SYNTHESES AND BIOEVALUATION OF NOVEL TRICYCLIC PYRONE COMPOUNDS AND OVALICIN AND ITS ANALOGUES

by

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B.Sc., Kakatiya University, India, 1997
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department Of Chemistry
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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ABSTRACT

The first part of this thesis deals with the syntheses of ovalicin and its analogues. Ovalicin inhibits the endothelial cell proliferation. Apart from being anti-angiogenic it also exhibits antibiotic, antitumor, and immunosuppressive properties. Unlike other syntheses, we started with an acyclic compound, ethyl propiolate (**1.66**). Our flexible route towards the synthesis used intramolecular Heck cyclization reaction to construct an appropriately functionalized 3-methylene-6-(*tert*-butyldimethylsilyloxy)cyclohexene (**1.63**) from **1.66** in four steps. A number of synthetic analogues were synthesized via this strategy. Upon selective epoxidation and dihydroxylation of **1.63,** a mixture of diols (3*S**,4*R**,5*S**,6*S**)-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octane-4,5-diol (**1.107**) and (3*S**,4*S**,5*R**,6*R**)-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octane-4,5-diol (**1.108**) were obtained. Subsequent functional group transformations of diols **1.107** and **1.108** gave ketones (3*S**,4*S**,5*R**,6*R**)-6-(*tert*-butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan-4-one (**1.112)** and (3*S**,5*S**,*6S**)-6-(*tert*-butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan -4-one (**1.117**). Addition of vinyl lithium to the ketones followed by functional group transformation gave ovalicin analogues. Several intermediates were subjected to biological activity test for inhibition of growth of *T. brucei.* Our synthetic efforts towards the synthesis of ovalicin are discussed.

The second part of my thesis deals with the synthesis of different tricyclic pyrone (TP) analogues which inhibit the aggregation of Aβ peptides. Alzhemier's disease (AD) is caused by accumulation of fibrillar amyloid deposits in the AD brain. We synthesized a series of tricyclic pyrone derivatives and evaluated their counteraction on amyloid toxicity. TP analogue, (5a*S*,7*S*)-7-[(1*R*) and (1*S*)-2-(N3-adenyl)-1-methylethyl]-3-methyl-1H,7H-5a,6,8,9-tetrahyro-1-oxopyranol[4,3 -b] [1] benzopyran (CP2) is nontoxic, small and permeable molecule prevents the death of human neuroblastoma MC65 cells that conditionally expressed SβC gene. We further found that CP2 ameliorates the toxicity and inhibits the formation of Aβ oligomeric complexes. Binding studies using surface plasma resonance and atomic force microscopy studies suggest that CP2 permeates into the cells and interacts with Aβ peptides and inhibits the Aβ oligomerization. To understand the mechanism of Aβ aggregation and toxicity, CP2 and its derivatives are

synthesized to evaluate their action. The key intermediate in the synthesis of CP2 is (5a*S**,7*S**)-7-[(1*R**) and (1*S**)-2-bromo-1-methylethyl]-3-methyl-1H,7H-5a,6,8,9-tetrahyro-1-oxopyranol[4,3-b][1] benzopyran (**2.9**), which in turn can be prepared from our previously reported method. Our aim is to synthesize a series of compounds and investigate the biological activities of different TP analogues.

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Table of Contents

Structure Number Correlation List ……...ix
List of Figures……xxiii
List of Tables…….xxvii
Acknowledgements……..xxviii
Dedication……..xxix
Chapter 1- Synthesis of ovalicin and its analogues……1
1.1 Introduction…….1
1.2 Previous reported total synthesis……3
1.2.1 Corey's Syntheses……..3
1.2.2 Barton-Quiclet-Sire-Samadi Syntheses…..……8
1.2.3 Hayashi Syntheses.……..11
1.3 Synthesis of Ovalicin……14
1.3.1 Retrosynthetic analysis…….……...14
1.3.2 Synthesis of methylidenecyclohexanesilane **1.63**…...…….15
1.3.3 Epoxidation of methylidenecyclohexanesilane **1.63**……...17
1.3.4 Changing the protection group from silyl to benzoyl……..18
1.3.5 Epoxidation followed by dihydroxylation……...19
1.3.6 Separation of diols……...22
1.3.7 Methylation and Oxidation ……23
1.3.8 Preparation of alkenyl lithium reagent……25
1.3.9 Addition of alkenyl lithium reagent to ketone **1.87** and the transformation of the resulting adduct to ovalicin analogues…….……...26
1.3.10 Changing the protective group from silyl to (-)-(1*S*,4*R*)-camphanoyl…….28
1.3.11 Using trifluoromethyldioxirane (TFDO) as an oxidizing agent to

epoxidize diene **1.63**……...……30
1.3.12 Dihydroxylation and separation of diastereomers……...31
1.3.13 Synthesis of ketone **112**……...34
1.3.14 Synthesis of ketone **1.117** and **1.118**……...35
1.3.15 Addition of alkenyl lithium to ketone **1.112**……36
1.3.16 Synthesis of ovalicin analogues……...37
1.3.17 Continuing study towards the total synthesis of ovalicin……38
1.4 Biological activity of ovalicin analogues……..……41
1.5 Experimental Section……44
References……...…..68
Chapter 2- Novel tricyclic pyrone compounds protects cell death induced with intracellular Aβ oligomeric complexes……...73
2.1 Introduction ……..73
2.2 Background and significance……73
2.3 Synthesis of TP compounds……..81
2.3.1 Synthesis of CP2…….81
2.3.2 Synthesis of CP2 analogues……....84
2.4 Results and discussion……..90
2.4.1 The death of MC65 cells depends on the production of Aβ……...90
2.4.2 TP analogues protect MC65 cells in a structure dependent manner and CP2's protective effect is intimately related to its inhibition of Aβ-oligomeric complexes…….94
2.4.3 CP2 directly binds to Aβ and inhibits Aβ fibrilization……...98
2.4.4 Atomic force microscopic studies showing CP2 inhibits the Aβ aggregation……...101
2.5 Experimental……...116
References……126
Appendices: ^{1}H Spectra and ^{13}C NMR spectra……131

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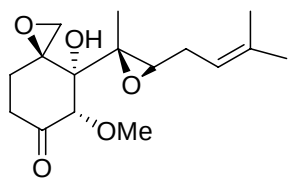

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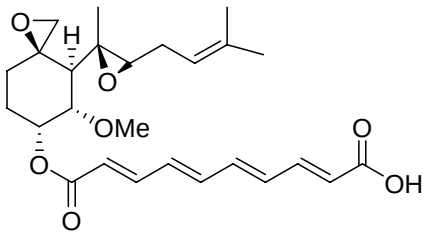

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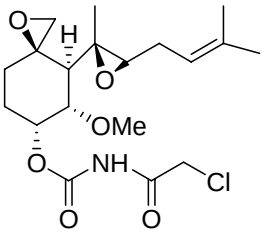

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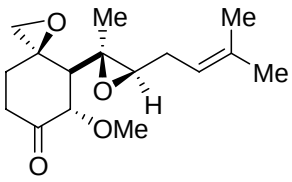

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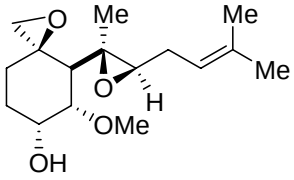

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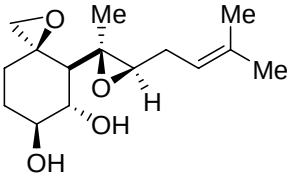

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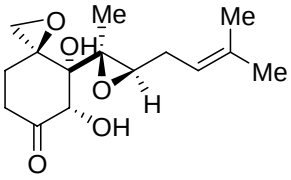

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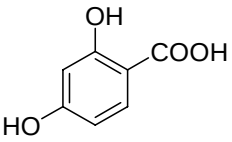

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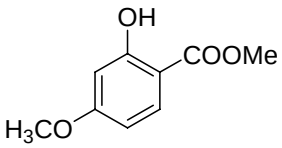

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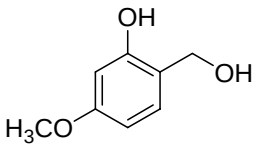

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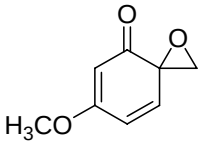

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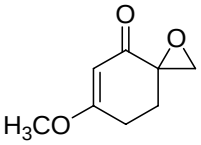

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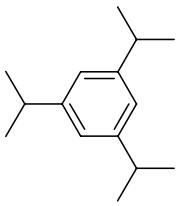

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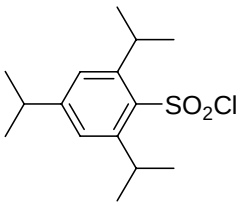

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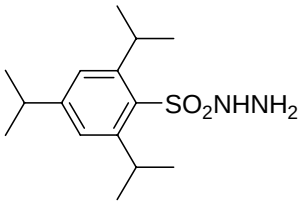

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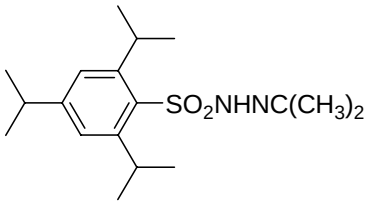

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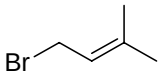

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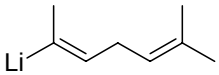

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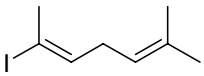

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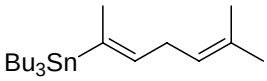

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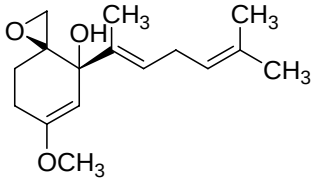

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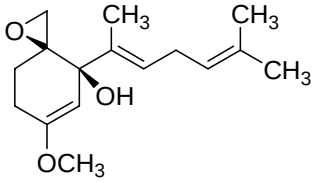

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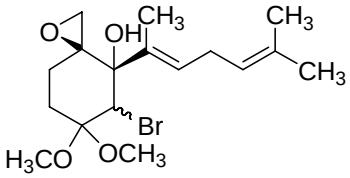

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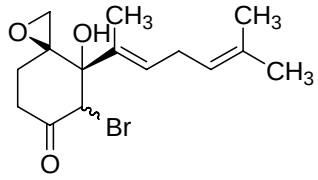

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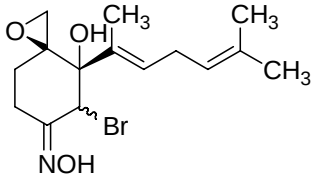

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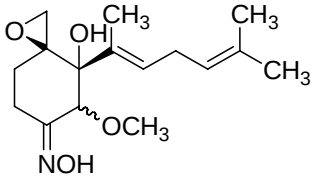

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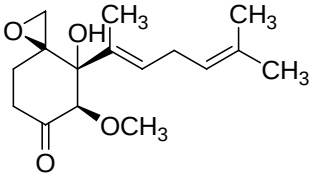

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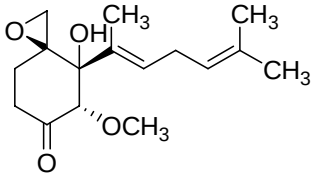
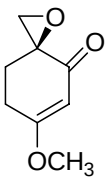
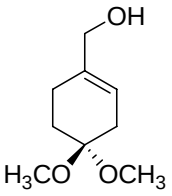
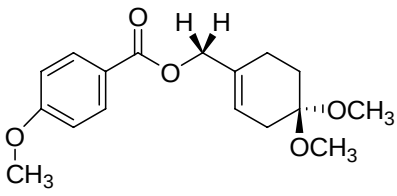
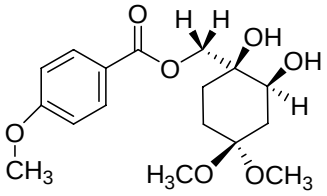
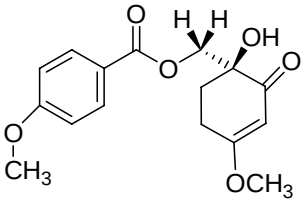


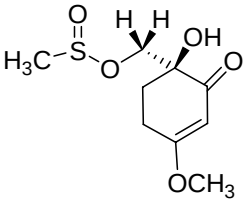
1.22 

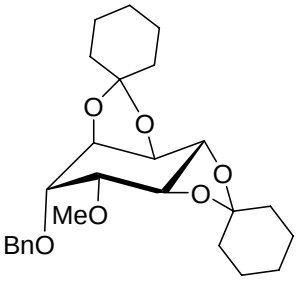
1.23 

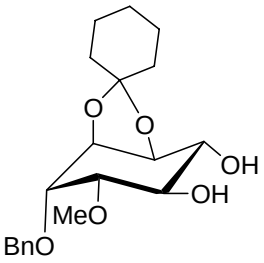
1.24 

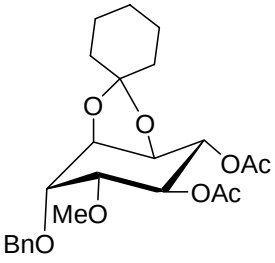
1.25 

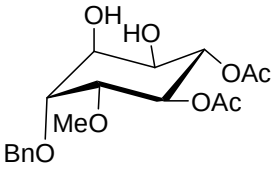
1.26 

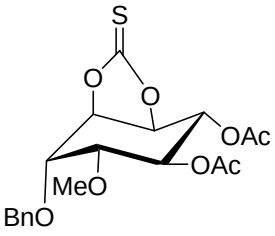
1.27 

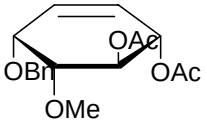
1.28 

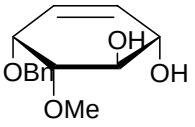
1.29 

1.30 

1.31 

1.32 

1.33 



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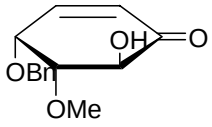

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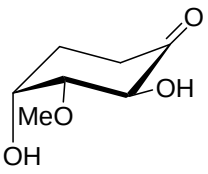

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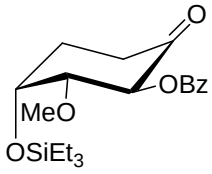

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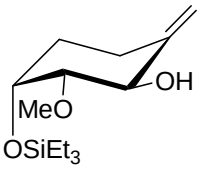

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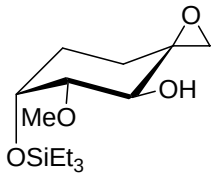

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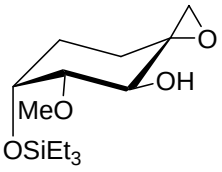

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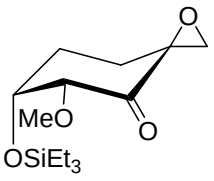

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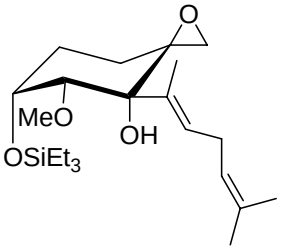

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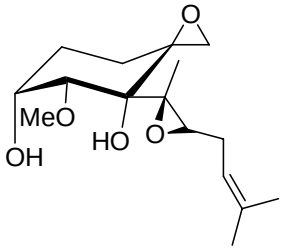

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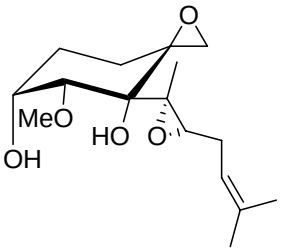

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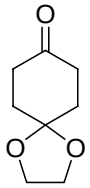

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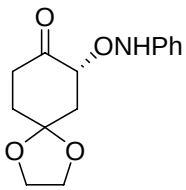

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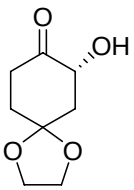

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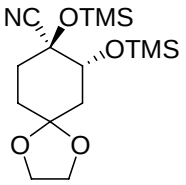

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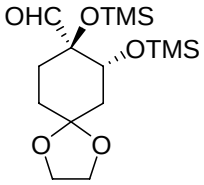

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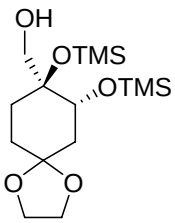

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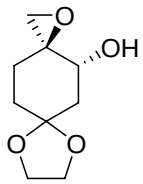

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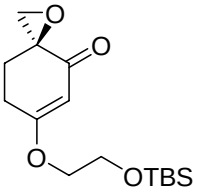

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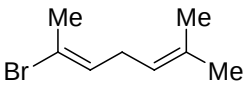

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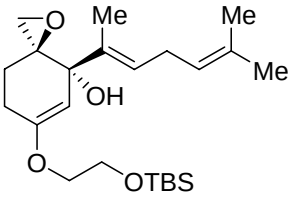

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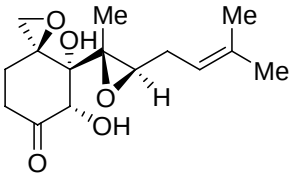

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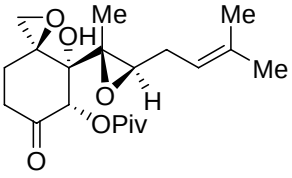

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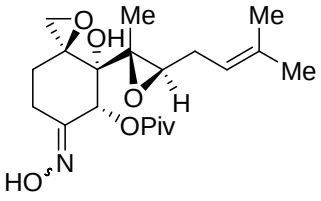

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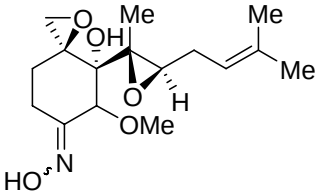

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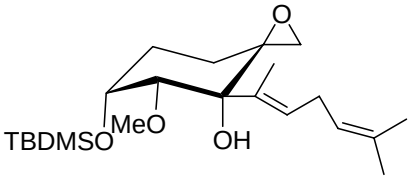

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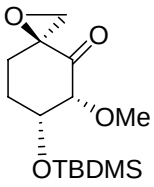

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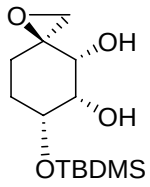
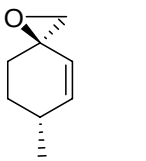
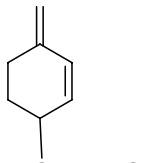
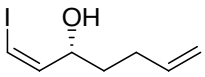
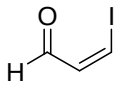
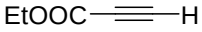


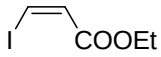
1.62 

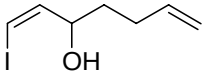
1.63 

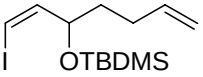
1.64 

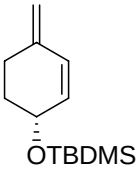
1.65 

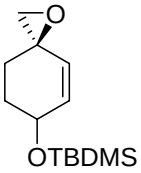
1.66 

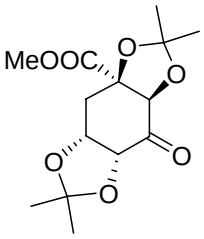
1.67 

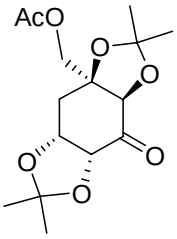
1.68 

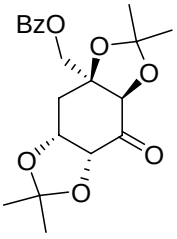
1.69 

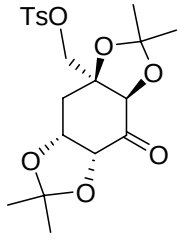
1.70 

1.71 

1.72 

1.73 

1.74 

1.75 

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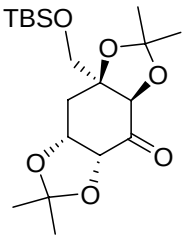

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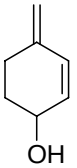

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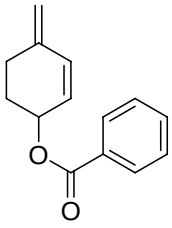

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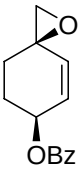

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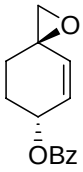

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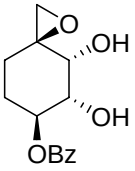

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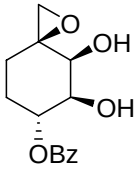

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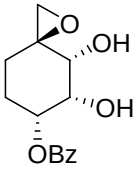

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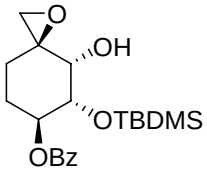

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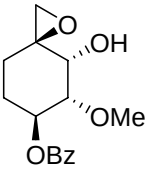

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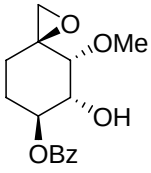

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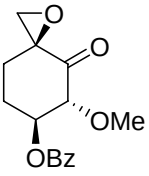

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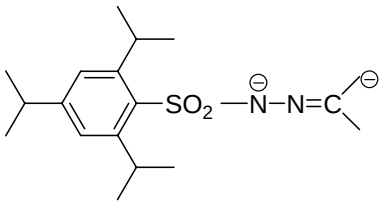

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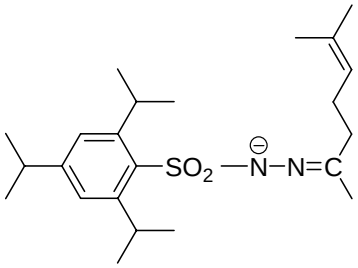

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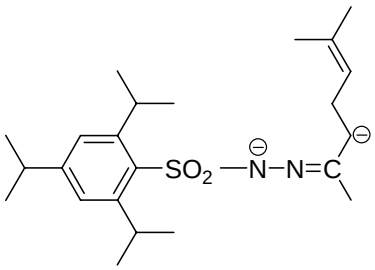

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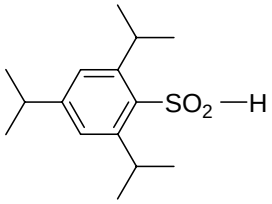

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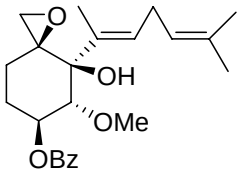

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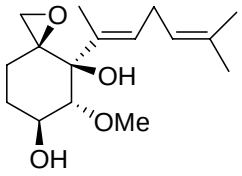

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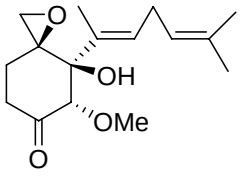

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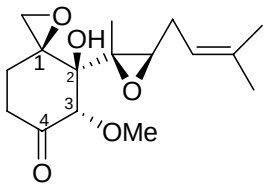

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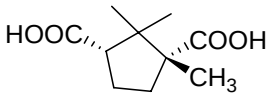

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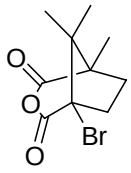

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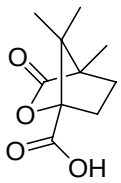

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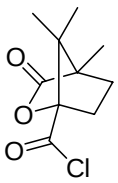
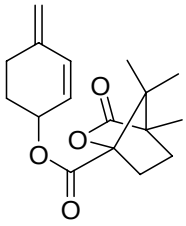
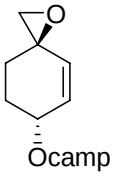
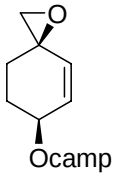
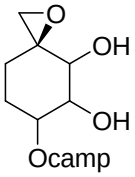
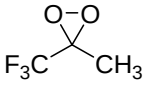


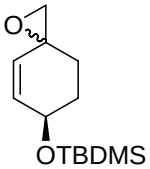
1.100 

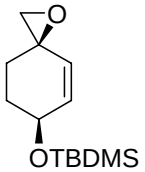
1.101 

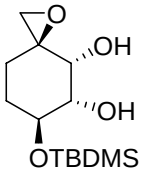
1.102 

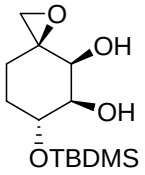
1.103 

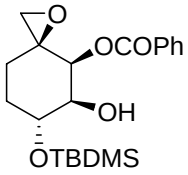
1.104 

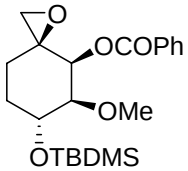
1.105 

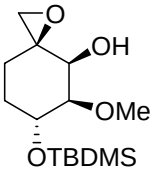
1.106 

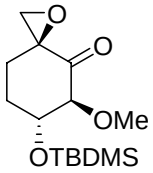
1.107 

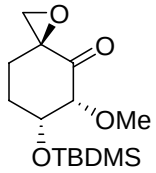
1.108 

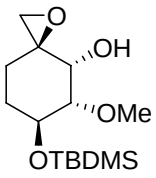
1.109 

1.110 

1.111 

1.112 

1.113 



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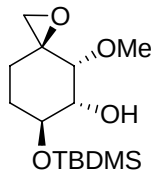

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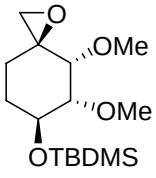

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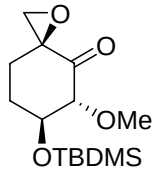

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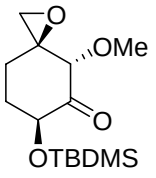

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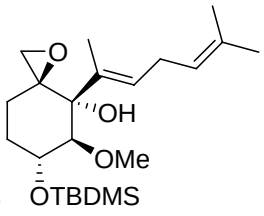

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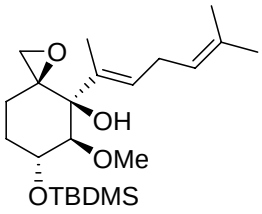

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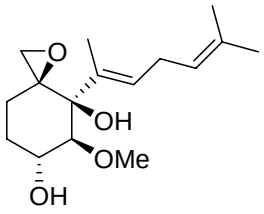

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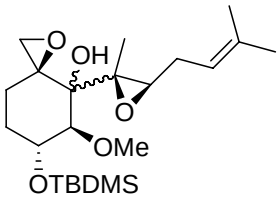

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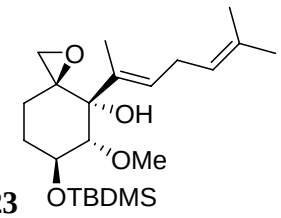

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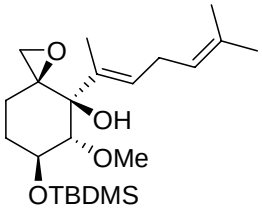

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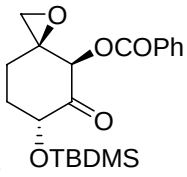

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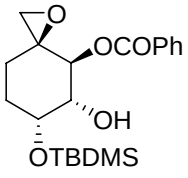

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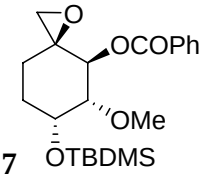

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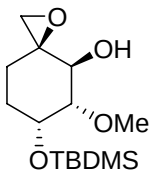

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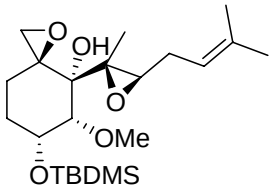

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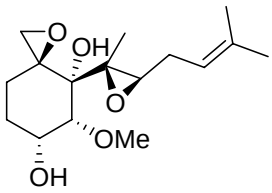

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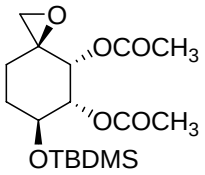

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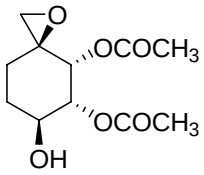

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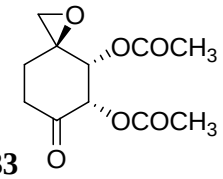

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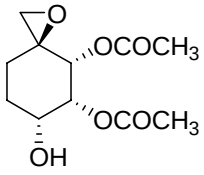

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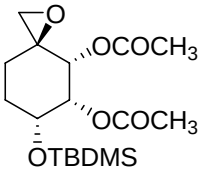

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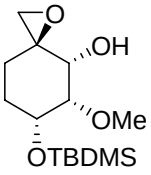

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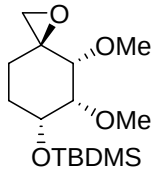

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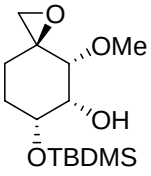

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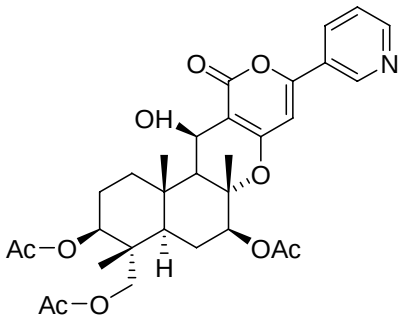

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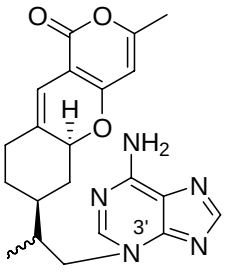
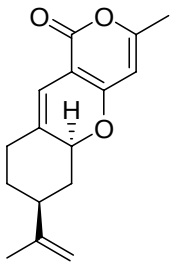
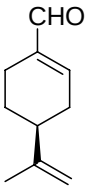
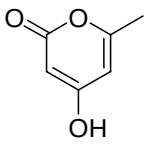
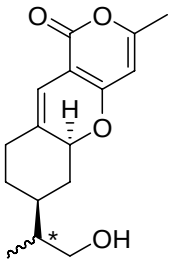
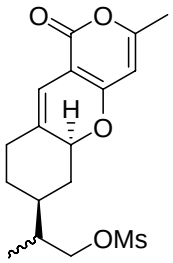
2.2 

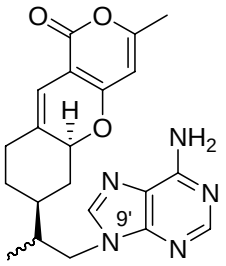
2.3 

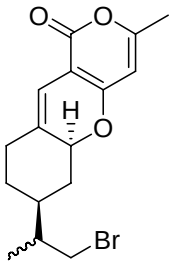
2.4 

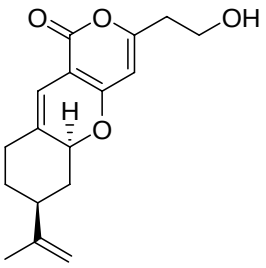
2.5 

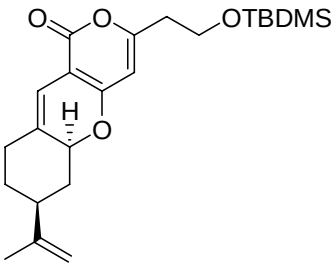
2.6 

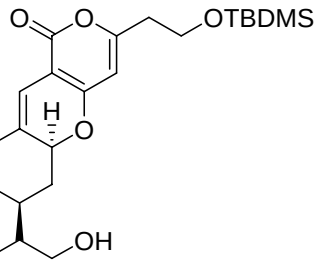
2.7 

2.8 

2.9 

2.10 

2.11 



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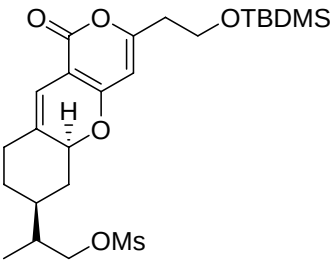

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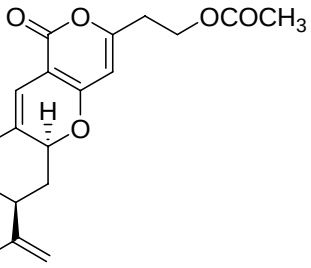

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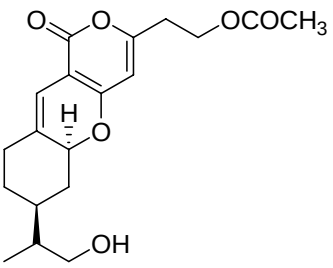

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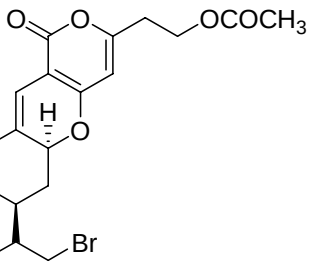

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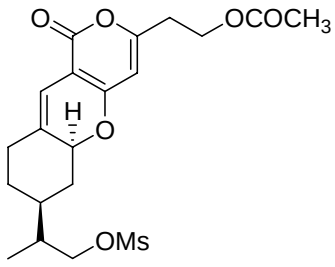

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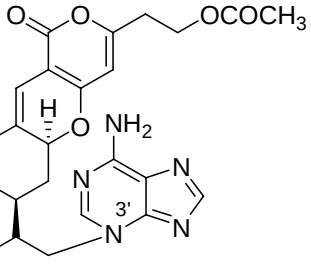

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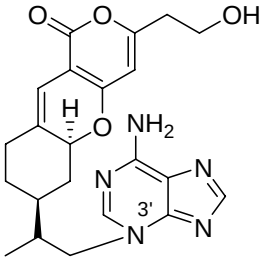

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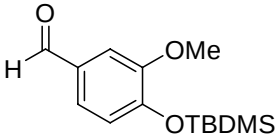

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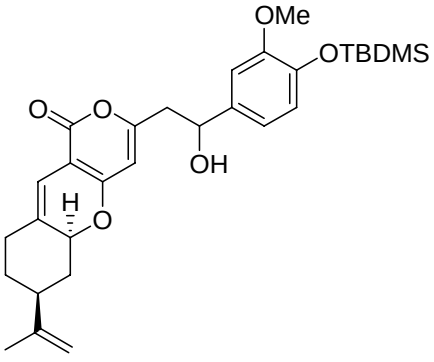

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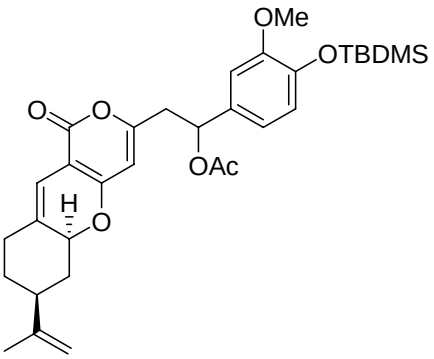

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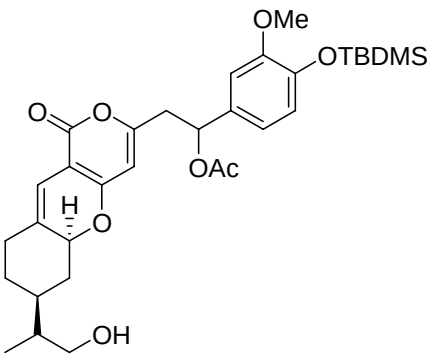

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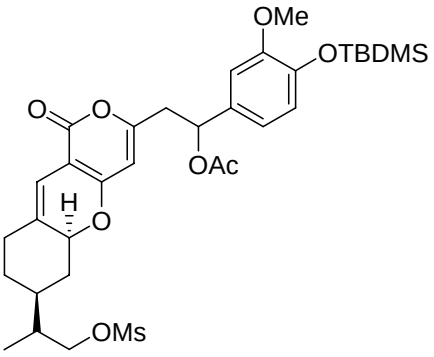

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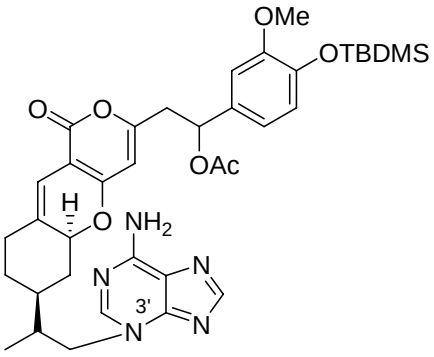

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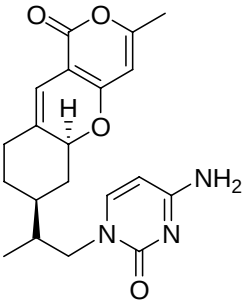

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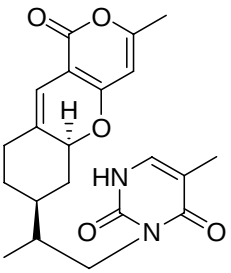

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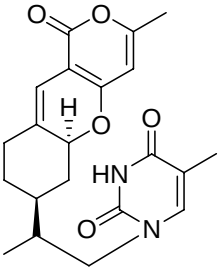

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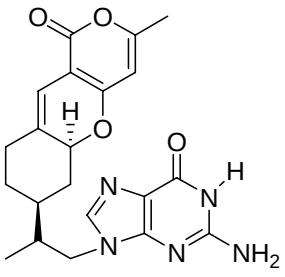

2.29

List of Figures

Figure 1.1 Structures of fumagillin, ovalicin and TNP-470.
Figure 1.2 Synthetic analogues of ovalicin and fumagillin.
Figure 1.3 Different chiral ketones.
Figure 1.4 X-ray crystal structure of compound 1.84.
Figure 1.5 X-ray crystal structure of 1.95.
Figure 1.6 Antiparasitic activity of analogue SB-5-87-F4-6.
Figure 1.7 Antiparasitic activity of analogue KL-1-71.
Figure 2.1 Amyloid plaques and neurofibrillary tangles shown in Alzhemiers case and these aggregrates are absent in normal case.
Figure 2.2 Comparison of different brain parts at different stages of AD.
Figure 2.3 β-Amyloid precursor protein (APP) and its metabolic derivatives.
Figure 2.4 Schematic diagram of conversion of a monomer to a fibril.
Figure 2.5 The hydrophobicity indexes of amyloid (1-43) sequence.
Figure 2.6 The symbol * indicates possible sites where CP2 can be modified.
Figure 2.7 C99 and other fragments induced by removal of TC
Figure 2.8 MC65 cells with TC and without TC. CP2 and TP17 compounds were also tested.
Figure 2.9 N2a-APPsw cells compared with control.
Figure 2.10 Cell viability was assessed after 72 hrs of removal of TC in L-685,458 at different concentrations.
Figure 2.11 Different concentrations of L-685,458 was added with TC removal, analyzed by western blot using 6E10.
Figure 2.12 MC65 cells were homogenized with and without formic acid extraction.
Figure 2.13 MC65 cells were homogenized in presence of Congo red and analyzed by western blot.
Figure 2.14 Comparison of cellular proteins. Analyzed with 6E10 and B994 antibodies.
Figure 2.15 MC65 protection assay with different concentrations of different TP analogues.

Figure 2.16 MC65 cells treated with different TP analogues. Western blots were analyzed with 6E10 and B994 antibodies.
Figure 2.17 MC65 cells were treated with different concentrations of CP2. Western blot was analyzed using 6E10 antibody.
Figure 2.18 MC65 cells were treated with TP17 and CP2 (2μM). Arrows indicate the deposition of Aβ-immunoreactive coarses.
Figure 2.19 Accumulation of CP2 inside the MC65 cells. The empty circles represent the cell surface CP2 and the dark circles represent the intracellular CP2.
Figure 2.20 TP compounds binding to Aβ 1-40 and Aβ 1-42 by SPR. Blue line is the response from the reference cell and the red line is the response from the experimental cell.
Figure 2.21 AFM image of Aβ40 in 50 mM phosphate buffer (no CP2) at day zero (upper panel). The heights and widths are shown in the bottom panel.
Figure 2.22 AFM image of Aβ40 in 50 mM phosphate buffer (no CP2) at day one (upper panel). The heights and widths are shown in the bottom panel (largest oligomer is 74nm high and 400nm wide).
Figure 2.23 AFM image of Aβ40 in 50 mM phosphate buffer (no CP2) at day three. Protofibrils and fibrils are formed and the lengths are 1μM.
Figure 2.24 AFM image of Aβ40 in 50 mM phosphate buffer (no CP2) at day 50. The material inside the solution is mainly fibrils and proto-fibrils. The largest fibril is 125 nm high and 1 μM wide.
Figure 2.25 AFM image of Aβ40 in 50 mM phosphate buffer, 25% TFE (no CP2) at day zero (upper panel). The lower panel represents the size and width of the oligomers.
Figure 2.26 AFM image of Aβ40 in 50 mM phosphate buffer, 25% TFE (no CP2) at day one.
Figure 2.27 AFM image of Aβ40 in 50 mM phosphate buffer, 25% TFE (no CP2) at day three. Protofibrils and fibrils were formed.
Figure 2.28 AFM image of Aβ40 in 50 mM phosphate buffer, in the presence of one equivalent of CP2 at day zero. The largest oligomer is about 30nm high and

40 nm wide.
Figure 2.29 AFM image of Aβ40 in 50 mM phosphate buffer, in the presence of one equivalent of CP2 at day one. The largest oligomer is about 35nm high and 40 nm wide.
Figure 2.30 AFM image of Aβ40 in 50 mM phosphate buffer, in the presence of one equivalent of CP2 at day three. Formation of fibrils or protofibrils was not found.
Figure 2.31 AFM image of Aβ40 in 50 mM phosphate buffer, in the presence of one equivalent of CP2 at day 50. No fibrils and protofibrils were found. Largest sizes of oligomers are about 25nm high and 30 nm wide.
Figure 2.32 AFM image of Aβ40 in 50 mM phosphate buffer, 25% TFE, in the presence of one equivalent of CP2 at day zero. The largest oligomers are about 10nm high and 30 nm wide.
Figure 2.33 AFM image of Aβ40 in 50 mM phosphate buffer, 25% TFE, in the presence of one equivalent of CP2 at day one. The largest oligomers are about 15nm high and 70 nm wide.
Figure 2.34 AFM image of Aβ40 in 50 mM phosphate buffer, 25% TFE, in the presence of one equivalent of CP2 at day three. No fibrils and protofibrils were found.
Figure 2.35 AFM image of Aβ42 in 50%TFE in the absence of CP2 at day zero. The largest oligomer is about 60 nm size.
Figure 2.36 A zoom in AFM image of the above Aβ42 in 50%TFE in the absence of CP2 at day zero.
Figure 2.37 AFM image of Aβ42 in 50% TFE in the absence of CP2 on day one. The arrows indicate the formation of protofibrils and large oligomers.
Figure 2.38 AFM image of Aβ42 in 50% TFE in the presenc of one equivalent of CP2 on day zero. The size of the oligomers is about 10-50 nm.
Figure 2.39 AFM image of Aβ42 in 50% TFE in the presence of one equivalent of CP2 on day one. The oligomers are about 10 nm-40 nm in sizes. The formation of protofibril is not observed.
Figure 2.40 A zoom in image of the above AFM image of Aβ42 in 50% TFE in the presence of one equivalent of CP2 on day one. The arrow shows the largest

oligomer of size 40 nm.
Figure 2.41 Cortical neurons were treated with different concentrations of Aβ42 oligomers. Data is shown as percentage viability after 48 hr treatment with Aβ42 oligomers.
Figure 2.42 Cortical neurons treated with Aβ monomers (M) and oligomers (O) in presence of CP2 (50 nM)and TP17 (50 nM).
Figure 2.43 Structures of quinoline compounds.

List of Tables

Table 1.1 IC_{50} values of different intermediates.

Table 2.1 Neurodegenerative diseases: proteins and pathology.

Table 2.2 K_D values for different TP compounds to Aβ peptides. NB = No binding.

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Dedication

"**SYNTHESIS AND BIOEVALUATION OF NOVEL TRICYCLIC PYRONE COMPOUNDS AND OVALICIN AND ITS ANALOGUES**"

Ph.D. Thesis by Srinivas K Battina dedicated

To,

My Mom & Dad
And
In loving memory of my grandparents

CHAPTER 1 – Synthesis of Ovalicin and its Analogues

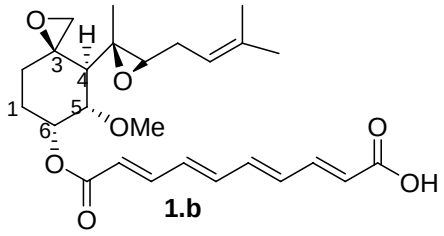
1.1 Introduction

Cancer is the second major cause of death, responsible for 2.5 million deaths every year in western countries.[1, 2] Over the years researchers developed a number of methods to treat cancer and a number of experimental treatments are evolving day by day. Currents methods like chemotherapy, radiation therapy and surgery are effective therapies to treat cancer. An approach to interrupt the tumor induced angiogenesis was first proposed by J. Folkman to treat cancer.[3, 4]

Angiogenesis is a process of involving the growth and formation of new blood vessels which plays an important role in healing wounds and repairing damaged tissues.[5] Angiogenesis plays a vital role in the growth of tumors.[5] Tumor cell requires nutrients and oxygen supply from the vascular system.[1, 5] So this strategy of inhibiting angiogenesis is proved to be an effective procedure to arrest the growth of tumor. Anti-angiogenic drugs (AAD) suppress the growth of tumor by inhibiting the growth of blood vessels, on which these cancer cells survive and grow.[6] Many of these drugs work by attacking the vascular endothelial growth factor (VEGF) pathway and some attach on the VEGF receptor.[6] Unlike chemotherapy drugs, AAD have no or less side effects. Side effects like bleeding in digestive tracts and increase in blood pressure are no exception.[6] In some cases AAD will shrink tumors and in some cases they will suppress the growth of tumors and will not allow them to grow further.[6] Patients with different physiological states may take varied time periods for treatment and some may even require long term treatment. The combination of AAD and chemotherapy drugs proved to be successful in some cases.[6]

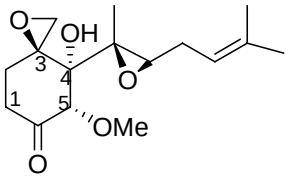
Natural products such as fumagillin and ovalicin were discovered to be potential anti-angiogenic sesquiterpene compounds due to their "inhibition of endothelial cell proliferation".[1, 5] Ovalicin (IC_{50} = 0.4 nM against human MetAP2)[7]and fumagillin are structurally similar compounds (Figure 1.1). TNP-470, O-(chloroacetylcarbamoyl)fumagillol is a synthetic derivative of fumagillin, is less toxic and more active than fumagillin with an IC_{50} value of 1.0 x 10^{-9} M against human MetAP2.[7, 8] TNP-470 is already in clinical trials used for treatment of cancers such as prostrate, kidney etc.[1] Like any other drugs, because of the presence of active functional groups, TNP-470 shows side effects like ataxia, vertigo, drowsiness and agitation.[1]

Figure 1.1



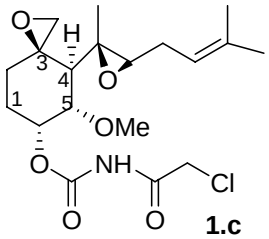
1.b

Fumagillin



1.a

Ovalicin



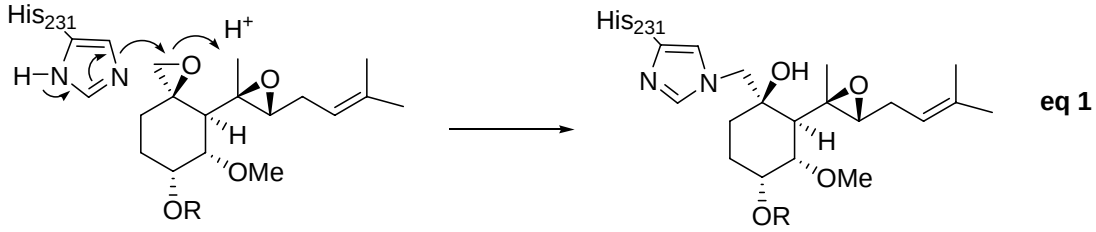
1.c

TNP-470

Ovalicin, structurally similar to fumagillin is isolated from fungus *Pseudorotium ovalis*.[9, 10] Ovalicin has a *tert*-hydroxy group at C-4 and a keto group at C-6, which not only differs in structure but also in activity with fumagillin. Ovalicin is stable at room temperature and remains crystalline for at least two years with no trace of decomposition whereas fumagillin decomposes gradually at room temperature.[8] Apart from being anti-angiogenic it also exhibits antibiotic, antitumor and immunosuppressive property.[5, 11, 12] The activity of ovalicin is comparable to TNP-470. "Both ovalicin and TNP-470 inhibit the proliferation of endothelial cells".[5] But it is not yet found whether the mode of action is the same for both compounds.[5]

Recently, the antimicrosporidial activity of these sesquiterpene compounds was demonstrated by Didier and coworkers.[13] More common microsporidia that infect humans are *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Encephalitozoon cuniculi* and *Encephalitozoon hellem*.[13] Currently albendazole is used to treat microsporidia infections in humans.[13] Fumagillin, TNP-470, ovalicin and ovalicin derivatives are screened *in vitro* on *Encephalitozoon intestinalis* and *Vittaforma corneae* assays and they were shown to inhibit the replication of these strains.[13] The authors found several synthetic analogues of ovalicin possessing greater activities. Trypanosoma brucei, parasitic protozoa causes sleeping sickness, malaria and other diseases.[14] *Trypasonoma brucei* contains a class of methionine aminopeptidases (MetAP2) enzymes.[15] Inhibition of this enzyme will result in death of the parasite. Ovalicin, an inhibitor of angiogenesis, is believed to be lethal to *Trypasonoma brucei* protozoa. We tested a series of ovalicin intermediates and the activities are discussed in the later part of this thesis.

Methionine aminopeptidases (MetAPs) specifically releases methionine residues from proteins. They exist in two types MetAP1 and MetAP2.[16] Addlagatta and Matthews reported MetAP2 as the molecular target for sequiterpenes like ovalicin and fumagillin. Both fumagillin and ovalicin have reactive epoxide groups which can coordinate and modify MetAP2.[17] To know which epoxide is more vital for the activity of these compounds, a number of analogues were synthesized with and without the epoxides. It was shown that the spiroepoxide is important and the side chain epoxide is not required for bioactivity.[17] It was also reported that the low affinity of these compounds was due to sterically hindered site in MetAP1.[18] This spiro epoxide binds to the MetAP and reacts with the histidine residue His(231), an active enzyme site (shown in the following equation 1).[18] It was determined by x-ray structure that fumagillin binds to His231 in MetAP2.[18]



eq 1

1.2 Previous reported total synthesis

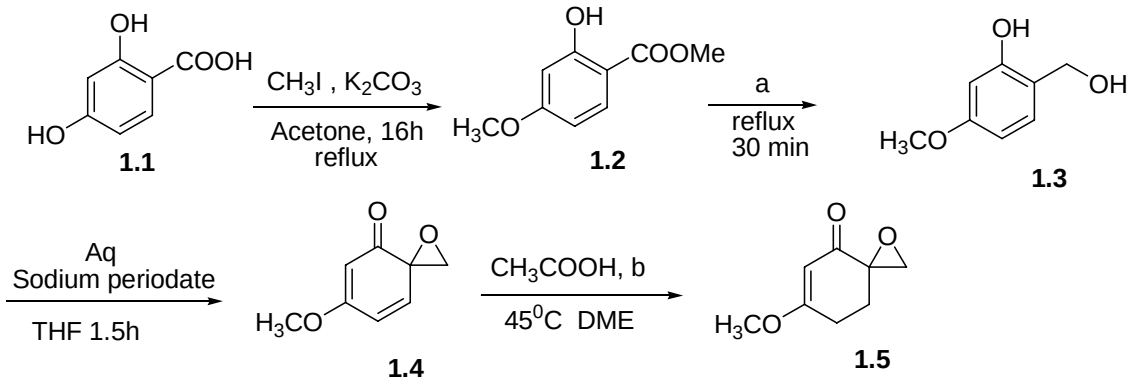
1.2.1 Corey's Synthesis

The first total synthesis of (±)-ovalicin was reported by E. J. Corey et al. in 1985.[19] Five total syntheses this compound sprouted up to date.[19-23] Though Corey's group synthesized fumagillin[24] earlier to the syntheses of ovalicin, they employed a completely different synthetic strategy for the synthesis of ovalicin. The design of this synthesis started with an epoxy enone from 2,4-dihydroxybenzoic acid and a stereoselective addition of a trisubstituted olefins resulting in an internally stereospecific epoxidized product which subsequently gave racemic ovalicin.

Reaction of 2,4-dihydrobenzoic acid (**1.1**) with methyl iodide and potassium carbonate yielded methyl 2-hydroxy-4-methoxybenzoate (**1.2**) in 83% yield and an immediate reduction with sodium bis(2-methoxyethoxy)aluminum dihydride gave phenol **1.3** in 97% yield (Scheme 1.1). Phenol was oxidized with aqueous solution of sodium periodate to give dienone epoxide **1.4** in 61% yield. Attempts to reduce γ,δ double bond selectively failed resulting in regeneration of

phenol **1.3**. However reduction was carried out following the procedure laid by Adam and Eggelte[25] by adding acetic acid and potassium azodicarboxylate. Diimide was generated *in situ* which reduces the γ,δ double bond to give epoxy enone **1.5** in 77% yield.[19]

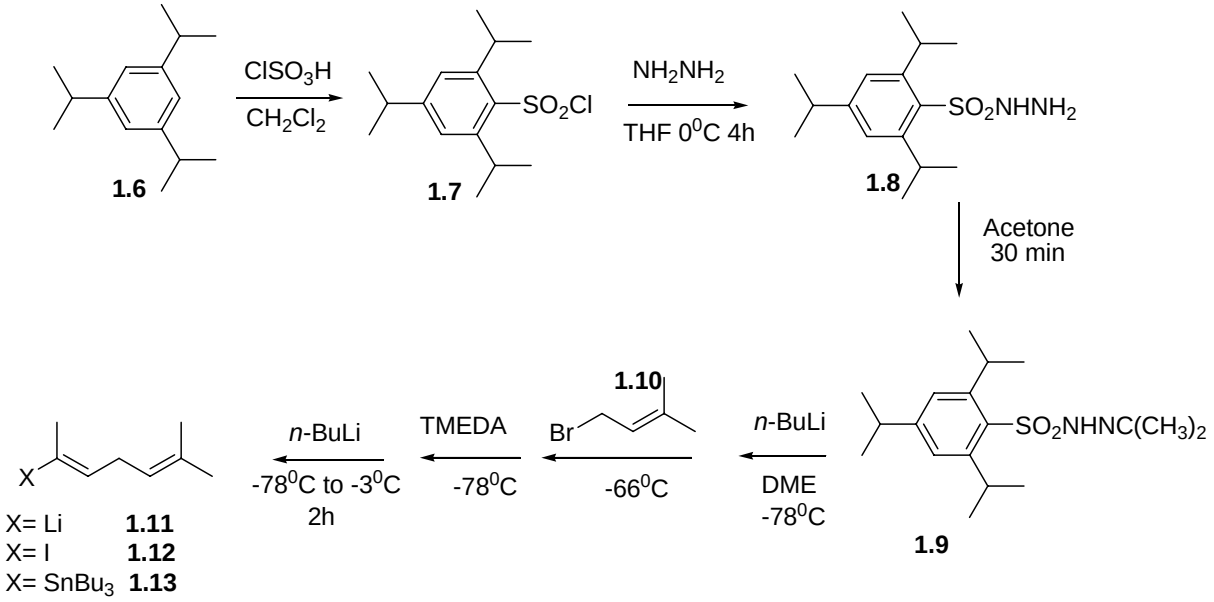
Scheme 1.1



a) Sodium bis(2-methoxyethoxy)aluminum dihydride b) Potassium azodicarboxylate

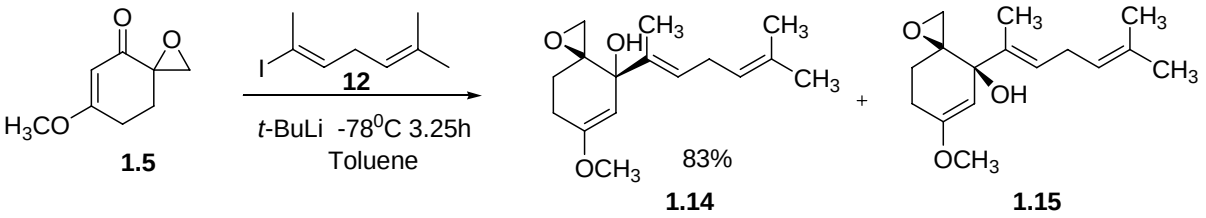
The next step is the addition of alkenyllithium reagent to the epoxy enone **1.5**. The preparation of alkenyllithium involves Shapiro reaction (the conversion of ketones to sulfonylhydrozones is known as Shapiro reaction) and its addition to **1.5** provided an intermediate for subsequent epoxidation. Compound 2,4,6-triisopropyl benzene (**1.6**) when treated with chlorosulfonic acid gives 2,4,6- triisopropylbenzenesulfonyl chloride (**1.7**) which reacts with hydrazine gave 2,4,6- triisopropylbenzenesulfonyl hydrazine (**1.8**). Acetone when treated with compound **1.8** gave acetone 2,4,6- triisopropylbenzenesulfonyl hydrazone (**1.9**).[26, 27]

Scheme 1.2



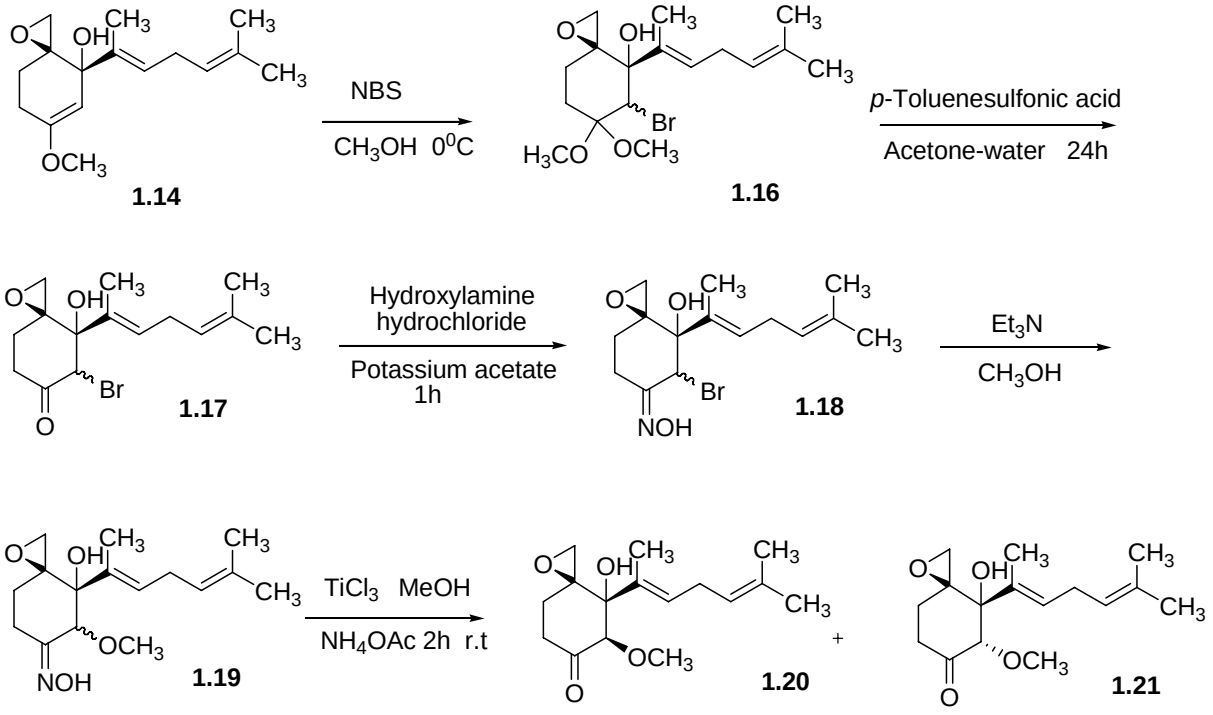
The solution of **1.9** and DME was cooled to -78 ^{0}C, and *n*-butyllithium was added to the mixture and allowed to reach -66 ^{0}C over 20 min. Then 1-bromo-3-methyl-2-butene (**1.10**) was added to the reaction mixture, and the temperature was maintained at -66 ^{0}C over a period of 20 minutes. N',N'-Tetramethylethylenediamine (TMEDA) was added to stabilize the anion formed at -78 ^{0}C followed by another equivalent of *n*-butyllithium. The reaction mixture vessel was allowed to warm to -3 ^{0}C over a period of 2 hours to obtain alkenyllithium reagent **1.11**. Compound **1.11** when treated with tri-*n*-butyltin chloride at -78 ^{0}C gave alkenyltin reagent **1.13**. The conversion of **1.13** to **1.12** can be carried by treatment of alkenyltin **1.13** followed by iodine (Scheme 1.2). Alkenyliodide **1.12** in ether was treated with *tert*-butyllithium at -78 ^{0}C, and to this reaction mixture epoxy enone **1.5** was added. A mixture of diastereomers **1.14** and **1.15** were obtained (Scheme 1.3). The addition reaction provided an intermediate, which can be converted to the epoxidized product through a stereoselective oxidation reaction.[19]

Scheme 1.3



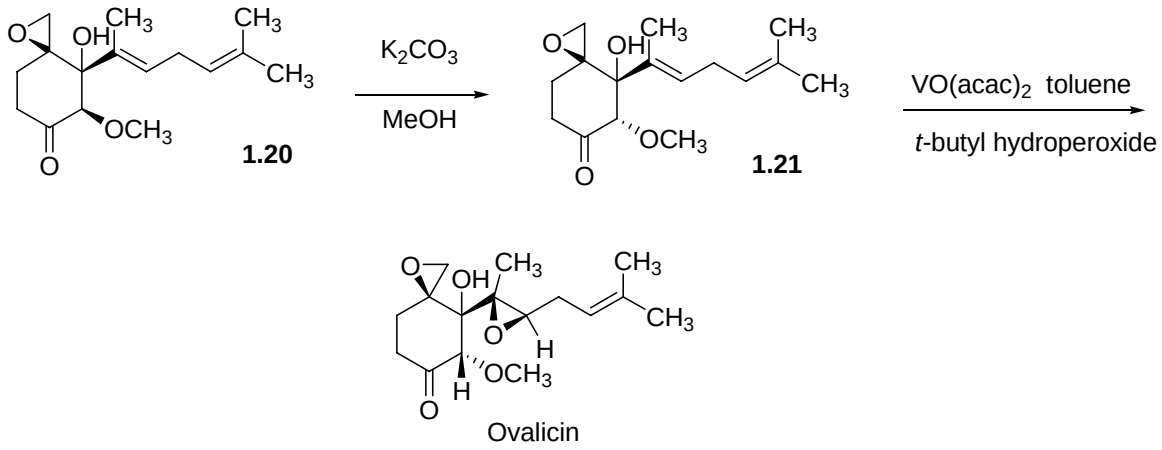
Reaction of **1.14** with *N*-bromosuccinimide (NBS) in methanol gave bromo ketal **1.16**. Compound **1.16** was treated with *p*-toluenesulfonic acid in acetone-water system to yield bromo ketone **1.17** in 94%. Bromo ketone **1.17** on treatment with hydroxylamine and potassium acetate for one hour gave bromo oxime **1.18**. Treatment of oxime **1.18** with triethylamine and methanol gave methoxy oxime **1.19** in greater than 90% yield. Hydrolysis of methoxy oxime **1.19** was performed in presence of titanium trichloride and methanol. Aqueous ammonium acetate served as a buffer in this conversion to yield a mixture of diastereomers **1.20** and **1.21** (Scheme 1.4). The characterization of these diasetreomers was performed by ^{1}H NMR spectroscopy.[19]

Scheme 1.4



Compound **1.20** was isomerized to **1.21** using potassium carbonate in presence of methanol. The conversion of compound **1.21** to ovalicin involves allylic epoxidation using vanadyl acetylacetonate along with *tert*-butylhydroperoxide in toluene (Scheme 1.5). Thus synthesized ovalicin was identified using ^{1}H NMR, IR, mass , ^{13}C NMR spectroscopy and found no different from the authentic sample.[19]

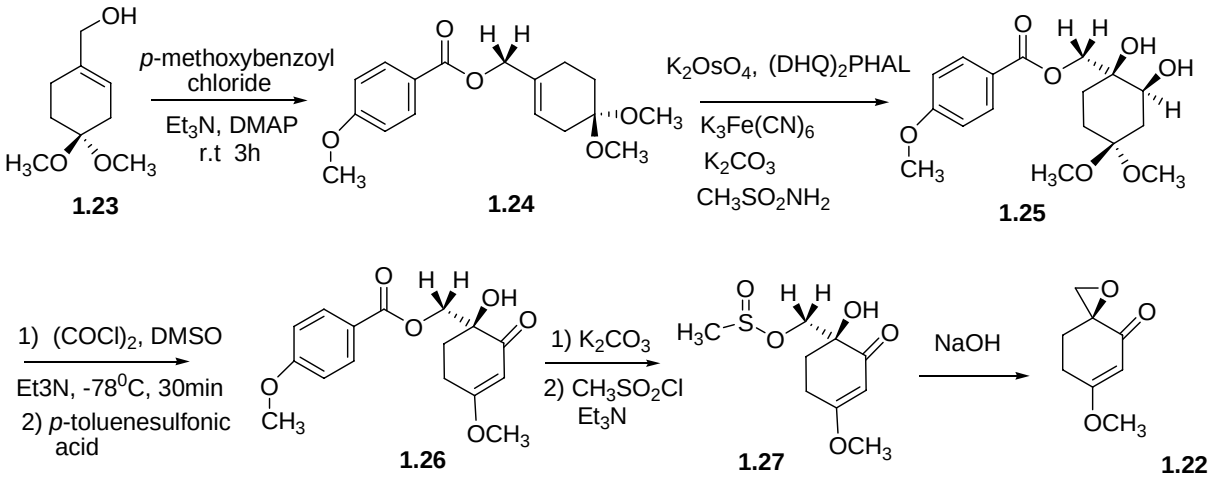
Scheme 1.5



Few years later, Corey et al. modified the original synthetic strategy and came up with a short enantioselective route using catalytic asymmetric dihydroxylation to synthesize ovalicin in large scale.[28] The key step in this strategy was the synthesis of a chiral intermediate **1.22**. Synthesis of ovalicin from this intermediate **1.22** followed the above cited procedure.[19] The esterification of readily available allylic alocohol **1.23** was carried with *p*-methoxybenzoyl chloride to give *p*-methoxybenzoate ester (**1.24**) in 98% yield. Intermediate **1.24** was considered to be a good substrate for OsO_4-biscinchona alkaloid catalyzed dihydroxylation. Compound **24** when treated 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, a dihydroxylated product **1.25** was obtained after 4 hours in 93% yield and 99% enantiomeric excess. Swern oxidation of intermediate **1.25** gave ketone in 87% yield, further treatment of this ketone with *p*-toluenesulfonic acid afforded β-methoxy α,β–enone **1.26** in 93% yield. Ester cleavage of **1.26** was carried out using K_2CO_3 followed by mesylation with methanesulfonyl chloride gave compound **1.27**. Treatment of compound **1.27**

with aqueous NaOH gave compound **1.22**, a key intermediate for the synthesis of ovalicin (Scheme 1.6).[28]

Scheme 1.6

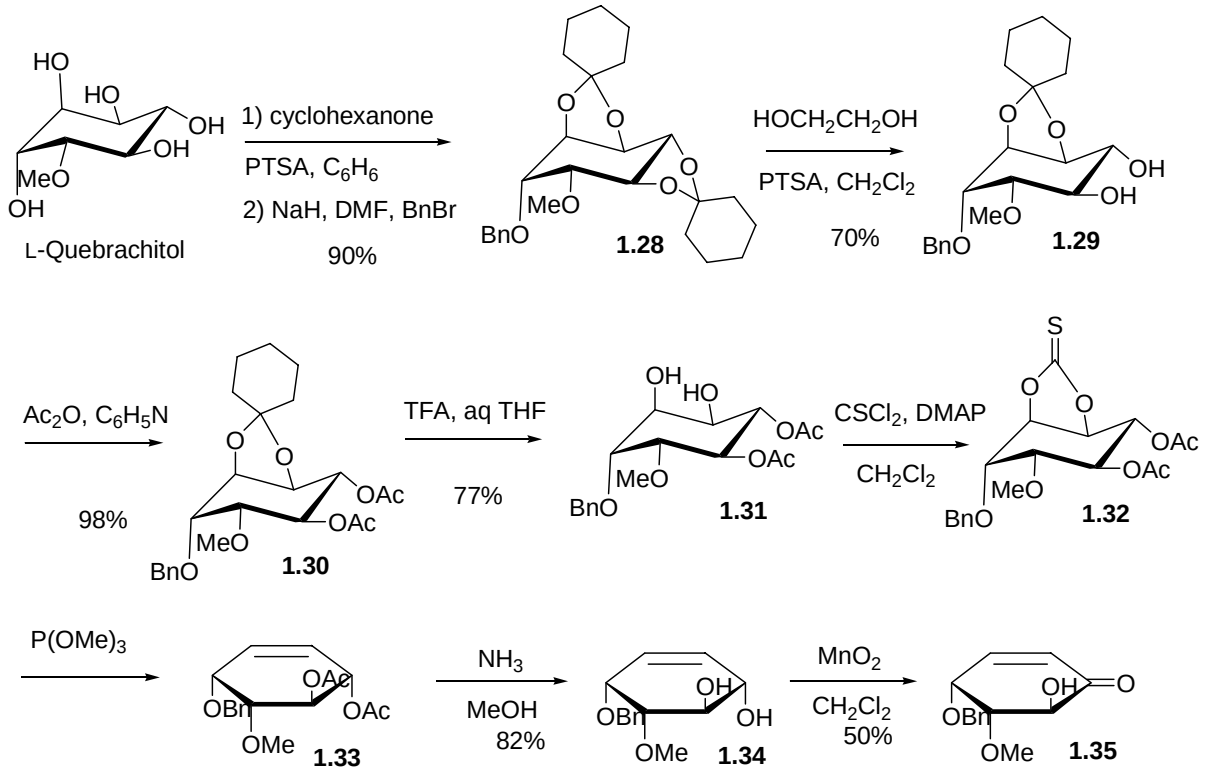


1.2.2 Barton-Quiclet-Sire-Samadi synthesis

Ovalicin and several other analogues were synthesized from a naturally occurring optically active cyclitol L-quebrachitol.[20] L-Quebrachitol was isolated from *Hevea brasiliensis*, considered to be a perfect compound. It has a methoxy group at C-2 and absolute stereochemistry required for conversion to ovalicin. This synthetic strategy slightly varies form the strategy reported by the same group[29] in 1994.

L-Quebrachitol on treatment with cyclohexanone and *p*-toluenesulfonic acid gave 1L-3,4:5,6-di-*O*-cyclohexylidene-2-*O*-methyl-*chiro*-insitol, further treatment with benzyl bromide gave **1.28**. The less stable *trans* ketal was cleaved readily using ethylene glycol in presence of *p*-toluenesulfonic acid to give a diol **1.29** in 70% yield. The diol was treated with acetic anhydride to give diacetylated product **1.30** in 98% yield. Deprotection of *cis*-ketal gave a crystalline diol **1.31** in 77% yield (Scheme 1.7).[20] Diol **1.31** was treated with thiophosgene (Corey-Winter *cis*-deoxygenation) and DMAP to give a thiocarbonate **1.32**. Compound **1.32** when heated to 120 ^{0}C with trimethyl phosphite gave a cyclohexene **1.33**. Removal of acetate groups in **1.33** was acheived using methanol ammonia solution to give ene-diol **1.34** in 82% yield. Selective oxidation of allylic alcohol was performed using MnO_2 to give enone **1.35** in 50% yield.[20]

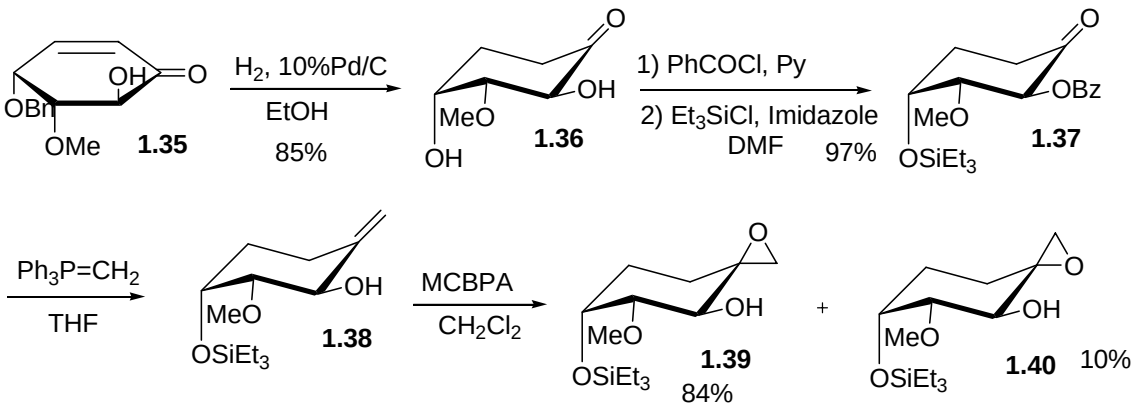
Scheme 1.7



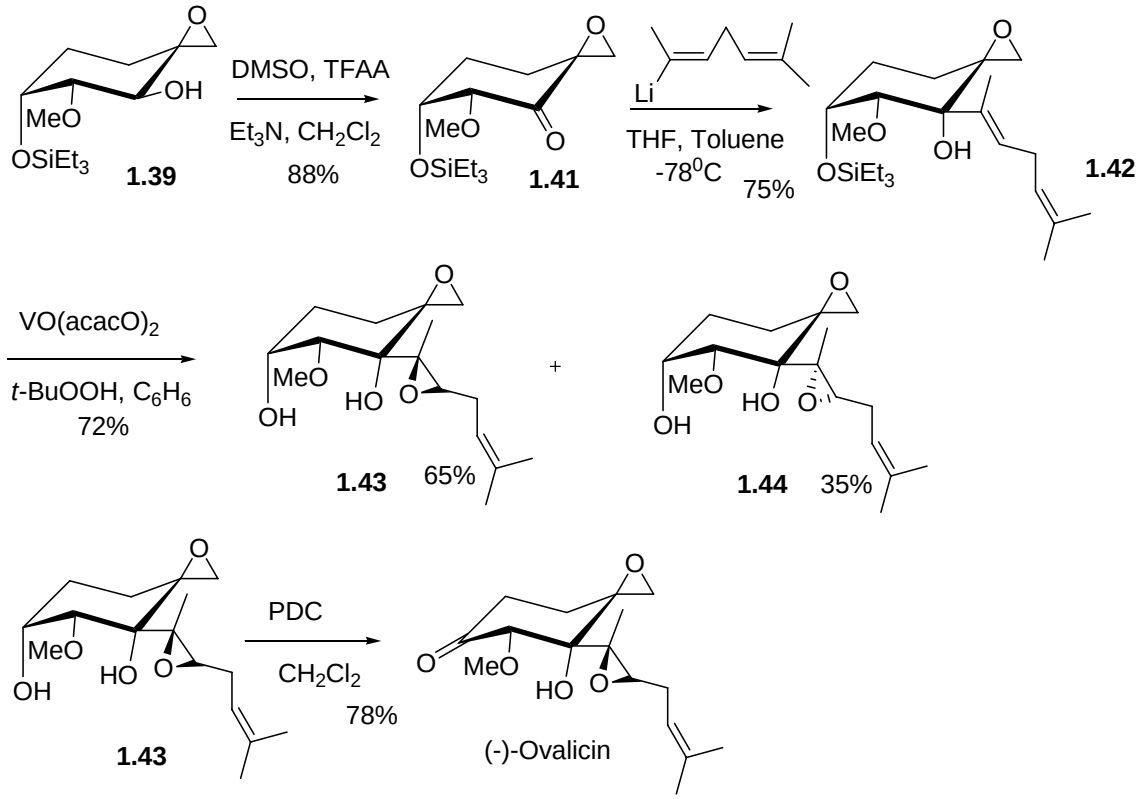
Compound **1.35** was a key intermediate in synthesis of (-)-ovalicin. The path to the synthesis of ovalicin followed Corey's strategy.[19] Hydrogenation of **1.35** using catalytic amount of palladium in ethanol afforded hexanonediol **1.36** in 85% yield. Selective benzylation of α-hydroxy alcohol and silylation of the γ-alcohol gave ketone **1.37**. The ketone was converted to an exocyclic olefin using a Wittig reagent, methylenetriphenylphosphorane to compound **1.38**. Oxidation of **1.38** was carried by using *meta*-chlorobenzoic acid (MCPBA). Epoxidation of exo double bond of **1.38** afforded a mixture of spiroepoxy isomers **1.39** (84%) and **1.40** (10%) (Scheme 1.8).[20] Swern oxidation of **1.39** gave epoxy ketone **1.41** in 88% yield. The synthesis of alkenyl side chain followed Corey's procedure[19], and addition of side chain to compound **1.41** gave exclusively one compound **1.42** in 75% yield. In this case the bottom face is being blocked by silyl group and methoxy group, allowing the side chain to come from the top face to give only one isomer. Sharpless epoxidation of adduct **1.42** using *tert*- butyl hydroperoxide and vanadium acetylacetonate yielded mixture of epoxides **1.43** and **1.44**. This mixture can be separated using silica gel column. Alcohol **1.43** was oxidized using pyridinium dichromate (PDC) to give (-)-

ovalicin in 78% (Scheme 1.9).[20] Thus synthesized (-)-ovalicin was characterized and matched to naturally occurring ovalicin.[20]

Scheme 1.8



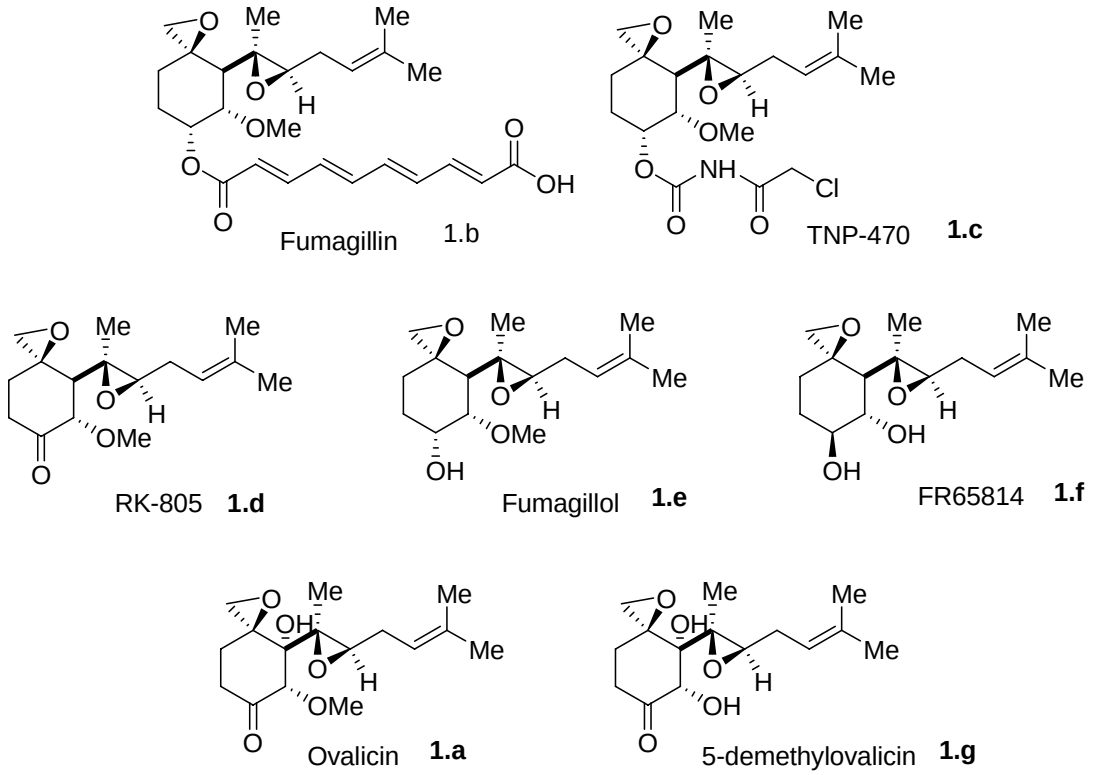
Scheme 1.9



1.2.3 Hayashi Synthesis

Another total synthesis was reported recently in 2006 by Hayashi et al.[21] Apart from synthesis of ovalicin their methodology was used to synthesize structurally similar compounds which belong to both the families of ovalicin and fumagillin (Figure 1.2). Herein authors developed proline-mediated α-aminoxylation of carbonyl compounds as a key step for the synthesis of RK-805 (isolated from fungus *Neosartorya* sp.), fumagillol, FR65814 (an immunosuppressant agent), ovalicin and 5-demethylovalicin.[21]

Figure 1.2

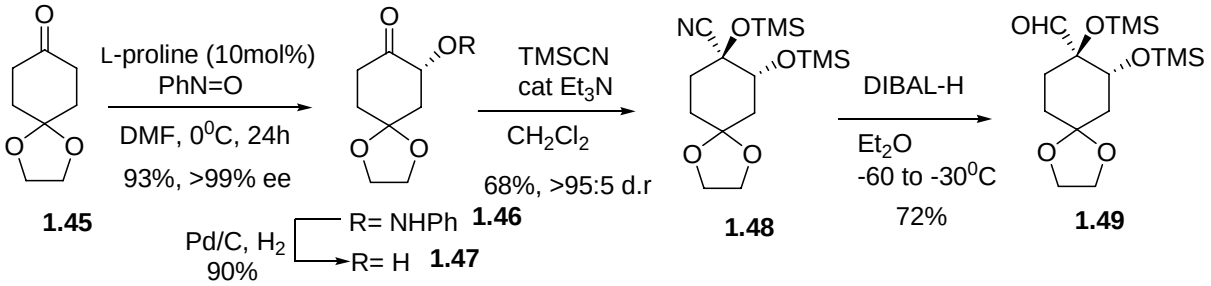


The synthesis of ovalicin starts with α-aminoxylation of 1,4-cyclohexanedione monoethylene ketal **1.45** using L-proline with a gradual addition of nitrosobenzene over 24h at 0 ^{0}C to give optically pure R-α-aminooxylated cyclohexanone **1.46** in 93% yield.[21] Reductive elimination of N-O bond in **1.46** was performed using H_2 at atmospheric pressure and catalytic amount of Pd/C to give **1.47**. After several experiments the authors found out that the cyanation

followed by silylation of compound **1.47** gave a highly diastereoselective compound **1.48** but in low yields. The reduction of cyanide **1.48** to aldehyde **1.49** was performed using diisobuytlaluminium hydride (DIBAL-H) (Scheme 1.10).[21]

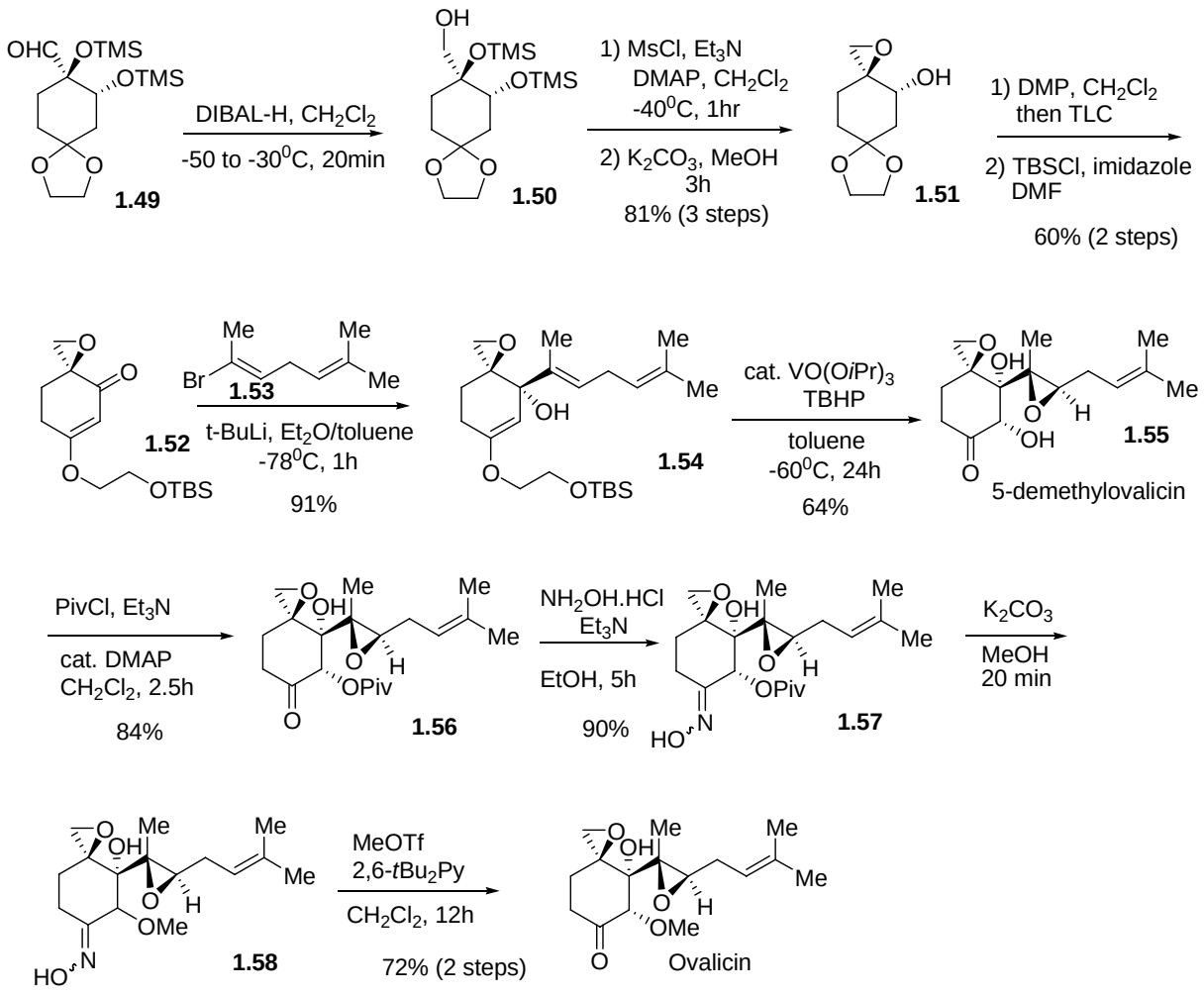
Further reduction of aldehyde **1.49** to alcohol **1.50** was carried using DIBAL-H. Intermediate **1.50** was converted to a spiroepoxide alcohol **1.51**. The total yield of conversion from **1.49** to **1.51** is 81%. Oxidation of alcohol **1.51** using Dess-Martin periodinane (DMP) followed by treatment with *tert*-butyldimethylsilyl chloride (TBSCl) gave cyclohexenone **1.52** in 60% yield. Substituted alkenyl bromide **1.53** addition to cyclohexenone epoxide **1.52** gave an adduct **1.54** in 91% yield. This addition is highly diastereoselective. Since this side chain was highly unstable and reactive, conversion to an epoxide **1.55** using a vanadium reagent along with *tert*-butylhydrogen peroxide (TBHP) was carried out quickly. The obtained compound **1.55** is 5-demethylovalicin (also a potent anti-angiogeneic inhibitor). The traditional methods of conversion of 5-demethylovalicin to ovalicin failed. Authors finally adapted a strategy by modifying Corey's method.[19] Alcohol **1.55** was protected with pivaloyl chloride and ketone **1.56** was transformed to an oxime **1.57**. Deprotection of pivaloyl group with K_2CO_3 and methanol gave a compound **1.58**. The oxime **1.58** was converted to ovalicin in good yield (Scheme 1.11).[21]

Scheme 1.10



TMS = trimethylsilyl DIBAL-H = diisobutylaluminium hydride

Scheme 1.11



DMP = Dess-Martin Periodinane; Piv = pivaloyl; OTf = trifluoromethanesulfonate, Py = pyridine
Ms = methanesulfonyl

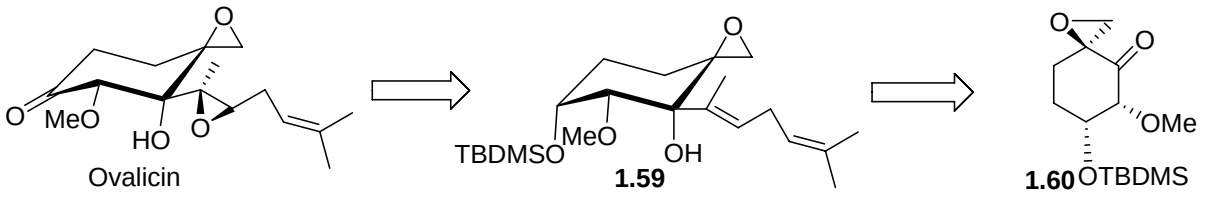
Two other total syntheses of ovalicin were reported by other researchers[22, 23] which are not discussed in this thesis.

