SPHAEROPSIDIN A FOR CANCER TREATMENT

by

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DEDICATION

The unsung heroes in this venture are my many family members and friends who have waited patiently, as well as supported me totally throughout the pursuit of these degrees. I would like to thank my mother for always supporting me in everything I do, always being there, and showing me that hard work truly pays off. Most of all, this is dedicated to my best friend, my fiancé, who is not only my inspiration for returning to my education, but the foundation of my support system for the last six years. Shelley, my love, you deserve more recognition for this endeavor than I do and I am forever grateful to have you as my life partner.

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LIST OF ABBREVIATIONS

Abbreviation	Description
Ac_2O	Acetic anhydride
Bcl-2	“B-cell lymphoma 2” gene
CH_2N_2	Diazomethane
CNS	Central nervous system
$CuSO_4$	Copper sulfate
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DMAP	Dimethyl aminopyridine
DNA	Deoxyribonucleic acid
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EtOAc	Ethyl acetate
EtOH	Ethanol
GI_{50}	Growth inhibition concentration
HRMS	High resolution mass spectrometry
MS	Mass spectrometry
$NaBH_4$	Sodium borohydride
$NaIO_4$	Sodium periodate
NCI	National Cancer Institute

NMR	Nuclear magnetic resonance
MeOH	Methanol
p53	Cellular tumor antigen p53
PCC	Pyridinium chlorochromate
PtO_2	Platinum oxide
PTSA	Toluenesulfonic acid
RVI	Regulatory volume increase
SAR	Structure activity relationship
TLC	Thin layer chromatography

I. INTRODUCTION

Cancer

According to NCI greater than 1.6 million new cases of cancer were diagnosed in the United States in 2016 with an expected expenditure of $156 billion by 2020.[1] While it has been suggested that as many as 10 qualities or "hallmarks" may exist for cancer, two important characteristics are apoptosis resistance and increased cell migration (**Figure 1**). Since apoptosis is a heavily regulated, programmed cell death, it is a common way for a cell population to be controlled and for irregular cells to be removed from that population.[2] This process must be highly regulated because over-active apoptosis mechanisms may result in immunodeficiency while under-active leads to carcinogenesis. While cancer cells inherently evade apoptosis, there are certain types of cancer which seem to have greater apoptosis resistance than others. This commonly occurs due to the collections of modifications in genes, like Bcl-2 and p53, which aid the evasion of apoptosis. Examples of such cancers include glioblastoma, melanoma, and advanced renal cell carcinoma. Besides genetic factors, this enhanced resistance to apoptosis can also result from the fact that it is highly regulated. Thus, feedback mechanisms have been developed by certain cancer cells to regulate this process and subsequently interfere with treatment-induced apoptotic cell death.[3] Cancer cells show greater instances of cell migration which can lead to metastasis and increase the severity of prognosis. This metastasis may occur due to changes in cell shape as well

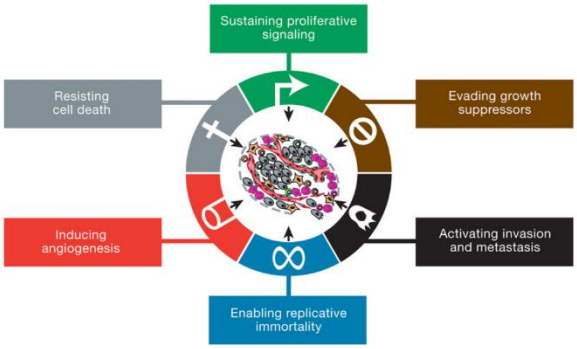


Figure 1: Hallmarks of cancer[2]

as changes to cellular adhesion to neighbor cells or the extracellular matrix. While it is reported that the cancer mortality rate is falling for men and staying steady for women[1], it is also emphasized that glioblastoma, melanoma, and advanced renal cancers have been resilient to many chemotherapies.[4,5] To compound the problem, if a chemotherapy fails to destroy the entire tumor the cells can gain resistance to the drug in the future. This process can lead to multidrug resistance and further complicate the treatment of the tumor. This resistance is achieved through up-regulation of efflux pumps and/or down-regulation of solute carriers which work in conjunction to reduce the concentration of small molecule drugs within the cancer cell.[6] Thus, cancer therapeutics which can overcome apoptosis resistance, metastasis and multidrug resistance are in great demand.

Natural Products

Natural products have long been a source for medicinal remedies, as far back as 460 B.C. Hippocrates of Kos was credited for the use of natural products to treat a variety of ailments.[7] Among the many examples, one notable instance was the use of opium as a postoperative pain killer, which was reported as early as 1784 (**Figure 2**). It was not until 1805 that morphine was extracted from opium and isolated by Friedrich Sertürner. This marked a turning point in pharmacology, although morphine was not widely used until the introduction of the hypodermic needle.[8]

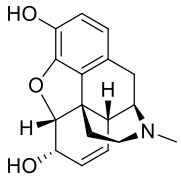


Figure 2: Morphine

From 1940-2010 it was found that 48% of small molecule drugs were natural products or directly derived therefrom with almost 75% being labeled at "Other than synthetic".[9] These natural products have scaffolds that allow for binding to many sites and many times have desirable effects, thus making them useful drug leads. Although

these drugs may have desirable effects at the out start, they generally have unwanted qualities as well. Analogs of these drug leads will allow for greater understanding of which portions for the molecule are involved in the binding to the target and which parts are not. If an analog is tested and proves to have greater potency than the natural product, then it begins to suggest that the portion of the molecule that was altered is involved in the binding to the target and is part of the pharmacophore. Also, if an analog is tested and proves to have a decrease in potency, then it also begins to suggest that this portion of the molecule should be left intact. Should the analog maintain potency, it indicates that this portion of the molecule is not involved in the binding to the target and is an auxophore. The auxophore region can be used to modify stability, absorption, metabolism, distribution, and excretion without drastically changing the potency of the molecule.

Fungal Metabolite Sphaeropsidin A

Sphaeropsidin A is a pimarane diterpene in a family of sphaeropsidins A-F (**Figure 3**). Its isolation, characterization, and biological evaluation have been reported in a number of publications. Sphaeropsidins A and B have more pronounced biological activities and specifically have been noted for phytotoxic and antifungal effects.[10]

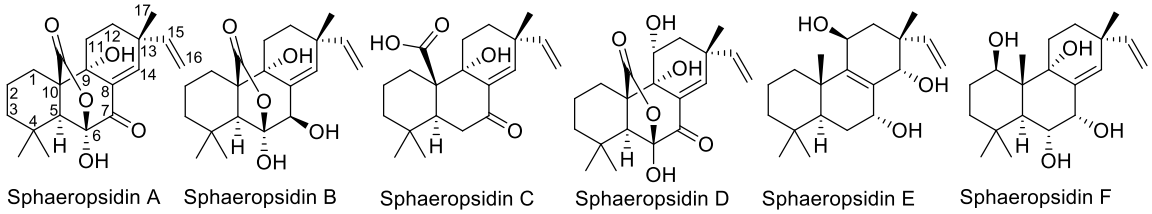
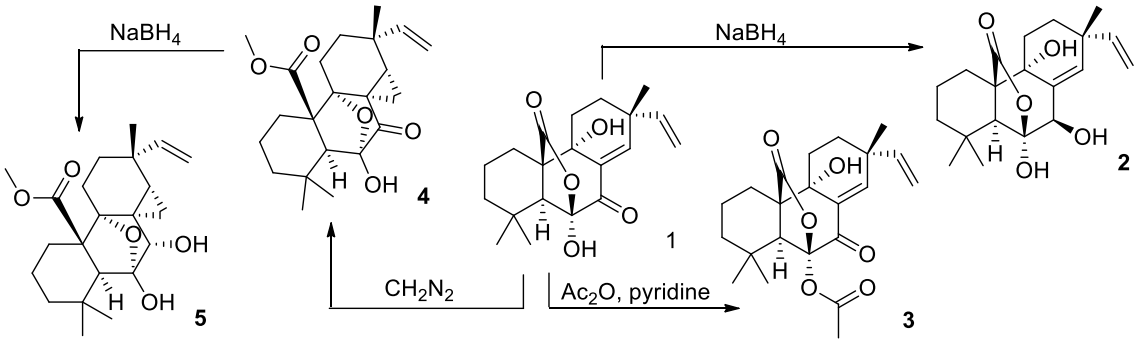


Figure 3: Structures of sphaeropsidins A-F. Numbering of sphaeropsidn A

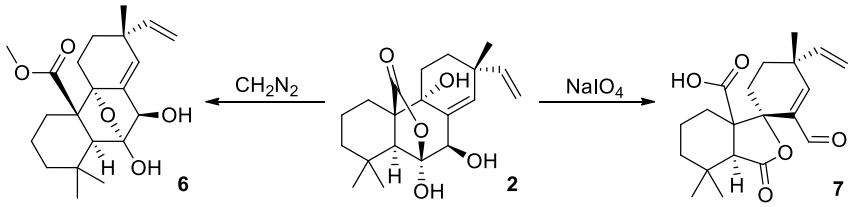
In a report dated 1972, sphaeropsidin A was first fermented from the fungus *Aspergillus chevalieri*, named LL-S491β, and found to have antibacterial properties

against gram-positive organisms. The authors also performed the synthesis of several analogs and their characterization. Sphaeropsidin A (**1**) was reduced with sodium borohydride and acetylated utilizing acetic anhydride and pyridine to produce sphaeropsidin B (**2**) and monoacetate **3**, respectively. Sphaeropsidin A undergoes esterification with diazomethane to produce methyl ester **4** which is reduced with sodium borohydride to give secondary alcohol **5** (**Scheme 1**).



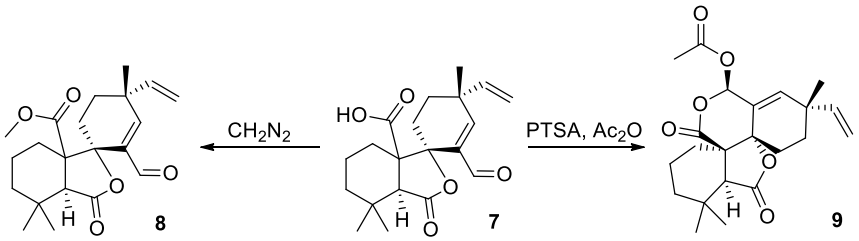
Scheme 1: Synthesis of analogs 1-5

Further, sphaeropsidin B undergoes esterification with diazomethane and can be oxidized using sodium periodate to give ester **6** and lactone **7**, respectively (**Scheme 2**).



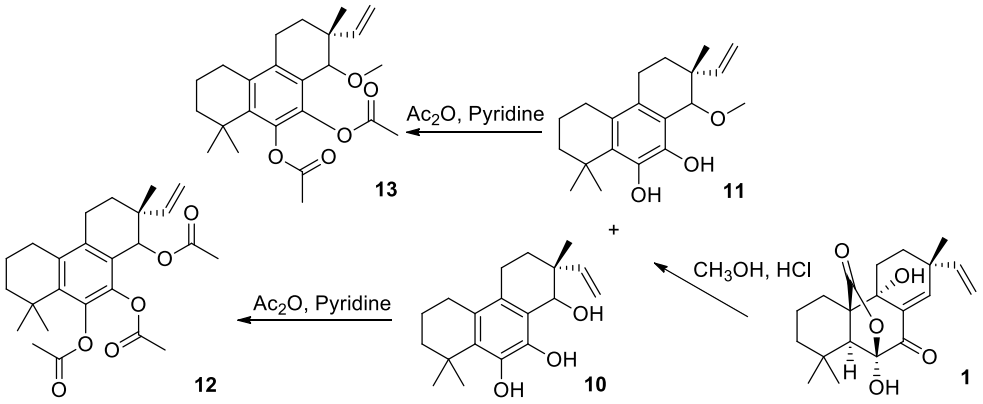
Scheme 2: Synthesis of analogs 6 and 7

Further, lactone **7** can then undergo esterification with diazomethane and reacts with acetic anhydride, with catalytic toluenesulfonic acid (PTSA), to give ester **8** and lactone **9**, respectively (**Scheme 3**).



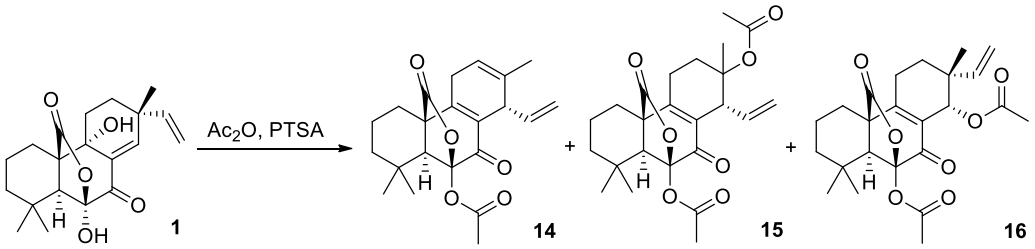
Scheme 3: Synthesis of analogs 8 and 9

Finally sphaeropsidin A can be treated with methanolic hydrochloric acid to provide aromatic compounds **10** and **11**, both of which can be acetylated to yield acetates **12** and **13** (**Scheme 4**).[11]



Scheme 4: Synthesis of analogs 11-13

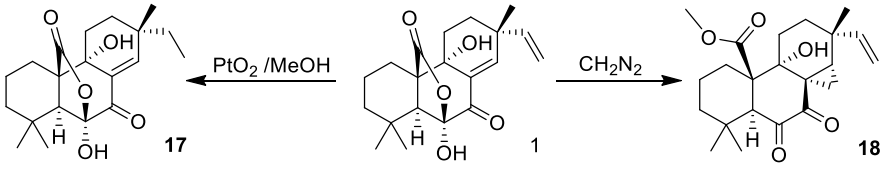
While this paper reported several analogs, the authors did not describe any biological evaluation of these compounds. This report demonstrated that sphaeropsidin A could tolerate many reaction conditions, but lacked a synthetic route that offered the ability to vary the substitutions greatly in order to investigate the structure activity relationship (SAR). These authors followed up by reporting synthesis and characterization of three more analogs in 1973 by treating sphaeropsidin A with acetic anhydride with catalytic amounts of PTSA. The biological activity of these analogs was not reported because they were synthesized for mechanistic studies and not for biological evaluation (**Scheme 5**).[12]



Scheme 5: Synthesis of analogs 14-16

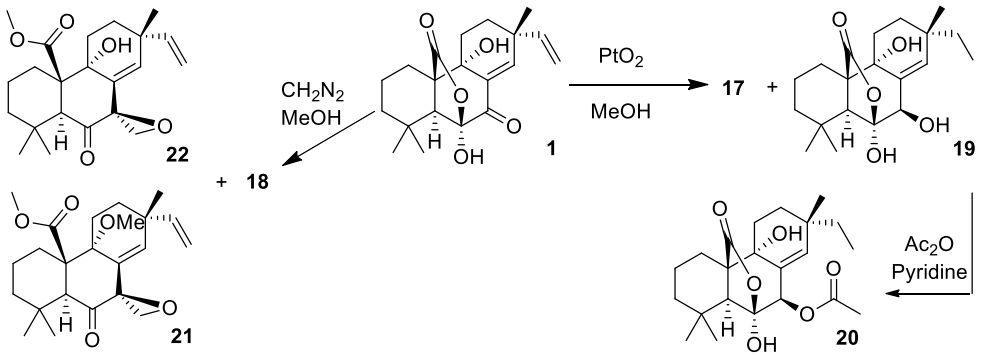
Later in 1996, sphaeropsidin A was reported to be extracted from the *Diplodia cupressi* by another group and when characterized it was found to be identical to LL-S491β. Sphaeropsidin A was evaluated biologically due to *Diplodia cupressi's* implication in the canker disease suffered by cypress trees. It was found to have antimicrobial activity when tested against 12 fungi cultures.[13]

In 2004, three new analogs were reported along with their synthesis. Sphaeropsidin A undergoes reduction with platinum oxide in methanol under nitrogen to give hydrogenated **17** and reacts with diazomethane to produce dione **18** (**Scheme 6**).[10]



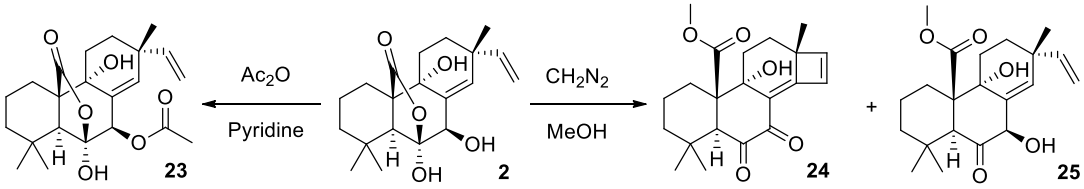
Scheme 6: Synthesis of analogs 17 and 18

In 2011, four new derivatives were synthesized and evaluated for antibacterial activity. Sphaeropsidin A was also reduced with platinum oxide in methanol under nitrogen to give **17,** but this time another analog (**19)** was isolated. Compound **19** was then acetylated with acetic anhydride catalyzed by pyridine to give acetate **20**. In this report, sphaeropsidin A reacted with diazomethane to provide **18**, **21**, and **22** (**Scheme 7**).



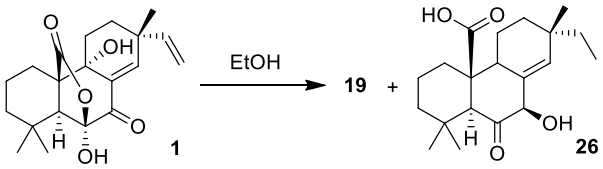
Scheme 7: Synthesis of analogs 17-22

In the same paper, sphaeropsidin B is acetylated using a previously described methodology to yield acetate **23**. In addition, sphaeropsidin B was reacted with diazomethane in methanol to generate dione **24** and acetate **25** (**Scheme 8**).[14]



Scheme 8: Synthesis of analogs 23-25

In a report also dated in 2011, a new analog and its synthesis were reported. It was found to undergo hydrogenation in ethanol to produce **19** and **26** (**Scheme 9**).



Scheme 9: Synthesis of analog 26

In addition, sphaeropsidin A, sphaeropsidin B, as well as sphaeropsidins C-F, (**19**, **3**, **26**, and **18**) were evaluated for cytotoxicity. Most compounds did not show cytotoxicity when tested at 10 µM concentrations and thus were not evaluated further, but sphaeropsidin A, sphaeropsidin D, and analog **3** were found active. Thus, sphaeropsidin

A (**1**), sphaeropsidin D (**Sph D**), and analog **3** were evaluated for cytotoxicity and were compared against doxorubicin (**DR)** (**Table 1**).

Table 1: Antiproliferative activities of sphaeropsidins and their analogs. Reported by Wang, *et al.*[15]

Cmpd	IC_{50} (μM)							
	NCI-H460[a]	SF-268[b]	MCF-7[c]	MDA-MB-231[d]	PC-3[e]	PC-3M[f]	MIAPa Ca-2[g]	WI-38[h]
1	1.9	2.1	3.0	1.4	2.5	2.4	2.0	3.7
Sph D	>10	7.5	>10	3.7	9.6	>10	9.0	>10
3	2.8	4.9	2.0	2.5	nt	2.7	nt	nt
DR	0.3	0.6	0.4	0.5	0.3	0.3	0.3	0.8

a: non-small cell lung cancer, b: CNS glioma, c: breast cancer, d: human metastatic breast adenocarcinoma, e: prostate adenocarcinoma, f: metastatic prostate adenocarcinoma, g: pancreatic cancer, h: normal human primary fibroblast cells, nt=not tested

Notably, sphaeropsidin A and analog **3** had very similar potency. In addition, sphaeropsidin A was used in a cell migration assay and found to have similar results as the positive control, LY294002, suggesting that it may be effective against metastasis.[15] This report showed for the first time that sphaeropsidin A had cytotoxic qualities and also suggested that derivatization at the 6-O position (See **Figure 3** for numbering) maintains potency.

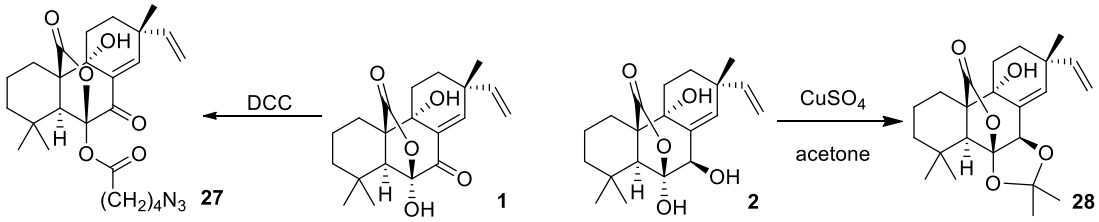
Again, in 2012, sphaeropsidin A as well as sphaeropsidin B, sphaeropsidin C, analog **3**, **7**, **16**, **17**, **19**, **20**, **23**, as well as three analogs of sphaeropsidin C were evaluated against a panel of six cancer cell lines. It was noted here that sphaeropsidin A and analogs **3** and **17** had similar cytotoxicity and compared favorably to known anticancer agents such as cisplatin (**Cis-p**), etoposide (**VP16**), carboplatin (**C-p**) and temozolomide (**Tz**) (**Table 2**).[16]

Table 2: Antiproliferative activities of sphaeropsidins and their analogs. Reported by Lallemand, *et al.*[16]

Name	IC_{50} (μM)					
	A549[a]	OE21[b]	Hs683[c]	U373[c]	SKMEL28[d]	B16F10[d]
Cis-p	0.5	n.d.	0.5	0.4	1.6	1.9
C-p	11	n.d.	20	19	149	44
VP16	3.2	n.d.	3.2	30.5	1.6	0.04
Tz	611	n.d.	763	676	905	234
1	1	0.3	n.d.	0.4	2	0.4
2	>100	78	>100	>100	>100	>100
3	2	1	3	1	3	2
7	26	78	9	61	>100	30
16	27	9	20	6	30	8
17	3	2	3	3	2	2
19	74	62	90	81	>100	99
20	>100	74	>100	>100	>100	>100
23	>100	77	>100	>100	>100	97

a: non-small cell lung cancer, b: esophageal, c: glioma, d: melanoma, n.d.=not determined

In 2013, two more analogs were reported along with their synthesis. Sphaeropsidin A was esterified using dicyclohexylcarbodiimide (DCC) and 5-azidopentanoic acid to provide azide **27**, while sphaeropsidin B was converted to **28** utilizing copper sulfate in acetone (**Scheme 10**).



Scheme 10: Synthesis of analogs 27 and 28

In this report, sphaeropsidin A was evaluated biologically and showed favorable qualities as a bite deterrent and larvicide for the purpose of controlling mosquito populations in connection with dengue fever.[17]

Recently, in 2015, sphaeropsidin A was evaluated by the NCI against the 60 cancer cell panel where it was found to be most active against renal cell cancer and melanoma sub-panels (**Figure 4**).

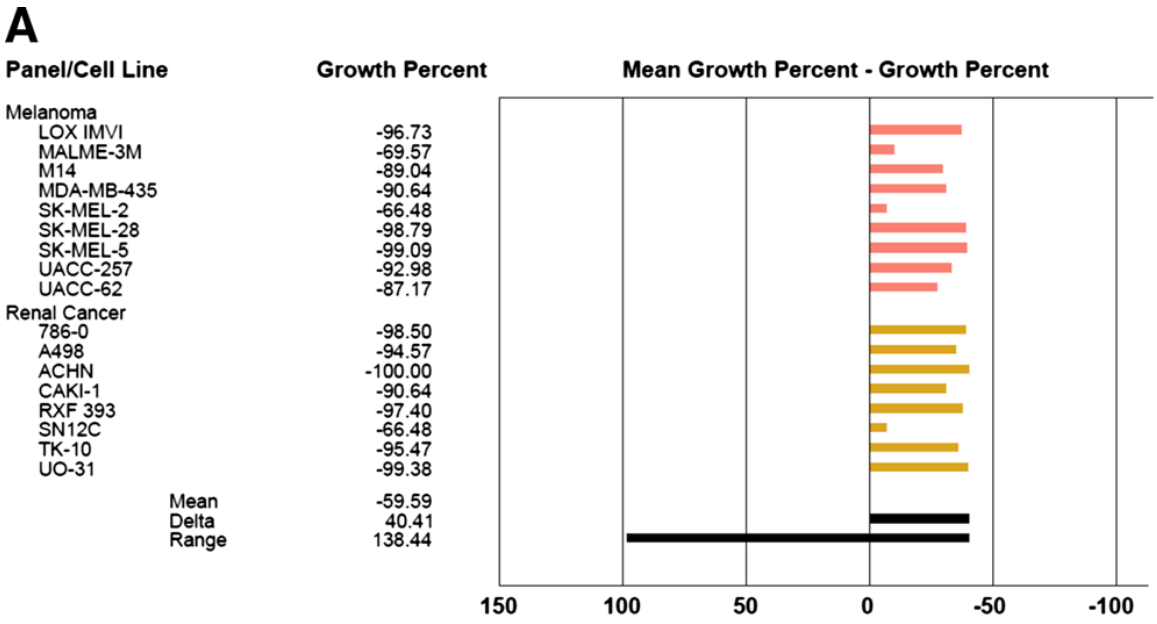


Figure 4: NCI renal cancer and melanoma sub-panels. Sphaeropsidin A was tested at 10 μM and "0" represents the mean inhibition (60%). Taken from Mathieu, *et al.*[18]

In the report by Mathieu, *et al.*,[18] sphaeropsidin A was biologically evaluated and found to induce irreversible cell shrinkage, in SKMEL-28, when treated for just six hours. The authors conclude that sphaeropsidin A most likely disrupts the cell ion homeostasis, inducing cell shrinkage, and culminating in cell death. Furthermore, sphaeropsidin A proved successful against drug resistant cancer cell lines and in some instances more effective against resistant cell lines than the parent cell lines (**Table 3**).[18]

Table 3: Evaluation of sphaeropsidin A against MDR cells. Reported by Mathieu, *et al.*[18]

Cell Line	Resistance Mechanism	**1** IC_{50} ± SD (μM)	Resistance Factor	Control drug resistance factor
KB-3-1[a]		4.13 ± 0.7		
KB-C1	ABCB1[e]	2.08 ± 0.1	0.5	>500
MDA 231[b]		1.72 ± 0.1		
MDA 231 bcrp	ABCG2[f]	1.09 ± 0.04	0.81	>100
GLC4[c]		1.09 ± 0.04		
GLC4/adr	ABCC1[g], MVP[h]	1.30 ± 0.03	1.20	>100
HL60[d]		1.36 ± 0.29		
HL60 vinc	ABCB1	1.32 ± 0.51	0.97	>1000

a: epidermal carcinoma, b: breast adenocarcinoma, c: small cell lung cancer, d: promyelocytic leukemia, e: p-glycoprotein, f: breast cancer resistance protein, g: major vault protein, h: major vault protien

RVI and Ion-Transport

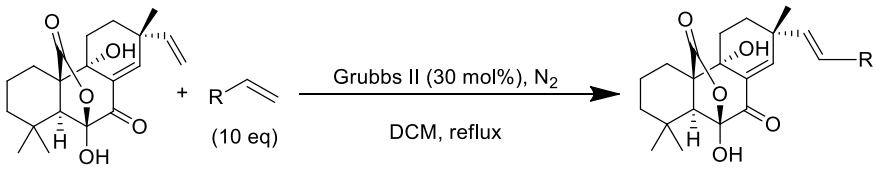
There are ion channels and transporters which respond to volume changes within the cell and induce cell shrinkage or increase cell volume. Although mammals normally maintain constant osmolarity, these mechanisms are involved in a myriad of physiological processes. Particularly, there are certain regulatory volume mechanisms which do not operate when at some steady state volume, but are only activated when the cell changes volume away from this "steady state".[19] These changes in volume have been hypothesized to be responsible for the ability of cancer cells to slip through spaces to achieve metastasis. By altering the expression of these ion transporters, a cancer cell can reduce its volume in order to pass through small gaps between cells or the extracellular matrix. Ion transporters have been explored as druggable targets for cancer chemotherapy due to their implication in several "hallmarks," such as cell migration and apoptosis evasion.[20] Both the up and down regulation of ion channels and transporters have been shown to be a mechanism for drug resistance to pro-apoptotic chemotherapies.[21]

Project Goals

Due to poor prognosis and rising mortality rates there is great demand for cancer therapeutics which address apoptosis resistance, metastasis and multidrug resistance. Also, ion transporters have shown promise as druggable targets for cancer treatment due to their involvement in many of the "hallmarks" of cancer. At the same time, sphaeropsidin A is a natural product which has shown promising results against resistant cancer cells, yet has not had its cytotoxic SAR fully explored. A small library of analogs of sphaeropsidin A has been generated but few have been tested for cytotoxicity, while none have shown greater activity than that of the natural product. Synthetic pathways have been limited to a few areas of the molecule and lacked the ability to generate diverse analogs. The goal of the current project is to generate novel sphaeropsidin A analogs through previously unexplored synthetic methodologies and extend the available SAR in this family of compounds.

