I. TOTAL SYNTHESIS OF (±) OVALICIN AND ITS ANALOGUES

II. BIO-BASED POLYMERS FROM VEGETABLE OIL

III. NEW SYNTHETIC METHODS OF DIACETYLENE FATTY ACIDS

by

HUIPING ZHAO

B.S., East China University of Science and Technology, 1998

M.S., East China University of Science and Technology, 2001

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry

College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2008

Abstract

I. Ovalicin is a natural product isolated from the culture of fungus *Pseudorotium ovalis* Stolk, it selectively inhibit type 2 methionine amino-peptidase (MetAP 2), which related to many physiological activities such as angiogenesis. Total synthesis of (±) ovalicin, its C4(S*), C4(S*)C5(S*) stereo-isomers, and C5 regio-isomer were synthesized via an intramolecular Heck reaction of (Z)-3-(t-butyldimethyl silyloxy)-1-iodo-1,6-heptadiene utilizing a catalytic amount of palladium acetate. Subsequent epoxidation, dihydroxylation, methylation (or stereochemistry inversion before or after methylation) and oxidation led to a variety of ketones, key intermediates for synthesis of ovalicin and its analogues. Introduction of side-chain to ketones by lithium (Z)-6-methylhepta-2,5-dien-2-ide and following functional group transformation led to ovalicin and its analogues. Anti-trypanosomal activities of various ovalicin analogues and synthetic intermediates were evaluated.

II. Bio-based polymers from vegetable oils are renewable and environment-friendly materials. Dihydroxylated, trihydroxylate, tetrahydroxylated and hexahydroxylated triglycerides, triamino and triisopropylamino glycerides were synthesized from model triglyceride glyceryl trioleoate. These monomers were cross-linked with 1, 4-phenylene diisocynate to make polyurethanes and polyureas. The physical properties of these polymers were examined by gel content and swelling value measurements, thermodynamic and viscoelastic properties were studied from TGA, DSC and DMA measurements. The structure-property relationship was discussed based on these measurements.

III. Diacetylenic fatty acids were widely applied in material science to regulate alignment on surface and stabilize self-assembled nanomaterials. A novel synthetic method of diacetylenic fatty acids from vegetable oils was developed. Its self-assembling properties on alumina surface were measured and discussed.

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Chapter 1 Total synthesis of (±) ovalicin and its analogues

Chapter 2 Biobased polymers from vegetable oils

Chapter 3 New synthetic methods of diacetylenic fatty acids

List of Abbreviations

Ac	acetate
AP	aminopeptidase
Bu (n-Bu)	normal butyl
Calcd.	calculated
d	doublet
DAM	diacetylenic acid mixture
DAP	diacetylenic acid pure
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DIBAL-H	diisobutylaluminum hydride
DMA	dynamic mechanical analysis
DMAP	4-(dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DSC	differential scanning calorimetry
Ec	Escherichia coli
Et	ethyl
HRMS	high resolution mass spectroscopy
HMPA	hexamethylphosphoramide
IC50	the half maximal inhibitory concentration
i-Pr	*iso*-Propyl
IR	infrared
M	molarity
m	multiplet
MetAP	methionine aminopeptidase
Me	methyl
mmol	millimole
MOMCl	methoxylmethoxyl chloride
Ms	methanesulfonyl, mesyl
NBS	N-bromosuccinimide
NMR	nuclear magnetic resonance

Nu	nucleophile
PDC	pyridinium dichromate
Ph	phenyl
q	quartet
quint	quintet
s	singlet
sept	septet
t	triplet
TBAF	tetrabutylammonium fluoride
TBDMS	tert-butyldimethylsiyl
TGA	thermogravimetric analysis
THF	tetrahydrofuran
TMEDA	*N*,*N*,*N'*,*N'*-Tetramethylethylenediamine

Structure-Number Correlation Lists

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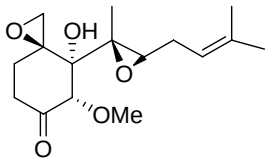

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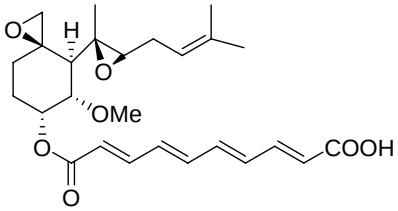

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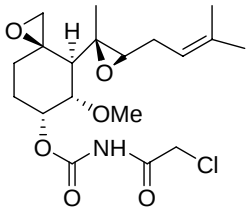

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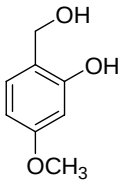

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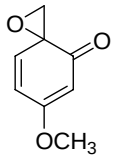

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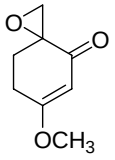

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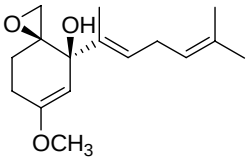

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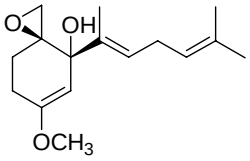

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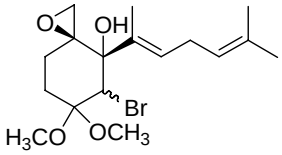

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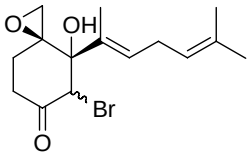

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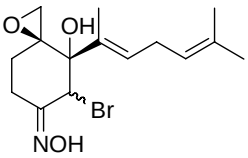

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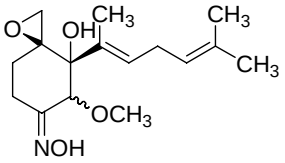

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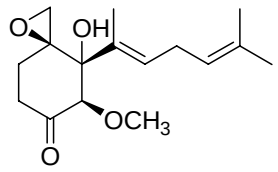

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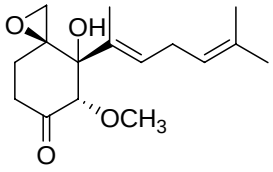

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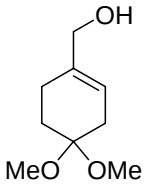

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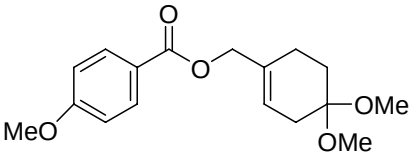

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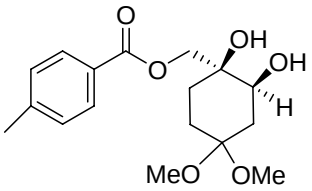

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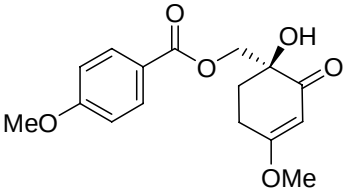

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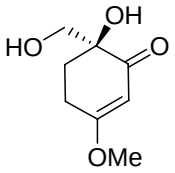

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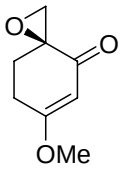

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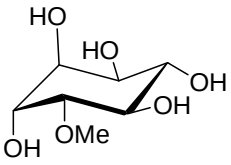

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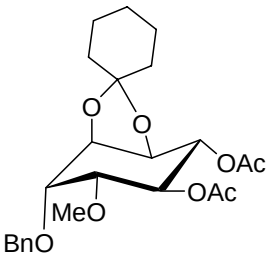

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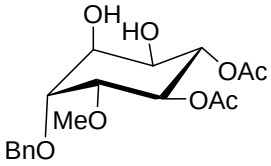

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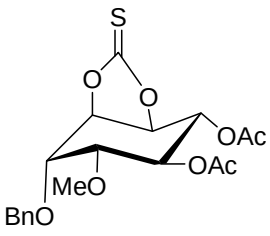

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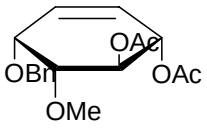

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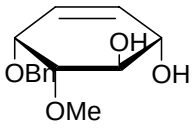

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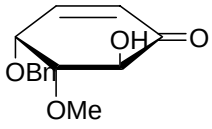

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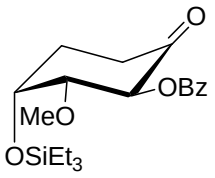

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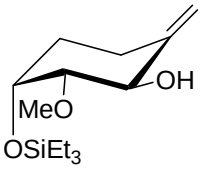

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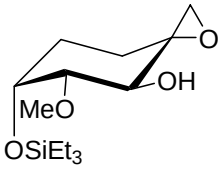

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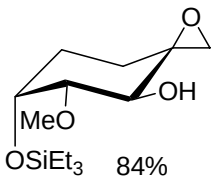

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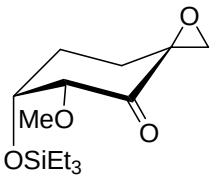

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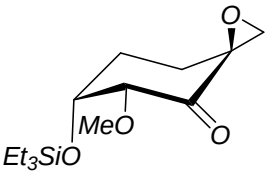

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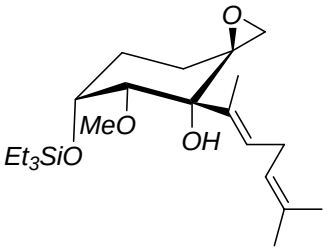

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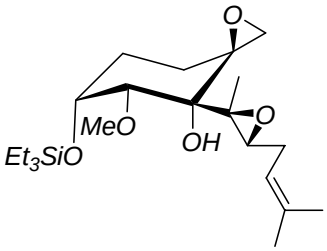

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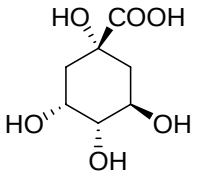

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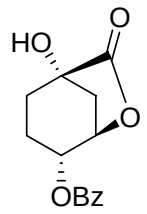

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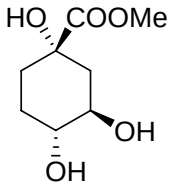

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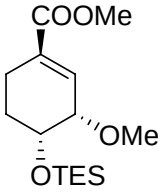

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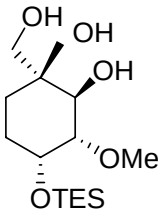

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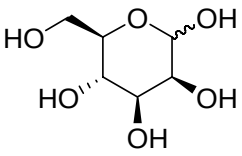

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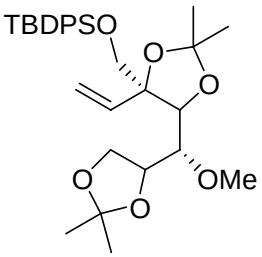

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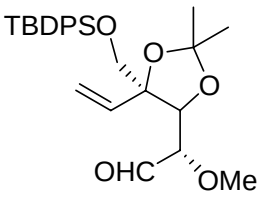

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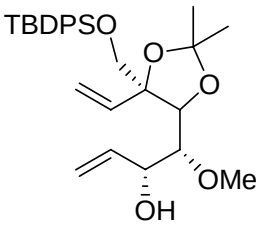

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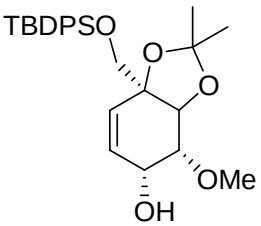

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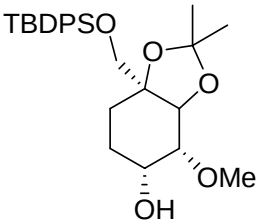

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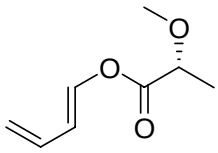

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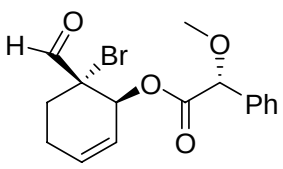

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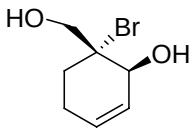

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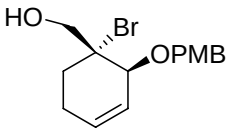

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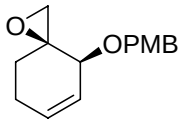

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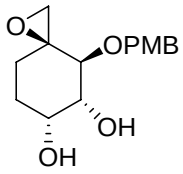

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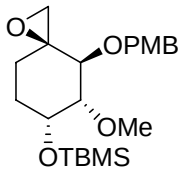

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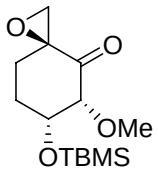

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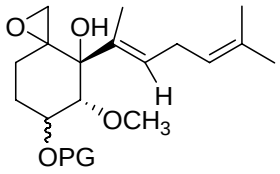

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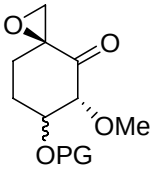

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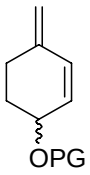

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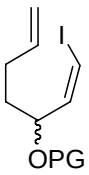

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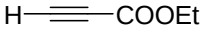

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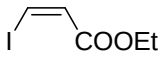

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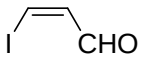

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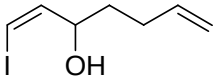

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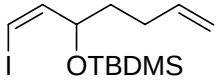

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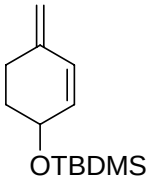

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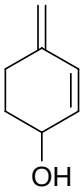

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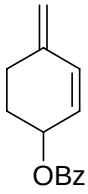

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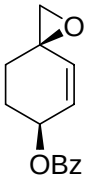

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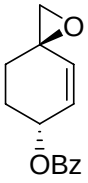

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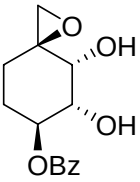

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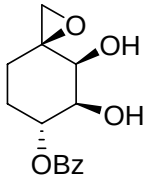

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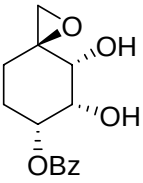

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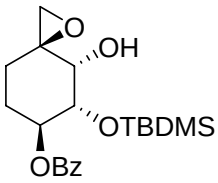

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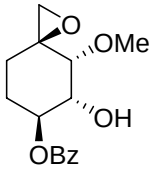

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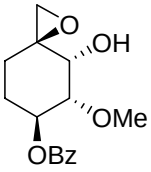

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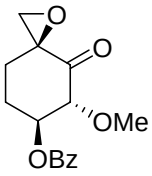

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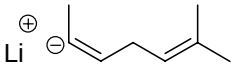

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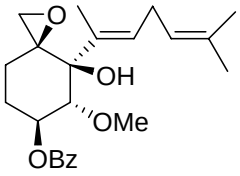

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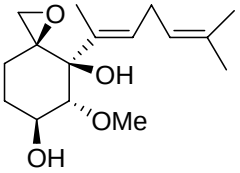

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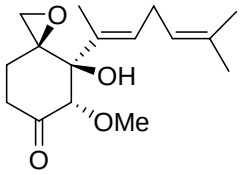

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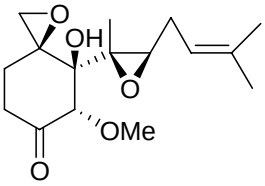

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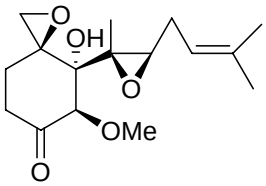

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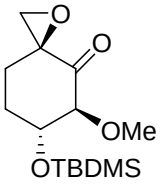

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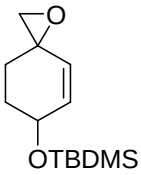

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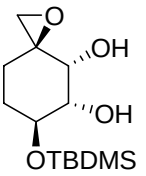

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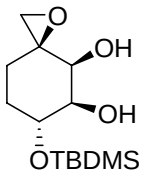

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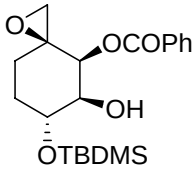

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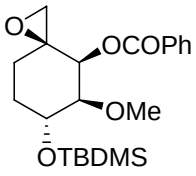

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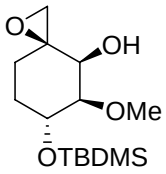

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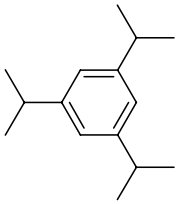

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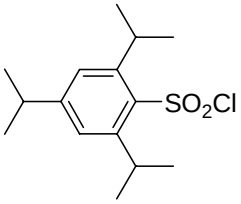

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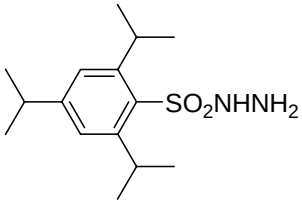

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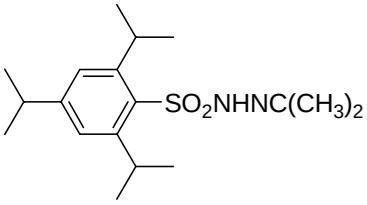

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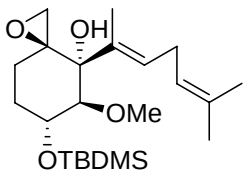

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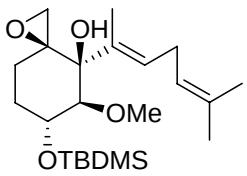

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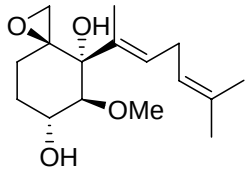

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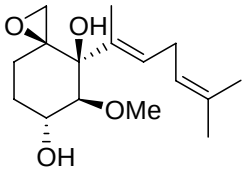

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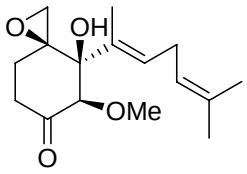

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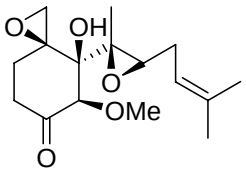

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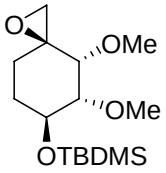

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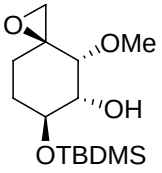

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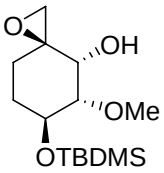

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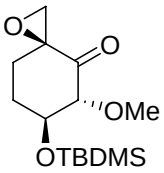

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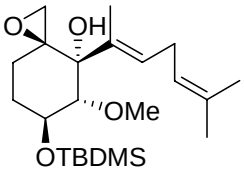

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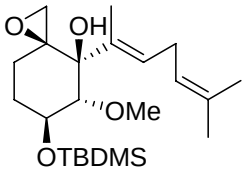

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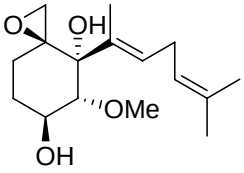

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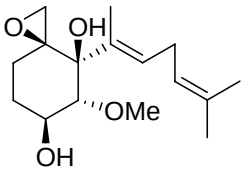

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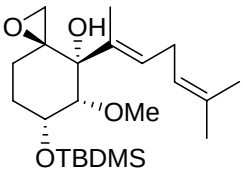

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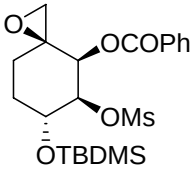

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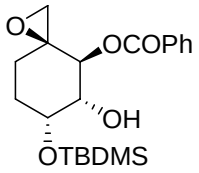

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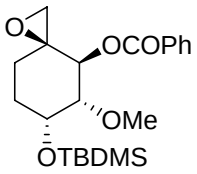

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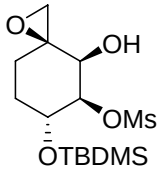

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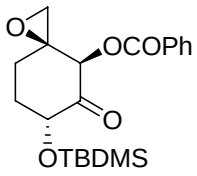

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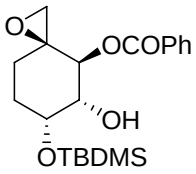

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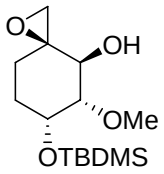

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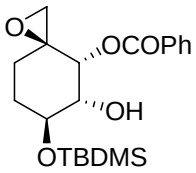

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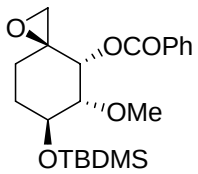

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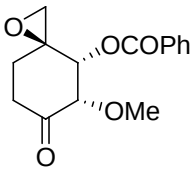

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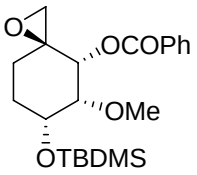

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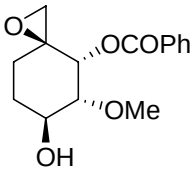

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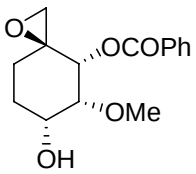

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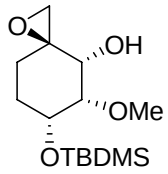

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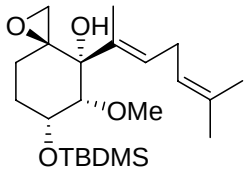

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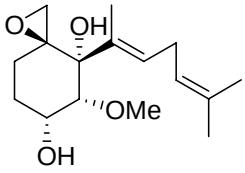

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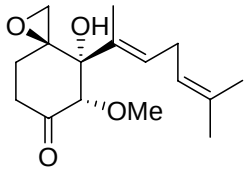

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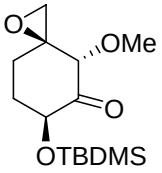

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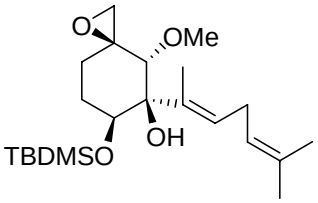

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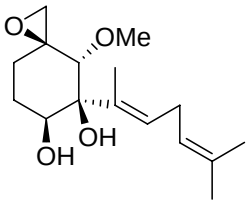

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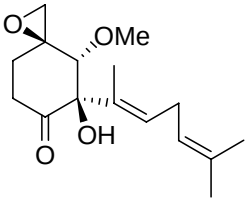

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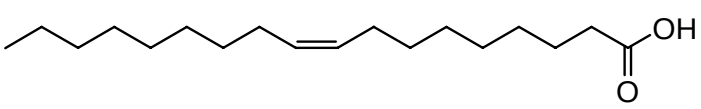

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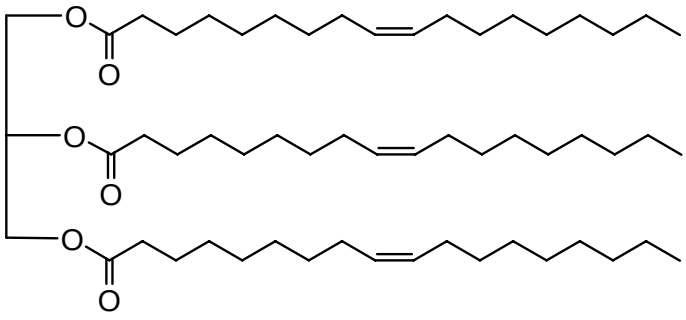

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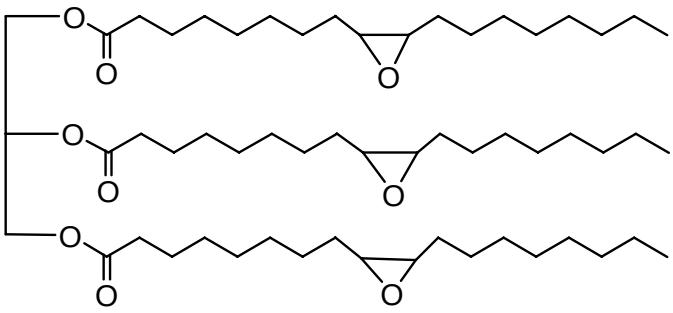

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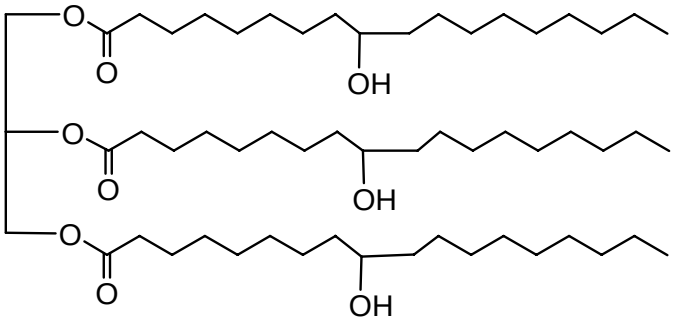

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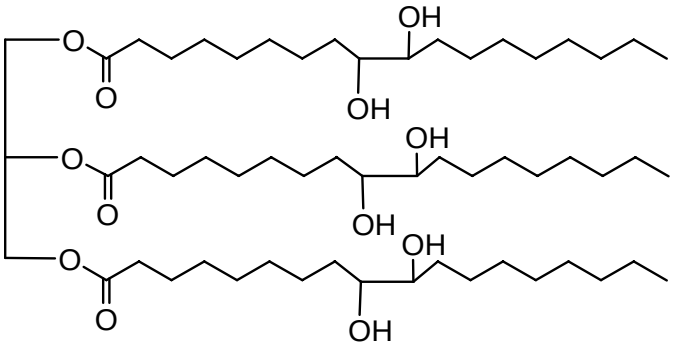

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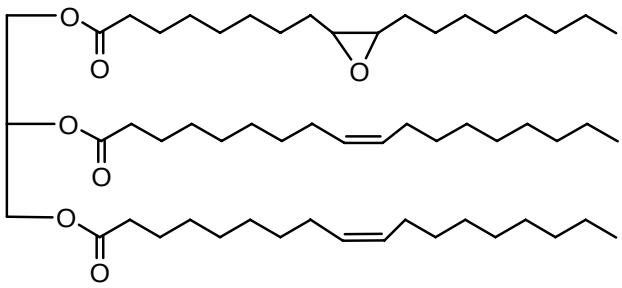

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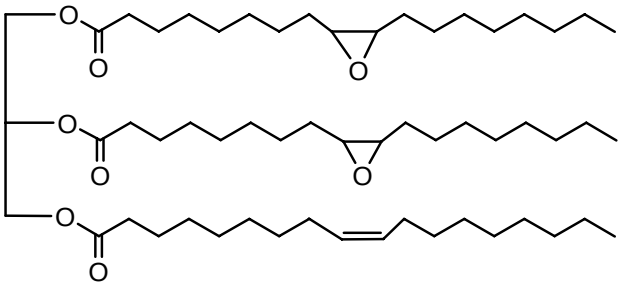

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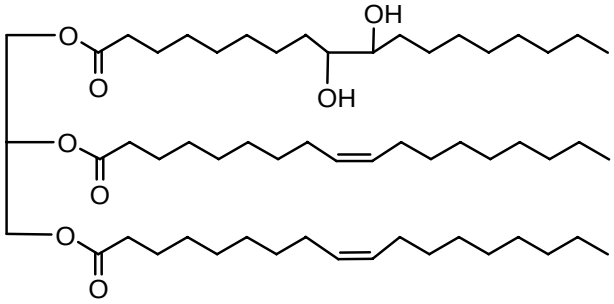

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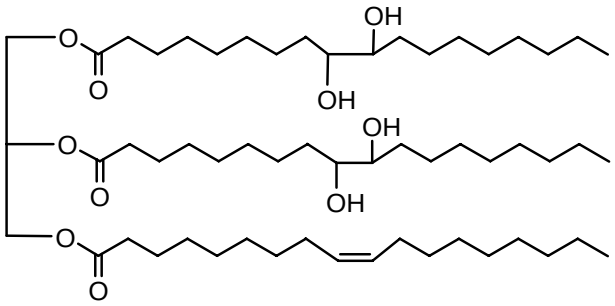

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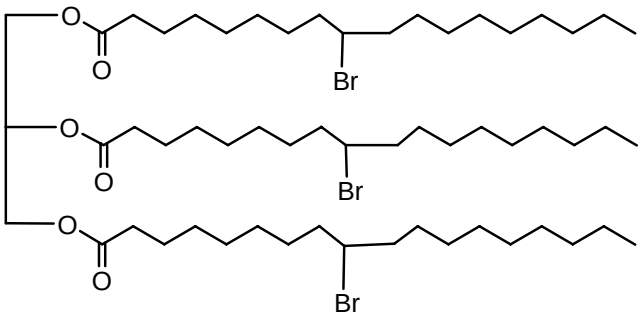

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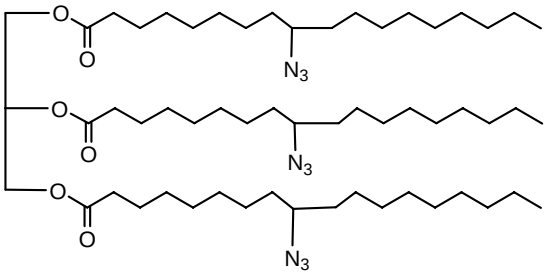

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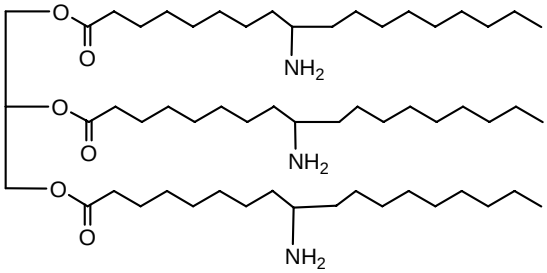

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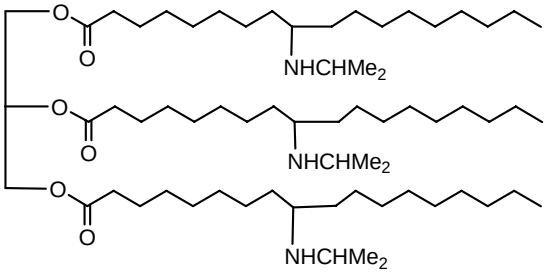

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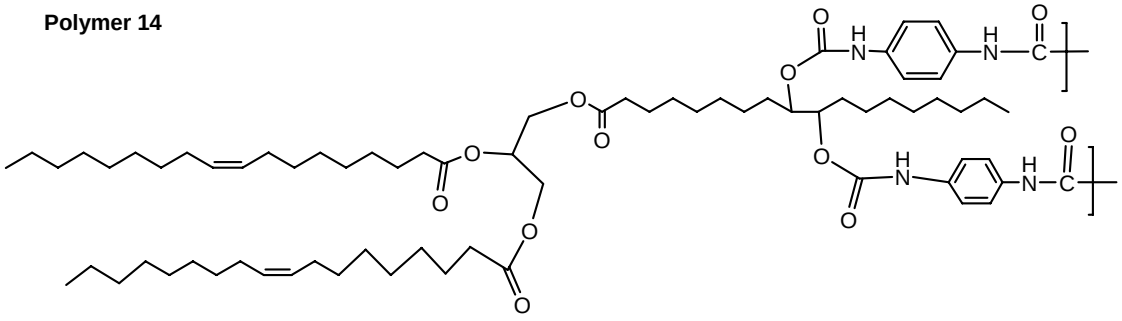

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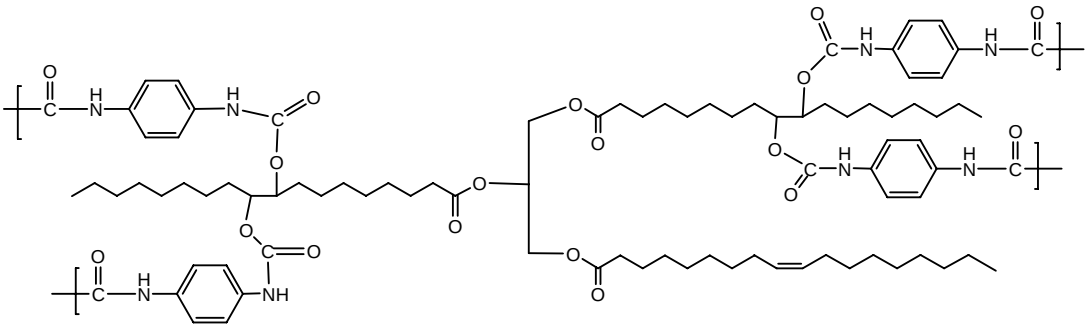

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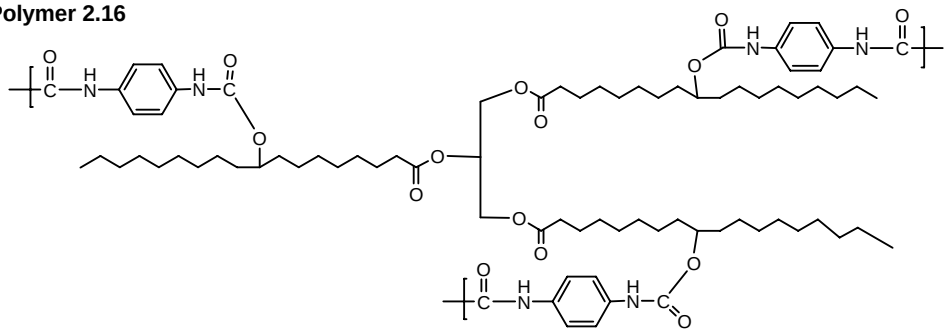
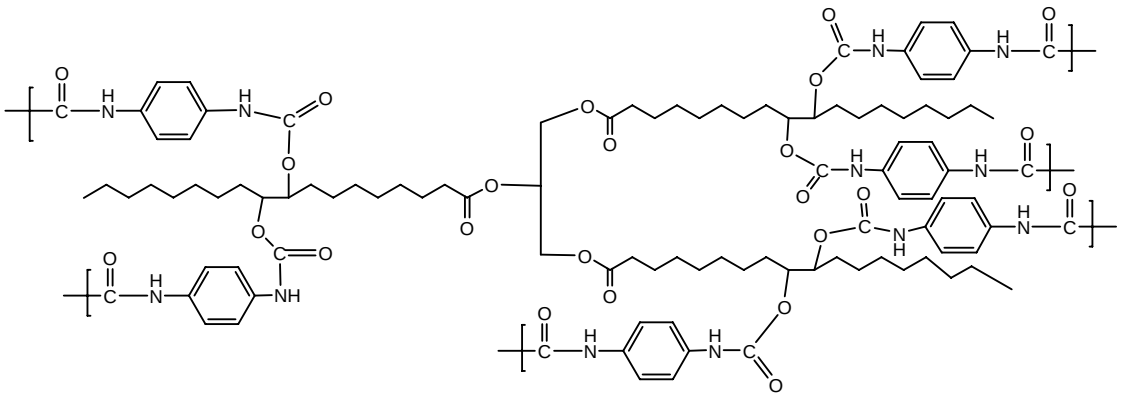


Polymer 2.17



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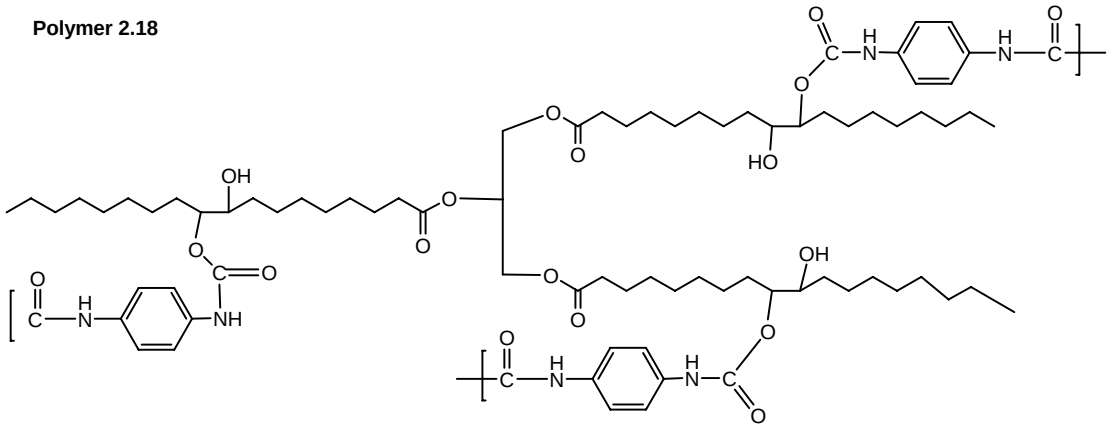

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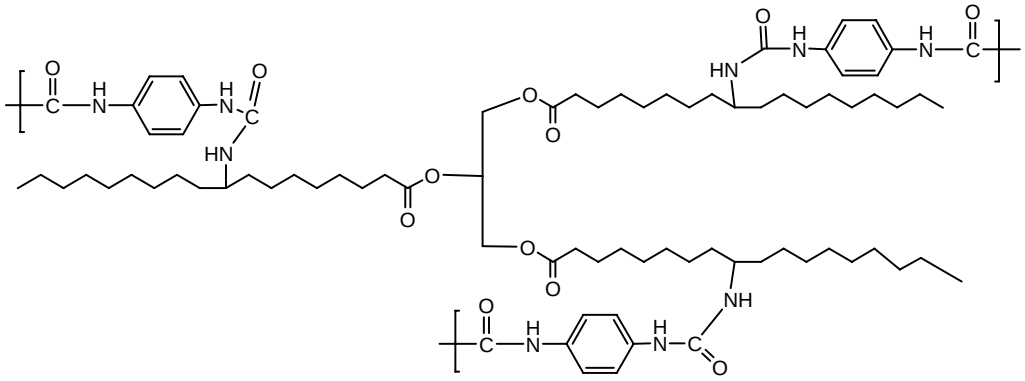
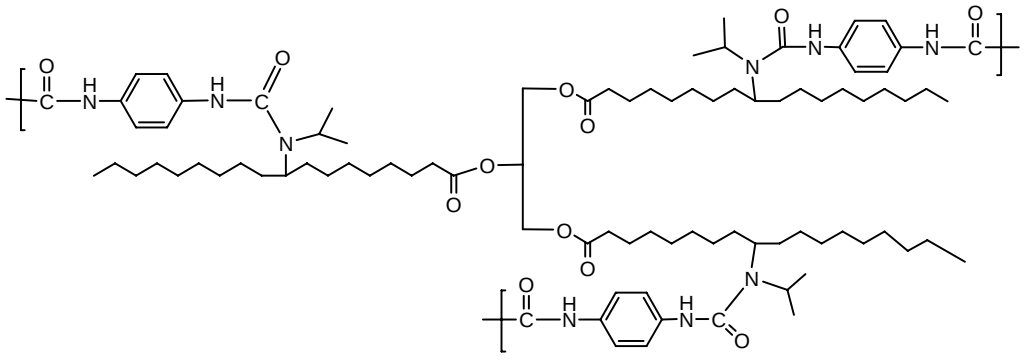


Polymer 2.20



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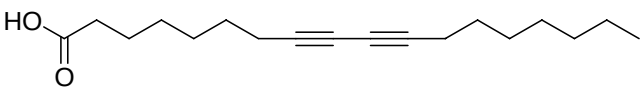

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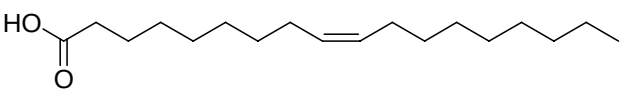

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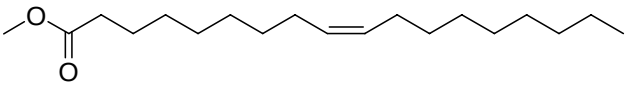

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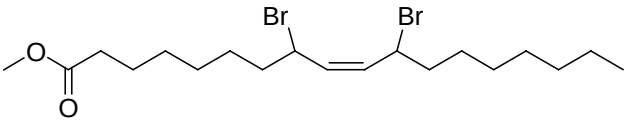

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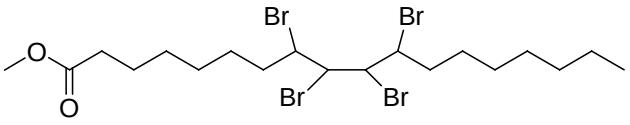

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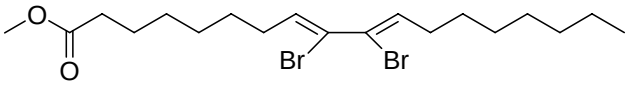

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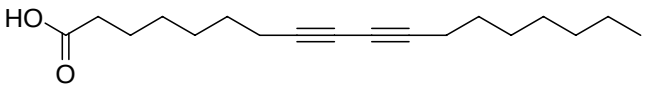

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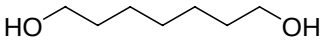

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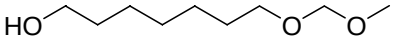

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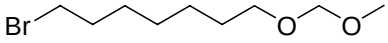

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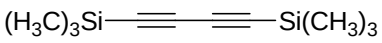

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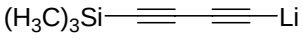

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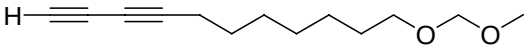

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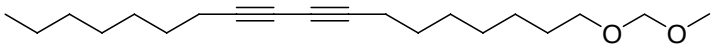

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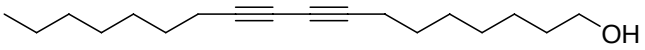

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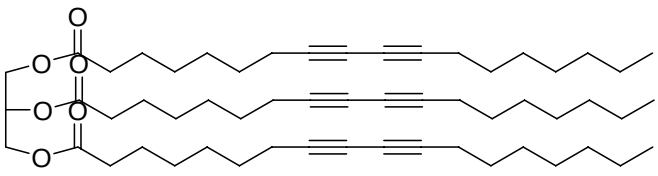

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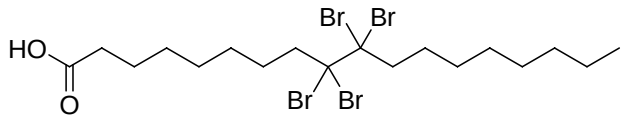

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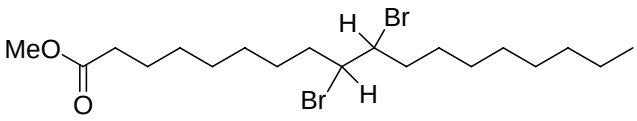

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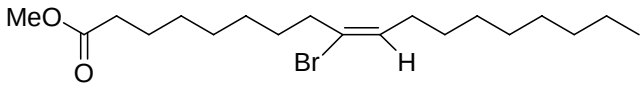

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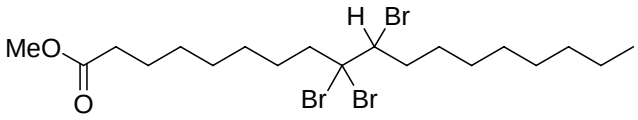

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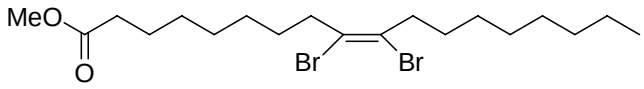

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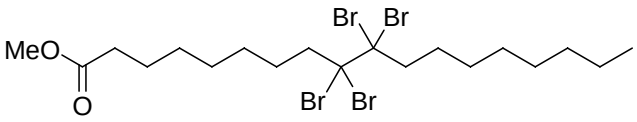

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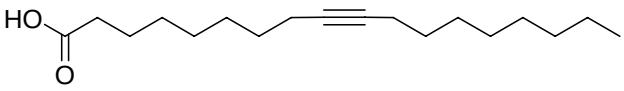

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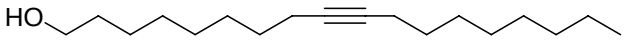

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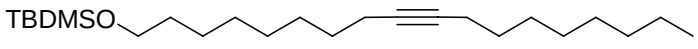

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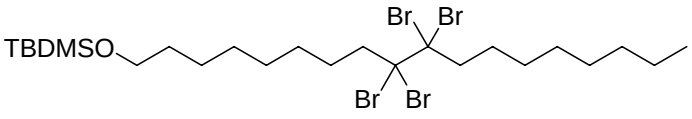

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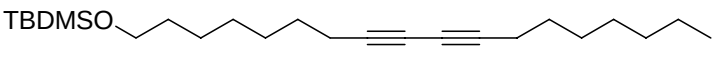


Acknowledgements

I would like to give my great appreciation to my research advisor, Dr. Duy H. Hua for his guidance and instruction throughout my graduate study. His enthusiastic commitment to chemistry and individual student achievement transformed me into an experienced chemist; his broadness and depth of knowledge in chemistry will always remind me to learn for a lifetime.

I would like to thank my committee members Dr. Kenneth Klabunde, Dr. Daniel Higgins and Dr. Jean-Pierre H. Perchellet for their suggestions and comments on my proposition oral and research project. I appreciate Dr. Sajid Alavi for being the outside chairperson for the examining committee of my final defense.

I am grateful to other faculty and staff member in the chemistry department for their help during the past five years. Special thanks to Dr. Kraft and Dr. Bossmann for their instruction and encouragement during the organic seminar, to James Hodgson for taking troubles to fix numerous glass-wares.

Sincere thanks to all former and current labmates in Dr. Hua's group for their help and friendship. Special thanks to Dr. Kaiyan Lou and Srinivas Battina for their selfless help and illuminating suggestions on my research.

Special thanks to Huaien Dai from biochemistry department and Yi Yang from biology department for their unconditional support and friendship.

No words can express my great thanks to my wife, Haihua Lu, for her sacrifice and dedication to me and my family. Without her constant support, I couldn't have done this job. Thanks to my son Zichen Zhao, his laughter is the power for me to go through difficulties and never lose confidence for my future.

Chapter 1 Total Synthesis of (±) Ovalicin and Its Analogues

1.1 Introduction

1.1.1 Methionine Aminopeptidase (MetAP)

Proteins are essential components of organisms and perform a variety of substantial functions within cells.[1] Many proteins have structural functions, such as offering matrix for bones and joint tissues, providing structure and form to the human organism. Other proteins may have dynamic functions such as catalysis of biochemical transformation, substance transportation and metabolic control.[1] Therefore, protein synthesis is the base of life. In eukaryotes, all protein synthesis is initiated by methionine, while in prokaryotes, mitochondria and chloroplasts, it is initiated by formylated methionine (formyl group can be hydrolyzed by a deformylase after protein synthesis). Protein maturation or degradation requires the removal of methionine residue from N-terminal of newly synthesized peptides. There are two families of aminopeptidases (APs) capable of removing N-terminal methionine residue from nascent peptides and proteins; one family is that acts nonspecifically and progressively removing methionine and other residues, it is usually believed to be involved in general degradation processes. Another family is that to be more highly regulated and have more specific physiological activities.[2]

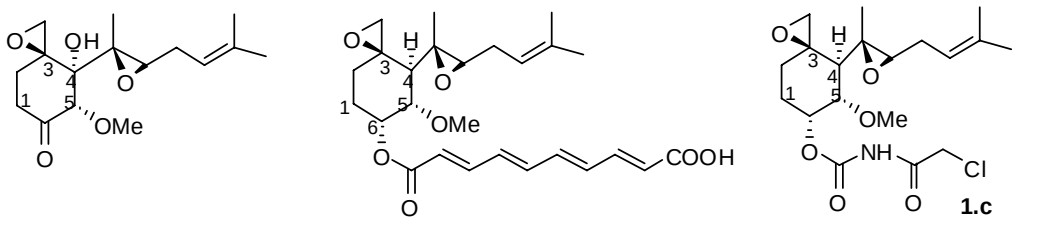
Methionine aminopeptidases belong to the latter family of APs, they selectively and non-processively removed the initiator methionine from the proteins in which the penultimate position is occupied by small and uncharged residues such as glycine, alanine, serine, threonine, valine, cysteine or proline. This specificity observed in cotranslational processing has been an distinguishing feature of the cobalt-containing metallo-enzyme MetAPs and presumably related to many important bioactivities.[2,3] Since the removal of the N-terminal methionine is an inevitable process for the life circle of prokaryotes and eukaryotes, methionine aminopeptidases are widely conserved from bacteria to humans.[4] The localization of methionine aminopeptidase gene on the *Escherichia coli* chromosome indicates that these enzymes are essential for cell growth.[5] MetAPs can be divided into two subfamilies, MetAP-1 and MetAP-2, differentiated by an extra helical subdomain of a 60-peptide residue inserted in the C-terminal domain of MetAP-2 as well as the smaller size of the active sites of MetAP-1 relative to the MetAP-2 enzymes.[6] MetAP-1s are found in prokaryotes, they only have the catalytic domain, the prototypical

examples are *Escherichia coli* MetAP (*Ec*MetAP) and yeast MetAP. MetAP-2s are found in eukaryotes and archea, the representative enzymes of this type are porcine MetAP-2 and human MetAP-2.[2] Both of the two subfamilies of methionine aminopeptidases are capable of removing methionine from nascent proteins and are considered as homologous, the difference may result from the evolutionary distance.[2] Their shared characterizations include catalytic specificity and mechanism (including cobalt dependence) and a similar 3-dimensional fold at the active site.[2, 4]

1.1.2 MetAP-2 Inhibitor Ovalicin and Its Mechanism of Inhibition

Ovalicin (**1.1**) and fumagillin (**1.2**) belong to a family of structurally related natural products isolated respectively from cultures of the fungus *Pseudorotium ovalis* Stolk[7] and *Aspergillus fumigatus*.[8] They are both promising therapeutic agents for treating cancer and other human diseases.[3] TNP-470 (AGM-1470, **1.3**), a synthetic analogue of fumagillin with less toxicity and higher potency, is the most promising compound among the >60 angiogenesis inhibitors in clinical trials to treat a variety of tumors including cervical cancer, pediatric solid tumors, lymphomas, acute leukemias, and AIDs-related Kaposi's sarcoma.[9,10]

Figure 1.1



Ovalicin **Fumagillin** **TNP-470 (AGM-1470)**

Ovalicin, fumagilin and TNP-470 specifically target type 2 Methionine aminopeptidases (MetAP2) and inhibit the enzyme activity by covalently binding to a histidine residue at the active site.[11] Determination of the crystal structure of MetAP2-ovalicin and MetAP2-fumagillin complexes undoubtedly supported this covalent binding and revealed the physiological mechanism at molecular level.[12] The interaction between MetAP2 and ovalicin could be demonstrated by a single-crystal X-ray analysis of fumagallin-MetAP2 complex depicted in

Figure 2.2 due to the similar binding mechanism. The X-ray analysis of the complex shows that the nitrogen atom on imidazole fragment of His231 is covalently linked with the carbon of the spiro-cyclic epoxide. The free oxygen generated from epoxide ring opening is coordinated with adjacent cobalt atom. The epoxide-containing side chain sits in the completed covered pocket near the active site and it affinities with His 331 through hydrophobic contacts at the brink of the pocket. The carboxylic acid side chain points out from the active site and have hydrophobic interaction with Leu^{238} and Leu^{447}.[12]

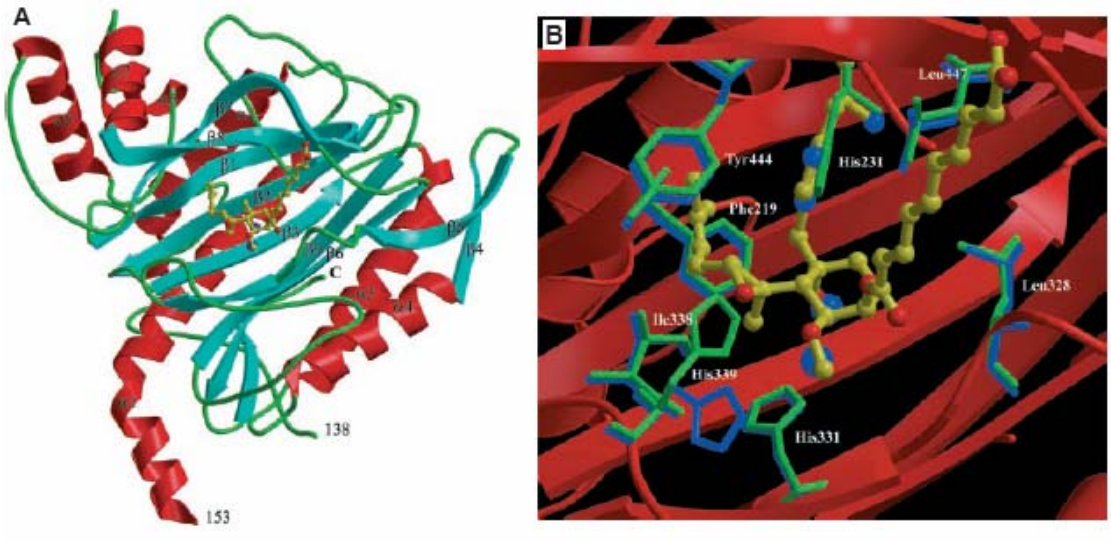
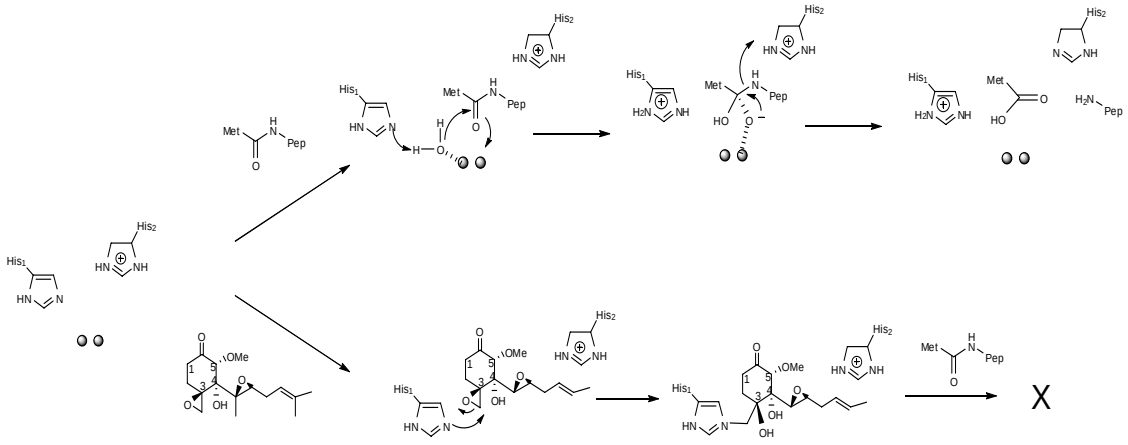


Figure 2.2 (A) overall structure of the complex: hsMetAP2 (red, green, and blue), fumagillin (yellow and red ball and stick) (B) Fumagillin in the active site of HsMetAP2: fumagillin and the covalently attached His231 are depicted as ball and stick with carbon yellow, oxygen red and nitrogen blue. The two cobalt atoms was described as two bigger blue dots.[12]

The inhibition mechanism could be chemically described in Scheme 1.1:[11] during the removal of methionine residue from nascent peptide catalyzed by MetAP2, at least two histidine residues were involved as well as the binuclear cobalt centers. Histidine 231 may serve as a general base to activate a water molecule with the assistance of the coordinated metal ions, the activated water molecule nucleophilically attacked the carbonyl of the terminal methionine to form peptide-metal adduct. The protonated histidine 339 acts as a general acid to facilitate the cleavage of peptide amide bond by protonating the nitrogen and turned it to a good leaving group, release of the oxygen-cobalt coordination eventually removes the methionine residue from the peptide and regenerates the enzyme. On the other hand, when the active site of MetAP2 was

occupied by inhibitor such as ovalicin, the nitrogen atom on imidazole side chain of His-231 nucleophilically attack the epoxide ring and irreversibly linked the compound with the enzyme, thus deactivated the enzyme permanently. Without active enzyme, the terminal methionine of nascent protein can not be removed, thus prevents the protein maturation and eventually inhibit the relevant physiological activities.[11] The growth inhibition or lethality of the deletion or inactivation of all MetAP genes in *E. coli* underscores the physiological importance of the MetAP enzymatic activity.[5]

Scheme 1.1 Illustration of proposed catalytic mechanism of MetAP2 and its inhibition by ovalicin[11]



1.1.3 MetAP-2 Enzyme Inhibition Related Physiological Activity

1.1.3.1 Anti-Angiogenesis

Angiogenesis, an important physiological process related to the growth and development of new capillary blood vessels, occurs in healthy body for wound healing and damaged tissue reparation. Abnormal angiogenesis is a well-acknowledged feature of many proliferative diseases and accumulating evidence shows that progressive tumor growth and metastasis is dependent on angiogenesis. Endothelial cells form the inner lining of all blood vessels and their proliferation plays a vital role in angiogenesis. Proliferated endothelial cells forms new blood

vessels that supply the tumor with oxygen and nutrients as well as provide a passage allowing tumor cell to escape into circulation and lodge in other organs (tumor metastasis).[13,14]

Ovalicin and fumagillin have shown potent anti-angiogenesis activity by specifically inhibit MetAP2 enzymes.[10] The strong correlation between their inhibition of MetAP2 enzyme activity and the inhibition of endothelial cell proliferative growth indicates the inhibition of MetAP2 is likely to be the molecular base for the inhibition of endothelial cell proliferation.[15]

Since angiogenesis is not only critical for the growth of tumors but also a dominant feature in a variety of angiogenesis-related diseases such as diabetic retinopathy, haemangiomas, arthritis and psoriasis,[13,14,16] angiogenesis inhibitors such as ovalicin and its analogues may have great potential in treating these notorious diseases. Ironically, despite their side effect of weight loss in treatment of cancer patients, angiogenesis inhibitors may have potential in treating obesity.[17,18]

1.1.3.2 Anti-parasitic Activities

Sleeping sickness, or "human African trypanosomiasis", is a widespread tropical disease that covers about 36 countries of sub-Saharan Africa and affected over 60 million African people.[19] It is caused by mono-flagellated parasitic protozoa named *Trypanosoma brucei*. The spread of this parasite between mammalian hosts is largely through the bites of infected tsetse flies (*Glossina* genus). The parasite replicates in the blood, passes blood-brain barrier, and invades the central nervous system resulting in changes in personality, alteration of the biological clock (the circadian rhythm), confusion, slurred speech, seizures and difficulty in walking and talking, and eventually death if left untreated.[20] Due to the devastating nature of this kind of parasite, a constant search for new therapeutic agents to kill the parasites and thus restrain this disease is obviously desired.

In the previous research reported by Chiang and his coworkers,[21] fumagilin and TNP-470 were found to block the in vitro growth of malaria parasite *Plasmodium falciparum* and *Leishmania donavani* (a trypanosomal disease caused by species of *Leishmania*). cDNA clone of *Plasmodium falciparum* identified the existence of the pfMetAP2 gene in the parasites and the sequence alignment analysis confirmed this enzyme belongs to the MetAP-2 class of methionine aminopeptidases, with 40% sequence identity with human MetAP2 and 45% identity with yeast MetAP-2. The IC_{50} values for fumigillin against four strain of *P. falciparum* were between 4 and

17 ng/ml, against *Leishmania donavani* was 22 μg/ml, while the average IC_{50} values for TNP470 against four strain of *P. falciparum* were 1.2 ng/ml, against *Leishmania donavani* was 1.3 μg/ml. The effectiveness of fumagillin and TNP-470 against malaria parasite could attribute to their inhibitory potency of pfMetAP2. The inhibition of this enzyme would be lethal to the survival of the plasmodial parasites.[5,22]

Ovalicin, fumagillin and TNP-470 are also effective to inhibit the replication of microsporidia *Encephalitozoon interstinalis* and *Vittaforma corneae,* both single-celled and obligated intracellular parasites, more than 70% in vitro. Although not specified, deletion or inactivation of MetAP enzymes was indicated to be the main responsible factor.[23,24]

Based on these previous anti-parasitic activities, we believe that ovalicin and its anologues may act as broad anti-parasitic agents. Therefore, ovalicin and its analogues and synthetic intermediates were subjected to *in vitro* bioactivity test against *Tryposomal brucei*, parasites causing African sleeping sickness, the result will be discussed in the bio-evaluation part.

1.2 Synthetic Background

(-)-Ovalicin, a sesquiterpene exhibiting antibiotic, anti-tumor and immuno-suppressive activities, was isolated from the culture of the fungus *Pseudorotium ovalis* Stolk.[25] The combination of chemical and X-ray crystallographic techniques revealed the structure and stereochemistry are identical to the structure of lettuce seed germination stimulant graphinone, a metabolite of the fungus *Graphium sp*.[26] The positive observation of a wide range of activities attracted a lot of synthetic chemists' interest to achieve its total syntheses from different chemical approaches.

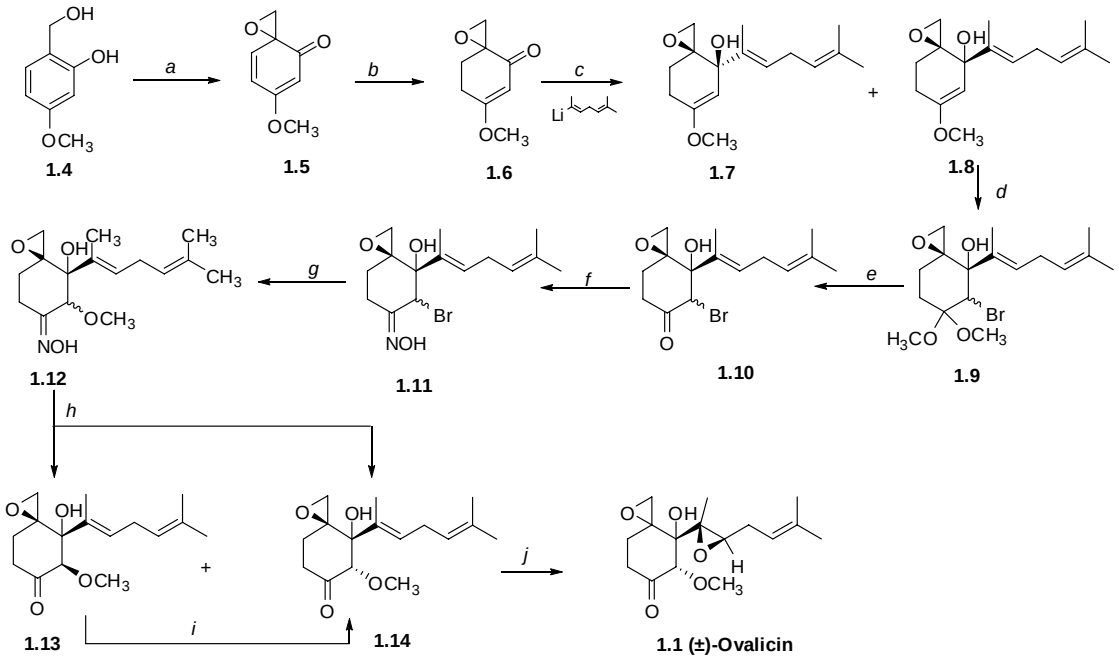
1.2.1 Starting from Cyclic Materials

1.2.1.1 Corey's Approach

The first total synthesis of racemic ovalicin was accomplished by E. J. Corey in 1985 (Scheme 1.2).[27] The cyclohexane ring was derived from Phenol **1.4.** The epoxide ring and ketone on compound **1.5** were introduced in one step by reaction of phenol **4** with aqueous sodium periodate solution in THF. Reduction with *in situ* generated diimide by adding acetic acid solution (1M in dimethoxyethane) to a mixture of **1.5** and potassium azodicarboxylate at 45^{o}C gives epoxy enone **1.6** in 77% yield. Reaction of **1.6** with vinyllithium in toluene at -78^{o}C gives a mixture of diastereoisomers **1.7** and **1.8** (1:17 ratio) which can be separated by silica gel column chromatography. The desired compound **1.8** was treated with *N*-bromosuccinimide (NBS) in methanol to give bromo ketal **1.9** and the corresponding α-bromo α'-enol ether isomer, then the mixture was treated with *p*-toluenesulfonic acid in acetone-water solvent mixture to yield bromo ketone **1.10** in 94%. Bromo ketone **1.10** was converted to bromo oxime **1.11** when treated with hydroxylamine and potassium acetate for one hour at room temperature. Treatment of oxime **1.11** with triethylamine in methanol at room temperature for 2 hours gave methoxy oxime **1.12** with >90% yield. The following hydrolysis of methoxy oxime **1.12** with titanium trichloride in methanol buffered by aqueous ammonium acetate (pH=6, 18 eq) yields a mixture of diastereomers **1.13** and **1.14** (**Scheme 2**). After the treatment of the mixture with potassium carbonate in methanol, **1.13** was completely isomerized to **1.14**. The final epoxidation with vanadyl acetylacetonate and *tert*-butyl hydroperoxide in toluene gave (±)Ovalicin in 89% yield.

This racimic mixture was indistinguishable from an authentic sample by ^{1}H NMR $^{13}CNMR$, IR, MS spectroscopies.

Scheme 1.2

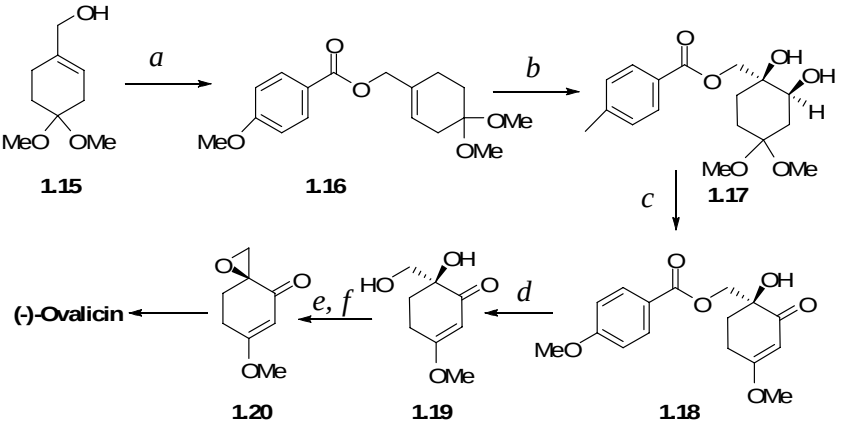


Reagents and conditions: *a*. aqueous sodium periodate(0.3M)/THF, 23°C, 1.5h; *b*. Acetic acid, potassium azodicarboxylate/DME, 45°C, 24h *c,* vinyllithium/ether, -78°C; *d*. NBS/methanol, 0°C; *e*. *p*-toluenesulfonic acid in acetone/water(1.5:1), r.t. 24h; *f*. $NH_2OH{\cdot}HCl$/acetic acid buffer, KOAc; *g*. Et_3N/Methanol; *h*. $TiCl_3$/Methanol, NH_4OAc buffer, r.t. 2.5 h; *i*. K_2CO_3/Methanol, 0°C, 2h; *j*. vanadyl acetylacetonate, *t*-butyl hydroperoxide/toluene, r.t. 2h

Recognized that the racemic ovalicin was resulted from non-optical pure epoxic ketone **1.6**, several years later, Corey et al. modified the original total synthesis and developed an elegant asymmetric syntheis of spirocyclic epoxide **1.20**, which led to (-) ovalicin (Scheme 1.3).[28] Starting with readily available allylic alcohol **1.15**, *p*-methoxylbenzoate ester **1.16** was obtained by treating **1.15** with 1.1 equiv of *p*-methoxylbenzoyl chloride in the presence of triethylamine and DMAP in 98% yield. Asymmetric dihydroxylation of *p*-methoxylbenzoate ester **1.16** with K_2OsO_4 (1 mol %), $(DHQ)_2PHAL$ (1 mol %), K_3FeCN_6, K_2CO_3 and $CH_3SO_2NH_2$ in *t*-BuOH-H_2O mixture gave compound **1.17** after 4 hours in 93% yield and 99% enantiomeric excess. The presence of *p*-Methoxylbenzoyl group in the substrate of this cinchona-OsO4 catalyzed dihydroxylation reaction dramatically enhanced the enantioselectivity. Swern oxidation of **1.17** followed by acid-catalyzed elimination gave the corresponding β-methoxy α,β–

enone **1.18**. Deprotection of **1.18** by K_2CO_3/methanol gave compound **1.19**. Mesylation of **1.19** followed by a S_N2 substitution in the presence of NaOH solution gave the enantiomer **1.20**, a key intermediate for the enantioselective synthesis of (-)-ovalicin. Synthesis of (-) ovalicin from this intermediate **1.20** followed the above reported procedure.[27]

Scheme 1.3



Reagents and conditions: *a* 1.1eq p-methoxybenzoyl chloride, 1.5 eq Et_3N, 0.05 eq DMAP 23°C, 3 hrs *b* 1 mol% K_2OsO_4, 1 mol% $(DHQ)_2PHAL$ biscinchona, 3 eq K_2CO_3, 1 eq $CH_3SO_2NH_2$, t-BuOH-H_2O, 0°C, 4 hrs *c* Swern Oxidation, then catalytic amount p- toluenesulfonic acid/CH_2Cl_2 *d* K_2CO_3/MeOH, r.t. 3 hrs *e* CH_3SO_2Cl, Et_3N/CH_2Cl_2 *f* NaOH/water 5 min

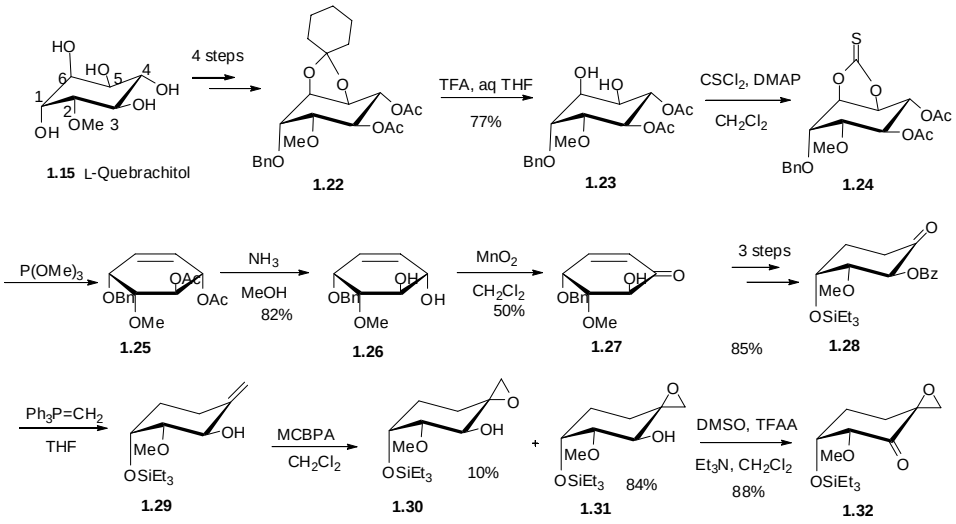
1.2.1.2 Bath-Barton's Approach

The first asymmetric total synthesis of (-)-ovalicin was reported by Bath, Samadi and their co-workers by using commercial available *L*-quebrachitol as a chiral pool[29,30] in 16 steps with an overall yield of 3.5%. This method involved double dehydroxylation at C6 and C5 positions and a Wittig reaction at C4 position (Scheme 1.4).

L-quebrachitol was first transformed to fully protected compound **1.22** in 4 steps. Acidic deprotection of cis-ketal **1.22** gave crystalline diol **1.23**. Treatment of **1.23** with thiophosgene in methylene chloride in the presence of DMAP gave thiocarbonate **1.24**, which underwent a Corey-Winter *cis*-desoxygenation to give compound **1.25**. Deprotection followed by selective oxidation of allylic alcohol **1.26** by using freshly prepared MnO_2 gave α,β-unsaturated ketone **1.27**. Catalytic hydrogenation of **1.27** by palladium/ethanol followed by selective benzoylation at the more reactive α–hydroxyl group and subsequently silylation gave fully protected ketone **1.28**.

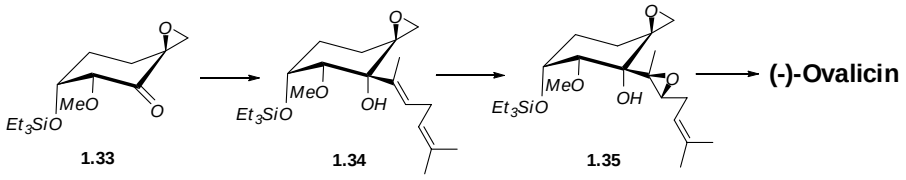
Wittig reaction of the ketone **1.28** allowed the introduction of the spirocyclic epoxide function, which was achieved by epoxidation of **1.29** with *m*-CPBA to give the cis spiro-epoxide **1.31** as the major product. Swern oxidation of **1.31** gave keto-epoxide **1.32**.

Scheme 1.4



The introduction of the side chain is similar to Corey's approach (Scheme 1.5). Vinyllithium was prepared *in situ* and then added into the solution of **1.33** in toluene to give compound **1.34**. Sharpless epoxidation of **1.34** by using tert-butyl hydroperoxide in the presence of vanadyl acetylacetonate gave compound **1.35** as the major product. Final deprotection and oxidation gave (-)-ovalicin.

Scheme 1.5

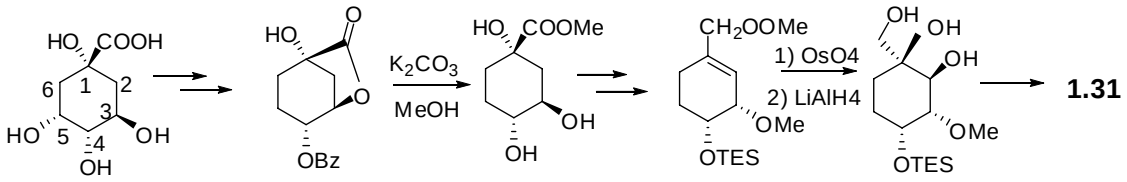


Reagents and conditions: *a.* vinyllithium(prepared *in situ*)/toluene, -78°C; *b.* $VO(acacO)_2$, tert-butyl hydroperoxide *c.* TBAF, THF; *d.* PDC, CH_2Cl_2

Compound **1.31** was also prepared from (-)quinic acid **1.36** after (1) dehydroxylation at C5 (compound **1.36** to compound **1.37**), (2) stereocenter inversion at C3 position by oxidation

and selective reduction and at C1 position by elimination and enantioselective dihydroxylation, (3) mesylation and substitution to form spiro-epoxide and (4) final Swern oxidation (Scheme 1.6)[31].

Scheme 1.6



1.36 **1.37** **1.38** **1.39** **1.40**

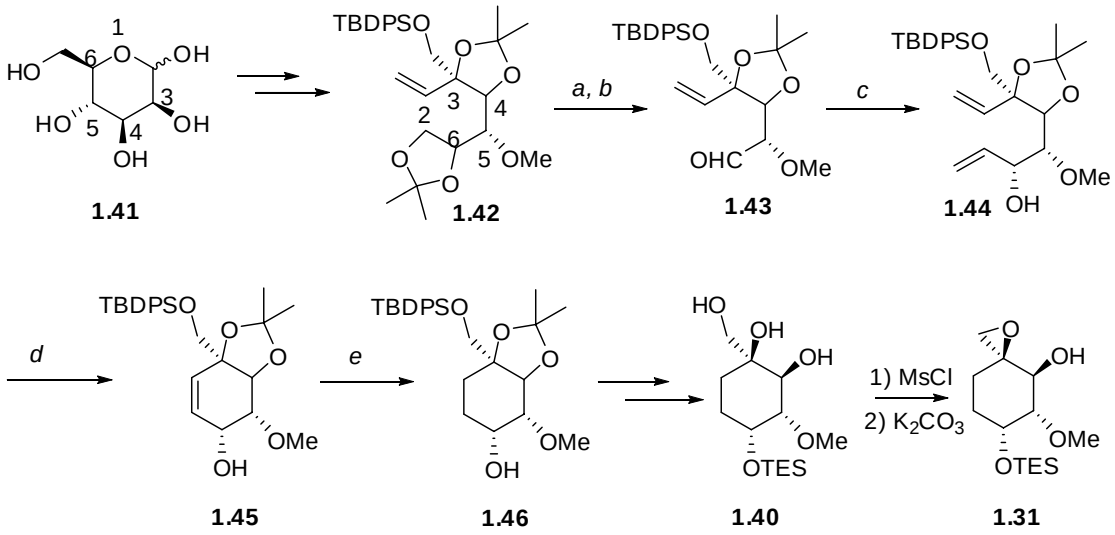
1.2.2 Ring Construction from Acyclic Starting Materials

1.2.2.1 Olefin Metathesis Approach[32]

The key intermediate of chiral epoxide ketone **1.31** in Bath-Baton's total syntheis of (-)ovalicin was also been synthesized from chiral pool compound *D*-Mannose utilizing a ring closing olefin metathesis strategy (Scheme 1.7).

D-Mannose **1.41** was transformed to compound **1.42** after introducing protected methylene alcohol on C3, Wittig reaction of aldehyde on C2 followed by methylation of hydroxyl group on C5. Selective deisopropylidenation of **1.42** and oxidation using sodium periodate result in compound **1.43**. Vinylation of aldehyde **1.43** gave allylic alcohol mixture **1.44**. The undesired alcohol can be transform to desired isomer by Mitsunobu reactions. Ring closing metathesis of **1.44** using Grubbs second generation catalyst gave desired cyclohexene **1.45**, which was hydrogenated to corresponding cyclohexanol **1.46.** Selectively deprotection and protection gave compound **1.40** and then transformed to **1.31** after mesylation and S_N2 cyclization. .

Scheme 1.7

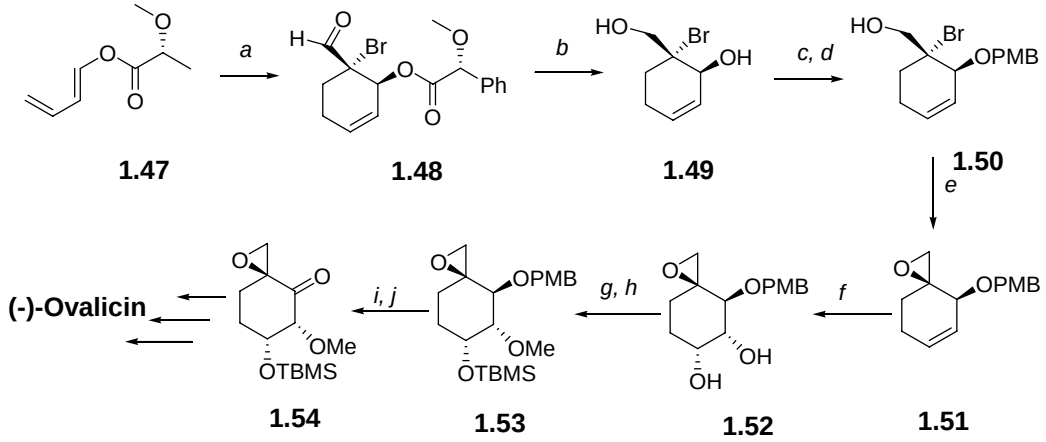


Reagents and Conditions: *a,* aq. AcOH, 50°C, 72%; *b,* $NaIO_4$, THF/H_20, r.t., and then $(CH_2OH)_2$, quant.; *c,* vinylmagnesium chloride, THF, -20°C, 80%, *d,* Grubbs second generation catalyst, toluene, 80°C, 94%; *e,* 10% Pd/c, H_2, EtOAC, r.t., 99%

1.2.2.2 Diels-Alder Approach

Most recently, a catalytic enantioselective Diels-Alder approach was reported to synthesize enantiomer (-)-ovalicin (Scheme 1.8).[33] The key step was the endo-Diels-Alder adduction between 2-bromoacrolein and butadiene **1.47,** which was assisted by a chiral auxiliary on **1.47**. Reduction of **1.48** by using borane-ammonia complex in ethyl ether afforded diol **1.49** with 89% yield. Diol **1.49** was selectively protected by using the para-methoxybenzylidene acetal formation and reduction procedure. The resulting PMB-alcohol **1.50** was converted to spiral cyclic epoxide **1.51** via intramolecular S_N2-type substitution in the presence of sodium hydride in THF/MeOH. The subsequent diastereoseletive dihydroxylation of compound **1.51** was guided by the up-pointing *p*-methoxybenzyl protecting group to give diol **1.52**. The selective protection of less sterically hindered and more reactive equatorial hydroxyl group on C6 by TBDMSCl followed by methylation of C3 alcohol by iodomethane in the presence of sodium hydride in THF gave compound **1.53**. Removal of the PMB group by DDQ in methylene chloride and following oxidation using Dess-Martin periodinane afforded the key intermediate **1.54**, which was transformed to optical (-)-ovalicin using the same procedure described above.

Scheme 1.8



a, 2-bromoacrolein, BF_3-OEt_3, CH_2Cl_2, -78°C, 75%; *b.* BH_3-NH_3, Et_2O, 89%; *c.* p-$MeOC_6H_4CH(OMe)_2$ CSA, CH_2Cl_2, 89%; *d.* DIBAL-H, CH_2Cl_2, -10°C, 94%; e. NaH, THF, MeOH, 98%; f. 1 mol%OsO_4, NMO, acetone/H2O, 92% *g*. TBDMSCl, imidazole, CH_2Cl_2, 89%; *h*. NaH, MeI, THF, 99%; *i.* DDQ, CH_2Cl_2, H_2O, 89%; *j.* Dess-Martin periodinane, $NaHCO_3$, CH_2Cl_2, 92%

1.3 Synthesis of (±)-Ovalicin via Intramolecular Heck Reactions (Research in Hua's Lab)

As mentioned above, ovalicin was observed as an non-toxic, non-inflammatory and more potent anti-angiogenesis agent than fumagillin,[28] the potency was the same as that of the most active analogue, TNP-470, which encountered some problem in the clinical trial.[34] Although there are several synthetic methods already reported, synthesis with more flexibility for analogue preparation is still highly desired for the future compound screening of their known and unknown therapeutic potentials. In connection with our studies on a modified asymmetric Heck reaction applied to screen a family of asymmetric phosphine ligands,[35] we realized that intramolecular Heck reaction could be applied to construct the cyclohexane framework with functionalities for further structural transformations.

This project was initiated by Srinivas K. Battina[36] with assistance of Anna Jimenez.[37] Kaiyan Lou contributed a lot to this project. Their work was briefly summarized in the following paragraphs.

1.3.1 Summary of previous work

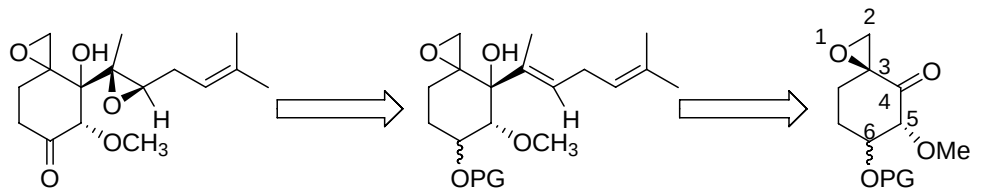
1.3.1.1 Retrosynthesis

The retrosynthetic analysis of (±)-ovalicin (**1**) was described in Scheme 1.9 and Scheme 1.10. We planned to synthesize ovalicin by introducing 1,5-dimethylhexa-1,4-dienyl side chain to ketone **1.56**, a key intermediate reported to form compound **1.55**.[27,30] It was initially expected that the addition of dieny side chain could be stereoselectively from the *beta*-face due to the steric effect of methoxyl group on C5 position. PG (protecting group) could be benzoyl or TBDMS group.

Structurally similar compounds of **1.56** were reported by fuctionality transformations of cyclic starting materials such as substituted phenol,[27,28] natural chiral pool compounds with defined stereochemistry,[29,31] or by constructing the ring from acyclic compounds via olefin metathesis[32] or Diels-Alder reaction.[33] In this lab, a novel strategy of intramolecular Heck reaction was applied to obtain the cyclic diene **1.57** from acyclic alkenyl iodide **1.58** (Scheme 1.10). Transformation from **1.57** to **1.56** can be accomplished via a selective epoxidation of the

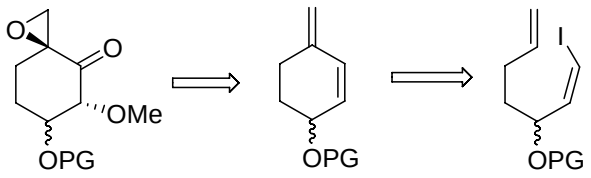
more labile exo-methylene function of diene **1.57** followed by dihydoxylation and subsequent functionility transformations.

Scheme 1.9



1.1 (±)-Ovalicin **1.55** **1.56**

Scheme 1.10

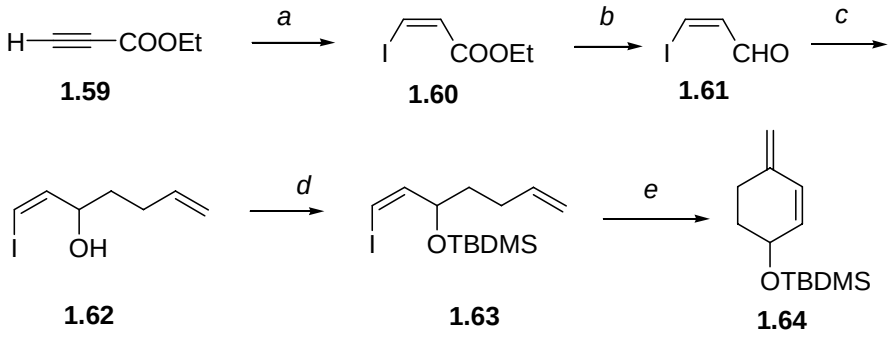


1.56 **1.57** **1.58**

1.3.1.2 3-Methylene-6-(tert-butyldimethylsilyloxy) cyclo hexene (1.64)

Alkenyl iodide **1.60** was readily synthesized from commercial available ethyl propiolate **1.59** by a sequence of reaction depicted in Scheme 1.11. Hydroiodination reaction of **1.59** with sodium iodide in acetic acid at 70 ^{o}C for 12 hours gave (Z)-ethyl 3-iodoacrylate (**1.60**) with 89.2% yield after purification of fractional distillation. The reduction reaction of iodoacrylate **1.60** by using diisobutylaluminium hydride (DIBAL-H) at -78 ^{o}C gave (Z)-iodoacrylaldehyde (**1.61**). Adding freshly prepared Grignard reagent slowly via cannula to a THF solution of **1.61** at -78 ^{o}C gave iodoalcohol **1.62** without any stereoselectivity in 74% yield. Silylation of alcohol **1.62** with *t*-butyldimethylsilyl chloride almost quantitatively gave silyl ether **1.63**. The intramolecular Heck reaction was carried out by treating silyl protected iodo compound with palladium acetate, silver phosphate, triphenylphosphine and proton sponge in dimethylforamide (DMF) at 50 ^{o}C for 7 hours, which gave 93% yield of cyclized product **1.64**.

Scheme 1.11

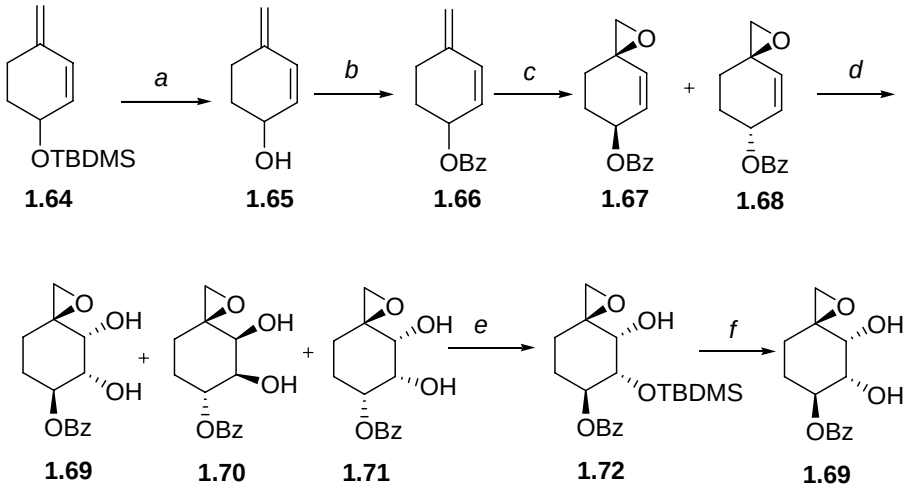


Reagents and conditions: *a*. AcOH, NaI, 70^{o}C, 12hr *b*. DIBAL-H, CH_2Cl_2, -78^{o}C, 45 min *c.* 3-butenylmagnesium bromide,-78^{o}C *d.*TBDMSCl, imidazole, DMAP, DMF, 0^{o}C- r.t, 12 hr.; *e*. $Pd(OAc)_2$, Ph_3P, Ag_3PO_4, proton sponge, DMF, 50^{o}C, 7 hr

1.3.1.3 (3S*,4R*,5S*,6S*)-6-(benzoyloxy)-1-oxaspiro[2.5]octane-4,5-diol

Initially, m-CPBA was used to oxidize the more labile external double bond to spiro epoxide, but the result was not encouraging, little or none of the desired spiro epoxide was obtained. This may due to the unstability of acid-sensitive silyl group in the presence of in situ generated *meta*-chlorobenzoic acid. In order to avoid this problem, silyl protected **1.64** was transformed to benzoyl protected **1.66**. This benzoyl protected compound (**1.66**) was successfully epoxidized by m-CPBA to a mixture of two inseparable epoxides **1.67** and **1.68**. Because the resulting epoxides were not very stable at room temperature, they were not purified and were directly treated with osmium tetraoxide (OsO_4) and N-methylmorpholine-N-oxide (NMO) in a solution mixture of *tert*-butanol and acetone-water system. Three inseparable diastereomers **1.69**, **1.70** and **1.71** were formed in 5:4:1 ratio based from ^{1}H NMR of the crude material. The mixture of three diols was treated with *tert*-butyldimethylsilyl chloride (TBDMSCl) expecting the possible separation. Fortunately, the major compound was isolated and identified by NMR spectrum (^{1}H NMR, ^{13}C NMR) and X-ray crystal analysis as **1.72**. Since the hydroxyl group on C5 needs to be methylated in the final product, compound **1.72** was then desilylated back to diol **1.69** (Scheme 1.12).

Scheme 1.12

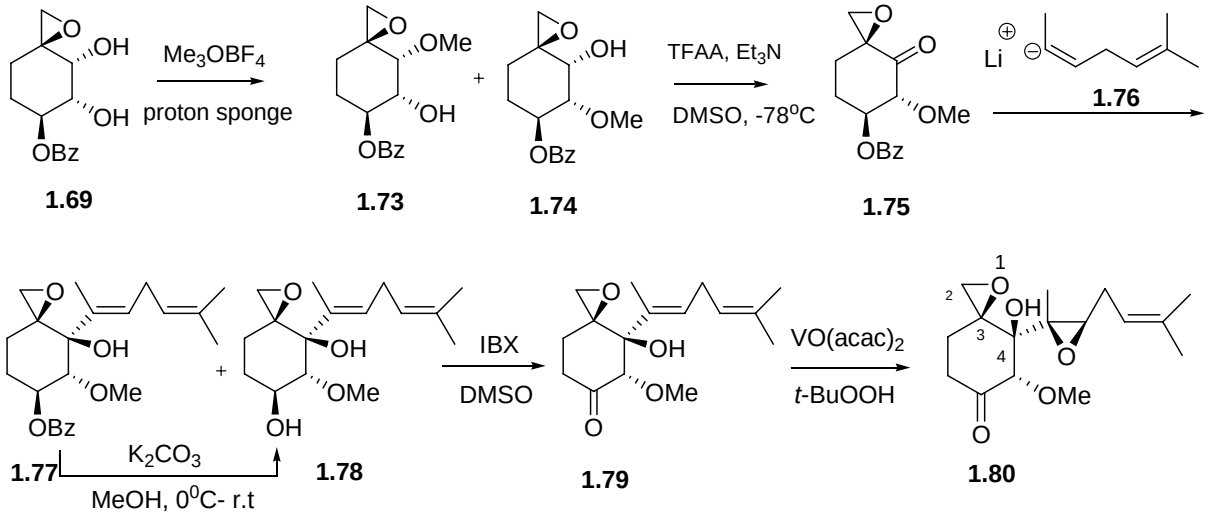


a. TBAF, THF, 0^0C, 10 hr *b.* C_6H_5COCl, Et_3N, 0^0C - r.t, 10 hr *c.* MCPBA, NaF, KF, CH_2Cl_2 *d.* NMO, OsO_4, *t*-BuOH, acetone-H_2O *e*. TBDMSCl, DMAP, Et_3N, 0^0C-r.t, 6 hr *f*. TBAF, THF, 0^0C,7hr

1.3.1.4 (3S*,4S*,5R*,6S*)-4-(2'-Methyl-3'-(3''-methylbut-2''-enyl) oxiran-2'-yl)-5-methoxy-6-oxo-1-oxaspiro[2.5]octan-4-ol (1.80)

Diol **1.69** was methylated unselectively with trimethyloxonium tetrafluoroborate (Me_3OBF_4) in the presence of proton sponge in methylene chloride. The resulting mixture of methylated compounds **1.73** and **1.74** was separated by column chromatography. Treatment of **1.74** with TFAA in the presence of triethylamine in DMSO (Swern oxidation) gave ketone **1.75** after flash column chromatography. Compound **1.75** reacted with freshly prepared alkenyl lithium **1.76** to give adduct products **1.77** (15%) and **1.78** (10%), both of which had wrong stereochemistry at C4 position. No other isomer was isolated and identified. Compound **1.77** was readily deprotected to give alcohol **1.78** by K_2CO_3 in methanol. Oxidation of compound **1.78** by IBX in DMSO gave ketone **1.79**. The selectively side chain epoxidation using vandyl acetoacetonate ($VO(acac)_2$) and *t*-butyl hydroperoxide in toluene at 0^oC gave compound **1.80**. Crystal X-ray analysis suggested that it is a C4 stereoisomer of ovalicin. The failure transformation from ketone **1.75** to desired ovalicin indicated that the stereochemistry of benzoyl group at C6 is the controlling factor of the addition reaction. We assume that because benzoyl group can rotate freely around C6-O axis, the *beta*-face of compound **1.75** was intermittently blocked, which resulted in bigger steric effect than that of methoxyl group on C5.

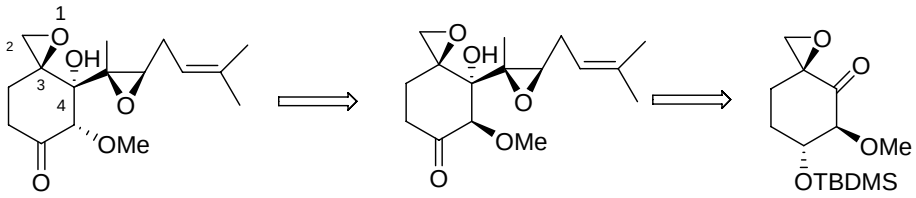
Scheme 1.13



1.3.1.5 Other Attempt Towards (±) Ovalicin

Although we could transform diol **1.70** and **1.71** to their corresponding ketones following the same synthetic route which convert diol **1.69** to ketone **1.75** and then introduce the side chain to see if we could get the desired (±) Ovalicin or not, we were not allowed to do so because of the difficulty related to isolation and identification of these two compounds. However, when we applied another epoxidation method (discussed later) at neutral conditions without changing the protecting group from TBDMS to benzoyl, we did obtained the similar diol which can be transformed to ketone **1.75**; we thought that the much more bulky TBDMS group should block the *alpha*-face and force the alkenyllithium side chain to attack the ketone from the *beta*-face, which could result in the right stereochemistry at C4 position (Scheme 1.14). Although this will give us the opposite stereochemistry at C5 position, it could be transformed to desired one according to E. J. Corey's procedure（compound **1.13** was isomerized to compound **1.14** when treated with K_2CO_3 in methanol）.[27]

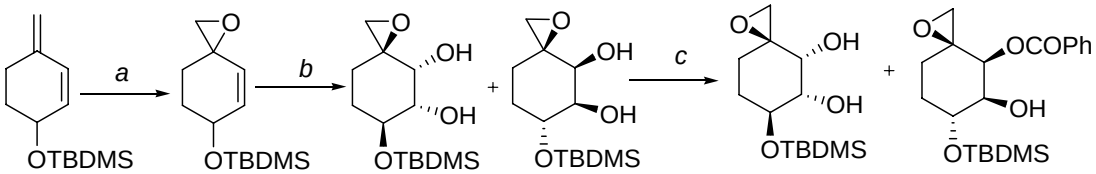
Scheme 1.14



1 **1.81** **1.82**

Therefore, our next effect was focused on the synthesis of ketone **1.82**. Diene **1.64** was epoxidized successfully by neutral oxidizing reagents dioxiranes (generated in situ by trifluroacetone and oxone) to give spiro epoxides. Since spiro epoxide **1.83** was unstable, it was treated with OsO_4 directly to dihydroxylate the internal double bond. The stereochemistry of the addition products was controlled by the adjacent bulky OTBDMS; the OsO4 had to approach the double bond from the opposite side of the OTBDMS group, which finally resulted in inseparable diol **1.84** and **1.85.** Luckily enough, when Kaiyan Lou treated the mixture with benzoyl chloride (C_6H_5COCl) in methylene chloride in the presence of triethylamine for two days, the result (by NMR) showed that only one diol was protected (**1.85**) and the other remained unreacted (**1.84**). These protected and unprotected compounds have big R_f value difference and can be separated easily by using silica column chromatography (Scheme 1.15).

Scheme 1.15

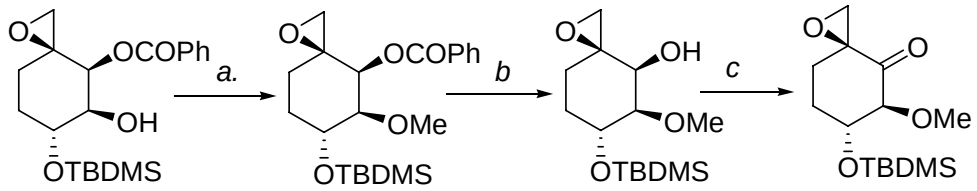


1.64 **1.83** **1.84** **1.85** **1.84** (unreacted) **1.86**

Reagents and conditions: *a.* EDTA, $NaHCO_3$, CH_3COCF_3, Oxone, THF, CH_3CN
b. OsO_4, NMO, *t*-BuOH, Acetone-H_2O *c.* PhCOCl, Et_3N, THF, r.t. 48h

The transformation of **1.86** to **1.82** can be described as following (Scheme 1.16): methylation of the remaining hydroxyl group on C5 using trimethoxyoxium tetrafluorideborane as methylating agent and proton sponge as base at 0 ^{o}C overnight gave compound **1.87** with 85.5% yield. Treatment of **1.87** by potassium carbonate in methanol at 0 ^{o}C quantitatively gave compound **1.88** (Isolation yield: 86.1%). Oxidation of **1.88** by IBX in DMSO gave ketone **1.82** with 70.0% yield after flash column chromatography.

Scheme 1.16



1.86 **1.87** **1.88** **1.82**

a. Me3OBF4, proton sponge, CH2Cl2, 0^{o}C, 48 h; *b*. K2CO3, MeOH, 0^{o}C, 10 h;
c. IBX, DMSO, r. t., 12 h.

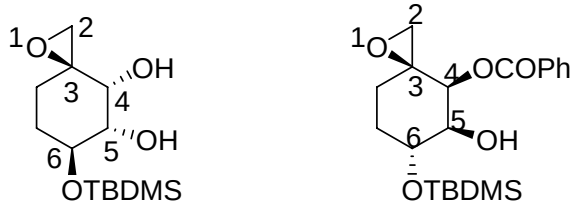
Following the procedure reported by E. J. Corey, the vinyl dialkene side chain was tried to introduce to ketone **1.82** a couple times, but the result was not so encouraging, the yield of this addition reaction is very low and in many cases, no desired product was obtained. Considering the reoccurrence of this procedure in literature, this method should be reliable. Since this problem of low yield was also observed in the side chain introduction to ketone **1.75** in this lab, it should most likely associate with the quality of intermediates or reagents. Therefore, extreme caution should pay to the purity of ketones and the quality of the freshly prepared alkenyllithium **1.76**.

1.3.2 Continuing Research

1.3.2.1 Structure and Conformation Determination of Two Diols

Although we had assigned the structures of compound **1.84** and **1.86** based on proton NMR (Figure 1.3), further spectra evidences are highly desired in order to assure the structure assignment. 2D NOESY was applied here on the expectation that if diol **1.84** was the one reacted, there shouldn't be any correlation between down-pointing proton on C2 position and up-pointing proton on C4 position because of the long distance between them; if diol **1.85** was the one reacted with benzoyl chloride, there should be correlation between one of the hydrogens on C2 and the one on C4 position because of the short distance between two down-pointing protons.

Figure 1.3

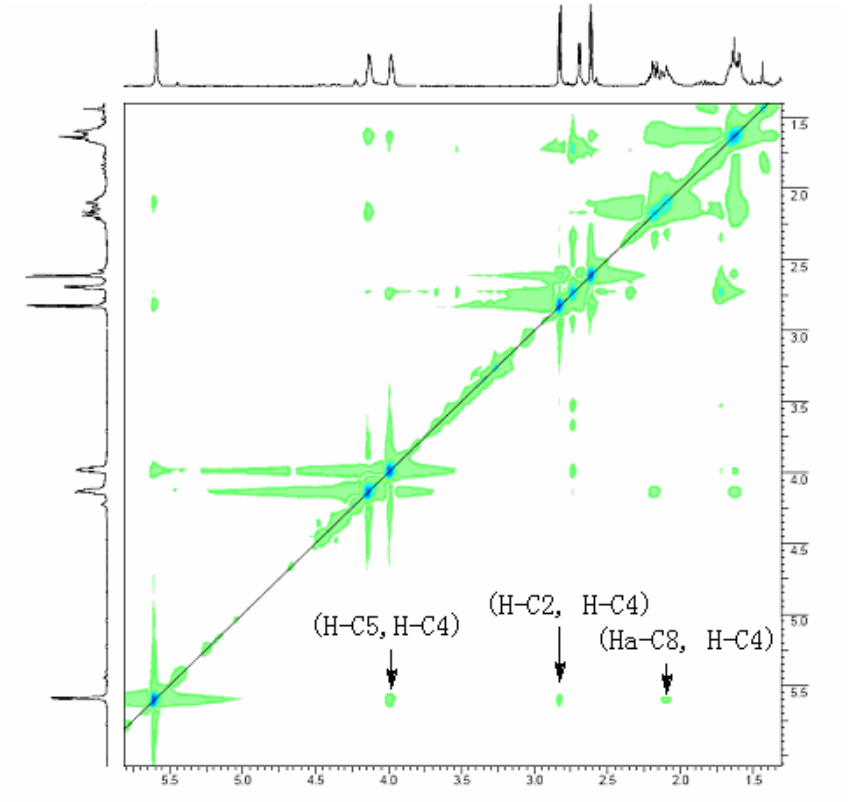


1.84 **1.86**

First, we took 2D NOESY spectroscopy of compound **1.86** (Figure 1.4) because of the big chemical shift difference among the protons on C2, C4, C5 and C6. Signal at δ 5.62 was assigned to C4-H because of the benzoyloxy group on C4 position. The small coupling constant (3 Hz) could help in locating the proton on C5 position, which should have a same small coupling constant with C4-H. However, it was not so easy to determine the coupling constants of the signals at 4.15 and 3.99, both of which seemed having a big coupling constant and a small coupling constant. From the peak splitting pattern, we attempted to assign the signal at δ 4.0 as C5-H because of the quartet shape and the signal at δ 4.15 to C6 proton because of the more complicated splitting pattern. 2D NOESY could approve this assignment right or wrong because only C5-H has correlation with C4-H. Signals at δ 2.8 (doublet, J = 4.4 Hz) and δ 2.6 (doublet, J = 4.4 Hz) were corresponding to protons C2 position. From 2D spectroscopy in Figure 5, the clear correlation peak at (5.62, 3.99) verified that the signal at 3.99 belongs to C5-H. It also clearly showed correlation peak at on the cross point of (δ 5.6, 2.8), which strongly indicated that

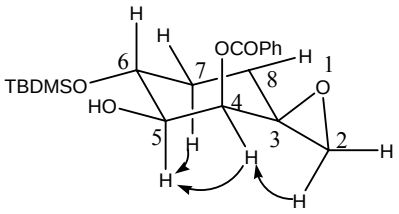
diol **1.85** was the one reacted with benzoyl chloride, so the structure of mono-protected diol was assigned as **1.86**.

Figure 1.4

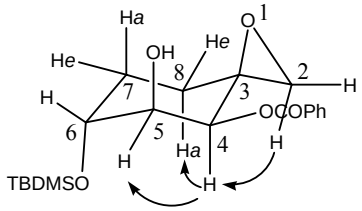


Previously, we assumed that the conformation of **1.86** is **1.86a** with the bulky TBDMS group on the equatorial position (Figure 1.5), but this conformation can not explain the correlation peak at (δ 5.6, 2.1). C4-H should only have two correlating protons on C2 and C5. Conformation **1.86b** can explain the correlation peak at (δ 5.6, 2.1), it could be the correlation between two *axial* protons on C4 and C8, but this conformation put the bulky TBDMS group on the *axial* position, which is thermodynamically unstable. Although this conformation is unusual, we found support from literature.[33] Moreover, we can trace this conformation back to diol **1.85** based on the reactivity difference between the two hydroxyl groups: when TBDMS group occupies the *axial* position on C6; the hydroxyl group on C4 position of **1.85** has to occupy the less hindered and more reactive equatorial position, and this perfectly explained why diol **1.85** readily reacted with benzoyl chloride at room temperature.

Figure 1.5



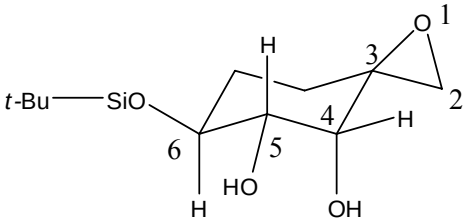
1.86a



1.86b

After the determination of compound **1.86**, along with ^{1}H NMR and ^{13}C NMR, the structure of unreacted diol was assigned as compound **1.84**. ^{1}HNMR shows a doublet at δ 3.2 with a small *J* value of 3.0, which should be the proton on C4, and two mutiplet at δ 3.7 and 3.8. Although it is not so easy to determine the coupling constants, both of the mutiplet looks like having a big coupling constant (*axial–axial* coupling) and a small coupling constant (axial-equatorial coupling). Therefore the conformation of **1.84** should be the one shown in Figure 1.6. The reactivity of this compound further support the conformation assignment that put the hydroxyl group on C5 on equatorial position: when this compound compete with diol **1.85** at room temperature with 1 equivalent of benzoyl chloride, the less reactive C4 hydroxyl group was not active enough at room temperature while the more reactive hydroxyl group was too hindered to react because of the adjacent bulky TBDMS group; moreover, when this compound reacted with 1 equivalent of benzoate cyanate or acetal anhydride at elevated temperature, the major protected hydroxyl group was the equatorial one on C5 postion.

Figure 1.6

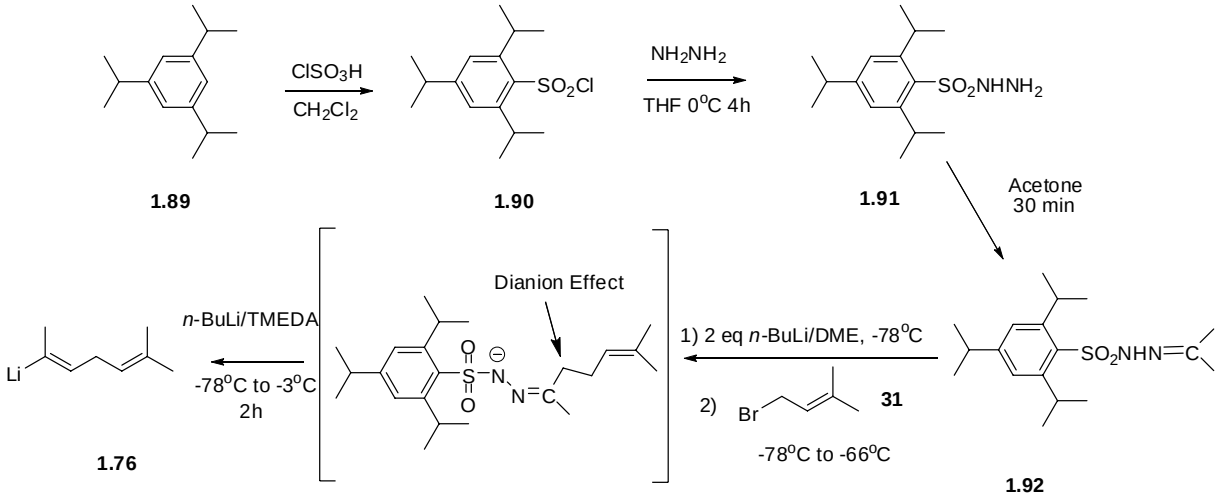


1.3.2.2 Synthesis of Alkenyllithium

We believe that the quality of benzenesulfonyl hydrazone **1.92** directly determine the result of side chain addition, it is important to make sure every intermediate is pure enough (Scheme 1.17). Treatment of 2,4,6-Triisopropyl benzene **1.89** with chlorosulfonic acid at 0^{o}C for one hour gave 2,4,6-triisopropyl benzenesulfonyl chloride **1.90**. In stead of column chromatography, it was purified by recrystallization using solvent mixture of prtroleum:ether (4:1) with 65% yield. A solution of 2,4,6-Triisopropyl benzenesulfonyl chloride **1.90** in THF was treated with distilled hydrazine at 0^{o}C for 4 hours to afford 2,4,6-Triisopropyl benzenesulfonyl hydrazine **1.91**. In order to avoid purification in next step, the resulting compound **1.91** was purified by recrystallization using ether as solvent. Treatment of 2,4,6-triisopropyl benzenesulfonyl hydrazine **1.91** with freshly distilled acetone for half an hour exclusively afforded benzenesulfonyl hydrazone **1.92** after simply evaporating excessive acetone and vacuum-dried overnight. This white powder was pure enough to be used in the next step of Shapiro reaction to afford the crucial alkenyllithium.[38,39]

The preparation of alkenyllithium was achieved by applying the method developed by E. J. Corey.[27] The reaction could be simply monitored by color change. Two equivalents of *n*-BuLi were added to a solution hydrazone **1.92** in dry dimethoxyl ether at -78 ^{o}C, the color should turn light yellow because of the formation of dianion on nitrogen and terminal carbon. Subsequent addition of allylic bromide made the yellow solution a little bit thicker (the carbon anion attacked the allylic bromide). After stirred at -23 ^{o}C for 1 hour, N,N,N',N'-Tetramethylethylenediamine (TMEDA) was added, followed by another equivalent of *n*-BuLi at -78 ^{o}C. At this stage, it was very important to see the color turned into orange. This was the indication of formation of second dianion intermediate. Then the temperature was slowly warmed to -3^{o}C from -78^{o}C to complete the transformation towards compound **1.76**. Since all of the above intermediates are sensitive to moisture and air, every operation was carried out under argon. Addition of alkenyllithium **1.76** to ketones was performed right after the reagent was prepared.

Scheme 1.17



1.3.2.3 (3S*,4S*,5S*,6S*)-4-(2'-Methyl-3'-(3''-methylbut-2''-enyl)oxiran-2'-yl)-5-methoxy-6-(tert-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-4-ol

Introduction of side chain to ketone **1.82** was achieved by adding freshly prepared alkenyllithium **1.76** to the solution of **1.82** in toluene at -78 ^{o}C and stirred at this temperature for 3 hours before warming to -3 ^{o}C (Scheme 1.18). Crude ^{1}H NMR showed that this addition was not stereospecific, vinyl anion attacked ketone **1.82** from both up and down sides and gave a mixture of isomers of **1.93** and **1.94** in a ratio of 1:1 (determined from the crude NMR show in Figure 1.7) with a total yield of 75%. The signal at δ 6.0 was assigned to the proton on C2' position (on side chain) of compound **1.94**, while the signal at δ 5.43 was assigned to the proton on C2' position of compound **1.93**. The overlapped signals at ~5.1-5.2 were assigned to the two protons on C4' positions of compound **1.93** and **1.94**. These assignments were consistent with that reported in the literature.[30,33] The chemical shift difference between the protons on C'2 positions of two isomers may attribute to the effect of lone pairs electrons on oxygen of spiro epoxides. When both of the epoxide and side chain are pointing upwards (**1.93**), the lone pair electrons on epoxide may push the π electrons on nearby double bond towards C2' postion, which put the proton on C2' position in more electron-rich environment and thus shifted to up-field. When the epoxide points upward while the side chain points downward (**1.94**), there is no such effect on the proton on C2' and it will appear down-field shifted. This mixture was not

separable by column chromatography. Removal of the the silyl protecting TBDMS group using TBAF gave two separabe alcohols **1.95** and **1.96** by using petroleum ether and ethyl actate (2:1).

Scheme 1.18

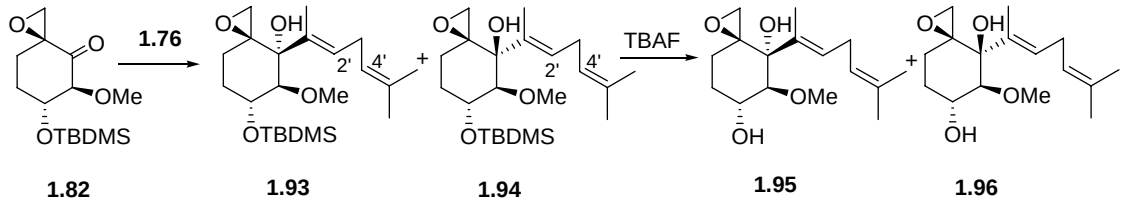
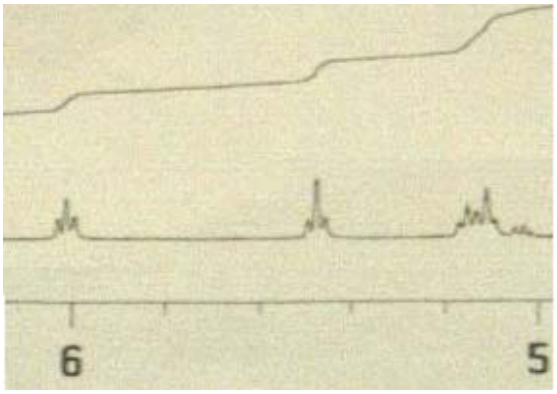
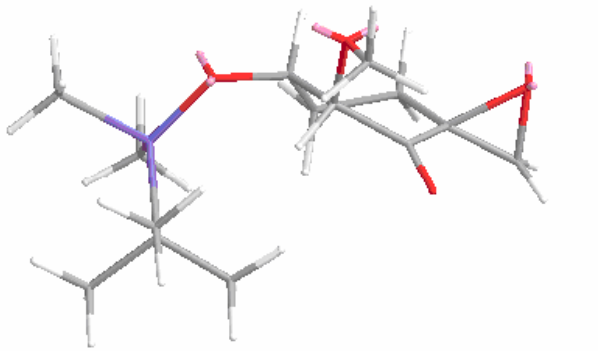


Figure 1.7. The expanded crude NMR of vinyl anion addition to ketone **1.82** (δ 5~6).



