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SYNTHESIS OF AMBROX AND ITS DERIVATIVES FROM NIDORELLOL

Miss Sunisa Suwancharoen

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By	Miss Sunisa Suwancharoen
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Thesis Advisor	Associate Professor Surachai Pornpakakul, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

…….. Dean of the Faculty of Science

(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

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(Assistant Professor Warinthorn Chavasiri, Ph.D.)

…….……. Thesis Advisor

(Associate Professor Surachai Pornpakakul, Ph.D.)

…….. Examiner

(Professor Sophon Roengsumran, Ph.D.)

…….. Examiner

(Associate Professor Amorn Petsom, Ph.D.)

…….. Examiner

(Associate Professor Nongnuj Muangsin, Ph.D.)

…….. External Examiner

(Assistant Professor Rungnapha Saeeng, Ph.D.)

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วิทยานิพนธ์นี้เป็นรายงานการสังเคราะห์ (+)-แอมบรอกซ์ จากสาร (−)-นิโดเรลลอล การสังเคราะห์มีขั้นตอนที่สำคัญคือ การปิดวงของ (−)-นิโดเรลลอล โดยใช้กรดเป็นตัวเร่งปฏิกิริยา การทำปฏิกิริยาโอโซโนไลซิส ของสารผลิตภัณฑ์ที่เกิดจากการปิดวง และขั้นตอนการกำจัดหมู่ไฮดรอกซีที่ตำแหน่งคาร์บอนที่ 7 โดยสามารถสังเคราะห์ (+)-แอมบรอกซ์ได้ใน 7 ขั้นตอน คิดเป็น 57% จาก (−)-นิโดเรลลอล เนื่องจากการสังเคราะห์แอมบรอกซ์จาก (−)-นิโดเรลลอล ได้เป็น (+)-แอมบรอกซ์ ซึ่งมีคอนฟิกิวเรชันแบบ เอ็นท์-แอมบรอกซ์ แทนที่จะได้ (−)-แอมบรอกซ์ ตามที่คาดหวังไว้ (−)-นิโดเรลลอล จึงมีคอนฟิกิวเรชันสัมบูรณ์ ตรงข้ามกับที่มีการรายงานไว้ เพราะฉะนั้น จึงพิสูจน์คอนฟิกิวเรชันสัมบูรณ์ ที่ถูกต้องของ (−)-นิโดเรลลอล ได้ว่า คือ *trans*-(5*R*,7*R*,8*R*,9*S*,10*R*)-labda-12,14-diene-7α,8β-diol

นอกจากนี้ ได้ศึกษาสมบัติการตรึงกลิ่นของ (−)-นิโดเรลลอล, (+)-แอมบรอกซ์และสารอนุพันธ์ โดยการทดสอบการดมกลิ่นด้วยแถบการดมกลิ่นและข้อมูลจาก TGA และใช้ (−)-แอมบรอกซ์เป็นชุดควบคุมบวก (−)-นิโดเรลลอล และ (−)-แอมบรอกซ์ มีสมบัติการตรึงกลิ่น ขณะที่ (+)-แอมบรอกซ์ และสารอนุพันธ์ ไม่แสดงให้เห็นสมบัติการตรึงกลิ่น

ภาควิชา.....เคมี.....ลายมือชื่อนิสิต.....
สาขาวิชา.....เคมี.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....
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Herein synthesis of (+)-ambrox from (–)-nidorellol is reported. The key reaction in the synthesis involved acid-promoted cyclization of (–)-nidorellol, ozonolysis of the acid-promoted cyclized products and dehydroxylation of the 7-hydroxyl group. (+)-Ambrox has been obtained by a seven-step procedure in 53% overall yield from (–)-nidorellol. Since the ambrox synthesis from (–)-nidorellol gave (+)-ambrox, an *ent*-ambrox configuration, instead of the expected product, (–)-ambrox, the absolute configuration of (–)-nidorellol has thus been shown to be opposite to that illustrated in previous report. Therefore, the correct absolute configuration of (–)-nidorellol has been proved to be *trans*-(5*R*,7*R*,8*R*,9*S*,10*R*)-labda-12,14-diene-7α,8β-diol.

In addition fixative property of (–)-nidorellol, (+)-ambrox and its derivatives were examined by smelling paper strip test and profiles of thermal gravimetric analysis (TGA) and (–)-ambrox was used as positive control. (–)-Nidorellol and (–)-ambrox exhibited fixative property while (+)-ambrox and its derivatives, did not show fixative property.

Department : Chemistry Student's Signature .....

Field of Study : Chemistry Advisor's Signature .....

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LIST OF ABBREVIATIONS

^{13}C-NMR	: carbon-13 nuclear magnetic resonance spectroscopy
^{1}H-NMR	: proton nuclear magnetic resonance spectroscopy
Acetone-d_6	: hexadeuterated acetone
Benzene-d_6	: deuterated benzene
$CDCl_3$	: deuterated chloroform
CD_3OD	: tetradeuterated methanol
CH_2Cl_2	: dichloromethane
COSY	: Correlated Spectroscopy
cm^{-1}	: unit of wavenumber (IR)
d	: doublet (NMR)
d	: day (s)
dd	: double of doublet (NMR)
ddd	: double of double of doublet (NMR)
DMAP	: *N*,*N*-dimethylaminopyridine
DMSO	: dimethyl sulfoxide
DMSO-d_6	: hexadeuterated dimethyl sulfoxide
eq	: equivalent (s)
EtOAc	: ethyl acetate
EtOH	: ethanol
Et_3N	: triethylamine
Et_2O	: diethyl ether
FT-IR	: Fourior transform- infrared resonance spectroscopy
g	: gram (s)
h	: hour (s)
Hz	: hertz (s)
^{1}H-NMR	: proton nuclear magnetic resonance spectroscopy
HMBC	: Heteronuclear Multiple Bond Correlation
HSQC	: Heteronuclear Single Quantum Correlation
HREIMS	: High Resolution Electro Spray Ionization Mass Spectrometry
IR	: infrared resonance spectroscopy
J	: coupling constant

KBr	: potassium bromide (IR)
M	: molar (s)
m	: multiplet (NMR)
m.p.	: melting point
m/z	: mass per charge ratio
MeCN	: acetonitrile
MeOH	: methanol
mg	: milligram (s)
MHz	: megahertz (s)
min	: minute (s)
mL	: milliliter (s)
mmol	: millimole (s)
Me_2CO	: acetone
MS	: mass spectroscopy
MsCl	: methansulfonyl chloride
NMR	: nuclear magnetic resonance spectroscopy
NOESY	: Nuclear Overhauser Enhancement Spectroscopy
$H_2NNH_2 \cdot H_2O$	: hydrazine hydrate
PCC	: pyridinium chlorochromate
Ph	: phenyl
ppm	: parts per million (unit of chemical shift)
q	: quartet (NMR)
R_f	: retardation factor
rt	: room temperature
s	: singlet (NMR)
st	: stretching vibration (IR)
t	: triplet (NMR)
td	: triple of doublet (NMR)
THF	: tetrahydrofuran
TLC	: thin layer chromatography
p-TsCl	: paratoluenesulfonyl chloride
p-$TsOH \cdot H_2O$	: paratoluenesulfonic acid monohydrate

p-$TsNHNH_2$	: paratoluenesulfonyl hydrazide
δ	: chemical shift
$[\alpha]_D^{20}$	: Specific rotation at 20 ^oC and Sodium D line (589 nm)
^oC	: degree Celsius
ν_{max}	: the reciprocating wavelength (IR)

CHAPTER I

INTRODUCTION

People have used fragrance materials from the ancient time for many purposes, on their body or religious ceremonies. Most of them were made from naturally occurring materials such as essential oils, exudates, balsams and resin. In the 19th century synthesis of some fragrance chemicals were started (Fráter, Bajgrowicz and Kraft, 1998).

Ambrox is one of the most valuable naturally fragrance materials which has excellent amber-like odor and it is reported that (–)-ambrox has stronger odor than its enatiomer (Ohloff *et al*., 1985). Ambrox is a degrading product from ambergris which is a metabolite of sperm whales (*Physeter macrocephalus* L.) (Ohloff, 1982; Tamimoto and Oritani, 1997; Koga *et al*., 1998; Gorbachov and Rossister, 1999; Shen *et al*., 2007). Due to increasing consumption of ambrox and encouragement of whale protection in 1946 (Fears, 2009) the synthetic routes of ambrox have been developed. Many synthetic routes of ambrox which have been appeared in literatures are developed from occurring natural products.

In fact, several routes to ambrox have been developed from naturally occurring diterpenes. Labdane-type diterpenes is a suitable synthon for ambrox synthesis due to its structural features and easy available from nature (Castro *et al*., 2002). Recently we could isolate (–)-nidorellol, a labdane-type diterpene, from *Croton oblongifolius* in good yield. (–)-Nidorellol was first isolated from *Nidorella auriculata* and from *Stevia sarensis* (Bohlmann and Fritz, 1978). Also it was isolated by our group from *Croton oblongifolius* collected from Kanchanaburi Province, Thailand (Roengsumran *et al*., 2002). In 1978 its configuration was established by Bohlmann and Fritz as *trans*-(5*S*,7*S*,8*S*,9*R*,10*S*)-labda-12,14-diene-7β,8α-diol which the configuration is a suitable synthon for (–)-ambrox. Therefore we outline our plan for a synthesis of ambrox. The key reaction in their 7-step synthesis involved

acid-promoted cyclization of (–)-nidorellol; ozonolysis of the acid-promoted cyclized products; and dehydroxylation of the 7-hydroxyl group.

Therefore, the main objectives of this research are

1. To synthesize ambrox and its derivatives from nidorellol.
2. To evaluate fixative property of ambrox and its derivatives.

CHAPTER II

LITERATURE REVIEWS

2.1 Classification of sense in perfume

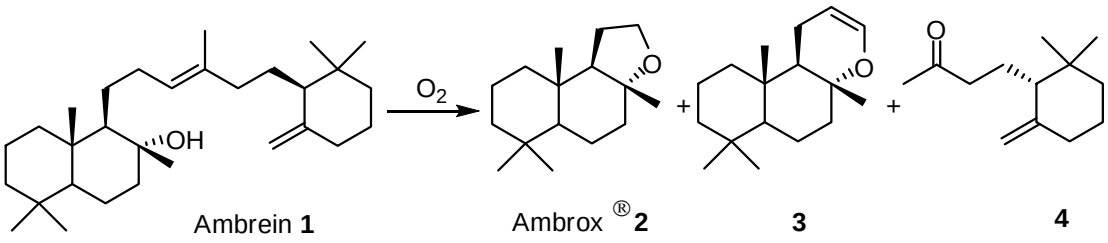
The scents in fragrance are classified in three groups, top note, middle note and base note (or end note) upon its volatility after application of a perfume (Poucher, 1974). Top note consist of substances which evaporate quickly. This scent is perceived immediately on application of perfume such as lemon, lavender, bergamot, lemongrass etc. Middle note is heart of perfume which are emerged when the top note dissipate such as eugenol, clary sage, hops etc. Additionally middle note is intermediate volatility components and usually appear about two minutes to one hour after application. The last one is base note which is the scents that are appeared close to the departure of the middle note such as pepper, vanillin, oakmoss, ambergris extract, etc. Compounds in this class evaporate slowly and often have fixative property. Scents in base note usually appear around 30 minutes after application and some of them can appear on wearer more than 24 hours ("Prefume" Wikipedia, the free encyclopedia[online], 2011).

2.2 Fixative components in perfume

In perfumery fixatives are important substances which were used to decrease the evaporation rate of the top and middle note and improve stability of more volatile substances to lasting stay odor on wearer (Poucher, 1974; "Fixative (Perfumery)" Wikipedia, the free encyclopedia[online], 2011). Fixative materials used in perfumery were both natural from plants or animals and synthetic substances. Natural fixatives usually have a fragrance considered a base note such as sandalwood, vetivert, orris root, musk, ambergris, etc. ("Fixative (Perfumery)" Wikipedia, the free encyclopedia[online], 2011). A few of synthetic fixative are, Glucam® (Seldner and Princeton, 1981), benzyl benzoate (Poucher, 1974; Baydar *et al*., 1994), cinnamic acid ester, vanillin, coumarin (Austin, 1984), etc.

2.3 Ambrox®

Ambrox is a degrading product from ambergris which has been one of the most highly valued perfume materials. It was first used in perfumery by Muslim Spain (Ohloff, 1982a). Ambergris is a metabolite of sperm whales (*Physeter macrocephalus* L.) which is secreted in the stomach or intestinal tract of the sperm whale and discarded into the sea in the form of a gray to black stone-like mass (Ohloff, 1982b; Gorbachov and Rossiter, 1999; Shen *et al*., 2007). The major constituent of ambergris is ambrein **1**, a triterpene alcohol, which was decomposed by the exposure to sea water, air and sunlight to give some odorous compounds (Tamimoto and Oritani, 1997; Koga *et al*., 1998; Gorbachov and Rossiter, 1999) as showed in scheme 2.1.



Scheme 2.1 Degradation of ambrein

From this process, ambrox® **2** is found and it has an excellent amber-like odor. In 1985 Ohloff has reported that the odor of (–)-**2** is much stronger than that of (+)-**2** (Ohloff *et al*., 1985). To date, ambrox is a valuable ingredient of many fine fragrances because of its unique scent and fixative property (Poucher, 1974; Barco *et al*., 1995).

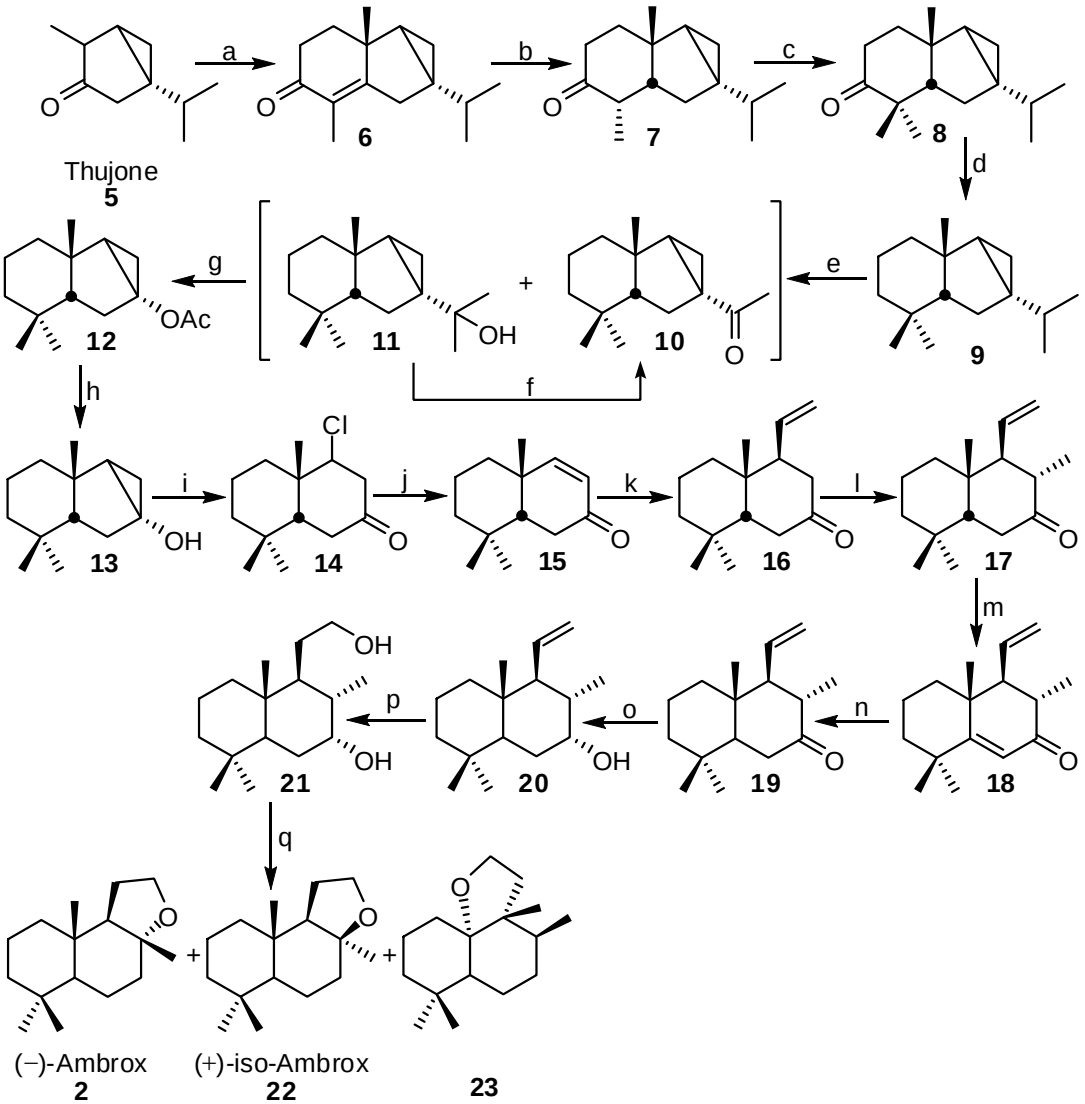
2.4 Synthesis of (–)-ambrox and its derivatives

Due to the increasing consumption of ambrox and the encouragement of whale protection in 1946 (Fears, 2009), many synthetic routes of ambrox have been developed. The synthetic routes for ambrox and its derivatives usually started from natural substances. However, numerous synthetic strategies for ambrox have been developed from naturally occurring terpenoid compounds. In 1950, the first synthesis of (–)-ambrox from (–)-sclareol was reported by Stoll and Hinder (Stoll and Hinder, 1950; Hider and Stoll, 1950; Barco *et al*., 1995; Fráter, 1998).

2.4.1 Synthesized from monoterpenes

Some of synthetic routes for ambrox have been developed from monoterpenoid compounds.

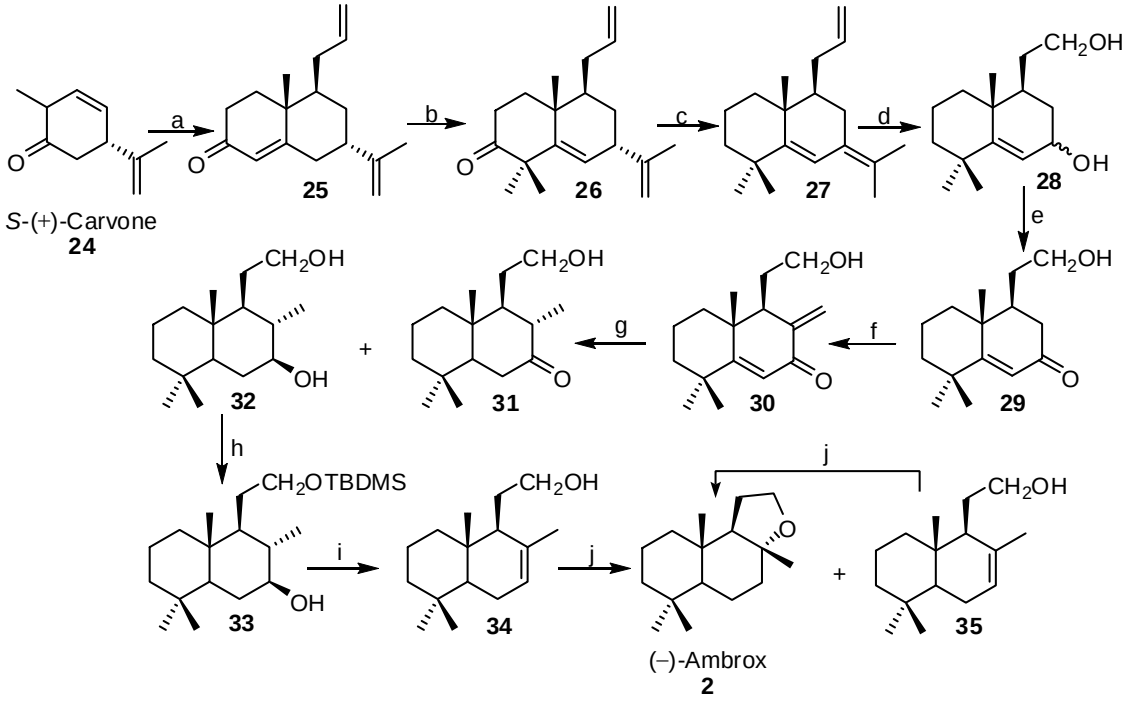
In 1994, Kutney and Chen synthesized (–)-ambrox via transformation of thujone **5** to enone **15** (Scheme 2.2). Following by 7-step from **15**, (–)-ambrox was obtained in 9.5% from thujone derivative **15** together with (+)-iso-ambrox and ionoxide principal (Kutney and Chen, 1994).



(a) EVK, EtOH, KOH, 60%; (b) H_2, Pd-C, EtOH, 98%; (c) CH_3I, KO^{t}Bu, HO-*t*-Bu, 84%; (d) NH_2NH_2, KOH, DEG, 65%; (e) O_3, EtOAc, -40°C, 69%; (f) i) PhH, HOTs; ii) $KMnO_4$, H_2O, *t*-BuOH; iii) $Pb(OAc)_4$; 83%; (g) *m*-CPBA, CH_2Cl_2, 82%; (h) KOH, EtOH, 100%; (i) $FeCl_3$, DMF; (j) NaOAc, MeOH, 80% from **12**; (k) CH_2=CHMgBr, CuI, THF, 70%; (l) LDA, DMF, CH_3I, 65%; (m) i) LDA, THF; ii) PhSeCl; iii) H_2O_2, Pyr., CH_2Cl_2, 62%; (n) Li, NH_3, NH_4Cl, 90%; (o) L-Selectride, THF, 95%; (p) i) BH_3-THF; ii) H_2O_2, 71%; (q) i) HOTs, Touene, 80°C, (**2**, 31%) or ii) HOTs, CH_3NO_2, 90°C, (**2**, 48%)

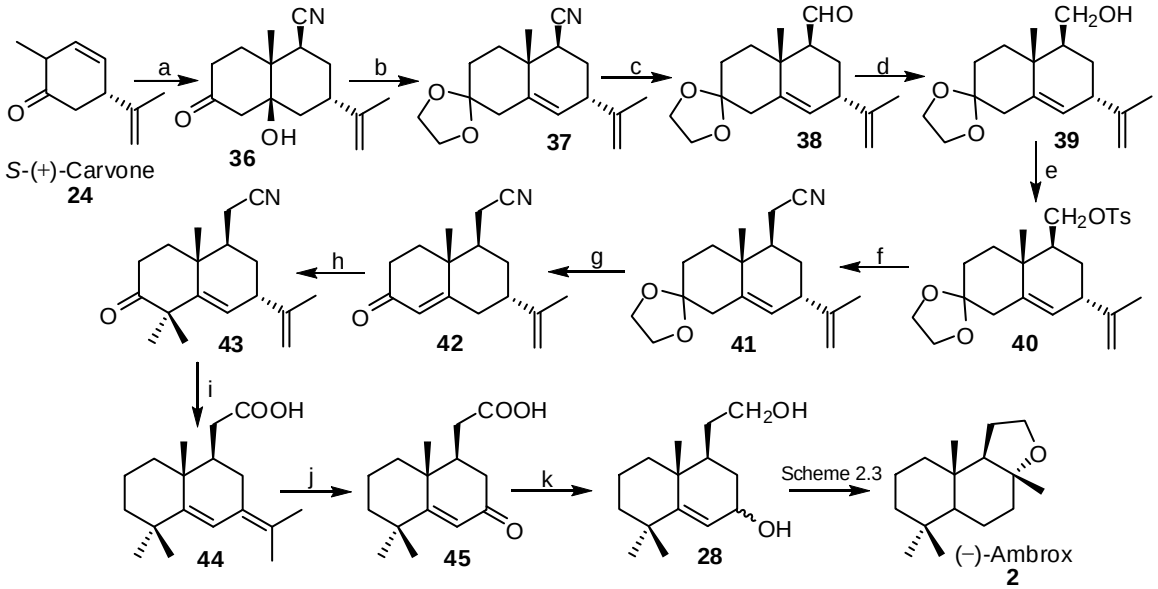
Scheme 2.2 Synthetic route for (–)-ambrox from thujone by Kutney and Chen

In the same year, Verstegen-Haaksma *et al*. could synthesize (–)-ambrox from **25** and **36** which were prepared from *S*-(+)-carvone **24**, a monoterpene. Transformation of **25** to (–)-ambrox was shown in scheme 2.3 and from **36** was showed in scheme 2.4 (Verstegen-Haaksma *et al*., 1994).



(a) see Verstegen-Haaksma et al., 1994; (b) MeI, KO-*t*-Bu, 88%; (c) Hydrazine, KOH, DEG, 220°C, 85%; (d) O_3, MeOH, -78°C; $NaBH_4$; 80%; (e) MnO_2, acetone, 90%; (f) $(Me)_2N\text{-}CH_2\text{-}N(Me)_2$, Ac_2O, 70%; (g) Li, NH_3, EtOH, 10% for **31**, 73% for **32**; (h) TBDMSCl, DMF, imidazole, 98%; (i) i) MsCl, DMAP, CH_2Cl_2; $LiCO_3$, LiBr, Δ; ii) HF, acetonitrile; 80%; (j) *p*-TsOH, nitromethane

Scheme 2.3 Synthetic route for (–)-ambrox from **25** by Verstegen-Haaksma *et al*.

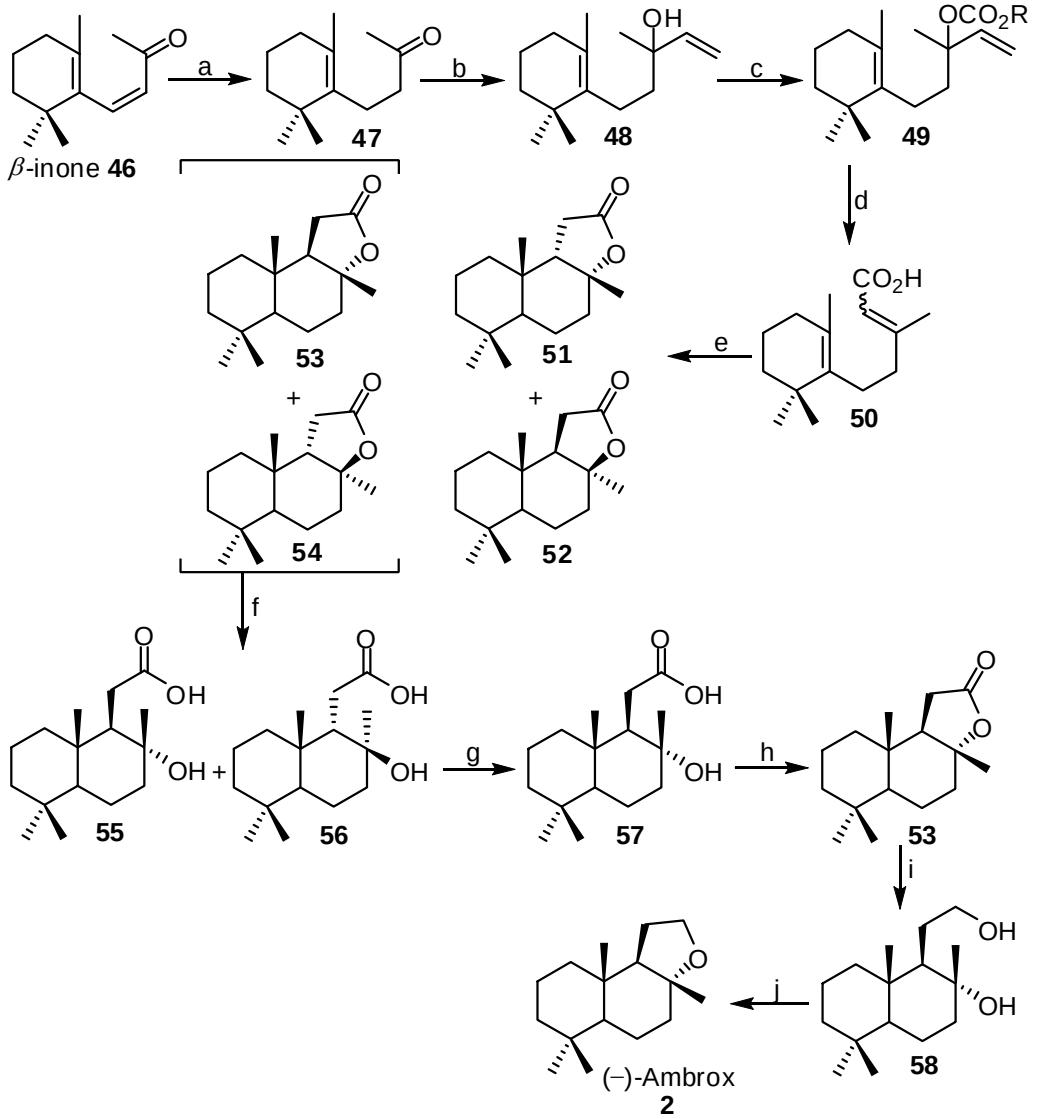


(a) see Verstegen-Haaksma et al., 1994; (b) *p*-TsOH, toluene, glycol, Δ, 90%; (c) DIBAH, toluene, 95%; (d) $NaBH_4$, 99%; (e) TsCl, pyridine, 96%; (f) NaCN, DMF, 99%; (g) HCl, H_2O, 93%; (h) MeI, KO^{t}Bu, HO-*t*-Bu, 80%; (i) Hydrazine, KOH, DEG, 220°C, 98%; (j) O_3, MeOH, 90%; (k) $NaBH_4$; 80%

Scheme 2.4 Synthetic route for (–)-ambrox from **36** by Verstegen-Haaksma *et al*.

2.4.2 Synthesized from sesquiterpenes

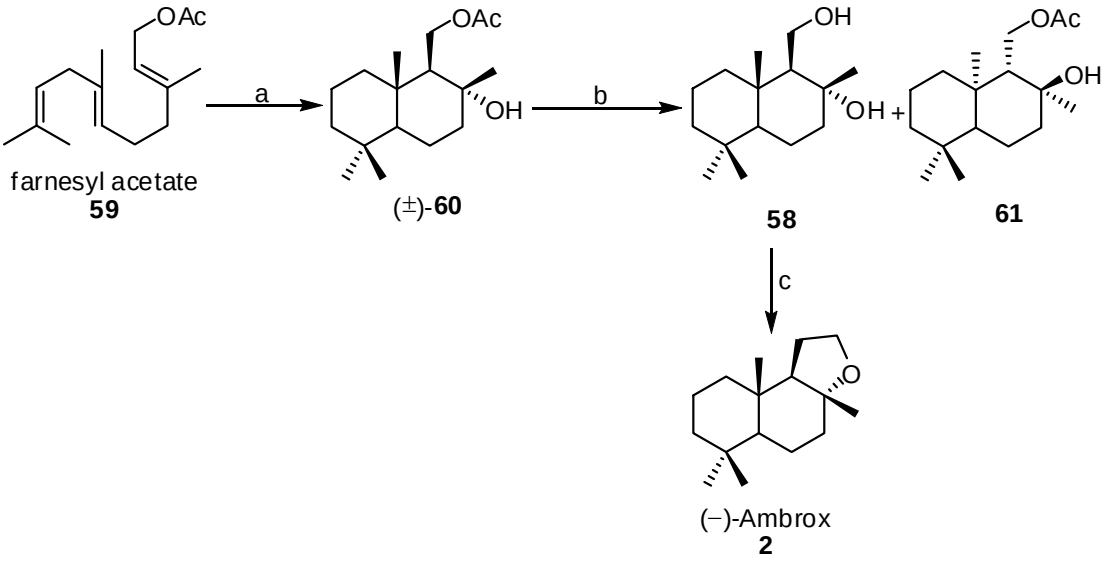
In 1994, Kurashiki and Shibata used *β*–inone **46** which was a derivative of sesquiterpene, *β*-monocyclohomofanesic acid, as a starting material. They succeeded to synthesize ambrox via 10 steps (Scheme 2.5) (Kurashiki and Shibata, 1994).



(a) nickel diatomaceous earth, EtOH, H_2 (10 atm), 150°C for 3h, 80°C for 5h, 89%; (b) vinyl magnesium chloride, THF, 85%; (c) i) NaH, toluene, reflux, 10h; ii) methyl chloroformate, rt, 2h; 96.3%; (d) PdC, triorthotolyl-phosphine, *i*-PrOH, CO, 50°-60°C, 5h; 71.2%; (e) $ClSO_3H$, CH_2Cl_2, -60°-70°C, 20 min, 83.2%; (f) MeOH, 30% NaOH, reflux; (g) i) MeOH, H_2O, (–)-1-(*p*-tolyl)ethylamine, aqueous 1N NaOH, rt, overnight; ii) 1N NaOH; 68%; (h) toluene, reflux, overnight, quantitative yield; (i) bis-(2-methoxy-ethoxy)-aluminium sodium hydride, toluene, 60°C, 2h, 99%; (j) pyridine, *p*-TsCl, rt, 24h, 71%

Scheme 2.5 Synthetic route for (–)-ambrox from *β*–inone by Kurashiki and Shibata

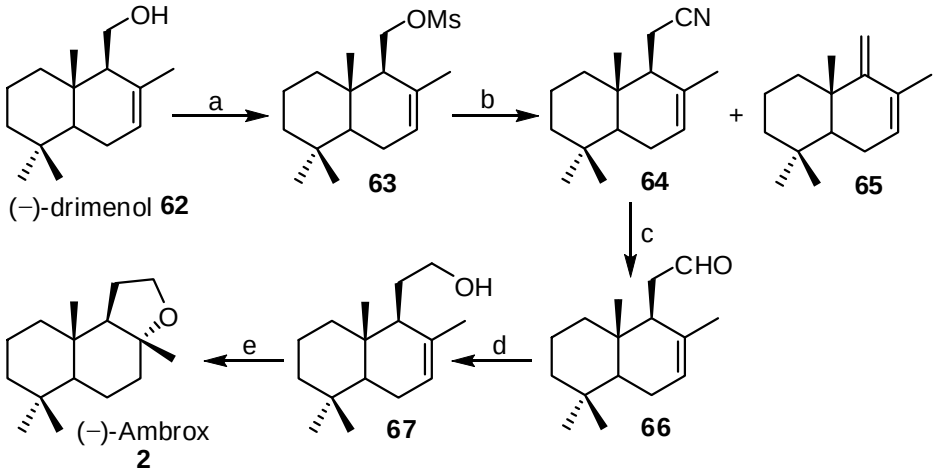
In 1996, Tamimoto and Oritani could develop an efficient synthesis of enantiomerically pure (–)-ambrox from farnesyl acetate **59** using lipase enzyme to prepared drimanediol **58** in key step (Scheme 2.6). Transformation of **58** gave **2** in 37% overall yield from **60** (Tamimoto and Oritani, 1996).



(a) $ClSO_3H$; (b) lipase PS-30, H_2O, 1day, (> 98%e.e. (37%) for **58**; 51%e.e. (60%) for **61**); (c) *p*-TsCl, pyridine, quantitative yield

Scheme 2.6 Synthetic route for (–)-ambrox from farnesyl acetate by Tamimoto and Oritani

In 2000, Akita *et al*. synthesized (–)-ambrox from a sesquiterpene, drimenol **62**. The synthetic sequence was developed via 5 steps and **2** was gave in 19% overall yield (scheme 2.7) (Akita *et al*., 2000).



(a) MsCl, pyridine, 99%; (b) NaCN, DMSO, 45%; (c) i) DIBAL-H, toluene; ii) 2M aqueous HCl; 81%; (d) $NaBH_4$, MeOH, 99%; (e) MeCN, *p*-TsOH·H_2O, 55%

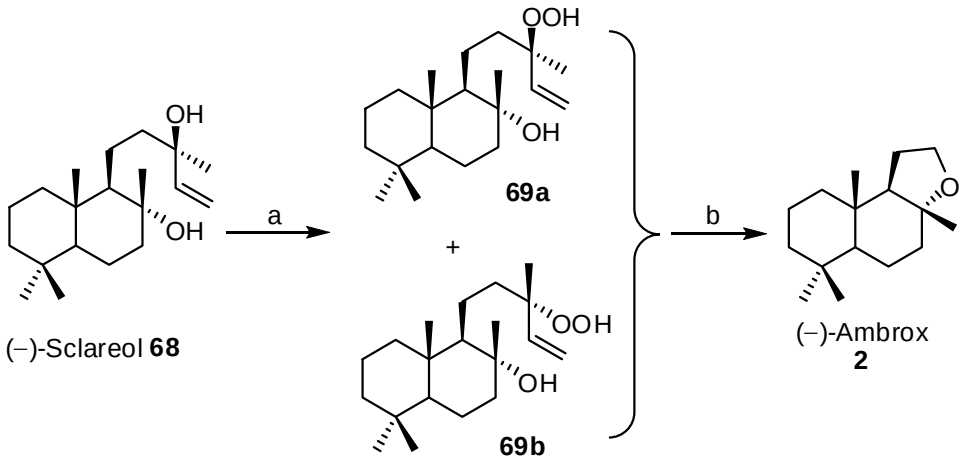
Scheme 2.7 Synthetic route for (–)-ambrox from drimenol by Akita *et al*.

2.4.3 Synthesized from diterpenes

Among numerous routes to ambrox, naturally occurring diterpenes were usually used as starting materials due to its structural features and easy available from nature.

Sclareol was mostly used as starting material in many synthetic routes due to its suitable synthon for ambrox synthesis. It was isolated from clary sage, *Salvia* sp., around 0.006-0.1% yield (Ulubelen *et al*., 1985; Ulubelen *et al*., 1994; Souleles and Argyriadou, 1997; Senatore, F. *et al*., 2005; Yadav *et al*., 2010).

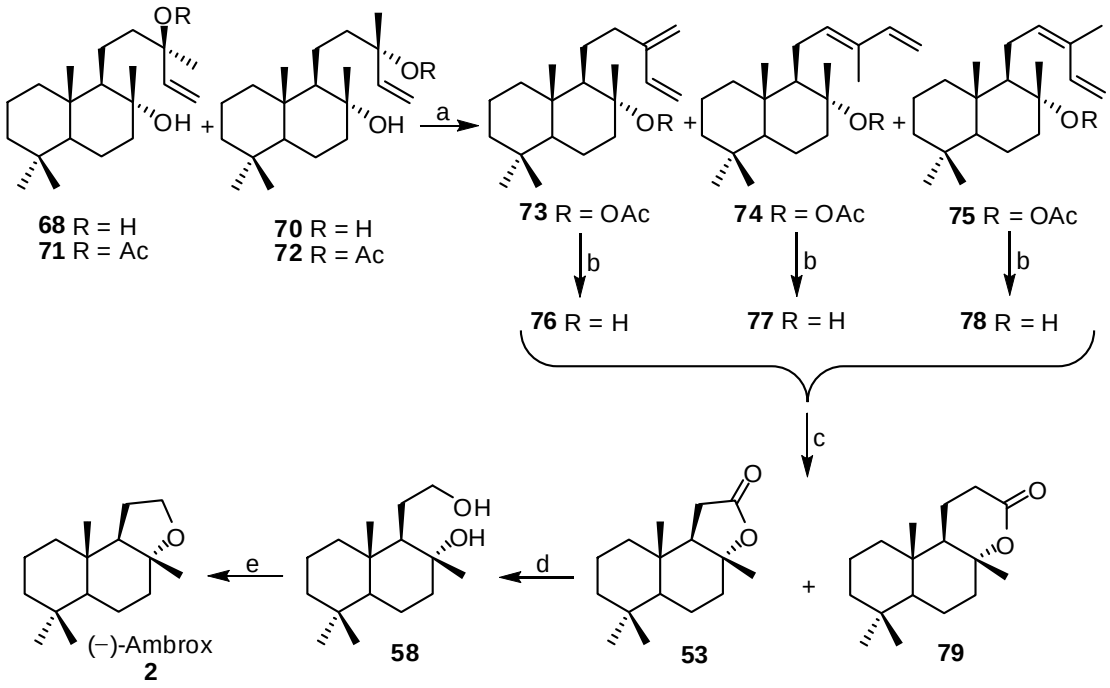
In 1987, Decorzant *et al*. presented the transformation of sclareol **68** to ambrox via radical process using Fe^{2+} and Cu^{2+} (Scheme 2.8). They succeeded to synthesize ambrox in 11% overall yield (Decorzant *et al*., 1987).



(a) 70% aqueous H_2O_2, *p*-TsOH, CH_2Cl_2, 38%; (b) $Cu(OAc)_2{\cdot}2H_2O$, $FeSO_4{\cdot}7H_2O$, CH_3OH, 2h, 50°C, 30%

Scheme 2.8 Synthetic route for (−)-ambrox from sclareol by Decorzant *et al*.

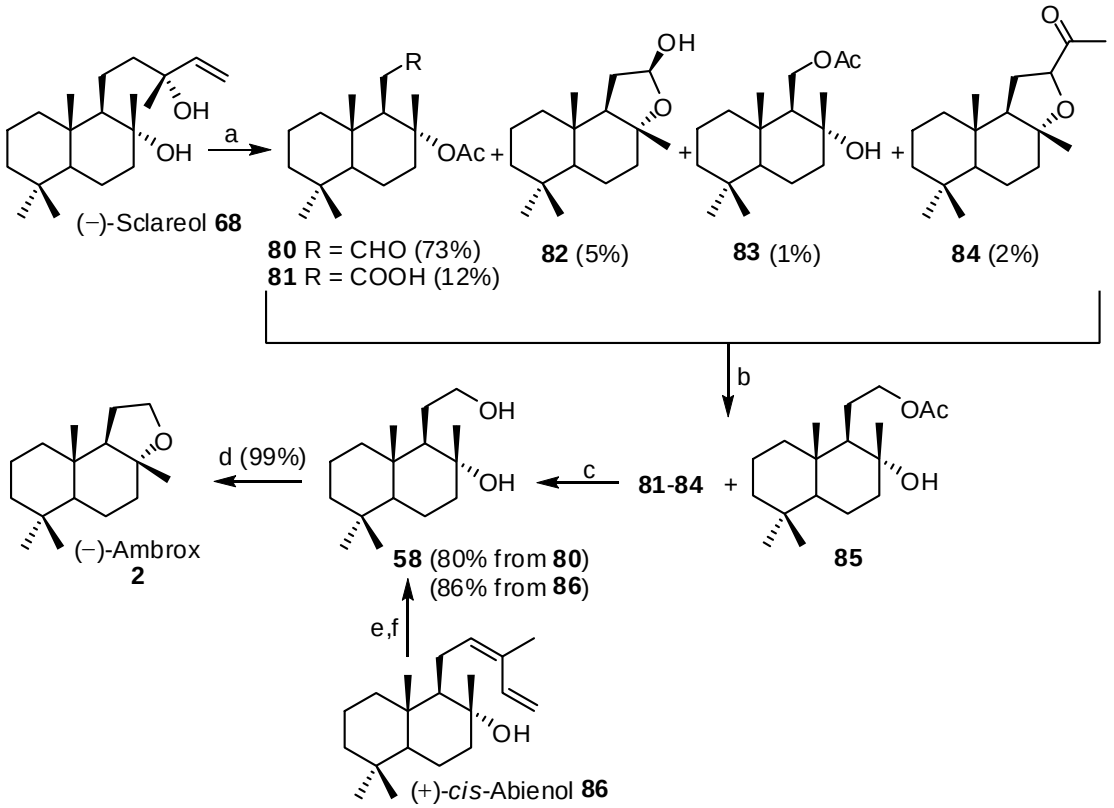
In 1988, Coste-Manière *et al*. could synthesize ambrox from sclareol (Scheme 2.9), involving palladium catalyzed key step. Five steps from mixture of sclareol **68** and its derivatives **70-72**, (−)-ambrox **2** was obtained (Coste-Manière *et al*., 1988).



(a) $Pd(OAc)_2$, dioxane, 100°C, 15 min, 100%; (b) $LiAlH_4$, Et_2O/H+, 2h, 96%; (c) $KMnO_4$, 1d, 80%; (d) $LiAlH_4$, THF, 25°C, 3h, 98%; (e) *p*-TsCl, CH_2Cl_2, 25°C, 2h, 90%

Scheme 2.9 Synthetic route for (–)-ambrox from sclareol by Coste-Manière *et al*.

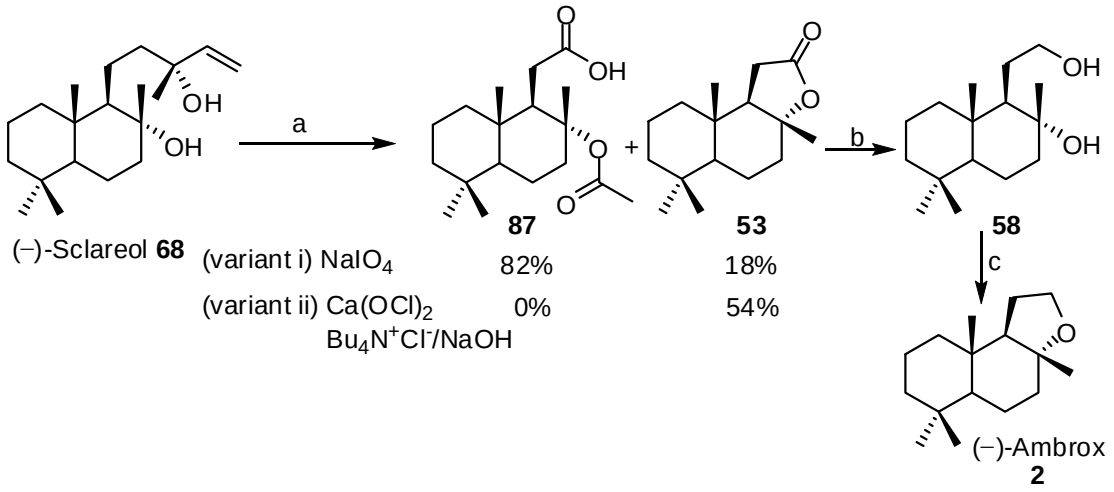
In 1993, Barrero *et al*. used (–)-sclareol **68** as starting material for (–)-ambrox synthesis. The oxidative degradation of side chain of **68** was the critical step which gave mixture of **80**-**84** (Scheme 2.10). In addition they have also presented the transformation of (+)-*cis*-abienol **86** which was prepared from a commercial sample of Canadian balsam into (–)-ambrox **2**, 84% overall yield (Barrero *et al*., 1993c).



(a) OsO_4, $NaIO_4$, THF, 45°C, 6h; (b) $NaBH_4$, THF, 30 min; (c) KOH, MeOH 10%, rt, 2h; (d) TsCl, Py; (e) O_3, CH_2Cl_2, -78°C, 4.5 h; (f) $LiAlH_4$, rt, 2h

Scheme 2.10 Synthetic route for (−)-ambrox from sclareol by Barrero *et al*.

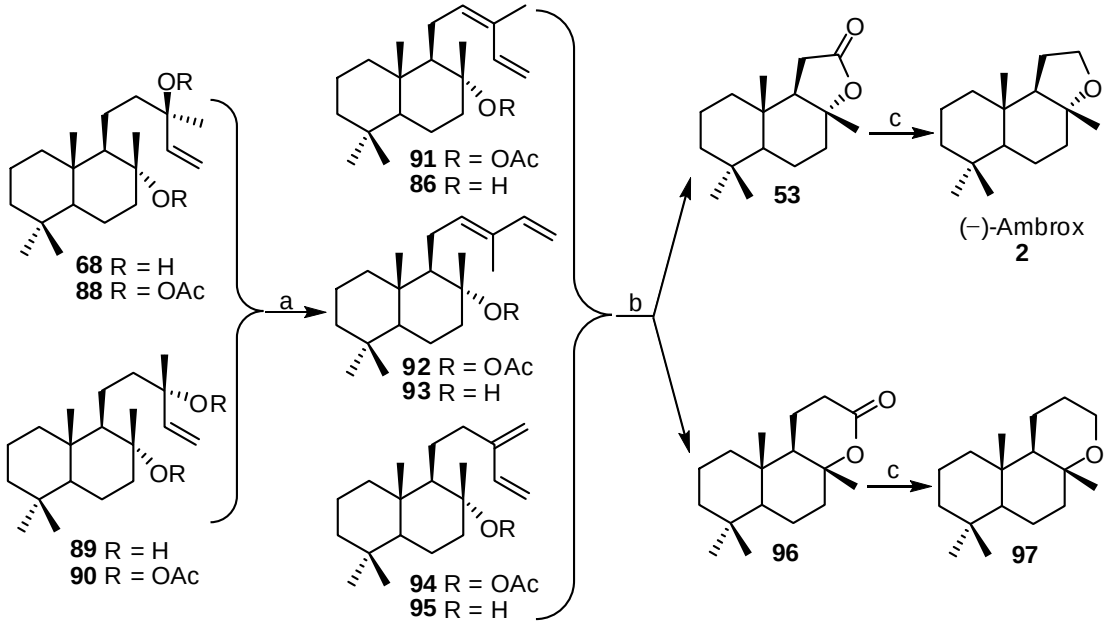
In that year, Martres *et al*. used ruthenium oxide as a catalyst for side chain degradation of (−)-sclareol **68** and followed by reduction and cyclization then (−)-ambrox **2** was obtained (Scheme 2.11) (Martress *et al*., 1993).



(a) $RuCl_3{\cdot}3H_2O$, CCl_4, CH_3CN, H_2O, 40°C; (b) $LiAlH_4$, Et_2O, 25°C, 95%; (c) NaH, *p*-TsCl, CH_2Cl_2, 25°C 94%

Scheme 2.11 Synthetic route for (−)-ambrox from sclareol by Martress *et al*., 1993

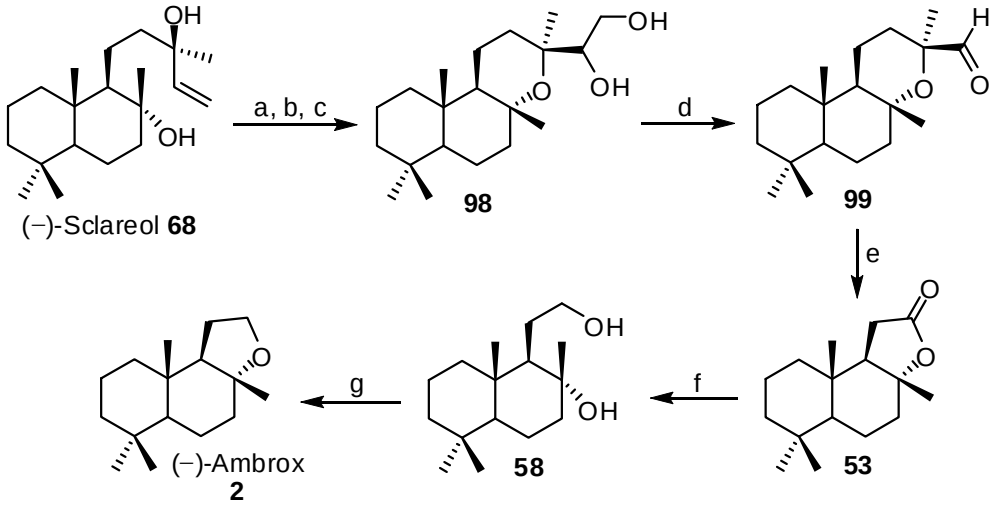
In 1997, Waegell studied on the transformation of a mixture of sclareol, episclareol and their acetate forms to yield a mixture of abienols, **86**, **93**, **95**, and abienol acetates, **91**, **92**, **94** (Scheme 2.12). Following by oxidative cleavage of a mixture with permanganate, reduction and cyclization, (–)-ambrox was obtained (Waegell, 1997).



(a) cat. $Pd(OAc)_2$, Phosphine dioxane, 100%; (b) $KMnO_4$, acetone, 80%; (c) i) $LiAlH_4$; ii) H^+; 90%

Scheme 2.12 Synthetic route for (–)-ambrox from sclareol by Waegell

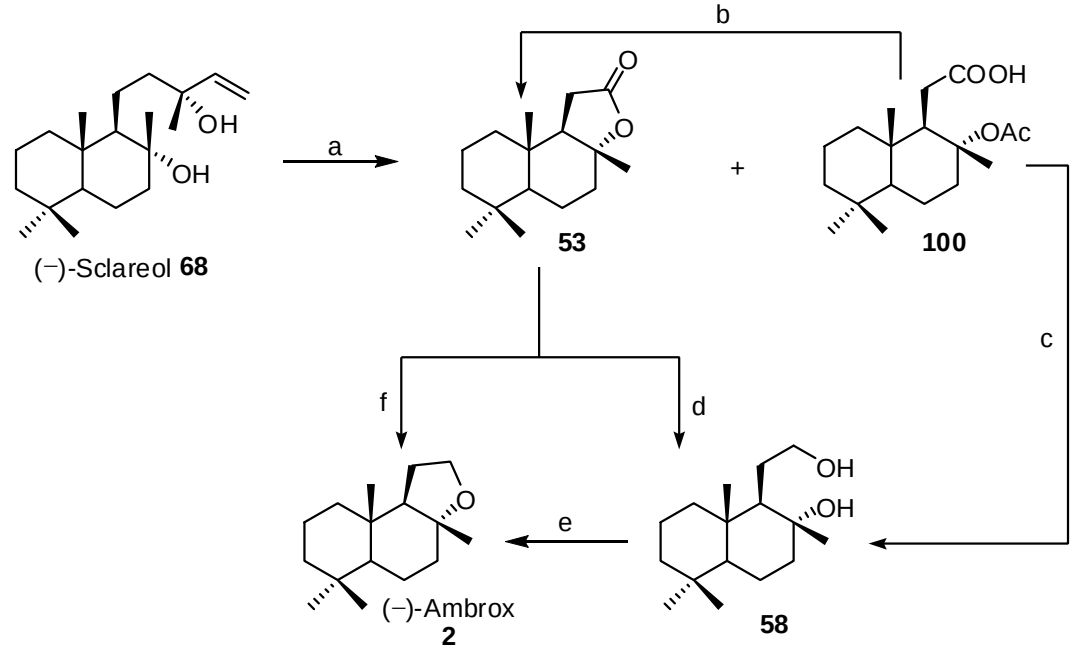
In 2001, Moulines *et al*. presented a practical synthesis of (–)-ambrox from (–)-sclareol **68** using no metallic oxidative degradation of side chain of sclareol (scheme 2.13) (Moulines *et al*., 2001).