II. RESULTS AND DISCUSSION

Grubbs II Cross Metathesis

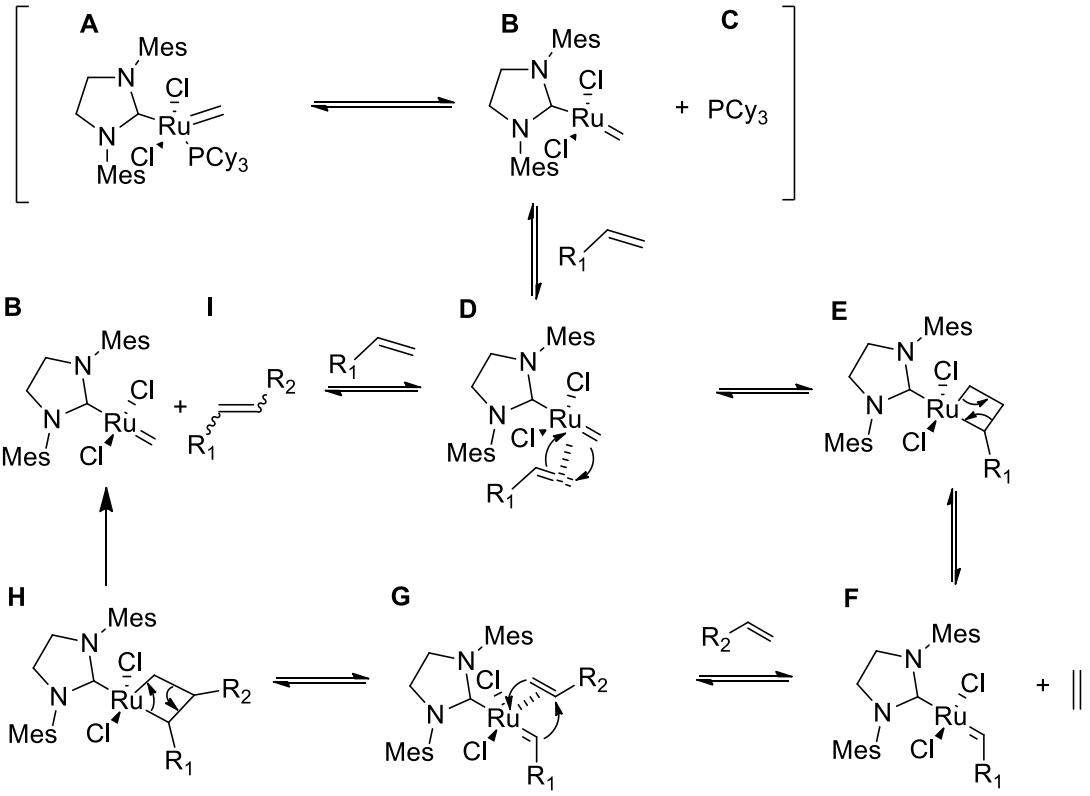


Scheme 11: Grubbs II cross metathesis general scheme

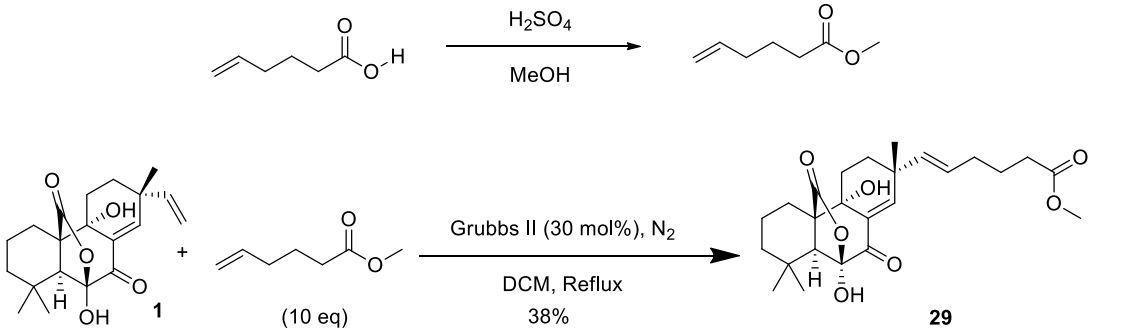
The first example of olefin metathesis was reported in 1958 while the widely accepted mechanism was proposed in 1970. It was in the 1970s that the first transition metal catalysts were synthesized to be single-component in order to move away from the days of multicomponent catalysts, made in situ, which commonly required rather harsh conditions. While these new catalysts allowed for simple set up and initiation times they were weakened by sensitivity to air and moisture. They were also limited in functional group compatibility, decreasing their effectiveness. While use of ruthenium has been reported as early as the 1960s as a catalyst, it was not until the 1980s that ruthenium was widely used. As opposed to the catalysts of the 1960s, ruthenium allowed for more flexibility for synthetic use. These new catalysts were more resilient to air, moisture, and a variety of functional groups, but it was not until 1992 that a ruthenium metathesis catalyst was reported. It was only effective for ring-opening metathesis and suffered from poor reactivity with many cyclic olefins. This first catalyst maintains a resemblance to more contemporary catalysts, but the more contemporary have markedly better functional group compatibility while having greater catalytic activity. The marked change between the first catalyst and the contemporaries is the substitution of the triphenyl phosphines for the tricyclohexyl phosphines with the subsequent catalyst substituting one of the

tricyclohexyl phosphines with a *N*-hetereocyclic carbenes. The introduction of *N*-heterocyclic carbenes as ligands for the catalyst marked a new era of olefin metathesis and opened up more synthetic routes. So much so that in 2005 Grubbs, Schrock and Chauvin were awarded the Nobel Prize in Chemistry for the development of metathesis catalysts. While the most common types of olefin metathesis may be ring-closing metathesis or ring-opening metathesis, we utilized a cross metathesis reaction to generate sphaeropsidin A analogs. Cross metathesis is more challenging than ring-closing or ring-opening metathesis. Ring-opening can benefit from the release of energy accompanied with opening a strained ring while ring-closing metathesis can benefit from entropic conditions.[22]

Sphaeropsidin A was not found to homo-couple when refluxed in DCM, most likely due to steric hindrance. To achieve cross metathesis the desired alkene is added in three portions in order to reduce the probability of homo-coupling of the added alkene in the refluxing mixture of sphaeropsidin A and Grubbs II catalyst. The postulated mechanism is shown in **Scheme 12**. The catalytic species (**A**) will undergo disassociation of tricyclohexylphosphine (**C**) which will leave a coordination spot on the ruthenium center (**B**). This allows for the first alkene to coordinate forming a π-complex (**D**) that will form a transient metallacyclobutane (**E**), the latter will quickly rearrange to release ethylene and produce a new metal carbene complex (**F**). Next, the second alkene will coordinate (**G**) and form a similar metallacyclobutane (**H**), this time rearranging to regenerate **B** and generating cross metathesis product (**I**). **Schemes 13-19** illustrate the specific sphaeropsidin A analogs prepared by this methodology.

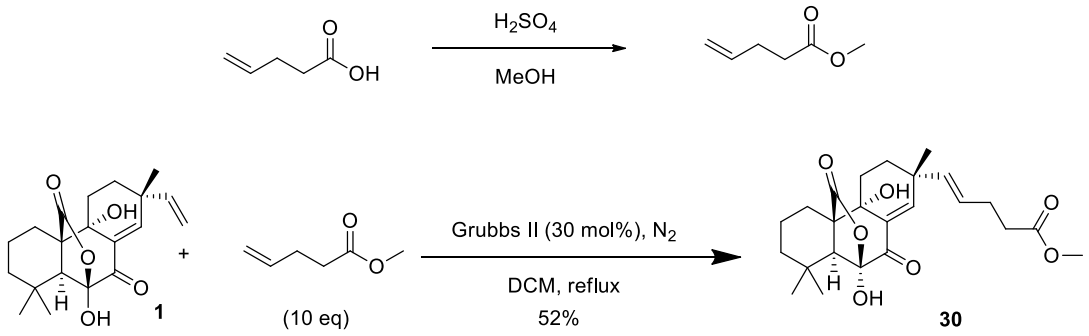


Scheme 12: Grubbs II cross metathesis proposed mechanism



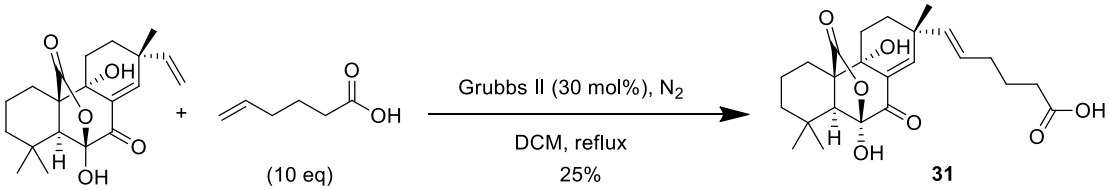
Scheme 13: Preparation of analog 29

Analog **29** was generated by first synthesizing the necessary alkene by Fisher esterification of 5-hexenoic acid in methanol, catalyzed with sulfuric acid. Using the general methodology listed in the experimental section methyl-5-hexenoate was reacted with sphaeropsidin A to afford **29** in a 38% yield. The analog is less polar than the natural product by TLC in 70/30 hexanes/EtOAc.



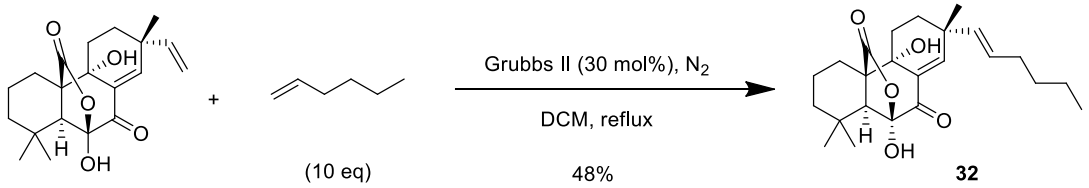
Scheme 14: Preparation of analog 30

Analog **30** was generated by first synthesizing the necessary alkene by Fisher esterification of 4-pentenoic acid in methanol, catalyzed with sulfuric acid. Using the general methodology listed in the experimental section methyl-4-pentenoate was reacted with sphaeropsidin A to afford **30** in a 52% yield. The analog is less polar than the natural product by TLC in 70/30 hexanes/EtOAc.



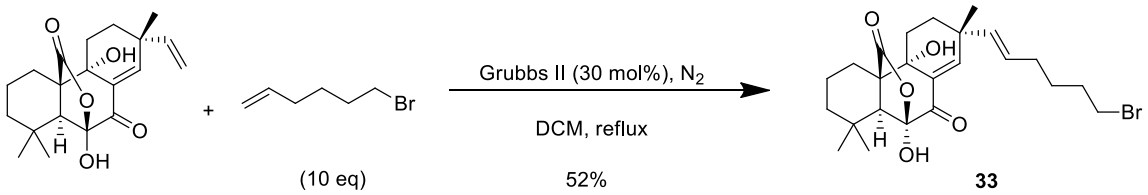
Scheme 15: Preparation of analog 31

Using the general methodology listed in the experimental section 5-hexenoic acid and sphaeropsidin A was reacted to afford **31** in a 25% yield. The analog is more polar than the natural product by TLC in 70/30 hexanes/EtOAc.



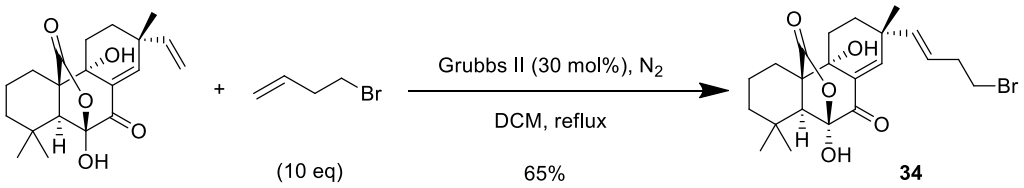
Scheme 16: Preparation of analog 32

Using the general methodology listed in the experimental section 1-hexene was reacted with sphaeropsidin A to afford **32** in a 48% yield. The analog is less polar than the natural product by TLC in 70/30 hexanes/EtOAc.



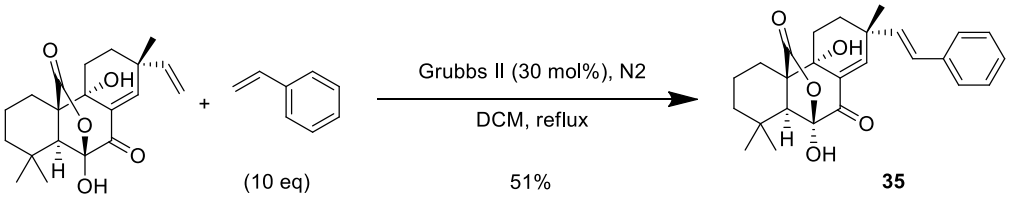
Scheme 17: Preparation of analog 33

Using the general methodology listed in the experimental section 6-bromo-1-hexene was reacted with sphaeropsidin A to afford **33** in a 52% yield. The analog is less polar than the natural product by TLC in 70/30 hexanes/EtOAc.



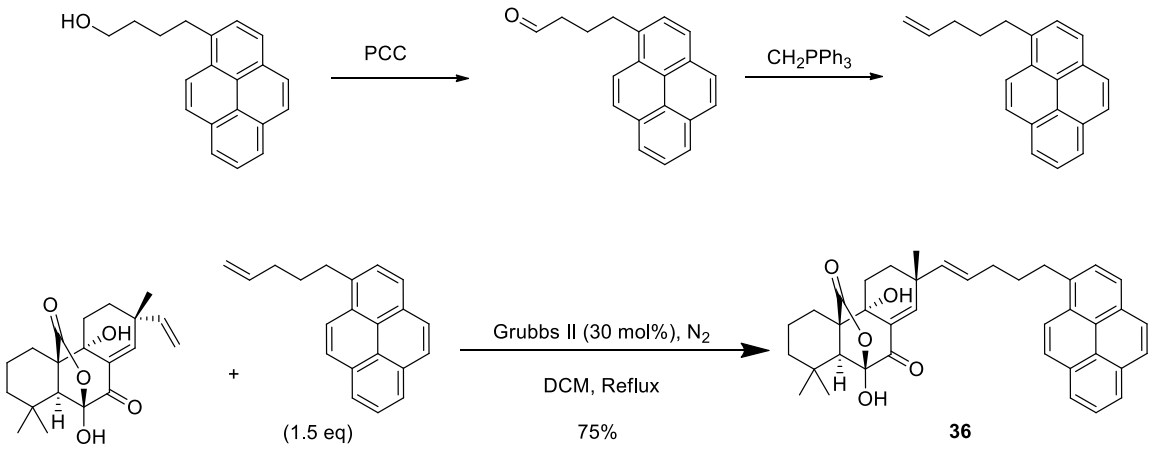
Scheme 18: Preparation of analog 34

Using the general methodology listed in the experimental 4-bromo-1-butene was reacted with sphaeropsidin A to afford **34** in a 65% yield. The analog is less polar than the natural product by TLC in 70/30 hexanes/EtOAc.



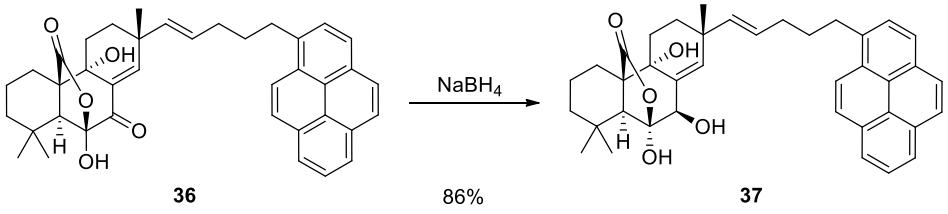
Scheme 19: Preparation of analog 35

Using the general methodology listed in the experimental section styrene was reacted with sphaeropsidin A to afford **35** in a 51% yield. The analog is less polar than the natural product by TLC in 70/30 hexanes/EtOAc.



Scheme 20: Preparation of analog 36

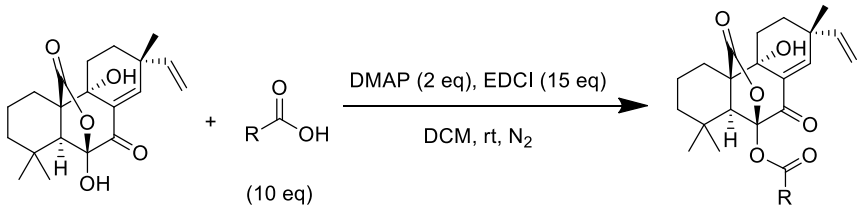
Analog **36** was generated by first synthesizing the necessary alkene by oxidation of pyrene butanol with PCC to the corresponding aldehyde and using methyltriphenyl phosphorane to perform a Wittig reaction, producing the desired alkene. Altering the general methodology listed in the experimental section by reducing the number of total equivalents of the prepared alkene to 1.5 equivalents affords **36** in a 75% yield. The analog is much more non-polar than the natural product sphaeropsidin A and most other analogs by TLC in 70/30 hexanes/EtOAc. Purification of this analog requires a different solvent system, 95/5 chloroform/ether, for purification by flash column chromatography.



Scheme 21: Preparation of analog 37

Analog **37** was generated by reducing **36** with sodium borohydride and affords **37** in an 86% yield. The analog is more polar than the parent analog but less polar than the natural product sphaeropsidin A by TLC in 70/30 hexanes/EtOAc.

Steglich Esterification

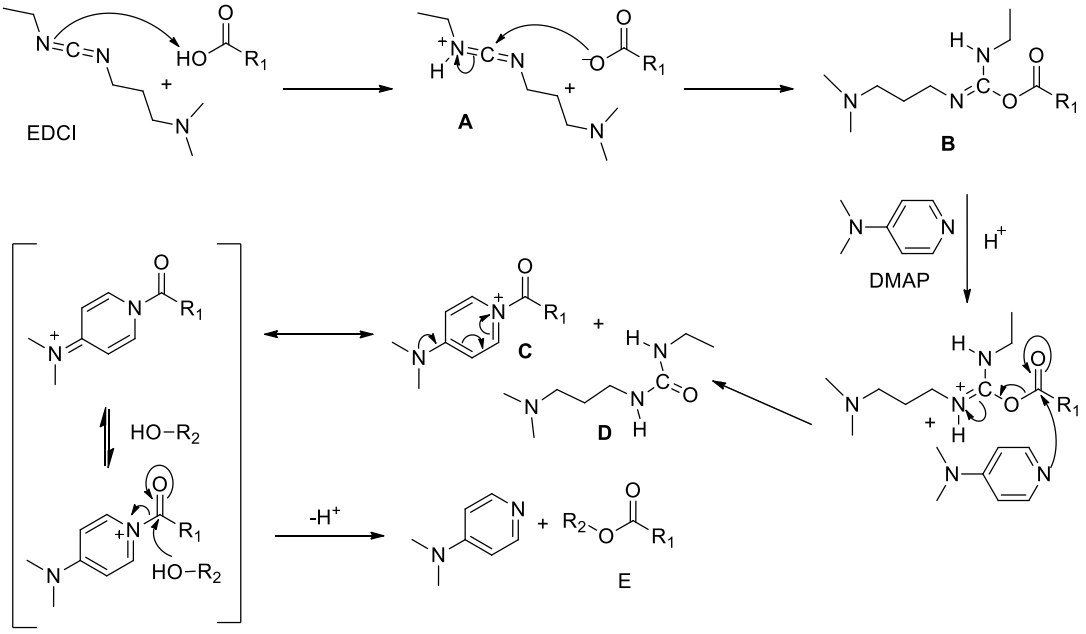


Scheme 22: Steglich esterification general scheme

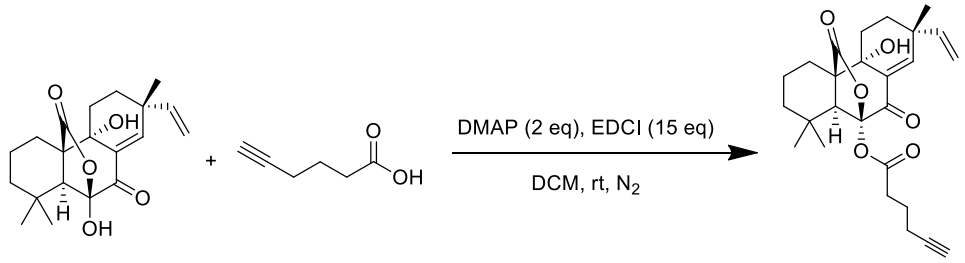
In 1978 Neises and Steglich reported a method to generate esters from carboxylic acids and alcohols. The method utilizes DCC and dimethyl aminopyridine (DMAP) to achieve the conversion as this was an improvement of the previous method using only DCC to synthesize amides and highlighted the limitations of this synthesis.[23] Furthermore, this process has been adapted due to the difficulty of removing DCC during the work-up of the reaction. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) is much more easily removed by extraction than DCC.

The EDCI is used as a base for the proton on the desired carboxylic acid, leaving the diimide carbon of intermediate **A** vulnerable for addition by the deprotonated acid. DMAP is then used for acyl substitution on **B**, while generating a urea (**D**) as a leaving group and acyl pyridinium salt **C**. The amino group on the pyridine acts as a lone pair donor in order to stabilize the positive charge on the nitrogen in the ring after attacking the acyl carbon, thus eliminating a yield reducing *N*-acyl urea compounds. The DMAP acts as a bulky leaving group, allowing for the hydroxyl group on sphaeropsidin A to

substitute easily, yielding the desired ester (**E**). The proposed mechanism is shown in **Scheme 23**. **Schemes 24-30** illustrate the specific analogs generated by this methodology.

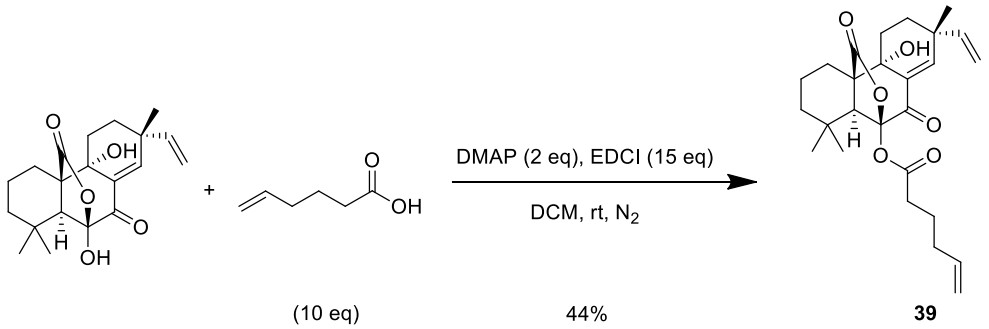


Scheme 23: Steglich esterification proposed mechanism



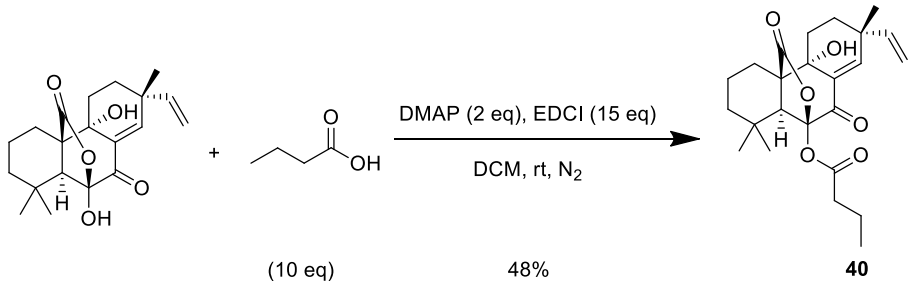
Scheme 24: Preparation of analog 38

Analog **38** is generated using the general procedure listed in the experimental chapter in a 45% yield. It is possible to monitor the reaction between the carboxylic acid with EDCI and DMAP by TLC but activation of the carboxylic acid is made more difficult to visualize by UV due to the lack of conjugated double bonds in the carboxylic acid. The analog is less polar than the natural product by TLC in chloroform.



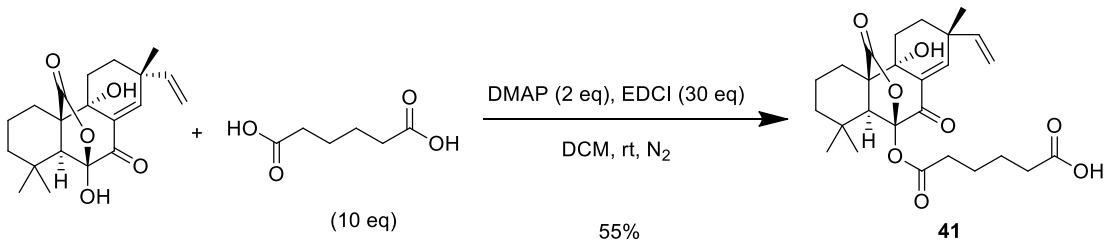
Scheme 25: Preparation of analog 39

Analog **39** is generated using the general procedure listed in the experimental chapter, in a 44% yield. Activation of the carboxylic acid is made more difficult to visualize due to the lack of conjugated double bonds in the carboxylic acid. The analog is less polar than the natural product by TLC in chloroform.



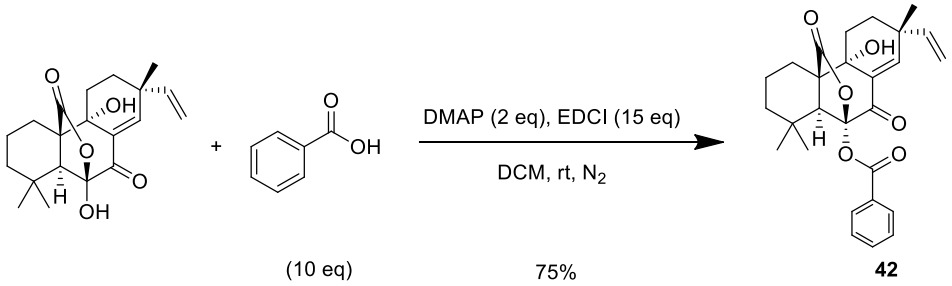
Scheme 26: Preparation of analog 40

Analog **40** is generated using the general procedure listed in the experimental chapter, in a 48% yield. Activation of the carboxylic acid is made more difficult to visualize due to the lack of conjugated double bonds in the carboxylic acid. The analog is less polar than the natural product by TLC in chloroform.