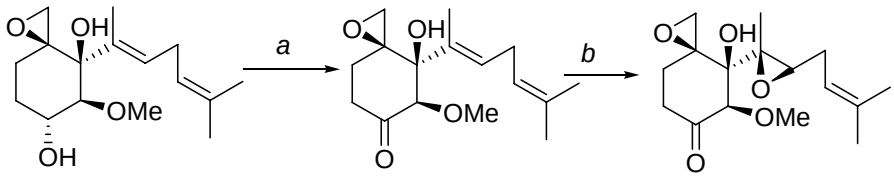
The result indicates that selectivity of side chain addition could be determined by two factors (Figure 1.8): the first one is the steric effect of silyl group, because the bulky silyl group can rotate around the oxygen-silicon axis, the *alpha*-face is blocked intermittently (when the t-butyl group rotate below the cyclohexone ring, it blocks the *alpha*-face, when it rotate away from the cyclohexone ring, the *alpha*-face is open to nucleophilic attack). The second one is the static effect caused by methoxyl group and cyclic epoxide, the lone pair electrons on methoxyl and epoxide functional groups act as a threshold again the neclophilic attack from the *beta*-face because of the static repulsion. However, when the bottom face is blocked by t-butyl group, the vinyl anion has to attack from the *beta*-face by breaking through the static threshold. The addition products indicate that these two factor played equal roles in determining the addition direction.

Figure 1.8 Energy minimized conformation of ketone **1.82**



Similarly, oxidation of the major alcohol isomer **1.96** using IBX gave ketone **1.97**, which was converted to ovalicin analogue **1.98** by Sharpless epoxidation using $VO(acac)_2$ and t-Bu-OOH (Scheme 1.19).

Scheme 19



1.96 **1.97** **1.98**

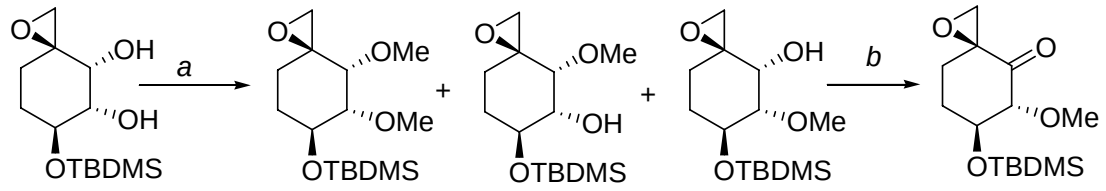
a. IBX / DMSO. *b.* Vanyl acetoneacetonate, t-BuOOH, toluene

1.3.2.4 (3S*,4S*,5R*,6S*)-4-(2'-Methyl-3'-(3''-methylbut-2''-enyl) oxiran-2'-yl)-5-methoxy-6-oxo-1-oxaspiro[2.5]octan-4-ol

Since TBDMS protected compound diol **1.84** has the same stereochemistry as benzoyl protected diol **1.69**, if it was transformed to ketone **1.102**, then introduce the side chain, the result maybe indicate why the yield of addition of vinyllithium **1.76** to ketone **1.75** was so low and if the protecting group is the major controlling factor for the addition reaction. Because the equatorial hydroxyl group on C5 is more reactive than the C4 axial hydroxyl group, C5 methylation was carried out before C4 protection. Unfortunately, among the conditions and reagents tried in this lab by Srinivas Battina, only the one using proton sponge as base and Me_3OBF_4 as methylation reagent gave desired product and this method was not selective; a

mixture of **1.99**, **1.100** and **1.101** was obtained at a ratio of 1:4:4 (Scheme 1.20) from crude NMR. The reason we didn't exclusively get compound **1.101** could be that hydroxyl group on C5 (equatorial position) is more reactive but also more hindered while the hydroxyl group on C4 is less reactive but also less hindered. Since one of our objectives is to synthesize ovalicin analogue, we didn't try other methods to selectively methylate the C5 hydroxyl group and compound **1.99** and **1.100** may lead to ovalicin analogues (discussed later). These compounds can be separated by subjecting to silica gel using petroleum ether and ether as eluting solvent. The structural assignment of compound **1.100** and **1.101** was base on 2D NOESY. We found that the less polar isomer had a correlation at (δ 3.4, 2.9) between the methoxyl proton on C4 and methylene proton on C2, so the structure of this compound was assigned as **1.100** (Figure 1.9). We didn't find any correlation between the methoxyl proton and methylene proton of the more polar isomer (**1.101**). The following oxidation of **1.101** by using IBX in DMSO exclusively gave ketone **1.102**, which was not purified and used directly in next step (Scheme 1.20).

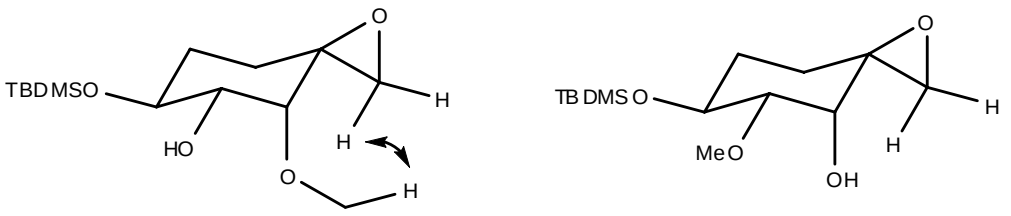
Scheme 1.20



1.84 **1.99** **1.100** **1.101** **1.102**

a. Me_3OBF_4, proton sponge, CH_2Cl_2, 0^oC; *b*. IBX / DMSO

Figure 1.9



1.100 **1.101**

Introduction of the side chain to ketone **1.102** was similar to that of ketone **1.82** (Scheme 1.21). Freshly prepared vinyl lithium **1.76** was added to the solution of **1.102** in toluene at -78 ^{o}C and stirred at this temperature for 3 hours before warming to -3 ^{o}C. This addition might give a mixture of two isomers in 73% yield with compound **1.104** as the major product (1:4) (Figure 1.10). This result showed that the bulky group at C6 position is the major controlling factor for the addition on C4 position. The proton assignment was based on the literature[29,33] of the chemical shift of proton on C2' of alkenyl side chain. We are not so sure if we should assign the chemical shift at δ ~5.42 to proton on C2' position of compound **1.103**, because it should give a triplet with a similar splitting pattern as compound **1.104** instead of a singlet-like peak. However, removal of the silyl protecting group using TBAF gave two separable alcohols **1.105** and **1.106**, which indicate the chemical shift at δ ~5.42 may belong to proton on C2' position of compound **1.103**.

Scheme 1.21

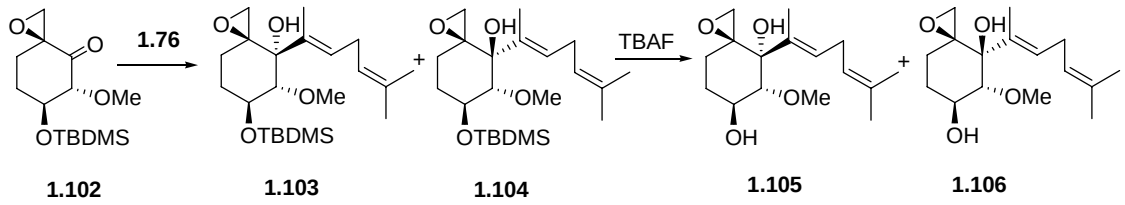
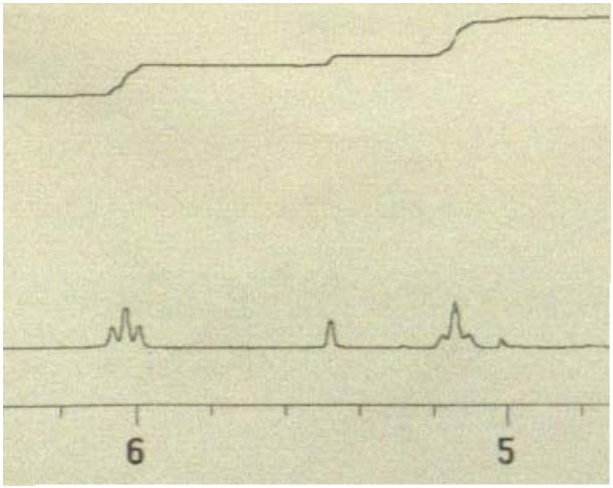
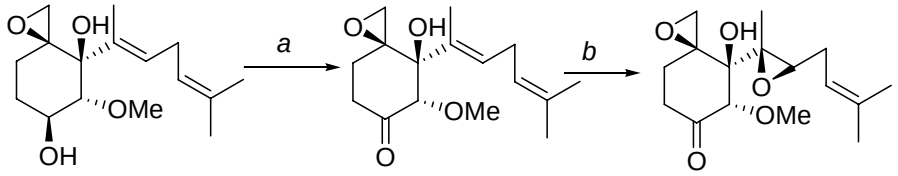


Figure 1.10 The expanded crude NMR of vinyl anion addition to ketone **1.102** (δ 5~6).



Oxidation of alcohol **1.105** using IBX in DMSO gave ketone **1.79**, which was converted to ovalin analogue by epoxidation using $VO(acac)_2$ and t-Bu-OOH (Scheme 1.22). This product had exact ^{1}HNMR and ^{13}CNMR as that of compound **1.80** obtained from another route by Battina, whose structure was confirmed by X-ray spectroscopy.

Scheme 1.22



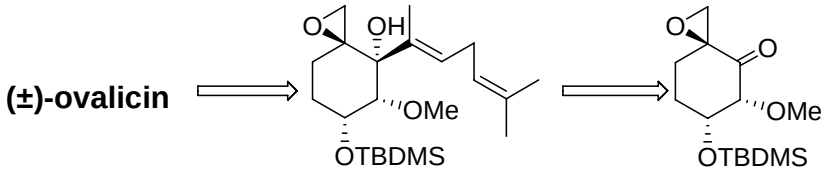
1.105 **1.79** **1.80**

a. IBX / DMSO. *b.* Vanyl acetoneacetonate, t-BuOOH, toluene

1.3.2.5 Transformation towards Compound 1.54

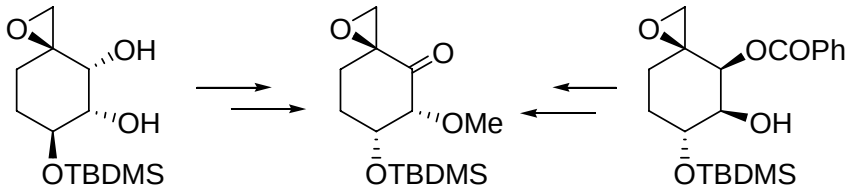
From our failed attempts and information from literature,[29,30,33] we can conclude that if both methoxy and OTBDMS groups are *cis* to each other and *anti* to the spiro epoxide functionality, the *alpha*-face was totally blocked by methoxy and OTBDMS groups ((Scheme 1.24, Figure 1.11), the addition of the side chain should be stereoselective, since the alkenyl anion (side chain) have to attack the ketone **1.54** from the unblocked *beta*-face, resulting in only one compound that has the right stereo-chemistry at C4 position. So the next effect was focused on the transformation of compounds **1.84** and **1.86** to compound **1.54** by inverting the stereo center at C5 position or C4 position, respectively.

Scheme 1.23



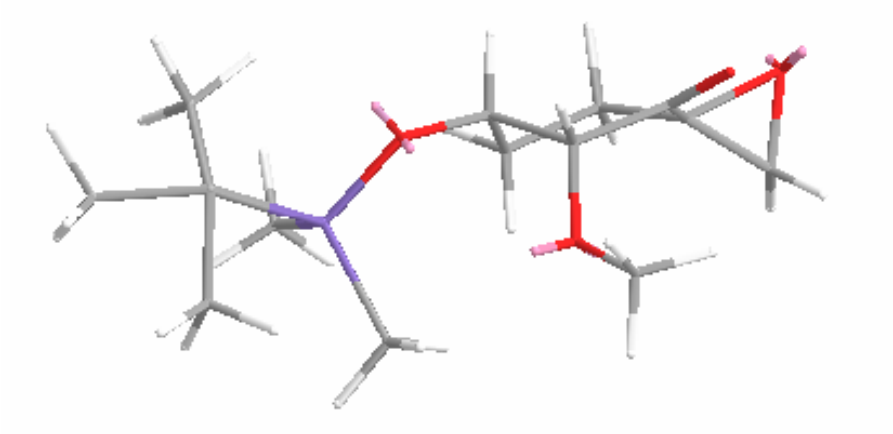
1.107 **1.54**

Scheme 1.24



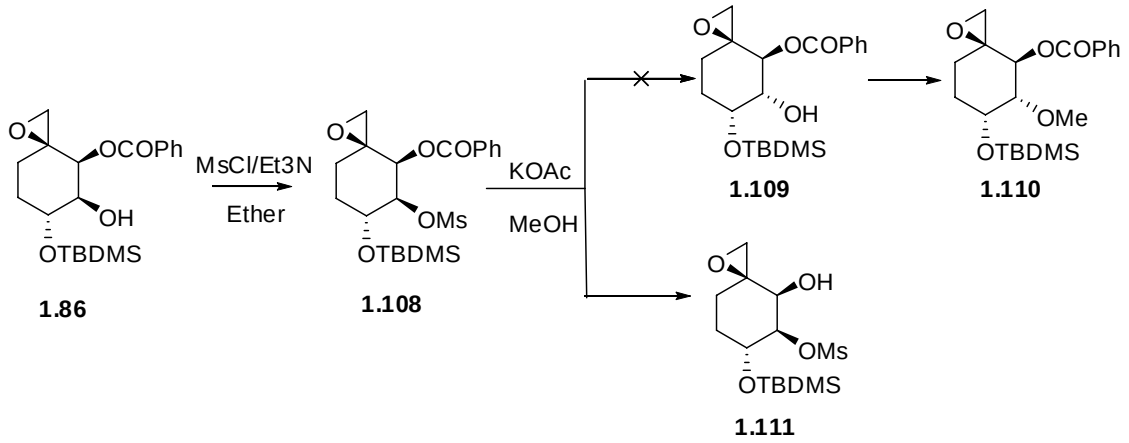
1.84 **1.49** **1.86**

Figure 1.11 Energy minimized conformation of ketone **1.54**.



Stereochemistry inversion of the OH functionality on C5 position was initially planned by a nucleophilic substitution reaction at sp^3 carbon (C5). When the OH group was converted to a good leaving group, for instance, OMs group, a direct S_N2 displacement of the good leaving group on sp^3 carbon could invert the configuration of C5 stereocenter. Although the hydroxyl group was transform to OMs (**1.108**) successfully, attempt of S_N2 substitution by using potassium acetate in methanol at 0 ^oC failed; instead, it hydrolyzed the benzoyl ester to alcohol **1.111** (Scheme 1.25). Compound **1.110** was not obtained from this route. Other conditions such as Mitsunobo inversion was not tried due to the spatial crowd around C5 position.

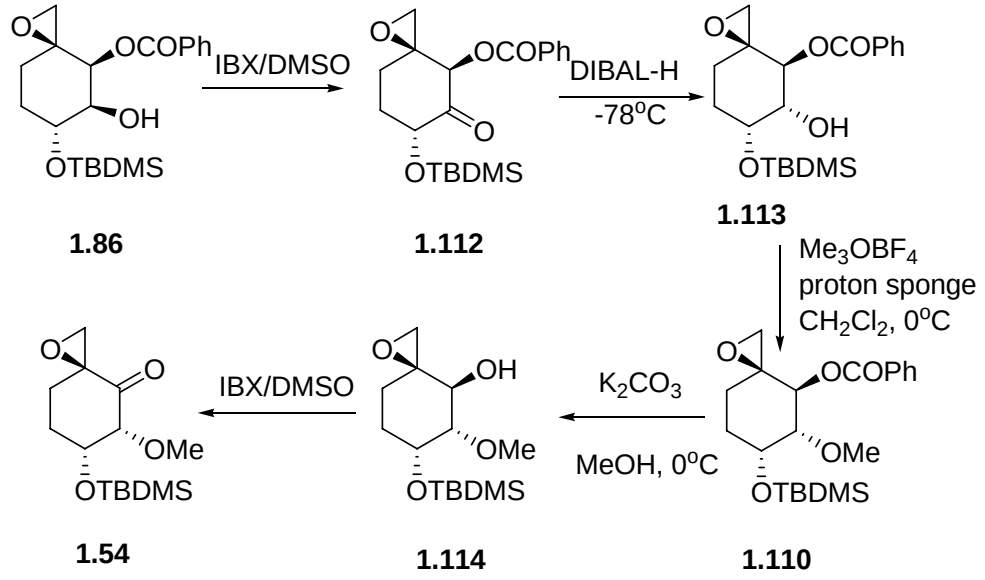
Scheme 1.25



Another possible approach for the configuration inversion of C5 is oxidation and selective reduction (Scheme 1.26). Since OTBDMS group is much more bulky than OCOPh functionality, when bulky reducing agent was used it should attack the carbonyl center from the opposite face of OTBDMS group, thus force the hydroxyl group posing into the same direction as that of OTBDMS. The inversion is described as following: Alcohol **1.86** was oxidized by IBX in DMSO; we found that this oxidation process was very slow; it took about 3 days to complete the reaction. The IBX had to be dissolved in DMSO before it was added into the solution of **1.86** in DMSO via cannula, otherwise the reaction would never complete because of the solubility of IBX in this system. Because this ketone can decompose slowly on silica gel, the crude material was not suitable to be purified on column. Therefore, the complete transformation from alcohol **1.86** to ketone **1.112** was highly desired. The reduction of **1.112** to **1.113** was assumed to be controlled by the hindrance of OTBDMS group. Because the OTBDMS group is more sterically hindered than OCOPh, the hydride should attack the ketone from the *beta*-face. However, when $NaBH_4$ was used, it attack the ketone from the *alpha*-face more easily than from the *beta*-face, thus it mainly reduced **1.112** back to compound **1.86**, although we did get the desire product **1.113**, the ratio is roughly **3:1** (**1.86:1.113**). When more bulky DIBAL-H was added to the solution of **1.112** in THF at -78 ^{o}C, the major product is **1.113** along with recovered starting material (the ratio is about 2:1) and the yield is 85% based on the recovered starting material. The structural difference between **1.86** and **1.113** is demonstrated by chemical shift and *J* coupling value. The big *J* value (~9 Hz) between hydrogen on C4 and C5 of **1.113** indicates an *axial-axial* coupling. Methylation of **1.113** was carried out with Me_3OBF_4 in the presence of

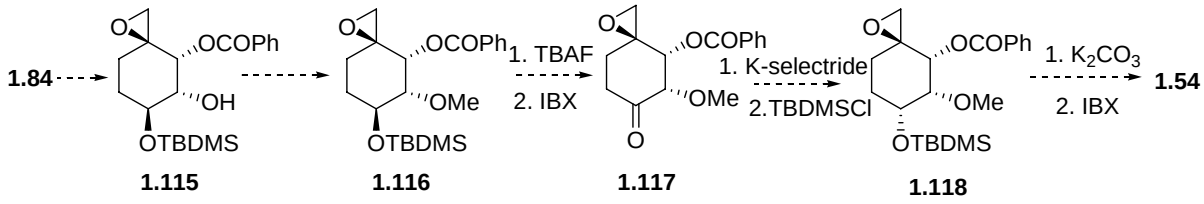
proton sponge as base in methylene chloride at 40 ^{o}C in 2 hours, which gave 86% yield. Deprotection of **1.110** with potassium carbonate in methanol at 0^{o}C gave compound **1.114** quantitatively. ^{1}H NMR and ^{13}C NMR spectra of compound **1.114** was identical to the reported one. Oxidation of **1.114** by IBX in DMSO gave compound **1.54** and this compound was used directly in the next step.

Scheme 1.26



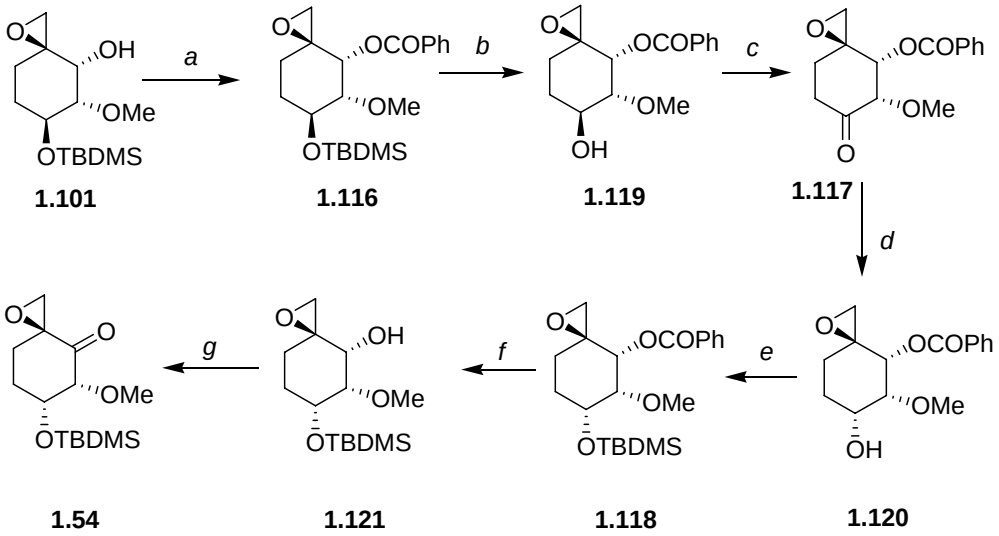
Transformation of compound **1.84** to **1.54** was initially planned to follow the route described in Scheme 1.27 in order to accomplish the transformation efficiently. We thought although compound **1.84** couldn't react with benzoyl chloride at room temperature, it is still possible to selectively protect the C4 OH by changing condition and reagents. Disappointedly, when we tried to protect the hydroxyl by using PhCOCl in the presence of triethylamine at elevated temperature, both of the hydroxyls were protected. Benzoyl cyanate and acetal anhydride were also tried, but the major product was the one with C5 OH protected. We believe that the reactivity difference between two hydroxyls of diol **1.84** can be attributed to its conformations. Because the hydroxyl group on C5 of Compound **1.84** occupied the equatorial position, it is more reactive than the hydroxyl on C4 position (Figure 1.7).

Scheme 1.27



Then we have to do the methylation first as mentioned in scheme 1.20. But before the stereochemistry inversion at C6, the hydroxyl group on C4 has to be protected. Although the protection of C4 hydroxyl by PhCOCl couldn't go smoothly at room temperature, when the temperature was raise to 45^{o}C in pyridine, compound **1.101** was successfully protected in 10 minutes to give compound **1.116** in 97% yield (Scheme 1.28). Higher temperature or prolonged reaction time caused compound to decompose. Compound **1.116** was deprotected by using TBAF in THF at 0 ^{o}C and then oxidized by IBX in DMSO to give **1.117**. Since the benzonyl ester and methoxy group blocked the *alpha*-face, bulky K-selectride attacked the ketone from the up face and gave compound **1.120** exclusively. The ^{1}HNMR and ^{13}CNMR spectra of **1.120** were different from compound **1.119**, which suggested compound **1.119** was converted to **1.120** successfully. Silylation of compound **1.120** by TBDMSCl in DMF at 0 ^{o}C in the presence of imidazole as base, DMAP as catalyst gave compound **1.118** in 98% yield. Treatment of **1.118** with potassium carbonate in methanol at 0 ^{o}C gave compound **1.121** in 96%. The following oxidation by IBX in DMSO at room temperature overnight converted **1.121** to compound **1.54**, which was used directly in the next step.

Scheme 1.28



1.101 **1.116** **1.119** **1.117** **1.120** **1.118** **1.121** **1.54**

Reagents and Conditions: *a*, PhCOCl, pyridine, 45^{o}C, 10 min; *b*. TBAF, THF, 0^{o}C, 4h; *c*. IBX/DMSO, r.t., overnight; *d*. K-selectride, -78^{o}C, 4h; *e*. TBDMSCl, imidazole, DMAP, DMF, 0^{o}C, 8h, *f*. K_2CO_3, MeOH, 0^{o}C, 8h; *g*. IBX/DMSO, r.t., overnight

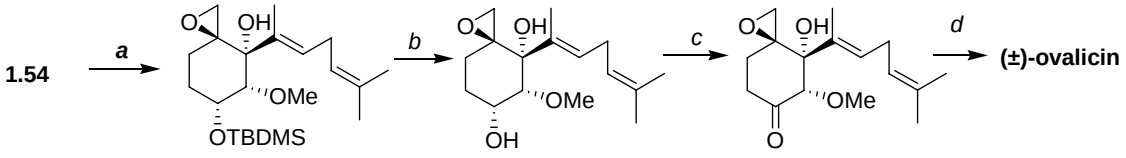
1.3.2.6 Synthesis of (±) Ovalicin

The introduction of side chain has two possibilities; one is the alkenyl anion attack the ketone from the up face, the other approach is from the bottom face. From the 3D structure of compound **1.54**, the bottom face was not only blocked by axial OMe group, but also by the OTBDMS group. Therefore, the vinyl lithium **1.76** has to attack the ketone **1.54** from the up face, which would result in the right stereo-chemistry at C4 position.

The addition of the side chain was carried out as following (Scheme 1.29): freshly prepared alkenyl lithium **1.76** was added to a solution of **1.54** in toluene at -78^{o}C, stirred at this temperature for 3 hours and then warmed gradually to 0 ^{o}C to give compound **1.122** in a 75% yield. ^{1}H NMR and ^{13}C NMR spectra were the same as the reported data for compound **1.122**,[30,33] Treatment of **1.122** with TBAF in THF at 0 ^{o}C gave alcohol **1.123**, ^{1}H NMR showed a clean deprotection. Oxidation of **1.123** by IBX in DMSO overnight gave compound **1.124** with a 92% yield. Selectively epoxidation of compound **1.124** using vanadyl acetylacetonate and *t*-BuOOH with the assistance of allylic alcohol on C4 position gave racemic (±)-ovalicin with

52.0% yield and recovered starting material (22.5%). ^{1}H NMR and ^{13}C NMR spectra were consistent with the reported data.[33]

Scheme 1.29



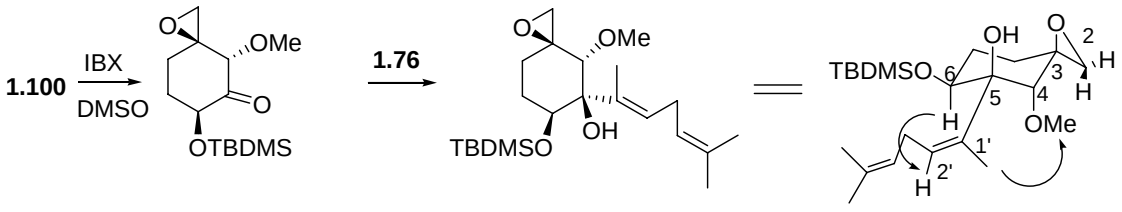
1.122 **1.123** **1.124**

Reagenta and Conditions: a. vinyllithium, tolune, -78^{o}C, 3h; b, TBAF/THF, 0^{o}C4h, c. IBX/DMSO, r.t., overnight, d. Vo(acetoneactonate)2, t-BuOOH, toluene, 0^{o}C, 8h

1.3.2.7 Synthesis of Ovalicin Analogues

We also used the monomethylated compound **1.100** to synthesize ovalicin C5 side chain analogues, which have not been reported previously. C5 alcohol **1.100** was oxidized by IBX in DMSO overnight at room temperature to give ketone **1.125** in a 90% yield. Since the *beta*-face was blocked by adjacent bulky TBDMS group, addition reaction with alkenyllithium **1.76** likely approached the ketone **1.125** from the *alpha*-face, which gave a single stereoisomer **1.126** in a 72% yield. The stereochemistry was supported by 2D NOESY spectroscopy: the cross-peak at (δ 3.29, δ 1.75 ppm) belongs to the correlation between C4- methoxy and C1'-methyl of C5- side chain; the cross peak at (δ 4.27, δ 5.75 ppm) belongs to C6-proton and the C2'olefinic proton (Scheme 1.30). These correlations suggest that the C5-side chain is on the opposite site of TBDMS group.

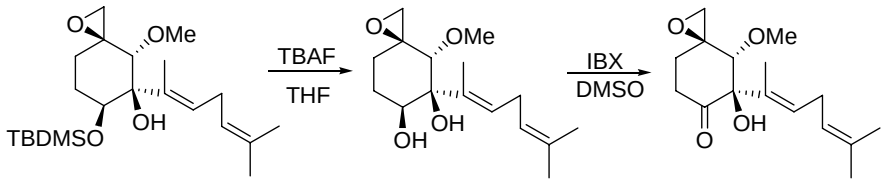
Scheme 1.30



1.125 **1.126**

Treatment of compound **1.126** with TBAF in THF gave compound **1.127** with 85% yield. Oxidation of **1.127** using IBX in DMSO at room temperature gave compound **1.128** with a yield of 64%. The result of the final epoxidation was not available because the reaction was conducted in a small scale (Scheme 1.31).

Scheme 1.31



1.126 **1.127** **1.128**

1.4 Bioevaluation of Ovalicin Analogues

1.4.1 Anti-tryponosomal Activity

This bio-evaluation was performed in Dr. Peter K. Chiang's laboratory at Pharmadyn Inc., 552 Del Rey Avenue, Sunnyvale, California.

Methionine aminopeptidase 2 inhibitors such as fumagillin and its synthetic analogue TNP-470 were reported to potently block in vitro growth of *P. falciparum* and *Leishmania donavani*, parasites causing most lethal form of malaria infection.[21] Since ovalicin is a more potent than fumagillin and as potent as TNP-470 MetAP2 inhibitor, it and its synthetic intermediates may also have the potential to inhibit diverse parasite growth. A variety of synthetic intermediates were tested against *Trypasonoma. brucei in vitro* following reported methods,[40,41] the result was summarized in Table 1.1. It is found that ovalicin C5-side chain analogue **1.126**, possesses the most potent inhibitory activity in vitro with an IC_{50} value of 0.28 ± 0.07 μM (Table 1). In comparison, ovalicin precursor **1.122**, a C4-side chain compound, is slightly less active with an IC_{50} of 0.40 ± 0.05μM. Surprising, a structurally simple diol synthetic intermediate **1.70**, has an IC_{50} of 0.72 ± 0.15μM and is 2.5 fold less active than **1.126**. Compounds **1.69**, **1.75**, **1.77** and **1.78** are less active. The anti-trypanosomal activities of

compounds **1.122** and **1.126** are greater than those previously reported for 5'-modified adenosine derivatives.[20]

Table 1.1 ***In vitro* antitrypanosomal activity of ovalicin analogs and synthetic intermediates.**

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1.122	0.40 ± 0.05	**1.77**	4.20 ± 0.40
1.69	325 ± 30	**1.78**	41.5 ± 4.0
1.70	0.72 ± 0.15	**1.126**	0.28 ± 0.07
1.75	47.3 ± 4.0		

The results demonstrate that ovalicin and its analogues and even its synthetic intermediates are effective to inhibit *Tryponosomal Bruei* parasites. Biological targets of this class of compounds and efficacy in animal model need to be studied in order to develop high-throughput screening methods and identify more effective ovalicin related compounds. Ovalicin analogs **1.126** could be explored as a leading compound for design and screening of wide-range of anti-parasitic agents.[21,23]

1.4.2 Anti-Cancer Activity

This anti-cancer activity screening was performed in Dr. Jean-Pierre H. Perchellet's laboratory at Department of Biology in Kansas State University.

Since anti-cancer activities of ovalicin, fumagillin, TNP-470 and their analogs are being investigated intensely, the anti-tumor effects of compounds **1.69** and **1.79** against HL-60 cells was studied in vitro. After 4 days in culture, 1.6-10 μM concentrations of **1.79** inhibit HL-60 tumor cell proliferation more effectively than 10 μM **1.69**. Although both compounds have significant antiproligerative effects, we can't obtain IC_{50} values because they cannot reduce the metabolic activity of HL-60 tumor cells below 50%. These weak growth inhibition activity of cultured tumor cells are consistent with those reported for the angiogenesis inhibitor TNP-470 in various tumor cell lines,[42,43] because the anti-tumor activity of these compounds are largely mediated by inhibition of angiogenesis, not directly targeting the cancer cell.[34]

1.5 Conclusion

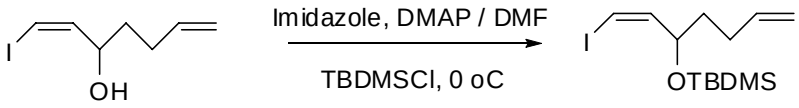
We successfully synthesized (±)-ovalicin, its C4(S*), C4(S*)C5(S*) stereo-isomers and C5(S*) regio-isomer by addition reactions of alkenyllithium with ketones **1.54**, **1.75**, **1.82**, and **1.102**, which were prepared via an intramolecular Heck reaction from a common intermediate **1.63**, and subsequent epoxidation, dihydroxylation, methylation, stereo-chemistry inversion before or after methylation, and oxidation. The bulky OTBDMS group on C6 position controlled the stereochemistry generated at C4 and C5 positions.

Bio-evaluation of various ovalicin analogues and synthetic intermediates shows that compound **1.122**, an ovalicin precursor, and compound **1.126**, a C5(S*) regio-isomer of ovalicin, have strong anti-trypanosomal activities. These two compounds may be used as leading compounds for the development of anti-parasitic drugs.

1.6 Experimental Information

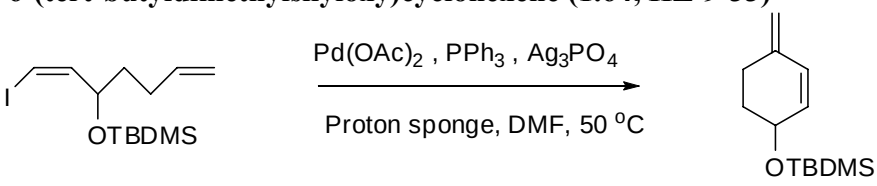
General Methods. Unless otherwise indicated, Nuclear Magnetic Resonance (NMR) spectra were obtained at 400 MHz for ^{1}H and 100 MHz for ^{13}C in $CDCl_3$. Chemical shifts were reported in δ ppm using tetramethylsilane as internal standard. High-resolution Mass spectra were obtained from Maldi and ESI spectrometers. Reagents and solvents are purchased from Aldrich or Acoros. Silica gel (200~400 mesh) were purchased from Natland corporation. Tetrahydrofuran and diethyl ether were distilled over sodium and benzophenone before use. Methylene chloride was distilled over CaH_2 and toluene and benzene were distilled over $LiAlH_4$.

(Z)-1-Iodohepta-3-(tert-butyldimethylsilyloxy)-1,6-diene (1.63, HZ-9-32)



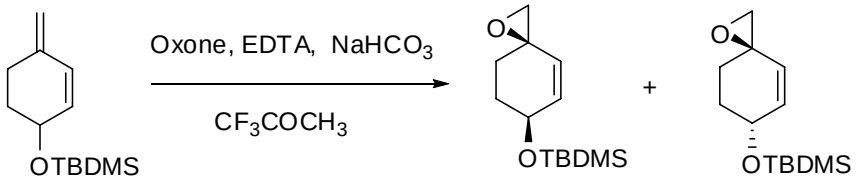
To a solution of (Z)-1-Iodohepta-1,6-dien-3-ol (380 mg, 1.6mmol) in anhydrous DMF (3ml), were added imidazole (326mg, 4.8mmol) and DMAP (39mg, 0.32mmol) under ice/water bath, follow by TBDMSCl (362mg, 2.4mmol). The solution was stirred at 0 ^{o}C for 10 hours, diluted with ether and washed by water and brine, dried over magnesium sulfate. After concentration and column chromatography using petroleum ether and ether (10:1), 417mg of DP was obtained (yield: 74%). **^{1}HNMR** ($CDCl_3$): δ 6.22~6.20 (m, 2H, HC=CH), 5.98~5.78 (m, 1H, =CH), 5.10~4.96 (m, 2H, $=CH_2$), 4.42~4.30 (m, 1H, OCH), 2.20-2.08 (m, 2H, CH_2), 1.70-1.50 (m, 2H, CH_2), 0.89 (s, 9H, $3CH_3$), 0.11 (s, 3H, CH_3), 0.10 (s, 3H, CH_3). **^{13}CNMR** δ 144.8, 138.6, 114.9, 80.1, 75.2, 36.2, 29.4, 26.1, 18.3, -4.0, -4.5. **HRMS** calcd for $C_{13}H_{26}IOSi$ ($M+H^+$) 353.0792, found 353.0799.

3-Methylene-6-(tert-butyldimethylsilyloxy)cyclohexene (1.64, HZ-9-33)



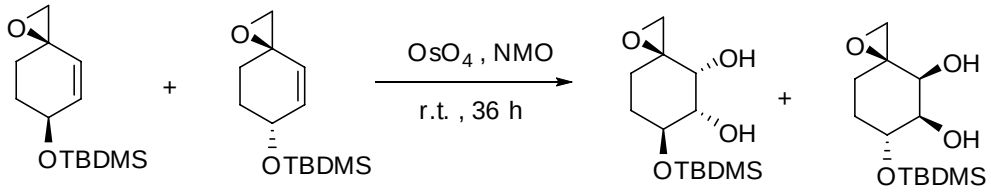
A mixture of (Z)-1-Iodohepta-3-(tert-butyldimethylsilyloxy)-1,6-diene (417 mg, 1.18 mmol), $Pd(OAc)_2$ (26.6 mg, 0.12 mmol) , PPh_3 (62.9 mg, 0.24 mmol), Ag_3PO_4(494 mg, 1.18 mmol) and Proton sponge (506 mg, 2.36 mmol) were vacuum-dried and purged with argon. After adding 5ml anhydrous DMF, the mixture was stirred at 50 ^oC for 8 hours. The reaction was quenched with water, extracted with ether, washed with water and brine and dried over magnesium sulfate. After concentration and column chromatography using petroleum ether as eluting solvent, 293 mg of 3-Methylene-6-(tert-butyldimethylsilyloxy)cyclohexene was obtained (yield: 91.3%) **^{1}HNMR** (CDCl3): δ 6.03 (d, J = 9 Hz, 1 H, =CH), 5.62 (d, J = 10 Hz, 1 H, =CH), 4.72 (d, J = 8 Hz, 2 H, =CH2), 4.25 (q, 1 H, HC-OSi), 2.4 (m, 1 H, CH2), 2.21 (m, 1 H, CH2), 1.82 (m, 1 H, CH2), 1.55 (m, 1 H, CH2), 0.82 (s, 9 H, t-Bu), 0.01 (s, 6H, 2x$SiCH_3$). **^{13}C NMR** (CDCl3): δ 142.6, 133.9, 130.2, 111.9, 67.1, 32.9, 27.9, 26.1, 18.4, -4.3.

(3S*, 6R*) and (3S*, 6S*)-6-(t-butyldimethylsilyloxy)-1-oxaspiro[2.5]oct-4-ene (1.83, HZ-9-34)



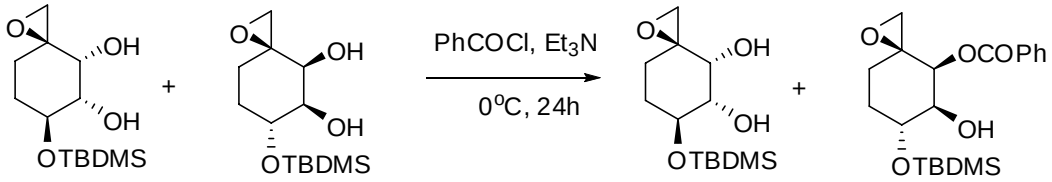
To a 25-ml-two-neck-round-bottom flask with a dewar condensor, was added diene **1.64** (300mg, 1.34 mmol) to a solution of EDTA (4.8 ml, $4x10^{-4}$ M) in MeCN/THF (8:2) and cooled to 0°C along with dry ice on top of the dewar condenser. Then 563 mg (6.70 mmol) of $NaHCO_3$ and 145 μl (1.54 mmol) of 1,1,1-trifluoroacetone were added via syringe, followed by 173 mg of Oxone® in portions. The resulting reaction mixture was stirred vigorously for 6 hours at 0°C. Then it was diluted with ether and washed with water and brine, dried over anhydrous magnesium sulfate, concentrated and purified by column chromatography on silica gel (deactivated by Et_3N) using petroleum ether-diethyl ether (4:1) as eluent to give 146 mg of the mixture **1.83** of two epoxides (yield: 45.3%) along with 97 mg of starting material. **^{1}H NMR** ($CDCl_3$): δ 5.90 (m, 1 H, HC=), 5.14 (m, 1 H, HC=), 4.17 (m, 1 H, HCOSi), 2.78 (d, *J* = 5 Hz, 1 H, OCH_2), 2.67 (d, *J* = 5 Hz, 1 H, OCH_2), 1.87 ~1.6 (m, 4 H, $2CH_2$), 0.95 (s, 9 H, $3CH_3$), 0.21 (s, 3 H, $SiCH_3$), 0.20 (s, 3 H, $SiCH_3$). **^{13}C NMR** ($CDCl_3$): δ 139.5, 138.2, 130.0, 128.8, 66.8, 66.0, 56.0, 55.4, 54.9, 31.8, 30.9, 27.9, 27.8, 26.0, 18.4, -4.4, -4.5. **HRMS** calcd for $C_{13}H_{25}O_2Si$ ($M+H^+$) 241.1624, found 241.1631.

(3S*,4R*,5S*,6S*)-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octane-4,5-diol and (3S*, 4S*, 5R*, 6R*)-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octane-4,5-diol (1.84 and 1.85, HZ-9-35)



A mixture of two epoxide compounds (100 mg, 0.42 mmol) were dissolved in 10 ml mixed solution (acetone: water: tert-butanol = 6: 2: 1). To it, was added NMO (147 mg, 1.26 mmol), followed by OsO_4 (10.7 mg, 0.042 mmol) and stirred at r. t. overnight. Acetone was evaporated under vacuum and the remaining solution was extracted with ether. The organic layer was washed with water, brine, dried over magnesium sulfate, concentrated to dryness. Column chromatography on silica gel using petroleum ether-ethyl acetate as eluent gave 91mg of a mixture of diol **1.84** and isomer **1.85** with a 85% overall yield. These two isomers are partially separable at this stage. **^{1}H NMR (1.84)** ($CDCl_3$): δ 3.98 (m, 1 H, H-COSi), 3.85 (m, 1 H, H-COH), 3.82 (m, 1 H, H-COH), 2.94 (d, *J* = 9 Hz, 1 H, OCH_2), 2.66 (d, *J* = 9 Hz, 1 H, OCH_2), 2.60 (m, 2 H, 2xOH), 2.0~1.6 (m, 4 H, 2xCH2), 0.91 (s, 9 H, t-Bu), 0.11 (s, 3 H, $SiCH_3$), 0.10 (s, 3 H, $SiCH_3$). **^{13}C NMR (1.84)** ($CDCl_3$): δ 74.8, 70.4, 70.01, 59.6, 51.1, 28.3, 26.3, 25.8, 18.0, -4.7,-4.8. **HRMS** calcd for $C_{13}H_{27}O_4Si$ ($M+H^+$) 275.1679, found 275.1676.

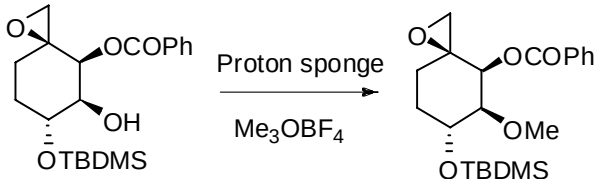
(3S*, 4S*, 5R*, 6R*)-6-(*tert*-butyldimethylsilyloxy)-5-hydroxy-1-oxaspiro[2.5]octa-4-yl benzoate (1.84 and 1.86, HZ-10-50)



To a cold solution of the mixture of diols (71.0 mg, 0.26mmol) in 5 ml of methylene chloride at 0°C, was added benzoyl chloride (36.3 mg, 0.26 mmol) and triethylamine (41µl, 0.26 mmol). The resulting solution was stirred for 48 hours at room temperature. The reaction solution was extracted with diethyl ether, washed water and brine. The organic layer was dried over anhydrous magnesium sulfate, concentrated and separated by column chromatography to give

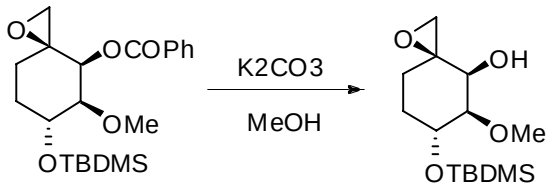
43.6 mg of compound **1.86** in 71.4% yield (based on the amount of its precursor) and 26 mg of unreacted diol. **^{1}H NMR (1.86)** ($CDCl_3$): δ 8.09 (d, *J* = 2 Hz, 2 H, Ar), 7.59 (t, *J* = 5 Hz, 1 H, Ar), 7.49 (t, *J* = 3 Hz, 2 H, Ar), 5.61 (d, J=3.1, 1H, H-COCOPh), 4.13 (m, 1 H, H-COSi), 4.0 (m, 1H, H-COH), 2.82 (d, *J* = 9 Hz, 1 H, OCH_2), 2.6 (d, *J* = 9 Hz, 1 H, OCH_2), 2.2 (d, *J* = 5 Hz, 1 H, CH_2), 2.1 (t, *J* = 8 Hz, 1 H, CH_2), 1.62 (m, 1 H, CH_2), 1.58 (m, 1 H, CH_2), 0.98 (s, 9 H, t-Bu), 0.21 (s, 3 H, $SiCH_3$), 0.21 (s, 3 H, $SiCH_3$). **^{13}C NMR (1.86)** ($CDCl_3$): δ 165.6, 133.5, 130.0, 129.9, 129.8, 128.6, 74.4, 71.3, 70.6, 58.7, 49.7, 27.1, 26.9, 25.9, 18.2, -4.6, -4.7. **HRMS** calcd for $C_{20}H_{30}O_5SiNa$ ($M+Na^+$) 401.1760, found 401.1754.

(3*S,4*S**,5*R**,6*R**)-6-(*tert*-Butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan-4-yl benzoate (1.87, HZ-10-59)**



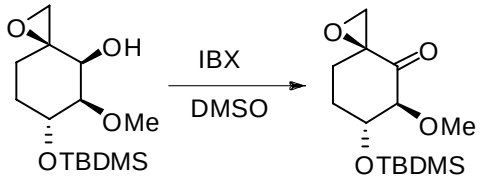
To a solution of compound **1.86** (35mg, 0.09 mmol) in 2 ml of methylene chloride, was added 28.5 mg (0.13 mmol) of proton sponge and 19.67 mg (0.13 mmol) of trimethyloxonium tetrafluoroborate at 0 ^oC. The reaction mixture was stirred at room temperature for 48 hours, diluted with ether and washed with saturated ammonium chloride solution, water and brine, dried over anhydrous magnesium sulfate, concentrated and purified by column chromatography (silica gel) using hexane and diethyl ether (1:1 v/v) to give 31mg (85.5 % yield) of compound **1.87**. **^{1}H NMR:** δ 8.07 (d, J = 7 Hz, 2H, Ar), 7.59 (t, J = 7 Hz, 1H, Ar), 7.45 (t, J = 8 Hz, 2H, Ar), 5.45 (d, J = 3 Hz, 1H, CHOCOPh), 4.09 (m, 1H, CHOTBDMS), 3.4 (s, 3H, OMe), 3.36 (m, 1H, CHOMe), 2.81 (d, J =5 Hz, 1H, CHO), 2.63 (d, J = 5 Hz, 1H, CHO), 2.12 (m, 1H, CH), 1.96 (m, 1H, CH), 1.62 (m, 1H, CH), 1.58 (m, 1H, CH), 0.92 (s, 9H, *t*-Bu), 0.097 (s, 3H, SiMe), 0.091 (s, 3H, SiMe); **^{13}C NMR** δ 165.8, 133.3, 130.3, 130.0, 128.6, 83.7, 71.7, 69.8, 58.7, 58.2, 50.7, 28.8, 27.0, 25.99, 18.3, -4.5, -4.8.

(3*S,4*S**,5*R**,6*R**)-6-(*tert*-Butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan-4-ol (1.88, HZ-10-60)**



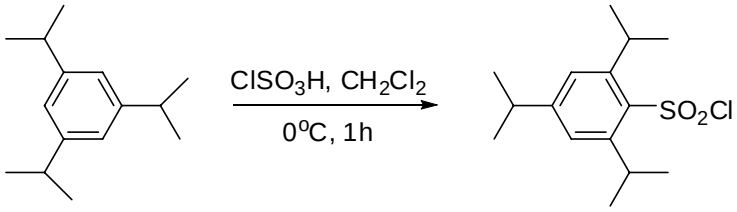
To a solution of compound **1.87** (31 mg, 0.079mmol) in anhydrous 0.2 ml of methanol was added 43 mg of potassium carbonate (0.32 mmol) at 0 ^{o}C. The solution was stirred at 0 ^{o}C for 8 hours and then diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. Column-chromatography on silica gel using petroleum-ethyl ether (2:1) as eluting solvent gave 19.8 mg of **1.88** (Yield: 86.1%). **^{1}H NMR:** δ 4.18 (m, 1 H, CHOTBDMS), 3.97 (dd, J = 4 Hz, 1 H, CHOH), 3.45 (s, 3 H, OMe), 3.34 (t, J = 4 Hz, 1 H, CHOMe), 2.89 (d, J = 6 Hz, 1 H, OCH_2), 2.43 (d, J = 5 Hz, 1 H, OCH_2), 2.2 (m, 2 H, CH_2), 1.48 (m, 2 H, CH_2), 0.9 (s, 9 H, *t*-Bu), 0.1 (s, 3 H, SiMe), 0.089 (s, 3 H, SiMe); **^{13}C NMR:** δ 83.9, 67.9, 66.3, 59.1, 58.7, 48.5, 26.4, 26.05, 25.9, 18.2, -4.7, -4.74.

(3*S,4*S**,5*R**,6*R**)-6-(*tert*-Butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan-4-one (1.82, HZ-10-61)**



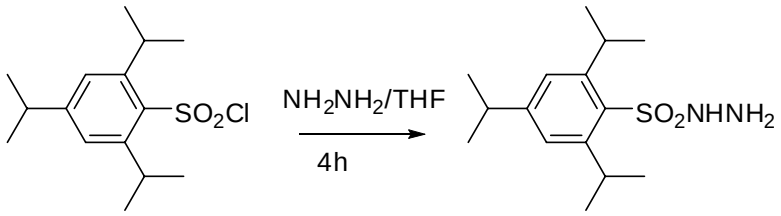
To a solution of 14.1 mg (0.046mmol) of compound **1.88** in 2 ml of DMSO, was added 37.2 mg (0.186 mmol) of IBX. The reaction mixture was stirred at room temperature overnight and then diluted with ether and washed with water and brine, dried over anhydrous magnesium sulfate and concentrated to dryness to provide 9.3 mg of compound **1.82** (Yield: 70.0%). It was used directly in next step. **^{1}H NMR:** δ 3.83 (m, 1 H, CHOTBDMS), 3.47 (d, J = 8 Hz, 1 H, CHOMe), 3.4 (s, 3 H, OMe), 2.8 (d, J = 6 Hz, 1 H, OCH_2), 2.67 (d, J = 6 Hz, 1 H, OCH_2), 2.1 (m, 2 H, CH_2), 1.7 (m, 2 H, CH_2), 0.8 (s, 9 H, *t*-Bu), 0.01 (s, 3 H, $SiCH_3$), 0.01 (s, 3 H, $SiCH_3$); **^{13}C NMR:** δ 206, 90.3, 73.5, 60.3, 53.8, 29.7, 27.1, 25.9, 18.2, -4.45, -4.83.

2,4,6-Triisopropylbenzenesulphonyl chloride (1.90, HZ-8-17)



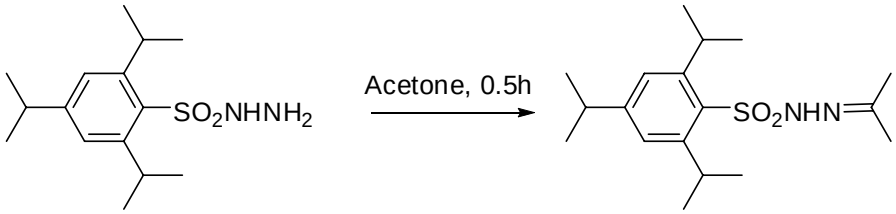
2,4,6-Triisopropylbenzene (10.0 g, 49.0 mmol) was dissolved in 50 ml of methylene chloride and cooled to 0 ^{o}C. To it, was added 14.3 ml sulfuric chloride dropwise and stirred at 0 ^{o}C for 1 hour. The reaction was quenched by pouring the solution into crushed ice slowly, and then extracted with methylene chloride. The organic layer was washed with water, brine, dried over magnesium sulfate and concentrated to dryness. The crude NMR showed more than 90% yield. Re-crystallization with ethyl ether gave 9.6g white solid (yield: 64.8%). **^{1}H NMR** ($CDCl_3$) δ 7.26 (s, 2 H, Ar), 4.23 (hept, 2 H, CH), 2.93(hept, 1 H, CH), 1.31 (d, J = 7 Hz, 12 H, CH_3), 1.27 (d, J = 7 Hz, 6 H, CH_3); **^{13}C NMR** ($CDCl_3$) δ 155.8, 150.56, 139.44, 124.5, 34.57, 29.9, 24.56, 23.64.

2,4,6-Triisopropylbenzenesulphonyl hydrazine (1.91, HZ-8-18)



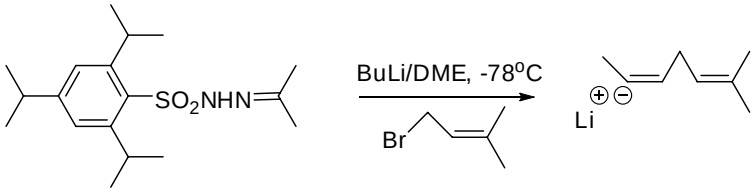
To a solution of compound **1.90** (3.9 g, 12.9 mmol) in 5 ml THF, was added a solution of NH_2NH_2 in 5 ml of THF dropwise via syringe at 0 ^{o}C. The resulting muggy white mixture was stirred at room temperature for 4 hours, and then diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. Crude NMR showed almost 100% yield. **^{1}H NMR** ($CDCl_3$) δ 7.23 (s, 2 H, Ar), 5.4 (bs, 3 H, NH), 4.15 (hept, 2 H, CH), 2.76 (hept, 1 H, CH_3), 1.28 (d, J = 5 Hz, 12 H, CH_3), 1.26 (d, J = 5 Hz, 6 H, CH_3); **^{13}C NMR** ($CDCl_3$) δ 151.99, 141.5, 124.2, 34.4, 29.96, 25.1, 23.7.

2,4,6-Triisopropylbenzenesulphonyl hydrazone (1.92, HZ-8-19)



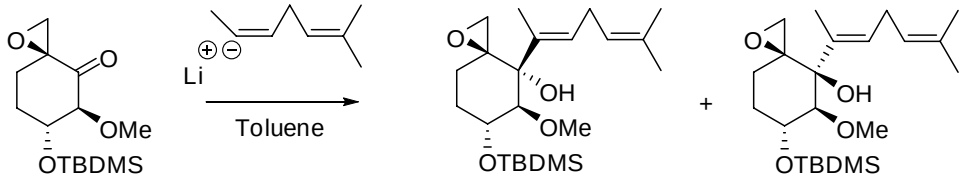
Compound **1.91** (0.8 g, 2.68 mmol) was dissolved in 10 ml of freshly dried acetone and stirred at room temperature for 0.5 hour. After evaporating solvent and dried overnight, 0.91 g of white powder was obtained (100%). **^{1}H NMR** ($CDCl_3$) δ 7.16 (s, 2 H, Ar), 7.08 (bs, 1 H, NH), 4.2 (hept, 2 H, CH), 2.76 (hept, 1 H, CH), 1.9 (s, 3 H, CH_3), 1.78 (s, 3 H, CH_3), 1.28 (d, J = 5 Hz, 12 H, CH_3), 1.26 (d, J = 5 Hz, 6 H, CH_3); **^{13}C NMR** ($CDCl_3$) δ 153.3, 151.5, 123.97, 34.35, 30.12, 24.98, 23.75, 16.59.

Lithium (Z)-6-methylhepta-2,5-dien-2-ide (**1.76**, HZ-8-20)



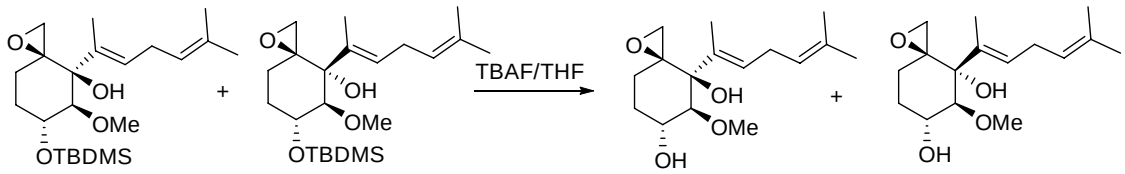
Compound **1.92** (230 mg, 0.68 mmol) was dried and dissolved in 4 ml of DME (freshly distilled). This resulting solution was stirred and cooled to -78 ^{o}C. Then 2.15 equivalents (0.91 ml) of *n*-BuLi was added to the above solution, the solution turns to yellow color. The resulting solution was monitored very carefully and slowly warmed to -66 ^{o}C over 20 minutes and again cooled to -78 ^{o}C. To this solution 93µl (0.82 mmol) of 1-Bromo-3-methyl-2-butene was added at -78 ^{o}C. The solution was warmed to -66 ^{o}C over 20 minutes and allowed to stir at -66 ^{o}C for one hour and again recooled to -78 ^{o}C. Then TMEDA (0.34 ml 2.2 mmol) was added and 0.46 ml (0.75 mmol) of *n*-BuLi was added. The above solution was allowed to warm to -3 ^{o}C over 2 hours and again cooled to -78 ^{o}C. Assuming 100% conversion to vinyl lithium, the concentration of this solution was assigned as 0.12 mol/L.

(3*S,4*R**,5*S**,6*S**)-4-(1',5'-Dimethylhexa-1',4'-dienyl)-5-methoxy-6-(*tert*-butyl dimethylsilyloxy)-1-oxaspiro[2.5]octan-4-ol and (3*S**,4*S**,5*S**,6*S**)-4-(1',5'-dimethyl hexa-1',4'-dienyl)-5-methoxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-4-ol (1.93 and 1.94, HZ-9-13)**



To a solution of ketone **1.82** (24 mg, 0.084 mmol) in 1 ml toluene was added 1.5ml of vinyl lithium 0.18mmol) at -78 ^{0}C under argon. This solution was stirred at -78 ^{0}C for 2 hours and slowly warmed to 0 ^{o}C, and then diluted with ether, washed with water and brine, dried over anhydrous magnesium sulfate. Column chromatography on silica gel using hexane and ethyl ether (2:1 v/v) afforded 21.3 mg (64.5% yield) of a mixture and this mixture can not be separates and used directly in next step.

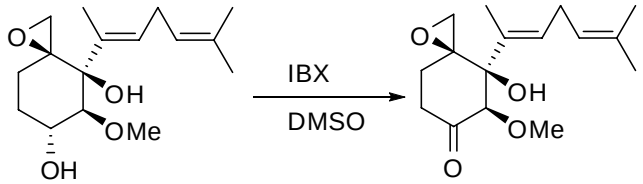
(3*S,4*S**,5*S**,6*S**)-4-(1',5'-Dimethylhexa-1',4'-dienyl)-5-methoxy-6-hydroxy-1-oxaspiro[2.5]octan-4-ol (1.96) and (3*S**,4*R**,5*S**,6*S**)-4-(1',5'-Dimethylhexa-1',4'-dienyl)-5-methoxy-6-hydroxy-1-oxaspiro[2.5]octan-4-ol (1.95) (HZ-8-27)**



A mixture of **1.93** and **1.94** (11 mg, 0.028 mmol) was dissolved in 0.5 ml of THF and cooled to 0 ^{o}C. To it, was added 56 µl of TBAF (1M, 0.056 mmol) and stirred at 0 ^{o}C for 6 hours. The solution was diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness and purified by column chromatography using petroleum ether and ethyl acetate (3:1) to give 6.1 mg of DP.(yield: 61%). **^{1}HNMR (1.96):** δ 5.96(td, J=7.0, 1.5, 1H, H-C=), 5.10 (td, J=7.3, 1.5), 3.80 (td, J=9.5, 4.7, 1H, H-C6), 3.66 (s, 3H, OCH_3), 3.12 (d, J=9.6, 1H), 3.06 (dd, J=5.5, 1.8, 1H, H-COMe), 2.75 (t, J=7.0, 2H, H_2C-C=), 2.38(d, J=5.5, 1H), 2.13-2.03(m, 2H), 1.79 (s, 3H, CH_3), 1.70 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 1.54-1.41(m, 1H), 1.31-

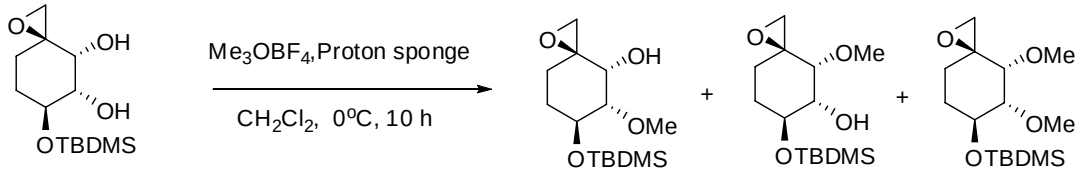
1.23(m, 1H). **^{13}CNMR (1.96)**: δ 134.0, 132.4, 131.0, 122.4, 92.0, 77.7, 70.8, 62.8, 62.4, 48.3, 29.5, 29.0, 27.3, 25.9, 18.1, 13.4.

(3*S,4*S**,5*S**,6*S**)-4-(1',5'-Dimethylhexa-1',4'-dienyl)-5-methoxy-6-oxo-1-oxaspiro [2.5]octan-4-ol (1.97, HZ-10-113)**



To a solution of compound **1.96** (10.1 mg, 0.036 mmol) in 1 ml of DMSO, was added 42 mg of IBX (0.15 mmol) and stirred at room temperature overnight. The reaction solution was diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. Column chromatography (silica gel) using hexane and diethyl ether (1:1 v/v) as eluent gave 8.2 mg product (yield: 82%). **^{1}H NMR** ($CDCl_3$) δ 5.66 (td, J = 7.2, 1.2 Hz, 1 H, =CH), 5.04 (td, J = 5.5, 1.2 Hz, 1 H, =CH), 3.84 (s, 1 H, CHOMe), 3.59 (s, 3 H, OMe), 3.43 (dd, J = 5.4, 1.7 Hz, 1 H, CH_2O), 2.61 (td, J = 8.1, 1.2 Hz, 2H, CH_2C=), 2.60 (d, J=5.4Hz, 1H, CH_2O), 1.6~1.4 (m, 2H), 2.22~2.18 (m, 1H) 1.72 (s, 3 H, CH_3), 1.67 (s, 3 H, CH_3), 1.52~1.44 (m, 1H), 1.43 (s, 3 H, CH_3). **^{1}CNMR:** δ 206.0, 132.8, 132.4, 130.9, 121.9, 91.5, 78.2, 61.7, 61.2, 47.6, 37.2, 27.7, 27.4, 25.9, 18.1, 12.6.

(3*S,4*R**,5*S**,6*S**)-6-(*tert*-butyldimethylsilyloxy)-4,5-dimethoxy-1-oxaspiro[2.5] octane(1.99), (3*S**,4*R**,5*S**,6*S**)-6-(*tert*-butyldimethylsilyloxy)-4-methoxy-1-oxaspiro [2.5]octan-5-o1(1.100), (3*S**,4*R**,5*S**,6*S**)-6-(*tert*-Butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan -4-o1 (1.101) (HZ-8-83)**



To a solution of diol **1.84** (266mg, 0.97mmol) in anhydrous methylene chloride (5ml), was added proton sponge (498mg, 2.33mmol) and Me_3OBF_4 (172 mg, 1.16 mmol) at 0 °C. The mixture was stirred at 0 °C for 10 hours, then diluted with ether, washed by water and brine, dried over anhydrous sodium sulfate, filtered and concentrated to dryness. Column

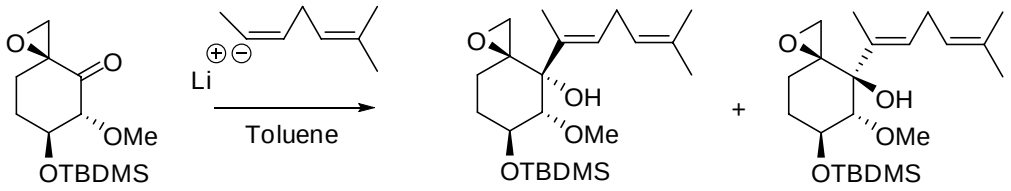
chromatography using hexane and ethyl acetate (2:1) gave 27 mg of dimethoxy **1.92**, two monomethylated products (228.8 mg). Based on proton NMR and 2D NMR, the less polar isomer was assigned as C4-OMe C5-OH, and the more polar isomer as desired C4-OH C5-OMe. Further purification using long slim column and petroleum ether/ethyl acetate (3:1) as eluting solvent can separate these two isomers. **^{1}H NMR** of **1.99** (CDCl3) δ 4.03 (m, 1 H, H-COSi), 3.45 (s, 3 H, OMe), 3.41 (s, 3 H, OMe), 3.37 (s, 1 H, H-COMe), 3.35 (s, 1 H, H-COMe), 2.95 (d, J = 5 Hz, 1 H, H-CO), 2.71 (d, J = 5 Hz, 1 H, H-CO), 1.85 ~1.65 (m, 4 H, 2CH2), 0.91 (s, 9 H, 3CH3), 0.11 (s, 3 H, CH3), 0.09 (s, 3 H, CH3); **^{13}C NMR** of **1.99** (CDCl3) δ 83.9, 79.7, 69.1, 59.1, 58.6, 58.3, 52.3, 29.3, 26.5, 26.0, 18.3, -4.54, -4.59. **HRMS of 1.99** calcd for $C_{15}H_{31}O_4Si$ ($M+H^+$) 303.1992, found 303.1997. **^{1}H NMR** of **1.100** (CDCl3) δ 3.95 (m, 1 H, H-COSi), 3.81 (s, 1 H, H-COH), 3.42 (s, 3 H, OMe), 3.34 (m, 1 H, H-COMe), 2.93 (d, J = 5 Hz, 1 H, H-CO), 2.68 (d, J = 5 Hz, 1 H, H-CO), 2.36 (d, 1 H, OH), 1.90 ~ 1.63 (m, 4 H, 2CH2), 0.91 (s, 9 H, 3CH3), 0.11 (s, 3 H, CH3), 0.089 (s, 3 H, CH3); **^{13}C NMR** of **1.100** (CDCl3) δ 80.4, 74.3, 70.6, 58.6, 58.0, 51.9, 29.1, 28.7, 26.6, 26.2, 25.9, 18.2, -4.4, -4.6. **HRMS** of **1.100** calcd for $C_{14}H_{29}O_4Si$ ($M+H^+$) 289.1830, found 289.1826. **^{1}H NMR 1.101** (CDCl3) δ 4.01 (m, 1 H, H-COSi), 3.82 (s, 1 H, H-COH), 3.45 (s, 3 H, OMe), 3.38 (dd, J=6.2, 3.3, 1 H, H-COMe), 2.91 (d, J = 4.4 Hz, 1 H, H-CO), 2.67 (d, J = 4.8 Hz, 1 H, H-CO), 2.32 (s, broad, 1 H, OH), 1.85 (m, 2 H, CH2), 1.90~1.60(m, 4 H, 2CH2), 0.91 (s, 9 H, 3CH3), 0.10 (s, 3 H, CH3), 0.09 (s, 3 H, CH3); **13C NMR** of **1.101** (CDCl3) δ 84.5, 70.3, 68.6, 59.2, 58.8, 51.5, 30.5, 29.1, 26.2, 25.9, 18.3, -4.6. **HRMS** of **1.101** calcd for $C_{14}H_{29}O_4Si$ ($M+H^+$) 289.1830, found 289.1841.

(3*S,5*S**,6*S**)-6-(*tert*-Butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan-4-one (1.102, HZ-8-31)**

To a solution of compound **1.101 (**32 mg, 0.11 mmol) in 1.0 ml of DMSO, was added 124 mg (0.44 mmol) of IBX. The reaction mixture was stirred at room temperature for overnight. The reaction mixture was diluted with ether and washed with water and brine, dried over anhydrous magnesium sulfate and concentrated to dryness. It was used directly in next step. **^{1}H NMR** ($CDCl_3$) δ 4.17 (m, 1 H, H-COTBDMS), 3.51 (d, J = 4.8 Hz, 1 H, H-OCH_3), 3.29 (s, 3 H, OMe),

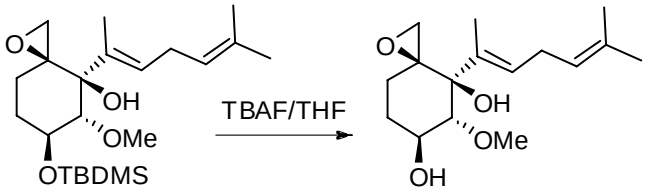
2.87 (d, J = 1.2 Hz, 1 H, CHO), 2.85 (d, J = 1.2 Hz, 1 H, CHO), 2.3 (m, 2 H, CH_2), 1.6 (m, 2 H, CH_2), 0.85 (s, 9 H, *t*-Bu), 0.66 (s, 6 H, 2 CH_3); **^{13}C NMR**: ($CDCl_3$) δ 203.6, 86.9, 71.4, 59.6, 58.3, 54.2, 27.4, 26.9, 25.8, 18.1, -4.8.

(3*S,4*S**,5*R**,6*S**)-4-(1',5'-Dimethylhexa-1',4'-dienyl)-5-methoxy-6--(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-4-ol (1.104) and (3*S**,4*R**,5*R**,6*S**)-4-(1',5'-Dimethylhexa-1',4'-dienyl)-5-methoxy-6--(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-4-ol (1.103, HZ-8-34)**



To a solution of Ketone **1.104** (23 mg, 0.080 mmol) in 1 ml toluene was added 1.0 ml of vinyl lithium (0.12 mmol) at -78 ^{o}C under argon. This solution was stirred at -78 ^{o}C for 2 hours and slowly warmed to 0 ^{o}C, and then diluted with ether, washed with water and brine, dried over anhydrous magnesium sulfate. Column chromatography on silica gel using hexane and ethyl ether (2:1 v/v) afforded 19.0 mg (60.0% yield) of **1.103** and **1.104**.

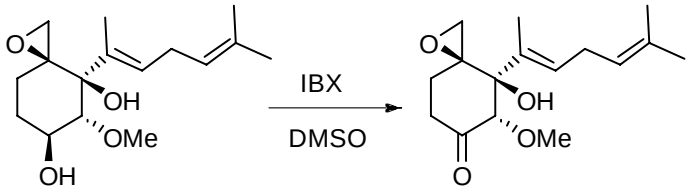
(3*S,4*S**,5*R**,6*S**)-4-(1',5'-Dimethylhexa-1',4'-dienyl)-5-methoxy-6-hydroxy-1-oxaspiro[2.5]octan-4-ol (1.106, HZ-8-35)**



A mixture of compound **1.103** and **1.104** (11 mg, 0.028 mmol) were dissolved in 0.5 ml of THF and cooled to 0^{o}C. To it, was added 56 µl of TBAF (1M, 0.056 mmol) and stirred at 0^{o}C for 6 hours. The solution was diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. **^{1}HNMR (1.106)** ($CDCl_3$) δ 5.90 (td , J = 4 Hz, J = 1 Hz, 1 H, =CH), 5.11 (m, 1 H, HC=), 3.89 (m, 1 H, CHO), 3.68 (s, 3 H, OMe), 3.13 (d, J = 8 Hz, 1 H, CHO), 2.74 (d, J = 6 Hz, 1 H, CHO), 2.01 (m, 2 H, CH_2), 1.72 (s, 3 H, CH_3), 1.69 (s, 3

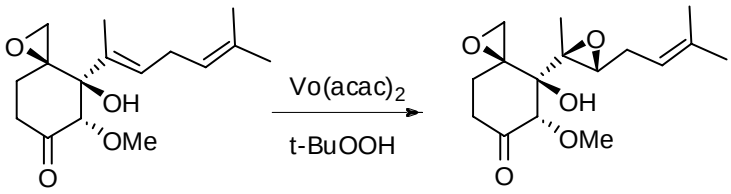
H, CH_3), 1.63 (s, 3 H, CH_3), 1.5 (m, 2 H, CH_2); **^{13}C NMR** δ 132.8, 132.5, 131.1, 122.5, 93.5, 70.8, 62.7, 62.4, 55.8, 51.3, 28.9, 28.0, 27.5, 25.9, 18.1, 13.7; **HRMS** calcd for $C_{16}H_{30}NO_4$ ($M+NH^+$) 300.2169, found 300.2173.

(3*S,4*S**,5*R**,6*S**)-4-(1',5'-Dimethylhexa-1',4'-dienyl)-5-methoxy-6-oxo-1-oxaspiro [2.5]octan-4-ol (1.79, HZ-8-36)**



To a solution of compound **1.106** (10.1 mg, 0.036 mmol) in 1 ml of DMSO, was added 42 mg of IBX (0.15 mmol) and stirred at room temperature overnight. The reaction solution was diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. Column chromatography (silica gel) using petroleum ether and diethyl ether (1:1 v/v) as eluent gave 8.2 mg product **1.79** (yield: 82%). **^{1}H NMR** ($CDCl_3$) δ 5.76 (t, J = 8 Hz, 1 H, =CH), 5.02 (t, J = 5 Hz, 1 H, =CH), 4.01 (s, 1 H, CHOMe), 3.58 (s, 3 H, OMe), 3.43 (d, J = 5 Hz, 1 H, CH_2O), 2.91 (d, J = 5 Hz, 1 H, CH_2O), 2.706 (m, 2 H, CH_2), 2.19 (m, J = 5 Hz, 2 H, CH_2), 1.67 (s, 3 H, CH_3), 1.63 (s, 3 H, CH_3), 1.59 (s, 3 H, CH_3), 1.45 (dd, J = 5 Hz, 2 H, CH_2). **^{13}C NMR** δ 209.2, 132.5, 132.3, 131.4, 121.7, 91.0, 79.2, 61.5, 61.1, 52.2, 36.8, 27.5, 27.2, 25.8, 18.1, 12.5; **HRMS** calcd for $C_{16}H_{28}NO_4$ ($M+NH^+$) 298.2018, found 298.2015.

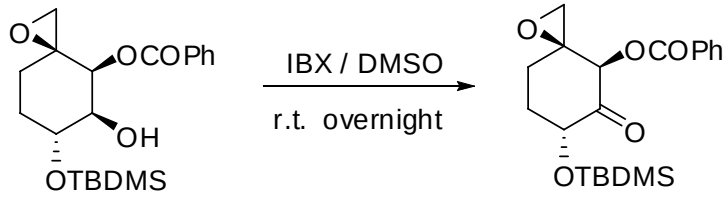
(3*S,4*S**,5*R**,6*S**)-4-(2'-Methyl-3'-(3''-methylbut-2''-enyl)oxiran-2'-yl)-5-methoxy-6-oxo-1-oxaspiro[2.5]octan-4-ol (1.80)**



Compound **1.79** (8.2 mg, 0.029 mmol) was dissolved in 0.5 ml of benzene and cooled to 5 ^oC. To it, was added vanadyl acetoacetonate (2.33 mg, 0.09 mmol) and *tert*-butylhydroperoxide (8.2 µl, 0.058 mmol). The mixture was warmed to room temperature and stirred for 3 hours. The reaction

mixture was loaded on to a silica gel column after evaporate solvent and separated using petroleum ether and ethyl ether (2:1 v/v) as an eluent to yield 4.6 mg (54% yield) of **1.80**. **^{1}HNMR:** δ 5.1 (t, J = 8 Hz, 1 H, =CH), 3.85 (s, 1 H, CHOMe), 3.62 (t, J = 6 Hz, 1 H, CHO), 3.51 (s, 3 H, OMe), 3.38 (d, J = 5 Hz, 1 H, CHO), 2.95 (d, J = 5 Hz, 1 H, CHO), 2.7 (m, 2 H, CH_2), 2.26 (m, 2 H, CH_2), 2.10 (m, 2 H, CH_2), 1.714 (s, 3 H, CH_3), 1.711 (s, 3 H, CH_3), 1.324 (s, 3 H, CH_3); **^{13}C NMR:** δ 212.49, 130.88, 128.04, 88.40, 86.45, 61.0, 60.43, 52.98, 51.84, 36.53, 29.92, 27.67, 22.21, 13.36. **HRMS** calcd for $C_{16}H_{24}O_5Na$ ($M+Na^+$) 319.1522, found 319.1518.

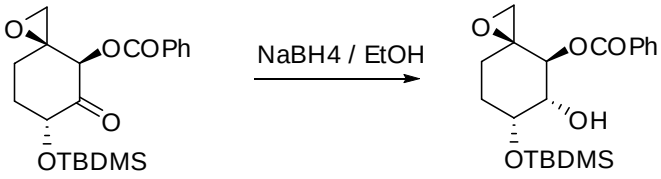
(3*S,4*R**,6*R**)-6-(*tert*-Butyldimethylsilyloxy)-5-one-1-oxaspiro[2.5]octan-4-yl Benzoate** (**1.112**, HZ-9-17)



Compound **1.86** (33 mg, 0.087 mmol) was vacuum-dried and purged with argon. To it was added a solution of IBX (96.7 mg, 0.35 mmol) in 2 ml of DMSO and stirred at room temperature for 3 days. The resulting solution was diluted with ether, washed with water and brine, dried over magnesium sulfate and evaporated to dryness. Flash column gave 31 mg **1.112** (yield: 94.5%). **^{1}HNMR:** δ8.09 (m, 2H, H-Ar) 7.62 (m, 1H, H-Ar) 7.48 (m, 2H, H-Ar), 6.24 (s, 1H, H-COCOPh), 4.37 (t, J=2.9Hz, 1H, H-COSi), 3.04 (d, J=4.4, 1H, CH_2O), 2.76 (d, J=4.8 Hz, 1H, CH_2O), 2.2~1.6 (m, 4H, $2CH_2$) **^{13}CNMR:** δ201.0, 165.2, 133.7, 130.2, 129.3, 128.7, 75.5, 72.7, 61.9, 50.1, 31.2, 26.7, 25.9, 18.3, -4.7, -5.0. **HRMS** calcd for $C_{20}H_{28}O_5SiNa$ ($M+Na^+$) 399.1598, found 399.1605.

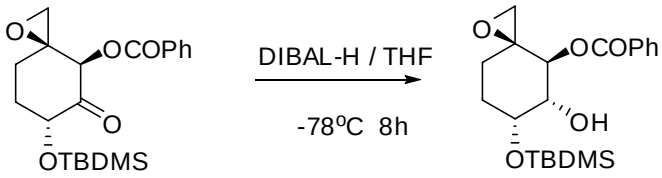
(3*S,4*S**,5*S**,6*R**)-6-(*tert*-Butyldimethylsilyloxy)-5-ol-1-oxaspiro[2.5]octan-4-yl Benzoate (1.113)**

Method 1 (HZ-9-37)



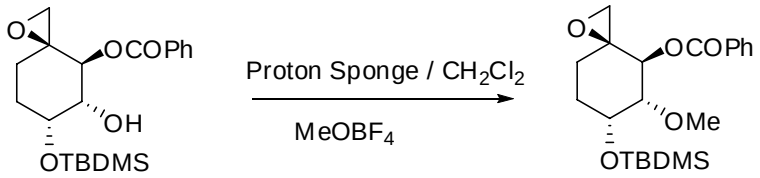
Compound **1.112** (11.1 mg, 0.03 mmol) was dissolved in 0.5 ml of ethanol and cooled to 0°C. To it was added sodium borohydride (1.1 mg, 0.029 mmol) and stirred at 0°C for 4 hours. The resulting solution was diluted with ether, washed with water and brine, dried over magnesium sulfate and evaporated to dryness. Column chromatography gave 1.3 mg of **1.113** (yield: 11.8%).**^{1}HNMR** δ 8.07 (m, 2H, H-Ar) 7.56 (m, 1H, H-Ar) 7.45 (m, 2H, H-Ar), 5.54 (d, J=8.8 Hz, 1H, H-COCOPh), 4.27 (m, 1H, H-COSi), 3.90 (m, 1H, H-COH), 2.87 (d, J= 4.8 Hz, 1H, CH_2O), 2.67 (d, J= 4.4 Hz, CH_2O), 2.2~1.6 (m, 4H, $2CH_2$). **^{13}CNMR:** δ141.6, 133.5, 130.1, 130.0, 128.7, 77.4, 73.7, 71.7, 71.5, 50.6, 28.5, 26.8, 26.0, 18.3, -4.8, -4.9. **HRMS** calcd for $C_{20}H_{30}O_5SiNa$ ($M+Na^+$) 401.1760, found 401.1752.

Method 2 (HZ-9-50)



Compound **1.112** (11 mg, 0.03 mmol) was dissolved in 0.5 ml of THF and cooled to -78°C. To it was added diisobutylaluminum hydride (in toluene, 46 μ l, 0.046 mmol) and stirred at -78°C for 8 hours. The reaction was quenched with acetic acid and diluted with ether, washed with water and brine, dried over magnesium sulfate and evaporated to dryness. Column chromatography gave 4.7 mg of **1.113** (yield: 42.4% or 84.8% based on recovered starting material).

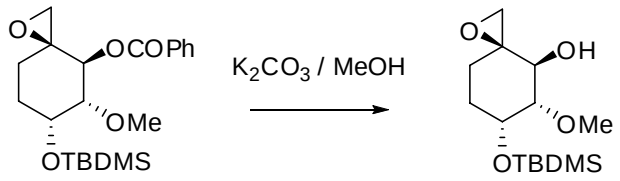
(3*S,4*S**,5*S**,6*R**)-6-(*tert*-Butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan-4-yl Benzoate** (**1.110**, HZ-9-43)



To a solution of compound **1.113** (2.7 mg, 0.0068 mmol) in anhydrous methylene chloride (0.2 ml), was added proton sponge (3.52mg, 0.0164 mmol) and Me_3OBF_4 (1.21 mg, 0.0082 mmol) at 0 °C. The mixture was stirred at 0 °C for 10 hours, then diluted with ether, washed by water and brine, dried over anhydrous sodium sulfate, filtered and concentrated to dryness. Column chromatography with petroleum ether and ethyl ether gave 2.3 mg of **1.110** (yield: 86.1%).

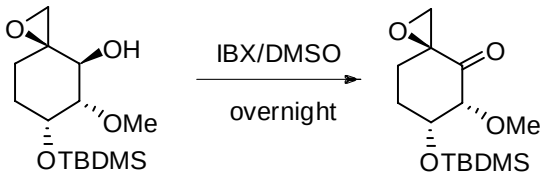
[1]HNMR δ 8.08 (m, 2H, H-Ar) 7.57 (m, 1H, H-Ar) 7.47 (m, 2H, H-Ar), 5.59 (d, J=8.8 Hz, 1H, H-COCOPh), 4.32 (m, 1H, H-COSi), 3.50 (dd, J= 9.2, 2.6 Hz, 1H, H-COH), 3.41 (s, 3H, CH_3O), 2.76 (d, J= 4.4 Hz, 1H, CH_2O), 2.65 (d, J= 4.4 Hz, CH_2O), 2.4~1.4 (m, 4H, $2CH_2$). **[13]CNMR:** δ 166.1, 133.3, 130.1, 130.0, 128.6, 82.7, 70.9, 68.6, 59.1, 58.9, 51.1, 28.9, 27.0, 26.0, 18.4, -4.4, -4.9. **HRMS** calcd for $C_{21}H_{32}O_5SiNa$ ($M+Na^+$) 415.1917, found 415.1906.

(3*S,4*S**,5*S**,6*R**)-6-(*tert*-Butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan-4-ol** (**1.114**, HZ-9-44)



To a solution of compound **1.110** (2.3 mg, 0.0059mmol) in anhydrous 0.2 ml of methanol was added 8.1 mg of potassium carbonate (0.059 mmol) at 0°C. The solution was stirred at 0°C for 8 hours and then diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. Column-chromatographed on silica gel using petroleum-ethyl ether (2:1) as eluting solvent to give 1.6 mg of **1.114** (Yield: 94.7%). **[1]HNMR** δ 4.32 (m, 1H, H-COH), 4.10 (dd, J= 9.1, 5.8 Hz, 1H, H-COMe), 3.44(s, 3H, OMe), 3.13(d, J=5.2, 1H, OCH_2), 3.07 (dd, J=9.5, 2.2 Hz, 1H, H-COH), 2.62(d, J=4.7, 1H, OCH_2) 2.4~2.2(m, 1H, CH_2), 1.98 (d, J= 6.2, 1H, OH), 1.8~1.7 (m, 2H, CH_2), 1.30~1.15 (m, 1H, CH_2) 0.91 (s, 9H, $3CH_3$), 0.11 (s, 3H, CH_3-Si), 0.08 (s, 3H, CH_3-Si) **[13]CNMR** δ 85.5, 67.6, 66.9, 60.2, 57.8, 50.1, 29.0, 26.5, 26.0, 18.3, -4.5, -4.8. **HRMS** calcd for $C_{14}H_{29}O_4Si$ ($M+H^+$) 289.1830, found 289.1837.

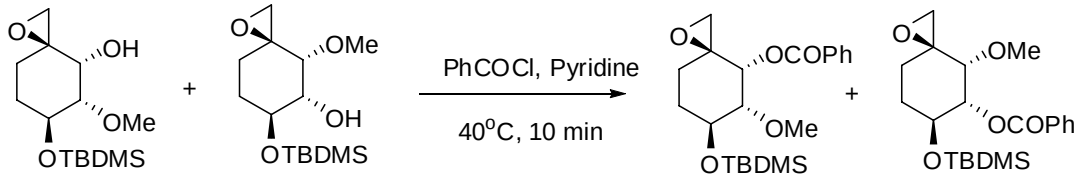
(3*S,5*R**,6*R**)-6-(*tert*-butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan-4-one** (**1.54**, HZ-9-15)



Compound **1.114** (22 mg, 0.076 mmol) was dissolved in 1 ml of DMSO. To it, was added 86 mg of IBX (0.31 mmol) and stirred at r. t. overnight. The reaction solution was diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. The crude NMR showed a clean oxidation with almost quantitative transformation. The

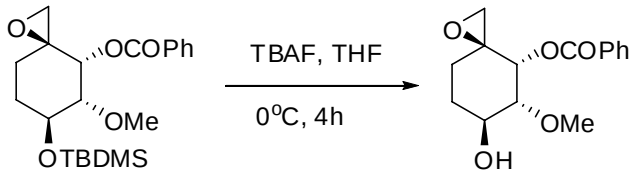
compound was used directly in next step. **^{1}H NMR** δ 4.44 (m, 1H, H-COSi), 3.98(d, J=2.5, 1H, H-COMe), 3.44(s, 3H, OMe), 3.40(m, 1H,), 3.29(d, J=4.7, 1H, OCH_2), 2..77(d, J=4.7, 1H, OCH_2), 2.6~2.4(m, 1H, CH_2), 2.2-2.0 (m, 2H, CH_2), 1.6~1.4 (m, 1H, CH_2) 0.92 (s, 9H, $3CH_3$), 0.08 (s, 3H, CH_3), 0.07 (s, 3H, CH_3) **^{13}C NMR** δ 202.48, 87.5, 72.3, 60.7, 58.5, 51.4, 28.9, 27.1, 25.9, 18.3, -4.4, -5.0. **HRMS** calcd for $C_{14}H_{26}O_4SiNa$ ($M+Na^+$) 309.1498, found 309.1499.

(3*S,4*R**,5*S**,6*S**)-6-(*tert*-Butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan -4-yl benzoate (1.116), and (3*S**,4*R**,5*S**,6*S**)-6-(*tert*-Butyldimethylsilyloxy)-4-methoxy- 1-oxaspiro [2.5]octan -5-yl benzoate (HZ-8-88)**



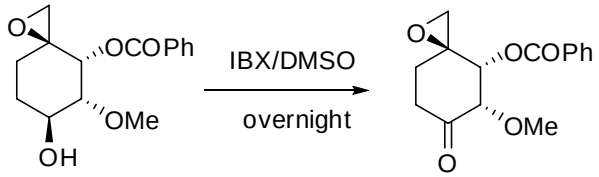
A mixture of two momomethoxyl compounds (179 mg, 0.62 mmol) was vacuum-dried and dissolved in 2 ml pyridine. After adding benzoyl chloride (144 µl, 1.24 mmol), the solution was heated to 40 ^{o}C for 15 minutes and diluted with ether, washed with saturated ammonium chloride, sodium bicarbonate, water and brine, dried over magnesium sulfate, filtered and evaporated to dryness. Column chromatography using petroleum ether and ethyl ether (4:1) gave 152.5 mg of **1.116** (two-step yield: 40.1%) and 68.6 mg region-isomer. **^{1}H NMR (1.109)**: δ 8.02 (d, J=7.0, 2H, Ar-H), 7.55 (t, 7.58, t, J=7Hz, 1H, Ar-H), 7.45 (t, J=7Hz, 2H, Ar-H), 5.34 (d, J=2.9Hz, 1H, H-COCOPh), 4.09 (td, J=6.6, 3.3, 1H, H-COSi), 3.50 (dd, J=6.96, 2.93, H-COMe), 3.42 (s, 3H, OMe), 3.04 (d, J=5.1, 1H, OCH), 2.68(d, J=4.8, 1H, OCH), 2.69-1.71(m, 4H, $2CH_2$), 0.93(s, 9H, $3CH_3$), 0.12(s, 3H, $SiCH_3$), 0.11(s, 3H, $SiCH_3$), **^{3}C NMR (1.116)**:δ 165.7, 133.3, 130.3, 129.9, 128.6, 83.4, 72.1, 69.5, 59.0, 57.7, 52.1, 29.3, 27.0, 26.0, 18.4, -4.5, -4.7. **HRMS (1.116)** calcd for $C_{21}H_{32}O_5SiNa$ ($M+Na^+$) 415.1917, found 415.1920. **^{1}H NMR (Isomer)** δ 8.02 (d, J=7.0, 2H, Ar-H), 7.55 (t, 7.58, t, J=7Hz, 1H, Ar-H), 7.45 (t, J=7Hz, 2H, Ar-H), 5.42 (dd, J=5.4, 2.9, 1H, H-COCOPh), 4.18 (m, 1H, H-COSi), 3.70 (d, J=2.9, 1H, H-COMe), 3.40(s, 3H, OMe), 3.05 (d, J=5.1, 1H, CH2O), 2.75 (d, J=5.5, CH2O), 2.2~1.5 (m, 4H, 2CH2), 0.89 (s, 9H, 3CH3), 0.11 (s, 3H, SiCH3), 0.10 (s, 3H, SiCH3). **^{13}C NMR (Isomer)** δ 165.7, 133.3, 130.3, 129.8, 128.6, 77.52, 74.8, 68.2, 58.8, 58.5, 51.1, 29.0, 26.8, 25.8, 18.1, -4.6, -4.7.

(3*S,4*R**,5*S**,6*S**)-6-ol-5-methoxy-1-oxaspiro[2.5]octan-4-yl benzoate(1.119,HZ-8-89)**



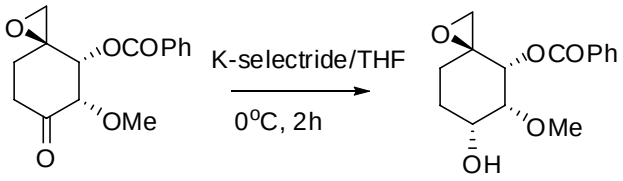
To a solution of compound **1.116** (153 mg, 0.39 mmol) in 3 ml of THF, was added 0.78 ml of TBAF (1M, 0.78 mmol) at 0°C and stirred at 0°C for 4 hours. The solution was diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. The crude NMR showed a clean deprotection with almost quantitative transformation. **^{1}H NMR** δ 8.02 (d, J=7.0, 2H, Ar-H), 7.55 (t, 7.58, t, J=7Hz, 1H, Ar-H), 7.45 (t, J=7Hz, 2H, Ar-H), 5.09 (m, 1H, H-COCOPh), 4.13-4.01(m, 1H, H-COH), 3.43 (s, 3H, OMe), 3.40(m, 1H, H-COMe), 2.96 (d, J= 4.4, 1H, H-CO), 2.73 (d, J=4.8, 1H, H-CO), 2.40-1.2 (m, 4H, $2CH_2$) **^{13}C NMR** δ165.6, 133.6, 130.0, 129.8, 128.7, 83.9, 72.1, 68.7, 57.6, 53.2, 28.2, 26.7. **HRMS** calcd for $C_{15}H_{19}O_5$ ($M+H^+$) 279.1227, found 279.1233.

(3*S,4*R**,5*S**)--5-methoxy-1-oxaspiro[2.5]octan-6-one -4-yl benzoate (1.117, HZ-8-117)**



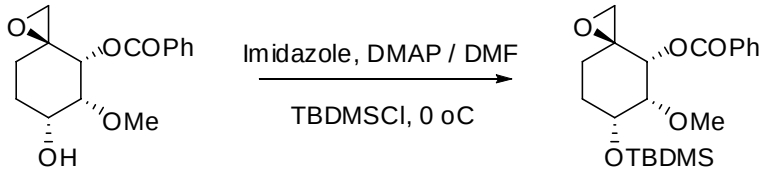
To a solution of compound **1.119** (71 mg, 0.25 mmol) in 2 ml of DMSO, was added 286 mg of IBX (1.0 mmol) and stirred at room temperature overnight. The reaction solution was diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. The crude NMR showed a clean oxidation with almost quantitative transformation. **^{1}H NMR** δ 7.98 (m, 2H, H-Ar), 7.54 (m, 1H, H-Ar), 7.43 (m, 2H, H-Ar), 5.25 (dd, J=3.7, 1.8, 1H, H-COMe), 4.33 (dd, J=3.6, 1.1, 1H, H-COCOPh), 3.48 (s, 3H, OMe), 3.21 (d, J=4.4, 1H, H-CO), 2.93 (dd, J=4.7, 1H, H-CO), 2.82-1.56 (m, 4H, $2CH_2$). **^{13}C NMR** δ 205.1, 165.4, 133.8, 130.1, 129.3, 128.7, 83.1, 77.2, 58.5, 57.2, 54.5, 37.1, 28.7. **HRMS** calcd for $C_{15}H_{17}O_5$ ($M+H^+$) 277.1071, found 277.1079.

(3*S,4*R**,5*S**,6*R**)-6-ol-5-methoxy-1-oxaspiro[2.5]octan -4-yl benzoate (1.120, HZ-8-96)**



To a cold solution (ice/water bath) of compound **1.117** (33 mg, 0.12 mmol) in 0.5 ml of THF, was added 143µl of K-selectride (1M, 0.14 ml) and stirred at 0°C for 2 hours. The reaction solution was diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. Column-chromatographed on silica gel using petroleum-ethyl acetate (3:1) as eluent to give 31 mg of **1.120** (Yield: 93%). **^{1}H NMR** δ 8.08 (m, 2H, H-Ar), 7.63 (m, 1H, H-Ar), 7.46 (m, 2H, H-Ar), 5.18 (d, J=3.3, 1H, H-COCOPh), 4.14 (m, 1H, H-COH), 3.71 (t, J=3.3 1H, H-COMe), 3.50 (s, 3H, OCH_3), 3.02 (d, J=4.7, 1H, OCH_2), 2.71(d, J=3.8, 1H, OCH_2) 2.2-0.8 (m, 4H, $2CH_2$). **^{13}C NMR** δ 165.7, 133.5, 130.0, 129.8, 128.8, 79.9, 72.5, 68.1, 58.8, 57.7, 52.4, 27.8, 24.8. **HRMS** calcd for $C_{15}H_{19}O_5$ ($M+H^+$) 279.1227, found 279.1235.

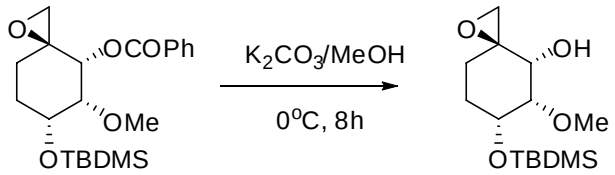
(3*S,4*R**,5*S**,6*R**)-6-(*tert*-butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan-4-yl benzoate (1.118, HZ-8-120)**



Compound **1.120** (40 mg, 0.14 mmol) was dissolved in 2ml of anhydrous DMF and cooled to 0°C. To it was added imidazole (29.4, 0.42 mmol), catalytic DMAP, followed by TBDMSCl (32.6 mg, 0.22 mmol). The solution was stirred at 0°C for 8 hours and then was diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. Column-chromatography on silica gel using petroleum-ethyl ether (4:1) as eluants to give 55 mg of **1.118** (Yield: 97.6%). **^{1}H NMR** δ 8.04 (m, 2H, H-Ar), 7.57 (m, 1H, H-Ar), 7.44 (m, 2H, H-Ar), 5.24 (d, J=2.5, 1H, H-COCOPh), 3.97 (m, 1H, H-COSi), 3.74 (t, J=2.2 1H, H-COMe), 3.54 (s, 3H, OCH_3), 3.10 (d, J=5.1, 1H, OCH_2), 2.65(d, J=5.2, 1H, OCH_2) 2.2-1.6 (m, 4H, $2CH_2$).0.92 (s, 9H, $3CH_3$), 0.11 (s, 3H, CH_3), 0.09 (s, 3H, CH_3); **^{13}C NMR** δ 152.6, 133.4,

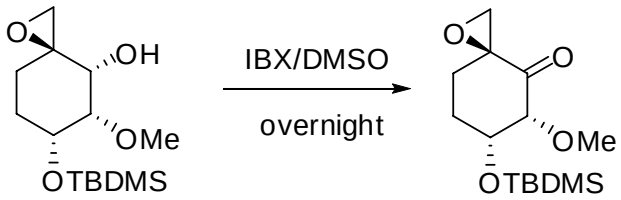
130.1, 130.0,128.6, 82.6, 71.6, 71.2, 61.0, 57.6, 51.3, 28.4, 27.9, 26.0, 18.3, -4.48, -4.53; **HRMS** calcd for $C_{21}H_{32}O_5SiNa$ ($M+Na^+$) 415.1917, found 415.1916.

(3*S,4*R**,5*S**,6*R**)-6-(*tert*-butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan-4-ol (1.121, HZ-8-100)**



To a solution of compound **1.118** (17.8 mg, 0.045mmol) in anhydrous methanol was added 62.6 mg of potassium carbonate at 0°C. The solution was stirred at 0°C for 8 hours and then diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. Column-chromatography on silica gel using petroleum-ethyl ether (2:1) as eluent gave 12.6 mg of **1.121** (Yield: 96%). **^{1}H NMR** δ 4.29 (m, 1H, OH), 4.20 (d, J=8.8, 1H, H-COH), 3.44(s, 3H, OMe), 3.40(m, 1H, H-COSi), 3.29(t, J=3.0, 1H, H-COMe), 2.83(d, J=4.4, 1H, OCH_2), 2.74(d, J=4.8, 1H, OCH_2) 2.6~2.4(m, 1H, CH_2), 2.0-1.7 (m, 2H, CH_2), 1.2~1.0 (m, 1H, CH_2) 0.92 (s, 9H, $3CH_3$), 0.15 (s, 3H, CH_3), 0.12 (s, 3H, CH_3) **^{13}C NMR** δ 79.0, 74.0, 70.5, 59.9, 56.7, 53.2, 29.0, 25.9, 22.3, 18.3, -4.8, -4.9. **HRMS** calcd for $C_{14}H_{29}O_4Si$ ($M+H^+$) 289.1830, found 289.1824.

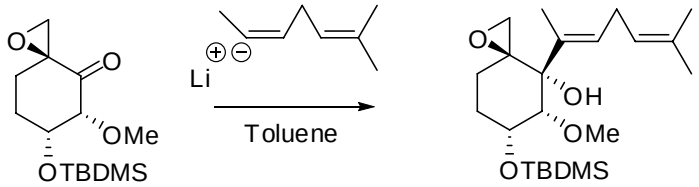
(3*S,5*R**,6*R**)-6-(*tert*-Butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan-4-one (1.54, HZ-9-15)**



Compound **1.121** (22 mg, 0.076 mmol) was dissolved in 1 ml of DMSO. To it, was added 86 mg of IBX (0.31 mmol) and stirred at r. t. overnight. The reaction solution was diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. The crude NMR showed a clean oxidation with almost quantitative transformation. The compound was used directly in next step. **^{1}H NMR** δ 4.44 (m, 1H, H-COSi), 3.98(d, J=2.5, 1H, H-COMe), 3.44(s, 3H, OMe), 3.40(m, 1H,), 3.29(d, J=4.7, 1H, OCH_2), 2..77(d, J=4.7, 1H, OCH_2), 2.6~2.4(m, 1H, CH_2), 2.2-2.0 (m, 2H, CH_2), 1.6~1.4 (m, 1H, CH_2) 0.92 (s, 9H, $3CH_3$),

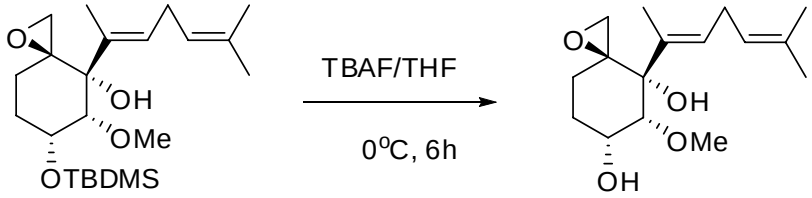
0.08 (s, 3H, CH_3), 0.07 (s, 3H, CH_3); **^{13}C NMR** δ 202.48, 87.5, 72.3, 60.7, 58.5, 51.4, 28.9, 27.1, 25.9, 18.3, -4.4, -5.0.

(3*S, 4*R**, 5*R**, 6*R**)-6-(*tert*-Butyldimethylsilyloxy)-4-((E)-1',5'-dimethylhexa- 1',4'–dienyl)-5-methoxy-1-oxaspiro[2.5]octan-4-ol (1.122, HZ-9-19)**



To a solution of ketone **1.54** (21 mg, 0.073 mmol) in 1 ml toluene was added 1.22ml of vinyl lithium (0.146 mmol) at -78 ^{0}C under argon. This solution was stirred at -78 ^{0}C for 2 hours and slowly warmed to 0 ^{o}C, and then diluted with ether, washed with water and brine, dried over anhydrous magnesium sulfate. Column chromatography on silica gel using hexane and ethyl ether (2:1 v/v) afforded 22.1 mg (75% yield) of **1.121**. **^{1}H NMR** δ 5.71 (m, 1 H, H-C=), 5.12 (m, 1 H, H-C=), 4.45 (m, 1H, H-COSi), 3.51 (d, J= 2.6, 1 H, HCOMe), 3.45 (s, 3 H, OMe), 2.81 (d, J=5.1, 1H H-CHO), 2.77 (m, 2H, CH_2C=), 2.42 (d, J=5.1, 1H, H-CHO) 2.0~1.8(m, 4H, $2CH_2$), 1.66 (s, 6H, $(CH_3)_2C$=), 1.61 (s, 3H, CH_3C=); **^{13}C NMR** δ 132.7, 131.7, 127.8, 123.0, 80.6, 79.3, 68.6, 62.2, 57.8, 50.7, 28.7, 27.3, 25.9, 25.8, 25.5, 20.9, 18.1, 18.0, 14.2, -4.68, -4.83; **HRMS** calcd for $C_{22}H_{44}O_4SiN$ ($M+NH_4^+$) 414.3040, found 414.3036.

(3*S, 4*R**, 5*R**, 6*R**)- 4-((E)-1', 5'-Dimethylhexa-1', 4'-dienyl)-5-methoxy-1-oxaspiro[2.5]octane-4,6-diol (1.123, HZ-9-25)**



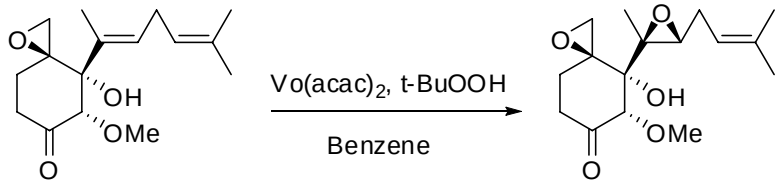
To a solution of compound **1.122** (12 mg, 0.030 mmol) in 1 ml of THF, was added 60 μl of TBAF (1M, 0.060 mmol) at 0^{o}C and stirred at 0^{o}C for 6 hours. The solution was diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. The crude NMR showed a clean deprotection with almost quantitative transformation. **^{1}H NMR** δ5.65 (t, J = 7.0 Hz, 1H, H-C=), 5.10 (t, J = 7.0 Hz, 1H, H-C=), 4.34 (m, 1H, H-COH),

3.61 (d, J = 3.3 Hz, 1H), 3.49 (s, 3H), 3.17 (bs, 1H), 3.07 (bs, 1H), 2.79 (d, J = 5.1 Hz, 1H), 2.79-2.69 (m, 2H), 2.45 (d, J = 5.1 Hz, 1H), 2.45-2.37 (m, 1H), 2.04-2.00 (m, 1H), 1.89-1.81 (m, 1H), 1.68 (s, 3H), 1.67 (s, 3H), 1.62 (s, 3H), 1.24-1.18 (m, 1H). **^{13}C NMR δ** 133.9, 132.3, 127.5, 122.6, 80.0, 79.9, 67.0, 61.5, 57.8, 50.4, 27.8, 27.3, 25.9, 25.2, 18.0, 14.3. **HRMS** calcd for $C_{16}H_{30}NO_4$ ($M+NH_4^+$) 300.2169, found 300.2171.

(3*S, 4*R**, 5*S**)- 4-((E)-1',5'-Dimethylhexa-1',4'-dienyl)-4-hydroxy-5-methoxy-1-oxaspiro[2.5]octan-6-one (1.124, HZ-9-29)**

To a solution of compound **1.123** (4.7 mg, 0.017 mmol) in 0.5 ml of DMSO, was added 18.7 mg of IBX (0.067 mmol) and stirred at room temperature overnight. The reaction solution was diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. The crude NMR showed a clean oxidation. Column chromatography using petroleum ether and ethyl ether (2:1) affords 4.3 mg of **1.117** (yield: 92.3%). **^{1}H NMR** δ 5.64 (m, 1H, H-C=), 5.10 (m, 1H, H-C=), 4.26 (s, 1H, H-COMe), 3.50 (s, 3H, OMe), 2.85 (d, J = 4.9 Hz, 1H, OCH_2), 2.80-2.62 (m, 4H), 2.61 (d, J = 4.9 Hz, 1H, OCH_2), 2.51-2.47 (m, 2H), 1.70 (s, 6H, $2CH_3C$=), 1.62 (s, 3H, CH_3C=), 1.52-1.48 (m, 1H). **^{13}C NMR** δ 207.7, 133.8, 132.6, 127.9, 122.3, 85.9, 83.1, 61.1, 59.7, 51.2, 37.1, 30.5, 27.2, 25.9, 18.0, 14.6. **HRMS** calcd for $C_{16}H_{28}NO_4$ ($M+NH_4^+$) 298.2018, found 298.2020.

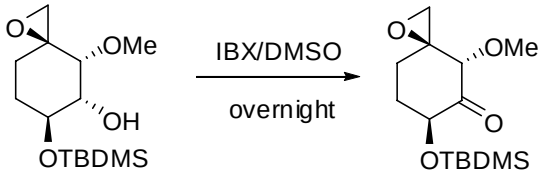
(±) Ovalicin (1, HZ-9-30)



To a solution of compound **1.124** (4.0 mg, 0.014mmol) 0.2 ml of benzene was added 1.1 mg of $Vo(acac)_2$ at 5 ^{o}C, followed by 4.0 μl t-BuOOH (0.028mmol). The resulting brown solution was stirred at room temperature for 1.5 hours and then loaded to column directly. Petroleum ether and ethyl ether (2:1) were used as eluting solvents to give 2.2 mg of (±) Ovalicin (52.0%) and

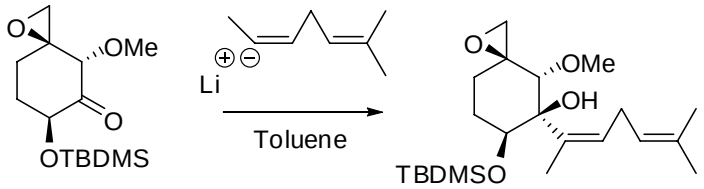
0.9 mg of recovered starting material. **^{1}H NMR** δ 5.18 (t, J=6.6Hz, 1H, H-C=), 4.23 (s, 1H, H-COMe), 3.57 (s, 3H, OMe), 3.14 (s, 1H), 3.10 (d, J = 4.2 Hz, 1H, OCH_2), 2.90 (t, J = 6.3 Hz, 1H), 2.73 (d, J = 4.2 Hz, 1H, OCH_2), 2.71-2.61 (m, 2H), 2.51-2.47 (m, 1H), 2.45-2.39 (m, 1H), 2.18-2.12 (m, 1H), 1.75 (s, 3 H, CH_3C=), 1.66 (s, 3H, CH_3C=), 1.45-1.41 (m, 1H), 1.37 (s, 3H, CH_3C=). **^{13}C NMR** δ 206.8, 135.7, 118.2, 86.3, 78.8, 60.7, 60.5, 59.5, 57.0, 51.5, 36.9, 30.5, 27.2, 25.9, 18.2, 14.6. **HRMS** calcd for $C_{16}H_{24}O_5Na$ ($M+Na^+$) 319.1522, found 319.1515.

(3*S,4*S**,6*S**)-6-(*tert*-Butyldimethylsilyloxy)-4-methoxy-1-oxaspiro[2.5]octan-5-one (1.125, HZ-9-28)**



To a solution of compound **1.100** (31 mg, 0.11 mmol) in 1 ml of DMSO, was added 183 mg of IBX (0.65 mmol) and stirred at room temperature overnight. The reaction solution was diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. The compound was used directly in next step. **^{1}H NMR** δ 4.34 (m, 1H, H-COSi), 4.24(s, 1H, H-COMe), 3.43(s, 3H, OMe), 3.02(dd, J=5.2, 1.1, 1H, OCH_2), 2..57(d, J=5.2, 1H, OCH_2), 2.4~1.6(m, 4H, $2CH_2$), 0.93 (s, 9H, $3CH_3$), 0.10 (s, 6H, $2CH_3$). **^{13}C NMR** δ 206.0, 83.2, 81.8, 61.6, 61.3, 49.9, 30.4, 26.7, 25.8, -4.67, -4.91. **HRMS** calcd for $C_{14}H_{26}O_4SiNa$ ($M+Na^+$) 309.1498, found 309.1506.

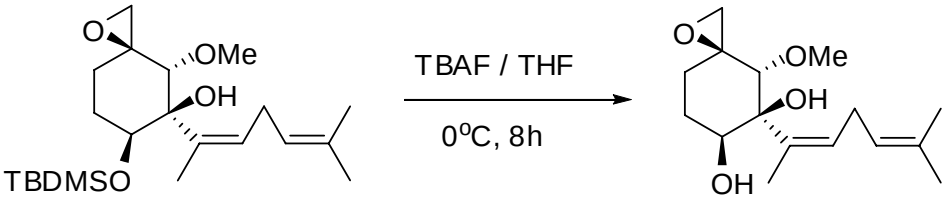
(3*S,4S*,5S*,6S*)-4-methoxy-5-(1',5'-Dimethylhexa-1',4'-dienyl)--6-(*tert*-butyldimethylsilyl oxy)-1-oxaspiro[2.5]octan-5-ol (1.126, HZ-9-20)**



To a solution of ketone **1.125** (25 mg, 0.087 mmol) in 1 ml toluene was added 1.46 ml of vinyl lithium (0.174mmol) at -78 ^{0}C under argon. This solution was stirred at -78 ^{0}C for 2 hours and slowly warmed to 0 ^{o}C, and then diluted with ether, washed with water and brine, dried over anhydrous magnesium sulfate. Column chromatography on silica gel using hexane and ethyl ether (2:1 v/v) afforded 24.8 mg (72.0% yield) of **1.126**. **^{1}H NMR** δ 5.75 (td, J= 7.0, 1.1Hz, 1 H,

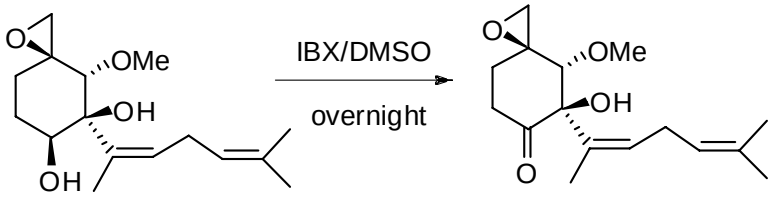
H-C=), 5.10 (m, 1 H, H-C=), 4.28 (m, 1H, H-COSi), 3.29 (s, 3 H, OMe), 2.75 (d, J=4.8, 1H, CH_2O), 2.74 (m, 2H, CH_2C=), 2.73 (s, 1 H, H-COMe), 2.32 (d, J=4.4, 1H, H-CHO), 2.28 (1H, OH), 2.26~1.84(m, 3H, $2CH_2$), 1.76 (s, 3H, CH_3C=), 1.67(s, 3H, CH_3C=), 1.62 (s, 3H, CH_3C=).1.30-1.10 (m, 1H, CH_2O). 0.84 (s, 9H, $3(CH_3)$-C), 0.08 (s, 3H, CH_3Si), 0.07 (s, 3H, CH_3Si); **^{13}C NMR** 138.1, 131.5, 126.1, 123.2, 88.5, 78.4, 70.1, 59.2, 56.4, 50.5, 28.0, 27.4, 26.6, 25.94, 25.87, 18.2, 17.9, 14.4, -3.11, -4.76; **HRMS** calcd for $C_{22}H_{44}O_4SiN$ ($M+NH_4^+$) 414.3040, found 414.3031.