(a) AcOOH, EtOAc, NaOAc, rt, 8 days, nearly quantitative yield; (b) *t*-BuOH, 30°C, NaOH 1N, rt, over night, nearly quantitative yield; (c) H_2SO_4, 0°C, EtOAc, 2 h, quantitative yield; (d) THF, $NaIO_4/H_2O$, rt, darkness, 16 h, 97%; (e) AcOOH, rt, overnight, 82%; (f) $LiAlH_4$, THF, rt, 3 h, 97%; (g) THF, 0°C, N_2, hexanic *n*-BuLi 1.6M, TsCl, 96%

Scheme 2.13 Synthetic route for (−)-ambrox from sclareol by Moulines *et al*.

In 2004, Barrero *et al.* presented the synthesis of nor-ambreinolide **53** from (−)-sclareol **68** by treatment with $KMnO_4$-Ac_2O followed by alkaline hydrolysis. Compound **53** was directly transformed into (−)-ambrox **2** by reduction with $NaBH_4$ in the presence of Lewis acid (scheme 2.14) (Barrero *et al*., 2004).

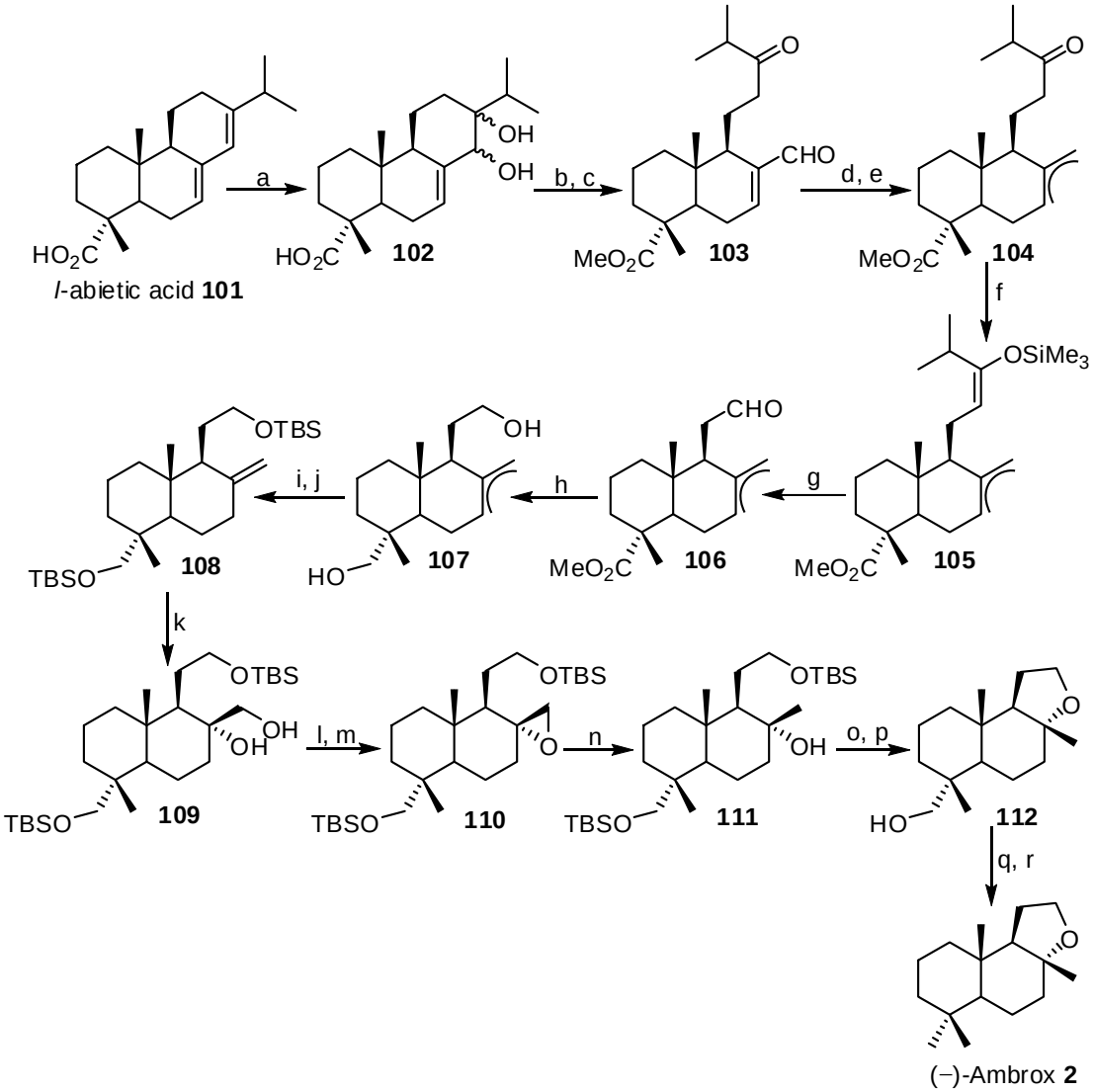


(a) $KMnO_4$, acetone, Ac_2O, 0°C, 2h, 94%; (b) 2N NaOH, reflux, 2h,97%, then HCl 2N; (c) $LiAlH_4$, THF, 0°C, 2h, 97%; (d) KBH_4, EtOH, reflux, 10h, 95% or red-Al (2.5 eq), toluene, reflux, 12h, 78%; (e) $CBrCl_3$, Ph_3P, $NaHCO_3$, CH_2Cl_2, reflux, 6h, 92%; (f) ZnI_2, $NaBH_4$, THF, rt, 1h, 85%

Scheme 2.14 Synthetic route for (−)-ambrox from sclareol by Barrero *et al.*

Furthermore, other diterpenes were used as starting materials for (–)-ambrox synthesis as the following.

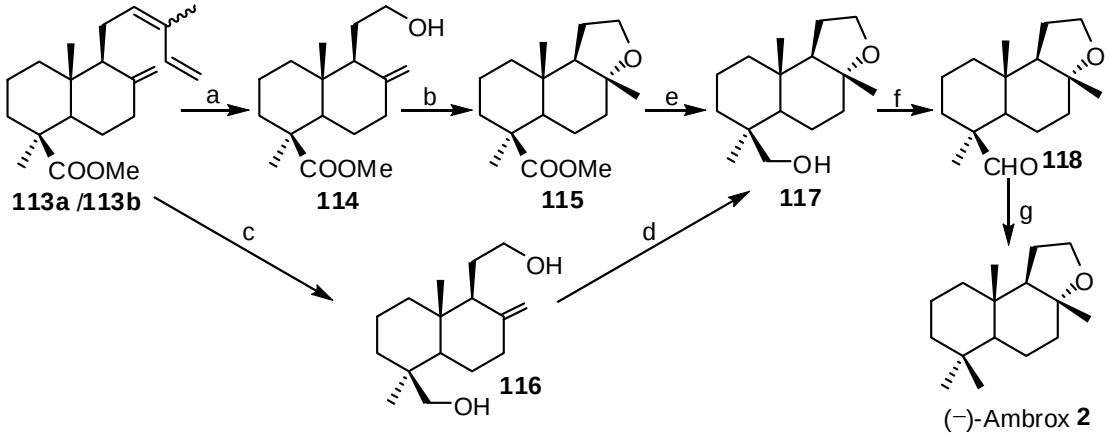
In 1987, Koyama *et al*. synthesized (–)-ambrox from *l*-abietic acid via 18 steps (Scheme 2.15) (Koyama *et al*., 1987).



(a) OsO_4, Me_3NO/*t*-BuOH-py-H_2O, reflux, 20h, 86%; (b) CH_2N_2, CH_2Cl_2-MeOH, quantitative yield; (c) $Pb(OAc)_4$, benzene, 90%; (d) $HSCH_2CH_2SH$, *p*-TsOH, rt; (e) Raney Ni(W1), AcOEt-EtOH, 85% from **104**; (f) TMSOTf, CH_2Cl_2, -78°C, quantitative yield; (g) O_3, AcOEt-py 1.3% v/v, -78°C, 49%; (h) $LiAlH_4$, 83%; (i) TBDMSOTf, quantitative yield; (j) UV-irradiation with high pressure mercury lamp, *i*-PrOH-xylene, 0°C, quantitative yield; (k) OsO_4-TBHP, acetone, Et_4NOH, 50°C, 71%; (l) MsCl; (m) Li-HMDS, THF, rt, quantitative yield from **110**; (n) reduction; (o) deprotection; (p) MsCl, py, 78% from **111**; (q) Irelan-Liu method; (r) Li, $EtNH_2$, 93% from **112**

Scheme 2.15 Synthetic route for (–)-ambrox from *l*-abietic acid by Koyama *et al*.

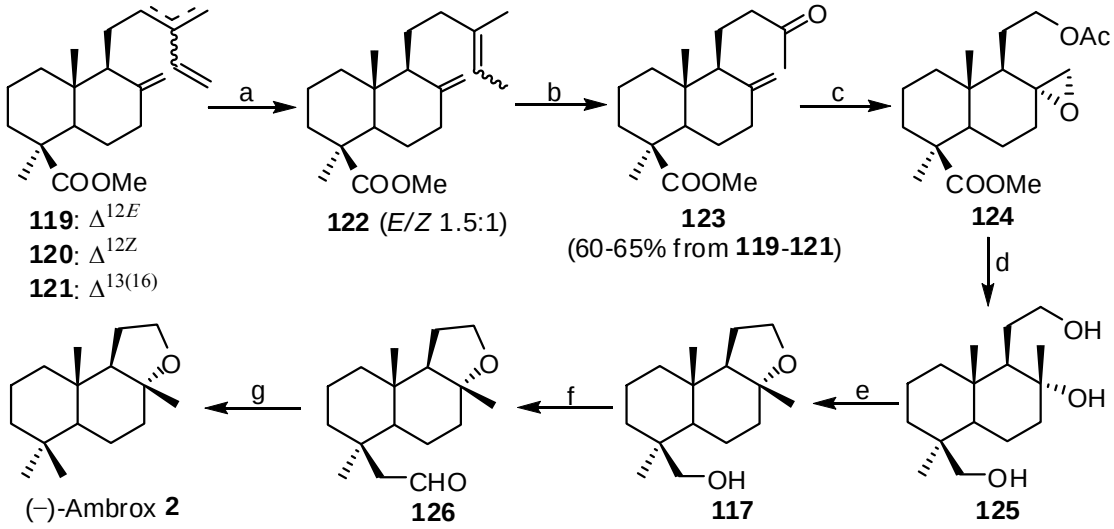
In 1993, Barrero *et al*. presented two routes for preparing (–)-ambrox from communic acid. The first route, they stated from methyl *trans*- (**113a**) or *cis*-communate (**113b**), or mixture of them (Scheme 2.16). Degradation of side chain, followed by formation of tetrahydrofurane ring, and then reduction of methoxycarbonyl group, compound **2** was obtained (Barrero *et al*., 1993a).



(a) O_3, CH_2Cl_2, -78°C; $LiAlH_4$, THF, rt, 40%; (b) *p*-TsOH, CH_3NO_2, rt, 1-1.2h, 85%; (c) O_3, CH_2Cl_2, -78°C; $LiAlH_4$, THF, reflux, 35-40%; (d) *p*-TsOH, CH_3NO_2, rt, 1-1.2h, 80%; (e) *p*-TsOH, CH_3NO_2, rt, 3h, 85%; (f) Jones reagent, acetone, 0°C, 90%; (g) $N_2H_4 \cdot H_2O$, KOH, TEG, reflux, 1h, 71%

Scheme 2.16 Synthetic route for (–)-ambrox from communic acid by Barrero *et al*. (1993a)

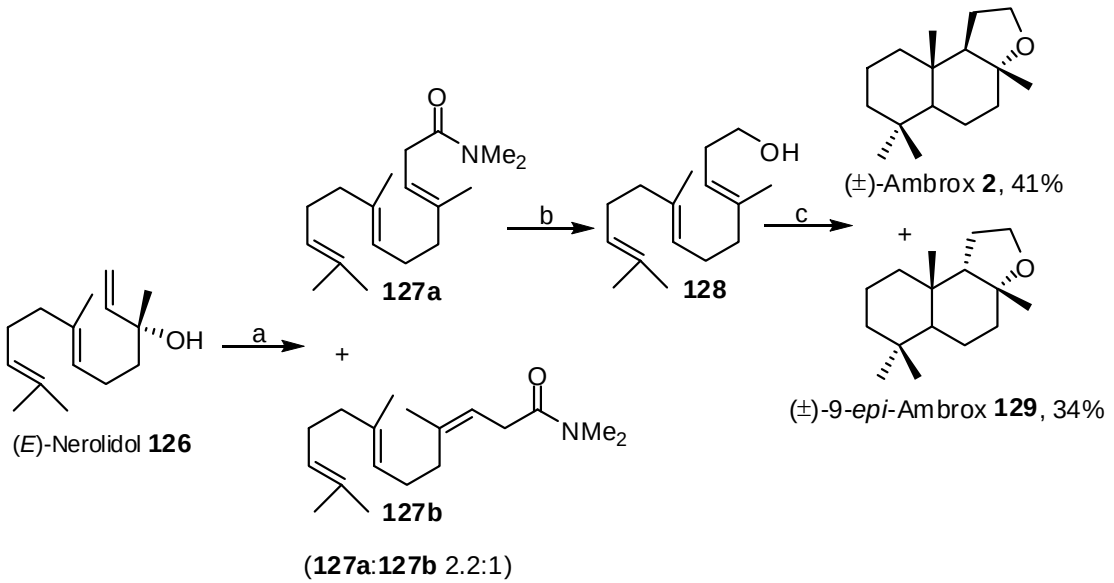
The second route, they started with degradation of side chain of a mixture of methyl communates (**119**, **120**, **121**), followed by formation of tetrahydrofurane ring, and then reduction of methoxycarbonyl group, compound **2** was also obtained (Scheme 2.17) (Barrero *et al*., 1993b).



(a) *t*-BuOH, Na, rt, overnight; (b) $NaIO_4$, 0.2% OsO_4, *t*-BuOH, H_2O, rt, 150h; (c) *m*-CPBA, CH_2Cl_2, rt, 5d, 90%; (d) $LiAlH_4$, THF, reflux, 1h, 85%; (e) *p*-TsOH, CH_3NO_2, rt, 1h, 87%; (f) Jones reagent, acetone, 0°C, 88%; (g) $N_2H_4{\cdot}H_2O$, KOH, TEG, reflux, 1h, 71%

Scheme 2.17 Synthetic route for (–)-ambrox from communic acid by Barrero *et al*. (1993b)

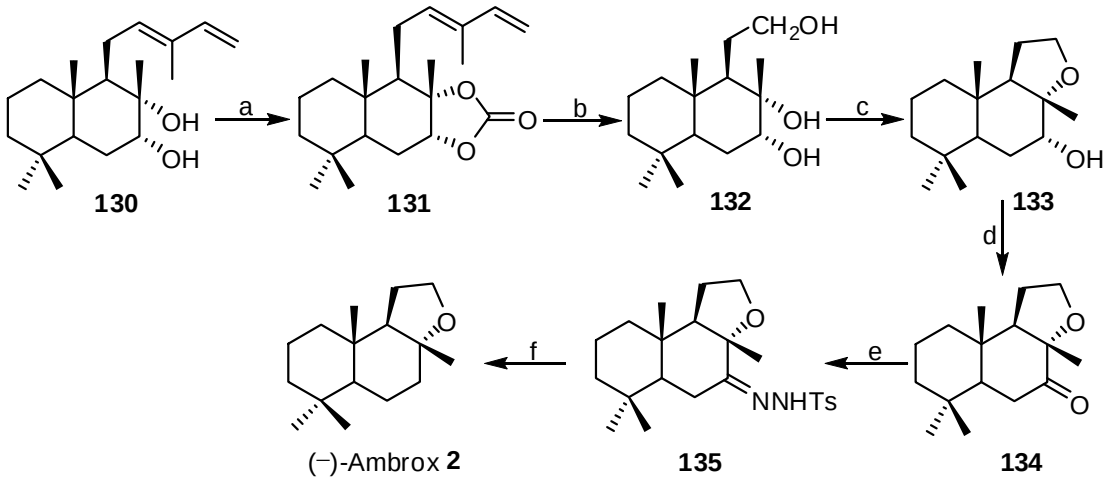
In 1996, Barrero *et al*. synthesized (±)-Ambrox® from (*E*)-nerolidol **126** in 3 steps (Scheme 2.18) (Barrero *et al*., 1996).



(a) DMFDMA, xylene, reflux, 13h, 79%; (b) $LiBEt_3H$, THF, -78°C, h, 75%; (c) $ClSO_3H$, $PrNO_2$, -78°C, 6 min

Scheme 2.18 Synthetic route for (–)-ambrox from (*E*)-nerolidol by Barrero *et al*.

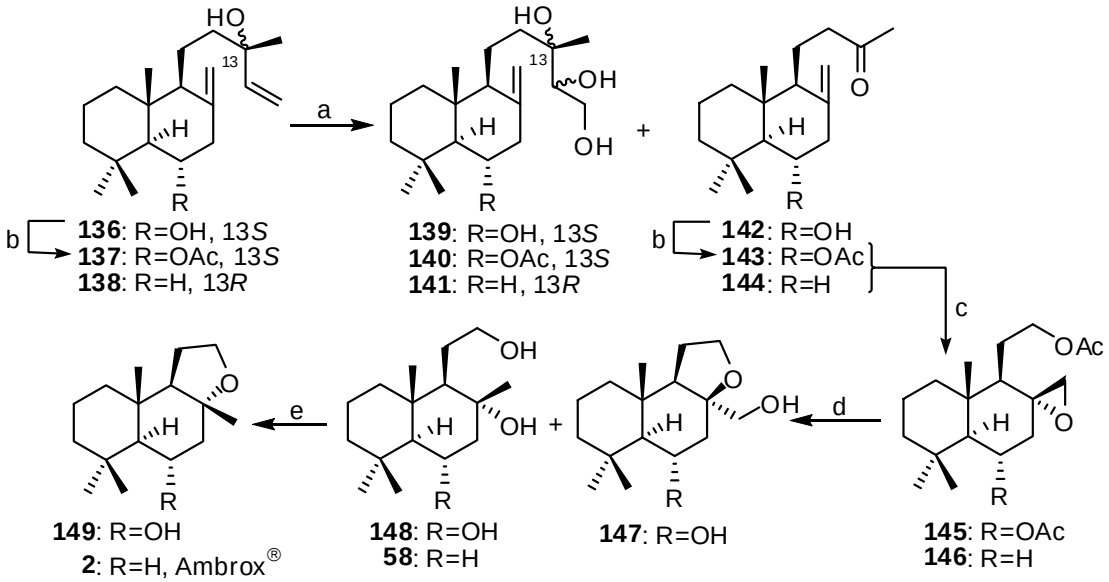
In 1998, Hashimoto *et al*. synthesized (–)-ambrox from labda-12,14-diene-7α,8α-diol **130** (Scheme 2.19). Protection of diol, following by oxidative degradation of side chain, cyclization and dehydroxylation at C-7, afforded **2** in 21% overall yield (Hashimoto *et al*., 1998).



(a) triphosgene, Py, CH_2Cl_2, 100%; (b) i) O_3, CH_2Cl_2; ii) $LiAlH_4$; 59%; (c) *p*-TsOH, CH_3NO_2, 76%; (d) CrO_3-H_2SO_4, 87%; (e) $TsNHNH_2$, 73%; (f) $NaBH_3CN$, 74%

Scheme 2.19 Synthetic route for (–)-ambrox from labda-12,14-diene-7α,8α-diol by Hashimoto *et al*.

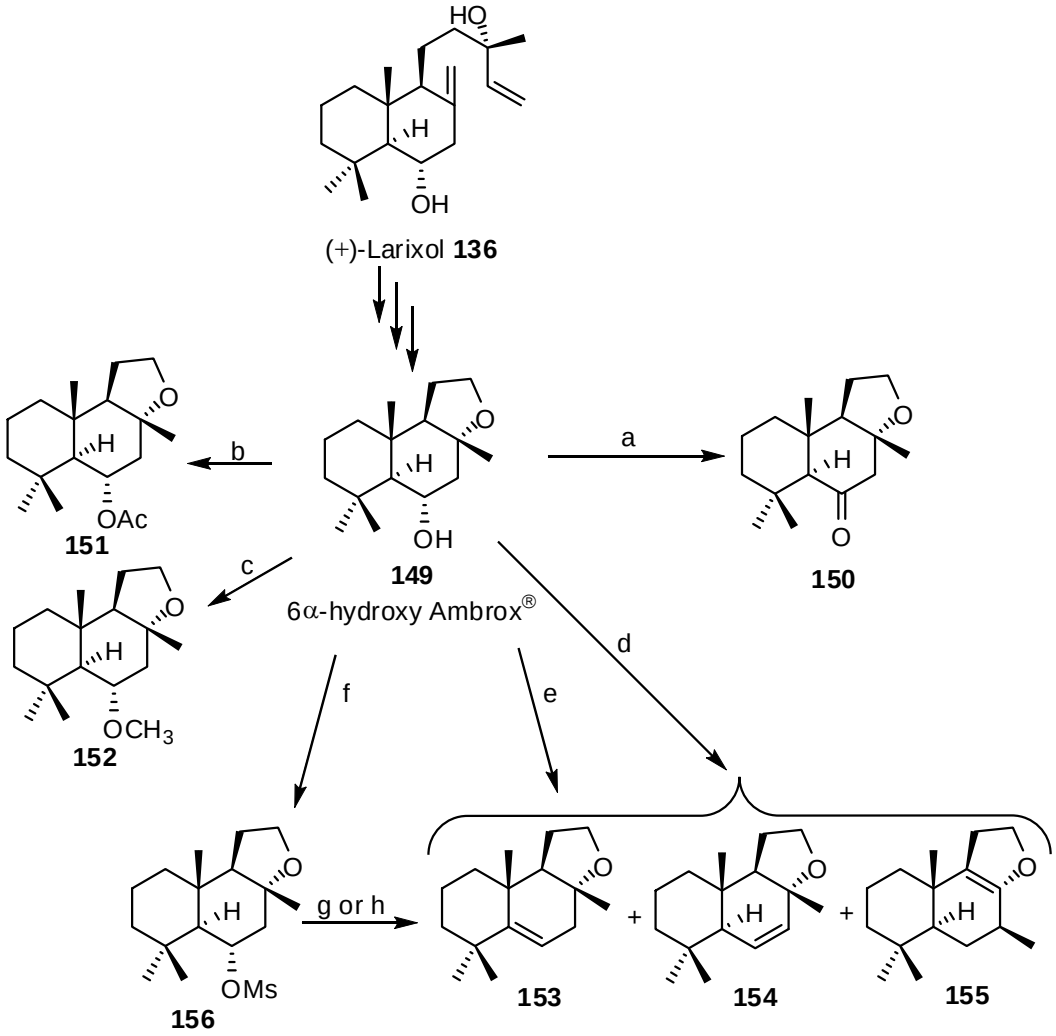
In 2001, Bolster *et al*. synthesized ambrox from (+)-larixol **136** using potassium permanganate for oxidative degradation of side chain (Scheme 2.20). Following by epoxidation and reduction diol **58** was formed. Cyclization of 58 gave **2** in 64% (Bolster *et al*., 2001a).



(a) $KMnO_4$, *N*,*N*,*N*′-triethylbenzenemethanaminiumchloride, CH_2Cl_2, rt, 88%; (b) Ac_2O, CH_2Cl_2, py, DMAP, 91%; (c) for R=OAc: *m*-CPBA, CH_2Cl_2, 84%; for R=H: see Bolster et al., 2001a; (d) for R=OH, OAc: $LiAlH_4$, THF, 0°C to rt, 95%, for R=H: see Bolster et al., 2001a; (e) for R=OH: p-TsOH, CH_3NO_2, 64%

Scheme 2.20 Synthetic route for (–)-ambrox and its derivatives from (+)-larixol by Bolster *et al*.

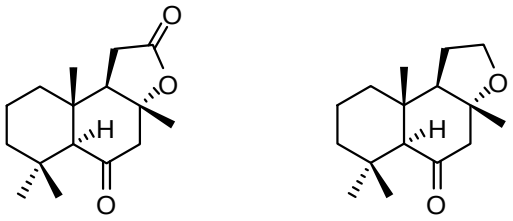
Furthermore, they synthesized Ambrox®-like compounds starting from (+)-larixol **136** (Scheme 2.21). The results showed that alkenes **153-155** had the typical ambergris fragrance properties, a pleasant smell (Bolster *et al*., 2001a).



(a) $H_2Cr_2O_7$, acetone, 91%; (b) Ac_2O, CH_2Cl_2, py, DMAP, 94%; (c) NaH, MeI, DMF, 100°C, 95%; (d) *p*-TsOH, benzene, Δ, 80%, **17**:**18**:**19**=2:1:1; (e) $SOCl_2$, py, 0°C to rt, 57%; (f) MsCl, 0°C to rt, 92%; (g) LiBr, Li_2CO_3, DMF, 120°C, 71%; (h) MgI_2, toluene, 67%

Scheme 2.21 Synthetic route for derivatives of ambrox from (+)-larixol by Bolster *et al.*

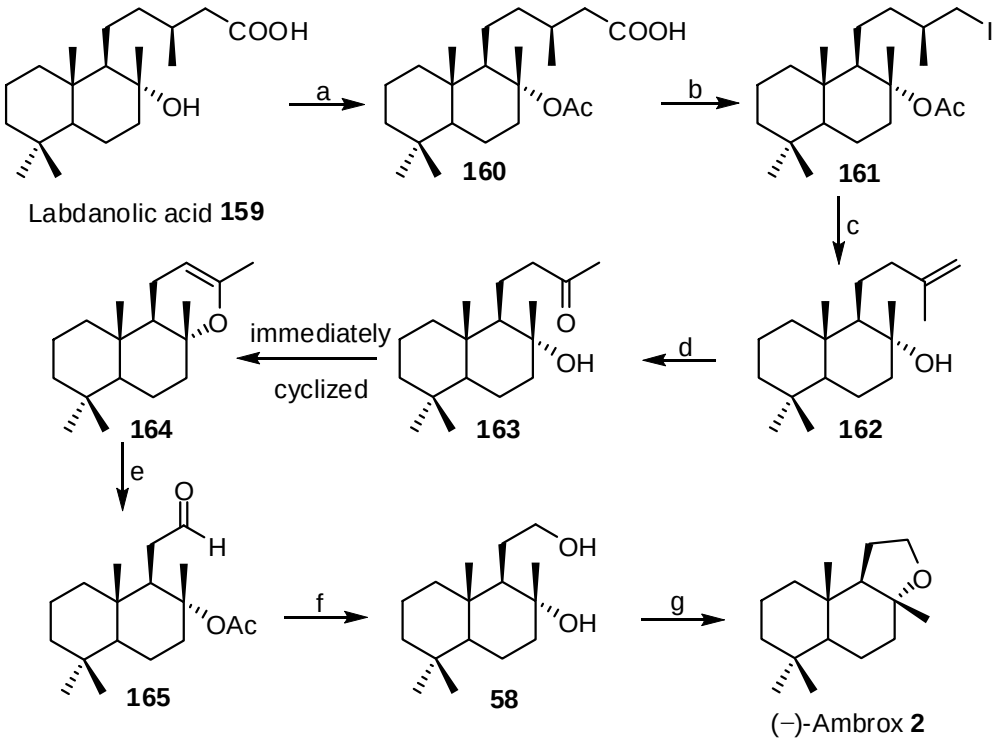
In addition, they reported that they could synthesize other Ambrox®-like compounds from (+)-larixol **136** (Figure 2.1). They found that both of them did not have any smell (Bolster *et al*., 2001b).



6-oxo-Sclareolide **157** 6-oxo-Ambrox® **158**

Figure 2.1 Ambrox-like compounds from (+)-larixol

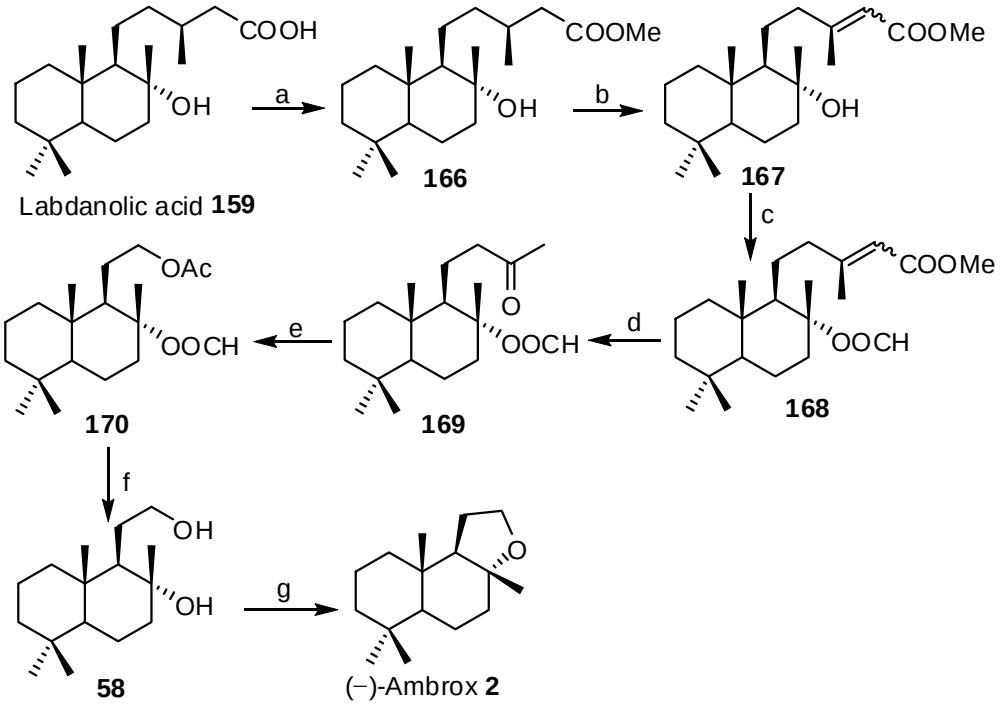
Moreover, Bolster *et al*. presented synthetic route to (–)-ambrox not only from (+)-larixol but also from labdanolic acid **159** (Scheme 2.22). They reported on the iododecarboxylation of labdanolic acid as the key step (Bolster *et al*., 2001c).



(a) AcCl, *N*,*N*-dimethylaniline, 35-45%; (b) IBDA, I_2, CCl_4, $h\nu$, Δ, 76%; c) *t*-BuOK, THF, 74%; (d) O_3, MeOH/CH_2Cl_2 1:5, -78°C, PPh_3, 44%; (e) O_3, CH_2Cl_2, Py, -78°C; (f) $LiAlH_4$, THF, 60%; (g) *p*-TsOH, CH_3NO_2, 87%

Scheme 2.22 Synthetic route for (–)-ambrox from labdanolic acid by Bolster *et al*.

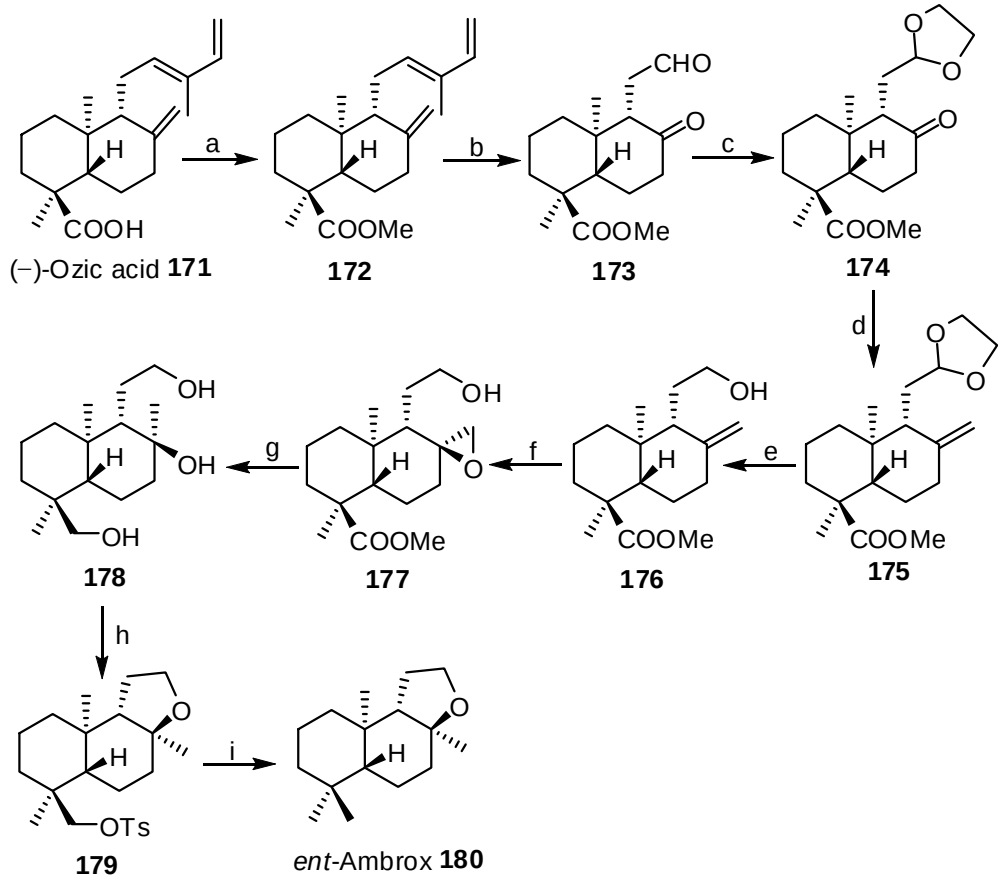
In 2002, Castro *et al*. also used labdanolic acid **159**, which is a major diterpenoid of the acid fraction of non-polar extracts of *Cistus ladaniferus* L., as starting substance for the synthesis of ambrox. The transformation consisted of α,β-dehydrogenation of methyl labdanolate, oxidative degradation of side chain and stereoselective tetrahydrofuran ring (Scheme 2.23) (Castro *et al*., 2002).



(a) CH_2N_2, 43%; (b) LDA, THF, Ph_2Se_2, -78°C, H_2O_2, 94%; (c) HCOOH-Ac_2O, 93%; (d) $KMnO_4$, $MgSO_4$, acetone, 88%; (e) *m*-CPBA, CH_2Cl_2, 96%; (f) KOH, MeOH, 59%; (g) *p*-TsOH, $MeNO_2$, 75%

Scheme 2.23 Synthetic route for (–)-ambrox from labdanolic acid by Castro *et al*.

In 2003, Giacomini *et al*. synthesized *ent*-ambrox **180** starting from (–)-ozic acid **171**, a constituent of *Hymenaea courbaril var*. *altissima*, and it was easily transformed to methyl ester **172**. Following the synthetic sequence, *ent*-ambrox **180** was obtained in a 58% overall yield (Scheme 2.24) (Giacomini *et al*., 2003).



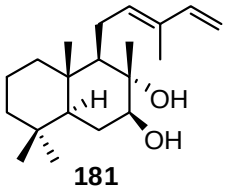
(a) CH_2N_2; (b) i) O_3, CH_2Cl_2, -78°C; ii) PPh_3, rt; 85%; (c) camphorsulfonic acid, benzene, reflux, 90%; (d) Zn, $TiCl_4$, CH_2Br_2, CH_2Cl_2, rt, 73%; (e) i) THF, HCl 1%, rt, ii) $NaBH_4$, MeOH, rt; 81%; f) *m*-CPBA, CH_2Cl_2, rt, 90%; (g) $LiAlH_4$, THF, reflux, 86%; (h) TsCl, Py, rt, 62%; (i) i) NaI, Zn, DMF, 120°C; ii) *m*-CPBA, CH_2Cl_2, rt; 58% (two steps)

Scheme 2.24 Synthetic route for (−)-ambrox from (−)-ozic acid by Giacomini *et al*.

2.5 Diterpenoid compounds from *Croton oblongifolius* in Thailand

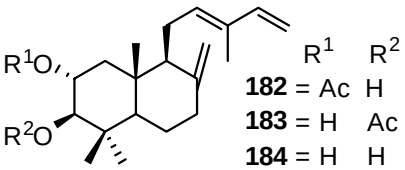
Plao Yai (*Croton oblongifolius*) in Euphorbiaceae family is widely distributed in Thailand. It has been used as a traditional medicine for many applications such as for dysmenorrhea, as a purgative, and to treat dyspepsia and dysenteria. Moreover, this plant had been used as folk-medicine in conjunction with *Croton sublyratus* to treat gastric ulcers and gastric cancers (Roengsumran *et al*., 2002). In literatures, many types of diterpenoids (Figure 2.2) have been isolated from *C. oblongifolius* from various parts of Thailand.

Labdane diterpenoids

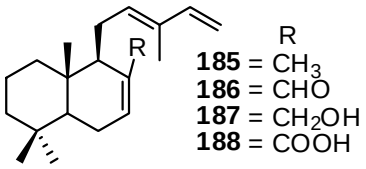


181

(Roengsumran *et al*., 2002)

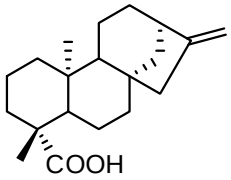


(Roengsumran *et al*., 2001)



(Roengsumran *et al*., 1999a)

Kaurane diterpenoid



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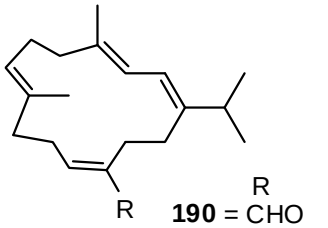
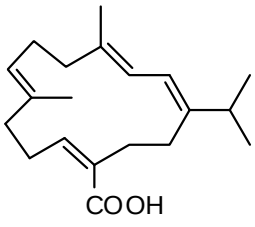
(Ngamrojanavanich *et al*., 2003)

Cembraniod diterpenoids



(Roengsumran *et al*., 1999b)

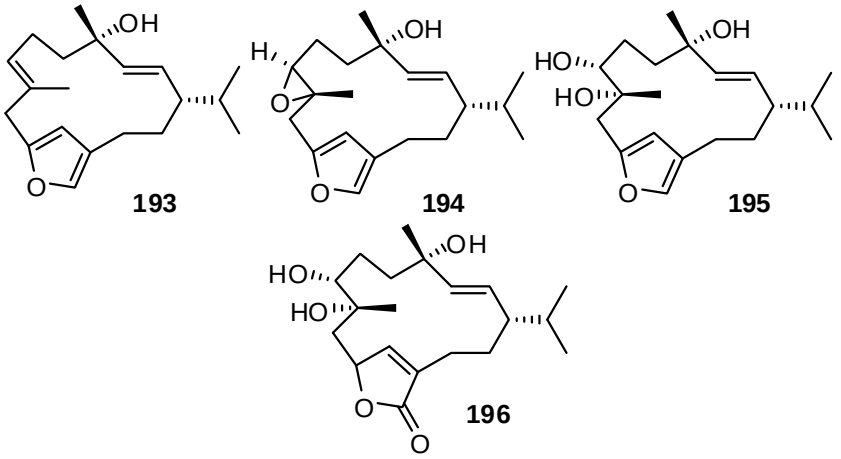


192

(Roengsumran *et al*., 1998)

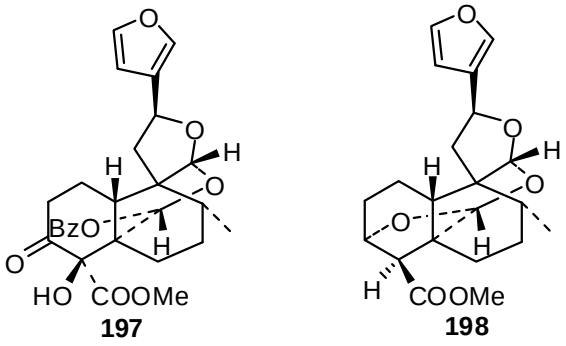
Figure 2.2 Isolated-ditepenoids from *C. oblongifolius* in Thailand

Furanocembranoid diterpenoids



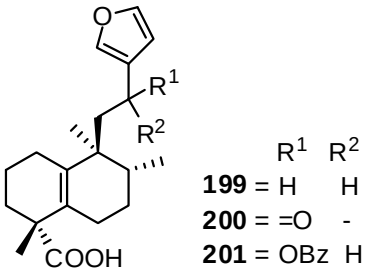
(Pudhom *et al*., 2007)

Clerodane diterpenoids



(Roengsumran *et al*., 2002)

Halimane diterpenoids

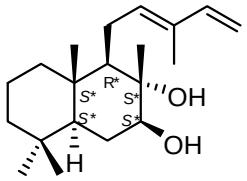


(Roengsumran *et al*., 2004)

Figure 2.2 Isolated-ditepenoids from *C. oblongifolius* in Thailand (Continued)

2.6 Isolation of (–)-nidorellol

The first isolation of (–)-Nidorellol **123** has been reported in 1978 by Bohlmann and Fritz. It was isolated from *Nidorella ayriculata* and its stereochemistry were performed as *trans*-(5*S*,7*S*,8*S*,9*R*,10*S*)-labda-12,14-diene-7β,8α-diol (Figure 2.3) (Bohlmann and Fritz, 1978).



(−)-nidorellol **181**

Figure 2.3 Structure and stereochemistry configuration of (–)-niorellol reported by Bohlmann and Fritz

In 1988, Zdero et al. could isolate (–)-nidorellol from the aerial parts of Bolivian *Stevia sarensis* (Zdero *et al*., 1988). Moreover, In 2002 Roengsumran *et al*. reported the isolation of chemical constituents from stem bark of *Croton oblongifolius* which collected from Kanchanaburi Province, Thailand. They found (–)-nidorellol together with a new furoclerodane and one known clerodane (Roengsumran *et al*., 2002).

CHAPTER III

EXPERIMENTS

3.1 Materials and methods

3.1.1 General remarks

For all moisture sensitive reactions were carried out in oven-dried glassware fitted with rubber septa under argon. The Buchi rotary evaporator was used for the rapid removal of large amounts of volatile organic solvents. Merck's TLC aluminium sheet, silica gel 60 F_{254} (layer 0.2 mm) was used to monitor reaction courses and products with detection by UV light and vanillin/H_2SO_4 reagent. For chromatographic separations, Merck's silica gel (230-400 mesh ASTM) and Merck's silica gel 60 RP-18 (40-60 μm) were used as adsorbent for normal column chromatography and for reverse phase column chromatography, respectively. All commercial grade solvents used in this research, such as hexane, dichloromethane, ethyl acetate and methanol, were distilled prior to use. The reagent grade solvents were used for synthesis, thin layer chromatography and crystallization. THF was freshly distilled from sodium benzophenone ketyl under argon and CH_2Cl_2 was freshly distilled from calcium hydride under argon for reaction with exclusion of moisture. Pyridine was dried over calcium hydride under argon before use.

3.1.2 Instrumentation

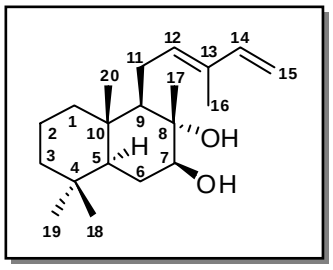
Optical rotations were determined at 589 nm on Perkin-Elmer Model 341 polarimeter. The melting points were recorded on a MEL-TEMP melting point apparatus. IR spectra were obtained on Thermo Scientific Model Nicolet6700 spectrometer. The ^{1}H and ^{13}C Nuclear magnetic resonance were recorded at 400 and 100 MHz, respectively, on a Varian Model Mercury (400 MHz) and Bruker Model AVANCE (400 MHz) spectrometers in deuterated chloroform ($CDCl_3$), dimethylsulfoxide-d_6 (DMSO-d_6), methanol-d_4 (CD_3OD), acetone-d_6 ($(CD_3)_2CO$), and benzene-d_6 (C_6D_6). Chemical shifts were reported relative to residual solvents peaks [$CDCl_3$, at 7.26 (^{1}H) and at 77.1 (^{13}C); C_6D_6, at 7.16 (^{1}H) and at 128.0 (^{13}C); DMSO-

d_6, at 3.33 (^{1}H) and at 39.5 (^{13}C); CD_3OD, at 3.31 (^{1}H of methyl), at 4.87 (^{1}H of hydroxyl) and at 49.0 (^{13}C); $(CD_3)_2CO$, at 2.05 (^{1}H), at 24.8 (^{13}C of methyl) and at 206.0 (^{13}C of carbonyl)]. HREIMS were recorded on Bruker Model micrOTOF spectrometer and Mass Spectrometer LCT, Micromass UK Limited. X-ray crystallographic analysis data were collected on Bruker SMART CCD diffractometer. The data from thermogravimetric analysis (TGA) were obtained on Perkin-Elmer Model Pyris diamond. Ozone was generated by OZZON Model 6501T for ozonolysis reaction.

3.2 Experimental procedures and characterization data

3.2.1 Plants collection, metabolite extraction and isolation of nidorellol from *Croton oblongifolius*

Croton oblongifolius barks were collected from Prachuap Khiri Khan Province in the southern part of Thailand. The powdered, air-dried stem bark (380.0 g) of *Croton oblongifolius* was extracted with hexane (500 ml x 6) at room temperature to give yellowish brown oil (24.9 g). The crude extract was fractionated by silica gel column chromatography eluted with hexane-EtOAc gradient in stepwise fashion (1-100% EtOAc). The fraction from elution with 3:7 EtOAc-hexane was re-chromatographed on reverse phase column eluting 20% H_2O in MeOH to afford **181** (1.6 g) as white solid in 0.42% yield.



Nidorellol (181)

mp. 78-80°C

R_f = 0.26 (EtOAc-hexane, 1:1).

Optical Rotation: $[\alpha]_D^{20}$ = -23.2^o (c = 0.40, $CHCl_3$).

IR (KBr): ν_{max} 3363, 3090, 2920, 1632, 1601, 1387, 1077 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80 (3H, s, H-18), 0.84 (3H, s, H-20), 0.88 (3H, s, H-19), 0.89 (1H, m, H-1a), 1.03 (1H, dd, *J*=1.6, 12.4 Hz, H-5), 1.11 (1H, m, H-3b), 1.14 (3H, s, H-17), 1.26 (1H, t, *J*=5.6, H-9), 1.28 (1H, ddd, *J*=12.0, 12.4, 12.8 Hz, H-6b), 1.33 (1H, m, H-3a), 1.42 (1H, m, H-2b), 1.58 (1H, m, H-2a), 1.60 (1H, m, H-1b), 1.78 (3H, s, H-16), 1.85 (1H, ddd, *J*=1.6, 4.8, 12.8 Hz, H-6a), 2.20 (1H, ddd, *J*=5.6, 6.8, 15.6 Hz, H-11a), 2.39 (1H, ddd, *J*=5.6, 6.8, 15.6 Hz, H-11b), 3.51 (1H, dd, *J*=4.8, 12.0 Hz, H-7), 4.91 (1H, d, *J*=10.8 Hz, H-15b), 5.06 (1H, d, *J*=17.2 Hz, H-15a), 5.55 (1H, d, *J*=6.8 Hz, H-12), 6.33 (1H, dd, *J*=10.8, 17.2 Hz, H-14) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 12.0 (C-16), 15.7 (C-20), 18.0 (C-17), 18.6 (C-2), 21.7 (c-19), 23.6 (C-11), 27.8 (C-6), 33.4 (C-4), 33.6 (C-18), 39.4 (C-10), 39.9 (C-1), 41.7 (C-3), 53.7 (C-5), 60.3 (C-9), 78.2 (C-8), 80.4 (C-7), 110.7 (C-15), 132.8 (C-13), 135.7 (C-12), 141.6 (C-14) ppm.

HRMS (ESI-TOF) *m/z* 329.2450 $[M+Na]^+$ calcd for $C_{20}H_{34}O_2Na$ 329.2451.

3.2.2 Effect of *p*-TsOH·H_2O on acid-promoted cyclization of (–)-nidorellol

Effect of *p*-TsOH·H_2O on acid-promoted cyclization of (–)-nidorellol (**181**) was examined. To each solution of **181** (30.0 mg, 0.098 mmol) in CH_2Cl_2 (10 ml), various amount of *p*-TsOH·H_2O (5% mol, 10% mol, 20% mol and 25% mol) were added and stirred at room temperature. The reaction courses and products were monitored by TLC using 30% EtOAc in hexane as mobile phase with detection by UV light and vanillin/H_2SO_4 reagent and also monitored by ^{1}H NMR. The optimal condition was determined from the reaction time of complete transformation of **181** into cyclization products.

3.2.3 Effect of solvents on cyclization of (–)-nidorellol

The acid-promoted cyclization of **181** with 20% mol of *p*-TsOH·H_2O were carried out in various deuterated solvents, chloroform ($CDCl_3$), dimethylsulfoxide-d_6 (DMSO-d_6), methanol-d_4 (CD_3OD), acetone-d_6 ($(CD_3)_2CO$) and benzene-d_6 (C_6D_6). To an NMR tube containing a solution of **181** (10.0 mg, 0.03 mmol) in deuterated solvent (0.7 ml) *p*-TsOH·H_2O (1.2 mg) was added and vigorously shaken for 30 seconds. Then a reaction mixture was stored at ambient

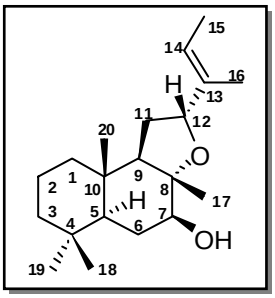
temperature and monitored by ^{1}H NMR spectrometer until the cyclization was completed.

3.2.4 Effect of temperature on the ratio of compounds 182 and 183

To examine the effects of temperature on the acid-promoted cyclization of **2**, 20% mol of *p*-TsOH·H_2O was used as acid catalyst. The reactions were carried out in deuterated chloroform ($CDCl_3$) at various temperature, -18°C, 4°C and room temperature. The reaction courses and ratio of **182** and **183** were monitored by ^{1}H NMR spectrometer.

3.2.5 Synthesis of compounds 182 and 183

To a solution of **181** (353.9 mg, 1.15 mmol) in CH_2Cl_2 (50 ml), 20% mol of *p*-TsOH·H_2O was added and stirred at room temperature for 3 hours. The reaction mixture was transferred to a separatory funnel and washed with saturated $NaHCO_3$ and water, respectively. The organic layer was separated and dried over anhydrous Na_2SO_4. Removal of dichloromethane by evaporation afforded a 3:1 mixture of **182** and **183** in quantitative yield as viscous light yellow oil. The mixture of **182** and **183** was isolated on silica gel preparative TLC using 3:7 EtOAc-Hexane as a mobile phase to give **182** and **183** in 14% and 8%, respectively.



Compound 182

Compound **182** was obtained as white solid.

mp. 120-122°C

R_f = 0.36 (EtOAc-hexane, 3:7).

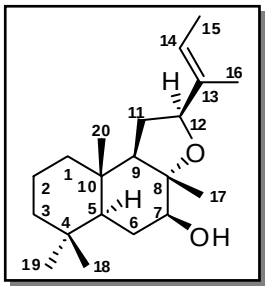
Optical Rotation: $[\alpha]_D^{20} = -5.7^o$ (*c* = 0.35, $CHCl_3$).

IR (KBr): ν_{max} 3422, 2950, 2920, 1046 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83 (3H, s, H-20), 0.83 (3H, s, H-18), 0.89 (3H, s, H-19), 0.95 (1H, dd, *J*=12.4, 12.4 Hz, H-1a), 1.05 (1H, dd, *J*=2.0, 12.8 Hz, H-5), 1.15 (3H, s, H-17), 1.16 (1H, m, H-3a), 1.32 (1H, ddd, *J*=11.2, 12.8, 12.8 Hz, H-6b), 1.37 (1H, m, H-9), 1.41 (1H, m, H-1b), 1.42 (1H, m, H-2a), 1.42 (1H, m, H-3b), 1.51 (1H, ddd, *J*=2.0, 7.6, 10.8 Hz, H-11a), 1.57 (3H, s, H-16), 1.59 (3H, d, *J*=6.8 Hz, H-15), 1.63 (1H, m, H-2b), 1.89 (1H, ddd, *J*=2.0, 4.8, 12.8 Hz, H-6a), 1.96 (1H, ddd, *J*=9.2, 10.8, 12.0 Hz, H-11b), 3.70 (1H, dd, *J*=4.8, 11.2 Hz, H-7), 4.42 (1H, dd, *J*=2.0, 9.2 Hz, H-12), 5.52 (1H, q, *J*=6.8 Hz, H-14) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 11.8 (C-16), 13.2 (C-15), 15.0 (C-20), 16.3 (C-17), 18.5 (C-2), 21.3 (C-19), 27.7 (C-11), 28.7 (C-6), 33.2 (C-4), 33.7 (C-18), 36.0 (C-10), 39.7 (C-1), 42.3 (C-3), 56.1 (C-5), 58.1 (C-9), 78.7 (C-7), 81.2 (C-12), 84.7 (C-8), 120.6 (C-14), 136.6 (C-13) ppm.

HRMS (ESI-TOF) *m/z* 329.2449 $[M+Na]^+$ calcd for $C_{20}H_{34}O_2Na$ 329.2451.



Compound 183

Compound **183** was obtained as colorless crystals.

mp. 140-142°C

R_f = 0.41 (EtOAc-hexane, 3:7).

Optical Rotation: $[\alpha]_D^{20}$ = +31.0^o (*c* = 0.29, $CHCl_3$).

IR (KBr): ν_{max} 3353, 2951, 2920, 1051 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.82 (3H,s, H-19), 0.84 (3H, s, H-20), 0.88 (3H, s, H-18), 0.99 (1H, td, *J*=4.0, 13.6 Hz, H-1a), 1.04 (1H, dd, *J*=2.0, 12.8 Hz, H-5), 1.13 (3H, s, H-17), 1.16 (1H, td, *J*=4.4, 14.4 Hz, H-3a), 1.32 (1H, ddd, *J*=11.2, 12.8, 12.8 Hz, H-6b), 1.42 (1H, m, H-3b), 1.44 (1H, m, H-2a), 1.46, (1H, m, H-9), 1.48 (1H, m, H-1b), 1.59 (3H, s, H-16), 1.61 (3H, d, *J*=6.8 Hz, H-15), 1.66 (1H, m, H-11a), 1.67 (1H, m, H-2b), 1.80 (1H, m, H-11b), 1.89 (1H, ddd, *J*=2.0, 4.8, 12.8 Hz, H6-a), 3.68

(1H, dd, *J*=4.8, 11.2 Hz, H-7), 4.27 (1H, dd, *J*=6.4, 9.2 Hz, H-12), 5.58 (1H, q, *J*=6.8 Hz, H-14) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 12.6 (C-16), 13.2 (C-15), 16.0 (C-20), 18.6 (C-2), 19.7 (C-17), 21.2 (C-19), 28.2 (C-11), 29.2 (C-6), 33.3 (C-4), 33.6 (C-18), 36.0 (C-10), 40.0 (C-1), 42.4 (C-3), 56.0 (C-5), 59.3 (C-9), 79.5 (C-7), 83.6 (C-12), 84.5 (C-8), 119.0 (C-14), 136.4 (C-13) ppm.