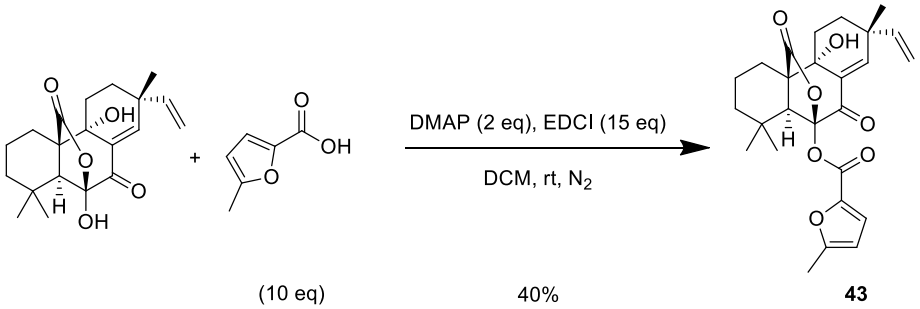
Scheme 27: Preparation of 41

Analog **41** was generated adapting the general procedure listed in the experimental chapter, increasing the equivalents of EDCI by a factor of two, in a 55% yield. The carboxylic acid is left in excess to avoid the presence of the dimer. Activation of the carboxylic acid is made more difficult to visualize due to the lack of conjugated double bonds in the carboxylic acid. The analog is more polar than the natural product by TLC is chloroform and thus is purified with a (2/98) of (MeOH/chloroform).



Scheme 28: Preparation of analog 42

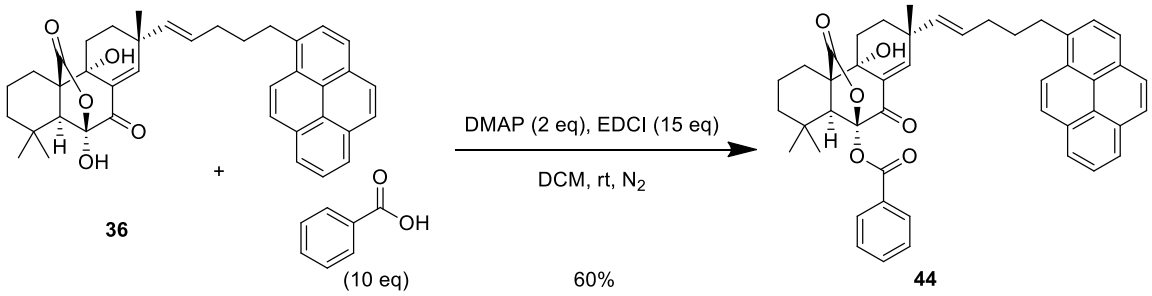
Analog **42** was generated using the general procedure listed in the experimental chapter, in a 75% yield. Activation of the carboxylic acid is made more facile to visualize due to the presence of conjugated double bonds in the carboxylic acid. The analog is less polar than the natural product by TLC in chloroform.



Scheme 29: Preparation of analog 43

Analog **43** is generated using the general procedure listed in the experimental chapter, in a 40% yield. Activation of the carboxylic acid is made more facile to visualize

due to the presence of conjugated double bonds in the carboxylic acid. The analog is less polar than the natural product by TLC in chloroform.



Scheme 30: Preparation of analog 44

This analog was generated by esterification of **36** with the experimental described here and affords **44** in a 60% yield. The analog is less polar than the parent analog and the natural product sphaeropsidin A by TLC in 70/30 hexanes/EtOAc.

Biological Evaluations

Table 4: Antiproliferative activities of sphaeropsidin A analogs

Cmpd	GI_{50} (μM)					
	B16F10[a]	SKMEL28[a]	A549[b]	MCF-7[c]	HS683[d]	U373n[d]
(**29**)	1.8	2.7	2.7	2.5	3.3	2.8
(**30**)	2	5	5	7	4	8
(**31**)	81	>100	>100	>100	85	>100
(**32**)	0.5	1.2	0.6	0.5	1.1	1.4
(**33**)	2.0	3.1	3.0	5.4	4.3	5.0
(**34**)	2.5	2.8	2	2.9	3	2.8
(**35**)	2.5	2.3	1.6	3.1	2.7	3.1
(**36**)	0.3	0.3	0.3	0.4	0.3	0.8
(**38**)	2.8	2.6	2.4	3.7	2.8	2.4
(**41**)	0.8	1.6	1.9	2.1	2.0	1.8
(**42**)	1.9	2.3	1.8	2	2.5	2.2

a: murine melanoma, b:epithelial adenocarcinoma, c:breast cancer , d:glioma

Most analogs seem to maintain similar potency as sphaeropsidin A, with the exception of **31** and **36** (**Table 4**). This indicates that the C-15,16 (See **Figure 3** for

position numbering) olefin does not tolerate polar moieties (**31**) but potency increases with large hydrophobic substitutions (**36**). All 6-O-ester analogs maintain potency identical to that of the natural product. During purification of the ester analogs, it was observed that most are hydrolytically labile and with **42** most susceptible. Thus, the hypothesis was formed that these analogs may behave as pro-drugs .To obtain evidence in support of this hypothesis we performed quantitative video microscopy of sphaeropsidin A as well as analogs **38** and **42** to monitor cell morphology. There were interesting differences in kinetics observed in the three compounds. A clear delay is seen in the two analogs **38** and **42** vs. the natural product, as well as clear differences between the two analogs (**Figure 5**).

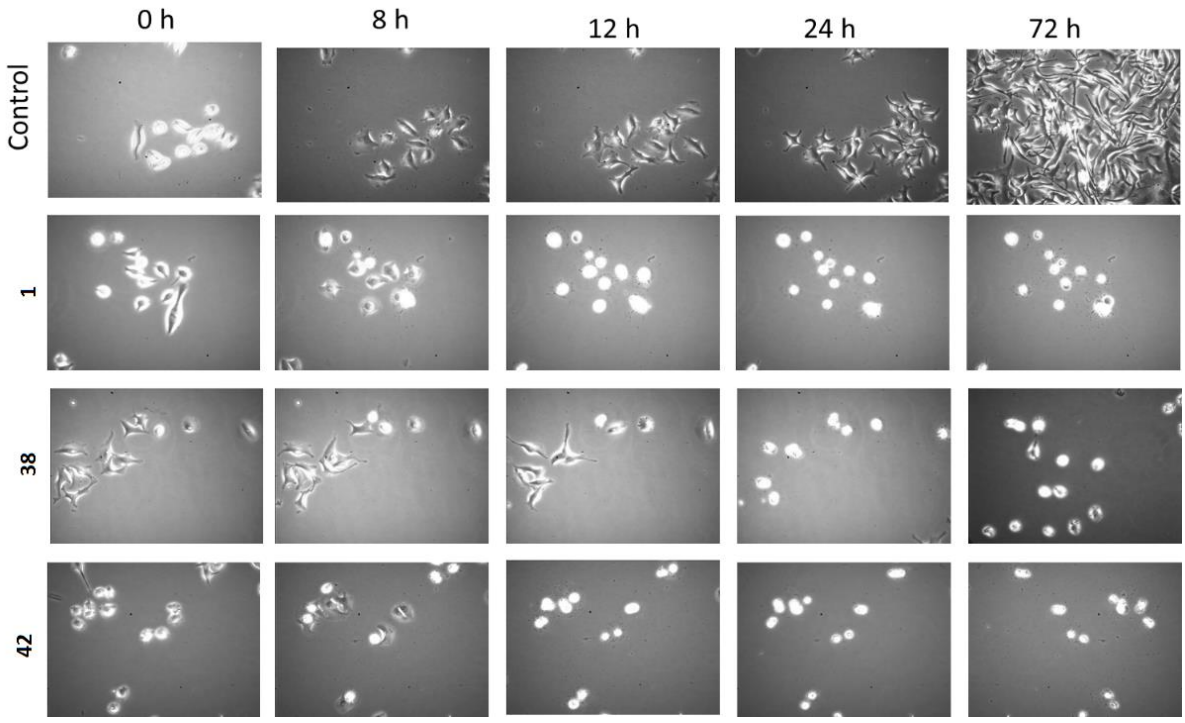
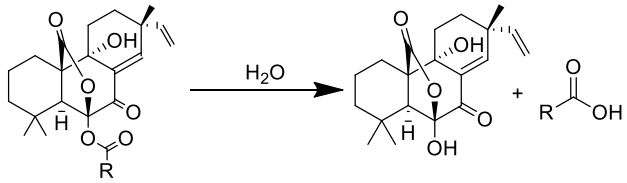


Figure 5: Video microscopy. Sphaeropsidin A and analogs 38 and 42

Looking at **Figure 5**, it appears that there is a delay in activation for the two analogs, **38** and **42**, in comparison to the natural product. This suggests that these

compounds may be pro-drugs and require hydrolysis in order to release the active natural product (**Scheme 31**).



Scheme 31: Proposed hydrolysis of ester analogs

It would follow that, hydrolysis of analog **42** should proceed faster than analog **38** because of the electron withdrawing nature of the benzene ring leaving the acyl carbon more electron deficient; implying that analog **42** should become active before analog **38**.

III. CONCLUSION

Sphaeropsidin A is a promising anticancer agent due the sensitivity of MDR cell lines to sphaeropsidin A. However, before its development as anticancer agent broad SAR data must be obtained. In the previous literature sphaeropsidin A has been derivatized in various positions and a number of analogs were prepared. However, only a few of them were evaluated for anticancer activity and analogs more potent than the natural product could not be found.

In the current thesis, we attempted to fill the gap in the literature by exploring two new derivatization methods, namely olefin cross metathesis and Steglich esterification. The latter derivatizes 6-O by introducing esters while the former allows one to incorporate modifications at C-15,16 olefin. It was found that derivatization at the alkene position with hydrophobic groups leads to compounds with retained or enhanced potency when compared to the natural product. Thus, this position appears to be ideal for generating analogs possessing better physical/chemical characteristics, which is an important aspect for drug development. Esterification of the O-6 oxygen also leads to analogs with retained potencies. However, in this case, we believe the synthesized analogs act as prodrugs that undergo hydrolysis which release sphaeropsidin A. This hypothesis received further support through quantitative video microscopy experiments showing the delay in cytotoxicity of the prodrugs compared with sphaeropsidin A. If this hypothesis is indeed correct, it provides an opportunity to design prodrugs with better physical/chemical properties and tumor site specificity. Future work will further investigate this hypothesis through the synthesis of an ester which is much less susceptible hydrolysis.

It is expected that the results obtained in the current thesis will be useful for further development of Spha as an anticancer agent and in the long run contribute to our ability to treat drug resistant cancers, such as: glioma, melanoma, and non-small cell lung cancer.

IV. EXPERIMENTAL

General

All reagents, solvents and catalysts were purchased from commercial sources (Acros Organics and Sigma-Aldrich) and used without purification. All reactions were performed in oven-dried flasks open to the atmosphere or under nitrogen and monitored by thin layer chromatography (TLC) on TLC precoated (250 µm) silica gel 60 F254 glass-backed plates (EMD Chemicals Inc.). Visualization was accomplished with UV light. Flash column chromatography was performed on silica gel (32-63 µm, 60 Å pore size). ^{1}H and ^{13}C NMR were recorded on a Bruker 400 spectrometer. Chemical shifts are reported in ppm relative to the TMS internal standard. Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). HRMS analyses were performed using Waters Synapt G2 LCMS. The >95% purity of the synthesized compounds was ascertained by UPLC/MS analyses.

General Procedure for Grubbs II Cross Metathesis

Sphaeropsidin A (10 mg, 28.9 mmol) and Grubbs II catalyst (7.4 mg, 8.7 mmol) are dissolved in DCM, under nitrogen, and brought to reflux. Desired alkene (289 mmol) is added in three portions in 1.5 hour intervals. Solvent is evaporated under reduced pressure and purified by flash column chromatography in 10% diethyl ether/hexanes or by preparatory TLC in 70/30 hexanes/EtOAc.

General Procedure for Steglich Esterification

The desired carboxylic acid (289 mmol), DMAP (7.1 mg, 57.8 mmol), and EDCI (67.3 mg, 433.5 mmol) are added to round bottom flask, under nitrogen and dissolved in 2.5 mL of DCM. Reaction is stirred for 1.5 hours and monitored on TLC to observe the activation of the acid. Sphaeropsidin A (10 mg, 28.9 mmol) is added and reaction is stirred overnight to produce the desired analog. Solvent is evaporated under reduced pressure, the crude material is dissolved in EtOAc, washed 3x with DI water, washed with brine, and dried over $MgSO_4$. The desired analog is purified by flash column chromatography with 2.5% hexanes/DCM or by preparatory TLC in chloroform.

Characterization of Compounds

29 (38%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.85 (d, *J* = 1.8 Hz, 1H), 5.46 (m, 2H), 5.21 (s, 1H), 3.69 (s, 3H), 2.75 (s, 1H), 2.40 – 1.00 (m, 51H). HRMS m/z (ESI+) calcd for $C_{25}H_{34}O_7$ ($M+Na^+$) 469.2202, found 469.2185.

30 (52%): ^{1}H NMR (400 MHz, CDCl3) δ 6.83 (d, *J* = 1.8 Hz, 1H), 5.50 (m, 2H), 5.21 (s, 1.5H), 3.70 (s, 3H), 2.73 (s, 1.5H), 2.49 – 1.03 (m, 68H). HRMS m/z (ESI+) calcd for $C_{24}H_{32}O_7$ ($M+H^+$) 433.2226, found 433.2223.

31 (25%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (d, *J* = 1.8 Hz, 1H), 5.50 (s, 7H), 5.32 (s, 2H), 5.21 (d, *J* = 5.4 Hz, 3H), 2.10 – 1.17 (m, 196H).HRMS m/z (ESI +) calcd for $C_{24}H_{32}O_7$ ($M+H^+$) 433.2226, found 433.2223.

32 (48%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (d, *J* = 1.8 Hz, 1H), 5.55 – 5.33 (m, 2H), 5.20 (s, 1H), 2.72 (s, 1H), 2.30-1.08 (m, 39H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.7, 174.5, 153.8, 136.0, 132.4, 129.6, 103.5, 71.0, 57.0, 51.2, 40.3, 38.5, 32.5, 32.4,

32.2, 31.5, 30.2, 26.9, 24.8, 22.9, 22.3, 22.2, 18.0, 13.9. HRMS m/z (ESI+) calcd for $C_{24}H_{34}O_5$ ($M+Na^+$) 425.2304, found 425.2283.

33 (52%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.85 (d, *J* = 1.8 Hz, 1H), 5.48 (m, 2H), 5.32 (s, 1H), 5.22 (s, 1H), 4.14 (q, *J* = 7.1 Hz, 1H), 3.43 (t, *J* = 6.7 Hz, 2H), 2.74 (s, 1H), 2.35 – 0.99 (m, 52H). MS m/z (ESI-) calcd for $C_{24}H_{33}BrO_5$ (M-H) 479.1433, found 479.4.

34 (65%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.85 (d, *J* = 1.8 Hz, 1H), 5.59 (dt, *J* = 15.7, 1.0 Hz, 1H), 5.48 (dt, *J* = 15.7, 6.5 Hz, 1H), 5.21 (s, 1H), 3.41 (t, *J* = 6.9 Hz, 2H), 2.74 (s, 1H), 2.67 – 2.56 (m, 2H), 2.30 – 1.07 (m, 53H). HRMS m/z (ESI+) calcd for $C_{22}H_{29}BrO_5$ ($M+Na^+$) 475.1096, found 475.1091.

35 (51%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (m, 5H), 6.96 (d, *J* = 1.8 Hz, 1H), 6.44 (d, *J* = 16.3 Hz, 1H), 6.20 (d, *J* = 16.3 Hz, 1H), 5.23 (s, 2H), 2.76 (s, 1H), 2.35 –1.24 (m, 49H). HRMS m/z (ESI+) calcd for $C_{20}H_{30}O_5$ ($M+Na^+$) 445.1991, found 445.1981.

36 (75%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 – 7.83 (m, 11H), 6.85 (d, *J* = 1.8 Hz, 1H), 5.52 (dt, *J* = 26.9, 12.6 Hz, 2H), 5.21 (s, 1H), 3.41 – 3.32 (m, 2H), 2.72 (s, 1H), 2.30 – 1.20 (m, 45H). HRMS m/z (ESI+) calcd for $C_{36}H_{40}O_5$ ($M+Na^+$) 611.2773, found 611.2766.

37 (86%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, *J* = 9.3 Hz, 1H), 8.22 – 8.16 (m, 2H), 8.13 (dd, *J* = 8.5, 2.3 Hz, 2H), 8.08 – 7.97 (m, 3H), 7.88 (d, *J* = 7.8 Hz, 1H), 5.91 – 5.82 (m, 1H), 5.54 – 5.44 (m, 2H), 4.82 (s, 1H), 4.22 (d, *J* = 1.7 Hz, 1H), 3.42 – 3.31 (m, 2H), 2.56 (s, 1H), 1.17 (dd, *J* = 74.6, 37.7 Hz, 48H). HRMS m/z (ESI+) calcd for $C_{39}H_{42}O_5$ ($M+Na^+$) 613.2930, found 613.2932.

38 (45%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.56 (d, *J* = 1.7 Hz, 1H), 5.82 (dd, *J* = 17.5, 10.7 Hz, 1H), 5.10 (dd, *J* = 6.9, 0.6 Hz, 1H), 5.06 (s, 1H), 2.89 (s, 1H), 2.69 (qt, *J* = 16.6, 7.2 Hz, 2H), 2.37 (td, *J* = 7.0, 2.6 Hz, 2H), 2.24 (d, *J* = 13.5 Hz, 1H), 2.13 – 1.07 (m, 25H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.4, 173.9, 169.2, 148.4, 144.6, 135.8, 113.0, 105.3, 83.0, 77.2, 71.6, 69.4, 56.0, 53.3, 50.8, 40.5, 38.8, 32.9, 32.7, 32.6, 29.6, 26.6, 24.4, 23.3, 22.6, 22.3, 17.7, 17.6. HRMS m/z (ESI+) calcd for $C_{26}H_{32}O_6$ ($M+Na^+$) 463.2097, found 463.2075.

39 (44%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.55 (d, *J* = 1.4 Hz, 1H), 5.92 – 5.70 (m, 2H), 5.16 – 4.96 (m, 4H), 2.88 (s, 1H), 2.63 – 2.46 (m, 2H), 2.28 – 1.06 (m, 39H). HRMS m/z (ESI+) calcd for $C_{26}H_{34}O_6$ ($M+Na^+$) 465.2253, found 465.2256.

40 (48%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.56 (d, *J* = 1.8 Hz, 1H), 5.83 (dd, *J* = 17.5, 10.7 Hz, 1H), 5.12 – 5.05 (m, 2H), 2.88 (s, 1H), 2.52 (qt, *J* = 16.1, 7.3 Hz, 2H), 2.29 – 0.97 (m, 44H). HRMS m/z (ESI+) calcd for $C_{24}H_{32}O_6$ ($M+Na^+$) 439.2097, found 439.2093.

41 (55%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.54 (d, *J* = 1.7 Hz, 1H), 5.80 (dd, *J* = 17.6, 10.6 Hz, 1H), 5.30 (s, 1H), 5.17 – 5.00 (m, 2H), 3.36 (s, 1H), 2.89 (s, 1H), 2.69 – 0.99 (m, 49H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.6, 178.0, 173.9, 169.3, 148.3, 144.6, 135.8, 113.0, 105.2, 71.6, 56.0, 53.3, 40.4, 38.8, 33.9, 33.4, 32.7, 32.6, 29.5, 26.5, 24.4, 23.9, 23.8, 22.6, 22.3, 17.7. HRMS m/z (ESI+) calcd for $C_{26}H_{34}O_8$ ($M+Na^+$) 497.2151, found 497.2154.

42 (75%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 – 8.02 (m, 2H), 7.70 – 7.56 (m, 1H), 7.56 – 7.42 (m, 2H), 6.61 (s, 1H), 5.81 (dd, *J* = 17.5, 10.6 Hz, 1H), 5.07 – 5.04 (m, 2H), 3.02 (s, 1H), 2.25 (d, *J* = 13.5 Hz, 1H), 2.05 – 1.00 (m, 21H). ^{13}C NMR (100 MHz,

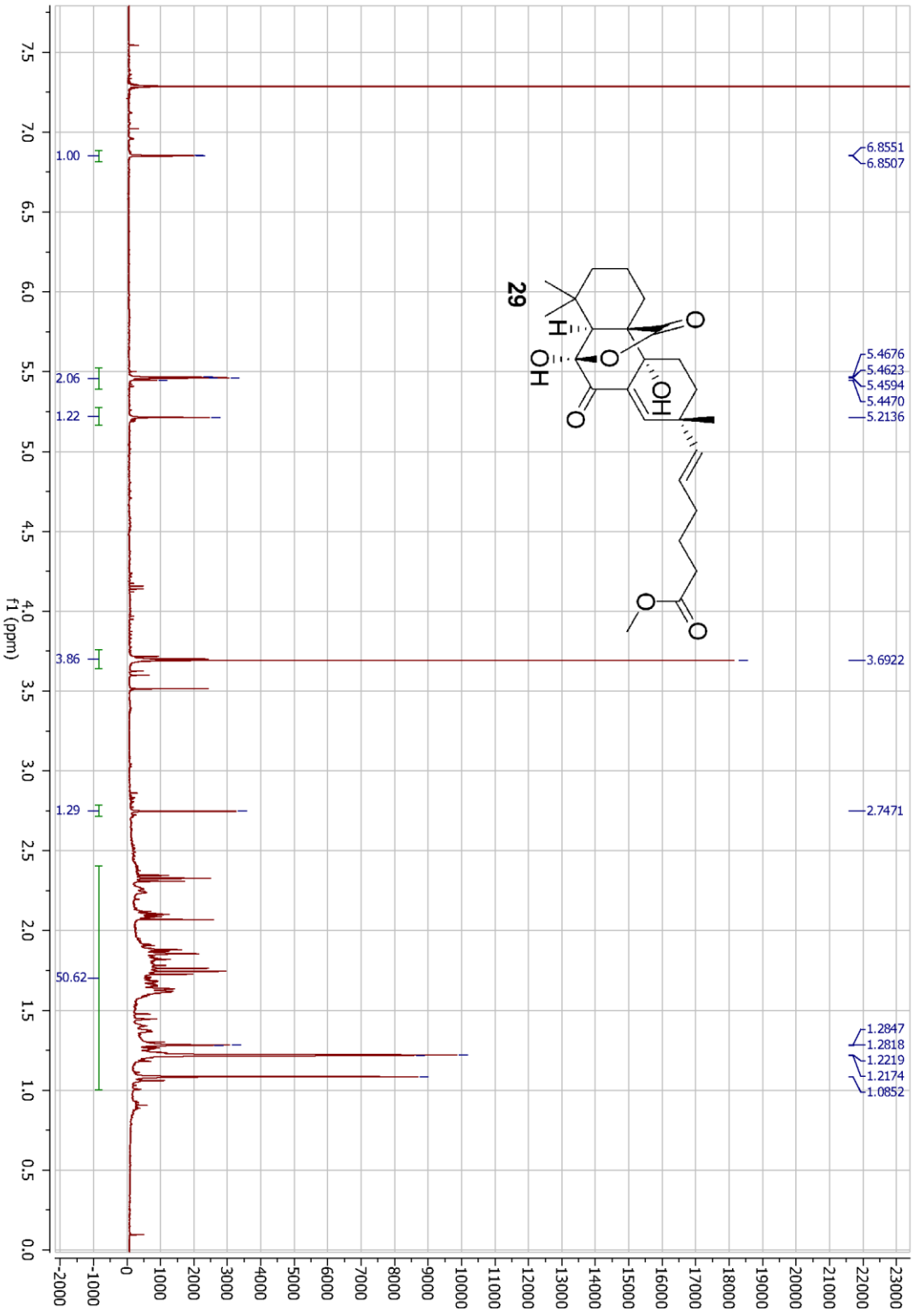
$CDCl_3$) δ 144.6, 133.8, 130.2, 129.0, 128.7, 113.0, 77.3, 77.0, 76.7, 40.6, 32.6, 29.6, 26.6, 24.39, 22.4, 17.7. HRMS m/z (ESI+) calcd for $C_{27}H_{30}O_6$ ($M+Na^+$) 473.1940, found 473.1920.

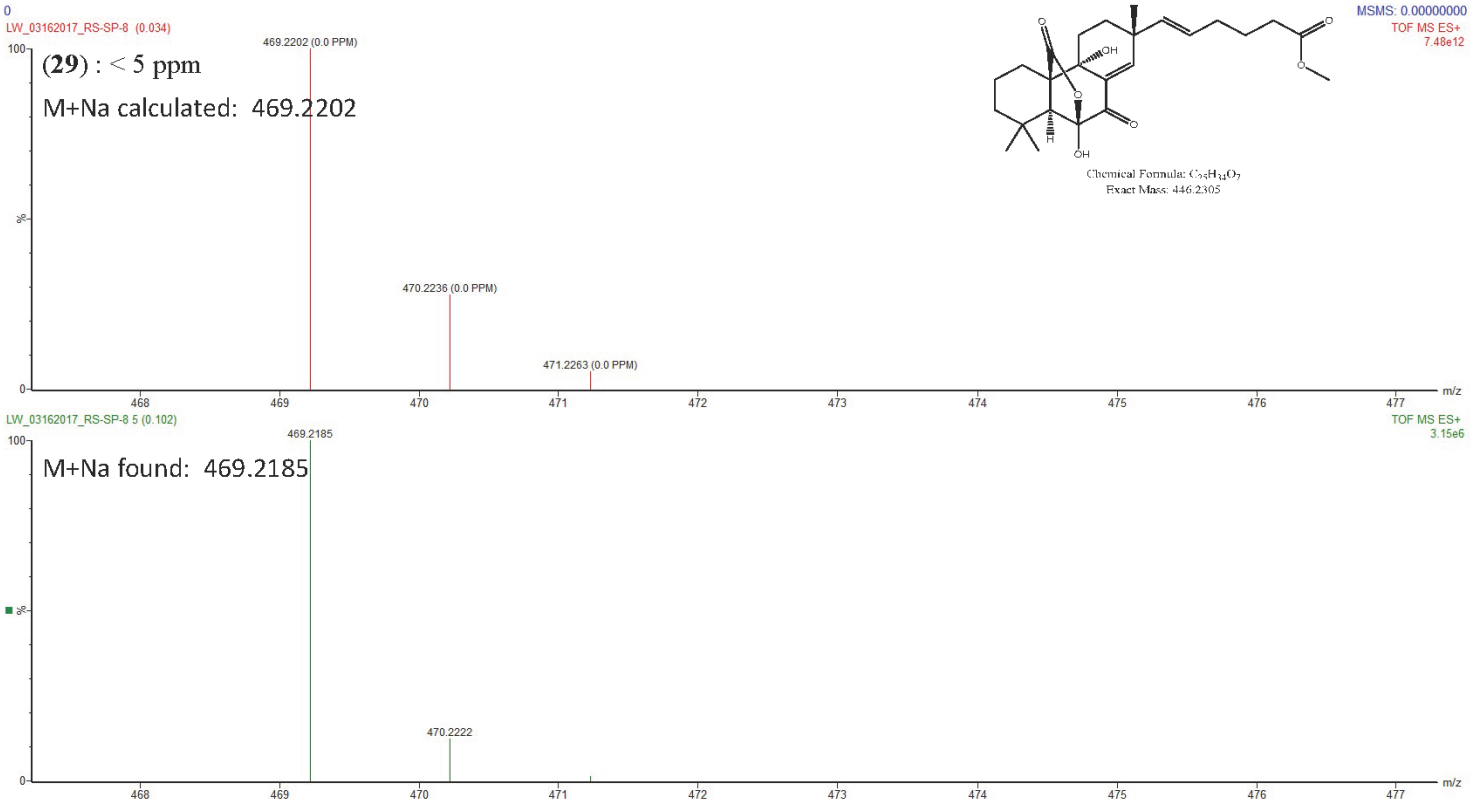
43 (40%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, *J* = 3.4 Hz, 1H), 6.60 (s, 1H), 6.20 (dd, *J* = 3.4, 0.9 Hz, 1H), 5.83 (dd, *J* = 17.5, 10.6 Hz, 1H), 5.08 (m, 2H), 2.99 (s, 1H), 2.42 (s, 3H), 2.32 – 1.03 (m, 32H). HRMS m/z (ESI+) calcd for $C_{26}H_{30}O_7$ ($M+Na^+$) 477.1889, found 477.1902.

