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Syntheses of natural products OSW-1, superstolide A and their derivatives

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SYNTHESES OF NATURAL PRODUCTS OSW-1, SUPERSTOLIDE A AND THEIR DERIVATIVES

by

Yan Mei

An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the Doctor of
Philosophy degree in Pharmacy
in the Graduate College of
The University of Iowa

May 2009

Thesis Supervisor: Associate Professor Zhendong Jin

ABSTRACT

OSW-1 is a natural saponin and its anticancer activities are 10- to 100-fold more potent than many well-known anticancer agents in clinical use. Its cytotoxicity profile suggests that it may have a unique mode of action different from other well-known anticancer agents. However, its mechanism still remains a mystery after years of study, and no paper has ever been published in this area. Extensive *in vitro* and *in vivo* testing has been conducted and toxicological experiments have also been carried out by our collaborator Prof. Huang's laboratory at MD Anderson Cancer Center. In order to identify the pharmacophore and mechanism of OSW-1 and increase its *in vivo* activity and selectivity, simplified analogs and amino analogue were synthesized for the SAR study.

Superstolide A (**1**) is a highly potent anti-tumor reagent that was isolated from deep water marine sponge in 1996. The potent anticancer activity, molecular complexity (11 chiral centers) and scarcity in natural resources make this molecule an attractive synthetic target. This dissertation includes a model study for the construction of the 16-membered macrolactone present in Superstolide A. More specially, there is a focus on the investigation of three crucial carbon-carbon bond-forming reactions in our synthetic strategy including Julia olefination, Suzuki coupling and Horner-Emmons olefination.

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Graduate College
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CERTIFICATE OF APPROVAL

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PH.D. THESIS

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Yan Mei

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for the thesis requirement for the Doctor of Philosophy
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To Lu and Maggie

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Superstolide A (**1**) is a highly potent anti-tumor reagent that was isolated from deep water marine sponge in 1996. The potent anticancer activity, molecular complexity (11 chiral centers) and scarcity in natural resources make this molecule an attractive synthetic target. This dissertation includes a model study for the construction of the 16-membered macrolactone present in Superstolide A. More specially, there is a focus on the investigation of three crucial carbon-carbon bond-forming reactions in our synthetic strategy including Julia olefination, Suzuki coupling and Horner-Emmons olefination.

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TABLE OF CONTENTS

LIST OF TABLES viii

LIST OF FIGURES ix

LIST OF SCHEMES xiii

CHAPTER 1 BIOLOGICAL ACTIVITY AND PREVIOUS SYNTHETIC EFFORTS OF OSW 1

1.1 Introduction 1
1.1.1 Structure and natural source of OSW-1 1
1.1.2 Biological activities 2
1.2 Synthesis of OSW-1 5
1.2.1 Fuchs approach for the synthesis of the protected aglycone of OSW-1 5
1.2.2 The first total synthesis of OSW-1 6
1.2.3 Jin approach for the total synthesis of OSW-1 7
1.2.4 Morzycki's approach to the total synthesis of OSW-1 8
1.2.5 Guo approach to the synthesis of OSW-1 9
1.3 Preliminary biological studies on OSW-1 10
1.3.1 Anticancer activity and selectivity of OSW-1 11
1.3.2 Structural damage to mitochondria by OSW-1 12
1.3.3 OSW-1 induced calcium-dependent apoptosis 14
1.3.4 Apoptosis in the Parental HL-60 Cells & the Mitochondrial Respiration-deficient C6F Cells 15
1.3.5 OSW-1 effectively killed drug-resistant primary leukemia cells from chronic lymphocytic leukemia patient 15

CHAPTER 2 PREVIOUS STUDIES ON THE SYNTHESIS OF ANALOGS OF OSW1 19

2.1 Introduction 19
2.2 Structure–activity relationships (SAR) studies of OSW-1 by Morzycki's group 19
2.3 Structure–activity relationships (SAR) of OSW-1 by Nemoto's group 22
2.4 Structure–activity relationships (SAR) of OSW-1 by Yu and Hui's group 24
2.4.1 Steroidal glycosides bearing the disaccharide moiety of OWS-1 25
2.4.2 OSW-1 analog with modified side chain 27
2.4.3 OSW-1 analogs with modified sugar residues 30
2.5 Structure–activity relationships (SAR) of OSW-1 by Jin group 31

CHAPTER 3 DESIGN AND SYNTHESIS OF OSW-1 ANALOGS 35

3.1 Introduction 35

3.2 17–des-oxo-OSW-1 35
3.3 Simplified OSW-1 analog without C-17 hydroxy group and C-22 keto group 37
3.4 Conclusion 40
3.5 Chemical experiments 41
3.5.1General 41
3.5.2 Experimental procedures and spectral data 42

CHAPTER 4 DESIGN AND SYNTHESIS AMINO ANALOGS OF OSW-1 47

4.1 Introduction 47
4.2 Retro-synthetic analysis of amino analog 48
4.3 First attempt toward to synthesis amino analog **4.1** 50
4.3.1 Synthesis of amino L-arabinose moiety 50
4.3.1.1 The first approach for the synthesis of L-arabinosamine moiety 50
4.3.1.2 The first approach for the synthesis of L-arabinosamine moiety 54
4.3.2 Synthesis of D-xylosamine moiety 57
4.3.3 Synthesis of disaccharide 58
4.3.4 The attempted glycosylation between the steroidal aglycone **3.8** and disaccharide **4.44** 59
4.4 A revised strategy for the synthesis of amino analog 61
4.4.1 Stepwise Retro-synthesis analysis of amino analog formation 61
4.4.2 Synthesis of monosaccharide donor **4.48** 61
4.4.3 Synthesis of mono amino saccharide fragment 62
4.4.4 Alternative attempt to mono amino saccharide fragment 63
4.4.5 Synthesis of armed-donor 64
4.4.6 Alternative approach to mono amino saccharide fragment 65
4.4.7 The attempted glycosylation with amino xylose component 68
4.4.8 Synthesis of armed D-xylosamine donor 70
4.4.9 The attempted glycosylation between the compound **4.45** and armed donor **4.73** 71
4.4.10 Synthesis of the amino-analog **4.77** 72
4.4.11 Future plan of synthesis of amino analog **4.1** 73
4.5 Conclusion 74
4.6 Experimental section 74
4.6.1 General 74
4.6.2 Experimental procedures and spectral data 75

CHAPTER 5 MODEL STUDY TOWARDS THE TOTAL SYNTHESIS OF ANTICANCER NATURAL PRODUCT SUPERSTOLIDE A 99

5.1 Introduction: Superstolides A and B 99
5.2 Roush First Synthesis of Superstolide A 101
5.3 Retrosynthetic Analysis of Superstolide A 105
5.4 Model study on the 16-membered macrolactone **5.28** 106

5.4.1 Retrosynthetic Analysis 106
5.4.2 Asymmetric synthesis of Fragment **5.29** 107
5.4.3 Synthesis of fragment **5.26** 109
5.5 Future plan 110
5.6 Experimental section 111
5.6.1 General 111
5.6.2 Experimental procedures and spectral data 113

CHAPTER 6 AN IMPROVED PROCEDURE FOR THE OXIDATIVE CLEAVAGE OF OLEFINS BY OsO_4-$NaIO_4$ 122

6.1 Introduction 122
6.2 Effects of Various Bases on the Rate of the Reaction and the Products Distribution 123
6.3 A Superior Improvement of the Oxidative Cleavage of Olefins 125
6.4 Conclusion 126
6.5 Experimental section 126

REFERENCES 128

APPENDIX A NMR DATA 134

LIST OF TABLES

Table

1.1 Cytostatic Activity of Compounds **1.1-1.5** and Controls on HL-60 Cells 2

1.2 The GI_{50}, TGI and LC_{50} against the NCI 60 Cell-line Tumor Panel 3

1.3 Comparison of IC_{50} of OSW-1 in malignant and non-malignant cells 12

1.4 Comparison of IC_{50} values of OSW-1 in F-ara-A-sensitive and F-ara-A-resistant primary leukemia cells isolated from patients (n = 26) 16

1.5 IC_{50} values of OSW-1 in primary leukemia cells isolated from CLL patients (n = 34) in relation to clinical characteristics 17

2.1 Antitumor Activities of the New OSW-1 Analogs (TSC_{50} in nM) in the Calcein AM Cytotoxicity Assay 21

2.2 Cytotoxic activity of OSW-1 analogs in vitro 23

2.3 Growth inhibition rate (%) of glycosides on tumor cells (P388 and A-549) 26

2.4 Growth inhibition rate (%) of analogs **2.30 - 2.34** on tumor cells (P388 and A-549) 26

2.5 Cytotoxicity of OSW-1, its analogs **2.35 (a-d)** and cisplatin against tumor cells 28

2.6 Antitumor activities of the analogs of OSW-1 with modified side chain 29

2.7 The Anti-proliferative activity of the OSW-1 analogs (**2.37a-h)** against tumor Cells 31

2.8 IC_{50} (nM) of OSW-1 and its analogs 33

3.1 IC_{50} (nM) of OSW-1 and its analogs 40

4.1 Reduction of the hydroxyl emine **4.9** 51

4.2 Investigate good method to remove PMB group 67

6.1 A Superior Improvement of the Oxidative cleavage of olefins 125

LIST OF FIGURES

Figures

A.1 300 MHz ^{1}H-NMR of compound **3.7** ($CDCl_3$) 135

A.2 75 MHz ^{13}C-NMR of compound **3.7** ($CDCl_3$) 136

A.3 300 MHz ^{1}H-NMR of compound **3.8** ($CDCl_3$) 137

A.4 75 MHz ^{13}C-NMR of compound **3.8** ($CDCl_3$) 138

A.5 300 MHz ^{1}H-NMR of compound **3.9** ($CDCl_3$) 139

A.6 75 MHz ^{13}C-NMR of compound **3.9** ($CDCl_3$) 140

A.7 300 MHz ^{1}H-NMR of compound **3.5** ($CDCl_3$) 141

A.8 75 MHz ^{13}C-NMR of compound **3.5** ($CDCl_3$) 142

A.9 300 MHz ^{1}H-NMR of compound **4.15** ($CDCl_3$) 143

A.10 75 MHz ^{13}C-NMR of compound **4.15** ($CDCl_3$) 144

A.11 300 MHz ^{1}H-NMR of compound **4.25** ($CDCl_3$) 145

A.12 75 MHz ^{13}C-NMR of compound **4.25** ($CDCl_3$) 146

A.13 300 MHz ^{1}H-NMR of compound **4.53** ($CDCl_3$) 147

A.14 75 MHz ^{13}C-NMR of compound **4.53** ($CDCl_3$) 148

A.15 300 MHz ^{1}H-NMR of compound **4.55** ($CDCl_3$) 149

A.16 75 MHz ^{13}C-NMR of compound **4.55** ($CDCl_3$) 150

A.17 300 MHz ^{1}H-NMR of compound **4.56** ($CDCl_3$) 151

A.18 75 MHz ^{13}C-NMR of compound **4.56** ($CDCl_3$) 152

A.19 300 MHz ^{1}H-NMR of compound **4.57β** ($CDCl_3$) 153

A.20 75 MHz ^{13}C-NMR of compound **4.57β** ($CDCl_3$) 154

A.21 300 MHz ^{1}H-NMR of compound **4.57α** ($CDCl_3$) 154

A.22 75 MHz ^{13}C-NMR of compound **4.57α** ($CDCl_3$) 156

A.23 300 MHz ^{1}H-NMR of compound **4.59** ($CDCl_3$) 157

A.24 75 MHz ^{13}C-NMR of compound **4.59** ($CDCl_3$) 158

A.25 300 MHz ^{1}H-NMR of compound **4.61** ($CDCl_3$) 159
A.26 75 MHz ^{13}C-NMR of compound **4.61** ($CDCl_3$) 160
A.27 300 MHz ^{1}H-NMR of compound **4.62** ($CDCl_3$) 161
A.28 75 MHz ^{13}C-NMR of compound **4.62** ($CDCl_3$) 162
A.29 300 MHz ^{1}H-NMR of compound **4.63** ($CDCl_3$) 163
A.30 75 MHz ^{13}C-NMR of compound **4.63** ($CDCl_3$) 164
A.31 300 MHz ^{1}H-NMR of compound **4.52** ($CDCl_3$) 166
A.32 75 MHz ^{13}C-NMR of compound **4.52** ($CDCl_3$) 167
A.33 300 MHz ^{1}H-NMR of compound **4.45** ($CDCl_3$) 168
A.34 75 MHz ^{13}C-NMR of compound **4.45** ($CDCl_3$) 169
A.35 300 MHz ^{1}H-NMR of compound **4.33** ($CDCl_3$) 170
A.36 75 MHz ^{13}C-NMR of compound **4.33** ($CDCl_3$) 171
A.37 300 MHz ^{1}H-NMR of compound **4.67** ($CDCl_3$) 172
A.38 75 MHz ^{13}C-NMR of compound **4.67** ($CDCl_3$) 173
A.39 300 MHz ^{1}H-NMR of compound **4.68** ($CDCl_3$) 174
A.40 75 MHz ^{13}C-NMR of compound **4.68** ($CDCl_3$) 175
A.41 300 MHz ^{1}H-NMR of compound **4.69** ($CDCl_3$) 176
A.42 75 MHz ^{13}C-NMR of compound **4.69** ($CDCl_3$) 177
A.43 300 MHz ^{1}H-NMR of compound **4.70α** ($CDCl_3$) 178
A.44 75 MHz ^{13}C-NMR of compound **4.70α** ($CDCl_3$) 179
A.45 300 MHz ^{1}H-NMR of compound **4.70β** ($CDCl_3$) 180
A.46 75 MHz ^{13}C-NMR of compound **4.70β** ($CDCl_3$) 181
A.47 300 MHz ^{1}H-NMR of compound **4.71** ($CDCl_3$) 182
A.48 75 MHz ^{13}C-NMR of compound **4.71** ($CDCl_3$) 183
A.49 300 MHz ^{1}H-NMR of compound **4.36** ($CDCl_3$) 184
A.50 75 MHz ^{13}C-NMR of compound **4.36** ($CDCl_3$) 185
A.51 300 MHz ^{1}H-NMR of compound **4.37** ($CDCl_3$) 186

A.52 75 MHz ^{13}C-NMR of compound **4.37** ($CDCl_3$) 187
A.53 300 MHz ^{1}H-NMR of compound **4.38** ($CDCl_3$) 188
A.54 75 MHz ^{13}C-NMR of compound **4.38** ($CDCl_3$) 189
A.55 300 MHz ^{1}H-NMR of compound **4.39** ($CDCl_3$) 190
A.56 75 MHz ^{13}C-NMR of compound **4.39** ($CDCl_3$) 191
A.57 300 MHz ^{1}H-NMR of compound **4.75** ($CDCl_3$) 192
A.58 75 MHz ^{13}C-NMR of compound **4.75** ($CDCl_3$) 193
A.59 300 MHz ^{1}H-NMR of compound **4.76** ($CDCl_3$) 194
A.60 75 MHz ^{13}C-NMR of compound **4.76** ($CDCl_3$) 195
A.61 300 MHz ^{1}H-NMR of compound **4.77** ($CDCl_3$) 197
A.62 75 MHz ^{13}C-NMR of compound **4.77** ($CDCl_3$) 198
A.63 300 MHz ^{1}H-NMR of compound **5.38** ($CDCl_3$) 199
A.64 75 MHz ^{13}C-NMR of compound **5.38** ($CDCl_3$) 200
A.65 300 MHz ^{1}H-NMR of compound **5.39** ($CDCl_3$) 201
A.66 75 MHz ^{13}C-NMR of compound **5.39** ($CDCl_3$) 202
A.67 300 MHz ^{1}H-NMR of compound **5.40** ($CDCl_3$) 203
A.68 75 MHz ^{13}C-NMR of compound **5.40** ($CDCl_3$) 204
A.69 300 MHz ^{1}H-NMR of compound **5.41** ($CDCl_3$) 205
A.70 75 MHz ^{13}C-NMR of compound **5.41** ($CDCl_3$) 206
A.71 300 MHz ^{1}H-NMR of compound **5.29** ($CDCl_3$) 207
A.72 75 MHz ^{13}C-NMR of compound **5.29** ($CDCl_3$) 208
A.73 300 MHz ^{1}H-NMR of compound **5.49** ($CDCl_3$) 209
A.74 75 MHz ^{13}C-NMR of compound **5.49** ($CDCl_3$) 210
A.75 300 MHz ^{1}H-NMR of compound **5.50** ($CDCl_3$) 211
A.76 75 MHz ^{13}C-NMR of compound **5.50** ($CDCl_3$) 212
A.77 300 MHz ^{1}H-NMR of compound **5.51** ($CDCl_3$) 213
A.78 75 MHz ^{13}C-NMR of compound **5.51** ($CDCl_3$) 214

A.79 300 MHz ^{1}H-NMR of compound **5.51'** ($CDCl_3$) 215

A.80 75 MHz ^{13}C-NMR of compound **5.51'** ($CDCl_3$) 216

A.81 300 MHz ^{1}H-NMR of compound **5.26** ($CDCl_3$) 217

A.82 75 MHz ^{13}C-NMR of compound **5.26** ($CDCl_3$) 218

LIST OF SCHEMES

Schemes

1.1	OSW-1 and its four natural analogs	1
1.2	Fuchs synthesis of the protected aglycone of OSW-1	5
1.3	Yu and Hui total synthesis of OSW-1	6
1.4	Jin synthesis of OSW-1	8
1.5	Morzycki synthesis of OSW-1	9
1.6	Guo synthesis of OSW-1	10
2.1	OSW-1 analogs made by Morzycki's group	20
2.2	Estrone analogs of OSW-1	22
2.3	Steroidal glycosides bearing the disaccharide moiety of OWS-1	24
2.4	OWS-1 analogs with modified side chains	28
2.5	OSW-1 analogs with modified sugar residues	31
2.6	Analogs of OSW-1 synthesized by Jin group	32
3.1	Structure of analog 17–des-oxo-OSW-1 **3.1**	35
3.2	Synthesis of analog **3.1**	36
3.3	Structure of analog **3.5**	38
3.4	Synthesis of analog **3.5**	38
4.1	Structure of amino-analog **4.1**	48
4.2	Retro-synthetic analysis of amino analog **4.1**	49
4.3	Synthesis of L-arabinosamine moiety	50
4.4	The attempted inversion of stereochemistry *via* S_N2 substitution	52
4.5	Synthesis of α -1- benzyl-2-phthaloylamino-arabinose **4.4**	53

4.6	Azidonitration of tri-*O*-acetyl-D-galactal	55
4.7	Mechanism of azidonitration	55
4.8	Azidonitration of compound **4.25**	55
4.9	The second approach to synthesis of L-arabinosamine **4.4**	56
4.10	Synthesis of D-xylosamine moiety	57
4.11	Synthesis of amino-disaccharide **4.4**	58
4.12	The attempted glycosylation between the steroidal aglycone **3.8** and disaccharide **4.44**	59
4.13	Retrosynthetic analysis of the stepwise formation of amino analog **4.1**	60
4.14	Synthesis of monosaccharide donor **4.48**	61
4.15	Synthesis of amino mono saccharide fragment **4.45**	62
4.16	Alternative attempt to mono amino saccharide fragment	63
4.17	Synthesis of armed-saccharide **4.60**	64
4.18	Alternative approach to mono amino saccharide fragment	65
4.19	PMB groups deprotection by DDQ	66
4.20	Regioselectively protect the diol **4.52**	68
4.21	Glycosylation with amino xylose component	68
4.22	Structure study of receptor **4.45** and donor **4.3**	69
4.23	Synthesis of armed amino-xylose donor **4.73**	70
4.24	Glycosylation with armed saccharide component	71
4.25	Glycosylation of diol **4.52**	72
4.26	Synthesis of amino **4.77**	72
4.27	Future plan of synthsis of amino analog **4.1**	73
5.1	Structure of Superstolides A and B	100

5.2	Synthesis of Phosphonium Salt **5.10**	102
5.3	Synthesis of Bimetallic Diene Linchpin **5.16**	103
5.4	Completion of the Total Synthesis of Superstolide A	104
5.5	Retrosynthetic analysis	105
5.6	Retro-synthesis of 16-membered macro-lactone **5.28**	106
5.7	Synthesis of chiral secondary alcohol **5.36**	108
5.8	Completion of the synthesis of Fragment **5.29**	109
5.9	Synthesis of fragment **5.26**	110
5.10	Proposed synthetic route for the model 16-membered macrolactone **5.28**	111
6.1	Oxidative cleavage reaction	122
6.2	Effects of Various Bases on the Rate of the Reaction and the Products Distribution	124

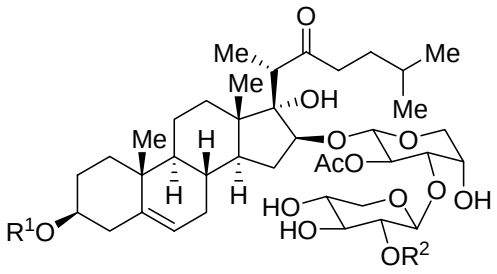
CHAPTER 1

BIOLOGICAL ACTIVITY AND PREVIOUS SYNTHETIC EFFORTS OF OSW-1

1.1 Introduction

1.1.1 Structure and natural source of OSW-1

The steroidal saponin OSW-1 (**1.1**), a highly potent anticancer natural product, and its four natural analogs (**1.2-1.5**), are members of the cholestane glycosides. They were isolated from the bulbs of *Ornithogalum saundersiae*, a perennial grown in southern Africa where it is cultivated as a garden plant and flowers (Scheme 1.1).[1] Their absolute structures were determined by extensive spectroscopic methods.[1] The structural novelty of compounds **1.1-1.3** is characterized by a disaccharide attached to a steroidal aglycone, whereas compounds **1.4** and **1.5** have another glycosyl sugar associated with the C-3 alcohol position of the steroid.



	R^1	R^2	
1.1	H	*p*-methoxybenzoyl	**OSW-1**
1.2	H	3,4-dimethoxybenzoyl	
1.3	H	(*E*)-cinnamoyl	
1.4	Glc	*p*-methoxybenzoyl	
1.5	Glc	(*E*)-cinnamoyl	

Scheme 1.1 OSW-1 and its four natural analogs

1.1.2 Biological activities

Compounds **1.1**-**1.5** strongly suppressed the growth of human promyelocytic leukemia HL-60 cells, showing IC_{50} values ranging between 0.1 and 0.3 nM. Thus, these compounds are much more potent against HL-60 cells than the clinically applied anticancer agents such as etoposide, adriamycin and methotrexate used as positive controls (Table 1.1).[2]

Table 1.1 Cytostatic Activity of Compounds **1.1**-**1.5** and Controls on HL-60 Cells

Compounds	IC_{50} (nM)
1.1	0.25
1.2	0.2
1.3	0.29
1.4	0.24
1.5	0.12
Etoposide	25
Adriamycin	7.2
Methotrexate	12

OSW-1, the main constituent of the *Ornithogalum saundersiae* bulbs, exhibited exceptionally potent cytotoxic activities against various human malignant tumor cells such as mouse mastrocarcinoma, human pulmonary adenocarcinoma, human pulmonary large cell carcinoma, and human pulmonary squamous cell carcinoma.[2] OSW-1 is extremely cytotoxic in the NCI 60 cell-line in *vitro* screen, with a mean IC_{50} of 0.75 nM.[2] Thus, its anticancer activities are 10 to 100 times more potent than many well-known

anticancer agents in clinical use, including mitomycin C, adriamycin, cisplatin, camptothecin, and even paclitaxel (Table 1.2). [2] But the toxicity of OSW-1 to normal human pulmonary cells is relatively low. *In vivo* tests showed that OSW-1 appeared to prolong the life span of mice bearing P388 leukemia by 59% with a single dosage at 10 μg/kg.[2] The adriamycin-resistant and camptothecin-resistant P388 leukemia cells are still sensitive to OSW-1, suggesting that OSW-1 may represent a new type of drug for cancer chemotherapy.[2] However, the anticancer mechanism of action of OSW-1 is still unknown at the present time.

Due to its exceedingly potent anticancer activities and a possible novel mechanism of action, OSW-1 has been an attractive synthetic target since the publication of its structure and potent anti-tumor activity.[3-14, 17] To determine the pharmacophore of OSW-1, conduct extensive structure-activity relationship study, and investigate its novel mechanism of action, a highly efficient total synthesis pathway that readily allows structural modification is most desirable to provide sufficient quantities of the target molecule for extensive biological studies.

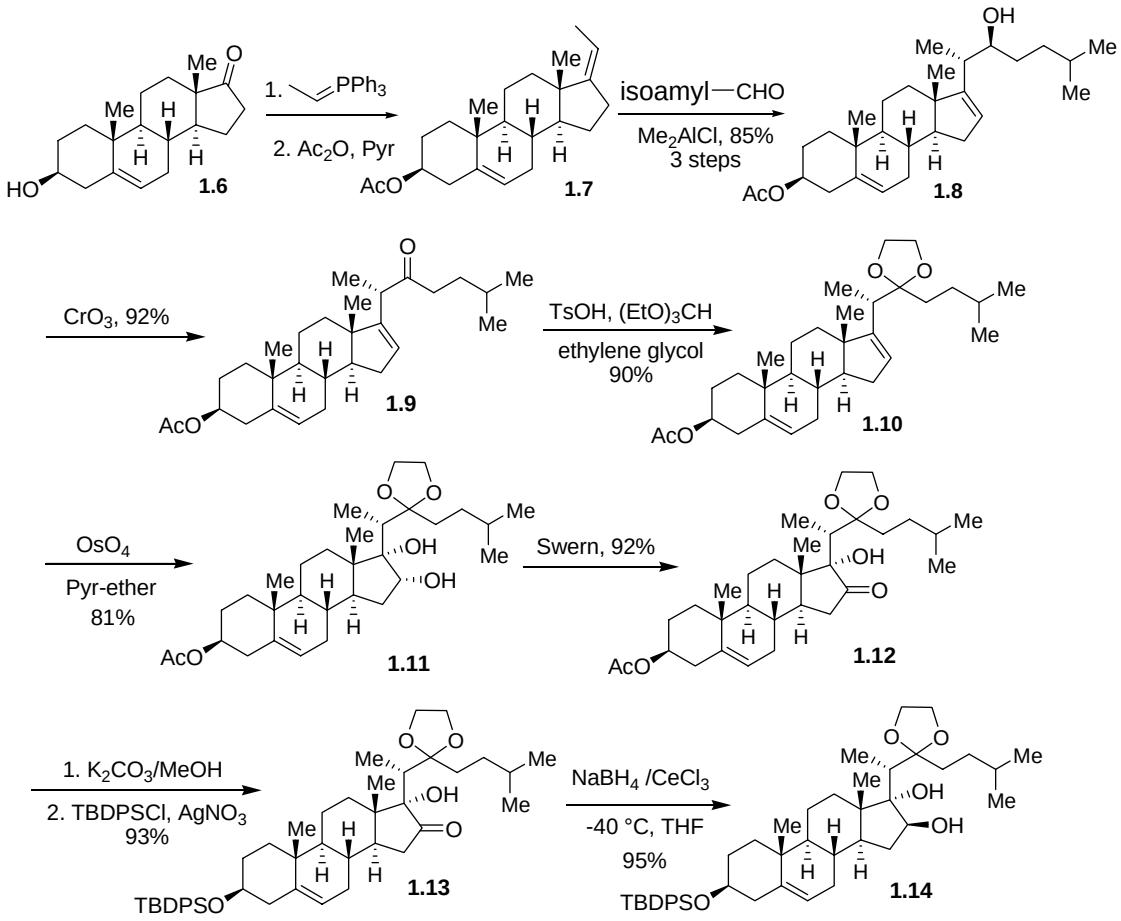
Table 1.2 The GI_{50}, TGI and LC_{50} against the NCI 60 Cell-line Tumor Panel

Cell line	-Lg GI_{50}	-Lg TGI	-Lg LC_{50}
CCRF-CEM	10.00	8.15	5.92
HL-60	10.46	6.74	5.00
K-562	9.66	7.85	5.00
MOLT-4	10.01	7.96	6.34
RPMI-8226	10.42	9.66	5.68
SR	9.92	6.89	5.20
A549/ATCC	9.34	5.68	5.09
EKVX	9.74	6.26	5.18
HOP-62	9.80	8.70	6.89
HOP-92	8.96	7.49	5.52
NCI-H23	9.44	6.96	5.89
NCI-H322M	7.57	5.89	5.30

HCI-H460	9.80	7.47	5.66
NCI-H522	9.44	8.27	6.49
COLO 205	9.00	8.80	6.54
HCC-2998	7.52	5.72	5.36
HCT-116	9.42	6.72	5.89
HCT-15	7.96	7.02	6.16
HT-29	9.44	6.52	5.43
KM-12	9.39	7.40	5.96
SW-620	9.89	9.26	7.66
SF-268	8.62	6.07	5.13
SF-295	10.32	8.64	5.52
Sf-539	10.00	8.35	6.74
SNB-19	7.89	6.48	5.54
SNB-75	10.38	8.49	5.52
U251	10.59	7.29	5.60
Lox IMVI	9.60	8.64	7.04
MALME-3M	9.20	8.64	5.62
M14	9.57	9.02	7.29
SK-MEL-2	9.46	7.77	5.62
UACC-257	9.03	8.89	7.39
UACC-62	9.51	8.89	8.44
IGROV1	8.82	5.72	5.16
OVCAR-3	8.02	5.70	5.33
OVCAR-4	8.44	5.66	5.24
OVCAR-5	7.30	5.70	5.23
OVCAR-8	7.82	5.80	5.27
SK-OV-3	8.40	5.92	5.00
786-0	10.21	5.92	5.46
A498	9.62	8.66	6.85
ACHN	8.10	5.66	5.14
CAKI-1	8.77	7.31	6.06
RXF-393	10.15	7.74	6.39
SN12C	5.89	5.54	5.17
TK-10	7.11	5.72	5.05
UO-31	9.21	8.85	7.52
PC-3	9.68	8.80	6.41
DU-145	8.43	5.82	5.37
MCF7	9.09	5.92	5.44
MCF7/ADR-RES	7.62	5.70	5.18
MDA-MB-231/ATCC	7.72	5.89	5.27
HS 578T	10.27	9.13	5.70
MDA-MB-435	9.24	8.05	6.39
MDA-N	9.11	8.05	6.31
BT-549	8.74	5.77	5.34
T-47D	8.29	5.82	5.11

1.2 Synthesis of OSW-1

1.2.1 Fuchs approach for the synthesis of the protected aglycone of OSW-1

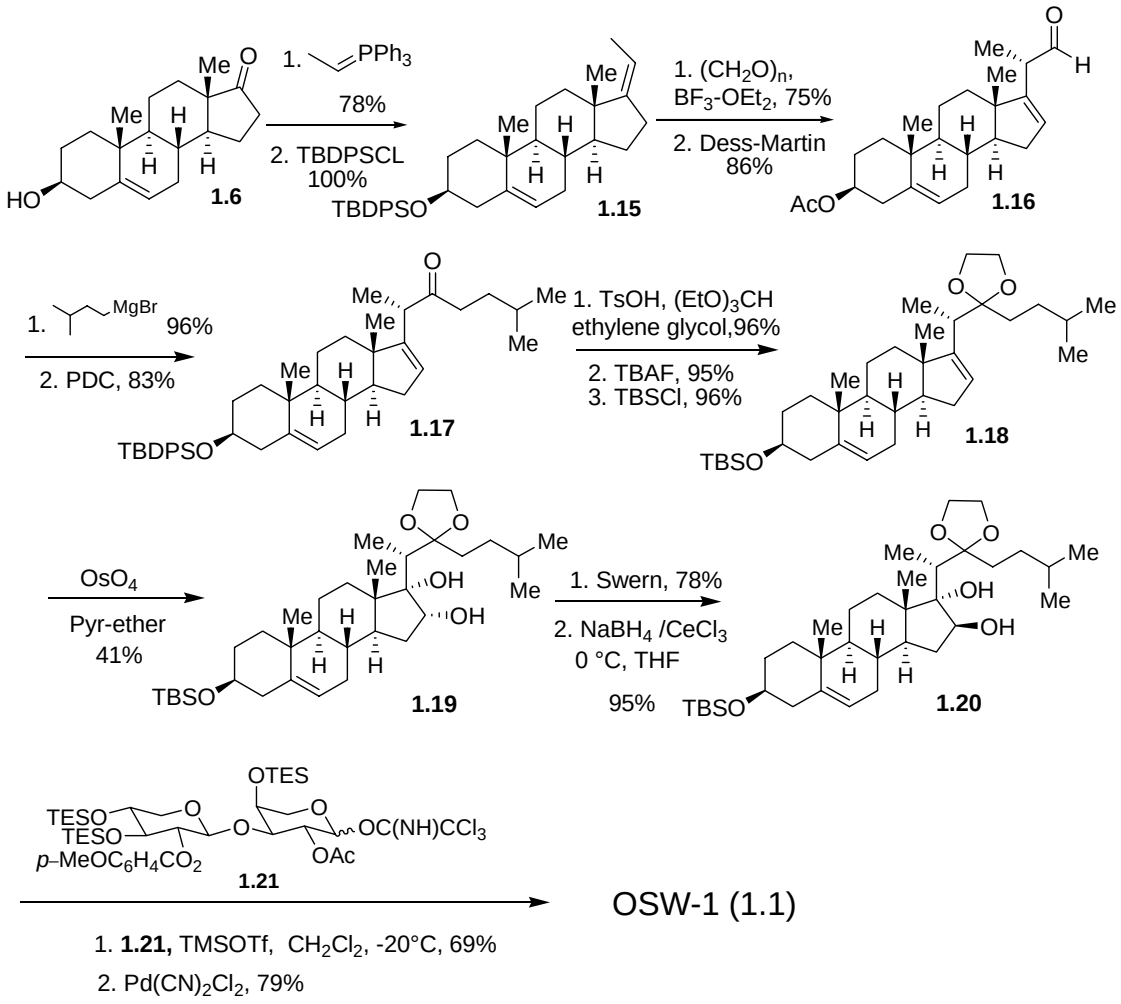


Scheme **1.2** Fuchs synthesis of the protected aglycone of OSW-1

In 1998 Fuchs and Gao published the first synthesis of the protected aglycone of OSW-1.[4] Their synthesis consists of 10 steps from 5-androsten-3b-ol-17one **1.6** and the overall yield is 46%.[4, 5] The key reactions in the synthesis are a stereospecific carbonyl-

ene reaction for the installation of the side chain, a regio- and stereo selective dihydroxylation, and a stereoselective reduction of the C-16 ketone (Scheme 1.2).

1.2.2 The first total synthesis of OSW-1



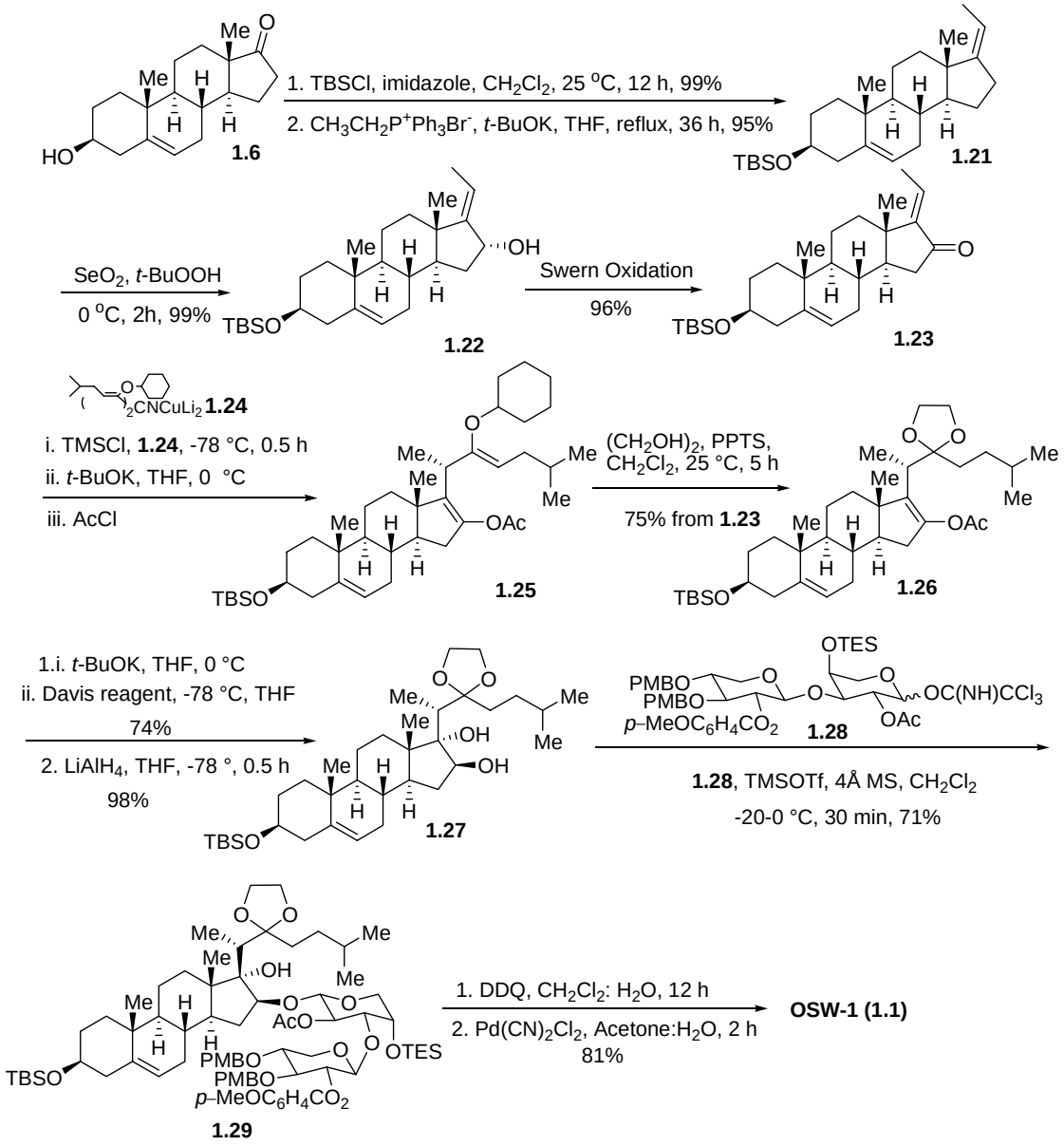
Scheme **1.3** Yu and Hui total synthesis of OSW-1

In 1999 Yu, Hui and their coworkers completed the first total synthesis of OSW-1.[6] They used a similar approach to prepare Fuchs' aglycone, but the yield of OsO_4 mediated dihydroxylation is only 41% compared to 81% in the same step in Fuchs synthesis. The total synthesis has 27 steps with the longest linear sequence of 14 steps and the overall yield is 6%. Their total synthesis is summarized in Scheme 1.3. It should be noted that in 2005, Yu published an improved synthesis of the aglycone in *J. Org. Chem*.[31]

1.2.3 Jin approach for the total synthesis of OSW-1

Both Fuchs and Hui syntheses of the protected aglycone require the use of stoichiometric amount of OsO_4, which is highly toxic and expensive. In addition, the overall yield in Hui's synthesis is only 6%. To supply a sufficient quantity of OSW-1 for pharmacological studies, a highly efficient and flexible strategy for the total synthesis of OSW-1 was successfully developed in the Jin's laboratory in 2001.[12,13]

Employing the α-halo vinyl ether chemistry developed in the Jin laboratory, the steroidal side chain was successfully installed *via* a stereoselective 1,4 addition of an α-alkoxy vinyl cuprate **1.24** to 17(20)-en-16-one **1.23**. The 17α-OH group was introduced by the stereoselective oxidation of the resulting 16, 17-enolate with a Davis reagent.[15] The Jin synthesis avoids the use of toxic OsO_4, and consists of only 10 linear operations from the commercially available starting material 5-androsten-3β-ol-17-one **1.6**. The overall yield of the total synthesis is 28% (Scheme 1.4). [12, 13]

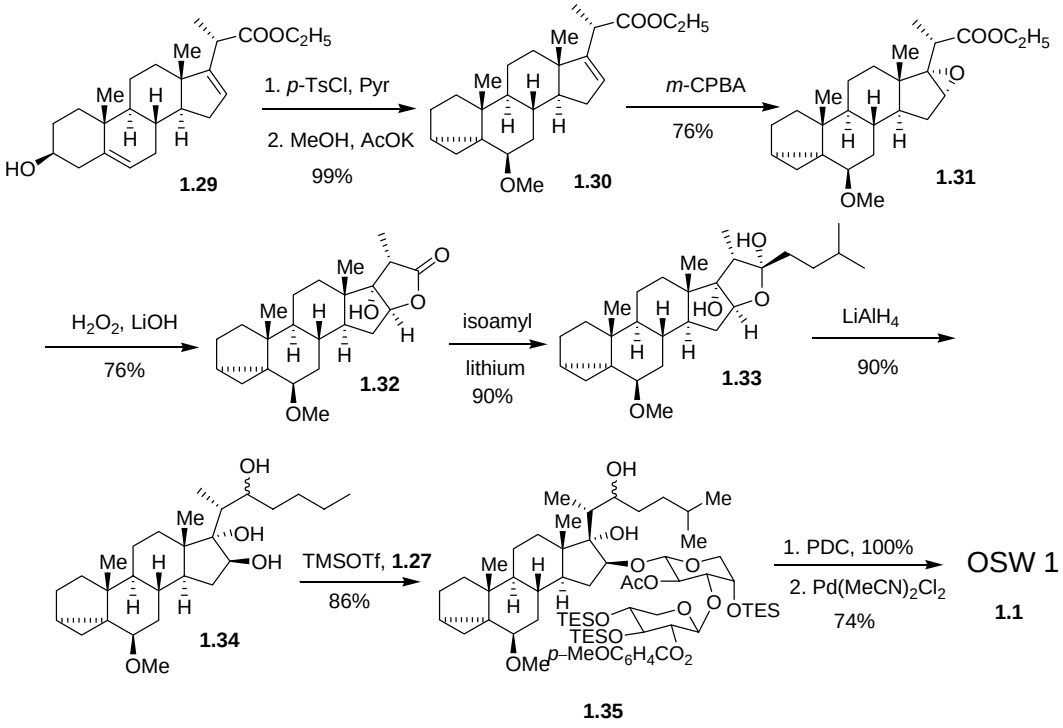


Scheme **1.4** Jin synthesis of OSW-1

1.2.4 Morzycki approach to the total synthesis of OSW-1

In 2002, Polish chemist Morzycki and his coworkers published their synthesis of OSW-1 employing the same disaccharide first prepared by Yu and Hui.[3] They reported the improved synthesis of the OSW-1 aglycone in the hemiketal form, with the ring B

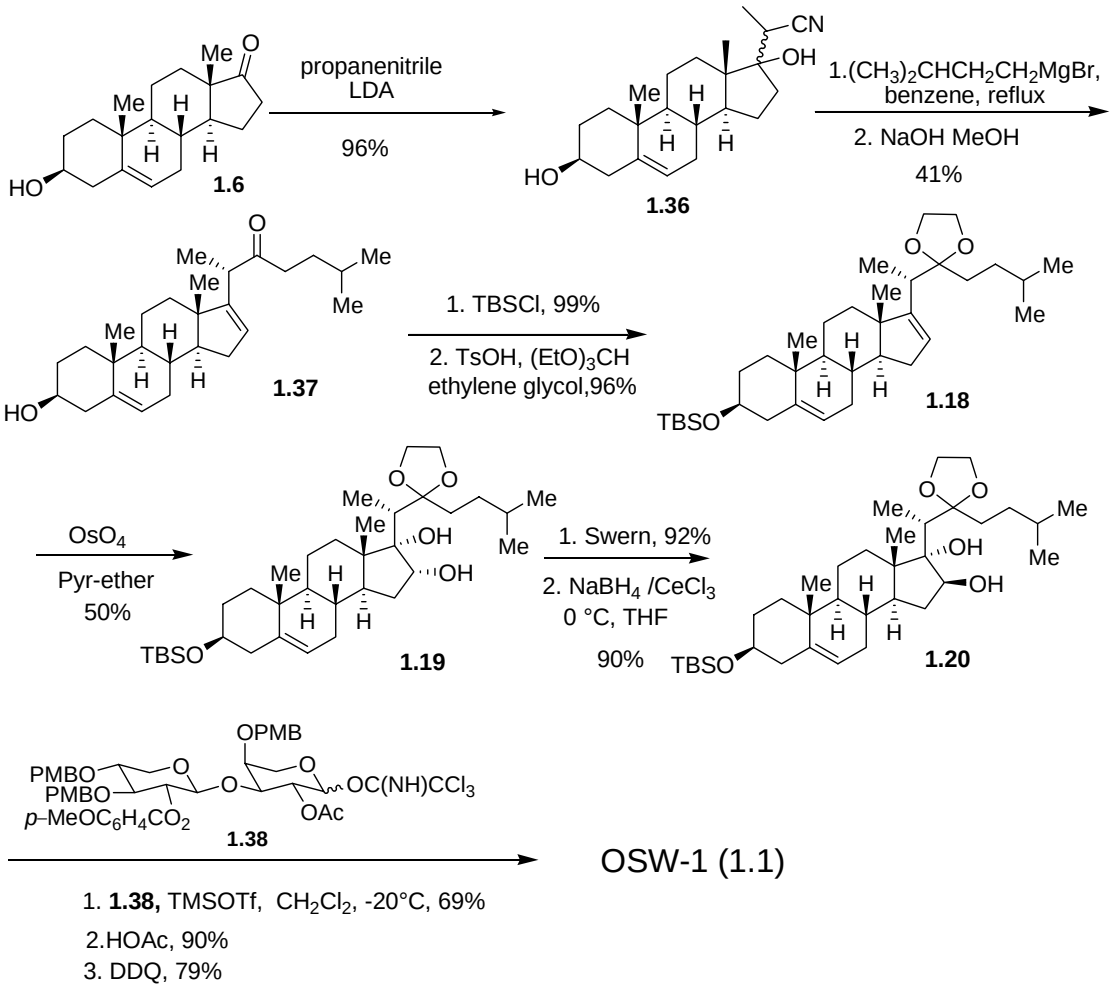
double bond and 3β-OH group protected as *i*-steroid ether. The total synthesis has the longest linear sequence of 9 steps and the overall yield is 29%. Their total synthesis is summarized in Scheme 1.5.



Scheme **1.5** Morzycki synthesis of OSW-1

1.2.5 Guo approach to the synthesis of OSW-1

In 2008, the Guo group from Wayne State University published their synthesis of OSW-1.[17] The total synthesis has the longest linear sequence of 10 steps and the overall yield is 6.4%. Their total synthesis is summarized in Scheme 1.6.



Scheme **1.6** Guo synthesis of OSW-1

1.3 Preliminary biological studies on OSW-1

Jin's total synthesis has provided a sufficient amount of OSW-1 for comprehensive studies on its cytotoxic activity, selectivity, and underlying mechanisms of action. The biological and pharmacological investigation (using OSW-1 synthesized in the Jin laboratory) was carried out in Prof. Peng Huang's laboratory at MD Anderson Cancer Center.

The studies by Prof. Huang's group showed that OSW-1 exhibited extremely potent activity against a variety of cancer cells *in vitro*. However, non-malignant human cells were statistically significantly less sensitive to OSW-1 than cancer cells, with IC_{50} values 40-150-fold greater than those observed in the malignant cells. OSW-1 was found to induce structural and functional damage to mitochondria membrane and cristae, leading to the loss of transmembrane potential, increase of cytosolic calcium and activation of calcium-dependent apoptosis. The therapeutic importance of this compound was further demonstrated by its ability to kill primary leukemia cells from patient refractory to conventional anticancer agents. The following is the summary of biological investigation conducted by Prof. Huang's group at MD Anderson Cancer Center.[18]

1.3.1 Anticancer activity and selectivity of OSW-1.

The MTT assay (Cell viability was determined by a 3-(4,5-dimethyl thiazol-2)-2,5-diphenyltetrazolium bromide) was used to determine the cytotoxic activity of OSW-1 in various cancer cell lines in comparison with several normal or non-malignant cells.[18] OSW-1 exhibited very potent cytotoxic effects in a broad spectrum of malignant tumor cells, including several human leukemia cell lines, ovarian cancer SKOV3 cells, malignant brain tumor U87 cells, and pancreatic cancer AsPC-1 cells. The IC_{50} values, defined as the drug concentrations that inhibited 50% cell viability, were less than 0.1 nM in all but pancreatic cell lines. The human pancreatic cancer cell line AsPC-1 was also highly sensitive to this compound with the IC_{50} value of 0.4 nM. Further experiments with primary leukemia cells freshly isolated from thirty four patients with chronic

lymphocytic leukemia (CLL) also showed that OSW-1 was very effective in killing these primary cancer cells with the IC_{50} values of less than 1 nM.[18]

Cancer cells were found to be approximately 40-150 times more sensitive to OSW-1 than the non-malignant cells, as determined by the ratios of their respective IC_{50} values, with comparison of malignant cells and non-malignant cells from similar tissue types (Table 1.3).[18] These results have shown that OSW-1 has a preferential cytotoxic activity against cancer cells *in vitro*.

Table 1.3 Comparison of IC_{50} of OSW-1 in malignant and non-malignant cells

	IC_{50} (nM)		
	Non-Malignant	Malignant	Ratio
Hematopoietic	1.59	0.040	39.75
Ovarian	0.83	0.021	39.52
Brain	7.13	0.047	151.70

Note: The effect of OSW-1 on cell viability was determined by MTT assay (72 h and expressed as IC_{50} for comparison. The IC_{50} value for malignant hematopoietic cells was the averaged value for ML-1 cells, HL-60 cells, and Raji cells. The IC_{50} value for non-malignant hematopoietic cells was the averaged value for normal lymphocytes and CFU-GM. Malignant ovarian cancer cells (SKOV3) were compared with normal primary human fibroblasts isolated ovarian tissue; Malignant brain tumor cells (U87) were compared with immortalized normal human astrocytes.

1.3.2 Structural damage to mitochondria by OSW-1

The potent *in vitro* cytotoxicity of OSW-1 and its preferential effect against cancer cells prompted further investigation on the underlying mechanism of the drug action. In order to identify its cellular target(s), DNA micro-array analysis was used to examine potential changes in gene expression after cells were incubated with OSW-1 *in*

vitro. This analysis revealed the expression of genes coding for mitochondrial metabolic enzymes were consistently increased after the drug treatment. These enzymes included NADH dehydrogenase (ubiquinone) 1β subcomplexes 1 and 4, NADH dehydrogenase (ubiquinone) 1α subcomplex 7, Cytochrome c oxidase subunit VIa polypeptide 1 (COX6A), Cytochrome c oxidase subunit VIIb (COX7B), and Cytochrome c oxidase subunit IV isoform 1. Because these enzymes are all involved in the mitochondrial metabolic function, it is speculated that OSW-1 might somehow disturb the mitochondrial function and subsequently lead to change in gene expression of the relevant molecules.

Experiments also showed that OSW-1 did not induce a substantial change in mitochondrial respiratory activity by direct analysis of mitochondrial respiratory rate using an oxygen consumption assay.[18] Consequently, transmission electron microscopic analysis was used to examine the possibility of mitochondrial structural damage by treatment with OSW-1. The mitochondria exhibited clear double layers of the outer and inner membranes, with well-organized mitochondrial cristae in control HL-60 cells. As early as 6 h after incubation with OSW-1, there was a significant change in mitochondrial morphology, including blurred membrane outlines, disappearance and/or disorganized cristae, and pale mitochondrial matrix. These structural changes became more apparent as incubation time was prolonged up to 14 hours. Interestingly, the nuclear membranes appeared intact even at 14th hour. It suggested that the damaging effect of OSW-1 was relatively specific to the mitochondrial membranes.

1.3.3 OSW-1 induced calcium-dependent apoptosis

Because mitochondria play an important role in maintaining a proper cytosolic calcium concentration [Ca^{2+}] level by its ability to uptake Ca^{2+} released from the endoplasmic reticulum, this organelle participates in the regulation of various Ca^{2+} signaling processes including apoptosis. Whether the damage of mitochondrial membranes by OSW-1 might change cytosolic calcium levels was examined.

HL-60 cell line was treated with various concentrations of OSW-1 for up to 6 hours. Flow cytometric analysis using a calcium-specific fluorescent dye (Calcium Green-1) measured cytosolic calcium. 1 nM OSW-1 significantly increased the cytosolic calcium. The positive control cells treated with thapsigargin, a high-affinity inhibitor of Ca^{2+}-ATPase which increased cytosolic calcium, was similar to that observed on cells treated with OSW-1. Similar cytosolic calcium increase was also observed in human pancreatic AsPC-1 cells treated with OSW-1.[18]

Based on the results described above, the increase of cytosolic calcium may be a consistent biochemical event by OSW-1 treatment. For further evaluation of the role of increased cytosolic calcium in mediating OSW-1-induced apoptosis, a cell-permeable calcium chelator BAPTA-AM (Molecular Probes) was used to test if chelation of cytosolic Ca^{2+} would affect the ability of OSW-1 to induce cell death. Incubation of HL-60 cells with 1 nM OSW-1 resulted in a loss of 61% cells in a day (39% cells remained intact). And for 24 hours, cell viability wasn't significantly altered by BAPTA-AM itself. However, addition of 1 μM BAPTA-AM significantly suppressed OSW-1-induced apoptosis, resulting in a loss of only 14% of the cells. These data suggest that the increase

of cytosolic calcium seems to be a critical event in mediating OSW-1 induced apoptosis.[18]

1.3.4 Apoptosis in the Parental HL-60 Cells & the Mitochondrial Respiration-deficient C6F Cells

Cells adapted to survive with mitochondrial defects should be less sensitive to OSW-1 than cells that rely on intact mitochondria, if damage to mitochondrial by OSW-1 were critical in causing apoptosis. In order to examine this assumption, a subclone of human leukemia cells (C6F), which had mitochondrial DNA mutations and functional defects, were used to compare with that of the parental HL-60 cells. Flow cytometric analysis showed that a large subpopulation of cells with sub-G_1 DNA content was formed in 24 hours after HL-60 cells were treated with 0.5 nM OSW-1. It indicated DNA fragmentation and apoptosis. But there were no substantial number of apoptotic cells generation when C6F cells were treated with the same concentration of OSW-1. A time-dependent loss of mitochondrial transmembrane potential was observed when OSW-1 was treated in HL-60 cells, but not in C6F cells. C6F cells were also substantially more resistant to OSW-1 than the parental HL-60 cells by colony formation assay.[18]

1.3.5 OSW-1 effectively killed drug-resistant primary leukemia cells from chronic lymphocytic leukemia patients

Due to its unique mechanism of action, OSW-1 might be effective in killing cancer cells that have become resistant to conventional anticancer agents. In order to test this possibility, Prof. Peng Huang's group evaluated the cytotoxic effect of OSW-1 in

primary leukemia cells isolated from patients with chronic lymphocytic leukemia (CLL) who were refractory to chemotherapy with the fludarabine-base regimen (a frontline treatment for CLL in clinic).[18] CLL cells isolated from two fludarabine-refractory patients were extremely resistant to F-ara-A (the active component of fludarabine for *in vitro* use). Strikingly, the IC_{50} values of these fludarabine-resistant CLL cells to OSW-1 were less than 0.3 nM. It proved that these cells were extremely sensitive to OSW-1.[18]

Further analysis of primary leukemia cells isolated from 26 CLL patients who were either sensitive (n=18) or resistant (n=8) to fludarabine showed that all fludarabine-resistant CLL cells were sensitive to OSW-1 (Table 1.4).[18]

Table 1.4 Comparison of IC_{50} values of OSW-1 in F-ara-A-sensitive and F-ara-A-resistant primary leukemia cells isolated from patients (n = 26)

	Fara-A Sensitive (n=18)	Fara-A Resistant (n=8)	P value
Fara-A (IC_{50}, μM)	0.57 ± 0.33	17.12 ± 18.15	0.00056
OSW-1(IC_{50}, nM)	0.23 ± 0.27	0.15 ± 0.19	0.47

Note: Primary leukemia cells freshly isolated from 26 CLL patients were treated with various concentrations of OSW-1 or F-ara-A for 72 hours. The IC_{50} values were determined by MTT assay. Data are shown as the mean ± SD. Statistic analysis using the Student's t-test, $P < 0.05$ is considered statistically significant.

For further analysis of the *in vitro* cytotoxic data, 26 of total 34 patient samples, which were tested for sensitivity to OSW-1, provided sufficient numbers of cells for determination of IC_{50} values for both F-ara-A and OSW-1. After detailed analysis of the *in vitro* cytotoxic data, there was no significant difference found in OSW-1 sensitivity with respect to patient's gender, RAI stage, or prior chemotherapeutic history (Table

1.5).[18] Because of the equal sensitivity to OSW-1 between the primary leukemia cells isolated from patients in advanced disease stages or with prior chemotherapeutic history and those in early disease stage or without prior chemotherapy, this novel compound may be potentially useful in treating patients in advanced stages who have relapsed from prior treatments.

Table 1.5 IC_{50} values of OSW-1 in primary leukemia cells isolated from CLL patients (n = 34) in relation to clinical characteristics

Patients		n	IC_{50}	P value
Sex				
	Male	20	0.64 ± 0.81	0.39*
	Female	14	0.41 ± 0.71	
RAI Stage				
	0	9	0.44 ± 0.75	
	1	12	0.33 ± 0.43	
	2	5	0.58 ± 1.00	0.47#
	3	2	1.08 ± 0.83	
	4	6	1.04 ± 1.09	
Prior RX				
	Yes	14	0.61 ± 0.69	0.70*
	No	20	0.50 ± 0.83	

Note: Primary leukemia cells freshly isolated from 34 CLL patients were treated with various concentration of OSW-1 for 72 hours. The IC_{50} values were determined by MTT assay. Data are shown as the mean ± SD. *Statistical analysis using the Student's t-test, # Statistical analysis using ANOVA. $P < 0.05$ is considered statistically significant.

In summary, OSW-1 possesses highly potent anticancer activity against several human malignant cell lines and primary leukemia cells from patients with CLL. The

unique mechanism action of OSW-1, which caused structural damage to mitochondria and to induce calcium-dependent apoptosis, is a novel pharmacological action different from the common anticancer agents currently used in clinical treatment of cancer. This novel compound seems to effectively kill cancer cells that have become resistant to conventional therapeutic agents. The ability of OSW-1 to potently kill fludarabine-resistant primary CLL cells isolated from patients in advanced disease stages or relapse from prior chemotherapy suggests that this compound may be potentially useful for treating drug-resistant patients.[18]

CHAPTER 2

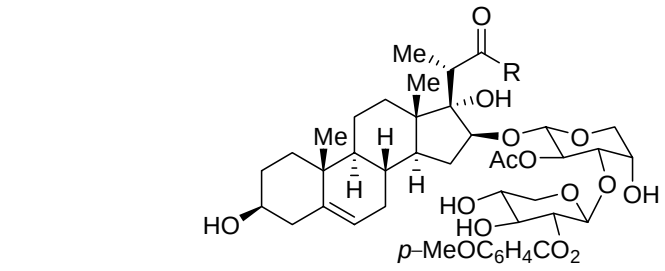
PREVIOUS STUDIES ON THE SYNTHESIS OF ANALOGS OF OSW-1

2.1 Introduction

The highly potent anticancer activity of OSW-1 prompted several research groups around the world to investigate its structure-activity relationship. Some preliminary studies on the structure–activity relationships (SAR) of this novel anticancer agent have been reported.[5-11, 19-22] It is found that the steroidal aglycone and the sugar moiety of OSW-1 both play important roles for its anticancer activity. The protected aglycone alone does not show any anticancer activity, nor does the disaccharide moiety alone.[7,8] Analogs with nonsteroidal aglycones attached with the same disaccharide exhibit very poor anticancer activity.[7] In addition, the dissacharide moiety has to be attached to the C-16 position of the steroidal aglycone.[7,8] Furthermore, the glycoside bond between 16β-hydroxysteroid and dissacharide moiety has to be α configuration. The following is the summary of the published structure-activity relationships carried out by several research groups.

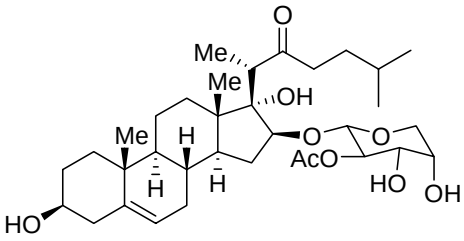
2.2 Structure–activity relationships (SAR) studies of OSW-1 by Morzycki's group

Morzycki's group published the synthesis of OSW-1 analogs with different sizes of a steroidal side chain.[11, 19] In addition, they also synthesized OSW-1 analogs containing a monosaccharide instead of a disacharide. The structures of their analogs are shown in Scheme 2.1 and their testing data are summarized in Table 2.1.

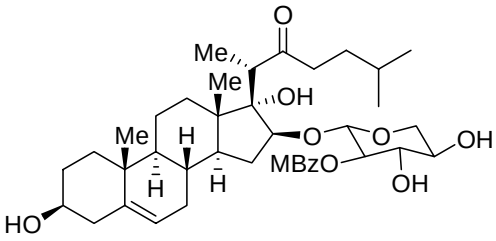


2.1a: R =*n*-C_3H_7
2.1b: R =*i*-C_3H_7
2.1c: R =*n*-C_4H_9
2.1d: R =*n*-C_5H_{11}
2.1e: R =*n*-C_7H_{15}
2.1 f: R =H

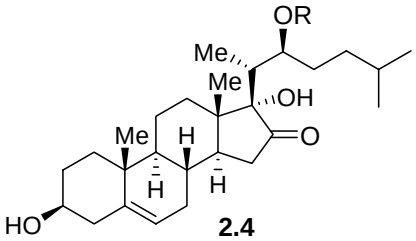
2.1



2.2

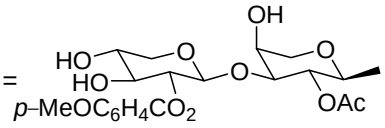


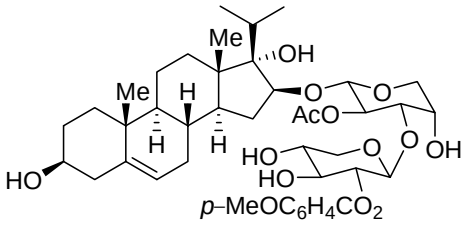
2.3



2.4

R =





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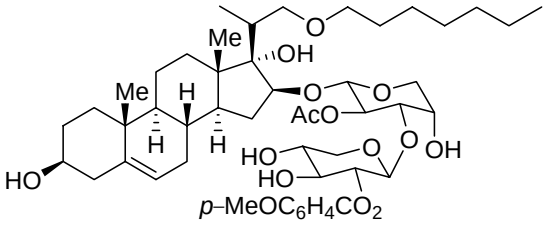


2.6

MBz=*p*-$MeOC_6H_4CO$

Scheme **2.1** OSW-1 analogs made by Morzycki's group

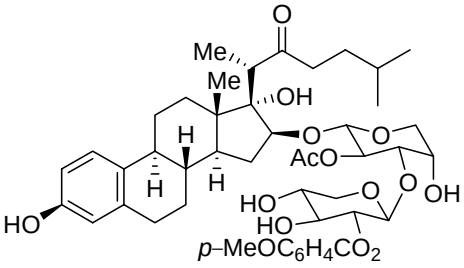
Table **2.1** Antitumor Activities of the New OSW-1 Analogs (TSC_{50} in nM) in the Calcein AM Cytotoxicity Assay

	Cell line (TCS_{50} in nM)								
Compound	CEM	MCF7	K526	ARN8	G361	HeLa	HOS	A549	NIH3T3
OSW-1	0.3 ± 0.03	50 ± 2	0.8 ± 0.05	4 ± 0.4	1000 ± 31	8 ± 0.4	40 ± 2	0.5 ± 0.01	>50,000
2.1a	0.5 ± 0.02	90 ± 4	1.4 ± 0.4	3 ± 0.2	3100 ± 26	12 ± 0.5	140 ± 11	0.9 ± 0.03	>50,000
2.1d	0.2 ± 0.01	45 ± 1.5	0.7 ± 0.05	0.6 ± 0.05	1400 ± 52	8 ± 0.15	42 ± 6	0.5 ± 0.025	>50,000
2.5	1300 ± 90	18200 ± 130	5100 ± 73	6800 ± 66	9300 ± 101	6400 ± 87	17400± 202	3200 ± 85	>50,000
2.4	280 ± 10	3400 ± 66	430 ± 17	500± 7.5	14400 ± 99	1000 ± 54	11700± 150	480 ± 39	>50,000
2.3	1000 ± 80	3500 ± 63	1800 ± 35	1500 ± 27	3500 ± 85	2300 ± 100	7200± 103	7200 ± 120	> 50,000
2.2	200 ± 20	1300 ± 15	430 ± 20	1950 ± 61	2200 ± 91	570 ± 31	4200± 92	4200 ± 96	> 50,000
2.6	<200	680 ± 32	<400	< 400	1900 ± 78	350 ± 25	1800± 63	< 200	> 50,000

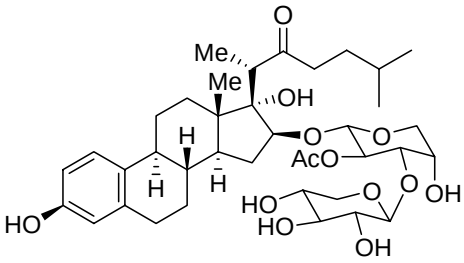
The anticancer activity of Morzycki's OSW-1 analogs was evaluated *in vitro* against eight cancer cell lines of different histopathological origins and normal mouse fibroblast NIH 3T3 cells. The results were summarized in Table 2.1. The analogs which were not included in Table 2.1 (compounds **2.1b, 2.1c, 2.1e** and **2.1f**) show very weak anticancer activities. Among the tested analogs, the most active compound was **2.1d**, which showed similar antitumor potency as OSW-1. And compound **2.1a**, which has a shortened side chain, exhibited a slight loss of activity. Monosaccharide analogs of OSW-1 (**2.2, 2.3**) were about 1000 times less active than OSW-1. Compound **2.4,** the structural isomer of OSW-1 was about 1000 times less active than OSW-1. Analog **2.5**, which did not contain the C22 carbonyl group, and had a shorter side chain, presented even less activity than compound **2.4**. Analog **2.6**, which had a long ether moiety to replace the side chain, showed almost 10,000 times less activity than OSW-1. Based on the results, Morzycki concluded that: 1. The small variations in the structure, for example, in the size of cholestane side chain, don't affect anticancer activies significantly.[11] 2. The disaccharide moiety is essential for the anticancer activities of

OSW-1, and the position of the disaccharide moiety is also important.[11] 3. The presence of C-22 carbonyl group also seems to be a pharmacophore of OSW-1.[11]

2.3 Structure–activity relationships (SAR) of OSW-1 by Nemoto's group



2.7



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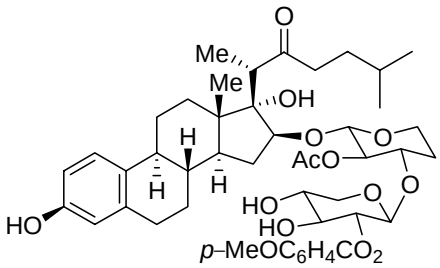

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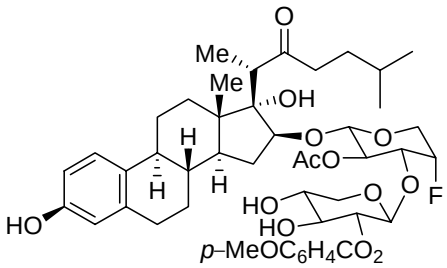
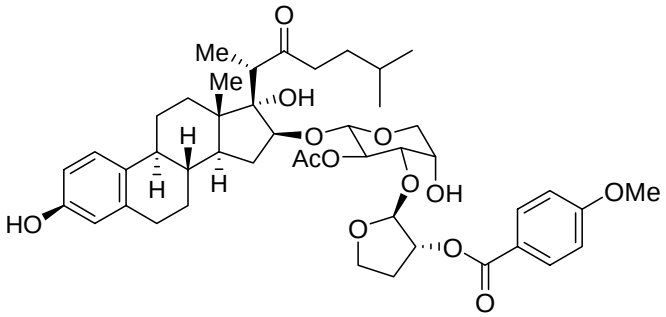


2.10



2.11

Scheme **2.2** Estrone analogs of OSW-1

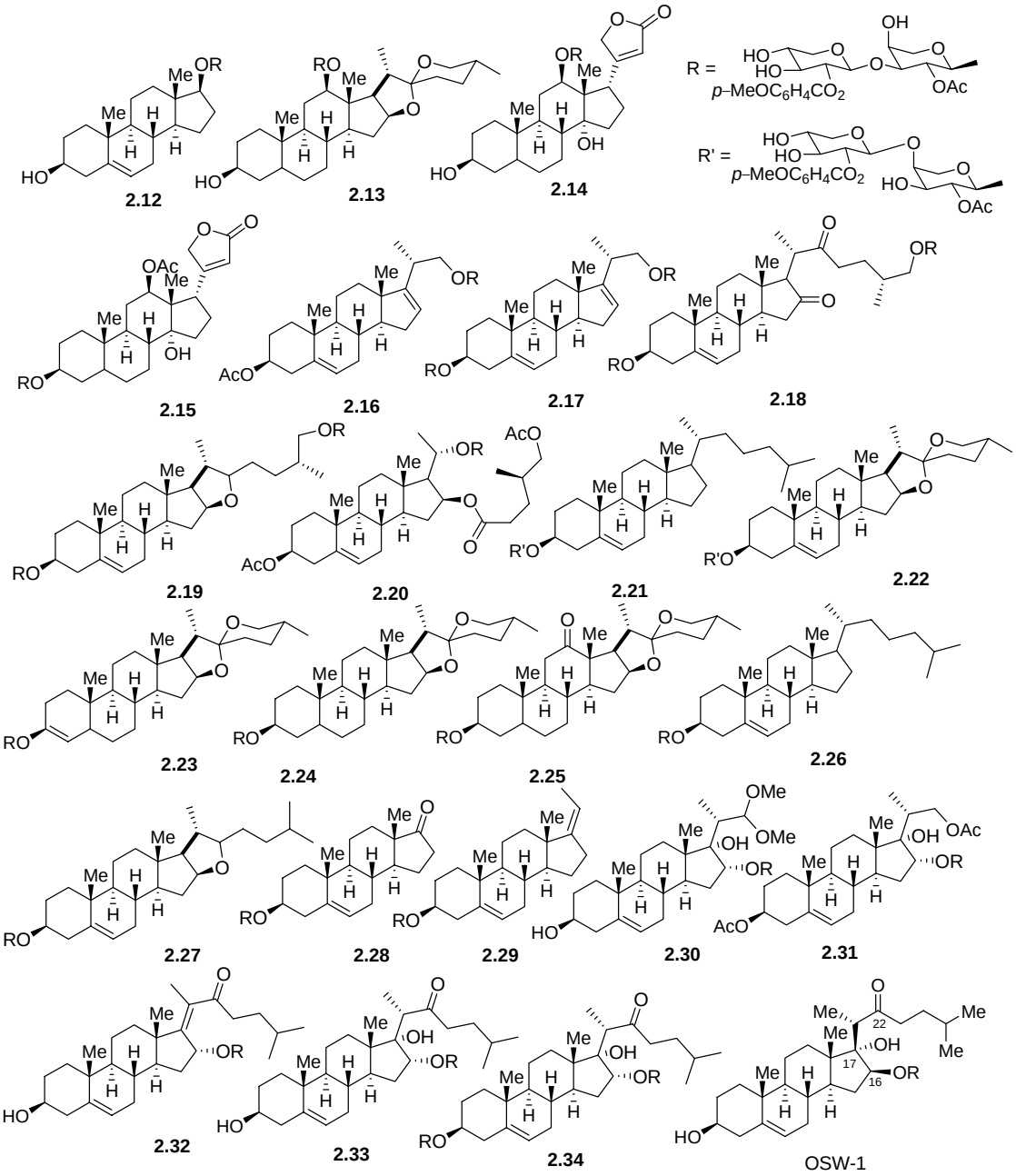
Several analogs of OSW-1 containing aromatic steroidal aglycones were synthesized by Nemoto's group in Japan. The aromatic steroidal aglycone was from commercially available estrone (Scheme 2.2). [20, 21]

Table **2.2** Cytotoxic activity of OSW-1 analogs *in vitro*

Compounds	IC_{50} Value (μM)	
	NCI-H460	MDA-MB-231
2.7	0.26	0.68
2.8	9.98	6.94
2.9	13.7	10.9
2.10	2.59	1.17
2.11	5.75	18.2
Cisplatin	0.66	7.85

The anticancer activity of OSW-1 analogs was evaluated *in vitro*. The cytotoxic effect of the estrone analogs in Table 2.2 were in the μM rang and much weaker than OSW-1 as reported in the literature. Therefore, the steroidal aglycone component must be essential for the growth-inhibitory activity of OSW-1. In Nemoto's paper, he also compared these estrone analogs and made some comments about the structure activity relationship of OSW-1. However, because all of these estrone analogs showed low cytotoxic activities, Nemoto's conclusions didn't provide much useful imformation.

2.4 Structure–activity relationships (SAR) of OSW-1 by Yu and Hui's group



Scheme **2.3** Steroidal glycosides bearing the disaccharide moiety of OSW-1

After completing the first synthesis of OSW-1, Yu and Hui's group conducted extensive investigation on the structure–activity relationships (SAR) of OSW-1.[7-9, 22-25] They designed the analogs with large variation of side chain and modified sugar residues. They also synthesized other steroidal glycosides bearing the disaccharide moiety of OSW-1.

2.4.1 Steroidal glycosides bearing the disaccharide moiety of OSW-1

Because the disaccharide moiety was essential to the antitumor activities of OSW-1, a number of steroidal glycosides bearing the disaccharide moiety of OWS-1 were synthesized and evaluated for their antitumor activities (Scheme 2.3).[7]

Analogs **2.12-2.29** contained the same disaccharide moiety, but had a large variety of different aglycone components. The *in vitro* anticancer activities of the glycosides **2.12-2.29** against P388 (mouse leukemia) and A-549 (human pulmonary adenocarcinoma) were evaluated, and the results were listed in Table **2.3**. The activities of all these compounds were much less potent than that of OSW-1. Once again, it demonstrates that the aglycone is essential to its antitumor activity.[7]

Table **2.3** Growth inhibition rate (%) of glycosides on tumor cells (P388 and A-549)

Compounds	P388			A-549		
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-4} M	10^{-5} M	10^{-6} M
2.12	99.6	41.3	18.8	98.2	19.3	0
2.13	100.0	39.6	5.7	87.8	10.2	0
2.14	98.1	0	3.8	79.6	0	0
2.15	98.1	98.1	7.5	89.8	71.4	0
2.16	100.0	30.2	3.8	89.8	24.5	4.1
2.17	100.0	100.0	0	98.2	57.0	0
2.18	100.0	96.9	15.9	98.5	39.7	0
2.19	83.8	100.0	3.7	98.7	88.7	0
2.20	100.0	100.0	0	98.1	0	0
2.21	39.4	1.8	1.9	43.6	0	0
2.22	100	20.9	11.9	98.6	8.6	0
2.23	100	51.9	16.6	98.6	61.3	1.6
2.24	68.7	13.9	9.7	97.9	0	0
2.25	51.0	10.9	4.7	98.7	0	9.9
2.26	38.9	6.2	4.8	15.1	17.2	0
2.27	80.4	6.8	7.7	98.7	0	0
2.28	60.5	16.3	11.6	96.2	2.4	10.9
2.29	85.4	36.1	6.5	98.2	20.7	0

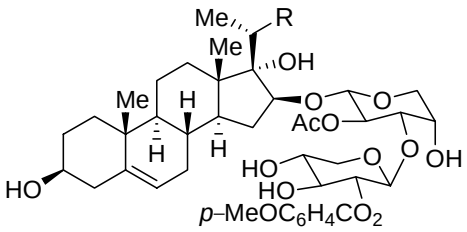
Table **2.4** Growth inhibition rate (%) of analogs **2.30 - 2.34** on tumor cells (P388 and A-549)

Compounds	P388			A-549		
	10^{-6} M	10^{-7} M	10^{-8}M	10^{-6} M	10^{-7} M	10^{-8}M
OSW-1	100	100	100	99.3	99.1	98.2
2.30	—	—	—	—	—	—
2.31	20.4	15.5	17.9	6.2	0.8	—
2.32	—	—	—	—	—	—
2.33	66.7	35.4	15.5	53.8	29.5	—
2.34	90.7	52.0	—	80.9	6.7	0.7

The *in vitro* anticancer activities of the glycosides **2.30-2.34** against P388 (mouse leukemia) and A-549 (human pulmonary adenocarcinoma) were evaluated by the standard MTT assay. The results were listed in Table **2.4**. The C16-epimer (**2.33**) of OSW-1 showed much lower activities than OSW-1. This result showed that the C-16 configuration was essential to the antitumor activities of OSW-1. It is obvious that analogs **2.30-2.33**, which had both an opposite configuration of C-16 and a modified C-17 side chain, were much less potent.[7]

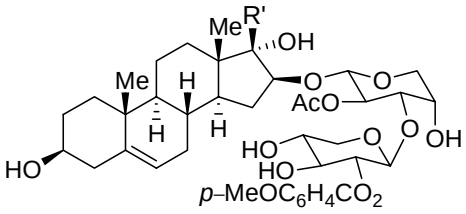
2.4.2 OSW-1 analogs with modified side chains

Because of the similarity of the cytotoxicity profile and molecular structure of the OSW-1 aglycone and cephalostatins,[22] Fuchs proposed a unified mechanism to explain the cytotoxic action of both OSW-1 and cephalostatins. He proposed the formation of the C-22 oxocarbenium ions as the key intermediat that might be highly toxic against cells. To examine how the side chain influence the cytotoxic action, especially of the C-22-one function, Yu's group synthesized a panel of analogs of OSW-1 with modified side chain on the steroidal skeleton and tested their anticancer activities (Scheme 2.4).[22, 25]



2.35

2.35a: R =-$CH_2CH_2CH_2CH(CH_3)_2$
2.35b: R =-$CH(OH)CH_2CH_2CH(CH_3)_2$
2.35c: R =-$CH(=O)CH_2CH_2CH_3$
2.35d: R =-$CH(=O)CH_3$



R' was shown in Table 2.6

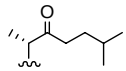
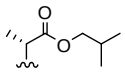
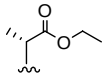
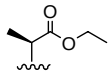
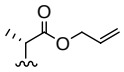
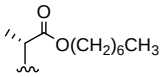
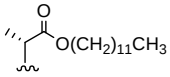
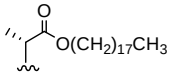
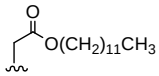
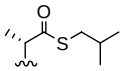
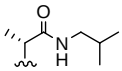
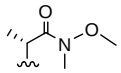
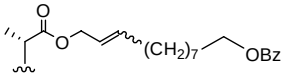
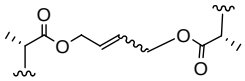
2.36a-m

Scheme **2.4** OWS-1 analogs with modified side chains

Table **2.5** Cytotoxicity of OSW-1, its analogs **2.35 (a-d)** and cisplatin against tumor cells

Compounds	IC_{50} Value (μM)		
	AGS	7404	MCF-7
OSW-1	1.42	0.10	0.27
2.35a	1.38	0.036	0.06
2.35b	7.26	1.86	1.79
2.35c	1.92	0.032	0.20
2.35d	6.98	2.90	6.61
Cisplatin	24.1	8.37	18.7

Table **2.6** Antitumor activities of the analogs of OSW-1 with modified side chain

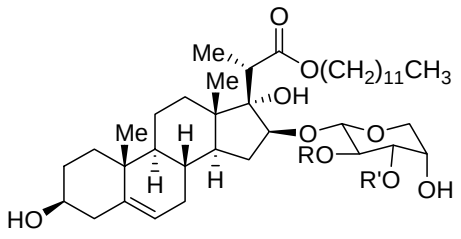
Compounds	C17 side chain R'	IC_{50} Value (μM)		
		HeLa	JurKat T	MCF-7
OSW-1		0.012	0.0022	0.094
2.36a		0.23	0.00068	0.9
2.36b		0.24	0.0053	>10
2.36c		0.034	0.042	0.13
2.36d		0.065	0.0027	0.1
2.36e		0.002	0.0014	2.6
2.36f		0.0033	0.0073	0.14
2.36g		>10	>10	>10
2.36h		10	0.073	1.4
2.36i		0.0013	0.0009	5.2
2.36j		0.0084	0.0053	1.8
2.36k		0.012	0.035	16.5
2.36l		0.04	0.03	0.19
2.36m		0.07	0.066	2.8

Based on the antitumor testing results of analogs **2.35a-2.35d**, Yu concluded that the side chain of OSW-1 can tolerate certain modification without losing significant antitumor potency. It appears that the antitumor activity of OSW-1 was independent of the C-22-one function (Table 2.5). He further concluded that these testing results suggest that Fuchs's postulation was incorrect. However, we had serious doubts about the accuracy of these testing data. Because, the control data (OSW-1) was obviously incorrect (for example, our IC_{50} value of OSW-1 against MCF-7 is around 0.1 nM). After personal communication with Prof. Yu, I believed that data in table 2.5 were not accurate and all conclusions in this paper are not convincing.

