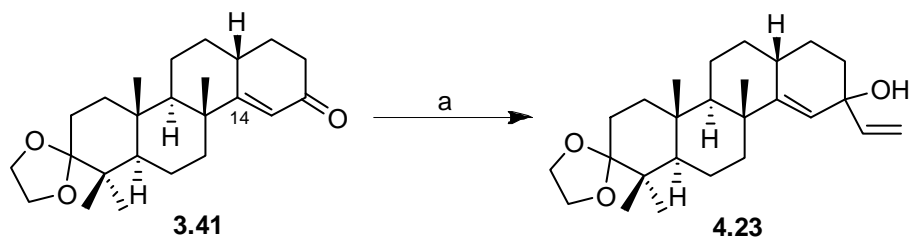
**Scheme 4.11: Proposed formation of the hydroxymethyl group by conjugate addition of a vinyl nucleophile**



a.) Michael addition of vinyl nucleophile b.) ozonolysis c.) hydride reduction.

$\alpha,\beta$ -Unsaturated ketone **3.41a** was treated with a mixture of vinylmagnesium bromide and catalytic copper iodide dimethylsulfide complex in THF at  $-78^\circ\text{C}$ . Consumption of the starting enone **3.41a** occurred very quickly to produce a new compound. After isolation it was determined that the new compound was not the desired  $\beta$ -vinylketone **4.21**; instead a 1,2-addition occurred producing compound **4.23**.

### Scheme 4.12: 1,2-addition reaction of vinyl cuprate

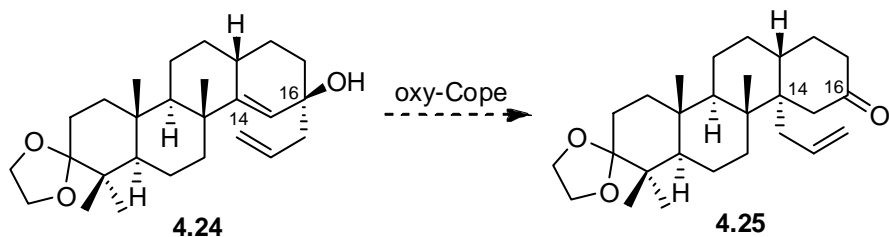


a.) vinylmagnesium bromide, cat. CuI.MeSMe, THF  $-78^{\circ}\text{C}$ .

Failure of the Michael addition may be caused by the high substitution of the C14 position and the steric hindrance of the adjacent quaternary center, which result in the preference for the competing 1,2-addition reaction, to produce **4.23**.

The preference for the 1,2-addition over the 1,4-addition resulted in the investigation of a different approach. In the new approach, an allyl Grignard would be used to accomplish a 1,2-addition to produce an adduct with the C16 configuration as in **4.24**. The 3-hydroxyl-1,5-diene **4.24** would be used for an oxy-Cope rearrangement reaction to stereoselectively functionalize the C14 position.

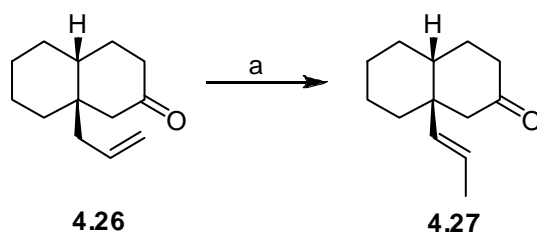
### Scheme 4.13: Proposed oxy-Cope rearrangement of compound 4.24



The oxy-Cope product **4.25** would have an allyl group at the C14 position. To convert this to the desired hydroxymethyl functionality, the double bond of the allyl group would be isomerized to an internal double bond. This would be accomplished by using the procedure employed by Sakurai et al<sup>56</sup>, as shown in the transformation of **4.26** to **4.27**. Ozonolysis followed by hydride reduction should result in the formation of the  $\beta$ -hydroxymethyl compound **4.20**.



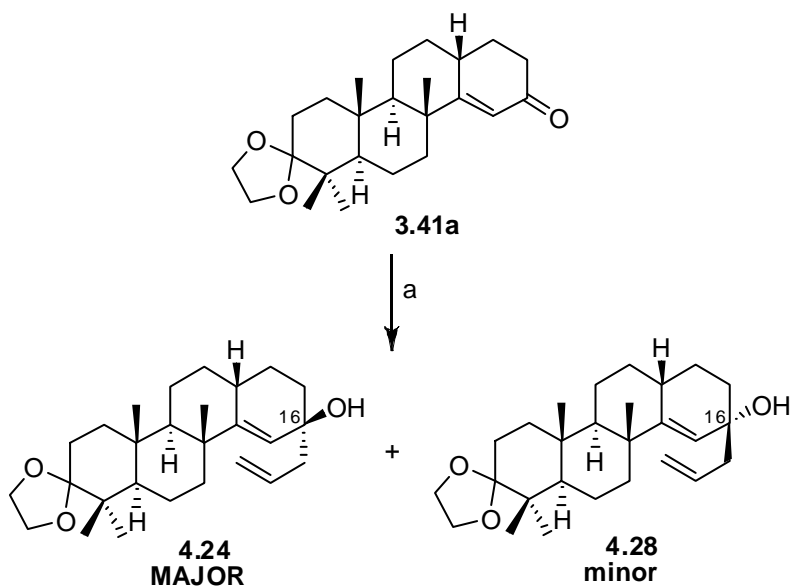
**Scheme 4.14: Isomerization of terminal double bond to an internal double bond<sup>56</sup>**



a.)  $(\text{PhCN})_2\text{PdCl}_2$ , benzene, reflux.

Burnell et al.<sup>54</sup> conducted a study, of 1,2-additions of allyl magnesium reagents to cyclohexenones, from which they determined that allyl magnesium reagents preferentially add axial to cyclohexenones. Treatment of  $\alpha,\beta$ -unsaturated ketone **3.41a** with allyl magnesium chloride at  $-78^\circ\text{C}$  produced two 1,2-addition products, diastereomers **4.24** and **4.28**. After isolation and  $^{13}\text{C}$  NMR analysis, it was determined that the major product was the desired diastereomer **4.24**. The stereo-chemistry of the carbinol centers were assigned based their chemical shifts in the  $^{13}\text{C}$  NMR spectra, in which the signal further downfield belongs to the axial-addition product<sup>54</sup>. The C16 chemical shift of **4.24** was more downfield relative to the C16 chemical shift in **4.28**, confirming our expectations of the reaction.

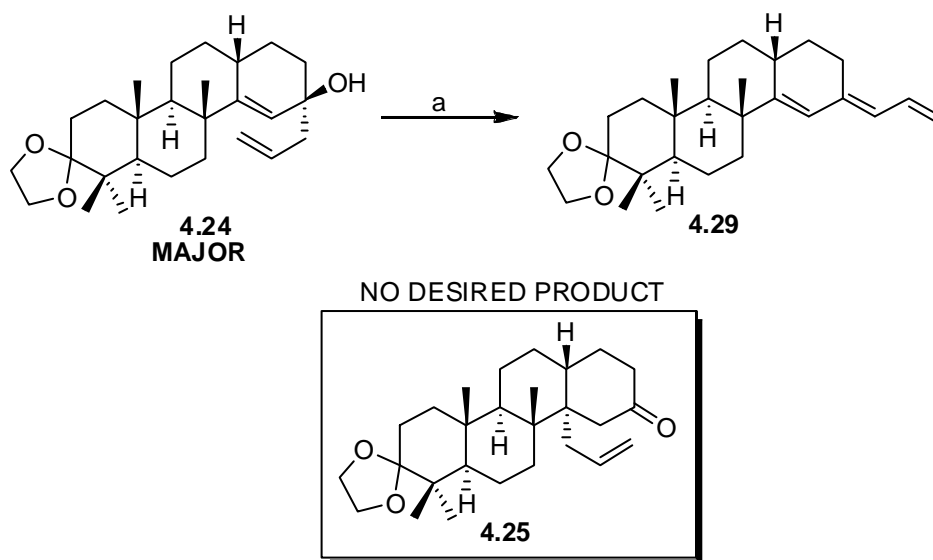
**Scheme 4.15: 1,2-addition of allyl magnesium chloride to enone 3.41a**



a.) allylmagnesiumchloride, THF,  $-78^\circ\text{C}$ , **4.24** (60 % yield), **4.28** (20 % yield)

Unexpectedly, attempts at a thermal oxy-Cope-rearrangement resulted in no product formation only recovery of the starting compound **4.24**. Formation of the anion of the hydroxyl group (anionic oxy-Cope) may result in the oxy-cope-rearrangement proceeding at lower temperatures<sup>49</sup>. As explained by Carey and Sunberg<sup>49</sup>, the best results are obtained when a large cation is used as the counter ion, the normal choice being the potassium cation.<sup>49</sup>

#### Scheme 4.16: Failed anionic oxy-Cope rearrangement



a.) KH, THF, rt – 50°C.

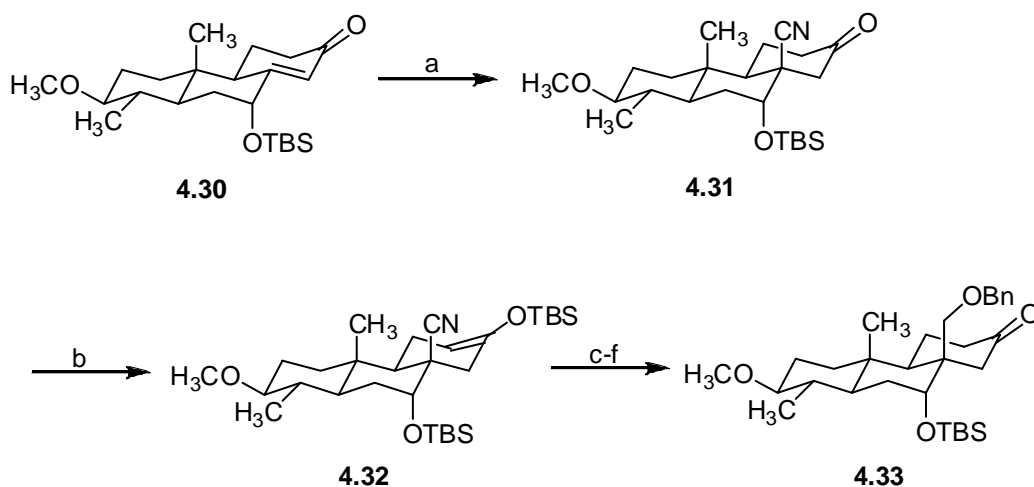
It was decided that potassium hydride was to be used as the base to deprotonate the hydroxyl group, resulting in the  $K^+$  as the counter ion. Initially, treatment of **4.24** with KH in THF at room temperature resulted in no product formation. The reaction temperature was then increased to 50°C, which resulted in complete consumption of the starting compound **4.24**. This produced an unexpected UV active product based on TLC. After isolation it was determined that the product was not the desired oxy-Cope-rearrangement product **4.25**, instead it was the elimination product **4.29**, which explained the UV activity on TLC.

Failure of the oxy-Cope-rearrangement may be because of the highly substituted C14 position and the rigidity of the tetracyclic compound preventing conformational change for favorable orbital overlap.

## 4.4 Conjugate addition of a nitrile group.

Fuchs et al.<sup>55</sup> demonstrated that an angular hydroxymethyl group can be accomplished from the conjugate addition of a nitrile group. They initially performed a Negata hydrocyanation reaction of the  $\alpha,\beta$ -unsaturated ketone **4.30** by treatment with diethylaluminumcyanide to produce the axial nitrile compound **4.31**. The ketone in **4.31** was protected as the *t*-butyldimethylsilyl (TBS) enol ether **4.32** by treatment with triethylamine and *t*-butyldimethylsilyl triflate. Treatment of the nitrile **4.32** with DIBAL-H in hexane with ether as the solvent and subsequent hydrolysis of the imine with buffered acetic acid resulted in the formation of an aldehyde. Further reduction with DIBAL-H produced the hydroxymethyl compound that was protected as its benzyl ether with benzyl bromide. Treatment of the enolsilyl ether with hydrochloric acid produced the benzyl protected hydroxymethyl compound **4.33**.<sup>55</sup>

**Scheme 4.17: Fuchs's synthesis of benzyl protected hydroxymethyl compound 4.33<sup>55</sup>**

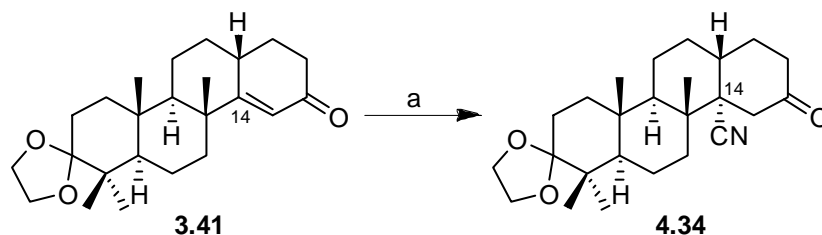


a.)  $\text{Et}_2\text{AlCN}$ , 60%. b.) TBS-Tf,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$  100%. c.) i. DIBAL(hexane), ether ii. AcOH 95%. d.) DIBAL (hexane), ether 90% e.) BnBr f.) HCl.

It was decided that this reaction series would be employed on enone **3.41a** to functionalize the C14 position with the desired hydroxymethyl group. Because of unavailability of diethylaluminum cyanide, a different cyanation approach was investigated. The  $\alpha,\beta$ -

unsaturated ketone **3.41a** was treated with ammonium chloride, potassium cyanide, in a 2:1 mixture of DMF and water<sup>4</sup> at 90°C to produce our desired  $\beta$ -cyanoketone **4.34**.

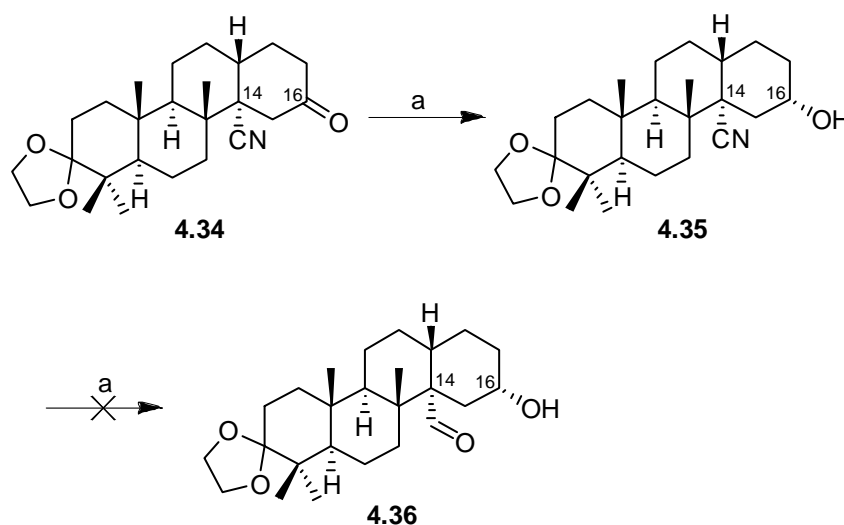
**Scheme 4.18: Cyanation of compound 3.41a to produce  $\beta$ -cyanoketone 4.34**



a.)  $\text{NH}_4\text{Cl}$ , KCN, 2:1 DMF:H<sub>2</sub>O, 91% yield.

Based on the steric effect of the axial methyl group adjacent to C14, it was presumed that the nitrile addition occurred from the opposite face giving the C14 configuration illustrated compound **4.34**. In an effort to shorten the synthetic steps, reduction of the nitrile was attempted without protection of the ketone, which should produce aldehyde **4.36**. When nitrile **4.34** was treated with excess DIBAL-H in THF, with THF as the solvent, the reaction resulted in reduction of only the ketone to form alcohol **4.35** - the nitrile remained intact.

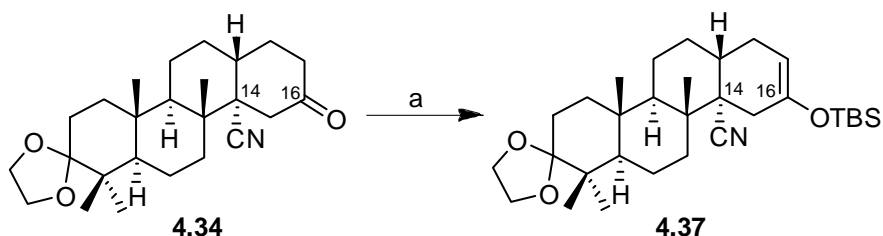
**Scheme 4.19: Attempted reduction of nitrile group without protection of the ketone**



a.) 1.0 M DIBAL in THF, THF; AcOH, 73% yield of **4.35**

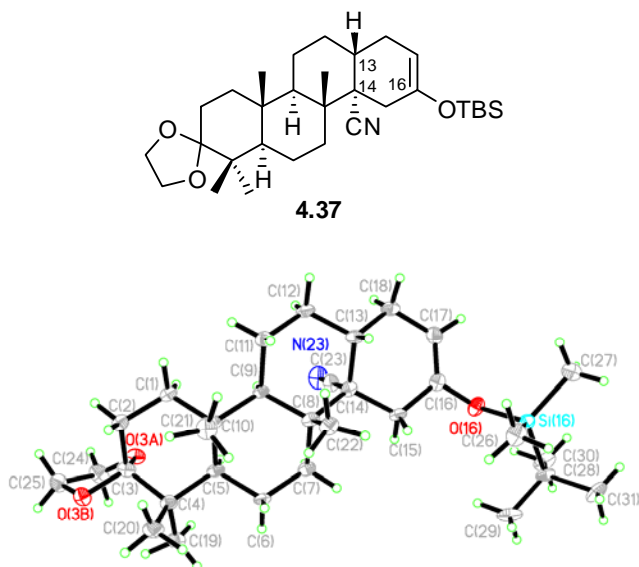
After isolation of alcohol **4.35**, it was retreated under the same conditions, which resulted in no formation of **4.36** only recovery of the starting alcohol **4.35**. Since the direct reduction approach was unsuccessful, the C16 ketone was protected as its TBS enol ether.  $\beta$ -ketonitrile **4.34** was treated with triethylamine and TBS-triflate in methylene chloride at 0°C to produce enol silyl ether **4.37** quantitatively.

**Scheme 4.20: Synthesis of TBS-enol ether 4.37**



a.) Et<sub>3</sub>N, TBS-Tf, CH<sub>2</sub>Cl<sub>2</sub> 0°C 1 h, 100% yield.

After purification of enol silyl ether **4.37**, by column chromatography, a colorless oil was obtained. Storage of the oil at 0°C overnight formed crystals from which the crystal structure was obtained by single-crystal X-ray analysis. This crystal structure confirmed the presence of all the functional groups (enolsilylether at C16, nitrile group at C14) and stereochemistry of all the stereocenters (C14 and C13 centers) of compound **4.37**.

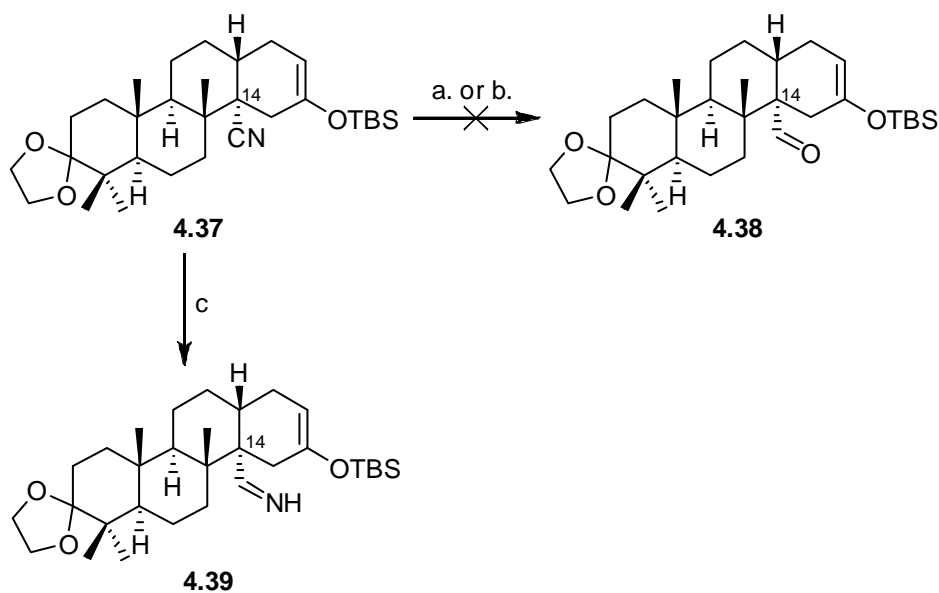


**Figure 4.1: Crystal structure obtained from single-crystal X-ray analysis of enol silyl ether 4.37**

Treatment of **4.37**, under similar conditions as Fuchs et al.<sup>55</sup>, with DIBAL-H in THF with THF as the solvent did not result in the reduction of the nitrile, instead compound **4.37** was recovered. We then tried treating **4.37** with lithium aluminum hydride in THF. This gave the same result, of no reduction, even after increasing the temperature of the reaction. Interestingly, by changing the DIBAL-H reagent from DIBAL-H in THF to DIBAL-H in toluene and using toluene as the solvent, the nitrile was consumed after 15 minutes at room temperature to form a very polar compound. After isolation it was determined that the product was not the desired aldehyde **4.38**, instead the intermediate imine **4.39** was isolated as the product.

Normally imines are hydrolyzed to their corresponding aldehydes during aqueous work up of the reactions. In the reaction described above the reaction was quenched with a buffered solution of 6% acetic acid that was saturated with potassium acetate. This neutral solution for hydrolysis of the imine is similar to what Fuchs et al.<sup>55</sup> used in their work up and is used to prevent the removal of the acid sensitive enol silyl ether protecting group.

**Scheme 4.21: Reduction of nitrile 4.37 to imine 4.39**

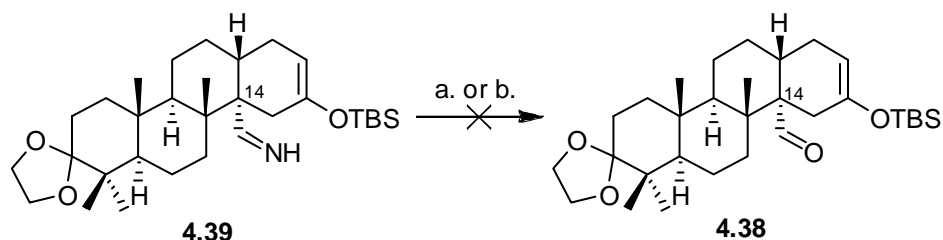


a.) 1 M DIBAL in THF, THF, KOAc/AcOH; b.) LAH, THF rt – 50°C, KOAc/AcOH; c.) 1 M DIBAL in toluene, toluene, rt 15 minutes, KOAc/AcOH, NaHCO<sub>3</sub>, 58% yield

Because of the steric hindrance of the imine, it was believed that the hydrolysis might be slow so the imine **4.39** was retreated with the buffered solution of acetic acid and potassium

acetate and left to proceed for a longer time. After 2 days it was determined that imine **4.39** was still intact. In their partial synthesis of myriceric acid A from the natural product oleanolic acid, Konoike et al.<sup>2</sup> report a similar problem. They had an imine on the same C14 position that was stable under usual hydrolysis conditions.<sup>2</sup> They accomplished the hydrolysis of their imine by treating it with sodium nitrite in aqueous dioxane-acetic acid to produce its corresponding aldehyde.<sup>2</sup>

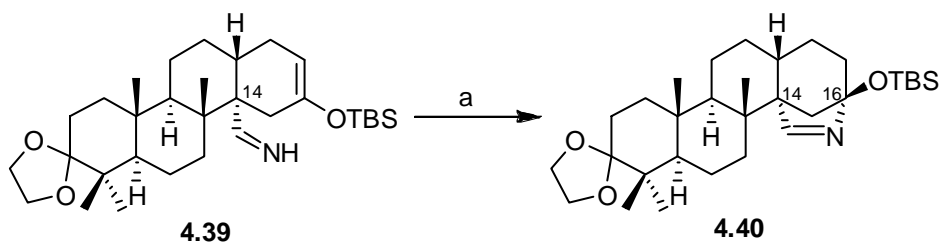
#### Scheme 4.22: Failed hydrolysis of imine



a.) KOAc/AcOH, THF, rt 2 days; b.) KOAc/AcOH, NaNO<sub>2</sub>, rt 2 days

To prevent removal of the enol silyl ether, Konoike et al.'s<sup>2</sup> procedure was modified. Instead of treating with acetic acid, imine **4.39** was treated with the buffered solution of acetic acid/potassium acetate and sodium nitrite for 2 days. Unfortunately, these conditions also failed to provide the aldehyde **4.38**. Since the neutral hydrolysis conditions failed, it was decided that the usual acid hydrolysis condition would be attempted. Imine **4.39** was treated with aqueous acetic acid in THF, which produced a new compound. After isolation it was determined that the product was not the aldehyde **4.38**, instead an interesting cyclic imine compound **4.40** was formed with the TBS group still on the C16 oxygen.

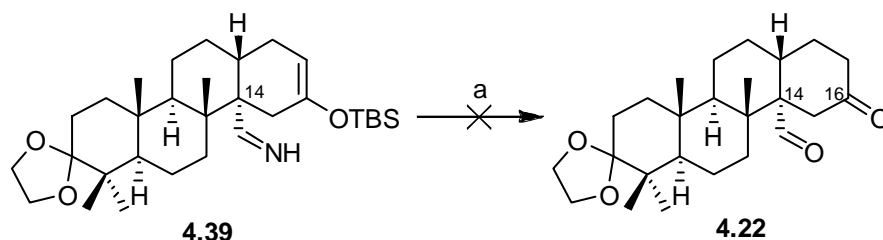
#### Scheme 4.23: Synthesis of cyclic imine 4.40



a.) AcOH, THF rt, 60% yield.

It was believed that removal of the TBS protecting group followed by re-treatment with acid should produce keto aldehyde **4.22**. However, in an effort to make the synthesis shorter, it was decided that deprotection and acid hydrolysis would be attempted in one step starting with imine **4.39**. It was expected that treatment of imine **4.39** with HF would result in removal of the silicon protecting group and acid hydrolysis of the imine. This condition resulted in complete decomposition of the compound, probably because of the harshness of the hydrofluoric acid, HF.

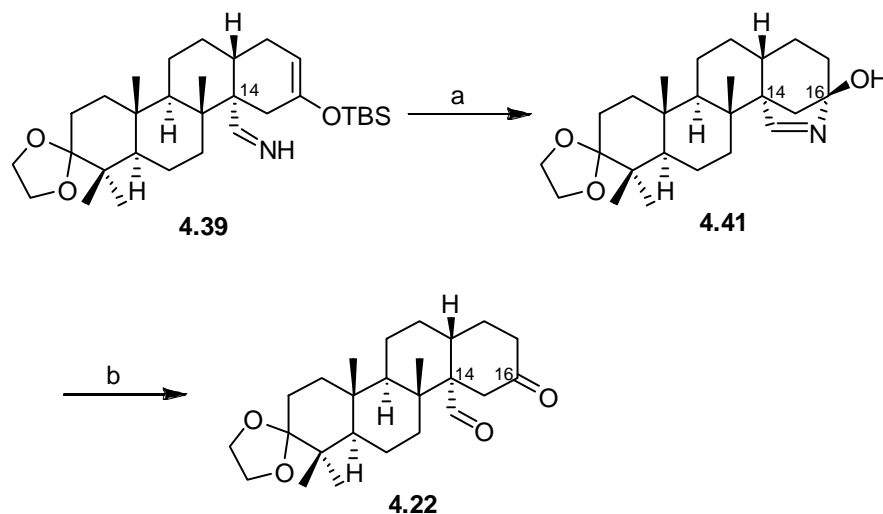
**Scheme 4.24: Decomposition of imine by HF**



a.) HF, THF, 0°C, 30 minutes, decomposition.

Interestingly, when imine **4.39** was treated with TBAF in THF at room temperature a new product formed within 30 minutes. This product was determined to be the cyclic imine alcohol **4.41**. Treatment of **4.41** with acetic acid in a 1:1 solution of THF and water under reflux produced the desired keto aldehyde **4.22** in high yield (80%).

**Scheme 4.25: Synthesis of aldehyde 4.22 through hemiiminal 4.41**



a.) TBAF, THF, rt, 69% yield after 2 steps from **4.39**; b.) AcOH, 1:1 THF:H<sub>2</sub>O, reflux 4 h, 80% yield.



To complete the formation of the hydroxymethyl functionality at C14, the aldehyde in **4.22** needs to be reduced. Because of the steric hindrance around the aldehyde the C16 ketone may need to be protected before reduction of the aldehyde is attempted.

## 4.5 Conclusion

In section 4.2 it was discovered that forming a hydroxy group at C13 could form the C12-C13 double bond. It was also discovered that this C13 hydroxy group facilitated the aromatization of the D ring. To prevent aromatization, functionalization of the C14 position was necessary. In section 4.2 and 4.3, attempts were made to add the C14 hydroxymethyl group: the intramolecular radical cyclization in section 4.2, the Michael addition of the vinyl cuprate approach, and the oxy-Cope-rearrangement approach in section 4.3, failed because of the high substitution at the C14 position and the steric hindrance caused by the quaternary center on C8 adjacent to the C14 position.

In section 4.4 the Michael addition to the C14 position was finally accomplished using a nitrile group as the nucleophile. Reduction of the nitrile turned out to be dependent on the solvent used for the DIBAL-H reaction, in which it only occurred when toluene was used as the solvent. The reduction of the nitrile resulted in the formation of the imine **4.39** which proved to be difficult to hydrolyze under normal hydrolysis conditions because of high steric hindrance in its surroundings. Removal of the protecting group of **4.39** resulted in a new cyclic imine alcohol compound **4.41**, which was cleanly hydrolyzed to aldehyde **4.22** when treated with acid.

From these results it is believed that functionalization of the C14 position was possibly the major hurdle in the functionalization of the D ring of (+)-myriceric acid A. Other reactions, not shown here, have been accomplished in which the other functionalities were constructed. So, a fully functionalized ABCD ring compound should be completed in the near future and this will facilitate the total synthesis of (+)-myriceric acid A.

## CHAPTER 5 - Activity studies of tetracyclic synthetic intermediates of (+)-myriceric acid A

### 5.1 Fujimoto's structure-activity relationship studies of myriceric acid and its derivatives

In their structure-activity relationship (SAR) studies of the myriceric acids and their derivatives, Fujimoto et al.<sup>1</sup>, suggested that four functional groups were important for the endothelin-A receptor antagonist activity.<sup>1</sup> These important functionalities are the carbonyl group at C3, the carboxylic acid group at C17, the *trans*-caffeoyloxy group at C27, and the dimethyl groups at C20 (Figure 5.1). Reduction of the carbonyl group at C3 or migration of one of the methyl groups at C20 resulted in reduction of the receptor antagonist activity. Removal of the carbonyl group at C3 or the *trans*-caffeoyloxy group at C27 or replacement of the C17 carboxylic acid group by a methyl ester group, resulted in complete loss of the binding affinity.<sup>1</sup>

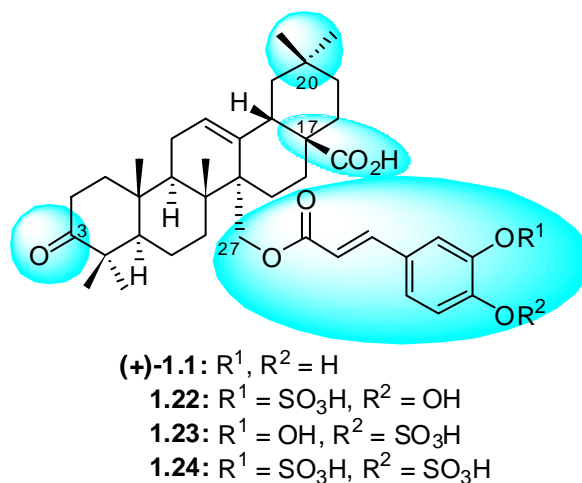


Figure 5.1: Myriceric acid A and sulfated derivatives

When the hydroxy groups on the *trans*-caffeoyloxy group were sulfated, as in compounds 1.22, 1.23, and 1.24, the binding affinities increased to 15 nM, 3.4 nM, and 41 nM, respectively.<sup>1</sup>

## 5.2 Synthetic intermediates of myriceric acid A which were tested as ET<sub>A</sub> receptor antagonists using SMA bioassay

In the course of our synthesis several interesting tetracyclic compounds were obtained (Figure 5.2). Many of these contained functionalities that were not present in the myriceric acid derivatives tested by Fujimoto et al.<sup>1</sup> To elaborate the SAR done by Fujimoto et al.<sup>1</sup>, we decided to test these tetracyclic compounds using the SMA bioassay described in chapter 1.

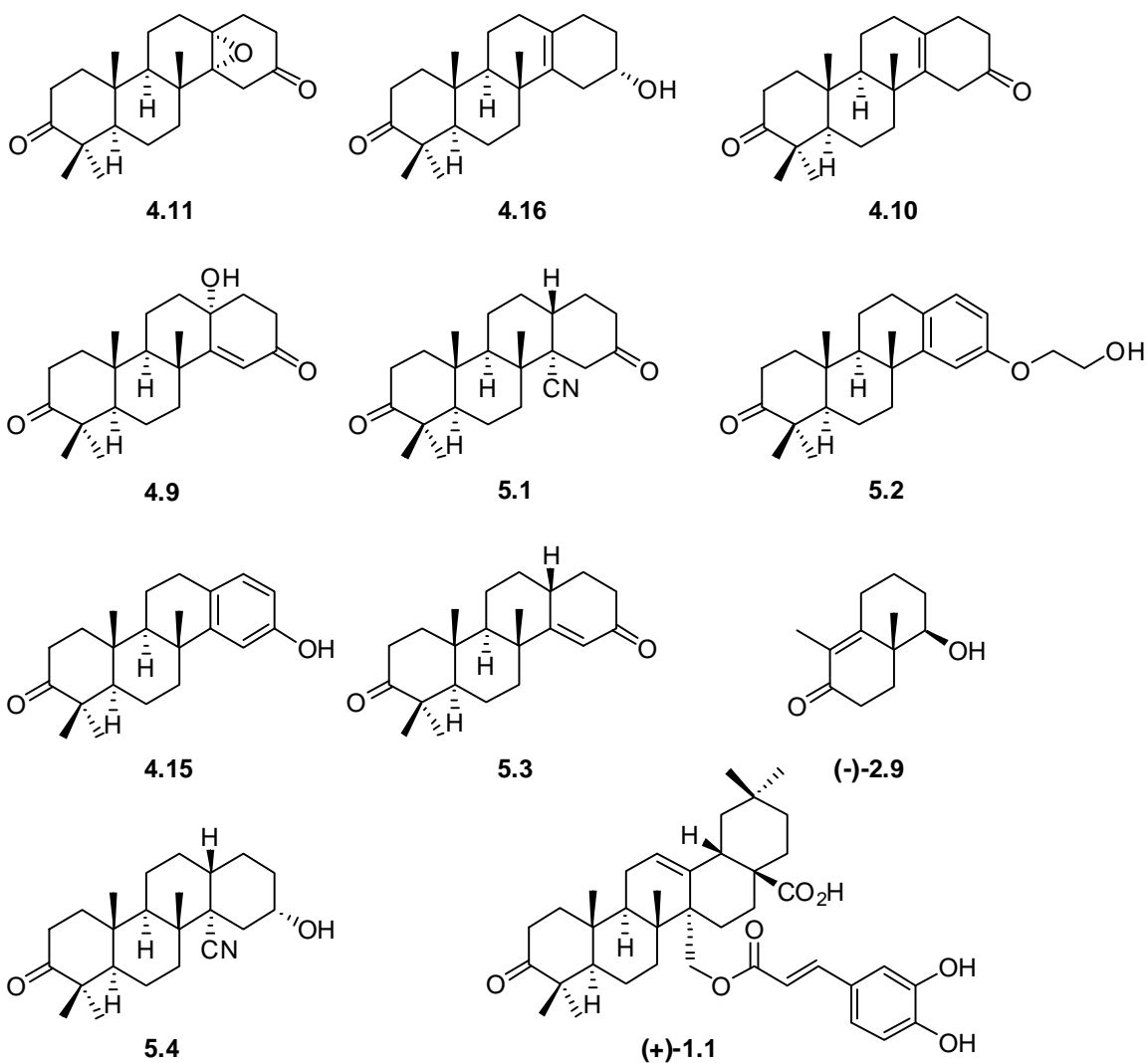
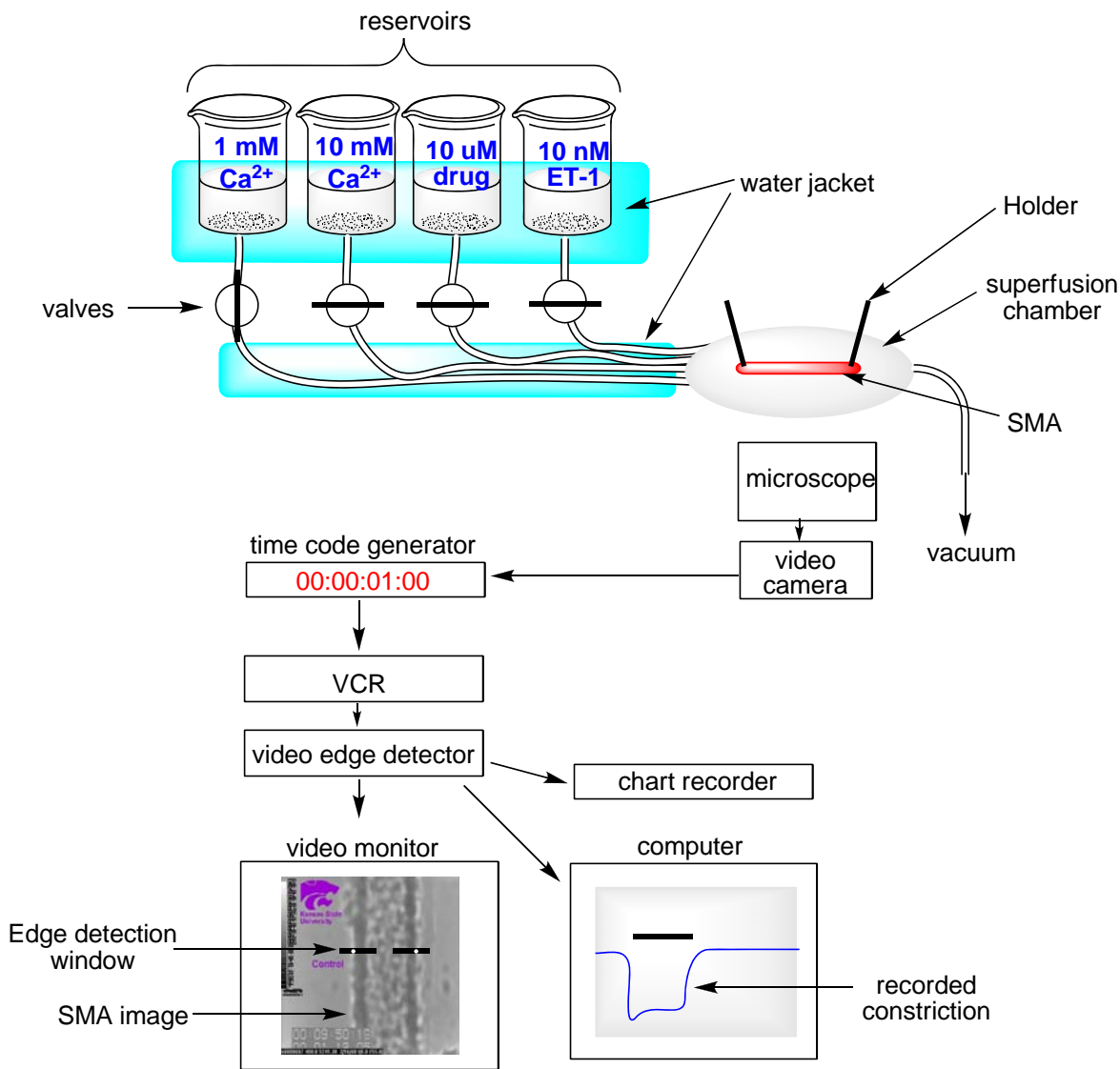


Figure 5.2: Synthetic intermediates that were tested

All the compounds listed were tested first for their antagonistic activity then for their ability to reverse the vasospasm. In these experiments, only the changes in the blood vessel diameter were recorded and the experiments were performed in the apparatus illustrated below.



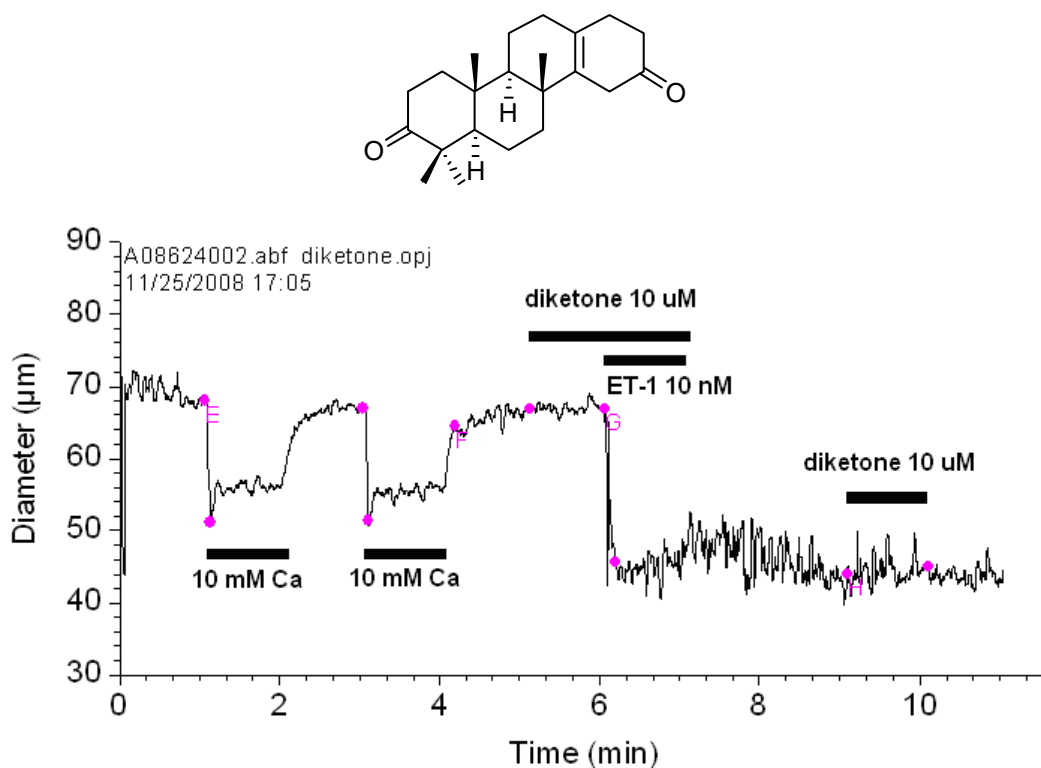
**Figure 5.3: Schematic of apparatus used for testing antagonistic activity of the compounds**

Four solutions were made for these experiments: 1.0 mM  $\text{Ca}^{2+}$  physiological salt solution (PSS); 10 mM physiological salt solution; 10  $\mu\text{M}$  drug solution, in which the drug is dissolved in the 1.0 mM  $\text{Ca}^{2+}$  PSS; and 10 nM ET-1 solution, in which the ET-1 is dissolved in the 1.0 mM  $\text{Ca}^{2+}$  PSS. All the solutions were at pH 7.4 and a full description on how to make them is

described in the experimental section. Each solution was poured into a separate reservoir as shown in the diagram above and kept at 37°C with a water jacket maintained at 37°C.

After the SMA has been isolated and cut into segments, one segment was transferred into the superfusion chamber, filled with 1 mM  $\text{Ca}^{2+}$  solution, and held in place by two blunted glass needles mounted on micromanipulators. After the segment was secure, it was superfused with 1 mM  $\text{Ca}^{2+}$  solution for 1 minute. To induce a constriction, the 1 mM  $\text{Ca}^{2+}$  valve is closed and the 10 mM  $\text{Ca}^{2+}$  valve is open and the SMA is superfused for 1 minute with 10 mM  $\text{Ca}^{2+}$  solution, which caused a constriction. This constriction was set to 100 %, and used as a control to compare to the constriction caused by ET-1. After the 1 minute of 10 mM  $\text{Ca}^{2+}$  was over, the valve was closed and the 1 mM  $\text{Ca}^{2+}$  valve was opened and the SMA was superfused for 1 minute with this solution. At 1.0 mM  $\text{Ca}^{2+}$  solution, the SMA diameter quickly returned to its normal diameter. This process was repeated once more.

To determine whether the drug is an antagonist, the 1 mM  $\text{Ca}^{2+}$  valve was closed and the drug valve was open and the SMA was superfused for 1 minute with the 10  $\mu\text{M}$  solution of the drug. This step is done to determine whether the drug alone has any effect on the SMA. After the SMA has been superfused for 1 minute with the drug alone, the ET-1 valve is also open and the SMA is superfused for 1 minute with both the drug solution and the 10 nM ET-1 solution. If the drug is an antagonist, no constriction should be observed when the SMA is superfused with both the drug solution and the ET-1 solution. If the drug is not an antagonist, then the ET-1 should induce the vasospasm even when the SMA is being superfused with both the drug and the ET-1 solutions. This was the case with all the compounds tested as is seen in the representative experiment illustrated in the figure below (Figure 5.4).



**Figure 5.4: Representative experiment – testing antagonistic activity of compounds and reversal of vasospasm**

After the SMA is superfused with the drug and ET-1 solutions for 1 minute, both valves are closed and the 1 mM  $\text{Ca}^{2+}$  valve is open and the SMA is superfused for 2 minutes with 1 mM  $\text{Ca}^{2+}$  solution. This is done to wash away the drug and the ET-1. For the drugs that were tested, they were unable to prevent the vasospasm, and even after washing away the ET-1 the constriction still remained while the SMA was superfused with the 1 mM  $\text{Ca}^{2+}$  solution. To test whether the drug can reverse the vasospasm, after the 2 minute washing of the SMA with 1 mM  $\text{Ca}^{2+}$  solution, the constricted SMA was superfused with 10  $\mu\text{M}$  drug solution for 1 minute. If it was unable to reverse the vasospasm, no effect would be seen during this time, as seen in figure 5.4. If it can reverse it, then an increase of the SMA diameter should be observed.

After the SMA is superfused for 1 minute with the drug solution, it is superfused once more with 1 mM  $\text{Ca}^{2+}$  solution to wash away the drug and the effect was observed. After this wash the experiment was ended, the SMA was washed away with 1 mM  $\text{Ca}^{2+}$  solution and the chamber was washed for 3 minutes with 1 mM  $\text{Ca}^{2+}$  solution before another experiment was started.

### 5.3 Results and discussion

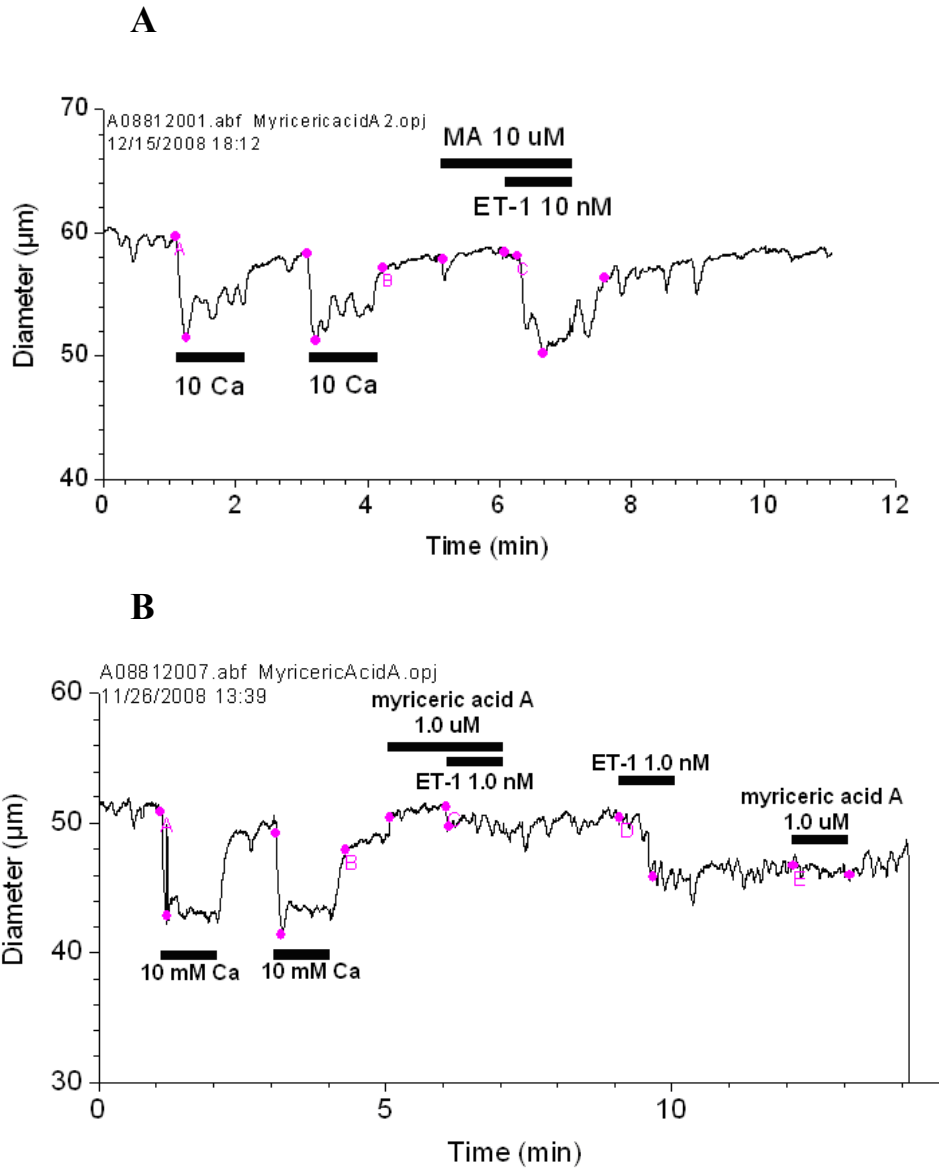
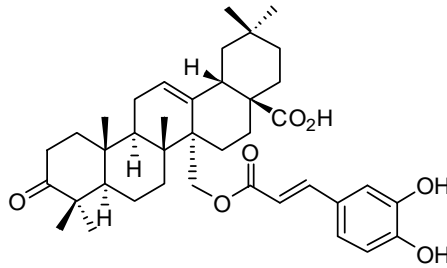
After testing all the compounds listed, entry 1 to 11, none showed any antagonistic activity (Figure 5.4). All showed a constriction greater than 100 % when compared to the constriction induced by the 10 mM Ca<sup>2+</sup> solution (Table 5.1).

**Table 5-1: Compounds tested and % constriction**

Entry	Compound	Control constriction: Average of 10 mM Ca Constriction (μm)	Constriction caused by 10 nM ET-1 in the presence of 10 μM Compound (μm)	% Constriction caused by 10 nM ET-1 in the presence of 10 μM Compound	Relaxation of constriction by addition of compound (μm)	% relaxation Of constriction by addition of compound
1	4.11	11.21	17.91	160	NA	NA
2	4.16	4.51	8.09	179	NA	NA
3	4.10	16.19	21.21	131	NA	NA
4	4.9	13.18	20.9	159	NA	NA
5	5.1	18.6	24.08	129	NA	NA
6	5.1	7.73	11.11	144	5.77	52
7	5.2	12.16	23.62	194	NA	NA
8	4.15	4.7	5.5	117	NA	NA
9	5.3	5.35	7.91	148	NA	NA
10	(-)-2.9	10.61	18.36	173	NA	NA
11	5.4	15.9	17.46	110	NA	NA

(+)-Myriceric acid A was also tested using similar conditions. First, its antagonistic activity was tested by superfusing the SMA with 10 μM myriceric acid A solution and 10 nM ET-1 solution. At these concentrations, constriction was still induced by the ET-1 in the presence of myriceric acid A. As seen in Figure 5.5-A, the constriction is not as significant as with the other drugs tested, so some prevention was observed. The antagonistic activity was seen more clearly when the test was repeated using lower concentrations. Myriceric acid A showed antagonistic activity by preventing the constriction of the SMA when the SMA was

superfused with both, a 1  $\mu\text{M}$  solution of (+)-myriceric acid A and 1 nM solution of ET-1 (Figure 5.5-B).

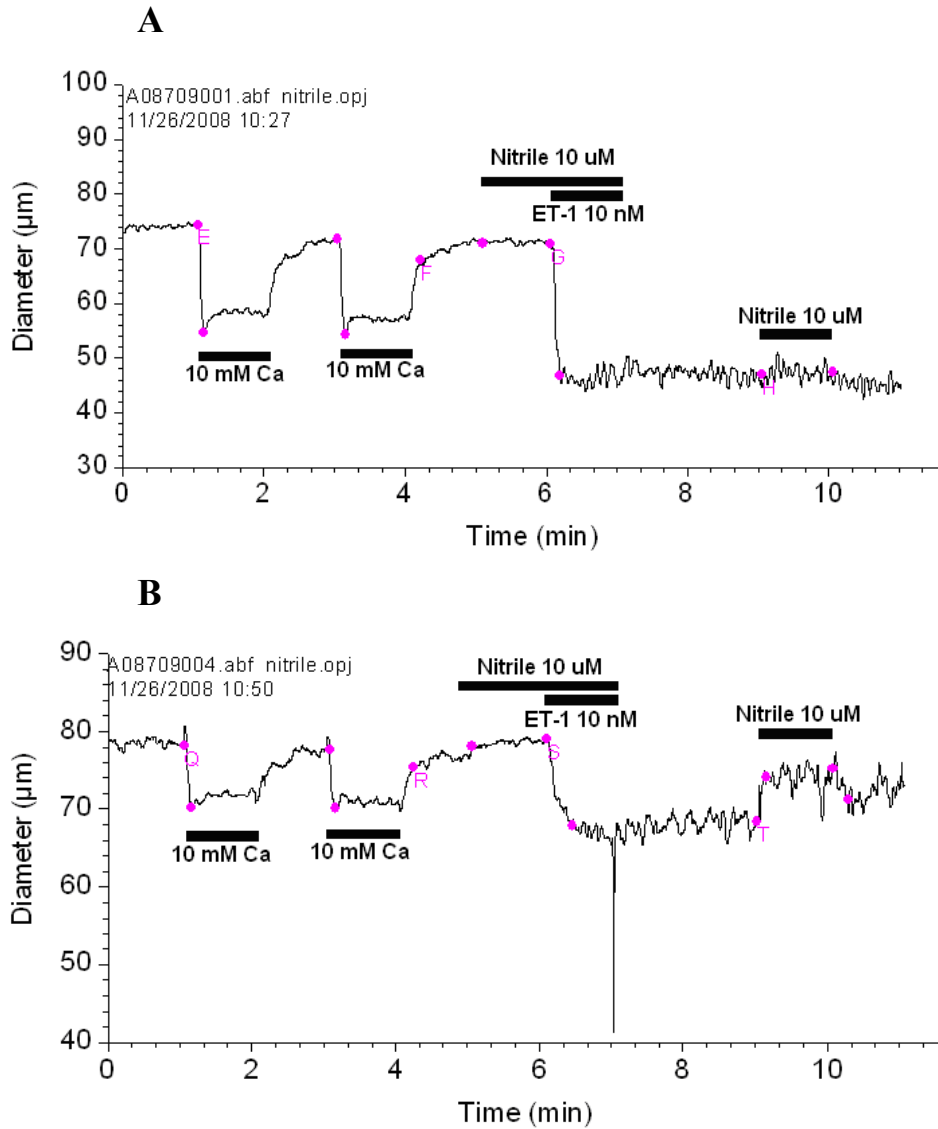
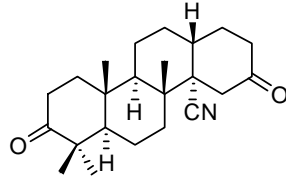


**Figure 5.5: A - failed antagonistic activity of (+)-myriceric acid A at higher concentrations; B – antagonistic activity of (+)-myriceric acid A at lower concentrations**



To determine whether myriceric acid A could reverse the vasospasm, the constriction of the SMA was induced with 1 nM ET-1 for 1 minute. After the vasospasm was induced with ET-1, the SMA was superfused for 2 minutes with 1.0 mM  $\text{Ca}^{2+}$  solution to wash away the ET-1. As expected, this did not reverse the vasospasm. After the SMA was superfused for 2 minutes with 1 mM  $\text{Ca}^{2+}$  solution, the constricted SMA was superfused with 1  $\mu\text{M}$  solution of myriceric acid A for 1 minute. As seen in Figure 5.5, (+)-myriceric acid A failed to reverse the vasoconstriction.

All the compounds were tested 3-7 times each, and the results obtained for each compound were normally consistent for the same compound, showing no antagonistic activity. When nitrile **5.1** was tested, two results were obtained as illustrated in Figure 5.6-A and 5.6-B, entry 5 and 6 respectively. In both experiments, nitrile **5.1** showed no antagonistic activity, by failing to prevent the ET-1 induced vasoconstriction. In the experiment summarized in entry 6 (Figure 5.6-B) and others (not shown), when the SMA was superfused with the nitrile **5.1** a slight increase in vessel diameter occurred, but it still failed to prevent the vasoconstriction when ET-1 was added. After the irreversible vasoconstriction was induced by ET-1, the nitrile was tested to see whether it could reverse it. When the constricted SMA was superfused for 1 minute with the nitrile, there was a 52% relaxation of the constriction (Figure 5.6-B). When the addition of nitrile **5.1** was terminated the constriction returned to the normal constriction.



**Figure 5.6: A-antagonistic activity of nitrile 5.1; B – antagonistic activity and reversal of vasospasm by nitrile 5.1**

These results indicate that nitrile 5.1 is not an endothelin receptor antagonist. The results illustrated in Figure 5.6-B (entry 6) indicate that nitrile 5.1 may be able to reverse the vasospasm. However, because of the inconsistency between the results illustrated in Figure 5.6-A and 5.6-B, it cannot be concluded that nitrile 5.1 can reverse the vasospasm. Repetition of this experiment

and further investigations are necessary to determine whether nitrile **5.1** can reverse the vasospasm.

## 5.4 Conclusion

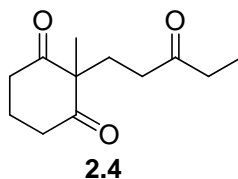
Although none of the compounds tested showed any antagonistic activity, they contribute to the structure-activity relationship by confirming the necessity of other functional groups like the *trans*-caffeoyloxy ester group and the carboxylic acid group. It is possible that addition of these functional groups may result in an active tetracyclic compound. Further more the nitrile compound **5.1**, needs to be investigated further to determine if it can reverse the vasoconstriction.

## CHAPTER 6 - Experimental

**General Procedures.** Unless otherwise stated, Nuclear Magnetic Resonance (NMR) spectra were recorded on a Varian 400 MHz and Varian 200 MHz instrument, for proton NMR; Varian 100 MHz and Varian 50 MHz instrument, for carbon NMR. The NMR spectra were obtained in chloroform-*d* (CDCl<sub>3</sub>), for proton NMR, the chemical shifts are given in parts per million (ppm) downfield from tetramethylsilane (TMS) as an internal standard, unless otherwise stated. Coupling constants (*J*) are given in hertz. Solvents were dried by distillation under argon from CaH<sub>2</sub> (CH<sub>2</sub>Cl<sub>2</sub>, pyridine, Et<sub>3</sub>N, DMF, diisopropylamine), Na-Ph<sub>2</sub>CO (THF, Et<sub>2</sub>O), Mg (EtOH, MeOH), and LAH (toluene, benzene). All other reagents were obtained from commercial suppliers and used without further purification. All reactions were performed under argon atmosphere, unless otherwise stated. Thin layer chromatography (TLC) was performed on plates obtained from Aldrich and column chromatography was performed on silica gel (200-400 mesh) from Natland corporation.

### Chapter 2, Experimental Section

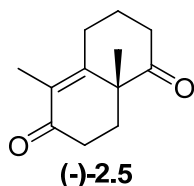
#### *2-Methyl-2-(3-oxopentyl)cyclohexane-1,3-dione (2.4)*



(AA-6-70) To a refluxing solution of 5 g (39.6 mmol) of 2-methyl-1,3-cyclohexanedione and 0.089 g (1.6 mmol) of potassium hydroxide dissolved in 30 mL of dry methanol was added dropwise, over five minutes, 5 g (59.4 mmol) of ethyl vinyl ketone. After refluxing overnight the reaction was quenched with saturated aqueous ammonium chloride and 1 M hydrochloric acid then extracted three times with ethyl acetate. The combined ethyl acetate layers were washed with brine, dried over magnesium sulfate, and concentrated to give 8.32 g (quantitative yield) of

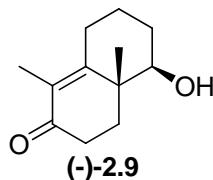
triketone **2.4** as a yellow oil, which was used directly for the next reaction.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  ppm 2.78-2.59 (m, 4 H), 2.39 (q,  $J = 7.3$  Hz, 2 H), 2.32 (t,  $J = 7.3$  Hz, 2 H), 2.07 (t,  $J = 7.9$  Hz, 2 H), 2.10-1.84 (m, 2 H), 1.24 (s, 3 H), 1.02 (t,  $J = 7.3$  Hz, 3 H);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  ppm 210.5, 210.3 (2 C), 64.6, 38.0 (2 C), 37.2, 36.2, 30.0, 20.0, 17.8, 7.9.

**(R)-5,8a-Dimethyl-3,4,8,8a-tetrahydronaphthalene-1,6(2H,7H)-dione [(-)-2.5]<sup>20</sup>**



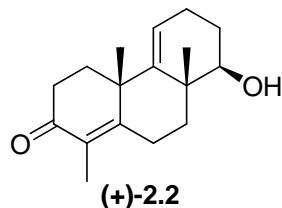
(AA-6-72) To a solution of 8.32 g (39.6 mmol) of triketone **2.4** in 200 mL of dry DMF was added 6.54 g (39.6 mmol) of D-phenylalanine and 4.6 g (19.8 mmol) of *d*-CSA. After 2 hours at room temperature, the reaction temperature was increased by 15°C every 2 hours until it got to 85°C and stirred overnight. After cooling to room temperature, cold saturated aqueous sodium bicarbonate was added to the reaction and it was extracted three times with diethyl ether. The combined diethyl ether layers was washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the product comes out at a solvent ratio of 6:1 – 4:1 hexane:ether to give 4.6 g (61% yield, after 2 steps) of the product **(-)-2.5** as a yellow oil. Rotation:  $[\alpha]_{\text{D}}^{25} = -124^\circ$  ( $c = 0.495$ ,  $\text{CHCl}_3$ ) (lit.<sup>21</sup>  $-125^\circ$   $c = 0.54$  after column, and  $-140^\circ$   $c = 0.200$  after recrystallization)  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  ppm 2.88 (dt,  $J = 15.6, 5.8$  Hz, 1 H), 2.73-2.64 (m, 1 H), 2.66 – 2.40 (m, 4 H), 2.20 - 2.04 (m, 3 H), 1.81 (s, 3 H), 1.80 – 1.71 (m, 1 H), 1.42 (s, 3 H);  $^{13}\text{C-NMR}$  (50 MHz NMR ( $\text{CDCl}_3$ ))  $\delta$  ppm 211.7, 197.2, 158.1, 130.4, 50.4, 37.1, 33.1, 29.4, 27.0, 23.1, 21.3, 11.0.

**(4aR,5R)-5-Hydroxy-1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one [(-)-2.9]<sup>20</sup>**



(AA-7-15) To a solution of 4.62 g (24.1 mmol) of ketone (-)-2.5 dissolved in 60 mL of dry ethanol at 0°C was added 227 mg (6.01 mmol) of sodium borohydride. After stirring for 1 hour the reaction was quenched by adding saturated ammonium chloride and 1 M hydrochloric acid. The solution was diluted and extracted three times with diethyl ether and the combined diethyl ether layers was washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the compound came out at a solvent ratio of 1:1 hexane:ether to give 4.1 g (87% yield) of (-)-2.9 as a yellow oil. Rotation:  $[\alpha]_{\text{D}}^{25} = -144^{\circ}$  (c = 0.25, CHCl<sub>3</sub>) (lit.,<sup>20</sup> after crystallization  $[\alpha]_{\text{D}}^{25} = -164.6^{\circ}$  (c = 2.16, CHCl<sub>3</sub>)) <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 3.42 (dt,  $J = 11.4, 4.7$  Hz, 1 H), 2.69 (dm,  $J = 14.9$  Hz, 1 H), 2.46-2.43 (m, 2 H), 2.14 (dt,  $J = 13.2, 4.4$  Hz, 1 H), 2.10-2.02 (m, 1 H), 1.95-1.81 (m, 3 H), 1.78 (d,  $J = 1.5$  Hz, 3 H), 1.70 (ddd,  $J = 24.6, 12.9, 4.1$  Hz, 1 H), 1.52 (bd,  $J = 5.6$ , 1 H), 1.37 (qt,  $J = 13.5, 4.4$  Hz, 1 H), 1.18 (s, 3 H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  ppm 200.1, 162.5, 130.2, 78.6, 42.6, 34.0 (2 C), 30.5, 27.7, 23.5, 16.4, 11.9.

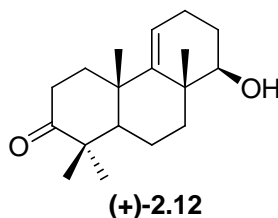
**(4aS,8R,8aR)-8-Hydroxy-1,4a,8a-trimethyl-4,4a,6,7,8,8a,9,10-octahydrophenanthren-2(3H)-one [(+)-2.2]<sup>17</sup>**



(AA-10-23) To a stirring solution of 4.18 g (21.5 mmol) of ketone (-)-2.9 dissolved in 60 mL of dry methanol was added 3.5 g (65 mmol) of sodium methoxide. After 3 hours at room temperature 6.47 mL (65 mmol) of ethyl vinyl ketone was added dropwise over 5 minutes and the solution was stirred at room temperature overnight then refluxed for six hours. After cooling,

saturated aqueous ammonium chloride and 1 M hydrochloric acid were added to the reaction, which was diluted and extracted three times with diethyl ether. The combined diethyl ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the compound came out at a 2:1 solvent ratio of hexane: diethyl ether to give 3.83 g (69% yield) of the enone **(+)-2.2** as an off white solid. Rotation:  $[\alpha]_D^{25} = +21^\circ$  ( $c = 0.145$ ,  $\text{CHCl}_3$ ), (lit.<sup>17</sup> after crystallization,  $[\alpha]_D^{27} = +50^\circ$  ( $c = 2.0$ ,  $\text{CHCl}_3$ ))  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 5.50 (t,  $J = 3.8$  Hz, 1 H) 3.48 (dd,  $J = 8.8, 7.7$  Hz, 1 H) 2.67 - 2.42 (m, 4 H) 2.28 - 2.20 (m, 2 H) 2.10 - 1.96 (m, 3 H) 1.81 - 1.73 (m, 2 H) 1.78 (s, 3H) 1.53 - 1.44 (m, 2 H) 1.42 (s, 3 H) 1.27 (s, 3 H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  ppm 198.7, 163.4, 148.3, 128.3, 120.2, 76.1, 41.4, 39.1, 35.1, 34.5, 34.1, 28.1, 26.1, 24.8, 24.6, 20.6, 11.36.

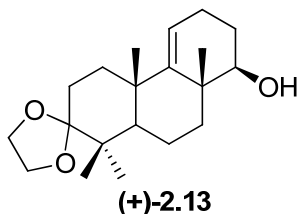
***(4aS,8R,8aR)-8-Hydroxy-1,1,4a,8a-tetramethyl-4,4a,7,8,8a,9,10,10a-octahydrophenanthren-2(1H,3H,6H)-one [(+)-2.12]***<sup>15</sup>



(AA-6-16) At  $-78^\circ\text{C}$ , 83 mL of liquid ammonia was condensed into a three neck round bottom flask equipped with a Birch condenser filled with dry ice and acetone. Addition of lithium wire 324 mg (47 mmol) in small pieces turned the solution dark blue. After stirring for 10 minutes at  $-78^\circ\text{C}$ , 3.3 g (12.3 mmol) of the enone **(+)-2.2** dissolved in 40 mL of dry THF was cannulaed into the reaction flask and the reaction was stirred at  $-35^\circ\text{C}$ . After 2 hours, the reaction was put at  $-78^\circ\text{C}$  and 6.6 mL (106 mmol) of dry methyl iodide was added and the reaction was put to  $-35^\circ\text{C}$ . After 2 hours the liquid ammonia was left to evaporate overnight. The solution was made acidic by the addition of 2 M HCl and it was extracted four times with methylene chloride. The combined methylene chloride layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of

methylene chloride, hexane, and diethyl ether in which the compound came out at 4:4:0.3 ratio of hexane : methylene chloride : diethyl ether to give 1.66 g (49% yield) of the compound **(+)-2.12** as a white solid. Rotation: lit.,<sup>17</sup>  $[\alpha]_D^{24} = +38^\circ$  (c = 0.45, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 5.35 (t,  $J = 4.1$  Hz, 1 H), 3.45 – 3.40 (m, 1 H), 2.74 – 2.65 (m, 1 H), 2.41 (ddd,  $J = 15.9, 6.0, 3.2$  Hz, 1 H), 2.21 – 2.01 (m, 4 H), 1.81 – 1.65 (m, 4 H), 1.62 – 1.56 (m, 2 H), 1.87 (dd,  $J = 12.2, 2.6$  Hz, 1 H), 1.29 (d,  $J = 0.9$  Hz, 3 H), 1.23 (dd,  $J = 13.0, 4.0$  Hz, 1 H), 1.18 (s, 3 H), 1.09 (s, 3 H), 1.08 (s, 3 H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  ppm 217.27, 151.42, 117.70, 78.54, 53.82, 47.91, 39.44, 39.03, 38.87, 38.16, 34.90, 26.28, 25.87, 24.99, 23.33, 21.80, 20.54, 19.49.