HRMS (ESI-TOF) *m/z* 329.2459 $[M+Na]^+$ calcd for $C_{20}H_{34}O_2Na$ 329.2457.

3.2.6 Ozonolysis of 182

To a solution of **182** (10.6 mg, 0.03 mmol) in 5% H_2O in acetone (20 ml) at -78^{o}C O_3 stream was bubbled for 3 hours. Then the solution was bubbled with N_2 for 30 min, diluted with water and extracted with dichloromethane. The organic phase was washed successively with saturated $NaHCO_3$ (10 ml) and water. Then organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation to give a mixture of ketone **184a** and lactone **185** as colorless viscous oil (8.0 mg).

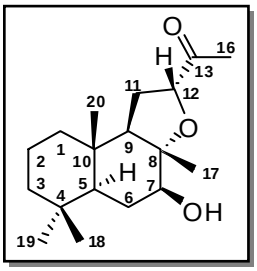
3.2.7 Ozonolysis of 183

To a solution of **183** (13.7 mg, 0.04 mmol) in 5% H_2O in acetone (20 ml) at -78^{o}C O_3 stream was bubbled for 3 hours. Then the solution was bubbled with N_2 for 30 min, diluted with water and extracted with dichloromethane. The organic phase was washed successively with saturated $NaHCO_3$ (10 ml) and water. Then organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation to give a mixture of ketone **184b** and lactone **185** as colorless viscous oil (10.1 mg).

3.2.8 Ozonolysis of a mixture of 182 and 183

To a solution of a 3:1 mixture of **182** and **183** (50.3 mg, 0.16 mmol) in 5% H_2O in acetone (20 ml) at -78^{o}C O_3 stream was bubbled for 3 hours. Then the solution was bubbled with N_2 for 30 min, diluted with water and extracted with dichloromethane. The organic phase was washed successively with saturated $NaHCO_3$ (10 ml) and water. Then organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation to give a mixture of ketones **184a** and **184b**, and

lactone **185** as colorless viscous oil. Isolation of ketones **184a** and **184b**, lactone **185** on silica gel column chromatography eluting with EtOAc-Hexane (4:6) led to white solid of **185** in 19%, colorless oil of **184a** in 15% and colorless oil of **184b** in 14%.



Compound 184a

Compound **184a** was obtain as colorless viscous oil

R_f = 0.16 (EtOAc-hexane, 4:6).

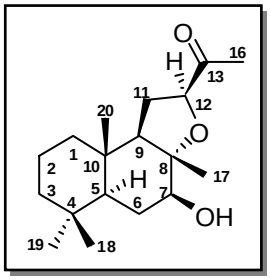
Optical Rotation: $[\alpha]_D^{20}$ = -14.7^o (*c* = 0.38, $CHCl_3$).

IR (KBr): ν_{max} 3432, 2923, 1715, 1044 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (3H, s, H-20), 0.84 (3H, s, H-19), 0.89 (3H, s, H-18), 0.95 (1H, dd, *J*=2.0, 11.2 Hz, H1a), 1.04 (1H, dd, *J*=2.0, 12.8 Hz, H-5), 1.15 (1H, m, H-3a), 1.17 (3H, s, H-17), 1.25 (1H, dd, *J*=7.6, 12.4 Hz, H-9), 1.34 (1H, ddd, *J*=11.2, 12.8, 12.8 Hz, H-6b), 1.43 (1H, m, H-3b), 1.43 (1H, m, H-2a), 1.44 (1H, m, H-1b), 1.65 (1H, m, H-2b), 1.91 (1H, m, H-11a), 1.93 (1H, ddd, *J*=2.0, 3.6, 12.8 Hz, H-6a), 2.11 (1H, ddd, *J*=10.4, 11.6, 12.4 Hz, H-11b), 2.21 (3H, s, H-16), 3.76 (1H, dd, *J*=3.6, 11.2 Hz, H-7), 4.42 (1H, dd, *J*=2.0, 10.4 Hz, H-12) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 15.1 (C-20), 16.3 (C-17), 18.4 (C-2), 21.2 (C-19), 26.7 (C-11), 26.9 (C-16), 29.1 (C-6), 33.2 (C-4), 33.6 (C-18), 35.9 (C-10), 39.7 (C-1), 42.2 (C-3), 56.0 (C-5), 57.7 (C-9), 78.7 (C-7), 81.3 (C-12), 86.2 (C-8), 210.4 (C-13) ppm.

HRMS (ESI-TOF) *m/z* 317.2089 $[M+H]^+$ calcd for $C_{16}H_{27}O_3$ 317.2087.



Compound 184b

Compound **184b** was obtain as colorless viscous oil

$\mathbf{R_f}$ = 0.16 (EtOAc-hexane, 4:6).

Optical Rotation: $[\alpha]_D^{20}$ = +4.0^o (*c* = 0.28, $CHCl_3$).

IR (KBr): ν_{max} 3425, 2927, 1715, 1047 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83 (3H, s, H-20), 0.83 (3H, s, H-19), 0.89 (3H, s, H-18), 1.00 (1H, dt, *J*=3.2, 9.2 Hz, H-1a), 1.05 (1H, dd, *J*=2.4, 12.8 Hz, H-5), 1.09 (3H, s, H-17), 1.17 (1H, dt, *J*=4.8, 13.2 Hz, H-3a), 1.33 (1H, m, H-6b), 1.43 (1H,m, H-3b), 1.46 (2H, m, H-2), 1.46 (1H, m, H-9), 1.50 (1H, m, H-1b), 1.92 (1H, m, H-11b), 1.92 (1H, m, H-6a), 2.09 (1H, ddd, *J*=6.4, 8.0, 14.0 Hz, H-11a), 2.25 (3H, s, H-16), 3.71 (1H, dd, *J*=4.8, 11.2 Hz, H-7), 4.33 (1H, dd, *J*=8.0, 8.8 Hz, H-12) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5 (C-20), 17.8 (C-17), 18.4 (C-2), 21.2 (C-19), 25.9 (C-11), 27.0 (C-16), 29.3 (C-6), 33.2 (C-4), 33.6 (C-18), 36.0 (C-10), 39.7 (C-1), 42.2 (C-3), 55.9 (C-5), 59.9 (C-9), 78.9 (C-7), 82.9 (C-12), 85.9 (C-8), 210.9 (C-13) ppm.

HRMS (ESI-TOF) *m/z* 317.2096 $[M+H]^+$ calcd for $C_{16}H_{27}O_3$ 317.2087.

3.2.9 Preparation of 185

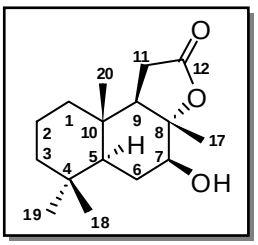
3.2.9.1 *Via* ozonolysis of a mixture of 182 and 183

To a solution of the mixture of **182** and **183** (351.8 mg, 1.15 mmol) in 5% H_2O in acetone (50 ml) at -78^{o}C O_3 stream was bubbled for 3 hours. Then the solution was bubbled with N_2 for 30 min, diluted with water and extracted with dichloromethane. The organic phase was washed successively with saturated $NaHCO_3$ (10 ml) and water. Then organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation to give colorless viscous oil (285 mg).

The viscous oil was exposed to air at ambient temperature for 14 days and it was crystallized itself to **185**. After recrystallization of **185** from acetone, colorless crystals of **185** were obtained in 90% yield.

3.2.9.2 *Via* ozonolysis of (–)-nidorellol

To a solution of **181** (50.1 mg, 0.16 mmol) in 5% H_2O in acetone (25 ml) at -78^oC O_3 stream was bubbled for 3 hours. Then the solution was bubbled with N_2 for 30 min, diluted with water and extracted with dichloromethane. The organic phase was washed successively with saturated $NaHCO_3$ (10 ml) and water. Then organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation to give colorless viscous oil (46.3 mg). The viscous oil was exposed to air at ambient temperature for 3 weeks and it was found as white solid in viscous oil. Isolation on silica gel column chromatography eluting with EtOAc-Hexane (3:7) led to white solids of **185** in 47%.



Compound 185

mp. 144-146°C

R_f = 0.19 (EtOAc-hexane, 3:7).

Optical Rotation: $[\alpha]_D^{20}$ = -25.0^o (*c* = 0.30, $CHCl_3$).

IR (KBr): ν_{max} 3408, 2953, 1781, 1133, 1053 cm^{-1}.

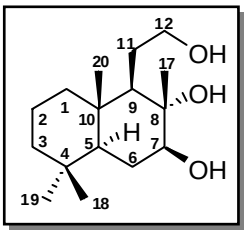
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (3H, s, H-19), 0.89 (3H, s, H-18), 0.91 (3H, s, H-20), 0.99 (1H, dt, *J*=3.6, 13.2 Hz, H-1a), 1.11 (1H, dd, *J*=2.8, 13.2 Hz, H-5), 1.17 (1H, dt, *J*=4.4, 14.0 Hz, H-3a), 1.32 (3H, s, H-17), 1.37 (1H, ddd, *J*=10.8, 13.2, 13.6 Hz, H-6b), 1.42 (1H, m, H-1b), 1.46 (1H, m, H-3b), 1.47 (1H, m, H-2a), 1.66 (1H, m, H-2b), 1.82 (1H, dd, *J*=6.8, 14.8 Hz, H-9), 2.01 (1H, ddd, *J*=2.8, 4.8, 13.6 Hz, H-6a), 2.26, (1H, dd, *J*=6.8, 16.0 Hz, H-11a), 2.48 (1H, dt, *J*=14.8, 16.0 Hz, H-11b), 3.88 (1H, dd, *J*=4.8, 10.8 Hz, H-7) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 15.2 (C-20), 16.5 (C-17), 18.2 (C-2), 21.0 (C-19), 28.4(C-11), 29.0 (C-6), 33.2 (C-4), 33.3 (C-18), 35.6 (C-10), 39.3 (C-1), 42.0 (C-3), 55.4 (C-5), 57.0 (C-9), 76.8 (C-7), 89.7 (C-8), 176.3 (C-12) ppm.

HRMS (ESI-TOF) *m/z* 267.1952 $[M+H]^+$ calcd for $C_{16}H_{27}O_3$ 267.1955.

3.2.10 Reduction of 185

To a suspension of $LiAlH_4$ (449.5 mg) in dried THF (10 ml) a solution of 7-hydroxy sclareolide **185** (262.9 mg, 0.99 mmol) in dried THF (10 ml) was solely added at 0^{o}C. The reaction mixture was stirred for 5 hours under argon at room temperature. To the reaction mixture an aqueous solution of 5% HCl (15 ml) was added and extracted with Et_2O (10 ml x 4). The organic layer was washed with brine and then dried over anhydrous Na_2SO_4. Removal of the solvent afforded a residue as white solid. The residue was crystallized from water/acetone to afford white solid of **186** in 91% yield.



Compound 186

mp. 210-211°C

R$_f$ = 0.24 (EtOAc).

Optical Rotation: $[\alpha]_D^{20}$ = +1.6^o (*c* = 0.31, MeOH).

IR (KBr): ν_{max} 3295, 2947, 1386, 1097, 1050,1014 cm^{-1}.

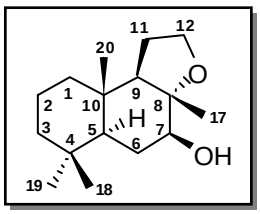
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.73 (3H, s, H-20), 0.74 (3H, s, H-19), 0.83 (1H, m, H-1a), 0.83 (3H, s, H-18), 0.97 (1H, dd, *J*=1.6, 12.4 Hz, H-5), 1.06 (3H, s, H-17), 1.08 (1H, m, H-3a), 1.12 (1H, t, *J*=3.6 Hz, H-9), 1.25 (1H, ddd, *J*=11.6, 12.4, 12.8 Hz, H-6a), 1.33 (1H, m, H-3b), 1.37 (1H, m, H-2a), 1.53 (1H, m, H-11a), 1.53 (1H, m, H-2b), 1.55 (1H, m, H-1b), 1.59 (1H, m, H-11b), 1.77 (1H, ddd, *J*=1.6, 4.4, 12.8 Hz, H-6b), 3.35 (1H, dt, *J*=3.6, 10.8 Hz, H-12b), 3.44 (1H, dd, *J*=4.4, 11.6 Hz, H-7), 3.67 (1H, td, *J*=4.4, 10.8 Hz, H-12a) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 15.4 (C-20), 17.9 (C-17), 18.3 (C-2), 21.5 (C-19), 27.1 (C-11), 27.9 (C-6), 33.2 (C-4), 33.3 (C-18), 39.1 (C-10), 39.2 (C-1), 41.7 (C-3), 53.8 (C-5), 57.4 (C-9), 63.7 (C-12), 76.6 (C-8), 80.3 (C-7) ppm.

HRMS (ESI-TOF) *m/z* 293.2094 $[M+Na]^+$ calcd for $C_{16}H_{30}O_3Na$ 293.2087.

3.2.11 Cyclization of 186

Triol **186** (8.6 mg, 0.032 mmol) was stirred with pyridine (0.8 ml) at room temperature for 20 min. To this solution TsCl (18.2 mg) was added and the mixture was continually stirred for 3 hours under argon. The reaction mixture was diluted with water and extracted with Et_2O (10 ml x 4). The organic phase was washed successively with 2M HCl (10 ml x 2), saturated $NaHCO_3$ (10 ml x 2) and water. Removal of the solvent afforded a crude product as white solid. Purification on silica gel column chromatography eluting with CH_2Cl_2-EtOAc (1:1) led to colorless needles of 7-hydroxy-ambrox **187** in 90% yield.



Compound 187

mp. 136-138°C

R_f = 0.33 (EtOAc-hexane1:1).

Optical Rotation: $[\alpha]_D^{20}$ = +31.7° (*c* = 0.34, $CHCl_3$).

IR (KBr): ν_{max} 3425, 2917, 1383, 1034, 1001 cm^{-1}.

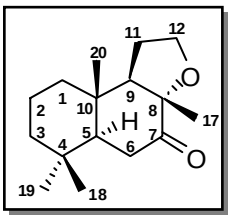
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83 (3H, s, H-19), 0.84 (3H, s, H-20), 0.89 (3H, s, H-18), 0.98 (1H, dd, *J*=3.6, 13.2 Hz, H-1a), 1.04 (1H, dd, *J*=2.8, 12.8 Hz, H-5), 1.09 (3H, s, H-17), 1.16 (1H, dt, *J*=4.4, 13.6 Hz, H-3a), 1.30 (1H, m, H-9), 1.32 (1H, dd, *J*=11.2, 12.8, 12.8 Hz, H-6a), 1.43 (1H, m, H-3b), 1.43 (1H, m, H-2a), 1.48 (1H, m, H-1b), 1.65 (1H, m, H-2b), 1.77 (1H, m, H-11a), 1.79 (1H, m, H-11b), 1.89 (1H, ddd, *J*=2.8, 4.8, 12.8 Hz, H-6b), 3.64 (1H, dd, *J*=4.8, 11.2 Hz, H-7), 3.82 (1H, dt, *J*=8.8, 8.8 Hz, H-12a), 3.93 (1H, dt, *J*=3.6, 8.8Hz, H-12b) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 15.2 (C-20), 15.9 (C-17), 18.5 (C-2), 21.3 (C-19), 22.5 (C-11), 29.1 (C-6), 33.2 (C-4), 33.7 (C-18), 35.9 (C-10), 39.8 (C-1), 42.3 (C-3), 56.1 (C-5), 58.6 (C-9), 65.5 (C-12), 78.9 (C-7), 83.8 (C-8) ppm.

HRMS (ESI-TOF) *m/z* 253.2154 $[M+H]^+$ calcd for $C_{16}H_{29}O_2$ 253.2162.

3.2.12 Oxidation of 187

To a solution of 7-hydroxy-ambrox **187** (28.3 mg, 0.22 mmol) in dried CH_2Cl_2 (5 ml) PCC (169.2 mg) was added and continually stirred for 4 hours under argon. The mixture was diluted with CH_2Cl_2 (5 ml), filtered through silica gel and flushed with Et_2O (5 ml x 2). The filtrate was evaporated under reduced pressure and colorless needles of 7-oxo-ambrox **188** were obtained in 98% yield.



Compound 188

mp. 130-132°C

R_f = 0.42 (EtOAc-hexane, 1:1).

Optical Rotation: $[\alpha]_D^{20}$ = +112.9° (*c* = 0.35, $CHCl_3$).

IR (KBr): ν_{max} 2923, 1721, 1070, 1007 cm^{-1}.

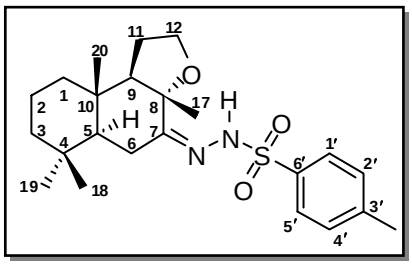
^{1}H NMR (400 MHz, $CDCl_3$ and 2 drops of MeOD): δ 0.86 (3H, s, H-18), 0.87 (3H, s, H-19), 1.05 (3H, s, H-20), 1.06 (1H, m, H-1a), 1.18 (1H, dt, *J*=4.4, 13.2 Hz, H-3a), 1.32 (3H, s, H-17), 1.35 (1H, dd, *J*=2.8, 14.0 Hz, H-5), 1.47 (1H, m, H-3b), 1.50 (1, m, H-2a), 1.60 (1H, d, *J*=13.2 Hz, H-1b), 1.70 (1H, m, H-2b), 1.70 (1H, dd, *J*=6.0, 12.8 Hz, H-9), 1.88 (2H, m, H-11), 2.39 (1H, dd, *J*=2.8, 13.6 Hz, H-6a), 2.51 (1H, dd, *J*=13.6, 14.0 Hz, H-6b), 3.88 (1H, dt, *J*=8.8, 8.8 Hz, H-12a), 3.98 (1H, dt, *J*=3.2, 8.8 Hz, H-12b) ppm.

^{13}C NMR (100 MHz, $CDCl_3$ and 2 drops of MeOD): δ 14.7 (C-20), 18.3 (C-2), 20.2 (C-17), 20.8 (C-19), 22.1 (C-11), 33.2 (C-18), 33.9 (C-4), 36.2 (C-10), 37.2 (C-6), 39.6 (C-1), 41.9 (C-3), 59.3 (C-5), 60.8 (C-9), 65.4 (C-12), 86.2 (C-8), 209.4 (C-7) ppm.

HRMS (ESI-TOF) *m/z* 251.2005 $[M+H]^+$ calcd for $C_{16}H_{27}O_2$ 251.2006.

3.2.13 Treatment of 188 with tosylhydrazine

To a solution of 7-oxo-ambrox **188** (26.5 mg, 0.11 mmol) in dried THF (5 ml) $TsNHNH_2$ (59.1 mg) was added and refluxed for 5 hours. The reaction mixture was cooled to room temperature and evaporated under reduced pressure to give a residue as white solid. Purification on silica gel column chromatography afforded **189** as colorless needles in 91% yield.



Compound 189

mp. 190-192°C

R_f = 0.40 (EtOAc-hexane, 3:7).

Optical Rotation: $[\alpha]_D^{20}$ = +62.0° (*c* = 0.30, $CHCl_3$).

IR (KBr): ν_{max} 3169 (NH), 3060, 2927, 1329, 1160, 1017 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80 (3H, s, H-19), 0.84 (3H, s, H-18), 0.87 (3H,s, H-20), 0.90 (1H, dt, *J*=3.2, 13.2 Hz, H-1a), 0.97 (1H, dd, *J*=2.0, 14.0 Hz, H-5), 1.10 (1H, dt, *J*=3.6, 14.8 Hz, H-3a), 1.16 (3H, s, H-17), 1.42 (1H, m, H-3b), 1.44 (1H, m, H-2a), 1.45 (1H, m, H-9), 1.48 (1H, m, H-1b), 1.64 (1H, m, H-2b), 1.72 (2H, m, H-11), 2.06 (1H, t, *J*=14.0 Hz, H-6a), 2.42 (3H, s, 4′-CH_3), 2.42 (1H, dd, *J*=2.0, 14.0 Hz, H-6b), 3.86 (1H, dt, *J*=8.0 Hz, H-12a), 3.96 (1H, dt, *J*=5.6, 8.0 Hz, H-12b), 7.28 (2H, d, *J*=8.0 Hz, H-3′,H-5′), 7.88 (2H, d, *J*=8.0 Hz, H-2′, H-6′) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.5(C-20), 18.3 (C-2), 20.1 (C-17), 20.8 (C-19), 21.1 (C-11), 21.7 (4′-CH_3), 31.0 (C-6), 33.3 (C-18), 33.3 (C-4), 35.6 (C-10), 39.4 (C-1), 42.1 (C-3), 57.5 (C-5), 60.5 (C-9), 66.1 (C-12), 85.7 (C-8), 127.9 (C-2′ and C-6′), 129.4 (C-3′ and C-5′), 136.5 (C-4′), 143.3 (C-1′), 158.0 (C-7) ppm.

HRMS (ESI-TOF) *m/z* 441.2183 $[M+Na]^+$ calcd for $C_{23}H_{34}N_2O_3SNa$ 441.2182.

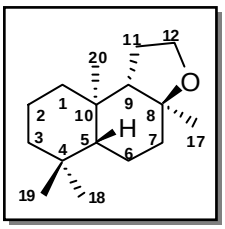
3.2.14 Preparation of 2

3.2.14.1 *Via* reduction of compound 189

To a solution of **189** (31.9 mg, 0.07 mmol) in dried THF (5 ml) $NaBH_4$ (57.6 mg) was added and refluxed overnight. After cooling to room temperature the mixture was diluted with Et_2O. The solution was then washed with water, aqueous $NaHCO_3$ (10ml), 2M HCl (10 ml) and water respectively. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give a residue as white solid. Purification on silica gel column chromatography afforded (+)-ambrox (**2**) as colorless needles in 33% yield.

3.2.14.2 *Via* Wolff-Kishner reduction of compound 188

To a solution of compound **188** (30.1 mg, 0.12 mmol) in diethylene glycol (3 ml) KOH (41.1 mg) and 98%, hydrazine hydrate (0.1 ml) were added and refluxed for 1 hour at 160°C. Then the mixture was refluxed at 200°C for 3 hours. After cooling room temperature, the mixture was diluted with water, neutralized with 2M HCl and extracted with EtOAc (10 ml x 4). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give a residue as viscous oil. Purification on silica gel column chromatography afforded (+)-ambrox (**2**) as colorless needles in 74% yield.



Compound 2

mp. 72-74°C

R_f = 0.59 (EtOAc-hexane, 3:7).

Optical Rotation: $[\alpha]_D^{20}$ = +23.0° (*c* = 0.25, $CHCl_3$)

IR (KBr): ν_{max} 2920, 1456, 1376, 1004 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83 (3H, s, H-19), 0.84, (3H, s, H-20), 0.88 (3H, s, H-18), 0.96 (1H, dd, *J*=2.0, 12.4 Hz, H-5), 1.03 (1H, dt, *J*=2.0, 12.8 Hz, H-1b), 1.09 (3H, s, H-17), 1.18 (1H, dt, *J*=4.4, 14.0 Hz, H-3a), 1.40 (1H, m, H-7b), 1.40 (1H, m,

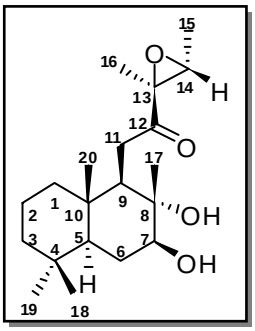
H-9), 1.41 (1H, m, H-3a), 1.42 (1H, m, H-2b), 1.45 (1H, m, H-1a), 1.65 (1H, m, H-2a), 1.74 (2H, m, H-11), 1.75 (2H, m, H-6), 1.94 (1H, td, *J*=3.2, 11.2 Hz, H-7a), 3.83 (1H, dt, *J*=8.4 Hz, H-12b), 3.92 (1H, dt, *J*=4.8, 8.4 Hz, H-12a) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 15.1 (C-20), 18.4 (C-2), 20.6 (C-6), 21.1 (C-17), 21.1 (C-19), 22.6 (C-11), 33.1 (C-4), 33.6 (C-18), 36.2 (C-10), 39.7 (C-7), 39.9 (C-1), 42.4 (C-3), 57.2 (C-5), 60.1 (C-9), 65.0 (C-12), 79.4 (C-8) ppm.

HRMS (ESI-TOF) *m/z* 259.2045 $[M+Na]^+$ calcd for $C_{16}H_{28}ONa$ 259.2032.

3.2.15 Air-auto oxidation of 183

Compound **183** was decomposed by exposure to air at ambient temperature for 5 months. A mixture of decomposed products (8.2 mg) was isolated by preparative silica gel TLC with 3 times development using 50% EtOAc in hexane as mobile phase. Seven fraction bands on the TLC were scratched off. Silica gel of each collected fraction was suspended in CH_2Cl_2, filtered through filter paper (Whatman No.1) and flushed with CH_2Cl_2 (10ml x 3). The filtrate was evaporated under reduced pressure. From the collected fractions F-3 (R_f = 0.31-0.37) and F-6 (R_f = 0.11-0.15) compounds **185** and **190a** were afford in 29% as white solid and 18% as colorless viscous oil, respectively. The collected fraction F-5 (R_f = 0.15-0.21) was further purified by reverse phase HPLC using 30% H_2O in MeOH to 100% MeOH to give **190b** as colorless viscous oil in 7% yield.



Compoud 190a

R_f = 0.51 (EtOAc).

Optical Rotation: $[\alpha]_D^{20}$ = +8.0^o (*c* = 0.14, $CHCl_3$)

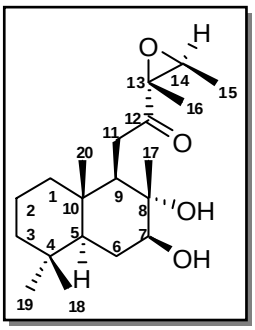
IR (film): ν_{max} 3421, 2924, 1706, 1063 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79 (3H, s, H-20), 0.79 (3H, s, H-18), 0.84 (1H, m, H-1a), 0.89 (3H, s, H-19), 1.05 (3H, s, H-17), 1.11 (1H, m, H-5), 1.16 (1H, m, H-3b),

1.30 (1H, m, H-6a), 1.36 (1H, m, H-1b), 1.38 (3H, d, *J*=5.2 Hz, H-15), 1.39 (1H, m, H-3b), 1.43 (1H, m, H-2a), 1.44 (3H, s, H-16), 1.58 (1H, m, H-2b), 1.89 (1H, m, H-6b), 1.90 (1H, dd, *J*=4.4, 5.6 Hz, H-9), 2.37 (1H, dd, *J*=5.6, 17.6 Hz, H-11a), 2.50 (1H, dd, *J*=4.4, 17.6 Hz, H-11b), 3.24 (1H, q, *J*=5.2 Hz, H-14), 3.52 (1H, dd, *J*=3.6, 11.2 Hz, H-7) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.0 (C-16), 13.9 (C-15), 15.9 (C-20), 17.2 (C-17), 18.5 (C-2), 21.6 (C-19), 28.1 (C-6), 30.3 (C-11), 33.4 (C-4), 33.5 (C-18), 38.85 (C-10), 39.2 (C-1), 41.8 (C-3), 53.7 (C-5), 54.2 (C-9), 57.3 (C-14), 64.1 (C-13), 77.4 (C-8), 81.3 (C-7), 212.2 (C-12) ppm.

HRMS (ESI-TOF) *m/z* 361.2337 $[M+Na]^+$ calcd for $C_{20}H_{34}O_4Na$ 361.2349.



Compound 190b

R_f = 0.56 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79 (3H, s, H-20), 0.80 (3H, s, H-19), 0.86 (1H, m, H-1a), 0.89 (3H, s, H-18), 1.10 (3H, s, H-17), 1.12 (1H, dd, *J*=4.4, 14.0 Hz, H-5), 1.14 (1H, m, H-3a), 1.31 (1H, m, H-6a), 1.34 (1H, m, H-1b), 1.38 (3H, d, *J*=5.2 Hz, H-15), 1.39 (1H, m, H-3b), 1.44 (1H, m, H-2a), 1.45 (3H, s, H-16), 1.57 (1H, m, H-2b), 1.84 (1H, dd, *J*=3.2, 5.2 Hz, H-9), 1.89 (1H, m, H6b), 2.24 (1H, *J*=3.2, 17.2 Hz, H-11a), 2.57 (1H, dd, *J*=5.2, 17.2 Hz, H-11b), 3.14 (1H, q, *J*=5.2 Hz, H-14), 3.50 (1H, dd, *J*=4.0, 10.8 Hz, H-7) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.2 (C-16), 13.8 (C-15), 16.1 (C-20), 17.2 (C-17), 18.5 (C-2), 21.6 (C-19), 27.9 (C-6), 30.5 (C-11), 33.4 (C-4), 33.5 (C-18), 33.9 (C-10), 39.5 (C-1), 41.7 (C-3), 53.7 (C-5), 54.8 (C-9), 57.2 (C-14), 64.1 (C-13), 77.4 (C-8), 81.0 (C-7), 211.9 (C-12) ppm.

3.3 Fixative property determination

(–)-Nidorellol, (+)-ambrox and its derivatives **182**, **183**, **185**, **187** and **188**, were determined their fixative property compared with standard (–)-ambrox. Testing solutions were prepared using a procedure as described by Seldner and Princeton (Seldner and Princeton, 1981). Each testing solution in EtOH consisted of 5% w/w of *D*-limonene and 1% w/w of testing compound.

3.3.1 Smelling paper strip test (Poucher, 1974)

This method was adopted from a classical method as described by Poucher. The smelling paper strips were cut into 1x10 cm. 20 μL of each testing solution was applied on the paper strip. Each applied-paper strip was smelled by 5 healthy 25-32 years old volunteers every hour until *D*-limonene odor could not be detected. Fixation time of the test was time that 60% of panelists could detect *D*-limonene odor.

3.3.2 Evaporation rate by TGA studies (Iimura *et al*., 2000)

All testing solutions were subjected to a TG-DTA instrument to determine rate of evaporation of each formula. All experiments were operated on aluminum pan with the same condition:

Pan: Alumina
Temperature range: 1°C/ 10 min
Gas: Air zero

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Isolation of nidorellol from *Croton oblongifolius*

(–)-Nidorellol **181** was obtained from stem bark of *Croton oblongifolius* as white solid in 0.42% yield. On basis of spectroscopic data including IR, 1D and 2D NMR and MS, structure of **181** was established. The FT-IR spectra showed signal of hydroxyl (3363 cm^{-1}) and conjugated diene (1632 and 1601 cm^{-1}). The ^{13}C NMR and HSQC spectral data indicated signals of 20 carbons comprising of five methyl carbons (δ_C 33.6, 21.7, 18.0, 15.7 and 12.0 ppm), five sp^3 methylene carbons (δ_C 41.7, 39.9, 27.8, 23.6 and 18.6 ppm), one sp^2 methylene carbon (δ_C 110.7 ppm), three sp^3 methine carbons (δ_C 80.4, 60.3 and 53.7 pmm), two sp^2 methine carbons (δ_C 141.6 and 135.7 ppm), three sp^3 quaternary carbons (δ_C 78.2, 39.4 and 33.4 ppm) and one sp^2 quaternary carbon (δ_C 132.8 ppm). From HMBC spectral data with assistance of ^1H-^1H correlations (COSY), the skeleton of **181** could be established (Figure 4.1a).

The careful analysis of NOESY spectra showed the correlation between H-12 and H-14. This data indicated that the conjugated diene was *E*-configuration. The correlations between H-7 and H-5 and between H-7 and H-9 suggested that these protons were occupied on the same face and they were de-noted as α proton. The correlation between H-17 of methyl proton and H-20 of methyl proton suggested that these methyl groups were occupied on the same face. The no observed correlations between these two methyl groups and the α protons (H-5, H-7 and H-9) indicated that both methyl groups and the α protons were situated on the opposite face (Figure 4.1b).

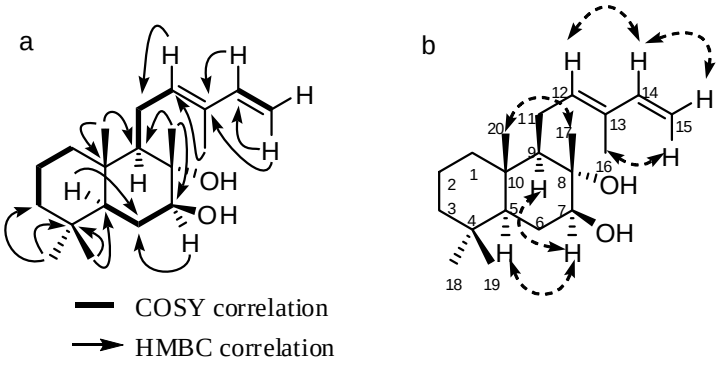
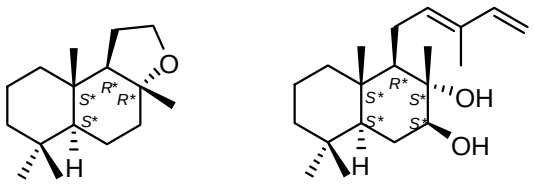


Figure 4.1 (a) COSY and HMBC correlation of (–)-nidorellol (b) Crucial NOESY correlation of (–)-nidorellol

Since specific rotation of **181** was $[\alpha]_D^{20}$ = -23.2° (lit. $[\alpha]_D^{24}$ = -21.0°) (Bohlmann and Fritz, 1978), its stereo correlation (Figure 4.2) was (–)-nidorellol as reported by Bohlmann and Fritz (Bohlmann and Fritz, 1978) which is a suitable synthon for synthesis of (–)-ambrox.

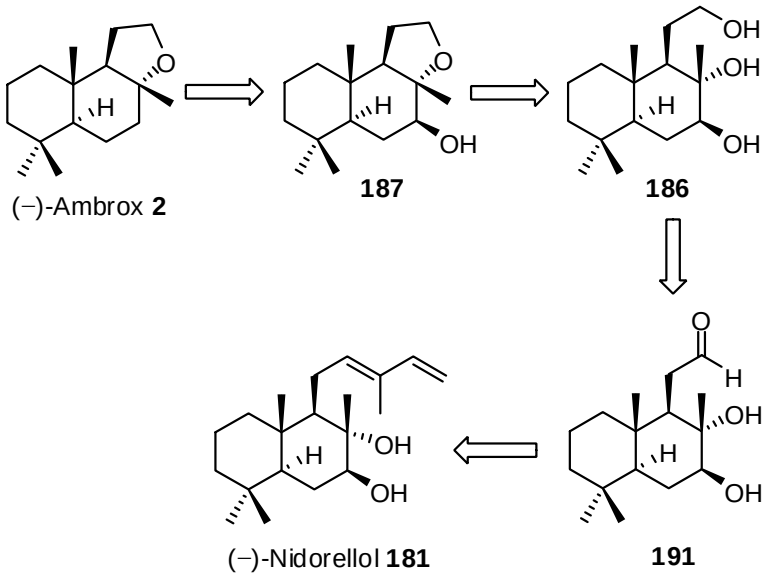


(–)-Ambrox **2** **181**

Figure 4.2 Structure of (–)-ambrox and (–)-nidorellol

4.2 Synthetic approach to ambrox

The retrosynthetic strategy for ambrox was performed as Scheme 4.1 which consisted of oxidative cleavage of conjugated diene, reduction of aldehyde, cyclization and dehydroxylation.



Scheme 4.1 Retrosynthesis of (–)-ambrox

Unfortunately, (–)-nidorellol was unstable. It was decomposed to many compounds at ambient temperature. We found that it was easily cyclized in acid condition. Monitoring a solution of (–)-nidorellol in $CDCl_3$ by ^1H NMR experiment. The decreasing H-7 signal of (–)-nidorellol was observed while H-7 signals of cyclization products were increased and the ratio of cyclization products and (–)-nidorellol was 2.9:1.6 at 2 days (see Figure 4.3).

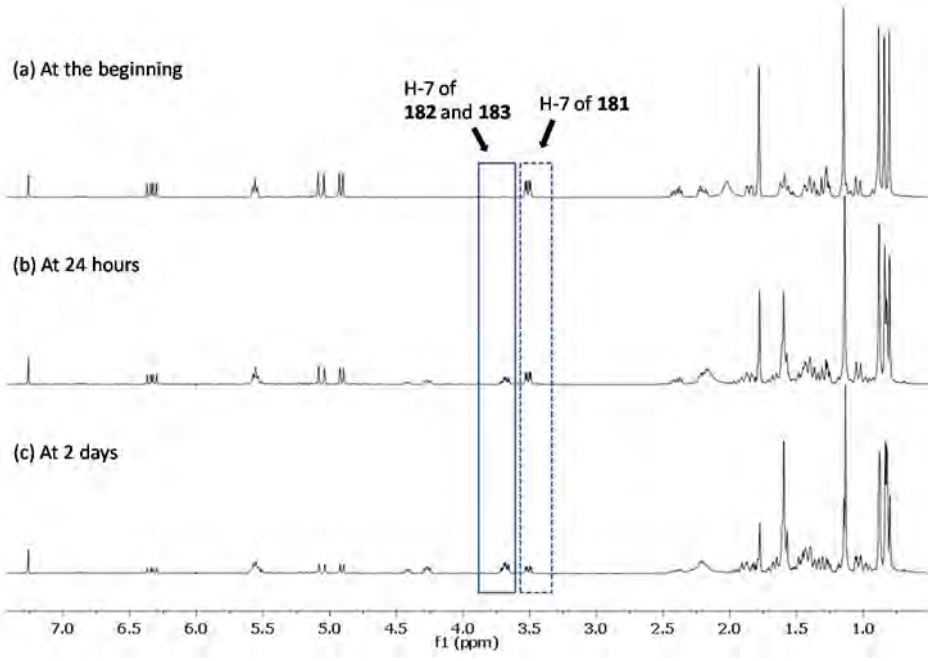
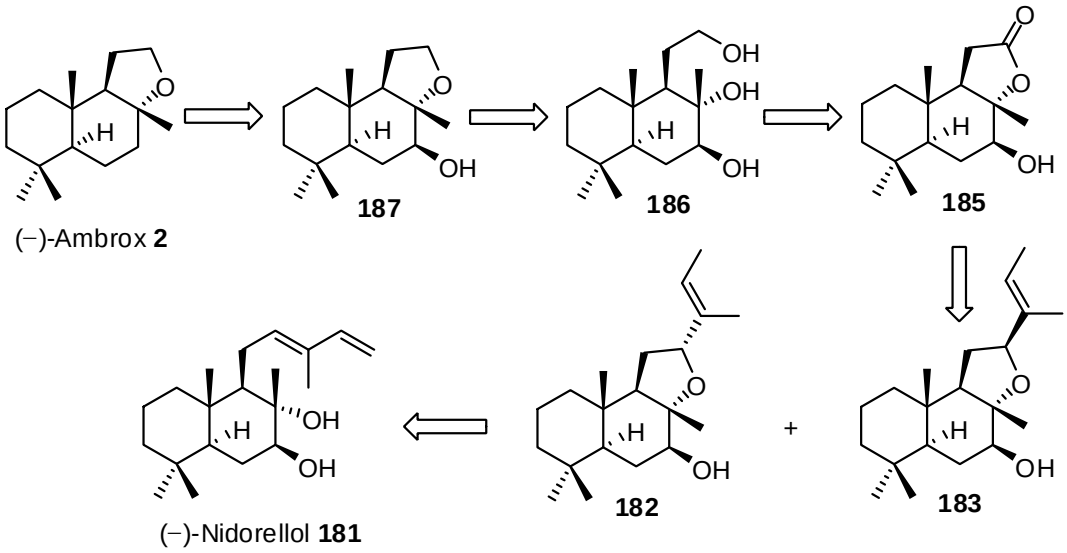


Figure 4.3 The comparative ^1H NMR spectra of (–)-nidorellol in $CDCl_3$ (a) at the beginning (b) at 24 hours (c) at 2 days.

Due to this phenomena the first step of ambrox synthesis was changed to cyclization of (–)-nidorellol then followed by oxidative cleavage, reduction, cyclization and dehydroxylation (Scheme 4.2).



Scheme 4.2 Plan for synthesis of (–)-ambrox

4.3 Effect of *p*-TsOH·H_2O on acid-promoted cyclization of (–)-nidorellol (181)

Effect of *p*-TsOH·H_2O on acid-promoted cyclization of **181** was studies in CH_2Cl_2 at various amounts of *p*-TsOH·H_2O. The results were shown in Table 4.1. TLC monitoring of reaction course showed that increasing amount of *p*-TsOH·H_2O could accelerate the cyclization reaction. The ^1H NMR experiment of each crude product showed the similar results. The optimum amount of *p*-TsOH·H_2O was found to be around 20% mol.

Table 4.1 Acid-promoted cyclization of compound **181** at various amounts of *p*-TsOH·H_2O

entry	*p*-TsOH·H_2O (% mol)	complete cyclization time (hours)
1	5	24
2	10	10
3	20	3
4	25	3

4.4 Effect of solvents on cyclization of (–)-nidorellol

The acid-promoted cyclization of **181** with 20% mol of *p*-TsOH·H_2O was carried out in various deuterated solvents to determined suitable solvent. ^{1}H NMR monitoring (Table 4.2) showed that fastest cyclization of **181** was carried out in $CDCl_3$, a moderate polar solvent. In non polar solvent, benzene-d_6, it could be cyclized slower than in chloroform-*d*. Cyclization of **181** in both polar protic (CD_3OD) and polar aprotic ($(CD_3)_2CO$) solvents were also performed the cyclization was very slow. Moreover we found that compound **181** in acetone-d_6 was decomposed at 10 hours and cyclization products was observed at 2 months but the reaction was not completed. In addition, compound **181** was stable in DMSO-d_6 and no cyclization was observed.

In this work, the moderate polar solvent, $CDCl_3$, was an optimum solvent for acid-promoted cyclization of **181**.

Table 4.2 Acid-promoted cyclization of compound **181** at various solvents

entry	Solvent	complete cyclization time
1	$CDCl_3$	6 hours
2	C_6D_6	24 hours
3	CD_3OD	2 months
4	$(CD_3)_2CO$	2 months*
5	DMSO-d_6	no cyclization[#]

*Compound was decomposed at 10 hours without cyclization and cyclization was observed at 2 months but the reaction was not completed.

[#]No cyclization was observed at 2 months.

4.5 Effect of temperature on the ratio of 182 and 183

Acid-promoted cyclization of **181** with 20% mol *p*-TsOH·H_2O in $CDCl_3$ at various temperatures including room temperature (RT), 4°C and -18°C was monitored by ^{1}H NMR. The results (Table 4.3) showed that complete cyclization time of **181** at RT, 4°C and -18°C were 10 hours, 4 days and 32 days, respectively. After complete

cyclization, the ratios of **182**:**183**, 1:0.9 at RT, 1:0.9 at 4°C and 1:1 at -18°C were changed until the ratios of **182**:**183** were constant. At RT, 4°C and-18°C the mixture of **182** and **183** was unchanged after 4 days, 32 days and 46 days, respectively.

Table 4.3 Acid-promoted cyclization of compound **181** in $CDCl_3$ at various temperatures

time	ratio of **182** and **183**[#]		
	room temperature	4°C	-18°C
10 min	1:2*	1:2*	1:2*
10 h	1:0.9**	1:2	1:2
24 h	1:0.5	1:1.8	1:2
2 days	1:0.4	1:1.4	1:1.9
4 days	1:0.3	1:0.9**	1:1.7
8 days	1:0.3	1:0.7	1:1.5
10 days	1:0.3	1:0.6	1:1.5
17 days	1:0.3	1:0.5	1:1.5
21 days	1:0.3	1:0.4	1:1.1
32 days	1:0.3	1:0.3	1:1**
46 days	1:0.3	1:0.3	1:0.6
145 days	1:0.3	1:0.3	1:0.6

[#]Ratios of **182** and **183** were determined by integration of H-12 (δ_H 4.42 for **182** and δ_H 4.27 for **183**) from ^{1}H NMR experiments.

*Ratios of **182** and **183** after 10 minutes at room temperature before stored at different temperature

**Complete cyclization time

Considering structure of **181** in chair-conformation A and B (Figure 4.4) conformation A should reacted faster than B because it has less steric repulsion between methyl group on conjugated diene moiety and hydroxyl group on C-8.

Therefore more amounts of compound **183** were observed at the early period of reaction course. By ^{1}H NMR monitoring we found that compound **182** and **183** could be isomerized in acid condition until the ratio of **182** and **183** was 1:0.3. Considering the conformation structure of compound **182** and **183** suggested that compound **183** has steric effect between alkenyl group on C-12 and methyl group on C-8 while **182** has less steric effect (Figure 4.4). Due to compound **183** was less stable thus it may be easy isomerized by protonation at oxygen atom on furan moiety to open ring then followed by ring closure to be more stable isomer **182** than **183** while compound **182** showed that it was less isomerized to **183**. This was the reason why compound **182** was a major product in the final reaction course.

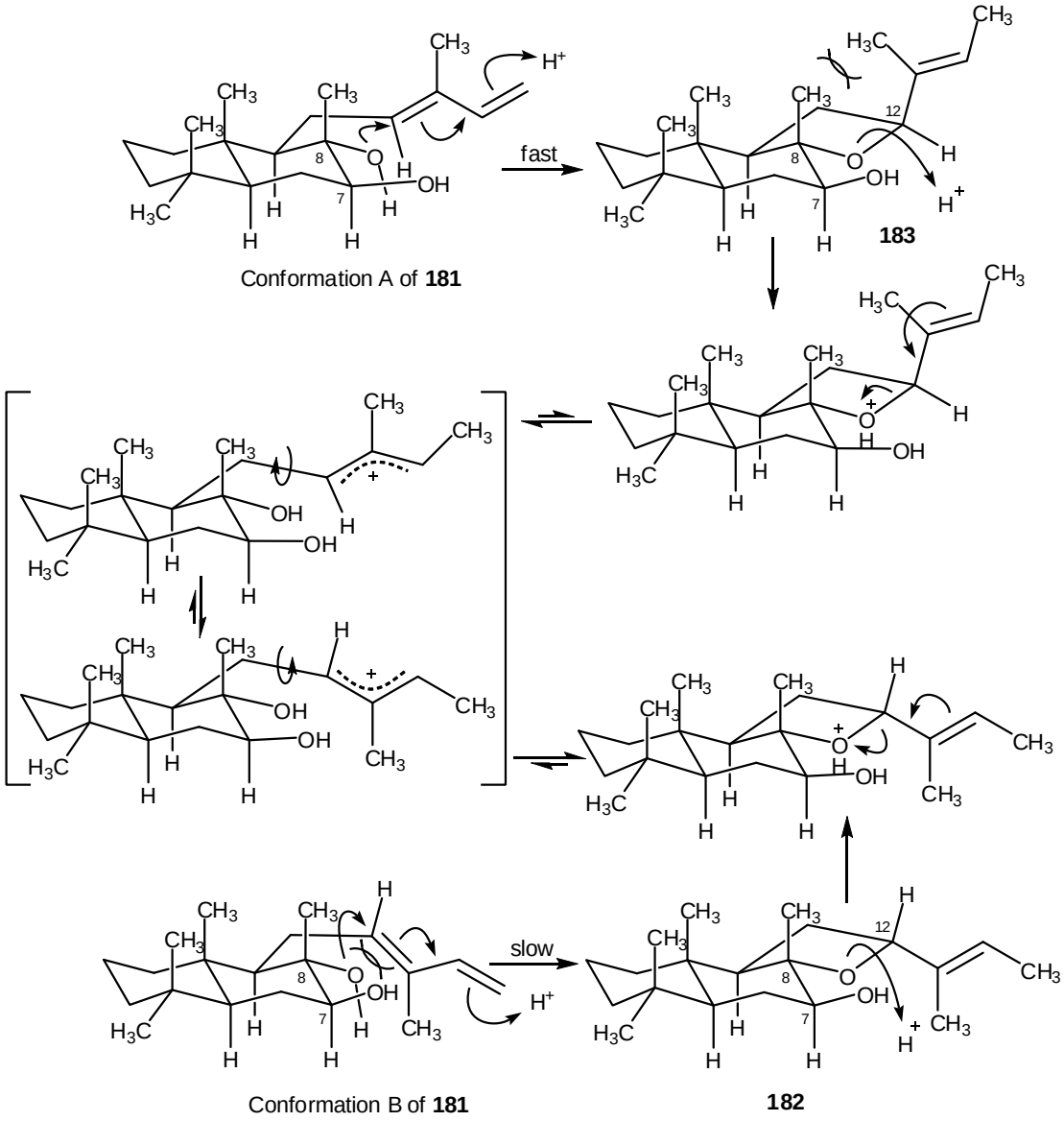
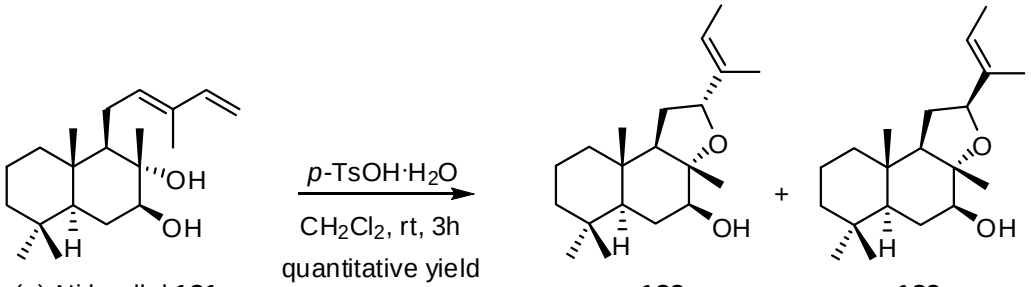


Figure 4.4 Proposed mechanism of acid-promoted cyclization of **181**

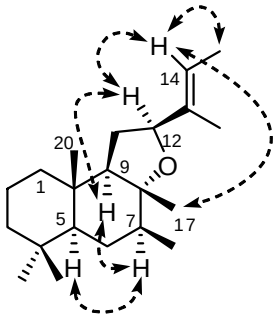
4.6 Cyclization of (–)-nidorellol



(−)-Nidorellol **181** **182** **183**

Scheme 4.3 Acid-promoted cyclization of **181**

Acid-promoted cyclization of **181** with 20% mol *p*-TsOH·H_2O in CH_2Cl_2 for 3 hours at room temperature afforded **182** and its epimers **183** in 3:1 ratio. By ^{1}H NMR data and weight of the products it should be presumed that the mixture of **182** and **183** obtained in quantitative yield. Isolation of **182** and **183** on silica gel column chromatography gave **182** in 14% yield and **183** in 8% yield. Due to poor yield from isolation of **182** and **183**, they may be decomposed during purification. Thus the mixture of **182** and **183** was directly used in the ozonolysis step. The relative configurations of **183** were determined by 1D NOESY experiment. Due to non-observation of between H-12 [δ_H 4.27 (dd, J=6.4, 9.2 Hz)] and the methyl protons of C-17 and between H-12 and the methyl protons of C-20 it was suggested that the methyl protons of C-17 and C-20 were on the opposite face to H-12. The observed NOEs between H-12 and H-14, between H-12 and H-9, between H-9 and H-7and between H-7 and H-5 indicated that these protons were occupied on the same face. Thus relative configuration of **183** was determined and also confirmed by x-ray crystallographic analysis (Figure 4.5 and Figure 4.6). ^{1}H and ^{13}C NMR spectra of **182** indicated that its structure was closely related to **183**. Moreover the observed NOEs of **182** between H-12 [δ_H 4.42 (dd, J=2.0, 9.2 Hz)] and the methyl protons of C-17 in the NOESY experiment suggested that H-12 occupied on the same face of the methyl group on C-8. Thus compound **182** was an epimer of **183**.



183

Figure 4.5 Crucial 1D NOESY correlation of **183**

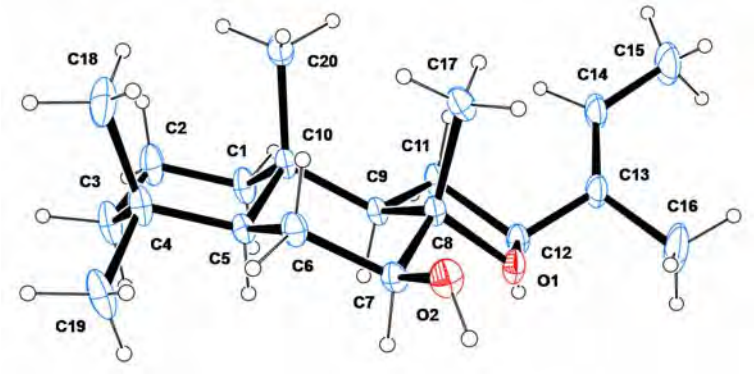


Figure 4.6 The ORTEP drawing of **183**

4.7 Synthesis of compound 185

4.7.1 *Via* ozonolysis of a mixture of compound 182 and 183

Since ^{1}H NMR monitoring of ozonolysis displayed degradation of compound **182** and **183** the products of degradation were isolated by TLC to afford a mixture of corresponding ketone **184a** and **184b** together with lactone **185**. When the reaction mixtures from ozonolysis of **182** (Figure 4.7a) and **183** (Figure 4.7c) exposed to air at ambient temperature ketone **184a** and **184b** could be further transformed to **185** (Figure 4.7b and 4.7d). Due to poor yield from isolation of **182** and **183**, the mixture of **182** and **183** was directly used in the ozonolysis step (Scheme 4.4).