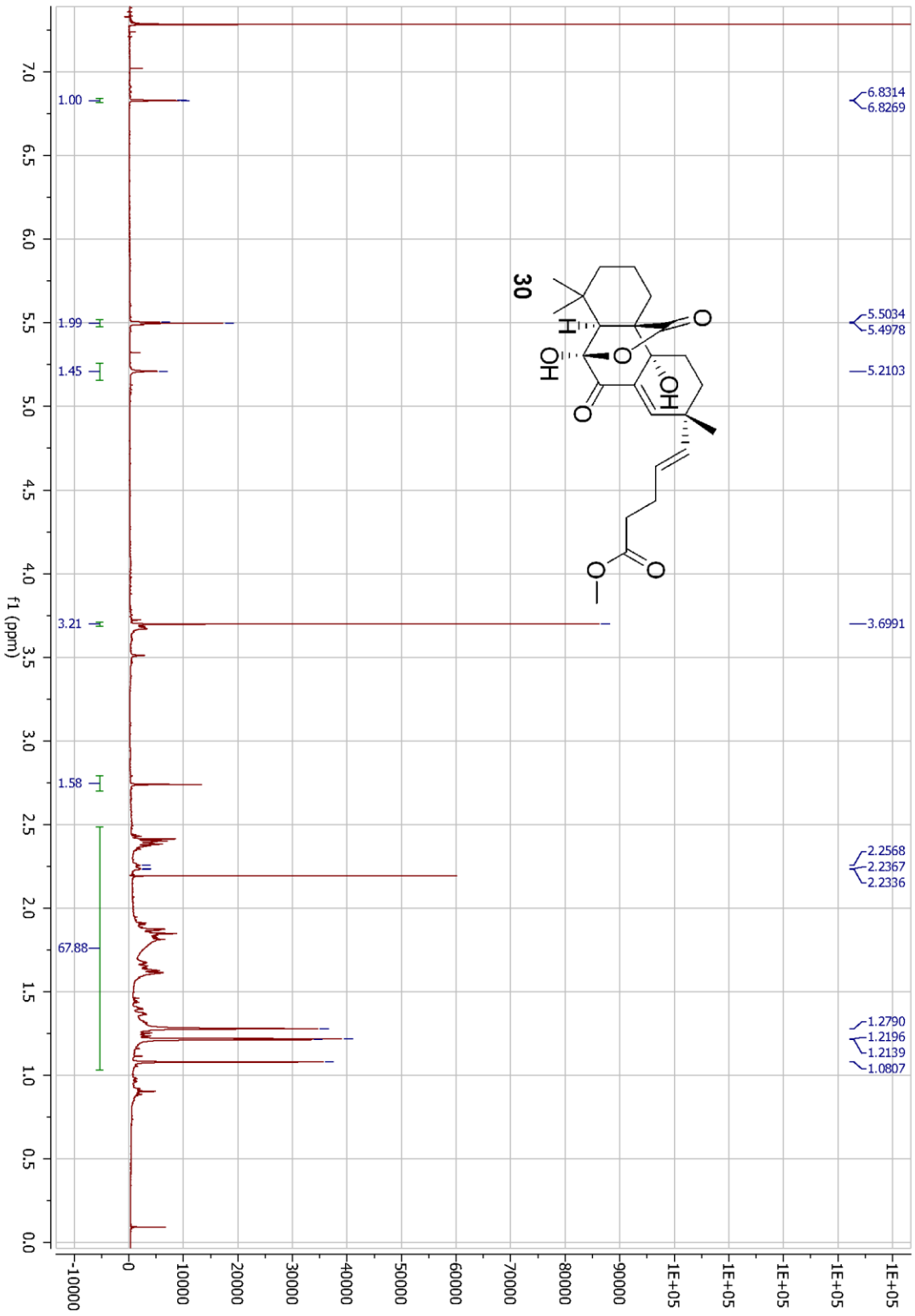
44 (60%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 – 7.96 (m, 9H), 7.87 (d, *J* = 7.8 Hz, 1H), 7.65 (m, 1H), 7.55 – 7.48 (m, 2H), 6.63 (s, 1H), 5.54 (m, 2H), 3.40 – 3.31 (m, 2H), 2.44 – 1.04 (m, 45H). HRMS m/z (ESI+) calcd for $C_{46}H_{44}O_6$ ($M+Na^+$) 715.3036, found 715.3030.

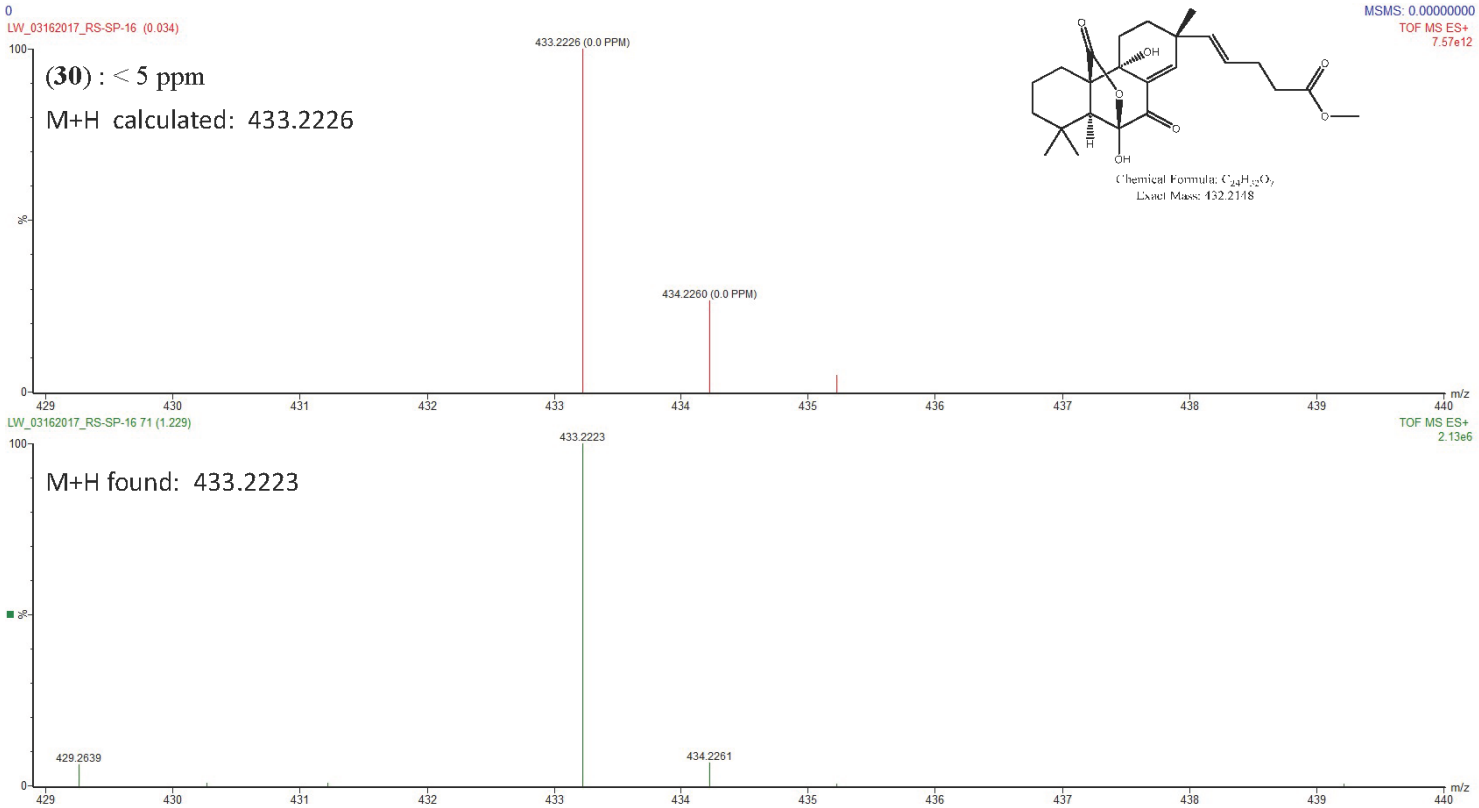
V. SUPPORTING INFORMATION

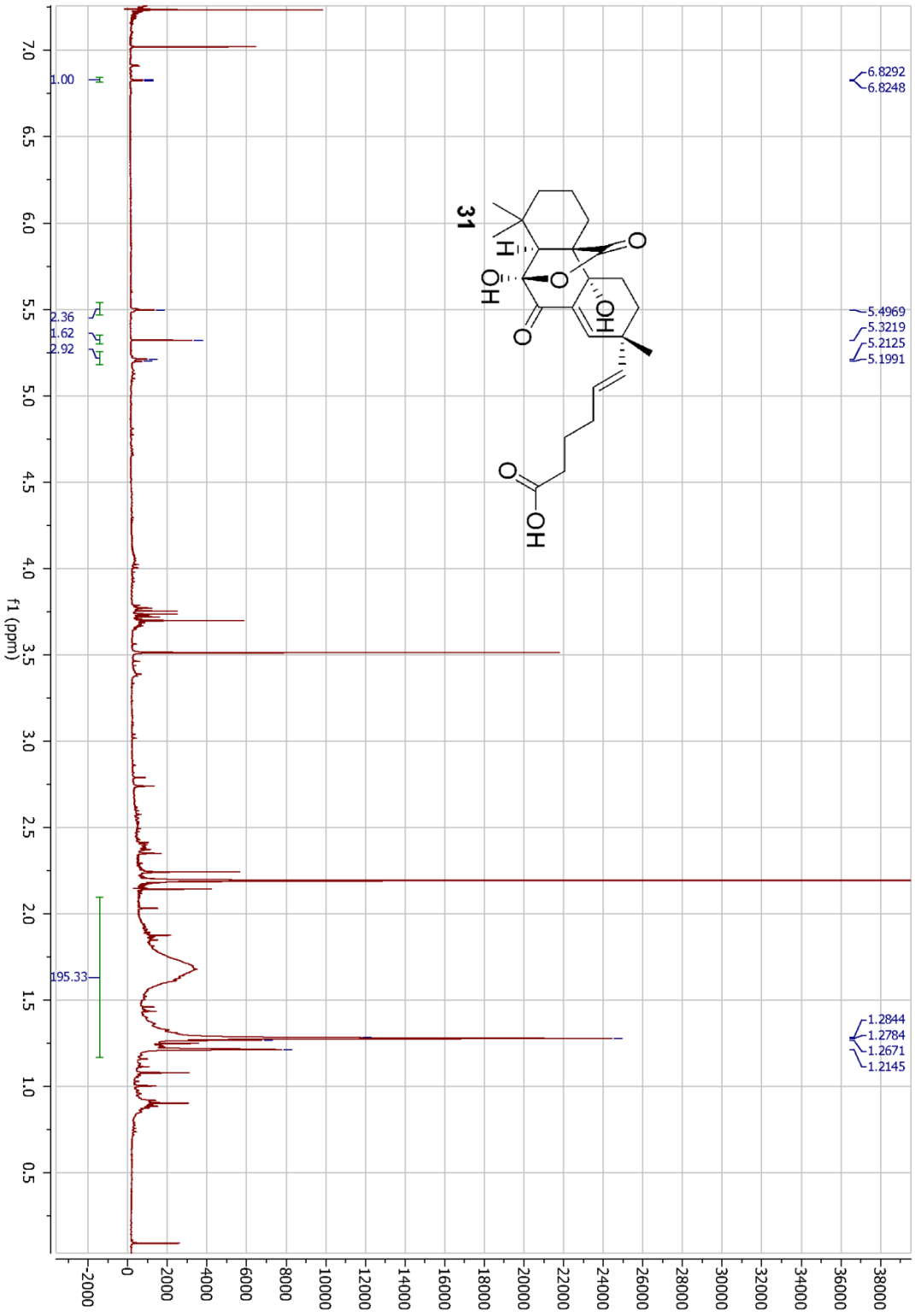
LIST OF SUPPORTING DATA

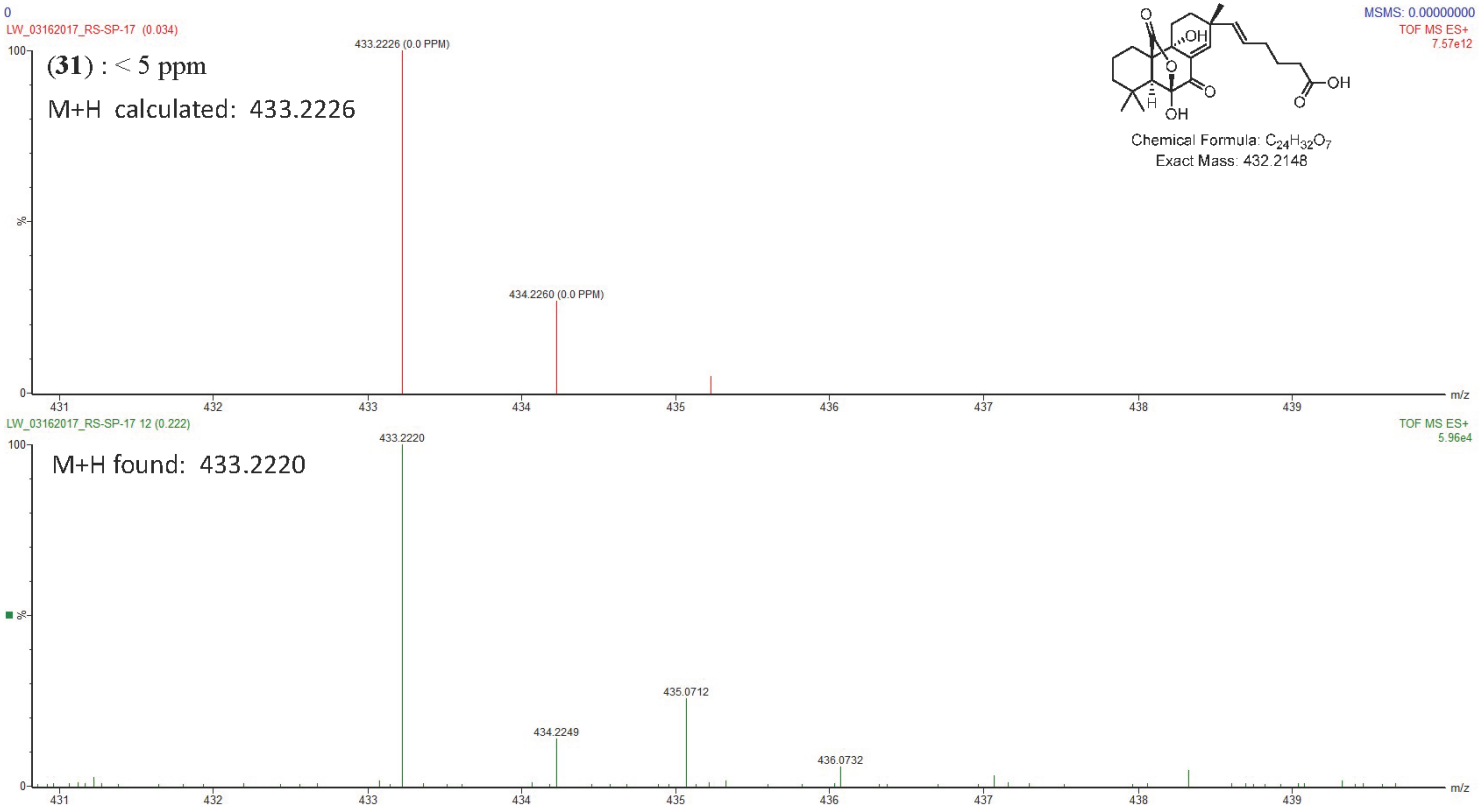

29
6.8551
6.8507
5.4676
5.4623
5.4594
5.4470
5.2136
3.6922
2.7471
1.2847
1.2818
1.2219
1.2174
1.0852
1.00
2.06
1.22
3.86
1.29
50.62
f1 (ppm)

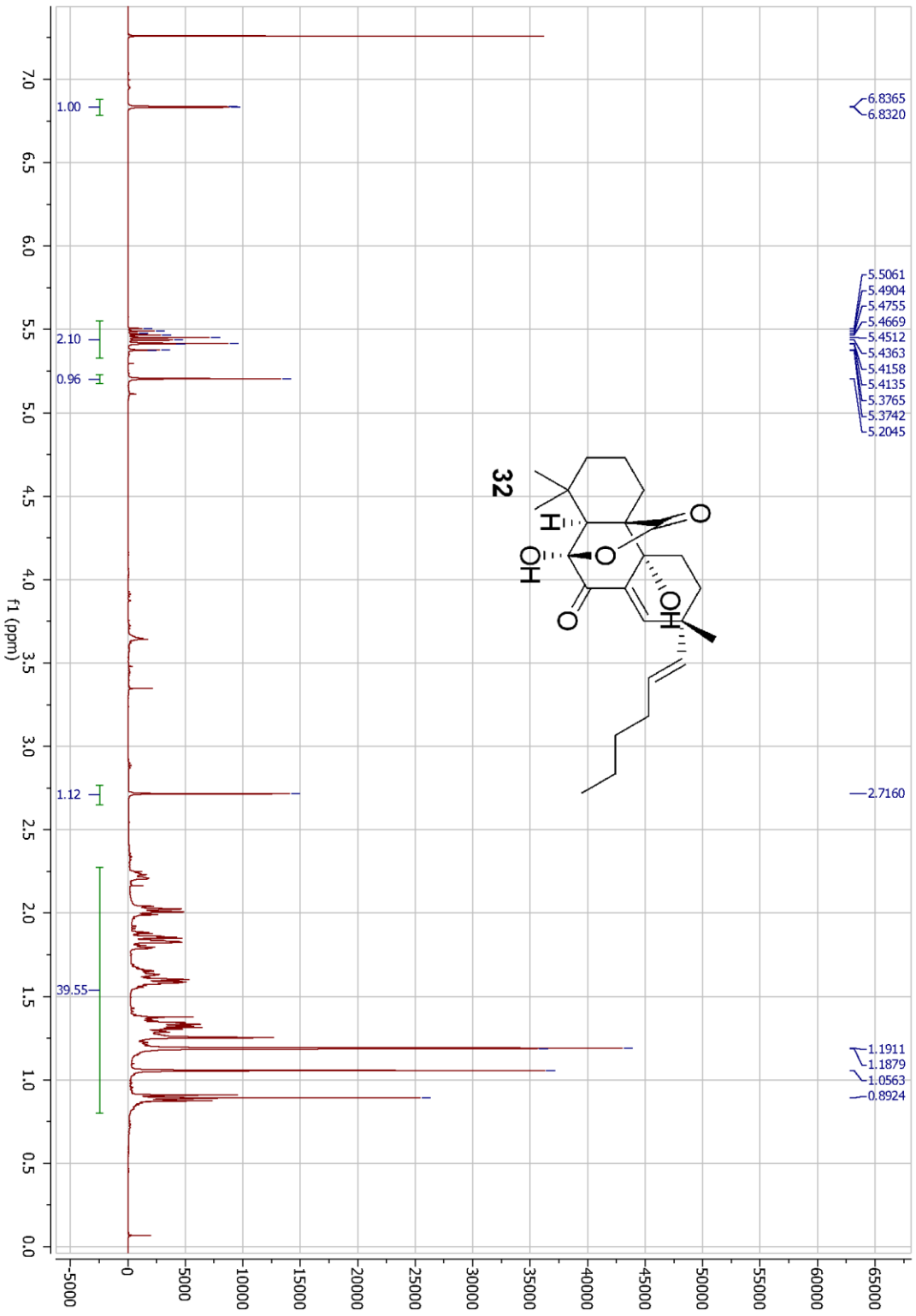

0
LW_03162017_RS-SP-8 (0.034)
MSMS: 0.00000000
TOF MS ES+
7.48e12
(29) : < 5 ppm
M+Na calculated: 469.2202
469.2202 (0.0 PPM)
470.2236 (0.0 PPM)
471.2263 (0.0 PPM)
Chemical Formula: C26H38O7
Exact Mass: 446.2305
LW_03162017_RS-SP-8 5 (0.102)
TOF MS ES+
3.15e6
M+Na found: 469.2185
469.2185
470.2222
m/z


30
1.00
1.99
1.45
3.21
1.58
67.88
6.8314
6.8269
5.5034
5.4978
5.2103
3.6991
2.2568
2.2367
2.2336
1.2790
1.2196
1.2139
1.0807
f1 (ppm)


0
LW_03162017_RS-SP-16 (0.034)
100
%
0
433.2226 (0.0 PPM)
434.2260 (0.0 PPM)
(30) : < 5 ppm
M+H calculated: 433.2226
MSMS: 0.00000000
TOF MS ES+
7.57e12
OH
OH
Chemical Formula: C24H32O7
Exact Mass: 432.2148
429 430 431 432 433 434 435 436 437 438 439 440
m/z
LW_03162017_RS-SP-16 71 (1.229)
TOF MS ES+
2.13e6
100
%
0
433.2223
M+H found: 433.2223
429.2639
434.2261
429 430 431 432 433 434 435 436 437 438 439 440
m/z


31
6.8292
6.8248
5.4969
5.3219
5.2125
5.1991
1.2844
1.2784
1.2671
1.2145
1.00
2.36
1.62
2.92
195.33
f1 (ppm)


0
LW_03162017_RS-SP-17 (0.034)
100
%
(31) : < 5 ppm
M+H calculated: 433.2226
433.2226 (0.0 PPM)
434.2260 (0.0 PPM)
0
431
432
433
434
435
436
437
438
439
m/z
MSMS: 0.00000000
TOF MS ES+
7.57e12
OH
O
O
O
OH
O
H
OH
Chemical Formula: C24H32O7
Exact Mass: 432.2148
LW_03162017_RS-SP-17 12 (0.222)
TOF MS ES+
5.96e4
100
%
M+H found: 433.2220
433.2220
435.0712
434.2249
436.0732
0
431
432
433
434
435
436
437
438
439
m/z