***(4a'S,8'R,8a'R)-1',1',4a',8a'-Tetramethyl-3',4',4a',6',7',8',8a',9',10',10a'-decahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-8'-ol [(+)-2.13]*<sup>17</sup>**

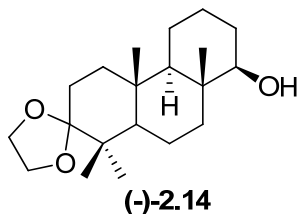


(AA-7-52) In a round-bottom flask equipped with a Dean-Stark apparatus, 1.1 g (4 mmol) of ketone **(+)-2.12** was dissolved in 125 mL of toluene. 322 mg (1.2 mmol) of pyridinium paratoluenesulfonate (PPTS) and 2.5 g (40 mmol) of ethylene glycol were added and the reaction was put to reflux. After 2 hours the reaction was determined to be complete by TLC. Saturated sodium bicarbonate was added to the reaction, which was extracted three times with diethyl ether. The combined ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the compound came out at a 4:1 ratio of hexane:diethyl ether to give 1.1 g (85% yield) of the ketal **(+)-2.13** as a white solid. Rotation:  $[\alpha]_D^{24} = +18^\circ$  (c = 0.40, CHCl<sub>3</sub>) lit.<sup>17</sup> after recrystallization,  $[\alpha]_D^{27} = +22^\circ$  (c = 0.26, CHCl<sub>3</sub>) <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 5.30 (t,  $J = 4.1$  Hz, 1 H), 4.00-3.87 (m, 4 H), 3.41 (dt,  $J = 10.8, 5.0$  Hz, 1 H), 2.19-2.10 (m, 2 H), 1.95 (dt,  $J = 12.6, 3.2$  Hz, 1 H), 1.92-1.83 (m, 1 H), 1.70-1.55 (m, 7 H), 1.30-1.20 (m, 3 H), 1.16 (s, 3 H), 1.13 (s, 3 H), 0.97 (s, 3 H), 0.86 (s, 3 H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  ppm



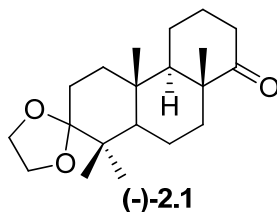
152.27, 116.36, 113.28, 78.80, 65.07, 65.00, 51.48, 42.60, 39.50, 39.42, 39.18, 36.12, 27.43, 26.35, 25.04, 23.78, 23.13, 20.66, 20.11, 18.53.

**(4a'R,4b'R,8'R,8a'R)-1',1',4a',8a'-Tetramethyldodecahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-8'-ol [(-)-2.14]<sup>18,19</sup>**



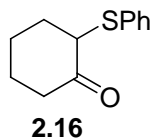
(AA-8-72) Alkene (+)-**2.13**, 720 mg (2.2 mmol), was dissolved in 40 mL of ethanol that was deoxygenated by bubbling argon through it for 10 minutes; heating was necessary for the alkene to dissolve. After cooling to room temperature 300 mg of 10% palladium on activated carbon was added then the reaction was put under hydrogen gas atmosphere by adapting a balloon full of hydrogen gas to the reaction flask. After stirring overnight under hydrogen the reaction was determined to be complete by TLC. The reaction was diluted with ethanol and diethyl ether, filtered through celite, concentrated and purified by flash silica gel column chromatography a 1:1 ratio of hexane:diethyl ether to give a mixture of the desired product and a little of the deprotected product which was subjected to the previously described protection procedure, from which the compound was obtained and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the compound came out at a 4:1 ratio of hexane:diethyl ether to give 700 mg (97% yield) of the alcohol (-)-**2.14** as a white solid. Rotation:  $[\alpha]_D^{24} = -9.4^\circ$  (c = 0.100, CHCl<sub>3</sub>) (lit.,<sup>19</sup> after enzymatic resolution  $[\alpha]_D^{26} = -44^\circ$  (c = 0.10, CHCl<sub>3</sub>)). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 3.99 – 3.86 (m, 4 H), 3.13 (dd, *J* = 11.4, 4.1 Hz, 1 H), 1.90 – 1.74 (m, 3 H), 1.64 – 1.33 (m, 10 H), 1.30 -1.05 (m, 7 H), 0.93 (s, 3 H), 0.88 (s, 6 H), 0.85 (s, 3 H) ; <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  ppm 113.50, 81.40, 65.09 (2 C), 56.86, 53.87, 42.46, 39.86, 39.43, 37.22, 37.07, 30.43, 27.11, 24.79, 23.15, 20.23, 20.13, 18.28, 16.48, 13.31.

***(4a'S,4b'R,8a'R)-1',1',4a',8a'-Tetramethyldecahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-8'(3'H)-one [(-)-2.1]*<sup>18,19</sup>**



(AA-8-34) Alcohol (-)-2.14, 510 mg (1.6 mmol), was dissolved in 30 mL of DMSO then 487 mg (1.7 mmol) of IBX was added to the reaction. After stirring overnight the reaction was determined to be complete by TLC. It was diluted with water and extracted three times with diethyl ether. The combined ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the compound came out between 5:1 and 4:1 ratio of hexane:diethyl ether to give 400 mg (78% yield) of the ketone (-)-2.1 as a white solid. Rotation:  $[\alpha]_D^{24} = -8^\circ$  ( $c = 0.150$ ,  $\text{CHCl}_3$ ) (lit.,<sup>19</sup> after enzymatic resolution  $[\alpha]_D^{26} = -26^\circ$  ( $c = 0.14$ ,  $\text{CHCl}_3$ ))  
 $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 3.98 – 3.86 (m, 4 H), 2.54 (td,  $J = 14.0, 6.9$  Hz, 1 H), 2.18 (dm, 1 H), 2.08 – 2.01 (m, 1 H), 1.82 (td,  $J = 13.9, 4.0$  Hz, 1 H), 1.75 – 1.35 (m, 10 H), 1.26 (dd,  $J = 12.3, 2.1$  Hz, 1 H), 1.18 (dd,  $J = 11.8, 3.0$  Hz, 1 H), 1.14 (s, 3 H), 0.98 (s, 3 H), 0.94 (s, 3 H), 0.84 (s, 3 H);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  ppm 215.93, 113.17, 65.12, 65.10, 57.62, 53.21, 49.36, 42.43, 38.25, 37.88, 37.11, 34.65, 27.03, 26.41, 23.09, 20.26, 20.07, 20.04, 18.02, 16.75.

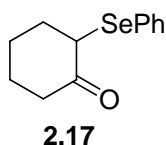
***2-(Phenylthio)cyclohexanone (2.16)***



(AA-3-60) At  $-20^\circ\text{C}$ , 31.8 mL (50.9 mmol) of 1.6 M n-butyllithium was added to 7.2 mL (50.9 mmol) of dry diisopropyl amine dissolved in 200 mL of dry THF and stirred for 30 minutes to make fresh lithium diisopropyl amine (LDA). At  $-78^\circ\text{C}$ , the fresh LDA (50.9 mmol) was added

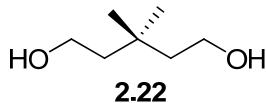
to 5 g (50.9 mmol) of cyclohexanone dissolved in 200 mL of dry THF. After 1.5 hours 13.3g (61.1 mmol) of phenyl disulfide was quickly added to the reaction. After 5.5 hours the reaction was quenched with saturated ammonium chloride and extracted three times with diethyl ether. The combined diethyl ether layers was washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the product came out at 9:1 ratio of hexane to diethyl ether to give 6.64 g (62 % yield ) of the product **2.16** as a lightly green oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 7.42 – 7.38 (m, 2 H), 7.31 – 7.22 (m, 3 H), 3.83 (t,  $J = 6.6$  Hz, 1 H), 2.95 – 2.88 (m, 1 H), 2.82 – 2.19 (m, 2 H), 2.12 -2.04 (m, 1 H), 2.00 – 1.91 (m, 2 H), 1.88 – 1.81 (m, 1 H), 1.74 – 1.65 (m, 1 H).

***2-(Phenylselenanyl)cyclohexanone (2.17)***



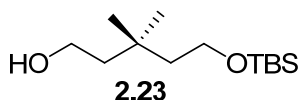
(AA-4-63) At  $-20^\circ\text{C}$ , 6.4 mL (10.2 mmol) of 1.6 M n-butyllithium was added to 1.43 mL (10.2 mmol) of dry diisopropyl amine dissolved in 50 mL of dry THF and stirred for 30 minutes to make fresh lithium diisopropyl amine (LDA). At  $-78^\circ\text{C}$ , the fresh LDA (10.2 mmol) was added to 1 g (10.2 mmol) of cyclohexanone dissolved in 50 mL of dry THF. After 1.5 hours 2.64 g (11.2 mmol) of phenylselenenylbromide was quickly added to the reaction. After 5.5 hours the reaction was quenched with saturated aqueous ammonium chloride and extracted three times with diethyl ether. The combined diethyl ether layers was washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the product came out at 19:1 ratio of hexane to diethyl ether to give 2.21 g (86 % yield ) of the product **2.17**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 7.56 – 7.52 (m, 2 H), 7.32 – 7.25 (m, 3 H), 3.93 (td,  $J = 3.9, 1.7$  Hz, 1 H), 3.03 – 2.95 (m, 1 H), 2.36 – 2.27 (m, 1 H), 2.25 – 2.17 (m, 2 H), 2.02 – 1.93 (m, 1 H), 1.91 – 1.67 (m, 3 H).

***3,3-Dimethylpentane-1,5-diol (2.22)***<sup>29</sup>



(AA-1-61) To a stirring suspension, at 0°C, of 1.42 g (37.4 mmol) of lithium aluminium hydride in 100 mL of dry THF was added, dropwise over 20 minutes, a solution of 3 g (18.7 mmol) of 3,3-dimethylglutaric acid in 30 mL of THF. After stirring overnight at room temperature the reaction was quenched slowly with 1 mL of water and stirred for 10 minutes, then 1 mL of 10% sodium hydroxide was added slowly and stirred for another 10 minutes. Another 3 mL of water was added and stirred for 10 minutes after which the reaction turned into a cloudy white mixture which was filtered and washed with ethyl acetate to give 2.5 g (quantitative yield) of the diol **2.22** which was used without further purification in the next step. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 3.73 (t, *J* = 7.1 Hz, 4 H), 1.58 (t, *J* = 7.1 Hz, 4 H), 0.95 (s, 6 H).

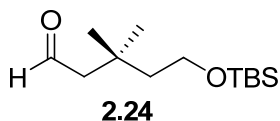
***5-(tert-Butyldimethylsilyloxy)-3,3-dimethylpentan-1-ol (2.23)***<sup>30</sup>



(AA-5-109) Sodium hydride (60% by weight in oil), 60.8 mg (1.52 mmol), was quickly added to a solution of 200 mg (1.52 mmol) of diol **2.22** in 3 mL of dry THF. After stirring at room temperature for 50 minutes, 229 mg (1.52 mmol) of *tert*-butyldimethylsilylchloride (TBSCl) was quickly added under argon. After 1 hour, the reaction was quenched with saturated aqueous ammonium chloride and extracted three times with diethyl ether. The combined diethyl ether layers was washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the product came out at 5:1 ratio of hexane to diethyl ether to give 266 mg (71 % yield) of the

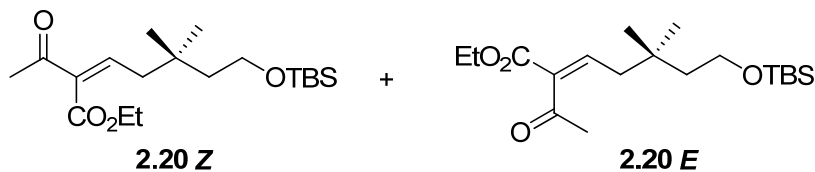
product **2.23**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 3.71 (dt,  $J = 13.0, 7.0$  Hz, 4 H), 1.55 (dt,  $J = 14.7, 7.3$  Hz, 4 H), 0.94 (s, 6 H), 0.90 (s, 9 H), 0.07 (s, 6 H).

**5-(tert-Butyldimethylsilyloxy)-3,3-dimethylpentanal (2.24)**



(AA-2-111) Alcohol **2.23**, 1 g (4.06 mmol), and 1.3 g (4.47 mmol) of IBX were dissolved in 5 mL of dimethyl sulfoxide. After stirring overnight the reaction was diluted with water at which time a white solid formed which was filter and washed several times with water and diethyl ether. The filtrate was then extracted three times with diethyl ether. The combined diethyl ether layers was washed with brine, dried over magnesium sulfate, and concentrated to give 0.8024 g (81 % yield ) of the aldehyde **2.24** which was used without further purification for the next step.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 9.85 (t,  $J = 3.1$  Hz, 1 H), 3.72 (t,  $J = 6.8$  Hz, 2 H), 2.33 (d,  $J = 3.1$  Hz, 2 H), 1.62 (t,  $J = 6.8$  Hz, 2 H), 1.08 (s, 6 H), 0.89 (s, 9 H), 0.05 (s, 6 H).

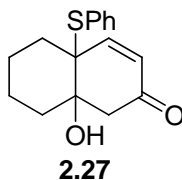
**(Z)-Ethyl 2-acetyl-7-(tert-butyldimethylsilyloxy)-5,5-dimethylhept-2-enoate (2.20 Z)** , &  
**(E)-ethyl 2-acetyl-7-(tert-butyldimethylsilyloxy)-5,5-dimethylhept-2-enoate (2.20 E)**



(AA-2-114) To a solution, at  $0^\circ\text{C}$ , of 802 mg (3.3 mmol) of aldehyde **2.24** and 473 mg (3.63 mmol) of ethyl acetoacetate in 1.8 mL of dry methylene chloride was added 28 mg (0.33 mmol) of piperidine and 20 mg (0.33 mmol) of acetic acid. After stirring for 30 minutes the reaction was warmed up to room temperature and stirred for another 30 minutes at which time it was

diluted with diethyl ether and water and extracted three times with diethyl ether. The combined diethyl ether layer was washed with saturated aqueous sodium bicarbonate, water, brine, dried over sodium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which one isomer came out at 19:1 hexane to diethyl ether to give 388 mg (33 % yield) of the *E* isomer **2.20E**, and the other came out at 9:1 hexane to diethyl ether to give 539 mg (46 % yield) of the *Z* isomer **2.20Z**. (*E* isomer, **2.20E**)  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 7.02 (t,  $J = 7.9$  Hz, 1 H), 4.25 (q,  $J = 7.2$  Hz, 2 H), 3.67 (t,  $J = 7.0$  Hz, 2 H), 2.36 (s, 3 H), 2.20 (d,  $J = 7.9$  Hz, 2 H), 1.51 (t,  $J = 7.0$  Hz, 2 H), 1.31 (t,  $J = 7.0$  Hz, 3 H), 0.95 (s, 6 H), 0.88 (s, 9 H), 0.05 (s, 6 H); (*Z* isomer, **2.20Z**)  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 6.96 (t,  $J = 7.9$  Hz, 1 H), 4.31 (q,  $J = 7.2$  Hz, 2 H), 3.69 (t,  $J = 7.0$  Hz, 2 H), 2.32 (s, 3 H), 2.27 (d,  $J = 7.9$  Hz, 2 H), 1.53 (t,  $J = 7.0$  Hz, 2 H), 1.34 (t,  $J = 6.7$  Hz, 3 H), 0.97 (s, 6 H), 0.89 (s, 9 H), 0.05 (s, 6 H).

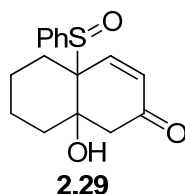
***8a-Hydroxy-4a-(phenylthio)-4a,5,6,7,8,8a-hexahydronaphthalen-2(1H)-one (2.27)***



(AA-3-83) A solution of ketosulfide **2.16**, 1 g (4.8 mmol), in 10 mL of THF was added to a suspension of 139 mg (5.8mmol) of pre-washed NaH in 20 mL of THF at  $-78^\circ\text{C}$ , then put to RT. After 1.5 hours the reaction was put at  $-78^\circ\text{C}$ , and 326 mg (4.8 mmol) of 3-butyn-2-one was added then it was left to slowly warm up to RT. After 2 hours, the reaction was diluted with ethyl acetate, quenched slowly with saturated ammonium chloride, and extracted three times with ethyl acetate. The combined ethyl acetate layer was washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography to give 400 mg (30% yield) of the enone **2.27**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 7.53 – 7.49 (m, 2 H), 7.41 – 7.28 (m, 3 H), 6.63 (d,  $J = 9.9$  Hz, 1 H), 5.95 (d,  $J = 9.5$  Hz, 1 H), 3.04 (s, 1 H), 2.77 (d,  $J = 16.6$  Hz, 1 H), 2.35 (d,  $J = 16.8$  Hz, 1 H), 2.06 – 1.98 (m, 1 H), 1.77 – 1.60 (m, 5 H), 1.51 – 1.43

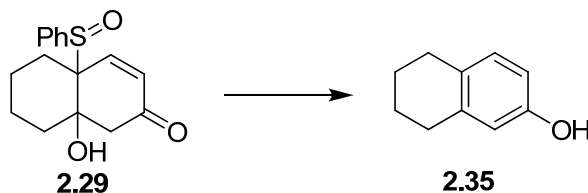
(m, 1 H), 1.34 – 1.24 (m, 1 H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  ppm 198.56, 152.57, 138.13 (2 C), 130.06, 129.30 (2 C), 128.72, 125.76, 73.50, 61.27, 35.36, 35.14, 30.60, 23.91, 20.97.

***8a-Hydroxy-4a-(phenylsulfinyl)-4a,5,6,7,8,8a-hexahydronaphthalen-2(1H)-one (2.29)***



(AA-3-105) To a solution of 170 mg (0.62 mmol) of enone **2.27** in 5 mL of 4:1 dioxane:water, at  $0^\circ\text{C}$ , was added 381 mg (0.62 mmol) of OXONE and left to warm up to RT. After 30 minutes the reaction was diluted with ethyl acetate, water and extracted three times with ethyl acetate. The combined ethyl acetate layer was washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography to give 71 mg (39% yield) of the enone sulfoxide **2.29**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 7.61 – 7.51 (m, 5 H), 6.30 (d,  $J = 9.9$  Hz, 1 H), 5.92 (d,  $J = 10.1$  Hz, 1 H), 5.08 (s, 1 H), 3.45 (d,  $J = 17.7$  Hz, 1 H), 2.78 (d,  $J = 17.6$  Hz, 1 H), 2.49 (td,  $J = 13.1, 3.5$  Hz, 1 H), 1.86 – 1.76 (m, 3 H), 1.64 – 1.59 (m, 2 H), 1.49 – 1.45 (m, 1 H), 1.26 – 1.14 (m, 1 H).

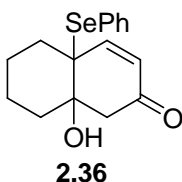
***5,6,7,8-Tetrahydronaphthalen-2-ol from enone sulfoxide (2.35)***



(AA-4-15) Enone sulfoxide **2.29**, 74.7 mg (0.26 mmol), was dissolved in 7 mL of toluene and refluxed. After 5 hours the reaction was cooled to RT, diluted with diethyl ether, water and extracted three times with diethyl ether. The combined diethyl ether layer was washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column

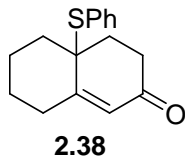
chromatography to give 11 mg (28 % yield) of the phenol **2.35**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 6.92 (d,  $J = 8.1$  Hz, 1 H), 6.58 (dd,  $J = 8.1, 2.6$  Hz, 1 H), 6.54 (d,  $J = 2.6$  Hz, 1 H), 4.53 (d,  $J = 4.1$  Hz, 1 H), 2.71 – 2.68 (m, 4 H), 1.76 (p,  $J = 3.2$  Hz, 4 H).

***8a-Hydroxy-4a-(phenylselanyl)-4a,5,6,7,8,8a-hexahydronaphthalen-2(1H)-one (2.36)***



(AA-4-56) Freshly prepared 0.1M LDA, 8.7 mL (0.87 mmol), was added to a solution of 200 mg (0.79 mmol) of phenyl selenide **2.17** in 5 mL of THF at  $-78^\circ\text{C}$ . After one hour, 93  $\mu\text{L}$  (1.19 mmol) of 3-butyne-2-one was added at  $-78^\circ\text{C}$ . After 2 hours, the reaction was diluted with ethyl acetate, quenched slowly with saturated aqueous ammonium chloride, and extracted three times with ethyl acetate. The combined ethyl acetate layer was washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography to give 25 mg (10 % yield) of enone **2.36**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 7.61- 7.58 (m, 2 H), 7.40 – 7.36 (m, 1 H), 7.31 – 7.27 (m, 2 H), 6.74 (d,  $J = 9.8$  Hz, 1 H), 5.85 (d,  $J = 9.9$  Hz, 1 H), 2.66 (d,  $J = 16.6$  Hz, 1 H), 2.29 (d,  $J = 16.5$  Hz, 1 H), 2.12 (td,  $J = 13.4, 3.7$  Hz, 1 H), 1.93 – 1.87 (m, 1 H), 1.76 – 1.65 (m, 5 H), 1.52 – 1.45 (m, 1 H), 1.36 – 1.27 (m, 1 H).

***4a-(Phenylthio)-4a,5,6,7,8-hexahydronaphthalen-2(3H)-one (2.38)***



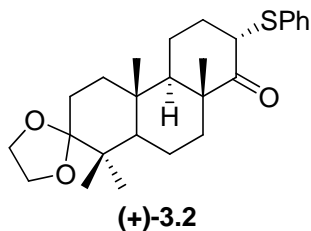
(AA-4-118) Sodium methoxide, 108 mg (2 mmol), was added to a solution of 200 mg (1 mmol) of phenyl sulfide **2.16** in 5 mL methanol at RT. After one hour, 170  $\mu\text{L}$  (2 mmol) of methyl vinyl ketone was added at RT. After 24 hours the reaction was diluted with diethyl ether, slowly quenched with saturated aqueous ammonium chloride, and extracted three times with diethyl



ether. The combined ether layer was washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography to produce 260 mg (100% yield) enone **2.38**.  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 7.61- 7.58 (m, 2 H), 7.40 – 7.36 (m, 1 H), 7.31 – 7.27 (m, 2 H), 6.74 (d,  $J = 9.8$  Hz, 1 H), 5.85 (d,  $J = 9.9$  Hz, 1 H), 2.66 (d,  $J = 16.6$  Hz, 1 H), 2.29 (d,  $J = 16.5$  Hz, 1 H), 2.12 (td,  $J = 13.4, 3.7$  Hz, 1 H), 1.93 – 1.87 (m, 1 H), 1.76 – 1.65 (m, 5 H), 1.52 – 1.45 (m, 1 H), 1.36 – 1.27 (m, 1 H).

### Chapter 3, Experimental Section

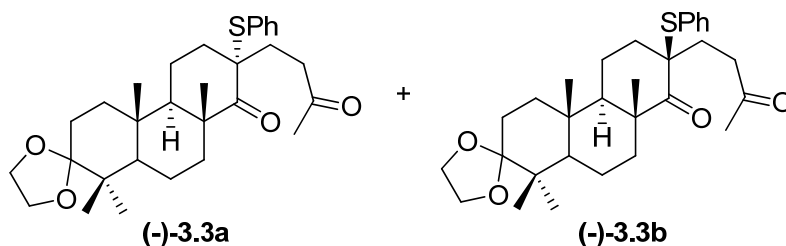
*4a'S,4b'R,7'S,8a'R*-1',1',4a',8a'-Tetramethyl-7'-(phenylthio)decahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-8'(3'H)-one [(+)-3.2]



(AA-10-62) To a solution of 460 mg (1.44 mmol) of ketone (-)-**2.1** in 10 mL of THF at  $-20^\circ\text{C}$  was added 43 mL (4.31 mmol) of freshly made 0.1 M lithium diisopropyl amine (LDA). Immediately after the addition of the LDA the reaction was put to room temperature. After stirring at room temperature for one hour a solution of 472 mg (2.16 mmol) of phenyl disulfide dissolved in 10 mL of THF was cannulaed into the reaction and left to stir overnight at room temperature. After stirring overnight the reaction was quenched with saturated aqueous ammonium chloride and extracted three times with diethyl ether. The combined diethyl ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane, methylene chloride and diethyl ether in which the product came out at a 4:4:0.1 solvent ratio of hexane:methylene chloride:diethyl ether to give 440 mg (71 % yield) of the  $\alpha$ -phenyl sulfide (+)-**3.2** as a white solid. Rotation:  $[\alpha]_D^{25} = +10.8^\circ$  ( $c = 0.130, \text{CHCl}_3$ )(AA-8-57).  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$

ppm 7.41 – 7.37 (m, 2 H), 7.30 – 7.21 (m, 3 H), 4.21 (dd,  $J = 13.1, 6.4$  Hz, 1 H), 3.97 – 3.86 (m, 4 H), 2.35 – 2.28 (m, 1 H), 1.84 – 1.36 (m, 10 H), 1.29 – 1.21 (m, 3 H), 1.19 (s, 3 H), 0.96 (s, 3 H), 0.93 (s, 3 H), 0.84 (s, 3 H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  ppm 209.91, 134.38, 132.62 (2 C), 129.09 (2 C), 127.32, 112.98, 65.09 (2 C), 57.93, 54.91, 53.03, 50.04, 42.36, 38.28, 36.95, 34.87, 34.74, 26.95, 23.06, 20.96, 20.03, 19.91, 17.93, 16.67; HRMS calcd. for  $\text{C}_{26}\text{H}_{36}\text{O}_3\text{S}$  ( $\text{M}+\text{H}^+$ ) 429.2463, found 429.2444.

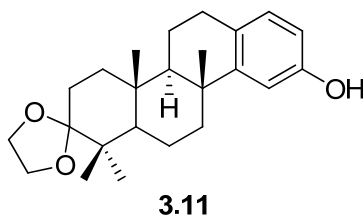
***(4a'S,4b'R,7'R,8a'R)-1',1',4a',8a'-Tetramethyl-7'-(3-oxobutyl)-7'-(phenylthio)decahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-8'(3'H)-one [(-)-3.3a] and (4a'S,4b'R,7'S,8a'R)-1',1',4a',8a'-tetramethyl-7'-(3-oxobutyl)-7'-(phenylthio)decahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-8'(3'H)-one [(-)-3.3b]***



(AA-10-29) The  $\alpha$ -ketosulfide (+)-**3.2**, 500 mg (1.17 mmol), and 126 mg (2.33 mmol) of sodium methoxide were dissolved in 8 mL of methanol and 16 mL of THF. After stirring for one hour at room temperature 292  $\mu\text{L}$  (3.51 mmol) of methyl vinyl ketone was syringed into the reaction and stirred for 15 minutes at room temperature then the reaction was heated to 55°C and stirred for an additional one hour. After cooling to room temperature the reaction was quenched by adding saturated aqueous ammonium chloride and the solution was extracted three times with diethyl ether. The combined diethyl ether layer was washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the first diastereomer comes out between 4:1 and 3:1 hexane to diethyl ether solvent ratio and the second diastereomer comes out between 2:1 and 1:1 hexane to diethyl ether solvent ratio to give 272 mg of (-)-**3.3a** (70 % yield) and (-)-**3.3b** 116 mg (30 % yield) (97% total yield) respectively, of the two diastereomers as white solids. **Less polar**

**[(-)-3.3a]** Rotation:  $[\alpha]_D^{25} = -32.3^\circ$  ( $c = 0.235$ ,  $\text{CHCl}_3$ )  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 7.40 (m, 5 H), 3.99 – 3.90 (m, 4 H), 2.55 – 2.35 (m, 2 H), 2.27 (dd,  $J = 11.8, 6.9$  Hz, 1 H), 2.90 (s, 3 H), 2.06 -1.78 (m, 7 H), 1.68 – 1.24 (m, 8 H), 1.06 (s, 3 H), 0.98 (s, 3 H), 0.95 (s, 3 H), 0.87 (s, 3 H);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  ppm 211.52, 208.18, 137.59 (2 C), 130.36, 129.66, 128.88 (2 C), 113.17, 65.06 (2 C), 58.01, 53.31, 49.07, 47.86, 42.35, 39.23, 38.11, 36.87, 36.19, 30.91, 30.56, 30.17, 26.87, 23.11, 20.70, 20.18, 18.27, 16.46, 16.10; HRMS calcd. for  $\text{C}_{30}\text{H}_{42}\text{O}_4\text{S}$  ( $\text{M}+\text{H}^+$ ) 499.2882, found 499.2884. **More polar [(-)-3.3b]** Rotation:  $[\alpha]_D^{25} = -50.4^\circ$  ( $c = 0.115$ ,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 7.23 -7.28 (m, 5 H), 3.99 – 3.87 (m, 4 H), 2.50 – 2.30 (m, 2 H), 2.09 (s, 3 H), 2.02 – 1.66 (m, 9 H), 1.50 (s, 3 H), 1.47 -1.12 (m, 7 H), 1.01 (s, 3 H), 0.94 (s, 3 H), 0.84 (s, 3 H);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  ppm 211.23, 208.24, 136.62 (2 C), 130.73, 129.58, 128.97 (2 C), 113.25, 65.14 (2 C), 58.21, 56.74, 53.17, 49.41, 42.42, 39.05, 38.01, 36.93, 36.84, 36.44, 31.07, 30.21, 27.01, 23.96, 23.03, 19.94, 18.09, 17.37, 17.06; HRMS calcd. for  $\text{C}_{30}\text{H}_{42}\text{O}_4\text{S}$  ( $\text{M}+\text{H}^+$ ) 499.2882, found 499.2886.

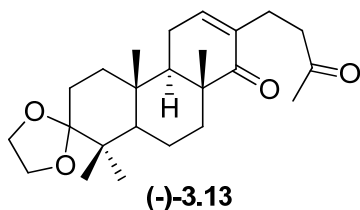
***(4aR,4bR,10bR)-1,1,4a,10b-Tetramethyl-3,4,4a,4b,5,6,10b,11,12,12a-decahydro-1H-spiro[chrysene-2,2'-[1,3]dioxolan]-9-ol (3.11)***



(AA-5-31) To a solution of 20 mg (0.04 mmol) of diketosulfide **(-)-3.3b** in 1.5 mL of methanol was added 4.3 mg (0.08 mmol) of sodium methoxide and the whole was put to reflux. After refluxing overnight, the reaction was cooled and then quenched with saturated aqueous ammonium chloride and extracted three times with diethyl ether. The combined diethyl ether layer was washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a gradient of hexane and diethyl ether to give 4.6 mg (31 % yield) of the compound **3.11** as a white solid.  $^1\text{H NMR}$  (200 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 6.87 (d,  $J = 8.1$

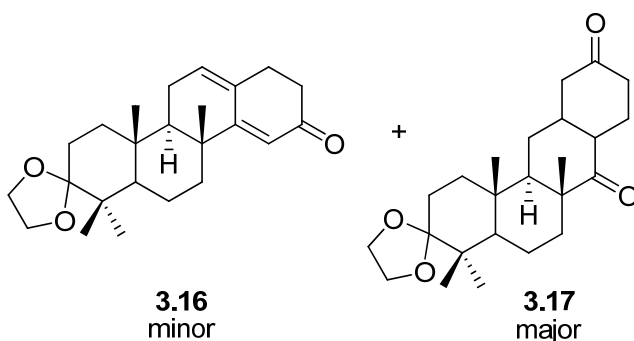
Hz, 1 H), 6.71 (d,  $J = 2.6$  Hz, 1 H), 6.55 (dd,  $J = 8.1, 2.6$  Hz, 1 H), 4.50 (bs, 1 H), 3.98 – 3.85 (m, 4 H), 2.90 – 2.61 (m, 2 H), 2.82 – 2.24 (m, 1 H), 1.95 – 1.25 (m, 11 H), 1.18 (s, 3 H), 0.96 (d,  $J = 1.5$  Hz, 6 H), 0.85 (s, 3 H).

**(4a'S,4b'R,8a'R)-1',1',4a',8a'-Tetramethyl-7'-(3-oxobutyl)-4',4a',4b',5',8a',9',10',10a'-octahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-8'(3'H)-one [(-)-3.13]**



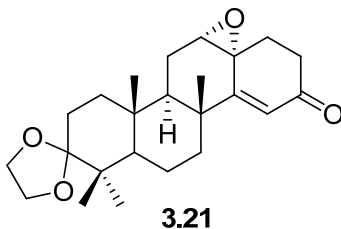
(AA-10-31) (NMR-aa-5-58) To a stirring solution, at 0°C, of 576 mg (1.16 mmol) of diketosulfide **(-)-3.3a+b** in 17 mL of methylene chloride was added 273 mg (1.16 mmol) of 73 % *m*-chloroperbenzoic acid and 195 mg (2.32 mmol) of sodium bicarbonate. After stirring for 30 minutes the reaction was quenched with 10 % aqueous sodium sulfite and saturated aqueous sodium bicarbonate and extracted three times with diethyl ether. The combined diethyl ether layers were washed with brined, dried with magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the compound came out at solvent ratio of 1:1 hexane to diethyl ether to give 388 mg (97 % yield) of the compound **(-)-3.13** as a white solid. Rotation:  $[\alpha]_D^{25} = -26.8^\circ$  ( $c = 0.340$ ,  $\text{CHCl}_3$ )(AA-8-57).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 6.69 (t,  $J = 4.1$  Hz, 1 H), 3.98 – 3.85 (m, 4 H), 2.54 – 2.32 (m, 4 H), 2.28 – 2.25 (m, 2 H), 2.11 (s, 3 H), 1.95 – 1.91 (m, 1 H), 1.82 (td,  $J = 13.4, 4.1$  Hz, 1 H), 1.63 – 1.39 (m, 6 H), 1.30 – 1.22 (m, 2 H), 1.02 (s, 3 H), 1.00 (s, 3 H), 0.94 (s, 3 H), 0.85 (s, 3 H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  ppm 208.61, 205.51, 144.63, 135.83, 113.17, 65.17, 65.13, 53.57, 53.14, 45.32, 42.89, 42.41, 37.60, 36.40, 34.82, 30.14, 26.87, 25.21, 23.75, 23.12, 20.19, 18.66, 18.30, 16.57; HRMS calcd. for  $\text{C}_{24}\text{H}_{36}\text{O}_4$  ( $\text{M}+\text{H}^+$ ) 389.2692, found 389.2677.

*(4aR,4bR,10bR)*-1,1,4a,10b-Tetramethyl-4,4a,4b,5,7,8,10b,11,12,12a-decahydro-1H-spiro[chrysene-2,2'-[1,3]dioxolan]-9(3H)-one (**3.16**) and *(6a'R,12a'R,12b'S)*-4',4',6a',12b'-tetramethyldodecahydro-1'H-spiro[[1,3]dioxolane-2,3'-tetraphene]-7',10'(2'H,4'H)-dione (**3.17**)



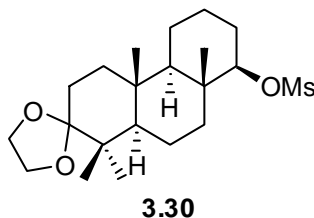
(AA-5-88) Diketoenone (-)-**3.13**, 11.1 mg (0.029 mmol), and 5.8 mg (0.145 mmol) of sodium hydride (60 % in oil) were dissolved in 1 mL of THF then put to reflux. After two hours the reaction was cooled then quenched with saturated aqueous ammonium chloride and extracted three times with diethyl ether. The combined diethyl ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether to give 2.3 mg of the 1,2 addition adduct **3.16** (21 % yield) as a white solid and 3.3 mg of the 1,4 addition adduct **3.17** (31 % yield) as a white solid. (**MINOR PRODUCT, 3.16**)  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 6.02 – 5.99 (m, 1 H), 5.77 (s, 1 H), 3.98 – 3.85 (m, 4 H), 2.64 – 2.56 (m, 2 H), 2.46 – 2.34 (m, 2 H), 2.27 – 2.10 (m, 2 H), 1.97 – 1.91 (m, 1 H), 1.81 (td,  $J = 13.6, 4.0$  Hz, 1 H), 1.67 – 1.60 (m, 2 H), 1.55 – 1.44 (m, 4 H), 1.33 – 1.21 (m, 4 H), 1.03 (s, 3 H), 1.00 (s, 3 H), 0.95 (s, 3 H), 0.86 (s, 3 H) (**MAJOR PRODUCT, 3.17**)  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 3.98 – 3.86 (m, 4 H), 3.01 (t,  $J = 5.7$  Hz, 1 H), 2.76 – 2.67 (m, 1 H), 2.62 (td,  $J = 14.3, 7.2$  Hz, 1 H), 2.45 (dt,  $J = 6.6, 2.1$  Hz, 1 H), 2.28 (t,  $J = 13.9$  Hz, 1 H), 2.23 – 2.01 (m, 3 H), 1.86 – 1.38 (m, 10 H), 1.30 (dd,  $J = 12.2, 3.0$  Hz, 1 H), 1.20 (s, 3 H), 1.15 (td,  $J = 13.6, 4.3$  Hz, 1 H), 1.01 (s, 3 H), 0.94 (s, 3 H), 0.85 (s, 3 H);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  ppm 214.80, 211.84, 113.04, 65.17, 65.14, 53.49, 51.54, 49.58, 44.56, 42.81, 42.52, 40.11, 38.16, 38.11, 37.16, 34.53, 27.01, 25.78, 25.44, 23.14, 20.46, 20.07, 18.04, 16.72.

**(4<sup>R</sup>,5a<sup>S</sup>,6a<sup>R</sup>,6b<sup>R</sup>,12a<sup>R</sup>)-6b,10,10,12a-Tetramethyl-3,4,6,6a,6b,7,8,10,10a,11,12,12a-dodecahydrospiro[chryseno[6-b]oxirene-9,2'-[1,3]dioxolan]-2(5aH)-one (3.21)**



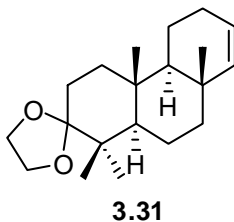
(AA-5-92) To a solution, at 0°C, of 3.2 mg (0.0086 mmol) of dienone **3.16** in 0.5 mL of methylene chloride was added 1.9 mg (0.0086 mmol) of m-chloroperbenzoic acid (77 % by weight). After one hour the reaction was put to room temperature and stirred for an additional six hour. The reaction was quenched with 10 % aqueous sodium sulfite and saturated aqueous sodium bicarbonate and extracted three times with diethyl ether. The combined diethyl ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the compound came out at a 3:1 – 2:1 solvent ratio of hexane to diethyl ether to give 2.5 mg (78 % yield) of the epoxide **3.21** as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 6.03 (s, 1 H), 3.97 – 3.84 (m, 4 H), 3.43 (s, 1 H), 2.86 – 2.77 (m, 1 H), 2.54 -2.33 (m, 3 H), 2.22 – 2.15 (m, 1 H), 2.04 – 1.97 (m, 1 H), 1.82 (q, *J* = 14.3 Hz, 3 H), 1.51 – 1.40 (m, 7 H), 1.07 (s, 3 H), 0.97 (s, 3 H), 0.93 (s, 3 H), 0.84 (s, 3 H).

**(4a'R,4b'R,8'R,8a'R,10a'R)-1',1',4a',8a'-Tetramethyldodecahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthrene]-8'-yl methanesulfonate (3.30)**



(AA-7-48) triethylamine, 109  $\mu\text{L}$  (0.78 mmol), was added to a solution of 84 mg (0.26 mmol) of alcohol (-)-**2.14** in 2.5 mL of methylene chloride. After 15 minutes at room temperature it was put to 0°C, 30.2  $\mu\text{L}$  (0.39 mmol) of methane sulfonyl chloride was added and the reaction was left to warm-up to RT. After 2 hours, some methanol and saturated aqueous sodium bicarbonate were added and stirred briefly. After extracting three times with diethyl ether, the combined layers were washed with brine, dried over magnesium sulfate, and concentrated to produce 104 mg (100 % yield) of the mesylate **3.30**. No further purification was done.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 4.20 (dd,  $J = 11.4, 4.8$  Hz, 1 H), 3.99 – 3.86 (m, 4 H), 2.98 (s, 3 H), 2.09 – 1.62 (m, 6 H), 1.50 – 1.11 (m, 10 H), 0.96 (s, 3 H), 0.92 (s, 3 H), 0.89 (s, 3 H), 0.84 (s, 3 H).

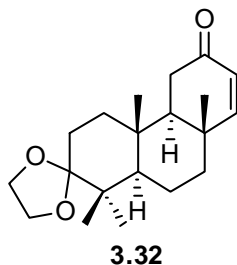
**(4a'R,4b'S,8a'S,10a'R)-1',1',4a',8a'-Tetramethyl-3',4',4a',4b',5',6',8a',9',10',10a'-decahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthrene] (3.31)**



(AA-7-49) To a solution of 104 mg (0.26 mmol) of mesylate **3.30** in 10 mL of DMF was added 96 mg (1.3 mmol) of lithium carbonate, 113 mg (1.3 mmol) of lithium bromide and put to reflux. After 5 hours the reaction was stopped, cooled to room temperature, diluted with diethyl ether

and 1 M HCl was added slowly and stirred briefly. After extracting three times with diethyl ether, the combined layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography to produce 60 mg (76 % yield after 2 steps) of tricyclic alkene **3.31**.  $^1\text{H NMR}$  (200 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 5.44-5.25 (m, 2 H), 4.01-3.39 (m, 4 H), 2.09-1.60 (m, 7 H), 1.45-1.07 (m, 7 H), 0.95 (s, 3 H), 0.94 (s, 3 H), 0.88 (s, 3 H), 0.84 (s, 3 H).

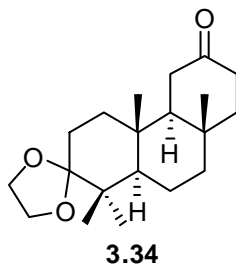
***(4a'R,4b'S,8a'S,10a'R)-1',1',4a',8a'-Tetramethyl-4',4a',4b',5',8a',9',10',10a'-octahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-6'(3'H)-one (3.32)***



(AA-7-89) To a solution of 21 mg (0.07 mmol) of alkene **3.31** in 0.6 mL of acetonitrile was added 0.15 mg (cat.) CuI and 74 mg (0.58 mmol) *tert*-butylhydrogenperoxide (90 %) then heated to 50°C. After running overnight the reaction was cooled to room temperature and a solution of 10 % aqueous sodium sulfite was added and stirred for 5 minutes. The mixture was diluted with sodium bicarbonate, diethyl ether and extracted three times with diethyl ether. The combined diethyl ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether to give 17 mg (77 % yield) of the enone **3.32**.  $^1\text{H NMR}$  (200 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 6.61 (d,  $J = 9.9$  Hz, 1 H), 5.75 (d,  $J = 9.5$  Hz, 1 H), 3.99 – 3.86 (m, 4 H), 2.44 – 2.23 (m, 2 H), 1.86 – 1.61 (m, 3 H), 1.51 – 1.45 (m, 3 H), 1.41 – 1.33 (m, 2 H), 1.31 -1.27 (m, 1 H), 1.24 – 1.20 (m, 1 H), 1.09 (s, 3 H), 0.96 (s, 3 H), 0.95 (s, 3 H), 0.86 (s, 3 H).

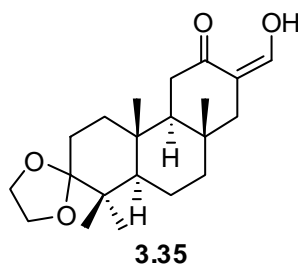


***(4a'R,4b'S,8a'S,10a'R)-1',1',4a',8a'-Tetramethyldecahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-6'(3'H)-one (3.34)***



(AA-7-92) To a solution of 37 mg (0.116 mmol) of enone **3.32** in 3 mL of ethanol was added 6 mg (cat.) Pd-C(10%) then put under 30 psi of hydrogen gas. After 1 hour the reaction was diluted with ethanol and diethyl ether then filtered through celite. The resulting solution was concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether to give 22 mg (59 % yield) of the ketone **3.34**. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 3.99-3.86 (m, 4 H), 2.46-2.20 (m, 4 H), 1.84-1.14 (m, 12 H) 1.11 (s, 3 H), 0.95 (s, 3 H), 0.89 (s, 3 H), 0.86 (s, 3 H).

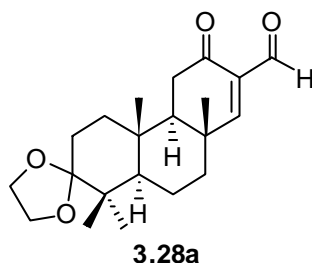
***(4a'R,4b'S,8a'S,10a'R,Z)-7'-(Hydroxymethylene)-1',1',4a',8a'-tetramethyldecahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-6'(3'H)-one (3.35)***



To a dispersion of 10 mg (0.24 mmol) of NaH in 0.2 mL of benzene was added a solution of 22 mg (0.08 mmol) of ketone **3.34** in 0.5 mL of benzene and 13 μL (0.16 mmol) of ethyl formate. After 24 hours the reaction was diluted with diethyl ether and water then extracted with 1 %

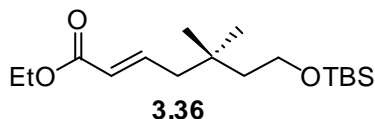
NaOH solution. The aqueous layer was acidified with 1 M HCl and extracted three times with diethyl ether. The combined ether layers were washed with brine, dried over magnesium sulfate and concentrated to produce 24 mg of the enol **3.35** without any further purification.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 8.43 (bd, 1 H), 4.00-3.86 (m, 4 H), 2.38-2.25 (m, 3 H), 2.09 (d,  $J = 14.5$  Hz, 1 H), 1.94 (d,  $J = 14.3$  Hz, 1 H), 1.85-1.67 (m, 4 H), 0.96 (s, 3 H), 0.91 (s, 3 H) 0.87 (s, 3 H), 0.87 (s, 3 H).

***(4a'R,4b'S,8a'S,10a'R)-1',1',4a',8a'-Tetramethyl-6'-oxo-3',4',4a',4b',5',6',8a',9',10',10a'-decahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthrene]-7'-carbaldehyde (3.28a)***



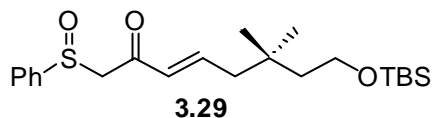
(AA-7-96) To a solution of 24 mg (0.08 mmol) of crude enol **3.35** in 0.5 mL of dioxane was added a solution of 18.2 mg (0.08 mmol) of DDQ and 1 drop of acetic acid in 0.5 mL of dioxane. After 1 hour the reaction was diluted with diethyl ether and washed with saturated aqueous sodium bicarbonate until the wash was colorless. The ether layer was washed with brine, dried over magnesium sulfate, and concentrated to give 16 mg (67 % yield, after 2 steps) of crude product **3.28a** without any further purification.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 10.05 (s, 1 H), 4.00-3.86 (m, 4 H), 2.56-2.34 (m, 2 H), 1.91-1.47 (m, 11 H), 1.15 (s, 3 H), 0.98 (s, 3 H), 0.96 (s, 3 H), 0.86 (s, 3 H).

**(E)-Ethyl 7-(tert-butyl dimethylsilyloxy)-5,5-dimethylhept-2-enoate (3.36)**



(AA-7-62) To a solution of 353 mg (1.45 mmol) of aldehyde **2.24** in 7 mL of methylene chloride was added 756 mg (2.2 mmol) of (carboethoxymethylene)-triphenylphosphorane then put to reflux. After 6 hours the reaction was cooled to room temperature, diluted with diethyl ether and water and extracted three times with diethyl ether. The combined ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether. The compound came out using a solvent ratio of 19:1 hexane to diethyl ether to give 368 mg (81 % yield) of the conjugated ethyl ester **3.36**.  $^1\text{H NMR}$  (200 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 6.99 (dt,  $J = 15.8, 8.1$  Hz, 1 H), 5.81 (d,  $J = 15.8$  Hz, 1 H), 4.19 (q,  $J = 7.3$  Hz, 2 H), 3.68 (t,  $J = 7.0$  Hz, 2 H), 2.13 (dd,  $J = 7.7, 1.5$  Hz, 2 H), 1.50 (t,  $J = 7.3$  Hz, 2 H), 1.29 (t,  $J = 7.0$  Hz, 3 H), 0.94 (s, 6 H), 0.89 (s, 9 H), 0.05 (s, 6 H).

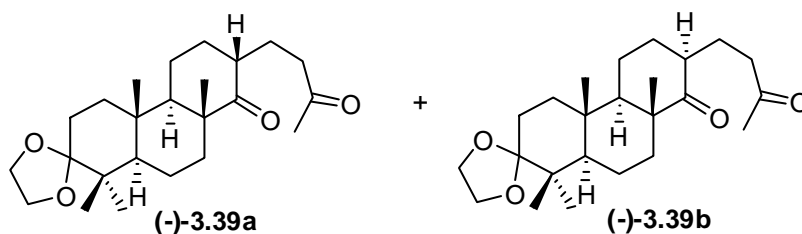
**(E)-8-(tert-Butyl dimethylsilyloxy)-6,6-dimethyl-1-(phenylsulfinyl)oct-3-en-2-one (3.29)**



(AA-7-67) To a solution of 54 mg (0.38 mmol) of methylphenyl sulfoxide **3.36** in 1 mL of THF at  $-20^\circ\text{C}$  was added 3.8 mL (0.38 mmol) of freshly prepared LDA. After 1 hour at  $-20^\circ\text{C}$ , a solution of 100 mg (0.32 mmol) of conjugated ester in 4 mL of THF was added and the reaction was left to slowly warm up to room temperature. After 2 hours, the reaction was diluted with diethyl ether, quenched with saturated aqueous ammonium chloride, and extracted three times

with diethyl ether. The combined ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether. The compound came out using a solvent ratio of 1:1 hexane to diethyl ether to give 80 mg (62 % yield) of the sulfoxide **3.29**.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 7.70-7.63 (m, 2 H), 7.66-7.50 (m, 3 H), 6.90 (dt,  $J = 15.8, 7.7$  Hz, 1 H), 6.11 (dt,  $J = 15.8, 1.1$  Hz, 1 H), 4.00 (dd,  $J = 41.0, 13.6$  Hz, 2 H), 3.67 (t,  $J = 7.0$  Hz, 2 H), 2.15 (dd,  $J = 7.7, 1.1$  Hz, 2 H), 1.47 (t,  $J = 7.3$  Hz, 2 H), 0.92 (s, 6 H), 0.88 (s, 9 H), 0.04 (s, 6 H).

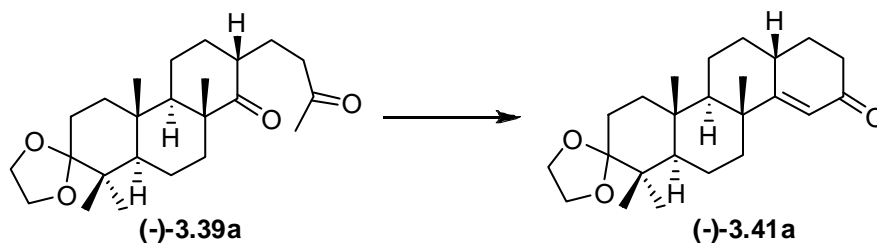
**(4a'S,4b'R,7'R,8a'R)-1',1',4a',8a'-Tetramethyl-7'-(3-oxobutyl)decahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-8'(3'H)-one [(-)-3.39a] and (4a'S,4b'R,7'S,8a'R)-1',1',4a',8a'-tetramethyl-7'-(3-oxobutyl)decahydro-1'H-spiro[[1,3]dioxolane-2,2'-phenanthren]-8'(3'H)-one [(-)-3.39b] from sulfides (-)-3.3a and (-)-3.3b**



(AA-10-71) A mixture of diketosulfides **(-)-3.3a+b**, 425 mg (0.85 mmol), was dissolved in 17 mL of dry toluene. To this solution, 14 mg (0.085 mmol) of AIBN was added and the mixture was put to reflux. At reflux, 0.460 mL of tributyltinhydride was added and refluxed overnight. After determining that the reaction was complete, by TLC, the reaction was stopped, cooled and the solvent was removed by vacuum and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether to give two diastereomers **(-)-3.39a+b** with a combined yield of 89% in which the first diastereomer came out at a 2:1 solvent ratio of hexane to diethyl ether to give 150 mg (45 % yield) of **(-)-3.39a** as a white solid. The other diastereomer came out between a 2:1 – 1:1 solvent ratio of hexane to diethyl ether to give 146 mg (44 % yield) of **(-)-3.39b** as a white solid. (**less polar (-)-3.39a**) Rotation:  $[\alpha]_{\text{D}}^{25} = -25^\circ$  ( $c = 0.160, \text{CHCl}_3$ );

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 3.97 – 3.85 (m, 4 H), 2.58 – 2.51 (m, 2 H), 2.41 – 2.33 (m, 1 H), 2.12 (s, 3 H), 2.10 – 2.04 (m, 1 H), 1.92 – 1.55 (m, 7 H), 1.51 – 1.12 (m, 8 H), 1.11 (s, 3 H), 0.97 (s, 3 H), 0.93 (s, 3 H), 0.84 (s, 3 H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  ppm 215.93, 209.40, 113.16, 65.14, 65.11, 58.47, 53.19, 49.26, 44.73, 42.43, 41.65, 38.32, 37.04, 34.58, 33.99, 30.10, 27.02, 24.18, 23.12, 20.58, 20.11, 20.07, 18.05, 16.68; HRMS calcd. for  $\text{C}_{24}\text{H}_{38}\text{O}_4$  ( $\text{M}+\text{H}^+$ ) 391.2848, found 391.2838. (**more polar (-)-3.39b**) Rotation:  $[\alpha]_{\text{D}}^{25} = -72^\circ$  ( $c = 0.05$ ,  $\text{CHCl}_3$ )  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 3.99 – 3.86 (m, 4 H), 2.47 (t,  $J = 7.3$  Hz, 2 H), 2.63 – 2.30 (m, 1 H), 2.13 (s, 3 H), 1.96 – 1.74 (m, 5 H), 1.70 – 1.40 (m, 7 H), 1.38 -1.15 (m, 4 H), 1.07 (s, 3 H), 0.95 (s, 3 H), 0.93 (s, 3 H), 0.84 (s, 3 H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  ppm 219.81, 209.06, 113.24, 65.13 (2 C), 53.61, 51.58, 48.04, 44.42, 42.36, 41.70, 37.66, 36.62, 36.33, 30.16, 26.92, 25.41, 25.02, 23.10, 20.30, 20.09, 16.24, 17.22, 16.14; HRMS calcd. for  $\text{C}_{24}\text{H}_{38}\text{O}_4$  ( $\text{M}+\text{H}^+$ ) 391.2848, found 391.2856.

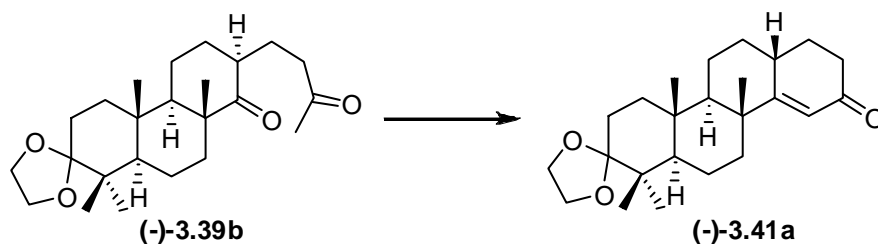
***(4aR,4bR,6aR,10bR)-1,1,4a,10b-Tetramethyl-4,4a,4b,5,6,6a,7,8,10b,11,12,12a-dodecahydro-1H-spiro[chrysene-2,2'-[1,3]dioxolan]-9(3H)-one [(-)-3.41a] from [(-)-3.39a]***



(AA-10-72) Diketone **(-)-3.39a**, 9.7 mg (0.025 mmol), and 4.5 mg (0.084 mmol) of sodium methoxide were dissolved in 0.5 mL of methanol and 0.25 mL of THF and stirred overnight at room temperature. The reaction was then refluxed for an additional 4 hours after which it was cooled down and quenched with saturated aqueous ammonium chloride and extracted three times with diethyl ether. The combined diethyl ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the compound came out at a 3:1 solvent ratio of hexane to diethyl ether to give 8.5 mg (92 % yield) of the enone **(-)-3.41a** as a white

solid. Rotation:  $[\alpha]_D^{25} = -30^\circ$  ( $c = 0.06$ ,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 5.82 (d,  $J = 1.8$  Hz, 1 H), 3.98 – 3.86 (m, 4 H), 2.58 – 2.47 (m, 1 H), 2.37 (dt,  $J = 16.4, 5.0$  Hz, 1 H), 2.28 – 2.19 (m, 1 H), 2.11 – 1.97 (m, 2 H), 1.88 – 1.75 (m, 2 H), 1.72 – 1.45 (m, 8 H), 1.30 – 1.16 (m, 4 H), 1.12 (s, 3 H), 0.94 (s, 6 H), 0.86 (s, 3 H);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  ppm 201.65, 176.89, 119.98, 113.21, 65.14, 65.09, 57.30, 53.37, 42.39, 41.39, 38.53, 38.06, 37.17, 36.23, 35.39, 34.65, 29.62, 27.09, 23.12, 22.81, 20.62, 20.14, 18.63, 16.66; HRMS calcd. for  $\text{C}_{24}\text{H}_{36}\text{O}_3$  ( $\text{M}+\text{H}^+$ ) 373.2743, found 373.2729.

***(4aR,4bR,6aR,10bR)-1,1,4a,10b-Tetramethyl-4,4a,4b,5,6,6a,7,8,10b,11,12,12a-dodecahydro-1H-spiro[chrysen-2,2'-[1,3]dioxolan]-9(3H)-one [(-)-3.41a] from [(-)-3.39b]***

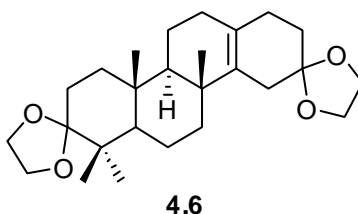


(AA-10-73) Diketone **(-)-3.39b**, 9.1 mg (0.023 mmol), and 4.5 mg (0.084 mmol) of sodium methoxide were dissolved in 0.5 mL of methanol and 0.25 mL of THF and stirred for three days at room temperature. The reaction was then refluxed for and additional 4 hours after which it was cooled down and quenched with saturated ammonium chloride and extracted three times with diethyl ether. The combined diethyl ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the compound came out at a 3:1 solvent ratio of hexane to diethyl ether to give 7 mg (81 % yield) of the enone **(-)-3.41a** as a white solid. Rotation:  $[\alpha]_D^{25} = -30^\circ$  ( $c = 0.06$ ,  $\text{CHCl}_3$ )  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 5.82 (d,  $J = 1.8$  Hz, 1 H), 3.98 – 3.86 (m, 4 H), 2.58 – 2.47 (m, 1 H), 2.37 (dt,  $J = 16.4, 5.0$  Hz, 1 H), 2.28 – 2.19 (m, 1 H), 2.11 – 1.97 (m, 2 H), 1.88 – 1.75 (m, 2 H), 1.72 – 1.45 (m, 8 H), 1.30 – 1.16 (m, 4 H), 1.12 (s, 3 H), 0.94 (s, 6 H), 0.86 (s, 3 H);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  ppm 201.65, 176.89, 119.98,

113.21, 65.14, 65.09, 57.30, 53.37, 42.39, 41.39, 38.53, 38.06, 37.17, 36.23, 35.39, 34.65, 29.62, 27.09, 23.12, 22.81, 20.62, 20.14, 18.63, 16.66; HRMS calcd. for C<sub>24</sub>H<sub>36</sub>O<sub>3</sub> (M+H<sup>+</sup>) 373.2743, found 373.2729.

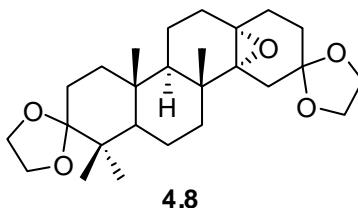
## Chapter 4, Experimental Section

### *Diketal (4.6)*



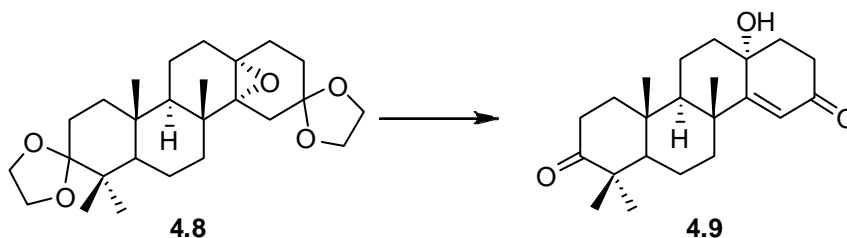
(AA-8-93) In a round bottom flask equipped with a Dean-Stark apparatus, 208.3 mg (0.56 mmol) of enone **(-)-3.41a** was dissolved in 27 mL of toluene. Pyridinium *para*-toluenesulfonate (PPTS), 45 mg (0.17 mmol), and 347 mg (5.6 mmol) of ethylene glycol were added and the reaction was put to reflux. After 2 hours the reaction was determined to be complete by TLC. Saturated aqueous sodium bicarbonate was added to the reaction, which was extracted three times with diethyl ether. The combined ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the compound came out between a 4:1 to 2:1 ratio of hexane:diethyl ether to give 210 mg (90 % yield) of the diketal **4.6** as a white solid. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ ppm 4.02-3.81 (m, 8 H), 2.25-1.10 (m, 20 H), 0.96 (s, 3 H), 0.92 (s, 3 H), 0.88 (s, 3 H), 0.82 (s, 3 H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ ppm 135.67, 125.40, 113.47, 109.30, 65.09, 65.00, 64.58, 64.39, 55.94, 53.37, 42.37, 38.21, 37.81, 37.16, 36.87, 33.80, 32.14, 30.86, 29.74, 27.09, 23.06, 20.21, 19.96, 18.64, 17.99, 16.54.

***Diketal epoxide (4.8)***



(AA-8-54) To a stirring solution, at 0°C, of 11.8 mg (0.028 mmol) of diketal **4.6** in 2 mL of methylene chloride was added 9 mg (0.037 mmol) of 73 % m-chloroperbenzoic acid and 3.5 mg (0.042 mmol) of sodium bicarbonate. After stirring for 1 hour the reaction was quenched with 10 % aqueous sodium sulfite and saturated sodium bicarbonate and extracted three times with diethyl ether. The combined diethyl ether layers were washed with brine, dried with magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the compound came out at a solvent ratio of 4:1 to 2:1 hexane to diethyl ether to give 12 mg (100 % yield) of the epoxide **4.8**. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ ppm 4.02-3.81 (m, 8 H), 2.11-1.13 (m, 20 H), 1.01 (s, 3 H), 0.90 (s, 3 H), 0.85 (s, 3 H), 0.82 (s, 3 H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ ppm 113.30, 107.88, 69.73, 65.04, 64.98, 64.52, 64.17, 62.37, 52.79, 46.48, 42.31, 37.58, 36.90, 36.78, 35.17, 35.05, 29.39, 28.23, 28.14, 27.01, 23.11, 20.07, 18.05, 17.76, 16.13, 15.97.

***(4aR,4bR,6aR,10bR)-6a-Hydroxy-1,1,4a,10b-tetramethyl-4,4a,4b,5,6,6a,7,8,10b,11,12,12a-dodecahydrochrysene-2,9(1H,3H)-dione (4.9)***

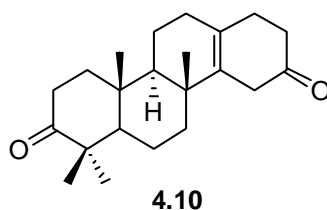


(AA-8-56) Epoxy diketal **4.8**, 12.5 mg (0.028 mmol), and 0.5 mg (0.003 mmol) of p-toluenesulfonic acid were dissolved in 0.5 mL of dry acetone and left to stir at room temperature.



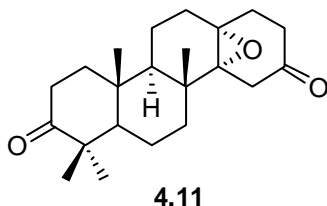
After 5 hours the reaction was diluted with diethyl ether, quenched with saturated aqueous sodium bicarbonate and extracted three times with diethyl ether. The combined ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether to produce 4.2 mg (44 % yield) of the  $\beta$ -hydroxy enone **4.9**.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 5.90 (s, 1 H), 2.90-2.30 (m, 4 H), 2.18-1.66 (m, 10 H), 1.55-1.34 (m, 5 H), 1.18 (s, 3 H), 1.09 (s, 6 H), 1.07 (s, 3 H)

***(4aR,4bR,10bR)-1,1,4a,10b-Tetramethyl-4,4a,4b,5,6,7,8,10,10b,11,12,12a-dodecahydrochrysene-2,9(1H,3H)-dione (4.10)***



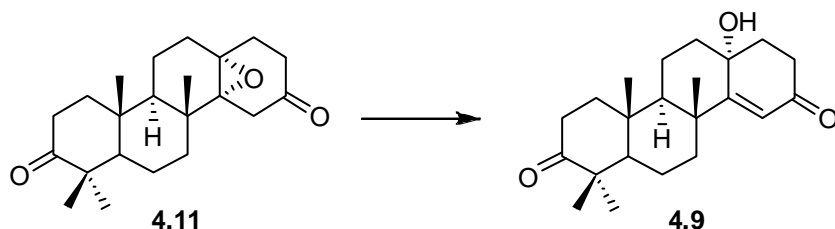
(AA-8-102) To a solution of 32 mg (0.77 mmol) of diketal **4.6** dissolved in 2.5 mL of THF was added 2.5 mL of 1 M HCl and the reaction heated to 40°C. After 1 hour the reaction was complete, as determined by TLC, so it was diluted with diethyl ether, quenched with saturated aqueous sodium bicarbonate and extracted three times with diethyl ether. The combined ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography. Using a solvent gradient of hexane and diethyl ether, 18.8 mg (74 % yield) of the dione **4.10** was isolated.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 2.81 (q,  $J = 20.2$  Hz, 2 H), 2.59-2.34 (m, 4 H), 2.32-2.25 (m, 2 H), 2.12-2.04 (m, 2 H), 2.03-1.97 (m, 1 H), 1.80 (dt,  $J = 12.6, 2.3$  Hz, 1 H), 1.72-1.62 (m, 2 H), 1.59-1.52 (m, 2 H), 1.51-1.42 (m, 3 H), 1.27-1.18 (m, 2 H), 1.09 (s, 3 H), 1.05 (s, 3 H), 1.01 (s, 3 H), 0.96 (s, 3 H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  ppm 218.01, 212.36, 135.63, 127.57, 97.04, 55.35, 54.84, 47.49, 39.15, 38.54, 38.43, 37.78, 37.58, 34.16, 32.16, 30.55, 26.85, 21.05, 20.15, 19.76, 18.37, 16.42.

***Diketone epoxide (4.11)***



(AA-10-115) To a stirring solution, at 0°C, of 6.7 mg (0.02 mmol) of diketone **4.10** in 0.5 mL of methylene chloride was added 6 mg (0.026 mmol) of 73 % m-chloroperbenzoic and stirred for 2 hours then warmed up to room temperature. After 2 more hours, the reaction was quenched with 10 % aqueous sodium sulfite and saturated aqueous sodium bicarbonate and extracted three times with diethyl ether. The combined diethyl ether layers were washed with brine, dried with magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the compound came out at a solvent ratio of 2:1 hexane to diethyl ether to give 5 mg (72 % yield) of the epoxide **4.11**. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 2.69 (q, *J* = 18.5 Hz, 2 H), 2.47 (dd, *J* = 8.5, 6.1 Hz, 2 H), 2.44-2.36 (m, 1 H), 2.20-1.96 (m, 4 H), 1.93-1.84 (m, 2 H), 1.77-1.73 (m, 1 H), 1.62-1.56 (m, 2 H), 1.50-1.33 (m, 6 H), 1.09 (s, 3 H), 1.08 (s, 3 H), 1.03 (s, 3 H), 0.91 (s, 3 H).

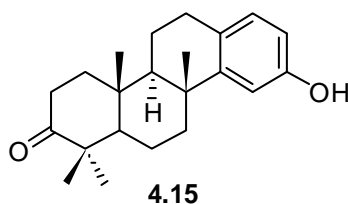
***(4aR,4bR,6aR,10bR)-6a-Hydroxy-1,1,4a,10b-tetramethyl-4,4a,4b,5,6,6a,7,8,10b,11,12,12a-dodecahydrochrysene-2,9(1H,3H)-dione (4.9)***



(AA-8-84) Epoxide **4.11**, 17 mg (0.05 mmol), and 14 mg (0.1 mmol) of potassium carbonate were dissolved in 1 mL of ethanol. After 4 hours the reaction was diluted with diethyl ether,

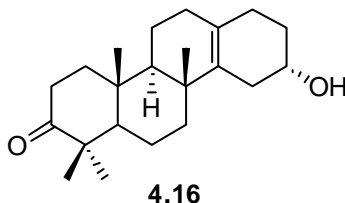
quenched with saturated aqueous ammonium chloride and extracted three times with diethyl ether. The combined ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography. Using a solvent gradient of hexane and diethyl ether, 17 mg (100 % yield) of  $\beta$ -hydroxyenone **4.9** was obtained.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 5.90 (s, 1 H), 2.90-2.30 (m, 4 H), 2.18-1.66 (m, 10 H), 1.55-1.34 (m, 5 H), 1.18 (s, 3 H), 1.09 (s, 6 H), 1.07 (s, 3 H).

***(4aR,4bR,10bR)-9-Hydroxy-1,1,4a,10b-tetramethyl-3,4,4a,4b,5,6,10b,11,12,12a-decahydrochrysen-2(1H)-one (4.15)***



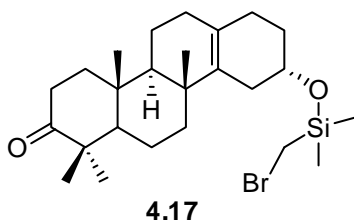
(AA-8-86) To a suspension of 4 mg (0.1 mmol) of 60% NaH in 0.1 mL of THF was added a solution of 8.7 mg (0.025 mmol) of  $\beta$ -hydroxy enone **4.9** in 0.5 mL of THF. After 10 minutes, 100  $\mu\text{L}$  (0.54 mmol) of bromomethyldimethylsilylchloride was added. After 30 minutes the reaction was determined to be complete by TLC, so it was diluted with diethyl ether, quenched with saturated aqueous ammonium chloride and extracted three times with diethyl ether. The combined ether layers were washed with brine, dried over magnesium sulfate, and concentrated. After crude NMR it was determined to be the phenol **4.15**, no further purification was done.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 6.90 (d,  $J = 8.2$  Hz, 1 H), 6.73 (d,  $J = 2.3$  Hz, 1 H), 6.58 (dd,  $J = 8.2, 2.7$  Hz, 1 H), 4.55 (s, 1 H), 2.92-2.84 (m, 1 H), 2.80-2.71 (m, 1 H), 2.60-2.44 (m, 2 H), 2.86 (dt,  $J = 12.5, 3.1$  Hz, 1 H), 2.09-2.03 (m, 1 H), 1.83-1.66 (m, 6 H), 1.52-1.46 (m, 4 H), 1.35-1.32 (m, 2 H), 1.22 (s, 3 H), 1.11 (s, 3 H), 1.09 (s, 3 H), 1.02 (s, 3 H).