1.3 Synthesis of Ovalicin

1.3.1 Retrosynthetic analysis

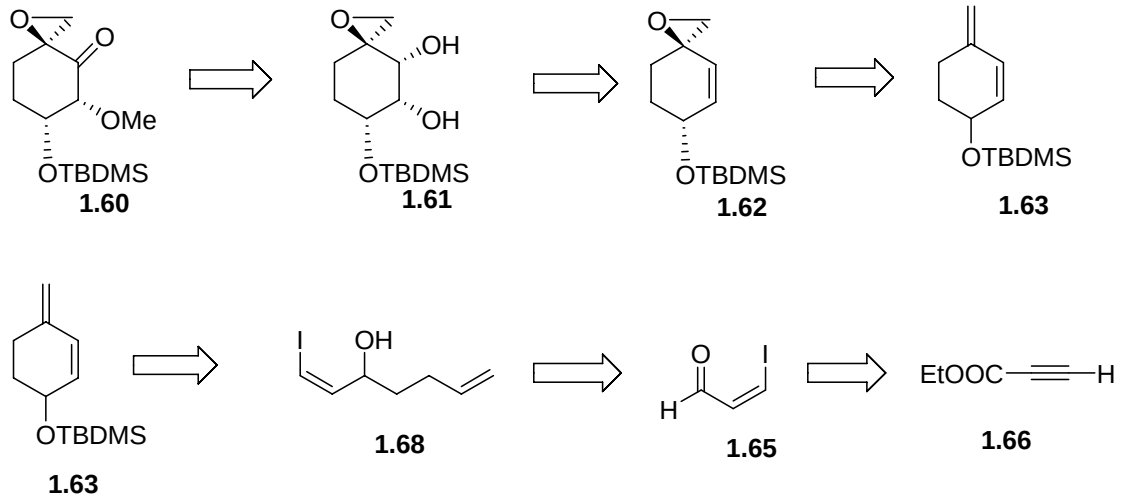
The target molecule ovalicin, a sesquiterpene comprises a cyclohexane ring with five stereogenic centers of which three of them are on the ring, two epoxides of which one is a spiro epoxide, a substituted olefin and a ketone.[21] Other researchers, as discussed in earlier sections started with a core six-membered ring compounds (substituted phenols, naturally occurring stereo-defined cyclohexane ring compounds, etc) as starting materials. We used a simple non-cyclic compound, ethyl propiolate to originate our synthetic scheme. A novel approach to synthesize a functionalized six membered ring was achieved via intramolecular Heck cyclization and a regioselective epoxidation are the key steps in our synthetic scheme. Generation of diastereomers in due course of our synthetic strategy led us to use this route to access a range of simpler and stable analogues for bioevaluation. The retrosynthesis of ovalicin is outlined in the following schemes. The later part of this total synthesis i.e. addition of the side chain and modifications on the cyclohexane ring follows the Corey[19] and Barton[20] strategy (Scheme 1.12). The addition of side chain is stereoselective, coming from the opposite side of the methoxy and silyloxy groups and these methoxy, silyloxy groups have to be *cis* to each other in compound **1.60** (Scheme 1.12). Stereochemical inversion is unavoidable in these groups if they are not *cis* to each other and is discussed later in this thesis. The synthesis of trisubstituted olefin was reported by Adlington and Barrett.[26, 27] This was converted to alkenyl lithium reagent, a very reactive species to react with the ketone in compound **1.60** (similar compound **1.41** was reported by Barton and coworkers[20]). Functional group transformations such as sharpless epoxidation, deprotection and oxidation are performed from compound **1.59** to obtain final product ovalicin.

Scheme 1.12



Synthesis of a structurally similar compound to **1.60** was already reported by Barton.[20] Naturally occurring compound with defined stereochemistry[20], substituted phenols[19] and functional group substituted cyclohexane rings[21] were proven to be essential starting materials to synthesize ovalicin. In our case none of those starting materials were used to synthesize compound **1.60**. Instead we obtained this compound by a series of transformations from a readily available alkyne ethyl propiolate (**1.66**). Ketone **1.60** was obtained from diol **1.61**. Methylation followed by oxidation of **1.61** yields ketone **1.60**. The diol **1.61** was readily obtained from selective epoxidation and dihydroxylation of appropriately functionalized methylidenecyclohexene **1.63**. The key steps in our total synthesis are the utilization of Heck reaction in construction of this functionalized methylidenecyclohexene **1.63** and regioselective epoxidation. Intramolecular Heck cyclization of **1.68** afforded methylidenecyclohexene **1.63**. Grignard addition to aldehyde **1.65** would provide **1.68**. Aldehyde **1.65** was readily obtained by reduction of alkyne **1.66** (Scheme 1.13).

Scheme 1.13

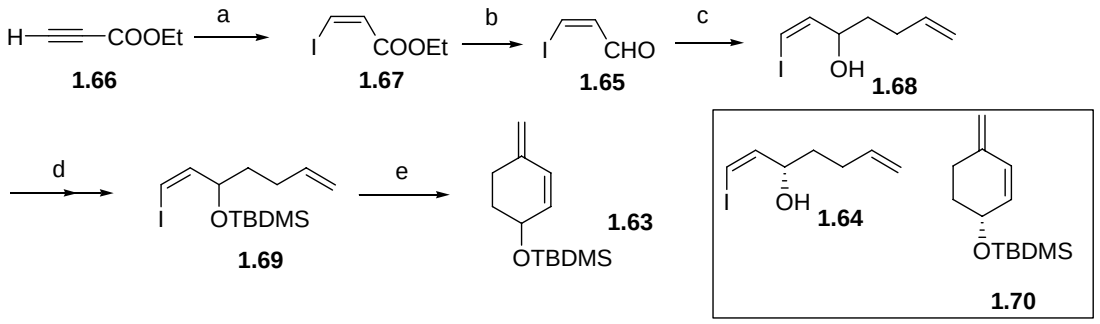


1.3.2 Synthesis of methylidenecyclohexenyloxysilane (1.63)

Synthesis of *tert*-butyldimethyl(4-methylenecyclohex-2-enyloxy)silane **1.63** is outlined in the Scheme 1.14. Commercially available ethyl propiolate (**1.66**) was used as the starting material. Authors Marek, Alexakis and Normant found out that methyl propiolate and substituted propiolates can be converted to (*Z*)-β-iodoacrylates by hydroiodination.[30] This conversion was

regio and stereospecific.[30] Dry sodium iodide and glacial acetic acid were stirred along with ethyl propiolate (**1.66**) and the resulting mixture was heated to 70 ^{0}C for 12 hours. Thus obtained product was distilled to get pure ethyl (*Z*)-β-iodoacrylate **1.67** in 89.2% yield.[31] The reaction of iodoacrylate **1.67** with diisobutylaluminium hydride (DIBAL-H) at -78 ^{0}C gave (*Z*)-iodoacrylaldehyde **1.65**.[30, 31] Aldehyde **1.65** was quite unstable and converts to (*E*)-isomer upon long standing or at higher temperatures. Addition of freshly prepared Grignard reagent (refluxing 4-bromo-1-butene with magnesium in THF at 85 ^{0}C for 2 hours gave Grignard reagent) to aldehyde **1.65** gave an iodoalcohol **1.68** (a racemic mixture) without any stereoselectivity. We did not obtain an enantio pure **1.64**. Iodoalcohol **1.68** was obtained in 74% yield. The side products of the this reaction were both (*E*)- and (*Z*)- isomers of 3-iodoprop-2-en-1-ol. Iodoalcohol **1.68** was protected with *tert*-butyldimethylsilyl chloride (TBDMSCl).[24] The reaction was carried in presence of base imidazole and a catalytic amount of 4-dimethylaminopyridine (DMAP) in dimethylforamide (DMF) and stirred for 12 hours at room temperature to give 98% yield of **1.69**.[32, 33] Intramolecular Heck cyclization was performed to obtain a cyclized compound **1.63** (a racemic mixture) not an enantiopure compound **1.70**. This intramolecular Heck cyclization was studied by a graduate student James McGill earlier in our laboratory.[34] He optimized the reaction conditions, found out suitable base, solvent and other reagents to obtain best results. Herein the same procedure was followed to obtain the cyclized product from **1.69**. This silyl protected iodo compound was treated with palladium acetate, silver phosphate, triphenylphosphine and proton sponge in presence of dimethylforamide (DMF) and the reaction mixture was heated to 50 ^{0}C for 7 hours to obtain 93% yield of cyclized product **1.63**. A doublet assigned to C2 hydrogen at δ 6.1, δ 5.7 for C1 hydrogen and a doublet at δ 4.8 for C7 hydrogen in proton NMR spectra confirms the cyclization of the product **1.63**. Attempts were made to cyclize the unprotected alcohol **1.68.** Cyclized alcohol was obtained in low yields and generation of side products was inevitable.

Scheme 1.14

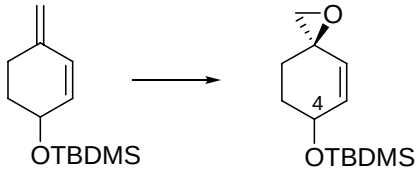


(a) AcOH, NaI, 70^{0}C, 12hr (b) DIBAL-H, CH_2Cl_2, -78^{0}C, 45 min (c)  MgBr, -78^{0}C

(d) TBDMSCl, imidazole, DMAP, DMF, 0^{0}C- r.t, 12 hr (e) $Pd(OAc)_2$, Ph_3P, Ag_3PO_4, proton sponge, DMF, 50^{0}C, 7 hr

1.3.3 Epoxidation of methylidenecyclohexanesilane 1.63

Scheme 1.15

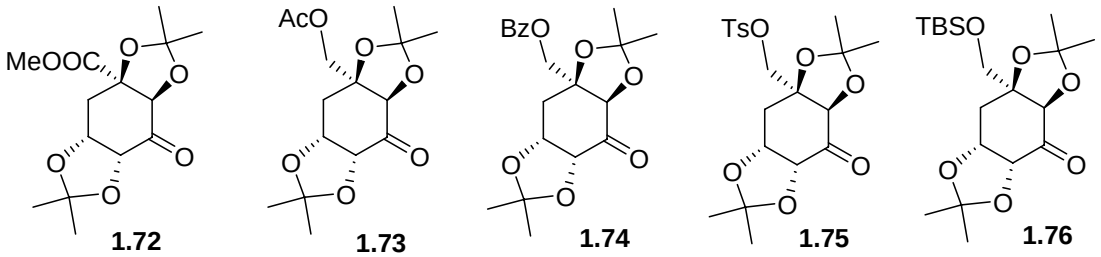


1.63 **1.71** (racemic 1:1 mixture at C4 position)

Epoxidation of **1.63** was initially carried out using *meta*-chloroperbenzoic acid (MCBPA) as an oxidizing agent (Scheme 1.15). Initially the reaction was carried out with 1 equivalent of MCBPA in CH_2Cl_2 at 0 ^{0}C. New spots were discovered on thin layer chromatography (TLC) and one of which was assumed to be the desired product. Subsequently on column chromatography (CC) the new spots weren't collected. Assumptions were made that new spots formed on TLC were decomposed on silica gel column chromatography. It is possible that *in situ* generated *meta*-chlorobenzoic acid, decomposes the desired epoxide. So as to neutralize the thus formed *meta*-chlorobenzoic acid, 2 equivalents of $NaHCO_3$ were added to the reaction mixture. The problem of decomposition could not be avoided. Reagents like sodium fluoride and potassium fluoride were also used along with MCPBA,[35] however only decomposed by-products were detected from nuclear magnetic resonance (NMR) spectrum of the crude product. Different solvent systems and varied reaction times didn't provide desired spiro epoxide. Other oxidizing agents were in search to overcome the problem.

Dioxiranes are neutral oxidizing reagents reported by Shi et.al[36] for asymmetric epoxidation of olefins. Dioxiranes were produced *in situ* from oxone (potassium peroxomonosulfate) and chiral ketones.[36] A few chiral ketones **1.72-1.76** are listed below.[37] High yields and enantiomeric excess are noticeable features of this oxidation.[36-38]

Figure 1.3



1.72 **1.73** **1.74** **1.75** **1.76**

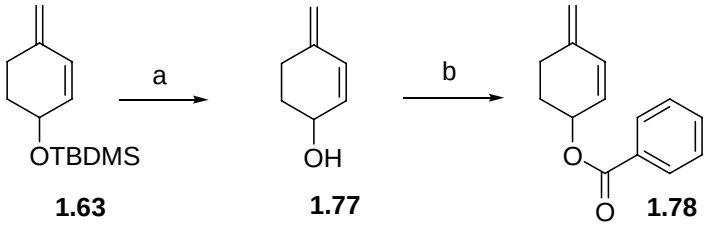
Chiral ketone wasn't used to oxidize **1.63** instead readily available cyclohexanone was used. Following the procedure cited by Shi,[38] compound **1.63** was mixed with cyclohexanone. To this mixture dipropylene glycol dimethyl ether (DMM), acetonitrile, aqueous K_2CO_3, acetic acid and catalytic amount of Bu_4NHSO_4 were added. This mixture was cooled to -10 ^{0}C. A solution of oxone-EDTA and aqueous K_2CO_3 was added to the above mixture. The reaction was stirred for 3 hrs at -10 ^{0}C. The TLC analysis shows the formation of product. The reaction was worked up and without further purification the crude material was treated with OsO_4 for dihydroxylation. After column chromatography about 5% of desired diol **1.61** was obtained. Use of chiral ketone might have increased the yield of this reaction. A different kind of dioxirane was used to obtain the spiro epoxide in high yields and is discussed in later parts of this thesis.

1.3.4 Changing the protection group from silyl to benzoyl

In our previous attempts the generation of *meta*-chlorobenzoic acid was affecting the silyl group resulting in low yields or absence of desired product. In order to overcome this problem benzoyl protecting group was replaced to silyl protecting group. The cleavage of *tert*-butyldimethylsilyl (TBDMS) group was achieved by treating **1.63** with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) at 0 ^{0}C for 10 hours. The progress of the reaction was monitored by TLC. On completion of the reaction, no work up was performed. The alcohol **1.77** was protected with benzoyl chloride. To the alcohol **1.77,** benzoyl chloride (C_6H_5COCl) and

triethylamine (Et_3N) were added at 0 ^{0}C. Warming up the reaction to room temperature and allowing the reaction to stir for 10 hours afforded benzoyl protected diene **1.78** in 98% yield (Scheme 1.16). Epoxidation of **1.78** with MCBPA led to no decomposition of the product. Therefore benzoyl protecting group was considered to be the best for this scheme.

Scheme 1.16



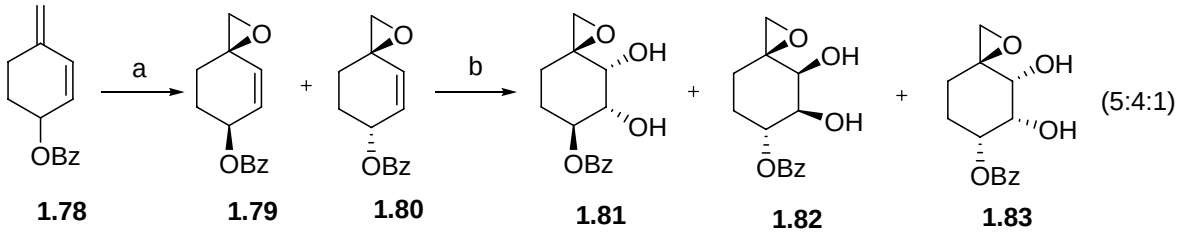
1.63 **1.77** **1.78**

(a) TBAF, THF, 0^{0}C, 10 hr (b) C_6H_5COCl, Et_3N, 0^{0}C - r.t, 10 hr

1.3.5 Epoxidation followed by dihydroxylation

Changing the protecting group from silyl to benzoyl was a major modulation in our synthetic scheme. Experiments in this section were carried out by Kaiyan in our group. He played a major role in separation and identification of the diol intermediates **1.81** ~ **1.83** (Scheme 1.17). The benzoyl protected diene **1.78** was oxidized using MCPBA as an oxidizing agent in presence of sodium fluoride (NaF) and potassium fluoride (KF). The resulting epoxide was stable for few hours without any considerable decomposition. The progress of the reaction was monitored by TLC. The organic layers were washed with sodium thiosulfate (to remove any excess oxidizing agents), dried over anhydrous sodium sulfate and concentrated. The NMR spectrum of the crude product confirms the formation of the product. This epoxide wasn't purified by column chromatography. Moreover this oxidation was not stereoselective. Both isomers **1.79** and **1.80** were formed. Without any further delay this mixture of ene-epoxides was treated with osmium tetraoxide (OsO_4), N-methylmorpholine-N-oxide (NMO) in the presence of *tert*-butanol and acetone-water system as a solvent.[39] Three inseparable diastereomers were formed **1.81**, **1.82** and **1.83** in 5:4:1 ratio (based on integration in crude proton NMR spectrum). The assignments of the stereochemistry were based on NMR J-couplings of the materials. Kaiyan was not able to separate these isomers. Appropriate solvent system to separate these isomers was not found (Scheme 1.17).

Scheme 1.17

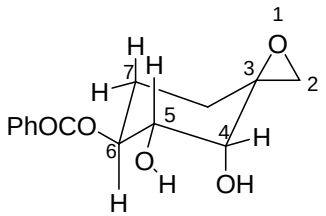


1.78 **1.79** **1.80** **1.81** **1.82** **1.83**

(a) MCPBA, NaF, KF, CH_2Cl_2 (b) NMO, OsO_4, *t*-BuOH, acetone-H_2O

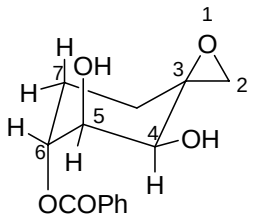
The three diols were identified by proton NMR spectra. Our assumptions were based on the coupling constants and coupling patterns and the structure of **1.84**. Compound **1.84** was obtained from a silylation reaction as described in scheme 1.18 (vide infra). The structure of the compound **1.84** was identified by a single crystal X-ray analysis. Compound **1.84** on desilylation regenerated pure diol **1.81**. Thus the structure of **1.81** was confirmed.

Compound **1.81**

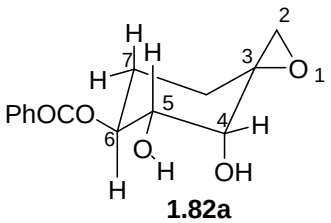


The proton NMR spectrum of the compound **1.81** shows signals at δ 8.06 (m, 2H), 7.59 (m, 1H) and 7.46 (m, 2H) for benzoyl group. Shifts at δ 5.35 is assigned to C6-H and appears as triplet of a doublet with J values of 8 Hz and 4 Hz respectively, which correspond to the coupling between C6-H and C5-H (J = 8Hz, axial-axial coupling), C6-H and C7-H (J = 8Hz, axial-axial coupling) and C6-H and C7-equatorial H (J = 4 Hz, axial-equatorial coupling). The intensities of the triplet-doublet resonance in the spectrum show 1:1:2:2:1:1. The coupling constant of 8 Hz suggests an axial-axial coupling. So the coupling constant of 8 Hz suggests that C6-H is oriented at the axial position Moreover the osmium tetraoxide dihydroxylation is a *syn* addition. So the two OH groups at C4 and C5 are *cis*. Because C5-OH is equatorial, consequently C4-OH is axial.

Compound **1.82**



1.82

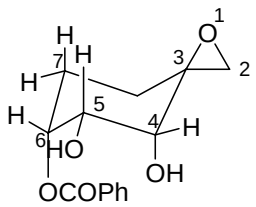


1.82a

Both are enantiomers

Compounds **1.82a** and **1.81** differ in the position of epoxide. The C3-O bond is axial in **1.81** and equatorial in **1.82a**. Both **1.82** and **1.82a** are enantiomers, so both the compounds show same δ values. The signals at δ 8.06, 7.59 and 7.46 for compound **1.82** correspond to the benzoyl group, which are similar in compound **1.81**. Since the compounds **1.81** and **1.82a** differ only in the position of epoxide, both the compounds must show the same splitting pattern for C6-H with C5-H, C7-axial H and C7-equatorial H. This is confirmed by observing splitting patterns and coupling constants in proton NMR spectrum. Signal at δ 5.35 (for C6-H), for compound **1.82** appears as a triplet of a doublet with J = 8 Hz and J = 4 Hz respectively. The coupling between C6-equatorial H with C5-equatorial H is J = 8Hz, C7-equatorial H is 8 Hz and C7-axial H is 4 Hz. The intensities of the triplet-doublet resonance in the spectrum show 1:1:2:2:1:1. The C2-H signals (doublets) at δ 2.60 and 2.86 for compound **1.82** differ from the C2-H signals (doublets) of compound **1.81** which show at δ 2.71 and 2.91. Thus the two compounds **1.81** and **1.82** can be differentiated by proton NMR spectra.

Compound **1.83**



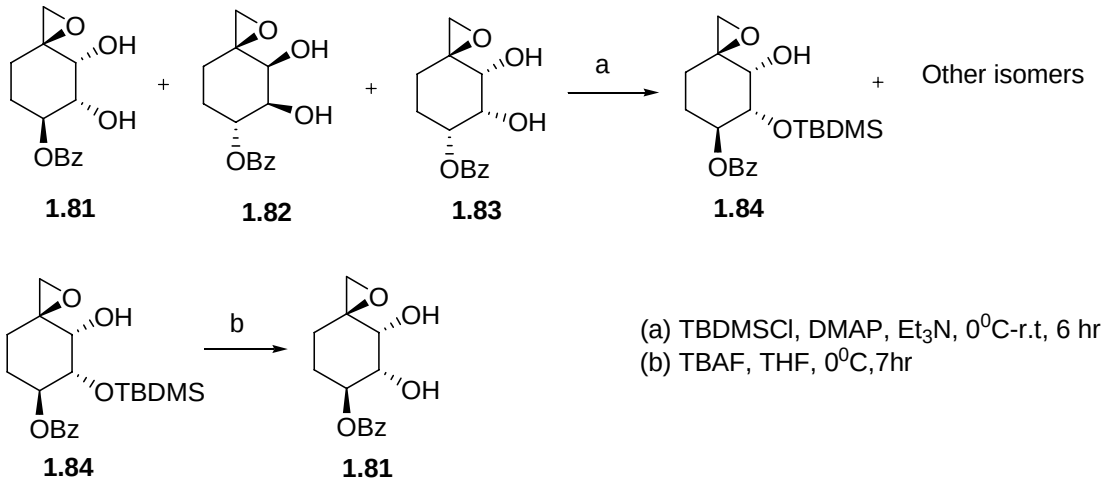
The proton NMR spectrum of compound **1.83** show signals at δ 8.06 (m, 2H), 7.59 (m, 1H), 7.46 (m, 2H) for benzoyl group, which are similar to compounds **1.81** and **1.82**. Signal at δ 5.18 is assigned to C6-H and appears as doublet of a triplet with J value of 3.1 Hz and 10 Hz respectively. The coupling between C6-H with C7-equatorial H has a coupling constant of J = 10 Hz (equatorial-equatorial). The coupling between C6-H and C7-axial H (equatorial-axial) and

with C5- axial H (equatorial-axial) has a coupling constant of J = 3.1 Hz. The intensities of the doublet of a triplet resonance in the spectrum show 1:2:1:1:2:1. The coupling constant of 3.1 Hz suggests that the C6-H is oriented at the equatorial position. Moreover osmium tetraoxide dihydroxylation is a *syn* addition. The two OH groups at C4 and C5 are *cis*. Since the C6-H is equatorial, implies that OH group at C5 is also equatorial.

1.3.6 Separation of diols

A novel idea was designed by Kaiyan to separate the diols **1.81**, **1.82** and **1.83**. The mixture of the diols was taken and silylation was performed using *tert*-butyldimethylsilyl chloride (TBDMSCl) with triethylamine as a base and DMAP as a catalyst (Scheme 1.18). Interestingly the major compound was isolated and identified by NMR spectrum and X-ray crystal analysis (Figure 1.4). The exact configuration of the silylated diol was unambiguously by X-ray single crystal analysis, which revealed the exact configuration of one diol **1.81**. There were other silylated products formed along with compound **1.84** but are not crystalline and couldn't be identified using NMR spectrum. After identifying the silylated product **1.84,** it was desilylated and converted back to diol **1.81** (Scheme 1.18) and are used for further conversion to the final product, illustrated in the following sections. The X-ray crystal structure of compound **1.84** is shown in Figure 1.4.

Scheme 1.18



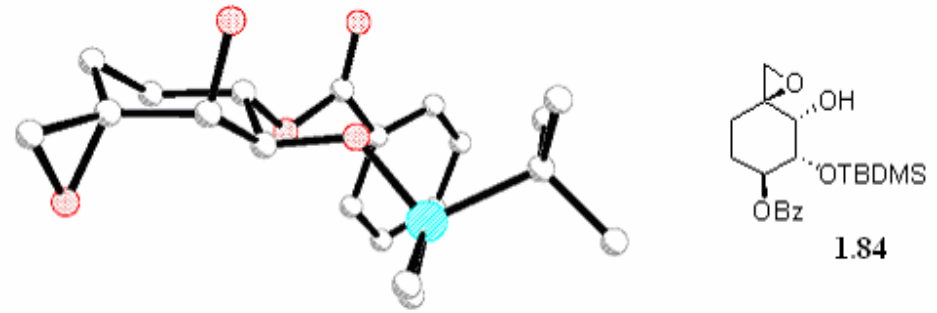
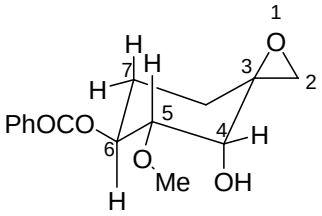


Figure 1.4 X-ray crystal structure of compound **1.84**

1.3.7 Methylation and Oxidation

Diol **1.81** was treated with trimethyloxonium tetrafluoroborate (Me_3OBF_4) in the presence of proton sponge as a base to give a methylated product.[40] This methylation was not selective, thus obtained a mixture of methylated compounds **1.85** and **1.86** in 1.3:1 ratio. The compound **1.85** has the methoxy group next to the benzoyl group and they are *trans* to each other.

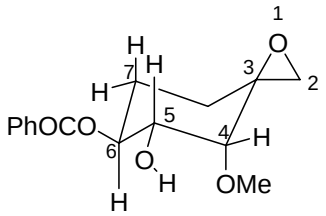
Compound **1.85**



The proton NMR spectrum of the compound **1.85** shows signals at δ 8.06 (m, 2 H), 7.59 (m, 1 H) and 7.47 (m, 2 H) for benzoyl group. Signal at δ 5.43 is assigned to C6-H and appears as triplet of a doublet with J values of 9 Hz and 4 Hz respectively. The Coupling between C6-axial H with C5-axial H is J = 9 Hz (axial-axial coupling), C7-axial H is J = 8 Hz (axial-axial coupling) and C7-equatorial H is J = 4 Hz (axial-equatorial coupling). The intensities of the triplet of a doublet resonance in the spectrum show 1:1:2:2:1:1. The J value of 8 Hz suggests an axial-axial coupling. The coupling constant of 8 Hz for C6-H with C5-H confirms that C6-H is oriented in the axial position. Signal at δ 3.66 is assigned to C5-H and appears as a doublet of a doublet with J values 3.2 Hz and 7.6 Hz respectively. The coupling constant J = 3.2 Hz correspond to C5-axial H with C4-equatorial H and coupling constant J =7.6 Hz correspond to

C5-axial with C6-axail H. The coupling constant of 7.6 Hz suggest that C5-H is oriented *trans* to the C6-H.

Compound **1.86**

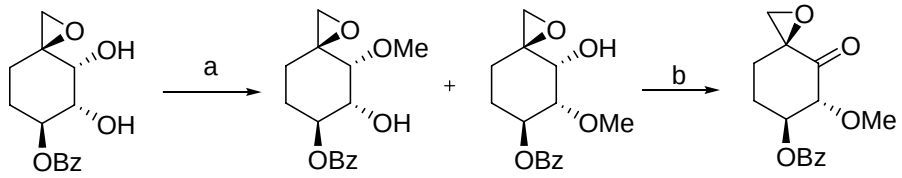


The proton NMR spectrum of the compound **1.86** show signals at δ 8.07, 7.56 and 7.45 for benzoyl group, which are similar to compound **1.85**. Signal at δ 5.32 is assigned to C6-H and it appears as a doublet of a triplet with J values 4 Hz and 9.2 Hz respectively. The coupling of C6-axail H with C5-axial H is J = 9.2 Hz, C7-axail H is J = 9.2 Hz and C7-equatorial H is J = 4 Hz. Signal at 3.99 is a doublet of a triplet is assigned to C5-H with J values of 3.2 Hz and 8 Hz respectively which correspond to the coupling between C5-axial H with C4-equatorial H (J = 3.2 Hz), C6-axail H (J = 8 Hz) and C5-OH (J = 8.4 Hz). Signal at δ 2.49 is assigned to C5-OH with C5-H with a coupling constant of 8.4 Hz appears as a doublet.

The two compounds **1.85** and **1.86** are differentiated by the splitting pattern of C5-axial H with C4-H and C6-H, a doublet of a doublet for the former and a triplet of a doublet for the later.

The alcohol **1.85** was oxidized to ketone **1.87** via swern oxidation (Scheme 1.19).[41] Silica gel column chromatographic separation was performed quickly because of fear of decomposition of the product on the silica gel column. The compound **1.87** was not stable. The silica gel column chromatography was washed with 1% triethylamine before loading the crude reaction mixture to avoid decomposition of the product. Thus obtained ketone **1.87** was used in next step for alkenyl lithium addition without any further delay.

Scheme 1.19



1.81 **1.86** **1.85** **1.87**

(a) Me_3OBF_4, proton sponge, CH_2Cl_2, 0^0C- r.t, 7 hr (b) TFAA, DMSO, Et_3N, -78^0C-0^0C, 2hr

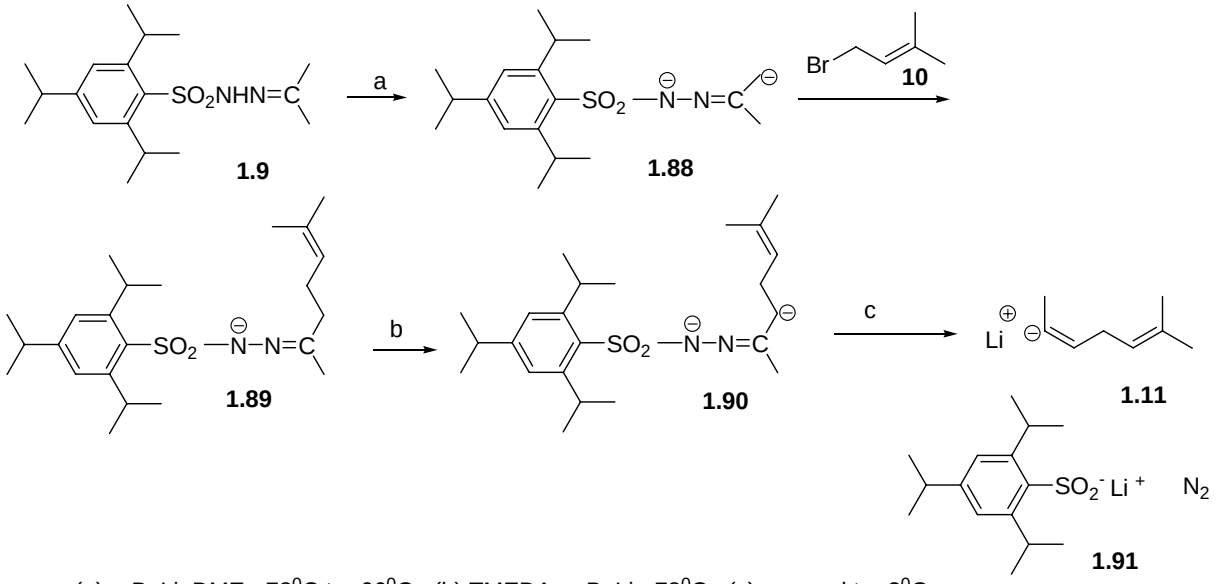
1.3.8 Preparation of Alkenyl Lithium reagent

The preparation of alkenyl lithium reagent was discussed earlier in Scheme 1.2 section 1.2.1. 2,4,6-Triisopropyl benzene (**1.6)** when treated with chlorosulfonic acid at 0 ^{0}C for one hour afforded 2,4,6-triisopropyl benzenesulfonyl chloride **1.7** in 88% yield after column chromatography. Chloride **1.7** can also be purified via crystallization using pentane as a solvent. 2,4,6-Triisopropyl benzenesulfonyl chloride **1.7** was treated with anhydrous hydrazine at 0 ^{0}C for 4 hours to produce benzenesulfonyl hydrazine **1.8**. The obtained benzenesulfonyl hydrazine **1.8** was pure and needs no purification from proton NMR spectrum analysis. Moreover hydrazine **1.8** decomposes on column when attempted to purify. 2,4,6-Triisopropyl benzenesulfonyl hydrazine **1.8** was dissolved in acetone and stirred for half an hour to obtain benzenesulfonyl hydrazone **1.9**, an application of Shapiro reaction (the synthesis of substituted and unfunctionalized alkenes from ketones is known as Shapiro reaction.).[26, 27] This hydrazone can be crystallized using aqueous methanol and should be dried over P_2O_5. Benezenesulfonyl hydrazone **1.9** should be absolutely dry to prepare alkenyl lithium reagent.[26, 27] Either the presence of acetone or water or both will quench the *n*-Buli, resulting in low yields of alkenyl lithium reagent.

Two equivalents of *n*-BuLi were added to hydrazone **1.9** at -78 ^{0}C to generate a dianionic species **1.88**. Subsequent addition of bromo compound **1.10** to the dianion gave a monoanionic species **1.89**. N,N,N',N'-Tetramethylethylenediamine (TMEDA) was added to stabilize the anionic species **1.89**. Another equivalent of *n*-BuLi was added to **1.89** to generate a dianionic species **1.90**. The solution of the dianionic species was a beautiful golden yellow color solution.

All the above intermediates are sensitive to moisture and air, and are always kept under inert atmosphere (argon). Intermediate **1.90** when slowly warmed to -3 ^{0}C from -78 ^{0}C, alkenyl lithium compound **1.11** was generated (Scheme 1.20).[26, 27] Addition of alkenyl lithium **1.11** to ketone **1.87** was performed without any delay.

Scheme 1.20



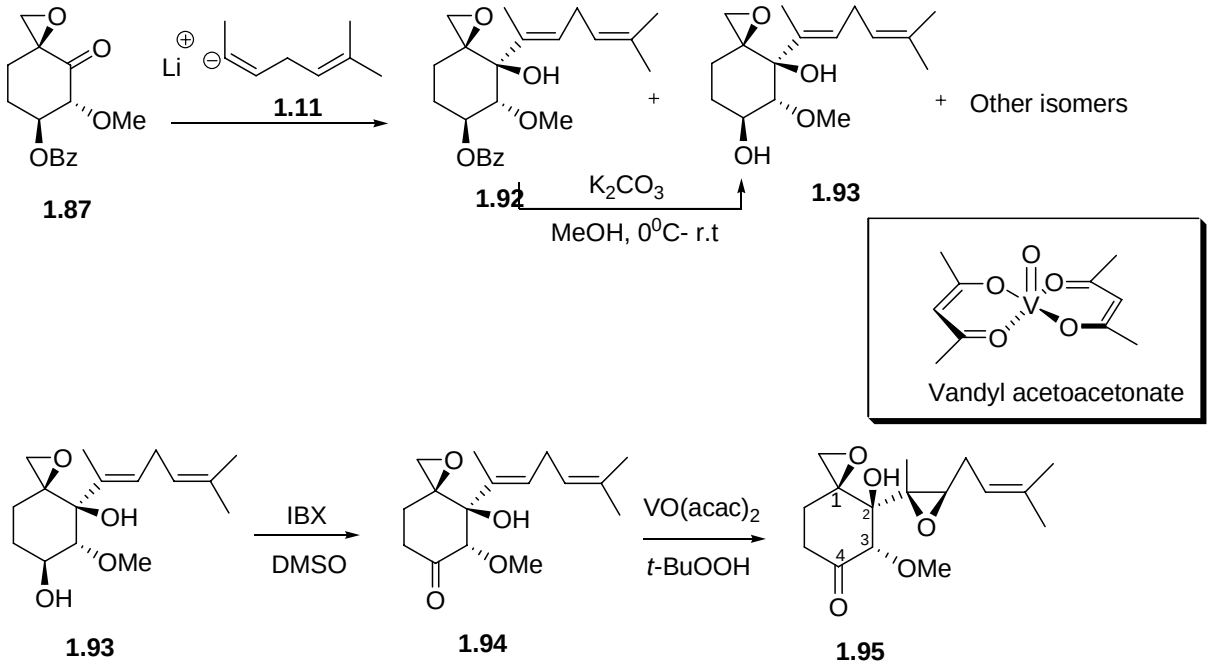
(a) n-BuLi, DME, -78^{0}C to -66^{0}C (b) TMEDA, *n*-BuLi, -78^{0}C, (c) warmed to -3^{0}C

1.3.9 Addition of akkenyl lithium reagent to ketone 1.87 and transformation of the resulting adduct to ovalicin analogues

The addition of alkenyl lithium reagent **1.11** to ketone **1.87** was no less similar to Corey's[19] and Barton's[20] strategy. The above prepared alkenyl lithium reagent was less stable and decomposes upon prolong standing. Without isolation the freshly prepared alkenyl lithium reagent was added to ketone **1.87**. Stereoselective addition was not observed. The addition of the organo lithium reagent was from both sides' *cis* and *anti* to methoxy group. Unlike in Bartons' synthesis, both the methoxy and silyl protected alcohol are *cis* to each other the former being equatorial and later being axial. In our case, the two major products were identified, **1.92** (15%) and **1.93** (10%). It was difficult to identify and characterize the remaining fractions from silica

gel column chromatography. Compound **1.93** was formed because the alkenyl lithum anion might have deprotected the benzoyl group. Compound **1.92** when treated with a base K_2CO_3 in presence of methanol deprotected the benzoyl group to give alcohol **1.93** in good yield. Alcohol **1.93** was oxidized to ketone **1.94** using *o*-iodoxybenzoic acid (IBX) in DMSO.[42] The final step was the Sharpless epoxidation, similar to that reported in Corey's and Barton's methodology. A metal-catalyzed reaction giving a selective epoxidation for olefinic alcohols was developed by Sharpless.[43] The epoxidation of **1.94** was carried out at 0 ^{0}C in presence of vandyl acetoacetonate ($VO(acac)_2$) and *t*-butyl hydroperoxide for 3 hours and toluene was used as a solvent. After silica gel column chromatographic separation and concentration of the fraction that contain the product, a nice crystalline compound appeared. On careful X-ray analysis, the obtained product, a compound **1.95**, a stereoisomer of ovalicin at C2 position (Scheme 1.21).

Scheme 1.21



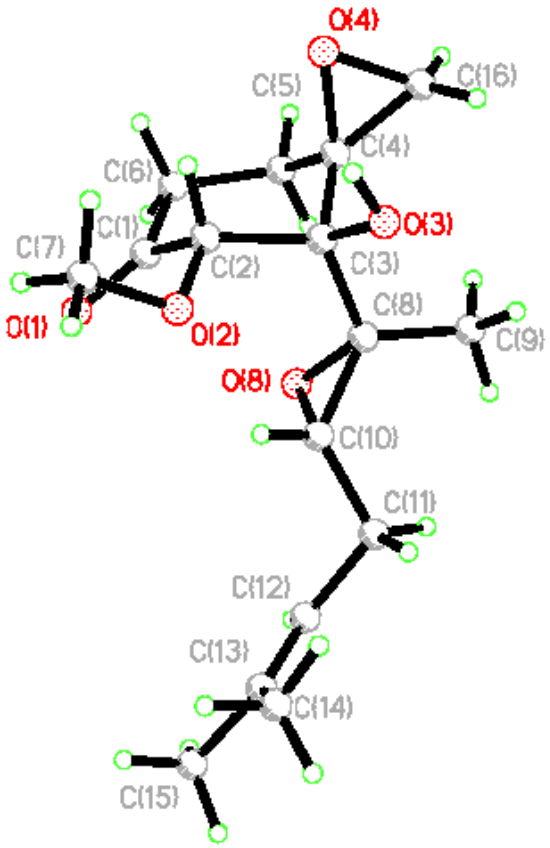


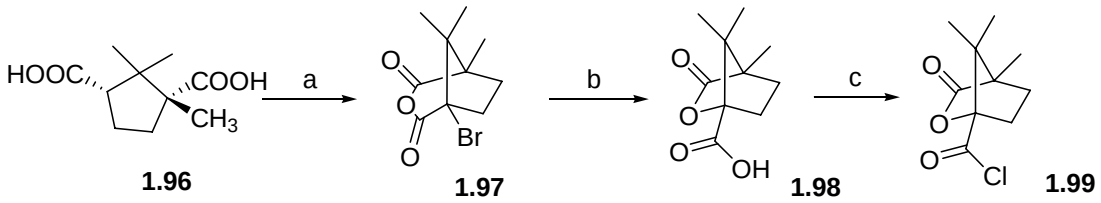
Figure 1.5 X-ray crystal structure of **1.95**

1.3.10 Changing the protective group from silyl to (-)-(1S,4R)- camphanoyl

The goal of using camphanyl chloride is to obtain an optically pure enantiomer. A chiral protecting group was used and tested. The silyl protecting group was cleaved and replaced with camphanoyl protecting group. The main reason for the choice of camphanyl chloride was to separate the diastereomers either by column chromatography or crystallization. The preparation of this compound (-)-(1*S*,4*R*)-camphanyl chloride followed a literature procedure.[44] Authors Kappes and Gerlach described a three step procedure for the preparation of (-)-(1*S*,4*R*)-camphanyl chloride.[44] (+)-Camphoric acid (**1.96**) was heated with phosphrous pentachloride on an oil bath for 5 hr at 85 ^{0}C. Then bromine was added to the above cooled mixture and again heated to 65 ^{0}C for 16 hr. After a careful workup we obtained (-)-bromocamphanic anhydride **1.97** as colorless crystals in 60% yield. Sodium carbonate and water were added to the anhydride

1.97 and the reaction solution was refluxed for 2 hr. The reaction mixture was neutralized with concentrated hydrochloric acid. Careful workup gave colorless crystals of (-)-camphanic acid **1.98** (59% yield). Acid **1.98** was converted to colorless acid chloride, (-)-camphanoyl chloride **1.99** using thionyl chloride in 87% yield (Scheme 1.22).[44]

Scheme 1.22

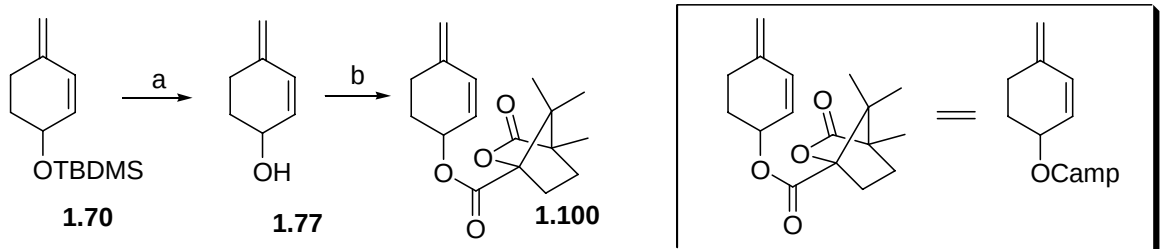


1.96 **1.97** **1.98** **1.99**

(a) PCl_5, Br_2, H_20 (b) Na_2CO_3, H_2O, 100^0C, H_3O^+ (c) $SOCl_2$ Reflux, 3hrs

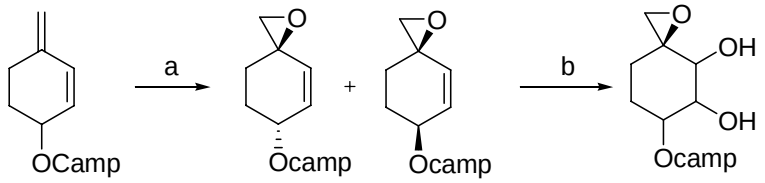
To produce the optically pure enantiomer, we planned to change the protecting group and replace the silyl with camphanoyl group. The alcohol was obtained on deprotecting **1.63** using TBAF as a reagent. Diene-alcohol **1.77** was formed in quantitative yield. Protecting alcohol **1.77** with (-)-camphanoyl chloride **1.99** was achieved in the presence of triethylamine. The obtained camphanic ester **1.100** has a pair of diastereomers. Theoretically they are separable using silica gel column chromatography. Attempts to separate the diastereomers failed and proper solvent system cannot be found. Attempts to crystallize the diastereomers also failed to provide a single diastereomer. Successful separation would allow us to continue with the total synthesis with one enantiomer leaving the other behind. This mixture of diastereomers were used in the following epoxidation and dihydroxylation (Scheme 1.23). The epoxidation was performed as that similar to those described in section 1.3.5. Ester **1.100** was treated with MCPBA to obtain a mixture of isomers **1.101** and **1.102**. This oxidation was not stereoselective. We tried to isolate this mixture, but couldn't succeed. Final bid to separate these mixtures atleast in the dihydroxylation step; we took the mixture and subjected to dihydroxylation conditions as discussed earlier. A mixture of diols **1.103** was obtained. We could not separate the mixture of diols using a silica gel column chromatography. Change in protection group wasn't assisting us for a better separation of diastereomers. This approach was not pursues further.

Scheme 1.23



1.70 **1.77** **1.100**

(a) TBAF, THF, 0^0C, 48hr (b) Et_3N, Camphanoyl Chloride(**1.99**)



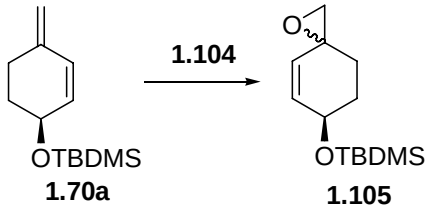
1.100 **1.101** **1.102** **1.103**

(a) MCPBA, NaF, KF, 0^0C (b) OsO_4, NMO, *t*-BuOH, Acetone-H_2O

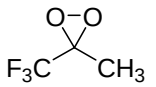
1.3.11 Using trifluoromethyldioxirane (TFDO) as a oxidizing agent to epoxidize diene 1.63

The oxidation of the diene **1.63** using MCPBA was not successful as discussed in section 1.3.3; search for alternate methods of oxidation was carried out. An (*S*)-isomer of compound **1.63** was reported by Fuchs[45] where methyl(trifluoromethyl)dioxirane (TFDO)[46] **1.104** was used as an oxidizing agent to successfully oxidize the exo-double bond (Scheme 1.23).[45]

Scheme 1.23



1.70a **1.105**



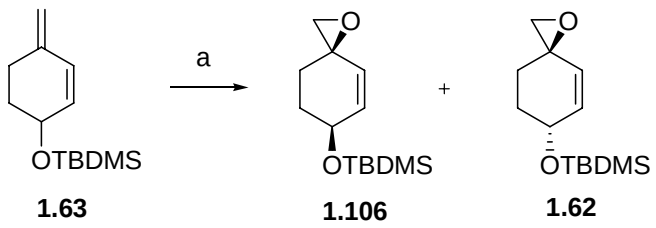
1.104

Methyl(trifluoromethyl)dioxirane

Methyl(trifluoromethyl)dioxirane was used as an oxidizing agent to convert diene **1.63** to an epoxide. We obtained a mixture of epoxides, **1.62** and **1.106**. The diene **1.63** was dissolved in

THF, acetonitrile and EDTA solution. Sodium bicarbonate was added followed by 1,1,1-trifluoroacetone (CH_3COCF_3) and oxone ($2KHSO_5.KHSO4.K_2SO_4$) in portions. Methyl(trifluoromethyl)dioxirane was generated *in situ*, which oxidizes the exo double bond to an epoxide. The reaction vessel was equipped with a dry ice condenser on the top and the reaction was carried out at 0 ^{0}C for 6 hrs. Workup was performed quickly followed by a flash silica gel column chromatography with 1% triethylamine as a co-solvent so as to avoid decomposition. After column chromatography 82% of desired mixture of epoxides **1.106** and **1.62** were obtained along with 9% of starting material **1.63(**recovered) (Scheme 1.25). The mixtures were not separable after column chromatography. A suitable solvent system was not found to separate them efficiently. On careful ^{1}H, ^{13}C NMR analysis, a mixture of isomers **1.106** and **1.62** in 1:1 ratio was found. Signals for compounds **1.62** and **1.106** overlap except the hydrogens associated with C-OTBDMS show at δ 4.26 and 4.34 in proton NMR spectra. But the assignment of which signal to which compound was difficult. Also two sets of peaks were observed in ^{13}C NMR spectrum. This clearly indicates the formation of two products **1.106** and **1.62**.

Scheme 1.25



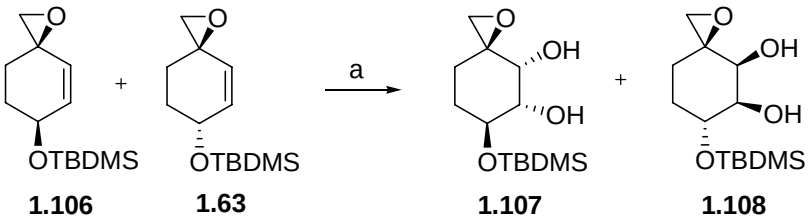
(a) EDTA, $NaHCO_3$, CH_3COCF_3, Oxone, THF, CH_3CN

1.3.12 Dihydroxylation and separation of diastereomers

The mixture of epoxides was taken and subjected to dihydroxylation conditions afforded diols **1.107** and **1.108**. The same reaction conditions were carried out for dihydroxylation as discussed above.[39] Dihydroxylation of a mixture of epoxides was performed using osmium tetraoxide (OsO_4) in catalytic amounts, in the presence of N-methylmorpholine-N-oxide (NMO), *tert*-butanol and acetone-water as solvent system. Overnight stirring followed by careful evaporation of acetone and silica gel column chromatography of the brown crude product gave a mixture of two *cis*-diols **1.107** and **1.108** in 65% yield in 1:1 ratio (Scheme 1.26). The reduced

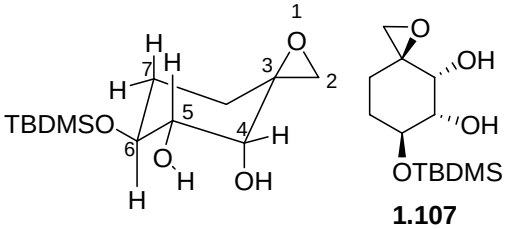
osmium is toxic in nature and a careful disposal of the waste was recommended. The separation of diols was difficult at this stage and it came as no surprise to us. We obtained the mixture of diols **1.107** and 1.**108** in 1:1 ratio from the integration of proton NMR spectrum. Characterization of the side products even after column chromatography was not possible.

Scheme 1.26



(a) OsO_4, NMO, *t*-BuOH, Acetone-H_2O

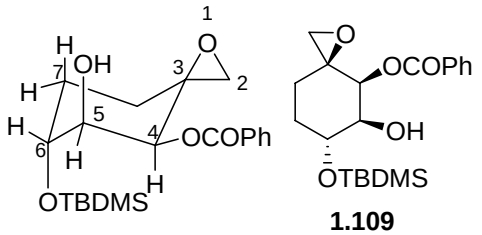
The separation of the diols was a difficult process even in this case. Earlier in section 1.3.6, one of the major isomer was identified by treating the mixture of diols with TBDMSCl. Surprisingly only one TBDMS protected isomer turned out to be a crystal and was proven by X-ray crystallography (Figure 1.4). Applying the same idea of protection of diols, Kaiyan took the mixture of the diols and treated with benzoyl chloride (C_6H_5COCl) instead of TBDMSCl. We were fortunate to get a better separation. Only one diol **1.108** reacted and the other **1.107** remained unreacted. The benzoyl protected alcohol **1.109** was obtained in 43% yield and recovered diol **1.107** was obtained in 49% yield (Scheme 1.27). The reason for the reaction of diol **1.108** might be the alcohol at C2 in **1.108** is less hindered and easier to react. The unprotected diol **1.107** is structurally similar to compound **1.81** except change in the protecting group of alcohol at C4 position.



1.107

The NMR spectrum of compound **1.107** shows a multiplet at δ 3.99 for C6-H. The proton NMR spectrum was taken in a 200 MHz spectrometer. The peak at δ 3.99 appears as a multiplet

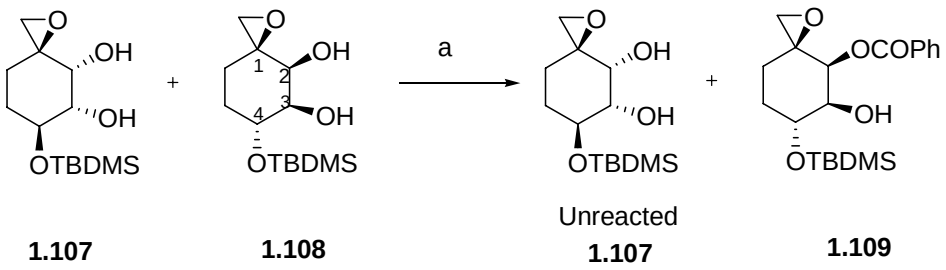
instead of a triplet of a doublet. Therefore the coupling constants were difficult to calculate. Both the signals for C5-H and C4-H appear at 3.82 as a multiplet. The two OH groups at C5 and C4 appear as doublets at δ 2.45 and 2.32. Signals at δ 2.92 (doublet, J = 4.4 Hz) and δ 2.67 (doublet, J = 4.4 Hz) correspond to C2-hydrogens.



1.109

The proton NMR spectrum of compound **1.109** shows signals at δ 8.07, 7.58 and 7.49 for benzoyl group. Signal at δ 5.49 is assigned to C4-H and appears as a doublet with J value of 3 Hz which correspond to the coupling between C4-axial H with C5-equatorial H. The coupling constant of 3 Hz suggests an axial-equatorial coupling. The coupling constant of 3 Hz suggests that C4-H is oriented at the axial position. Signal at δ 4.13 is assigned to C6-H and appears as a triplet of a doublet with J values 11 Hz and 3.6 Hz respectively, which corresponds to the coupling between C6-equatorial H with C5-equatorial H (J = 11 Hz, equatorial-equatorial coupling), C7-equatorial H (J = 11 Hz, equatorial-equatorial coupling) and C7-axial H (J = 3.6 Hz, equatorial-axial coupling). The intensities of the triplet of a doublet resonance in the spectrum show 1:1:2:2:1:1. The coupling constant of 11 Hz suggests that C6-H is in equatorial position. Signal at δ 3.98 is assigned to C5-H and appears as a multiplet because of coupling with C4-H, C6-H and hydrogen of C5-OH. Signals at δ 2.817 (doublet, J = 4.4 Hz) and δ 2.623 (doublet, J = 4.4 Hz) correspond to C2-hydrogens.

Scheme 1.27



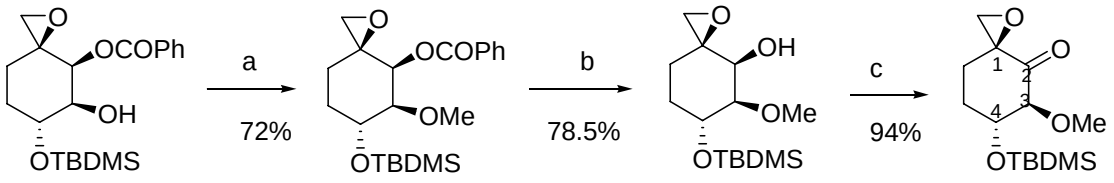
1.107 **1.108** Unreacted **1.107** **1.109**

(a) PhCOCl, Et_3N, THF, 0^0C-r.t, 48hr

1.3.13 Synthesis of ketone 112

Benzoyl diol **1.109** was methylated, followed by deprotection and oxidation is shown below in Scheme 1.28. The compound **1.109** was treated with trimethyloxonium tetrafluoroborate (Me_3OBF_4) in the presence of 1,8-bis(dimethylamino)naphthalene (proton sponge) as a base to yield methylated product **1.110** in 72% yield. There was a little decomposition of the product observed during the course of the reaction. The epoxide underwent ring opening. Thus formed side products couldn't be characterized. Methylated product **1.110** was treated with potassium carbonate to cleave the benzoyl protected group to give back the alcohol **1.111**. The cleavage was easy and quick to afford the alcohol **1.111** in 78% yield. The purification at each step of this long synthetic scheme was done by silica gel column chromatography. Many methods were reported for oxidation of alcohols. Swern oxidation was used earlier resulting the opening the epoxide and decomposition. So this procedure was discarded. Readily available *o*-iodoxybenzoic acid (IBX) was used to oxidize alcohols to ketones. This method successfully oxidized alcohols. Hence, alcohol **1.111** was oxidized to ketone **1.112** in 94% yield (Scheme 1.28).

Scheme 1.28



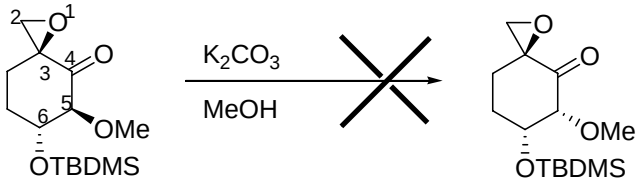
1.109 **1.110** **1.111** **1.112**

(a) Me_3OBF_4, proton sponge, CH_2Cl_2, 0^0C-r.t, 48hrs (b) K2CO3, MeOH, 0^0C-r.t, 5hrs
(c) IBX, DMSO, 12hrs.

Ketone **1.112** was not the preferred compound for the conversion to ovalicin because in the subsequent step, the addition of the side chain at C2, alkenyl lithium is not stereoselective. We obtained a mixture of isomers. The attack of alkenyl lithium was from both side's *cis* and *anti* to methoxy group at C3. In order to obtain a single stereoisomer, we planned to invert the methoxy group at C3 so that it is *cis* to the existing OTBDMS group at C4. This kind of isomerization was reported by Corey on a different intermediate in this total synthesis of ovalicin.[19] In Barton's synthesis,[20] compound **1.41** (Scheme 1.9) has the correct stereo and

regiochemistry to produce only one adduct **1.42**. The methoxy group and silyl ether are *cis* allowing the addition reaction takes place from the opposite face. Thus giving only one adduct **1.42**, where the alkenyl lithium side chain was coming opposite to these groups. We followed Corey's procedure by using potassium carbonate and methanol to invert the stereocenter at C5. Decomposition of the product was observed and the most probable reason could be the steric repulsion from the silyl ether group that prevent the deprotonation of C5 hydrogen (Scheme 1.29).

Scheme 1.29



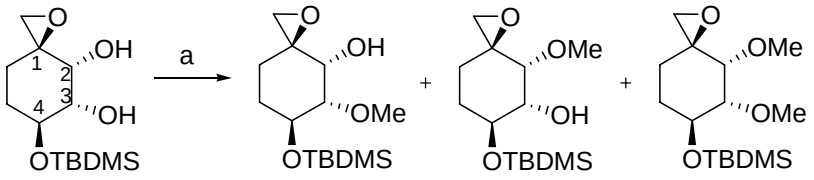
1.112 **1.113**

1.3.14 Synthesis of ketone1.117and 1.118

Diol **1.107** which was unreactive towards benzoylation in Scheme 1.27 was methylated and oxidized to obtain ketone **1.117**. The diol **1.107** was methylated using trimethyloxonium tetrafluoroborate (Me_3OBF_4) and proton sponge as a base. This methylation gave three products. Two mono methylated compounds **1.114** and **1.115** in 1:1 ratio in 80% (combined) yield and one dimethylated product **1.116** in 15% yield (Scheme 1.30). The obtained ratio is 2.6:2.6:1 for compounds **1.114**, **1.115** and **1.116** respectively. The separation of these methylated products was performed by silica gel column chromatography. The compounds were characterized using proton NMR and ^{13}C NMR spectrum. The dimethoxy compound **1.116** showed two methoxy signals at δ 3.41 and 3.45, two CH-OMe peaks at δ 3.35 and 3.37 and CH-OSi signal at δ 4.03. Using proton NMR spectrum we can differentiate the two monomethoxy compounds as they differ in signals for C4 proton δ 4.0 and 3.95 for compounds **1.114** and **1.115** respectively. After the separation the compounds **1.114** and **1.115** were oxidized to corresponding ketones. The oxidation was carried out using IBX in DMSO to obtain ketones **1.117**, **1.118** from alcohols **1.114**, **1.115** respectively in quantitative yields (Scheme 1.31). Monomethoxy compounds **1.114** and **1.115** are differentiated even after oxidizing them to subsequent ketones. The two ketones

show distinct signals in proton NMR spectra. Compound **1.117** shows a doublet for C3 proton at δ 3.52 and multiplet for C4 proton at δ 4.19. Compound **1.118** shows a singlet for C2 proton at δ 4.2 and a multiplet for C4 proton at δ 4.33.

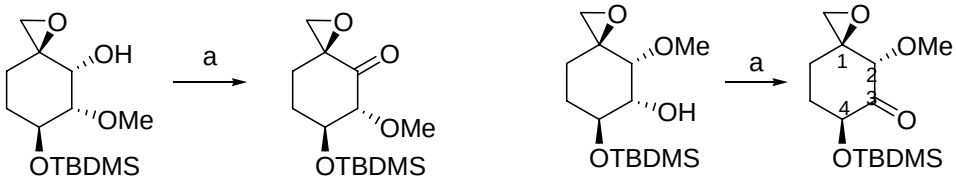
Scheme 1.30



1.107 **1.114** **1.115** **1.116**

(a) Me_3OBF_4, proton sponge, CH_2Cl_2, 0^0C- r.t, 48hr

Scheme 1.31



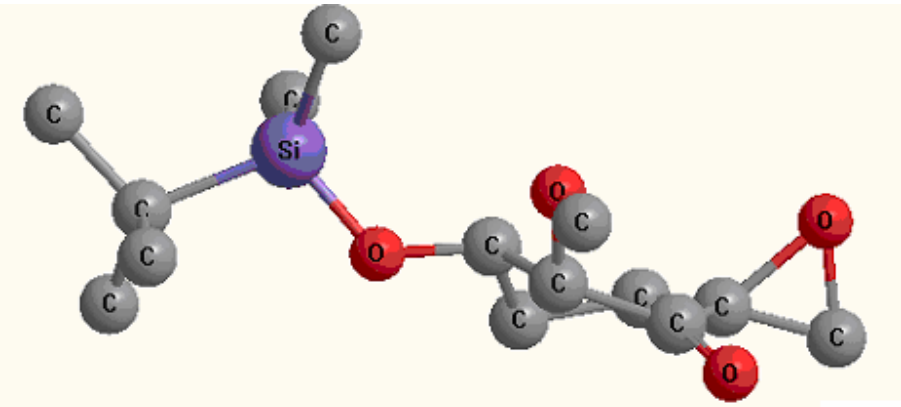
1.114 **1.117** **1.115** **1.118**

(a) IBX, DMSO, r.t, 12hr

1.3.15 Addition of alkenyll lithium reagent to ketone 1.112

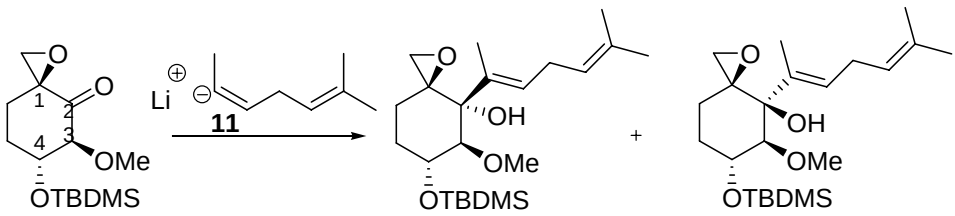
The preparation of alkenyl lithium reagent was discussed in section 1.3.8. The same procedure was carried in preparing and adding alkenyl lithium to the ketone **1.112**. Ketone **1.112** was dissolved in ether and small amount of toluene was added. This mixture was cooled to -78 ^{0}C and the freshly prepared alkenyl lithium **1.11** was added to the ketone. This addition did not give a stereoselective addition, instead gave a mixture of isomers, **1.119** and **1.120** in 75% yield (Scheme 1.32). A computer generated chem3D model (shown below) may explain the lack of stereoselective addition to the ketone. The methoxy group at C3 is axial, offering some resistance to the attack of alkenyl lithium. Also the *tert*-butyl on TBDMS group offers some resistance to the alkenyl lithum attack. The alkenyl lithium addition to the ketone **1.112** is not seteroselective

giving a mixture of two compounds **1.119** and **1.120**. If both the methoxy and OTBDMS groups are *cis* to each other and *anti* to the C-O bond of epoxide on C-1 then the addition is expected to be stereoselective resulting in one compound which should lead to ovalicin. The thus formed mixtures are not separable by silica gel column chromatography. We couldn't find proper solvent to separate them efficiently. From proton NMR spectrum data we assume that the ratio of **1.119** and **1.120** is 1:1. We assumed both the structures based on the methylene group protons of the spiroepoxide. One of the two compounds shows signal at δ 2.9 and 3.1 and the other shows signal at δ 2.38 and 2.39. It was difficult to assign which signal corresponds to which compound. The ambiguity still prevails.



Picture generated from Chem3D using MM2 minimization program of compound **1.112**

Scheme 1.32



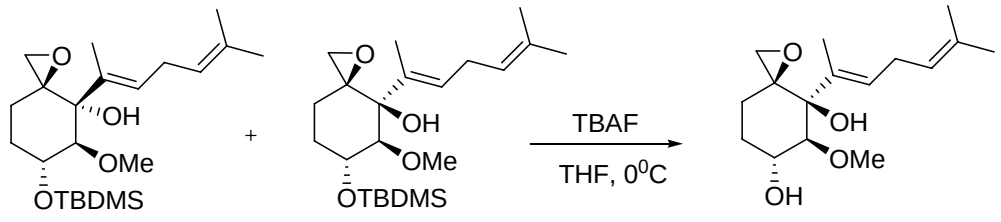
1.112 **1.119** **1.120**

1.3.16 Synthesis to ovalicin analogues

All our attempts to separate the mixture of adducts **1.119** and **1.120** failed. Separation in the next step was another resort. First we tried to deprotect the silyl protecting group using TBAF. After column chromatographic separation only one compound was characterized and assumed to be **1.121**. Further derivatization of this compound could help us to reveal the exact

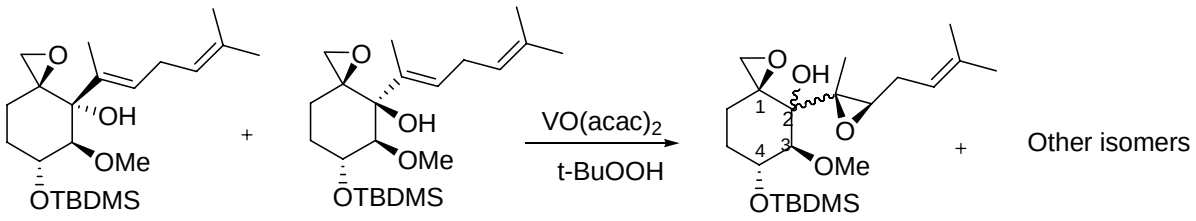
stereochemistry and structure of the compound. The remaining fractions cannot be characterized using NMR spectrum, thus remained unidentified. Small amounts of starting material was recovered (Scheme 1.33). The other approach was to epoxidize the double bond which is allylic to alcohol at C2 position. Sharpless epoxidation procedure was used to oxidize the double bond. The only identificable product was assumed to be **1.122** (Scheme 1.34). Two dimensional NOE experiments must be performed to provide sufficient information to confirm the stereochemistry at C2 position.

Scheme 1.33



1.119 **1.120** **1.121**

Scheme 1.34



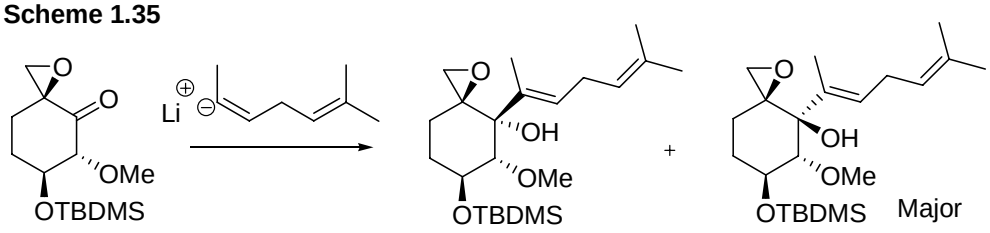
1.119 **1.120** **1.122**

1.3.17 Continuing study towards the total synthesis of ovalicin

A lot of time has been invested in fixing the stereochemistry, isolation of isomers; using various protecting groups and different reagents, to synthesize an intermediate that leads to ovalicin. Although we got a structurally similar compound, an isomer of ovalicin but our goal wasn't achieved. The major problem is making the methoxy position at C-3 and OTBDMS group at C-4 *cis* to each other in ketones **1.112** and **1.117**. This can be achieved by converting compounds **1.109** and **1.107** to ketone **1.60**. The on going experiments conducted by Huiping Zhao in our lab reveal that the total synthesis can be achieved only if the inversion is successful making both the C-3 methoxy and C-4 OTBDMS groups *cis* to each other and both of them are

trans to the C-O bond at C-1 position. Huiping added side chain to the other ketone **1.117**. The addition produced an unwanted isomer **1.124** as a major product (Scheme 1.35). The stereochemical assignment was done based on the reported values.[23] The proton NMR spectrum of compound **1.123** shows signals δ 5.48 and 5.1, whereas compound **1.124** shows signals at δ 5.98 and 5.1 for alkenyl protons on the side chain.

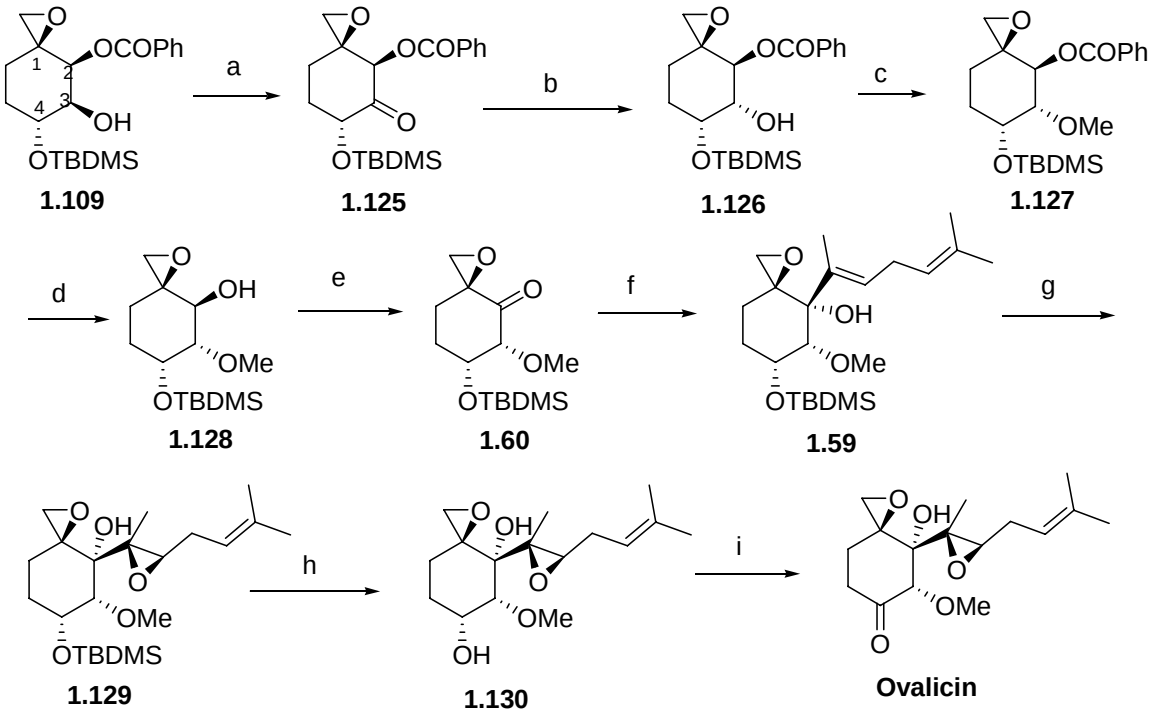
Scheme 1.35



1.117 **1.123** **1.124**

This result wasn't encouraging. The only alternative to achieve the final compound was to change our strategy. This can be achieved several ways. The most easiest and convenient way to achieve this is to invert the stereochemistry at C3 position. Compound **1.109** was prepared form the method discussed in earlier sections. The benzoylated diol **1.109** should be inverted at C3 position. To achieve this, alcohol **1.109** at C3 position should be oxidized using IBX. Ketone **1.125** will be reduced either using sodium borohydride or lithium selectride to obtain an alcohol **1.126,** *cis* to OTBDMS group. Further, methylation of **1.126** using Me_3OBF_4 should lead us to a methylated product **1.127**. Deprotecting the benzoyl group should give us an alcohol **1.128** and oxidation of alcohol **1.128** to ketone **1.60** will be performed using IBX. The addition of side chain **1.11** to ketone **1.60** should afford only one adduct **1.59**. Sharpless epoxidation followed by protection of TBDMS and oxidation of **1.130** will afford ovalicin (Scheme 1.36). This route to ovalicin should be effective and is carried by Huiping Zhao.

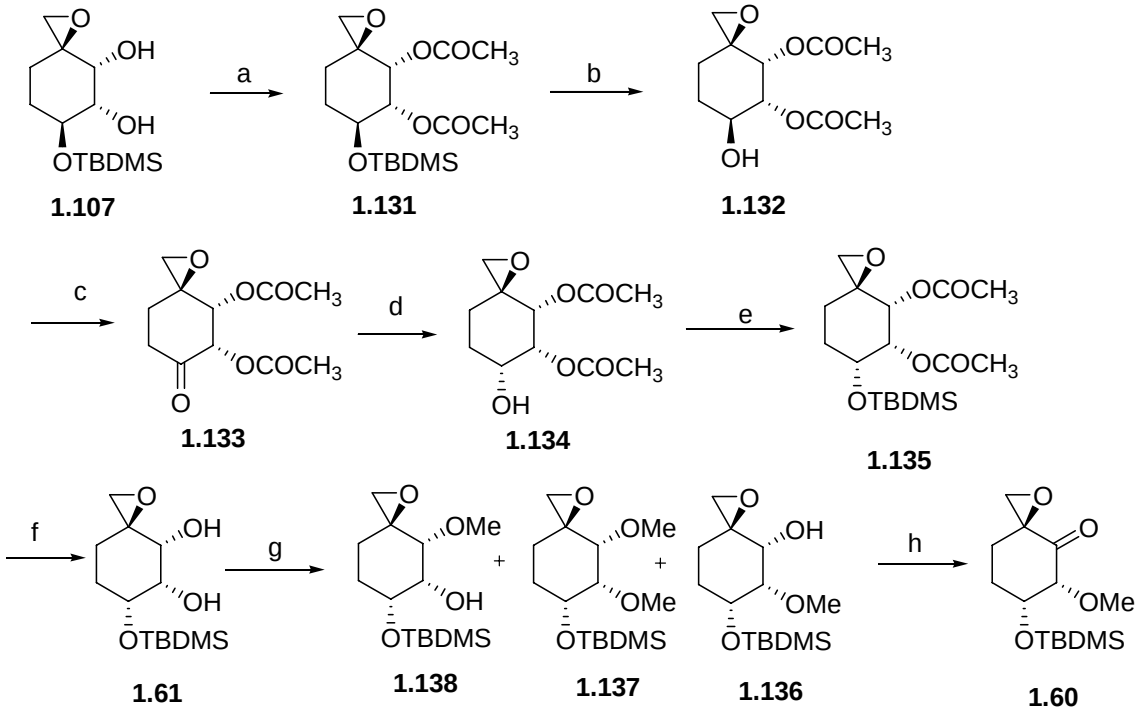
Scheme 1.36



(a) IBX, DMSO, 12 hr, r.t (b) $NaBH_4$ / Li Selectride (c) Me_3OBF_4, proton sponge, CH_2Cl_2
(d) K_2CO_3, MeOH (e) IBX, DMSO (f) **11**, Toluene (g) $VO(acac)_2$, *t*-BuOOH (h) TBAF, THF
(i) IBX, DMSO

Diol **1.107** can also be converted to the desired ketone **1.60**. A series of protection, functional group interconversions and deprotection will lead to desired ketone **1.60**. Diol **1.107** will be treated with acetyl chloride to protect both alcohols. Desilylation will be done using TBAF. Oxidation of **1.132** followed by selective reduction using lithium selectride should afford an alcohol **1.134**. Protection of alcohol **1.134** using TBDMSCl followed by cleavage of acetyl groups should give us compound **1.136**. Methylation of the diol **1.61** should give three compounds **1.136**, **1.137** and **1.138**. Oxidation of the compound **1.136** to **1.60** will be carried out using IBX (Scheme 1.37). The Ketone **1.60** is a vital intermediate to in the synthesis of ovalicin. The later steps to ovalicin are similar to the one in Scheme 1.36. The synthetic reaction described in the following scheme is carried by Huiping Zhao in our group.

Scheme 1.37



1.107 **1.131** **1.132** **1.133** **1.134** **1.135** **1.61** **1.138** **1.137** **1.136** **1.60**

(a) CH_3COCl, Et_3N, THF (b) TBAF, 0^0C (c) IBX, DMSO (d) NaBH4 or Li selectride
(e) TBDMSCl, DMAP, Imidazole, DMF (f) K_2CO_3, MeOH (g) Me_3OBF_4, proton sponge, CH_2Cl_2
(h) IBX, DMSO

1.4 Biological activity of ovalicin analogues

During the synthesis of ovalicin we obtained a number of intermediates and a few of them were submitted to test the bioacivity. The tests were performed by Dr. Peter K. Chaing at University of California; SanFransisco. Detailed description of the experiments[47, 48] is out of scope of this thesis. In order to evaluate the activity of these analogues, Luciferase assay tests were screened against *T. brucei in vitro*.[47, 48]

Luciferase assay is used to measure the binding activity of transcription factor. Luciferase reporter vector is first introduced into the cells and then the cells are lysed. Lucifern is added to this extract along with magnesium and ATP.[49] A catalytic oxidative carboxylation is observed when the enzyme and the substrates are combined. During this process light is emitted and is measured by luminometer. The light emitted is a direct measurement of the binding activity of the transcription factor.[49]

As we know that the TNP-470, a synthetic analogue of fumagillin inhibits the growth of *Trypasonoma brucei*, so the quest for synthetic analogues of structurally similar compounds is on its way. During our studies to the total synthesis, we discovered some novel compounds that inhibit the growth of *T. brucei*. The activity of these analogues was screened against *T. brucei* is summarized in the following table 1 (the results were provided by Dr. Peter Chiang).

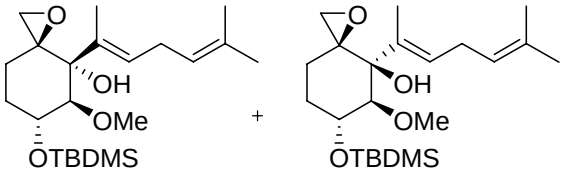
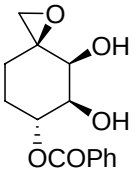
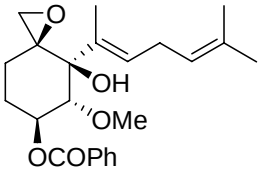
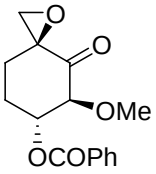
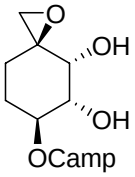
	Ovalicin Analogs	**IC_{50} (µg/mL)**
1 SB-5-87-F4-6		0.16 ± 0.02
2 KL-1-71		0.19 ± 0.04
3 SB-3-15-F7		1.64 ± 0.19
4 KL-2-26		13.06
5 Ovalicin 2		110.61

Table 1.1 IC_{50} values of different intermediates screened against *T.brucei*.

From the above table it is clearly evident that the compounds in entry **1** (SB-5-87-F4-6) is the most potent drug and compound in entry **2** (KL-1-71) is the next with IC_{50} values of 160

ng/mL and 190 ng/mL respectively. Especially compound in entry **2** which was synthesized in 6 steps can serve as a lead compound to inhibit *T.brucei*. Compound in entry **3** (SB-3-15-F7) also inhibits the growth of *T.brucei* but less compared to compounds in entries **1** and **2**. The compounds in entries **4** (KL-2-26) and **5** (ovalicin 2) are less active.

The antiparasitic activity of the two potent drugs, compounds in entries 1 and 2 is shown in the following figures 1.6 and 1.7 respectively. Both compounds inhibit the *T.brucei* growth

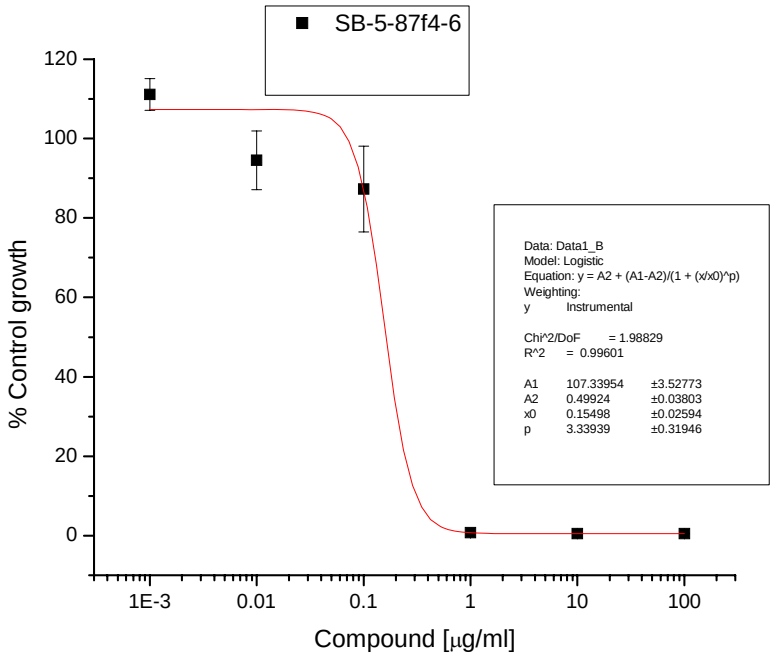


Figure 1.6 Antiparasitic activity of analogue **SB-5-87-F4-6**

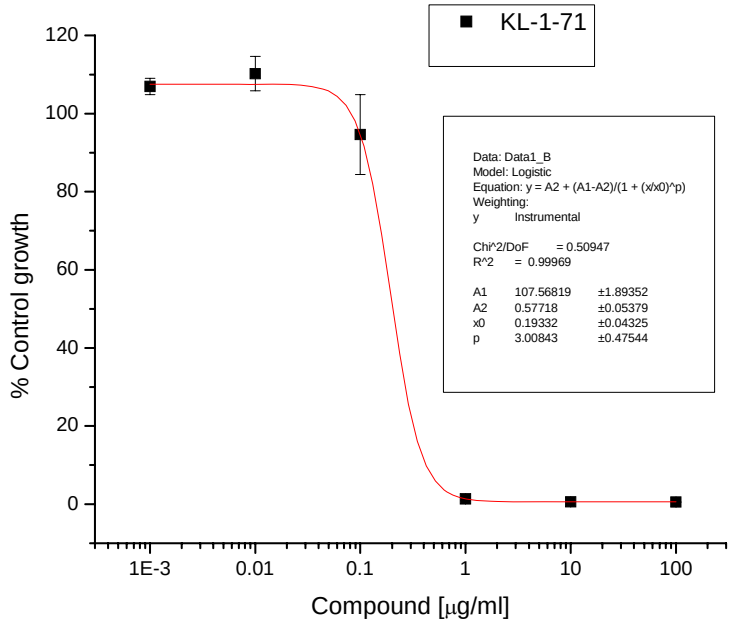


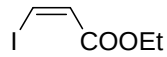
Figure 1.7 Antiparasitic activity of analogue **KL-1-71**

TNP 470 is a synthetic drug which is currently in clinical trials has some issues like short physiological half-life and severe side effects, so our compounds should be promising candidates and can best serve as agents to inhibit the growth of these parasites.

1.5 Experimental section

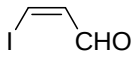
General Methods. Nuclear Magnetic Resonance (NMR) spectra were recorded on a 400 MHz and 100 MHz Varian spectrometer in $CDCl_3$ unless otherwise specified. The chemical shifts are given in parts per million (ppm) from downfield from tetramethylsilane as internal standard. Coupling constants (J) are given in hertz. TLC analyses were performed on thin-layer analytical plates from Aldrich. All the reactions were performed in argon atmosphere unless otherwise specified. Silical gel (200~400 mesh) from Natland corporation was used for column chromatographic separations. Solvents like THF and diethyl ether were distilled over sodium and benzopheneone, methylene chloride was distilled over CaH_2 and finally benzene and toluene were distilled over $LiAlH_4$.

Ethyl (*Z*)-β-Iodoacrylate (1.67)[30, 31] (SB-3-45)



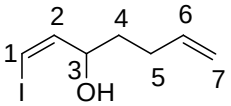
To a round bottom flask, 12.8 g (0.085 mol) of sodium iodide and 50 mL of glacial acetic acid was added. Then 8.37 g (0.085 mol) of ethyl propiolate was added to the above mixture. The resulting mixture was heated to 70 ^{0}C for 12 hours. The resultant yellow solution was washed with 3 M NaOH and extracted with diethyl ether. The organic layer was washed with brine and dried over anhydrous sodium sulfate, filtered and concentrated. Distillation was performed under reduced pressure to obtain pure 17.2 g (89.2% yield) of **1.67**. ^{1}H NMR ($CDCl_3$): δ 7.44 (d, J = 9 Hz, 1 H, =CH), 6.89 (d, J = 8 Hz, 1 H, =CHCOOEt), 4.25 (q, J = 7 Hz, 2 H, OCH_2), 1.32 (t, J = 8 Hz, 3 H, CH_3); ^{13}CNMR: δ 164.7 (C=O), 130.2, 94.5, 60.9, 14.3.

(*Z*)-β-iodoacrolein (1.65)[30, 31] (SB-3-43)



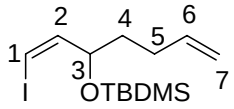
A solution of 3.74 g (0.017 mol) of iodoacrylate **1.67** in 40 mL of freshly distilled CH_2Cl_2 was cooled to – 78 ^{0}C. To this solution 1 M DIBAL-H in toluene was added dropwise via syringe and stirred at -78 ^{0}C for one hour. The reaction was monitored by TLC using hexane and diethyl ether as solvent. The resultant solution was not allowed to warm to room temperature because of fear of conversion to (*E*)-siomer. The formation of aldehyde was confirmed by taking a ^{1}H NMR. So no work up was performed and this aldehyde **1.65** was directly used in the next step. On long standing this compound will isomerizes to form a *trans* aldehyde. ^{1}H NMR ($CDCl_3$): δ 9.5 (d, J = 6 Hz, 1 H, CHO), 8.0 (d, J = 8 Hz, 1 H, =CH), 7.2 (dd, J = 8 Hz, J = 6 Hz, 1 H, =CHCHO).