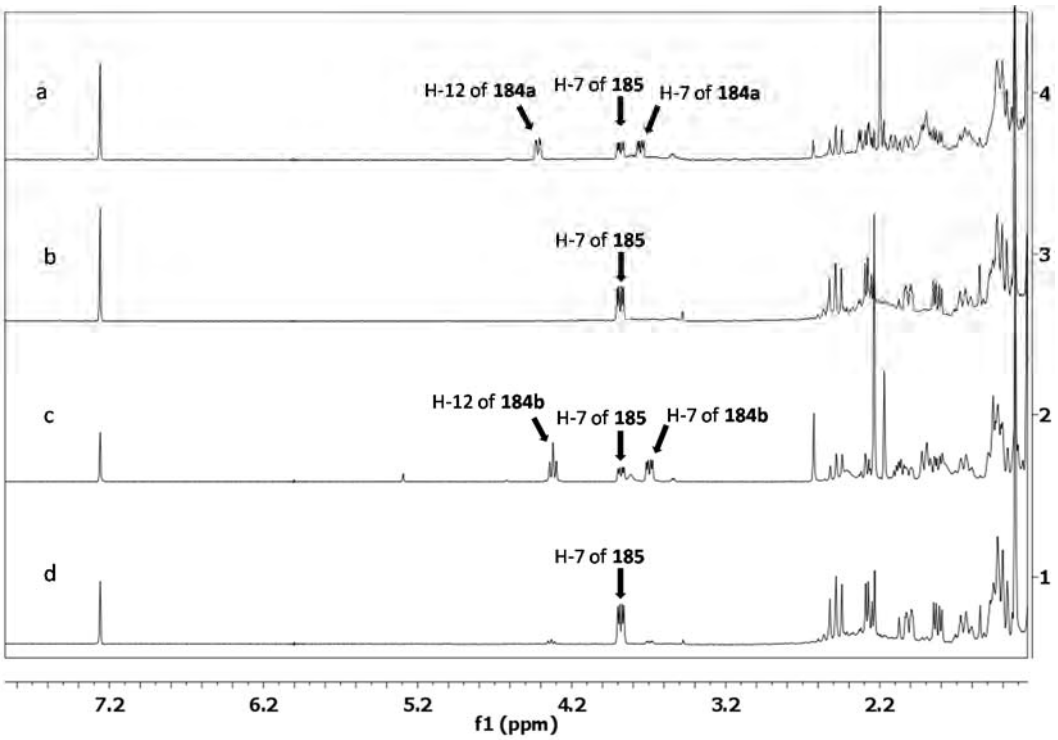
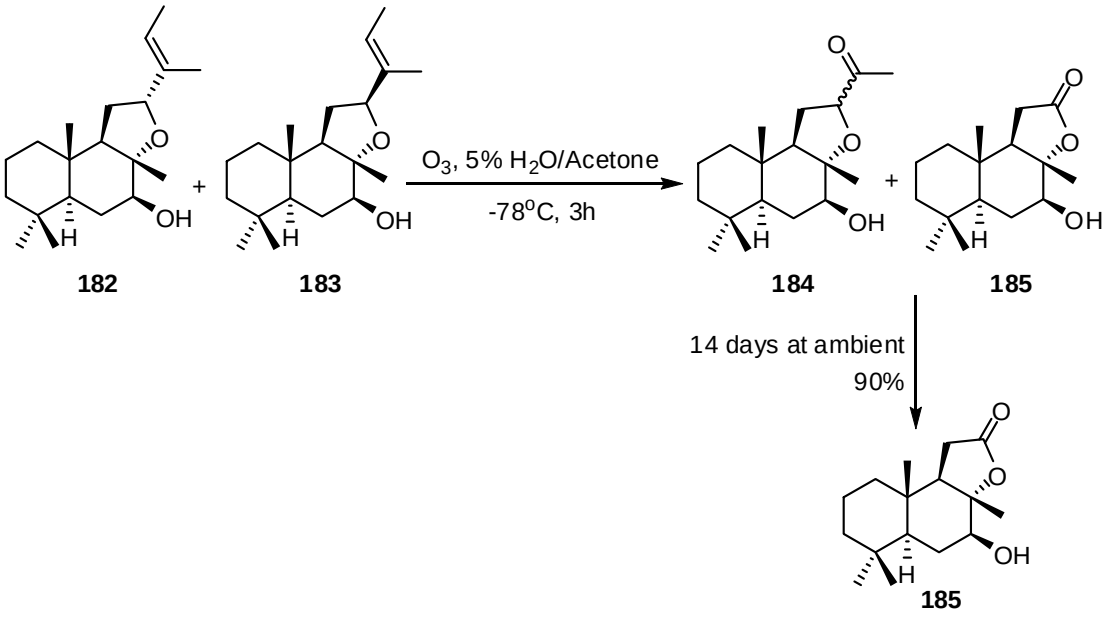


Figure 4.7 ^{1}H NMR spectra of products from (a) ozonolysis of **182** at after working-up; (b) ozonolysis of **182** at 4 days; (c) ozonolysis of **183** at after working-up and (d) ozonolysis of **183** at 4 days



Scheme 4.4 Ozonolysis of a mixture of **182** and **183**

Oxidative cleavage of **182** and **183** with ozone steam in 5% water in acetone (Schiaffo and Dussault, 2008) at -78^{o}C for 3 hours gave the mixture of **184** and **185** in 3:1 ratio as shown in Figure 4.8a.

The isolation of **184** and **185** on silica gel column chromatography gave two diastereomers of **184** in 29% yield and **185** in 19% yield. Additionally we found that the ratio of **184** and **185** was changed when the viscous oil mixture of **184** and **185** was stored and exposed to air at ambient temperature. Thus the viscous mixture of **184** and **185** was monitored by ^{1}H NMR. The results showed that ketone **184** was slowly transformed to **185** and it was crystallized itself to **185** completely after 14 days (Figure 4.8a, c and d). Recrystallization from acetone gave lactone **185** as colorless crystals in 90% yield.

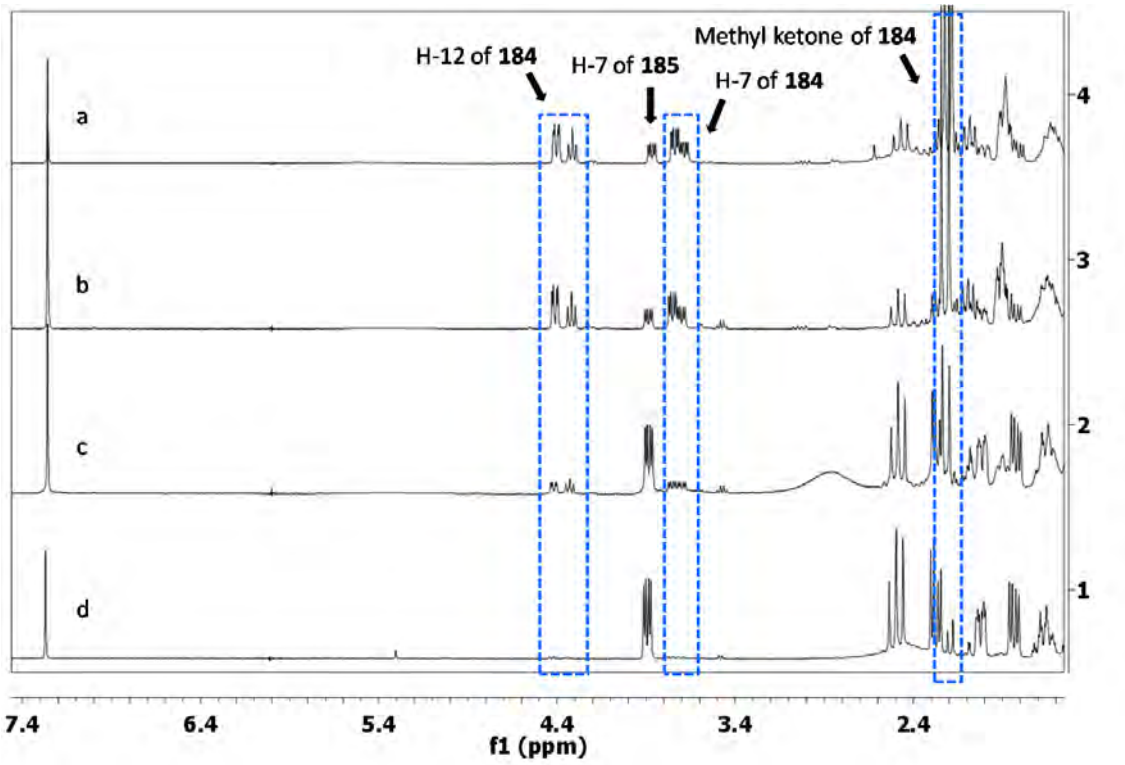


Figure 4.8 ^{1}H NMR spectra of products from ozonolysis of a 3:1 mixture of **182** and **183** (a) after working-up; (b) under no O_2 (stored in desiccators under anaerobic condition) for 3 days; (c) exposed to air for 3 days and (d) exposed to air for 14 days

To known how ketone **184** was converted itself to lactone **185**, the mixture of **184** and its epimer, exposed to atmospheric air and kept under anaerobic condition, were examined by ^{1}H NMR. The results (Figure 4.8b and 4.8c) revealed that the ketone **184** was transformed to **185** under atmospheric air while the ketone **184** was unchanged under anaerobic condition. This indicated that both diastereomers of **184** could be converted to **185** by air-auto oxidation. Structure of **185** was

characterized by 1D and 2D NMR (Figure 4.9a). Its relative configurations were determined by NOESY experiment (Figure 4.9b) and also confirmed by X-ray crystallographic analysis as shown in Figure 4.10.

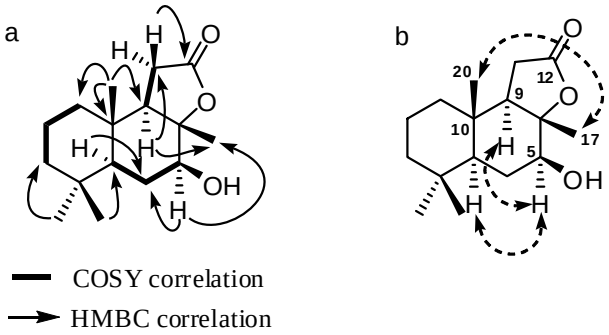


Figure 4.9 (a) COSY and HMBC correlation of **185** (b) Crucial NOESY correlation of **185**

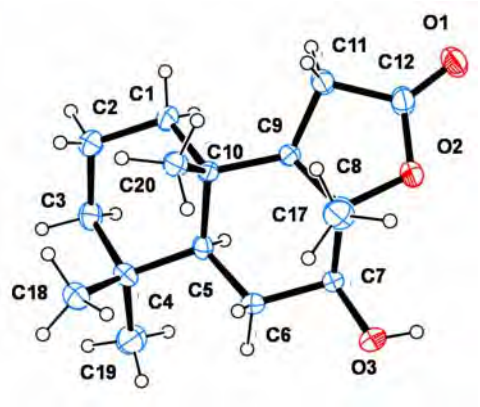
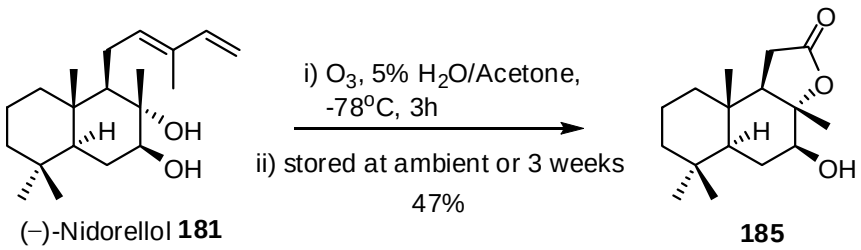


Figure 4.10 The ORTEP drawing of **185**

4.7.2 *Via* ozonolysis of (–)-nidorellol



Scheme 4.5 Ozonolysis of (–)-nidorellol **181**

We also found that compound **182** and **183** underwent air oxidative degradation to give **185** but the degradation of **182** and **183** was slower than of the ketone **184**. Because **182** and **183** could be converted to **185,** this encouraged us to

investigate the direct ozonolysis of **181** under the same condition. In fact by ^{1}H NMR monitoring, it was found signal of aldehyde protons, olefinic protons and small H-7 signal of **185** (Figure 4.11a). Attempt to isolate a reaction mixture was not successful because it consisted of too many compounds and they were unstable.

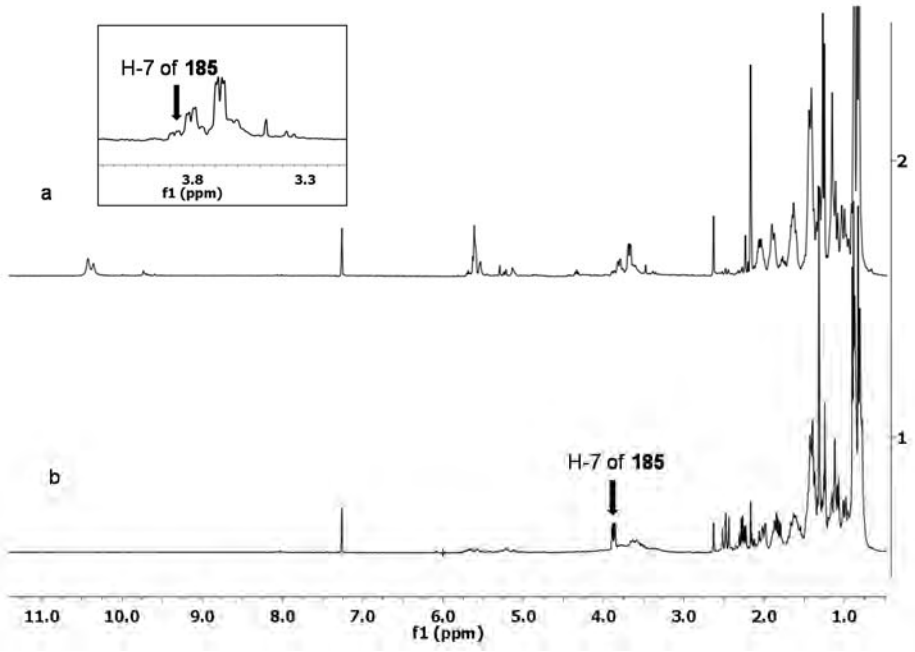


Figure 4.11 ^{1}H NMR spectra of reaction mixture of ozonolysis of **181** (a) After working-up and (b) exposed to air for 3 weeks

Moreover it was found that H-7 proton signal (δ_H 3.88 ppm) of **185** in ^{1}H NMR experiment was increased when a reaction mixture from the ozonolysis of **181** was stored under atmospheric air for 3 weeks (Figure 4.11b). This suggested that the mixture of product from ozonolysis of **181** also underwent air-auto oxidation. From isolation of the air-auto oxidation mixture, lactone **185** was obtained in 47% yield.

In case of air oxidation of compound **183**, it was found that it could be oxidized by O_2 molecule when it was stored and exposed to air for 5 months. The isolation of a mixture of air oxidation products of **183** was performed using preparative TLC and reverse phase HPLC, lactone **185** was obtained in 29% together with compound **190a** in 18% and compound **190b** in 7%. Since **190a** and **190b** were isolated from the mixture of air oxidation of **183** they were suggested that compound **183** also underwent air-auto oxidation and **185** was obtained via **190a** and **190b**

(Figure 4.12). It was believed that the mechanism of air oxidation of compound **183** and ketone **184** should be started by substitution of a reactive proton at C-12 with O_2 molecule via radical process to generate corresponding hydroperoxide **193** and **192**, respectively (Bäcktorp *et al.*, 2008). Substitution of O_2 molecule at C-12 of ketone **184** followed by degradation of the corresponding hydroperoxide **192** gave lactone **185** as end product. For air-auto oxidation of **183** via substitution of O_2 molecule at C-12, hydroperoxide **193** should be converted to epoxy ketone **190a** and **190b** and tetrahydrofuran ring **194** was formed via ring closure of **190a** and **190b**. Degradation of epoxy side chain **194** was led to lactone **185**.

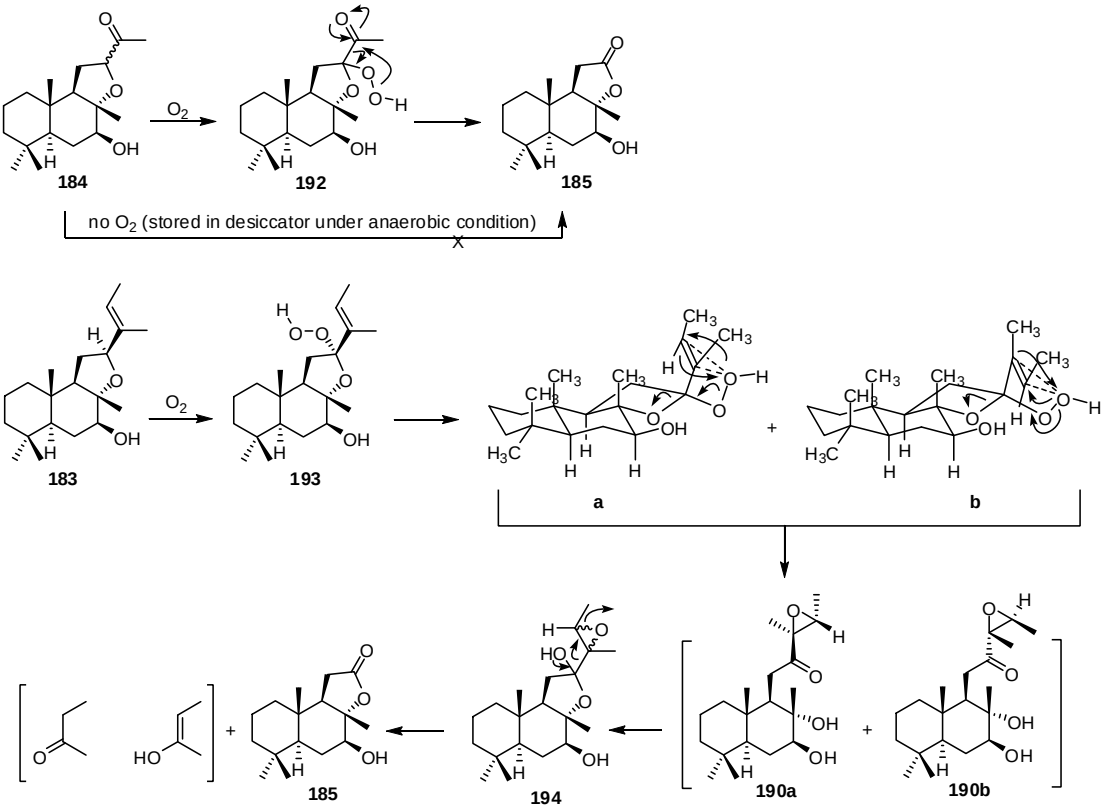


Figure 4.12 Proposed air oxidative degradation mechanism of **183** and **184**

Structure elucidation of **190a** was characterized by 1D and 2D NMR (Figure 4.13a). Correlations between H-7 and H-5, between H-7 and H-9, between H-9 and H-11a and between H-11a and methyl protons on C-14 by NOESY experiment indicated that theses protons were occupied on the same face. Due to no observation in NOESY experiment between those protons (H-5, H-7, H-9, H-11a and methyl

protons on C-14) and the methyl protons on C-8 and C-10 it was suggested that H-5, H-7, H-9, H-11a and methyl protons on C-14 were on the opposite face of the methyl protons on C-8 and C-10. In additionally, the observed correlation between methyl protons on C-8 and H-14 indicated that H-14 was on the same face of methyl protons on C-8 (Figure 4.13b).

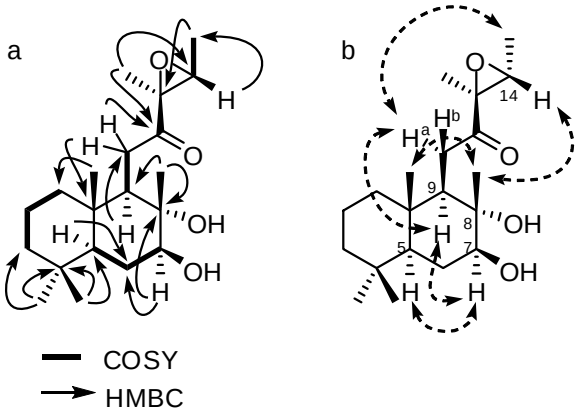


Figure 4.13 (a) COSY and HMBC correlation of **190a** (b) Crucial NOESY correlation of **190a**

Structure elucidation of **190b** was characterized by 1D and 2D NMR (Figure 4.13a). ^{1}H and ^{13}C NMR spectra of **192b** indicated that its structure was closely related to **190a**. Correlations between H-7 and H-5, between H-7 and H-9 and between H-9 and H-11a by NOESY experiment indicated that theses protons were occupied on the same face. Due to no observation in NOESY experiment between those protons (H-5, H-7, H-9 and H-11a) and the methyl protons on C-8 and C-10 it was suggested that H-5, H-7, H-9 and H-11a were on the opposite face of the methyl protons on C-8 and C-10. In additionally, the no observed correlation between methyl protons on C-8 and H-14 indicated that H-14 was on the opposite face of methyl protons on C-8 (Figure 4.13b). Thus **190b** should be an isomer of **190a**.

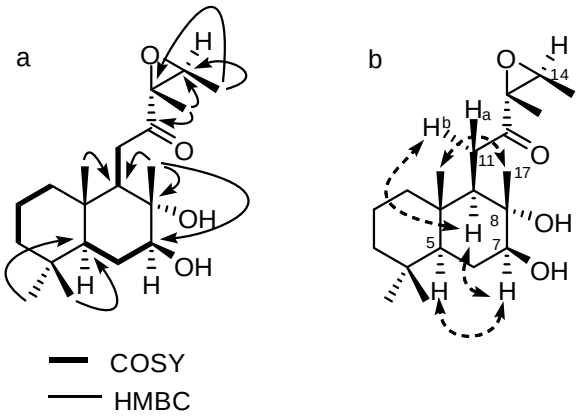
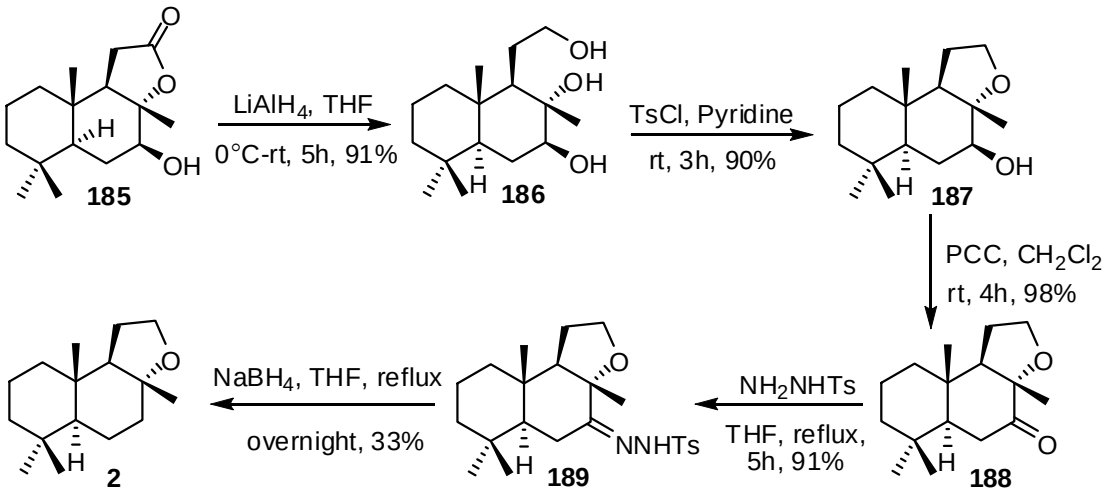


Figure 4.12 (a) COSY and HMBC correlation of **190b** (b) Crucial NOESY correlation of **190b**

4.8 Synthetic approach to compound 2

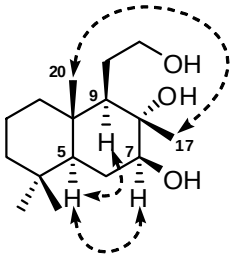
4.8.1 Using a procedure as described by Hashimoto and Caglioti



Scheme 4.6 The first synthetic route to ambrox

Reduction of lactone **185** with $LiAlH_4$ afforded triol **186** in excellent yield (91%). Correlations between H-9 and H-5 and between H-5 and H-7 by NOESY experiment indicated that theses protons were situated on the same face. Due to no observation in NOESY experiment between those protons (H-5, H-7 and H-9) and the methyl protons of C-17 and C-20 it was suggested that H-5, H-7 and H-9 were on the opposite face of the methyl protons of C-17 and C-20 (Figure 4.14). All data which

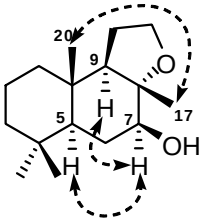
described above revealed that reduction of **185** with $LiAlH_4$ gave the retention configuration product.



186

Figure 4.14 Crucial NOESY correlation of **186**

Tosylation at 1° alcohol of **186** using TsCl in pyridine (Barrero *et al*., 1993c) gave a corresponding tosylate ester and it was immediately cyclized to be **187** in a good yield (90%). The relative configurations of **187** were determined by NOESY spectra. The observed NOEs between H-7 and H-5, between H-7 and H-9 and between methyl group on C-8 and methyl group on C-10 revealed that stereochemistry of **187** was retained (Figure 4.15).

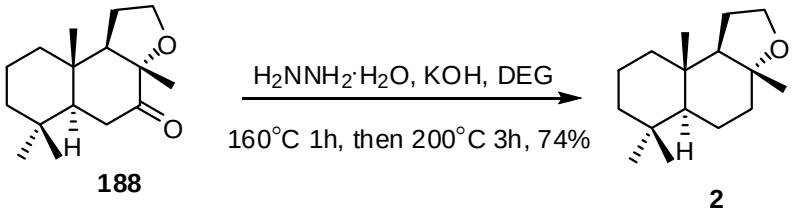


187

Figure 4.15 Crucial NOESY correlation of **187**

Dehydroxylation at C-7 was started by oxidation of hydroxyl group using PCC then reaction with *p*-toluenesulfonylhydrazide using a procedure as described by Hashimoto (Hashimoto *et al*., 1998) and finally reduction with $NaBH_4$ using a procedure as described by Caglioti (Caglioti, 1988) gave **2** in 29% from **187**.

4.8.2 *Via* Wolff-Kishner reduction of 188

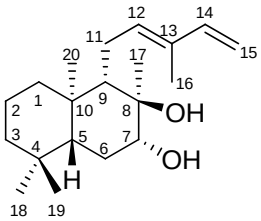


Scheme 4.7 Wolff-Kishner reduction of **188**

Since the procedure as described by Hashimoto and Caglioti gave poor yield of **2**, Wolff-Kishner reduction of carbonyl compound **188** on heating with hydrazine hydrate in the presence of KOH was performed (Alvarez-Manzaneda *et al*., 2005). This reduction process gave better result and compound **2** was obtained in good yield (74% yield) after purification.

4.9 Absolute configuration of (–)-nidorellol

^{1}H, ^{13}C and 2D NMR data of **2** were identical to standard (–)-ambrox but its specific rotation ($[\alpha]_D^{20}$ = +23.0^{o}) indicated that the synthesized ambrox was (+)-ambrox. Moreover, ^{1}H and ^{13}C NMR data and specific rotation of **2** were in good agreement with (+)-ambrox synthesized by Giacomini (Giacomini, *et al*., 2003). Since the synthesis of ambrox from (–)-nidorellol **181** gave *ent*-ambrox configuration instead of the expected product, (–)-ambrox, the absolute configuration of (–)-nidorellol has been showed to be opposite to that illustrated in previous report (Bohlmann and Fritz, 1978). Therefore, the correct absolute configuration of (–)-nidorellol has been proved to be *trans*-(5*R*,7*R*,8*R*,9*S*,10*R*)-labda-12,14-diene-7α,8β-diol (Figure 4.16).



trans-(5*R*,7*R*,8*R*,9*S*,10*R*)-labda-12,14-diene-7α,8β-diol

Figure 4.16 Absolute structure of (–)-nidorellol

4.10 Fixative property determination

4.10.1 Smelling paper strip test

In smelling paper strip studies, the periods ranging of *D*-limonene odor on paper strips which 60% of panelists could detect are shown in table 4.4. The results indicated that standard (–)-ambrox and (–)-nidorellol had fixative property. They could retard the evaporation rate of the testing solution more than other compounds. Evaporation rate of testing solution which consisted of (+)-ambrox, compounds **182**, **183**, **185** and **187** suggested that those compounds did not have fixative property for *D*-limonene. Moreover, it was found that compound **188** could accelerate evaporation of testing solution.

Table 4.4 Fixation time of *D*-limonene odor on smelling paper strip (60% of panelists could detect)

Sample	**Time (hour)**
D-limonene	6
(–)-Ambrox	9
(+)-Ambrox	6
(–)-Nidorellol	8
Compound **182**	5
Compound **183**	4
Compound **185**	4
Compound **187**	5
Compound **188**	1

4.10.2 Analysis by TGA studies

The themogravimetric analysis of testing solution which consisted of 1% of fixative (1% w/w), 5% of *D*-limonene (5% w/w) in EtOH are shown in Figure 4.17. The TGA plot showed that volatilization of testing solution began at about 20°C and temperature at 50% weight loss for *D*-limonene, (–)-ambrox, (+)-ambrox, (–)-nidorellol, **182**, **183**, **185**, **187**, **188** were 35.6, 38.8, 35.4, 26.6, 37.2, 36.0, 36.0, 37.2 and 30.7°C [156, 188, 154, 66, 172, 160, 160, 172 and 100 min (from starting temperature)]. These results indicated that only (–)-ambrox could decrease rate of volatilization of testing solution. (+)-Ambrox, compound **182**, **183**, **185** and **187** showed the similar rate of weight loss to *D*-limonene while compound **188** could accelerate evaporation of testing solution. These results showed same trend with smelling paper strip method. In case of (–)-nidorellol, TGA data showed opposite result to smelling paper strip result. This suggested that (–)-nidorellol may be decomposed due to air of the testing condition during measurement of TGA and fixative property of (–)-nidorellol was then lost.

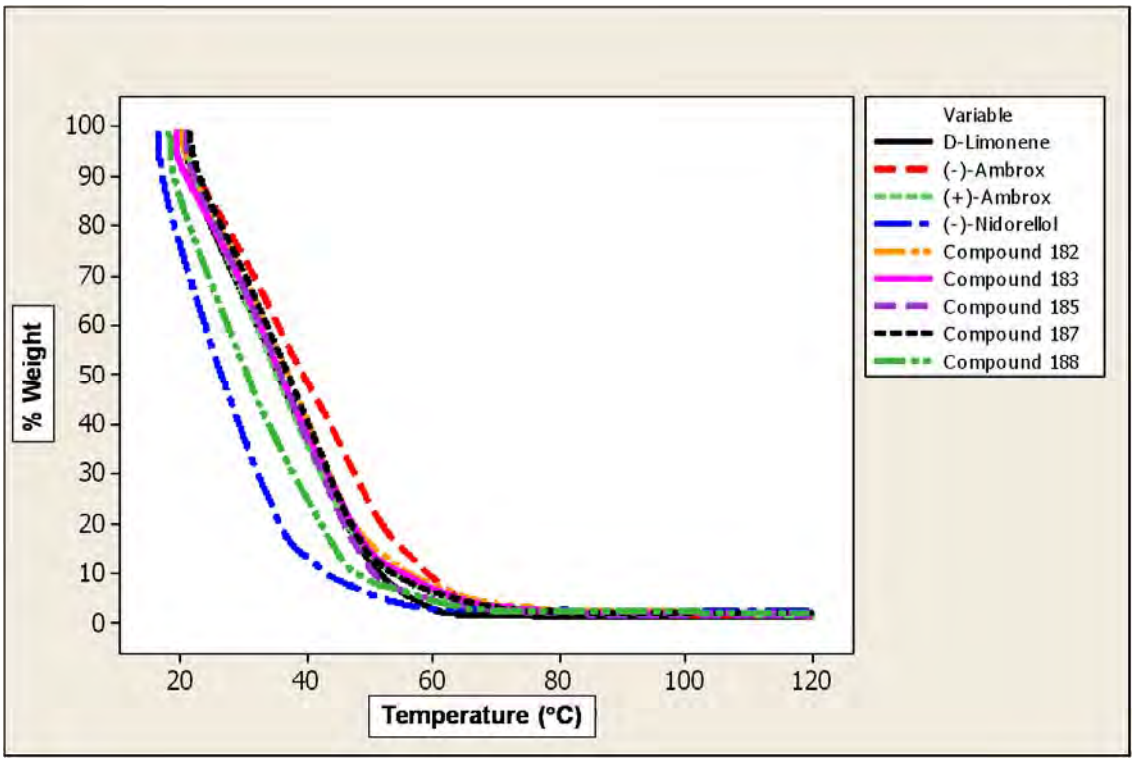


Figure 4.17 Thermogram of TGA

CHAPTER V

CONCLUSION

In conclusion, a successive synthesis of (+)-ambrox (*ent*-**2**) has been achieved in 53% overall yield via acid-promoted cyclization of (–)-nidorellol **181**; ozonolysis of **182** and **183**; and reductive dehydroxylation of the 7-hydroxyl group as key steps. Since (+)-ambrox was synthesized from (–)-nidorellol, the absolute configuration of (–)-nidorellol **181** has been proved and identified as *ent*-labdane type diterpene, *trans*-(5*R*,7*R*,8*R*,9*S*,10*R*)-labda-12,14-diene-7α,8β-diol. (–)-Nidorellol, compound **182**, **183** and **184** could be converted to **185** via ozonolysis and air auto-oxidation process. Moreover (–)-nidorellol showed fixative property at ambient condition while (+)-ambrox and its derivatives, compound **182**, **183**, **185**, **187** and **188** did not showed any fixative property.