**(4aR,4bR,9S,10bR)-9-Hydroxy-1,1,4a,10b-tetramethyl-3,4,4a,4b,5,6,7,8,9,10,10b,11,12,12a-tetradecahydrochrysen-2(1H)-one (4.16)**



(AA-8-103) K-selectride, 57  $\mu$ L (0.057 mmol), was added to a solution of 18.8 mg (0.057 mmol) of diketone **4.10** in 1.4 mL of THF at  $-78^{\circ}\text{C}$ . After 30 minutes the reaction was diluted with diethyl ether, quenched with saturated aqueous ammonium chloride and extracted three times with diethyl ether. The combined ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography. Using a solvent gradient of hexane and diethyl ether the compound came out between a 4:1 to 2:1 solvent ratio of hexane to diethyl ether producing, 17.3 mg (91 % yield) of keto alcohol **4.16**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 4.07 (bs, 1 H), 2.57-2.41 (m, 2 H), 2.22 (bd,  $J = 16.7$  Hz, 1 H), 2.07-1.90 (m, 6 H), 1.85 (dt,  $J = 12.6, 3.2$  Hz, 2 H), 1.75-1.40 (m, 8 H), 1.19 (dd,  $J = 12.0, 2.1$  Hz, 2 H), 1.08 (s, 3 H), 1.05 (s, 3 H), 1.00 (s, 3 H), 0.95 (s, 3 H).

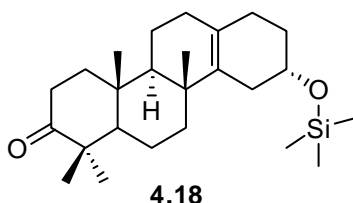
**(4aR,4bR,9S,10bR)-9-((Bromomethyl)dimethylsilyloxy)-1,1,4a,10b-tetramethyl-3,4,4a,4b,5,6,7,8,9,10,10b,11,12,12a-tetradecahydrochrysen-2(1H)-one (4.17)**



(AA-8-104) Alcohol **4.16** 17 mg (0.052 mmol), 14.3 mg (0.21 mmol) of imidazole, and 3.2 mg (0.026 mmol) of DMAP were dissolved in 0.5 mL of methylene chloride. After 5 minutes, 15

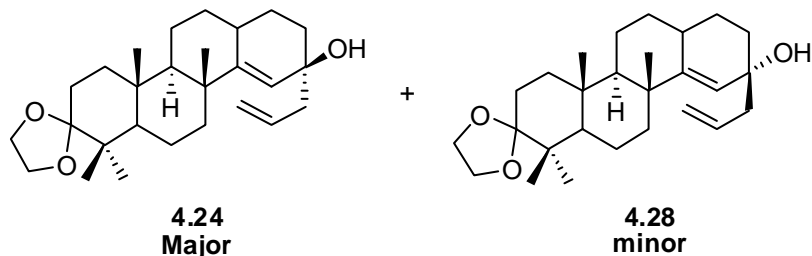
$\mu\text{L}$  (0.1 mmol) of bromomethyldimethylsilylchloride was added to the solution at room temperature. After 1 hour the reaction was diluted with diethyl ether and water and extracted three times with diethyl ether. The ether layers were washed with brine, dried over magnesium sulfate, concentrated and immediately purified by silica gel column chromatography. The silica was neutralized by washing with 1 % triethyl amine in hexane then a solvent gradient of hexane and ethyl ether was used and the compound came out between a solvent ratio of 19:1 to 9:1 hexane to diethyl ether to produce 15 mg (60 % yield) of the bromomethyldimethylsilylether **4.17**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 4.02-3.97 (m, 1 H), 2.57-2.40 (m, 4 H), 2.22 (bd,  $J = 16.1$  Hz, 1 H), 2.04-1.82 (m, 7 H), 1.73-1.65 (m, 1 H), 1.62-1.51 (m, 4 H), 1.49-1.39 (m, 3 H), 1.21-1.13 (m, 2 H), 1.08 (s, 3 H), 1.05 (s, 3 H), 0.99 (s, 3 H), 0.95 (s, 3 H), 0.27 (s, 6 H).

***(4aR,4bR,9S,10bR)-1,1,4a,10b-Tetramethyl-9-(trimethylsilyloxy)-3,4,4a,4b,5,6,7,8,9,10,10b,11,12,12a-tetradecahydrochrysen-2(1H)-one (4.18)***



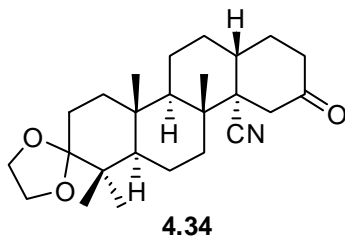
(AA-8-105) Tributyltin hydride, 17  $\mu\text{L}$  ( 0.062 mmol), was added to a refluxing solution of 0.5 mg (0.0031 mmol) of AIBN, and 15 mg ( 0.031 mmol) of bromo-methyldimethylsilylether compound **4.17** in 4 mL of benzene. After 5 hours the reaction was diluted with diethyl ether and water and extracted three times with diethyl ether. The ether layers were washed with brine, dried over magnesium sulfate, concentrated and immediately purified by silica gel column chromatography. Using a solvent gradient of hexane and diethyl ether the compound came out between a solvent ratio of 19:1 to 9:1 of hexane to diethyl ether to produce 6 mg (50 % yield) of the trimethyl silylether **4.18**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 3.90-3.82 (m, 1 H), 2.57-2.39 (m, 2 H), 2.22 (bd,  $J = 14.6$  Hz, 1 H), 2.01-1.80 (m, 7 H), 1.73-1.59 (m, 2 H), 1.55-1.37 (m, 6 H), 1.21-1.13 (m, 2 H), 1.08 (s, 3 H), 1.05 (s, 3 H), 1.00 (s, 3 H), 0.95 (s, 3 H), 0.12 (s, 9 H).

**(4aR,4bR,9S,10bR)-9-Allyl-1,1,4a,10b-tetramethyl-  
3,4,4a,4b,5,6,6a,7,8,9,10b,11,12,12a-tetradecahydro-1H-spiro[chrysene-2,2'-  
[1,3]dioxolan]-9-ol (4.24) and (4aR,4bR,9R,10bR)-9-allyl-1,1,4a,10b-tetramethyl-  
3,4,4a,4b,5,6,6a,7,8,9,10b,11,12,12a-tetradecahydro-1H-spiro[chrysene-2,2'-  
[1,3]dioxolan]-9-ol (4.28)**



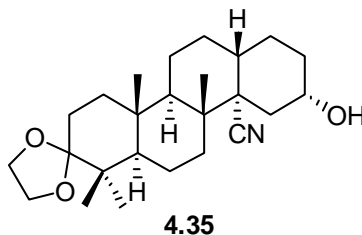
(AA-10-55) A 1.7 M allyl magnesium chloride, 110  $\mu\text{L}$  (0.185 mmol), solution was added to a solution of 46 mg (0.123 mmol) of enone (-)-**3.41a** in 2 mL of THF at  $-78^\circ\text{C}$ . After 30 minutes the reaction was diluted with diethyl ether, quenched with saturated aqueous ammonium chloride and extracted three times with diethyl ether. The combined ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography. Using a solvent gradient of hexane and diethyl ether, 30 mg (59% yield) of the Major product **4.24** (less polar), and 10 mg (20% yield) of the minor product **4.28** (more polar) were obtained. Major product (**4.24**):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 5.95-5.84 (m, 1 H), 5.22 (s, 1 H), 5.18-5.11 (m, 2 H), 3.99-3.87 (m, 4 H), 2.36-2.19 (m, 3 H), 1.90-1.74 (m, 3 H), 1.69-1.40 (m, 12 H), 1.29-1.12 (m, 4 H), 1.04 (s, 3 H), 0.94 (s, 3 H), 0.90 (s, 3 H), 0.86 (s, 3 H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  ppm 153.61, 134.25, 120.70, 118.81, 113.45, 70.75, 65.06 (2 C), 58.27, 53.89, 46.82, 42.35, 39.93, 38.98, 37.80, 37.14, 35.56, 33.53, 33.26, 27.38, 27.07, 23.11, 22.13, 21.18, 20.16, 18.76, 16.36; minor product (**4.28**):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 5.88-5.77 (m, 1 H), 5.30 (s, 1 H), 5.10 (s, 1 H), 5.08-5.05 (m, 1 H), 3.98-3.85 (m, 4 H), 2.27 (d,  $J = 7.3$  Hz, 2 H), 2.15-2.07 (m, 1 H), 1.94-1.87 (m, 1 H), 1.84-1.68 (m, 3 H), 1.64-1.10 (m, 15 H), 1.00 (s, 3 H), 0.93 (s, 3 H), 0.90 (s, 3 H), 0.84 (s, 3 H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  ppm 155.45, 134.45, 120.45, 118.24, 113.34, 70.14, 65.09, 65.04, 57.58, 53.56, 47.65, 42.35, 39.89, 39.18, 37.72, 37.12, 35.98, 34.04, 33.97, 27.51, 27.08, 23.10, 23.07, 20.86, 20.13, 18.71, 16.46.

**(4aR,4bR,6aR,10aS,10bR,12aR)-1,1,4a,10b-Tetramethyl-9-oxohexadecahydro-1H-spiro[chrysene-2,2'-[1,3]dioxolane]-10a-carbonitrile (4.34)**



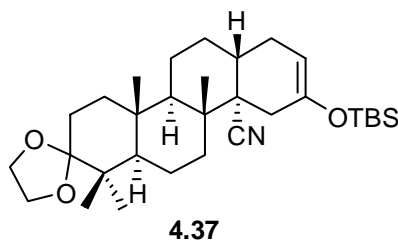
(AA-10-92) Enone (-)-**3.41a**, 45 mg (0.12 mmol), was dissolved in 0.6 mL of THF and 5 mL of 2:1 DMF:H<sub>2</sub>O. To this solution was added 39 mg (0.72 mmol) of ammonium chloride, 117 mg (1.8 mmol) potassium cyanide and the reaction was heated to 85 – 90°C overnight. After determining, by TLC, that the starting material was completely consumed the reaction was cooled to room temperature, diluted with water and extracted three times with diethyl ether. The combined diethyl ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography using a solvent gradient of hexane and diethyl ether in which the compound came out at a 2:1 solvent ratio of hexane to diethyl ether to give 40 mg (83 % yield) of the β-nitrile compound **4.34** as a white solid. Rotation:  $[\alpha]_D^{25} = -27^\circ$  (c = 0.045, CHCl<sub>3</sub>) <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 3.97 – 3.86 (m, 4 H), 2.54 (d, *J* = 14.1 Hz, 1 H), 2.46 (dm, *J* = 14.6 Hz, 1 H), 2.40 (d, *J* = 14.1 Hz, 1 H), 2.29 (td, *J* = 14.1, 6.4 Hz, 1 H), 2.00 – 1.94 (m, 2 H), 1.87 – 1.74 (m, 3 H), 1.68 – 1.57 (m, 6 H), 1.52 – 1.26 (m, 9 H), 1.05 (s, 3 H), 0.91 (s, 3 H), 0.83 (s, 3 H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ ppm 207.55, 120.50, 112.88, 65.15, 65.00, 55.39, 53.79, 52.43, 43.95, 42.36, 40.64, 40.49, 37.27, 37.15, 36.92 (2 C), 30.71 (2 C), 27.07, 23.02, 20.12, 20.10, 18.28, 16.52, 15.41. HRMS calcd. for C<sub>25</sub>H<sub>37</sub>NO<sub>3</sub> (M+H<sup>+</sup>) 400.2852, found 400.2851.

**(4aR,4bR,6aR,9S,10aS,10bR,12aR)-9-Hydroxy-1,1,4a,10b-tetramethylhexadecahydro-1H-spiro[chrysene-2,2'-[1,3]dioxolane]-10a-carbonitrile (4.35)**



(AA-10-84) Sodium borohydride, 0.2 mg (0.0042 mmol), was added to a solution of 6.8 mg (0.017 mmol) of  $\beta$ -cyanoketone **4.34** in 0.5 mL of ethanol at 0°C. After 30 minutes the reaction was diluted with diethyl ether, quenched with saturated aqueous ammonium chloride and extracted three times with diethyl ether. The combined ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography. Using a solvent gradient of hexane and diethyl ether the compound came out at a 2:1 solvent ratio of hexane to diethyl producing, 5 mg (73 % yield) of cyano alcohol **4.35**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 4.22-4.17 (m, 1 H), 3.96-3.85 (m, 4 H), 2.10-2.05 (m, 2 H), 1.93-1.87 (m, 1 H), 1.82-1.24 (m, 19 H), 0.96 (s, 3 H), 0.91 (s, 3 H), 0.88 (s, 3 H), 0.83 (s, 3 H).

**(4aR,4bR,6aR,10aS,10bR,12aR)-9-(tert-Butyldimethylsilyloxy)-1,1,4a,10b-tetramethyl-3,4,4a,4b,5,6,6a,7,10,10a,10b,11,12,12a-tetradecahydro-1H-spiro[chrysene-2,2'-[1,3]dioxolane]-10a-carbonitrile (4.37)**

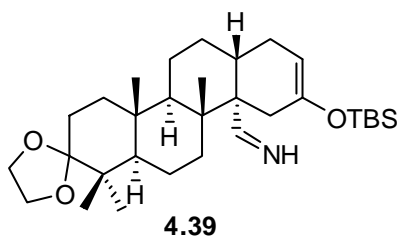


(AA-11-20)  $\beta$ -Cyanoketone **4.34**, 25 mg (0.063 mmol), was dissolved in 2.5 mL of methylene chloride followed by the addition of 0.070 ml of triethylamine and stirred at room temperature



for 15 minutes. The solution was put to 0°C, and then 0.050 ml of *tert*-butyldimethylsilyltriflate (TBSOTf) was added. After 2 hours the reaction was diluted with saturated aqueous sodium bicarbonate and extracted three times with diethyl ether. The combined diethyl ether layers were washed with brine, dried over magnesium sulfate, concentrated and immediately purified by silica gel column chromatography in which the silica was deactivated by washing with 1 % triethylamine. Using a solvent gradient of hexane and diethyl ether the compound came out at a 9:1 solvent ratio of hexane to diethyl ether to give 32 mg (100 % yield) of the silyl enol ether **4.37** as colorless crystals. Rotation:  $[\alpha]_D^{25} = -16^\circ$  ( $c = 0.055$ ,  $\text{CHCl}_3$ )  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 4.87 – 4.84 (m, 1 H), 3.97 – 3.85 (m, 4 H), 2.82 (dm,  $J = 17.3$  Hz, 1 H), 2.12 – 1.93 (m, 3 H), 1.83 – 1.22 (m, 15 H), 0.99 (s, 3 H), 0.91 (s, 12 H), 0.90 (s, 3 H), 0.83 (s, 3 H), 0.15 (s, 3 H), 0.14 (s, 3 H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  ppm 147.11, 122.14, 113.01, 102.75, 65.13, 65.00, 54.14, 52.43, 51.27, 42.35, 40.03, 37.47, 37.27, 36.84, 33.09, 33.01, 31.06, 30.01, 27.07, 25.92 (3 C), 23.07, 20.16 (2 C), 18.22, 18.17, 16.62, 15.55, -4.10, -4.18; HRMS calcd. for  $\text{C}_{31}\text{H}_{51}\text{NO}_3\text{Si}$  ( $\text{M}+\text{H}^+$ ) 514.3716, found 514.3718.

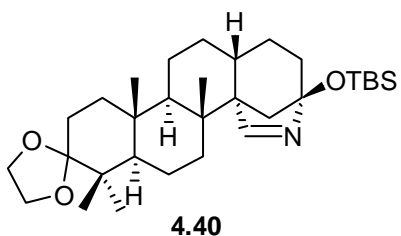
***((4aR,4bR,6aR,10aS,10bR,12aR)-9-(tert-Butyldimethylsilyloxy)-1,1,4a,10b-tetramethyl-3,4,4a,4b,5,6,6a,7,10,10a,10b,11,12,12a-tetradecahydro-1H-spiro[chrysene-2,2'-[1,3]dioxolane]-10a-yl)methanimine (4.39)***



(AA-10-101) Silylenolether **4.37**, 10mg (0.0195 mmol), was dissolved in 1ml of dry toluene at room temperature. To this solution was added 100  $\mu\text{l}$  (0.100mmol) of 1.0 M diisobutylaluminum hydride in toluene. After 30 minutes the reaction was determined to be complete, by TLC (15:1  $\text{CH}_2\text{Cl}_2$ :MeOH), and it was diluted with saturated aqueous sodium bicarbonate and extracted three times with diethyl ether. The combined diethyl ether layers were washed with brine, dried

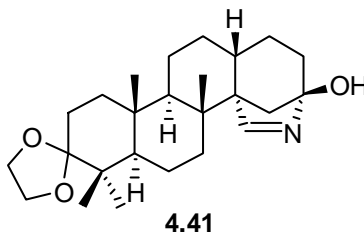
over magnesium sulfate, concentrated and purified by silica gel column chromatography in which the silica was deactivated by washing with 1 % triethylamine. Using a solvent gradient of hexane and diethyl ether the compound came out at a 3:1 solvent ratio of hexane to diethyl ether to give 5.8 mg (58 % yield) of the imine **4.39**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 8.38 (bs, 1 H), 4.69 – 4.68 (m, 1 H), 3.98 – 3.87 (m, 4 H), 2.19 – 2.03 (m, 2 H), 1.96 – 1.88 (m, 1 H), 1.85 – 1.77 (m, 2 H), 1.72 – 1.64 (m, 4 H), 1.51 – 1.46 (m, 5 H), 1.41 – 1.30 (m, 4 H), 1.23 – 1.14 (m, 2 H), 1.07 (s, 3 H), 0.93 (s, 3 H), 0.92 (s, 9 H), 0.90 (s, 3 H), 0.80 (s, 3 H), 0.14 (s, 3 H), 0.12 (s, 3 H); HRMS calcd. for  $\text{C}_{31}\text{H}_{53}\text{NO}_3\text{Si}$  ( $\text{M}+\text{H}^+$ ) 516.3828, found 516.3865.

***Iminal (4.40)***



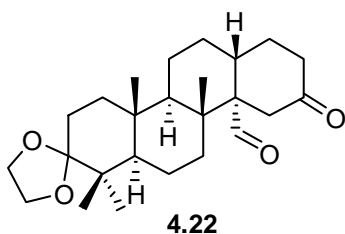
(AA-11-25) Imine **4.39**, 4 mg (0.0078 mmol), was dissolved in a 9:1:0.05 solution of dioxane to water to glacial acetic acid and left to stir at room temperature. After 24 hour, the reaction was diluted with diethyl ether, quenched slowly with saturated aqueous sodium bicarbonate and extracted three times with diethyl ether. The combined ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography. Using a solvent gradient of hexane and diethyl ether the compound came out at a 3:1 solvent ratio of hexane to diethyl producing, 2.4 mg (60 % yield) of cyclic siloxy iminal **4.40**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 7.70 (s, 1 H), 4.05-3.85 (m, 4 H), 2.03-1.64 (m, 6 H), 1.38-1.19 (m, 9 H), 0.93 (s, 3 H), 0.89 (bs, 15 H), 0.85 (s, 3 H), 0.14 (s, 3 H), 0.09 (s, 3 H).

### *Hemiminal (4.41)*



(Aibin's notebook – AS-4-62) Crude imine **4.39**, 46 mg (0.089 mmol), was dissolved in 3 mL of dry THF at room temperature followed by the addition of 178  $\mu\text{L}$  of 1.0M tetrabutylammonium fluoride (TBAF) in THF. After 1 hour the reaction was determined to be complete, by TLC (15:1  $\text{CH}_2\text{Cl}_2$ :MeOH), and it was diluted with water and extracted three times with diethyl ether. The combined diethyl ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography which produced 25 mg (69 % yield after 2 steps from the nitrile) of iminal **4.41** Rotation:  $[\alpha]_D^{25} = -43^\circ$  ( $c = 0.05$ ,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 7.76 (bs, 1 H), 4.00-3.84 (m, 4 H), 1.97 (bd,  $J = 10.5$  Hz, 1 H), 1.83 (td,  $J = 14.1, 3.5$  Hz, 1 H), 1.73-1.47 (m, 11 H), 1.41-1.15 (m, 9 H), 0.95 (s, 3 H), 0.93 (s, 3 H), 0.90 (s, 3 H), 0.85 (s, 3 H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  ppm 169.80, 113.29, 98.75, 65.11, 65.07, 54.63, 53.65, 47.47, 47.36, 42.41, 37.30, 37.18, 36.32, 33.92, 33.64, 32.08, 30.52, 28.92, 27.11, 23.07, 20.71, 20.11, 18.32, 18.21, 16.67; HRMS calcd. for  $\text{C}_{25}\text{H}_{39}\text{NO}_3$  ( $\text{M}+\text{H}^+$ ) 402.3008, found 402.3021.

**(4aR,4bR,6aR,10aS,10bR,12aR)-1,1,4a,10b-Tetramethyl-9-oxohexadecahydro-1H-spiro[chrysene-2,2'-[1,3]dioxolane]-10a-carbaldehyde (4.22)**



(Aibin's notebook – AS-4-65) Hemiiminal **4.41**, 10 mg (0.025 mmol), was dissolved in 1.25 mL (0.125 mmol) of 0.1 M acetic acid in THF and 1.25 mL of water then put to reflux. After refluxing for 5 hours the reaction was cooled to room temperature, diluted with diethyl ether and quenched with saturated aqueous sodium bicarbonate. The aqueous layer was extracted three times with diethyl ether. The combined ether layers were washed with brine, dried over magnesium sulfate, concentrated and purified by silica gel column chromatography to produce 8 mg (80 % yield) of aldehyde **4.22**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 10.31 (s, 1 H), 4.01-3.84 (m, 4 H), 2.49 (d,  $J = 14.1$  Hz, 1 H), 2.39 (dd,  $J = 16.0, 5.1$  Hz, 1 H), 2.27-2.18 (m, 1 H), 2.15-2.00 (m, 3 H), 1.92-1.16 (m, 15 H), 1.11 (s, 3 H), 0.98 (s, 3 H), 0.92 (s, 3 H), 0.81 (s, 3 H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  ppm 209.79, 209.07, 113.07, 65.15, 65.09, 60.04, 53.52, 53.13, 42.43, 42.40, 40.71, 39.89, 37.72, 37.61, 36.25, 31.16, 30.56, 28.99, 27.12, 23.03, 21.28, 20.09, 18.32, 17.28, 17.05 HRMS calcd. for  $\text{C}_{25}\text{H}_{38}\text{O}_4$  ( $\text{M}+\text{H}^+$ ) 403.2848, found 403.2856.

## Chapter 5, Experimental Section

All solutions were at pH 7.4.

### ***1 mM $\text{Ca}^{2+}$ physiological salt solution (PSS)***

The physiological salt solution (PSS) contained (in mmol/L) 150 NaCl, 3.6 KCl, 1.0  $\text{MgCl}_2$ , 1.0  $\text{CaCl}_2$ , 5.0 HEPES, and 5.0 glucose dissolved in purified water  $18 \Omega$ .<sup>13,14</sup>

### ***10 mM Ca<sup>2+</sup> PSS***

For the normalization of the constriction, a 10 mM Ca<sup>2+</sup> solution was made by increasing the CaCl<sub>2</sub> amount added to the physiological salt solution.<sup>13,14</sup>

### ***10 μM drug solution***

All the compounds tested were made by first dissolving the compound in no more than 0.1% volume of anhydrous DMSO relative to the total volume of solution made. Then the DMSO solution was added to the PSS solution making a 10 μM solution.

### ***10 nM ET-1 solution***

10 μg of ET-1 was dissolved in 400 μL of anhydrous DMSO. 200 μL of the ET-1 solution was added to 200 mL of PSS to make a 10 nM solution of ET-1.

### ***SMA preparation:***

The SMA was isolated from a Mongolian gerbil using a protocol approved by the Institutional Animal Care and Use Committee at Kansas State University. The gerbils were anesthetized with 4 % tribromoethanol (TBE) (10 μL/g) and decapitated. Temporal bones were removed, opened, and placed into a microdissection chamber containing PSS at 4°C. All the excess tissue and bone fragments around the cochlea were carefully removed; care was taken not to break the cochlea. Removing the otic capsule opened the cochlea, the bone surrounding the modiolus was carefully removed, the cochlear nerve was severed, and the SMA was isolated, carefully untangled and cut into 7-10 segments (approximately 200 μm long).<sup>13,14</sup>

### ***Superfusion of SMA:***

Vessel segments were transferred into a superfusion chamber mounted on the stage of an inverted microscope. The vessel was held in place by two blunted glass needles mounted on micromanipulators, and superfused. All experiments were performed at 37°C. The images of the vessel were displayed on a monitor and recorded on videotape. The outer vascular diameter was

measured by video edge detectors and recorded on a chart recorder as illustrated in Figure 5.3.<sup>13,14</sup>

## References

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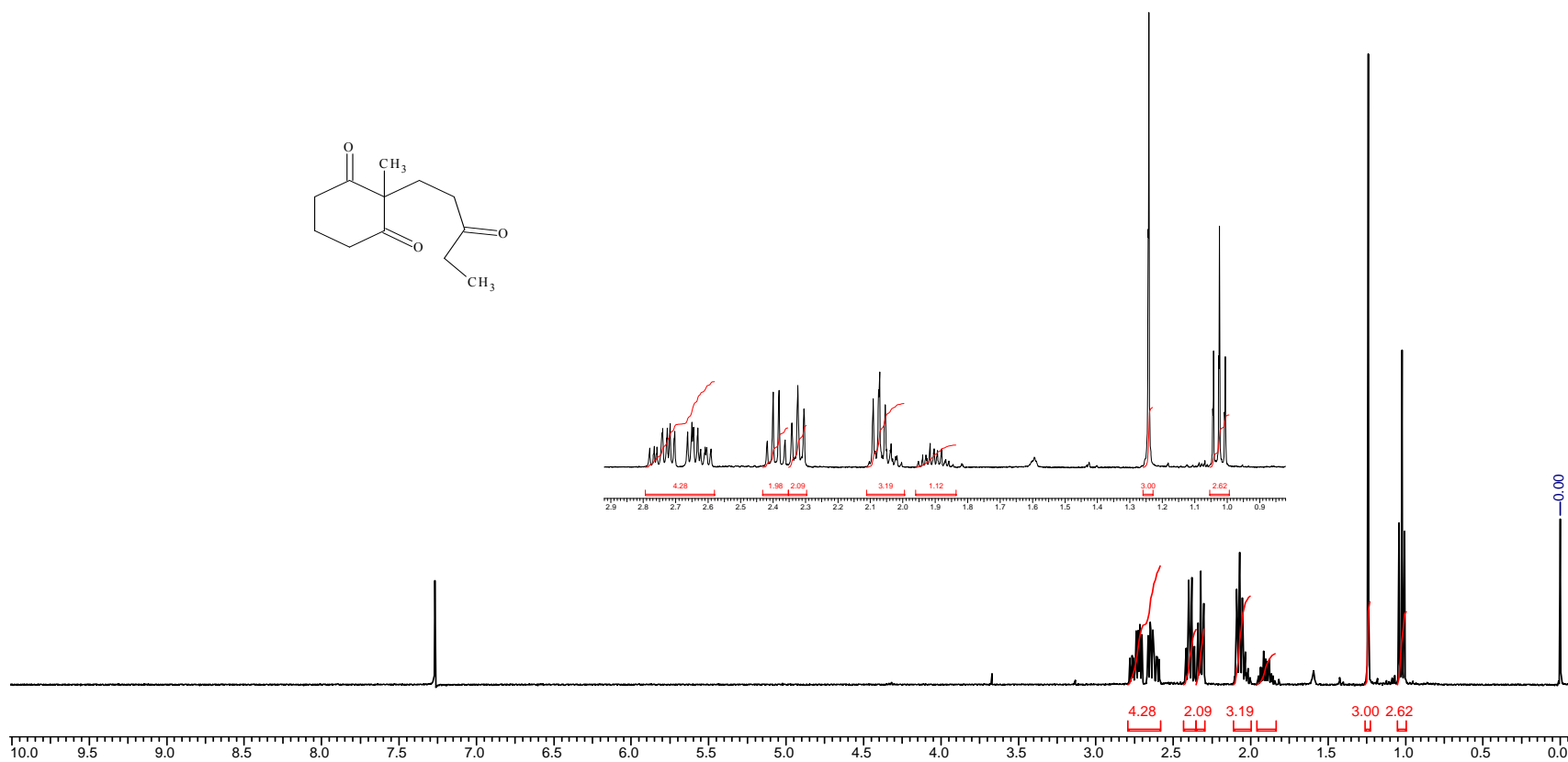
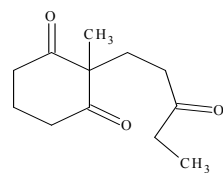
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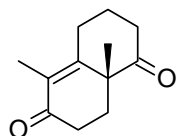
# Appendix A - Chapter 2 $^1\text{H}$ and $^{13}\text{C}$ NMR spectras

## $^1\text{H}$ NMR of Compound 2.4

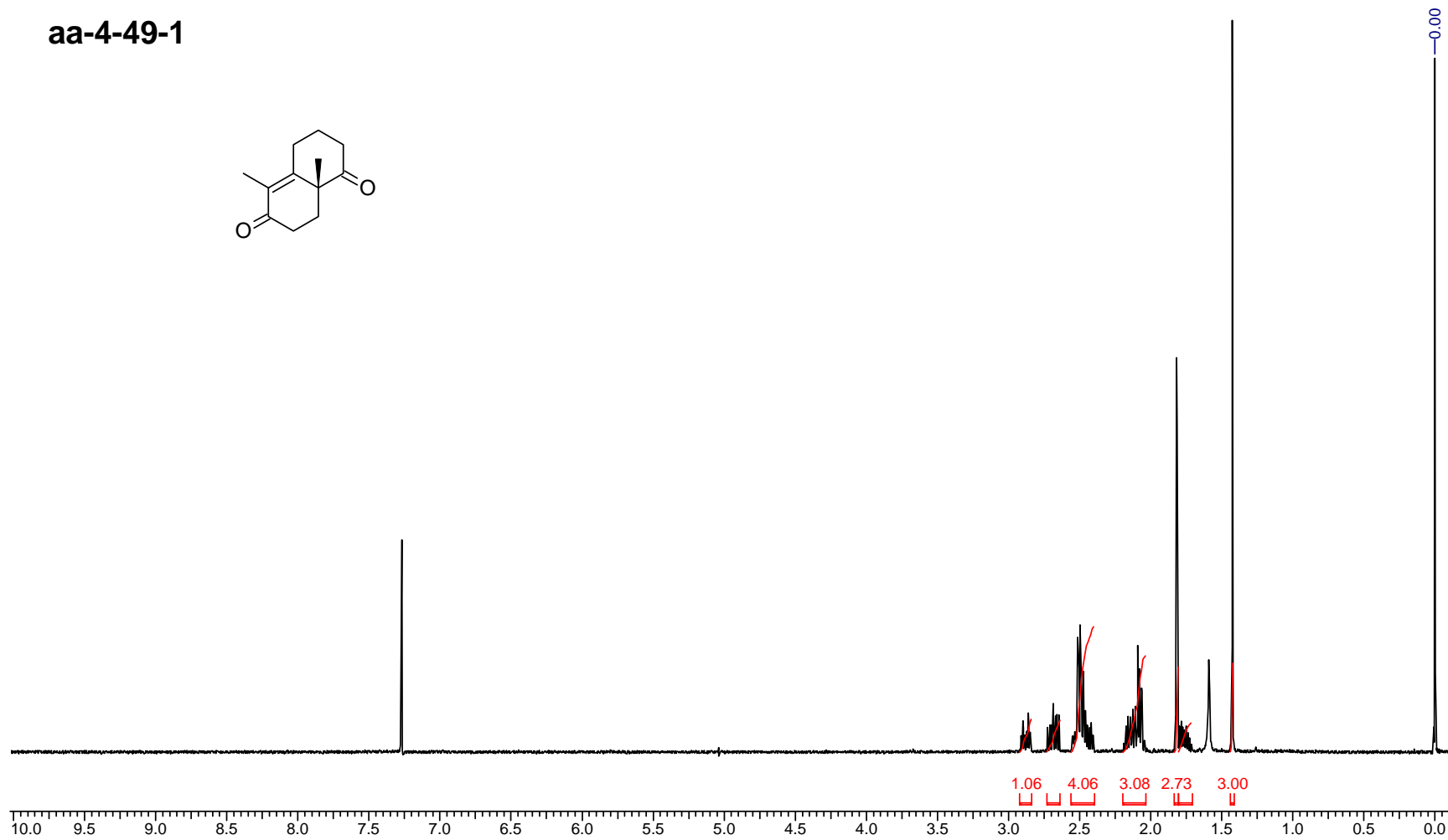


# <sup>1</sup>H NMR of Compound (-)-2.5

aa-4-49-1

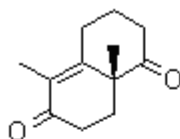


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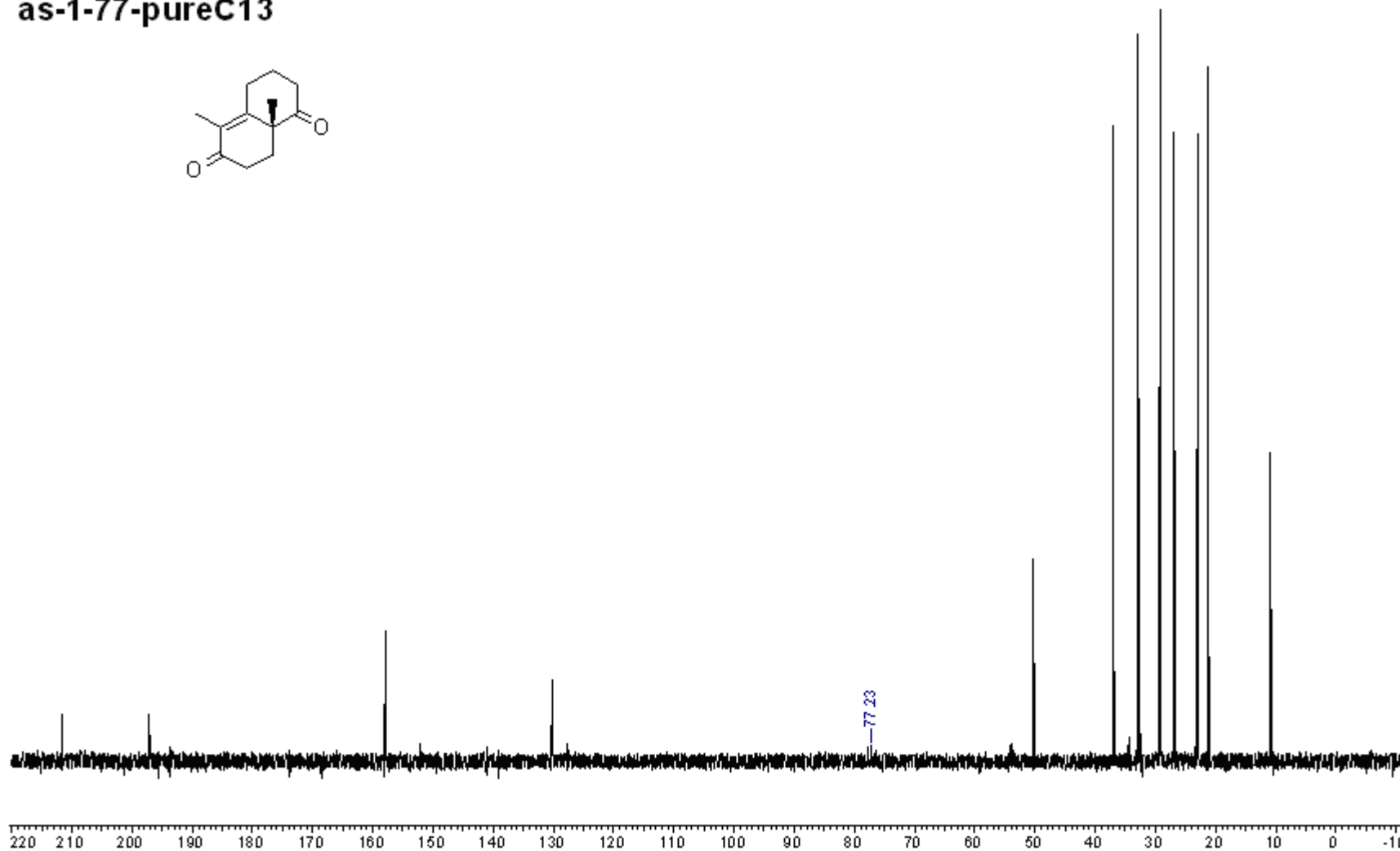


<sup>13</sup>C NMR of Compound (-)-2.5

as-1-77-pureC13



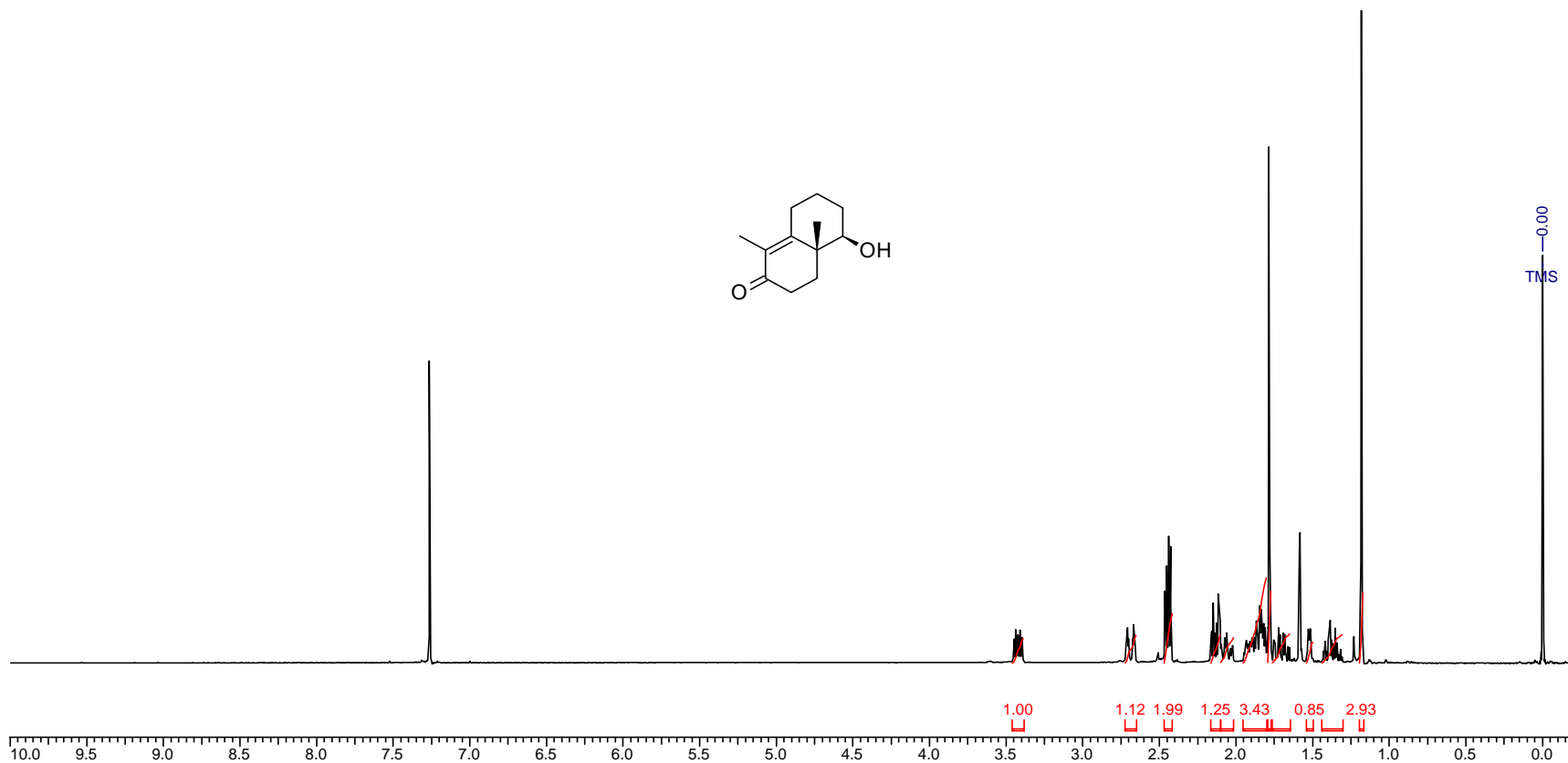
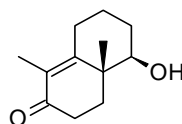
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# <sup>1</sup>H NMR of Compound (-)-2.9

## AA-11-14-1pure

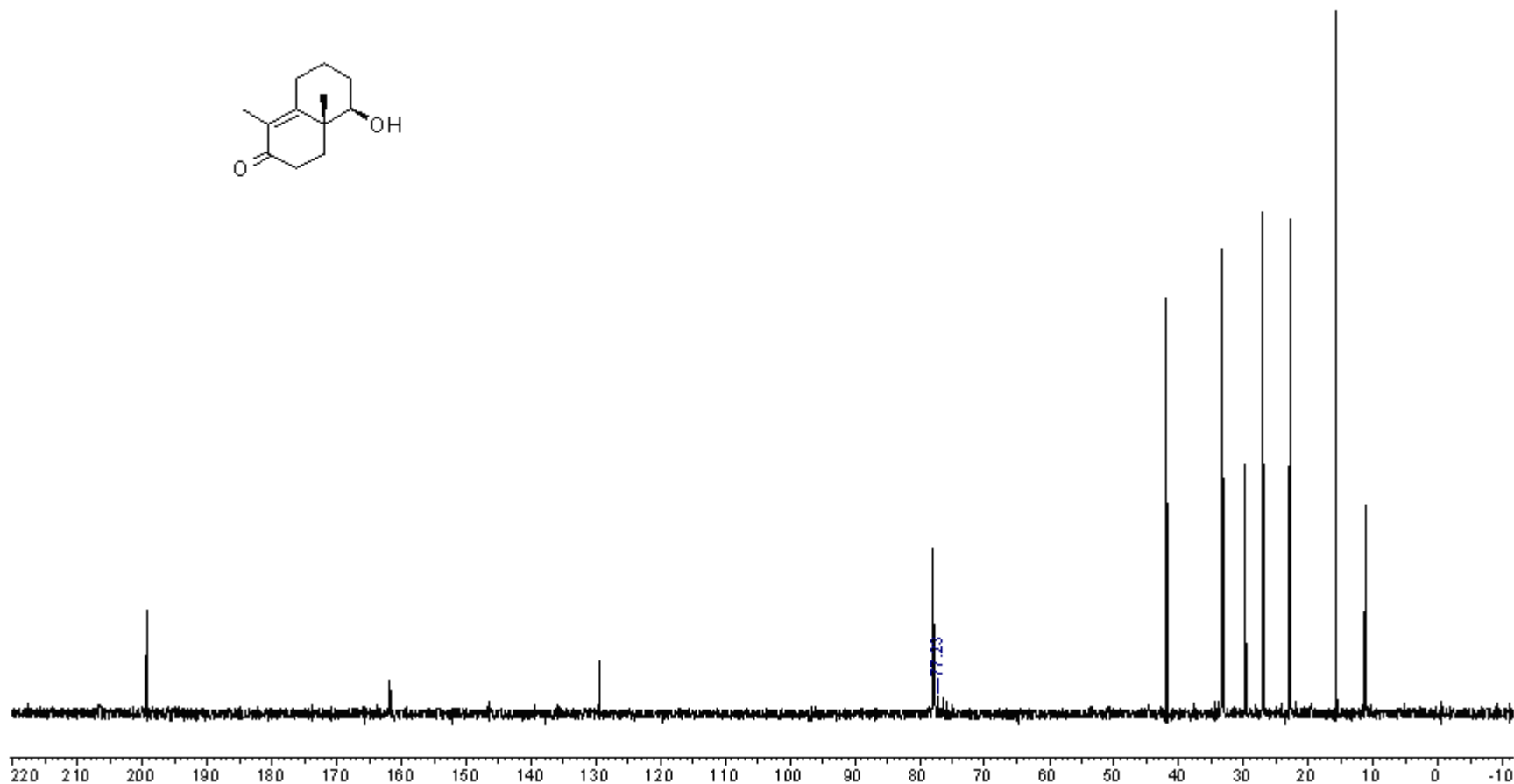
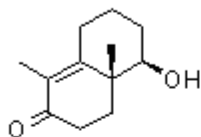
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Solvent	cdcl3	Sweep Width (Hz)	4797.03		Temperature (grad C)	25.000			



# <sup>13</sup>C NMR of Compound (-)-2.9

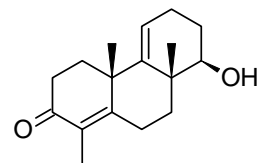
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Frequency (MHz)	50.29	Nucleus	13C	Number of Transients	500	Original Points Count	18720	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	12500.00	Temperature (grad C)	29.000				

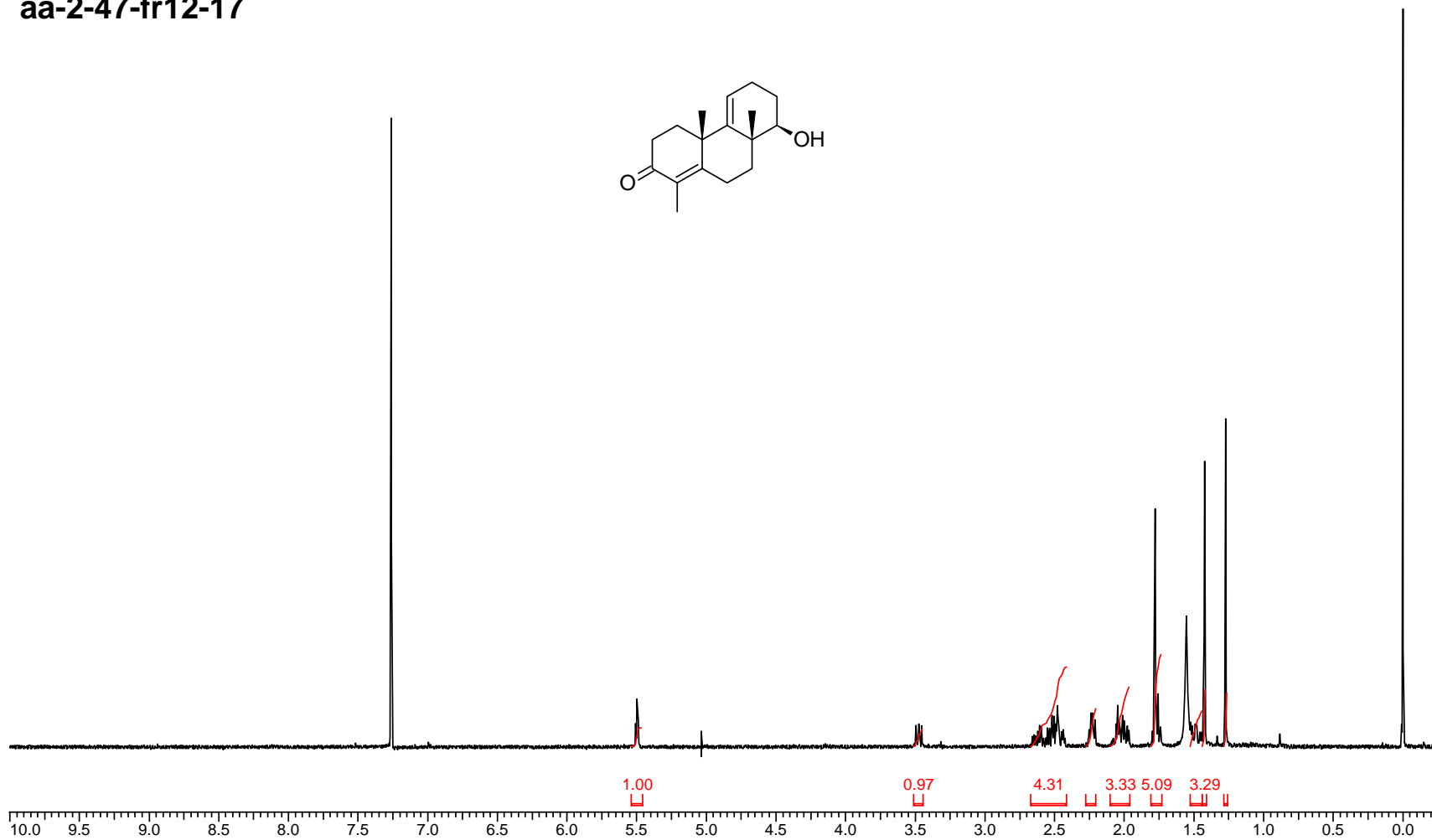


# <sup>1</sup>H NMR of Compound (+)-2.2

aa-2-47-fr12-17



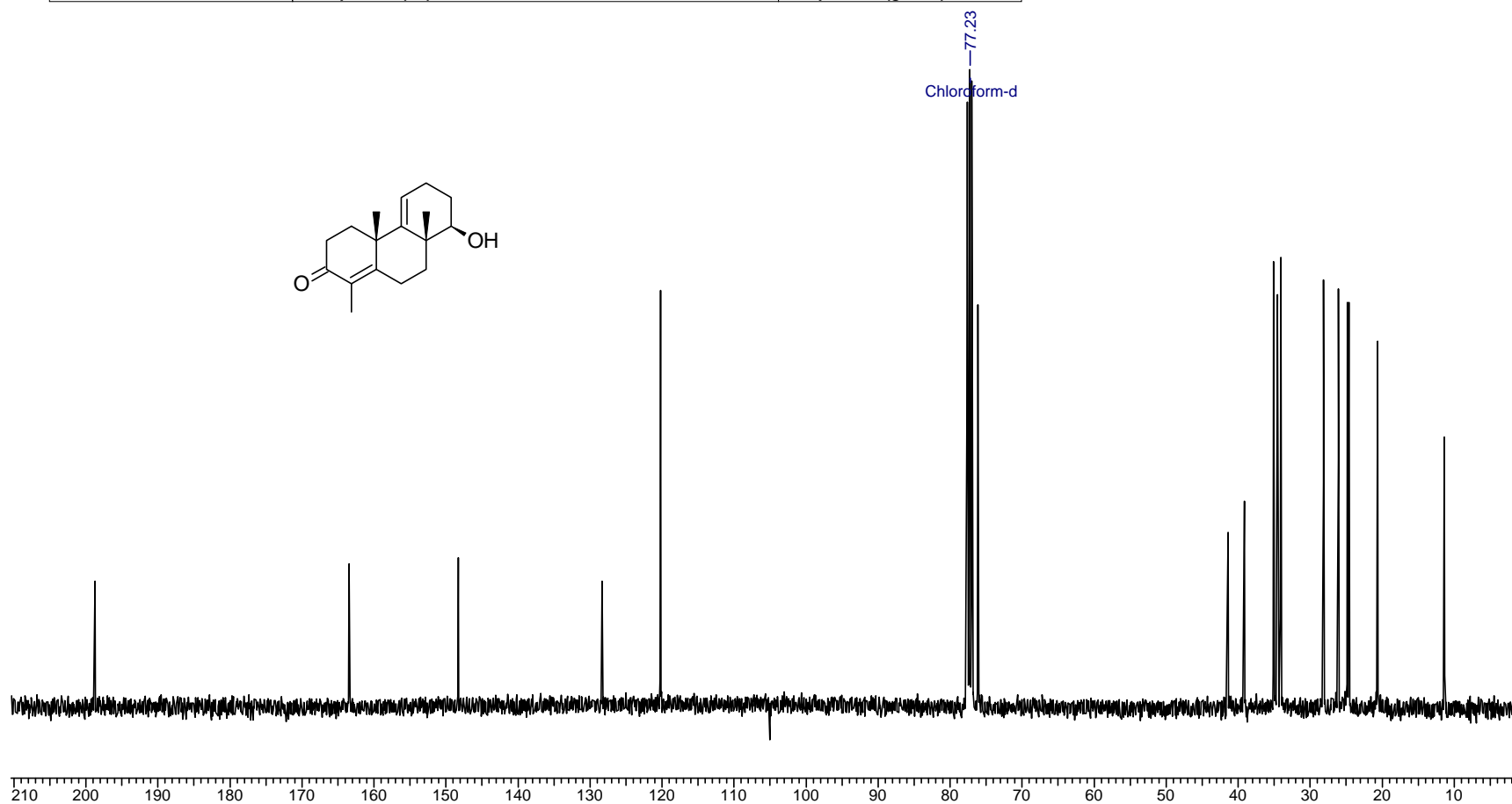
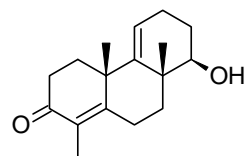
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# <sup>13</sup>C NMR of Compound (+)-2.2

## AA-11-18-pure-C13

<b>Acquisition Time (sec)</b>	1.3582	<b>Comment</b>	Std proton	<b>Date</b>	Aug 27 2008				
<b>Frequency (MHz)</b>	100.53	<b>Nucleus</b>	13C	<b>Number of Transients</b>	2000	<b>Original Points Count</b>	31375	<b>Points Count</b>	32768
<b>Solvent</b>	cdcl3	<b>Sweep Width (Hz)</b>	24125.45	<b>Temperature (grad C)</b>	25.000				





**Table 2: Crystal data and structure refinement for compound (+)-2.2**

Table 1. Crystal data and structure refinement for aa0301m.

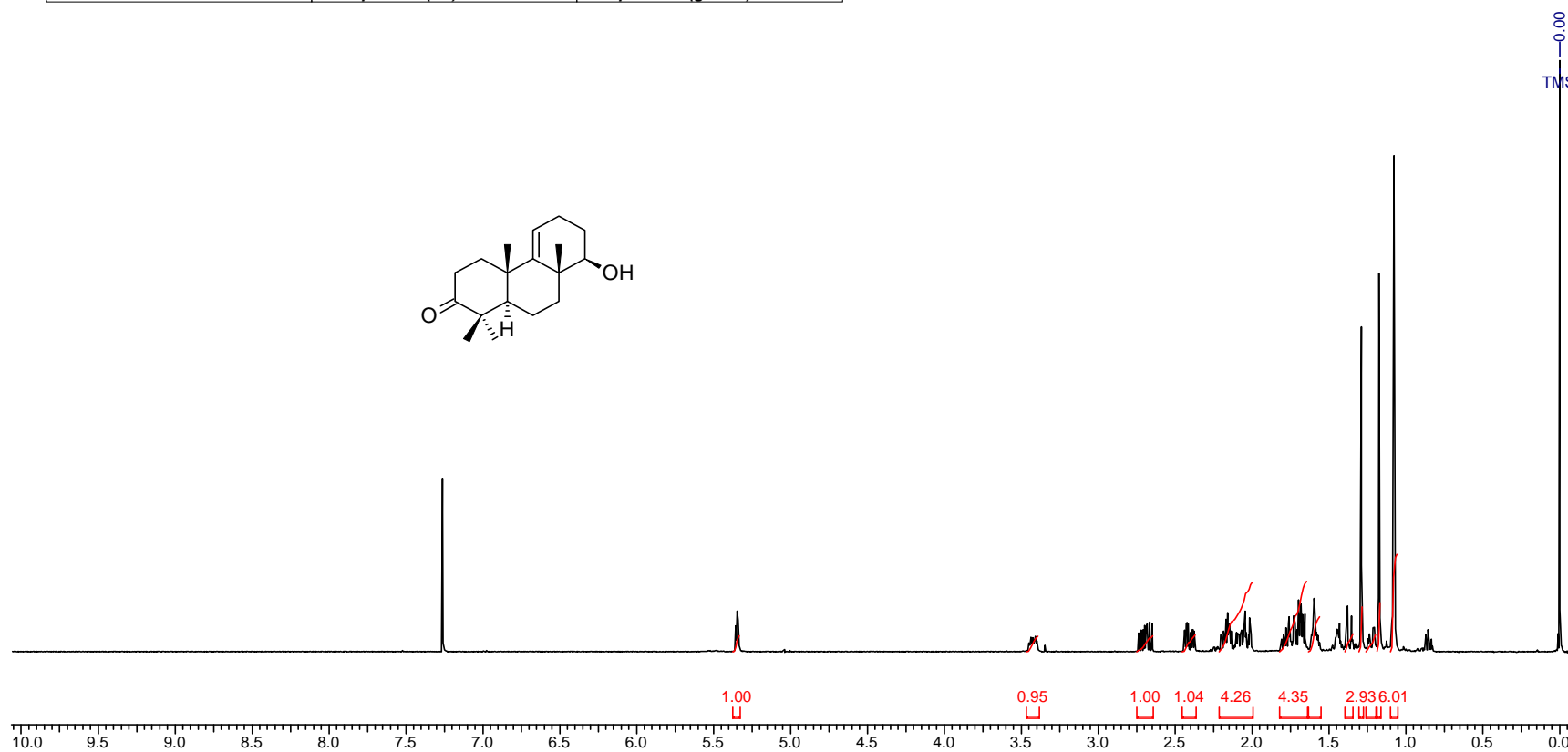
Identification code	aa0301m	
Empirical formula	C <sub>17</sub> H <sub>24</sub> O <sub>2</sub>	
Formula weight	260.36	
Temperature	203(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)	
Unit cell dimensions	a = 7.3026(8) Å	α = 90°.
	b = 19.157(3) Å	β = 105.620(6)°.
	c = 10.5171(12) Å	γ = 90°.
Volume	1416.9(3) Å <sup>3</sup>	
Z	4	
Density (calculated)	1.220 g/cm <sup>3</sup>	
Absorption coefficient	0.078 mm <sup>-1</sup>	
F(000)	568	
Crystal size	0.40 x 0.35 x 0.15 mm <sup>3</sup>	
Theta range for data collection	2.27 to 28.33°.	
Index ranges	-8 ≤ h ≤ 9, -25 ≤ k ≤ 25, -13 ≤ l ≤ 13	
Reflections collected	16238	
Independent reflections	5907 [R(int) = 0.0876]	
Completeness to theta = 28.33°	94.6 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F <sup>2</sup>	
Data / restraints / parameters	5907 / 1 / 350	
Goodness-of-fit on F <sup>2</sup>	0.930	
Final R indices [I > 2σ(I)]	R1 = 0.0551, wR2 = 0.1354	
R indices (all data)	R1 = 0.0841, wR2 = 0.1548	
Absolute structure parameter	-1.9(15)	
Extinction coefficient	0.060(6)	
Largest diff. peak and hole	0.234 and -0.264 e.Å <sup>-3</sup>	

# <sup>1</sup>H NMR of Compound (+)-2.12

AA-4-50-fr40

18 Aug 2008

Acquisition Time (sec)	6.5536	Comment	STANDARD 1H OBSERVE		Date	Aug 24 2004			
Frequency (MHz)	399.81	Nucleus	1H	Number of Transients	100	Original Points Count	18505	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	5000.00	Temperature (grad C)	29.000				

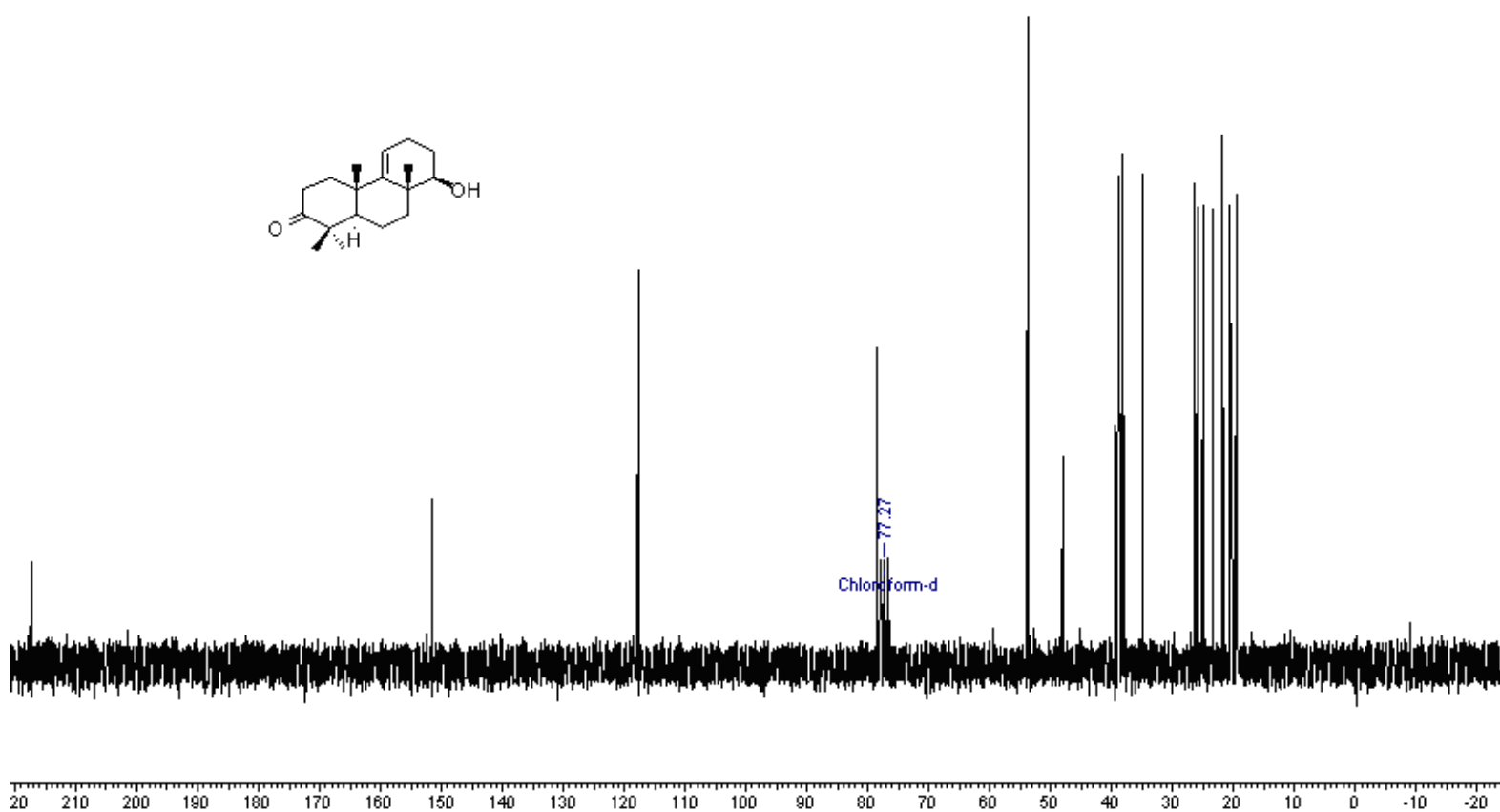
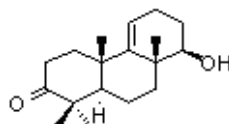


# <sup>13</sup>C NMR of Compound (+)-2.12

## AA-4-50-fr40

18 Aug 2008

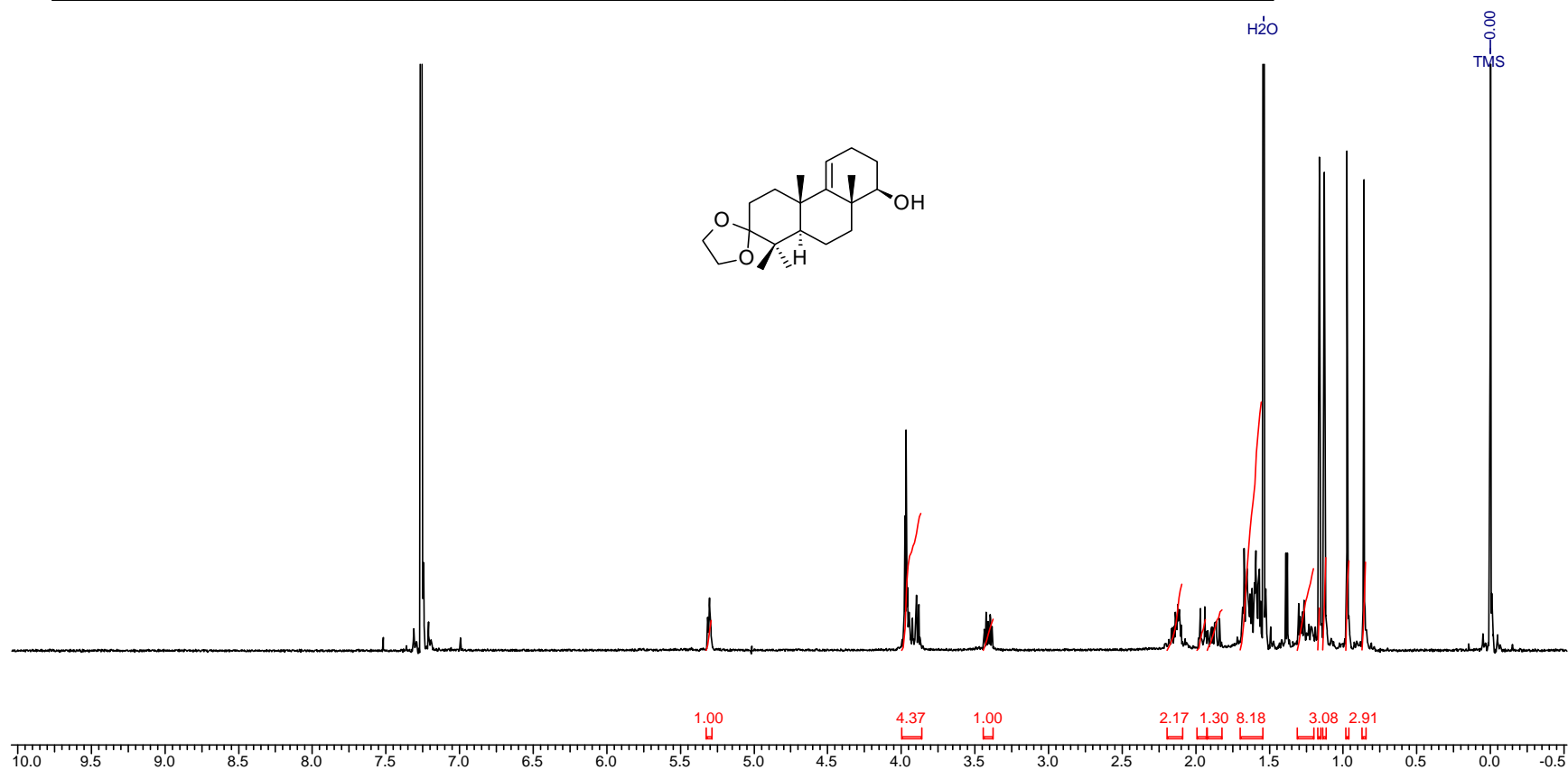
Acquisition Time (sec)	2.6214	Comment	13C OBSERVE	Date	Aug 3 2007				
Frequency (MHz)	50.79	Nucleus	13C	Number of Transients	1000	Original Points Count	18720	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	12500.00	Temperature (grad.C)	29.000				



# <sup>1</sup>H NMR of Compound (+)-2.13

AA-10-47-fr21-40

Acquisition Time (sec)	3.4152	Comment	Std proton		Date	Dec 20 2007			
Frequency (MHz)	399.77	Nucleus	1H	Number of Transients	64	Original Points Count	9828	Points Count	16384
Solvent	cdcl3	Sweep Width (Hz)	4797.31		Temperature (grad C)	25.000			

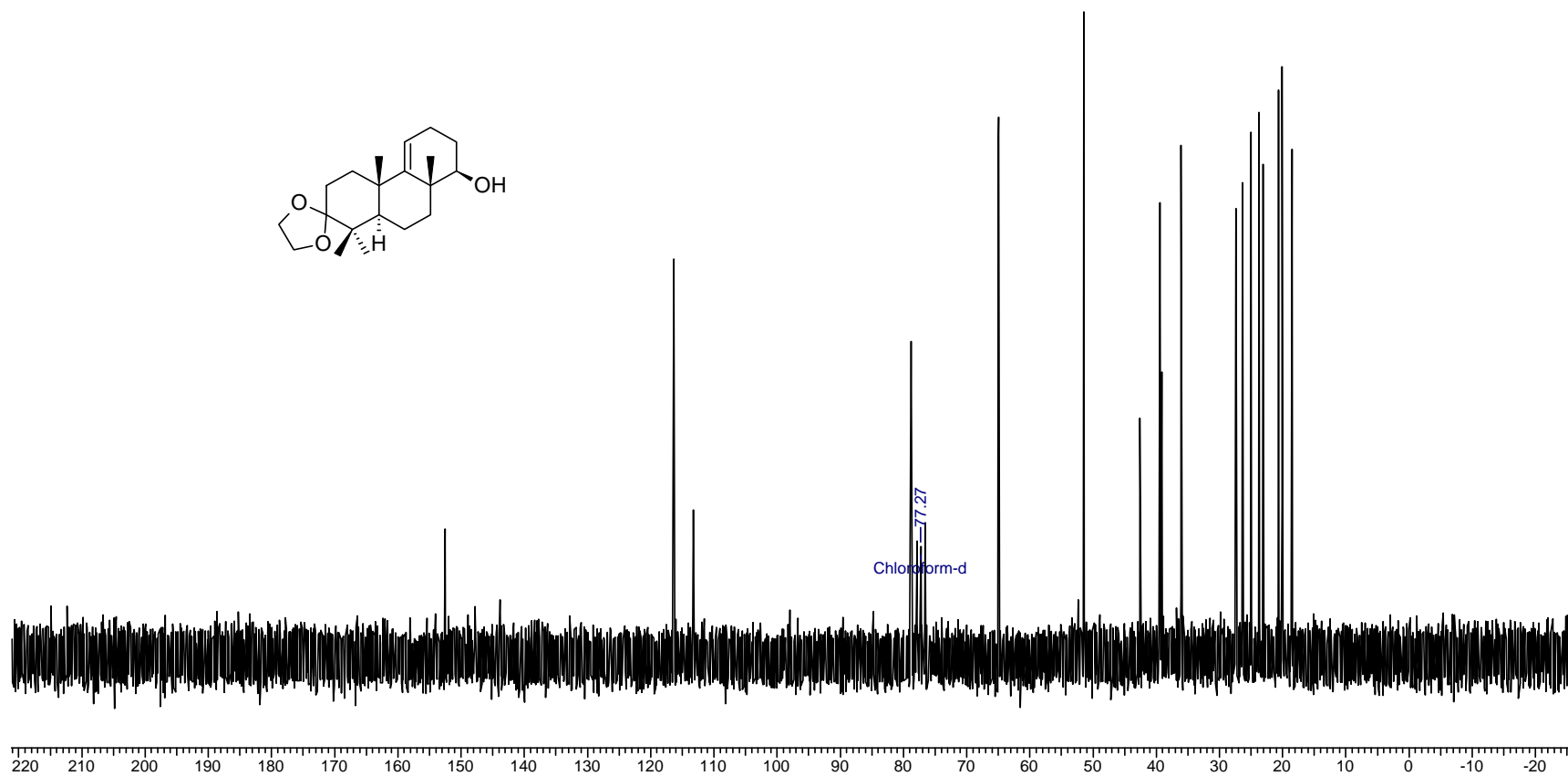
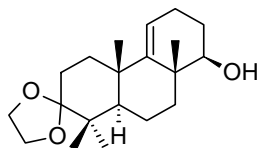