32
f1 (ppm)
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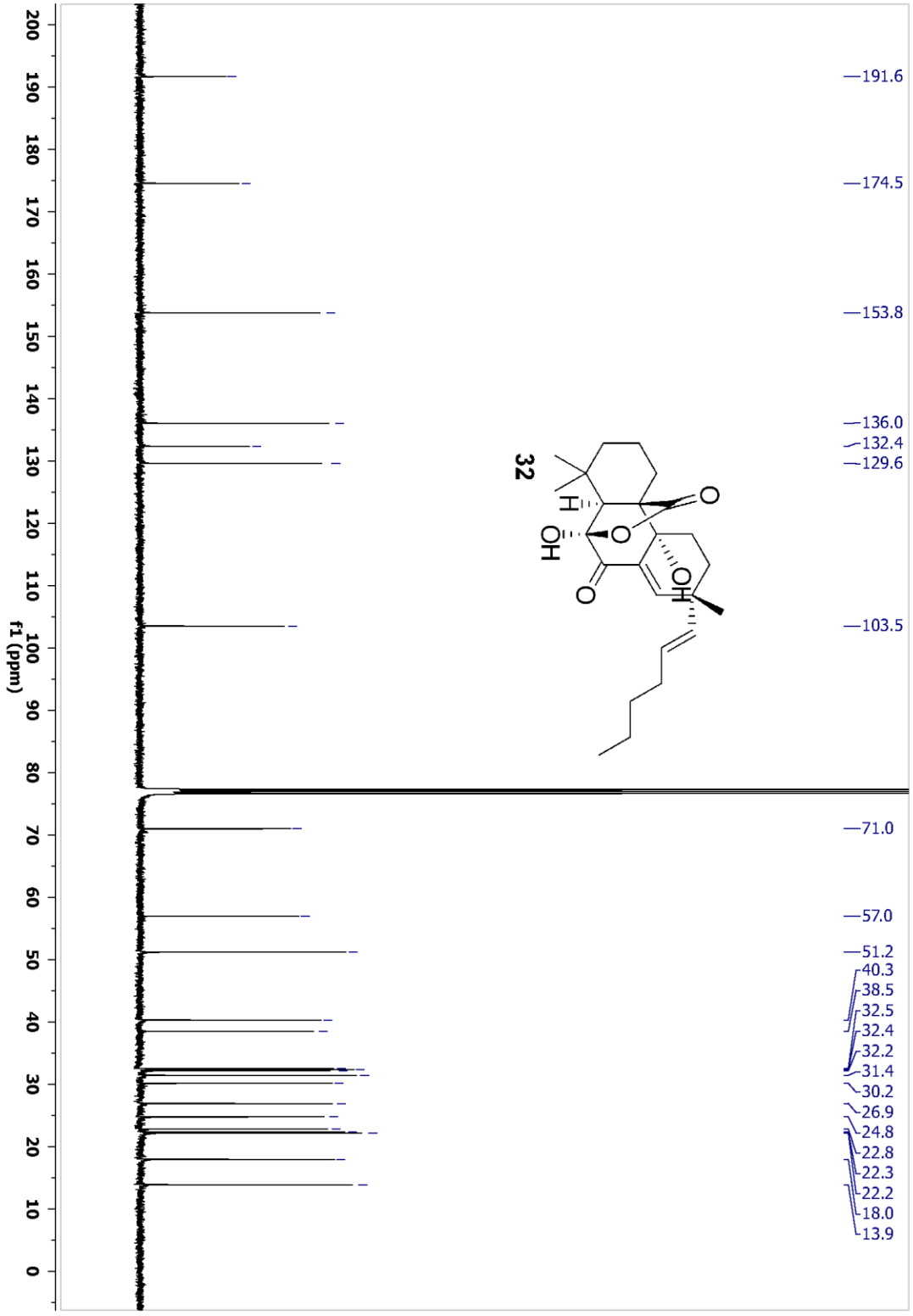
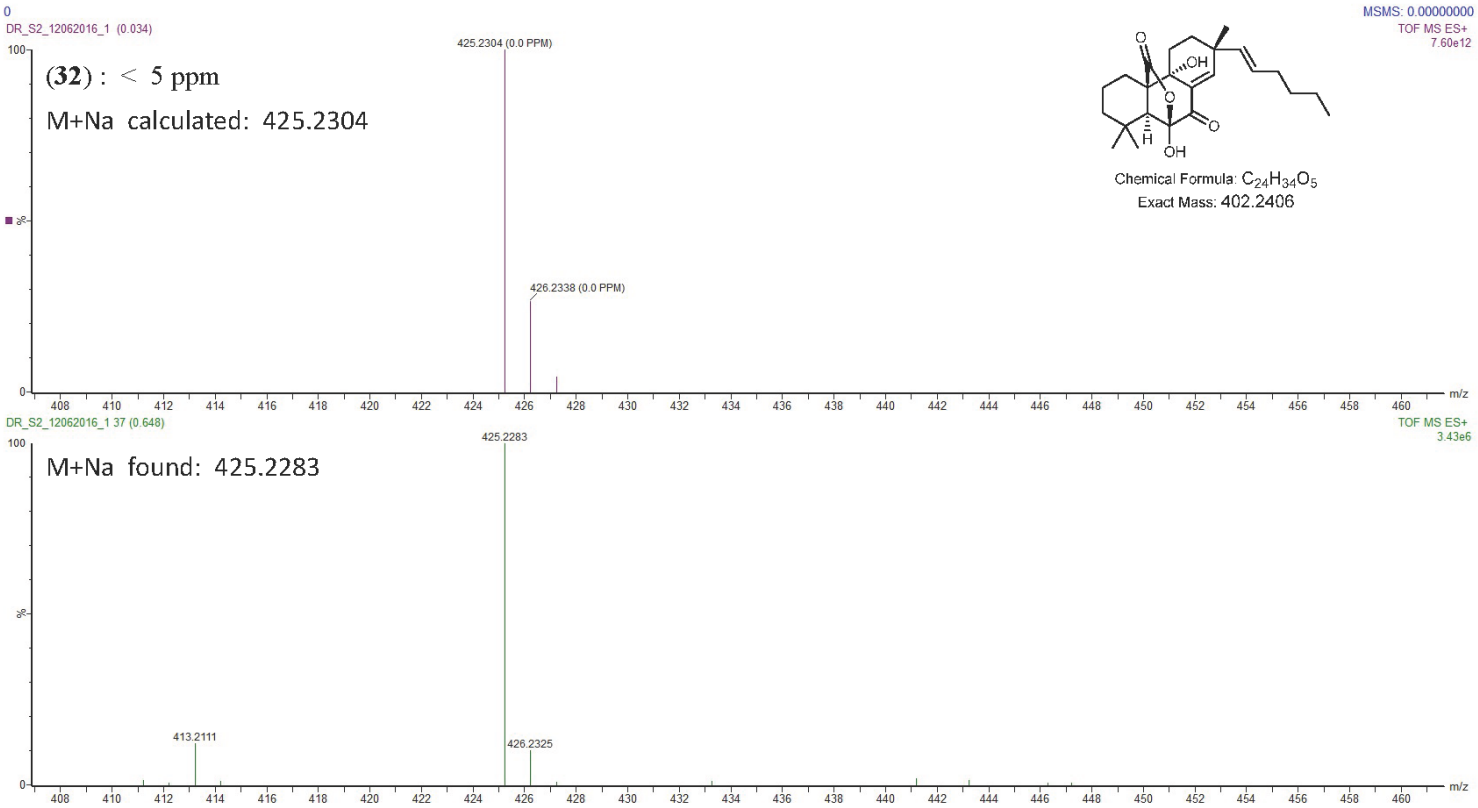
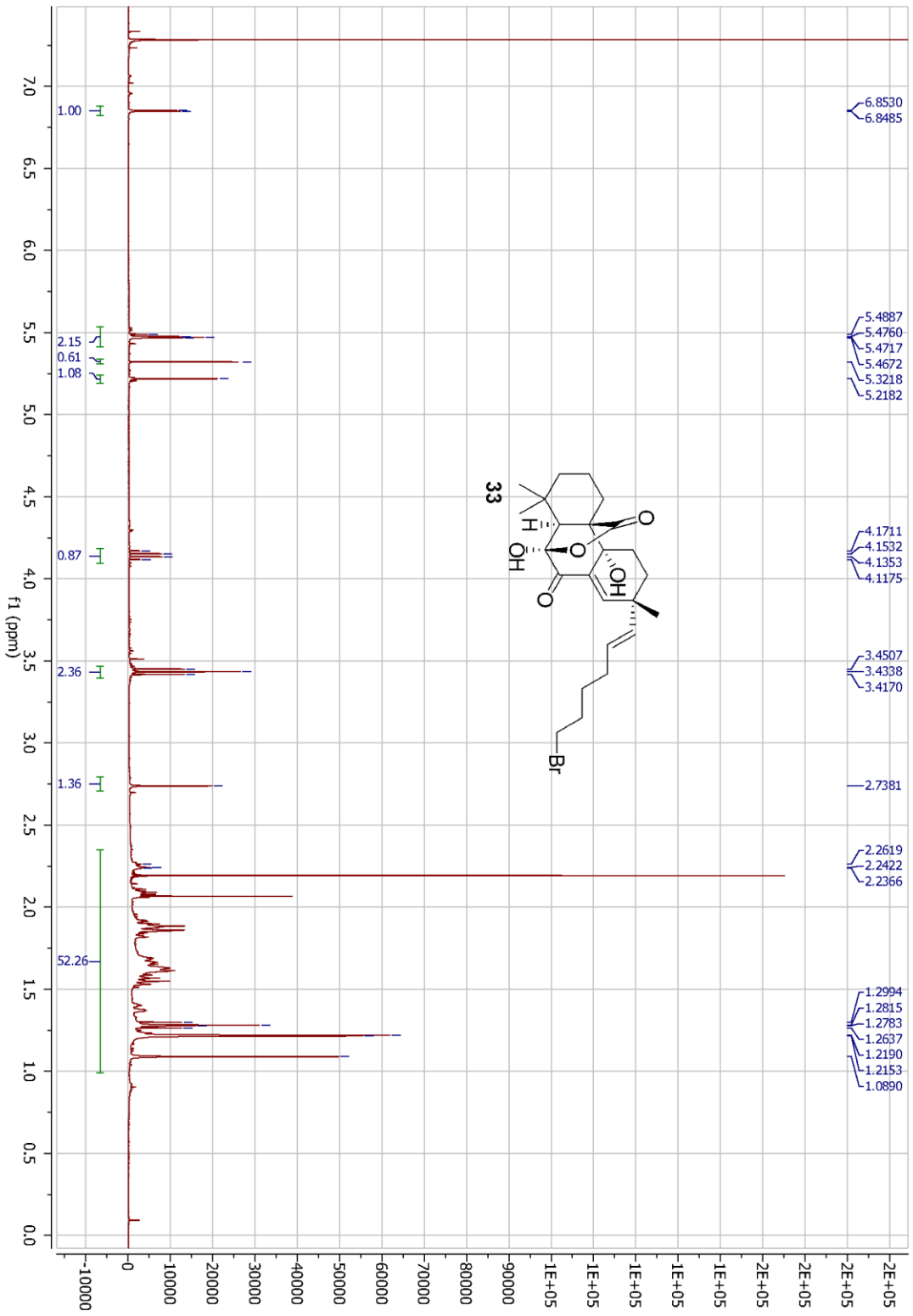
2.10
0.96
1.12
39.55
6.8365
6.8320
5.5061
5.4904
5.4755
5.4669
5.4512
5.4363
5.4158
5.4135
5.3765
5.3742
5.2045
2.7160
1.1911
1.1879
1.0563
0.8924


f1 (ppm)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
191.6
174.5
153.8
136.0
132.4
129.6
103.5
71.0
57.0
51.2
40.3
38.5
32.5
32.4
32.2
31.4
30.2
26.9
24.8
22.8
22.3
22.2
18.0
13.9
32
H
OH
O
O
O
OH


0
DR_S2_12062016_1 (0.034)
MSMS: 0.00000000
TOF MS ES+
7.60e12
100
(32) : < 5 ppm
M+Na calculated: 425.2304
425.2304 (0.0 PPM)
426.2338 (0.0 PPM)
%
0
m/z
408 410 412 414 416 418 420 422 424 426 428 430 432 434 436 438 440 442 444 446 448 450 452 454 456 458 460
Chemical Formula: C24H34O5
Exact Mass: 402.2406
DR_S2_12062016_1 37 (0.648)
TOF MS ES+
3.43e6
100
M+Na found: 425.2283
425.2283
413.2111
426.2325
%
0
m/z
408 410 412 414 416 418 420 422 424 426 428 430 432 434 436 438 440 442 444 446 448 450 452 454 456 458 460


f1 (ppm)
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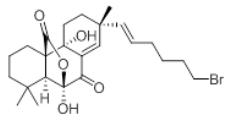
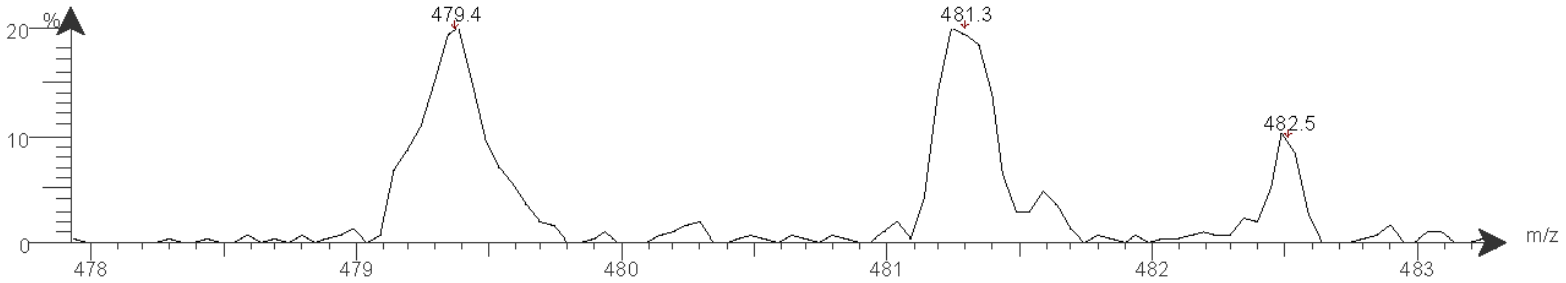
0.61
1.08
0.87
2.36
1.36
52.26
6.8530
6.8485
5.4887
5.4760
5.4717
5.4672
5.3218
5.2182
4.1711
4.1532
4.1353
4.1175
3.4507
3.4338
3.4170
2.7381
2.2619
2.2422
2.2366
1.2994
1.2815
1.2783
1.2637
1.2190
1.2153
1.0890
33
OH
H
O
O
OH
O
Br

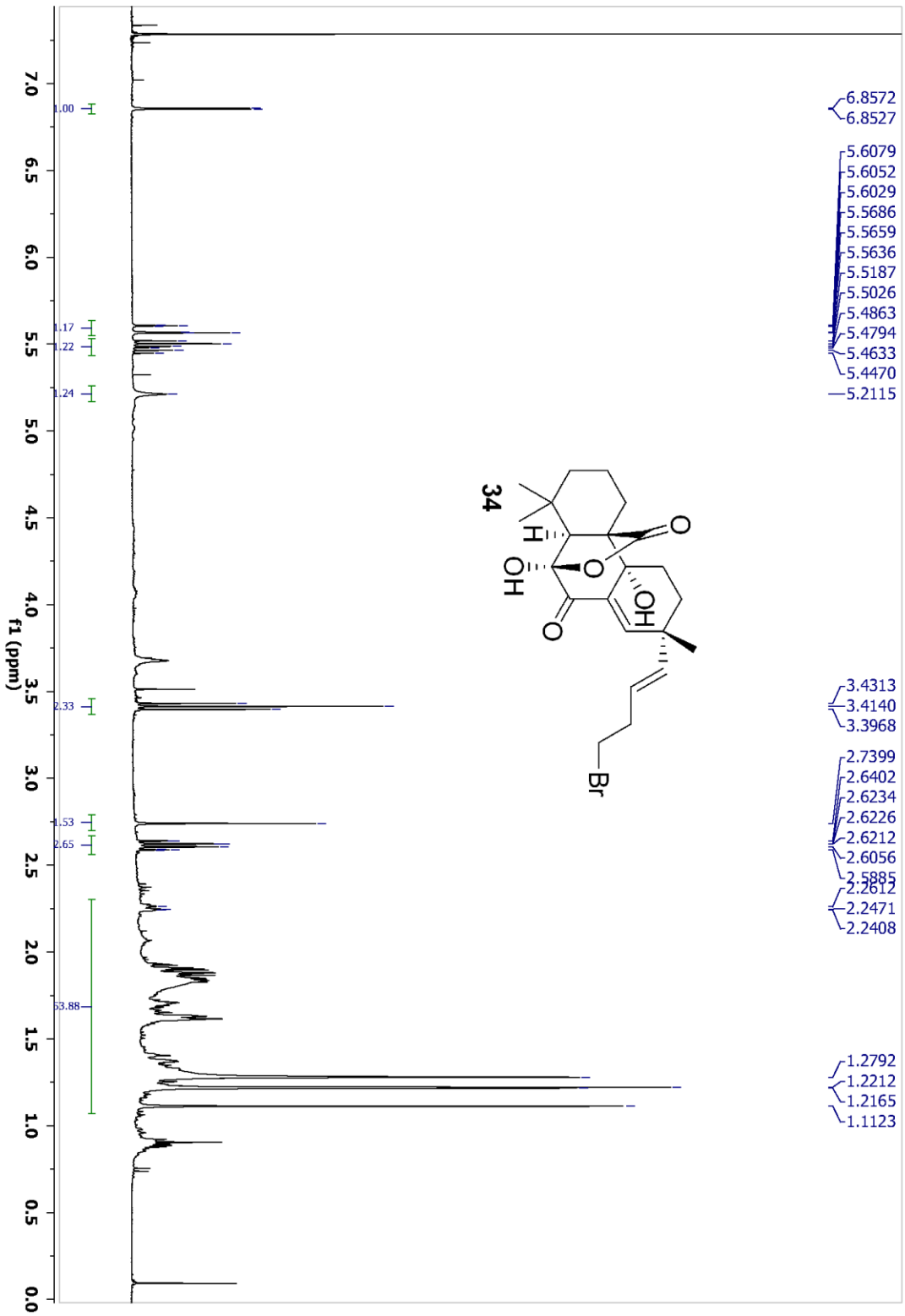


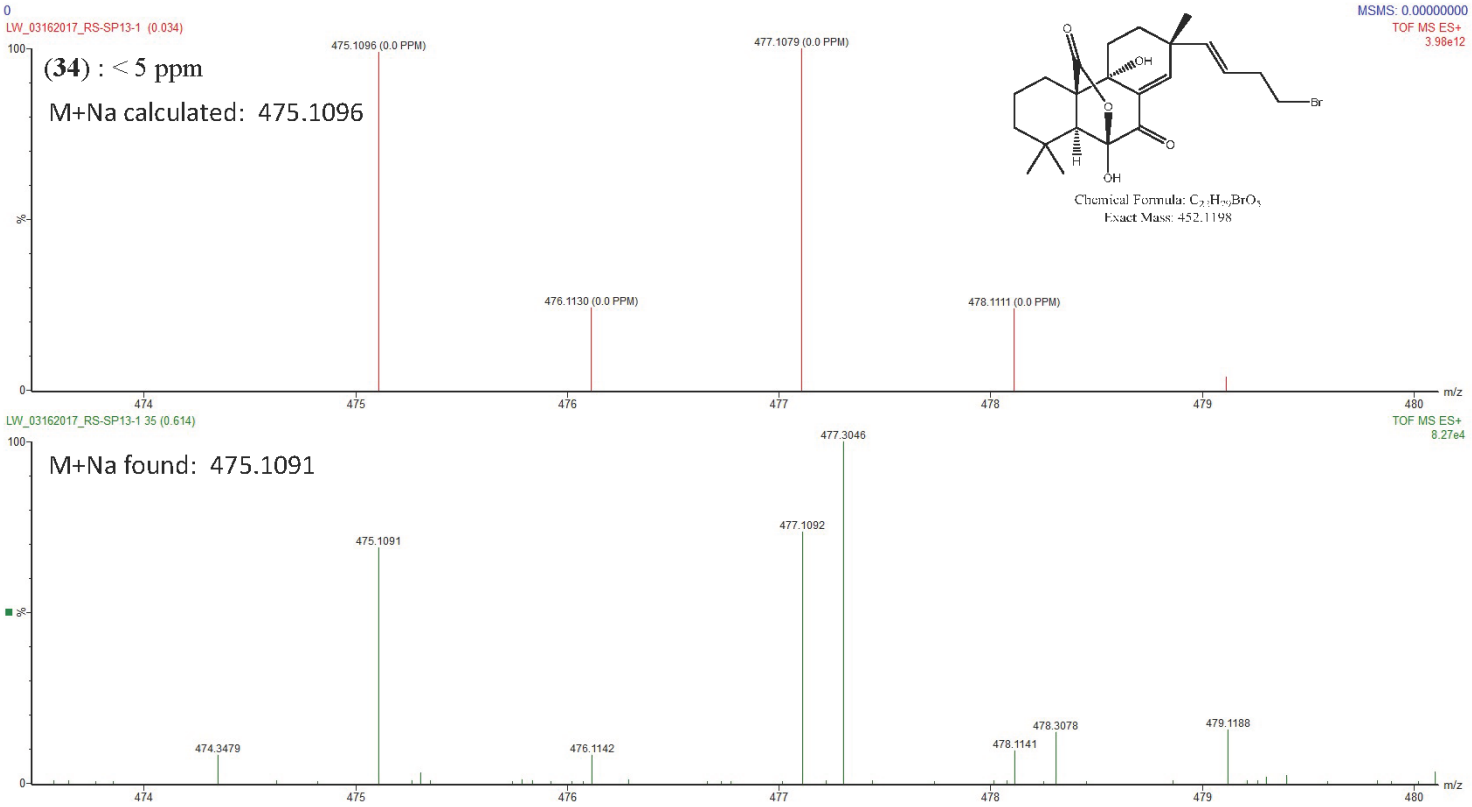
Chemical Formula: $C_{24}H_{33}BrO_5$
Exact Mass: 480.1511

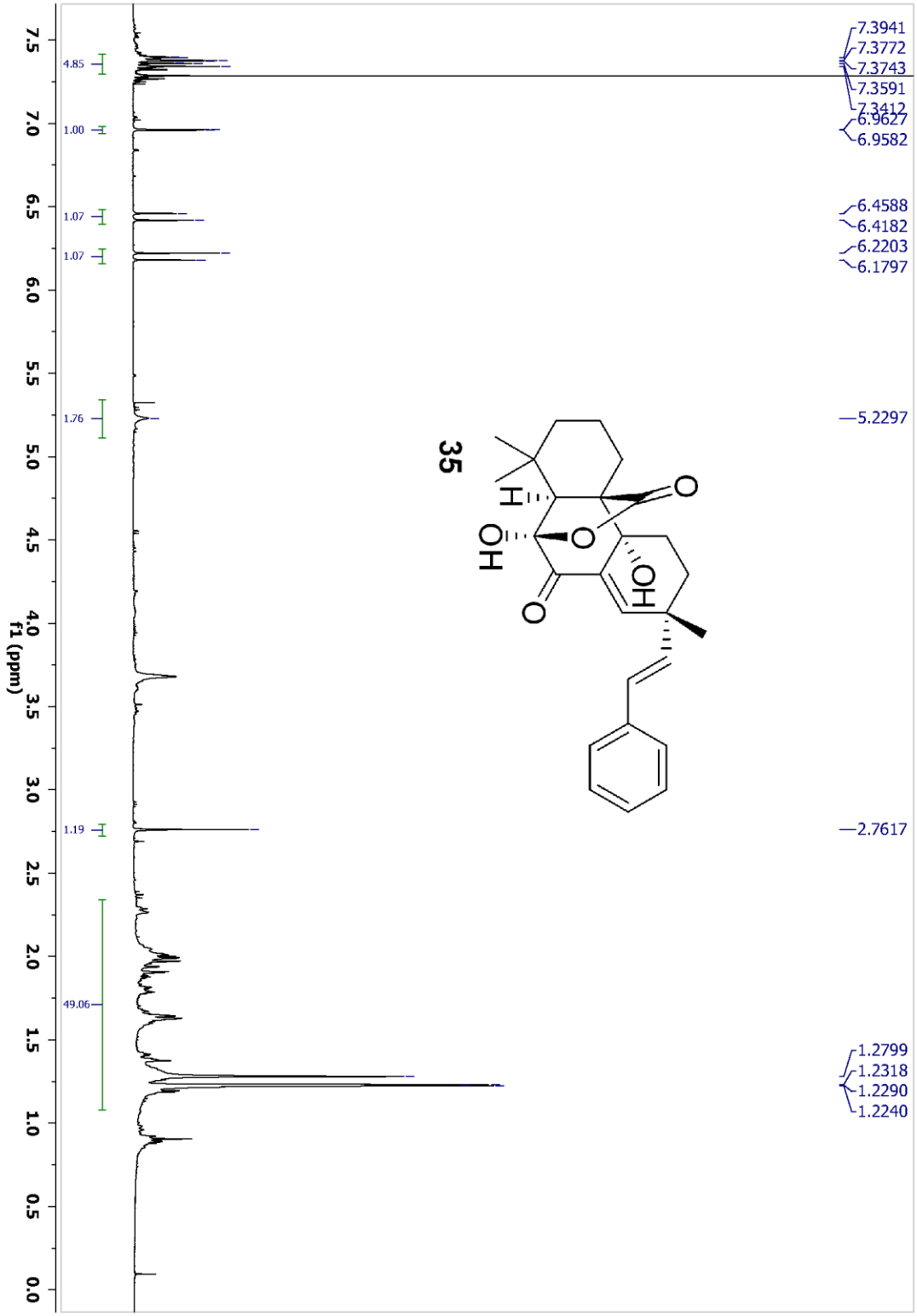
33

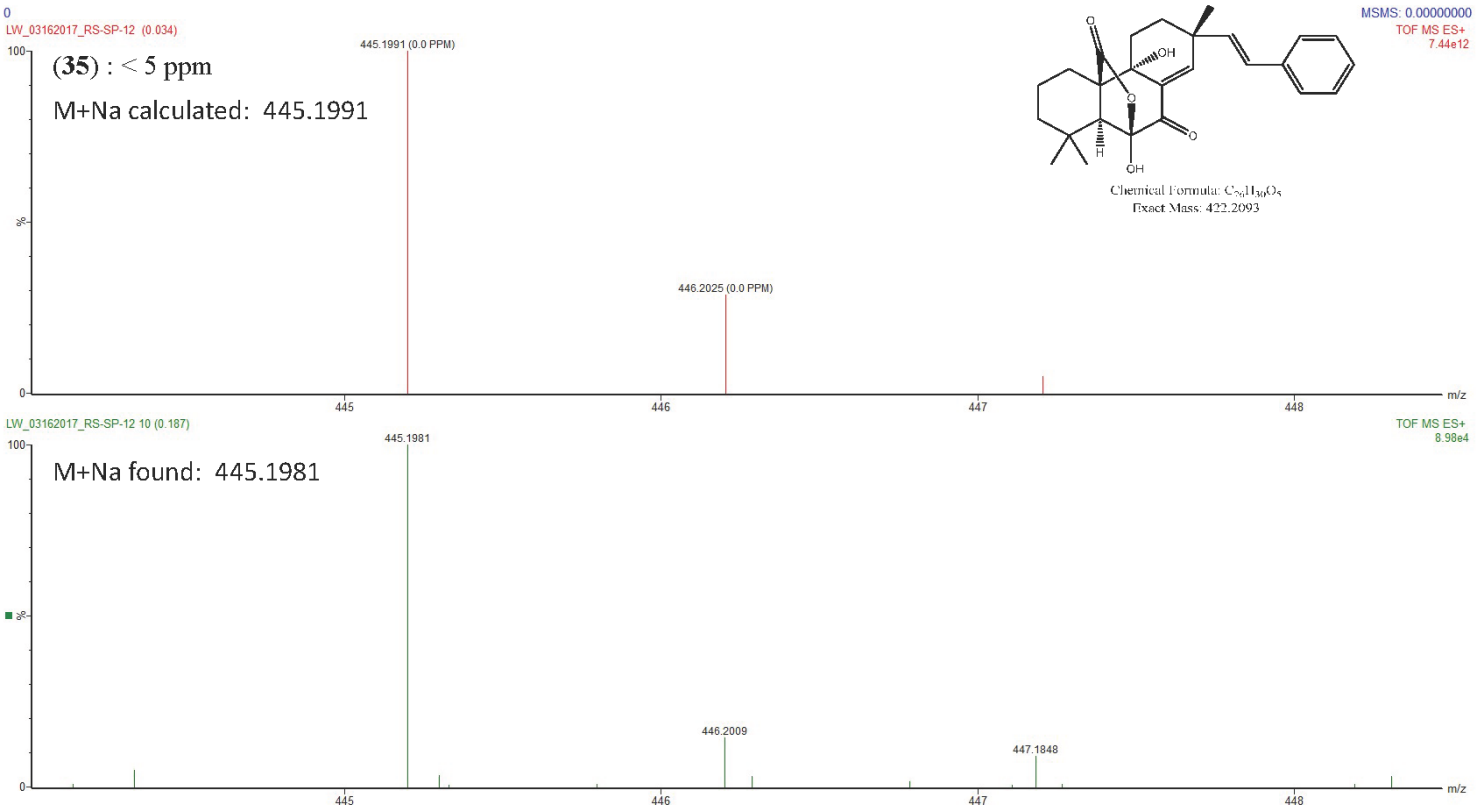
Spectrum RT 0.83 - 0.96 {7 scans} - Background Subtracted 0.34 - 0.75
RS-SP-14-6_is2.datx 2017.04.11 15:22:28 Bobby spha for Bills paper;
Intensity ESI - Max: 2.6E6