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APPENDICES

Appendix A
chemical data

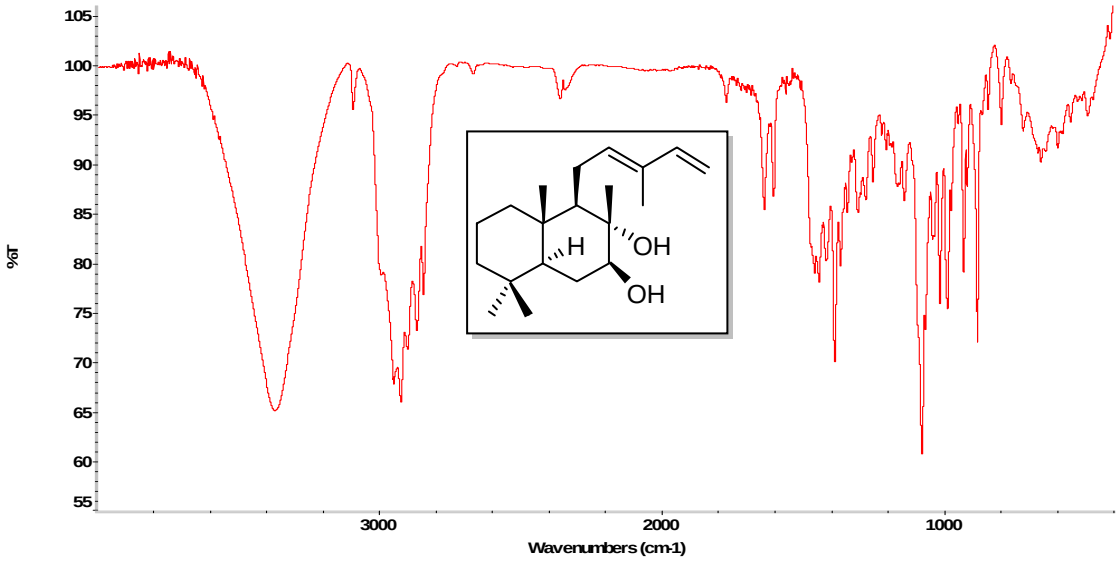

Figure A1 IR spectrum of (−)-nidorellol

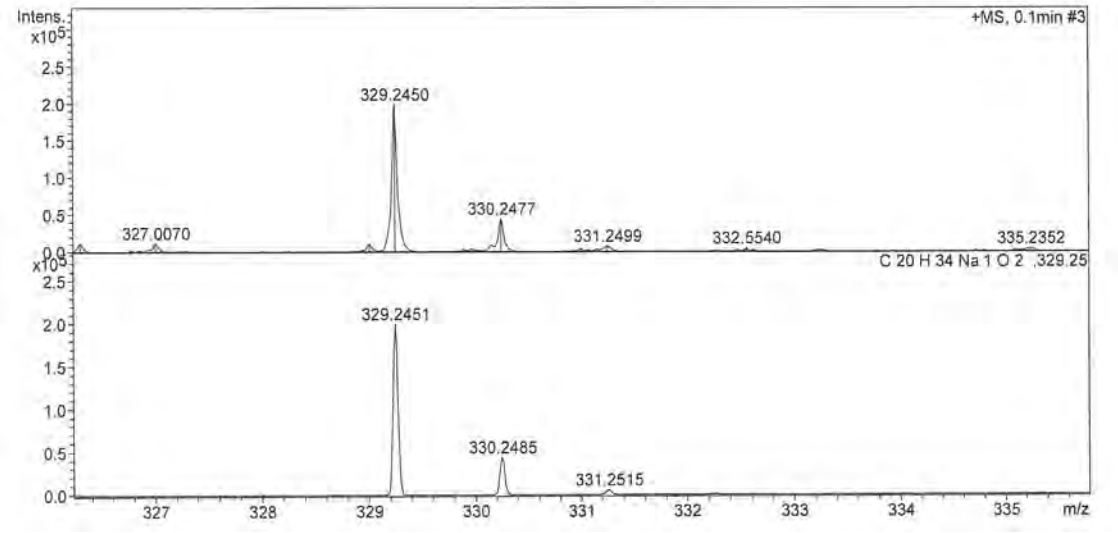

Figure A2 HRMS (ESI-TOF) spectrum of (−)-nidorellol

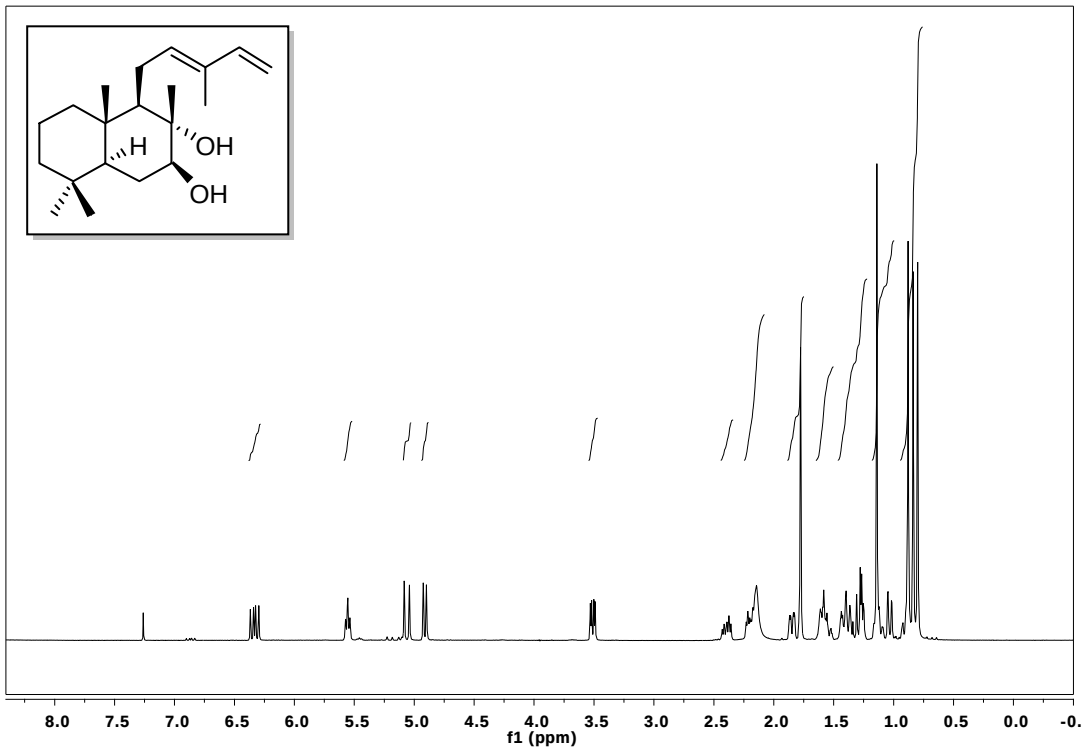

Figure A3 ^{1}H NMR spectrum of (–)-nidorellol

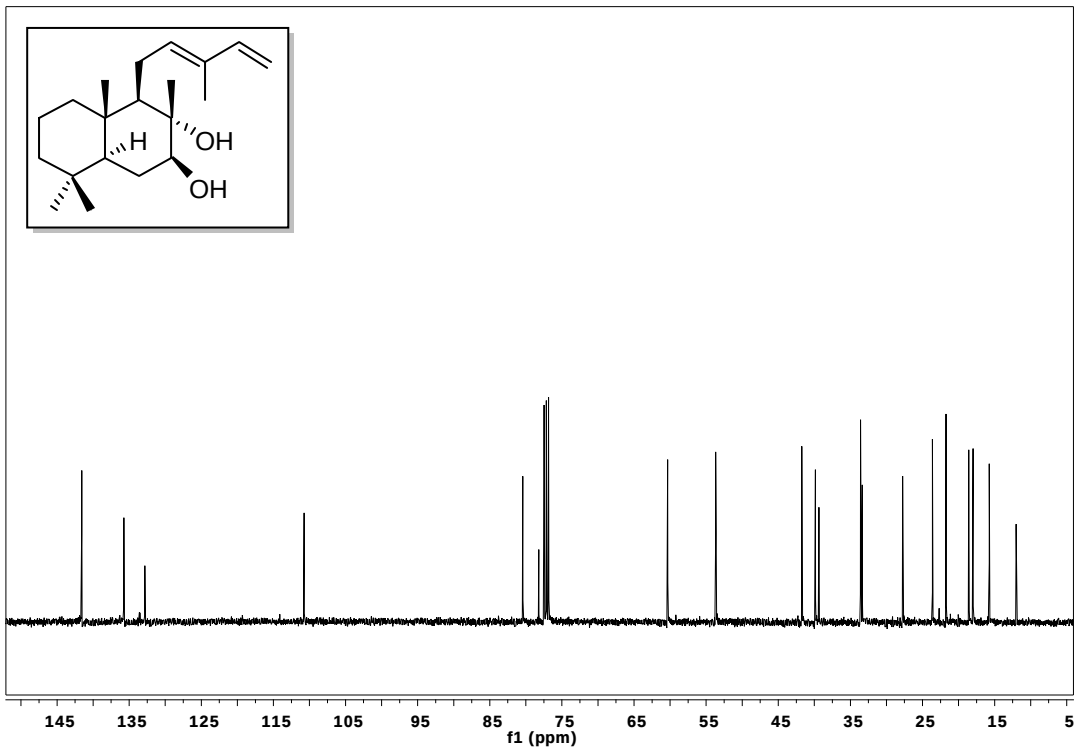

Figure A4 ^{13}C NMR spectrum of (–)-nidorellol

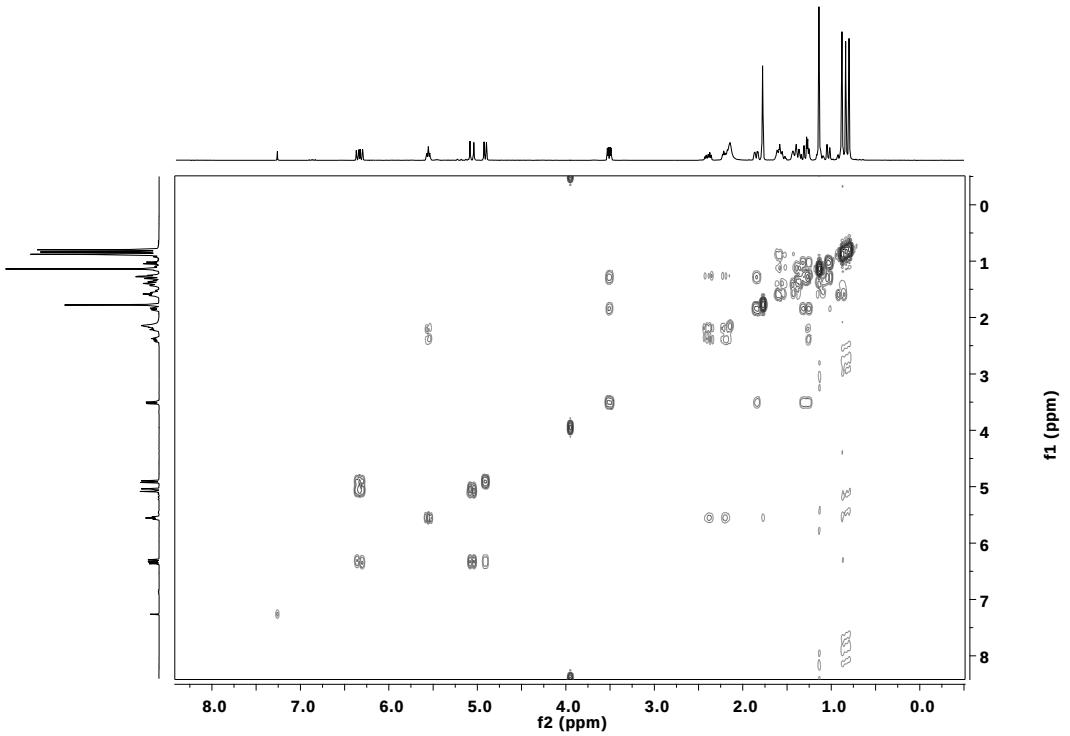

Figure A5 COSY spectrum of (–)-nidorellol

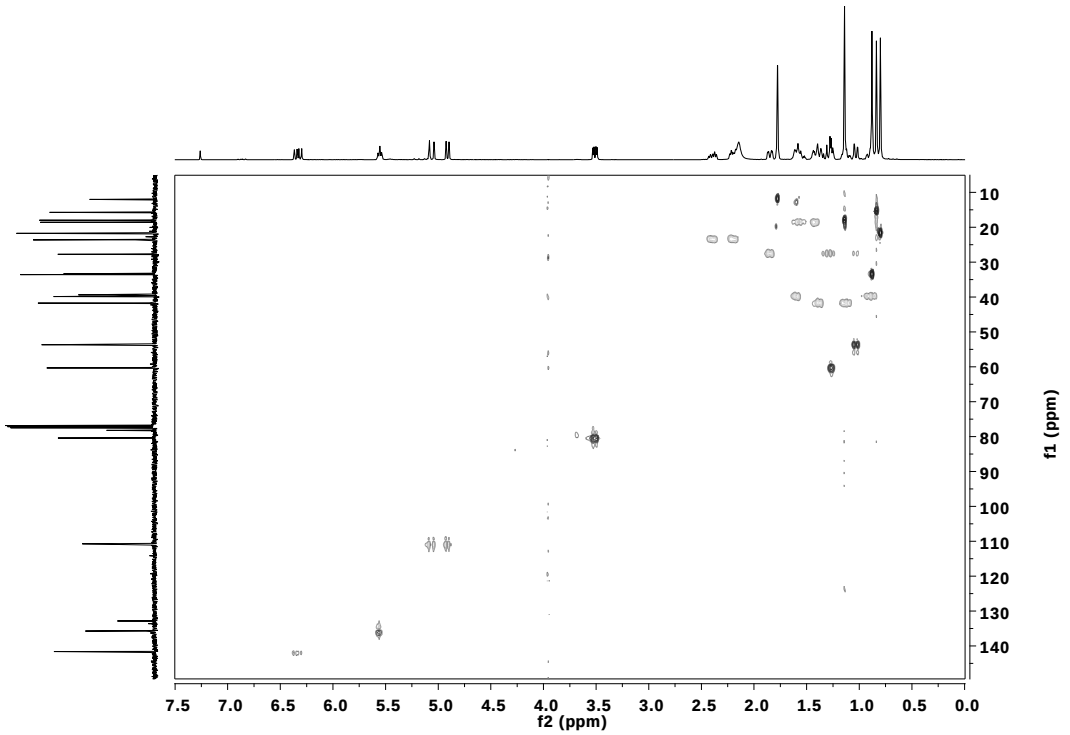

Figure A6 HSQC spectrum of (–)-nidorellol

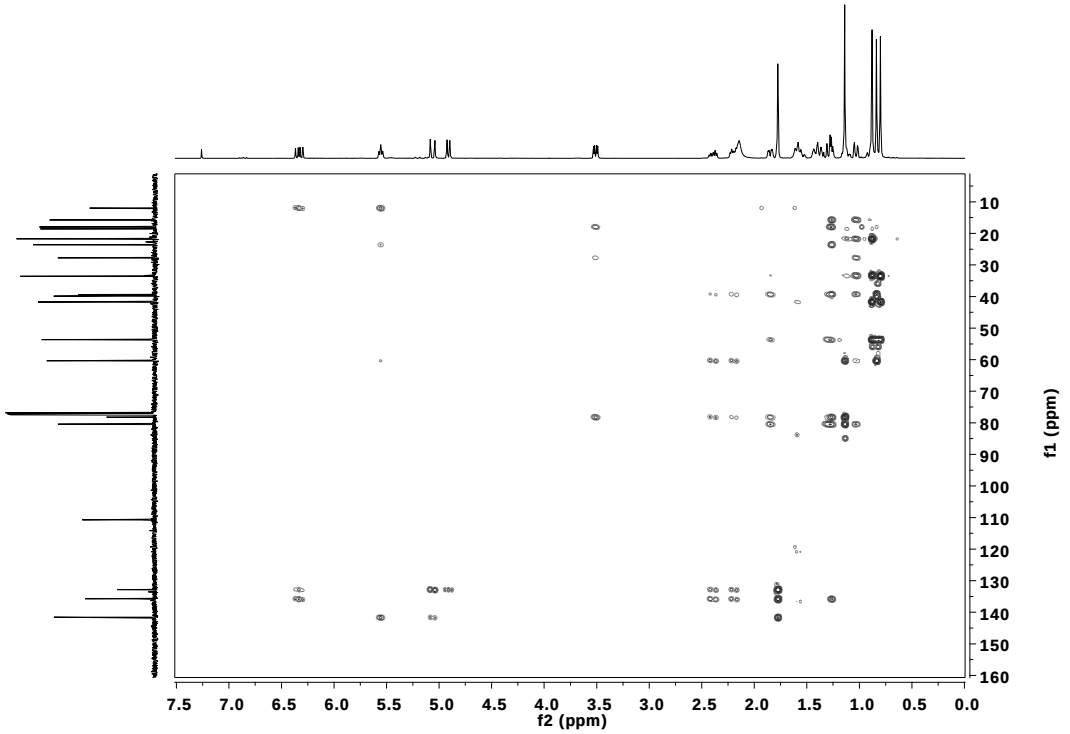

Figure A7 HMBC spectrum of (–)-nidorellol

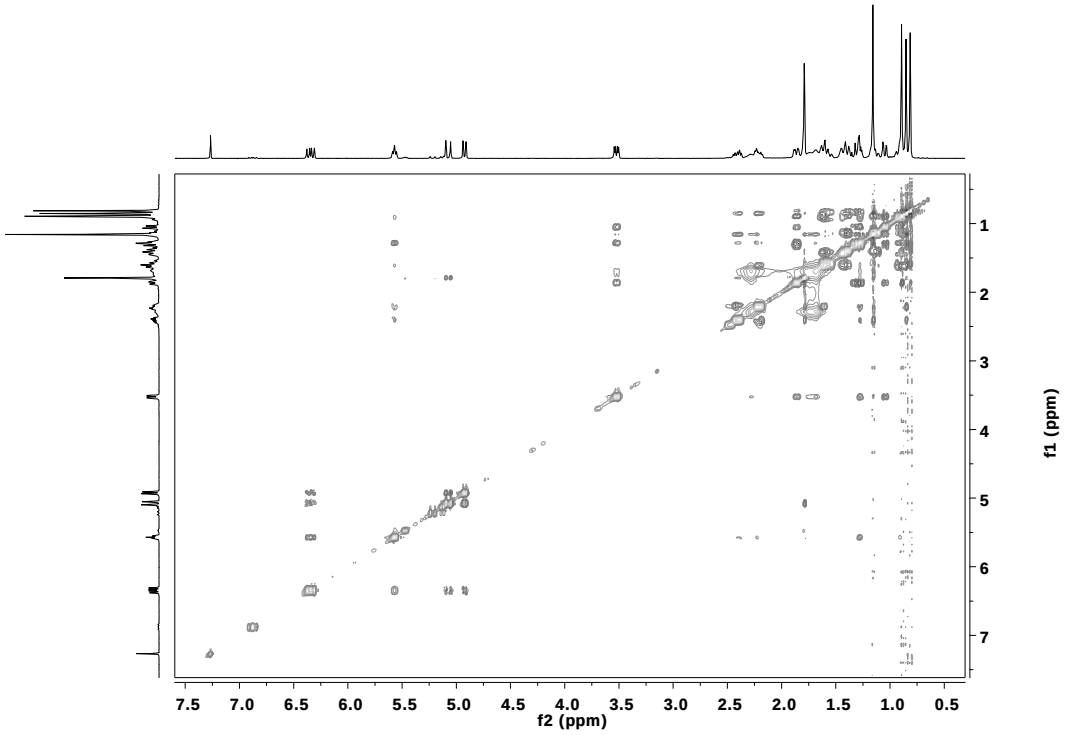

Figure A8 NOESY spectrum of (–)-nidorellol

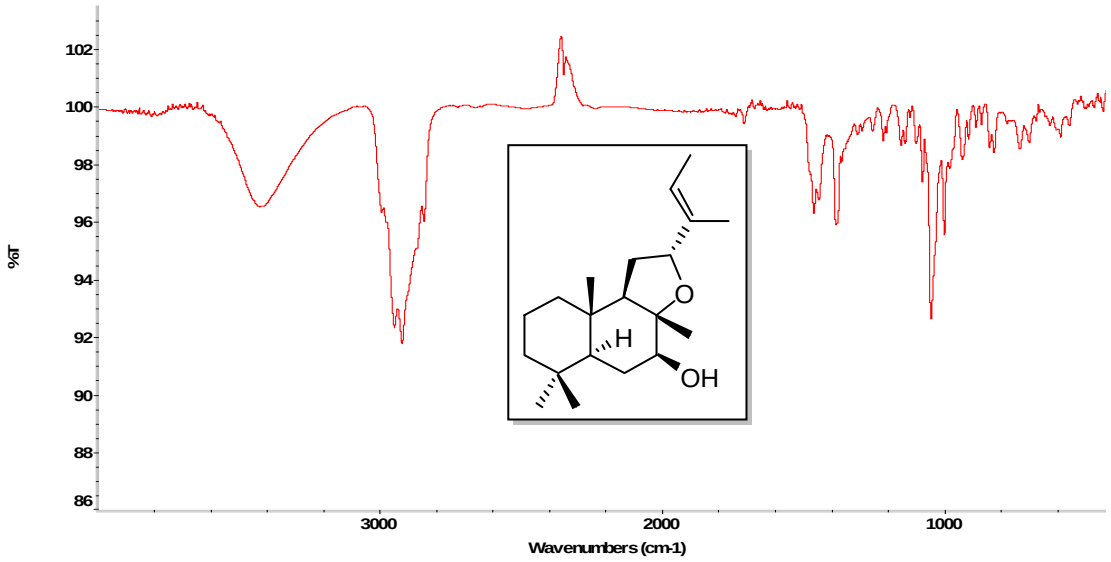

Figure A9 IR spectrum of compound **182**

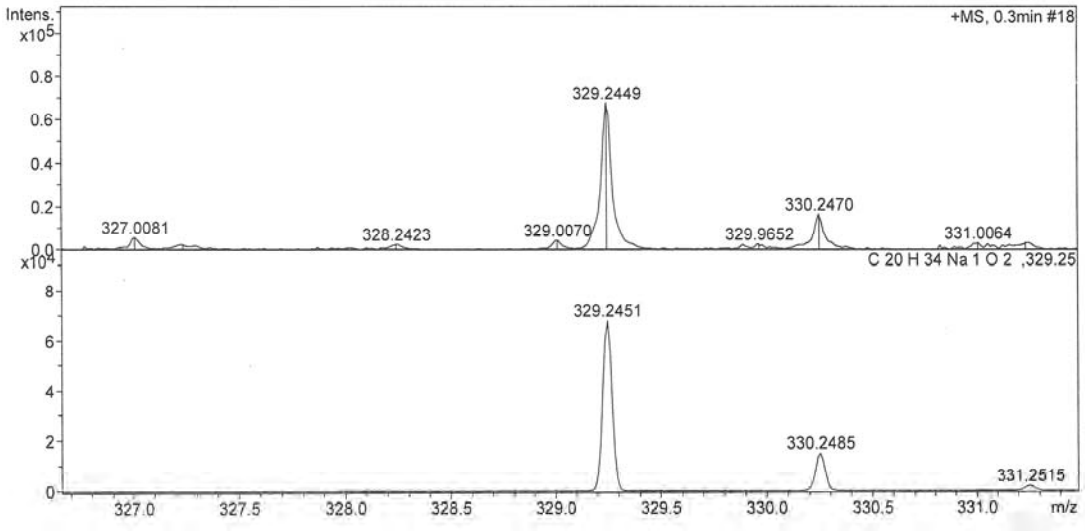

Figure A10 HRMS (ESI-TOF) spectrum of compound **182**

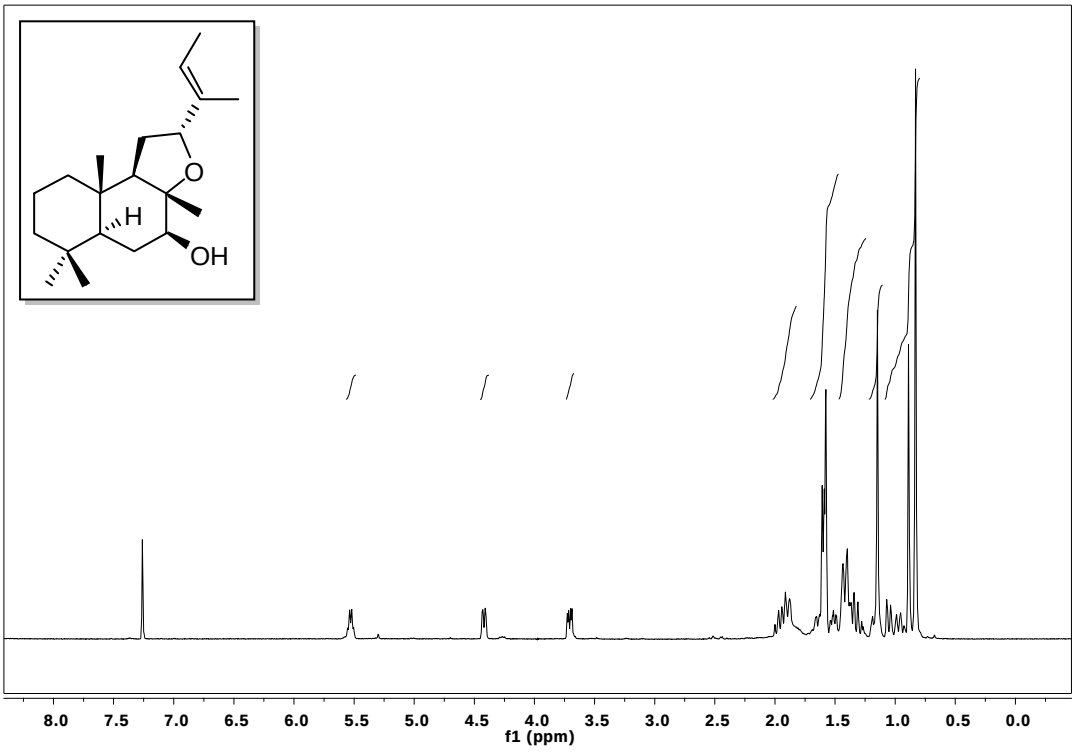

Figure A11 ^{1}H NMR spectrum of compound **182**

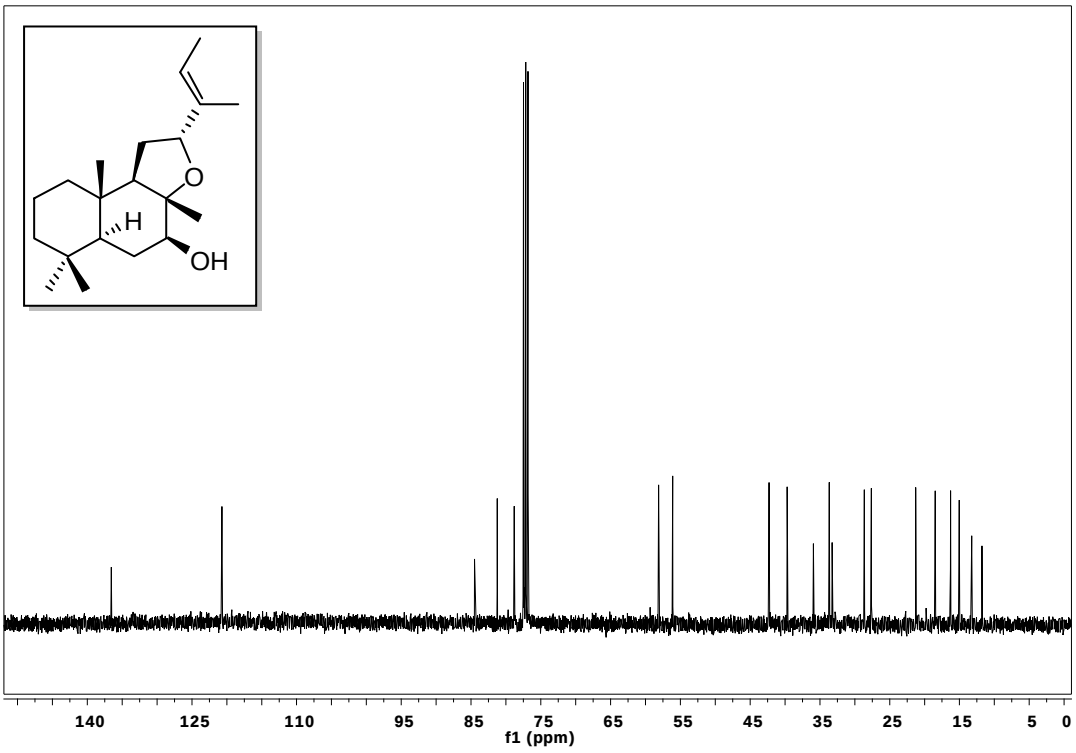

Figure A12 ^{13}C NMR spectrum of compound **182**

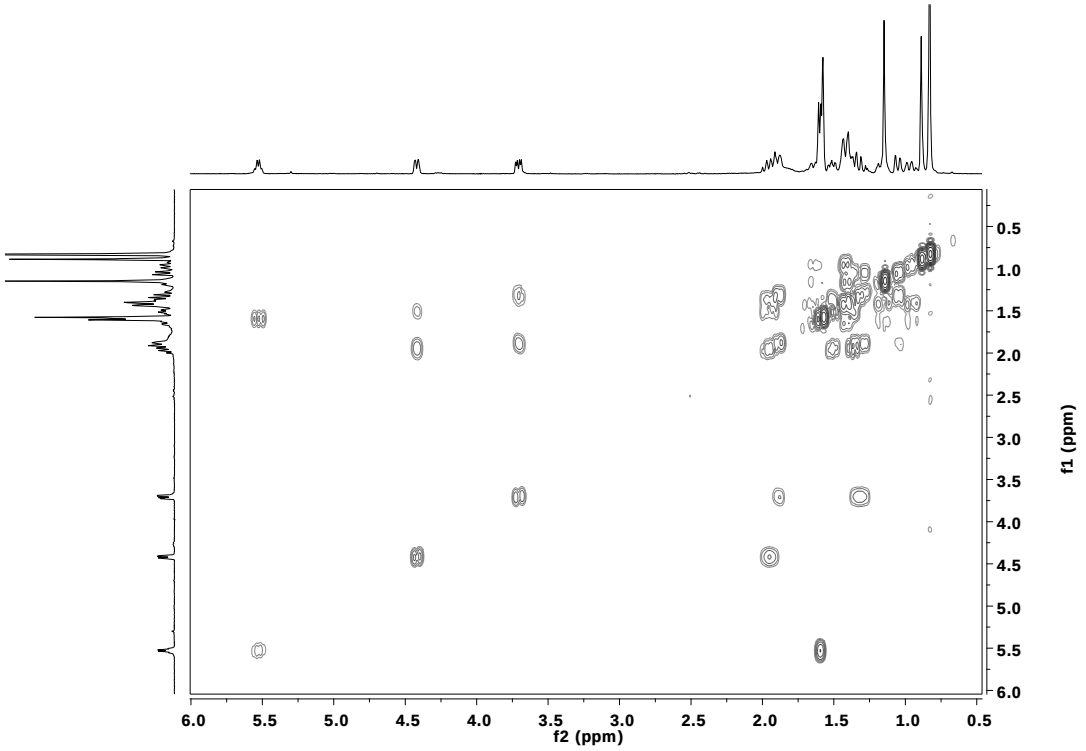

Figure A13 COSY spectrum of compound **182**

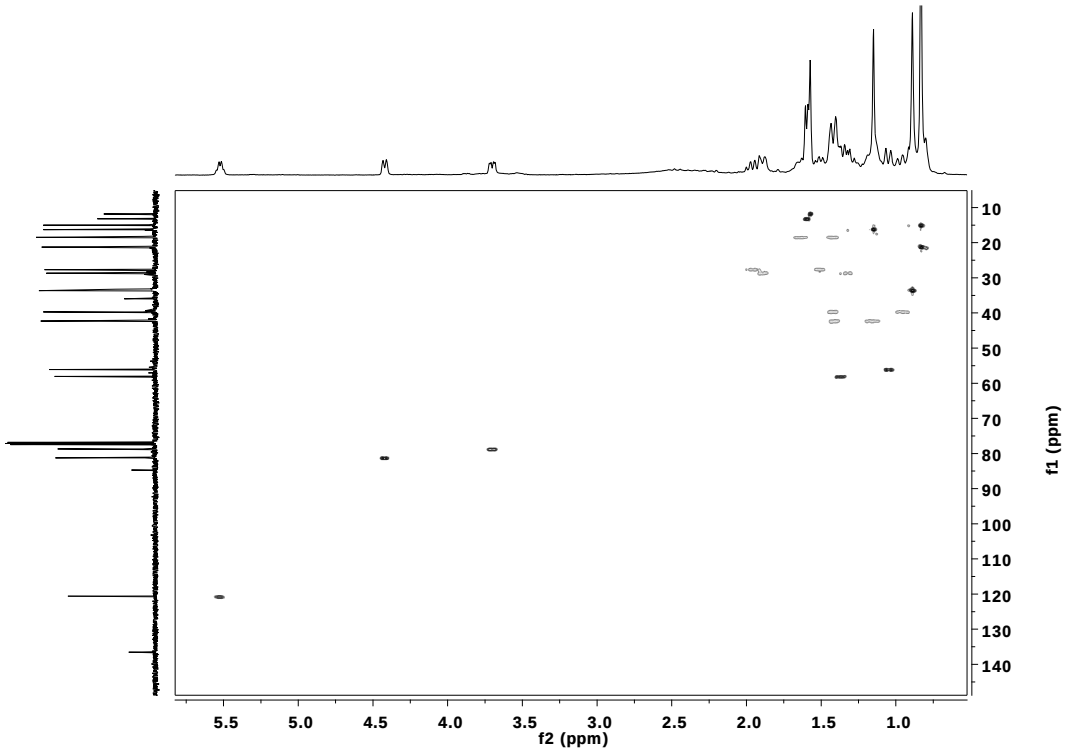

Figure A14 HSQC spectrum of compound **182**

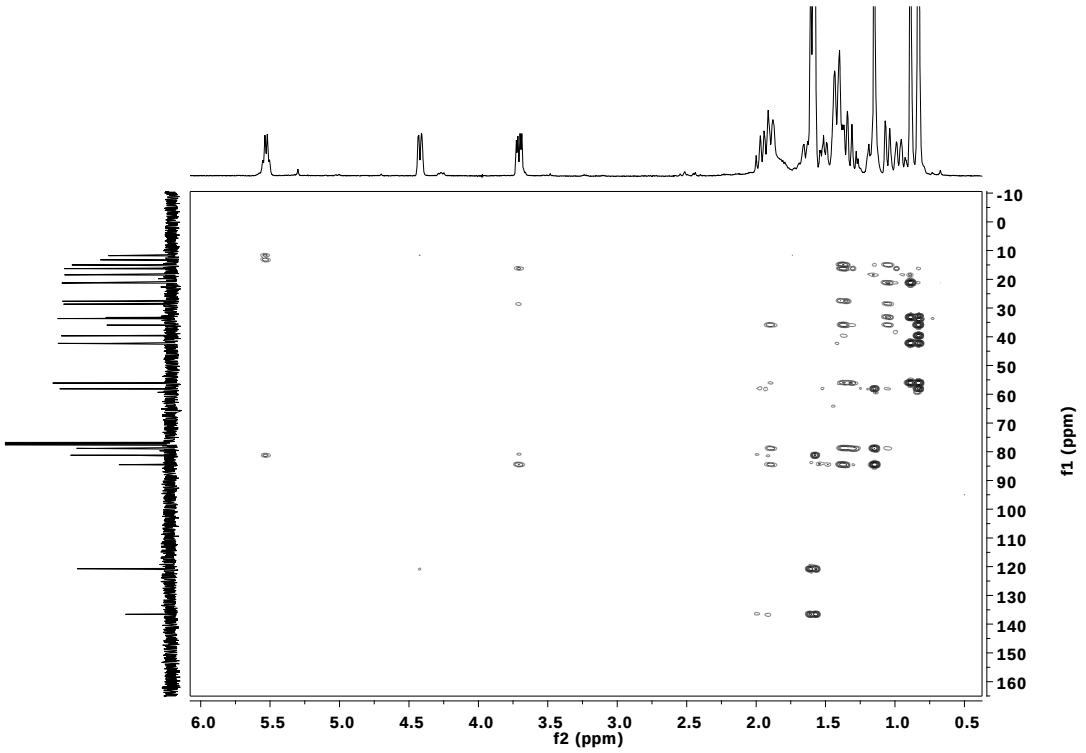

Figure A15 HMBC spectrum of compound **182**

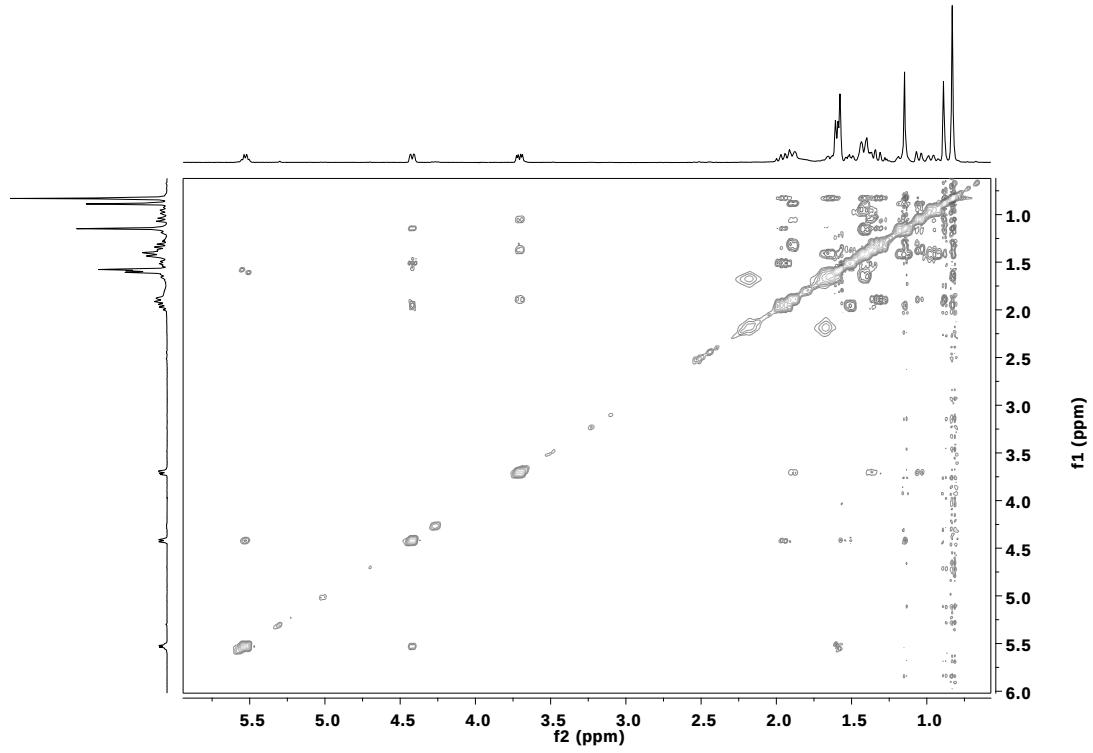

Figure A16 NOESY spectrum of compound **182**

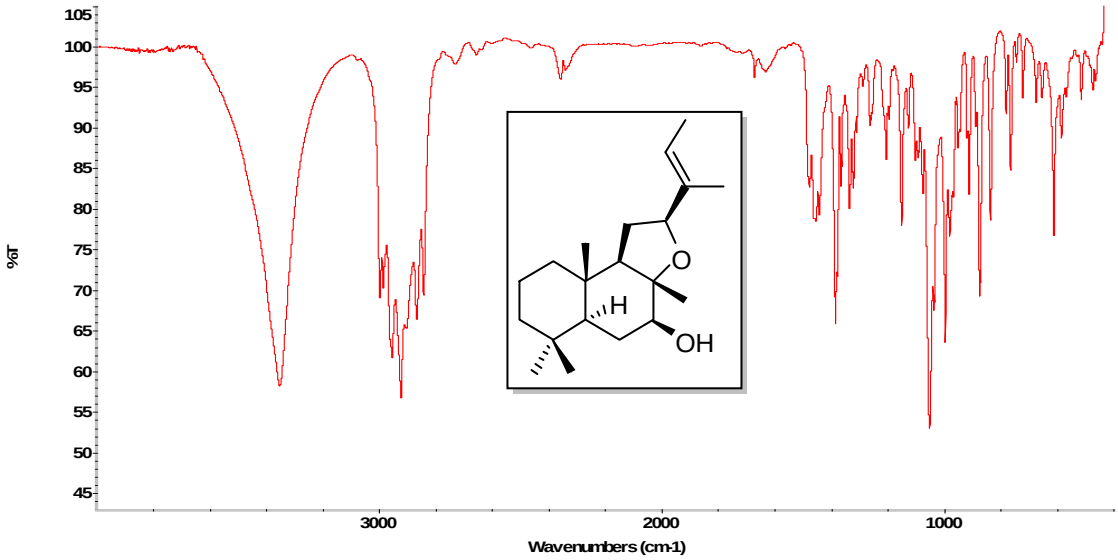

Figure A17 IR spectrum of compound **183**

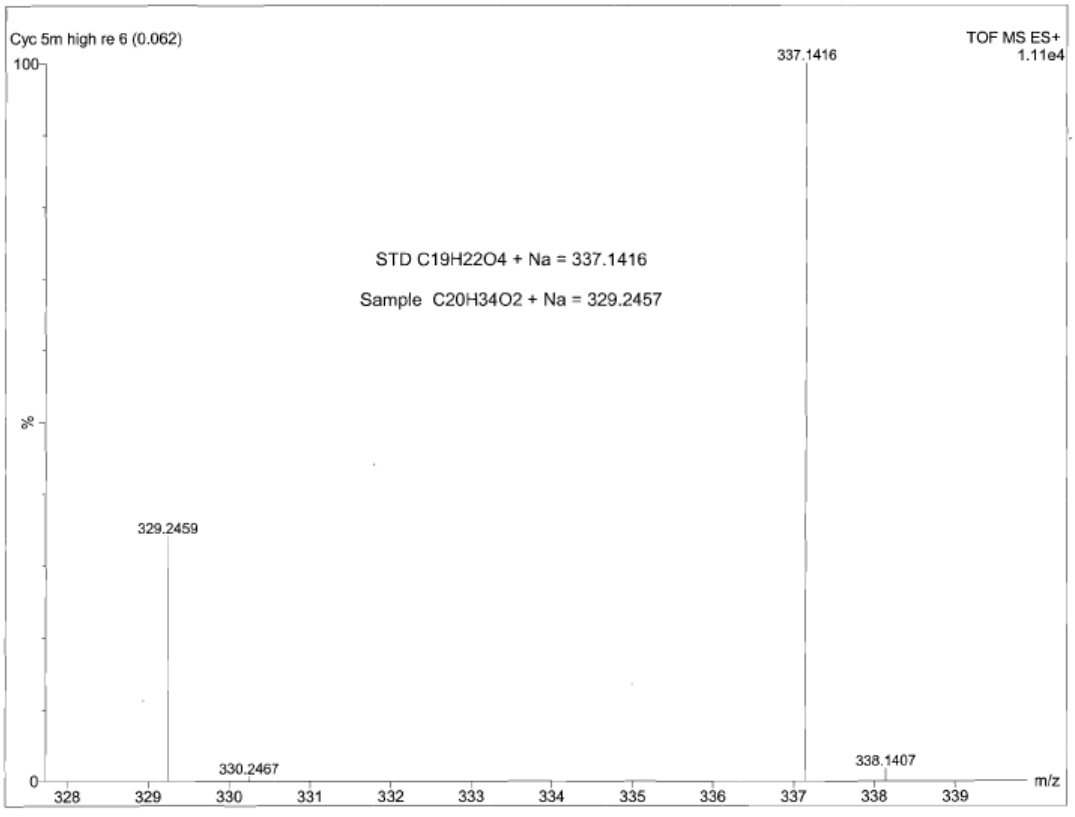

Figure A18 HRMS (ESI-TOF) spectrum of compound **183**

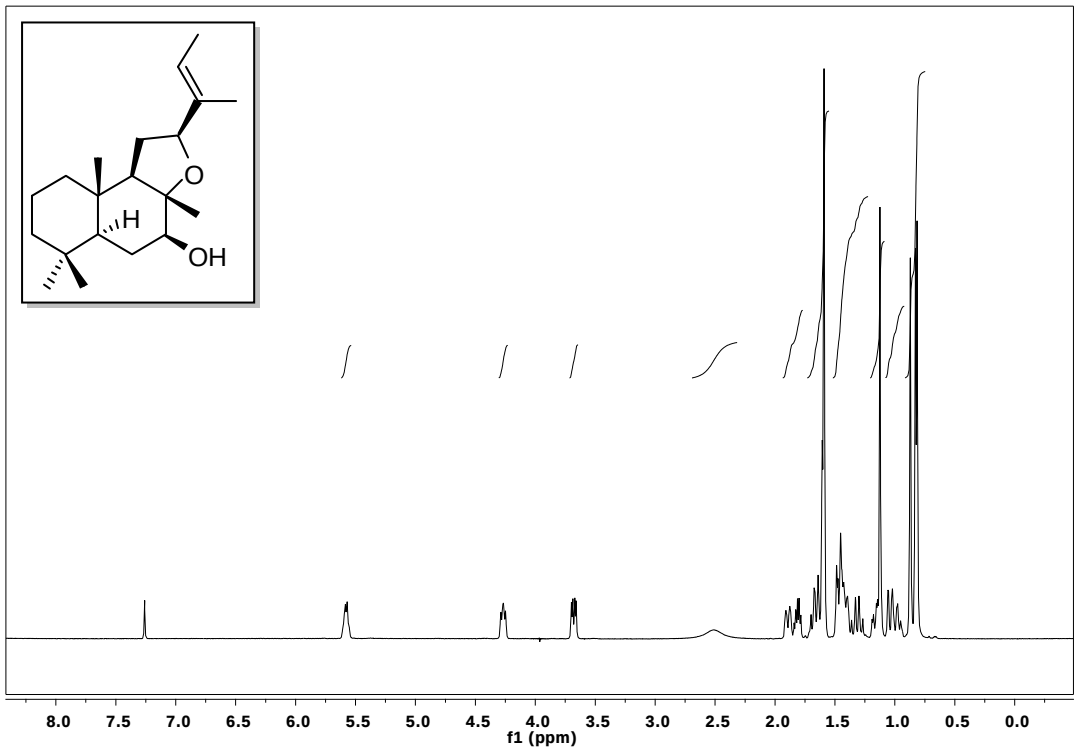

Figure A19 ^{1}H NMR spectrum of compound **183**

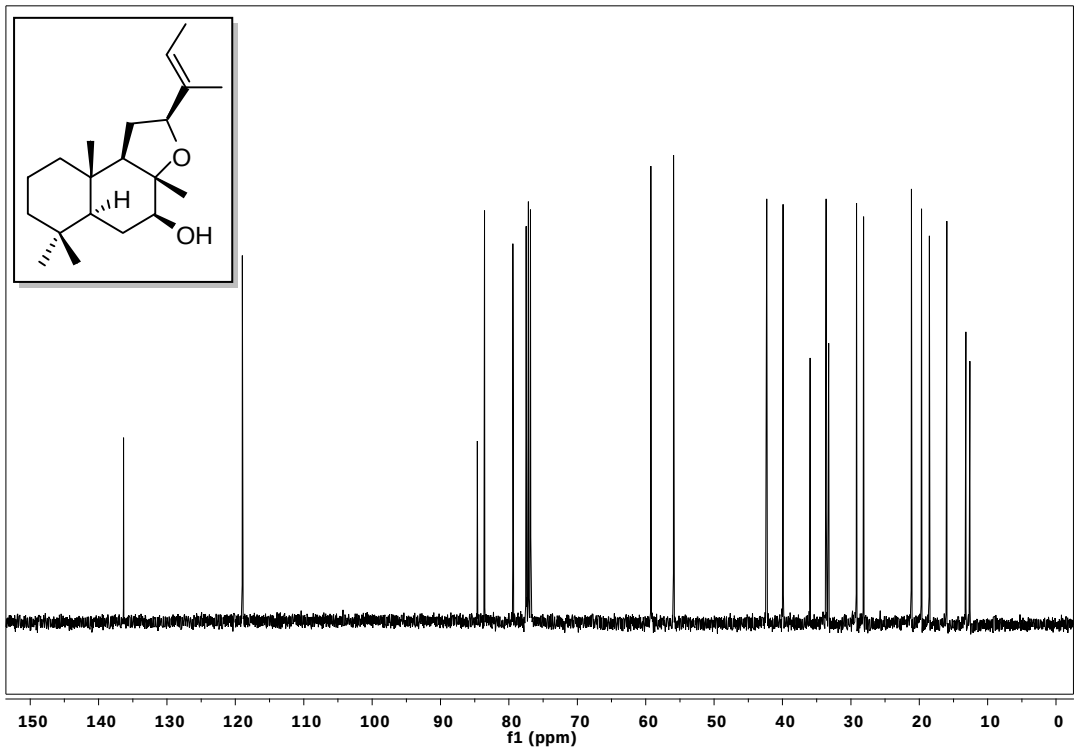

Figure A20 ^{13}C NMR spectrum of compound **183**

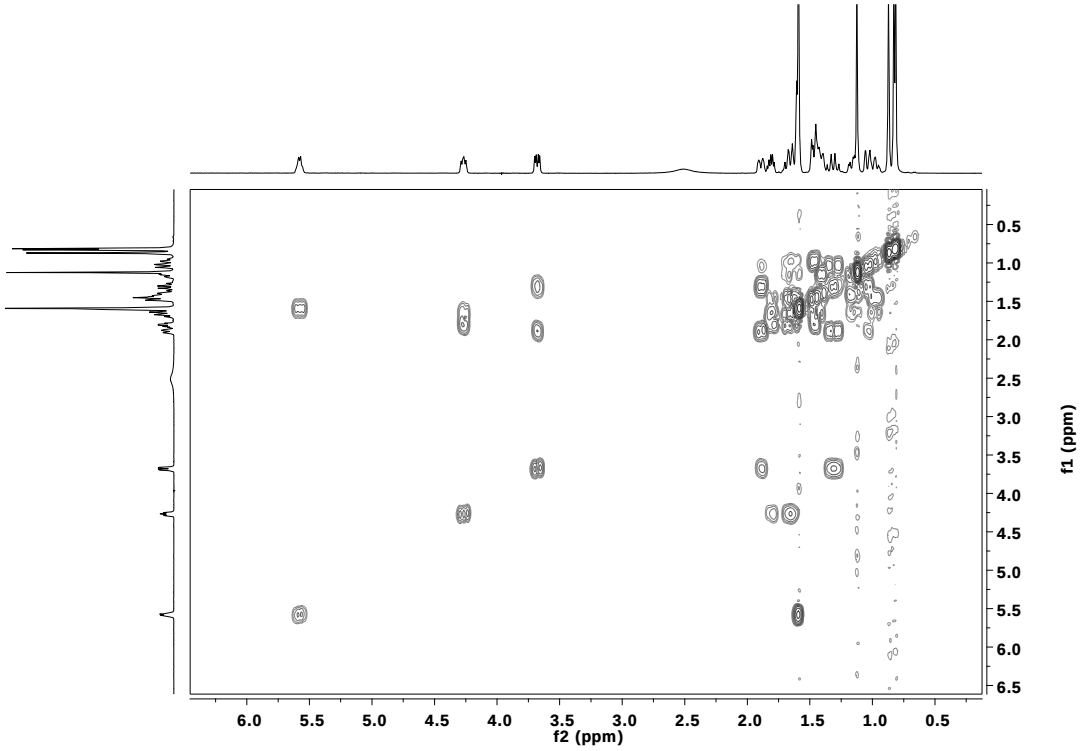

Figure A21 COSY spectrum of compound **183**

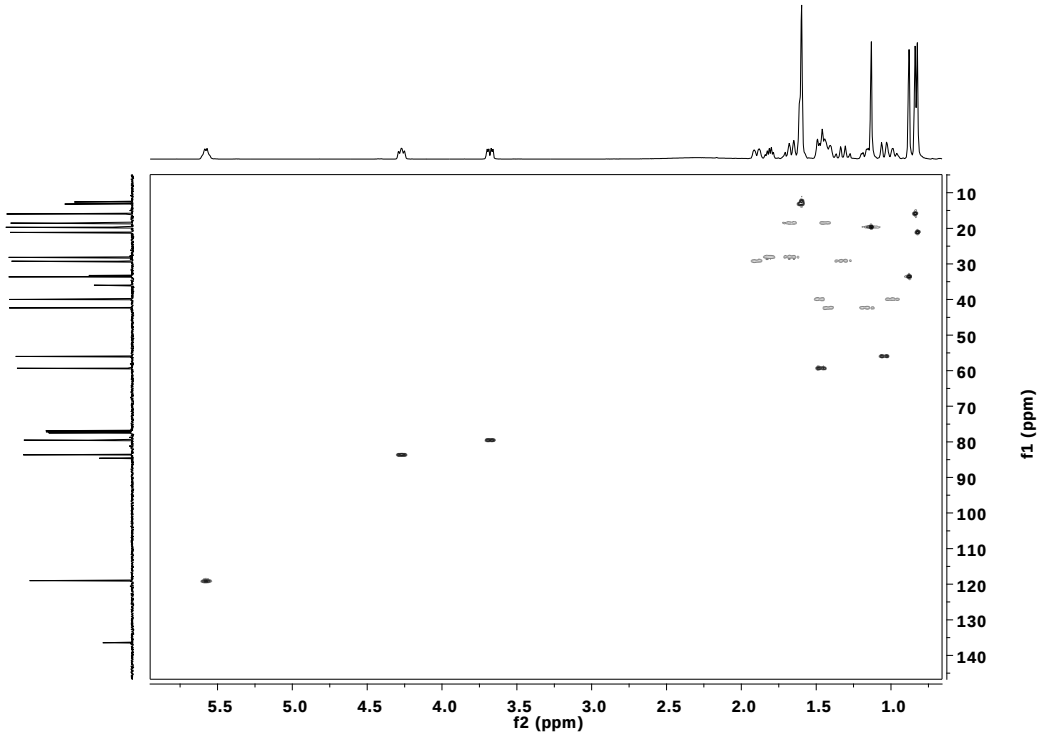

Figure A22 HSQC spectrum of compound **183**

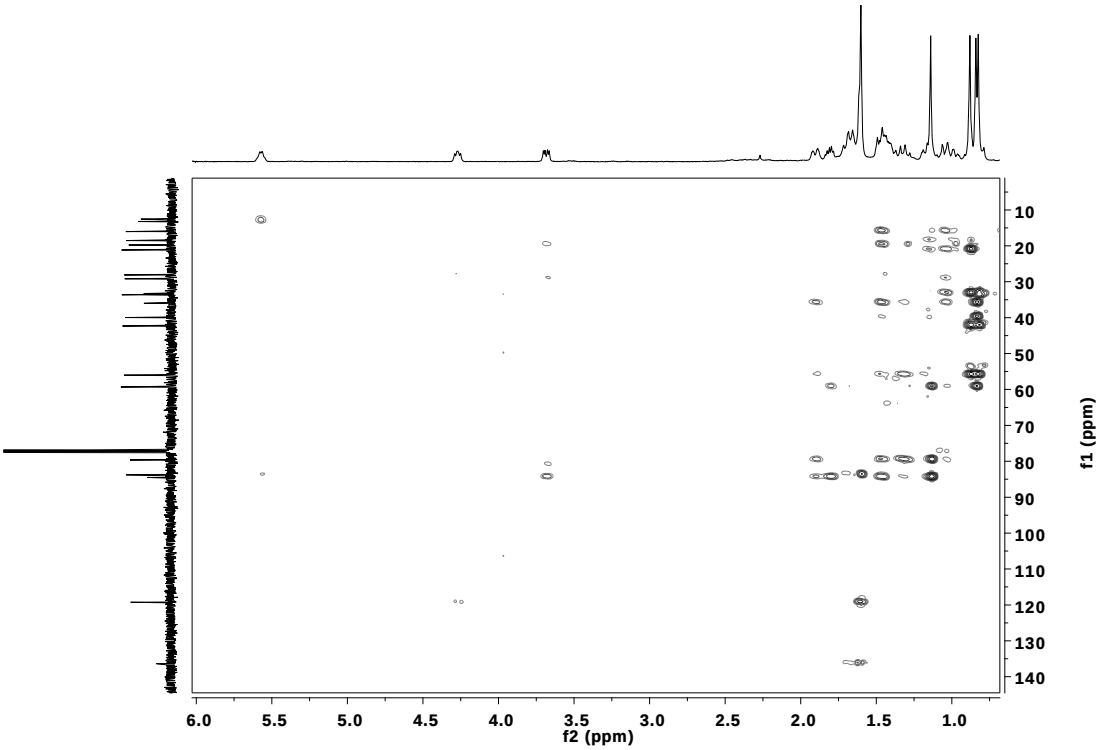

Figure A23 HMBC spectrum of compound **183**

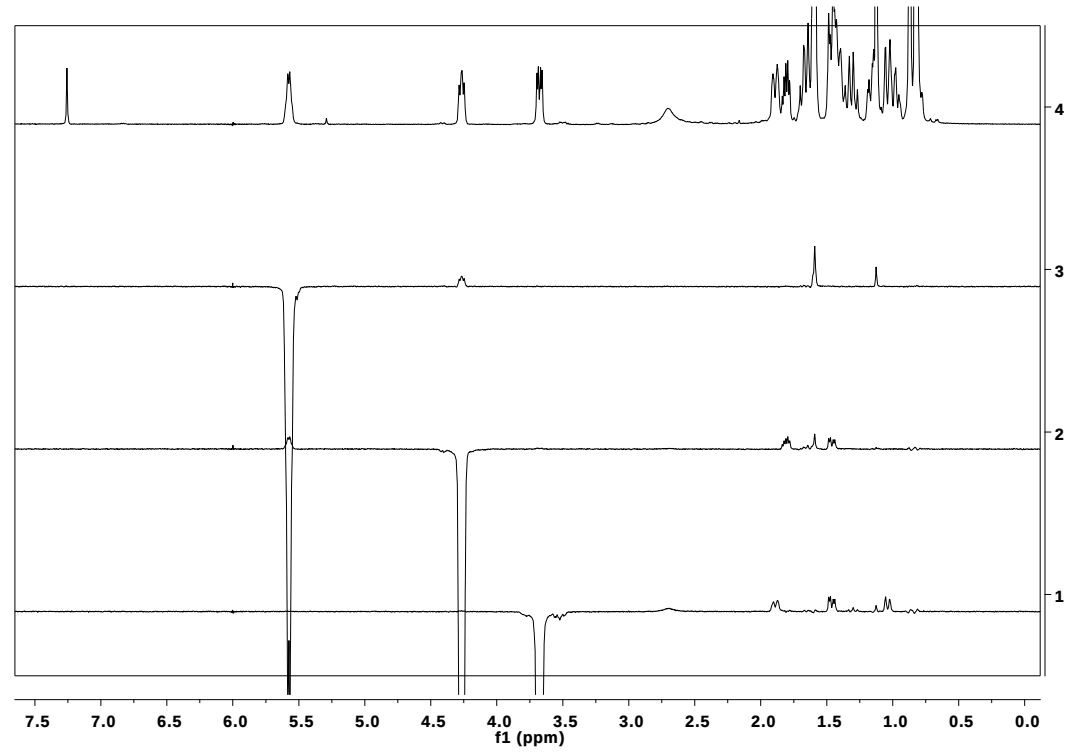

Figure A24 1D NOESY spectrum of compound **183**

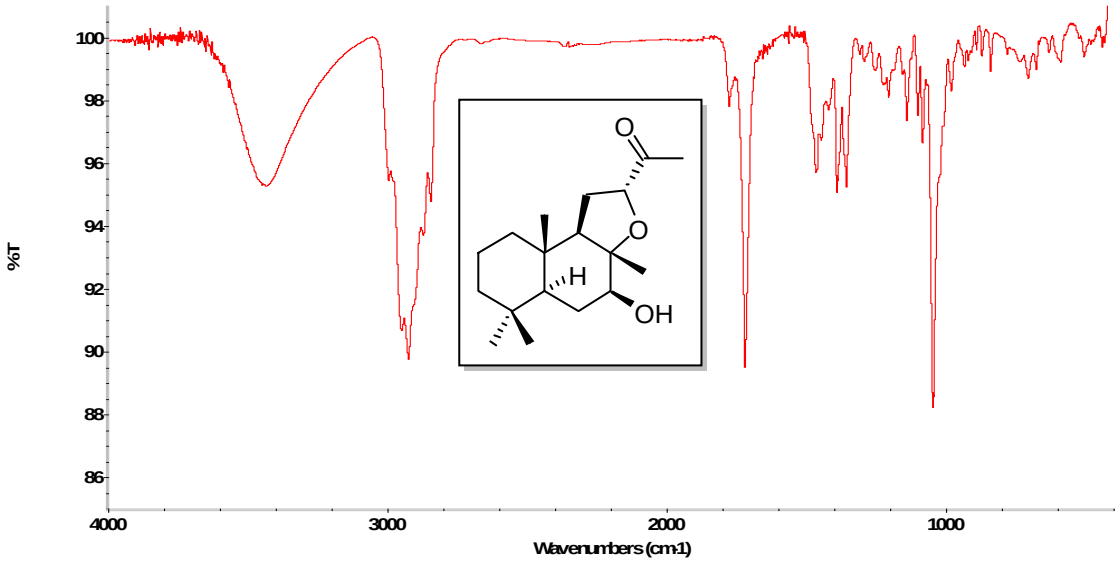

Figure A25 IR spectrum of compound **184a**

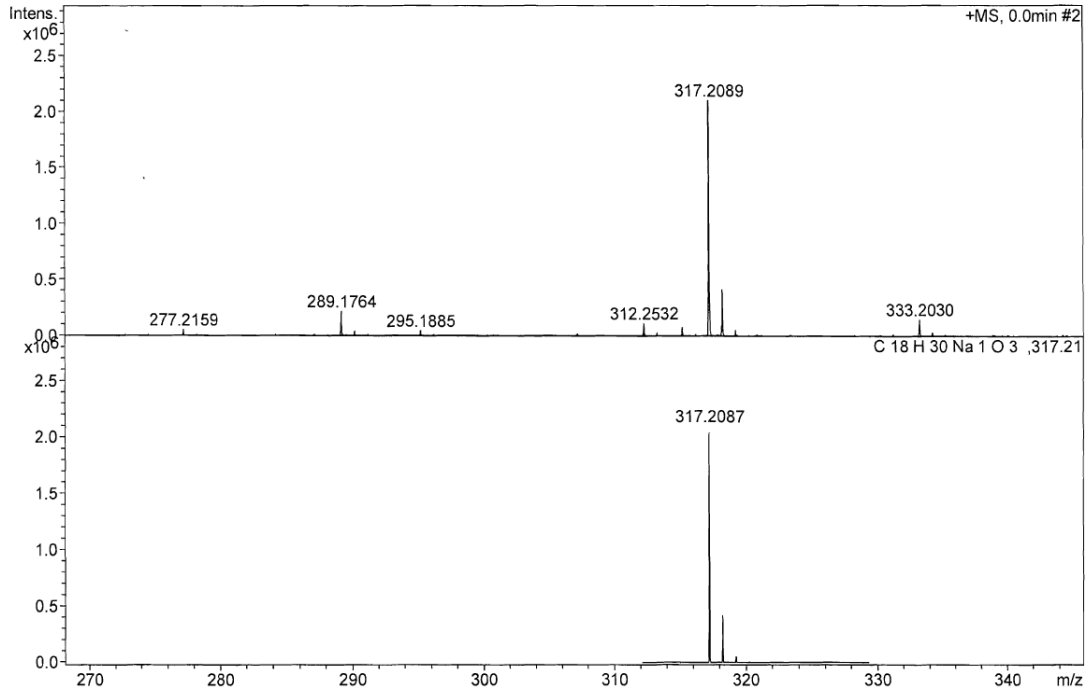

Figure A26 HRMS (ESI-TOF) spectrum of compound **184a**

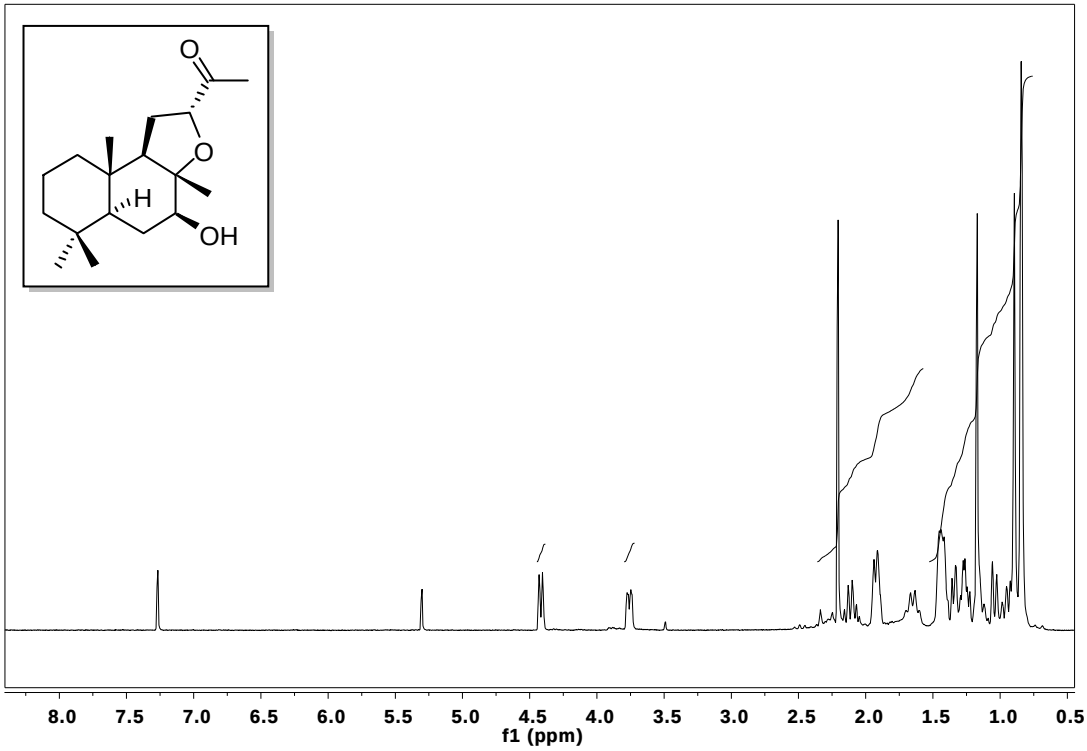

Figure A27 ^{1}H NMR spectrum of compound **184a**

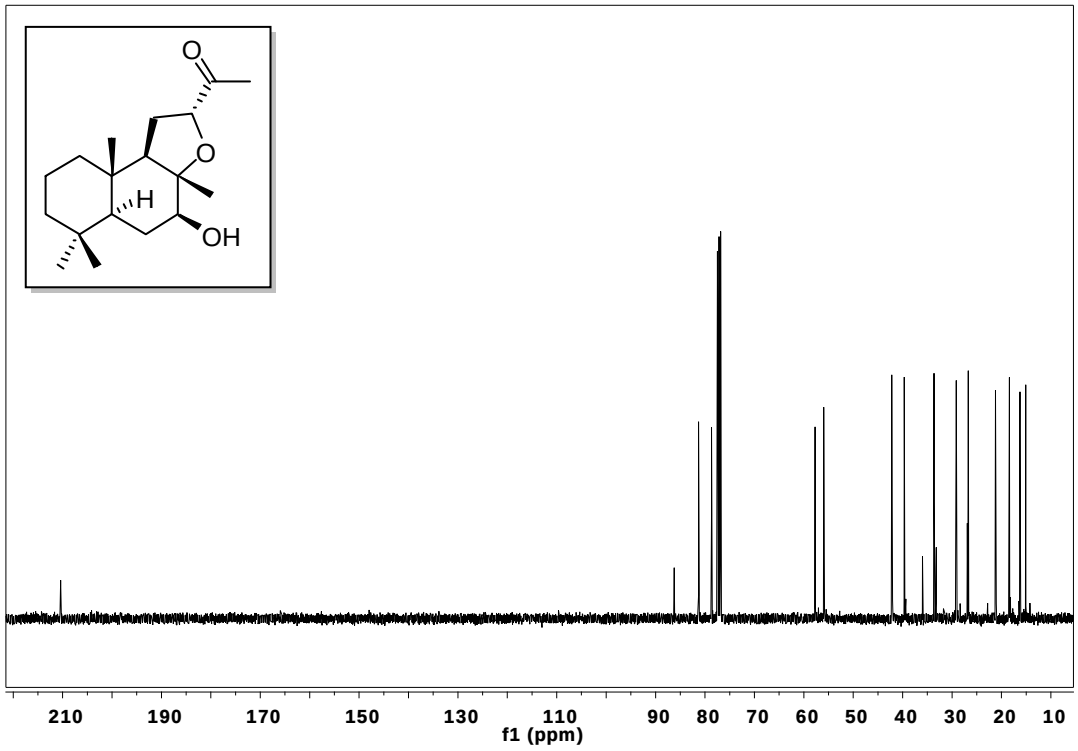

Figure A28 ^{13}C NMR spectrum of compound **184a**

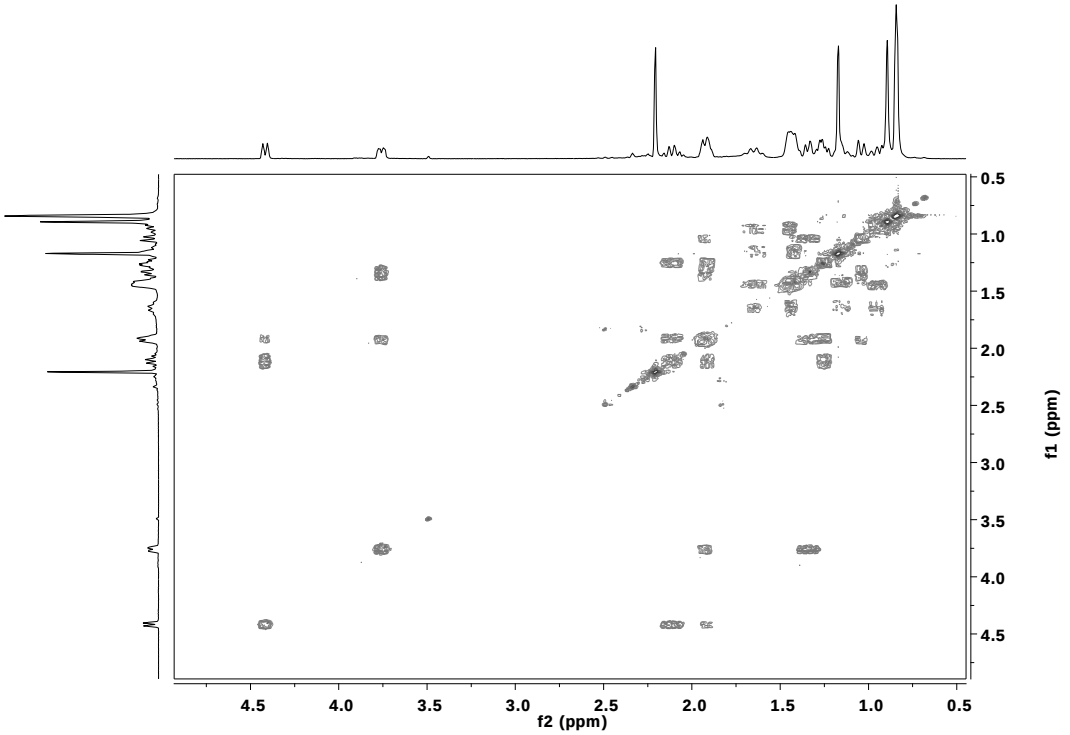

Figure A29 COSY spectrum of compound **184a**

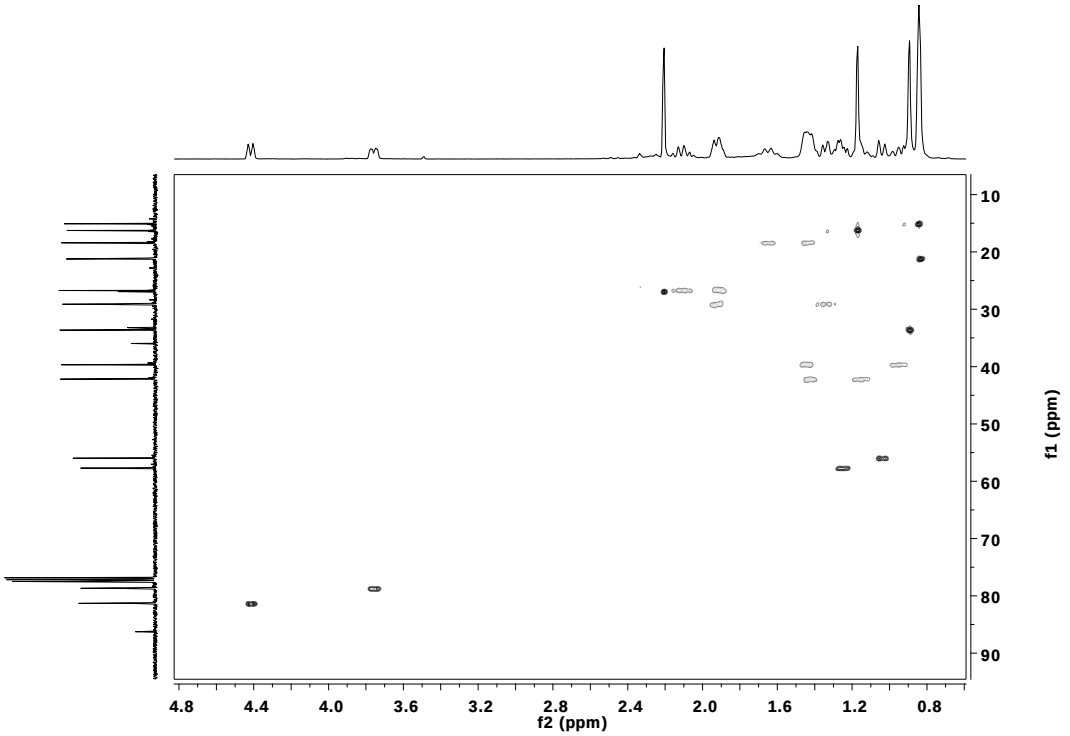

Figure A30 HSQC spectrum of compound **184a**

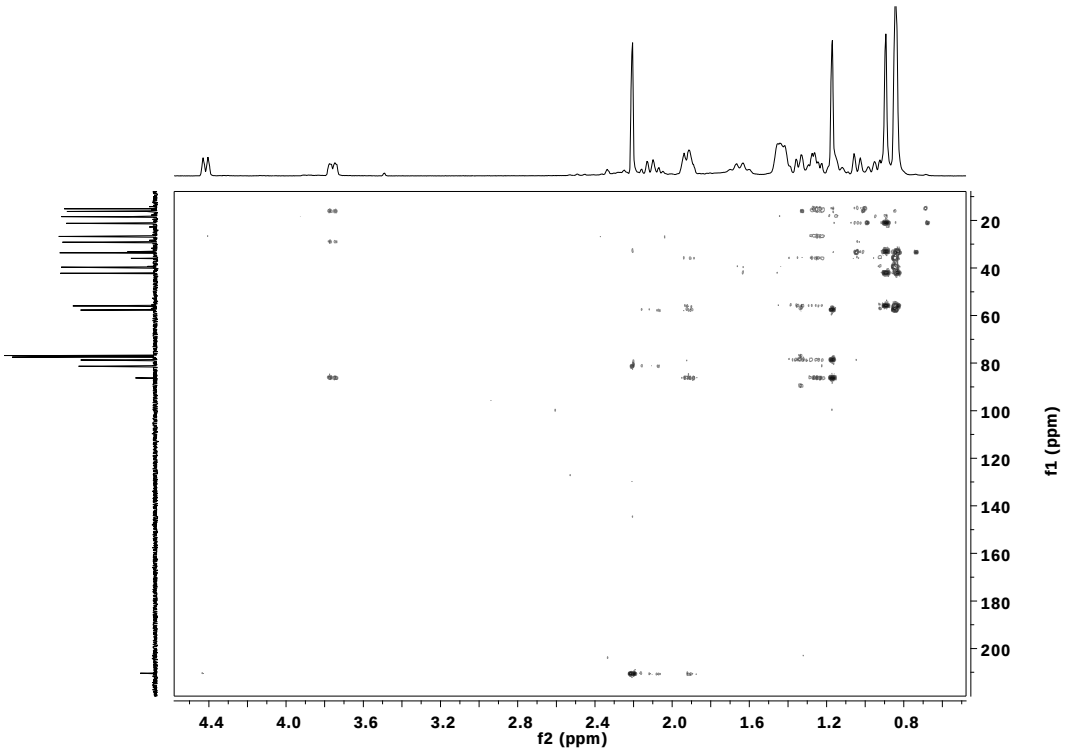

Figure A31 HMBC spectrum of compound **184a**

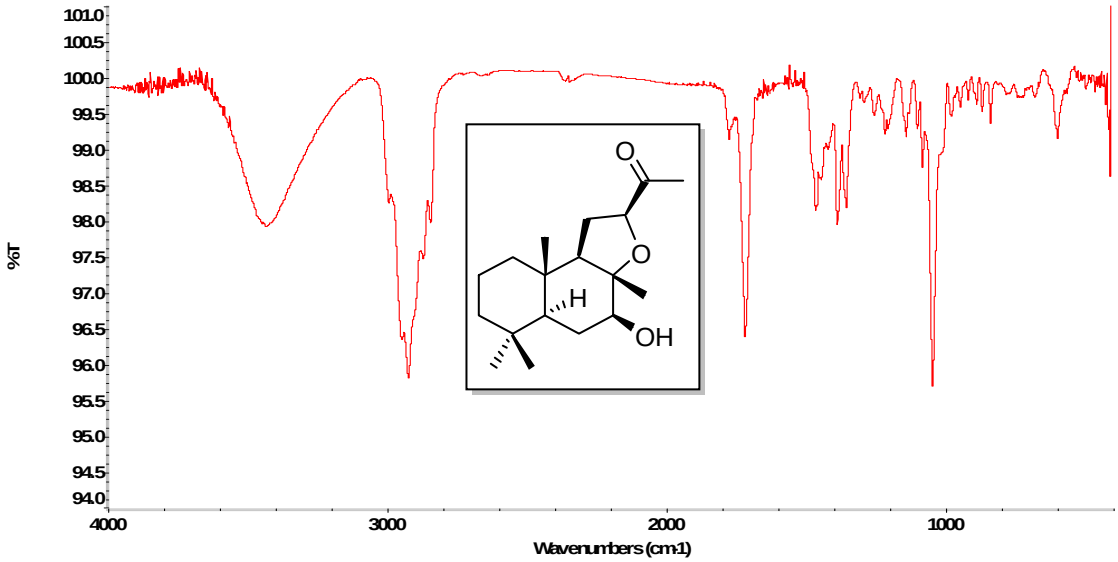