# $^{13}\text{C}$ NMR of Compound (+)-2.13

## AS-1-27-pure-C13

18 Aug 2008

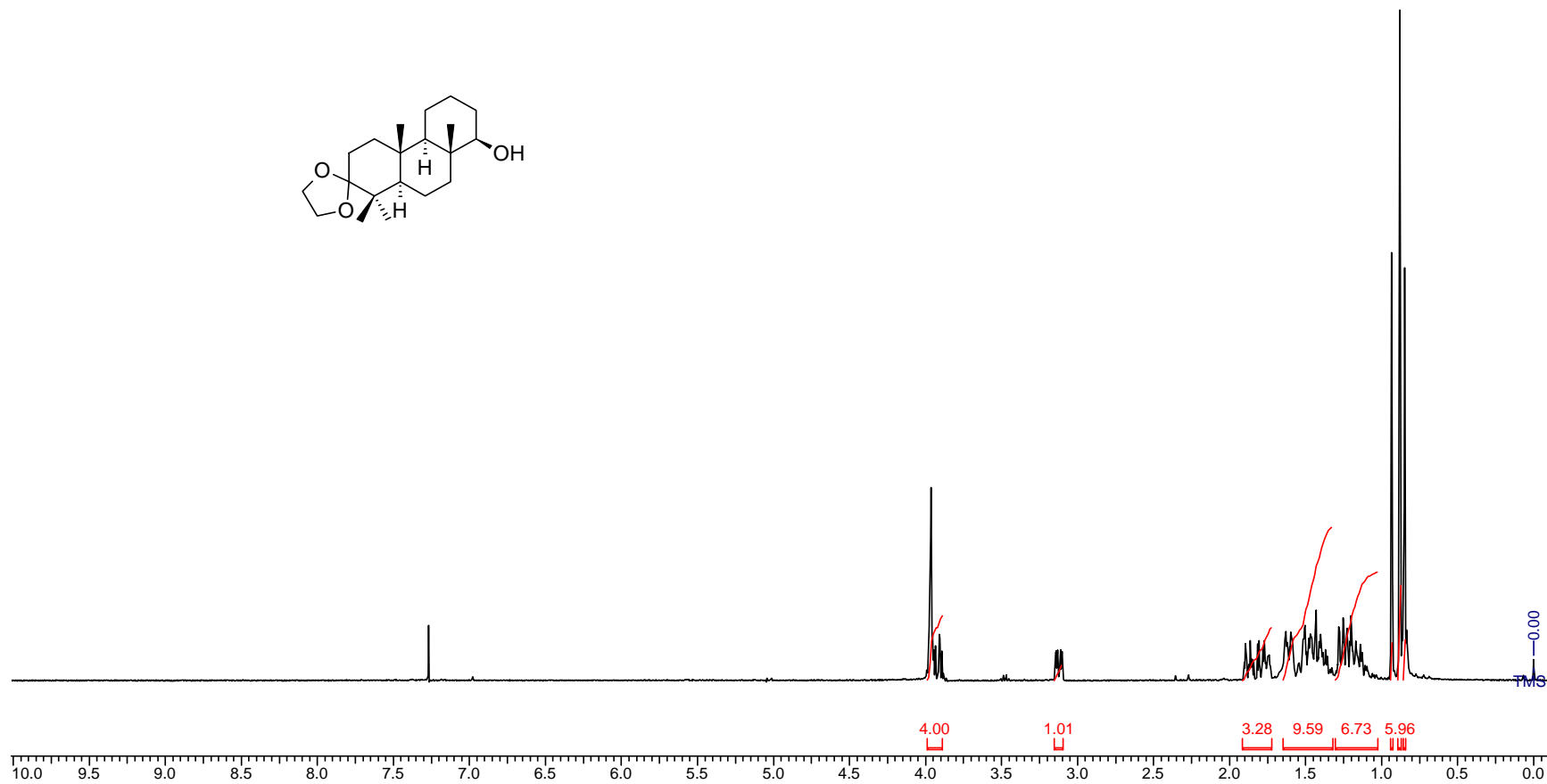
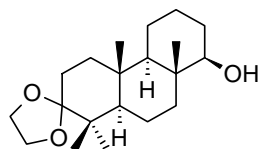
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<b>Solvent</b> CDCl3	<b>Sweep Width (Hz)</b> 12500.00	<b>Temperature (grad C)</b> 29.000		



# $^1\text{H}$ NMR of Compound (-)-2.14

## AA-4-88-1crude

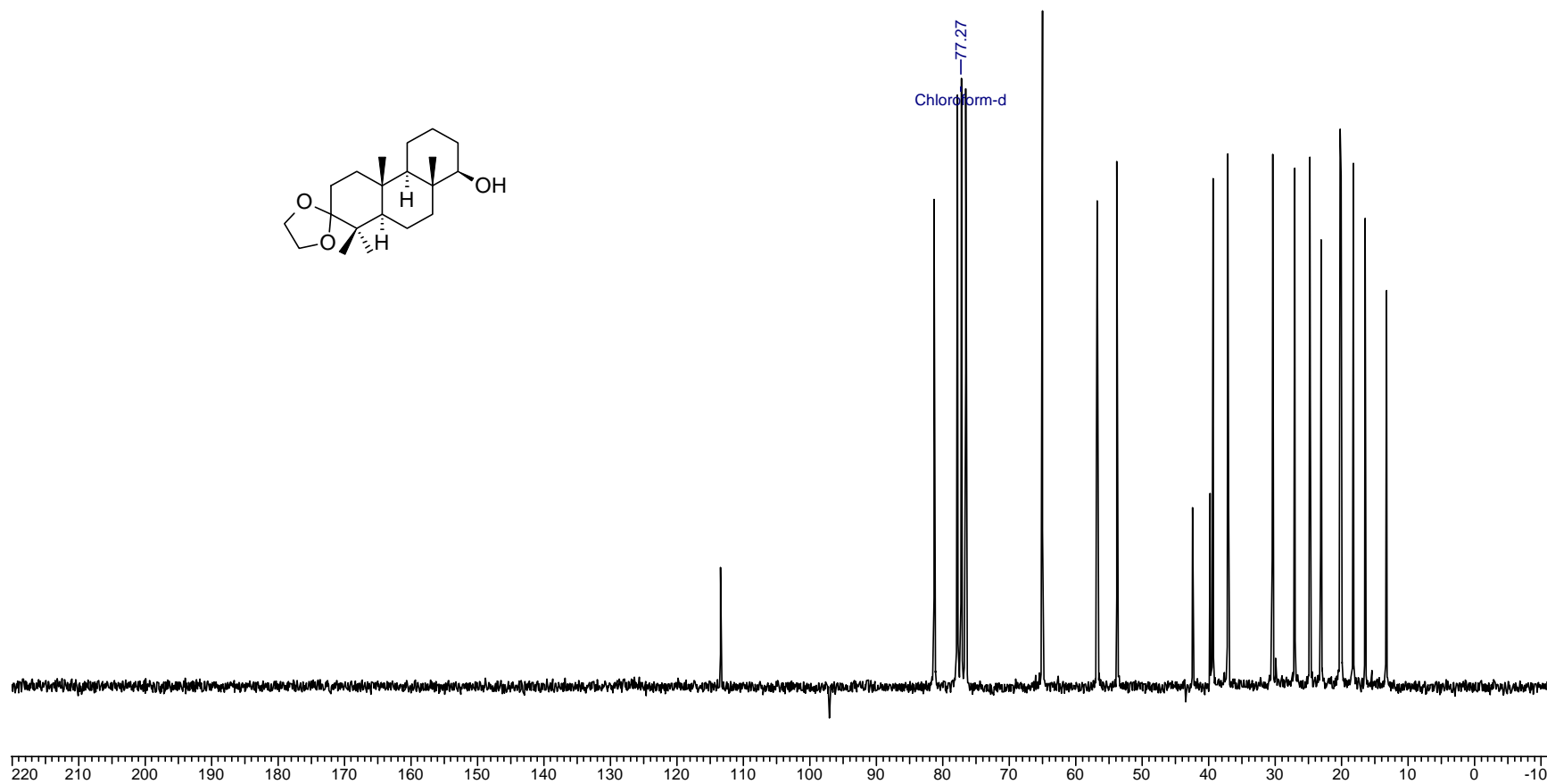
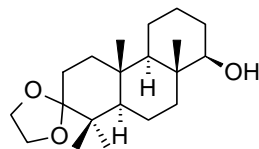
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Frequency (MHz)	399.81	Nucleus	1H	Number of Transients	300	Original Points Count	18505	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	5000.00	Temperature (grad C)	29.000				



# $^{13}\text{C}$ NMR of Compound (-)-2.14

## AA-4-88-1crude-C13

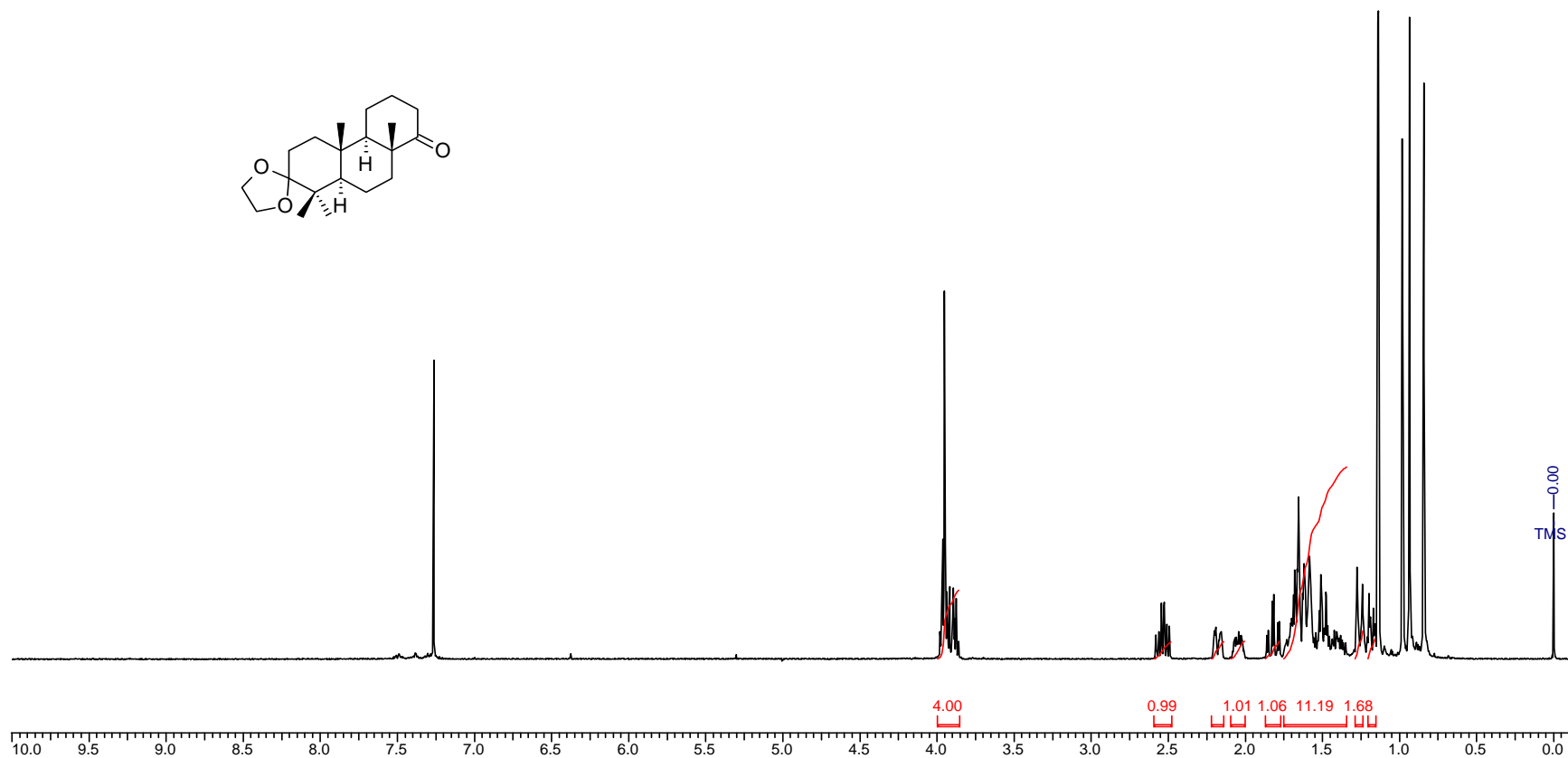
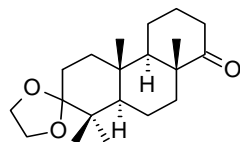
<b>Acquisition Time (sec)</b> 2.6214	<b>Comment</b> 13C OBSERVE	<b>Date</b> Nov 1 2004		
<b>Frequency (MHz)</b> 50.29	<b>Nucleus</b> 13C	<b>Number of Transients</b> 20000	<b>Original Points Count</b> 18720	<b>Points Count</b> 32768
<b>Solvent</b> CDCl3	<b>Sweep Width (Hz)</b> 12500.00	<b>Temperature (grad C)</b> 29.000		



# <sup>1</sup>H NMR of Compound (-)-2.1

AA-6-33-fr22

Acquisition Time (sec)	6.5536	Comment	STANDARD 1H OBSERVE		Date	Oct 27 2005			
Frequency (MHz)	399.79	Nucleus	1H	Number of Transients	100	Original Points Count	18505	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	5000.00	Temperature (grad C)	29.000				

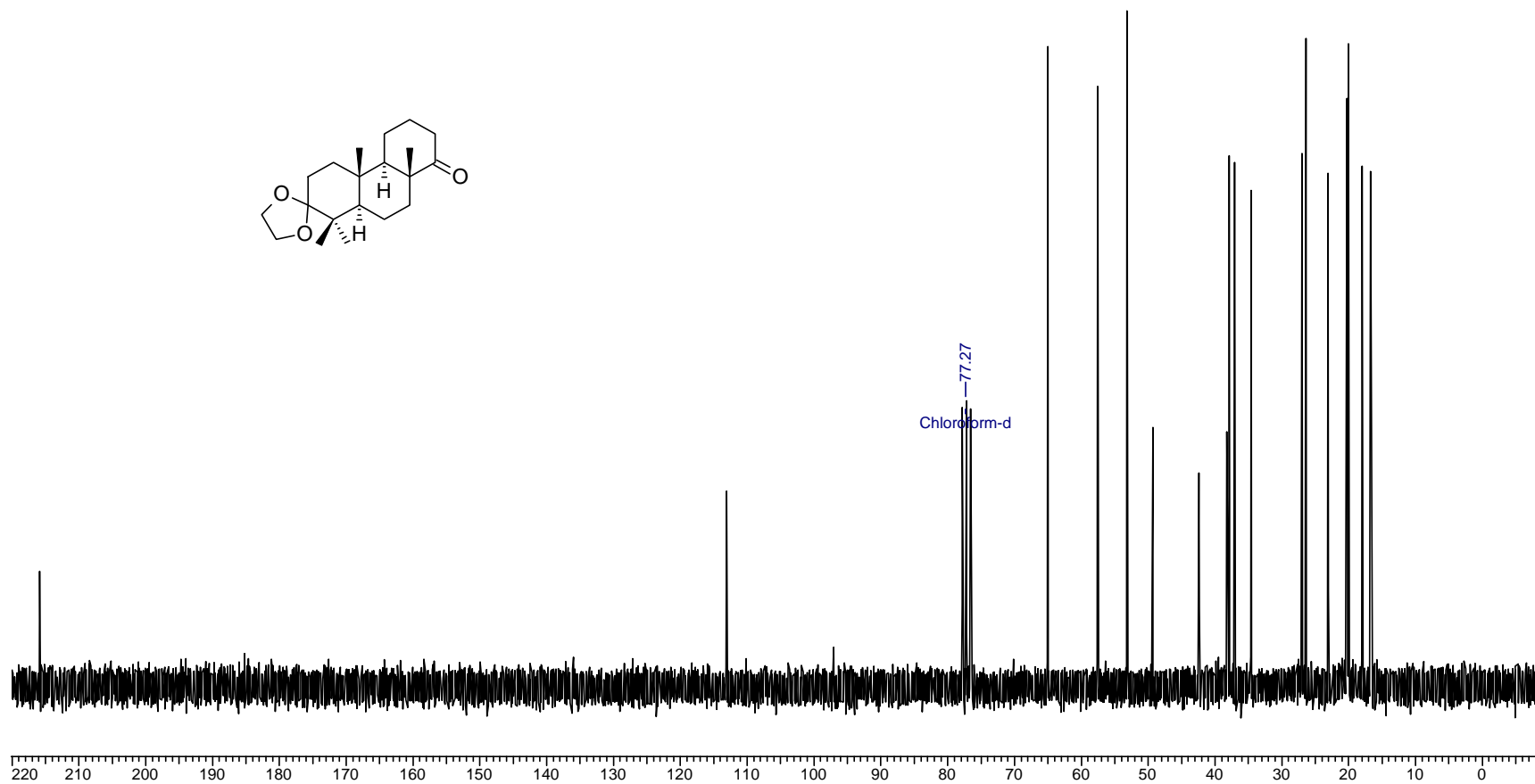
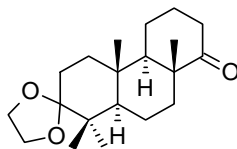




# <sup>13</sup>C NMR of Compound (-)-2.1

AA-4-89-fr1-8-C13

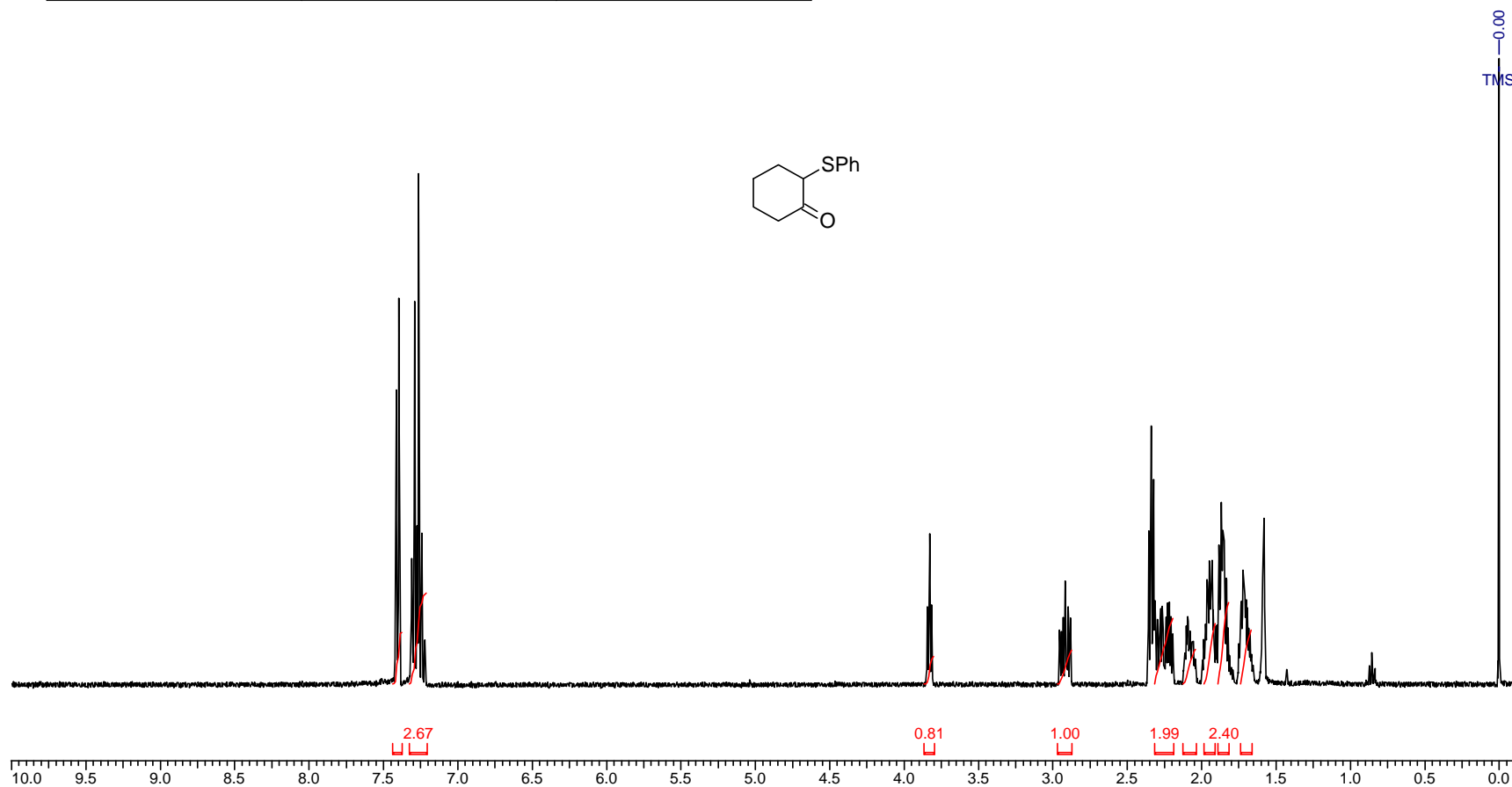
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<b>Frequency (MHz)</b> 50.29	<b>Nucleus</b> 13C	<b>Number of Transients</b> 20000	<b>Original Points Count</b> 18720	<b>Points Count</b> 32768
<b>Solvent</b> CDCl3	<b>Sweep Width (Hz)</b> 12500.00	<b>Temperature (grad C)</b> 29.000		



# <sup>1</sup>H NMR of Compound 2.16

AA-4-22-fr4-7

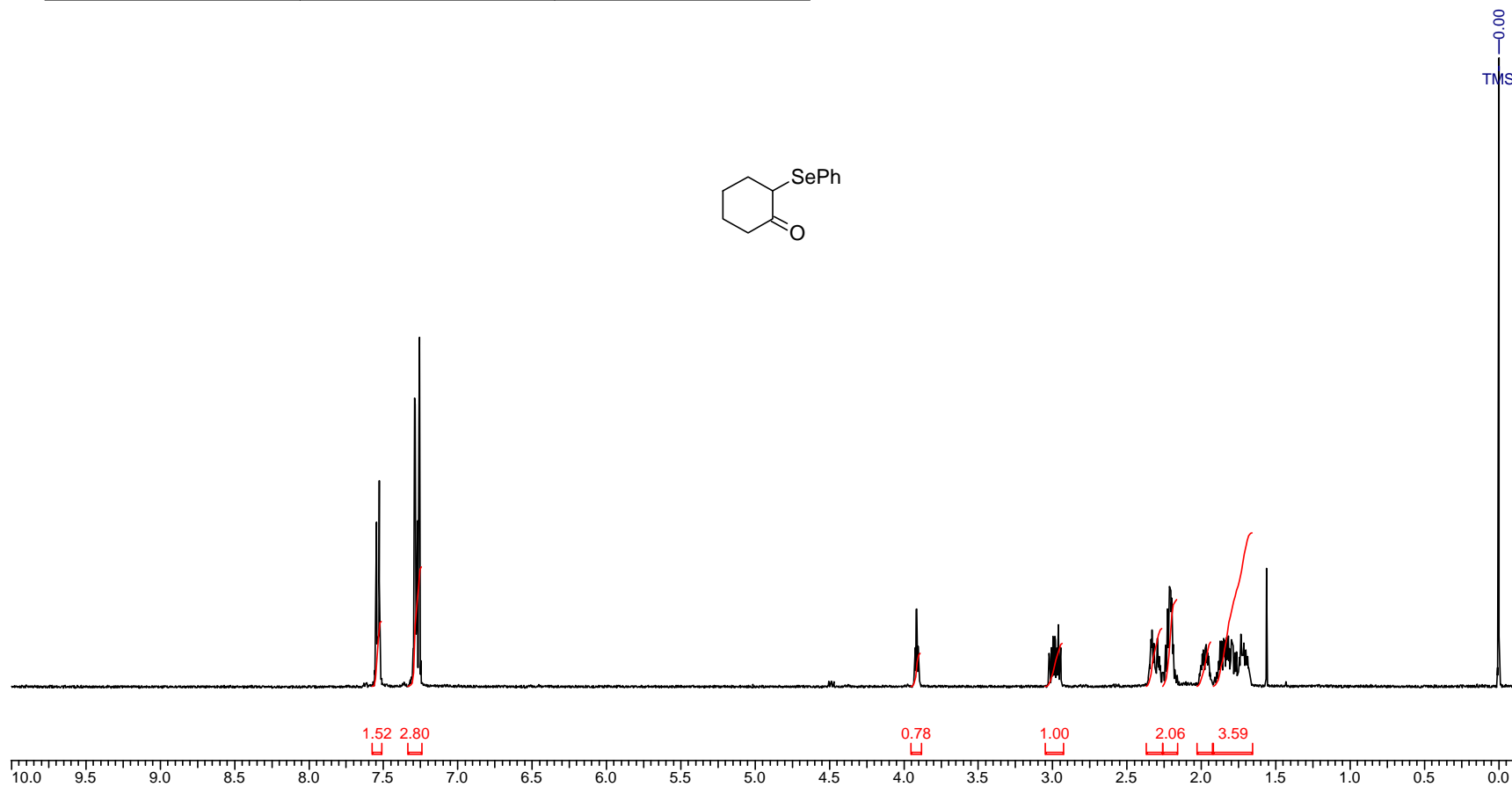
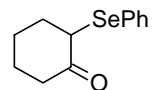
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# <sup>1</sup>H NMR of Compound 2.17

BW-2-100-fr28-32

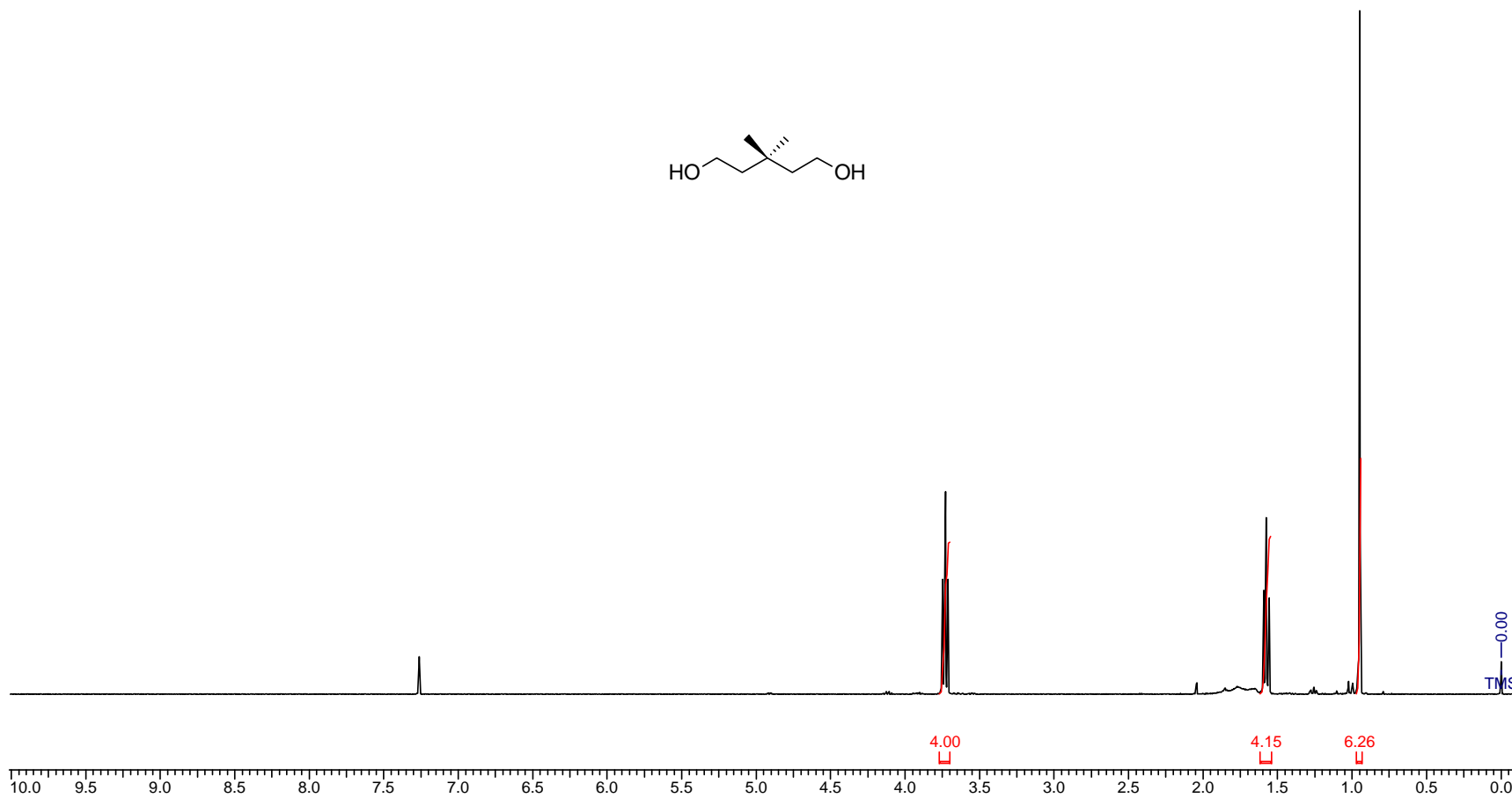
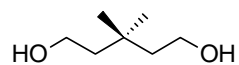
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Frequency (MHz)	399.81	Nucleus	1H	Number of Transients	32	Original Points Count	18505	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	5000.00	Temperature (grad C)	29.000				



# <sup>1</sup>H NMR of Compound 2.22

## AA-3-59-1crude

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<b>Solvent</b> CDCl <sub>3</sub>	<b>Sweep Width (Hz)</b> 5999.70	<b>Temperature (grad C)</b> 29.000		

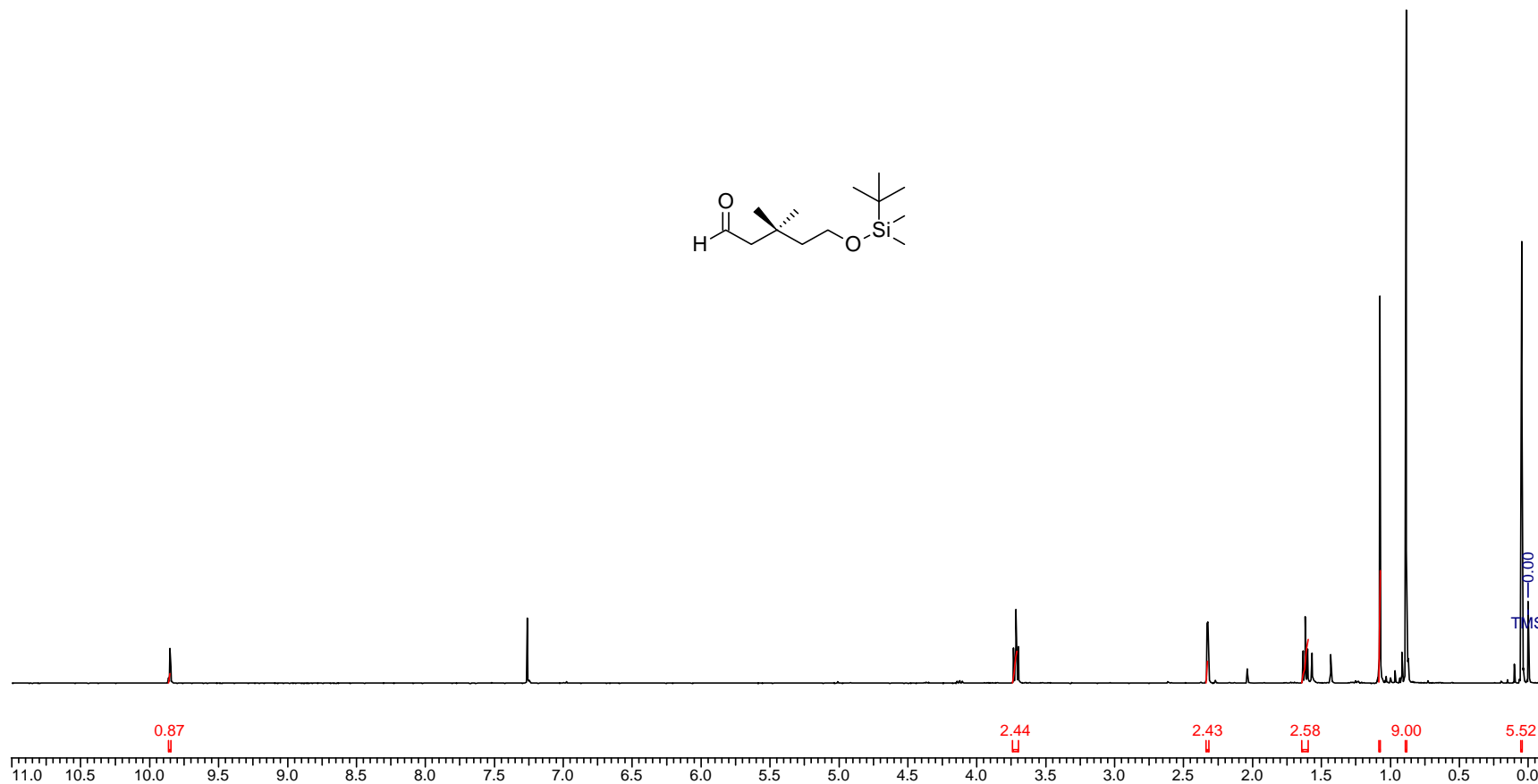
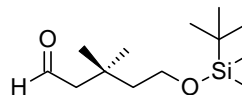




# <sup>1</sup>H NMR of Compound 2.24

## AA-2-58-1crude

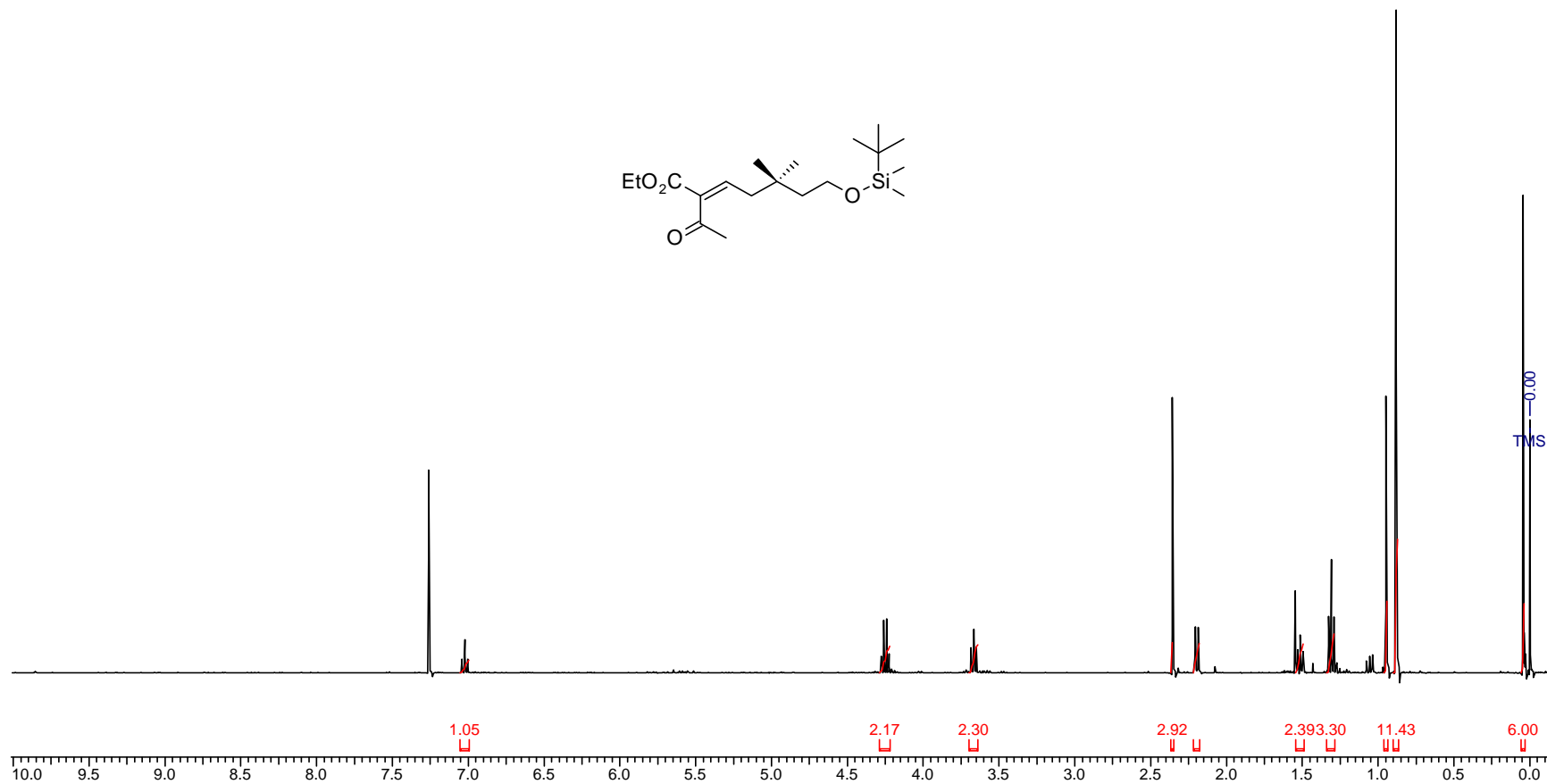
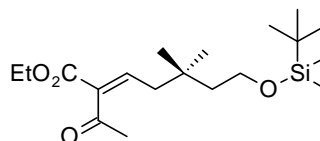
<b>Acquisition Time (sec)</b> 5.4616	<b>Comment</b> STANDARD 1H OBSERVE	<b>Date</b> Oct 20 2003		
<b>Frequency (MHz)</b> 399.82	<b>Nucleus</b> 1H	<b>Number of Transients</b> 100	<b>Original Points Count</b> 22205	<b>Points Count</b> 32768
<b>Solvent</b> CDCl3	<b>Sweep Width (Hz)</b> 5999.70	<b>Temperature (grad C)</b> 29.000		



# <sup>1</sup>H NMR of Compound 2.20 E

AA-2-114-fr27-44

Acquisition Time (sec)	6.5536	Comment	STANDARD 1H OBSERVE		Date	Jan 26 2004			
Frequency (MHz)	399.81	Nucleus	1H	Number of Transients	100	Original Points Count	18505	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	5000.00	Temperature (grad C)	29.000				



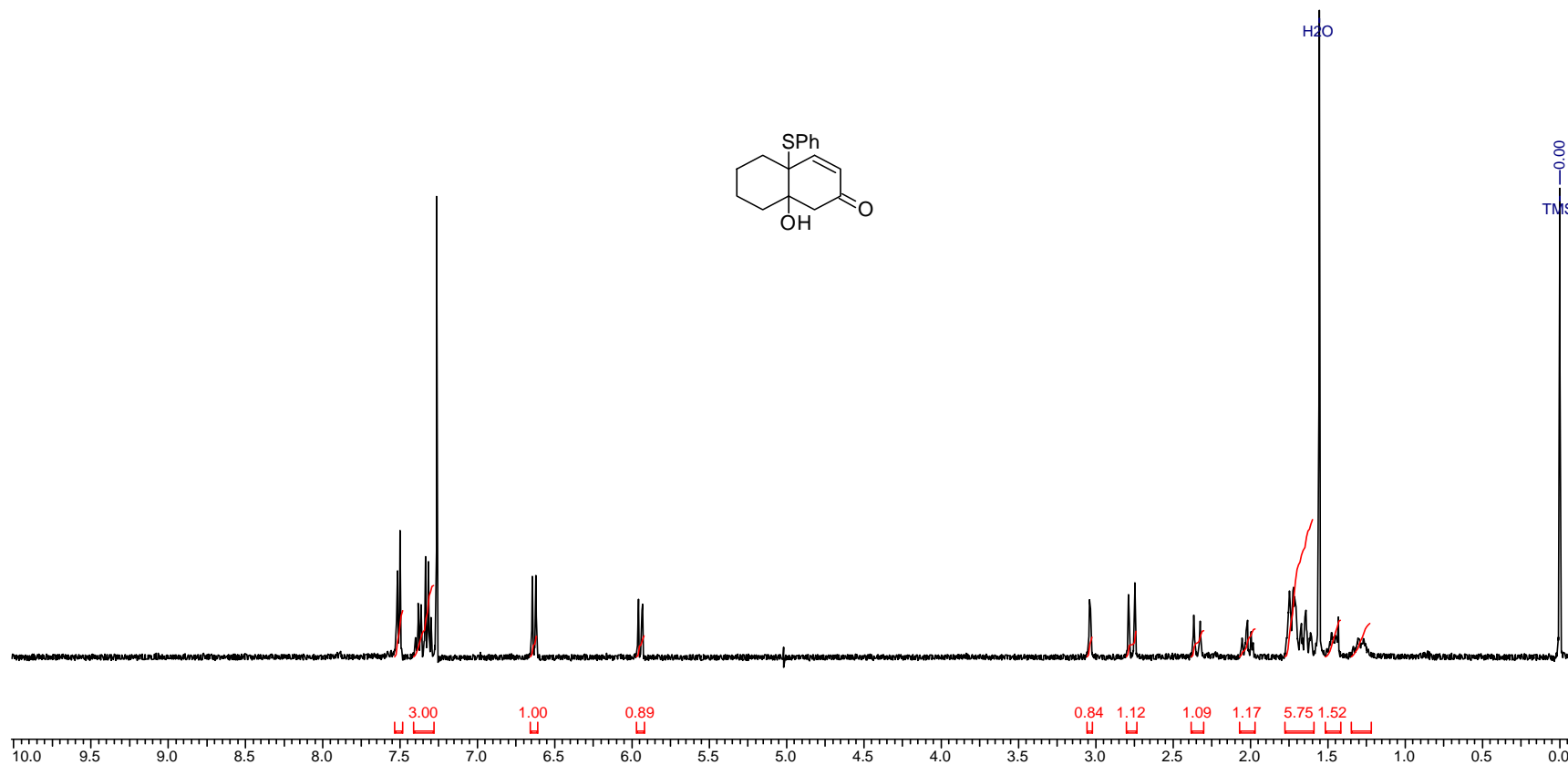




# <sup>1</sup>H NMR of Compound 2.27

AA-3-80-fr34

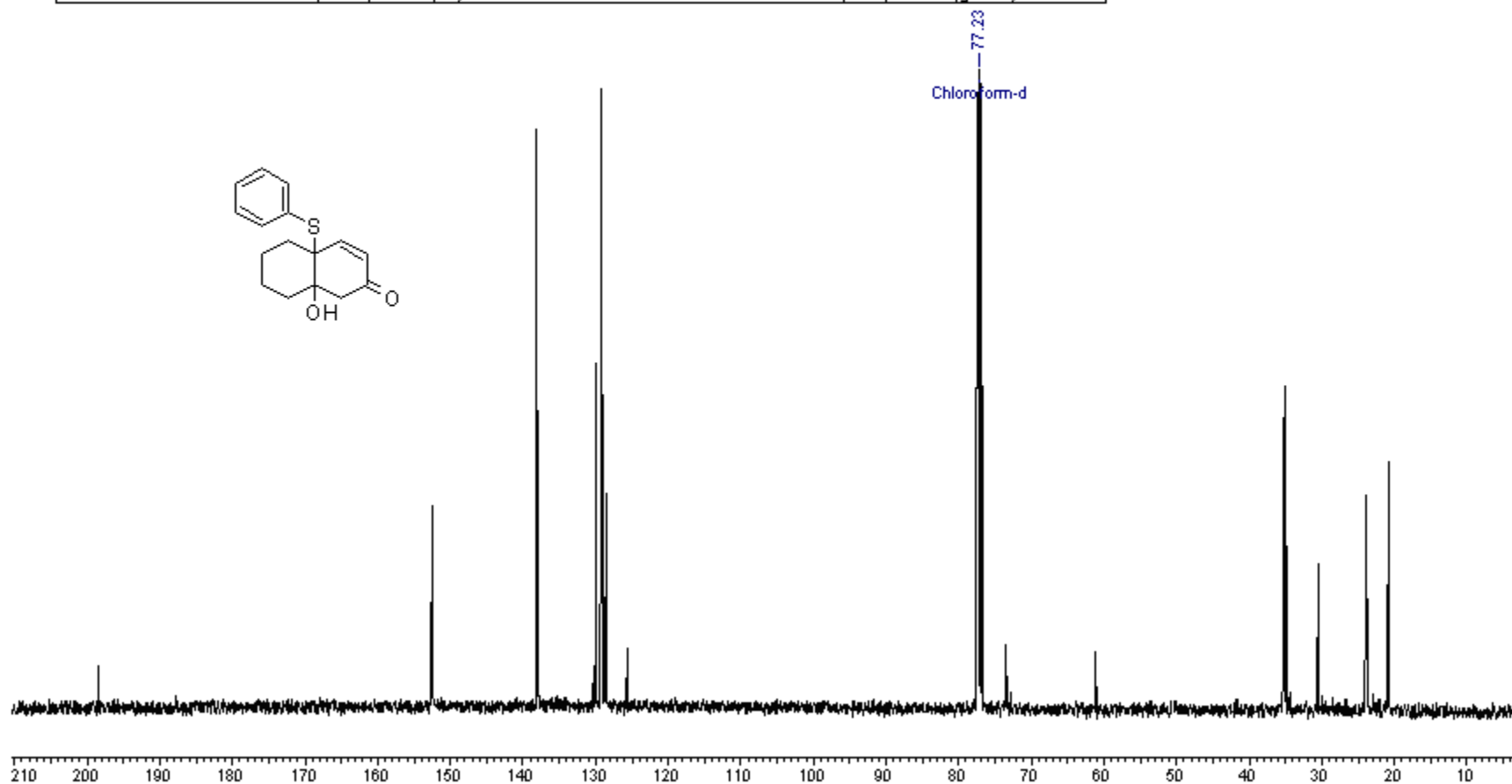
Acquisition Time (sec)	6.5536	Comment	STANDARD 1H OBSERVE		Date	Apr 16 2004			
Frequency (MHz)	399.81	Nucleus	1H	Number of Transients	200	Original Points Count	18505	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	5000.00	Temperature (grad C)	29.000				



# <sup>13</sup>C NMR of Compound 2.27

## AA-3-80-fr32-48C13

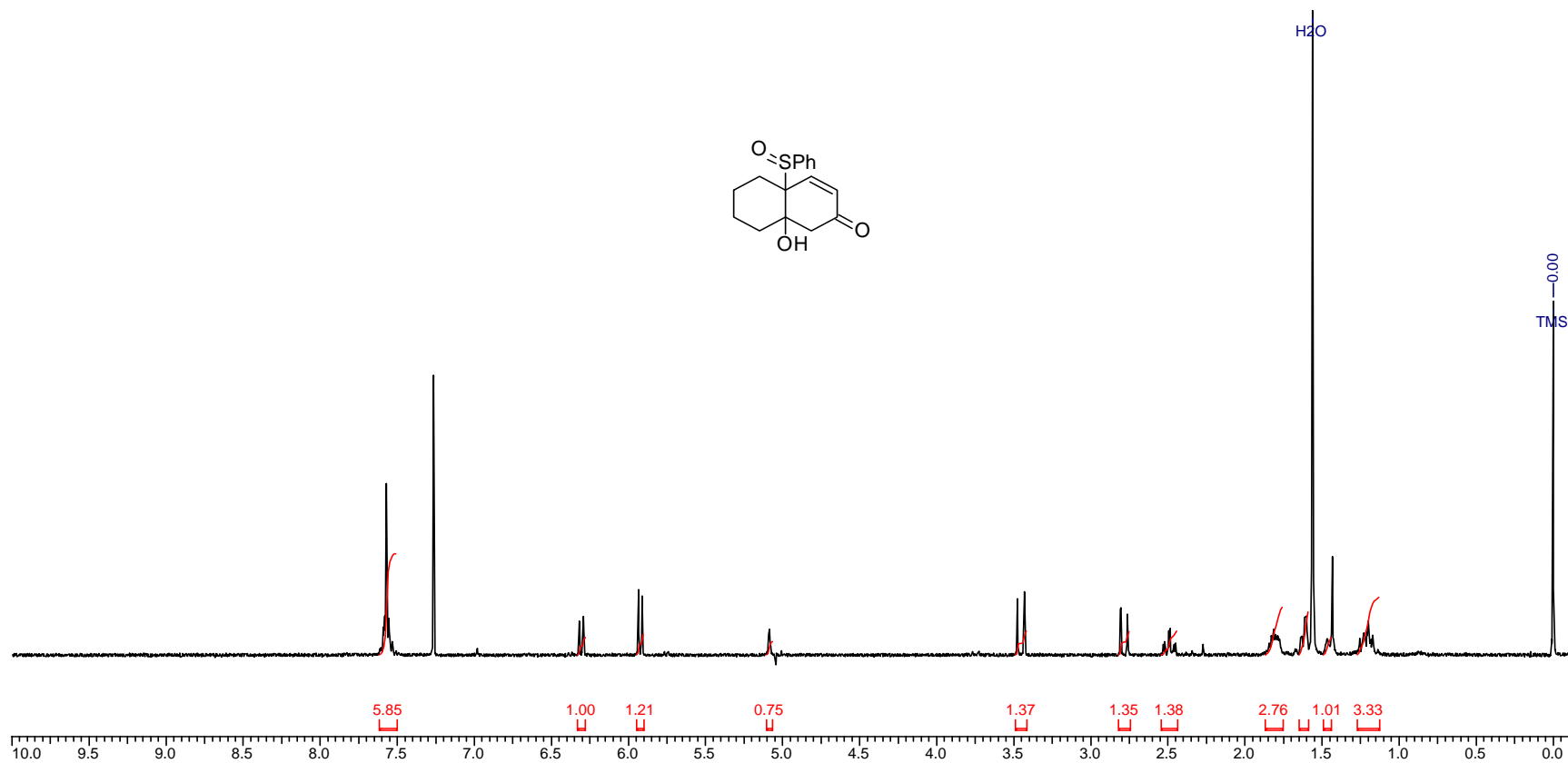
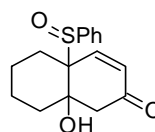
Acquisition Time (sec)	1.3107	Comment	13C OBSERVE	Date	Apr 19 2004
Frequency (MHz)	100.54	Nucleus	13C	Number of Transients	20000
Solvent	CDCl3	Sweep Width (Hz)	25000.00	Original Points Count	29984
				Points Count	32768
				Temperature (grad C)	29.000



# <sup>1</sup>H NMR of Compound 2.29

AA-3-105-fr40

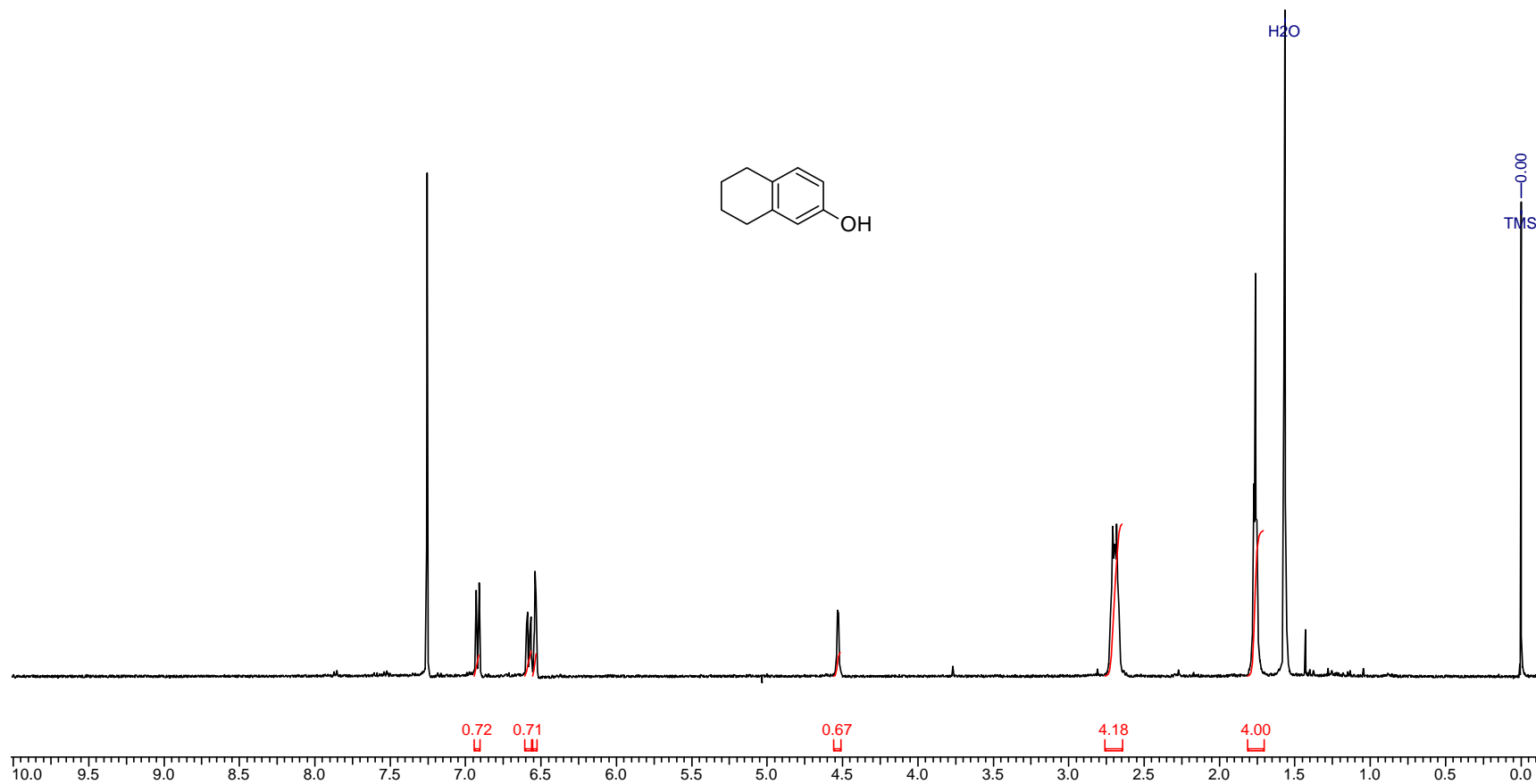
Acquisition Time (sec)	6.5536	Comment	STANDARD 1H OBSERVE	Date	May 18 2004				
Frequency (MHz)	399.81	Nucleus	1H	Number of Transients	200	Original Points Count	18505	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	5000.00	Temperature (grad C)	29.000				



# <sup>1</sup>H NMR of Compound 2.35

AA-4-15-fr5

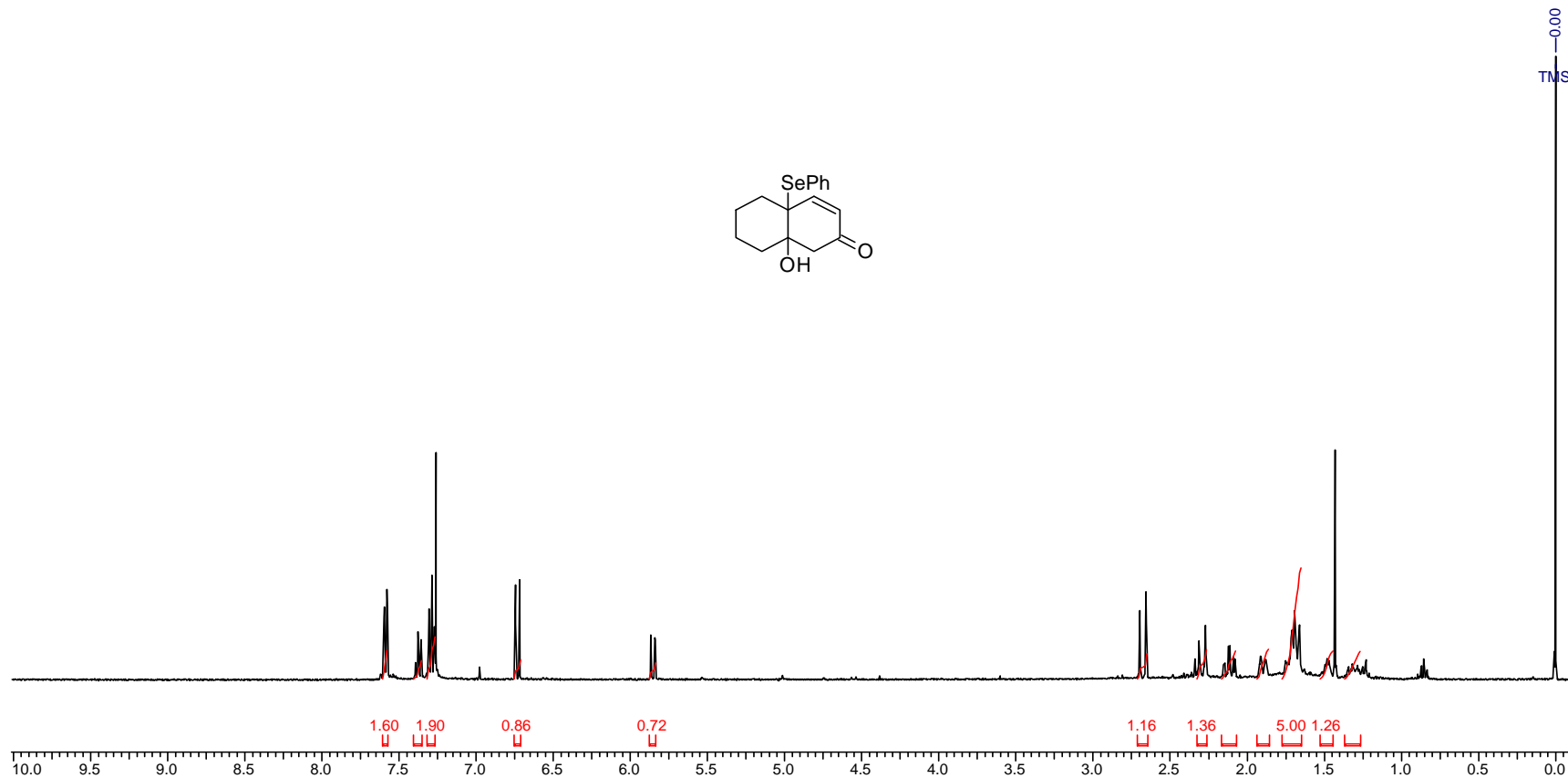
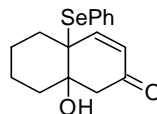
Acquisition Time (sec)	6.5536	Comment	STANDARD 1H OBSERVE		Date	Jun 16 2004			
Frequency (MHz)	399.81	Nucleus	1H	Number of Transients	100	Original Points Count	18505	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	5000.00	Temperature (grad C)	29.000				



# <sup>1</sup>H NMR of Compound 2.36

AA-4-56-fr51-57

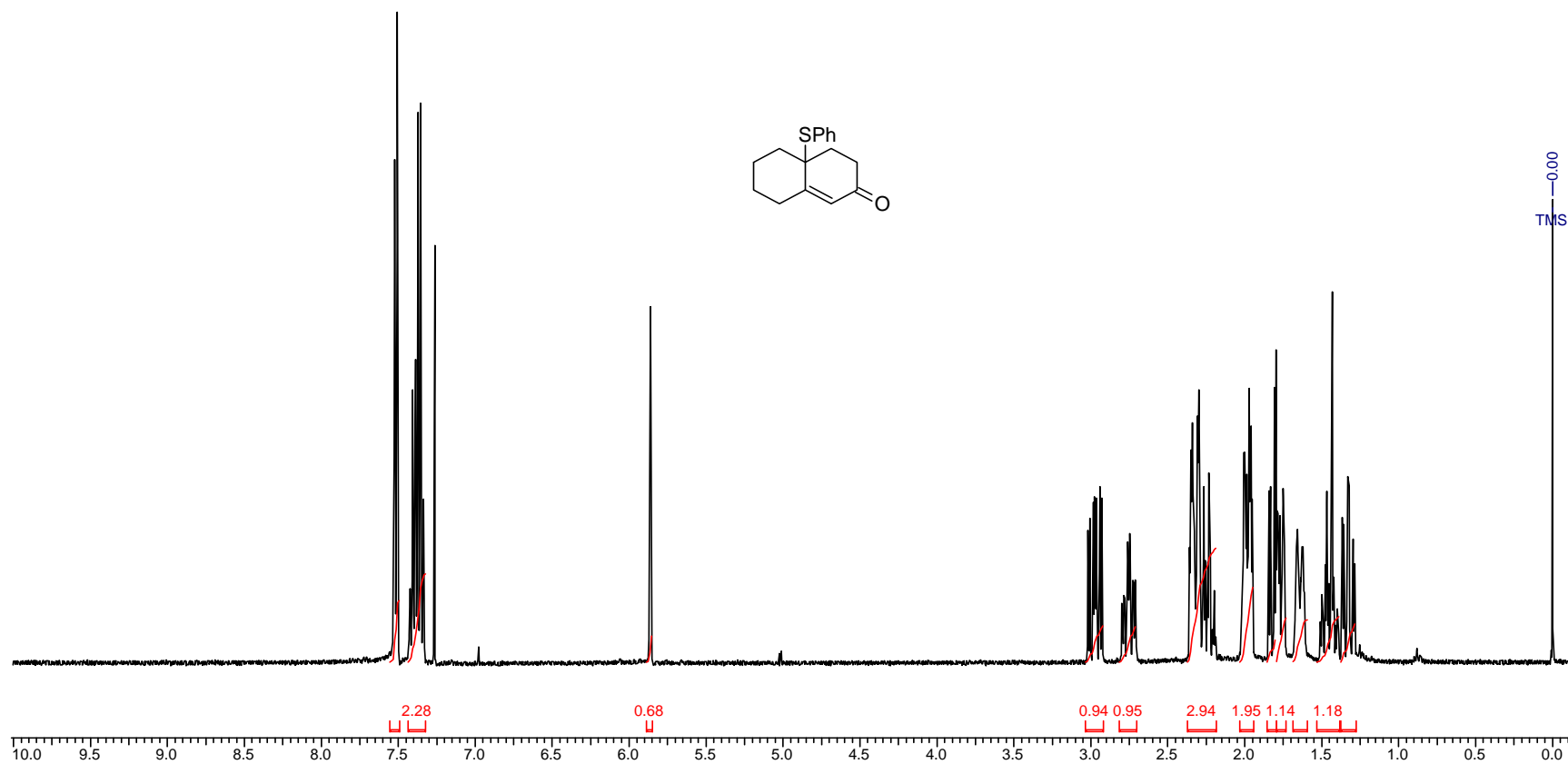
Acquisition Time (sec)	6.5536	Comment	STANDARD 1H OBSERVE		Date	Sep 6 2004			
Frequency (MHz)	399.81	Nucleus	1H	Number of Transients	100	Original Points Count	18505	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	5000.00	Temperature (grad C)	29.000				



# <sup>1</sup>H NMR of Compound 2.38

## AA-4-enonesulfide

Acquisition Time (sec)	6.5536	Comment	STANDARD 1H OBSERVE		Date	Dec 21 2004			
Frequency (MHz)	399.80	Nucleus	1H	Number of Transients	300	Original Points Count	18505	Points Count	32768
Solvent	CDCl <sub>3</sub>	Sweep Width (Hz)	5000.00	Temperature (grad C)	29.000				

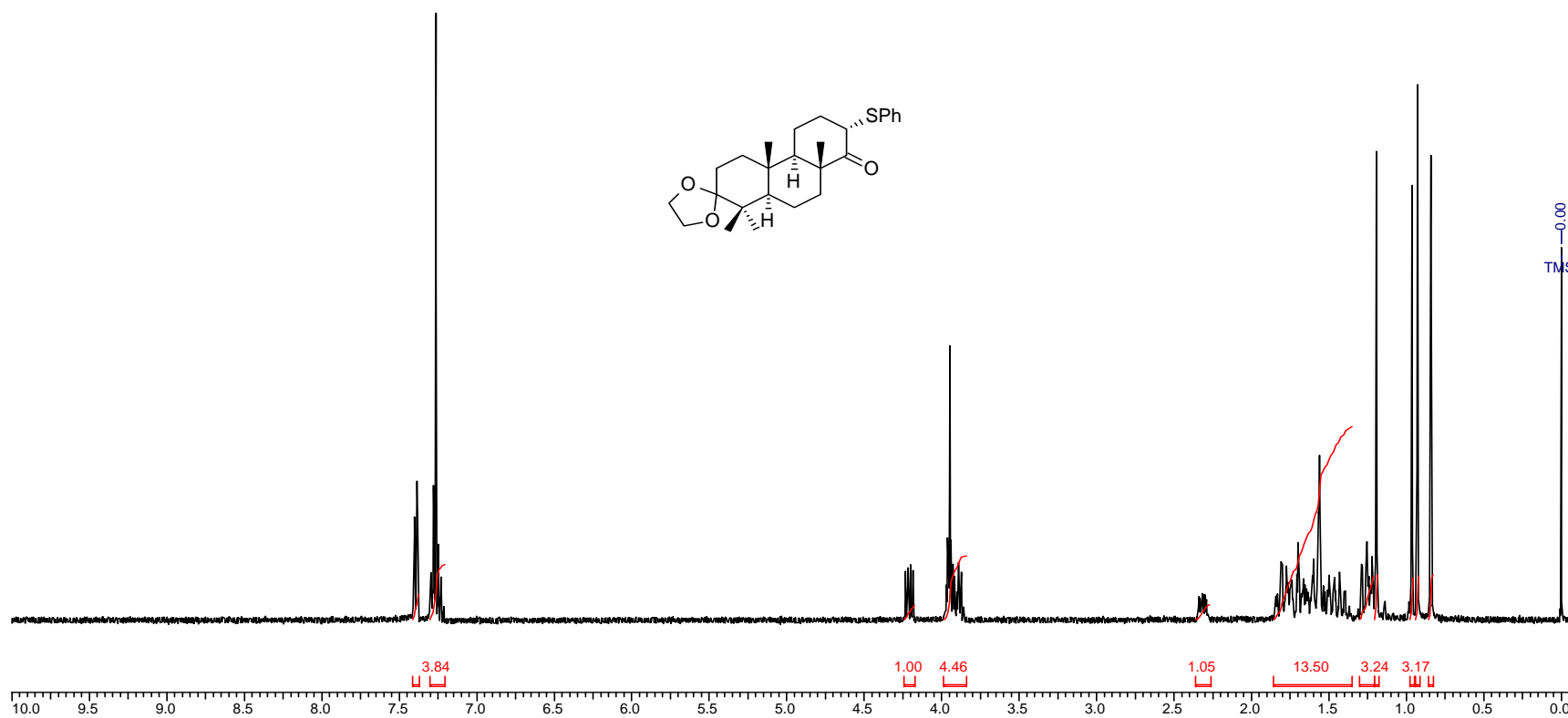


# Appendix B - Chapter 3 $^1\text{H}$ and $^{13}\text{C}$ NMR spectras

## $^1\text{H}$ NMR of Compound (+)-3.2

AA-4-97-fr19-22

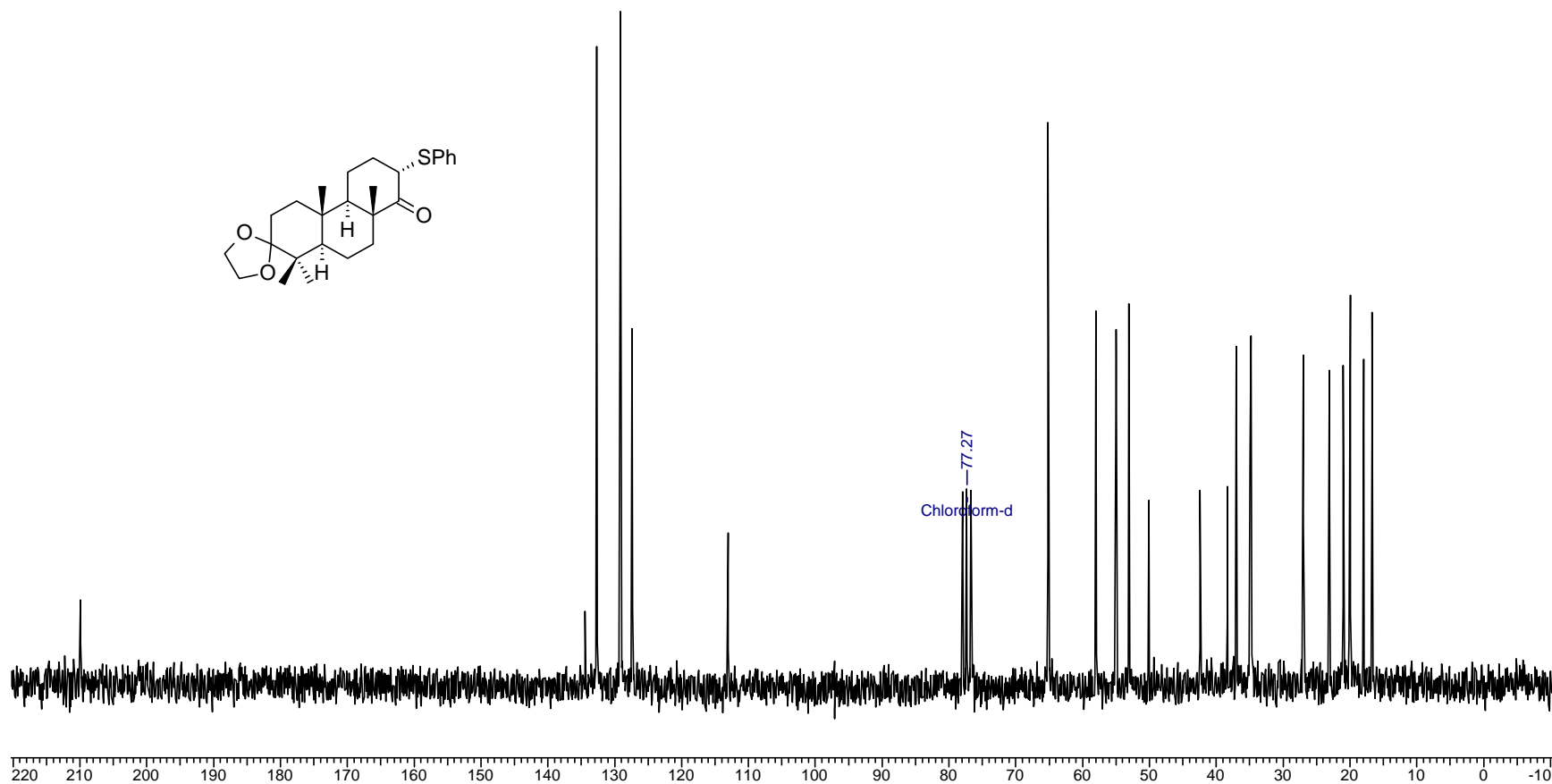
Acquisition Time (sec)	6.5536	Comment	STANDARD 1H OBSERVE		Date	Nov 27 2004			
Frequency (MHz)	399.81	Nucleus	$^1\text{H}$	Number of Transients	300	Original Points Count	18505	Points Count	32768
Solvent	$\text{CDCl}_3$	Sweep Width (Hz)	5000.00	Temperature (grad C)	29.000				