(3*S, 4S*, 5R*, 6*S**)-5-((E)-1', 5'-Dimethylhexa-1', 4'-dienyl)-4-methoxy-1-oxaspiro[2.5]octane-5,6-diol (1.127, HZ-9-26)**



Compound **1.126** (11 mg, 0.028 mmol) was dissolved in 0.5 ml of THF and cooled to 0°C. To it, was added 56 µl of TBAF (1M, 0.056 mmol) and stirred at 0oC for 6 hours. The solution was diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. The crude NMR showed a clean deprotection with almost quantitative transformation. **^{1}H NMR** δ 5.74 (td, J= 7.3, 1.5 Hz, 1H, H-C=), 5.13 (tt, J = 7.3, 1.5 Hz, 1H, H-C=), 4.20 (m, 1H, H-COH), 3.29 (s, 3H), 2.80 (t, J=5.5 Hz, 2H, CH_2-C=), 2.79 (d, J = 4.4 Hz, 1H, CH_2O), 2.73 (d, J=4.4 Hz, 1H, CH_2O), 2.68 (s, 1H, H-COMe) 2.32-2.12 (m, 1H, CH_2), 1.95-1.83 (m, 2H, CH_2), 1.81 (s, 3H, CH_3), 1.69 (s, 3H, CH_3), 1.63 (S, 3H, CH_3), 1.22-1.12 (m, 1H, CH_2). **^{13}C NMR δ** 136.3, 132.1, 127.3, 122.9, 87.2, 78.9, 68.2, 58.9, 57.1, 51.4, 27.5, 27.0, 26.3, 25.9, 18.0, 13.8;

(3*S, 4S*, 5R*, 6*S**)-5-((E)-1', 5'-Dimethylhexa-1', 4'-dienyl)-4-methoxy-1-oxaspiro[2.5]octane-5-hydroxy-6-one (1.128)**



To a solution of compound **1.127** (6.0 mg, 0.021 mmol) in 0.5 ml of DMSO, was added 24.0 mg of IBX (0.084 mmol) and stirred at room temperature overnight. The reaction solution was diluted with ethyl ether, washed with water and brine, dried over magnesium sulfate and concentrated to dryness. The crude NMR showed a clean oxidation. Column chromatography using petroleum ether and ethyl ether (2:1) affords 4.1 mg of **1.128** (yield: 68.3%). **^{1}H NMR** δ 5.84 (t, J=5.3, 1H, H-C=), 5.08 (t, J=6.5, 1H, H-C=), 4.11 (s, 1H, H-COMe), 3.53 (s, 3H, OMe), 3.29 (d, J=5.3, OH), 2.83-2.60(m, 4H), 2.59-2.56 (m, 1H), 2.08-1.95(m, 1H), 1.86-1.80 (m, 1H), 1.70 (s, 3H, CH_3C=), 1.59 (s, 6H, $2CH_3C$=), 1.32-1.20 (m, 1H). **^{13}C NMR** δ 207.7, 132.8, 131.8, 130.7, 121.8, 86.4, 62.2, 58.7, 52.6, 51.5, 43.5, 35.0, 32.8, 28.9, 27.5, 25.9.

Reference

(1) Devlin, T. M. *Biochemistry*; Willey-Liss: New York, 2002.

(2) Bradshaw, R. A.; Arfin, S. M. *Methionine aminopeptidase: structure and function*; Aminopeptidases **1996**, 91-106.

(3) Lowther, W. T.; Matthews, B. W. *Structure and function of the methionine aminopeptidases*. Biochimica et biophysica acta **2000**, *1477*, 157-67.

(4) Brdlik, C. M.; Crews, C. M. *A Single Amino Acid Residue Defines the Difference in Ovalicin Sensitivity between Type I and II Methionine Aminopeptidases*; Journal of Biological Chemistry **2004**, *279*, 9475-9480.

(5) Chang, S. Y.; McGary, E. C.; Chang, S. *Methionine Aminopeptidase Gene of Escherichia coli is Essential for Cell Growth*; Journal of Bacteriology. **1989**, *171*, 6979-6983.

(6) Addlagatta, A.; Matthews, B. W. *Structure of the angiogenesis inhibitor ovalicin bound to its noncognate target, human Type 1 methionine aminopeptidase*; Protein Science **2006**, *15*, 1842-1848.

(7) Cane, D. E.; King, G. G. S. *The biosynthesis of ovalicin: isolation of b-trans-bergamotene*; Tetrahedron Letters **1976**, 4737-40.

(8) McCowen, M. C., Callender, M. E., Lawlis, J. F. *Fumagillin (H-3), a New Antibiotic with Amebicidal Properties*; Science **1951**, *113*, 202-203.

(9) Ingber, D., Fujita, T., Kishimoto, S., Sudo, K., Kanamaru, T., Brem, H., Folkman, J. *Synthentic Analogues of Fumagillin That Inhibit Angiogenesis and Suppress Tumour Growth*; Nature **1990**, *348*, 555-557.

(10) Lowther, W. T.; McMillen, D. A.; Orville, A. M.; Matthews, B. W. *The anti-angiogenic agent fumagillin covalently modifies a conserved active-site histidine in the Escherichia coli methionine aminopeptidase*. Proceedings of the National Academy of Sciences of the United States of America **1998**, *95*, 12153-12157.

(11) Griffith, E. C.; Su, Z.; Niwayama, S.; Ramsay, C. A.; Chang, Y.-H.; Liu, J. O. *Molecular recognition of angiogenesis inhibitors fumagillin and ovalicin by methionine aminopeptidase 2*. Proceedings of the National Academy of Sciences of the United States of America **1998**, *95*, 15183-15188.

(12) Liu, S. W., Joanne.; Kemp, Christopher W.; Crews, Craig M.; Clardy, Jon *structure of Human Methionine Aminopeptidase-2 Complexed with Fumagillin*. Science **1998**, *282*, 1324-1327.

(13) Folkman, M. J. *Angiogenesis in cancer, vascular, rheumatoid and other disease.* Nature medicine **1995**, *1*, 27-31.

(14) Folkman, M. J. *Angiogenesis and angiogenesis inhibition: an overview.* EXS **1997**, *79*, 1-8.

(15) Zhou, G.; Tsai, C. W.; Liu, J. O. *Fumagalone, a Reversible Inhibitor of Type 2 Methionine Aminopeptidase and Angiogenesis*. Journal of Medicinal Chemistry **2003**, *46*, 3452-3454.

(16) Bernier, S. G.; Lazarus, D. D.; Clark, E.; Doyle, B.; Labenski, M.T.; Thompson, C.D.; Westlin, W.; Hannig, G. *A Methionine Aminopeptidase-2 Inhibitor, PPI-2458, for the Treatment of Rheumatoid Arthritis*. Proceeding of National Academic Science **2004**, *101*, 10768-10773.

(17) Rupnick, M. A.; Panigrahy, D.; Zhang, C. Y.; Dallabrida, S. M.; Lowell, B. B.; Langer, R.; Folkman, M. J. *Adipose tissue mass can be regulated through the vasculature* Proceedings of the National Academy Sciences **2002**, *99*, 10730-10735.

(18) Brakenhielm, E.; Cao, R. H.; Gao, B. H.; Angelin, B.; Cannon, B.; Parini, P.; Cao, Y. H. *Angiogenesis Inhibitor, TNP-470, Prevents Diet-Induced and Genetic Obesity in Mice*; Circulation Research **2004**, *94*, 1579-1587.

(19) http://www.who.int/features/qa/52/en/.

(20) Magdalena, R.; Haubrich, T.A.; Perrault, J.; Mackey, Z.B.; McKerrow, J. H.; Chiang, P. K.; Wnuk, S. F. *Antitrypanosomal Activity of 5'-Deoxy-5'-(iodomethylene)adenosine and Related 6-N-Cyclopropyladenosine Analogues*; Journal of Medicinal Chemistry **2006**, *49*, 2096-2102.

(21) Zhang, P. N., Diarmuid E.; Bujnicki, J. M.; Su, X. Z.; Brendle, J. J.; Ferdig, M.; Kyle, D. E.; Milhous, W.; Chiang, P. K. *Angiogenesis Inhibitors Specific for Methionine Aminopeptidases 2 as Drugs for Malaria and Leishmaniasis*; Journal of Biomedical Science **2002**, *9*, 34-40.

(22) Chiu, C. H.; Lee, C. Z.; Lin, K. S.; Tam, M. F.; Lin, L. Y. *Amino Acid Residues Involoved in the Functional Intergrity of Escherichia coli Methionine Aminopeptidase.*; Journal of Bacteriology. **1999**, *181*, 4689-4689.

(23) Didier, P. J.; Phillips, J. N.; Kuebler, D. J.; Nasr, M.; Brindley, P. J.; Stovall, M. E.; Bowers, L. C.; Didier, E. S. *Antimicrosporidial activities of fumagillin, TNP-470, ovalicin, and ovalicin derivatives in vitro and in vivo*; Antimicrobial Agents and Chemotherapy **2006**, *50*, 2146-2155.

(24) Upadhya, R.; Zhang, H. S.; Weiss, L. M. *System for expression of microsporidian methionine amino peptidase type 2 (MetAP2) in the yeast Saccharomyces cerevisiae*; Antimicrobial Agents and Chemotherapy **2006**, *50*, 3389-3395.

(25) Sigg, H. P.; Weber, H. P. *Isolation and structural elucidation of ovalicin*; Helvetica Chimica Acta **1968**, *51*, 1395-1408.

(26) Sassa, T.; Kaise, H.; Munakata, K. *Structure of graphinone*; Agricultural and Biological Chemistry **1970**, *34*, 649-51.

(27) Corey, E. J.; Dittami, J. P. *Total synthesis of (+-)-ovalicin*; Journal of the American Chemical Society **1985**, *107*, 256-7.

(28) Corey, E. J.; Guzman-Perez, A.; Noe, M. C. *Short Enantioselective Synthesis of (-)-Ovalicin, a Potent Inhibitor of Angiogenesis, Using Substrate-Enhanced Catalytic Asymmetric Dihydroxylation*; Journal of the American Chemical Society **1994**, *116*, 12109-10.

(29) Barton, D. H. R.; Bath, S.; Billington, D. D.; Gero, S. D.; Quiclet-Sire, B.; Samadi, M. *Total synthesis of (-)-ovalicin and analogs from L-quebrachitol*; Journal of the Chemical Society, Perkin Transactions 1: Organic and Bio-Organic Chemistry **1995**, 1551-8.

(30) Bath, S.; Billington, D. C.; Gero, S. D.; Quiclet-Sire, B.; Samadi, M. *Total synthesis of (-)-ovalicin from L-quebrachitol*; Journal of the Chemical Society, Chemical Communications **1994**, 1495-6.

(31) Barco, A.; Benetti, S.; De Risi, C.; Marchetti, P.; Pollini, G. P.; Zanirato, V. *Enantioselective formal synthesis of (-)-ovalicin using quinic acid as a chiral template*; Tetrahedron: Asymmetry **1998**, *9*, 2857-2864.

(32) Takahashi, S.; Hishinuma, N.; Koshino, H.; Nakata, T. *Synthesis of Ovalicin Starting from D-Mannose*; Journal of Organic Chemistry **2005**, *70*, 10162-10165.

(33) Tiefenbacher, K.; Arion, V. B.; Mulzer, J. *A Diels-Alder approach to (-)-ovalicin*; Angewandte Chemie, International Edition **2007**, *46*, 2690-2693.

(34) Zhang, Y.; Griffith, E. C.; Sage, J.; Jacks, T.; Liu, J. O. *Cell cycle Inhibition by the anti-angiogenic agent TNP-470 is mediated by p53 and p21$^{WAF/CIPI}$* Proceeding of National Academic Science **2000**, *97,* 6427-6432.

(35) McGill, J. W. *Design and synthesis of optically pure 8,8'-disubstituted 1,1'-binaphthyls and assessment of their applicability as chiral ligands for asymmetric Heck cyclization reactions* Kansas State University, Manhattan, KS, **2005**.

(36) Battina, S. K. *Syntheses and bioevaluation of Novel Tricyclic Pyrone Compounds and Ovalicin and its Analogues*; Kansas State University: Manhattan, KS, **2007**.

(37) Jimenez-Garate, A. L. *Syntheses and bioevaluation of Novel Tricyclic Pyrone Compounds and Ovalicin and its synthetic intermediates* Kansas State University: Manhattan Kansas, **2006**.

(38) Adlington, R. M.; Barrett, A. G. M. *Concise syntheses of 3-methylenetetrahydrofuran-2-one derivatives and related systems.* ; Journal of the Chemical Society, Perkin Transactions 1: Organic and Bio-Organic Chemistry **1981**, *11*, 2848-2863.

(39) Adlington, R. M.; Barrett, A. G. M. *Recent applications of the Shapiro reaction.*; Accounts of Chemical Research **1983**, *16*, 55-59.

(40) Mackey, Z. B.; Baca, A. M.; Mallari, J. P.; Apsel, B.; Shelat, A.; Hansell, E. J.; Chiang, P. K.; Wolff, B.; Guy, K. R.; Williams, J.; McKerrow, J. H. *Discovery of trypanocidal compounds by whole cell HTS of Trypanosoma brucei*; Chemical Biology & Drug Design **2006**, *67*, 355-363.

(41) Chiang, P. K.; Bujinicki, J. M.; Su, X. Z.; Lanar, D. E. *Malaria: therapy, genes and vaccines.* ; Current Molecular Medicine **2006**, *6*, 309-326.

(42) Miki, T.; Nonomura, N.; Nozawa, M.; Harada, Y.; Nishimura, K.; Kojima, Y.; Takahara, S.; Okuyama, A. *Angiogenesis inhibitor TNP-470 inhibits growth and metastasis of a hormone-independent rat prostatic carcinoma cell line*; J. Urology, 1998, 160, 210-213 **1998**, *160*, 210-213.

(43) Farinelle, S.; Malonne, H.; Chaboteaux, C.; Decaestecker, C.; Dedecker, R.; Gras, T.; Darro, F.; Fontaine, J.; Atassi, G.; Kiss, R. *Characterization of TNP-470-induced modifications to cell functions in HUVEC and cancer cells*; J. Pharmacol. & Toxicol. Methods **2000**, *43*, 15-24.

Chapter 2 Bio-Based Polymers from Vegetable Oil

2.1 Introduction

2.1.1 Polymers and Bio-based polymers

Polymers have been with us since the beginning of time. Natural polymers such as proteinaceous fibers of wool and silk, carbohydrate fibers of flax and cotton, as well as tree saps that produce amber and latex have a long history of applications.[1] As a science, it can be traced back to the mid-19th century when Charles Goodyear transformed sticky latex of natural rubber to an elastomer applied in tire use. After World War II, with the explosive development of modern synthetic chemistry and analytical instruments, petroleum-based synthetic polymers and polymeric composites such as plastics, adhesives, foams, elastomers and coating materials are penetrating into every corner of the modern society. They are extensively applied in the aerospace, automotive, infrastructure, marine, sports, military, and other industrial fields and have no counterpart in terms of weight, ease of fabrication, utilizing efficiency and economics.[1,2] However, the conflict between the expanding applications of polymeric materials and the finite petroleum oil reserve cause the oil price to soar in an astonishing manner. Combined with the environmental issues resulted from petroleum-based chemistry, such as pollution and global warming, more and more renewed interest is shifting back to natural and renewable resources to support a sustainable development.[3-5]

Plant oils, or vegetable oils, are mass-produced renewable resources derived from many crops.[6] These refined oils are predominantly made up of triglyceride molecules whose structure is shown in Figure 2.1. These molecules are composed of three fatty acids and a glycerol linked through ester functionalities. The structures of fatty acids vary from 14 to 22 carbons in length and 0 to 3 double bonds per molecule. Table 2.1 shows the distribution of fatty acids in various plant oils.[3] Due to the structural diversity of fatty acids, plant oils are composed of a variety of different triglycerides with many levels of unsaturation. With the development of genetic engineering techniques, the unsaturation level in plant oils, such as soybean, flax and corn, can been controlled according to the desire of applications. For example, High oleic developed genetically by Dupont consists mainly of 82.6% of oleic acid.

Figure 2.1 The representative triglyceride structure in plant oils

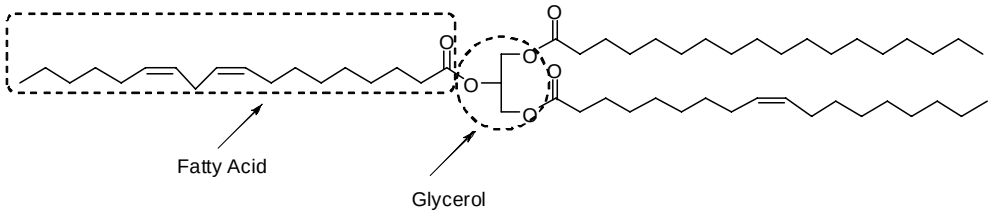


Table 2.1 Fatty acid distribution in various plant oils[3]

Oil	C/C=C	Canola	Corn	Cottonseed	Linseed	Olive	Palm	Rapeseed	Soybean	High Oleic[a]
Myristic	14:00	0.1	0.1	0.7	0.0	0.0	1.0	0.1	0.1	0.0
Myristoleic	14:01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Palmitic	16:00	4.1	11.5	21.6	5.5	16.9	46.8	3.0	10.5	6.4
Margaric	17:00	0.1	0.1	0.1	0.0	0.0	0.0	0.2	0.0	0.0
Margarolic	17:01	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Stearic	18:00	1.8	2.2	2.6	3.5	2.7	3.8	1.0	3.2	**3.1**
Oleic	**18:01**	**60.9**	**26.6**	**18.6**	**19.1**	**61.9**	**37.6**	**13.2**	**22.3**	**82.6**
Linoleic	**18:02**	**21**	**58.7**	**54.4**	**15.3**	**14.8**	**10**	**13.2**	**54.5**	**2.3**
Linolenic	**18:03**	**8.8**	**0.8**	**0.7**	56.6	**0.6**	**0.4**	**9.0**	**8.3**	**3.7**
Arachidic	20:00	0.7	0.2	0.3	0.0	0.4	0.2	0.5	0.2	0.2
Gadoleic	**20:01**	**1.0**	**0.0**	**0.0**	0.0	**0.1**	**0.3**	**9.0**	**0.0**	**0.4**
Eicosanoic	**20:02**	**0.0**	**0.0**	**0.0**	0.0	0.0	**0.0**	**0.7**	**0.0**	**0.0**
Behenic	**22:00**	**0.3**	**0.1**	**0.2**	0.0	0.0	0.0	**0.5**	**0.1**	**0.3**
Erucic	**22:01**	**0.7**	**0.0**	**0.0**	0.0	0.0	0.0	**49.2**	**0.0**	**0.1**
Lignoceic	**24:00**	**0.2**	**0.0**	**0.0**	0.0	0.0	0.0	**1.2**	**0.0**	**0.0**
Average #		**3.9**	**4.5**	**3.9**	**6.6**	**2.8**	**1.80**	**3.8**	**4.6**	**3.0**

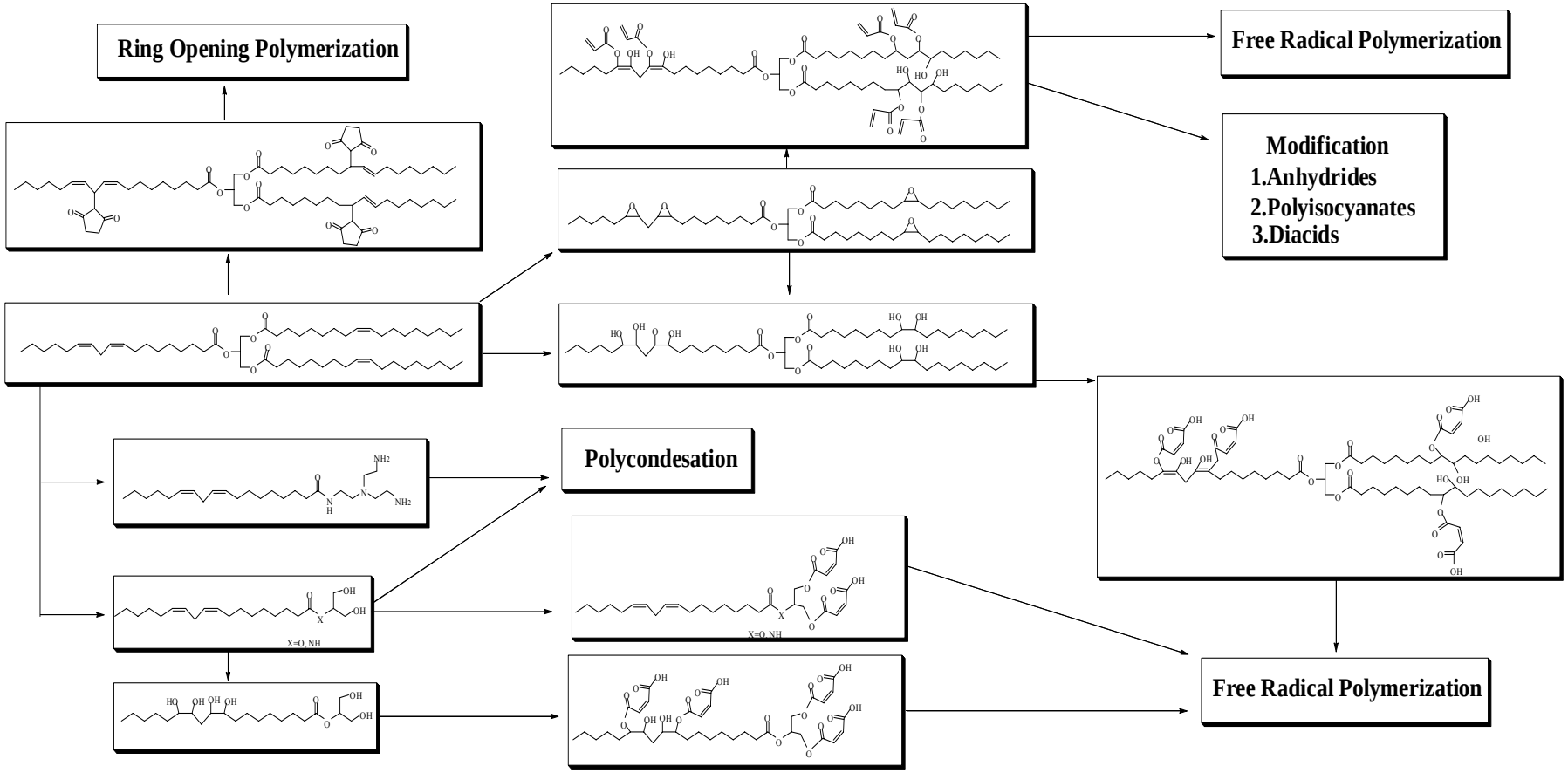
[a] Genetically engineered soybean with high oleic acid content (Dupond)

Currently, the major use of plant oils is in food industry as essential component of human diet.[6] They also found applications in paints, inks, resins, plasticizers/stabilizers, lubricants, thermoplastics, agrochemicals and pharmaceuticals[6]. In order to find more application in polymer fields, these natural plant oils need to be functionalized. The active sites liable to chemical reaction include the double bond, the allylic carbons, the ester group and the carbon next to the ester group. The strategy of these structural modifications is to introduce polymerizable groups on the triglyceride and the purpose of these modifications is to reach a

higher level of average molecular weight (Mw) and cross-link density, along with the incorporation of chemical functionalities known to improve stiffness in a polymer network. Currently, the structural modification mainly focused on the double bonds.[3]

Figure 2.2 illustrates several synthetic pathways from different labs to achieve this purpose. The internal doubles of triglycerides can be chemically functionalized the to maleates[7,8] or epoxy or hydroxyl functionalities[9] to go through polymerization via ring-opening or polycondensation. The derived epoxy can be further transformed to acrylates by acrylic acid[10,11] while the hydroxyl groups can be transformed to maleate half-esters and esters.[7,8] These monomers then can be blend with reactive diluent and cured by free radical polymerization.[3] The three-chain triglyceride was also hydrolyzed to monoglyceride and then similarly, go through polycondensation after attaching dihydroxyl or diamine groups, or free radical polymerization after introducing maleic half-esters.[9] Petrovic *et al* functionalized the olefin on the side chains into a family of polyols[12-14] which further reacted with isocyanates to form polyurethanes.[14,15] These polyurethanes have potential applications such as rigid materials or foam insulation, carpet backing etc. Cured with different chemicals such as diacids, different anhydrides, polyamines, and citric acids, epoxidized oils are transformed to soft rubber, soft to rigid thermosets or composites, and coating materials in labs around the world.[3] Many of the plant oil-based polymers are now used commercially or are in the process of commercialization.[3,16]

Figure 2.2 Chemical modification of triglycerides leading to polymerizations[3]



2.1.2 Plant oil Based Polyurethanes

Plant oil based polyurethanes are attracting growing world-wide interest driven by economical and environmental incentives. Comparing to the finite reserve and soaring price of crude fossil oil, plant oils are renewable resource and the price is relatively inexpensive. In addition, due to the hydrophobic nature of triglyceride, these bio-based polyurethanes have comparable chemical and physical properties with petroleum-based polyurethanes. They can be applied in the production of polyurethane foams for insulation, elastomers, coatings, *etc*. [12-14,17]

In order to produce these bio-based polyurethanes, plant oils have to be transformed to triglyceride derived polyols before reacting with different diisocyanates in the presence of suitable catalysts. In most cases, hydroxyl groups are introduced at the internal olefin positions using different strategies such as direct ozonolysis,[18] hydroformylation[12] or hydrogenation of epoxidized plant oil,[13] *et al*.

As mentioned above, plant oils such as vegetable oils are heterogeneous materials composed predominantly by unsaturated fatty acids, the composition of these fatty acids varies not only from oil to oil, but also within the same oil, depending on the weather condition, type of soil, and so on.[6] When these complicated materials are converted to polyols, the heterogeneity of polyols could be even more complicated. Therefore, it is not so easy to elucidate the relationship between the chemical structures of polyols and properties of the resulting polymer networks.

In order to gain insight into the structure and properties of plant oil based polyurethanes and initiate our study, we simplified the triglyceride by esterifying oleic acid with glycerol. This synthetic model triglyceride has evenly distributed unsaturation in the middle of every side chain. Through the study of this model compound, we expect to get preliminary information on the relationship between polyol structure and polymer properties and then apply this knowledge in the study of authentic plant oil based polyurethanes.

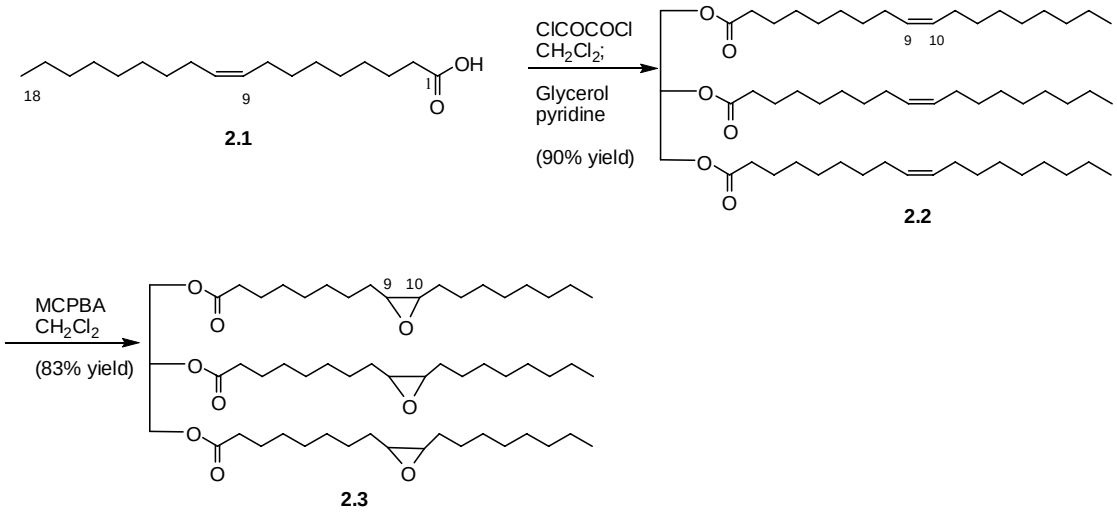
2.2 Synthesis and Property Analysis

2.2.1 Syntheses of Functionalized Triglycerides.

The strategies we applied were to transform the alkenyl groups on fatty acid chains of glycerol trioleoate to hydroxyl and amino functionalities, then these polyols or polyamines reacted with 1,4-phenylene diisocyanate to produce cross-linked polymers.

The model triglyceride **2.2** (glycerol trioleoate) was prepared as following: oleic acid (**2.1**) was transformed to carbonyl chloride with oxalyl chloride in dichloromethane, the freshly prepared chloride was vacuum-dried to remove the excessive oxalyl chloride before esterification with glycerol and pyridine (90%, 2 steps) (Scheme 2.1). Epoxidation of triglyceride **2.2** was initially achieved by using 3.3 equivalents of *m*-chloroperbenzoic acid (MCPBA) in dichloromethane at 0^{o}C, which afforded triepoxide **2.3** with 83% yield. Epoxidation with formic acid and hydroperoxide in methylene chloride at 0^{o}C gave similar result while the later method using much cheaper reagents.

Scheme 2.1

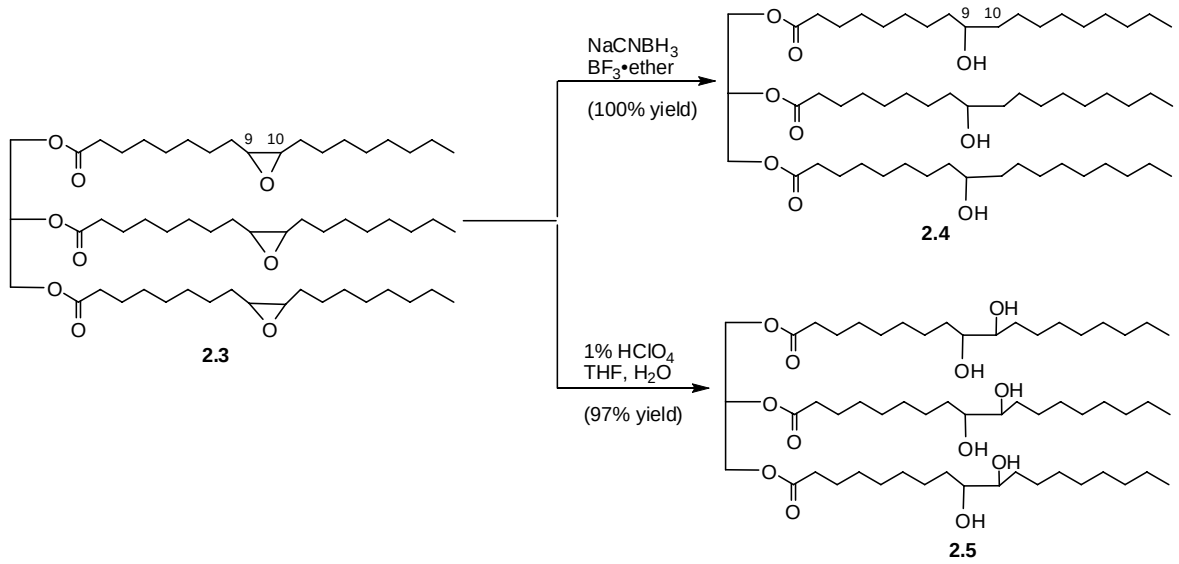


The triepoxide **2.3** was reduced to triol **2.4** with sodium cyanoborohydride in THF in the presence of boron trifluoride as Lewis acid (Scheme 2.2).[19,20] It's very difficult to determine the regiochemistry of the hydroxyl functions of triol **2.4** from ^{1}H and ^{13}C NMR, because the difference of chemical environment between the hydrogen and carbon of C9 and C10 is so similar. Most likely, the triol is a mixture of several regio-isomers with hydroxyl groups

distributed on C9 or C10 position of three side chain randomly. It is worth mentioning that during the synthesis of triol **2.4**, the adding sequence of reagents to the solution of epoxy **2.3** in THF is very important. If Lewis acid boron trifluoride was added to the solution before sodium cyanoborohydride, little or no desired triol can be obtained. ^{1}HNMR of crude material indicated that THF might attack the epoxy with the assistance of Lewis acid BF_3 and result in a sticky liquid. It could not develop on TLC or be purified by column chromatography, which suggested that it could be a polymeric material.

The epoxide-ring opening with diluted hydrochloride and sulfuric acid did not give an acceptable result, partially because of the nucleophilic attack of the ester by chlorine and sulfuric anions in the presence of hydrogen cations. However, when 1 % perchloric acid in THF-H_2O was used to open the epoxide ring, it exclusively gave hexaol **2.5** (Scheme 2.2).[19] Both of triol **2.4** and hexaol **2.5** can be purified by recrystallization using a solution mixture of petroleum ether and ethyl acetate.

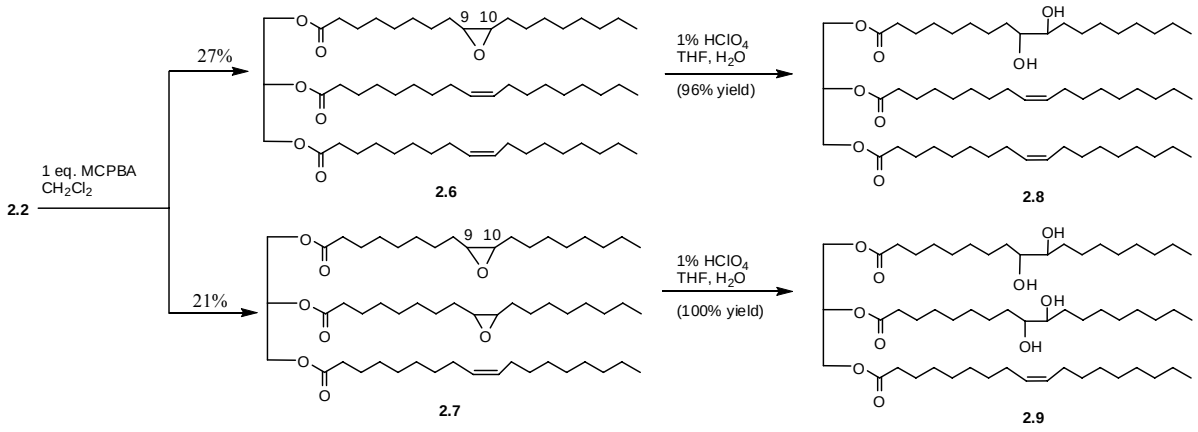
Scheme 2.2



It should be of interest to study the effect of the number of unpolymerized side-chain(s) to the properties of resulting polymers. For this purpose, diol **2.8** with two alkenyl side chain and tetraol **2.9** with one alkeny side chain were synthesized. Hence, reaction of compound **2.2** with 1 equivalent of MCPBA in dichloromethane afforded monoepoxide **2.6** and diepoxide **2.7** along

with small amount of triepoxides and recovered starting material **2.2**. Column chromatographic separation of the crude product gave pure monoepoxide **2.6** and diepoxide **2.7**. The regio-chemistry of the epoxy functions of **2.6** and **2.7** were assumed, it could be on either external or internal side-chain. Hydrolytic ring opening of **2.6** with perchloric acid in THF and water (3:2) provided diol **2.8** with 96% yield. Diol **2.8** possesses two hydroxyl functions on one of the three side chains and no hydroxyl function on the remaining two side chains. Hydrolytic ring opening of **2.7** with 1% perchloric acid in THF and water (3:2) provided tetraol **2.9** with 100% yield. Tetraol **2.9** possesses two hydroxyl functions on each of the two side chains and no hydroxyl function on the remaining one side chain. Since the regio-chemistry of epoxides **2.6** and **2.7** were not determined, the distribution of dihydroxyl groups on compound **2.8** and **2.9** may not represent the real structures, they could be on glyceride C1 or C2 side-chain.

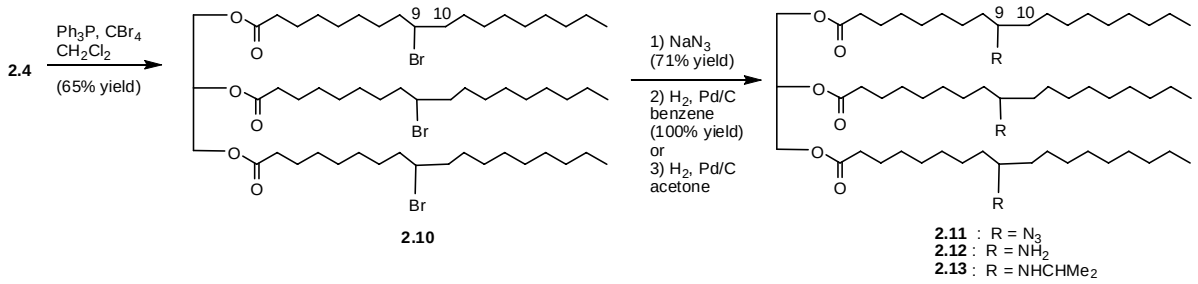
Scheme 2.3



Triamines **2.12** and **2.13** were also synthesized in order to produce polymers containing urea functionality. Initially, we tried to use direct olefin hydroamination to introduce the amine functionalities, but we didn't get satisfactory result and then we turn to stepwise tansformation (Scheme 2.4). Bromination of triol **2.4** with 3 equivalents of triphenylphosphine and 3 equivalents of carbon tetrabromide[21] afforded triboromide **2.10**. The following bromo-displacement with sodium azide gave triazido triglyceride **2.11**. Treatment of azide **2.11** with a catalytic amount of 10% Pd/C in benzene or ethyl acetate under 30 psi of H_2 gave triamine **2.12** in quantitative yield. A one-pot reduction-amination-reduction of azide **2.11** by the treatment with 30 psi of H_2 in the presence of 10% Pd/C in acetone gave triisopropylamine **2.13**. The azide

2.11 was first reduced to amine, then condensed with acetone (solvent) to form imine followed by reductive hydrogenation to afford amine **2.13**. Since the position of hydroxyl group of compound **2.4** was not determined, all of the amine functionalities of compound **2.12** and **2.13** could be on C9 or C10 positions.

Scheme 2.4

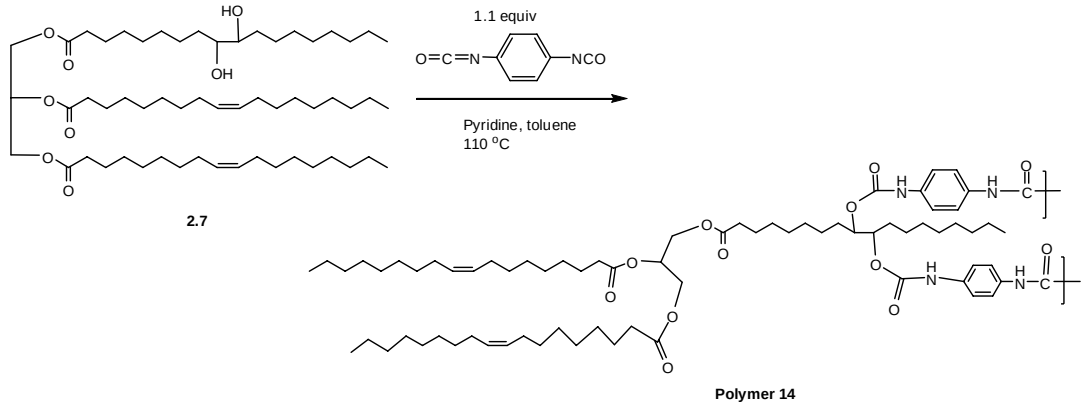


2.2.2. Syntheses of Polymers.

There were a variety of diisocyantes reported to form urethane by reacting with hydroxyl compounds,[15] in this lab, we used 1,4-phenylene diisocyanate (PDI)[22] as the cross-linking agent to synthesize polyurethane polymers from aforementioned triglyceride monomers with multi-hydroxyl or multi-amine functionalities. The presence of aromatic segment in PDI linker could enhance the stiffness in the resulting polymers.[3]

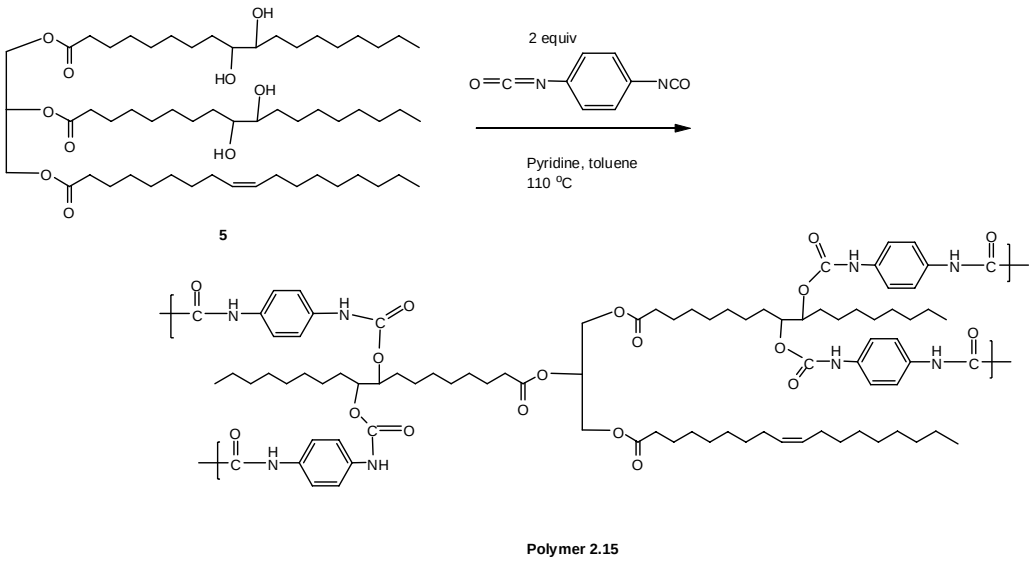
Treatment of diol **2.7** with 1 equiv of PDI and a catalytic amount of pyridine in refluxing toluene (Scheme 2.5) provided a light brown solid polymer **2.14 a**fter removal of pyridine and toluene, being washed with methylene chloride. The soluble portion of the lower molecular weight polymer of **2.14** was subjected to NMR study, its proton NMR spectrum showed a broad CH signal attached to carbamate function (polymer **2.14**) while the signature signals of the starting material **2.7** (CH attached to OH) cannot be observed in the ^{1}H NMR spectrum. The broadened peak (comparing to that of starting material **2.7**) at ~1680 of FTIR spectroscopy of insoluble polymer also indicated the formation of carbamate function.

Scheme 2.5



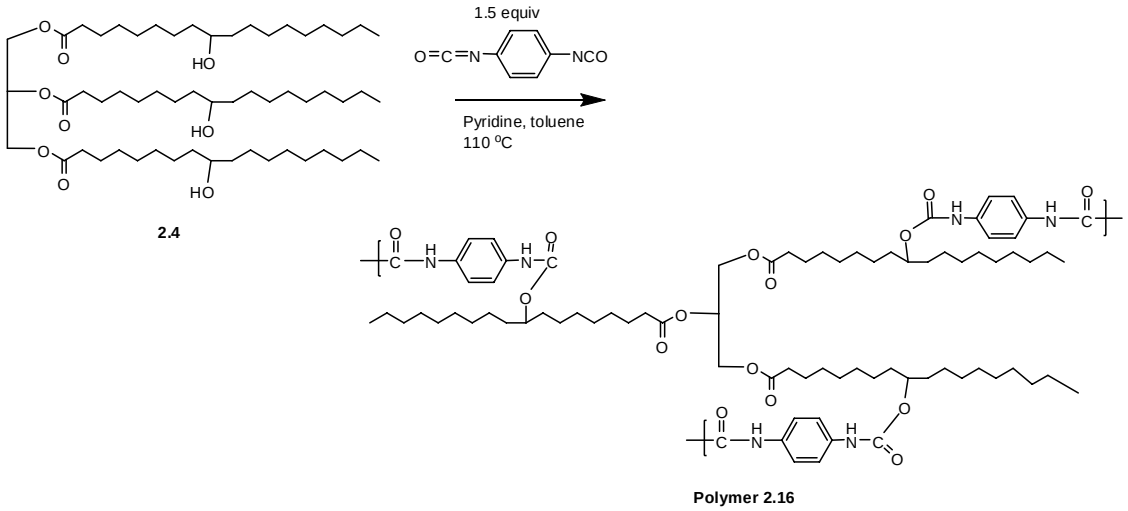
Polymer **2.15** derived from tetraol **2.8** was synthesized by the treatment of compound **2.8** with 2 equivalents of PDI and a catalytic amount of pyridine under refluxing toluene for 4 hours (Scheme 2.6). The crude material was filtered from toluene mixture after cooled to room temperature and immerse in chloroform to get rid of the soluble portion. Again, ^{1}H NMR spectrum of the soluble materials (lower molecular weight polymers; formed in a very small amounts) showed the presence of a broad CH signal attached to carbamate function and the disappearance of CH(OH) signal (at ~ δ 3.56 ppm). The broadened peak at ~1680 of FTIR spectroscopy was also been observed.

Scheme 2.6



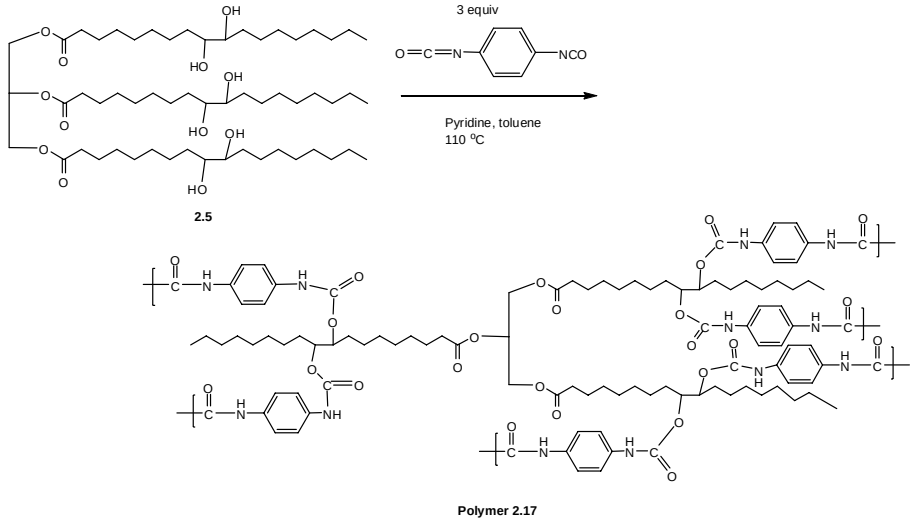
Polymer **2.16** was derived from triol **2.4**. It was synthesized by the treatment of compound **2.4** with 1.5 equivalents of PDI and a catalytic amount of pyridine under refluxing toluene for 4 hours (Scheme 2.7). The crude polymer was separated from toluene solvent by filtration after cooling the mixture to room temperature, then it was immerse in chloroform overnight to wash out the remaining soluble portion. Just as expected, ^{1}H NMR spectrum of the soluble materials (lower molecular weight polymers) showed the presence of a broad CH signal attached to carbamate function and the absence of CH(OH) signal (at ~ δ 3.56 ppm). Similar, the broadened peak at ~1680 was observed by FTIR.

Scheme 2.7



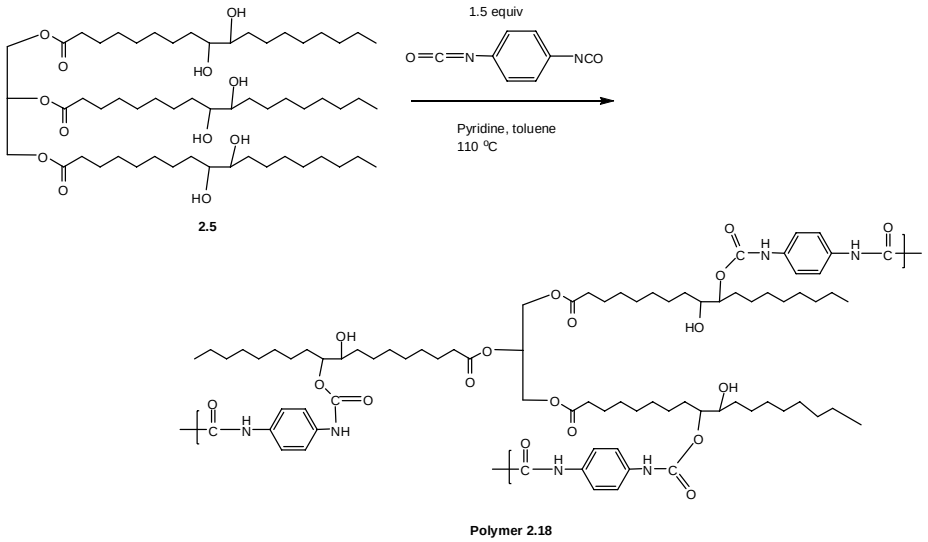
Polymer **2.17** was prepared by treating hexaol **2.5** with 3 equivalents of PDI in the presence of catalytic amount of pyridine in refluxing toluene for 4 hours (Scheme 2.8). The insoluble polymer was observed shortly after the beginning of refluxing. After the similar workup of filtration and overnight extraction, the highly cross-linked polymer **2.17** was obtained. This material was highly fragile. The tiny amount of soluble portion was also subjected to NMR study and showed same result as that of other polymers. The solid FTIR of the polymer showed a broaden peak at ~1680 cm^{-1} without surprise.

Scheme 2.8



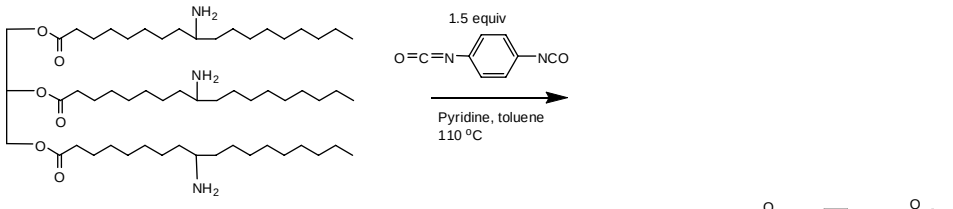
Following the same procedure as that of preparation of polymer **2.17,** polymer **2.18** was also synthesized in order to study the effect of OH/NCO molar ratio on properties of resulting polyurethane networks.[23] In this case only 1.5 equivalent of PDI was used and thus only half of the hydroxyl functions were expected to be consumed and another half would remain in the consequent polymer system. The structure of polymer with evenly distributed linkers on the side chains in Scheme 2.9 was arbitrary assigned and may not be consistent to the real structure of the resulting polymer **2.18**, since it was highly possible that both of the hydroxyl groups on the same side chain could react with PDI linkers.

Scheme 2.9



Formation of polyurea **2.19** and **2.20** were carried out by treating triamines **2.12** and **2.13** with 1.5 equivalent of PDI (Schemes 2.10 and 2.11). Due to the presence of amine functionality in the monomers, no pyridine catalyst was used in these two reactions. ^{1}HNMR analysis of the soluble potion (lower molecular weight polymers) of polymer **2.19** and **2.20** showed no proton signals of the methine CH hydrogens attached to amino functions (δ 2.67 and 2.88 ppm, respectively), instead, we observed proton signals at δ ~3.7 ppm, attributed to the methine hydrogens attached to urea functions. These observations indicated the formation of urea functionalities in polymer **2.19** and **2.20**.

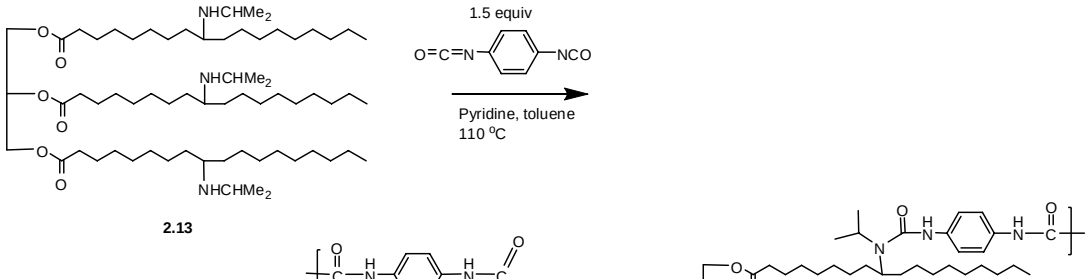
Scheme 10



2.12

Polymer 2.19

Scheme 11



2.13

Polymer 2.20

2.2.3 Measurement of Physical Properties:

Before the thermal and viscoelastic properties measurements of these polymers, we first measured gel content and swelling value to qualitatively compare the cross-linking density of these polymers since polymer properties are largely related to the cross-linking density.

The gel content and swelling value measurements were only carried out once, hence significant digit can not be reported. Experimental errors may exist.

2.2.3.1 Gel Content Measurement

Gel fraction refers to the insoluble portion of the crude polymers. The number of gel fraction may suggest the degree of cross-linking in polyurethane and polyurea networks. The general procedure could be described as following: [24,25] *x* mg of monomer and *y* mg of PDI linker was refluxed in toluene (with or without pyridine) for 4 hours, cooled to room temperature, filter, filtrate was reserved; solid was immersed in methylene chloride overnight and filtered, the filtrate was combined with reserved filtrate obtained from the reactions, concentrated and vacuum-dried overnight and got the weight *z* mg, solid was vacuum-dried overnight and got the weight *m* mg and then refluxed in chloroform for 3 h. The mixture was cooled to room temperature and filtered; filtrate was concentrated, vacuum-dried and got the weight *n* mg, solid was vacuum-dried and reserved for property test. The polymer yield and gel content were measured according the following equations:

$$\text{Polymer yield} = (x+y-z)/(x+y)*100\%$$

$$\text{Gel content} = (m-n)/m*100\%$$

Overall yield is calculated based on the final gel material against starting materials; it may indicate the polymerization efficiency to form insoluble gel materials.

$$\text{Overall yield} = \text{Polymer yield} * \text{Gel content}$$

Table 2.2 Polymer yields and gel contents

	Polymer **2.14**	Polymer **2.15**	Polymer **2.16**	Polymer **2.17**	Polymer **2.18**	Polymer **2.19**	Polymer **2.20**
Polymer yield	64%	90%	83%	95%	94%	90%	86%
Gel content	77%	94%	91%	96%	97%	95%	92%
Overall yield	49%	84%	76%	91%	91%	85%	79%

Table 2.2 indicates that the gel content is largely related to the number of functionality presented in the monomers. The diolpolymer **2.14** had the lowest gel content (overall yield: 49.1%) because the monomer **2.8** only have two hydroxyl functionalities, the polymerization can be easily terminated by forming dimer (two diol molecules reacted with two PDI molecules) and other oligomers. These low molecular weight portions could have bigger solubility in solvents such as toluene and chloroform and could be removed from the polymer network with ease. The gel content of polymer **2.15**, **2.16**, **2.17** seemed related to the number of hydroxyl functionality in the monomer, the more hydroxyl groups; the higher is the overall gel content. Particularly for polymer **2.15**, although it has a free side chain tangling in the polymer network, the four hydroxyl groups on two side chains ensure the resulting polymer large enough to be insoluble in most solvent via extended cross-linking. For polymer **2.18**, although only half of the hydroxyl groups of monomers were polymerized, it had almost the similar gel content as that of polymer **2.17**, this result further indicated that the gel content related to the number of hydroxyl functionality presenting in monomers, the more the hydroxyl functionality, the harder is the quench of the polymerization. For polyurea **2.20**, the polymer yield, gel content and overall yield are similar to that of polymer **2.16**, this may due to the fact that both starting monomers **2.16** and **2.20** only have one functionality on each side chain. A little surprisingly, polymer **2.19** has 85% overall yield, which is higher than that of the similar polymer **2.20**.

2.2.3.2 Swelling Value Measurements

The swelling value of a polymer is applied to measure the cross-linking density qualitatively; there is an inversed relationship between the swelling value and the cross-linking density, the higher the cross-linking density, the lower the polymer swelling capability. The swelling value is calculated as the ratio between the weight of swollen gel polymer and the dry weight of the dry gel polymer. However, swelling value is highly solvent dependent, different solvent system gives different swelling valve. In this lab, swelling experiments were carried out in toluene at room temperature at defined period[26] Since the diol polymer formed sticky gel-like mixture in toluene, partially because of solubility of the two dangling hydrophobic side chain in the nonpolar solvent, the swelling value is not available. The results of the swelling value of other polymers are summarized in Table 2.3.

Table 2.3 Swelling Values of Polymer Networks in Toluene

Swelling Value	Polymer **2.15**	Polymer **2.16**	Polymer **2.17**	Polymer **2.18**	Polymer **2.19**	Polymer **2.20**
Day 1	3.2	9.4	3.0	3.0	4.8	9.7
Day 2	3.4	9.8	3.0	3.0	4.8	9.7
Day 4	3.4	9.6	3.1	2.9	4.7	9.9

Among all the polymers measured in this lab, triol polymer **2.16** and secondary triamine polymer **2.20** have the highest swelling value of 9.4 and 9.7, this may because both of the monomers only have one functionality on each of the side chain, thus each arm can spread out freely when the monomer is polymerized and the resulting network extends as wide as it can to avoid steric confliction. Therefore, there is much more free space in the polymer network to accommodate toluene solvent, which results in the bigger swelling value. If there are two hydroxyl groups on each side chain (polymer **2.15**, **2.17**), the inner space of the network was reduced by extra cross-linking groups, which results less free space to accommodate toluene solvent and gives smaller swelling value. The one tangling side chain in polymer **2.15** seems only slightly affect the cross-linking density comparing to polymer **2.17**. The similar swelling value of polymer **2.18** as that of **2.17** may suggest that considerable portion of the cross-linking happened on the same side-chain (both of the two hydroxyl groups on the same side chain participate in the cross-linking). Surprisingly, polymer **2.19**, which should have the similar polymeric structure as polymer **2.16** and **2.20**, has a swelling value of 4.8 between that of polymer **2.16** (9.4) and **2.17** (3.0). The possible explanation may attribute to the extra NH group in polymer **2.19**, which could form extra hydrogen bonding between the urea back-bone, thus resist the invasion of toluene molecule into the polymer network and result in the lower swelling value than that of polymer **2.20**.

The small difference of swelling values between day 1 and day 2 or day 4 indicates that all the polymers reach their maximum swelling capacity after 24 hours. The irregular fluctuations of swelling values of polymer **2.16** and **2.18** may be caused by experimental errors.

2.2.3.3 Thermal and Viscoelastic Analysis:

Thermal and viscoelastic properties of these polymers were measured by Dr. Jiangfeng Zhang in Dr. Susan Sun's lab in the Department of Grain Science, Kansas State University using differential scanning colorimetry (DSC), thermogravimetric analysis (TGA) and dynamic mechanical analysis (DMA). These data provide useful information of the polymers for future material applications. We acknowledge the helpful collaboration of Dr. Zhang and Professor Sun.

1. Thermogravimetric Analysis:

TGA is commonly employed in polymer characterization to reveal the thermal stability of polymer samples by measuring the relation between changes in weight and changes in temperature.

The thermogravimeter (for example: PerkinElmer Pyris 1 TGA) usually consists of a high accurate balance with a small pan. The pan loaded with sample (usually 3~5 mg) is placed in a small electrically heated furnace with a thermocouple to precisely measure the temperature. The atmosphere in the furnace is purged with inert gas such as nitrogen or argon during the process to prevent oxidation or other undesired reactions. This instrument is usually controlled by a computer program and experiment is carried out by gradually raising the temperature. The analysis is accomplished by plotting the remaining weight against temperature.

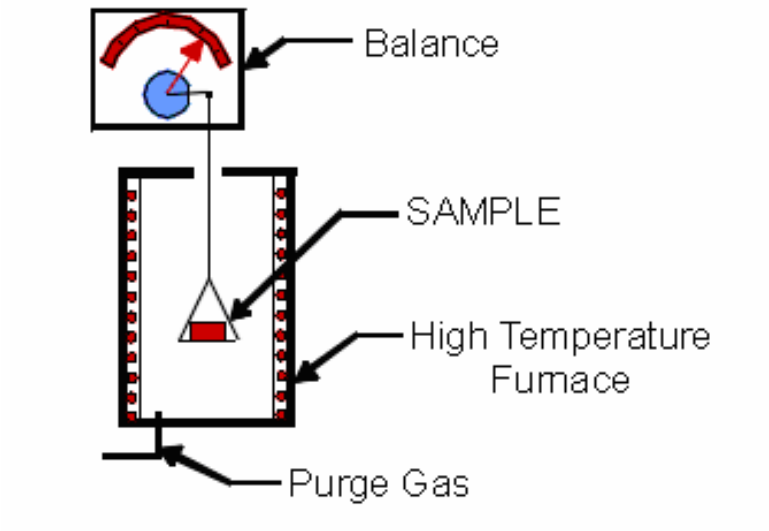


Figure 2.3 Schematic illustration of thermogravimetric instrument. (Taken from reference 25 without permission)[27]

As a family, polyurethanes usually show relatively low stability. This may associate to the chemical property of urethane bonds. Following is the three possible mechanisms proposed for decomposition of urethane bonds:[13]

1. Dissociated to isocyanate and alcohol:

   $RHNCOOR' \rightarrow RNCO + HOR'$

2. Decomposition to primary amine and alkene, along with carbon dioxide

   $RHNCOOCH_2CH_2R' \rightarrow RNH_2 + CH_2{=}CHR' + CO_2$

3. Formation of secondary amine

   $RHNCOOR' \rightarrow RHNR' + CO_2$

Polyurethane degradation usually initiated with dissociation mechanism, followed by evaporation of carbon dioxide and isocyanate.[28] From Figure 2.4, the early stage of the weight loss may relate to evaporation of carbon dioxide considering the feasibility and the low molecular weight. The first dramatic weight loss may related to the evaporation of dissociated diisocyanate at high temperature.

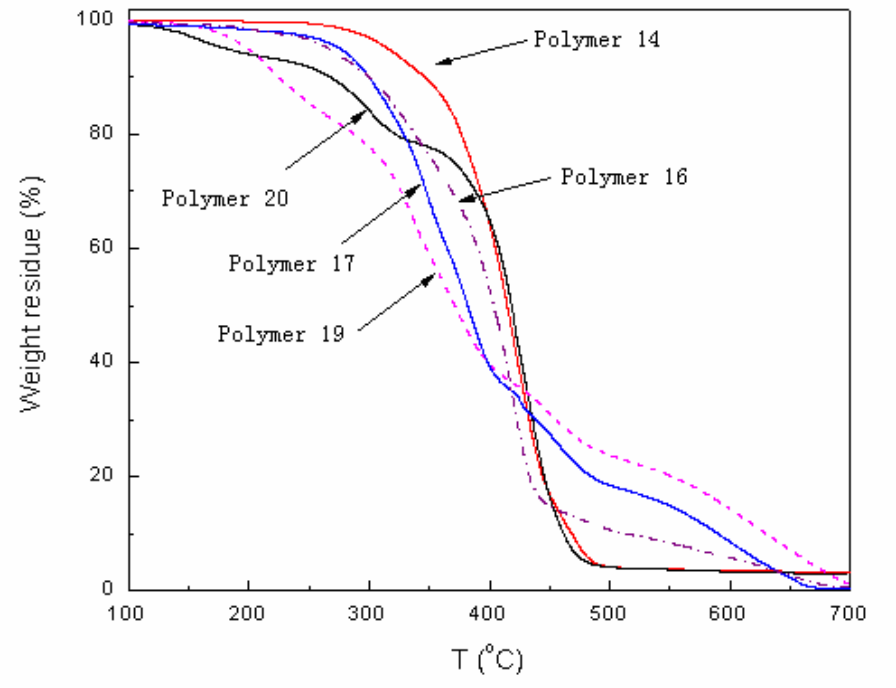


Figure 2.4 TGA thermograms of triglyceride polymers.

Polymer **2.14** (Figure 2.4) appeared as the most thermal stable polymer among all the investigated polymers as its thermal decomposition temperature was 282.6 °C. Its stability may relate to the low number of decomposable urethane bonds present in the polymer network and the two dangling alkene chain may act as good heat stabilizers. Polymer **2.20** was sensitive to high temperature as it started to decompose around 150 °C. The poor stability of urea segments in the polymer structures as shown in Scheme **2.11** might contribute to the low thermal stability. Polymers **2.16** and **2.17** have comparable thermal stability in nitrogen; they are less stable than polymer **14** but more stable than polymer **2.19** and **2.20** below 350 ^{o}C. For example, at ~305 °C, polymer **2.16** and **2.17** have about 10 % weight loss, polymer **2.14** only has around 4 % weight loss, comparing to the 18 % weight loss of polymer **2.20** and 23 % weight loss of polymer **2.19**.

2. Differential Scanning Colorimetry (DSC) is a thermoanalytical technique. In this method involves two small platinum holders, one with a small polymer sample sealed in a small aluminum pan, the other contain an empty pan as a reference. Two individual heaters were used to maintain the identical temperatures (by two identical platinum sensors) for the two small pans. The different heat flow needed to maintain the identical temperatures of the sample pan and the reference pan during a programmed heating cycle is then plotted as a function of temperature.

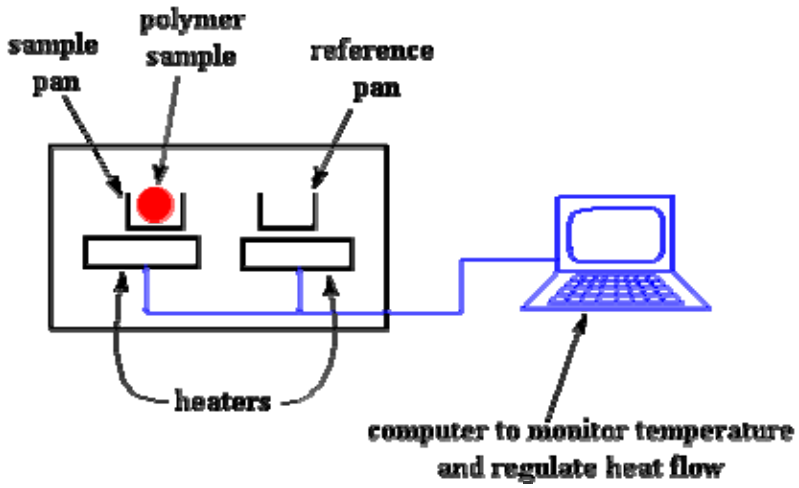


Figure 2.5 Schematic illustration of DSC analysis. (Picture was taken from internet without permission).

DSC was used here to determine the glass transition temperature, "at which there is an absorption or release of energy as the temperature is raised or lowered",[1] of aforementioned polymers. These temperatures (*T*g) indicate the subtle phase transition of the amorphous solid become soft on heating or brittle on cooling. Generally speaking, polymers with flexible backbone and small substituent groups have relatively low *T*g while those with rigid backbones (*eg*: aromatic groups on main Chain) have high *T*g, rubbery materials usually have low glass transition temperatures than glassy materials.[3]

Thermal behavior of two triglyceride monomers, compounds **2.4** and **2.5**, were characterized as controls in DSC measurement. The prominent endothermic peaks showed in the DSC thermograms (Figure 2.6) demonstrated that they were highly crystalline monomers, which were crystallized from petroleum ether and ethyl acetate solution. In the presence of pyridine as catalyst, the monomers reacted aggressively with 1,4-phenylene diisocyanate under similar conditions and resulted in cross-linked polymers **2.14-2.20**. The DSC curves of cross-linked polymers did not show melting peaks, which indicate no monomers was present in the polymer network and these polymers were most likely in amorphous states (Figure 2.7).

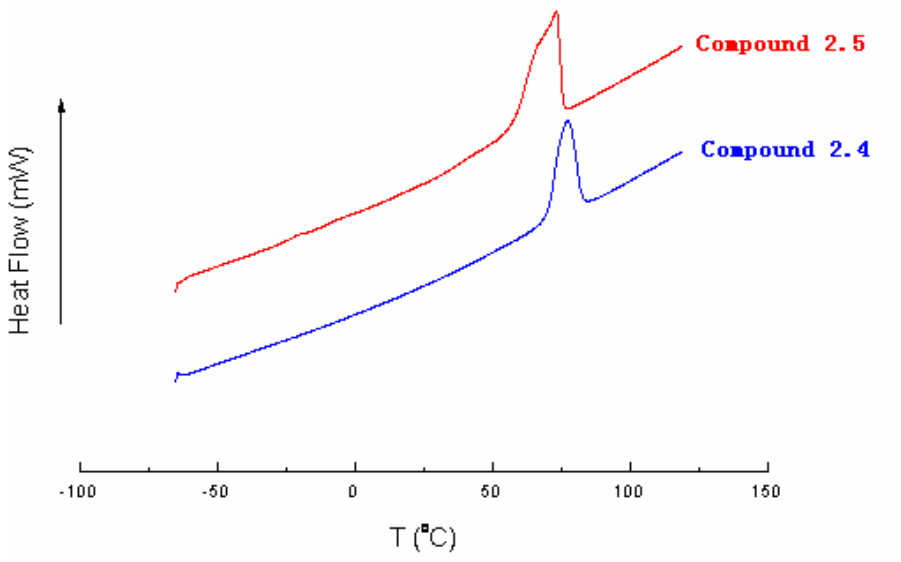


Figure 2.6 DSC thermograms of triglyceride monomers **2.4** and **2.5**.

From DSC measurements (Figure 2.7), Glass transition temperatures *Tg* of the cross-linked polymers **2.14 -2.20** were determined and listed in Table 2.4.

Table 2.4 Glass transition temperatures of the triglyceride polymers.

Polymer	**2.14**	**2.15**	**2.16**	**2.17**	**2.18**	**2.19**	**2.20**
Tg (°C)	-2.8	52.2	10.0	52.6	46.9	-20.7	10.2

Polymer **2.14**, derived from diol **2.8**, has the second lowest glass transition temperature at -2.8 °C. Considering the monomer structure in Scheme 2.2 (diol **2.8**), only one of the three alkyl chains was functionalized as hydroxyl groups, it is not uncommon that the linear polymer resulted from 2 + 2 polymerization had a low glass transition temperature. The other two dangling alkyl chains might act as internal plasticizers in the bulky polymer system due to the steric hindrance and enhanced chain movement. The similar glass transition temperatures of polymer **2.17** and **2.20** may trace back to the similarity of their monomers, both of which only have one functionality on every side chain. The backbones of the resulting polymers are consisted with rigid aromatic segments and long and flexible polymethylene chains. Comparing to the backbone of polymer **2.17** composed by rigid aromatic segments and short polymethylene chains, *Tg* at ~10 °C of polymer **2.16** or polymer **2.20** is reasonable. The glass transition temperatures of highly cross-linked polymers **2.17** (52.6 °C) and **2.18** (46.9 °C) are consistent with reported ones.[15]

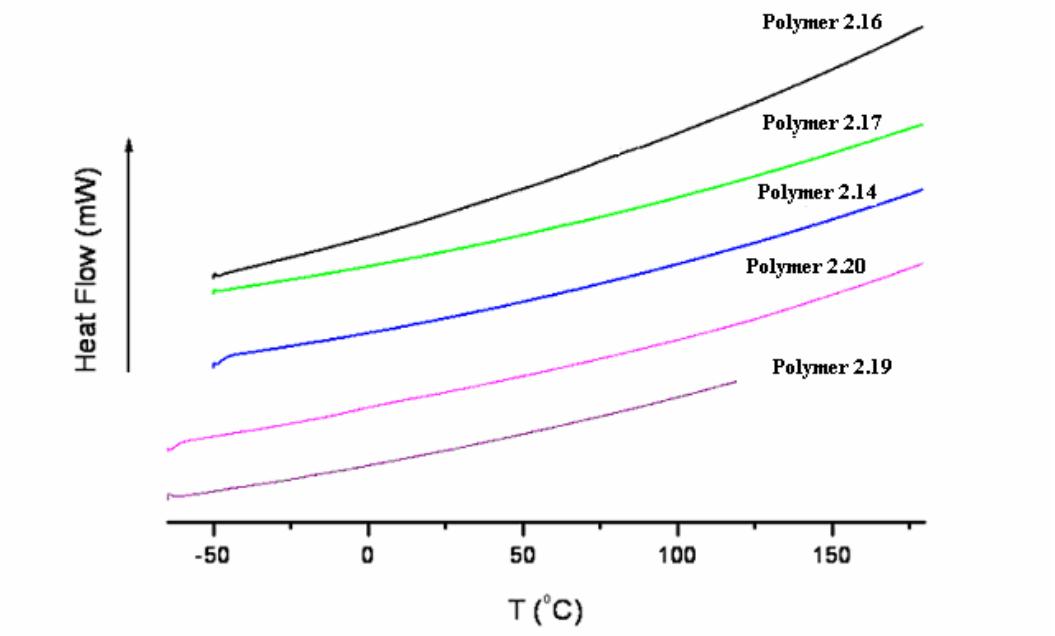


Figure 2.7 DSC thermograms of triglyceride polymers

3. Viscoelastic Characteristics: At extreme circumstances, a polymer may present mechanic characteristic as elastic solid or viscous liquid, while under usual conditions, a polymer will exhibit viscoelastic property, which is in between an ideal elastic and viscous liquid.[2] The most commonly used technique to measure viscoelastic properties is dynamic mechanical analysis. This technique records the stress in response to an applied sinusoidal strain and may be used to determine the cross-link degree of a polymer.[29]

Dynamic storage moduli of polymer **14**, **16**, **17** were measured by varying frequencies at different temperatures and results are shown in Figures 2.8-2.10. Polymer **14** exhibited a linear viscoelastic characteristic at low frequency (below 10 rad/s), which is similar to that of most thermoplastics (Figure 2.8). Hence, it likely can be used as a thermoplastic. Polymer **17** behaved as a rigid polymer as the modulus was independent of frequency and remained almost unchanged over the entire frequency range. At 25 °C, polymer **16** exhibited similar relaxation behavior as that of polymer **14**. Under elevated temperatures (50 °C and 80 °C), no steady increase in dynamic storage modulus is found (Figure 2.9), it may because above 50 ^{o}C (above Tg), all the segments already reach their maximum capacity of movement and they will have no response to outside stress. The highly cross-linked backbone structure of polymer **17** derived from a rigid 1,4-phenylene spacer between cross-linking points (Scheme 2.8), which greatly restricts the chain response to frequency even at elevated temperatures (Figure 2.10). 10^{-2}

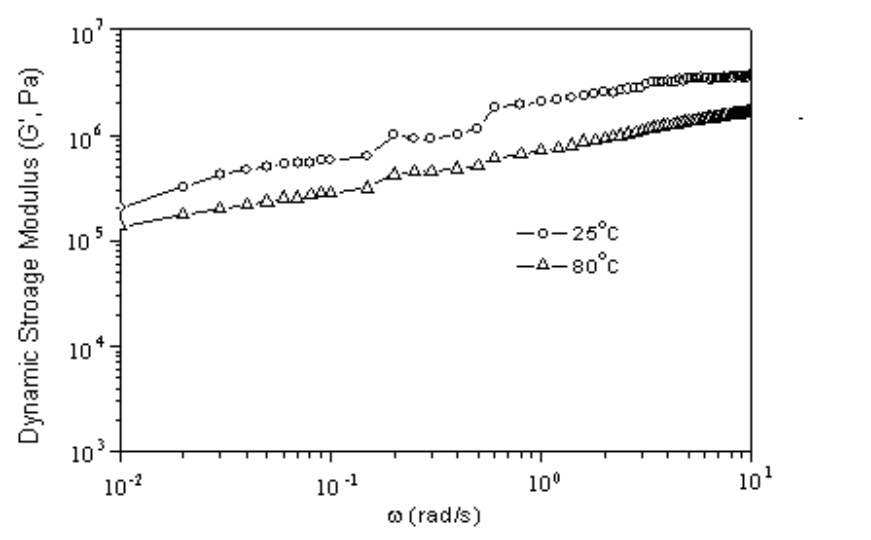


Figure 2.8 Dynamic mechanical properties of polymer **2.14.**

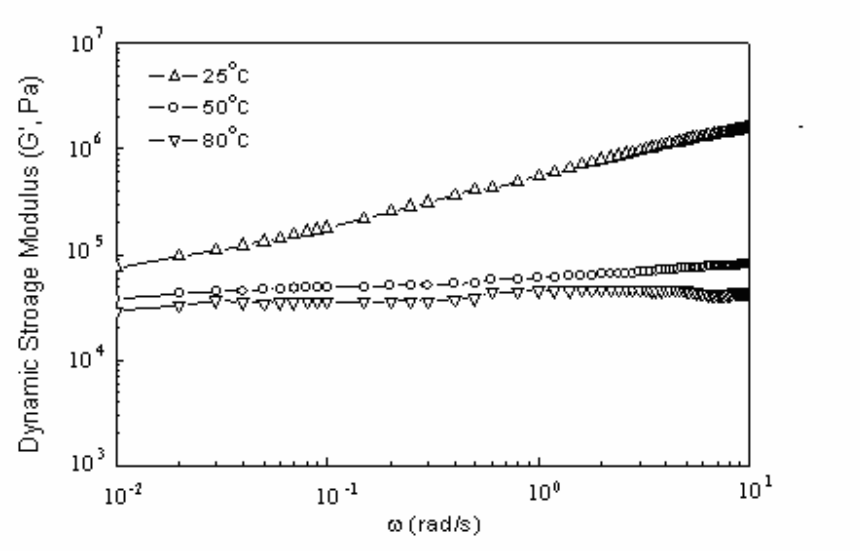


Figure 2.9 Dynamic mechanical properties of polymer **2.16**.

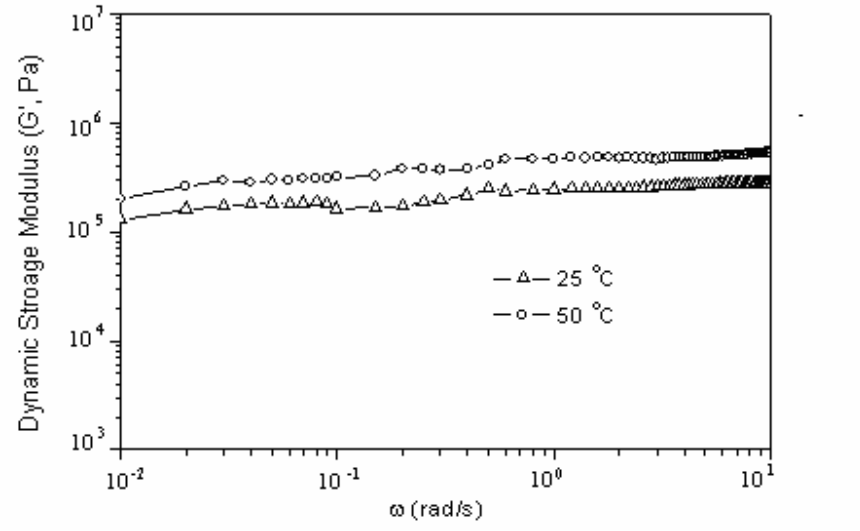


Figure 2.10 Dynamic mechanical properties of polymer **2.17**.

2.3 Conclusion

Model triglyceride glyceryl trioleoate (**2.2**) was functionalized via mono-, di-, and tri-epoxidations followed by hydrolytic ring opening and reduction to provide dihydroxylated, trihydroxylate, tetrahydroxylated and hexahydroxylated triglycerides, which were cross-linked with PDI. The trihydroxylated triglyceride was converted to triamino and triisopropylamino glycerides separately followed by cross-linkages with PDI.

The physical properties of these polymers were examined by gel content and swelling value measurements, thermodynamic properties were studied from TGA, DSC and DMA. These results indicate that the more functionality presents in monomer, the higher the cross-linking density; the higher the cross-linking density, the higher the gel content and glass transition temperatures, the lower the swelling values.

The results also suggest that the presence of free dangling side chain in polymer network can act as extra plasticizer and make the material more suitable for rubber applications (polymer **2.14** and **2.15**). The flexible polymethylene units present in polymer backbone also soften the material. The difunctionalization of double bond on side chains resulted in highly cross-linked materials with strong rigidity and fragility (polymer **2.17**), which may found potential application as rigid composites in material science.

2.4 Experimental

Materials

Chemicals such as oleic acid and reagents were purchased either from Aldrich Chemical Company or Fisher Chemical Company, and were used without purification. Silica gel, grade 643 (200~425 mesh), was used for the flash column chromatographic separation. Tetrahydrofuran and diethyl ether were distilled over sodium and benzophenone before use. Methylene chloride was distilled over CaH_2 and toluene and benzene were distilled over $LiAlH_4$.

Instrumentation

Unless otherwise indicated, nuclear magnetic resonance (NMR) spectra were obtained at 400 MHz for ^{1}H and 100 MHz for ^{13}C in deuteriochloroform, and chemical shifts were reported in ppm. Infrared spectra were obtained from a Nicolet Protege 460 FT-IR spectrometer and are reported in wavenumbers (cm^{-1}). Mass spectra were taken from a Bruker Esquire 3000 Plus HPLC-UV-Electrospray Ionization Mass Spectrometer and a MALDI-TOF/TOF MS instrument, Ultraflex II (Bruker Daltonics) model. Melting points are uncorrected.

The following DSC, TGA, and DMA measurements were carried out by Dr. Jianfeng Zhang in Prof. Susan Sun's labotory.

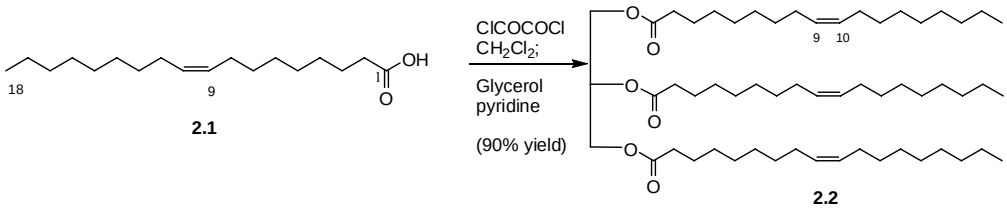
Differential Scanning Calorimetry (DSC): Thermal transitions of diols, triols, hexaols, triamines, and their cross-linked polymers were determined using a DSC instrument (Perkin-Elmer Pyris 1, Norwalk, CT) measuring from -60 to 180 °C at a scanning rate of 10 °C/min in an inert atmosphere of nitrogen with the nitrogen flow rate at 20mL/min. All results were obtained from the second DSC run to remove any prior thermal history.

Thermogravimetric Analysis (TGA): A thermal gravimetric analyzer (TGA) (Perkin-Elmer Pyris1 TGA, Norwalk, CT) was used to determine the thermal degradation temperatures of the triglyceride polymers. The sample was heated from room temperature to 700 °C at a ramp rate of 10 °C/min with nitrogen as the purge gas.

Dynamic Mechanical Analysis (DMA): Parallel-plate geometry was used to evaluate the triglyceride polymers' dynamic mechanical behavior with a dynamic mechanical analyzer (DMA, Perkin-Elmer Pysis DMA7e, Norwalk, CT). The circular sample with 10 mm diameter and 1 mm thickness was mounted between the parallel plates and subsequently was compressed by the two plates. A strain sweep was initially performed to determine the linear viscoelastic region of the samples. A dynamic frequency sweep test was carried out with a 3% strain and 0.01-50 rad/s frequency range.

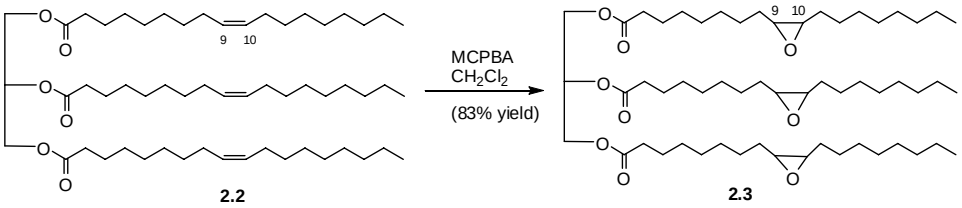
Synthesis of Monomers and Polymers

Glyceryl Trioleoate (2.2).



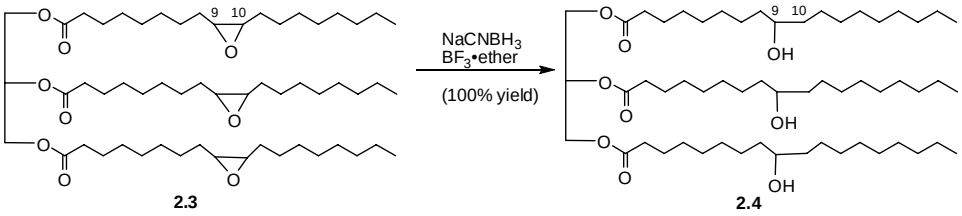
To a solution of 10.0 g (35.4 mmol) of oleic acid (**2.1**) in 50 mL of dichloromethane under argon was added 6.74 g (53.1mmol) of oxalyl chloride, and the solution was stirred at 25 ^{o}C for 2 hours. After removal of the excess of oxalyl chloride and solvent over a rotary evaporator, the oily residue, oleoyl chloride, was dried under vacuum and used in the next operation without purification. To a solution of 1.09 g (11.8 mmol) of glycerol and 5.72 mL (70.8 mmol) of pyridine in dichloromethane (20 mL) at 0 ^{o}C under argon, was added a solution of the above oleoyl chloride in 50 mL of dichloromethane via cannula. The solution was stirred at 25 ^{o}C for 2 hours, diluted with 50 mL of dichloromethane, washed twice with water (50 mL each) and saturated aqueous sodium chloride (50 mL) solution, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using hexane:ethyl acetate (20:1) as eluant to give 9.40 g (90.2% yield) of compound **2.2**, as an oil.[23] **^{1}H NMR** δ 5.34 (m, 6 H), 5.32 (m, 1 H), 4.09 - 4.34 (m, 4 H), 2 .31 (t, *J* = 7.3 Hz, 6 H), 2.01(m, 12 H), 1.61 (m, 6 H), 1.19 - 1.42 (m, 60 H), 0.88 (t, *J* = 5.9 Hz, 9 H); **^{13}C NMR** δ 173.4, 173.0, 130.2, 129.9, 69.1, 62.3, 34.4, 34.2, 32.1, 30.0, 29.9, 29.8, 29.7, 29.5, 29.45, 29.4, 29.3, 29.29, 27.4, 27.37, 25.1, 25.0, 22.9, 14.3; **HRMS** m/z 907.7893 (907.7725, calcd for $C_{57}H_{104}O_6Na^+$, M+Na^+).

Glyceryl Tris(9,10-epoxyoleoate) (2.3).



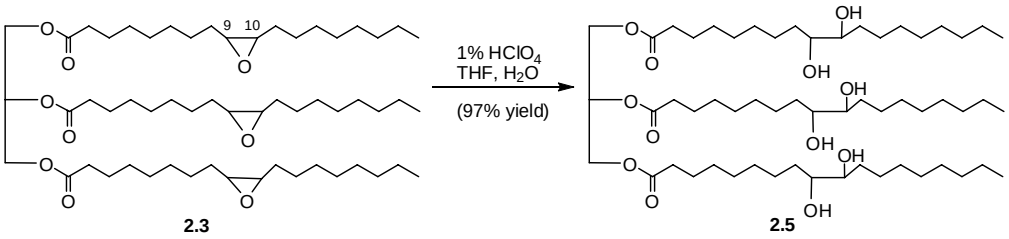
To a solution of 2.4 g (2.7 mmol) of glycerol trioleoate (**2.2**) in 50 mL of dichloromethane under argon, was added 3.6 g (16.3mmol) of *m*-chloroperbenzoic acid (MCPBA) at 0 ^{o}C. After stirring the reaction mixture for 4 hours, 30 mL of 10% $NaHCO_3$ was carefully added, the organic layer was separated, washed with water twice (30 mL each), and 30 mL of brine, dried ($MgSO_4$), and concentrated to give 2.1 g (83% yield) of triepoxide **2.3** as white solids. **^{1}H NMR** δ 5.26 (m, 1 H), 4.12 - 4.32 (m, 4 H), 2.90 (broad s, 6 H), 2.32 (m, 6 H), 1.61 (t, *J* = 7 Hz, 6 H), 1.27-1.49 (m, 72 H), 0.88 (t, *J* = 6.6 Hz, 9 H); **^{13}C NMR** δ 173.2, 173.0, 68.9, 62.1, 57.2, 34.2, 34.0, 31.9, 29.5, 29.4, 29.2, 29.0, 27.8, 26.6, 24.8, 22.7, 14.1; **HRMS** m/z 955.7618 (955.7573, calcd for $C_{57}H_{104}O_9Na^+$, M+Na^+).

Glyceryl Tris(9-hydroxyoleoate) (2.4).



To a cold (0 ^{o}C) solution of 0.50 g (0.53 mmol) of triepoxide **2.3** in 20 mL of THF, were added 0.40 g (6.4 mmol) of sodium cyanoborohydride and 0.41 mL (3.2 mmol) of BF_3•ether. After stirring at 25 ^{o}C for 8 hours, the reaction solution was diluted with 15 mL of water and extracted twice with ethyl acetate (20 mL each). The organic layers were combined and washed with water twice (20 mL each), and brine, dried ($MgSO_4$), concentrated to give 0.50 g (100% yield) of triol **2.4**. Mp. 70 – 71 ^{o}C; **^{1}H NMR** δ 5.24 - 5.28 (m, 1 H, CHOCO), 4.31 - 4.13 (m, 4 H, CH_2OCO), 4.1 (bs, 3 H, OH), 3.56 (bs, 3 H, CHO), 2.31 (m, 6 H), 1.27 - 1.61 (m, 84 H), 0.88 (t, *J* = 6.2 Hz, 9 H); **^{13}C NMR** δ 173.3, 172.9, 72.6, 69.5, 62.7, 38.2, 38.1, 34.8, 34.7, 32.5, 30.4, 30.3, 30.2, 30.1, 30.06, 30.0, 29.9, 29.8, 29.7, 29.66, 26.3, 26.26, 25.5, 23.3, 14.7; **FTIR** (KBr) ν 3344 (broad, OH), 2925, 2850, 1740 (sharp s, C=O), 1467, 1215 (C-O), 1102 cm^{-1}. **HRMS** m/z 962.0140 (961.8048, calcd for $C_{57}H_{110}O_9Na$, M+Na^+).

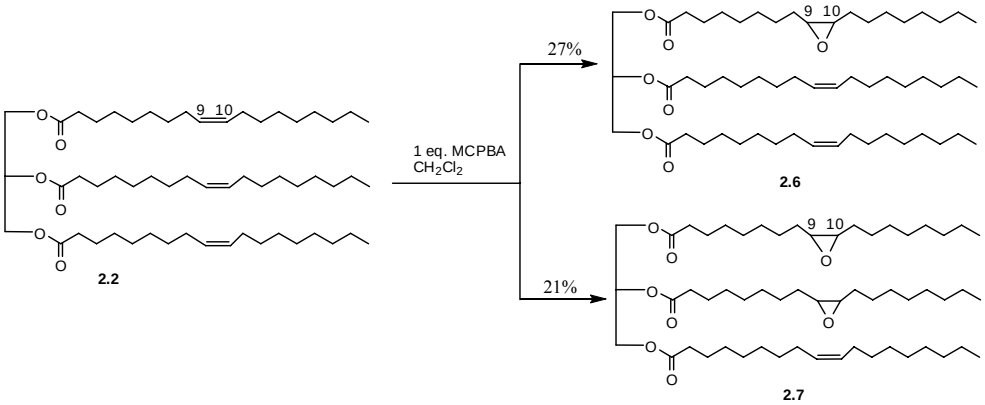
Glyceryl Tris(9,10-dihydroxyoleoate) (2.5).



2.3 **2.5**

A solution of 0.80 g (0.86 mmol) of triepoxide **2.3** in 70 mL of 1% $HClO_4$ in THF/H_2O (3:2) was stirred at 25 ^oC for 12 hours, diluted with 100 mL of ethyl acetate, washed twice with water (50 ml each), and brine (50 ml), and concentrated to give 0.82 g (97% yield) of compound **2.5**. Mp. 65 – 66 ^oC; **^{1}H NMR** δ 5.24 - 5.28 (m, 1 H), 4.12 - 4.32 (m, 4 H), 3.58 (bs, 6 H), 2.32 (t, *J* = 7.33 Hz, 6 H), 1.27 - 1.66 (m, 78 H), 0.88 (t, *J* = 6.23 Hz, 9 H); **^{13}C NMR** δ 173.3, 172.9, 74.5, 74.46, 68.9, 62.1, 34.2, 34.0, 33.7, 33.6, 31.9, 29.7, 29.6, 29.5, 29.45, 29.3, 29.2, 29.18, 29.0, 25.7, 25.6, 24.9, 24.8, 22.7, 14.1; **FTIR** (KBr) ν 3400 (broad, OH), 2918, 2849, 1741 (sharp s, C=O), 1466, 1241, 1168 cm^{-1}; **HRMS** m/z 1009.824 (1009.7890, calcd for $C_{57}H_{110}O_{12}Na$, $M+Na^+$).

Glyceryl Trioleoate Mono(epoxide) (2.6) and Di(epoxide) (2.7).

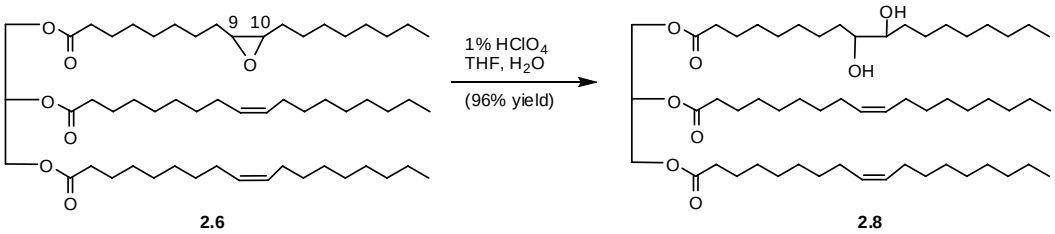


2.2 **2.6** **2.7**

To a solution of 4.0 g (4.52 mmol) of glycerol trioleoate (**2.2**) in 50 mL of dichloromethane under argon, was added 1.02 g (4.52 mmol) of MCPBA (77%) at 0 ^oC. After stirring the reaction mixture for 4 hours, 30 mL of 10% $NaHCO_3$ was added, the organic layer was separated, washed twice with water (30mL each), and 30 mL of brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using hexane:ethyl acetate (30:1) as eluant to give 1.06 g (27% yield) of monoepoxide **2.6**, and 0.87 g (21% yield) of diepoxide **2.7**. Both of the two compounds

were oily liquids. **^{1}H NMR** (**2.6**) δ 5.33 - 5.36 (m, 4 H), 5.25 - 5.28 (m, 1 H), 4.12 - 4.32 (m, 4 H), 2.90 (m, 2 H), 2.31 (m, 6 H), 1.99 - 2.03 (m, 8 H), 1.21 - 1.62 (m, 70 H), 0.83 - 0.90 (m, 9 H); **^{13}C NMR** (**2.6**) δ 173.3, 172.8, 130.0, 129.7, 68.9, 62.1, 57.2, 57.15, 34.2, 34.0, 31.9, 31.87, 29.8, 29.7, 29.5, 29.3, 29.2, 29.1, 29.0, 27.9, 27.2, 27.19, 26.6, 24.9, 24.8, 22.7, 14.1; **HRMS** (**2.6**) m/z 923.7783 (923.7674, calcd for $C_{57}H_{104}O_7Na^+$, M+Na^+). **^{1}HNMR** (**2.7**) δ 5.33 - 5.36 (m, 2 H), 5.25 - 5.28 (m, 1 H), 4.12 - 4.32 (m, 4 H), 2.90 (m, 4H), 2.31 (m, 6 H), 1.99 - 2.03 (m, 8 H), 1.21 - 1.62 (m, 70 H), 0.83 - 0.90 (m, 9 H); **^{13}CNMR** (**2.7**) δ 173.4, 172.9, 130.0, 129.8, 68.9, 62.1, 57.2, 34.2, 34.0, 31.9, 31.8, 29.8, 29.7, 29.5, 29.3, 29.2, 29.1, 29.0, 27.9, 27.2, 26.6, 24.9, 24.8, 22.7, 14.1;

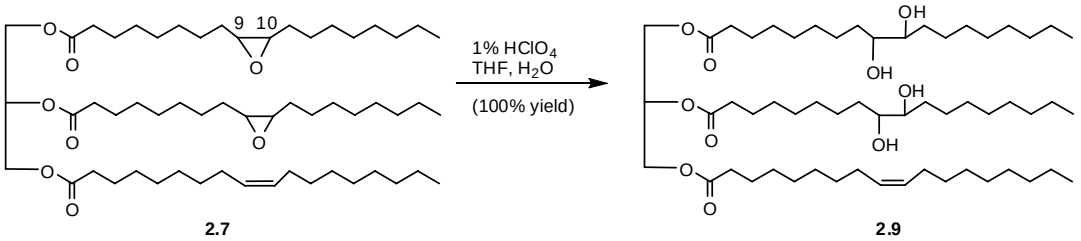
Glyceryl 9,10-Dihydroxytrioleoate (2.8).



2.6 **2.8**

To a solution of 20 mL of 1% $HClO_4$ in THF/H_2O (3:2), was added 1.0 g (1.11 mmol) of epoxide **2.6**. After stirring for 12 hours, the reaction solution was diluted with 50 mL of ethyl acetate, washed twice with water (30 mL each), and 30 mL of brine, and concentrated to give 0.98 g (96% yield) of diol **2.8**. **^{1}H NMR** δ 5.32 - 5.37 (m, 4 H), 5.20 - 5.30 (m, 1 H), 4.10 - 4.34 (m, 4 H), 3.37 - 3.43 (m, 2 H), 2.32 (t, *J* = 5.9 Hz, 6 H), 1.96 - 2.26 (m, 8 H), 1.27 - 1.68 (m, 70 H), 0.88 (t, J = 6.7 Hz, 9 H); **^{13}C NMR** δ 173.4, 173.0, 130.2, 129.9, 74.7, 69.2, 62.3, 34.4, 34.2, 33.9, 33.87, 32.1, 32.07, 30.0, 29.9, 29.8, 29.7, 29.65, 29.5, 29.49, 29.45, 29.4, 29.3, 29.1, 27.4, 27.38, 25.9, 25.8, 25.1, 25.0, 22.9, 14.2; **FTIR** (KBr) ν 3466 (broad, OH), 2925, 2854, 1745 (sharp s, C=O of ester), 1464, 1164 (C-O) cm^{-1}; **HRMS** m/z 941.7580 (941.7780, calcd for $C_{57}H_{106}O_8Na^+$, M+Na^+).

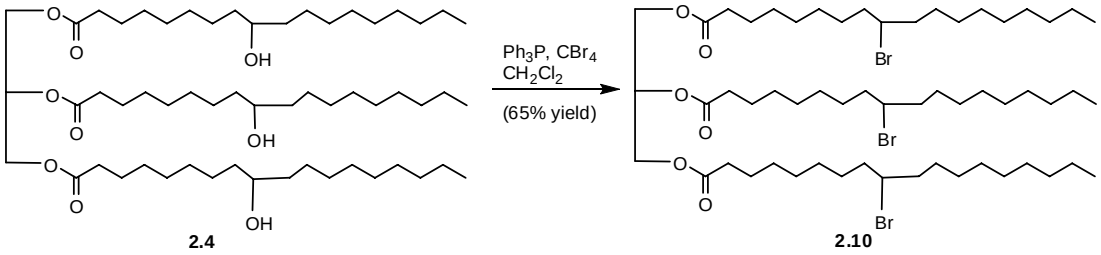
Glyceryl 9,10-Dihydroxytrioleoate (2.9).



2.7 2.9

To a solution of 20 mL of 1% $HClO_4$ in THF/H_2O (3:2), was added 0.87 g (0.92 mmol) of epoxide **2.7**. After stirring for 12 hours, the reaction solution was diluted with 50 mL of ethyl acetate, washed twice with water (30 mL each), and 30 mL of brine, and concentrated to give 0.98 g (96% yield) of tetraol **2.9**. **^{1}H NMR** δ 5.32 - 5.37 (m, 2 H), 5.20 - 5.30 (m, 1 H), 4.10 - 4.34 (m, 4 H), 3.37 - 3.43 (m, 4 H), 2.32 (t, *J* = 5.9 Hz, 6 H), 1.96 - 2.26 (m, 8 H), 1.27 - 1.68 (m, 70 H), 0.88 (t, J = 6.7 Hz, 9 H); **^{13}C NMR** δ 173.5, 173.1, 130.2, 129.9, 74.7, 74.6, 69.1, 62.3, 34.4, 34.2, 33.8, 32.1, 30.5, 30.0, 29.9, 29.75, 29.72, 29.63, 29.5, 29.47, 29.4, 29.1, 27.4, 27.36, 25.9, 25.8, 25.0, 22.9, 14.3.

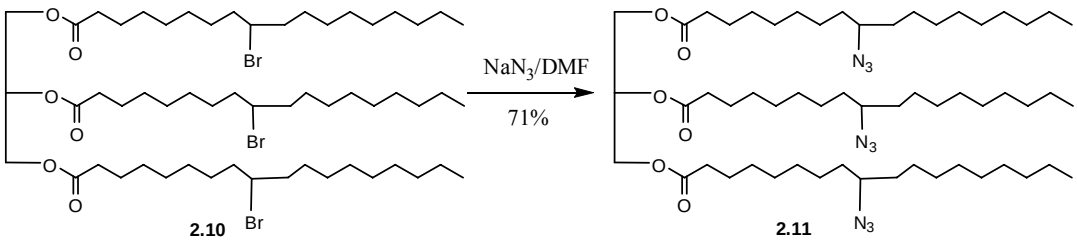
Glyceryl Tris(9-bromooleoate) (2.10).



2.4 2.10

To a cold (0 ^{o}C) solution of 0.58 g (0.62 mmol) of triol **2.4** and 0.98 g (3.7 mmol) of triphenylphosphine in 40 mL of dichloromethane under argon, was added 1.24 g (3.71 mmol) of carbon tetrabromide. After stirring at 0^{o}C for 2 hours, the mixture was concentrated and column chromatographed on silica gel using hexane:ethyl ether (4:1) as eluant to give 0.46 g (65% yield) of compound **2.10**. **^{1}H NMR** δ 5.24 (m, 1 H), 4.12 - 4.32 (m, 4 H), 4.02 (m, 3 H), 2.32 (t, *J* = 7.7 Hz, 6 H), 1.77 - 1.84 (m, 12 H), 1.27 - 1.64 (m, 72 H), 0.88 (t, *J* = 6.6 Hz, 9 H); **^{13}C NMR** δ 173.4, 173.39, 173. 0, 69.2, 62.3, 59.1, 59.05, 39.5, 39.4, 39.37, 34.4, 34.39, 34.2, 32.1, 32.07, 29.8, 29.7, 29.68, 29.5, 29.45, 29.4, 29.38, 29.35, 29.33, 29.3, 29.2, 29.2, 29.12, 29.1, 27.8, 27.78, 27.75, 25.1, 25.0, 22.9, 22.87, 14.3; **HRMS** m/z 907.8130 (907.7725, calcd for $C_{57}H_{107}Br_3O_6Na$-3HBr$^+$).

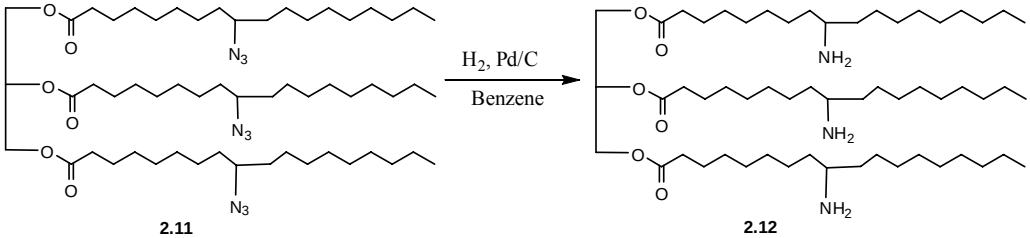
Glyceryl Tris(9-azidooleoate) (2.11).



2.10 2.11

A solution of 0.12 g (0.11 mmol) of tribromide **2.10** and 42 mg (0.66 mmol) of sodium azide in 15 mL of DMF was heated to reflux for 4 hours, diluted with 15 mL of water, and extracted twice with ethyl acetate (30 mL each). The combined organic layer was washed with water, brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using hexane:ethyl ether (4:1) as eluant to give 77 mg (71% yield) of compound **9**. **^{1}H NMR** δ 5.24 (m, 1 H), 4.10 - 4.32 (m, 4 H), 3.19 (m, 3 H), 2.29 (t, *J* = 7.3 Hz, 6 H), 1.27 - 1.64 (m, 84 H), 0.88 (t, *J* = 6.6 Hz, 9 H); **^{13}C NMR** δ 173.3, 172.9, 69.1, 63.3, 62.3, 34.6, 34.3, 34.2, 32.04, 32.01, 29.7, 29.6, 29.55, 29.5, 29.45, 29.41, 29.4, 29.3, 29.2, 29.15, 26.3, 25.0, 22.8, 14.2; **FT-IR** (neat) ν 1743.74 (C=O), 2096.73 (N_3); **HRMS** m/z 1036.8147 (1036.8242, calcd for $C_{57}H_{107}N_9O_6Na^+$).

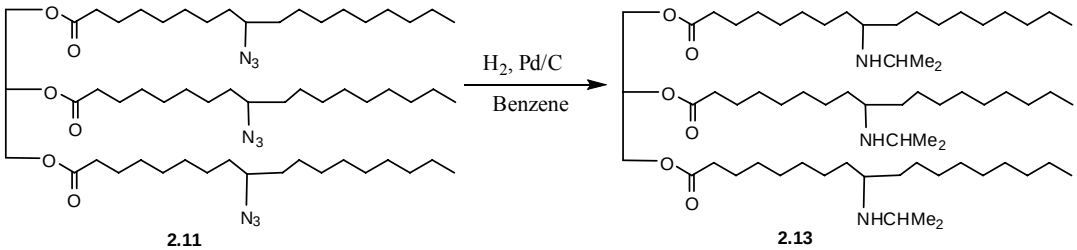
Glyceryl Tris(9-aminooleoate) (2.12).



2.11 2.12

To a solution of 0.44 g (0.43 mmol) of azide **2.11** in 3 mL of benzene under argon, was added 40 mg of 10% Pd/C. The mixture was maintained under 30 psi atmosphere of hydrogen on a hydrogenator for 12 hours, filtered, and the filtrate was concentrated to give 0.39 g (100% yield) of compound **2.12**. **^{1}H NMR** δ 5.27 (m, 1 H), 4.10 - 4.34 (m, 4 H), 2.67 (m, 3 H), 2.31 (t, *J* = 7.3 Hz, 6 H), 1.20 - 1.70 (m, 84 H), 0.88 (t, *J* = 6.4 Hz, 9 H); **^{13}C NMR** δ 173.4, 173.0, 69.0, 62.3, 51.4, 38.4, 34.4, 34.2, 32.1, 30.0, 29.9, 29.8, 29.6, 29.5, 29.3, 26.4, 25.0, 22.8, 14.3; **HRMS** m/z 958.8931 (958.8527, calcd for $C_{57}H_{113}N_3O_6Na^+$).

Glyceryl Tris[9-(*N*-isopropylamino)oleoate] (2.13).



2.11 **2.13**

To a solution of 0.31 g (0.31 mmol) of azide **2.11** in 5 mL of acetone under argon, was added 15 mg of 10% Pd/C. The mixture was maintained under 30 psi atmosphere of hydrogen for 12 hours at 25 ^{o}C, filtered, and the filtrate was concentrated to give 0.33 g (100% yield) of compound **2.13**. **^{1}H NMR** δ 5.24 (m, 1 H), 4.10 - 4.32 (m, 4 H), 3.19 (m, 3 H), 2.88 (m, 3 H), 2.31 (t, J = 7.7 Hz, 6 H), 1.27 - 1.64 (m, 84 H), 0.96 (d, *J* = 14.6 Hz, 18 H), 0.88 (t, *J* = 6.6 Hz, 9 H); **^{13}C NMR** δ 173.5, 173.1, 69.1, 62.3, 54.7, 45.9, 34.9, 34.3, 32.1, 30.2, 30.1, 29.9, 29.85, 29.6, 29.3, 26.0, 25.1, 23.8, 22.9, 14.3. **HRMS** m/z 1084.9760 (1084.9936, calcd for $C_{66}H_{131}N_3O_6Na^+$).

Cross-linked Polymer 2.14. To a solution of 0.90 g (1.01 mmol) of diol **2.8** and 0.16 g (1.01 mmol) of 1,4-phenylene diisocyanate (PDI) in 3 mL of toluene under argon, was added 15 mg of pyridine. After refluxing for 12 hours, the reaction solution was filtered. The solid was immersed in 20 mL of dichloromethane and the mixture was allowed to sit for 12 h. The insoluble material was filtered and dried under vacuum to give 0.68 g (64% yield) of cross-linked polymer **2.14** as a rubbery solid. **FT-IR** (KBr) ν 3319 (broad, NH), 3004 (Ar C-H), 2925, 2854, 1738 (broad s, C=O), 1605 (Ar C=C), 1549, 1514, 1377, 1210 cm^{-1}.

Cross-linked Polymer 2.15. To a solution of 0.87 g (0.91 mmol) of tetraol **2.9** and 0.29 g (1.82 mmol) of 1,4-phenylene diisocyanate (PDI) in 3 mL of toluene under argon, was added 15 mg of pyridine. After refluxing for 4 hours, the reaction solution was filtered. The solid was immersed in 20 mL of dichloromethane and the mixture was allowed to sit for 12 h. The insoluble material was filtered and dried under vacuum to give 1.0 g (89.5% yield) of cross-linked polymer **2.15** as a rubbery solid.

Cross-linked Polymer 2.16. A solution of 0.30 g (0.32 mmol) of triol **2.4**, 79 mg (0.49 mmol) of PDI and 15 mg of pyridine in 15 mL of toluene was heated to reflux for 4 hours. The solvent and pyridine was removed by filtration. The solid was immersed in 10 mL of dichloromethane for 12 hours and filtered. The insoluble material was filtered and dried under vacuum to give 0.31 g (83% yield) of polymer **2.16**, which is insoluble in most organic and inorganic solvents. **FT-IR** (KBr) ν 3330 (broad, NH), 3005 (Ar C-H), 2923, 2853, 1744 (broad s, C=O), 1608 (Ar C=C), 1520, 1466, 1408, 1310, 1214 (C-O) cm^{-1}.

Cross-linked Polymer 2.17. A solution of 0.35 g (0.36 mmol) of hexaol **2.5**, 0.17 g (1.06 mmol) of PDI, and 15 mg of pyridine in 15 mL of toluene was heated to reflux for 4 hours. The solvent and pyridine were removal by filtration, and the resulting solid was immersed in 10 mL of dichloromethane and allowed to sit for 12 h. The insoluble material was collected by filtration and dried under vacuum to give 0.49 g (95% yield) of polymer **2.17**, as a brown solid. Polymer **2.17** is insoluble in most organic and inorganic solvents. **FT-IR** (KBr) ν 3367 (broad, NH), 3004 (Ar C-H), 2925, 2853, 1736 (broad s, C=O), 1608 (Ar C=C), 1513, 1409, 1309, 1214 cm^{-1}.

Cross-linked Polymer 2.18. A solution of 0.35 g (0.36 mmol) of hexaol **2.5**, 92 mg (0.58 mmol) of PDI, and catalytic amount of pyridine in 2 mL of toluene was heated to reflux for 4 hours. The solvent and pyridine were removal by filtration, and the resulting solid was immersed in 10 mL of dichloromethane and allowed to sit for 12 h. The insoluble material was collected by filtration and dried under vacuum to give 0.42 g (94% yield) of polymer **2.18**, as a light brown solid. Polymer **2.18** is insoluble in most organic and inorganic solvents.

Cross-linked Polymer 2.19. A solution of 154 mg (0.16 mmol) of triamine **2.12** and 39.5 mg (0.24 mmol) of PDI in 10 mL of toluene was heated to reflux for 4 hours. After cooling to 25^{o}C, the reaction mixture was filtered, and the solid was immersed in 10 mL of dichloromethane for 12 h. The insoluble solid was collected by filtration and dried under vacuum to give 0.182 g (94% yield) of polymer **2.19** as a brown solid.

Cross-linked Polymer 2.20. A solution of 321 mg (0.34 mmol) of trisisopropylamine **2.13** and 82 mg (0.51 mmol) of PDI in 15 mL of toluene was heated to reflux for 4 hours. The solvent

was removed by filtration, and the solid was immersed in 10 mL of dichloromethane for 12 h. The insoluble solide was collected by filtration and dried under vacuum to give 0.39 g (97% yield) of polymer **2.20** as a brown solid.

References

(1) Carraher, C. E. *Polymer Chemistry*; Marcel Dekker, Inc.: New York, 2003.

(2) Fried, J. R. *Polymer Science & Technology, second edition* Upper saddle River, New Jersy, 2003.

(3) Wool, R. P.; Sun, Xiuzhi *Bio-Based Polymers and Composite*; Elsever Academic Press: Boston, 2005.

(4) Wool, R. P.; Khot, S. N.; LaScala, J. L.; Bunker, S. P.; Lu, J.; Thielemans, W.; Can, E.; Shantaram, S. M.; Williams, G. I. Affordable composites and plastics from renewable resources: Part I: synthesis of monomers and polymers. ACS Symposium Series, "Advancing sustainability through green chemistry and engineering"; Lankey, R. L.; Anastas, P. T. Eds., ACS Washington, DC 2002, 823, 177-204.

(5) Wool, R. P.; Khot, S. N.; LaScala, J. L.; Bunker, S. P.; Lu, J.; Thielemans, W.; Can, E.; Shantaram, S. M.; Williams, G. I. In *ACS Washington, DC* Lankey, R. L. A., P. T. Eds.,, Ed. 2002; Vol. 823, p 205-224.

(6) Salunkhe, D. K.; Chavan, J. K.; Adsule, R. N.; Kadam, S. S. *World Oilseeds Chemistry, Technology, and Utilization*; Van Nostrand Reinhold: New York, 1992.

(7) Eren, T. K.; Selim, H.; Wool, R. *Polymerization of maleic anhydride -modified plant oils with polyols.*; Journal of Applied Polymer Science **2003**, *90*, 197-202.

(8) Esen, H. K.; Selim, H.; Wool, R. *Photolytic and free-radical polymerization of monomethyl maleate esters of epoxidized plant oil triglycerides.*; Journal of Applied Polymer Science **2007**, *103*(1), 626-633.

(9) Wool, R. P., Kusefoglu, S. H.; Palmese, G. R. Patent, U. S., Ed. USA, 2000.