Figure A32 IR spectrum of compound **184b**

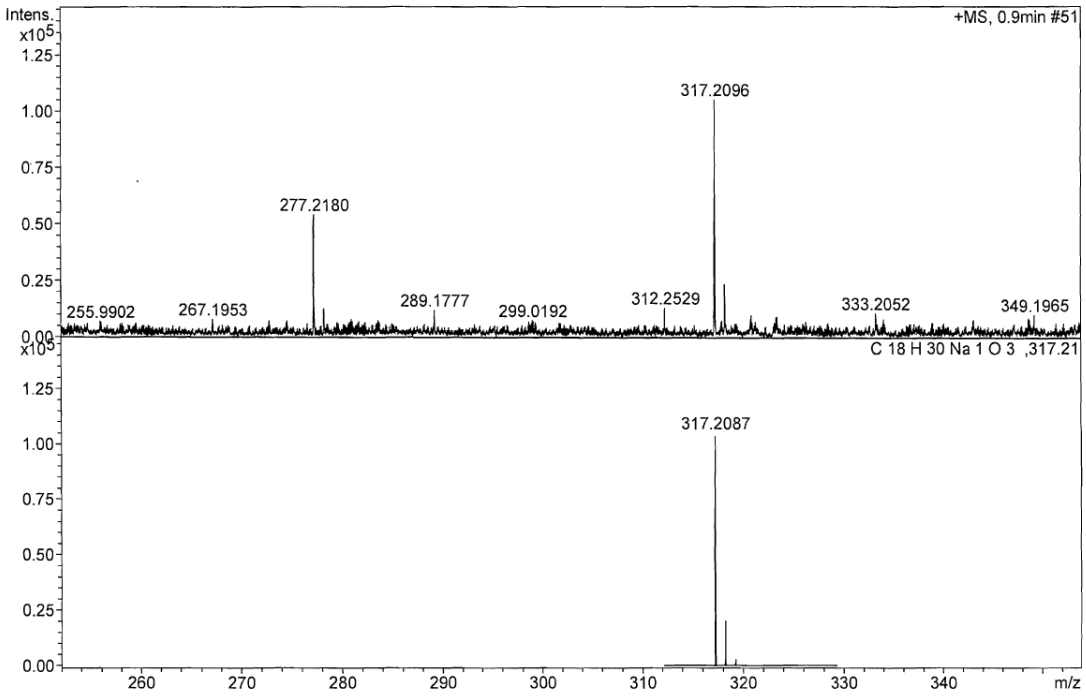

Figure A33 HRMS (ESI-TOF) spectrum of compound **184b**

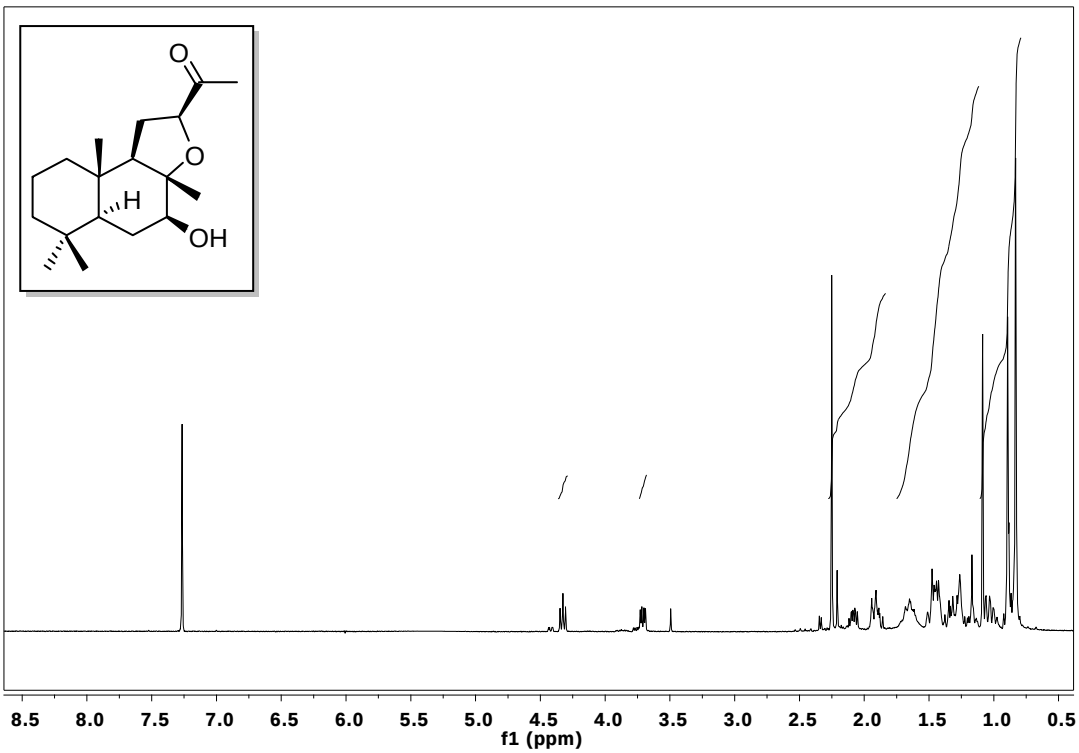

Figure A34 ^{1}H NMR spectrum of compound **184b**

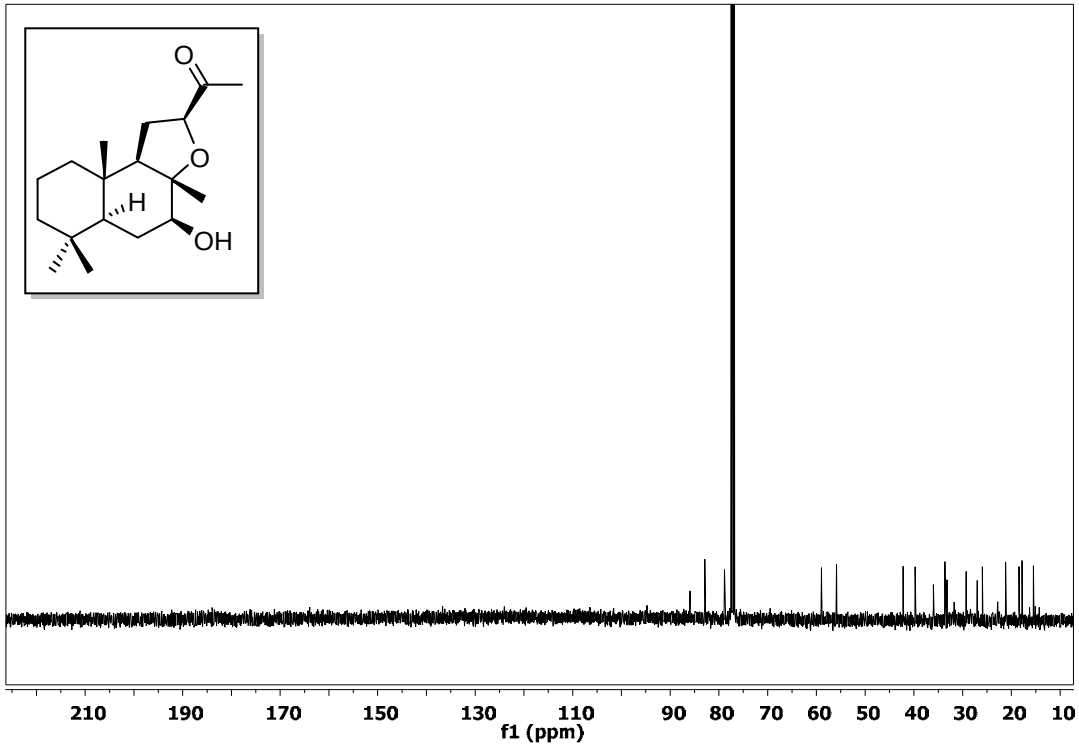

Figure A35 ^{13}C NMR spectrum of compound **184b**

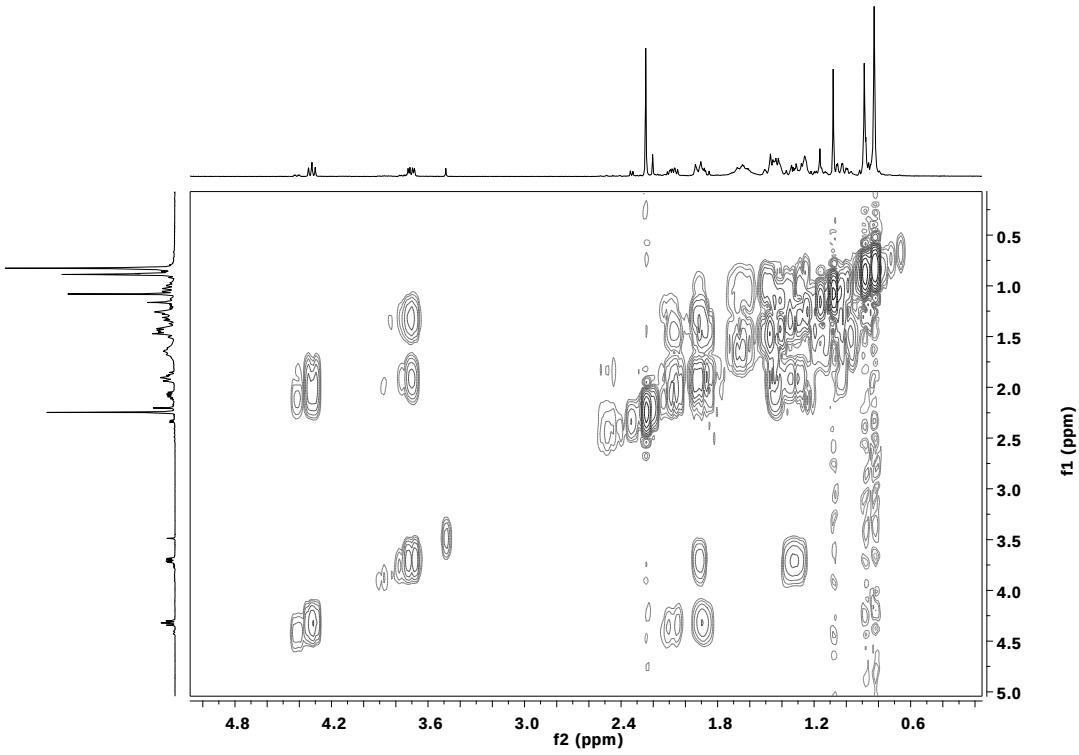

Figure A36 COSY NMR spectrum of compound **184b**

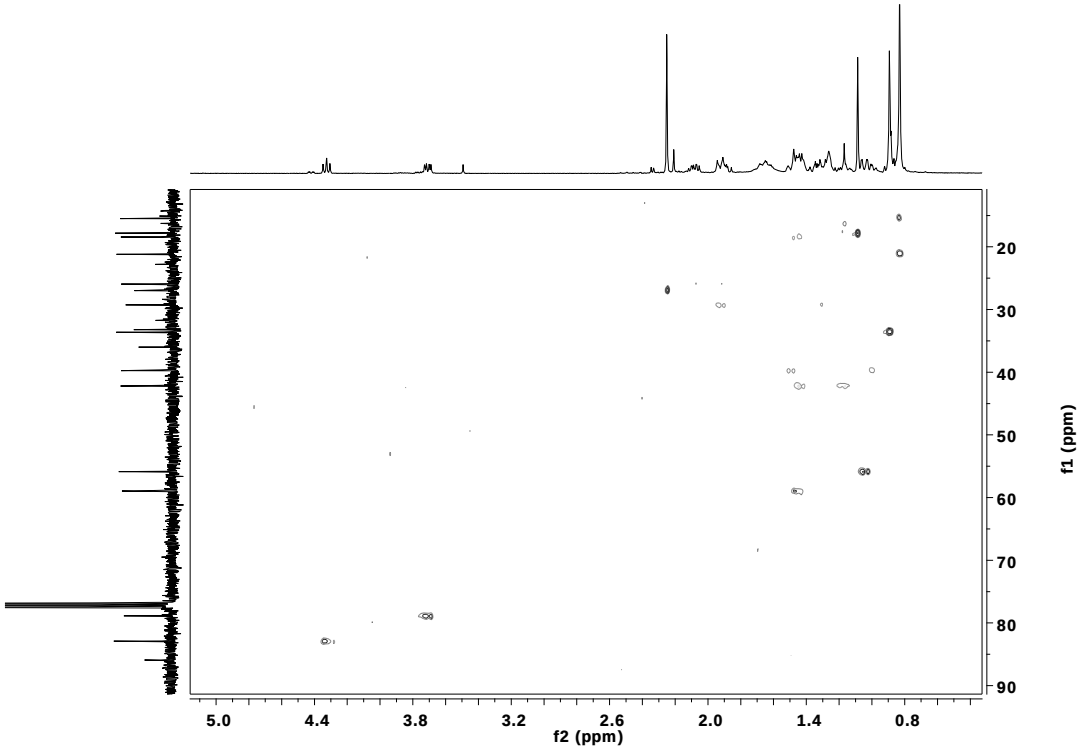

Figure A37 HSQC NMR spectrum of compound **184b**

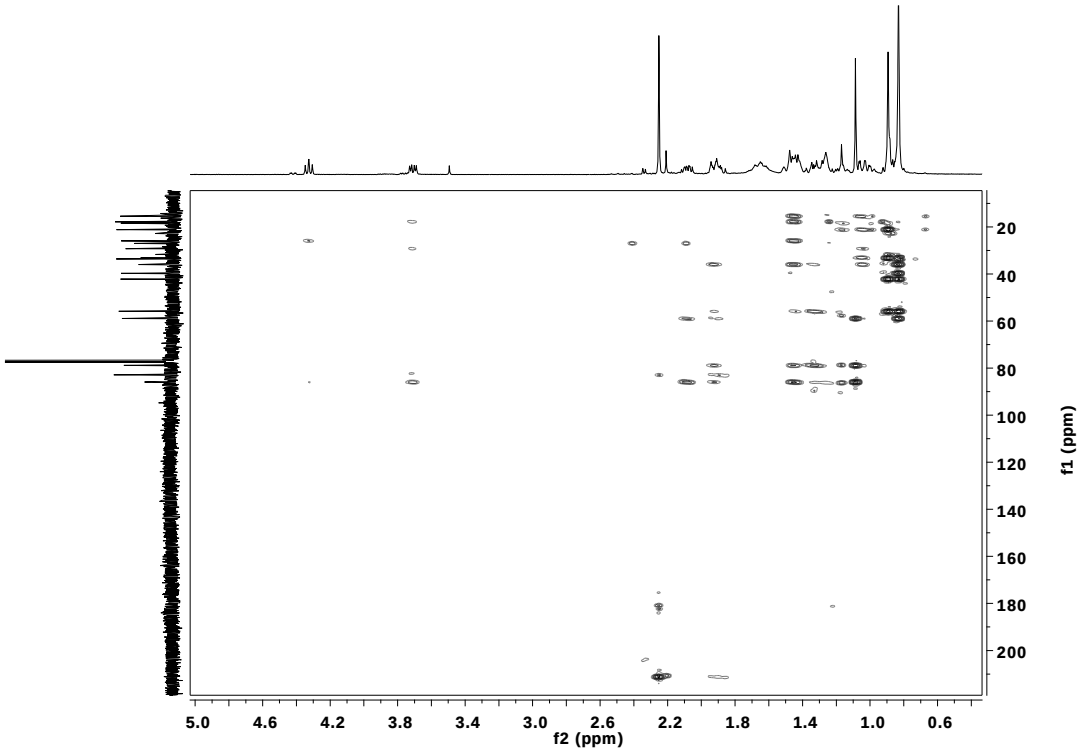

Figure A38 HMBC NMR spectrum of compound **184b**

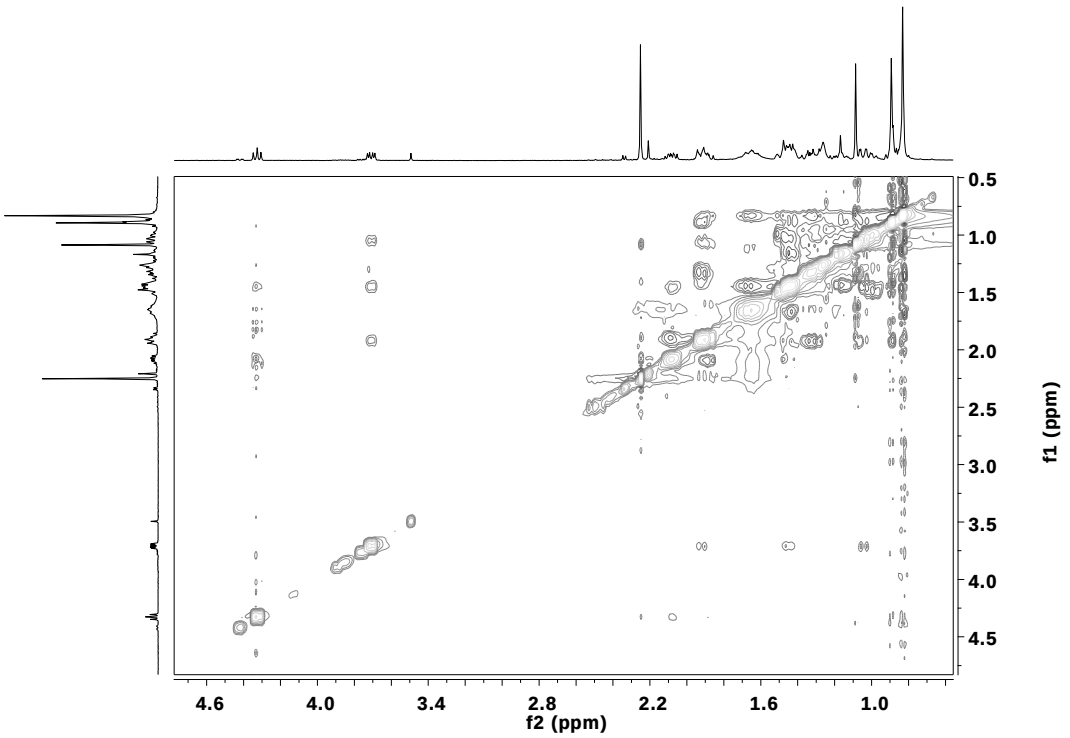

Figure A39 NOESY spectrum of compound **184b**

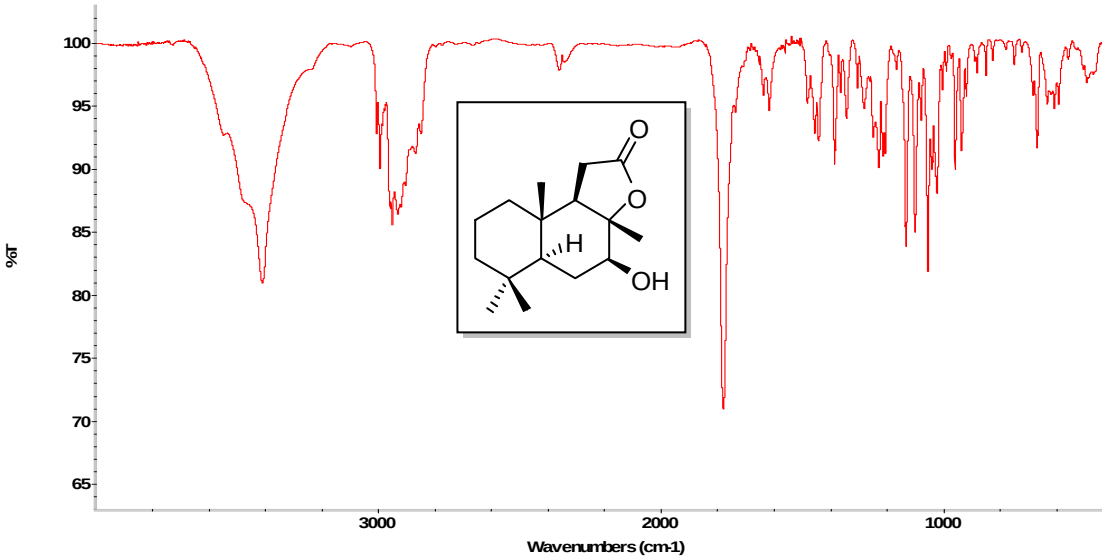

Figure A40 IR spectrum of compound **185**

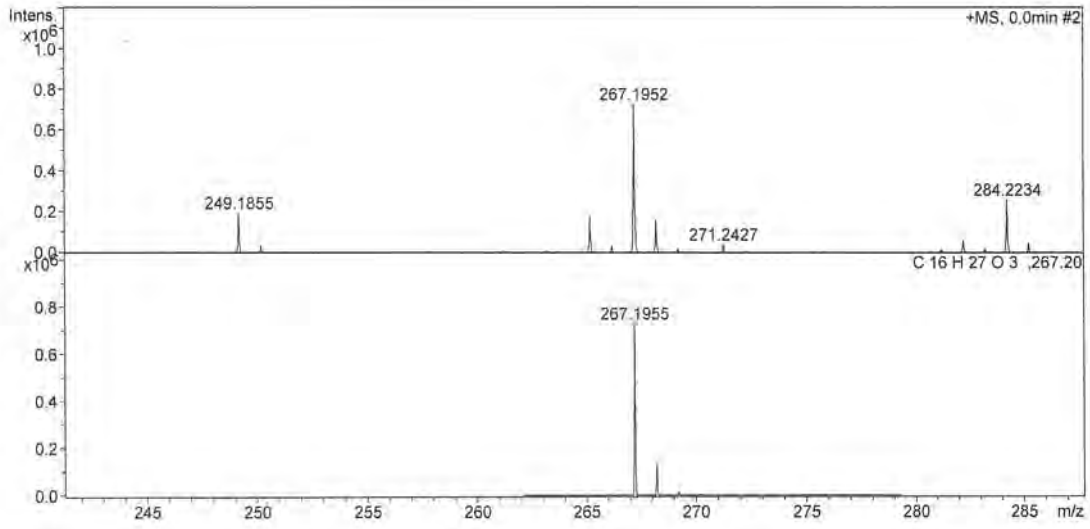

Figure A41 HRMS (ESI-TOF) spectrum of compound **185**

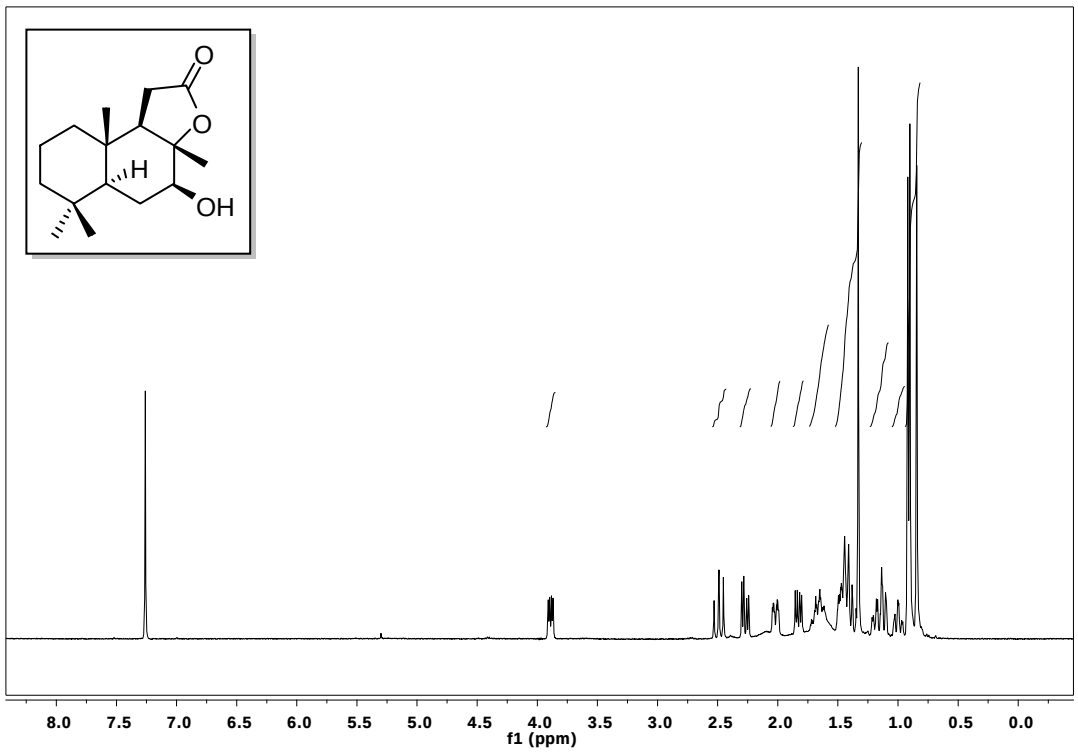

Figure A42 ^{1}H NMR spectrum of compound **185**

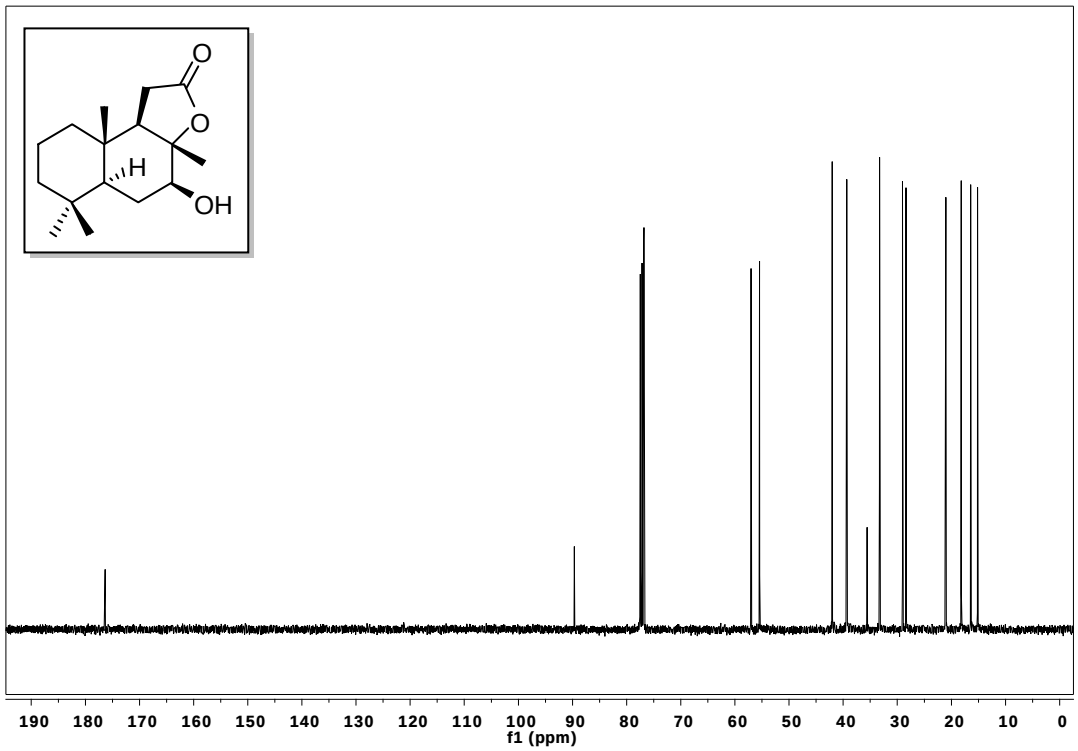

Figure A43 ^{13}C NMR spectrum of compound **185**

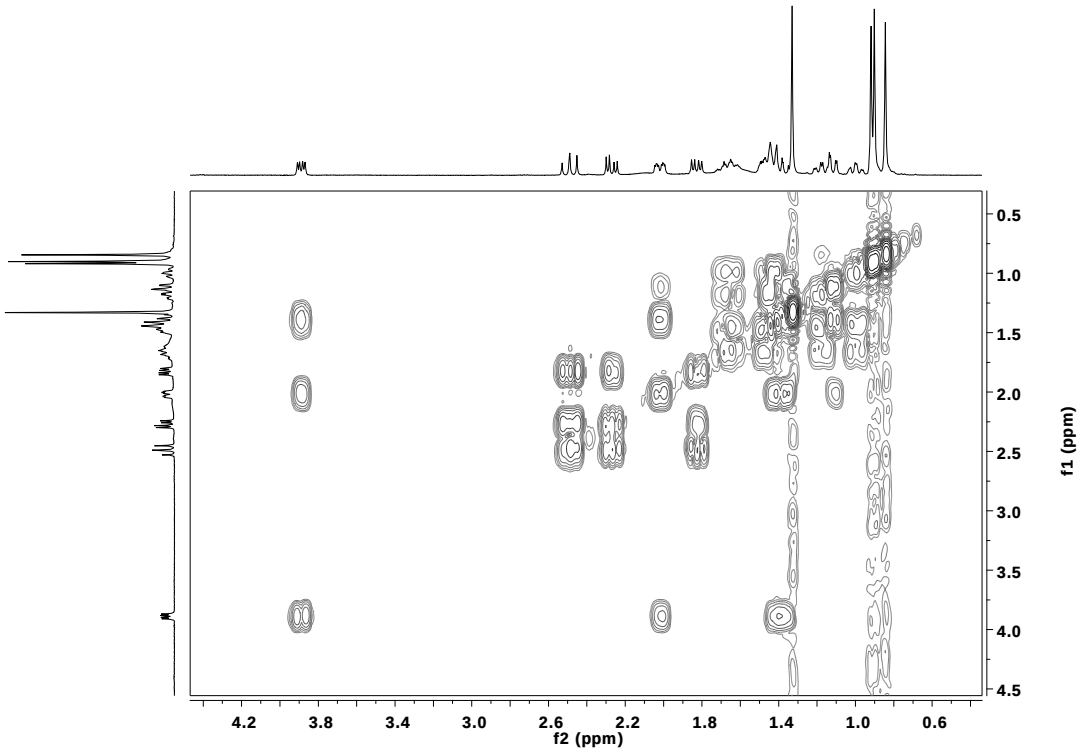

Figure A44 COSY spectrum of compound **185**

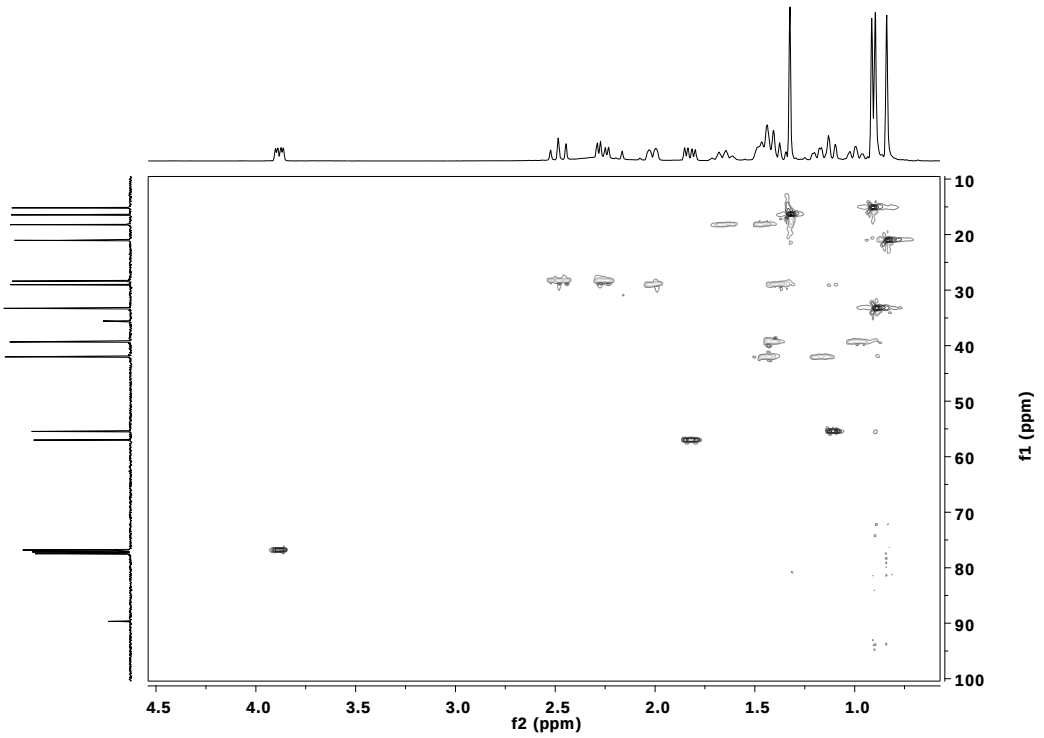

Figure A45 HSQC spectrum of compound **185**

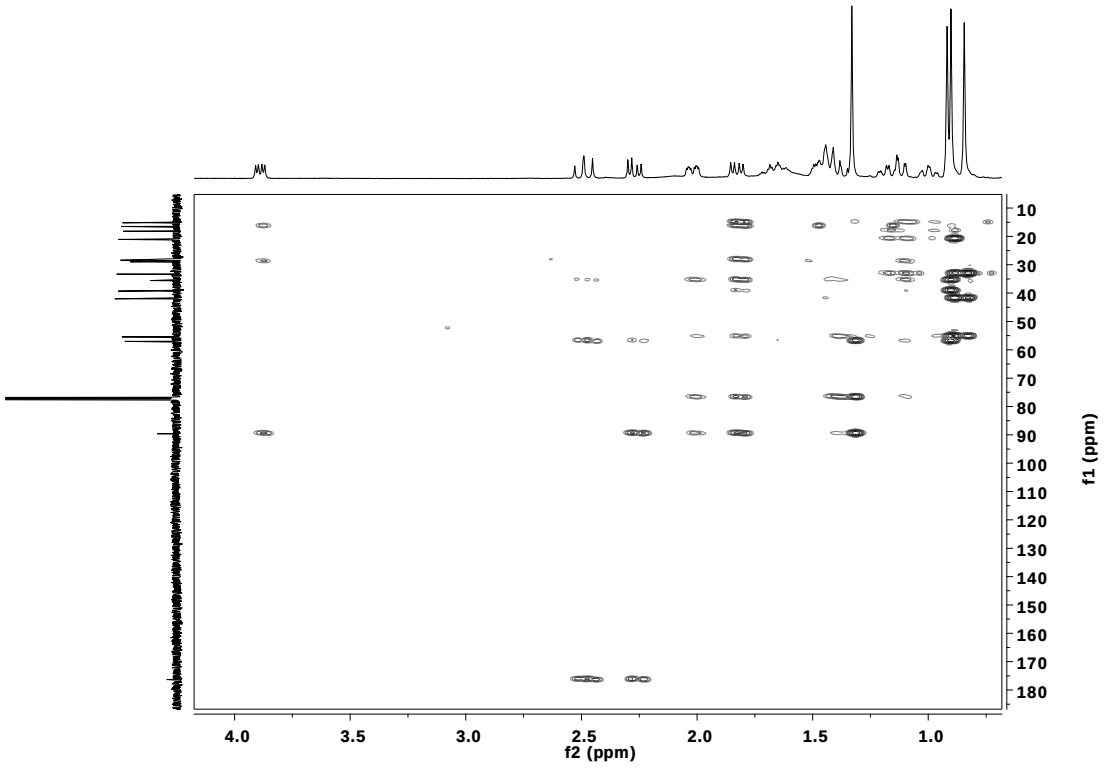

Figure A46 HMBC spectrum of compound **185**

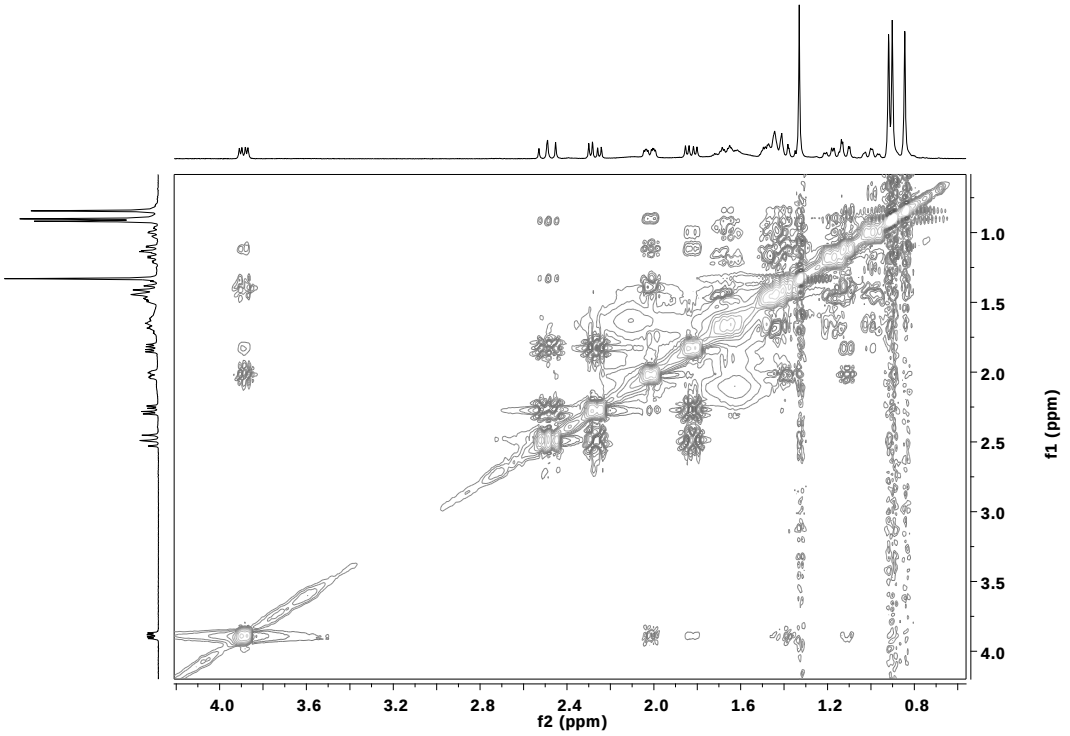

Figure A47 NOESY spectrum of compound **185**

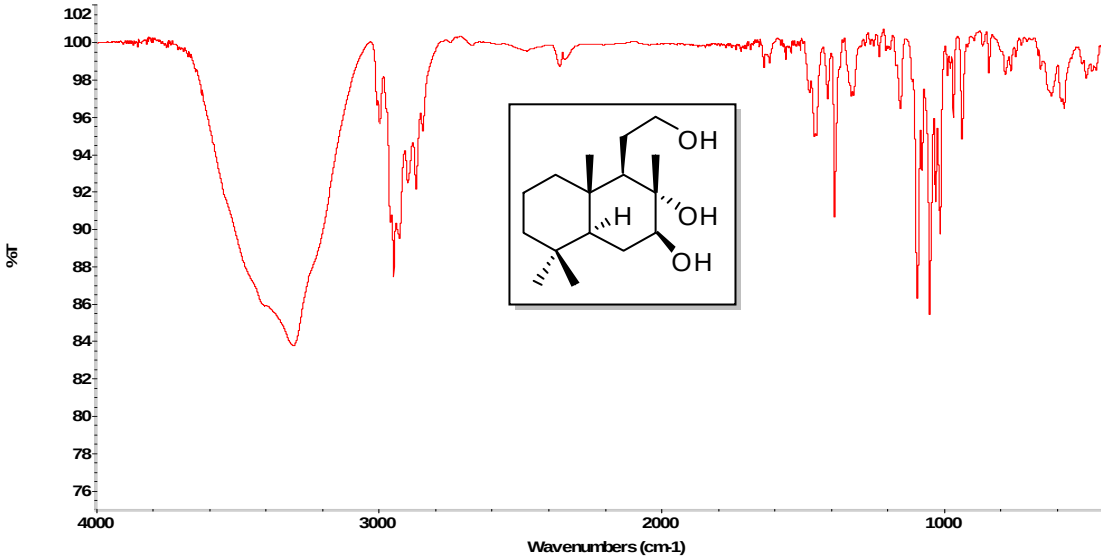

Figure A48 IR spectrum of compound **186**

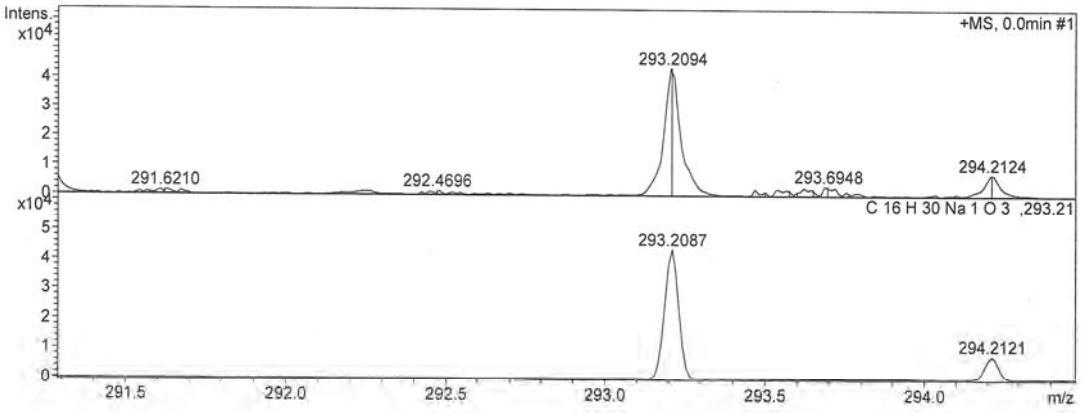

Figure A49 HRMS (ESI-TOF) spectrum of compound **186**

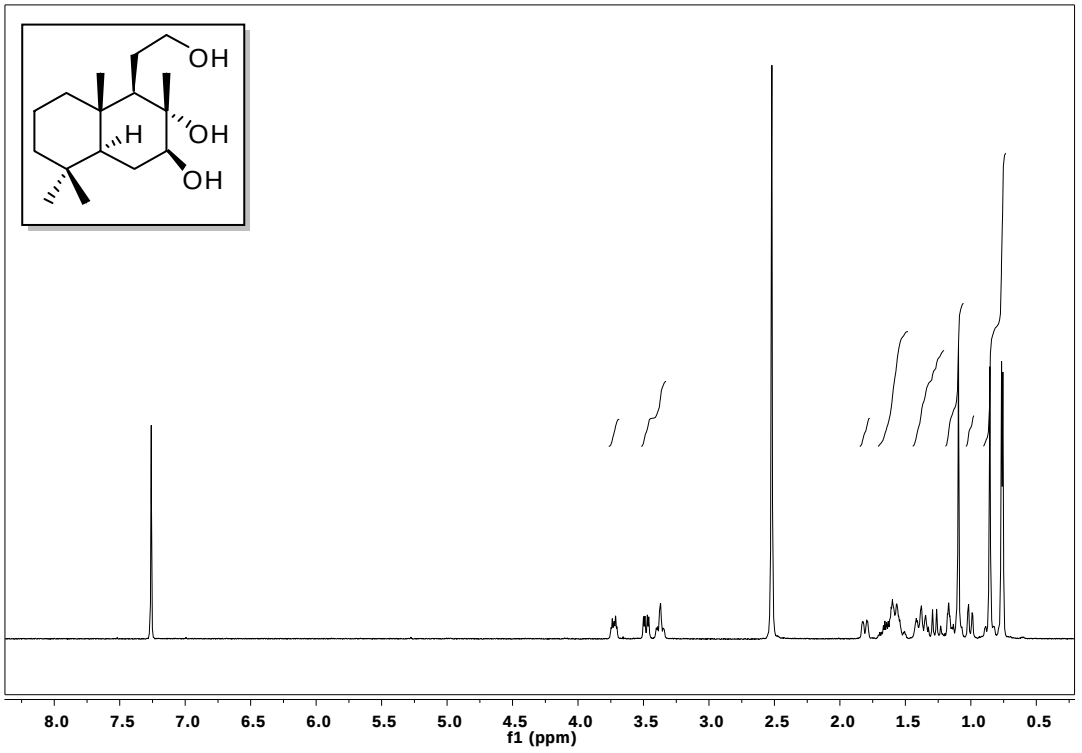

Figure A50 ^{1}H NMR spectrum of compound **186**

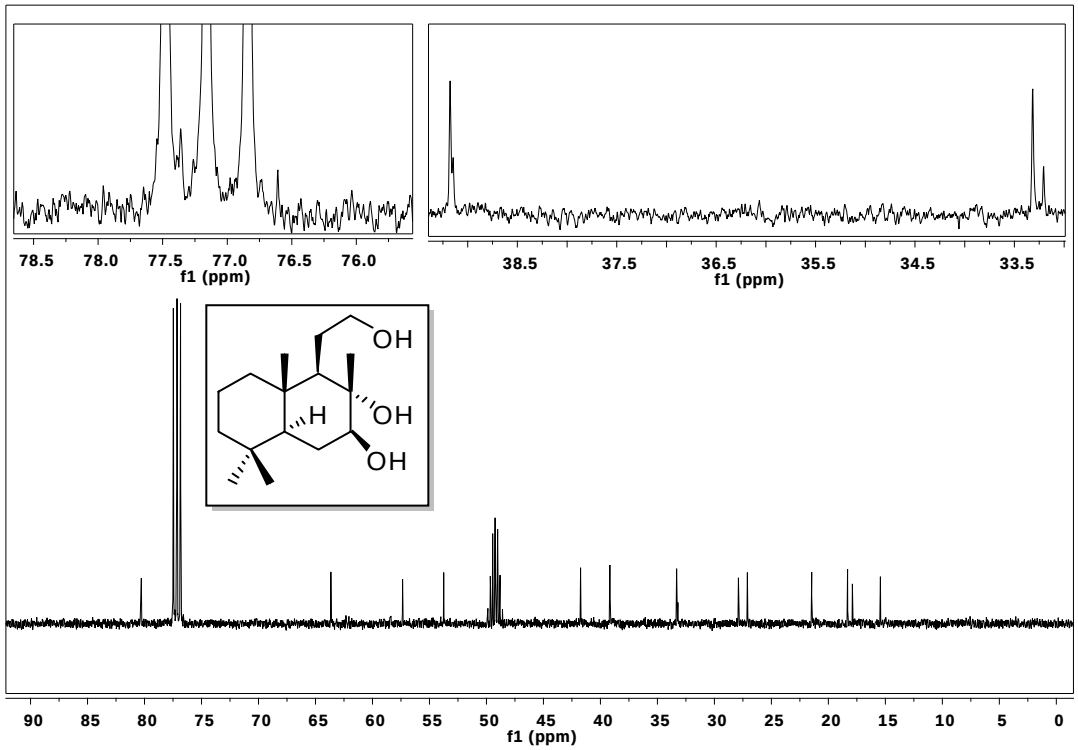

Figure A51 ^{13}C NMR spectrum of compound **186**

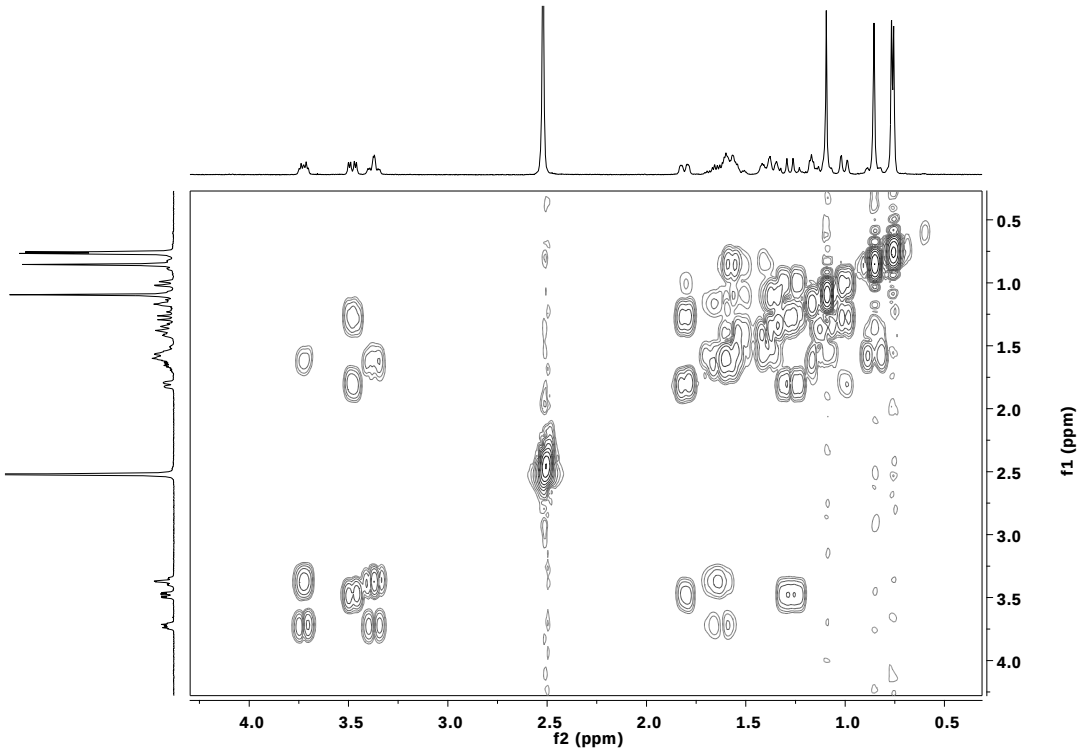

Figure A52 COSY spectrum of compound **186**

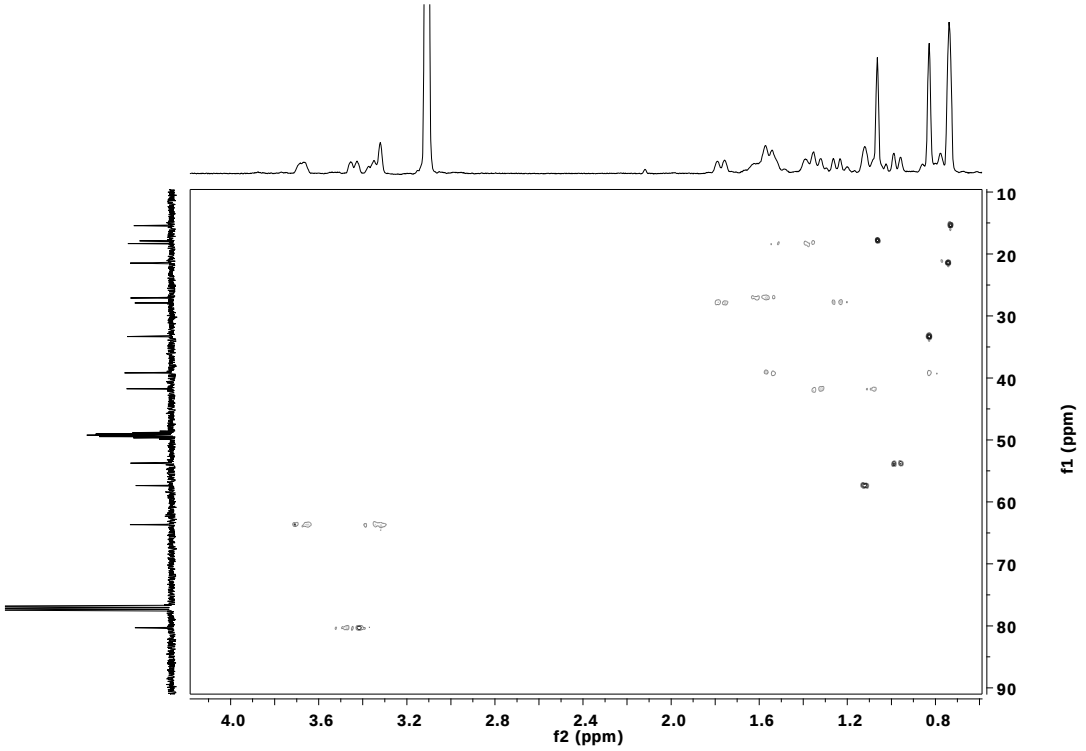

Figure A53 HSQC spectrum of compound **186**

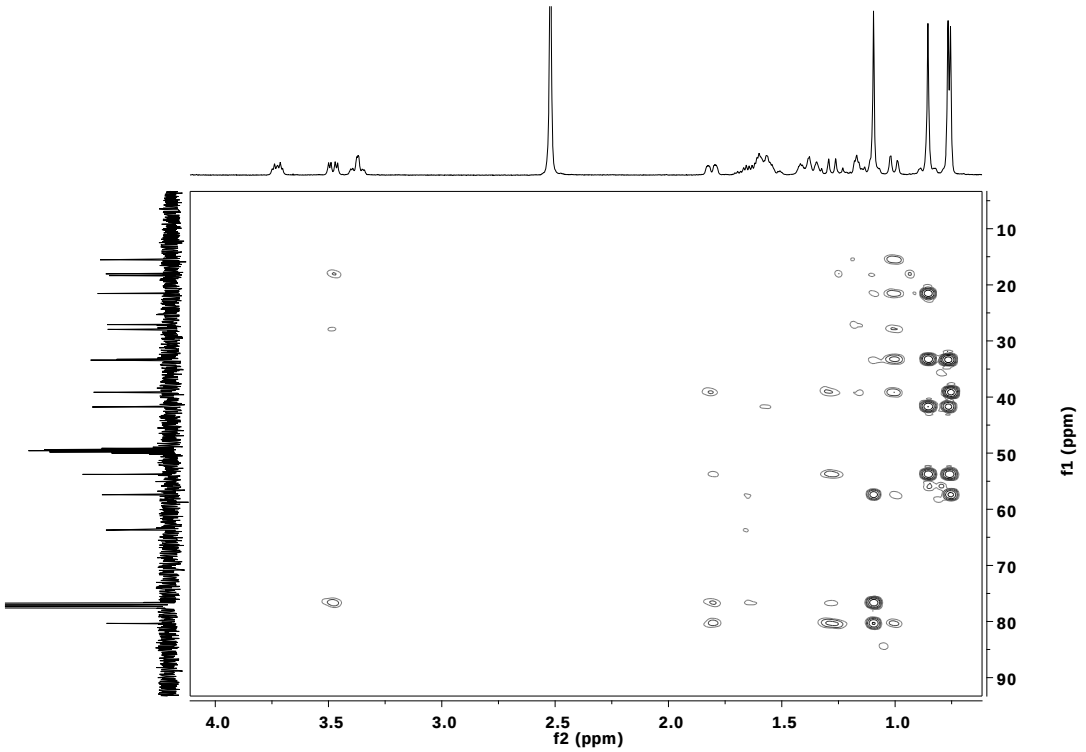

Figure A54 HMBC spectrum of compound **186**

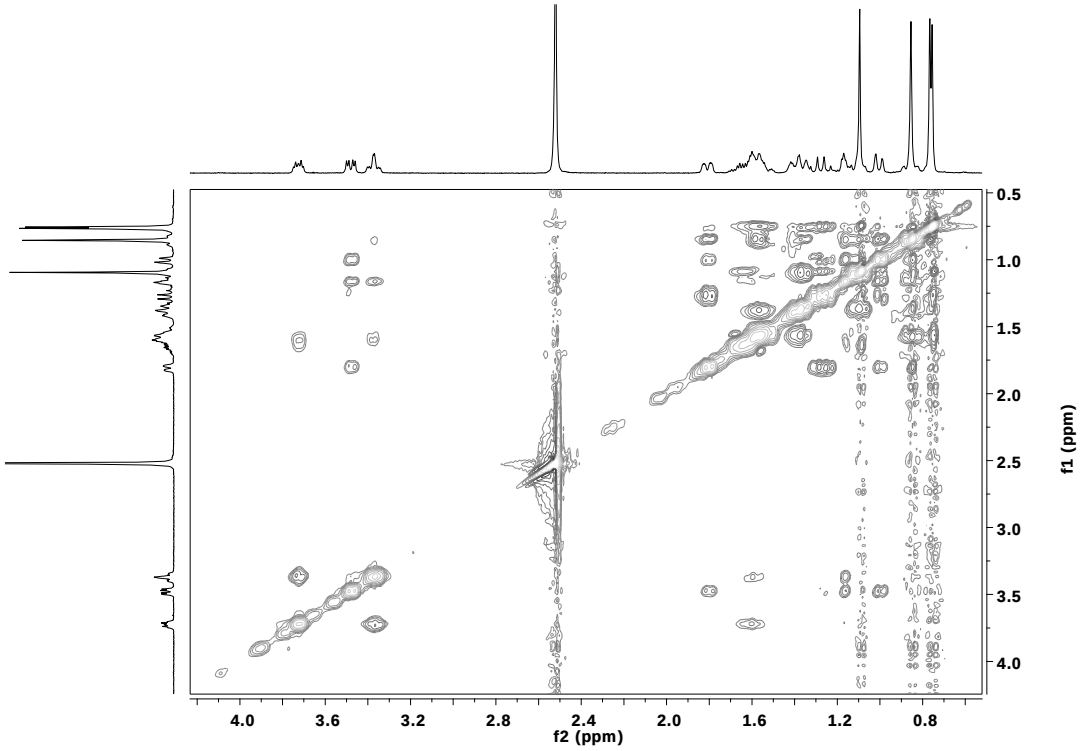

Figure A55 NOESY spectrum of compound **186**

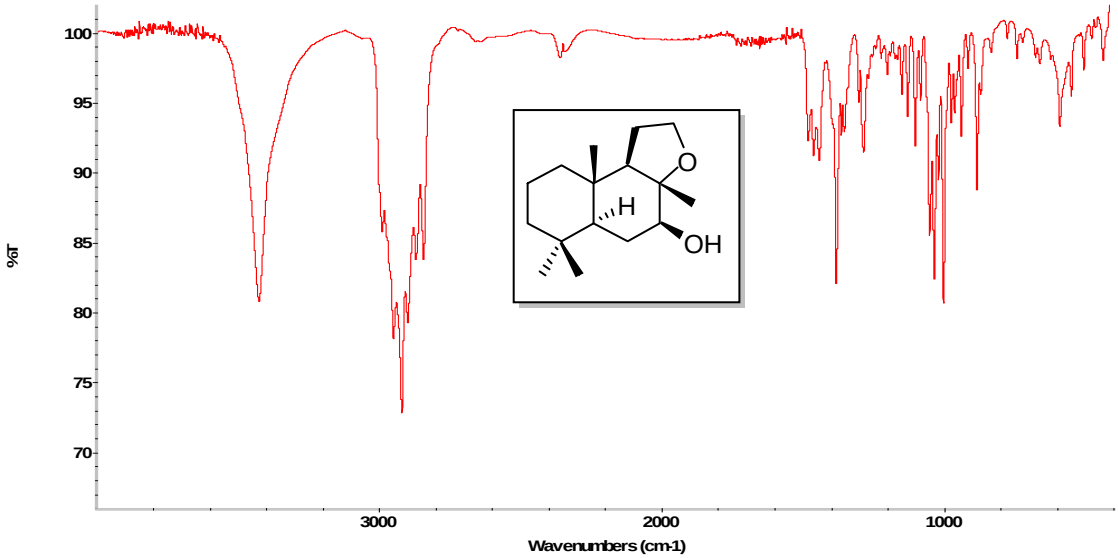

Figure A56 IR spectrum of compound **187**

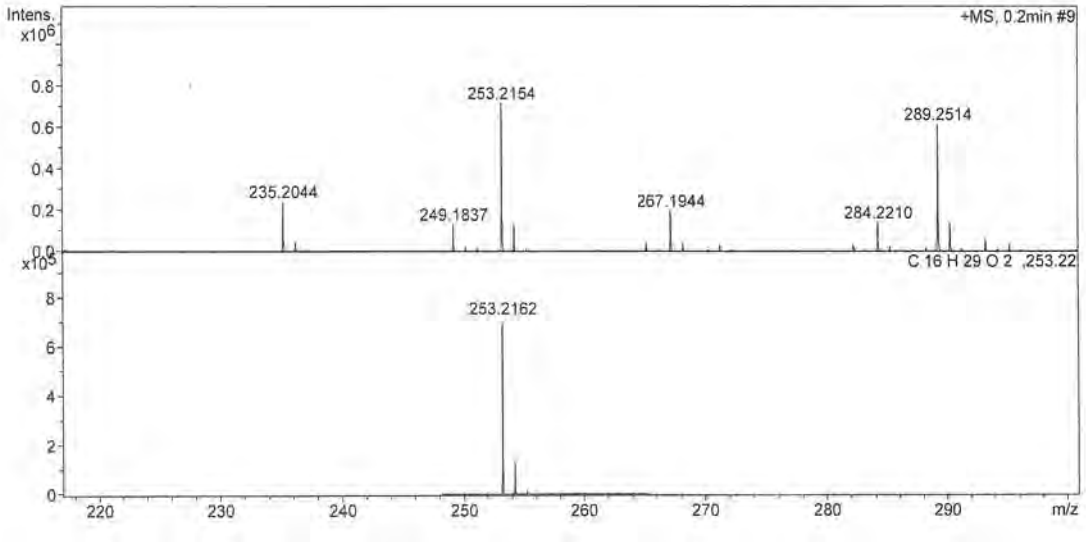

Figure A57 HRMS (ESI-TOF) spectrum of compound **187**

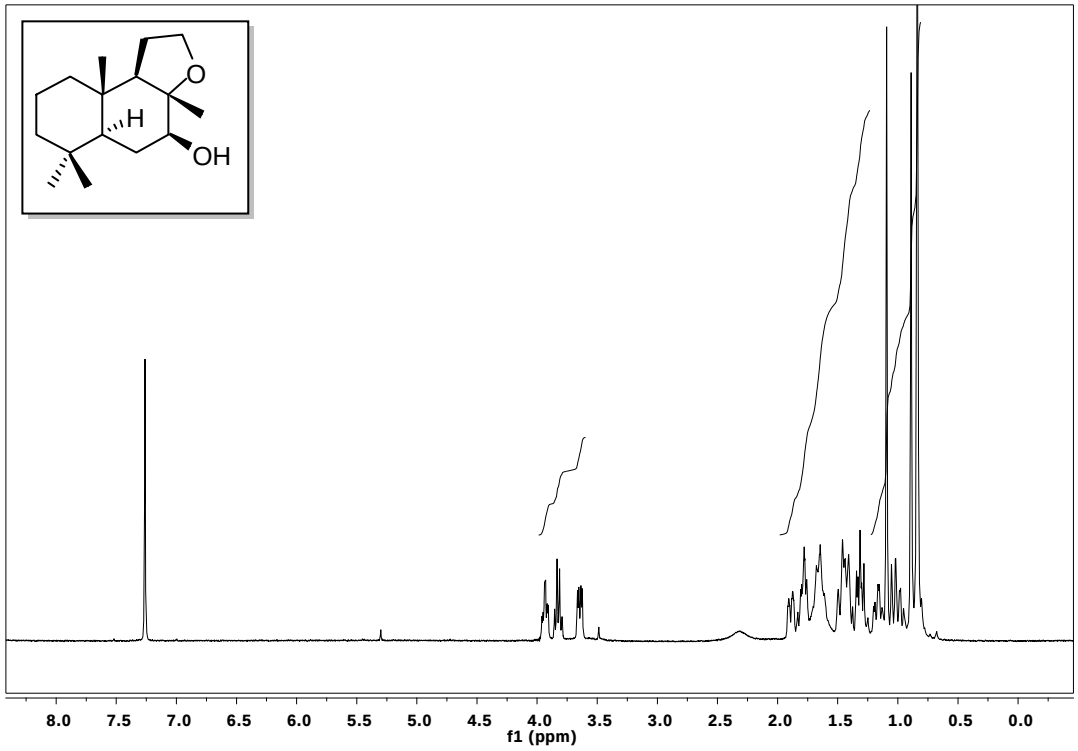

Figure A58 ^{1}H NMR spectrum of compound **187**

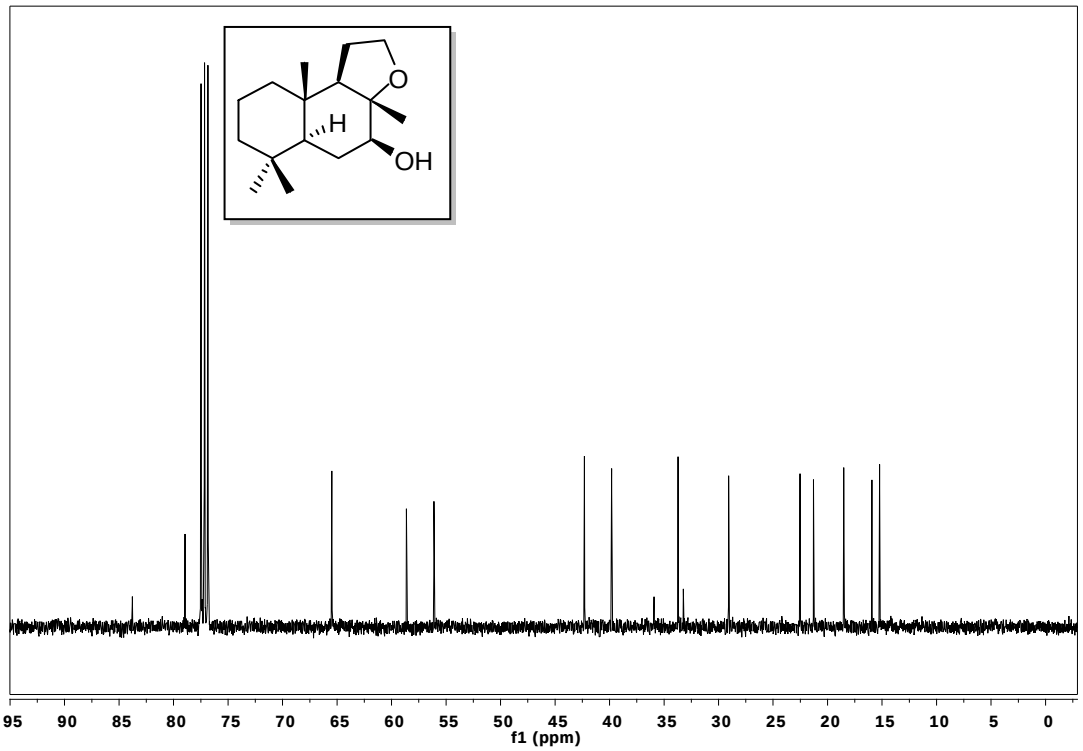

Figure A59 ^{13}C NMR spectrum of compound **187**

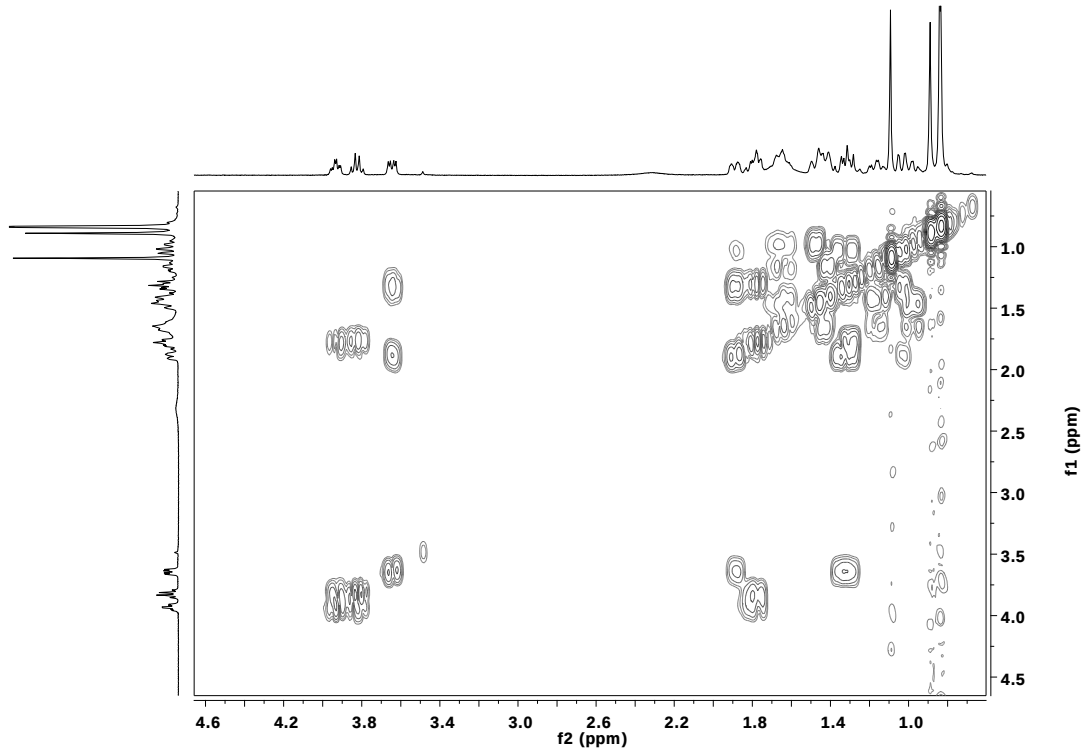

Figure A60 COSY spectrum of compound **187**

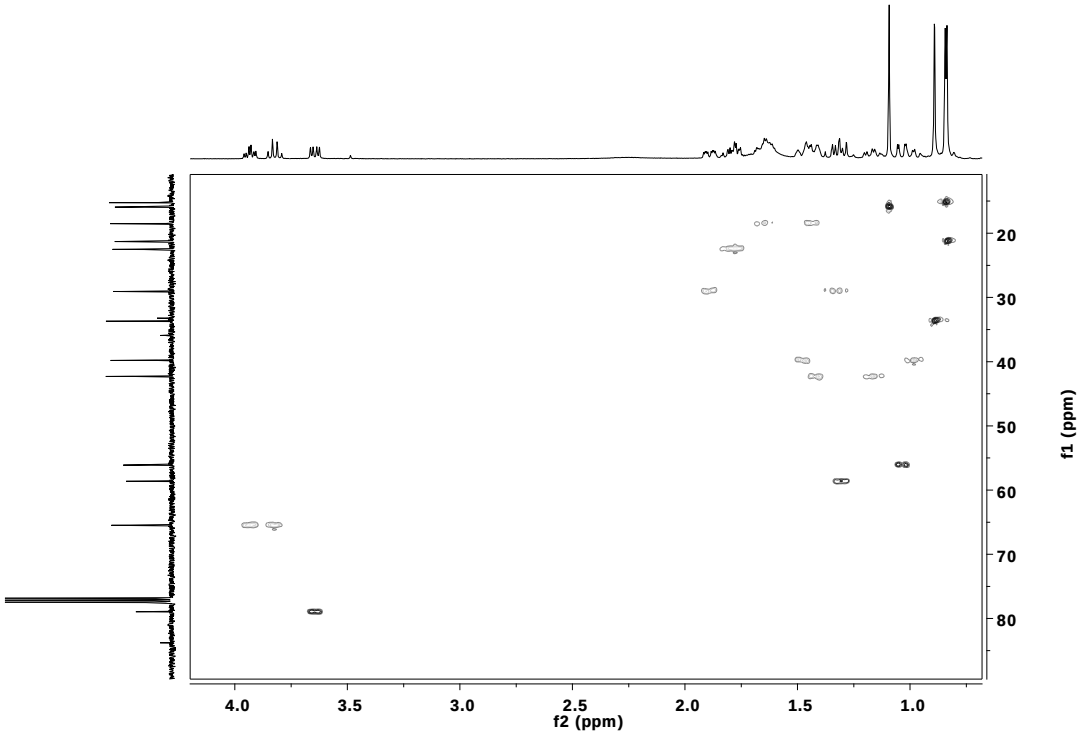

Figure A61 HSQC spectrum of compound **187**

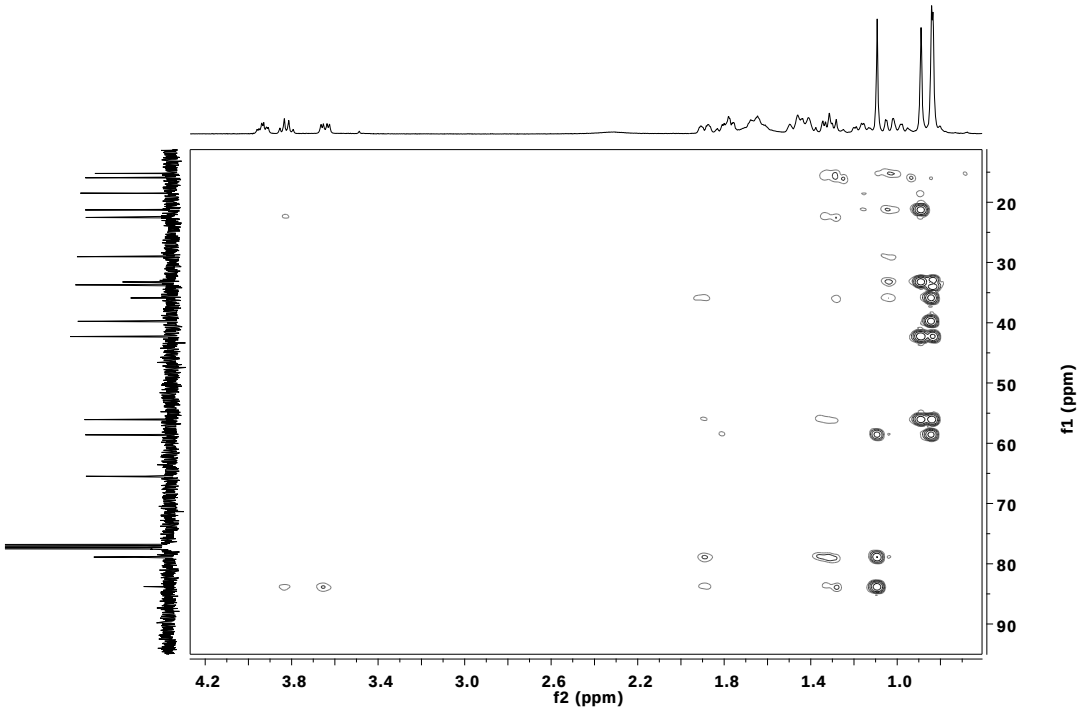

Figure A62 HMBC spectrum of compound **187**

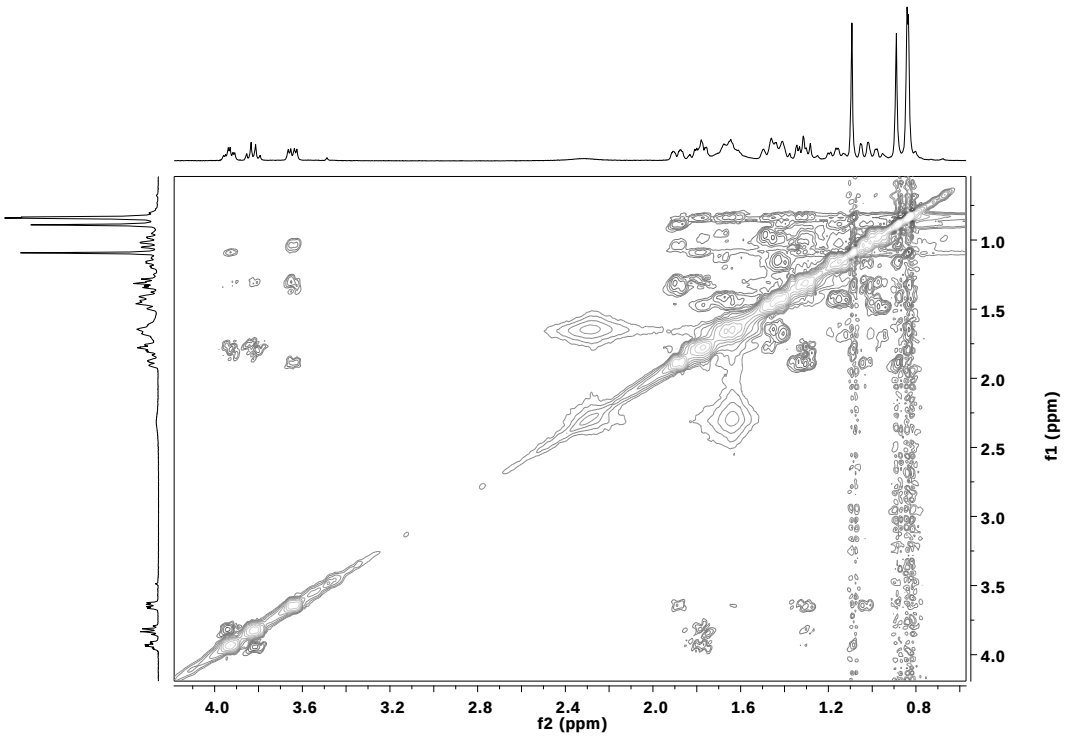

Figure A63 NOESY spectrum of compound **187**

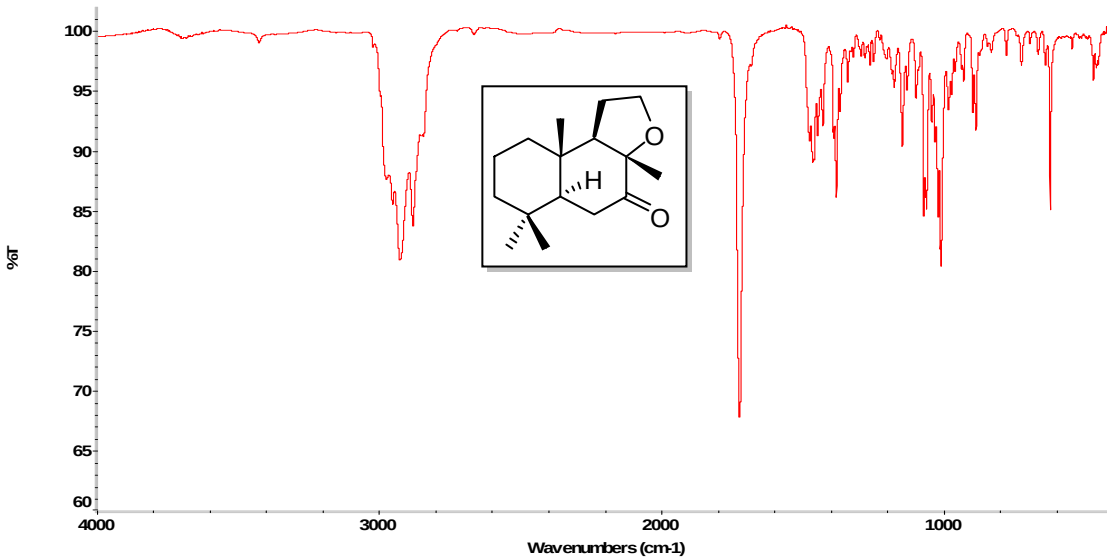

Figure A64 IR spectrum of compound **188**