Other analogs **2.36a-2.36m** made by Yu's group were shown in Table 2.6. **2.36a-2.36m** contained side chains with different length and different functional group. It was found that **2.36f** remained the similar anticancer activity of OSW-1.

2.4.3 OSW-1 analogs with modified sugar residues

In an attempt to identify structurally simpler analogs of OSW-1 with full antitumor activity, Yu's group also made modifications on the disaccharide moiety. Because of the similar anticancer activity, **2.36f** was used as parental compound. A panel of analogs was synthesized with modification on the sugar moiety **2.37a-h** (Scheme 2.5).[23]



2.37

2.37a: R = Ac, R' = -$(CH_2)_2$OMBz
2.37b: R = Ac, R' = H
2.37c: R = Ac, R' = All
2.37d: R =MBz, R' = H
2.37e: R =MBz, R' = All
2.37f: R = MBz, R' = -$(CH_2)_2$OMBz
2.37g: R = MBz, R' = -CH_2CH=$CH(CH_2)_8$OMBz
2.37h: R = MBz, R' = -CH_2CH=CHC(=O)OCH_2CH_3

Scheme **2.5** OSW-1 analogs with modified sugar residues

Table **2.7** The Anti-proliferative activity of the OSW-1 analogs (**2.37a-h)** against tumor cells

Tumor cells	IC_{50} Value (μM)			
	2.37a	**2.37b**	**2.37c**	**2.37d-h**
RKO	ND	ND	1.7	ND
JurKat	ND	0.078	ND	ND
HeLa	ND	1.2	1.1	ND

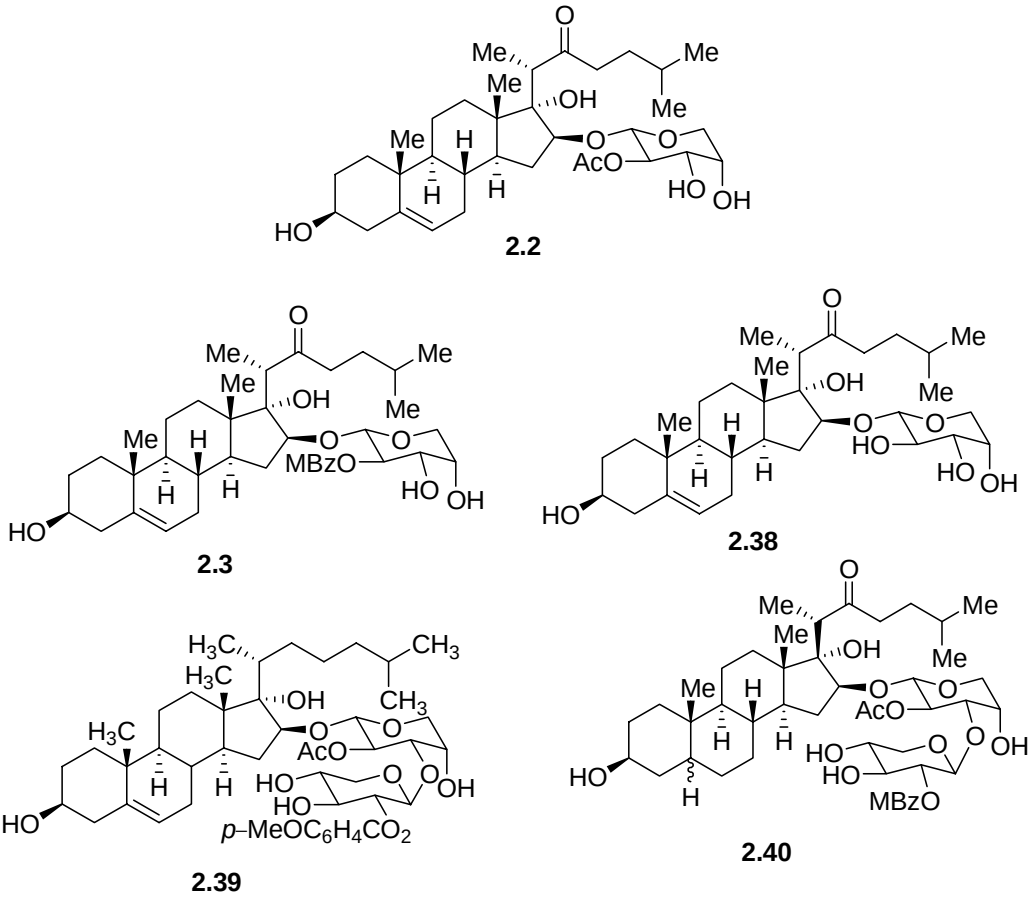
ND: not determined

The results of *in vitro* activities of analogs **2.37a-h** were summarized in Table 2.7. The designed analog **2.37a** and **2.37d-h** did not show any anti-proliferative activity against cancer cell lines tested. Only **2.37b** showed activity against Jurkat cells (IC_{50} = 0.078 μM).[23]

2.5 Structure–activity relationships (SAR) of OSW-1 by Jin group

In order to determine the minimum pharmacophore present in OSW-1, our group also designed and synthesized a number of OSW-1 analogs. The structures of these

analogs are shown in Scheme 2.6. Compounds **2.2**, **2.3** and **2.38** are monosaccharide analogs of OSW-1. Analog **2.39** has a modified side chain, and **2.40** is a mixture of 5, 6-dihydro-OSW-1 with 3 to 1 ratio of *trans*-fused AB rings and *cis*-fused AB rings.



Scheme **2.6** Analogs of OSW-1 synthesized by Jin group

Their *in vitro* anticancer activities are summarized in Table 2.8. Biological testing showed analogs **2.2**, **2.3** and **2.38** exhibit slightly cytotoxic activity *in vitro* against cancer cell KBM-3 (IC_{50}=20 nM), which were much lower than that of OSW-1.

Monosaccharide OSW-1 analogs lost cytotoxicity dramatically. Even the addition of an acetyl or *p*-methoxybenzoyl group would not improve cytotoxicity. These results showed that the disaccharide moiety was a pharmacophore for the anticancer activity of OSW-1. Our results are consistent with what were observed by other groups.[11, 19]

Table **2.8** IC_{50} (nM) of OSW-1 and its analogs

Cells	OSW-1	**2.2**	**2.3**	**2.38**	**2.39**	**2.40**
Malignant:						
ML-1	0.019					
KBM-3	0.052	20.000	20.000	20.000		
HL-60	0.044				0.307	0.090
Raji	0.058					0.251
SKOV3	0.021				0.760	0.250
A2008						0.601
C13						0.424
U87	0.047					
AsPC-1	0.391					14.290
Non-malignant:						
Normal Lymphocyte	1.730					
Fibroblast	0.830					1.846

In addition, the C5-C6 double bond of OSW-1 was reduced to give analog **2.40**. The *in vitro* anti-tumor activities of the synthetic 5, 6-dihydro-OSW-1 **2.60** were evaluated by standard MTT assay. The results showed that 5, 6-dihydro-OSW-1 **2.60** was slightly less potent than OSW-1.[29] The saturation of the C5-C6 double bond and the

resulting conformation change in the molecular structure of OSW-1 has a very slight effect on OSW-1's cytotoxic activity. Further studies will be conducted in the future.

Our study also showed that analog **2.39** were about 10 fold less potent than OSW-1 in human leukemia HL-60 cells. When SKOV3 cell line was used, analog **2.39** was about 30 fold less potent than OSW-1. These testing data suggest that the C22- ketone moiety has some influence on cytotoxic action of OSW-1, but does not play a crucial role.

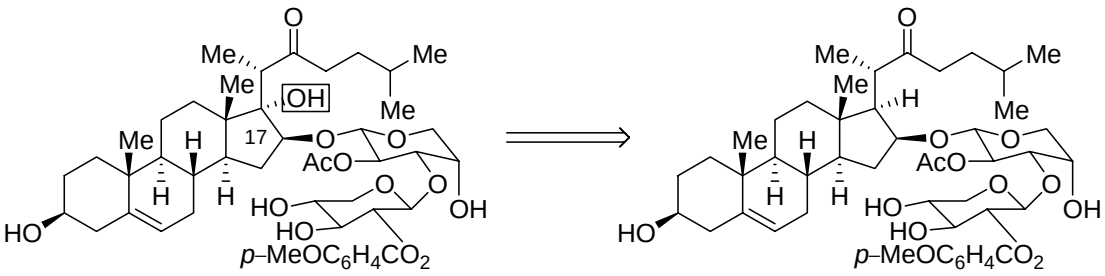
CHAPTER 3

DESIGN AND SYNTHESIS OF OSW-1 ANALOGS

3.1 Introduction

Although some structure–activity relationships (SAR) information of OSW-1 has been obtained, the mininium pharmacophore has yet to be determined. Therefore, a panel of OSW-1 analogs with simplified structures was designed, and their syntheses are summarized in this chapter.

3.2 17–des-oxo-OSW-1

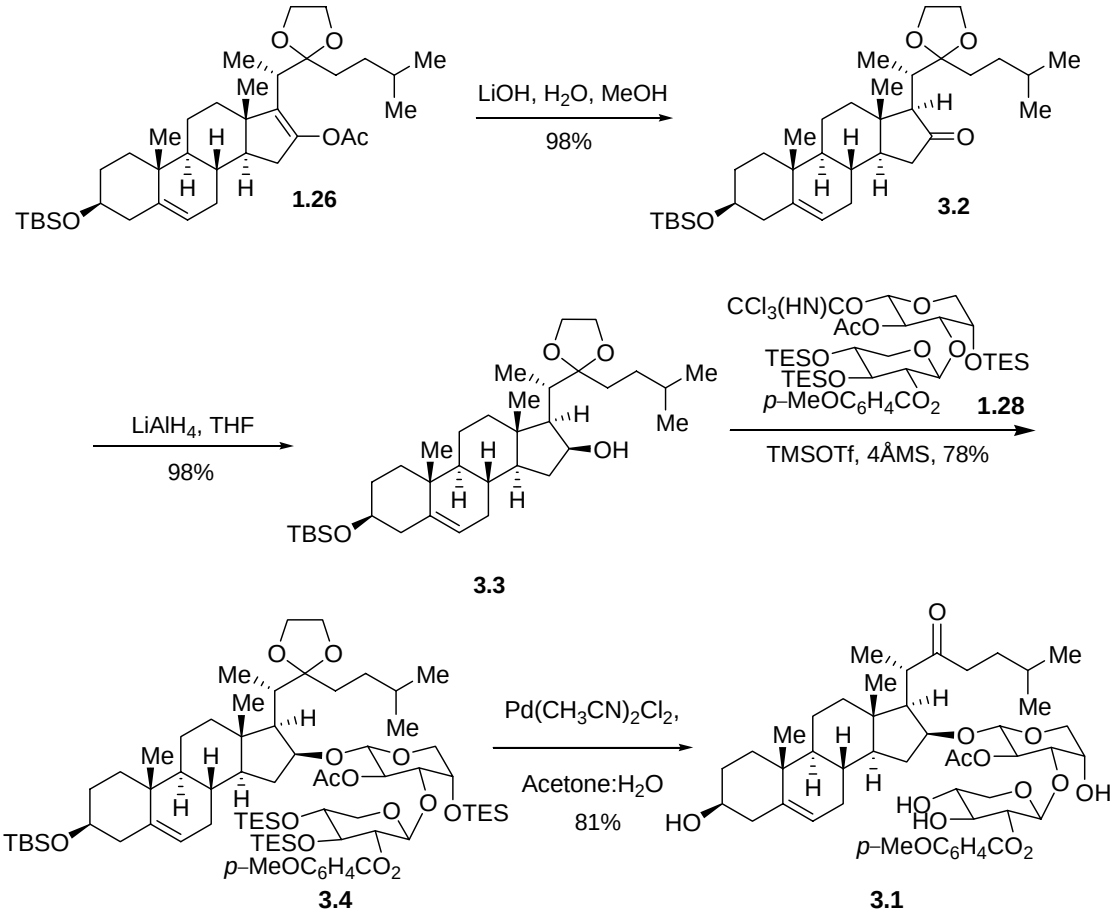


OSW-1 **3.1**

Scheme 3.1 Structure of analog 17–des-oxo-OSW-1 **3.1**

The C-17 hydroxy group of OSW-1 is a tertiary alcohol. It is very hindered, because it is blocked by both the disaccharide moiety and the steroidal side chain. Therefore, we hypothesize that the C-17 hydroxy group is not an important binding site

for OSW-1's potent anticancer activity. To confirm our hypothesis, the simplified analog **3.1**, which doesn't contain a C-17 hydroxy group, was proposed to be synthesized.



Scheme 3.2 Synthesis of analog **3.1**

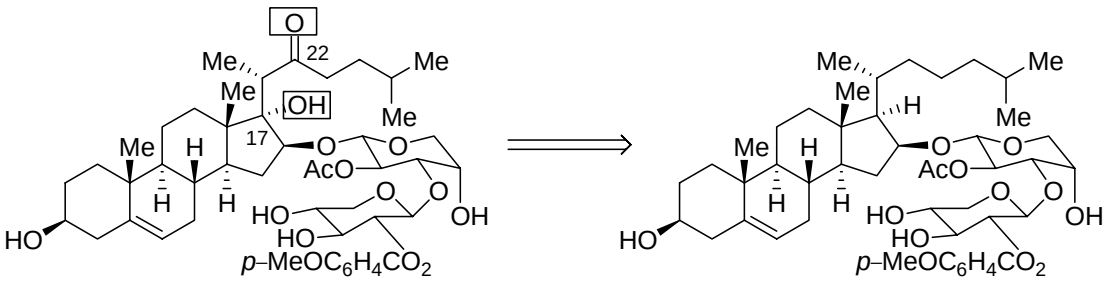
Our strategy employed in the total synthesis of OSW-1 was modified to be adapted to the synthesis of analog **3.1** (Scheme 3.2). Enol acetate **1.26**, an advanced intermediate in our total synthesis, was hydrolyzed by LiOH to give its corresponding ketone, which was stereoselectively reduced by $LiAlH_4$. TMSOTf promoted

glycosylation between TES protected disaccharide **1.28** and the steroid aglycone **3.3** provided compound **3.4** in 78% yield. Removal of all the protecting groups by the treatment of compound **3.4** with $Pd(CH_3CN)_2Cl_2$ afforded analog **3.1** in 81% yield.[30]

The cytotoxic activity of analog **3.1** was evaluated by Prof. Peng Huang at MD Anderson Cancer Center. It is highly cytotoxic against human HL-60 cell with an IC_{50} value of 0.041 nM, which is very similar to that of OSW-1 (0.044 nM). It also exhibited potent cytotoxicity against ML-1 cells with an IC_{50} value of 0.021 nM, and Raji cells with an IC_{50} value of 0.073 nM, respectively. Comparing with the corresponding IC_{50} values of OSW-1 (0.044 nM for HL-60 cells and 0.058 nM for Raji cells), we can draw a conclusion that analog **3.1** is as potent as OSW-1 in terms of *in vitro* anticancer activity. These results comfirmed our hypothesis that C-17 hydroxy group is indeed not an important function group in OSW-1's cytotoxic action.

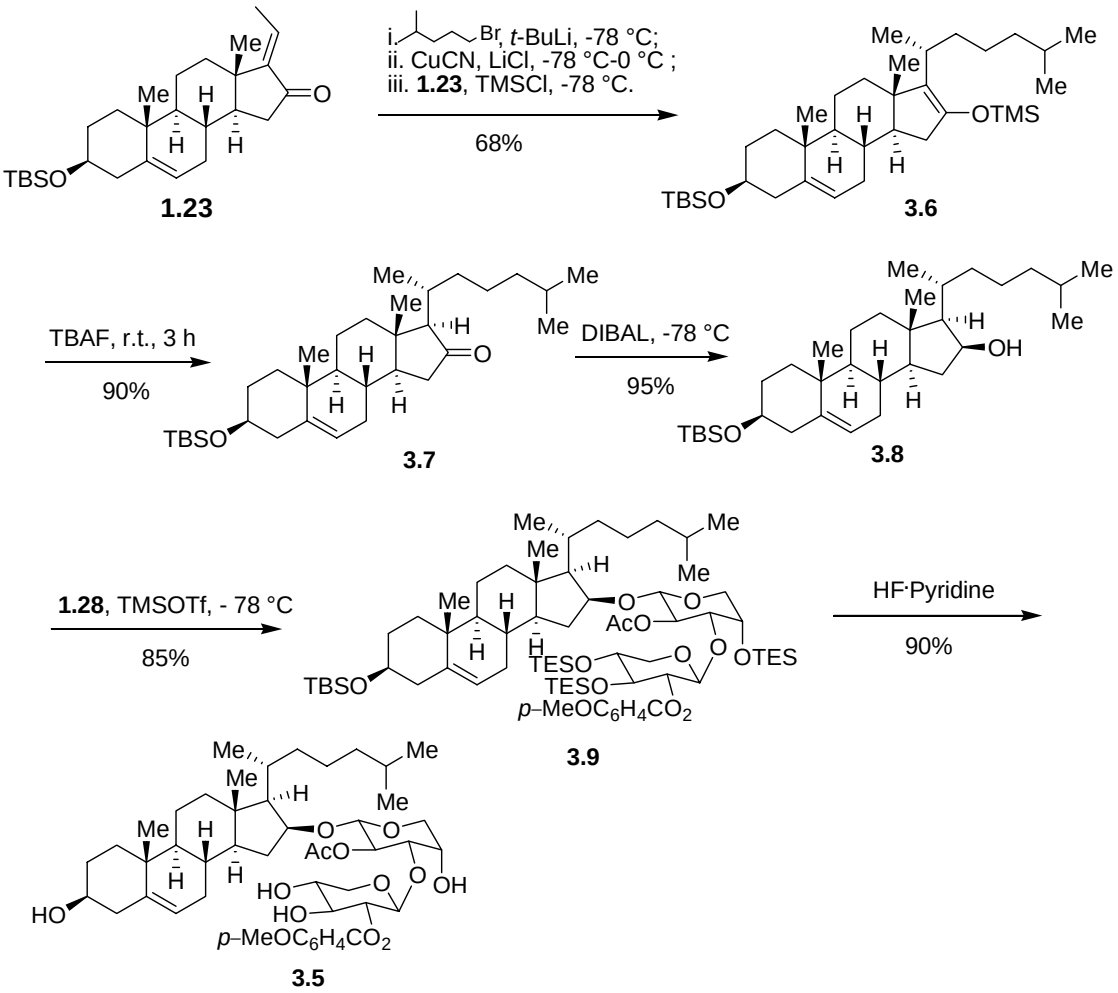
3.3 Simplified OSW-1 analog without C-17 hydroxy group and C-22 keto group

Our biological evaluation of analog **2.39** showed that the removal of C-22 ketone moiety only slightly reduced its antitumor activity. Since we have shown that the C-17 hydroxyl group is not important in its cytotoxic action, a new simplified analog **3.5** was designed (Scheme 3.3).



OSW-1 **3.5**

Scheme **3.3** Structure of analog **3.5**



Scheme **3.4** Synthesis of analog **3.5**

Again, our strategy for the total synthesis of OSW-1 was adapted to the synthesis of analog **3.5**. The synthesis of this analog is shown in Scheme 3.4. The simplified steroidal side chain was introduced by TMSCl activated 1,4-addition of 4-methylpentyl cuprate to enone **1.23** to give the silyl enol ether **3.6**, which was easily converted to ketone **3.7** by removal of the TMS group. The Ketone **3.7** was stereoselectively reduced to alcohol **3.8** by DIBAL. Glycosylation between **3.8** and the disaccharide **1.28** provided α-glycoside **3.9** in 85% yield. Global deprotection by HF·Pyridine afforded analog **3.5** in 90% yield.

The cytotoxicity of analog **3.5** was tested in Prof. Peng Huang's laboratory at MD Anderson Cancer Center. The testing result showed that compound **3.5** was still very cytotoxic against malignant tumor cells. IC_{50} values were 0.449 nM for HL-60 cells, and 2.767 nM for SKOV3. Comparing IC_{50} values, analog **3.5** was similar to analog **2.39** (Table 3.1). Although it is less active than OSW-1, its IC_{50} values are still at nM level, which was very potent. Once again, it comfirmed our hypothesis that C-17 hydroxy group was not a pharmacophore, and C-22 keto group was not the key functional group for the anticancer activity. To the best of our knowledge, compound **3.5** is the most simplified analog with similar cytotoxicity of OSW-1 that has ever been synthesized.

Table **3.1** IC_{50} (nM) of OSW-1 and its analogs

Cells	OSW-1	**3.1**	**3.5**	**2.39**
Malignant:				
ML-1	0.019	0.021		
HL-60	0.044	0.041	0.449	0.307
C6F		0.044		
Raji	0.058	0.073		
DA-1		0.038		
DA-1 3b		0.032		
SKOV3	0.021	0.054	2.767	0.760
HCT+/+		0.586		
HCT-/-		1.144		
U87	0.047	0.047		
AsPC-1	0.391	1.293		

3.4 Conclusion

The simplified designed analogs **3.1** and **3.5** were successfully synthesized by employing the similar synthetic strategy of OSW-1. The biologic testing results have shown that both compounds **3.1** and **3.5** still have similar potent anticancer activity to OSW-1. These results proved that C-17 hydroxy group and C-22 keto group were not the absolute structure requriments for its cytotoxic action and could be removed. Compound **3.5** is the most simplified synthetic analog that retains the highly potent *in* vitro anticancer activity.

3.5 Chemical experiments

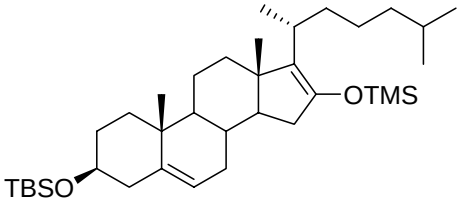
3.5.1 General

Reagents were all purchased from the following companies and were used as received unless indicated: Aldrich Chemical Company, Fluka, Fisher Scientific and ACROS Organics. Tetrahedrofuran (THF) and diethyl ether (Et_2O) were distilled over sodium benzophenone ketal; benzene, toluene, methylene chloride (CH_2Cl_2) and acetonitrile (CH_3CN) were distilled over calcium hydride. Methanol (CH_3OH), dimethylsulfoxide (DMSO) and dimethylformamide (DMF) were purchased from Aldrich Company in anhydrous form and used as received. Acetone, ethyl acetate (EtOAc) and hexanes were purchased from Fisher Company and used as received. ^{n}BuLi, ^{t}BuLi was titrated by diphenyl acetic acid prior to use. Sodium sulfate (Na_2SO_4), magnesium sulfate ($MgSO_4$) and potassium carbonate (K_2CO_3) were purchased from Fisher Company as anhydrous form and used as received.

All moisture sensitive reactions were performed in flame-dried glassware under a positive pressure of nitrogen or argon. All reactions were monitored by thin layer chromatography (TLC) with co-spots of the starting material(s). Products were isolated or purified by flash column chromatography, preparative TLC, or distillation under reduced pressure. Optical rotations were measured with Jasco P-1020 polarimeter. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with Bruker Avance 300 (300 MHz) instrument, Bruker WM360 (360 MHz) instrument, or Bruker DRX400 (400 MHz) instrument. The deuterated solvents for NMR spectroscopy were chloroform-d_1 ($CDCl_3$), benzene-d_6 (C_6D_6), and are reported in parts per million (ppm) with residual protonated solvent peak or solvent ^{13}C-NMR peak as internal standard ($CDCl_3$: 7.26 ppm for ^{1}H-NMR and 77.0

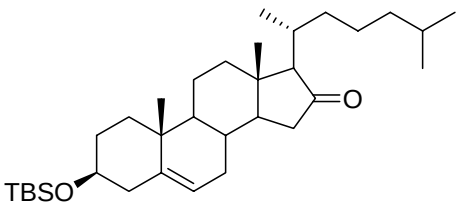
ppm for ^{13}C-NMR; C_6D_6: 7.15 ppm for ^{1}H-NMR and 128.0 ppm for ^{13}C-NMR. When peak multiplicity is reported, the following abbreviations are used: s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet), m (multiplet).

3.5.2 Experimental procedures and spectral data



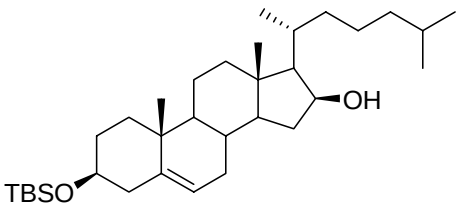
3.6

A solution of 1-bromo-4-methyl-pentane (100.0 μL, 0.687 mmol) in dry THF (1 mL) was cooled to -78 °C, then *t*-BuLi (1.45 M, 1.14 mL, 1.66 mmol) was added dropwise. The reaction mixture was stirred at -78 °C for 40 min, and was then cannulated to a clear solution of CuCN (31.0 mg, 0.344 mmol) and LiCl (29.0 mg, 0.687 mmol) in dry THF (1 mL) at -78 °C. After the reaction was stirred at -78 °C for 40 min and allowed to warm up to 0 °C, the reaction mixture was cooled to -78 °C, and a solution of **1.23** (49.2 mg, 0.114 mmol) and TMSCl (72.7 μL, 0.573 mmol) in dry THF (0.7 mL) (which was pre-cool to -78 °C.) was cannulated to the cuprate at -78 °C. The reaction was stirred at -78 °C for 30 min, and was quenched with triethylamine (1 mL). The solution was diluted with hexanes (20 mL) and passed through a short silica gel pad which was pretreated with 3% triethylamine-hexane. The silica gel was washed with ether (3×10 mL). The solvent was removed and the product was isolated by NEt_3 deactivated silica gel column chromatography (Hexane:EtOAc 30:1) to afford silyl enol ether **3.6** (55.8 mg, 83%) as colorless oil.



3.7

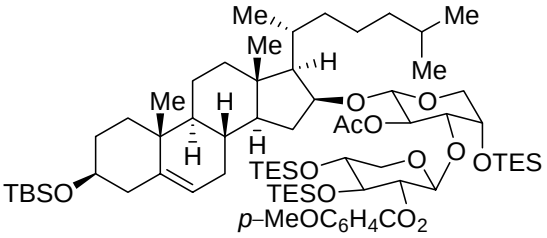
Silyl enol ether **3.6** (20.2 mg, 0.0343 mmol) was dissolved in CH_2Cl_2 (1 mL). TBAF (34.3 μL, 1M in THF) was added to the solution at 25 °C and the reaction mixture was stirred for 3h. The reaction was quenched with water (0.3 mL), and the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 2 mL). The combined organic layer was washed with brine and dried over Na_2SO_4. After the filtration, the solvent was removed and the product was isolated by silica gel column chromatography (Hexane:EtOAc 30:1) to afford **3.7** (19 mg, 95%) as white solid; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 5.30 (d, *J* = 5.1 Hz, 1H), 3.47 (m, 1H), 2.16 (m, 4H), 1.81-1.56 (overlap, 6H), 1.53-1.24 (overlap, 9H), 1.25-0.82 (overlap, 25H), 0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 219.6, 141.7, 120.4, 72.5, 67.9, 50.9, 49.9, 42.9, 42.7, 39.1, 38.95, 38.91, 37.0, 37.2, 36.6, 35.8, 32.0, 31.8, 31.3, 30.7, 27.9, 25.9, 25.0, 22.7, 22.6, 20.5, 19.4, 18.7, 18.2, 13.6, -4.6.



3.8

To a solution of **3.7** (20.6 mg, 0.0388 mmol) in anhydrous THF (1 mL) was added DIBAL (1M, 77.6 μL, 0.0776 mmol) at -78 °C. After the reaction was stirred at -78 °C

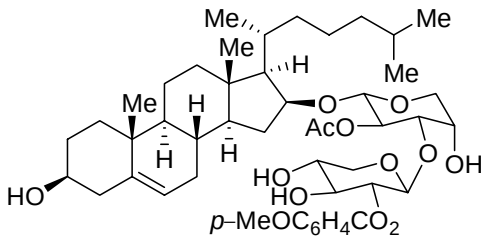
for 3 h, it was quenched with ethyl acetate (1 mL). Saturated aqueous potassium sodium tartrate (2 mL) was added. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 2 mL). The combined organic layer was washed with brine and dried over Na_2SO_4. After filtration, the solvent was removed and the product was isolated by silica gel column chromatography (Hexane:EtOAc 5:1) to afford **3.8** (20.2mg, 95%) as white solid; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 5.30 (d, J = 5.1 Hz, 1H), 4.35 (m, 1H), 3.47 (m, 1H), 2.16 (m, 4H), 1.81-1.56 (overlap, 6H), 1.53-1.24 (overlap, 9H), 1.25-0.82 (overlap, 25H), 0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 141.7, 120.9, 72.6, 72.5, 61.4, 54.5, 50.2, 42.8, 42.2, 39.9, 39.5, 37.3, 37.0, 36.6, 36.5, 36.3, 32.0, 31.8, 31.5, 29.8, 28.1, 25.9, 24.2, 22.8, 22.6, 20.7, 19.4, 18.3, 18.2, 13.0, -4.6.



3.9

The mixture of **3.8** (47.0 mg, 0.085 mmol) and **1.28** (100.8 mg, 0.11 mmol) was pre-dried by benzene for 3 times and dissolved in anhydrous CH_2Cl_2 (1.5 mL). Dry 4Å MS powder (120 mg) was added and the mixture was stirred at 25 °C for 15 min, and was then cooled to -20 °C and TMSOTf (0.02 M in CH_2Cl_2, 235 μL, 0.0047 mmol) was added dropwise. The reaction mixture was stirred at -20 °C for 30 min and quenched with 0.15 mL of Et_3N. After filtration, the filtrate was concentrated by rotavop and purified by silica gel column chromatography (Hexane:EtOAc 40:1) to afford **3.9** (95.3 mg, 85%

yield) as colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.98 (d, *J* = 9.0 Hz, 2H), 6.98 (d, *J* = 9.0 Hz, 2H), 5.28 (d, *J* = 4.8 Hz, 1H), 5.02 (m, 1H), 4.88 (t, *J* = 6.1Hz, 1H), 4.80 (d, *J* = 4.8 Hz, 1H), 4.22 (d, *J* = 4.8 Hz, 1H), 4.13 (m, 1H), 3.85 (overlap, 5H), 3.74 (m, 2H), 3.62 (m, 1H), 3.45 (m, 1H), 3.32 (m, 1H), 3.22 (m, 1H), 2.16 (m, 4H), 1.81-1.56 (overlap, 9H), 1.53-1.24 (overlap, 9H), 1.25-0.82 (overlap, 25H), 0.53 (m, 18H), 0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 168.6, 164.5, 163.2, 141.6, 131.9, 122.8, 121.0, 113.3, 102.4, 81.8, 72.9, 72.7, 70.8, 70.4, 60.9, 55.3, 55.0, 50.2, 42.8, 42.1, 39.8, 39.6, 37.3, 36.6, 36.3, 36.1, 32.1, 31.8, 31.5, 29.6, 28.0, 25.9, 23.3, 22.8, 22.6, 20.9, 20.8, 19.3, 18.3, 18.2, 13.9, 6.88, 6.81, 6.79, 5.0, 4.9, 4.8, -4.6.



3.5

To a solution of **3.9** (24.0 mg, 0.0183 mmol) in dry THF (0.5 mL) was added HF·Pyr (10mg, 5eq.). After the reaction was stirred at 25 °C for 3 h, saturated sodium bicarbonate was used to quench the reaction. The mixture was extracted by $CHCl_3$ (3 x 5ml). The combined organic phase was dried over Na_2SO_4 and concentrated by vaccum. The product was purified by silica gel column chromatography ($CHCl_3$: MeOH, 15 : 1) to afford **3.5** (13.3 mg, 90%) as form solid; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.98 (d, *J*= 8.7 Hz, 2H), 6.90 (d, *J* = 8.7 Hz, 2H), 5.31 (d, *J* = 4.2 Hz, 1H), 5.02 (m, 1H), 4.91 (t, *J* = 5.9, 5.9 Hz, 1H), 4.70 (d, *J* = 6.6 Hz, 1H), 4.25 (d, *J* = 4.8 Hz, 1H), 4.12 (m, 1H), 3.85

(overlap, 10H), 3.40 (m, 4H), 2.16 (m, 4H), 1.81-1.56 (overlap, 9H), 1.53-1.24 (overlap, 9H), 1.25-0.74 (overlap, 25H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 169.6, 166.4, 164.1, 140.9, 132.4, 121.7, 121.6, 114.0, 102.1, 81.8, 80.3, 74.8, 74.2, 72.0, 70.9, 69.9, 66.9, 64.9, 63.4, 61.0, 55.6, 55.2, 50.4, 42.5, 42.3, 40.3, 39.8, 37.4, 36.7, 36.4, 36.3, 31.9, 31.8, 31.6, 29.7, 28.1, 23.2, 23.0, 22.8, 21.0, 20.8, 19.6, 18.4, 12.8.

CHAPTER 4

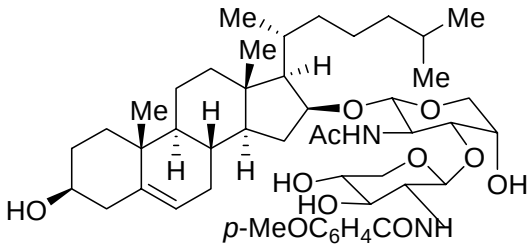
DESIGN AND SYNTHESIS OF AMINO ANALOGS OF OSW-1

4.1 Introduction

Although OSW-1 exhibits extremely potent *in vitro* cytotoxicity against various human cancer cells including cancer cells resistant to other anticancer agents, the promise of this potent natural product is dampened by its relatively weak *in vivo* anticancer activity. In the animal study conducted in our collaborator Prof. Huang's laboratory, no tumor shrinkage was observed although OSW-1 prolonged the life span of mice injected with the P388 leukemia cell line.[32] At the end, all mice were dead. To develop OSW-1 into a drug candidate, chemical modification of the original structure will be absolutely necessary.

We speculate that its weak *in vivo* anticancer activity is due to its poor pharmacokinetics (PK). It is known that the removal of two ester groups in the disaccharide portion of OSW-1 reduces its *in vitro* anticancer activity by more than 1,000 fold. We hypothesize that the relatively weak *in vivo* anticancer activity is due to the cleavage of two ester groups by enzymes in mice. To develop OSW-1 into a drug candidate, it is absolutely essential to improve its pharmacokinetics. Although it has been reported that encapsulating OSW-1 into the GM3 liposomes can slow down the hydrolysis of the benzoyl ester by 18 times,[33] we believe that encapsulation by liposomes alone will not make a significant difference in its pharmacokinetics. It is our conviction that chemical modification of the original chemical structure of OSW-1 will be necessary to make a significant improvement in its pharmacokinetics and *in vivo* activity.

From a medicinal chemistry point of view, amide groups are bioisosteric to ester groups, and hydrolysis of an amide group is usually slower than its corresponding ester.[33] Therefore, replacing both ester groups in the disaccharide portion of OSW-1 with bioisosteric amides is expected to reduce the rate of metabolism of the compound by hydrolysis and prolong the action of OSW-1, which would in turn significantly improve its *in vivo* anticancer activity.

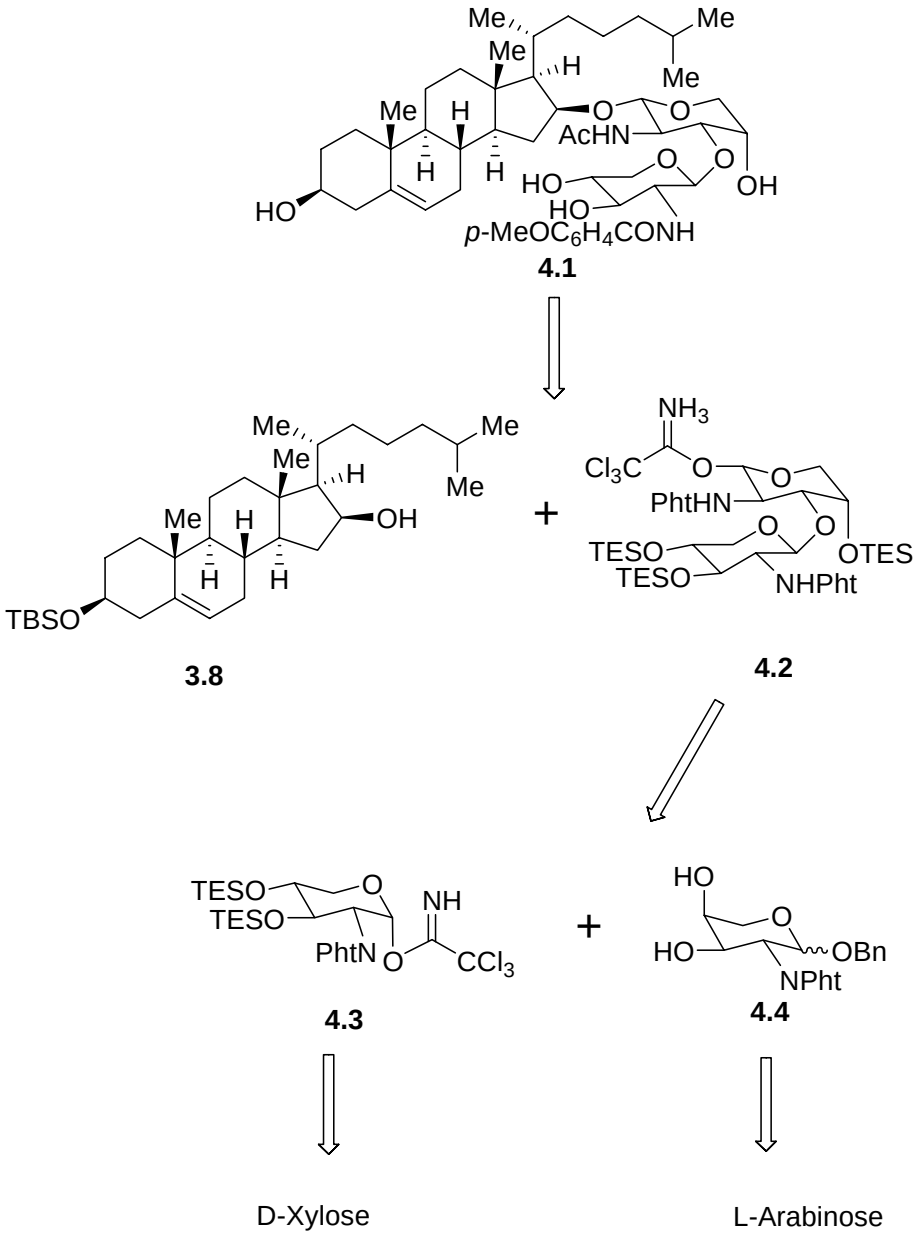


4.1

Scheme **4.1** Structure of amino-analog **4.1**

4.2 Retrosynthetic analysis of amino analog

To test our hypothesis a designed analog **4.1** is proposed to be synthesized employing the total synthesis approach developed in our laboratory followed by comprehensive pharmacological evaluation. We decided to use the simplified steroidal aglycone **3.8** prepared in the previous chapter as the aglycone of the designed amide analog. The original retrosynthetic analysis is shown in scheme 4.2.



Scheme **4.2** Retrosynthetic analysis of amino analog **4.1**

Amino analog **4.1** would be synthesized by glycosylation between aglycone **3.8** and disaccharide **4.2**. Glycosylation of monosaccharide **4.3** and **4.4** would provide the disaccharide **4.2**. D-Xylose would be employed as the starting material for the preparation

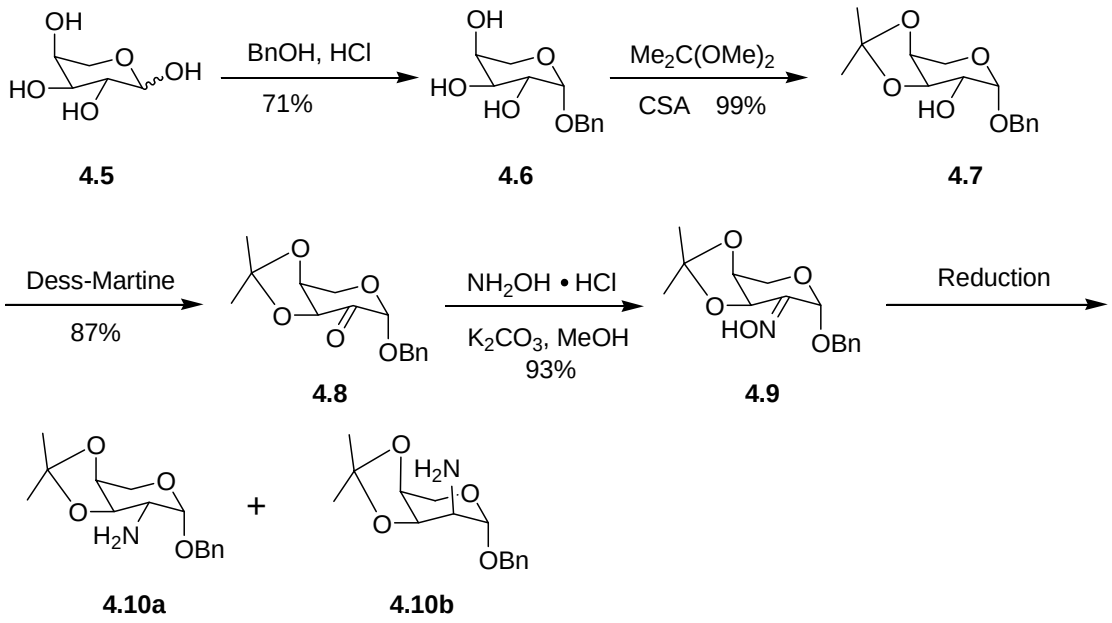
of amino xylose **4.3**, and L-arabinose would be the starting material for the preparation of amino arabinose **4.4**.

4.3 The first attempt toward the synthesis amino analog 4.1

Due to the structural similarity of OSW-1 and its amino analog **4.1**, the total synthesis approach of OSW-1 developed in our laboratory was employed. Based on the retro-synthetic strategy we proposed, the amino saccharides **4.3** and **4.4** were required to be synthesized from D-xylose and L-arabinose, respectively.

4.3.1 Synthesis of L-arabinosamine moiety

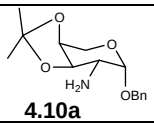
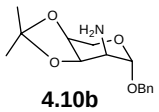
4.3.1.1 The first approach for the synthesis of L-arabinosamine moiety



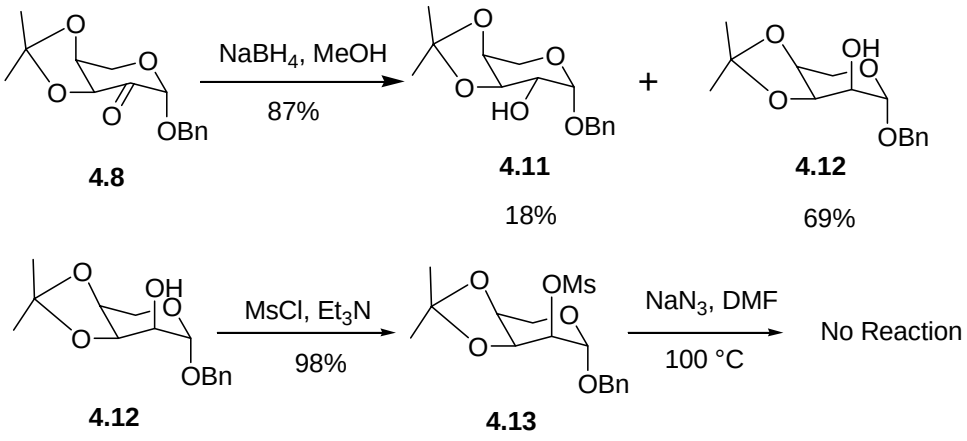
Scheme **4.3** Synthesis of L-arabinosamine moiety

L-Arabinose **4.5** was dissolved in BnOH with anhydrous HCl bubbled to generate β-*O*-benzyl arabinose **4.6** in 71% yield. The *cis*-3,4 hydroxy groups of **4.6** were regio-selectively protected by 2,2-dimethoxypropane to give compound **4.7** in 99% yield. Dess-Martin oxidation of compound **4.7** provided ketone **4.8** which was treated with hydroxyl-amine to form the oxime **4.9** in 93% yield.

Table **4.1** Reduction of the oxime **4.9**

Entry	Condition	Yield	 **4.10a**	 **4.10b**
1	$LiAlH_4$, THF, 0 °C, 12h		20%	30%
			30% starting material recovery	
2	$LiAlH_4$, THF, RT, 48h		trace	major product based on NMR
3	$LiAlH_4$, THF, reflux, 12h		trace	major product based on NMR
4	$NaBH_4$, $NiCl_2$, MeOH, 0 °C, 48h		trace	major product based on NMR
5	$NaBH_4$, MoO_3, MeOH, 0 °C, 48h		trace	major product based on NMR

We then investigated the stereoselective reduction of oxime **4.9**. $LiAlH_4$ mediated reduction at 0 °C provided 20% yield of the desired product **4.10a**, and 30% of the **4.10b** with wrong stereochemistry along with the recovery of 30% of starting material (Table 4.1). These experimental results suggested that the reducing agents preferred to attack the oxime from β face which was relatively less stereohindered. To obtain the desired compound **4.10a**, an alternative approach was studied.

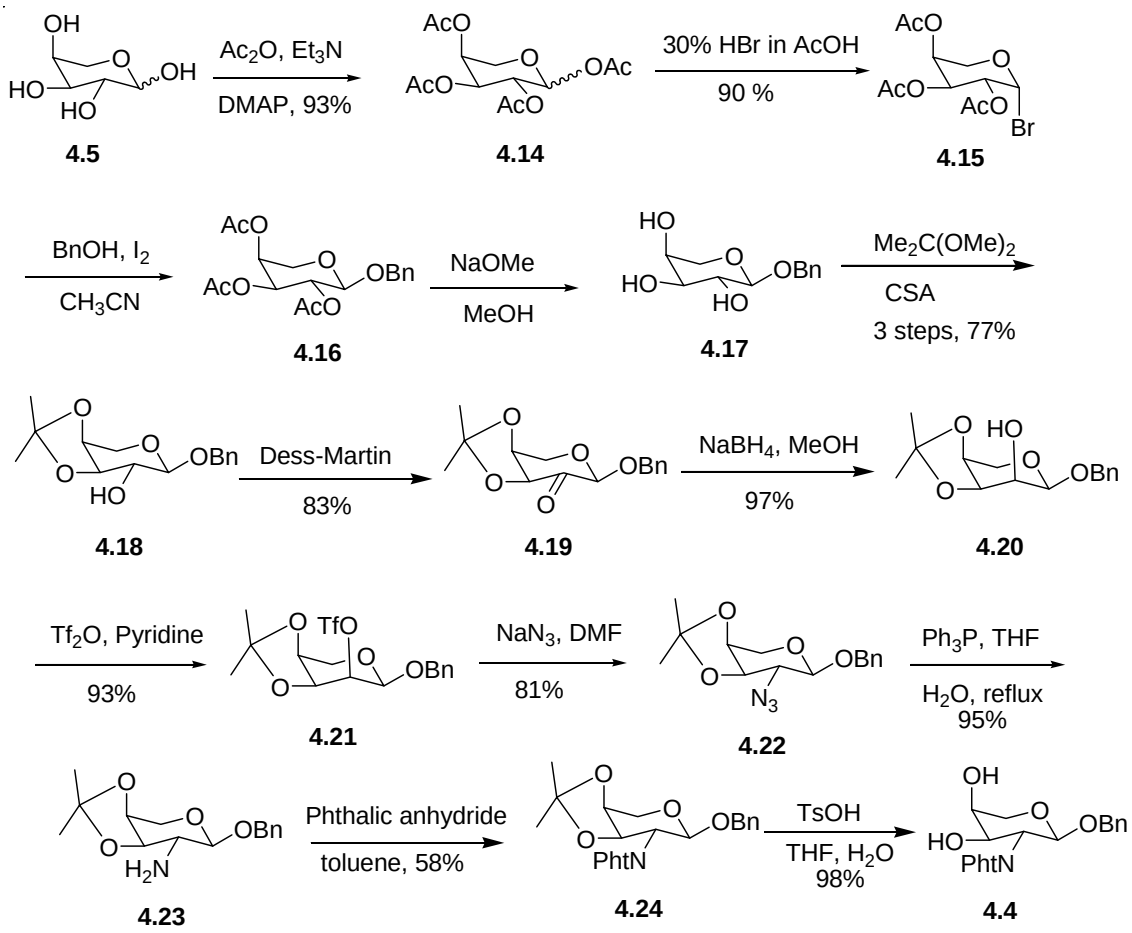


Scheme **4.4** The attempted inversion of stereochemistry *via* S_N2 substitution

Compound **4.8** was stereoselectively reduced to alcohol **4.12** in 69% yield. Then alcohol **4.12** was converted to mesylate **4.13** in 98% yield. Unfortunately, no S_N2 reaction was observed when compound **4.13** was treated with NaN_3 in DMF at 100 ^oC. We then tried higher temperature and different leaving groups, but it appeared that the benzyl group blocked the bottom side of the C-2 position and S_N2 substitution was not observed.

In order to invert the stereochemistry at C-2 position, the anomeric benzyl group had to be at equatorial position. Therefore, we modified our orginal approach. Acetylation of arabinose provided the fully protected L-arabinose **4.14**. Compound **4.14** was treated with 30% HBr in AcOH to obtain β-bromide **4.15**. Iodine catalyzed BnOH substitution, followed by hydrolysis and regioselective protection of *cis*-3,4-diol, afforded compound **4.18** in 77% yield. Compound **4.18** underwent Dess-Martin oxidation

to provide ketone **4.19**, which was stereoselectively reduced to alcohol **4.20** by $NaBH_4$. As expected, the reaction was completely selective and only one product was obtained. Alcohol **4.20** was converted to its corresponding triflate **4.21** in 93% yield, which underwent a facile S_N2 substitution by sodium azide to give compound **4.22** with inversion of C-2 stereochemistry. Staudinger reduction of azide **4.22** gave amine **4.23** in 95% yield. Amine **4.23** reacted with phthalic anhydride to provide **4.24**, which underwent deprotection to afford compound **4.4** in 98% yield (Scheme 4.5).

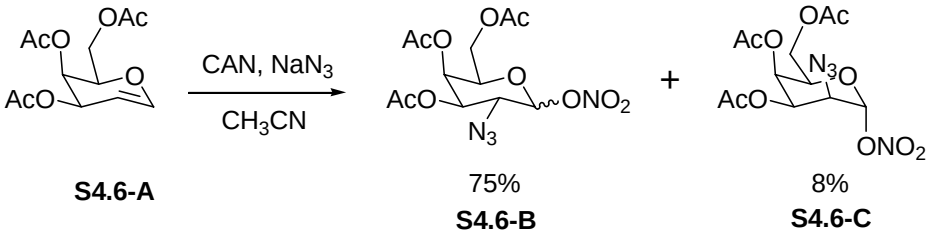


Scheme **4.5** Synthesis of α -1- benzyl-2-phthaloylamino-arabinose **4.4**

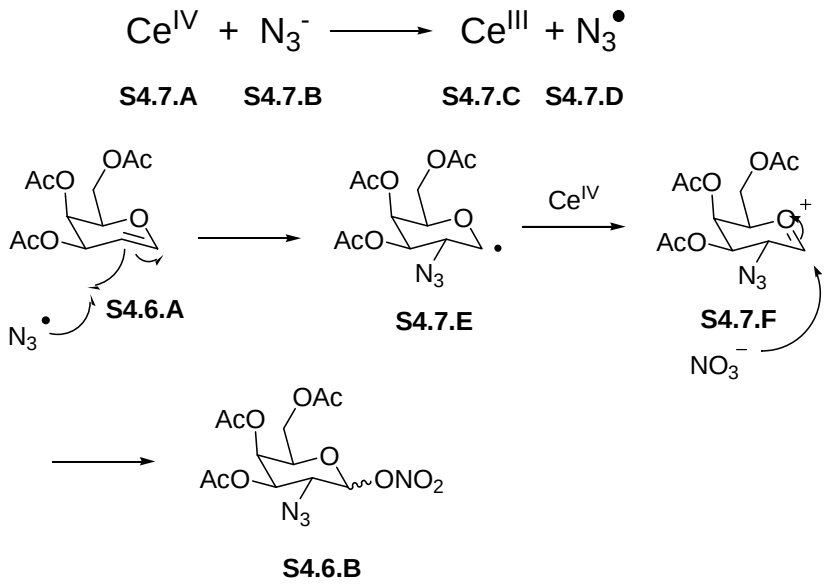
Although we successfully synthesized the functionalized L-arabinosamine **4.4**, this synthetic route was very long. Therefore, we had to search for a more efficient alternative approach.

4.3.1.2 The 2nd approach for the synthesis of L-arabinosamine moiety

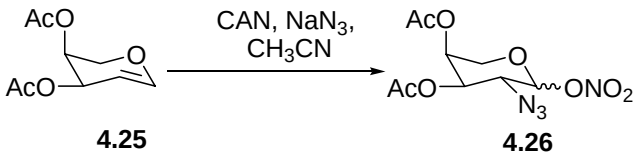
Introducing the amino group C-2 position of L-arabinose with the right stereochemistry was the key step in the synthesis of L-arabinosamine moiety. The reported stereoselective azidonitration of tri-*O*-acetyl-D-galactal[34] appeared to be quite promising to install the requisite C-2 azido group in arabinose (Scheme 4.6). In the azidonitration of tri-*O*-acetyl-D-galactal, the major product was the compound with C-2 azide group at the equatorial orientation. The mechanism of this addition was shown in Scheme 4.7. Oxidation of the azide anion by CAN leads to the corresponding radical **S4.7-D**. The radical induced addition follow the anti-Markovnikov route to attack the electron rich carbon from the least hinder side, leading to the formation of compound **S4.7-E** with C-2 azido group at the equatorial orientation. Based on the mechanism suggested by Trahanovsky and Robbins[34], the initially formed azido-alkyl radical was oxidized to a carbonium ion which, in turn, accepted nitrate to form azidonitrate. During the radical induced addition, the neighboring acetyl group at C3-position plays the major role in the stereoselective azidonitration. Because the C-3 alcohol in L-arabinose has the same stereo configuration as that of D-galactal, we expected that the azidonitration of compound **4.25** could provide the desired product with azido group at equatorial position (Scheme 4.8).



Scheme **4.6** Azidonitration of tri-*O*-acetyl-D-galactal

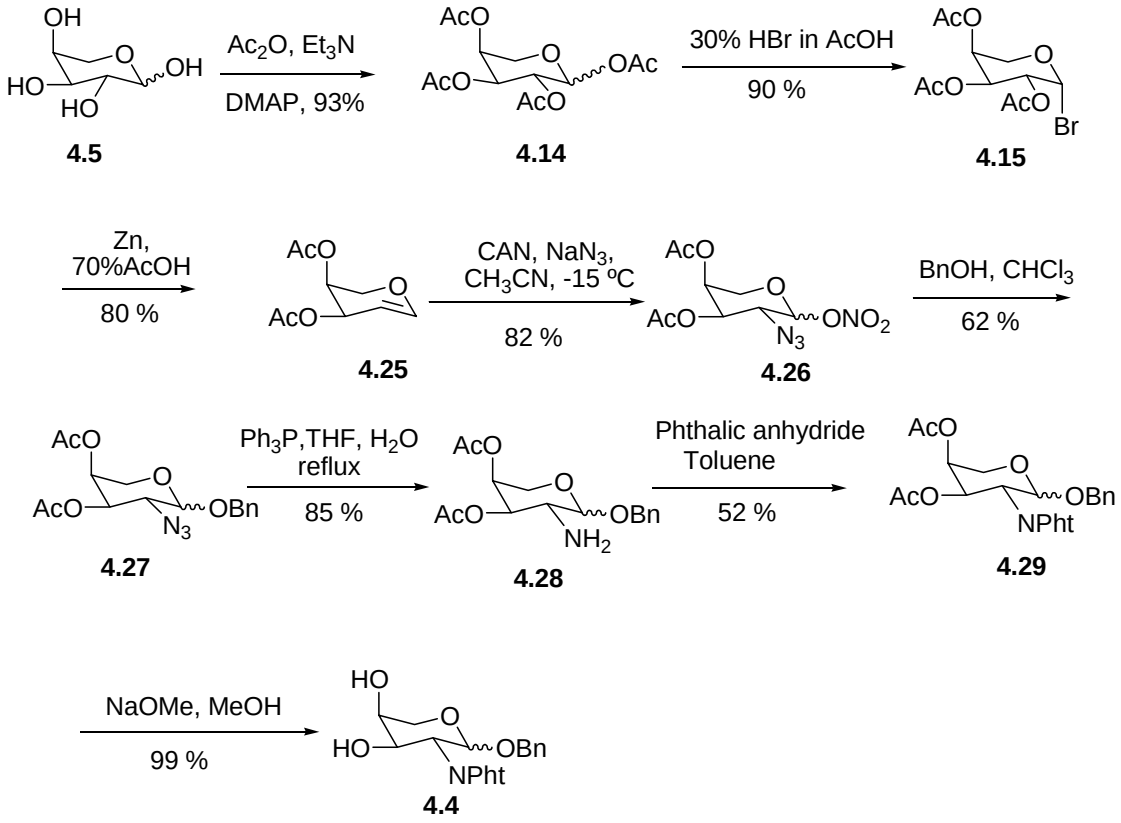


Scheme **4.7** Mechanism of azidonitration



Scheme **4.8** Azidonitration of compound **4.25**

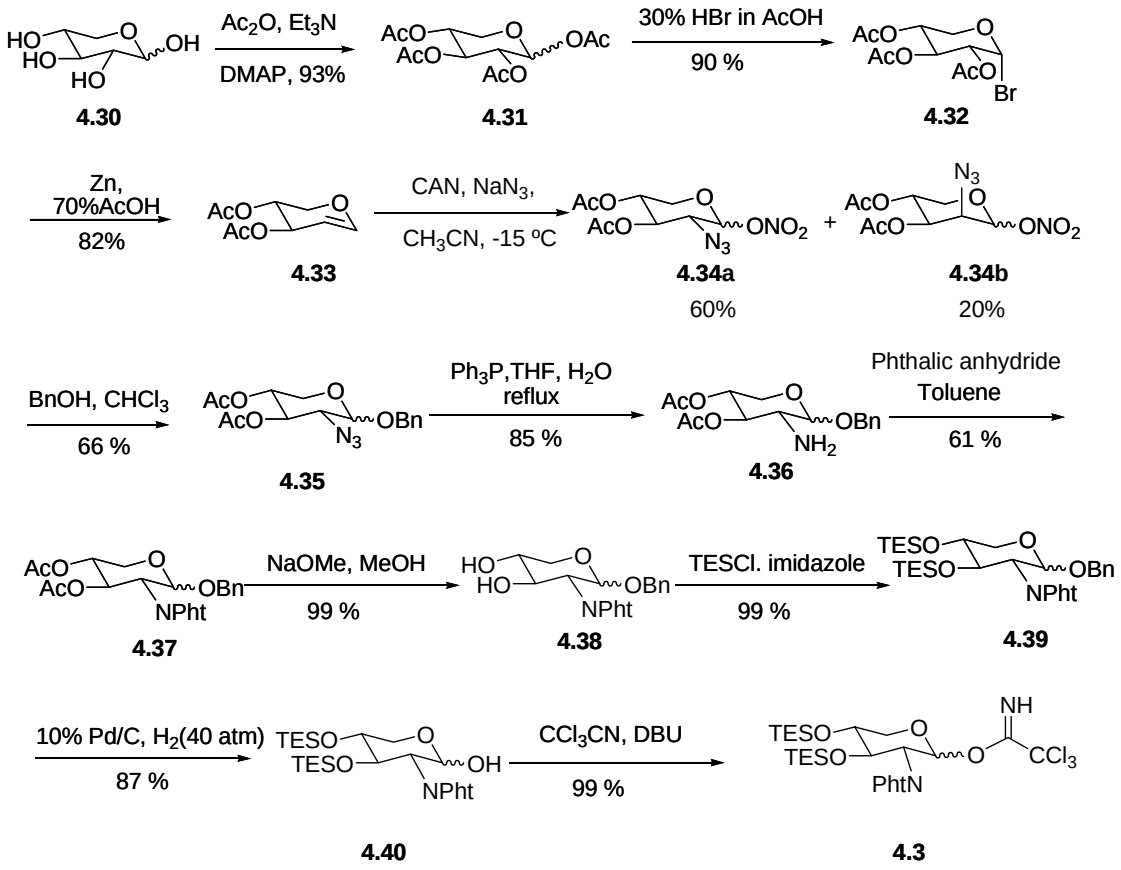
Acetylation of arabinose provided the tetra acetyl L-arabinose **4.14**. Compound **4.14** was treated with 30% HBr in AcOH to obtain β-bromide **4.15**. Zn-mediated β elimination provided compound **4.25**. As expected, stereoselective oxidative addition gave azide **4.26** in 82% yield. No axial product was detected. This result proved our assumption. Then, compound **4.26** was converted to **4.27** by benzyl alcohol substitution. Staudinger reduction followed by the protection of the amine **4.28** with a phthaloyl group afforded compound **4.29**. Hydrolysis of **4.29** under basic condition generated diol **4.30** (scheme 4.8).



Scheme **4.9** The second approach to synthesis of L-arabinosamine **4.4**

This second approach for the synthesis of the L-arabinosamine moiety consisted of only 8 steps, which was 4 steps shorter than the first approach. We then decided to apply the same approach to synthesize D-xylosamine moiety.

4.3.2 Synthesis of D-xylosamine moiety.

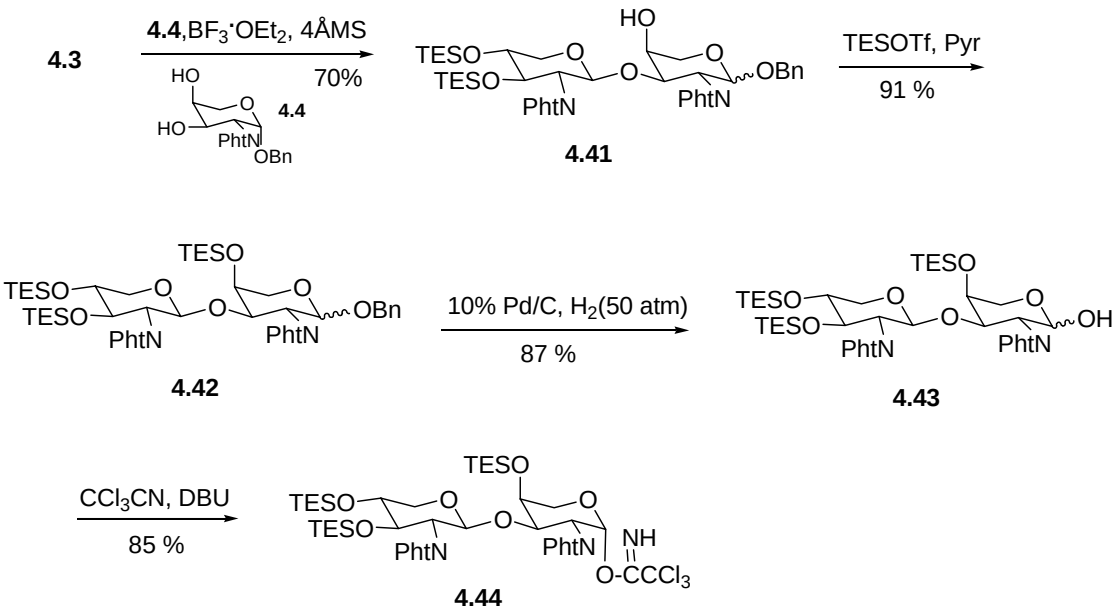


Scheme **4.10** Synthesis of D-xylosamine moiety

In the event, the oxidative azidonitration of compound **4.33** gave azide **4.34a** with desired stereochemistry in 60% yield (Scheme 4.10). Compound **4.34b** with the azide

group at the axial position was isolated in only 20% yield. Staudinger reaction was employed to reduce azide **4.35** to amine **4.36** which was protected by phthalic anhydride. Hydrolysis of **4.37** under basic conditions generated diol **4.38**, which was protected by TES groups. Hydrogenolysis of the TES protected compound **4.39** provided hemiacetal **4.40** which was converted to the corresponding trichloroacetimidate **4.3**. D-Xylosamine was successfully synthesized, and it was ready for the glycoslation.

4.3.3 Synthesis of disaccharide

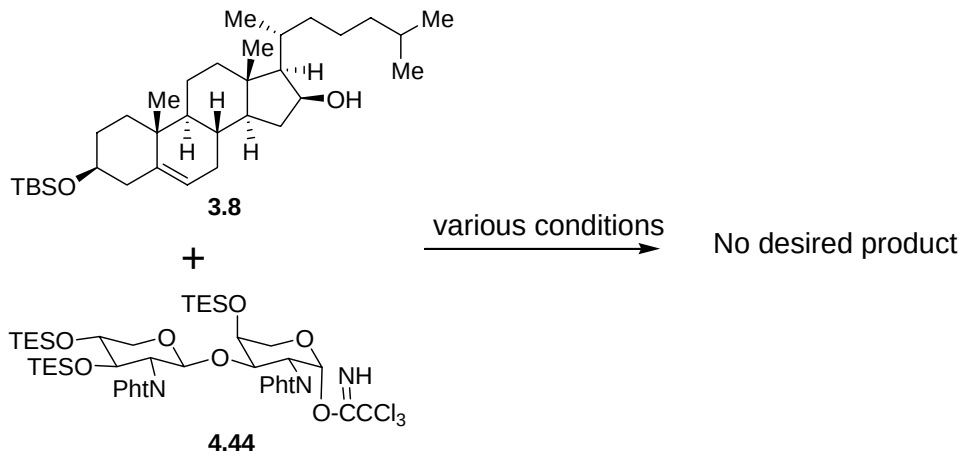


Scheme **4.11** Synthesis of amino-disaccharide **4.44**

Glycosylation of diol **4.4** with trichloroacetimidate **4.3** proceeded smoothly in the presence of a catalytic amount of TMSOTf to provide disaccharide **4.41** regio- and

stereoselectively. Then the free alcohol of compound **4.41** was protected by a TES group, and the benzyl group was cleaved under hydrogenation conditions. The disaccharide **4.43** reacted with trichloroacetonitrile in the presence of a catalytic amount of DBU to form the donor **4.44** (Scheme 4.11).

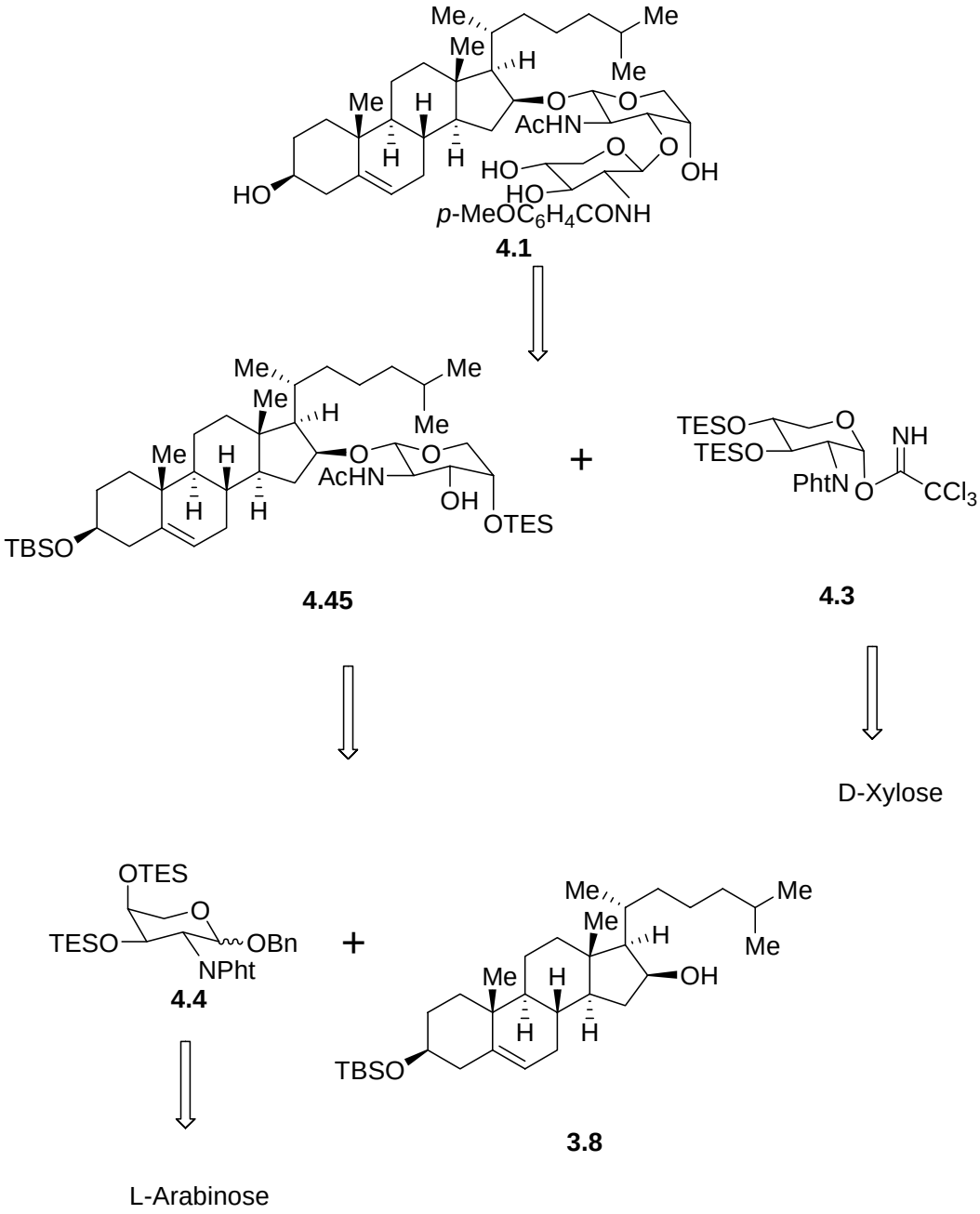
4.3.4 The attempted glycosylation between the steroidal aglycone **3.8** and disaccharide **4.44**



Scheme **4.12** The attempted glycosylation between the steroidal aglycone **3.8** and disaccharide **4.44**

We studied many different conditions, including different Lewis acid activation, the amount of the Lewis acid, different reaction temperature, different solvents, and reaction concentration (Scheme 4.12). Unfortunately, we were not able to isolate any desired product. In most case, there were no reactions. But, using larger amounts of Lewis acid or higher reaction temperature caused the starting material to decompose and

made the reactions very messy. This failed glycosylation forced us to change our original synthetic scheme. We decided to introduce one sugar at a time.



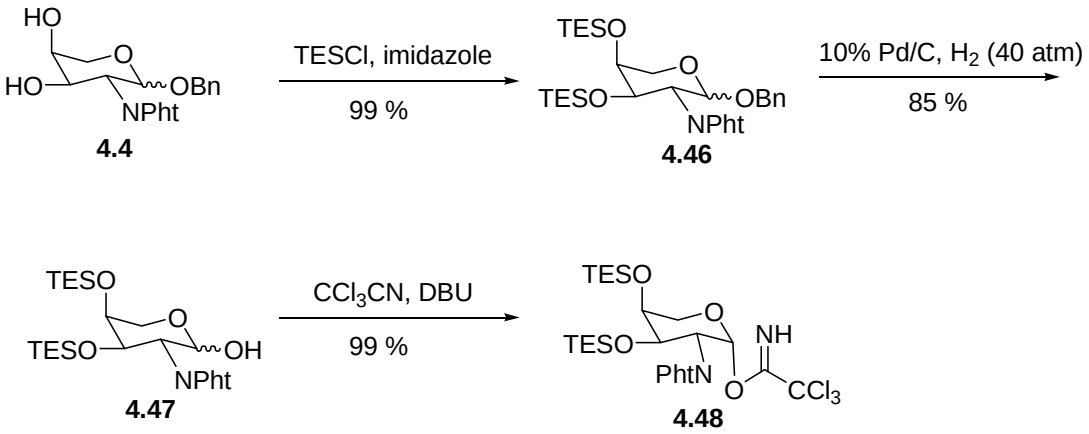
Scheme **4.13** Retrosynthetic analysis of the stepwise formation of amino analog **4.1**

4.4 A revised strategy for the synthesis of amino analog

4.4.1 Stepwise Retro-synthesis analysis of amino analog formation

A revised synthetic strategy was proposed, and the new retrosynthetic analysis is outlined in scheme 4.13. In this case, we decided to introduce one sugar moiety at a time.

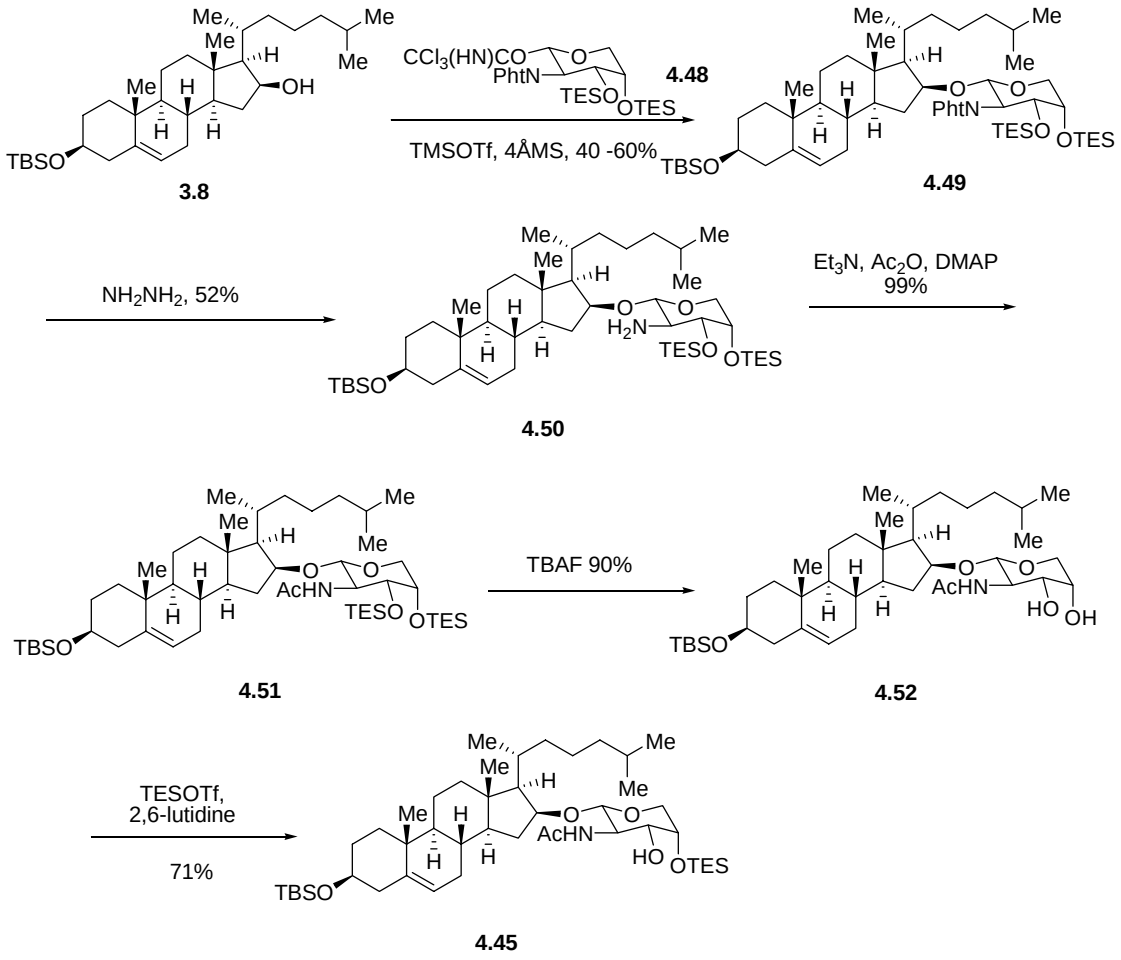
4.4.2 Synthesis of monosaccharide donor **4.48**



Scheme **4.14** Synthesis of monosaccharide donor **4.48**

L-Arabinosamine diol **4.4**, which was previously synthesized in scheme 4.9, was protected by a TESCl group to give **4.46** (Scheme 4.14). Deprotection of the benzyl group under the standard hydrogenation conditions provided hemiacetal **4.47**, which was then converted to the corresponding trichloroacetimidate **4.48**.