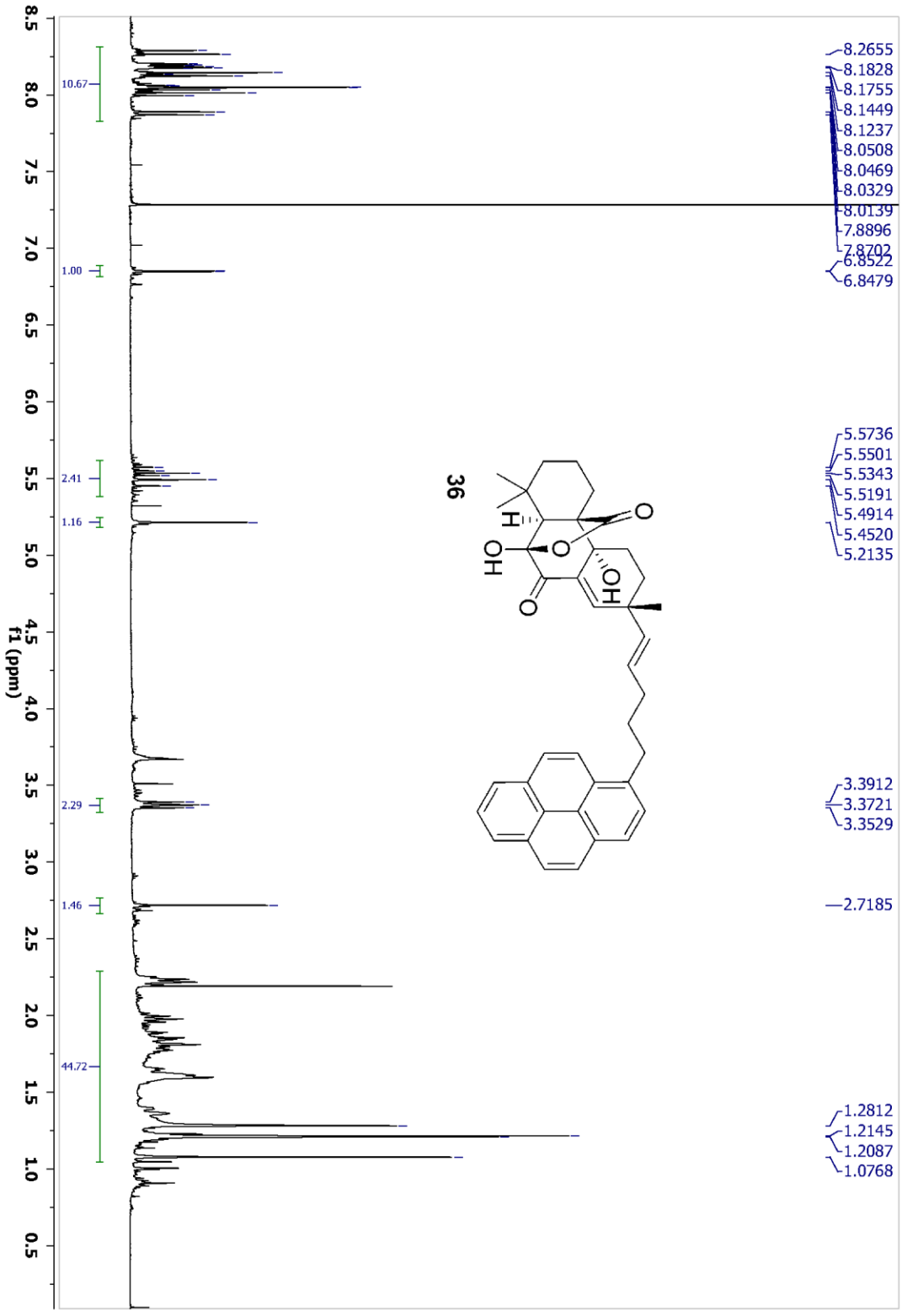


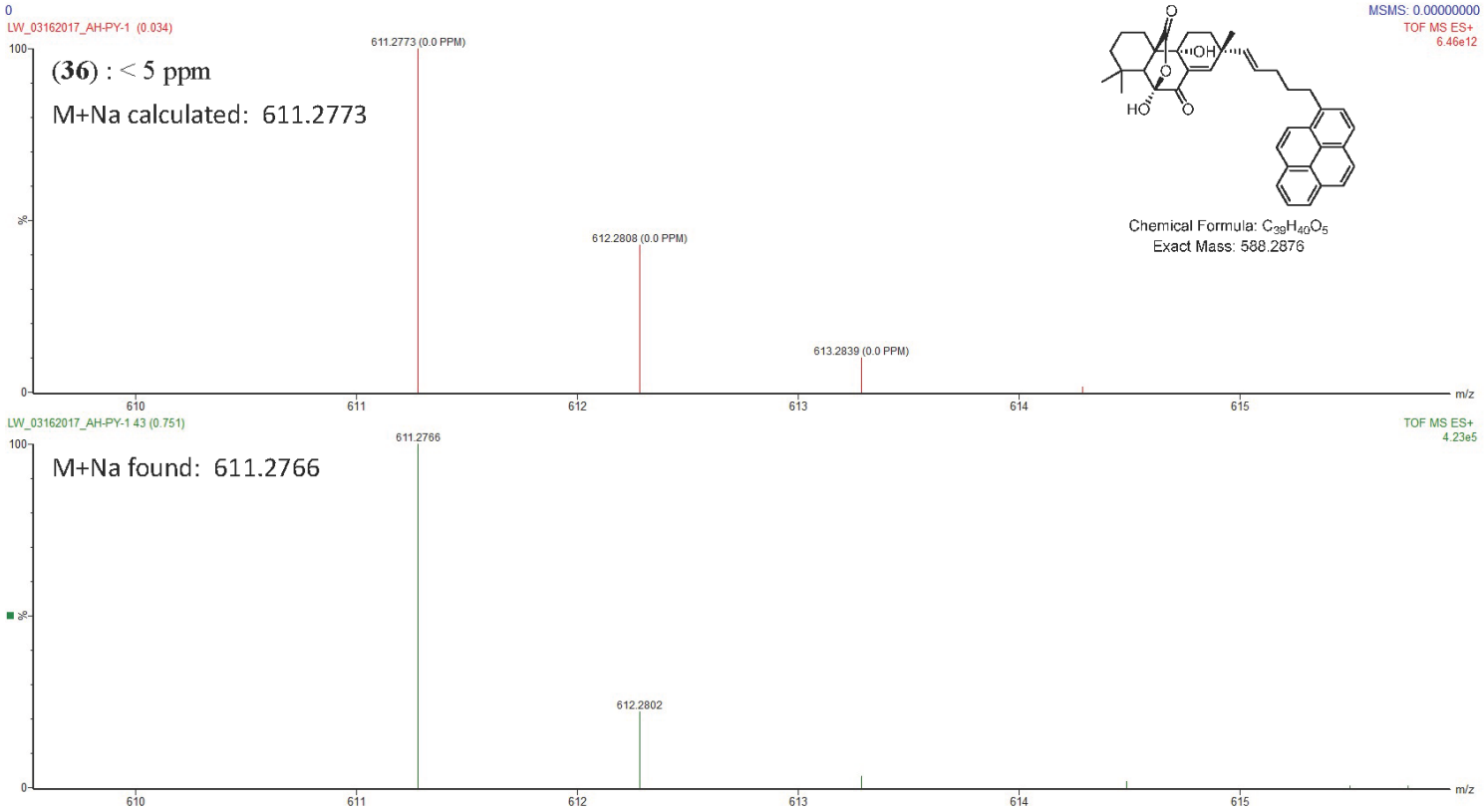

6.8572
6.8527
5.6079
5.6052
5.6029
5.5686
5.5659
5.5636
5.5187
5.5026
5.4863
5.4794
5.4633
5.4470
5.2115
3.4313
3.4140
3.3968
2.7399
2.6402
2.6234
2.6226
2.6212
2.6056
2.5885
2.2612
2.2471
2.2408
1.2792
1.2212
1.2165
1.1123
1.00
1.17
1.22
1.24
2.33
1.53
2.65
53.88
f1 (ppm)
34
O
H
OH
O
O
OH
Br

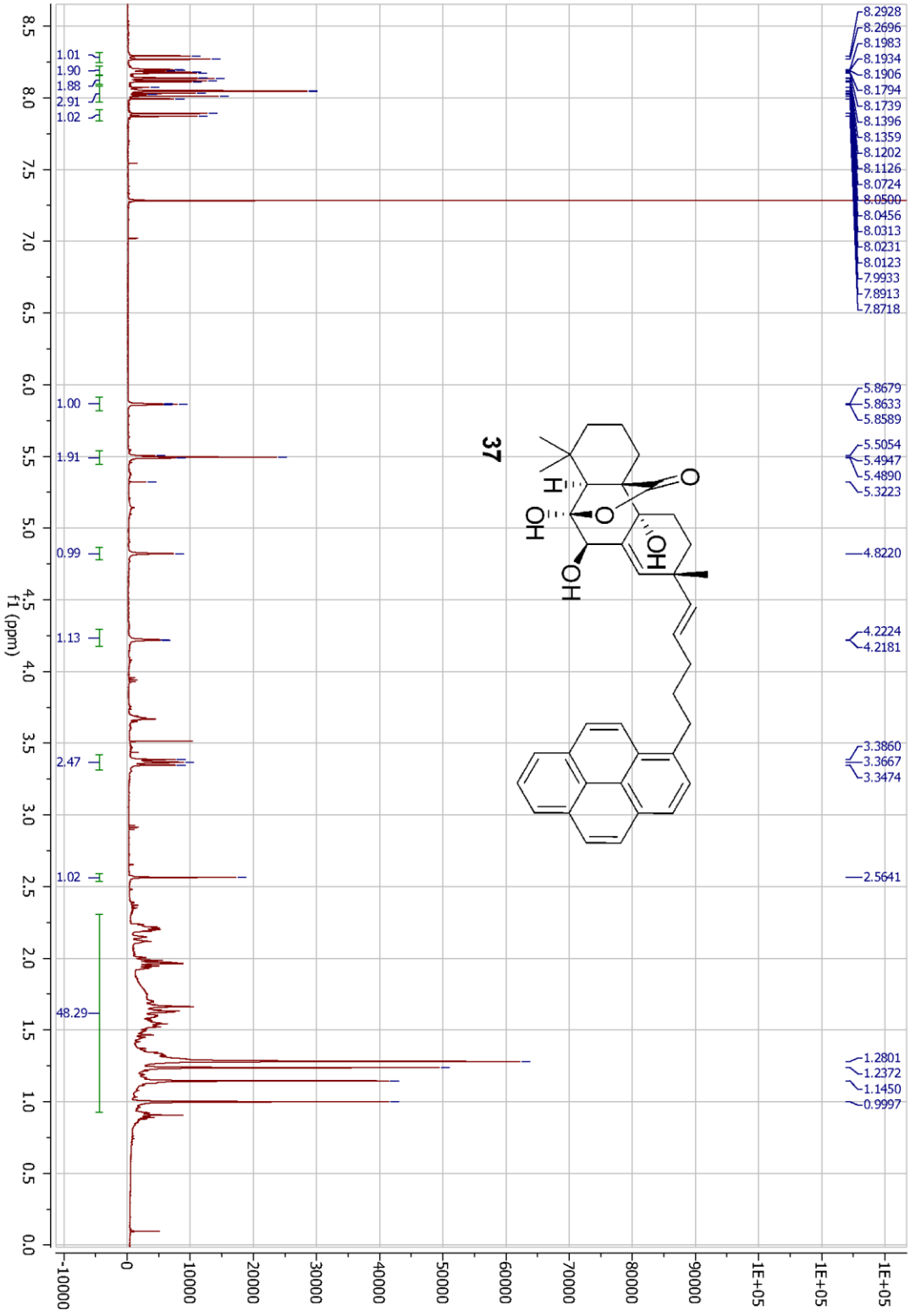

0
LW_03162017_RS-SP13-1 (0.034)
MSMS: 0.00000000
TOF MS ES+
3.98e12
100
%
(34) : < 5 ppm
M+Na calculated: 475.1096
475.1096 (0.0 PPM)
477.1079 (0.0 PPM)
476.1130 (0.0 PPM)
478.1111 (0.0 PPM)
OH
Br
O
OH
Chemical Formula: C21H29BrO5
Exact Mass: 452.1198
0
474
475
476
477
478
479
480
m/z
LW_03162017_RS-SP13-1 35 (0.614)
TOF MS ES+
8.27e4
100
%
M+Na found: 475.1091
477.3046
477.1092
475.1091
474.3479
476.1142
478.1141
478.3078
479.1188
0
474
475
476
477
478
479
480
m/z


7.3941
7.3772
7.3743
7.3591
7.3412
6.9627
6.9582
6.4588
6.4182
6.2203
6.1797
5.2297
2.7617
1.2799
1.2318
1.2290
1.2240
4.85
1.00
1.07
1.07
1.76
1.19
49.06
f1 (ppm)
35
H
OH
O
O
OH
O


0
LW_03162017_RS-SP-12 (0.034)
445.1991 (0.0 PPM)
(35) : < 5 ppm
M+Na calculated: 445.1991
446.2025 (0.0 PPM)
100
%
0
445
446
447
448
m/z
MSMS: 0.00000000
TOF MS ES+
7.44e12
OH
O
O
O
H
OH
Chemical Formula: C26H30O5
Exact Mass: 422.2093
LW_03162017_RS-SP-12 10 (0.187)
TOF MS ES+
8.98e4
445.1981
M+Na found: 445.1981
446.2009
447.1848


8.2655
8.1828
8.1755
8.1449
8.1237
8.0508
8.0469
8.0329
8.0139
7.8896
7.8702
6.8522
6.8479
5.5736
5.5501
5.5343
5.5191
5.4914
5.4520
5.2135
3.3912
3.3721
3.3529
2.7185
1.2812
1.2145
1.2087
1.0768
10.67
1.00
2.41
1.16
2.29
1.46
44.72
f1 (ppm)
36


0
LW_03162017_AH-PY-1 (0.034)
611.2773 (0.0 PPM)
(36) : < 5 ppm
M+Na calculated: 611.2773
612.2808 (0.0 PPM)
613.2839 (0.0 PPM)
MSMS: 0.00000000
TOF MS ES+
6.46e12
Chemical Formula: C39H40O5
Exact Mass: 588.2876
LW_03162017_AH-PY-1 43 (0.751)
TOF MS ES+
4.23e5
M+Na found: 611.2766
611.2766
612.2802
m/z


8.2928
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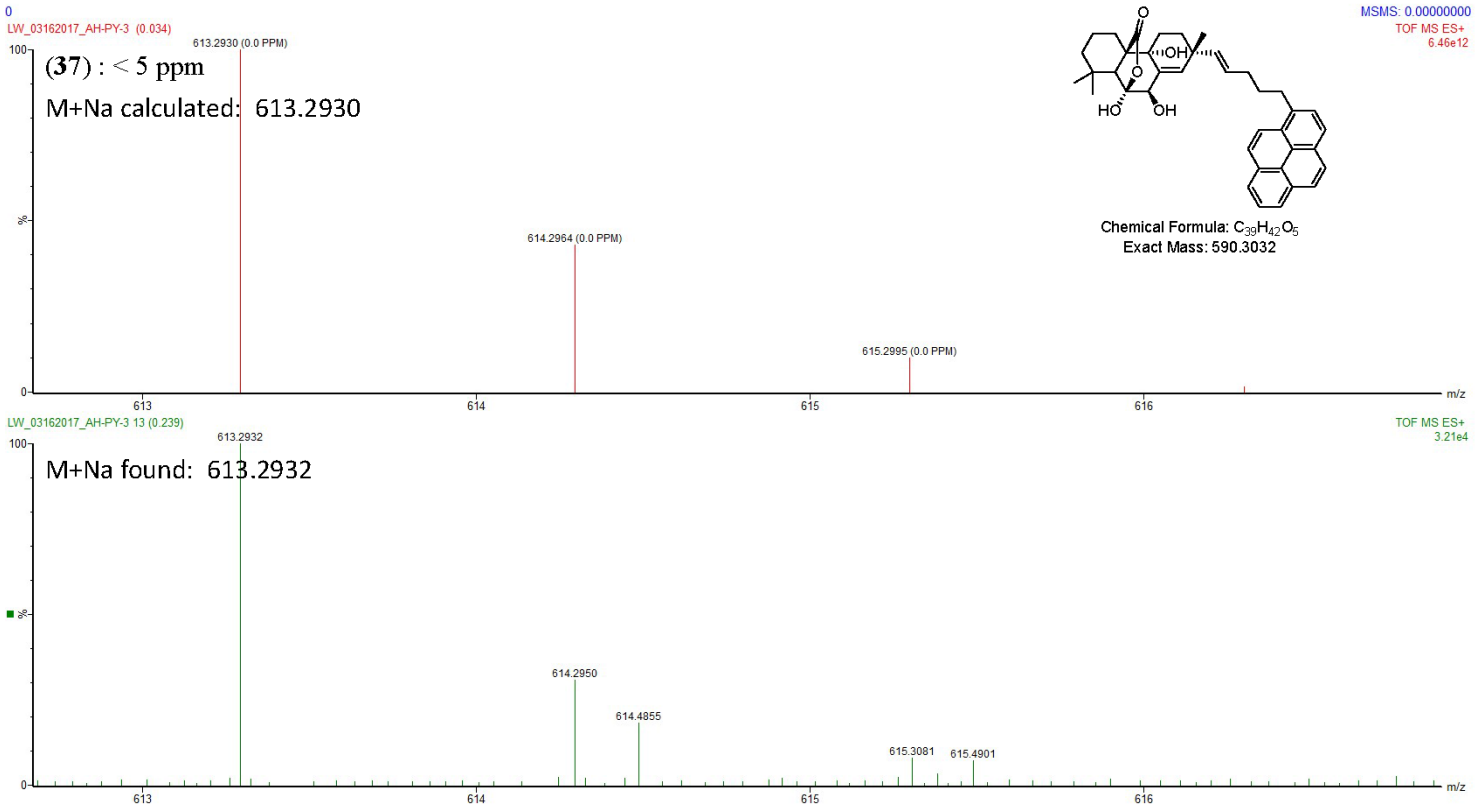
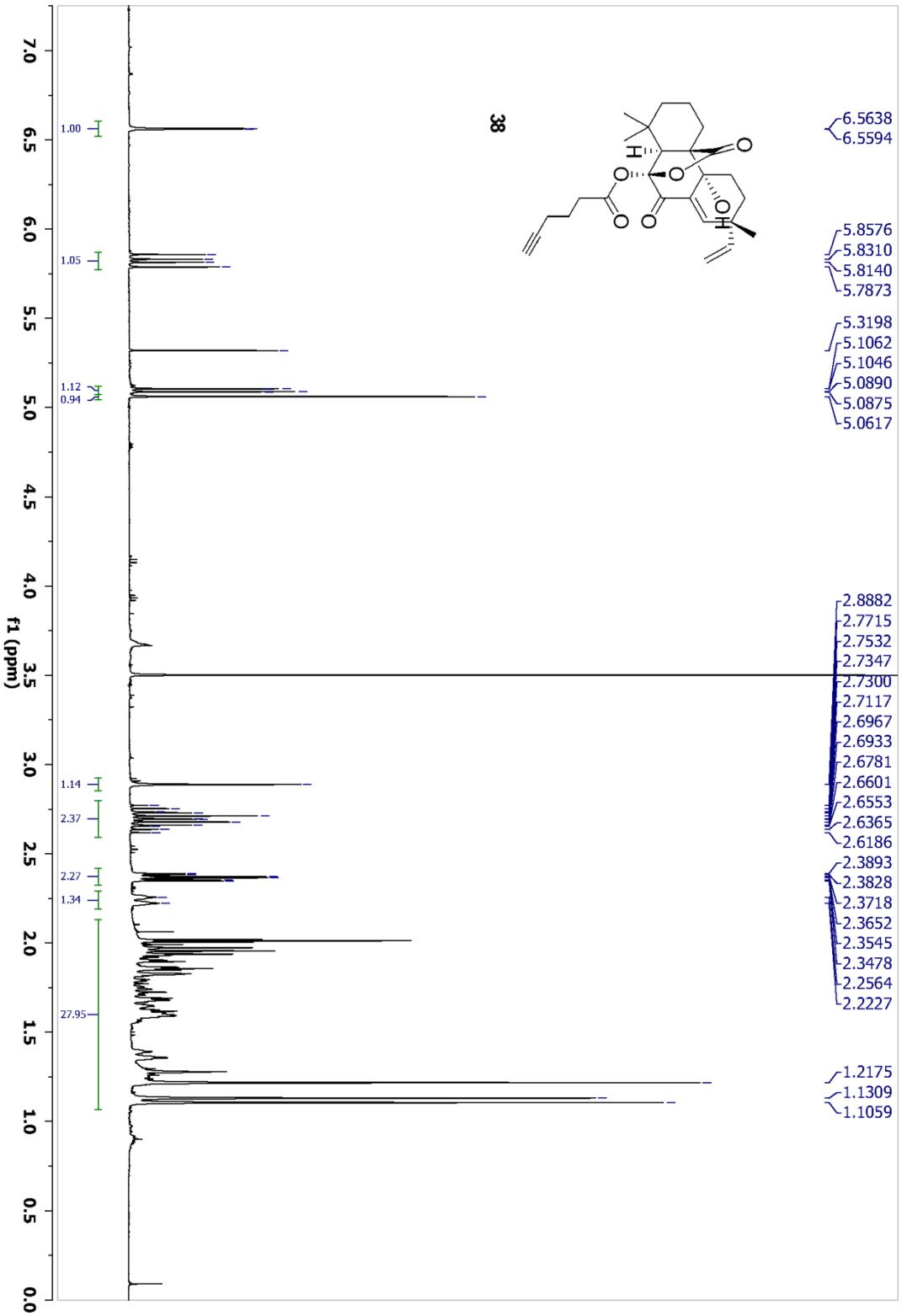
8.1934
8.1906
8.1794
8.1739
8.1396
8.1359
8.1202
8.1126
8.0724
8.0500
8.0456
8.0313
8.0231
8.0123
7.9933
7.8913
7.8718
5.8679
5.8633
5.8589
5.5054
5.4947
5.4890
5.3223
4.8220
4.2224
4.2181
3.3860
3.3667
3.3474
2.5641
1.2801
1.2372
1.1450
0.9997
1.01
1.90
1.88
2.91
1.02
1.00
1.91
0.99
1.13
2.47
1.02
48.29
37
OH
OH
OH
O
O
f1 (ppm)


0
LW_03162017_AH-PY-3 (0.034)
613.2930 (0.0 PPM)
(37) : < 5 ppm
M+Na calculated: 613.2930
614.2964 (0.0 PPM)
615.2995 (0.0 PPM)
100
%
0
613
614
615
616
m/z
MSMS: 0.00000000
TOF MS ES+
6.46e12
O
OH
O
HO
OH
Chemical Formula: C39H42O5
Exact Mass: 590.3032
LW_03162017_AH-PY-3 13 (0.239)
TOF MS ES+
3.21e4
613.2932
M+Na found: 613.2932
614.2950
614.4855
615.3081
615.4901


38
6.5638
6.5594
5.8576
5.8310
5.8140
5.7873
5.3198
5.1062
5.1046
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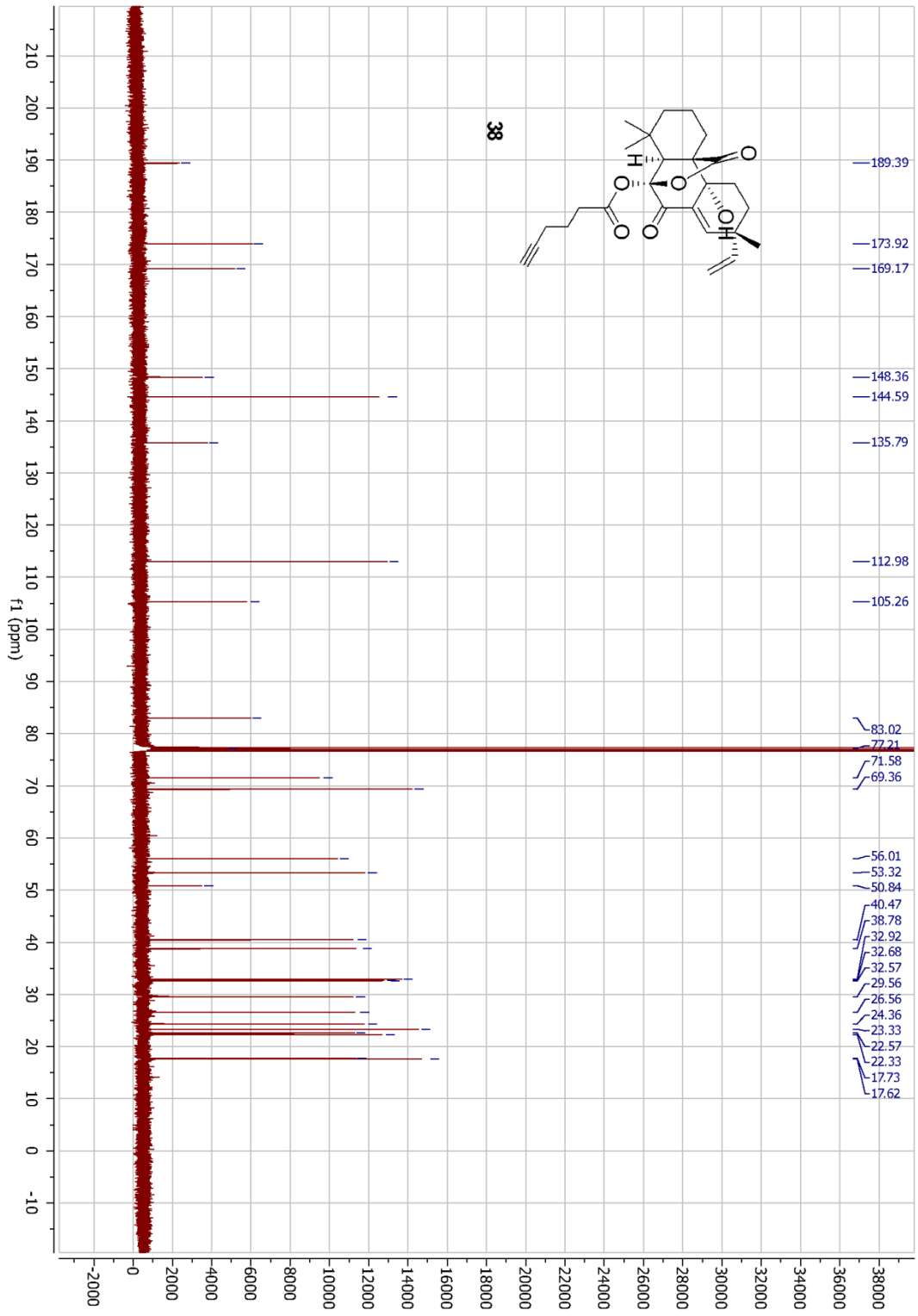
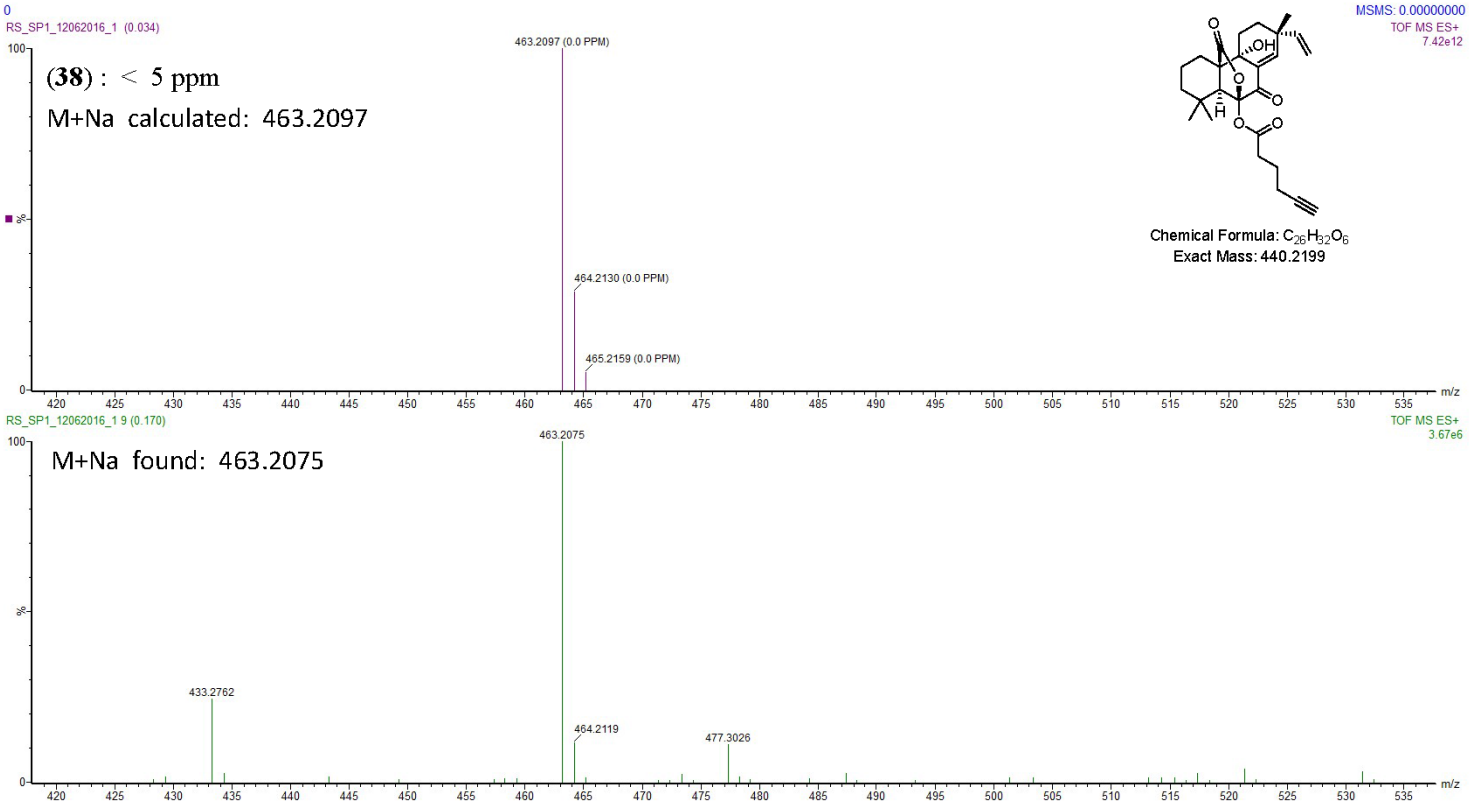
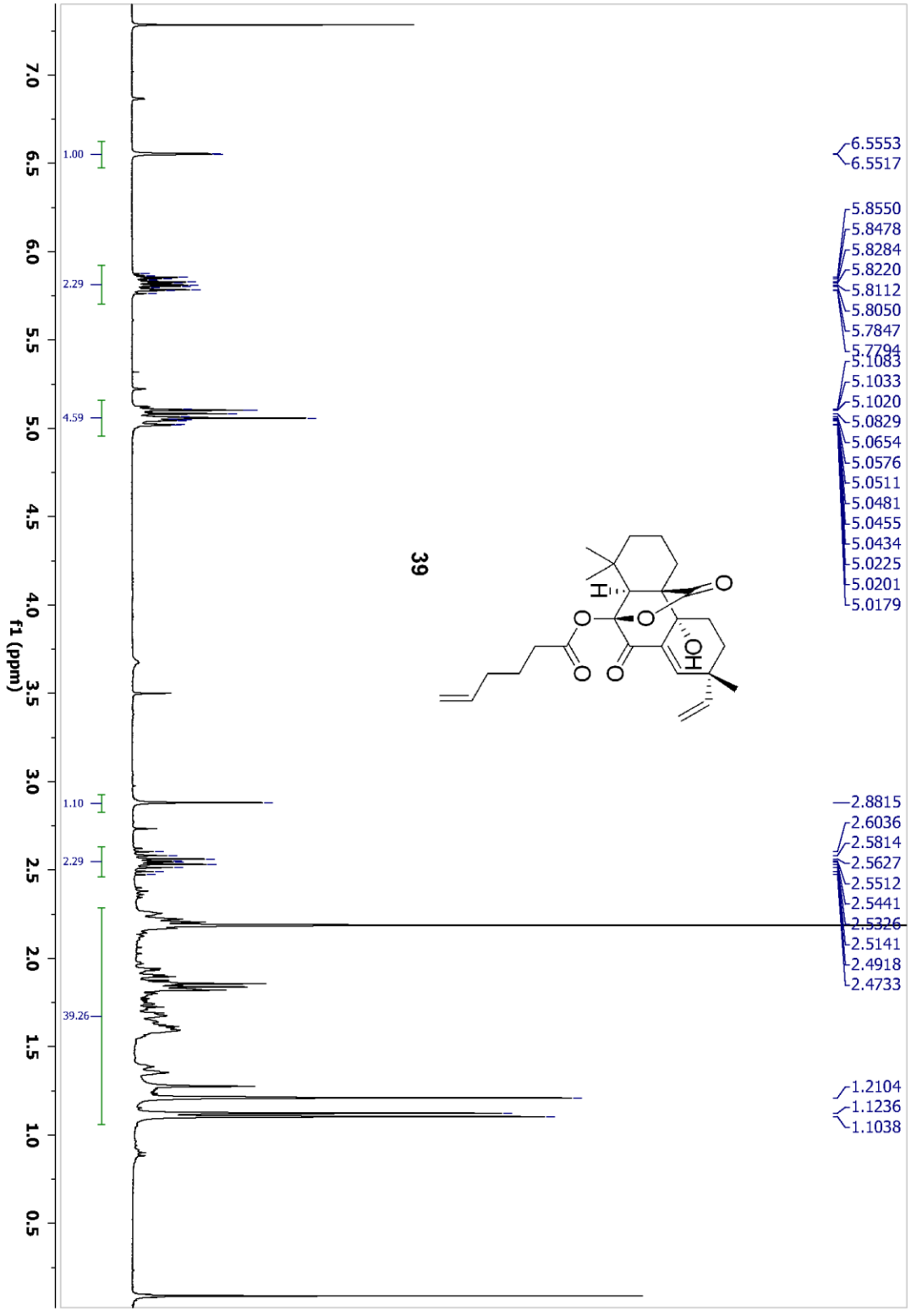
5.0875
5.0617
2.8882
2.7715
2.7532
2.7347
2.7300
2.7117
2.6967
2.6933
2.6781
2.6601
2.6553
2.6365
2.6186
2.3893
2.3828
2.3718
2.3652
2.3545
2.3478
2.2564
2.2227
1.2175
1.1309
1.1059
1.00
1.05
1.12
0.94
1.14
2.37
2.27
1.34
27.95
f1 (ppm)