(*Z*)-1-Iodohepta-1,6-dien-3-ol (1.68) (SB-3-44)



A three neck 200 mL round bottom equipped with a dropping funnel and a water condenser was charged with 1.58 g (0.067 mol) of magnesium turnings and 30 mL THF under argon. This mixture was stirred vigorously and heated to reflux at 70 ^{0}C. 4-Bromo-1-butene was added to this heated mixture drop wise via dropping funnel. Then this whole mixture was heated to reflux for 2 hours. This reaction mixture was cooled to room temperature to give alkenyl magnesium bromide, a Grignard reagent. This mixture was kept under argon. Without any further delay this freshly prepared Grignard solution was added to aldehyde **1.65** slowly via cannula at -78 ^{0}C. This solution was stirred at -78 ^{0}C for one hour and then slowly warmed to room temperature. The resulting solution was diluted with water and extracted with diethyl ether. The organic layer was washed with saturated solution of $NaHCO_3$ and brine, dried over anhydrous sodium sulfate, filtered and concentrated. Column chromatographic separation on silica gel using hexane and diethyl ether (1:1 v/v) afforded 2.95 g (74% yield) of a colorless liquid. ^{1}H NMR ($CDCl_3$) δ 6.35 (d, J = 8 Hz, 1 H, C1-H), 6.27 (t, J = 8 Hz, 1 H, C2-H), 5.86 (m, 1 H, C6-H), 4.9 (dd, 1 H, C7-H), 5.1 (dd, 1 H, C7-H), 4.4 (q, J = 8 Hz, 1 H, C3-H), 2.2 (m, 2 H, C5-H), 1.65 (m, 2 H, C4-H); ^{13}C NMR 143.4, 138.2, 115.4, 82.7, 74.2, 35.1, 29.5.

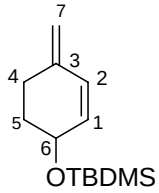
(*Z*)-1-Iodo-3-(*tert*-butyldimethylsilyloxy)-1,6-heptadiene (1.69) (SB-3-57)



A solution of 5.6 g (0.023 mol) of alcohol **1.68** and distilled DMF, 40 mL was stirred at 0 ^{0}C. Then to this solution 4.8 g (0.071 mol) of imidazole was added. Catalytic amount of 4-dimehtylaminopyridine (DMAP) (2 g) was added to the above mixture. This mixture was stirred for 10 min at 0 ^{0}C. To this mixture 4.61 g (0.031 mol) of *tert*-butyldimethylsilyl chloride (TBDMSCl) was added. Slowly the reaction mixture was warmed to room temperature and stirred overnight. The progress of the reaction was monitored by TLC using hexane and diethyl ether (50:1 v/v) as a developing solvent. The resulting solution was diluted with water and extracted with diethyl ether. The organic layer was washed with 1H HCl, aqueous $NaHCO_3$ and brine. The organic extract was dried over anhydrous sodium sulfate and concentrated to dryness.

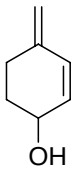
Column chromatographic separation on silica gel using mixture of hexane and diethyl ether (50:1 v/v) afforded 8.114 g (98% yield) of title compound **1.69**. ^{1}H NMR ($CDCl_3$) δ 6.19 (s, 2 H, HC-1=C-2H), 5.85 (m, 1 H, C6-H), 5.03 (d, J = 1 Hz, I H, =C-7H), 4.9 (d, J = 10 Hz, 1 H, =C-7H), 4.3 (q, J = 6 Hz, 1 H, C3-H), 2.08 (m, 2 H, C-5H_2), 1.67 (m, 2 H, C-4H_2), 0.88 (s, 9 H, *t*-Bu), 0.09 (s, 3 H, $SiCH_3$), 0.05 (s, 3 H, $SiCH_3$): ^{13}C NMR δ 144.8, 138.6, 114.9, 80.07, 75.2, 36.21, 29.4, 26.06, 18.3, -3.98.

3-Methylene-6-(*tert*-butyldimethylsilyloxy)cyclohexene (1.63) (SB-3-58)



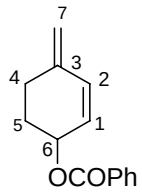
A round bottom flask was charged with 0.52 g (2.3 mmole) of palladium acetate (II), 9.7 g (0.023 mole) of silver phosphate and 1.21 g (4.6 mmol) of triphenyl phosphene. The flask was vaccum and flame dried and kept under argon. Then 10.8 g (0.051 mol) of proton sponge was added to the round bottom and the resulting mixture was kept under argon. Then 100 mL of distilled DMF was added to the reaction mixture and the resulting solution was heated to 70 ^{0}C for 12 hours. This mixture was filtered through a pad of celite 545, diluted with water and extracted with diethyl ether. The organic layer was washed with 1 M HCl, aqueous sodium bicarbonate and brine. Organic layer was dried over anhydrous sodium sulfate and concentrated to dryness. Column chromatographic separation on silica gel using a gradient mixture of hexane and diethyl ether (50:1 v/v) afforded 4.81 g (93% yield) of **1.63**.^{1}H NMR ($CDCl_3$) δ 6.1 (d, J = 10 Hz, 1 H, C-2H), 5.7 (d, J = 10 Hz, 1 H, C-1H), 4.8 (d, J = 5 Hz, 2 H, C-7H_2), 4.3 (q, 1 H, C-6H), 2.4 (m, 1 H, C-4H), 2.22 (m, 1 H, C-4H), 1.9 (m, 1 H, C-5H), 1.63 (m, 1 H, C-5H), 0.9 (s, 9 H, *t*-Bu), 0.09 (s, 3 H, $SiCH_3$), 0.09 (s, 3 H, $SiCH_3$) ^{13}C NMR δ 142.6, 133.9, 130.2, 111.9, 67.1, 32.9, 27.9, 26.1, 18.4, -4.34, -4.39.

3-Methylene-6-(hydroxy)cyclohexene (1.77) (SB-2-38)



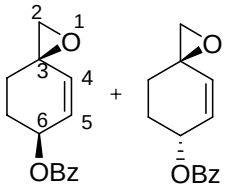
To a solution of 1.3 g (5.8049 mmol) of **1.63** in 20 mL of distilled THF was added 4.2 mL of TBAF (1 M in THF) at 0 ^{0}C. The reaction mixture was stirred at 0 ^{0}C for 10 hours. The resulting solution was washed with water. The organic layer was washed with brine and extracted with ethyl acetate, dried over anhydrous sodium sulfate and concentrated to dryness. The resulting solution was not purified by column chromatography and directly used for next reaction. ^{1}H NMR ($CDCl_3$) δ 6.209 (d, J = 9 Hz, 1 H, =CH), 5.808 (d, J = 10 Hz, 1 H, =CH), 4.878 (s, 2 H, CH_2), 4.33 (m, 1 H, CHOH), 2.6-2.2 (m, 2 H, CH_2), 2.1-1.6 (m, 2 H, CH_2); ^{13}C NMR ($CDCl_3$) δ 142.1, 132.2, 131.4, 112.9, 66.1, 32.4, 27.3.

3-Methylene-6-(benzoyloxy)cyclohexene (1.78) (SB-2-39)



To a 5.81 mmol of alcohol **1.77**, 4.8 mL (34.8 mmol) of distilled triethylamine was added and stirred at 0 ^{0}C for 10 minutes. Then 2.6 mL (14.51 mmol) of benzoyl chloride was added and stirred at 0 ^{0}C. The resulting solution was slowly allowed to warm to room temperature. Then the reaction was stirred at room temperature for 6 hours. The progress of the reaction was monitored by TLC using hexane and diethyl ether (3:1 v/v) as a developing solvent. The resulting solution was washed with water and extracted with ether. The organic layer was washed with 1 M HCl, aqueous sodium bicarbonate and brine. The organic layer was dried over anhydrous sodium sulfate, concentrated and applied to column chromatography (silica gel) using gradient mixture of hexane and diethyl ether (3:1 v/v) afforded 1.09 g (88%) of **1.78**. ^{1}H NMR ($CDCl_3$) δ 8.1 (m, 2 H, Ar), 7.5 (m, 1 H, Ar), 7.4 (m, 2 H, Ar), 6.31 (d, J = 9 Hz, 1 H, C2-H), 5.9 (dd, J = 4 Hz, J = 4 Hz, 1 H, C1-H), 5.6 (m, 1 H, C6-H), 4.95 (s, 2 H, C7-H), 2.6 (m, 1 H, C4-H), 2.4 (m, 1 H, C4-H), 2.16 (m, 1 H, C5-H), 1.97(m, 1 H, C5-H). ^{13}C NMR δ 166.3, 141.5, 133.3, 133.0, 129.7, 128.4, 127.5, 113.9, 68.7, 28.5, 26.9.

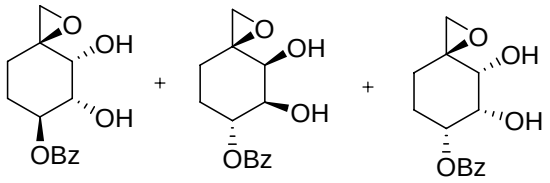
(3*R,8*R**) and (3*R**,8*S**)-6-(Benzoyloxy)-1-oxaspiro[2.5] oct-4-ene (1.79 & 1.80) (SB-2-47)**



1.79 **1.80**

To a solution of 1 g (4.673 mmol) of diene 1.78 in 25 mL of methylene chloride was added 334 mg (7.944 mmol) of sodium fluoride and 190 mg (3.27 mmol) of potassium fluoride. The reaction vessel was cooled to 0 ^{0}C. Then 1.21 g (7.01 mmol) of *meta*-chloroperbenzoic acid (MCPBA) was added to the reaction mixture. This mixture was stirred at 0 ^{0}C for 5 hours. Using TLC (hexane, diethyl ether (9:1)) the reaction progress was monitored. The resulting mixture was washed with water and extracted with diethyl ether. The organic layer was washed with aqueous sodium thiosulfate, aqueous sodium bicarbonate and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated to dryness under vacuum. Without further purification the residue was dihydroxylation in the next step. We did not observe any starting material diene **1.78** in the crude proton NMR, so we assumed a complete conversion. We obtained a mixture of two compounds **1.79** and **1.80** in 1:1 ratio (based on integration in proton NMR). The assignments either to the proton signals or ^{13}C signals are based on our assumptions. It was difficult to assign a set of signals to its respective compound. ^{1}H NMR ($CDCl_3$) δ (for one compound) 8.062 (m, 2 H, CH of Ph), 7.569 (m, 1 H, CH of Ph), 7.447 (m, 2 H, CH of Ph), 6.184 (dd, J = 3 Hz, J = 4 Hz, 1 H, =CH), 5.632 (m, 1 H, CHOCOPh), 5.498 (t, J = 10 Hz, 1 H, =CH), 2.895 (d, J = 5 Hz, 1 H, CHO), 2.875 (d, J = 2 Hz, 1 H, CHO), 2.86-2.96 (m, 2 H, CH_2), 1.83-2.34 (m, 2 H, CH_2); ^{1}H NMR ($CDCl_3$) δ (for another compound) 8.06 (m, 2 H, CH of Ph), 7.573 (m, 1 H, CH of Ph), 7.452 (m, 2 H, CH of Ph), 6.159 (dd, J = 3 Hz, J = 4 Hz, =CH), 5.598 (m, 1 H, CHOCOPh), 5.495 (t, J = 10 Hz, 1 H, =CH), 2.952 (d, J = 5 Hz, 1 H, CHO), 2.863 (d, J = 2 Hz, 1 H, CHO), 2.86-2.96 (m, 2 H, CH_2), 1.83-2.34 (m, 2 H, CH_2); ^{13}C NMR ($CDCl_3$) (for one compound) δ 166.3, 133.6, 133.3, 133.3, 130.5, 129.9, 128.6, 68.3, 55.2, 55.1, 27.4, 27.3; ^{13}C NMR ($CDCl_3$) (for another compound) δ 166.2, 133.2, 133.0, 132.8, 130.4, 129.8, 128.6, 67.7, 55.2, 55.1, 27.3, 27.1.

(3*S,4*S**,5*R**,6*R**)-6-(Benzoyloxy)-1-oxaspiro[2.5]octane-4,5-diol (1.82), (3*S**,4*R**,5*S**,6*S**)-6-(benzoyloxy)-1-oxaspiro[2.5]octane-4,5-diol (1.81) & (3*S**,4*R**,5*S**,6R*)-6-(benzoyloxy)-1-oxaspiro[2.5]octane-4,5-diol (1.83) (SB-2-48)**

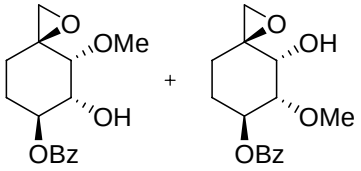


1.81 **1.82** **1.83**

To a solution of 1g (4.3478 mmol) of mixture of epoxides (**1.79** and **1.80**) in 32 mL of acetone and water (3:1) and 5 mL of *t*-butanol was added 1.528 g of NMO and 221 mg of OsO_4. The reaction was stirred at room temperature for 30 hours. The resulting solution was extracted with ether and washed with water and brine. The water layer was washed with ether three times. The organic layers were dried on anhydrous sodium sulfate and concentrated to dryness. The resulting residue was purified by column chromatography using a mixture of hexane and ethyl acetate (1:1 v/v). After column we obtained 0.526 g (46%) of mixture of diols in 5:4:1 ratio. It was difficult to identify the other fractions by NMR. Herein we assumed that the mixture of diols consists of three diastereomers mainly. The isomers were identified based on the splitting pattern and J coupling values by Kaiyan. Later Kaiyan silylated this mixture of diols, only one crystalline product **1.84** was obtained and isolated. On desilylation compound **1.81** was obtained and characterized by NMR. ^{1}H NMR ($CDCl_3$) (for compound **1.81**) δ 8.06 (m, 2 H, Ph), 7.59 (m, 1 H, Ph), 7.46 (m, 2 H, Ph), 5.35 (td, J = 8 Hz, 2 Ha-Ha, J = 4 Hz, Ha-He, 1 H, CH-OCOPh), 4.11 (m, 1 H, CHOH), 3.73 (s, 1 H, CHOH), 3.21 (d, J = 4 Hz, 1 H, CHO), 3.05 (d, J = 2 Hz, CHO), 2.22- 2.0 (m, 2 H, CH_2), 1.9- 1.6 (m, 2 H, CH_2); ^{13}C NMR ($CDCl_3$) (for compound **1.81**) δ 166.6, 133.3, 129.8, 129.7, 128.4, 73.0, 72.6, 72.8, 58.7, 51.7, 26.0, 25.7; ^{1}H NMR ($CDCl_3$) (for compound **1.82**) δ 8.05 (m, 2 H, Ph), 7.59 (m, 1 H, Ph), 7.46 (m, 2 H, Ph), 5.41 (td, J = 7.2 Hz, 2 Ha- Ha, J = 4 Hz, Ha-He 1 H, CH-OCOPh), 4.00 (m, 1 H, CHOH), 3.86 (dd, J = 6 Hz, J = 3 Hz, 1 H, CHOH), 2.91 (d, J = 5 Hz, 1 H, CHO), 2.65 (d, J = 5 Hz, 1 H, CHO), 2.3-1.7 (m, 4 H, 2 CH_2); ^{1}H NMR ($CDCl_3$) (for compound **1.83**) δ 8.07 (m, 2 H, Ph), 7.58 (m, 1 H, Ph), 7.46 (m, 2 H, Ph), 5.18 (dt, J = 10 Hz, He-He, J = 3 Hz, 2 Ha-He, 1 H, CH-OCOPh), 4.35 (bs, 1 H, CHOH), 3.81 (d, J = 2.5 Hz, 1 H, CHOH), 3.07 (d, J = 5 Hz, 1 H, CHO), 2.71 (d, J = 4.8 Hz, 1 H,

CHO), 2.22-1.60 (m, 4 H, 2 CH_2); ^{13}C NMR ($CDCl_3$) (for compound **1.83**) δ 166.3, 133.5, 130.3, 130.0, 128.7, 72.9, 71.3, 71.1, 58.9, 51.1, 26.7, 24.7.

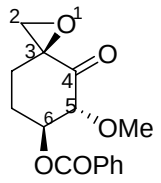
(3*S,4*R**,5*S**,6*S**)-6-(Benzoyloyz)-5-methoxy-1-oxaspiro[2.5]octan -4-o1 (1.85) & (3*S**,4*R**,5*S**,6*S**)-6-(benzoyloxy)-4-methoxy-1-oxaspiro[2.5]octan -5-o1 (1.86) (LK-2-24)**



1.86 **1.85**

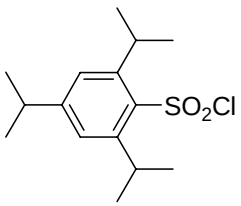
To a solution of 194 mg (0.733 mmol) of compound **1.81** in 3 mL of methylene chloride, 314 mg (1.465 mmol) of 1,8-bis(dimethylamino)naphthalene (proton sponge) and 108 mg (0.732 mmol) of trimethyloxonium tetrafluoroborate was added. The reaction vessel was cooled to 0 ^{0}C. The reaction was stirred at 0 ^{0}C for nine hours. The resultant solution was washed with water and extracted using ether. The organic layer was washed with 1 M HCl, aqueous sodium bicarbonate and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated to dryness. The residue was applied to column chromatography for separation using hexane and ethyl acetate (3:2 v/v). Obtained mixture of compounds **1.86** and **1.85** in 1:1.3 ratio. The ratio of compounds was calculated based on the column chromatographic separation. This experiment was taken from Kaiyan with his permission. ^1H NMR ($CDCl_3$) (for compound **1.86**) δ 8.07 (m, 2 H, Ph), 7.56 (m, 1 H, Ph), 7.45 (m, 2 H, Ph), 5.32 (m, 1 H, CH-OCOPh), 3.99 (dt, J = 3.2 Hz, J = 8 Hz, 1 H, CHOH), 3.46 (s, 3 H, OMe), 3.18 (d, J = 3 Hz, 1 H, CHOMe), 2.93 (d, J = 5 Hz, 1 H, CHO), 2.81 (d, J = 5 Hz, 1 H, CHO), 2.51 (d, J = 4 Hz, 1 H, OH) 2.56-1.36 (m, 4 H, 2 CH_2); ^{13}C NMR ($CDCl_3$) (for compound **1.86**) δ 166.6, 133.2, 130.0, 129.4, 128.6, 83.0, 73.3, 72.8, 58.4, 57.2, 52.7, 26.3, 26.2; ^1H NMR ($CDCl_3$) (for compound **1.85**) δ 8.06 (m, 2 H, Ph), 7.59 (m, 1 H, Ph), 7.47 (m, 2 H, Ph), 5.43 (dt, J = 9 Hz, J = 4 Hz, J = 8 Hz, 1 H, CH-OCOPH), 3.66 (t, J = 3.2 Hz, J = 7.6 Hz, 1 H, CHOMe), 3.49 (s, 3 H, OMe), 2.93 (d, J = 5 Hz, 1 H, CHO), 2.76 (d, J = 5 Hz, 1 H, CHO), 2.51 (d, J = 3 Hz, 1 H, OH), 2.17-1.52 (m, 4 H, 2 CH_2); ^{13}C NMR ($CDCl_3$) (for compound **1.85**) δ 166.0, 133.2, 129.9, 129.8, 128.6, 81.8, 71.3, 71.3, 58.8, 58.7, 51.9, 26.1, 26.1.

(3*S,5*R**,6*S**)-6-(benzoyloxy)-5-methoxy-1-oxaspiro[2.5]octan-4-one (1.87) (SB-2-67)**



To a solution of 16 mg (0.058 mmol) of alcohol **1.85** in 2 mL of methylene chloride was added 73.14 mg (0.1725 mmol) of Dess Martin periodinane at 0 ^{0}C. The reaction mixture was allowed to reach room temperature and allowed stirred for 2 hours. The reaction mixture was washed with aqueous sodium thiosulfate and extracted using diethyl ether. The organic layer was washed with aqueous sodium bicarbonate and brine, dried over anhydrous sodium sulfate, filtered and concentrated. Column chromatographic separation on silica gel using hexane and ethyl acetate as eluent (1:1 v/v) afforded 6 mg (38% yield) of title compound **1.87**. ^{1}H NMR ($CDCl_3$) δ 7.99 (d, J = 8 Hz, 2 H, Ph), 7.58 (t, J = 7 Hz 1 H, Ph), 7.45 (t, J = 8 Hz, 2 H, Ph), 5.5 (q, 1 H, C-6H), 3.88 (d, J = 5Hz, 1 H, C-5 HOMe), 3.41 (s, 3 H, OMe), 3.01 (d, 1 H, J = 6 Hz, OCH_2), 2.9 (d, 1 H, J = 6 Hz, OCH_2), 2.4 (m, 2 H, CH_2), 2.2 (m, 2 H, CH_2); ^{13}C NMR ($CDCl_3$) δ 203, 165, 137, 133.5, 129.7, 128.6, 83.9, 72.7, 59.0,54.04, 29.9, 27.3, 24.4.

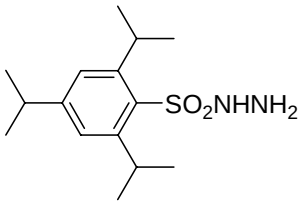
2,4,6-Triisopropylbenzenesulphonyl chloride (1.7)[26, 27] (SB-2-53)



A round bottom flask was charged with 5 g (0.025 mol) of 1,3,5-triisopropyl benzene and dissolved in 25 mL of CH_2Cl_2. This vessel was cooled to 0 ^{0}C and 7.16 mL (0.1078 mol) chlorosulfonic acid ($ClSO_3H$) was added dropwise to the above solution. This solution was stirred at 0 ^{0}C for 1 hour. The resultant solution was poured into crushed ice and washed with water and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over anhydrous sodium sulfate. Column chromatographic separation on silica gel using mixture of hexane and diethyl ether (1:1 v/v) afforded 7.29 g (98% yield) of **1.7**. ^{1}H NMR ($CDCl_3$) δ 7.26 (s,

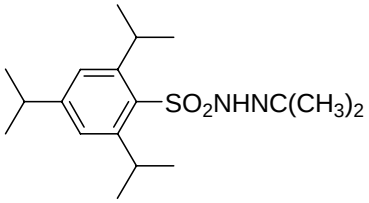
2 H, Ar), 4.23 (hept, 2 H, CH), 2.93(hept, 1 H, CH), 1.31 (d, J = 7 Hz, 12 H, CH_3), 1.27 (d, J = 7 Hz, 6 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 155.8, 150.56, 139.44, 124.5, 34.57, 29.9, 24.56, 23.64.

2,4,6-Triisopropylbenzenesulphonyl hydrazine (1.8)[26, 27] (SB-2-54)



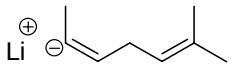
A round bottom flask was charged with 7.29 g (0.0241 mol) of **1.7** and was dissolved in 10 mL of THF. The resulting solution was cooled to 0 ^0C and 2.17 mL (0.0602 mol) of hydrazine was added and stirred at 0 ^0C for 4 hours. The resultant solution was washed with water and extracted with diethyl ether three times. The organic layer was washed with brine and dried over anhydrous sodium sulfate and concentrated to give 6 g (83 % yield) of title compound **1.8**. ^1H 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 (1.9)[26, 27] (SB-2-117)



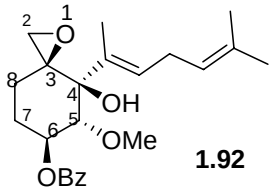
To compound 1 g (3.305 mmol) of **1.8** was dissolved in 15 mL of distilled acetone. The reaction was stirred at room temperature for half an hour. The excess acetone was pumped off using a rotavapour. The 5 mL of CH_2Cl_2 and 5 mL of toluene was added to the above solution and pumped off using a rotavapor. Then the resultant light yellow solid was dried over P_2O_5 in a desiccator under high vaccum for one day to get rid of any moisture or traces of water. Obtained 1.0 g (91% yield) of **1.9**. ^1H 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.

Preparation of akenyl lithium (1.11)[19, 26, 27] (SB-2-118)

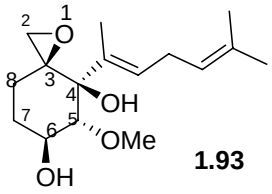


A round bottom flask was charged with 350 mg (1.036 mmol) of **1.9** in freshly distilled 4 mL of DME. This resulting solution was stirred and cooled to -78 ^{0}C. Then 2.15 equivalents (1.4 mL) of *n*-BuLi was added to the above solution, the solution turns to yellow color. The resulting solution was monitored very carefully and slowly warmed to -66 ^{0}C over 20 minutes and again recooled to -78 ^{0}C. To this solution 0.143 mL (1.243 mmol) of 1-Bromo-3-methyl-2-butene was added at -78 ^{0}C. The solution was warmed to -66 ^{0}C over 20 minutes and allowed to stir at -66 ^{0}C for one hour and again recooled to -78 ^{0}C. Then a chelating base TMEDA (0.52 mL 3.42 mmol) was added and 0.712 mL (1.139 mmol) of *n*-BuLi was added. The above solution was allowed to warm to -3 ^{0}C over 2 hours and recooled to -78 ^{0}C. Assuming 100% conversion to alkenyl lithium, this solution was added to ketone **1.87**.

(3*S,4*S**,5*R**,6*S**)-4-(1',5'-Dimethylhexa-1',4'-dienyl)-5-methoxy-6-benzoyloxy-1-oxaspiro[2.5]octan-4-ol (1.92) and (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 (1.93) (SB-3-15)**



1.92

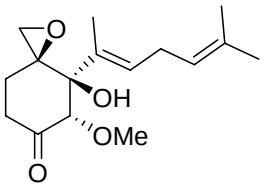


1.93

To a solution of 49 mg (0.1775 mmol) ketone **1.87** in 0.5 mL diethyl ether and 0.5 mL of toluene two equivalents of alkenyl lithium **1.11** was added at -78 ^{0}C. The resulting solution was stirred for one hour at -78 ^{0}C and slowly allowed to reach room temperature. The progress of the reaction was monitored by TLC using hexane and diethyl ether (2:1 v/v) as a developing solvent. Then the reaction was quenched with aqueous ammonium chloride and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to dryness under vaccum. Column chromatographic separation on silica gel using gradient mixture of hexane and ethyl acetate (1:1 v/v) afforded 10 mg of **1.92** (15%) and 5 mg of

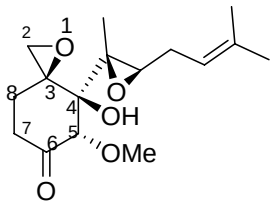
1.93 (10%).It was difficult to characterize the remaining fractions. The stereoisomer at C4 of compound **1.93** was not identified. The stereochemistry of both the structures was confirmed based on the derivatization in the later steps. ^{1}H NMR (**1.92**) ($CDCl_3$) δ 8.08 (d, J = 9 Hz, 2 H, Ar), 7.59 (t, J = 4 Hz, 1 H, Ar), 7.562 (t, J = 2 Hz, 2 H, Ar), 6.13 (t, J = 7 Hz, 1 H, =CH), 5.58 (m, 1 H, CHOCOPh), 5.14 (t, J = 5 Hz, 1 H, =CH), 3.6 (s, 3 H, OMe), 3.56 (m, 1 H, CHOMe), 3.3 (d, J = 5 Hz, 1 H, CHO), 2.82 (d, J = 5 Hz, 1 H, CHO), 2.78 (m, 2 H, CH_2), 1.714 (s, 3 H, CH_3), 1.712 (s, 3 H, CH_3), 1.65 (s, 3 H, CH_3). ^{1}H NMR (**1.93**) ($CDCl_3$) δ 5.90 (doublet of a triplet , 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).

(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 (1.94) (SB-3-33)**

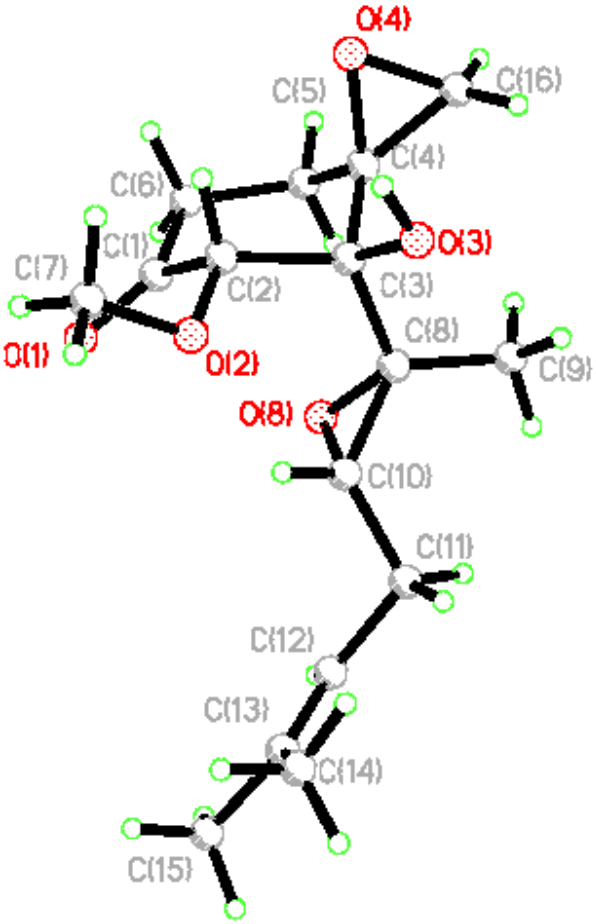


A round bottom was charged with 7 mg (0.0247 mmol) of **1.93** and 1 mL DMSO at room temperature. To the above mixture 14 mg (0.0495 mmol) of IBX was added and stirred at room temperature for 5 hours. The reaction mixture was washed with water and extracted with diethyl ether. The organic layer was washed with brine and dried over anhydrous sodium sulfate, concentrated and applied to column chromatography (silica gel). Hexane and diethyl ether (1:1 v/v) was used as an eluent to afford 4 mg (58% yield) of title compound **1.94**. The stereochemistry of **1.94** was confirmed based on the derivatization in the later step. ^{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).

(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 (1.95) (SB-3-37)**

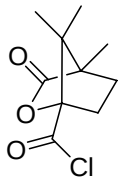


To a solution of 3.5 mg (0.0125 mmol) of ketone **1.94** and 0.5 mL of toluene, 0.019 M in toluene of vanadyl acetoacetonate (0.0025 mmol) was added at 0 ^{0}C. Then 0.1 M in toluene (0.04 mmol) of *tert*-butylhydroperoxide was added to the above mixture. After 3 hours stirring at 0 ^{0}C there was a new spot on TLC. Without any workup the reaction mixture was loaded on to a silica gel column chromatography using hexane and ethyl acetate (1:1 v/v) as an eluent to yield 2 mg (48% yield) of **1.95** (a crystalline solid).The structure was confirmed by taking X-ray. Based on this X-ray structure the earlier products (**1.94** and **1.93**) were characterized. ^{1}H NMR ($CDCl_3$) δ 5.1 (tt, 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), 1.53 (m, 1 H, CH).



X-ray crystal structure of compound **1.95.**

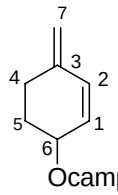
(-)-Camphanoyl Chloride (1.99) (SB-3-83)[44]



A round bottom flask was equipped with a drying tube and was charged with 4.58 g (0.0232 mol) of (-)-camphanic acid (**1.98**). To this flask 13.5 mL (0.197 mmol) of thionyl chloride was added dropwise. Then a reflux condenser was attached to the flask and the resulting solution was heated to reflux for three hours. The flask was cooled to room temperature and excess thionyl chloride

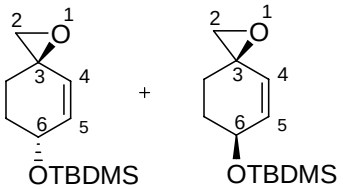
was removed under vaccum. Later 2 mL of benzene was added to the mixture and azeotropic off the remaining thionyl chloride leaving 4.37 g (87% yield) of the pure chloride **1.99**. ^{1}H NMR ($CDCl_3$) δ 2.48 (m, 1 H, CH), 2.19 (m, 1 H, CH), 1.99 (m, 1 H, CH), 1.77 (m, 1 H, CH), 1.15 (s, 3 H, Me), 1.14 (s, 3 H, Me), 1.13 (s, 3 H, Me); ^{13}C NMR ($CDCl_3$) δ 176.9, 171.9, 95.1, 90.1, 55.13, 31.6, 30.8, 28.9, 16.8, 9.8.

3-Methylene-6-((*-*)-*(1S,4R)-camphanoyl*oxy)cyclohexene (1.70) (SB-3-99)



To a solution of 1.6 g (0.0145 mol) of alcohol **1.70** in 50 mL CH_2Cl_2 was added 4.69 mL (0.058 mol) of pyridine and cooled to 0 ^{0}C. Then 0.1772 g (0.00145 mol) of DMAP was added to the above mixture and allowed to stir for 10 min at 0 ^{0}C. Then 3.782 g (0.01745 mol) of (-)-(1*S*,4*R*)-camphanoyl chloride was added and slowly allowed the reaction mixture to warm to room temperature and stirred the reaction mixture at room temperature for 12 hours. The progress of the reaction was monitored by TLC using hexane and ethyl acetate (1:1 v/v) as a developing solvent. This reaction mixture was washed with 1 M HCl and extracted using diethyl ether. Then the organic layer was washed with aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. Column chromatographic separation on silica gel using hexane and ethyl acetate (1:1) as an eluent afforded 3.6 g (86% yield) of **1.100**. ^{1}H NMR ($CDCl_3$) δ 6.3 (dd, J = 3 Hz, 1 H, =CH), 5.8 (q, J = 6 Hz, 1 H, =CH), 5.5 (m, 1 H, CHO), 4.95 (s, 2 H, $=CH_2$), 2.42 (m, 2 H, CH_2), 2.05 (m, 2 H, CH_2), 1.95 (m, 2 H, CH_2), 1.67 (m, 2 H, CH_2), 1.08, (s, 3 H, CH_3), 1.02 (s, 3 H, CH_3), 0.931 (s, 3 H, CH_3), 0.927 (s, 3 H, CH_3); ^{13}C NMR 178, 167.1, 141, 133.89, 133.86, 126.2, 126.1, 114.38, 114.35, 91.149, 69.56, 69.47, 54.85, 54.22, 30.66, 30.58, 29.09, 29.06, 28.35, 26.12, 26.58, 16.91, 16.84, 16.78, 9.76.

(3*R,8*R**) and (3*R**,8*S**)-6-(t-Butyldimethylsilyloxy)-1-oxaspiro[2.5] oct-4-ene (1.106 & 1.62) (SB-5-96)**

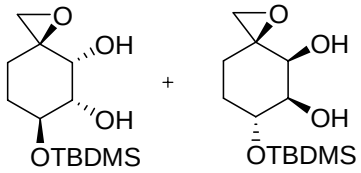


1.62 **1.106**

A round bottom flask was charged with 10.5 g (0.0468 mol) of **1.63** in 125 mL of EDTA (4 x10^{-4} M) to CH_3CN + THF (8:2) in 9:11 ratio and the round bottom was cooled to 0 ^{0}C. The round bottom was equipped with a dewar condenser on the top. It is very necessary to cool the dewar flask to -78 ^{0}C throughout the reaction period to avoid the escape of low boiling liquids (1,1,1-trifluoroacetone) which are added in due course (two additions of 1,1,1-trifluoroacetone). Then 19.67 g (0.234 mol) of sodium bicarbonate was added to the above mixture at 0 ^{0}C. Then 2.53 mL (0.0269 mol) of 1,1,1-trifluoroacetone was added via syringe followed by 14.38 g (0.0234 mol) of oxone was added over 20 minutes in portions. The resulting mixture was stirred at 0 ^{0}C for one hour. Then another half portion of 2.53 mL (0.0269 mol) of 1,1,1-trifluoroacetone was added and the reaction was maintained at 0 ^{0}C. This mixture was stirred at 0 ^{0}C for 6 hours. The dry ice dewar is to be maintained at -78 ^{0}C always. The progress of the reaction was monitored by TLC using hexane and diethyl ether (1:1 v/v) as a developing solvent. The reaction mixture doesn't go to completion. If we run it for a long time there is always a risk of decomposition of epoxide. The reaction mixture was diluted with CH_2Cl_2 and washed with aqueous sodium sulfate. The organic layer was washed with brine and dried over anhydrous sodium sulfate. The silica gel was used to perform column chromatography. Hexane was mixed with silica gel along with 1% triethylamine and sonicated for one hour. Column chromatographic separation on silica gel using hexane and diethyl ether (1:1 v/v) as an eluent afforded 9.1 g (82%) of mixture of epoxides **1.106** and **1.62** in 1:1 ratio. The ratio of the two isomers was based on the integration from the proton NMR. The starting material the diene **1.63** (9%) was recovered. It is difficult to assign which set of proton and ^{13}C NMR's belong to which isomer. ^{1}H NMR ($CDCl_3$) (of one isomer): δ 6.0 (d, J = 10Hz, 1 H, =CH), 5.22 (t, *J* = 10 Hz, 1 H, =CH), 4.35 (1 H, HC-OSi), 2.84 (d, J = 5 Hz, 1 H, OCH_2), 2.76 (d, *J* = 5 Hz, 1 H, OCH_2), 1.91 (m, 2 H, CH_2), 1.7 (m, 2 H, CH_2), 0.89 (s, 9 H, t-Bu), 0.03 (s, 3 H, $SiCH_3$), 0.03 (s, 3 H, $SiCH_3$); ^{1}H NMR ($CDCl_3$) (of another isomer) δ 6.0 (d, J = 10Hz, 1 H, =CH), 5.22 (t, *J* = 10 Hz, 1 H, =CH), 4.28 (1 H, HC-OSi), 2.84 (d, J = 5 Hz, 1 H,

OCH_2), 2.76 (d, *J* = 5 Hz, 1 H, OCH_2), 1.91 (m, 2 H, CH_2), 1.7 (m, 2 H, CH_2), 0.89 (s, 9 H, t-Bu), 0.03 (s, 3 H, $SiCH_3$), 0.03 (s, 3 H, $SiCH_3$); ^{13}C NMR ($CDCl_3$) (of one isomer): δ 139.4, 129.99, 66.8, 55.3, 31.7, 27.8, 26.0,18.3, -4.4, -4.48. ^{13}C NMR ($CDCl_3$) (of another isomer): δ 138.2, 128.8, 65.9, 54.9, 30.9, 27.8, 26.0, 18.3, -4.4, -4.48.

(3*S,4*S**,5*R**,6*R**)-6-(*tert*-Butyldimethylsilyloxy)-1-oxaspiro[2.5]octane-4,5-diol (1.108) & (3*S**,4*R**,5*S**,6*S**)-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octane-4,5-diol (1.107) (SB-5-97)**

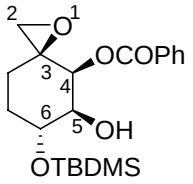


1.107 **1.108**

To a solution of 9 g (0.0374 mol) of mixture of epoxides (**1.106** and **1.62**) in 665 mL of acetone-water (3:1) 90 mL of *tert*-butanol was added. To this mixture 13.14 g (0.1123 mol) of NMO was added followed by catalytic amount of 0.5 g of osmium tetraoxide was added and stirred at room temperature. This mixture was stirred at room temperature for 36 hours. The progress of the reaction was monitored by TLC using hexane and ethyl acetate as a developing solvent. In order to get rid of acetone in the solution, distillation was performed. Then the resulting solution was washed with water and extracted with CH_2Cl_2. The organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated. Column chromatographic separation on silica gel using mixture of hexane and diethyl ether (1:1 v/v) afforded 65 % of two *cis* diols **1.107** and **1.108** in 1:1 ratio. The ratio of the two compounds was based on integration in proton NMR. These two isomers were not separable by column chromatography at this stage. We couldn't find suitable solvent system to separate them efficiently. The identification of the two alcohols is carried by derivatization in the next step. ^1H NMR ($CDCl_3$) (of one isomer) δ 3.99 (m, 1H, CHOTBDMS), 3.82 (m, 1 H, CHOH), 2.94 (d, J = 5 Hz, 1 H, OCH_2), 2.6 (d, J = 5 Hz, OCH_2), 2.42 (d, J = 6 Hz, 1 H, CHOH), 1.85 (m, 2 H, CH_2), 1.5 (m, 2 H, CH_2), 0.91 (s, 9 H, *t*-Bu), 0.11 (s, 3 H, CH_3), 0.10 (s, 3 H, CH_3); ^1H NMR ($CDCl_3$) (of another isomer) δ 3.99 (m, 1H, CHOTBDMS), 3.82 (m, 1 H, CHOH), 3.02 (d, J = 3 Hz, 1 H, OCH_2), 2.91 (d, J = 4 Hz, OCH_2),

2.83 (d, J = 5 Hz, 1 H, CHOH), 1.85 (m, 2 H, CH_2), 1.5 (m, 2 H, CH_2), 0.91 (s, 9 H, *t*-Bu), 0.11 (s, 3 H, CH_3), 0.10 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) (of one isomer) δ 75.8, 71.4, 70.62, 59.6, 51.4, 28.8, 26.91, 26.07, 18.18, -4.29, -4.62; ^{13}C NMR ($CDCl_3$) (of another isomer) δ 74.6, 71.16, 70.62, 59.0, 49.7, 28.8, 26.39, 25.96, 14.41, -4.48, -4.62.

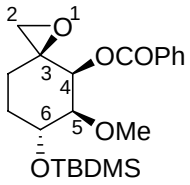
(3*S,4*S**,5*R**,6*R**)-6-(*tert*-Butyldimethylsilyloxy)-5-hydroxy-1oxaspiro[2.5]octan -4-yl benzoate (1.109) (SB-5-99)**



To a solution of 6.7 g (0.0245 mol) of mixture of **1.107** and **1.108** (in 1:1 ratio) in 150 mL THF was added 10.2 mL (0.073 mol) of triethylamine and the solution was stirred at 0 ^{0}C for 10 minutes. Then 3.1 mL (0.027 mol) of benzoyl chloride was added to the solution and stirred at 0 ^{0}C for one hour and allowed to reach room temperature slowly. This solution was stirred at room temperature for 12 hours. The resulting solution was washed with 1 M HCl and extracted with methylene chloride. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over anhydrous sodium sulfate, filtered and concentrated. Column chromatographic separation on silica gel using mixture of hexane and ethyl acetate (1:1 v/v) afforded unreacted **1.107** in 49% yield and benzoylated product **1.109** in 43% yield. The alcohol at C4 position is benzoylated in compound **1.108**, which is confirmed from proton NMR as we see a doublet signal at δ 5.49. The absence of a multiplet in the same region confirms that the C5 alcohol is not benzoylated. ^{1}H NMR of **1.109** ($CDCl_3$) δ 8.07 (d, J = 7 Hz, 2 H, Ar), 7.58 (d, J = 3 Hz, 1 H, Ar), 7.49 (t, J = 7 Hz, 2 H, Ar), 5.49 (d, J = 3 Hz, 1 H, CHOCOPh), 4.13 (m, 1 H, CHOTBDMS), 3.98 (m, 1 H, CHOH), 2.82 (d, J = 5 Hz, 1 H, CHO), 2.61 (d, J = 5 Hz, 1 H, CHO), 2.14 (t, J = 8 Hz, 2 H, CH_2), 1.66 (m, 2 H, CH_2), 0.94 (s, 9 H, *t*-Bu), 0.126 (s, 3 H, CH_3), 0.119 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 152.5, 141.5, 133.5, 130.0, 128.7, 74.5, 71.2, 70.6, 58.8, 49.6, 27.1, 26.8, 25.9, 18.2, -4.5, -4.7. ^{1}H NMR of **1.107** ($CDCl_3$) δ 3.99 (m, 1H, CHOTBDMS), 3.82 (m, 2 H, CHOH), 2.94 (d, J = 5 Hz, 1 H, OCH_2), 2.69 (d, J = 5 Hz, OCH_2), 2.42 (m, 1 H, CH), 2.33 (m, 1 H, CH), 1.75

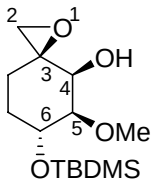
(m, 2 H, CH_2), 0.91 (s, 9 H, *t*-Bu), 0.11 (s, 3 H, CH_3), 0.10 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 74.8, 70.36, 70.01, 59.6, 51.1, 28.3, 26.3, 25.8, 17.9, -4.7, -4.8.

(3*S,4*S**,5*R**,6*R**)-6-(*tert*-Butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan -4-yl benzoate (1.110) (SB-5-102)**



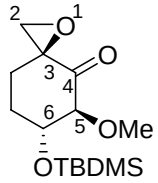
To a solution of 2.23 g (5.65 mmol) of **1.109** in 50 mL of CH_2Cl_2 was added 2.9 g (13.57 mmol) of proton sponge at 0 ^{0}C. The reaction mixture was stirred for 15 minutes at 0 ^{0}C and then 1 g (6.78 mmol) of trimethyloxonium tetrafluoroborate was added in portions and stirred at 0 ^{0}C for half an hour. The reaction mixture was allowed to reach room temperature was stirred for 48 hours. The progress of the reaction was monitored by TLC using hexane and diethyl ether (1:1 v/v) as a developing solvent. The reaction mixture was washed with 1 M HCl and extracted with diethyl ether. The organic layer was washed with aqueous sodium bicarbonate and brine, dried over anhydrous sodium sulfate, concentrated and the residue was applied to column chromatography (silica gel). Mixture of hexane and diethyl ether (1:1 v/v) was used as an eluent to obtain 1.25 g (72 % yield) of title compound **1.110**. ^1H NMR of ($CDCl_3$) δ 8.07 (d, J = 7 Hz, 2 H, Ar), 7.59 (t, J = 7 Hz, 1 H, Ar), 7.45 (t, J = 8 Hz, 2 H, Ar), 5.45 (d, J = 3 Hz, 1 H, CHOCOPh), 4.09 (m, 1 H, CHOTBDMS), 3.4 (s, 3 H, OMe), 3.36 (m, 1 H, CHOMe), 2.81 (d, J =5 Hz, 1 H, CHO), 2.63 (d, J = 5 Hz, 1 H, CHO), 2.12 (m, 1 H, CH), 1.96 (m, 1 H, CH), 1.62 (m, 1 H, CH), 1.58 (m, 1 H, CH), 0.92 (s, 9 H, *t*-Bu), 0.097 (s, 3 H, SiMe), 0.091 (s, 3 H, SiMe); ^{13}C NMR ($CDCl_3$) 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 (1.111) (SB-5-104)**



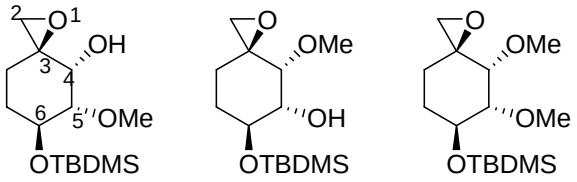
To a solution of 1.25 g (3.061 mmol) of **1.110** in 60 mL of distilled anhydrous methanol was added 1.06 g (7.65 mmol) of potassium carbonate at 0 ^{0}C. The reaction mixture was stirred at 0 ^{0}C for 5 hours. The solid reside in the mixture from potassium carbonate was filtered through a pad of celite and the resulting mixture was evapourated using a rotavapor to get rid of methanol. Then the residue was diluted with CH_2Cl_2 and washed with 1 M HCl, aqueous $NaHCO_3$ and brine. The organic layers were dried over anhydrous sodium sulfate, filtered and evapourated. Column chromatographic separation on silica gel using mixture of hexane and ethyl acetate (1:1 v/v) afforded 730 mg (79% yield) of **1.111**. ^{1}H NMR ($CDCl_3$) 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 ($CDCl_3$) 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 (1.112) (SB-6-22)**



To a solution of 305 mg (1.0 mmol) of **1.111** in a solution of 20 mL of distilled DMSO was added 422.2 mg (1.508 mmol) of *o*-iodoxybenzoic acid (IBX) at room temperature. The reaction was stirred at room temperature for 12 hours. The resultant solution was washed with water and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over anhydrous sodium sulfate and concentrated to dryness under vacuum. Column chromatographic separation on silica gel using mixture of hexane and ethyl acetate (1:1) afforded 283 mg (94% yield) of **1.112**. ^{1}H NMR ($CDCl_3$) 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, -4.45, -4.83.

(3*S,4*R**,5*S**,6*S**)-6-(*tert*-Butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan -4-o1 (1.114), (3*S**,4*R**,5*S**,6*S**)-6-(*tert*-butyldimethylsilyloxy)-4-methoxy-1-oxaspiro[2.5]octan -5-o1 (1.115), (3*S**,4*R**,5*S**,6*S**)-6-(*tert*-butyldimethylsilyloxy)-4,5-dimethoxy-1-oxaspiro[2.5]octane (1.116). (SB-8-94)**



1.114 **1.115** **1.116**

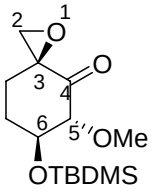
To a solution of 900 mg (3.279 mmol) of **1.107** in 15 mL of methylene chloride was added 1.83 g (8.526 mmol) of 1,8-bis(dimethylamino)naphthalene (proton sponge) and the reaction mixture was stirred at 0 ^{0}C for 15 minutes. Then 0.727 g (4.919 mmol) of trimethyloxonium tetrafluoroborate was added to this solution at 0 ^{0}C. The resulting solution was stirred at room temperature for 48 hours. The reaction mixture was washed with 1 M HCl and extracted with CH_2Cl_2. The organic layer was washed with aqueous sodium bicarbonate and brine, dried over anhydrous sodium sulfate, filtered and concentrated to dryness. Column chromatographic separation on silica gel using gradient mixture of hexane and ethyl acetate (1:1 v/v) afforded 149 mg (15% yield) of **1.116**, two monomethylated products **1.114** and **1.115** in 80% yield (in 1:1 ratio based on the crude proton NMR)and recovered 45 mg (5%) of starting material **1.107** was recovered. Based on proton NMR we can distinguish the two compounds **1.114** and **1.115**. Compound **1.115** shows a doublet at δ 3.33 for C4 hydrogen where as compound **1.114** shows a doublet of a doublet at δ 3.34 for C4 hydrogen. Another column chromatographic separation using hexane and ethyl acetate (1:1 v/v) was used as an eluent to separate the monomethylated products but couldn't separate them efficiently. ^{1}H NMR of **1.114** ($CDCl_3$) δ 3.99 (m, 1 H, CHOTBDMS), 3.82 (s, 1 H, CHOH), 3.45 (s, 3 H, OMe), 3.4 (m, 1 H, CHOMe), 2.9 (d, J = 5 Hz, 1 H, CHO), 2.67 (d, J = 5 Hz, 1 H, CHO), 2.32 (d, 1 H, OH), 1.85 (m, 2 H, CH_2), 1.67 (m, 2 H, CH_2), 0.91 (s, 9 H, *t*-Bu), 0.1 (s, 3 H, CH_3), 0.089 (s, 3 H, CH_3); ^{13}C NMR of **1.114** ($CDCl_3$) 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.

^{1}H NMR of **1.115** ($CDCl_3$) δ 3.95 (m, 1 H, CHOTBDMS), 3.81 (s, 1 H, CHOH), 3.42 (s, 3 H, OMe), 3.34 (m, 1 H, CHOMe), 2.93 (d, J = 5 Hz, 1 H, CHO), 2.68 (d, J = 5 Hz, 1 H, CHO), 2.36

(d, 1 H, OH), 1.90 (m, 2 H, CH_2), 1.63 (m, 2 H, CH_2), 0.91 (s, 9 H, *t*-Bu), 0.11 (s, 3 H, CH_3), 0.089 (s, 3 H, CH_3); ^{13}C NMR of **1.114** ($CDCl_3$) 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.

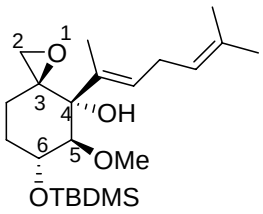
^{1}H NMR of **1.116** ($CDCl_3$) δ 4.03 (m, 1 H, CHOTBDMS), 3.45 (s, 3 H, OMe), 3.41 (s, 3 H, OMe), 3.37 (s, 1 H, CHOMe), 3.35 (s, 1 H, CHOMe), 2.95 (d, J = 5 Hz, 1 H, CHO), 2.71 (d, J = 5 Hz, 1 H, CHO), 1.85 (m, 2 H, CH_2), 1.65 (m, 2 H, CH_2), 0.91 (s, 9 H, *t*-Bu), 0.11 (s, 3 H, CH_3), 0.09 (s, 3 H, CH_3); ^{13}C NMR of **1.116** ($CDCl_3$) 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.

(3*S,5*S**,6*S**)-6-(*tert*-Butyldimethylsilyloxy)-5-methoxy-1-oxaspiro[2.5]octan -4-one (1.117) (SB-8-87)**

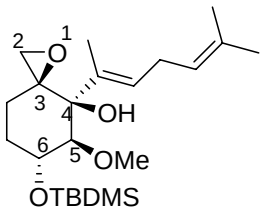


To a solution of 10 mg (0.0329 mmol) of **1.114** in 0.5 mL of DMSO under argon, was added 12 mg (0.043 mmol) of IBX. The reaction mixture was stirred at room temperature for 12 hours. The reaction mixture was washed with water and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated to dryness. Column chromatographic separation on silica gel using gradient mixture of hexane and ethyl acetate (1:1 v/v) afforded 9 mg (90% yield) of **1.117**. ^{1}H NMR ($CDCl_3$) δ 4.17 (m, 1 H, CHOTBDMS), 3.51 (d, J = 4.8 Hz, 1 H, C5-H), 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 of **1.117** ($CDCl_3$) 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*R**,5*S**,6*S**)-4-(1',5'-Dimethylhexa-1',4'-dienyl)-5-methoxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-4-ol (1.119) and (3*S**,4*S**,5*S**,6*S**)-4-(1',5'-dimethylhexa-1',4'-dienyl)-5-methoxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-4-ol (1.120) (SB-6-48)**



1.119

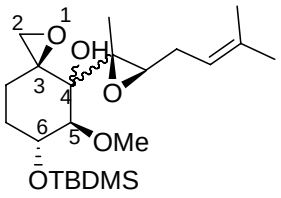


1.120

To a solution of 70 mg (0.2326 mmol) of **1.112** in 1 mL ether and 1 mL toluene was added 0.9302 mmol of alkenyl lithium **1.11** at -78 ^{0}C under argon. This solution was stirred at -78 ^{0}C for one hour and slowly allowed to reach to room temperature. The progress of the reaction was monitored by TLC using hexane and ethyl acetate as a developing solvent. The resulting solution was washed with aqueous ammonium chloride and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous sodium sulfate. Column chromatographic separation on silica gel using gradient mixture of hexane and ethyl acetate (1:1 v/v) afforded 72 mg (75% yield) of a mixture of compounds **1.119** and **1.120**. These two isomers were inseparable by column chromatography. From the proton NMR it is clear that there are two compounds in about ~1:2 ratio (based on proton NMR integration). Two distinct signals appear for C2 hydrogen's and C5 hydrogen's. Apart from that two sets of signals appear for methyl groups on side chain (δ 1.743, 1.689, 1,625 for one set and δ 1.7303, 1.679, 1.613), two signals for *tert*-Si group (δ 0.909 for one compound and 0.884 for another compound) and two sets of signals for silyl-methyl groups (δ 0.113, 0.0867 for one compound and δ 0.0794, 0.0684 for another compound). Two sets of doublets are also observed for methylene group of epoxide. This confirm the presence of two compounds. It is hard to assign peaks which correlate to the respective compound. It is also difficult to decide which compound is in larger portion in this mixture. So here in we report two sets of proton NMR data. ^{1}H NMR ($CDCl_3$) (one compound which is a major fraction in the mixture) δ 5.99 (m, 1 H, C=C-H), 5.13 (m, 1 H, H-C=C), 4.09 (m, 1 H, CHOTBDMS), 3.54 (s, 3 H, OMe), 3.48 (s, 1 H, CHOMe), 3.08 (d, J = 12 Hz, 1 H, CHO), 2.345 (d, J = 7 Hz, 1 H, CHO), 2.05-1-95 (m, 2 H, CH_2), 1.743 (s, 3 H, Me), 1.689 (s, 3 H, Me), 1.625 (s, 3 H, Me), 1.30-1.20 (m, 2 H, CH_2), 0.909 (s, 9 H, *t*-Bu), 0.113 (s, 3 H, Me), 0.0867 (s, 3 H, Me); ^{1}H NMR ($CDCl_3$) (another compound which is a minor fraction in the mixture) δ 5.45 (m, 1 H, C=C-H), 5.1 (m, 1 H, H-C=C), 4.09 (m, 1 H, CHOTBDMS), 3.54 (s, 3 H, OMe), 3.5 (s, 1 H, CHOMe), 2.965 (d, J = 2 Hz, 1 H, CHO), 2.729 (d, J = 7 Hz, 1 H, CHO), 2.05-1-95 (m, 2 H, CH_2),), 1.7303 (s, 3 H,

Me), 1.679 (s, 3 H, Me), 1.613 (s, 3 H, Me), 1.30-1.20 (m, 2 H, CH_2), 0.884 (s, 9 H, *t*-Bu), 0.079 (s, 3 H, Me), 0.068 (s, 3 H, Me).

(3*S,4*S**,5*S**,6*S**)-4-(2'-Methyl-3'-(3''-methylbut-2''-enyl)oxiran-2'-yl)-5-methoxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-4-ol (1.122) (SB-6-60)**



To a solution of 24 mg (0.0584 mmol) of mixture of **1.119** and **1.120** in 1 mL of benzene was added 2.167 mg (0.0082 mmol) of vanadly acetylacetonate (a solution in 2 mL of benzene) and 1.16 mL of 0.1 M *tert*-butyl hydroperoxide (commercially available from aldrich) at room temperature. The reaction mixture was stirred at room temperature for 2 hours. The organic layer was washed with 10% sodium thiosulfate and extracted with ether three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated to dryness. The crude NMR shows the presence of some starting material but reveals the formation of product. Column chromatographic separation on silica gel using a mixture of hexane and ethyl acetate (1:1 v/v) afforded 2 mg of compound **1.122** (8%). Besides the above product 12 mg (50%) of starting material **1.119** and **1.120** and some unidentified materials (8%) were recovered. We were unable to determine the stereochemistry of this compound at C4 position at this stage. Removal of TBDMS group and oxidation of thus formed alcohol to ketone may help us to determine the stereochemistry by comparing with compound **1.95**. ^{1}H NMR ($CDCl_3$) δ 5.14 (m, 1 H, H-C=C), 4.02 (m, 1 H, CHOTBDMS), 3.6 (s, 3 H, OMe), 3.23 (d, J = 8 Hz, 1 H, CHOMe), 3.11 (t, J = 8 Hz, 1 H, O-CH-CH_2), 3.01 (s, 1 H, OH), 2.77 (m, 1H, CHO), 2.50 (d, J = 5 Hz, 1H, CHO), 2.27 (m, 2H, CH_2), 2.11 (m, 1H, CH), 1.87 (m, 1H, CH), 1.683 (s, 3 H, CH_3), 1.68 (s, 3H, CH_3), 1.29 (s, 3 H, CH_3), 0.877 (s, 9 H, *t*-Bu), 0.092 (s, 3 H, SiMe), 0.07 (s, 3 H, SiMe).

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CHAPTER 2 – Novel tricyclic pyrone compounds protect cell death induced with intracellular Aβ oligomeric complexes

2.1 Introduction

Protein aggregation may lead to many neurodegenerative diseases such as Alzhemier's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotropic lateral sclerosis (ALS) and prion diseases.[1] Most active research has been carried out in these areas as these diseases are characterized by loss of synaptic connections and aggregation of proteins both intracellular and extracellular.[1] The deposition of aggregated protein in different areas of brain can cause different kinds of neurodegenerative diseases. Deposition of different type of proteins and pathology is depicted in the following table (shown in Table 2.1).[1] Each and every disease has its own reasons and symptoms and can occur in either in middle age or in old age of a person and eventually leading to death. There is no effective treatment or cure.[2] Early detection is not easy as the symptoms are exhibited only after death of many neurons and certain parts of brain are weakened.[2] Factors that attribute to this neuron death are "excitotoxicity, cellular calcium overload, mitochondrial dysfunction, oxidative stress" etc.[2]

As per statistics there are 4 million people who suffer from AD, 1 million from PD, 350,000 from multiple sclerosis and 20,000 from ALS in United States.[2] There are 20 million patients who suffer from these four diseases worldwide.[2] "AD, a irreversible disease with no cure is the third most costly disease in US after heart disease and cancer".[3] "Federal government spent $647 million for AD research in the year 2005".[3] After viewing this it is very important to address the exact cause and prevention and curative measures for this AD disease.

2.2 Back ground and Significance

Alzhemier's disease (AD) is characterized by loss of memory, speech, recognition of people and objects, etc.[1, 4] This is because of fibrillar amyloid deposits in the brains of alzhemier's patients. An AD patient's brain has two kinds of classical lesions, extracellular senile (neuritic) plaques and intracellular nuerofibrillary tangles.Extracellular deposits (plaques) consist of Aβ peptides and intracellular deposits (tangles) are Aβ tau protein.[5] Aβ peptide is a fragment of another protein called amyloid precursor protein (APP). In a normal persons brain

these deposits are eliminated whereas in AD patient they accumulate as plaques.[6] Tau proteins are insoluble proteins inside neurons, a subset of microtubles (helps transport of nutrients within neurons) aggregates in a AD patient (Figure 2.1).[6]

Disease	**Etiology**	**Regions most affected**	**Characteristic pathology**	**Disease proteins deposited**
Huntington's Disease	Huntingtin (dominant)	Striatum, other basal ganglia, cortex	Intranuclear inclusions and cytoplasmic aggregates	Huntingtin with polyglutamine expansion
Other polyglutamine diseases (DRPLA, SCA1-3, etc., SBMA)	Atrophin-1, ataxin-1-3, etc.; androgen receptor (AR) (dominant)	Basal ganglia, brain stem cerebellum and spinal cord	Intranuclear inclusions	Atrophin-1, ataxins or AR
Alzheimer's Disease (AD)	Sporadic (ApoE risk factor)	Cortex, hippocampus, basal forebrain, brain stem	Neuritic plaques & neurofibrillary tangles	Aβ peptide(from APP)&,hyper phopphorylate d tau
	Amyloid precursor protein (APP) (dominant)	Same as sporadic	Same as sporadic	Same as sporadic
	Presenilin1,2 (dominant)	Same as spordaic	Same as spordaic	Same as spordaic
Fronto-temporal dementia with parkinsonism	Tau mutations (dominant)	Frontal and temporal cortex, hippocampus	Pick bodies	Hyperphosph orylated tau protein
Parkinson's Disease (PD)	Spordaic	Substantia nigra, cortex,locus ceruleus,etc	Lewy bodies and Lewy neuritis	α-Synuclein
	α-Synuclein (dominant)	Similar to sporadic, but more widespread	Same as sporadic	α-Synuclein

	Parkin (also DJ-1, PINK1) recessive (some dormant)	Substantia nigra	Lewy bodies absent (or much less frequent)	α-Synuclein (when present)
Amyotropic lateral sclerosis (ALS)	Spordaic Superoxide dismutase-1 (dominant)	Spinal motor neurons and motor cortex Same as spordaic	Bonina bodies and axonal spheroids Same	Unknown (neurofilaments) Unknown
Prion diseases (kuru, CJD, GSS disease, fatal familial insomnia, new variant CJD)	Spordaic, genetic and infectious	Cortex, thalamus, brain stem, cerebellum, other regions	Spongiform degeneration amyloid, other aggregates	Prion protein

ApoE, apolipoproteinE; APP, amyloid precursor protein; CJD, Creutzfeldt-Jakob disease; DRPLA, dentate-rubral and pallido-Luysian atrophy; GSS, Gerstmann-Straussler-Scheinker; SBMA, spinal and bulbar muscular atrpphy; SCA, spino-cerebellar ataxia

Table 2.1 Neurodegenerative diseases: proteins and pathology [1]

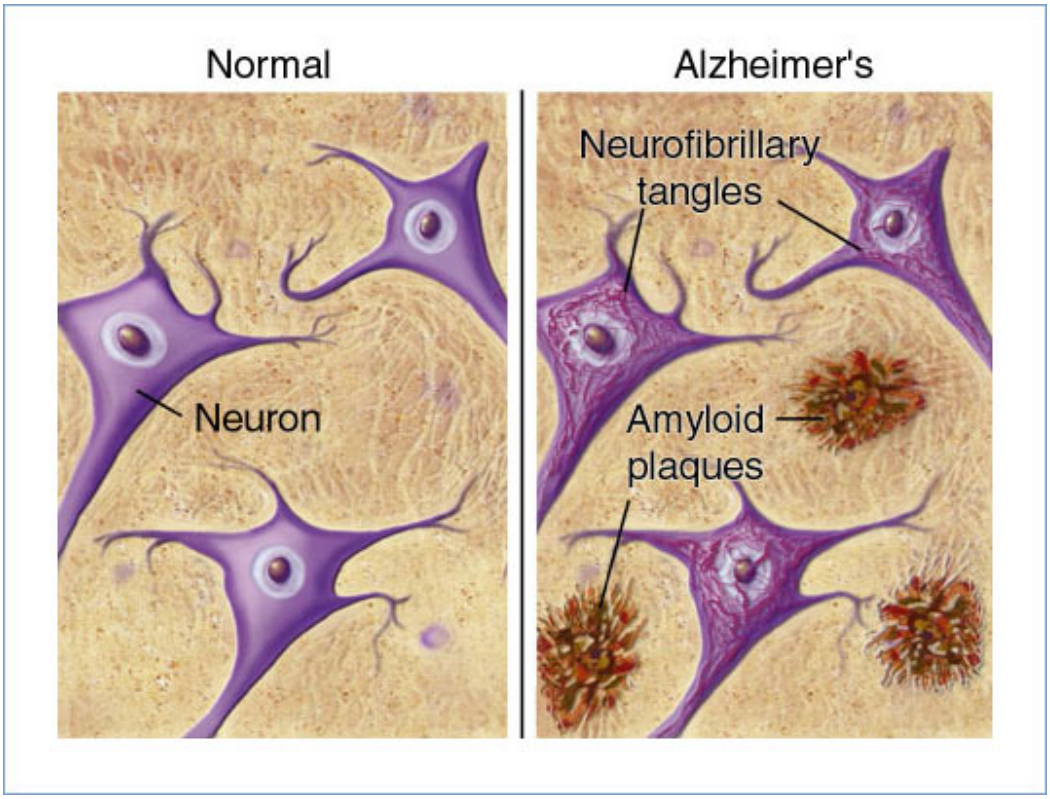


Figure 2.1 Amyloid plaques and neurofibrillary tangles shown in Alzhemier's case and these aggregrates are absent in normal case (This picture is taken from reference without permission).[6]

The following figure 2.2 depicts how the some parts of brain shrinks as the disease progresses.[7] In early stages of this disease ventricles are enlarged and the hippocampus cells begin to degenerate and shrink which markedly shows symptoms like relapse of short term memory etc.[7] As the disease advances the cerebral cortex region shrinks along with hippocampus leaving patient with emotional outbursts and partial impairment.[7] As the disease advances, the patient loses control over some vital organs and eventually leads him to death.[7]

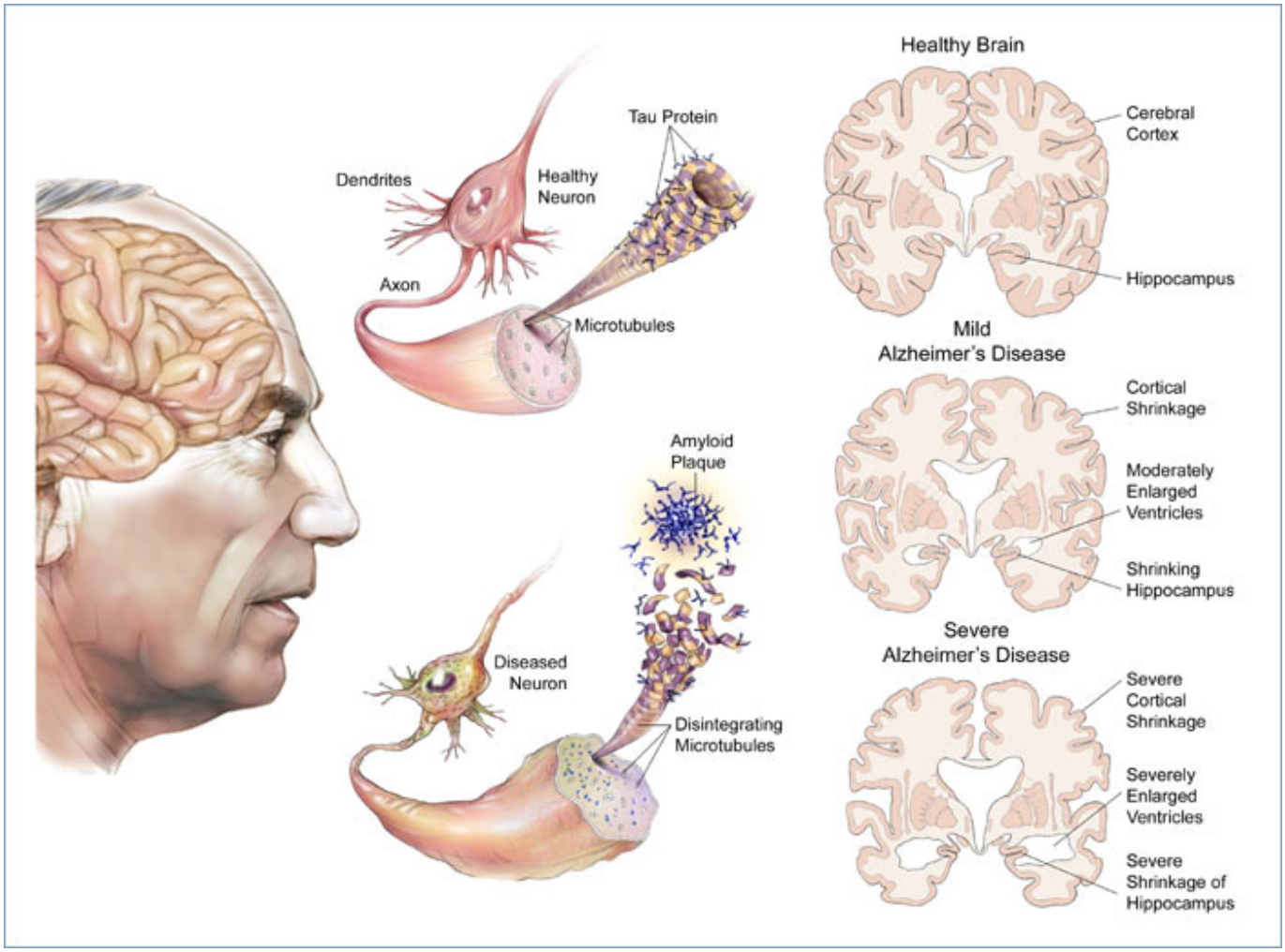


Figure 2.2 Comparison of different brain parts at different stages of AD (This picture is taken from reference without permission).[7]

Amyloid β-protein (Aβ) is the major constituent of fibrillar deposits in AD patients. It is hypothesized that the production of Aβ in the brain is increased by disease-associated mutations and risk factors which is proven when Aβ fibrils killed the neuron cells when added *in vitro*. Therefore AD pathogenesis is caused by generation of extracellular Aβ fibrils. It is also hypothesized that the intraneuronal accumulation of Aβ is the first step of the amyloid cascade.[8] Also it is proven that intracellular Aβ induces more toxicity, atleast 10,000 times more toxic than extracellular Aβ.[9, 10] Therefore it is important to decipher the mechanisms of the intraneuronal Aβ aggregates that induces toxicity.

Aβ is a subunit of a large precursor called amyloid-β precursor protein (APP) consisting of 770 amino acids. APP is first cleaved by β-secretase to produce a 99-residue COOH-terminal membrane fragment called C99. Later this C99 fragment is cleaved by γ-secretase to produce either a 40 reside (Aβ40) or a 42 residue (Aβ42) peptide. This Aβ fragment has 28 amino acids outside the membrane and 12-14 amino acids of transmembrane domain. C99 fragment can further be cleaved by α-secretase to generate a 83-residue COOH-terminal membrane fragment called C83 (Figure 2.3).[5] Both Aβ40 and Aβ42 are hydrophobic and toxic and aggregate quickly. Based on biochemical and immunohistochemical studies, it is proven that Aβ42 has higher propensity for aggregation and fibrillogenesis.[11]

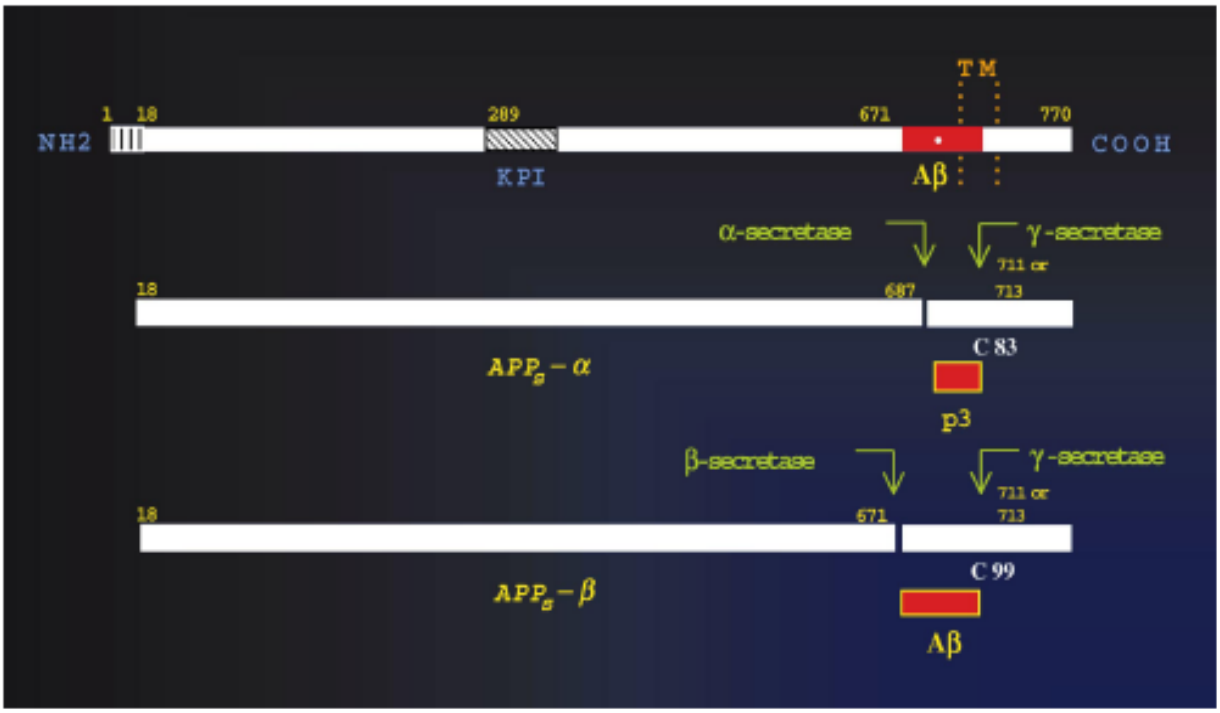


Figure 2.3 β-Amyloid precursor protein (APP) and its metabolic derivatives (This picture was taken from the reference without permission).[5]

Aβ aggregation is a multitep process involving metastable intermediates including oligomeric complexes.[12] Monomers quickly oligomerize to paranuclei. This paranuclei look like beads on a circular string comprising 5 to 6 monomers units. The Aβ aggregation starts with the formation of paranuclei. This paranuclei can oligomerize to form large oligomers. There is always equilibrium between monomers, paranuclei and large oligomers. Monomers, paranuclei

and large oligomers do not have any β sheets or turns, so are unstructured (U). On long standing these unstructured oligomers are converted to protofibrils (~ 5 nm). Protofibrils have β-sheets and turns. Finally step is the conversion of protofibrils to fibrils (~10 nm) which is an irreversible transformation (Figure 2.4).[12]

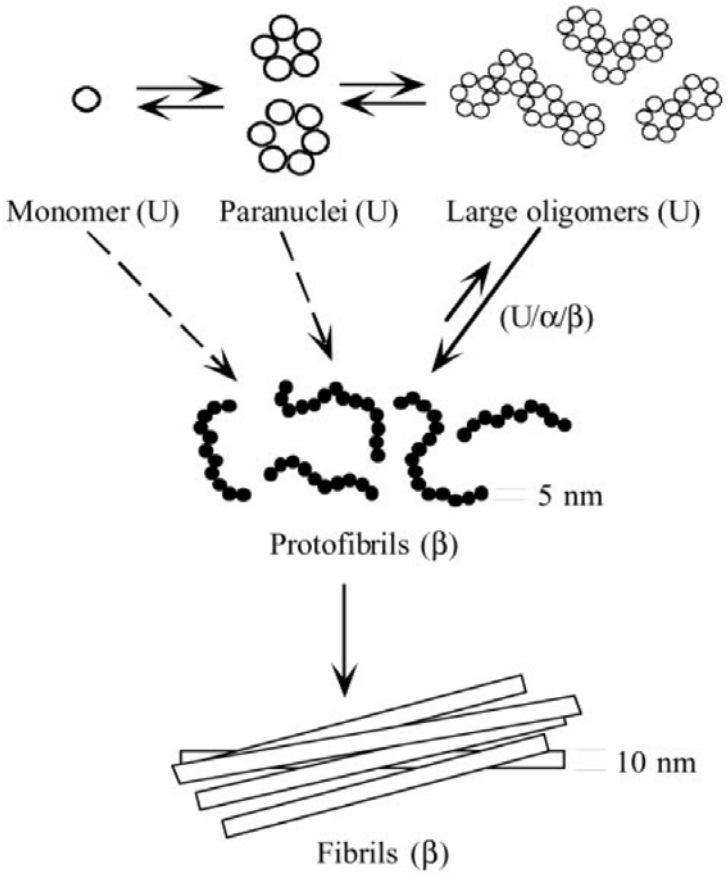


Figure 2.4 Schematic diagram of conversion of a monomer to a fibril (This picture is taken from the reference without permission).[12]

The following diagram shows the hydrophobicty indexes of Aβ1 -43 peptide. The more positive units refer to the more hydrophobic β-sheet forming fragments. The more negative units refer to the more hydrophilic fragments (Figure 2.5).[13]

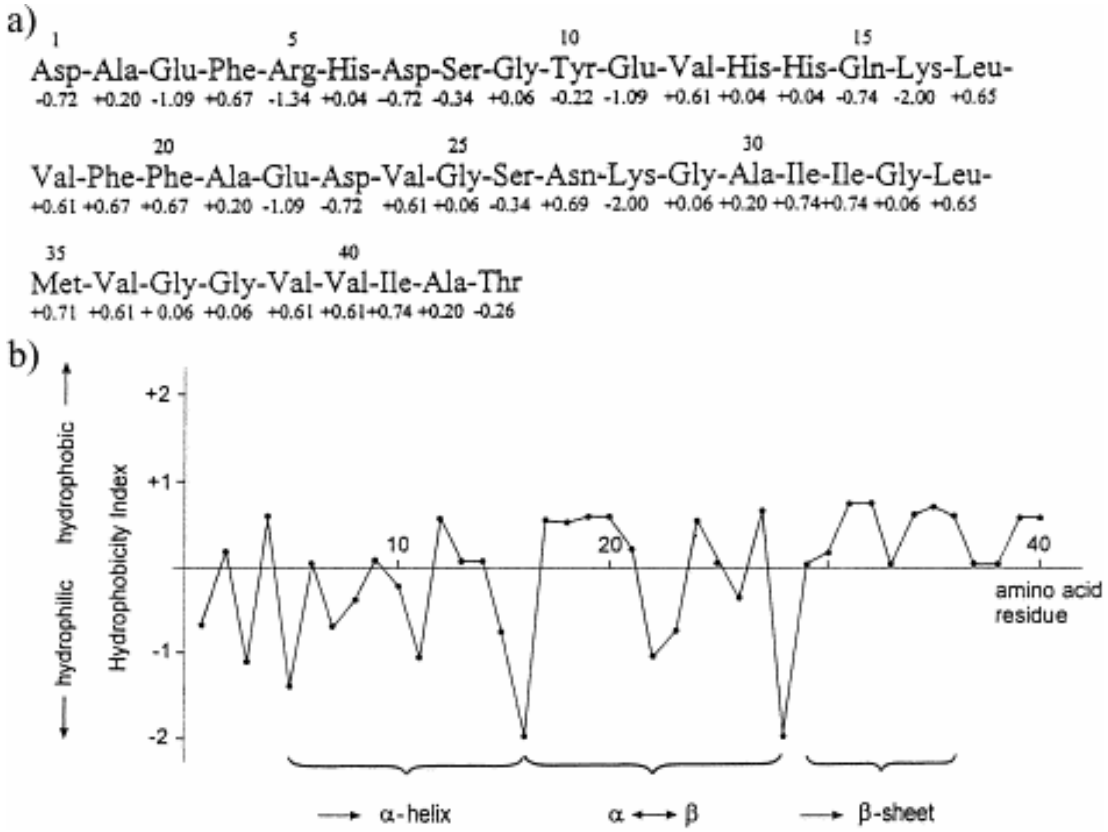


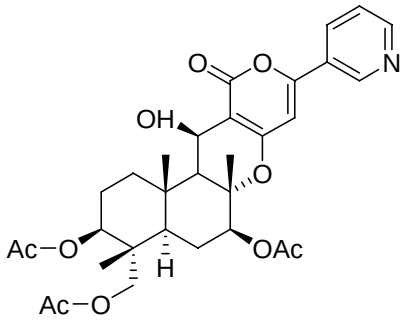
Figure 2.5 The hydrophobicity indexes of amyloid (1-43) sequence (This picture is taken from the reference without permission).[13]

The Aβ oligomers is considered to be the most toxic form of oligomers[14], accumulate in process of cultured APP transgenic mouse (Tg2576), within the endosomal vesicles and along microtubles.[14-16] A recent report demonstrated that accumulation of Aβ in abnormal endosomes is because of increase in Aβ levels in AD.[17] The development of Aβ oligomeric forms in neurons are responsible for neuritic damage and synaptic alterations.[15, 16] It is also proven that intraneuronal Aβ accumulation occurs before the appearance of amyloid plaques.[15, 18-20] From this data it is evident that Aβ accumulation and oligomerization in the endosomal compartments is toxic to neurons. So compounds that are cell permeable are indispensable to target intraneuronal Aβ.[21]

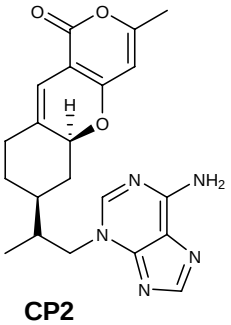
In the current study, we used MC65 cell lines as a convenient model for study of Aβ aggregation and toxicity and to screen bioactive compounds.[22] MC65 is human neuroblastoma cell line that conditionally express a partial APP fusion protein termed SβC (C99) and die on

third day after transgene induction by removal of tetracycline (TC) from the media.[23, 24] Later C99 is cleaved by γ-secretase to produce Aβ.[15] Moreover MC65 cell toxicity is easily measured by simple MTT assay.[15]

It is been proven that several tricyclic pyrone (TP) compounds protects MC65 cells from death.[25] TP compounds are structurally similar to pyripyropene A[26] and arisugacin[27], a cholesterol O-acyl-transferase inhibitor and a acetylcholineesterase inhibitor respectively. Using MC65 cell we screened many compounds, we selected a small molecule CP2 that protects MC65 cells from death. CP2 was found to be lipophilic and cell permeable and block Aβ aggregation intracellularly.[15]



Pyripyropene A **2.1**



CP2 **2.2**

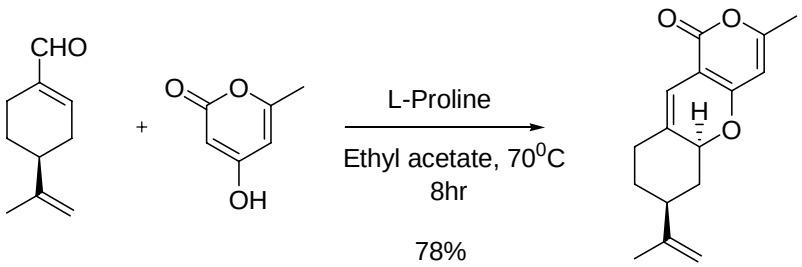
2.3 Synthesis of TP compounds

2.3.1 Synthesis of CP2

All the TP compounds are structurally similar to ACAT inhibitor, pyripyropene A **2.1**. Several compounds were synthesized containing various functionalities.[24] The key intermediate **2.3** was synthesized by Dr. Yi Chen, a previous researcher in our group.[28] Several analogues were designed by functionalizing at C3 and C7 positions. Dr. Yi Chen synthesized a TP compound via condensation. A cyclic enal and a pyrone were condensed in presence of L-proline. The condensation reaction afforded a tricyclic compound. Continuous efforts to synthesize an optically active pyrone were finally successful. Dr. Chen chose an asymmetric molecule (*S*)-(-)-perillaldehyde to induce chirality in the tricyclic compounds. This aldehyde has an isopropylene group which is believed to induce stereochemistry in the TP product. Treatment of pyrone **2.5** with (*S*)-(-)-perillaldehyde **2.4** with L-proline in ethyl acetate at 70 ^{0}C gave desired TP

compound in 78% yield. The structure was determined by single X-ray crystallographic analysis.[28, 29] Surprisingly only one diastereomer was obtained (Scheme 2.1). Compound **2.3** was purified via recrystallization using ethyl acetate as a solvent.

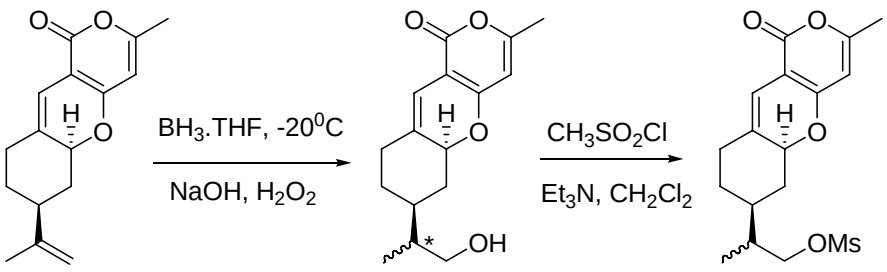
Scheme 2.1



2.4 **2.5** **2.3**

Hydroxylation was carried out using 0.3 equiv. of BH_3.THF followed by oxidation with hydrogen peroxide and hydrolysis of resultant borate with sodium hydroxide gave an inseparable mixture of two diastereomers alcohol **2.6** in 1:1 ratio. Hydroboration has to be performed at -20 ^{0}C to selectively hydroborate the terminal double bond (Scheme 2.2). The presence of two isomers was confirmed by taking ^{13}C NMR spectrum. This mixture was not separable by silica gel column chromatography. The mixture of alcohols **2.6** were mesylated. Treating compound **2.6** with methanesulfonyl chloride in the presence of triethylamine afforded a mesylated product **2.7** in 94% yield (Scheme 2.2).