4.4.3 Synthesis of mono amino saccharide fragment



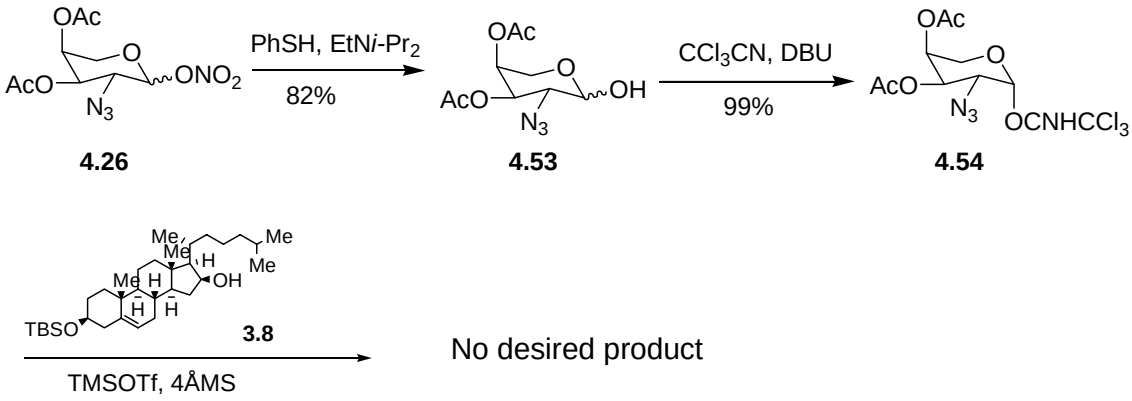
Scheme **4.15** Synthesis of amino mono saccharide fragment **4.45**

In the presence of a catalytic amount of TMSOTf, glycosylation between steroid **3.8** with **4.48** gave compound **4.49** stereoselectively. Due to the neighboring phthaloyl group participation, only α product was obtained. This result indicated that monosaccharide could be successfully installed to aglycone **3.8** with excellent stereoselectivity. But the yield was between 40-60%. Hydrazine was used to remove the

phthaloyl group, followed by acylation to generate **4.51**. However, the cleavage of the phthaloyl group only gave 52% yield. Both TES groups in compound **4.51** were removed by TBAF to afford diol **4.52**. Then, the C-4 axial alcohol of compound **4.52** was regioselectively protected by TSE group again (Scheme 4.15). Although we successfully installed the first sugar, the yield of both the glycosylation and deprotection of phthaloyl group were not satisfactory. We needed to find a better approach.

4.4.4 Alternative attempt to mono amino saccharide fragment

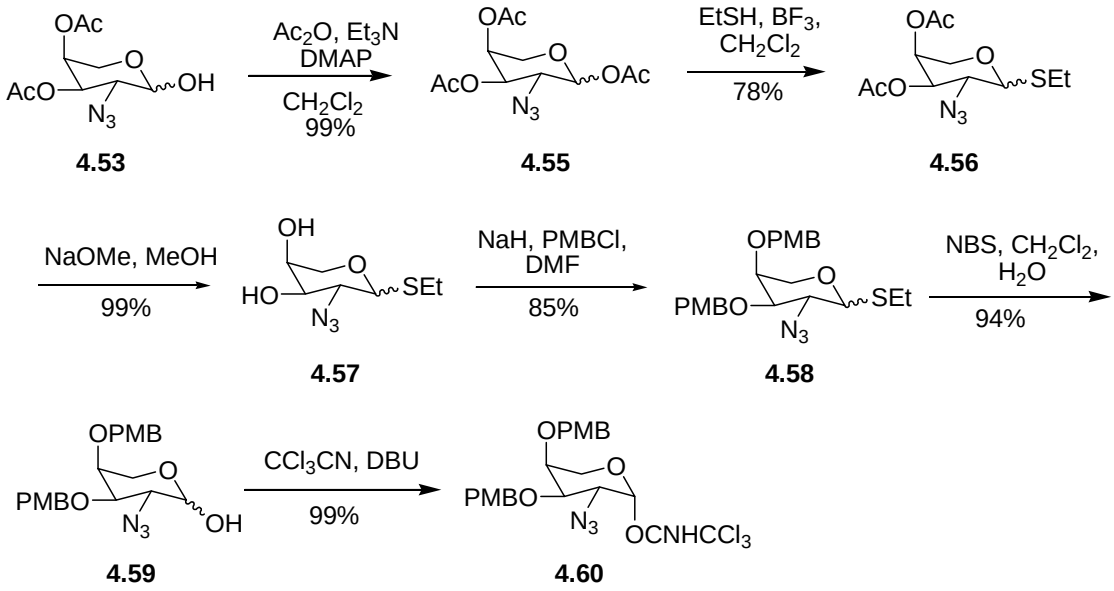
After careful analysis, we hypothesized that the bulkiness of the phthaloyl group had caused the low yield of the glycosylation. In addition, removal of the phthaloyl group was also troublesome. Therefore, we decided not to use sugars with a phthaloyl group. Instead, we decided to employ a donor with an azido group at C-2 position.



Scheme **4.16** Alternative attempt to mono amino saccharide fragment

Di-acetate protected arabinose **4.26** which was an intermediate in Scheme 4.9 was treated with PhSH and EtN*i*-Pr_2 to form the hemiacetal **4.53.** Compound **4.53** was converted to donor **4.54** under the standard conditions. However, donor **4.54** failed to react with **3.8** due to the deactivation by both acetate groups in the donor. Therefore, we needed to arm the donor with electron –donating groups (Scheme 4.16).

4.4.5 Synthesis of armed-donor

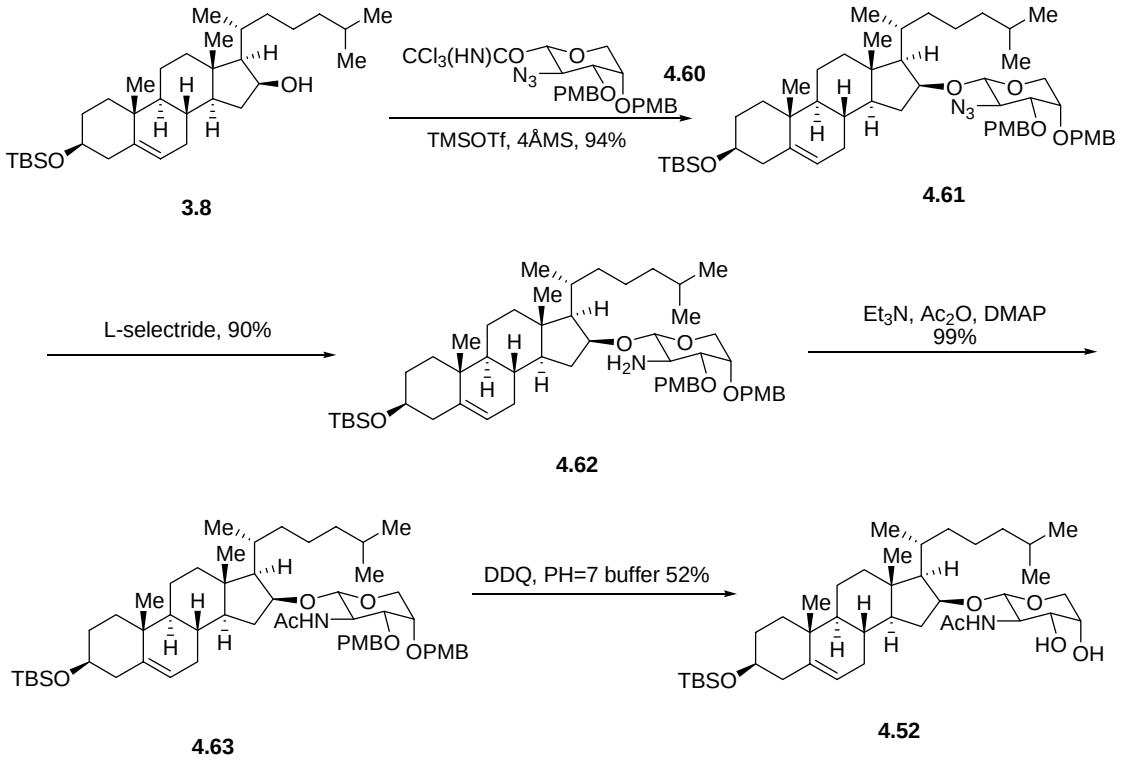


Scheme **4.17** Synthesis of armed-saccharide **4.60**

In order to obtain the armed donor for the glycosylation, electron donation groups were required to protect the two hydroxyl groups at C-3 and C-4 positions of azido arabinose. We decided to use *para*-methoxyl benzyl (PMB) group armed the sugar (Scheme 4.17). The acetylated compound **4.55** was treated with ethylthiol and boron

trifluoride-ethyl ether complex gave the thioglycoside **4.56** as an α, β mixture.[42] Deacetylation followed by the installation of PMB groups under basic conditions afforded compound **4.58**. NBS was used to cleave the carbon-sulfide bond followed by CCl_3CN activation to provide armed-donor **4.60**.

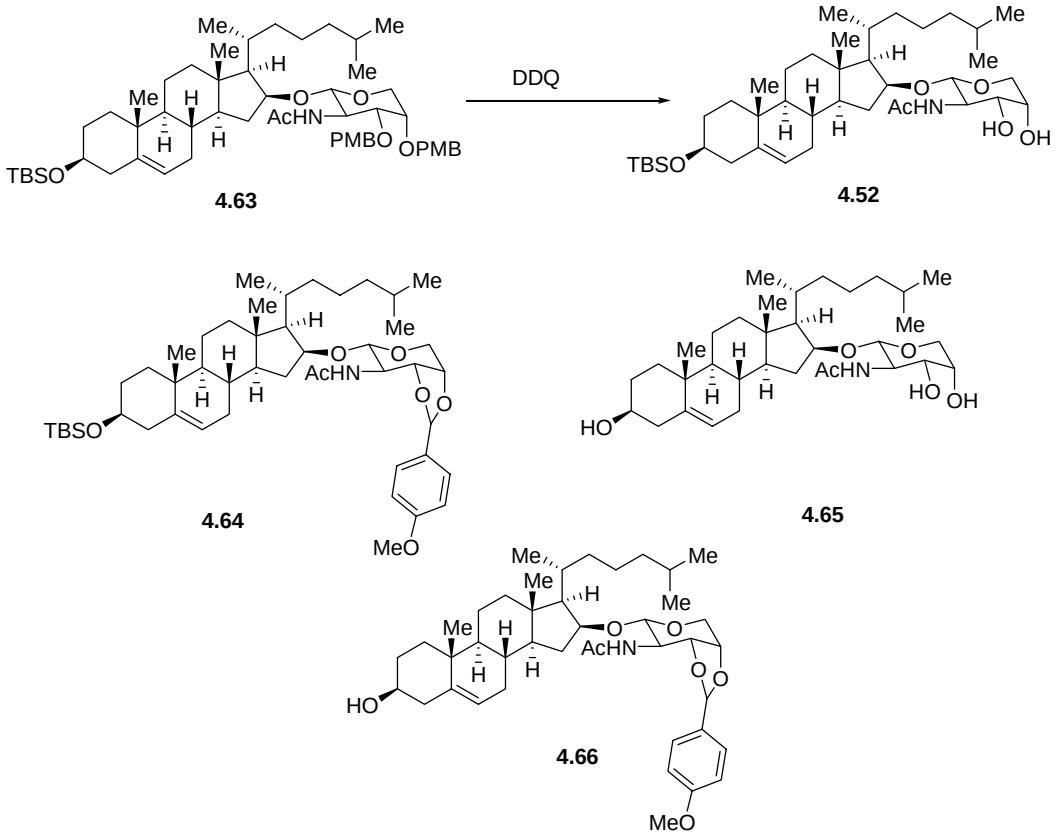
4.4.6 Alternative approach to mono amino saccharide fragment



Scheme **4.18** Alternative approach to mono amino saccharide fragment

The glycosylation between the steroid **3.8** and the armed-donor **4.60** provided compound **4.61** in 94% yield with complete stereoselectivity. Staudinger reduction of the azide **4.61** provided amine **4.62** in only 63% yield. But we found that the yield of

compound **4.62** could be improved to 90% if L-selectride was used as a reducing agent. Acetylation of amine **4.62** afforded amide **4.63**. However, the deprotection of two PMB groups by DDQ provided diol **4.52** in only 52% yield (Scheme 4.18). The low yield was mainly due to the formation of the PMB acetal by the neighboring hydroxyl group. The improvement in yield was not observed even though pH 7 buffer was used. Therefore, a better method to cleave PMB group had to be found (scheme 4.19).

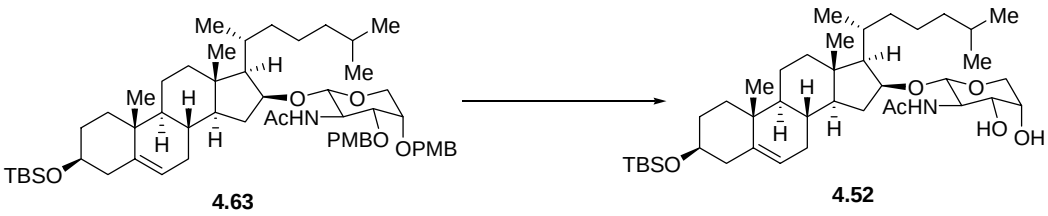


4.63 **4.52** **4.64** **4.65** **4.66**

Entry	Condition	result
1	DDQ, CH_2Cl_2, H_2O, 12h	**4.52** 52%, **4.64** 33%, also **4.65** and **4.66** were formed, but the yields were not determined
2	DDQ, CH_2Cl_2, pH=7 buffer, 12h	**4.52** 42%, **4.64** 40%, also **4.65** and **4.66** were formed, but the yields were not determined

Scheme **4.19** PMB groups deprotection by DDQ

Table **4.2** Investigate good method to remove PMB group

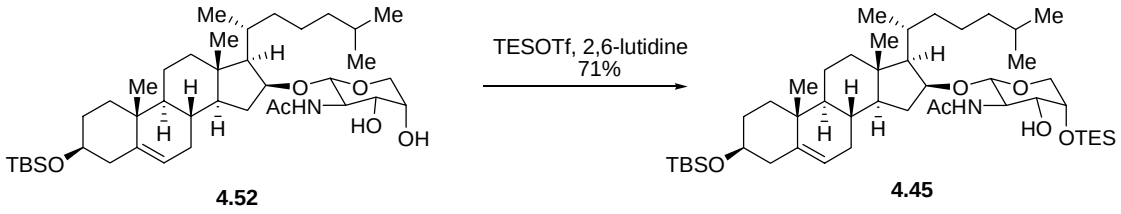


4.63 **4.52**

Entry	Condition	result
1	CAN, CH_2Cl_2, PH=7 buffer, 30min	messy reaction, TBS has been cleaved. all product went to the baseline
2	$SnCl_4$, PhSH, CH_2Cl_2, -78°C	messy reaction
3	TEA, PMBOMe, CH_2Cl_2,12h	no reaction
4	BF_3-OEt_2,Et_3SiH, CH_2Cl_2,12h	no reaction
5	TMSCl, anisole, $SnCl_2$, CH_2Cl_2	messy reaction
6	$SnCl_2$, EtSH, CH_2Cl_2	no reaction
7	$AlCl_3$, EtSH, CH_2Cl_2	no reaction
8	BCl_3, Me_2S	messy reaction, TBS has been cleaved. all product went to the baseline
9	$MgBr_2$-Et_2O, Me_2S(3eq),CH_2Cl_2	clean reaction, but never finished
10	**$MgBr_2$-Et_2O, Me_2S-CH_2Cl_2 (1:1), reflux**	**88%**

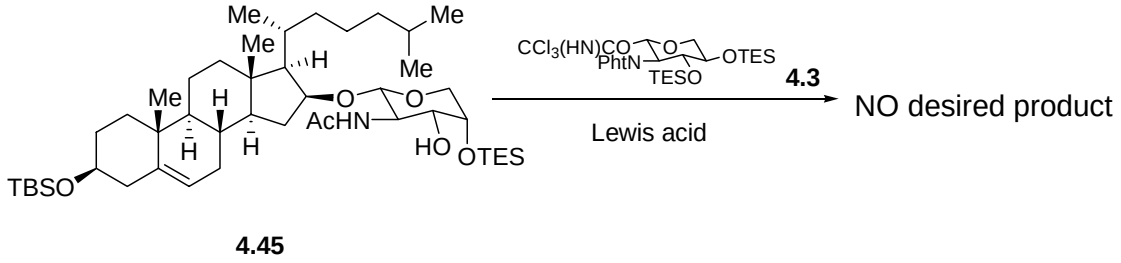
In order to improve the yield of the deprotection of PMB groups, a number of reaction conditions were carefully studied (Table 4.2). However, most of these conditions resulted in either very messy reactions or no reaction. We were delighted to observe that treatment of compound **4.63** with $MgBr_2$-Et_2O in the present of Me_2S (3eq.) and CH_2Cl_2 provided a very clean reaction (Entry 9 in Table 4.3).[33] However, the reaction couldn't go to completion if only 3 eq. of Me_2S was used according to the literature procedure. [33] We found that excess of dimethyl sulfide or using dimethyl sulfide as a co-solvent with methylene chloride could eventually drive the reaction to the completion, and the yield of

diol **4.52** was 88%. The diol **4.52** was regioselectively protected by TES group to afford compound **4.42** in 71% yield (scheme 4.20).



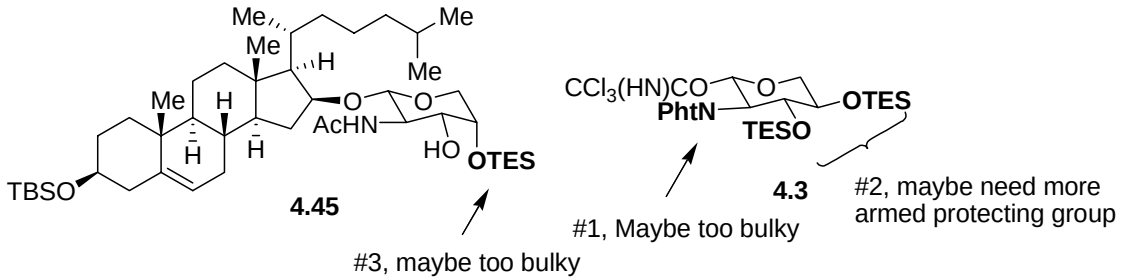
Scheme **4.20** Regioselectively protect the diol **4.52**

4.4.7 The attempted glycosylation with amino xylose component



Scheme **4.21** Glycosylation with amino xylose component

Unfortunately, the glycosylation between compound **4.45** and donor **4.3** didn't provide any desired product, even though a lot of conditions had been tried (Scheme 4.21).

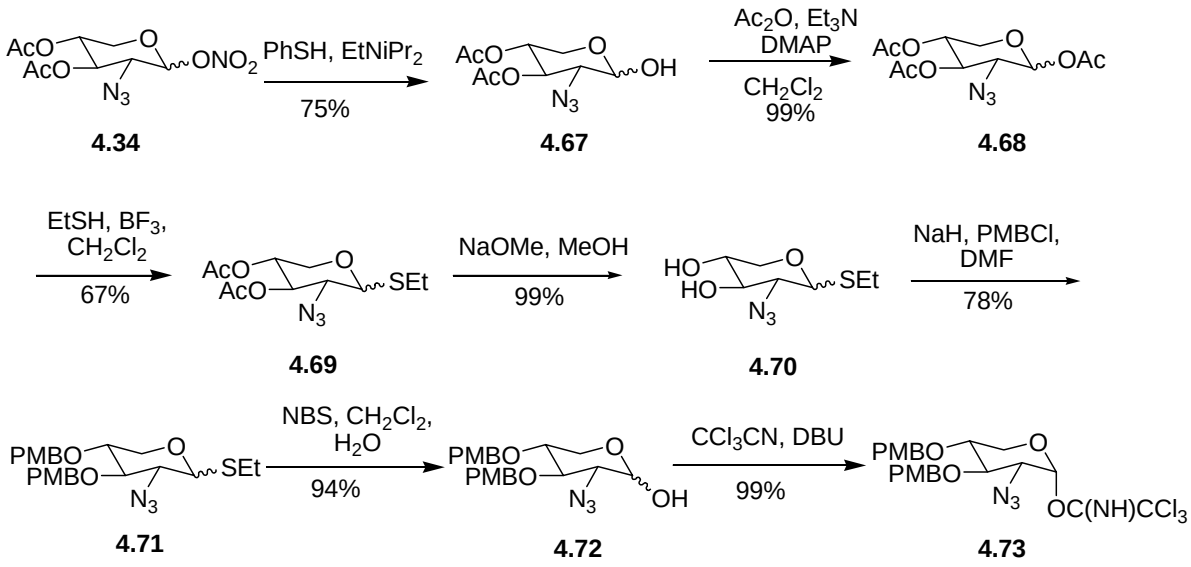


Scheme **4.22** Structure study of receptor **4.45** and donor **4.3**

The failed glycosylation between compound **4.45** and donor **4.3** was devastating. We had to think really hard about the reasons behind this failed glycosylation. We thought there might be three possibilities (Scheme 4.22). First of all, it is possible that the Pht protecting group at C-2 position of the donor **4.3** was too sterically bulky to prevent the reaction between donor **4.3** and compound **4.45**, which was also quite sterically hindered due to the presence of a TES group on the neighboring alcohol. To solve this problem, we could avoid the Phthaloyl protecting group at C-2 position of donor **4.3**. The second reason might be that donor **4.3** needed to be armed to facilitate the glycosylation. Therefore, it might be necessary to use PMB protecting groups to replace TES protecting groups. The third reason might be simply due to the neighboring bulky TES group in the alcohol **4.45** blocking the donor **4.3** completely. However, we know that the TES group at the same position didn't pose any problem in our synthesis of the disaccharide of OSW-1. Therefore, we decided to modify the xylose donor first.

4.4.8 Synthesis of armed D-xylosamine donor

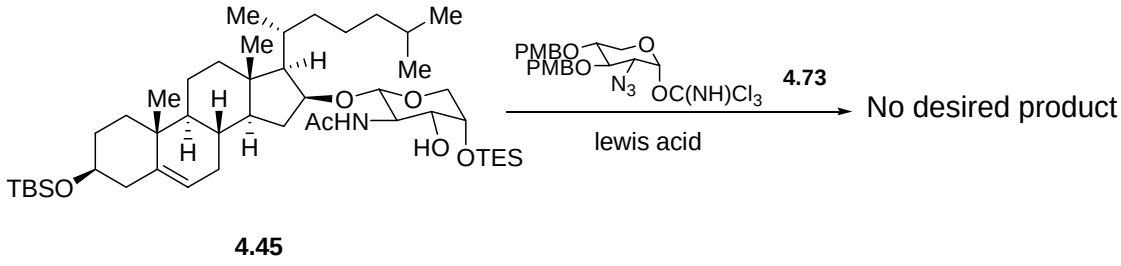
In order to reduce the bulkiness of the Phthaloyl group in compound **4.3**, an azide substituted xylose donor would be employed. The preparation of modified xylose **4.73** was carried out in a concise and straightforward manner shown in Scheme 4.23. PhSH and EtN*i*-Pr_2 were used to cleave the nitrite to provide hemiacetal **4.67**, followed by acetylation to afford **4.68**. The acetylated compound **4.68** was treated with ethylthiol and boron trifluoride-ethyl ether complex to give the thioglycoside **4.69**.[36] Hydrolysis followed by the protection by PMB groups under basic conditions afforded compound **4.71**. NBS was used to cleave the carbon-sulfide bond followed by CCl_3CN activation to provide armed-donor **4.73**. With this synthetic approach, compound **4.73** was successfully synthesized in 7 steps with 36% overall yield.



Scheme **4.23** Synthesis of armed amino-xylose donor **4.73**

4.4.9 The attempted glycosylation between the compound **4.45** and armed donor **4.73**

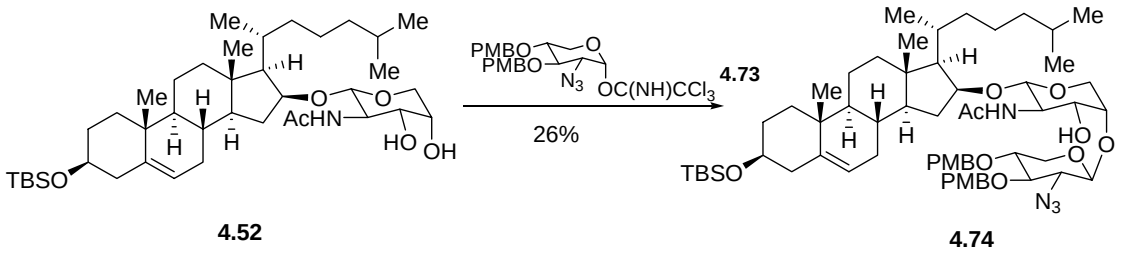
The armed azido-xylose doner **4.73** was employed in the glycosylation (scheme 4.24). After a lot of different conditions were carefully investigated, unfortunately no desired product was isolated. This result showed our assumption that the bulkiness of xylosamine **4.3** was the key reason for the failure of the requisite glycosylation was incorrect. Therefore, the difficulty of this glycosylation should be caused by the bulkiness of the TES group in compound **4.45**.



4.45

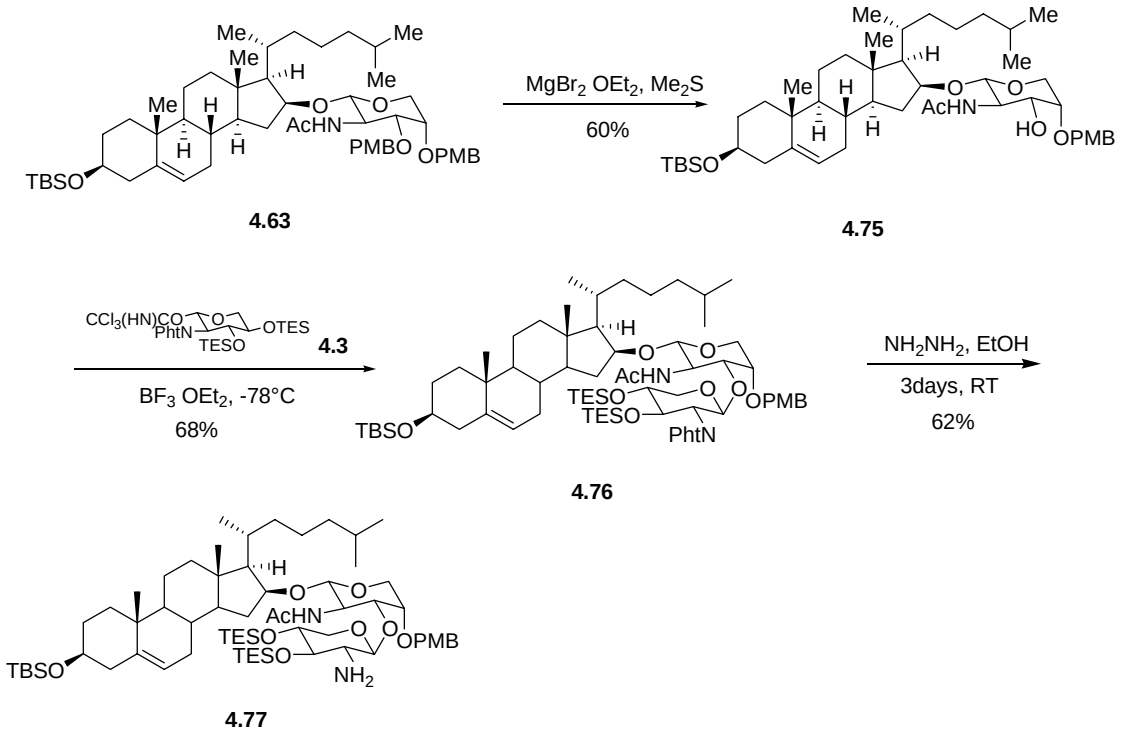
Scheme **4.24** Glycosylation with armed saccharide component

To prove the difficulty of the glycosylation was caused by the bulkiness of the TES group in compound **4.45**, diol **4.52** was employed in the glycosylation (Scheme 4.25). The reaction was not very clean, but product **4.74** was isolated in 26% yield. This result indicated that without the bulky TES group, the glycosylation could occur except the C-4 hydroxyl group was more reactive than C-3. To successfully carry out the requisite glycosylation, the C-4 hydroxy group was still needed to be protected, but with a smaller group than TES group.



Scheme **4.25** Glycosylation of diol **4.52**

4.4.10 Synthesis of the amino **4.77**

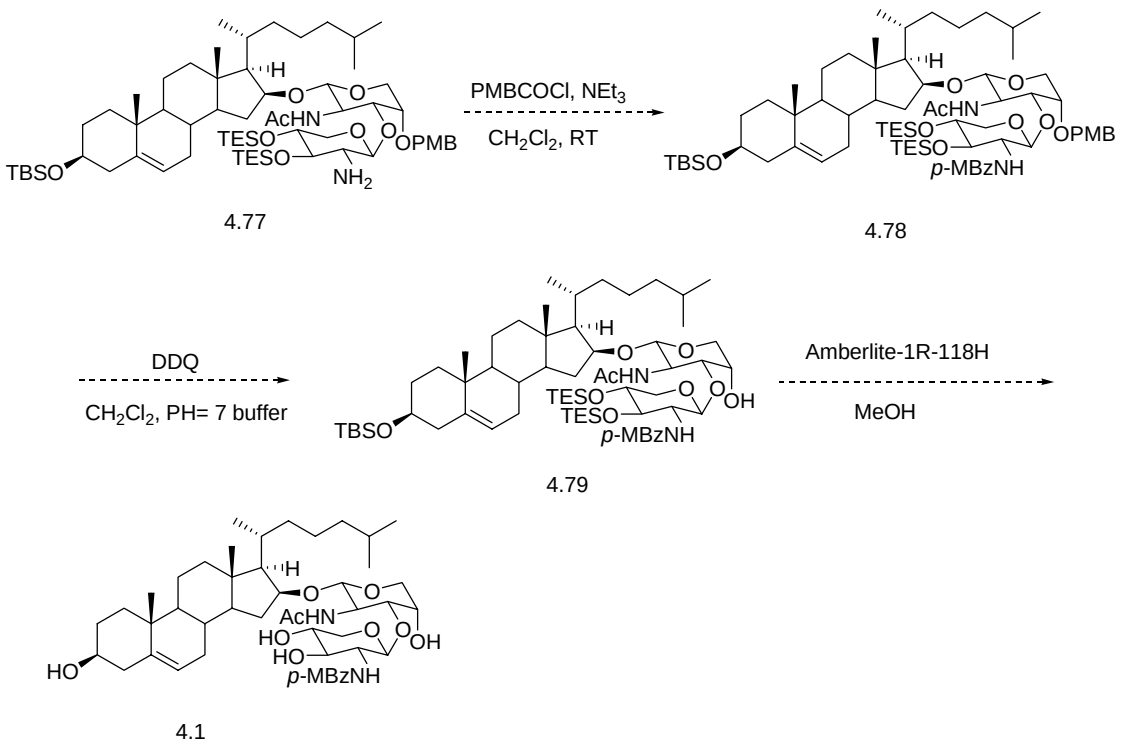


Scheme **4.26** synthesis of amino **4.77**

We were able to optimize the $MgBr_2$-Et_2O-Me_2S mediated deprotection so that only the PMB group attached to the C-3 position was regioselectively cleaved. The yield of this reaction was 60%.

As expected, the glycosylation between compound **4.75** and donor **4.3** provided desired disaccharide **4.77** with the desired stereochemistry, and the convertion yield was around 68% based on the recovery of 50% of the starting material **4.75**. This result confirmed that the steric hindrance of the TES group in **4.45** was the major reason for the failure of the glycosylation. Hydrazine was used to remove the phthaloyl group to give amine **4.77** (Scheme 4.26)

4.4.11 Future plan of synthesis of amino analog **4.1**



Scheme **4.27** Future plan of synthesis of amino analog **4.1**

Amine **4.77** will be converted to amide **4.78**. After the deprotection of two PMB groups by DDQ in buffer, all silyl groups will be cleaved by Amberlite-1R-118H, and the target molecule **4.1** will be successfully synthesized (Scheme 4.27).

4.5 Conclusion

In conclusion, amine **4.77** was successfully synthesized. And target amino analog **4.1** will be successfully synthesized in 3 steps soon. It should be noted that the synthetic strategy was quite different from that of OSW-1, and more study is needed to further optimize the synthetic procedures involved in this approach so that it will provide a sufficient amount of the material for biological testing.

4.6 Experimental section

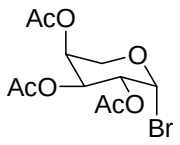
4.6.1 General

Reagents were all purchased from the following companies and were used as received unless indicated: Aldrich Chemical Company, Fluka, Fisher Scientific and ACROS Organics. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled over sodium benzophenone ketyl; benzene, toluene, methylene chloride (CH_2Cl_2) and acetonitrile (CH_3CN) were distilled over calcium hydride. Methanol (CH_3OH), dimethylsulfoxide (DMSO) and dimethylformamide (DMF) were purchased from Aldrich Company in anhydrous form and used as received. Acetone, ethyl acetate (EtOAc) and hexanes were purchased from Fisher Company and used as received. ^{n}BuLi and ^{t}BuLi were titrated by diphenyl acetic acid prior to use. Sodium sulfate (Na_2SO_4),

magnesium sulfate ($MgSO_4$) and potassium carbonate (K_2CO_3) were purchased from Fisher Company as anhydrous forms and used as received.

All moisture sensitive reactions were performed in flame-dried glassware under a positive pressure of nitrogen or argon. All reactions were monitored by thin layer chromatography (TLC) with co-spots of the starting material(s). Products were isolated or purified by flash column chromatography, preparative TLC, or distillation under reduced pressure. Optical rotations were measured with Jasco P-1020 polarimeter. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with Bruker Avance 300 (300 MHz) instrument, Bruker WM360 (360 MHz) instrument, or Bruker DRX400 (400 MHz) instrument. The deuterated solvents for NMR spectroscopy were chloroform-d_1 ($CDCl_3$), benzene-d_6 (C_6D_6) or water-d_2 (D_2O), and are reported in parts per million (ppm) with residual protonated solvent peak or solvent ^{13}C-NMR peak as internal standard ($CDCl_3$: 7.26 ppm for ^{1}H-NMR and 77.0 ppm for ^{13}C-NMR; C_6D_6: 7.15 ppm for ^{1}H-NMR and 128.0 ppm for ^{13}C-NMR; When peak multiplicity is reported, the following abbreviations are used: s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet), m (multiplet).

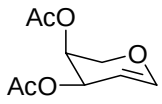
4.6.2 Experimental procedures and spectral data



4.15

Dry 1,2,3,4-*O* tetraacetyl-L-arabinose (985 mg, 3.1mmol) was dissolved in dry CH_2Cl_2 and was cooled to 0 ^oC. HBr (30% in AcOH, 2.3mL, 8.7 mmol) was added

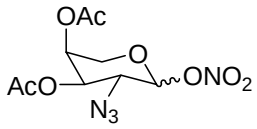
slowly by additional funnel. The reaction mixture was stirred at 0 ^{o}C for one hour and at 25 ^{o}C for three hours. The reaction solution was washed with water (5 mL) first, then the organic layer was poured into cold saturated aqueous $NaHCO_3$ with stirring. The organic layer was separated, and the water layer was extracted with CH_2Cl_2 (5 mL) three times. The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed to afford **4.15** (953 mg, 91%) as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 6.60 (d, J = 3.6 Hz, 1H), 5.30 (m, 2H), 4.97 (m, 1H), 4.11 (d, J = 13.2 Hz, 1H), 3.83 (d, J = 13.2 Hz, 1H), 2.07 (s, 3H), 2.05 (s, 3H), 1.95 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 169.8, 169.7, 169.6, 89.6, 67.9, 67.8, 67.6, 64.7, 20.8, 20.7, 20.6.



4.25

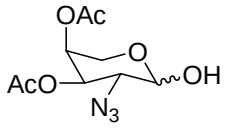
$CuSO_4.5H_2O$ (20 g) was dissolved in water (200 mL). And zinc (100 g) was added. The mixture was stirred at room temperature for 20 mins, then filtered. The solid was washed with water (3 x 100 mL) and 200 proved ethanol (2 x 100 ml) to provide dust containing 5% Zn-Cu (100 g). Pyranosyl bromide **4.15** (50g, 0.148 mol) and zinc dust containing 5% Zn-Cu (100 g) was each divided into 5 equal portions. Portions of **4.15** and zinc mixtures were added to a mechanically stirred solution of 70% acetic acid (200 mL) kept between – 10 ^{o}C and - 5 ^{o}C at 15 minutes intervals. After the last portions of reagents were added, stirring was continued until **4.15** was consumed (30 min).The reaction mixture was filtered and the zinc pellet was rapidly washed with CH_2Cl_2 (500 mL). The zinc was then immediately quenched with cold water. The filtrate was extracted with CH_2Cl_2 (3 x 200 mL). The combined organic layers were then washed with water (3

x 200 mL), with saturated $NaHCO_3$ (300 mL), with brine (300 mL), and dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded crude **4.25**. Purification by silica gel chromatography eluting with Hexane: Ethyl Acetate (10 : 1) afforded 23.6 g of **4.25** as a clear oil (80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 6.40 (d, J = 6 Hz, 1H), 5.34 (t, J = 4.7 Hz, 1H), 5.09 (m, 1H), 4.75 (t, J = 5.6 Hz, 1H), 3.91 (m, 2H), 1.98(s, 3H), 1.97 (s, 3H): ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 170.6, 170.0,150.0, 97.7, 66.2, 63.1, 63.0, 21.3, 21.0.



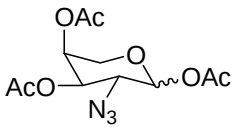
4.26

The compound **4.25** (8 g, 40 mmol) was dissolved in dry CH_3CN (250 mL) and was cooled to - 20 ^{o}C. Sodium azide (6 g, 91.9 mmol) and CAN (94 g, 172 mmol) were added to the mechanically stirred solution of **4.25**. The reaction flask was equipped with a drying tube. After 6 hours at - 20 ^{o}C, the reaction mixture was poured into 200 mL water and 300 mL ethyl ether. The organic layer was separated and the water layer was extracted by ethyl ether (100 mL) three times. The combined organic layer was washed with water, brine and dried over $MgSO_4$. The solvent was removed to afford crude **4.26** as a clear oil (9.4 g, 81%).



4.53

The compound **4.26** (130 mg, 0.043 mmol) was dissolved in dry CH_3CN (2 mL) and was cooled to 25 ^oC. After *i*-Pr_2NEt (131 μL, 0.128 mmol) and PhSH(76 μL, 0.043 mmol) were added to the reaction mixture, stirring was continued until **4.26** was consumed (3 hours). The solvent was removed by reduced pressure. The product was purified by silica gel column chromatography (Hexane:EtOAc 3:1) to afford **4.53** (90.1 mg, 82% yield) as a clear oil. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 5.31 (overlap, 2H), 5.24 (m, 1H), 5.11 (m, 1H), 4.72 (dd, J = 12.6, 4.2 Hz, 1H), 4.52 (m, 1H), 4.10 (d, J = 13.2 Hz, 1H), 3.92 (dd, J = 13.2, 3.0 Hz, 1H), 3.69 (dd, J = 11.7, 3.0, Hz, 1H), 3.59 (overlap, 3H), 3.38 (d, J = 8.1 Hz, 1H), 2.81 (d, J = 4.2 Hz, 1H), 2.08(s, 3H), 2.07 (s, 3H), 1.99 (s, 6H): ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 170.1, 169.8, 96.8, 92.5, 70.9, 68.8, 68.0, 67.3, 64.5, 62.3, 60.5, 20.8, 20.7, 20.5, 20.4



4.55

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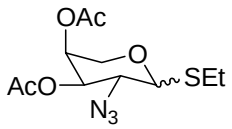
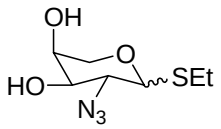
To a solution of **4.53** (100 mg, 0.386 mmol) and triethylamine (53.8 μL, 0.579 mmol) in CH_2Cl_2 (2 mL) was added Ac_2O (51.2 mg, 0.502 mmol) and DMAP (4.7 mg, 0.038 mmol). The reaction was stirred at 25 ^oC for 3 hours until the compound **4.53** was completely consumed. The reaction was then quenched with saturated aqueous $NaHCO_3$. The organic layer was separated, and the water layer was extracted with CH_2Cl_2 (2 mL) three times. The combined organic layer was washed with water, brine and dried over Na_2SO_4. The solvent was removed and the product was purified by silica gel column chromatography (Hexane: EtOAc 3:1) to afford **4.55** (117.1 mg, 99% yield) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 6.18 (d, J = 3.6 Hz, 1H), 5.36 (d, J = 7.6 Hz, 2H), 5.26 - 5.16 (overlap, 4H), 4.78 (dd, J = 10.5, 3.0 Hz, 2H), 3.99 – 3.6 (overlap, 9H), 2.06 – 1.91 (overlap, 27H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 170.3, 170.2, 170.1, 169.9, 169.1, 169.0, 93.6, 91.1, 71.2, 68.5, 68.1, 67.3, 65.4, 62.9, 60.1, 57.3, 21.1, 21.1, 20.9, 20.8.



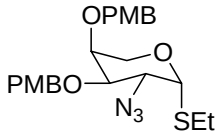
4.56

A solution of **4.55** (90.0 mg, 0.31 mmol), EtSH (36 μL, 0.56 mmol) and dry 4Å MS powder (120 mg) in anhydrous CH_2Cl_2 (1.5 mL) was stirred at 25 °C for 15 min, then the $BF_3{\cdot}OEt_2$ (0.1 M in CH_2Cl_2, 310 μL, 0.031 mmol) was added dropwise. The reaction mixture was stirred at 25 °C for 3 hours and quenched with 0.15 mL Et_3N. The solid was filtered, and the solvent was removed. The crude product was purified by silica gel column chromatography (Hexane:EtOAc 4:1) to afford **4.56** (70.7 mg, 78% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 5.34 (d, J = 5.1 Hz, 1H), 5.21 (m, 1H), 5.16 (m, 1H), 5.01 (dd, J = 10.8, 3.3 Hz, 1H), 4.78 (dd, J = 10.5, 3.0 Hz, 1H), 4.26 – 4.13 (overlap, 3H), 3.97 (dd, J = 13.2, 2.4 Hz, 1H), 3.68 – 3.51 (overlap, 3H), 2.70 (m, 2H), 2.55 (m, 1H), 2.06 (overlap, 6H), 2.00 (s, 3H), 1.95 (s, 3H), 1.21 (overlap, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 170.4, 170.3, 170.0, 169.8, 85.2, 83.8, 72.7, 70.0, 68.5, 67.8, 61.2, 60.9, 58.7, 25.2, 24.8, 21.1, 20.93, 20.9, 15.1, 14.8.



4.57

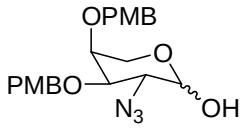
The compound **4.56** (70.7 mg, 0.24 mmol) was dissolved in MeOH (1 mL) and sodium methoxide (1.3 mg, 0.024 mmol) was added. The reaction was stirred at room temperature for 4 hours. The solvent was removed and the product was isolated by silica gel column chromatography (Hexane:EtOAc 1:2) to afford **4.57** as a clear oil (52.6 mg, 99%). α – arabinose: ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 4.18 (m, 1H), 3.98 (dd, J = 12.6, 2.4 Hz, 1H), 3.87 (broad, 1H), 3.46 (overlap, 3H), 2.67 (m, 2H), 1.20 (m, 3H); ^{13}C NMR (75MHz, $CDCl_3$): δ (ppm) 84.9, 73.5, 69.7, 68.1, 64.2, 24.9, 15.2; β – arabinose: ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 5.39 (d, J = 4.8 Hz, 1H), 4.20 (dd, J = 12.9, 1.5 Hz, 1H), 4.04 (dd, J = 9.6, 2.1 Hz, 1H), 3.97 (broad, 1H), 3.83 (dd, J = 9.6, 3.3 Hz, 1H), 3.72 (dd, J = 12.6, 2.4 Hz, 1H), 2.63 (m, 2H), 1.27 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 83.4, 69.4, 68.4, 62.9, 61.4, 24.7, 14.7.



4.58

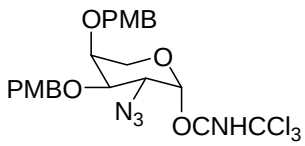
To a solution of **4.57** (26 mg, 0.119 mmol) in dry DMF (1 mL) was added NaH (60% in mineral oil, 12 mg, 0.392 mmol) at 0 ^{o}C. The reaction solution was stirred at 25 ^{o}C for 10 min. Then, *p*-methoxybenzyl chloride (36 μL, 0.261 mmol) and tetrabutylammonium iodide (1 mg, 0.003 mmol) were added. The reaction was stirred at room temperature for 12 hours and quenched with saturated aqueous $NaHCO_3$. The

organic layer was separated and the water layer was extracted with ethyl acetate (5 ml) three times. The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed and the product was purified by silica gel column chromatography (Hexane:EtOAc 4:1) to afford **4.58** as a clear oil (46.4 mg, 85%).[1]H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.17 (m, 4H), 6.77 (m, 4H), 5.22 (d, J = 4.8 Hz, 1H), 4.47 (d, J = 17.1 Hz, 4H), 4.12 (m, 1H), 3.85 (m, 1H), 3.71 (overlap, 6H), 3.62 (overlap, 3H) 2.51 (m, 2H), 1.17 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 130.2, 129.9, 129.7, 114.0, 83.8, 72.0, 71.9, 71.5, 61.5, 60.8, 55.5, 24.9, 15.0.



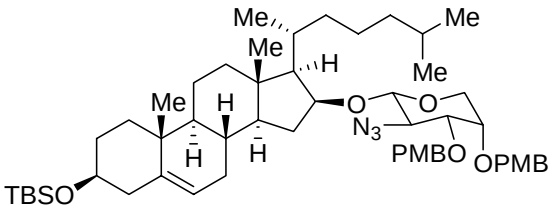
4.59

To a solution of **4.58** (188.2 mg, 0.41 mmol) in CH_2Cl_2 (2.5 mL) and water (0.3 mL) was added NBS (79.5 mg, 0.446 mmol) in one portion. After the reaction was stirred at room temperature for 1 hour, saturated aqueous Na_2SO_3 was added. The organic layer was separated and the water layer was extracted with CH_2Cl_2 (5 ml) three times. The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed and the product was purified by silica gel column chromatography (Hexane:EtOAc 5:1) to afford **4.59** as a clear oil (159.9 mg, 94%).



4.60

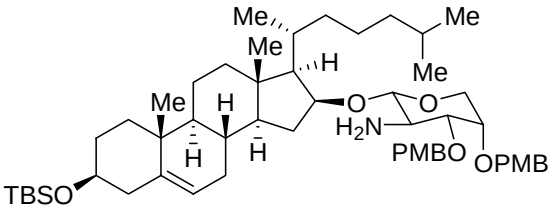
Compound **4.59** (159.9 mg, 0.39 mmol) was dried by benzene and dissolved in dry CH_2Cl_2 (2 mL). Then, trichloroacetonitrile (0.2 mL, 1.97 mmol) and DBU (1 drop) were added. The reaction was stirred at 25 ^oC for 1 hour. The solvent was removed and the product was purified by Et_3N deactived silica gel column chromatography (Hexane: EtOAc 5:1) to afford **4.60** as a clear oil (217.6 mg, 99%), which was used without identification.



4.61

A solution of **3.8** (68.0 mg, 0.132 mmol), **4.60** (110.5 mg, 0.198 mmol) and dry 4Å MS powder (120 mg) in anhydrous CH_2Cl_2 (1.5 mL) was stirred at 25 °C for 15 min. Then the reaction mixture was cooled to -20 °C and TMSOTf (0.02 M in CH_2Cl_2, 235 μL, 0.0047 mmol) was added dropwise. The reaction mixture was stirred at -20 °C for 30 min and quenched with 0.15 mL Et_3N. The solid was filtered and the solvent was removed. The product was purified by silica gel column chromatography (Hexane:EtOAc 40:1) to afford **4.61** (113.4 mg, 94% yield) as a form solid; ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.17 (m, 4H), 6.76 (d, J = 8.4 Hz, 4H), 5.20 (m, 1H), 4.60 – 4.38 (overlap, 4H), 3.87 (overlap, 3H), 3.71 (s, 6H), 3.61 (dd, J = 7.8, 9.9 Hz, 1H), 3.47 (broad, 1H), 3.38 (m, 1H), 3.13 (dd, J = 3.3, 9.9 Hz, 1H), 3.06 (d, J = 12.6 Hz, 1H), 2.16 (m, 4H), 1.81-1.56 (overlap, 9H), 1.53-1.24 (overlap, 9H), 1.25-0.82 (overlap, 25H), -0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 141.8, 130.5, 130.0. 129.8, 129.6, 121.2, 114.0, 113.99,

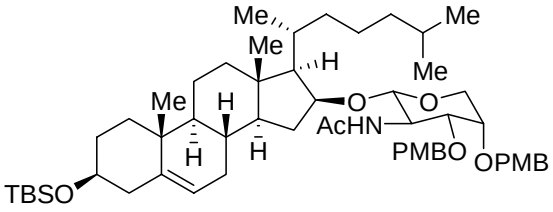
104.4, 82.5, 78.9, 72.9, 71.7, 71.2, 70.9, 64.1, 63.7, 62.0, 55.49, 55.46, 55.3, 50.5, 43.3, 42.5, 40.2, 40.0, 37.6, 36.8, 36.6, 36.5, 32.3, 32.1, 31.6, 30.2, 29.9, 29.6, 28.4, 26.2, 24.2, 23.2, 22.9, 22.8, 21.1, 19.6, 18.5, 18.2, 13.0, -4.4; $[\alpha]_D^{25}$ -11.24 (*c* 2.9, $CHCl_3$). $C_{54}H_{83}N_3O_7Si$, HRMS (m/z) 913.6346; Calculated MS: 913.6000.



4.62

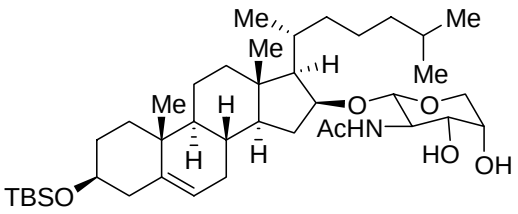
To a solution of **4.61** (1.1 g, 1.20 mmol) in dry THF (25 mL) was added L-selectride (1 M in THF, 4.8 mg 4.8 mmol) in one portion. After the reaction was stirred at room temperature for 3 hours, it was quenched with ethyl acetate (5 mL). Water (50 mL) was added. The organic layer was separated and the water layer was extracted with ethyl acetate (3 × 20 mL). The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed and the crude product was purified by silica gel column chromatography (Hexane:EtOAc 2:1) to afford **4.62** as a form soild (959 mg, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.17 (m, 4H), 6.75 (d, J = 8.4 Hz, 4H), 5.20 (m, 1H), 4.61 – 4.20 (m, 4H), 3.94 (overlap, 3H), 3.70 (s, 3H), 3.69 (s, 3), 3.41 (broad, 1H), 3.37 (m, 1H), 3.21 (m, 3H), 2.16 (m, 4H), 1.81-1.56 (overlap, 9H), 1.53-1.24 (overlap, 9H), 1.25-0.82 (overlap, 25H), -0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 141.8, 130.7, 130.4. 129.8, 129.6, 121.3, 114.0, 113.9, 107.1, 82.2, 81.2,72.9, 71.0, 70.1, 63.7, 61.6, 55.5, 55.2, 53.7, 50.5, 43.0, 42.4, 40.0, 37.5, 36.8, 36.4, 36.1, 32.3, 32.1,

32.0, 31.6, 30.2, 29.9, 29.6, 28.4, 26.2, 24.1, 23.1, 22.9, 22.7, 21.1, 19.6, 18.5, 18.3, 13.2, -4.4; $[\alpha]_D^{25}$ +7.7 (*c* 1.5, $CHCl_3$).



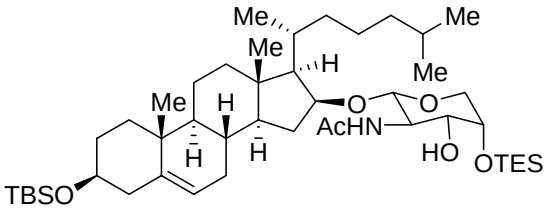
4.63

To a solution of **4.62** (342.8 mg, 0.386 mmol) in CH_2Cl_2 (3 mL) was added Ac_2O (51.2 mg, 0.502 mmol) and triethylamine (53.8 μL, 0.579 mmol). After the reaction mixture was stirred at 25 ^oC for 3 hours, it was quenched with saturated aqueous $NaHCO_3$. The organic layer was separated and the water layer was extracted with CH_2Cl_2 (3 mL) three times. The combined organic layer was washed with water, brine and dried over Na_2SO_4. The solvent was removed and the crude product was purified by silica gel column chromatography (Hexane: EtOAc 3:1) to afford **4.63** as a form solid (358.1 mg, 99% yield). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.23 (d, J = 7.2 Hz, 2H), 7.07 (d, J = 6.9 Hz, 2H), 6.75 (t, J = 8.7 Hz, 4H), 5.38 (d, J = 8.1 Hz, 1H), 5.20 (m, 1H), 4.55 – 4.22 (overlap, 5H), 4.01 - 3.78 (overlap, 4H), 3.70 (s, 3H), 3.69 (s, 3), 3.46 (broad, 1H), 3.37 – 3.29 (overlap, 2H), 2.16 (overlap, 7H), 1.81-1.56 (overlap, 9H), 1.53-1.24 (overlap, 9H), 1.25-0.82 (overlap, 25H), -0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 169.4, 141.8, 130.6, 130.3. 130.1, 129.6, 124.3, 121.2, 113.9, 113.8, 102.1, 82.2, 81.2,72.9, 72.6, 70.8, 70.1, 60.5, 60.2, 55.5, 55.2, 51.2, 50.4, 43.0, 42.4, 40.1, 39.9, 37.5, 36.8, 36.5, 35.5, 32.3, 32.1, 32.0, 31.7, 30.1, 29.9, 29.6, 28.3, 26.2, 24.1, 23.7, 23.0, 22.9, 22.8, 21.0, 19.6, 18.5, 18.4, 12.3, -4.4; $[\alpha]_D^{25}$ -34.4 (*c* 1.5, $CHCl_3$).



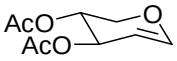
4.52

The compound **4.63** (372 mg, 0.4 mmol) was dissolved in dry CH_2Cl_2 and dimethyl sulfide (1:1, 20 mL). $MgBr_2 \cdot Et_2O$ (530.4 mg, 1.2 mmol) was added to the reaction mixture. After the reaction mixture was refluxed for 12 hours, it was quenched with saturated aqueous $NaHCO_3$. The organic layer was separated and the water layer was extracted with CH_2Cl_2 (10 mL) three times. The combined organic layer was washed with water, brine and dried over Na_2SO_4. The solvent was removed and the crude product was purified by silica gel column chromatography (Hexane : EtOAc 1:2) to afford **4.52** as a form solid (240.1 mg, 87% yield). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 5.59 (d, J = 5.4 Hz, 1H), 5.21 (m, 1H), 4.24 (d, J = 4.8 Hz, 1H), 3.95 - 3.78 (overlap, 4H), 3.69 (broad, 3H), 3.48 (m, 1H), 3.37 (m, 1H), 2.88 (m, 1H), 2.16 (overlap, 7H), 1.81-1.56 (overlap, 9H), 1.53-1.24 (overlap, 9H), 1.25-0.82 (overlap, 25H), -0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 171.6, 141.8, 121.0, 102.4, 83.1, 72.8, 71.3, 65.9, 63.4, 61.1, 55.1, 54.4, 53.1, 50.4, 43.0, 43.4, 40.1, 39.7, 37.5, 36.8, 36.6, 36.0, 32.3, 31.9, 31.6, 30.5, 29.9, 28.3, 26.1, 23.9, 23.6, 23.0, 22.8, 21.3, 19.6, 18.5, 18.3, 13.8,13.1, -4.4; $[\alpha]_D^{25}$ -11.4 (*c* 1.3, $CHCl_3$).



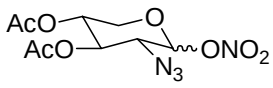
4.45

To a solution of **4.52** (22 mg, 0.033 mmol) in dry CH_2Cl_2 (1 mL) was added 2,6-lutidine (6 μL, 0.05 mmol) at -78 ^oC. The reaction solution was stirred at -78 ^oC for 10 min and TESCl (6.9 μL, 0.033 mmol) was added. The reaction was stirred at -78 ^oC for 0.5 hours and quenched with saturated aqueous NH_4Cl. The organic layer was separated and the water layer was extracted with ethyl acetate (1 ml) three times. The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed and the crude product was purified by silica gel column chromatography (Hexane:EtOAc 4:1) to afford **4.45** as a form solid (15.8 mg, 71%). ^1H NMR (300 MHz, C_6D_6): δ (ppm) 5.36 (d, J = 4.8 Hz, 1H), 5.15 (d, J = 6.9 Hz, 1H), 4.45 (d, J = 4.8 Hz, 1H), 4.28 (m, 1H), 4.05 - 3.95 (overlap, 3H), 3.81 (m, 2H), 3.64 (m, 1H), 3.40 (dd, J = 11.7, 3.0 Hz, 1H), 2.52 -2.41 (overlap, 2H), 2.20 – 1.61 (overlap, 10H), 1.57 (s, 3H), 1.51-1.20 (overlap, 12H), 1.06-0.87 (overlap, 36H), 0.62 (m, 6H), 0.13 (s, 3H), 0.12 (s, 3); ^{13}C NMR (75MHz, C_6D_6): δ (ppm) 169.7, 141.6, 121.4, 103.0, 82.4, 72.9, 71.5, 68.4, 64.0, 61.5, 55.1, 54.7, 50.6, 43.5, 42.4, 40.4, 40.0, 37.7, 36.8, 36.2, 32.7, 32.2, 31.7, 30.5, 28.6, 26.1, 24.4, 23.1, 23.0, 22.8, 21.1, 19.5, 18.42, 18.35,13.1, 7.1, 5.4 -4.4; $[\alpha]_D^{25}$ -5.78 (*c* 1.3, $CHCl_3$).



4.33

$CuSO_4.5H_2O$ (20 g) was dissolved in water (200 mL). And zinc (100 g) was added. The mixture was stirred at room temperature for 20 mins, then, filtered. The solid was washed with water (3 x 100 mL) and absolute ethanol (2 x 100 ml) to provide dust containing 5% Zn-Cu (100 g). Pyranosyl bromide **4.32** (50g, 0.148 mol) and zinc dust containing 5% Zn-Cu (100 g) was each divided into 5 equal portions. Portions of the **4.32** and zinc mixture were added to a mechanically stirred solution of 70% acetic acid (200 mL) kept between – 10 ^oC and - 5 ^oC at 15 minutes intervals. After the last portions of reagents were added, stirring was continued until **4.32** was consumed (30 min).The reaction mixture was filtered and the zinc pellet was rapidly washed with CH_2Cl_2 (500 mL). The zinc was then immediately quenched with cold water. The filtrate was extracted with CH_2Cl_2 (3 x 200 mL). The combined organic layers were then washed with water (3 x 200 mL), with saturated $NaHCO_3$ (300 mL), with brine (300 mL), and they were then dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded crude **4.33**. Purification by silica gel chromatography eluting with Hexane: Ethyl Acetate (10 : 1) afforded 24.2g of **4.33** as a clear oil (82%). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 6.58 (d, J = 5.7 Hz, 1H), 4.95 (overlap, 3H), 4.17 (m, 1H), 3.96 (dd, J = 12.3, 1.8 Hz, 1H), 2.08(s, 3H), 2.05 (s, 3H): ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 169.9, 169.7, 148.0, 97.4, 67.2, 63.6, 63.4, 21.2, 21.0

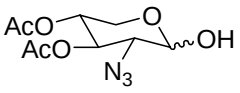


4.34

.

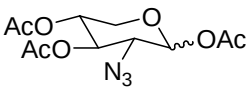
The compound **4.33** (8 g, 40 mmol) was dissolved in dry CH_3CN (250 mL) and was cooled to - 20 ^oC. Sodium azide (6 g, 91.9 mmol) and CAN (94 g, 172 mmol) were

added to a mechanically stirred solution of **4.33**. The reaction flask was equipped with drying tube. After 6 hours at - 20 ^{o}C, the reaction mixture was poured into 200 mL water and 300 mL ethyl ether. The organic layer was separated and the water layer was extracted with ethyl ether (100 mL) three times. The combined organic layer was washed with water, brine and dried over $MgSO_4$. The solvent was removed to afford crude **4.34** as a clear oil (9.9 g, 85%).



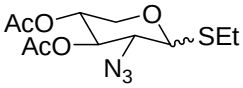
4.67

The compound **4.34** (130 mg, 0.043 mmol) was dissolved in dry CH_3CN (2 mL) and was cooled to 25 ^{o}C. After *i*-Pr_2NEt (131 μL, 0.128 mmol) and PhSH(76 μL, 0.043 mmol) were added to the reaction mixture, stirring was continued until **4.34** was consumed (3 hours). The solvent was removed by reduced pressure. The product was isolated by silica gel column chromatography (Hexane: EtOAc 3:1) to afford **4.67** as a clear oil (82.3 mg, 75% yield) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 5.51 (t, J = 9.6 Hz, 1H), 5.32 (d, J = 3.2 Hz, 1H), 5.05 - 4.91 (overlap, 3H), 4.66 (d, J = 8 Hz, 1H), 4.07 (q, J = 5.6, 11.7 Hz, 1H), 3.92 – 3.78 (overlap, 4H), 3.43 (q, J = 8.0, 9.6 Hz, 1H), 3.33 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 171.3, 171.23, 171.22, 171.19, 97.8, 93.2, 74.1, 91.0, 70.3, 70.1, 65.8, 63.9, 62.6, 59.8, 21.83, 21.81, 21.77, 21.71.



4.68

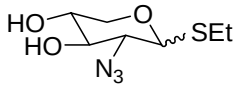
To a solution of **4.67** (100 mg, 0.386 mmol) and triethylamine (53.8 μL, 0.579 mmol) in CH_2Cl_2 (2 mL) was added Ac_2O (51.2 mg, 0.502 mmol) and DMAP (4.7 mg, 0.038 mmol). The reaction was stirred at 25 ^oC for 3 hours. The reaction mixture was quenched with saturated aqueous $NaHCO_3$. The organic layer was separated and the water layer was extracted with CH_2Cl_2 (2 mL) three times. The combined organic layer was washed with water, brine and dried over Na_2SO_4. The solvent was removed and the product was isolated by silica gel column chromatography (Hexane: EtOAc 3:1) to afford **4.68** as a clear oil (117.1 mg, 99% yield). ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 5.50 (d, J = 8.0 Hz, 1H), 5.08 (t, J = 9.6 Hz, 1H), 4.95 (m, 1H), 4.08 (dd, J = 17.2, 5.6 Hz, 1H), 3.59 (dd, J = 8.4, 9.8 Hz, 1H), 3.44 (dd, J = 9.8, 11.8 Hz, 1H), 2.17 (s, 3H), 2.10 (s, 3H), 2.02 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 170.9, 170.7, 169.7, 94.2, 72.9, 69.6, 64.4, 63.1, 21.9, 21.7, 21.66.



4.69

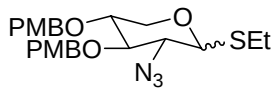
A solution of **4.68** (90.0 mg, 0.31 mmol), PhSH (36 μL, 0.56 mmol) and dry 4Å MS powder (120 mg) in anhydrous CH_2Cl_2 (1.5 mL) was stirred at 25 °C for 15 min, then the $BF_3{\cdot}OEt_2$ (0.1 M in CH_2Cl_2, 310 μL, 0.031 mmol) was added dropwise. The reaction mixture was stirred at 25 °C for 3 hours and quenched with 0.15 mL Et_3N. The solid was filtered and the solvent was removed. The crude product was purified by silica gel column chromatography (Hexane: EtOAc 4:1) to afford **4.69** (60.7 mg, 67% yield) as colorless oil. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 5.33 (d, J = 5.2 Hz, 1H), 5.27 (t, J = 9.2 Hz, 1H), 5.07 (t, J = 9.0 Hz, 1H), 4.95 (overlap, 2H), 4.41 (d, J = 9.4 Hz, 1H), 4.17

(dd, J = 5.2, 11.6 Hz, 1H), 4.17 (dd, J = 9.8, 11.4 Hz, 1H), 3.85 (m, 2H), 3.46 (dd, J = 9.2 Hz, 1H), 3.35 (q, J = 9.5, 11.6 Hz, 1H), 2.76 (m, 2H), 2.64 (m, 2H), 2.12 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.31 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 171.0, 170.8, 170.7, 170.5, 85.8, 84.4, 74.5, 72.2, 70.3, 69.9, 67.1, 64.5, 62.5, 60.9, 25.9, 25.8, 21.65, 21.63, 21.61, 21.57, 15.0, 15.7.



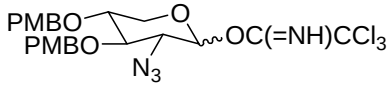
4.70

The compound **4.69** (70.7 mg, 0.24 mmol) was dissolved in MeOH (1 mL) and sodium methoxide (1.3 mg, 0.024 mmol) was added. The reaction was stirred at room temperature for 4 hours. The solvent was removed and the crude product was purified by silica gel column chromatography (Hexane: EtOAc 1:2) to afford **4.70** as a clear oil (52.6 mg, 99%).α – xylose: ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 5.24 (d, J = 3.9 Hz, 1H), 3.95 (broad, 1H), 3.83 (t, J = 14.1 Hz, 1H), 3.61 (overlap, 5H), 2.51 (m, 2H), 1.20 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 83.8, 73.8, 70.8, 63.9, 62.2, 25.0, 15.0; β –xylose: ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 4.21 (d, J = 9.9 Hz, 1H), 3.97 (q, J = 5.1, 11.4 Hz, 1H), 3.63 (m, 1H), 3.31 (t, J = 9.0, 9.0 Hz, 1H), 3.15 (m, 2H), 2.67 (m, 2H), 2.46 (broad, 1H), 1.53 (broad, 1H), 1.23 (t, J = 7.5, 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 85.4, 77.5, 77.4, 69.8, 69.3, 66.0, 25.1, 15.2.



4.71

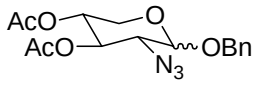
To a solution of **4.70** (26 mg, 0.119 mmol) in dry DMF (1 mL) was added NaH (60% in mineral oil, 12 mg, 0.392 mmol) at 0 ^{o}C. After the reaction solution was stirred at 25 ^{o}C for 10 min, *p*- methoxybenzyl chloride (36 µL, 0.261 mmol) and tetrabutylammonium iodide (1 mg, 0.003 mmol) were added. The reaction was stirred at room temperature for 12 hours and quenched with saturated aqueous $NaHCO_3$. The organic layer was separated and the water layer was extracted with ethyl acetate (5 ml) three times. The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed and the product was isolated by silica gel column chromatography (Hexane: EtOAc 4:1) to afford **4.71** as a clear oil (42.6 mg, 78%).^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.30 (m, 4H), 6.90 (m, 4H), 5.26 (d, J = 4.8 Hz, 1H), 4.80 (m, 4H), 4.67 – 4.54 (m, 4H), 4.47 (s, 1H), 4.20 (d, J = 10.0 Hz, 1H), 3.95 (overlap, 2H), 3.80 (overlap, 12H), 3.73 - 3.58 (overlap, 4H), 3.42 (t, J = 8.8 Hz, 1H), 3.33 (dd, J = 5.2, 14.8Hz, 1H), 3.15 (dd, J = 10.4, 11.5 Hz 1H), 2.71 (m, 2H), 2.58 (m, 2H), 1.29 (overlap, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 132.82, 132.77, 132.74, 132.68, 132.64, 132.63, 132.24, 132.16, 132.11,116.66, 116.63, 116.57, 116.50, 87.4, 86.4, 86.2, 82.9, 80.9, 77.93, 77.87, 75.66, 75.64, 74.1, 70.5, 68.3, 66.2, 63.6, 58.0, 27.4, 27.3, 17.7, 17.4.



4.73

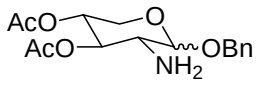
To a solution of **4.71** (188.2 mg, 0.41 mmol) in CH_2Cl_2 (2.5 mL) and water (0.3 mL) was added NBS (79.5 mg, 0.446 mmol) in one portion. After the reaction was stirred at room temperature for 1 hour, saturated aqueous Na_2SO_3 was added. The organic layer

was separated, and the water layer was extracted with CH_2Cl_2 (5 ml) three times. The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed and the crude product was purified by silica gel column chromatography (Hexane: EtOAc 5:1) to afford **4.72** as a clear oil (159.9 mg, 94%). Compound **4.72** (159.9 mg, 0.39 mmol) was dried by benzene and dissolved in dry CH_2Cl_2 (2 mL). Trichloroacetonitrile (0.2 mL, 1.97 mmol) and DBU (1 drop) were added. The reaction was stirred at 25 ^oC for 1 hour. The solvent was removed and the product was isolated by Et_3N deactived silica gel column chromatography (Hexane: EtOAc 5:1) to afford **4.73** as a clear oil (217.6 mg, 99%), which was used without identification.



4.35

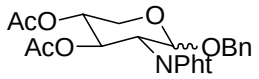
The compound **4.34** (9 g, 29.6 mmol) was dissolved in BnOH (26 ml) and $CHCl_3$ (26 ml). After adding 4 Å MS (4 g), the reaction mixture was stirred at room temperature for 7days. The solid was filtered and filtrate was washed by $CHCl_3$ (20ml). Organic solvent has been removed by reducing pressure distillation and the crude product **4.35** as a clear oil was used for the next reaction without further purification.



4.36

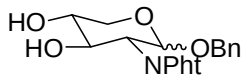
The compound **4.35** (29.6 mmol) was dissolved in THF (100 ml). After adding PPh_3 (15.5 g, 59.2mmol) and water (1.06 ml, 59.2mmol), the reaction mixture was refluxed for 12h. Then, organic solvent has been removed by rotovap, the crude product

was dissolved in 10% HCl (100ml) and washed by Et_2O (2 x 50ml). Aqueous phase was neutralized by saturated Na_2CO_3 and extracted with CH_2Cl_2 (3 x 50ml). The combined organic layers were then washed with brine (100 mL), and dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded crude **4.36** as a clear oil (56%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.33 (m, 5H), 5.06 (t, J = 9.6 Hz, 1H), 4.80 (m, 2H), 4.63 (d, J = 11.7 Hz, 1H), 4.39 (d, J = 11.7 Hz, 1H), 3.68 (m, 1H), 3.55 (t, J = 10.2 Hz, 1H), 2.77 (dd, J= 10.2, 3.3 Hz, 1H), 2.12 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 170.7, 170.0, 137.0, 128.4, 129.9, 127.89, 98.9, 74.1, 69.6, 69.5, 59.1, 54.6, 20.9, 20.7.



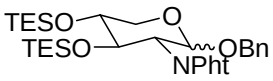
4.37

To a 100 ml flask charging DeanStark trap and condenser was added compound **4.36** (5 g, 16 mmol), phthalic anhydride (4.7 g, 32 mmol) and toluene (60 ml). The reaction mixture was refluxed for 24h. After cooling to room temperature, toluene was removed by rotovap, and the crude product was purifed by silica gel chromatography eluting with hexane: ethyl acetate (5: 1) afforded 4.4 g of **4.37** as clear oil (61%): ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.81 (m, 2H), 7.73 (m, 2H), 7.07 (m, 5H), 6.66 (dd, J = 11.7, 11.7 Hz, 1H), 5.05 (m, 1H), 4.93 (d, J = 3.6 Hz, 1H), 4.73 (d, J = 12.6 Hz, 1H), 4.47 (dd, J = 11.7, 3.3 Hz, 1H), 4.42 (d, J = 12.6 Hz, 1H), 3.82(m, 2H), 2.05 (s, 3H), 1.89(s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 170.5, 169.6, 167.8, 136.9, 134.3, 131.7, 128.4, 127.8, 127.6, 123.7, 96.3, 71.1,69.7, 67.3, 60.6, 59.4, 54.3, 21.0, 14.4; $[\alpha]_D^{25}$ 154.7^o (*c* 0.51, $CHCl_3$).



4.38

The compound **4.37** (108.7 mg, 0.24 mmol) was dissolved in MeOH (1 mL), and sodium methoxide (1.3 mg, 0.024 mmol) was added. The reaction was stirred at room temperature for 4 hours. The solid was filtered and the solvent was removed by rotovap to afford **4.38** as a clear oil (87.6 mg, 99%) without further purification.

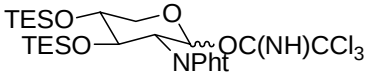


4.39

To a solution of **4.38** (87.6 mg, 0.237 mmol) in dry CH_2Cl_2 (1 mL) was added imidazole (32 mg, 0.474 mmol) and TESCl (71.1 g 0.474mmol) at 0 ^oC. The reaction was stirred at room temperature for 2 hours and quenched with water (2 ml). The organic layer was separated and the water layer was extracted with CH_2Cl_2 (5 ml) three times. The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed and the crude product was purified by silica gel column chromatography (Hexane:EtOAc 5:1) to afford **4.39** as a clear oil (140.2 mg, 99%).^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.87 (m, 1H), 7.74 (m, 3H), 7.12 (m, 2H), 7.05 (m, 3H), 5.10 (m, 1H), 4.75 (overlap, 2H), 4.36 (d, J = 12.9, Hz, 1H), 4.20 (dd, J = 10.8, 4.2 Hz, 1H), 3.6(m, 3H), 0.45-0.98 (m, 30H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 169.1, 167.9, 137.5, 134.3, 133.8, 132.9, 131.2, 128.4, 127.6, 127.5, 123.2, 97.1, 74.2, 70.1, 69.4, 68.7, 62.8, 57.4, 29.9, 7.14, 7.02, 5.6, 1.2; $[\alpha]_D^{25}$ 105.0 (*c* 0.52, $CHCl_3$). $C_{32}H_{47}NO_6Si_2$, HRMS (m/z): 598.303; Calculated MS: 597.2942.

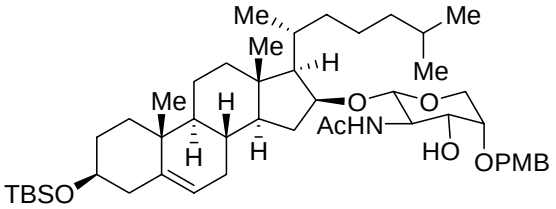
4.40

To a 10 ml 2 neck flask charging condenser and H_2 balloon was added compound **4.39** (120 mg, 0.2 mmol), Pd/C (120 mg), Et_3N (23.8 mg, 0.2 mmol) and ethanol (5 ml). Reaction mixture was heated to 40 ^oC for 12h. After cooling to room temperature, the Pd/C was removed by filtration and solvent was evaporated by rotovap. The crude product was purified by silica gel chromatography eluting with Hexane: Ethyl Acetate (5: 1) afforded 88.2 mg of **4.40** as a clear oil (87%).



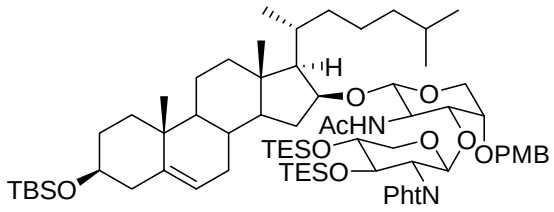
4.3

Compound **4.40** (88.2 mg, 0.174 mmol) was dried by benzene and dissolved in dry CH_2Cl_2 (2 mL). Then, trichloroacetonitrile (0.035 mL, 0.35 mmol) and DBU (1 drop) were added. The reaction was stirred at 25 ^oC for 1 hour. The solvent was removed and the crude product was purified by Et_3N deactived silica gel column chromatography (Hexane:EtOAc 5:1) to afford **4.3** as a clear oil (112.2 mg, 99%), which was used without identification.