(10) Trecker, D. J.; Borden, G. W.; Smith, O. W.; *Acrylated Epoxidized Soybean Oil Amine Composition and Method*; USP 3931075 **1976**.

(11) Trecker, D. J.; Borden, G. W.; Smith, O. W. *Method for Curing Acrylated Epoxided Soybean Oil Amine Compositions*; US Patent 3979270 **1976**.

(12) Guo, A.; Demydov, D.; Zhang, W.; Petrovic, Z. S. *Polyols and Polyurethanes from Hydroformylation of Soybean Oil.* Journal of Polymers and the Environment **2002**, *10*, 49-52.

(13) Petrovic, Z. S.; Yang, Liting; Zlatanic, A.; Zhang, W.; Javni, I. *Network structure and properties of polyurethanes from soybean oil.* Journal of Applied Polymer Science **2007**, *105*, 2717-2727.

(14) Vlcek, T., Petrovic, Z. S. *Optimization of the chemoenzymatic epoxidation of soybean oil.*; Journal of the American Oil Chemists' Society **2006**, *83*, 247-252.

(15) Javni, I.; Zhang, W.; Petrovic, Z. S. *Effect of different isocyanates on the properties of soy-based polyurethanes.* Journal of Applied Polymer Science **2003**, *88*, 2912-2916.

(16) Wool, R. P., *Development of affordable soy-based plastics, resins, and adhesives.* ; CHEMTECH **1999**, 29(6), 44-48.

(17) Petrovic, Z. S.; Ferguson, J. *Polyurethane elastomers.* Progress in Polymer Science **1991**, *16(5)*, 695-836.

(18) Petrovic, Z. S.; Zhang, W.; Javni, I. *Structure and Properties of Polyurethanes Prepared from Triglyceride Polyols by Ozonolysis.* Biomacromolecules **2005**, *6*, 713-719.

(19) Tone, H.; Nishi, T.; Oikawa, Y.; Hikota, M.; Yonemitsu, O. *A stereoselective total synthesis of (9S)-9-dihydroerythronolide A from D-glucose.* ; Tetrahedron Lett. **1987**, *28*, 4569-4572.

(20) Hutchins, R. O.; Taffer, I. M.; Burgoyne, W.; *Regio- and stereoselective Cleavage of Epoxides with Cyanoborohydride and Boron Trifluride Etherate*; Journal of Organic Chemistry **1981**, *46*, 5214-5215.

(21) Axelrod, E.; Milne, G. M.; Van Tamelen, E. E. *A general 1,5-diene synthesis involving overall allyl alcohol coupling with geometrical and positional control.* ; Journal of the American Chemistry Soceity **1970**, *92*, 2139-2141.

(22) Singh, A. *p-Phenylene diisocyanate based polyurethane elastomers.* ; Advances in Urethane Science and Technology **1996**, *13*, 112-139.

(23) Petrovic, Z. S., Zhang, W.; Zlatanic, A.; Lava, C. C.; Ilavskyy, M. *Effect of OH/NCO Molar Ratio on Properties of Soy-Based Polyurethane Networks.* Journal of Polymers and the Environment **2002**, *10*, 5-12.

(24) Chauvet, J. A.; Jose M.; Leiza, J. R. *Independent Control of Sol Molar Mass and Gel Content in Acrylate Polymer/latexes*. Polymer **2005**, *46*, 9555-9561.

(25) Percec, V.; Wang, J. H.; *Synthesis of Aromatic Polyethers by Scholl Reaction*; Polymer Bulletin **1991**, *25*, 9-16.

(26) Zlatanic, A.; Lava, C.; Zhang, W.; Petrovic, Z. S. *Effect of structure on properties of polyols and polyurethanes based on different vegetable oils.* Journal of Polymer Science, Part B: Polymer Physics. **2004**, *42*, 809-819.

(27) http://radchem.nevada.edu/chem455/lecture_22__thermal_methods.htm.

(28) Petrovic, Z. S.; Guo, A.; Zhang, W. *Structure and properties of polyurethanes based on halogenated and nonhalogenated soy-polyols*; Journal of Polymer Science, Part A: Polymer Chemistry **2000**, *38*, 4062-4069.

(29) Cai H.; Ait-Kadi, A.; Brisson, J. *Dynamic rheological analysis of a miscible blend showing strong interactions*. Polymer **2003**, *44*, 1481-1489.

Chapter 3 New Synthetic Methods of Diacetylenic Fatty Acids

3.1 Background

Diacetylenes are unique polymerizable functional groups that require a highly ordered state for their polymerization. The compatibility of the distance of self-assembled polymerizable lipid molecules and the repeated distance of the polydiacetylene chain resulted from diacetylene 1,4-addition is crucial factor for this "topchemical polymerization" (Figure 3.1).[1] Moreover, the conjugated ene-yne polydiacetylene (PDA) backbones not only stabilize the nanostructures, their unique blue-to-red chromatic change resulted from the perturbation of PDA backbone by heat,[2] pH changes,[3] mechanical stress,[4] or interactions with reagents (including analytes) indicates potential applications in sensor designs.[5,6]

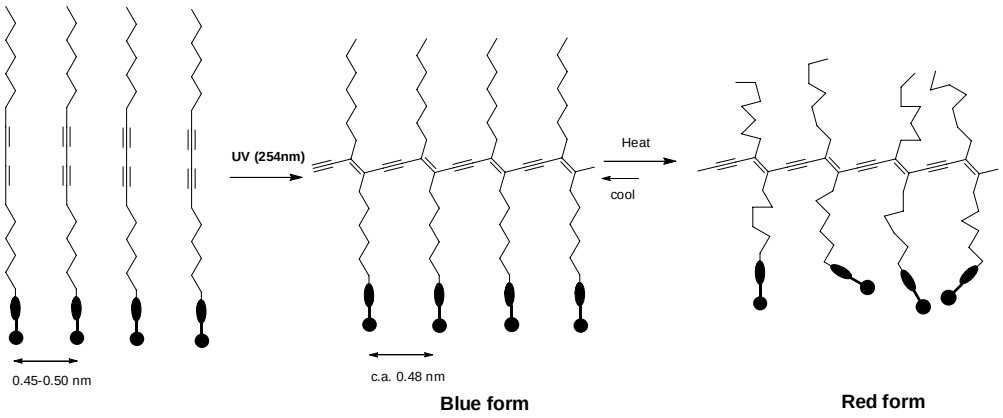


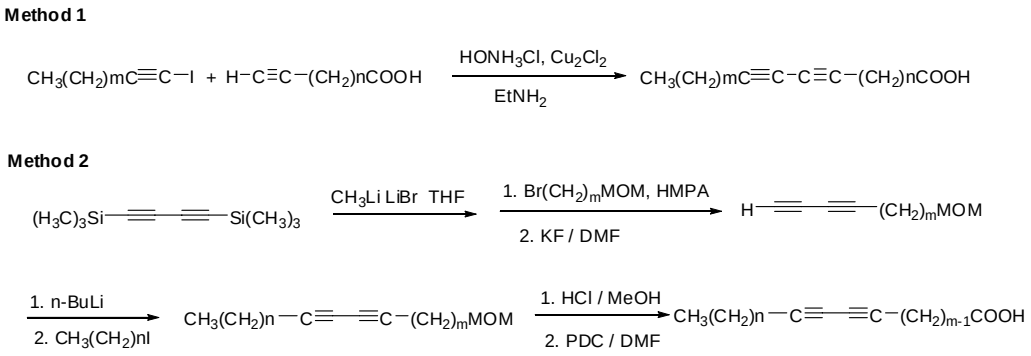
Figure 3.1 Polymerization scheme and schematic illustration of chromatic property using side chain disorder model,[7] if the side chains are tightly packed in ordered alignment, the material shows blue color; if the side chains are in a random manner, the material shows red color. (Reproduced without permission)

Because of their unique property of solid state polymerization and chromatic change, diacetylenic moieties are widely incorporated into self-assembling amiphiphile design, particularly, in the middle of fatty acid hydrocarbon chains.[1,8] Because of the terminal carboxylic acid groups, these diacetylene incorporated fatty acids are readily coupled with glycerol-3-phosphocholine to construct micropatterned biomimetic membranes[9,10] and nanotubules;[11] with sugar to self assemble into nanofibers;[12] with amine salt to self-assemble into

biocidal nanotubes;[13] with oligoethylene glycol to afford self-assembly of mesoscopically ordered chromatic polydiacetylene/silica nanocomposites[2] and so on. There are several excellent reviews on widely diverse nanomaterials formed by diacetylene incorporated fatty acids lipids[1,8]

Although diacetylene incorporated fatty acids and their derivatives found and continually to find novel applications in material sciences, there are mainly two synthetic methods reported so far (Scheme 3.1), one is coupling of a haloalkyne with a metal alkynoic acid via Cadiot-Chodkiewicz reaction[14,15] (**Method 1**), the other is started from 1, 4-bis (trimethylsilyl) butadiyne and put on the two side chains stepwise[16] (**Method 2**). Unfortunately, **method 1** has several limitations: first, it is not suitable for longer ω-alkynoic acids because of the low yield (generally <50%), second, it requires 1-haloalkynes, which is not so easy to make; third, unsymmetrical ω-diynoic acids are frequently contaminated by symmetrical diynoic acids. **Method 2** is flexible to make long chains with diacetylenic moiety at different position. However, this method has a much longer synthetic route and requires more harsh reaction conditions as well as expensive reagents.

Scheme 3.1



Therefore, it is of interest to find a convenient method to synthesize these diacetylene incorporated fatty acids. Since natural fatty acids are abundant, cheap and have varied chain-length (16-24 carbons), it should be of interest if we can transform these renewable material into the unique diacetylenic fatty acids.

3.2 Result and Discussion

Although there are numerous of fatty acids presenting in natural,[17] saturated fatty acids have no internal functional group for the structural transformation while multi-unsaturated fatty acids are too complicated and too expensive to be transformed, the best candidates for this purpose are these fatty acids with one unsaturation in the middle of the molecules (Table 3.1). Among them, oleic acid is the most abundant and accessible fatty acid derived from natural vegetable oils

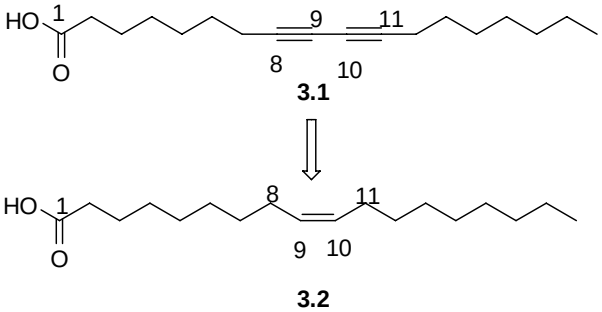
Table 3.1. Fatty acids with one unsaturation.

Myristoleic	Margarolic	Oleic	Gadoleic	Erucic
14:01*	17:01	18:01	20:01	22:01

*Number of carbon present on the fatty acid chain: number of double bond

Since these fatty acids are only differed in the number of the methylene groups between the carboxylic group and double bond as well as that between double bond and terminal methyl group, it is reasonable to focus the functional transformation on one particular fatty acid, and then apply the same synthetic methodology to all other fatty acids. Here, commercial available oleic acid (**3.2**) was chosen as the model compound for the structural transformation (Figure 3.2).

Figure 3.2

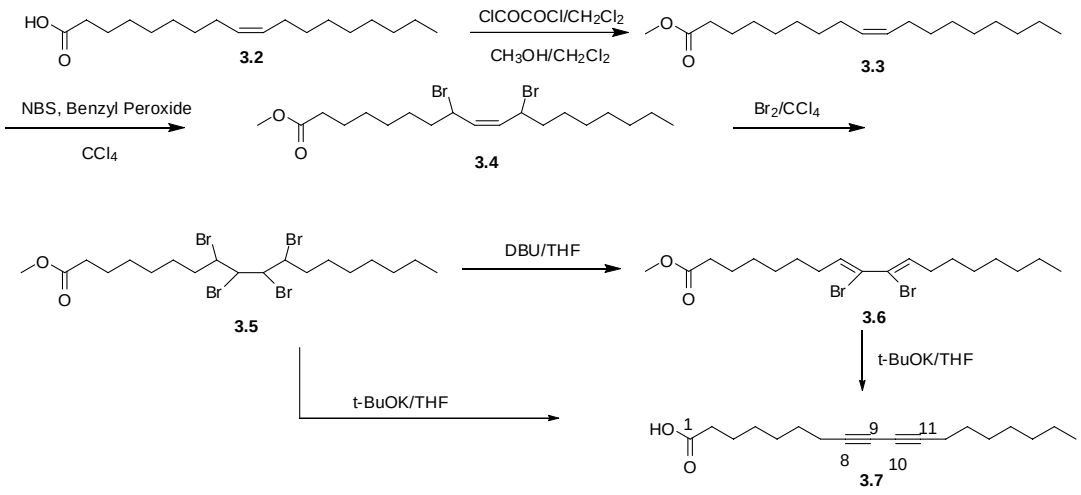


Apparently, in order to transform the olefin fatty acid to diacetylenic acid, protons on C8, C9, C10 and C11 of oleic acid should be involved in the transformation, and the transformation can

take advantage of the olefin functionality.

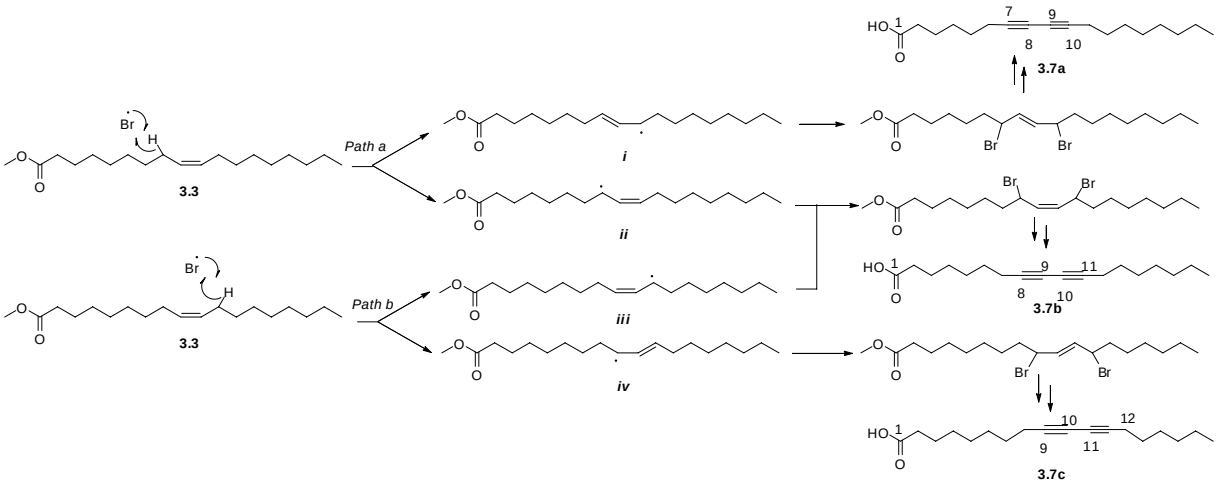
In order to involve the proton on C8 and C11, we first applied radical allylic bromination reaction to form the dibromide olefin (Scheme 3.2). In order to avoid the complexity caused by terminal carboxyl acid, oleic acid was transformed to corresponding ester **3.3** by reaction with oxalyl chloride and then methanol in the presence of pyridine (2 steps in 91% yield). Allylic bromination was carried out in benzene by using NBS as bromination agent and benzoic peroxide as initiator,[18] which gave dibromo **3.4** in 39% yield after column chromatography by using petroleum ether: ether (10:1) as eluent. In the following step, the internal double bond was brominated quantitatively by liquid bromine in carbon tetrachloride to give tetrabromide compound **3.5**.[19] Elimination of hydrobromides to make diacetylenic fatty acid was initially achieved stepwise: treatment of tetrabromide with DBU in methylene chloride or THF gave Methyl 9,10-dibromooctadeca- 8,10-dienoate (**3.6**), which was isolated by re-crystallization (79% yield); further treatment of **3.6** with potassium tert-butoxide in THF yielded final diacetylene **3.7** (mixture, 72% yield). Later, we found that by treatment of tetrabromide **3.5** with 10 fold of potassium tert-butoxide in THF at 0^{o}C could give the compound **3.7**. The crude material was purified by column chromatography using methylene chloride and methanol (50:1) as eluting solvent.

Scheme 3.2



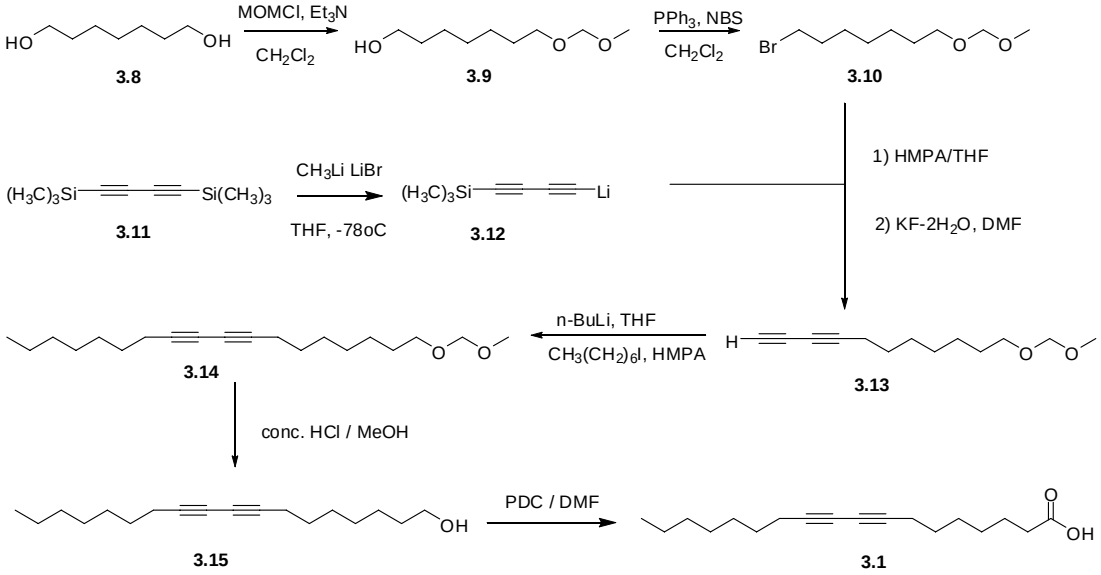
^{1}HNMR spectroscopy indicated that we could have successfully made diacetylenic oleic acid, but ^{13}CNMR spectroscopy didn't agree with it: there're more than enough carbon signals shown on the spectra for the dibromide **3.4**, tetrabromide **3.5** and diacetylene **3.7**. After carefully analyzing the reaction scheme and ^{13}CNMR spectra, we assumed that the diacetylene we got was a mixture of three compounds with a calculated ratio of 1: 2:1 (**3.7a**:**3.7b**:**3.7c**). The triple-bond shift was most likely the consequence of the allylic radical resonances during the allylic bromination, because there were four allylic intermediate structures for the allylic alkene (Scheme 3.3): if the radical attack initiated from C8 (*path a*), then once the radical (*i*) was formed, it was stabilized by its resonance structure with the radical on C10 (*ii*), therefore, the following radical attack could be accomplished by the radical on C8 or C10, which finally resulted in compound **3.7 a** and **3.7 b** after second allylic bromination. Based on the same argument, if the radical attack started on C11, the final diacetylene should be **3.7b** and **3.7c**. Since the initiation of the radical reaction was random, the ratio of these allylic bromination products was calculated as 1: 2: 1. The diacetylenic acid made from this method was labeled as **DAM** (diacetylenic acid mixture).

Scheme 3.3



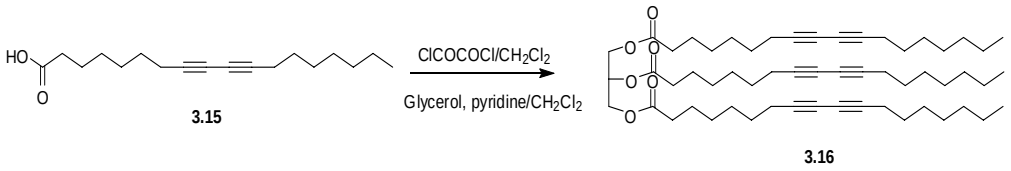
In order to further support this analysis and more importantly to observe the effect of the diacetylene triple-bond shift on surface self-assembly behavior, we synthesize the pure diacetylenic fatty acid according to the reported procedure (Scheme 3.4).[16] We started with 1,7-dihydroxylheptane, which was first monoprotected by MOMCl in the presence with triethylamine in methylene chloride to give compound **3.9** in 37.1% yield along with recovered starting material (43.7%). Bromination of compound **3.9** with triphenylphosphate and NBS in methylene chloride afforded compound **3.10** in 61.2%. Monolithiation of 1,4-bis(trimethylsilyl)-l,3-butadiyne (**3.11**) with methyllithium-lithium bromide complex in THF gave 1-lithium-4-(trimethylsilyl) butadiyne (**3.12**), which was directly coupled with the MOM-protected 7-bromoheptane **3.10** to yield 11-(methoxymethoxy)undeca- 1,3-diyne **3.13** after further desilylation with potassium fluoride dihydrate in DMF (yield 75.9% after column chromatography). Coupling of the freshly prepared lithium salt of **3.13** with 7-bromoheptane in the presence of HMPA as an anion stabilizer gave unsymmetrical 1-(methoxymethoxy) octadeca-8,10-diyne (**3.14**) in 64.3% yield. The MOM protecting group was quantitatively removed by treating the compound **3.14** with a solution of concentrated hydrochloride in methanol (1: 10, V/V) at room temperature. The final oxidation of the alcohol **3.15** by using PDD(pyridinium dichromate) in DMF at room temperature gave conjugated diacetylenic fatty acid **3.1** in 72.4% yield. The ^{13}C NMR spectrum clearly showed 18 carbons, which indicated compound **3.1** was a pure compound. The diacetylenic acid made from this method was labeled as **DAP** (Diacetylenic acid pure)

Scheme 3.4



We also synthesized two triglycerides (propane-1,2,3-triyl trioctadeca-8,10- diynoate) with diacetylenic fatty acids prepared through two different methods discussed above (Scheme 3.5). We labeled them as **TDAP** (triglyceride from diacetylenic acid pure compound) and **TDAM** (triglyceride from diacetylenic acid mixture).

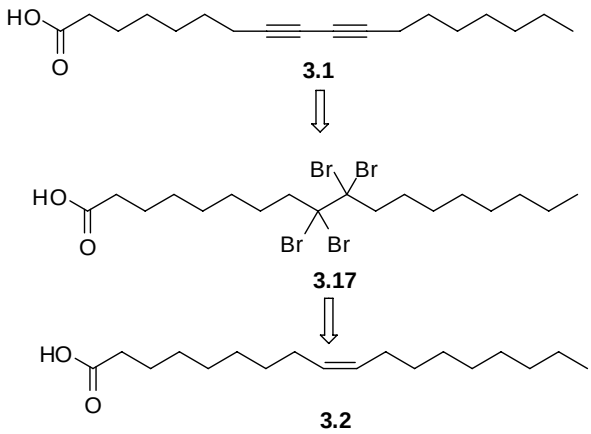
Scheme 3.5



Since the method described in Scheme 3.2 involved random triple bond shifts caused by the radical allylic bromination and gave a mixture of diacetylenic fatty acids, if we want to synthesize pure diacetylene, intermediates that have resonance structures have to be avoided. Therefore, we designed a new strategy shown in Scheme 3.6, we expected that oleic acid could be transformed to tetrabromide **3.17** and then convert compound **3.17** to diacetylene **3.1** by

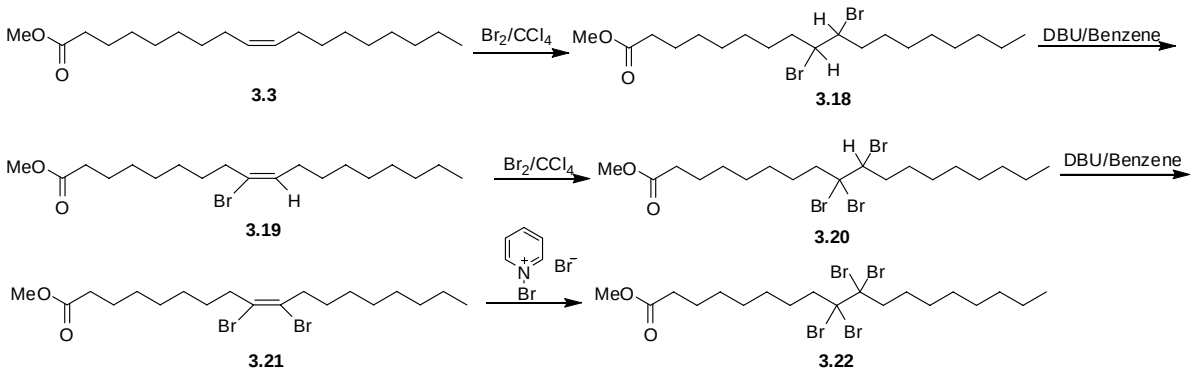
eliminations (Figure 3.3).

Figure 3.3



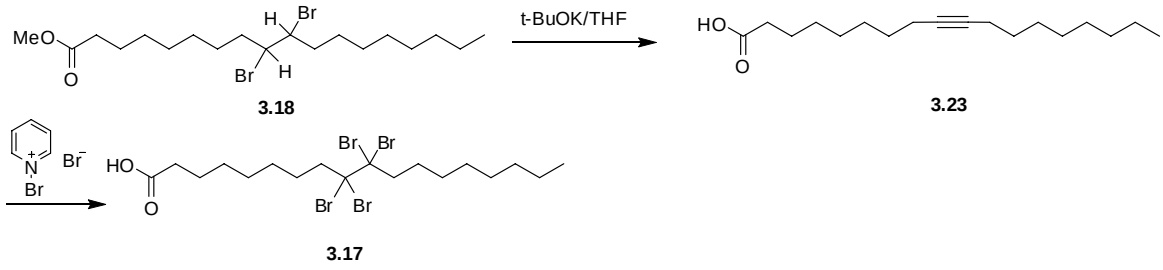
In order to avoid unexpected complexity, methyl oleate **3.3**, instead of oleic acid, was used as starting material. Preparation of tetrabromide was accomplished by following addition-elimination strategy (Scheme 3.6): first, methyl oleate was directly brominated to dibromo **3.18** by liquid bromine in carbon tetrachloride in quantitative yield,[20] ^{1}HNMR and ^{13}CNMR showed it is a single compound. The resulting dibromo **3.18** was subjected to a basic elimination by using DBU in benzene which clearly giving the vinylbromo **3.19** (with regio-isomer).[21] Repeated bromination and elimination gave vinydibromo methyl oleate **3.21** in excellent yield. This vinyldibromo **3.21** was supposed to give crucial tetrabromide fatty acid. However, treatment of vinyldibromo **3.21** with liquid bromine in carbon tetrachloride didn't give desired product. Fortunately, mild reagent such as pyridinium bromide perbromide quantitatively gave tetrabromide **3.22**.

Scheme 3.6



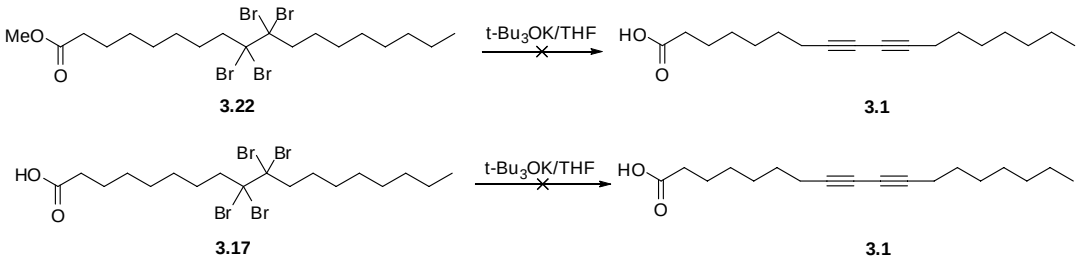
Later, a short synthesis was developed to synthesize the tetrabromide (Scheme 3.7): the dibromide **3.18** was first double-eliminated to acetylene **3.23**,[22] and then the resulting acetylene was brominated to tetrabromide **3.17** by using mild bromination reagent (pyridinium bromide perbromide).

Scheme 3.7



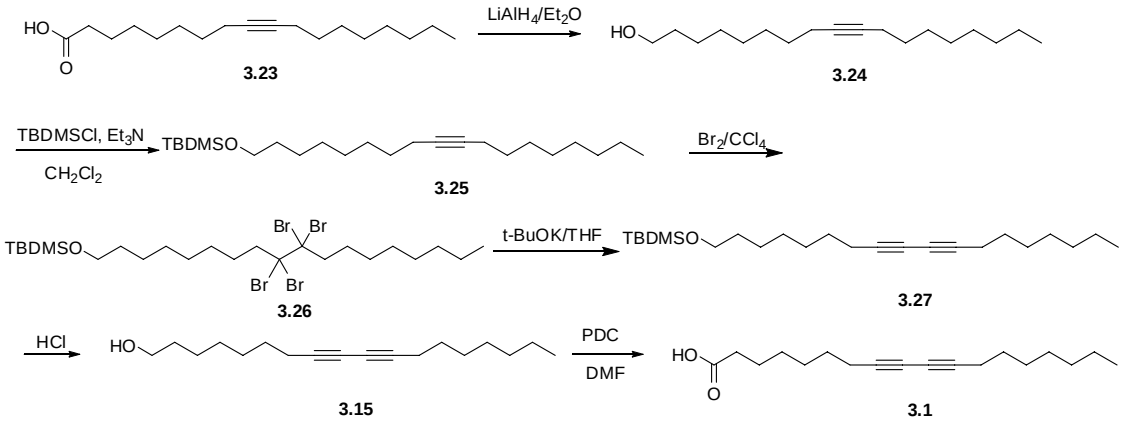
Disappointedly, when tetrabromides were treated with t-BuOK in THF, either of compound **3.17** and **3.22** afforded the desired diacetylene (Scheme 3.8). Most likely, the acidity of the protons on the carbon adjacent to tetrabromides were weak than those on the carbon next to the carboxylic ester and carboxylic acid proton.

Scheme 3.8



Therefore, carboxylic acid or ester groups have to be transformed in order to reduce the acidity of the protons on the adjacent carbon. Here, we reduced acetylene **3.23** to alcohol **3.24** and protected the alcohol with bulky TBDMS group before the elimination reaction. Strong bases such as n-butyllithium and tert-Butyllithium may deprotonate the adjacent α-proton of the tetrabromo function and make the elimination feasible (Scheme 3.9). Deprotection of **3.27** by solution of hydrochloride /methanol should give compound **3.15**, which could be oxidized to compound **3.1**. These works are not pursued.

Scheme 3.9



3.3 Studies of photopolymerization of diacetylenes

3.3.1. Chromatic Change

The diacetylene made from oleic acid is a mixture (**DAM**) and it is a liquid at room temperature and it would become solid when it was stored in refrigerator after several days. Once the temperature rise to room temperature, it becomes liquid. When it was stored in refrigerator for several months, the color will turn blue and when the solid turn into liquids, red-solid will be seen floating on the surface.

The diacetylene made from 1,4-bis(trimethylsilyl)-l,3-butadiyne is a pure compound (**DAP**). It is solid at room temperature and readily turns into blue color if left on bench for a couple of hours. When dissolved in solvent, red-solid can be observed on the surface of the solution. The color change must relate to the polymerized diacetylene.

Triglyceride **TDAM** is a liquid while **TDAP** is a solid. Both of the two triglycerides can not be polymerized as bulk materials, even with prolonged UV exposure or high temperatures.

3.3.2. Contact Angle Measurements

Water contact angle measurement was used to evaluate the adsorption of octadeca-8,10-diynoic acids (**DAM** and **DAP**) and glyceryl tris(octadeca- 8,10-diynoate)s (**TDAM** and **TDAP**) on vapor-deposited alumina surface. This measurement could reveal the effect of the triple-bond shift on their self-assembly behaviors. The adsorption (self-assembly) of the compounds may through the interaction between the polar carboxylic acid or ester groups and the alumina surface, the resulting monolayer is supposed to be stabilized by van der Waal interaction between the adjacent long hydrocarbon chains. Moreover, because of the hydrocarbon chain, the alumina surface changed to hydrophobic, thus by measuring the water contact angle, we can evaluated the monolayer formation. The results are summarized in Table 3.2. The water contact angle on blank alumina surface was measured as a control with a θ^{water} of 8^{o}, which means hydrophilic.

Table 3.2 Water Contact Angles of Chemically Modified Alumina Substrates

	DAM		DAP		TDAM		TDAP	
	BP	AP	BP	AP	BP	AP	BP	AP
Contact Angle	45	61	68	91	46	67	53	78

* BP: Before UV Light Polymerization AP: After UV Light Polymerization

1. Contact Angle (θ^{water}) of Alumina Surfaces Deposited with DAM and DAP

Diacetylenic fatty acids synthesized from two methods can self-assemble on the alumina surface. However, from the contact angle data (68^o vs 45^o), it is apparently that the pure diacetylenic acid (**DAP**) can modify the surface more hydrophobic than the mixture (**DAM**). This is not too surprising, because the pure material molecules can pack on a surface in a more ordered way than the mixture. The increase of the contact angle after polymerization indicates that topochemical polymerization occurred (the change of molecular orientation and free volume is very small) and it can improve the orderliness of molecular self-assembly. Since the alignment of diacetylene moieties of **DAP** is more ordered, long range polymerization is more efficient, which result in bigger increase of water contact angle.

2. Contact Angle (θ^{water}) of Alumina Surfaces Deposited with TDAM and TDAP

Triglycerides with three diacetylenic side-chains also adsorbed on the alumina surface. Surprisingly, the much less polar ester group (comparing to carboxylic acid) didn't affect the adsorption too much (46^o of **TDAM** vs 45^o of **DAM**), this may because the three ester groups on one triglyceride molecule, the binding energy of three esters contacting the alumina surface may be comparable to that of one carboxylic acid group. As for **TDAP**, the measured contact angle is smaller than that of **DAP** (53^o vs 68^o), that may be caused by the increase of intra-molecular distance among the three side-chains on the same triglyceride molecule due to the out-reaching glycerol linker, which make the side-chains be less tightly packed than diacetylenic fatty acids do (Figure 3.4). Because diacetylene polymerization is highly distance dependant, the prolonged

distance of intramolecular side chains make the adjacent side chain polymerization in the same molecule impossible. However, inter-penetrating molecular alignment of **TDAM** or **TDAP** molecules on the alumina surface still can meet the distance requirement for intermolecular polymerization, which improves the surface packing order (46^o to 67^o, 53^o to 78^o).

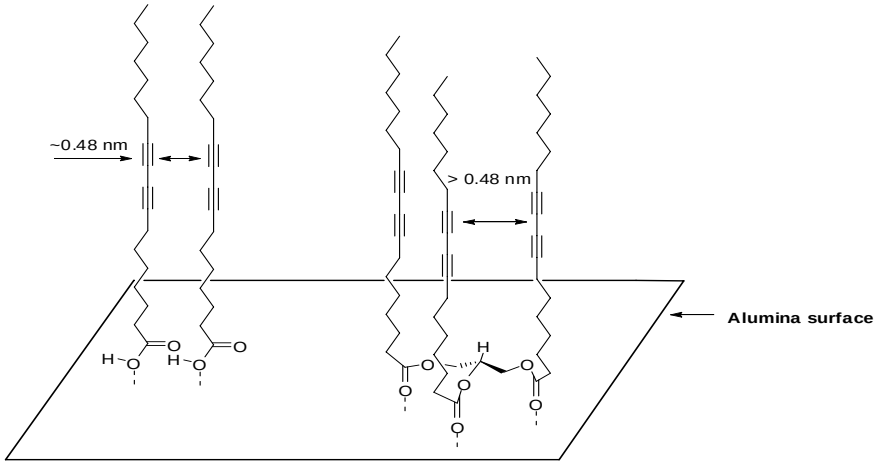


Figure 3.4 Schematic illustration of DAP and TDAP assembly on alumina surface. The self-assembly of DAP molecules on alumina surface can meet the distance requirement for solid state polymerization while self-assembly of TDAP molecules can not meet the distance requirement for intramolecular polymerization.

3.4 Conclusion

A new synthetic method of octadecadiynoic acid from oleic acid was developed. The pure octadeca-8,10-diynoic acid was also synthesized according to reported procedure in order to compare the self-assembly behavior on alumina surface. From the water contact angle measurement, we found that although the final octadecadiynoic acid is a mixture, it still can improve the self-assembly after photo-polymerization.

3.5 Experimental

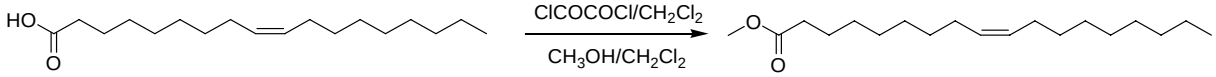
Sample Preparation and Contact Angle Measurement:

Alumina deposited glass slides were cut to small pieces (~1.5 x 1.5 mm^2). The small alumina substrates were soaked in a 5 mM toluene solution of fatty acid or triglyceride for 1 hour, thoroughly washed with toluene and then dried in argon flow. The water contact angle measurement was achieved by using a PG-1 pocket contact angle goniometer by measuring values of the two sides of a drop within 30 seconds after depositing the water drop.

UV polymerization of these self-assembled monolayers was carried out as following: sample deposited alumina substrate was loaded on the holder inside the chamber of UV irradiator. The chamber was flushed with argon for 10 minutes before turn on the UV light for a variable time. The measurement of contact angle on these polymerized surfaces was obtained following the same method mentioned above.

Synthesis

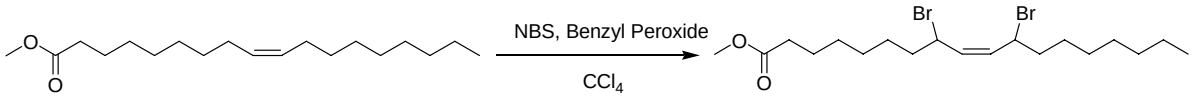
Methyl oleate (3.3)



To a solution of oleic acid (10.0 g, 35.4 mmol) in 50 mL of dichloromethane under argon was added oxalyl chloride (6.74 g, 53.1mmol), and the solution was stirred at 25 ^oC for 2 hours. After removal of excess oxalyl chloride and solvent over a rotary evaporator, the oily residue, acid chloride, was dried under vacuum and dissolved in 20 mL of dichloromethane. To this solution, was added methanol (1.68 g, 53.1 mmol) and pyridine (5.72 ml, 70.8 mmol) at 0 ^oC under argon. The solution was stirred at 25^oC for 2 hours, diluted with 50 mL of dichloromethane, washed twice with water (50 mL each) and saturated aqueous sodium chloride (50 mL) solution, dried (magnesium sulfate), concentrated, and and column chromatographed on silica gel using hexane:ethyl acetate (20:1) as eluant to give ester 9.58g g (91.3 % yield). **^{1}H NMR** δ 5.36 (m, 2H), 3.67 (s, 3H), 2.30(t, J=7.3, 2H), 2.03 (m, 4H), 1.62 (m, 2H), 1.24-1.36(m, 18H), 0.88 (t, J=6.6, 3H). **^{13}C NMR**: δ 130.2, 129.9, 51.6, 34.29, 32.08, 29.95, 29.86, 29.70, 29.50,

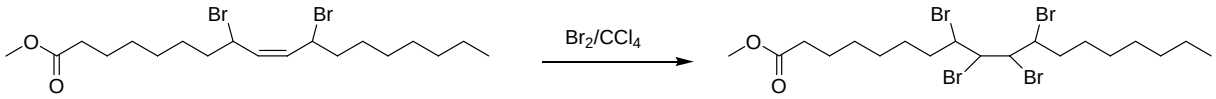
29.33, 29.30, 29.27, 27.39, 27.34, 25.13, 22.87, 14.28.

Methyl 8,11-dibromooctadec-9-enoate (3.4)



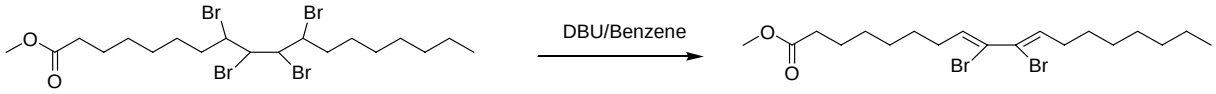
To a solution of methyl ester (5.0g, 16.9mmol) in 20 ml carbon tetrachloride, were added NBS (6.0g, 33.8mmol) and benzyl peroxide (153mg, 0.6mmol) at 0 ^{o}C. The resulting mixture was refluxed for 2 hours, diluted with 20 mL of dichloromethane, washed with water and brine, dried over magnesium sulfate, concentrated, and column chromatographed on silica gel using hexane:ether (10:1) as eluant to give dibromide 3.0g (39.0 % yield). **^{1}H NMR** δ 5.80-5.83 (m, 2H), 4.43-4.50 (m, 2H), 2.29-2.34 (m, 2H), 1.82-1.98 (m, 4H), 160-1.66 (m, 2H), 1.25-1.44(m, 18H), 0.88 (t, J=6.6, 3H). **^{13}C NMR**: δ 174.35, 133.73, 133.68, 133.48, 133.41, 133.35, 133.33, 133.21, 133.17, 54.24, 54.08, 53.86, 51.66, 39.16, 39.05, 38.99, 38.89, 34.26, 34.21, 34.11, 32.03, 31.94, 31.84, 29.58, 29.39, 29.28, 29.25, 29.21, 29.11, 29.04, 28.88, 28.74, 28.71, 28.55, 27.97, 27.92, 27.86, 27.76, 27.66, 27.50, 25.08, 25.01, 24.90, 22.85, 22.81, 22.74, 14.26. MS: 452.1.

Methyl 8,9,10,11-tetrabromooctadecanoate (3.5)



To a solution of dibromide (100mg, 0.22mmol) in 8ml carbon tetrachloride, was added liquid bromine (35.2mg, 0.22mmol) dropwise at room temperature. The mixture was stirred for another half an hour then solvent and excessive bromine were evaporated. The crude material was used directly in next step MS: 611.9.

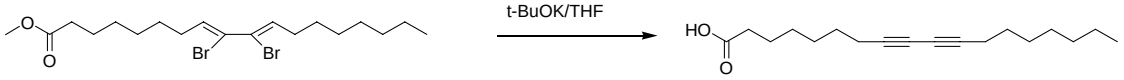
Methyl 9,10-dibromooctadeca-8,10-dienoate (3.6)



To a solution of tetrabromide (79mg, 0.13mmol) in 5ml benzene, was added DBU(78mg, 0.52mmol) at room temperature. The resulting mixture was stirred for another 4 four hours, then diluted with ethyl acetate, washed with saturated ammonium chloride, water, and brine, dried over magnesium sulfate and concentrated. Column chromatography gives 46.2mg product (79% yield). **^{1}H NMR** 6.40-6.52 (m, 2H), 2.26-2.36 (m, 6H), 1.22-1.70 (m, 18H), 0.88 (t, J=6.6, 3H). **^{13}C NMR** δ 174.36, 135.77, 135.69, 135.64, 135.40, 135.27, 135.06, 126.07, 125.84, 68.16, 51.61, 42.31, 34.26, 32.47, 32.34, 32.06, 31.97, 31.83, 29.47, 29.11, 29.0728.49, 28.28, 25.12, 25.04, 24.95, 22.83, 14.26.

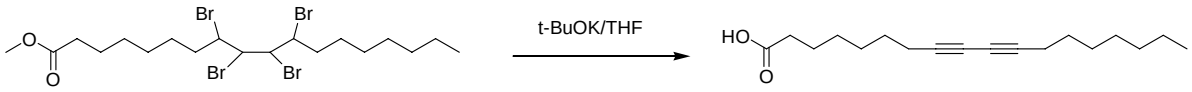
Octadeca-8,10-diynoic acid (3.7)

Method 1



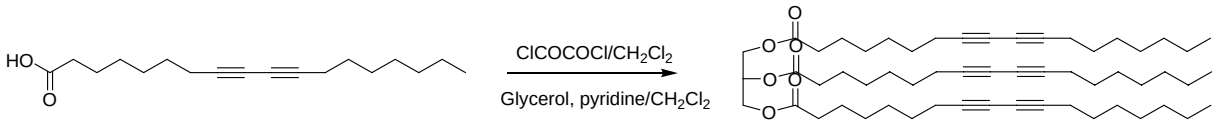
To a solution of dibromide-diene (58mg, 0.13mmol) in 3 ml of THF, was added potassium tert-butoxy (75mg, 1.3mmol). The resulting mixture was stirred overnight and diluted with ethyl acetate, washed with saturated ammonium chloride, water, and brine, dried over magnesium sulfate and concentrated. Column chromatographed on silica gel using dichloridemethane:methanol (50:1) as eluant to give diacetylene acid 26mg (72.1% yield). **^{1}H NMR** δ 2.33-2.38 (m, 2H), 2.23-2.67 (m, 4H), 1.26-1.66 (m, 18H), 0.88 (t, J=6.9, 3H). **^{13}C NMR** δ 179.91, 77.91, 77.54, 77.38, 65.65, 65.56,65.38, 34.15, 34.10, 34.00, 32.02, 31.88, 31.50, 31.12, 29.34, 29.26, 29.06, 29.02, 28.97, 28.91, 28.81, 28.70, 28.60, 28.54, 28.44, 28.40, 28.16, 24.80, 24.69, 24.34, 22.81, 19.40, 19.33, 14.26. MS $[M+1]^{+}$: 277.5.

Method 2



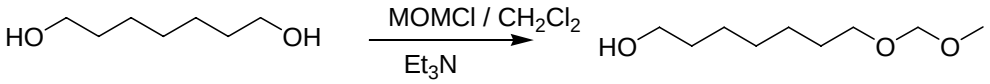
To a solution of tetrabromide (58mg, 0.13mmol) in 3 ml of THF, was added potassium tert-butoxy (75mg, 1.3mmol). The resulting mixture was stirred overnight and diluted with ethyl acetate, washed with saturated ammonium chloride, water, and brine, dried over magnesium sulfate and concentrated. Column chromatographed on silica gel using dichloridemethane:methanol (50:1) as eluant to give diacetylene acid 26mg (72.1% yield). ^{1}H NMR and ^{13}CNMR are the same as above.

Propane-1,2,3-triyl trioctadeca-8,10-diynoate (3.16)



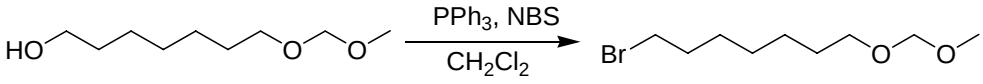
To a solution of diacetylene acid (32m g, 0.12 mmol) in 1.5 mL of dichloromethane under argon was added oxalyl chloride (17.6m g, 0.14mmol), and the solution was stirred at 25^{o}C for 2 hours. After removal of excess oxalyl chloride and solvent over a rotary evaporator, the oily residue, acid chloride, was dried under vacuum and used in the next operation without purification. To a solution of glycerol (3.2mg, 0.04 mmol) and pyridine (10mg , 0.12 mmol) in dichloromethane (1 ml) at 0 ^{o}C under argon was added a solution of the above acid chloride in 2mL of dichloromethane via cannula. The solution was stirred at 25 ^{o}C for 2 hours, diluted with 15 mL of dichloromethane, washed twice with water (15 mL each) and saturated aqueous sodium chloride (15 mL) solution, dried (magnesium sulfate), concentrated, and column chromatographed on silica gel using petroleum ether : ether (4:1) as eluant to give triglyceride 18mg (52 % yield): **^{1}H NMR** δ 5.27 (m, 1H), 4.31 (d, J=12, 2H), 4.14 (dd, J=12, 6, 2H), 2.34 (m, 6H), 2.25 (t, J=6.9, 12H), 1.21-1.68 (m, 54H), 0.88 (t, J=7.7, 9H). **^{13}C NMR** δ 173.40, 77.91, 77.40, 69.11, 65.75, 65.49, 62.32, 34.17, 32.03, 31.89, 31.50, 29.91, 29.26, 29.07, 29.03, 28.97, 28.73, 28.63, 28.55, 24.98, 24.87, 22.81, 19.41, 14.27.

7-(methoxymethoxy)-1-Heptanol (3.9)



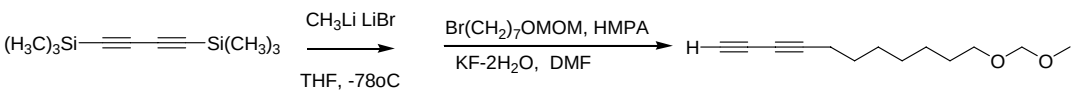
To a solution of 1,7-dihydroxyheptane (1.60g, 12.1mmol) in 20 ml dichloromethane, were added triethylamine(1.85ml, 13.3mmol) and dimethoxylchloride (1.07g, 13.3 mmol) at 0 ^{o}C. The resulting solution was stirred at room temperature overnight and diluted with ethyl acetate, washed with water and brine, dried over magnesium sulfate and concentrated. Column chromatography on silica gel using petroleum: ethyl acetate (3:2) as eluant to give 7-(methoxymethoxy)-1-Heptanol 0.79g (37.1 % yield). **^{1}H NMR** δ 4.62 (s, 2H), 3.65 (t, J=6.6, 2H), 3.52 (t, J=6.6, 2H), 3.37 (s, 3H), 1.3-1.7(m, 10H); **^{13}C NMR** δ 96.67, 68.08, 63.24, 55.32, 32.97, 29.91, 29.44, 26.42, 25.93.

1-bromo-7-(methoxymethoxy)-Heptane (3.10)



To a solution of dried 7-(methoxymethoxy)-1-Heptanol (344mg, 1.95mmol) and triphenylphosphite (769mg, 2.93mmol) in 10 ml dichloromethane, was added NBS (519mg, 2.93mmol) at 0^{o}C. The resulting mixture was stirred at room temperature for 20 minutes and concentrated. Column chromatography on silica gel using petroleum: ether (10:1) as eluant to give 1-bromo-7-(methoxymethoxy)-Heptane 285mg (61.2 % yield). **^{1}H NMR** δ 4.62 (s, 2H), 3.52 (t, J=6.6, 2H), 3.41 (t, J=6.9, 2H), 3.36 (s, 3H), 1.83-1.90 (m, 2H), 1.3-1.62(m, 8H); **^{13}C NMR** δ 96.66, 67.96, 55.30, 33.99, 32.94, 29.85, 28.75, 28.31, 26.24.

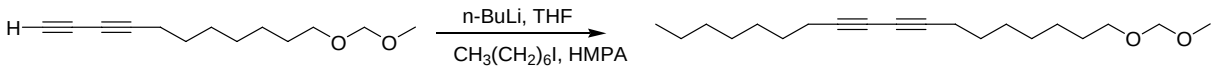
11-(methoxymethoxy)-1,3-undecadiyne (3.13)



To a solution of 1,4-bis(trimethylsily)-1,3-butadiyne (545mg, 2.80mmol) in 10 ml of THF was

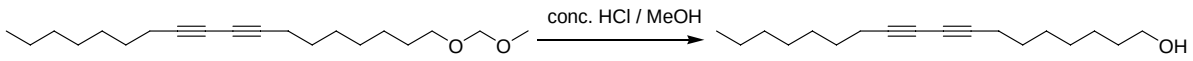
added methyllithium-lithium bromide (1.87 ml, 2.80mmol) at -78°C and stirred at room temperature for 4 hours. After adding a solution of 1-bromo-7-(methoxy methoxy) –Heptane (670mg, 2.8mmol) in 2 ml of HMPA at -78°C, the black solution was stirred at room temperature for another 1 hour, then adjust pH to 7 by 2mol/ml HCl, diluted with ethyl acetate, washed with water and brine, dried over magnesium sulfate and concentrated. Column chromatography on silica gel using petroleum: ethyl acetate (12:1) as eluant to give 11-(methoxymethoxy)-1,3-Undecadiyne 440mg (75.9 % yield). **^{1}H NMR** δ 4.62 (s, 2H), 3.52(t, J=6.6, 2H), 3.36 (s, 3H), 2.26 (m, 2H), 1.90 (s, 1H), 1.32-1.61(m, 10H). **^{13}C NMR** δ 96.54, 78.51, 68.65, 67.89, 64.90, 64.65, 55.17, 29.80, 28.95, 28.85, 28.06, 26.17, 19.11.

1-(methoxymethoxy)-8,10-Octadecadiyne (3.14)



To a solution of 11-(methoxymethoxy)-1,3-Undecadiyne (533mg, 2.56mmol) in 8 ml of THF was added n-butyl-lithium (1.92 ml, 3.07mmol) at -23°C and stirred at this temperature for 1 hours. After adding a solution of 1-bromo–Heptane (0.48ml, 3.07mmol) in 8 ml of HMPA at -23°C, the black solution was stirred at -23°C for half an hour and room temperature for 1 hour, then adjust pH to 7 by adding 2mol/ml HCl, diluted with ethyl acetate, washed with water and brine, dried over magnesium sulfate and concentrated. Column chromatography on silica gel using petroleum:ethyl acetate (12:1) as eluant to give 504mg of 1-(methoxymethoxy)-8,10-Octadecadiyne (yield: 64.3%). **^{1}H NMR** δ 4.62 (s, 2H), 3.52 (t, J=6.6, 2H), 3.36 (s, 3H), 2.25(t, J=6.6, 4H), 1.27-1.62(m, 20H), 0.88(t, J=6.3, 3H). **^{13}C NMR**: 96.58, 77.78, 77.57, 67.97, 65.52, 65.41, 55.28, 31.88, 29.87, 29.08, 29.00, 28.96, 28.54, 28.45, 26.26, 22.81, 19.37, 14.26.

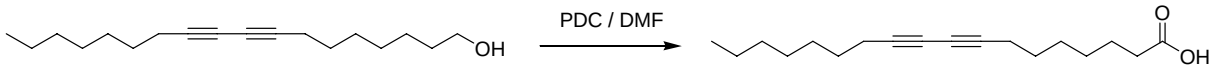
8,10-Octadecadiyn-1-ol (3.15)



To a solution of 164mg of 1-(methoxymethoxy)-8,10-Octadecadiyne in 10ml of methanol, was

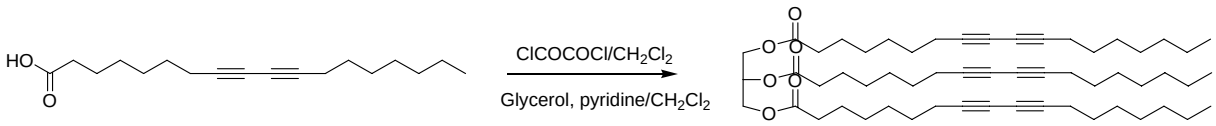
added 1.5 ml of concentrated HCl, the solution was stirred at room temperature overnight, diluted with ethyl acetate, washed with water and brine, dried over magnesium sulfate and concentrated. The colorless to light brown oil was used directly in the next step. **^{1}H NMR** δ 3.59 (t, J=6.6, 2H), 2.20 (t, J=6.6, 4H), 0.82-1.56 (m, 20H), 0.84 (t, J=6.6, 3H). **^{13}C NMR** δ 77.74, 77.51, 65.61, 65.48, 63.09, 32.88, 31.85, 29.04, 28.98, 28.94, 28.92, 28.55, 28.44, 25.76, 22.77, 19.37, 19.35, 14.18.

8,10-Octadecadiynoic acid (3.1)



To a solution of 110 mg of 8,10-Octadecadiyn-1-ol in 5ml of DMF was added 1.25g of pyridium dichromate, the resulting mixture was stirred at room temperature over night. Then the mixture was acidified with 2 mol/L of HCl, and then extracted with petroleum ether twice. The organic layer combined and washed with water and brine, dried over magnesium sulfate and concentrated. Column chromatography on silica gel using chloroform:methanol (50:1) as eluant to give 8,10-Octadecadiynoic acid (84mg, yield: 72.4%). **^{1}H NMR** δ 2.38 (t, J=7.7, 2H), 2.24 (t, J=6.9, 4H), **^{13}C NMR** δ 179.91, 77.91, 77.40, 65.75, 65.49, 34.03, 31.91, 29.04, 28.97, 28.73, 28.63, 28.59, 24.74, 22.83, 19.44, 19.37, 14.25.

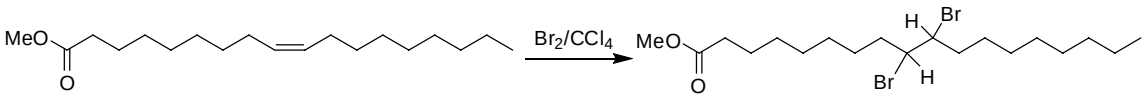
Propane-1,2,3-triyl trioctadeca-8,10-diynoate (3.16 pure)



To a solution of diacetylene acid (220mg, 0.79mmol) in 5mL of dichloromethane under argon was added oxalyl chloride (0.21ml, 1.2mmol), and the solution was stirred at 25^{o}C for 2 hours. After removal of excess oxalyl chloride and solvent over a rotary evaporator, the oily residue, acid chloride, was dried under vacuum and used in the next operation without purification. To a solution of glycerol (23mg, 26mmol) and pyridine (64mg , 0.80mmol) in

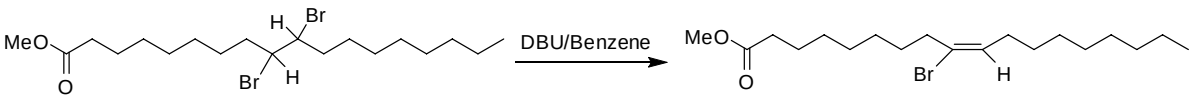
dichloromethane (1 ml) at 0 ^{o}C under argon was added a solution of the above acid chloride in 2mL of dichloromethane via cannula. The solution was stirred at 25 ^{o}C for 2 hours, diluted with 15 mL of dichloromethane, washed twice with water (15 mL each) and saturated aqueous sodium chloride (15 mL) solution, dried (magnesium sulfate), concentrated, and column chromatographed on silica gel using petroleum ether : Ether (4:1) as eluant to give triglyceride 18mg (52 % yield): **^{1}H NMR:** δ 5.27 (m, 1H), 4.31 (d, J=12, 2H), 4.14 (dd, J=12, 6, 2H), 2.34 (m, 6H), 2.25 (t, J=6.9, 12H), 1.21-1.68 (m, 54H), 0.88 (t, J=7.7, 9H). **^{13}C NMR:** δ 173.40, 77.91, 77.40, 69.11, 65.75, 65.49, 62.32, 34.16, 31.92, 29.05, 29.00, 28.78, 28.66, 28.58, 28.35, 24.88, 22.84, 19.43, 19.37, 14.29.

Methyl 9,10-dibromooctadecanoate (3.18, HZ-6-97)



To a solution of oleic acid (1.0 g, 3.38 mmol) in carbon tetrachloride (15 ml) was added liquid bromine (0.81 g, 5.06 mmol) at room temperature. The solution was stirred for half an hour and evaporated to dryness. The crude material was pure enough to be used directly in next step. **^{1}H NMR** δ 4.20 (m, 2H), 3.65 (s, 3H), 2.29 (t, J=7.7Hz, 3H), 2.10~1.90 (m, 2H), 1.89~1.75 (m, 2H), 1.65~1.50 (m, 4H), 1.40~1.20 (m, 18H), 0.87 (t, J=5.1 Hz, 3H); **^{13}C NMR** δ 174.45, 59.97, 59.89, 51.64, 35.04, 35.01, 34.23, 32.00, 29.54, 29.37, 29.17, 29.01, 28.79, 27.99, 27.91, 25.04, 22.82, 14.27.

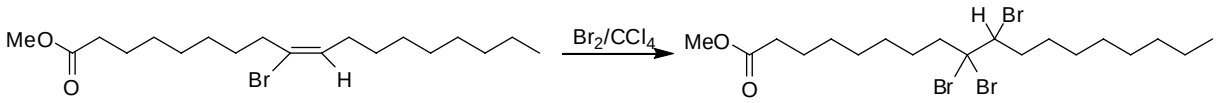
Methyl 9-bromooctadec-9-enoate (3.19)



To a solution of dibromooleic acid (0.5 g, 1.10 mmol) in benzene (10 ml) was added 0.25 g of DBU (1.64 mmol) at room temperature and stirred overnight. The solution was diluted with ethyl acetate, washed with 2N HCl, water and brine, dried over magnesium sulfate and evaporated to

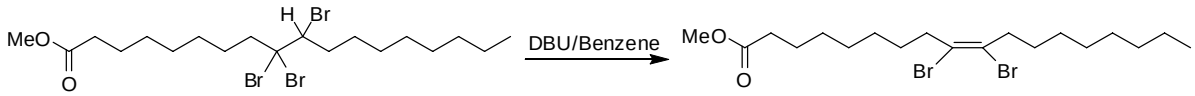
dryness. **^{1}H NMR** δ 5.60 (t, J=6.0Hz, 1H), 3.67 (s, 3H), 2.42~2.24 (m, 4H), 2.20~2.08 (m, 2H), 1.65~1.50 (m, 6H), 1.40~1.20 (m, 16H), 0.88 (t, J=6.4Hz, 3H). **^{13}C NMR** δ 174.4, 128.8, 128.6, 128.5, 128.4, 51.6, 41.7, 41.6, 34.2, 21.0, 31.43, 31.36, 29.6, 29.5, 29.43, 29.41, 29.35, 29.21, 29.1, 28.68, 28.58, 28.56, 28.30, 28.19, 25.1, 22.8.

Methyl 9,9,10-tribromooctadecanoate (3.20)



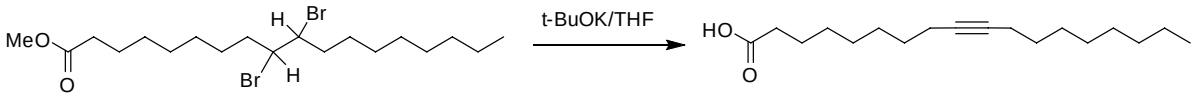
To a solution of oleic acid (0.3 g, 0.8 mmol) in carbon tetrachloride (8 ml) was added liquid bromine (0.19 g, 1.20 mmol) at room temperature. The solution was stirred for half an hour and evaporated to dryness. The crude material was not purified and was used directly in next step. **^{1}H NMR** δ 4.16 (m, 1H), 3.67 (s, 3H), 2.63~2.51 (m, 2H), 2.35 (t, J=7.6, 2H), 2.15~1.95 (m, 2H), 1.80~1.60 (m, 4H), 1.40~1.20 (m, 18H), 0.89 (t, J=6.5Hz, 3H); **^{13}C NMR** δ 174.4,78.7, 78.6, 64.9, 64.8, 51.6, 50.4, 37.6, 34.2, 32.0, 31.9, 29.5, 29.43, 29.36, 29.31, 29.15, 29.10, 28.9, 27.92, 27.85, 27.1, 27.0, 25.02, 24.99, 22.8, 14.3.

(Z)-Methyl 9,10-dibromooctadec-9-enoate (3.21)



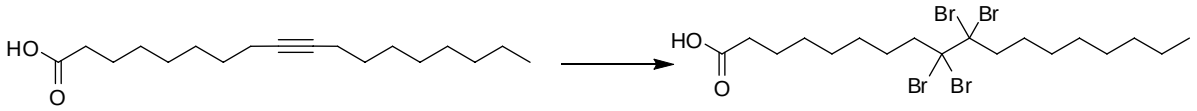
To a solution of dibromooleic acid (0.43 g, 0.8 mmol) in benzene (8 ml) was added 0.18 g of DBU (1.2 mmol) at room temperature and stirred overnight. The solution was diluted with ethyl acetate, washed with 2N HCl, water and brine, dried over magnesium sulfate and evaporated to dryness. **^{1}H NMR** δ 3.67 (s, 3H), 2.66 (t, J=7.0, 4H), 2.35 (t, J=7.6, 2H), 1.80~1.60 (m, 4H), 1.40~1.20 (m, 18H), 0.89 (t, J=6.5Hz, 3H); **^{13}C NMR** δ 174.4, 121.9，121.7，51.6，40.9，34.2，32.0，29.5，29.4，29.2，28.6，28.4，27.5, 27.4, 25.0, 22.8.

Octadec-9-ynoic acid (3.23, HZ-10-40)



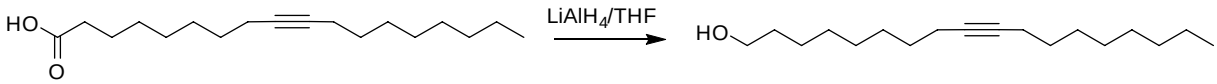
To a solution of dibrobo (2.0 g, 4.39 mmol) in 30 ml of THF was added t-BuOK (2.46 g, 21.93 mmol) and stirred overnight, the mixture was diluted with ether, washed with saturated ammonium chloride, water and brine, concentrated to dryness. The crude material was recrystallized in petroleum ether. **^{1}H NMR** δ 2.36 (t, J=7.4, 2H), 2.16 (t, J=5.5, 4H), 1.65 (m, 2H), 1.47 (m, 4H), 1.40-1.20 (m, 16H), 0.89 (t, J=6.4, 3H). **^{13}C NMR** δ 180.26, 80.58, 80.28, 34.23, 32.06, 29.44, 29.38, 29.35, 29.27, 29.17, 29.10, 28.98, 28.83, 24.84, 22.88, 18.97, 18.93, 14.32.

9,9,10,10-Tetrabromooctadecanoic acid (3.17, HZ-10-42)



To a solution of acetylene (30 mg, 0.11 mmol) in 3 ml of carbon tetrachloride was added pyridinium bromide (56.2 mg, 0.24 mmol) and stirred at room temperature for 4 hours. The mixture was diluted with ether, washed with water and brine, concentrated to dryness. The proton NMR showed a clean reaction result. **^{1}H NMR** δ 2.66 (t, J=7.4, 4H), 2.36 (t, J=7.4, 2H), 1.64 (m, 2H), 1.56 (m, 4H), 1.40-1.24 (m, 16H), 0.88 (t, J=6.8, 3H).

Octadec-9-yn-1-ol (3.24)



To a solution of acetylene acid (450mg， 1.59 mmol) in 15 mL of THF was added $LiAlH_4$ (302.3 mg, 7.97 mmol) at 0^{o}C. The resulting mixture was stirred at room temperature for 4 hours and quenched with water. The mixture was extracted with ether twice (25 ml x2) and the organic layers were combined, washed with water and brine, dried over magnesium sulfate and

concentrated to dryness. Column chromatography with petroleum ether and ether (3:1) gave alcohol (yield 96%). **^{1}H NMR** δ 3.64 (t, J=6.4, 2H), 2.14 (t, J=5.6, 4H), 1.57~1.54 (m, 2H), 1.49~1.46 (m, 4H), 1.40~1.28 (m, 20H), 0.88 (t, J=6.4, 3H); **^{13}C NMR** 80.48, 80.34, 63.19, 32.95, 32.04, 29.88, 29.51, 29.42, 29.31, 29.06, 28.97, 25.89, 22.86, 18.93, 14.30.

References

(1) Shimizu, T.; Masuda, M.; Minamikawa, H., *Supramolecular Nanotube Architectures Based on Amphiphilic Molecules.* Chemical Reviews **2005**, *105(4)*, 1401-1443.

(2) Lu, Y. F.; Yang, Y.; Sellinger, A.; Lu, M. C.; Huang, J. M.; Fan, H.Y.; Haddad, R.; Lopez, G.; Burns, A. R.; Sasaki, D. Y.; Shelnutt, J.; Brinker, J. *Self-Assembly of Mesoscopically Ordered Chromatic polydiacetylene/Silica Nanocomposites*; Nature **2001**, *410*, 913-917.

(3) Song, J.; Cheng, Q.; Kopta, S.; Stevens, R. C., Modulating *Artificial Membrane Morphology: pH-Induced Chromatic Transition and Nanostructural Transformation of a Bolaamphiphilic Conjugated Polymer from Blue Helical Ribbons to Red Nanofibers*. Journal of the American Chemical Society **2001**, *123(14)*, 3205-3213.

(4) Tomioka, Y.; Tanaka, N.; Imazeki, S. *Surface-pressure-induced reversible color change of a polydiacetylene monolayer at a gas-water interface*. Journal of Chemical Physics **1989**, *91(9)*, 5694-5700.

(5) Bommarito, G. M.; Lakshmi, B. B. *Colorimetric sensors constructed of diacetylene materials* US 20060134796A1

(6) Dvornic, P. R.; Kaganove, S. N.; Sarkar, A. US20050106572A1.

(7) Eckhardt, H.; Boudreaux, D. S.; Chance, R. R. *Effects of substituent-induced strain on the electronic structure of polydiacetylenes.* Journal of Chemical Physics **1986**, *85(7)*, 4116-4119.

(8) Fuhrhop, J. H.; Helfrich, W., *Fluid and solid fibers made of lipid molecular bilayers.* ; Chemical Reviews **1993**, *93*, 1565-82.

(9) Morigaki, K.; Baumgart, T.; Jonas, U.; Offenhaeusser, A.; Knoll, W. *Photopolymerization of Diacetylene Lipid Bilayers and Its Application to the Construction of Micropatterned Biomimetic Membranes.* Langmuir **2002**, *18(10),* 4082-4089.

(10) Morigaki, K.; Baumgart, T.; Offenhaeusser, A.; Knoll, W. *Patterning Solid-Supported Lipid Bilayer Membranes by Lithographic Polymerization of a Diacetylene Lipid*; Angew. Chem. Int. Ed. **2001**, *40*, 172-174.

(11) Yager, P.; Schoen, P. E., *Formation of tubules by a polymerizable surfactant. Molecular Crystals and Liquid Crystals* **1984**, *106(3-4)*, 371-81.

(12) Nie, X. P.; Wang, G. J. *Synthesis of Self-Assembling Properties of Diacetylene-Containing Glycolipids*; Journal of Organic Chemistry **2005**, *71*, 4734-4741.

(13) Lee, S. B.; Koepsel, R.; Stolz, D. B.; Warriner, H. E.; Russell, A. J. Self-Assembly of Biocidal Nanotubes from a Single-Chain Diacetylene Amine Salt; Journal of the American Chemistry Society **2004**, *126*, 13400-13405.

(14) Chodkiewicz, W.; Cadiot, P., *New synthesis of symmetrical and asymmetrical conjugated polyacetylenes.* Compt. rend. **1955**, *241,* 1055-1057.

(15) Tieke, B.; Wegner, G.; Naegele, D.; Ringsdorf, H., *Polymerization of tricosa-10,12-diyn-1-oic acid in multilayers.* Angewandte Chemie **1976**, *88(23)*, 805-806.

(16) Xu, Z. C.; Byun, H. S.; Bittman, R. *Synthesis of photopolymerizable long-chain*

conjugated diacetylenic acids and alcohols from butadiyne synthons. Journal of Organic Chemistry **1991**, *56 (25)*, 7183-7186.

(17)Salunkhe, D. K.; Chavan, J. K.; Adsule, R. N.; Kadam, S. S. *World Oilseeds Chemistry, Technology, and Utilization*; Van Nostrand Reinhold: New York, **1992**.

(18)Menzek, A.; Saracoglu, N.; Krawiec, M.; Watson, W. H.; Balci, M. *Synthesis of a New System Containing a Pyramidalized Double Bond: cis-Dicarbomethoxy dihydroheptalene and Its Reaction with Benzyne.* Journal of Organic Chemistry **1995**, *60*, 829-32.

(19)McMillen, D. W.; Grutzner, J. B. *Radical Bromination of Cyclohexene in CCl4 by Bromine: Addition versus Substitution*. Journal of Organic Chemistry **1994**, *59*, 4516-28.

(20)Gomez-Sanchez, E.; Soriano, E.; Marco-Contelles, J. *Synthesis of 7-Azabicyclo[2.2.1]heptane and 2-Oxa-4-azabicyclo[3.3.1]non-3-ene Derivatives by Base-Promoted Heterocyclization of Alkyl N-(cis(trans)-3,trans(cis)-4-Dibromocyclohex-1-yl)carbamates and N-(cis(trans)-3,trans(cis)-4-Dibromocyclohex-1-yl)-2,2,2-trifluoro acetamides.* Journal of Organic Chemistry **2007**, *72*, 8656-8670.

(21)Yan, Z.; McCracken, T.; Xia, S.; Maslak, V.; Gallucci, J.; Hadad, C. M.; Badjic, J. D. *Supramolecular Catalysis at Work: Diastereoselective Synthesis of a Molecular Bowl with Dynamic Inner Space*. Journal of Organic Chemistry **2008**, *73*, 355-363.

(22)Shukla, R.; Lindeman, S. V.; Rathore, R. *Binding of an acetonitrile molecule inside the ethereal cavity of a hexaarylbenzene-based receptor via a synergy of C-H...O/C-H...pi interactions.* Chemical Communications (Cambridge, United Kingdom) **2007**, 3717-3719.

Appendix

^{1}H-NMR AND ^{13}C-NMR SPECTRA

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Points Count	8192	*Pulse Sequence*	s2pul	*Receiver Gain*	28.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	1001.6563	*Sweep Width (Hz)*	3000.30	*Temperature (degree C)*	29.000		

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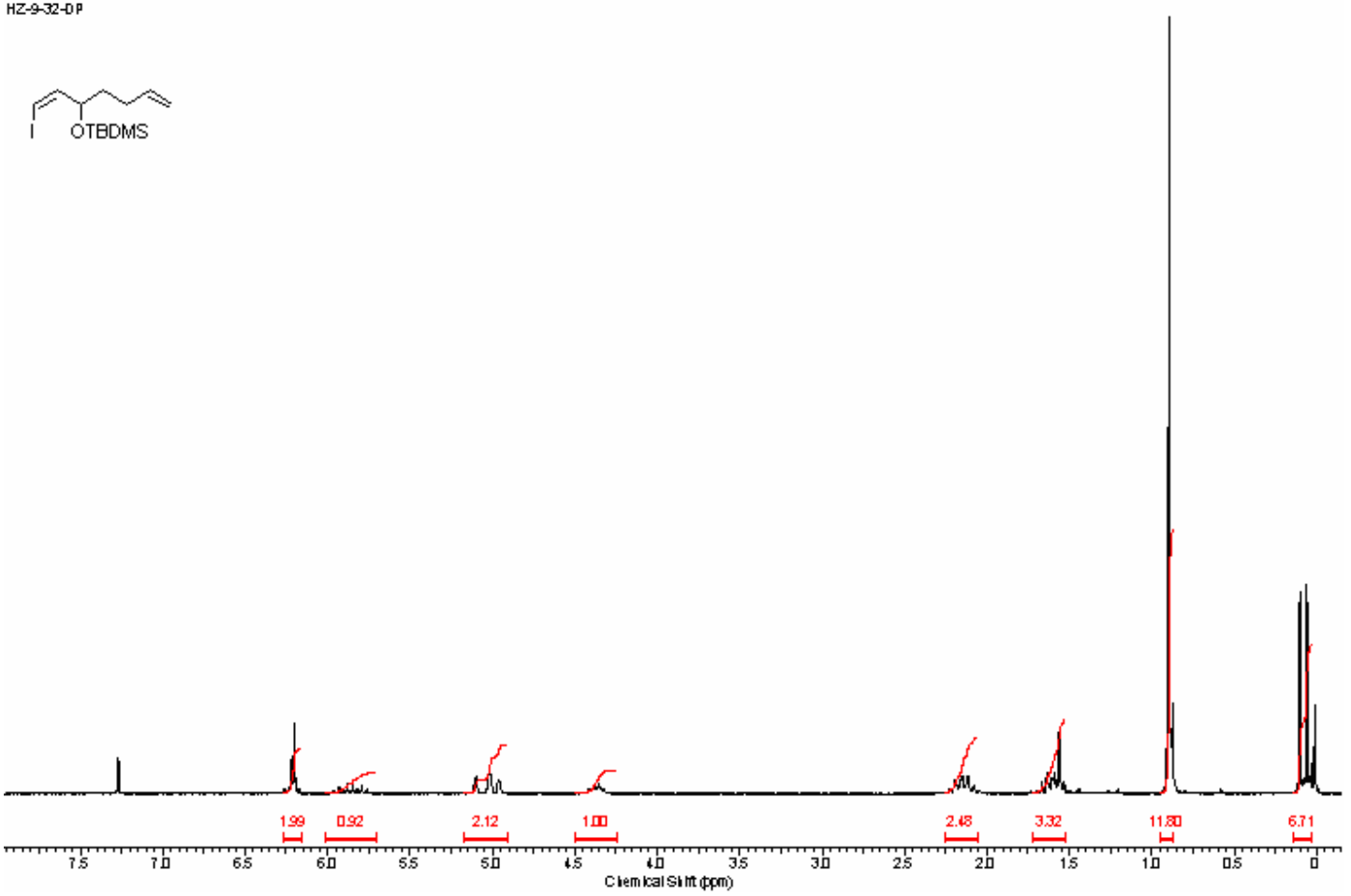

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Pulse Sequence	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d		
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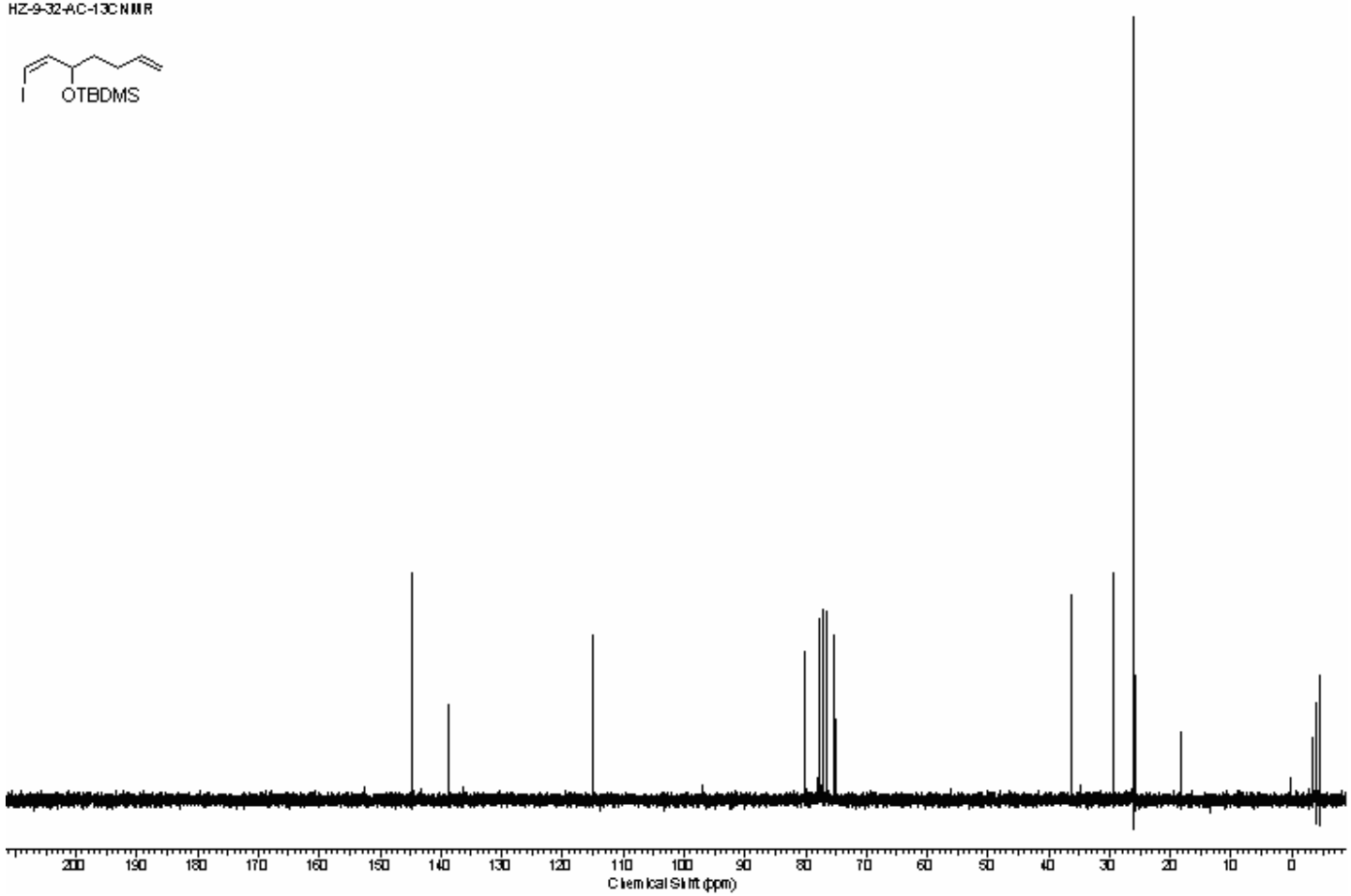
HZ-9-32-AC-13C NMR



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Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	4.00	Solvent	CHLOROFORM-d
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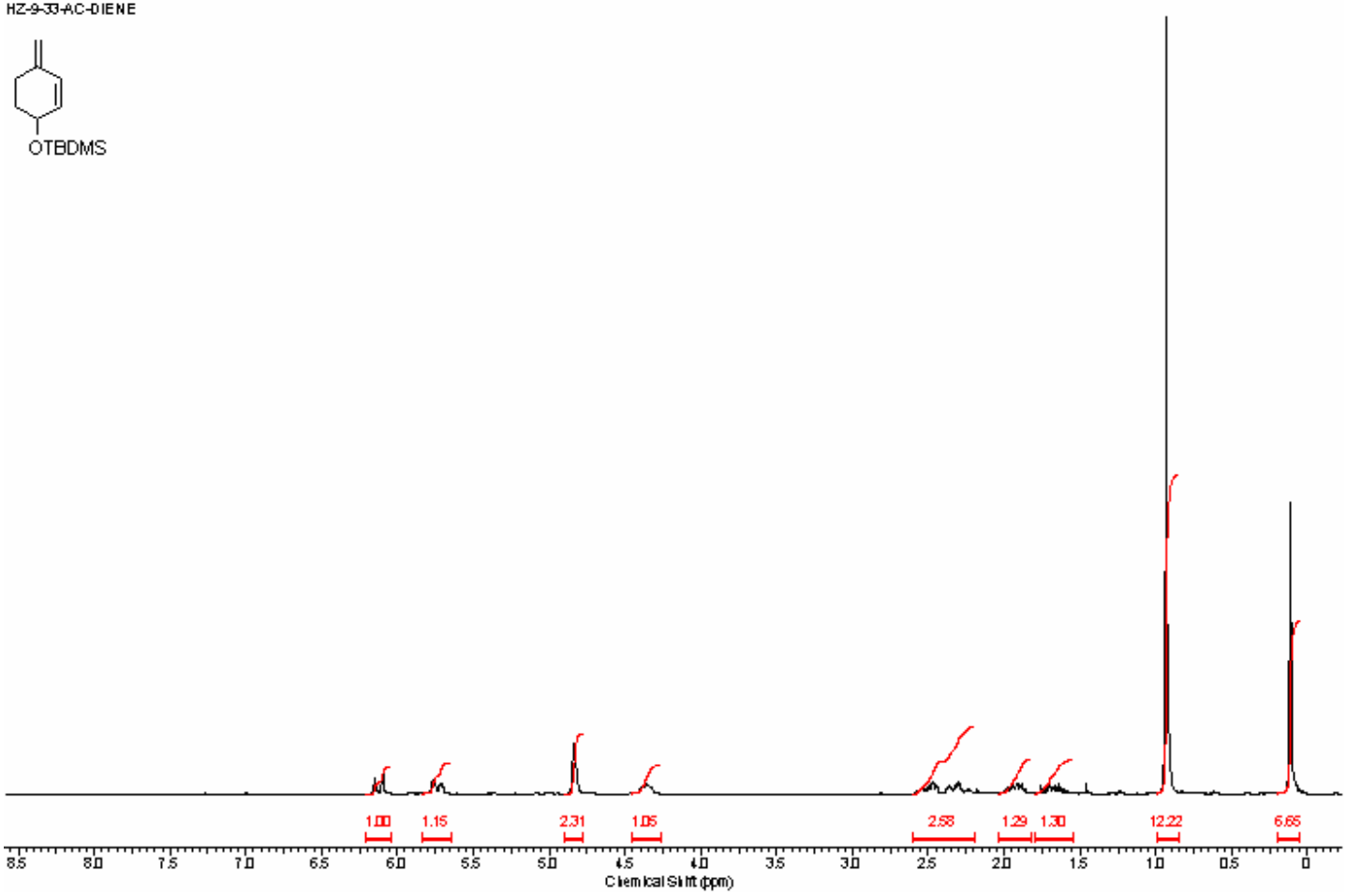
HZ-9-33-AC-DIENE



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Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d		
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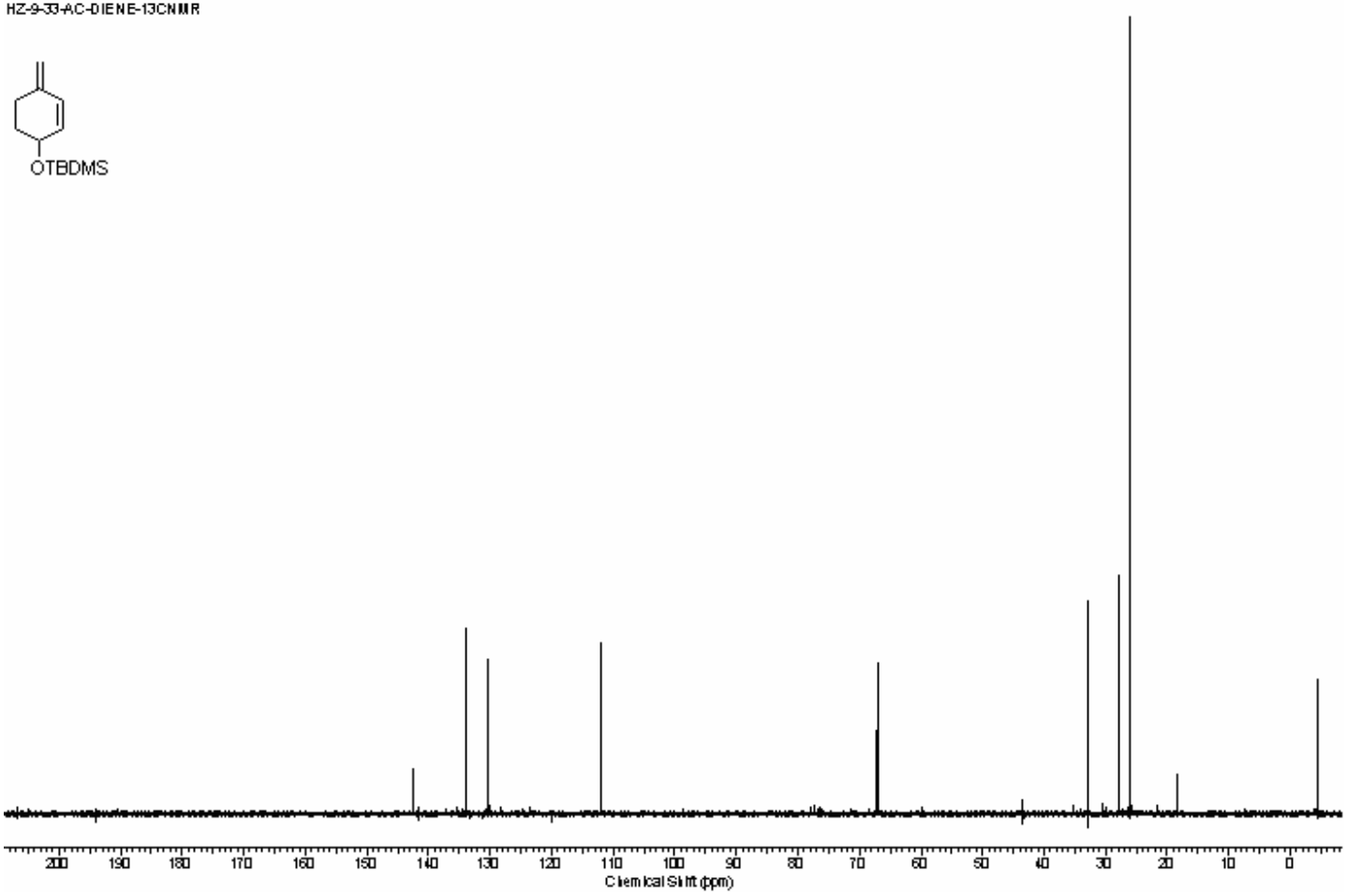
HZ-9-33-AC-DIENE-13CNMR



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Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	10.00	Solvent	CHLOROFORM-d
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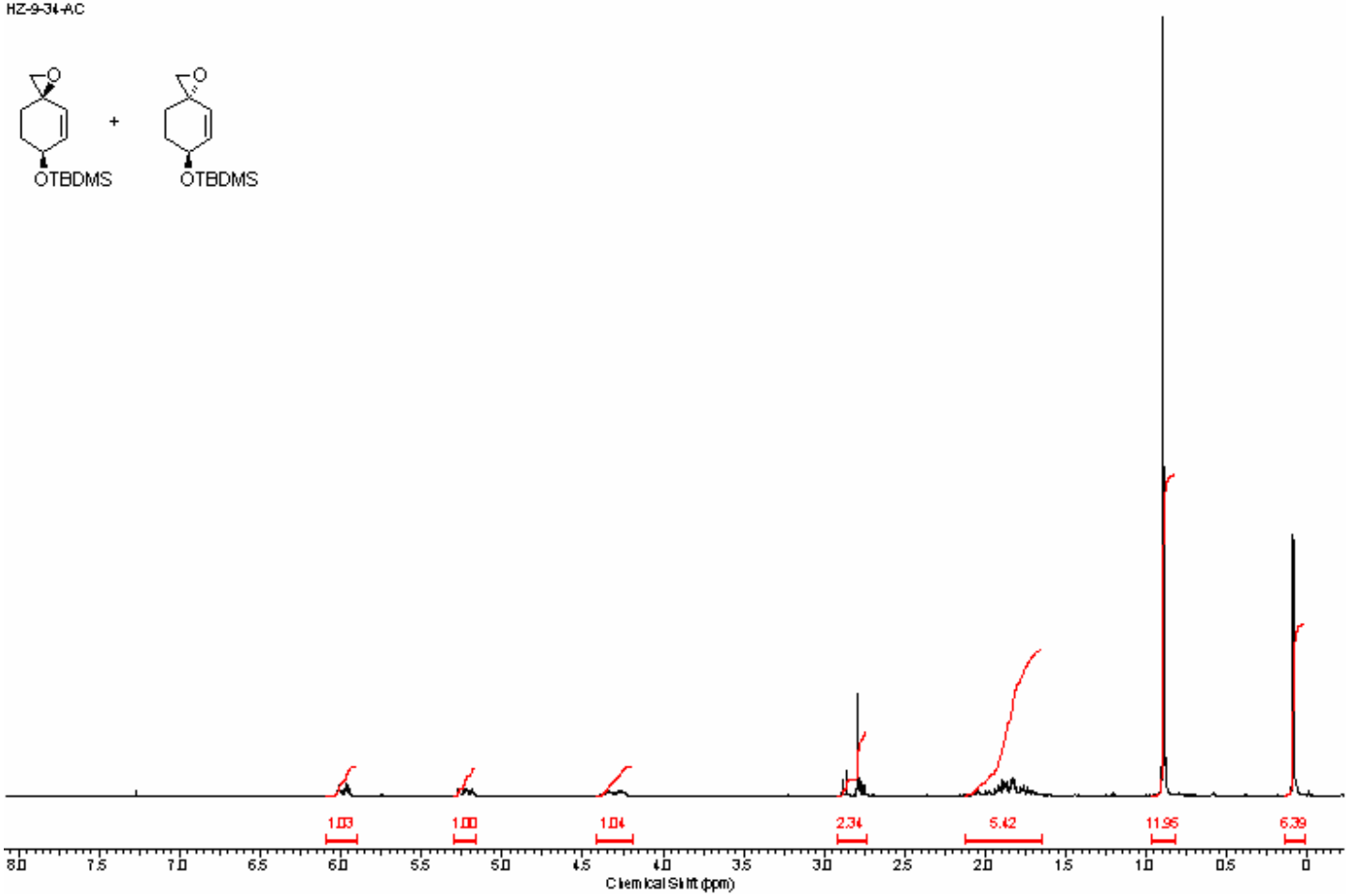

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Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d		
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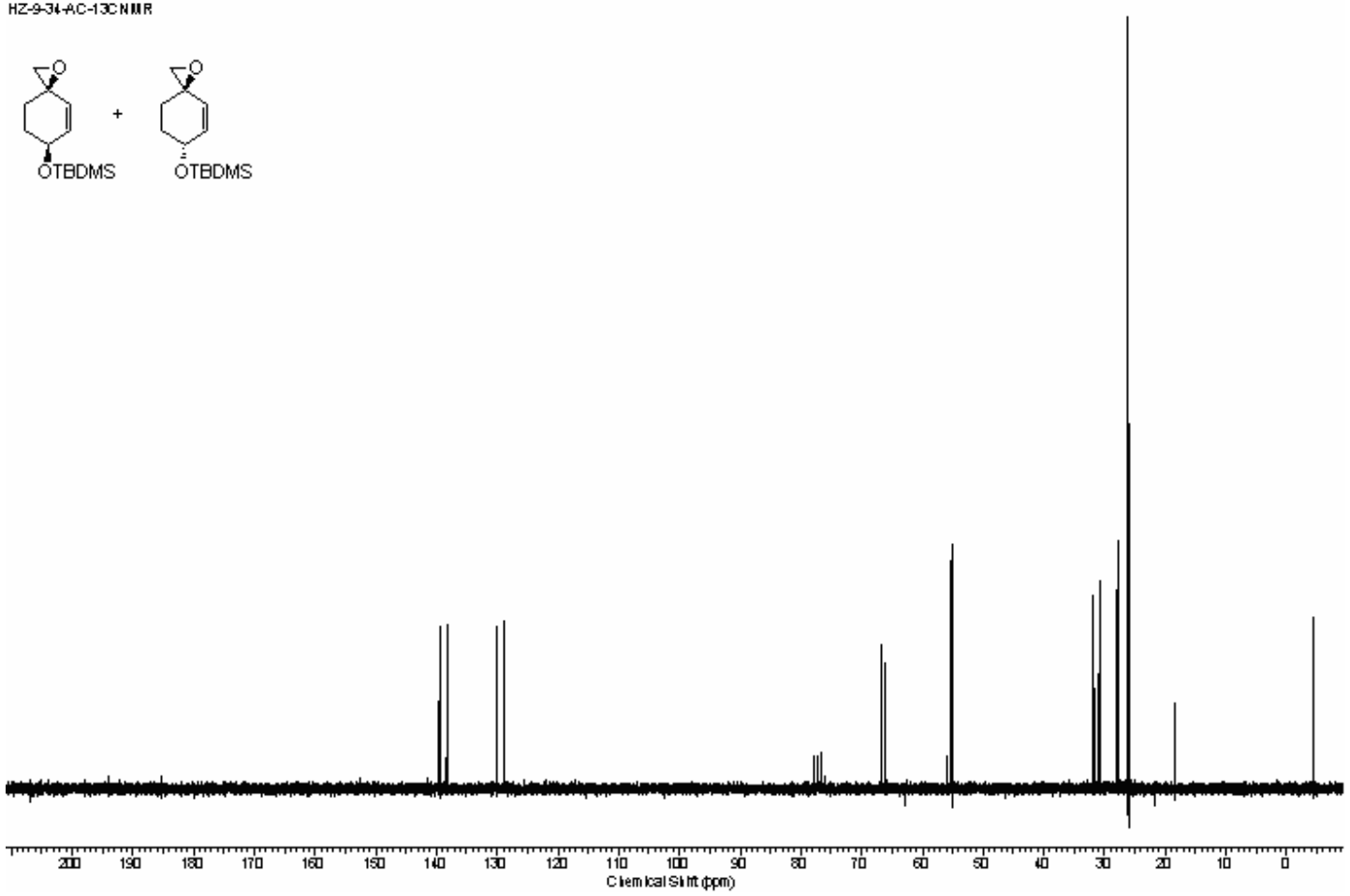
HZ-9-34-AC-13C NMR



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Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	22.00	Solvent	CHLOROFORM-d
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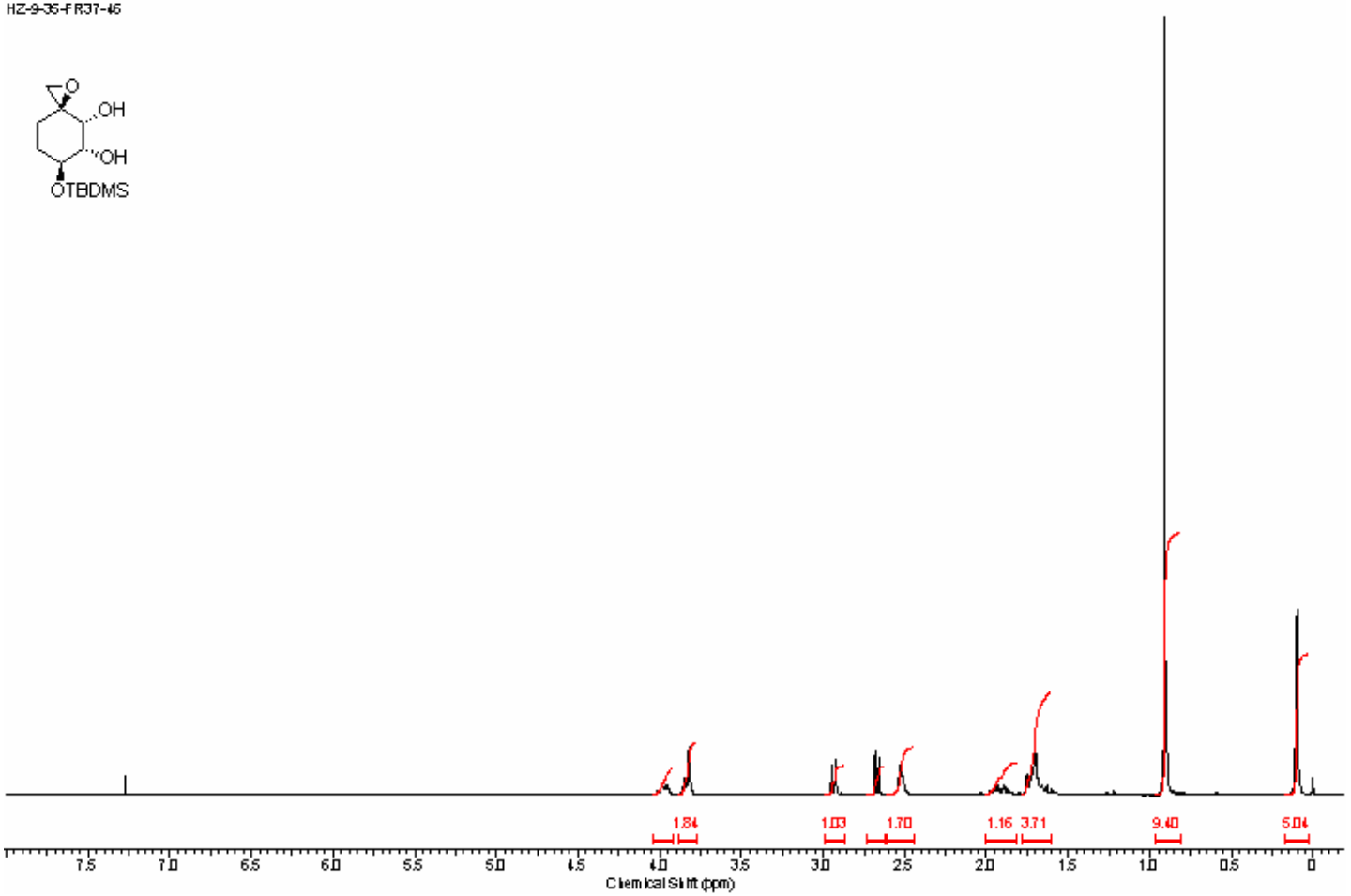
HZ-9-35-FR37-46



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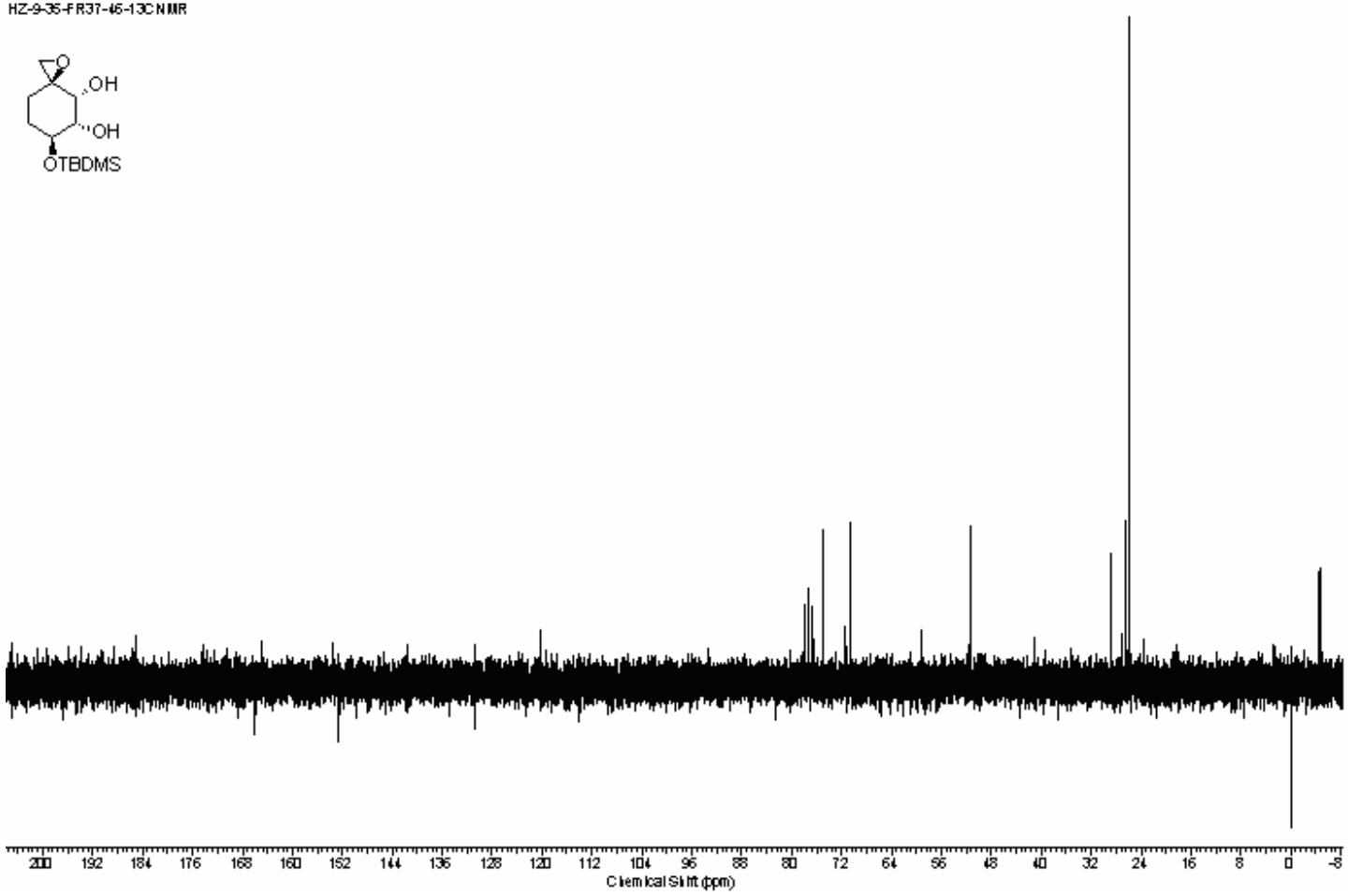
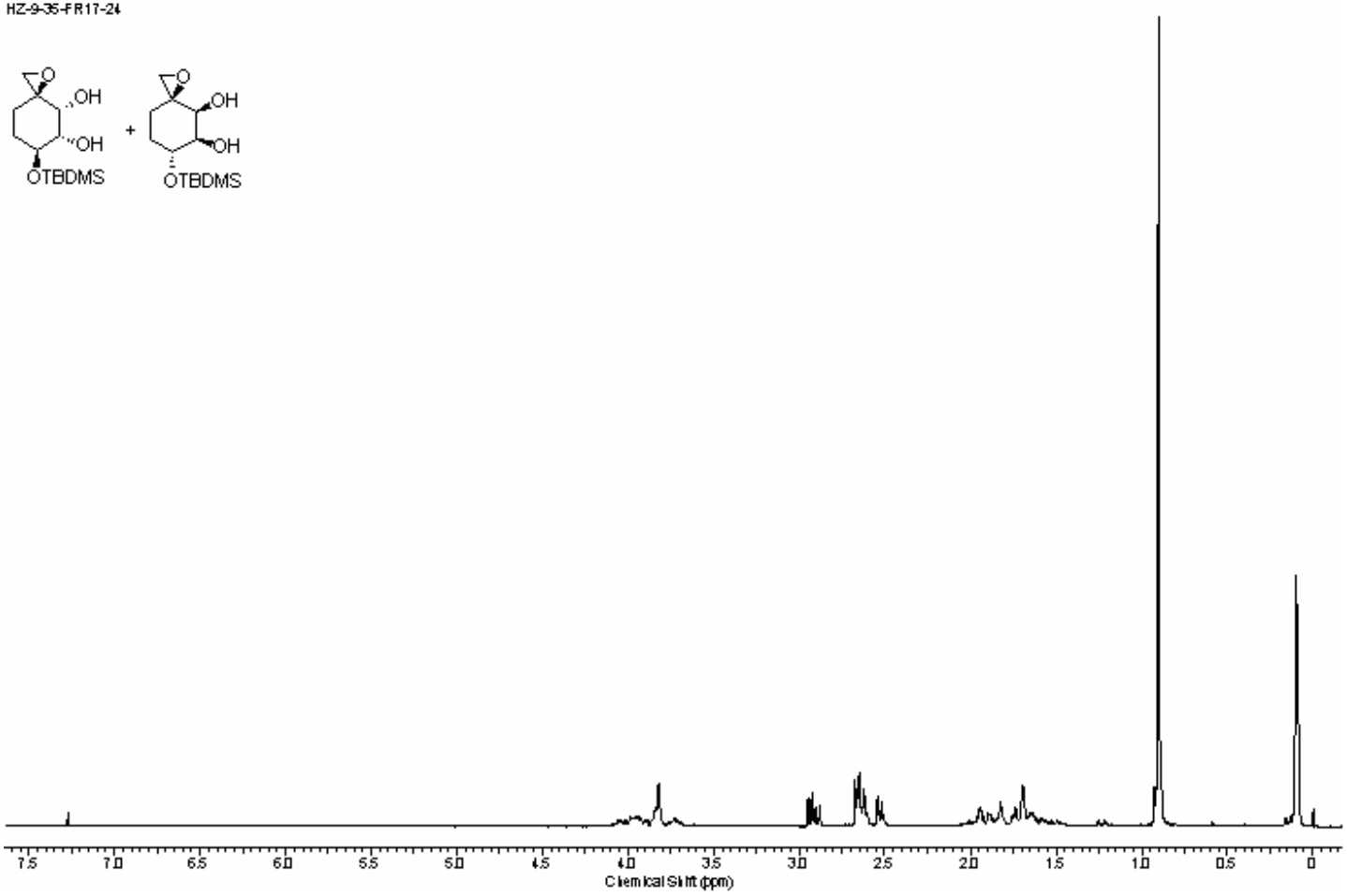
Spectrum Offset (Hz)	4880.8467	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-9-35-FR37-46-13C NMR



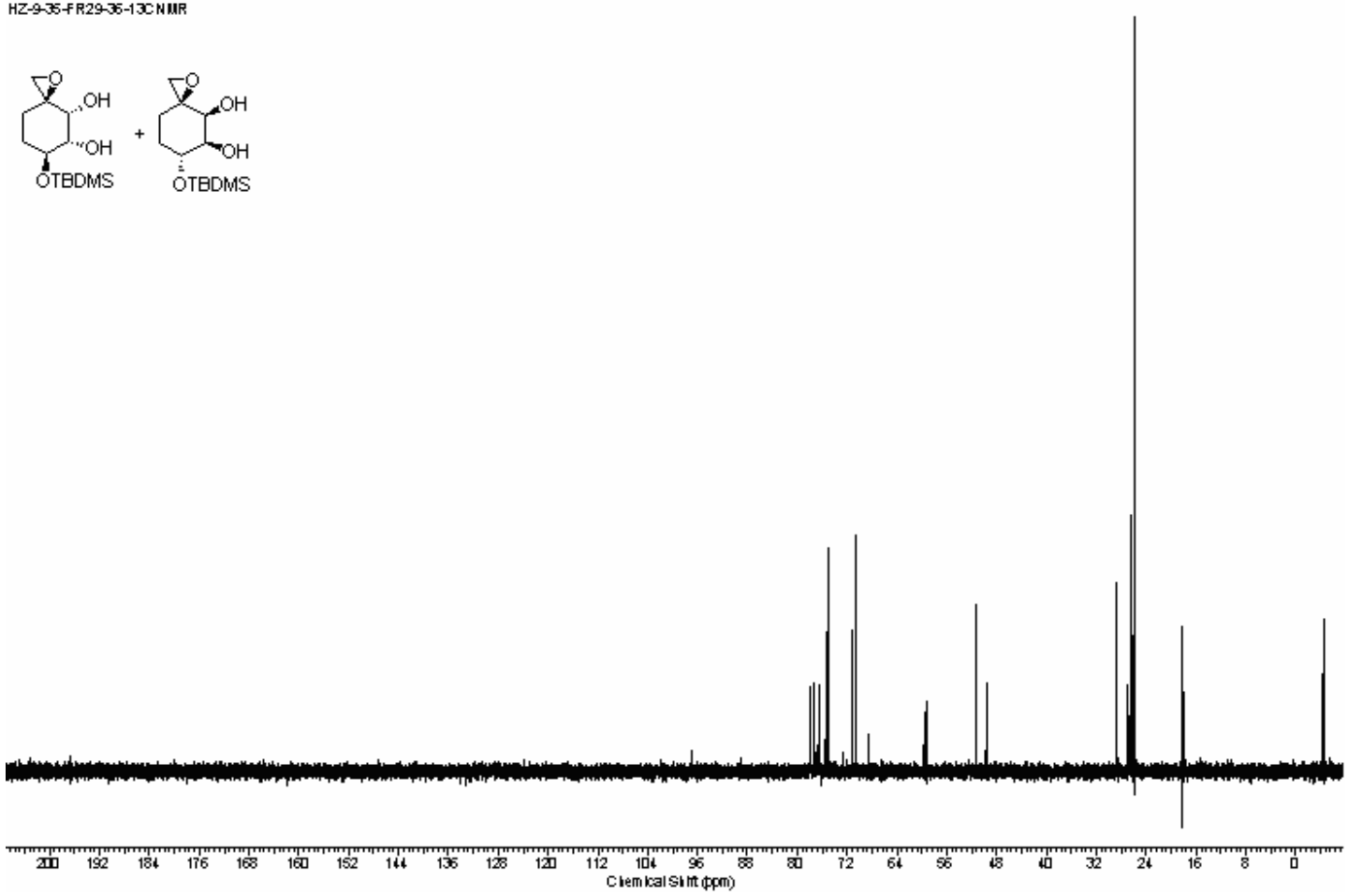
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	May 24 2007
Date Stamp	May 24 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-35-FR17-24				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	16.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.6490	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-9-35-FR17-24



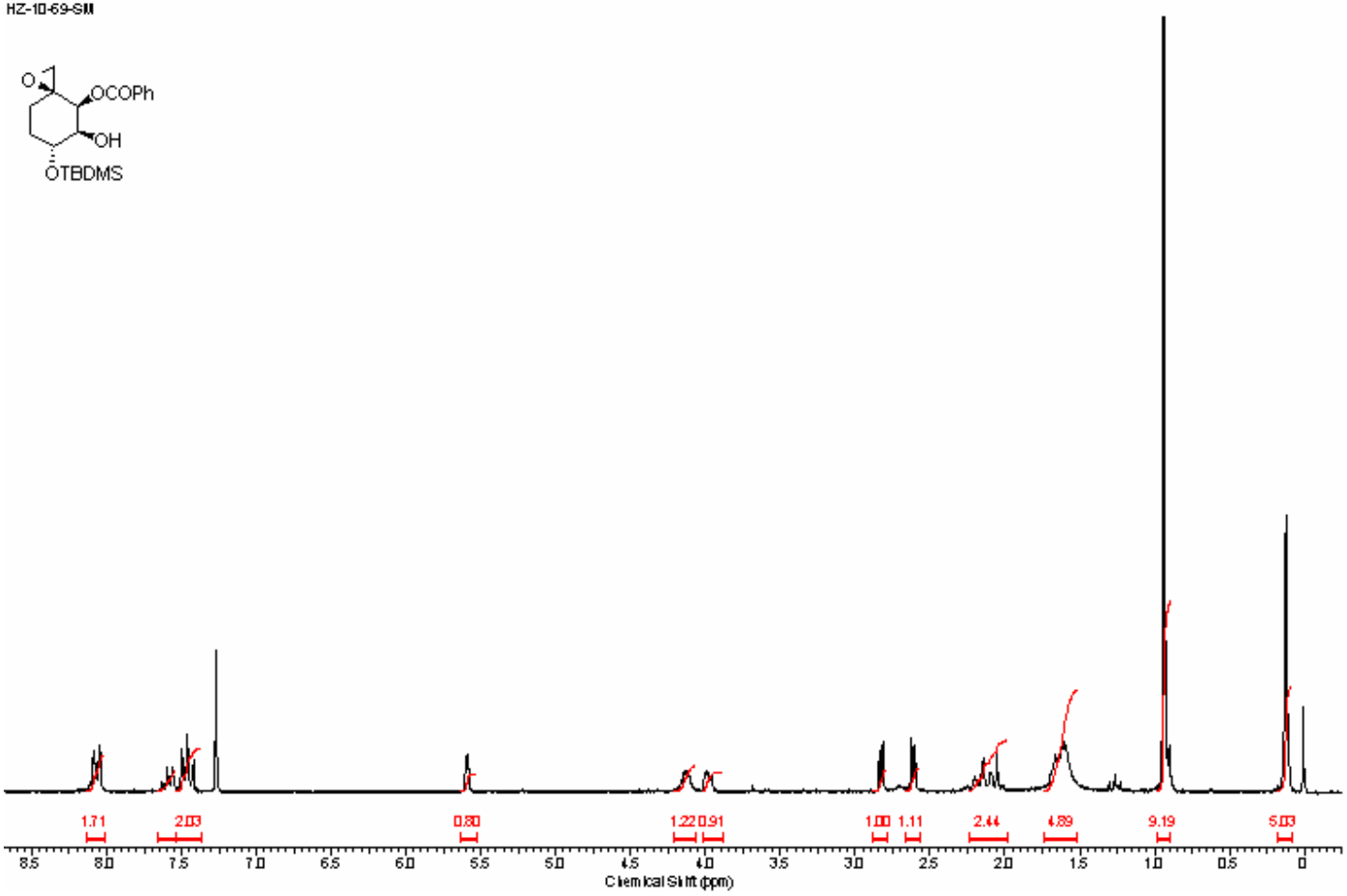
Acquisition Time (sec)	1.4976	Comment	13C OBSERVE	Date	May 25 2007	Date Stamp	May 25 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-35-FR29-36-13C NMR					Frequency (MHz)	50.29
Nucleus	13C	Number of Transients	640000	Original Points Count	18720	Points Count	32768
Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d		
Spectrum Offset (Hz)	4880.8467	Sweep Width (Hz)	12500.00	Temperature (degree C)	29.000		