# <sup>13</sup>C NMR of Compound (+)-3.2

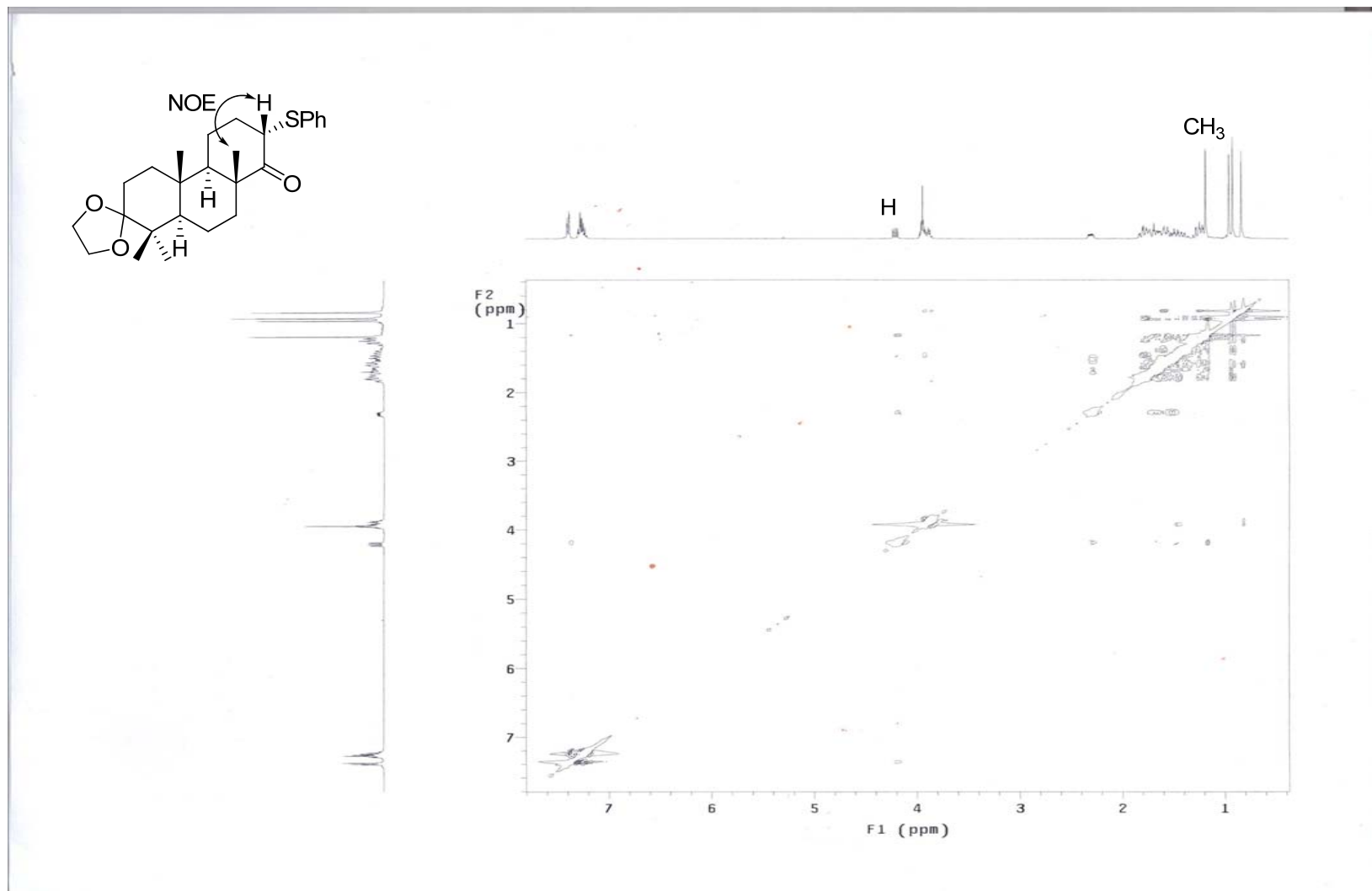
AA-10-26-fr21-64-C13

Acquisition Time (sec)	2.6214	Comment	13C OBSERVE		Date	Nov 4 2007			
Frequency (MHz)	50.29	Nucleus	13C	Number of Transients	1000	Original Points Count	18720	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	12500.00		Temperature (grad C)	29.000			





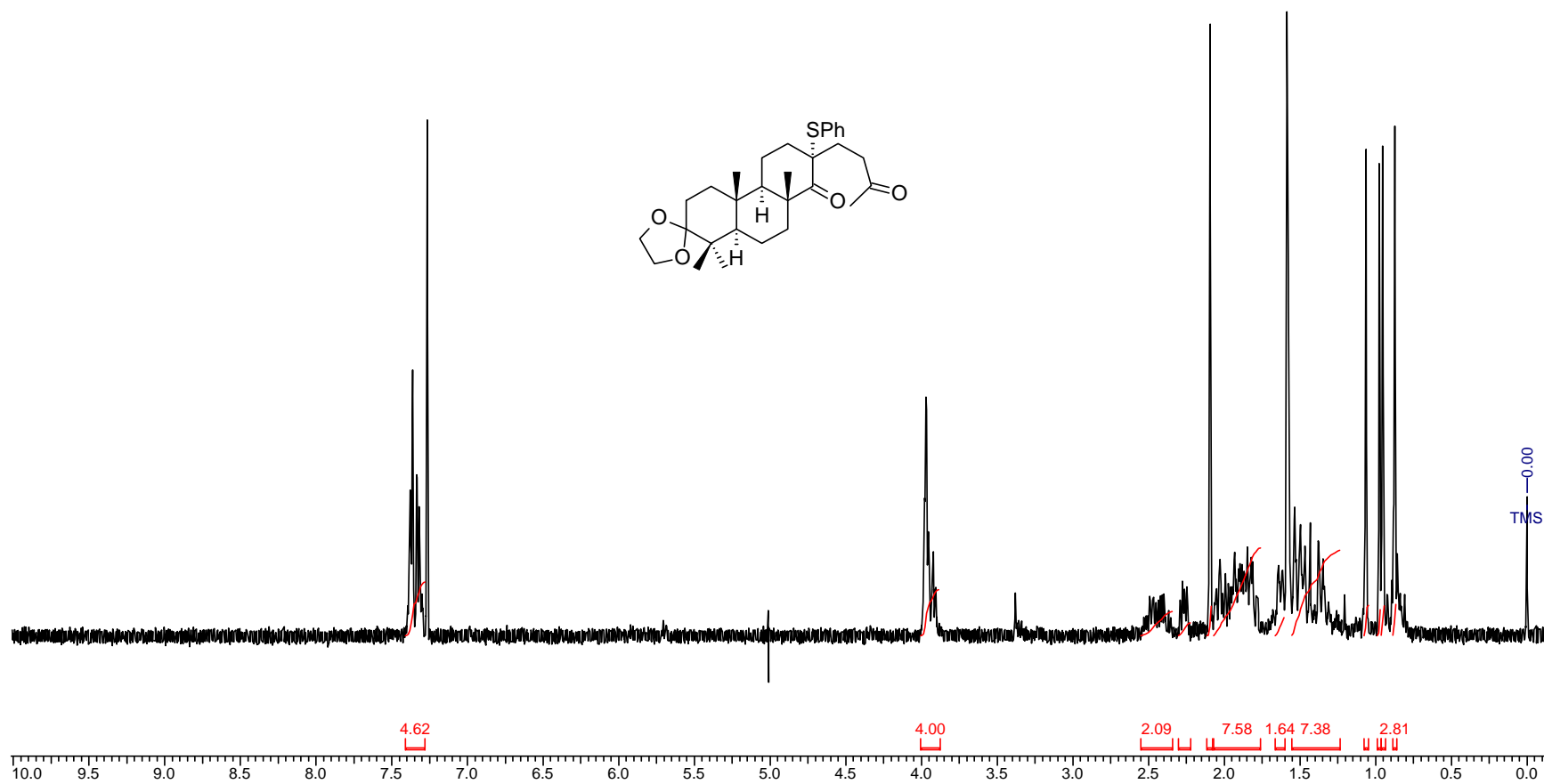
### NOESY of compound (+)-3.2



# $^1\text{H}$ NMR of Compound (-)-3.3a

AA-6-12-fr20-34

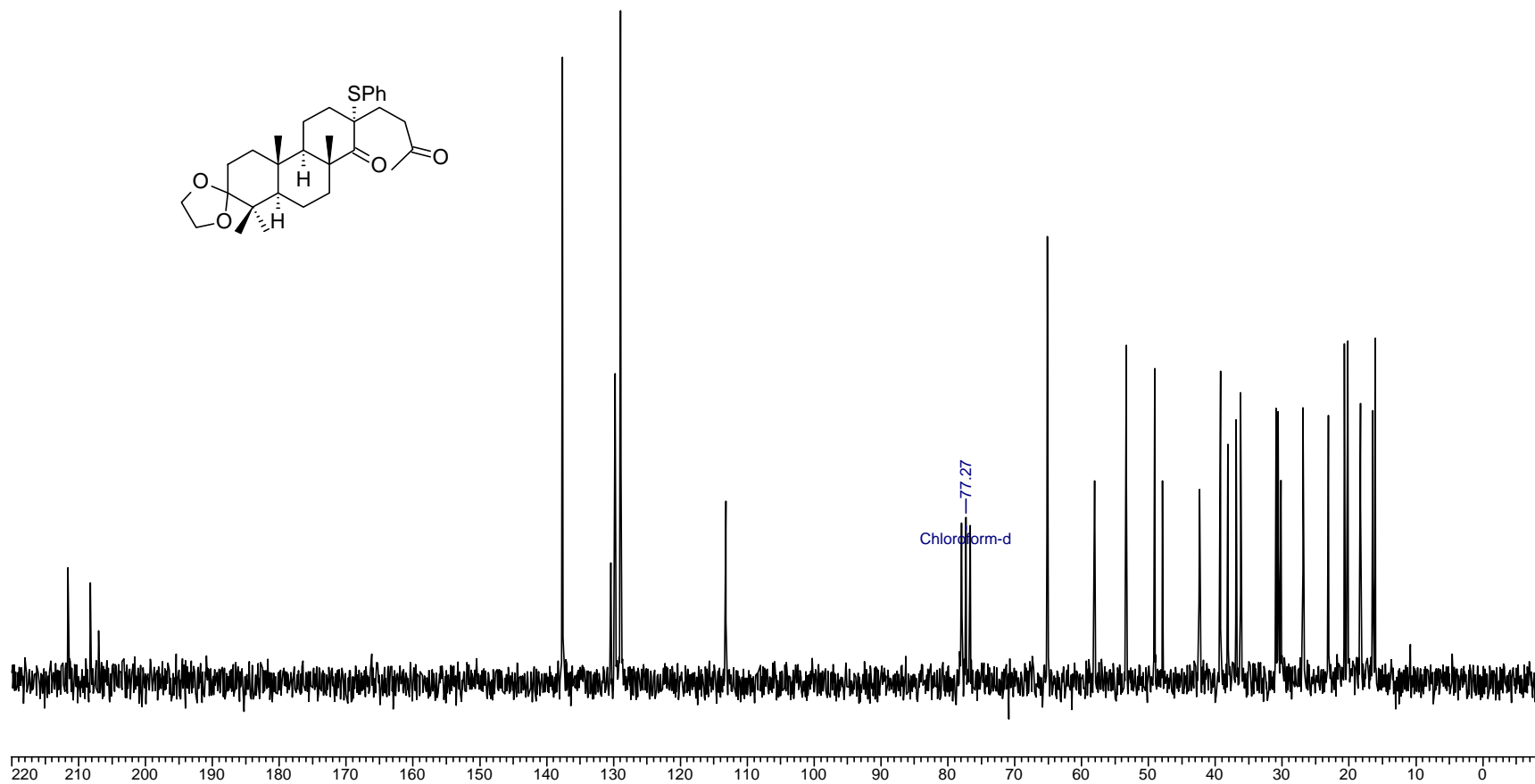
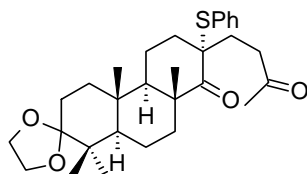
Acquisition Time (sec)	6.5536	Comment	STANDARD 1H OBSERVE	Date	Aug 18 2005				
Frequency (MHz)	399.79	Nucleus	$^1\text{H}$	Number of Transients	300	Original Points Count	18505	Points Count	32768
Solvent	$\text{CDCl}_3$	Sweep Width (Hz)	5000.00	Temperature (grad C)	29.000				



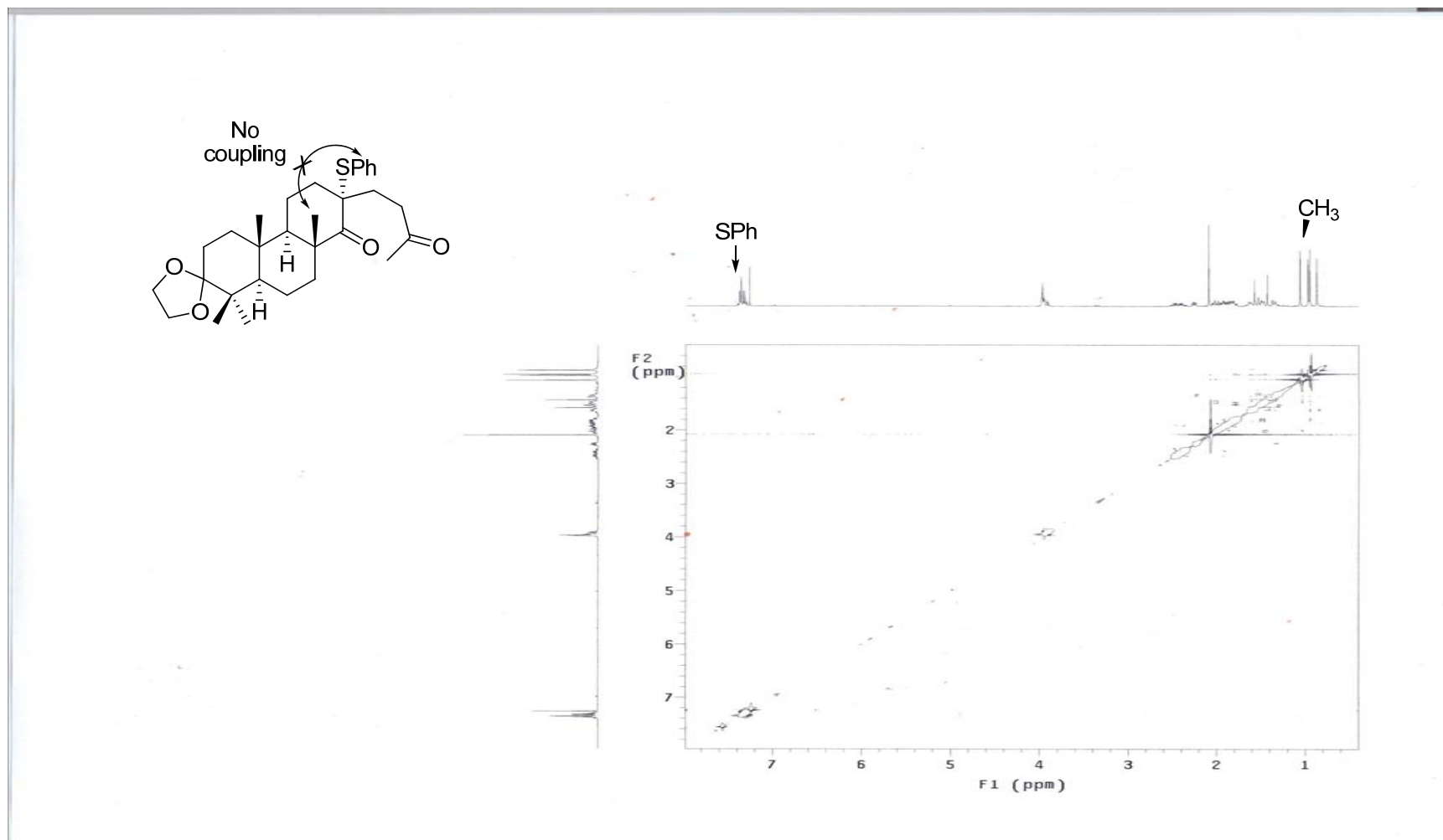
# <sup>13</sup>C NMR of Compound (-)-3.3a

AA-8-60-fr40-46-C13

Acquisition Time (sec)	2.6214	Comment	13C OBSERVE	Date	Jun 26 2007				
Frequency (MHz)	50.29	Nucleus	13C	Number of Transients	1000	Original Points Count	18720	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	12500.00	Temperature (grad C)	29.000				



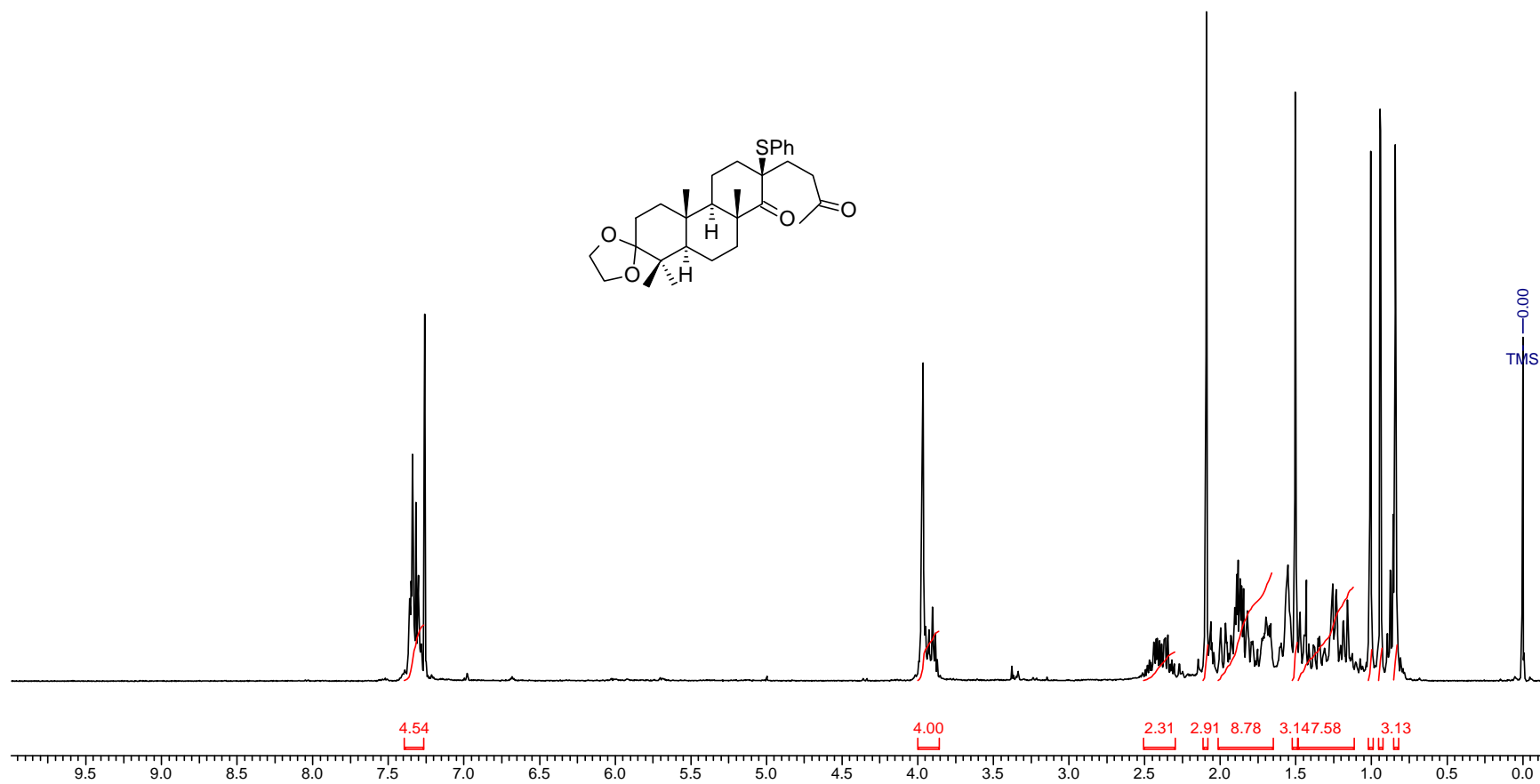
### NOESY of compound (-)-3.3a



# <sup>1</sup>H NMR of Compound (-)-3.3b

AA-10-29-fr31

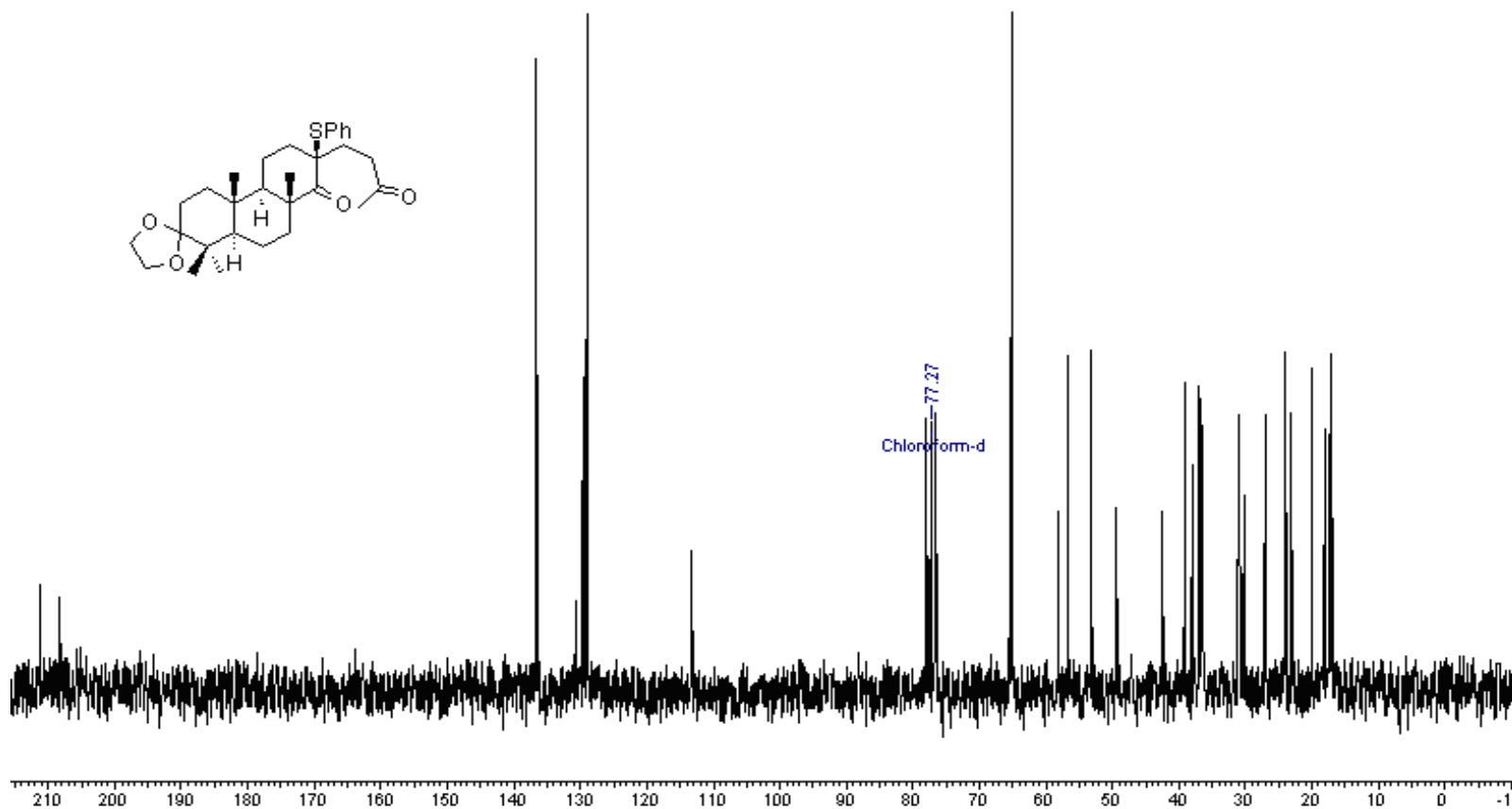
Acquisition Time (sec)	2.5614	Comment	Std proton	Date	Nov 6 2007	Frequency (MHz)	399.77				
Nucleus	<sup>1</sup> H	Number of Transients	32	Original Points Count	13104	Points Count	16384	Solvent	cdcl3	Sweep Width (Hz)	6396.42
Temperature (grad C)	30.000										



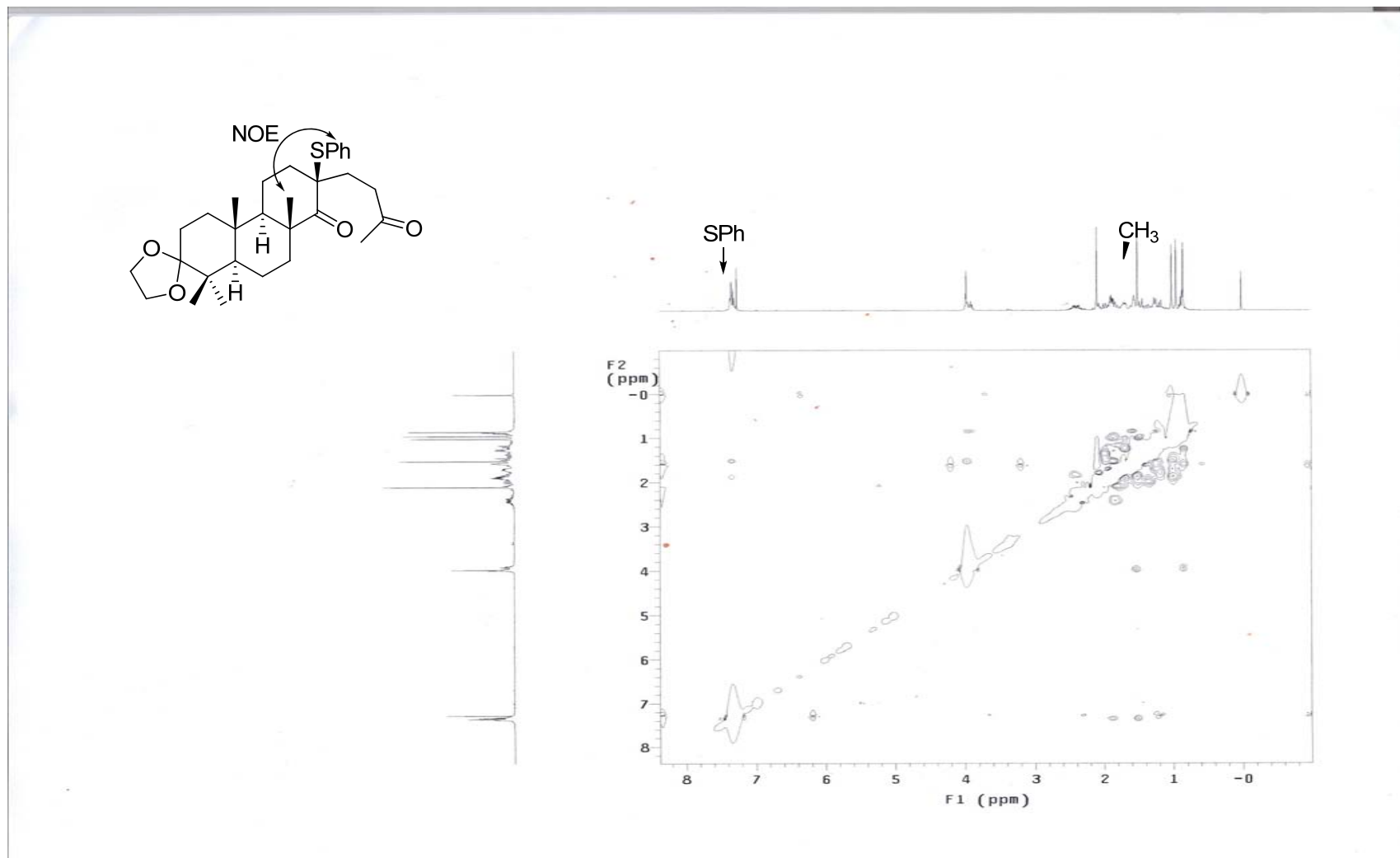
# <sup>13</sup>C NMR of Compound (-)-3.3b

## AA-8-60-fr55-59-C13

Acquisition Time (sec)	2.6214	Comment	13C OBSERVE	Date	Jun 26 2007
Frequency (MHz)	50.29	Nucleus	13C	Number of Transients	1000
Solvent	CDCl3	Sweep Width (Hz)	12500.00	Original Points Count	18720
				Points Count	32768
				Temperature (grad C)	29.000



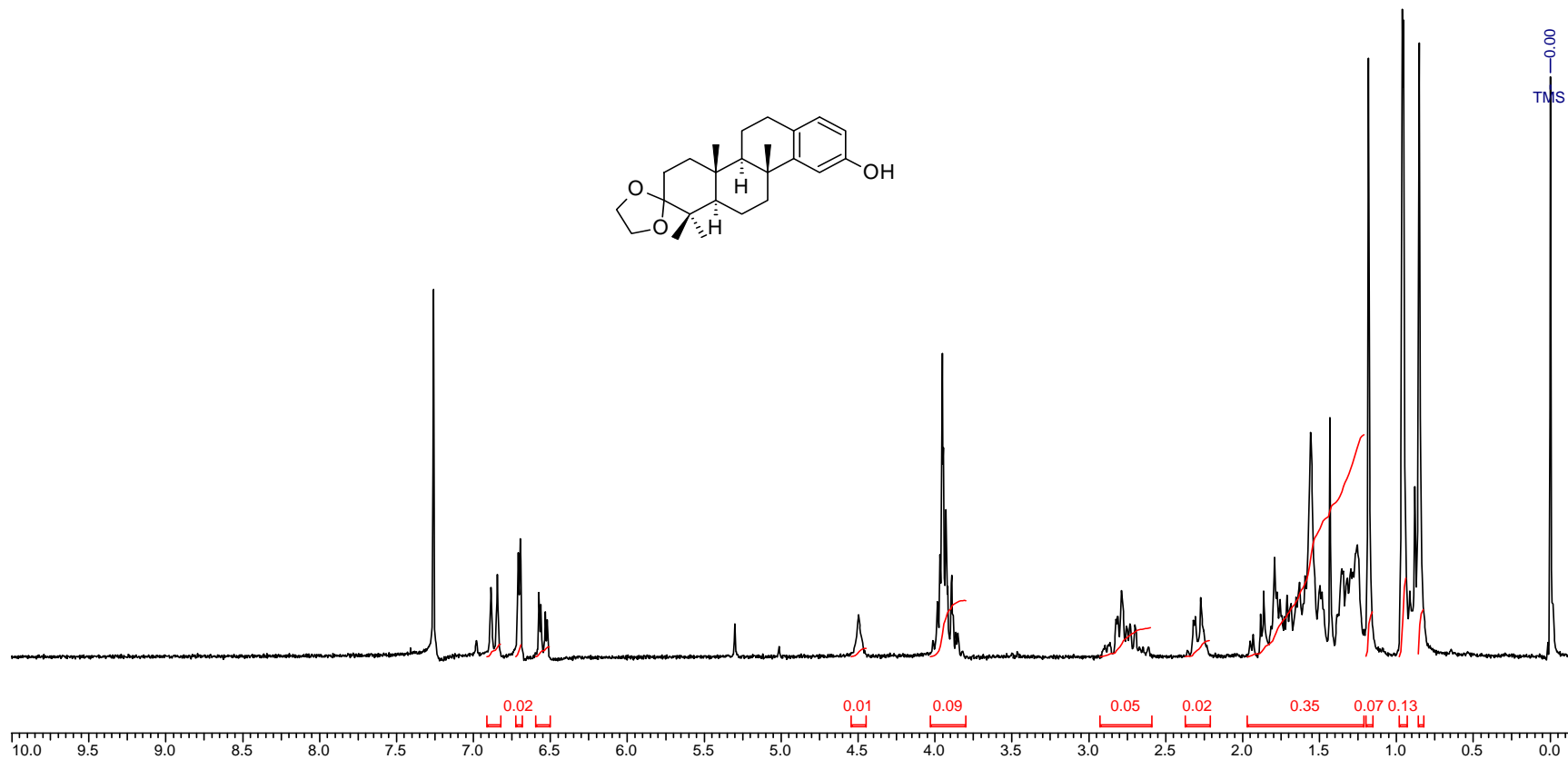
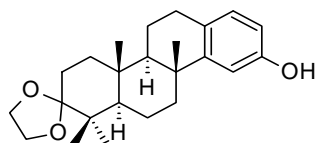
### NOESY of compound (-)-3.3b



# <sup>1</sup>H NMR of Compound 3.11

AA-5-31-fr13-20

Acquisition Time (sec)	2.7307	Comment	STANDARD 1H OBSERVE		Date	Mar 31 2005			
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	400	Original Points Count	5984	Points Count	8192
Solvent	CDCl3	Sweep Width (Hz)	3000.00	Temperature (grad C)	29.000				

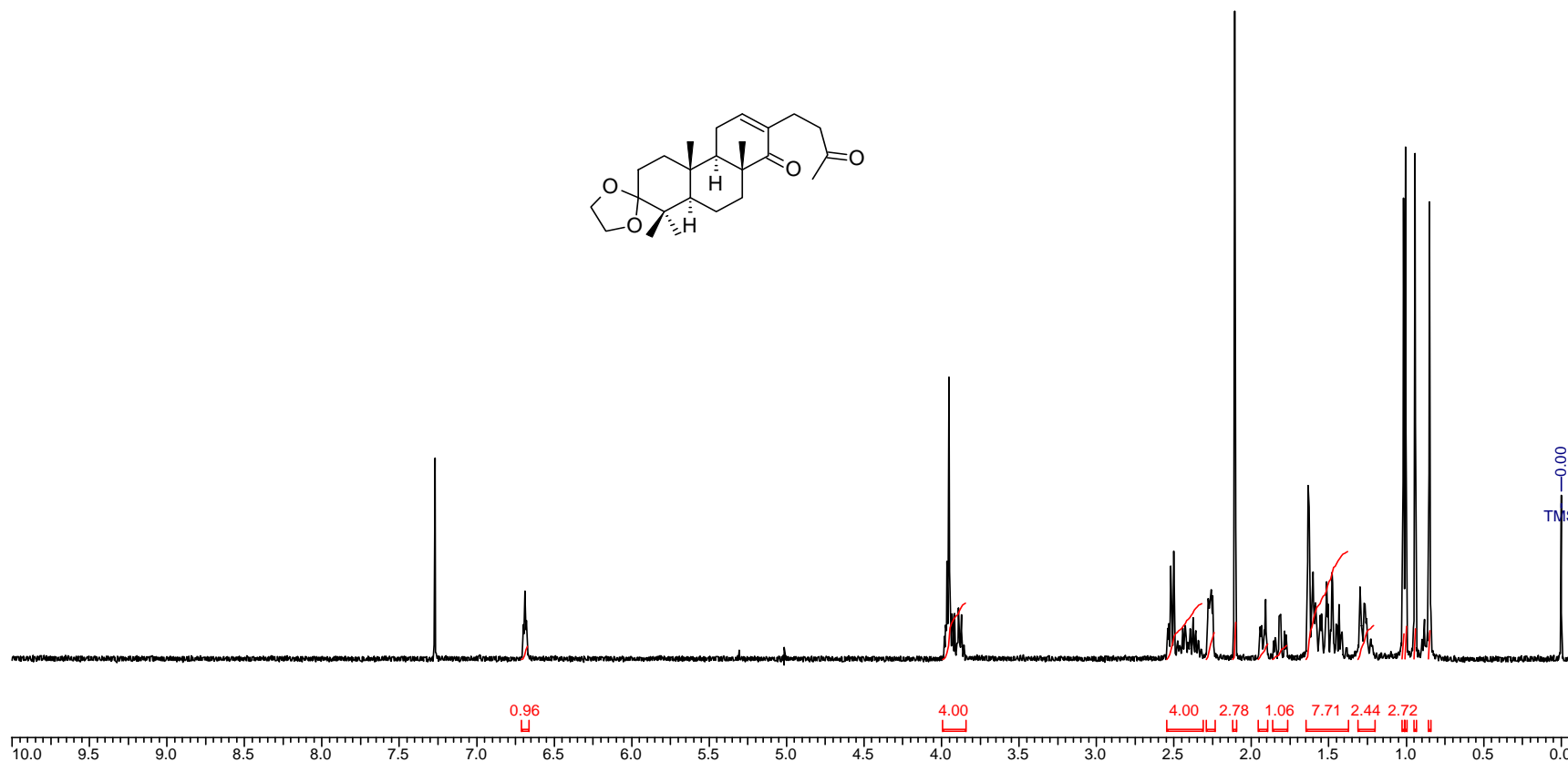
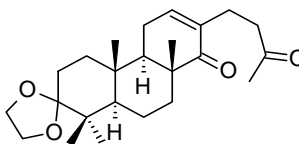




# <sup>1</sup>H NMR of Compound (-)-3.13

AA-5-58-fr15-24

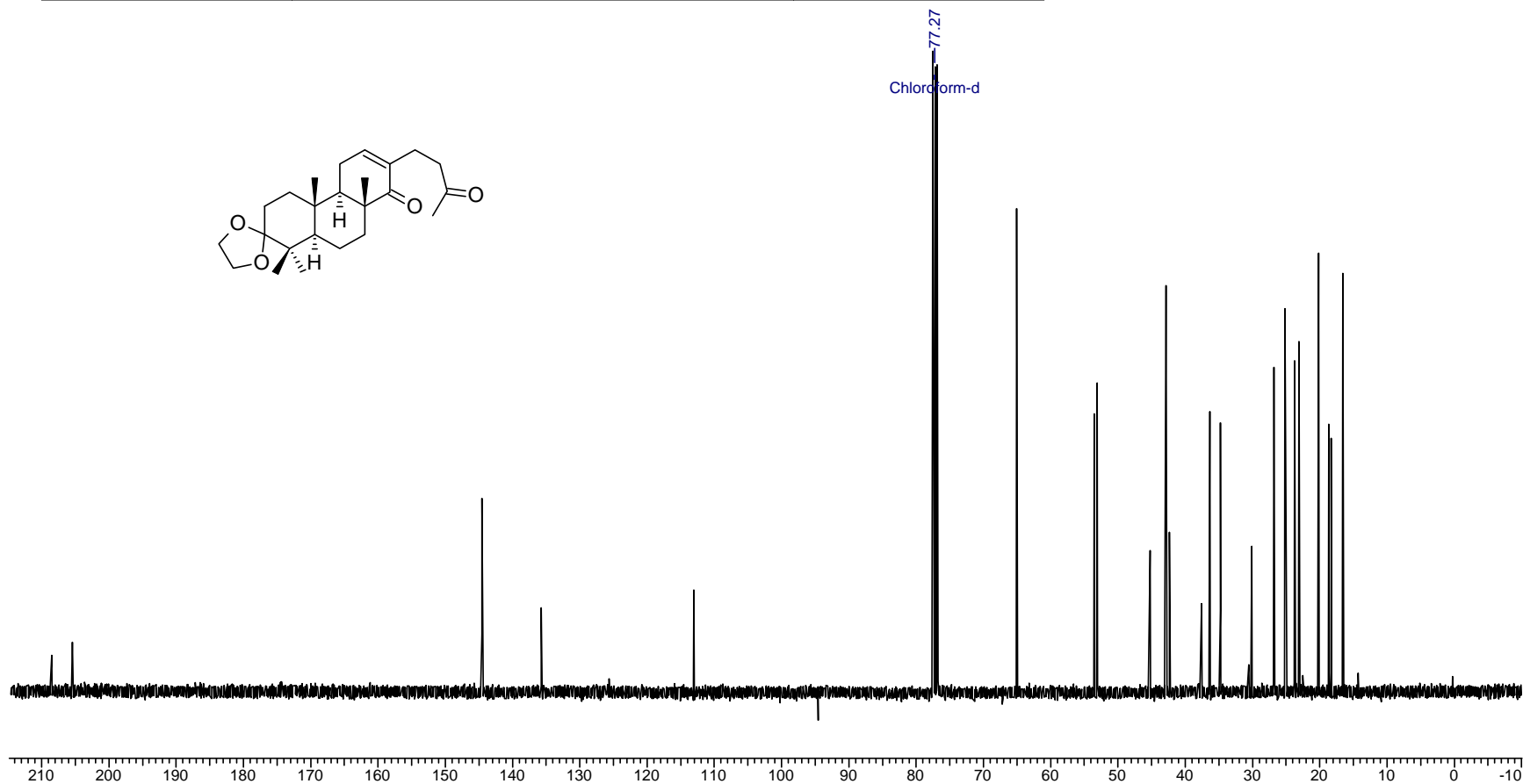
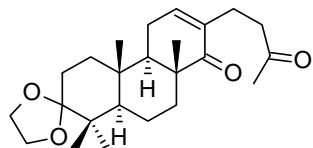
Acquisition Time (sec)	6.5536	Comment	STANDARD 1H OBSERVE		Date	May 13 2005			
Frequency (MHz)	399.79	Nucleus	1H	Number of Transients	300	Original Points Count	18505	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	5000.00	Temperature (grad C)	29.000				



# $^{13}\text{C}$ NMR of Compound (-)-3.13

## AA-5-58-fr15-24-C13

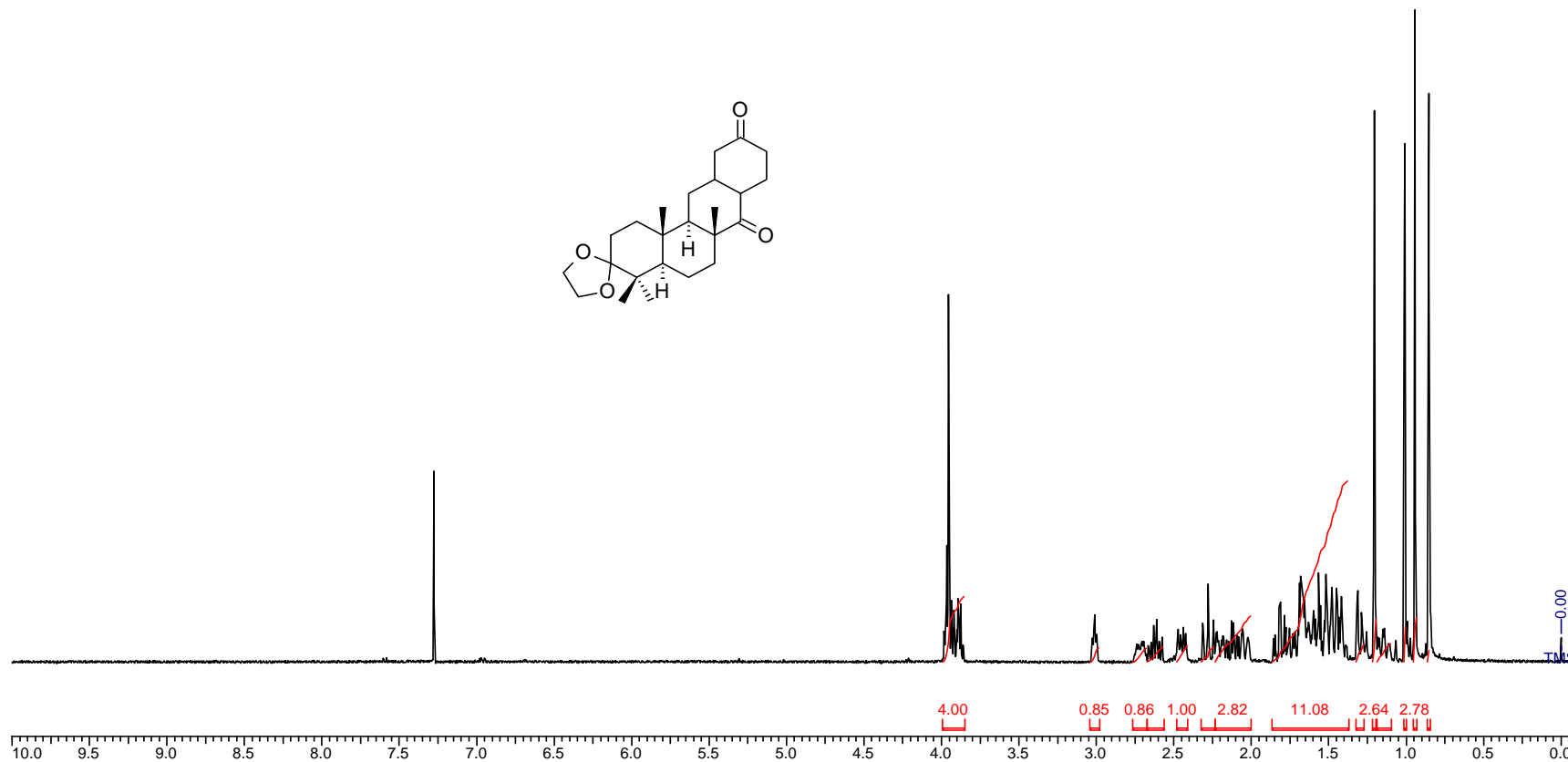
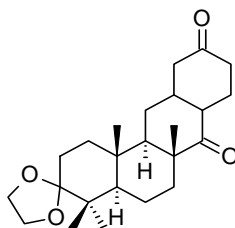
<b>Acquisition Time (sec)</b> 1.3107	<b>Comment</b> 13C OBSERVE	<b>Date</b> May 13 2005		
<b>Frequency (MHz)</b> 100.54	<b>Nucleus</b> 13C	<b>Number of Transients</b> 20000	<b>Original Points Count</b> 29984	<b>Points Count</b> 32768
<b>Solvent</b> CDCl3	<b>Sweep Width (Hz)</b> 25000.00	<b>Temperature (grad C)</b> 29.000		



# $^1\text{H}$ NMR of Compound 3.17

## AA-5-90-undprod

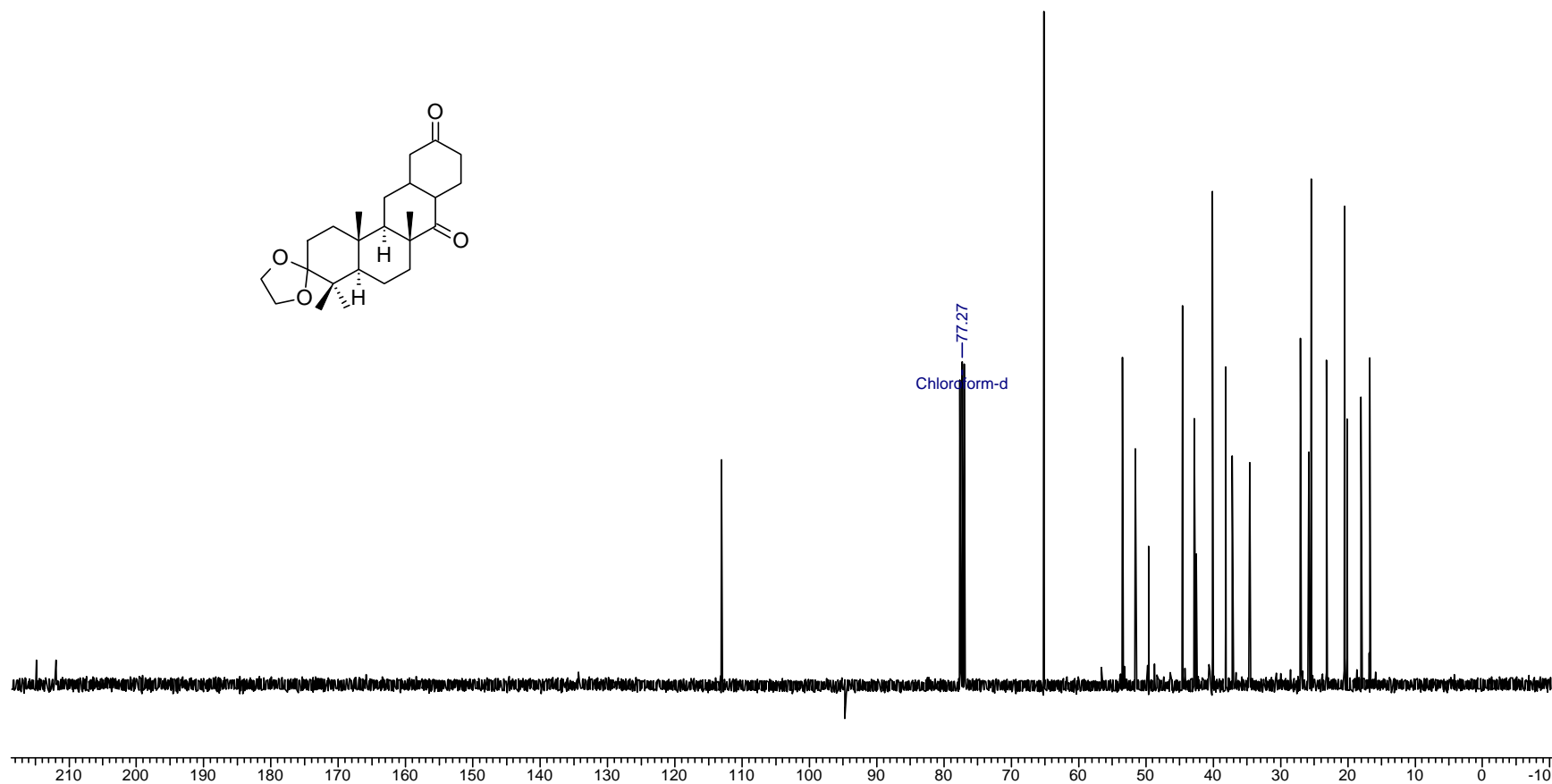
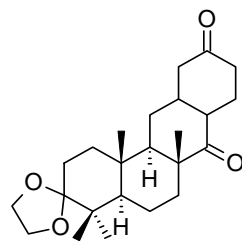
<b>Acquisition Time (sec)</b> 6.5536	<b>Comment</b> STANDARD 1H OBSERVE	<b>Date</b> Jun 28 2005		
<b>Frequency (MHz)</b> 399.79	<b>Nucleus</b> 1H	<b>Number of Transients</b> 32	<b>Original Points Count</b> 18505	<b>Points Count</b> 32768
<b>Solvent</b> CDCl <sub>3</sub>	<b>Sweep Width (Hz)</b> 5000.00	<b>Temperature (grad C)</b> 29.000		



# $^{13}\text{C}$ NMR of Compound 3.17

## AA-5-90-undprodC13

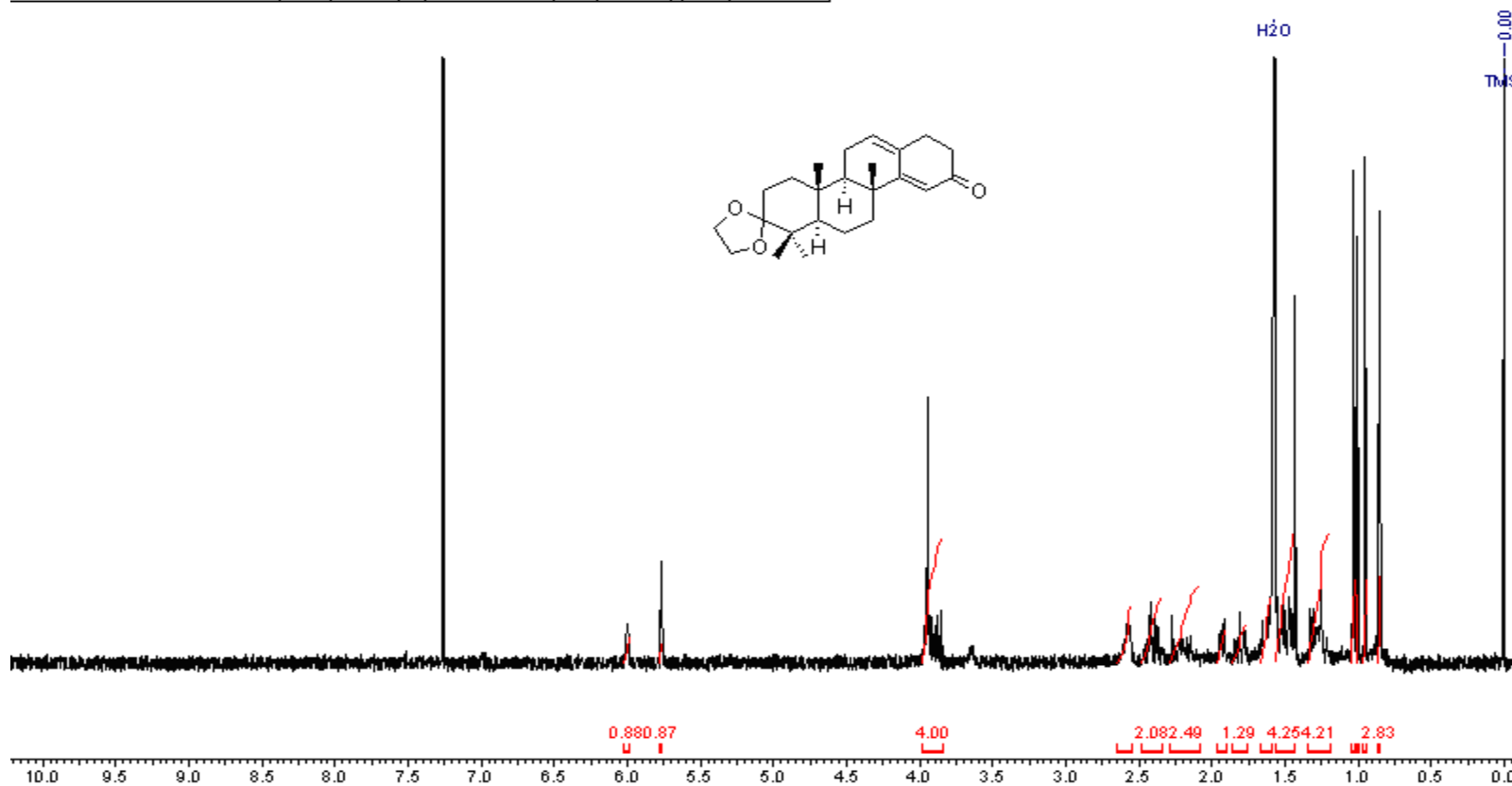
<b>Acquisition Time (sec)</b> 1.3107	<b>Comment</b> 13C OBSERVE	<b>Date</b> Jun 28 2005		
<b>Frequency (MHz)</b> 100.54	<b>Nucleus</b> 13C	<b>Number of Transients</b> 20000	<b>Original Points Count</b> 29984	<b>Points Count</b> 32768
<b>Solvent</b> CDCl3	<b>Sweep Width (Hz)</b> 25000.00	<b>Temperature (grad C)</b> 29.000		



# <sup>1</sup>H NMR of Compound 3.16

## AA-5-90-fr41-60

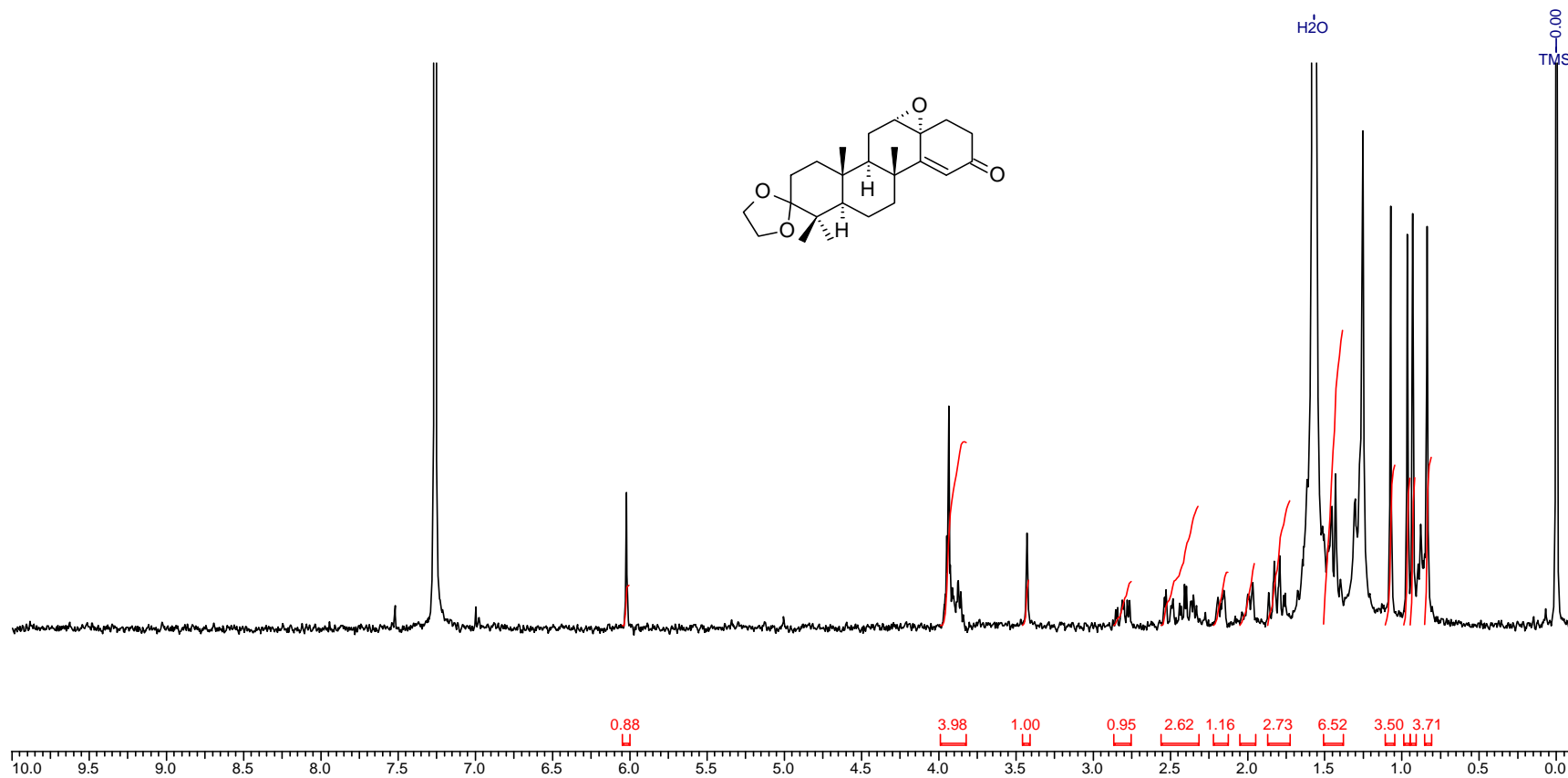
Acquisition Time (sec)	6.5536	Comment	STANDARD 1H OBSERVE	Date	Jun 25 2005
Frequency (MHz)	399.79	Nucleus	1H	Number of Transients	300
Solvent	CDCl <sub>3</sub>	Sweep Width (Hz)	9000.00	Original Points Count	18505
		Temperature (grad C)	29.000	Points Count	32768



# <sup>1</sup>H NMR of Compound 3.21

## AA-5-92-pure

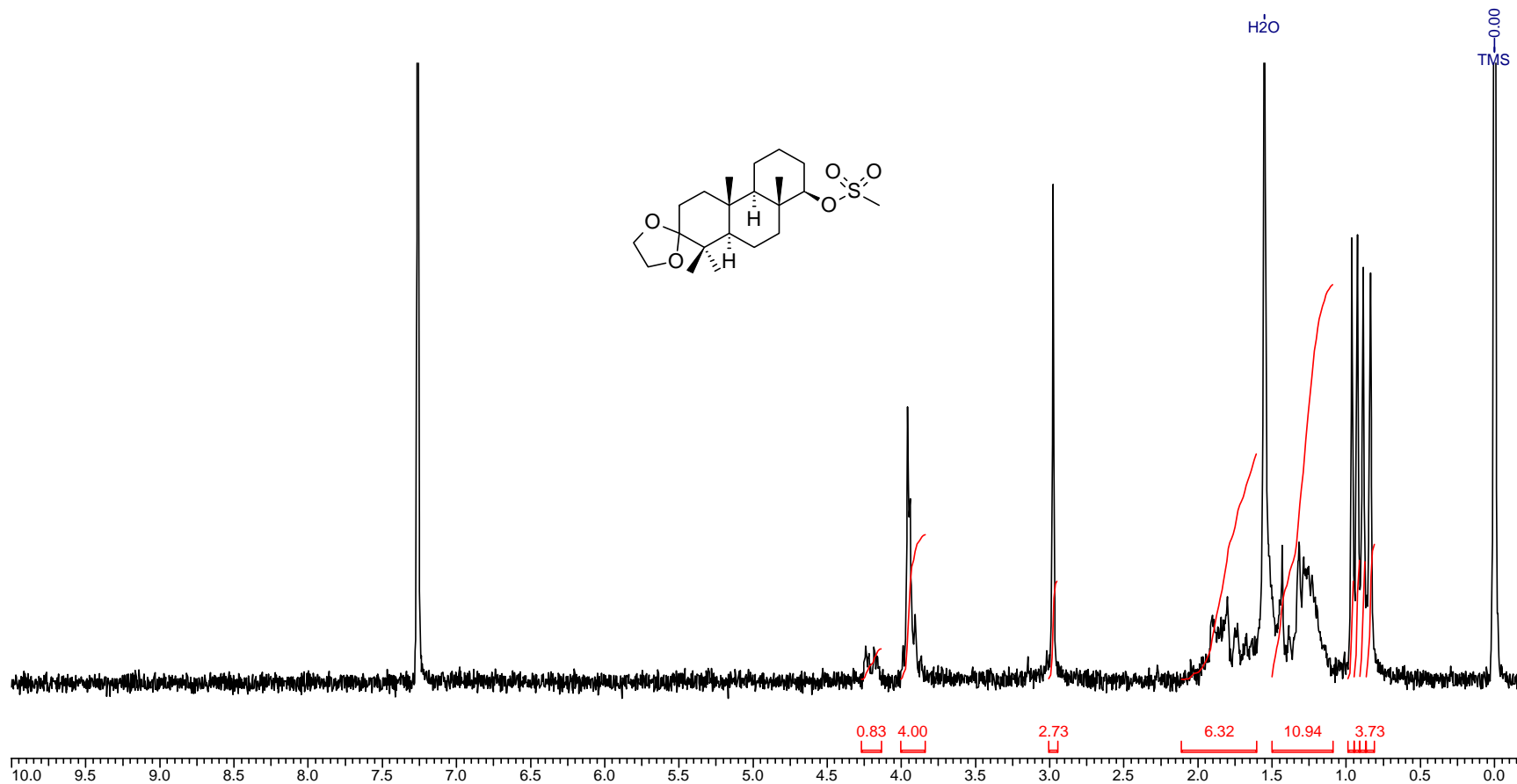
<b>Acquisition Time (sec)</b> 6.5536	<b>Comment</b> STANDARD 1H OBSERVE	<b>Date</b> Jun 27 2005		
<b>Frequency (MHz)</b> 399.79	<b>Nucleus</b> 1H	<b>Number of Transients</b> 300	<b>Original Points Count</b> 18505	<b>Points Count</b> 32768
<b>Solvent</b> CDCl <sub>3</sub>	<b>Sweep Width (Hz)</b> 5000.00	<b>Temperature (grad C)</b> 29.000		



# <sup>1</sup>H NMR of Compound 3.30

## AA-6-116-1crude

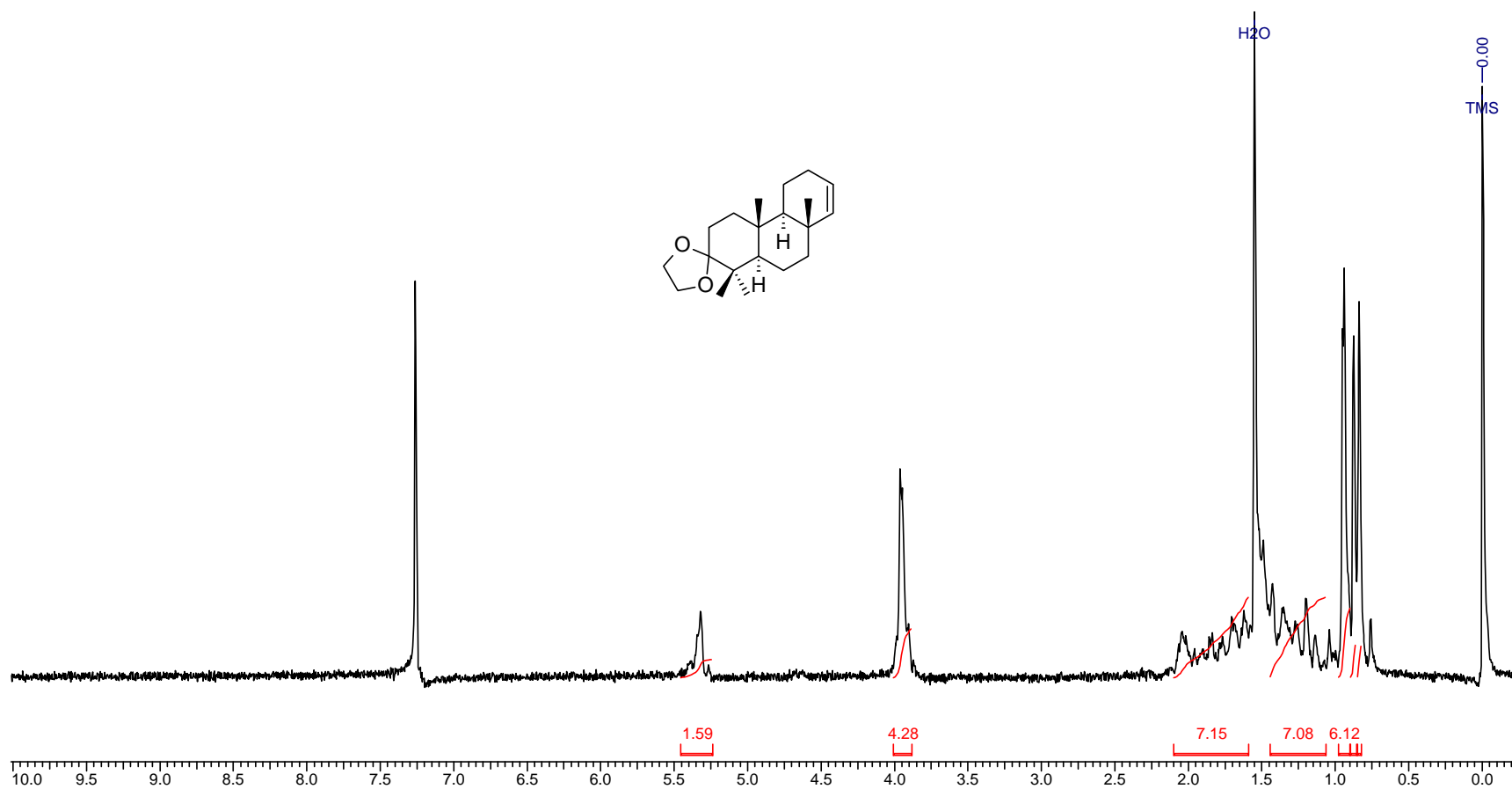
<b>Acquisition Time (sec)</b> 2.7307	<b>Comment</b> STANDARD 1H OBSERVE	<b>Date</b> Jul 10 2006		
<b>Frequency (MHz)</b> 199.98	<b>Nucleus</b> 1H	<b>Number of Transients</b> 300	<b>Original Points Count</b> 5984	<b>Points Count</b> 8192
<b>Solvent</b> CDCl <sub>3</sub>	<b>Sweep Width (Hz)</b> 3000.00	<b>Temperature (grad C)</b> 29.000		



# <sup>1</sup>H NMR of Compound 3.31

AA-6-119-f7-13

<b>Acquisition Time (sec)</b>	2.7307	<b>Comment</b>	STANDARD 1H OBSERVE		<b>Date</b>	Jul 20 2006			
<b>Frequency (MHz)</b>	199.98	<b>Nucleus</b>	1H	<b>Number of Transients</b>	50	<b>Original Points Count</b>	5984	<b>Points Count</b>	8192
<b>Solvent</b>	CDCl3	<b>Sweep Width (Hz)</b>	3000.00	<b>Temperature (grad C)</b>	29.000				