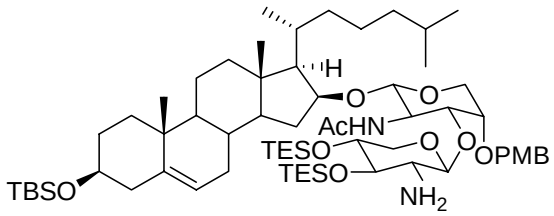
4.75

The compound **4.63** (185 mg, 0.2 mmol) was dissolved in dry CH_2Cl_2 (10 mL). Dimethyl sulfide (0.077 ml, 0.8 mmol) and $MgBr_2 \cdot Et_2O$ (133 mg, 0.4 mmol) were added to the reaction mixture. The reaction had been stirred at room temperature for 2 hours. Then, the reaction mixture was quenched with saturated aqueous $NaHCO_3$. The organic layer was separated and the water layer was extracted with CH_2Cl_2 (10 mL) three times. The combined organic layer was washed with water, with brine, and then dried over Na_2SO_4. The solvent was removed and the product was isolated by silica gel column chromatography (Hexane: EtOAc 1:2) to afford **4.75** as a form solid (86 mg, 60% conversion yield 25 mg starting material had been recovered). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.17 (d, J = 9.6 Hz, 2H), 6.86 (d, J = 8.4 Hz, 2H), 5.37 (d, J = 7.8 Hz, 1H), 5.21 (m, 1H), 4.51 (m, 2H), 4.33 (d, J = 3.3 Hz, 1H), 4.01 (m, 1H), 3.85 (overlap, 4H), 3.70 (s, 3H), 3.48 (m, 2H), 3.37 (m, 2H), 2.23 (overlap, 7H), 1.81-1.56 (overlap, 9H), 1.53-1.24 (overlap, 9H), 1.25-0.82 (overlap, 25H), -0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 170.1, 159.6, 141.9, 130.2, 129.7, 121.0, 114.1, 102.6, 83.1, 72.0, 71.8, 70.9, 69.0, 61.3, 60.2, 55.5, 55.1, 53.5, 53.2, 50.4, 43.0, 43.4, 40.3, 39.7, 37.5, 36.8, 36.6, 35.7, 32.3, 32.0, 31.6, 30.8, 30.7, 30.3, 29.9, 29.6, 28.3, 26.1, 24.0, 23.6, 23.2, 23.0, 22.9, 22.8, 21.0, 19.6, 18.5, 18.3, 14.3,13.2, -4.4; $[\alpha]_D^{25}$ -19.3 (*c* 1.9, $CHCl_3$). $C_{48}H_{79}NO_7Si$, HRMS (m+H/z): 810.5698; Calculated MS: 809.5626.



4.76

A solution of **4.65** (20.0 mg, 0.025 mmol), **4.3** (32.8 mg, 0.05 mmol) and dry 4Å MS powder (20 mg) in anhydrous CH_2Cl_2 (1.5 mL) was stirred at 25 °C for 15 min, cooled to -78 °C, and then $BF_3{\cdot}OEt_2$ (0.02 M in CH_2Cl_2, 125 μL, 0.0025 mmol) was added dropwise. The reaction mixture was stirred at -78 °C for 1.5 h and quenched with 0.15 mL Et_3N. The solid was filtered and the solvent was removed. The product was isolated by silica gel column chromatography (Hexane: EtOAc 4:1) to afford **4.76** as a clear oil (13.1 mg, 68% yield, recover 8 mg starting material **4.75**). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.76 (m, 2H), 7.66 (m, 2H), 7.27 (m, 2H), 6.81 (m, 2H), 5.21 (m, 1H), 5.02 (d, J = 8.1 Hz, 1H), 4.94 (d, J = 6.9 Hz, 1H), 4.59 (d, J = 10.2 Hz, 1H), 4.40 (m, 1H), 4.15 (m, 1H), 4.00 (m, 1H), 3.81-3.58 (overlap, 9H), 3.35-3.13 (overlap, 4H), 2.23 (overlap, 7H), 1.81-1.56 (overlap, 9H), 1.53-1.24 (overlap, 9H), 1.25-0.27 (overlap, 40H), -0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 170.7, 164.8, 159.2, 141.7, 134.3, 132.2, 131.9, 131.4, 130.1, 121.3, 113.7, 101.6, 99.6, 81.5, 75.9, 75.2,73.7, 73.5, 72.9, 72.8, 66.4, 65.4, 59.9, 57.8, 55.9, 55.4, 55.2, 50.5, 43.0, 42.3, 42.2, 40.2, 39.8, 37.5, 36.7, 35.9, 32.3, 31.9, 31.5, 29.9, 29.7, 29.2, 28.3, 26.2, 24.0, 23.0, 22.9, 22.8, 22.4, 21.0, 19.6, 18.5, 18.4, 14.5, 13.7, 7.1, 6.9, 5.5, 5.4 -4.4; $[\alpha]_D^{25}$ -18.7 (*c* 0.88, $CHCl_3$). $C_{73}H_{118}N_2O_{12}Si_3$, HRMS (m+Na/z): 1321.7862; Calculated MS: 1298.7993.



4.77

To a solution of **4.76** (50.0 mg, 0.038 mmol) in EtOH (1 mL) was added hydrazine (0.5 ml). The reaction was stirred at room temperature for 3 days. The solvent was removed and the crude product was purified by silica gel column chromatography (Hexane:EtOAc 5:1) to afford **4.77** as a form solid (28.0 mg, 62%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.20 (d, J = 7.8 Hz, 2H), 7.74 (d, J = 8.4, 2H), 5.43 (d, J = 8.1 Hz, 1H), 5.18 (m, 1H), 4.62 (d, J = 12 Hz, 1H), 4.49 (d, J = 11.7 Hz, 1H), 4.43 (d, J = 5.4 Hz, 1H), 4.14 (d, J = 7.8 Hz, 1H), 4.08 (m, 1H), 3.85-3.70 (overlap, 9H), 3.59-3.47 (overlap, 3H), 3.36- 3.22 (overlap, 4H), 3.02 (t, J = 7.5, 7.5 HZ, 1H), 2.61 (t, J = 8.4, 8.4 Hz, 1H), 2.23 (overlap, 7H), 1.81-1.56 (overlap, 9H), 1.53-1.24 (overlap, 9H), 1.25-0.27 (overlap, 40H), -0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 170.2, 159.3, 141.7, 131.0, 129.9, 121.2, 113.8, 105.8, 102.7, 82.2, 78.8, 72.9, 71.9, 71.2, 66.5, 60.8, 58.0, 55.4, 55.1, 53.9, 50.5, 43.0, 42.4, 40.2, 39.8, 37.6, 36.8, 36.4, 36.0, 32.3, 32.1, 32.0, 31.6, 31.1, 29.9, 29.6, 28.3, 26.2, 23.9, 23.2, 23.0, 22.8, 21.3, 21.0, 19.6, 18.5, 18.4, 14.3, 13.2, 7.2, 7.1, 5.4, 5.2, -4.4; $[\alpha]_D^{25}$ -16.1 (*c* 0.36, $CHCl_3$). $C_{65}H_{116}N_2O_{10}Si_3$, HRMS (m/z): 1168.7997; Calculated MS: 1168.7938.

CHAPTER 5

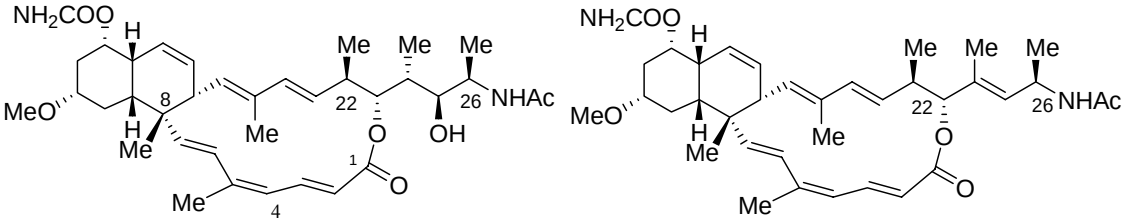
MODEL STUDY TOWARDS THE TOTAL SYNTHESIS OF ANTICANCER NATURAL PRODUCT SUPERSTOLIDE A

5.1 Introduction: Superstolides A and B

Neosiphonia superstes was isolated from Phymatellidae lithistides, one of the deep-water lithistid sponge families that are regarded as "living fossils". Superstolides A (**5.1**) and B (**5.2**) are marine natural products that were isolated from the deep-water marine sponge *Neosiphonia superstes*, collected off New Caledonia, a pacific island group which lies in the southwestern Pacific Ocean about 1,500 km east of Australia (Scheme 2.1).[38-41] The isolation and structural determination of Superstolides A (**5.1**) and B (**5.2**) were first reported by D'Auria and Zampella in 1994.[42, 43] 31.2 mg of Superstolide A and 3.0 mg of Superstolide B, as colorless amorphous solid, were isolated from 1.0 kg of sponge *Neosiphonia superstes*, collected of New Caledonia at 500-515 m depth.

The partial structural units and relative stereo-structures of Superstolides A and B were determined by extensive spectroscopic methods, including LSIMS, FABMS, UV and extensive 1D and 2D NMR.[42] Employing the gas chromatographic modification of Horeau's method,[31] and high field NMR-modified Mosher's method,[44-47] the absolute configuration was established. The structural novelty of these two molecules is characterized by a unique 16-membered macrolactone attached to a highly functionalized *cis*-decalin with six chiral centers, including one quaternary carbon chiral center (C8).

There are five consecutive chiral centers in the polypropionate chain portion (C22-C26) of Superstolide A.



Superstolide A (5.1) **Superstolide B (5.2)**

Scheme 5.1 Structure of Superstolides A and B

Both Superstolides A and B are highly cytotoxic against human bronchopulmonary non-small cell lung carcinoma NSCLC-N6-L16 cells with IC_{50} of 0.04 μg/mL and 0.039 μg/mL respectively.[38, 39] They also exhibited highly potent cytotoxicity against murine leukemia P388 cells with an IC_{50} of 3 ng/mL, and human nasopharyngeal carcinoma KB cells with an IC_{50} of 20 ng/mL and 5 ng/mL, respectively. In addition, Superstolide A is highly cytotoxic against human colon carcinoma HT29 cells with an IC_{50} of 0.04 μg/mL and murine leukemia cells expressing resistance toward doxorubicin P388 Dox with an IC_{50} value of 0.02 μg/mL.[38, 39]

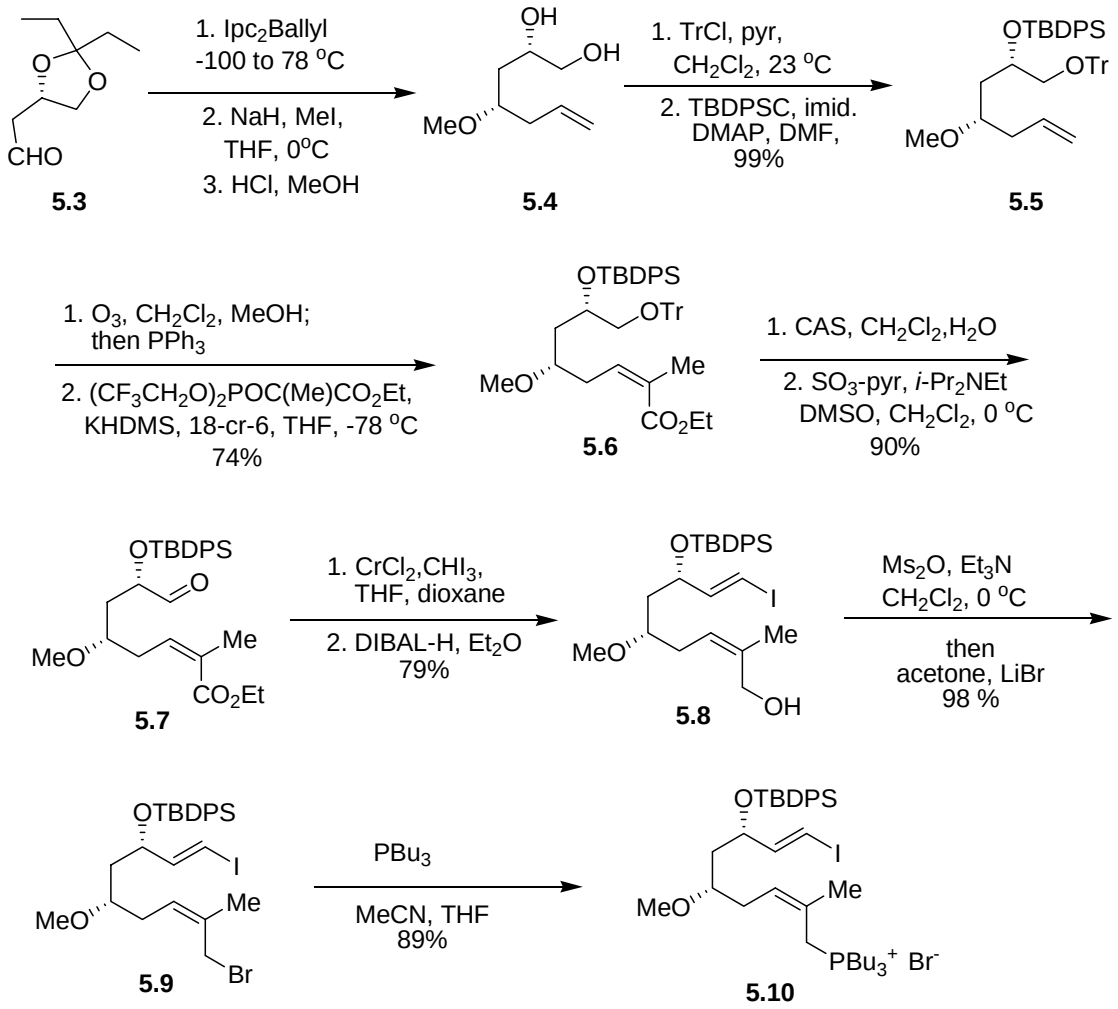
As the leading cause of cancer related mortality for both men and women in North America,[56] lung cancer has two distinct phenotypes. Non-small cell lung cancer (NSCLC), which accounts for large majority of lung carcinomas,[57] is less responsive to anticancer agents than Small-cell lung.[56] Chemotherapy and surgical resection[58-60] are the two

choices for patient with NSCLC. Chemotherapy shows the only marginal benefit and the impact of treatment on the survival and quality of life of patients with NSCLC remains controversial.[61, 62] Due to the limitations of controversial therapy, the development of new anti-NSCLC agents is highly desired. The potent cytotoxicity of Superstolides A and B against NSCLC make both molecules attractive synthetic targets.[48-55, 59-68]

Because of the scarcity of Superstolides A and B, extremely low isolation yield (0.003% for Superstolide A and 0.0003% for Superstolide B), and difficulty of sponge collection from 500-515 meters depth ocean, a highly efficient synthesis of these complex molecules (11 stereocenters, 6 double bonds, 3 rings, and 5 different functionalities) is required to supply sufficient quantities of the target material for future biological studies. Many analogs will need to be prepared for pharmacological evaluations to determine the minimum pharmacophore present in these two natural products. An efficient total synthesis pathway that readily allows structural modification is most desirable in this regard.

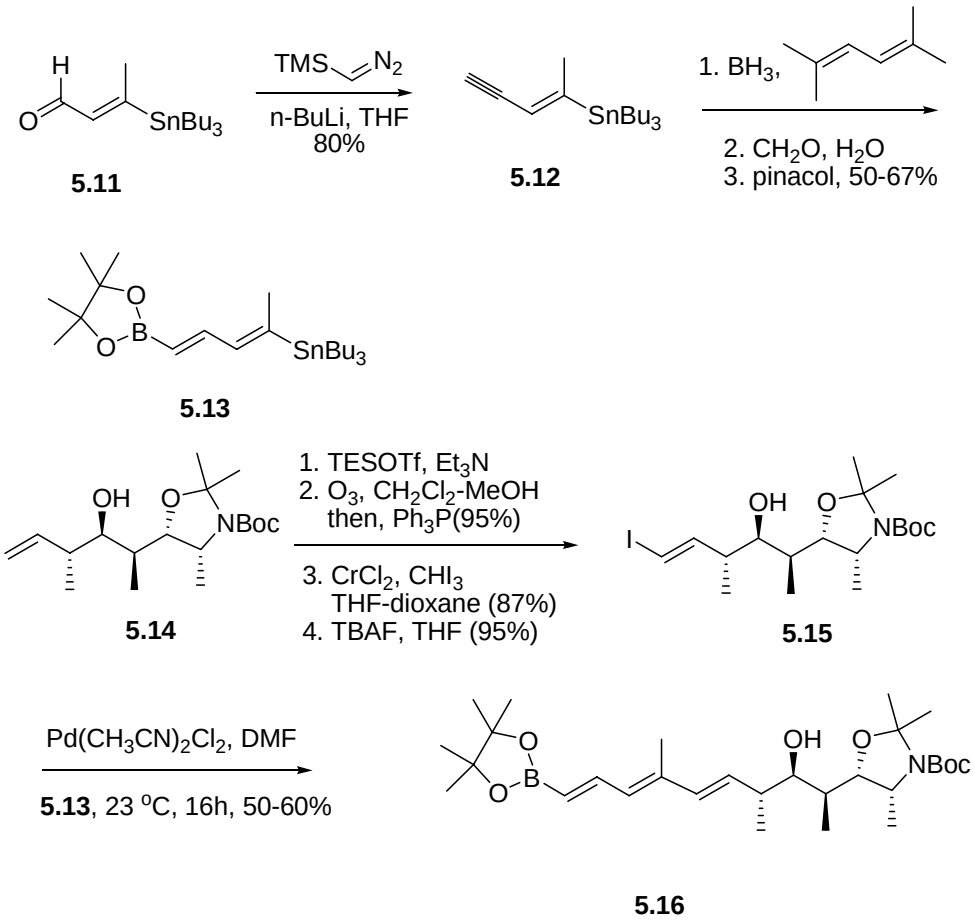
5.2 Roush First Synthesis of Superstolide A

Due to the potent anticancer activity of Superstolide A, a number of groups around the world have been working on the total synthesis of this molecule. Recently, Roush and his coworkers published their first total synthesis of Superstolide A.[63, 65] They reported a stereoselective total synthesis of Superstolide A by a route that was biomimetic in nature, and proceeded via the transannular Diels-Alder reaction of 24-membered octene. Their synthetic route was summarized in scheme 5.2, 5.3 and 5.4.

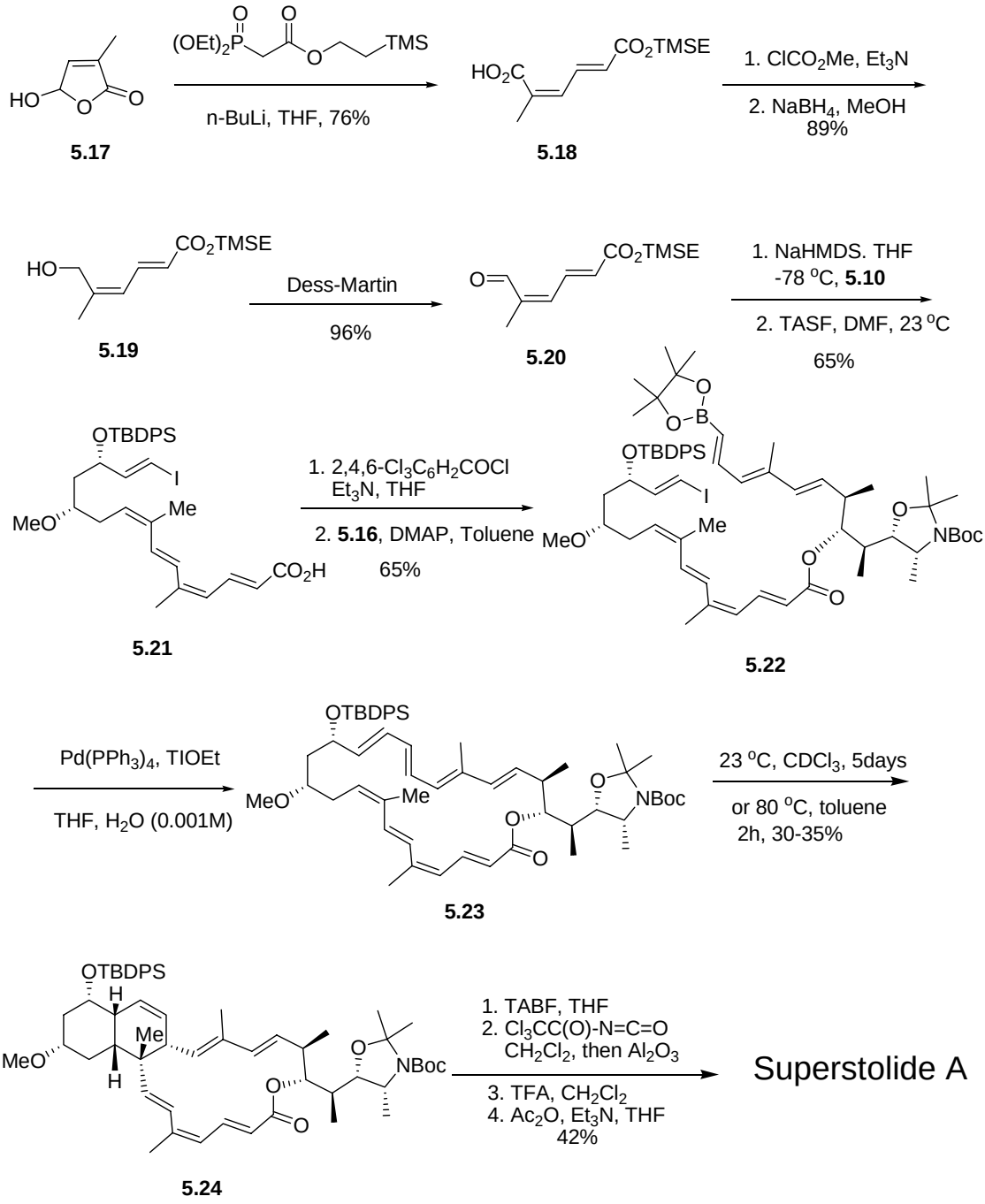


Scheme 5.2 Synthesis of Phosphonium Salt **5.10**

The longest linear route of Roush synthesis of Superstolide A consists of 23 steps with only 1% overall yield. The transannular Diels-Alder approach, which is the key reaction of the total synthesis is low yielding, and is hard to be adapted to the synthesis of analogs of Superstolide A. Therefore, a better total synthesis is needed.



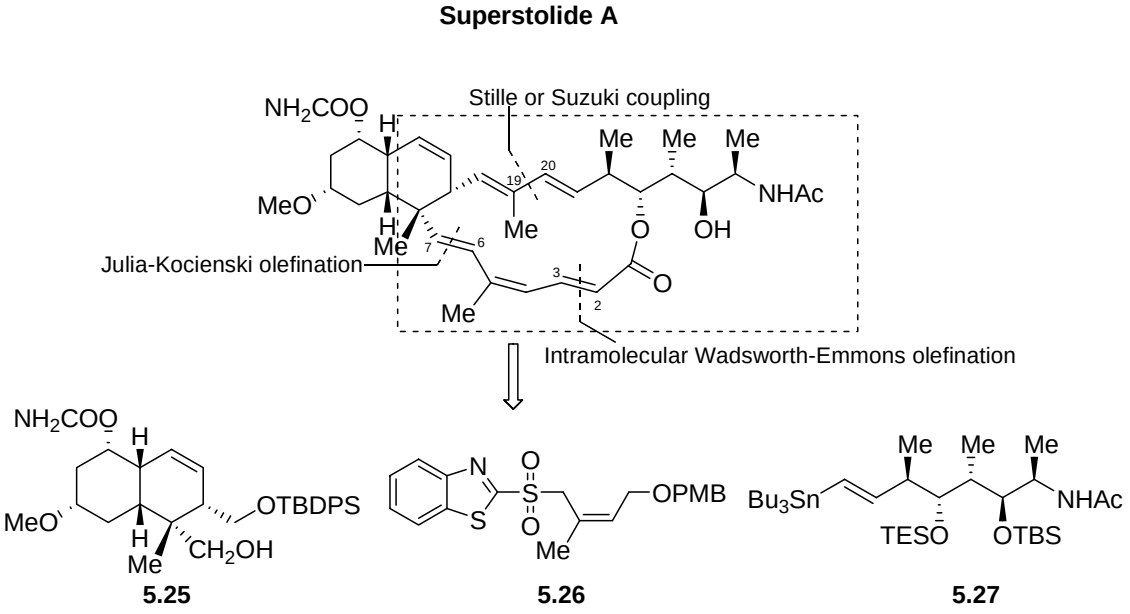
Scheme 5.3 Synthesis of Bimetallic Diene Linchpin **5.16**



Scheme 5.4 Completion of the Total Synthesis of Superstolide A

5.3 Retrosynthetic Analysis of Superstolide A

Our careful analysis of the targets has led to a synthetic strategy that is outlined in Scheme 5.5. The formation of the 16-membered macrolactone ring and the formation of the *cis*-decalin core are important steps in the total synthesis. The intramolecular Wadsworth-Emmons olefination is one of the most promising methods to form a macrolactone, making disconnection at C2-C3 obvious. Sequential disconnections at C6-C7 and C19-C20 reveal fragments **5.25**, **5.26** and **5.27** as potential key intermediates, with Julia-Kocienski olefination and Suzuki or Stille coupling playing crucial roles in the synthetic strategy.



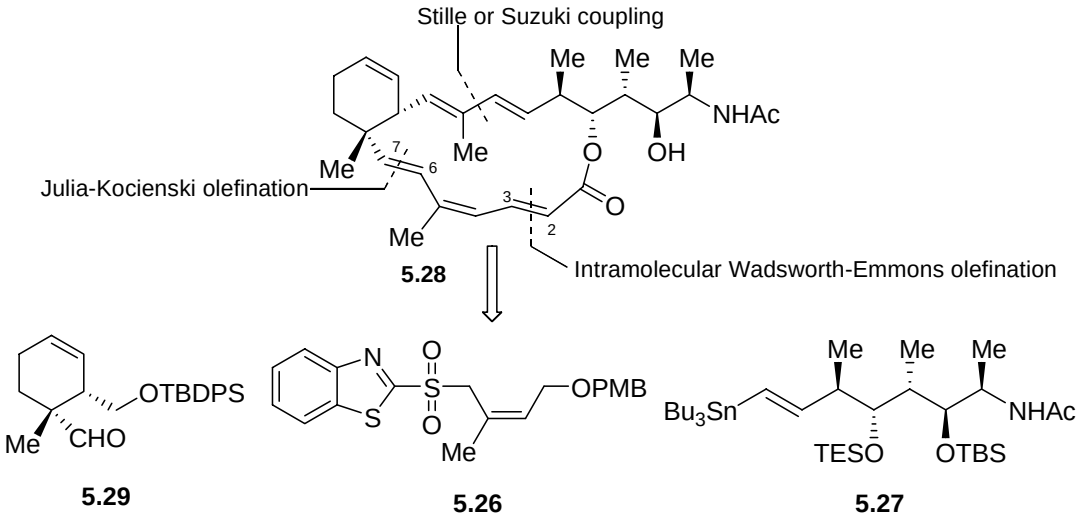
Scheme 5.5 Retrosynthetic analysis

The linear polypropionate **5.27** has been successfully synthesized by Jin and his coworkers.[59] In addition, a model study for the asymmetric synthesis of fragment **5.25** was successfully carried out by the Jin group.[69]

5.4 Model study on the 16-membered macrolactone 5.28

5.4.1 Retrosynthetic Analysis

Since the construction of the 16-membered macrolactone is critical in our synthetic strategy, a model compound **5.28** was designed. The *cis*-fused highly functionalized decalin of compound **5.28** is simplified to a cyclohexene ring whereas the 16-membered macrolactone remains intact (Scheme 5.6).



Scheme 5.6 Retro-synthesis of 16-membered macro-lactone **5.28**

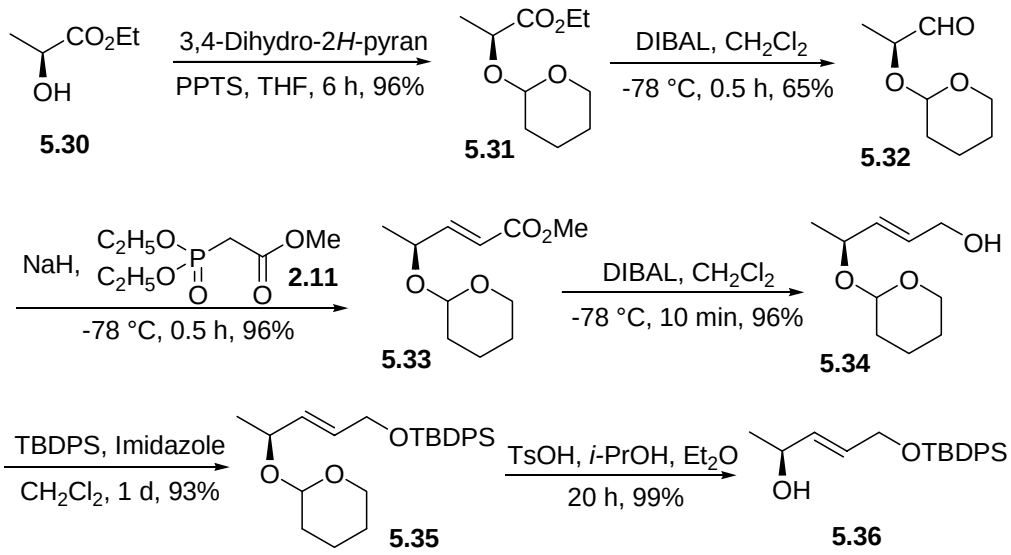
This modification will simplify the synthesis substantially, but at the same time, the basic templates of the molecule remain intact. It will provide a great deal of

information for several key coupling reactions, including intra-molecular Wadsworth-Emmons olefination, Julia-Kocienski olefination and Suzuki or Stille coupling. In addition, we speculate that the *cis*-fused decalin might serve to lock the macrolide into a certain conformation and the 16-membered macrolactone might be the key pharmacophore that interacts with cellular target(s).[69-74] Therefore, simplified analog **5.28** will test our hypothesis and provide important information regarding the structure-activity-relationship.

It should be emphasized that this simplification is mainly based on the assumption that the macrolactone might be the pharmacophore of the molecule and on the ease of synthesis. Due to the change from a *cis*-fused decalin to a simple cyclohexene ring based on molecular modeling, the conformation of the macrolactone will have significant differences from Superstolide A. Nevertheless, we still believe that it will provide valuable chemical and biological information. And my work focused on synthesize the six membered ring **5.29** and fragment **5.26**.

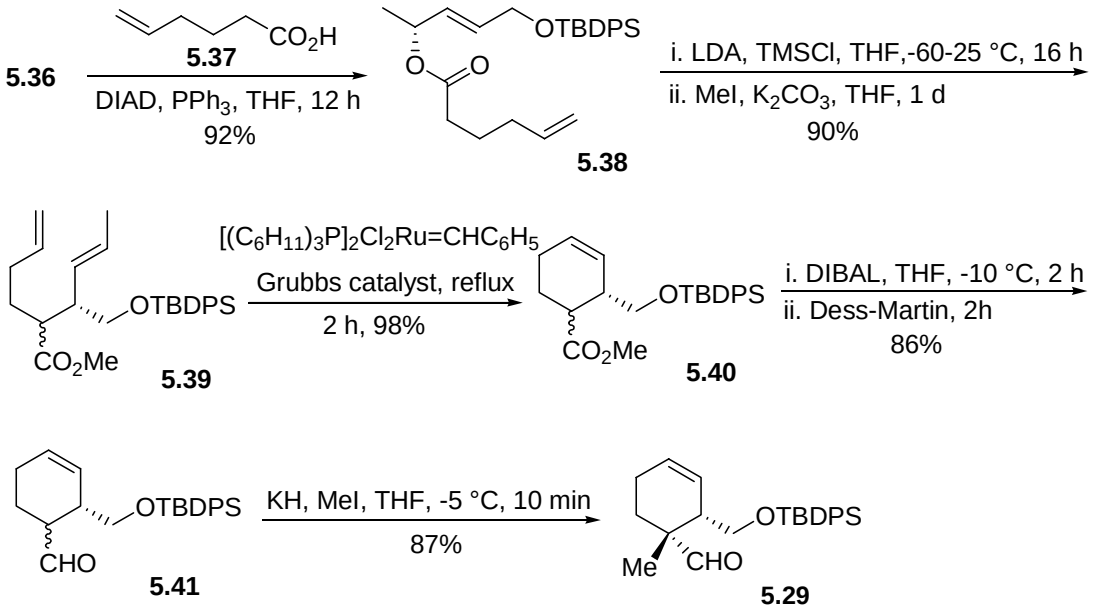
5.4.2 Asymmetric synthesis of fragment **5.29**

The fragment **5.29** was synthesized by employing an Ireland-Claisen rearrangement as the key reaction. The commercially available starting material ethyl (*S*)-(-)-lactate **5.30** was protected by dihydropyran, followed by DIBAL reduction to convert to the corresponding aldehyde **5.32**. Wadsworth-Emmons olefination provided *Z*-olefin **5.33** in 96% yield. The ester **5.33** was reduced to alcohol **5.34** by DIBAL, followed by TBDPS protection and deprotection of THP to generate compound **5.36**. The overall yield for the synthesis of chiral secondary alcohol **5.36** from **5.30** was 53% (Scheme 5.7).



Scheme 5.7 Synthesis of chiral secondary alcohol **5.36**

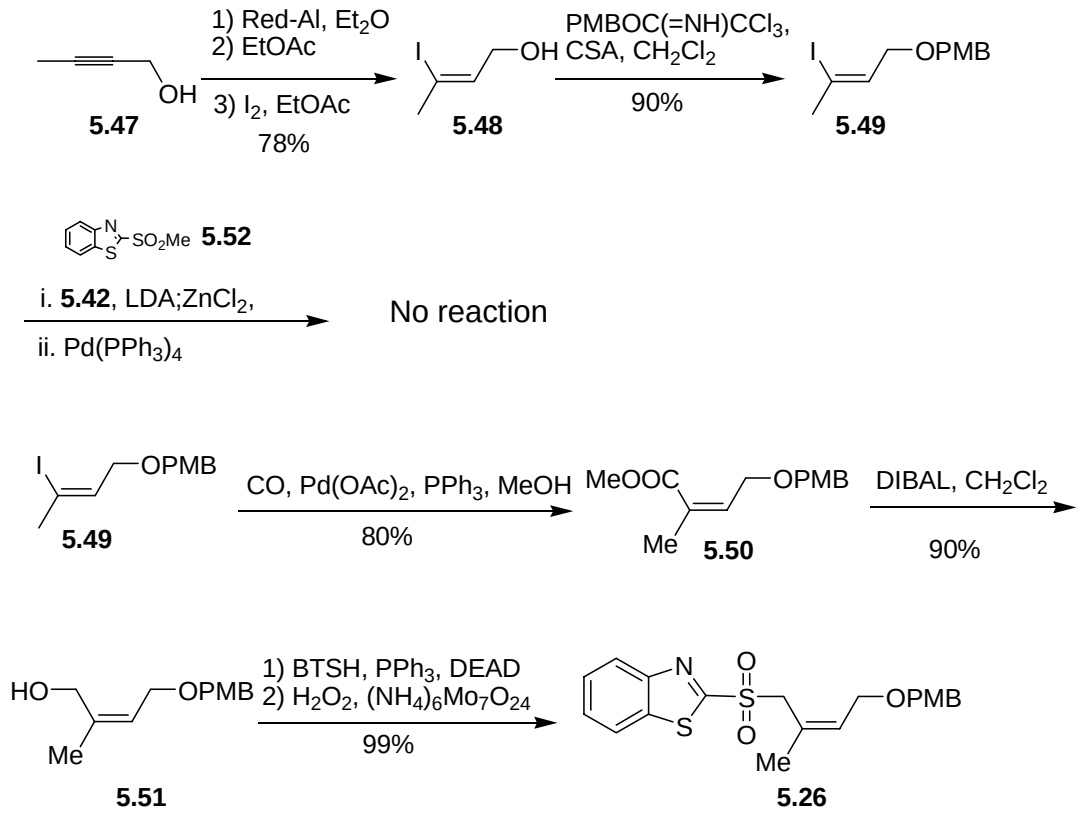
Under Mitsunobu conditions, alcohol **5.36** reacted with carboxylic acid **5.37** to give ester **5.38** with reversed chiral center in 92% yield (Scheme 5.8). Compound **5.38** underwent an Ireland-Claisen rearrangement[75] followed by methylation to afford **5.39** (a mixture of diastereomers) in 90% yield. RCM with Grubbs catalyst furnished nearly quantitative yield of cyclohexene derivative **5.40**.[77] Ester **5.40** was treated with DIBAL at -78 ^{o}C to give to an alcohol, which was then oxidized to aldehyde **5.41** using Dess-Martin oxidation. Treatment of compound **5.41** with KH and MeI in THF at -5 °C provided the desired product **5.29** which was comfirmed by NOESY spectroscopy.



Scheme 5.8 Completion of the synthesis of Fragment **5.29**

5.4.3 Synthesis of fragment **5.26** .

Stereoselective reductive addition of Red-Al to alkyne **5.47** followed by the addition iodine afford *Z*-vinyl iodide **5.48** in 78% yield.[79] However, no reaction was observed between compound **5.49** and benzothiazole sulfonyl zinc chloride under Negishi coupling conditions. Therefore, vinyl iodide **5.49** was converted to vinyl ester **5.50** in 80% yield under palladium-catalyzed carbonylation. Ester **5.50** was reduced to allylic alcohol **5.51** by DIBAL, followed by Mitsunobu reaction to form the sulfide, which was subsequently oxidized to allyl sulfone **5.26**. Employing the new approach fragment **5.26** was stereoselectively synthesized in 6 steps with 44% overall yield.

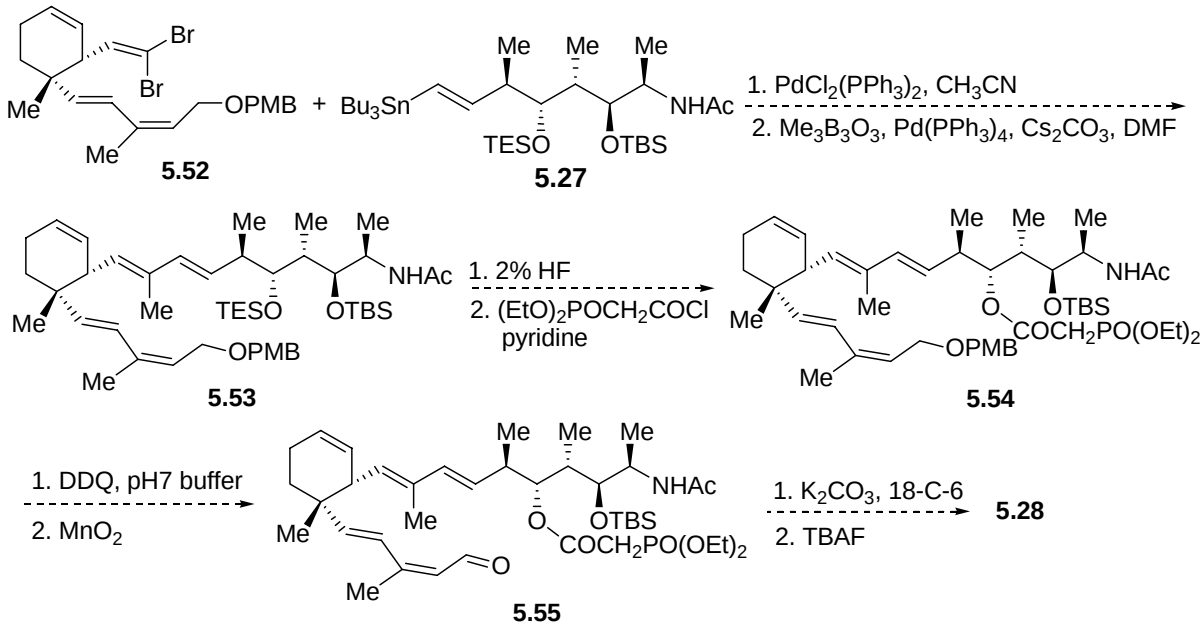


Scheme **5.9** Study toward to synthesis of fragment **5.26**

5.5 Future plan

Compound **5.52** has been synthesized by Kang, Ying.[81] It is known that Stille coupling between vinyl stannane **5.27** and the *trans*-bromide is much faster than the *cis*-bromide (Scheme 4.11).[82] Thus, Stille coupling followed by Suzuki methylation by using the recently developed procedure of Gary *et al.*[83] will afford compound **5.53**. Chemoselective deprotection of the TES group followed by an esterification should provide compound **5.54**.[84] Removal of the PMB group followed by MnO_2-mediated oxidation should provide compound **5.55**. Intra-molecular Wadsworth-Emmons

olefination[85] followed by the removal of the TBS group should afford the 16-membered macrolactone **5.28** with the requisite *trans* geometry of the double bond.



Scheme 5.10 Proposed synthetic route for the model 16-membered macrolactone **5.28**

5.6 Experimental section

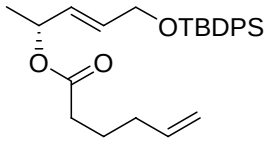
5.6.1 General

Reagents were all purchased from the following companies and were used as received unless indicated: Aldrich Chemical Company, Fluka, Fisher Scientific and ACROS Organics. Tetrahedrofuran (THF) and diethyl ether (Et_2O) were distilled over sodium benzophenone ketyl; benzene, toluene, methylene chloride (CH_2Cl_2) and acetonitrile (CH_3CN) were distilled over calcium hydride. Methanol (CH_3OH), dimethylsulfoxide (DMSO) and dimethylformamide (DMF) were purchased from

Aldrich Company in anhydrous form and used as received. Acetone, ethyl acetate (EtOAc) and hexanes were purchased from Fisher Company and used as received. ^{n}BuLi and ^{t}BuLi were titrated by diphenyl acetic acid prior to use. Sodium sulfate (Na_2SO_4), magnesium sulfate ($MgSO_4$) and potassium carbonate (K_2CO_3) were purchased from Fisher Company as anhydrous form and used as received.

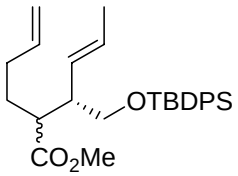
All moisture sensitive reactions were performed in flame-dried glassware under a positive pressure of nitrogen or argon. All reactions were monitored by thin layer chromatography (TLC) with co-spots of the starting material(s). Products were isolated or purified by flash column chromatography, preparative TLC, or distillation under reduced pressure. Optical rotations were measured with Jasco P-1020 polarimeter. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with Bruker Avance 300 (300 MHz) instrument, Bruker WM360 (360 MHz) instrument, or Bruker DRX400 (400 MHz) instrument. The deuterated solvents for NMR spectroscopy were chloroform-d_1 ($CDCl_3$), benzene-d_6 (C_6D_6) or water-d_2 (D_2O), and are reported in parts per million (ppm) with residual protonated solvent peak or solvent ^{13}C-NMR peak as internal standard ($CDCl_3$: 7.26 ppm for ^{1}H-NMR and 77.0 ppm for ^{13}C-NMR; C_6D_6: 7.15 ppm for ^{1}H-NMR and 128.0 ppm for ^{13}C-NMR; When peak multiplicity is reported, the following abbreviations are used: s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet), m (multiplet).

5.6.2 Experimental procedures and spectral data



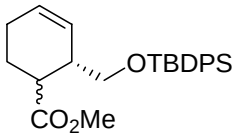
5.38

To a solution of alcohol **5.36** (4.96 g, 14.7 mmol) and acid **5.37** (2.01 g, 17.6 mmol) in THF (150 mL) was added triphenylphosphine (7.71 g, 29.4 mmol) at 25 °C. The reaction solution was cooled to 0 °C and DIAD (5.69 mL, 29.4 mmol) was added dropwise at this temperature. Then the reaction mixture was warmed up gradually to 25 °C and stirred for 12 h. The solvent was removed by vacuum, then CH_2Cl_2 (100 mL) and saturated aqueous $NaHCO_3$ (100 mL) were added. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layer was washed with brine and dried over Na_2SO_4. Then, it was concentrated by vaccum and purified by silica gel column chromatography (Hexanes:EtOAc 10:1) to afford **5.38** (5.86 g, 92% yield) as colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.68 (m, 4H), 7.41 (m, 6H), 5.80-5.75 (m, 3H), 5.40 (m, 1H), 5.03 (d, J = 17.1 Hz, 1H), 4.97 (d, J = 8.9 Hz, 1H), 4.31 (m, 2H), 2.32 (m, 2H), 2.12 (m, 2H), 1.72 (m, 2H), 1.30 (d, J = 6.4 Hz, 3H), 1.07-1.05 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ(ppm) 172.8, 137.7, 135.5, 133.6, 130.7, 129.6, 129.3, 127.6, 115.3, 70.2, 63.6, 33.9, 33.0, 26.8, 24.1, 20.3, 19.2.



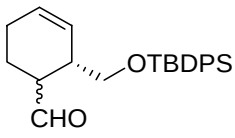
5.39

A solution of diisopropylamine (1.36 mL, 9.68 mmol) in THF (20 mL) was added ^{n}BuLi (2.35 M, 3.68 mL, 8.64 mmol) dropwise at -60 °C. The mixture was warmed up gradually to -20 °C in 20 minutes then cooled to -60 °C again. A solution of **5.38** (1.50 g, 3.46 mmol) and trimethylsilyl chloride (2.19 mL, 17.28 mmol) in THF (15 mL) was cannulated to the reaction solution at -60 °C, then the reaction mixture was warmed up slowly to 25 °C and stirred at this temperature for 16 h. The solvent was removed; then CH_2Cl_2 (30 mL) and saturated aqueous NH_4Cl (30 mL) were added. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed to give crude product. The crude product and methyl iodide (2.15 mL, 34.6 mmol) were dissolved in THF (20 mL); then K_2CO_3 (4.77 g, 34.6 mmol) was added at 25 °C. The reaction mixture was stirred for 1d. The solid was filtered, and the solvent was removed. The product was purified by silica gel column chromatography (Hexanes:EtOAc 10:1) to afford **5.39** (1.43 g, 90% yield) as colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.65 (m, 4H), 7.39 (m, 6H), 5.76 (m, 1H), 5.46 (m, 1H), 5.26 (m, 1H), 5.01 (d, J=16.3 Hz, 1H), 4.95 (d, J=10.2 Hz, 1H), 3.60 (s, 3H), 3.67-3.50 (m, 1H), 2.64-2.51 (m, 1H), 1.97 (m, 2H), 1.63-1.54 (m, 4H), 1.05-1.02 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ(ppm) 176.0, 138.1, 135.6, 135.5, 133.7, 133.6, 129.5, 128.2, 127.6, 114.9, 77.2, 65.7, 51.3, 51.0, 47.8, 45.7, 31.9, 28.7, 26.8, 19.3, 18.1, -1.1.



5.40

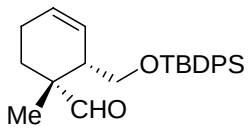
A solution of **5.39** (12.3 g, 28.3 mmol) and first generation Grubbs catalyst (500 mg, 0.602 mmol) in CH_2Cl_2 (3 L) was refluxed for 2 h. The reaction was quenched with MeOH at reflux then cooled down to room temperature. After 30 min, DMSO (2 mL) was added, and most of the solvent was removed by rotovap. The resulting concentrated reaction solution was stirred for 24h. All the solvents were removed by rotovap, and the product was isolated by silica gel column chromatography (Hexanes:EtOAc 20:1) to afford **5.40** (11.2 g, 98% yield) as yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.65 (m, 4H), 7.37 (m, 6H), 5.76 (m, 1H), 5.60 (m, 1H), 3.61 (s, 3H), 3.55 (t, J = 3.9 Hz, 2H), 2.75 (m, 1H), 2.59 (m, 1H), 2.07 (m, 2H), 1.87 (m, 1H), 1.75 (m, 1H), 1.05-1.03 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ(ppm) 176.3, 136.6, 133.7, 129.6, 128.0, 127.3, 77.2, 66.4, 51.6, 41.9, 40.4, 26.8, 25.0, 24.4, 19.3.



5.41

To a solution of compound **5.40** (885.4 mg, 2.18 mmol) in THF (25 mL) was slowly added DIBAL (1M, 7.63 mL, 7.63 mmol) at -10 °C. The reaction mixture was stirred at this temperature for 2h. The reaction was quenched with EtOAc and warmed up to 25 °C. Saturated aqueous sodium potassium tatrate (25 mL) was added, and the mixture was stirred for 20 min. The organic layer was separated, and the aqueous layer

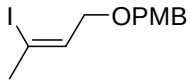
was extracted with EtOAc (3 × 25 mL). The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed by vacuum and the product was dissolved in CH_2Cl_2 (25 mL). Dess-Martin periodinane (1.29 g, 3.05 mmol) was added to the CH_2Cl_2 solution and stirred at 25 °C for 2h. The reaction was quenched with saturated aqueous $NaHCO_3$ (5 mL) and $Na_2S_2O_3$ (10 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed by vaccum and the product was purified by silica gel column chromatography (Hexanes:EtOAc 20:1) to afford **5.41** (705 mg, 10:1, 86% yield) as colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 9.69 (s, 1H), 7.63 (m, 4H), 7.41 (m, 6H), 5.77-5.73 (m, 1H), 5.53-5.49 (m, 1H), 3.66 (dd, J = 10.0, 5.3 Hz, 1H), 3.49 (dd, J = 10.0, 8.1 Hz, 1H), 2.78 (m, 1H), 2.52 (m, 1H), 2.06 (m, 2H), 1.76 (m, 2H), 1.04 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ(ppm) 153.3, 135.6 (two peaks), 133.9, 129.5 (two peaks), 128.0, 127.6, 126.6, 124.8, 65.5, 47.2, 38.8, 33.8, 26.9, 25.1, 22.9, 22.8, 19.2, -9.6.



5.29

A solution of **5.41** (240.3 mg, 0.639 mmol) and methyl iodide (398 μL, 6.39 mmol) in THF (6 mL) was cannulated to a suspension of oil free KH (102 mg, 2.56 mmol) in THF (6 mL) at -78 °C. The reaction mixture was warmed up to -5 °C gradually in 1h. The reaction was stirred at -5 °C for 10 min then quenched with isopropyl alcohol (5 mL) carefully. The organic layer was separated and the aqueous layer was extracted

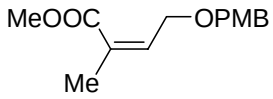
with EtOAc (3 × 5 mL). The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed and the product was purified by silica gel column chromatography (Hexanes:EtOAc 30:1) to afford **5.29** (216.2 mg, 87% yield) as colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 9.83 (s, 1H), 7.64 (m, 4H), 7.41 (m, 6H), 5.75 (m, 1H), 5.58 (m, 1H), 3.59 (m, 2H), 2.39 (m, 1H), 2.10 (m, 2H), 1.83 (m, 1H), 1.57 (m, 1H), 1.10 (s, 3H), 1.04 (s, 9H); ^{13}C NMR (75MHz, $CDCl_3$): δ (ppm) 205.6, 135.6, 133.2, 133.1, 129.7, 128.2, 127.7, 127.4, 125.6, 64.16, 46.3, 46.2, 26.6, 26.5, 22.2, 19.6, 19.1.



5.49

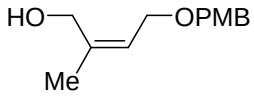
To ethyl ether (500 ml) was added Red-Al (3.4 M in toluene, 76 mL, 266 mmol). To this mechanically stirred solution was added **5.47**(10 mL, 133 mmoL) in ether (50 mL) dropwise at 0 ^{o}C. Then the reaction mixture was allowed to warm up to room temperature and stirred for 1 hour. After the mixture was cooled to -78 ^{o}C, iodine (50 g, 197 mmol) was added in one portion, and the reaction mixture was allowed to warm to room temperature over 2 hours. The reaction was quenched by slow addition of saturated Na_2SO_3 (aq), and the organic layer was separated and successively washed with Na_2SO_3 (aq), water and saturated NaCl (aq). The resulting organic solution was dried by $MgSO_4$, filtered and concentrated in vacuo. Flash silica gel chromatography (2:1 hexane/ ethyl acetate) gave vinyl iodide **5.48** 20.5 g (78%). $Cl_3CC(NH)OPMB$ (0.53 M, 254.4 mL, 134.8 mmol) was added to solution of **5.48** (20.5 g, 103 mmol) in 500 ml CH_2Cl_2 at room temperature. CSA (1.21 g, 7.725 mmol) was added in one portion and the reaction mixture was stirred at 25 ^{o}C for 3 hours. The reaction was quenched by slow addition of

saturated Na_2CO_3 (aq), and the organic layer was separated and successively washed with water and saturated NaCl (aq). The resulting organic solution was dried by Na_2SO_4, filtered and concentrated in vacuo. Flash silica gel chromatography (2:1 hexane/ ethyl acetate) gave **5.49** 29.5 g as a clear oil (90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.26 (d, J = 7.2 Hz, 2H), 6.89 (d, J = 7.2 Hz, 2H), 5.76 (m, 1H), 4.46 (s, 2H), 4.05 (dd, J = 4.8, 0.9 Hz, 2H), 3.78(s, 3H), 2.54 (d, J = 0.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 159.3, 132.4, 130.2, 129.5, 113.8, 102.4, 74.3, 72.2, 55.3, 33.8.



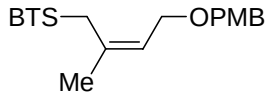
5.50

To a 10 mL two-neck flask was charged with **5.49** (94 mg, 0.3 mmol), Et_3N (96 μL, 0.6 mmol), MeOH (0.64 mL, 12 mmol), PPh_3 (8 mg, 0.033 mmol), $Pd(OAc)_2$ (3.2 mg, 0.045 mmol) and DMF (1 mL). A double-layer balloon which was filled with CO was connected to the reaction flask. The system was flushed with a gentle steam of CO for 1 min and placed in an oil bath at 70 ^{o}C. After 12 hours, the reaction mixture was poured into Hexanes/ether (1:1, 10 mL) and washed with water (2 mL, twice) and saturated NaCl (aq). The resulting organic solution was dried by $MgSO_4$, filtered and concentrated in vacuo. Flash SiO_2 chromatography (10:1 hexane/ ethyl acetate) gave **5.50** 59 mg as a clear oil (80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.27 (d, J = 7.2 Hz, 2H), 6.88 (d, J = 7.2 Hz, 2H), 6.16 (m, 1H), 4.46 (s, 2H), 4.45 (m, 2H), 3.80 (s, 3H), 3.72 (s, 3H), 1.92 (q, J = 1.5, 1.7, 1.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 168.8, 159.2, 142.2, 130.2, 129.4, 137.2, 113.8, 72.4, 68.6, 55.3, 51.5, 19.8.



5.51

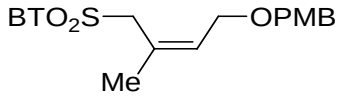
To a solution of compound **5.50** (970.1 mg, 3.85 mmol) in CH_2Cl_2 (25 mL) was slowly added DIBAL (1M, 11.5 mL, 11.5 mmol) at -78 °C. The reaction mixture was stirred at this temperature for 2 hours. The reaction was quenched with EtOAc and warmed up to 25 °C. Saturated aqueous sodium potassium tatrate (25 mL) was added, and the mixture was stirred for 20 min. The organic layer was separated, and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic layer was washed with brine and dried over Na_2SO_4. The crude product was concentrated in vacuum snf purified by flash silica gel chromatography (10:1 hexane/ ethyl acetate) tto afford **5.51** 769 mg as a clear oil (90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.27 (d, J = 7.2 Hz, 2H), 6.88 (d, J = 7.2 Hz, 2H), 5.57 (m, 1H), 4.46 (s, 2H), 4.09 (s, 2H), 4.01 (d, J = 6.9 Hz, 2H), 3.80 (s, 3H), 1.96 (s, 1H), 1.84 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 159.3, 141.0, 130.0, 129.5, 123.8, 113.9, 72.1, 65.4, 62.0, 55.3, 21.7.



5.51'

To a solution of compound **5.51** (659mg, 2.94 mmol), 2-mercaptobenzothiazole (0.59mL, 3.53 mmol) and PPh_3 (1.56g, 5.58 mmol) in THF (20 mL) was added DIAD (1.1 mL, 5.58 mmol) dropwise at 0 °C. The mixture was warmed up gradually to room temperature and kept at room temperature for 4 hours. The reaction was quenched by addition of saturated Na_2CO_3 (aq), and the organic layer was separated. The aqueous

layer was extracted by CH_2Cl_2 (3 x 20 mL). The organic layers were combined and successively washed with water and saturated NaCl (aq). The resulting organic solution was dried by Na_2SO_4, filtered, and concentrated in vacuo. The resulting crude product was purified by silica gel chromatography (10:1 hexane/ ethyl acetate) to afford **5.51'** 1.04g as a clear oil (95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.85 (d, J = 8.1 Hz, 1H), 7.74 (d, J = 8.1 Hz, 1H), 7.40 (t, J = 1.2, 1.2 Hz, 1H) 7.32 (m, 1H), 7.27 (d, J = 7.2 Hz, 2H), 6.85 (d, J = 7.2 Hz, 2H), 5.61 (m, 1H), 4.43 (s, 2H), 4.11 (d, J = 6.9 Hz, 2H), 4.08 (s, 2H), 3.76 (s, 3H), 1.90 (d J = 0.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 166.6, 159.6, 153.3, 135.6, 134.2, 130.5, 129.7, 127.4, 126.3, 124.5, 121.7, 121.2, 114.03, 72.4, 66.3, 55.5, 35.3, 23.1.



5.26

A solution of compound **5.51'** (38 mg, 0.102 mmol) in EtOH (1 mL) was added H_2O_2 (0.204 mL, 0.204 mmol) and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ at 0 °C. The mixture was warmed gradually to room temperature and kept at room temperature for 12 hours. The reaction was quenched by addition of saturated Na_2CO_3 (aq). The aqueous layer was extracted by CH_2Cl_2 (3 x 20 mL). The organic layers were combined and successively washed with water and saturated NaCl (aq). The resulting organic solution was dried by Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography (3:1 hexane/ ethyl acetate) to afford **5.26** 41 mg as a clear oil (99%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 8.20 (d, J = 7.5 Hz, 1H), 7.99 (d, J = 7.5 Hz, 1H), 7.61 (m, 2H) 7.15 (d, J = 8.7 Hz, 2H), 6.82 (d, J = 8.7 Hz, 2H), 5.81 (m, 1H), 4.30 (s, 2H),

4.27 (s, 2H) 3.86 (dd, J = 7.2, 1.2 Hz, 2H), 3.78 (s, 3H), 1.95 (d J = 1.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 165.6, 159.2, 152.6, 132.8, 129.8, 129.3, 129.2, 128.0, 127.7, 125.4, 125.0, 122.3, 113.7, 72.2, 65.9, 58.0, 55.2, 24.4.

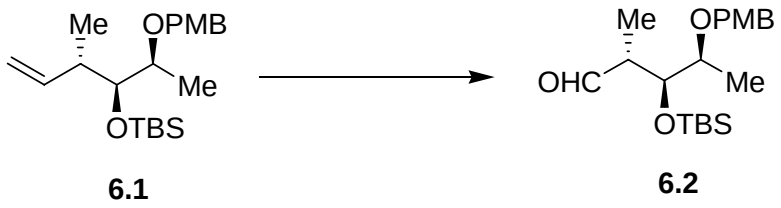
CHAPTER 6

AN IMPROVED PROCEDURE FOR THE OXIDATIVE CLEAVAGE OF OLEFINS BY OsO_4-$NaIO_4$

6.1 Introduction

This work has been published in Organic Letters.[87] It should be noted that Dr. Wensheng Yu made the most important contribution to this project.

To synthesize the polypropionate portion of Superstolides A, olefin **6.1** was needed to be converted to aldehyde **6.2** by an oxidative cleavage reaction (Scheme 6.1).[84, 85]



Scheme 6.1 Oxidative cleavage reaction

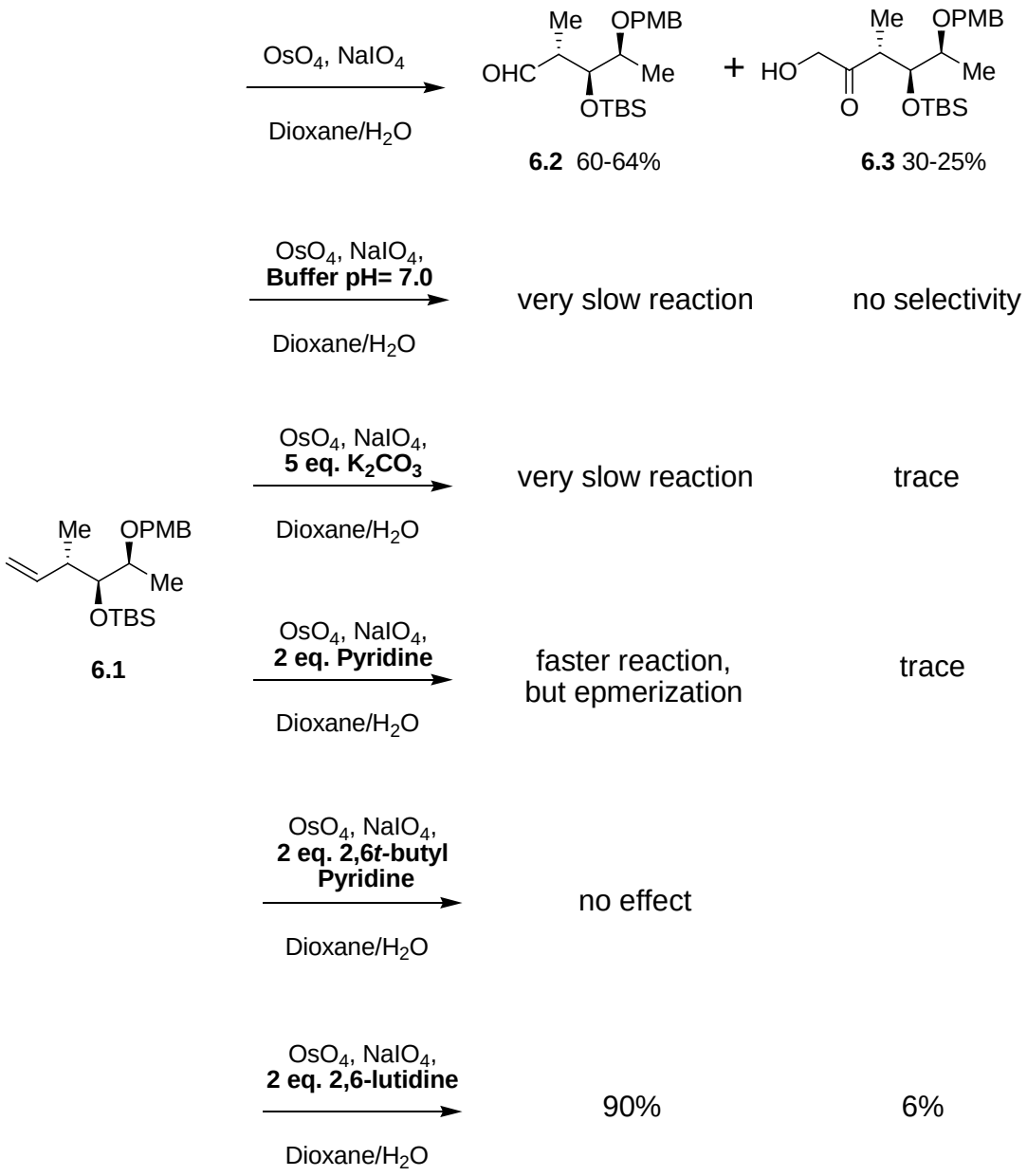
Under the standard Johnson-Lemieux oxidation conditions of OsO_4-$NaIO_4$ protocol,[86] the oxidative cleavage of the double bond of compound **6.1** provided the desired aldehyde **6.2** in only 60-64% yield (Scheme 6.2). The unexpected side product of α-hydroxy ketone **6.3** was isolated from the reaction mixture in 25-30% yield. The formation of the α-hydroxy ketones as side products under the Johnson-lemieux procedure was previously reported. [88] And we also noticed that the tedious two-step procedure (dihydroxylation with OsO_4-NMO or Sharpless asymmetric dihydroxylation

followed by oxidative cleavage by $NaIO_4$) was used much more often than the one-step Johnson-lemieux procedure.[88-92] Therefore, we decided to investigate the possibility of improving this classic oxidative cleavage reaction.

6.2 Effects of Various Bases on the Rate of the Reaction and the Product Distribution.

Because the reaction media was acidic (pH=2), we thought that the acid might be the reason to form the α-hydroxy ketone side-product, and buffer solution might change the result of the reaction. Thus phosphate buffer (pH=7) was used to replace deionized water. However, no improvement was observed. We speculated that the addition of a base might suppress the formation of compound **6.3** and improve the yield of **6.2**. To our delight, TLC showed very good selectivity and only a trace amount of **6.3** was formed when powdered K_2CO_3 was added. But the reaction rate decreased significantly and it didn't reach completion after 24 hours. This result indicated that the addition of a weak base could suppress the formation of compound **6.3**. However, we needed to find the a base that could inhibit the formation of **6.3**, but not slow the reaction rate. It is well known that the addition of a tertiary amine such as pyridine can dramatically increase the rate of formation of osmium (IV) ester complexes.[93, 94] We found that 2 eq. pyridine indeed prevented the formation of **6.3**, and at the same time increased the reaction rate. Unfortunately, the methyl group at the α-position of aldehyde **6.2** was epimerized via enolization of the aldehyde promoted by pyridine. To avoid the epimerization, a hindered base, 2, 6-di-*t*-butyl pyridine, was used. But no effect was observed. Therefore, the next

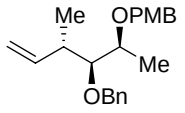
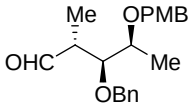
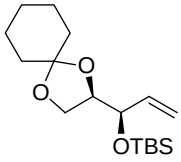
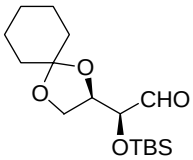
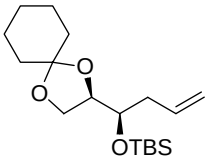
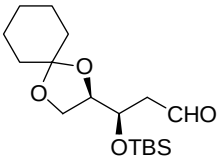
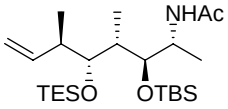
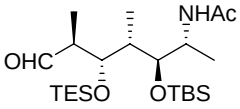
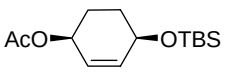
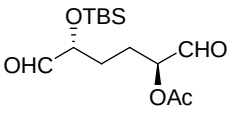
logical choice of base was 2, 6-lutidine. Indeed, 2, 6-lutidine provided the best result, and compound **6.3** was isolated in 90% yield.



Scheme 6.2 Effects of Various Bases on the Rate of the Reaction and the Products Distribution

6.3 A Superior Improvement of the Oxidative Cleavage of Olefins

Table 6.1 A Superior Improvement of the Oxidative cleavage of olefins

entry	substrate	product	time, yield (without 2,6-lutidine)	time, yield (with 2,6-lutidine)
1	 **6.4**	 **6.5**	1h, 44%	1h, 83%
2	 **6.6**	 **6.7**	20h, 34%	20h, 99%
3	 **6.8**	 **6.9**	3h, 21%	3h, 81%
4	 **6.10**	 **6.11**	3h, 42%	3h, 71%
5	 **6.12**	 **6.13**	24h, 28%	24h, 77%

The superior improvement by 2, 6-lutidine led us to study the broad scope of this reaction (Table 6.1). The oxidative cleavage reaction of compound **6.4** under the literature protocol (without 2, 6-lutidine) was very messy, and only gave 44% yield of the desired aldehyde **6.5** along with many uncharactized side products. Amazingly, with the addition of 2, 6-lutidine, the yield was improved to 83%. It indicated that the 2, 6-lutidine could suppress several uncharacterized side reactions at the same time. Under the literature protocol, the oxidative cleavage reaction of compound **6.6** was quite slow and afforded only 34% of the desired product **6.7** along with the recovery of 48% **6.6** after stirring at room temperature for 20 hours. We found longer reaction time caused lower yield due to the cleavage of TBS group. With our modified conditions (with 2, 6-lutidine), the reaction afforded 99% yield of **6.7** in 20 hours. In this reaction, 2, 6-lutidine not only inhibited the formation of side products, but also accelerated the reaction rate. The similar observations were also obtained in compounds **6.8, 6.10** and **6.12**.

6.4 Conclusion

The classic Johnson-Lemiux oxidation often suffers low yield due to the formation of side products. We found that the addition of 2, 6-lutidine can suppress the side reactions, accelerate the reaction rate and dramatically improve the yield of this very important reaction.

6.5 Experimental section

Typical Procedure for the improved OsO_4-$NaIO_4$ oxidative cleavage reaction follows: To a solution of olefin **6.1** (296mg, 0.812 mmol) in dioxane-water (3:1, 8 mL) was added 2,6-lutidine (0.189 mL, 1.62 mmol), OsO_4 (2.5% in 2-methyl-2-propanol,

165mg, 0.016 mmol), and $NaIO_4$ (695 mg, 3.25 mmol). The reaction mixture was stirred at 25 °C and monitored by TLC. After the reaction was complete, water (10 mL0 and CH_2Cl_2 (20 mL) was added. The organic layer was separated and the water later was extracted by CH_2Cl_2 (10 mL) 3 times. The combined organic layer was washed with brine and dried over Na_2SO_4. After removal of the solvent, the product was purified by flash column chromatography to obtain aldehyde **6.2** as colorless iol.