HZ-9-35-FR29-36-13C NMR



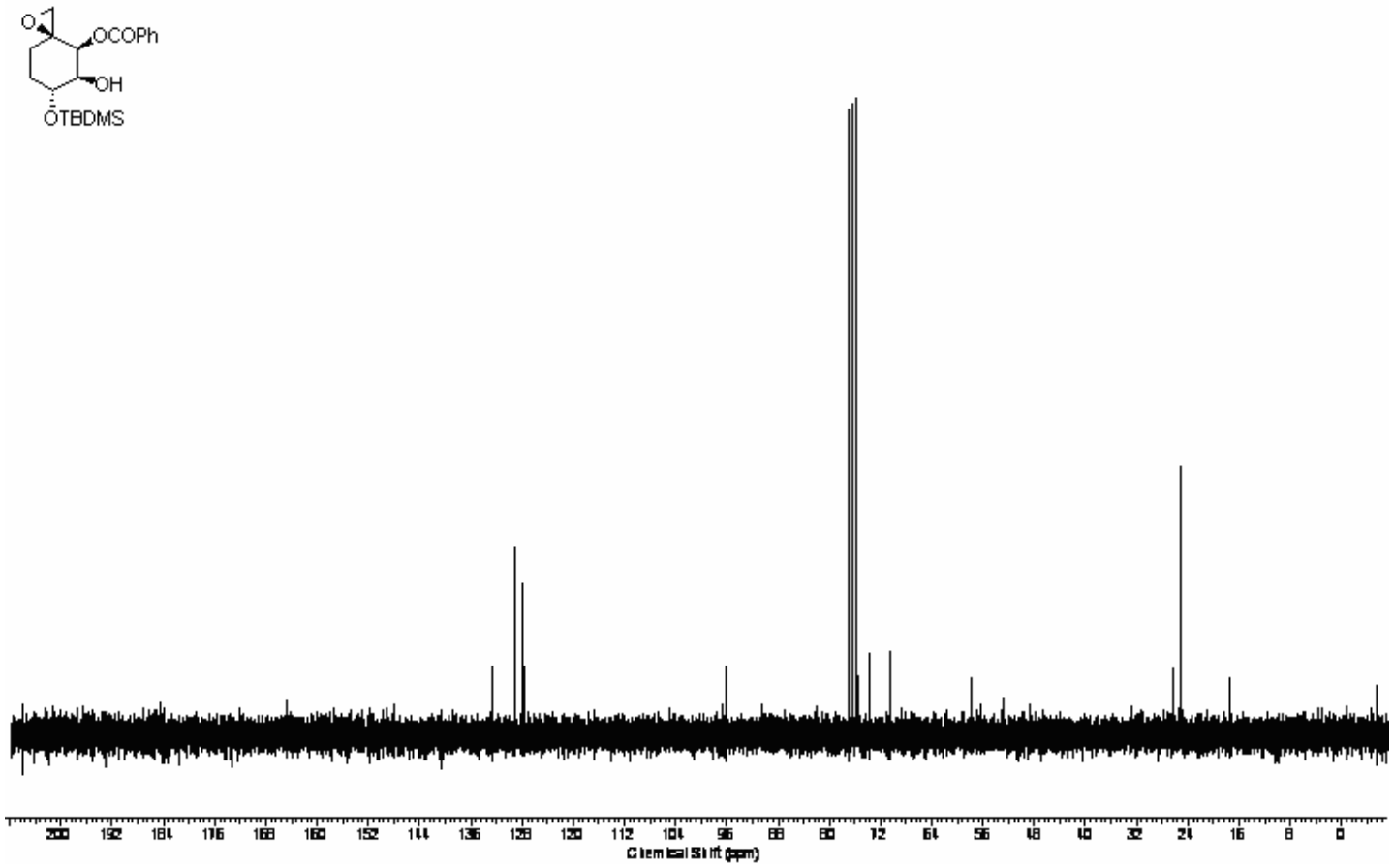
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Jul 11 2006		
Date Stamp	Jul 11 2006			File Name	E:\HZ-10-69-SM			Frequency (MHz)	199.98
Nucleus	1H	Number of Transients	32	Original Points Count	5984	Points Count	8192	Pulse Sequence	s2pul
Receiver Gain	36.00	Solvent	CHLOROFORM-d			Spectrum Offset (Hz)	1002.0226	Sweep Width (Hz)	3000.30
Temperature (degree C)	29.000								

HZ-10-69-SM



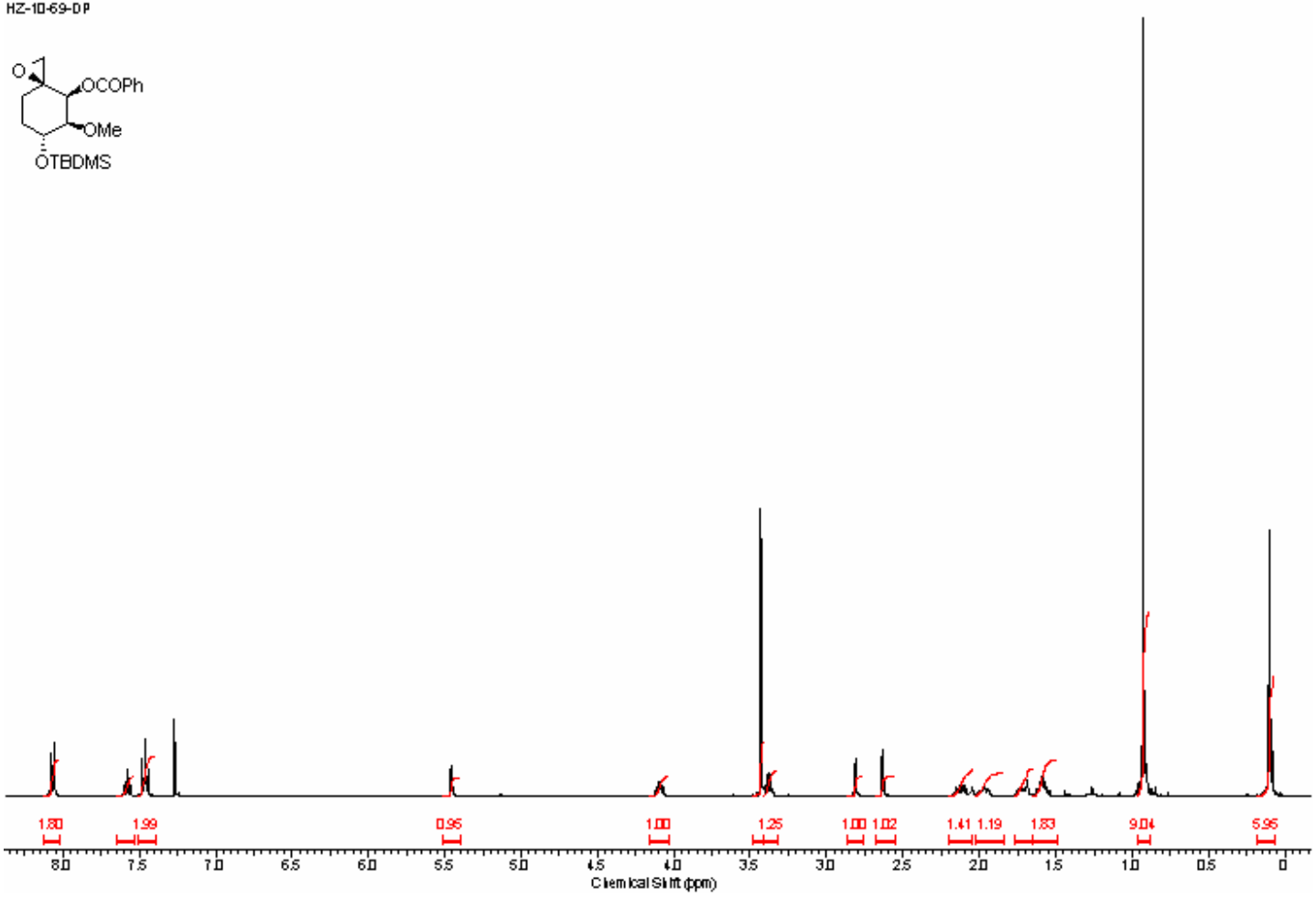
Acquisition Time (sec)	1.9316	Comment	13C OBSERVE			Date	Jul 22 2006		
Date Stamp	Jul 22 2006			File Name	E:\HZ-10-59-13CNMR			Frequency (MHz)	50.29
Nucleus	13C	Number of Transients	30000	Original Points Count	16120	Points Count	32768	Pulse Sequence	s2pul
Receiver Gain	4000	Solvent	CHLOROFORM-d			Spectrum Offset (Hz)	6880.0840	Sweep Width (Hz)	12500.00
Temperature (degree C)	29.000								

HZ-10-59-13CNMR



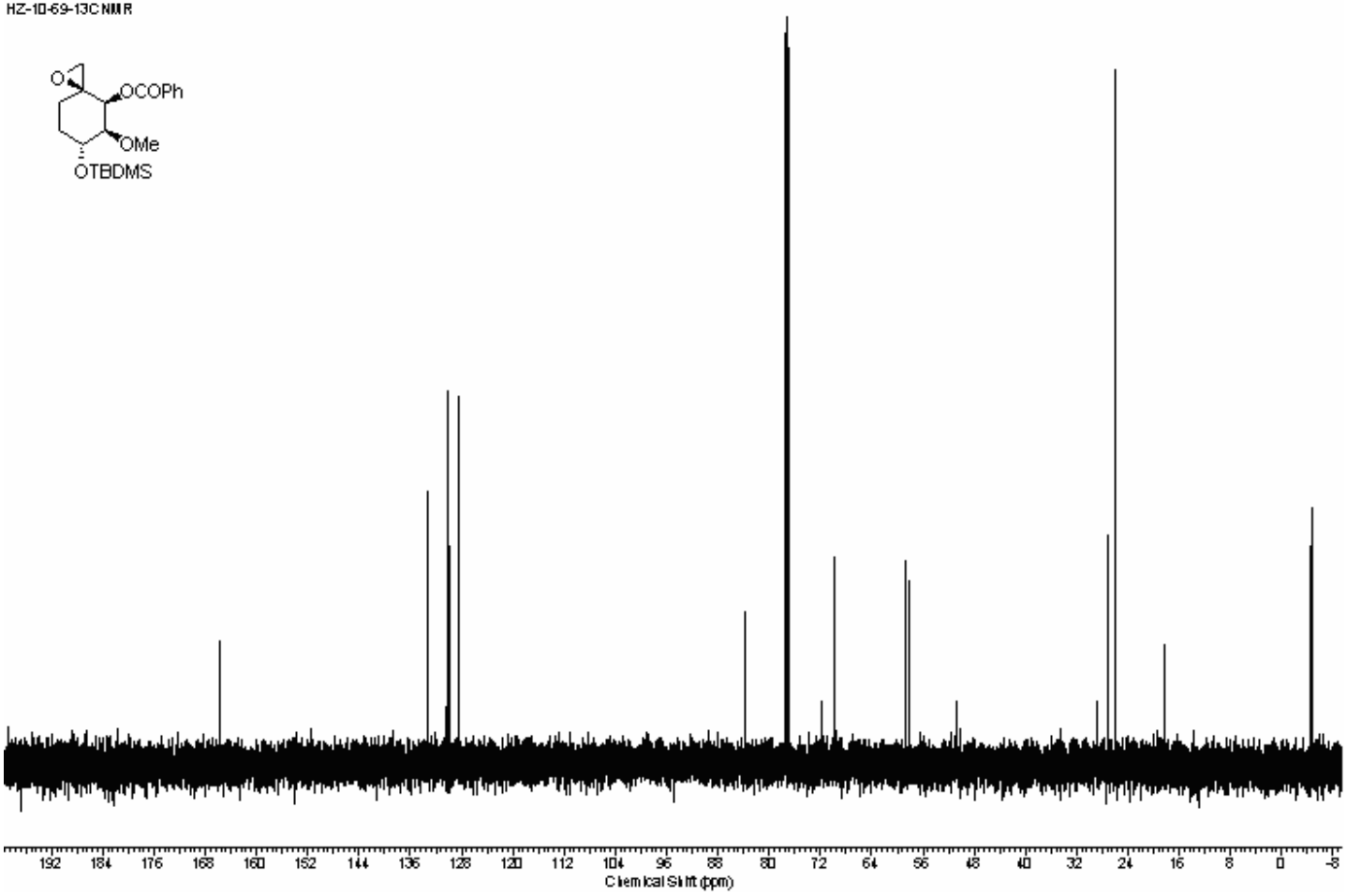
Acquisition Time (sec)	2.0486	Comment	Std proton	Date	Oct 31 2007			Date Stamp	Oct 31 2007
File Name	E:\ORIGINAL NMR-400\HZ-10-69-DP			Frequency (MHz)	399.77	Nucleus	1H	Number of Transients	8
Original Points Count	13104	Points Count	16384	Pulse Sequence	s2pul	Receiver Gain	38.00		
Solvent	CHLOROFORM-d			Spectrum Offset (Hz)	2406.1902	Sweep Width (Hz)	6396.42	Temperature (degree C)	25.000

HZ-10-69-DP



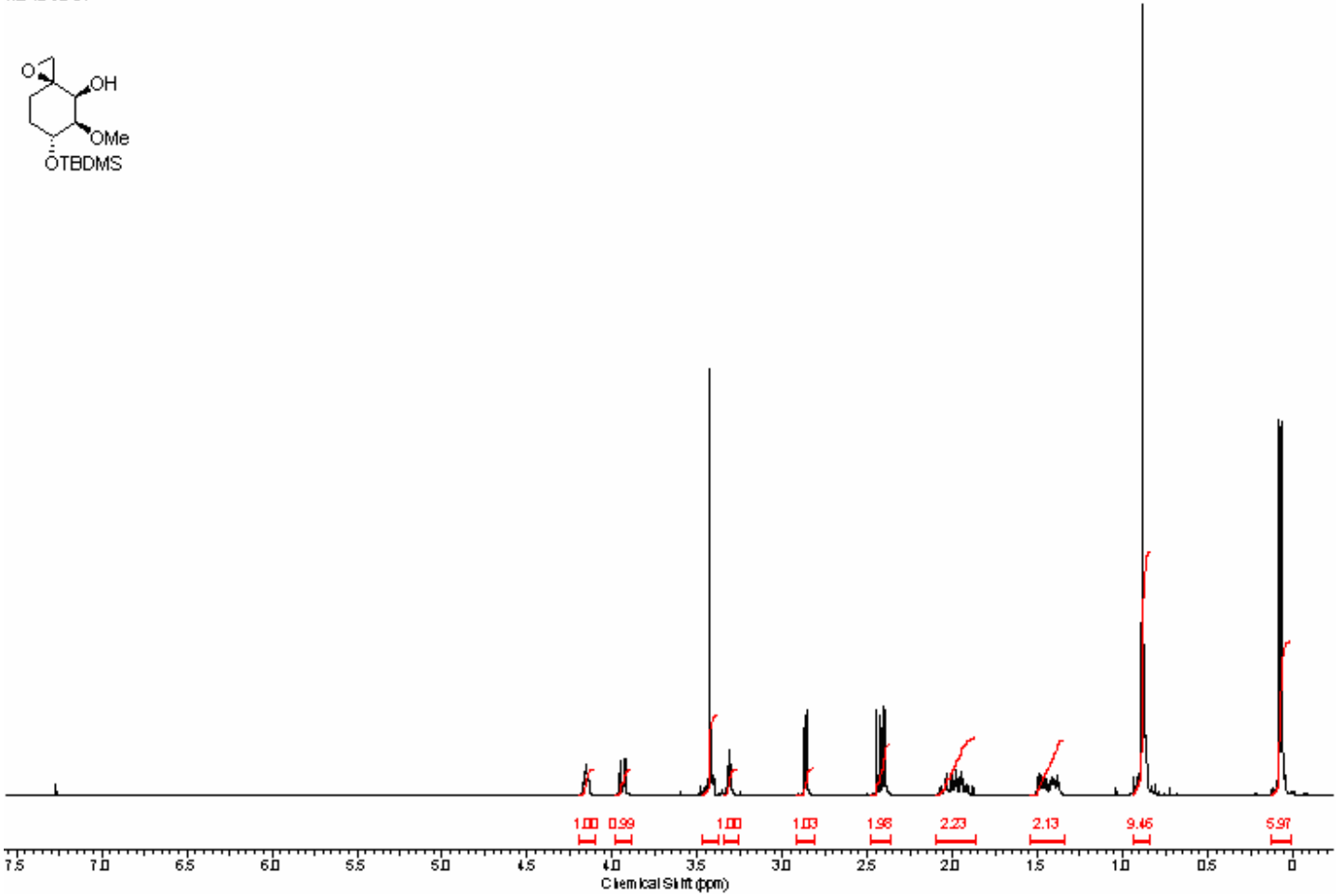
Acquisition Time (sec)	1.3005	*Comment*	Std proton	*Date*	Oct 31 2007	*Date Stamp*	Oct 31 2007
File Name	E:\ORIGINAL NMR-400\HZ-10-69-13C NMR			*Frequency (MHz)*	100.53	*Nucleus*	13C
Number of Transients	66256	*Original Points Count*	31315	*Points Count*	32768	*Pulse Sequence*	s2pul
Receiver Gain	30.00	*Solvent*	CHLOROFORM-d			*Spectrum Offset (Hz)*	10554.3848
Sweep Width (Hz)	24125.45	*Temperature (degree C)*	25.000				

HZ-10-69-13C NMR



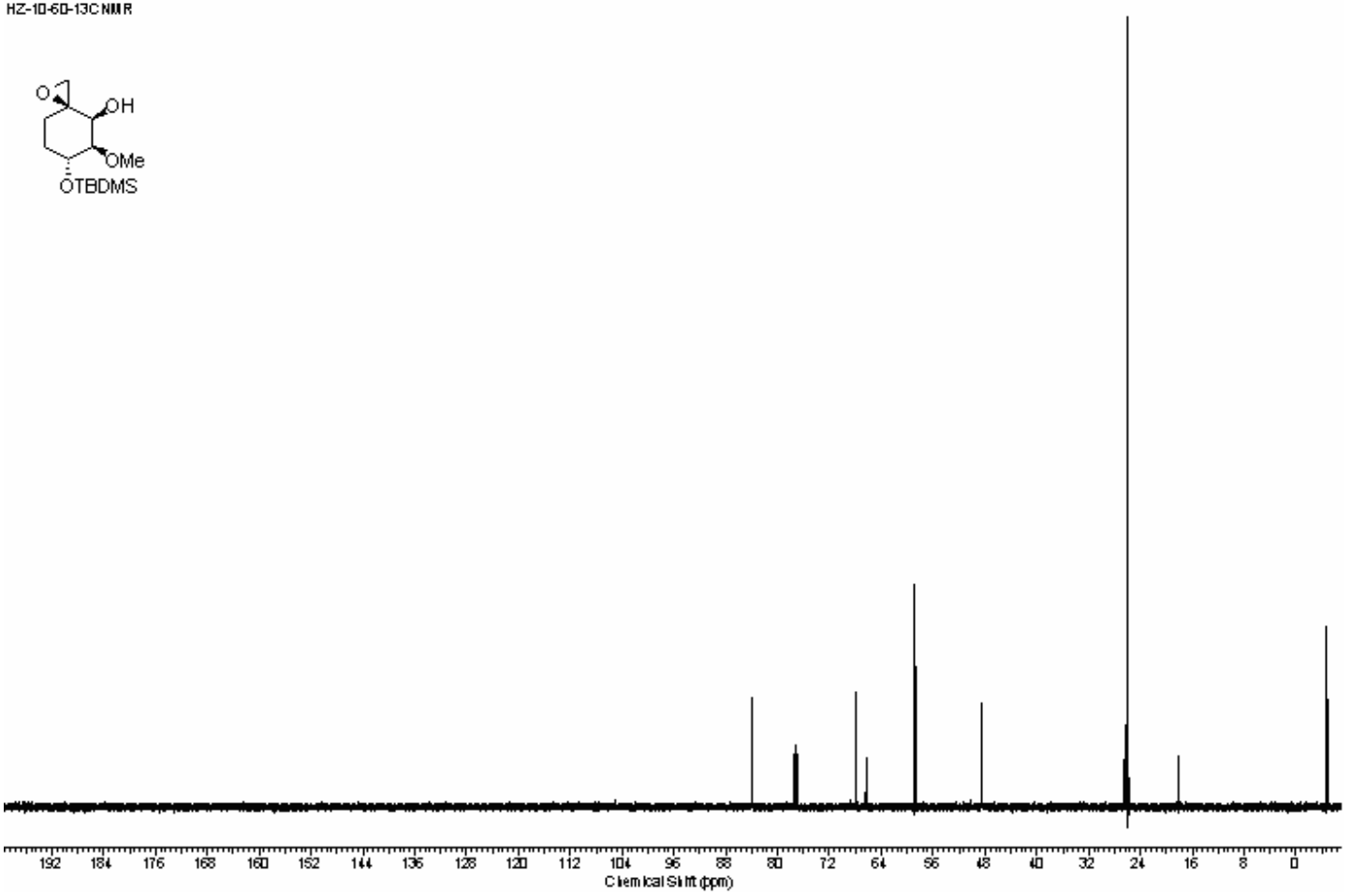
Acquisition Time (sec)	2.0486	Comment	Std proton	Date	Oct 31 2007			Date Stamp	Oct 31 2007
File Name	E:\O RIGINAL NMR-400\HZ-10-60-DP			Frequency (MHz)	399.77	Nucleus	1H	Number of Transients	8
Original Points Count	13104	Points Count	16384	Pulse Sequence	s2pul	Receiver Gain	20.00		
Solvent	CHLOROFORM-d			Spectrum Offset (Hz)	2405.9951	Sweep Width (Hz)	6396.42	Temperature (degree C)	25.000

HZ-10-60-DP



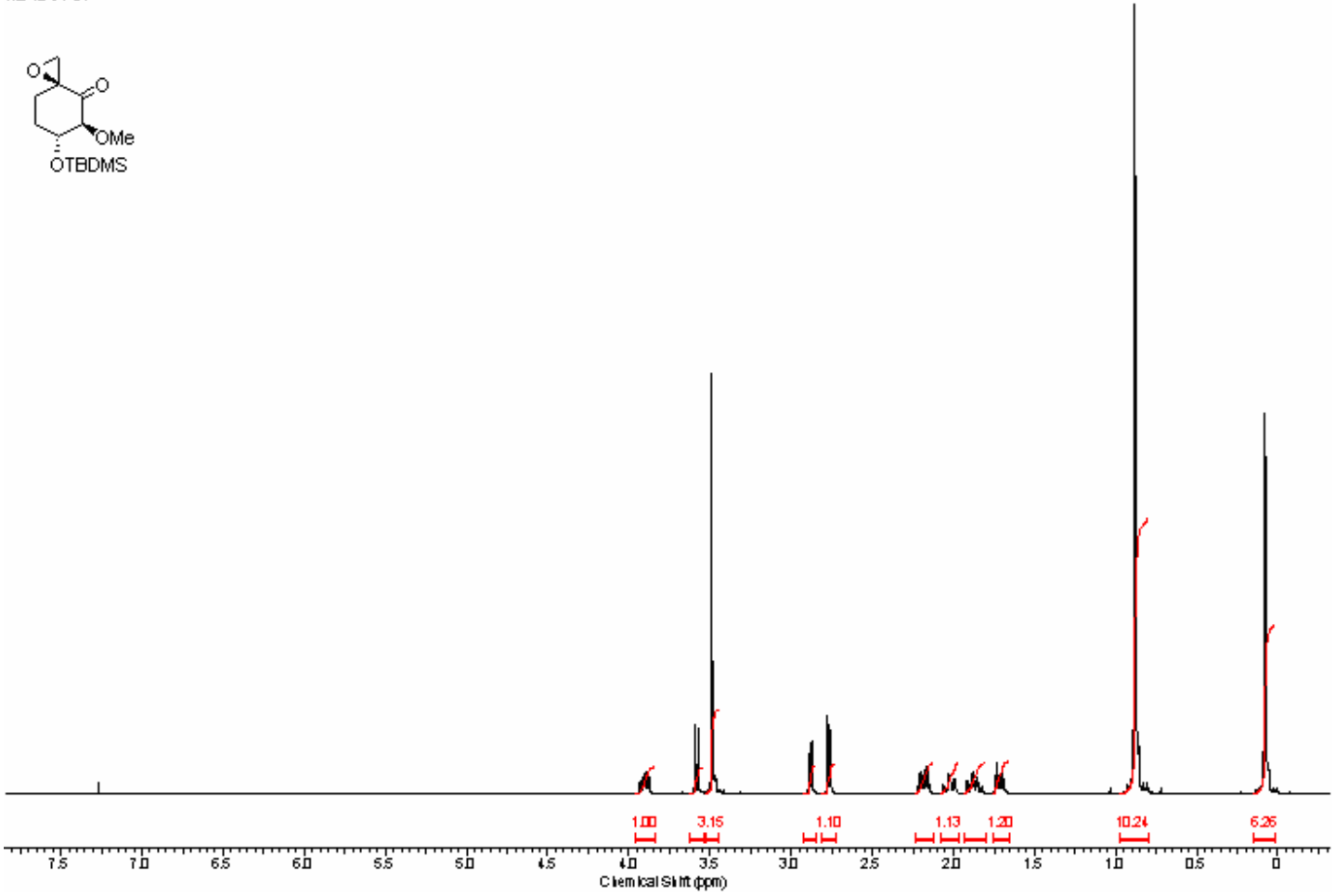
Acquisition Time (sec)	1.3005	*Comment*	Std proton	*Date*	Oct 31 2007	*Date Stamp*	Oct 31 2007
File Name	E:\O RIGINAL NMR-400\HZ-10-60-13C NMR			*Frequency (MHz)*	100.53	*Nucleus*	13C
Number of Transients	66256	*Original Points Count*	31315	*Points Count*	32768	*Pulse Sequence*	s2pul
Receiver Gain	30.00	*Solvent*	CHLOROFORM-d			*Spectrum Offset (Hz)*	10549.9668
Sweep Width (Hz)	24125.45	*Temperature (degree C)*	25.000				

HZ-10-60-13C NMR



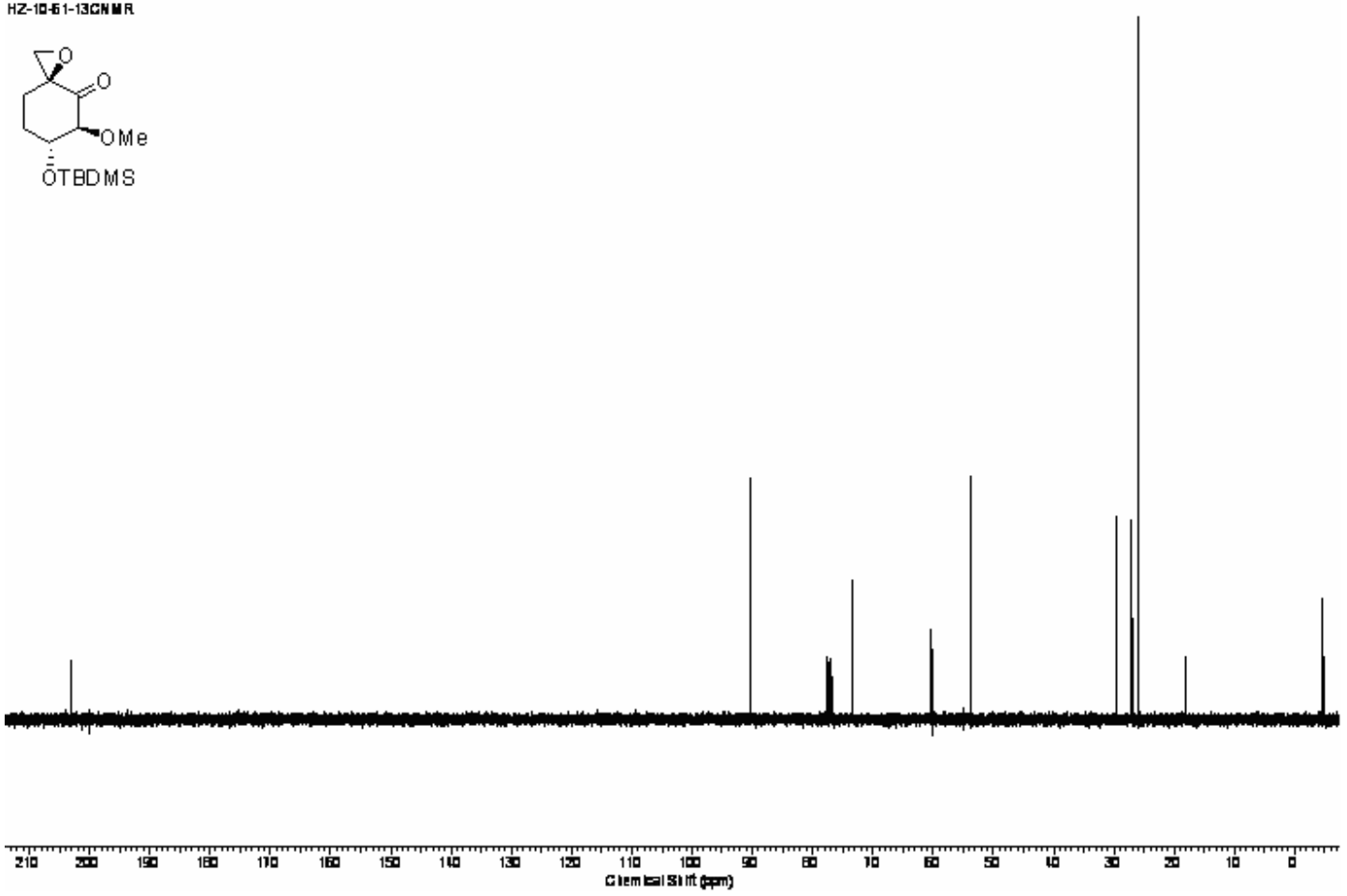
Acquisition Time (sec)	2.0486	Comment	Std proton	Date	Oct 31 2007			Date Stamp	Oct 31 2007
File Name	E:\ORIGINAL NMR-400\HZ-10-61-DP			Frequency (MHz)	399.77	Nucleus	1H	Number of Transients	16
Original Points Count	13104	Points Count	16384	Pulse Sequence	s2pul	Receiver Gain	20.00		
Solvent	CHLOROFORM-d			Spectrum Offset (Hz)	2405.9951	Sweep Width (Hz)	6396.42	Temperature (degree C)	25.000

HZ-10-61-DP



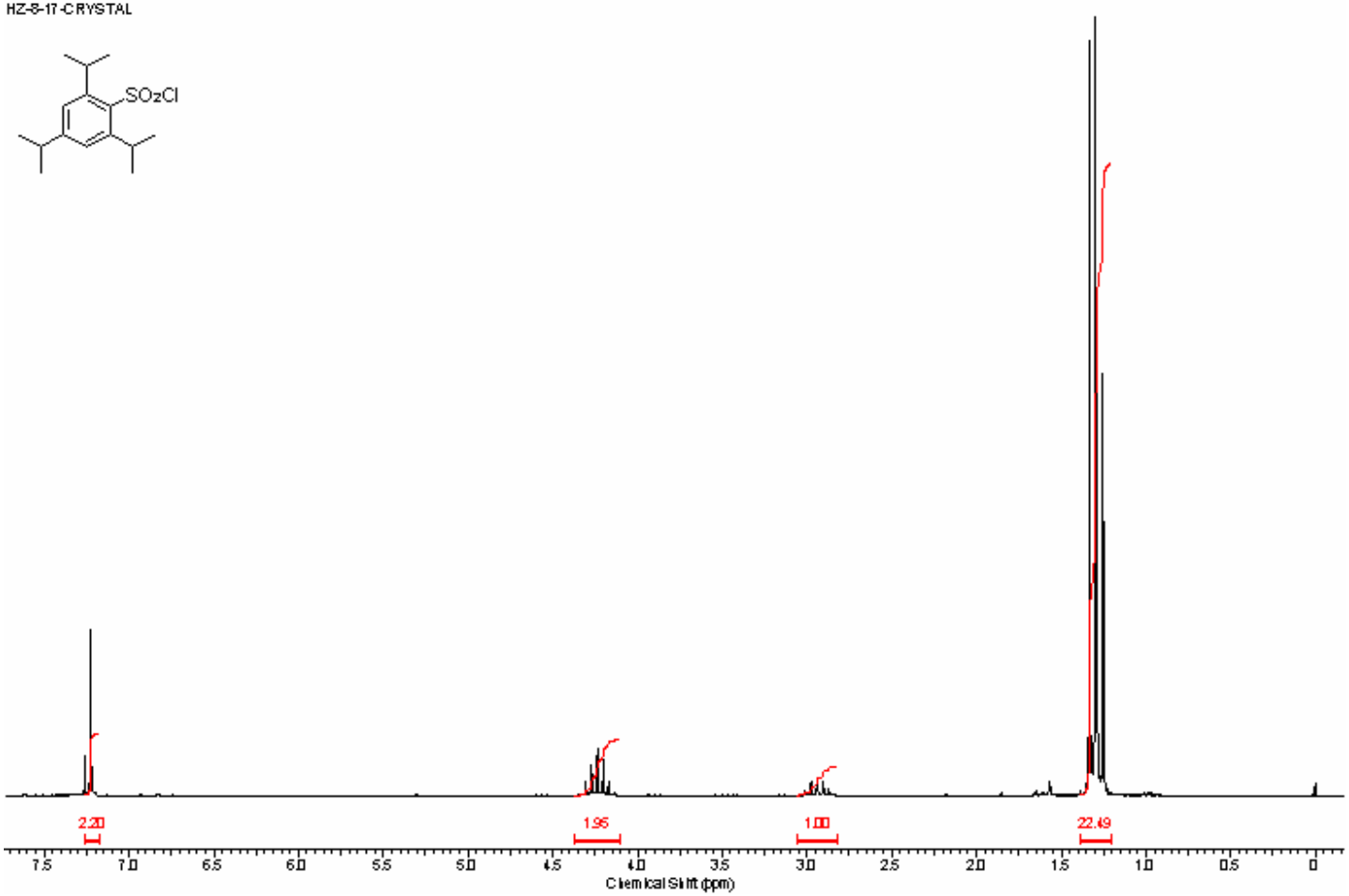
Acquisition Time (sec)	1.3005	Comment	Std proton	Date	Oct 31 2001	Date Stamp	Oct 31 2001
File Name	E:\0 RIGINAL NMR-400\HZ-10-61-13CNMR			Frequency (MHz)	100.53	Nucleus	13C
Number of Transients	256	Original Points Count	31315	Points Count	32768	Pulse Sequence	s2pul
Receiver Gain	30.00	Solvent	CHLOROFORM-d			Spectrum Offset (Hz)	10550.4990
Sweep Width (Hz)	24125.45	Temperature (degrees C)	25.000				

HZ-10-61-13CNMR



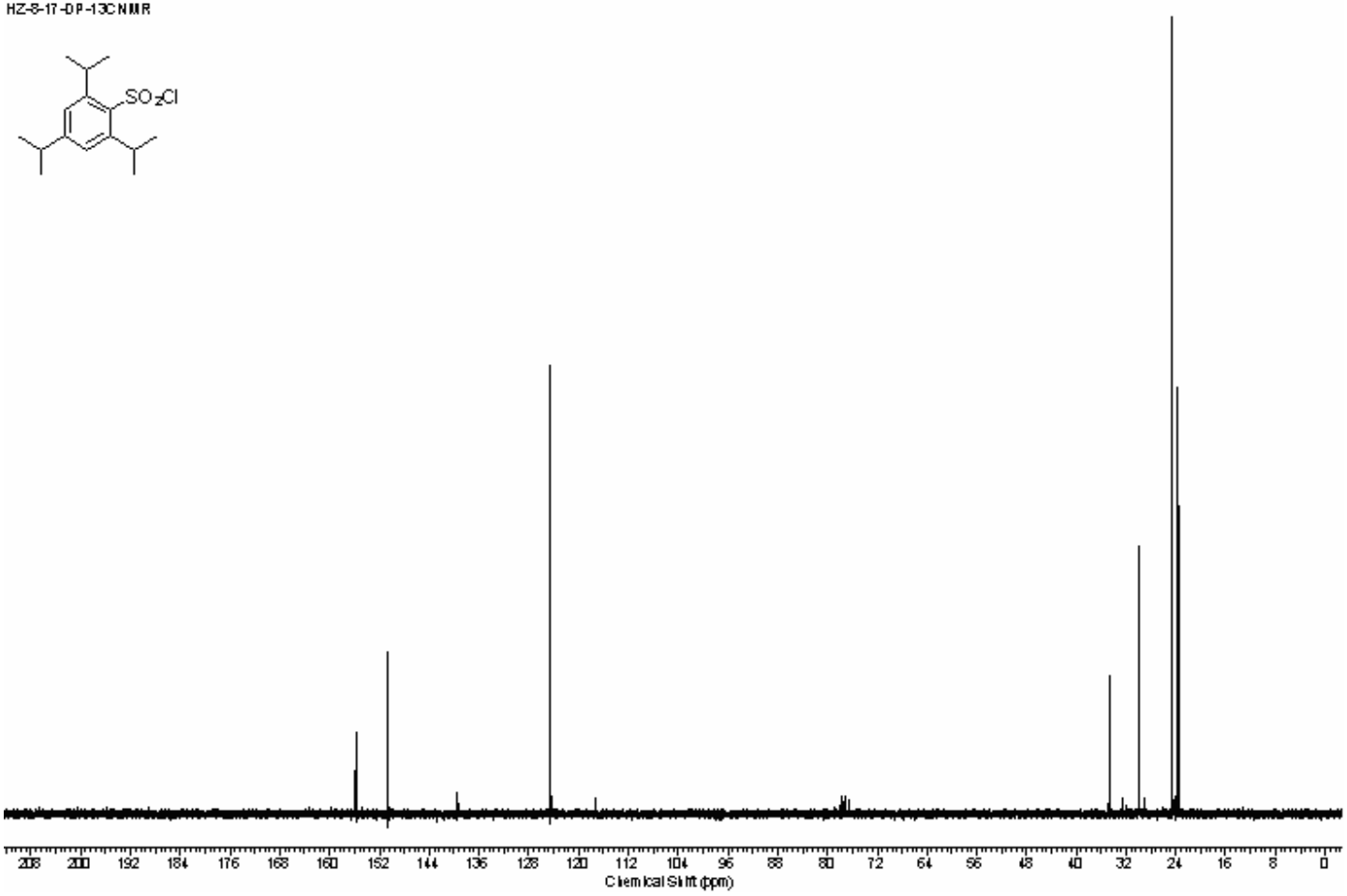
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Jan 31 2007
Date Stamp	Jan 31 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-8\HZ-8-17-CRYSTAL				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	22.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1000.5273	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-8-17-CRYSTAL



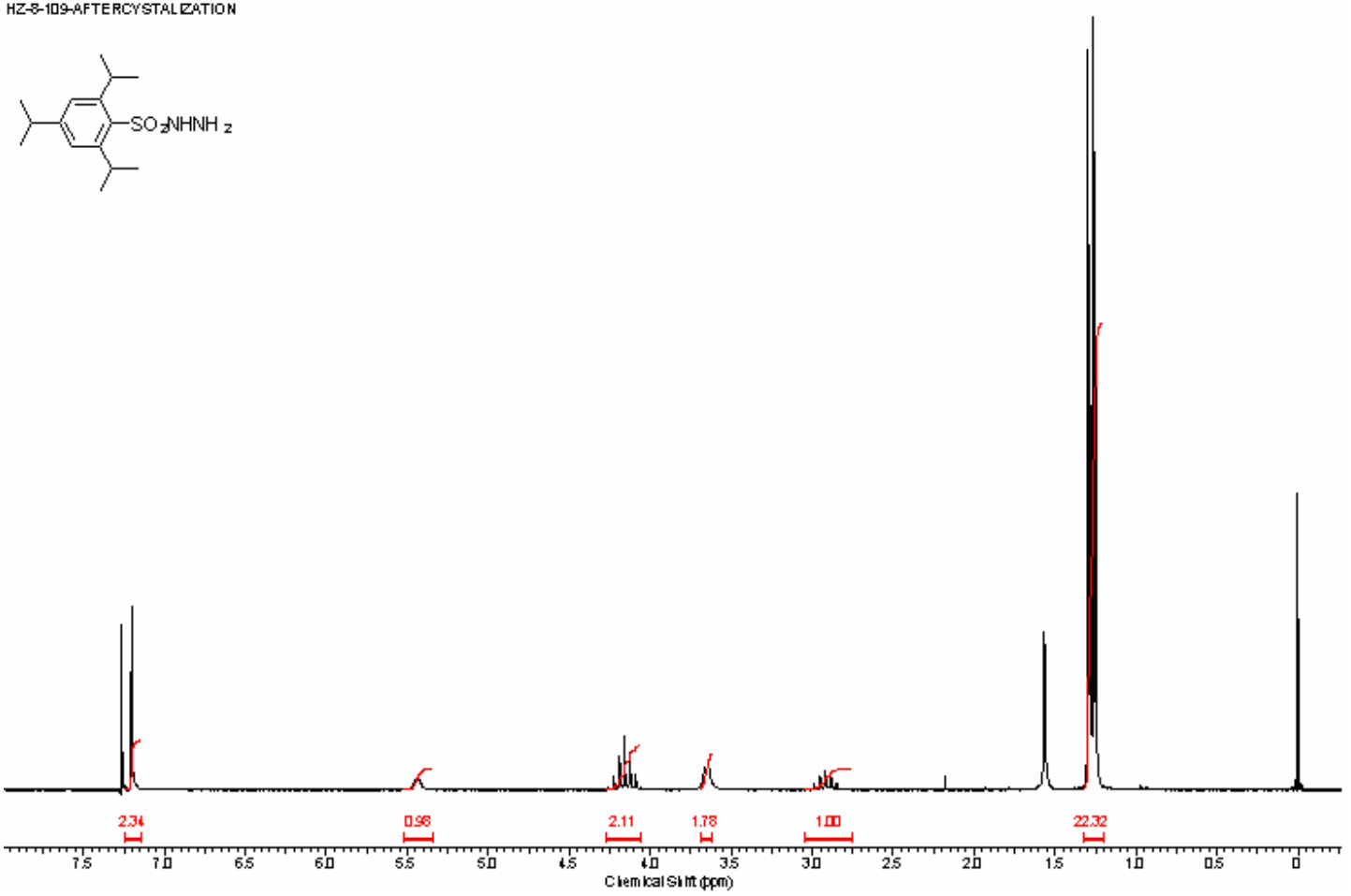
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Mar 14 2007	*Date Stamp*	Mar 14 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-S\HZ-S-17-DP-13C NMR					*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	64000	*Original Points Count*	18720	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	4879.7026	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-S-17-DP-13C NMR



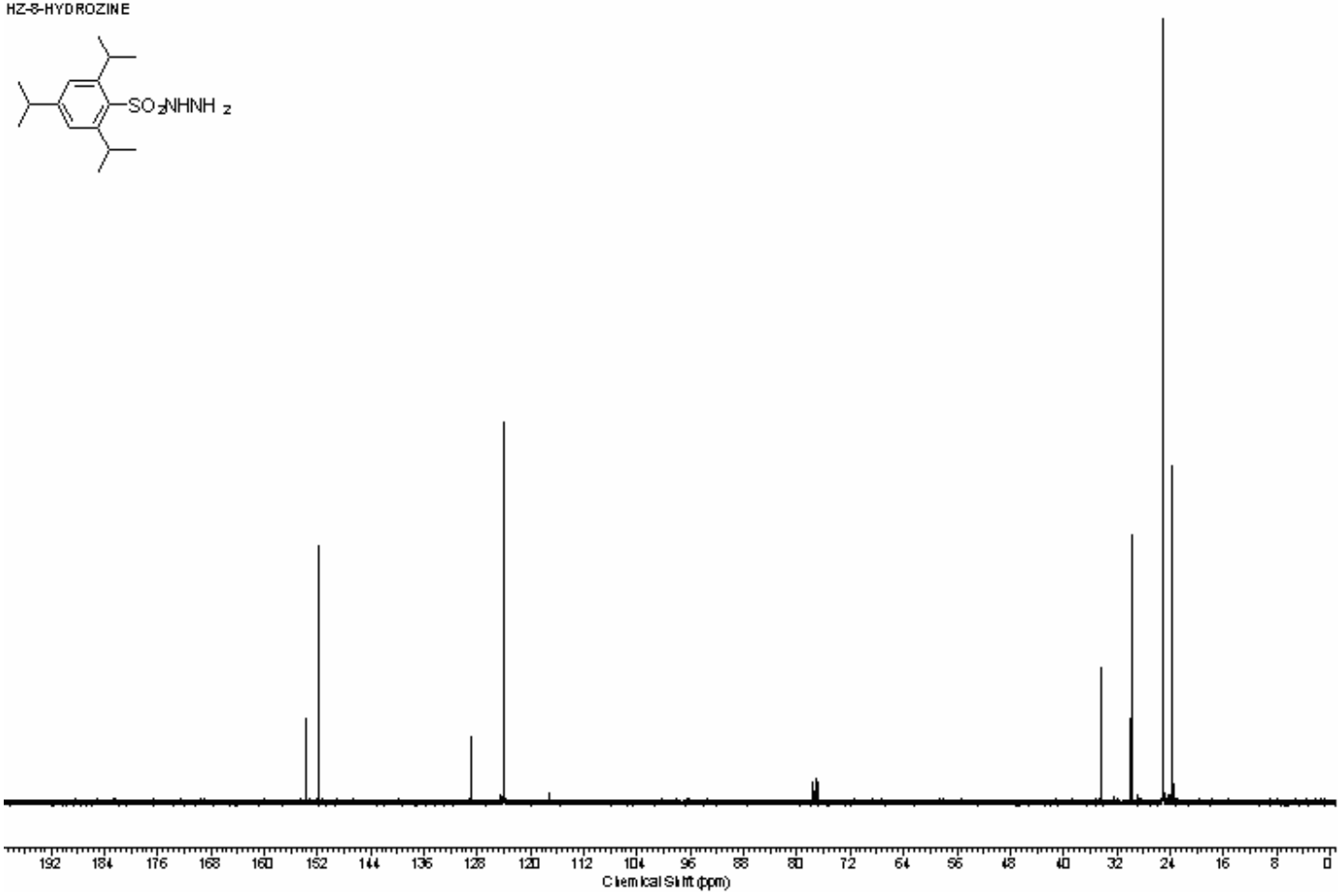
Acquisition Time (sec)	1.9945	*Comment*	STANDARD 1H OBSERVE			*Date*	Apr 24 2007
Date Stamp	Apr 24 2007	*File Name*	E:\HZ-8\HZ-8-109-AFTERCYSTALIZATION			*Frequency (MHz)*	199.98
Nucleus	1H	*Number of Transients*	64	*Original Points Count*	5984	*Points Count*	8192
Pulse Sequence	s2pul	*Receiver Gain*	34.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	1000.5273	*Sweep Width (Hz)*	3000.30	*Temperature (degree C)*	29.000		

HZ-8-109-AFTERCYSTALIZATION



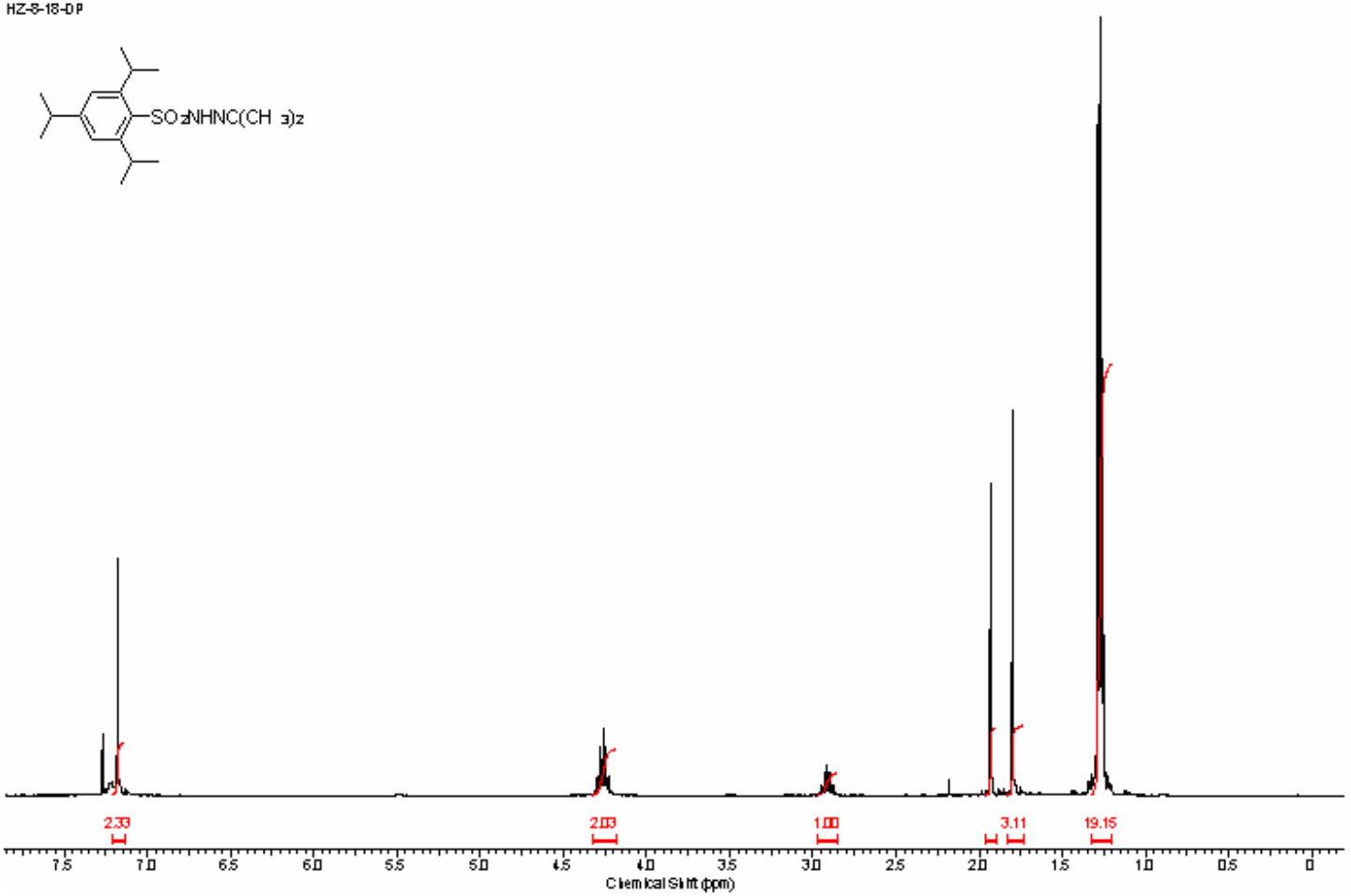
Acquisition Time (sec)	1.3005	*Comment*	Std proton	*Date*	Nov 2 2007			*Date Stamp*	Nov 2 2007
File Name	E:\HZ-S-HYDROZINE			*Frequency (MHz)*	100.53	*Nucleus*	13C	*Number of Transients*	5000
Original Points Count	31375	*Points Count*	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	30.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	10537.4512			*Sweep Width (Hz)*	24125.45	*Temperature (degree C)*	25.000		

HZ-S-HYDROZINE



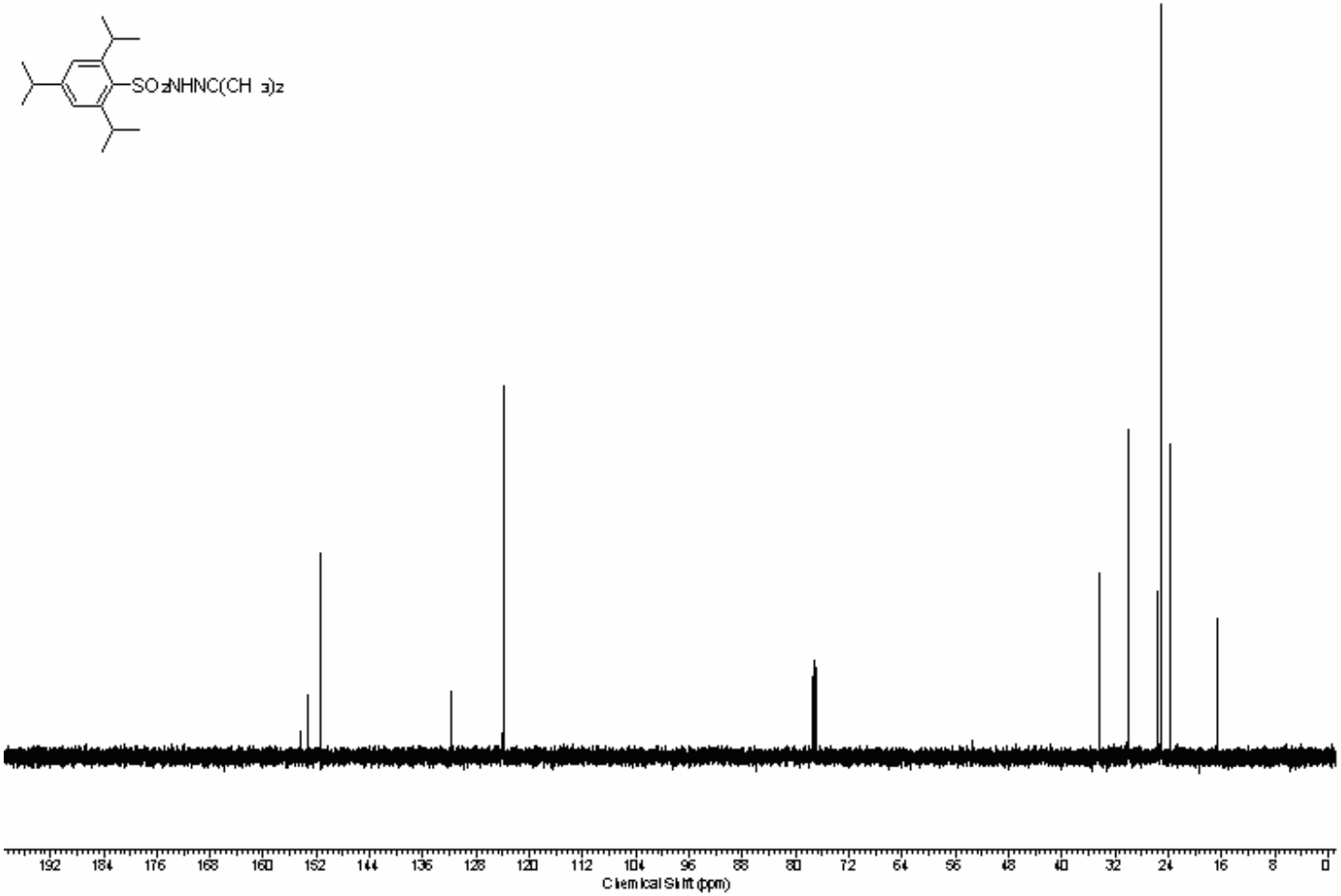
Acquisition Time (sec)	2.0486	*Comment*	Std proton			*Date*	Nov 15 2007		
Date Stamp	Nov 15 2007			*File Name*	E:\HZ-8-18-DP			*Frequency (MHz)*	399.77
Nucleus	1H	*Number of Transients*	8	*Original Points Count*	13104	*Points Count*	16384	*Pulse Sequence*	s2pul
Receiver Gain	32.00	*Solvent*	CHLOROFORM-d			*Spectrum Offset (Hz)*	2409.7039		
Sweep Width (Hz)	6396.42	*Temperature (degree C)*	25.000						

HZ-8-18-DP



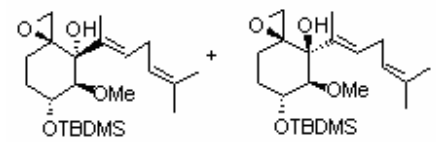
Acquisition Time (sec)	1.3005	Comment	Std proton	Date	Nov 15 2007			Date Stamp	Nov 15 2007
File Name	E:\HZ-8-19-HYDROZONE-13CNMR			Frequency (MHz)	100.53	Nucleus	13C	Number of Transients	1256
Original Points Count	31375	Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	30.00		
Solvent	CHLOROFORM-d			Spectrum Offset (Hz)	10552.7070	Sweep Width (Hz)	24125.45	Temperature (degree C)	25.000

HZ-8-19-HYDROZONE-13CNMR



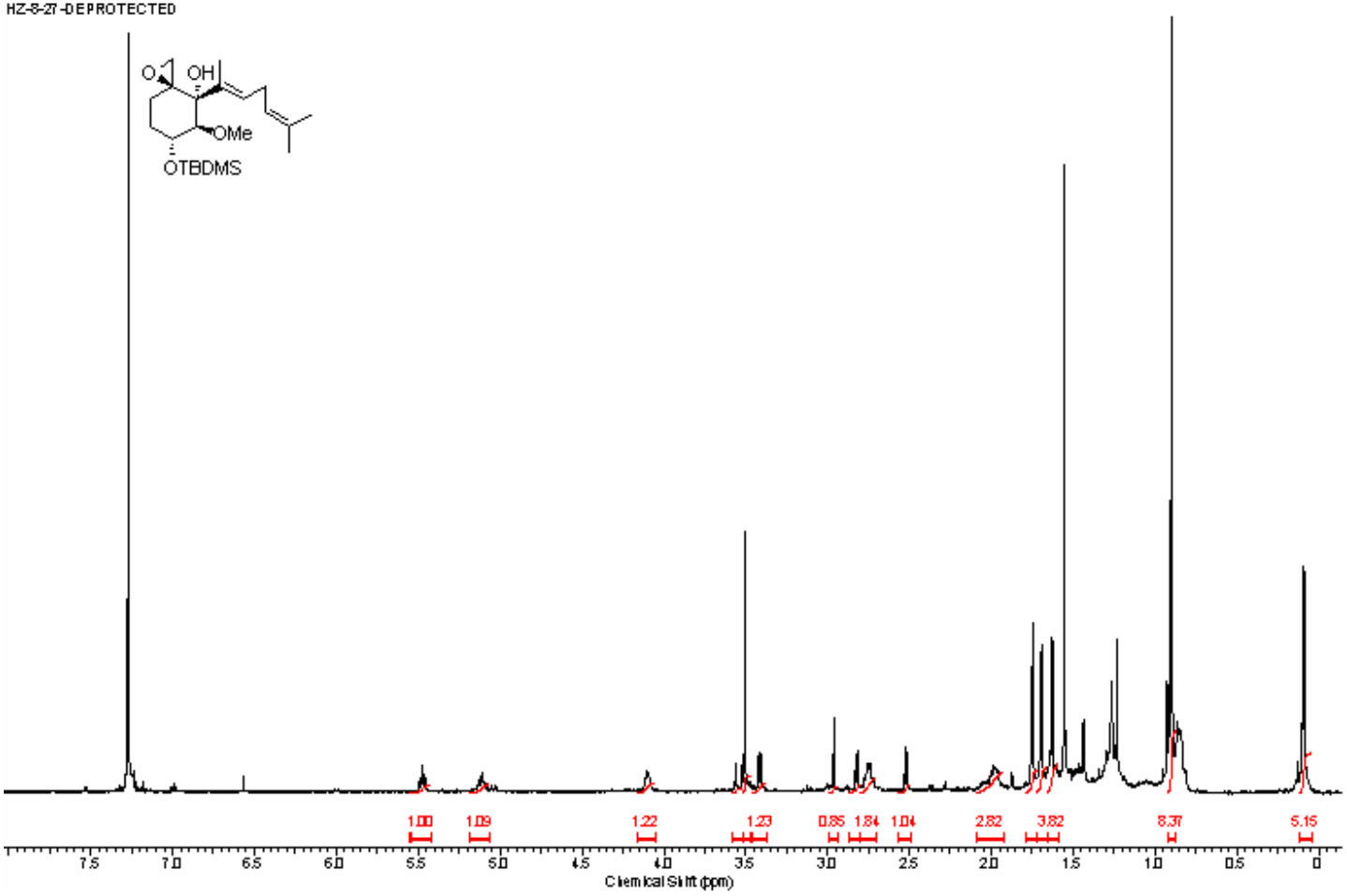
Acquisition Time (sec)	2.0486	*Comment*	Std proton	*Date*	Nov 1 2007			*Date Stamp*	Nov 1 2007
File Name	E:\ORIGINAL NMR-400\HZ-9-13-AC			*Frequency (MHz)*	399.77	*Nucleus*	1H	*Number of Transients*	8
Original Points Count	13104	*Points Count*	16384	*Pulse Sequence*	s2pul	*Receiver Gain*	4200		
Solvent	CHLOROFORM-d			*Spectrum Offset (Hz)*	2406.3726	*Sweep Width (Hz)*	6396.42	*Temperature (degree C)*	25.000

HZ-9-13-AC

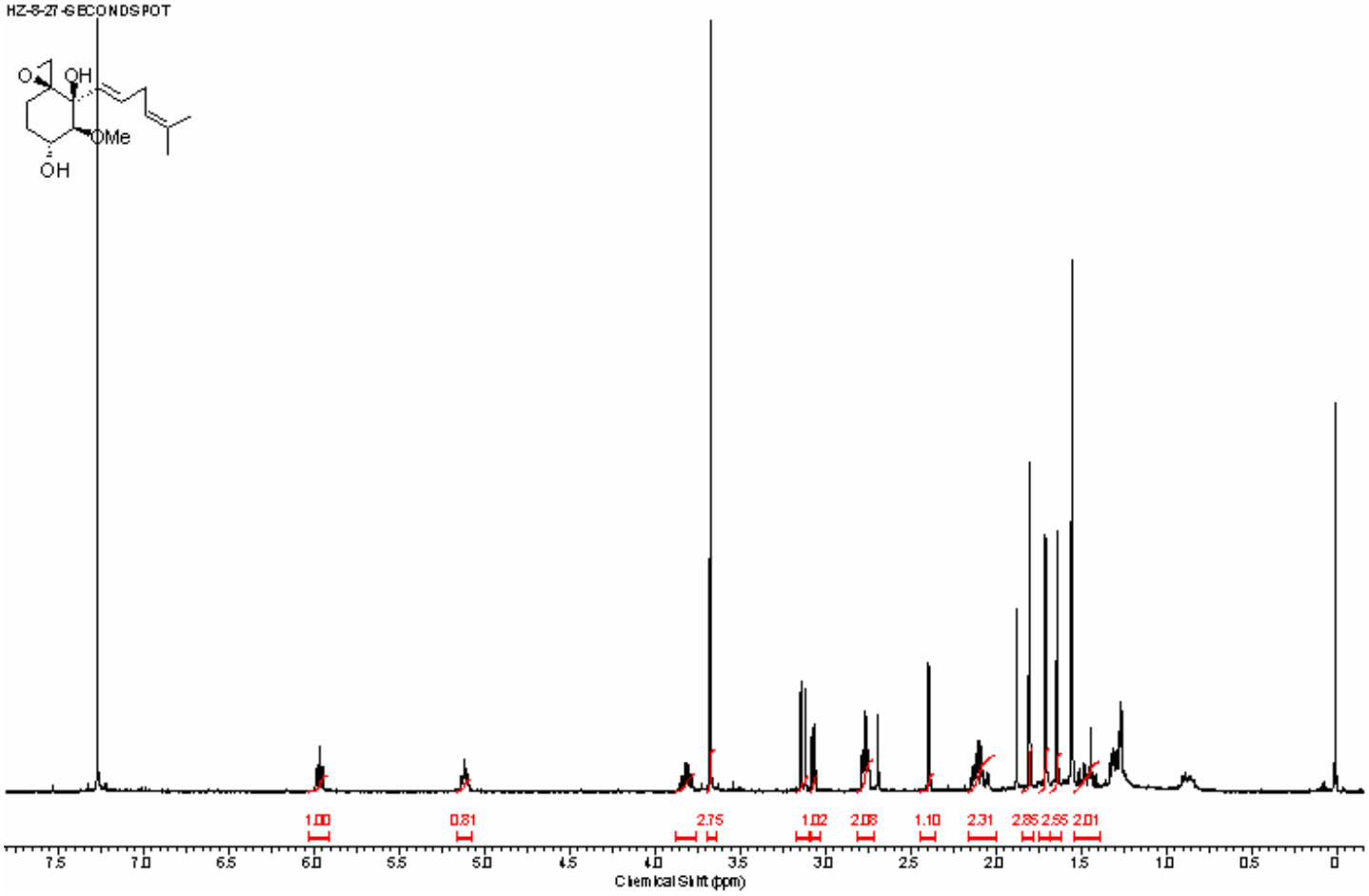


Acquisition Time (sec)	2.0486	*Comment*	Std proton	*Date*	Nov 2 2007	*Date Stamp*	Nov 2 2007
File Name	E:\ORIGINAL NMR-400\HZ-8-27-DEPROTECTED			*Frequency (MHz)*	399.77	*Nucleus*	1H
Number of Transients	16	*Original Points Count*	13104	*Points Count*	16384	*Pulse Sequence*	s2pul
Receiver Gain	50.00	*Solvent*	CHLOROFORM-d			*Spectrum Offset (Hz)*	2405.9951
Sweep Width (Hz)	6396.42	*Temperature (degree C)*	25.000				

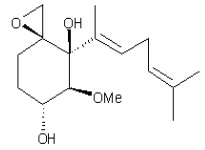
HZ-8-27-DEPROTECTED



Acquisition Time (sec)	3.7010	*Comment*	STANDARD 1H OBSERVE			*Date*	Feb 13 2007
Date Stamp	Feb 13 2007	*File Name*	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-8-400-OLD\HZ-8-27-SECONDSPOT				
Frequency (MHz)	399.78	*Nucleus*	1H	*Number of Transients*	64	*Original Points Count*	22208
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	2007.8860	*Sweep Width (Hz)*	6000.60	*Temperature (degree C)*	25.000		

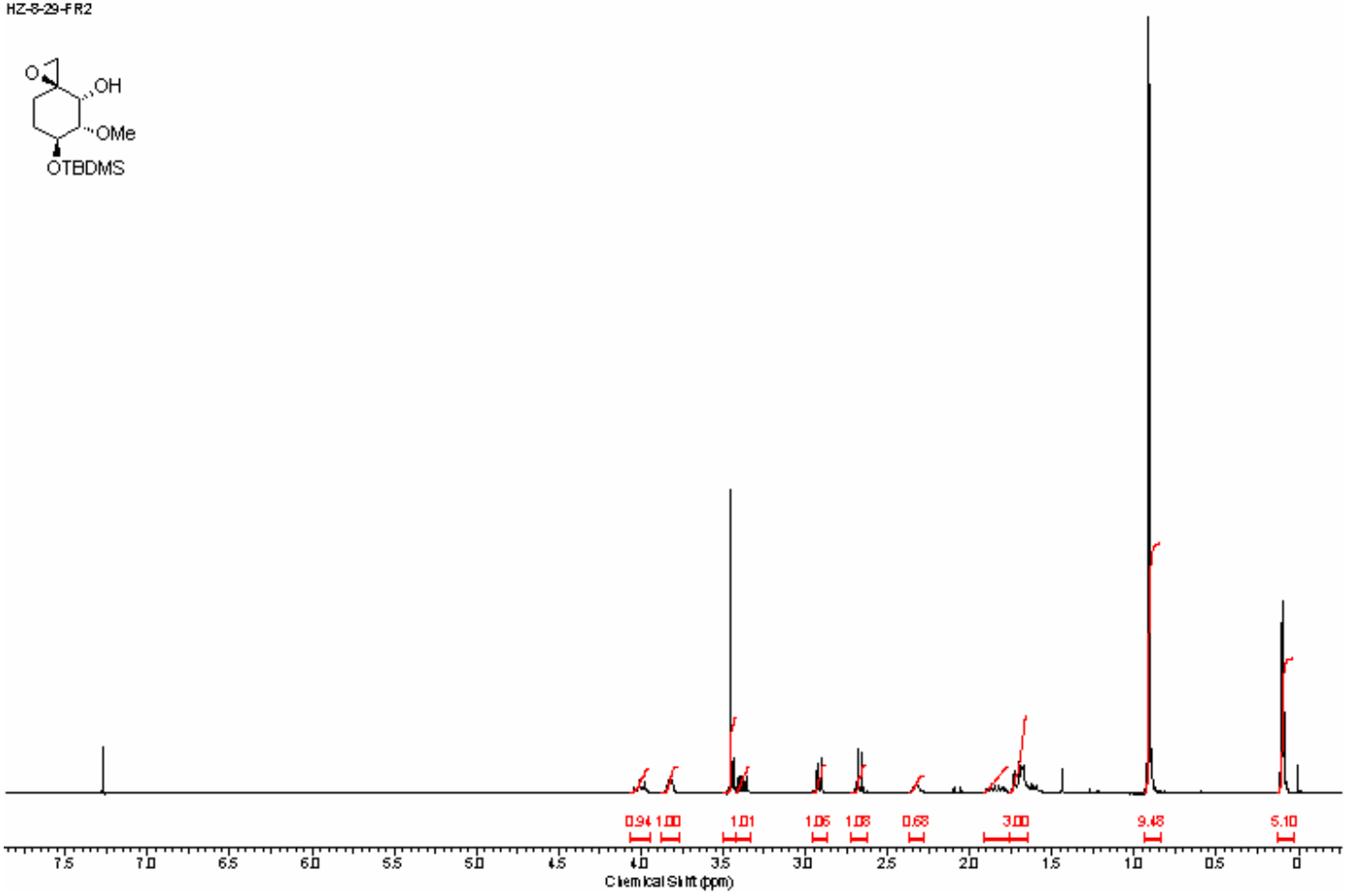


Acquisition Time (sec)	2.6214	*Comment*	13C OBSERVE			*Date*	Jan 29 2008		*Frequency (MHz)*	50.29
Nucleus	13C	*Original Points Count*	18720	*Points Count*	32768	*Solvent*	CDCl3	*Sweep Width (Hz)*	12500.00	
Temperature (grad C)	29.000									



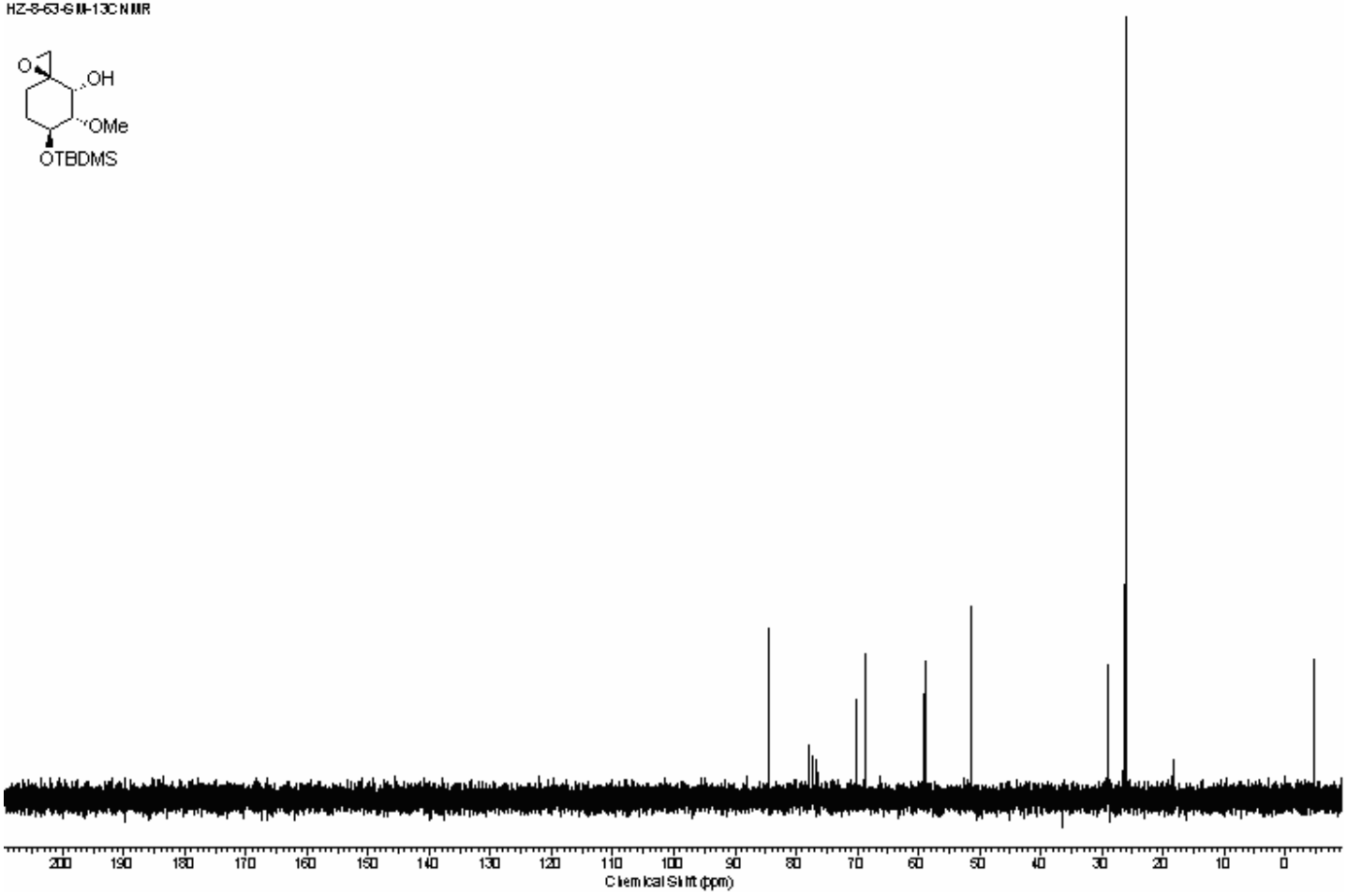
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Feb 14 2007
Date Stamp	Feb 14 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-S\HZ-S-29-FR2				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	24.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1002.0153	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-S-29-FR2



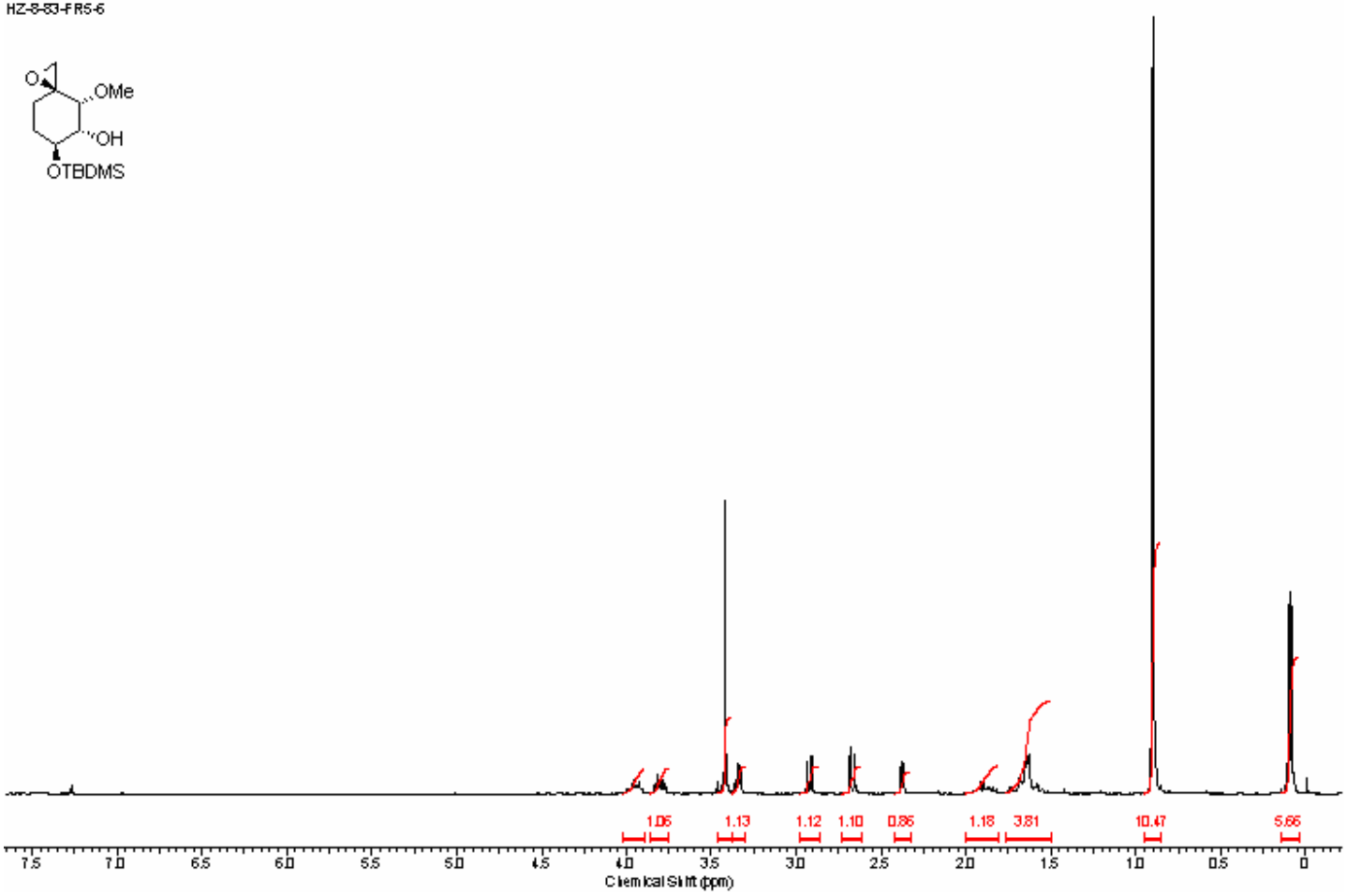
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Mar 17 2007	*Date Stamp*	Mar 17 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-S\HZ-8-63-SM-13C NMR					*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	64000	*Original Points Count*	18720	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	4880.4648	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-8-63-SM-13C NMR



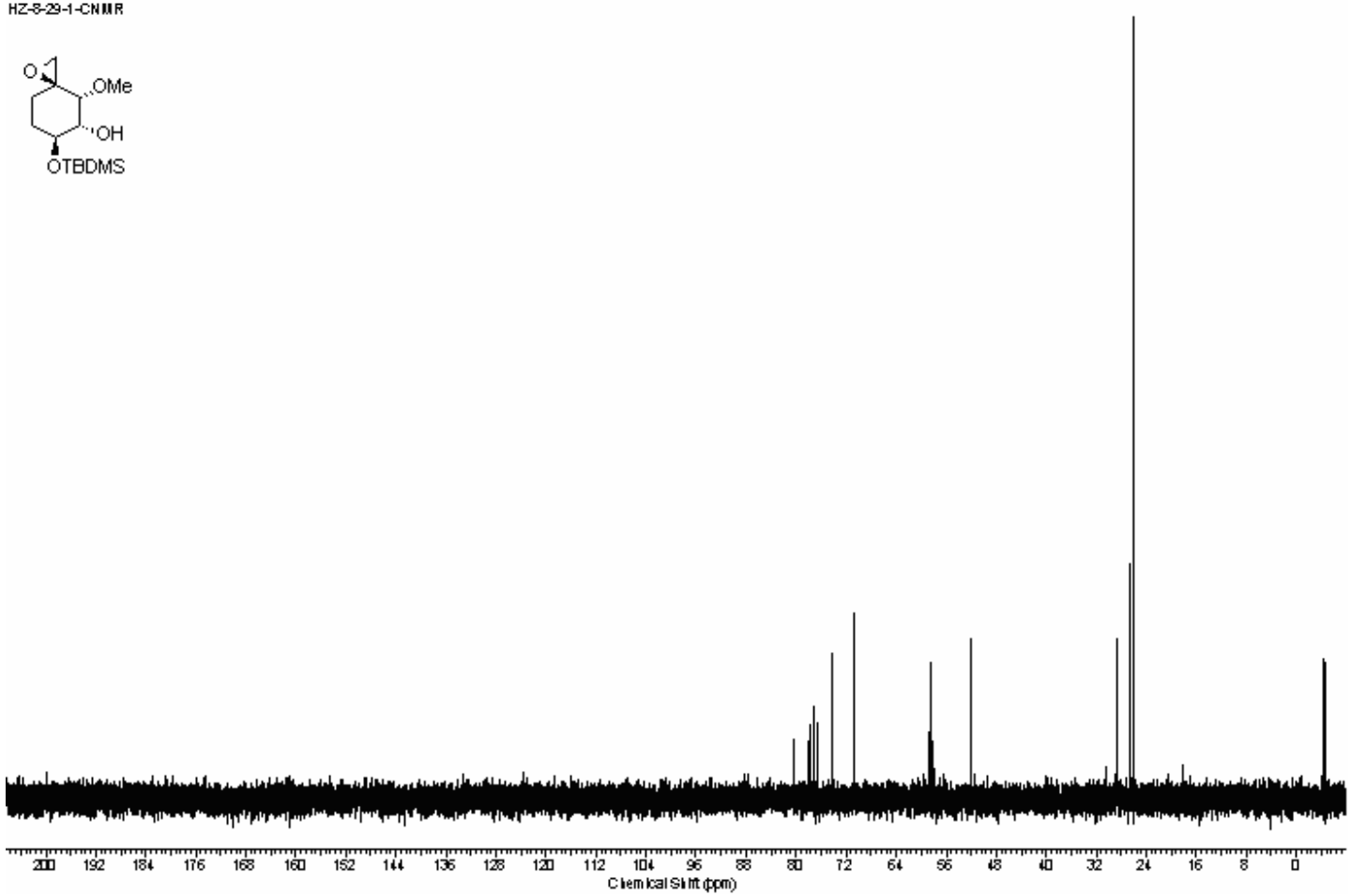
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Apr 5 2007
Date Stamp	Apr 5 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-S\HZ-8-83-FRS-6				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	16.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.6563	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-8-83-FRS-6



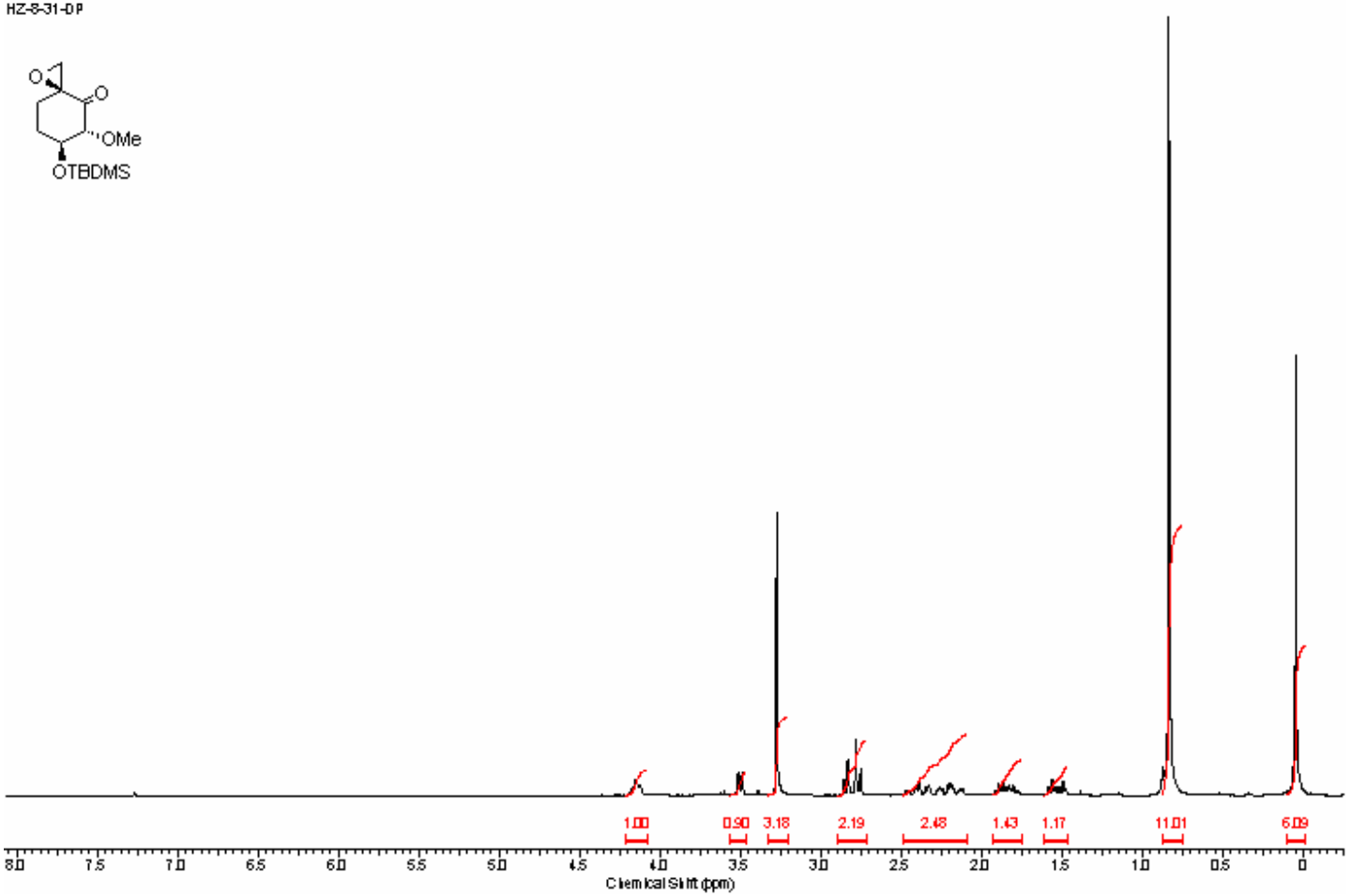
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Feb 13 2007	*Date Stamp*	Feb 13 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-8\HZ-8-29-1-CNMR					*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	64000	*Original Points Count*	16720	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	4880.8467	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-8-29-1-CNMR



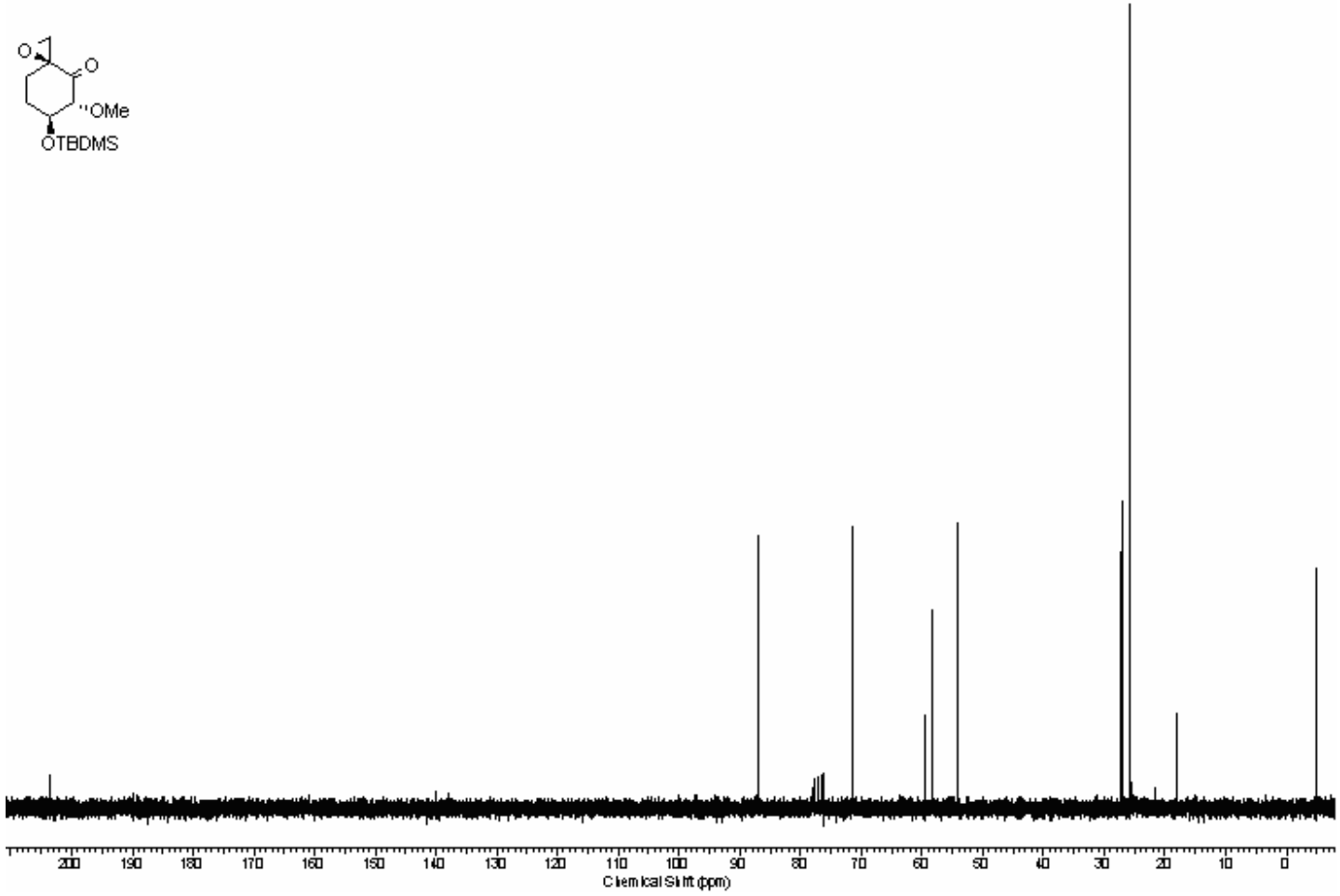
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Apr 19 2007		
Date Stamp	Apr 19 2007			File Name	E:\HZ-8\HZ-8-31-DP			Frequency (MHz)	199.98
Nucleus	1H	Number of Transients	64	Original Points Count	5984	Points Count	8192	Pulse Sequence	s2pul
Receiver Gain	10.00	Solvent	CHLOROFORM-d			Spectrum Offset (Hz)	1001.6563	Sweep Width (Hz)	3000.30
Temperature (degree C)	29.000								

HZ-8-31-DP



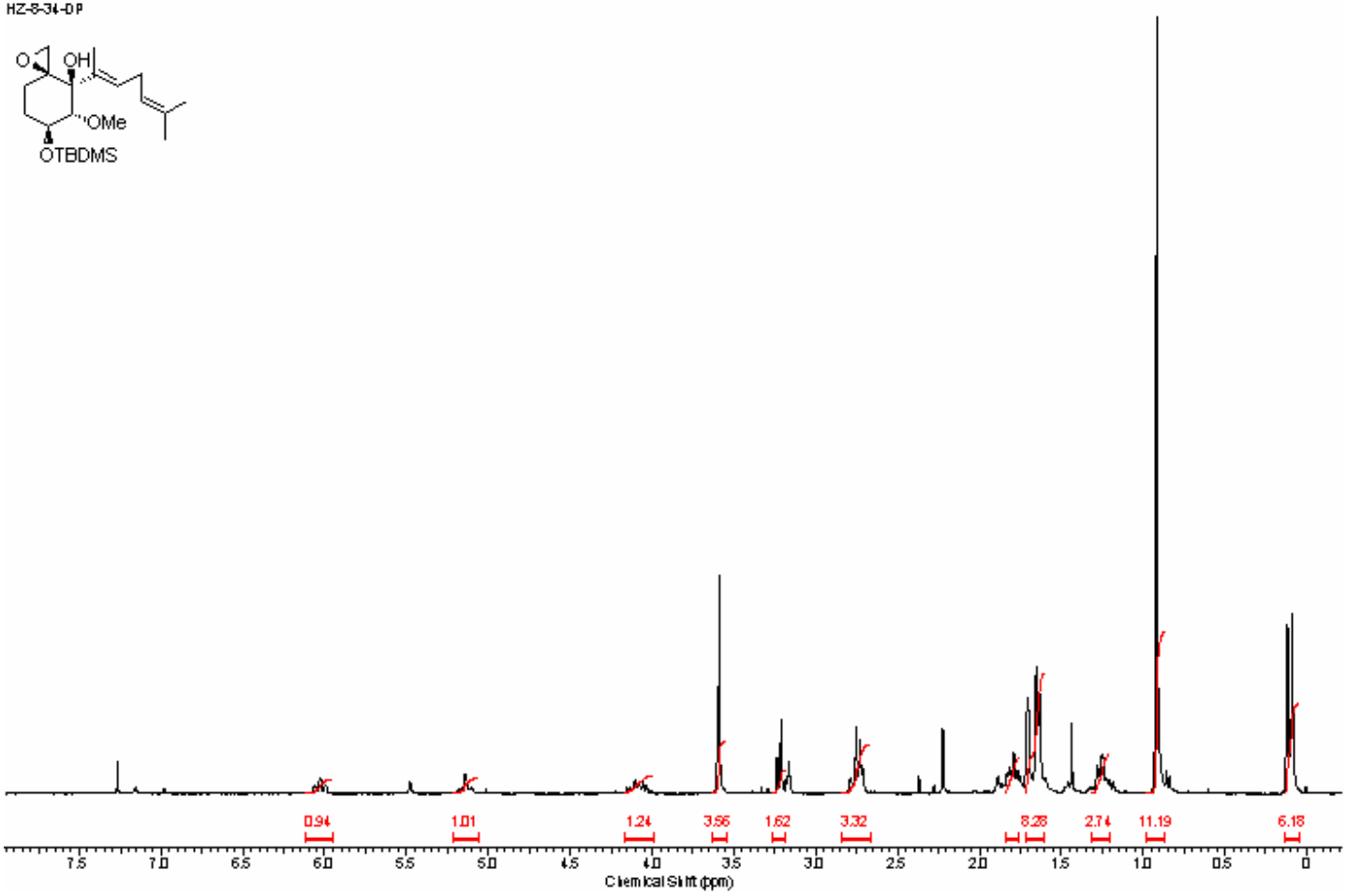
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE			*Date*	Apr 19 2007	*Date Stamp*	Apr 19 2007
File Name	E:\HZ-8\HZ-8-31-DP-13C NMR			*Frequency (MHz)*	50.29	*Nucleus*	13C	*Number of Transients*	64000000
Original Points Count	16720	*Points Count*	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	40.00		
Solvent	CHLOROFORM-d			*Spectrum Offset (Hz)*	4818.1768	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000

HZ-8-31-DP-13C NMR



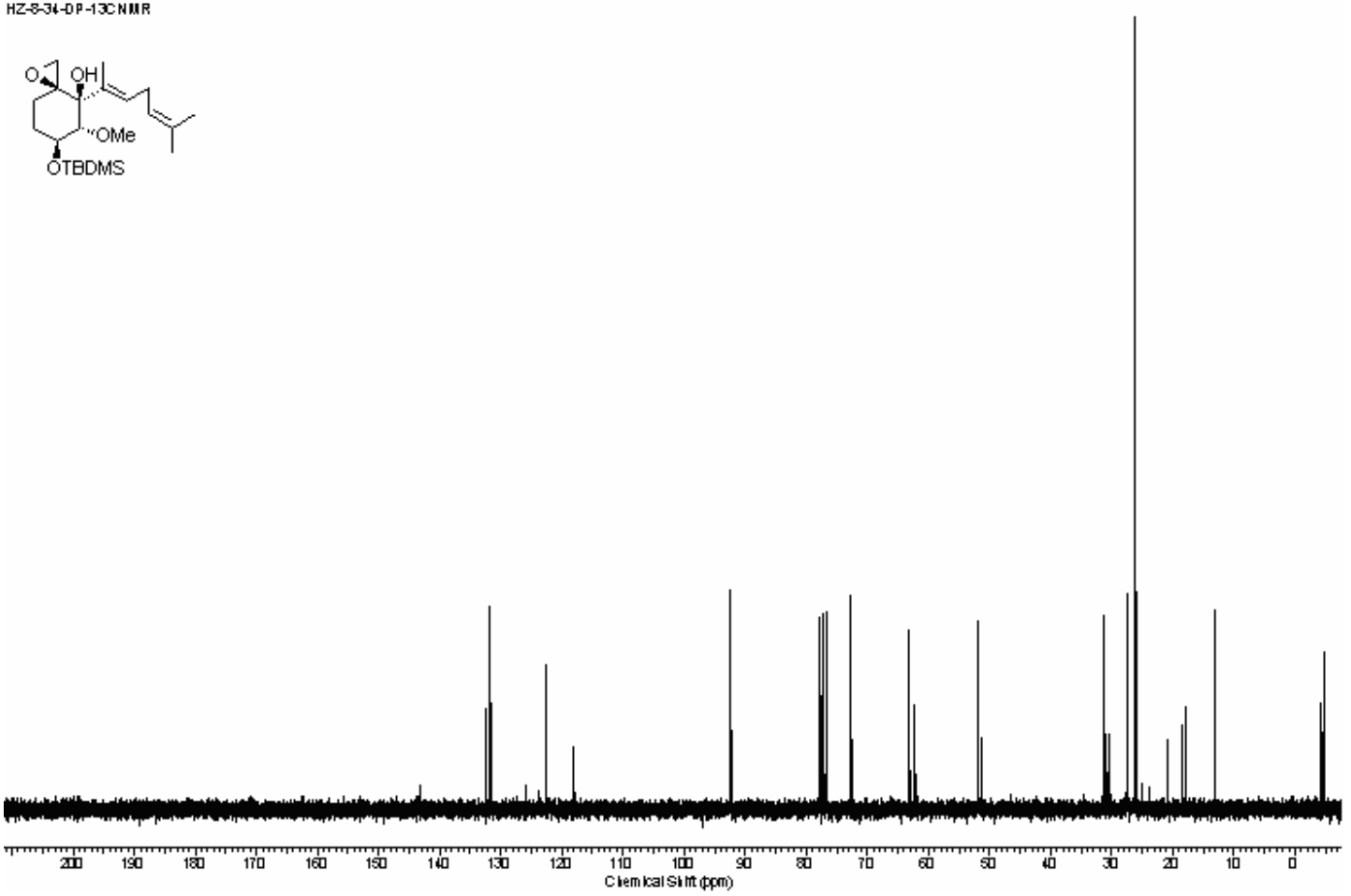
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Feb 19 2007
Date Stamp	Feb 19 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-S\HZ-S-34-DP				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	22.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.2900	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-S-34-DP



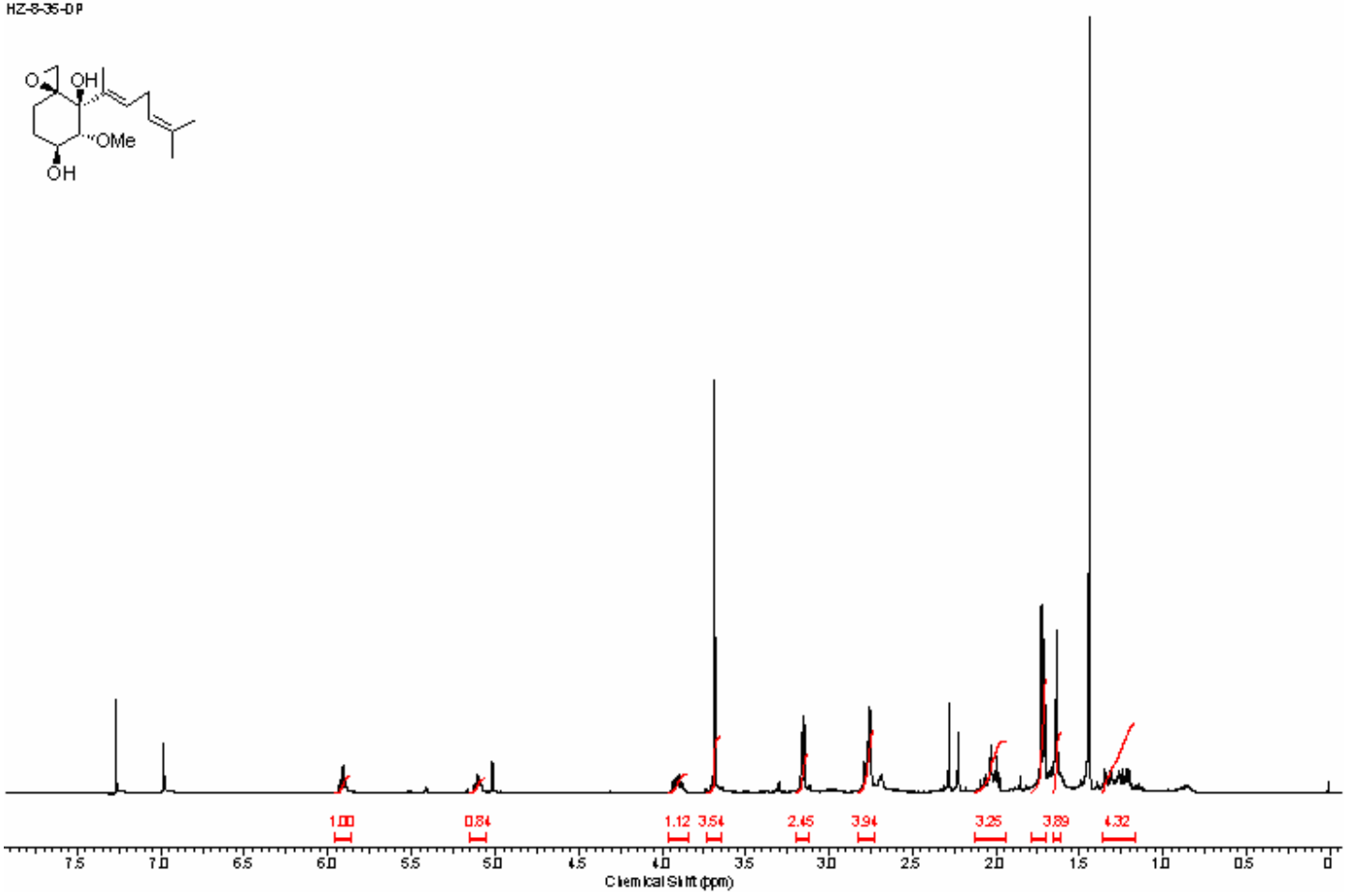
Acquisition Time (sec)	1.4976	Comment	13C OBSERVE	Date	Feb 19 2007	Date Stamp	Feb 19 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-S\HZ-S-34-DP-13C NMR					Frequency (MHz)	50.29
Nucleus	13C	Number of Transients	64000.00	Original Points Count	16720	Points Count	32768
Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d		
Spectrum Offset (Hz)	4881.2236	Sweep Width (Hz)	12500.00	Temperature (degree C)	29.000		

HZ-S-34-DP-13C NMR



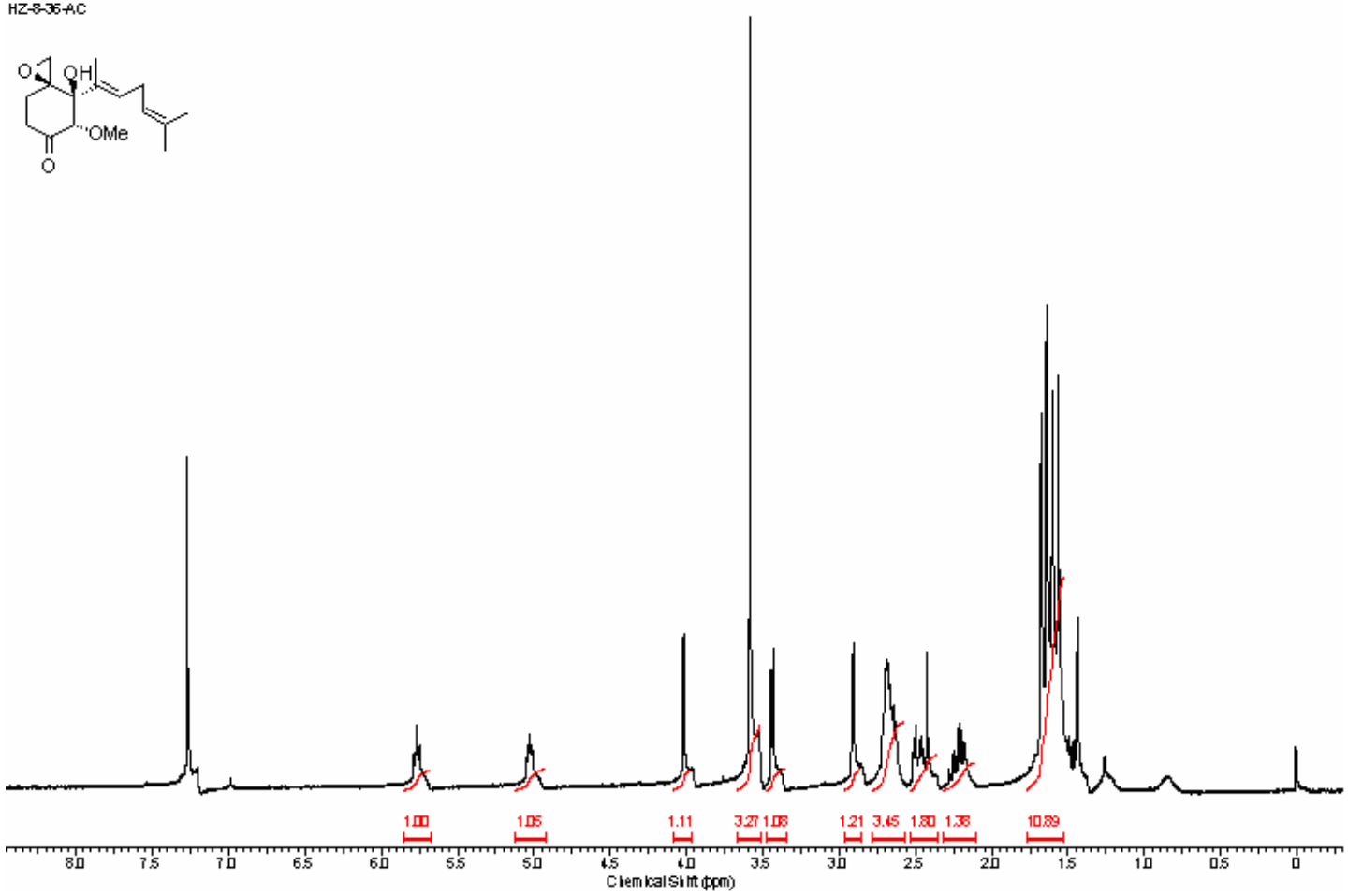
Acquisition Time (sec)	3.7010	Comment	STANDARD 1H OBSERVE			Date	Feb 19 2007
Date Stamp	Feb 19 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-8-400-OLD\HZ-8-35-DP				
Frequency (MHz)	399.78	Nucleus	1H	Number of Transients	64	Original Points Count	22208
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	40.000	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2008.4353	Sweep Width (Hz)	6000.60	Temperature (degree C)	25.000		

HZ-8-35-DP



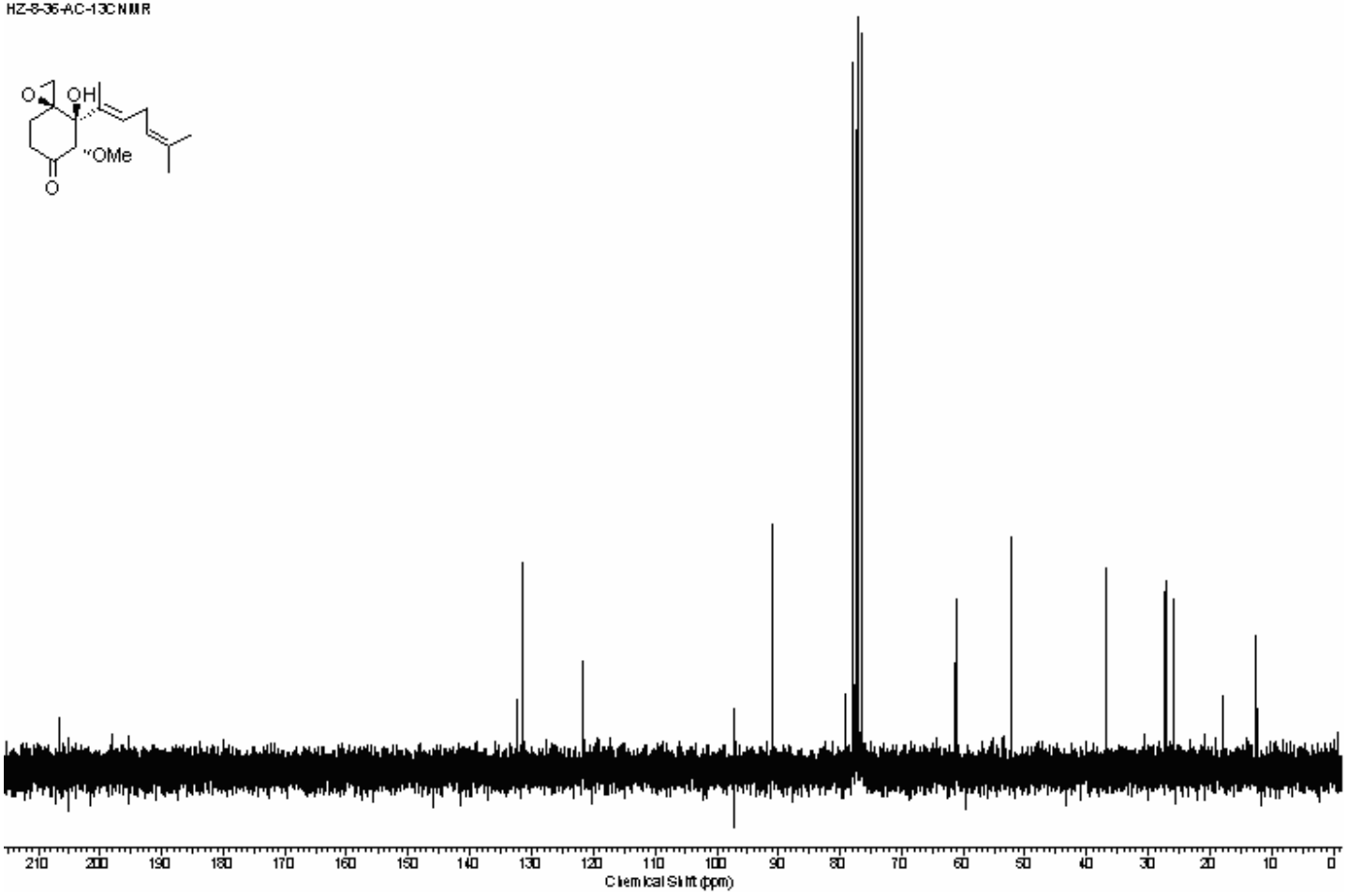
Acquisition Time (sec)	3.7010	Comment	STANDARD 1H OBSERVE			Date	Feb 20 2007
Date Stamp	Feb 20 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-8-400-OLD\HZ-8-36-AC				
Frequency (MHz)	399.78	Nucleus	1H	Number of Transients	64	Original Points Count	22208
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	53.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2009.9004	Sweep Width (Hz)	6000.60	Temperature (degree C)	25.000		

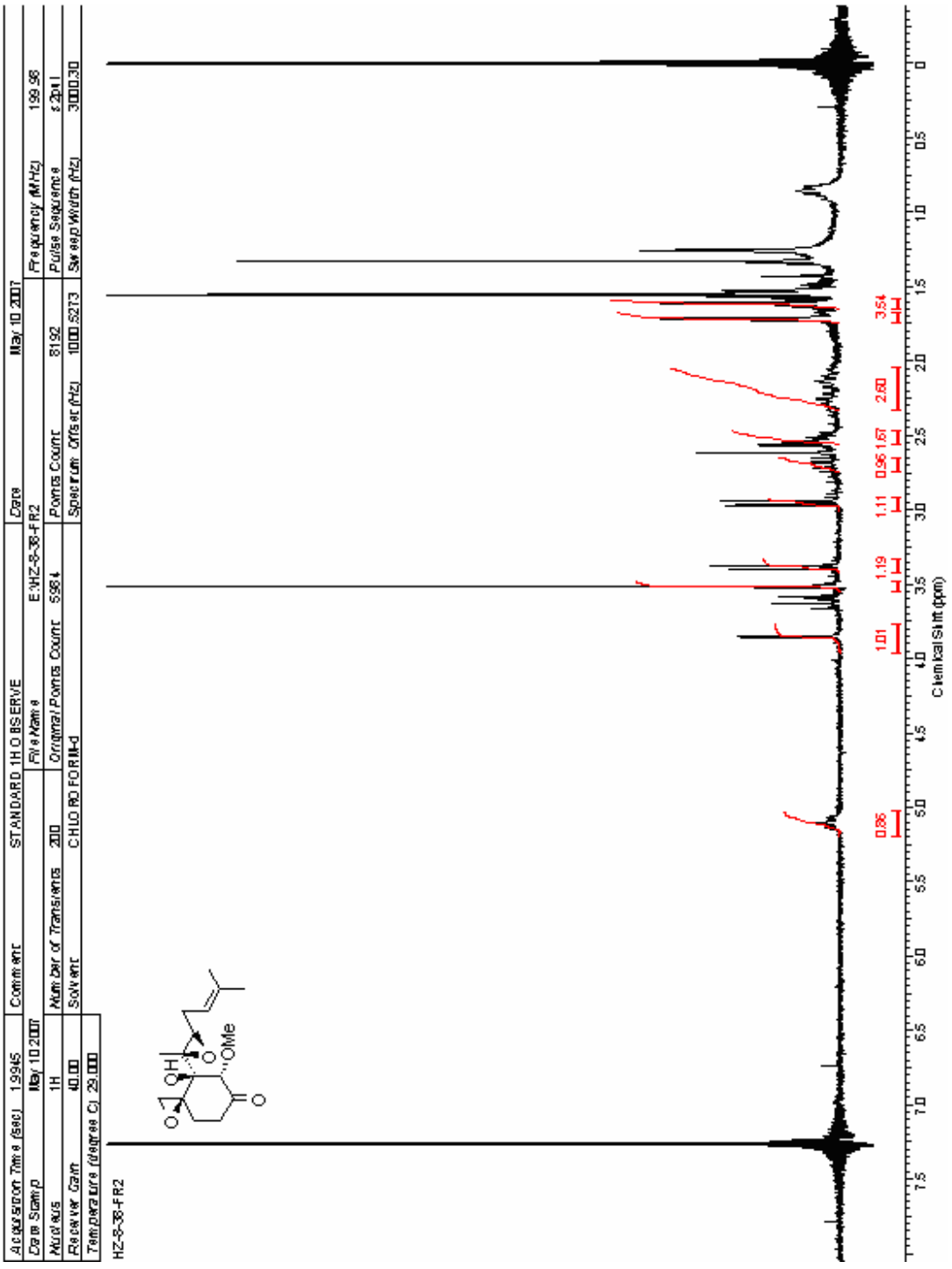
HZ-8-36-AC



Acquisition Time (sec)	1.4976	Comment	13C OBSERVE	Date	Feb 24 2007	Date Stamp	Feb 24 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-S\HZ-S-36-AC-13C NMR					Frequency (MHz)	50.29
Nucleus	13C	Number of Transients	64000.00	Original Points Count	16720	Points Count	32768
Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d		
Spectrum Offset (Hz)	4881.2285	Sweep Width (Hz)	12500.00	Temperature (degree C)	29.000		