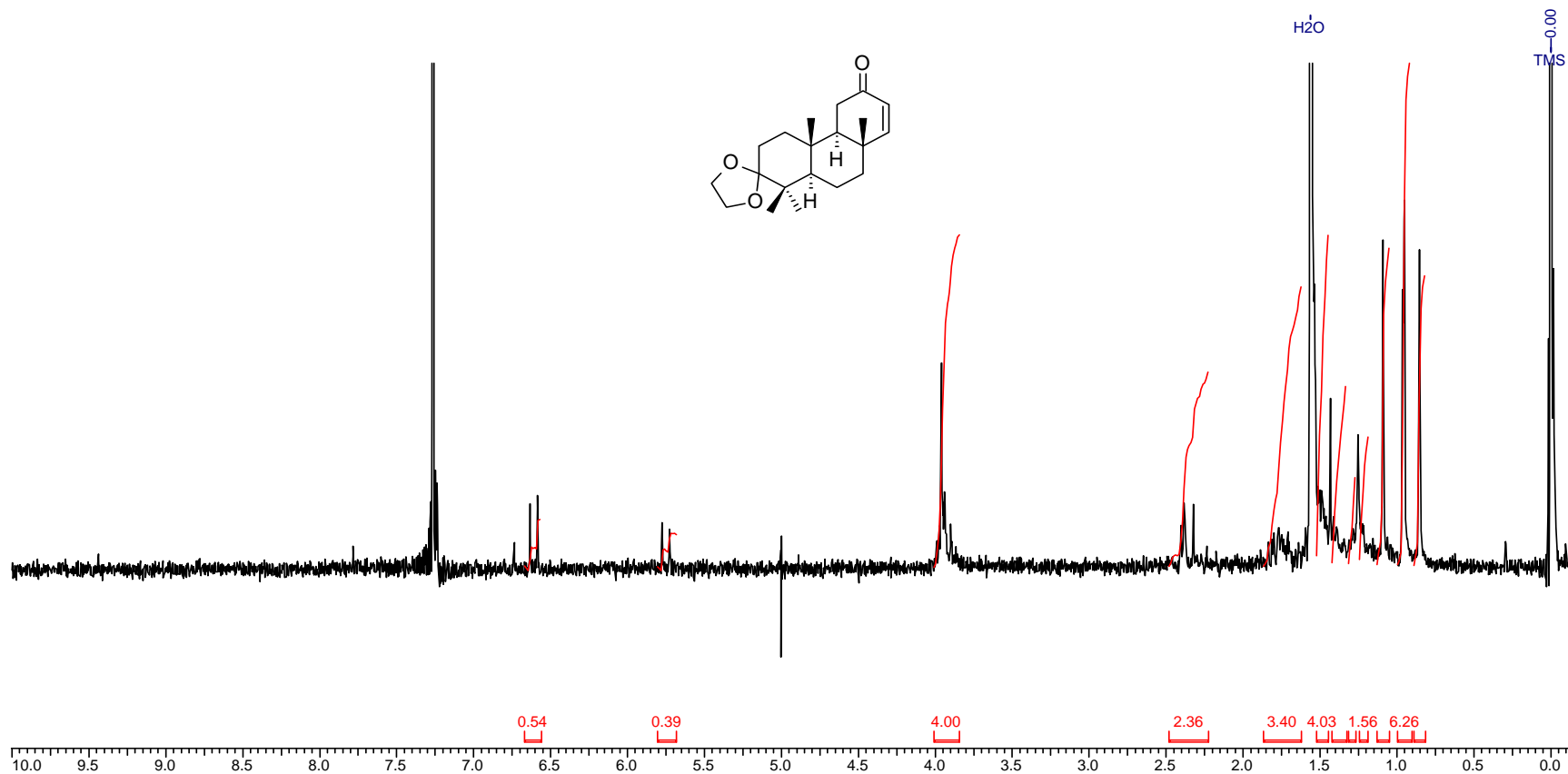




# <sup>1</sup>H NMR of Compound 3.32

AA-7-13-fr29-35

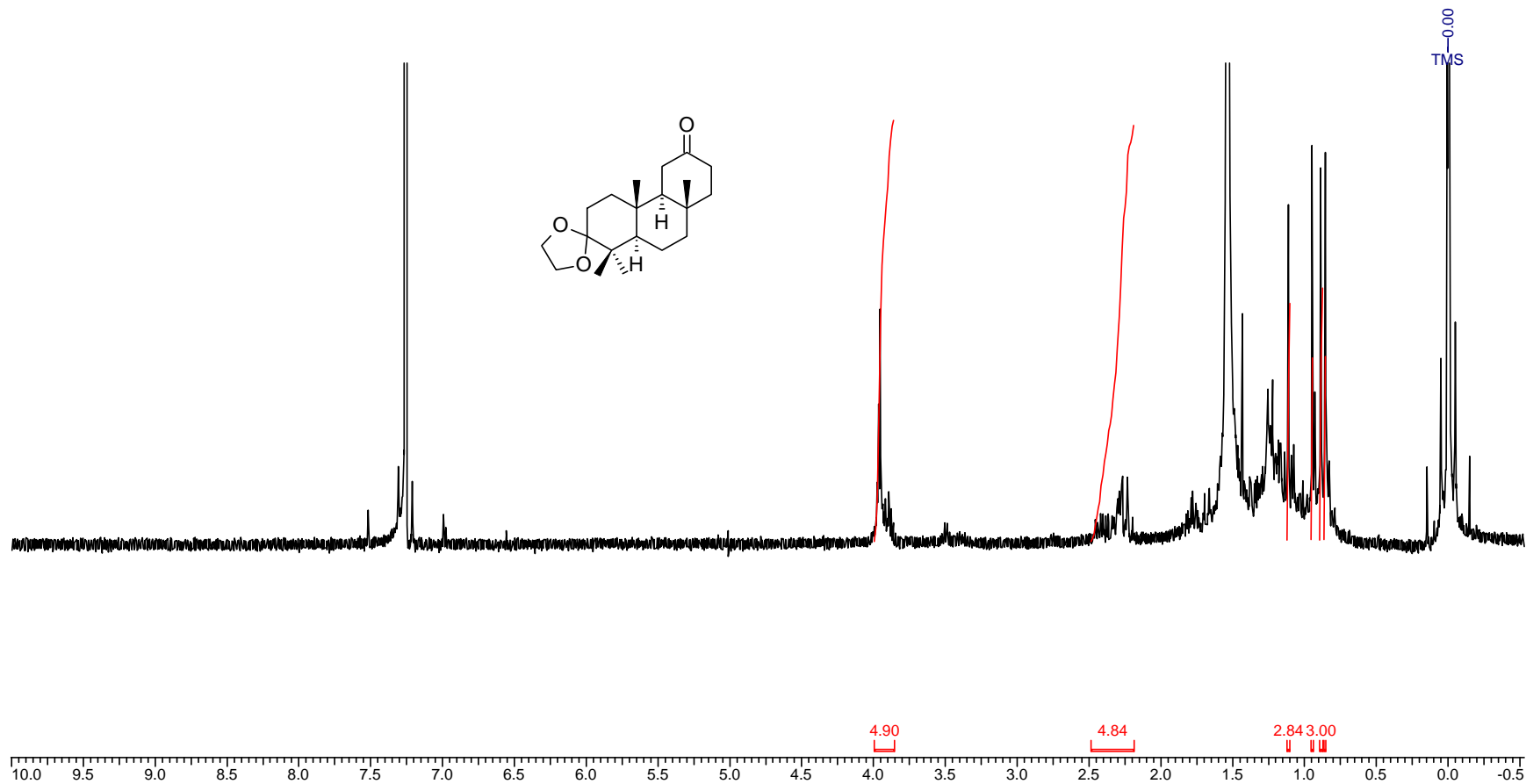
Acquisition Time (sec)	2.7307	Comment	STANDARD 1H OBSERVE		Date	Jul 27 2006			
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	300	Original Points Count	5984	Points Count	8192
Solvent	CDCl3	Sweep Width (Hz)	3000.00	Temperature (grad C)	29.000				



# <sup>1</sup>H NMR of Compound 3.34

## AA-7-92-1crude

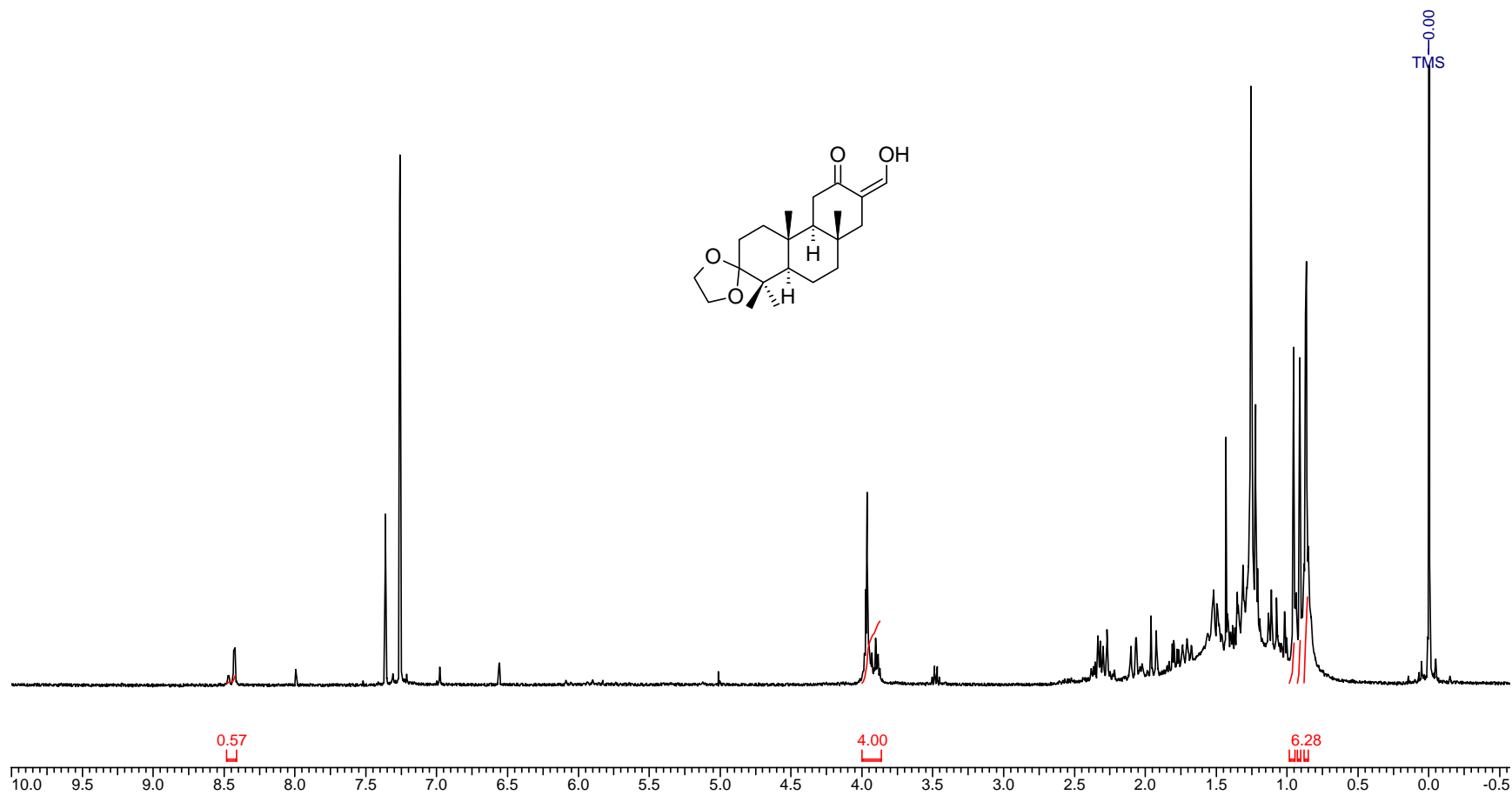
Acquisition Time (sec)	6.5536	Comment	STANDARD 1H OBSERVE	Date	Jan 4 2007				
Frequency (MHz)	399.78	Nucleus	1H	Number of Transients	300	Original Points Count	18505	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	5000.00	Temperature (grad C)	25.000				



# <sup>1</sup>H NMR of Compound 3.35

## AA-7-95-1crude

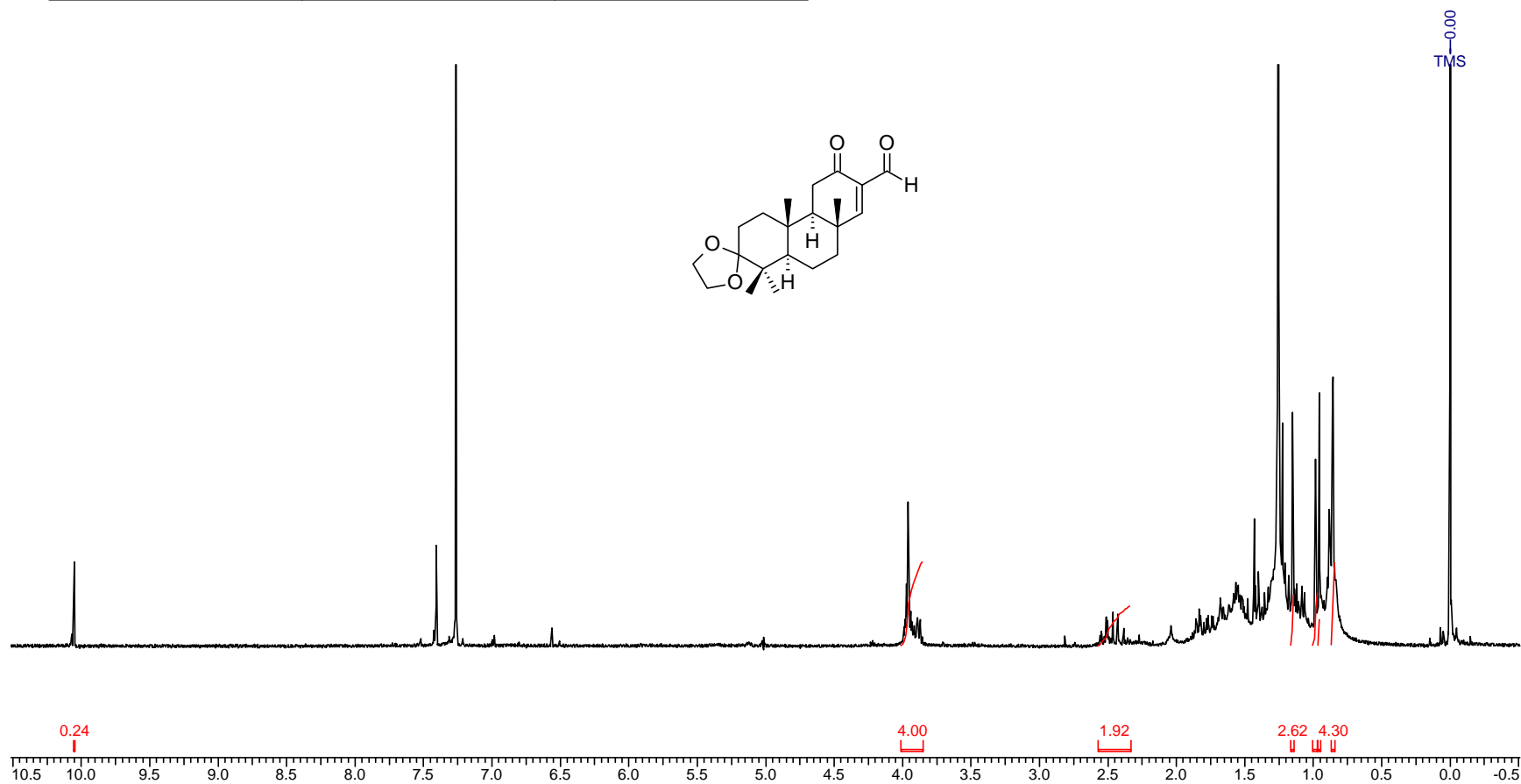
<b>Acquisition Time (sec)</b>	6.5536	<b>Comment</b>	STANDARD 1H OBSERVE		<b>Date</b>	Jan 15 2007			
<b>Frequency (MHz)</b>	399.78	<b>Nucleus</b>	1H	<b>Number of Transients</b>	300	<b>Original Points Count</b>	18505	<b>Points Count</b>	32768
<b>Solvent</b>	CDCl3	<b>Sweep Width (Hz)</b>	5000.00	<b>Temperature (grad C)</b>	25.000				



# <sup>1</sup>H NMR of Compound 3.28a

## AA-7-96-1crude

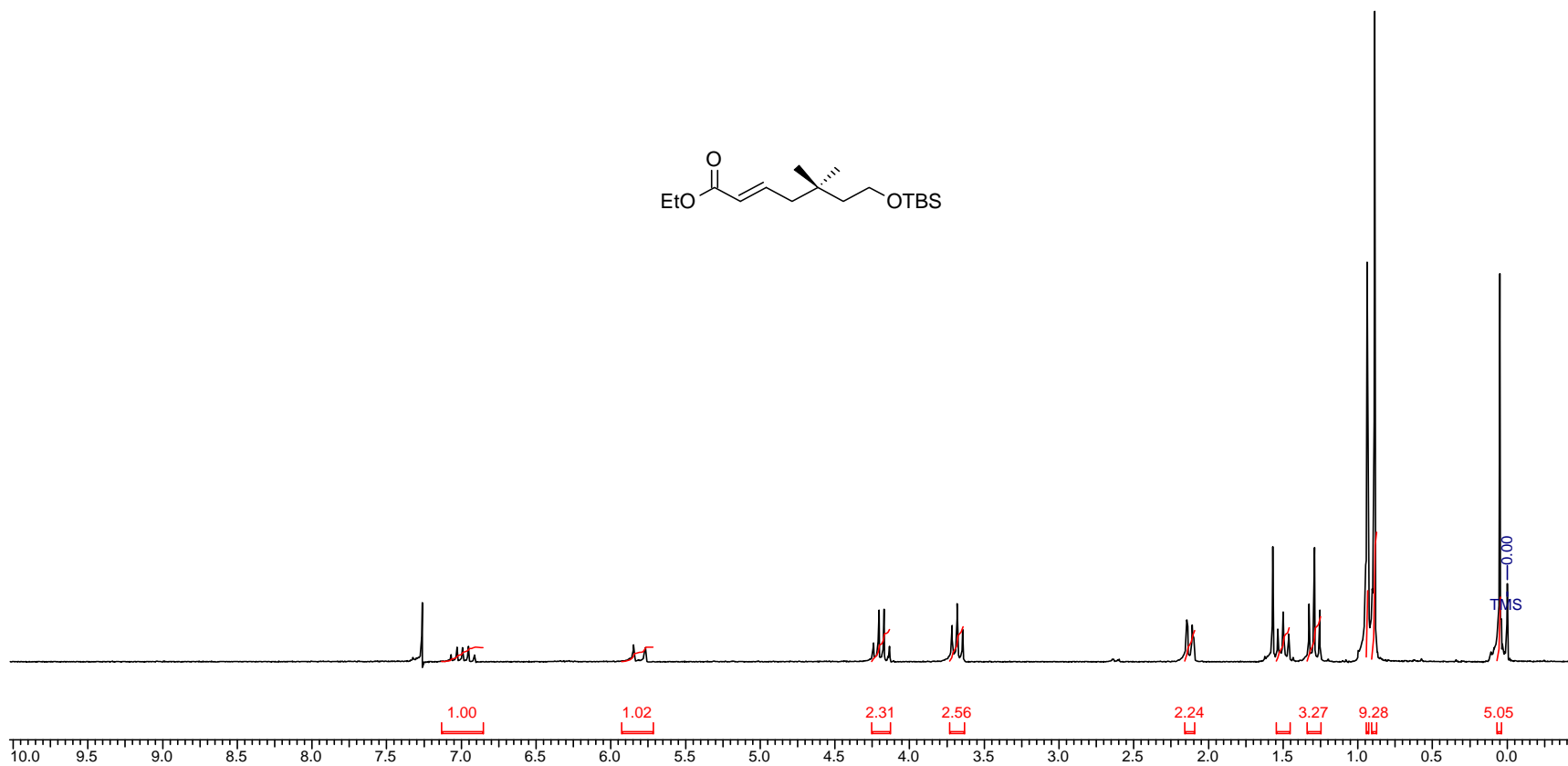
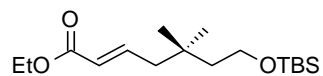
Acquisition Time (sec)	6.5536	Comment	STANDARD 1H OBSERVE	Date	Jan 18 2007				
Frequency (MHz)	399.78	Nucleus	1H	Number of Transients	300	Original Points Count	18505	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	5000.00	Temperature (grad C)	25.000				



# <sup>1</sup>H NMR of Compound 3.36

AA-7-62-fr1-10

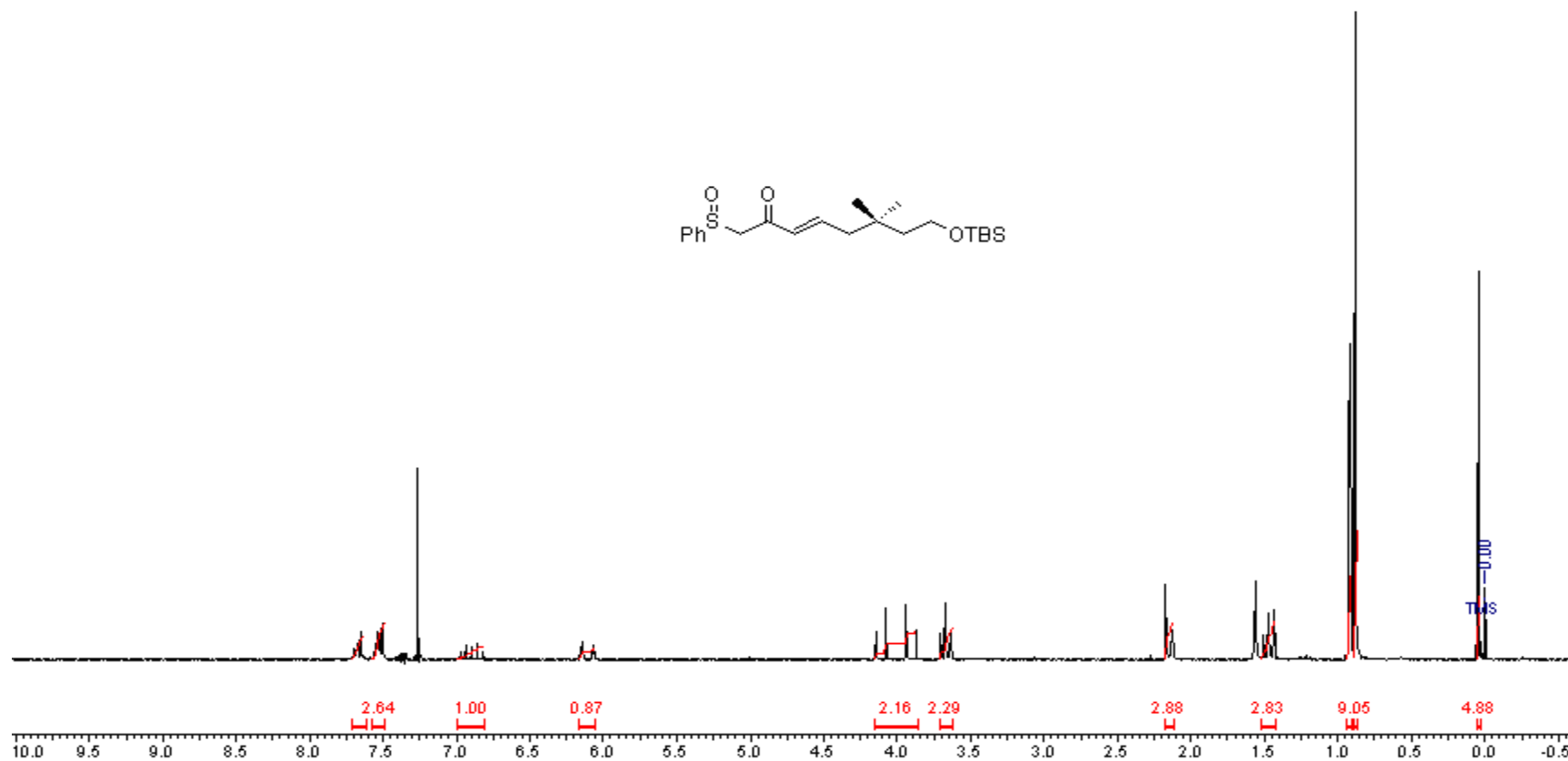
Acquisition Time (sec)	2.7307	Comment	STANDARD 1H OBSERVE		Date	Oct 19 2006			
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	300	Original Points Count	5984	Points Count	8192
Solvent	CDCl3	Sweep Width (Hz)	3000.00	Temperature (grad C)	29.000				



# <sup>1</sup>H NMR of Compound 3.29

AA-7-67-fr11-24

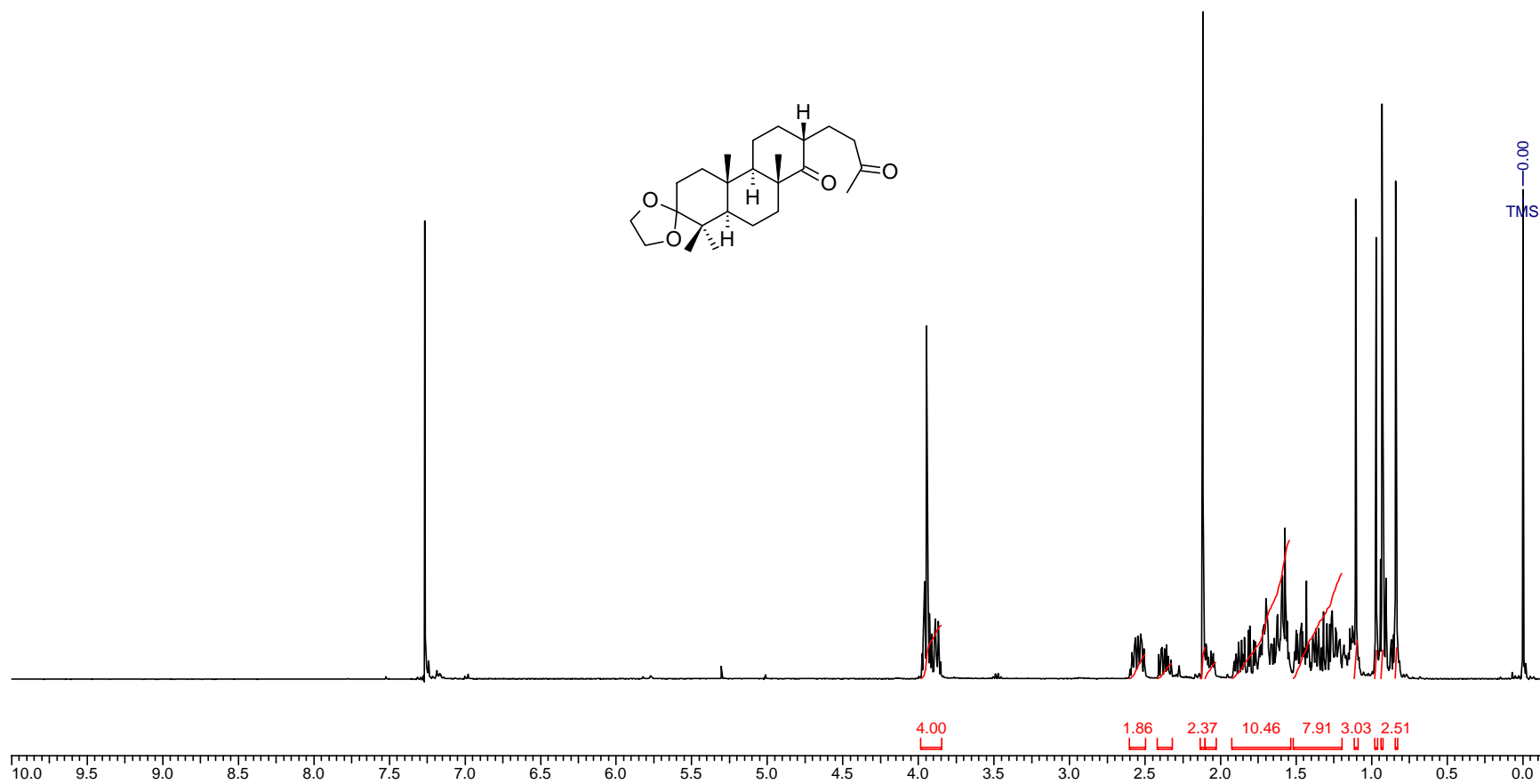
Acquisition Time (sec)	2.7307	Comment	STANDARD 1H OBSERVE	Date	Nov 22 2006
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	300
Solvent	CDCl3	Sweep Width (Hz)	3000.00	Original Points Count	5984
		Temperature (grad C)	29.000	Points Count	8192



# <sup>1</sup>H NMR of Compound (-)-3.39a

AA-10-69-fr17-21

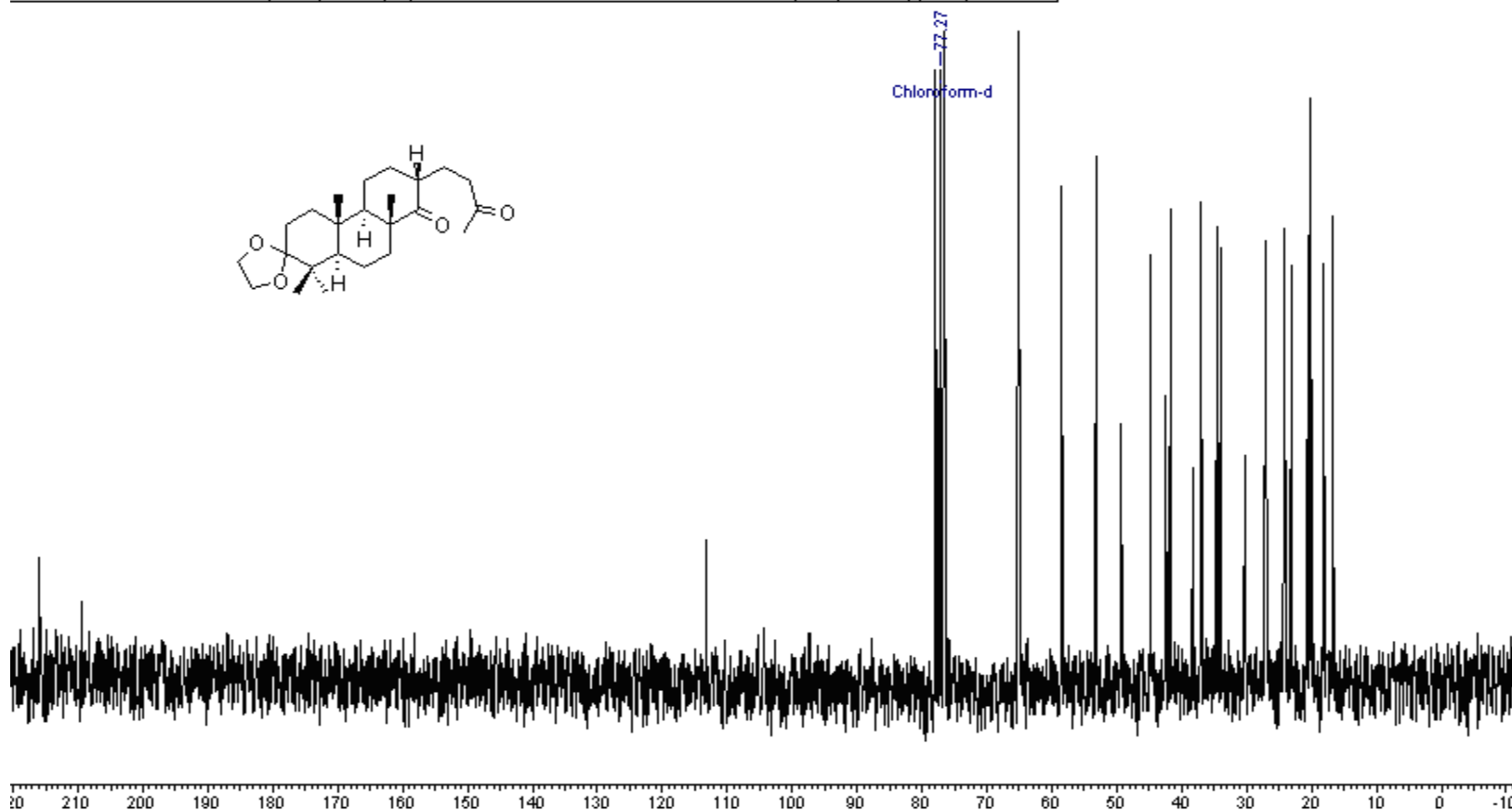
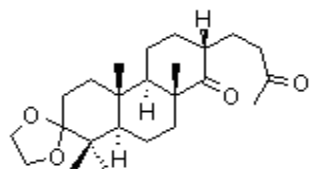
Acquisition Time (sec)	3.4152	Comment	Std proton	Date	Jan 30 2008	Frequency (MHz)	399.77				
Nucleus	<sup>1</sup> H	Number of Transients	100	Original Points Count	9828	Points Count	16384	Solvent	cdcl3	Sweep Width (Hz)	4797.31
Temperature (grad C)	25.000										



# <sup>13</sup>C NMR of Compound (-)-3.39a

## AA-8-46-fr19-28-C13

Acquisition Time (sec)	2.6214	Comment	13C OBSERVE	Date	May 23 2007				
Frequency (MHz)	50.29	Nucleus	13C	Number of Transients	10000	Original Points Count	18720	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	12500.00	Temperature (grad C)	29.000				

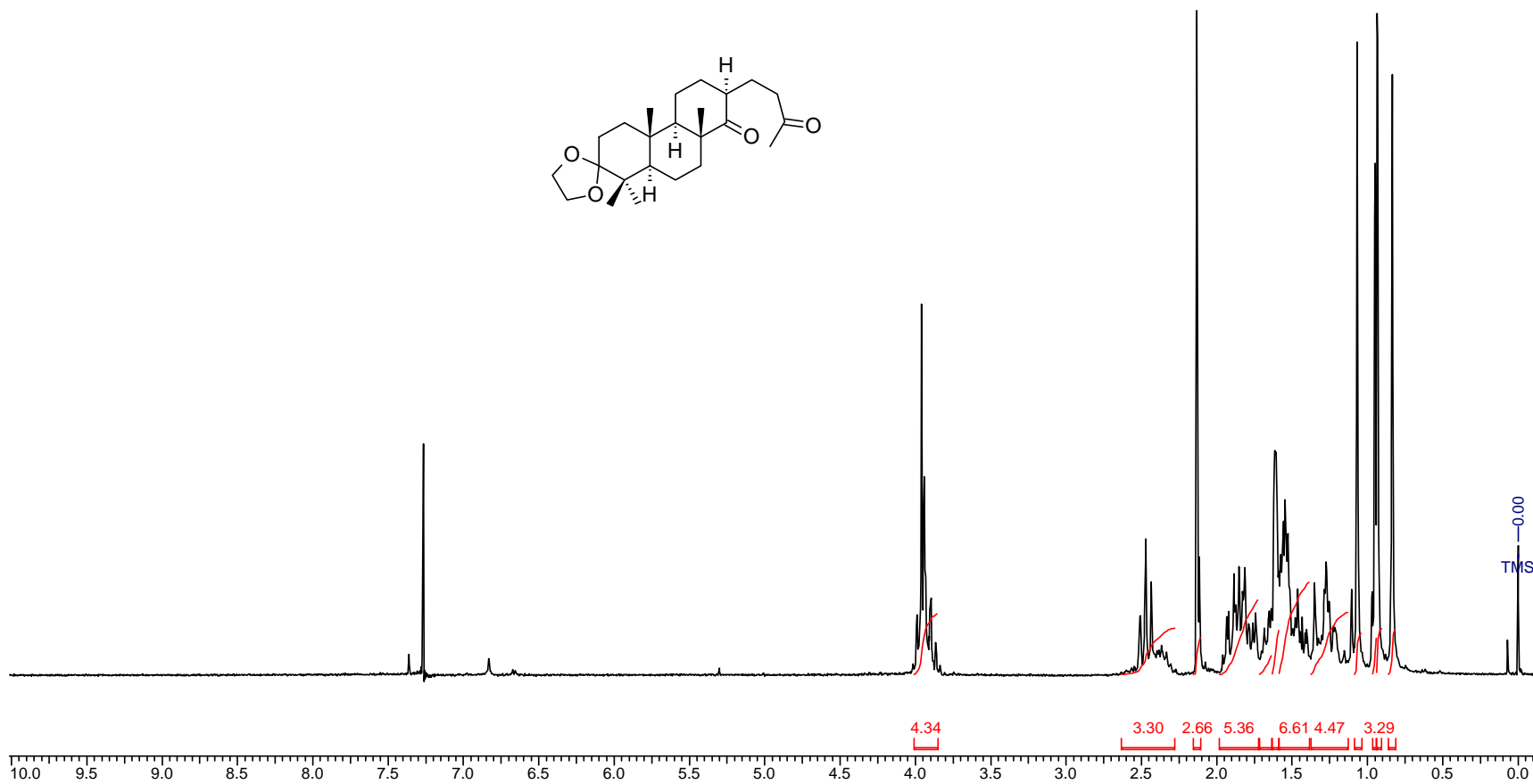
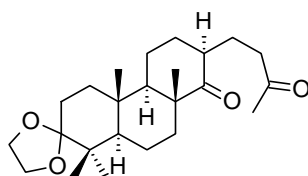




# <sup>1</sup>H NMR of Compound (-)-3.39b

AA-8-46-fr39-48

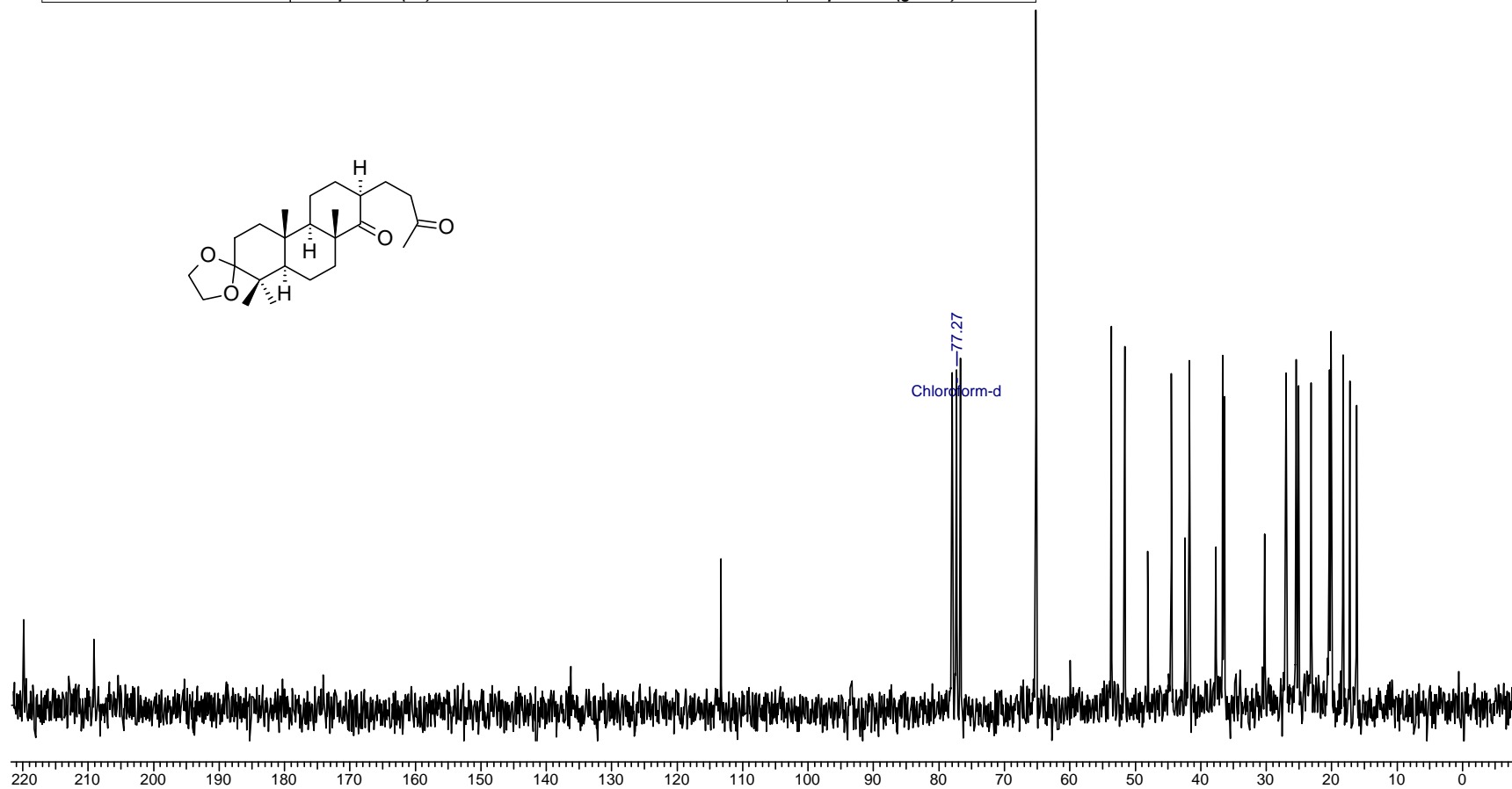
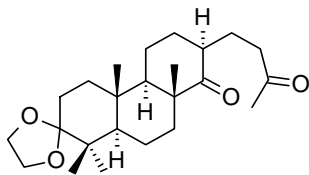
Acquisition Time (sec)	2.7307	Comment	STANDARD 1H OBSERVE		Date	May 23 2007			
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	32	Original Points Count	5984	Points Count	8192
Solvent	CDCl3	Sweep Width (Hz)	3000.00	Temperature (grad C)	29.000				



# <sup>13</sup>C NMR of Compound (-)-3.39b

## AA-8-46+63-fr39-48-C13

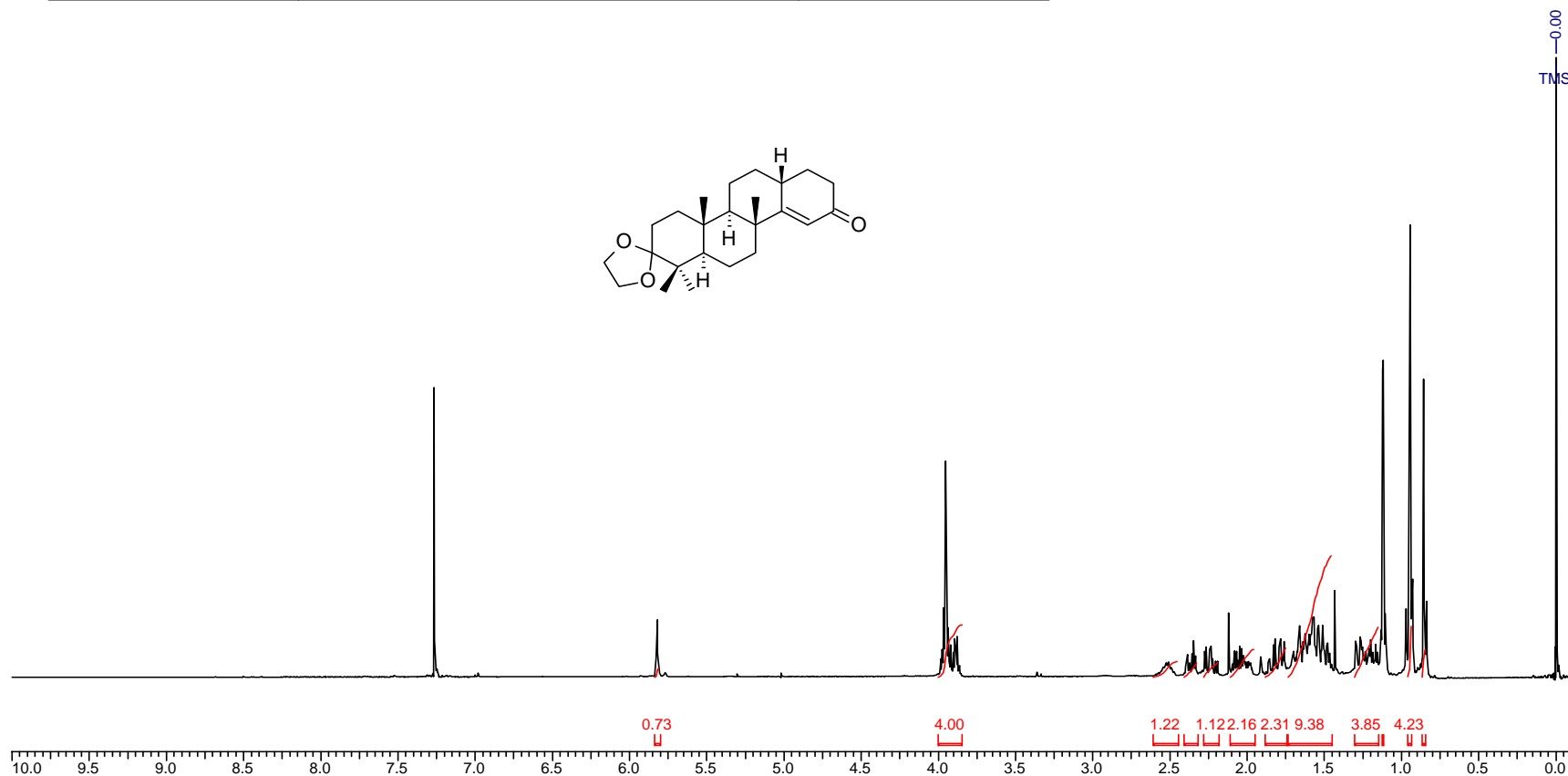
<b>Acquisition Time (sec)</b> 2.6214	<b>Comment</b> 13C OBSERVE	<b>Date</b> Jul 3 2007		
<b>Frequency (MHz)</b> 50.29	<b>Nucleus</b> 13C	<b>Number of Transients</b> 1000	<b>Original Points Count</b> 18720	<b>Points Count</b> 32768
<b>Solvent</b> CDCl3	<b>Sweep Width (Hz)</b> 12500.00	<b>Temperature (grad C)</b> 29.000		



# <sup>1</sup>H NMR of Compound 3.41a

AA-10-72+73-fr3-9

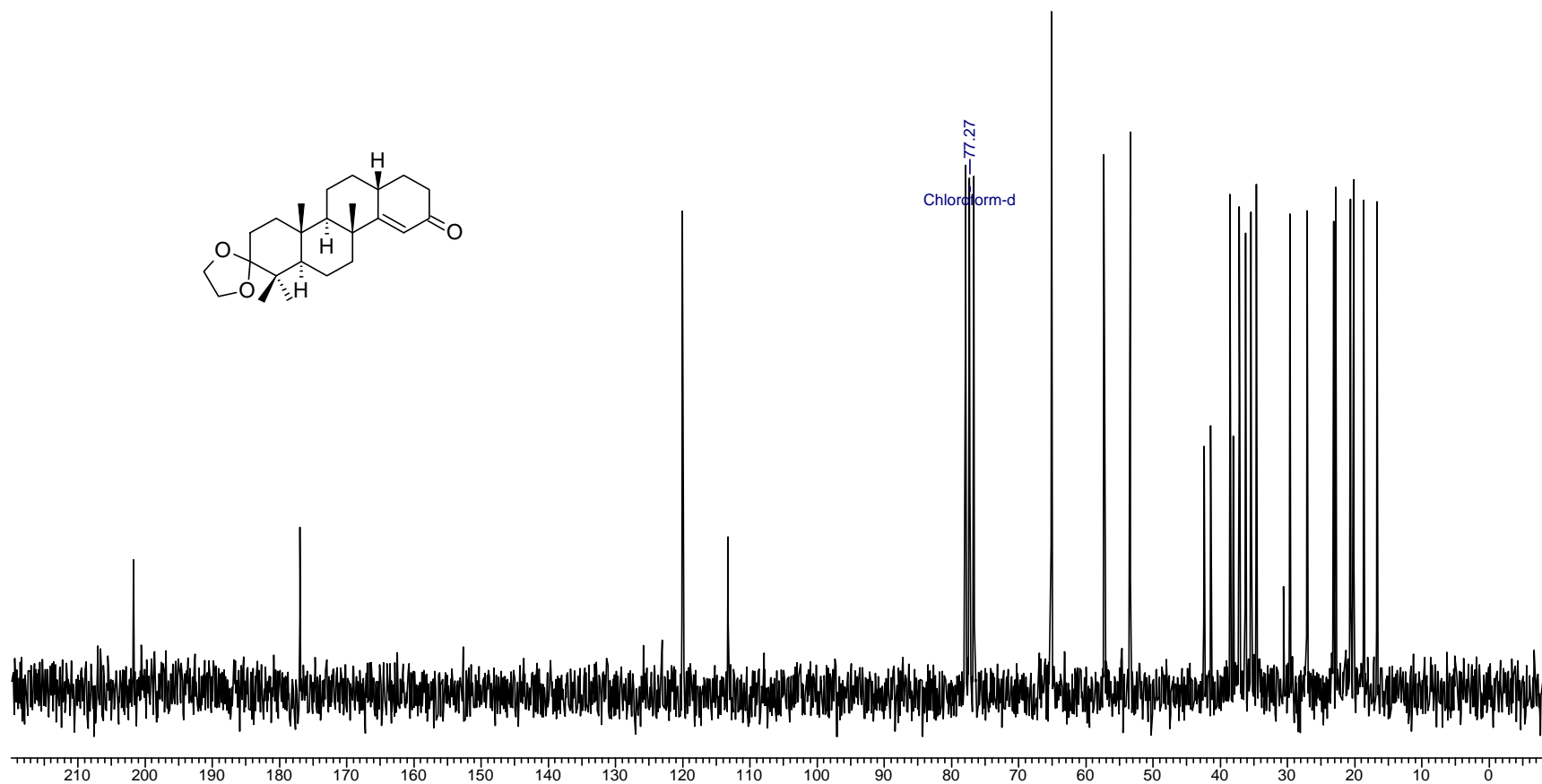
Acquisition Time (sec)	3.4152	Comment	Std proton	Date	Feb 4 2008				
Frequency (MHz)	399.77	Nucleus	1H	Number of Transients	100	Original Points Count	9828	Points Count	16384
Solvent	CDCl3	Sweep Width (Hz)	4797.31	Temperature (grad C)	20.000				



# <sup>13</sup>C NMR of Compound 3.41a

## AA-8-47-fr6-20-C13

<b>Acquisition Time (sec)</b> 2.6214	<b>Comment</b> 13C OBSERVE	<b>Date</b> May 28 2007		
<b>Frequency (MHz)</b> 50.29	<b>Nucleus</b> 13C	<b>Number of Transients</b> 10000	<b>Original Points Count</b> 18720	<b>Points Count</b> 32768
<b>Solvent</b> CDCl3	<b>Sweep Width (Hz)</b> 12500.00	<b>Temperature (grad C)</b> 29.000		

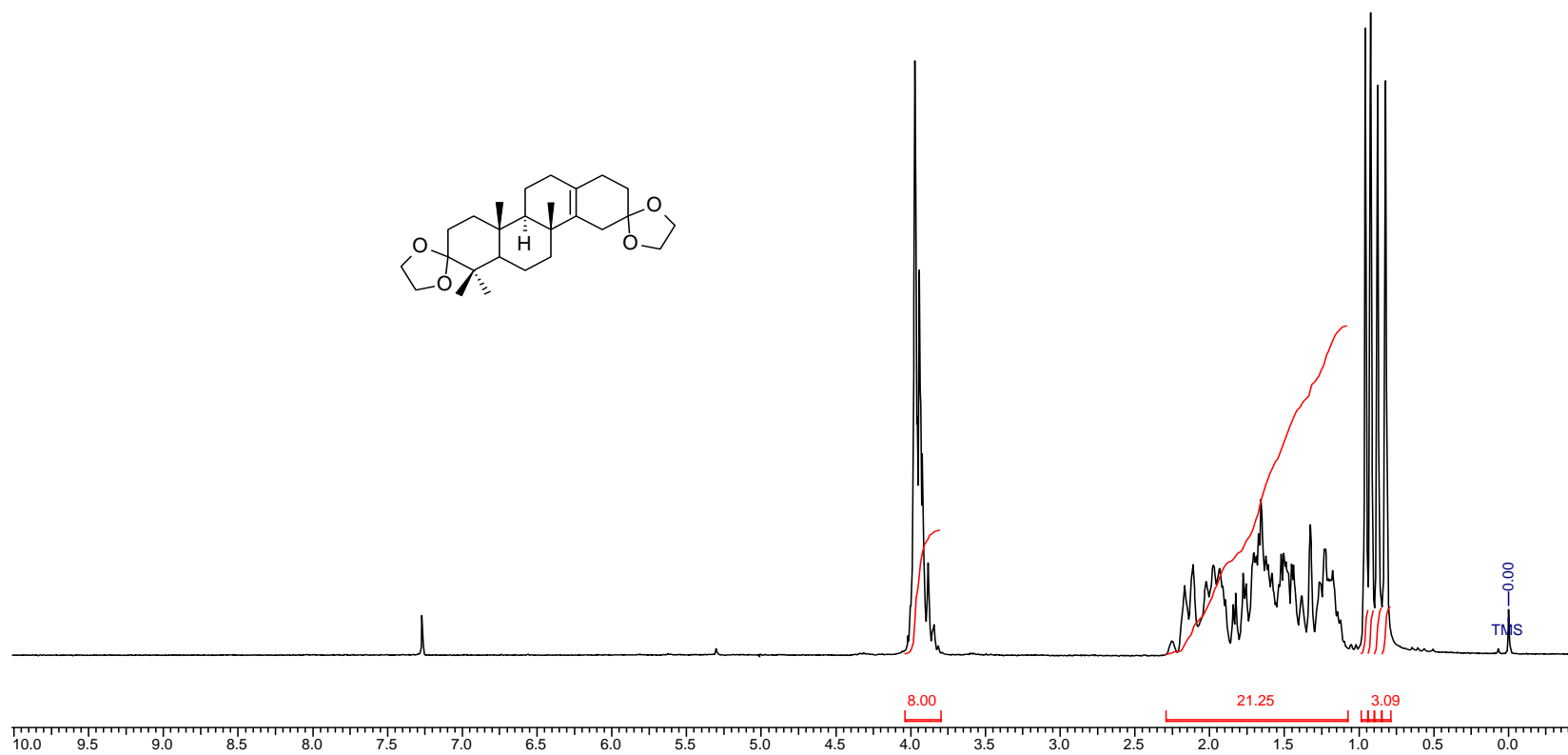


# Appendix C - Chapter 4 $^1\text{H}$ and $^{13}\text{C}$ NMR spectras

## $^1\text{H}$ NMR of Compound 4.6

AA-8-74-fr1-3

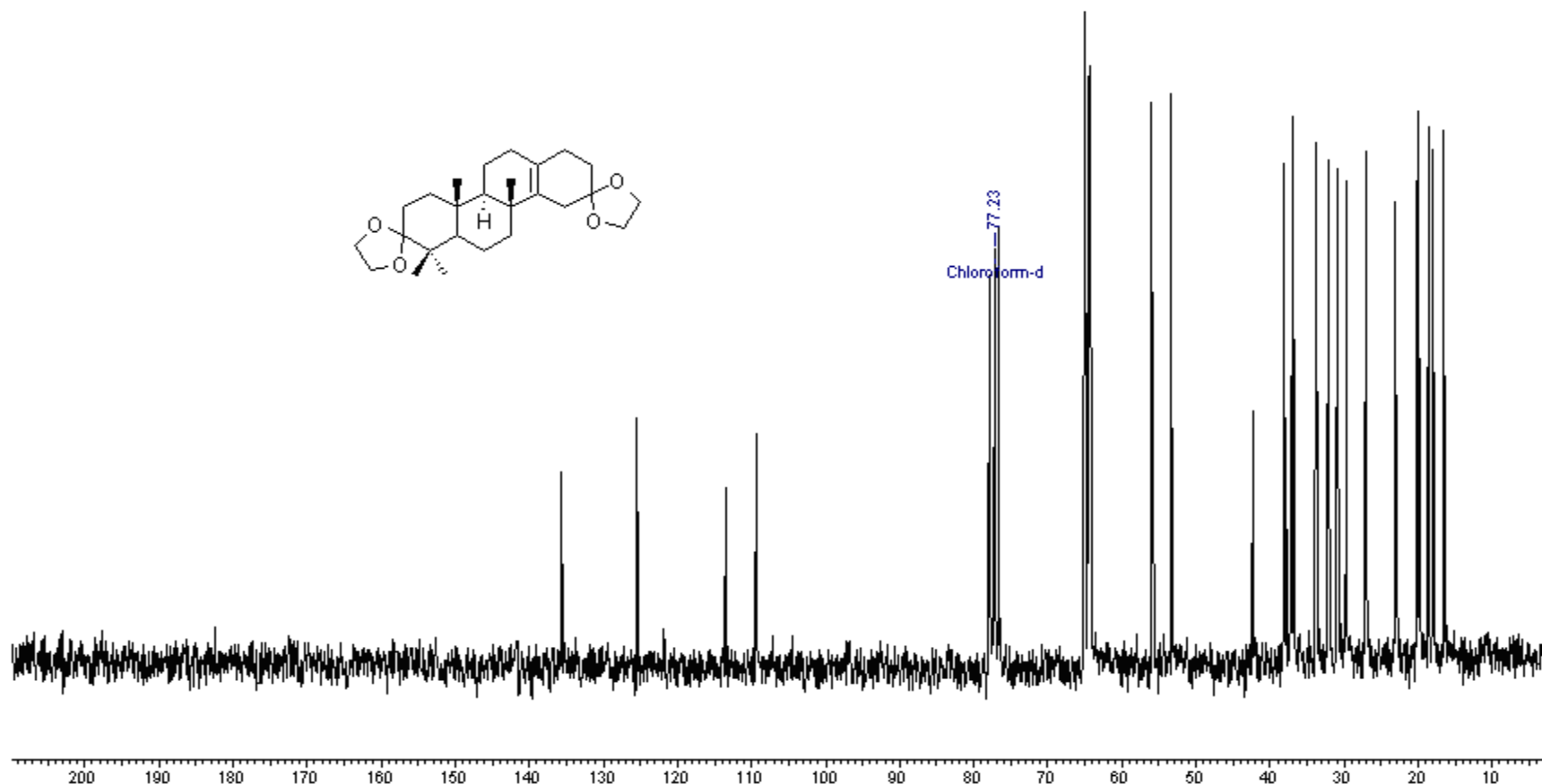
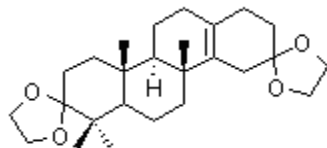
<b>Acquisition Time (sec)</b> 2.7307	<b>Comment</b> STANDARD 1H OBSERVE	<b>Date</b> Aug 3 2007
<b>Frequency (MHz)</b> 199.98	<b>Nucleus</b> 1H	<b>Number of Transients</b> 32
<b>Solvent</b> CDCl3	<b>Sweep Width (Hz)</b> 3000.00	<b>Temperature (grad C)</b> 29.000
		<b>Original Points Count</b> 5984
		<b>Points Count</b> 8192



# <sup>13</sup>C NMR of Compound 4.6

AA-8-74-fr1-3-C13

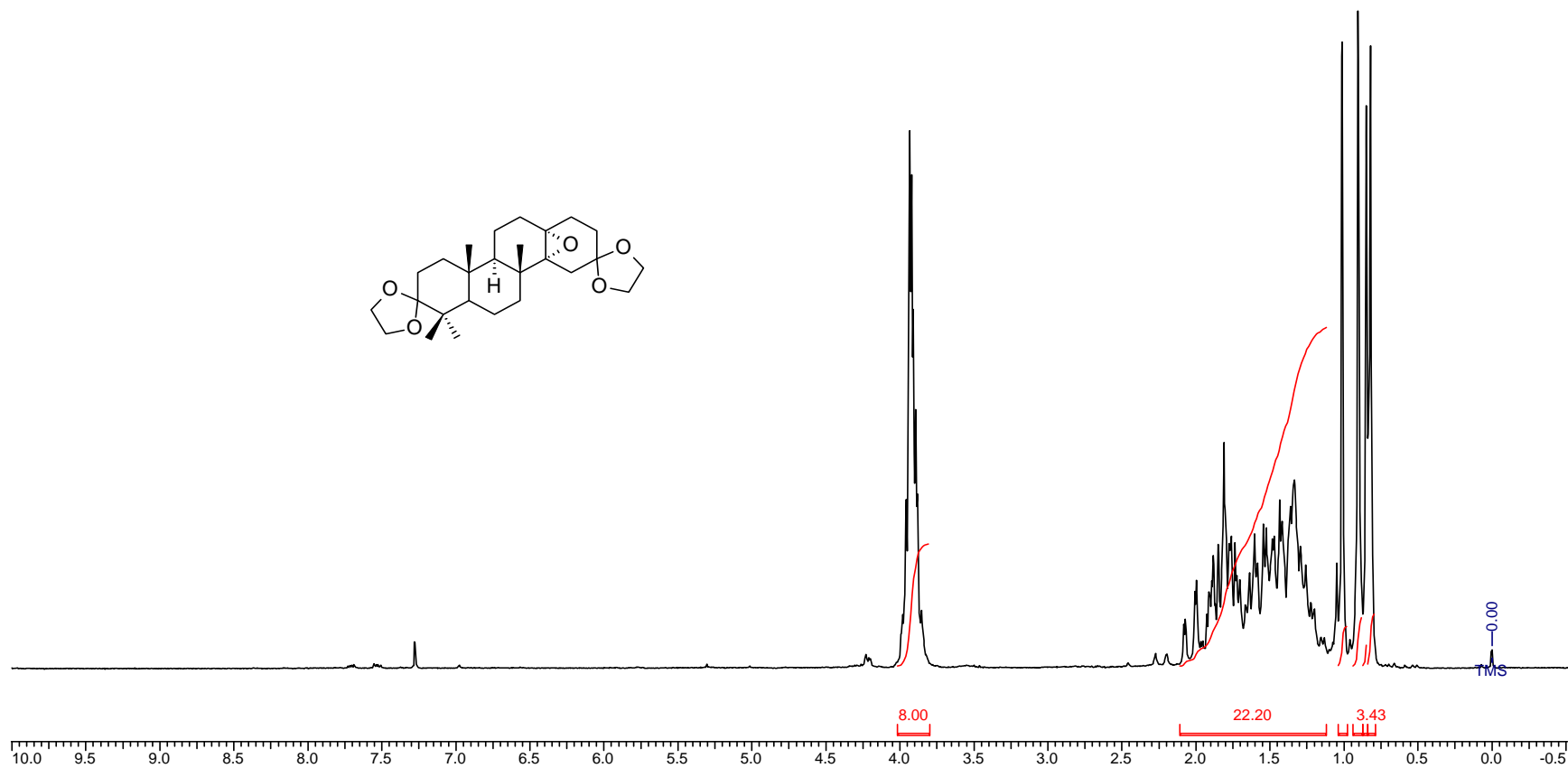
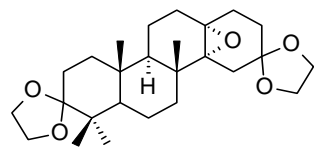
Acquisition Time (sec)	2.6214	Comment	13C OBSERVE	Date	Aug 3 2007				
Frequency (MHz)	50.29	Nucleus	13C	Number of Transients	1000	Original Points Count	18720	Points Count	32768
Solvent	CDCl3	Sweep Width (Hz)	12500.00	Temperature (grad C)	29.000				



# $^1\text{H}$ NMR of Compound 4.8

AA-8-77-fr3-8

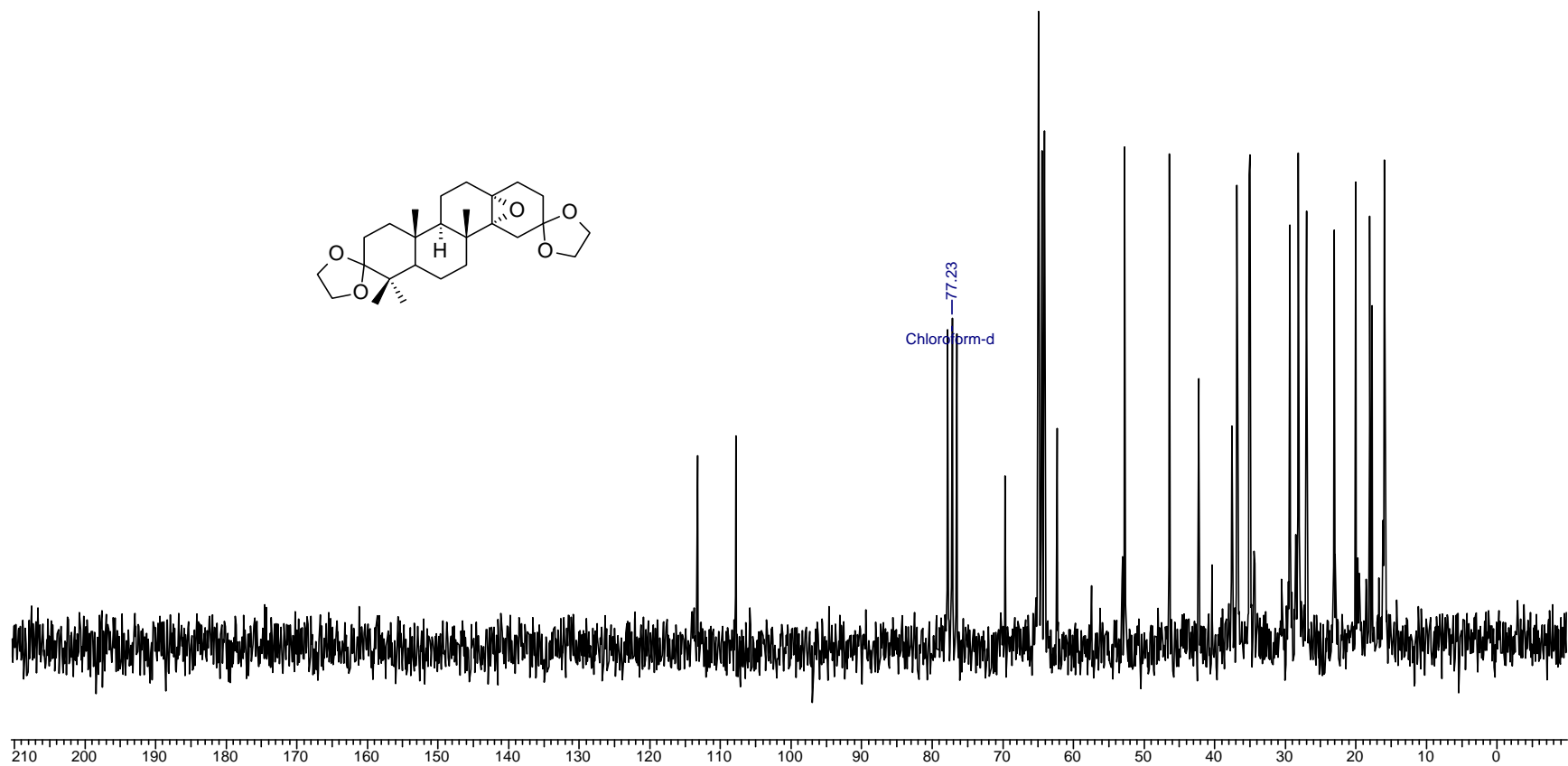
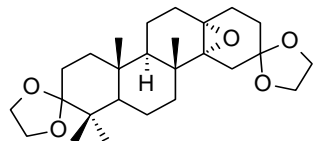
<b>Acquisition Time (sec)</b> 2.7307	<b>Comment</b> STANDARD 1H OBSERVE	<b>Date</b> Aug 7 2007		
<b>Frequency (MHz)</b> 199.98	<b>Nucleus</b> $^1\text{H}$	<b>Number of Transients</b> 32	<b>Original Points Count</b> 5984	<b>Points Count</b> 8192
<b>Solvent</b> $\text{CDCl}_3$	<b>Sweep Width (Hz)</b> 3000.00	<b>Temperature (grad C)</b> 29.000		



# <sup>13</sup>C NMR of Compound 4.8

## AA-8-77-fr3-8-C13

<b>Acquisition Time (sec)</b> 2.6214	<b>Comment</b> 13C OBSERVE	<b>Date</b> Aug 7 2007		
<b>Frequency (MHz)</b> 50.29	<b>Nucleus</b> 13C	<b>Number of Transients</b> 1000	<b>Original Points Count</b> 18720	<b>Points Count</b> 32768
<b>Solvent</b> CDCl3	<b>Sweep Width (Hz)</b> 12500.00	<b>Temperature (grad C)</b> 29.000		