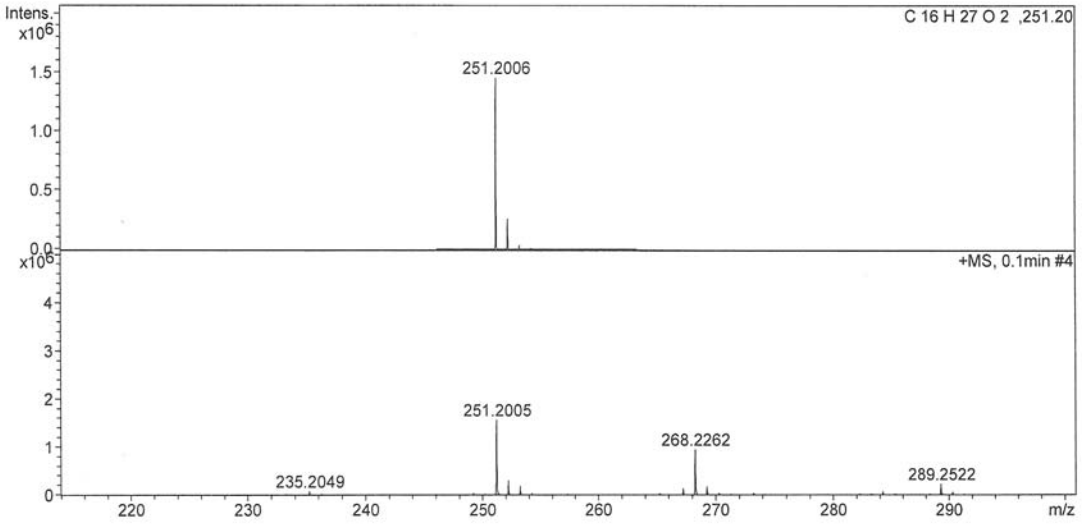

Figure A65 HRMS (ESI-TOF) spectrum of compound **188**

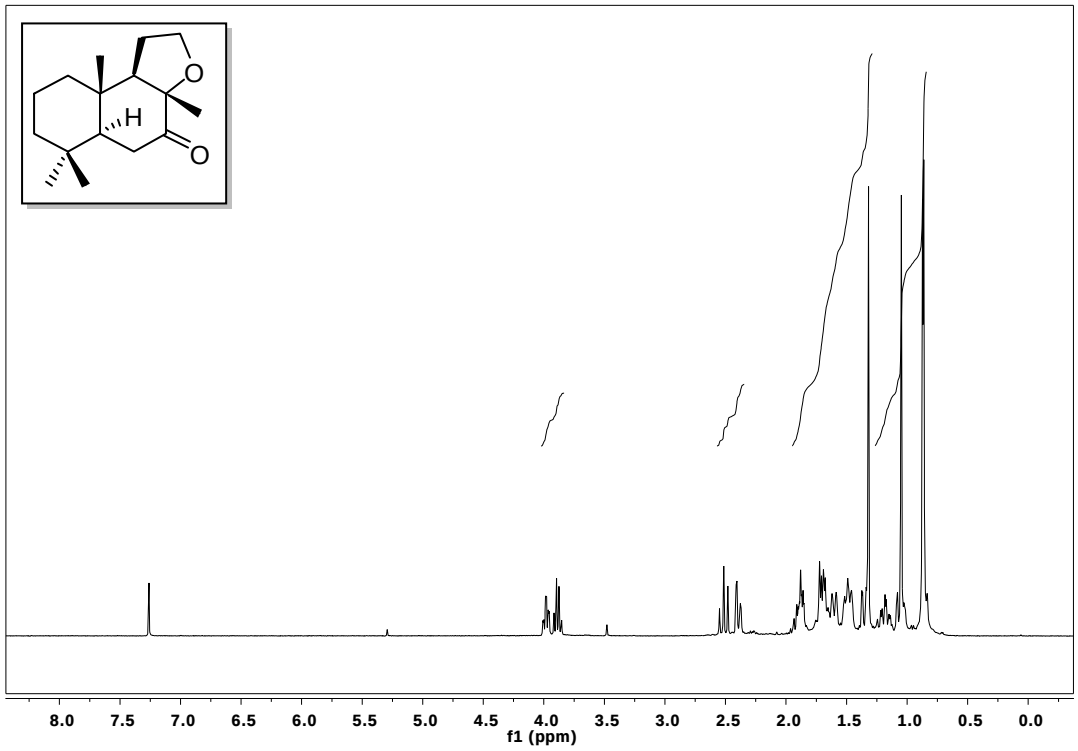

Figure A66 ^{1}H NMR spectrum of compound **188**

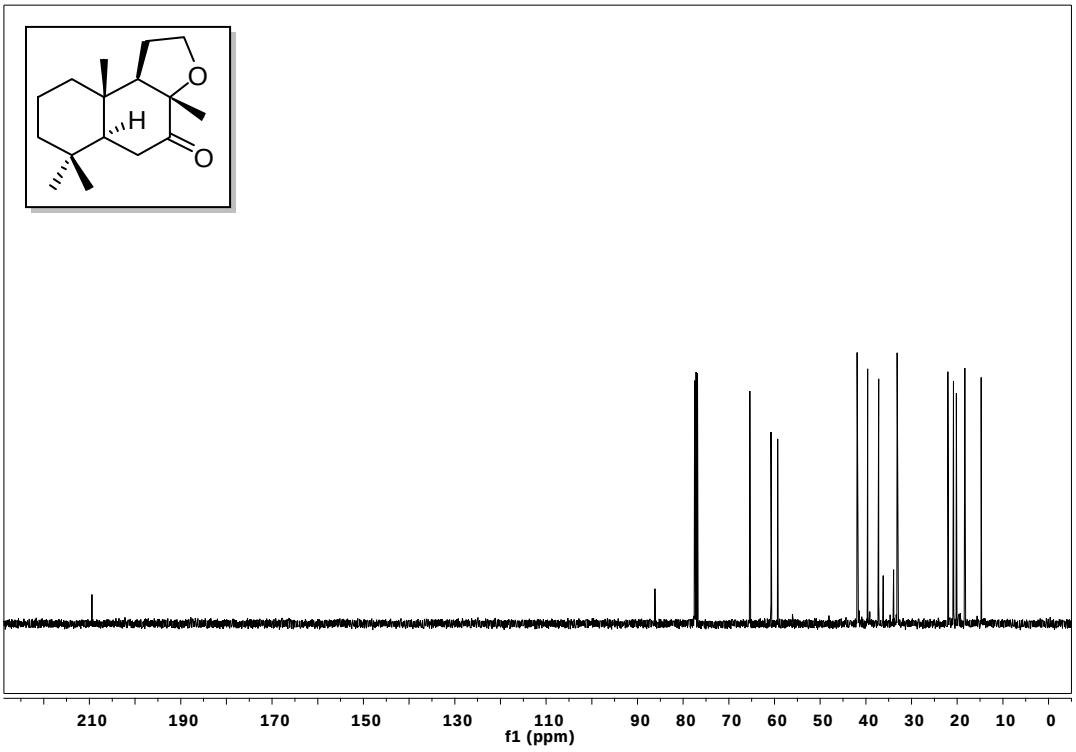

Figure A67 ^{13}C NMR spectrum of compound **188**

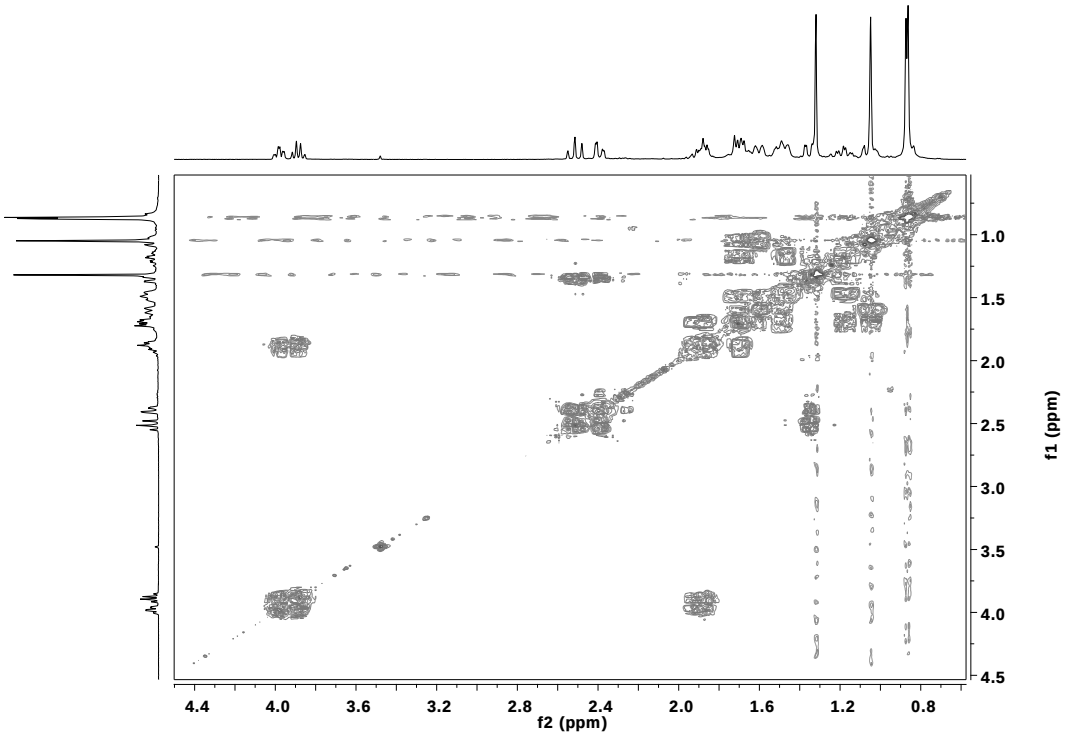

Figure A68 COSYspectrum of compound **188**

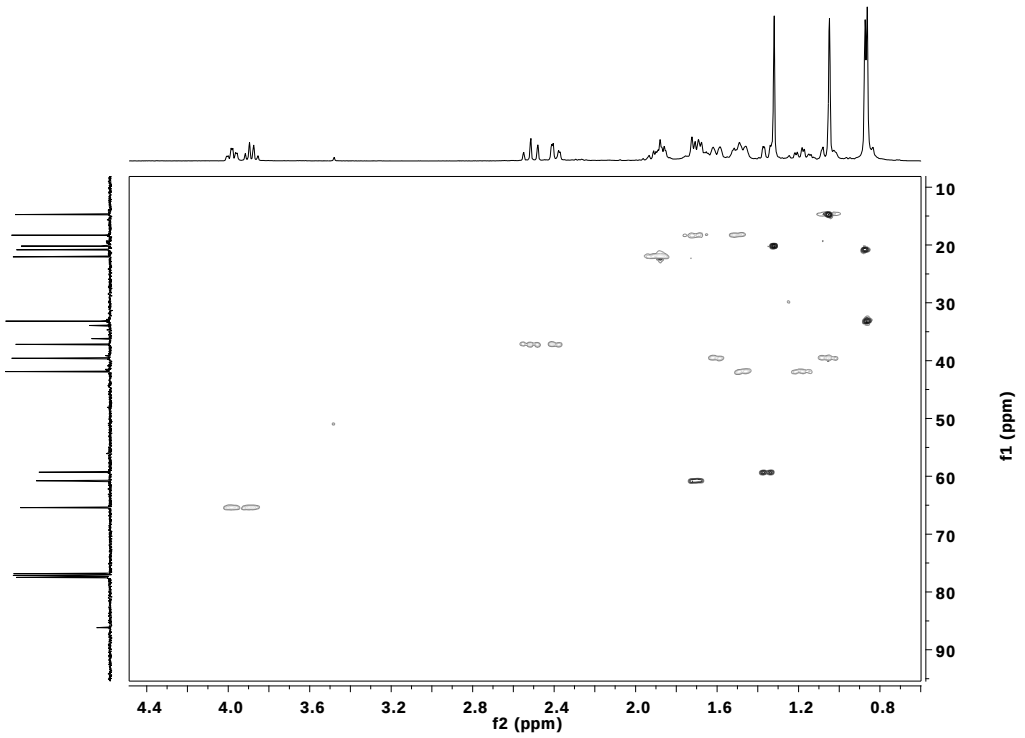

Figure A69 HSQC spectrum of compound **188**

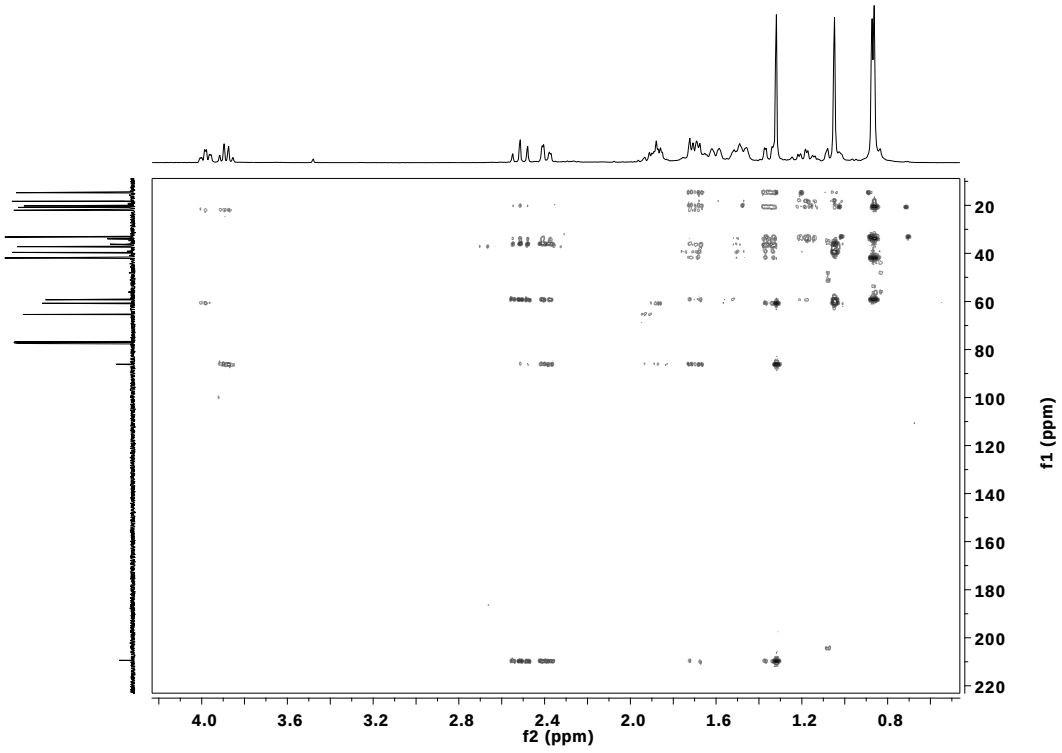

Figure A70 HMBC spectrum of compound **188**

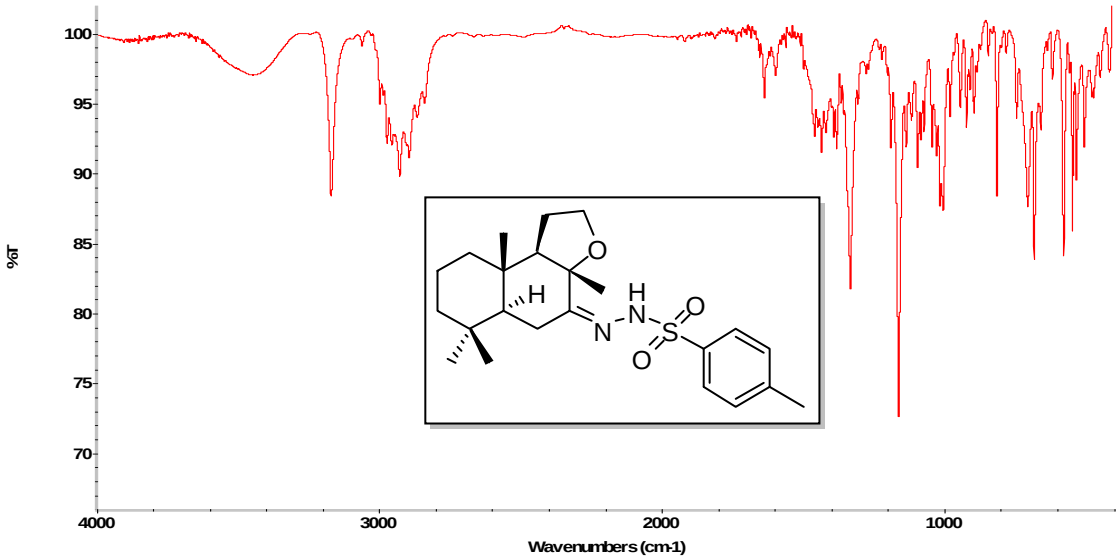

Figure A71 IR spectrum of compound **189**

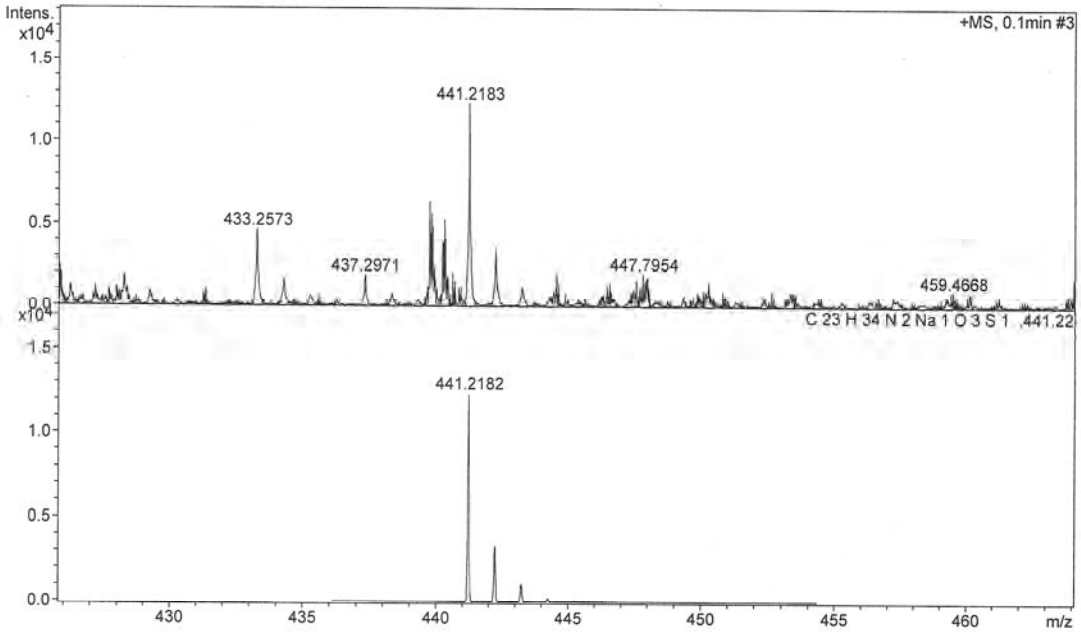

Figure A72 HRMS (ESI-TOF) spectrum of compound **189**

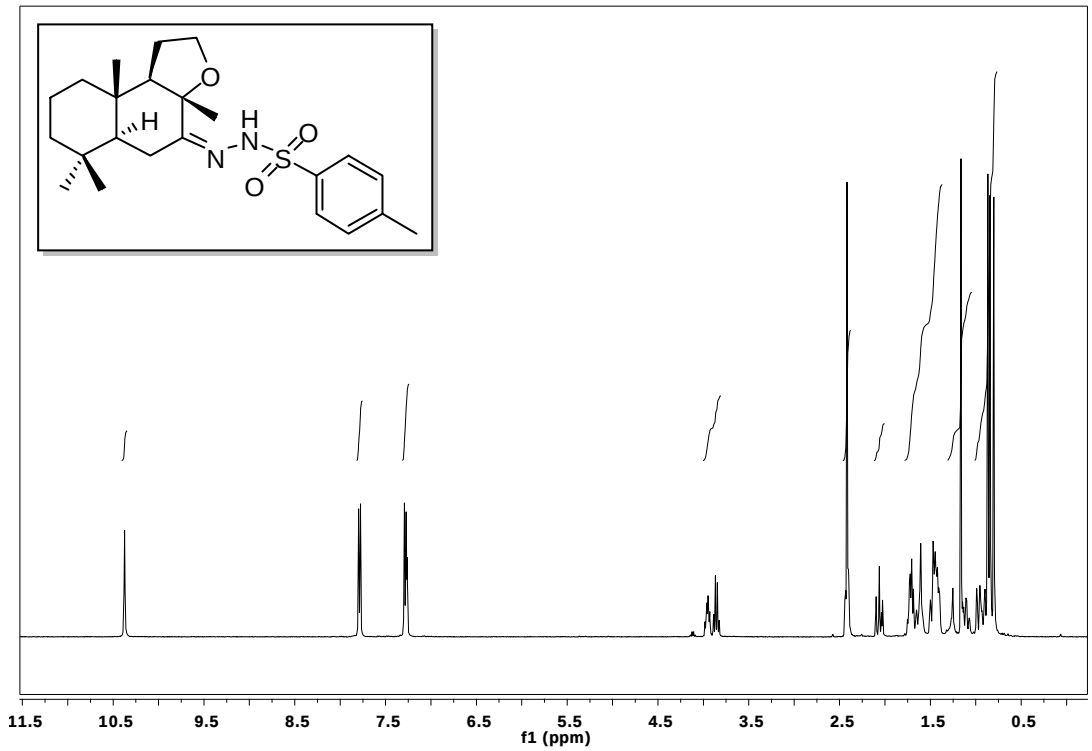

Figure A73 ^{1}H NMR spectrum of compound **189**

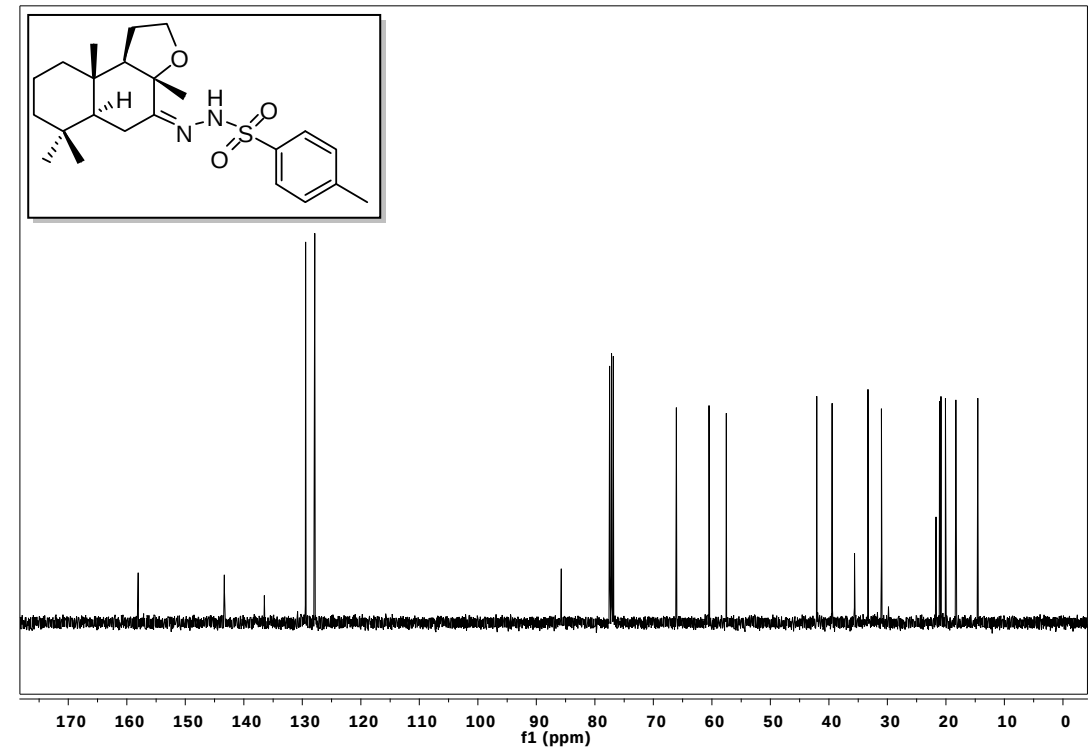

Figure A74 ^{13}C NMR spectrum of compound **189**

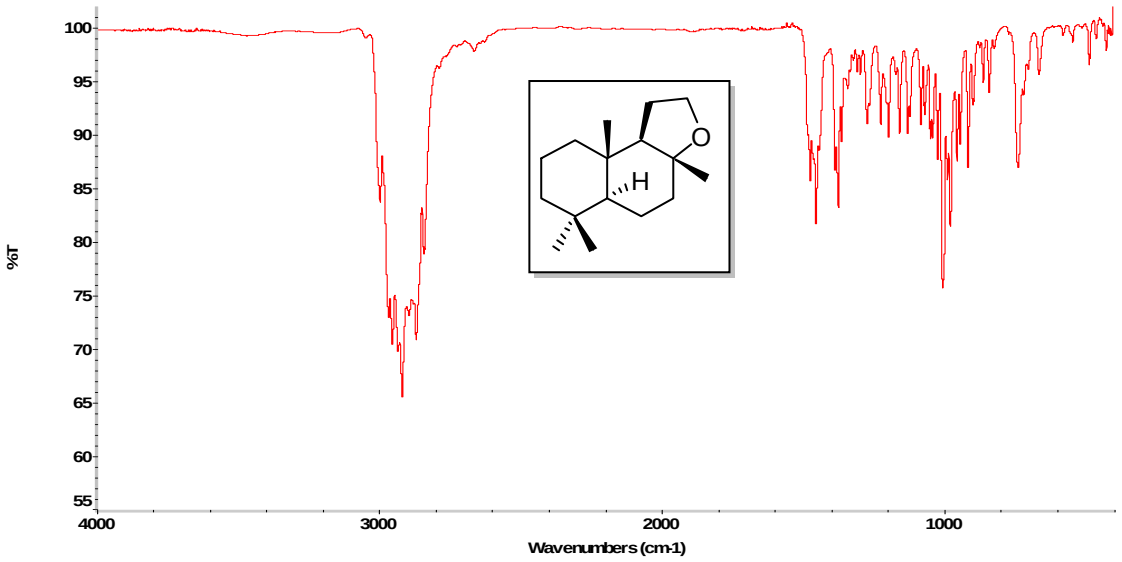

Figure A75 IR spectrum of (+)-ambrox

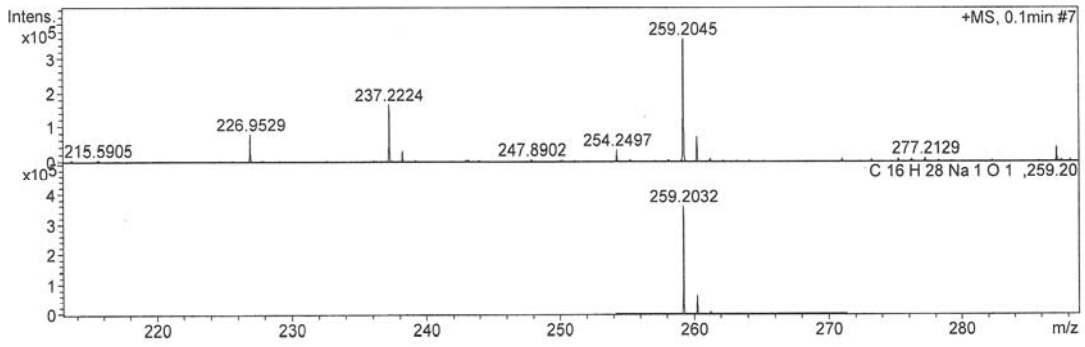

Figure A76 HRMS (ESI-TOF) spectrum of (+)-ambrox

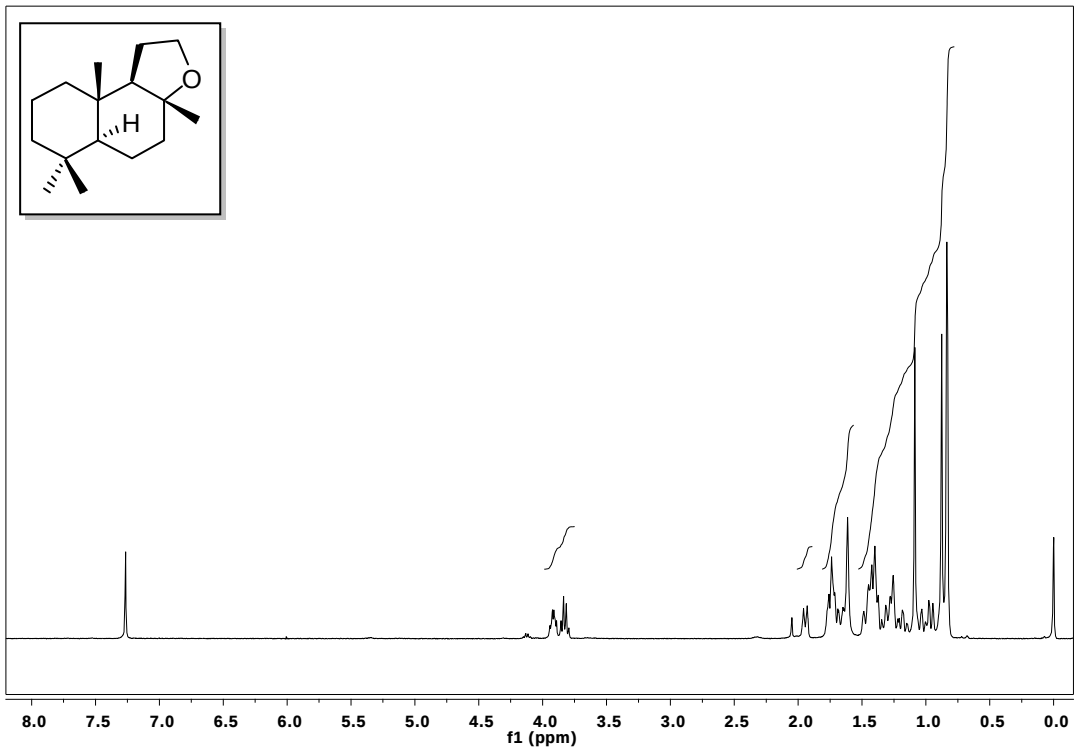

Figure A77 ^{1}H NMR spectrum of (+)-ambrox

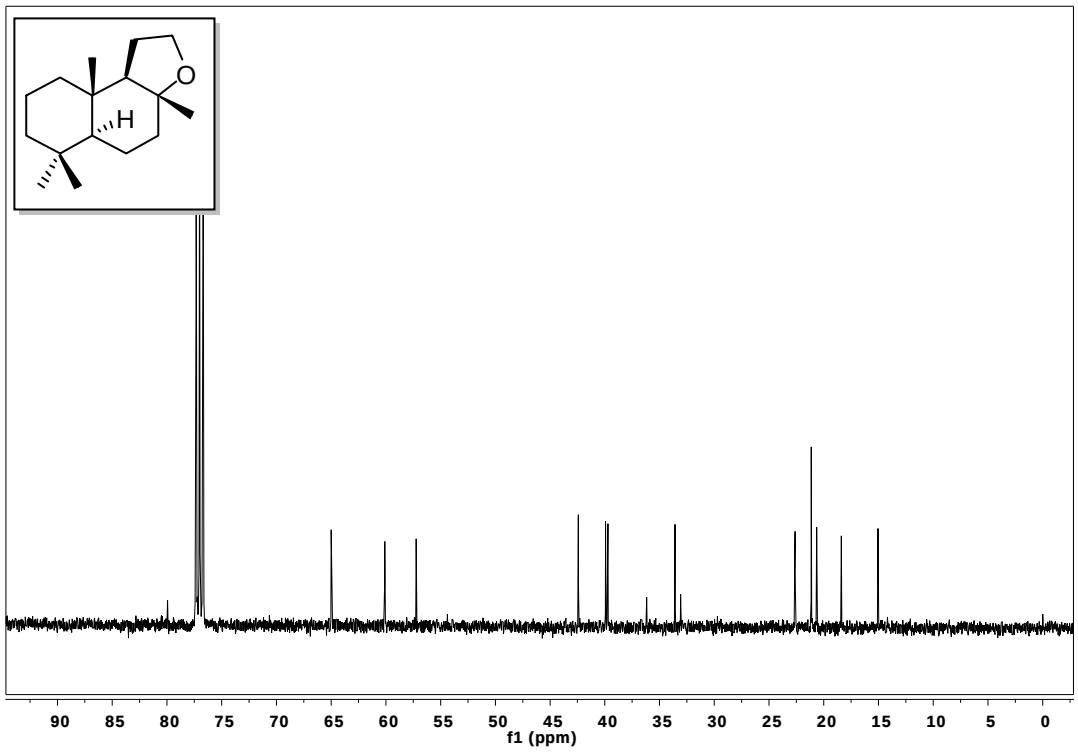

Figure A78 ^{13}C NMR spectrum of (+)-ambrox

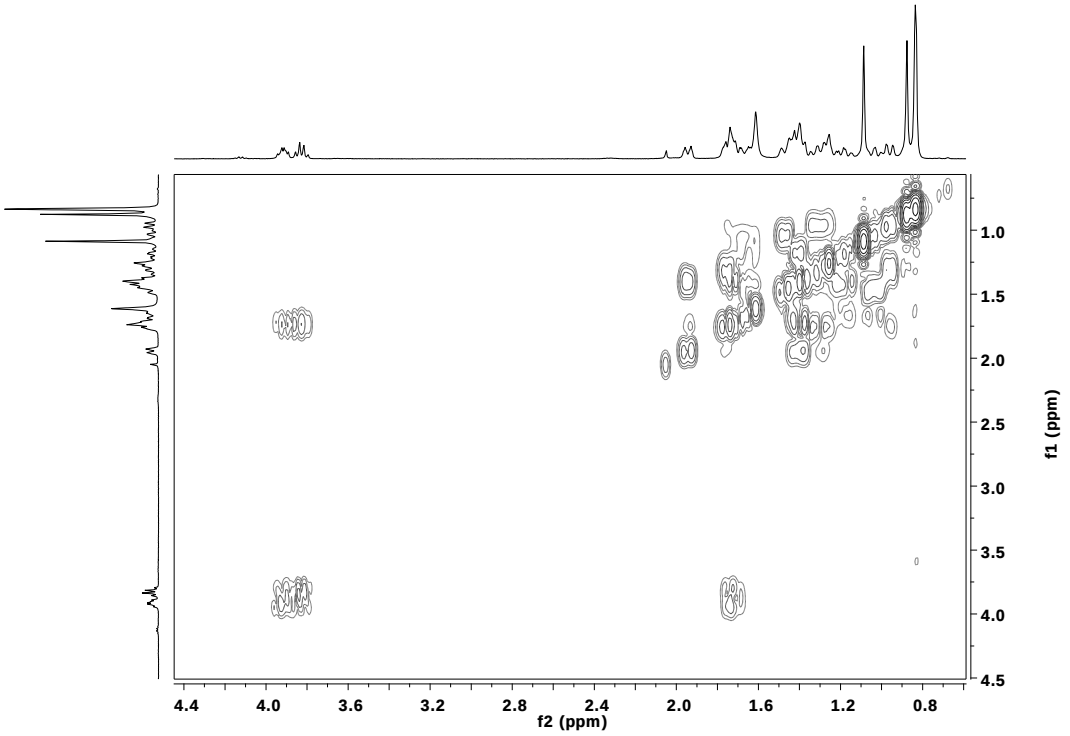

Figure A79 COSY spectrum of (+)-ambrox

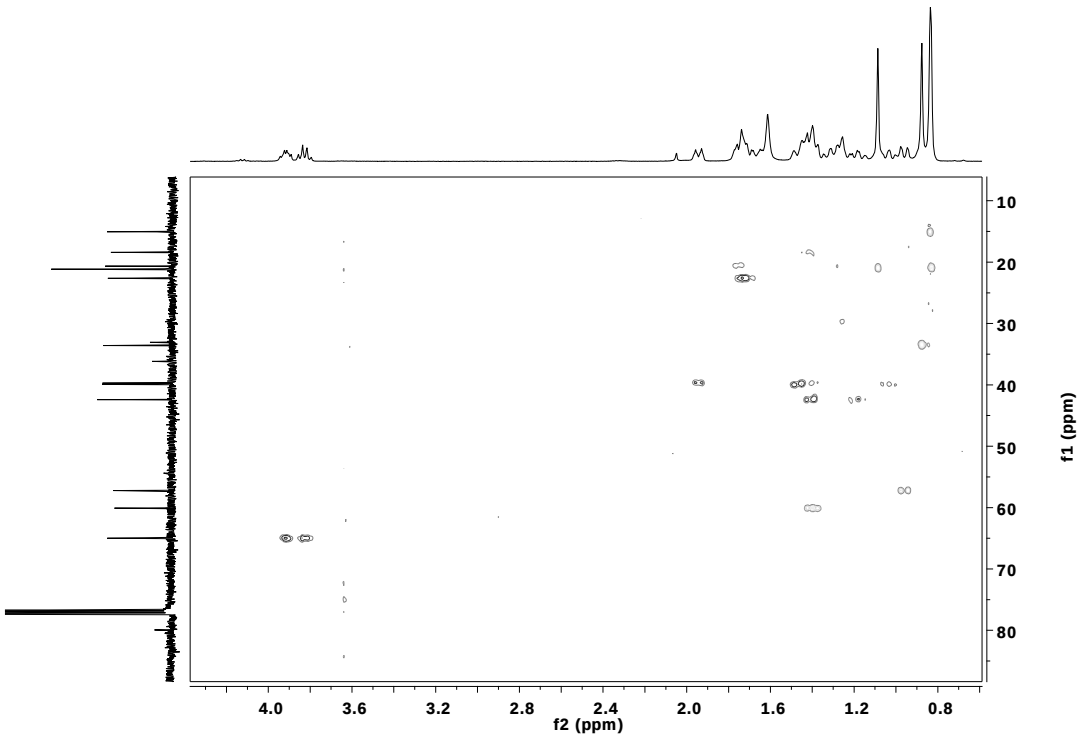

Figure A80 HSQC spectrum of (+)-ambrox

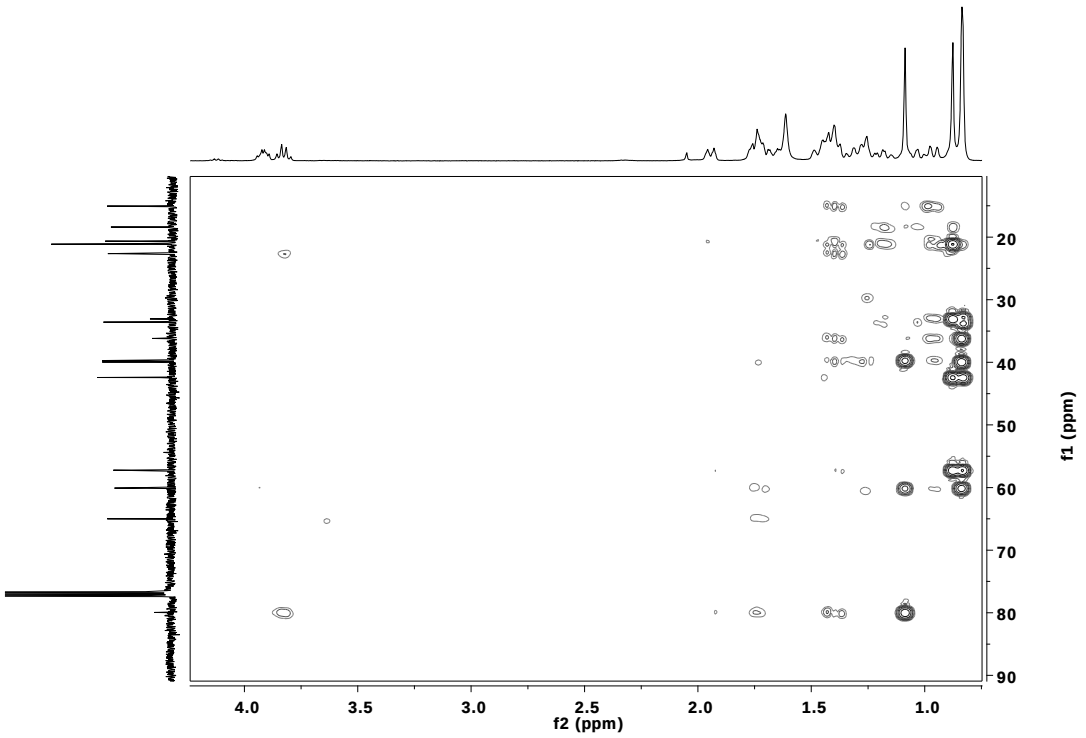

Figure A81 HMBC spectrum of (+)-ambrox

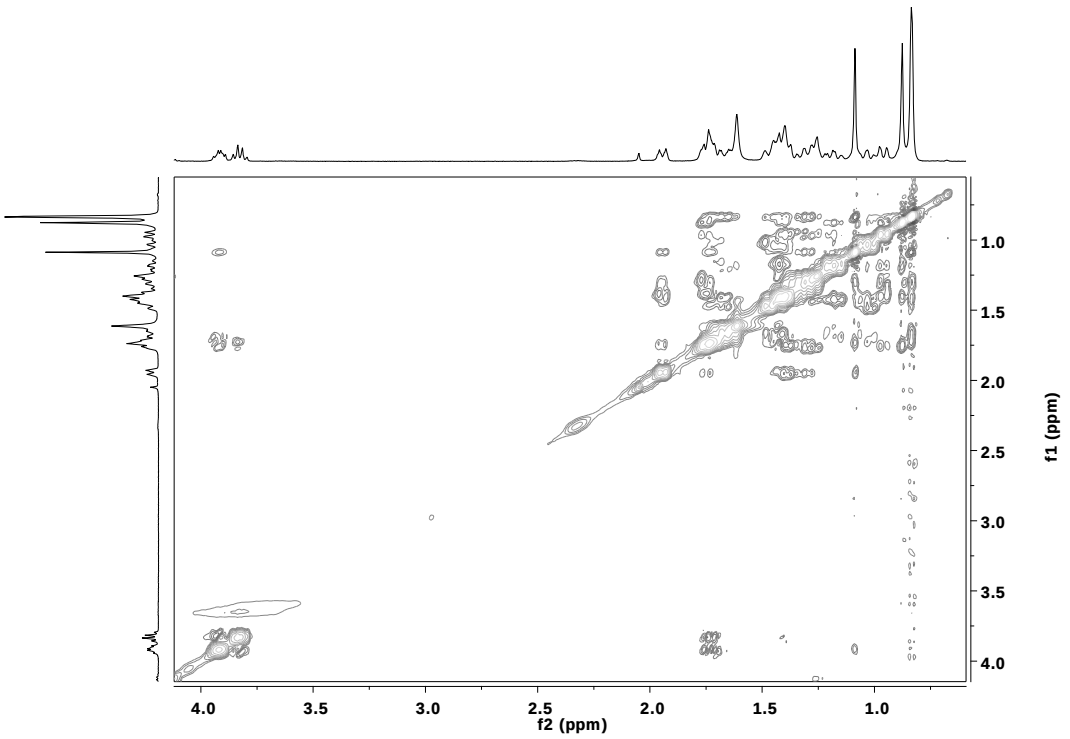

Figure A82 NOESY spectrum of (+)-ambrox

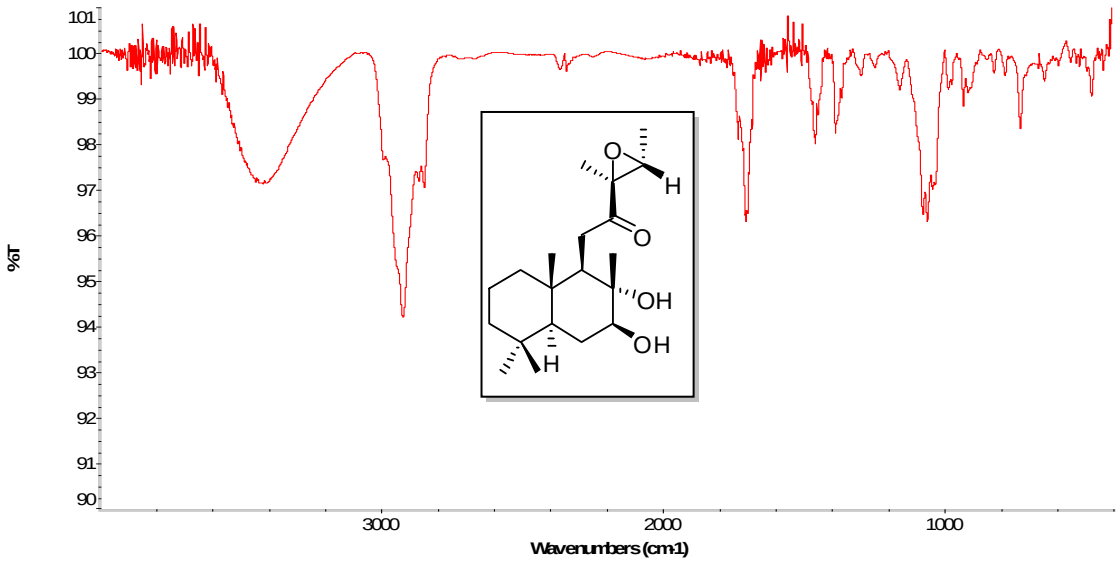

Figure A83 IR spectrum of **190a**

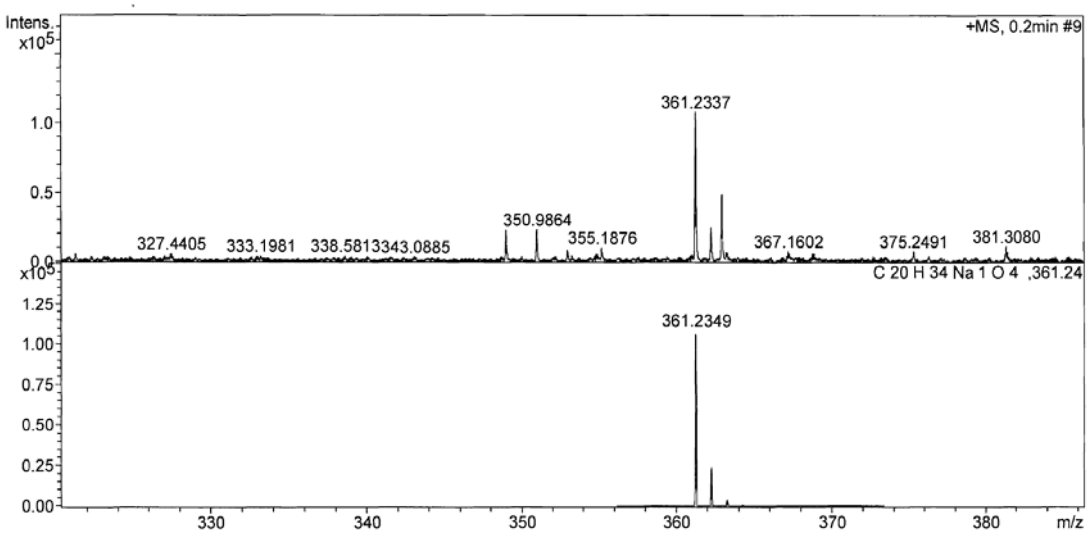

Figure A84 HRMS (ESI-TOF) spectrum of **190a**

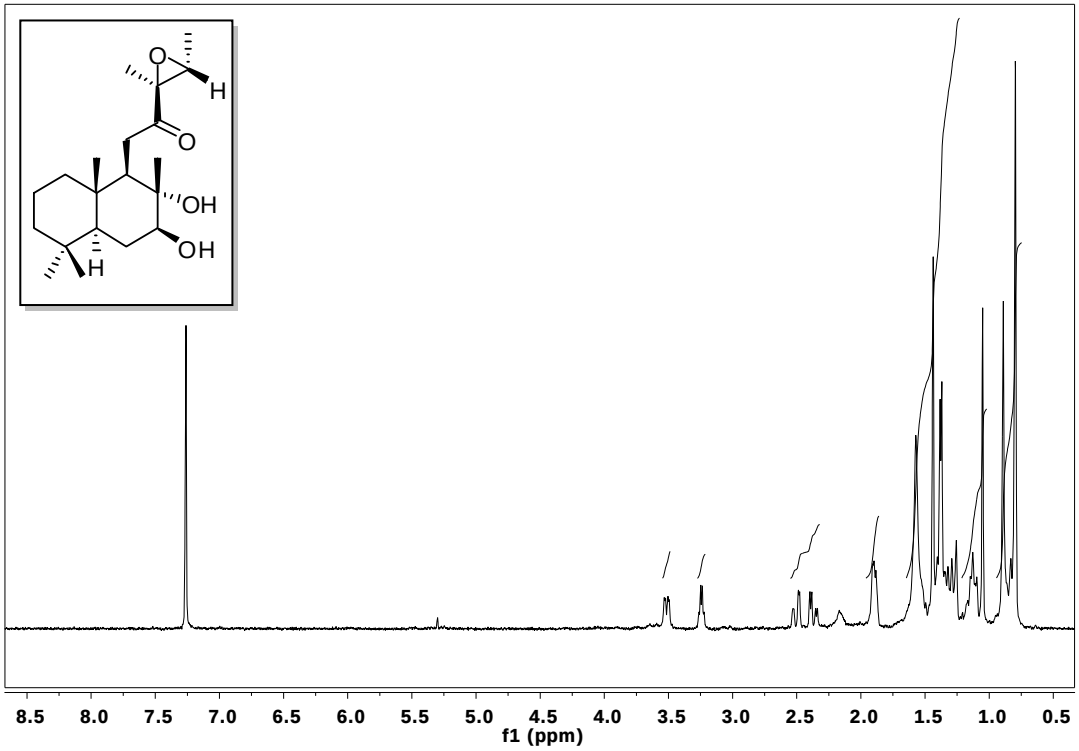

Figure A85 ^{1}H NMR spectrum of **190a**

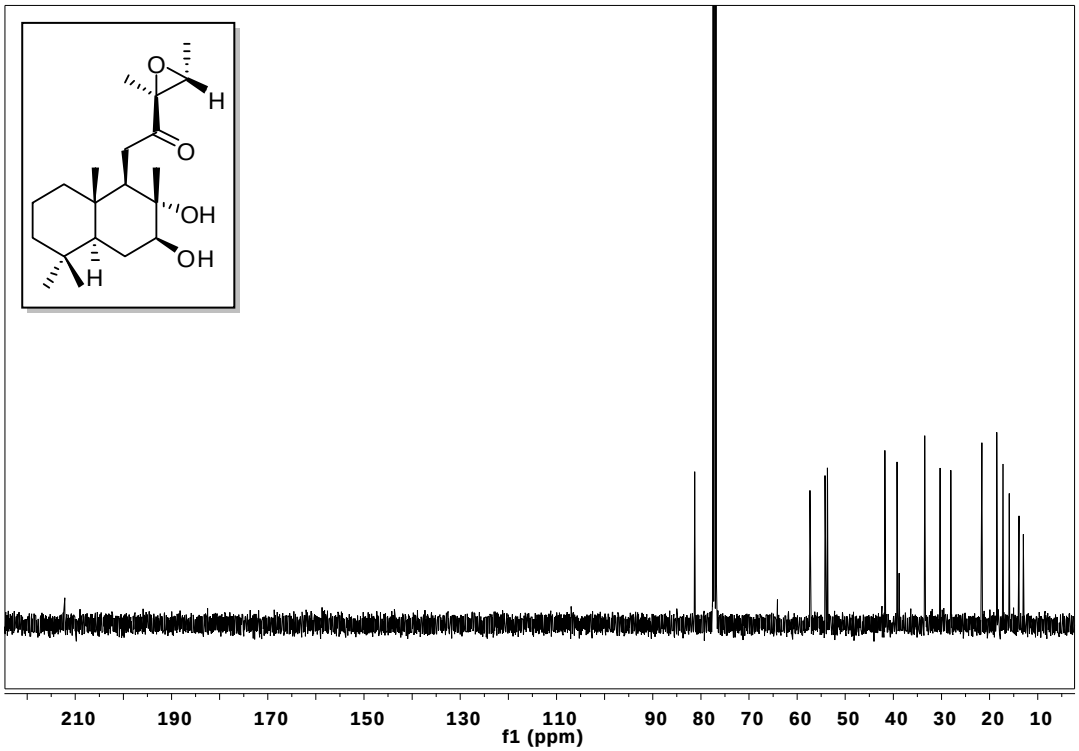

Figure A86 ^{13}C NMR spectrum of **190a**

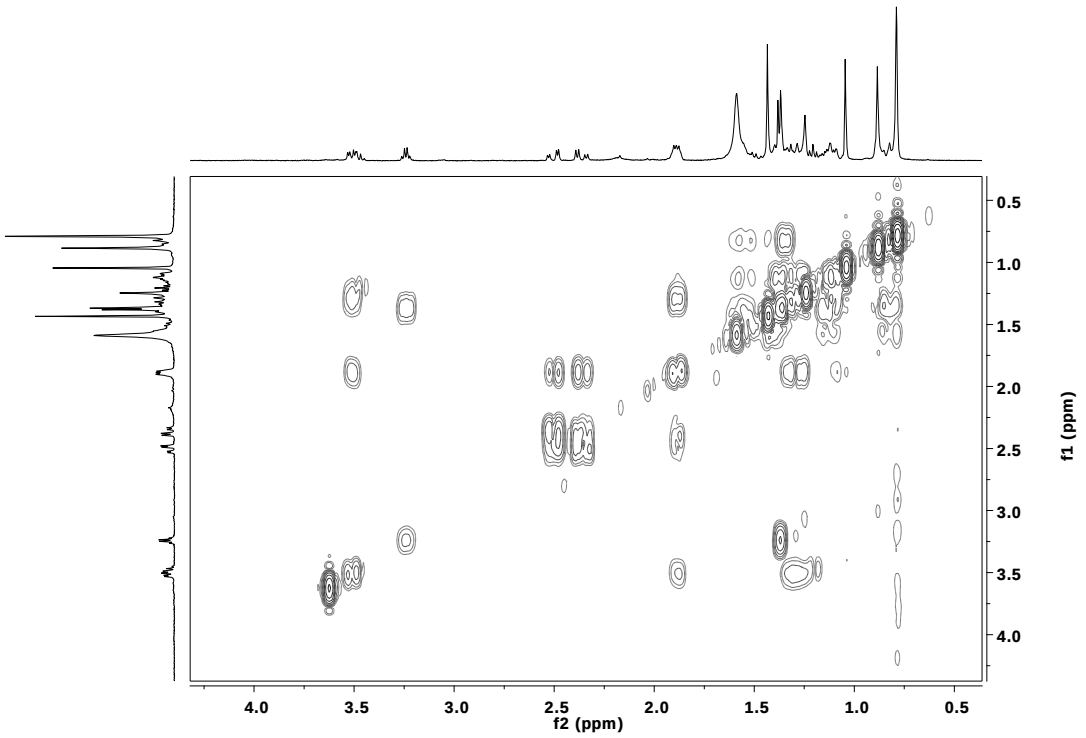

Figure A87 COSY spectrum of **190a**

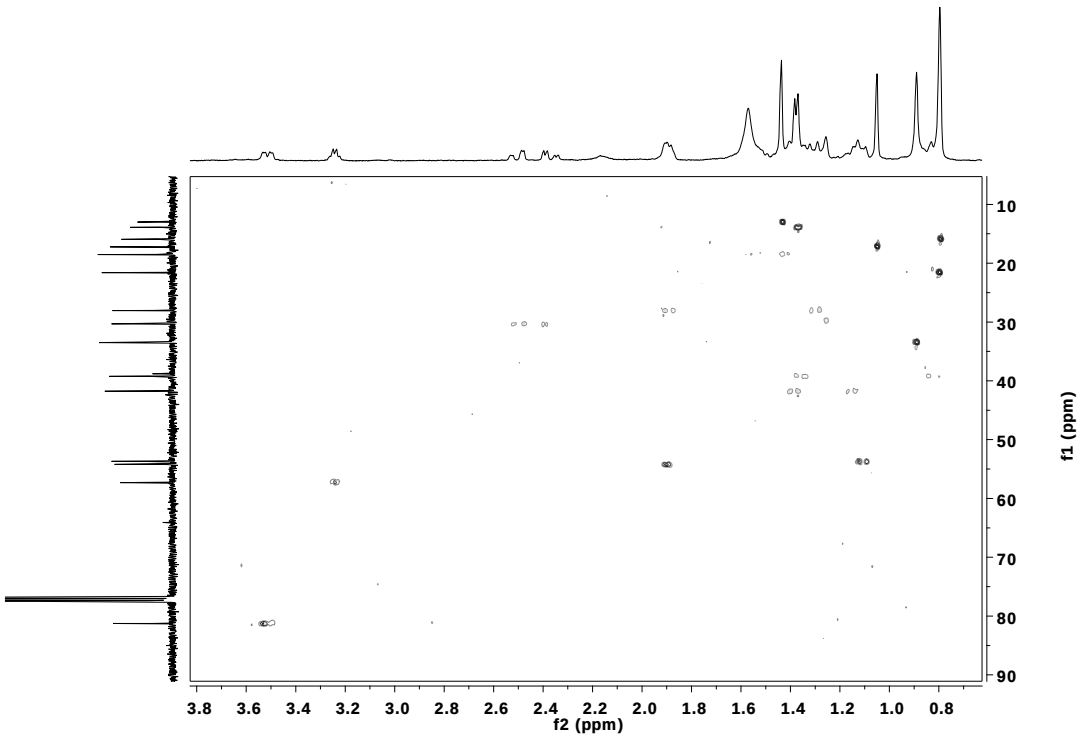

Figure A88 HSQC spectrum of **190a**

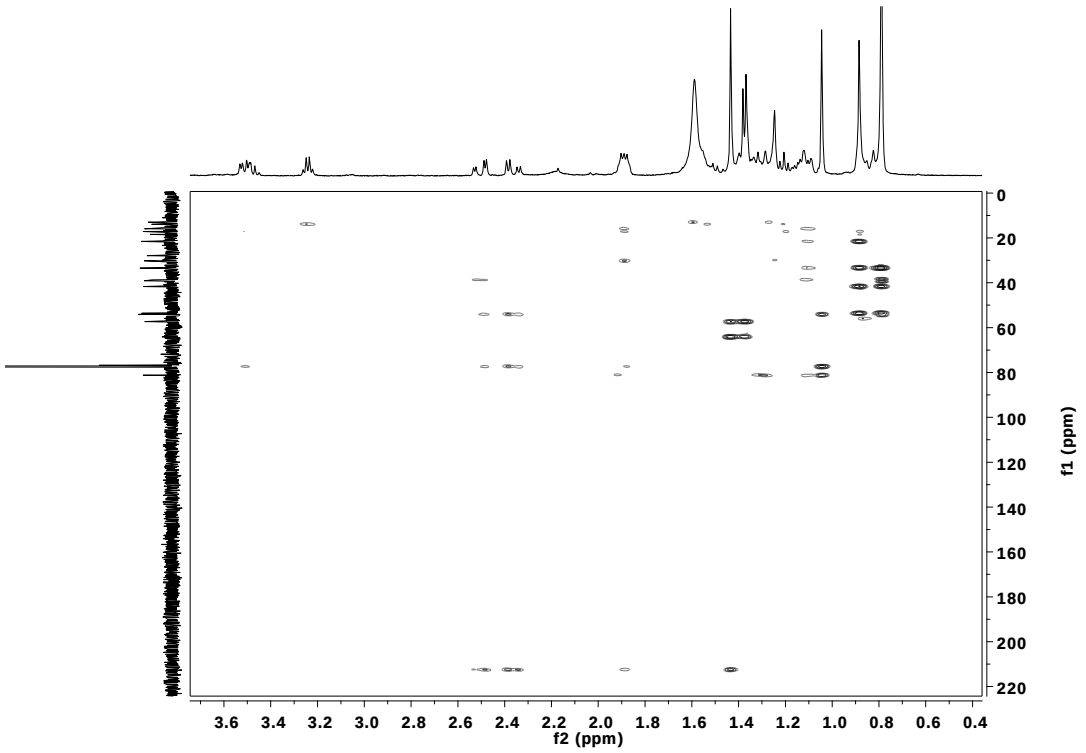

Figure A89 HMBC spectrum of **190a**

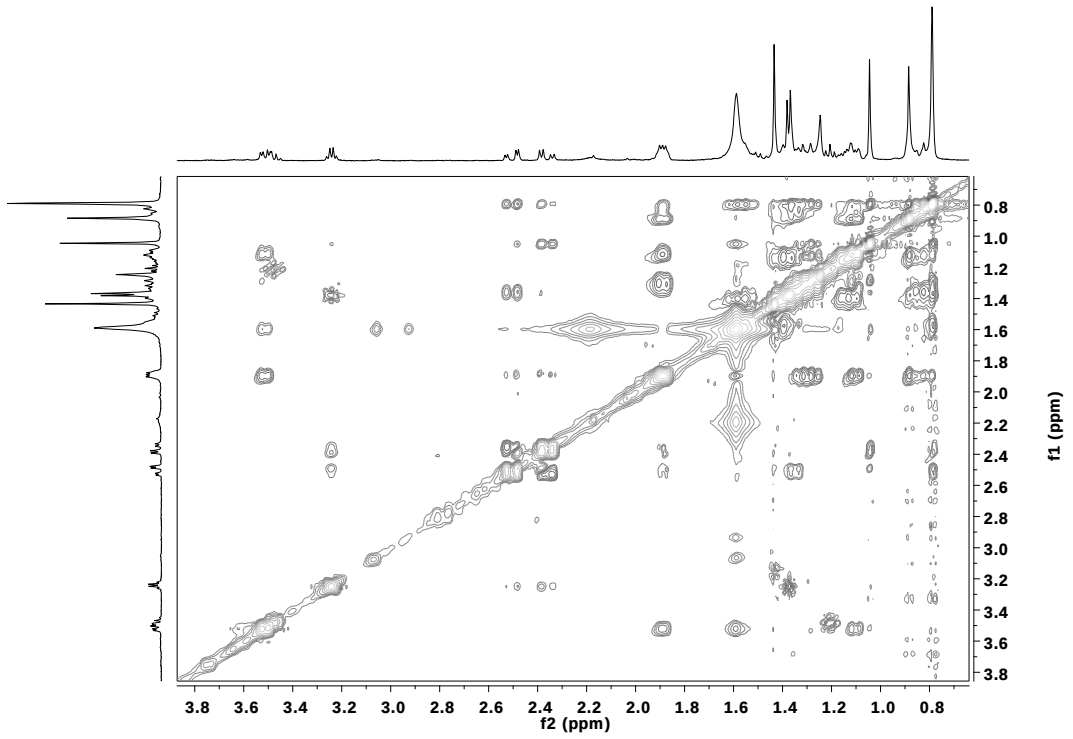

Figure A90 NOESY spectrum of **190a**

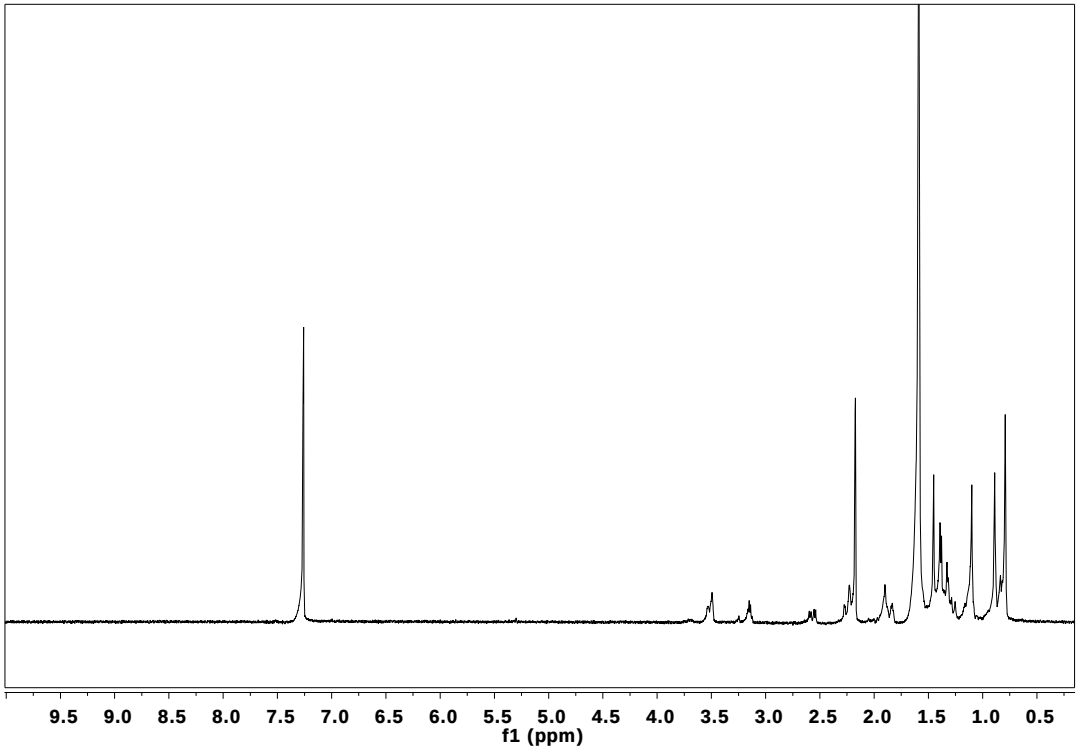

Figure A91 ^{1}H NMR spectrum of **190b**

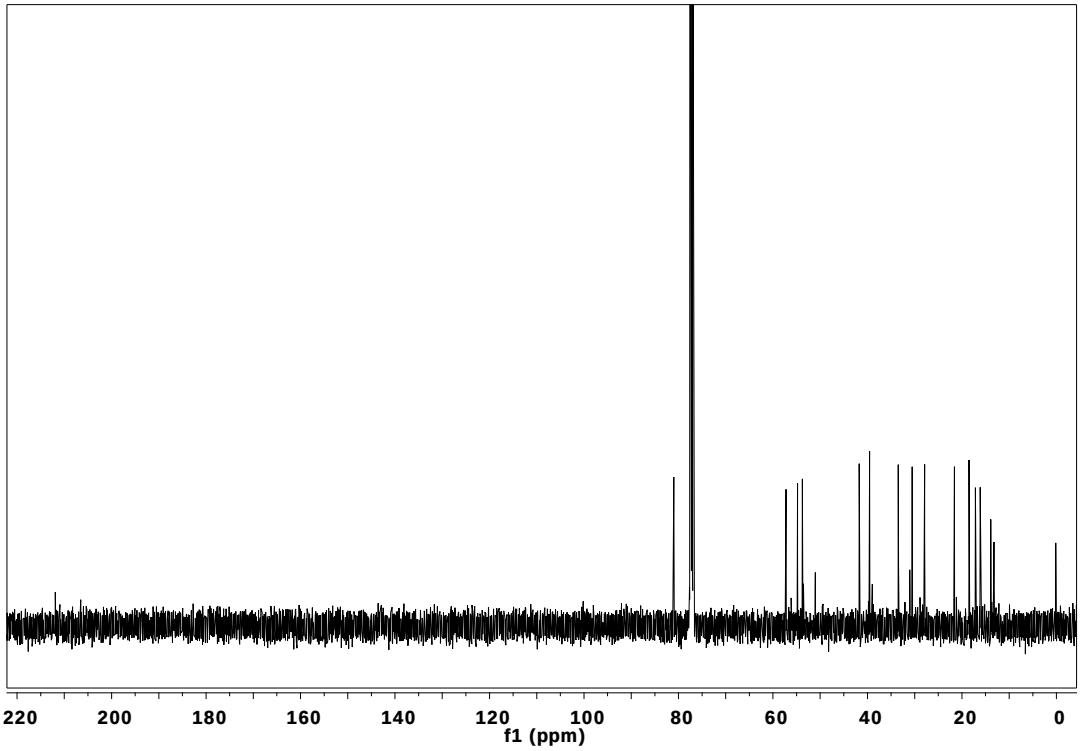

Figure A92 ^{13}C NMR spectrum of **190b**

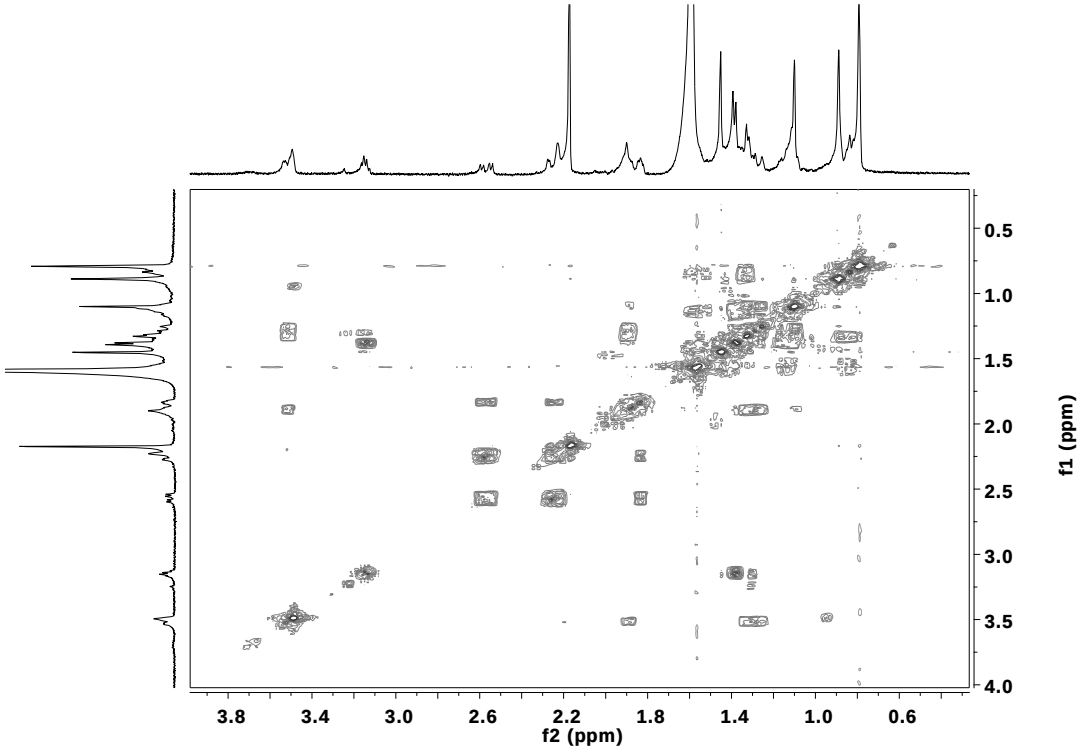

Figure A93 COSY spectrum of **190b**

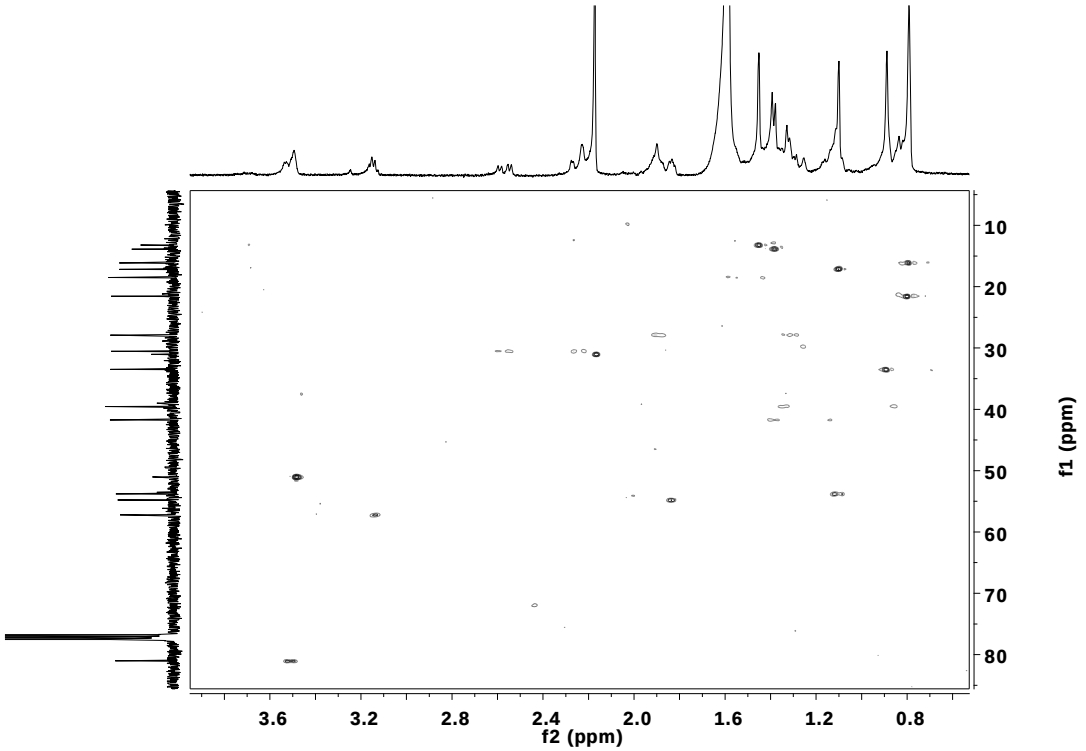

Figure A94 HSQC spectrum of **190b**

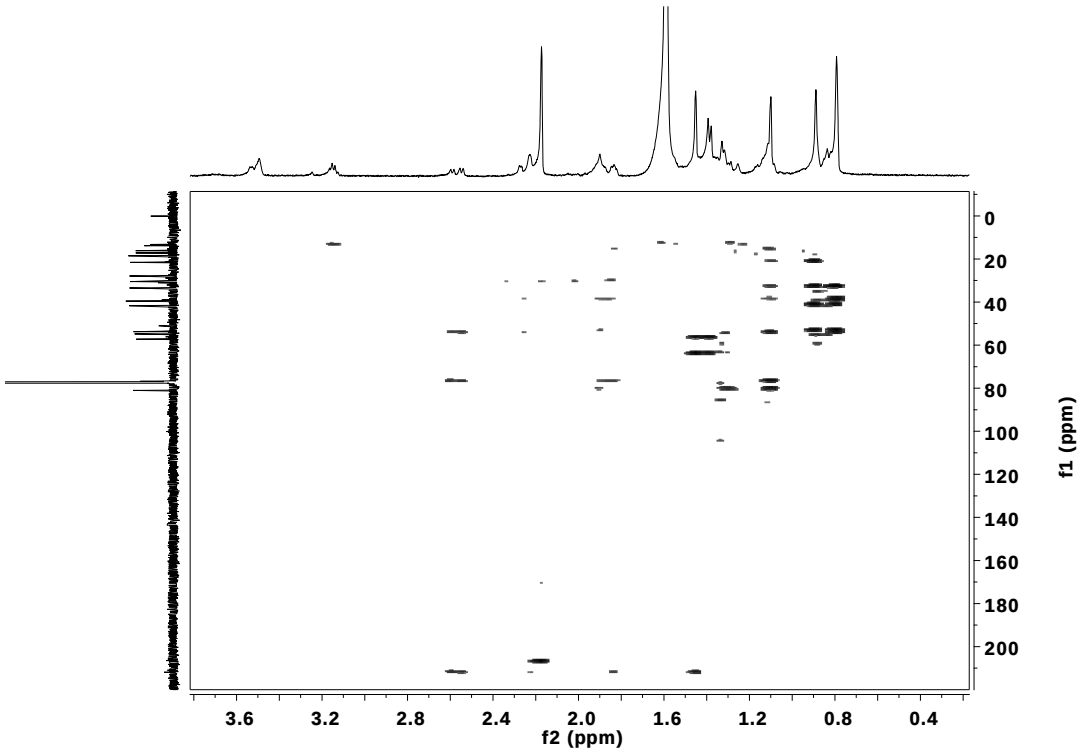

Figure A95 HMBC spectrum of **190b**

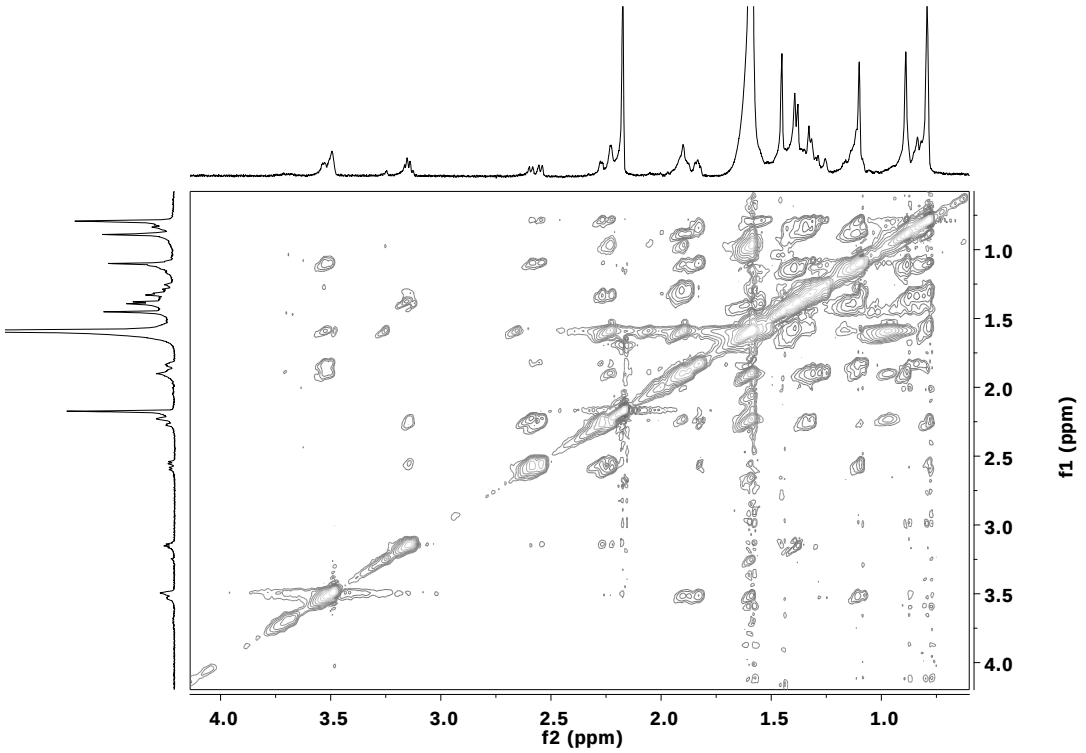

Figure A96 NOESY spectrum of **190b**

Appendix B
crystallography data

Table B1 Crystal data and structure refinement for compound **183**

Empirical formula	$C_{20}\ H_{34}\ O_2$
Formula weight	306.47