HZ-S-36-AC-13C NMR




Acquisition Time (sec) 1.9945
Comment STANDARD 1H OBSERVE
Date May 10 2007
Date Stamp May 10 2007
File Name E:\HZ-8-38-FR2
Frequency (MHz) 199.98
Nucleus 1H
Number of Transients 200
Original Points Count 5984
Points Count 8192
Pulse Sequence s2pul
Receiver Gain 40.00
Solvent CHLOROFORM-d
Spectrum Offset (Hz) 1000.5273
Sweep Width (Hz) 3000.30
Temperature (degree C) 29.000
HZ-8-38-FR2
OH
OMe
O
O
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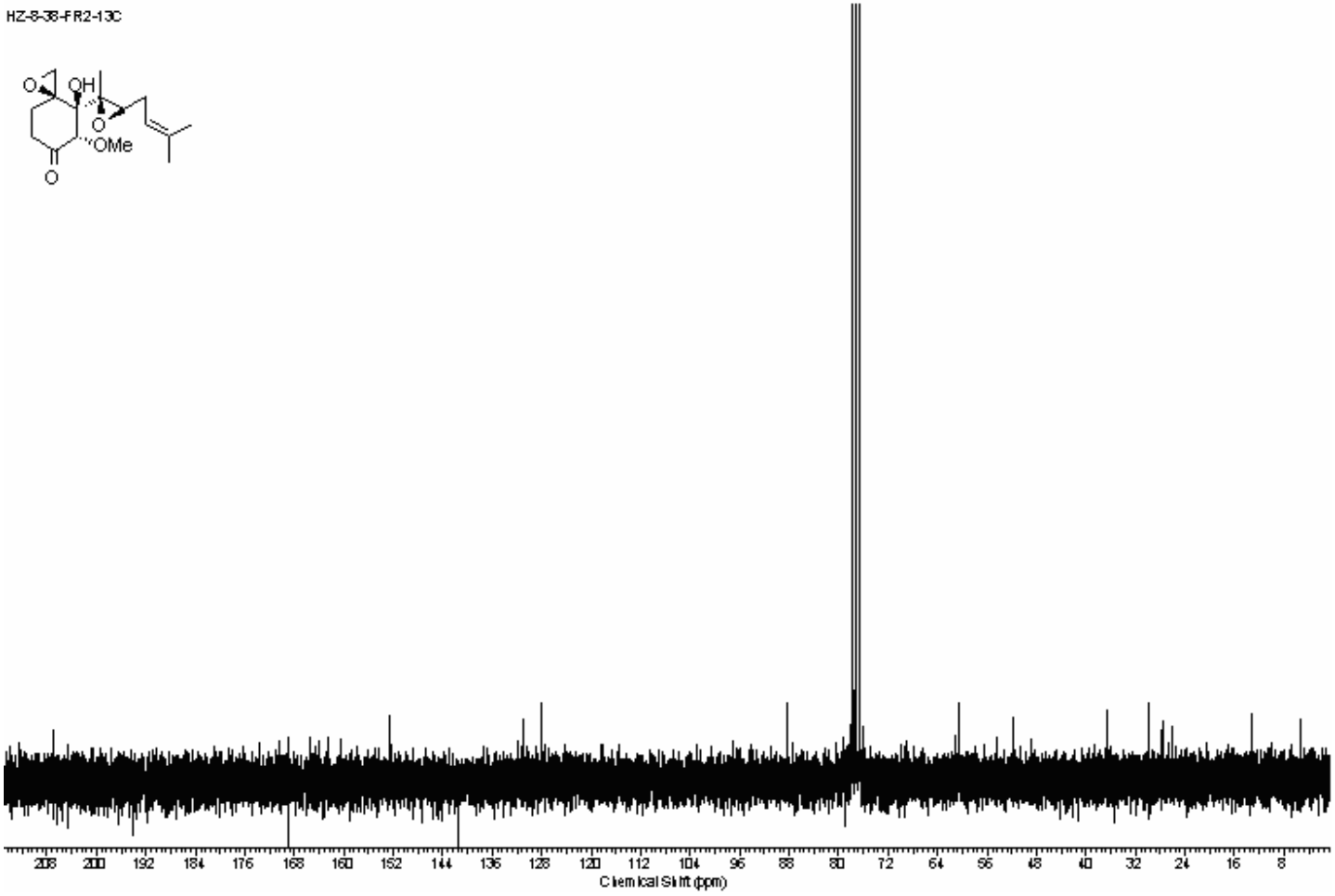
1.01
1.19
1.11
0.96
1.67
2.60
3.54
Chemical Shift (ppm)

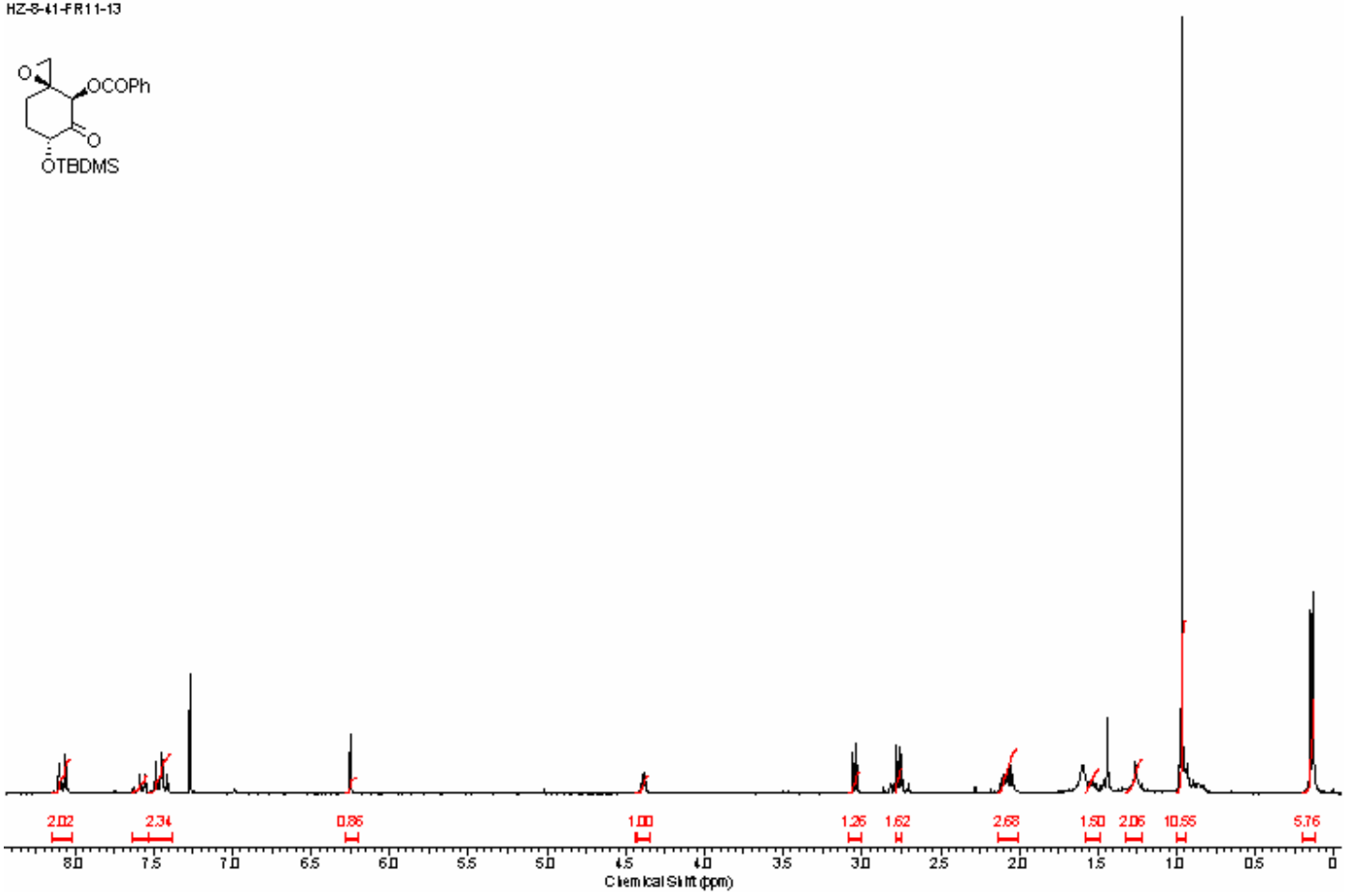
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE			*Date*	May 19 2007		
Date Stamp	May 19 2007			*File Name*	E:\HZ-8-38-FR2-13C			*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	20000000	*Original Points Count*	18720	*Points Count*	32768	*Pulse Sequence*	s2pul
Receiver Gain	40.00	*Solvent*	CHLOROFORM-d			*Spectrum Offset (Hz)*	4881.6094	*Sweep Width (Hz)*	12500.00
Temperature (degree C)	29.000								

HZ-8-38-FR2-13C



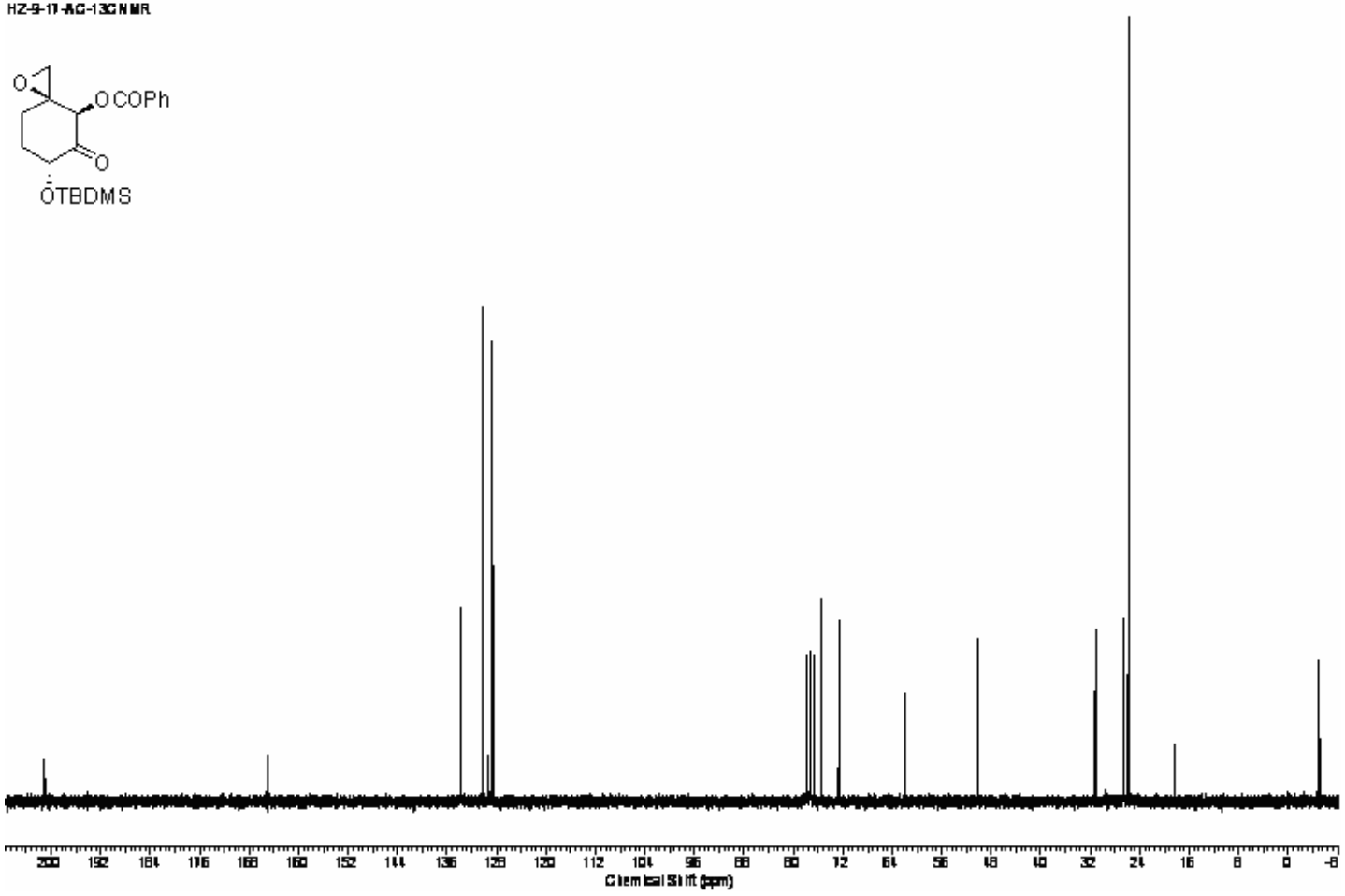
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Feb 23 2007
Date Stamp	Feb 23 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-S\HZ-S-41-FR11-13				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	35.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.6563	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-S-41-FR11-13



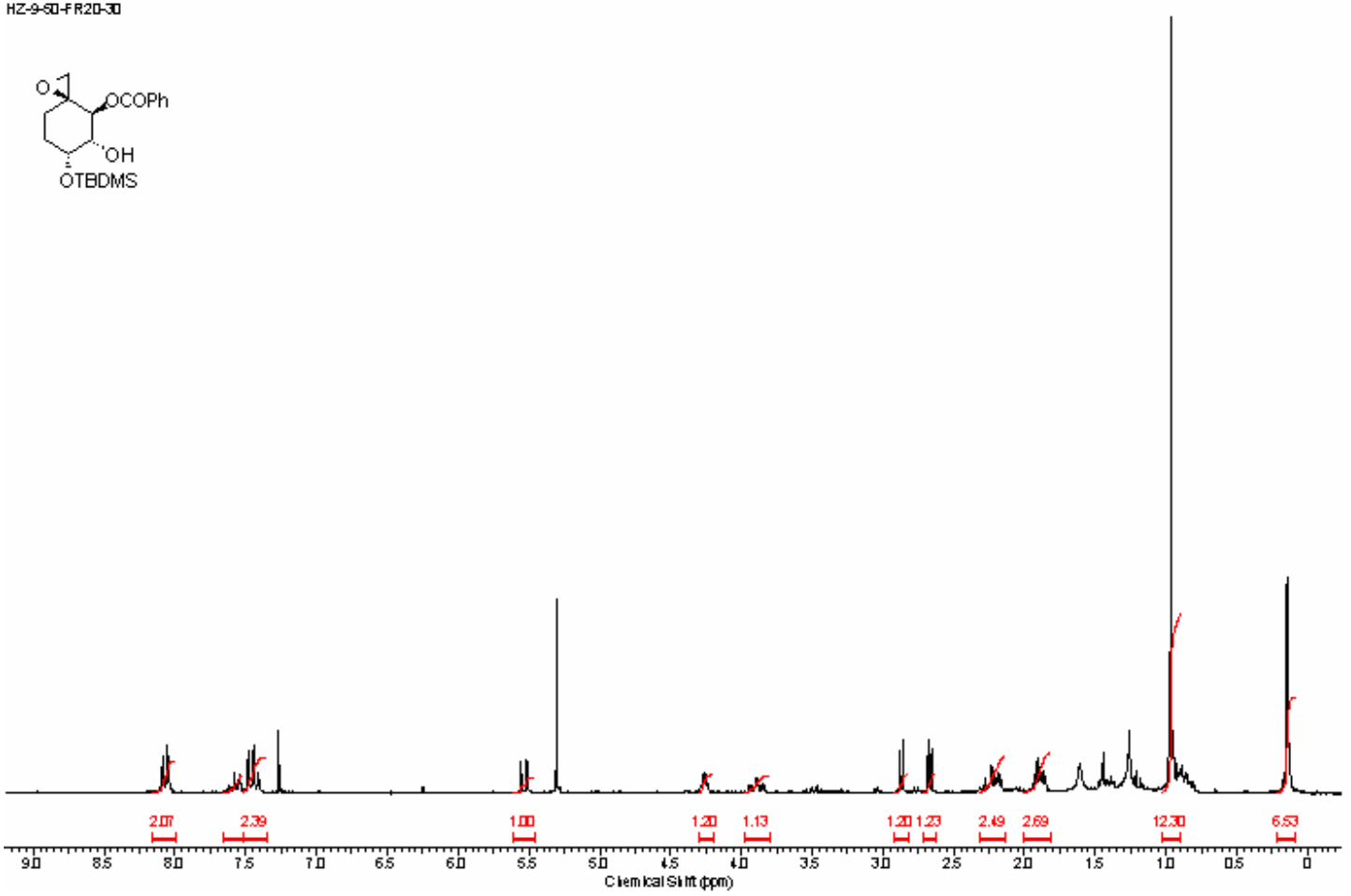
Acquisition Time (sec)	1.4976	Comment	13C OBSERVE	Date	May 28 2001	Date Stamp	May 28 2001
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-11-AC-13CNMR					Frequency (MHz)	50.29
Nucleus	13C	Number of Transients	640000	Original Points Count	16120	Points Count	32168
Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d		
Spectrum Offset (Hz)	4881.2285	Sweep Width (Hz)	12500.00	Temperature (degree C)	29.000		

HZ-9-11-AC-13CNMR



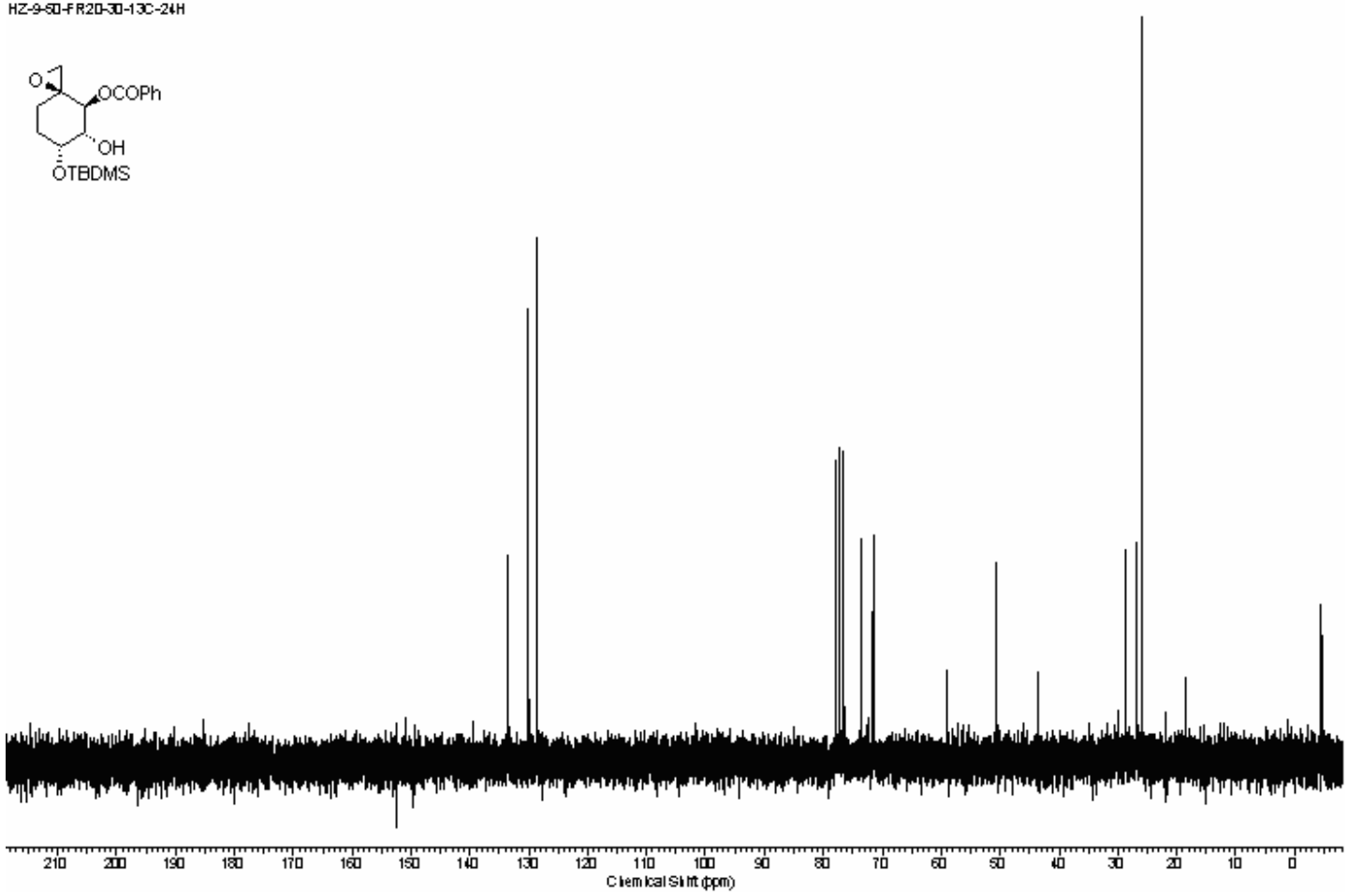
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Jun 22 2007
Date Stamp	Jun 22 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-60-FR20-30				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.2900	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-9-60-FR20-30



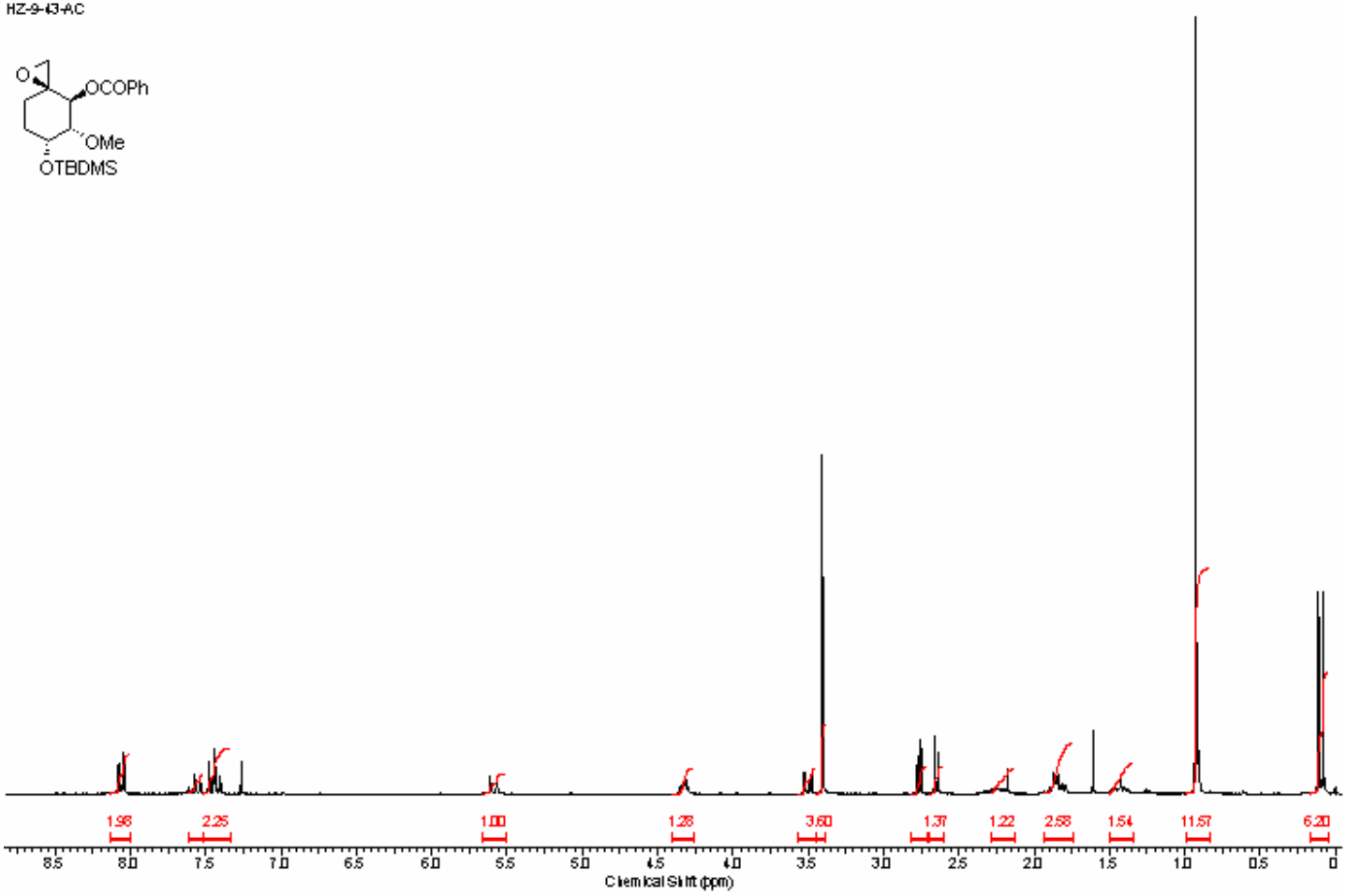
Acquisition Time (sec)	1.4976	Comment	13C OBSERVE	Date	Jun 22 2007	Date Stamp	Jun 22 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-50-FR20-30-13C-24H					Frequency (MHz)	50.29
Nucleus	13C	Number of Transients	64000000	Original Points Count	18720	Points Count	32768
Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d		
Spectrum Offset (Hz)	4881.2285	Sweep Width (Hz)	12500.00	Temperature (degree C)	29.000		

HZ-9-50-FR20-30-13C-24H



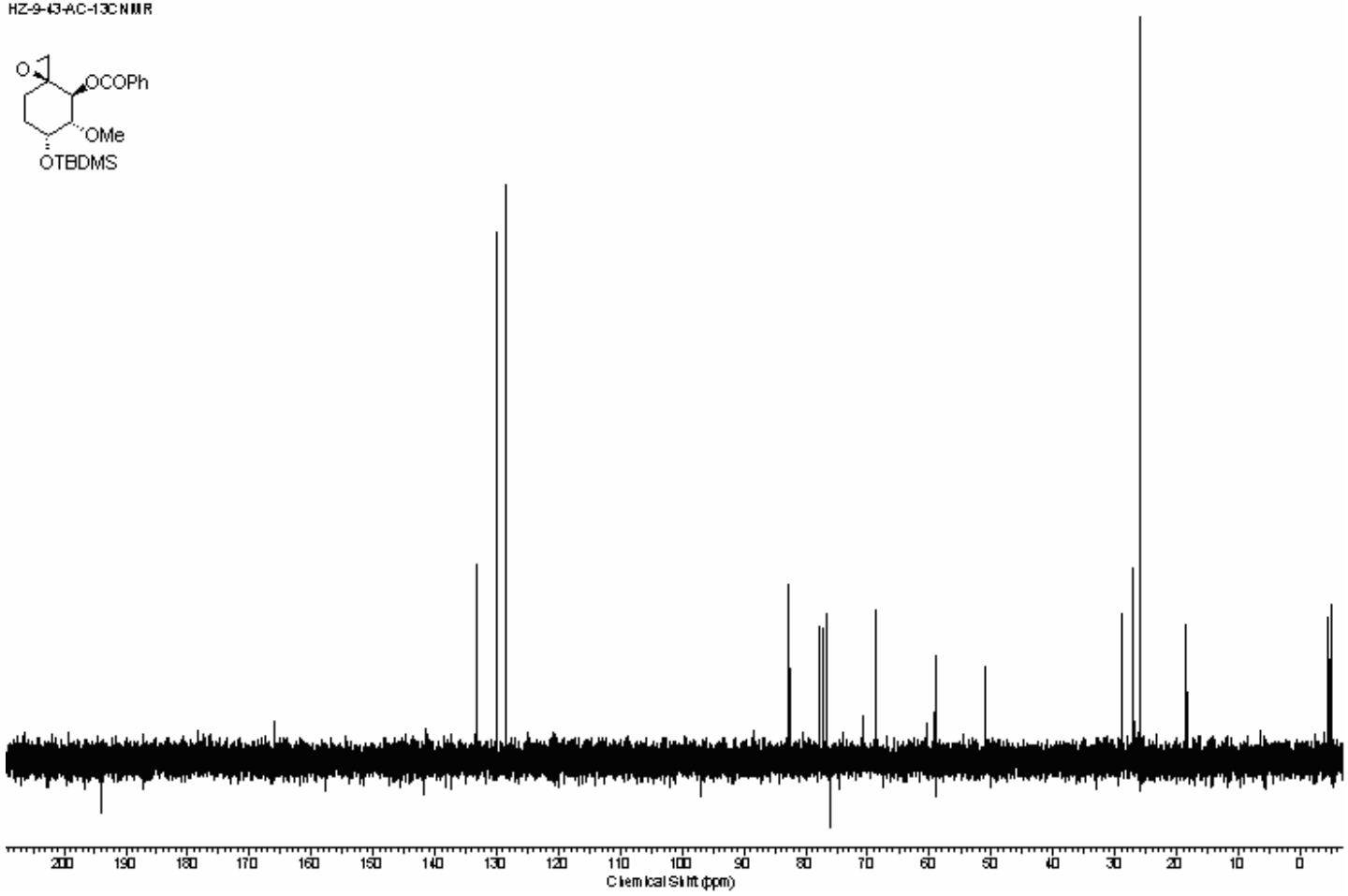
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	May 2 2007
Date Stamp	May 2 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-43-AC				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	24.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1000.8937	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-9-43-AC



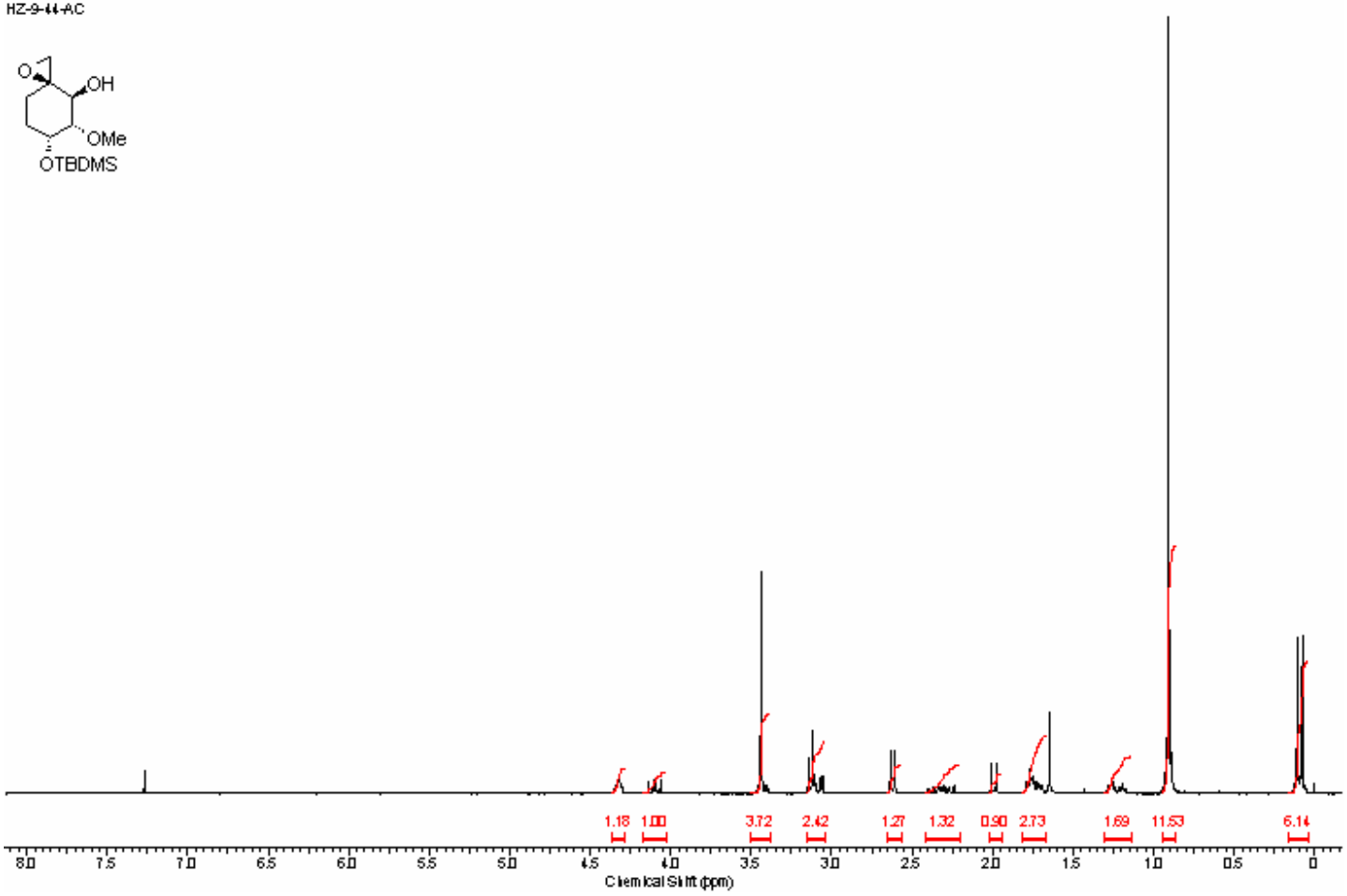
Acquisition Time (sec)	1.4976	Comment	13C OBSERVE	Date	May 2 2007	Date Stamp	May 2 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-43-AC-13C NMR					Frequency (MHz)	50.29
Nucleus	13C	Number of Transients	64000000	Original Points Count	18720	Points Count	32768
Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d		
Spectrum Offset (Hz)	4880.8467	Sweep Width (Hz)	12500.00	Temperature (degree C)	29.000		

HZ-9-43-AC-13C NMR



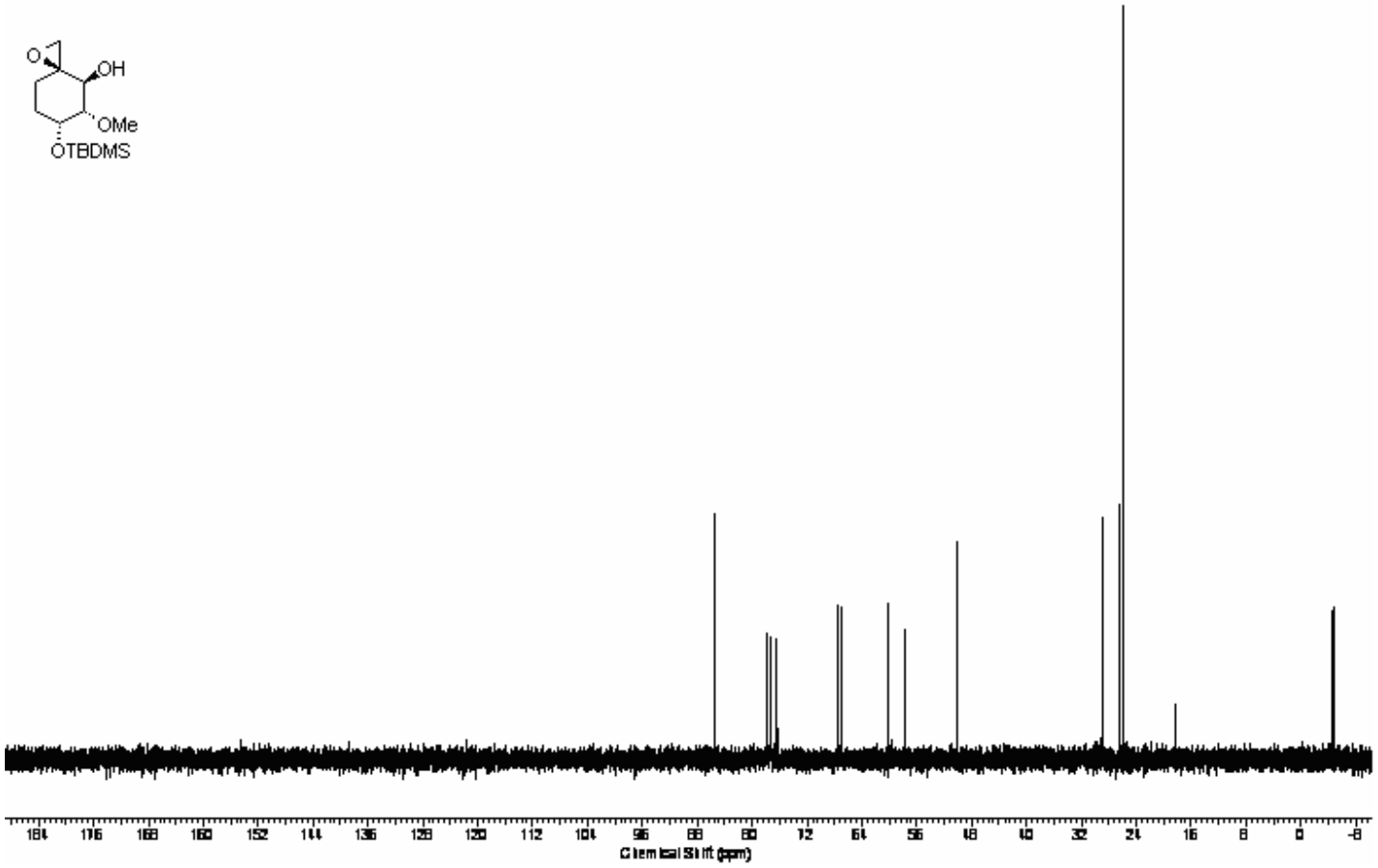
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Jun 4 2007
Date Stamp	Jun 4 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-44-AC				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	27.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.6563	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-9-44-AC



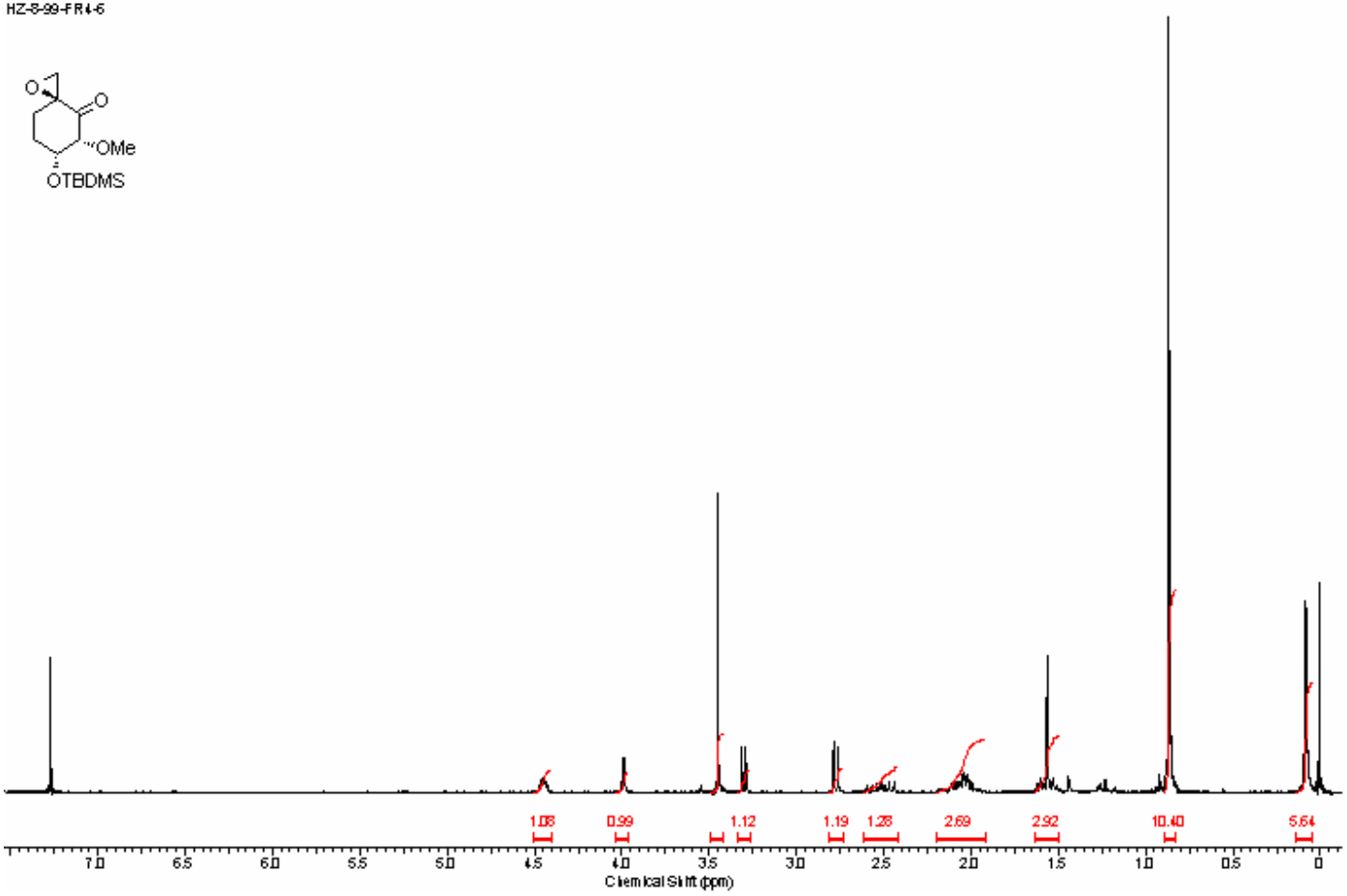
Acquisition Time (sec)	1.9316	Comment	13C OBSERVE	Date	Jul 4 2007	Date Stamp	Jul 4 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-44-AC-13CNMR					Frequency (MHz)	50.29
Nucleus	13C	Number of Transients	640000	Original Points Count	16120	Points Count	32768
Pulse Sequence	s2pul	Receiver Gain	4.000	Solvent	CHLOROFORM-d		
Spectrum Offset (Hz)	4881.2285	Sweep Width (Hz)	12500.00	Temperature (degree C)	29.000		

HZ-9-44-AC-13CNMR



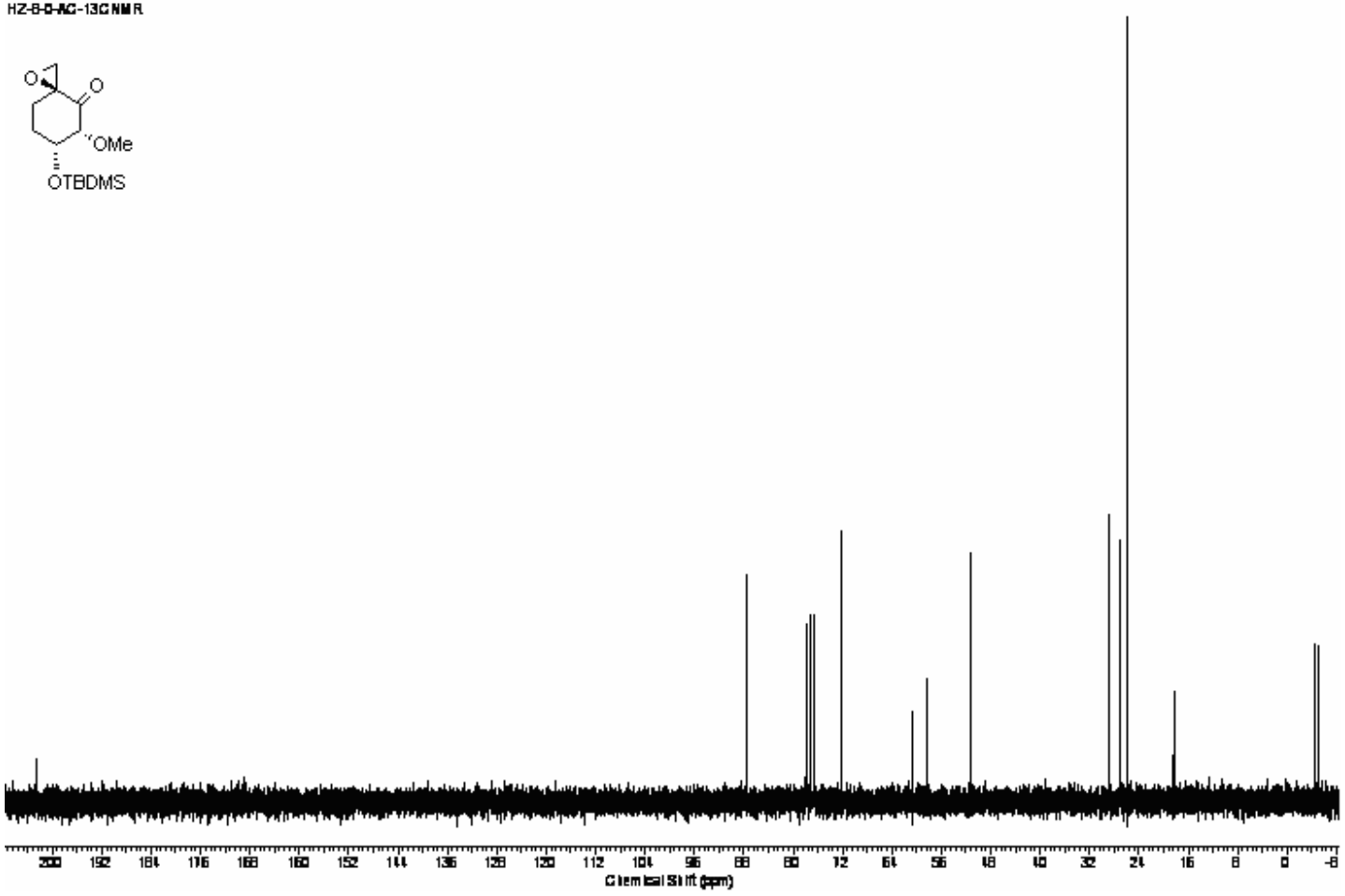
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Apr 13 2007
Date Stamp	Apr 13 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-8\HZ-8-99-FR4-6				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	3900	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.6563	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-8-99-FR4-6



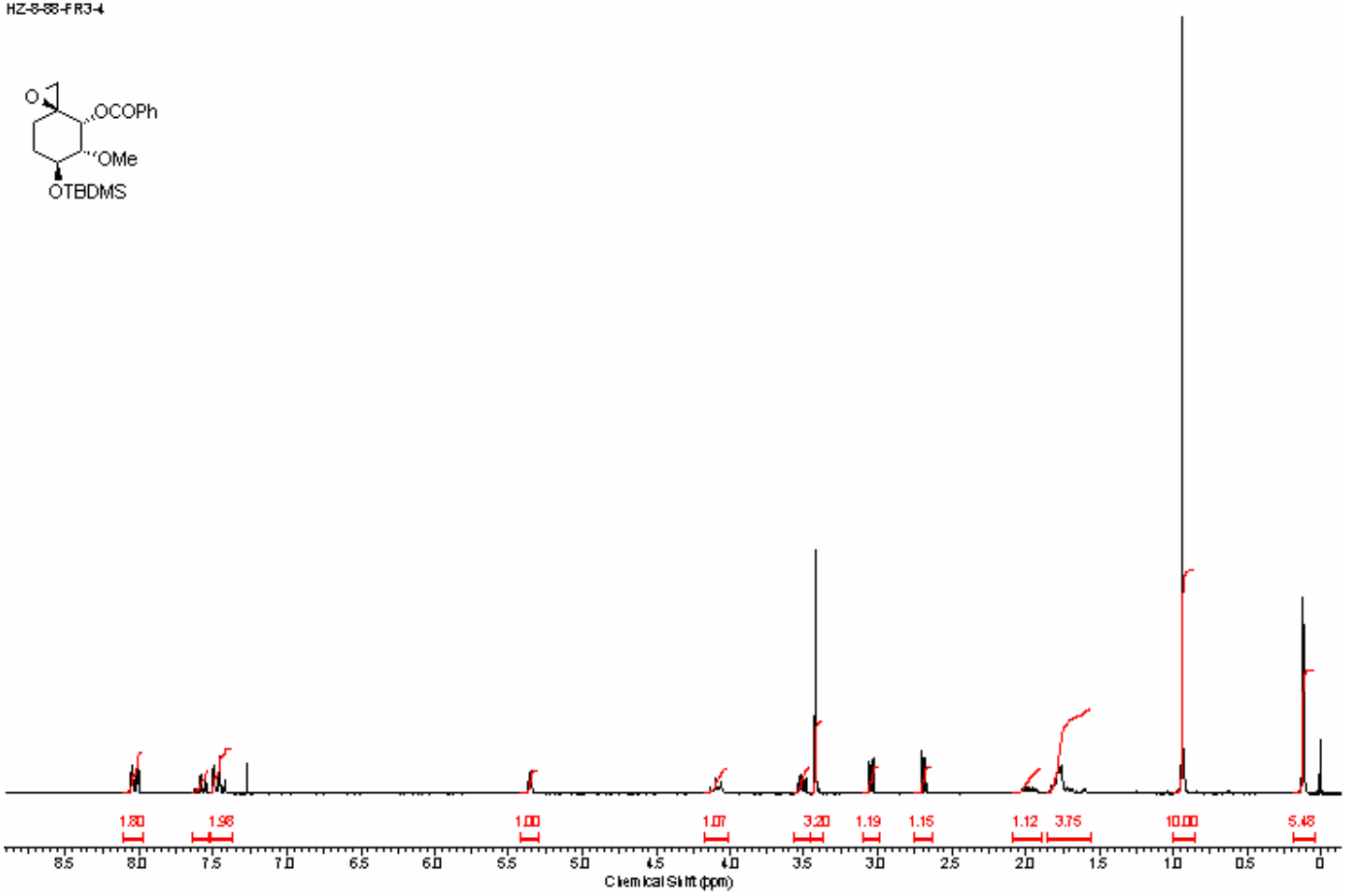
Acquisition Time (sec)	1.9316	Comment	13C OBSERVE			Date	Apr 15 2007		
Date Stamp	Apr 15 2007			File Name	E:\HZ-8-0-AC-13C.NMR			Frequency (MHz)	50.29
Nucleus	13C	Number of Transients	640000	Original Points Count	16120	Points Count	32768	Pulse Sequence	s2pul
Receiver Gain	4000	Solvent	CHLOROFORM-d			Spectrum Offset (Hz)	6881.2285	Sweep Width (Hz)	12500.00
Temperature (degree C)	29.000								

HZ-8-0-AC-13C.NMR



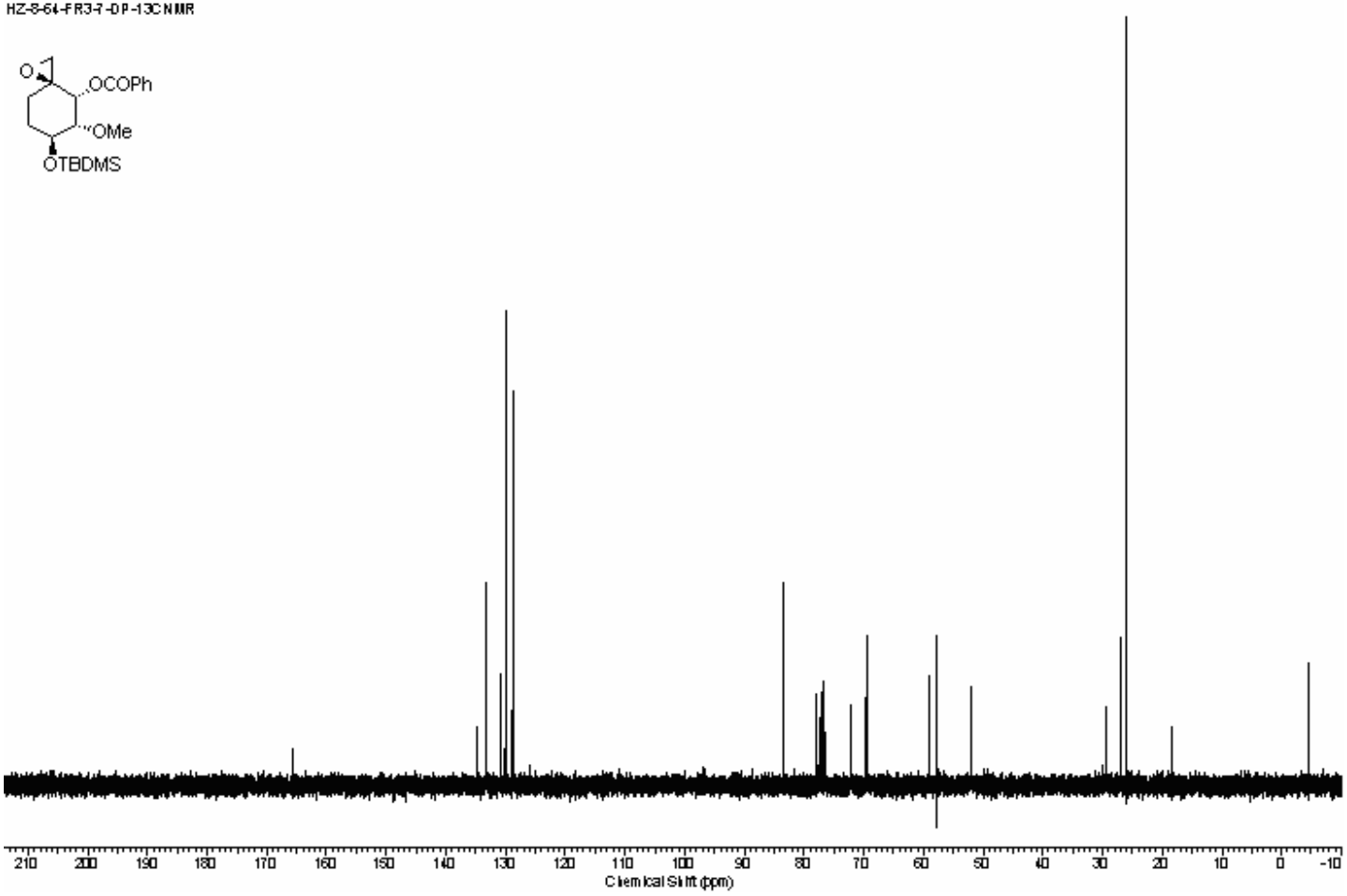
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Apr 6 2007
Date Stamp	Apr 6 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-S\HZ-S-88-FR3-4				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	28.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.6563	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-S-88-FR3-4



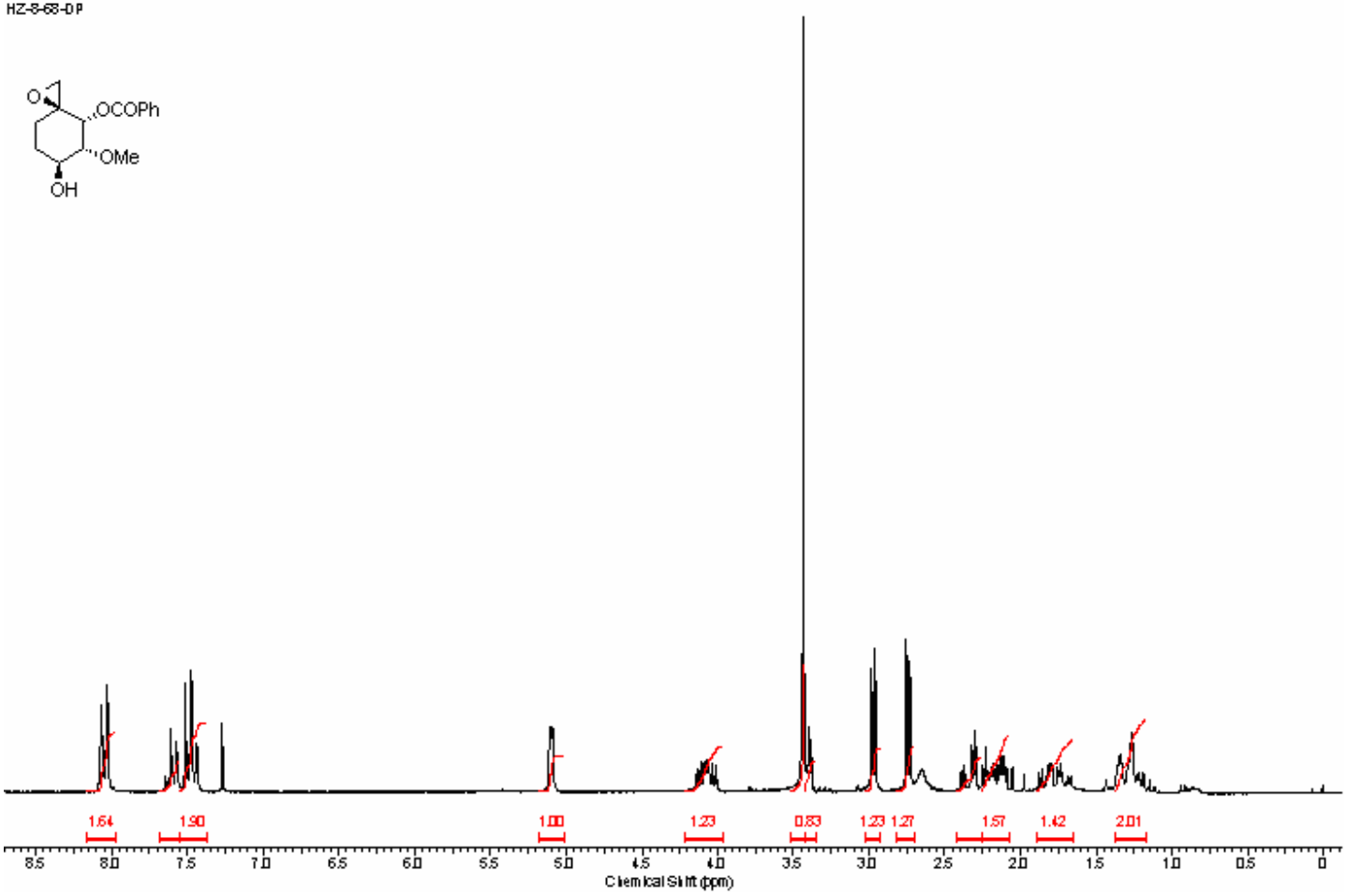
Acquisition Time (sec)	1.4976	Comment	13C OBSERVE	Date	Mar 17 2007	Date Stamp	Mar 17 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-S\HZ-8-64-FR3-7-DP-13C NMR					Frequency (MHz)	50.29
Nucleus	13C	Number of Transients	64000	Original Points Count	18720	Points Count	32768
Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d		
Spectrum Offset (Hz)	4880.4648	Sweep Width (Hz)	12500.00	Temperature (degree C)	29.000		

HZ-8-64-FR3-7-DP-13C NMR

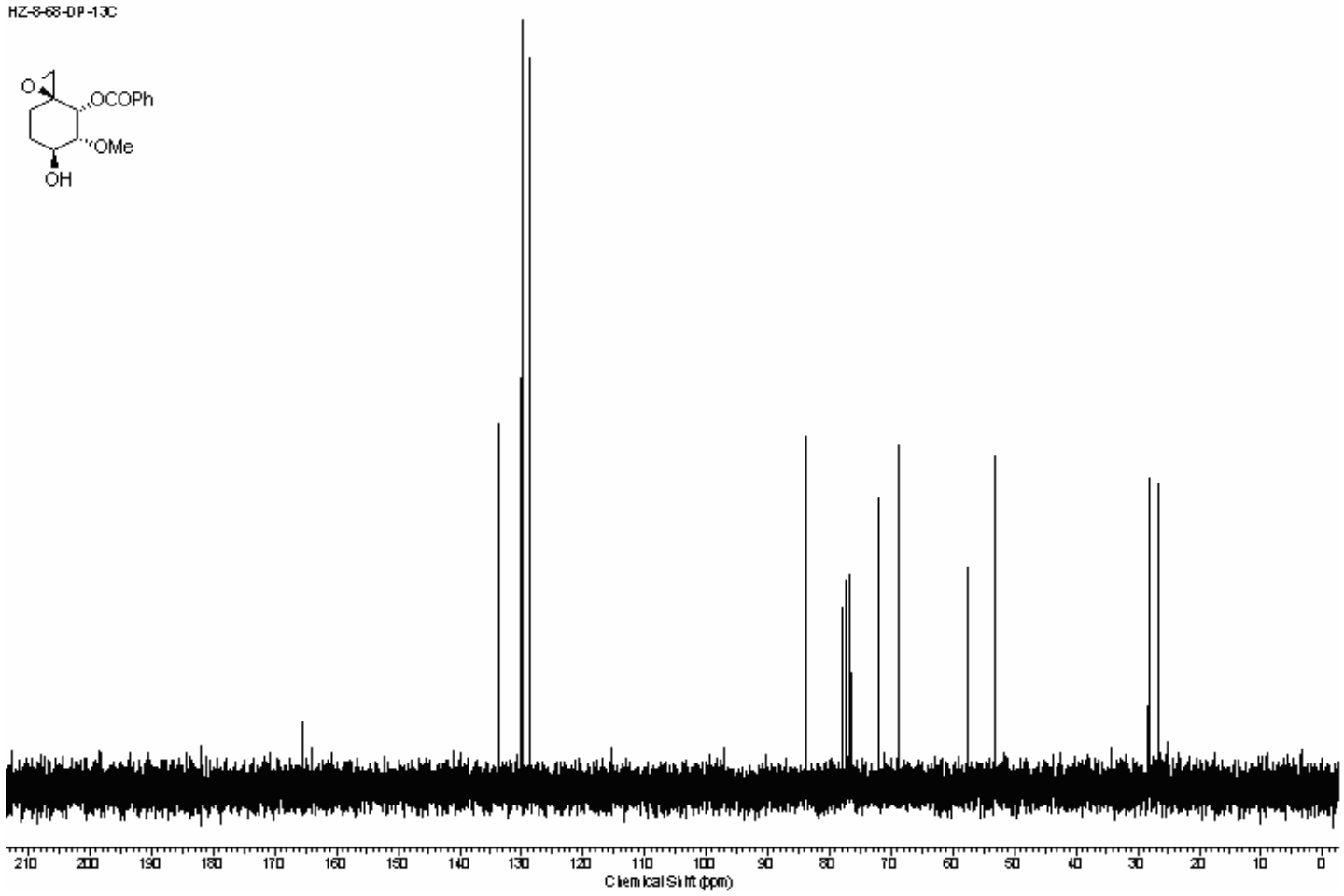


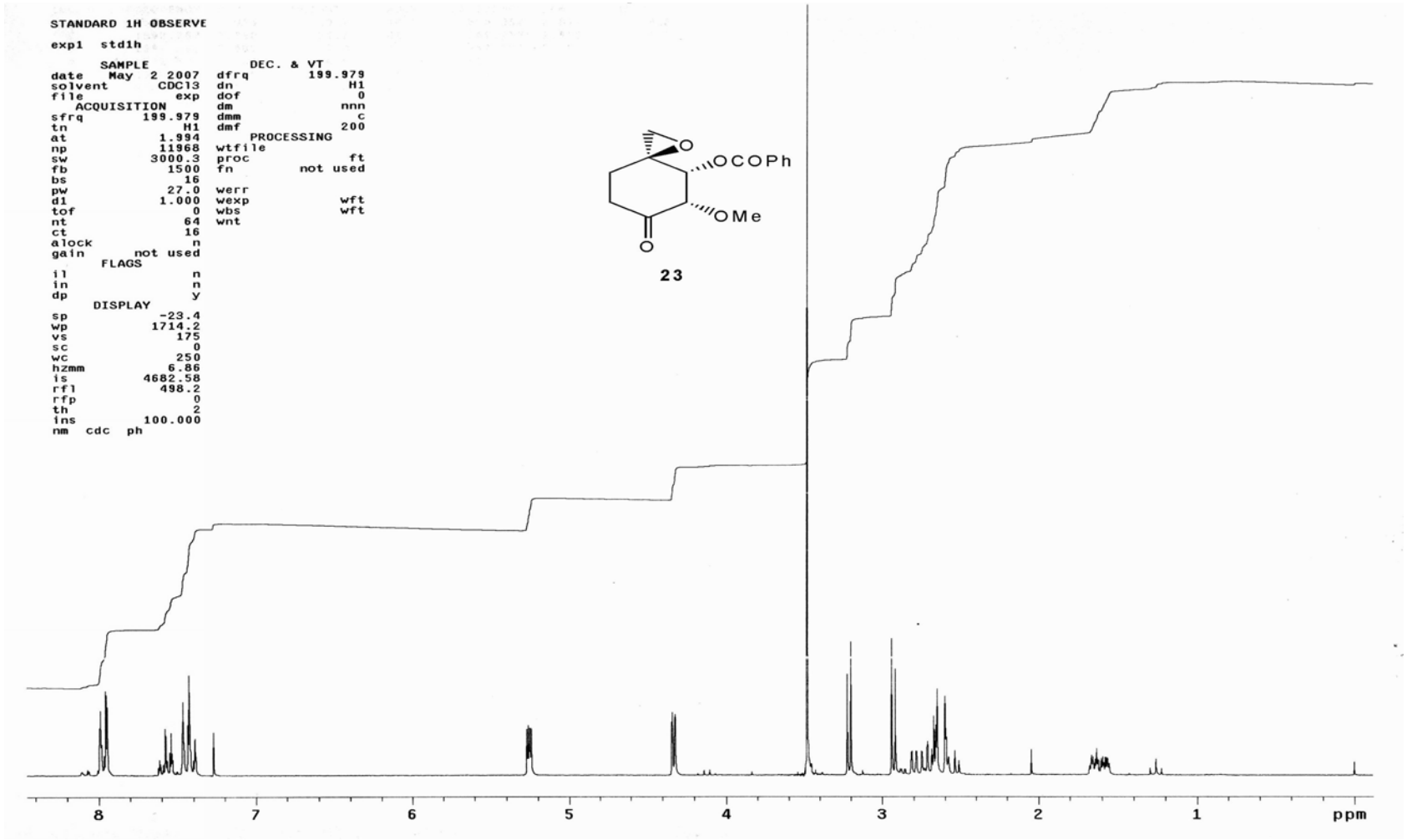
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Mar 26 2007
Date Stamp	Mar 26 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-S\HZ-S-68-DP				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	28.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1002.0226	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-S-68-DP



Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Mar 26 2007	*Date Stamp*	Mar 26 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-8\HZ-8-68-DP-13C					*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	64000000	*Original Points Count*	18720	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	4880.8467	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

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date May 2 2007
solvent CDCl3
file exp
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fb 1500
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pw 27.0
d1 1.000
tof 0
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ct 16
alock n
gain not used
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in n
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wp 1714.2
vs 175
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is 4682.58
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DEC. & VT
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dof 0
dm nnn
dmm c
dmf 200
PROCESSING
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proc ft
fn not used
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wexp wft
wbs wft
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4
3
2
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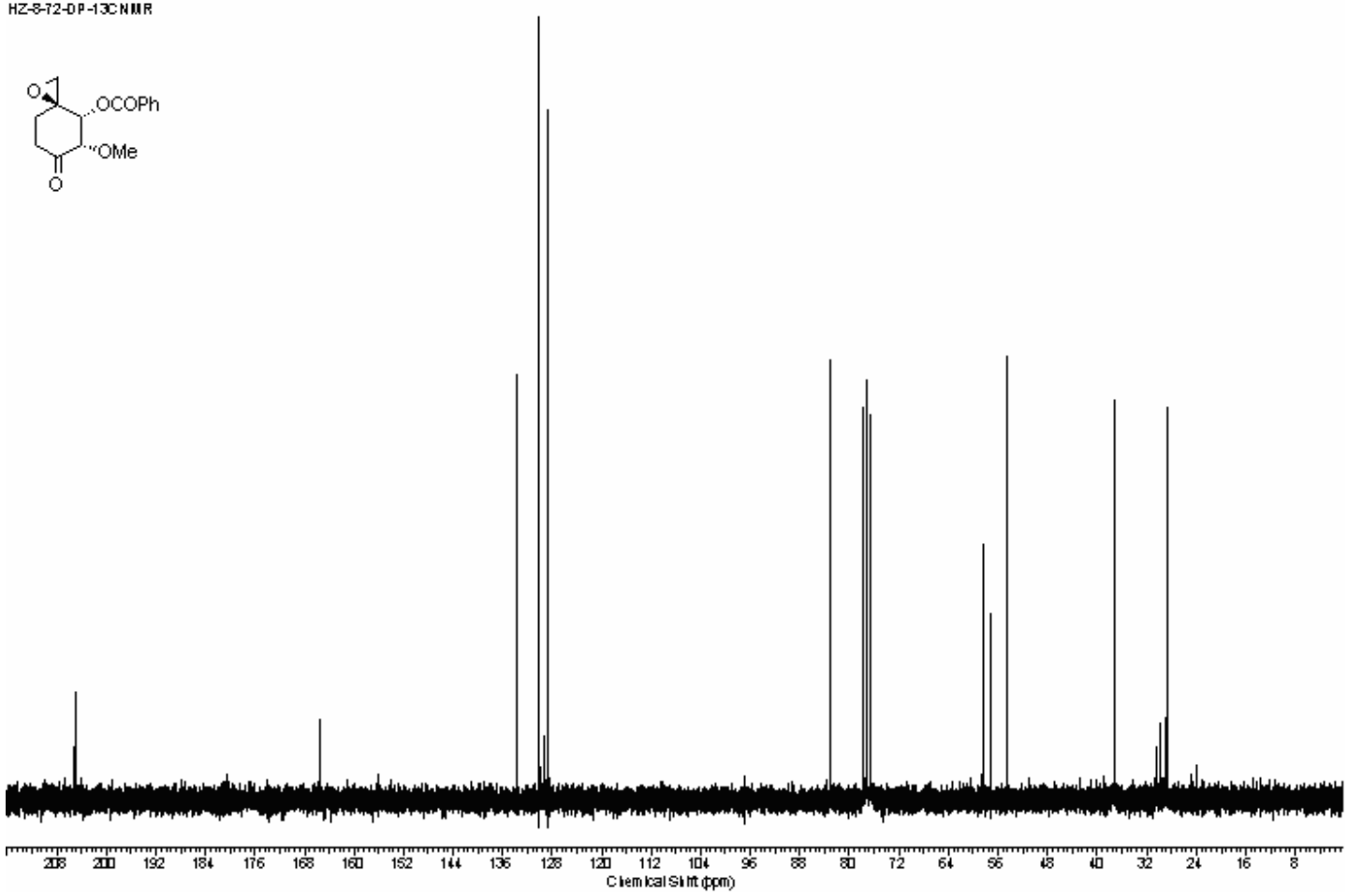
ppm

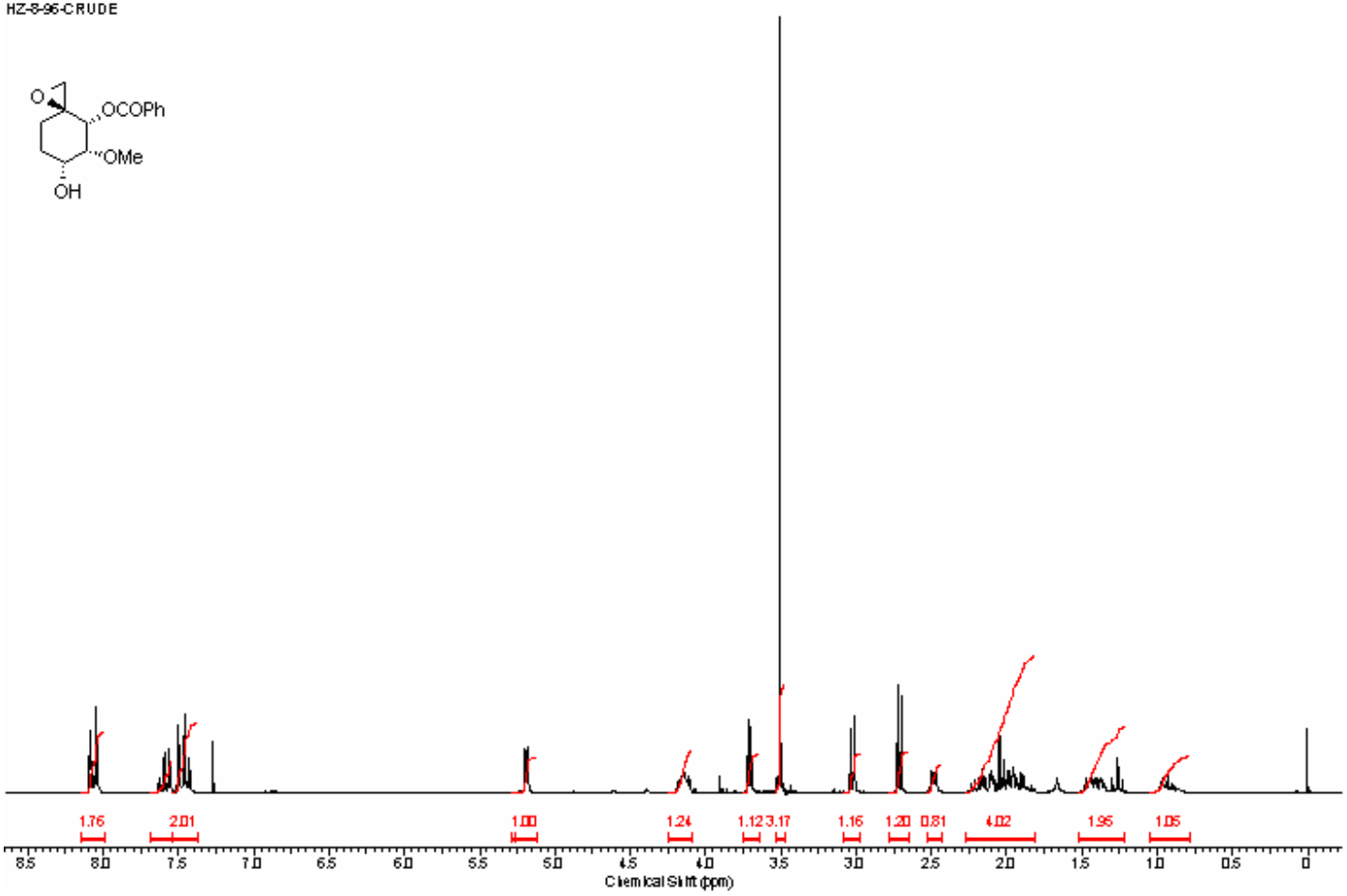
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Mar 31 2007	*Date Stamp*	Mar 31 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-S\HZ-S-72-DP-13C NMR					*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	6400.00	*Original Points Count*	16720	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	4881.2285	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-S-72-DP-13C NMR



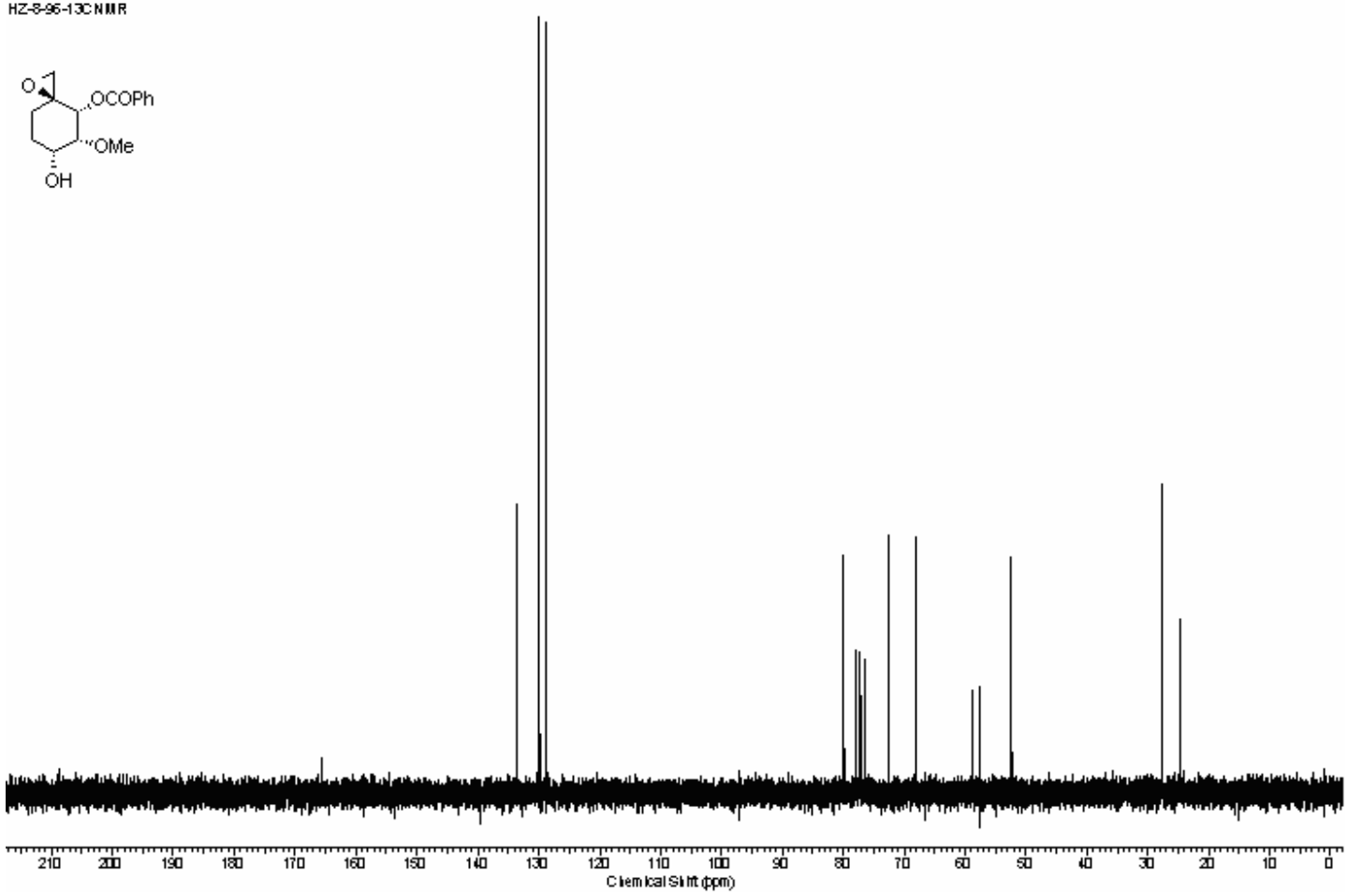
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Apr 11 2007
Date Stamp	Apr 11 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-S\HZ-S-96-CRUDE				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	28.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.6563	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-S-96-CRUDE



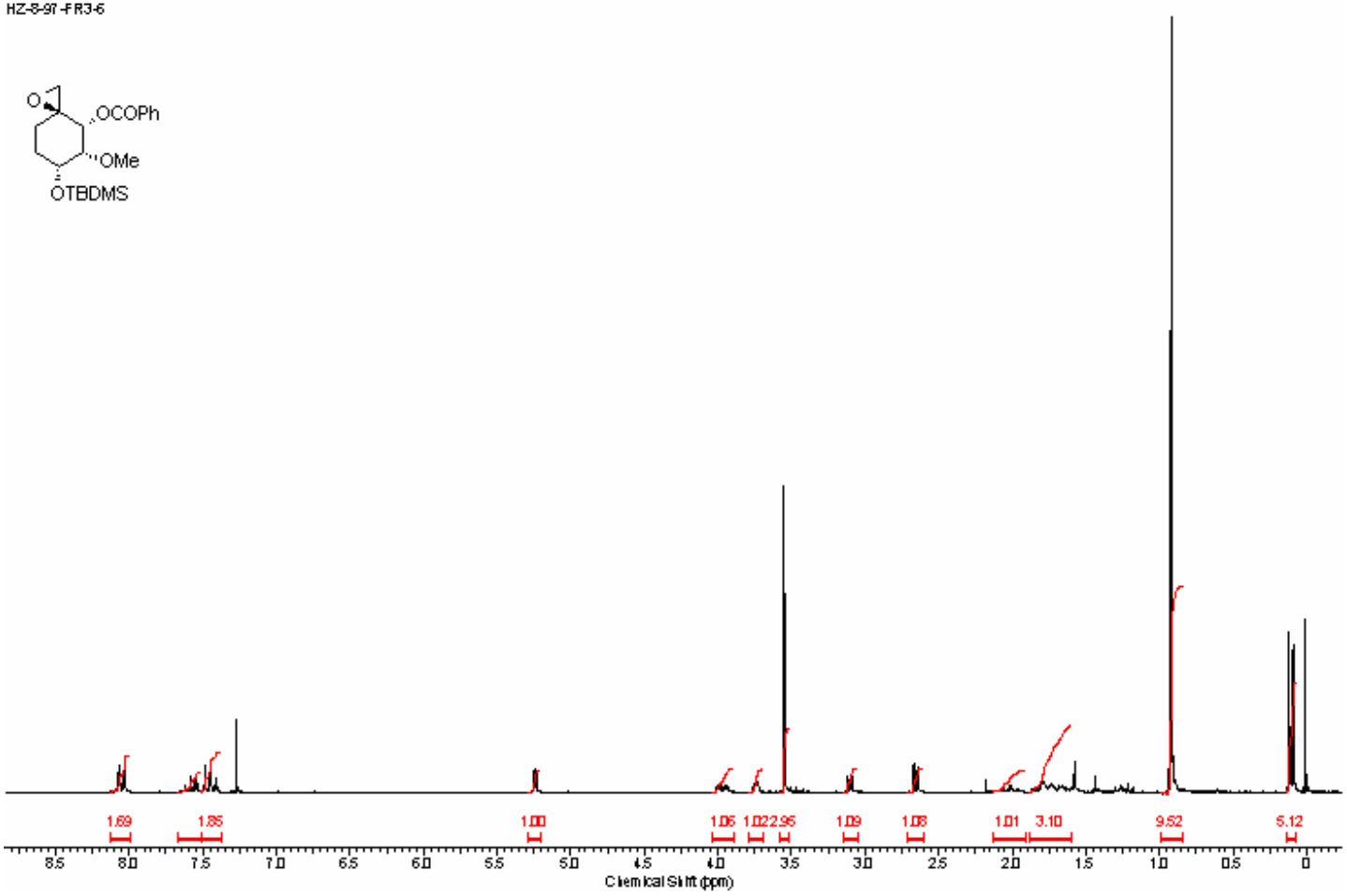
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Apr 11 2007	*Date Stamp*	Apr 11 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-S\HZ-S-96-13CNMR					*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	640000	*Original Points Count*	18720	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	4880.8467	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-S-96-13CNMR



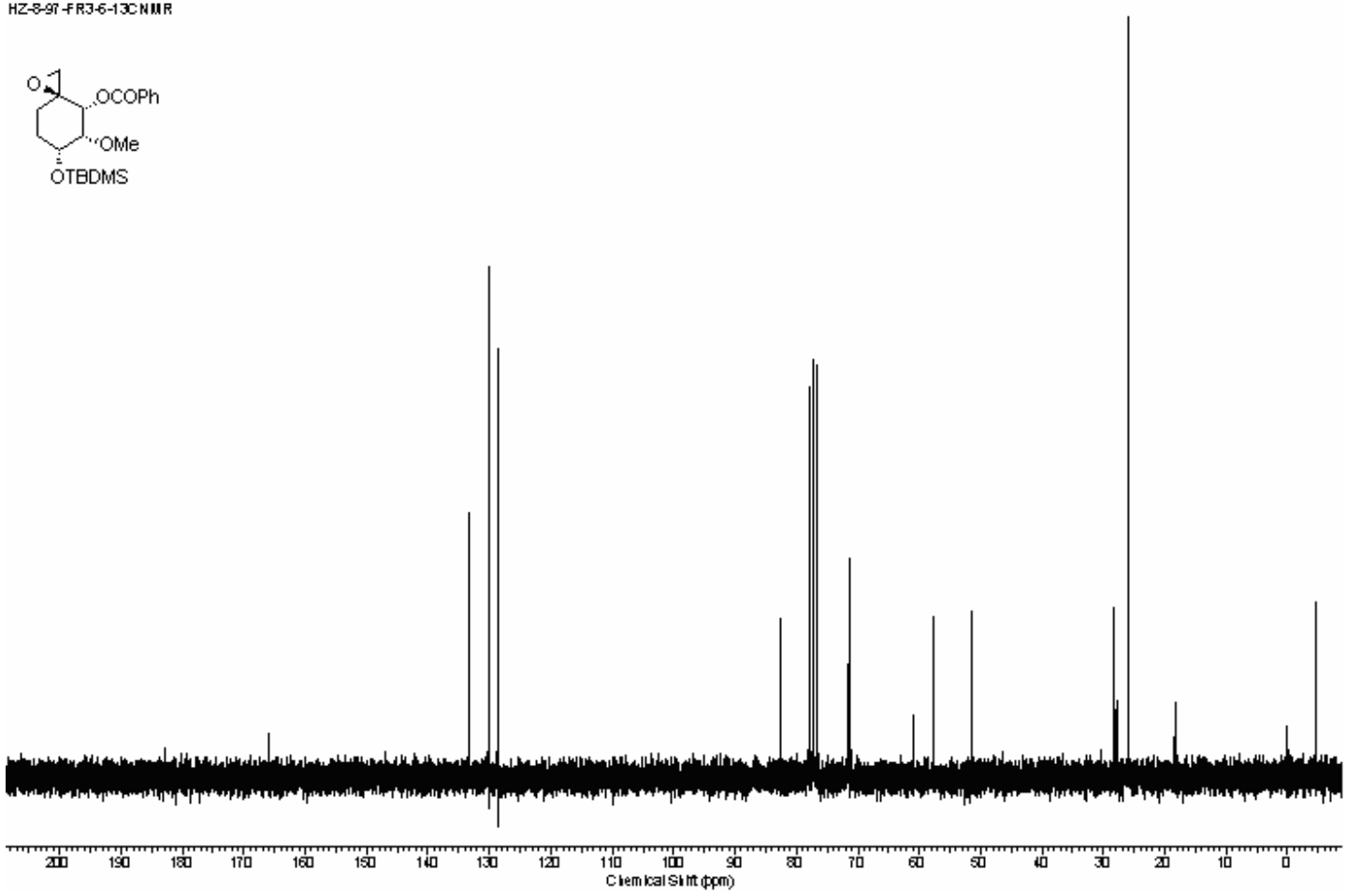
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Apr 12 2007
Date Stamp	Apr 12 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-8\HZ-8-97-FR3-6				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	34.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.6563	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-8-97-FR3-6



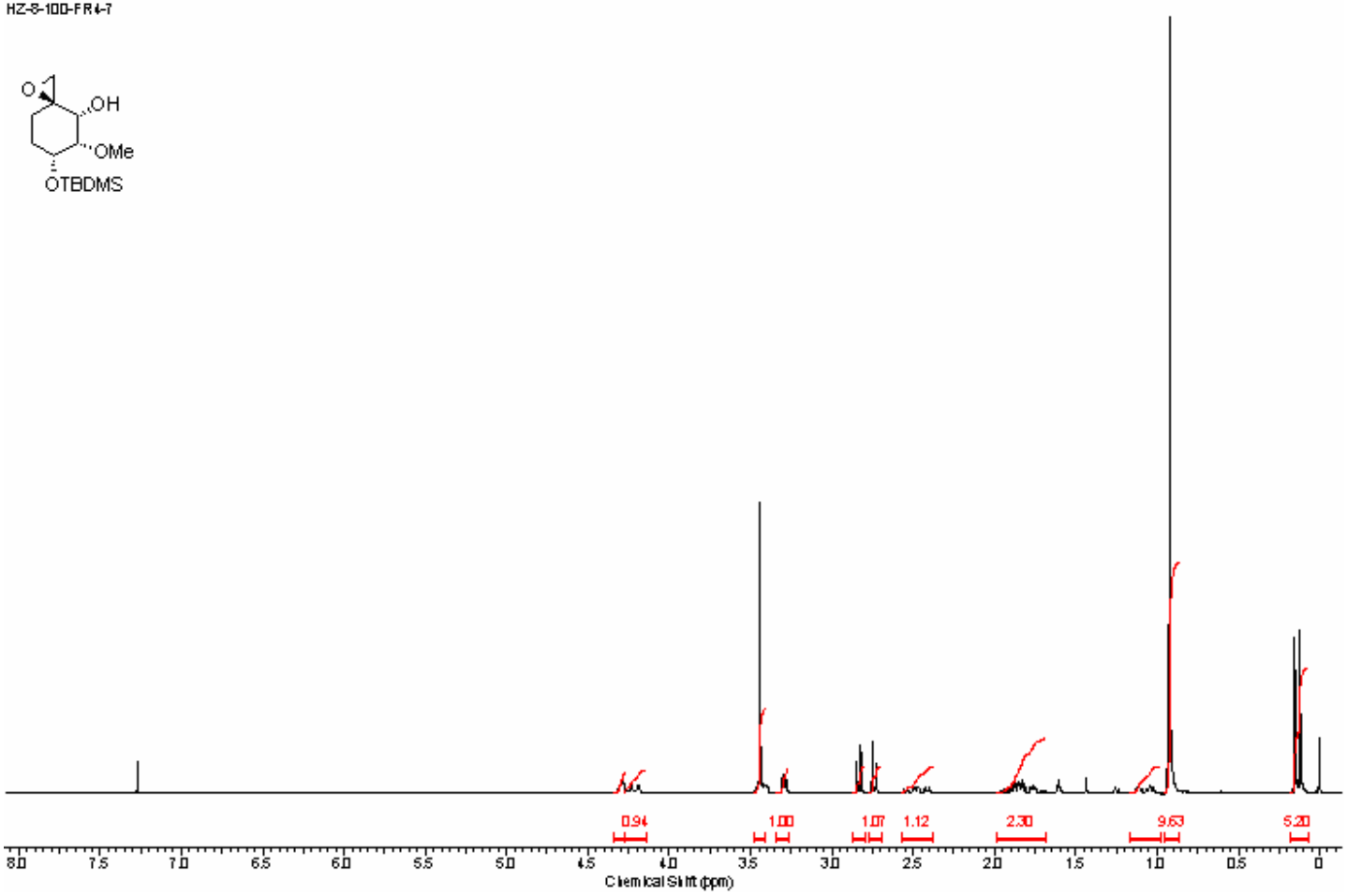
Acquisition Time (sec)	1.4976	Comment	13C OBSERVE	Date	Apr 13 2007	Date Stamp	Apr 13 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-S\HZ-S-97-FR3-6-13C NMR					Frequency (MHz)	50.29
Nucleus	13C	Number of Transients	640000	Original Points Count	18720	Points Count	32768
Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d		
Spectrum Offset (Hz)	4881.2236	Sweep Width (Hz)	12500.00	Temperature (degree C)	29.000		

HZ-S-97-FR3-6-13C NMR



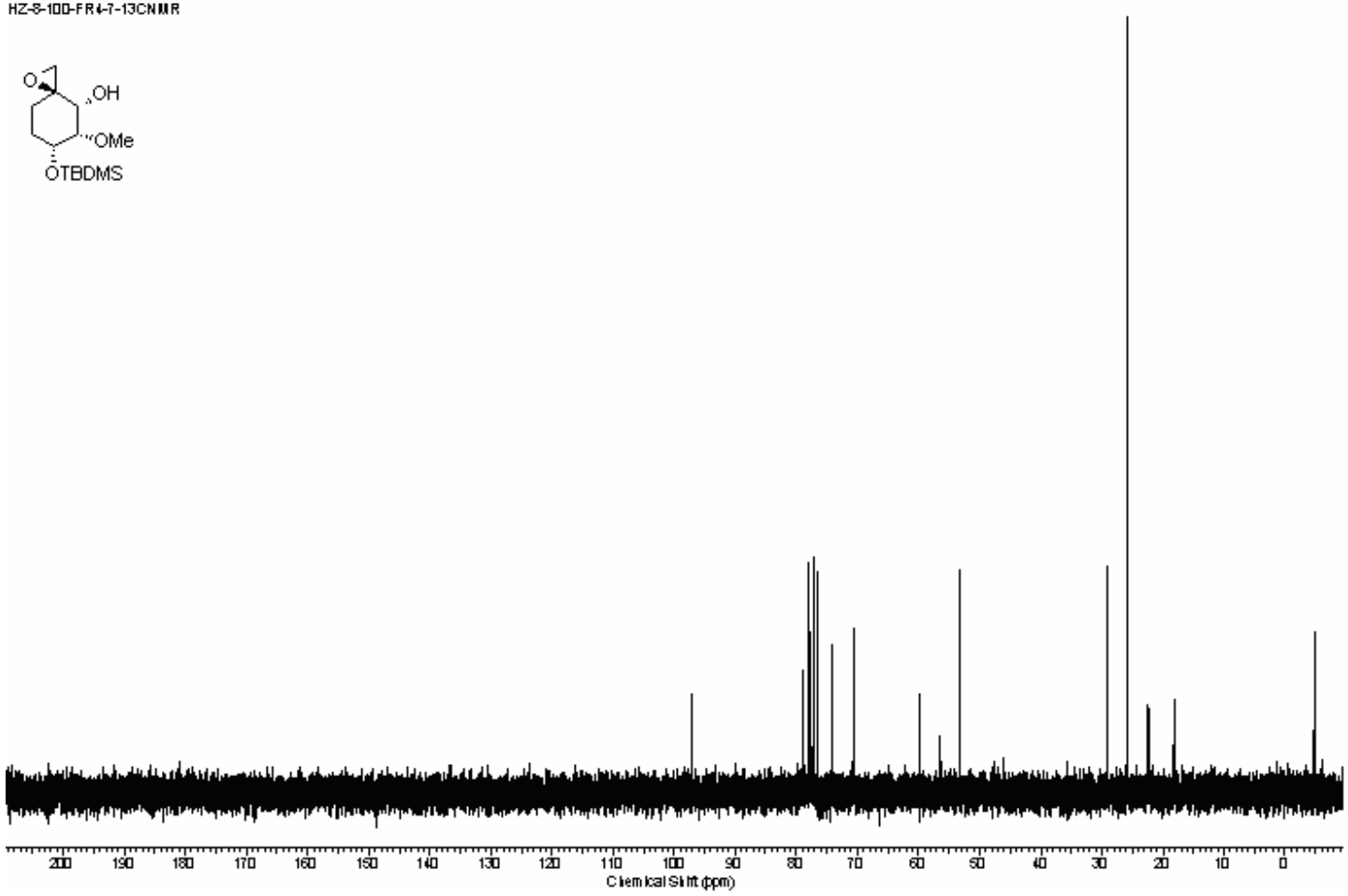
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Apr 14 2007
Date Stamp	Apr 14 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-8\HZ-8-100-FR4-7				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	28.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.6564	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-8-100-FR4-7



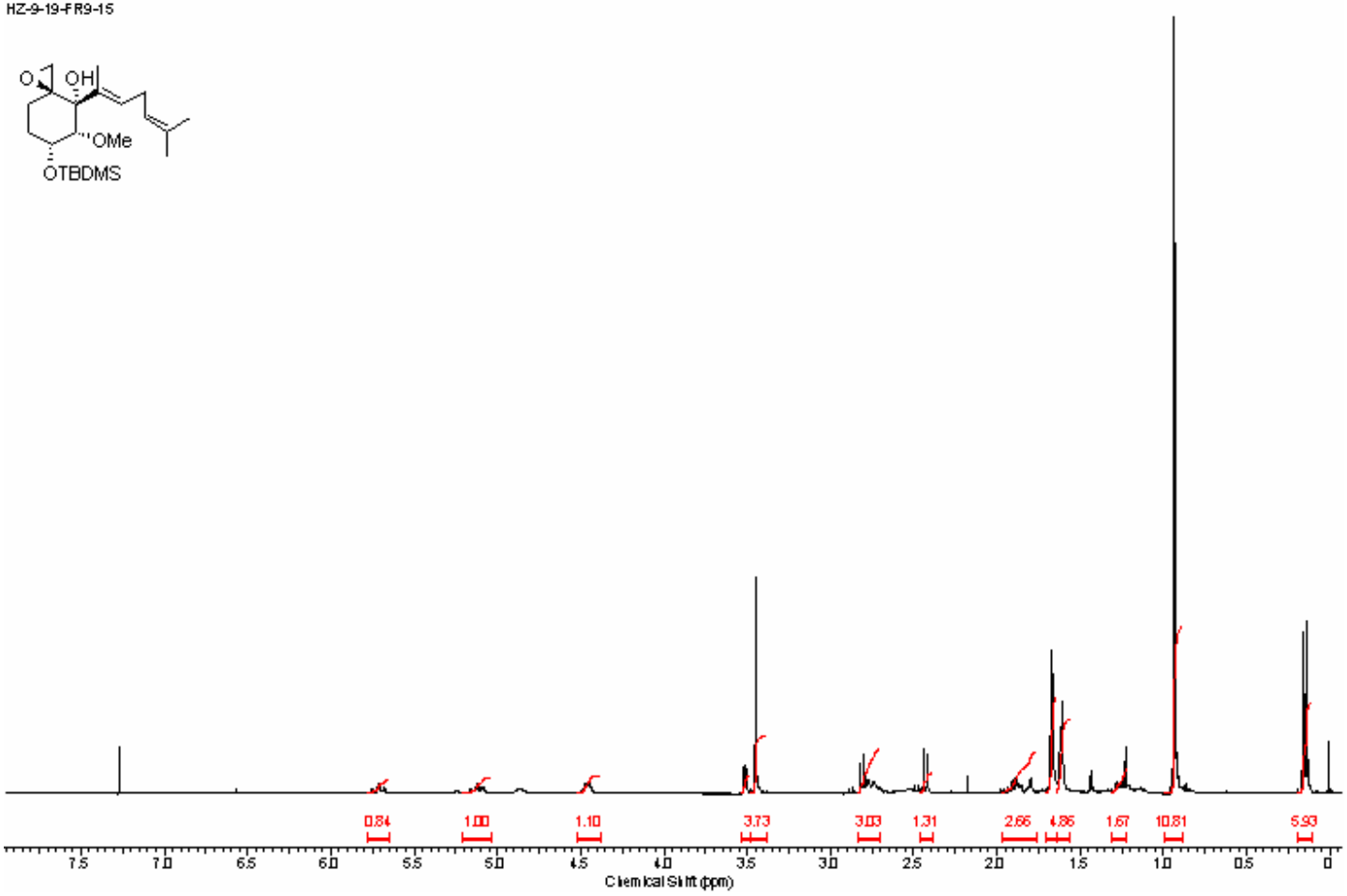
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Apr 14 2007	*Date Stamp*	Apr 14 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\MY DOCUMENTS\HZ-8\HZ-8-100-FR4-7-13CNMR					*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	6400000	*Original Points Count*	18720	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	4881.2241	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-8-100-FR4-7-13CNMR



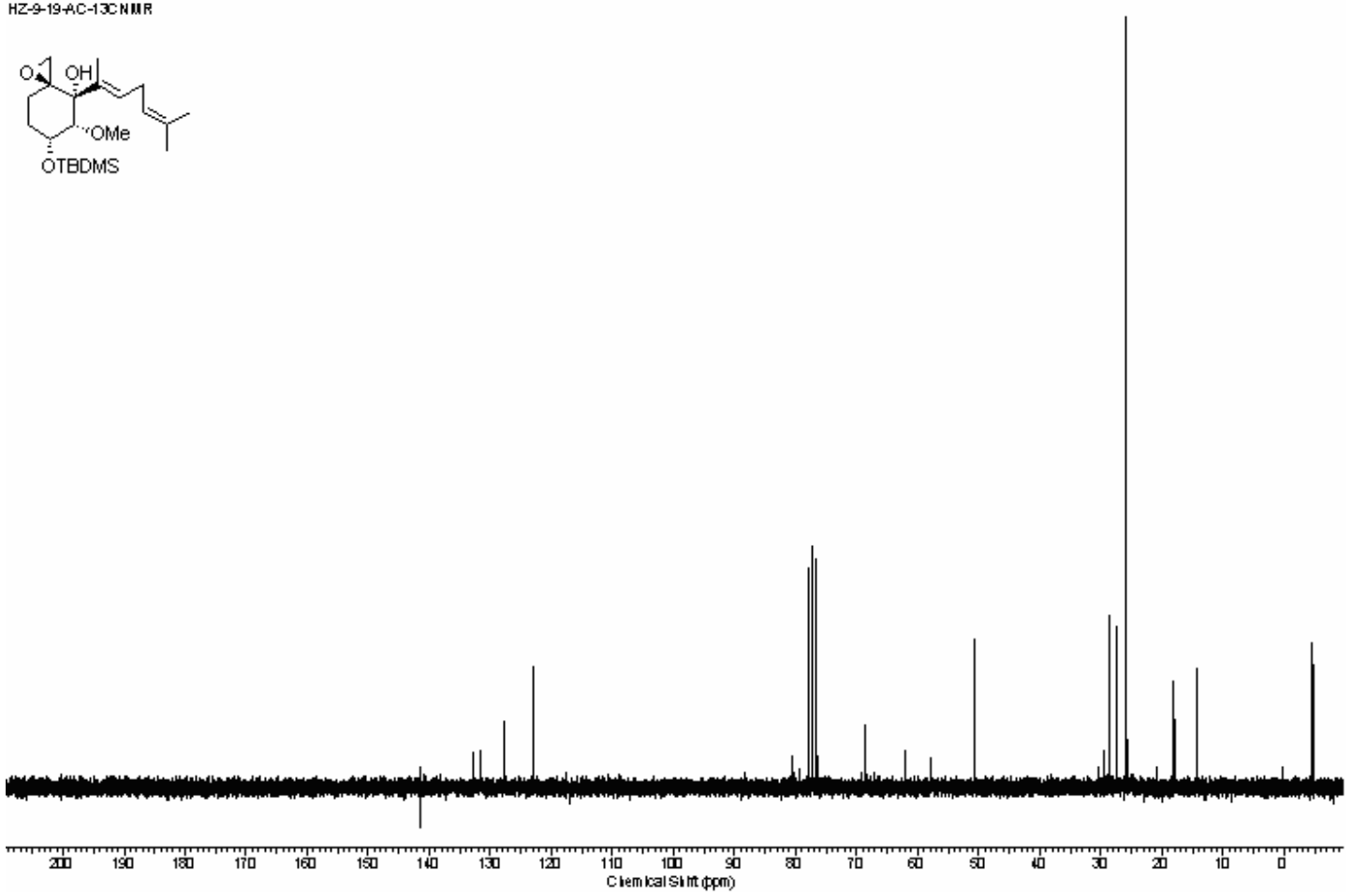
Acquisition Time (sec)	1.9947	Comment	STANDARD 1H OBSERVE			Date	May 8 2007
Date Stamp	May 8 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-19-FR9-15				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	22.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.7015	Sweep Width (Hz)	3000.00	Temperature (degree C)	29.000		

HZ-9-19-FR9-15



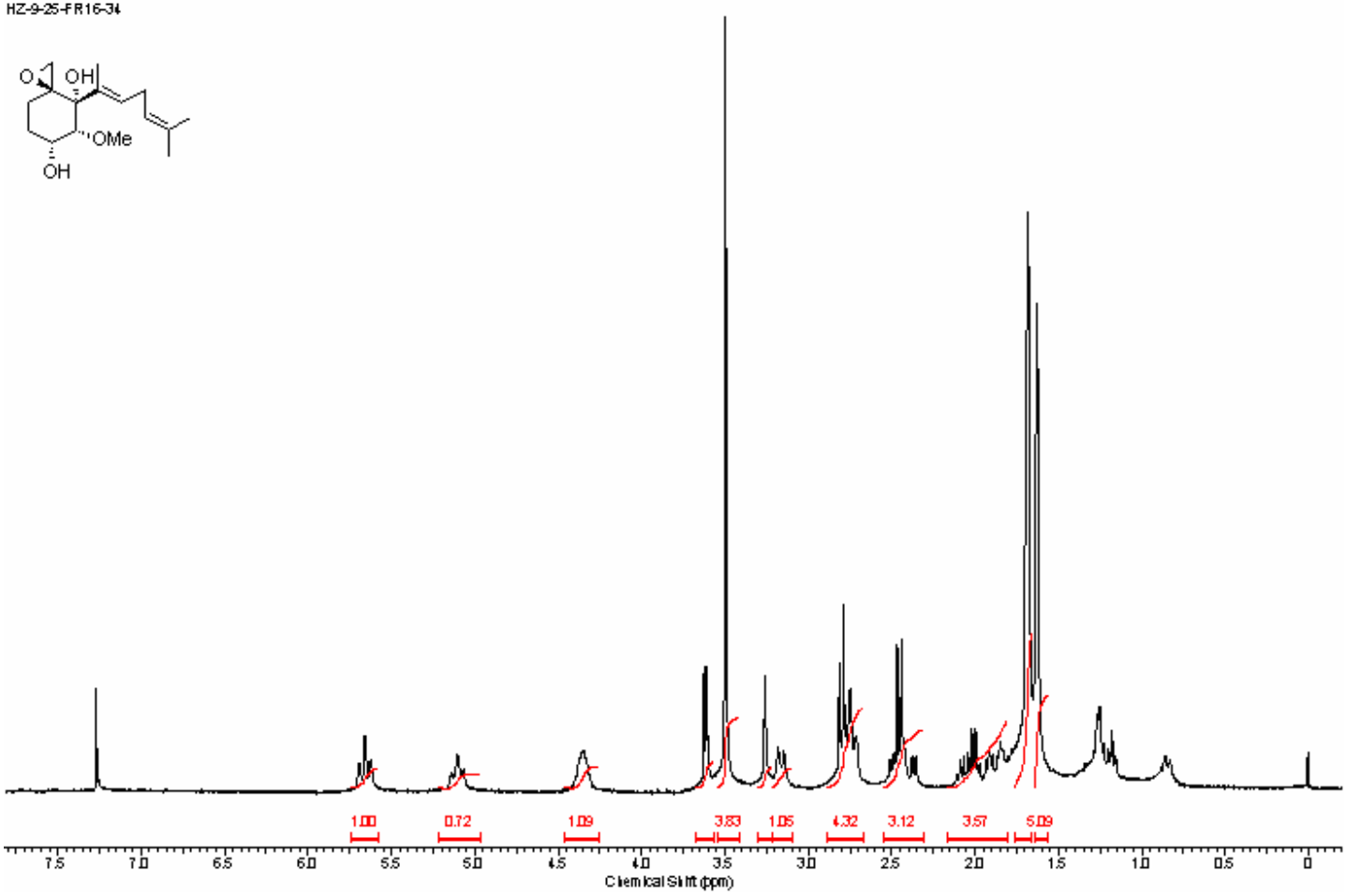
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	May 9 2007	*Date Stamp*	May 9 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-19-AC-13C NMR					*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	64000.00	*Original Points Count*	16120	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	4880.8467	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-9-19-AC-13C NMR



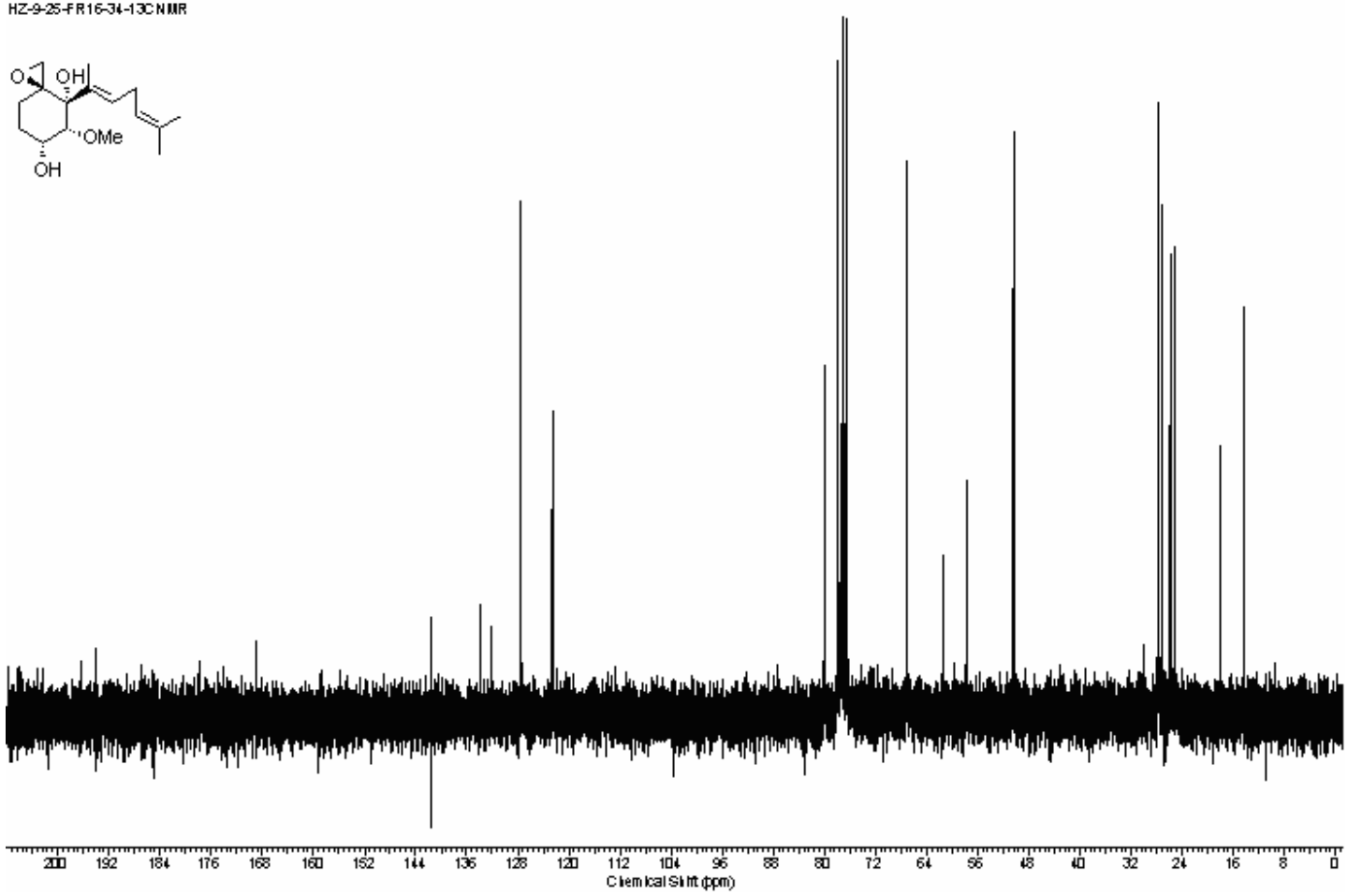
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	May 10 2007
Date Stamp	May 10 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-25-FR16-34				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	28.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1002.0226	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-9-25-FR16-34



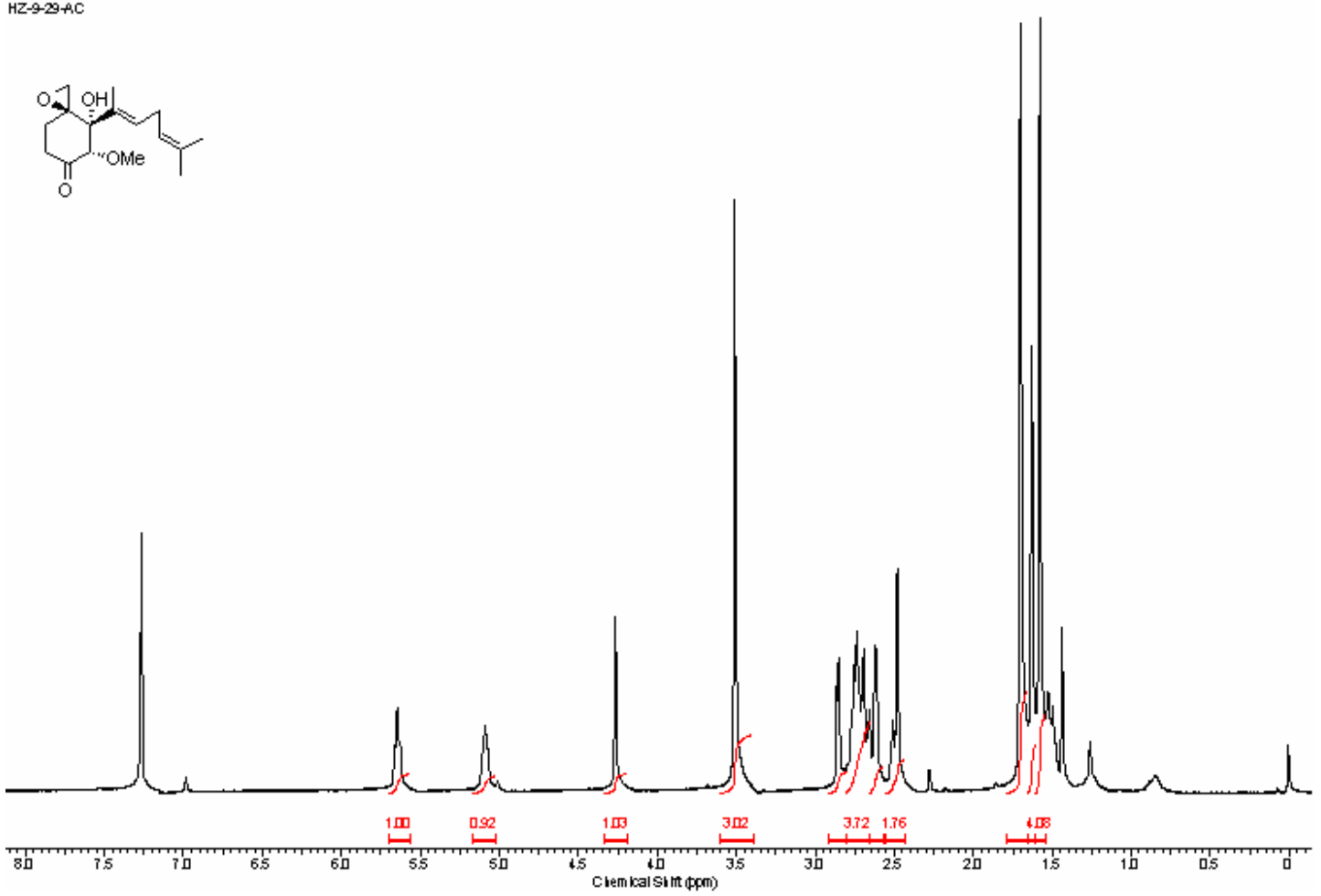
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	May 10 2007	*Date Stamp*	May 10 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-25-FR16-34-13C NMR					*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	64000000	*Original Points Count*	18720	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	4882.3730	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-9-25-FR16-34-13C NMR