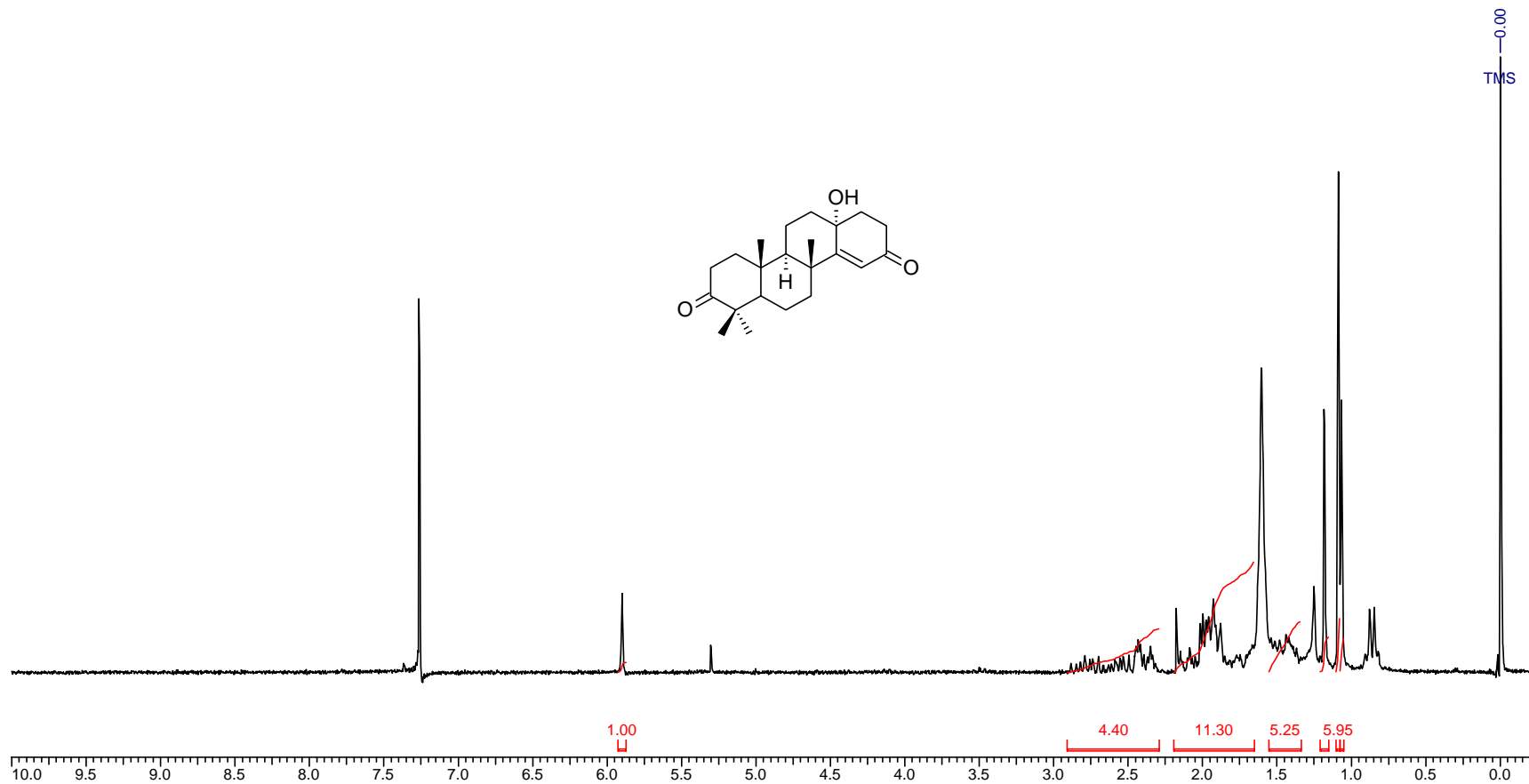




# <sup>1</sup>H NMR of Compound 4.9

AA-11-12-fr19-24

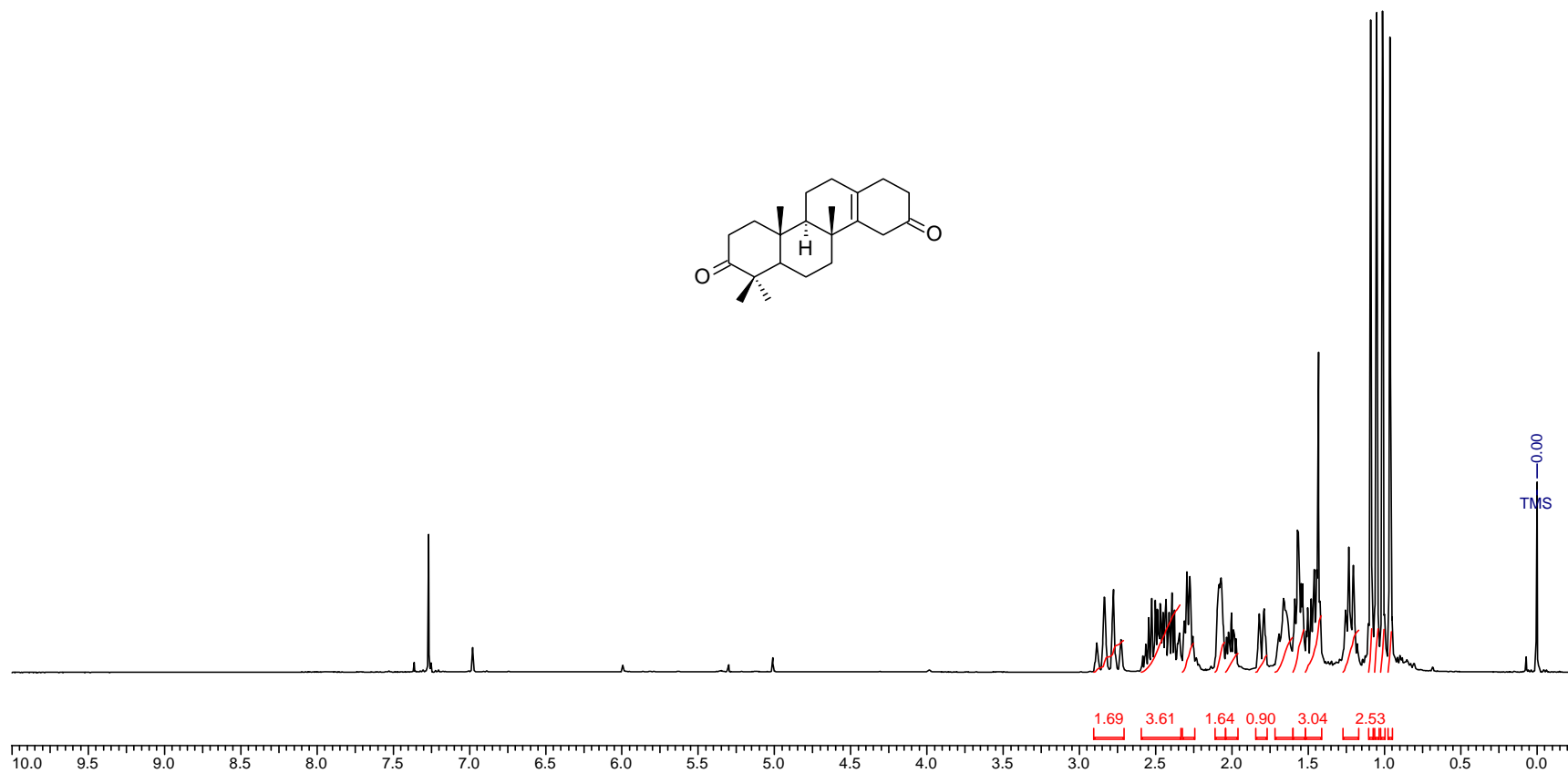
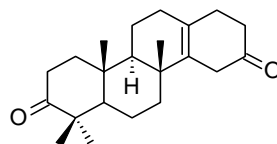
Acquisition Time (sec)	2.7307	Comment	STANDARD 1H OBSERVE		Date	Jun 18 2008			
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	300	Original Points Count	5984	Points Count	8192
Solvent	CDCl3	Sweep Width (Hz)	3000.00	Temperature (grad C)	29.000				



# <sup>1</sup>H NMR of Compound 4.10

AA-8-102-fr18-33

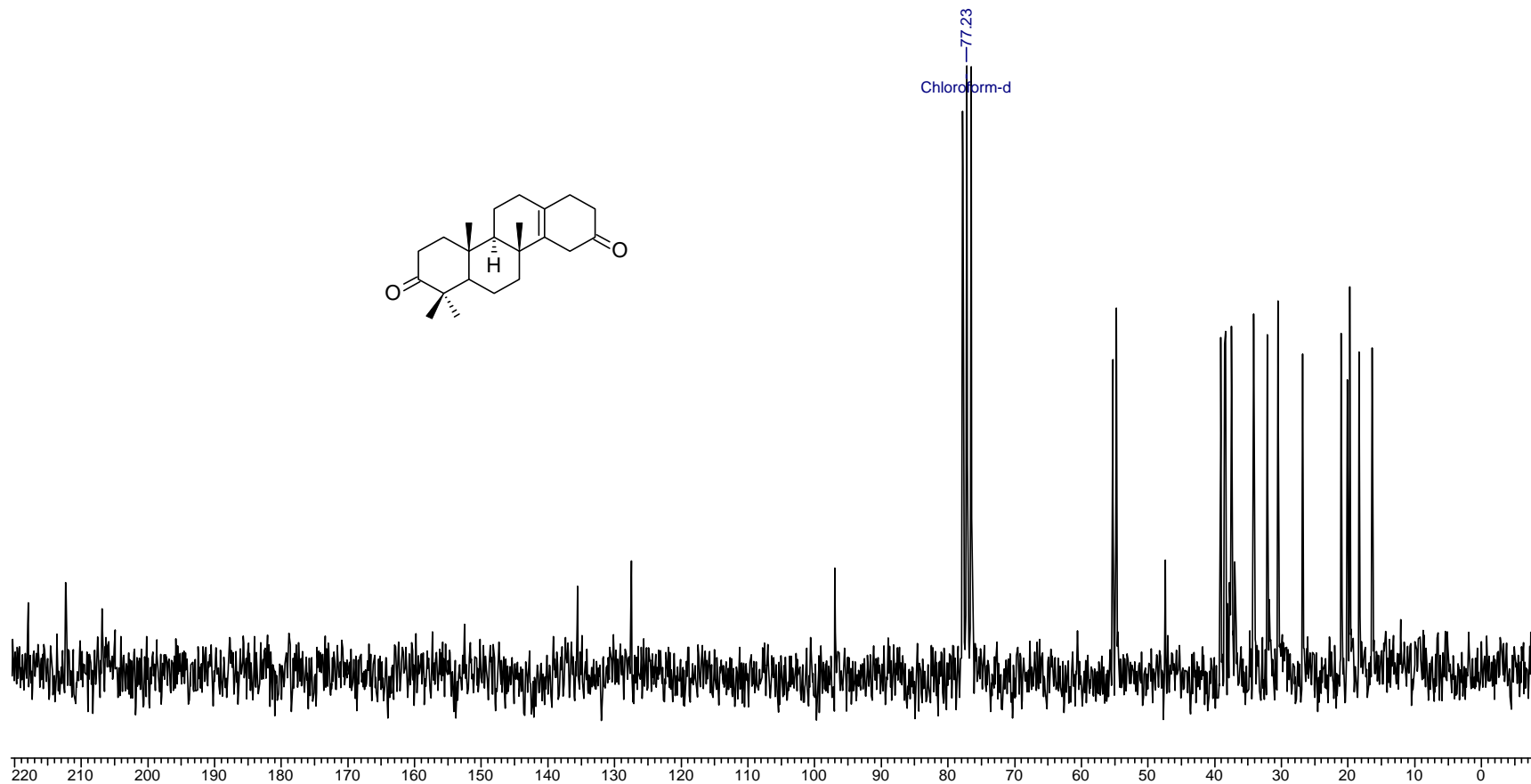
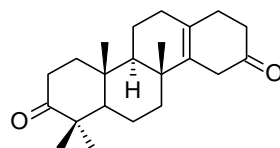
Acquisition Time (sec)	3.4152	Comment	Std proton	Date	Sep 10 2007				
Frequency (MHz)	399.77	Nucleus	1H	Number of Transients	300	Original Points Count	9828	Points Count	16384
Solvent	CDCl3	Sweep Width (Hz)	4797.31	Temperature (grad C)	25.000				



# <sup>13</sup>C NMR of Compound 4.10

## AA-8-102-fr18-33-C13

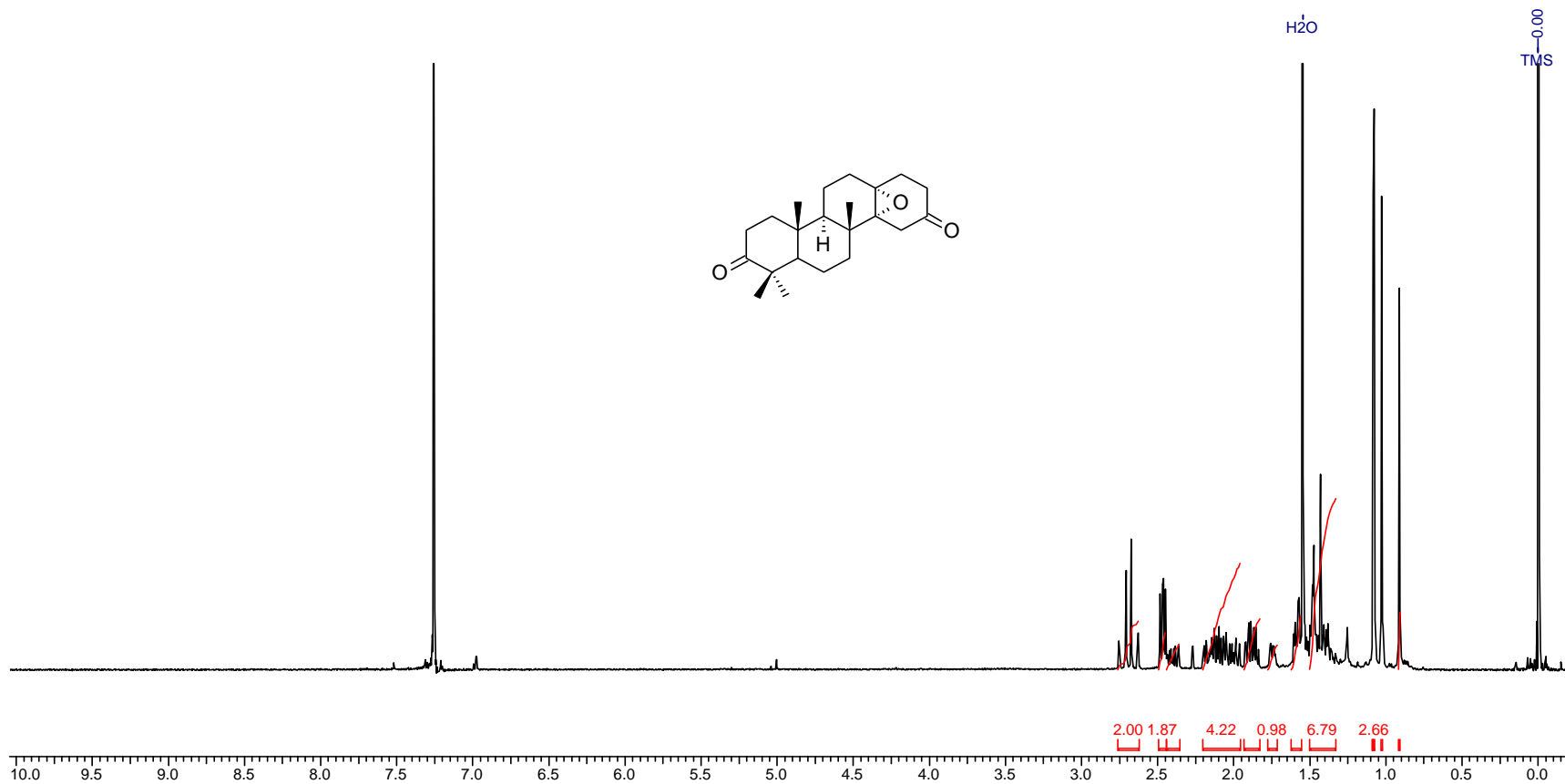
<b>Acquisition Time (sec)</b> 2.6214	<b>Comment</b> 13C OBSERVE	<b>Date</b> Sep 10 2007		
<b>Frequency (MHz)</b> 50.29	<b>Nucleus</b> 13C	<b>Number of Transients</b> 1000	<b>Original Points Count</b> 18720	<b>Points Count</b> 32768
<b>Solvent</b> CDCl3	<b>Sweep Width (Hz)</b> 12500.00	<b>Temperature (grad C)</b> 29.000		



# <sup>1</sup>H NMR of Compound 4.11

AA-10-115-fr20-28

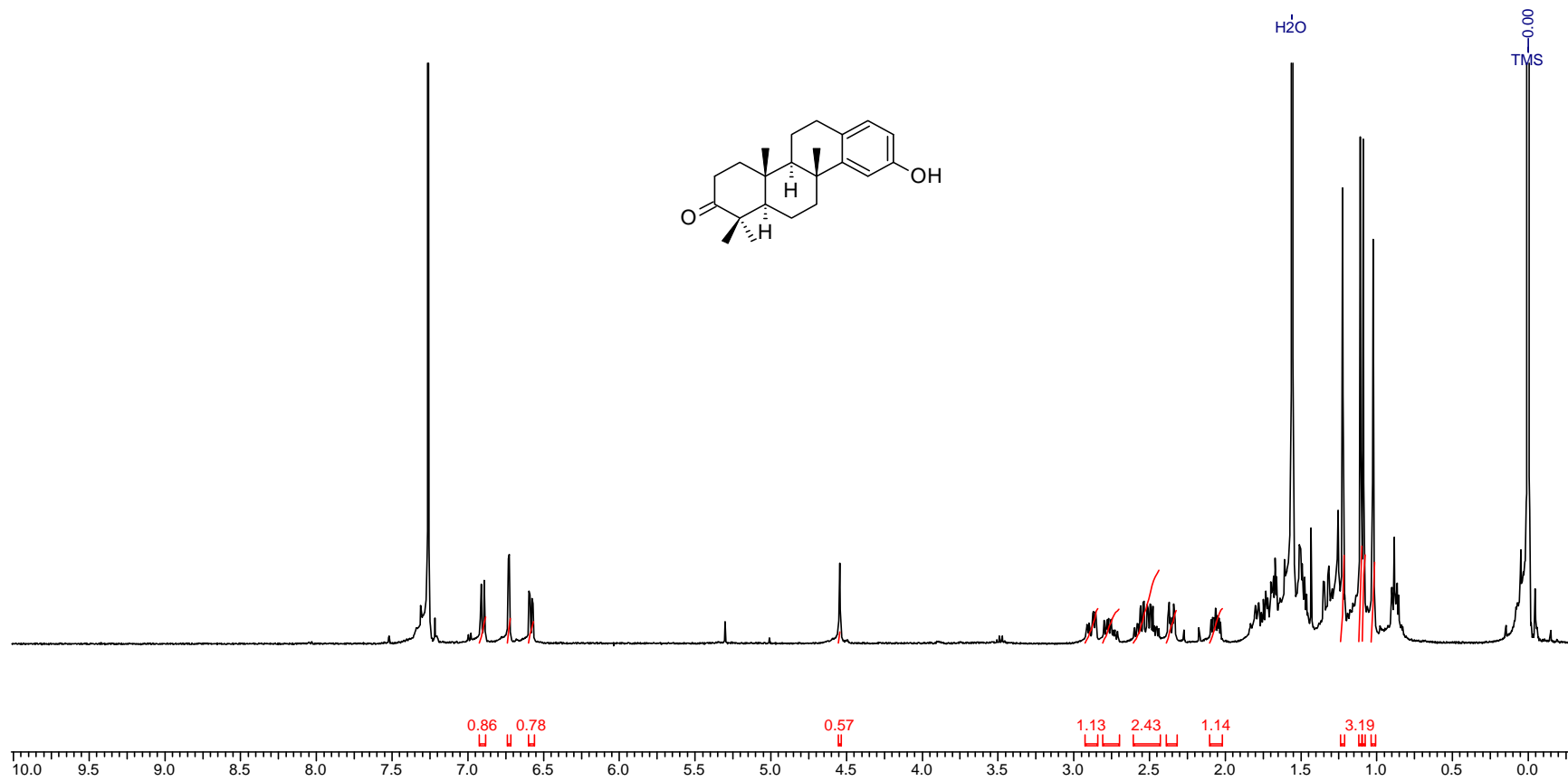
Acquisition Time (sec)	3.4154	Comment	Std proton		Date	Jun 3 2008			
Frequency (MHz)	399.76	Nucleus	1H	Number of Transients	300	Original Points Count	9827	Points Count	16384
Solvent	cdcl3	Sweep Width (Hz)	4797.03		Temperature (grad C)	25.000			



# <sup>1</sup>H NMR of Compound 4.15

## AA-11-phenol

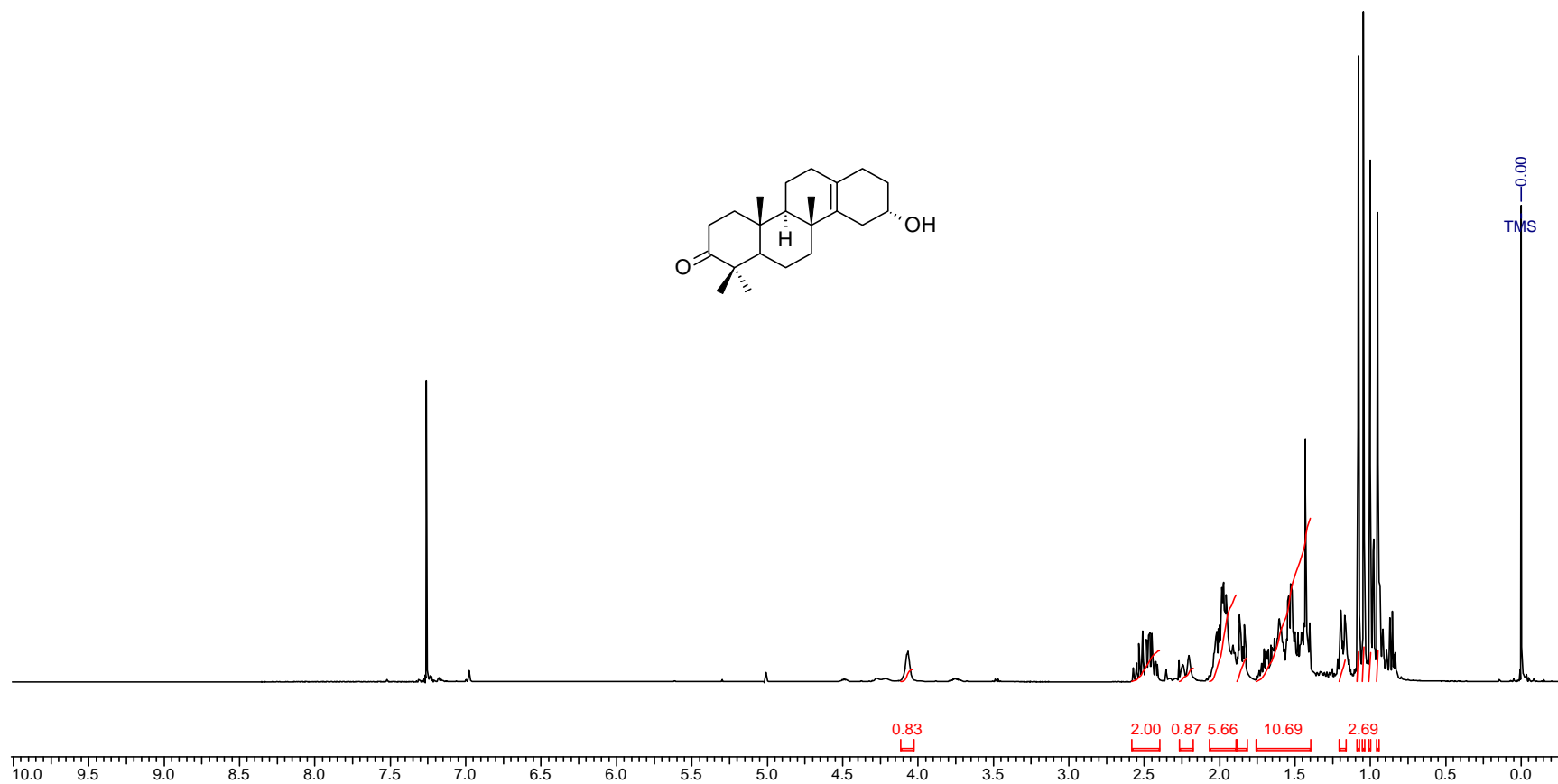
Acquisition Time (sec)	2.5616	Comment	Std proton	Date	Jul 28 2008	Frequency (MHz)	399.76				
Nucleus	1H	Number of Transients	100	Original Points Count	13103	Points Count	16384	Solvent	cdcl3	Sweep Width (Hz)	6395.91
Temperature (grad C)	25.000										



# <sup>1</sup>H NMR of Compound 4.16

## AA-8-103-fr8-14

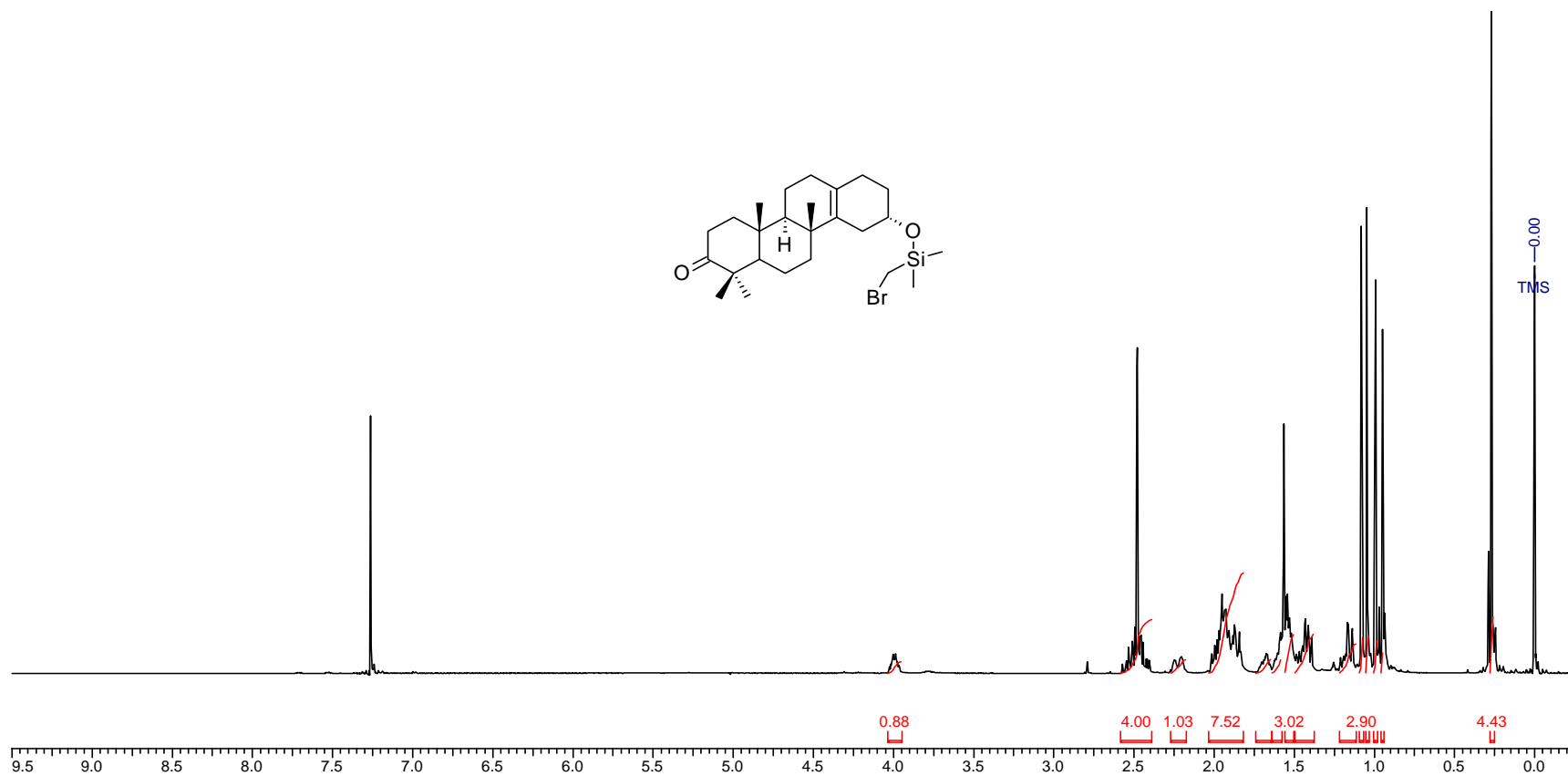
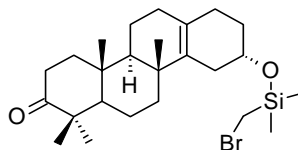
Acquisition Time (sec)	3.4152	Comment	Std proton	Date	Sep 11 2007				
Frequency (MHz)	399.77	Nucleus	1H	Number of Transients	200	Original Points Count	9828	Points Count	16384
Solvent	CDCl3	Sweep Width (Hz)	4797.31	Temperature (grad C)	25.000				



# <sup>1</sup>H NMR of Compound 4.17

AA-8-104-fr2-5

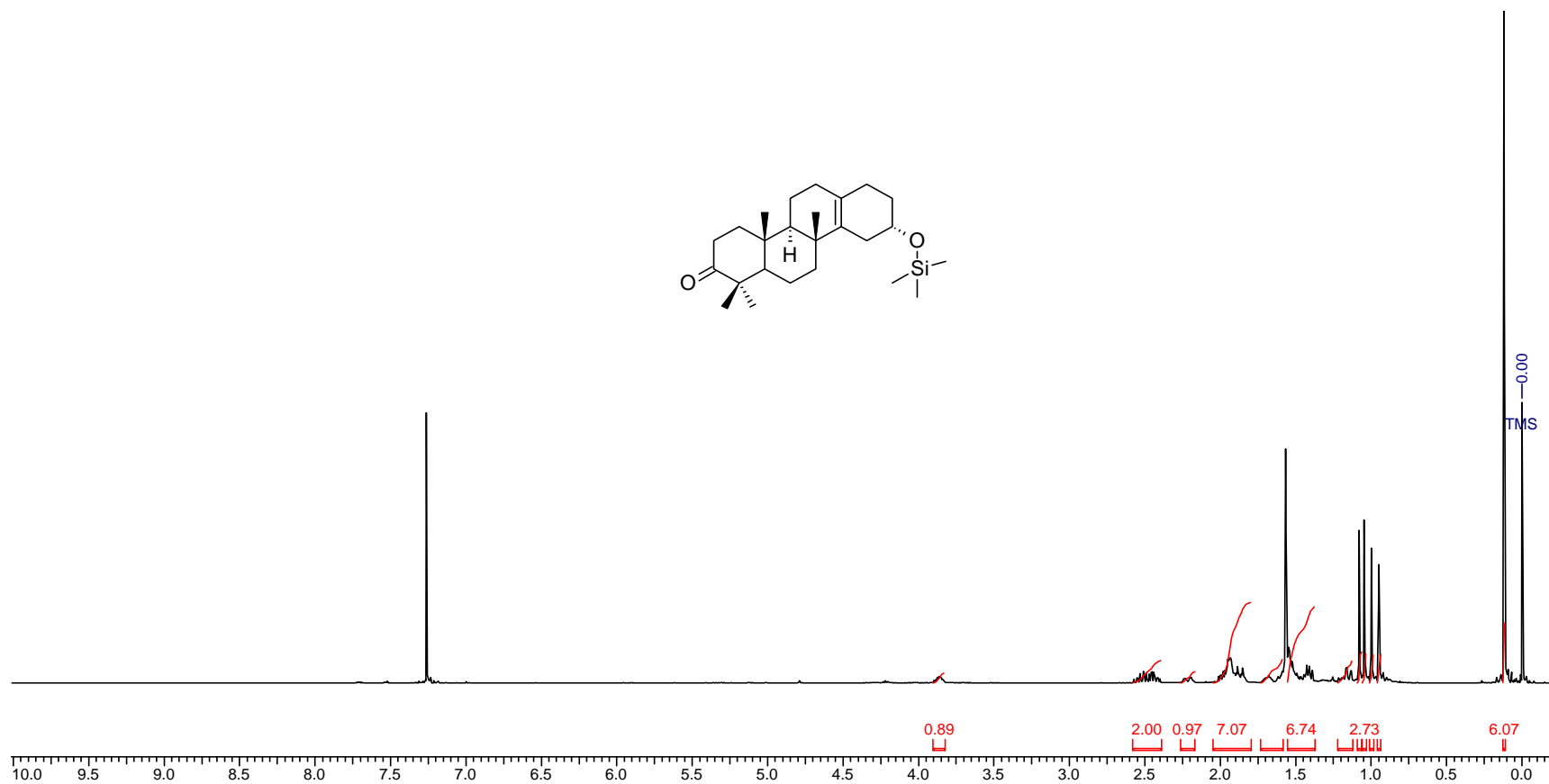
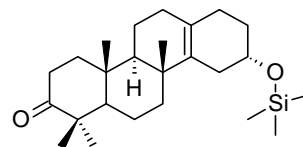
Acquisition Time (sec)	3.4152	Comment	Std proton	Date	Sep 12 2007				
Frequency (MHz)	399.77	Nucleus	1H	Number of Transients	200	Original Points Count	9828	Points Count	16384
Solvent	CDCl3	Sweep Width (Hz)	4797.31	Temperature (grad C)	25.000				



# <sup>1</sup>H NMR of Compound 4.18

AA-8-105-fr3-5

Acquisition Time (sec)	3.4152	Comment	Std proton	Date	Sep 13 2007				
Frequency (MHz)	399.77	Nucleus	1H	Number of Transients	200	Original Points Count	9828	Points Count	16384
Solvent	CDCl3	Sweep Width (Hz)	4797.31	Temperature (grad C)	25.000				

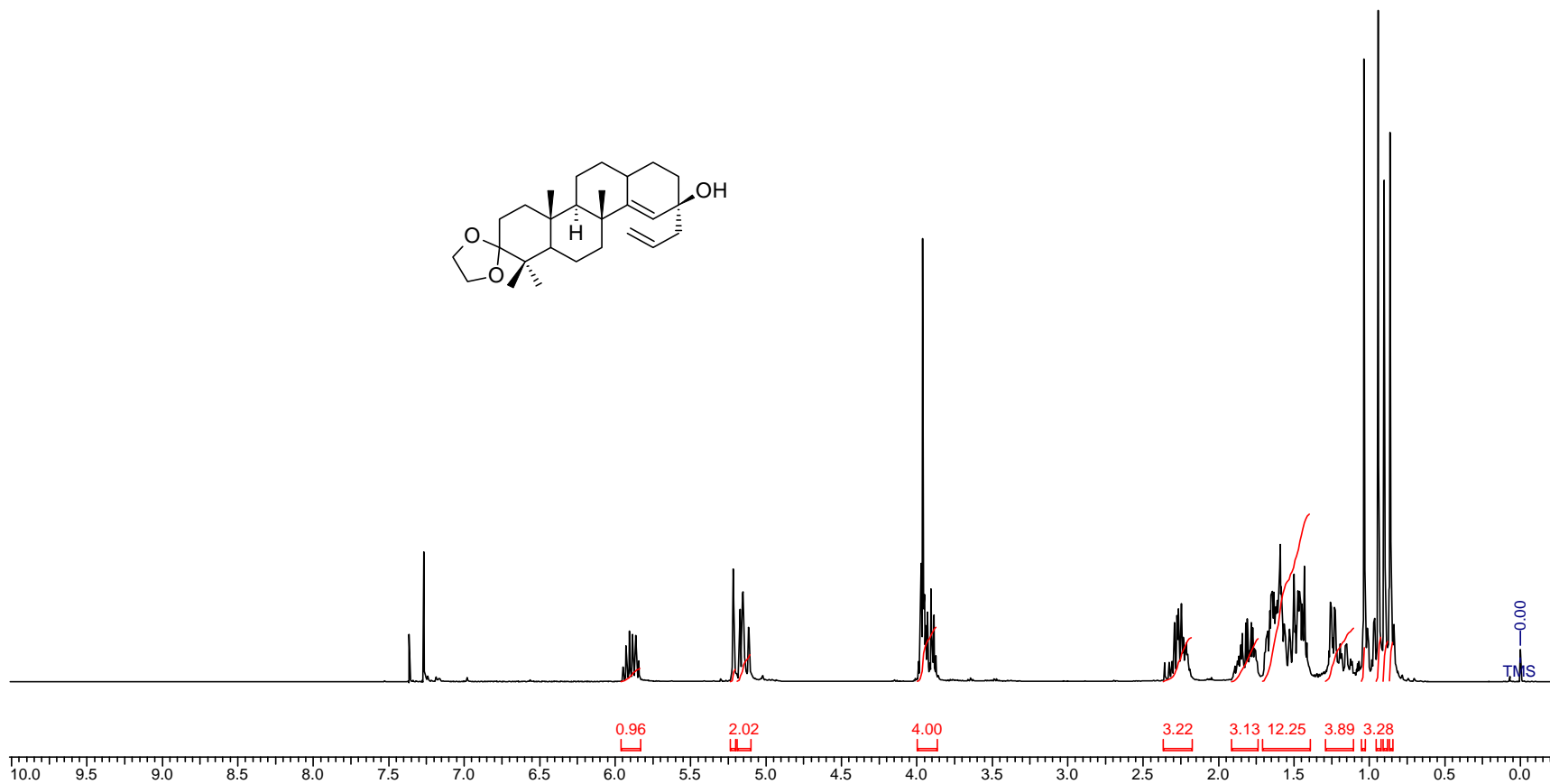
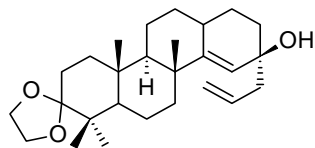




# <sup>1</sup>H NMR of Compound 4.24

## AA-10-55-fr1-12

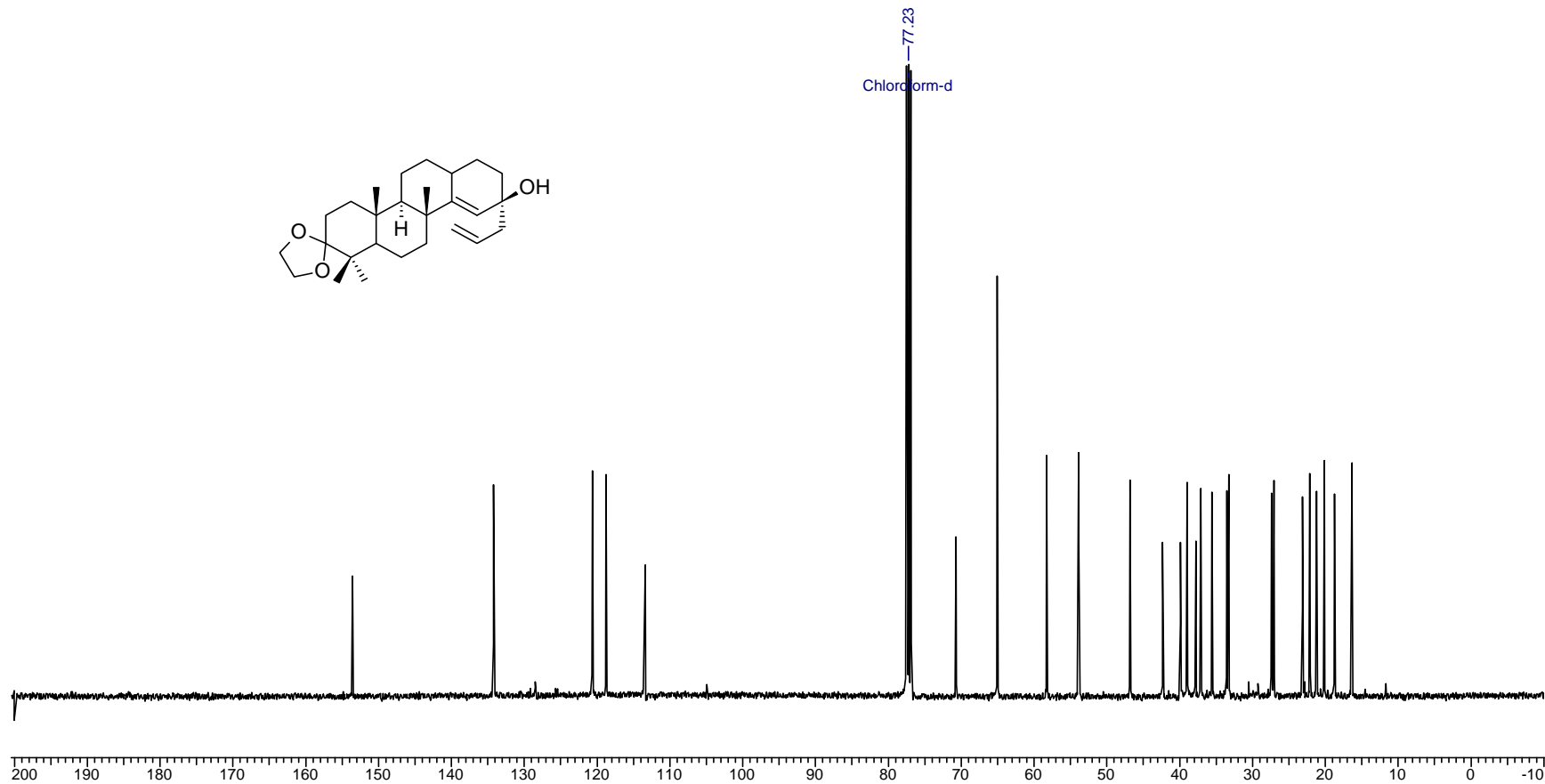
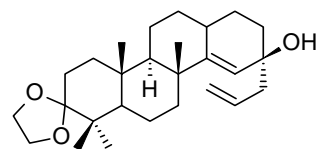
<b>Acquisition Time (sec)</b> 3.4152	<b>Comment</b> Std proton	<b>Date</b> Jan 9 2008		
<b>Frequency (MHz)</b> 399.77	<b>Nucleus</b> 1H	<b>Number of Transients</b> 32	<b>Original Points Count</b> 9828	<b>Points Count</b> 16384
<b>Solvent</b> cdcl3	<b>Sweep Width (Hz)</b> 4797.31	<b>Temperature (grad C)</b> 25.000		



# <sup>13</sup>C NMR of Compound 4.24

## AA-10-55-fr1-12-C13

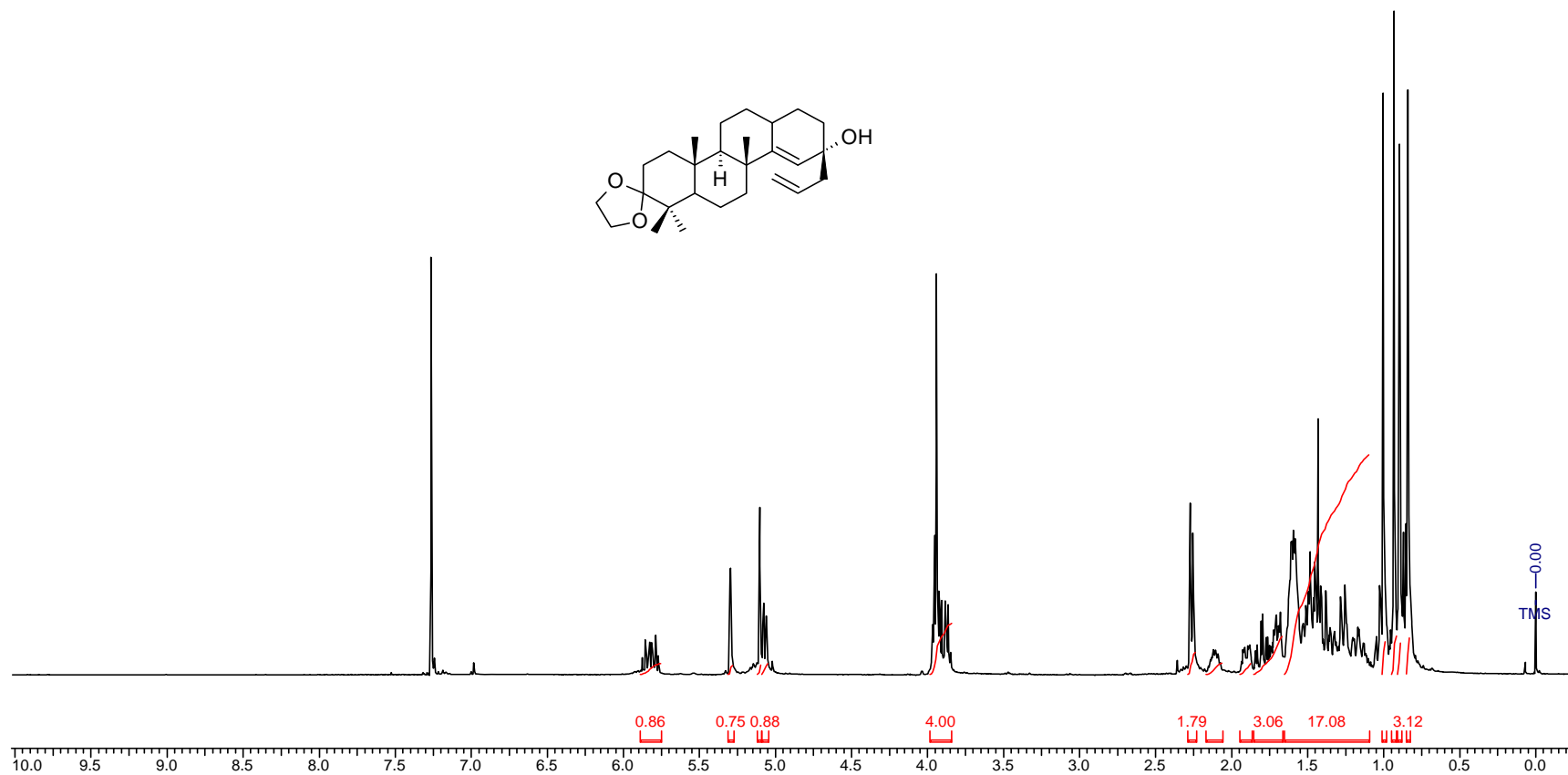
<b>Acquisition Time (sec)</b> 1.3582	<b>Comment</b> Std proton	<b>Date</b> Jan 9 2008		
<b>Frequency (MHz)</b> 100.53	<b>Nucleus</b> 13C	<b>Number of Transients</b> 20000	<b>Original Points Count</b> 31375	<b>Points Count</b> 32768
<b>Solvent</b> cdcl3	<b>Sweep Width (Hz)</b> 24125.45	<b>Temperature (grad C)</b> 25.000		



# <sup>1</sup>H NMR of Compound 4.28

AA-10-55-fr19-28

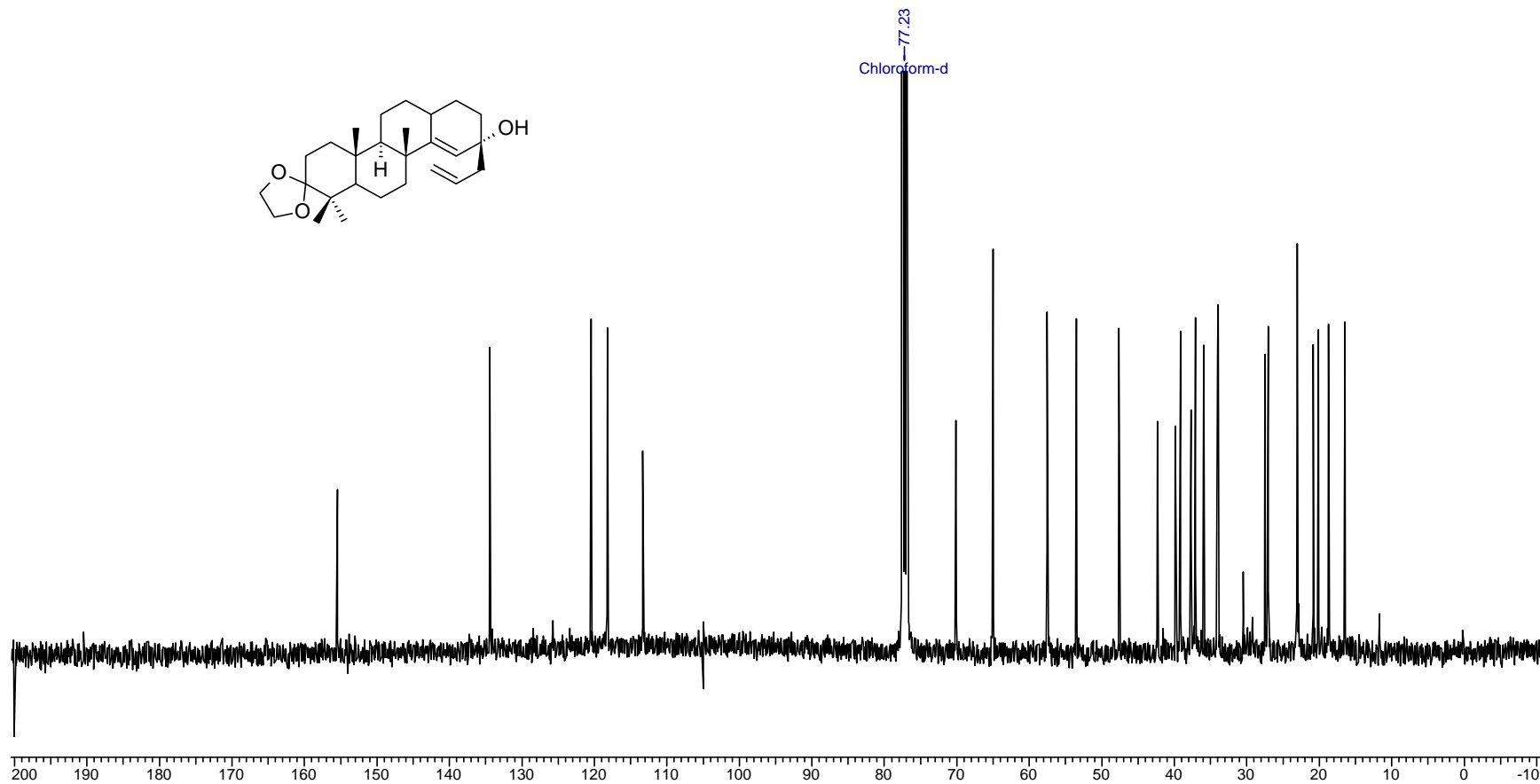
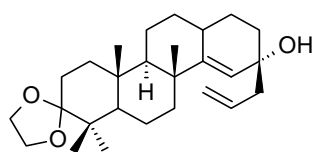
Acquisition Time (sec)	3.4152	Comment	Std proton	Date	Jan 10 2008	Frequency (MHz)	399.77				
Nucleus	1H	Number of Transients	100	Original Points Count	9828	Points Count	16384	Solvent	cdcl3	Sweep Width (Hz)	4797.31
Temperature (grad C)	25.000										



# <sup>13</sup>C NMR of Compound 4.28

AA-10-55-fr19-28-C13

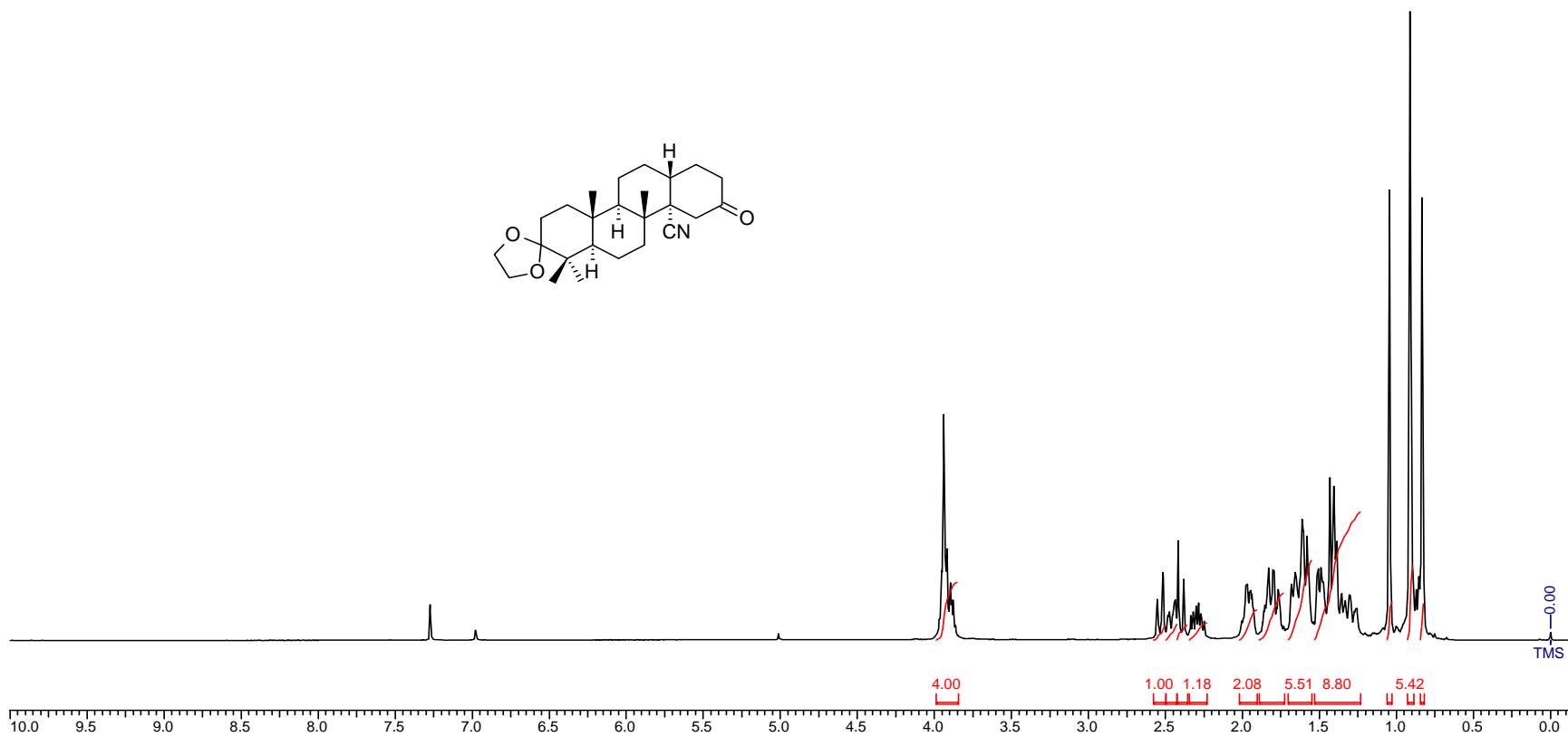
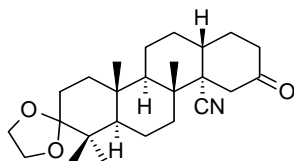
<b>Acquisition Time (sec)</b> 1.3582	<b>Comment</b> Std proton	<b>Date</b> Jan 10 2008		
<b>Frequency (MHz)</b> 100.53	<b>Nucleus</b> <sup>13</sup> C	<b>Number of Transients</b> 20000	<b>Original Points Count</b> 31375	<b>Points Count</b> 32768
<b>Solvent</b> cdcl3	<b>Sweep Width (Hz)</b> 24125.45	<b>Temperature (grad C)</b> 25.000		



# <sup>1</sup>H NMR of Compound 4.34

AA-10-83-fr7-28

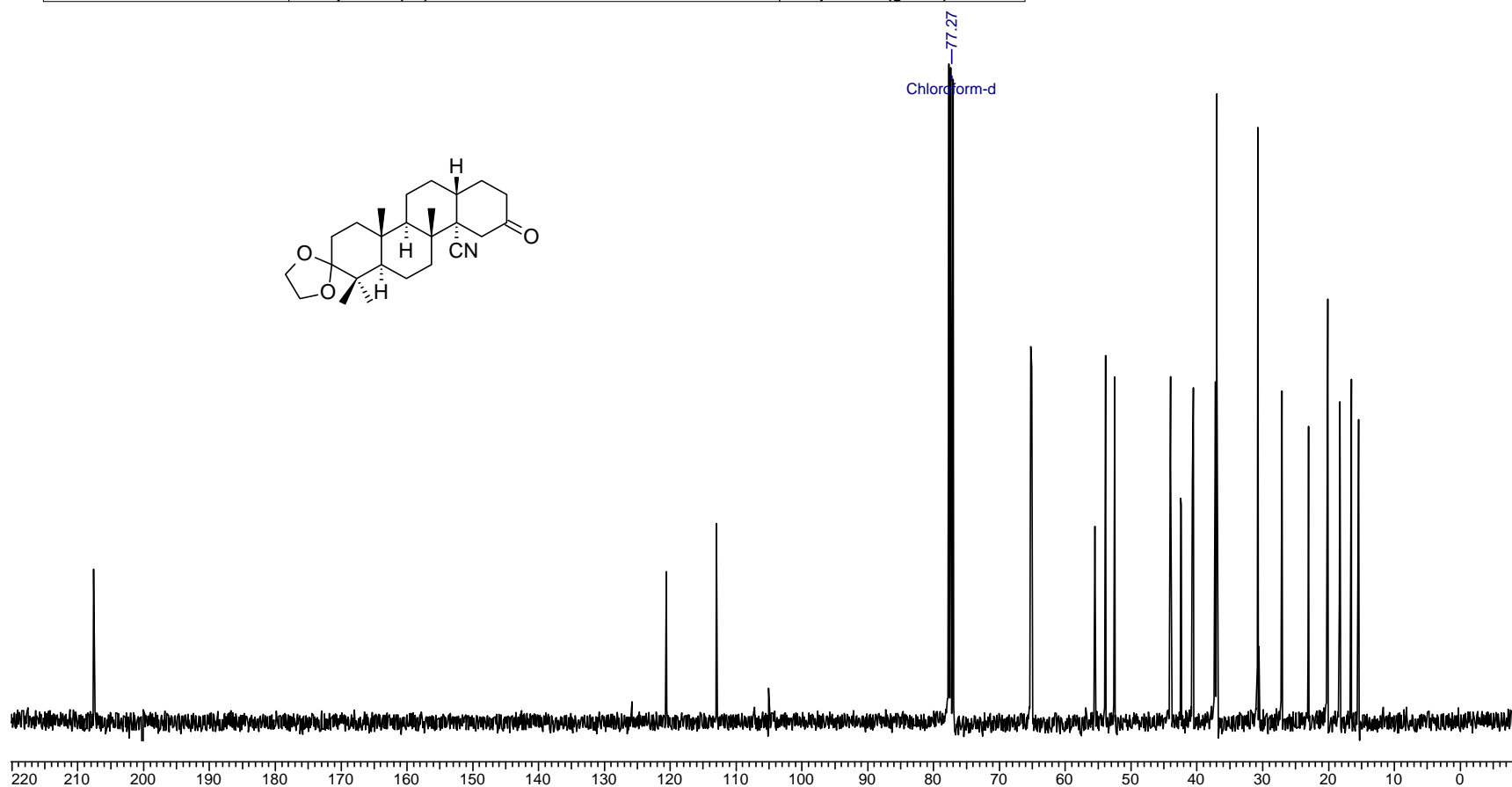
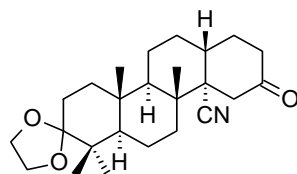
Acquisition Time (sec)	3.4152	Comment	Std proton	Date	Feb 26 2008	Frequency (MHz)	399.77				
Nucleus	1H	Number of Transients	32	Original Points Count	9828	Points Count	16384	Solvent	CDCl3	Sweep Width (Hz)	4797.31
Temperature (grad C)	25.000										



# $^{13}\text{C}$ NMR of Compound 4.34

## AA-10-83-fr7-28-C13

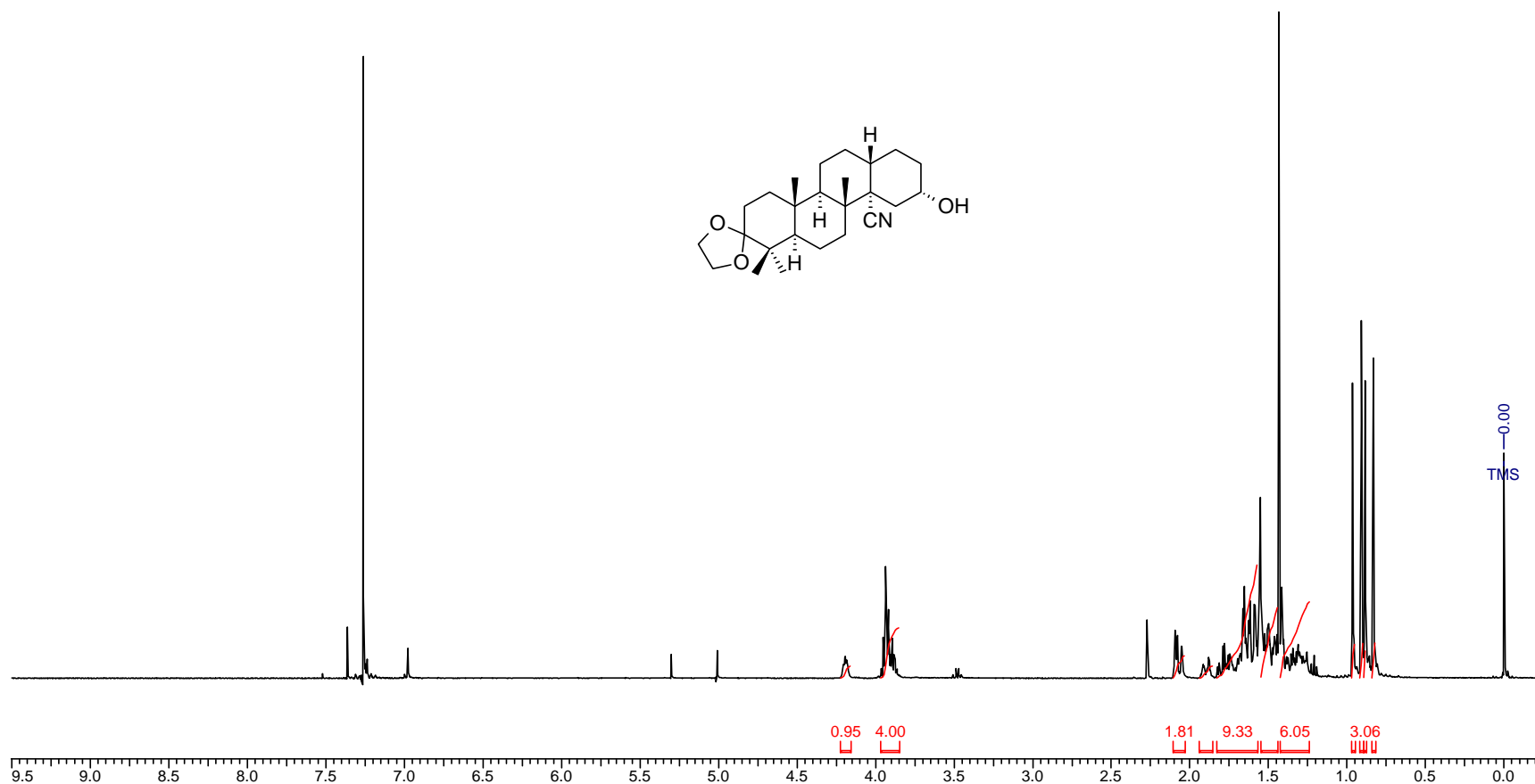
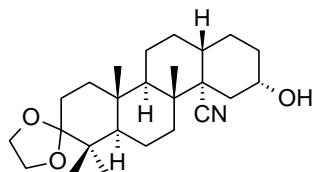
Acquisition Time (sec)	1.3582	Comment	Std proton	Date	Feb 26 2008				
Frequency (MHz)	100.53	Nucleus	$^{13}\text{C}$	Number of Transients	2000	Original Points Count	31375	Points Count	32768
Solvent	$\text{CDCl}_3$	Sweep Width (Hz)	24125.45	Temperature (grad C)	25.000				



# $^1\text{H}$ NMR of Compound 4.35

AA-10-84-fr7-14

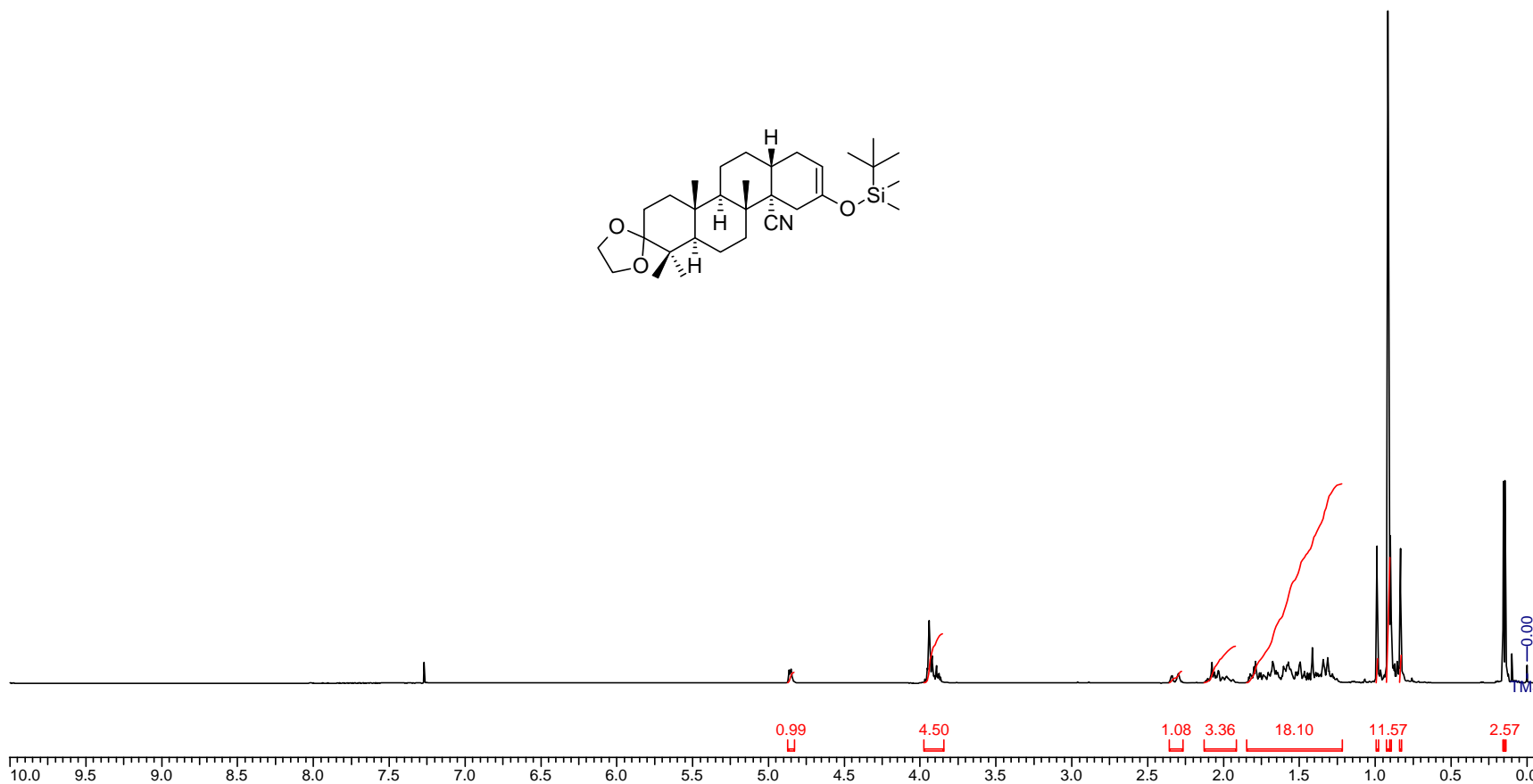
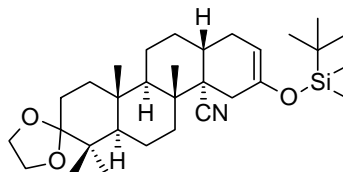
Acquisition Time (sec)	3.4152	Comment	Std proton	Date	Feb 25 2008				
Frequency (MHz)	399.77	Nucleus	$^1\text{H}$	Number of Transients	300	Original Points Count	9828	Points Count	16384
Solvent	$\text{CDCl}_3$	Sweep Width (Hz)	4797.31	Temperature (grad C)	25.000				



# <sup>1</sup>H NMR of Compound 4.37

AA-10-88-fr11-16

Acquisition Time (sec)	3.4152	Comment	Std proton	Date	Mar 5 2008				
Frequency (MHz)	399.77	Nucleus	1H	Number of Transients	300	Original Points Count	9828	Points Count	16384
Solvent	CDCl3	Sweep Width (Hz)	4797.31	Temperature (grad C)	25.000				

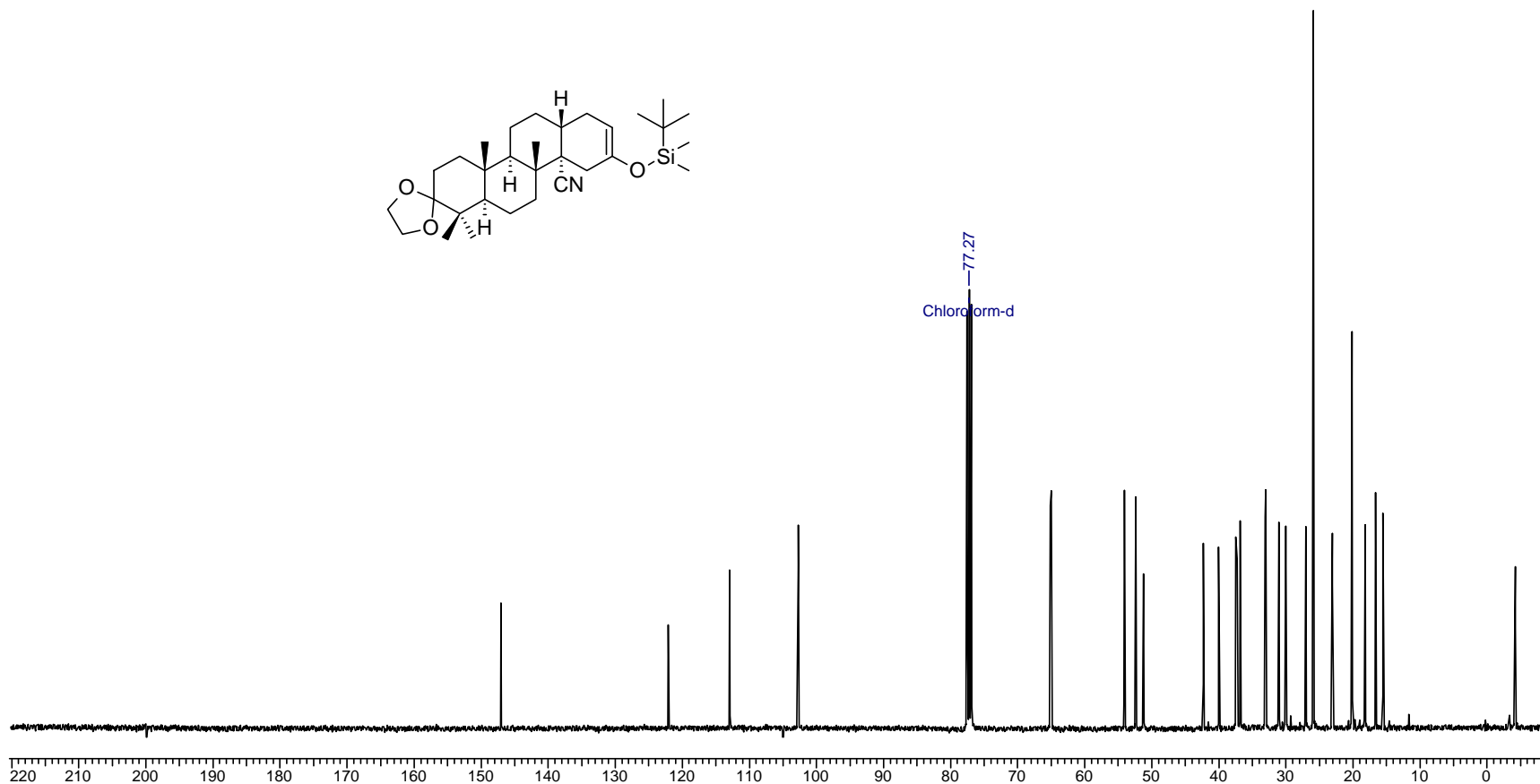
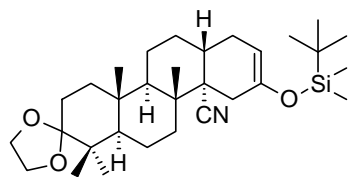




# $^{13}\text{C}$ NMR of Compound 4.37

AA-10-88-fr11-16-C13

Acquisition Time (sec)	1.3582	Comment	Std proton	Date	Mar 5 2008				
Frequency (MHz)	100.53	Nucleus	$^{13}\text{C}$	Original Points Count	31375	Points Count	32768	Solvent	$\text{CDCl}_3$
Sweep Width (Hz)	24125.45	Temperature (grad C)	25.000						



**Table 3: Crystal data and structure refinement for compound 4.37**

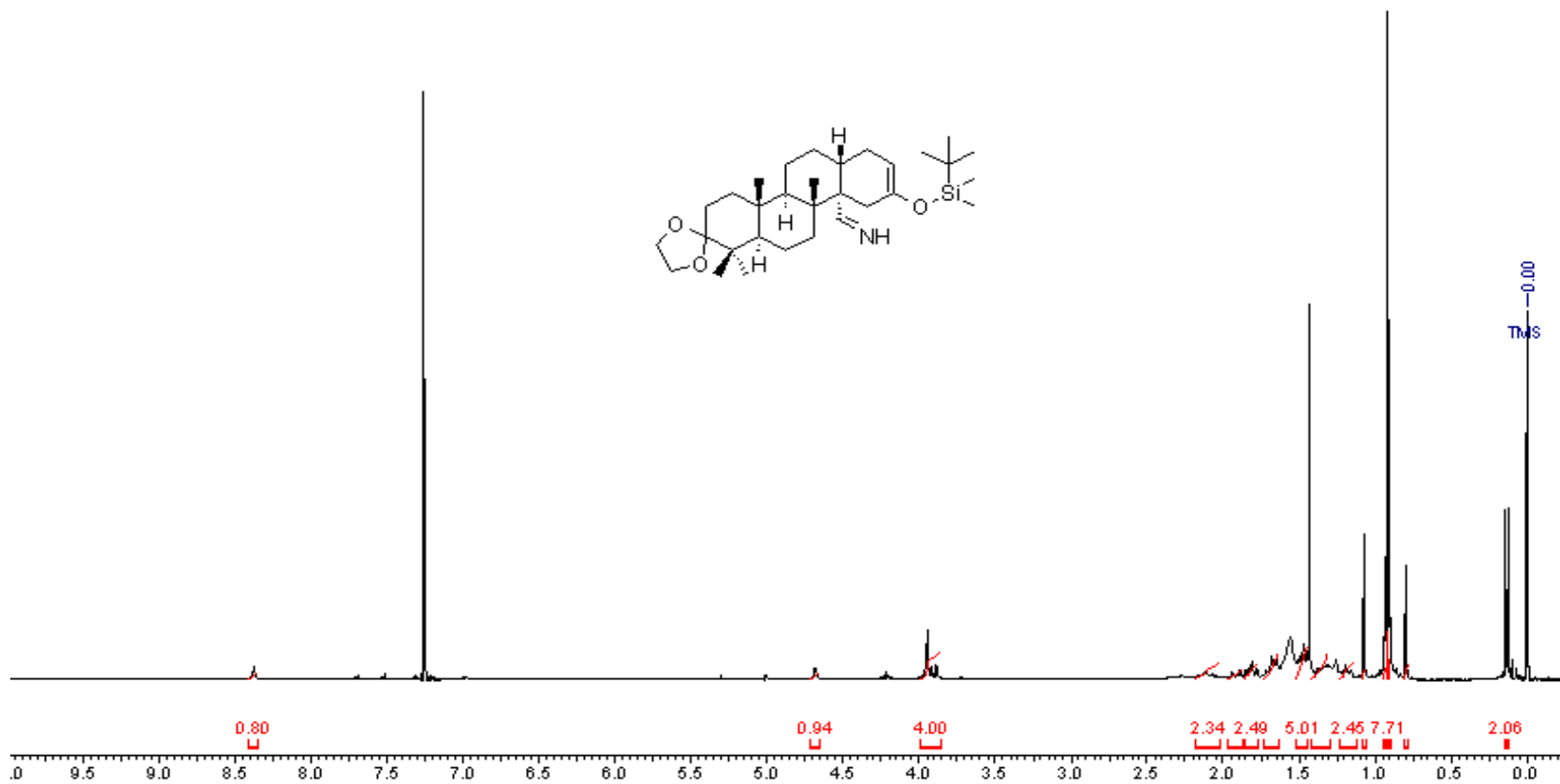
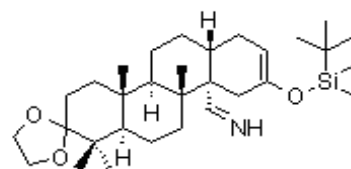
Table 1. Crystal data and structure refinement for aa0801m.