38
189.39
173.92
169.17
148.36
144.59
135.79
112.98
105.26
83.02
77.21
71.58
69.36
56.01
53.32
50.84
40.47
38.78
32.92
32.68
32.57
29.56
26.56
24.36
23.33
22.57
22.33
17.73
17.62
f1 (ppm)


0
RS_SP1_12062016_1 (0.034)
100
%
(38) : < 5 ppm
M+Na calculated: 463.2097
463.2097 (0.0 PPM)
464.2130 (0.0 PPM)
465.2159 (0.0 PPM)
0
420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495 500 505 510 515 520 525 530 535
m/z
MSMS: 0.00000000
TOF MS ES+
7.42e12
OH
O
H
Chemical Formula: C26H32O6
Exact Mass: 440.2199
RS_SP1_12062016_1 9 (0.170)
TOF MS ES+
3.67e6
100
%
M+Na found: 463.2075
463.2075
433.2762
464.2119
477.3026
0
420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495 500 505 510 515 520 525 530 535
m/z


39
f1 (ppm)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1.00
2.29
4.59
1.10
2.29
39.26
6.5553
6.5517
5.8550
5.8478
5.8284
5.8220
5.8112
5.8050
5.7847
5.7794
5.1083
5.1033
5.1020
5.0829
5.0654
5.0576
5.0511
5.0481
5.0455
5.0434
5.0225
5.0201
5.0179
2.8815
2.6036
2.5814
2.5627
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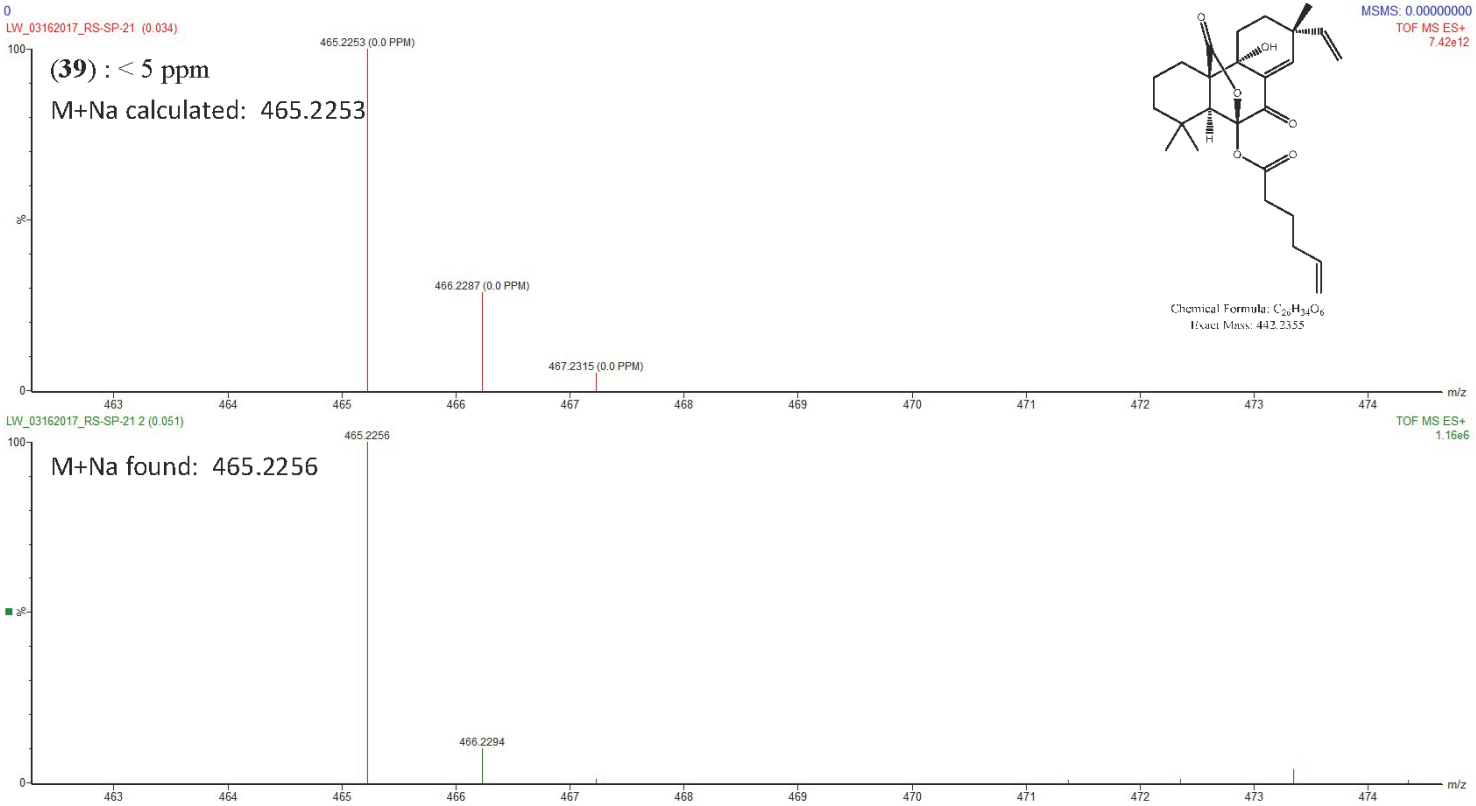
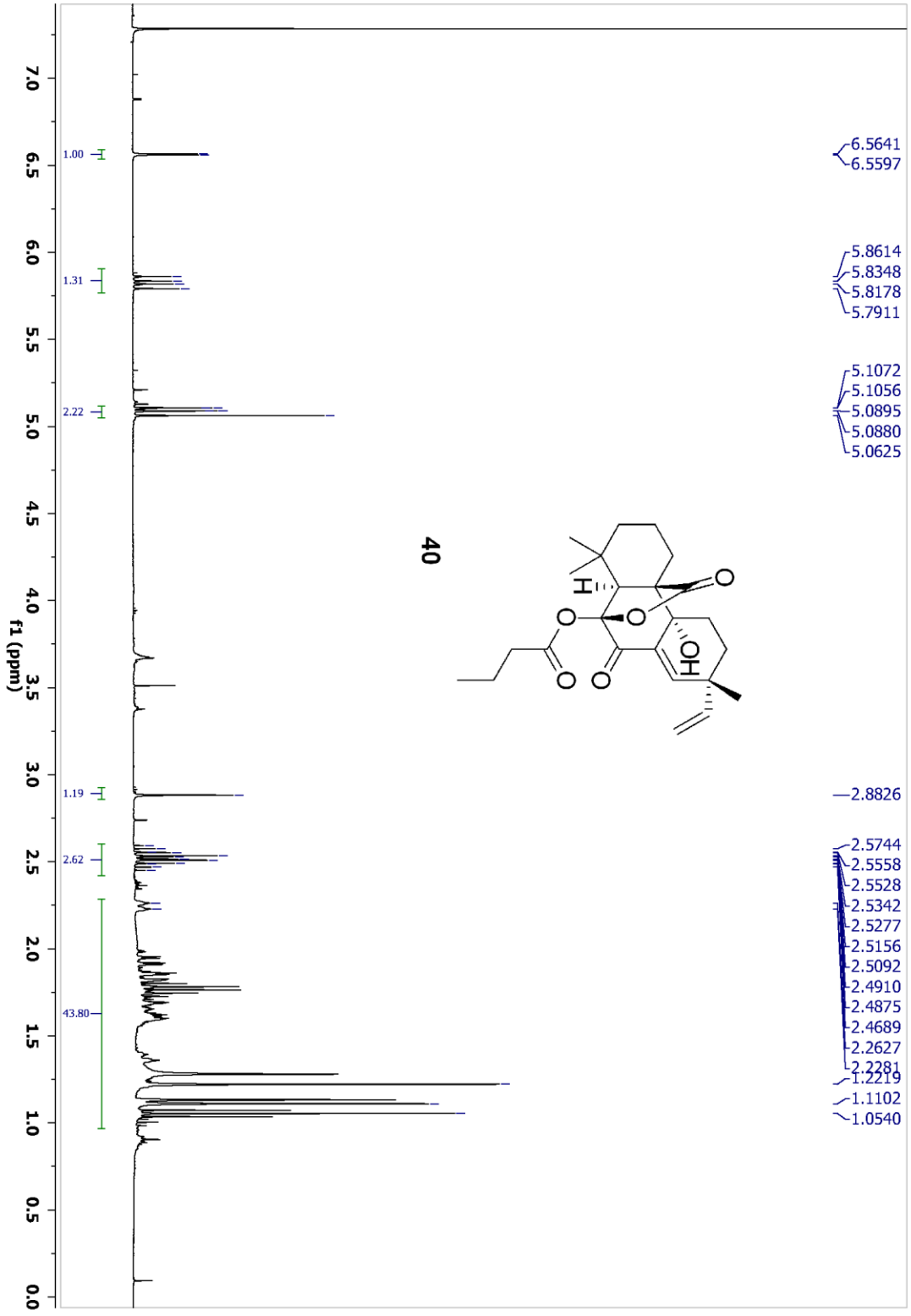
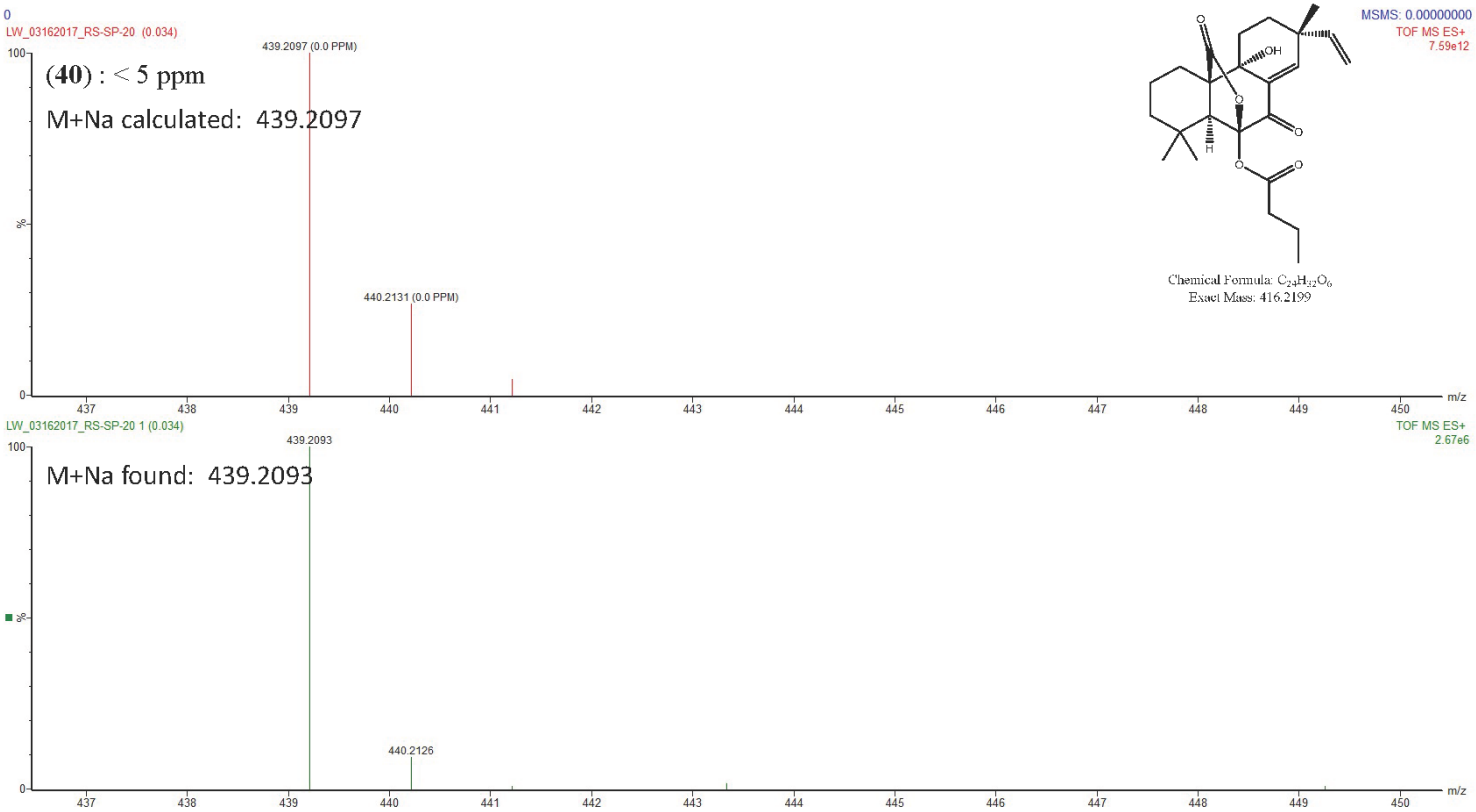
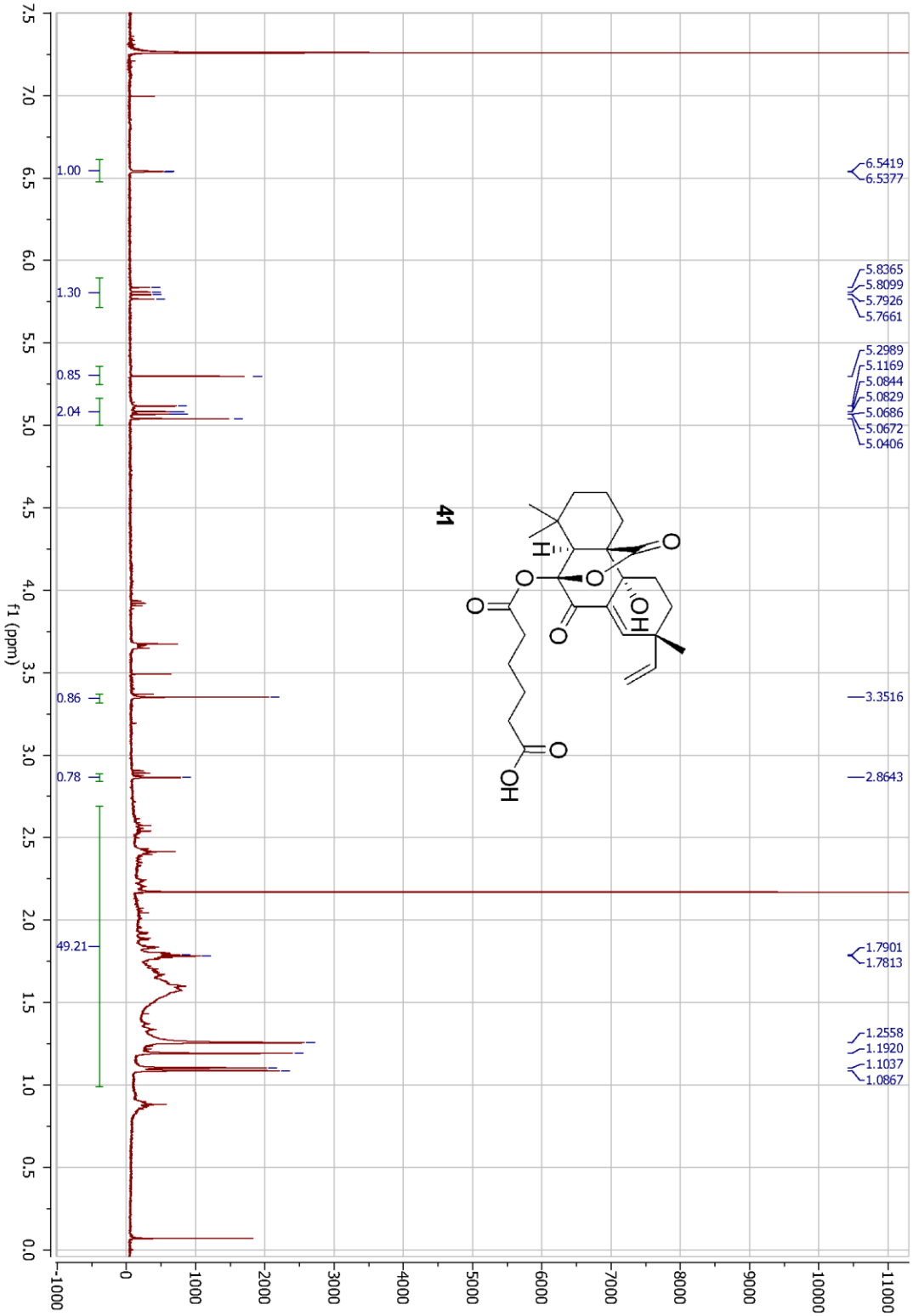
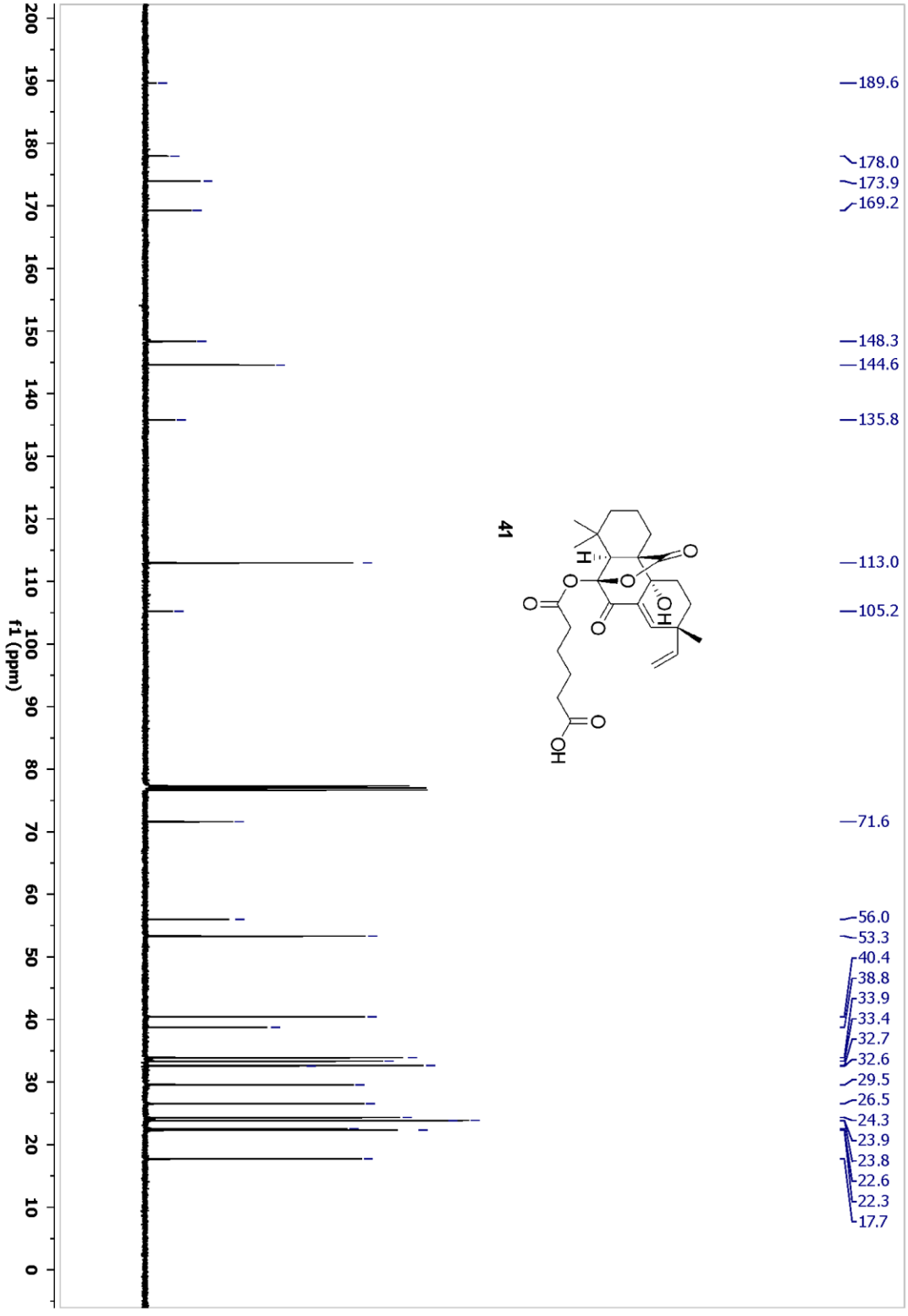
2.4918
2.4733
1.2104
1.1236
1.1038

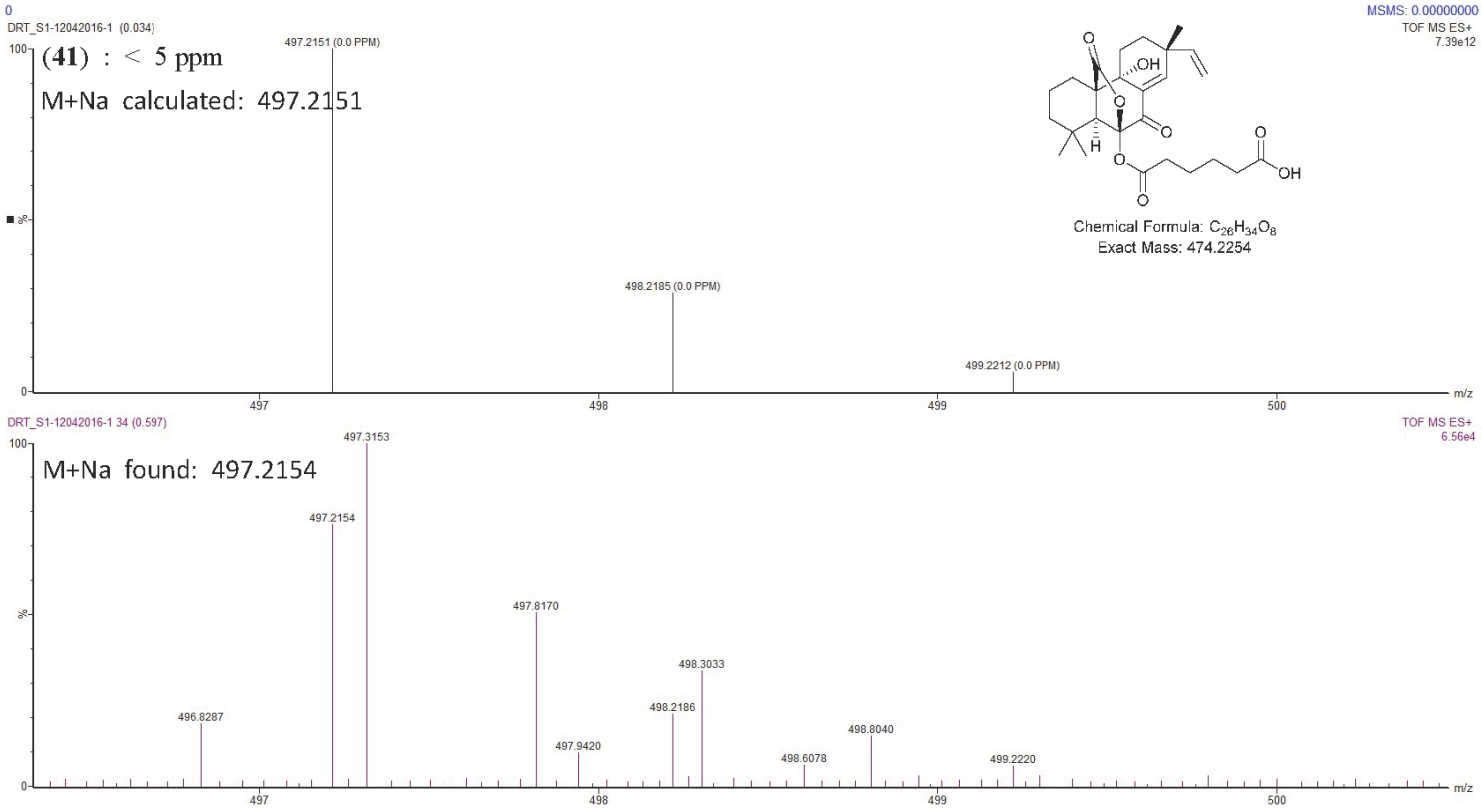

0
LW_03162017_RS-SP-21 (0.034)
100
%
465.2253 (0.0 PPM)
(39) : < 5 ppm
M+Na calculated: 465.2253
466.2287 (0.0 PPM)
467.2315 (0.0 PPM)
0
463
464
465
466
467
468
469
470
471
472
473
474
m/z
MSMS: 0.00000000
TOF MS ES+
7.42e12
OH
Chemical Formula: C26H34O6
Exact Mass: 442.2355
LW_03162017_RS-SP-21 2 (0.051)
TOF MS ES+
1.16e6
100
%
465.2256
M+Na found: 465.2256
466.2294
0
463
464
465
466
467
468
469
470
471
472
473
474
m/z