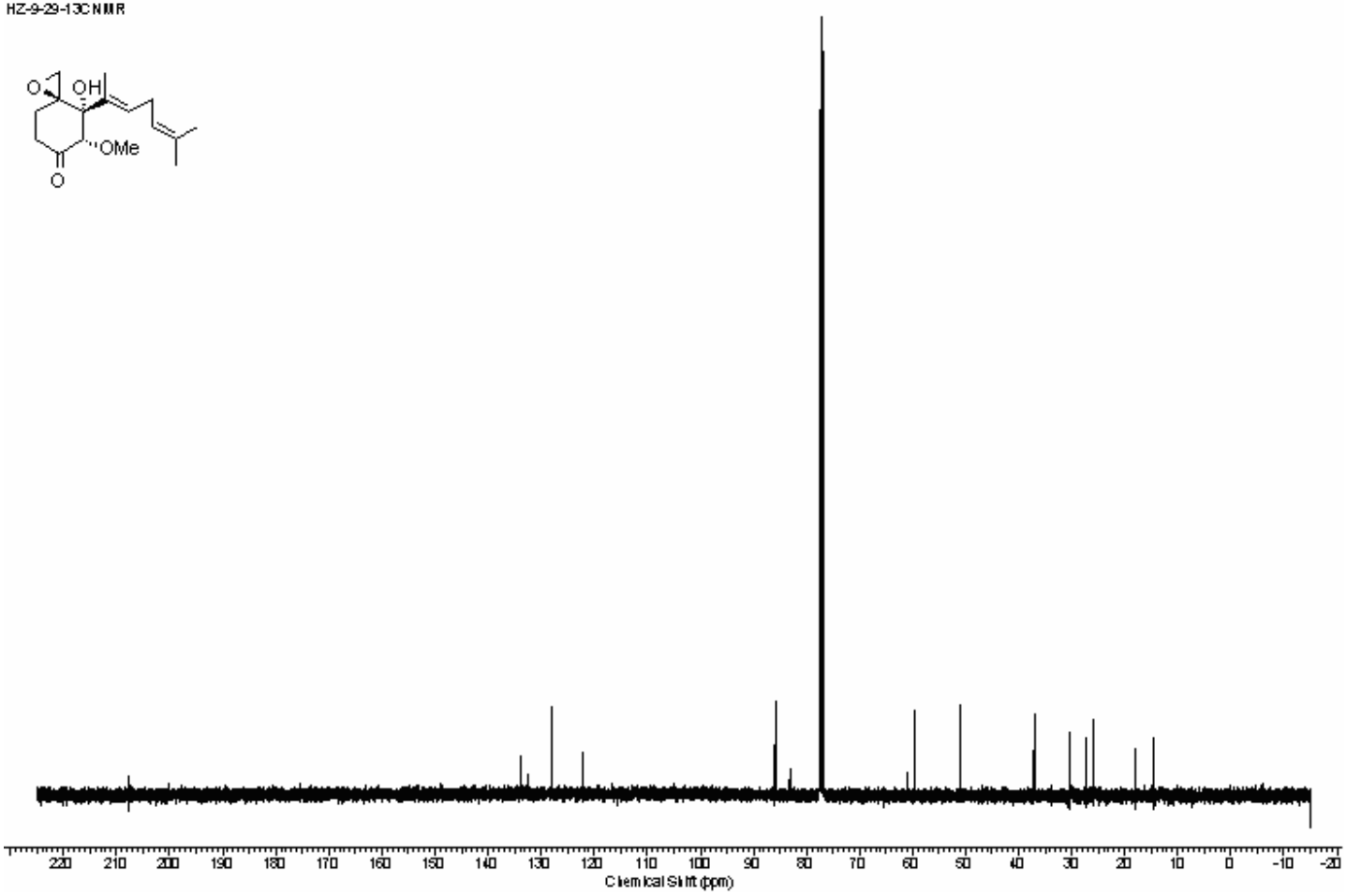
Acquisition Time (sec)	2.0486	*Comment*	Std proton	*Date*	May 13 2007			*Date Stamp*	May 13 2007
File Name	E:\DATA\ORIGINAL NMR-400\HZ-9-29-AC			*Frequency (MHz)*	399.78	*Nucleus*	1H	*Number of Transients*	64
Original Points Count	13104	*Points Count*	16384	*Pulse Sequence*	s2pul	*Receiver Gain*	48.00		
Solvent	CHLOROFORM-d			*Spectrum Offset (Hz)*	2411.8984	*Sweep Width (Hz)*	6396.42	*Temperature (degree C)*	25.000

HZ-9-29-AC



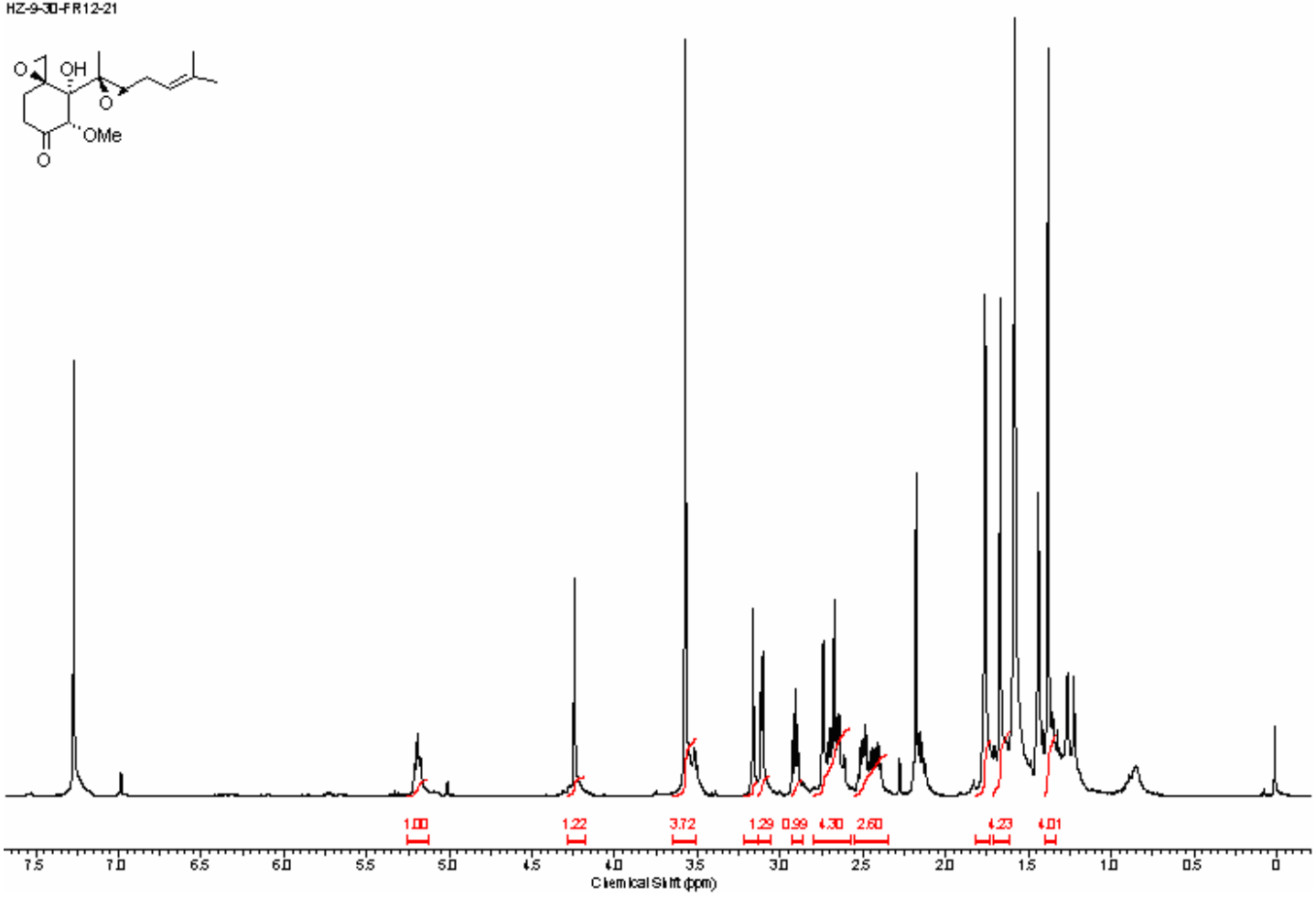
Acquisition Time (sec)	1.3005	*Comment*	Std proton	*Date*	May 12 2007	*Date Stamp*	May 12 2007
File Name	E:\O RIGINAL NMR-400\HZ-9-29-13C NMR			*Frequency (MHz)*	100.53	*Nucleus*	13C
Number of Transients	64000000	*Original Points Count*	31375	*Points Count*	32768	*Pulse Sequence*	s2pul
Receiver Gain	30.00	*Solvent*	CHLOROFORM-d			*Spectrum Offset (Hz)*	10556.7188
Sweep Width (Hz)	24125.45	*Temperature (degree C)*	30.00				

HZ-9-29-13C NMR



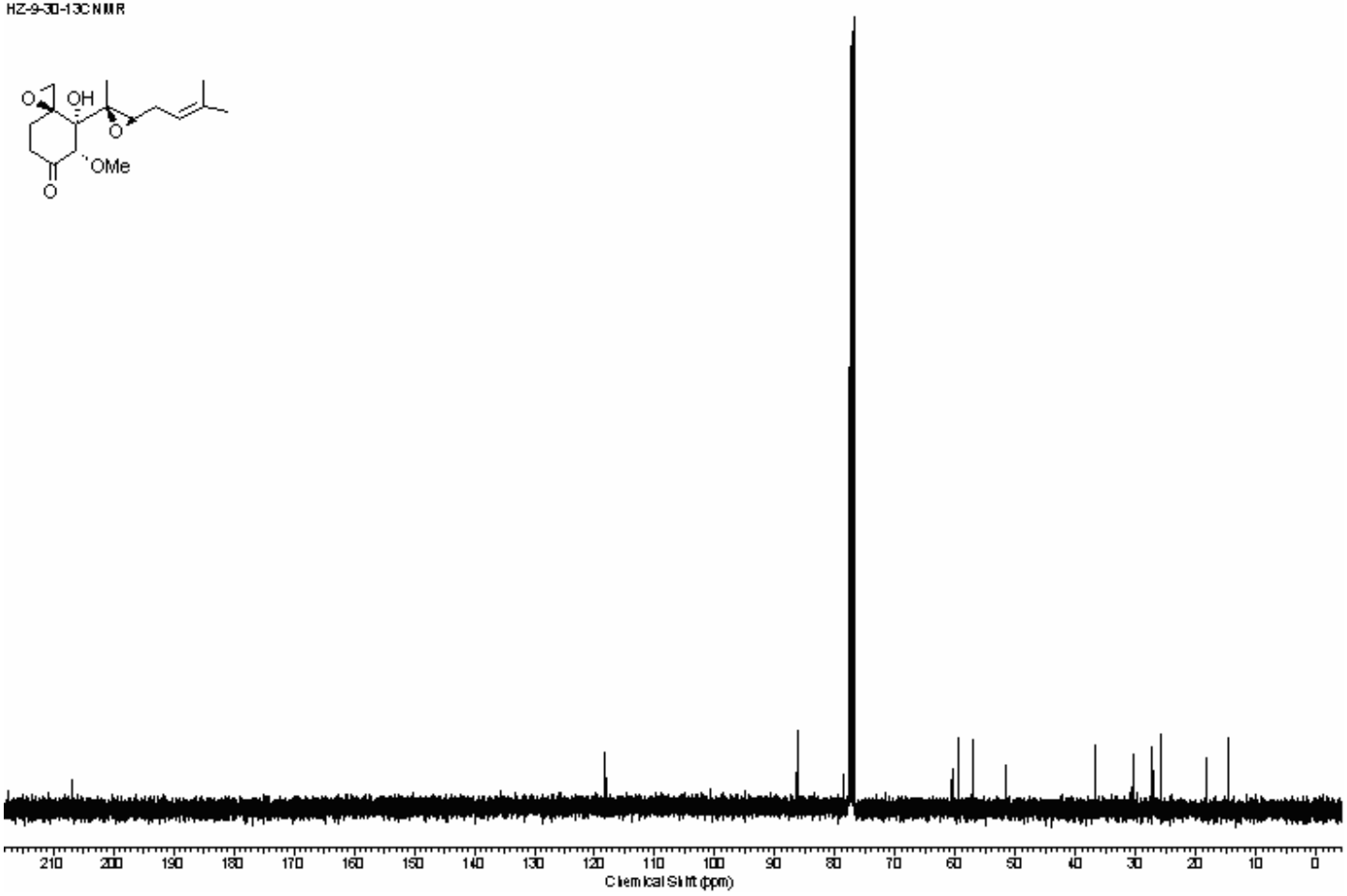
Acquisition Time (sec)	2.0486	*Comment*	Std proton	*Date*	May 13 2007			*Date Stamp*	May 13 2007
File Name	E:\O RIGINAL NMR-400\HZ-9-30-FR12-21			*Frequency (MHz)*	399.78	*Nucleus*	1H	*Number of Transients*	64
Original Points Count	13104	*Points Count*	16384	*Pulse Sequence*	s2pul	*Receiver Gain*	44.00		
Solvent	CHLOROFORM-d			*Spectrum Offset (Hz)*	2416.1804	*Sweep Width (Hz)*	6396.42	*Temperature (degree C)*	25.000

HZ-9-30-FR12-21



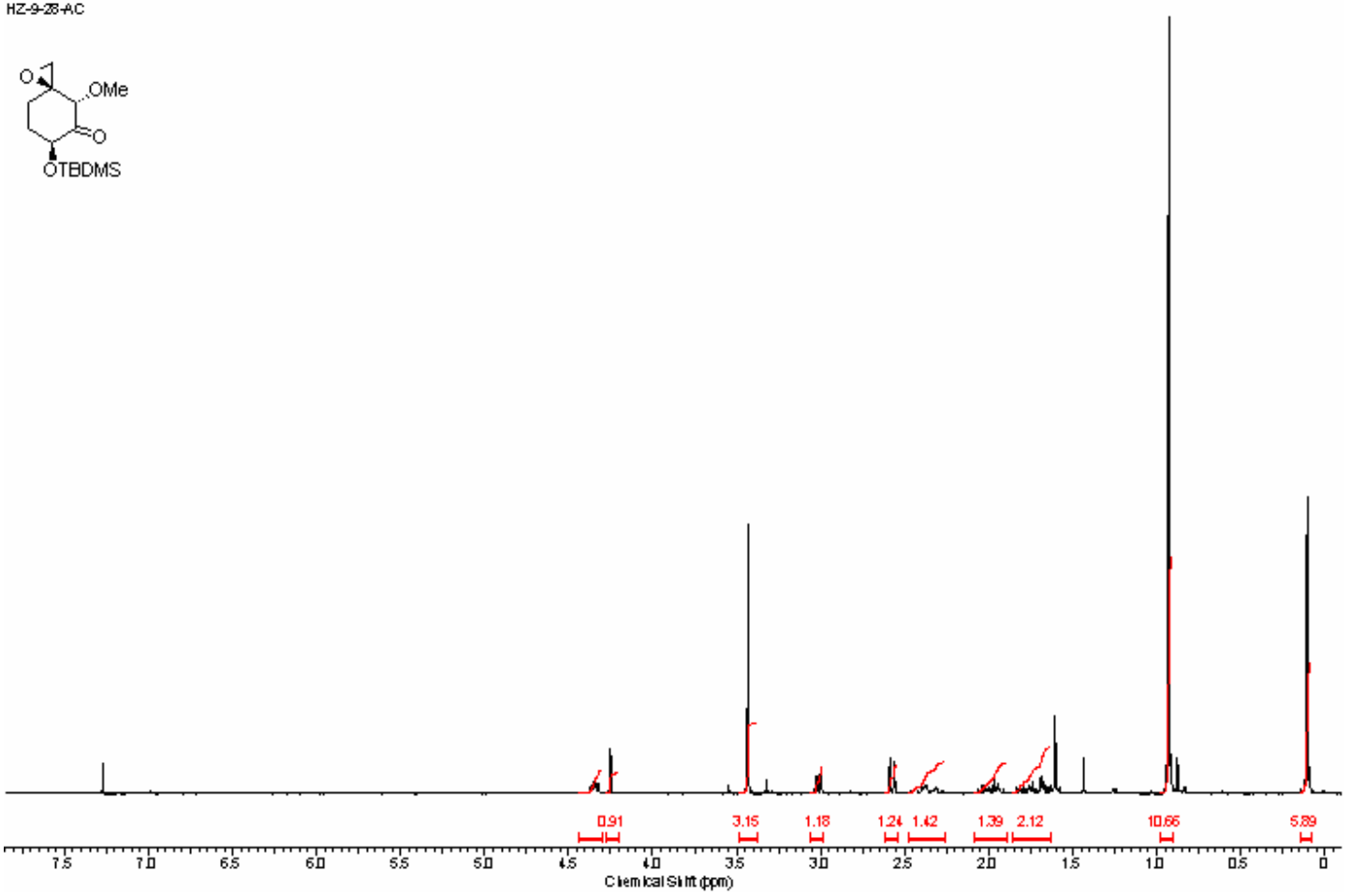
Acquisition Time (sec)	1.3005	*Comment*	Std proton	*Date*	May 13 2007	*Date Stamp*	May 13 2007
File Name	E:\O RIGINAL NMR-400\HZ-9-30-13C NMR			*Frequency (MHz)*	100.53	*Nucleus*	13C
Number of Transients	1000000000	*Original Points Count*	31375	*Points Count*	32768	*Pulse Sequence*	s2pul
Receiver Gain	30.00	*Solvent*	CHLOROFORM-d			*Spectrum Offset (Hz)*	10554.9062
Sweep Width (Hz)	24125.45	*Temperature (degree C)*	25.000				

HZ-9-30-13C NMR



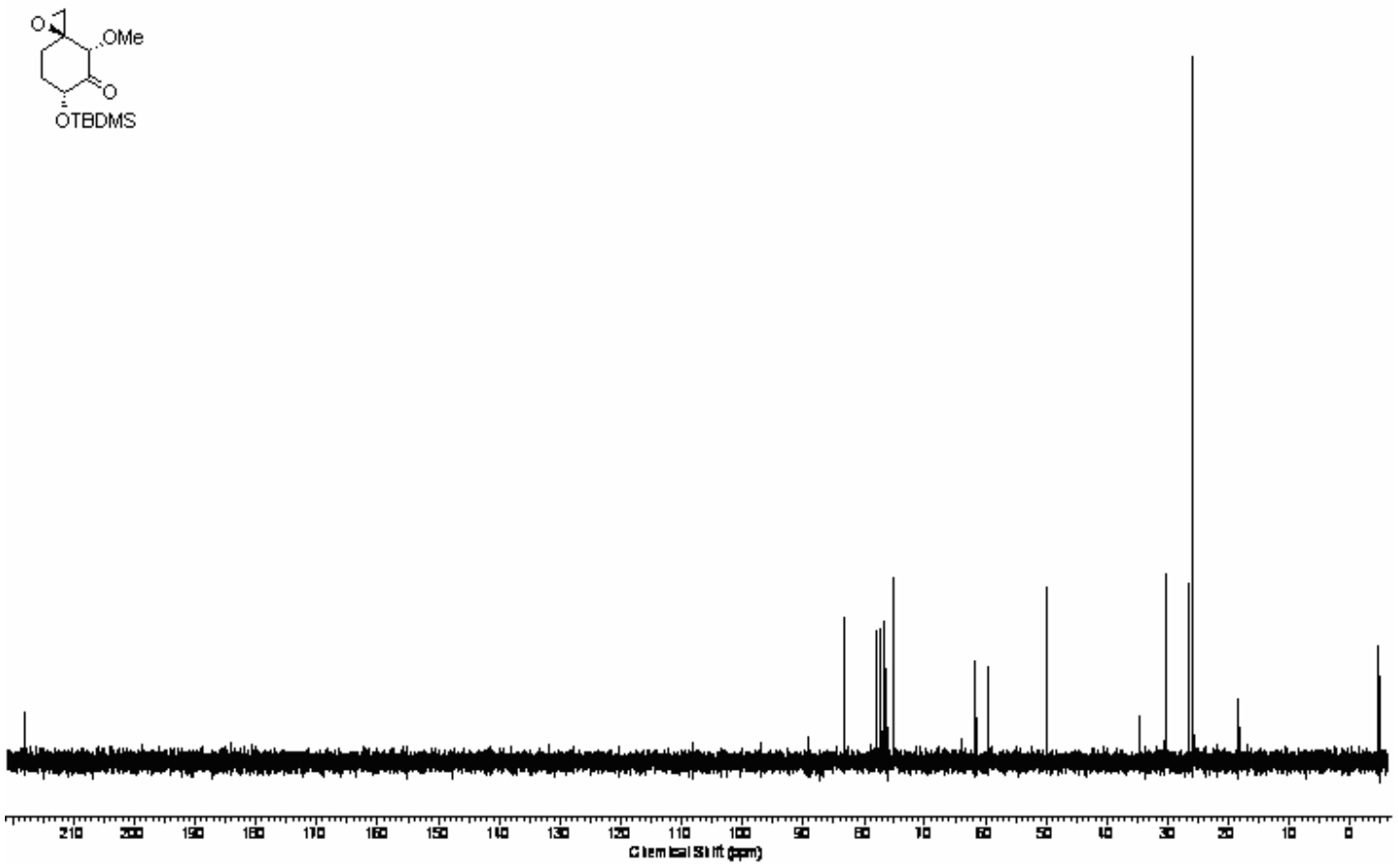
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Jul 4 2007
Date Stamp	Jul 4 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-28-AC				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	2800	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.6563	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-9-28-AC



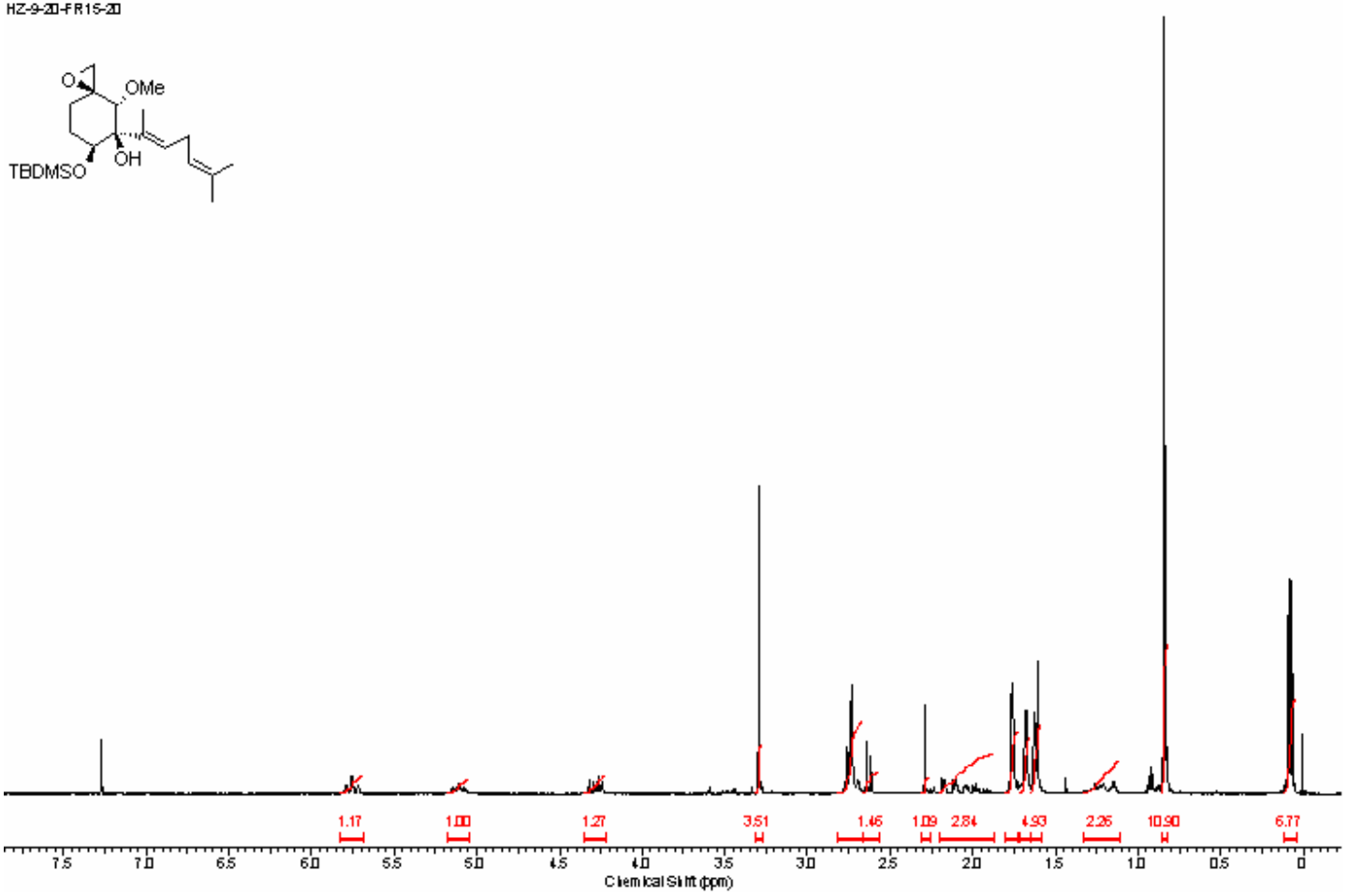
Acquisition Time (sec)	1.4976	Comment	13C OBSERVE	Date	Jul 4 2007	Date Stamp	Jul 4 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-28-13CNMR					Frequency (MHz)	50.29
Nucleus	13C	Number of Transients	64.0000	Original Points Count	16120	Points Count	32768
Pulse Sequence	s2pul	Receiver Gain	10.00	Solvent	CHLOROFORM-d		
Spectrum Offset (Hz)	4881.6050	Sweep Width (Hz)	12500.00	Temperature (degree C)	29.000		

HZ-9-28-13CNMR



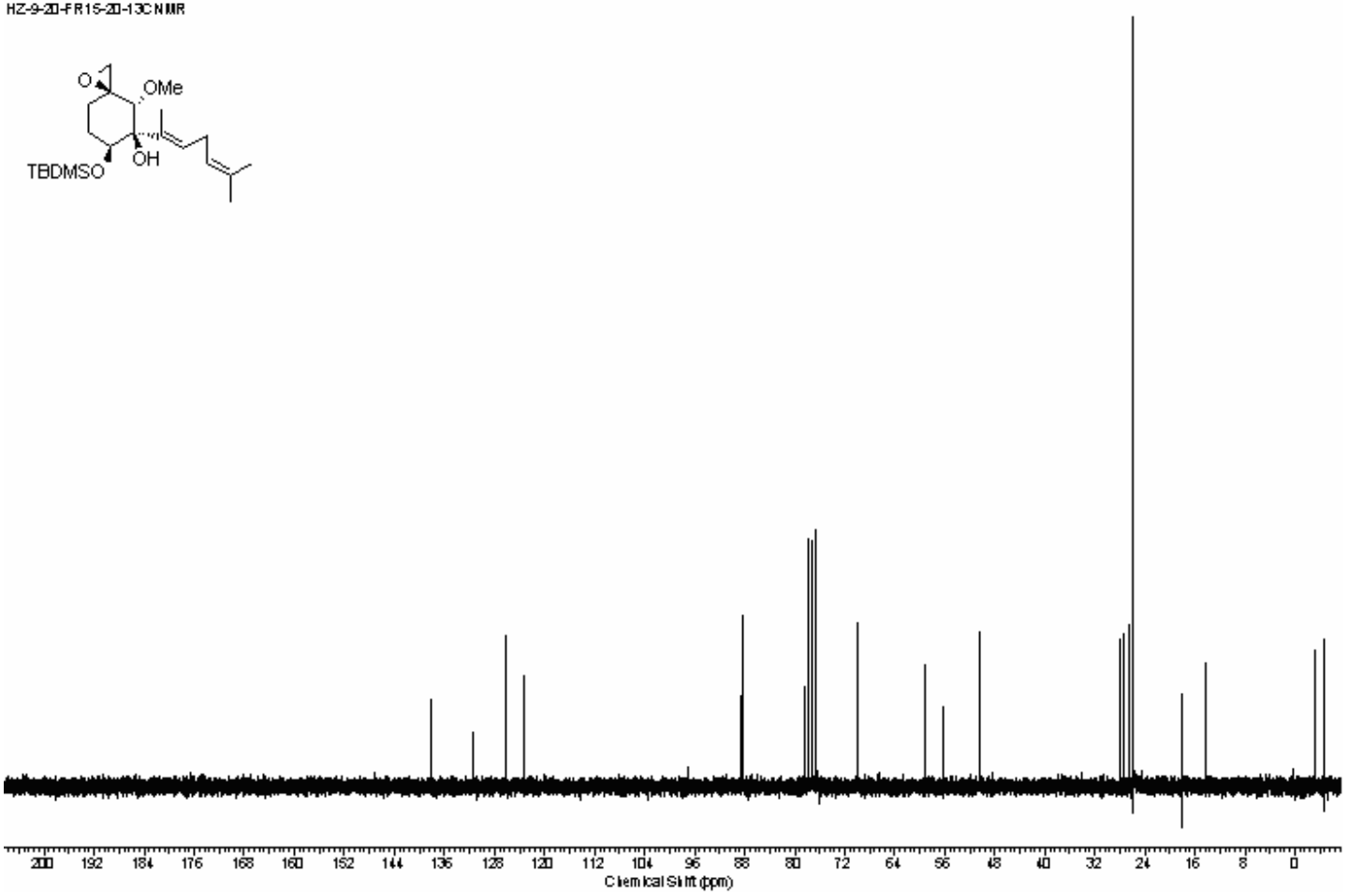
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	May 8 2007
Date Stamp	May 8 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-20-FR15-20				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	24.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.6563	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-9-20-FR15-20



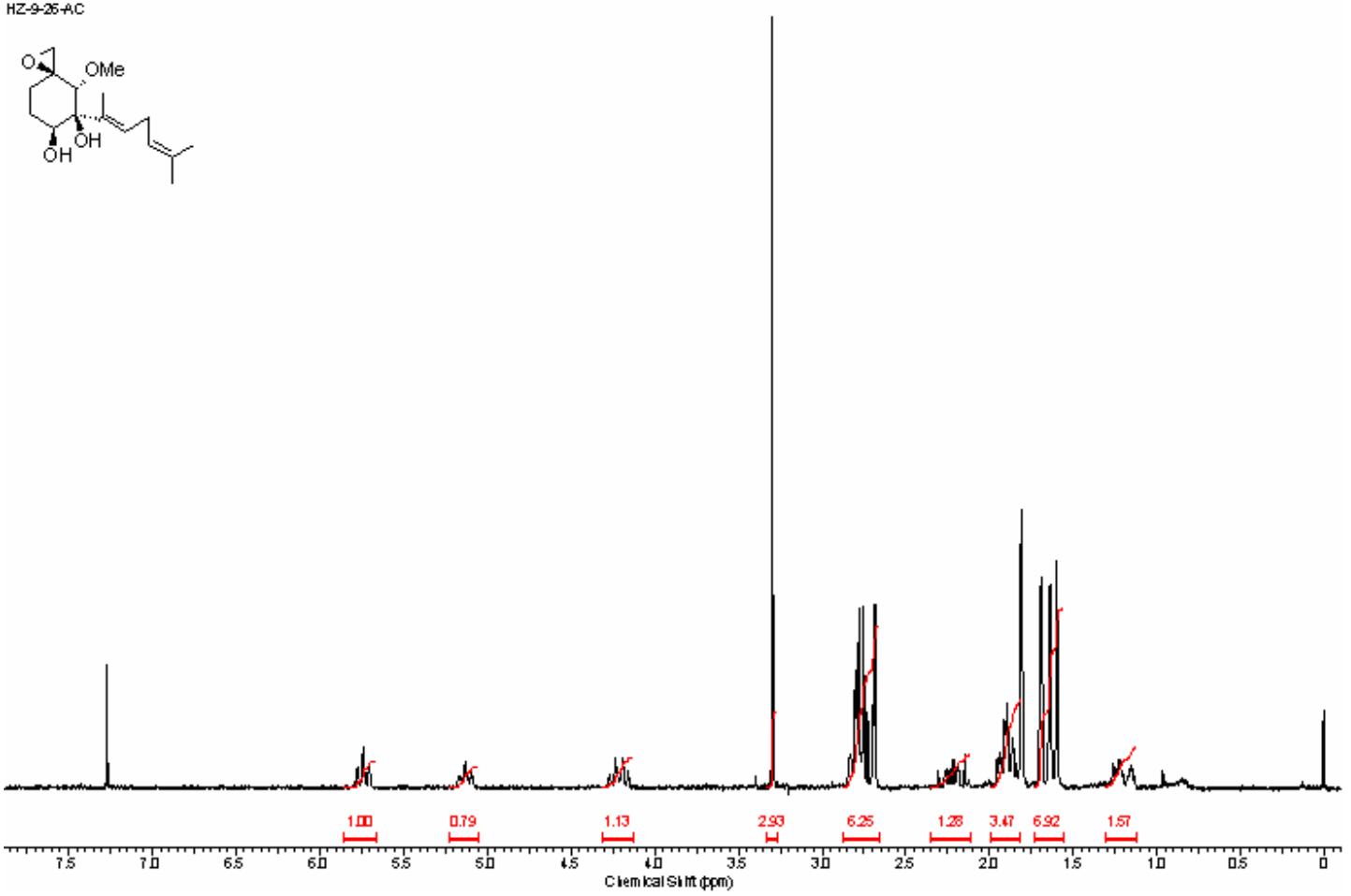
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	May 10 2007	*Date Stamp*	May 10 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-20-FR1S-20-13C NMR					*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	64000	*Original Points Count*	18720	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	4881.6055	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-9-20-FR1S-20-13C NMR



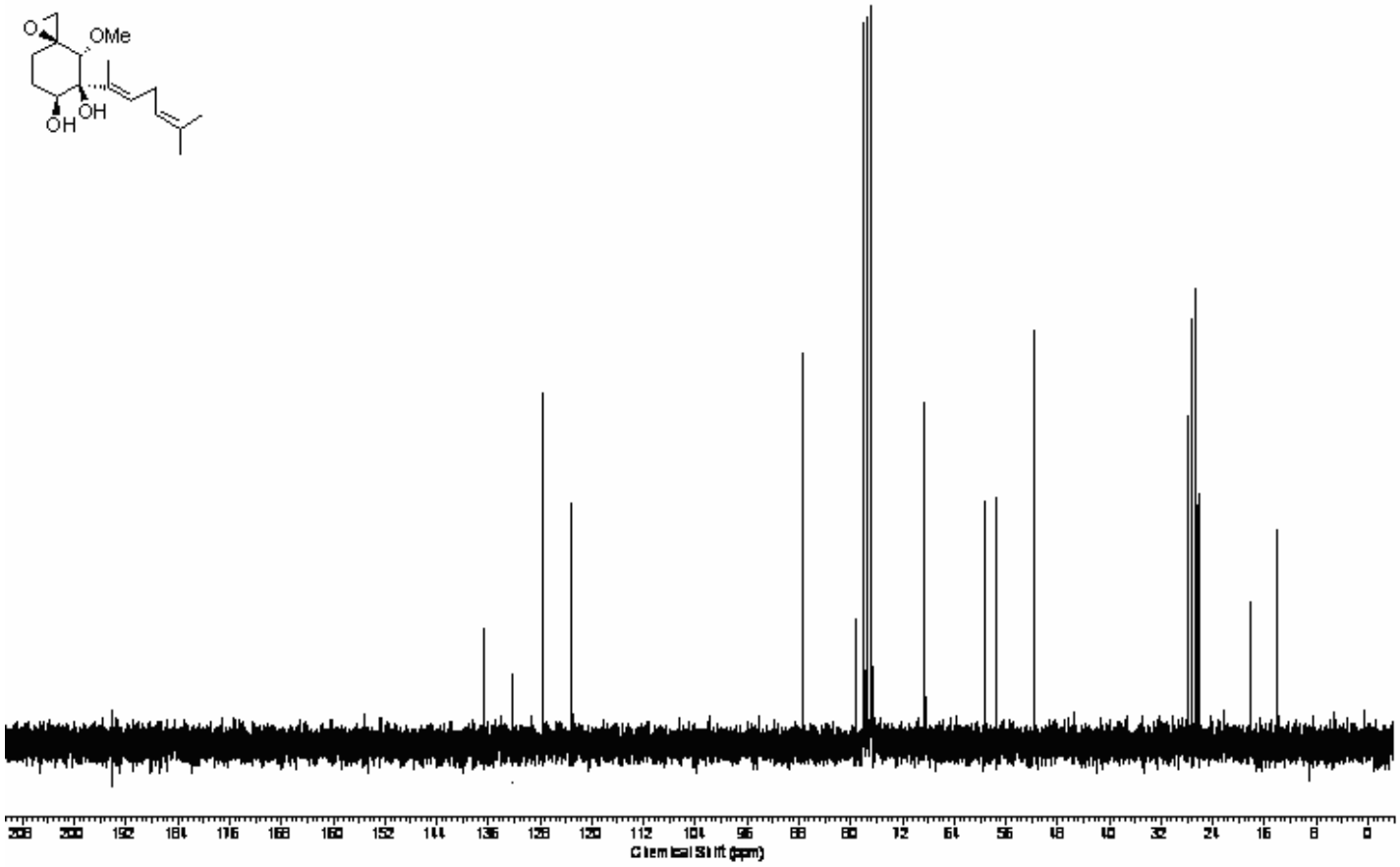
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Jun 3 2007
Date Stamp	Jun 3 2007	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-26-AC				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	4	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	29.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.2901	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-9-26-AC



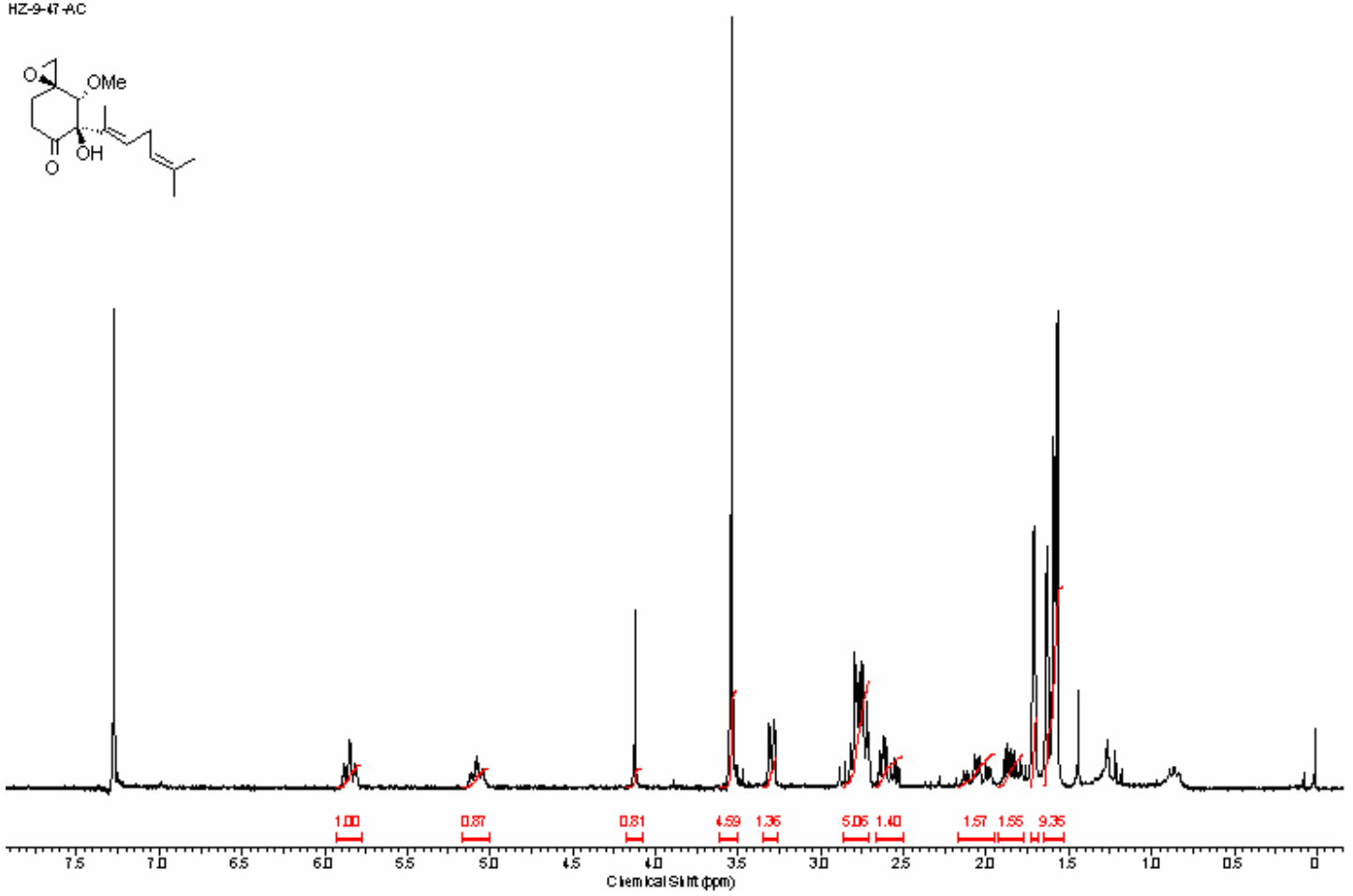
Acquisition Time (sec)	1.4916	Comment	13C OBSERVE	Date	Jul 2 2001	Date Stamp	Jul 2 2001
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-26-DP-13CNMR					Frequency (MHz)	50.29
Nucleus	13C	Number of Transients	61000.00	Original Points Count	16128	Points Count	32768
Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d		
Spectrum Offset (Hz)	6812.2695	Sweep Width (Hz)	12500.00	Temperature (degree C)	29.000		

HZ-9-26-DP-13CNMR



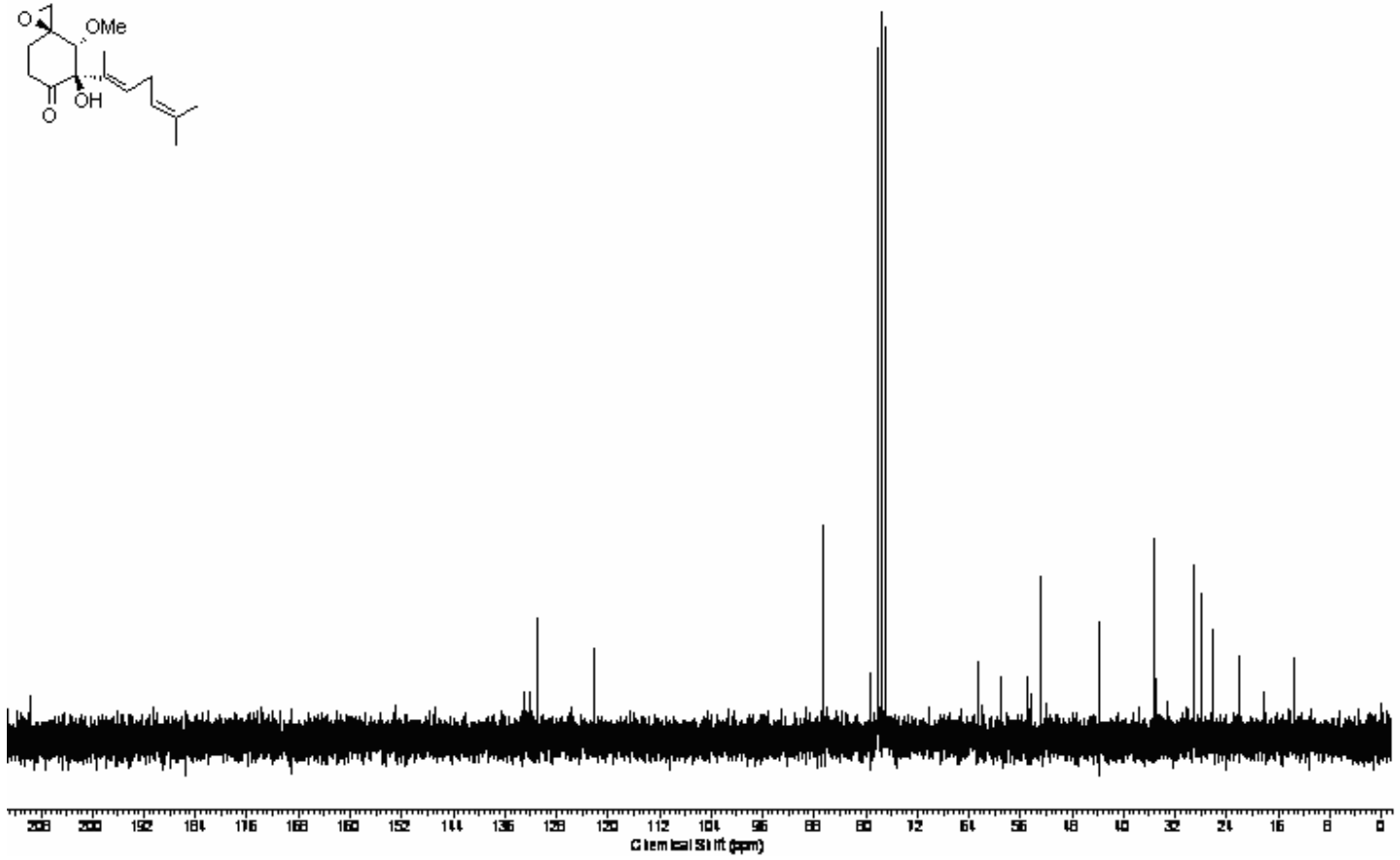
Acquisition Time (sec)	1.9945	*Comment*	STANDARD 1H OBSERVE			*Date*	Jun 8 2007
Date Stamp	Jun 8 2007	*File Name*	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-47-AC				
Frequency (MHz)	199.98	*Nucleus*	1H	*Number of Transients*	64	*Original Points Count*	5984
Points Count	8192	*Pulse Sequence*	s2pul	*Receiver Gain*	39.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	1002.3816	*Sweep Width (Hz)*	3000.30	*Temperature (degree C)*	29.000		

HZ-9-47-AC



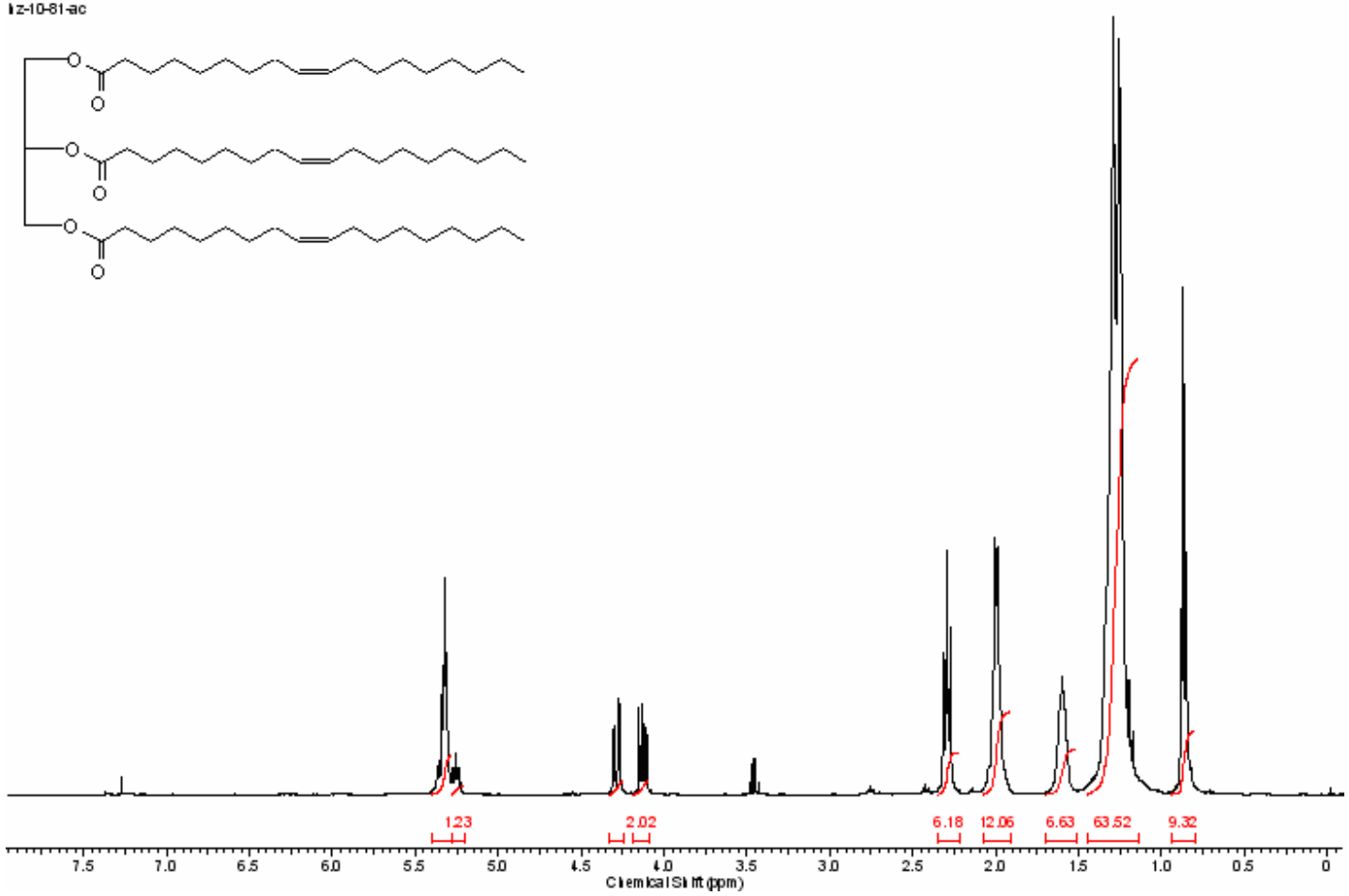
Acquisition Time (sec)	1.4916	Comment	13C OBSERVE	Date	Jul 17 2007	Date Stamp	Jul 17 2007
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\HZ-9\HZ-9-41-AC-13C					Frequency (MHz)	50.29
Nucleus	13C	Number of Transients	5400000	Original Points Count	16120	Points Count	32768
Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d		
Spectrum Offset (Hz)	4861.2285	Sweep Width (Hz)	12500.00	Temperature (degree C)	29.000		

HZ-9-41-AC-13C



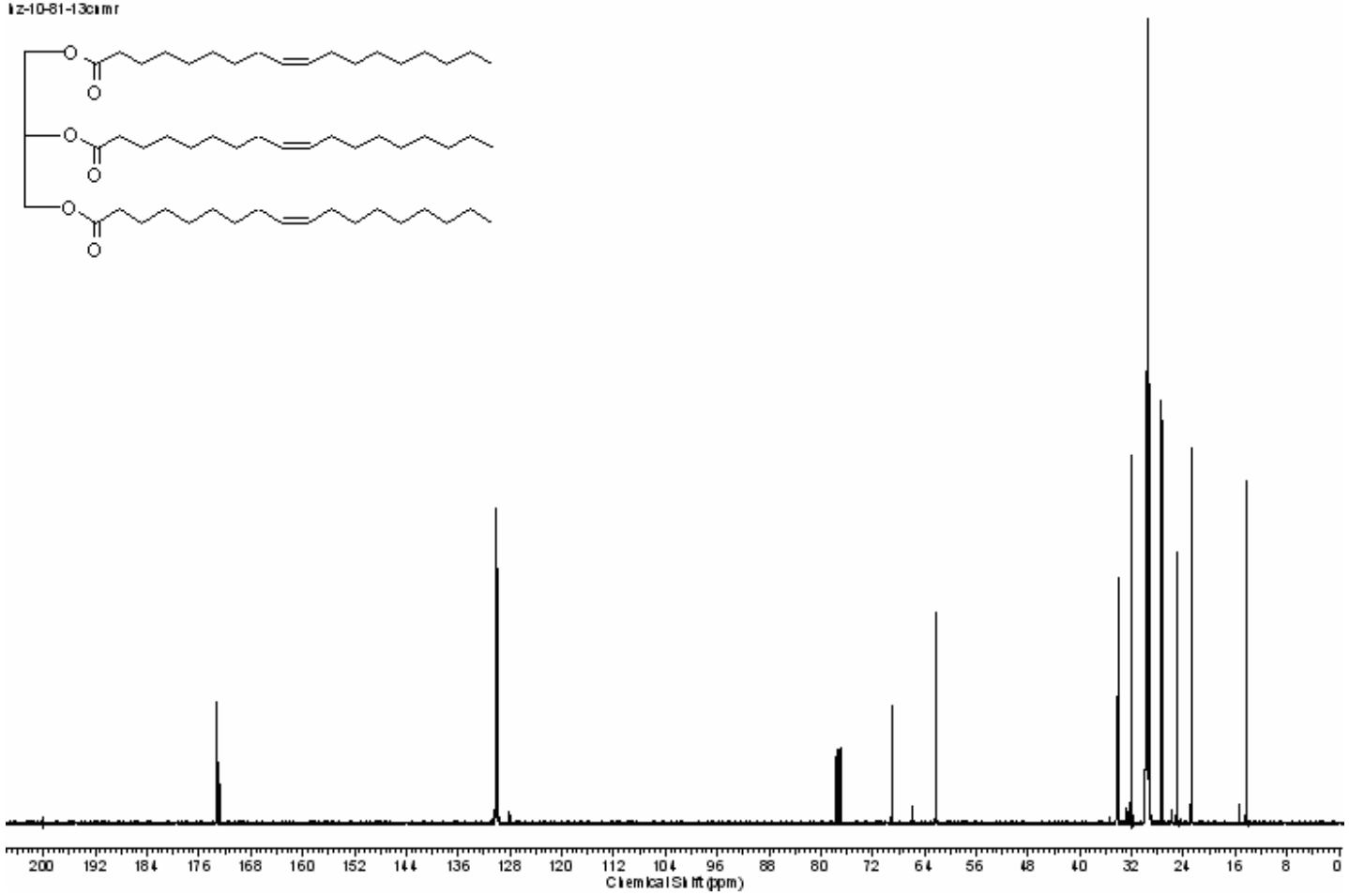
Acquisition Time (sec)	2.0486	*Comment*	Std proton	*Date*	Jan 3 2008	*Date Stamp*	Jan 3 2008
File Name	C:\Documents and Settings\Huiping\Desktop\liz-10-81-ac					*Frequency (MHz)*	399.77
Nucleus	1H	*Number of Transients*	8	*Original Points Count*	13104	*Points Count*	131072
Pulse Sequence	s2pul	*Receiver Gain*	8.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	2409.0586	*Sweep Width (Hz)*	6396.42	*Temperature (degree C)*	25.000		

liz-10-81-ac



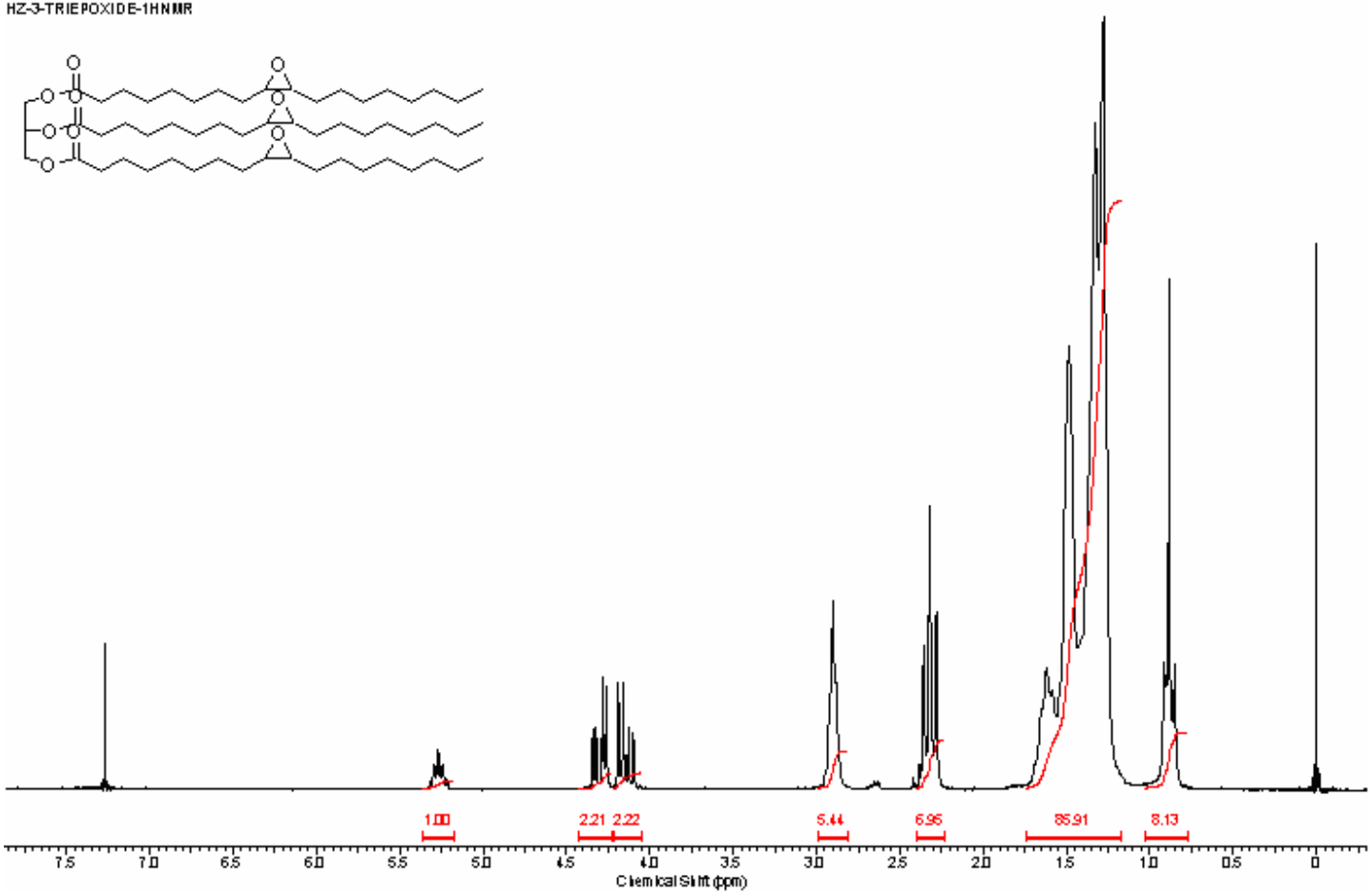
Acquisition Time (sec)	1.3005	*Comment*	Std proton	*Date*	Jan 3 2008	*Date Stamp*	Jan 3 2008
File Name	C:\Documents and Settings\Hulplug\Desktop\liz-10-81-13c.mr					*Frequency (MHz)*	100.53
Nucleus	13C	*Number of Transients*	1000	*Original Points Count*	31375	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	30.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	10551.2354	*Sweep Width (Hz)*	24125.45	*Temperature (degree C)*	25.000		

liz-10-81-13c.mr



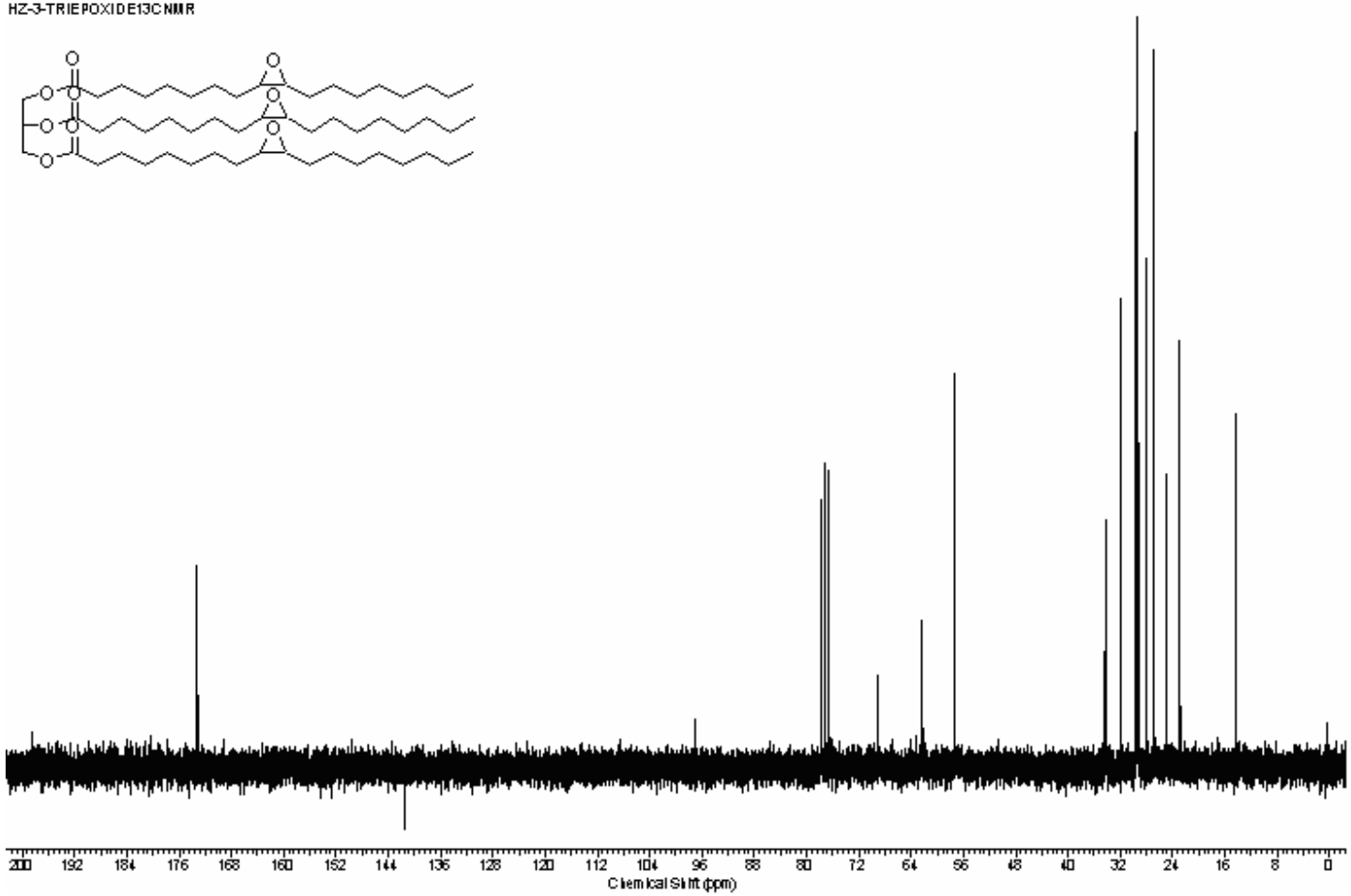
Acquisition Time (sec)	1.9945	*Comment*	STANDARD 1H OBSERVE			*Date*	Mar 7 2005
Date Stamp	Mar 7 2005	*File Name*	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-200MHZ\HZ-3-TRIEPOXIDE-1HNMR				
Frequency (MHz)	199.98	*Nucleus*	1H	*Number of Transients*	64	*Original Points Count*	5984
Points Count	8192	*Pulse Sequence*	s2pul	*Receiver Gain*	22.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	1002.0226	*Sweep Width (Hz)*	3000.30	*Temperature (degree C)*	29.000		

HZ-3-TRIEPOXIDE-1HNMR

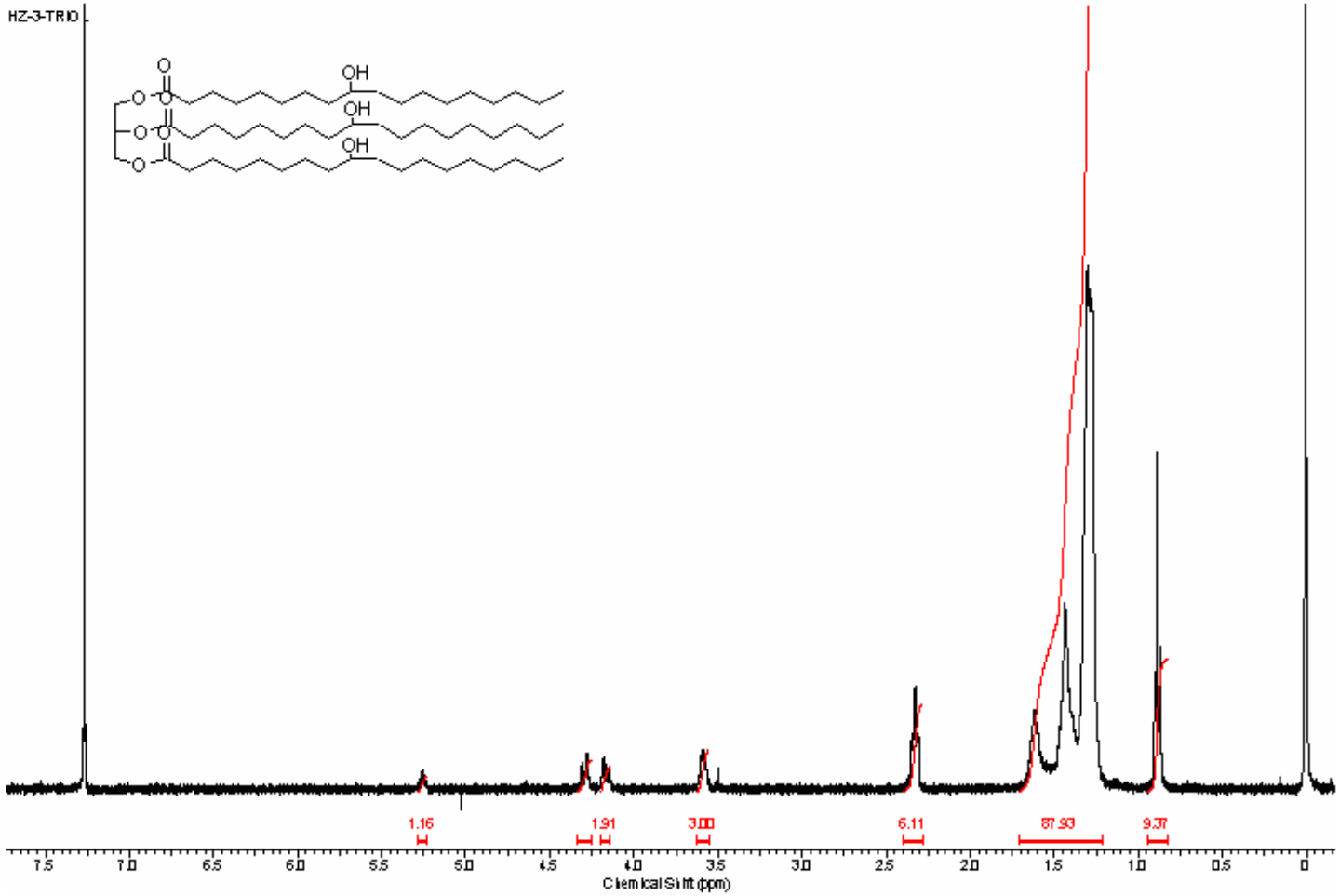


Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Feb 22 2005	*Date Stamp*	Feb 22 2005
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-200MHZ\HZ-3-TRIEPOXIDE13CNMR						
Frequency (MHz)	50.29	*Nucleus*	13C	*Number of Transients*	20000	*Original Points Count*	16720
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	4000	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	4881.9912	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-3-TRIEPOXIDE13CNMR

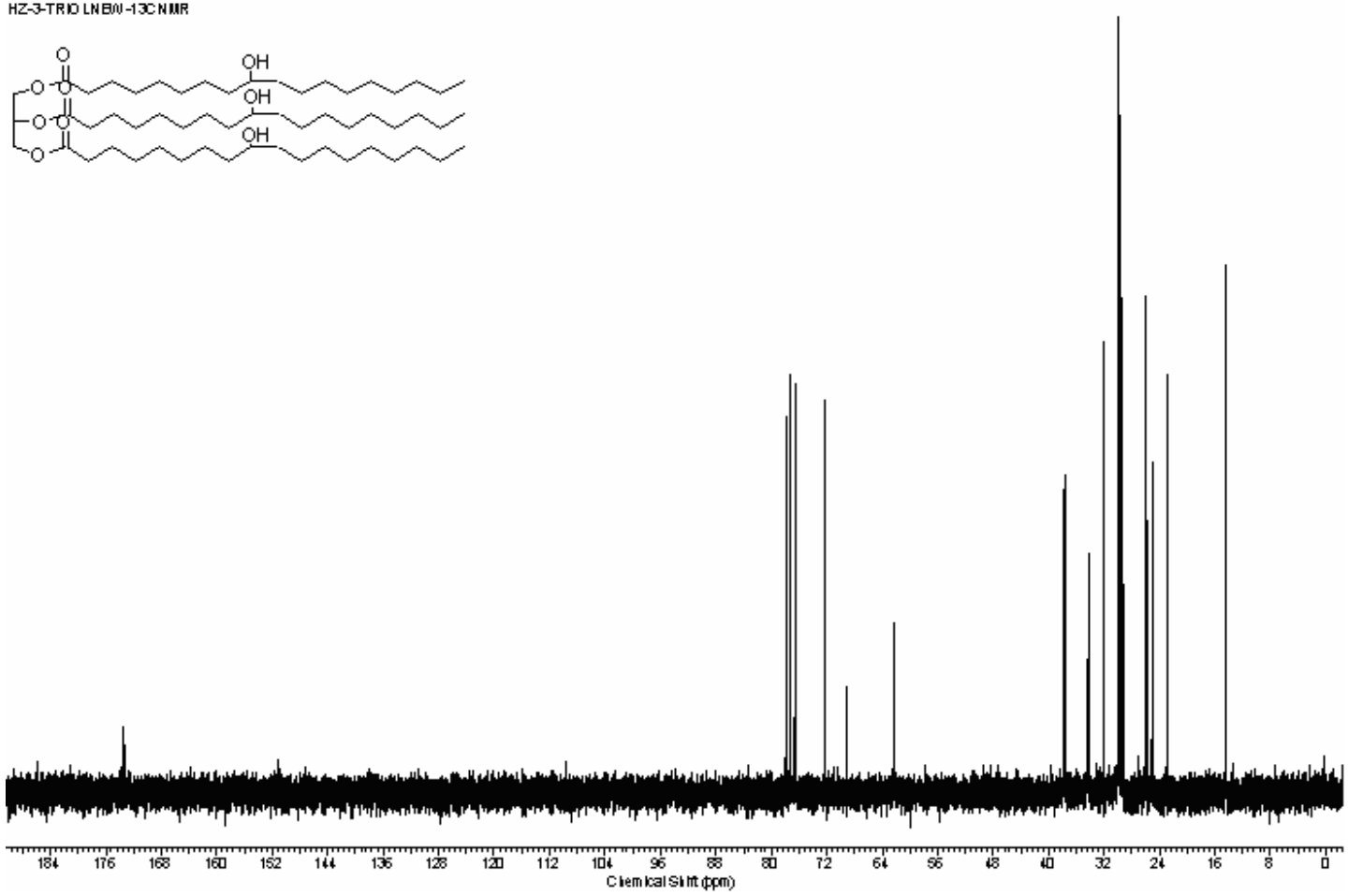


Acquisition Time (sec)	3.7010	*Comment*	STANDARD 1H OBSERVE			*Date*	Feb 23 2005
Date Stamp	Feb 23 2005	*File Name*	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-3-TRIOL				
Frequency (MHz)	399.80	*Nucleus*	1H	*Number of Transients*	64	*Original Points Count*	22208
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	2011.8737	*Sweep Width (Hz)*	6000.60	*Temperature (degree C)*	29.000		



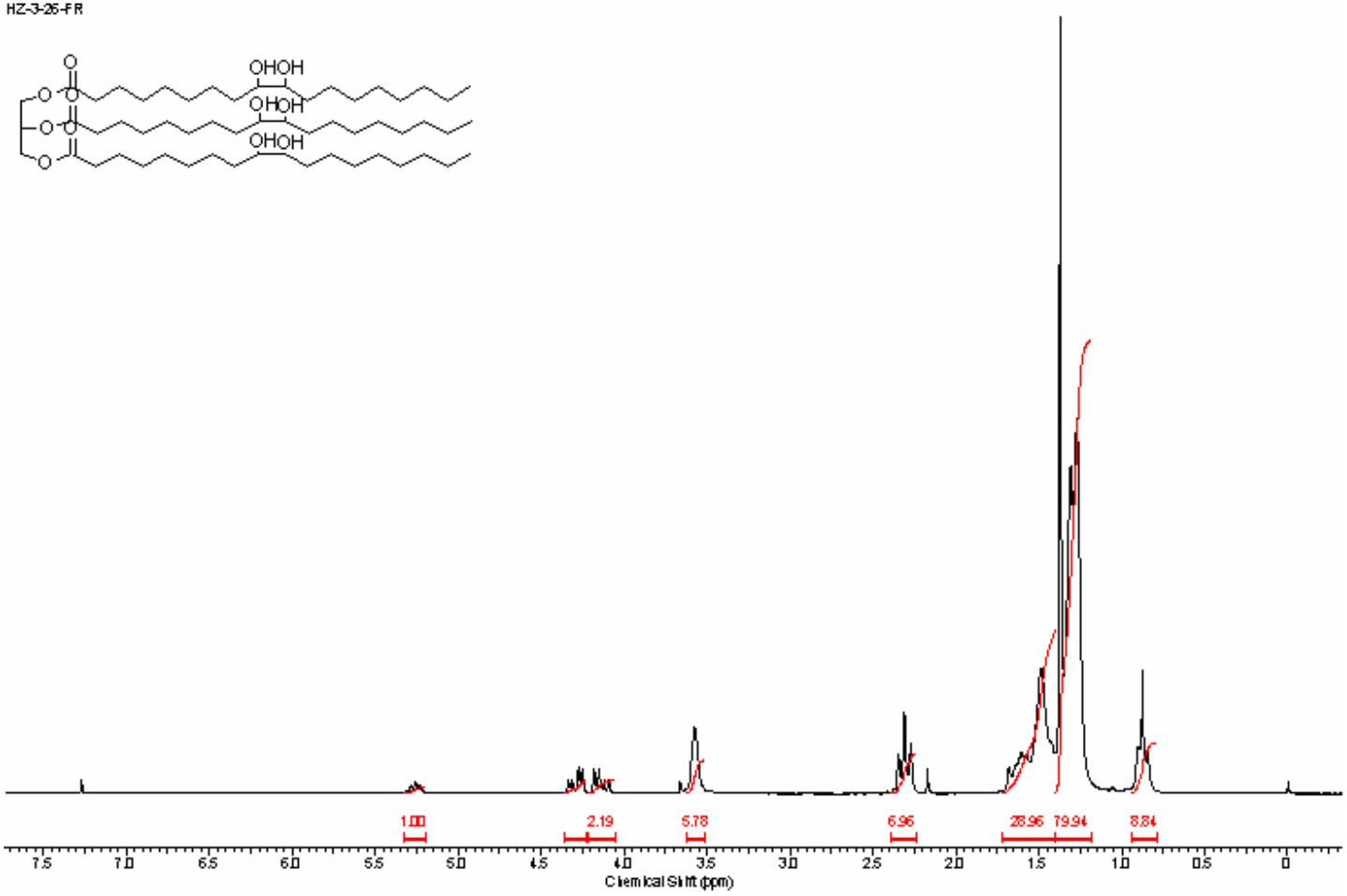
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Mar 2 2005	*Date Stamp*	Mar 2 2005
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-200MHZ\HZ-3-TRIOLNEW-13C.NMR						
Frequency (MHz)	50.29	*Nucleus*	13C	*Number of Transients*	20000	*Original Points Count*	18720
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	4881.6050	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-3-TRIOLNEW-13C.NMR



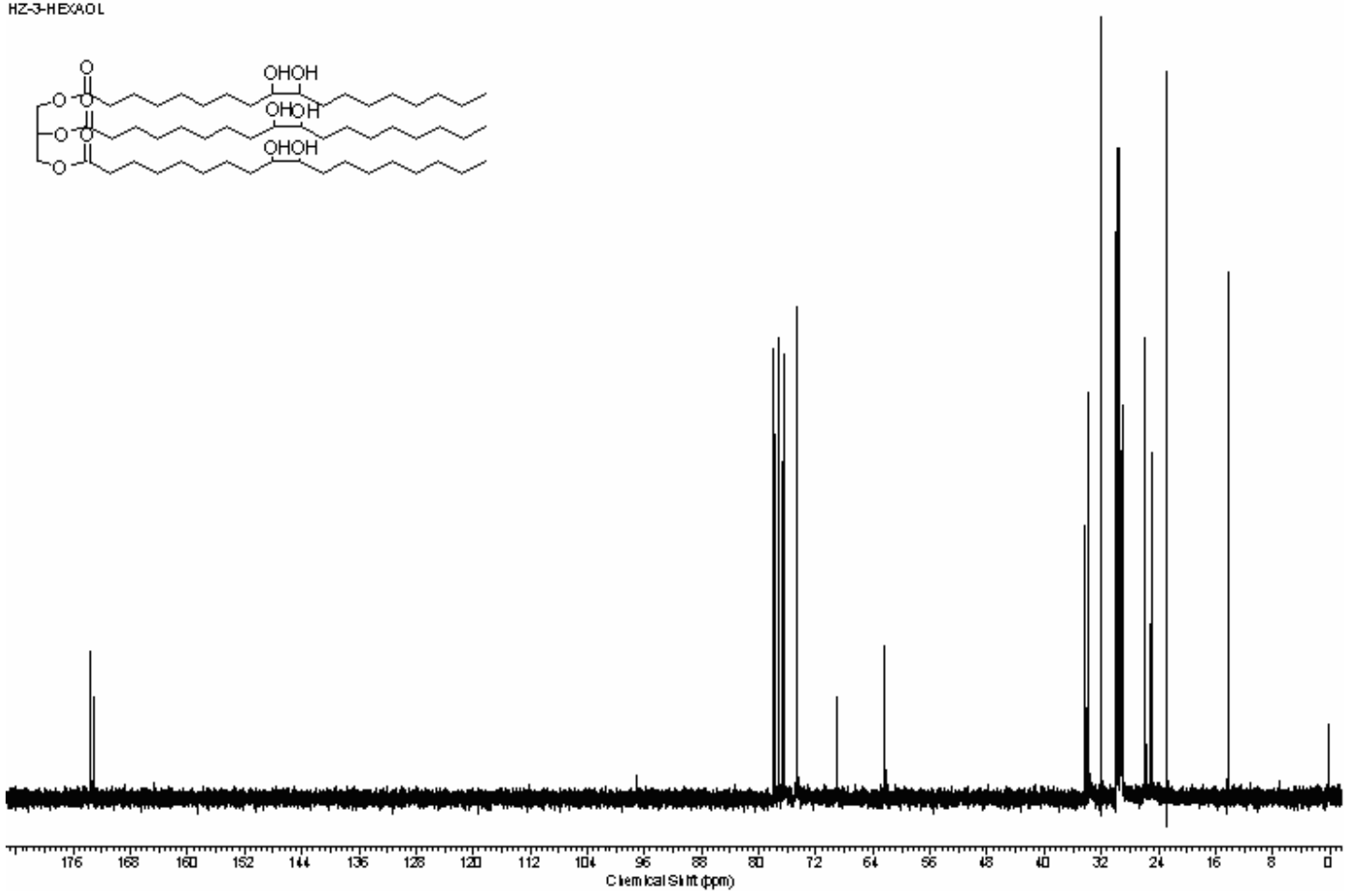
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Jan 26 2005
Date Stamp	Jan 26 2005	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-200MHZ\HZ-3-26-FR				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	11.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.6563	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-3-26-FR



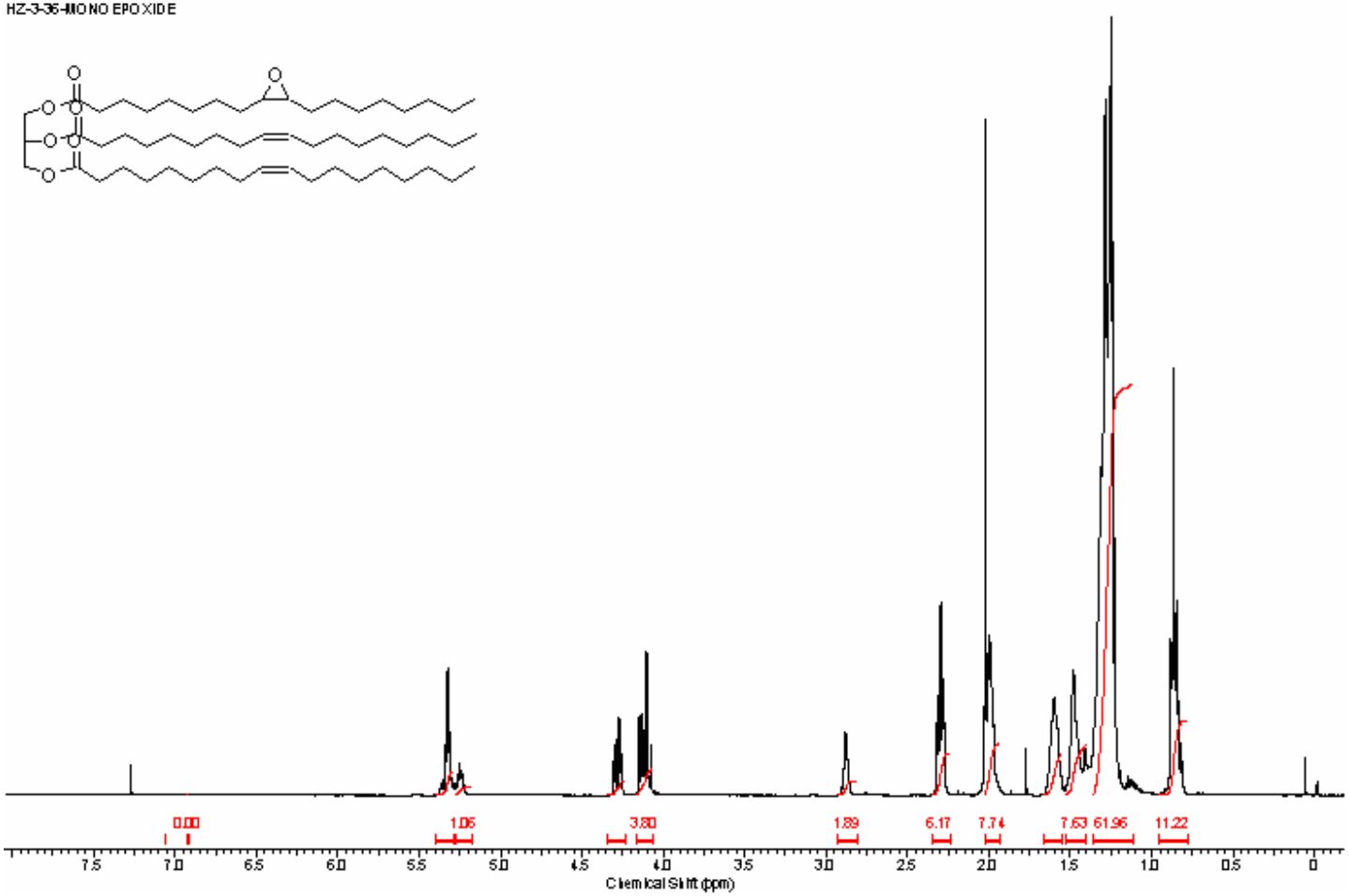
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Feb 22 2005	*Date Stamp*	Feb 22 2005
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-200MHZ\HZ-3-HEXAOL						
Frequency (MHz)	50.29	*Nucleus*	13C	*Number of Transients*	20000	*Original Points Count*	18720
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	4881.6050	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-3-HEXAOL



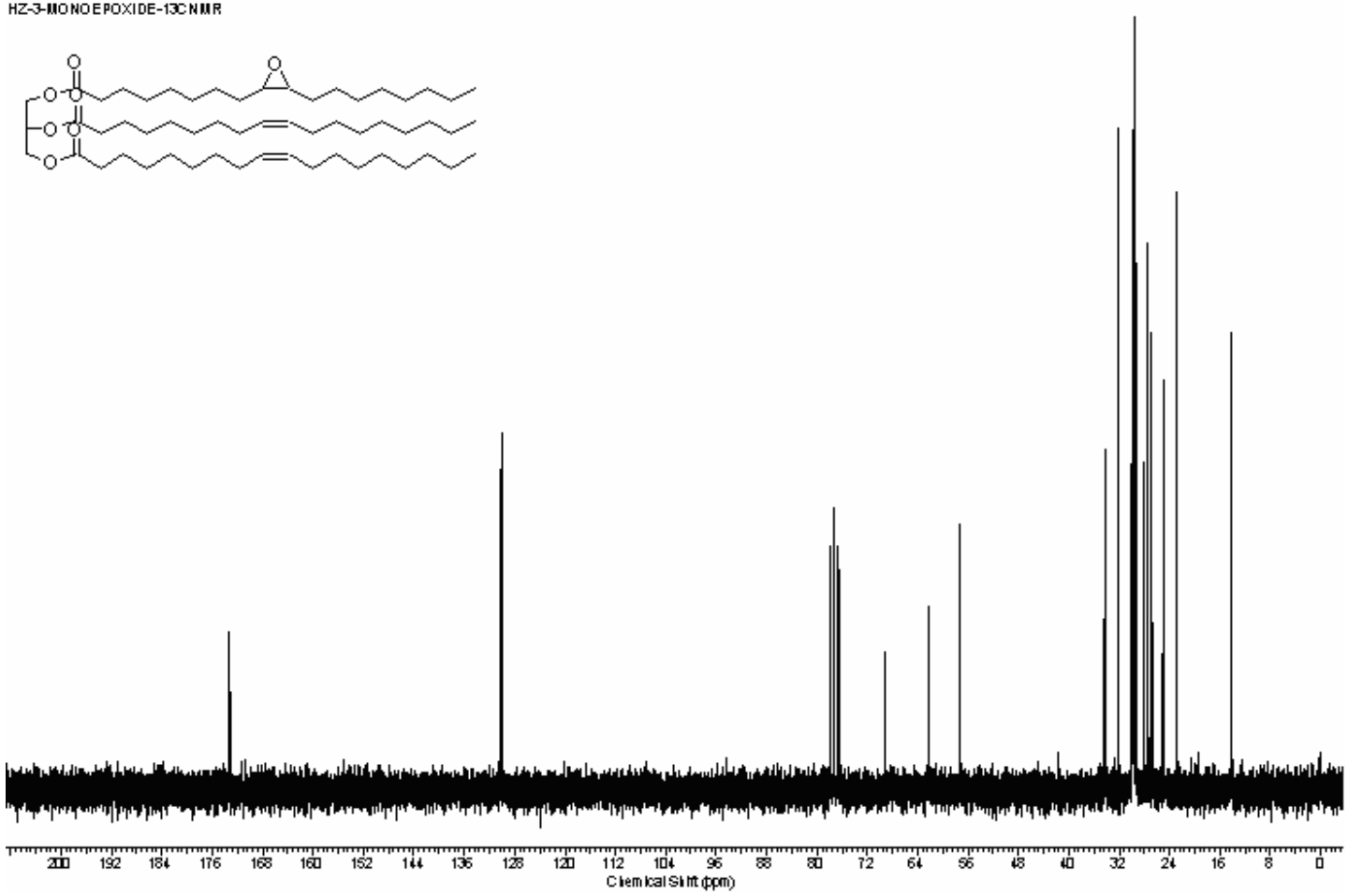
Acquisition Time (sec)	3.7010	Comment	STANDARD 1H OBSERVE			Date	Jan 30 2005
Date Stamp	Jan 30 2005	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-3-36-MONOEPOXIDE				
Frequency (MHz)	399.80	Nucleus	1H	Number of Transients	64	Original Points Count	22208
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	21.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2011.3241	Sweep Width (Hz)	6000.60	Temperature (degree C)	29.000		

HZ-3-36-MONOEPOXIDE



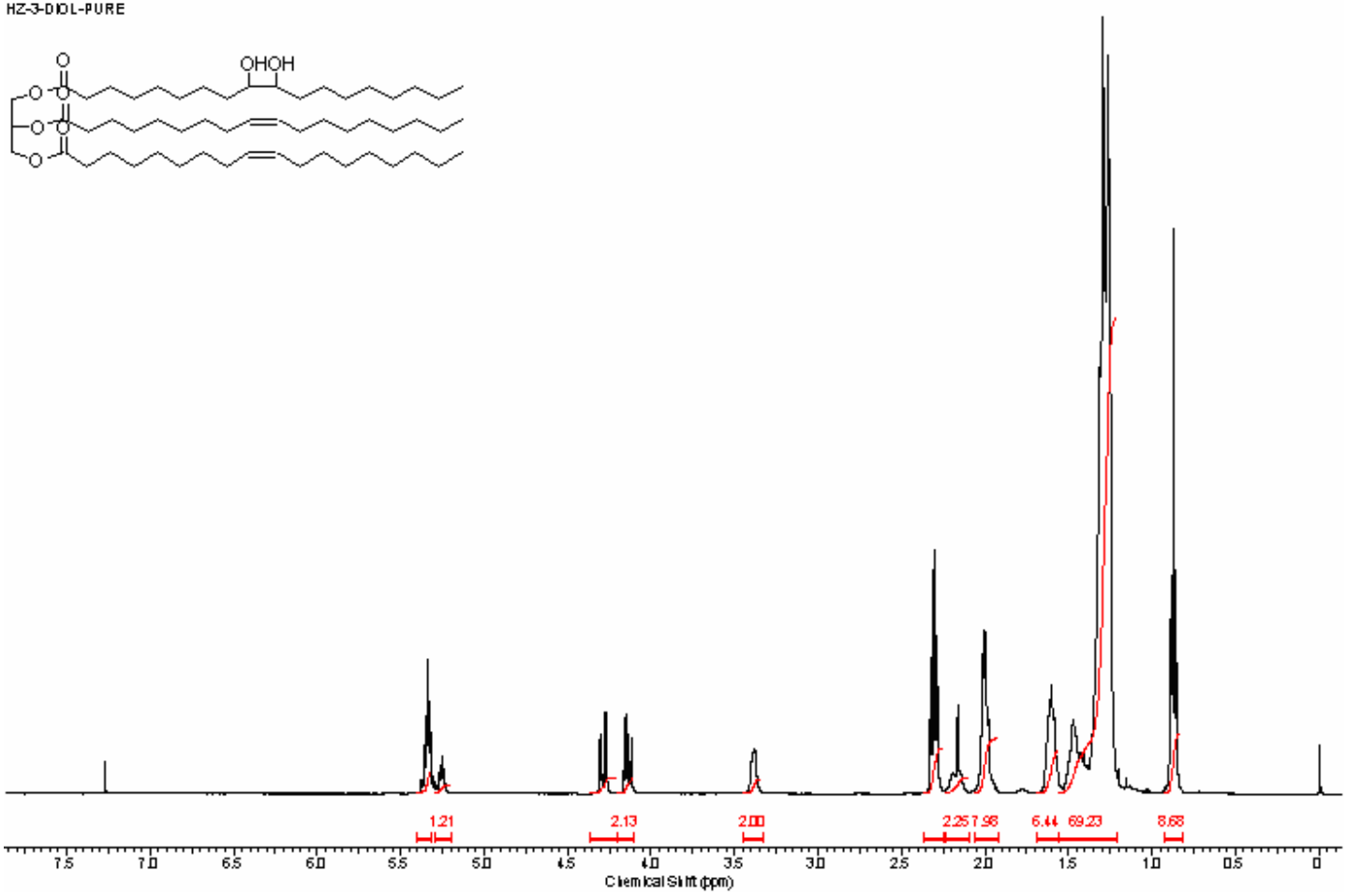
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Mar 2 2005	*Date Stamp*	Mar 2 2005
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-200MHZ\HZ-3-MONO EPOXIDE-13C NMR						
Frequency (MHz)	50.29	*Nucleus*	13C	*Number of Transients*	20000	*Original Points Count*	18720
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	4881.6050	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-3-MONO EPOXIDE-13C NMR



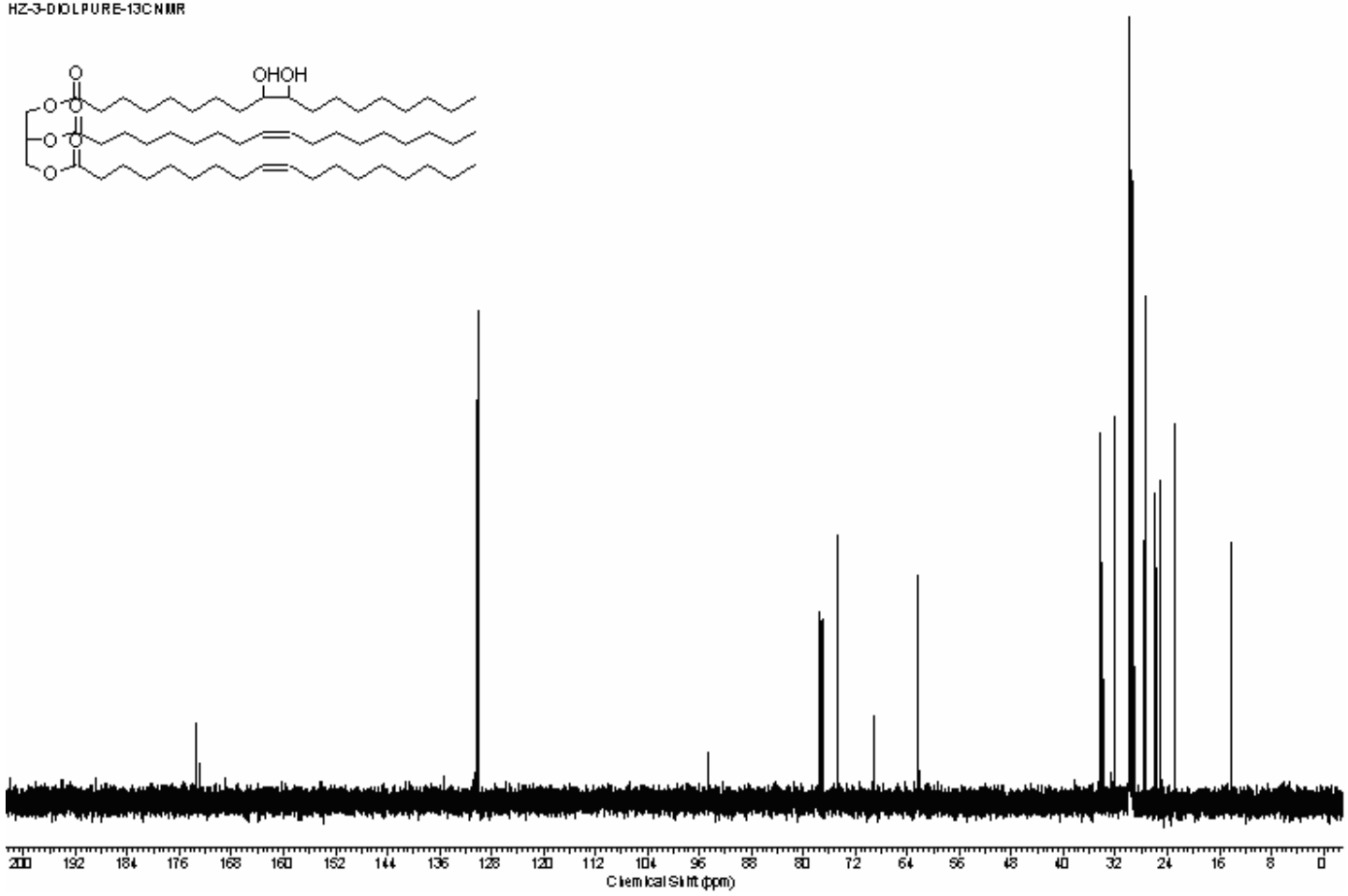
Acquisition Time (sec)	3.7010	Comment	STANDARD 1H OBSERVE			Date	Mar 4 2005
Date Stamp	Mar 4 2005	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-3-DIOL-PURE				
Frequency (MHz)	399.80	Nucleus	1H	Number of Transients	64	Original Points Count	22208
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	4.000	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2013.5219	Sweep Width (Hz)	6000.60	Temperature (degree C)	29.000		

HZ-3-DIOL-PURE



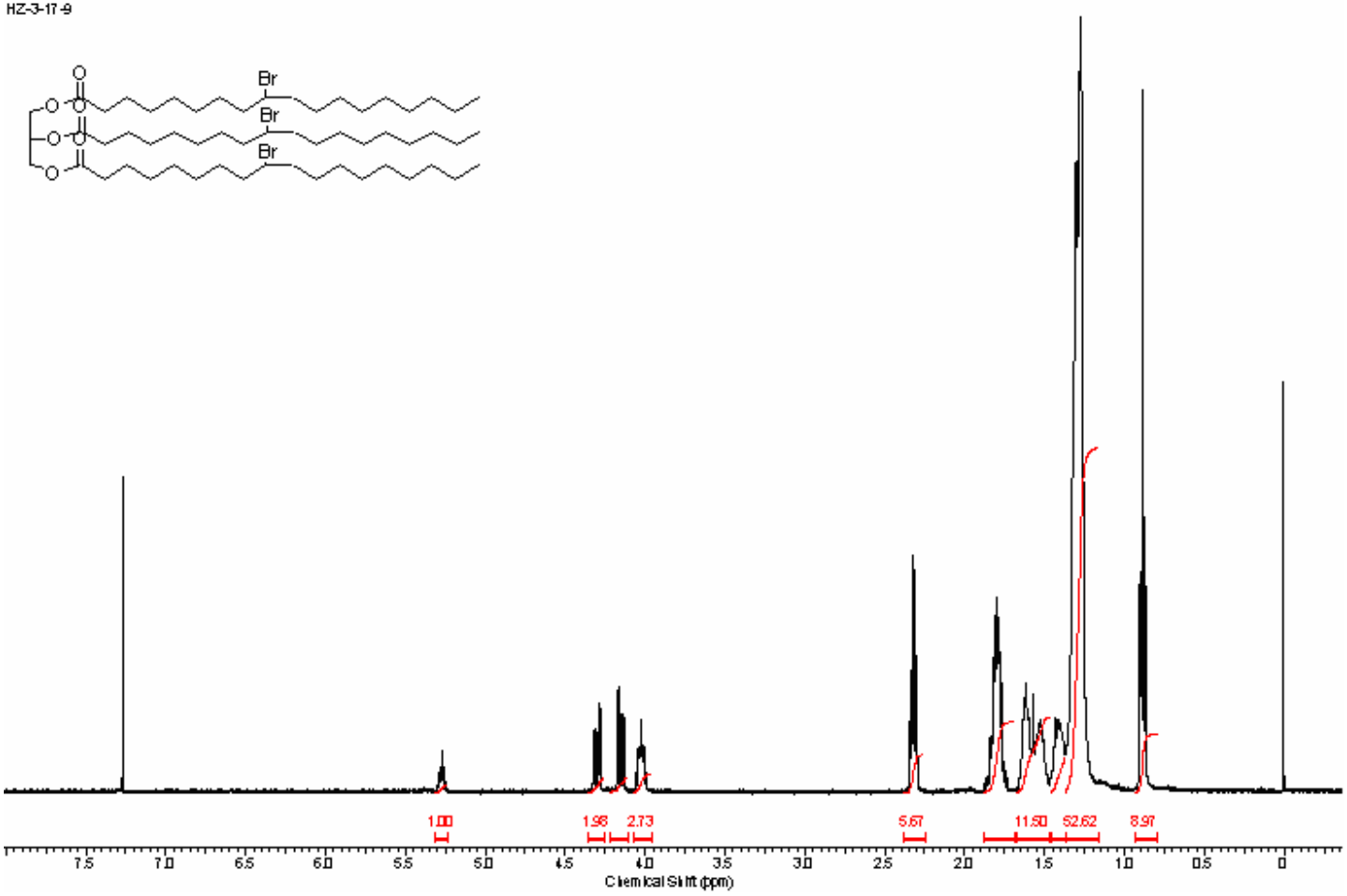
Acquisition Time (sec)	1.1994	Comment	13C OBSERVE	Date	Mar 4 2005	Date Stamp	Mar 4 2005
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-3-DIOLPURE-13CNMR						
Frequency (MHz)	100.54	Nucleus	13C	Number of Transients	20000	Original Points Count	29984
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	20.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	9617.5332	Sweep Width (Hz)	25000.00	Temperature (degree C)	29.000		

HZ-3-DIOLPURE-13CNMR



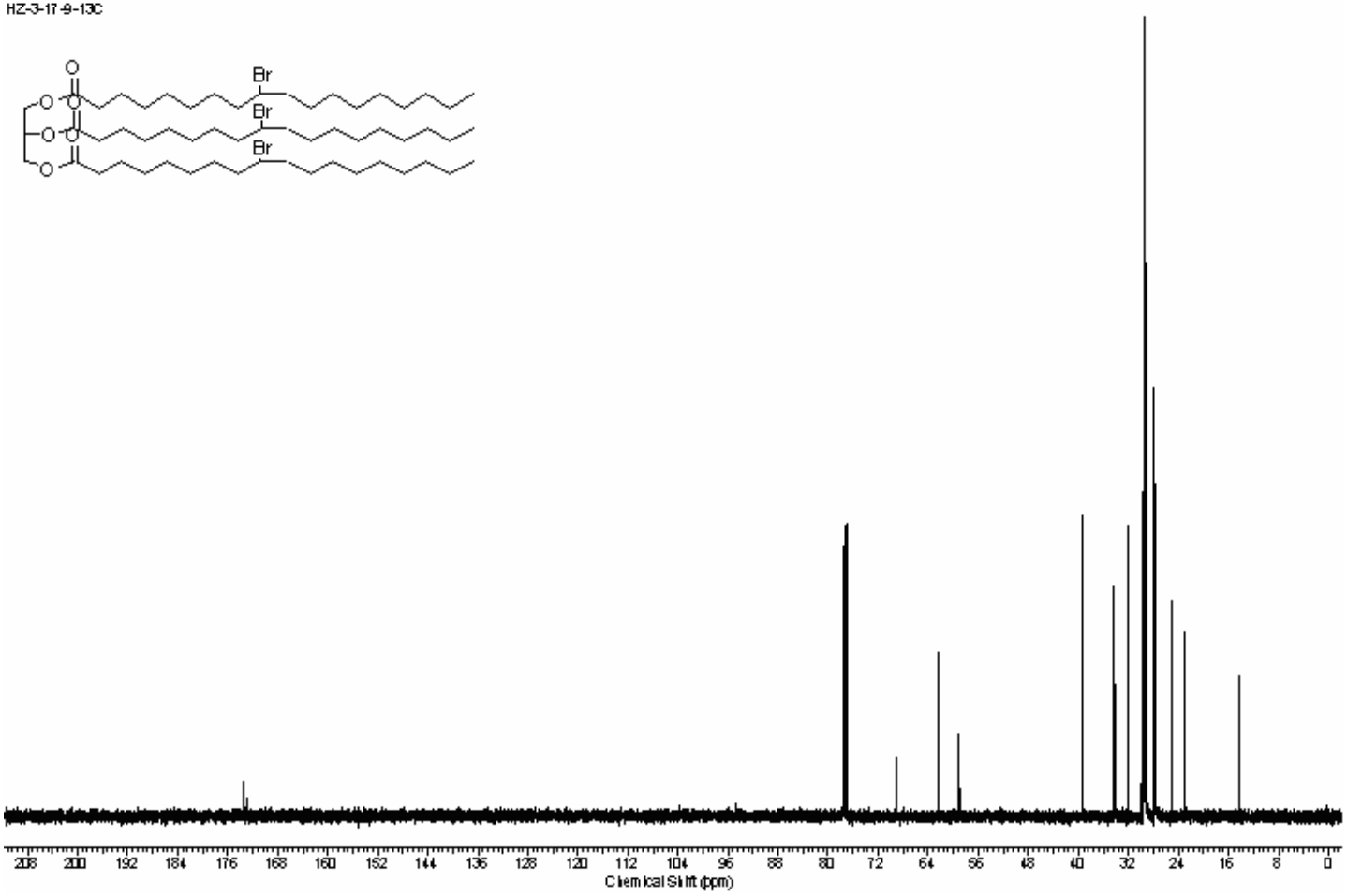
Acquisition Time (sec)	3.7010	Comment	STANDARD 1H OBSERVE			Date	Jan 13 2005
Date Stamp	Jan 13 2005	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-3-17-9				
Frequency (MHz)	399.80	Nucleus	1H	Number of Transients	64	Original Points Count	22208
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2011.8737	Sweep Width (Hz)	6000.60	Temperature (degree C)	29.000		

HZ-3-17-9



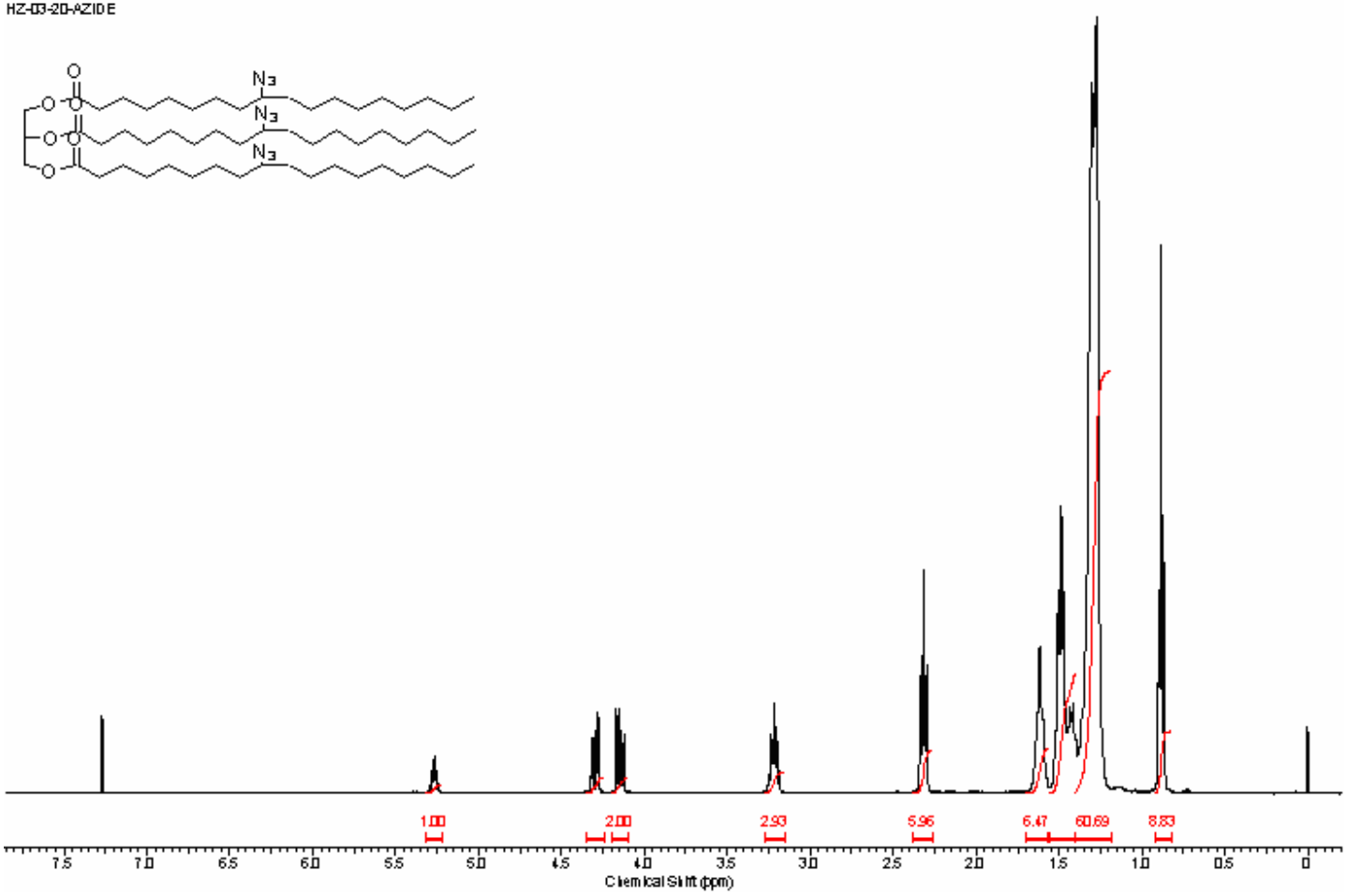
Acquisition Time (sec)	1.1994	*Comment*	13C OBSERVE	*Date*	Jan 13 2005	*Date Stamp*	Jan 13 2005
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-3-17-9-13C					*Frequency (MHz)*	100.54
Nucleus	13C	*Number of Transients*	20000	*Original Points Count*	29984	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	20.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	9516.7988	*Sweep Width (Hz)*	25000.00	*Temperature (degree C)*	29.000		

HZ-3-17-9-13C



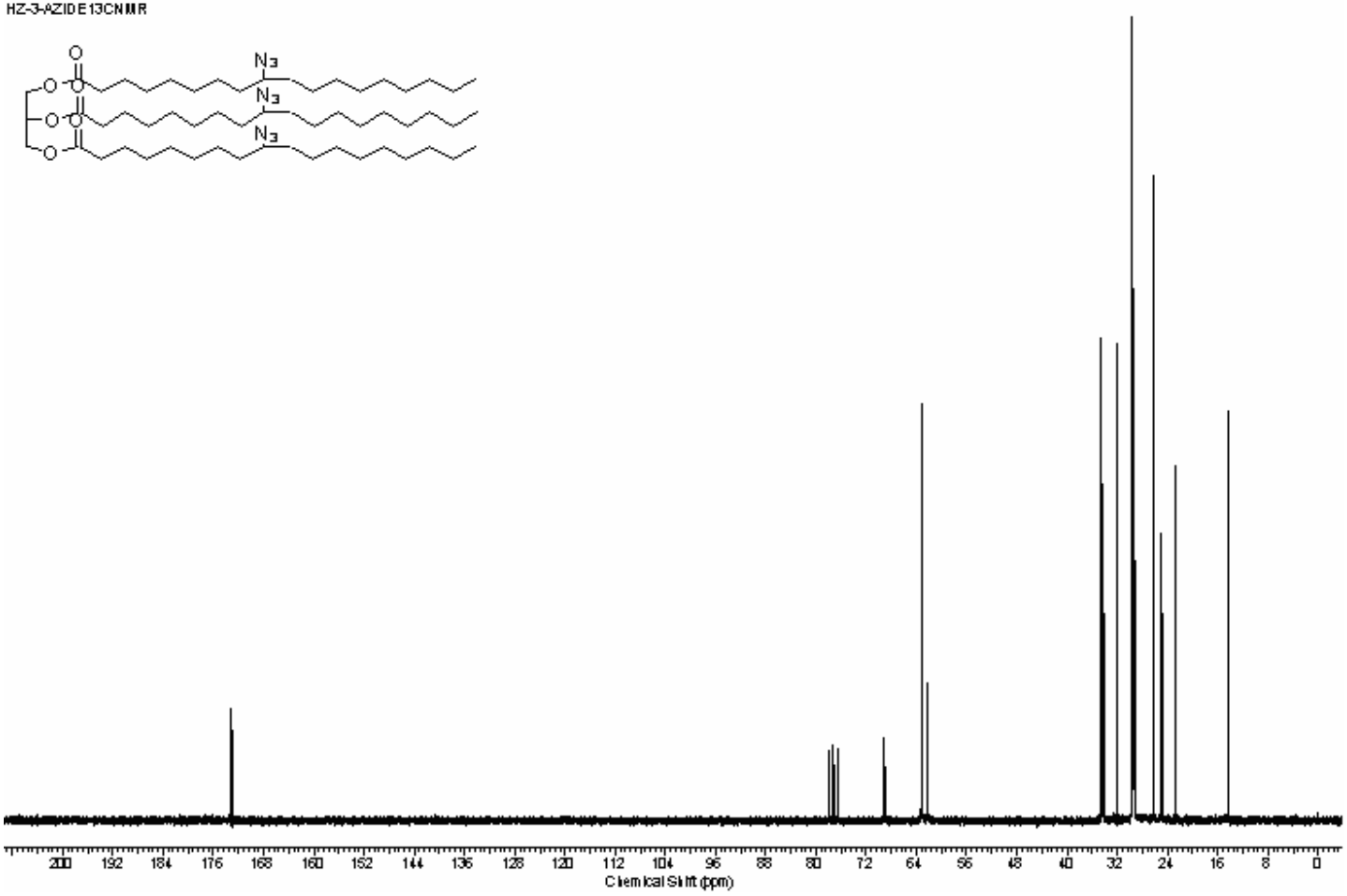
Acquisition Time (sec)	3.7010	Comment	STANDARD 1H OBSERVE			Date	Jan 18 2005
Date Stamp	Jan 18 2005	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-03-20-AZIDE				
Frequency (MHz)	399.80	Nucleus	1H	Number of Transients	64	Original Points Count	22208
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2010.2255	Sweep Width (Hz)	6000.60	Temperature (degree C)	29.000		