Identification code	aa0801m	
Empirical formula	C <sub>31</sub> H <sub>51</sub> N O <sub>3</sub> Si	
Formula weight	513.82	
Temperature	120(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 7.5193(3) Å	$\alpha = 90^\circ$ .
	b = 12.2929(5) Å	$\beta = 90^\circ$ .
	c = 32.1251(13) Å	$\gamma = 90^\circ$ .
Volume	2969.5(2) Å <sup>3</sup>	
Z	4	
Density (calculated)	1.149 g/cm <sup>3</sup>	
Absorption coefficient	0.110 mm <sup>-1</sup>	
F(000)	1128	
Crystal size	0.30 x 0.25 x 0.15 mm <sup>3</sup>	
Theta range for data collection	2.09 to 30.50°.	
Index ranges	-10 ≤ h ≤ 10, -16 ≤ k ≤ 17, -45 ≤ l ≤ 45	
Reflections collected	36941	
Independent reflections	9003 [R(int) = 0.0718]	
Completeness to theta = 30.50°	99.8 %	
Absorption correction	None	
Max. and min. transmission	0.9837 and 0.9678	
Refinement method	Full-matrix least-squares on F <sup>2</sup>	
Data / restraints / parameters	9003 / 0 / 334	
Goodness-of-fit on F <sup>2</sup>	1.151	
Final R indices [I > 2σ(I)]	R1 = 0.0888, wR2 = 0.2123	
R indices (all data)	R1 = 0.1140, wR2 = 0.2246	
Absolute structure parameter	0.1(2)	
Largest diff. peak and hole	1.002 and -0.572 e.Å <sup>-3</sup>	

# <sup>1</sup>H NMR of Compound 4.39

## AA-10-101-fr1-10

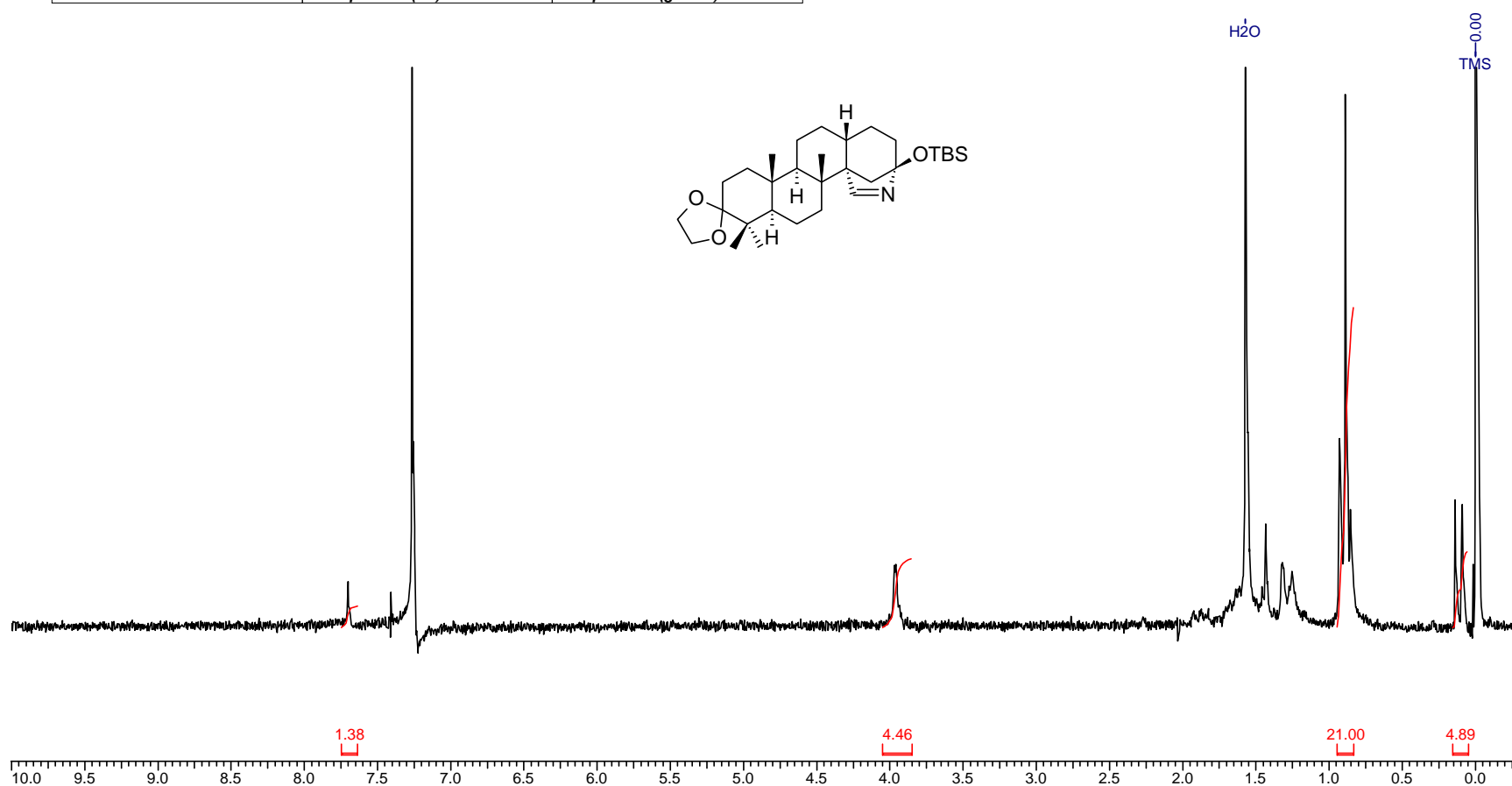
Acquisition Time (sec)	3.4152	Comment	Std proton	Date	Apr 9 2008	Frequency (MHz)	399.77				
Nucleus	1H	Number of Transients	300	Original Points Count	9828	Points Count	16384	Solvent	CDCl3	Sweep Width (Hz)	4797.31
Temperature (grad C)	25.000										



# <sup>1</sup>H NMR of Compound 4.40

AA-11-25-fr7-12

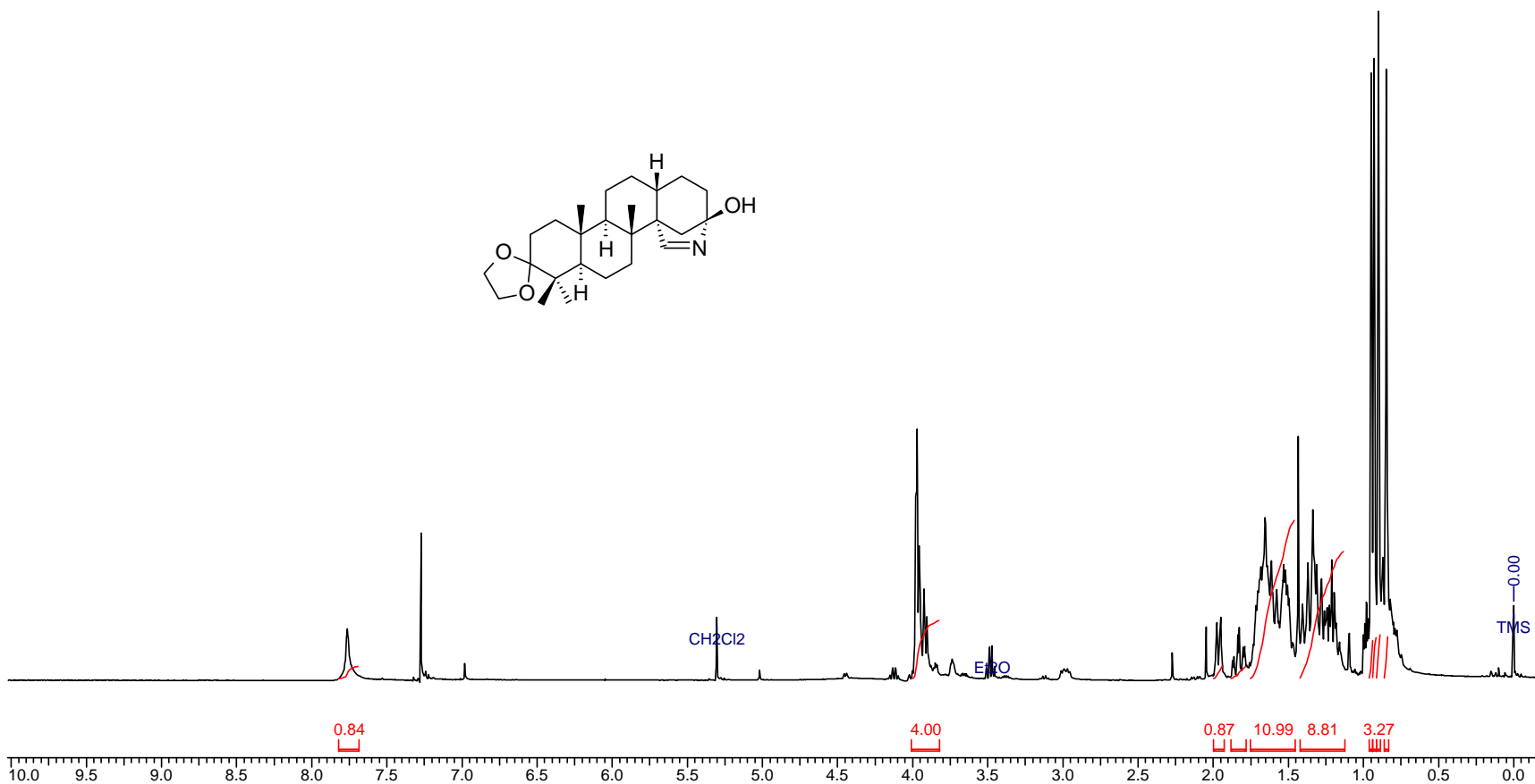
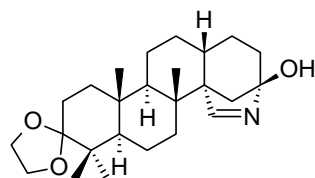
Acquisition Time (sec)	2.7307	Comment	STANDARD 1H OBSERVE		Date	Jul 21 2008			
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	300	Original Points Count	5984	Points Count	8192
Solvent	CDCl3	Sweep Width (Hz)	3000.00	Temperature (grad C)	29.000				



# <sup>1</sup>H NMR of Compound 4.41

## AS-4-62-pure

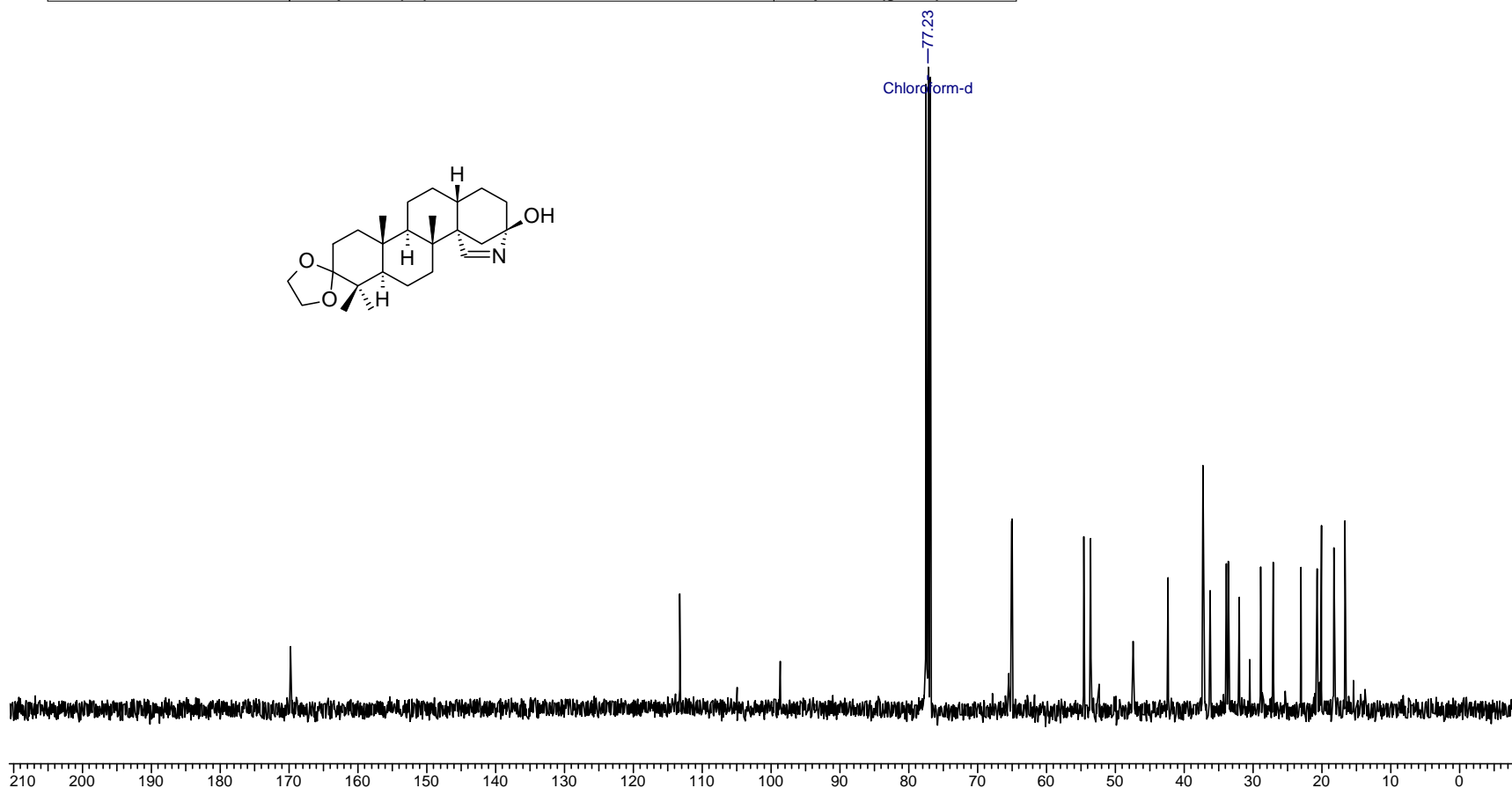
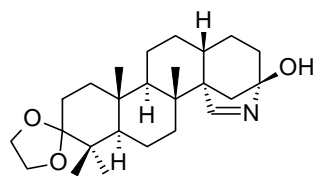
<b>Acquisition Time (sec)</b> 2.5616	<b>Comment</b> Std proton	<b>Date</b> Oct 29 2008		
<b>Frequency (MHz)</b> 399.76	<b>Nucleus</b> 1H	<b>Number of Transients</b> 300	<b>Original Points Count</b> 13103	<b>Points Count</b> 16384
<b>Solvent</b> cdcl3	<b>Sweep Width (Hz)</b> 6395.91	<b>Temperature (grad C)</b> 25.000		



# $^{13}\text{C}$ NMR of Compound 4.41

## AS-4-62-pure-C13

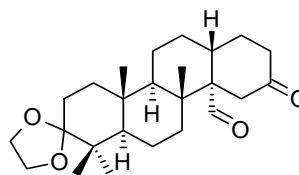
<b>Acquisition Time (sec)</b> 1.3582	<b>Comment</b> Std proton	<b>Date</b> Oct 29 2008		
<b>Frequency (MHz)</b> 100.53	<b>Nucleus</b> $^{13}\text{C}$	<b>Number of Transients</b> 3000	<b>Original Points Count</b> 31375	<b>Points Count</b> 32768
<b>Solvent</b> cdcl3	<b>Sweep Width (Hz)</b> 24125.45	<b>Temperature (grad C)</b> 25.000		



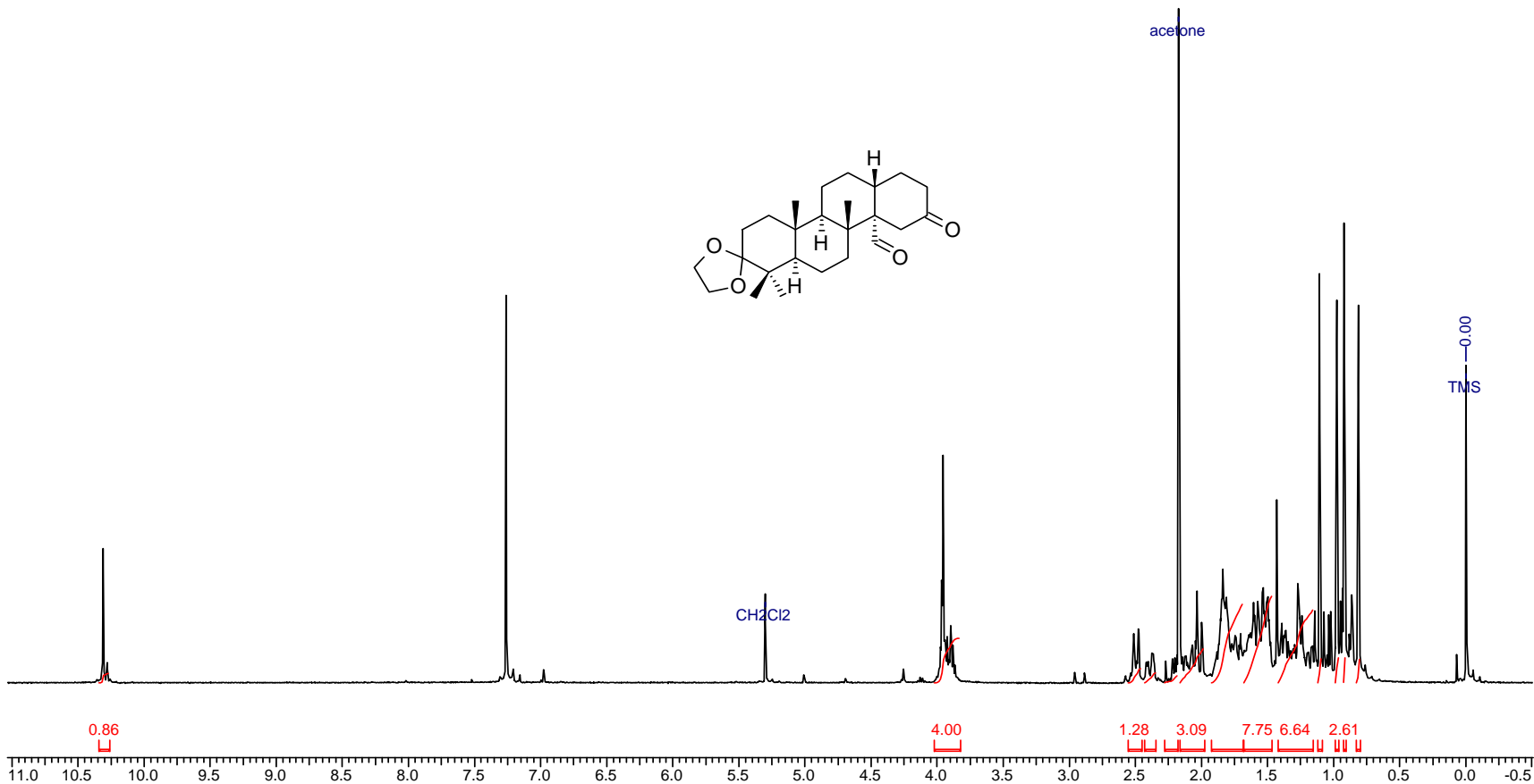
# <sup>1</sup>H NMR of Compound 4.22

## AA-11-purealdehyde

Acquisition Time (sec)	2.5616	Comment	Std	Date	Nov 11		
Frequency (MHz)	399.76	Nucleus	<sup>1</sup> H	Original Points Count	49983	Points Count	16384
Solvent	cdcl3	Sweep Width (Hz)	6395.91	Temperature (grad C)	25.000		



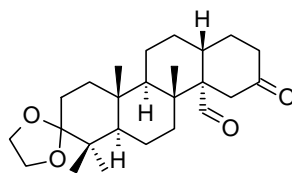
2



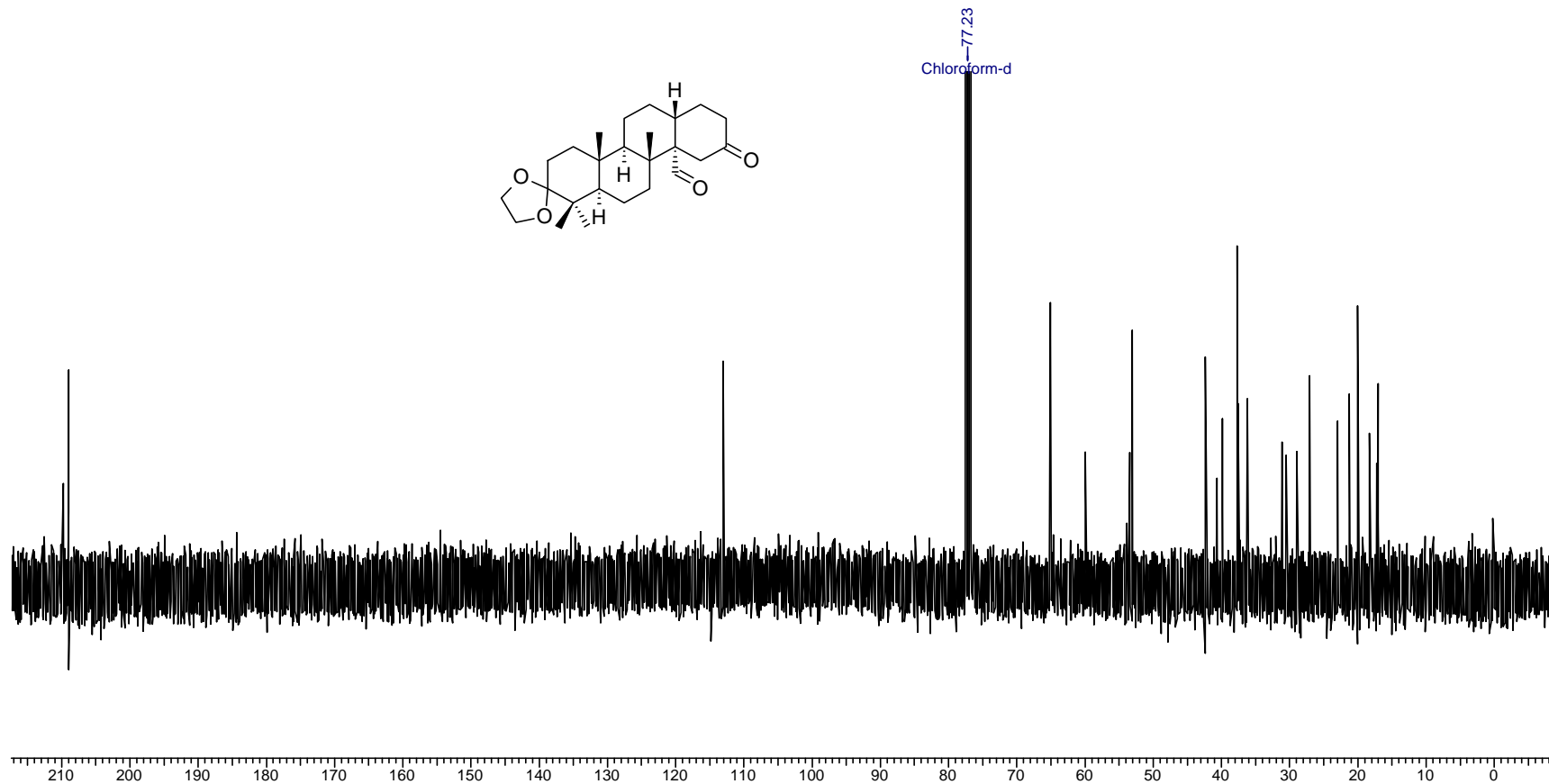
# $^{13}\text{C}$ NMR of Compound 4.22

## AA-11-purealdehyde-C13

<b>Acquisition Time (sec)</b> 1.3582	<b>Comment</b> Std proton	<b>Date</b> Nov 11 2008		
<b>Frequency (MHz)</b> 100.53	<b>Nucleus</b> $^{13}\text{C}$	<b>Number of Transients</b> 30000	<b>Original Points Count</b> 31375	<b>Points Count</b> 32768
<b>Solvent</b> cdcl3	<b>Sweep Width (Hz)</b> 24125.45	<b>Temperature (grad C)</b> 25.000		



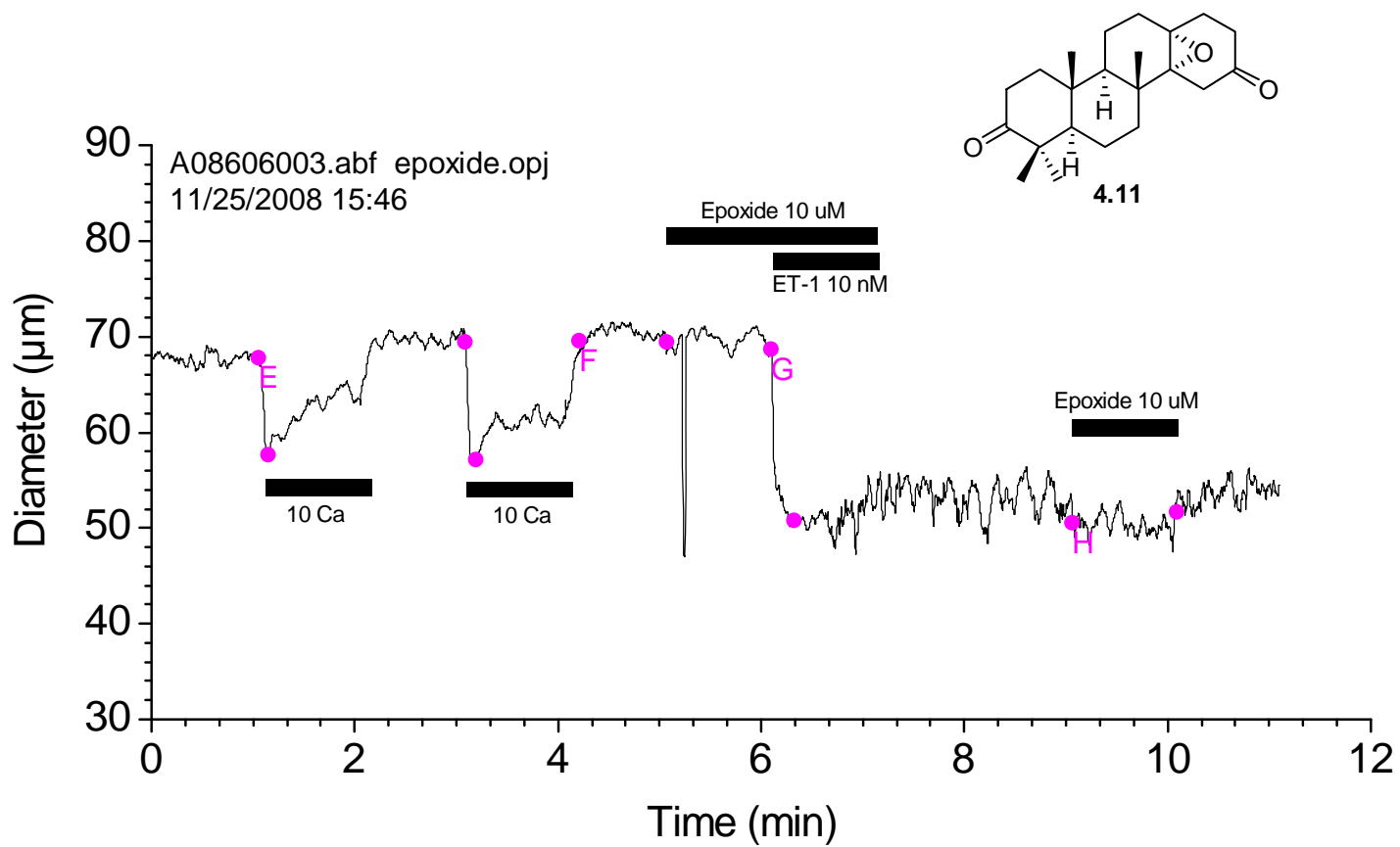
2



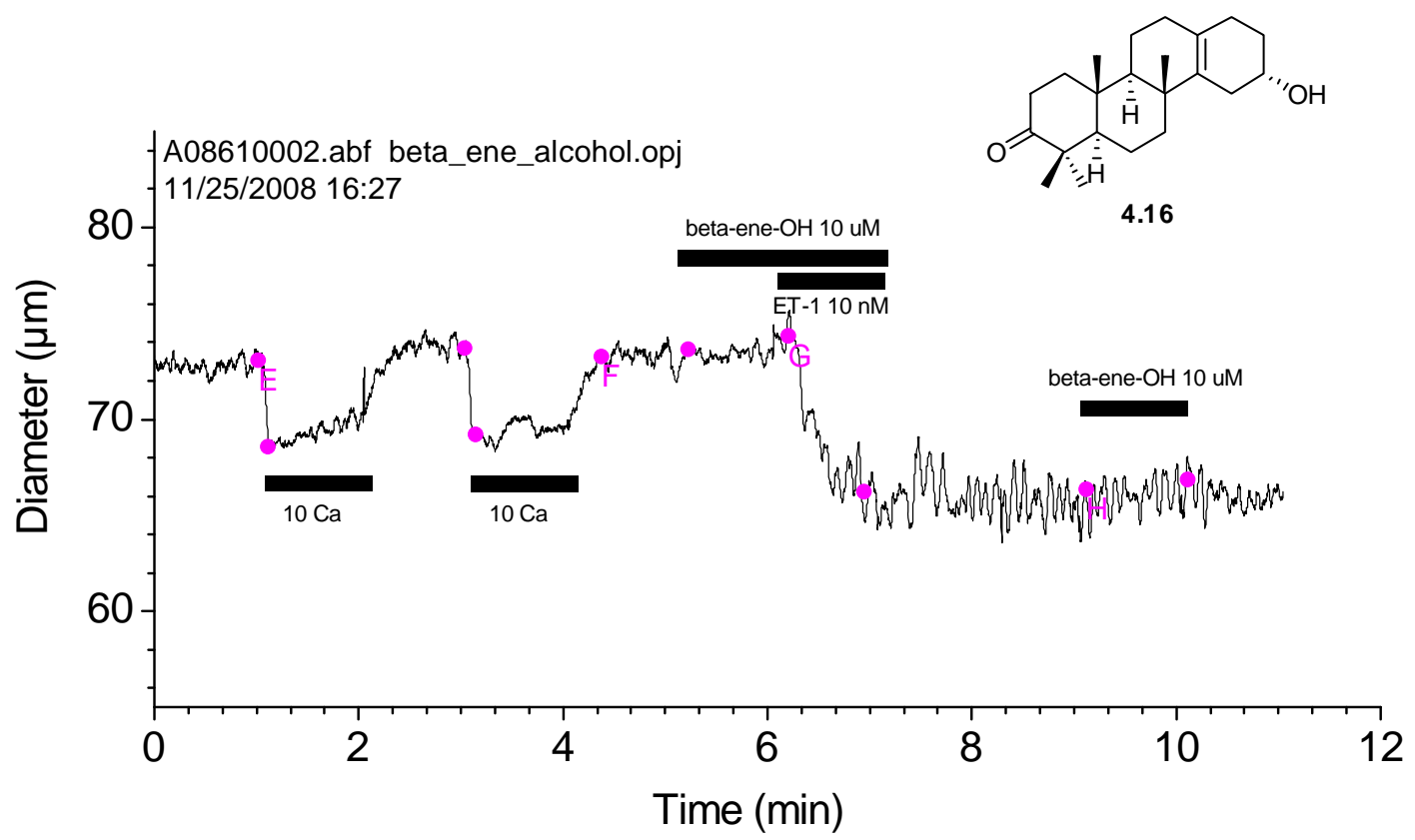


## Appendix D - Chapter 5, Blood vessel diameter graphs

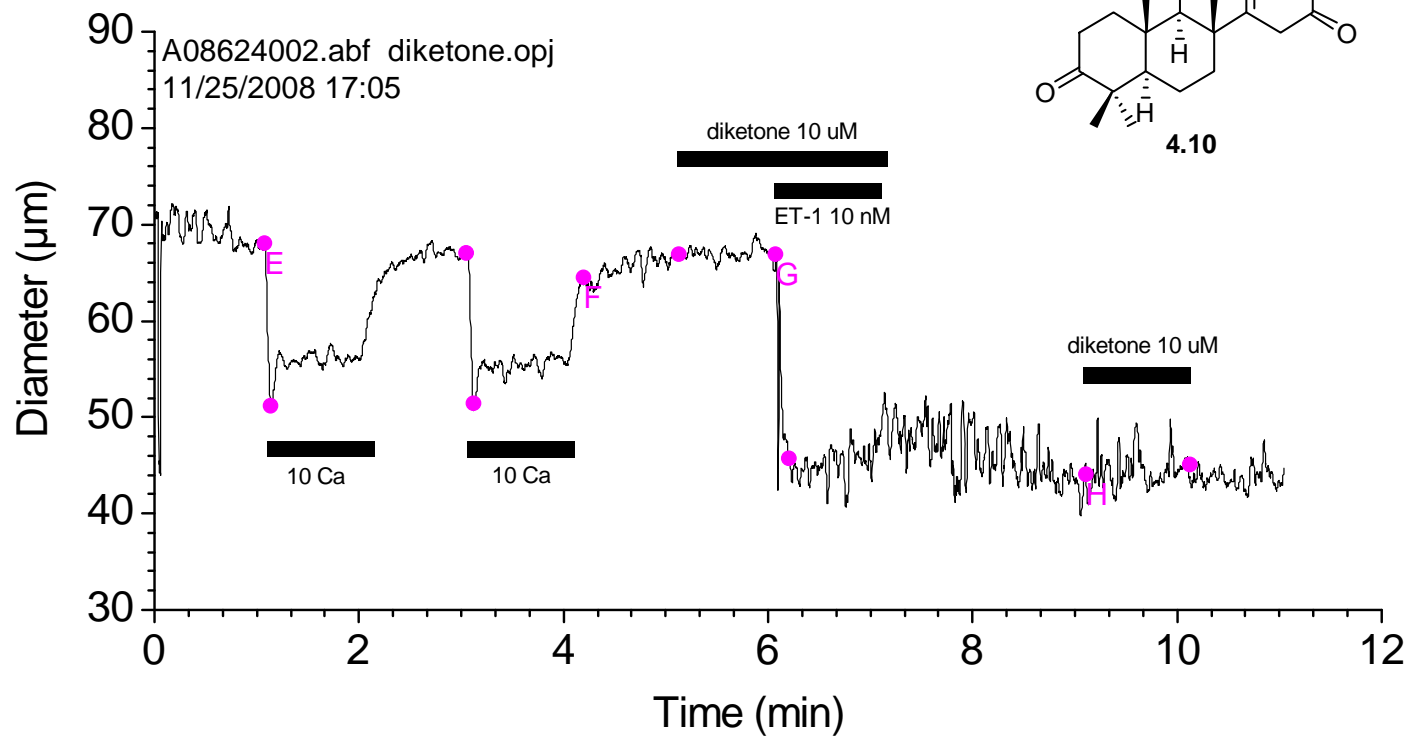
### Diameter graph of compound 4.11



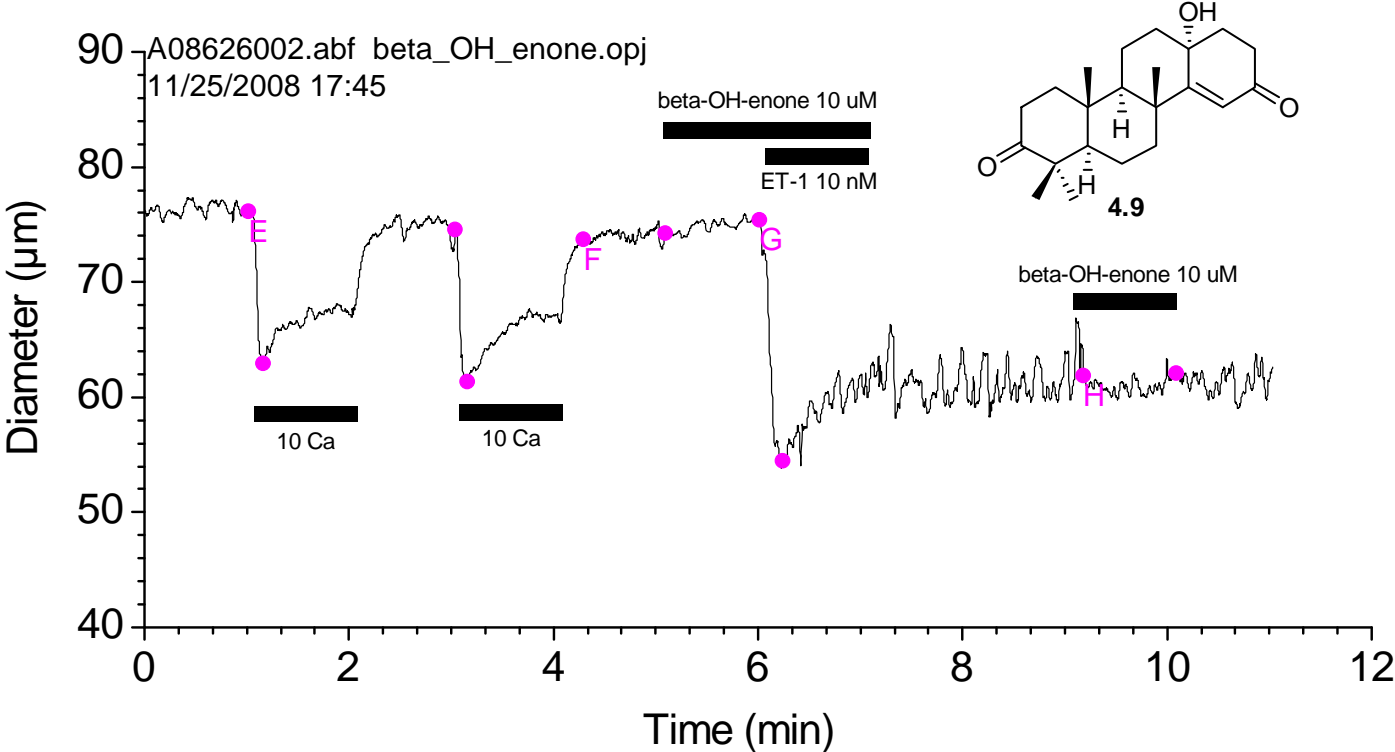
### Diameter graph of compound 4.16



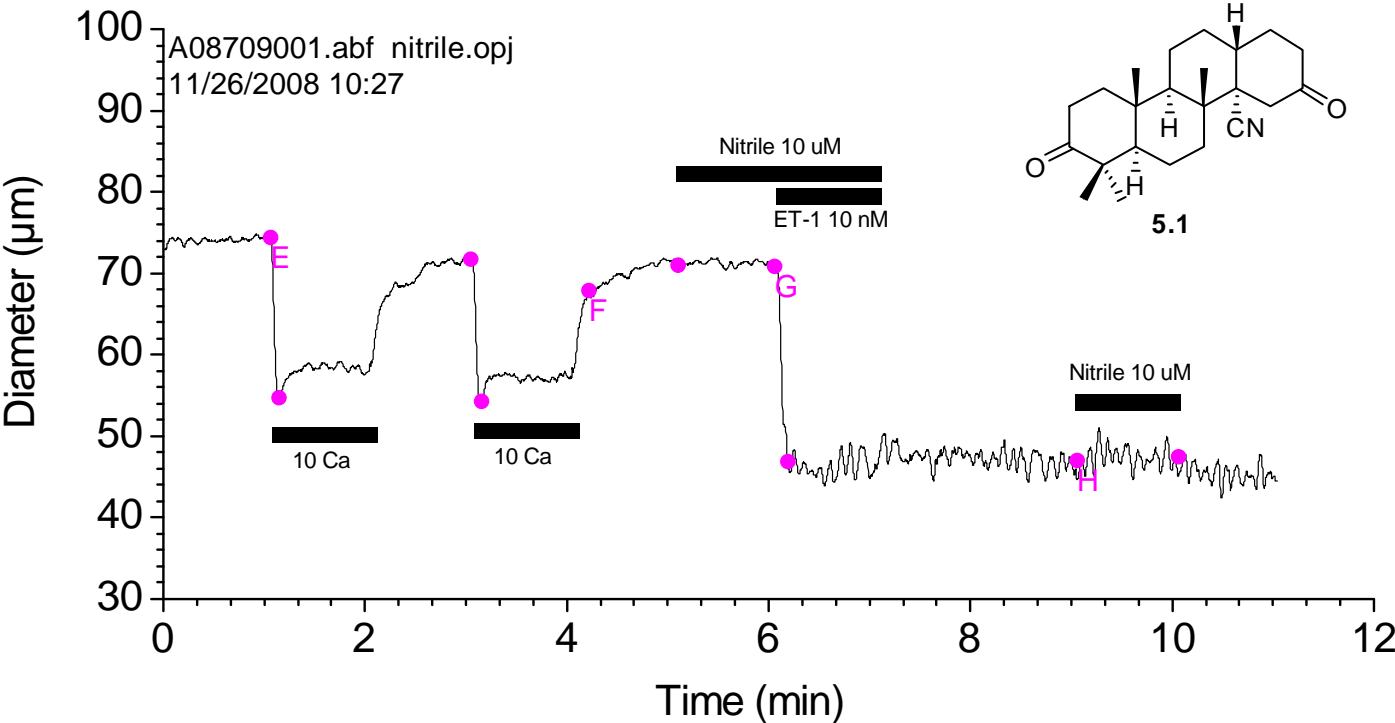
### Diameter graph of compound 4.10



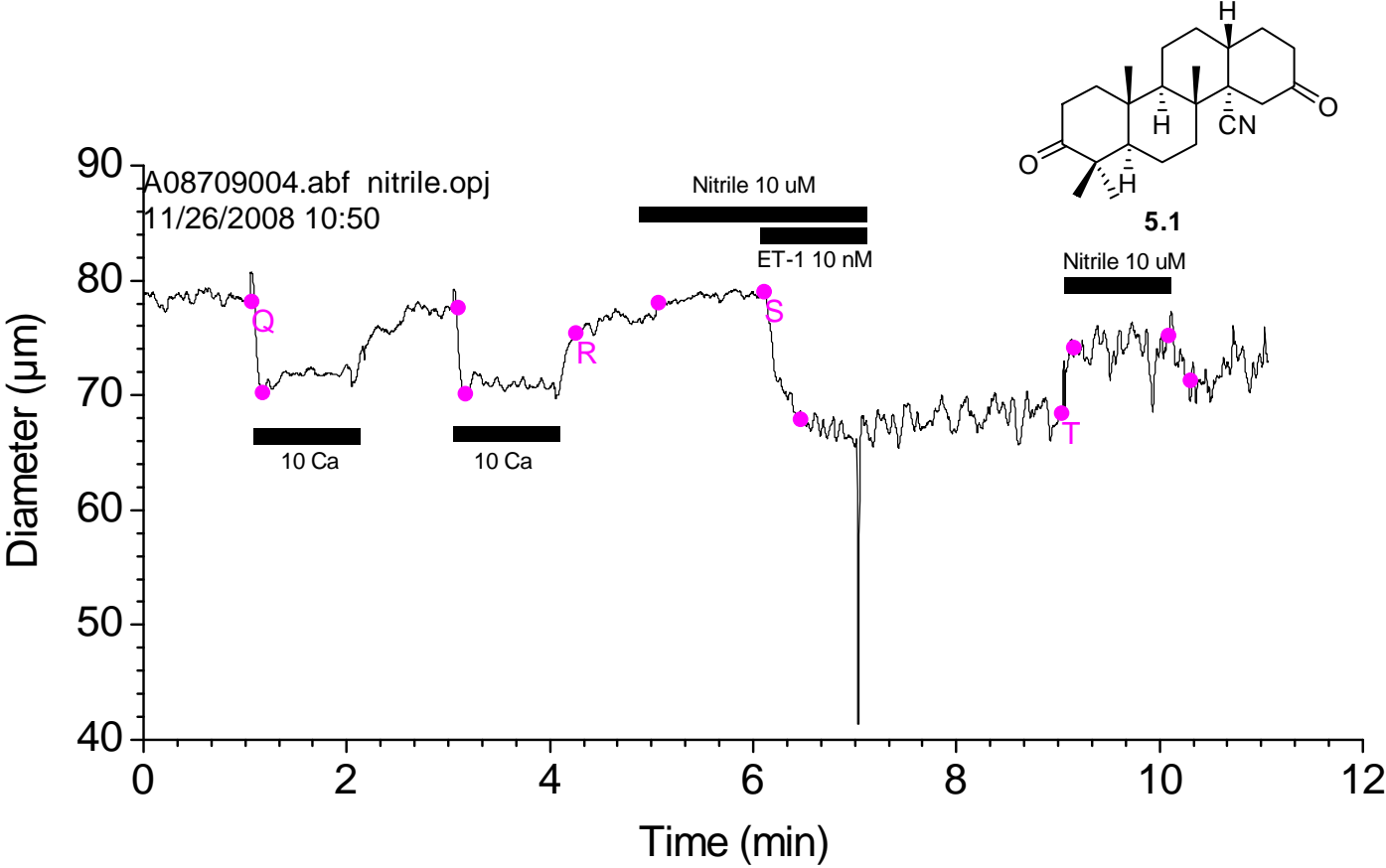
### Diameter graph of compound 4.9



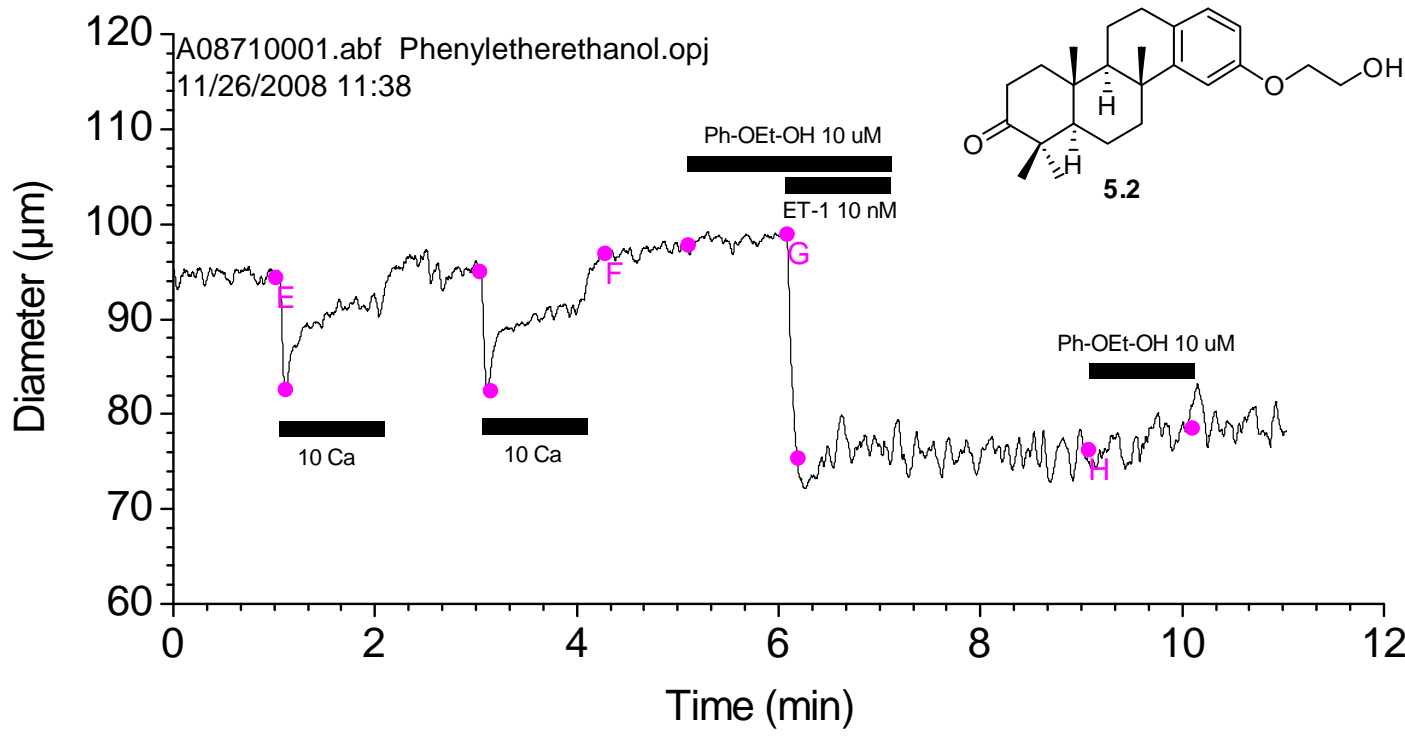
Diameter graph of compound 5.1



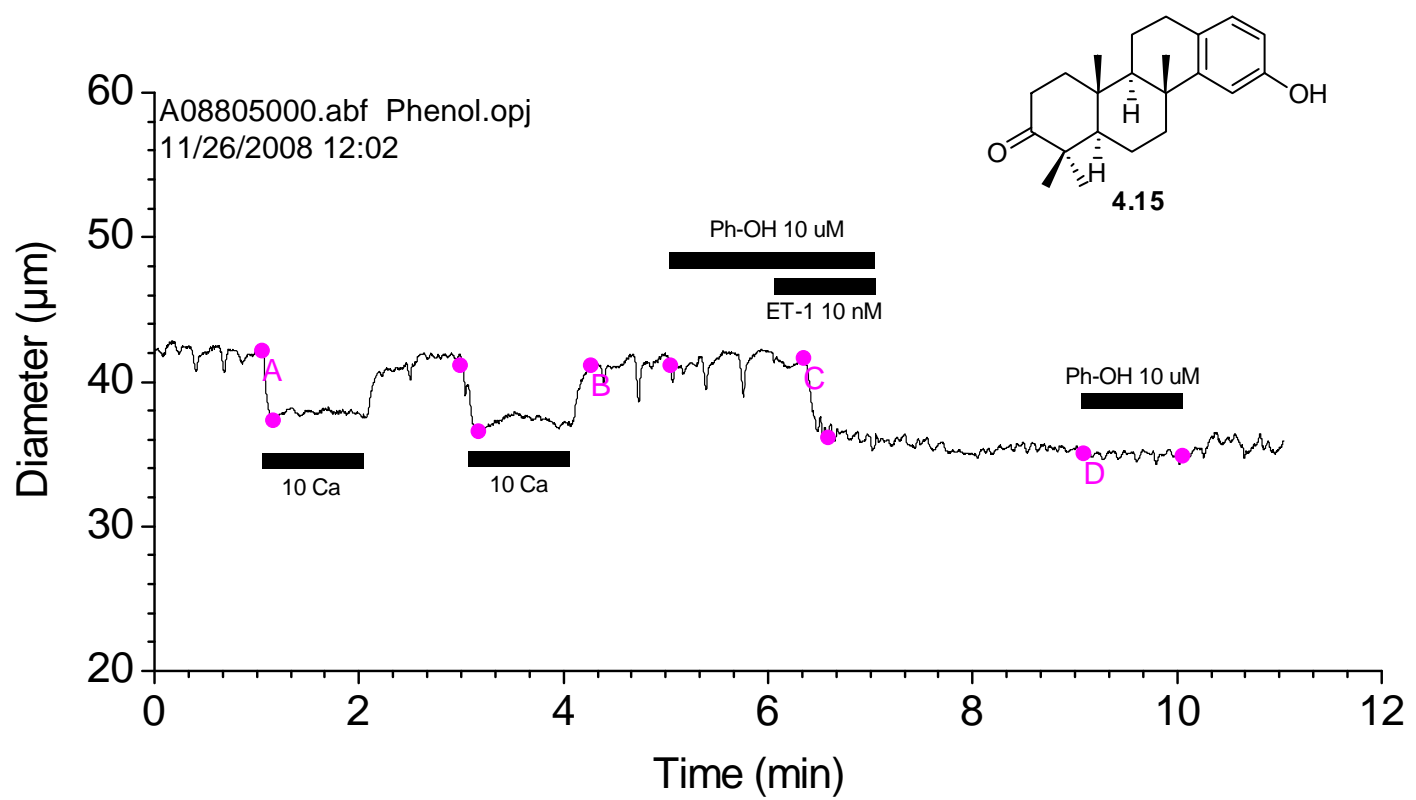
Diameter graph of compound 5.1



### Diameter graph of compound 5.2

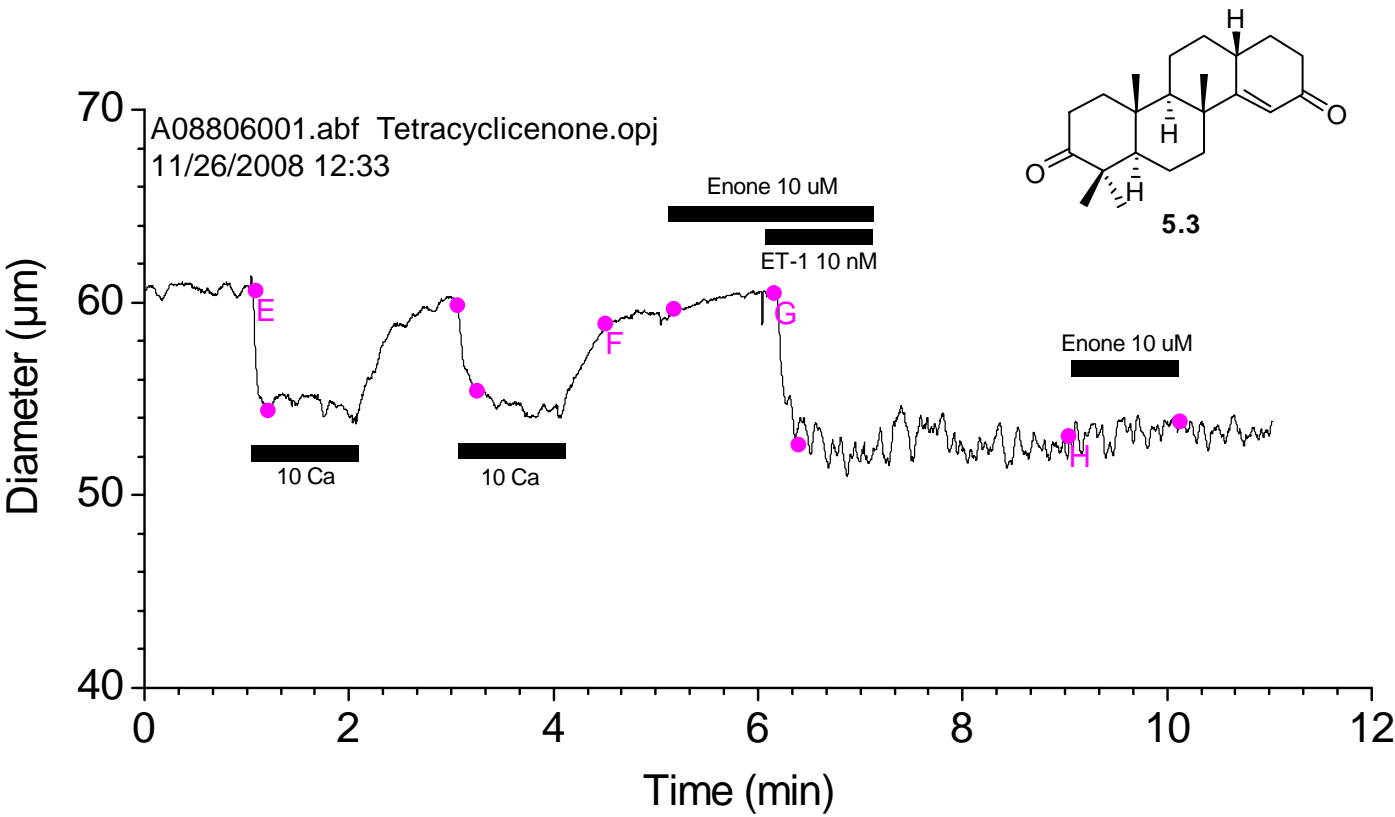


### Diameter graph of compound 4.15

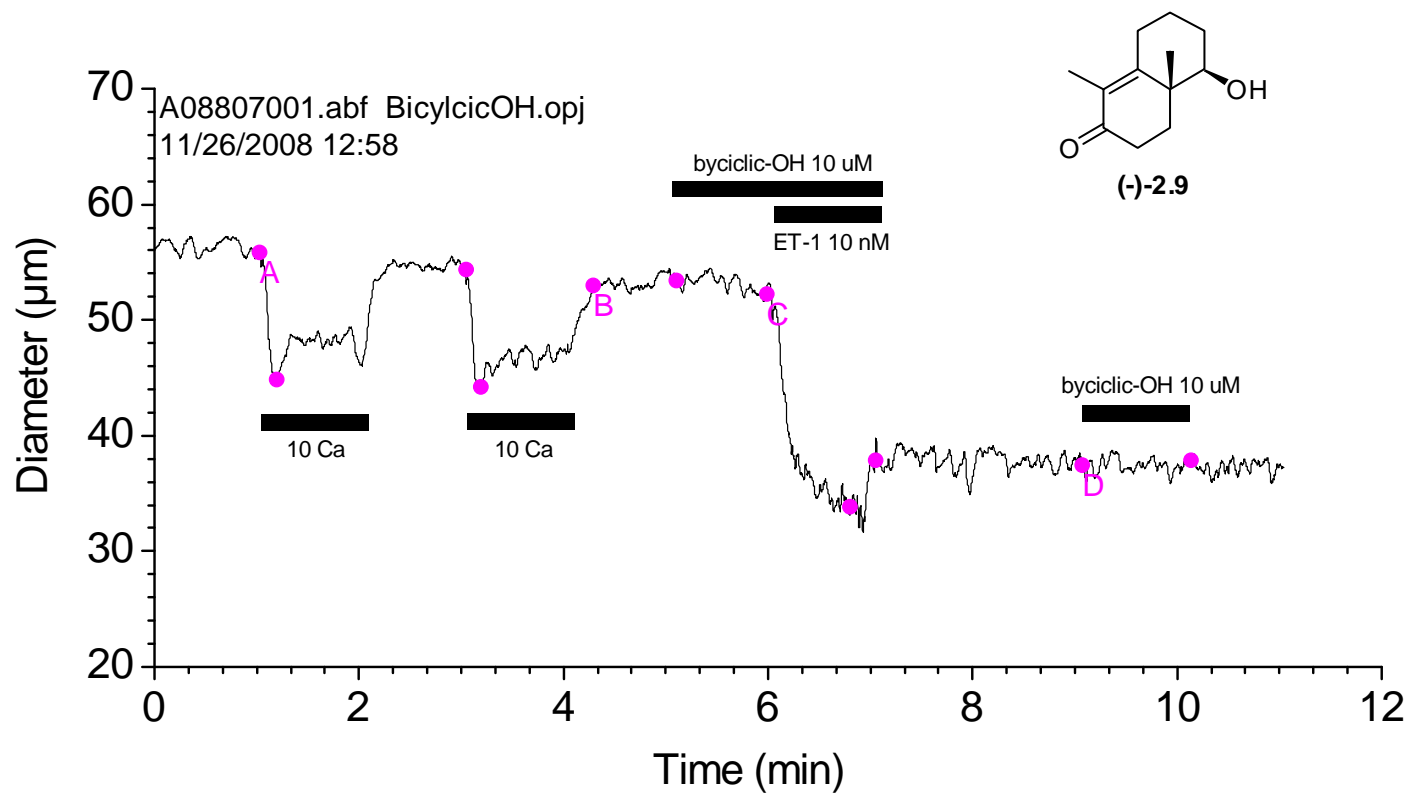




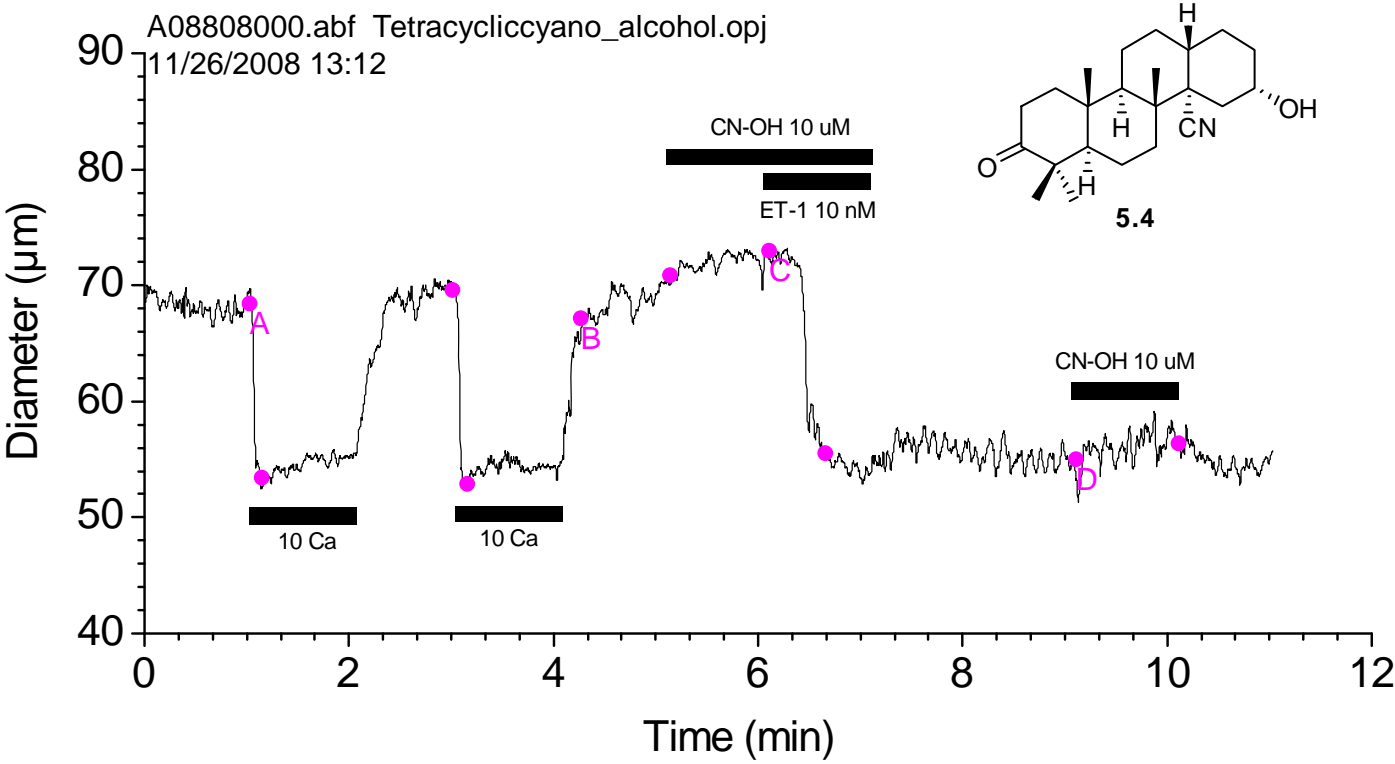
Diameter graph of compound 5.3



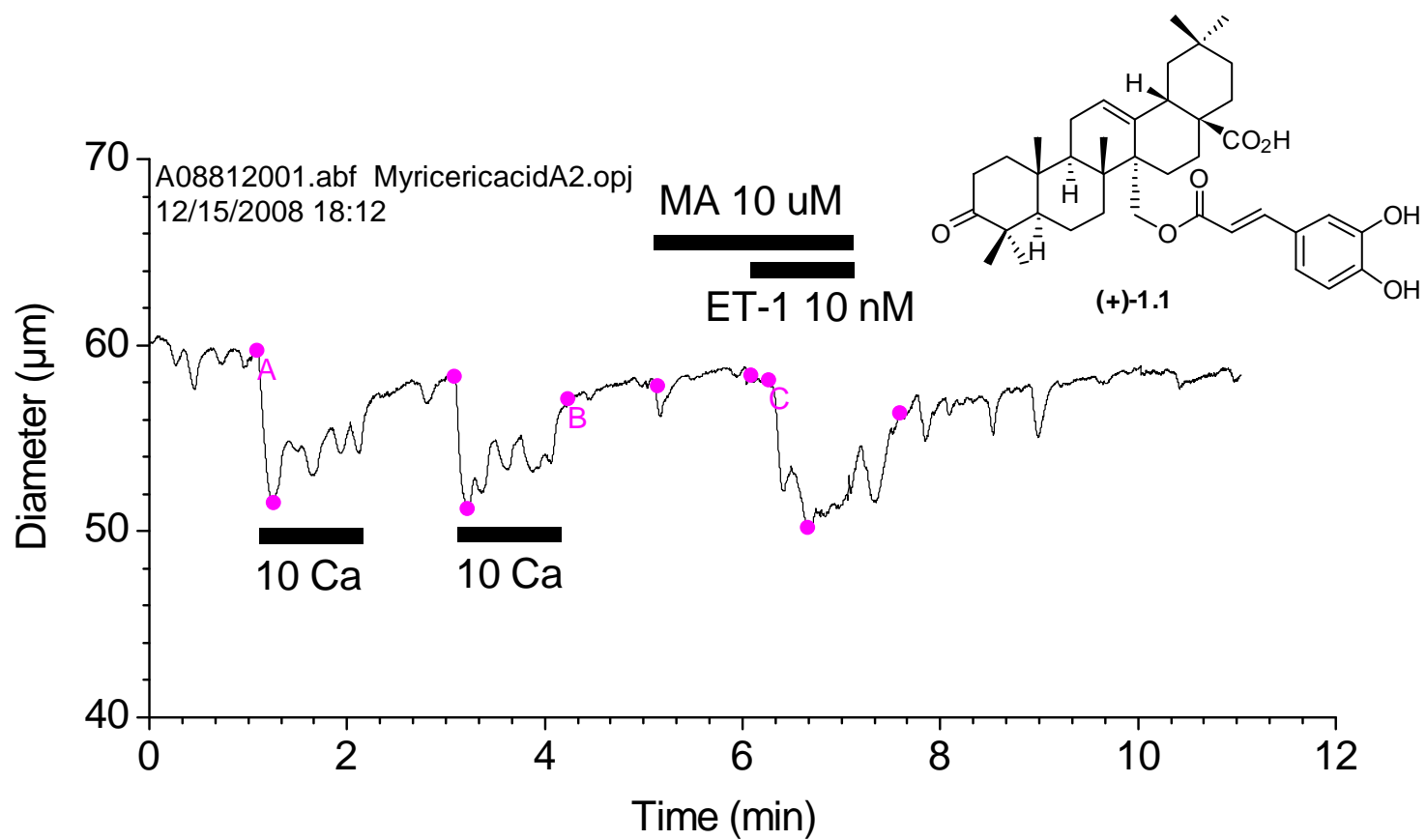
### Diameter graph of compound (-)-2.9



# Diameter graph of compound 5.4



### Diameter graph of compound (+)-1.1



### Diameter graph of compound (+)-1.1