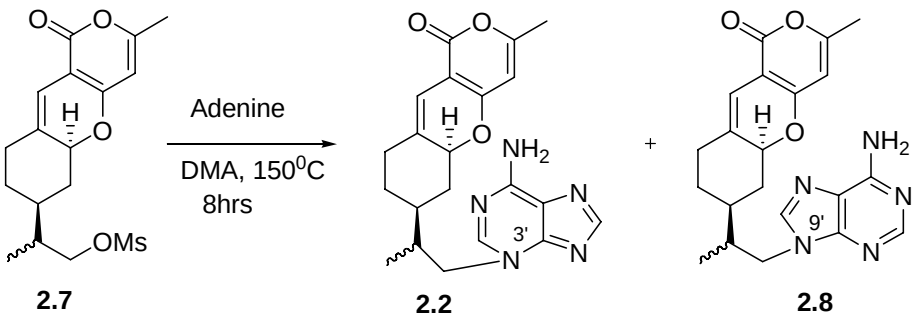
Scheme 2.2



2.3 **2.6** **2.7**

Mesylated product was heated with a highly polar compound adenine in the presence of DMA. Moreover adenine was known to be an active motif in some HIV agents. Mesylate **2.7** was heated with dimethyl acetamide (DMA) and 1 equiv. of adenine to 150 ^{0}C for 7 hrs. DMA in the reaction mixture was distilled off. Thus obtained brown residue was directly added to the silica gel column chromatography without any aqueous workup. Silica gel column chromatography yielded two compounds **2.2** and **2.8** in 10:1 ratio (Scheme 2.3). The compounds were characterized using ^{1}H NMR and ^{13}C NMR spectroscopy.[24]

Scheme 2.3

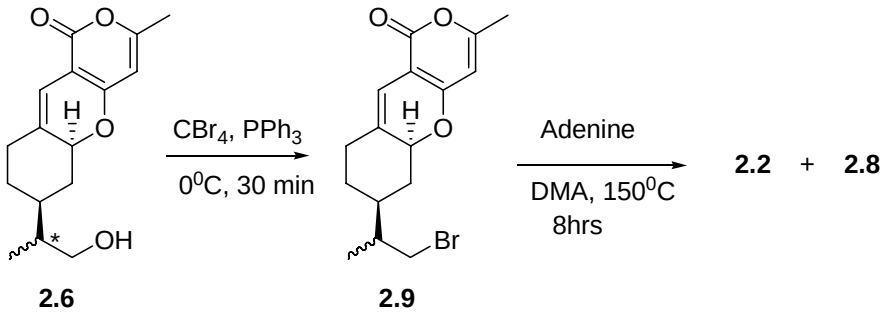


2.7 **2.2** **2.8**

Silica gel column chromatographic separation did not yield pure CP2. We purified by using HPLC (Jupiter C18, 10-μ column (Phenomenex, Torrance, CA, USA)). The running solvent system was water and acetonitrile with 0.1% trifluoroacetic acid. After several attempts using different programs we finally able to separate the N-9' analogue and CP2 in very pure form. We obtained CP2 as a trifluoracetic acid salt. This was later proved by ^{13}C NMR and mass spectroscopy. We are unsure that how many trifluoro acetic acid molecules are adhering to one each molecule of CP2. Recently CP2 was structurally defined by using HMBC and HMQC experiments (unpublished results).

Mixture of alcohols **2.6** were brominated using carbon tetrabromide and triphenyl phosphine to obtain bromide **2.9**. Bromide was stable on column and was purified by silica gel column chromatography (Scheme 2.4). Bromide **2.9** was heated to 150 ^{0}C in the presence of adenine and DMA. We obtained both N-3'and N-9' analogues.

Scheme 2.4



2.6 **2.9**

Following the same procedure radio labeled CP2 was prepared from ^{14}C- labeled adenine (was bought from Amersham Pharmacia Biotech, Piscataway, NJ, USA). We took 50 mg of non labeled adenine and dissolved in 10 mL ethanol and 10 mL double distilled water and heated on a hot plate. To the above solution 1 mL of labeled adenine (approximately 0.024 mg of ^{14}C adenine) was added. This mixture was cooled to -78 ^{0}C and lyophilized. Thus obtained adenine was used to prepare radio labeled CP2 by similar method followed in scheme 2.4.

2.3.2 Synthesis of CP2 analogues

There are a number of sites in CP2 where modification can be done to increase the activity, increase the binding ability. Introduction of a lipophilic group at C-11 will allow the molecule to penetrate into blood brain barrier. The following figure shows the possible sites where a CP2 molecule can be modified. Modification should be done before introducing adenine group since the purification process after addition is a bit tedious. Different paths are designed starting from the vital intermediate compound **2.3** to construct different analogues. Functional group modification can be made at sites shown in figure 2.6. There are a number of synthetic analogues made by Dr. Chen[28] and are tested. We synthesized different analogues and were tested for inhibition of Aβ aggregation.

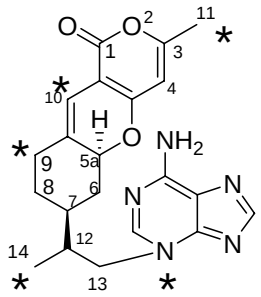
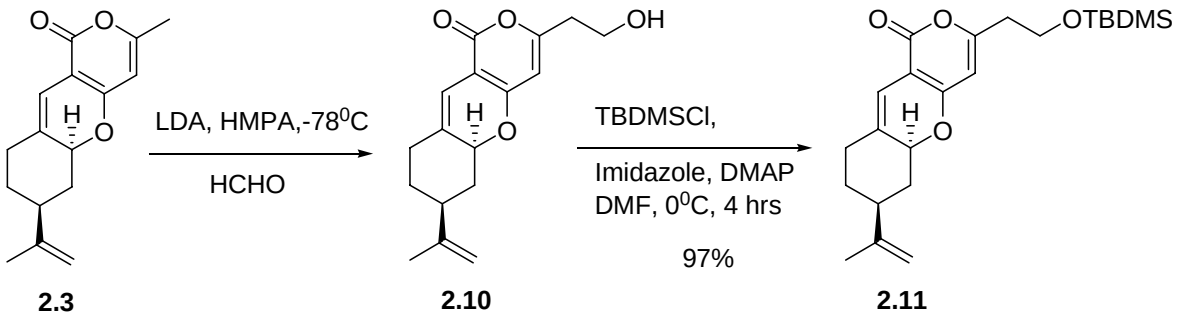


Figure 2.6 The symbol * indicates possible sites where CP2 can be modified.

First in the series was adding an extra carbon atom at C-11. Treatment of compound **2.3** with LDA at -78 ^{0}C yields a blue anionic species. Selective deprotonation of the C11 methyl group was done using LDA or *n*-BuLi and a subsequent addition of formaldehyde gave 25% (based on reacted **2.3**) of alcohol **2.10**. Anion generated at C11 will attack the aldehyde giving an alcohol **2.10**. The low yield of the reaction can be attributed to many reasons. The primary reason is the insolubility of formaldehyde in any solvent. Efforts were made to increase the yield of the reaction but we weren't successful. Formaldehyde was dried over P_2O_5 under vacuum before using. During the reaction, appearance of blue color solution confirms the formation of anion. HMPA was added to stabilize the anion. Anion was made to cannula to a formaldehyde-THF solution and allowed to warm to room temperature. Aqueous workup and silica gel column chromatography yielded an alcohol **2.10** in 25% yield and a small amount of starting material compound **2.3** was recovered (Scheme 2.5). Alcohol is not stable at room temperature and soon decomposes to an unknown compound. Next step is carried out quickly without any further delay. Alcohol **2.10** was silylated using *tert*-butyldimethylsilyl chloride using imidazole as a base and DMAP as a catalyst to obtain a silylated product **2.11** in 97% yield (Scheme 2.5).

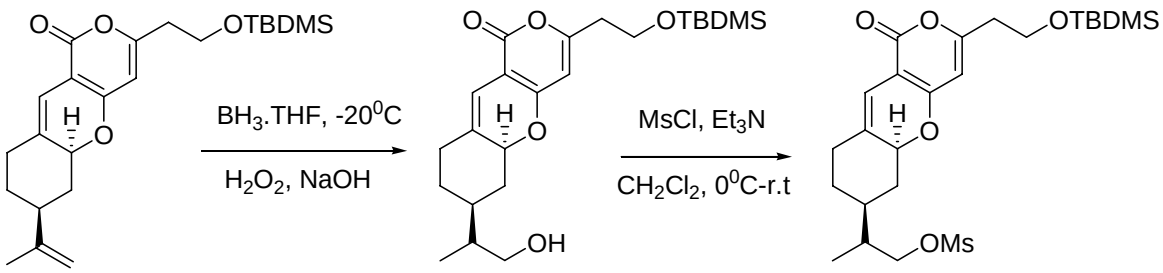
Scheme 2.5



2.3 **2.10** **2.11**

Silylated product was selectively hydroxylated using BH_3.THF at -20 ^{0}C followed by oxidation with hydrogen peroxide and hydrolysis of resultant borate with sodium hydroxide gave an inseparable mixture of two diastereomers alcohol **2.12** in 51% yield with recovery of some starting material **2.11**. TBDMS protecting group was stable to these conditions. At this juncture we did not convert alcohol to bromide using carbon tetrabromide as this reaction releases acid HBr which will deprotect the TBDMS group. Alcohol was converted to a mesylate **2.13** using methanesulfonyl chloride and triethylamine as a base at 0 ^{0}C to give 98% yield (Scheme 2.6). Mesylate **2.13** was heated with adenine to 150 ^{0}C in presence of DMA. Desired product with an adenine substitution was expected. On silica gel column chromatography we did not obtain the desired product. We concluded that silyl protecting group is not an efficient protecting group and changed to the protecting group to acetate.

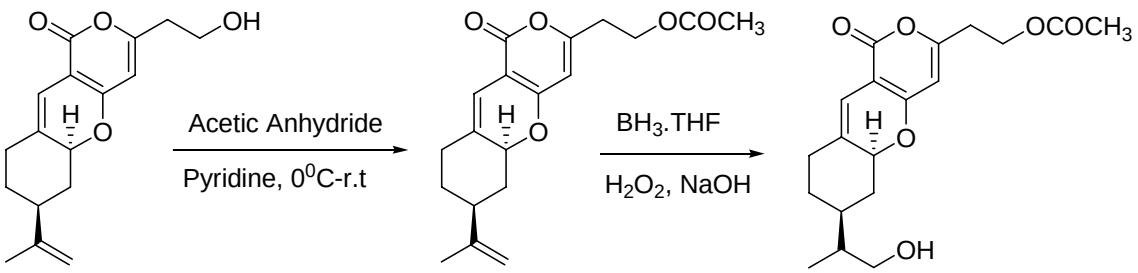
Scheme 2.6



2.11 **2.12** **2.13**

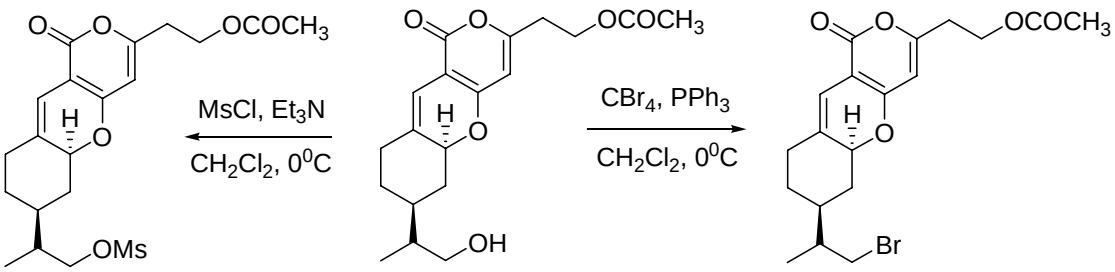
We protected alcohol **2.10** with acetate protecting group. Treatment of alcohol **2.10** with acetic anhydride and pyridine as a base afforded to give compound **2.14** in quantitative yield. Selective hydroxylation using BH_3.THF at -20 ^{0}C followed by oxidation with hydrogen peroxide and hydrolysis of resultant borate with sodium hydroxide gave an inseparable mixture of two diastereomers alcohol **2.15** with some starting material **2.14** recovery. Quick work up procedure was recommended. Sodium hydroxide (5% M) is recommended to keep the acetate group intact (Scheme 2.7). Alcohol **2.15** is converted both to mesylate **2.17** using methanesulfonyl chloride and bromide **2.16** using carbon tetrabromide (Scheme 2.8). Mesylated product **2.17** was proven to give best results on addition of adenine in the final step.

Scheme 2.7



2.10 **2.14** **2.15**

Scheme 2.8

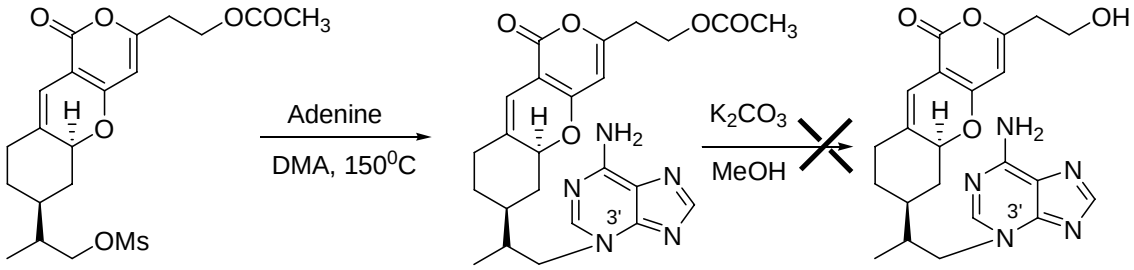


2.17 **2.15** **2.16**

Mesylate **2.17** was heated to 150 ^{0}C with adenine and DMA for 7 hours. The reaction mixture was subjected to vacuum distillation to remove DMA. Resultant brown residue was

added to silica gel column chromatography for purification but obtained an impure product **2.18** (Scheme 2.9). Compound **2.18** was purified by HPLC and the running solvent system was water and acetonitrile with 0.1% trifluoroacetic acid. Compound **2.18** was exhibiting similar activity when compared with CP2 and was also proven to inhibit the Aβ aggregation. Based on molecular modeling studies we concluded that the alcohol **2.19** will enhance the activity. Compound **2.18** was treated with potassium carbonate and methanol to cleave the acetate group. We were not able to deprotect the acetate group instead we obtained some unidentified compounds.

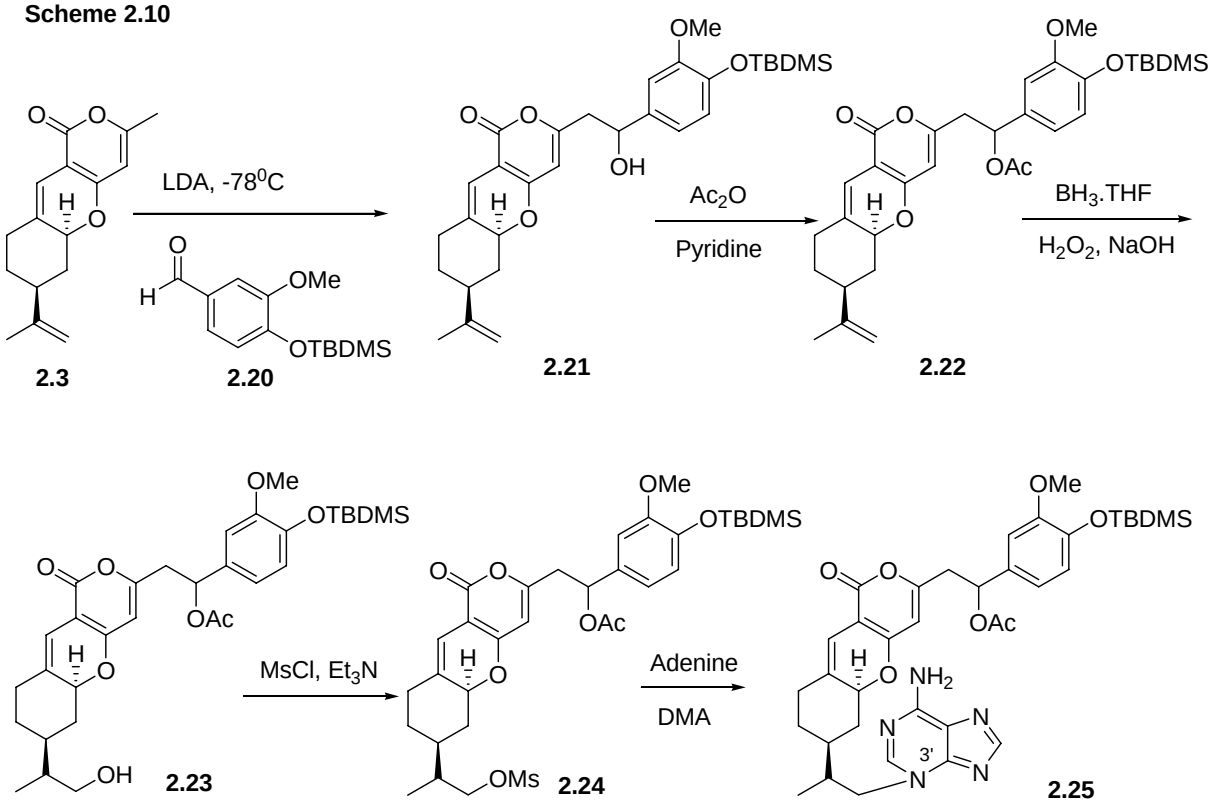
Scheme 2.9



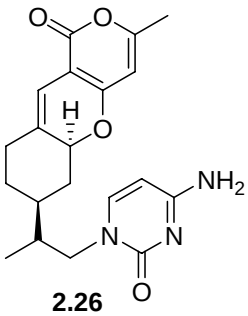
2.17 **2.18** **2.19**

Another analogue was made following the same procedure. Compound **2.3** was treated with LDA to deprotonate C11 methyl group. Hence, treatment of **2.3** with LDA followed by a substituted benzaldehyde **2.20** gave alcohol **2.21. A**lcohol was protected with acetate to give compound **2.22**. Selective hydroxylation using BH_3.THF at -20 ^{0}C followed by oxidation with hydrogen peroxide and hydrolysis of resultant borate with sodium hydroxide gave an inseparable mixture of two diastereomers alcohol **2.23**. Alcohol **2.23** was mesylated using methanesulfonyl chloride. Mesylated product **2.24** was heated with adenine and DMA at 150 ^{0}C. Silica gel column chromatography resulted in impure **2.25** (Scheme 2.10). Further purification was carried out using HPLC but obtained very less compound and couldn't analyze this further for structural characterization.

Scheme 2.10

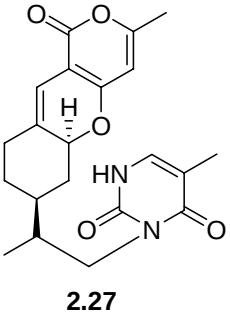


Apart from adenine the other nucleic acid bases are guanine, thymine and cytosine. The intermediate bromide **2.9** was used to prepare CP2 on treatment with adenine. Following the same procedure, bromide **2.9** was treated with guanine, thymine and cytosine in presence of NaH and DMF as a solvent. The resulting compounds **2.26**, **2.27**, **2.28** and **2.29** were purified by column chromatography and HPLC.



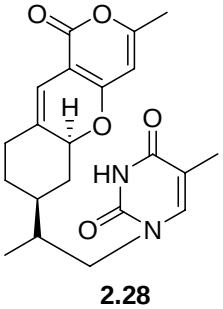
2.26

with Cytosine

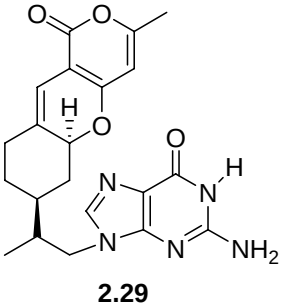


2.27

with Thymine



2.28



2.29

With Guanine

2.4 Results and Discussion

2.4.1 The death of MC65 cells depends on the production of Aβ

MC65 cells conditionally express a partial APP fusion protein called SβC and die on the third day after transgene induction by removal of tetracycline (TC) from the media.[22, 23, 25, 30] The death of MC65 results from the production of C99 and its derivatives. C99 is first expressed followed by subsequent appearance of other Aβ containing fragments of which 8KDa, 16.5KDa and 25KDa were the major species (Figure 2.7) (This work was done in Lee-Way Jin's Laboratory, University of California, Davis).[15, 23]

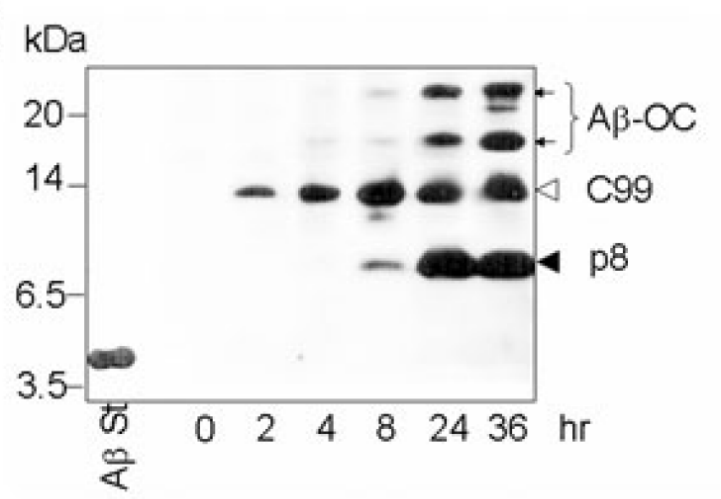


Figure 2.7 C99 and other fragments induced by removal of TC[15]

Pulse chase experiments failed to prove that CP2 did not affect the rate of C99 production. Then Aβ levels were measured in the conditioned media from cells (for 48 hrs) treated with drugs. Neither CP2 (10 μM) nor TP17 (10 μM) significantly altered the level of Aβ in conditioned media. By contrast γ-secretase inhibitor L-685,458 (2 μM) significantly reduced the amount of Aβ secreted in both MC65 cells (Figure 2.8)[15] (This work was carried out by Lee-Way Jin Laboratory, University of California, Davis) and N2a-APPsw (Figure 2.9)[15] (This work was done in Lee-Way Jin's Laboratory, University of California, Davis) cells. Collectively this illustrates that CP2 does not affect Aβ production or degradation effectively.

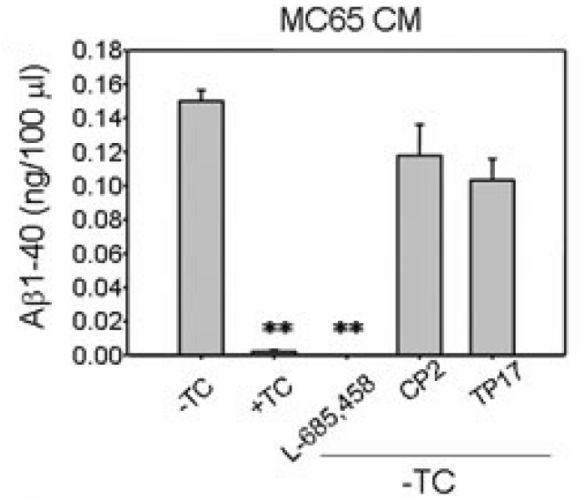


Figure 2.8 MC65 cells with TC and without TC. CP2 and TP17 compounds were also tested[15]

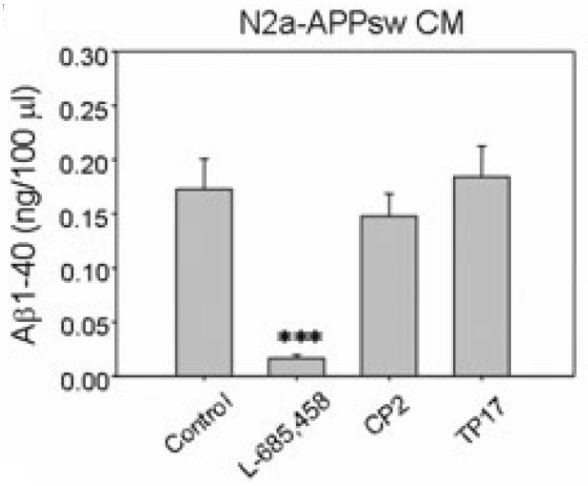


Figure 2.9 N2a-APPsw cells compared with control[15]

Treatments with L-685,458 blocked the death of TC-MC65 cells in a dose dependant manner, with an EC_{50} of ~110nm (Figure 2.10) (This work was done in Lee-Way Jin's Laboratory, University of California, Davis) which clearly indicates that the generation of Aβ is necessary for toxicity.[15] At the rescuing concentrations, L-685,458 significantly reduced or completely blocked the 16.5 kDa and 25 kDa in a dose dependant fashion (Figure 2.11)[15] (This work was done in Lee-Way Jin's Laboratory, University of California, Davis) clearly indicating that these bands are nothing but homo- and hetero oligomeric complexes of Aβ.[15] This is also

proven by quantification of these bands by measuring the optical density of the bands by NIH image.

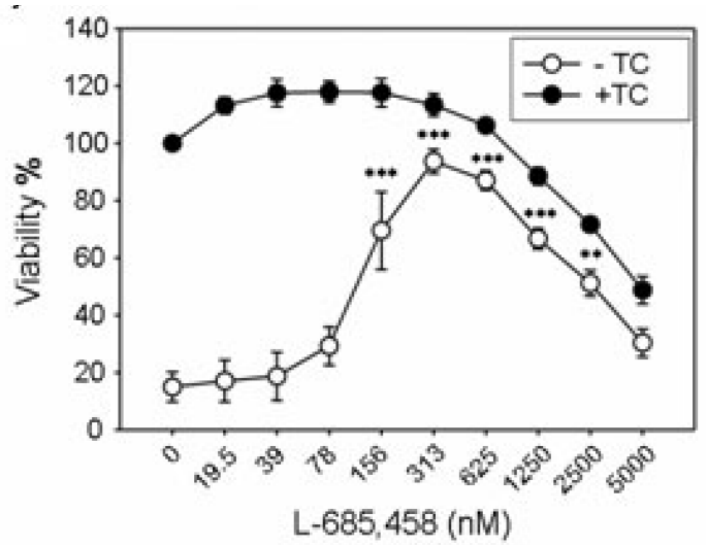


Figure 2.10 Cell viability was assessed after 72 hrs of removal of TC in L-685,458 at different concentrations[15]

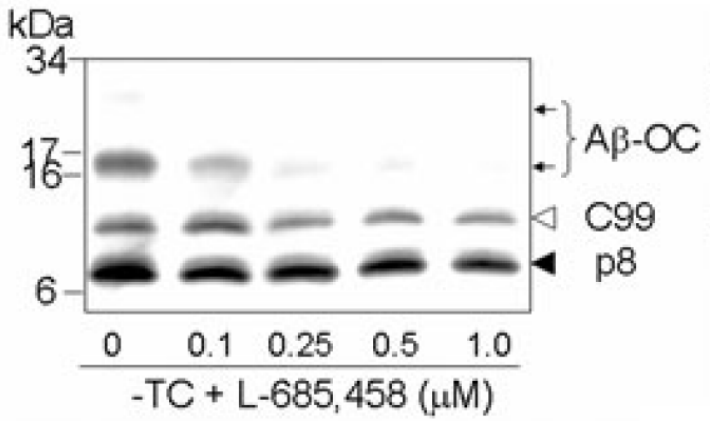


Figure 2.11 Different concentrations of L-685,458 was added with TC removal, analyzed by western blot using 6E10[15]

ELISA assays failed to detect Aβ in untreated cell homogenates, which clearly detected monomeric Aβ40 and trace amount of Aβ42 after formic acid extraction (Figure 2.12) (This work was done in Lee-Way Jin's Laboratory, University of California, Davis).[15] It was believed that during the process of homogenization Aβ oligomeric complexes (Aβ-OCs) were formed. To test this possibility MC65 cells were homogenized in presence of a dye, congo red. Thus it was proven that during the process of homogenization Aβ quickly aggregated into Aβ-OCs (Figure 2.13) (This work was carried out by Lee-Way Jin Laboratory, University of California, Davis).[15]

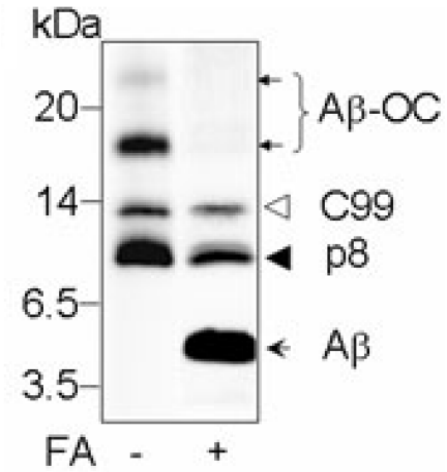


Figure 2.12 MC65 cells were homogenized with and without formic acid extraction[15]

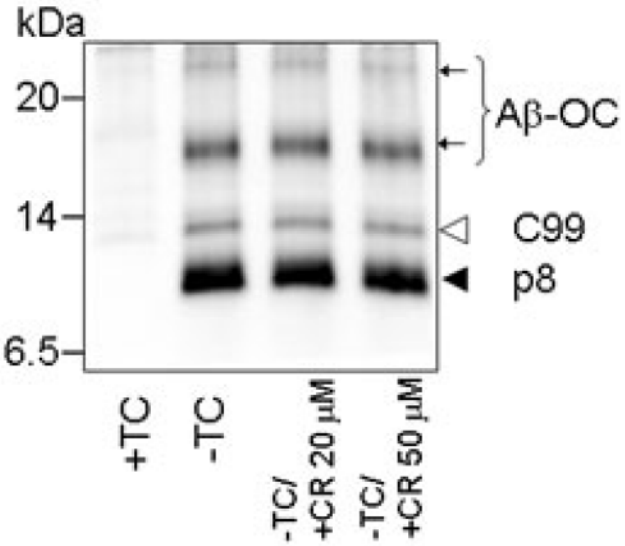


Figure 2.13 MC65 cells were homogenized in presence of Congo red and analyzed by western blot[15]

Woltjer and coworkers proved that 8-kDa band mainly comprises of Aβ dimers.[23] From the above experiments it is proven that 8-kDa band was not completely reduced either by L-685,458 treatment or formic acid disaggregation, proves that this band is not fully composed of Aβ peptides.[15] This band was not also recognized by antibody B994 (Figure 2.14) (This work was done in Lee-Way Jin's Laboratory, University of California, Davis).[15] So the composition of this band is not known, most likely this may be combination of Aβ dimers and COOH-terminal membrane fragment (CTF) Δ31, a caspase cleavage product of C99. When the proteins of TC-MC65 cells were separated by tricine/SDS PAGE, C99 was recognized both by 6E10 and B994 antibodies (Figure 2.14). Band C83, α-secretase cleavage product of C99 was not recognized by 6E10. Surprisingly L-685,458 treatment increased the level of C83.[15]

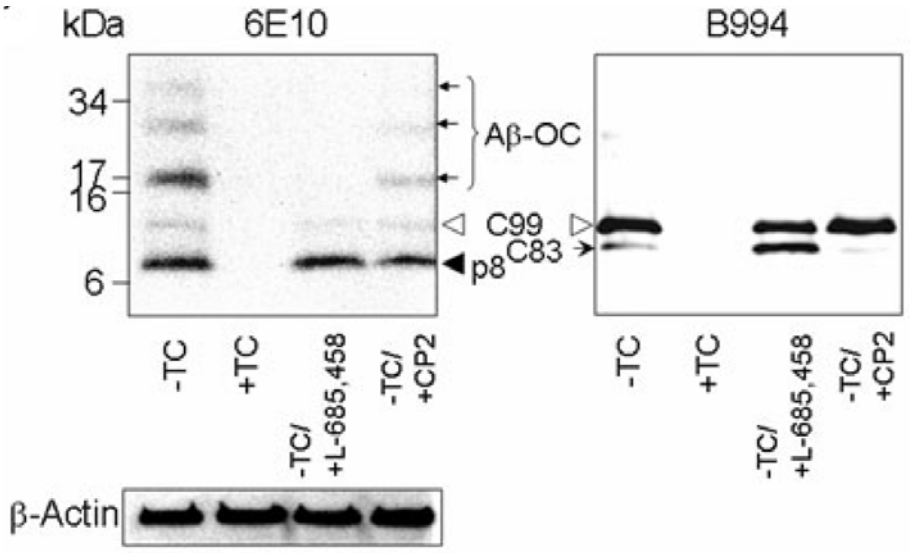
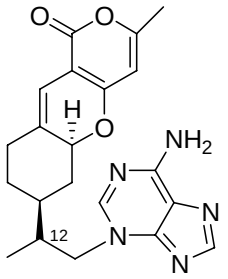


Figure 2.14 Comparison of cellular proteins. Analyzed with 6E10 and B994 antibodies[15]

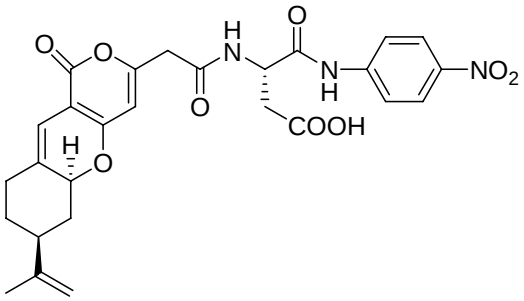
2.4.2 TP analogues protect MC65 cells in a structure dependent manner and CP2's protective effect is intimately related to its inhibition of Aβ-OCs

TP analogues protected MC65 cells with various potencies.[24, 25] TPs were synthesized based on the structures of pyripyropene A, apotent acyl-CoA: cholesterol O-acyltransferase (ACAT) inhibitor and arisugasin, a potent acetylcholineesterase inhibitior.[29] Both of them affect Aβ production and aggregation.[31-33] Surprisingly TPs protect MC65 cells at concentrations 1000

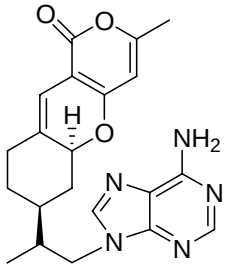
fold lower than those required for ACAT and acetylcholineesterase inhibition. However we found out that CP2 **2.2** was selected for further studies due to its high potency and low cellular toxicity. This CP2 is readily synthesized in 4 steps. CP2 protects MC65 cells at nanomolar concentrations (EC_{50} ≈0.15 μM). This CP2 contains both isomers 12-R and 12-S. They are unseparable even by HPLC. N9' analogue of CP2 **2.31** was less active (EC_{50} ≈3.0 μM) compared to CP2. The other isomer N10'-analogue **2.32** was not active at all. TP compound **2.33** was also inactive. CP1 also protected MC65 cells but less effective (EC_{50} ≈2.6 μM) than CP2. A graphical representation of MC65 cell survival over different concentrations of different TP compounds is shown in figure 2.15 (This work was done in Lee-Way Jin's Laboratory, University of California, Davis).[15]



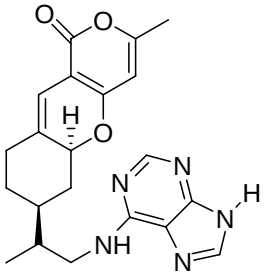
C12R - CP2
C12S - CP2 **2.2**



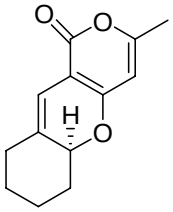
CP1 **2.30**



N9'-analog **2.31**



N10'-analog **2.32**



TP17 **2.33**

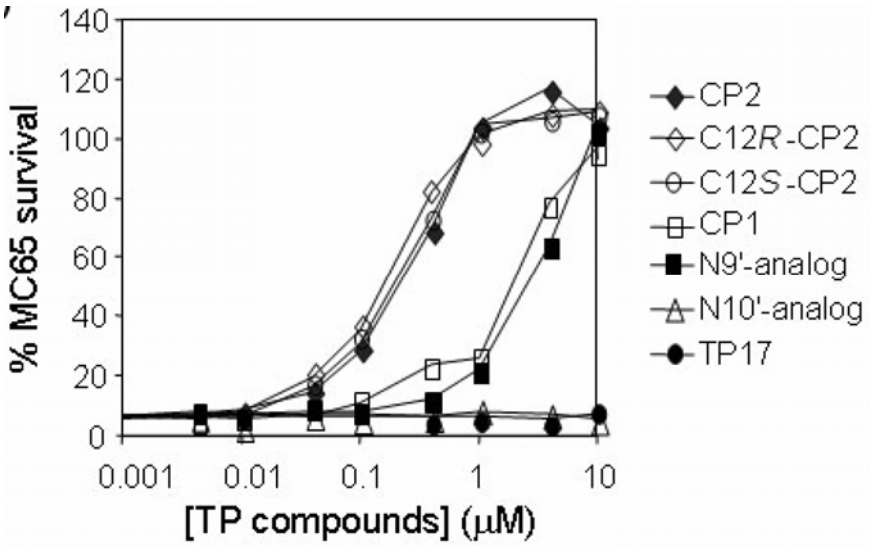


Figure 2.15 MC65 protection assay with different concentrations of different TP analogues[15]

We compared the effect of CP2, N9'-analogue and TP17 analogue on the accumulation of intracellular Aβ-OCs. Western blots demonstrated that only CP2 selectively reduces the levels of cellular Aβ-OCs (in a dose dependant manner) (Figure 2.17) (This work was done in Lee-Way Jin's Laboratory, University of California, Davis) not N9' analogue or TP17 (Figure 2.16). None of the drugs affected the levels of C99 and C83 (Figure 2.16 right panel). The western blots in figure 2.16 (This work was carried out by Lee-Way Jin Laboratory, University of California, Davis) demonstrated that 16.5 and 25 kDa bands were reduced when treated with 2 μM of CP2. "There was a one third reduction in p8 band similar to the effect of L-658,458 and formic acid disaggregation".[15] "This implies that CP2 treatment also resulted in diaggregation of Aβ isomers".[15] Figure 2.14 shows a comparison between the effect of CP2 and L-685,458. Since the effect of CP2 was not through inhibition of γ-sectretase as of L-685,458, so CP2 might interfere with the formation of Aβ-OCs.

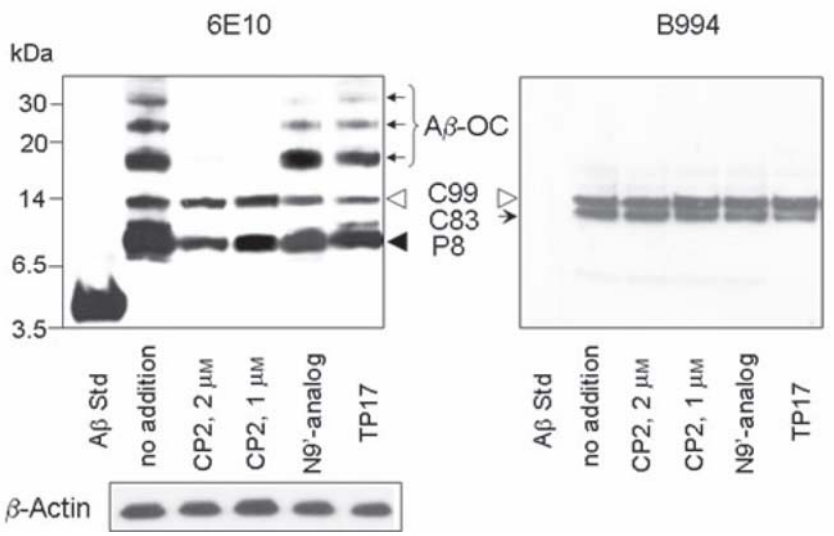


Figure 2.16 MC65 cells treated with different TP analogues. Western blots were analyzed with 6E10 and B994 antibodies.[15]

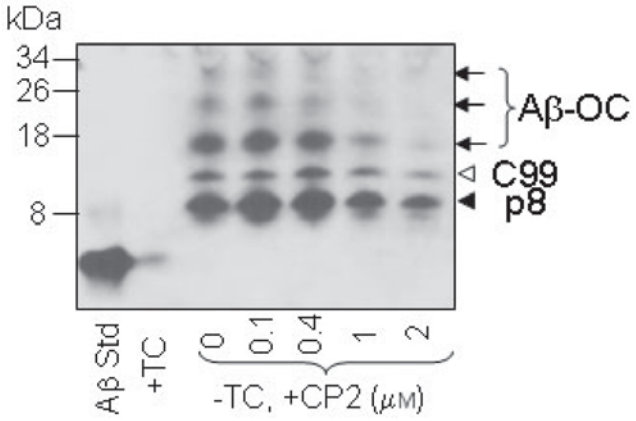


Figure 2.17 MC65 cells were treated with different concentrations of CP2. Western blot was analyzed using 6E10 antibody[15]

Parallel immunocytochemical studies revealed that CP2 reduced the level of cytoplasmic Aβ-immunoreacive deposits whereas N9' analogue and TP17 didn't. There was no visible immunoreactivity seen in +TC cells. Twenty four hours after TC removal the cells showed cytoplasmic Aβ-immunoreacive deposits (Figure 2.18) (This work was done in Lee-Way Jin's Laboratory, University of California, Davis).[15] The experiment was followed by confocal microscopy. This confirms that CP2 prevents the formation of intracellular Aβ aggregates. It also suggests that Aβ-OCs, demonstrated by Western blots (Figure 2.17) may be the major constituents of the cytoplasmic deposits.[15]

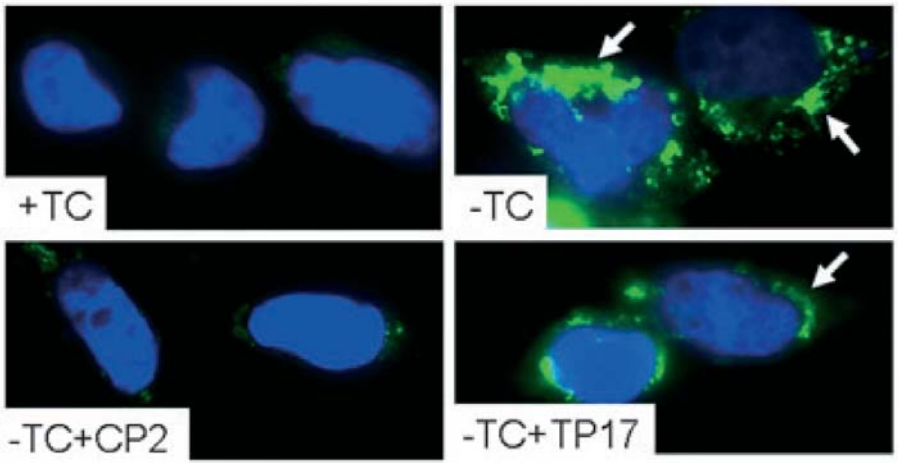


Figure 2.18 MC65 cells were treated with TP17 and CP2 (2 μM). Arrows indicate the deposition of Aβ-immunoreactive coarses.[15]

2.4.3 CP2 directly binds to Aβ and inhibits Aβ fibrilization

The above results strongly suggest that one of the cellular targets of CP2, either directly or indirectly, is the intracellular Aβ containing aggregates and aggregation process. To determine the lipophilicity of CP2, we measured the octonol/water partition coefficient (P_{Oct}) of CP2. A favourable P_{Oct} value for membrane penetration is 10. The P_{Oct} value for CP2 was 159, indicating its lipophilicity. According to Lipinski's rule,[34] CP2 has one hydrogen bond donor, seven hydrogen hydrogen bond acceptors, a MW of 393 and a calculated Log P_{Oct} value of 2.2, so is predicted that CP2 has good adsorption and permeation properties. Therefore it is likely that CP2 may permeate the cells and interact with intracellular accumulated Aβ.[15] Also when MC65 cells were incubated with [^{14}C]CP2, there is an accumulation of radioactivity inside the cells (Figure 2.19) (This work was done in Lee-Way Jin's Laboratory, University of California, Davis).[15]

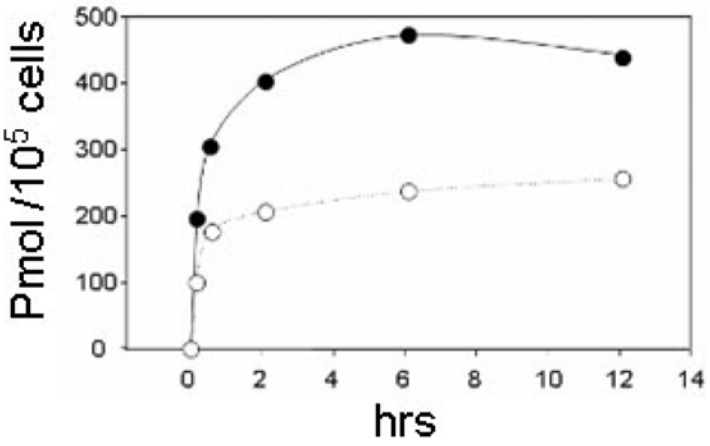


Figure 2.19 Accumulation of CP2 inside the MC65 cells. The empty circles represent the cell surface CP2 and the dark circles represent the intracellular CP2.[15]

Biacore's surface plasma resonance spectroscopy (SPR) was used to know if CP2 interacts with Aβ (this experiment was performed by Hui-Chuan Wu and Sandeep Rana). The binding of CP2 to Aβ1-40 and Aβ1-42 was clearly demonstrated, inspite of small responses due to small size of CP2. A real time biosensogram shown in figure 2.20 (This experiment was performed by Hui-Chuan Wu and Sandeep Rana) demonstrated that CP2 bound to the sensor CM5 chip immobilized with Aβ1-40, but not nonspecifically to the chip without immobilized Aβ1-40. "Approximately 1.5 equivalents of CP2, 0.46 equivalents of N9'-analogue, no TP17 were found to bind one equivalent of Aβ1-40 respectively. Similarly, 1 equivalent of of CP2, 0.2 equivalent of the N9'-analogue, but no TP17 were found to bind one equivalent of Aβ1-42 respectively".[15] These results demonstrate that the SPR binding studies of TP compounds appear parallel to their MC65 protection activities. The kinetic analysis of sensograms is shown in the table 2.2 (This work was done in Lee-Way Jin's Laboratory, University of California, Davis). CP2 shows high binding affinity to both Aβ40 and Aβ42 peptides. N9'-analogue shown an average binding and TP17 doesn't show any binding towards Aβ peptides.[15]

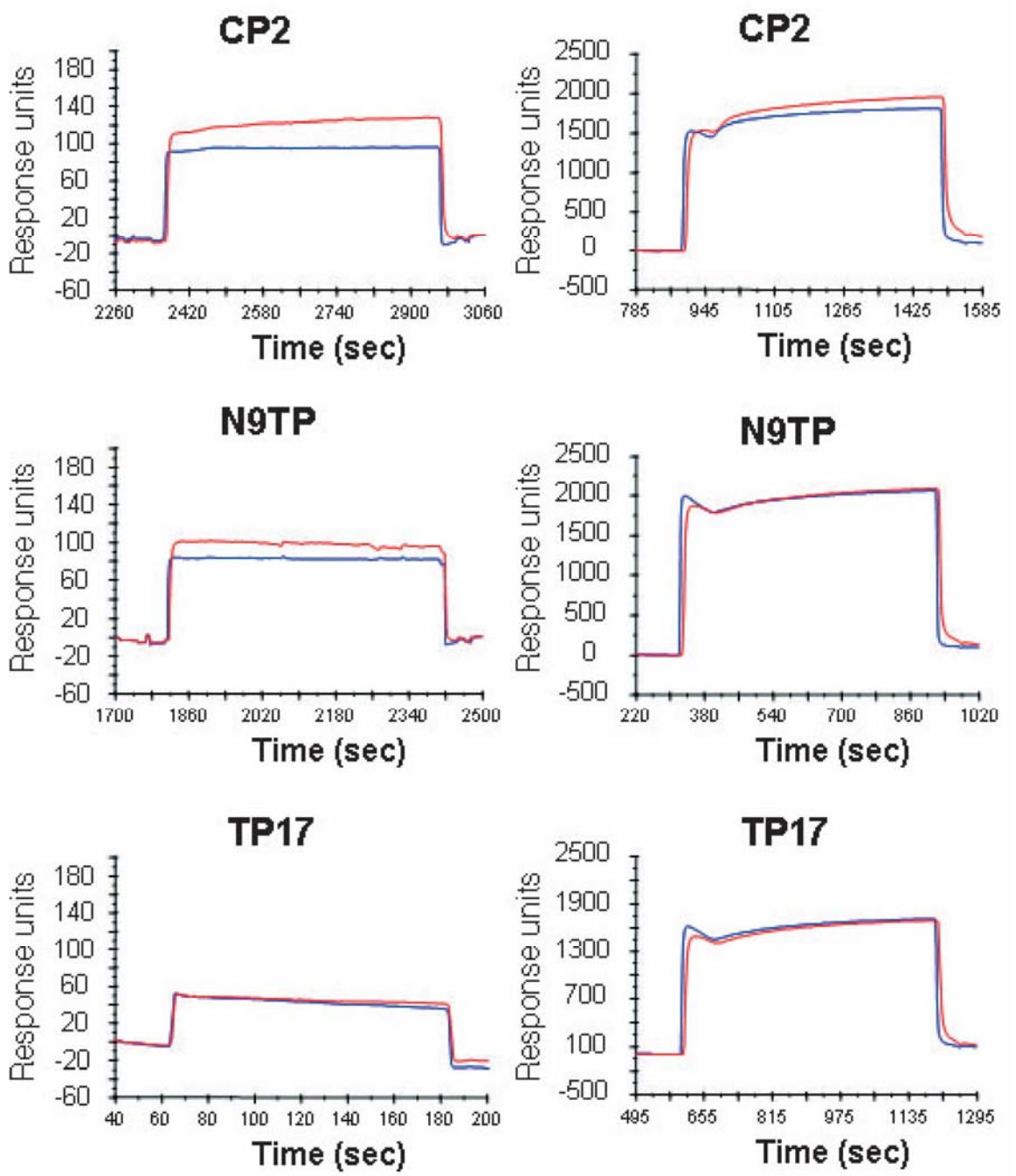


Figure 2.20 TP compounds binding to Aβ 1-40 (all left panels) and Aβ 1-42 (all right panels) by SPR. Blue line is the response from the reference cell and the red line is the response from the experimental cell.[15]

Analyte	Aβ40	Aβ42
CP2	5.05×10^{-9}	2.69×10^{-7}
N9 analog	9.96×10^{-9}	2.44×10^{-8}
TP17	NB	NB

Table 2.2 KD values for different TP compounds to Aβ peptides. NB = No binding[15]

2.4.4 Atomic Force Microscopic (AFM) studies showing CP2 inhibits the Aβ aggregation

Tapping mode AFM (Nanoscope IIIa SPM, Digital instruments Inc., SB, CA, USA) was used to obtain AFM images of the oligomerization and deoligomerization of Aβ peptides with CP2. Since Aβ40 aggregates at a slower rate than Aβ42, so Aβ40 was studied first. The Aβ40 peptide was dissolved in double distilled water, diluted with 50 mM phosphate buffer saline solution, pH 7.4, and with and without 25% 2,2,2-trifluoroethanol (TFE). Two separate set of experiments were performed with and without CP2. The prepared samples were centrifuged to remove any un-dissolved aggregates. The aliquot (50 μL) was taken and loaded on freshly cleaved mica. The samples are air dried and washed with double distilled water to remove any presence of salts. The samples were air dried again and AFM images were taken.

Atomic Force Microscopy (AFM) Studies of Aβ40 peptides

AFM images (All the AFM experiments were performed by Hui-Chuan Wu and Erik Pettersson) of Aβ40 peptides are shown in the following figures. Intially images were taken at different days without CP2 and TFE.

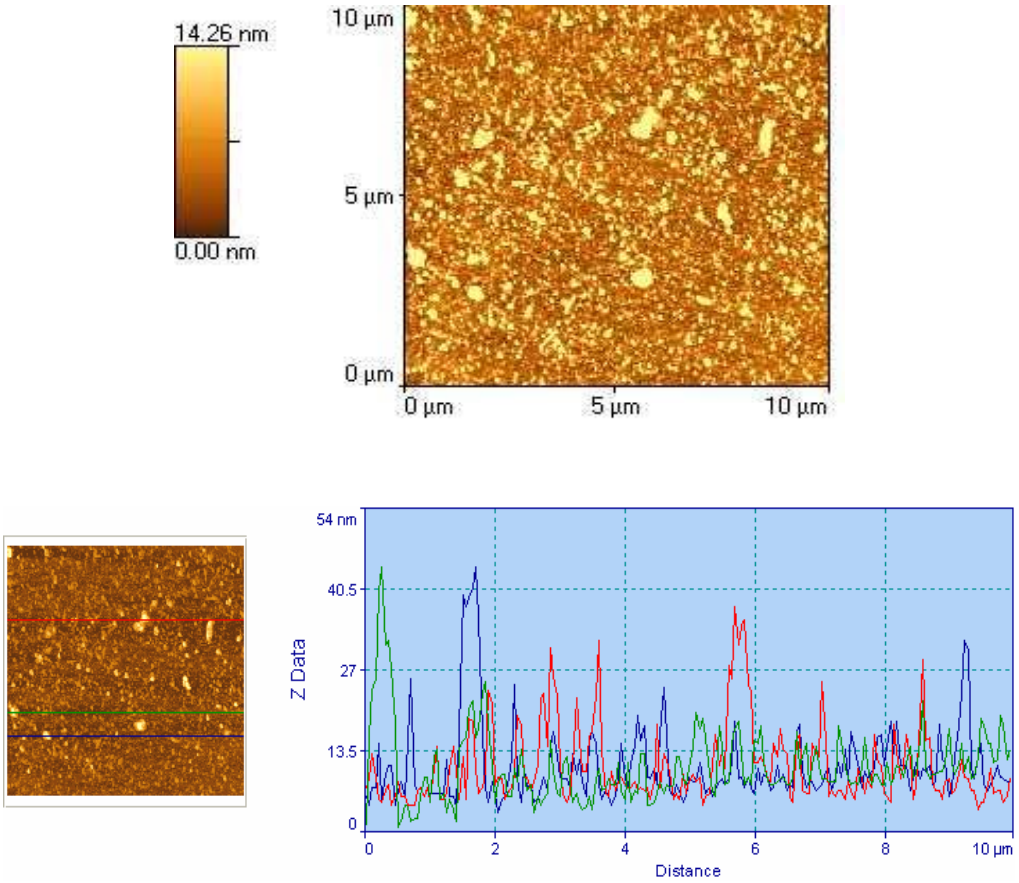
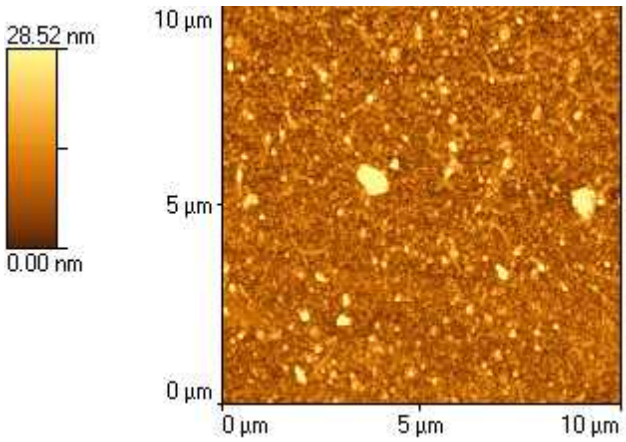


Figure 2.21 AFM image of Aβ40 in 50 mM phosphate buffer (no CP2) at day zero (upper panel). The heights and widths are shown in the bottom panel.



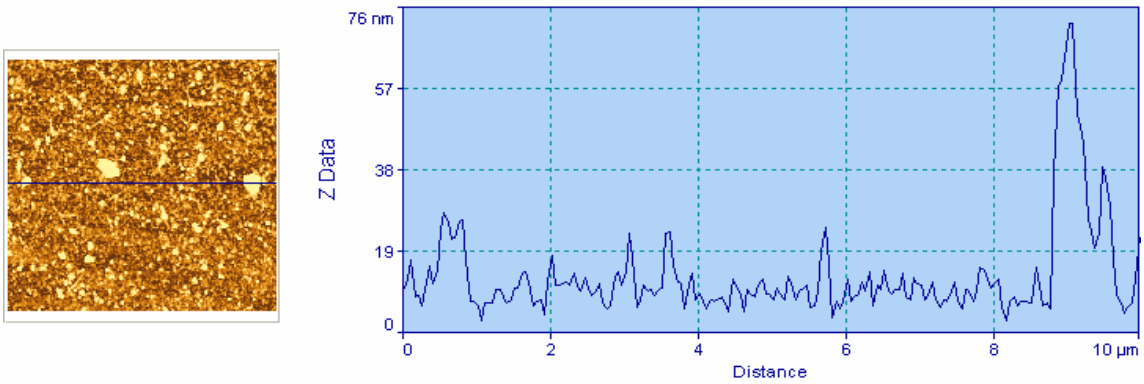


Figure 2.22 AFM image of Aβ40 in 50 mM phosphate buffer (no CP2) at day one (upper panel). The heights and widths are shown in the bottom panel (largest oligomer is 74 nm high and 400 nm wide).

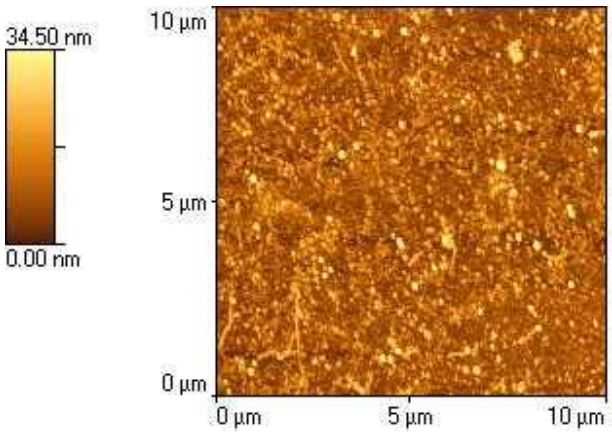


Figure 2.23 AFM image of Aβ40 in 50 mM phosphate buffer (no CP2) at day three. Protofibrils and fibrils are formed and the lengths are 1µM.

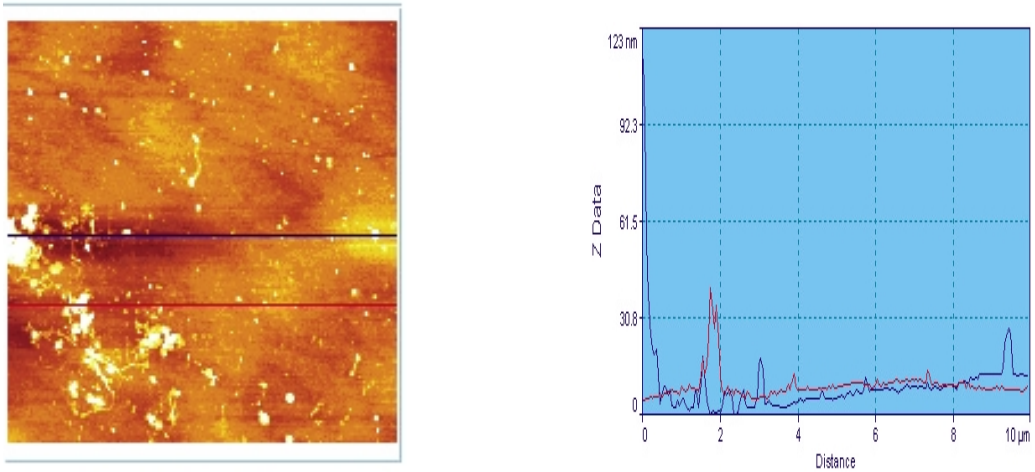


Figure 2.24 AFM image of Aβ40 in 50 mM phosphate buffer (no CP2) at day 50. The material inside the solution is mainly fibrils and proto-fibrils. The largest fibril is 125 nm high and 1 µM wide.

The following figures show AFM images, taken at different days with 25% TFE and without CP2.

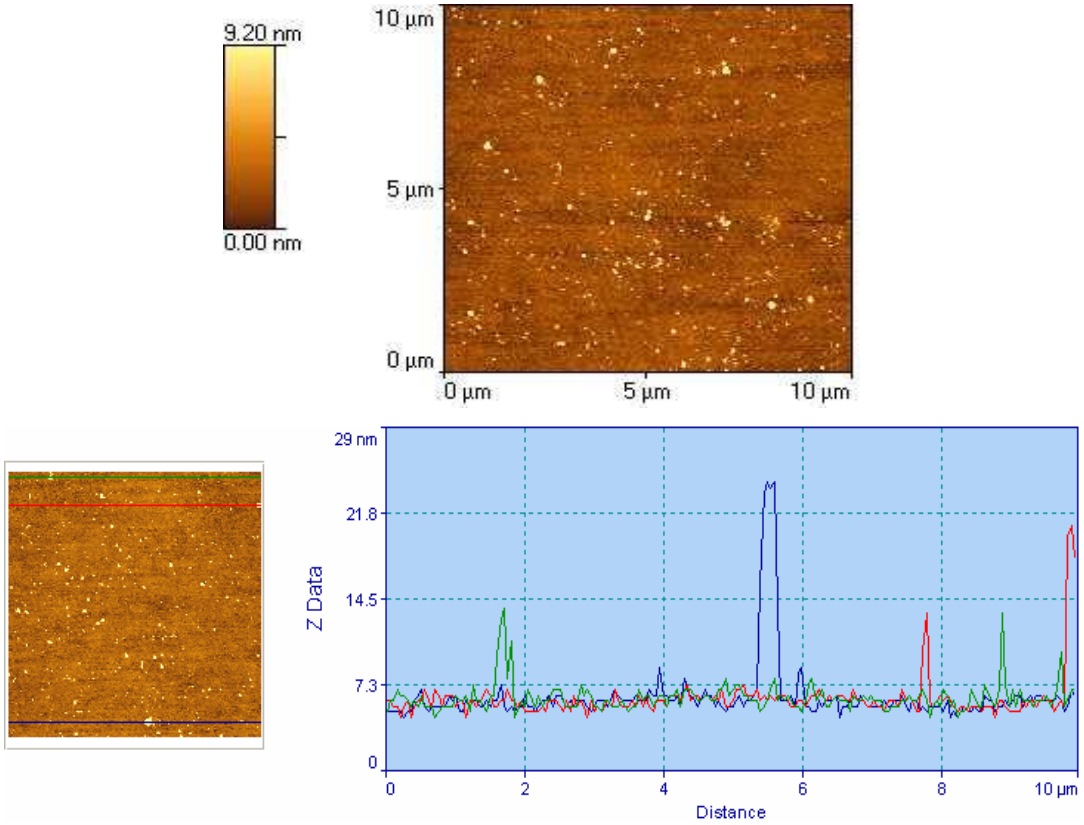


Figure 2.25 AFM image of Aβ40 in 50 mM phosphate buffer, 25% TFE (no CP2) at day zero (upper panel). The lower panel represents the size and width of the oligomers.

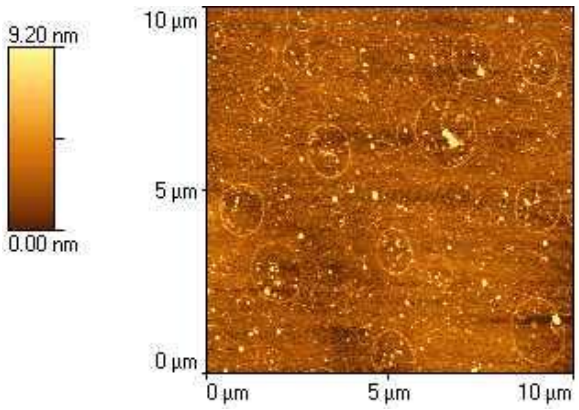


Figure 2.26 AFM image of Aβ40 in 50 mM phosphate buffer, 25% TFE (no CP2) at day one.

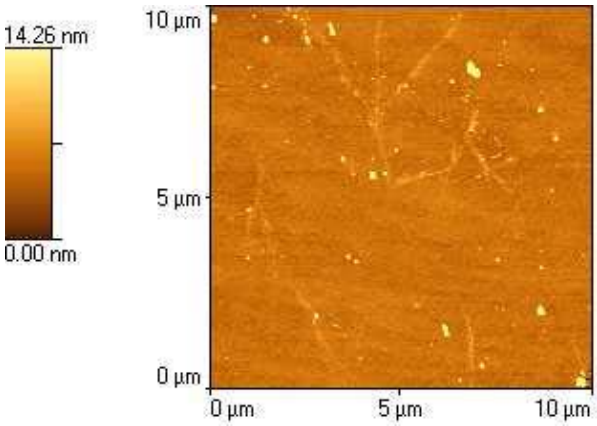


Figure 2.27 AFM image of Aβ40 in 50 mM phosphate buffer, 25% TFE (no CP2) at day three. Protofibrils and fibrils were formed.

The following figures show AFM images, taken at different days without TFE and with one equivalent of CP2.

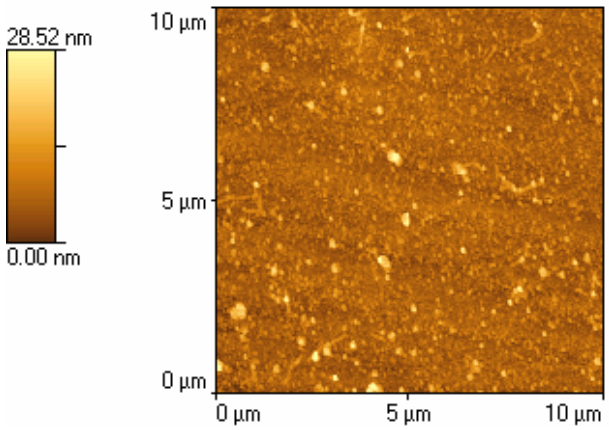


Figure 2.28 AFM image of Aβ40 in 50 mM phosphate buffer, in the presence of one equivalent of CP2 at day zero. The largest oligomer is about 30 nm high and 40 nm wide.

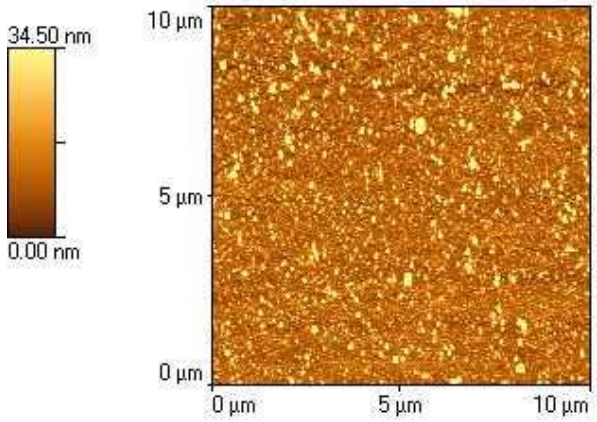


Figure 2.29 AFM image of Aβ40 in 50 mM phosphate buffer, in the presence of one equivalent of CP2 at day one. The largest oligomer is about 35 nm high and 40 nm wide.

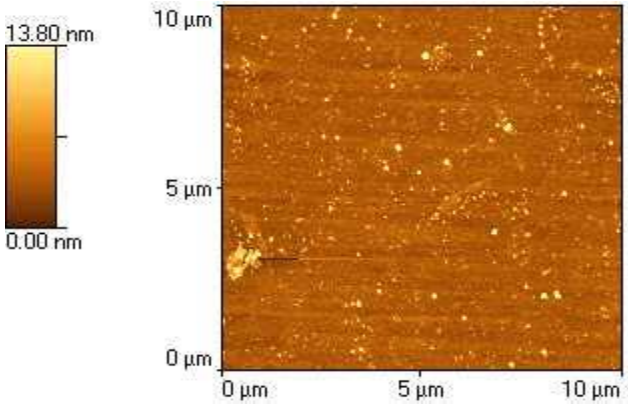


Figure 2.30 AFM image of Aβ40 in 50 mM phosphate buffer, in the presence of one equivalent of CP2 at day three. Formation of fibrils or protofibrils was not found.

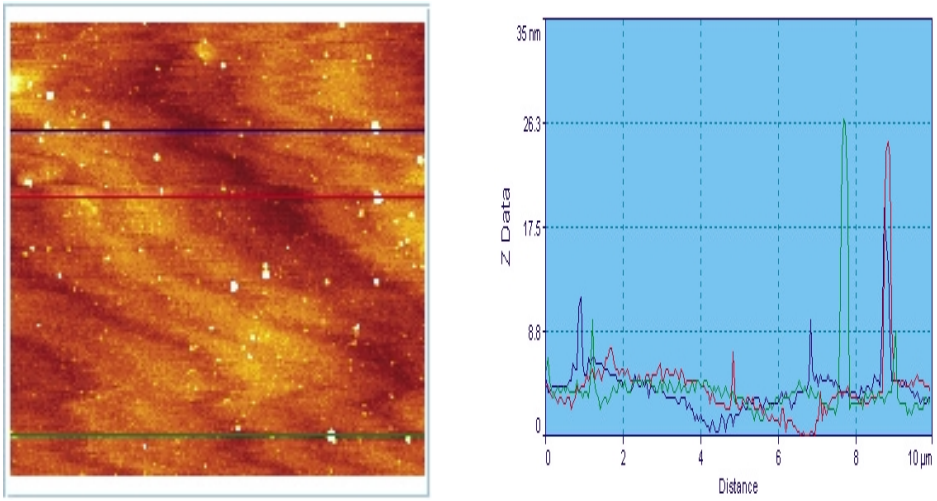


Figure 2.31 AFM image of Aβ40 in 50 mM phosphate buffer, in the presence of one equivalent of CP2 at day 50. No fibrils and protofibrils were found. Largest sizes of oligomers are about 2 nm high and 30 nm wide.

The following figures show AFM images, taken at different days with 25% TFE and with one equivalent of CP2.

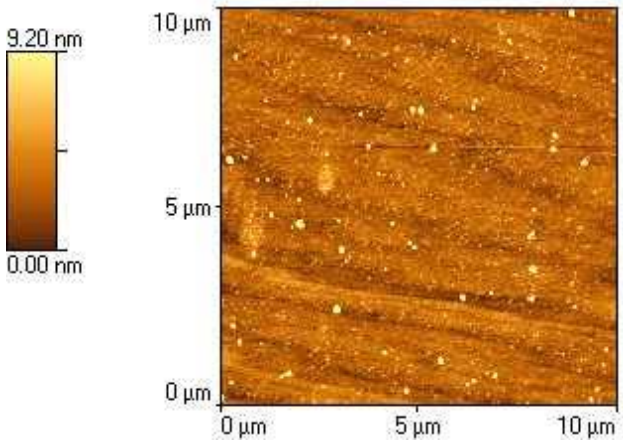


Figure 2.32 AFM image of Aβ40 in 50 mM phosphate buffer, 25% TFE, in the presence of one equivalent of CP2 at day zero. The largest oligomers are about 10 nm high and 30 nm wide.

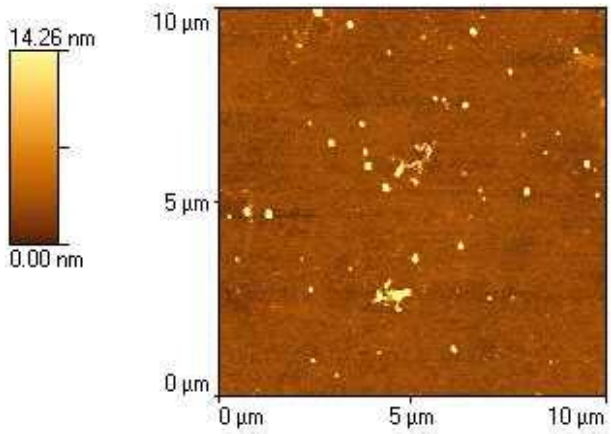


Figure 2.33 AFM image of Aβ40 in 50 mM phosphate buffer, 25% TFE, in the presence of one equivalent of CP2 at day one. The largest oligomers are about 15 nm high and 70 nm wide.

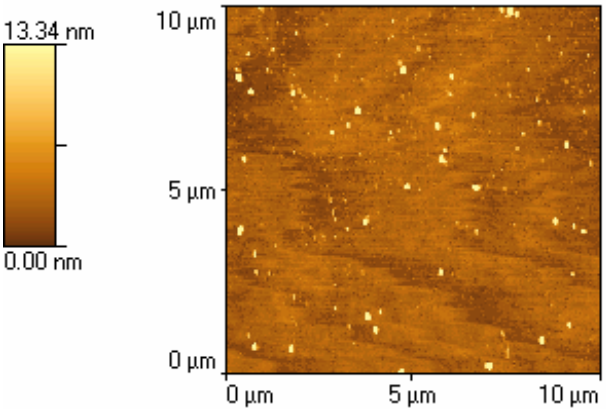


Figure 2.34 AFM image of Aβ40 in 50 mM phosphate buffer, 25% TFE, in the presence of one equivalent of CP2 at day three. No fibrils and protofibrils were found.

From the above AFM images we can conclude that the rate of aggregation is slower in presence of TFE than without TFE. In any case in presence of TFE and one equivalent of CP2, we never observed any fibrils and protofibrils or even presence of large oligomers. From this data we can conclude that CP2 inhibits the aggregation of Aβ40 peptides and possibly protect against MC65 cell death.

Similarly we studied the aggregation of Aβ42 in the presence and absence of CP2 using AFM. As we know that the Aβ42 aggregates much faster than Aβ40, so 50% TFE was added to decrease the rate of aggregation. Aβ42 peptide is a mixture of random coil, α-helix and β-sheet.

So Aβ42 peptide is dissolved in phosphate buffer and 50% TFE, pH 7.4, with and without one equivalent of CP2. AFM images were taken on day 1 and day zero.

The first three figures **2.35**, **2.36** and **2.37** are the AFM images (All the AFM experiments were performed by Hui-Chuan Wu and Erik Pettersson) in the absence of CP2 in day zero and day one. The very next day we can see the formation of large oligomers and protofibrils. The next three images **2.38**, **2.39**, **2.40** are the AFM images in the presence of one equivalent of CP2 in day zero and day one. After treatment with CP2 the largest oligomer was found to be in 40 nm size.

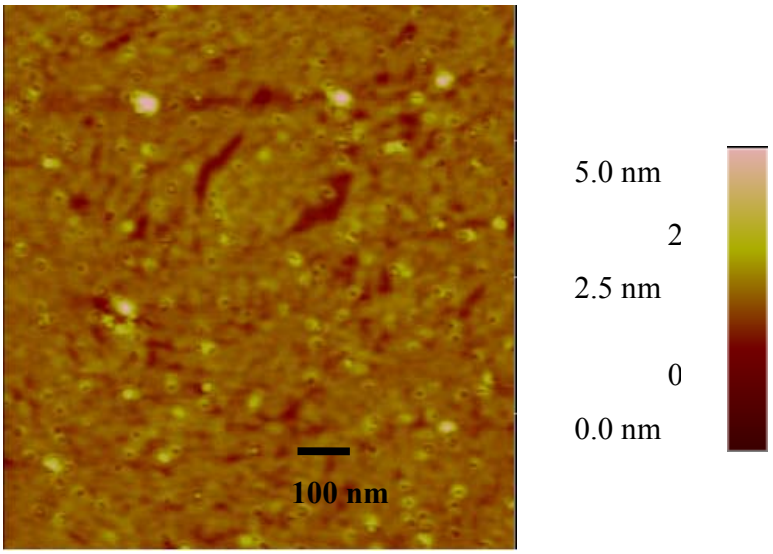


Figure 2.35 AFM image of Aβ42 in 50%TFE in the absence of CP2 at day zero. The largest oligomer is about 60 nm size.

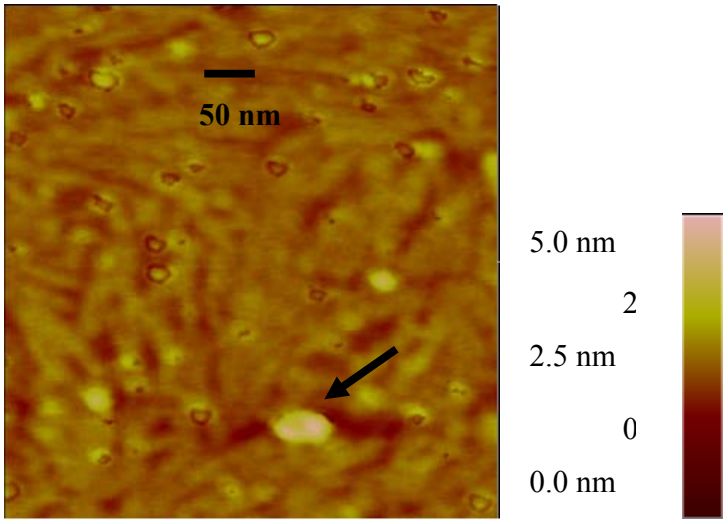


Figure 2.36 A zoom in AFM image of the above Aβ42 in 50%TFE in the absence of CP2 at day zero.

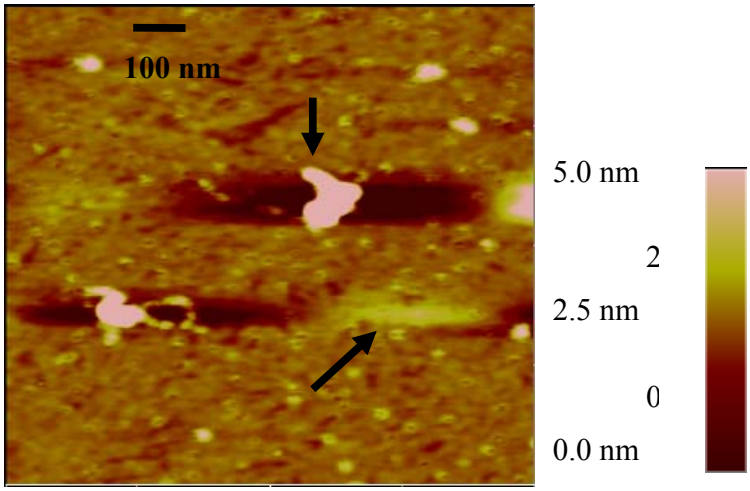


Figure 2.37 AFM image of Aβ42 in 50% TFE in the absence of CP2 on day one. The arrows indicate the formation of protofibrils and large oligomers.

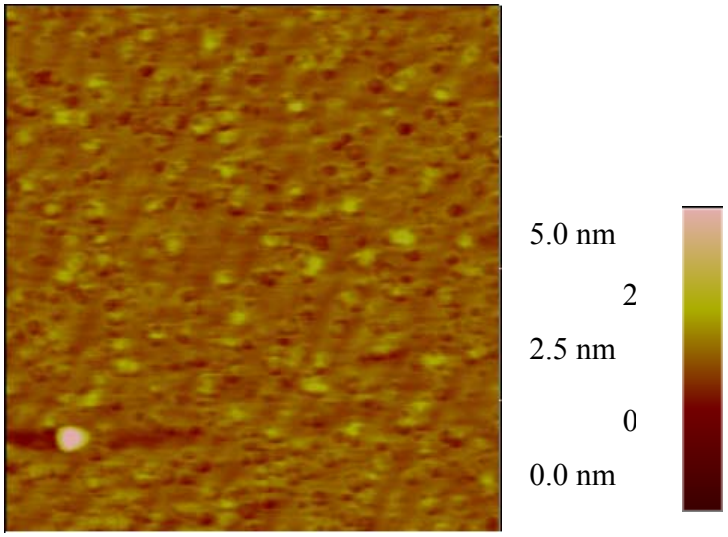


Figure 2.38 AFM image of Aβ42 in 50% TFE in the presenc of one equivalent of CP2 on day zero. The size of the oligomers is about 10-50 nm.

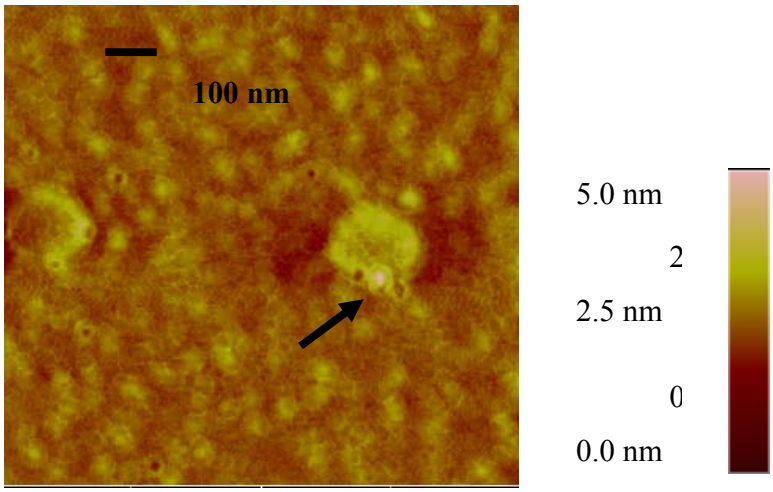


Figure 2.39 AFM image of Aβ42 in 50% TFE in the presence of one equivalent of CP2 on day one. The oligomers are about 10 nm-40 nm in sizes. The formation of protofibril is not observed.

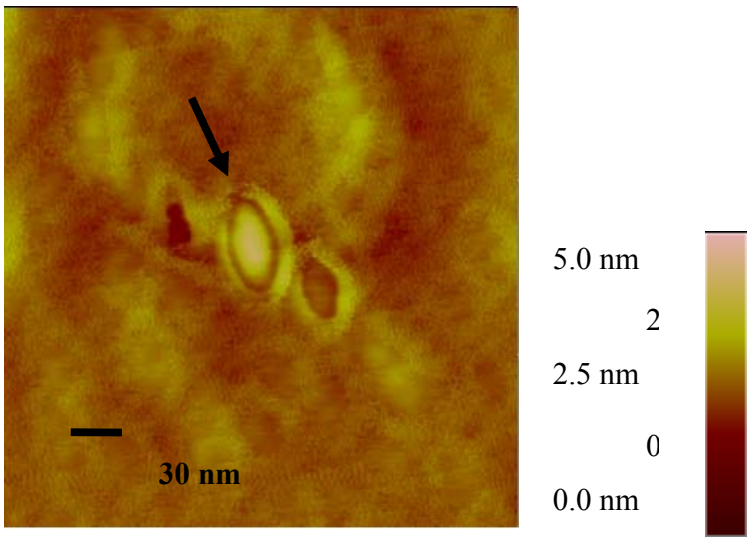


Figure 2.40 A zoom in image of the above AFM image of Aβ42 in 50% TFE in the presence of one equivalent of CP2 on day one. The arrow shows the largest oligomer of size 40 nm.

In the presence of one equivalent of CP2, the Aβ oligomers remained the same or decreased slightly to 40 nm and only 5% of peptide aggregated. In conclusion the aggregation of Aβ42 peptide was inhibited and there is no formation of fibrils and protofibrils was observed.

To test the toxicity of CP2, Aβ42 oligomers was applied to primary cortical neurons.[15] Aβ42 oligomers showed low levels of toxicity at 10 nM and showed a considerable amount of toxicity starting from 50 nM (Figure 2.41) (This work was carried out by Lee-Way Jin Laboratory, University of California, Davis). But unaggregated Aβ42 showed no significant toxicity.[15] Also the figure 2.42 (This work was carried out by Lee-Way Jin Laboratory, University of California, Davis) shows how CP2 blocks the toxicity when co administered Aβ42 oligomers. So CP2 not only protects the cell intracellularly but also blocks the toxicity of the extacellular applied Aβ oligomers.[15]

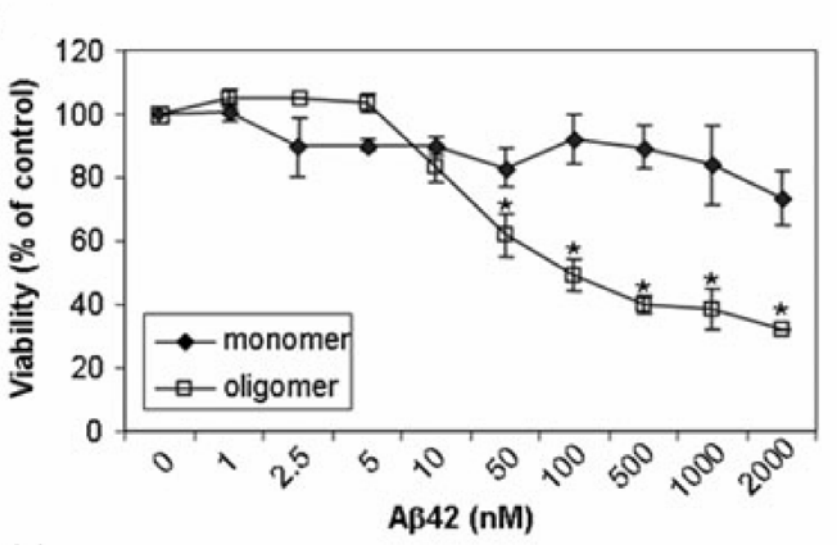


Figure 2.41 Cortical neurons were treated with different concentrations of Aβ42 oligomers. Data is shown as percentage viability after 48 hr treatment with Aβ42 oligomers.[15]

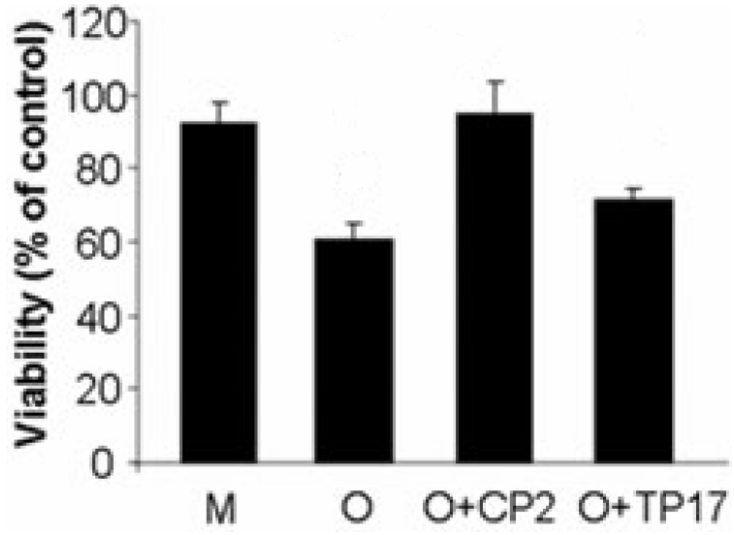


Figure 2.42 Cortical neurons treated with Aβ monomers (M) and oligomers (O) in presence of CP2 (50 nM)and TP17 (50 nM).[15]

It is believed that protein aggregation leads to many neurodegenerative diseases. Different types of proteins are responsible for different types of diseases as shown in table 2.1.[6] The formation of Aβ fibrils in the brain lead to neuron death. With the increase in number of deaths every year, it is very important to address the mechanism of Aβ aggregation which

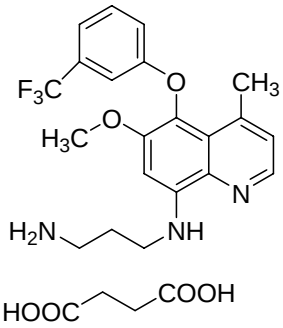
induces toxicity and eventually leads to patient's death. To study the mechanism we used MC65 cells, human neuroblastoma cells as a model to study the aggregation and toxicity. We synthesized different TP analogues to test the activity on MC65 cells.[15] Compound **2.2** (CP2), a TP analogue was studied extensively in this regard. We prepared CP2 in four steps starting form readily available compounds perillaldehyde and pyrone.

CP2 is a hydrogen donor and a good hydrogen acceptor, with molecular weight 393 and an octanol/water partition coefficient of 159. So according to Lipinski rules[34] CP2 is predicted to have good permeation property. This was also proved by radio labeling studies (Figure 2.19) and CP2 favors the blood brain barrier passage. We demonstrated that, the production of Aβ protein is responsible for the death of MC65 cells. It was also proven that the CP2 protects the cell death and inhibits the generation of Aβ oligomers (by SPR and AFM studies).

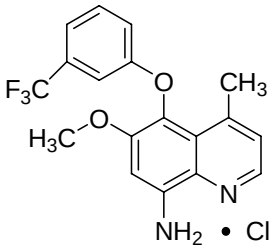
It was reported that some azodyes like congo red[15, 35, 36] can also inhibit the Aβ aggregation. Physical and structural aspects of these compounds will not allow them to permeate into the cells and also exhibit cell toxicity. Compounds like curcumin[15, 37] also inhibit the formation of Aβ oligomers. Unlike, above compounds CP2 is a small molecule that exhibit same properties without showing any toxicity in our assays.

CP2 has a structural scaffold that is accessible for optimization. Small molecules, compared to macromolecules such as β-sheet breaker peptides, have the advantage of lower cost, higher membrane permeability, higher stability, and high resistance to degradation. This versatility of small compounds is important for studying protein aggregation diseases like AD. As shown in figure 2.6 there are number of sites where modifications on CP2 can be performed. To understand the mechanism of intraneuronal Aβ aggregation and toxicity it is necessary to have a structure activity relationship study on CP2 derivatives.[15] Apart form CP2 recently we synthesized quinoline deivatives, structurally different to that TP compounds which also exhibits the inhibition of aggregation. The synthesis of the compounds **2.30**, **2.31**, **2.32** is not discussed in this thesis. These are a series of quinoline compounds where they have varied activity. Similar studies on these compounds are yet to be performed.