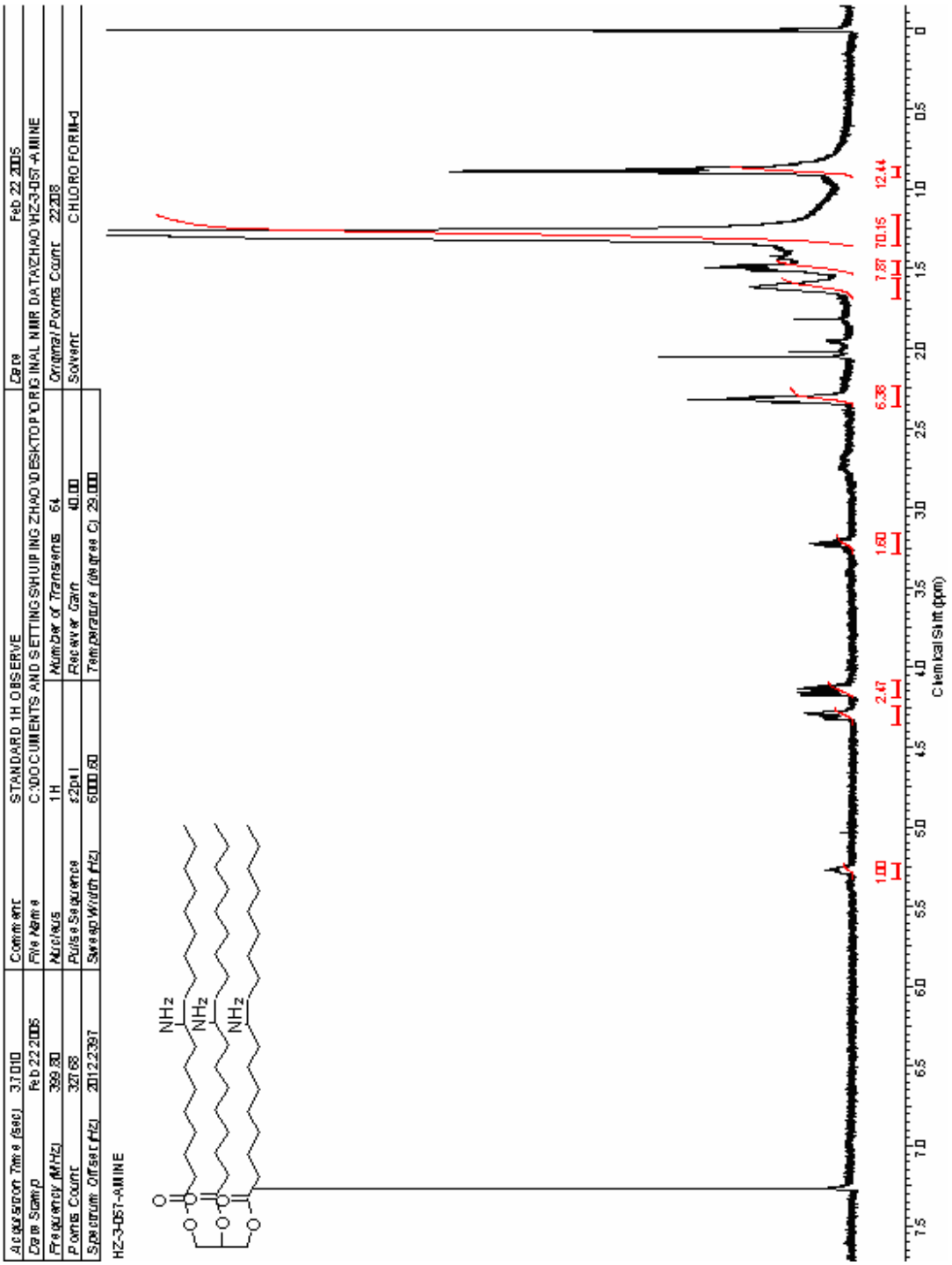
HZ-03-20-AZIDE



Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Mar 1 2005	*Date Stamp*	Mar 1 2005
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-200MHZ\HZ-3-AZIDE13CNMR						
Frequency (MHz)	50.29	*Nucleus*	13C	*Number of Transients*	20000	*Original Points Count*	16720
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	4879.6978	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

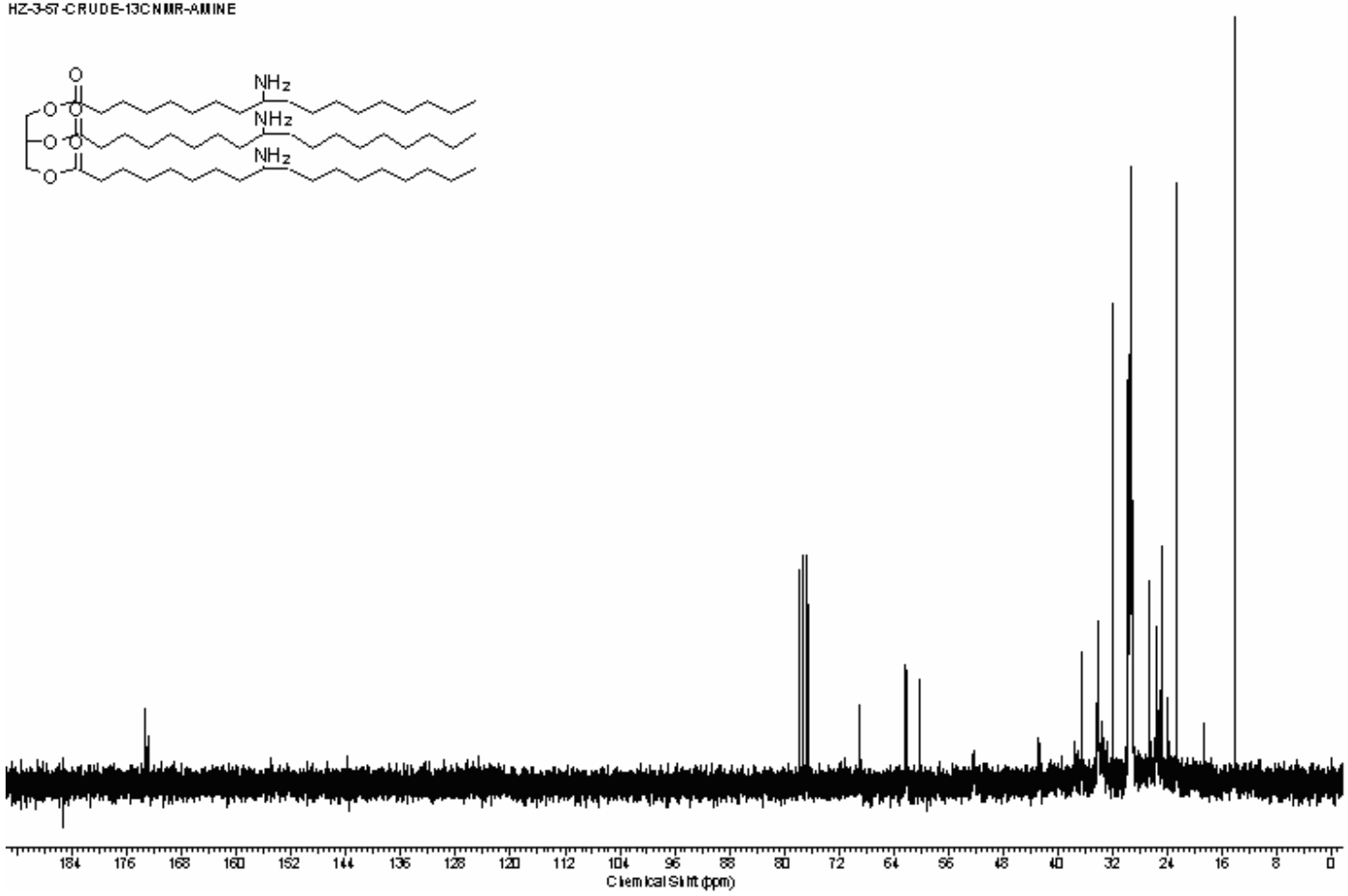
HZ-3-AZIDE13CNMR




Acquisition Time (sec) 3.7010
Comment STANDARD 1H OBSERVE
Date
Feb 22 2005
Date Stamp Feb 22 2005
File Name C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO HZ-3-057-4-MINE
Frequency (MHz) 399.80
Nucleus 1H
Number of Transients 64
Original Points Count 22308
Points Count 32768
Pulse Sequence s2pul
Receiver Gain 40.00
Solvent CHLOROFORM-d
Spectrum Offset (Hz) 2012.2397
Sweep Width (Hz) 6000.60
Temperature (degree C) 29.000
HZ-3-057-4-MINE
NH2
NH2
NH2
1.00
2.47
1.60
6.38
7.87
10.15
12.44
Chemical Shift (ppm)

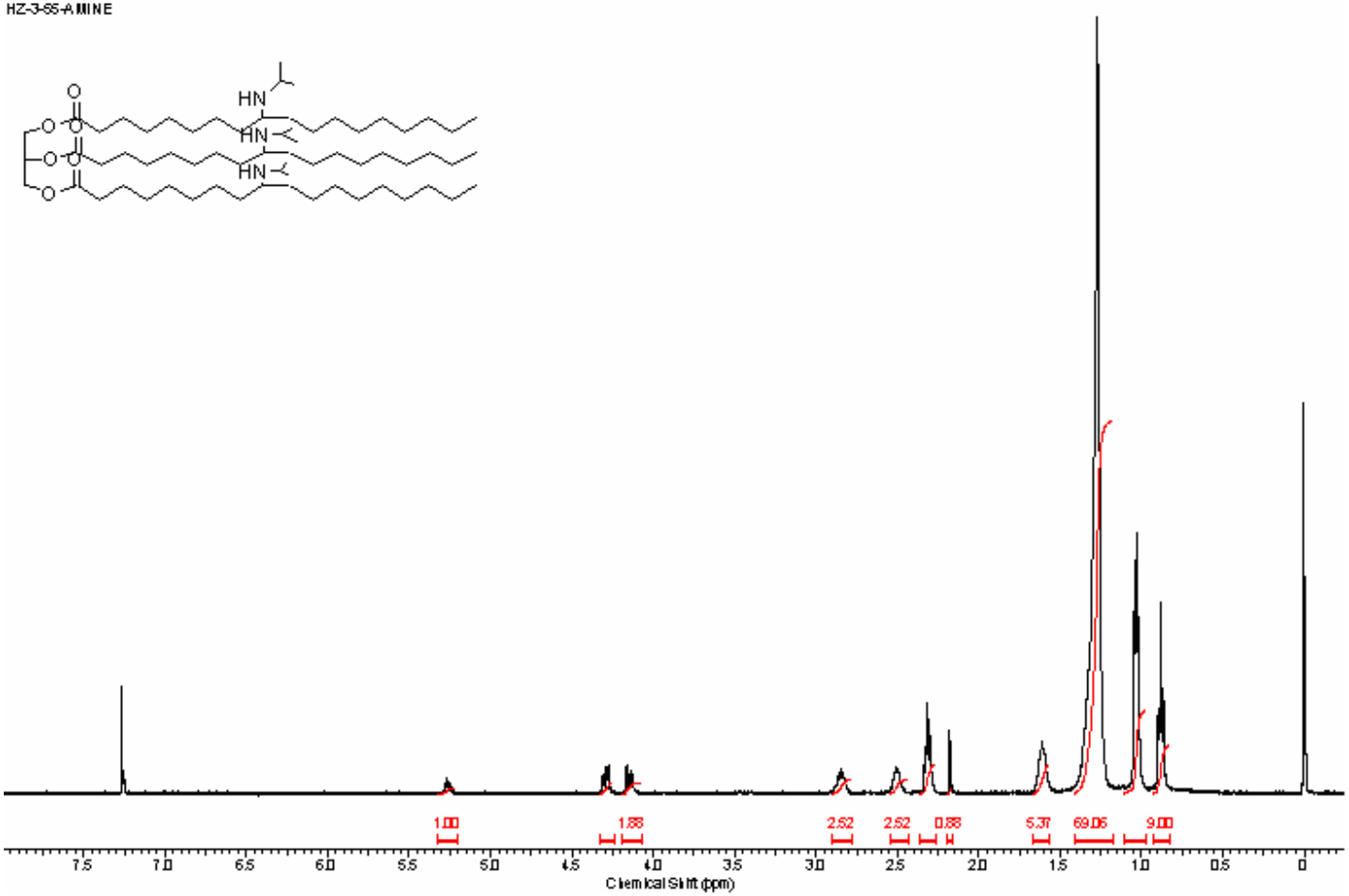
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Feb 24 2005	*Date Stamp*	Feb 24 2005
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-200MHZ\HZ-3-67-CRUDE-13C NMR-AMINE						
Frequency (MHz)	50.29	*Nucleus*	13C	*Number of Transients*	20000	*Original Points Count*	18720
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	4878.5537	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-3-67-CRUDE-13C NMR-AMINE



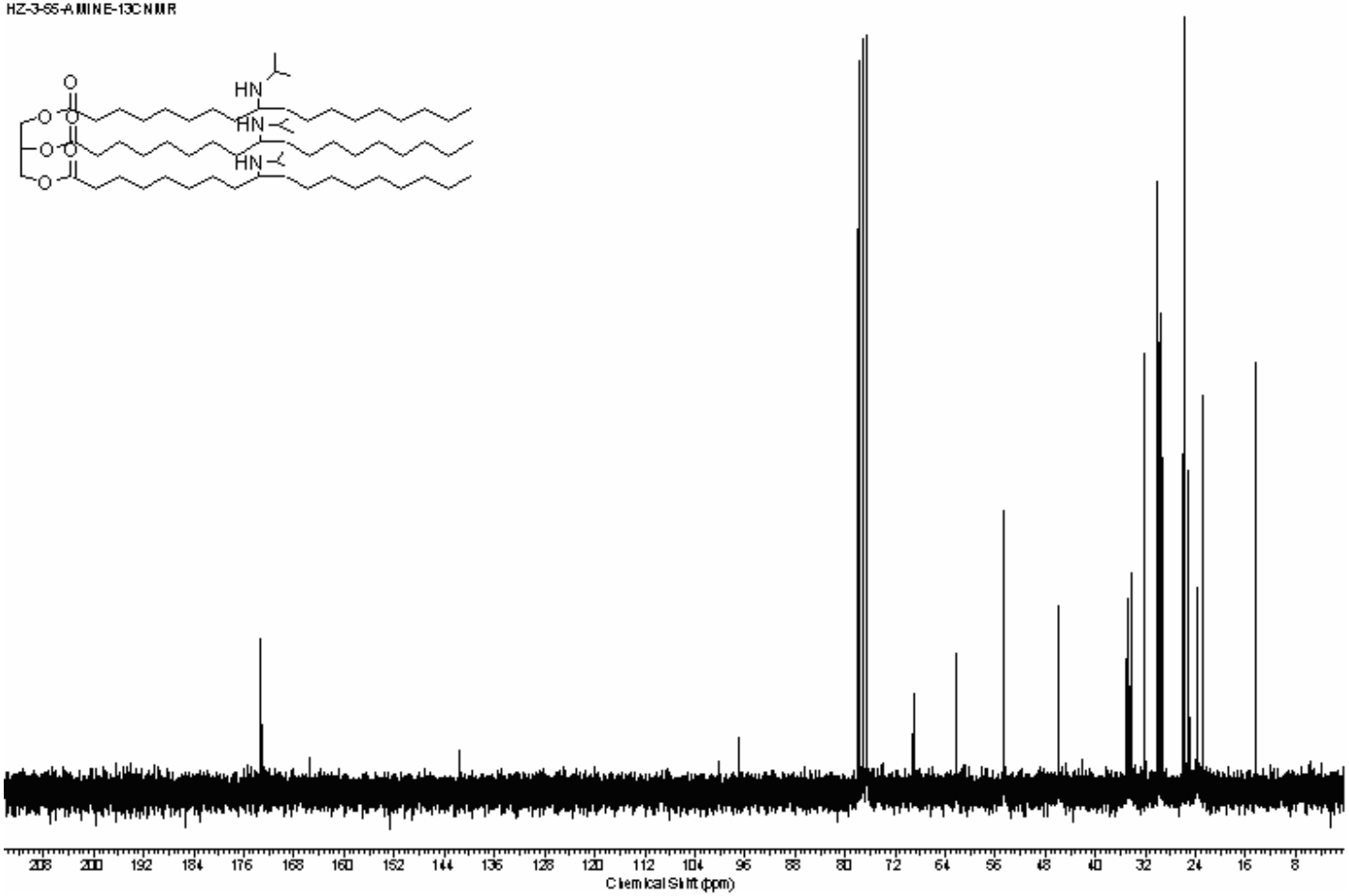
Acquisition Time (sec)	3.7010	Comment	STANDARD 1H OBSERVE			Date	Feb 21 2005
Date Stamp	Feb 21 2005	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-3-65-AMINE				
Frequency (MHz)	399.80	Nucleus	1H	Number of Transients	64	Original Points Count	22208
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2015.1698	Sweep Width (Hz)	6000.60	Temperature (degree C)	29.000		

HZ-3-65-AMINE



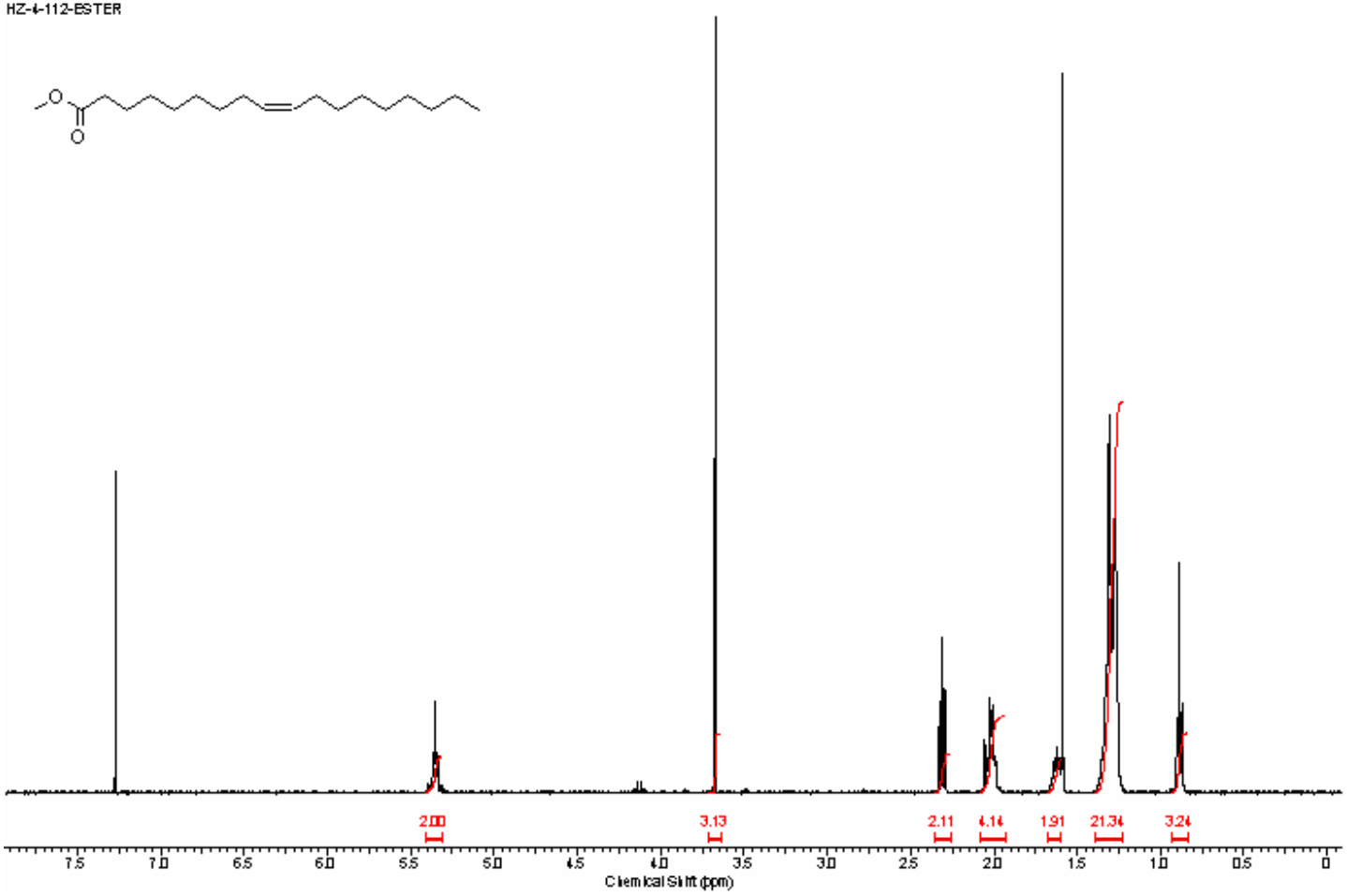
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Feb 21 2005	*Date Stamp*	Feb 21 2005
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-200MHZ\HZ-3-65-AMINE-13C NMR						
Frequency (MHz)	50.29	*Nucleus*	13C	*Number of Transients*	20000	*Original Points Count*	18720
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	4881.9868	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-3-65-AMINE-13C NMR



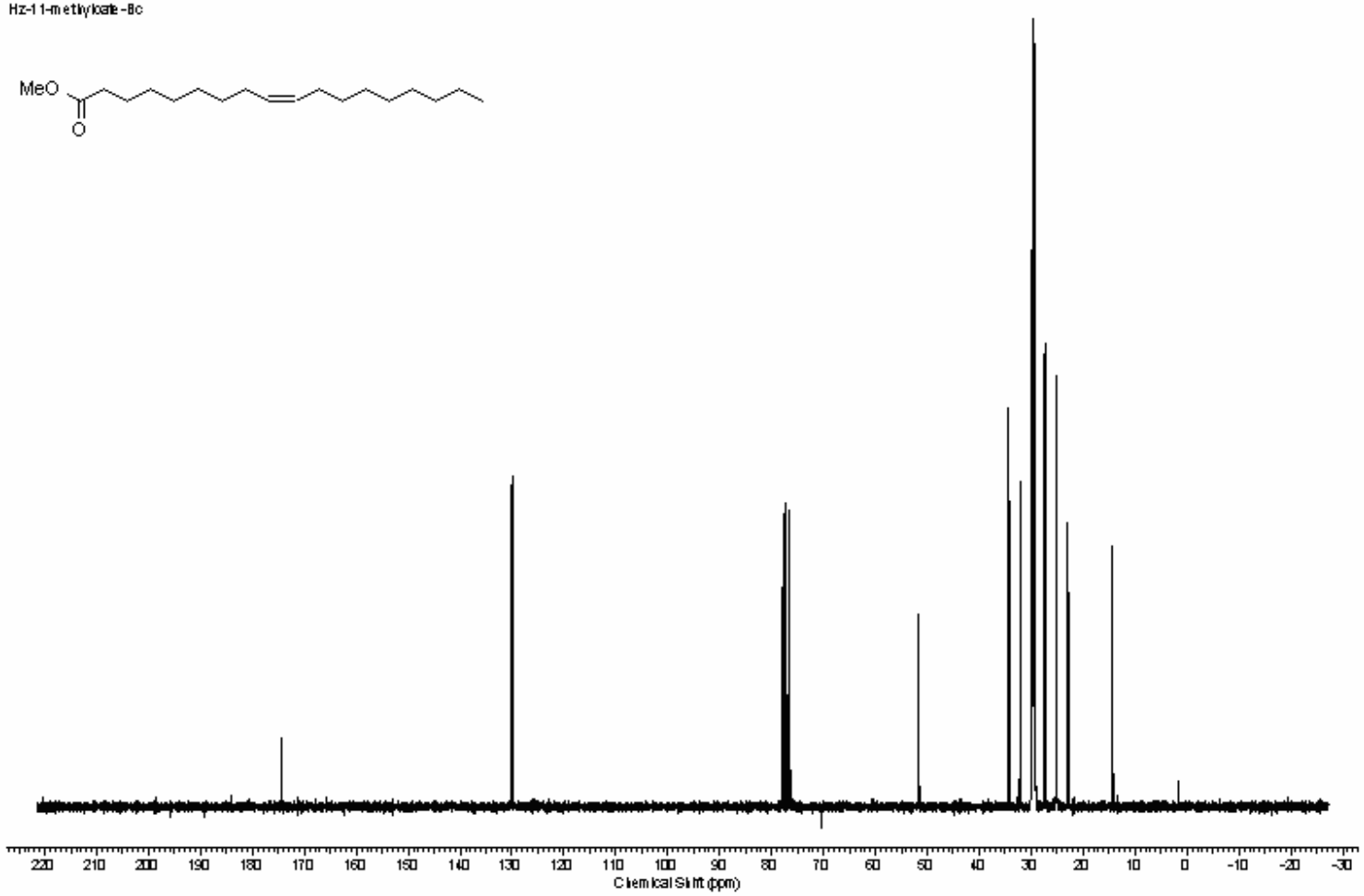
Acquisition Time (sec)	3.7010	Comment	STANDARD 1H OBSERVE			Date	Sep 20 2005
Date Stamp	Sep 20 2005	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-4-112-ESTER				
Frequency (MHz)	399.79	Nucleus	1H	Number of Transients	64	Original Points Count	22208
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2005.2832	Sweep Width (Hz)	6000.60	Temperature (degree C)	29.000		

HZ-4-112-ESTER



Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE			*Date*	Apr 1 2008	*Date Stamp*	Apr 1 2008
File Name	E:\HZ-11\Hz-11-methylcate-8c			*Frequency (MHz)*	50.29	*Nucleus*	13C	*Number of Transients*	64000
Original Points Count	18720	*Points Count*	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	40.00		
Solvent	CHLOROFORM-d			*Spectrum Offset (Hz)*	4881.2241	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000

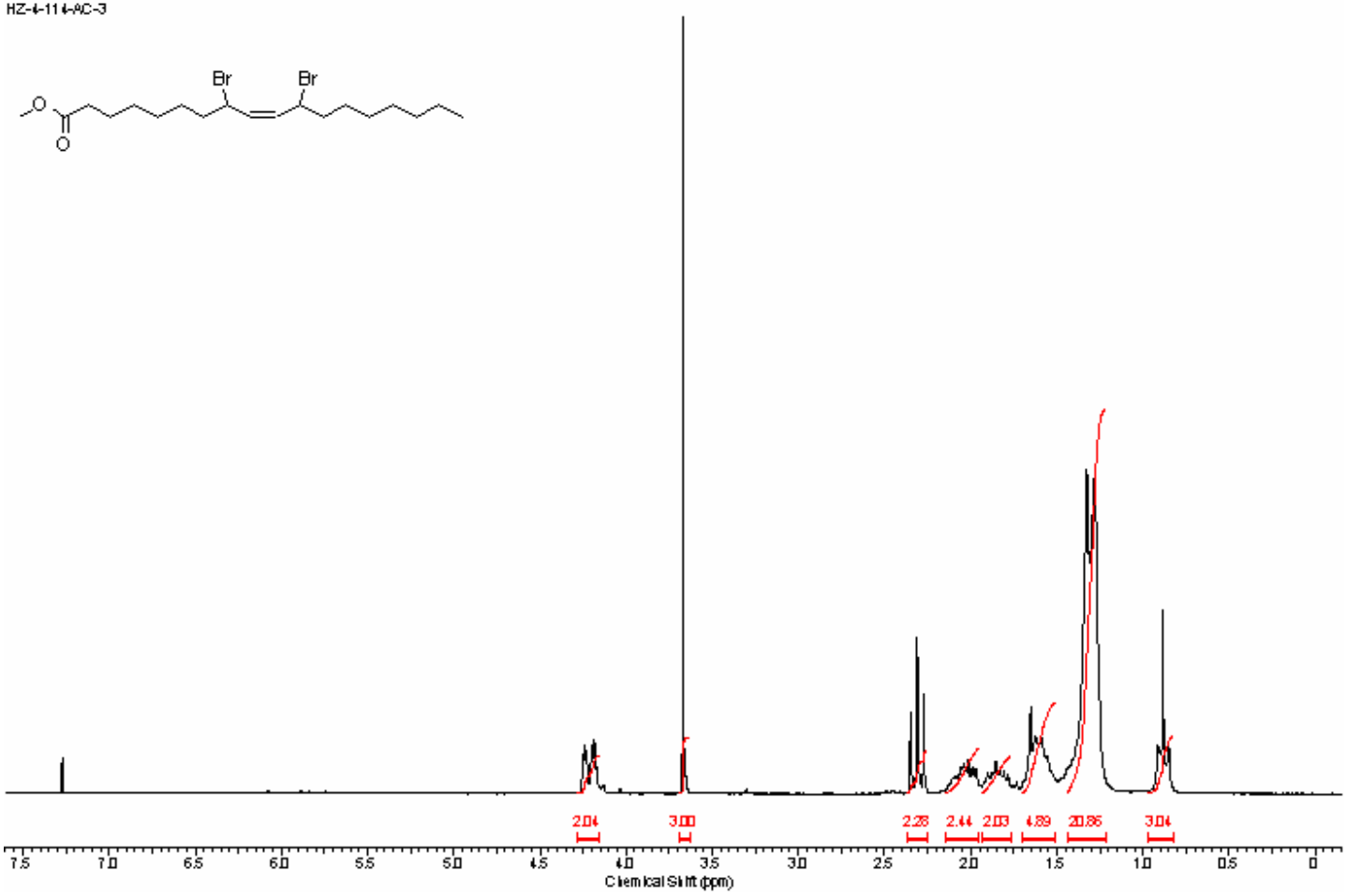
Hz-11-methylcate-8c



E:\HZ-11\Hz-11-methylcate-8c

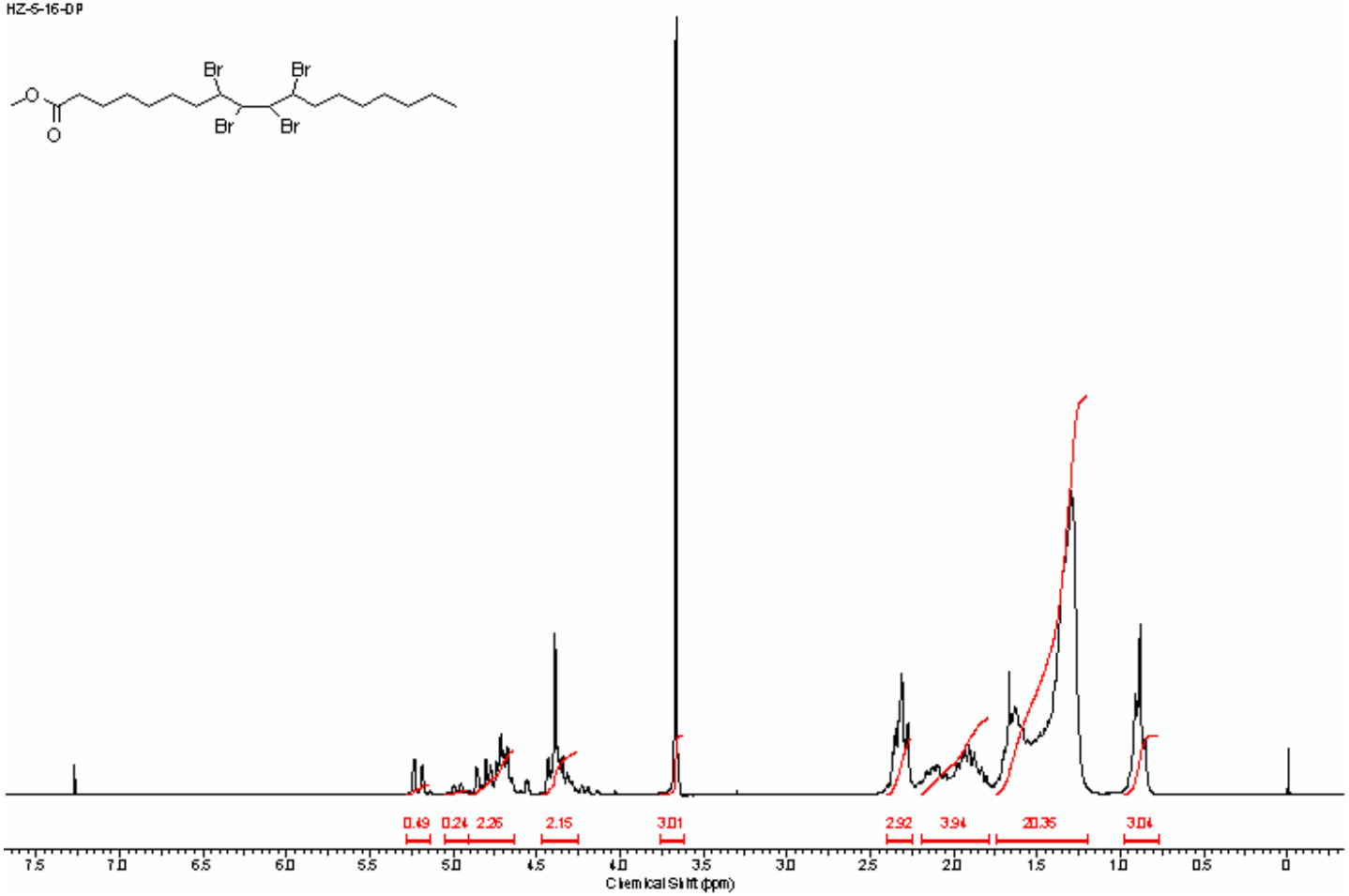
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Sep 23 2005
Date Stamp	Sep 23 2005	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-200MHZ\HZ-4-114-AC-3				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	12.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1002.3816	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-4-114-AC-3



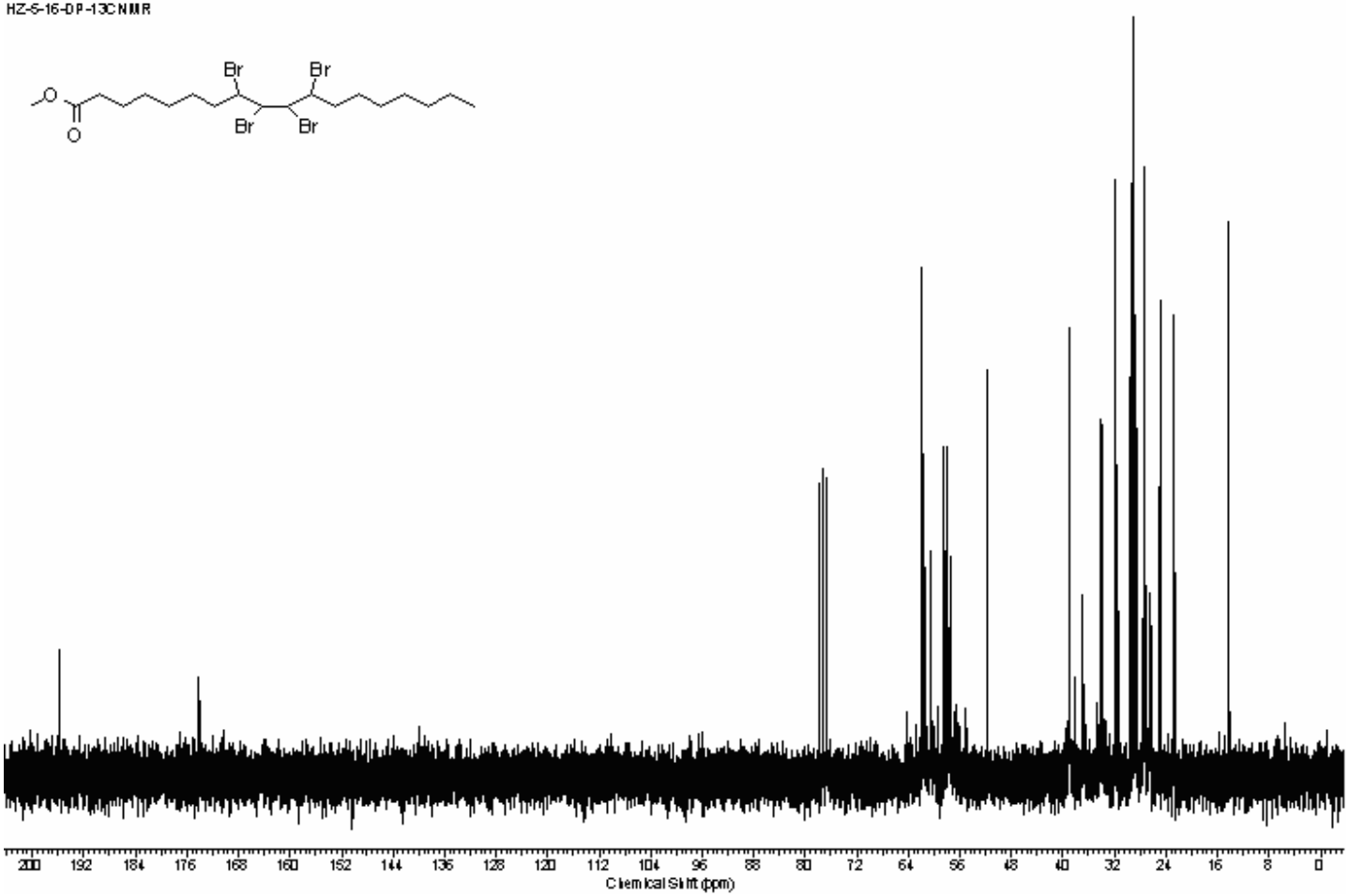
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Oct 10 2005
Date Stamp	Oct 10 2005	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-200MHZ\HZ-5-16-DP				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	128	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	11.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1002.0226	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-5-16-DP

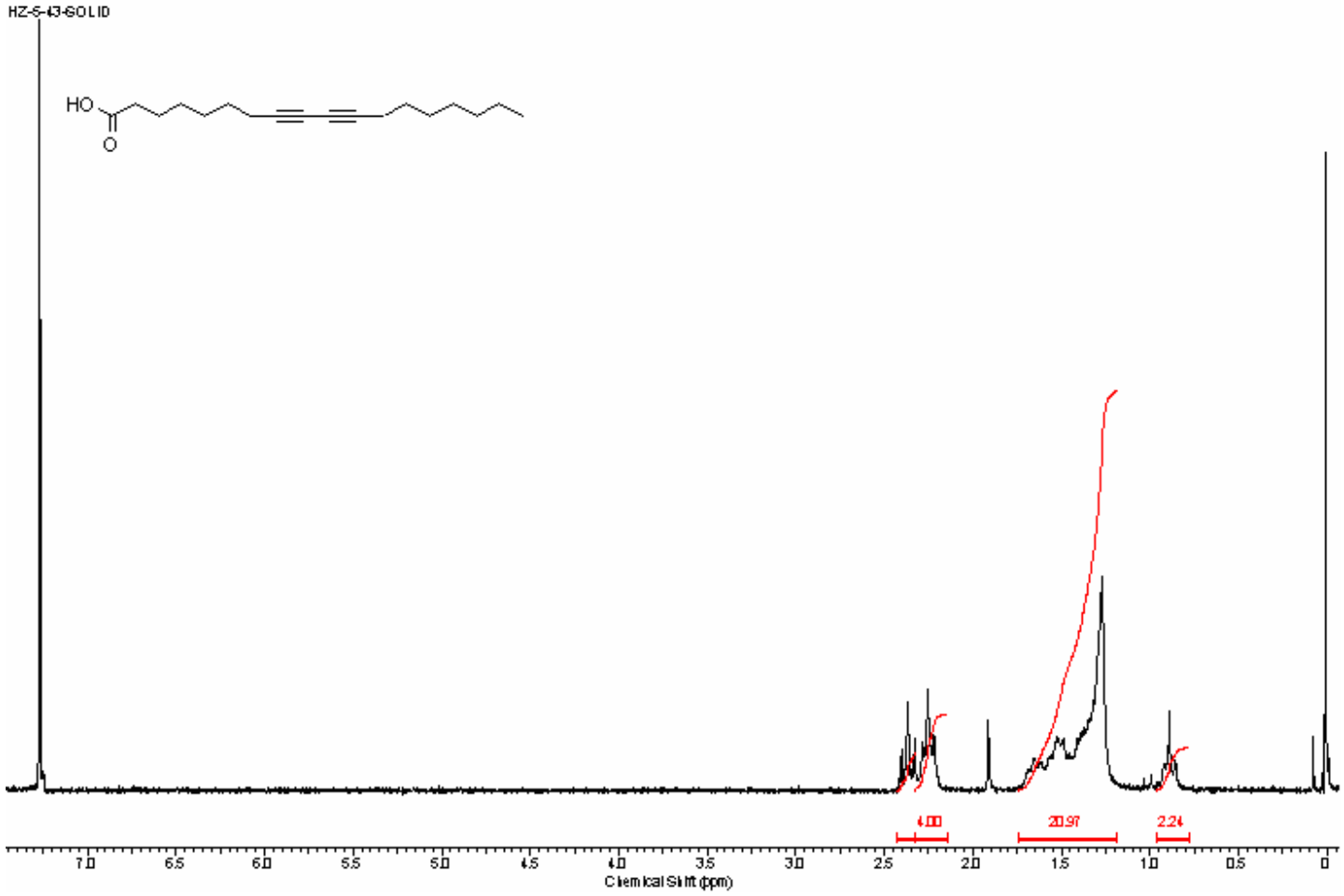


Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Oct 12 2005	*Date Stamp*	Oct 12 2005
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-200MHZ\HZ-6-16-DP-13CNMR						
Frequency (MHz)	50.29	*Nucleus*	13C	*Number of Transients*	60000	*Original Points Count*	18720
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	4879.3164	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-6-16-DP-13CNMR

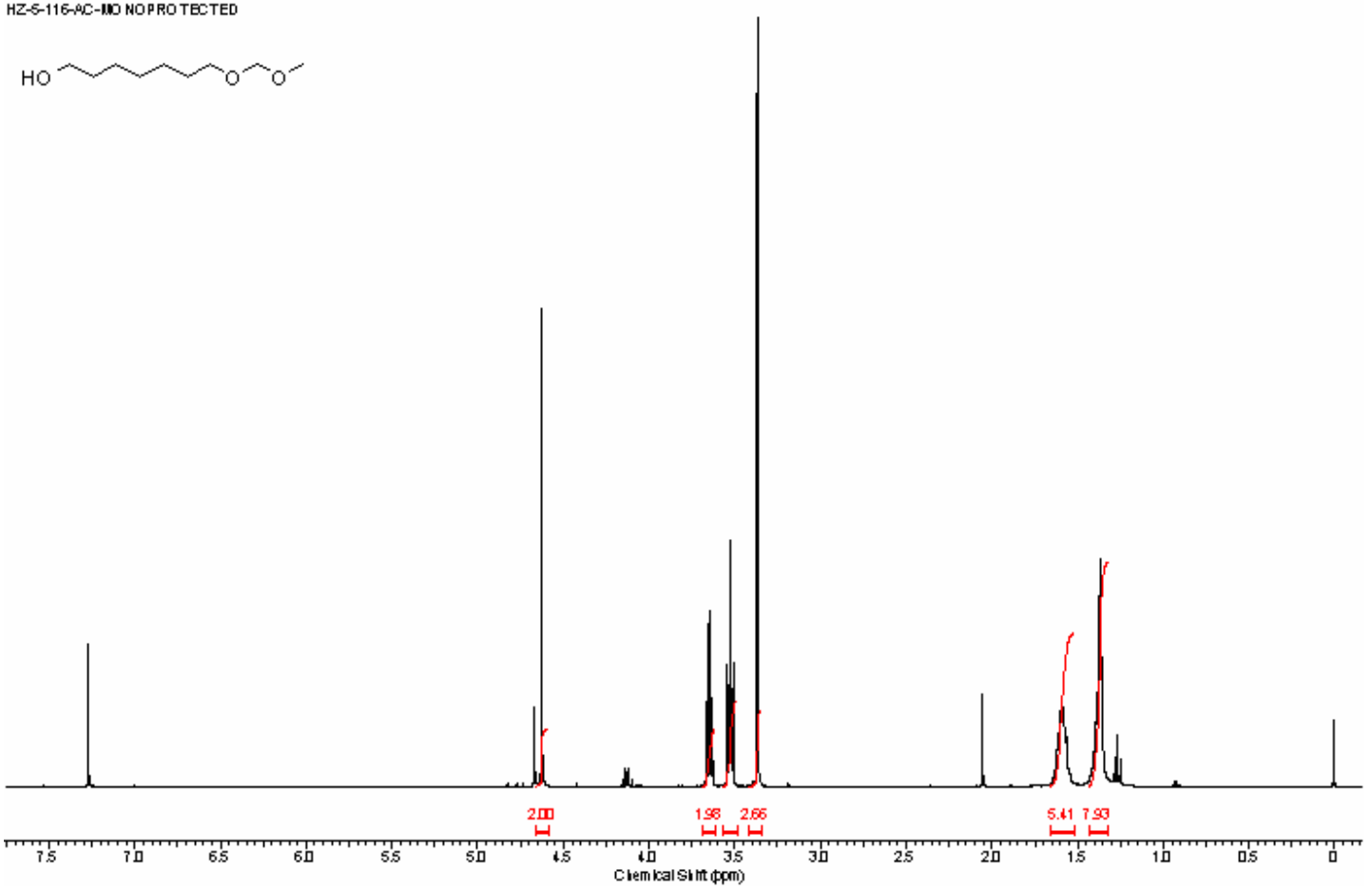


Acquisition Time (sec)	1.9945	*Comment*	STANDARD 1H OBSERVE			*Date*	Dec 7 2005
Date Stamp	Dec 7 2005	*File Name*	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\HZ-200MHZ\HZ-6-43-SOLID				
Frequency (MHz)	199.98	*Nucleus*	1H	*Number of Transients*	64	*Original Points Count*	5984
Points Count	8192	*Pulse Sequence*	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	1002.0226	*Sweep Width (Hz)*	3000.30	*Temperature (degree C)*	29.000		



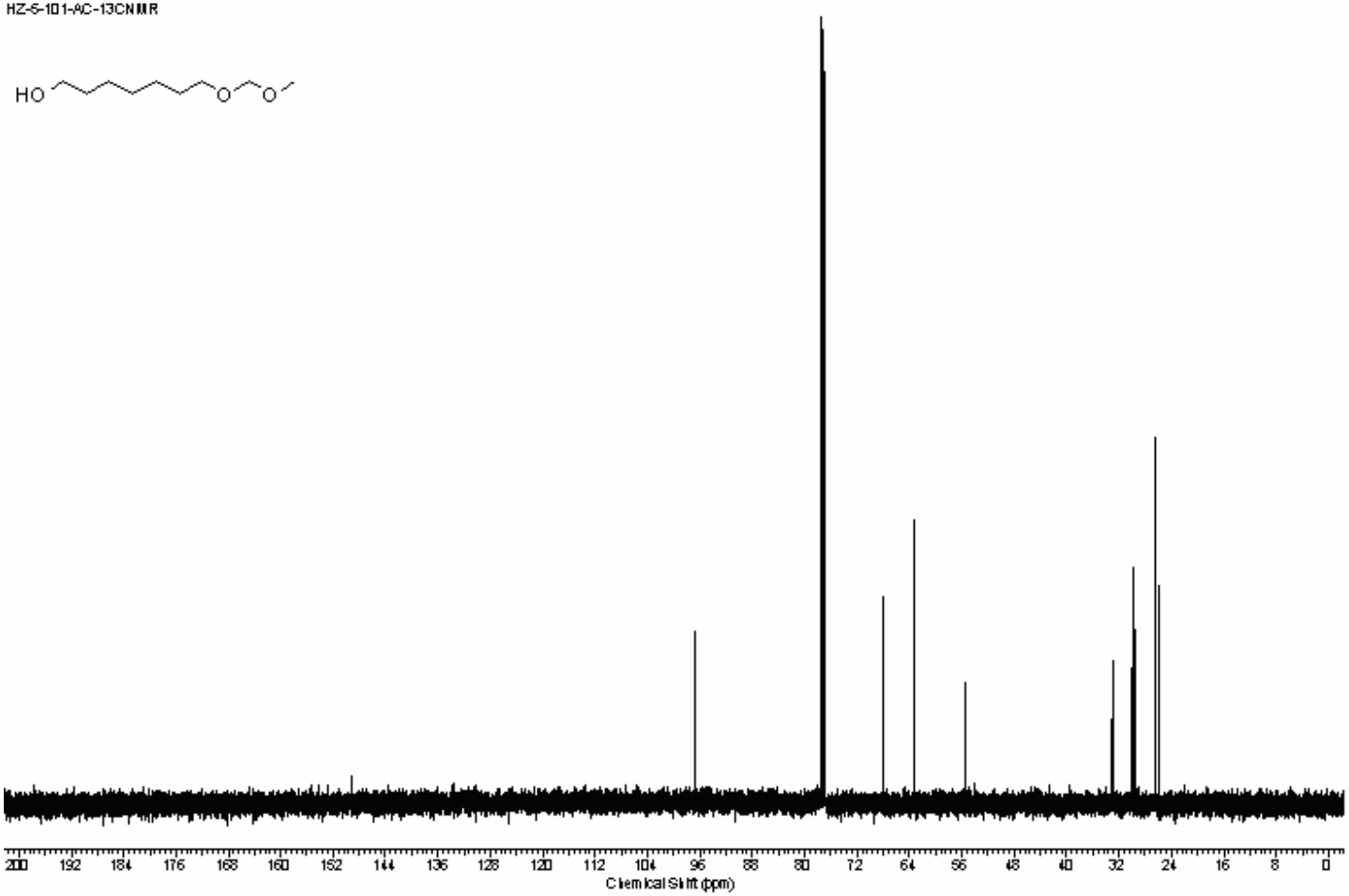
Acquisition Time (sec)	3.7010	Comment	STANDARD 1H OBSERVE			Date	Feb 6 2006
Date Stamp	Feb 6 2006						
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-6-116-AC-MO NO PROTECTED						
Frequency (MHz)	399.78	Nucleus	1H	Number of Transients	64	Original Points Count	22208
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2007.3364	Sweep Width (Hz)	6000.60	Temperature (degree C)	29.000		

HZ-6-116-AC-MO NO PROTECTED



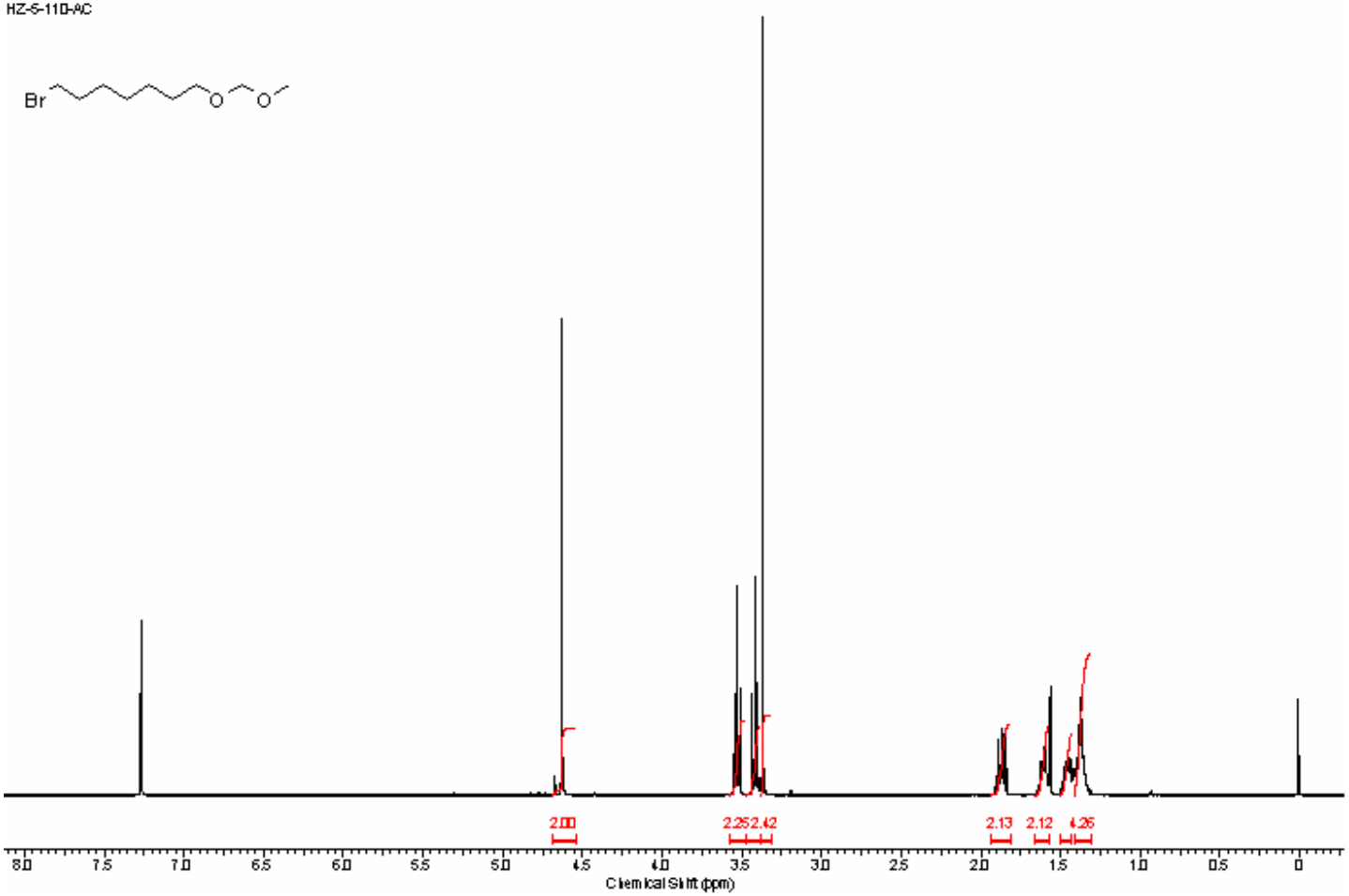
Acquisition Time (sec)	1.1994	Comment	13C OBSERVE	Date	Feb 13 2006	Date Stamp	Feb 13 2006
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-6-101-AC-13C NMR						
Frequency (MHz)	100.53	Nucleus	13C	Number of Transients	64000	Original Points Count	29984
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	20.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	9617.1533	Sweep Width (Hz)	29000.00	Temperature (degree C)	29.000		

HZ-6-101-AC-13CNMR



Acquisition Time (sec)	3.7010	Comment	STANDARD 1H OBSERVE			Date	Feb 3 2006
Date Stamp	Feb 3 2006	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-6-110-AC				
Frequency (MHz)	399.78	Nucleus	1H	Number of Transients	64	Original Points Count	22208
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2007.1533	Sweep Width (Hz)	6000.60	Temperature (degree C)	29.000		

HZ-6-110-AC

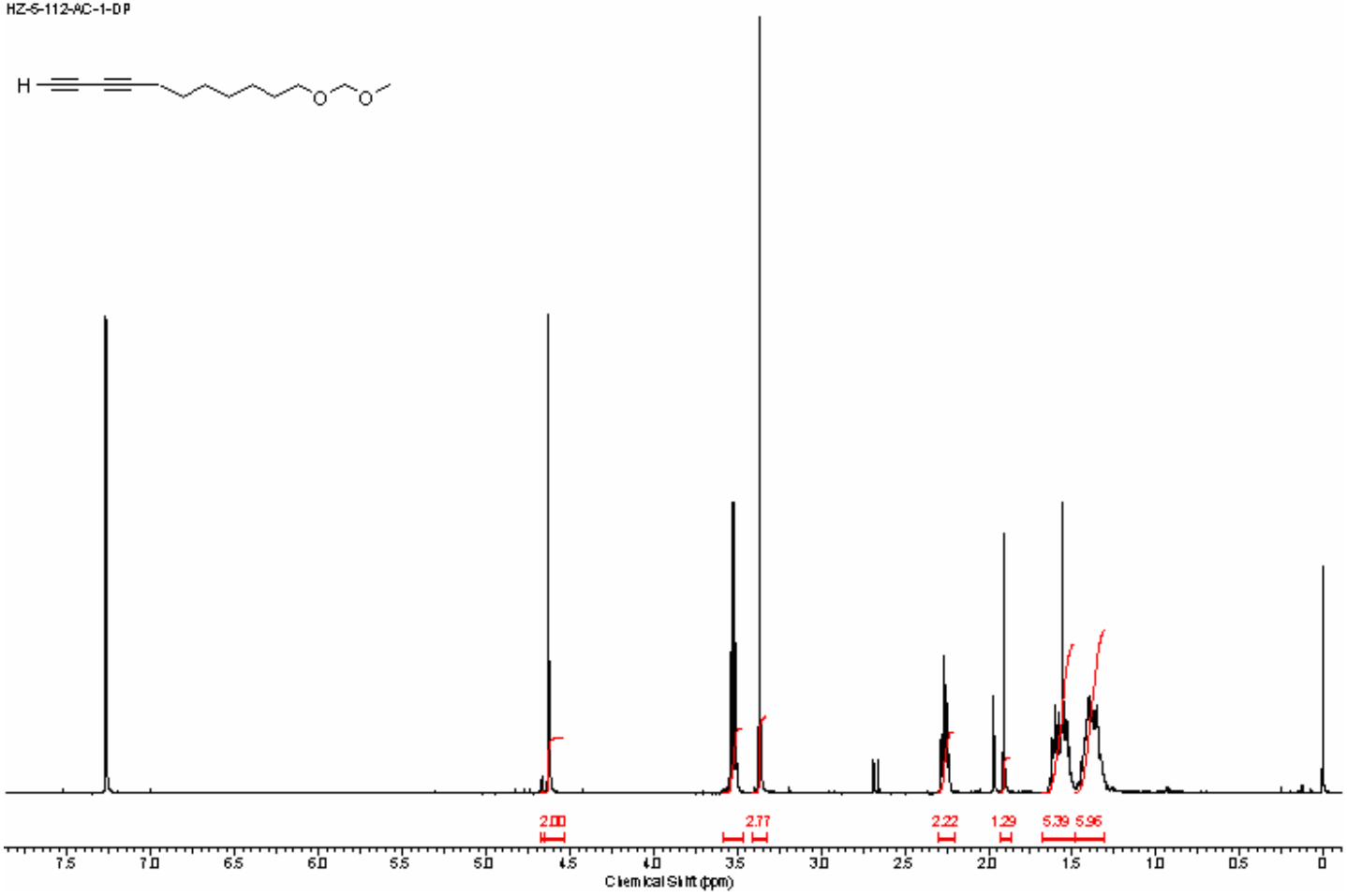


Acquisition Time (sec)	1.1994	*Comment*	13C OBSERVE	*Date*	Feb 12 2006	*Date Stamp*	Feb 12 2006
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-6-108-AC-13C						
Frequency (MHz)	100.53	*Nucleus*	13C	*Number of Transients*	64000	*Original Points Count*	29984
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	20.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	9517.9160	*Sweep Width (Hz)*	25000.00	*Temperature (degree C)*	29.000		

HZ-6-108-AC-13C

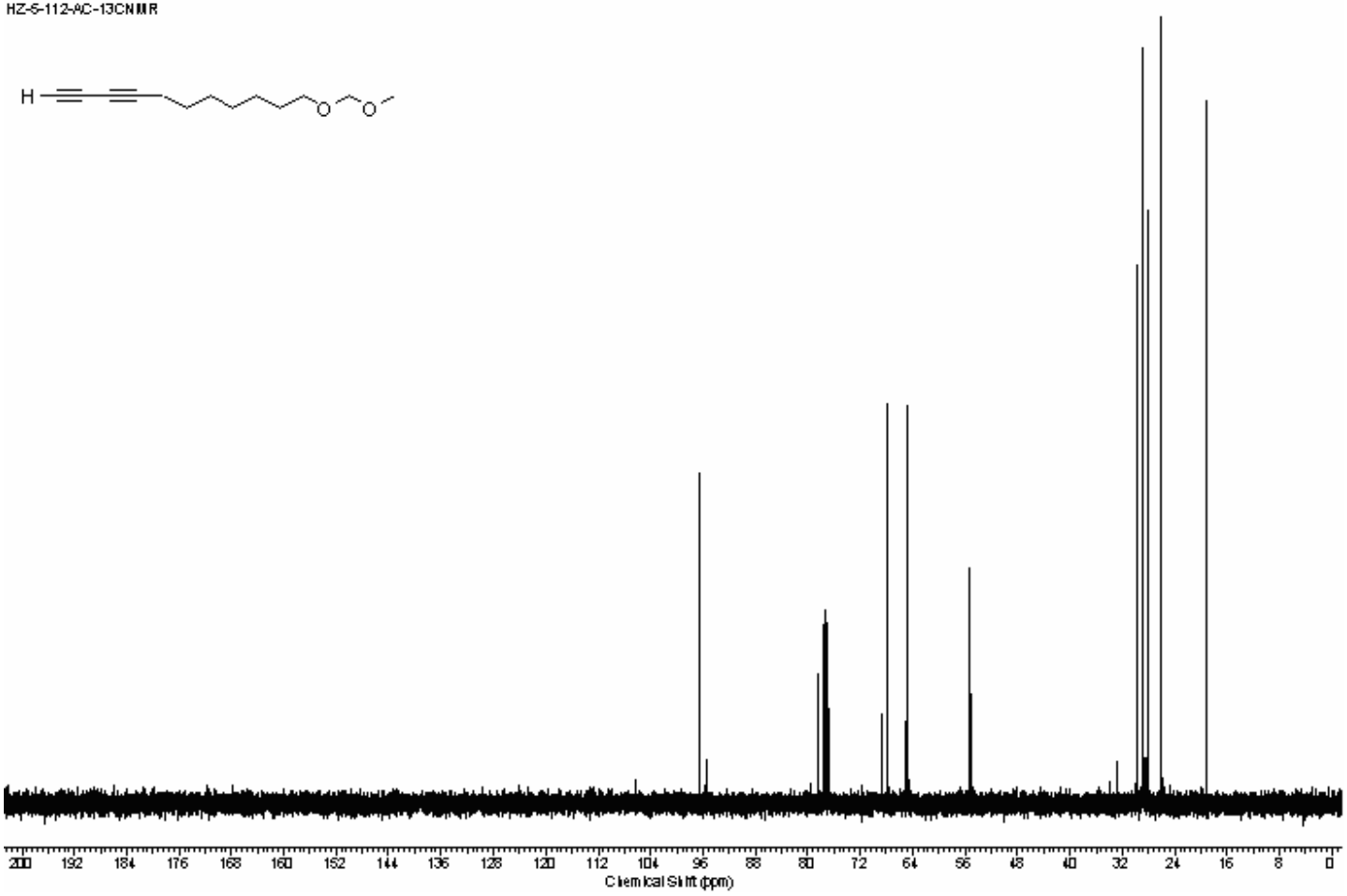
Acquisition Time (sec)	3.7010	*Comment*	STANDARD 1H OBSERVE			*Date*	Feb 5 2006
Date Stamp	Feb 5 2006	*File Name*	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-6-112-AC-1-DP				
Frequency (MHz)	399.78	*Nucleus*	1H	*Number of Transients*	64	*Original Points Count*	22208
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	2006.9702	*Sweep Width (Hz)*	6000.60	*Temperature (degree C)*	29.000		

HZ-6-112-AC-1-DP



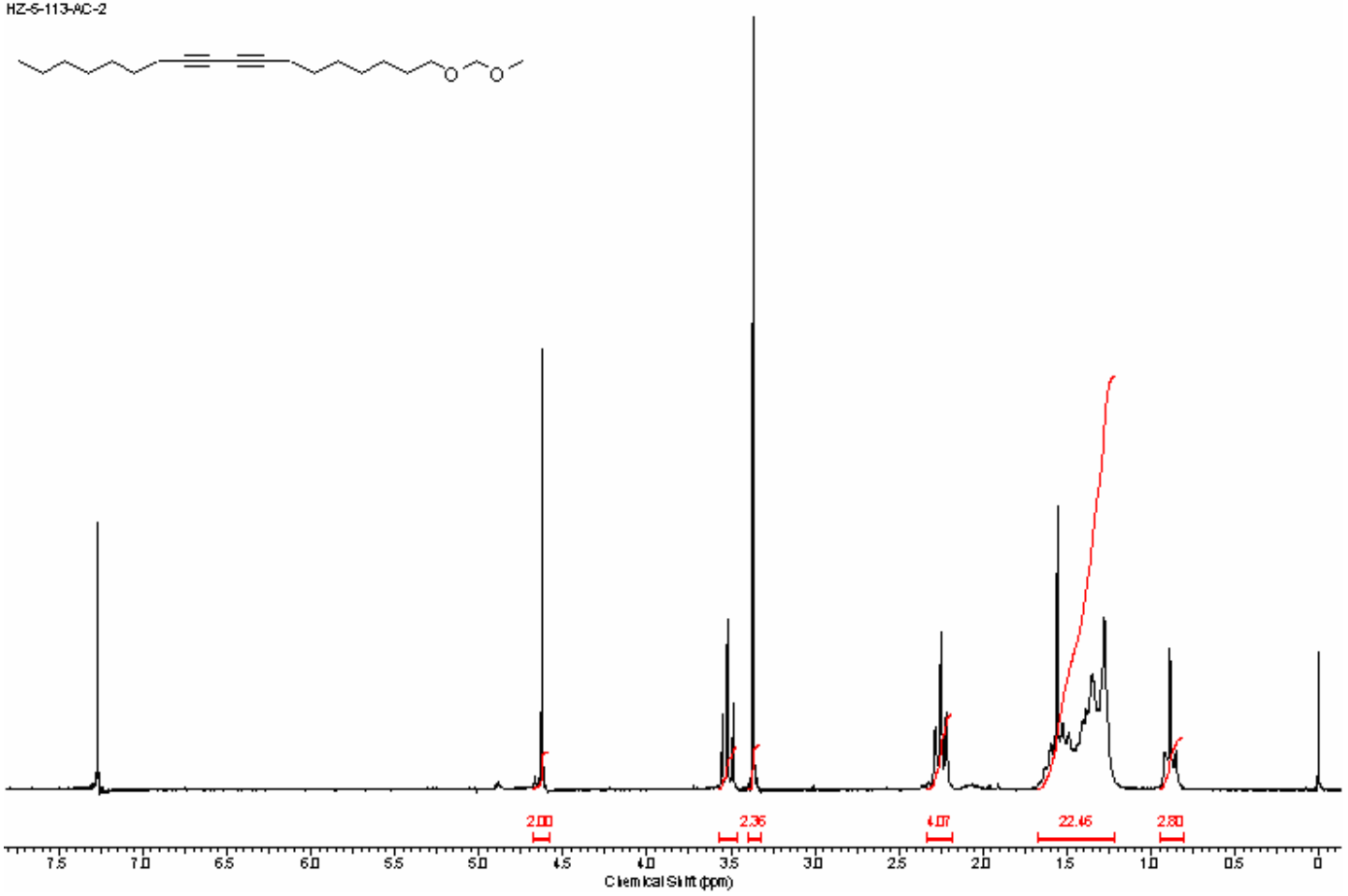
Acquisition Time (sec)	1.1994	Comment	13C OBSERVE	Date	Feb 13 2006	Date Stamp	Feb 13 2006
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-6-112-AC-13CNMR						
Frequency (MHz)	100.53	Nucleus	13C	Number of Transients	640.00	Original Points Count	29984
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	20.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	9511.8125	Sweep Width (Hz)	29000.00	Temperature (degree C)	29.000		

HZ-6-112-AC-13CNMR



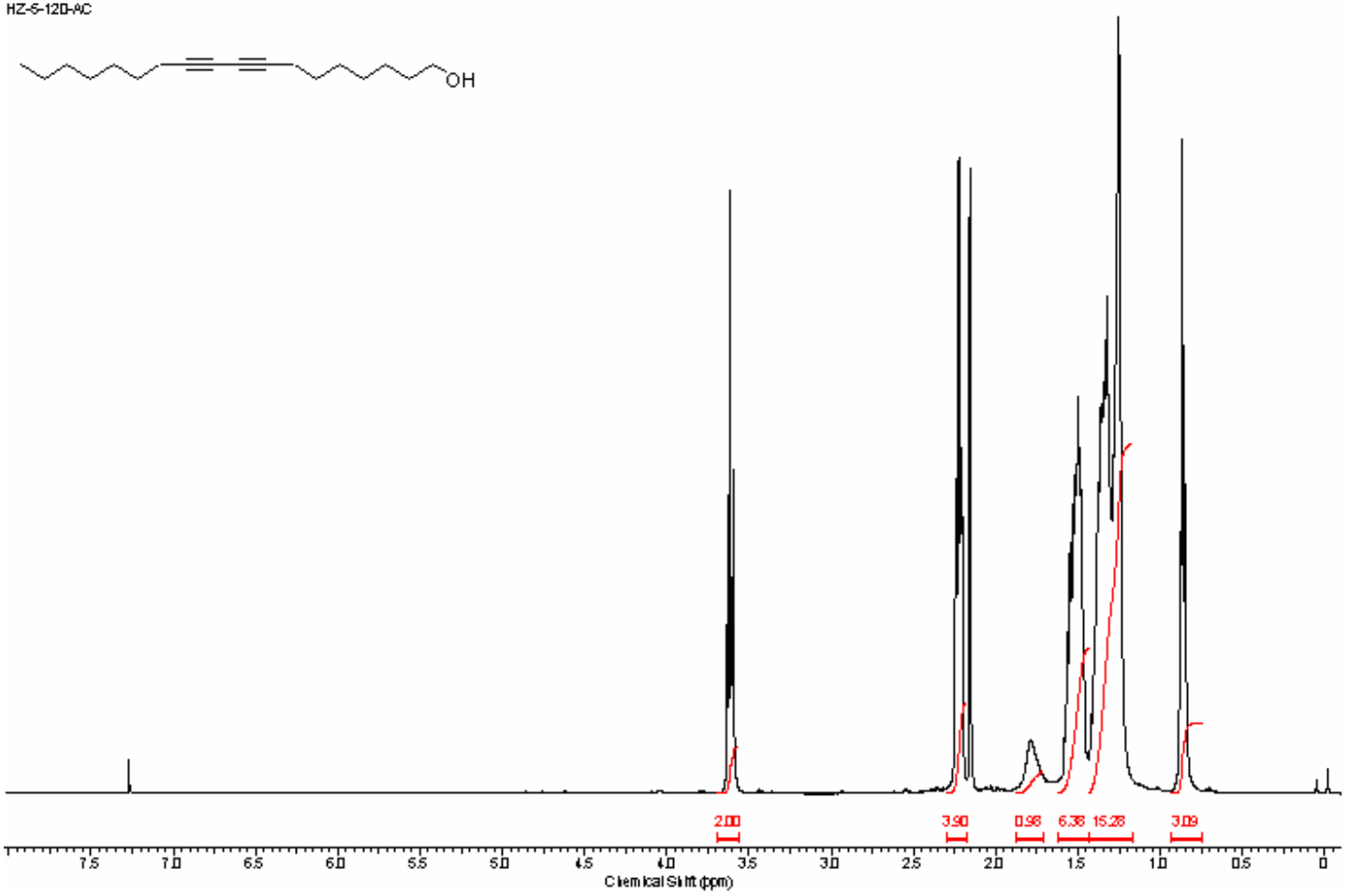
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Feb 6 2006
Date Stamp	Feb 6 2006	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-6-113-AC-2				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	8192	Pulse Sequence	s2pul	Receiver Gain	34.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1001.6563	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

HZ-6-113-AC-2



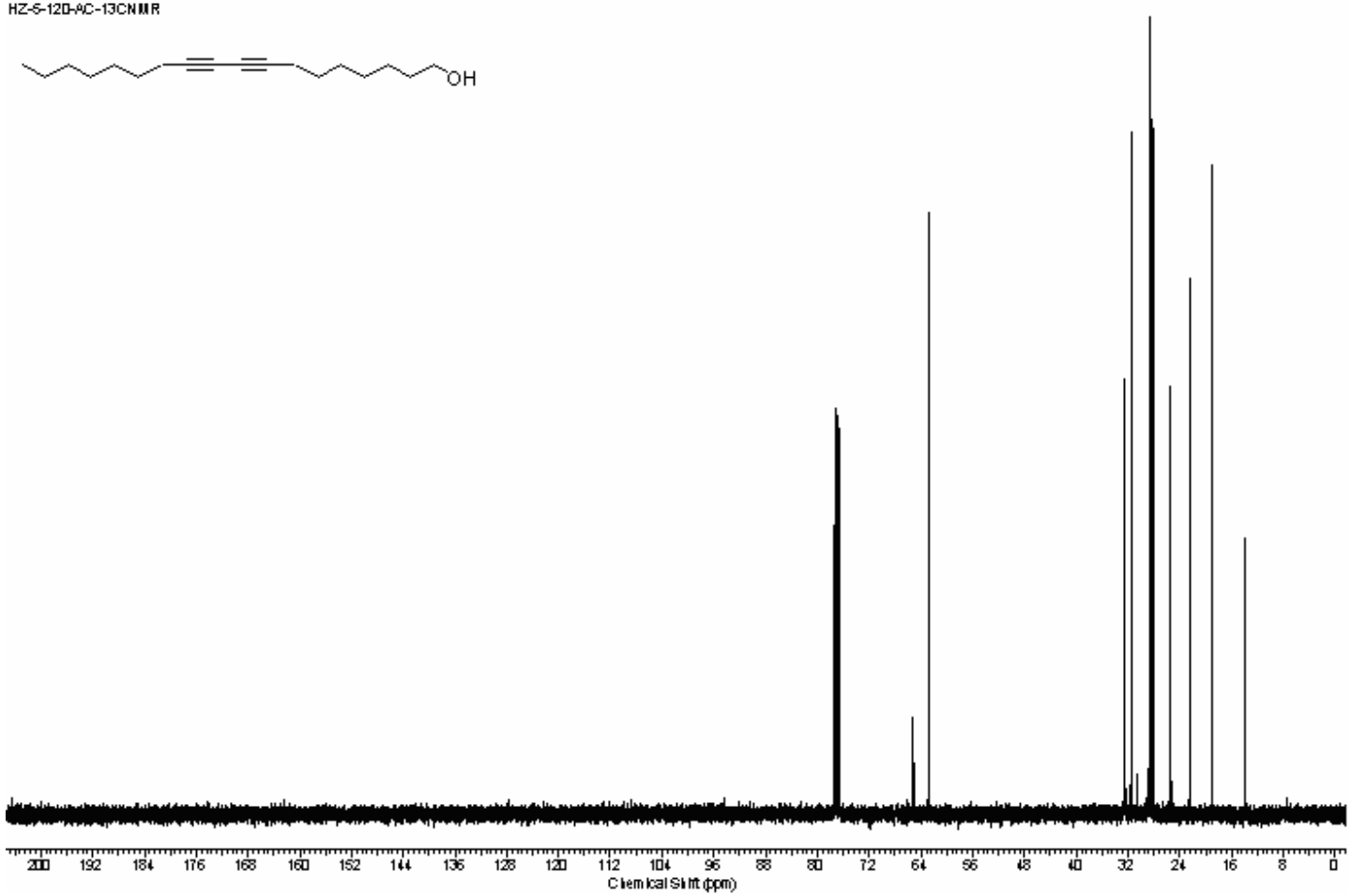
Acquisition Time (sec)	3.7010	*Comment*	STANDARD 1H OBSERVE			*Date*	Feb 12 2006
Date Stamp	Feb 12 2006	*File Name*	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-5-120-AC				
Frequency (MHz)	399.78	*Nucleus*	1H	*Number of Transients*	64	*Original Points Count*	22208
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	2008.2521	*Sweep Width (Hz)*	6000.60	*Temperature (degree C)*	29.000		

HZ-5-120-AC



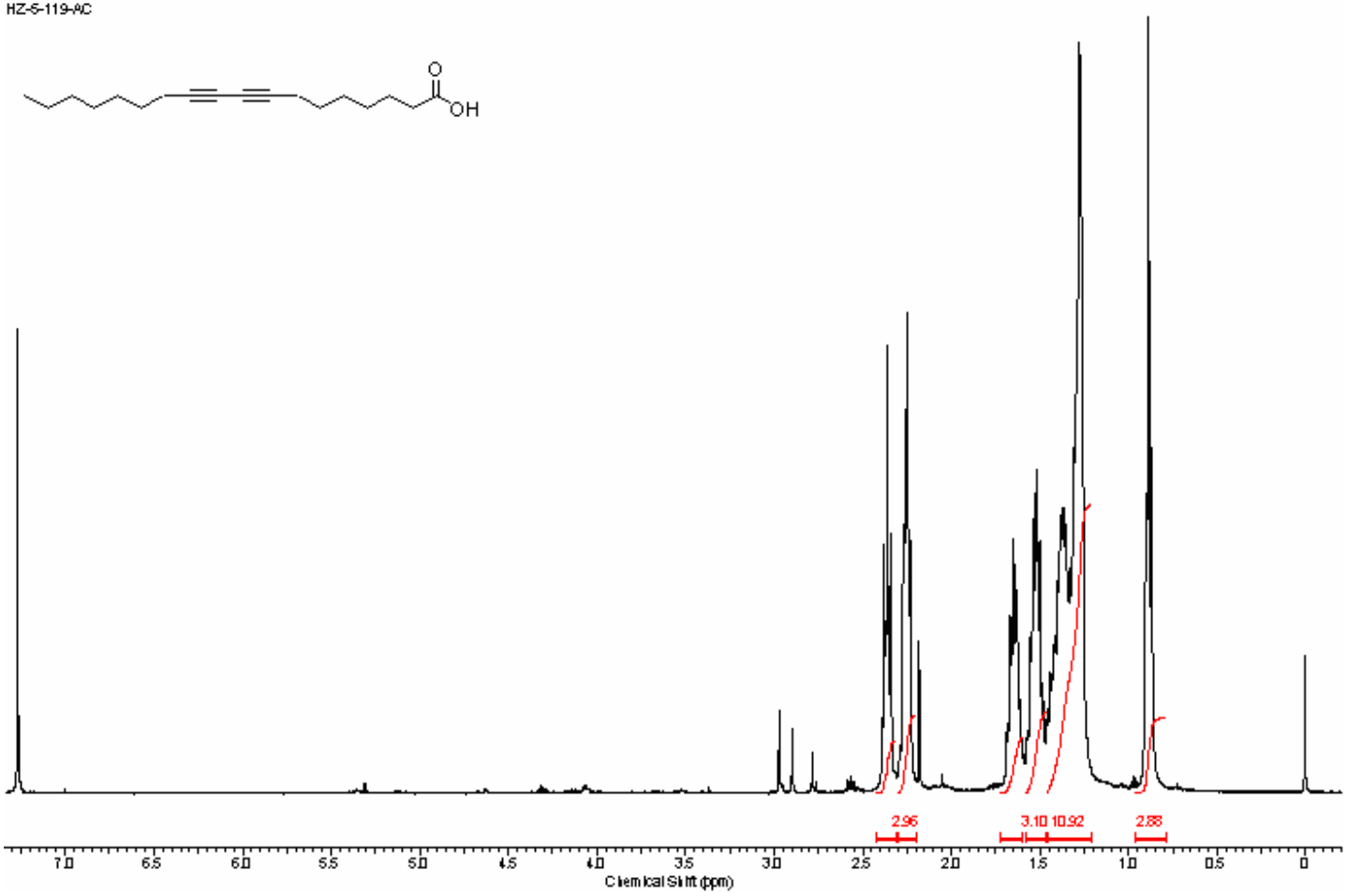
Acquisition Time (sec)	1.1994	Comment	13C OBSERVE	Date	Feb 12 2006	Date Stamp	Feb 12 2006
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-6-120-AC-13CNMR						
Frequency (MHz)	100.53	Nucleus	13C	Number of Transients	64000	Original Points Count	29984
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	20.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	9481.2949	Sweep Width (Hz)	25000.00	Temperature (degree C)	29.000		

HZ-6-120-AC-13CNMR



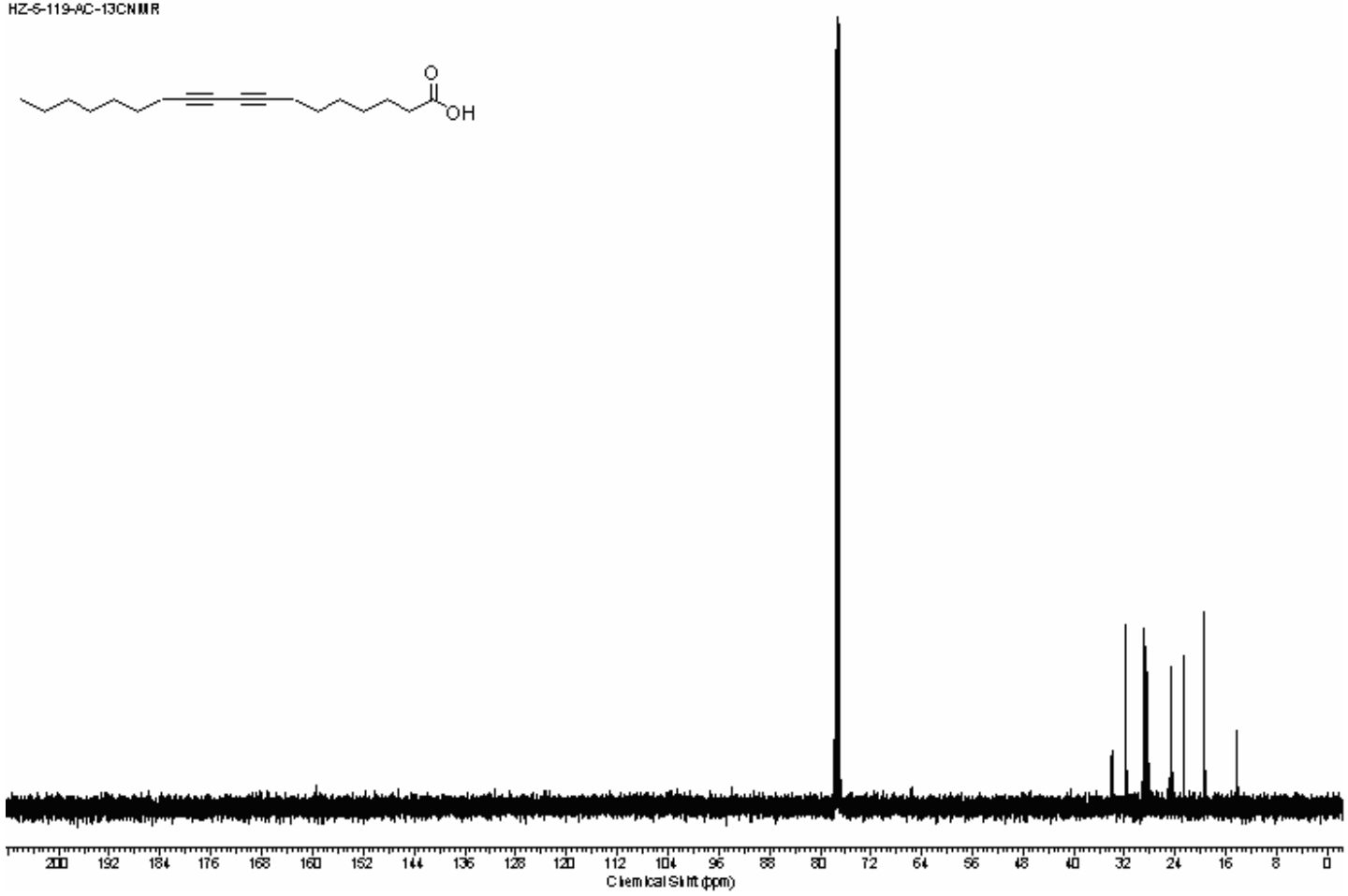
Acquisition Time (sec)	3.7010	Comment	STANDARD 1H OBSERVE			Date	Feb 8 2006
Date Stamp	Feb 8 2006	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-6-119-AC				
Frequency (MHz)	399.78	Nucleus	1H	Number of Transients	64	Original Points Count	22208
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2007.7029	Sweep Width (Hz)	6000.60	Temperature (degree C)	29.000		

HZ-6-119-AC



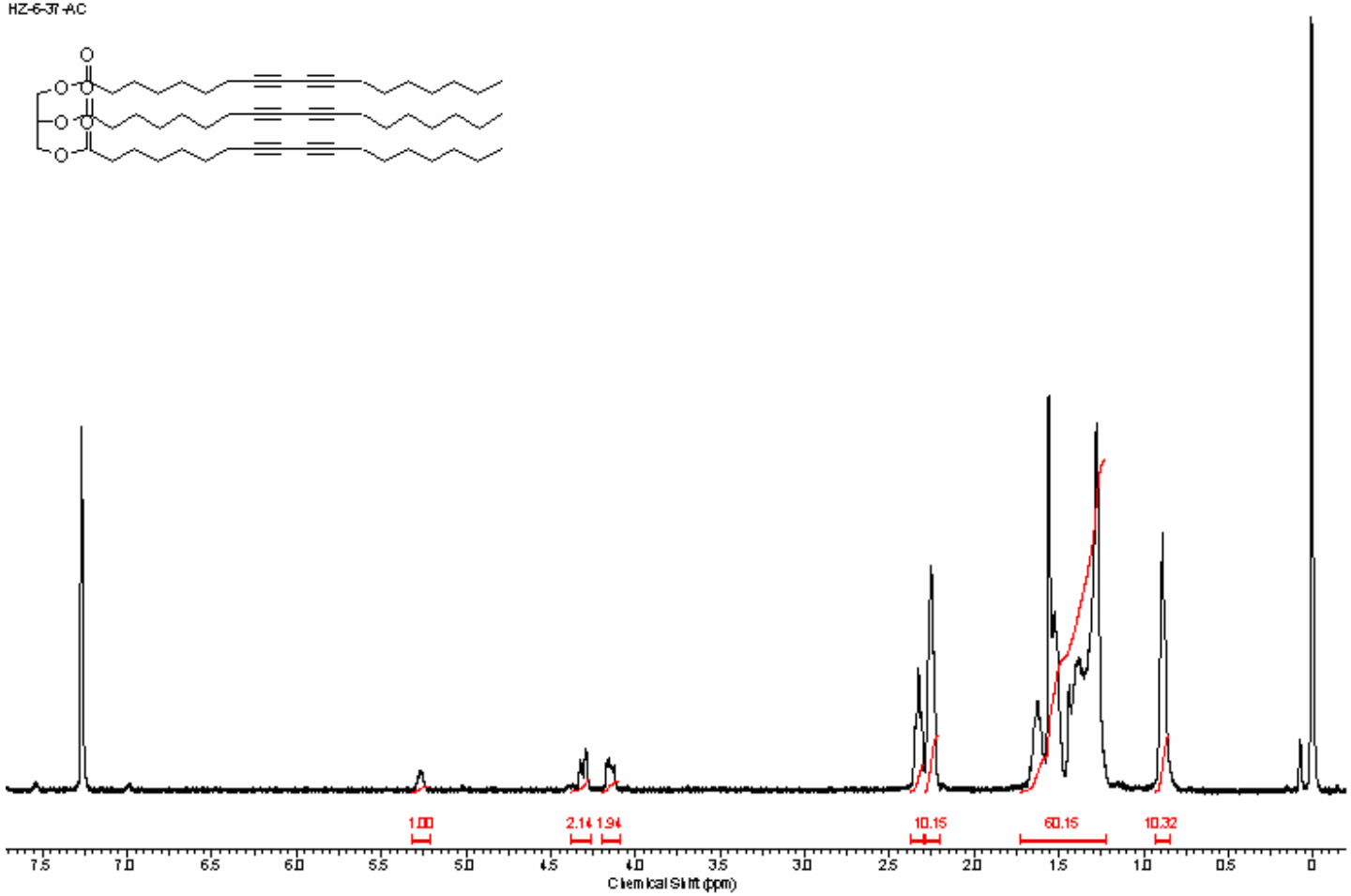
Acquisition Time (sec)	1.1994	*Comment*	13C OBSERVE	*Date*	Feb 9 2006	*Date Stamp*	Feb 9 2006
File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-6-119-AC-13C NMR						
Frequency (MHz)	100.53	*Nucleus*	13C	*Number of Transients*	20000	*Original Points Count*	29984
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	20.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	9517.1816	*Sweep Width (Hz)*	26000.00	*Temperature (degree C)*	29.000		

HZ-6-119-AC-13CNMR



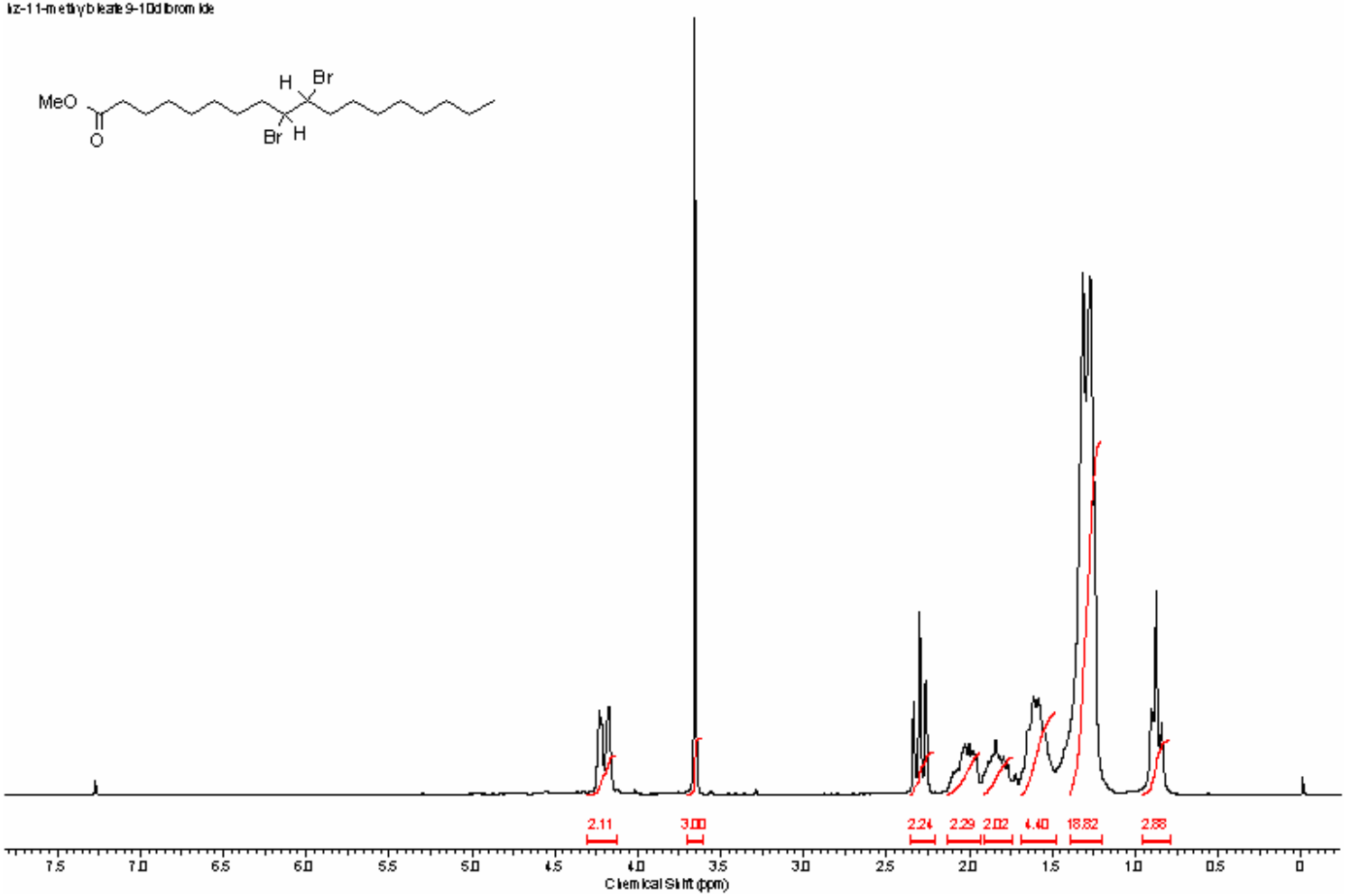
Acquisition Time (sec)	3.7010	Comment	STANDARD 1H OBSERVE			Date	Mar 28 2006
Date Stamp	Mar 28 2006	File Name	C:\DOCUMENTS AND SETTINGS\HUIPING ZHAO\DESKTOP\ORIGINAL NMR DATA\ZHAO\HZ-6\HZ-6-37-AC				
Frequency (MHz)	399.78	Nucleus	1H	Number of Transients	64	Original Points Count	22208
Points Count	32768	Pulse Sequence	s2pul	Receiver Gain	40.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2009.1678	Sweep Width (Hz)	6000.60	Temperature (degree C)	29.000		

HZ-6-37-AC



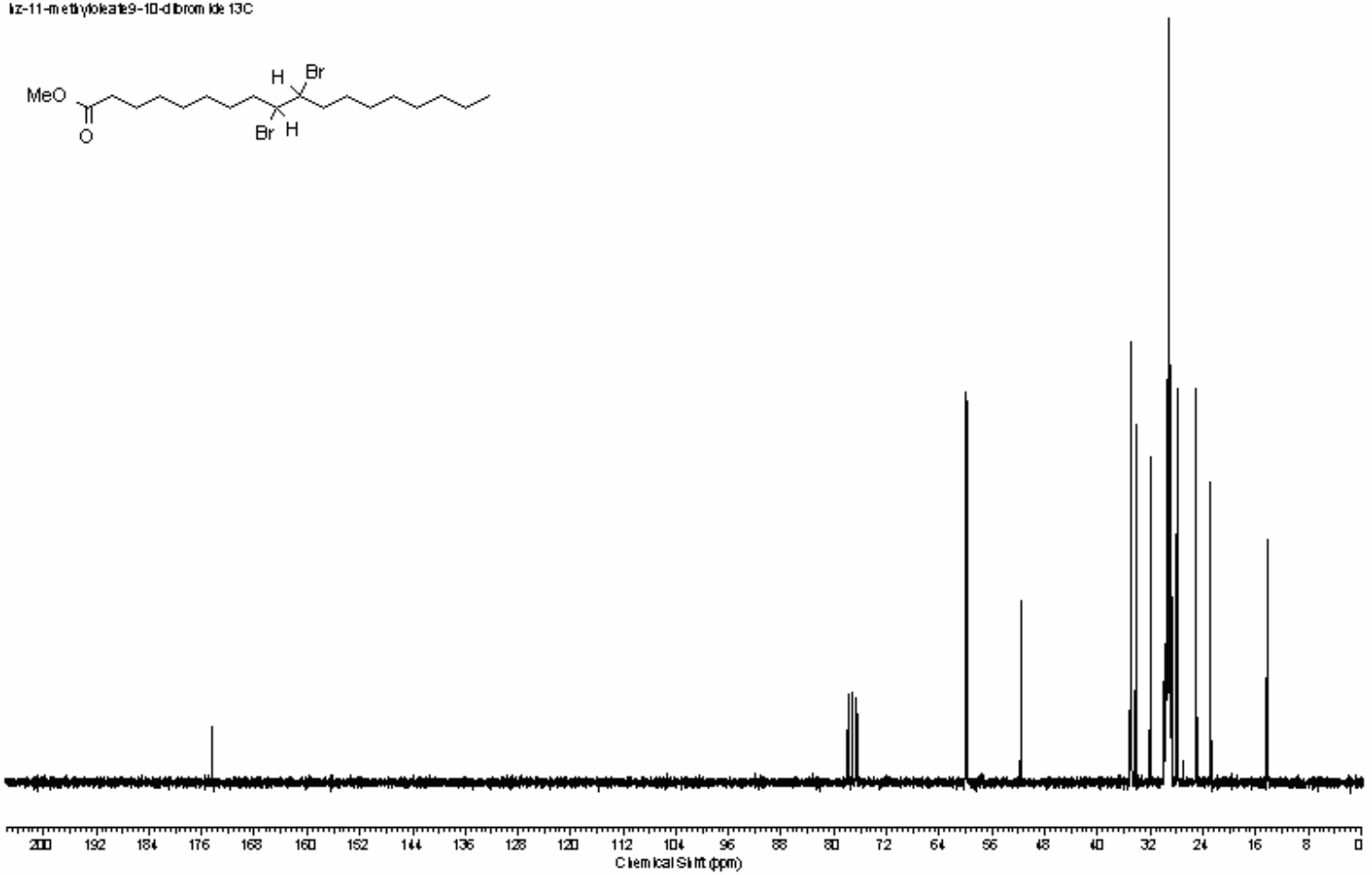
Acquisition Time (sec)	1.9945	Comment	STANDARD 1H OBSERVE			Date	Apr 1 2008
Date Stamp	Apr 1 2008	File Name	C:\Documents and Settings\Weiping\Desktop\HZ-11\liz-11-methyloleate9-10dibromide				
Frequency (MHz)	199.98	Nucleus	1H	Number of Transients	64	Original Points Count	5984
Points Count	131072	Pulse Sequence	s2pul	Receiver Gain	4.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	1003.3677	Sweep Width (Hz)	3000.30	Temperature (degree C)	29.000		

liz-11-methyloleate9-10dibromide



Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE			*Date*	Apr 1 2008
Date Stamp	Apr 1 2008	*File Name*	E:\HZ-11\hz-11-methyloleate9-10-dibromide 13C			*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	6400	*Original Points Count*	18720	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	4878.5581	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

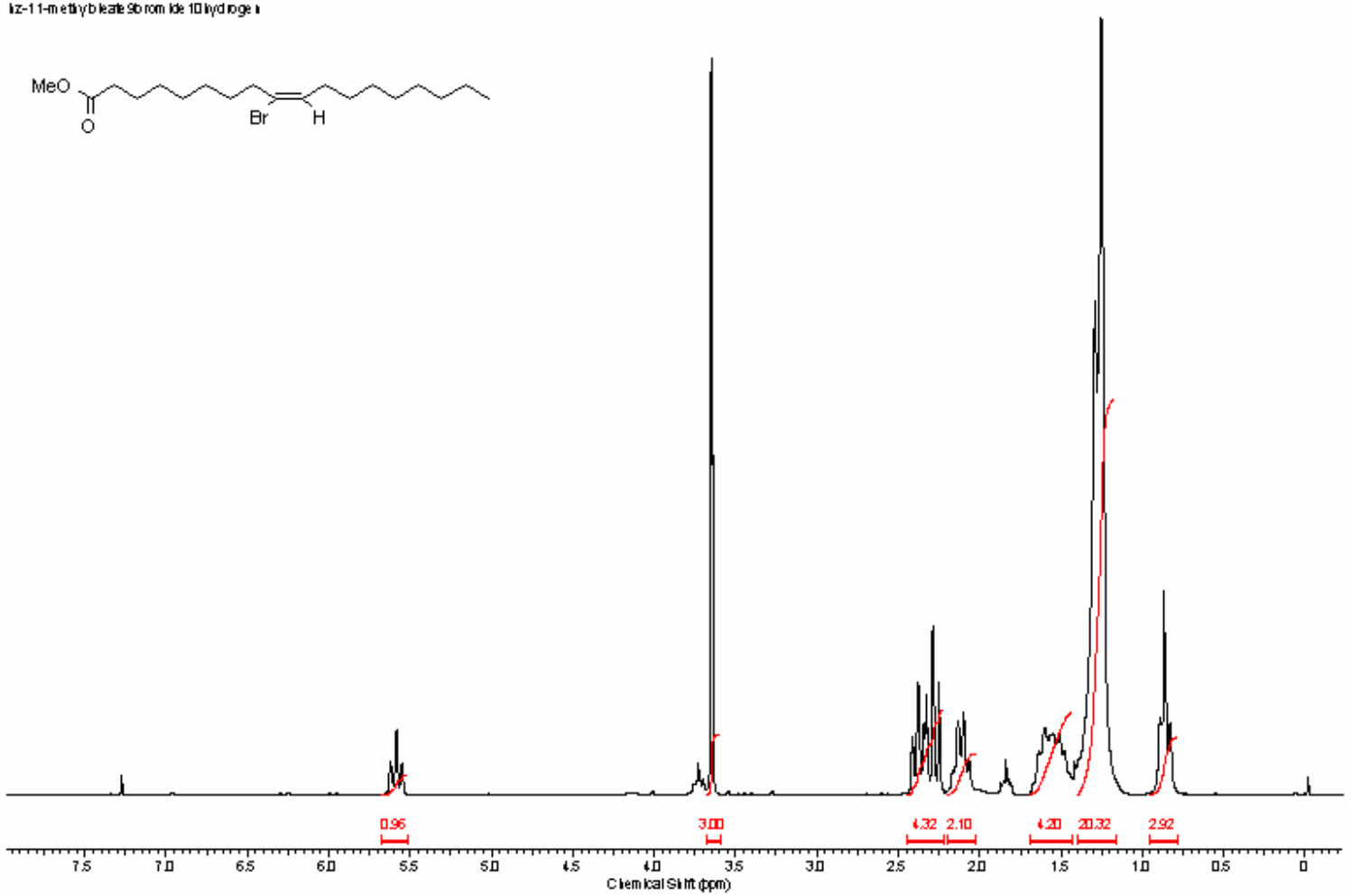
hz-11-methyloleate9-10-dibromide 13C



E:\HZ-11\hz-11-methyloleate9-10-dibromide 13C

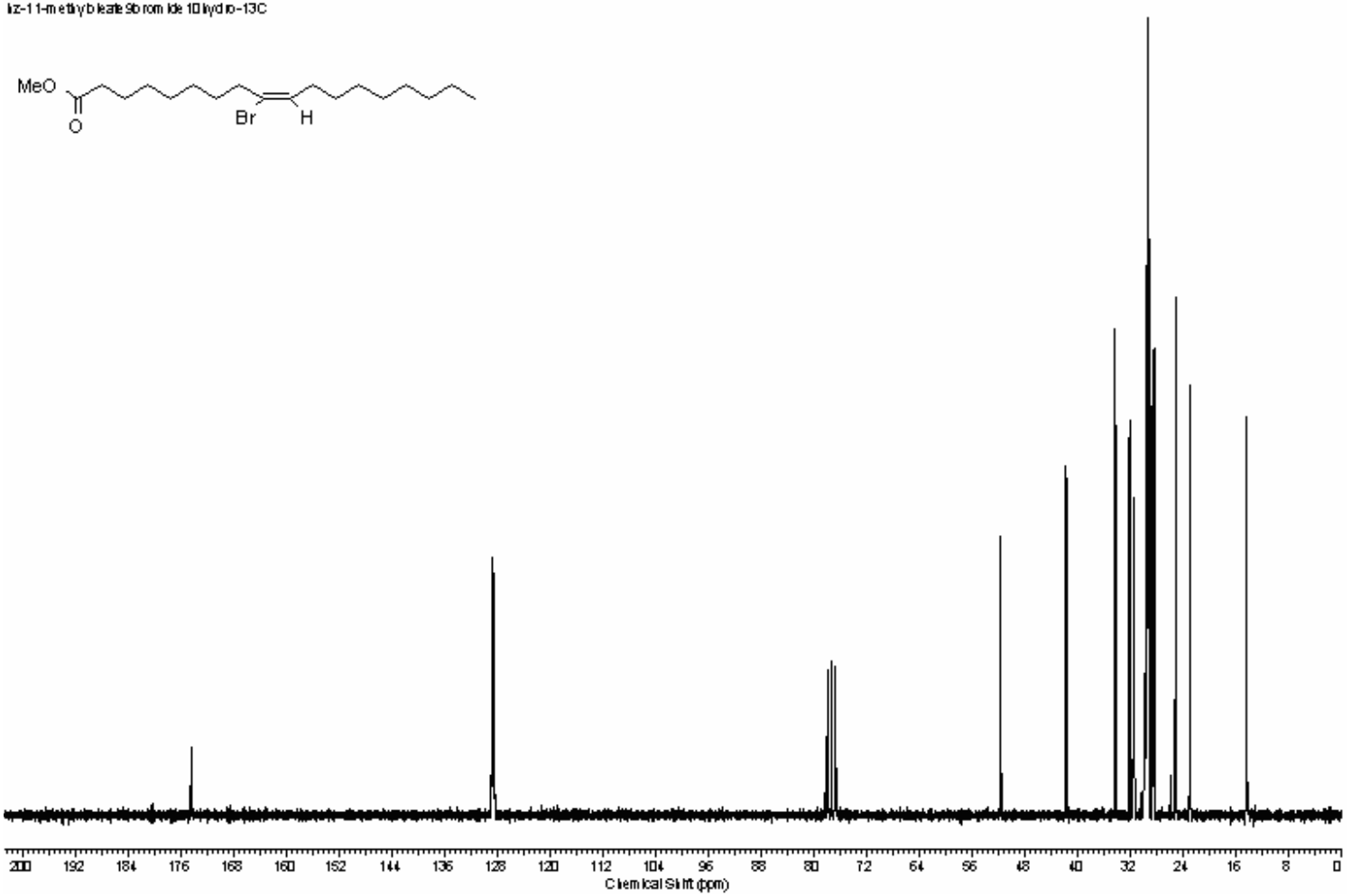
Acquisition Time (sec)	1.9945	*Comment*	STANDARD 1H OBSERVE			*Date*	Apr 2 2008
Date Stamp	Apr 2 2008	*File Name*	C:\Documents and Settings\Hsiping\Desktop\HZ-11\hz-11-methylbleatebromide1Hhydrogen				
Frequency (MHz)	199.98	*Nucleus*	1H	*Number of Transients*	64	*Original Points Count*	5984
Points Count	131072	*Pulse Sequence*	s2pul	*Receiver Gain*	4.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	1002.3834	*Sweep Width (Hz)*	3000.30	*Temperature (degree C)*	29.000		

hz-11-methylbleatebromide1Hhydrogen



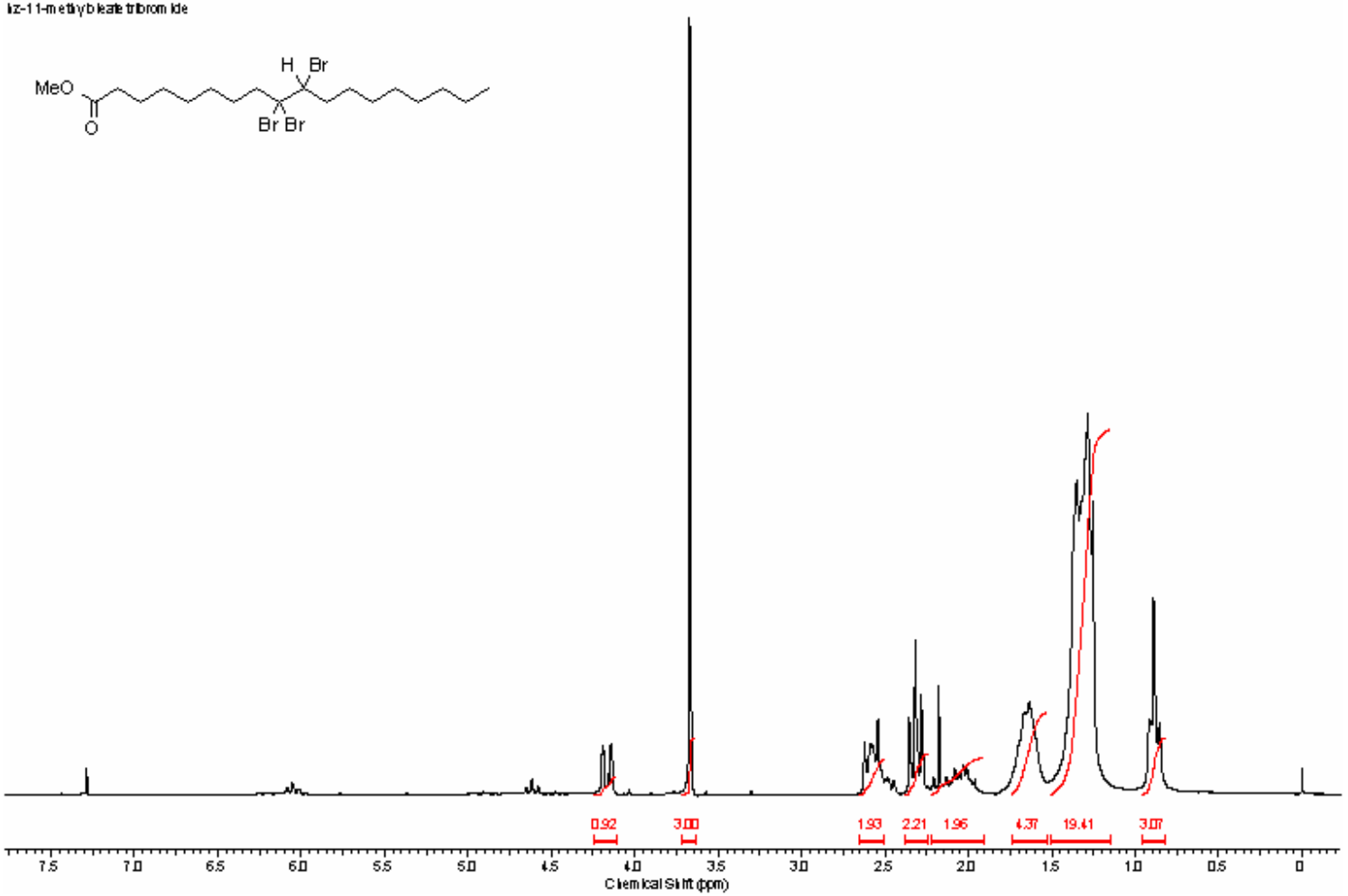
Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Apr 2 2008	*Date Stamp*	Apr 2 2008
File Name	C:\Documents and Settings\Huiping\Desktop\HZ-11\hz-11-methylbleatebromide10hydro-13C					*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	6400	*Original Points Count*	18720	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	4878.9351	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

hz-11-methylbleatebromide10hydro-13C



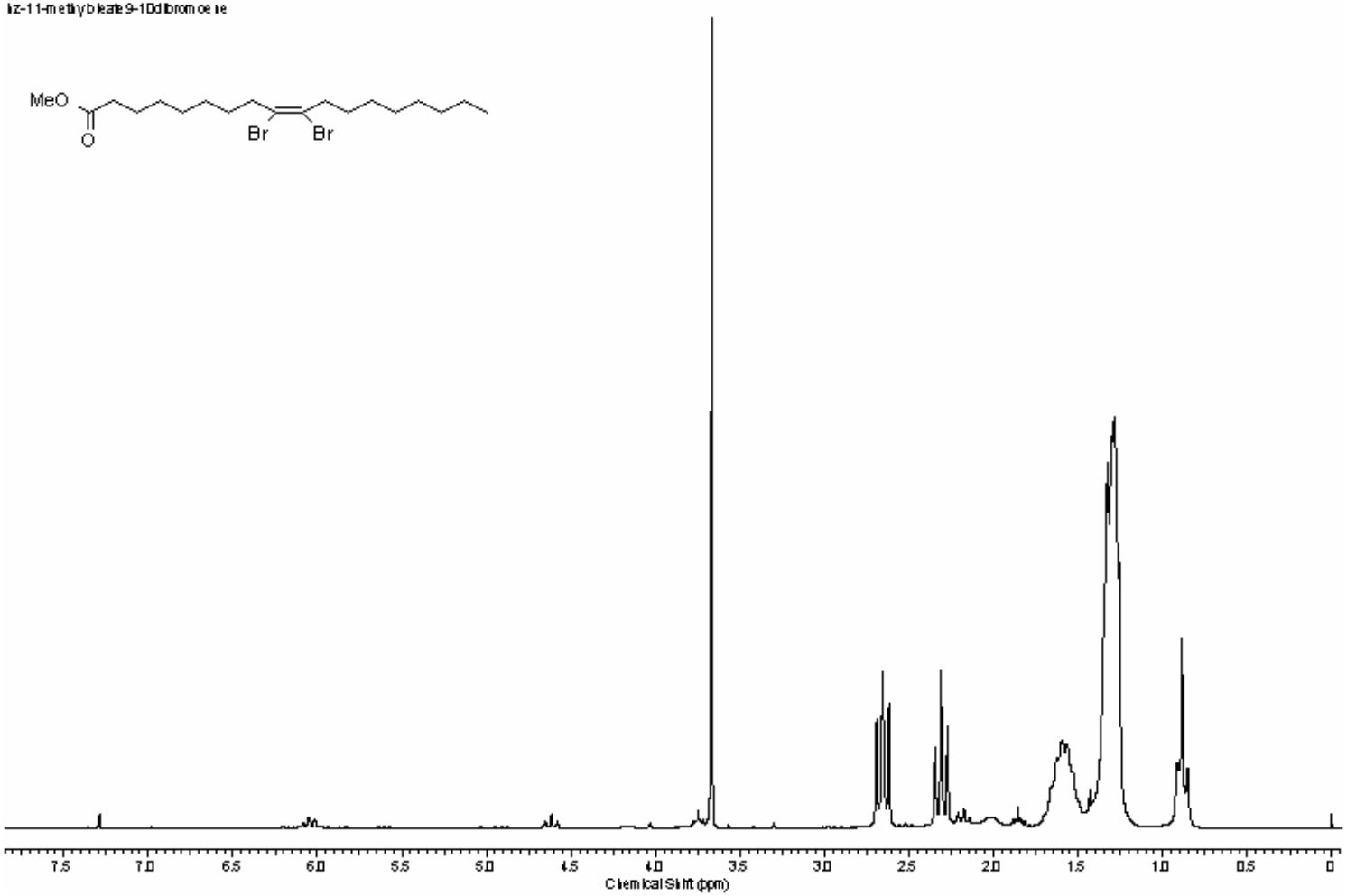
Acquisition Time (sec)	1.9945	*Comment*	STANDARD 1H OBSERVE			*Date*	Apr 2 2008
Date Stamp	Apr 2 2008	*File Name*	C:\Documents and Settings\Hualig\Desktop\HZ-11\hz-11-methyloleate tribromide				
Frequency (MHz)	199.98	*Nucleus*	1H	*Number of Transients*	64	*Original Points Count*	5984
Points Count	131072	*Pulse Sequence*	s2pul	*Receiver Gain*	4.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	1005.2892	*Sweep Width (Hz)*	3000.30	*Temperature (degree C)*	29.000		

hz-11-methyloleate tribromide



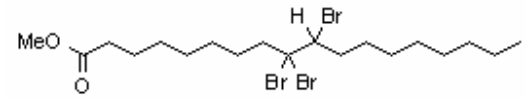
Acquisition Time (sec)	1.9945	*Comment*	STANDARD 1H OBSERVE			*Date*	Apr 2 2008
Date Stamp	Apr 2 2008	*File Name*	C:\Documents and Settings\Huplig\Desktop\HZ-11\hz-11-methyloleate9-10dibromoeie				
Frequency (MHz)	199.98	*Nucleus*	1H	*Number of Transients*	64	*Original Points Count*	5984
Points Count	131072	*Pulse Sequence*	s2pul	*Receiver Gain*	4.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	1006.7543	*Sweep Width (Hz)*	3000.30	*Temperature (degree C)*	29.000		

hz-11-methyloleate9-10dibromoeie



Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Apr 2 2008	*Date Stamp*	Apr 2 2008
File Name	C:\Documents and Settings\Huiping\Desktop\HZ-11\hz-11-methyl oleate tribromide-13C					*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	64000	*Original Points Count*	18720	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	4877.7949	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

hz-11-methyl oleate tribromide-13C



Acquisition Time (sec)	1.4976	*Comment*	13C OBSERVE	*Date*	Apr 2 2008	*Date Stamp*	Apr 2 2008
File Name	C:\Documents and Settings\Huiping\Desktop\HZ-11\HZ-11-methyloleatedibromoene					*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	64.000	*Original Points Count*	18720	*Points Count*	32768
Pulse Sequence	s2pul	*Receiver Gain*	40.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	4878.9351	*Sweep Width (Hz)*	12500.00	*Temperature (degree C)*	29.000		

HZ-11-methyloleatedibromoene