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APPENDIX A NMR DATA

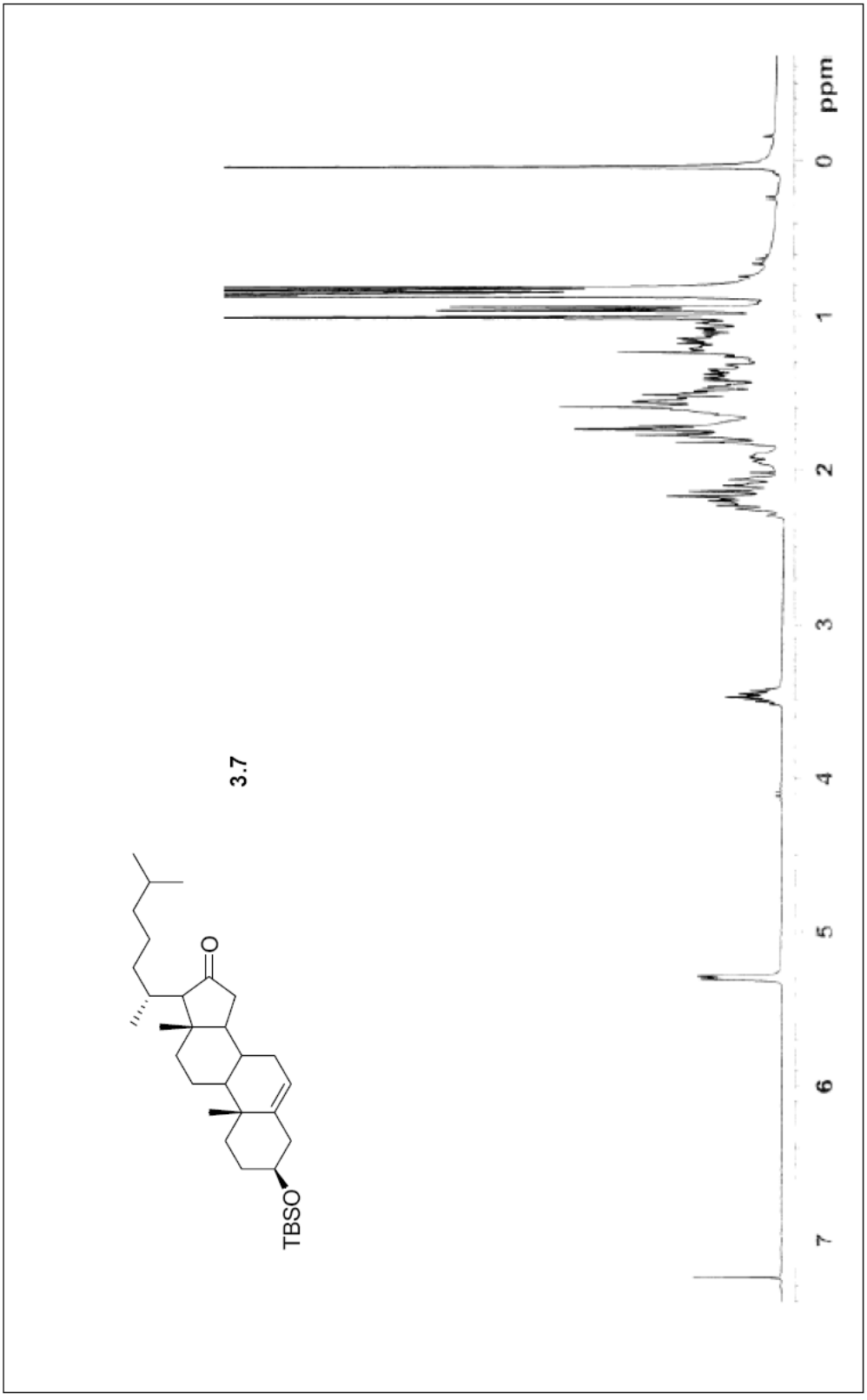

Figure A.1 300 MHz ^{1}H-NMR of compound 3.7 ($CDCl_3$)

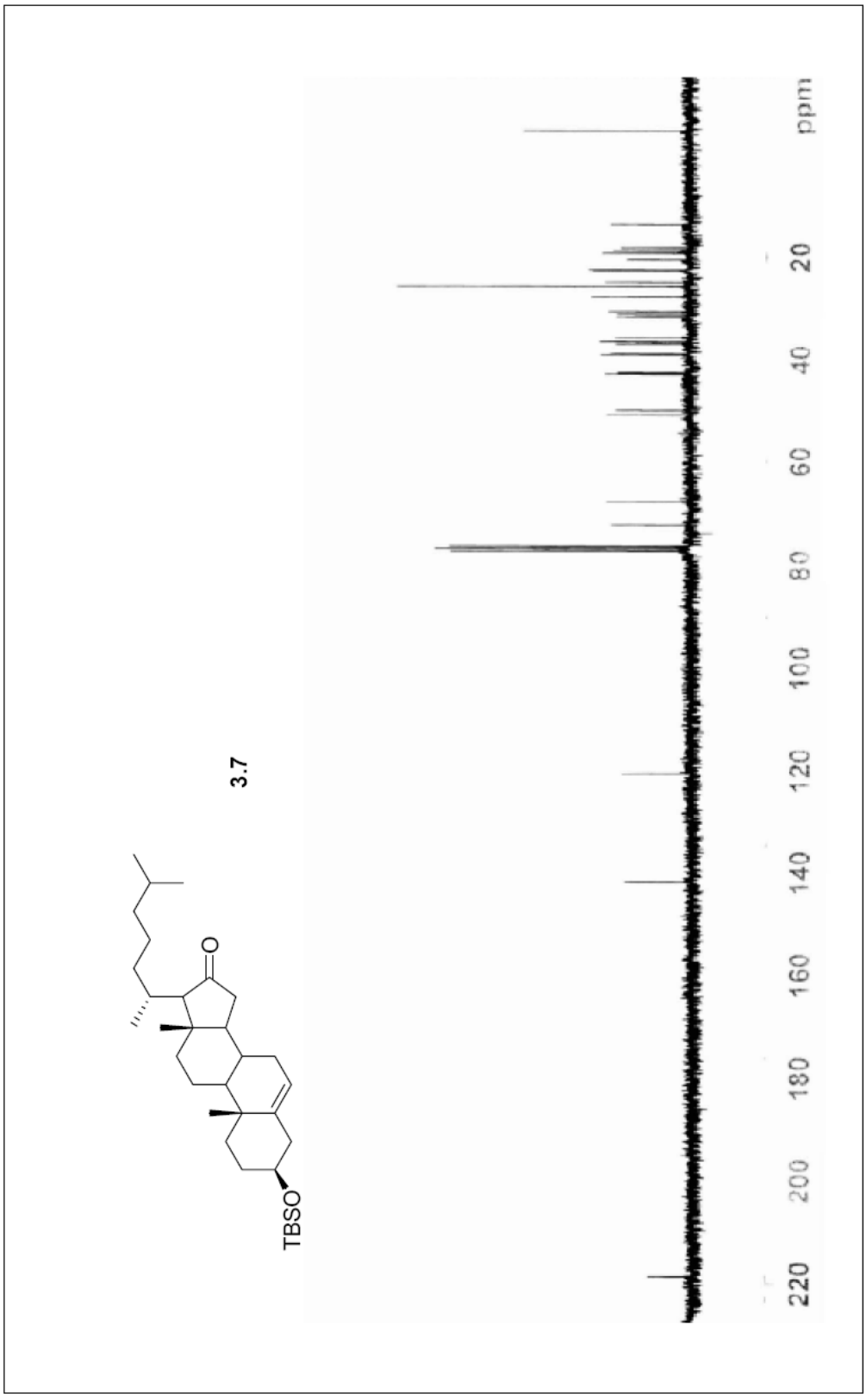

Figure A.2 75 MHz ^{13}C-NMR of compound 3.7 ($CDCl_3$)

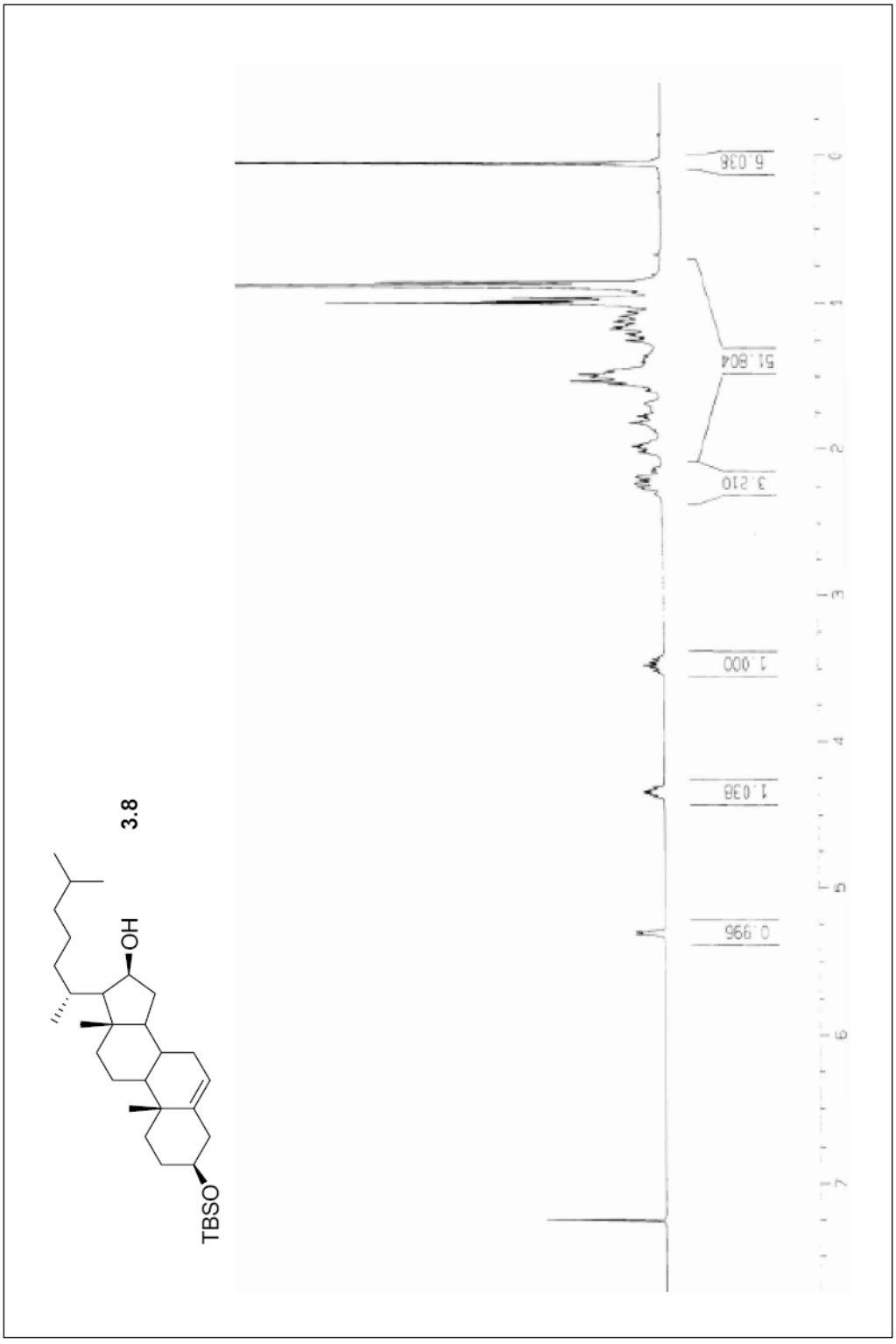

Figure A.3 300 MHz ^{1}H-NMR of compound 3.8 ($CDCl_3$)

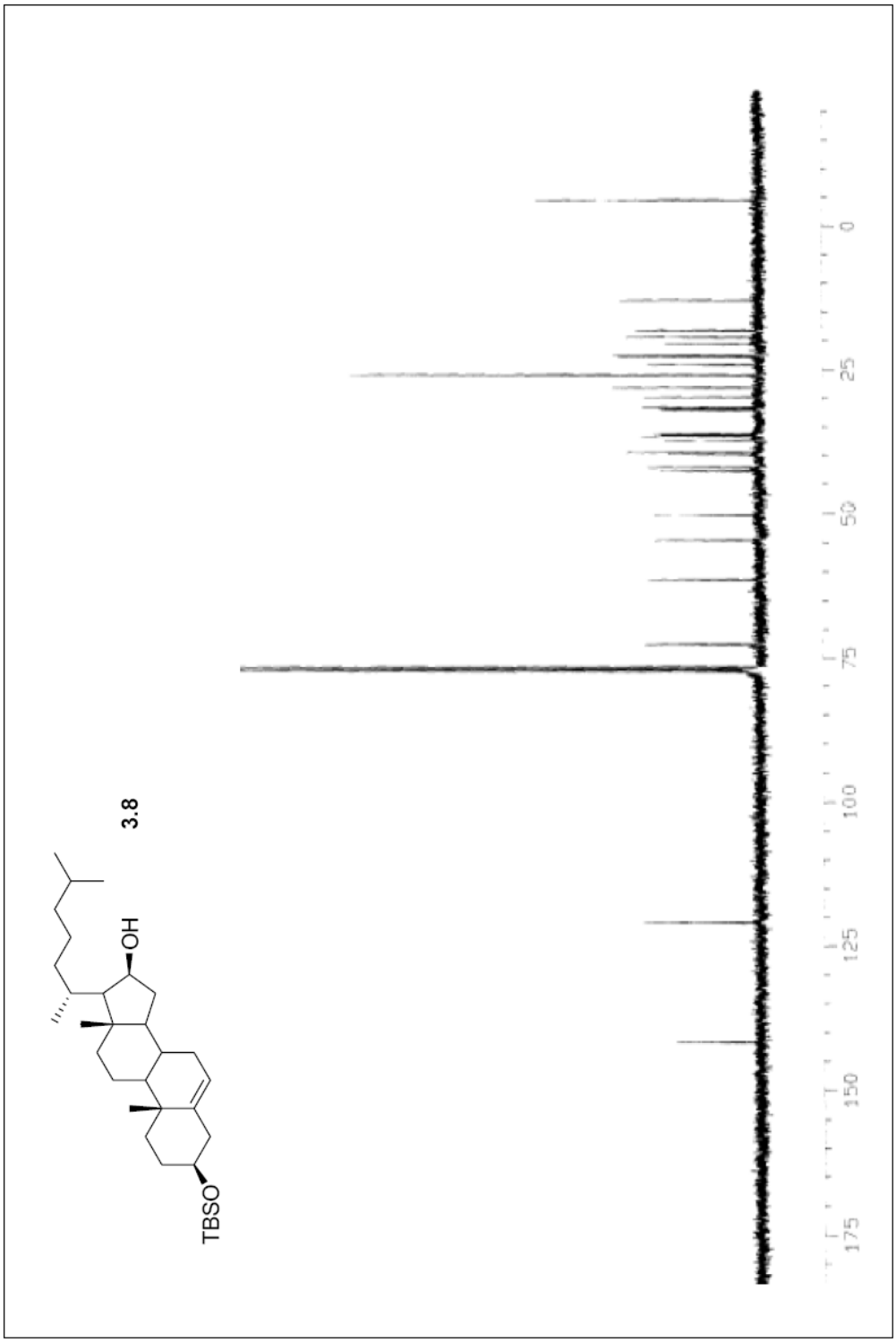

Figure A.4 75 MHz ^{13}C-NMR of compound 3.8 ($CDCl_3$)

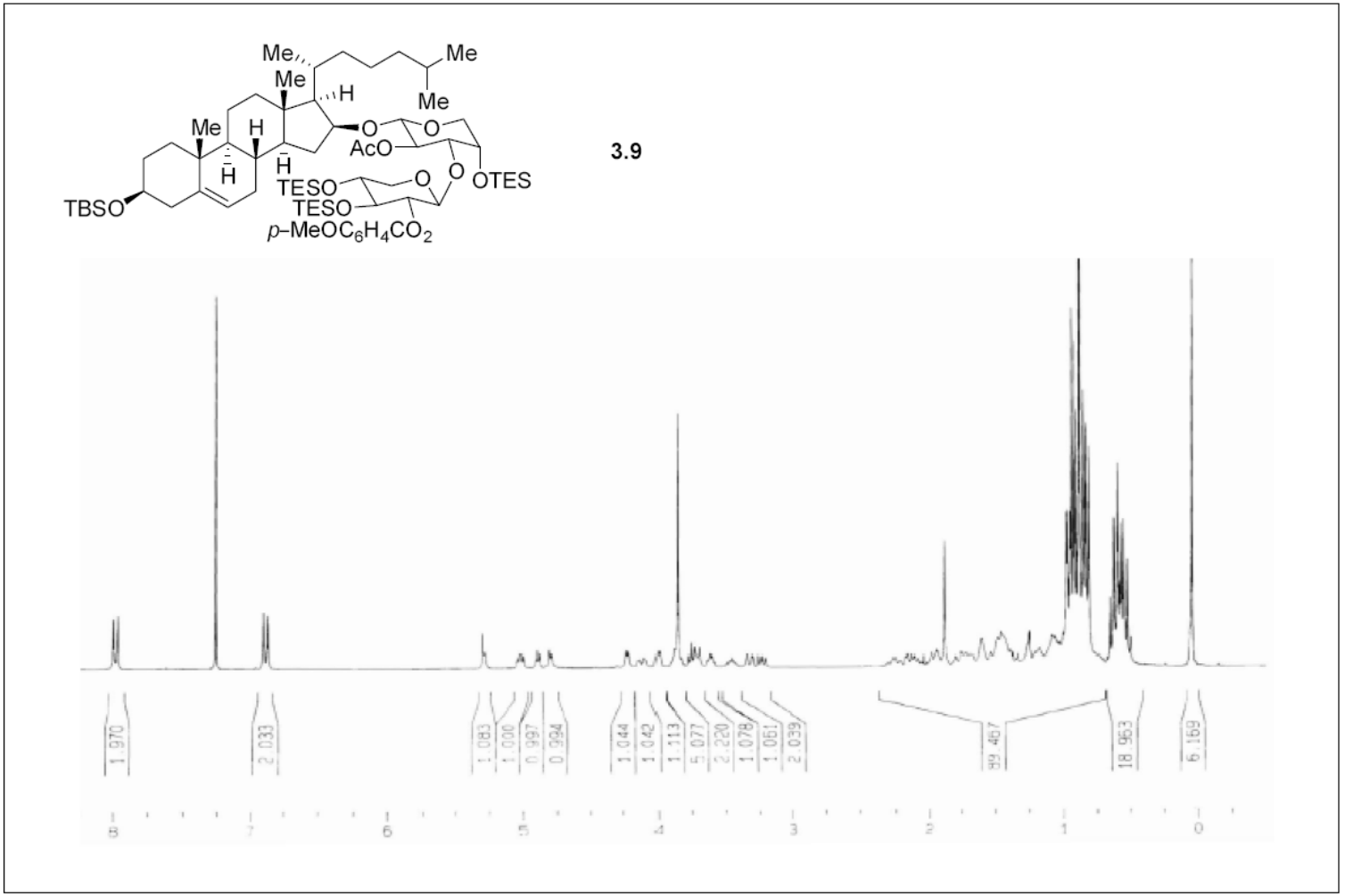

Figure A.5 300 MHz ^{1}H-NMR of compound 3.9 ($CDCl_3$)

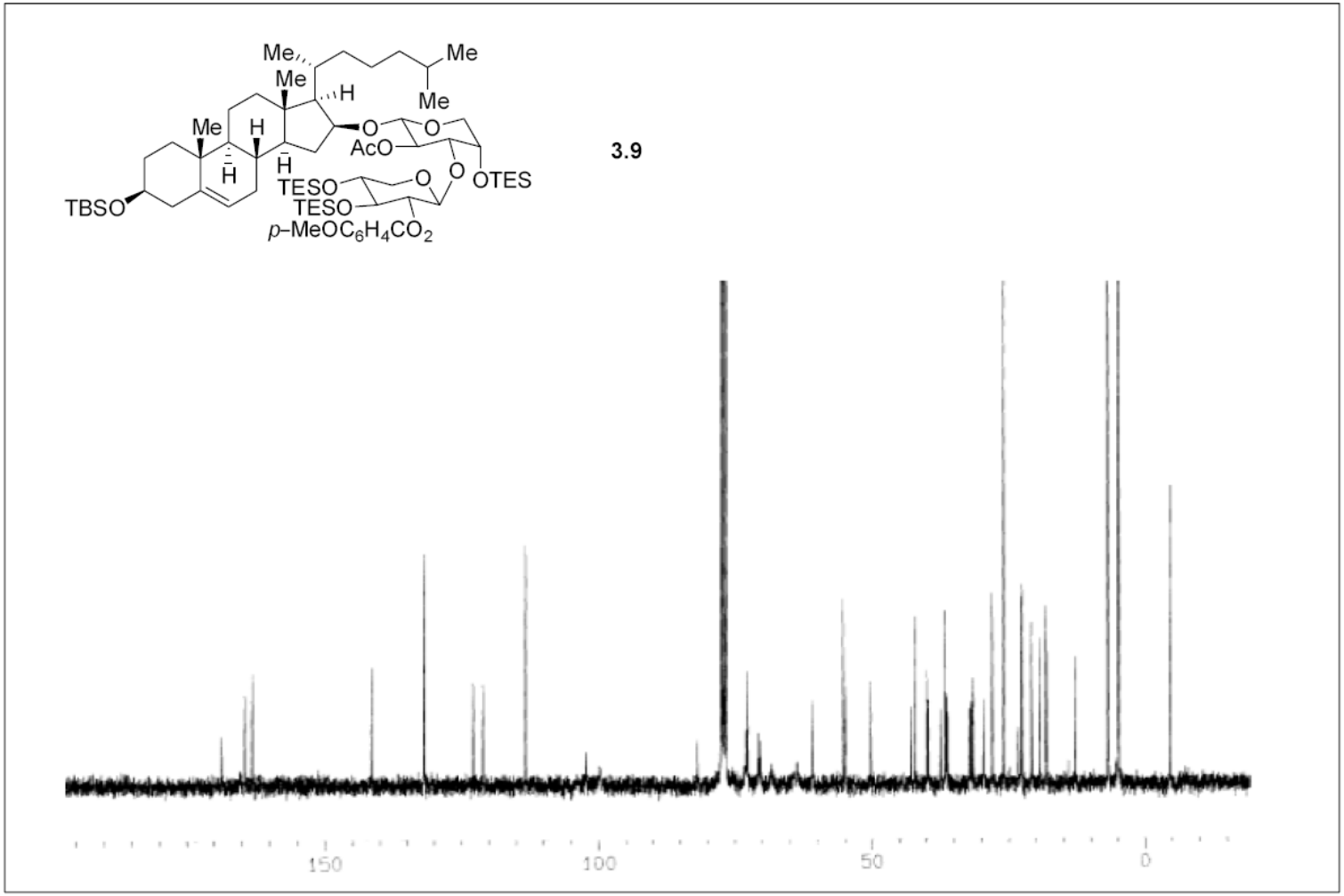

Figure A.6 75 MHz ^{13}C-NMR of compound 3.9 ($CDCl_3$)

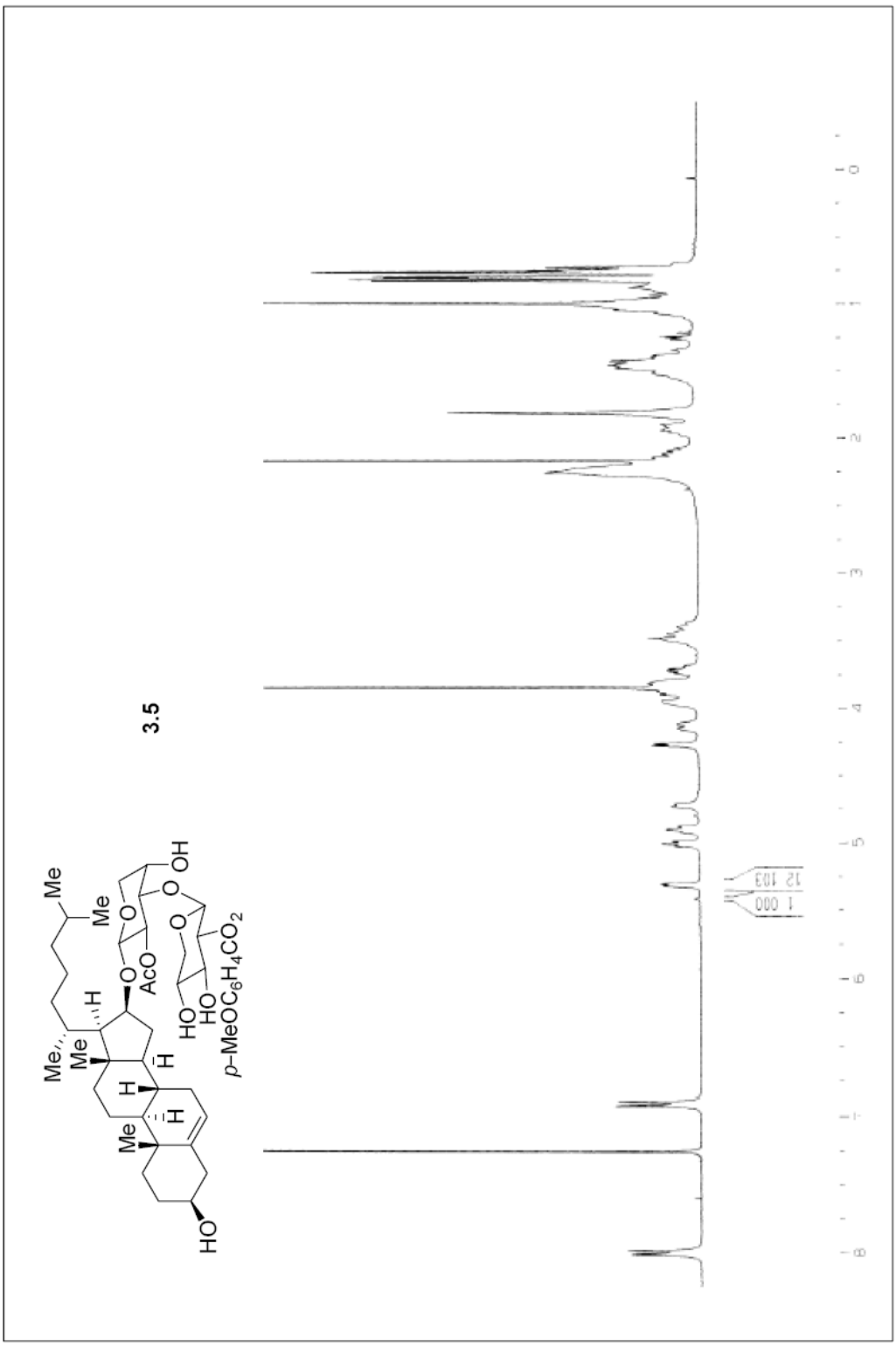

Figure A.7 300 MHz ^{1}H-NMR of compound 3.5 ($CDCl_3$)

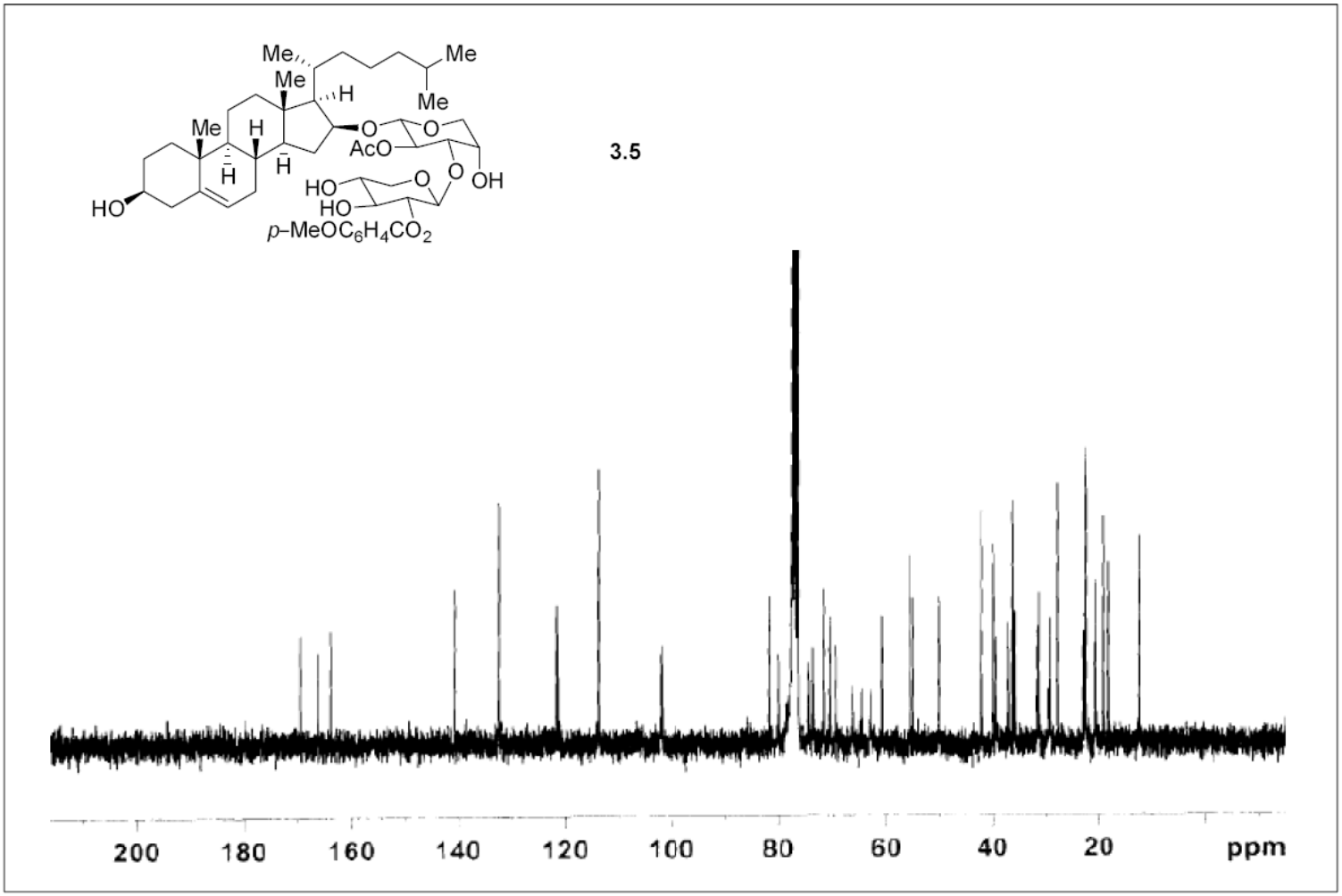

Figure A.8 75 MHz ^{13}C-NMR of compound 3.5 ($CDCl_3$)

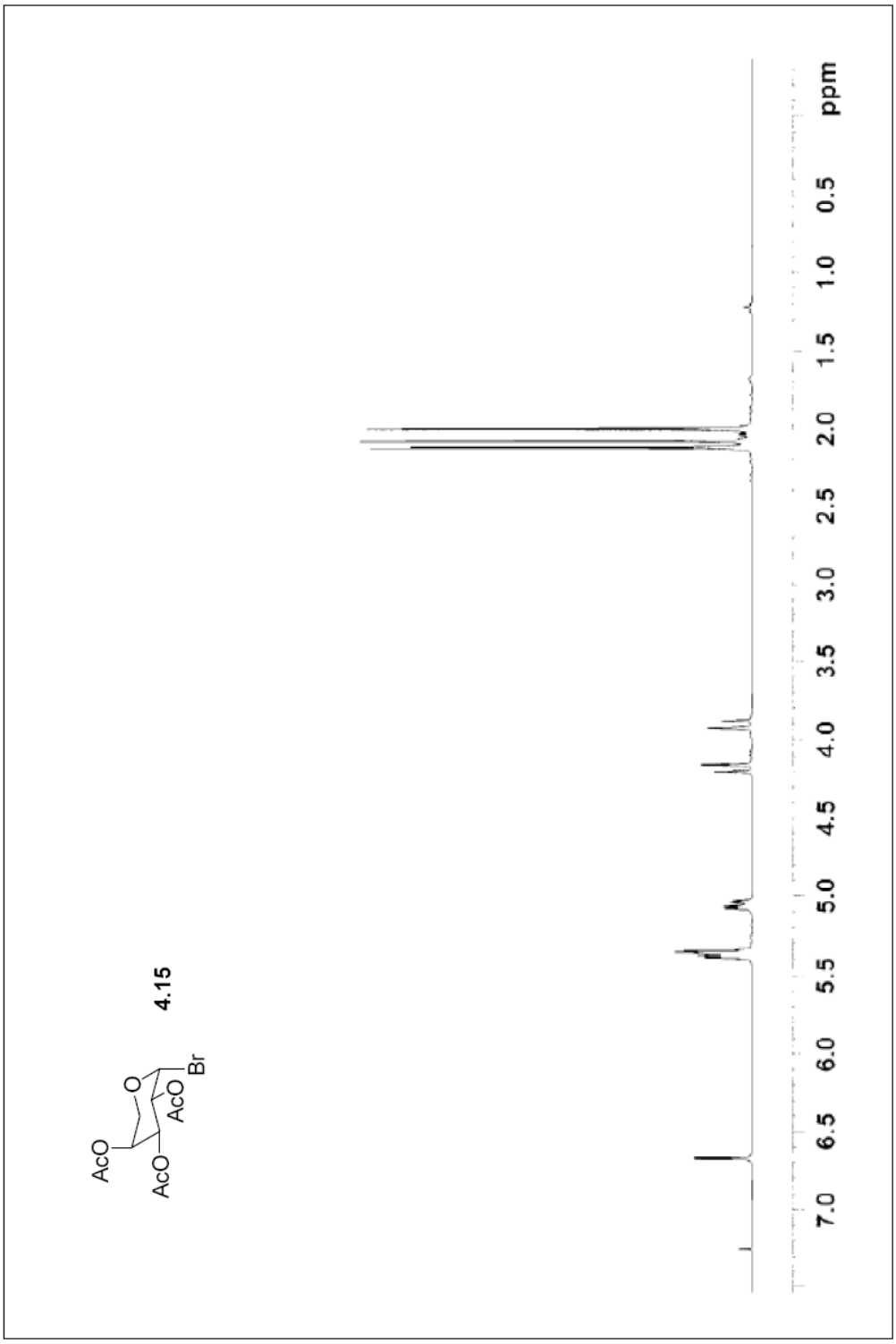

Figure A.9 300 MHz ^{1}H-NMR of compound 4.15 ($CDCl_3$)

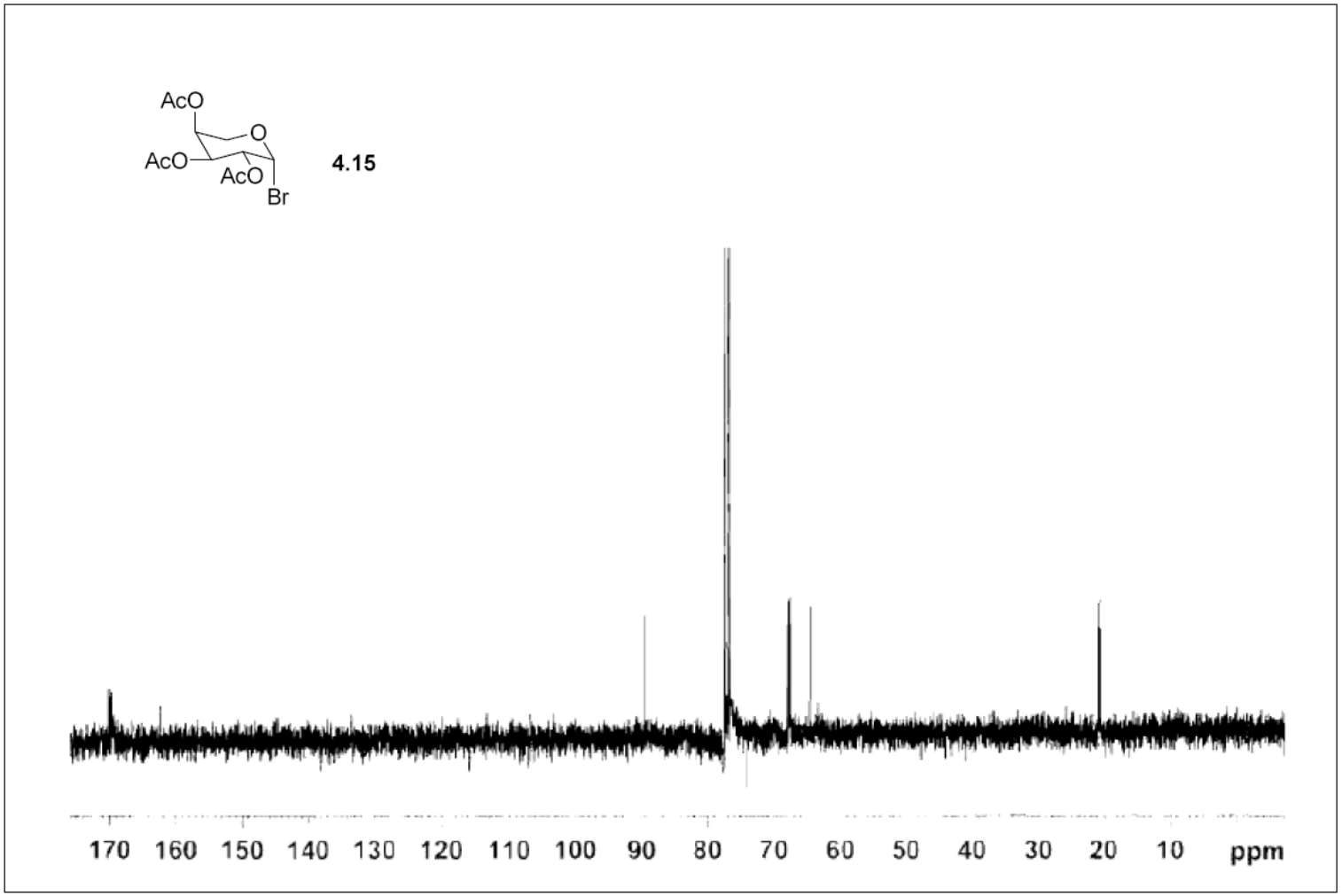

Figure A.10 75 MHz ^{13}C-NMR of compound 4.15 ($CDCl_3$)

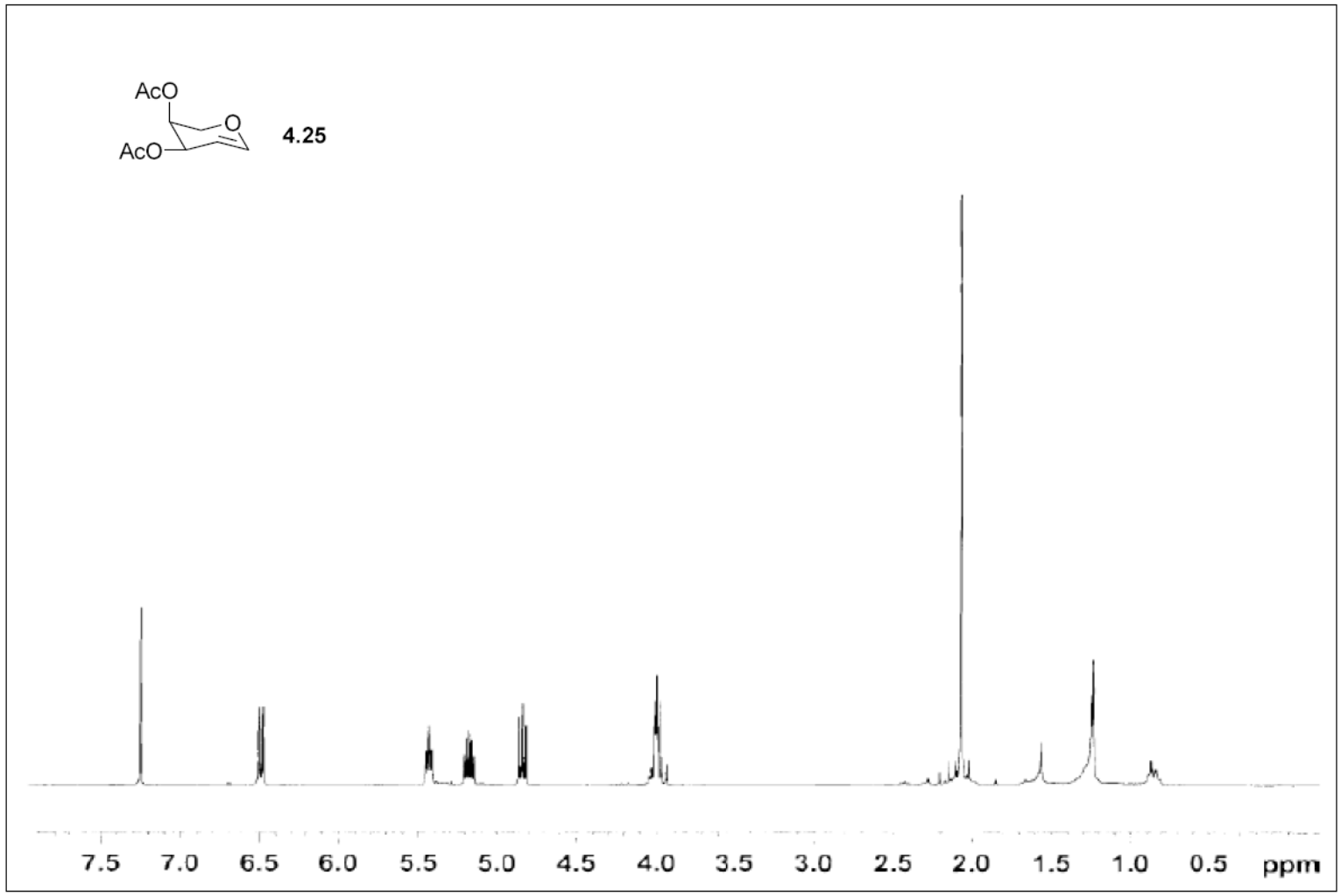

Figure A.11 300 MHz ^{1}H-NMR of compound 4.25 ($CDCl_3$)

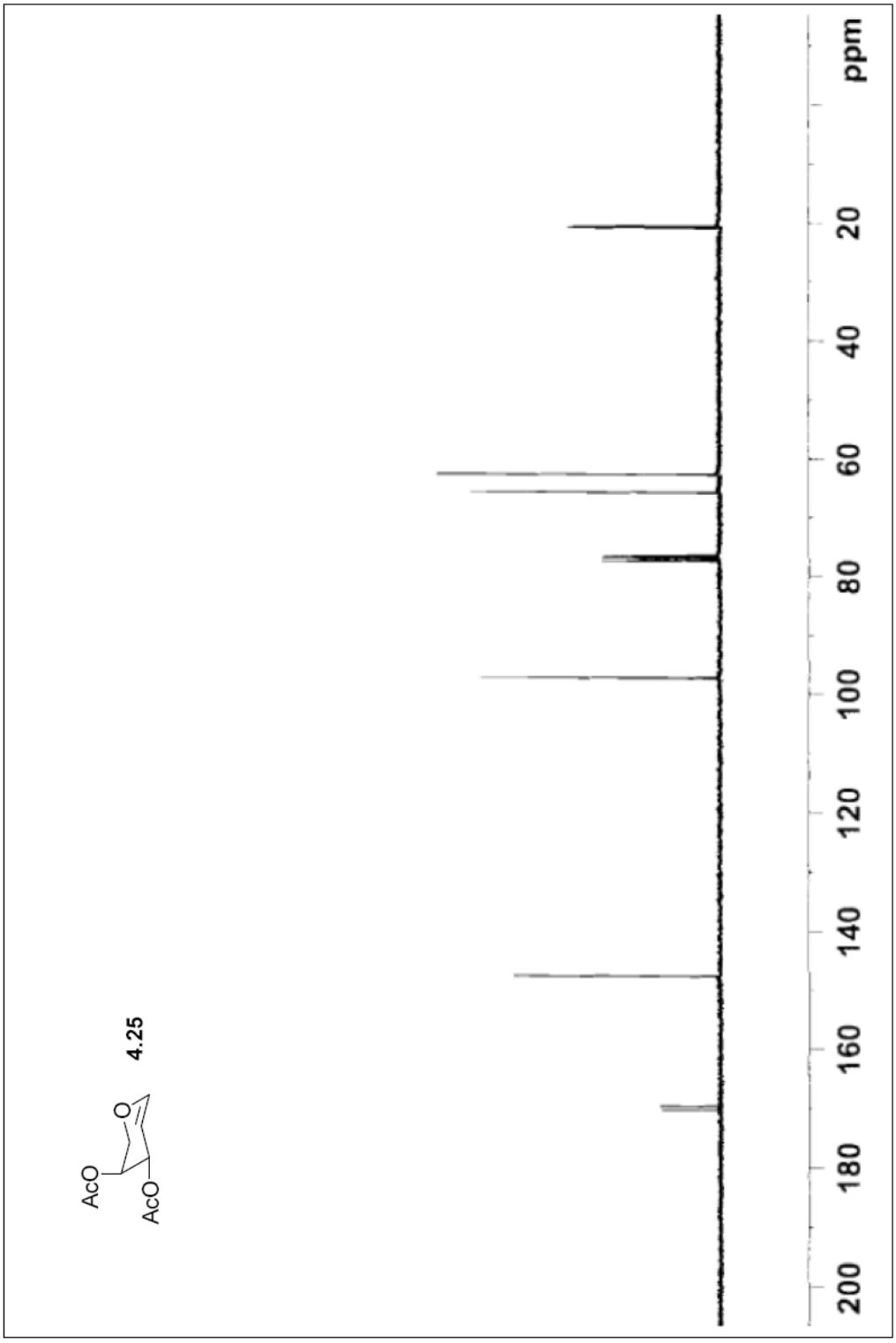

Figure A.12 75 MHz ^{13}C-NMR of compound 4.25 ($CDCl_3$)

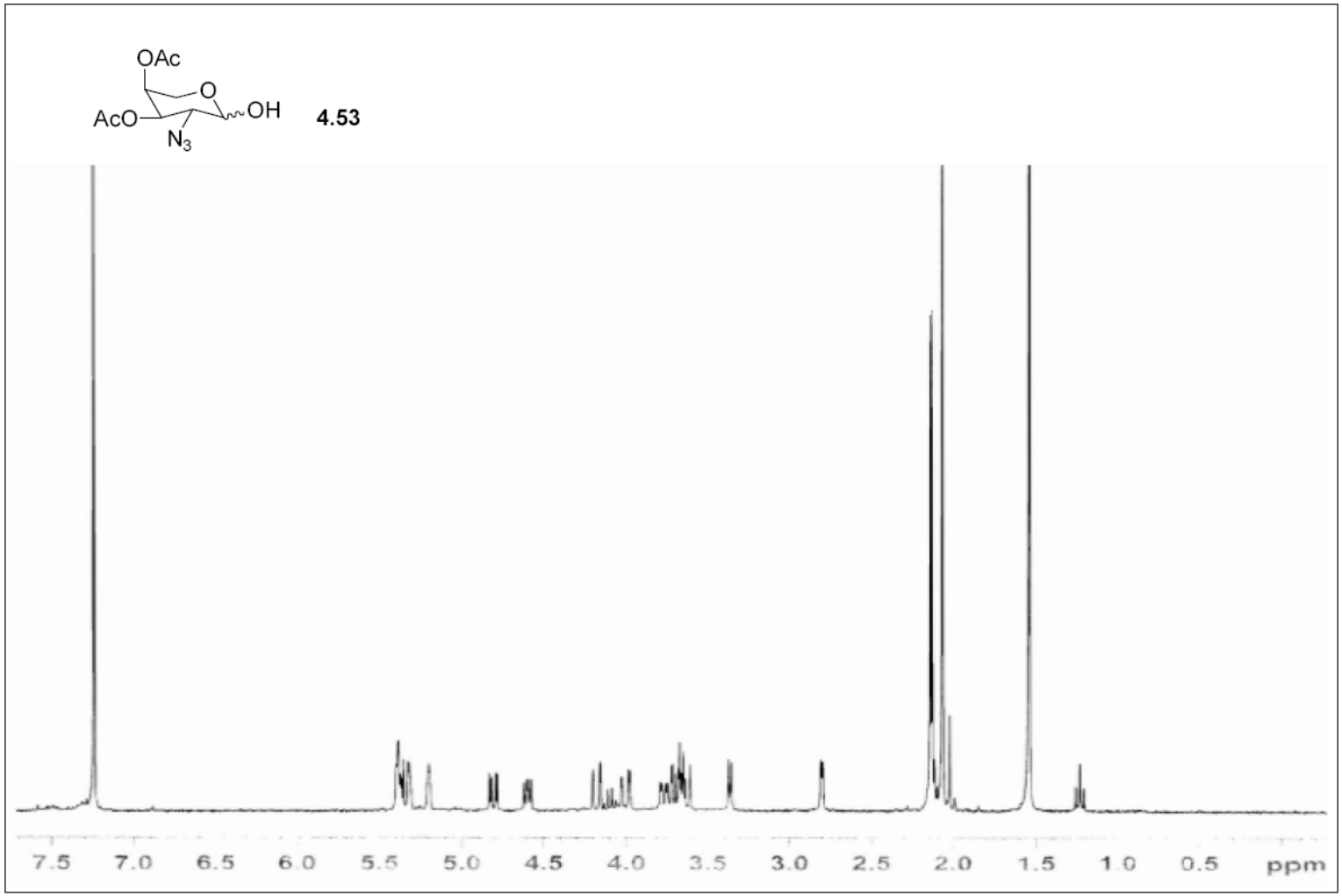

Figure A.13 300 MHz ^{1}H-NMR of compound 4.53 ($CDCl_3$)

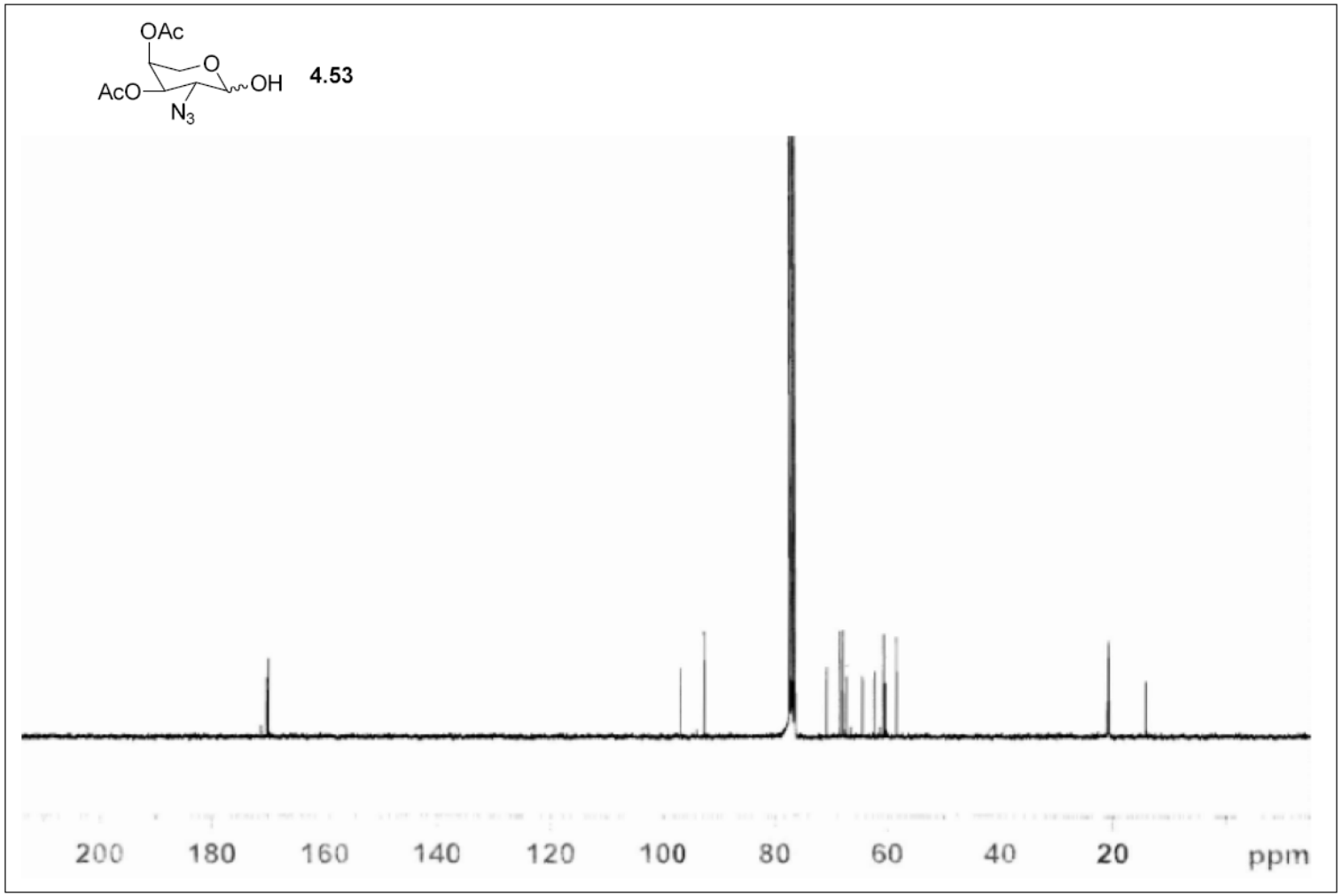

Figure A.14 75 MHz ^{13}C-NMR of compound 4.53 ($CDCl_3$)

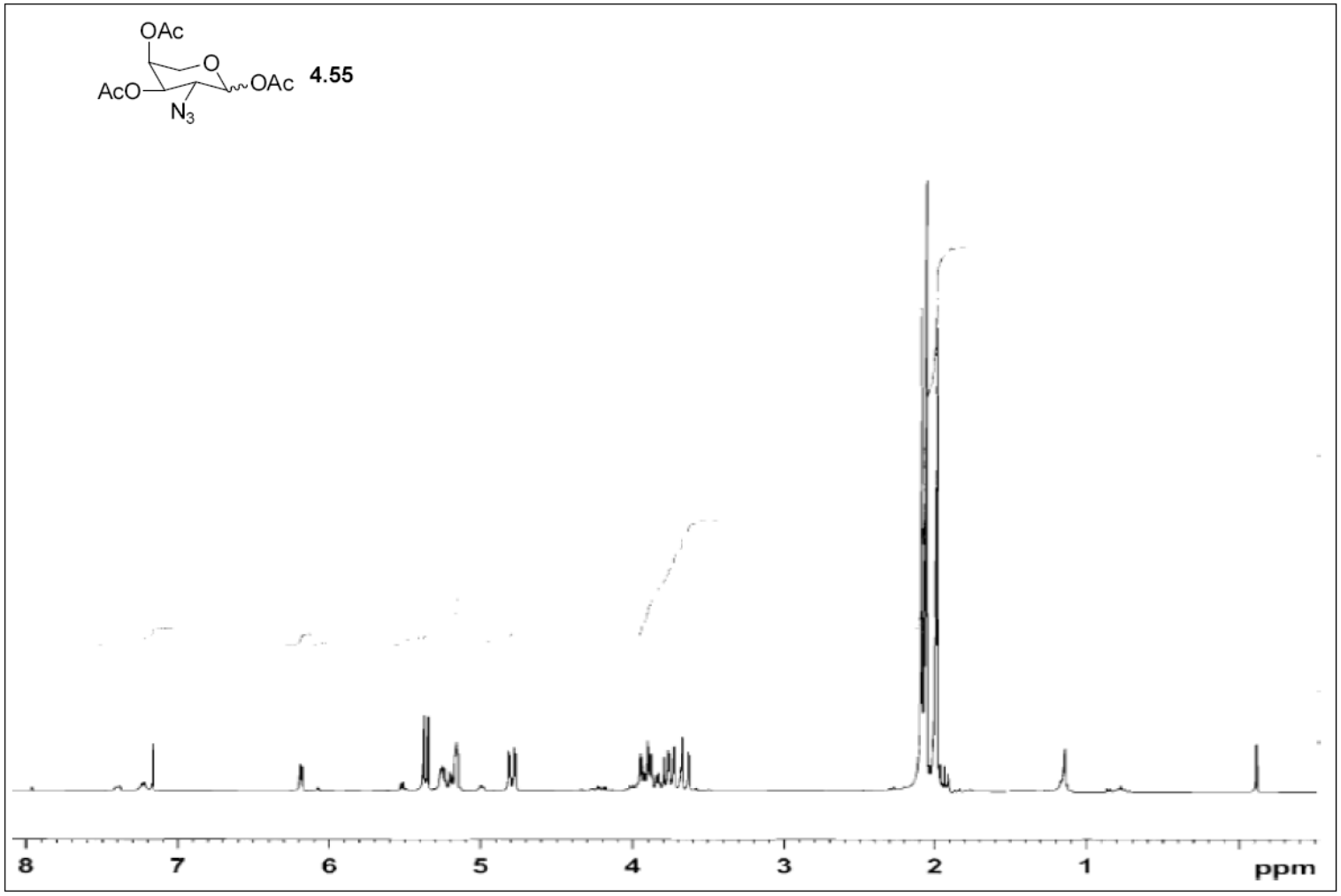

Figure A.15 300 MHz ^{1}H-NMR of compound 4.55 ($CDCl_3$)

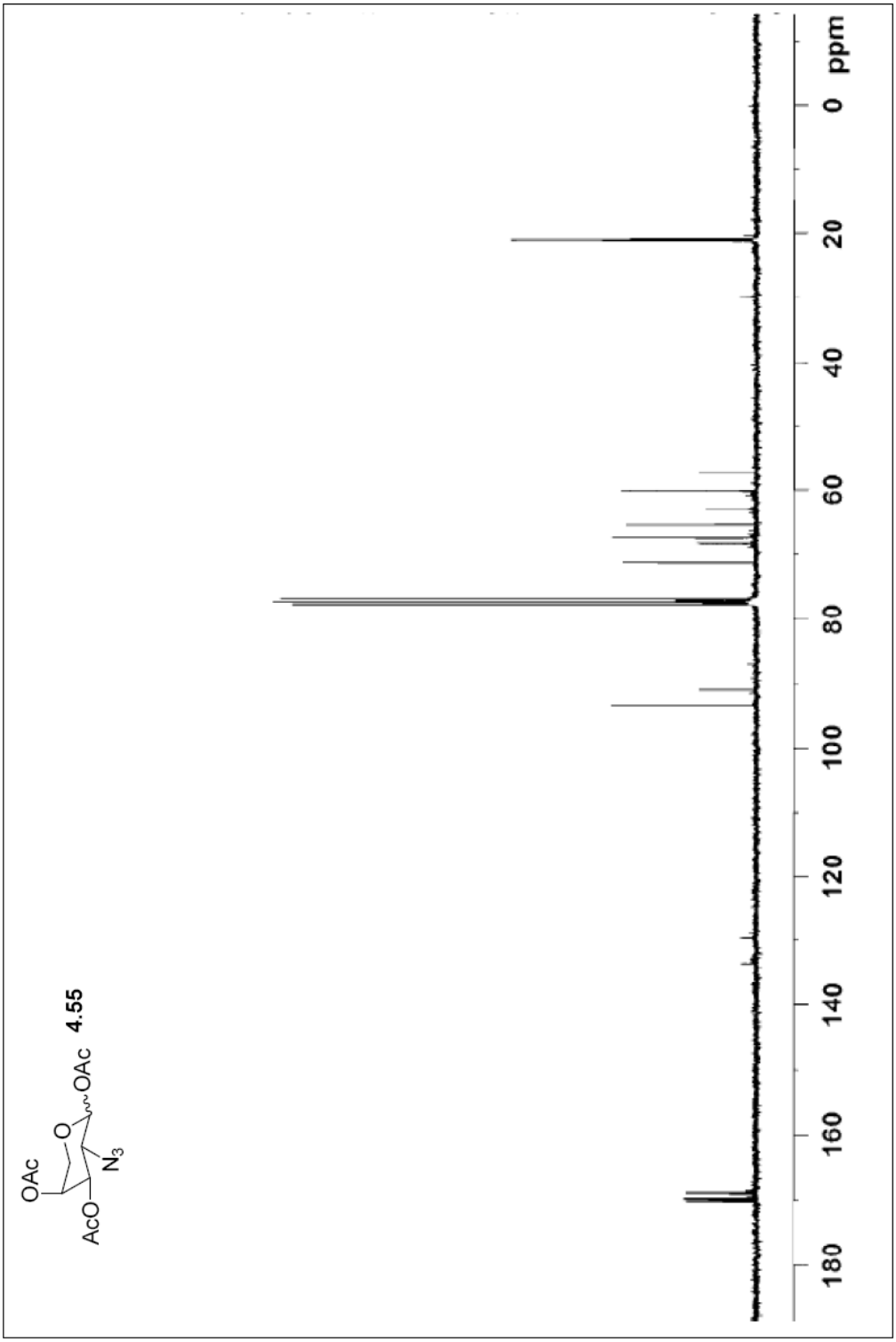

Figure A.16 75 MHz ^{13}C-NMR of compound 4.55 ($CDCl_3$)

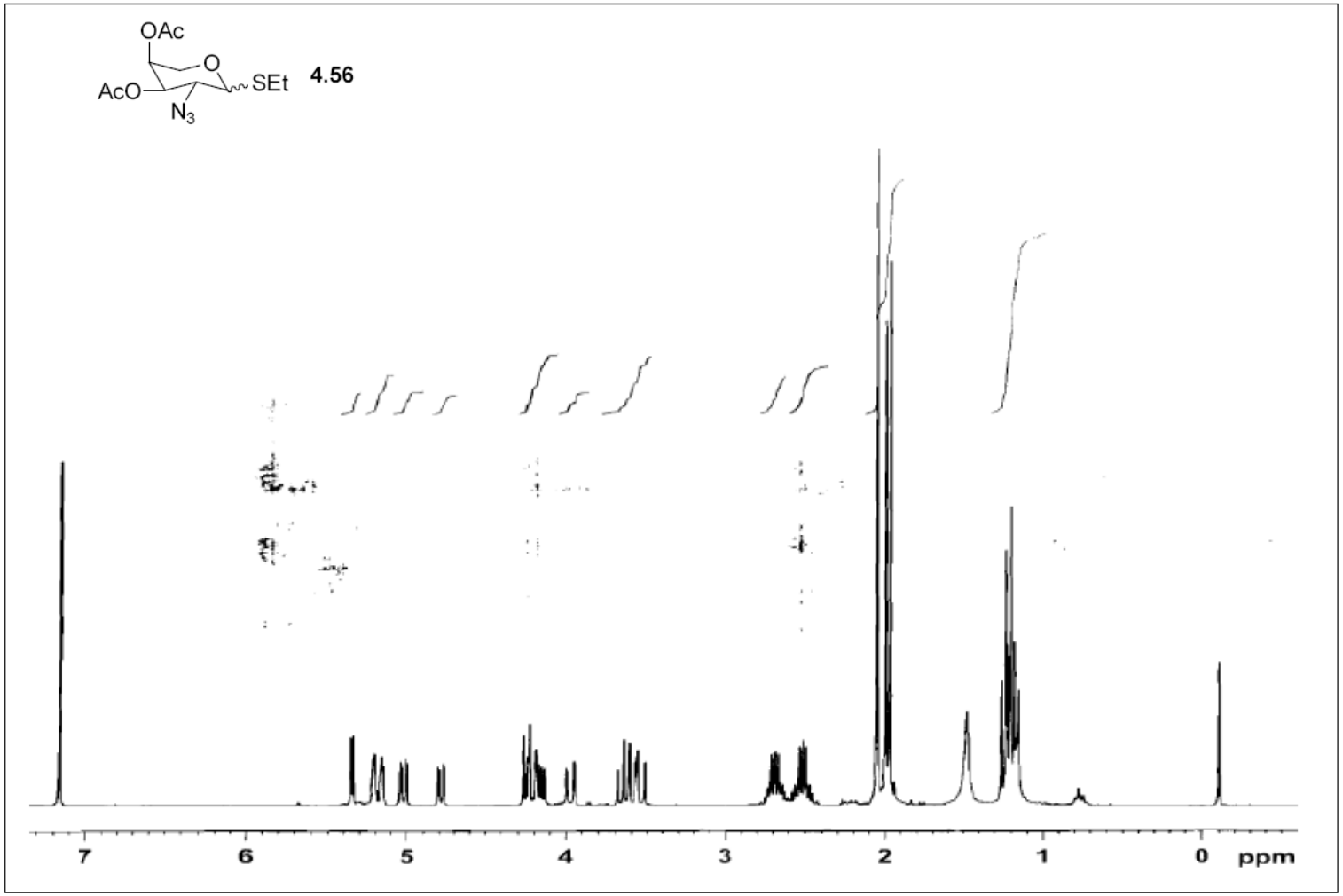

Figure A.17 300 MHz ^{1}H-NMR of compound 4.56 ($CDCl_3$)

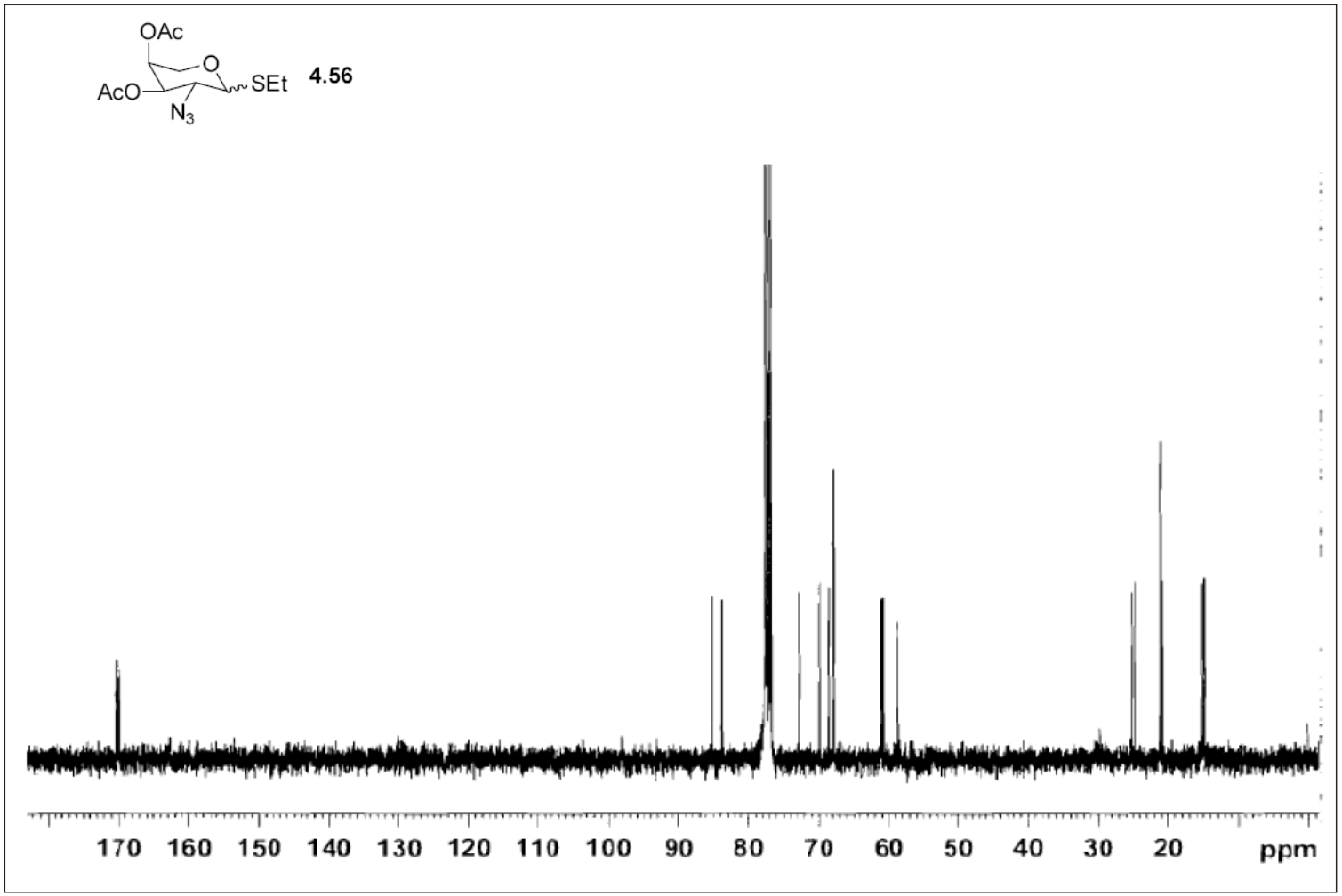

Figure A.18 75 MHz ^{13}C-NMR of compound 4.56 ($CDCl_3$)

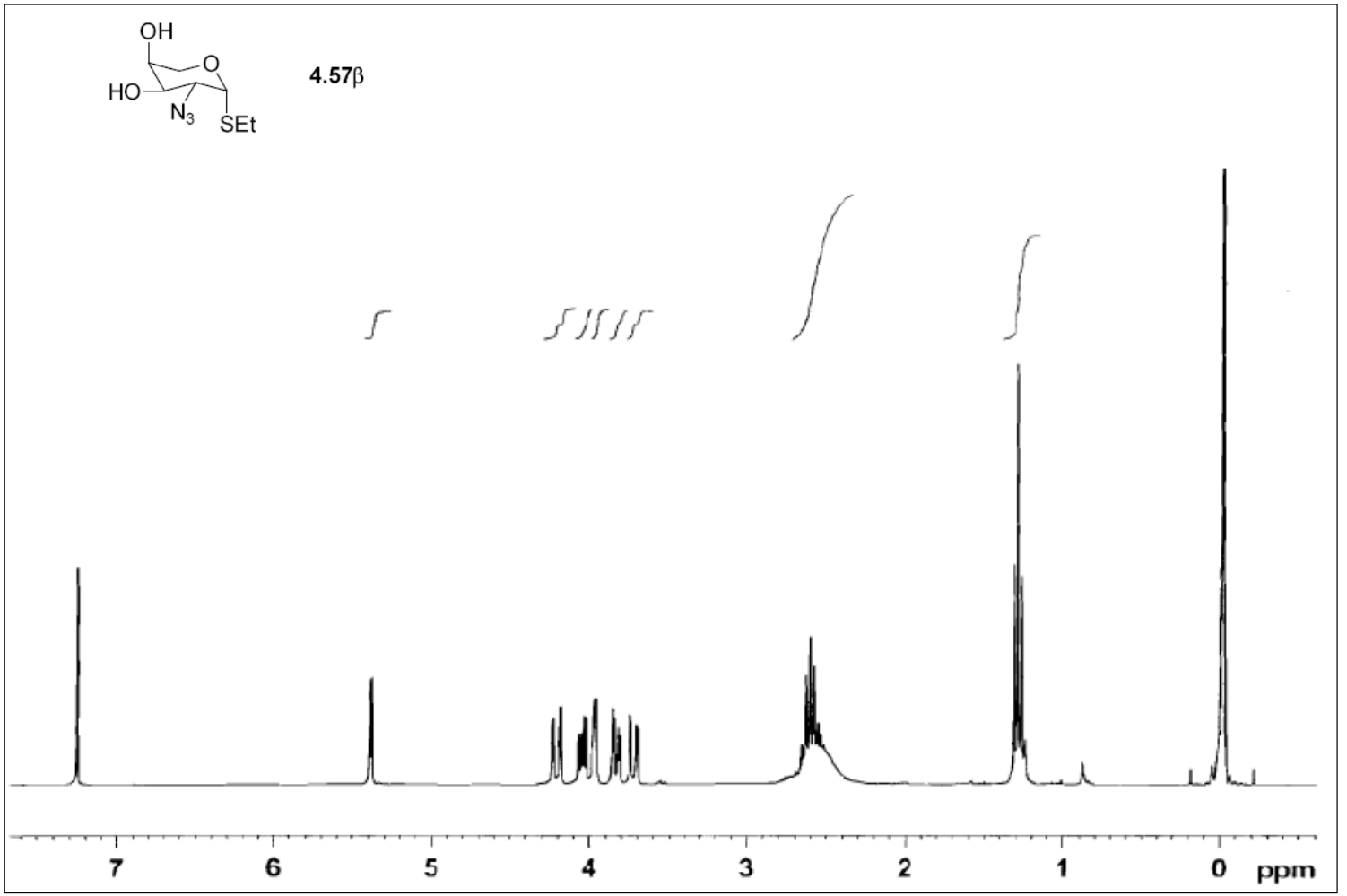

Figure A.19 300 MHz ^{1}H-NMR of compound 4.57β ($CDCl_3$)

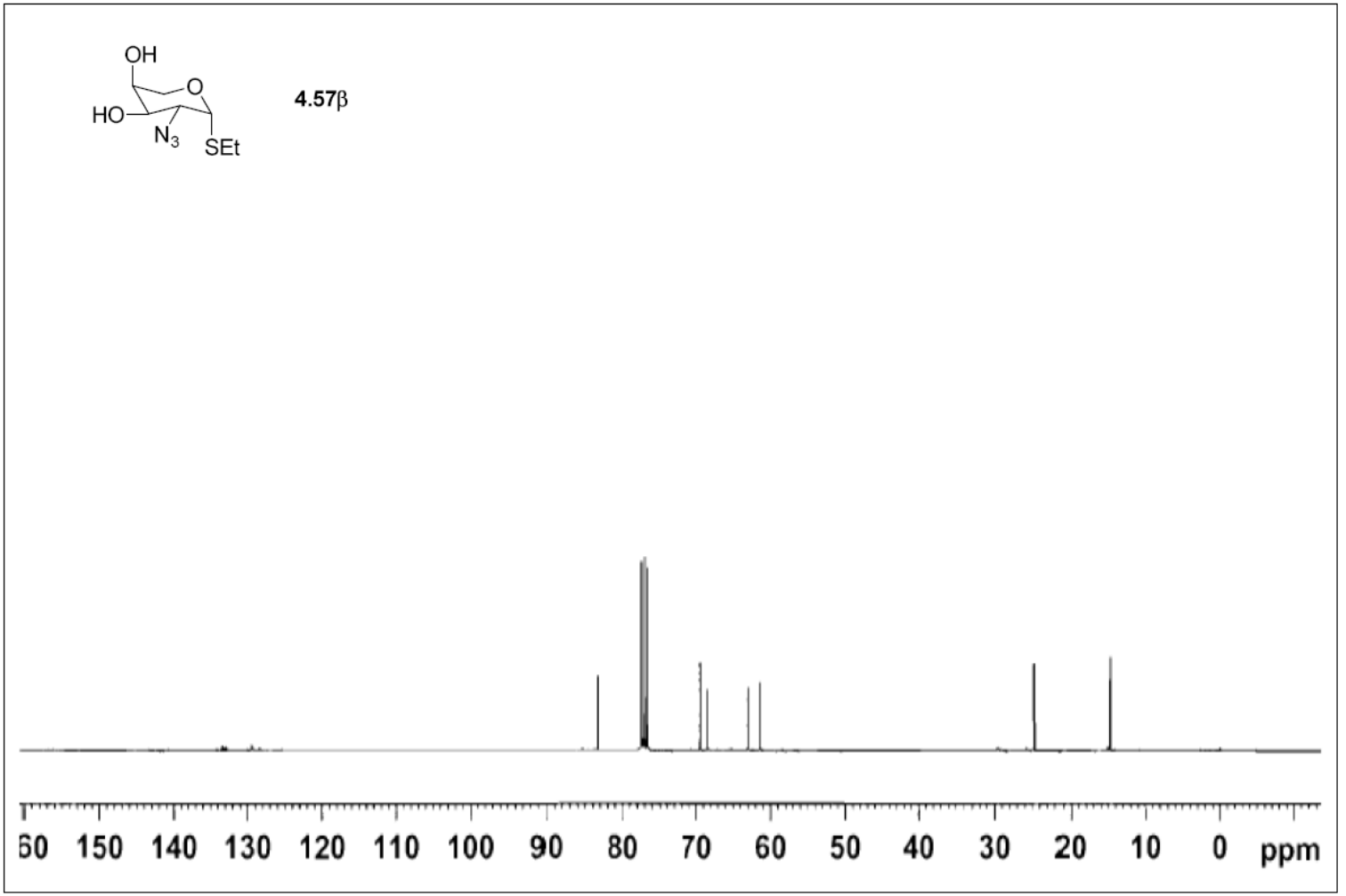

Figure A.20 75 MHz ^{13}C-NMR of compound 4.57β ($CDCl_3$)

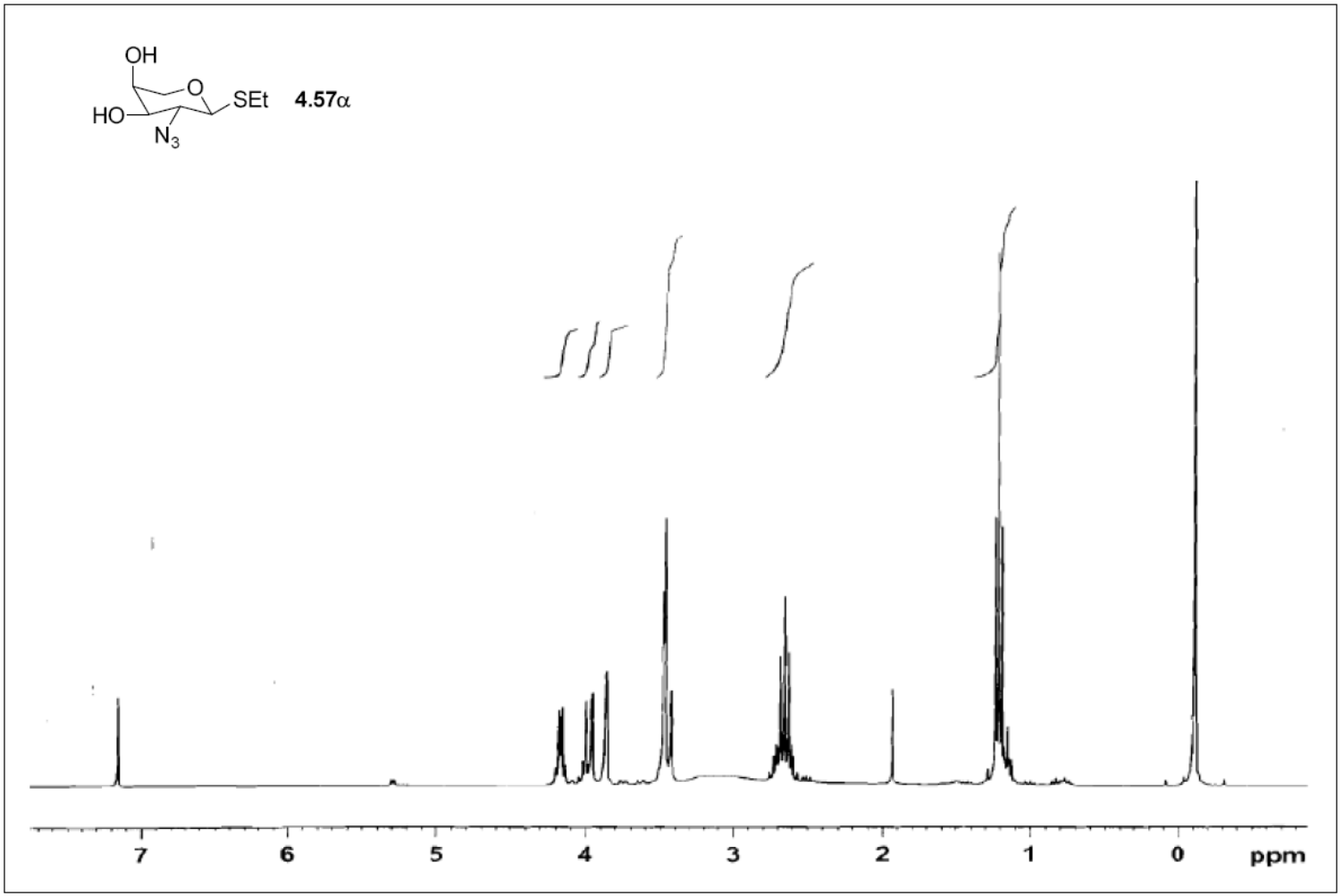

Figure A.21 300 MHz ^{1}H-NMR of compound 4.57α ($CDCl_3$)

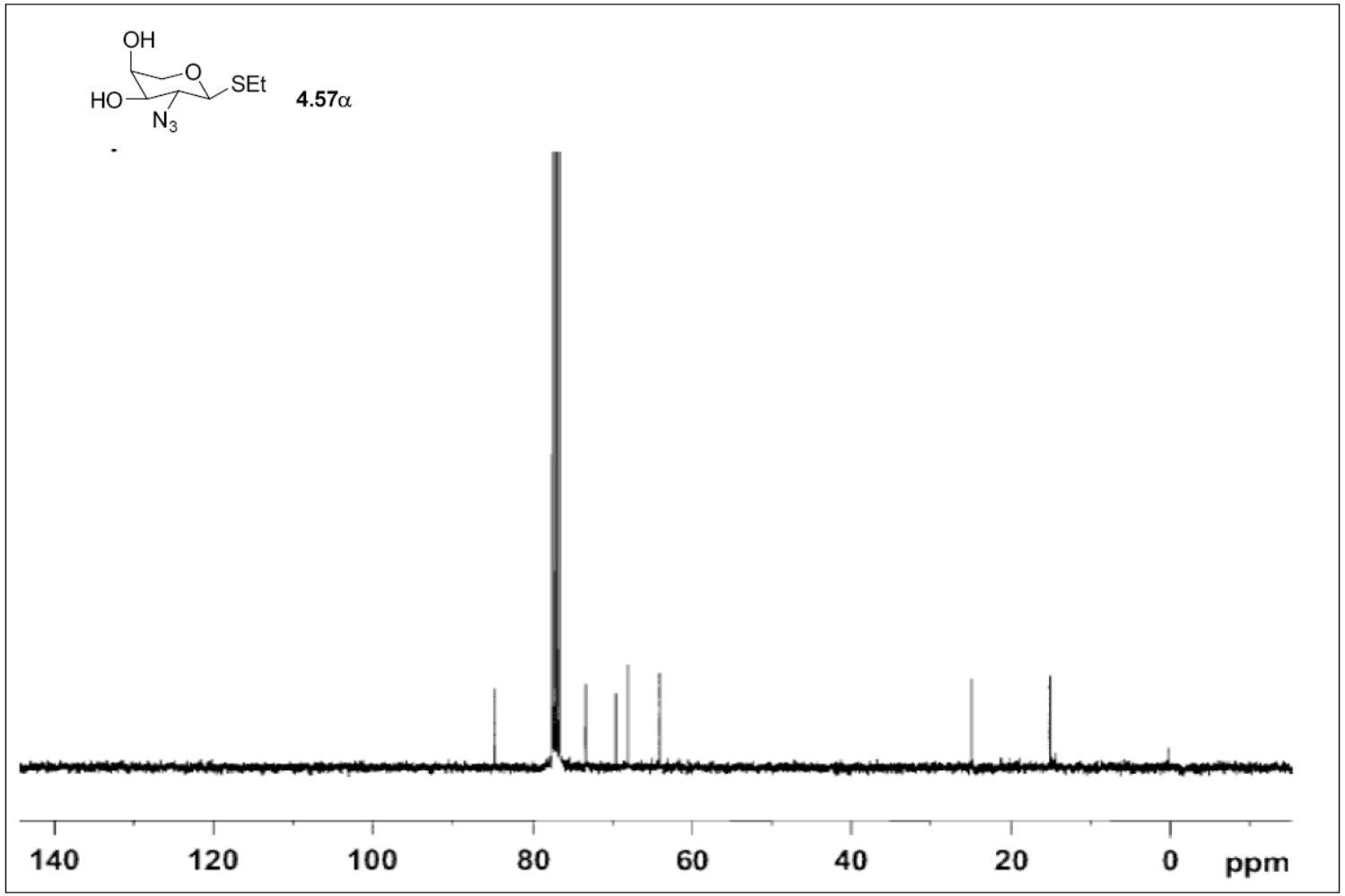

Figure A.22 75 MHz ^{13}C-NMR of compound 4.57*α* ($CDCl_3$)

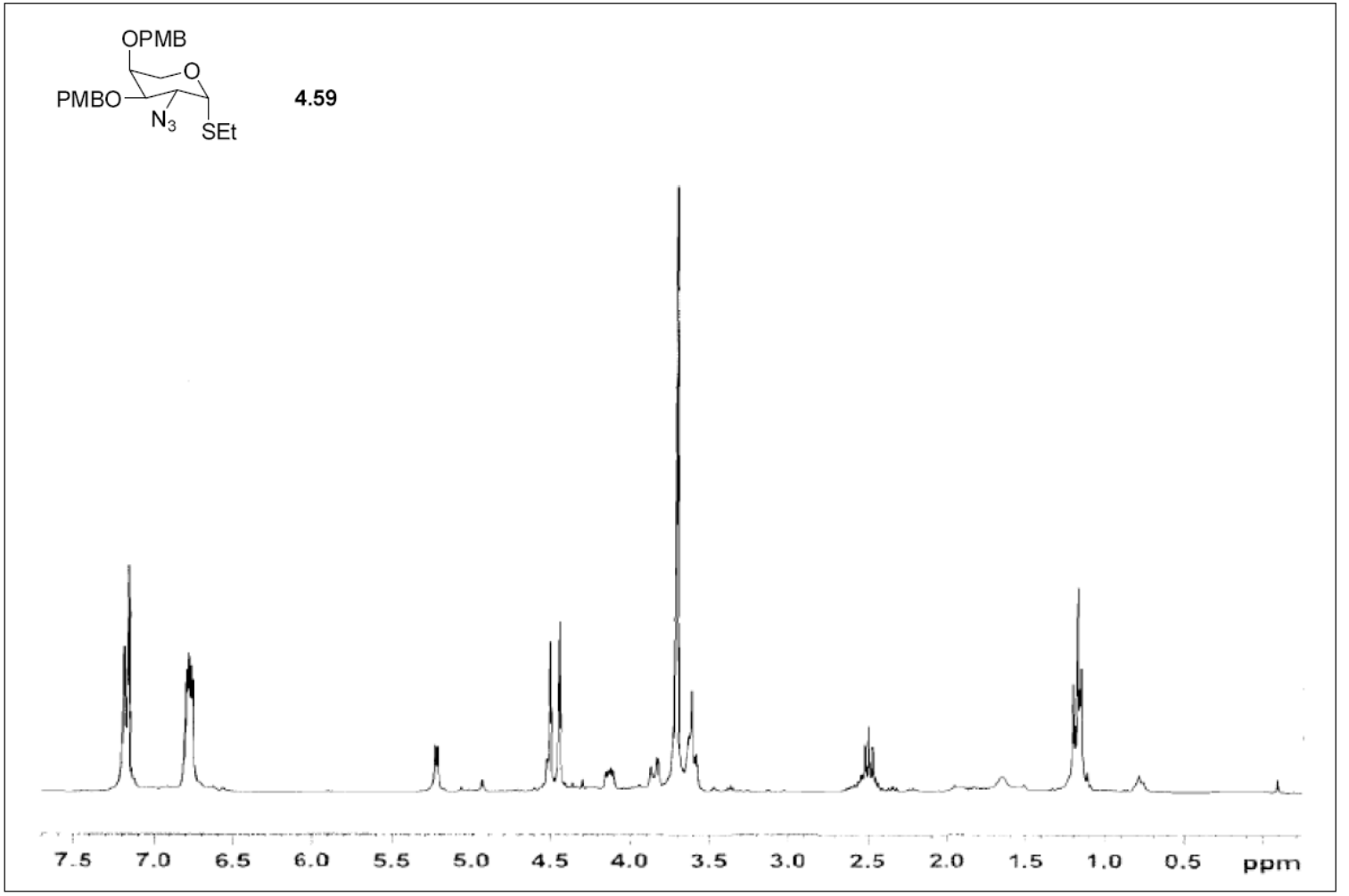

Figure A.23 300 MHz ^{1}H-NMR of compound 4.59 ($CDCl_3$)