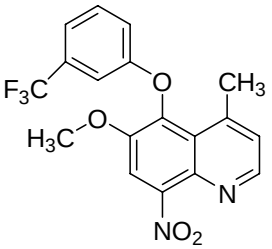
Figure 2.43



2.30



2.31

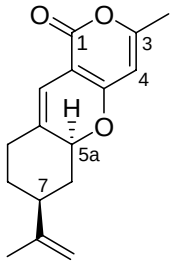


2.32

2.5 Experimental

General Methods. Nuclear Magnetic Resonance (NMR) spectra were recorded on a 400 MHz and 100 MHz Varian spectrometer in $CDCl_3$ unless otherwise specified. The chemical shifts are given in parts per million (ppm) from downfield from tetramethylsilane as internal standard. Coupling constants (J) are given in hertz. TLC analyses were performed on thin-layer analytical plates from Aldrich. All the reactions were performed in argon atmosphere unless otherwise specified. Silical gel (200~400 mesh) from Natland corporation was used for column chromatographic separations. Solvents like THF and diethyl ether were distilled over sodium and benzopheneone, methylene chloride was distilled over CaH_2 and finally benzene and toluene were distilled over $LiAlH_4$.

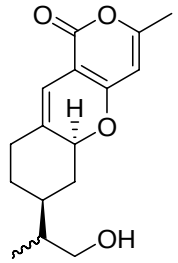
(5a*S*,7*S*)-7-Isopropenyl-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahyro-1-oxopyranol[4,3-b][1] benzopyran (2.3) (SB-8-65)[24, 29]



To a solution of 5 g (0.03968 mol) of **2.5** in 200 mL of ethyl acetate under argon at room temperature was added 6.55 g (0.04367 mol) of aldehyde **2.4** and 2.28 g (0.01984 mol) of L-proline. The reaction mixture was heated to reflux for 5 hrs. The reaction mixture was washed with water and brine. The organic layer was extracted with ethyl acetate. The organic layer was dried with anhydrous sodium sulfate, filtered and concentrated. Column chromatographic separation (on silica gel) using mixture of hexane and ethyl acetate (3:1 v/v) afforded 9.1 g of yellow solid, **2.3** in 78% yield. Purification was also performed by recrystillazation using ethyl acetate as a solvent. $[\alpha]^{22}_{D} = +31.9^{0}$ (*c* 0.75, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.1 (s, 1 H, C10 H), 5.72 (s, 1 H, C4 H), 5.1 (dd, J = 11 Hz, J = 5 Hz, 1 H, C5a H), 4.75 (m, 1 H, =CH), 4.73 (m, 1 H, =CH), 2.48 (ddd, J=14 Hz, J = 4 Hz, J = 2.4 Hz, 1 H), 2.22-2.02 (m, 3 H, CH_2), 2.19 (s, 3 H, C4-

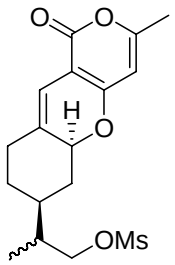
Me), 1.88-1.72 (m, 2 H, CH_2), 1.74 (s, 3 H, MeC=), 1.31 (ddd, J = 25 Hz, J = 12.8 Hz, J = 4 Hz, 1 H); ^{13}C NMR δ 163.4, 162.6, 161.7, 147.9, 132.3, 109.8, 109.6, 99.9, 97.5, 79.4, 43.6, 40.0, 32.5, 32.1, 20.9, 20.3.

(5a*S*,7*S*)-7-[(1*R*) and (1*S*)-2-Hydroxy-1-methylethyl]-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahyro-1-oxopyranol[4,3-b][1] benzopyran (2.6) (SB-4-100)[24, 29]



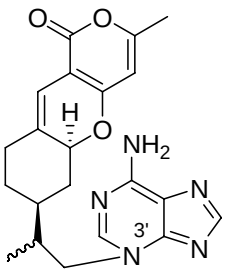
To a solution of 2.13 g (8.256 mmol) of **2.3** in 30 mL THF was added 8.25 mL (8.256 mmol) of 1 M BH_3.THF complex at -25 ^{0}C. After stirring the solution at -25 ^{0}C for 12 hours, 17 mL of 0.5% aqueous NaOH and 7 mL of 30% hydrogen peroxide were added at 0 ^{0}C. The solution was stirred at 0 ^{0}C for 4 hours and diluted with water and washed with dichloromethane three times. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated to dryness. The resulting solution was applied to silica gel column chromatography using gradient mixture of hexane and ethyl acetate afforded 1.25 g (80% yield; based on amount of reacted **2.3**) of **2.6** as a mixture of two diastereomers at C12 and 23 % recovery of starting material **2.3**. ^{1}H NMR ($CDCl_3$) δ 6.08 (s, 1 H, C4 H), 5.71 (s, 1 H, C10 H), 5.07 (t, J = 5.2 Hz, 1 H, C5a H), 3.58 (m, 2 H, CH_2O), 2.46 (m, 1 H), 2.19 (s, 3 H, Me), 2.11 (m, 2 H), 1.73-1.51 (m, 3 H), 1.16 (m, 2 H), 0.92 (d, J = 7 Hz, 3 H, Me); ^{13}C NMR δ 163.5, 162.8, 161.6, 133.0, 109.0, 100.0, 97.4, 79.7, 79.6, 65.6, 39.9, 39.8, 39.4, 37.2, 37.1, 36.9, 32.4, 32.3, 31.1, 30.4, 28.5, 20.1, 13.2, 13.1.

(5a*S*,7*S*)-7-[(1*R*) and (1*S*)-2-(Methanesulfonyloxy)-1-methylethyl]-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahyro-1-oxopyranol[4,3-b][1] benzopyran (2.7) (SB-4-104)[24, 29]

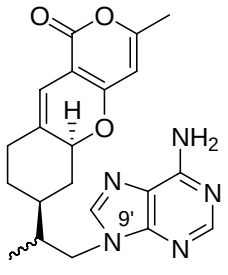


To a solution of 600 mg (2.1739 mmol) of **2.6** in 18 mL of methylene chloride 0.91 mL of triethylamine was added at 0 ^{0}C. The resulting solution was stirred at 0 ^{0}C for 10 minutes. To this solution 0.253 mL (3.261 mmol) of methane sulfonyl chloride was added at 0 ^{0}C. The resulting solution was stirred at 0 ^{0}C for 3 hours and diluted with water. The progress of the reaction was monitored using TLC using hexane and diethyl ether (2:1 v/v) as a developing solvent. The mixture was extracted with methylene chloride. The organic layer was washed with aqueous solution of sodium bicarbonate and brine, dried over sodium sulfate. Column chromatographic separation on silica gel using mixture of hexane and diethyl ether (2:1 v/v) afforded 550 mg of **2.7** in 91% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$) δ 6.08 (s, 1 H, C4 H), 5.71 (s, 1 H, C10 H), 5.06 (m, 1 H, CHO), 4.14 (m, 2 H, CH_2O), 3.03 (s, 3 H, MeS), 2.49 (d, J = 2.8 Hz, 1 H), 2.19 (s, 3 H, Me), 2.14-1.11 (m, 7 H), 0.98 (d, J = 6.8 Hz, 3 H, Me); ^{13}C NMR δ 163.2, 162.4, 161.7, 132.1, 109.6, 105.2, 99.8, 79.2, 79.1, 72.3, 38.9, 37.5, 37.4, 37.3, 37.2, 36.9, 32.2, 32.1, 30.8, 28.6, 20.2, 13.3, 13.2.

(5a*S*,7*S*)-7-[(1*R*) and (1*S*)-2-(N9-Adenyl)-1-methylethyl]-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahyro-1-oxopyranol[4,3-b][1] benzopyran (2.8) and (5a*S*,7*S*)-7-[(1*R*) and (1*S*)-2-(N3-adenyl)-1-methylethyl]-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahyro-1-oxopyranol[4,3-b][1] benzopyran (2.2) (SB-4-105)[24, 29]



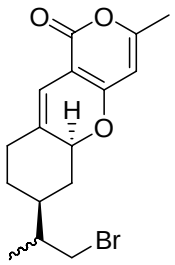
2.2



2.8

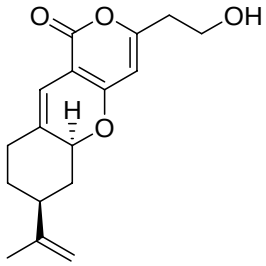
To a solution of 550 mg (1.554 mmol) of mesylate **2.7** in 10 mL of N,N-dimethyl acetamide (DMA) was added 209.7 mg (1.554 mmol) of adenine. The reaction mixture was heated to 150 ^{0}C for 8 hours. Then the vessel was cooled to room temperature and was added 1.554 mmol of sodium bicarbonate. Then the DMA in the reaction mixture was vacuum distilled to obtain a brown residue. Without any further workup the residue was made to dissolve in minimum amount of methanol (2 mL) and directly applied to silica gel Column chromatography. A mixture of chloroform and methanol (4:1 v/v) was used as eluent to give 4 % yield of **2.8** and 43 % yield of **2.2**. The obtained product was not pure after column chromatographic separation. So the combined fractions consisting of compound (**2.2**) (decided based on the proton NMR of the fractions after column chromatographic separation) were subjected to HPLC (Jupiter C18, 10-μ column (Phenomenex, Torrance, CA, USA)) separation. The running solvent system was water and acetonitrile with 0.1% trifluoroacetic acid (TFA). We ran several programs with varying amounts of solvents from both the pumps with a flow rate of 4 mL/minute. Finally were we able to separate both the compounds **2.8** and **2.2**. The obtained fractions were collected separately and lyophilized to obtain compounds **2.2** and **2.8** as TFA salts. We are uncertain about the number of TFA molecules adhering to one molecule of each compound. ^{1}H NMR of **2.2** ($CDCl_3$) δ 8.07 (s, 1 H, C8'H of adenine), 7.98 and 7.97 (2s, 1 H, C2'H of adenine; 2diastereomers), 6.10 (s, 1 H, C10 H), 5.72 and 5.71 (2s, 1 H, C4 H), 5.02 (m, 1 H, C5a H), 4.50 (dd, J = 14, 7 Hz, 1 H, CHN), 4.08 (2dd, J = 14 Hz, J = 8 Hz, 1 H, CHN), 2.46 (m, 2 H), 2.20 and 2.19 (2s, 3 H, Me), 2.10-1.22 (m, 6 H), 0.91 (d, J = 7 Hz, 3 H, Me); ^{13}C NMR δ 163.2, 163.1, 162.4, 161.7, 154.4, 154.0, 150.7, 142.3, 131.7, 131.6, 121.0, 199.8, 99.7, 97.3, 79.0, 78.8, 54.5, 54.4, 38.9, 38.1, 38.0, 37.1, 36.9, 36.1, 32.0, 31.9, 30.7, 27.6, 20.1, 13.3, 13.2. ^{1}H NMR of **2.8** ($CDCl_3$) δ 8.36 (s, 1 H, C2' H), 7.78 (s, 1 H, C8' H), 6.09 (s, 1 H, C4 H), 5.89 (bs, 2 H, NH_2), 5.72 (s, 1 H, C10 H), 5.01 (m, 1 H, C5a H), 4.24 (dd, J = 14 Hz, J = 7 Hz, 1 H, CHN), 4.01 (dd, J = 14 Hz, J = 7 Hz, 1 H, CHN), 2.5-1.2 (m, 8 H), 2.19 (s, 3 H, Me), 0.90 (d, J = 7 Hz, 3 H, Me); ^{13}C NMR δ 163.4, 162.7, 161.9, 155.6, 153.5, 150.6, 140.9, 132.1, 119.8, 109.9, 99.9, 97.5, 79.4, 79.2, 47.9, 39.3, 38.4, 38.3, 38.1, 38.0, 36.2, 32.3, 32.1, 31.1, 27.8, 20.3, 13.8.

(5a*S*,7*S*)-7-[(1*R*) and (1*S*)-2-Bromo-1-methylethyl]-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahyro-1-oxopyranol[4,3-b][1] benzopyran (2.9) (SB-8-16)[38]



To a solution of 500 mg (1.825 mmol) of **2.6**, 956 mg (3.65 mmol) of triphenylphosphine and 10 mL of methylene chloride was added. The reaction mixture was cooled to 0 ^{0}C. 1.52 g (4.56 mmol) of carbon tetrabromide was added in portions to this reaction mixture.[38] The reaction was stirred at 0 ^{0}C for half an hour. Then the solvent was evapourated using rotavapor. Then the brown residue was applied to the column directly without any aqueous workup. Column chromatographic separation on silica gel using mixture of hexane and diethyl ether (1:1 v/v) afforded 90% of **2.9**. ^{1}H NMR of **2.9** ($CDCl_3$) δ 6.09 (s, 1 H, C4 H), 5.71 (s, 1 H, C10 H), 5.08 (m, 1 H, C5a H), 3.41 (d, J = 4.4 Hz, 2 H, CH_2Br), 2.46 (m, 1 H), 2.19 (s, 3 H, Me), 2.11 (m, 2 H), 1.73-1.51 (m, 3 H), 1.16 (m, 2 H), 1.03 (d, J = 7 Hz, 3 H, Me); ^{13}C NMR δ 163.3, 162.5, 161.7, 132.3, 109.5, 99.8, 97.5, 79.34, 79.21, 39.6, 39.5, 39.1, 38.83, 38.80, 36.7, 32.17, 32.10, 30.9, 28.7, 20.2, 15.8.

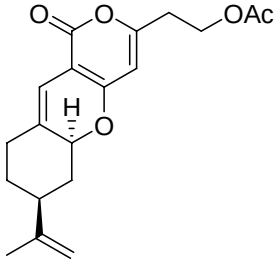
(5a*S*,7*S*)-3-(2-Hydroxyethyl)-7-isopropenyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (2.10) (SB-7-106)



To a solution of 0.4 mL of diisopropylamine in 28 mL of THF was cooled to -20 ^{0}C. To this solution 1.8 mL of 1.6 M *n*-Buli was added and stirred for 30 minutes. Thus obtained solution is the freshly prepared LDA and the reaction vessel is maintained at -20 ^{0}C to reduce the decomposition. In another flask, to a solution of 250 mg (0.969 mmol) of **2.3** in 5 mL THF was cooled to -78 ^{0}C. To this solution 19.4 mL (1.9372 mmol) of freshly prepared LDA was added via syringe. After five minutes of stirring at -78 ^{0}C the solution turned deep blue in color

indicating the formation of anion. This solution was stirred at -78 ^{0}C for 2 hours. In another flask a suspended solution of 290 mg (9.6899 mmol) of paraformaldehyde in 10 mL THF was taken and cooled to 0 ^{0}C. The anion formed in another flask is transferred to the paraformaldehyde-THF mixture via cannula and allowed to stir at 0 ^{0}C and slowly warmed to room temperature. The color of the solution turned reddish brown after reaching to room temperature. The reaction mixture was washed with water and extracted using methylene chloride. The organic layer was washed with aqueous sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, filtered and concentrated. Column chromatographic separation on silica gel using mixture of hexane and diethyl ether (1:1 v/v) gave a moderate yield 28% of **2.10** and 15% of starting material **2.3** was recovered. $[\alpha]_D^{23}$ = +23.7^o (C 3.5, $CHCl_3$); ^{1}H NMR $CDCl_3$ δ 6.06 (s, 1 H), 5.86 (s, 1 H), 5.11 (dd, *J* = 11 Hz, J = 4.4 Hz, 1 H, C5a H), 4.75 (s, 1 H, =CH_2), 4.73 (s, 1 H, =CH_2), 3.90 (t, *J* = 6.2 Hz, 2 H, CH_2O), 2.69 (t, *J* = 6.2 Hz, CH_2), 2.48 (m, 1 H), 2.20 ~ 1.70 (a series of m, 5 H), 1.73 (s, 3 H, Me), 1.34 ~ 1.25 (m, 1 H); ^{13}C NMR δ 163.3, 162.1, 148.0, 132.8, 109.9, 109.1, 101.1, 98.2, 79.6, 59.6, 43.6, 40.0, 37.3, 32.6, 32.1, 30.5, 20.9. HRMS calculated for $C_{17}H_{21}O_4$ (M+H) 289.1440, found 289.1411.

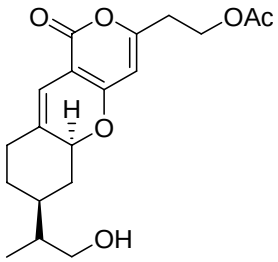
(5a*S*,7*S*)-3-(2-Acetoxyoxyethyl)-7-isopropenyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (2.14) (SB-8-29)



To a solution of 202 mg (0.7014 mmol) of **2.10** in 3 mL of distilled pyridine was added 143 mg (1.402 mmol) of acetic anhydride at 0 ^{0}C. The reaction mixture was stirred at 0 ^{0}C for 7 hours. The progress of the reaction was monitored using TLC and diethyl ether was used as a developing solvent. The reaction mixture was washed with water and extracted with ethyl acetate. The organic layer was washed with 1 M HCl, aqueous sodium bicarbonate and brine, dried over anhydrous sodium sulfate and concentrated to dryness under vacuum. Column chromatographic separation on silica gel using diethyl ether afforded 140 mg of **2.14** in 62% yield. $[\alpha]_D^{23}$ = +16.9^o

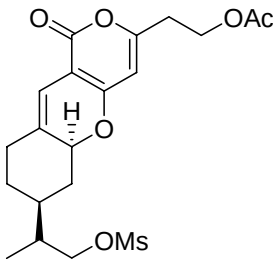
(C 0.15, CHCl3); ^{1}H NMR $CDCl_3$ δ 6.10 (s, 1 H), 5.79 (s, 1 H), 5.13 (dd, J = 11.2 Hz, 4.8 Hz, 1 H, C5a H), 4.75 (s, 1 H, =CH_2), 4.73 (s, 1 H, =CH_2), 4.33 (t, J = 6.4 Hz, 2 H, CH_2O), 2.76 (t, J = 6.4 Hz, CH_2), 2.48 (m, 1 H), 2.20 ~ 1.70 (m, 5 H), 1.74 (s, 3 H, Me), 1.28 (qd, J = 12.8, 4 Hz, 1 H); ^{13}C NMR δ 170.9, 163.0, 160.9, 147.9, 132.9, 110.0, 109.6, 100.8, 98.5, 79.6, 60.8, 43.6, 40.0, 33.6, 32.5, 32.1, 30.5, 20.1, 20.9. HRMS calculated for $C_{19}H_{23}O_5$ (M+H) 331.1545, found 331.1536.

(5a*S*,7*S*)-3-(2-Acetoxyethyl)-7-(2-hydroxy-1-methylethyl)-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (2.15) (SB-8-34)



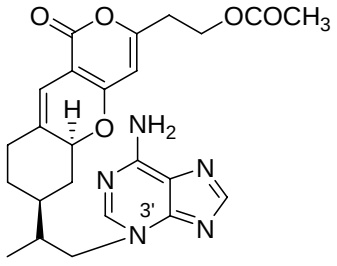
To a solution of 143 mg (0.433 mmol) of **2.14** in 3 mL THF under argon was added 0.43 mL (0.433 mmol) of 1 M BH_3.THF and the solution was stirred at -78 ^{0}C for 30 minutes and stirred at -20 ^{0}C for two days. To the solution at 0^{o}C, 1 mL of water, 0.5 m of 0.1% NaOH aqueous solution, and 0.5 mL of 30% H_2O_2 were added, and the resulting solution was stirred for 1 h. The solution was washed with water and extracted with methylene chloride. The organic layer was washed with aqueous solution of ammonium chloride and brine. The residue obtained after drying was applied to silica gel column chromatography. Diethyl ether was used as a solvent to give 72 mg of **2.15** in 56% yield along with 14% recovery of starting material **2.14**. ^{1}H NMR $CDCl_3$ δ 6.07 (s, 1 H), 5.78 (s, 1 H), 5.10 (m, 1 H, C5a H), 4.32 (t, J = 6.2 Hz, 2 H, CH_2O), 3.56 (m, 2 H, CH_2OH) 2.76 (t, J = 6.2 Hz, CH_2), 2.47 (m, 1 H), 2.17 ~ 1.10 (m, 7 H), 0.91 (d, J = 7 Hz, 1 H); ^{13}C NMR δ 170.9, 163.0, 160.9, 133.4, 118.1, 109.3, 100.8, 98.5, 79.9, 66.0, 60.8, 40.2, 39.5, 37.6, 37.5, 37.3, 33.6, 31.2, 21.0. HRMS calculated for C19H25O6 (M+H) 349.1651, found 349.1649.

(5a*S*,7*S*)-3-(2-Acetoxyethyl)-7-[(2-methanesulfonyloxy)-1-methylethyl]-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (2.17) (SB-8-48)



To a solution of 85 mg (0.2442 mmol) of **2.15** in 3 mL of methylene chloride was added 0.109 mL (0.7326 mmol) of triethylamine at 0 ^{0}C. To the above solution 28 μL (0.3664 mmol) of methanesulfonyl chloride was added at 0 ^{0}C, the resulting mixture was stirred at room temperature for 3 hours. The reaction mixture was washed with water and extracted with methylene chloride. The organic layer was washed with aqueous solution of sodium bicarbonate and brine, dried over anhydrous sodium sulfate and concentrated to dryness under vacuum. Column chromatographic separation on silica gel using diethyl ether as a solvent afforded 94 mg of **2.17** in 90% yield. ^{1}H NMR $CDCl_3$ δ 6.05 (s, 1 H), 5.76 (s, 1 H), 5.07 (m, 1 H, C5a H), 4.29 (t, J = 6.3 Hz, 2 H, CH_2O), 4.10 (m, 2 H, CH_2OS), 3.00 (s, 3 H, CH_3S), 2.73 (t, J = 6.3 Hz, CH_2), 2.47 (d, J = 14 Hz, 1 H), 2.17 ~ 1.10 (m, 7 H), 2.02 (s, 3 H, CH_3CO), 0.96 (d, J = 7 Hz, 1 H); ^{13}C NMR δ 170.9, 162.9, 162.3, 161.1, 132.7, 109.7, 100.7, 98.4, 79.4, 72.3, 60.8, 39.1, 37.6, 37.5, 37.4, 33.6, 32.3, 30.9, 13.3. HRMS calculated for $C_{20}H_{27}O_8S$ (M+H) 427.1426, found 427.1434.

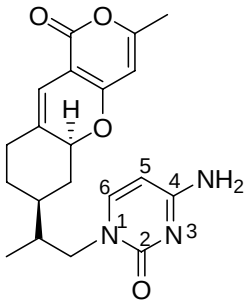
(5a*S*,7*S*)-3-(2-Acetoxyethyl)-7-[(1*R*) and (1*S*)- 2-(*N*3-adenyl)-1-methylethyl]-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (2.18) (SB-8-50)



To a solution of 94 mg (0.22 mmol) of **2.17** in 1.5 mL of DMA was added 33 mg (0.2442 mmol) of adenine and the resulting mixture was heated to 150 ^{0}C for 7 hours. The reaction mixture was cooled to room temperature and 50 mg (0.59 mmol) of sodium bicarbonate was added and DMA was distilled out under reduced pressure to obtain a brown residue. This residue was made to

dissolve in minimum amount of methanol (2 mL) and applied to column directly. Column chromatographic separation on silica gel using mixture of chloroform and methanol (4:1 v/v) afforded 5 mg of **2.18** in low yields. The obtained product after column chromatographic separation was not pure. So we resorted to HPLC (Jupiter C18, 10-μ column (Phenomenex, Torrance, CA, USA)) to obtain pure compound. The running solvent system was water and acetonitrile with 0.1% trifluoroacetic acid (TFA). The flow rate was maintained at 4 mL/minute. The obtained fractions were lyophilized to obtain pure **2.18** as a TFA salt. The number of TFA molecules adhering to compound **2.18** remained uncertain. ^{1}H NMR $CDCl_3$ ẟ 8.07 (s, C8'H of adenine), 8.01 & 8.00 (2s, 1 H, C2'H of adenine; 2 diastereomers), 6.10 (s, 1 H, C10H), 5.79 & 5.78 (2s, 1 H, C4 H), 5.05 (m, 1 H, C5a H), 4.54 (2dd, *J* = 13.5 Hz, J = 6.5 Hz, 1 H, CHN; 2 diastereomers), 4.34 & 4.33 (2t, *J* = 6.2 Hz, 2 H, CH_2O; 2 diasteromers), 4.07 (dd, *J* = 13.5, J = 8 Hz, 1 H, CHN), 2.78 & 2.76 (2t, *J* = 6.2 Hz, 2 H, CH_2; 2 diastereomers), 2.07 & 2.06 (2s, 3 H, Me; 2 diastereomers), 2.60 ~ 1.22 (m, 8 H), 0.91 (d, *J* = 7.0 Hz, 3 H, Me). ^{13}C NMR δ 182.0, 181.6, 164.8, 163.3, 161.1, 155.8, 152.9, 148.0, 141.6, 131.1, 129.6, 129.1, 121.3, 100.7, 79.1, 78.9, 63.8, 59.2, 50.5, 33.6, 29.9, 21.1, 15.1. HRMS calculated for $C_{24}H_{28}N_5O_5$ (M+H) 466.2090, found 466.2081.

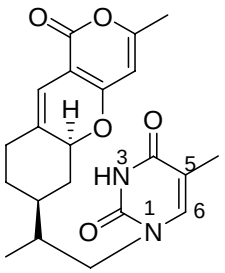
(5a*S*,7*S*)-7-[(1*R*) and (1*S*)-2-(N1-Cytosinyl)-1-methylethyl]-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahyro-1-oxopyranol[4,3-b][1] benzopyran (2.26) (LZ-2-73)



To a solution of 100 mg (0.295 mmol) of **2.9** in 3 mL of DMF was added 13 mg of sodium hydride and 36 mg (0.3245 mmmol) of cytosine. The resulting solution was stirred at room temperature for 12 hours and then heated to 80 ^{0}C and stirred for 20 hours. Then the reaction mixture was cooled to room temperature and 9 μL of acetic acid was added to the reaction mixture. DMF was distilled out under reduced pressure to obtain a brown residue which was

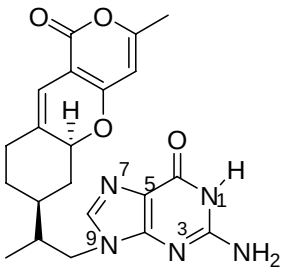
loaded on to a silica gel column chromatography without any aqueous workup. Mixture of chloroform and methanol (4:1 v/v) was used as an eluent to give 13 mg of **2.26** in 12 % yield. Other fractions were difficult to characterize. ^{1}H NMR $CDCl_3$ δ 7.17-7.19 (4 s, C5 H and C6 H of cytosine), 6.07 (s, 1 H, C10 H), 5.71 and 5.72 (2s, 1 H, C4 H), 5.05 (m, 1 H, C5a H), 3.95 (m, 1 H, CHN), 3.37 (m, 1 H, CHN), 2.19 (s, 3 H, Me), 2.60 ~ 1.22 (a series of m, 8 H), 0.87 (d, J = 2 Hz, 3 H, Me). ^{13}C NMR (CD_3OD) δ 166, 165.5, 163.9, 151.3, 137, 134.8, 109.6, 101.2, 94.5, 80.98, 80.83, 67.1, 54.5, 40.2, 39.5, 38.3, 37, 33.1, 32, 29.5, 19.9, 15.6, 13.7.

(5a*S*,7*S*)-7-[(1*R*) and (1*S*)-2-(N1-Thymineyl)-1-methylethyl]-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahyro-1-oxopyranol[4,3-b][1] benzopyran (2.28) (SB-9-95)



To a solution of 100 mg (0.29 mmol) of **2.9** in 5 mL of DMF was added 13 mg of sodium hydride and 40 mg (0.319 mmol) of thymine. The resulting solution was stirred at room temperature for 24 hours. To this reaction mixture 17.4 μL of acetic acid was added. DMF was distilled out under reduced pressure to obtain a brown residue. This brown residue was loaded on to a silica gel column chromatography without any aqueous workup. Column chromatographic separation using mixture of chloroform and methanol (4:1 v/v) gave 22 mg (20 % yield) of **2.28**. The remaining fractions obtained after column chromatography were difficult to characterize. ^{1}H NMR $CDCl_3$ δ 6.9 (s, 1 H, C6 H of thymine), 6.09 (d, J = 5.2 Hz, 1 H, C10 H), 5.7 (s, 1 H, C4 H), 5.04 (m, 1 H, C5a H), 3.9 (m, 2 H, CH_2N), 2.60 ~ 1.22 (m, 8 H), 2.19 (s, 3 H, Me), 1.94 (s, 3 H, Me of Thymine), 0.82-0.91 (4s, 3H, Me from two diastereomers). ^{13}C NMR ($CDCl_3$) δ 181.0, 178.8, 150.1, 141.6, 140, 134.5, 109.9, 109.5, 99.9, 99.9, 97.1, 72.1, 45.3, 39.2, 36.1, 32.2, 28.3, 20.3, 13.4.

(5a*S*,7*S*)-7-[(1*R*) and (1*S*)-2-(N9-Guanineyl)-1-methylethyl]-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahyro-1-oxopyranol[4,3-b][1] benzopyran (2.29) (SB-9-93)



To a solution of 200 mg (0.59 mmol) of **2.9** in 8 mL of DMF was added 26 mg of sodium hydride and 107 mg (0.71 mmol) of guanine. The resulting solution was stirred at room temperature for 16 hours. DMF was distilled out under reduced pressure to obtain a brown residue, which was loaded on to a silica gel column chromatography. No aqueous workup was performed. A mixture of chloroform and methanol (4:1 v/v) was used as an eluent to give 35 mg (15% yield) of title compound **2.29**. ^{1}H NMR $CDCl_3$ δ 7.54 (bs, 1 H, C8 H of guanine), 6.11 (s, 1 H, C10 H), 5.7 (s, 1 H, C4 H), 5.02 (m, 1 H, C5a H), 3.99 (m, 2 H, CH_2N), 2.60 ~ 1.22 (m, 8 H), 2.08 (s, 3H, Me), 0.87 (s, 3 H, Me). ^{13}C NMR (CD_3OD) δ 176.9, 162.0, 161.01, 155, 157, 134.9, 109.6, 101.3, 84.6, 80.9, 67.1, 45.0, 39.4, 29.9, 29.3, 29.2, 22.6, 19.9, 15.6.

References

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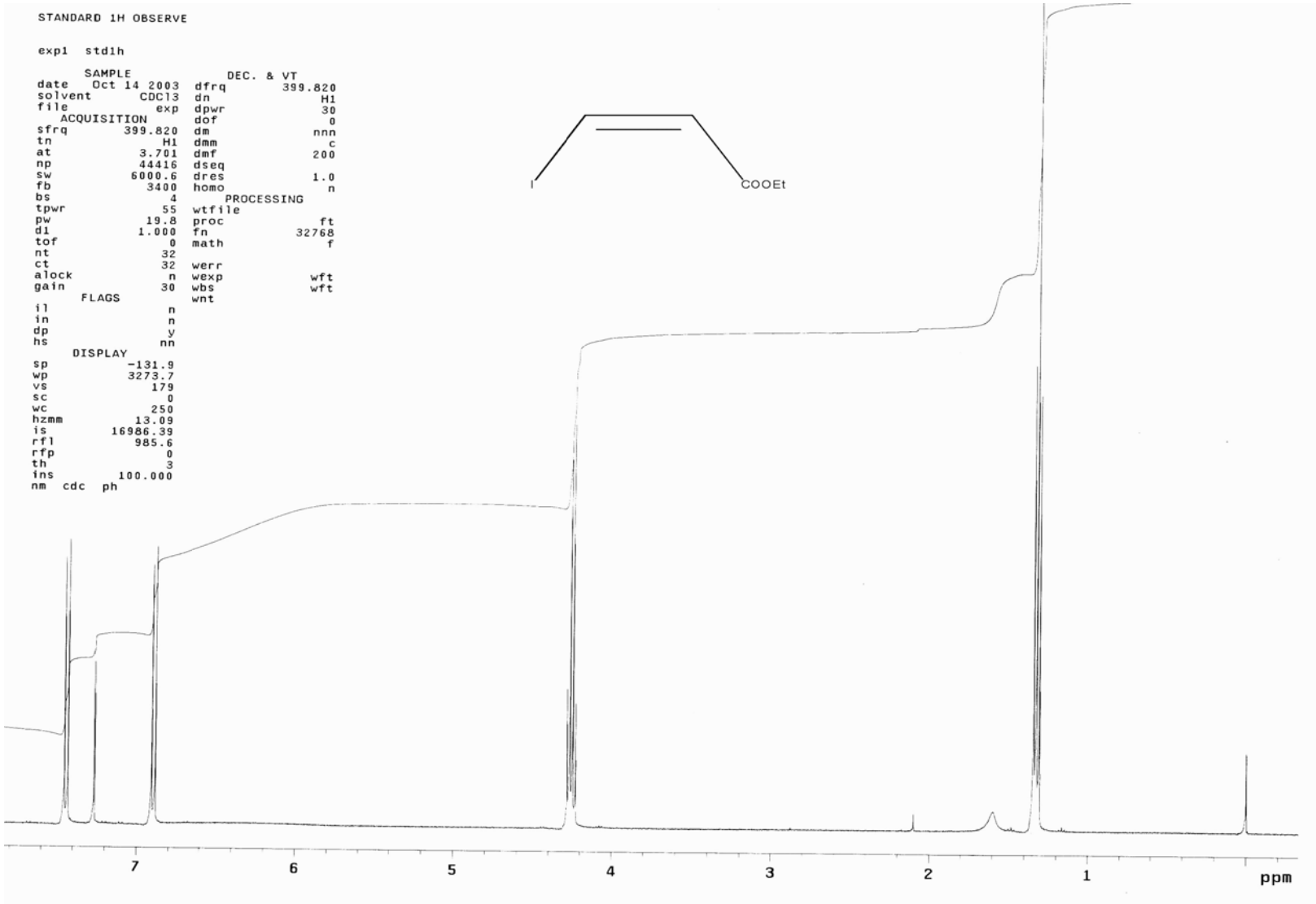
Appendix

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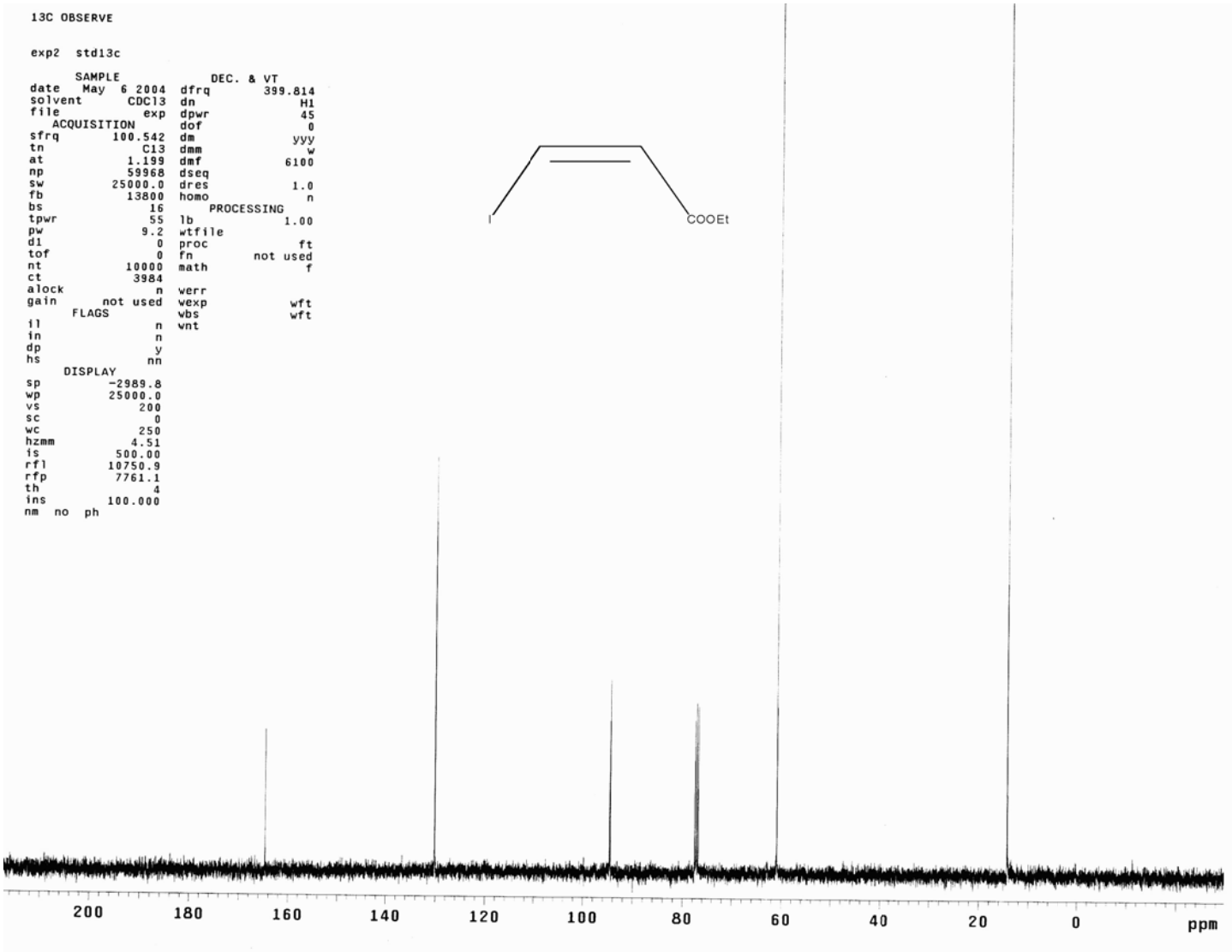
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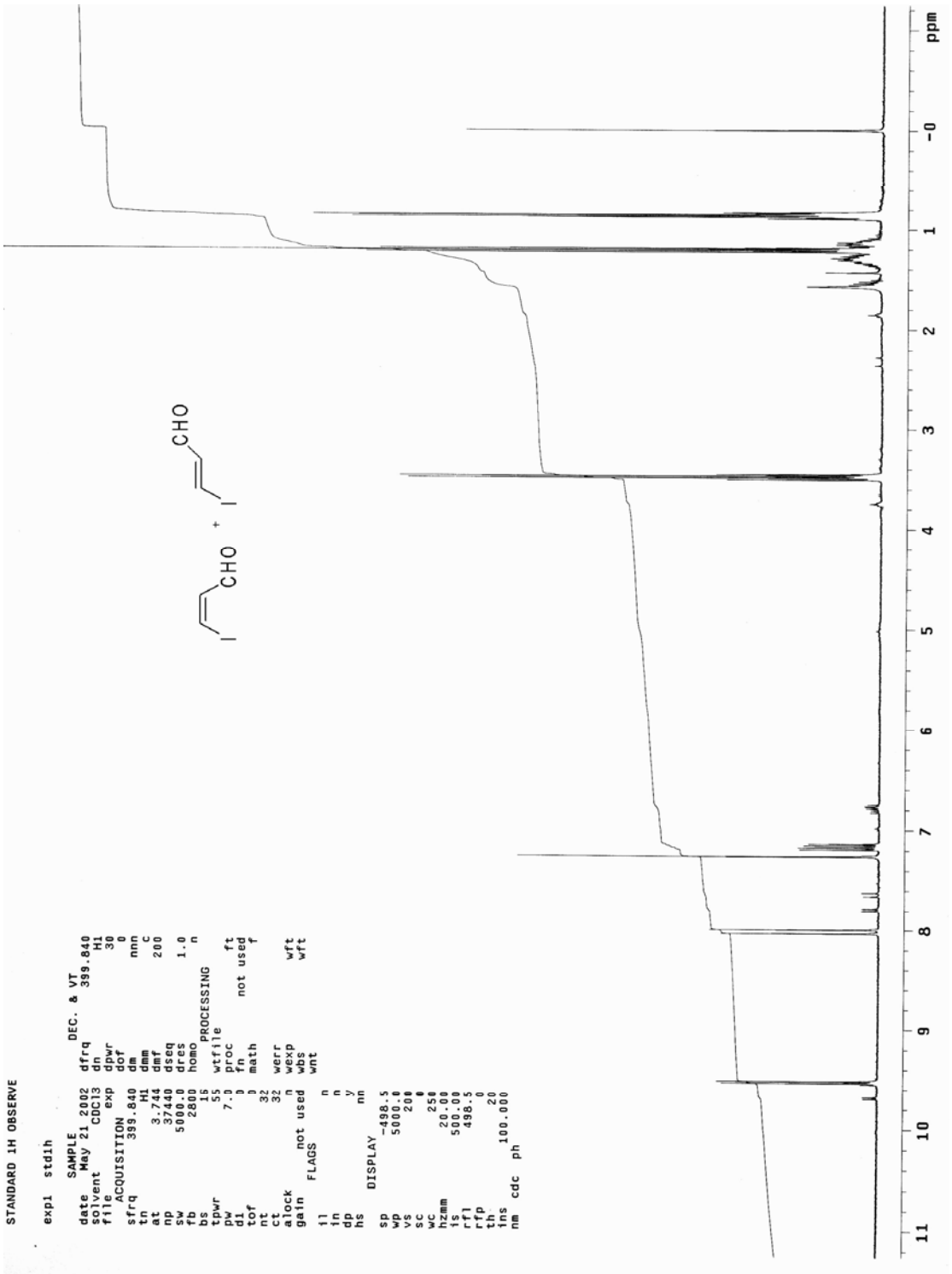
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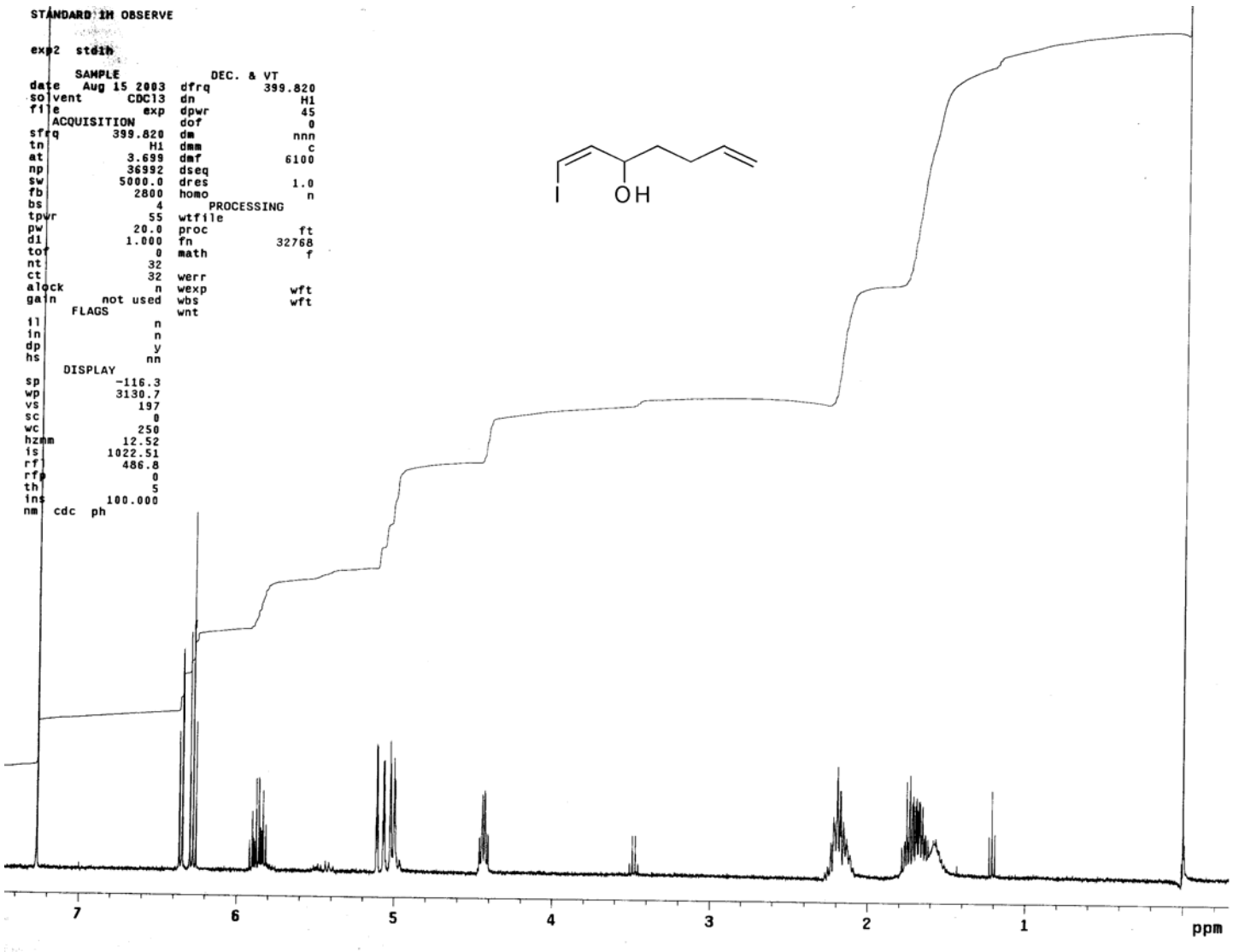
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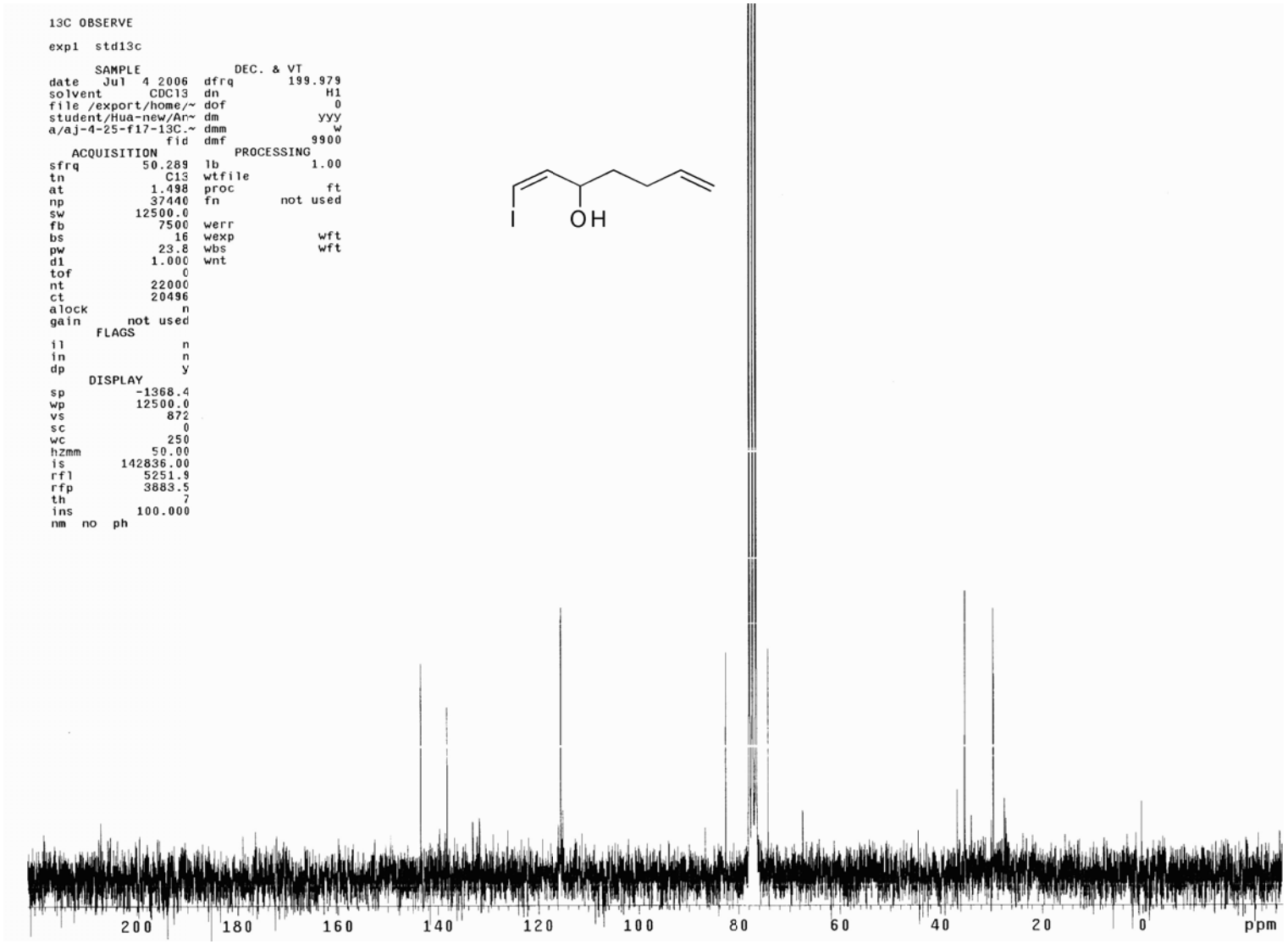
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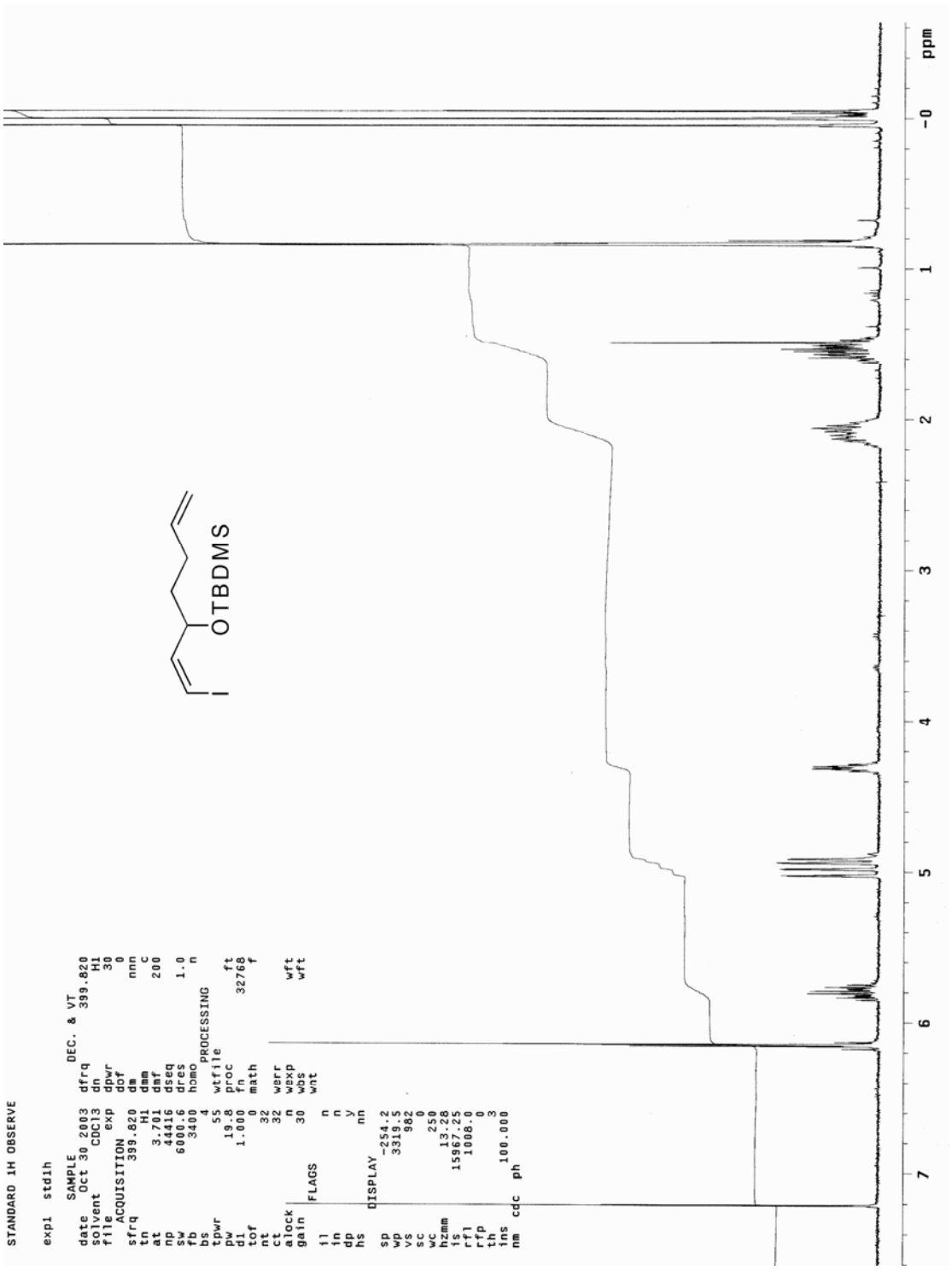
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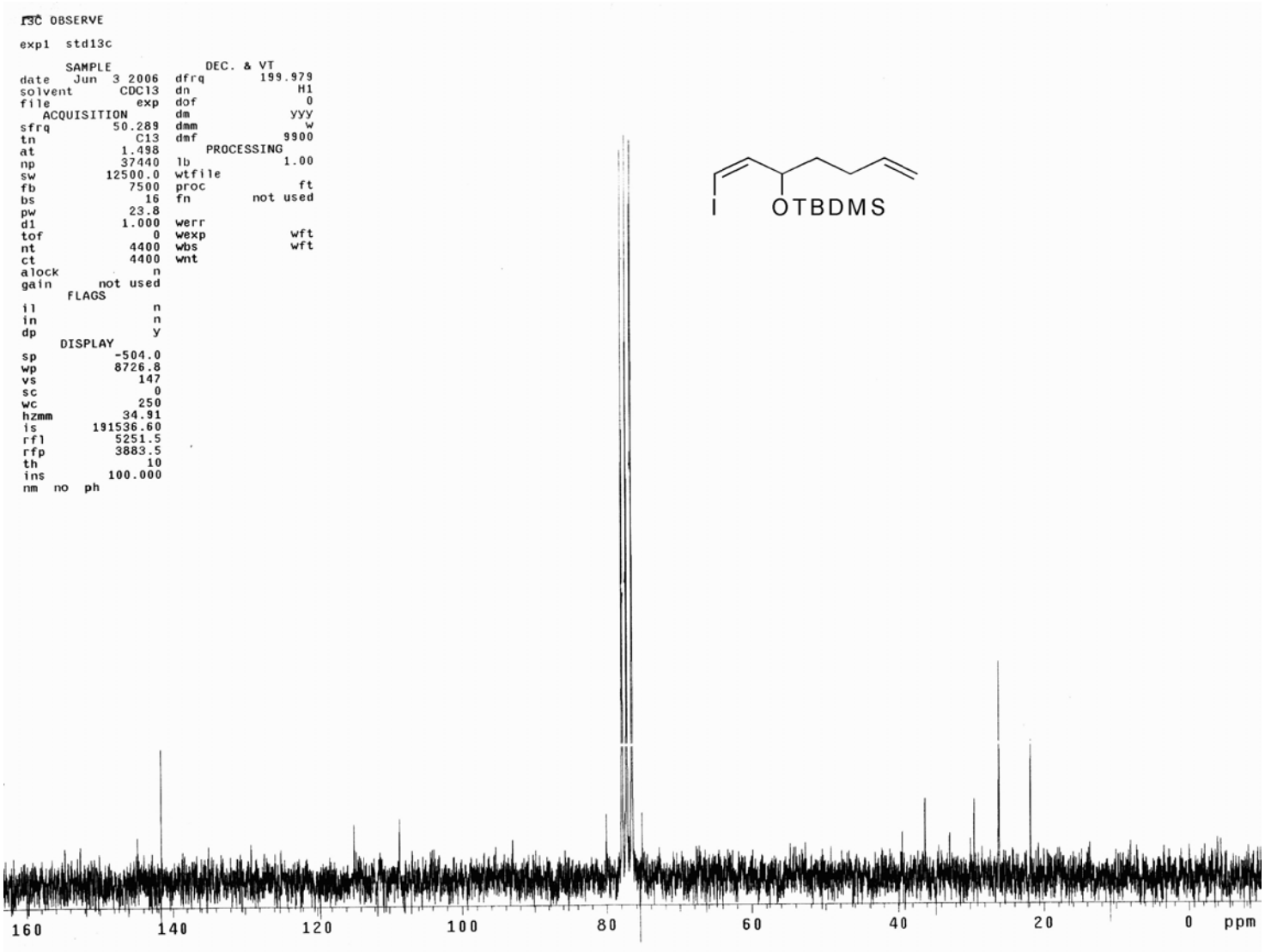
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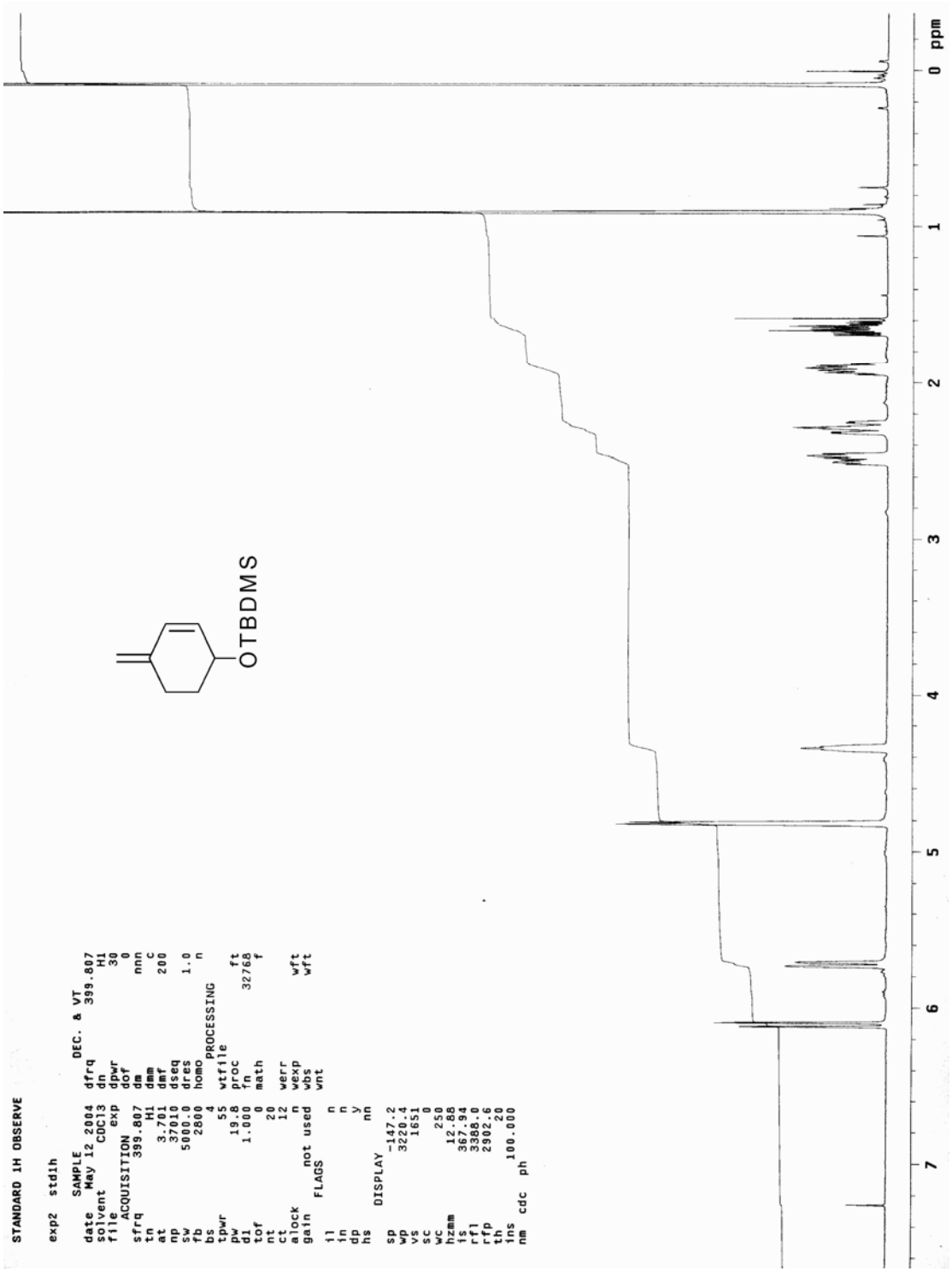
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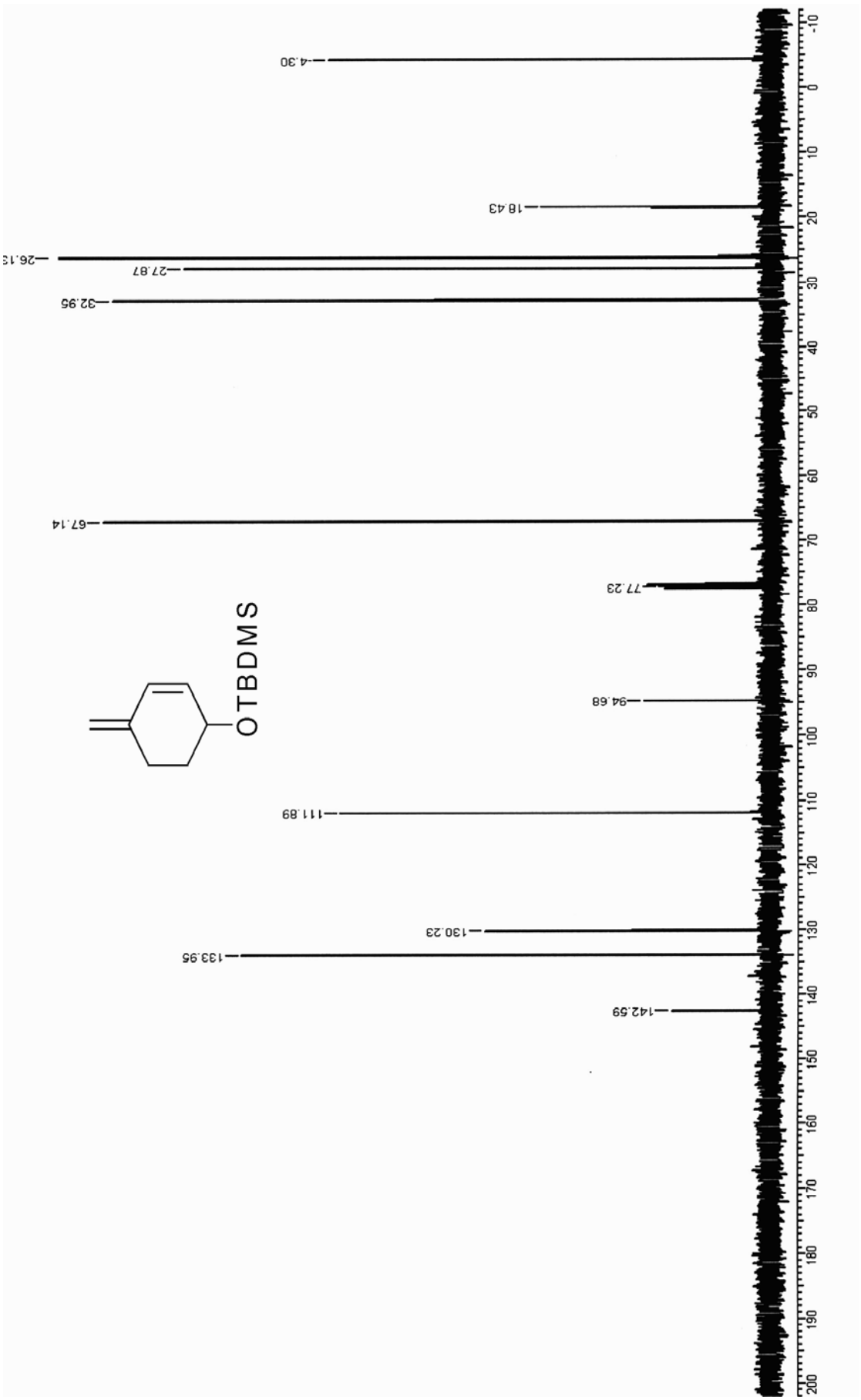
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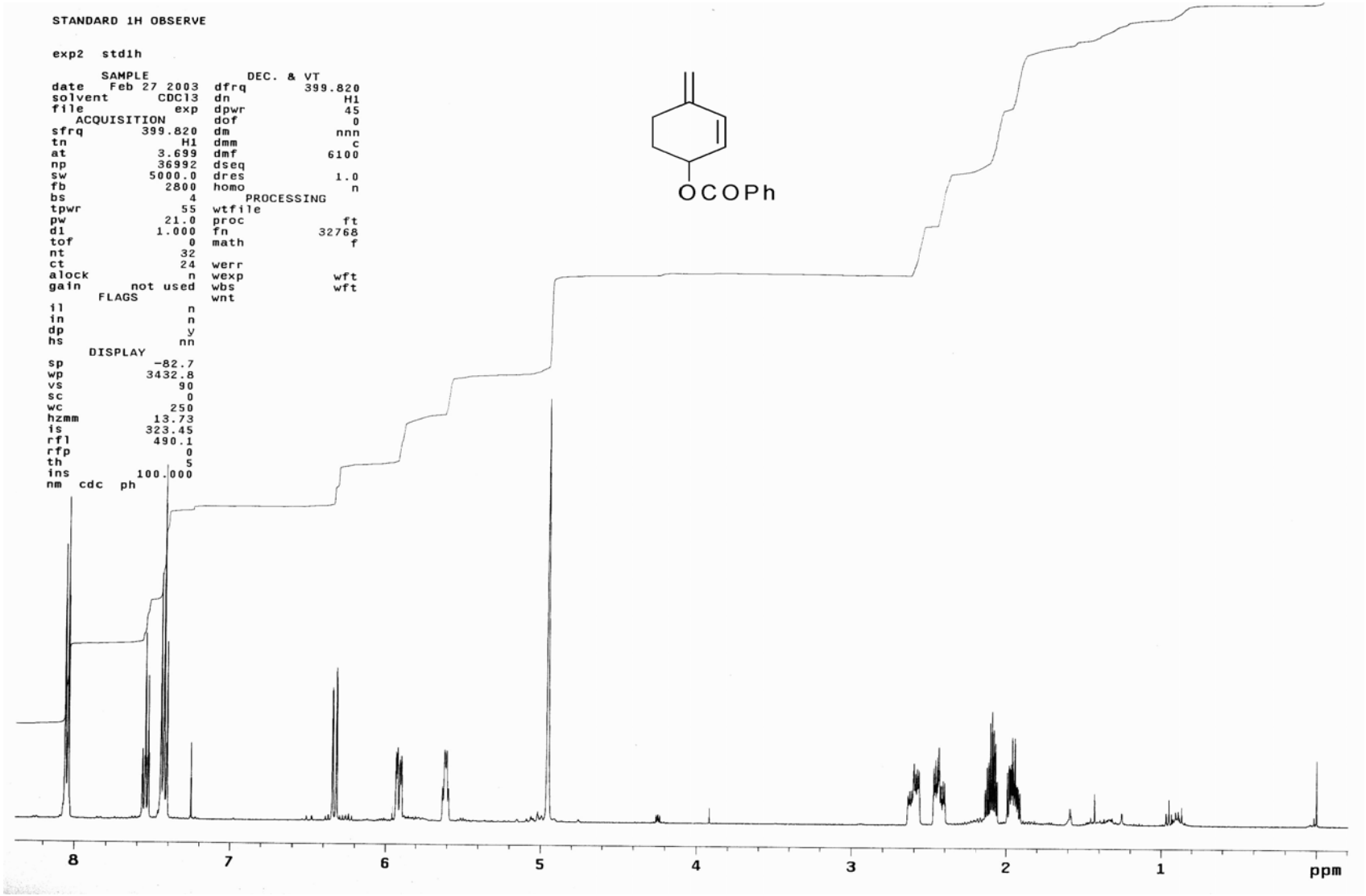
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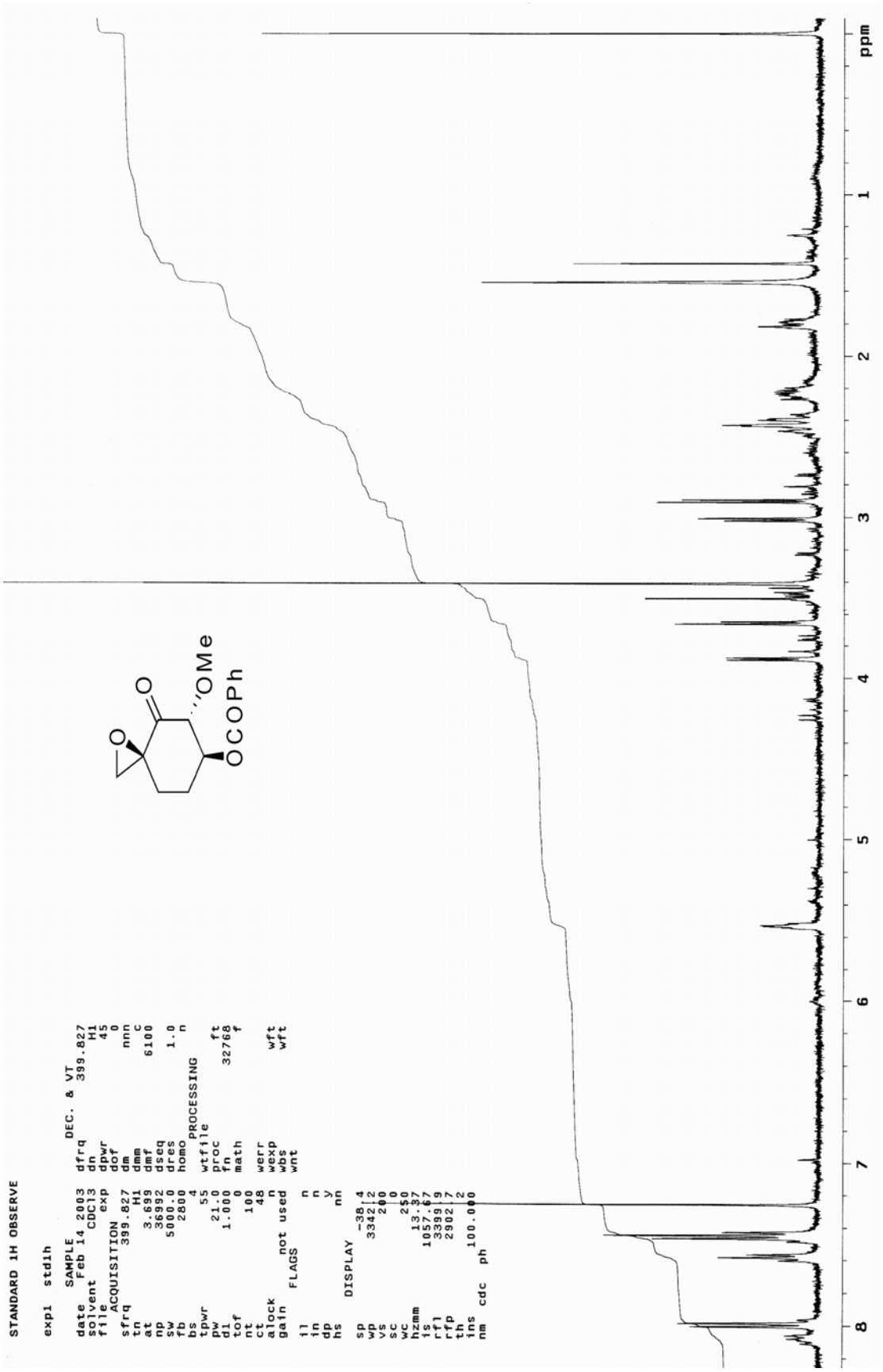
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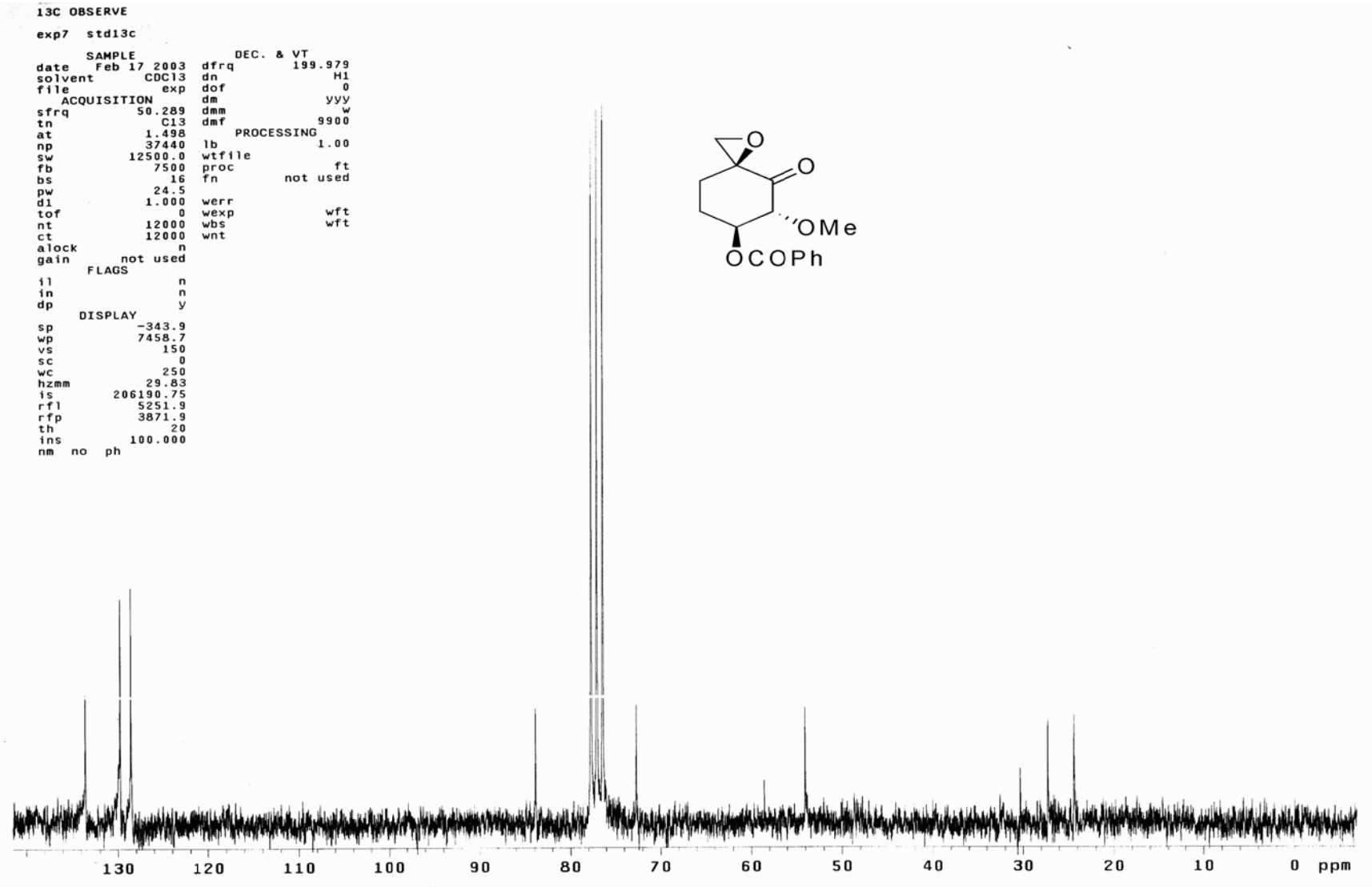
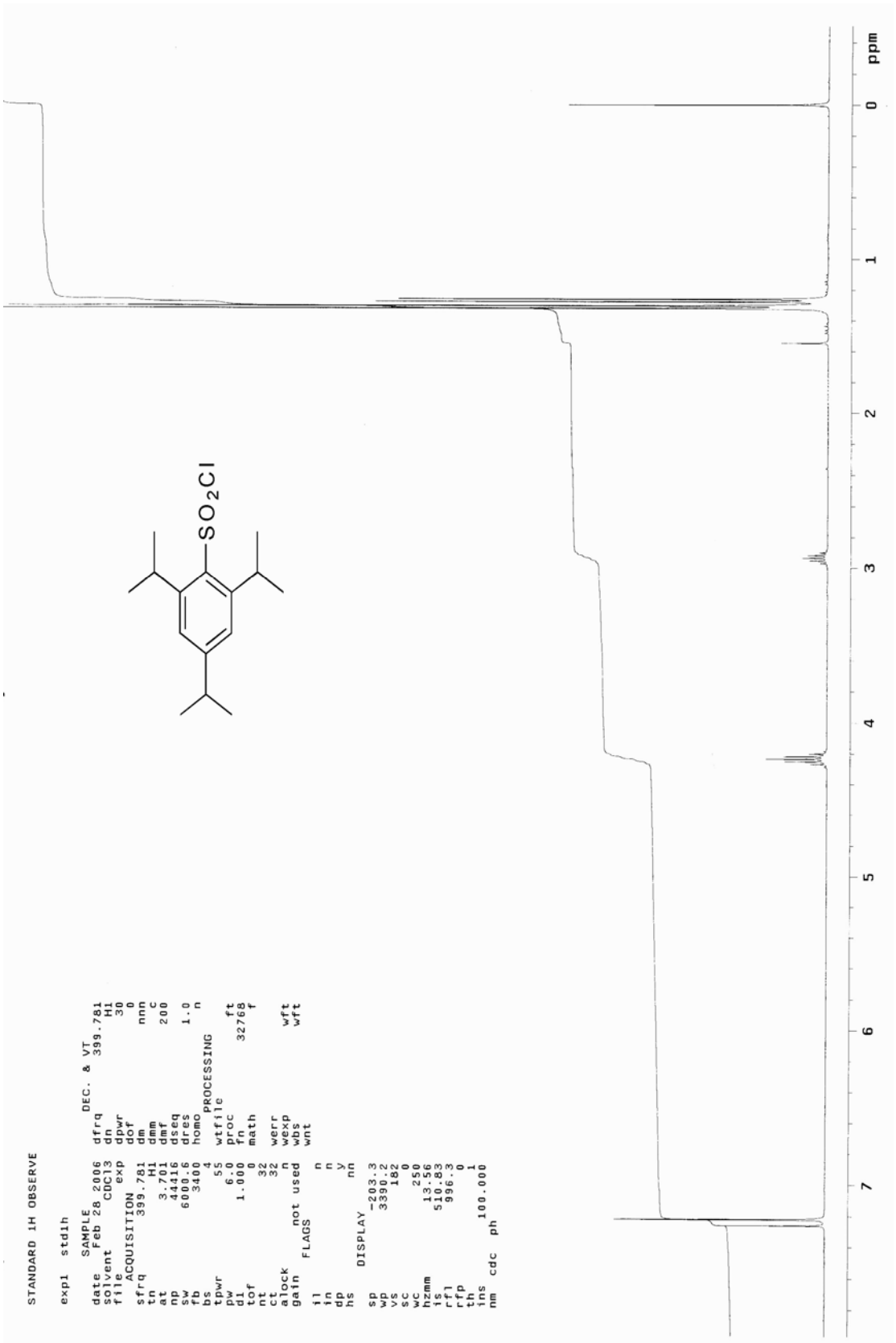
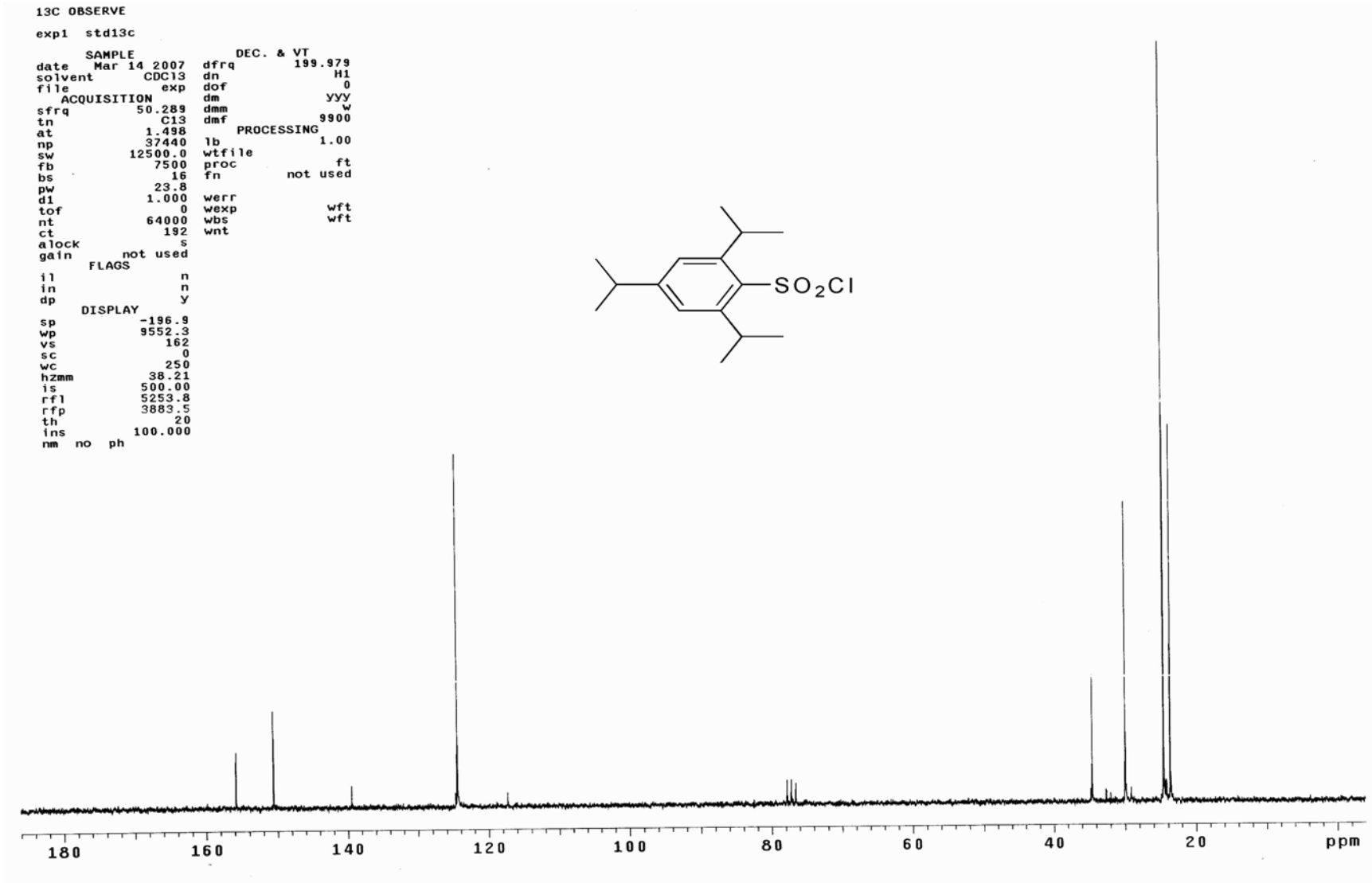
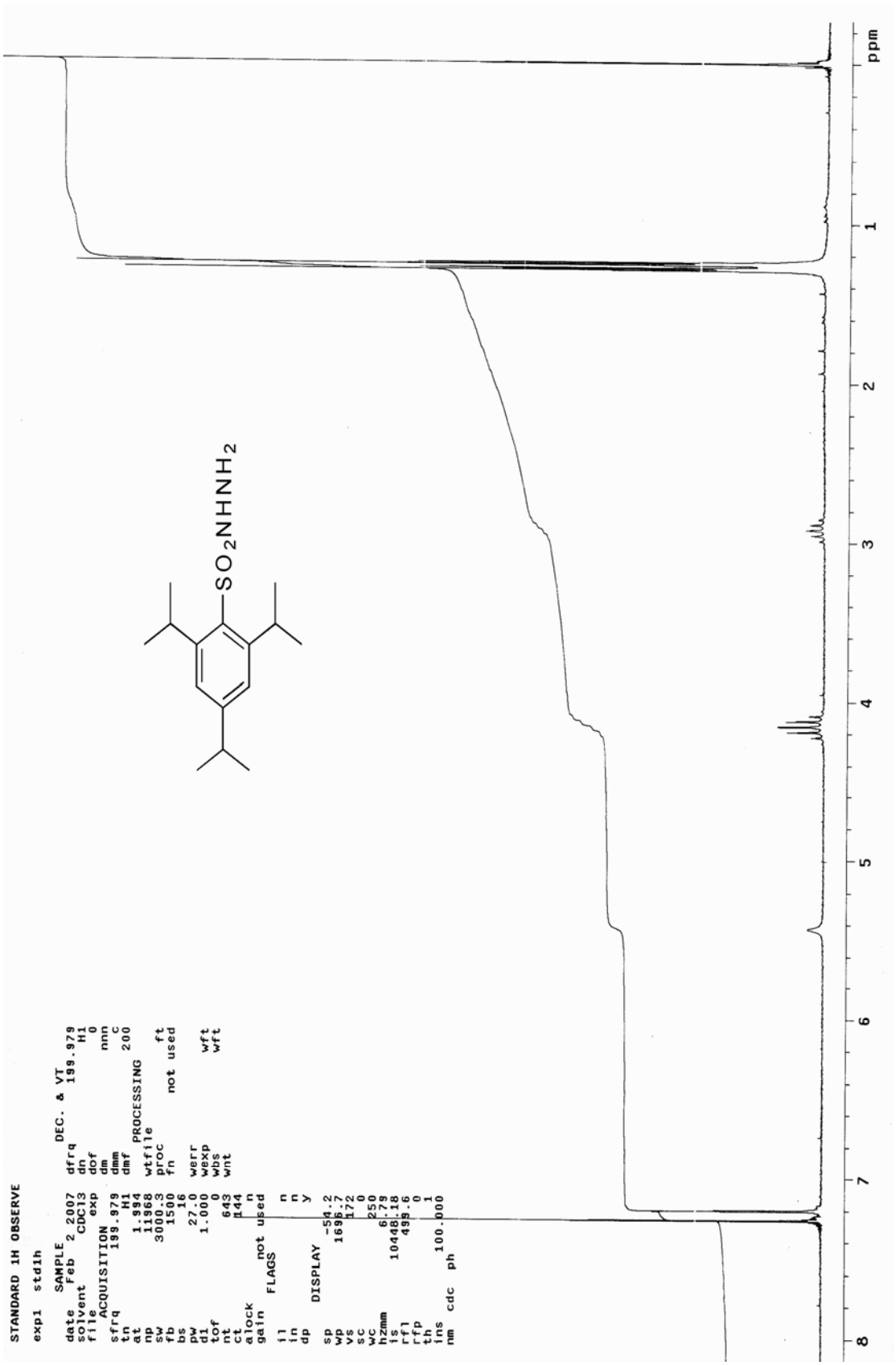
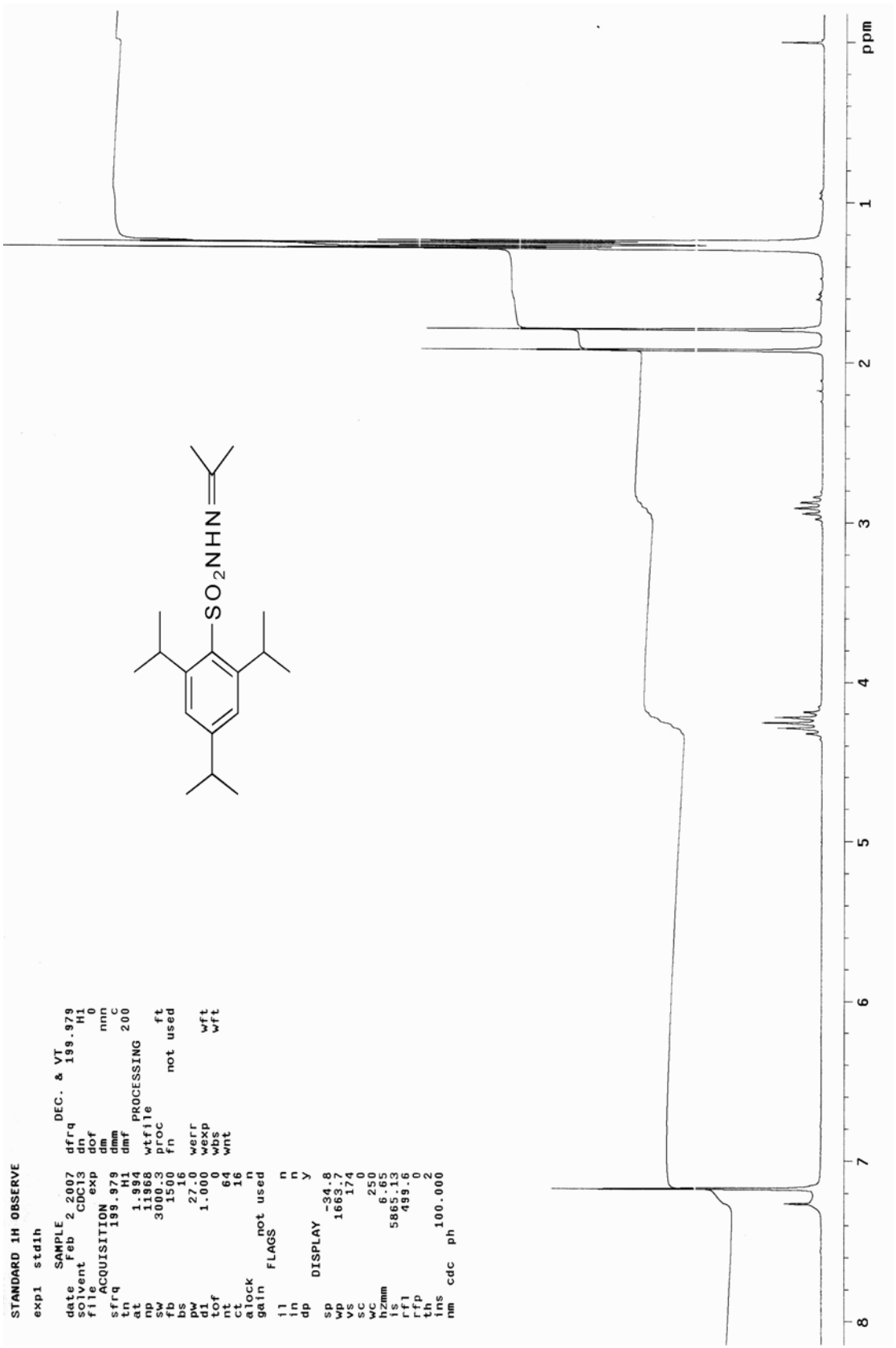
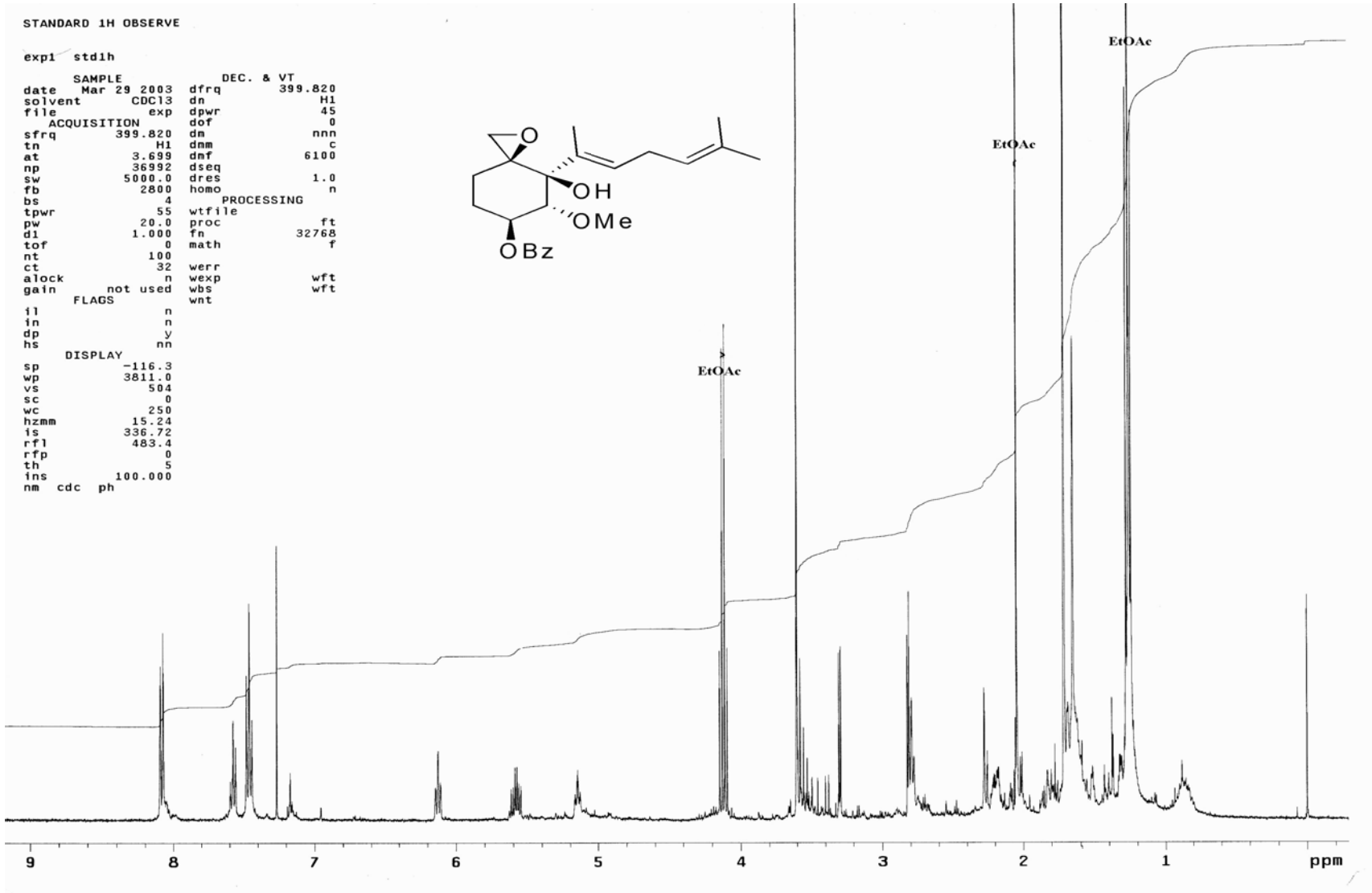