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	monoclinic, P21/c
Unit cell dimensions	*a* = 12.0921(6) Å alpha = 90 deg.
	b = 11.7469(6) Å beta = 105.696(2) deg.
	c = 13.6286(7) Å gamma = 90 deg.
Volume	1863.68(16) $Å^3$
Z, Calculated density	4, 1.092 Mg/m^3
Absorption coefficient	0.068 mm^{-1}
F(000)	680
Crystal size	0.30 x 0.25 x 0.18 mm
Theta range for data collection	1.75 to 34.82 deg.
Limiting indices	-19<=h<=19, -13<=k<=16, -17<=l<=11
Reflections collected / unique	14997 / 5648 [R(int) = 0.0307]
Completeness to theta	= 34.82 69.9 %
Max. and min. transmission	0.9879 and 0.9799
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5648 / 215 / 203
Goodness-of-fit on F^2	1.024
Final R indices [*I*>2sigma(*I*)]	R_1 = 0.0905, wR_2 = 0.2542
R indices (all data)	R_1 = 0.1316, wR_2 = 0.3091
Largest diff. peak and hole	0.844 and -0.723 $e.Å^{-3}$

Table B2 Crystal data and structure refinement for compound **185**

Empirical formula	$C_{16}H_{25}O_3$
Formula weight	265.37
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	monoclinic, P2(1)
Unit cell dimensions	a = 6.2810(5) Å alpha = 90 deg.
	b = 35.810(4) Å beta = 115.123(3) deg.
	c = 7.3289(9) Å gamma = 90 deg.
Volume	1492.5(3) A^3
Z, Calculated density	4, 1.181 Mg/m^3
Absorption coefficient	0.080 mm^{-1}
F(000)	580
Crystal size	0.18 x 0.2 x 0.3 mm
Theta range for data collection	2.27 to 26.40 deg.
Limiting indices	-7<=h<=4, -44<=k<=44, -8<=l<=9
Reflections collected / unique	11060 / 5864 [R(int) = 0.0714]
Completeness to theta	= 26.40 98.4 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5864 / 1 / 353
Goodness-of-fit on F^2	1.062
Final R indices [I>2sigma(I)]	R_1 = 0.1077, wR^2 = 0.2957
R indices (all data)	R_1 = 0.1268, wR^2 = 0.3140
Absolute structure parameter	0(3)
Largest diff. peak and hole	0.515 and -0.412 e.$Å^{-3}$

VITA

Miss Sunisa Suwancharoen was born on June 10, 1979 in Trat Province, Thailand. She is the oldest daughter of agriculturist family and she has one brother. When she was 6 years old, she attended to elementary school, Banpong School. Because her home so far from the city, when she was 12 years old she leaved her home to stay with her uncle and studied intermediate and high school at Streeprasertsin School.

When she finished high school, she moved to Bangsan, Chonburi and began undergraduate student at Burapha University. She graduated with a Bachelor Degree of Science in Chemistry in 2001. Later year, she was graduated with Graduate Diploma in Teaching from Burapha University.

In 2003 she moved to Bangkok to admit into a Master Degree program in organic chemistry at Department of Chemistry, Faculty of Science Chulalongkorn University and she graduated in 2005. In the same year, she admitted into Ph.D. program in organic chemistry at Department of Chemistry, Faculty of Science Chulalongkorn University.

She married to Mr. Thanachai Moonwong in 2008. He was a nice husband and encouraged me though out the study.