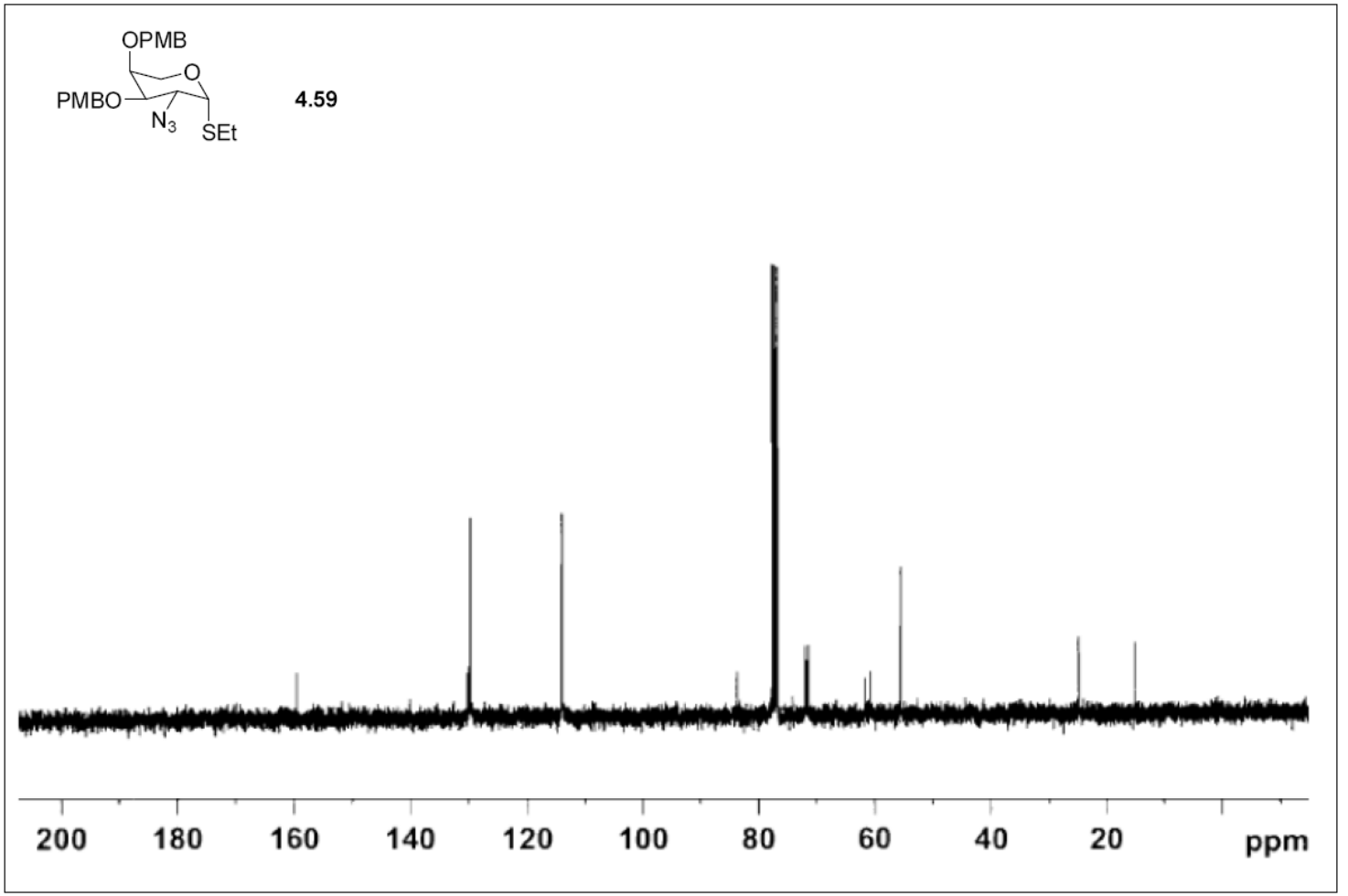

Figure A.24 75 MHz ^{13}C-NMR of compound 4.59 ($CDCl_3$)

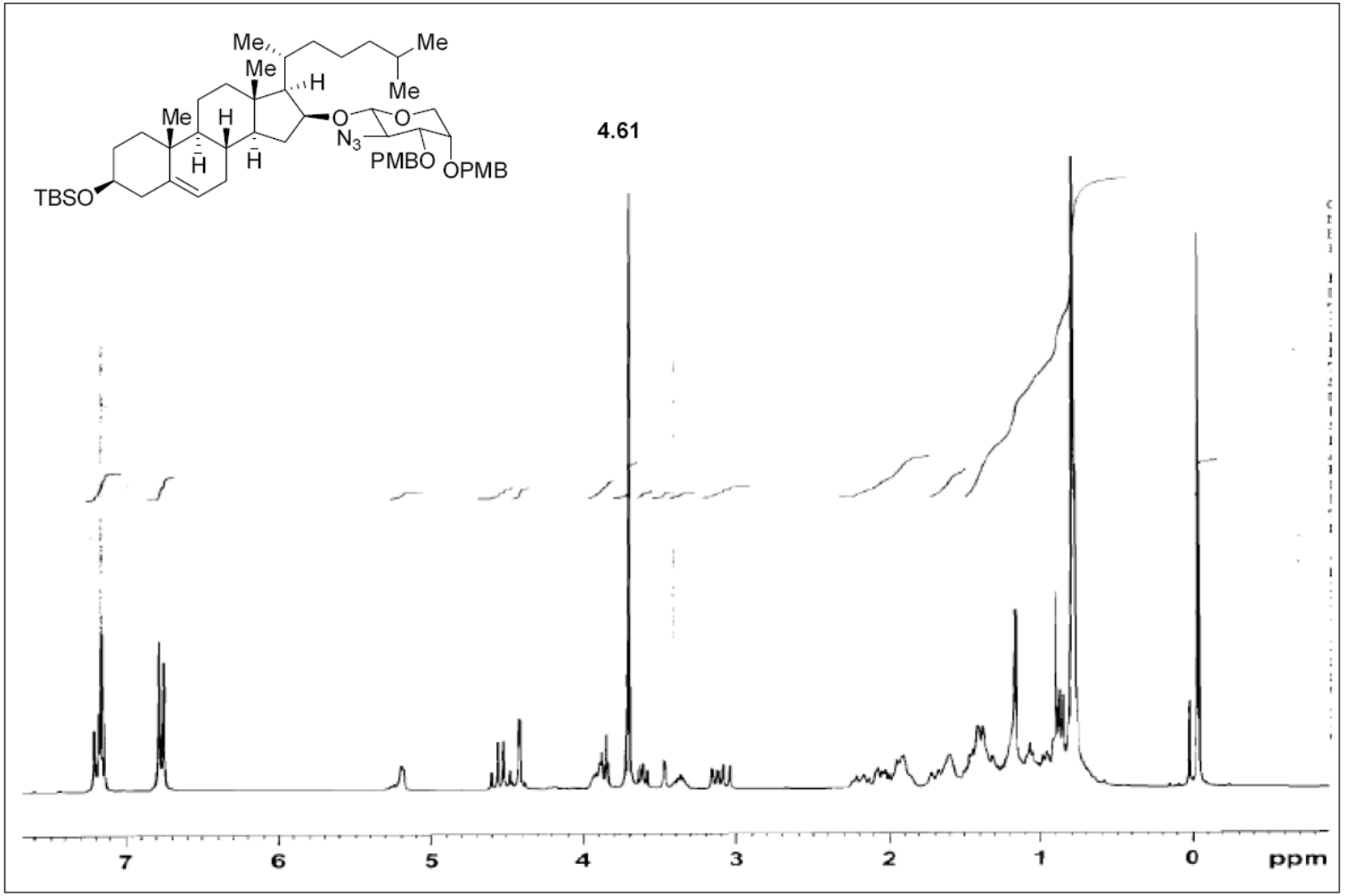

Figure A.25 300 MHz ^{1}H-NMR of compound 4.61 ($CDCl_3$)

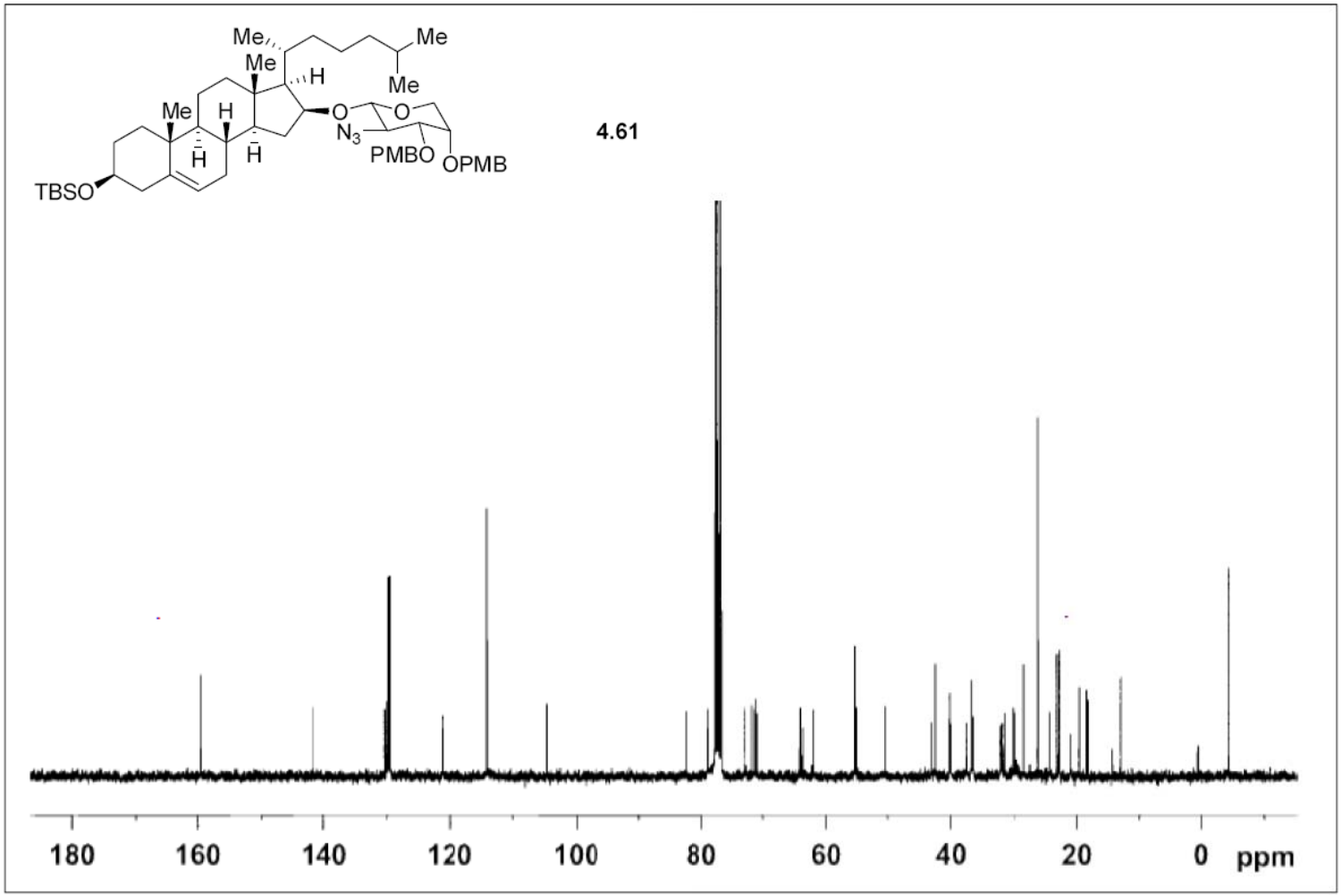

Figure A.26 75 MHz ^{13}C-NMR of compound 4.61 ($CDCl_3$)

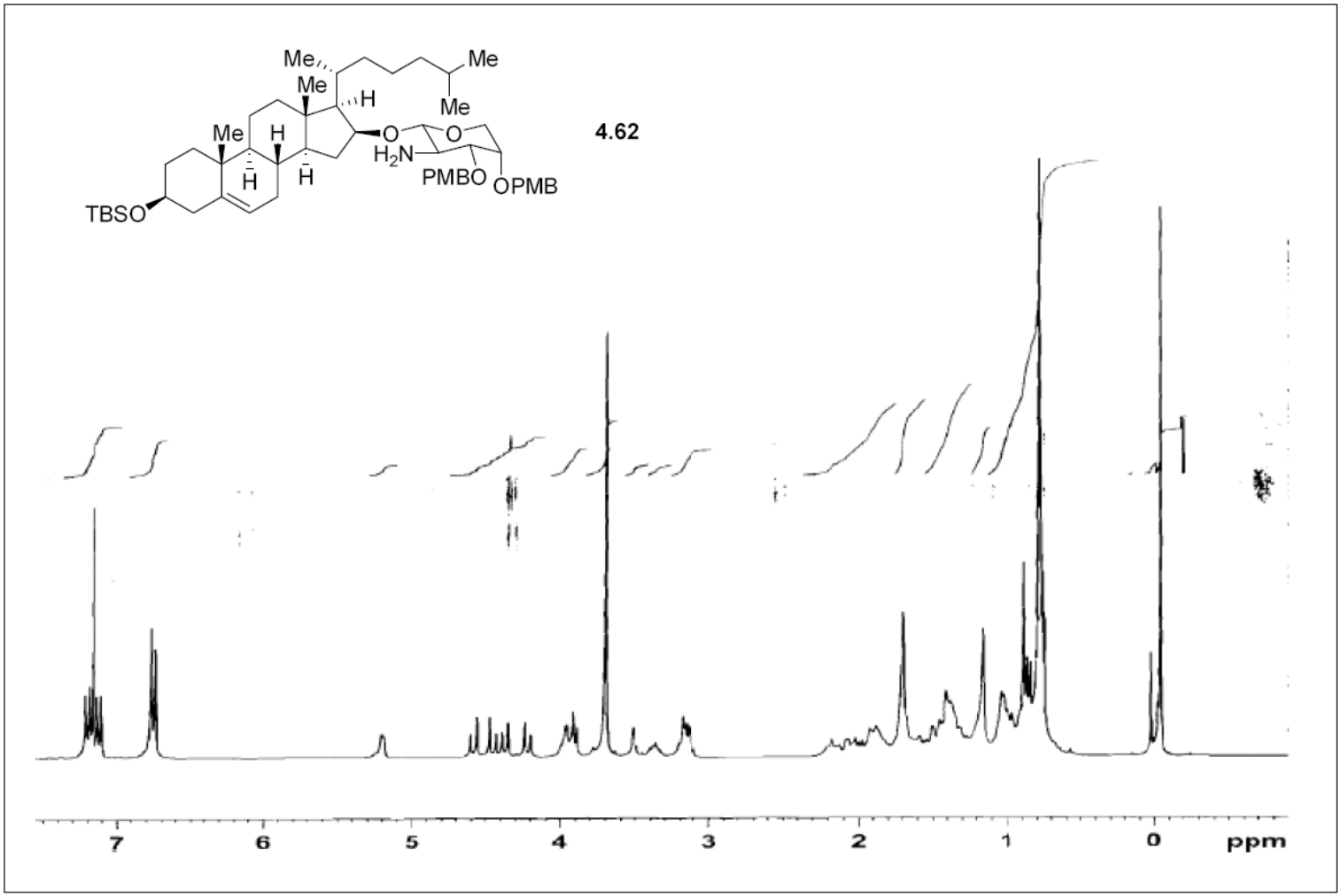

Figure A.27 300 MHz ^{1}H-NMR of compound 4.62 ($CDCl_3$)

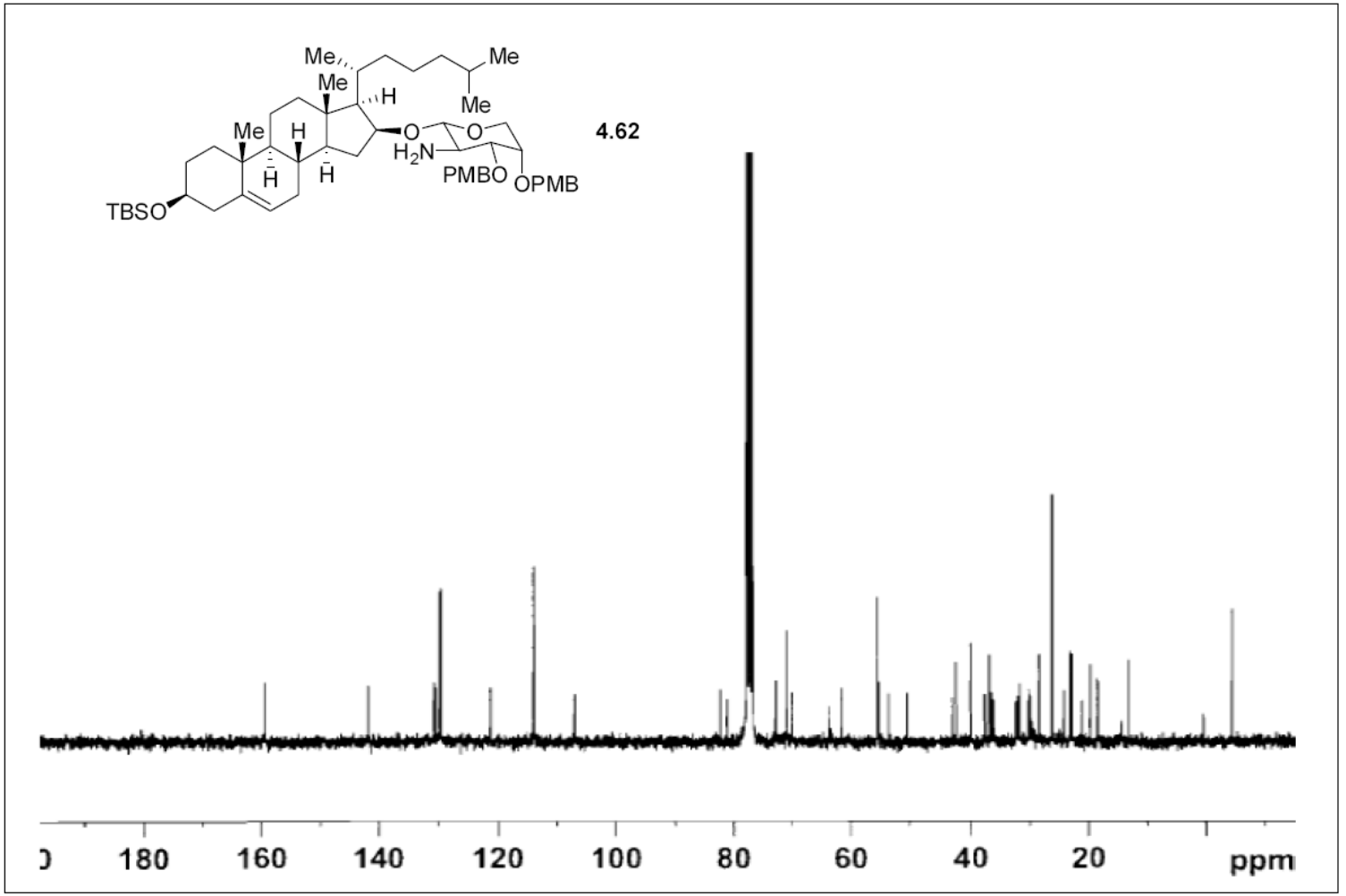

Figure A.28 75 MHz ^{13}C-NMR of compound 4.62 ($CDCl_3$)

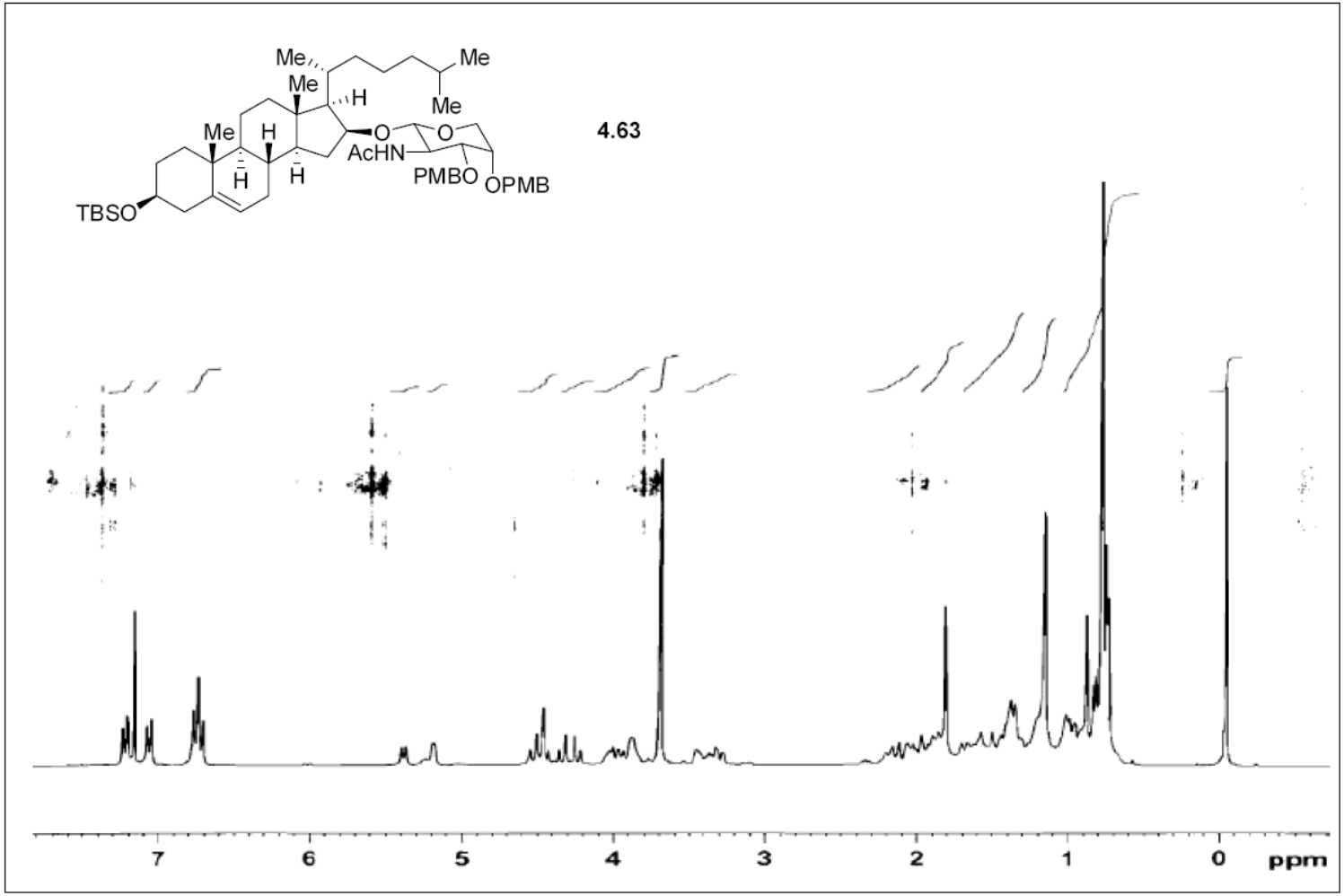

Figure A.29 300 MHz ^{1}H-NMR of compound 4.63 ($CDCl_3$)

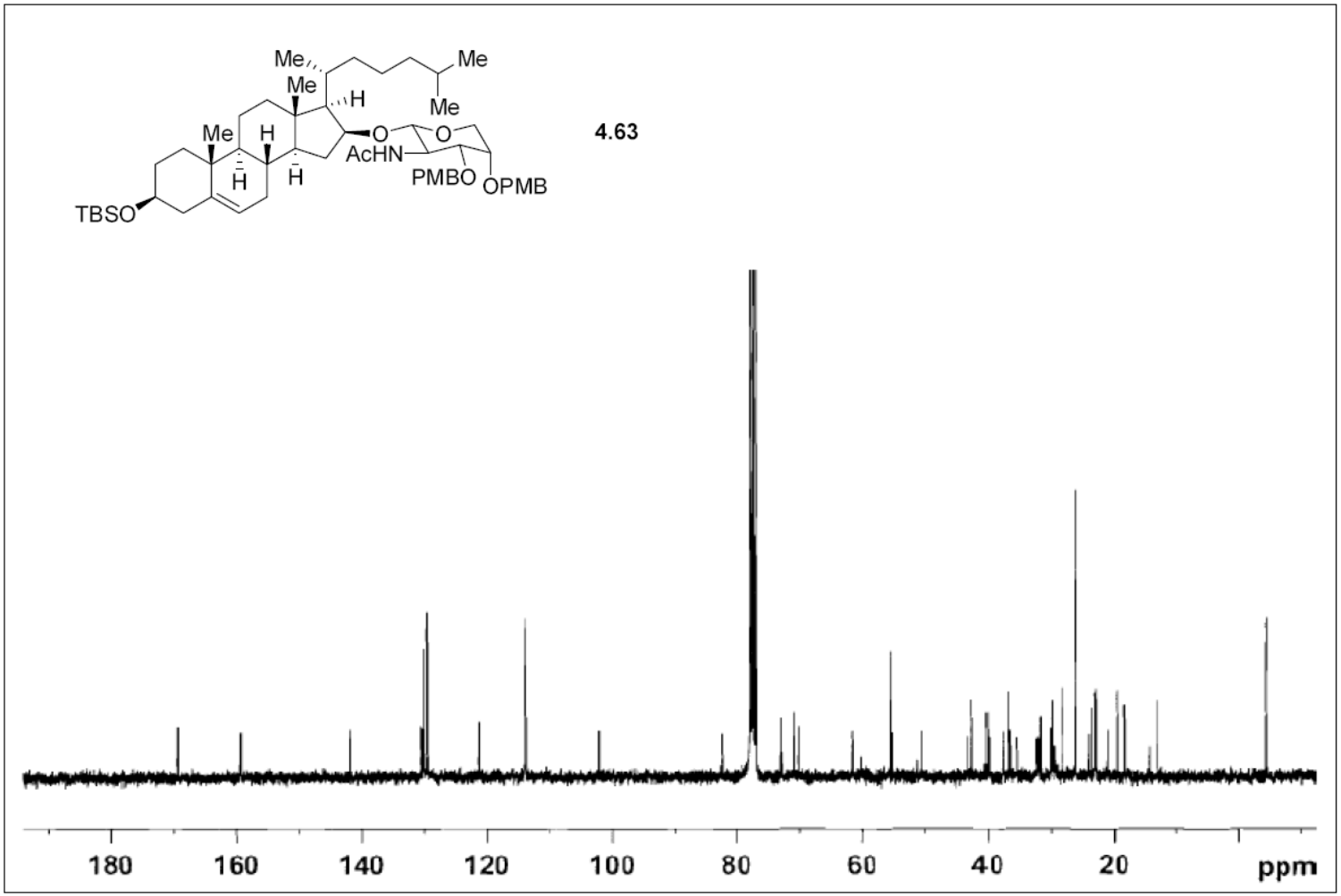

Figure A.30 75 MHz ^{13}C-NMR of compound 4.63 ($CDCl_3$)

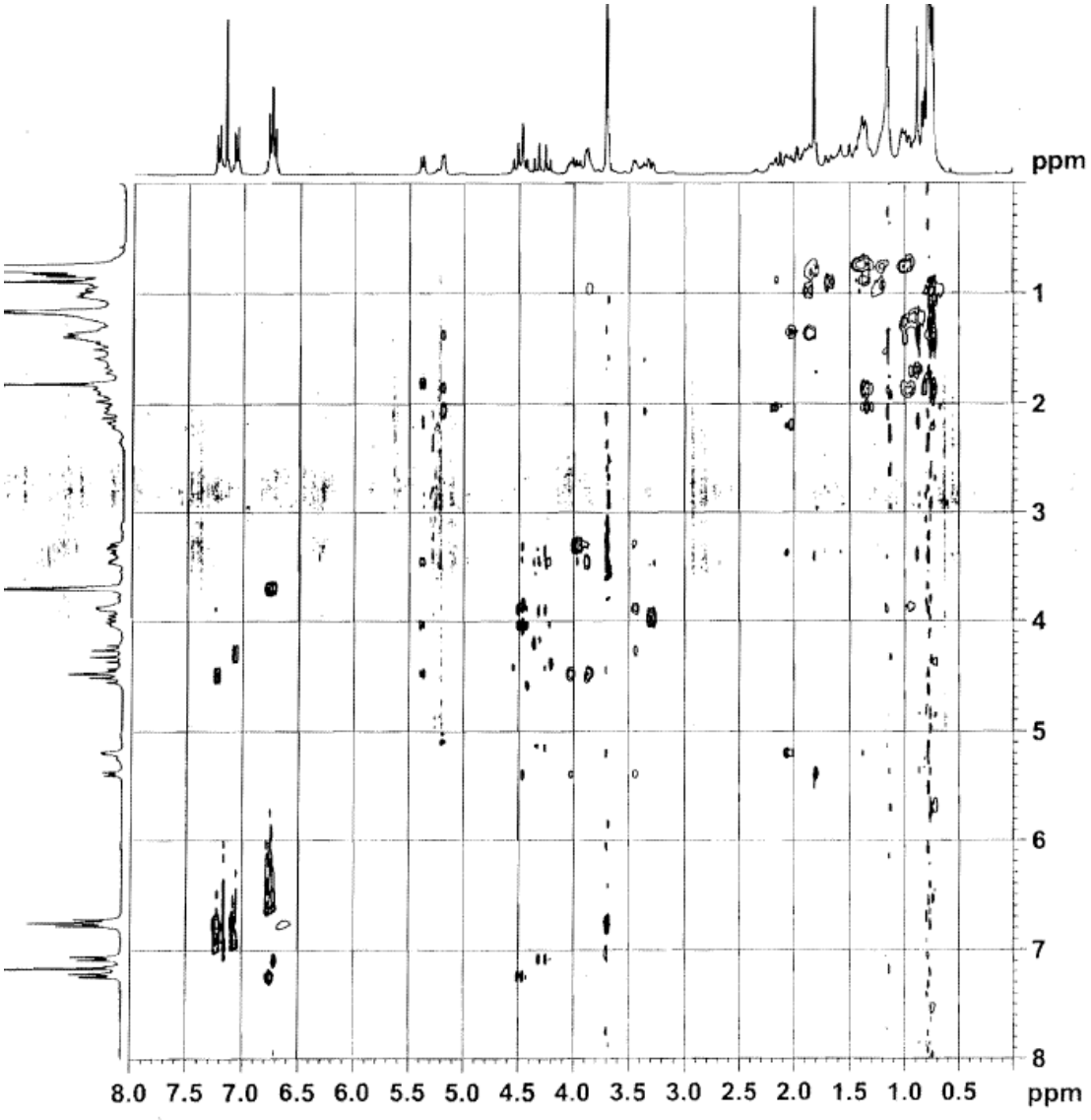

Figure A.30-CONTINUED NOE of compound **4.63**

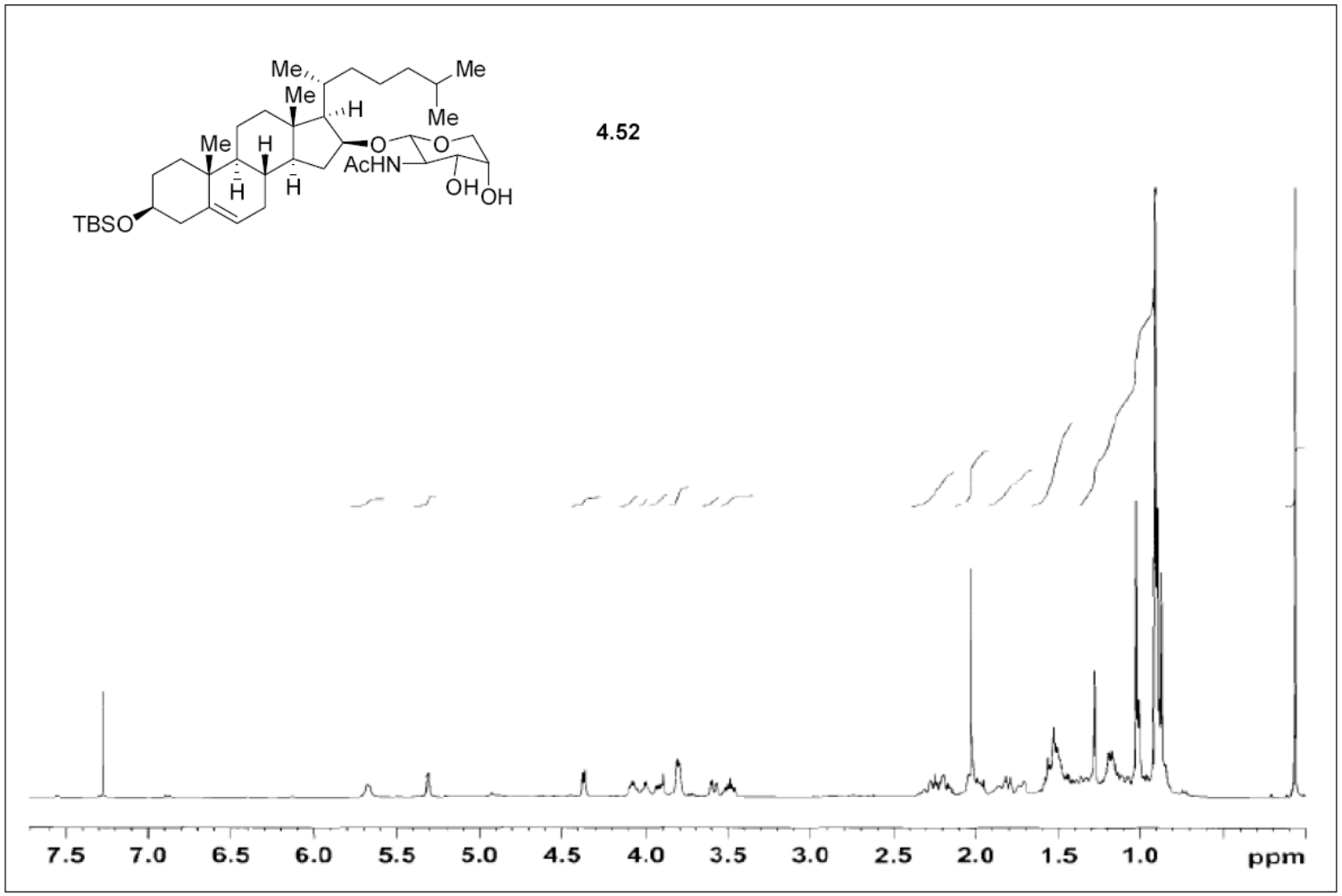

Figure A.31 300 MHz ^{1}H-NMR of compound 4.52 ($CDCl_3$)

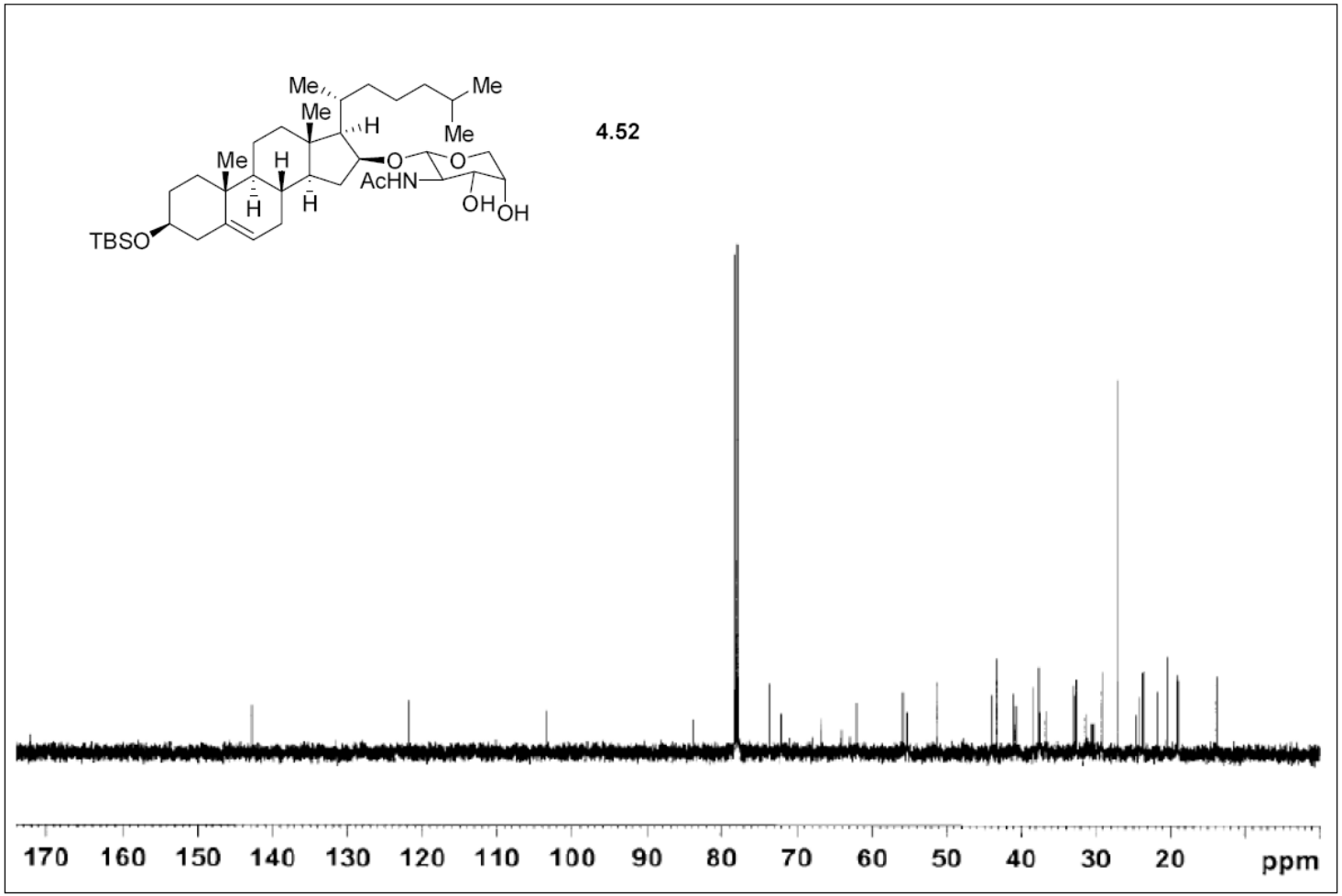

Figure A.32 75 MHz ^{13}C-NMR of compound 4.52 ($CDCl_3$)

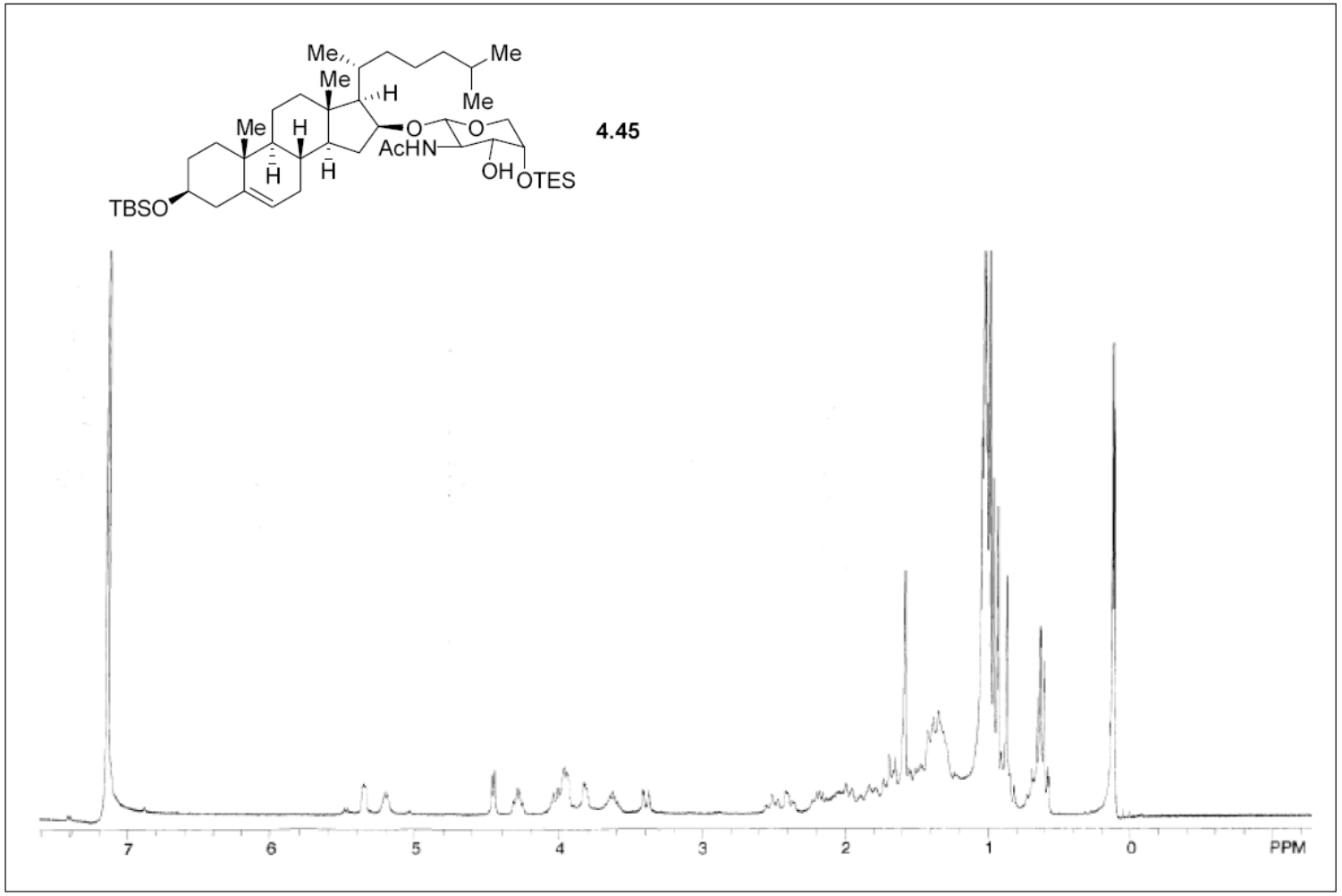

Figure A.33 300 MHz ^{1}H-NMR of compound 4.45 ($CDCl_3$)

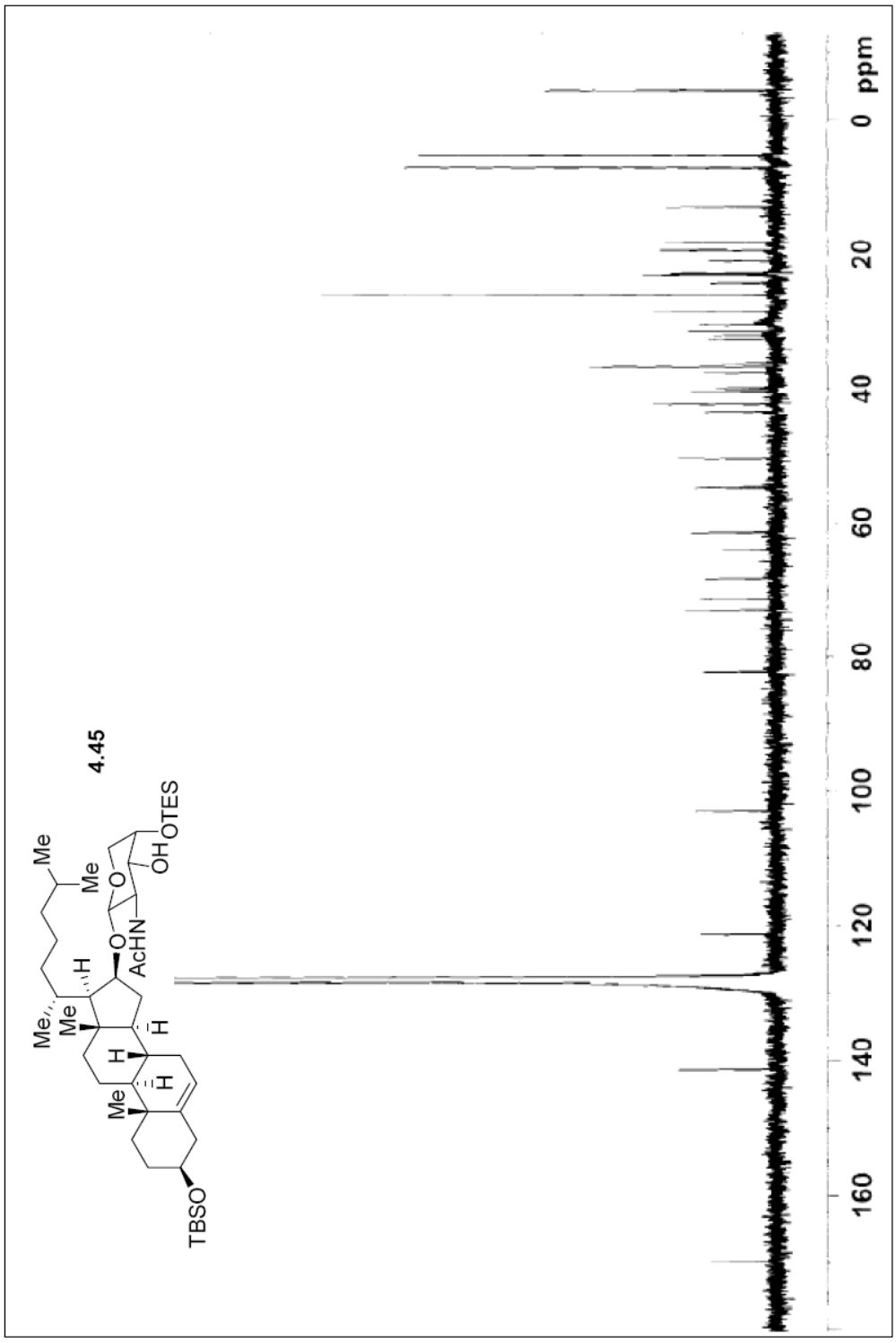

Figure A.34 75 MHz ^{13}C-NMR of compound 4.45 ($CDCl_3$)

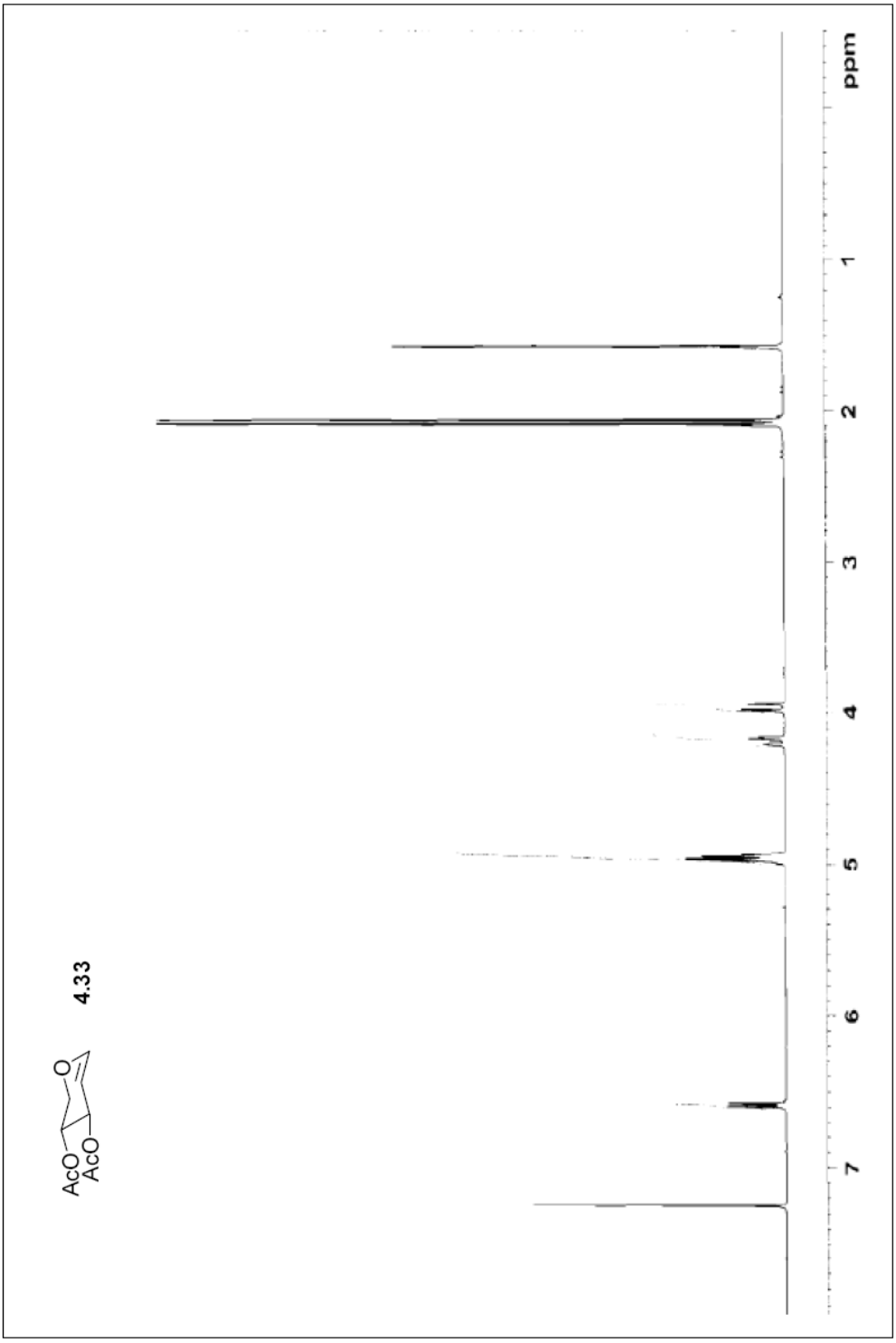

Figure A.35 300 MHz ^{1}H-NMR of compound 4.33 ($CDCl_3$)

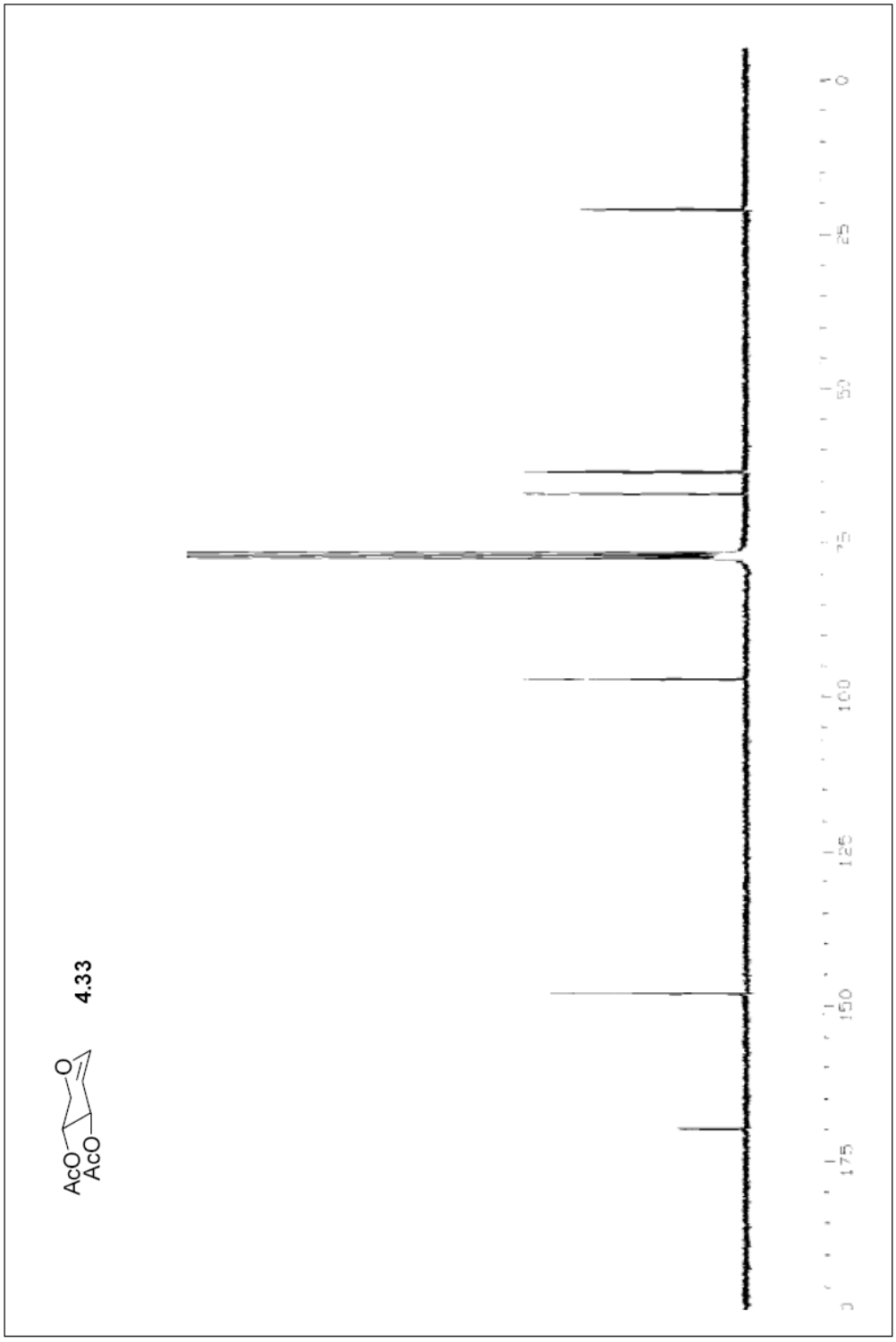

Figure A.36 75 MHz ^{13}C-NMR of compound 4.33 ($CDCl_3$)

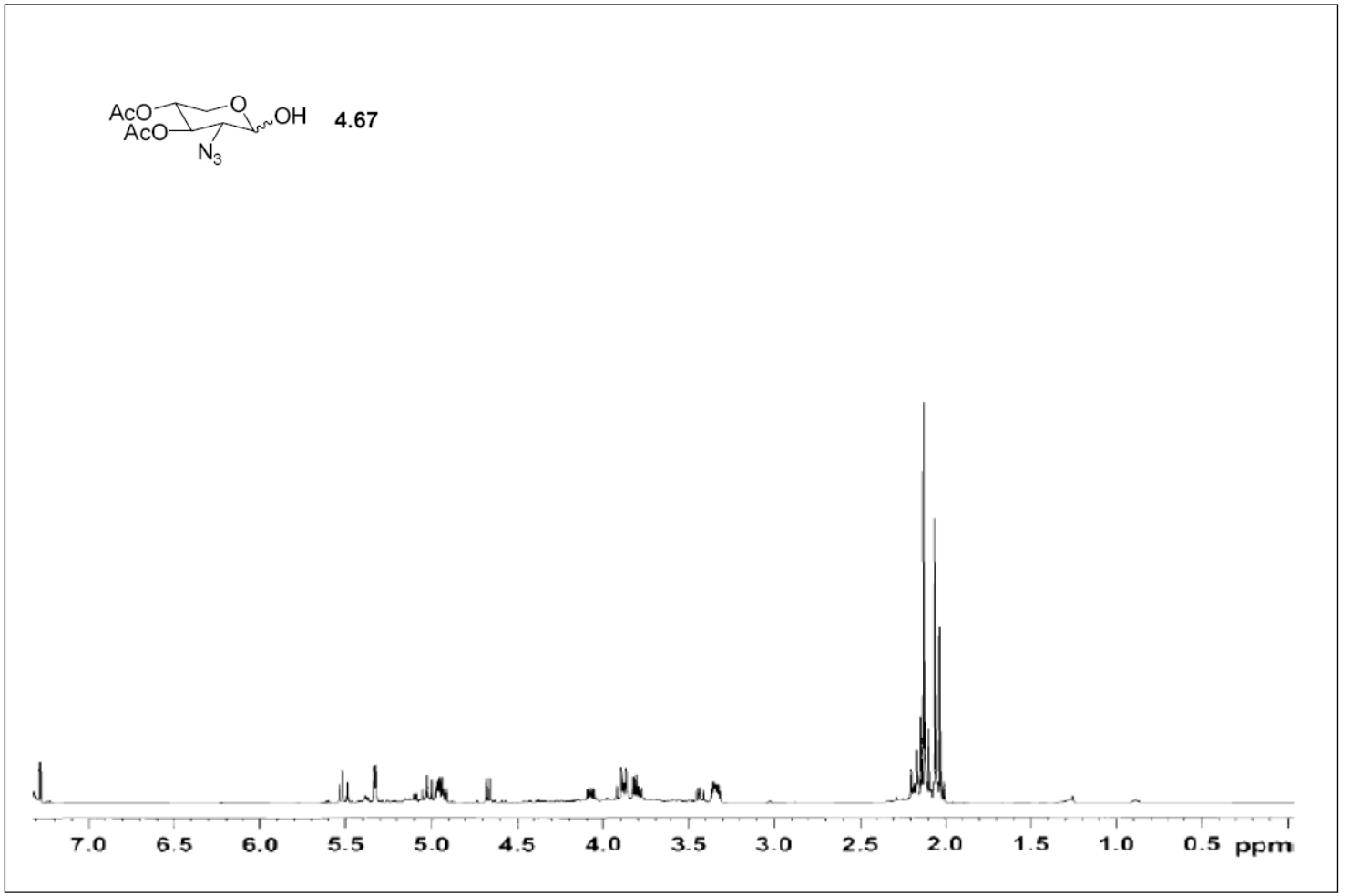

Figure A.37 300 MHz ^{1}H-NMR of compound 4.67 ($CDCl_3$)

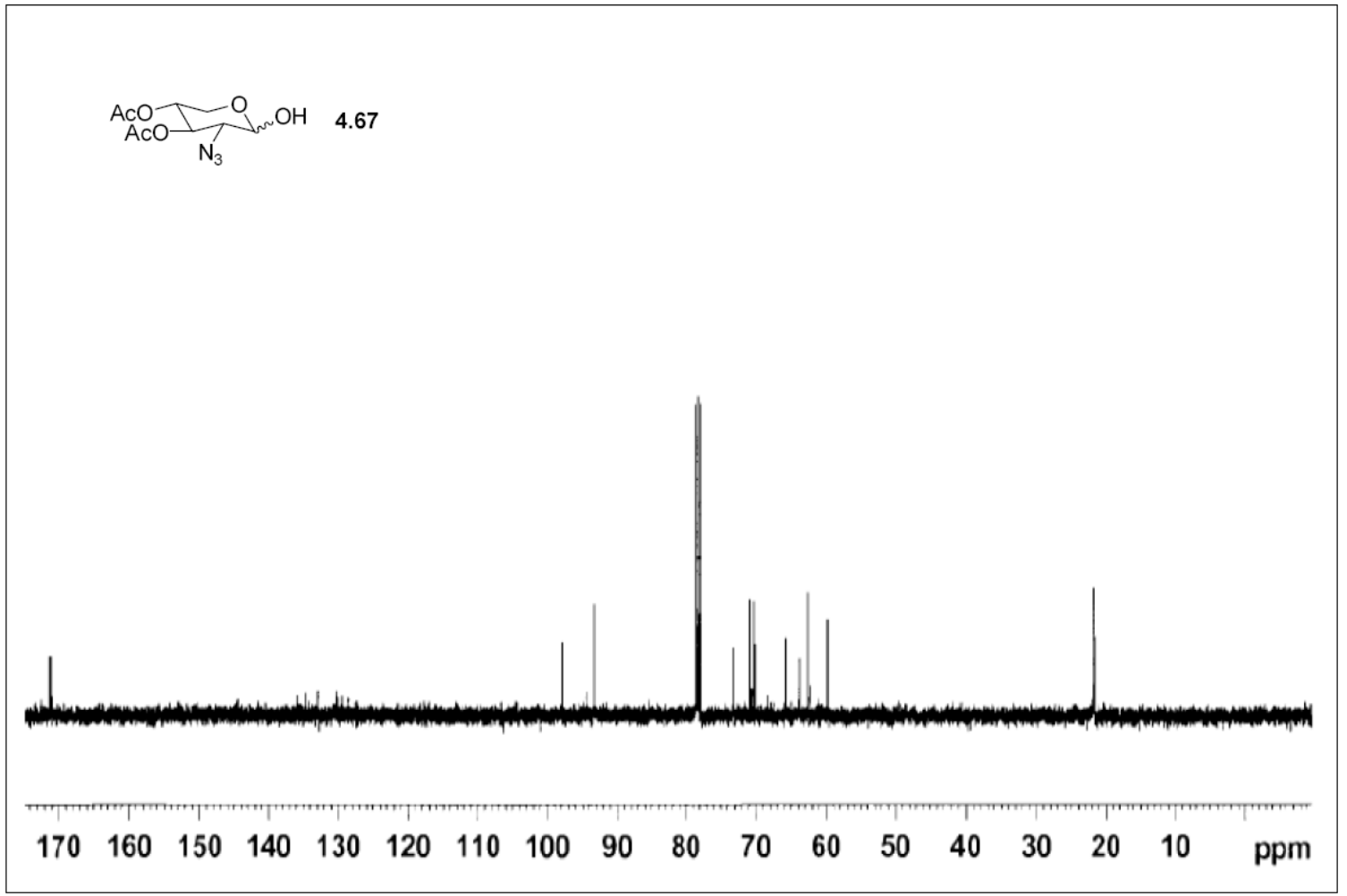

Figure A.38 75 MHz ^{13}C-NMR of compound 4.67 ($CDCl_3$)

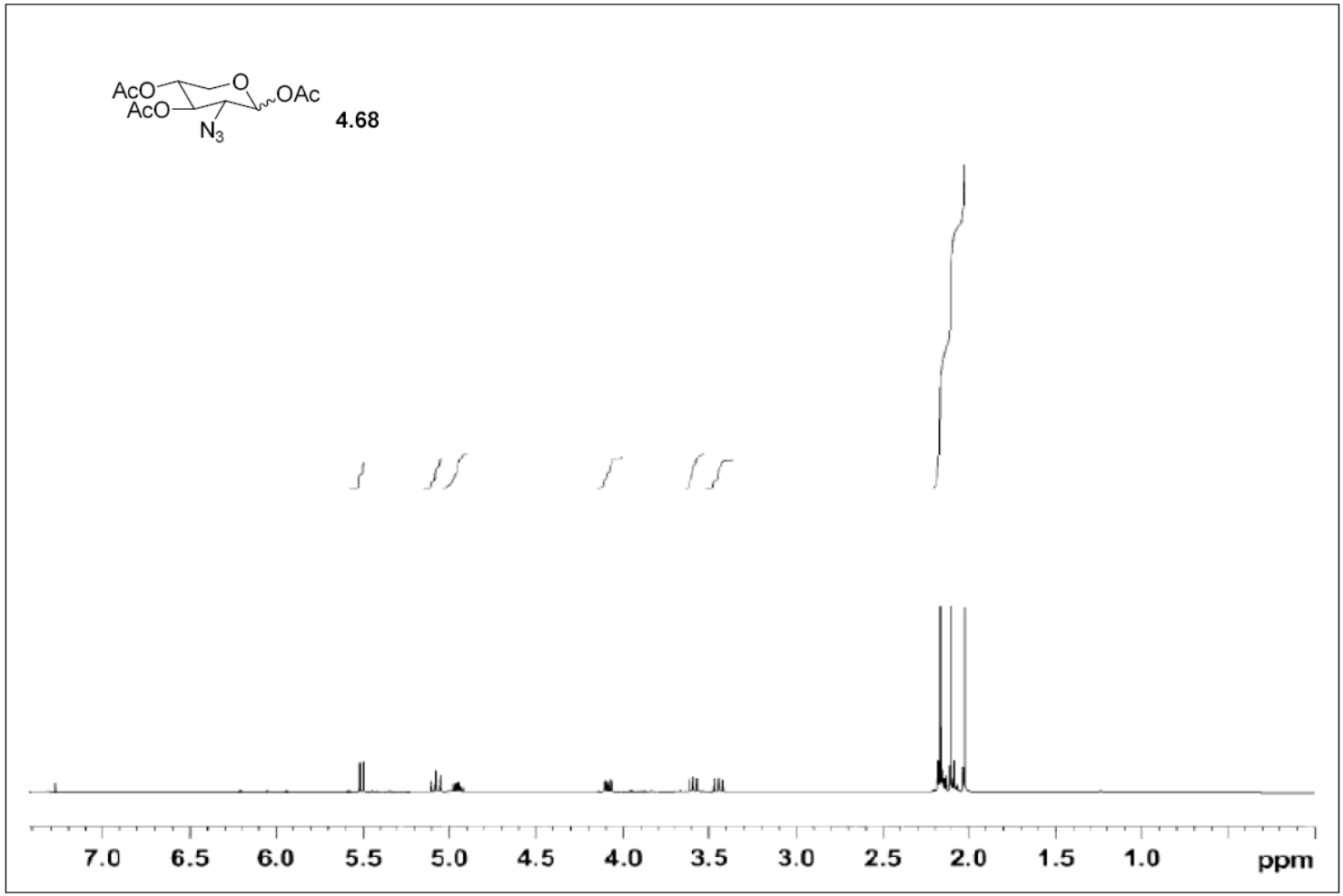

Figure A.39 300 MHz ^{1}H-NMR of compound 4.68 ($CDCl_3$)

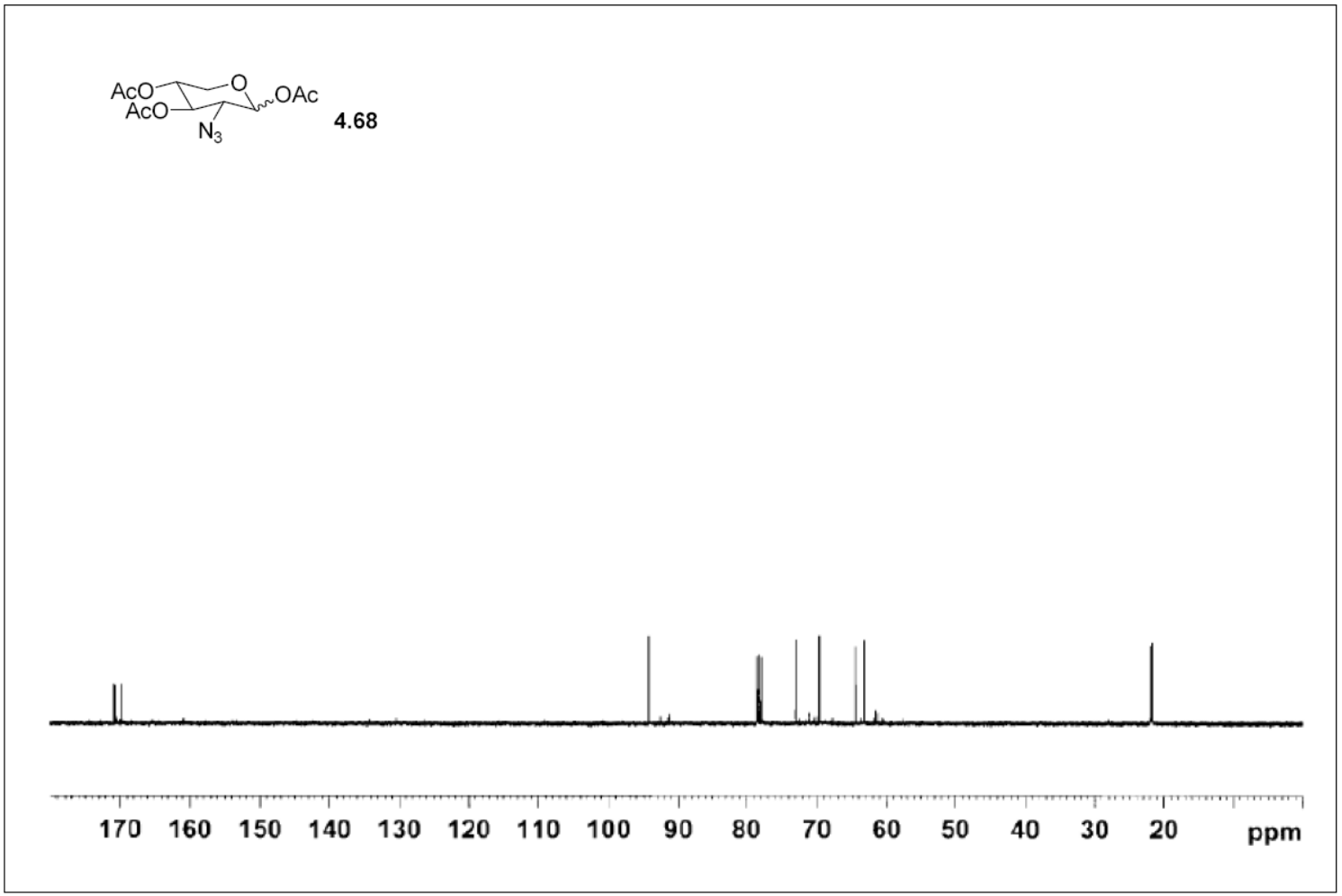

Figure A.40 75 MHz ^{13}C-NMR of compound 4.68 ($CDCl_3$)

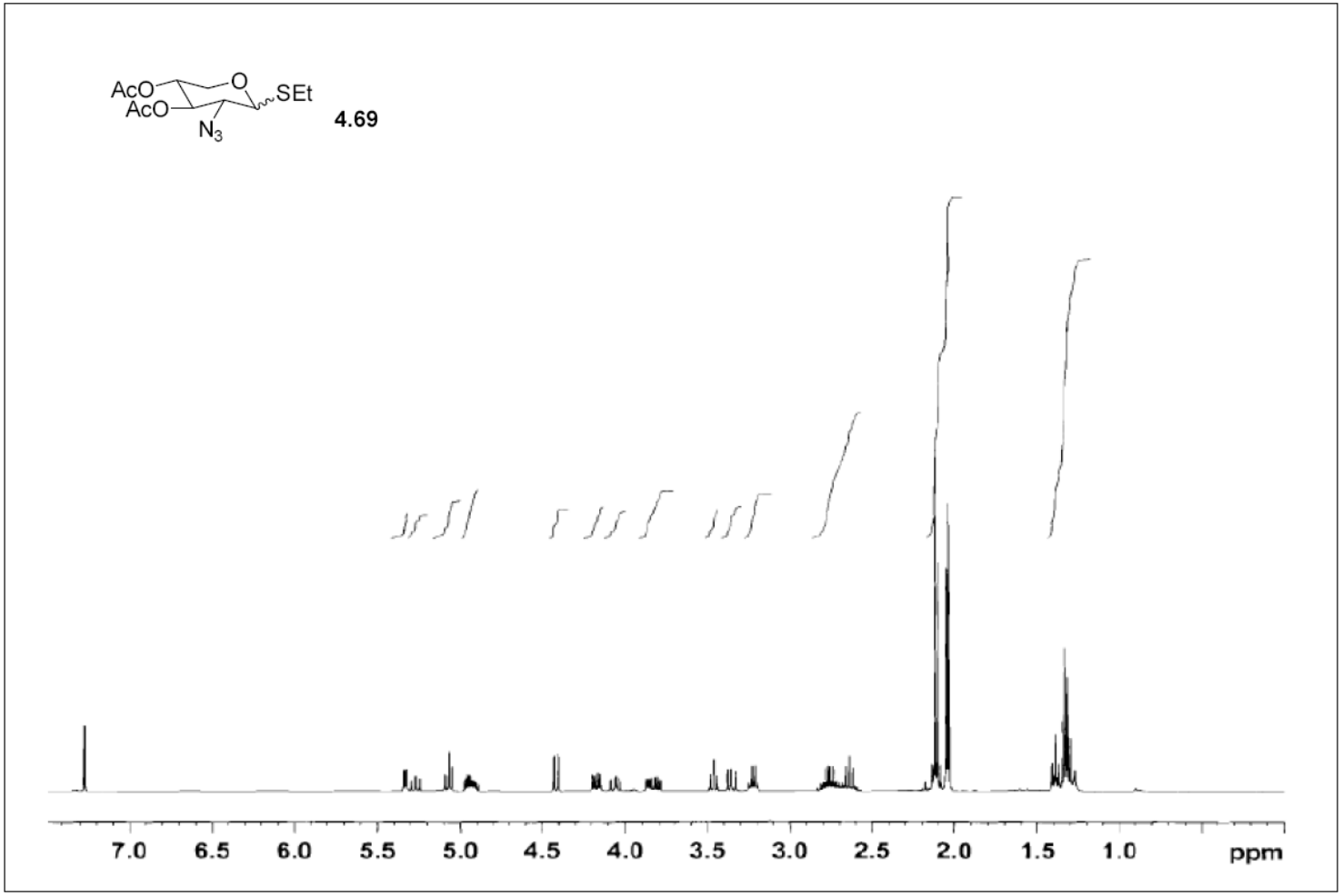

Figure A.41 300 MHz ^{1}H-NMR of compound 4.69 ($CDCl_3$)

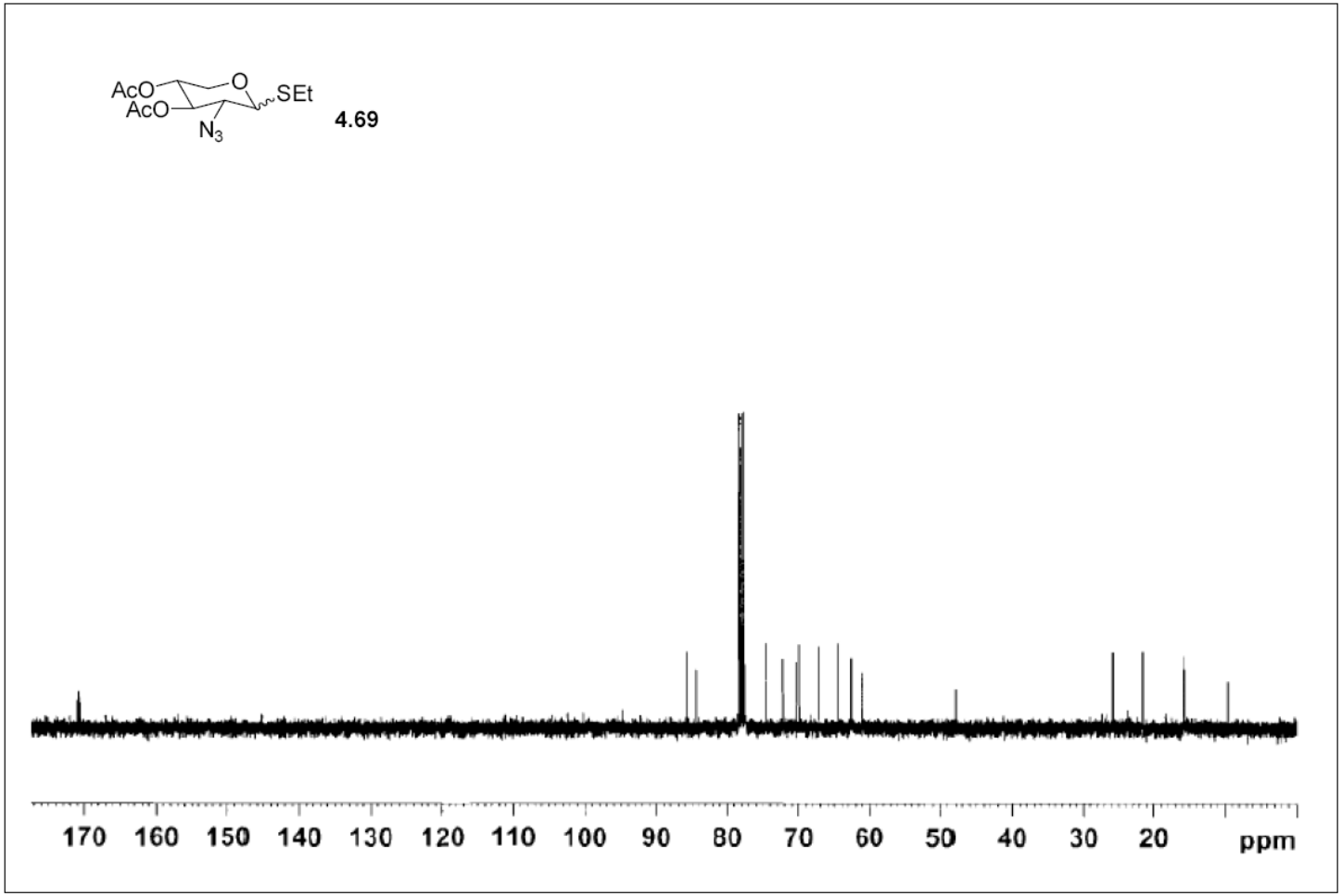

Figure A.42 75 MHz ^{13}C-NMR of compound 4.69 ($CDCl_3$)