13C OBSERVE
exp7 std13c
SAMPLE
date Feb 17 2003
solvent CDCl3
file exp
ACQUISITION
sfrq 50.289
tn C13
at 1.498
np 37440
sw 12500.0
fb 7500
bs 16
pw 24.5
d1 1.000
tof 0
nt 12000
ct 12000
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -343.9
wp 7458.7
vs 150
sc 0
wc 250
hzmm 29.83
is 206190.75
rfl 5251.9
rfp 3871.9
th 20
ins 100.000
nm no ph
DEC. & VT
dfrq 199.979
dn H1
dof 0
dm yyy
dmm w
dmf 9900
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
werr
wexp wft
wbs wft
wnt
O
O
OMe
OCOPh
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

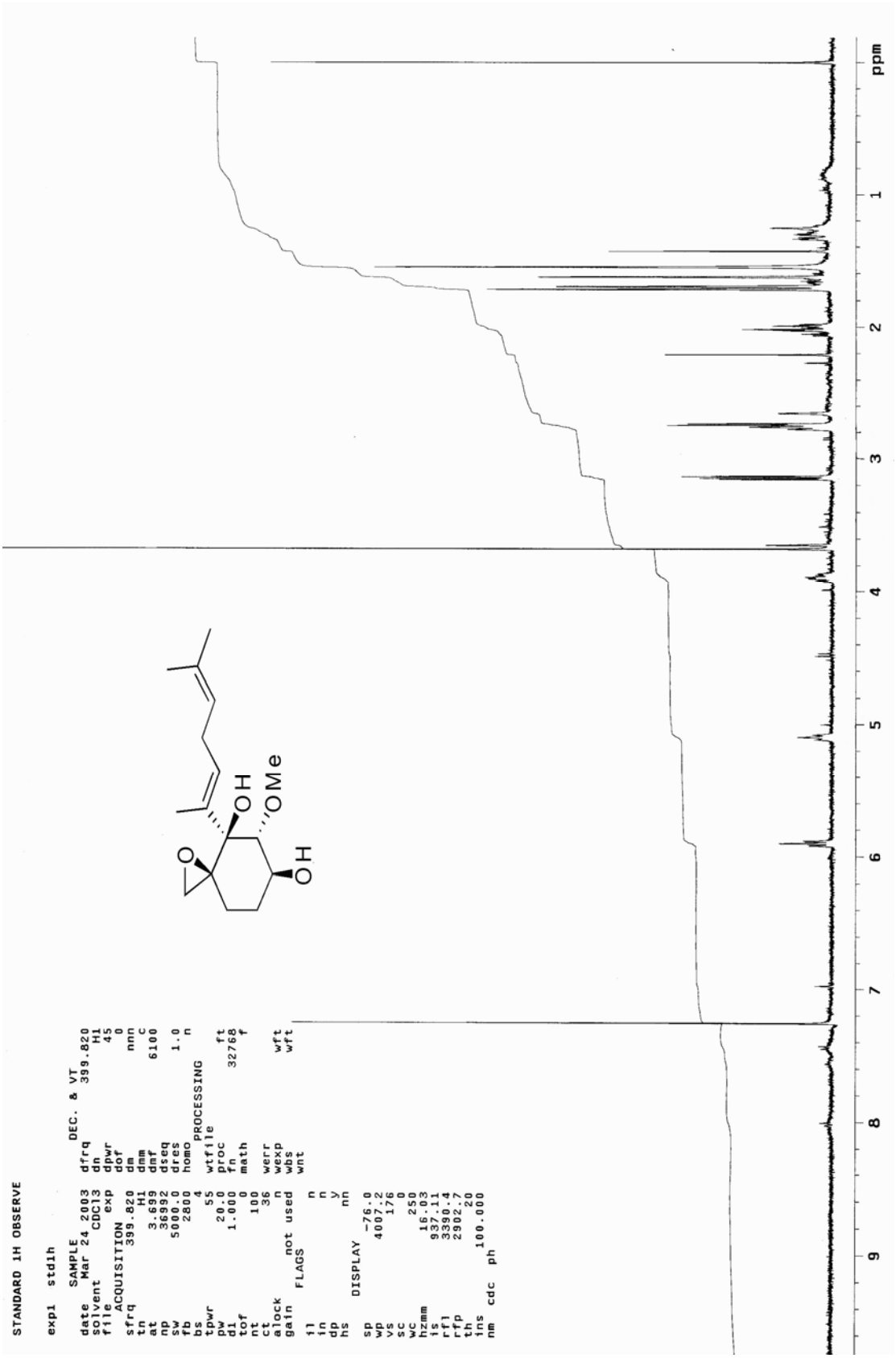

STANDARD 1H OBSERVE
expl stdih
SAMPLE
date Feb 28 2006
solvent CDCl3
file exp
ACQUISITION
sfrq 399.781
tn H1
at 3.701
np 44416
sw 6000.6
fb 3400
bs 4
tpwr 55
pw 6.0
d1 1.000
tof 0
nt 32
ct 32
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -203.3
wp 3390.2
vs 182
sc 0
wc 250
hzmm 13.56
is 510.83
rfl 996.3
rfp 0
th 1
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.781
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
SO2Cl
7
6
5
4
3
2
1
0
ppm


13C OBSERVE
exp1 std13c
SAMPLE
date Mar 14 2007
solvent CDCl3
file exp
ACQUISITION
sfrq 50.289
tn C13
at 1.498
np 37440
sw 12500.0
fb 7500
bs 16
pw 23.8
d1 1.000
tof 0
nt 64000
ct 192
alock s
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -196.9
wp 9552.3
vs 162
sc 0
wc 250
hzmm 38.21
is 500.00
rfl 5253.8
rfp 3883.5
th 20
ins 100.000
nm no ph
DEC. & VT
dfrq 199.979
dn H1
dof 0
dm yyy
dmm w
dmf 9900
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
werr
wexp wft
wbs wft
wnt
SO2Cl
180
160
140
120
100
80
60
40
20
ppm


STANDARD 1H OBSERVE
exp1 std1h
SAMPLE
date Feb 2 2007
solvent CDCl3
file exp
ACQUISITION
sfrq 199.979
tn H1
at 1.984
np 11968
sw 30003
fb 1500
bs 16
dw 27.0
d1 1.000
tof 0
nt 643
ct 144
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -54.2
wp 1695.7
vs 172
sc 0
wc 250
hzmm 6.79
is 10448.18
rfl 495.6
rfp 0
th 1
ins 100.000
nm cdc ph
DEC. & VT
dfrq 199.979
dn H1
dof 0
dm nnn
dmm c
dmf 200
PROCESSING
wtfile
proc ft
fn not used
werr
wexp wft
wbs wft
wnt
SO_2NHNH_2
8
7
6
5
4
3
2
1
ppm


STANDARD 1H OBSERVE
exp1 std1h
SAMPLE DEC. & VT
date Feb 2 2007 dfrq 199.979
solvent CDCl3 dn H1
file exp dof 0
ACQUISITION dm nnn
sfrq 199.979 dmm c
tn H1 dmf 200
at 1.984 PROCESSING
np 11968 wtfile ft
sw 3001.3 proc not used
fb 1500 fn
bs 16
pw 27.0 werr wft
d1 1.000 wexp wft
tof 0 wbs
nt 64 wnt
ct 16
alock n
gain n
FLAGS not used
il n
in n
dp y
DISPLAY
sp -34.8
wp 1663.7
vs 174
sc 0
wc 250
hzmm 6.65
is 5865.13
rfl 499.6
rfp 0
th 2
ins 100.000
nm cdc ph
SO2NHN
8
7
6
5
4
3
2
1
ppm


STANDARD 1H OBSERVE
exp1 std1h
SAMPLE
date Mar 29 2003
solvent CDCl3
file exp
ACQUISITION
sfrq 399.820
tn H1
at 3.699
np 36992
sw 5000.0
fb 2800
bs 4
tpwr 55
pw 20.0
d1 1.000
tof 0
nt 100
ct 32
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -116.3
wp 3811.0
vs 504
sc 0
wc 250
hzmm 15.24
is 336.72
rfl 483.4
rfp 0
th 5
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.820
dn H1
dpwr 45
dof 0
dm nnn
dmm c
dmf 6100
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
O
OH
OMe
OBz
EtOAc
EtOAc
EtOAc
9
8
7
6
5
4
3
2
1
ppm


STANDARD 1H OBSERVE
expl stdlh
SAMPLE
date Mar 24 2003
solvent CDCl3
file exp
ACQUISITION
sfrq 399.820
tn H1
at 3.699
np 36992
sw 5000.0
fb 2800
bs 4
tpwr 55
pw 20.0
d1 1.000
tof 0
nt 100
ct 36
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -76.0
wp 4007.2
vs 176
sc 0
wc 250
hzmm 16.03
is 937.11
rfl 3390.4
rfp 2902.7
th 20
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.820
dn H1
dpwr 45
dof 0
dm nnn
dmm c
dmf 6100
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
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OMe
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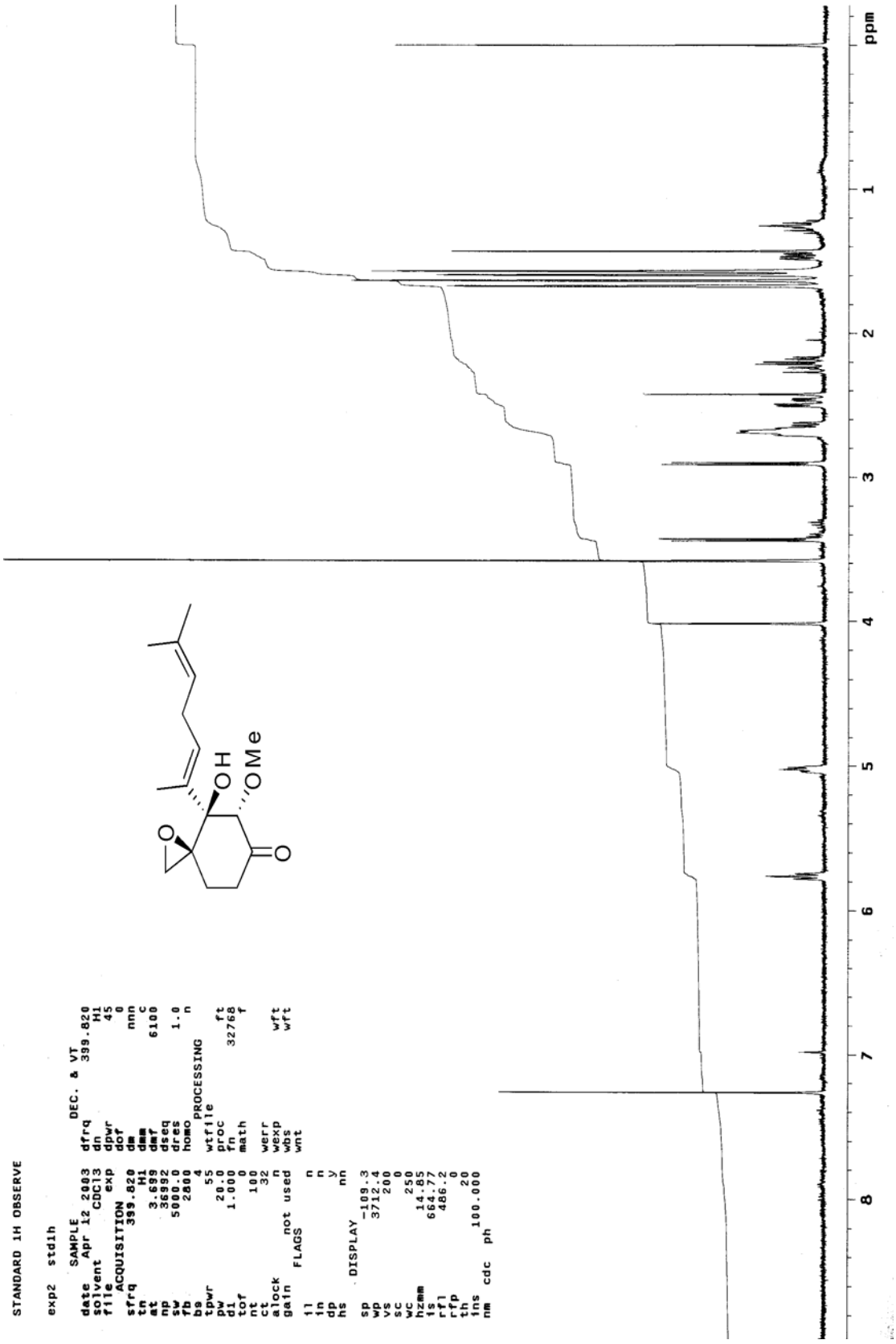
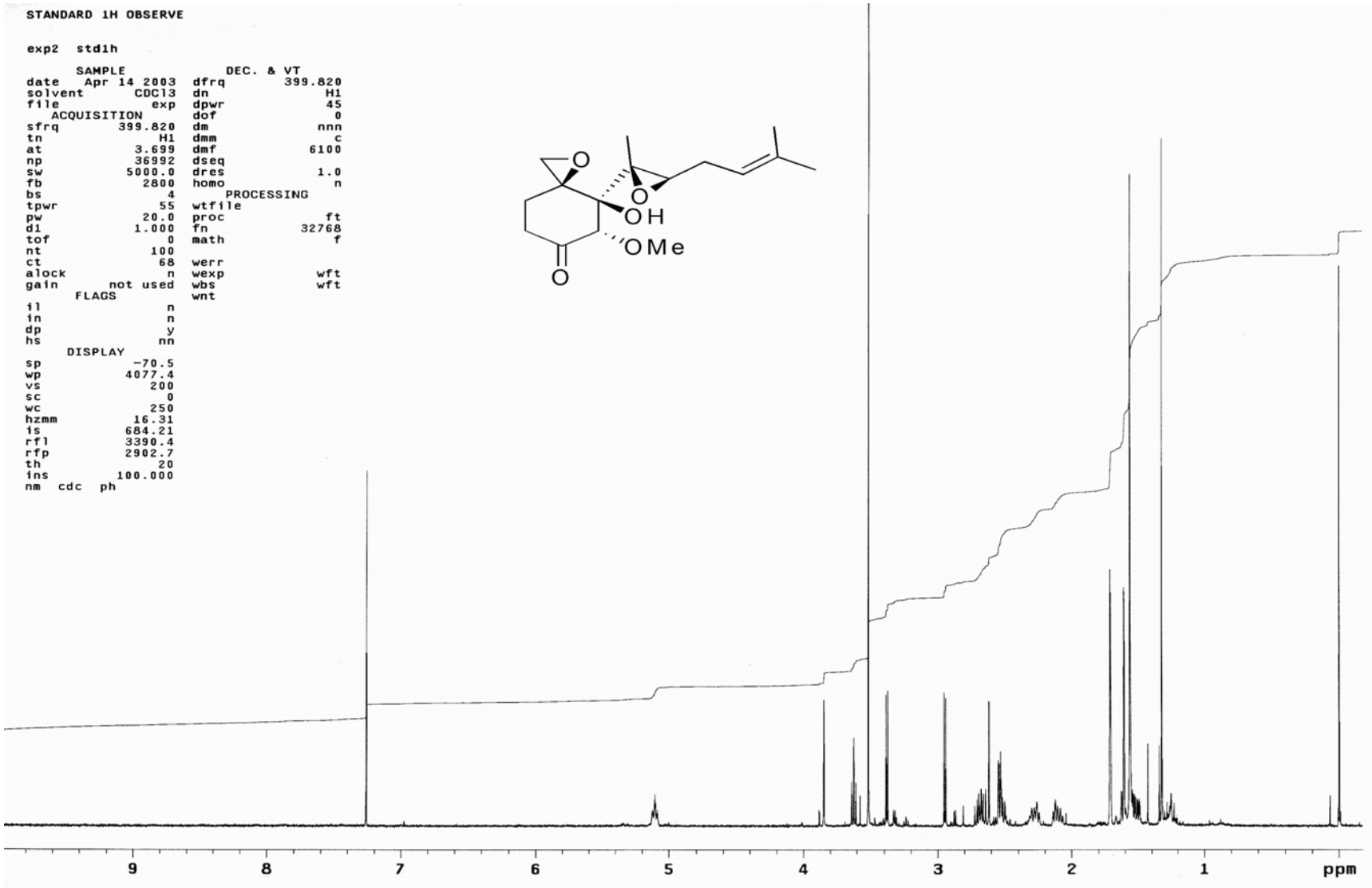
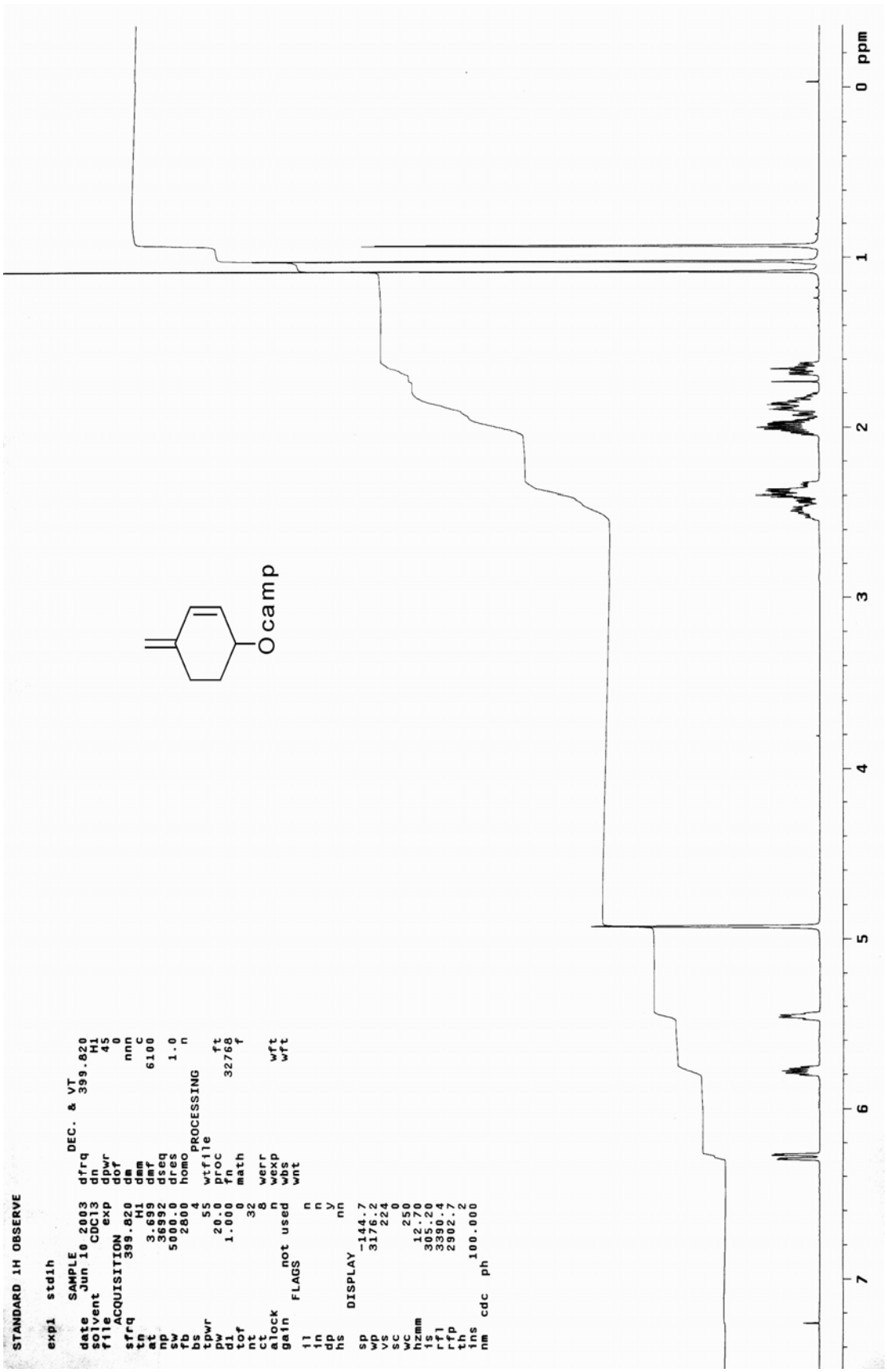
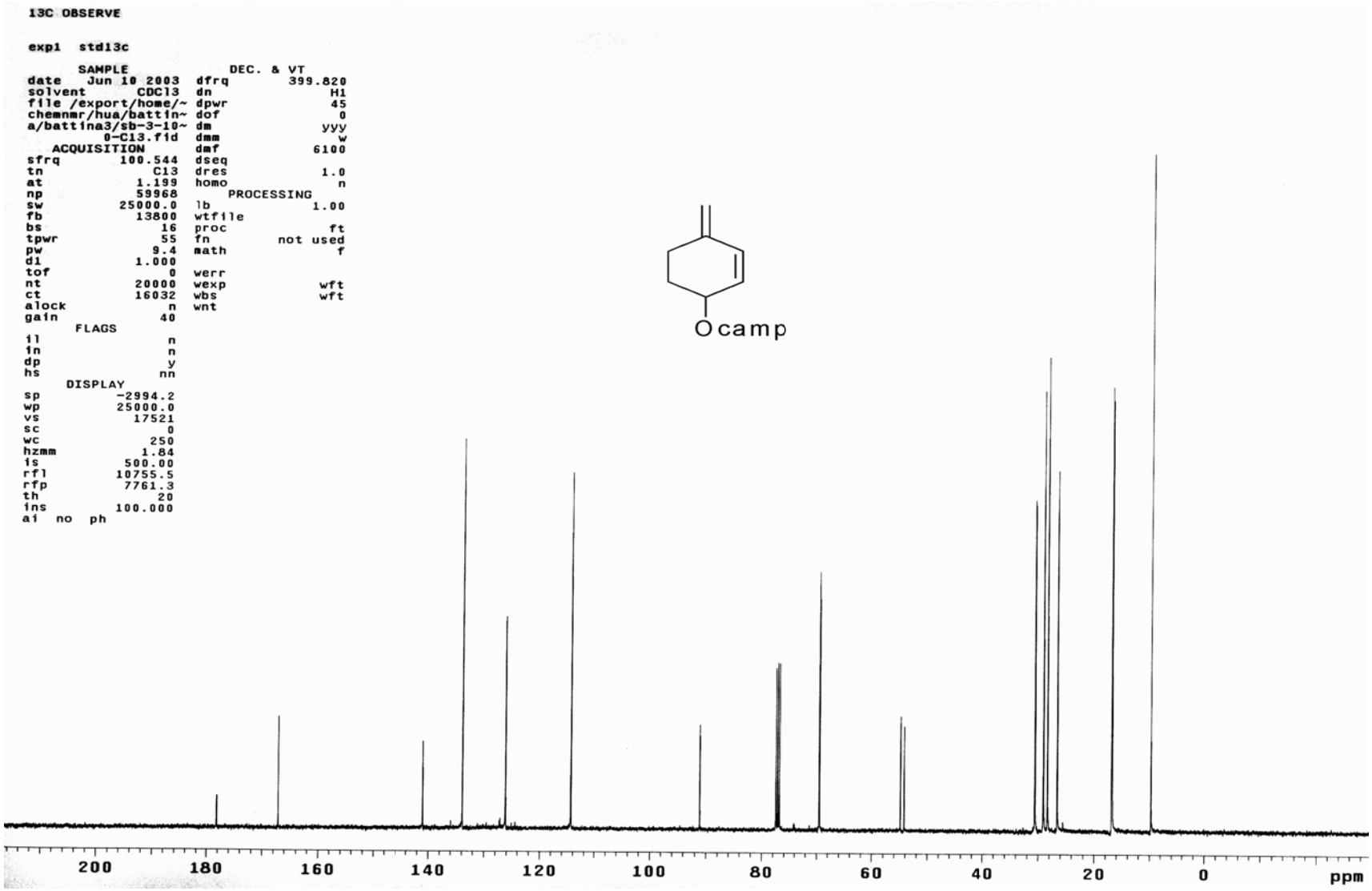
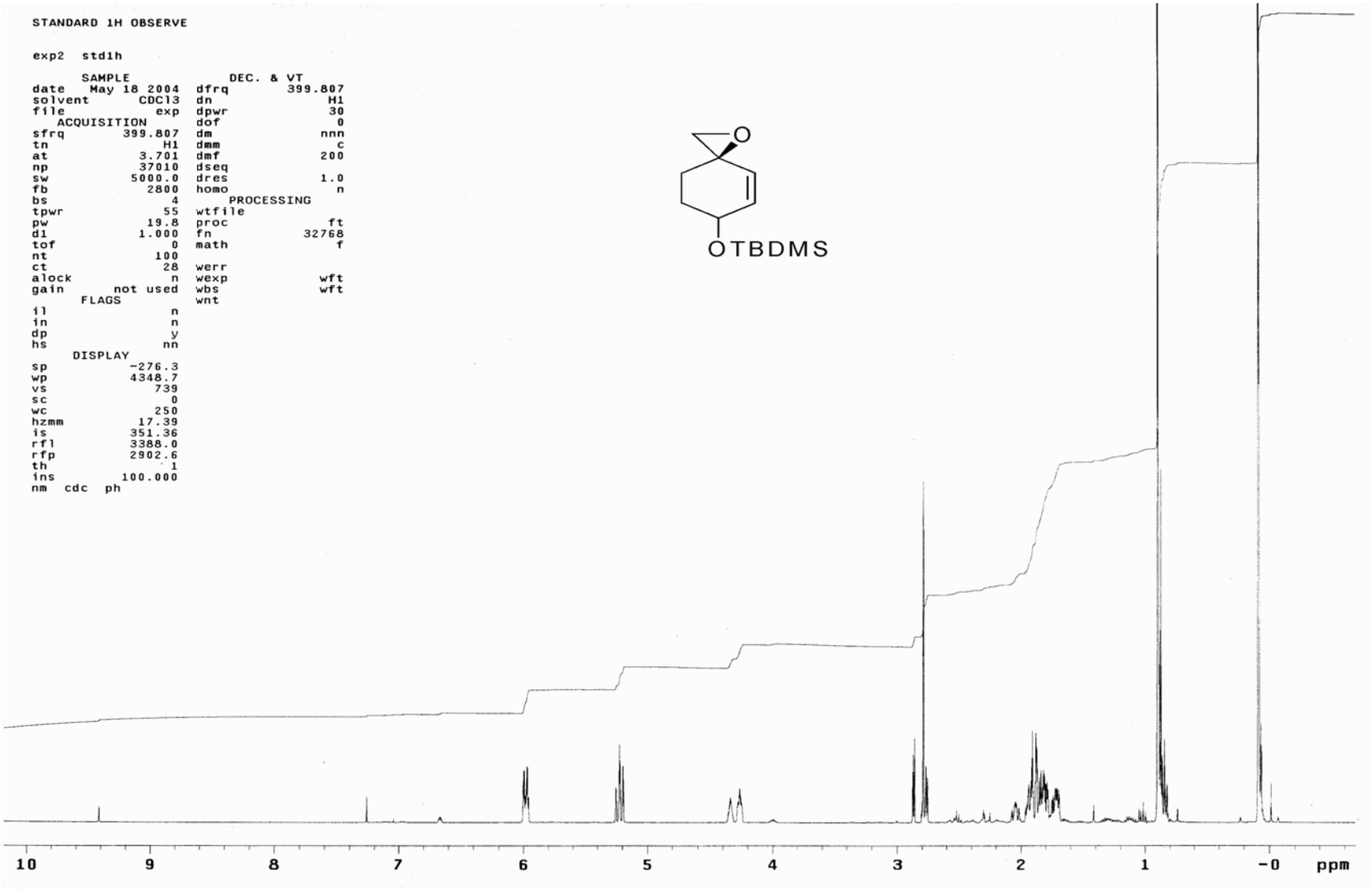
ppm
9
8
7
6
5
4
3
2
1

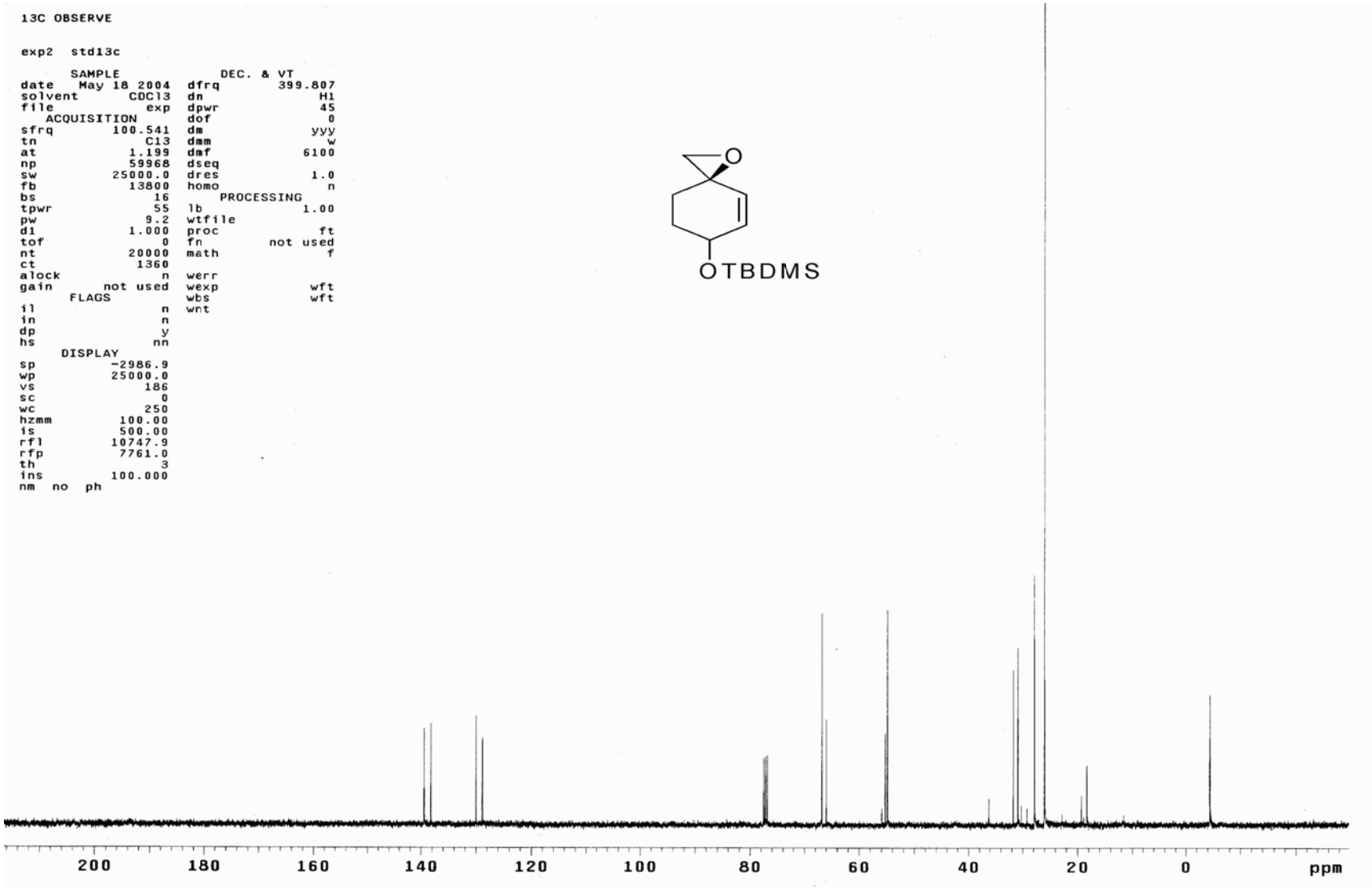

STANDARD 1H OBSERVE
exp2 stdih
SAMPLE
date Apr 12 2003
solvent CDCl3
file exp
ACQUISITION
sfrq 399.820
tn H1
at 3.699
np 36992
sw 5000.0
fb 2800
bs 4
tpwr 55
pw 20.0
d1 1.000
tof 0
nt 100
ct 32
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -109.3
wp 3712.4
vs 200
sc 0
wc 250
hzmm 14.85
is 664.77
rfl 486.2
rfp 0
th 20
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.820
dn H1
dpwr 45
dof 0
dm nnn
dmm c
dmf 6100
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
OH
OMe
O
O
8
7
6
5
4
3
2
1
ppm

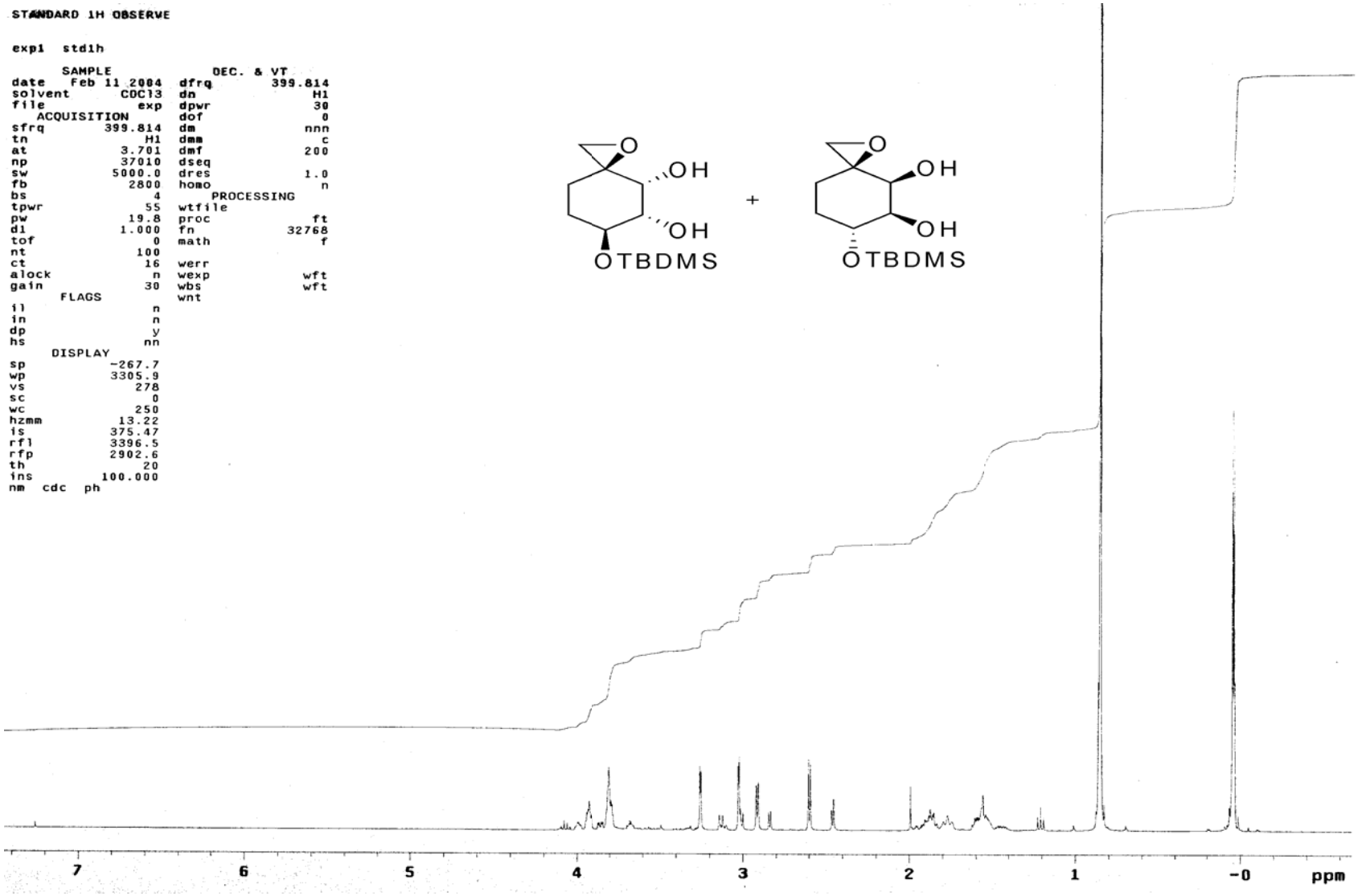

STANDARD 1H OBSERVE
exp2 std1h
SAMPLE
date Apr 14 2003
solvent CDCl3
file exp
ACQUISITION
sfrq 399.820
tn H1
at 3.699
np 36992
sw 5000.0
fb 2800
bs 4
tpwr 55
pw 20.0
d1 1.000
tof 0
nt 100
ct 68
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -70.5
wp 4077.4
vs 200
sc 0
wc 250
hzmm 16.31
is 684.21
rfl 3390.4
rfp 2902.7
th 20
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.820
dn H1
dpwr 45
dof 0
dm nnn
dmm c
dmf 6100
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
O
O
OH
OMe
O
9
8
7
6
5
4
3
2
1
ppm

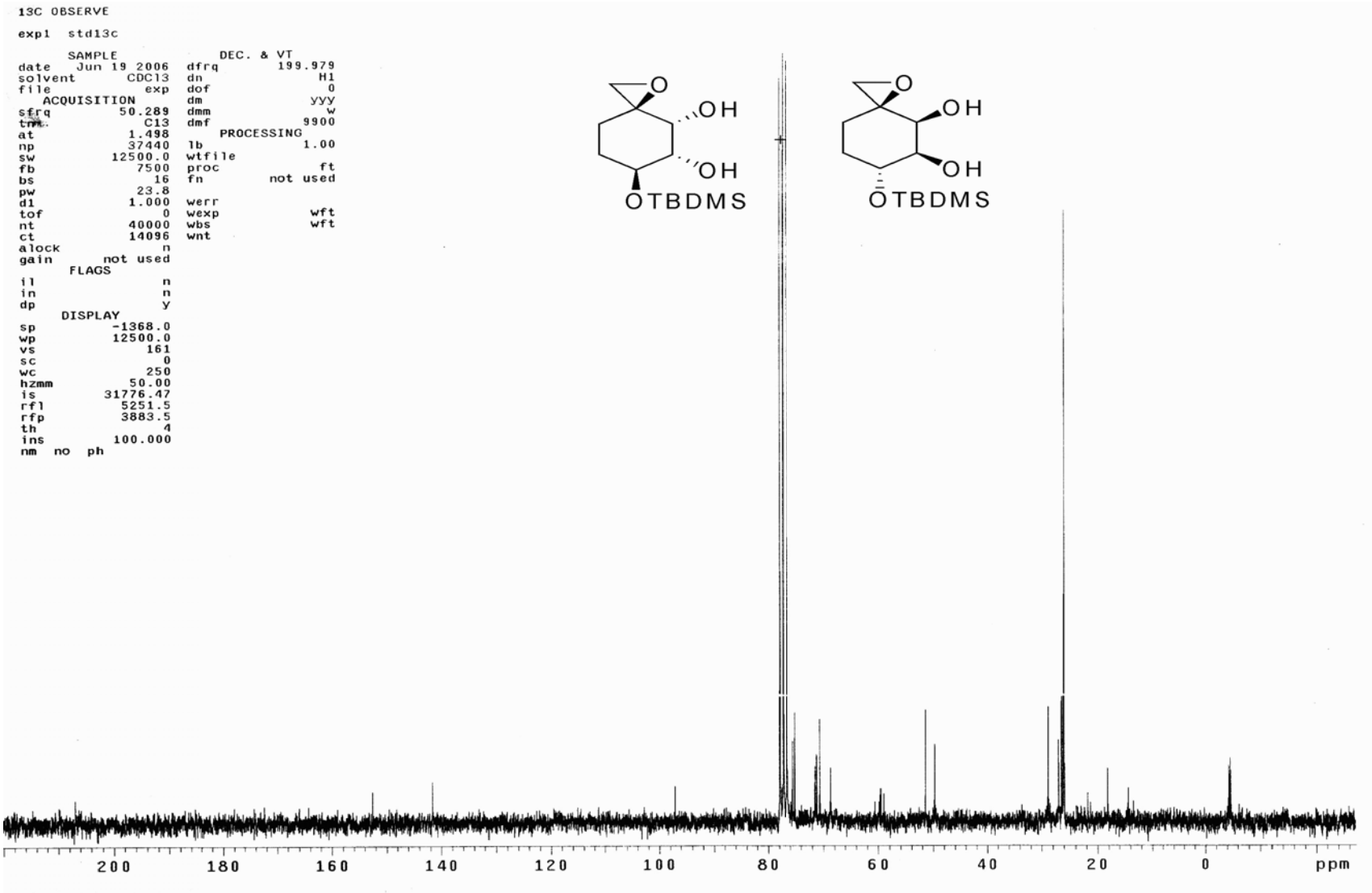

STANDARD 1H OBSERVE
expl stdih
SAMPLE
date Jun 10 2003
solvent CDCl3
file exp
ACQUISITION
sfrq 399.820
tn H1
at 3.699
np 36992
sw 5000.0
fb 2800
bs 4
tpwr 55
pw 20.0
d1 1.000
tof 0
nt 32
ct 8
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -144.7
wp 3176.2
vs 224
sc 0
wc 250
hzmm 12.70
is 305.20
rfl 3390.4
rfp 2902.7
th 2
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.820
dn H1
dpwr 45
dof 0
dm nnn
dmm c
dmf 6100
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
Ocamp
0 ppm

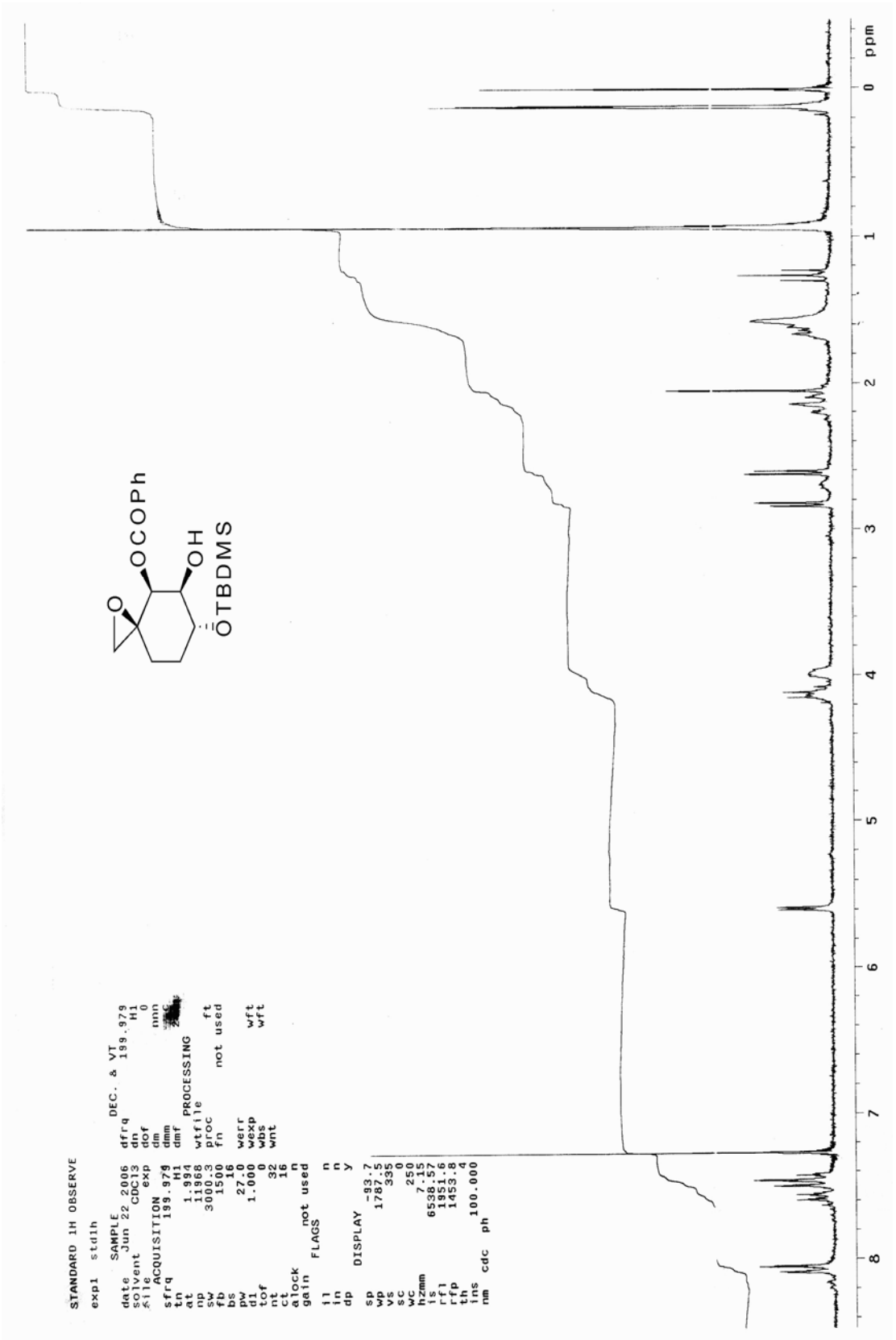

13C OBSERVE
expl std13c
SAMPLE
date Jun 10 2003
solvent CDCl3
file /export/home/~
chemnmr/hua/battin~
a/battina3/sb-3-10~
0-C13.fid
ACQUISITION
sfrq 100.544
tn C13
at 1.199
np 59968
sw 25000.0
fb 13800
bs 16
tpwr 55
pw 9.4
d1 1.000
tof 0
nt 20000
ct 16032
alock n
gain 40
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -2994.2
wp 25000.0
vs 17521
sc 0
wc 250
hzmm 1.84
is 500.00
rfl 10755.5
rfp 7761.3
th 20
ins 100.000
ai no ph
DEC. & VT
dfrq 399.820
dn H1
dpwr 45
dof 0
dm yyy
dmm w
dmf 6100
dseq
dres 1.0
homo n
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
math f
werr
wexp wft
wbs wft
wnt
Ocamp
200
180
160
140
120
100
80
60
40
20
0
ppm

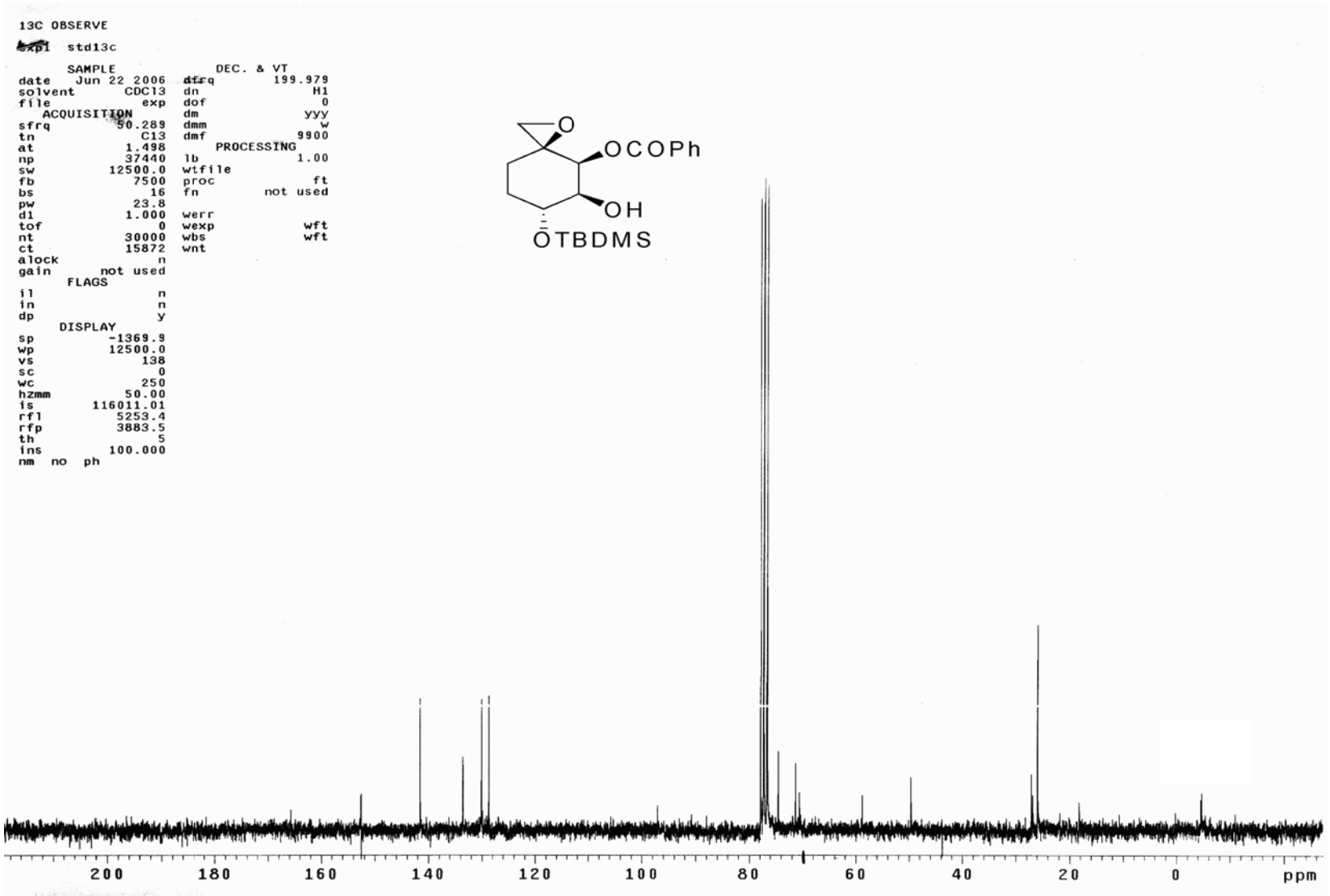

STANDARD 1H OBSERVE
exp2 std1h
SAMPLE
date May 18 2004
solvent CDCl3
file exp
ACQUISITION
sfrq 399.807
tn H1
at 3.701
np 37010
sw 5000.0
fb 2800
bs 4
tpwr 55
pw 19.8
d1 1.000
tof 0
nt 100
ct 28
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -276.3
wp 4348.7
vs 739
sc 0
wc 250
hzmm 17.39
is 351.36
rfl 3388.0
rfp 2902.6
th 1
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.807
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
O
OTBDMS
10
9
8
7
6
5
4
3
2
1
-0
ppm

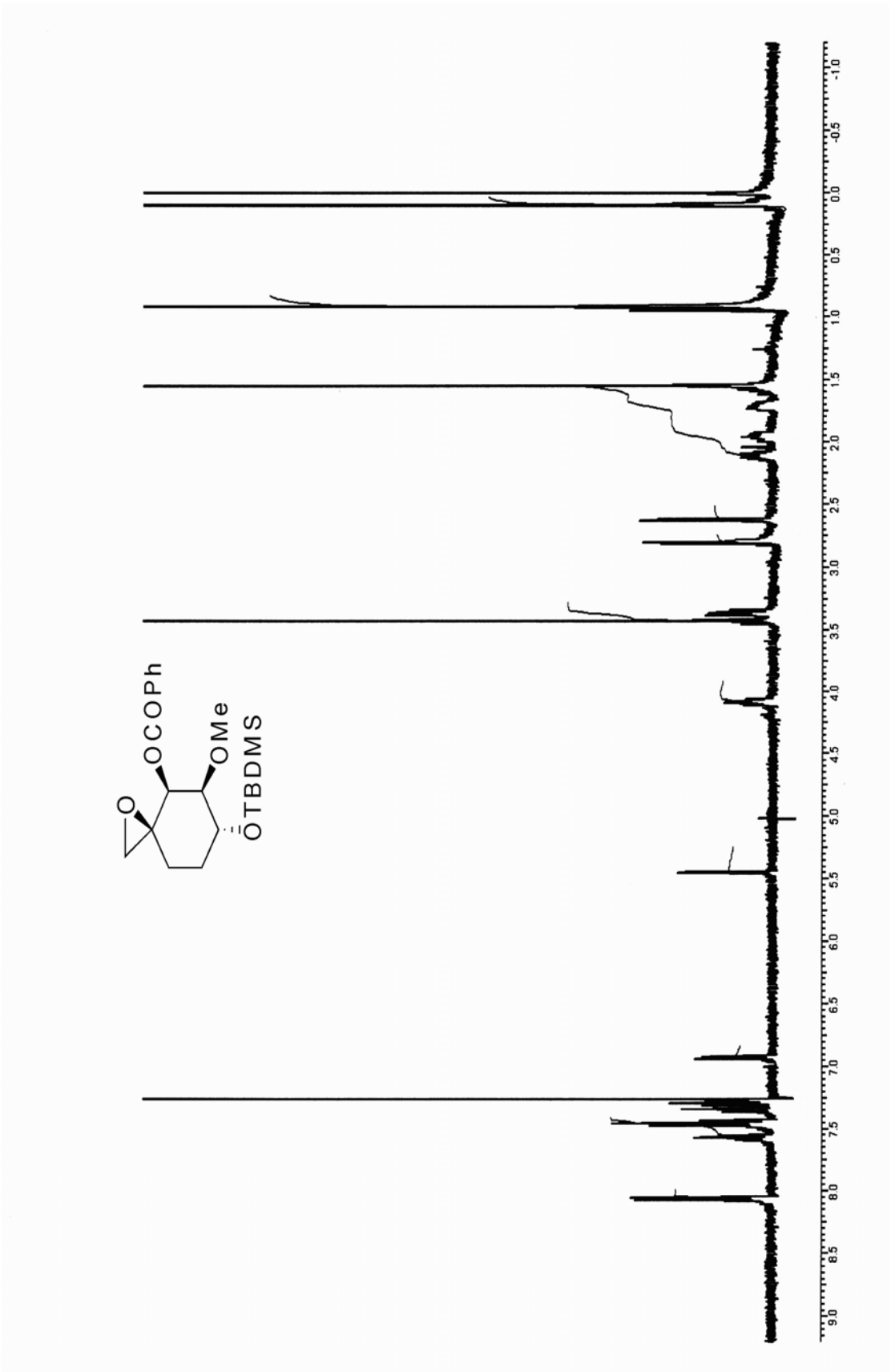

13C OBSERVE
exp2 std13c
SAMPLE
date May 18 2004
solvent CDCl3
file exp
ACQUISITION
sfrq 100.541
tn C13
at 1.199
np 59968
sw 25000.0
fb 13800
bs 16
tpwr 55
pw 9.2
d1 1.000
tof 0
nt 20000
ct 1360
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -2986.9
wp 25000.0
vs 186
sc 0
wc 250
hzmm 100.00
is 500.00
rfl 10747.9
rfp 7761.0
th 3
ins 100.000
nm no ph
DEC. & VT
dfrq 399.807
dn H1
dpwr 45
dof 0
dm yyy
dmm w
dmf 6100
dseq
dres 1.0
homo n
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
math f
werr
wexp wft
wbs wft
wnt
O
OTBDMS
200
180
160
140
120
100
80
60
40
20
0
ppm

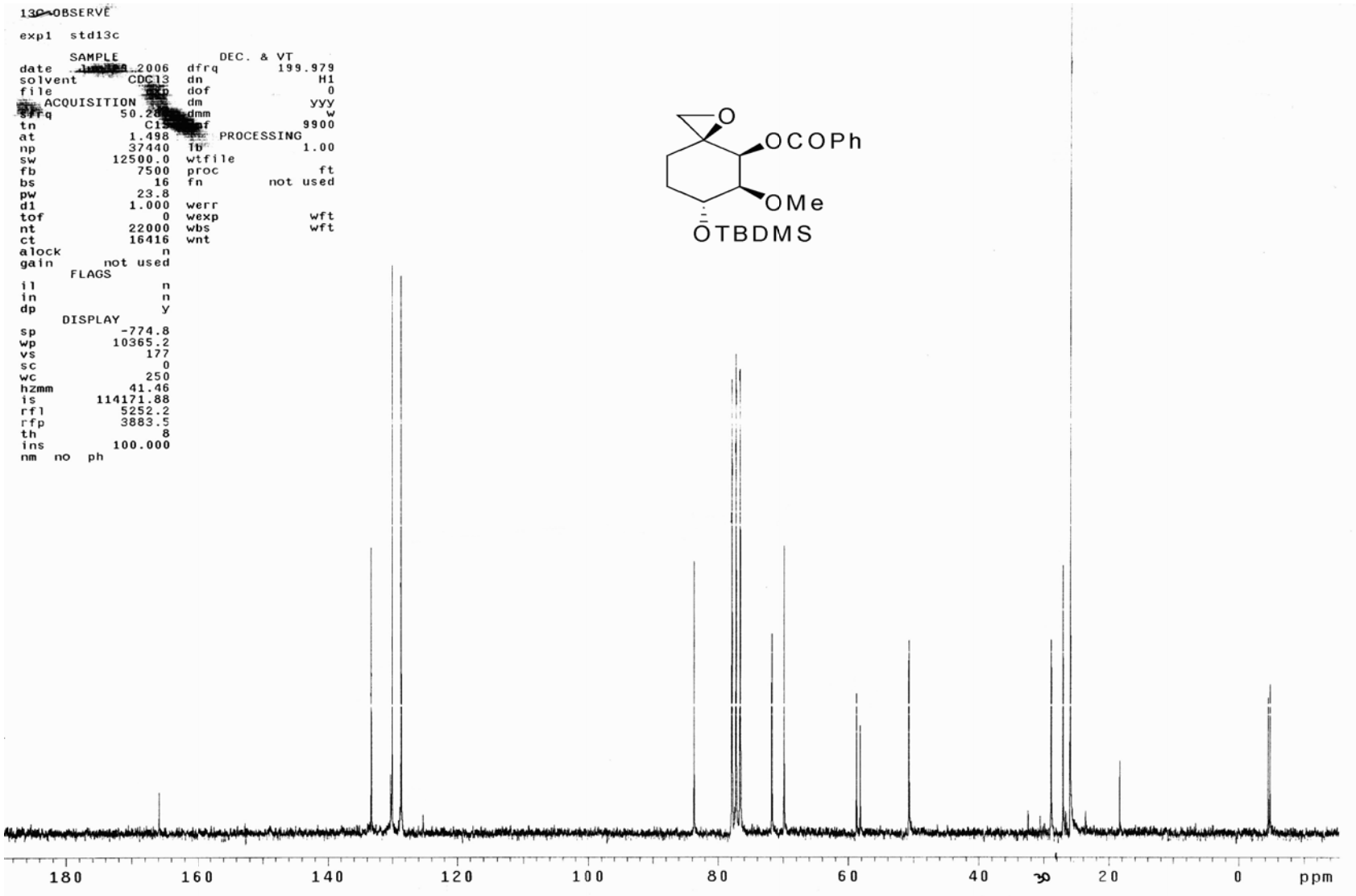

STANDARD 1H OBSERVE
exp1 std1h
SAMPLE
date Feb 11 2004
solvent CDCl3
file exp
ACQUISITION
sfrq 399.814
tn H1
at 3.701
np 37010
sw 5000.0
fb 2800
bs 4
tpwr 55
pw 19.8
d1 1.000
tof 0
nt 100
ct 16
alock n
gain 30
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -267.7
wp 3305.9
vs 278
sc 0
wc 250
hzmm 13.22
is 375.47
rfl 3396.5
rfp 2902.6
th 20
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.814
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
O
OH
OH
OTBDMS
+
O
OH
OH
OTBDMS
7
6
5
4
3
2
1
-0
ppm

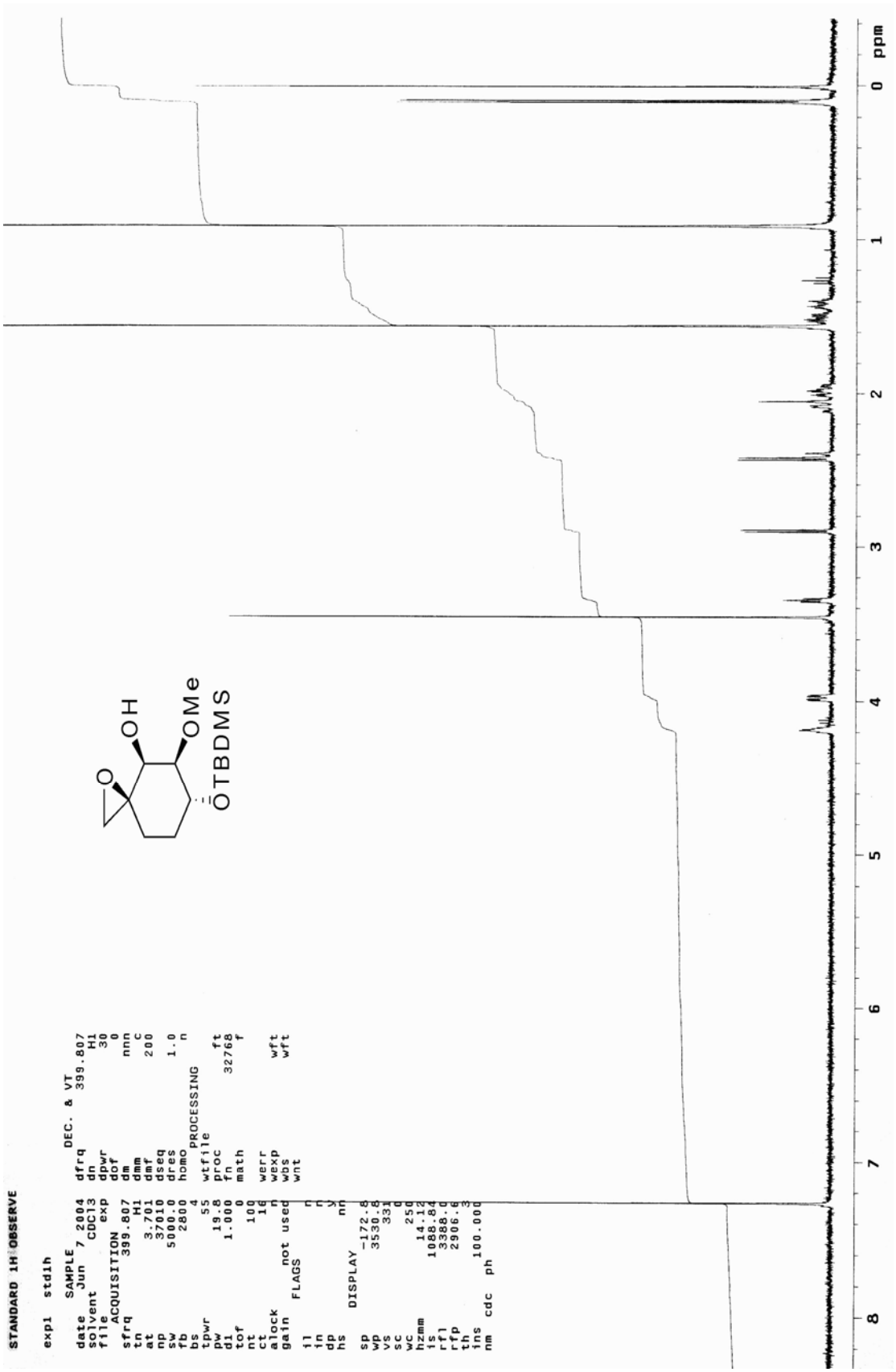

13C OBSERVE
expl std13c
SAMPLE
date Jun 19 2006
solvent CDCl3
file exp
ACQUISITION
sfrq 50.289
tn C13
at 1.498
np 37440
sw 12500.0
fb 7500
bs 16
pw 23.8
d1 1.000
tof 0
nt 40000
ct 14096
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -1368.0
wp 12500.0
vs 161
sc 0
wc 250
hzmm 50.00
is 31776.47
rfl 5251.5
rfp 3883.5
th 4
ins 100.000
nm no ph
DEC. & VT
dfrq 199.979
dn H1
dof 0
dm yyy
dmm w
dmf 9900
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
werr
wexp wft
wbs wft
wnt
O
OH
OH
OTBDMS
O
OH
OH
OTBDMS
200
180
160
140
120
100
80
60
40
20
0
ppm

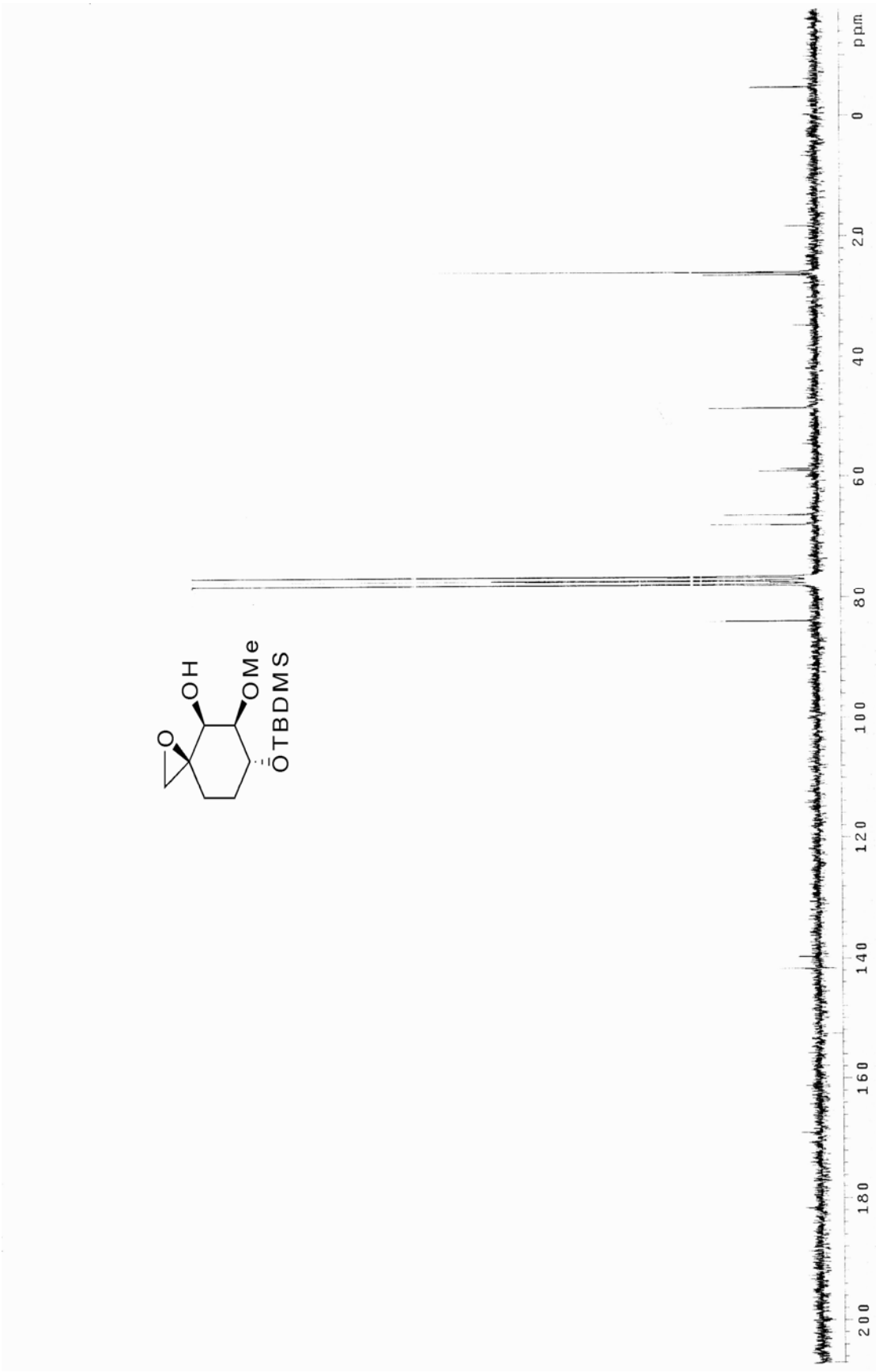

STANDARD 1H OBSERVE
OCOPh
OH
OTBDMS
ppm

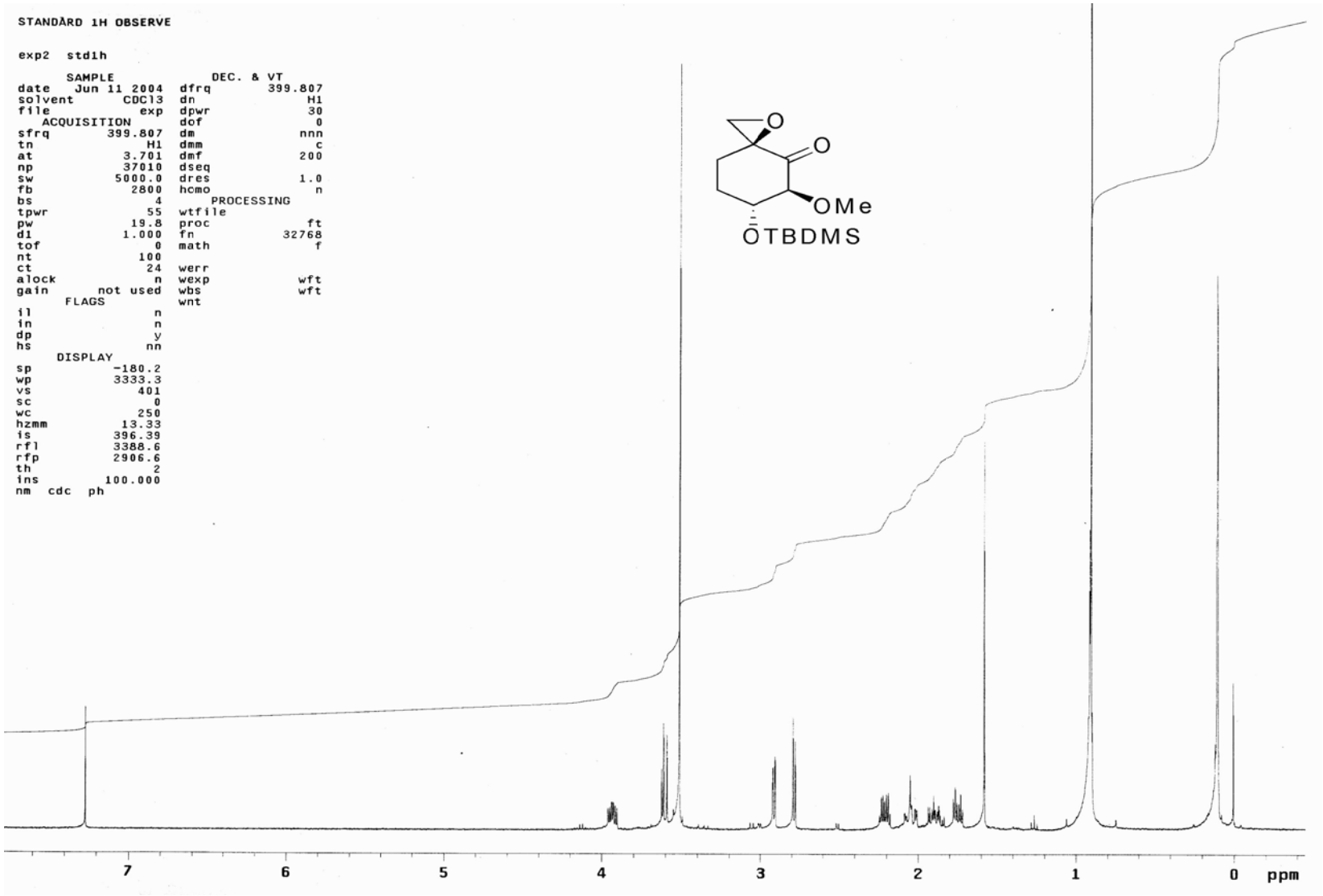

13C OBSERVE
exp1 std13c
SAMPLE
date Jun 22 2006
solvent CDCl3
file exp
ACQUISITION
sfrq 50.289
tn C13
at 1.498
np 37440
sw 12500.0
fb 7500
bs 16
pw 23.8
d1 1.000
tof 0
nt 30000
ct 15872
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -1369.9
wp 12500.0
vs 138
sc 0
wc 250
hzmm 50.00
is 116011.01
rfl 5253.4
rfp 3883.5
th 5
ins 100.000
nm no ph
DEC. & VT
dfrq 199.979
dn H1
dof 0
dm yyy
dmm w
dmf 9900
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
werr
wexp wft
wbs wft
wnt
O
OCOPh
OH
OTBDMS
200
180
160
140
120
100
80
60
40
20
0
ppm

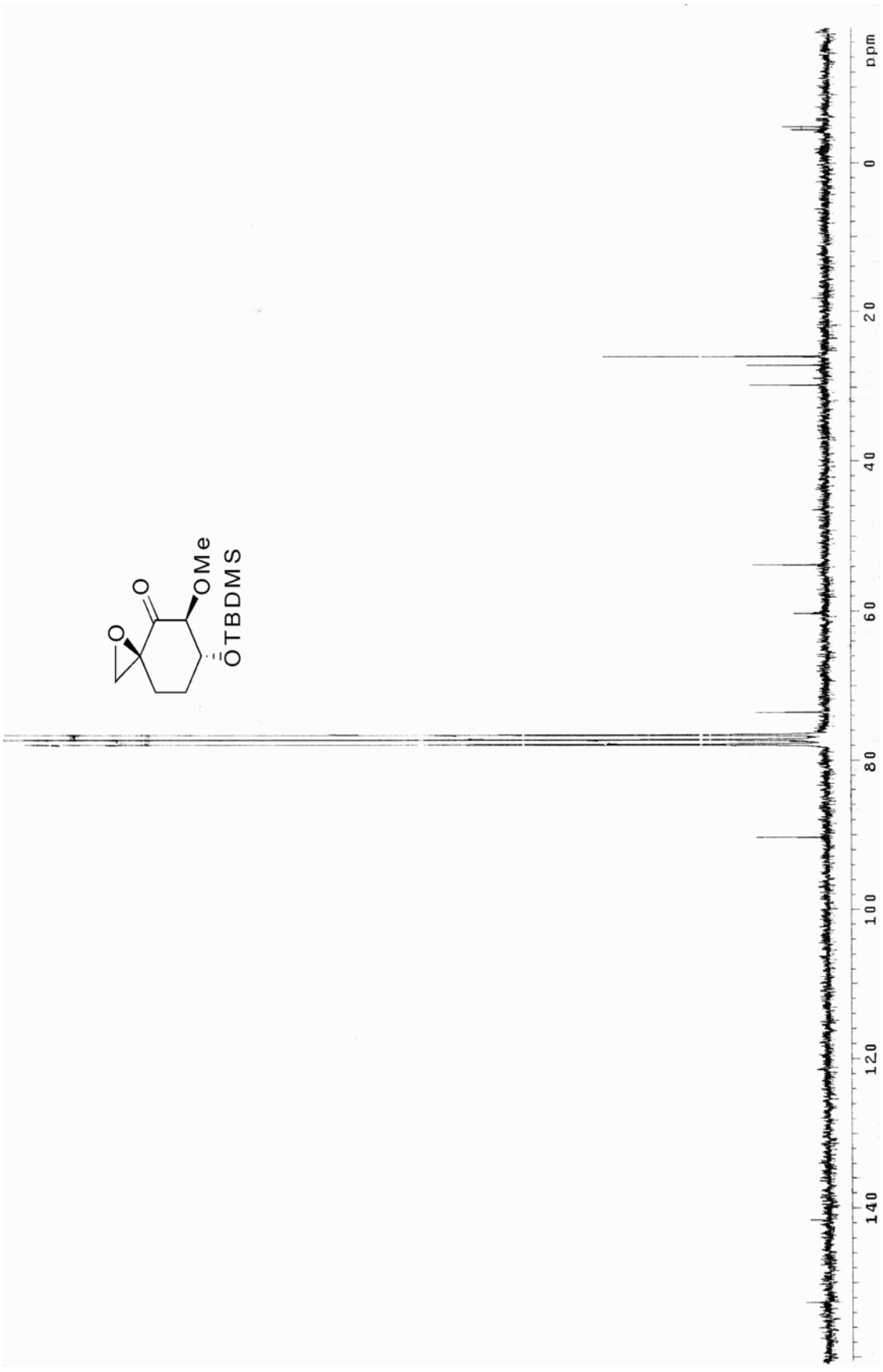

OCOPh
OMe
OTBDMS
O

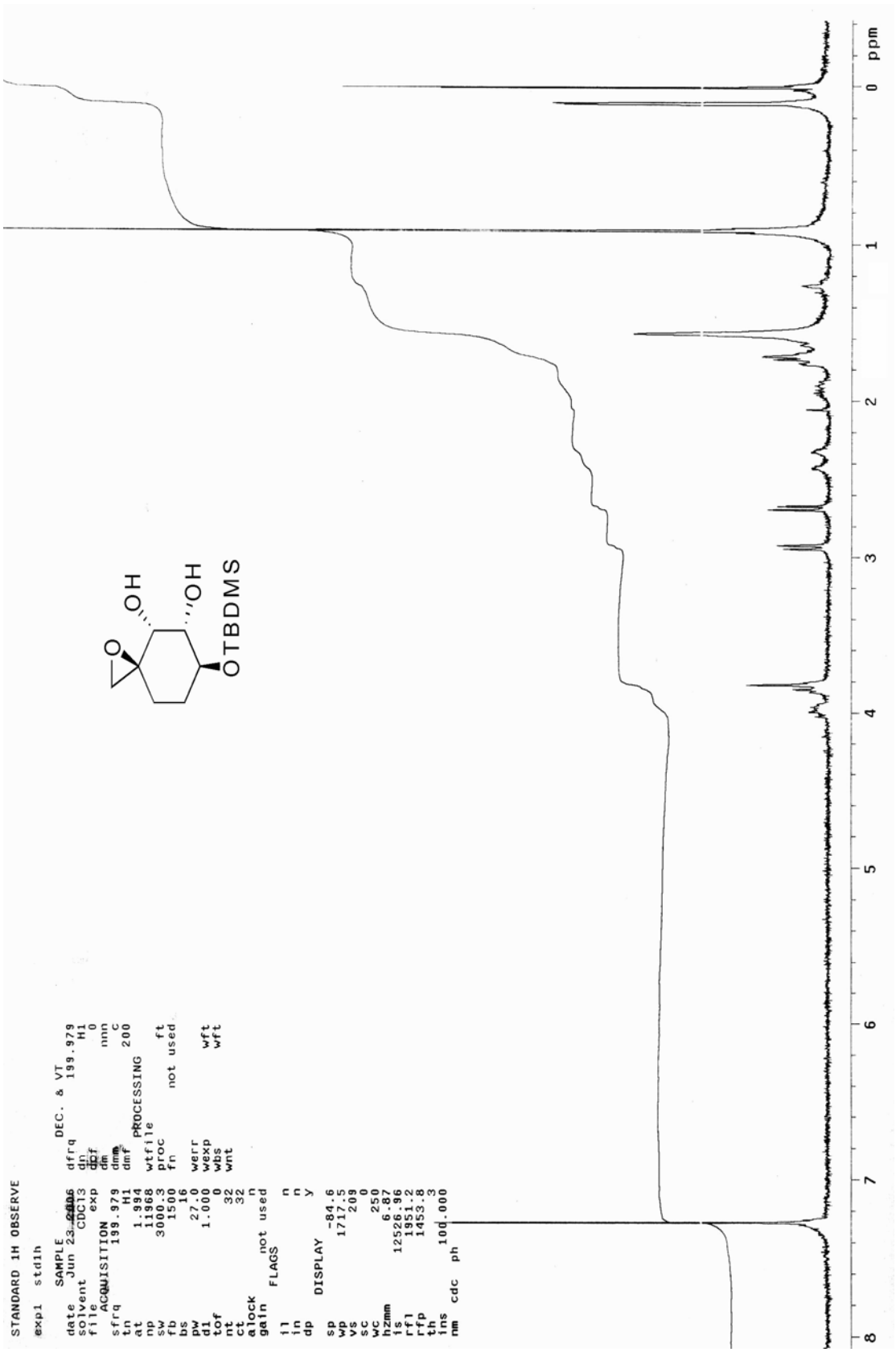

13C~OBSERVE
exp1 std13c
SAMPLE
date 2006
solvent CDCl3
file
ACQUISITION
sfrq 50.28
tn C13
at 1.498
np 37440
sw 12500.0
fb 7500
bs 16
pw 23.8
d1 1.000
tof 0
nt 22000
ct 16416
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -774.8
wp 10365.2
vs 177
sc 0
wc 250
hzmm 41.46
is 114171.88
rfl 5252.2
rfp 3883.5
th 8
ins 100.000
nm no ph
DEC. & VT
dfrq 199.979
dn H1
dof 0
dm yyy
dmm w
dmf 9900
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
werr
wexp wft
wbs wft
wnt
O
OCOPh
OMe
OTBDMS
180
160
140
120
100
80
60
40
20
0
ppm