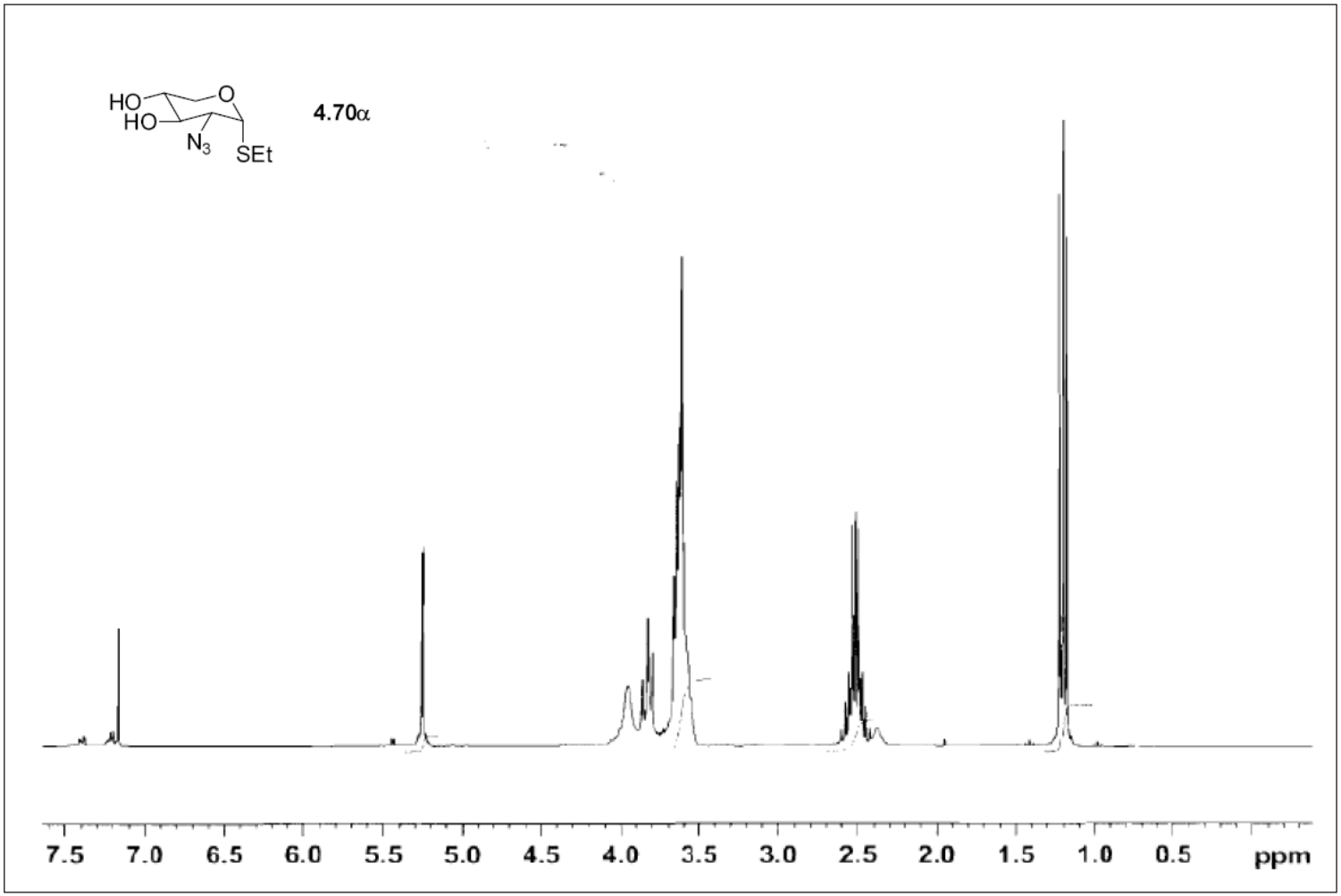

Figure A.43 300 MHz ^{1}H-NMR of compound 4.70α ($CDCl_3$)

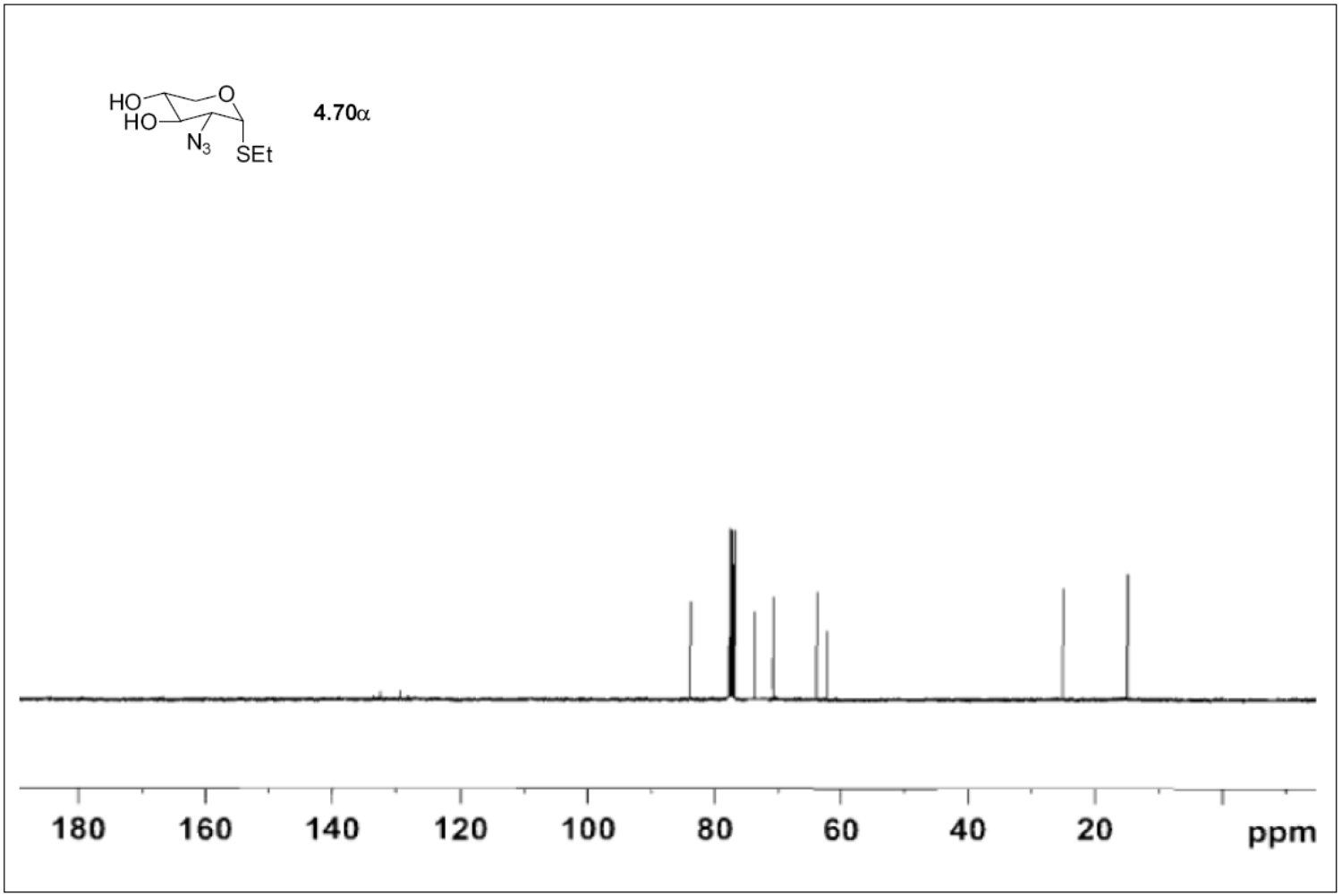

Figure A.44 75 MHz ^{13}C-NMR of compound 4.70α ($CDCl_3$)

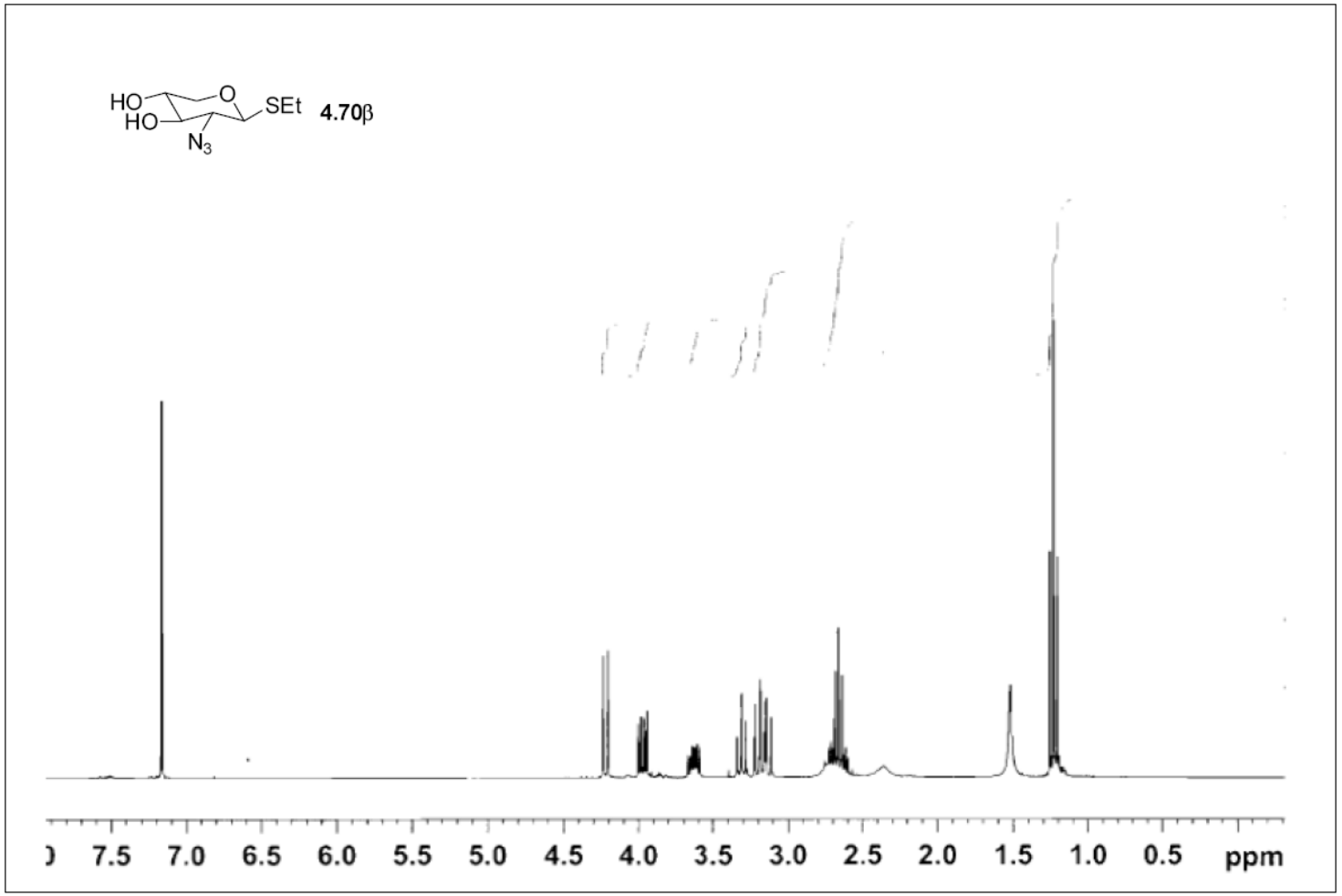

Figure A.45 300 MHz ^{1}H-NMR of compound 4.70β ($CDCl_3$)

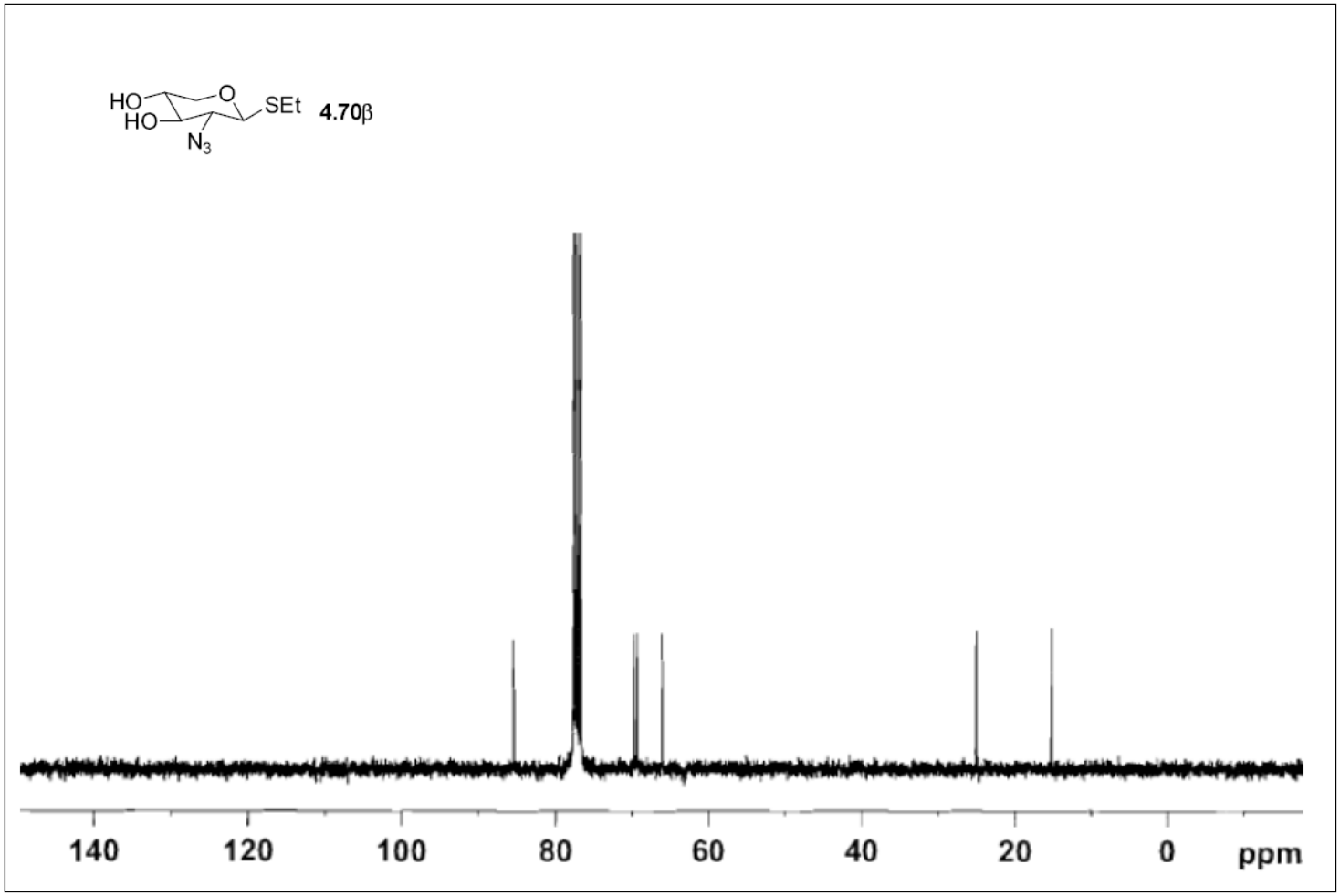

Figure A.46 75 MHz ^{13}C-NMR of compound 4.70β ($CDCl_3$)

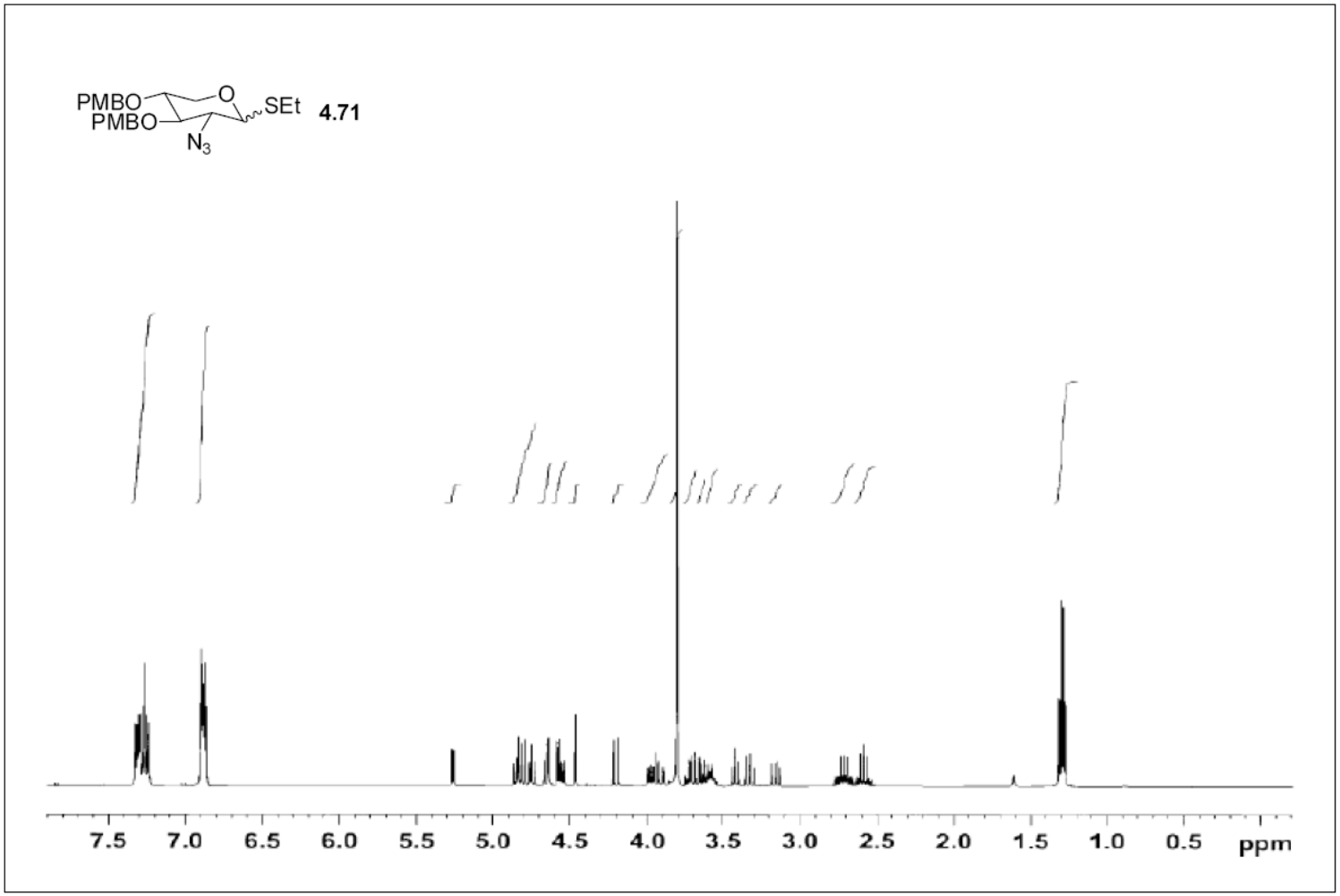

Figure A.47 300 MHz ^{1}H-NMR of compound 4.71 ($CDCl_3$)

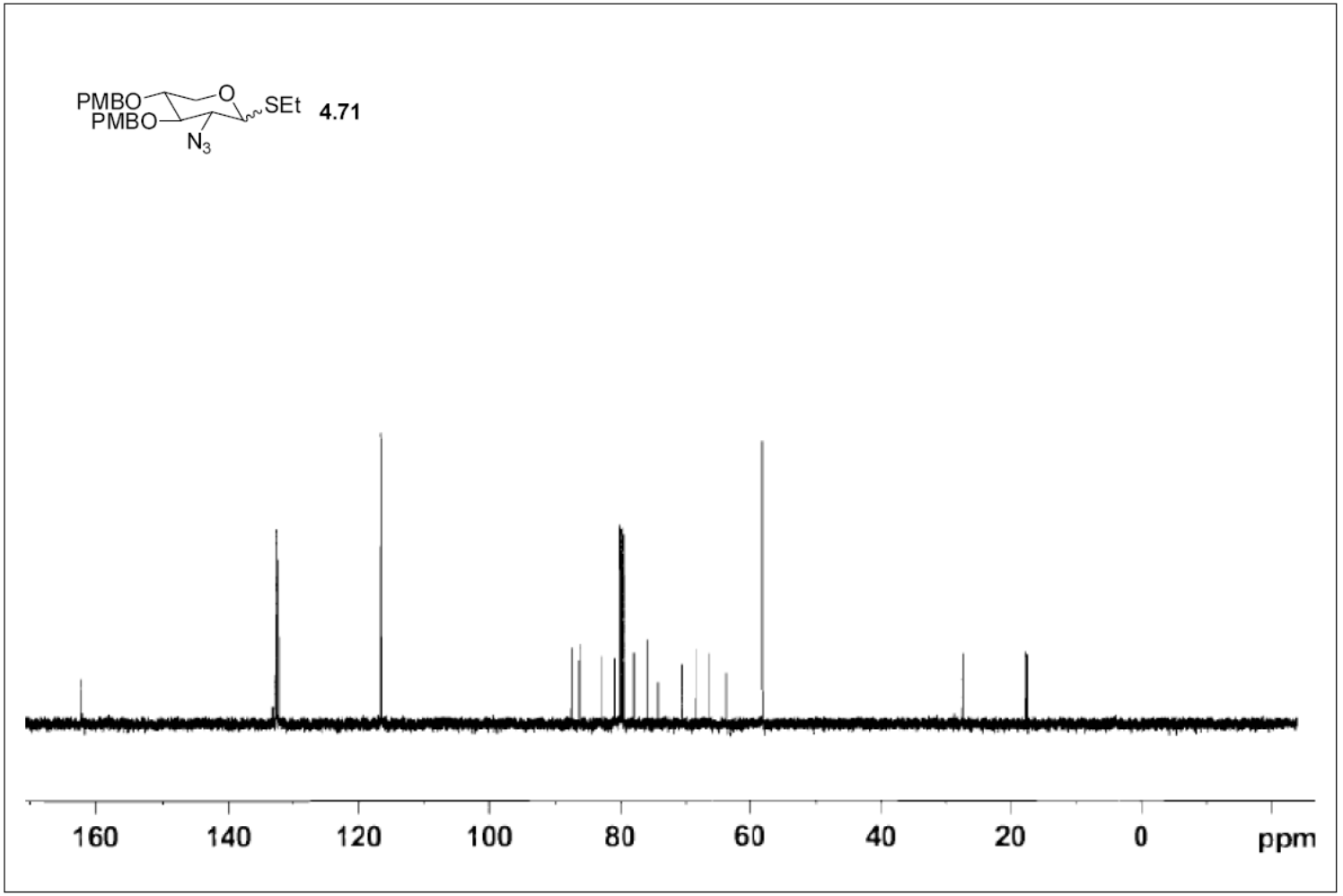

Figure A.48 75 MHz ^{13}C-NMR of compound 4.71 ($CDCl_3$)

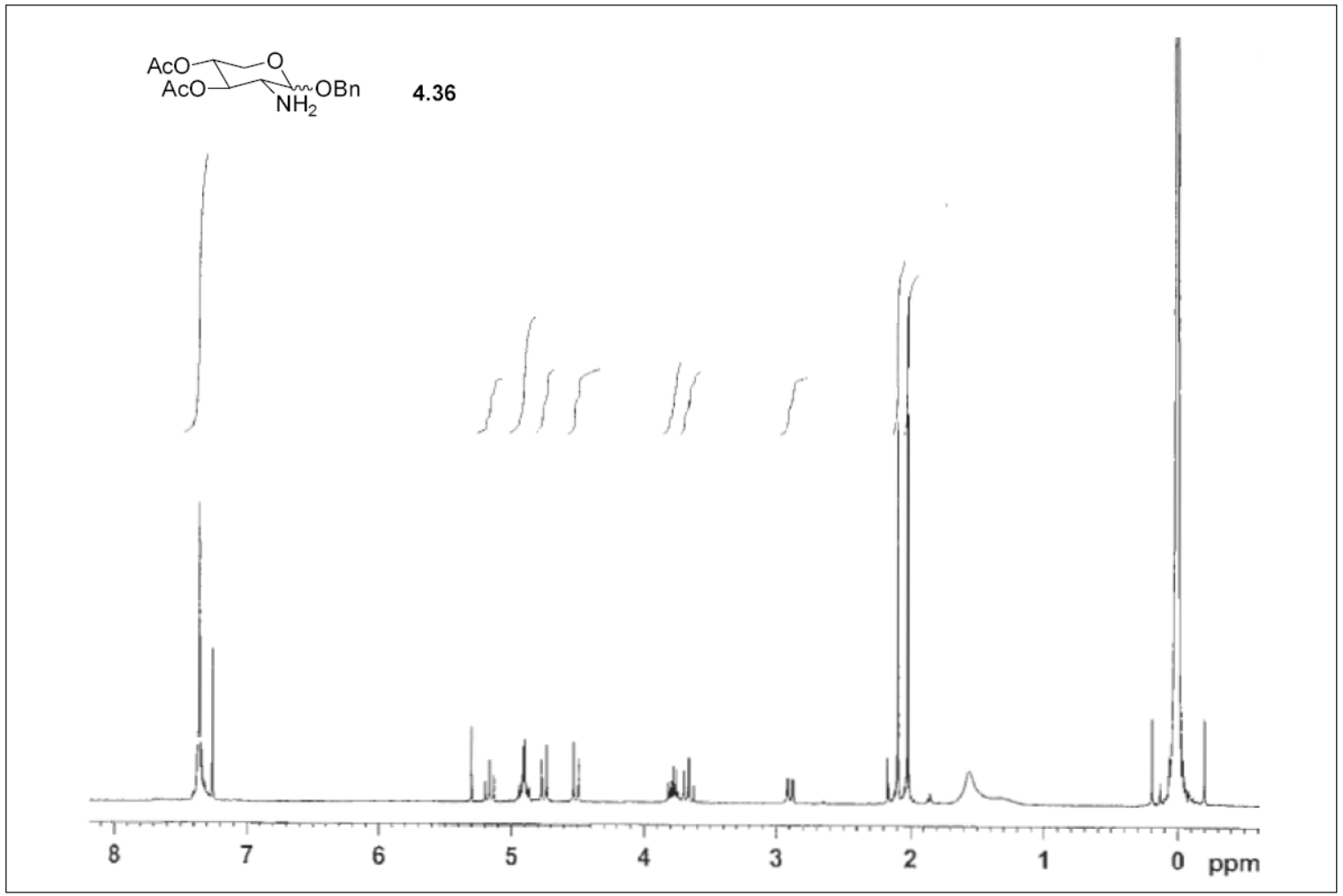

Figure A.49 300 MHz ^{1}H-NMR of compound 4.36 ($CDCl_3$)

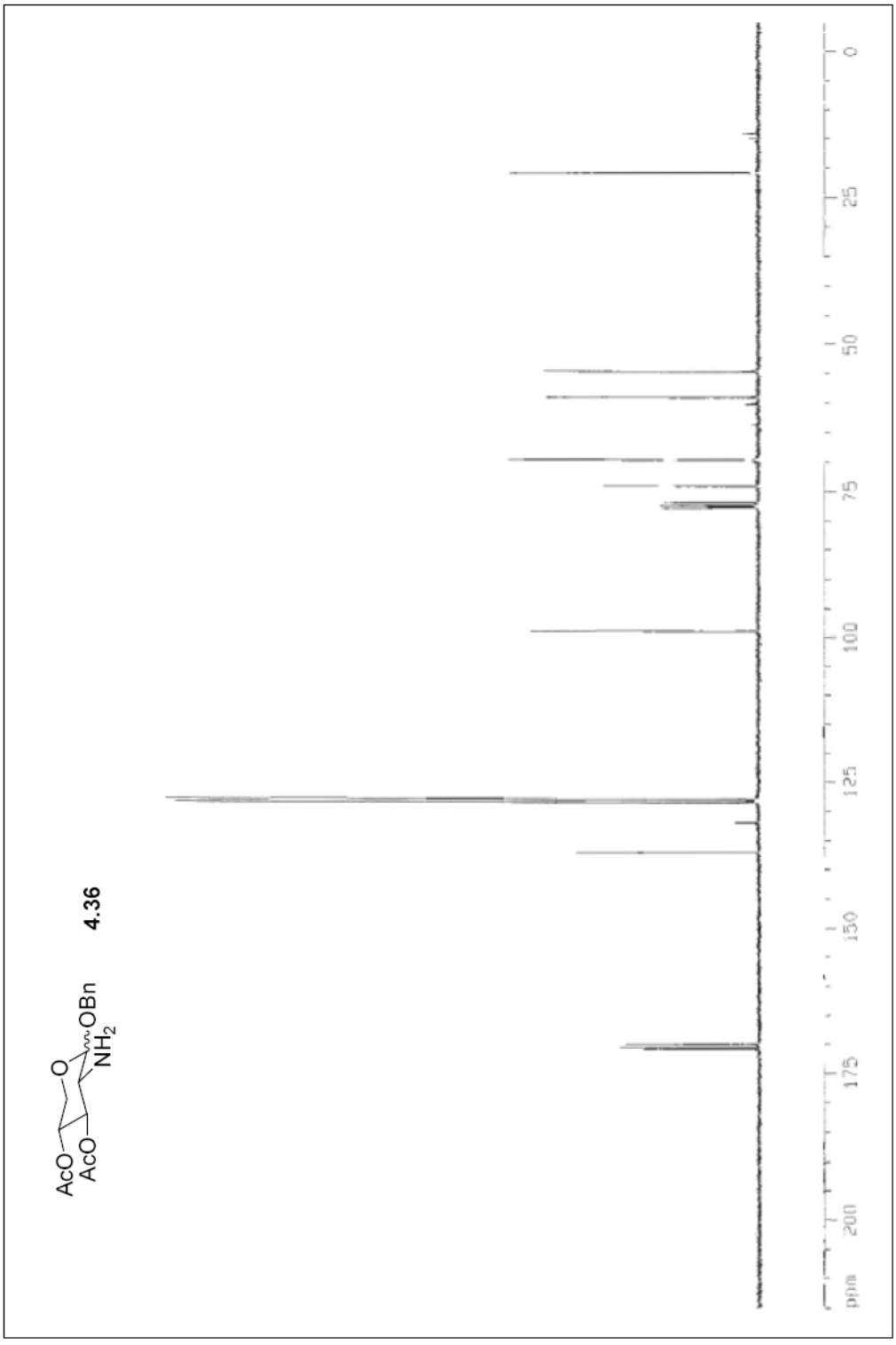

Figure A.50 75 MHz ^{13}C-NMR of compound 4.36 ($CDCl_3$)

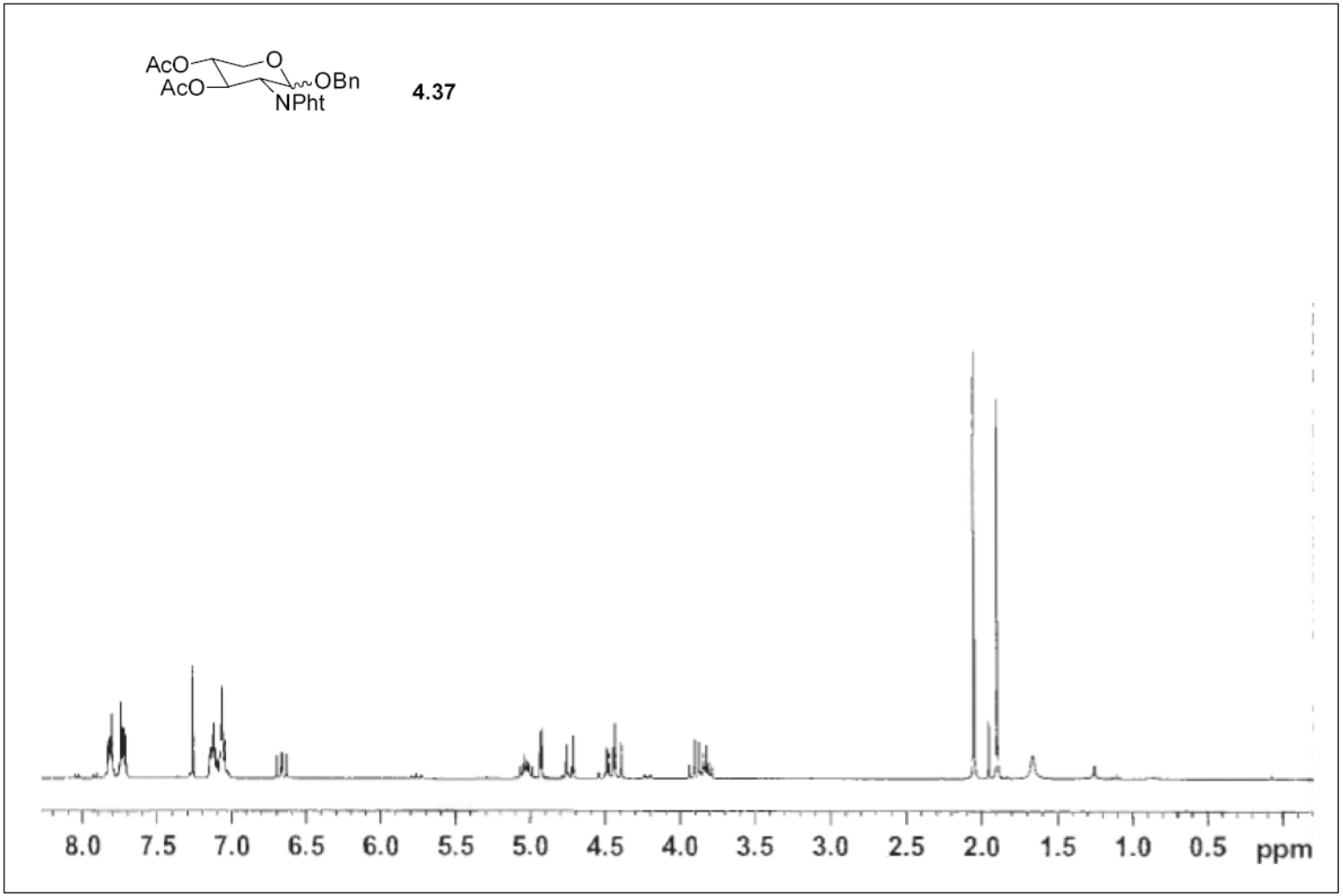

Figure A.51 300 MHz ^{1}H-NMR of compound 4.37 ($CDCl_3$)

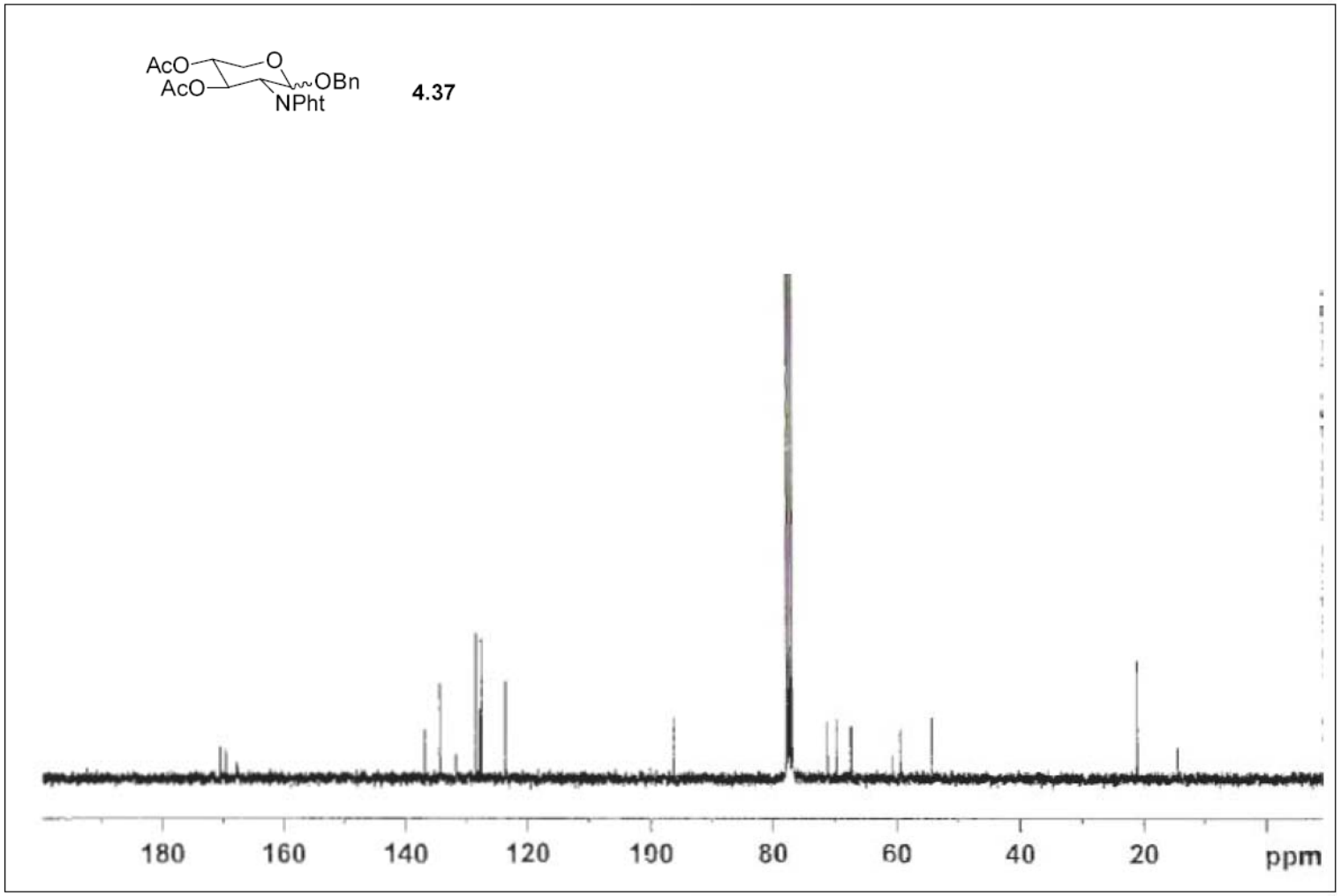

Figure A.52 75 MHz ^{13}C-NMR of compound 4.37 ($CDCl_3$)

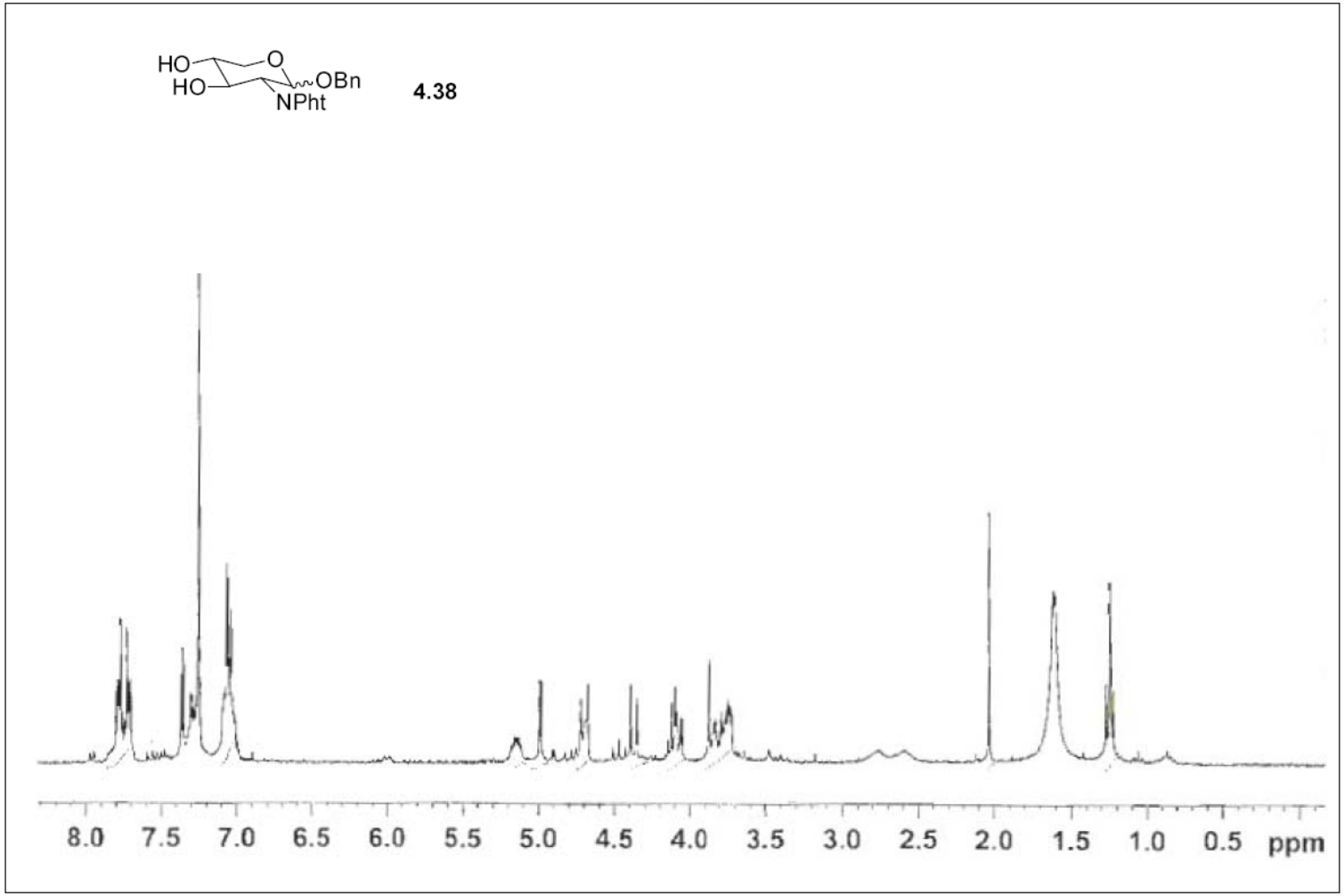

Figure A.53 300 MHz ^{1}H-NMR of compound 4.38 ($CDCl_3$)

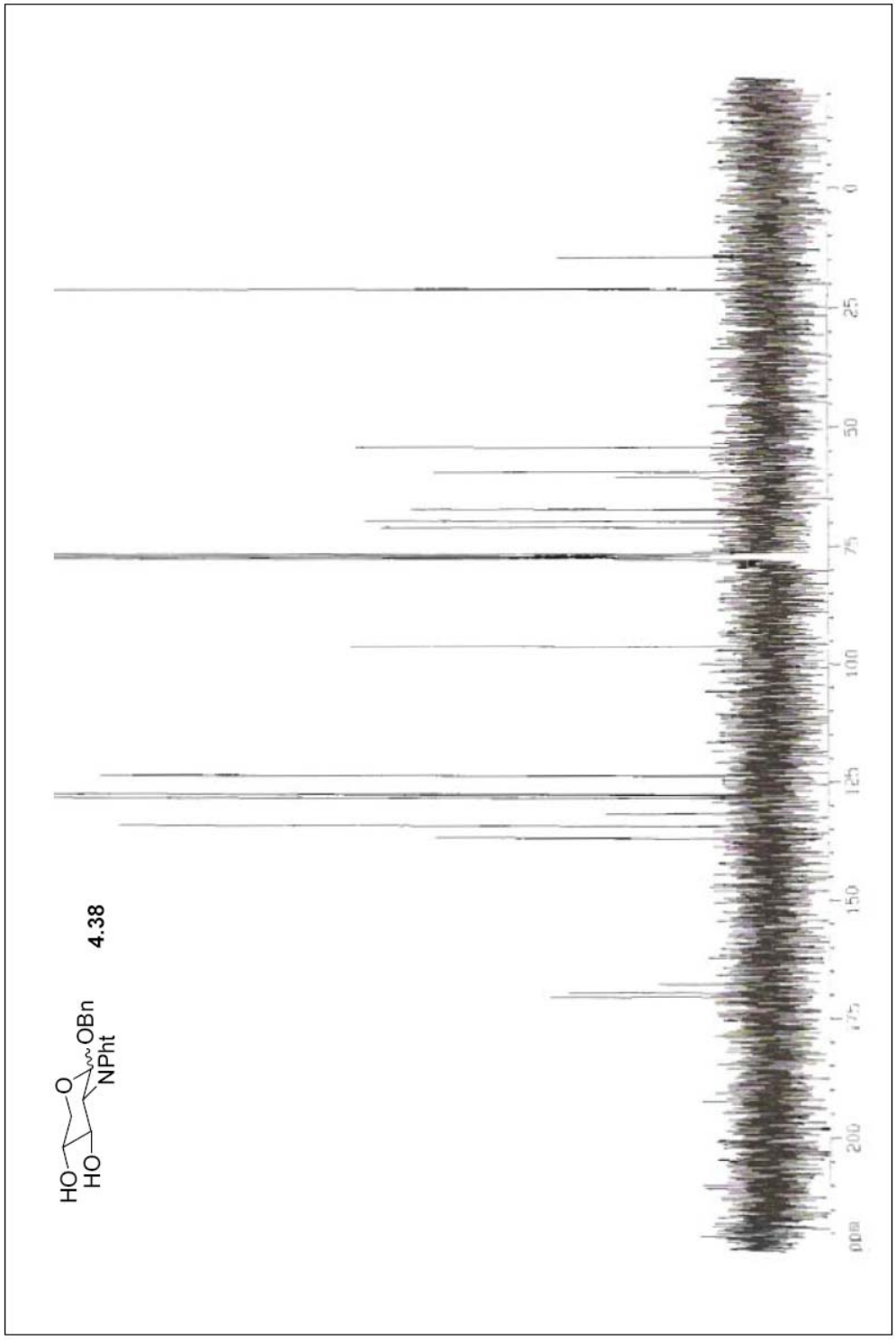

Figure A.54 75 MHz ^{13}C-NMR of compound 4.38 ($CDCl_3$)

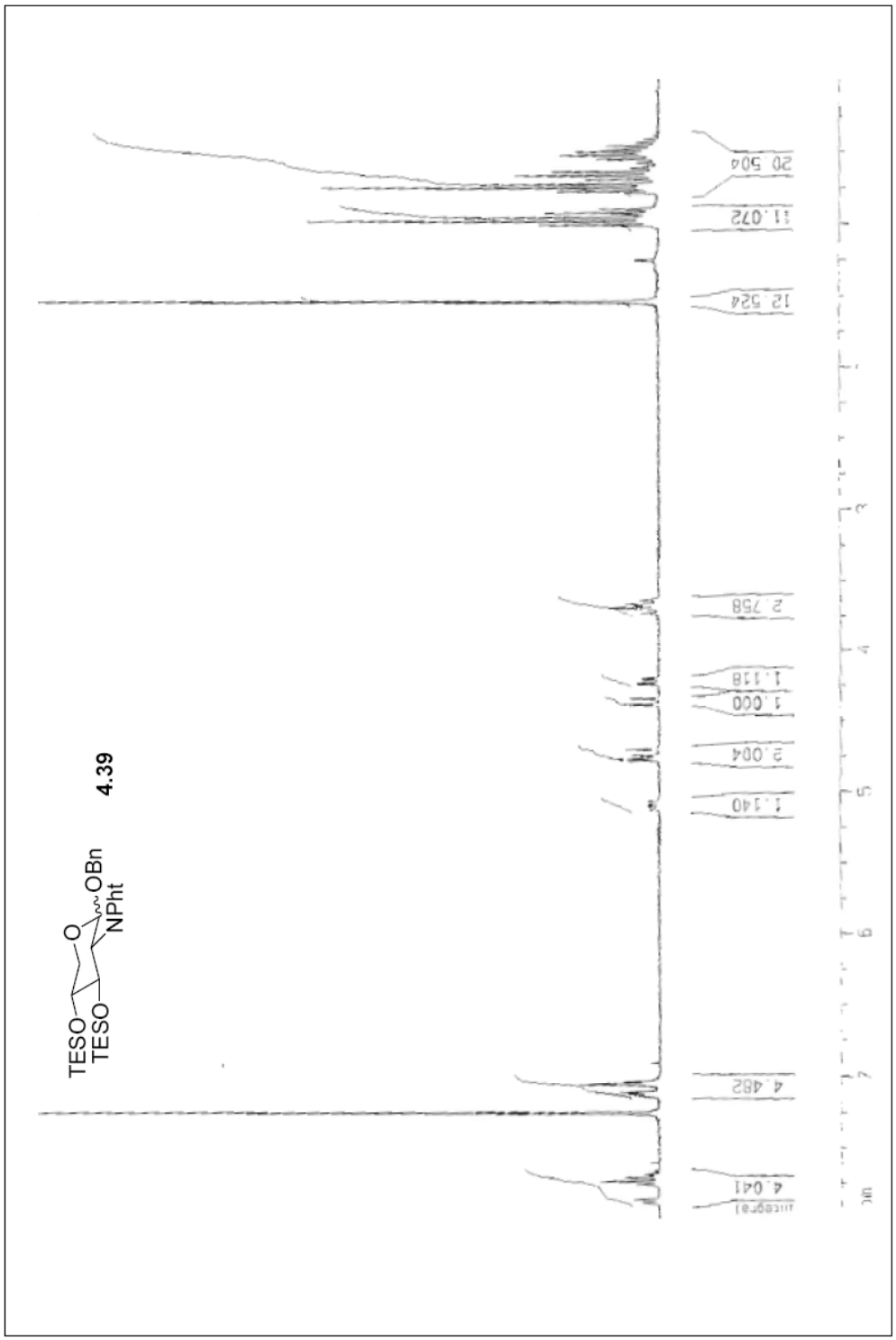

Figure A.55 300 MHz ^{1}H-NMR of compound 4.39 ($CDCl_3$)

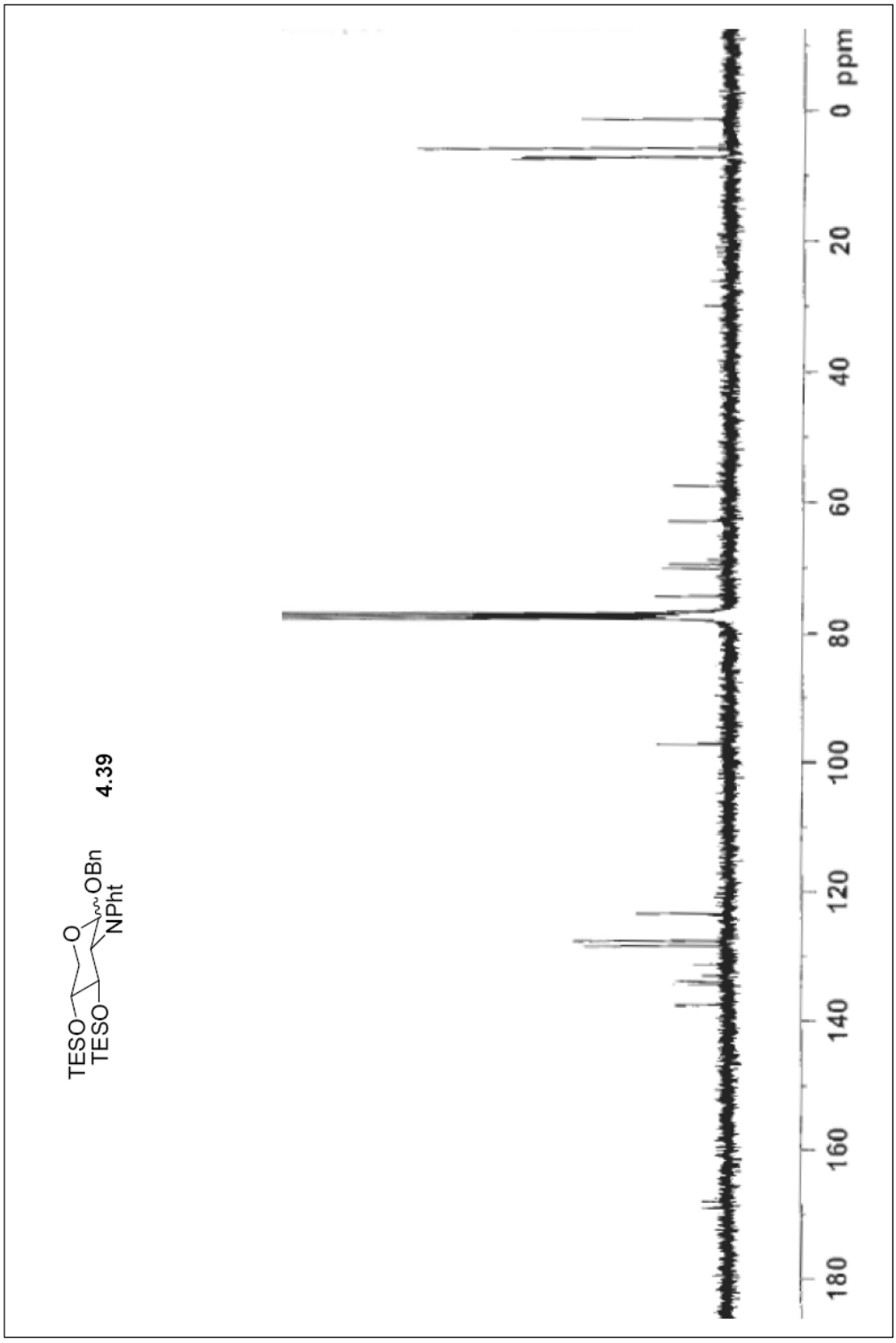

Figure A.56 75 MHz ^{13}C-NMR of compound 4.39 ($CDCl_3$)

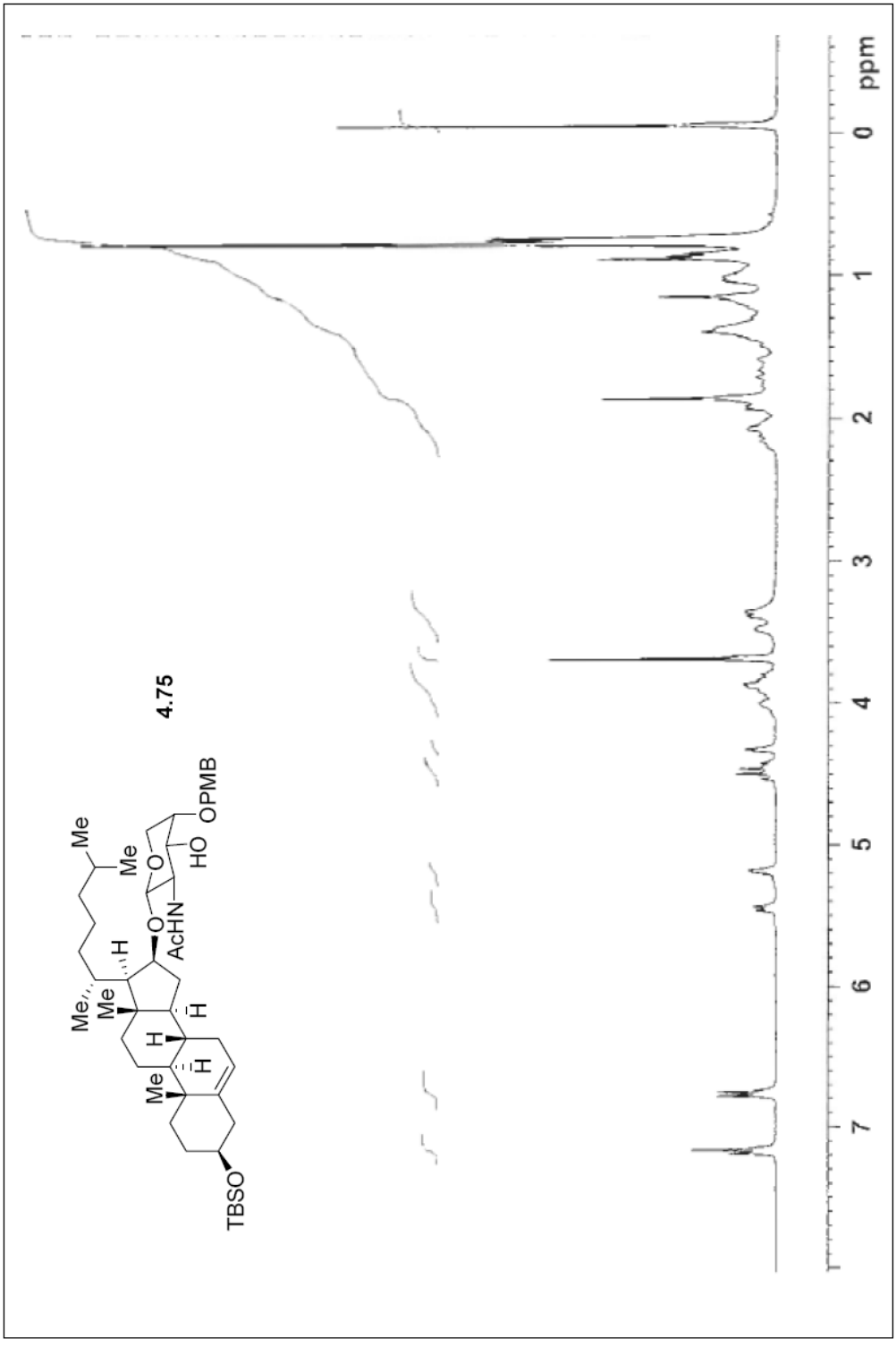

Figure A.57 300 MHz ^{1}H-NMR of compound 4.75 ($CDCl_3$)

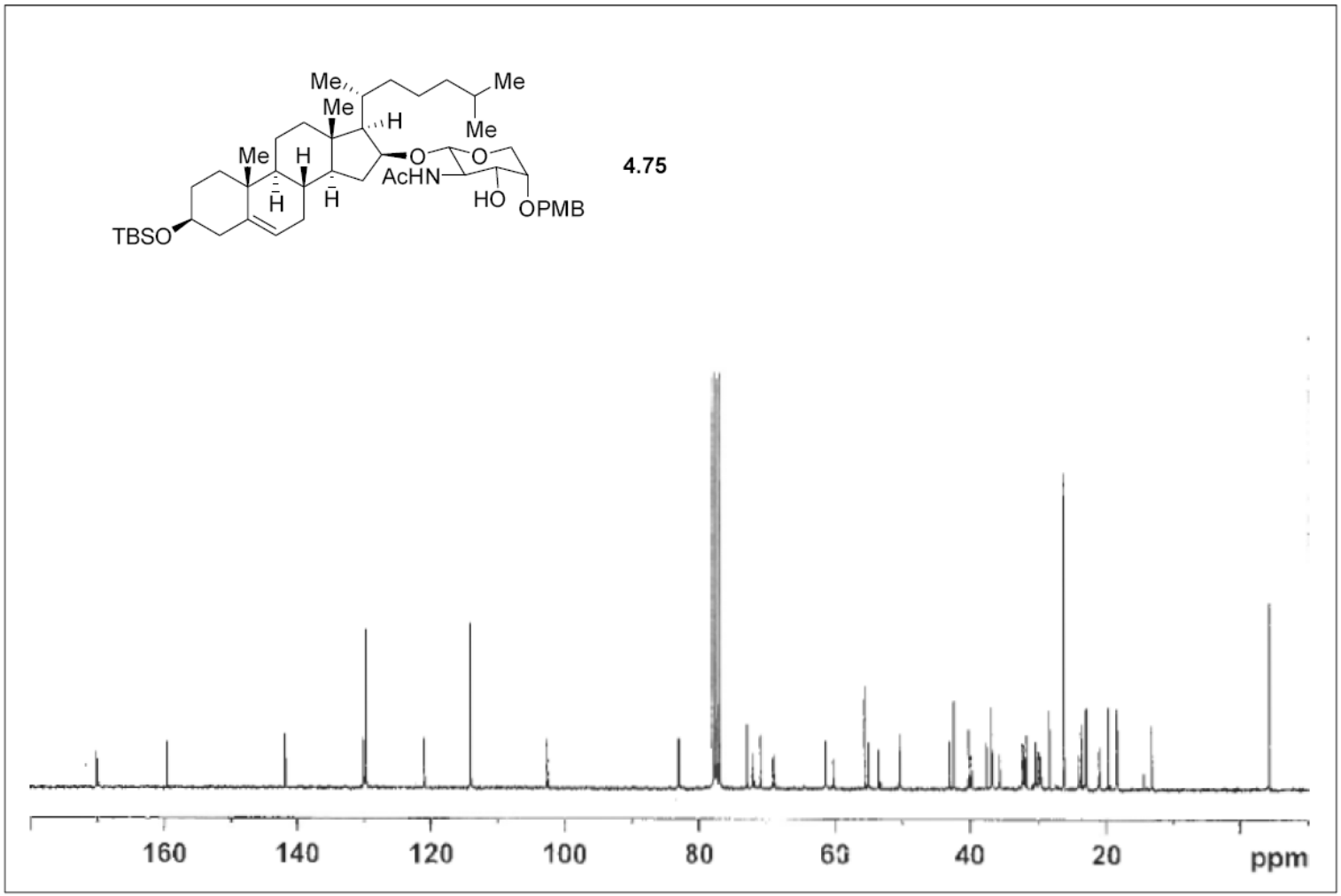

Figure A.58 75 MHz ^{13}C-NMR of compound 4.75 ($CDCl_3$)

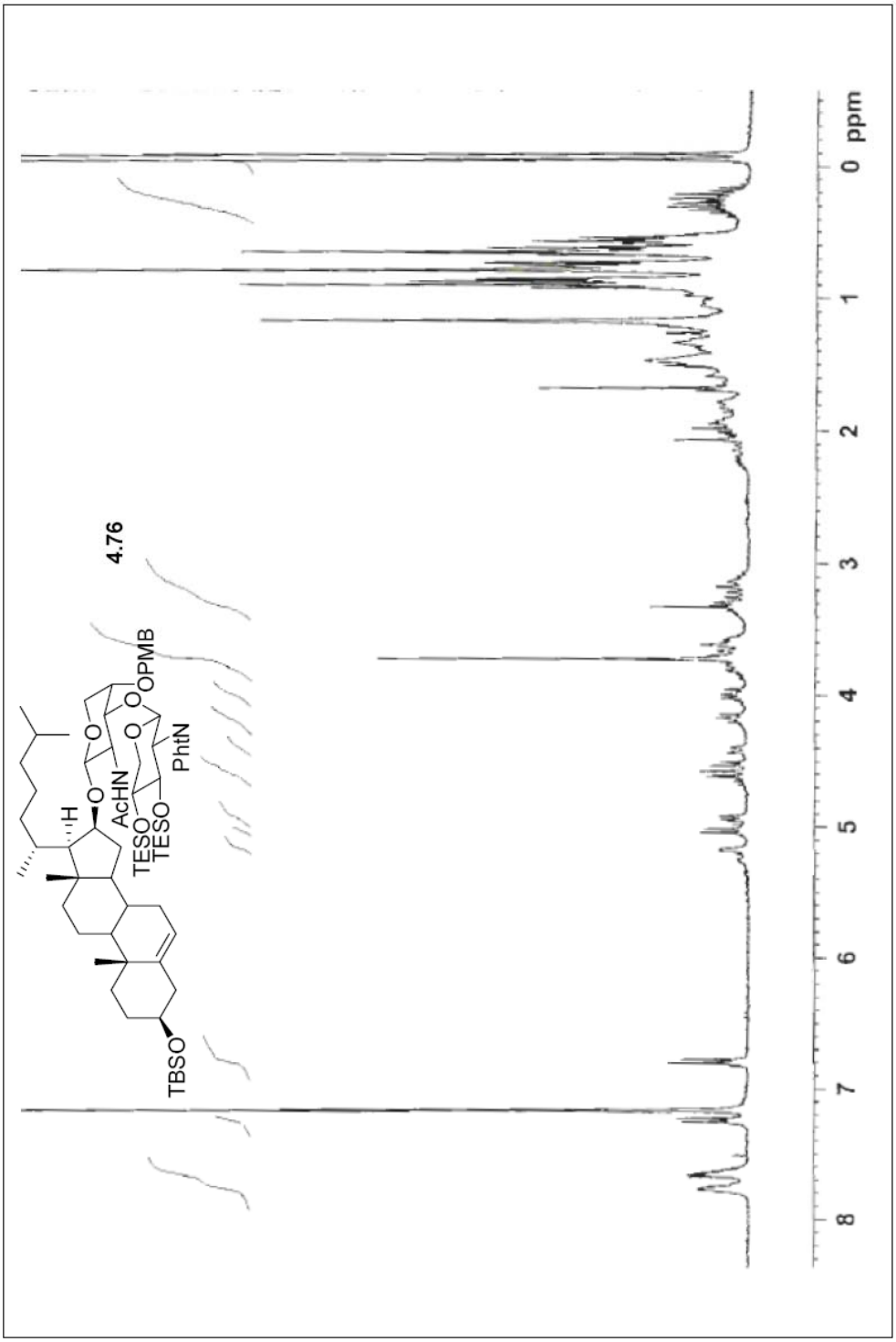

Figure A.59 300 MHz ^{1}H-NMR of compound 4.76 ($CDCl_3$)

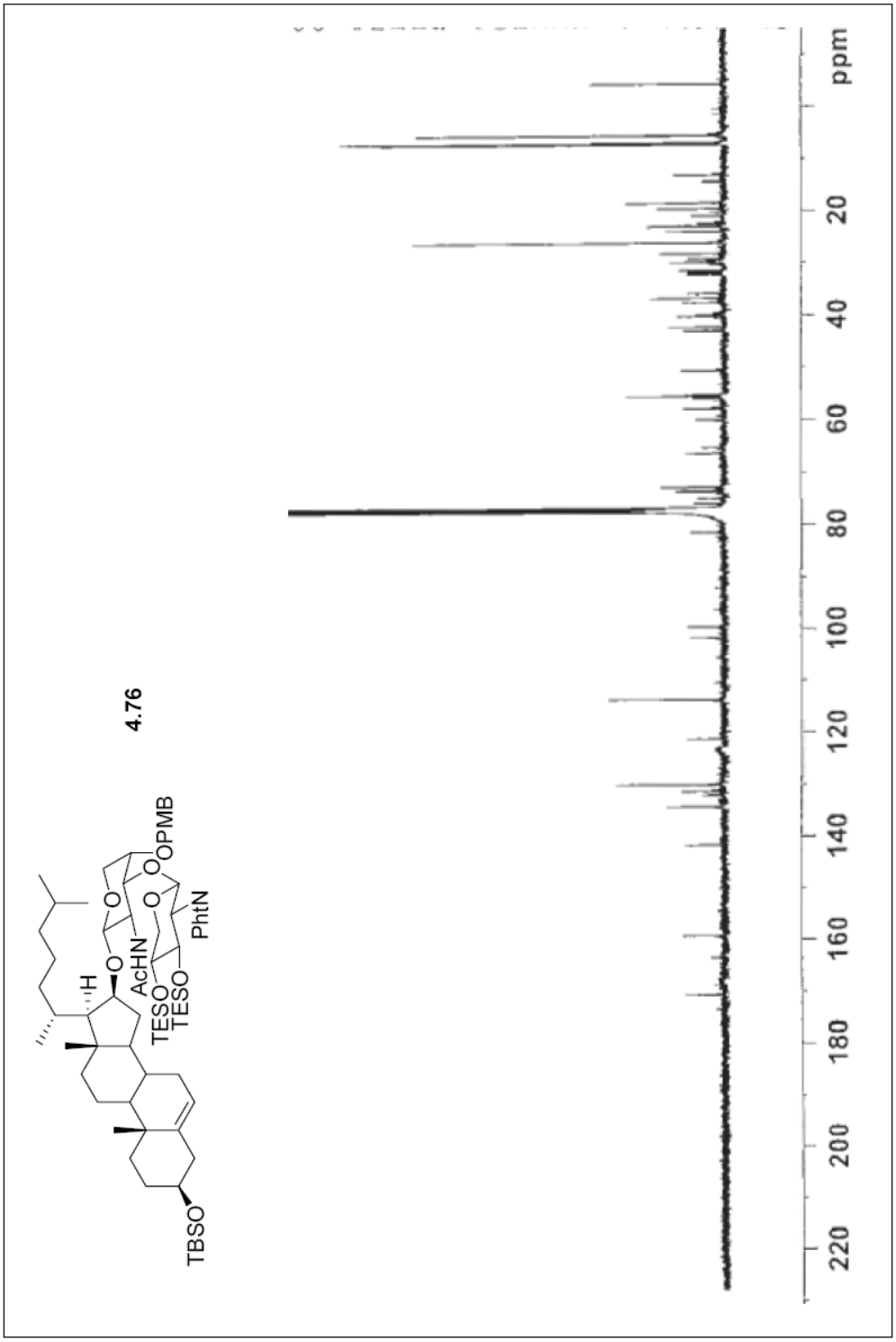

Figure A.60 75 MHz ^{13}C-NMR of compound 4.76 ($CDCl_3$)

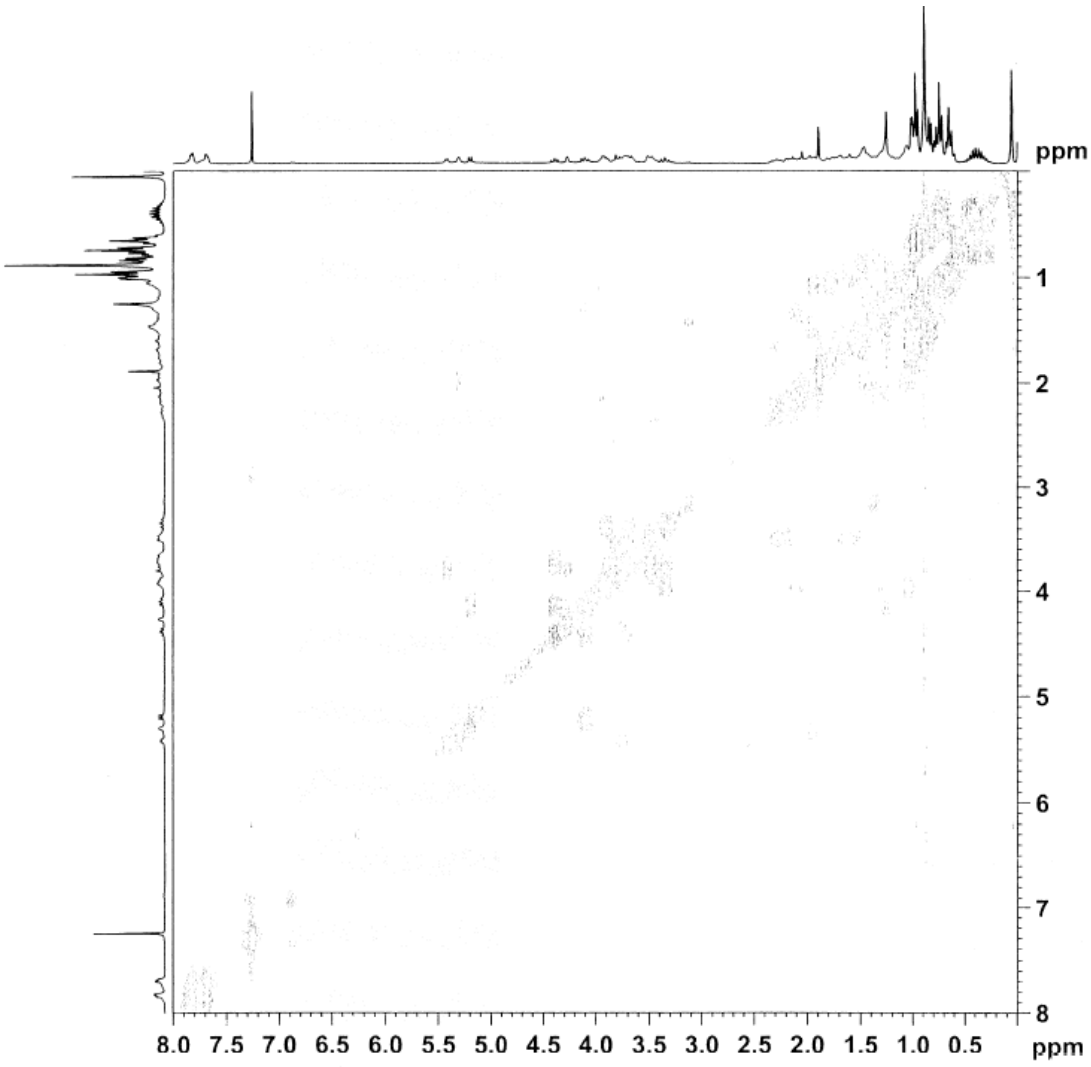

Figure A.60-CONTINUED NOE of compound **4.76**

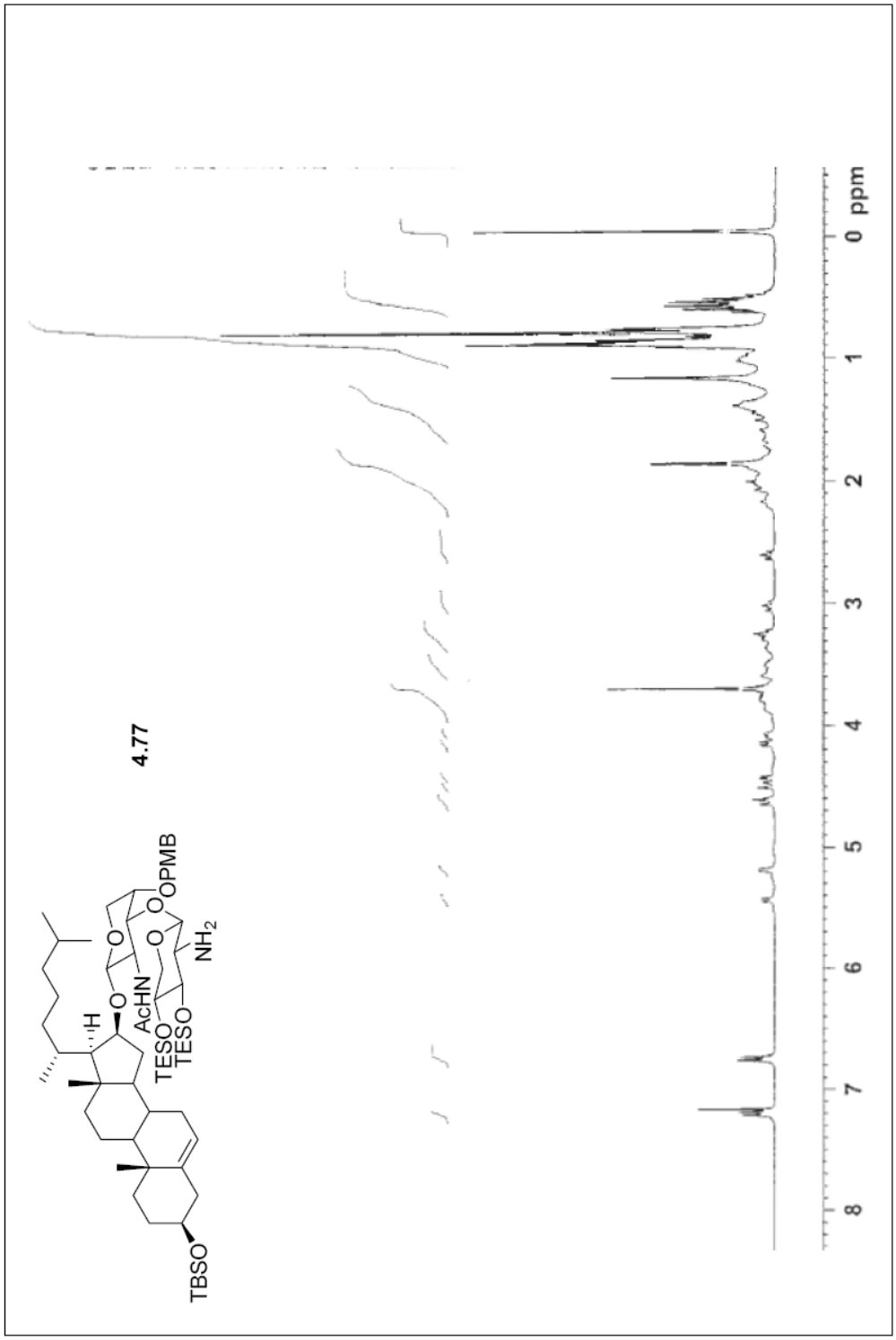

Figure A.61 300 MHz ^{1}H-NMR of compound 4.77 ($CDCl_3$)

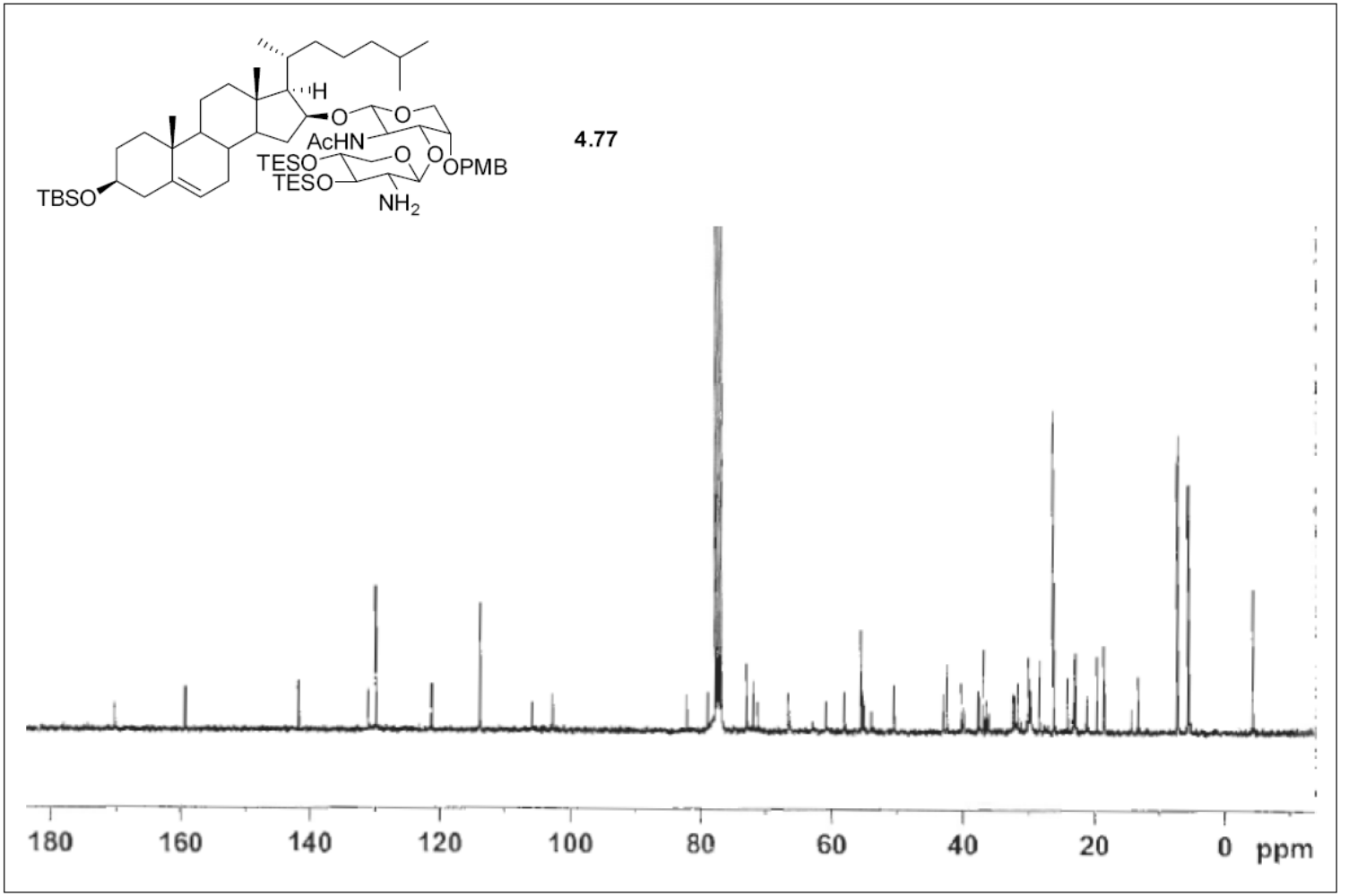

Figure A.62 75 MHz ^{13}C-NMR of compound 4.77 ($CDCl_3$)