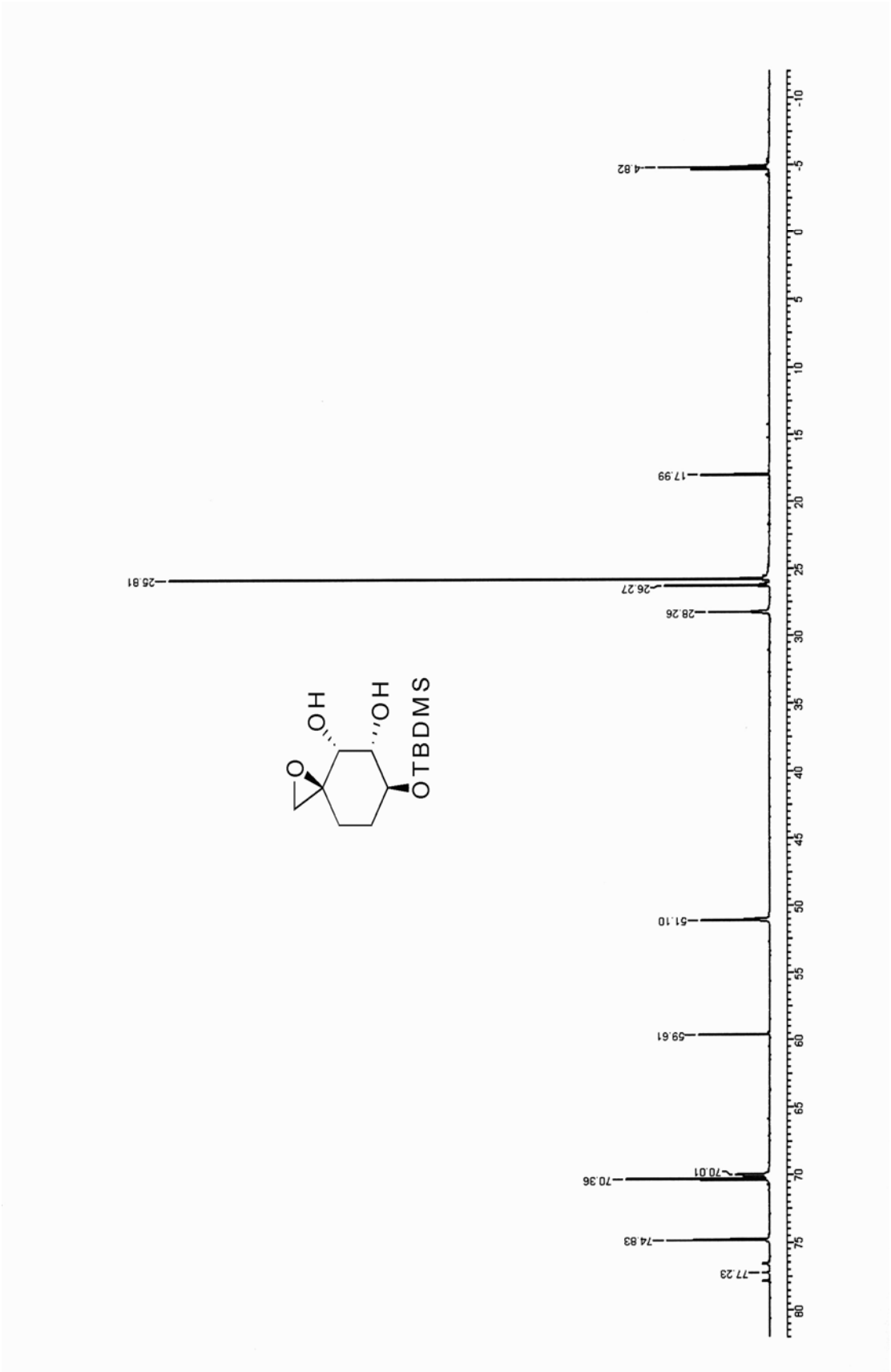

STANDARD 1H OBSERVE
expl stdih
SAMPLE
date Jun 7 2004
solvent CDCl3
file exp
ACQUISITION
sfrq 399.807
tn H1
at 3.701
np 37010
sw 5000.0
fb 2800
bs 4
tpwr 55
pw 19.8
d1 1.000
tof 0
nt 100
ct 16
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -172.8
wp 3530.8
vs 331
sc 0
wc 250
hzmm 14.12
is 1088.84
rfl 3388.0
rfp 2906.6
th 3
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.807
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
PROCESSING
wtfile ft
proc 32768
fn f
math
werr
wexp wft
wbs wft
wnt
OH
OMe
OTBDMS
8
7
6
5
4
3
2
1
0 ppm


OH
OMe
OTBDMS
O
ppm
0
20
40
60
80
100
120
140
160
180
200

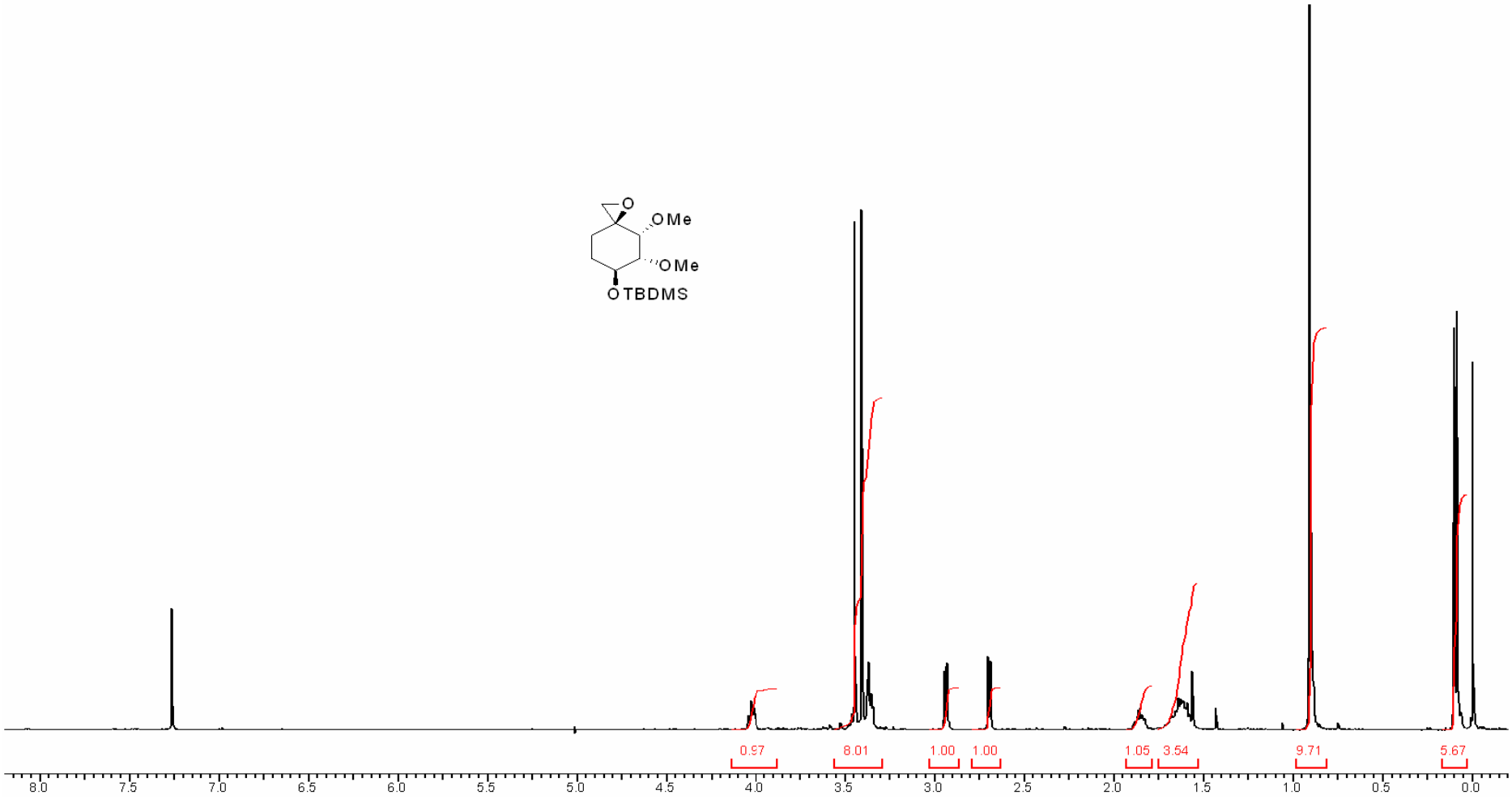

STANDARD 1H OBSERVE
exp2 std1h
SAMPLE
date Jun 11 2004
solvent CDCl3
file exp
ACQUISITION
sfrq 399.807
tn H1
at 3.701
np 37010
sw 5000.0
fb 2800
bs 4
tpwr 55
pw 19.8
d1 1.000
tof 0
nt 100
ct 24
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -180.2
wp 3333.3
vs 401
sc 0
wc 250
hzmm 13.33
is 396.39
rfl 3388.6
rfp 2906.6
th 2
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.807
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
O
O
OMe
OTBDMS
7
6
5
4
3
2
1
0
ppm

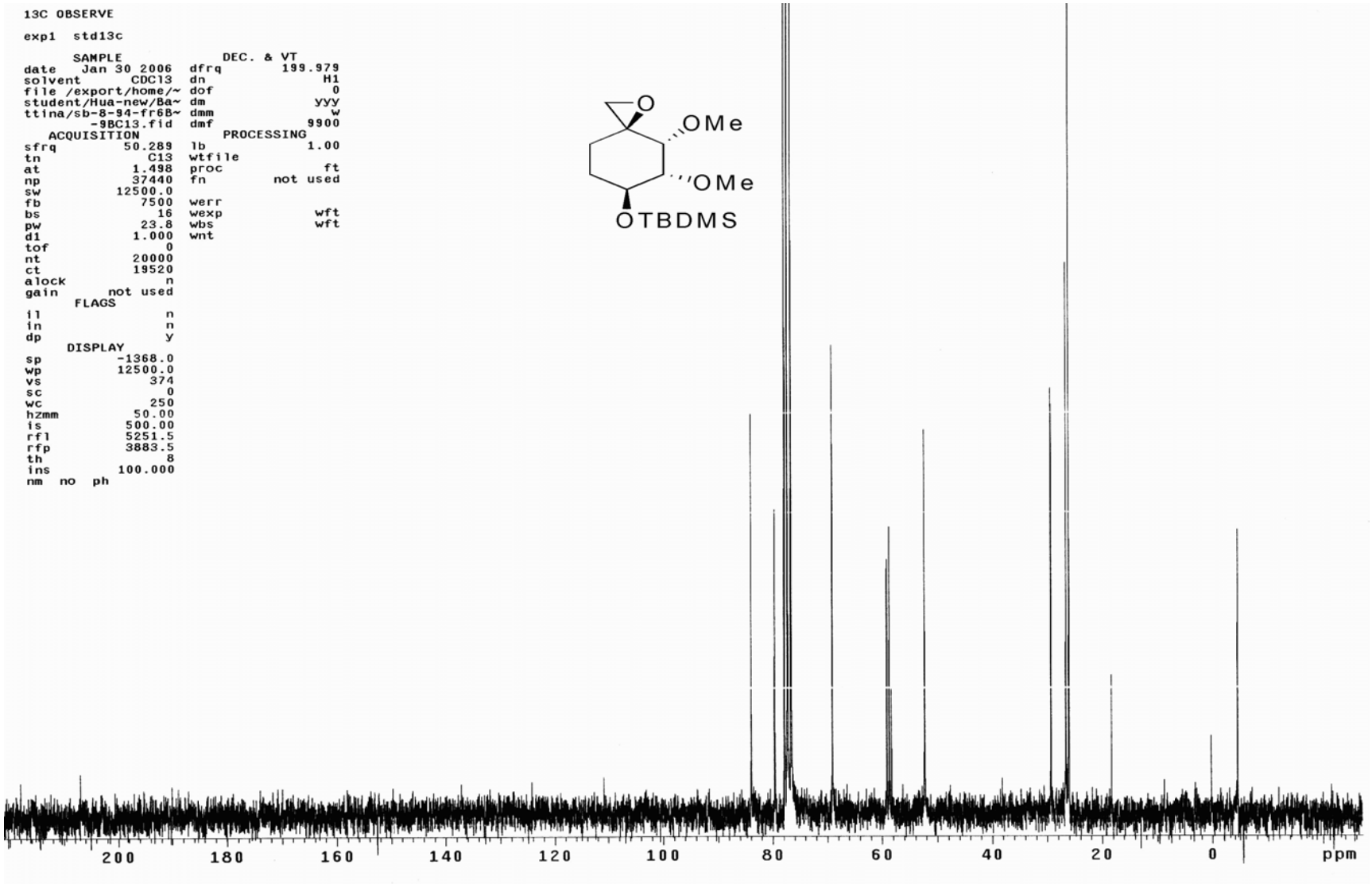

OMe
OTBDMS
O
O
ppm
0
20
40
60
80
100
120
140


STANDARD 1H OBSERVE
OH
OH
OTBDMS
0 ppm

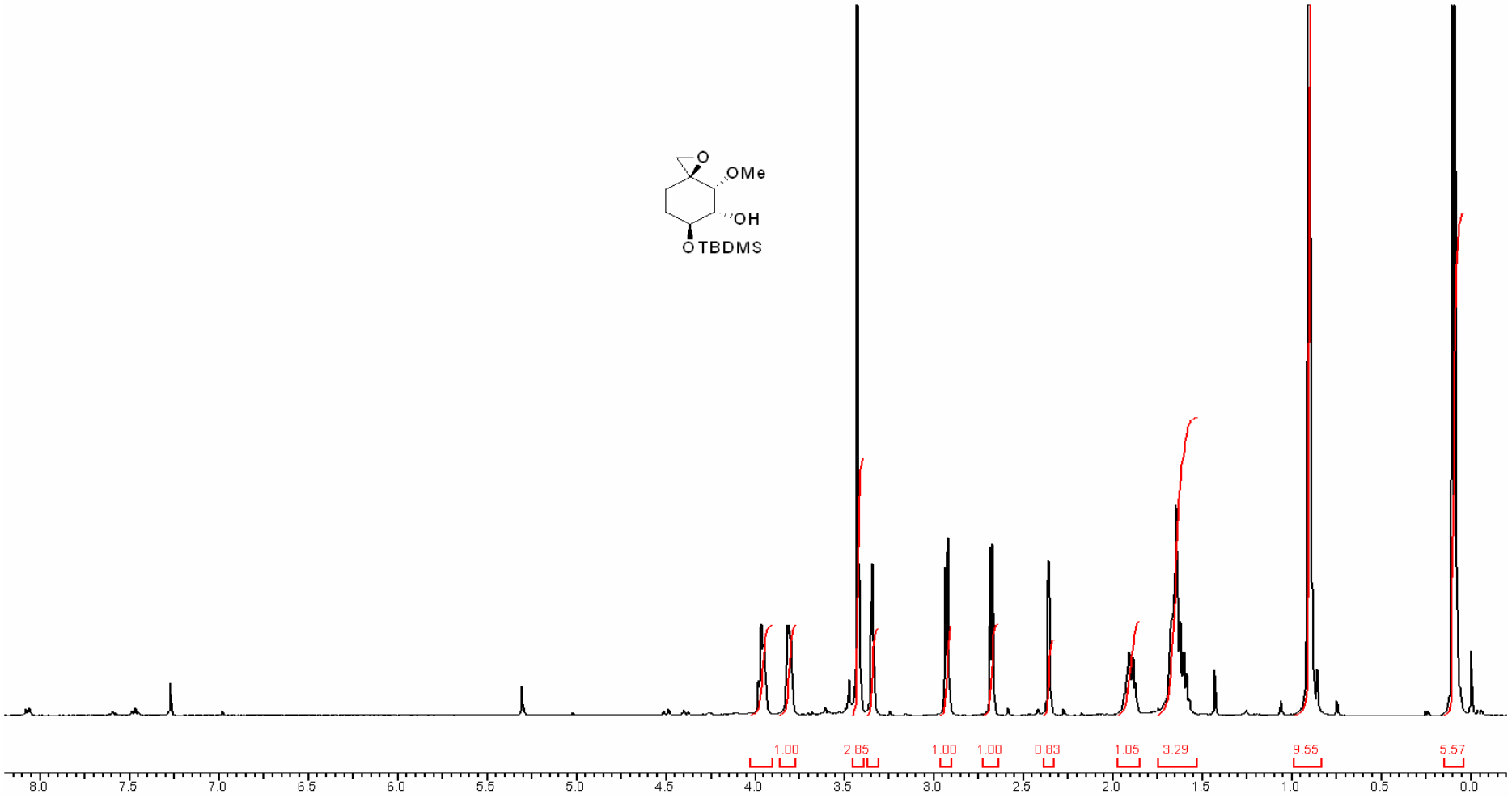

OH
OH
OTBDMS
O
-10
-5
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
4.82
17.99
25.81
26.27
28.26
51.10
59.61
70.01
70.36
74.83
77.23

Acquisition Time (sec)	5.4608	*Comment*	SB-8-94-fr6B-9B		*Date*	Jan 19 2006		*Frequency (MHz)*	399.78
Nucleus	1H	*Number of Transients*	100	*Original Points Count* 22208	*Points Count*	32768	*Solvent* CDCl3	*Sweep Width (Hz)*	6000.60
Temperature (grad C)	29.000								

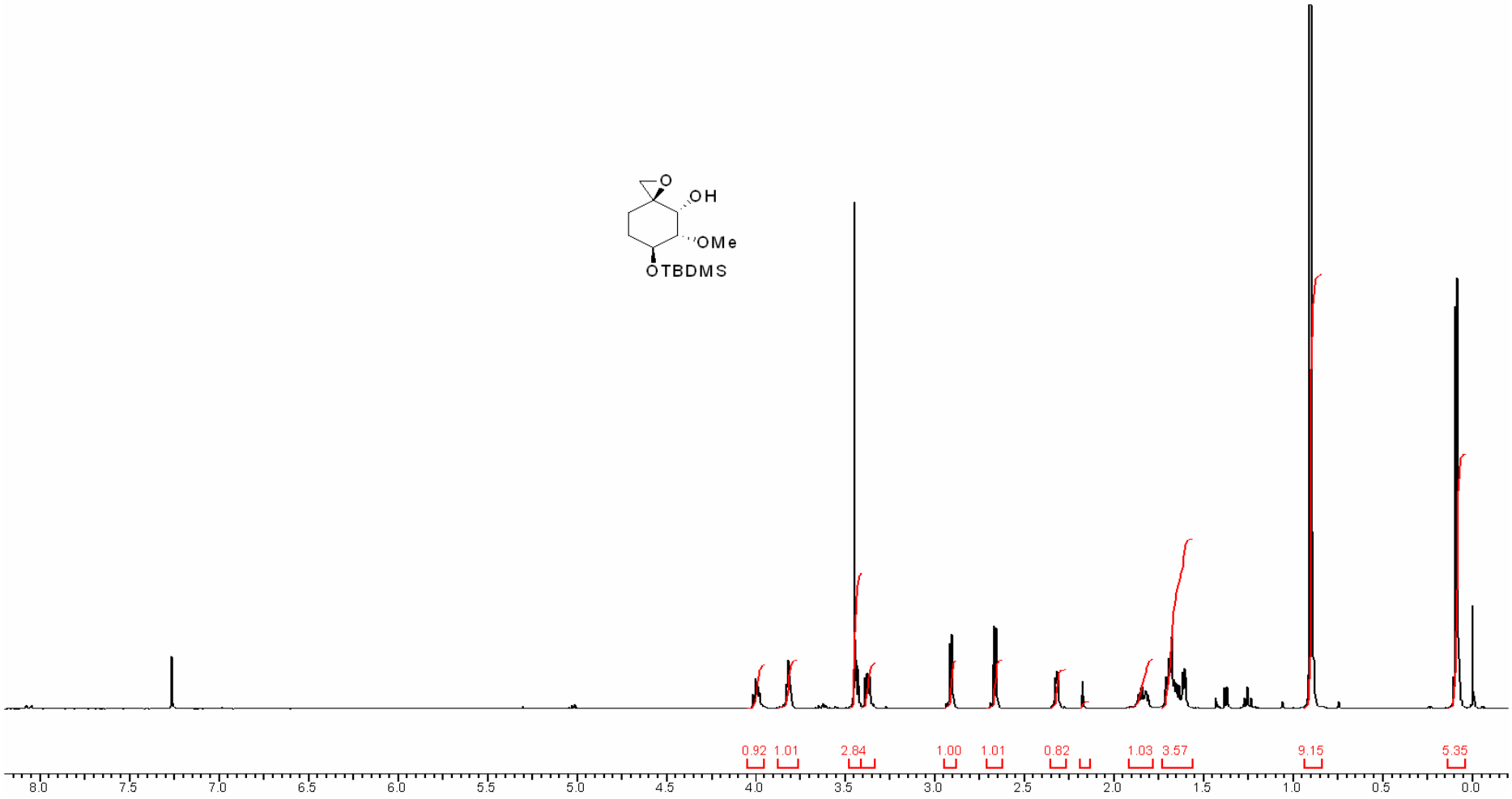


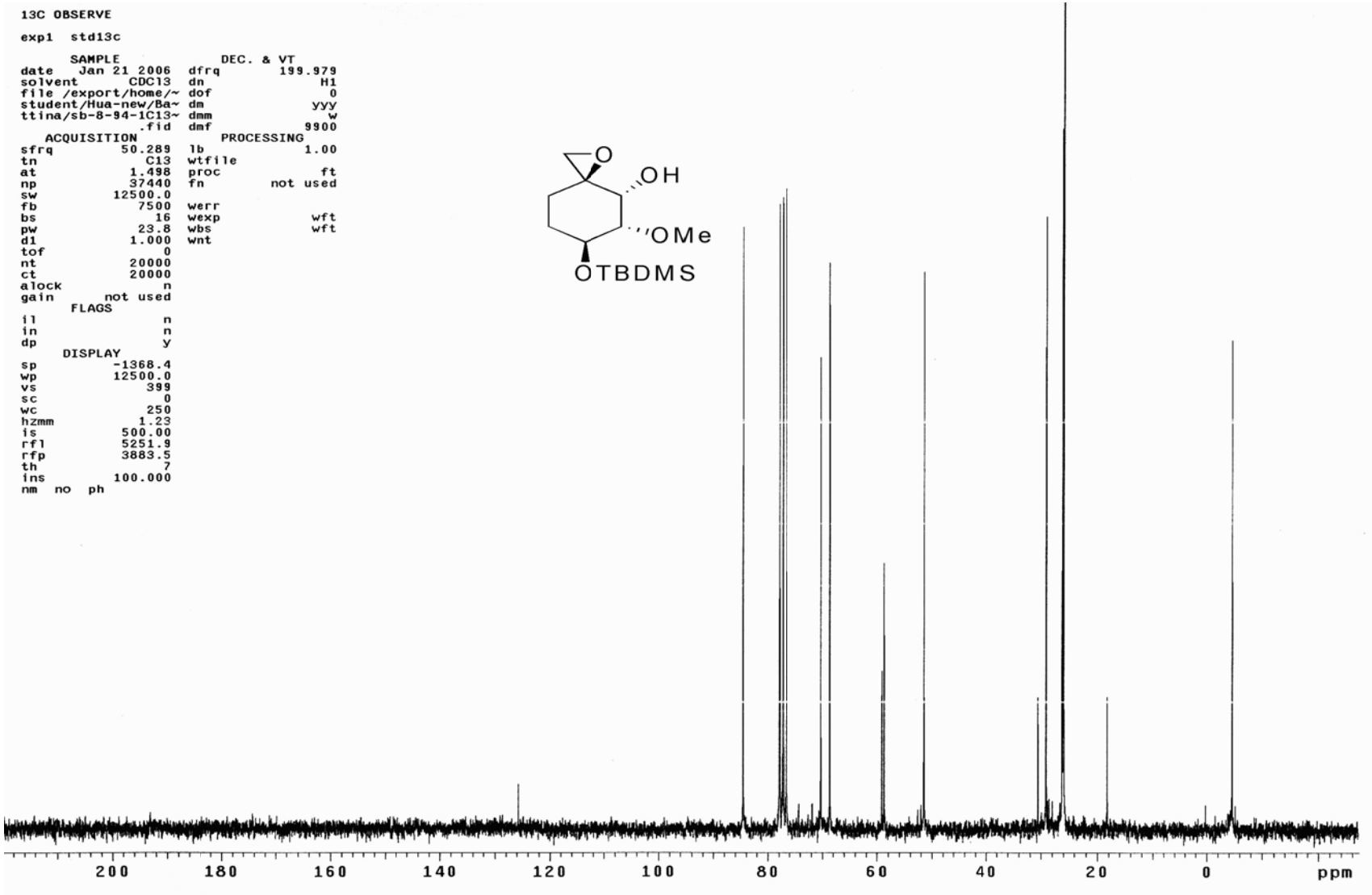

13C OBSERVE
exp1 std13c
SAMPLE
date Jan 30 2006
solvent CDCl3
file /export/home/~
student/Hua-new/Ba~
ttina/sb-8-94-fr6B~
-9BC13.fid
ACQUISITION
sfrq 50.289
tn C13
at 1.498
np 37440
sw 12500.0
fb 7500
bs 16
pw 23.8
d1 1.000
tof 0
nt 20000
ct 19520
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -1368.0
wp 12500.0
vs 374
sc 0
wc 250
hzmm 50.00
is 500.00
rfl 5251.5
rfp 3883.5
th 8
ins 100.000
nm no ph
DEC. & VT
dfrq 199.979
dn H1
dof 0
dm yyy
dmm w
dmf 9900
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
werr
wexp wft
wbs wft
wnt
O
OMe
OMe
OTBDMS
200
180
160
140
120
100
80
60
40
20
0
ppm

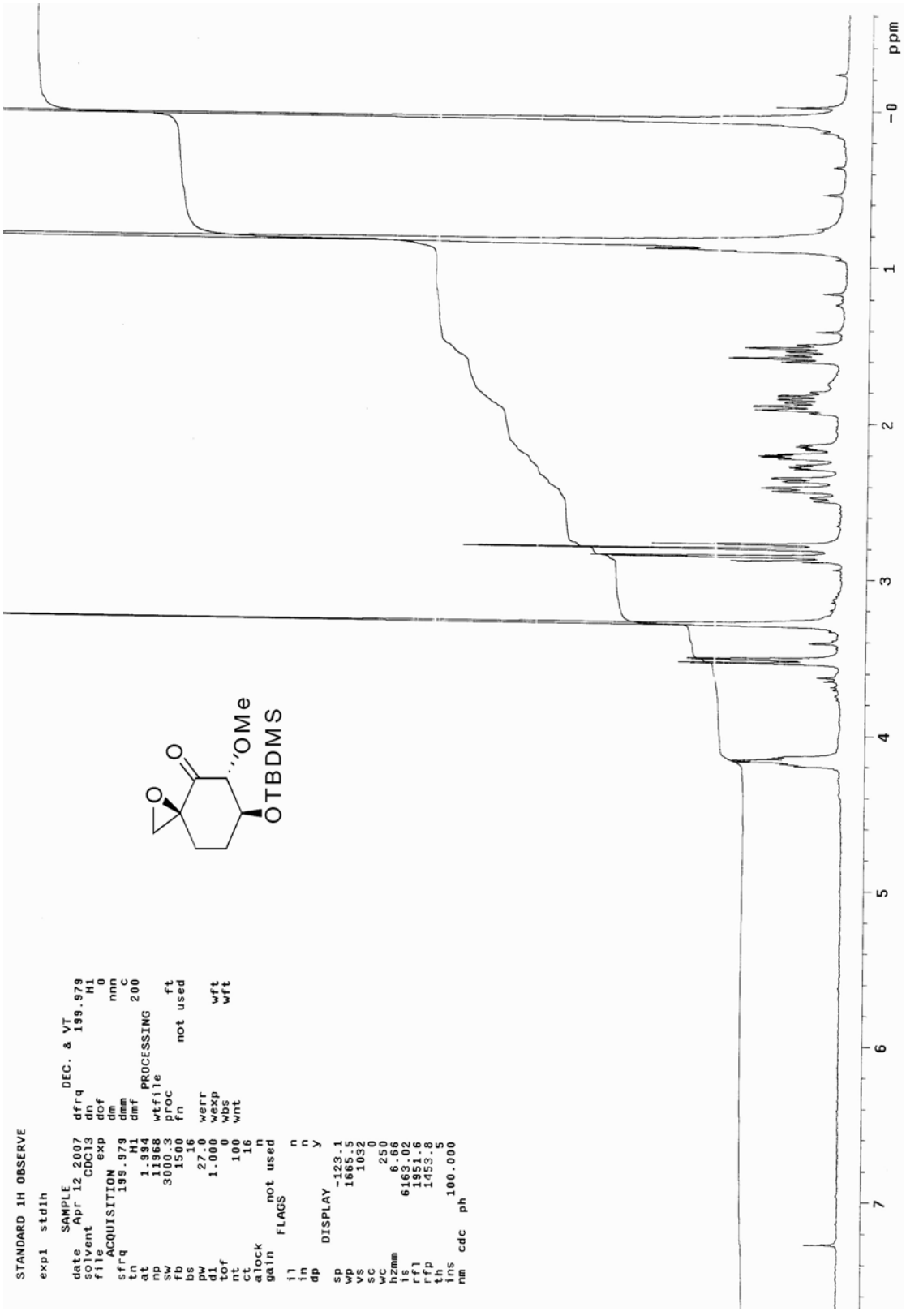
Acquisition Time (sec)	5.4608	*Comment*	sb-8-94-fr2-5		*Date*	Jan 18 2006		*Frequency (MHz)*	399.78
Nucleus	1H	*Number of Transients*	100	*Original Points Count* 22208	*Points Count*	32768	*Solvent* CDCl3	*Sweep Width (Hz)*	6000.60
Temperature (grad C)	29.000								

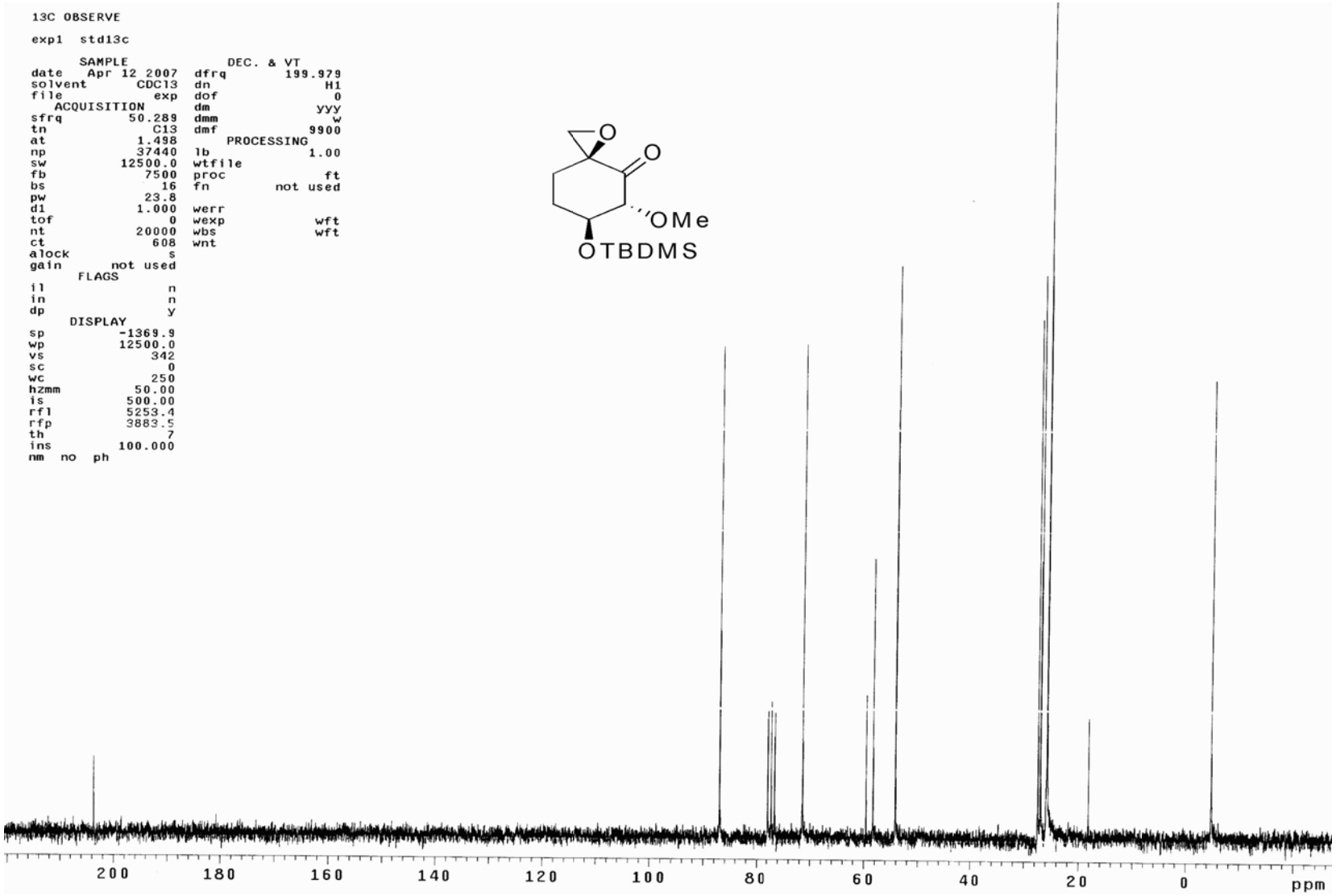


Acquisition Time (sec)	5.4608	*Comment*	sb-8-94-fr7-11		*Date*	Feb 1 2006		*Frequency (MHz)*	399.78
Nucleus	1H	*Number of Transients*	32	*Original Points Count* 22208	*Points Count*	32768	*Solvent* CDCl3	*Sweep Width (Hz)*	6000.60
Temperature (grad C)	29.000								

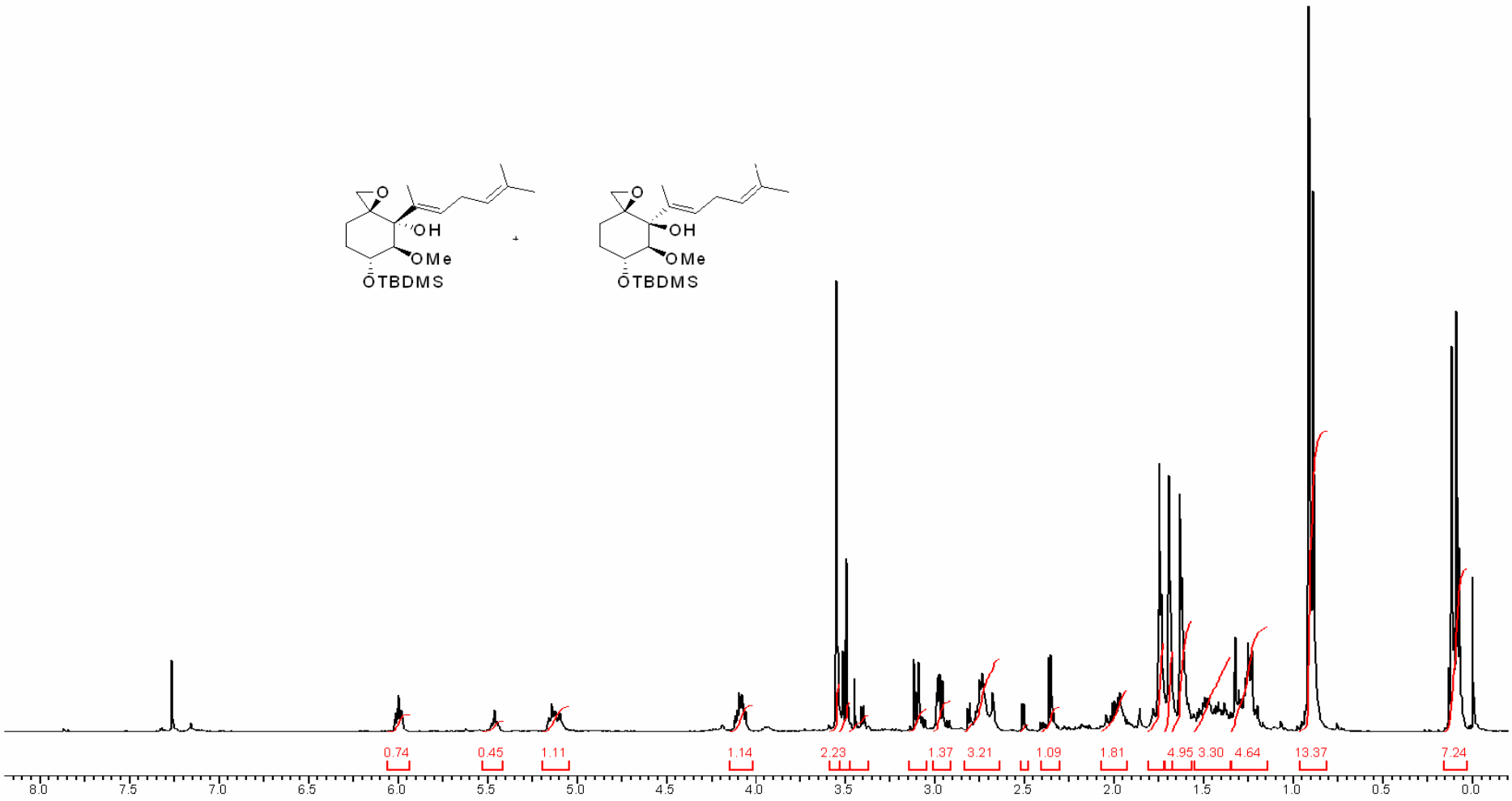


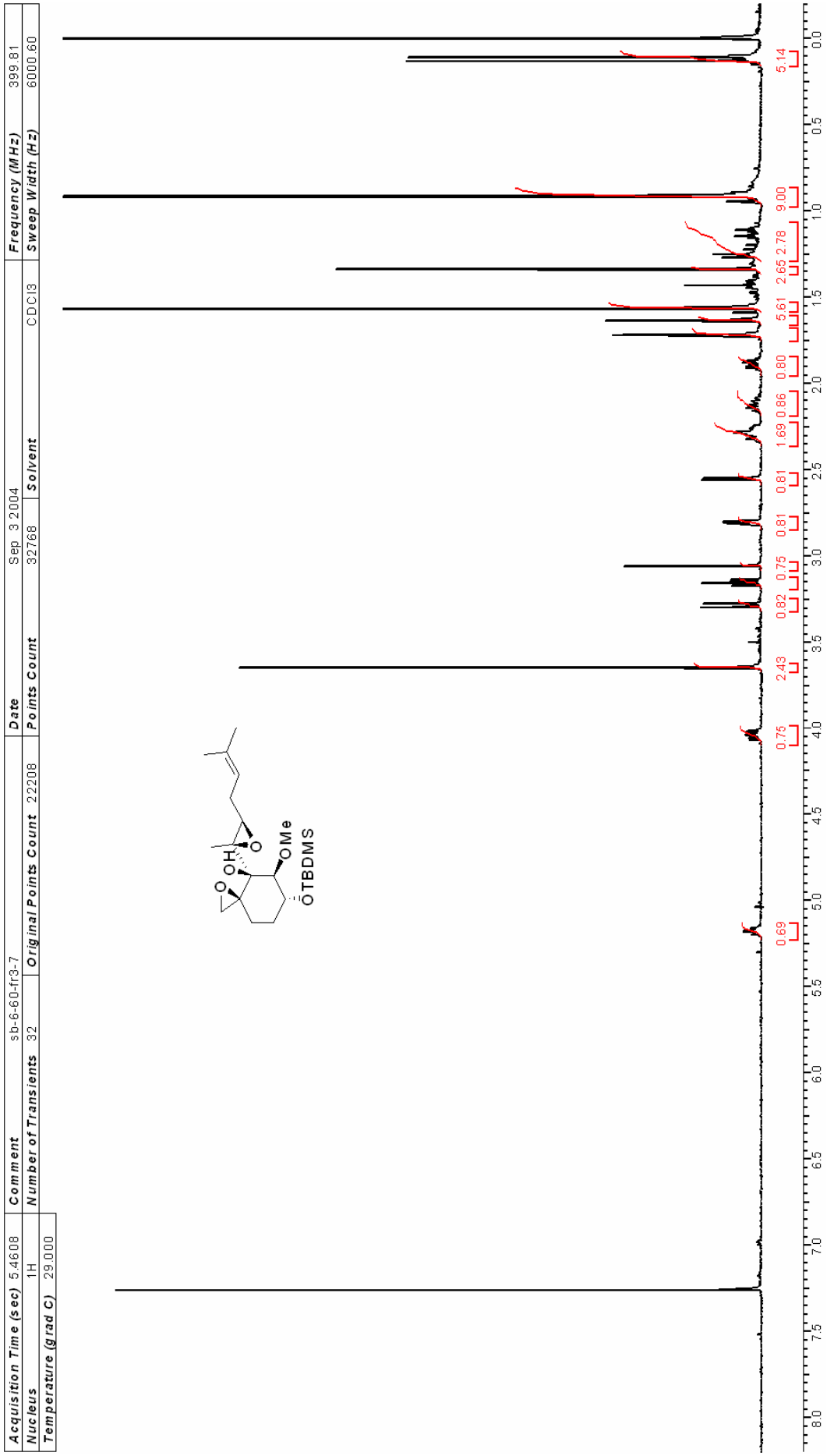

13C OBSERVE
exp1 std13c
SAMPLE
date Jan 21 2006
solvent CDCl3
file /export/home/~
student/Hua-new/Ba~
ttina/sb-8-94-1C13~
.fid
ACQUISITION
sfrq 50.289
tn C13
at 1.498
np 37440
sw 12500.0
fb 7500
bs 16
pw 23.8
d1 1.000
tof 0
nt 20000
ct 20000
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -1368.4
wp 12500.0
vs 399
sc 0
wc 250
hzmm 1.23
is 500.00
rfl 5251.9
rfp 3883.5
th 7
ins 100.000
nm no ph
DEC. & VT
dfrq 199.979
dn H1
dof 0
dm yyy
dmm w
dmf 9900
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
werr
wexp wft
wbs wft
wnt
O
OH
OMe
OTBDMS
200
180
160
140
120
100
80
60
40
20
0
ppm

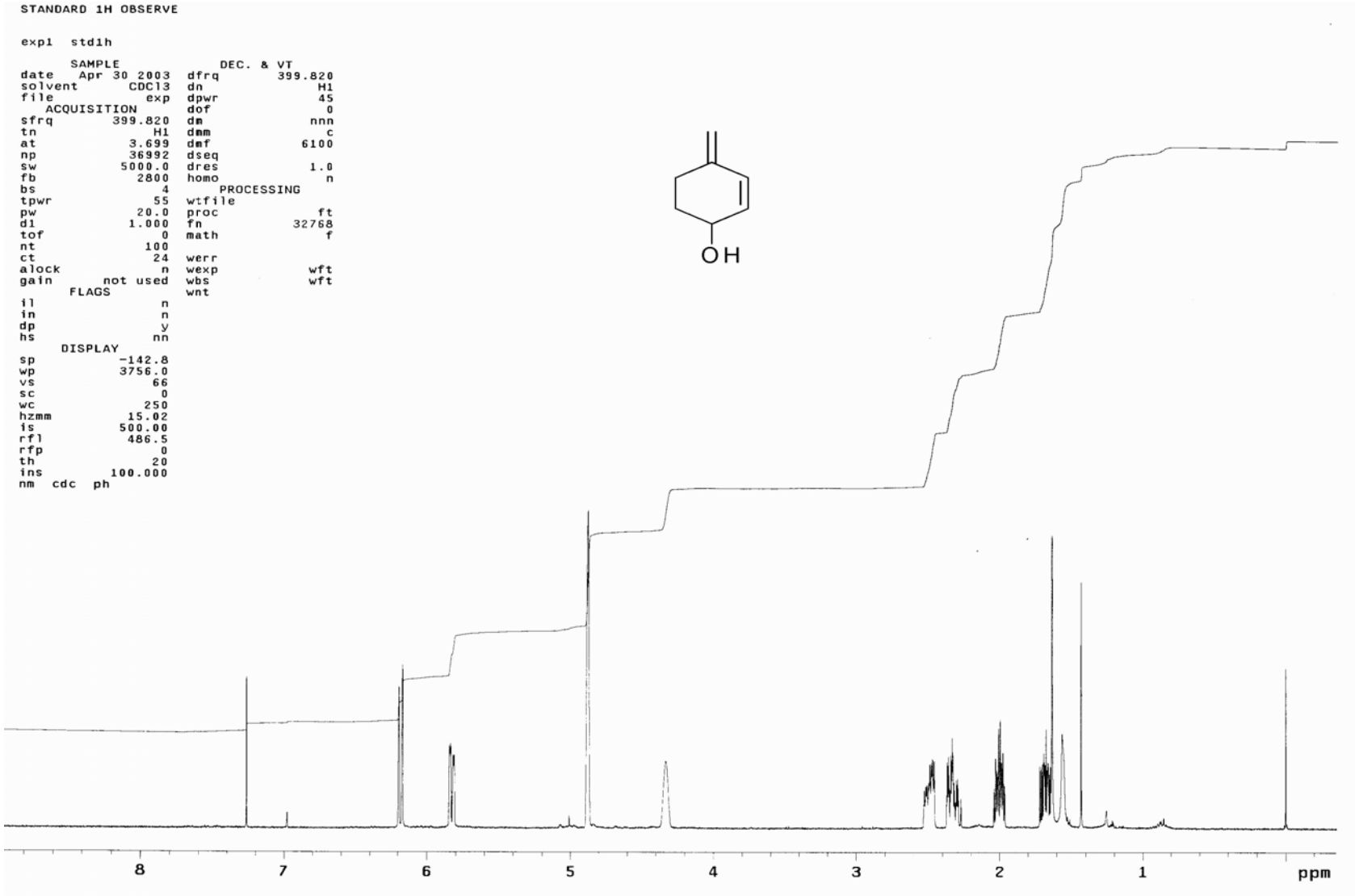

STANDARD 1H OBSERVE
expl stdih
SAMPLE
date Apr 12 2007
solvent CDCl3
file exp
ACQUISITION
sfrq 199.979
tn H1
at 1.994
np 11968
sw 3000.3
fb 1500
bs 16
pw 27.0
d1 1.000
tof 0
nt 100
ct 16
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -123.1
wp 1665.5
vs 1032
sc 0
wc 250
hzmm 6.66
is 6163.02
rfl 1951.6
rfp 1453.8
th 5
ins 100.000
nm cdc ph
DEC. & VT
dfrq 199.979
dn H1
dof 0
dm nnn
dmm c
dmf 200
PROCESSING
wtfile
proc ft
fn not used
werr
wexp wft
wbs wft
wnt
OMe
OTBDMS
7
6
5
4
3
2
1
-0
ppm

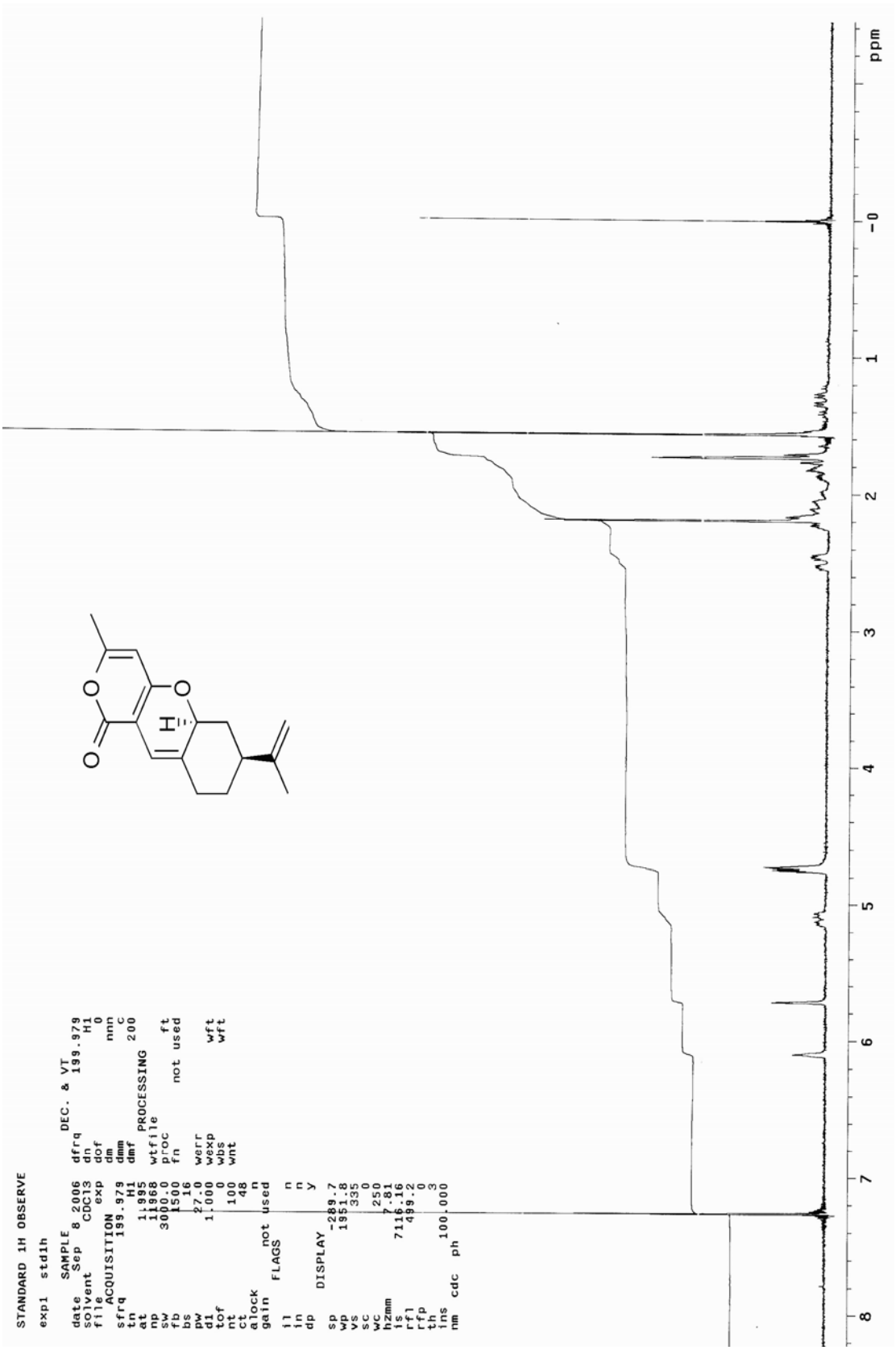

13C OBSERVE
exp1 std13c
SAMPLE
date Apr 12 2007
solvent CDCl3
file exp
ACQUISITION
sfrq 50.289
tn C13
at 1.498
np 37440
sw 12500.0
fb 7500
bs 16
pw 23.8
d1 1.000
tof 0
nt 20000
ct 608
alock s
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -1369.9
wp 12500.0
vs 342
sc 0
wc 250
hzmm 50.00
is 500.00
rfl 5253.4
rfp 3883.5
th 7
ins 100.000
nm no ph
DEC. & VT
dfrq 199.979
dn H1
dof 0
dm yyy
dmm w
dmf 9900
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
werr
wexp wft
wbs wft
wnt
OMe
OTBDMS
200
180
160
140
120
100
80
60
40
20
0
ppm

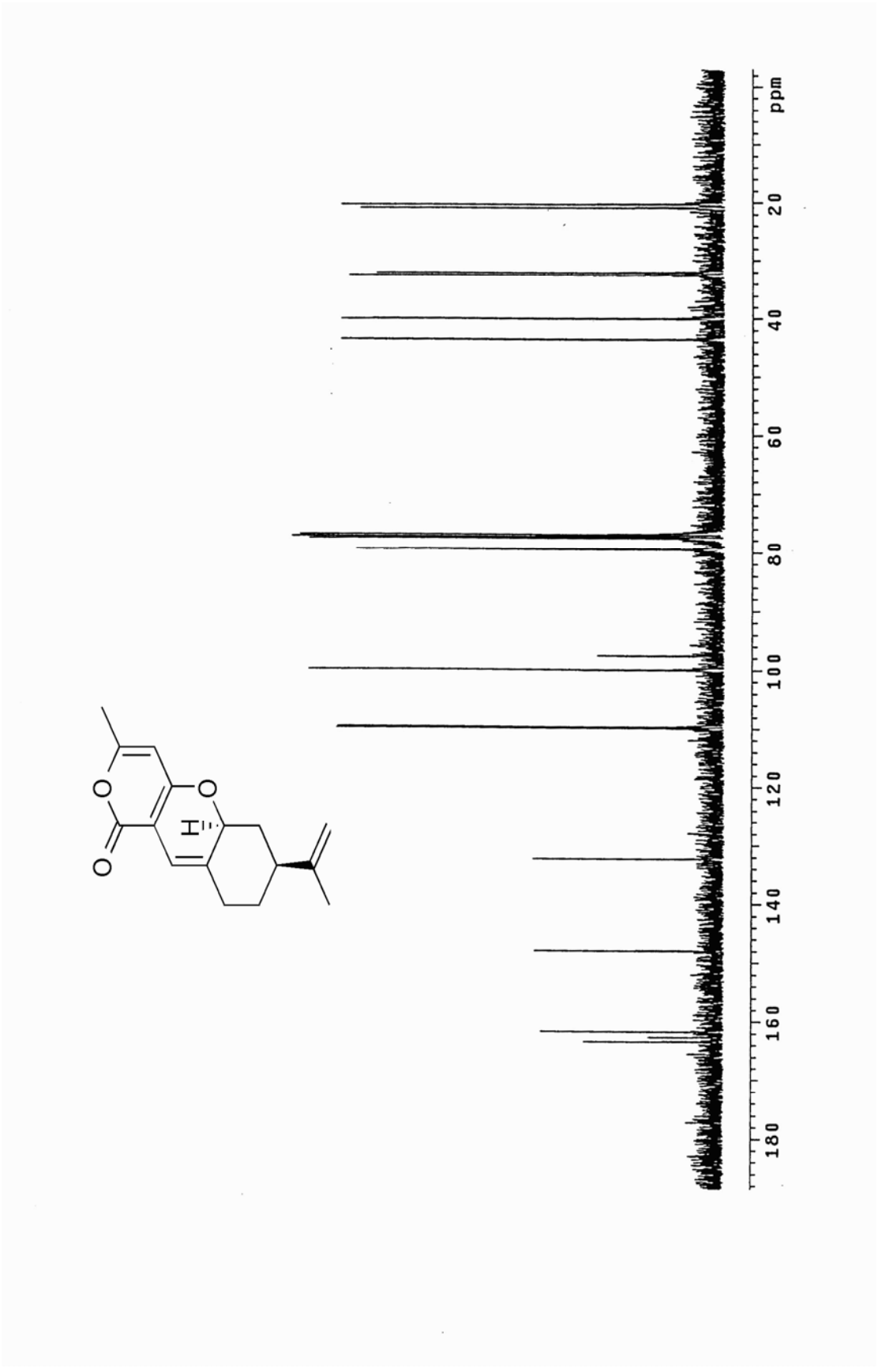
Acquisition Time (sec)	5.4608	*Comment*	sb-6-48-fr4-8		*Date*	Aug 10 2004		*Frequency (MHz)*	399.81
Nucleus	1H	*Number of Transients*	32	*Original Points Count* 22208	*Points Count*	32768	*Solvent* CDCl3	*Sweep Width (Hz)*	6000.60
Temperature (grad C)	29.000								

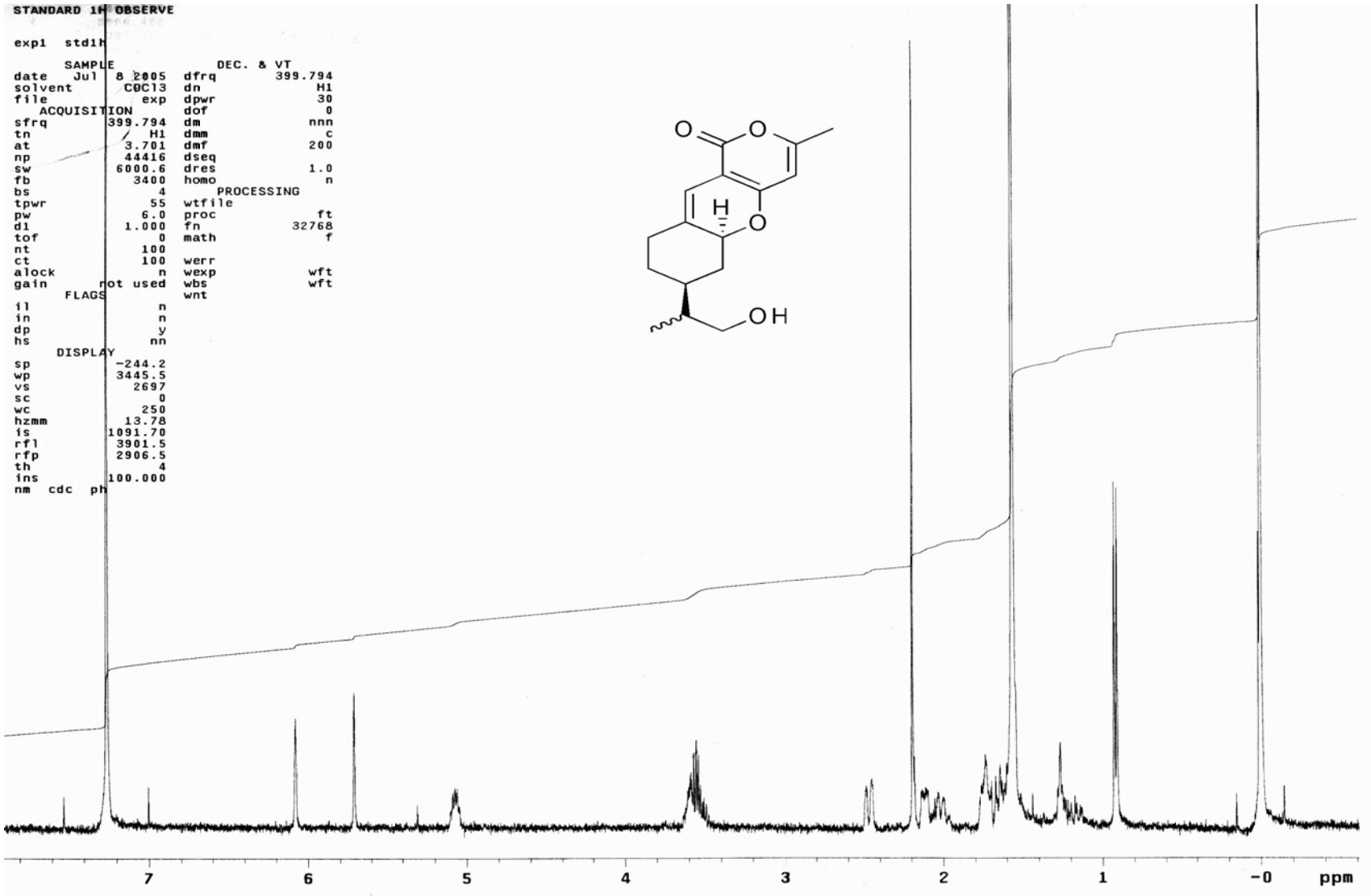


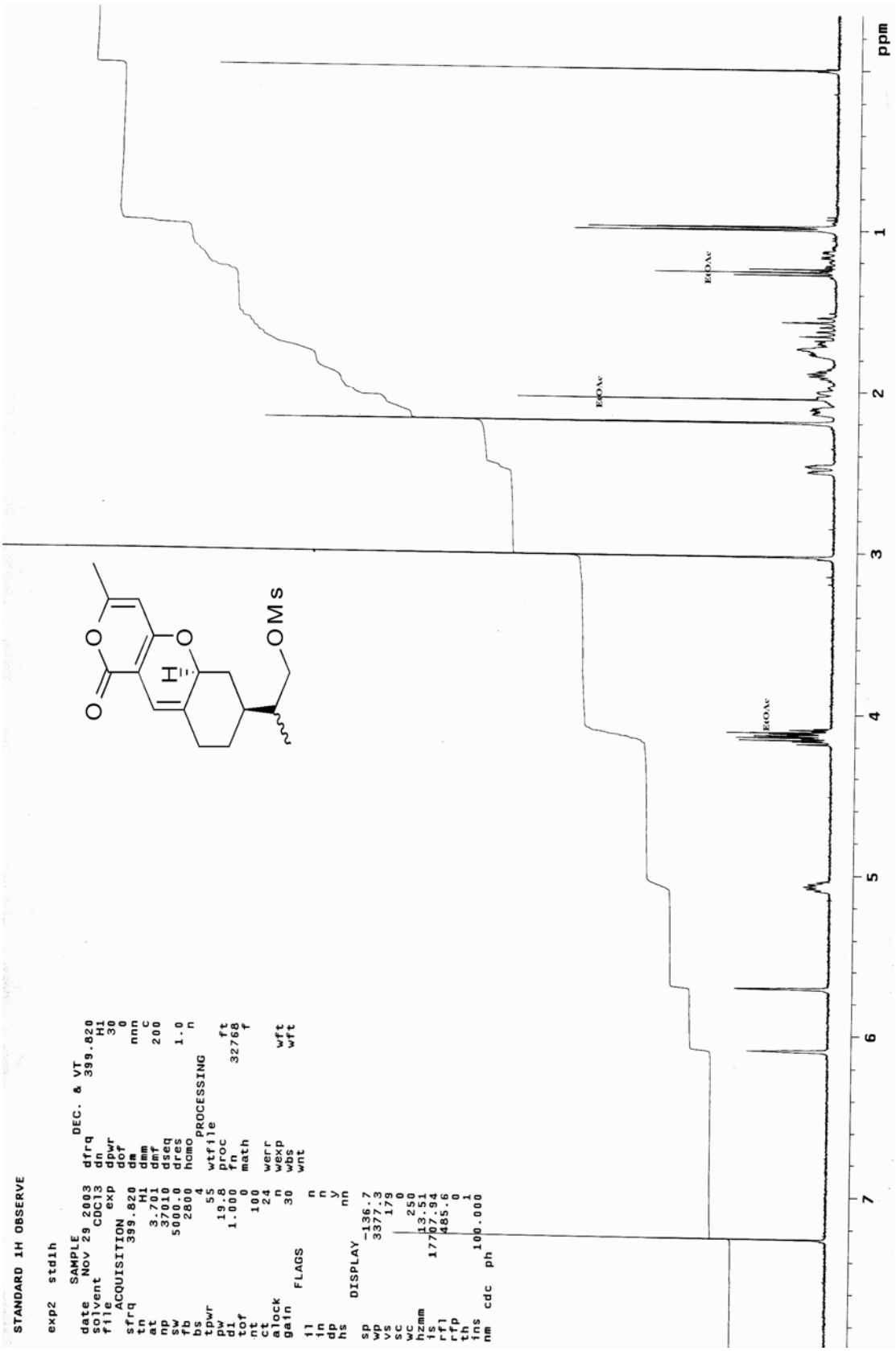

Acquisition Time (sec) 5.4608
Comment
Date Sep 3 2004
Frequency (MHz) 399.81
Nucleus 1H
Number of Transients 32
Original Points Count 22208
Points Count 32768
Solvent CDCl3
Sweep Width (Hz) 6000.60
Temperature (grad C) 29.000
sb-6-60-fr3-7
OMe
OTBDMS
0.69
0.75
2.43
0.82
0.75
0.81
0.81
1.69
0.86
0.80
5.61
2.65
2.78
9.00
5.14

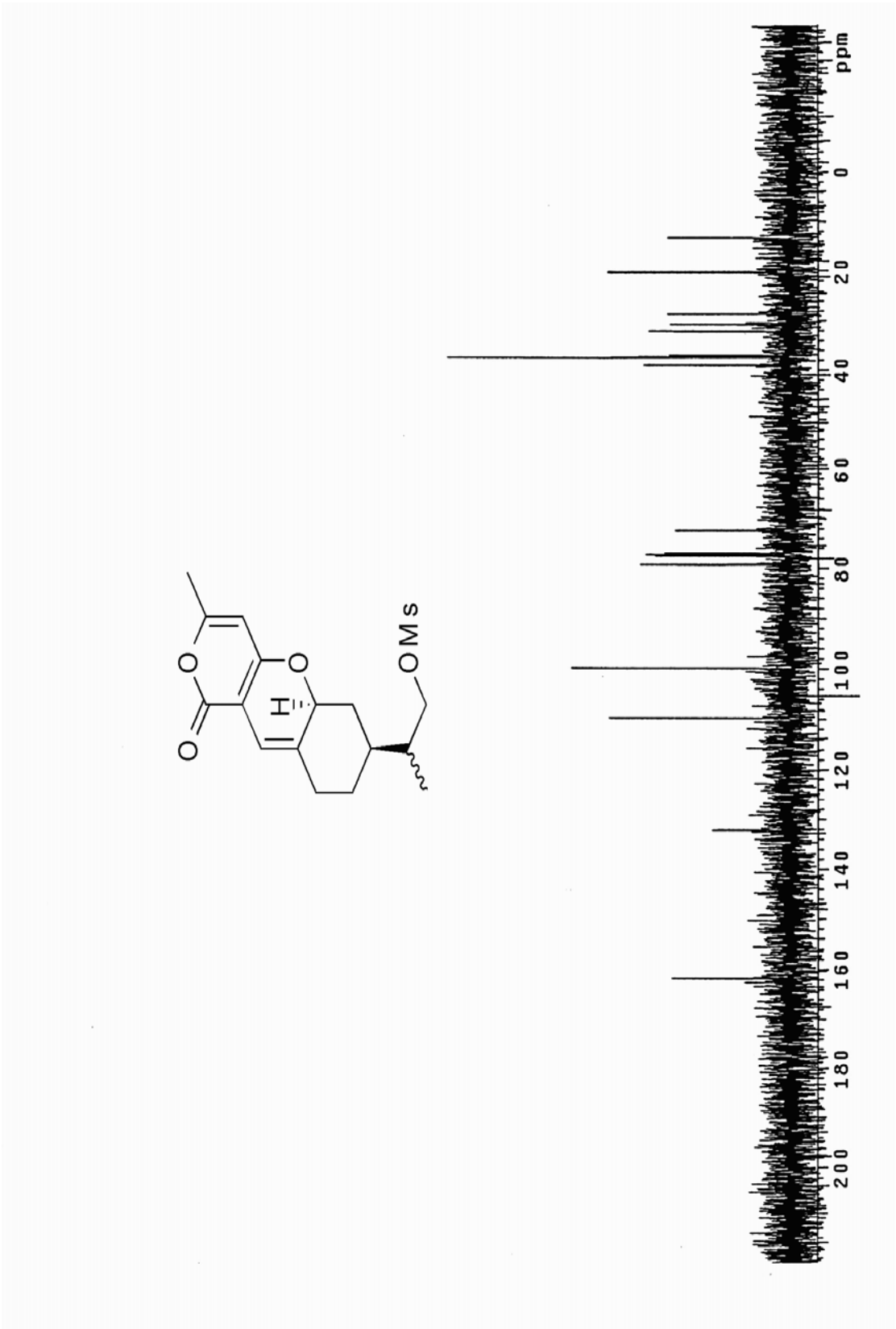

STANDARD 1H OBSERVE
exp1 std1h
SAMPLE
date Apr 30 2003
solvent CDCl3
file exp
ACQUISITION
sfrq 399.820
tn H1
at 3.699
np 36992
sw 5000.0
fb 2800
bs 4
tpwr 55
pw 20.0
d1 1.000
tof 0
nt 100
ct 24
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -142.8
wp 3756.0
vs 66
sc 0
wc 250
hzmm 15.02
is 500.00
rfl 486.5
rfp 0
th 20
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.820
dn H1
dpwr 45
dof 0
dm nnn
dmm c
dmf 6100
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
OH
8
7
6
5
4
3
2
1
ppm


STANDARD 1H OBSERVE
expl std1h
SAMPLE
date Sep 8 2006
solvent CDCl3
file exp
ACQUISITION
sfrq 199.979
tn H1
at 1.995
np 11968
sw 3000.0
fb 1500
bs 16
pw 27.0
d1 1.000
tof 0
nt 100
ct 48
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -289.7
wp 1951.8
vs 335
sc 0
wc 250
hzmm 7.81
is 7116.16
rfl 499.2
rfp 0
th 3
ins 100.000
nm cdc ph
DEC. & VT
dfrq 199.979
dn H1
dof 0
dm nnn
dmm c
dmf 200
PROCESSING
wtfile
proc ft
fn not used
werr
wexp wft
wbs wft
wnt
ppm

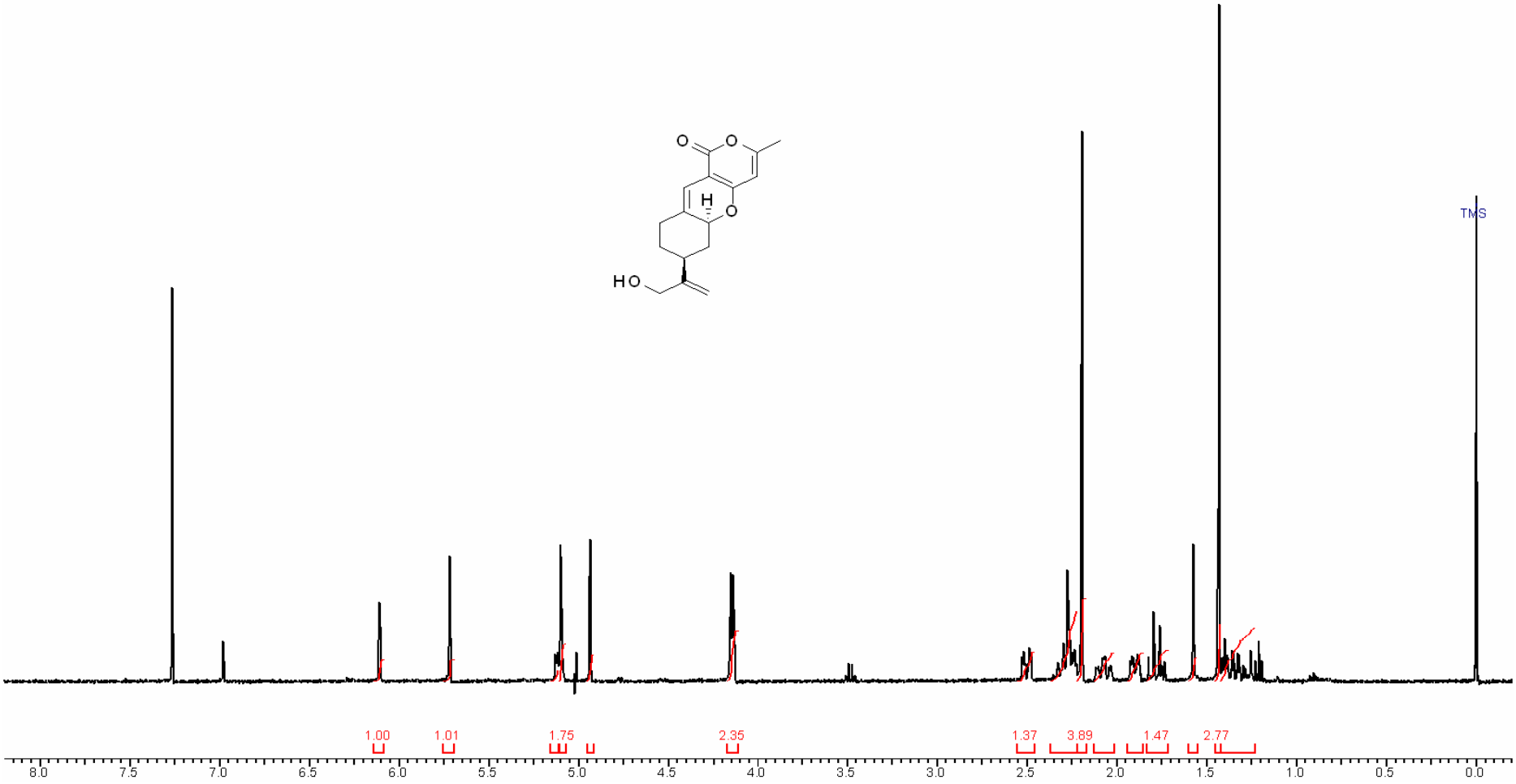

O
O
O
H
ppm
20
40
60
80
100
120
140
160
180

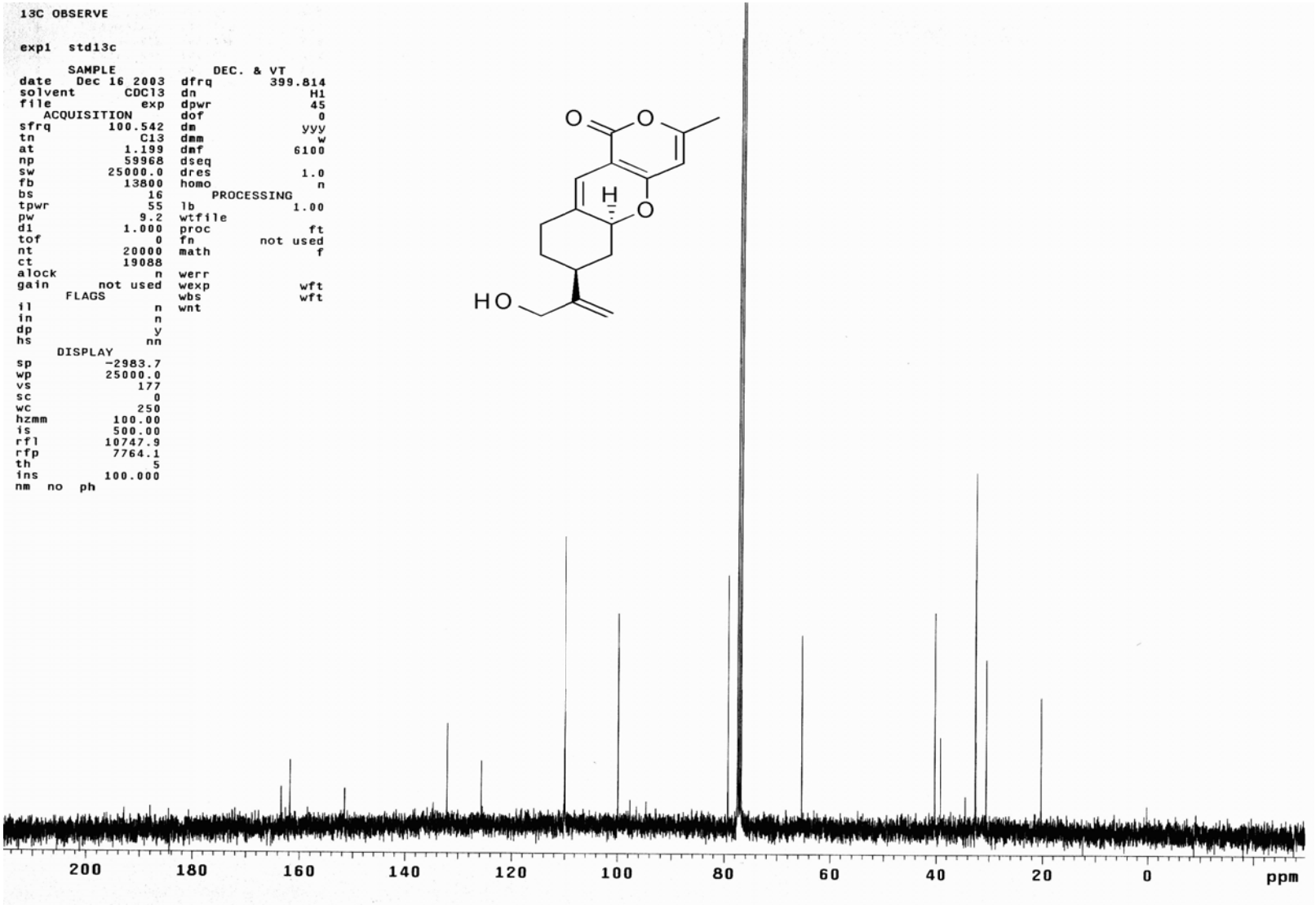

STANDARD 1H OBSERVE
exp1 std1h
SAMPLE
date Jul 8 2005
solvent CDCl3
file exp
ACQUISITION
sfrq 399.794
tn H1
at 3.701
np 44416
sw 6000.6
fb 3400
bs 4
tpwr 55
pw 6.0
d1 1.000
tof 0
nt 100
ct 100
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -244.2
wp 3445.5
vs 2697
sc 0
wc 250
hzmm 13.78
is 1091.70
rfl 3901.5
rfp 2906.5
th 4
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.794
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
O
O
H
O
OH
7
6
5
4
3
2
1
-0
ppm

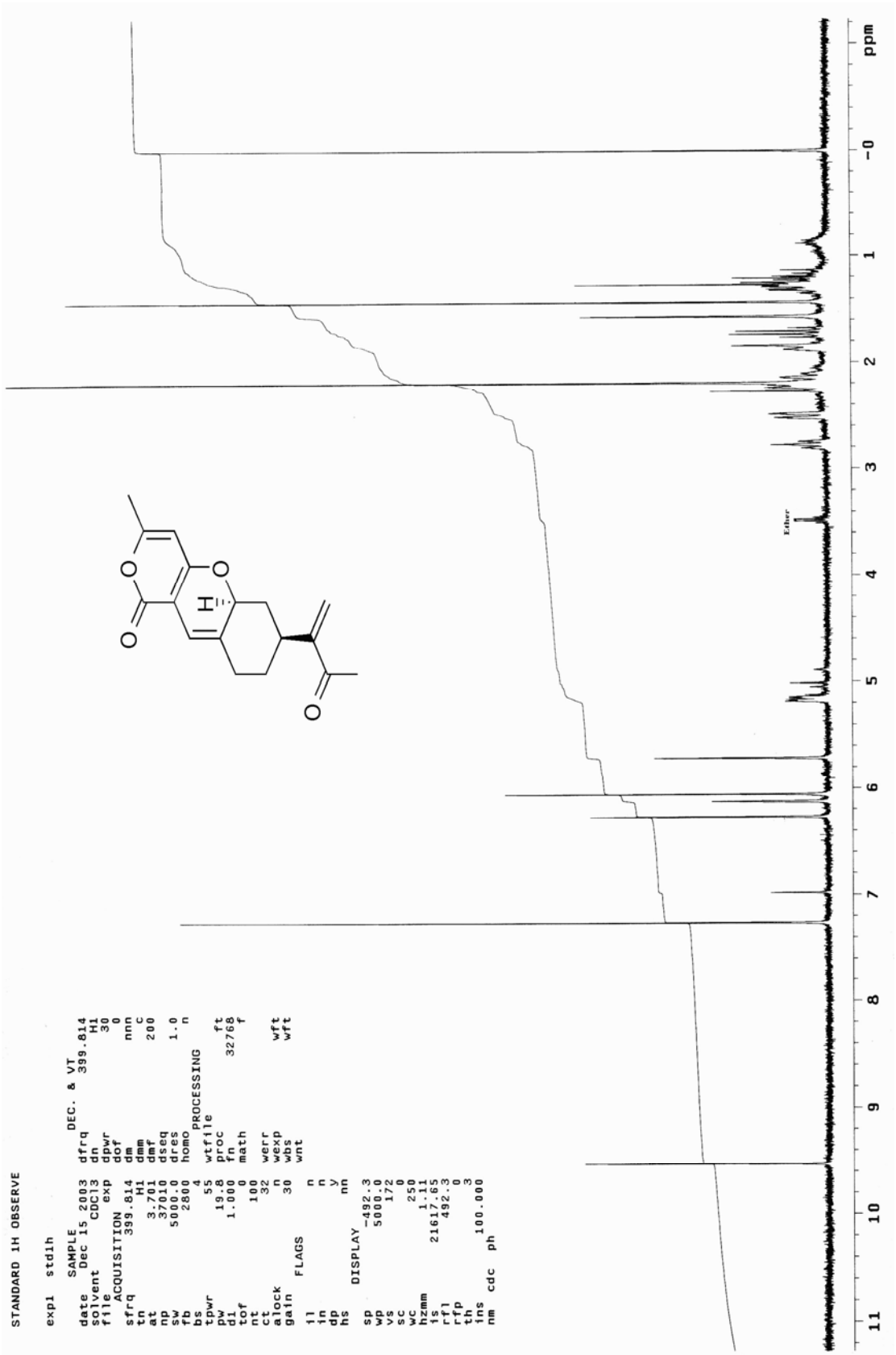

STANDARD 1H OBSERVE
exp2 stdih
SAMPLE
date Nov 29 2003
solvent CDCl3
file exp
ACQUISITION
sfrq 399.820
tn H1
at 3.701
np 37010
sw 5000.0
fb 2800
bs 4
tpwr 55
pw 19.8
d1 1.000
tof 0
nt 100
ct 24
alock n
gain 30
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -136.7
wp 3377.3
vs 179
sc 0
wc 250
hzmm 13.51
is 17707.94
rfl 485.6
rfp 0
th 1
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.820
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
OMs
EtOAc
EtOAc
EtOAc
ppm
7
6
5
4
3
2
1

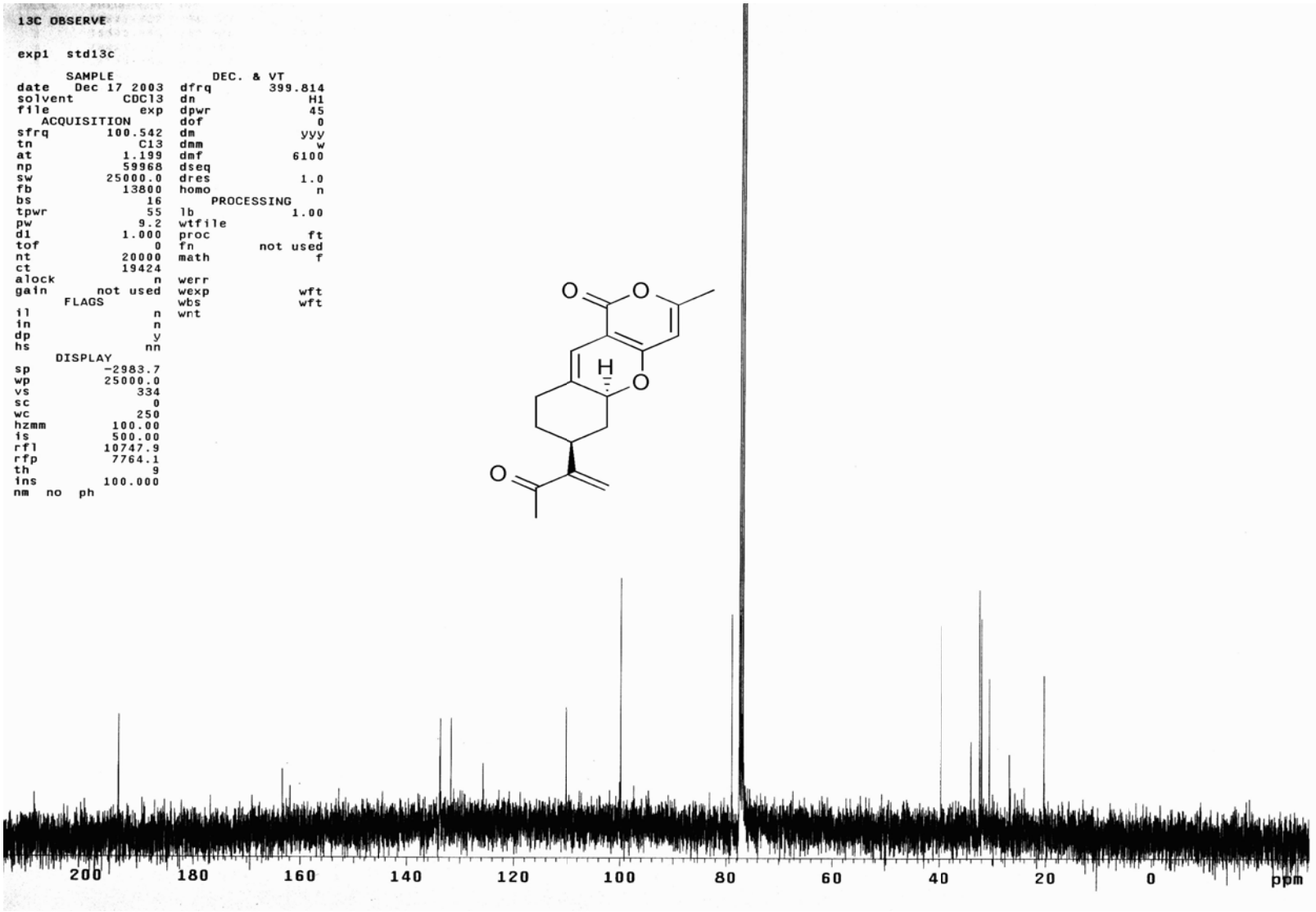

O
O
O
H
OMs
ppm
0
20
40
60
80
100
120
140
160
180
200

Acquisition Time (sec)	6.5536	*Comment*	sb-4-103-fr27-30		*Date*	Dec 15 2003		*Frequency (MHz)*	399.81
Nucleus	1H	*Number of Transients*	100	*Original Points Count* 18505	*Points Count*	32768	*Solvent* CDCl3	*Sweep Width (Hz)*	5000.00
Temperature (grad C)	29.000								

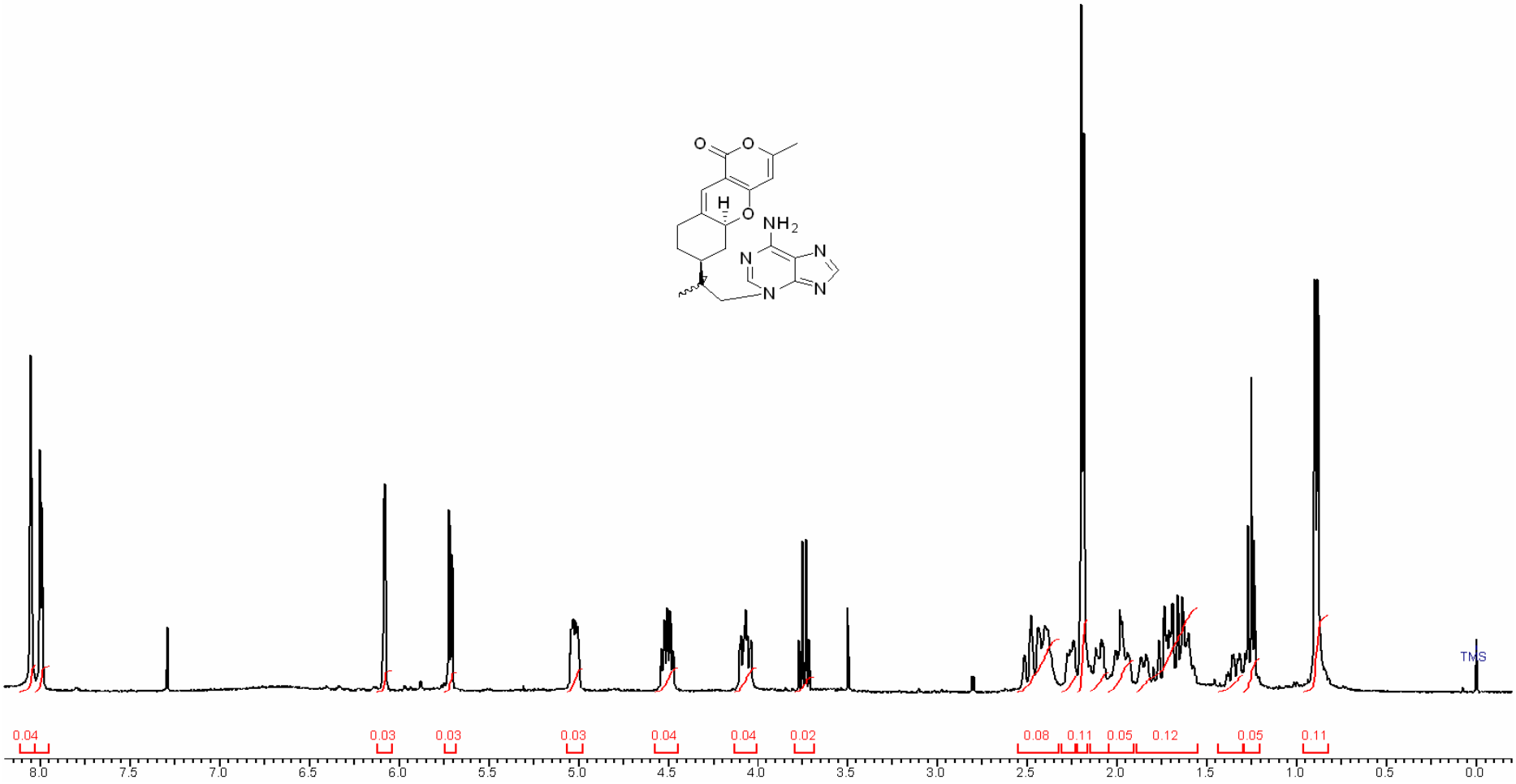


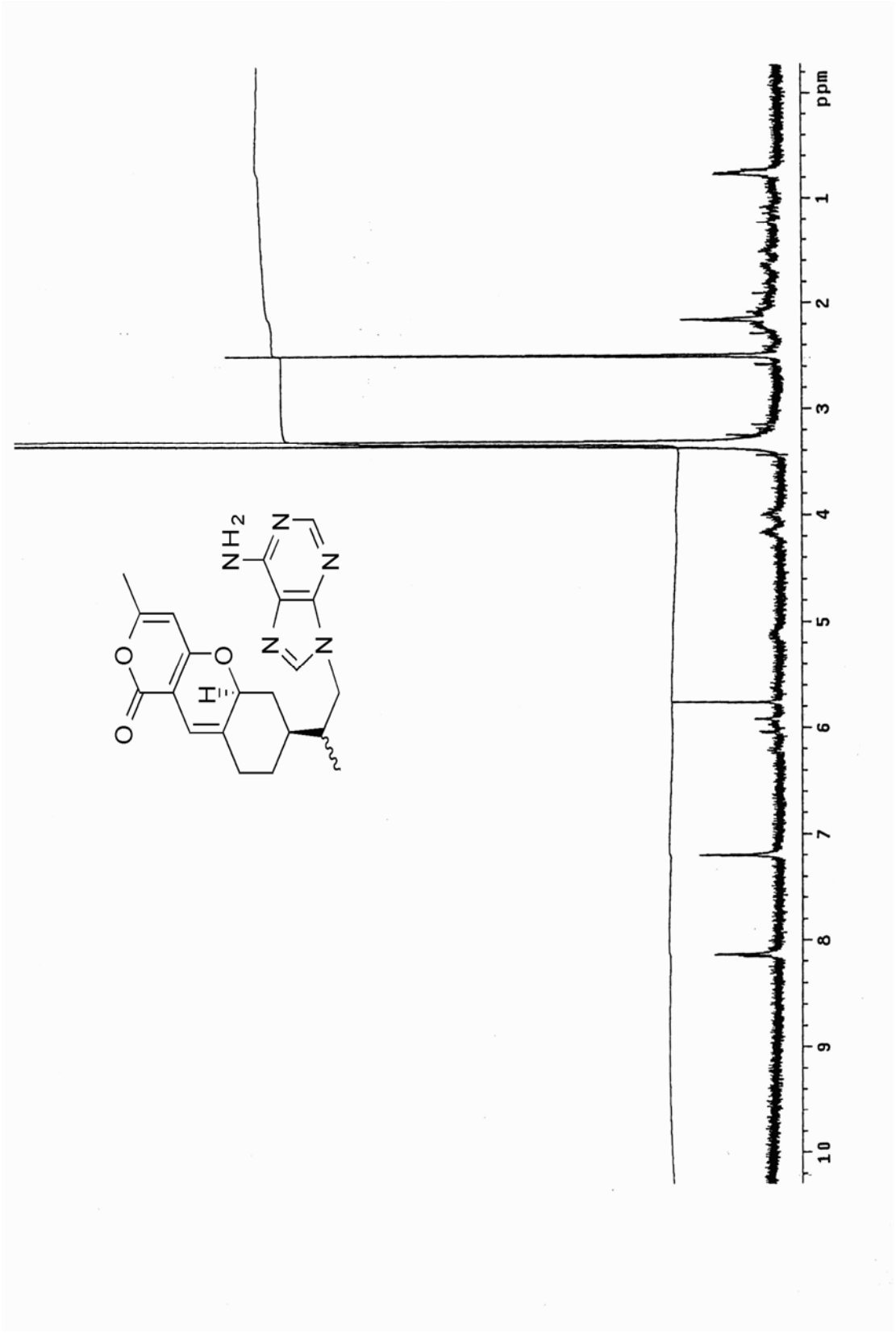

13C OBSERVE
exp1 std13c
SAMPLE
date Dec 16 2003
solvent CDCl3
file exp
ACQUISITION
sfrq 100.542
tn C13
at 1.199
np 59968
sw 25000.0
fb 13800
bs 16
tpwr 55
pw 9.2
d1 1.000
tof 0
nt 20000
ct 19088
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -2983.7
wp 25000.0
vs 177
sc 0
wc 250
hzmm 100.00
is 500.00
rfl 10747.9
rfp 7764.1
th 5
ins 100.000
nm no ph
DEC. & VT
dfrq 399.814
dn H1
dpwr 45
dof 0
dm yyy
dmm w
dmf 6100
dseq
dres 1.0
homo n
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
math f
werr
wexp wft
wbs wft
wnt
O
O
H
O
HO
200
180
160
140
120
100
80
60
40
20
0
ppm