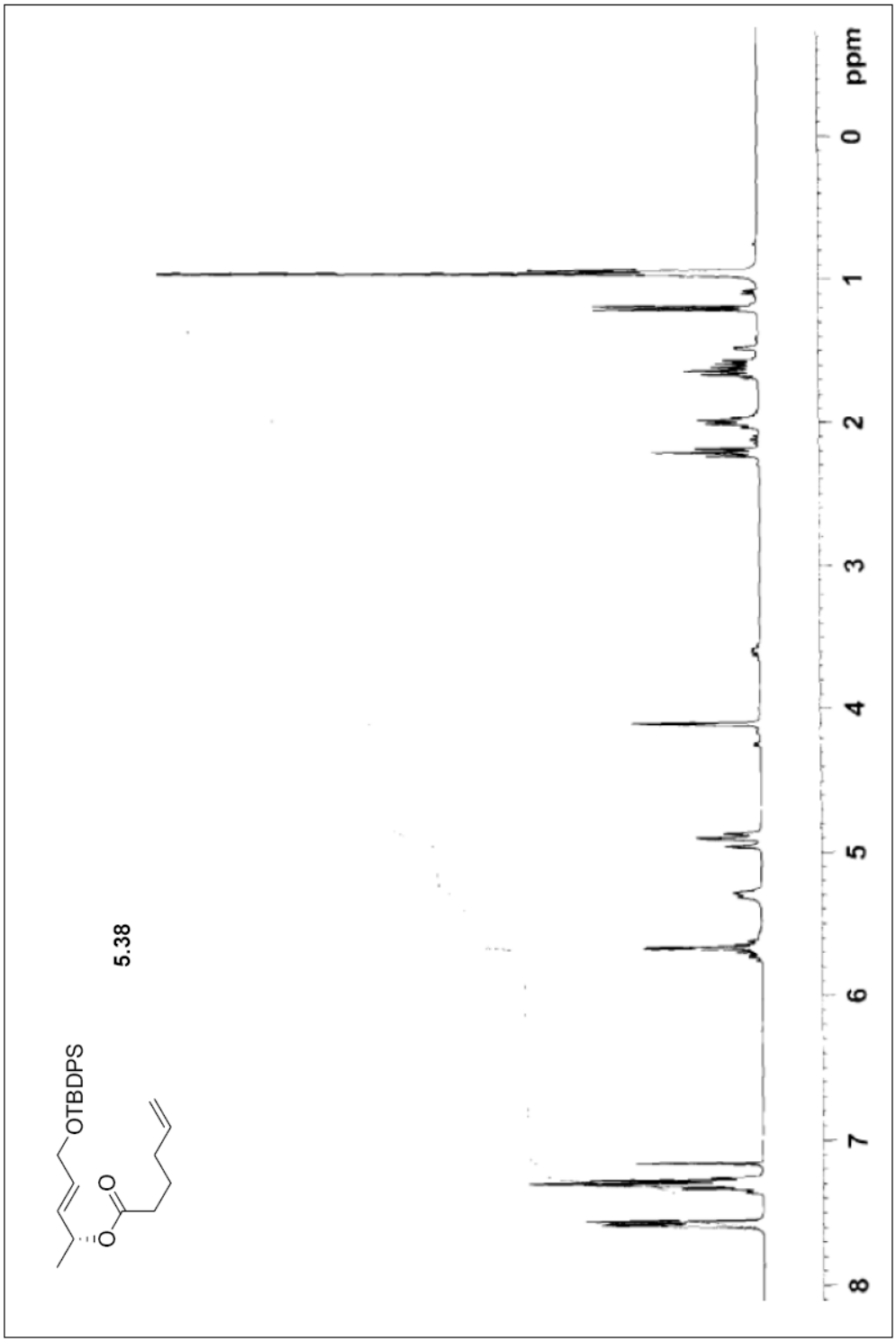

Figure A.63 300 MHz ^{1}H-NMR of compound 5.38 ($CDCl_3$)

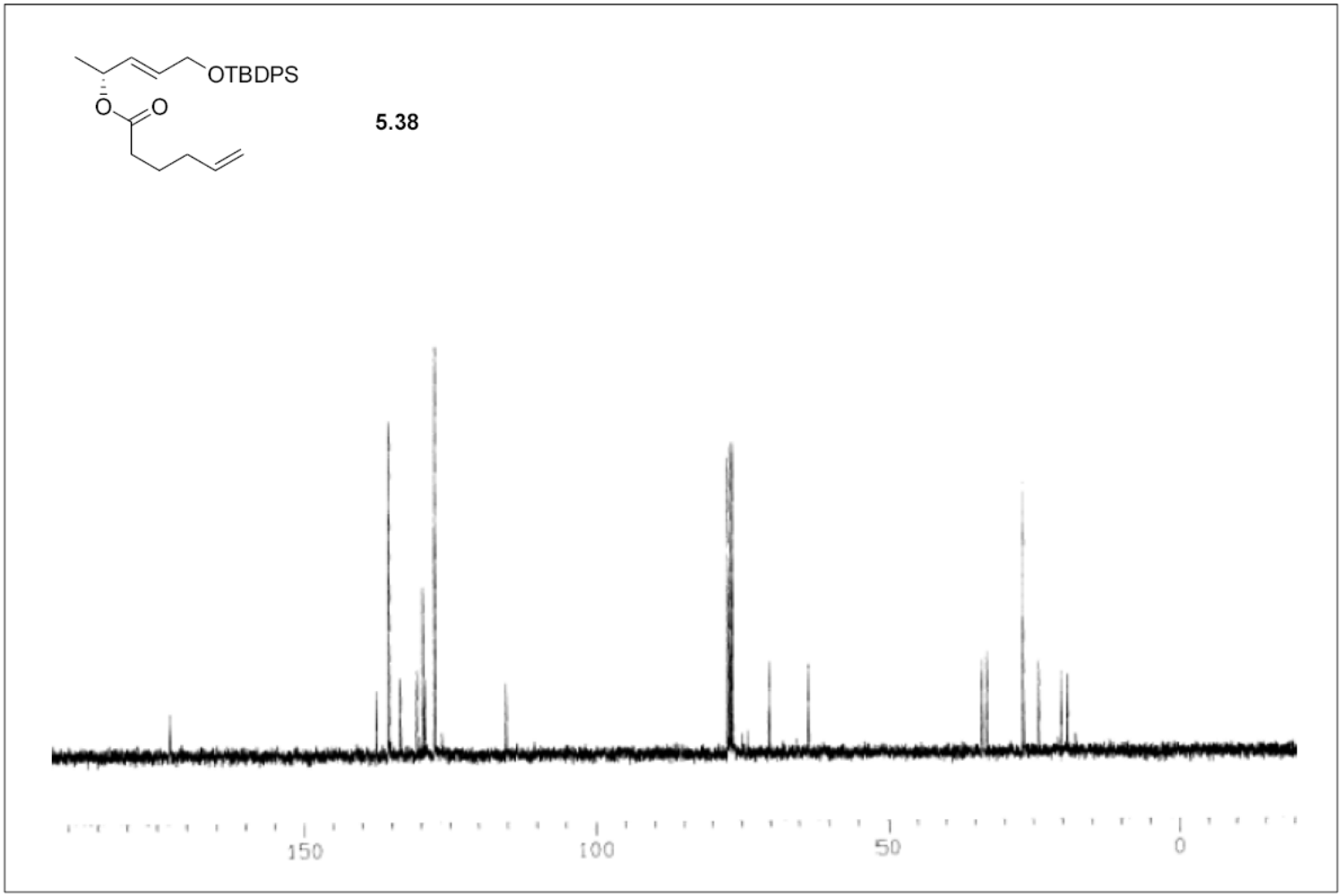

Figure A.64 75 MHz ^{13}C-NMR of compound 5.38 ($CDCl_3$)

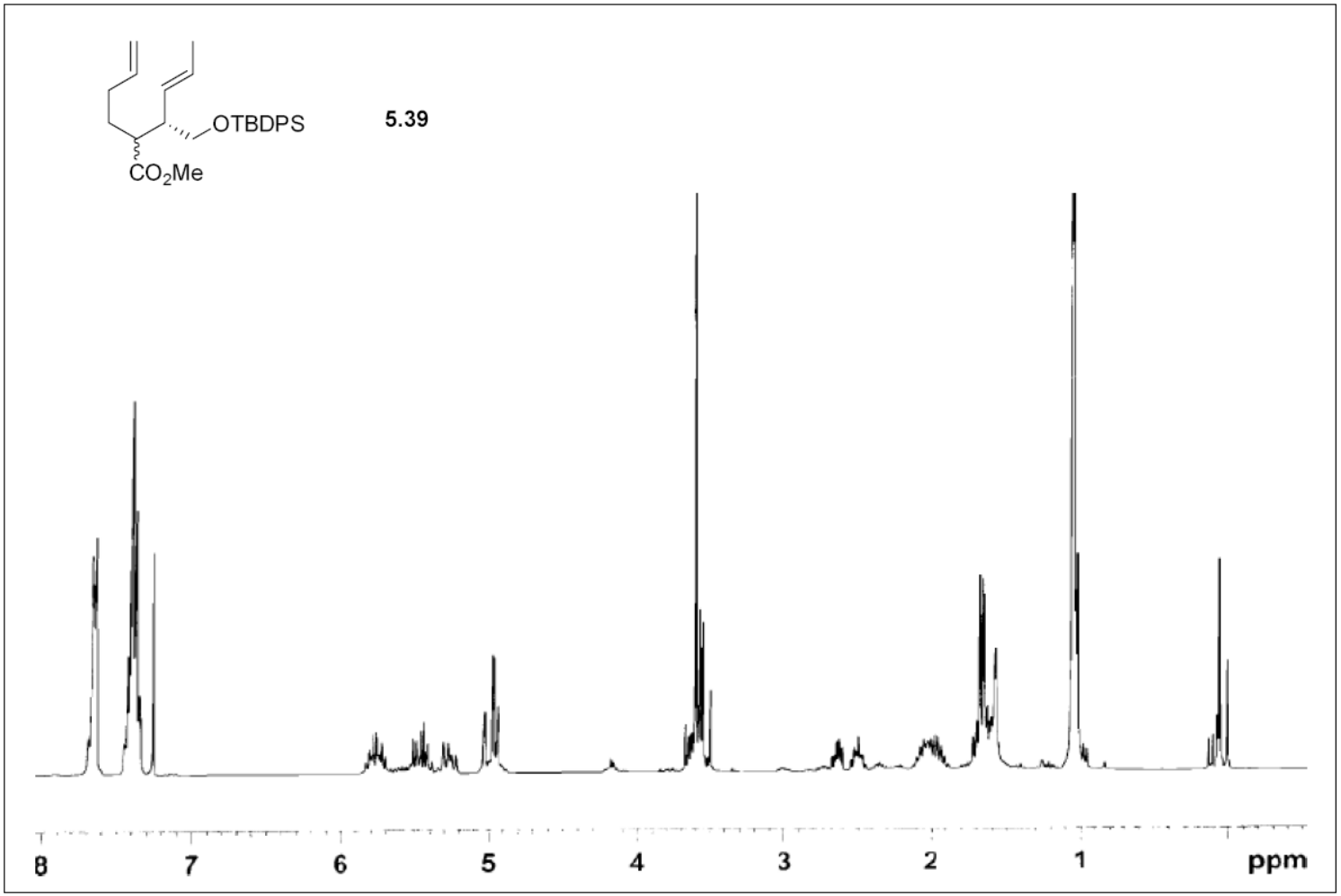

Figure A.65 300 MHz ^{1}H-NMR of compound 5.39 ($CDCl_3$)

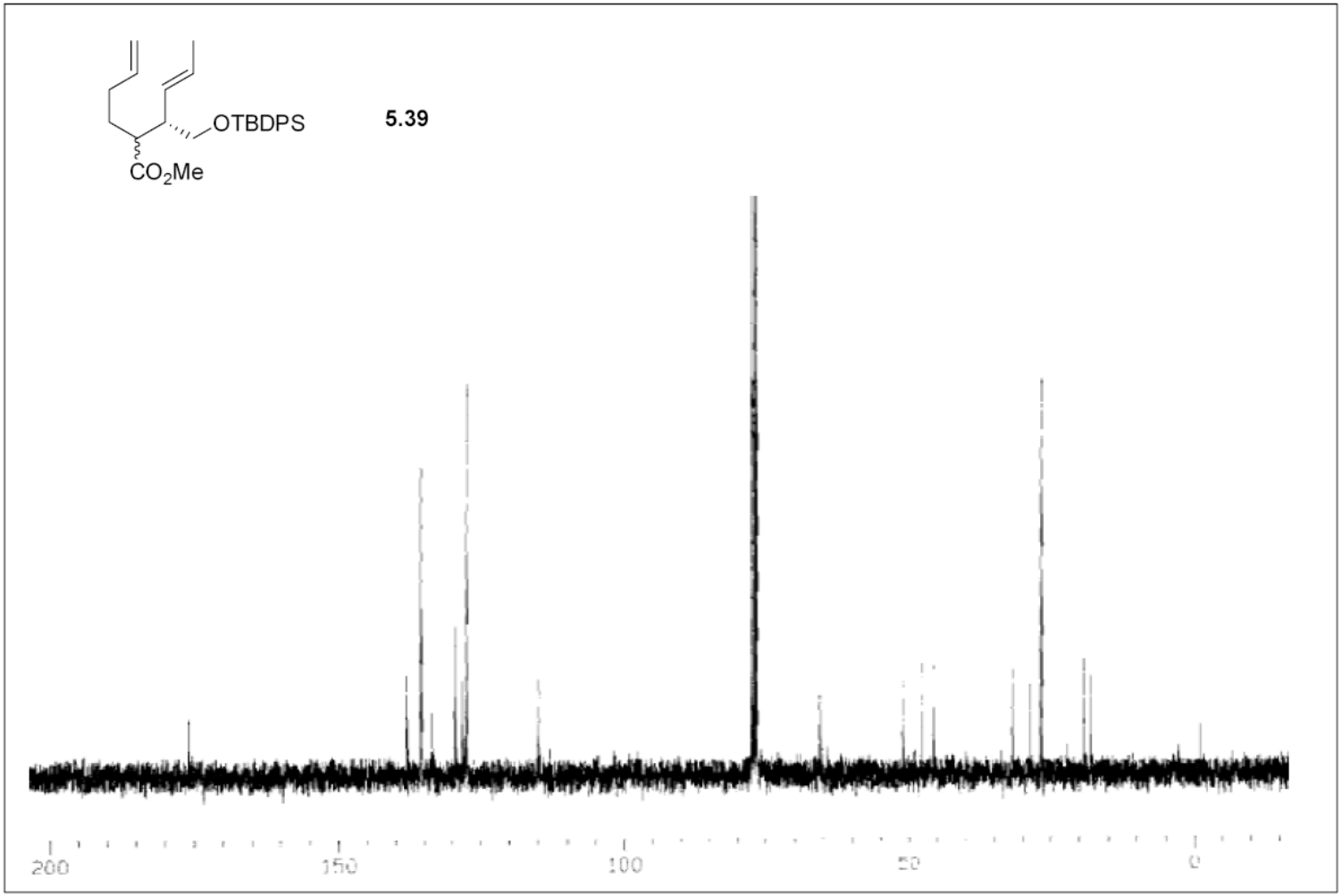

Figure A.66 75 MHz ^{13}C-NMR of compound 5.39 ($CDCl_3$)

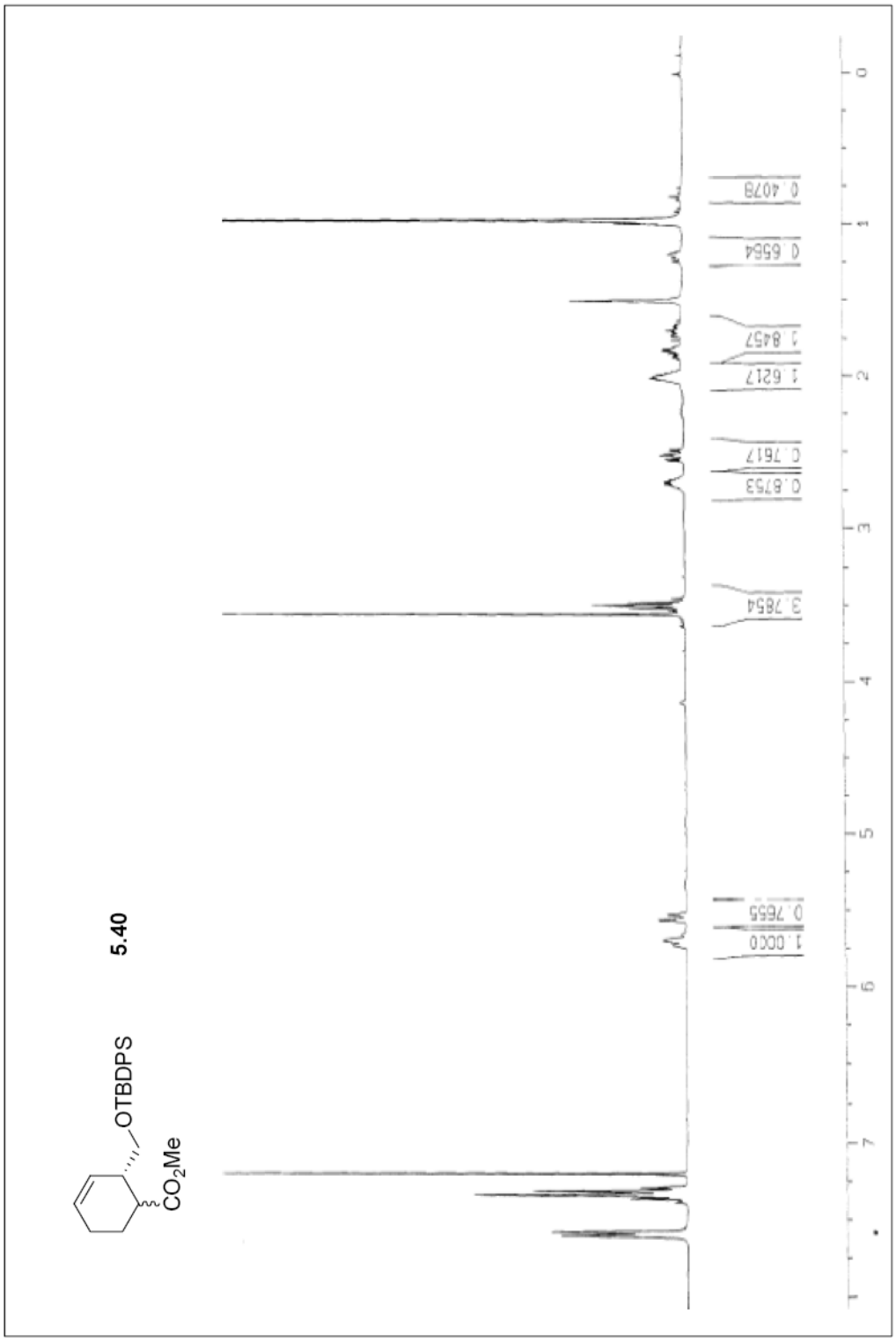

Figure A.67 300 MHz ^{1}H-NMR of compound 5.40 ($CDCl_3$)

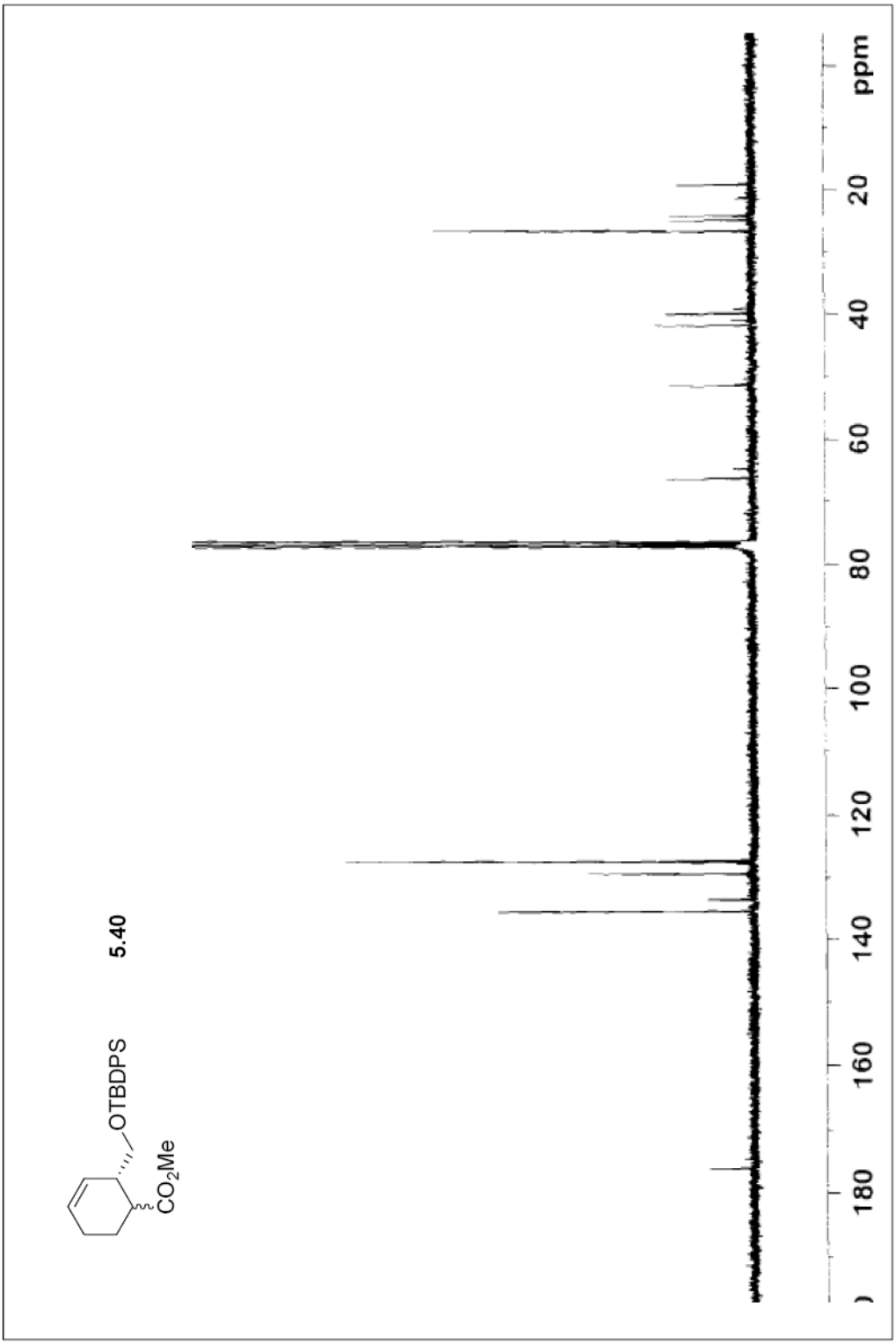

Figure A.68 75 MHz ^{13}C-NMR of compound 5.40 ($CDCl_3$)

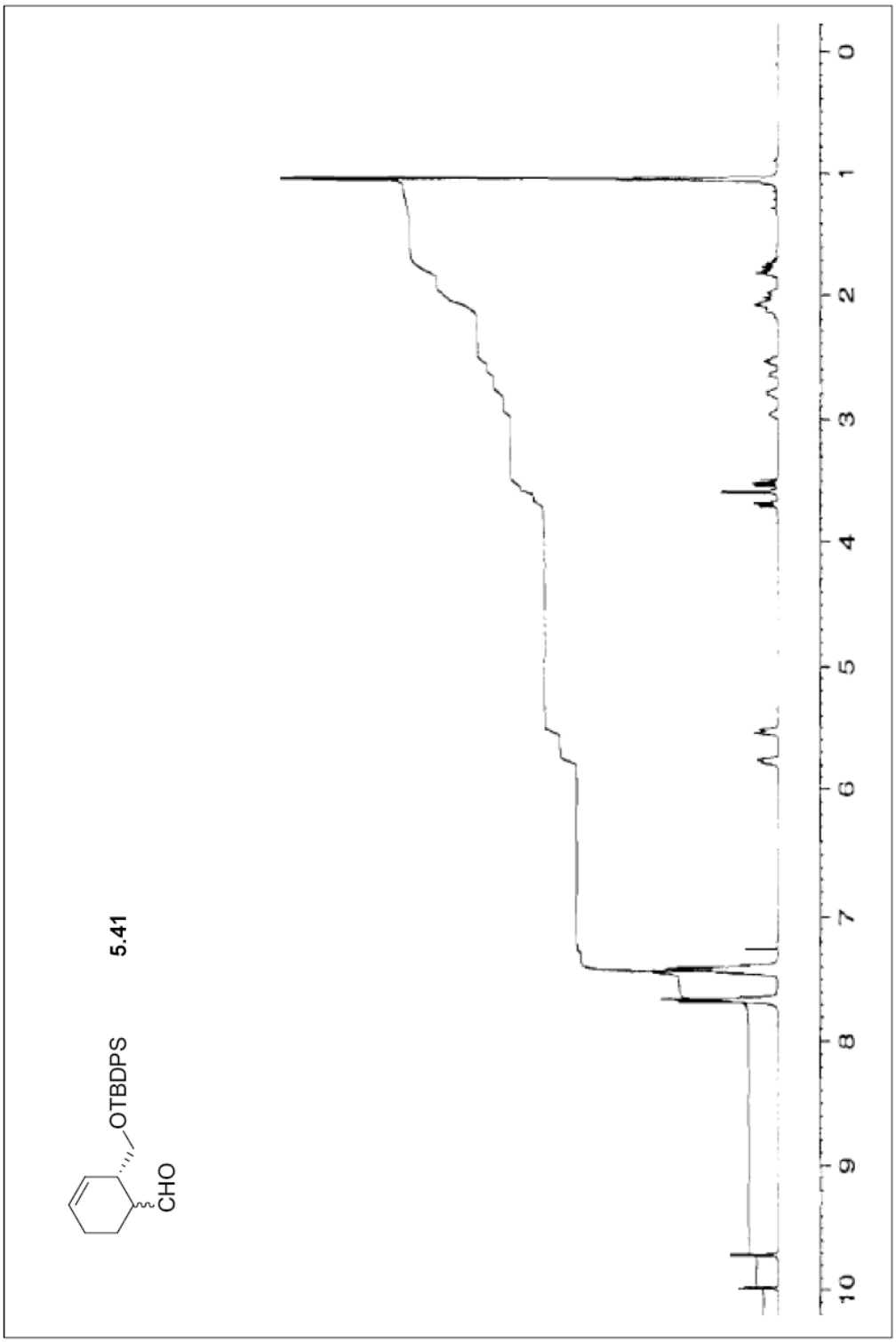

Figure A.69 300 MHz ^{1}H-NMR of compound 5.41 ($CDCl_3$)

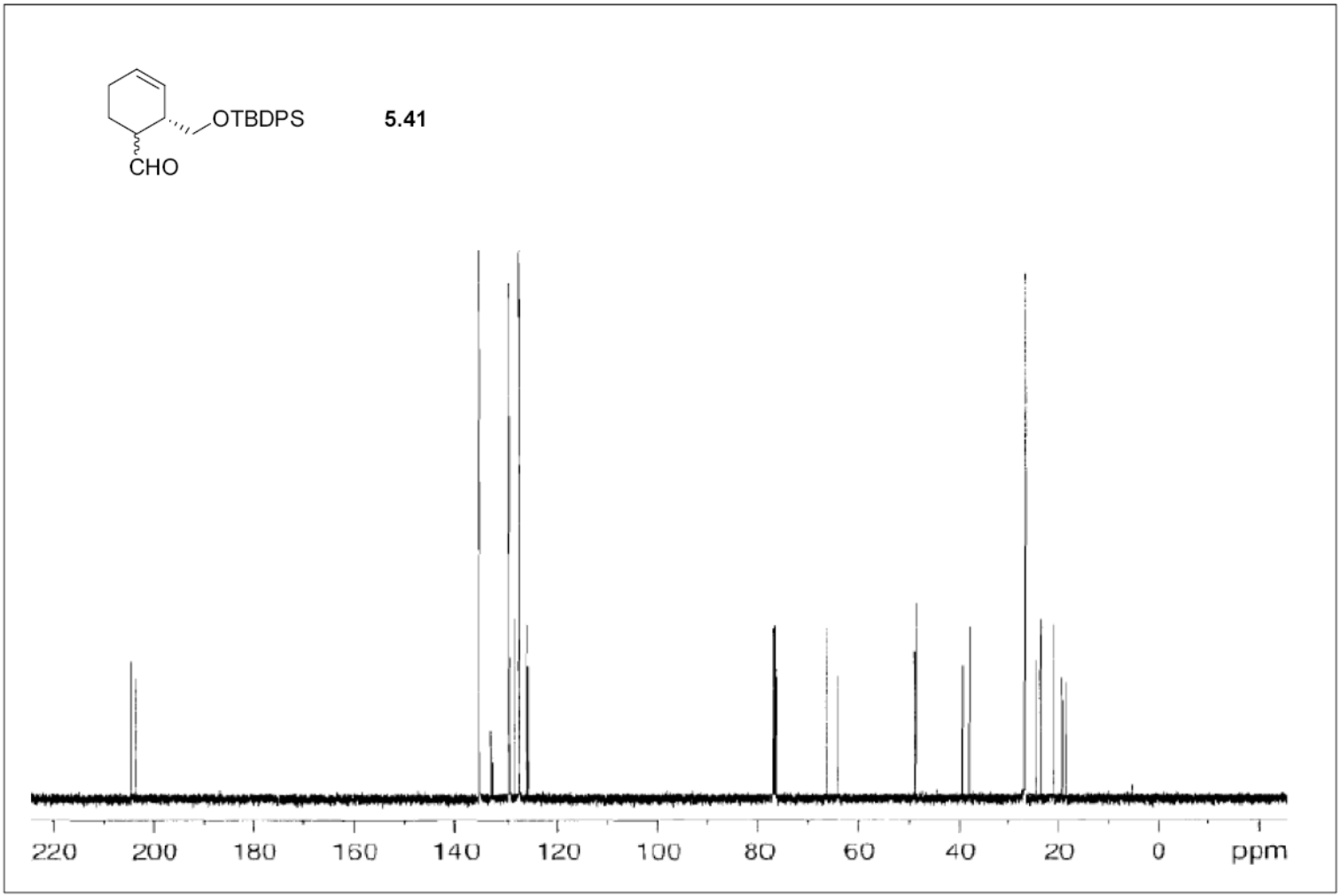

Figure A.70 75 MHz ^{13}C-NMR of compound 5.41 ($CDCl_3$)

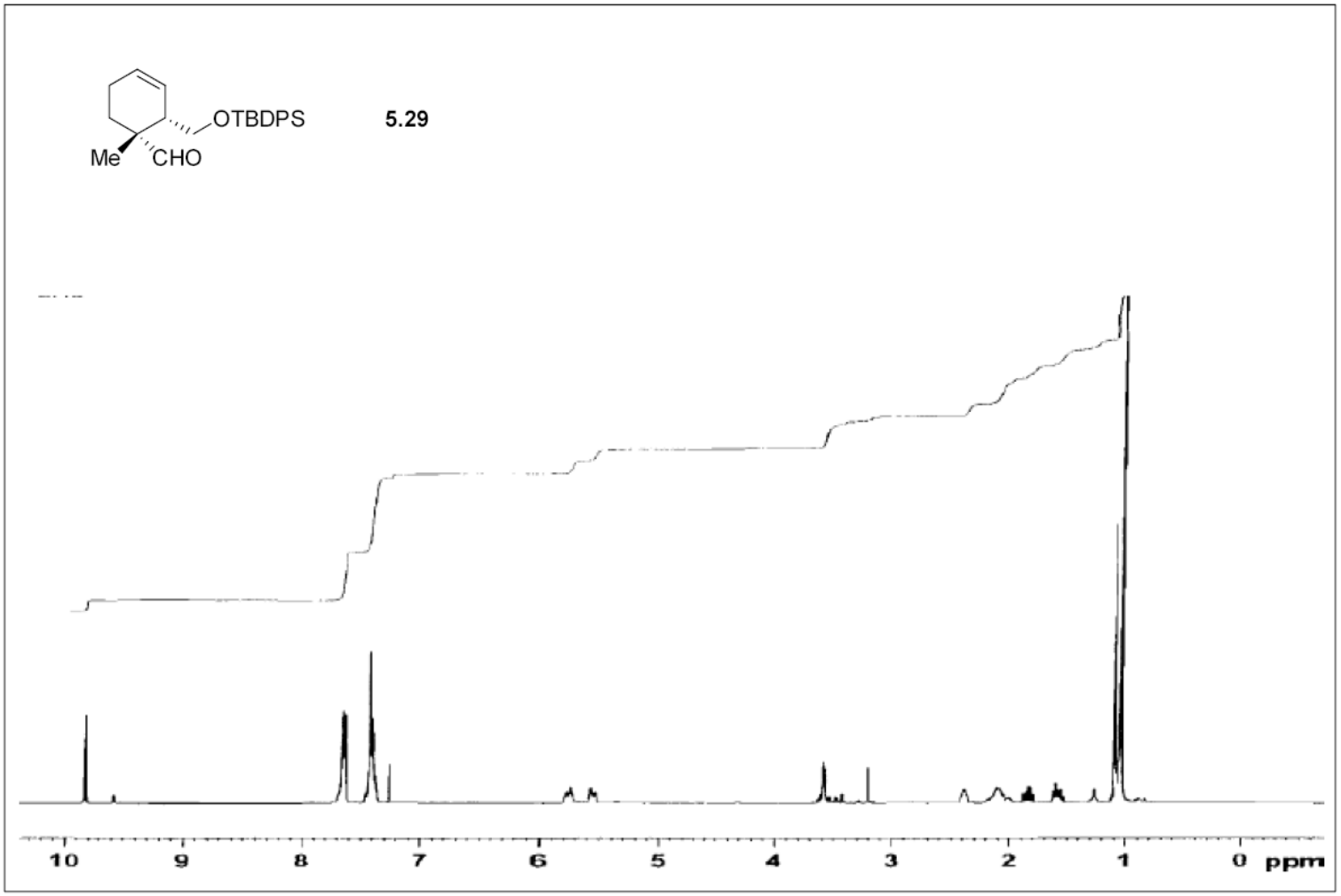

Figure A.71 300 MHz ^{1}H-NMR of compound 5.29 ($CDCl_3$)

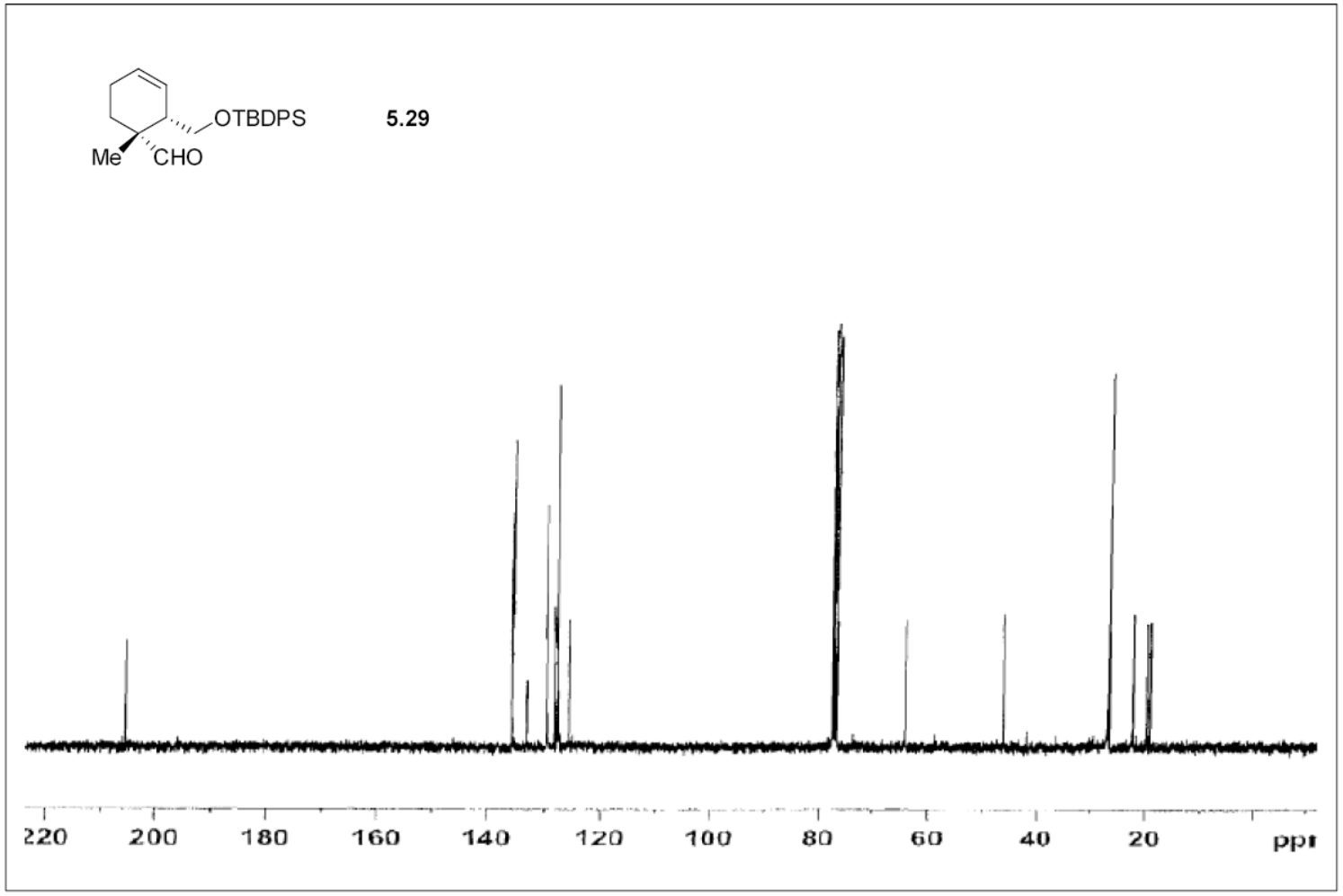

Figure A.72 75 MHz ^{13}C-NMR of compound 5.29 ($CDCl_3$)

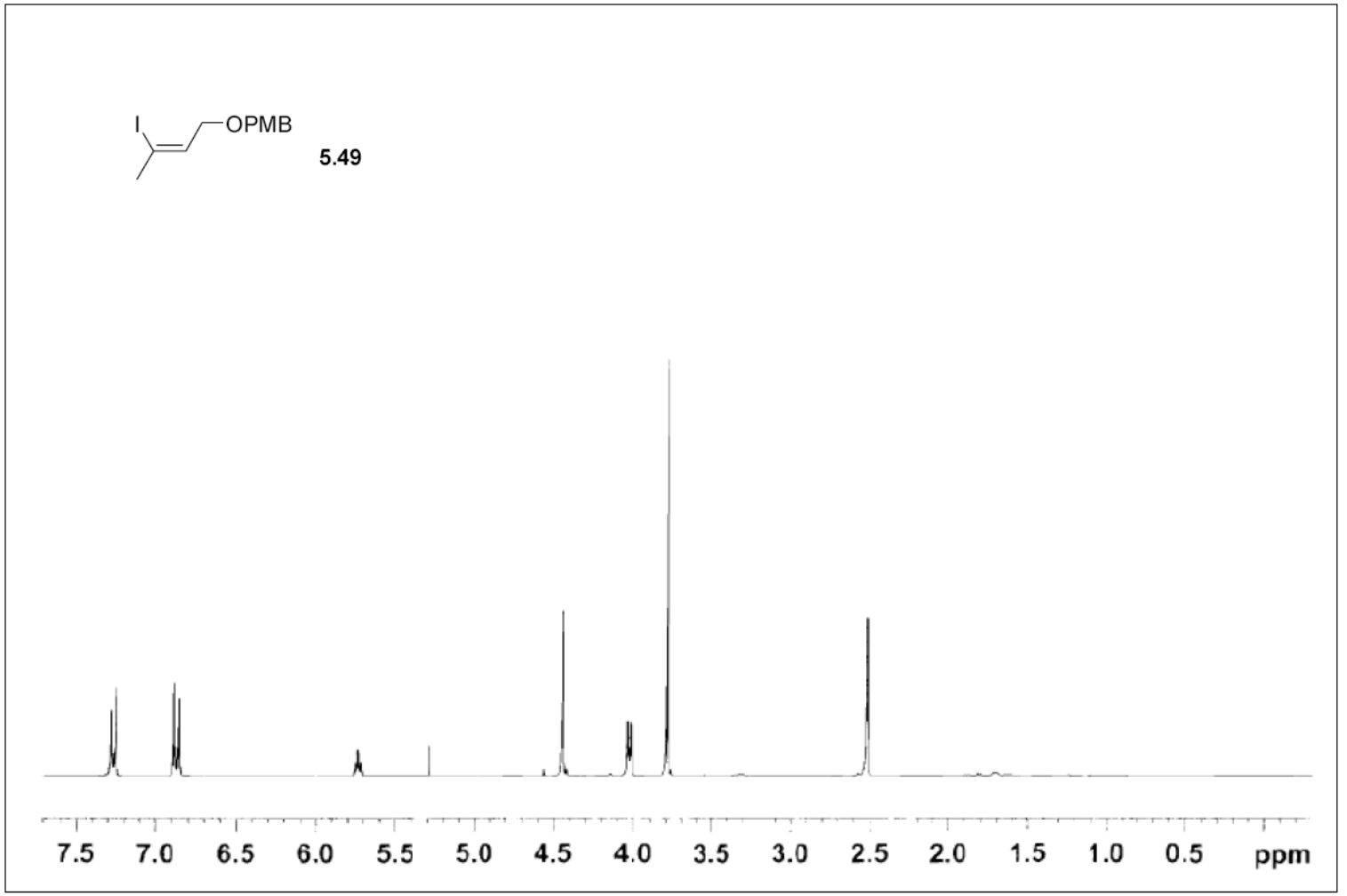

Figure A.73 300 MHz ^{1}H-NMR of compound 5.49 ($CDCl_3$)

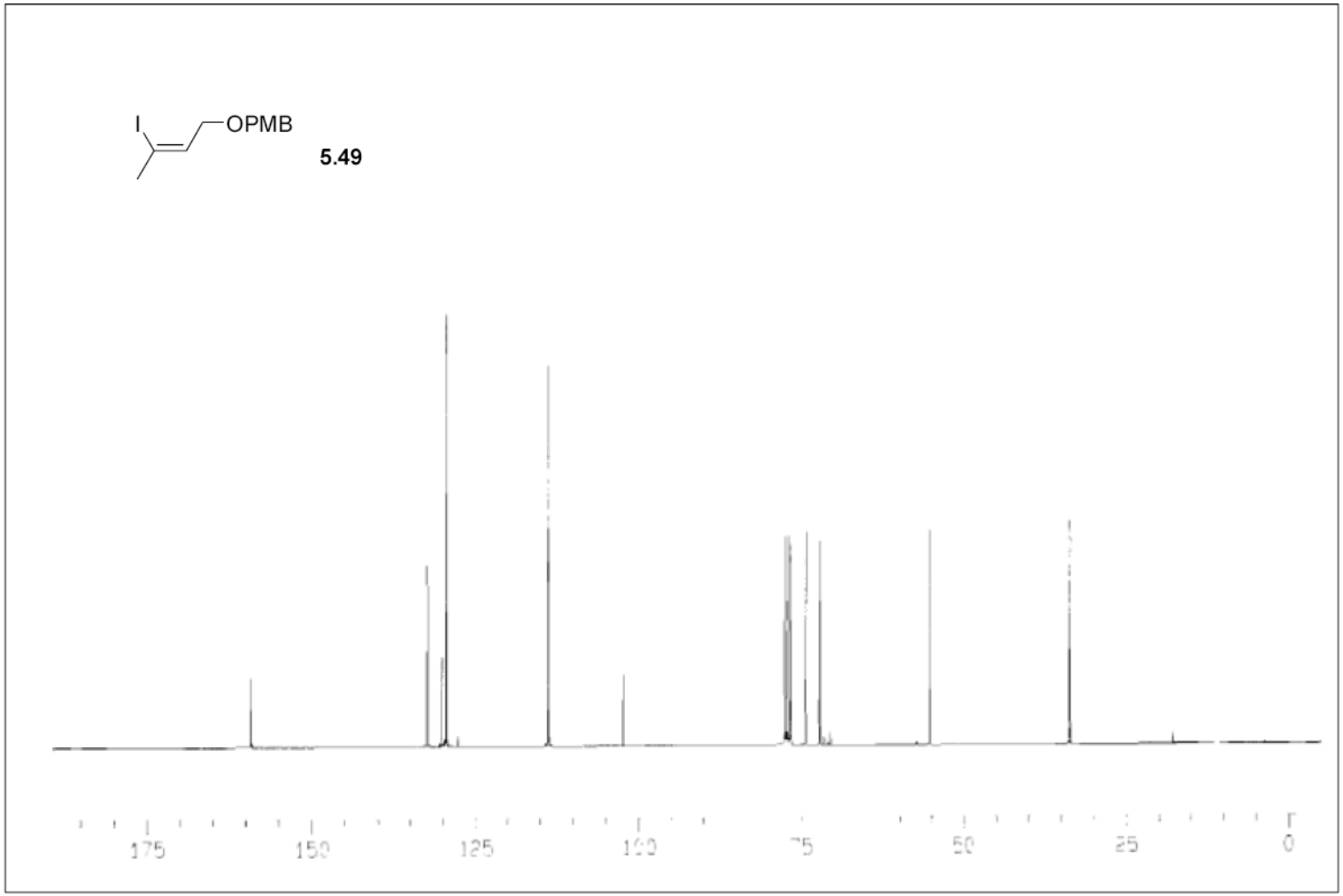

Figure A.74 75 MHz ^{13}C-NMR of compound 5.49 ($CDCl_3$)

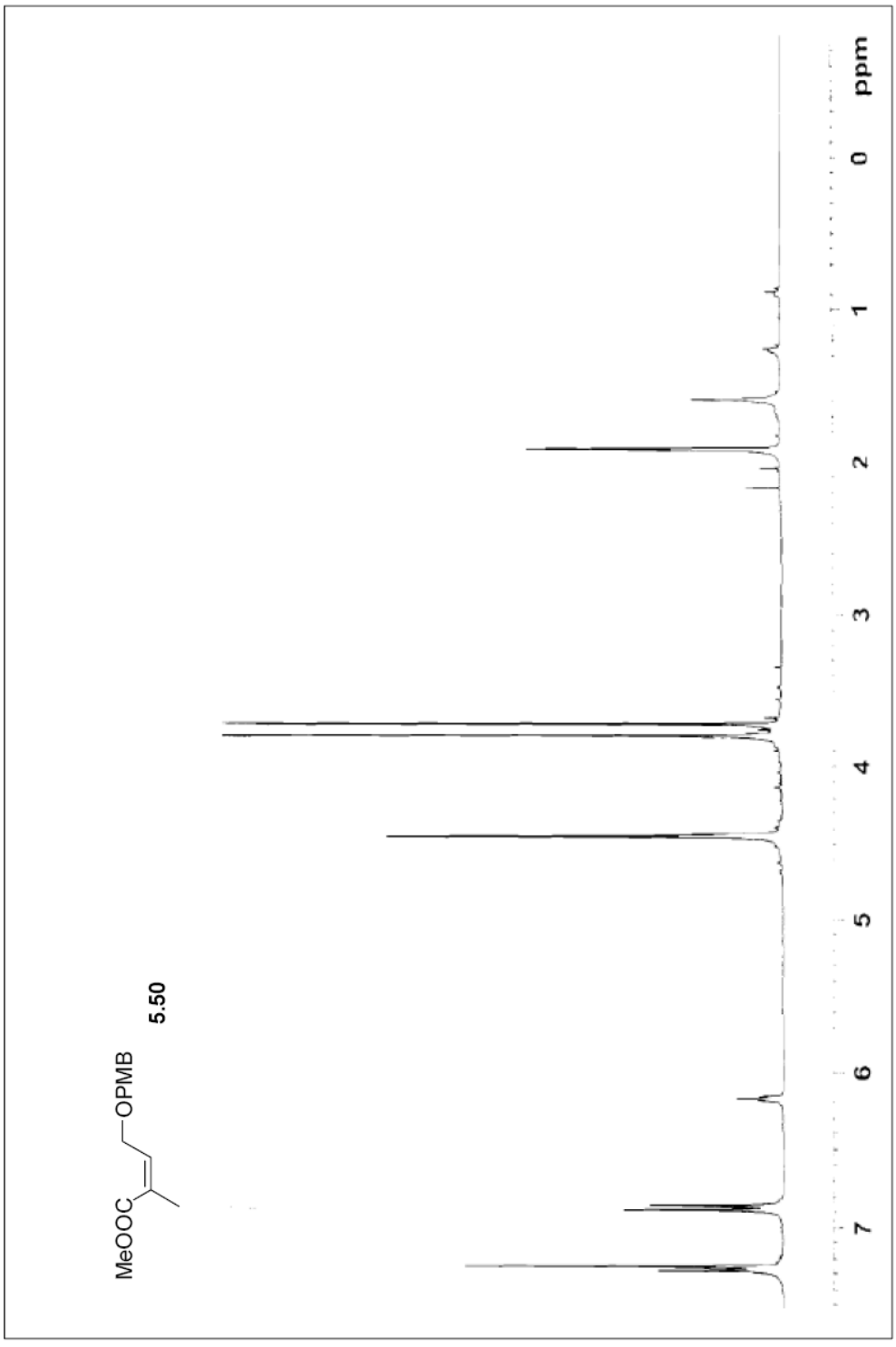

Figure A.75 300 MHz ^{1}H-NMR of compound 5.50 ($CDCl_3$)

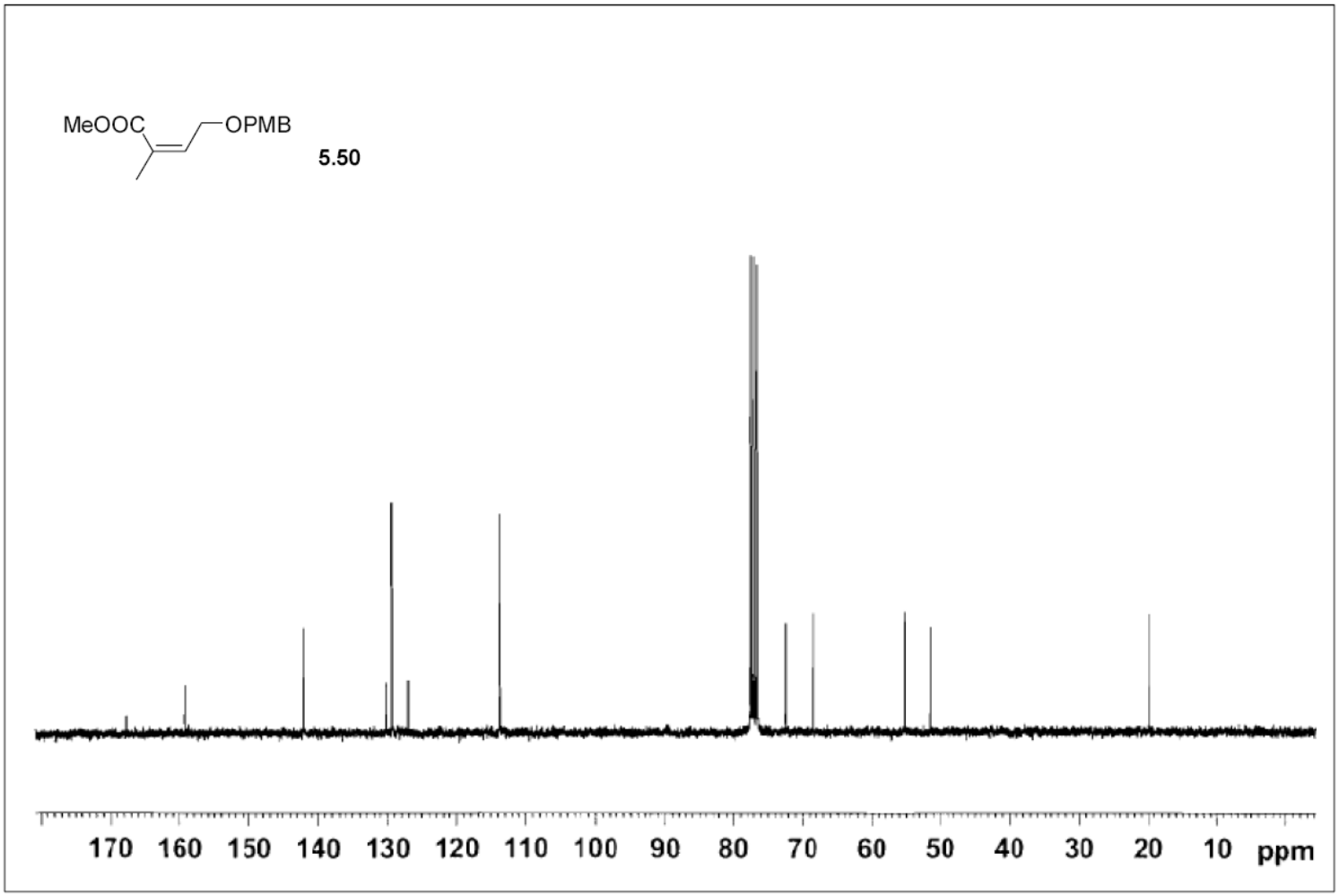

Figure A.76 75 MHz ^{13}C-NMR of compound 5.50 ($CDCl_3$)

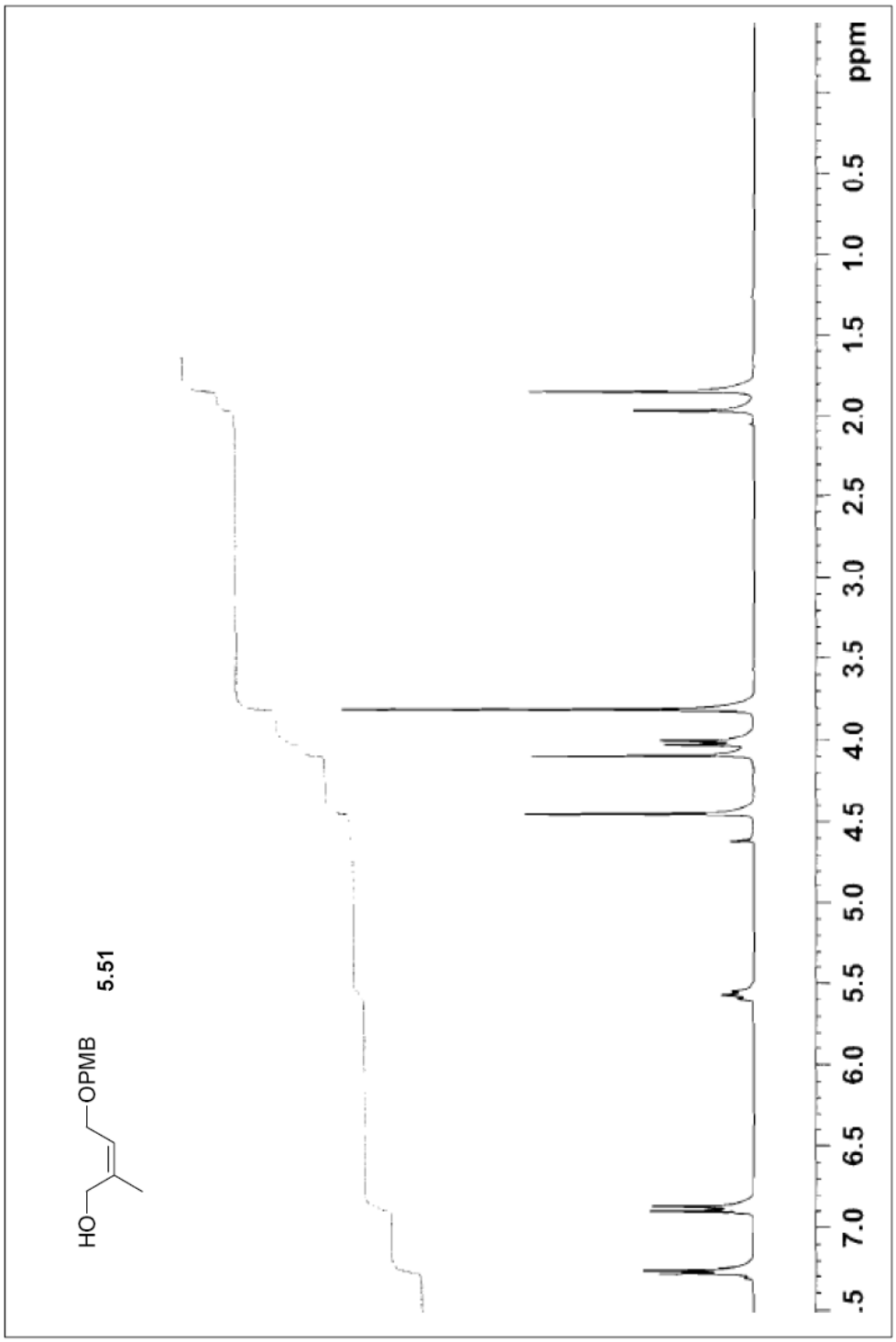

Figure A.77 300 MHz ^{1}H-NMR of compound 5.51 ($CDCl_3$)

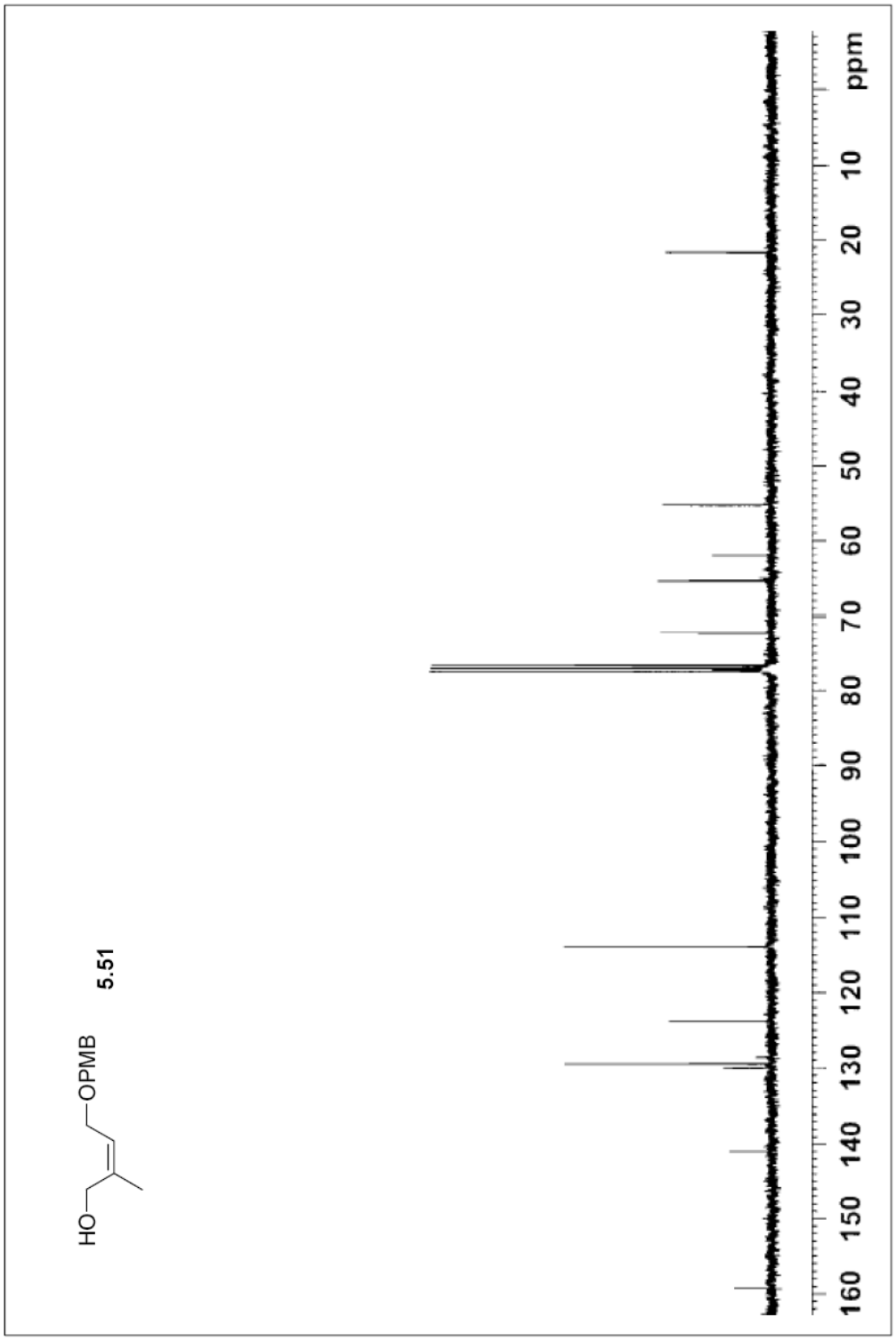

Figure A.78 75 MHz ^{13}C-NMR of compound 5.51 ($CDCl_3$)

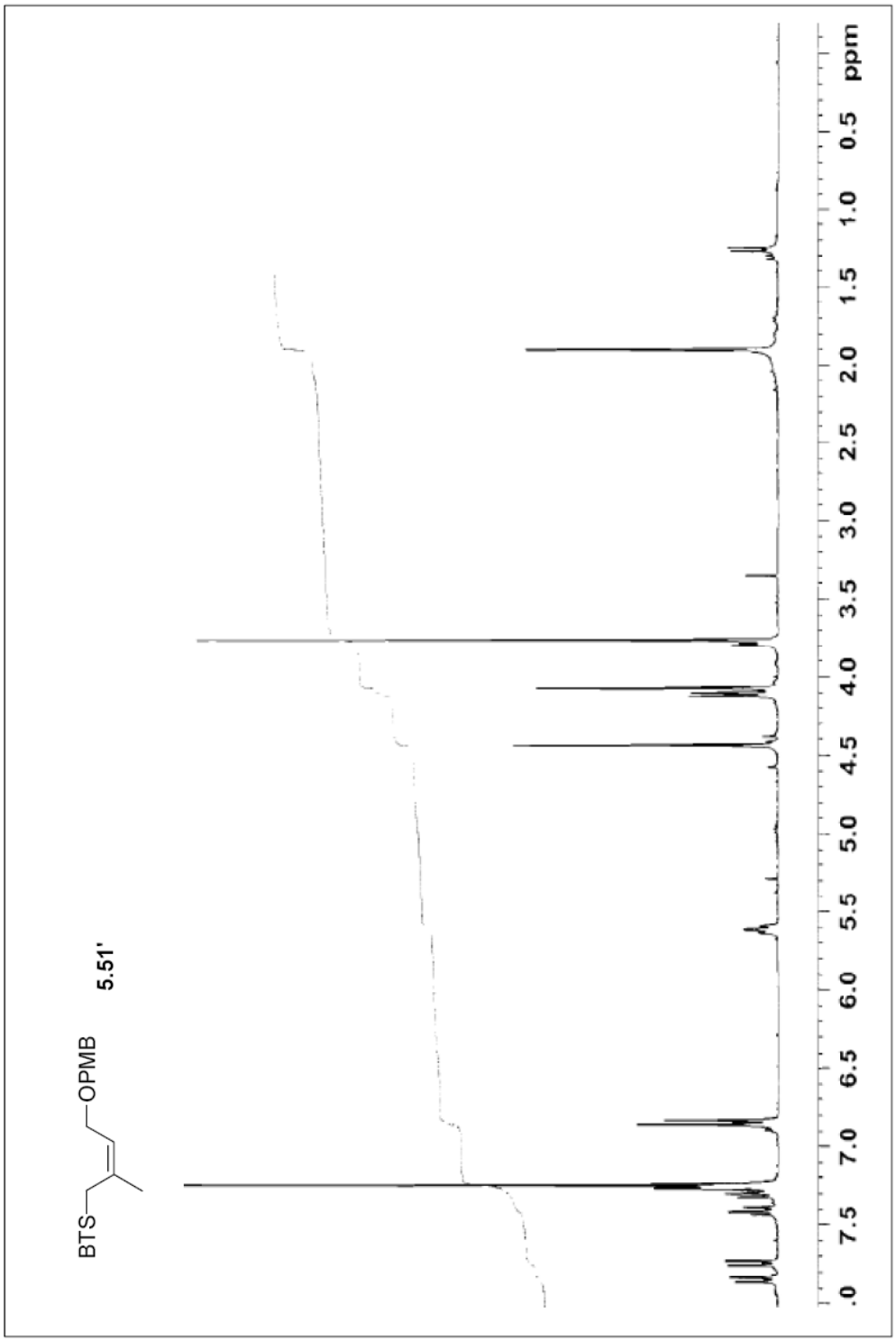

Figure A.79 300 MHz ^{1}H-NMR of compound 5.51' ($CDCl_3$)

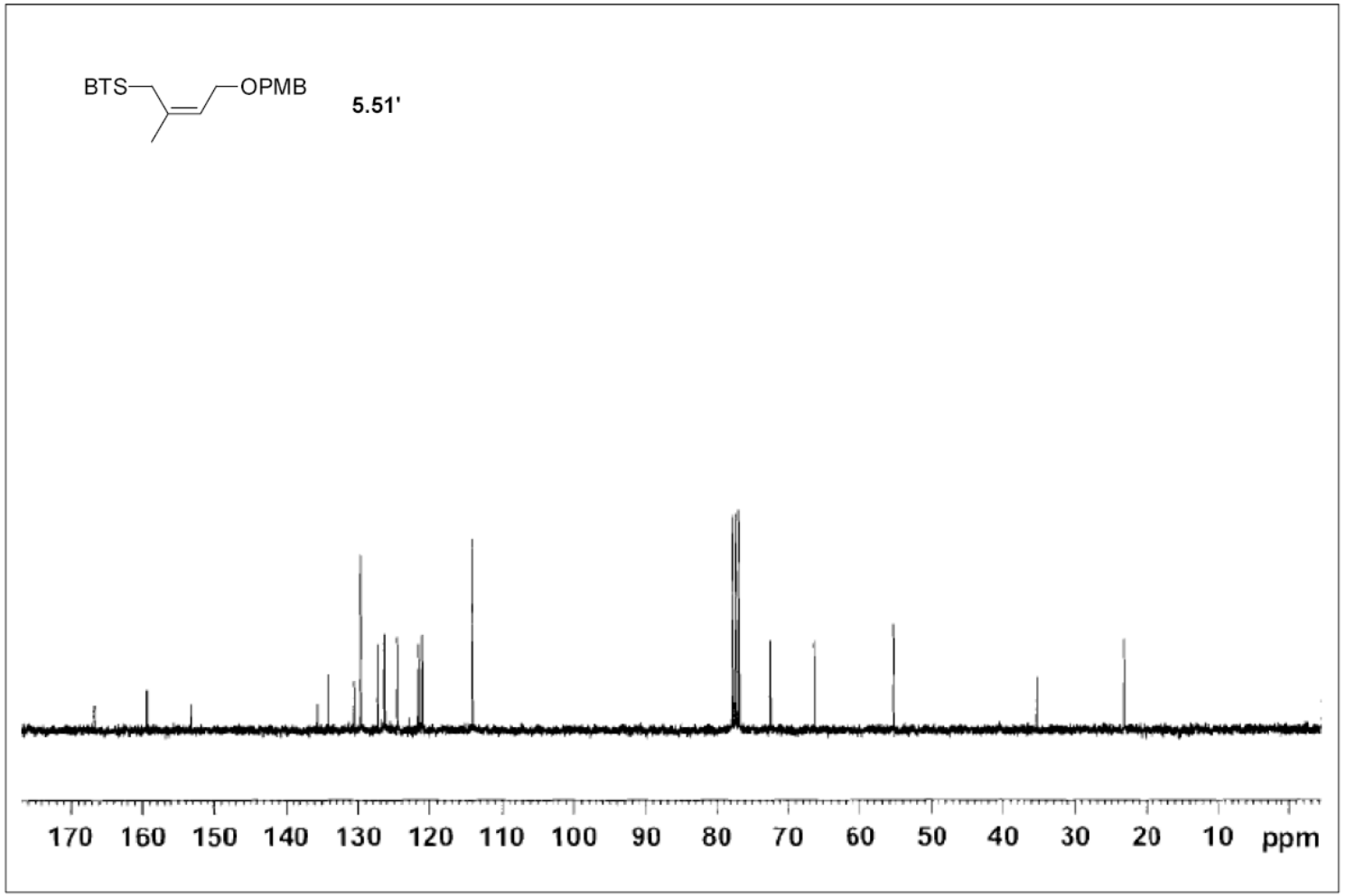

Figure A.80 75 MHz ^{13}C-NMR of compound 5.51' ($CDCl_3$)

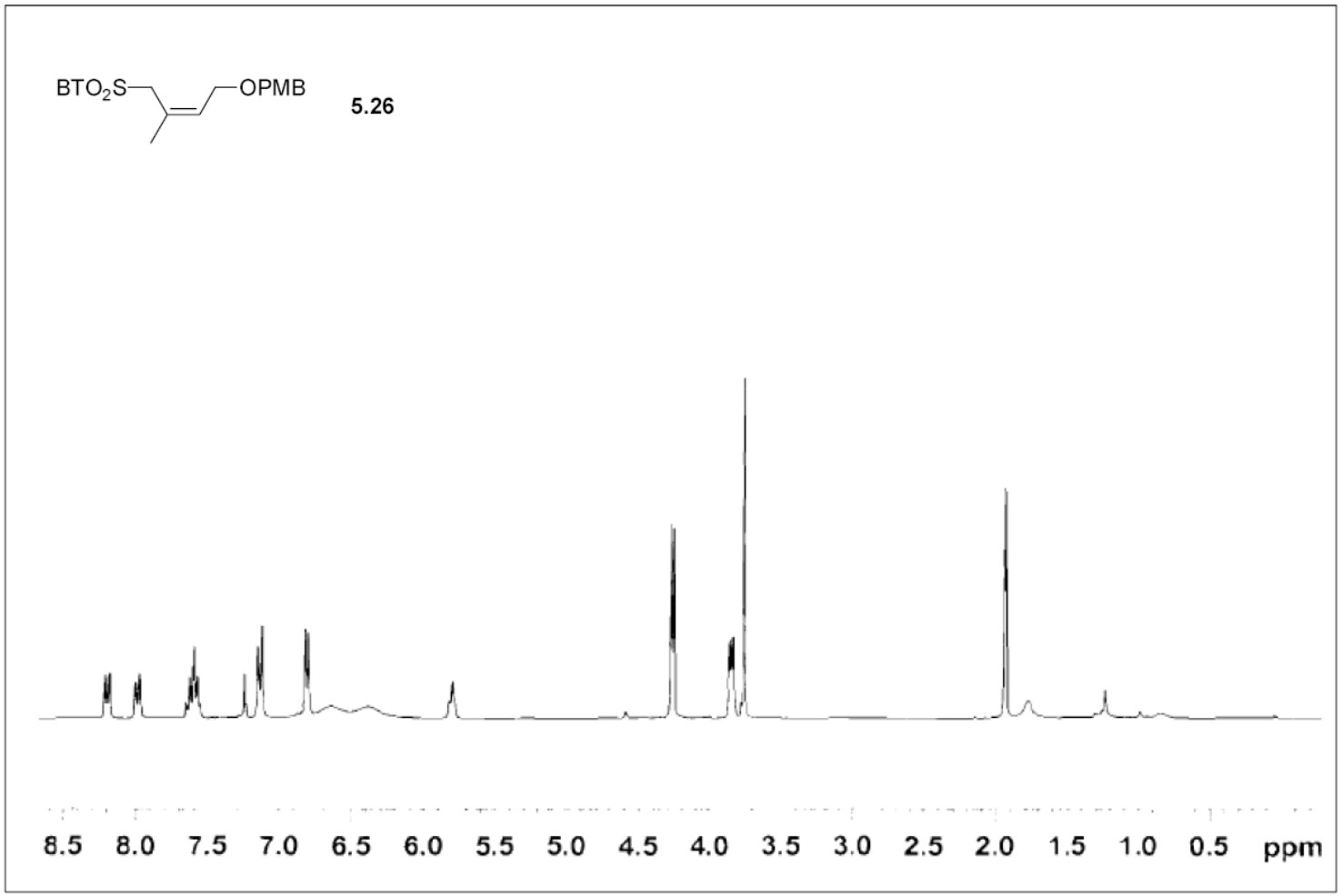

Figure A.81 300 MHz ^{1}H-NMR of compound 5.26 ($CDCl_3$)

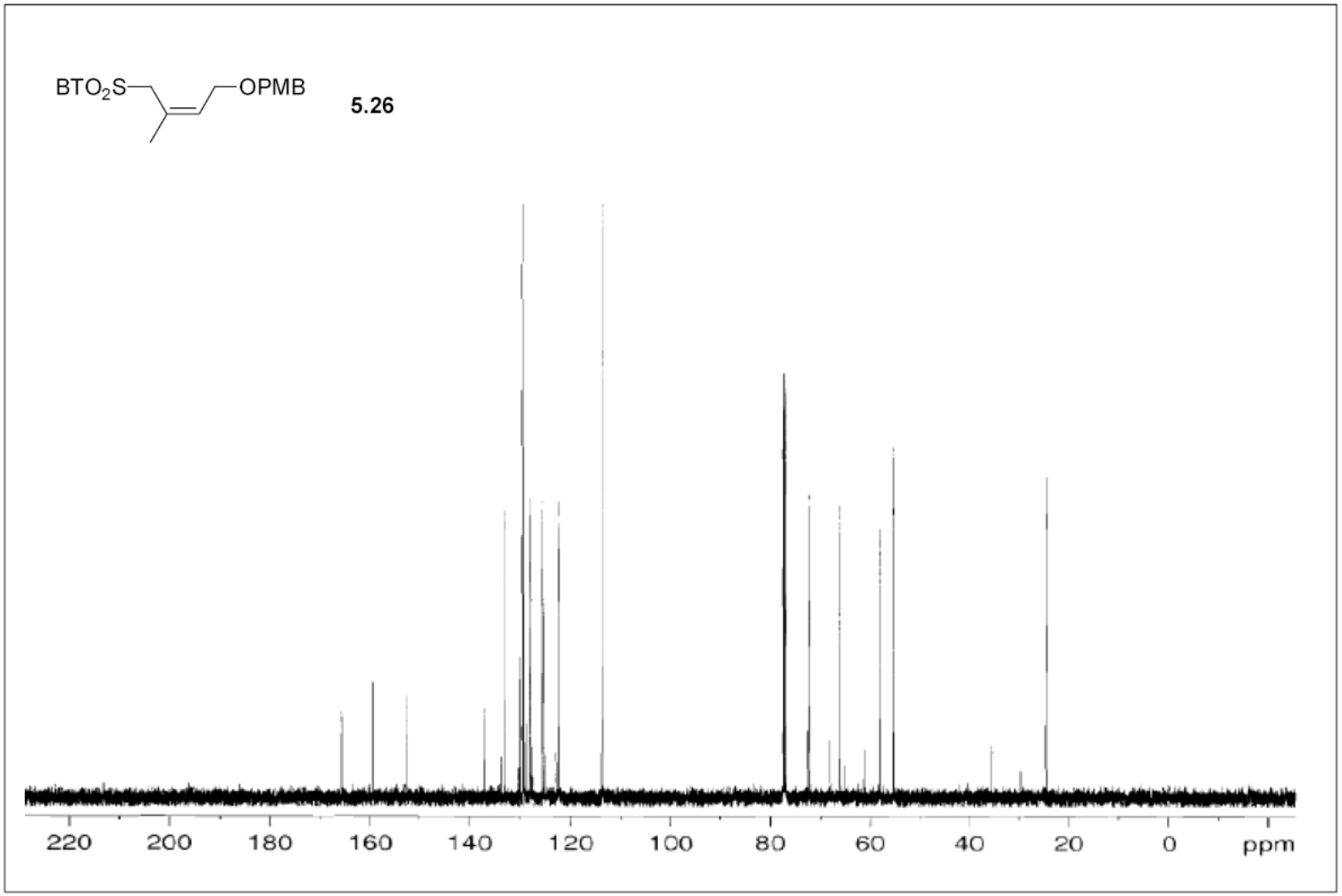

Figure A.82 75 MHz ^{13}C-NMR of compound 5.26 ($CDCl_3$)