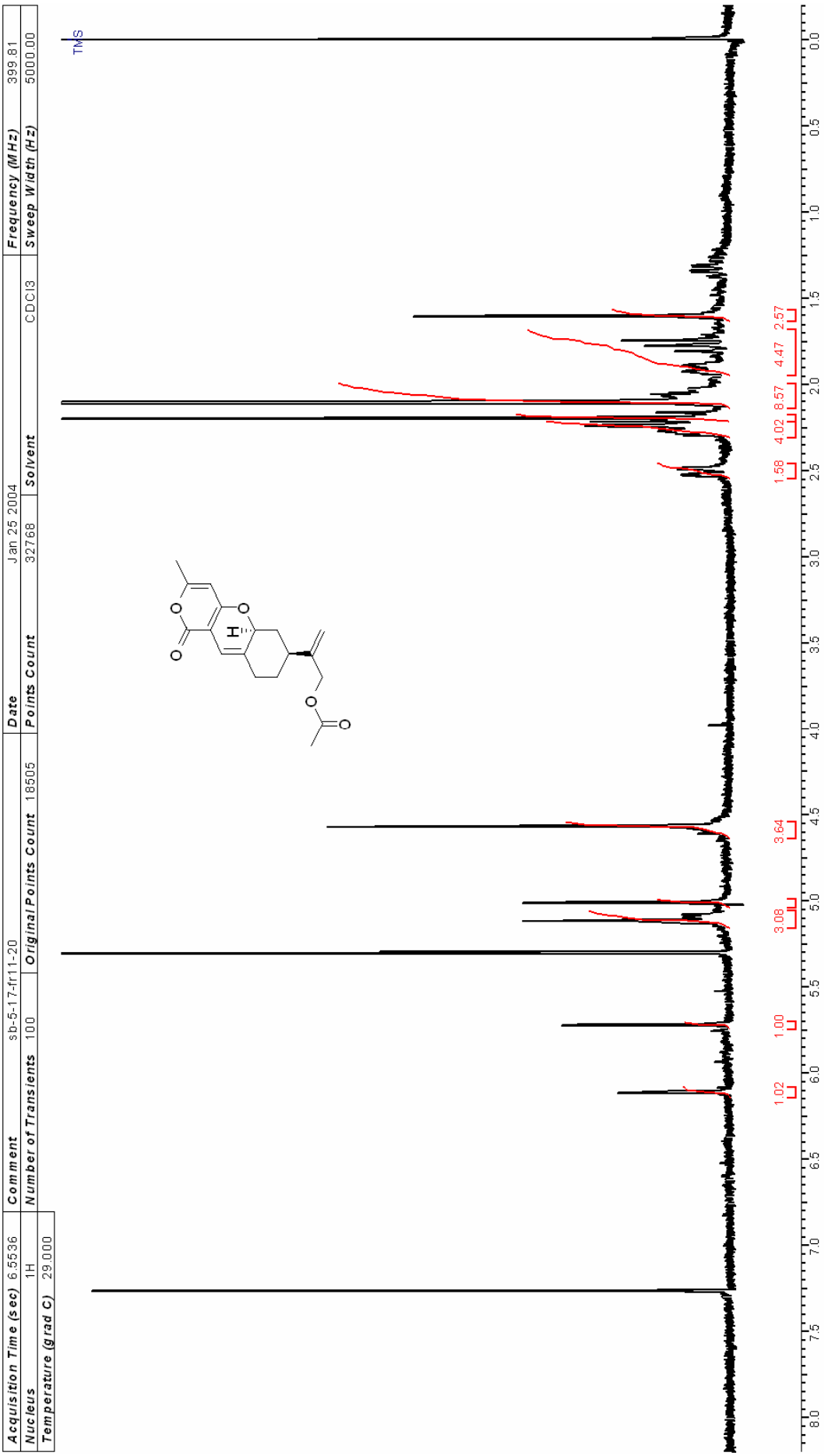

STANDARD 1H OBSERVE
expl stdlh
SAMPLE DEC. & VT
date Dec 15 2003 dfrq 399.814
solvent CDCl3 dn H1
file exp dpwr 30
ACQUISITION dof 0
sfrq 399.814 dm nnn
tn H1 dmm c
at 3.701 dmf 200
np 37010 dseq
sw 5000.0 dres 1.0
fb 2800 homo n
bs 4 PROCESSING
tpwr 55 wtfile
pw 19.8 proc ft
d1 1.000 fn 32768
tof 0 math f
nt 100
ct 32 werr
alock n wexp wft
gain 30 wbs wft
FLAGS wnt
il n
in n
dp y
hs nn
DISPLAY
sp -492.3
wp 5000.0
vs 172
sc 0
wc 250
hzmm 1.11
is 21617.65
rfl 492.3
rfp 0
th 3
ins 100.000
nm cdc ph
Ether
ppm

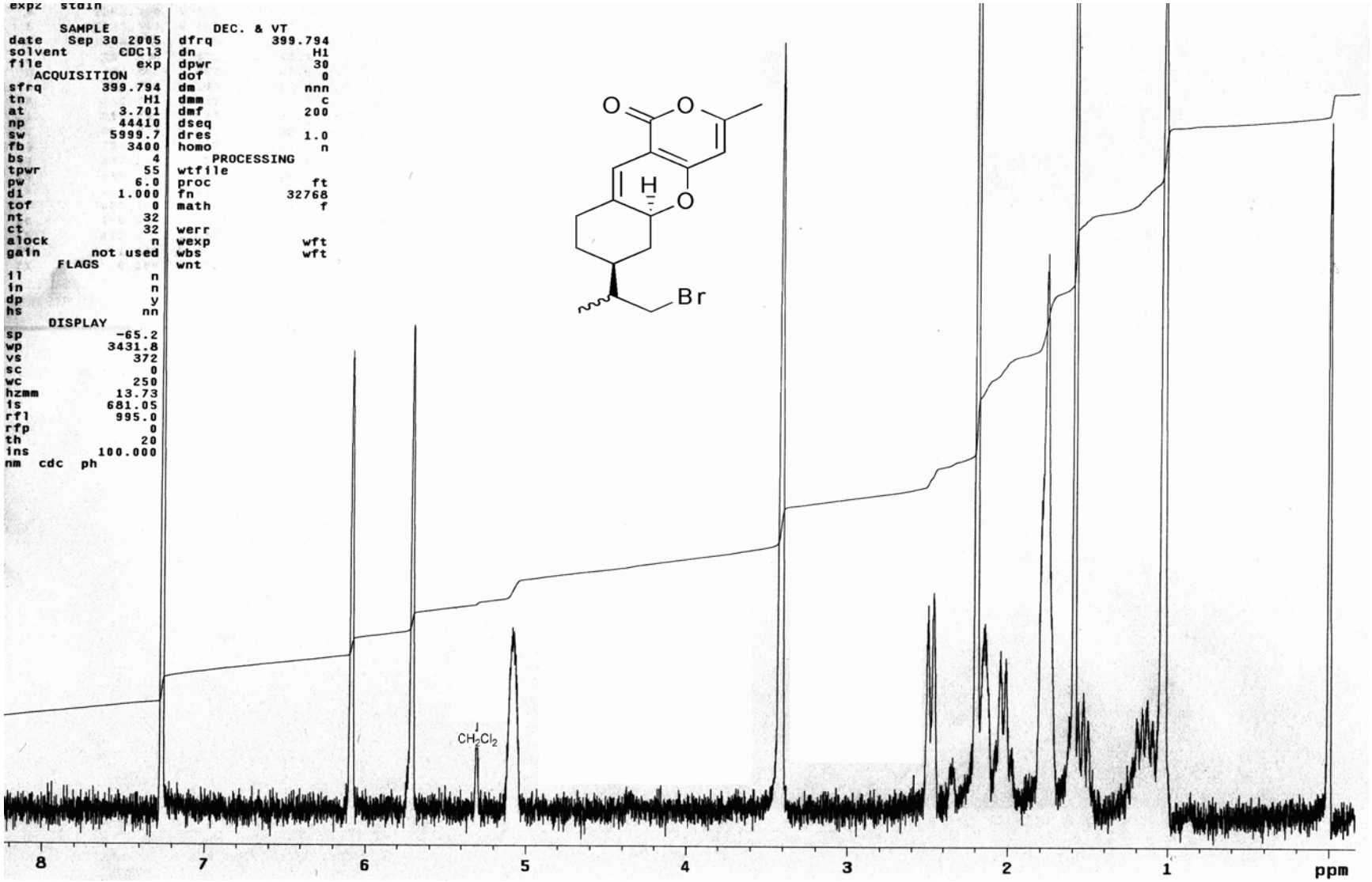

13C OBSERVE
exp1 std13c
SAMPLE
date Dec 17 2003
solvent CDCl3
file exp
ACQUISITION
sfrq 100.542
tn C13
at 1.199
np 59968
sw 25000.0
fb 13800
bs 16
tpwr 55
pw 9.2
d1 1.000
tof 0
nt 20000
ct 19424
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -2983.7
wp 25000.0
vs 334
sc 0
wc 250
hzmm 100.00
is 500.00
rfl 10747.9
rfp 7764.1
th 9
ins 100.000
nm no ph
DEC. & VT
dfrq 399.814
dn H1
dpwr 45
dof 0
dm yyy
dmm w
dmf 6100
dseq
dres 1.0
homo n
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
math f
werr
wexp wft
wbs wft
wnt
O
O
H
O
O
200
180
160
140
120
100
80
60
40
20
0
ppm

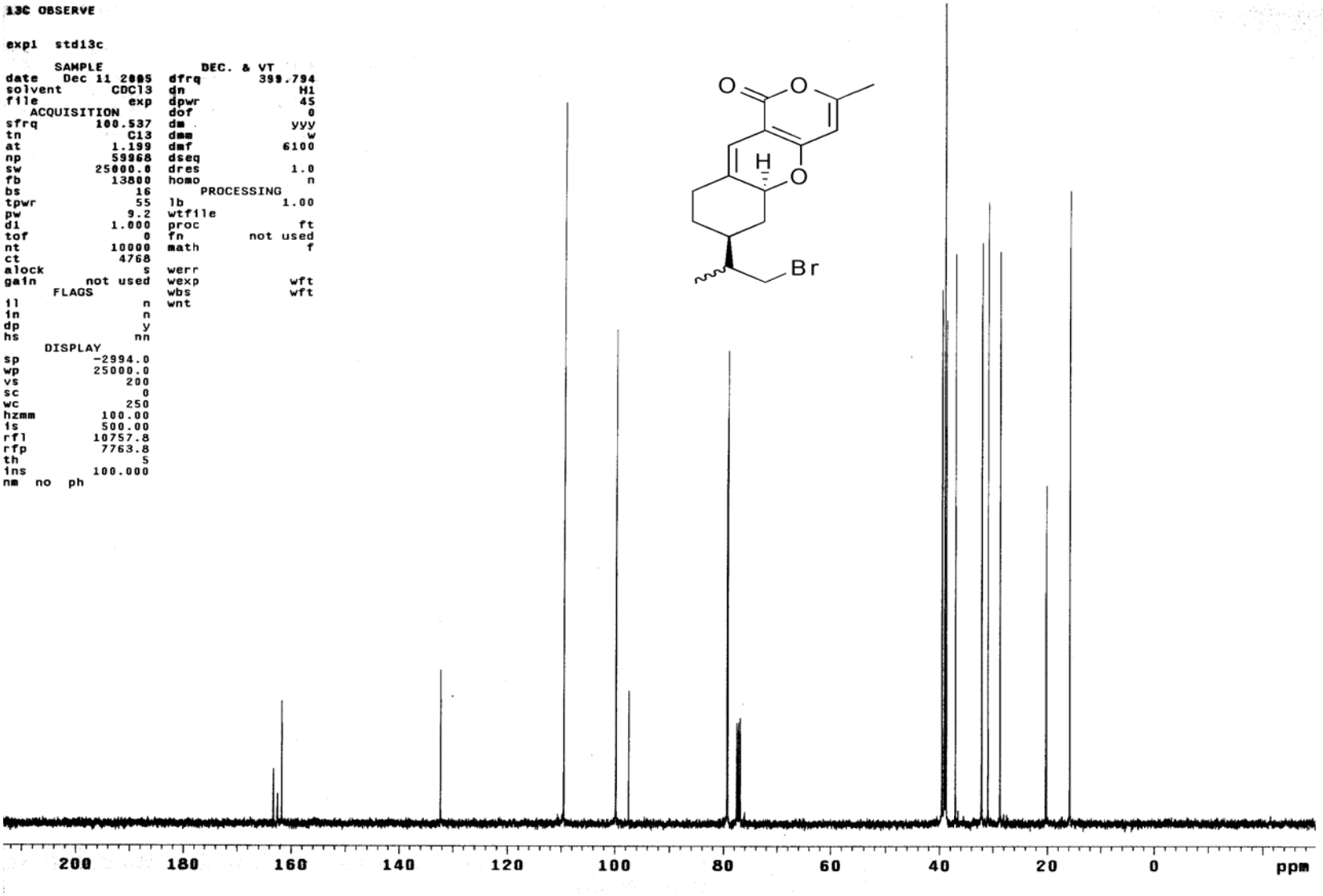
Acquisition Time (sec)	6.5536	*Comment*	sb-4-106-fr9-10b		*Date*	Dec 18 2003		*Frequency (MHz)*	399.81
Nucleus	1H	*Number of Transients*	100	*Original Points Count* 18505	*Points Count*	32768	*Solvent* CDCl3	*Sweep Width (Hz)*	5000.00
Temperature (grad C)	29.000								



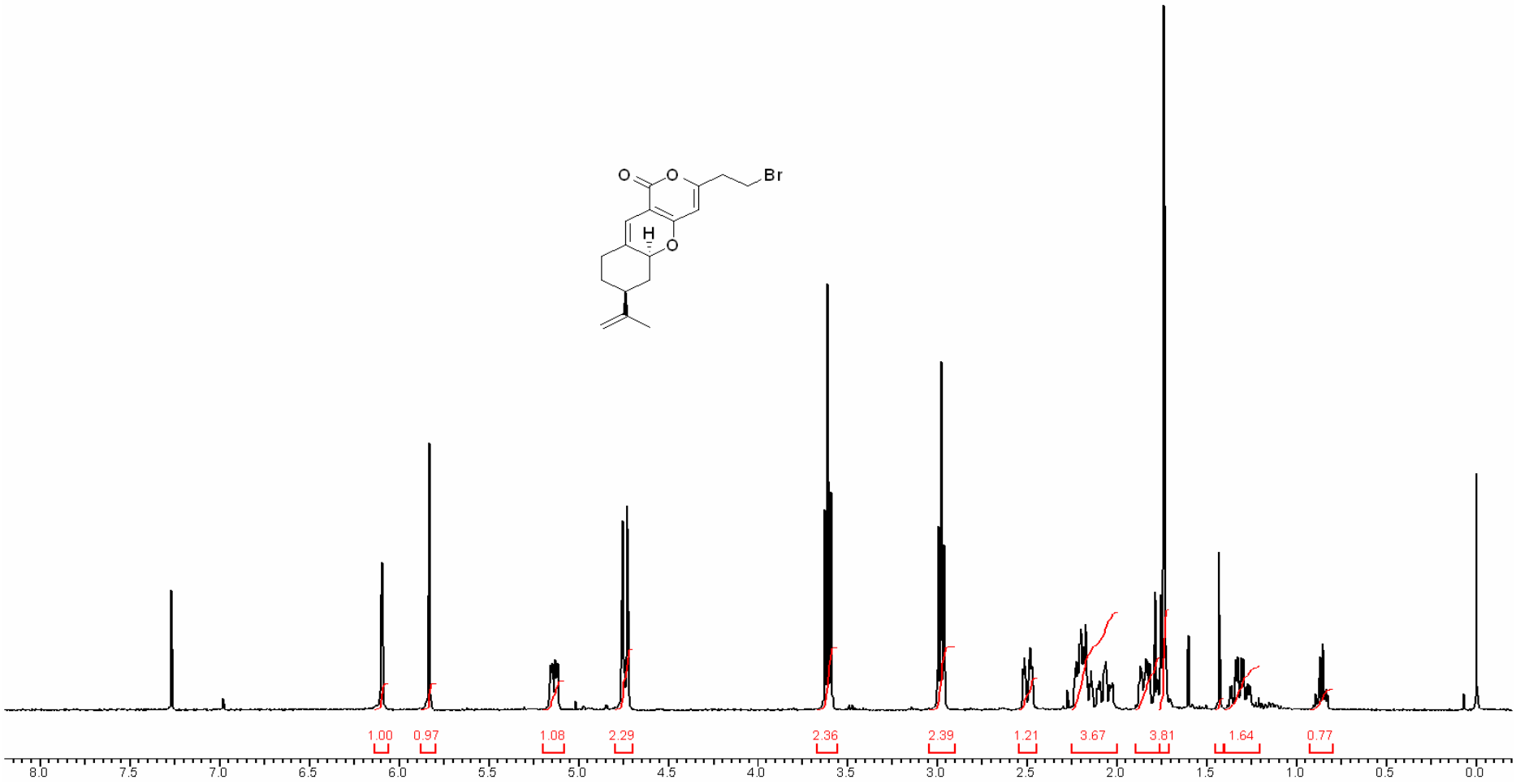

NH2
N
N
N
N
O
O
O
H
ppm
1
2
3
4
5
6
7
8
9
10

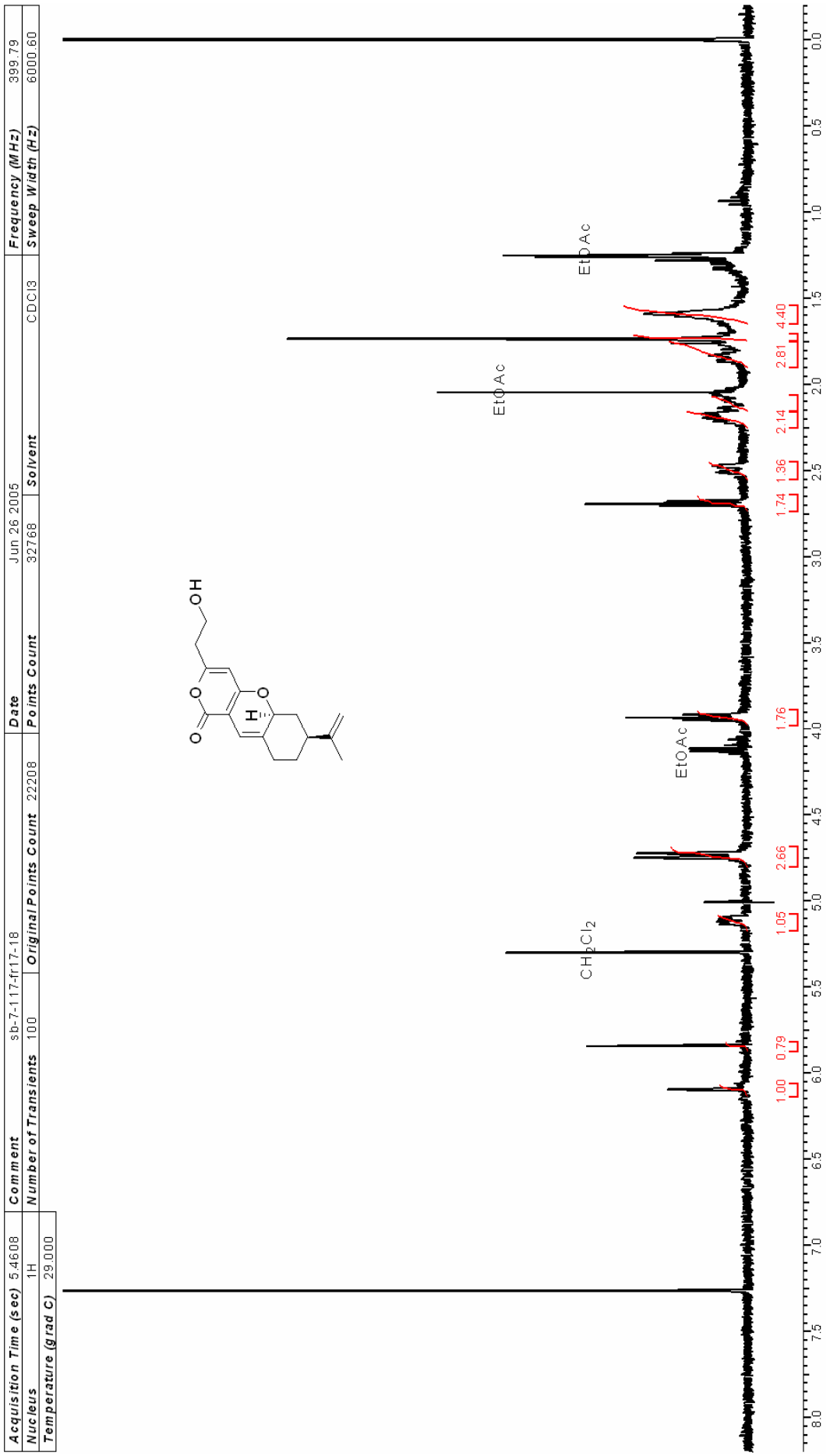

Acquisition Time (sec) 6.5536
Comment sb-5-17-fr11-20
Date Jan 25 2004
Frequency (MHz) 399.81
Nucleus 1H
Number of Transients 100
Original Points Count 18505
Points Count 32768
Solvent CDCl3
Sweep Width (Hz) 5000.00
Temperature (grad C) 29.000
TMS
1.02
1.00
3.08
3.64
1.58
4.02
8.57
4.47
2.57


expz stain
SAMPLE
date Sep 30 2005
solvent CDCl3
file exp
ACQUISITION
sfrq 399.794
tn H1
at 3.701
np 44410
sw 5999.7
fb 3400
bs 4
tpwr 55
pw 6.0
d1 1.000
tof 0
nt 32
ct 32
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -65.2
wp 3431.8
vs 372
sc 0
wc 250
hzmm 13.73
is 681.05
rfl 995.0
rfp 0
th 20
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.794
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
O
O
H
O
Br
CH2Cl2
8
7
6
5
4
3
2
1
ppm

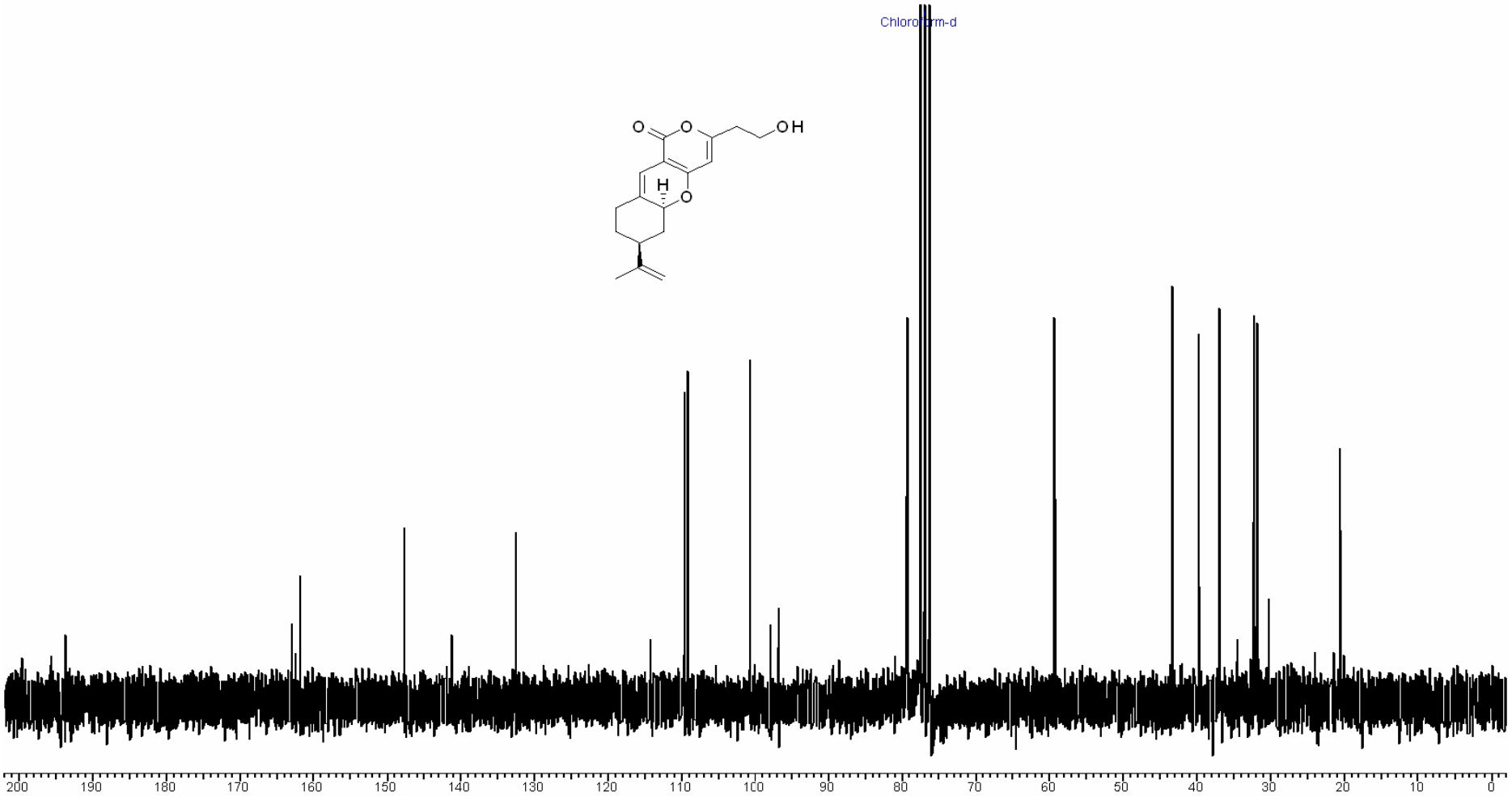

13C OBSERVE
expl std13c
SAMPLE
date Dec 11 2005
solvent CDCl3
file exp
ACQUISITION
sfrq 100.537
tn C13
at 1.199
np 59968
sw 25000.0
fb 13800
bs 16
tpwr 55
pw 9.2
d1 1.000
tof 0
nt 10000
ct 4768
alock s
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -2994.0
wp 25000.0
vs 200
sc 0
wc 250
hzmm 100.00
is 500.00
rfl 10757.8
rfp 7763.8
th 5
ins 100.000
nm no ph
DEC. & VT
dfrq 399.794
dn H1
dpwr 45
dof 0
dm yyy
dmm w
dmf 6100
dseq
dres 1.0
homo n
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
math f
werr
wexp wft
wbs wft
wnt
O
O
H
O
Br
200
180
160
140
120
100
80
60
40
20
0
ppm

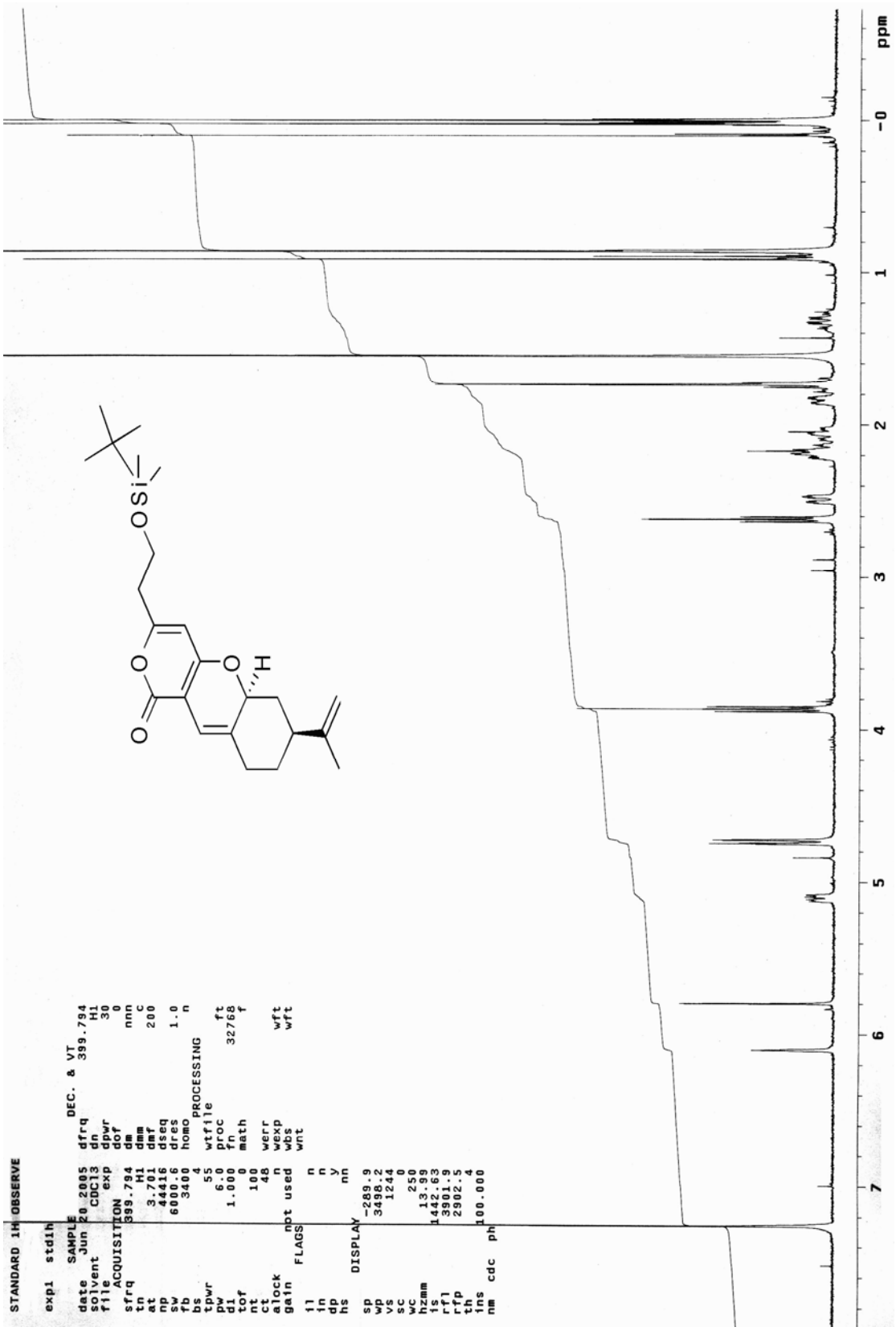
Acquisition Time (sec)	5.4608	*Comment*	sb-8-112-fr8		*Date*	Feb 16 2006		*Frequency (MHz)*	399.78
Nucleus	1H	*Number of Transients*	100	*Original Points Count* 22208	*Points Count*	32768	*Solvent* CDCl3	*Sweep Width (Hz)*	6000.60
Temperature (grad C)	29.000								

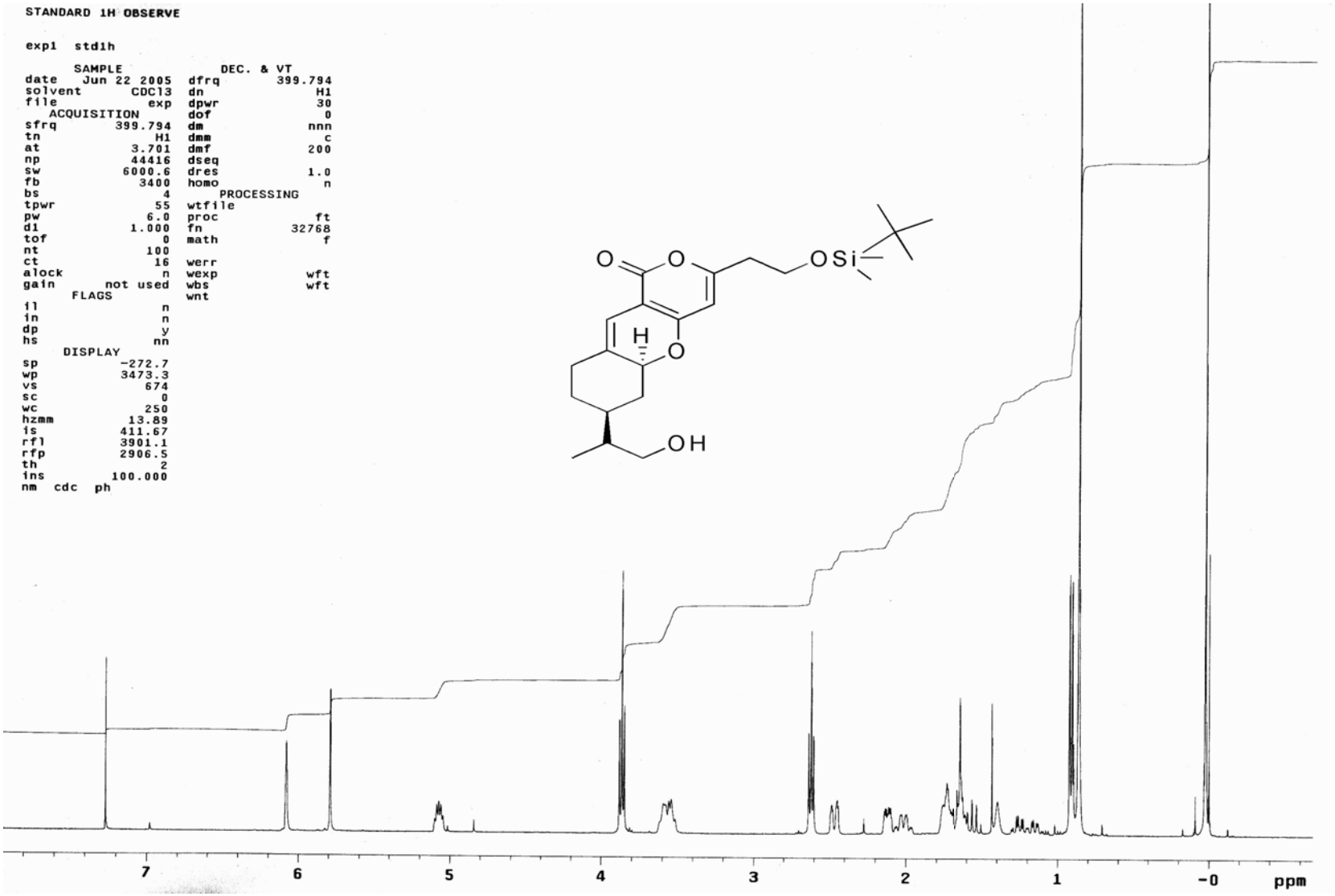


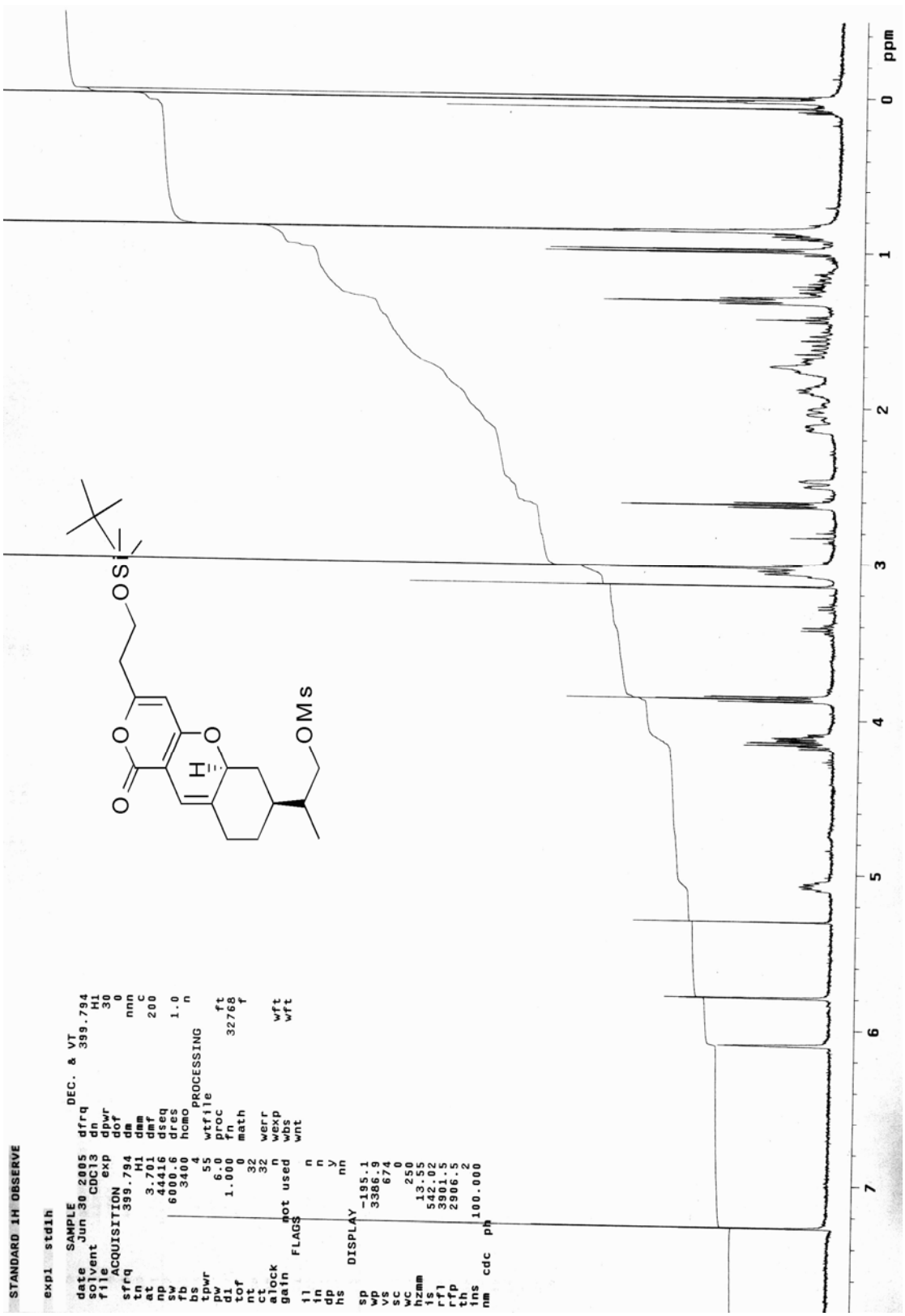

Acquisition Time (sec) 5.4608
Comment
Date
Jun 26 2005
Frequency (MHz) 399.79
Nucleus 1H
Number of Transients 100
Original Points Count 22208
Points Count 32768
Solvent CDCl3
Sweep Width (Hz) 6000.60
Temperature (grad C) 29.000
sb-7-117-fr17-18
OH
O
O
O
H
CH2Cl2
EtOAc
EtOAc
EtOAc
1.00
0.79
1.05
2.66
1.76
1.74
1.36
2.14
2.81
4.40
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

Acquisition Time (sec)	2.6214	*Comment*	sb-9-57-1C13		*Date*	Jul 20 2006		*Frequency (MHz)*	50.29
Nucleus	13C	*Number of Transients*	20000	*Original Points Count* 18720	*Points Count*	32768	*Solvent* CDCl3	*Sweep Width (Hz)*	12500.00
Temperature (grad C)	29.000								

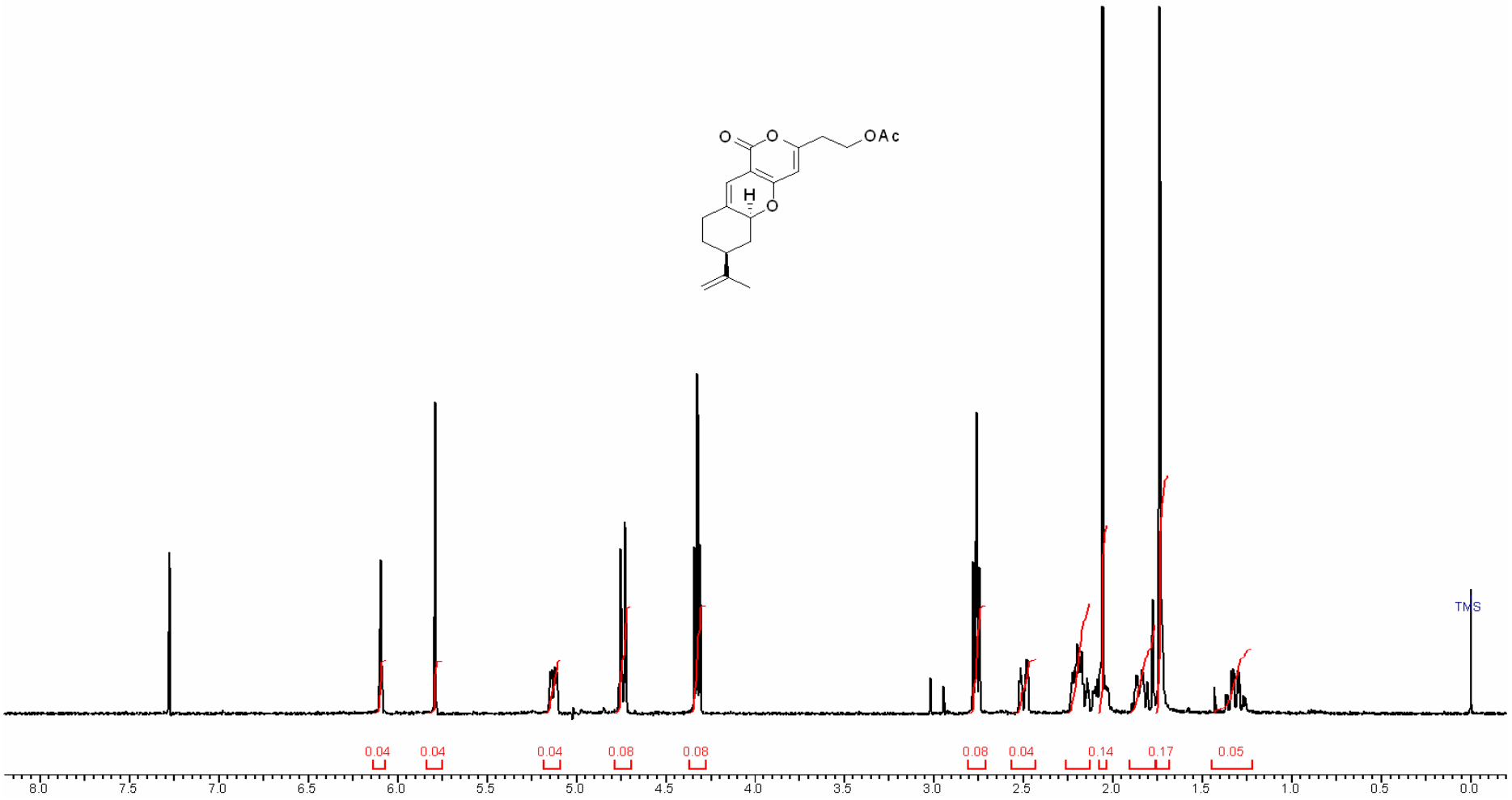


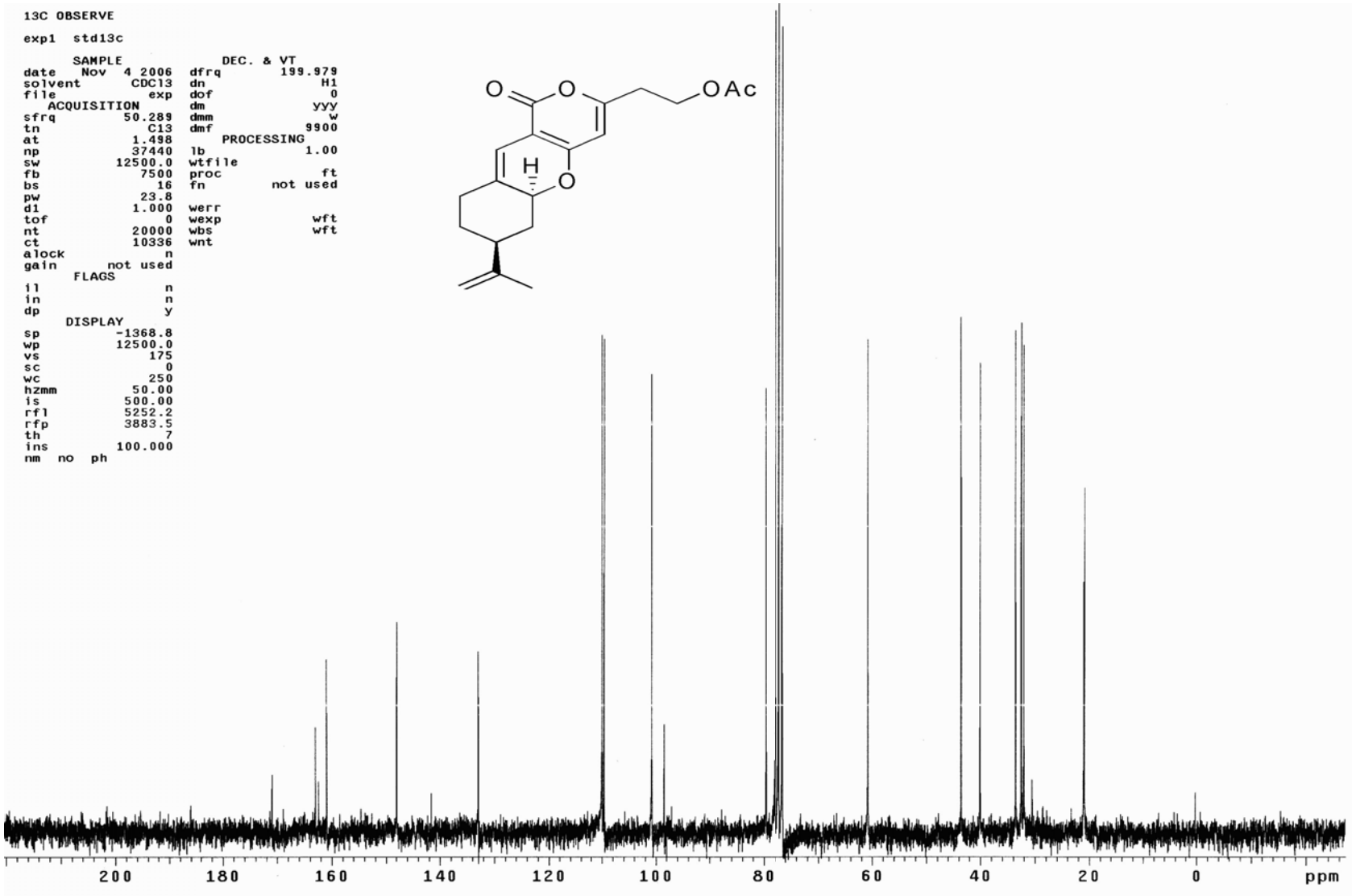

STANDARD 1H OBSERVE
DEC. & VT
ppm


STANDARD 1H OBSERVE
expl stdlh
SAMPLE
date Jun 22 2005
solvent CDCl3
file exp
ACQUISITION
sfrq 399.794
tn H1
at 3.701
np 44416
sw 6000.6
fb 3400
bs 4
tpwr 55
pw 6.0
dl 1.000
tof 0
nt 100
ct 16
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -272.7
wp 3473.3
vs 674
sc 0
wc 250
hzmm 13.89
is 411.67
rfl 3901.1
rfp 2906.5
th 2
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.794
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
O
O
OSi
H
O
OH
7
6
5
4
3
2
1
-0
ppm

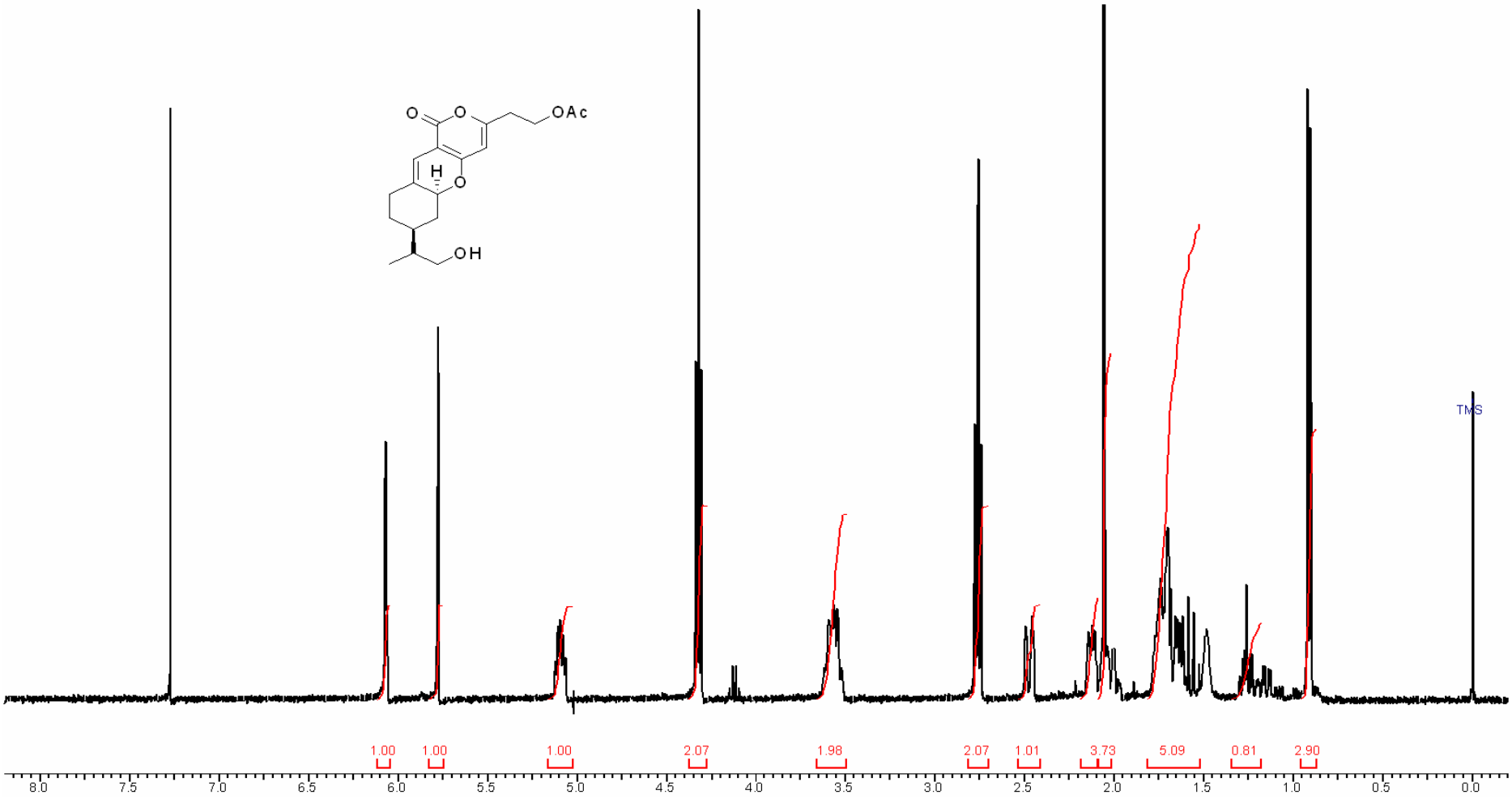

STANDARD 1H OBSERVE
exp1 std1h
DEC. & VT
SAMPLE
date Jun 30 2005
solvent CDCl3
file exp
ACQUISITION
sfrq 399.794
tn H1
at 3.701
np 44416
sw 60000.6
fb 3400
bs 4
tpwr 55
pw 6.0
d1 1.000
tof 0
nt 32
ct 32
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -195.1
wp 3386.9
vs 674
sc 0
wc 250
hzmm 13.55
is 542.02
rfl 3901.5
rfp 2906.5
th 2
ins 100.000
nm cdc ph
dfrq 399.794
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
hcmo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
OSi
OMs
H
O
O
O
7
6
5
4
3
2
1
0 ppm

Acquisition Time (sec)	5.4608	*Comment*	sb-8-29-fr11-14		*Date*	Sep 21 2005		*Frequency (MHz)*	399.79
Nucleus	1H	*Number of Transients*	100	*Original Points Count* 22208	*Points Count*	32768	*Solvent* CDCl3	*Sweep Width (Hz)*	6000.60
Temperature (grad C)	29.000								

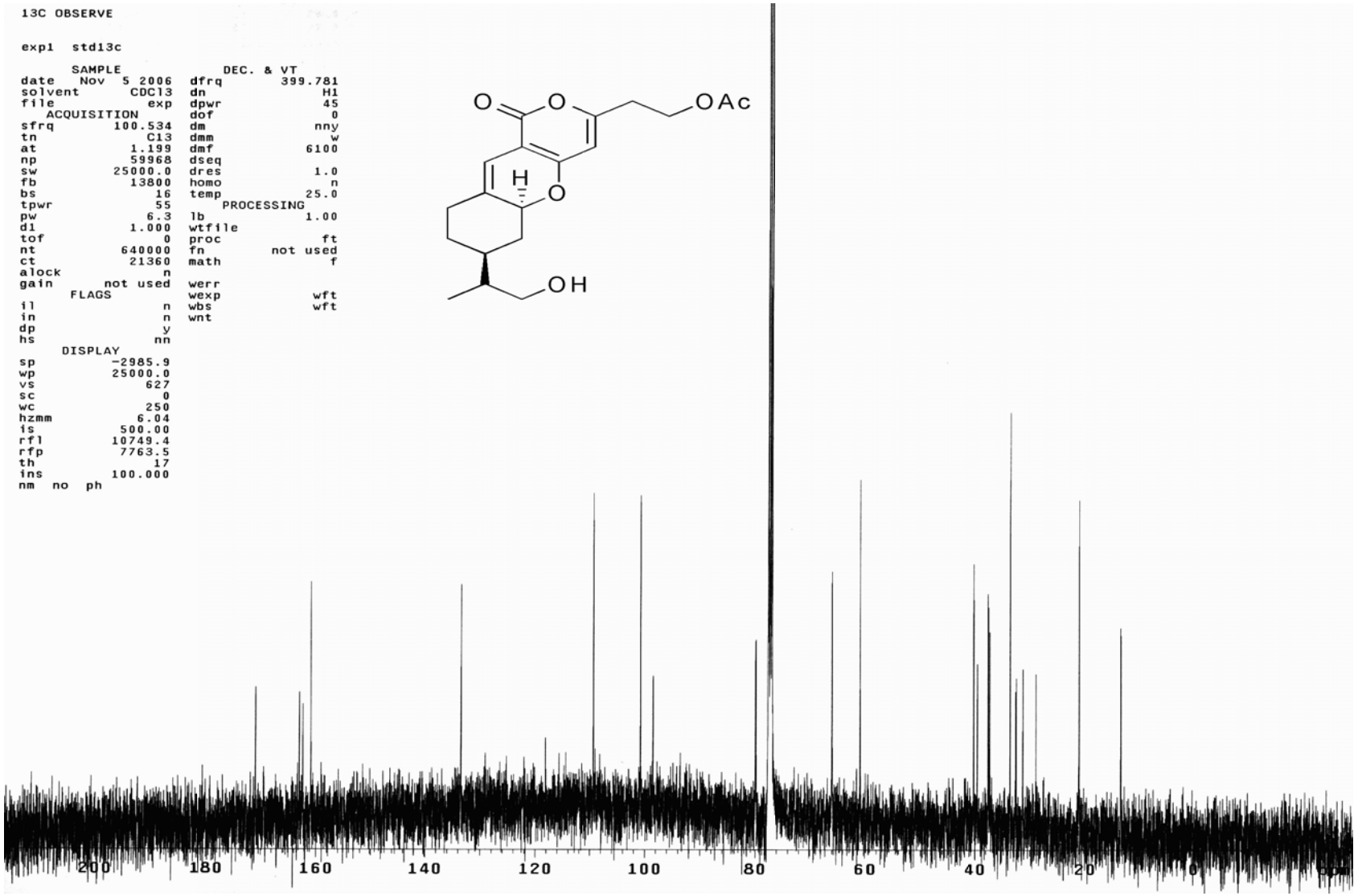


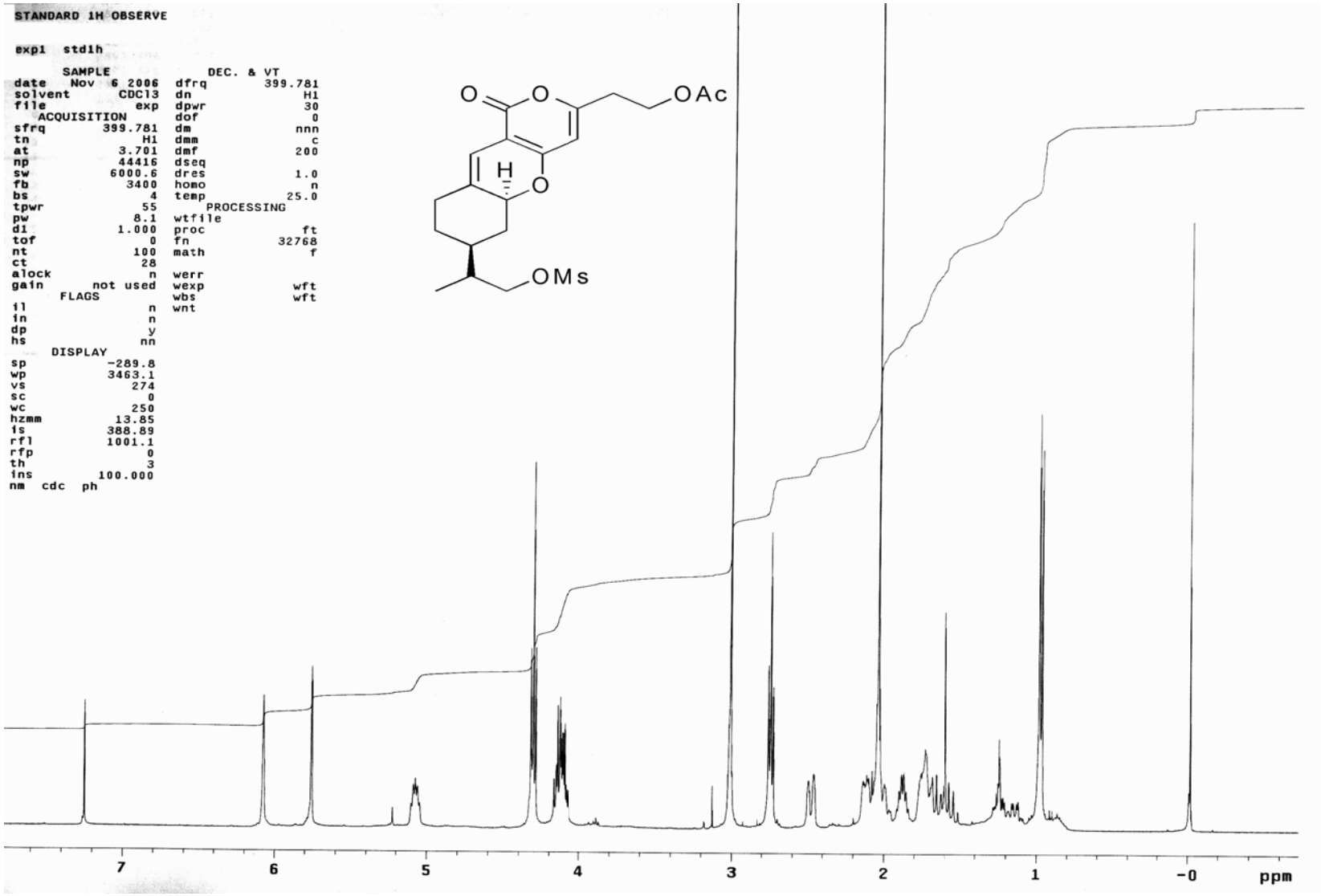

13C OBSERVE
exp1 std13c
SAMPLE
date Nov 4 2006
solvent CDCl3
file exp
ACQUISITION
sfrq 50.289
tn C13
at 1.498
np 37440
sw 12500.0
fb 7500
bs 16
pw 23.8
d1 1.000
tof 0
nt 20000
ct 10336
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -1368.8
wp 12500.0
vs 175
sc 0
wc 250
hzmm 50.00
is 500.00
rfl 5252.2
rfp 3883.5
th 7
ins 100.000
nm no ph
DEC. & VT
dfrq 199.979
dn H1
dof 0
dm yyy
dmm w
dmf 9900
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
werr
wexp wft
wbs wft
wnt
O
O
OAc
H
O
200
180
160
140
120
100
80
60
40
20
0
ppm

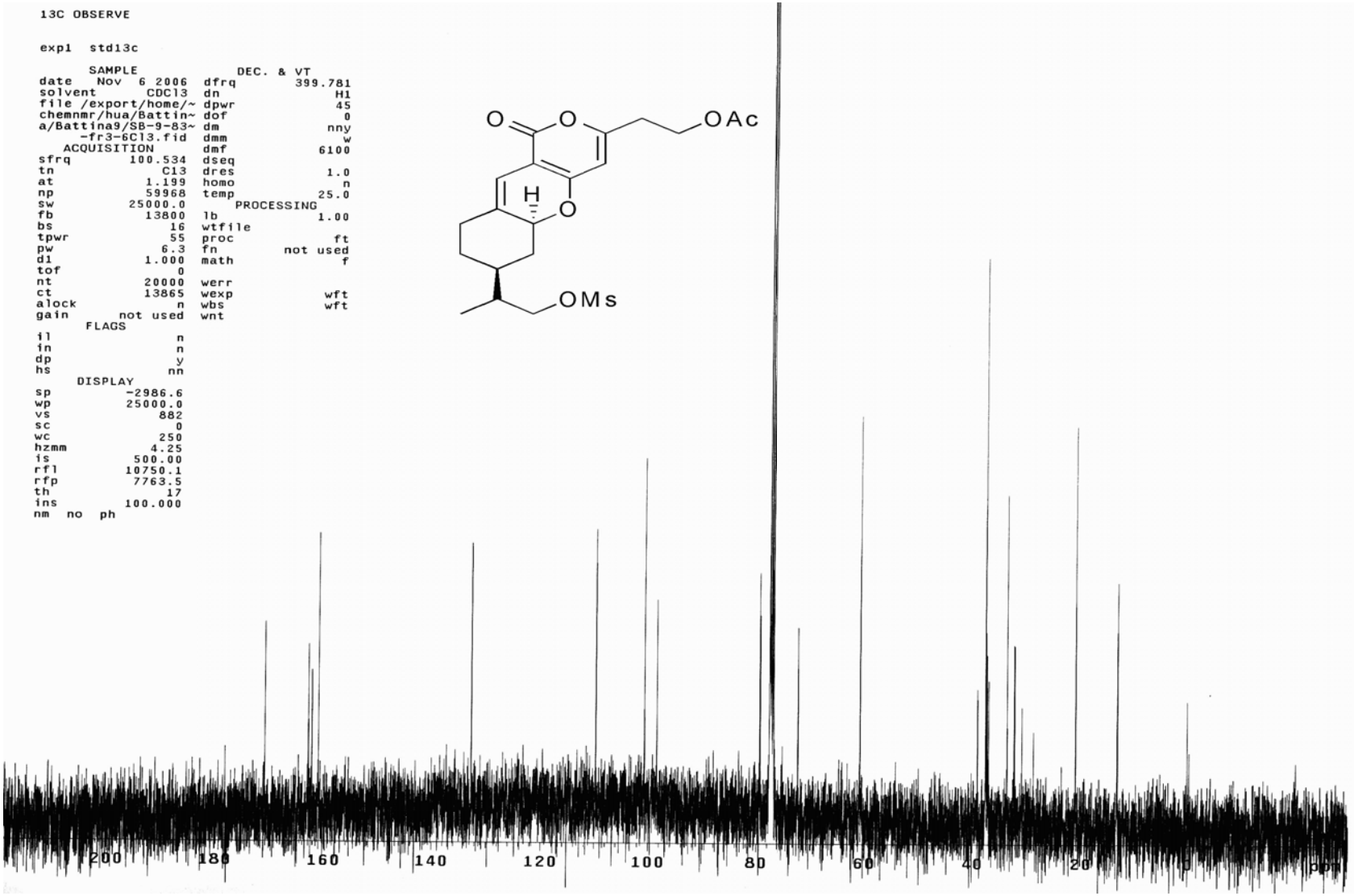
Acquisition Time (sec)	5.4608	*Comment*	sb-8-34-fr21		*Date*	Sep 29 2005		*Frequency (MHz)*	399.79
Nucleus	1H	*Number of Transients*	100	*Original Points Count* 22208	*Points Count*	32768	*Solvent* CDCl3	*Sweep Width (Hz)*	6000.60
Temperature (grad C)	29.000								

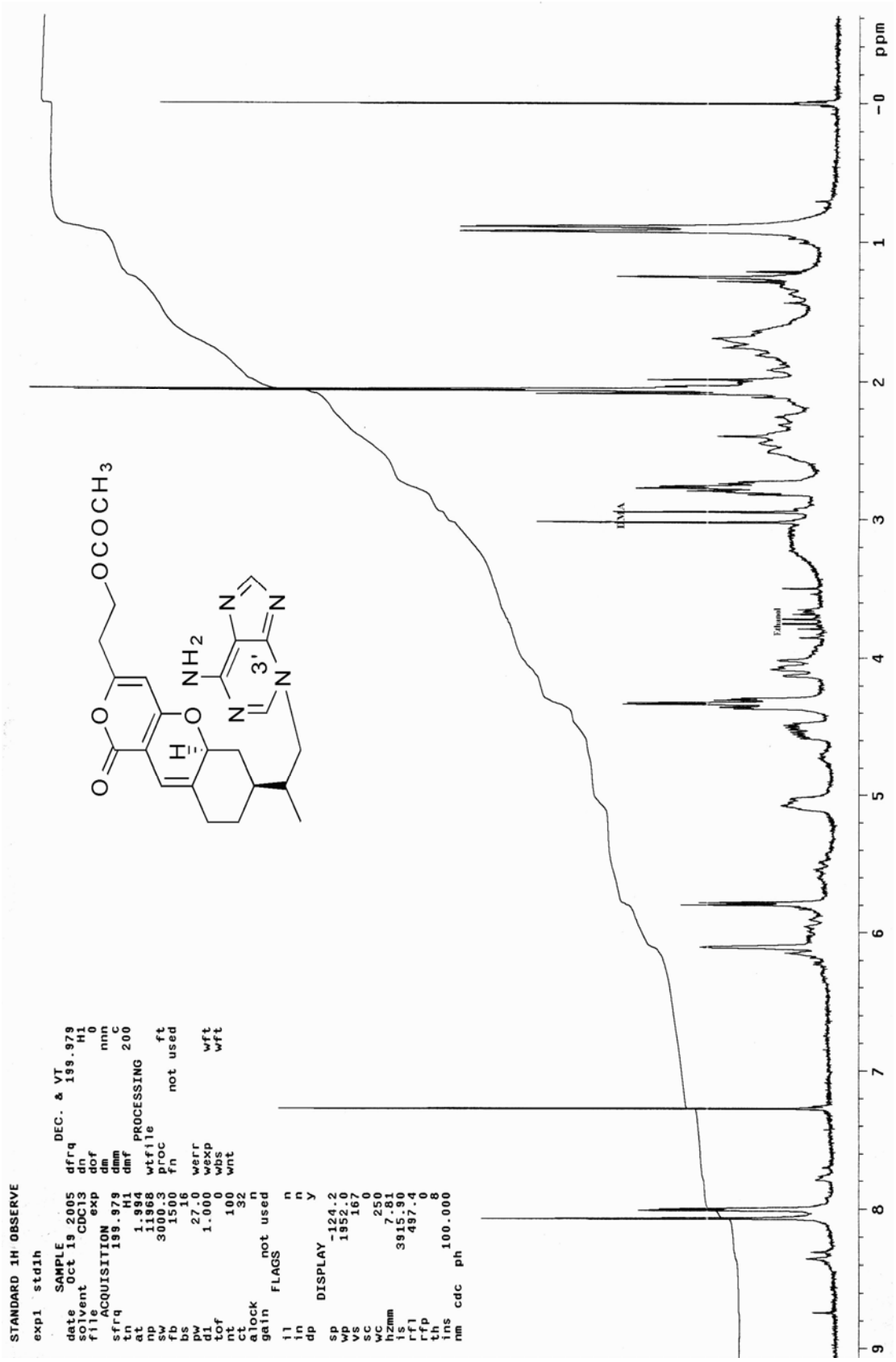


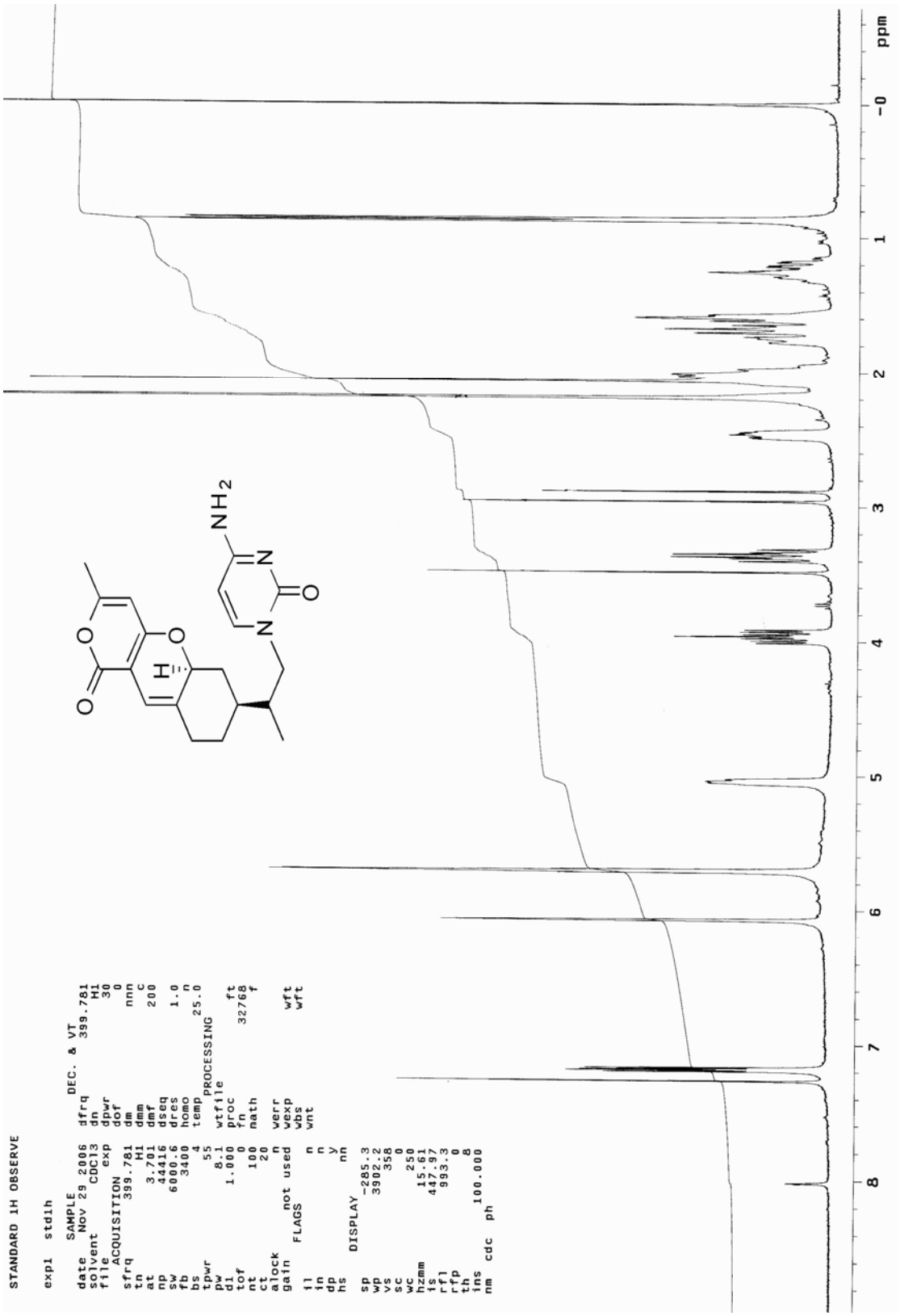
200

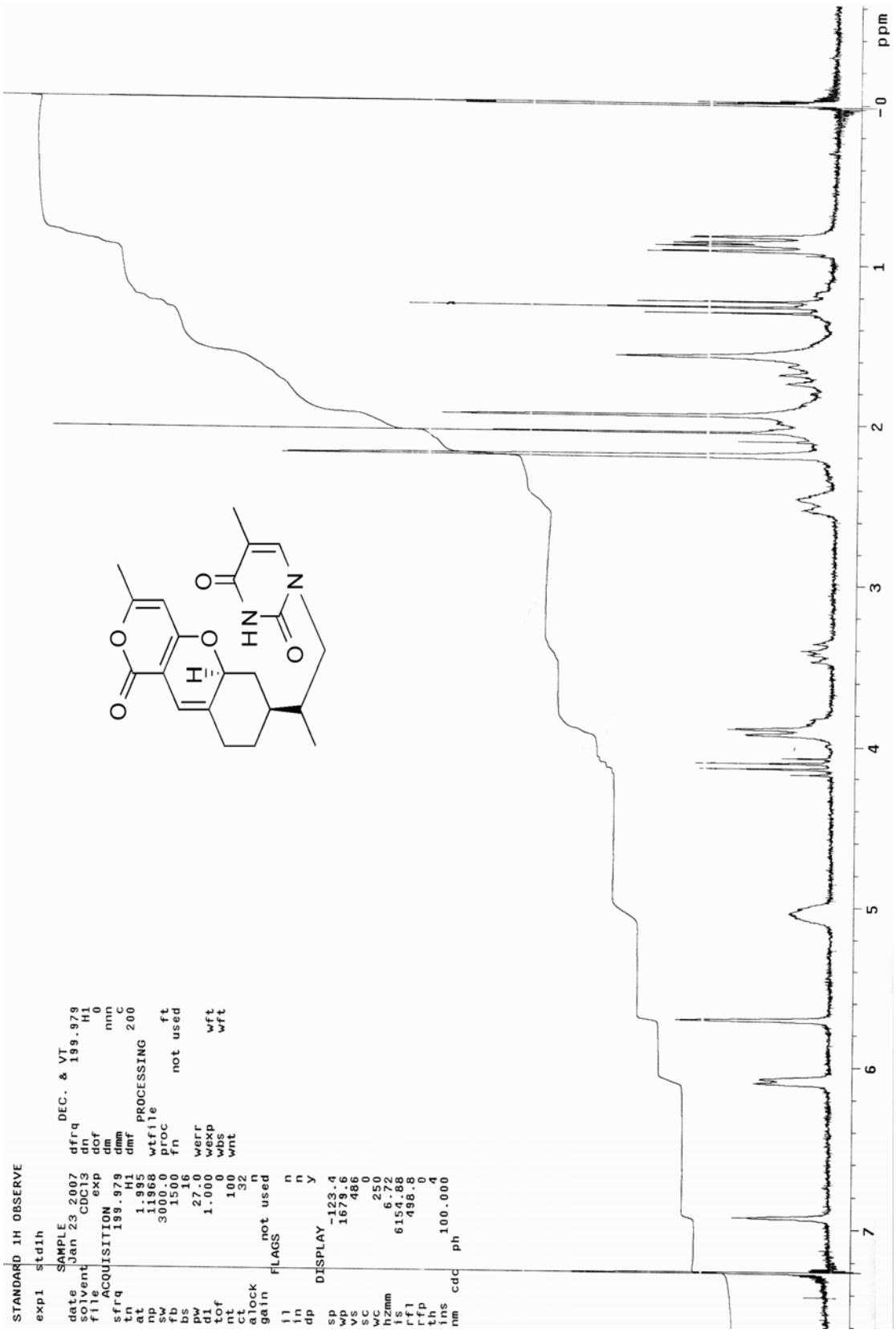

13C OBSERVE
exp1 std13c
SAMPLE
date Nov 5 2006
solvent CDCl3
file exp
ACQUISITION
sfrq 100.534
tn C13
at 1.199
np 59968
sw 25000.0
fb 13800
bs 16
tpwr 55
pw 6.3
d1 1.000
tof 0
nt 640000
ct 21360
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -2985.9
wp 25000.0
vs 627
sc 0
wc 250
hzmm 6.04
is 500.00
rfl 10749.4
rfp 7763.5
th 17
ins 100.000
nm no ph
DEC. & VT
dfrq 399.781
dn H1
dpwr 45
dof 0
dm nny
dmm w
dmf 6100
dseq
dres 1.0
homo n
temp 25.0
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
math f
werr
wexp wft
wbs wft
wnt
O
O
OAc
H
O
OH
200
180
160
140
120
100
80
60
40
20
0

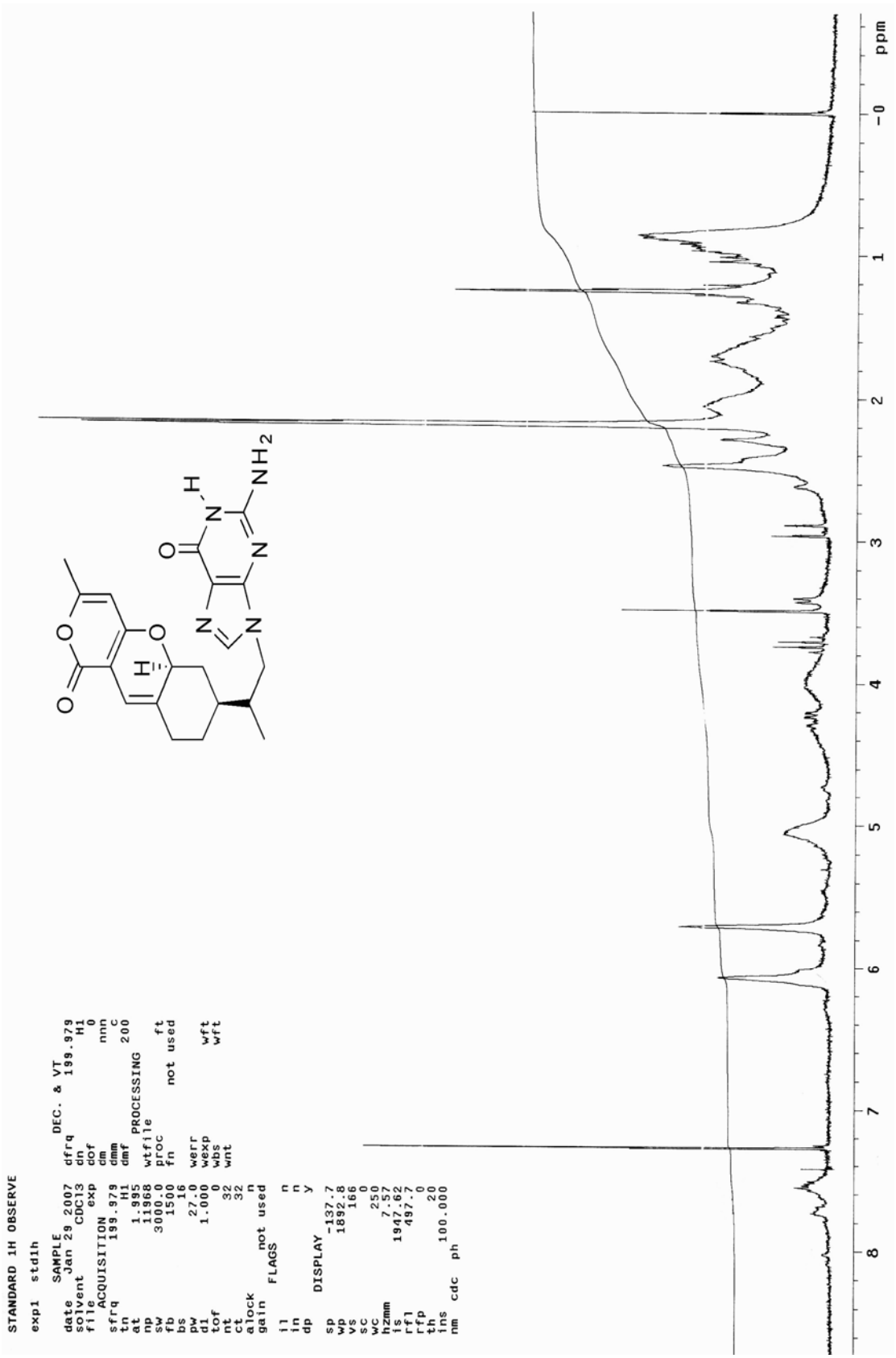

STANDARD 1H OBSERVE
exp1 std1h
SAMPLE
date Nov 6 2006
solvent CDCl3
file exp
ACQUISITION
sfrq 399.781
tn H1
at 3.701
np 44416
sw 6000.6
fb 3400
bs 4
tpwr 55
pw 8.1
d1 1.000
tof 0
nt 100
ct 28
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -289.8
wp 3463.1
vs 274
sc 0
wc 250
hzmm 13.85
is 388.89
rfl 1001.1
rfp 0
th 3
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.781
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
temp 25.0
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
O
O
OAc
H
O
OMs
7
6
5
4
3
2
1
-0
ppm


13C OBSERVE
exp1 std13c
SAMPLE
date Nov 6 2006
solvent CDCl3
file /export/home/~
chemnmr/hua/Battin~
a/Battina9/SB-9-83~
-fr3-6Cl3.fid
ACQUISITION
sfrq 100.534
tn C13
at 1.199
np 59968
sw 25000.0
fb 13800
bs 16
tpwr 55
pw 6.3
d1 1.000
tof 0
nt 20000
ct 13865
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -2986.6
wp 25000.0
vs 882
sc 0
wc 250
hzmm 4.25
is 500.00
rfl 10750.1
rfp 7763.5
th 17
ins 100.000
nm no ph
DEC. & VT
dfrq 399.781
dn H1
dpwr 45
dof 0
dm nny
dmm w
dmf 6100
dseq
dres 1.0
homo n
temp 25.0
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
math f
werr
wexp wft
wbs wft
wnt
O
O
OAc
H
O
OMs
200
180
160
140
120
100
80
60
40
20
0


STANDARD 1H OBSERVE
exp1 std1h
SAMPLE
date Oct 19 2005
solvent CDCl3
file exp
ACQUISITION
sfrq 199.979
tn H1
at 1.994
np 11968
sw 3000.3
fb 1500
bs 16
pw 27.0
d1 1.000
tof 0
nt 100
ct 32
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -124.2
wp 1952.0
vs 167
sc 0
wc 250
hzmm 7.81
is 3915.90
rfl 497.4
rfp 0
th 8
ins 100.000
nm cdc ph
DEC. & VT
dfrq 199.979
dn H1
dof 0
dm nnn
dmm c
dmf 200
PROCESSING
wtfile
proc ft
fn not used
werr
wexp wft
wbs wft
wnt
OCOCH3
NH2
3'
DMF
Ethanol
9
8
7
6
5
4
3
2
1
-0
ppm


STANDARD 1H OBSERVE
expl std1h
SAMPLE
date Nov 29 2006
solvent CDCl3
file exp
ACQUISITION
sfrq 399.781
tn H1
at 3.701
np 44416
sw 6000.6
fb 3400
bs 4
tpwr 55
pw 8.1
d1 1.000
tof 0
nt 100
ct 20
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -285.3
wp 3902.2
vs 358
sc 0
wc 250
hzmm 15.61
is 447.97
rfl 993.3
rfp 0
th 8
ins 100.000
nm cdc ph
DEC. & VT
dfrq 399.781
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
temp 25.0
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp wft
wbs wft
wnt
NH2
N
N
O
O
O
O
H
8
7
6
5
4
3
2
1
-0
ppm


STANDARD 1H OBSERVE
exp1 std1h
SAMPLE
date Jan 23 2007
solvent CDCl3
file exp
ACQUISITION
sfrq 199.979
tn H1
at 1.995
np 11968
sw 3000.0
fb 1500
bs 16
pw 27.0
d1 1.000
tof 0
nt 100
ct 32
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -123.4
wp 1679.6
vs 486
sc 0
wc 250
hzmm 6.72
is 6154.88
rfl 498.8
rfp 0
th 4
ins 100.000
nm cdc ph
DEC. & VT
dfrq 199.979
dn H1
dof 0
dm nnn
dmm c
dmf 200
PROCESSING
wtfile
proc ft
fn not used
werr
wexp wft
wbs wft
wnt
H
O
O
O
HN
O
N
7
6
5
4
3
2
1
0
ppm


STANDARD 1H OBSERVE
expl std1h
SAMPLE
date Jan 29 2007
solvent CDCl3
file exp
ACQUISITION
sfrq 199.979
tn H1
at 1.995
np 11968
sw 3000.0
fb 1500
bs 16
pw 27.0
d1 1.000
tof 0
nt 32
ct 32
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -137.7
wp 1892.8
vs 166
sc 0
wc 250
hzmm 7.57
is 1947.62
rfl 497.7
rfp 0
th 20
ins 100.000
nm cdc ph
DEC. & VT
dfrq 199.979
dn H1
dof 0
dm nnn
dmm c
dmf 200
PROCESSING
wtfile
proc ft
fn not used
werr
wexp wft
wbs wft
wnt
ppm