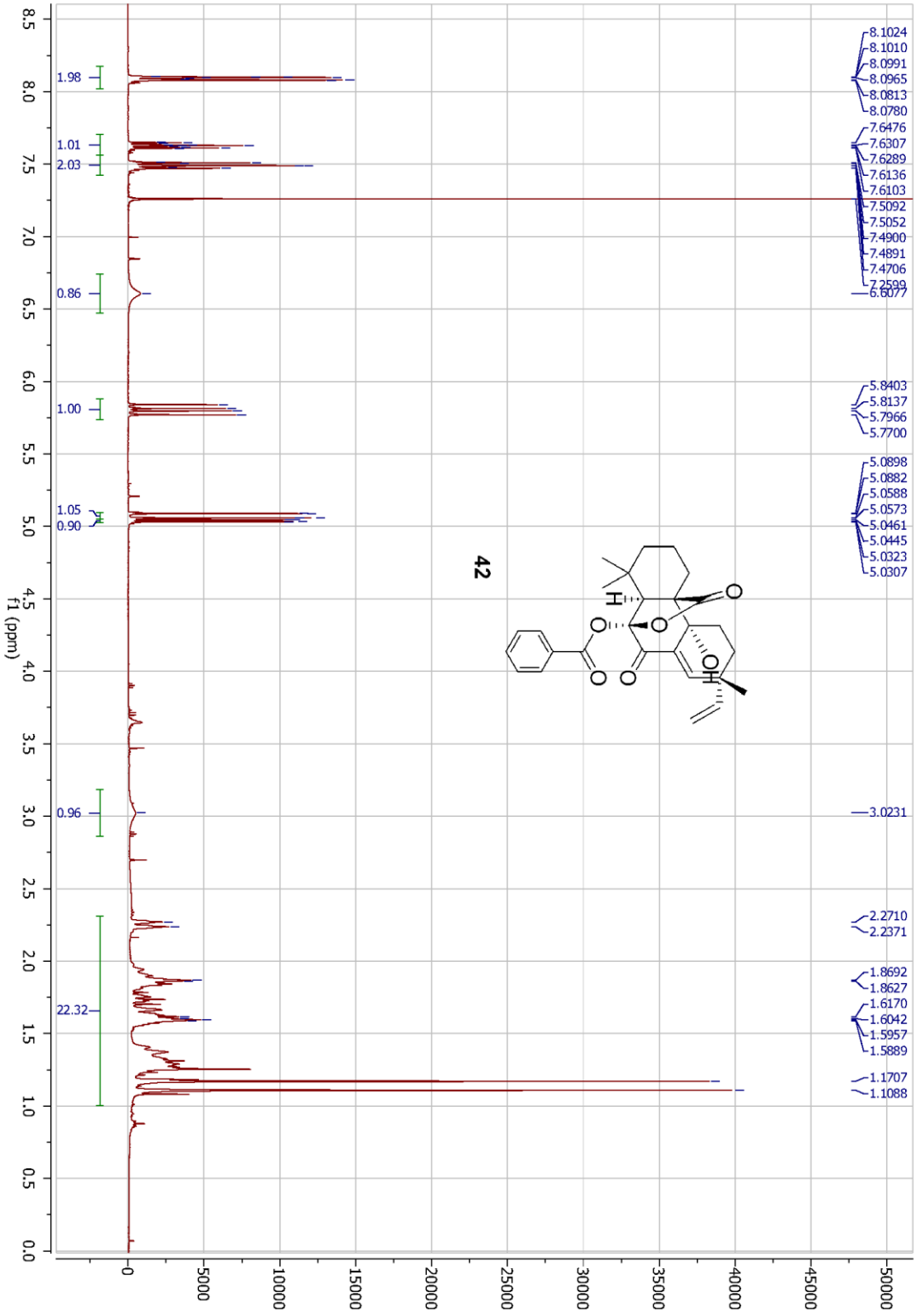

40
f1 (ppm)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1.00
1.31
2.22
1.19
2.62
43.80
6.5641
6.5597
5.8614
5.8348
5.8178
5.7911
5.1072
5.1056
5.0895
5.0880
5.0625
2.8826
2.5744
2.5558
2.5528
2.5342
2.5277
2.5156
2.5092
2.4910
2.4875
2.4689
2.2627
2.2281
1.2219
1.1102
1.0540
O
H
O
O
O
O
OH

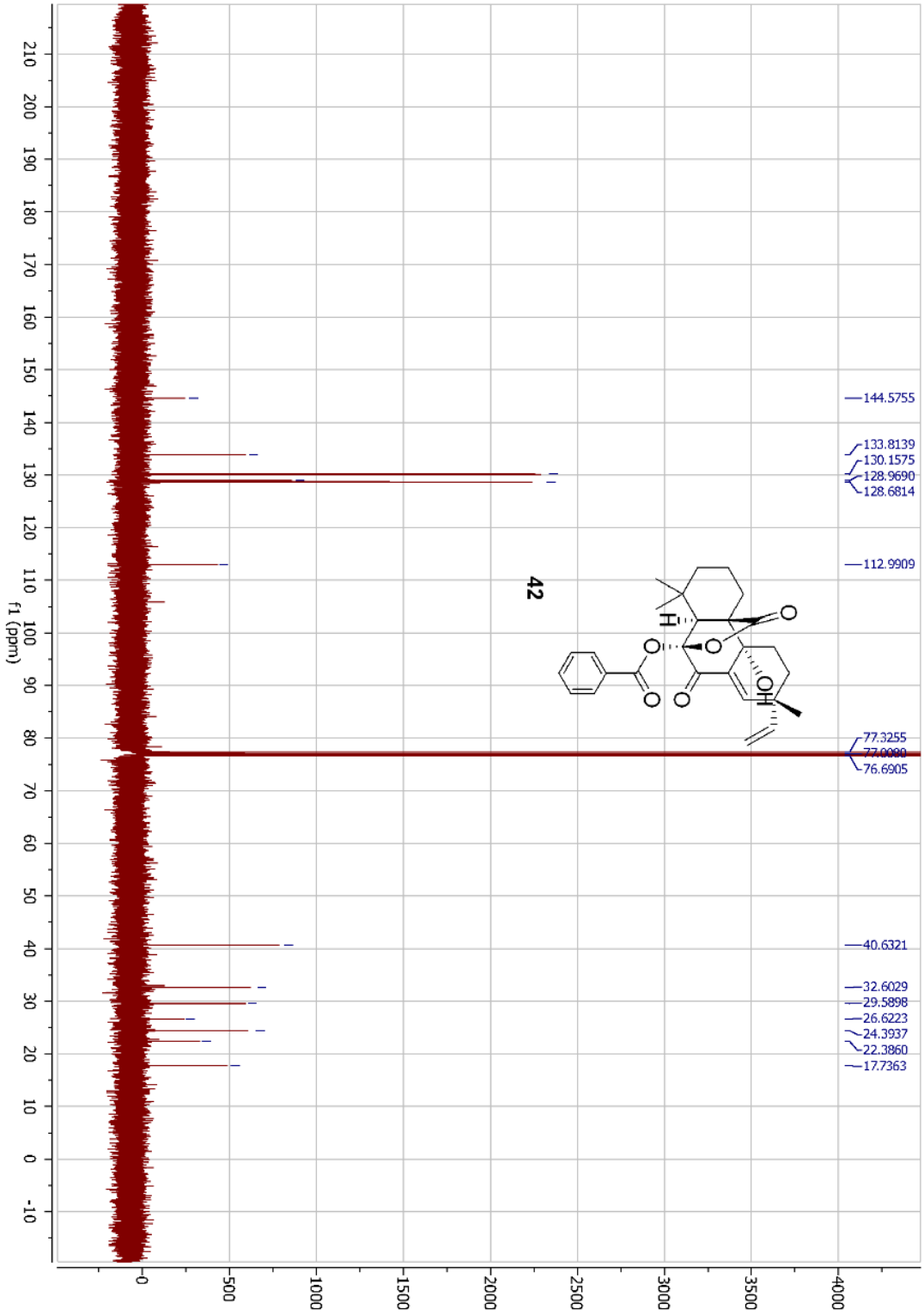

0
LW_03162017_RS-SP-20 (0.034)
439.2097 (0.0 PPM)
(40) : < 5 ppm
M+Na calculated: 439.2097
440.2131 (0.0 PPM)
MSMS: 0.00000000
TOF MS ES+
7.59e12
OH
Chemical Formula: C24H32O6
Exact Mass: 416.2199
100
%
0
437
438
439
440
441
442
443
444
445
446
447
448
449
450
m/z
LW_03162017_RS-SP-20 1 (0.034)
TOF MS ES+
2.67e6
439.2093
M+Na found: 439.2093
440.2126

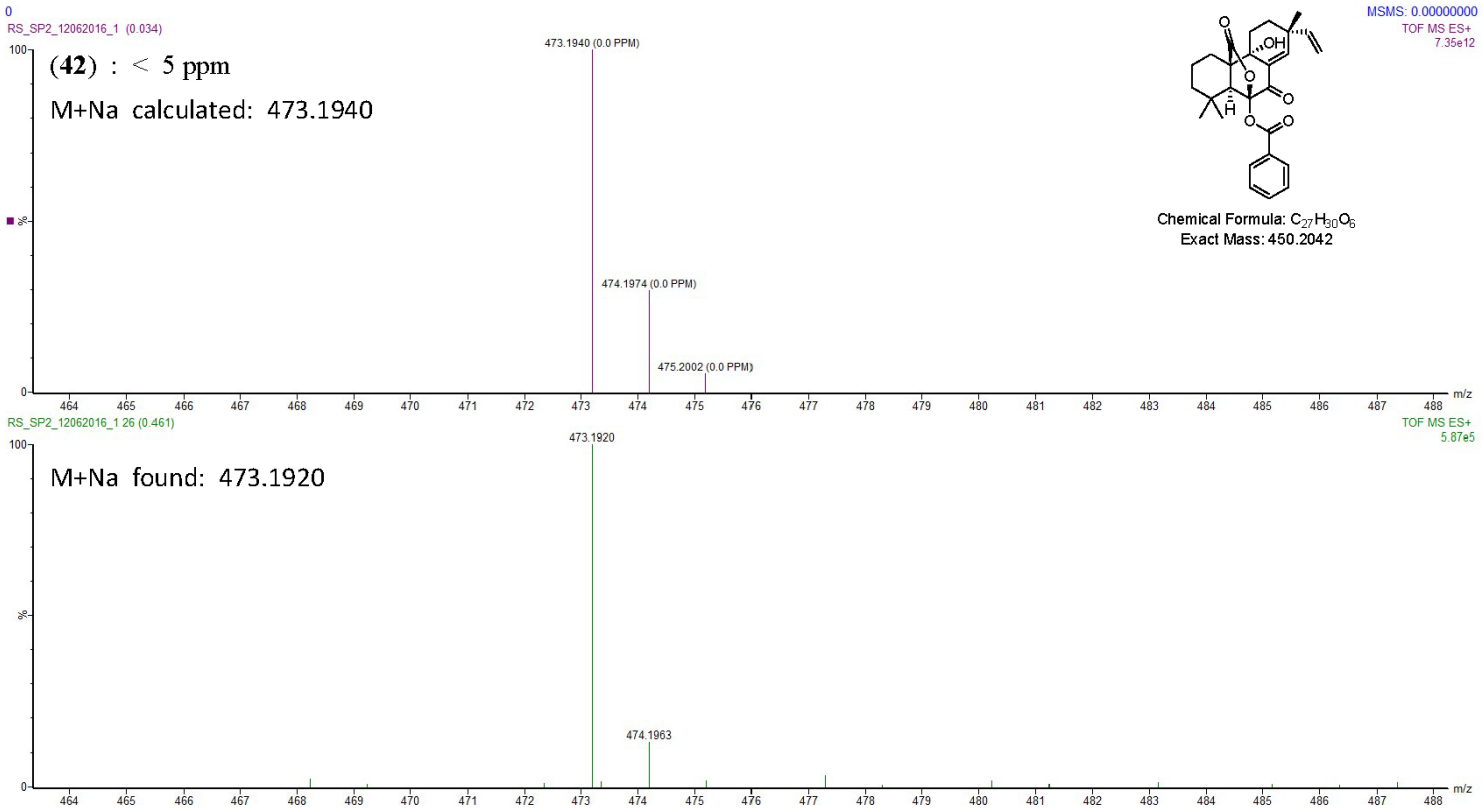

41
f1 (ppm)
1.00
1.30
0.85
2.04
0.86
0.78
49.21
6.5419
6.5377
5.8365
5.8099
5.7926
5.7661
5.2989
5.1169
5.0844
5.0829
5.0686
5.0672
5.0406
3.3516
2.8643
1.7901
1.7813
1.2558
1.1920
1.1037
1.0867

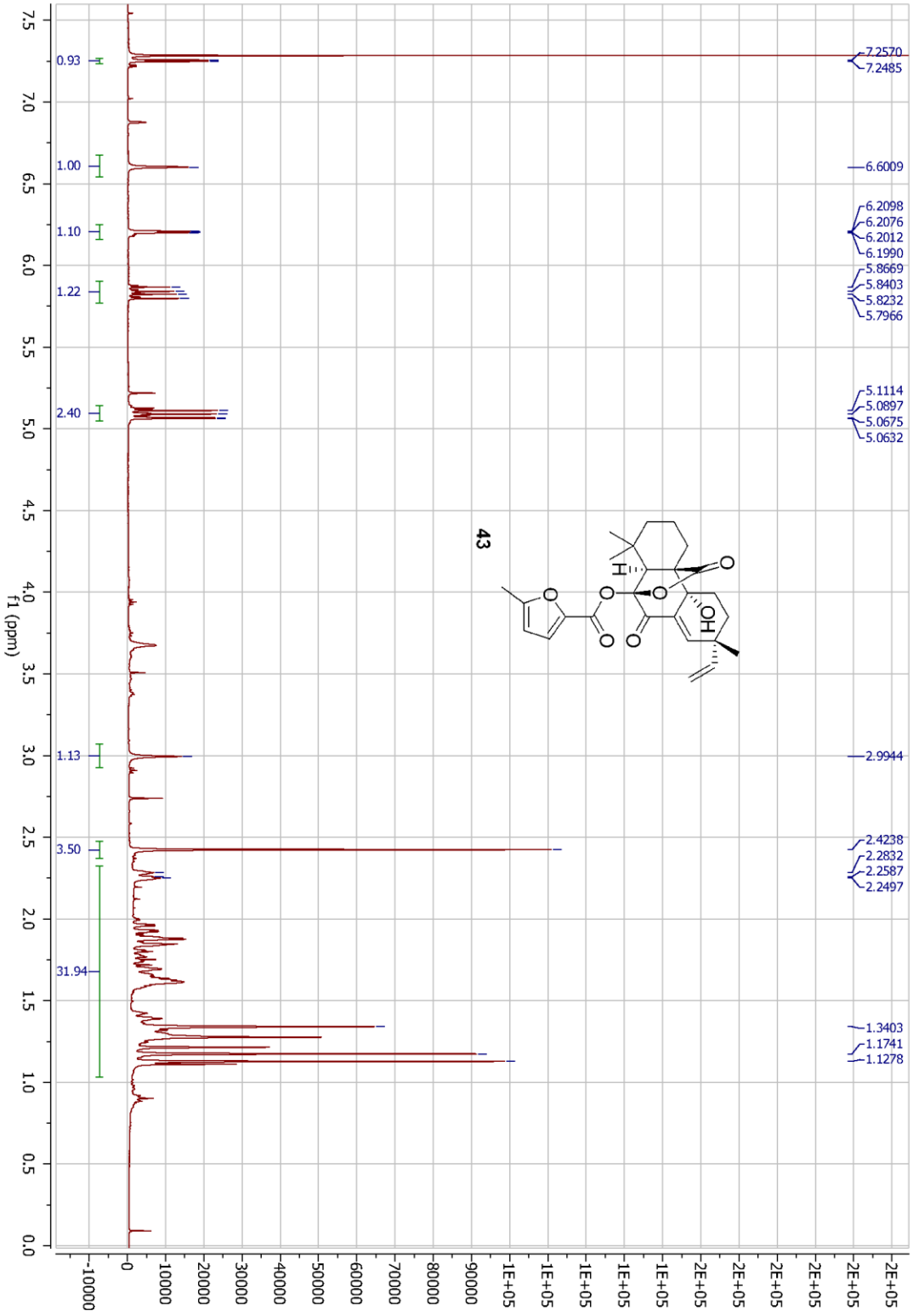

f1 (ppm)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
189.6
178.0
173.9
169.2
148.3
144.6
135.8
113.0
105.2
71.6
56.0
53.3
40.4
38.8
33.9
33.4
32.7
32.6
29.5
26.5
24.3
23.9
23.8
22.6
22.3
17.7
41
H
O
O
O
O
O
HO
HO
O

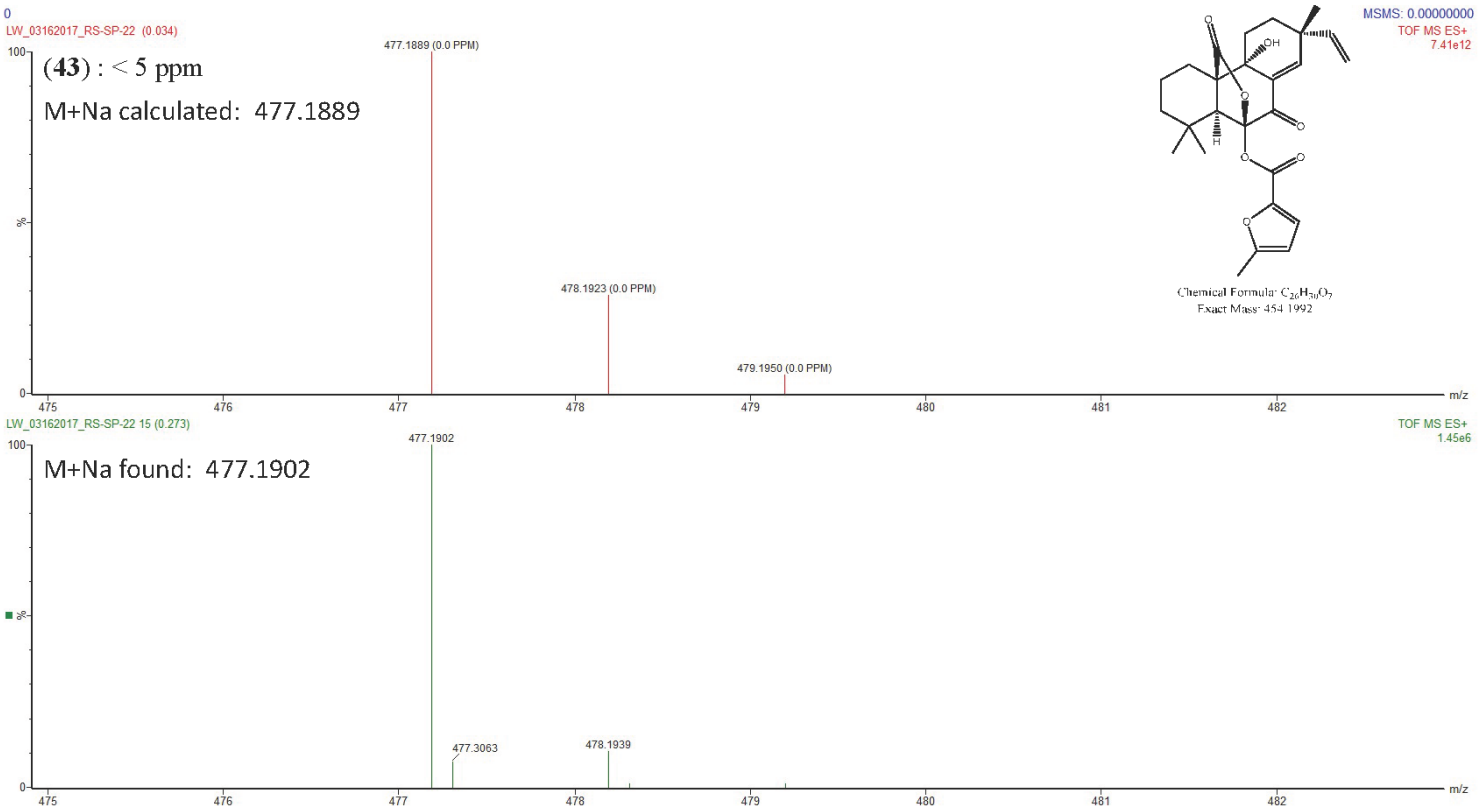

0
DRT_S1-12042016-1 (0.034)
MSMS: 0.00000000
TOF MS ES+
7.39e12
100
(41) : < 5 ppm
M+Na calculated: 497.2151
497.2151 (0.0 PPM)
%
498.2185 (0.0 PPM)
499.2212 (0.0 PPM)
0
497
498
499
500
m/z
OH
O
H
OH
Chemical Formula: C26H34O8
Exact Mass: 474.2254
DRT_S1-12042016-1 34 (0.597)
TOF MS ES+
6.56e4
100
M+Na found: 497.2154
497.3153
497.2154
497.8170
498.3033
498.2186
496.8287
497.9420
498.8040
498.6078
499.2220
%
0
497
498
499
500
m/z

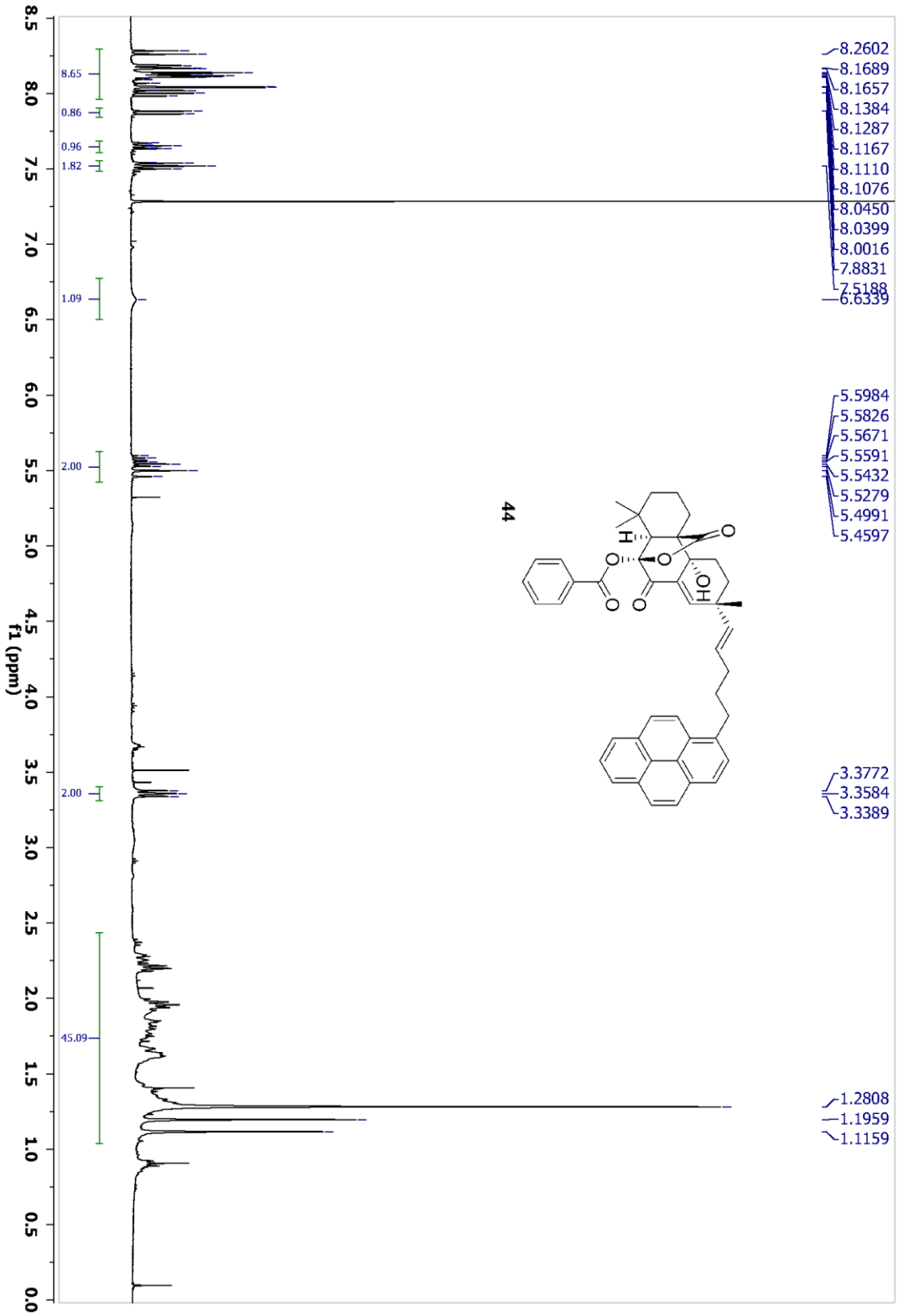

42
f1 (ppm)
1.98
1.01
2.03
0.86
1.00
1.05
0.90
0.96
22.32
8.1024
8.1010
8.0991
8.0965
8.0813
8.0780
7.6476
7.6307
7.6289
7.6136
7.6103
7.5092
7.5052
7.4900
7.4891
7.4706
7.2599
6.6077
5.8403
5.8137
5.7966
5.7700
5.0898
5.0882
5.0588
5.0573
5.0461
5.0445
5.0323
5.0307
3.0231
2.2710
2.2371
1.8692
1.8627
1.6170
1.6042
1.5957
1.5889
1.1707
1.1088

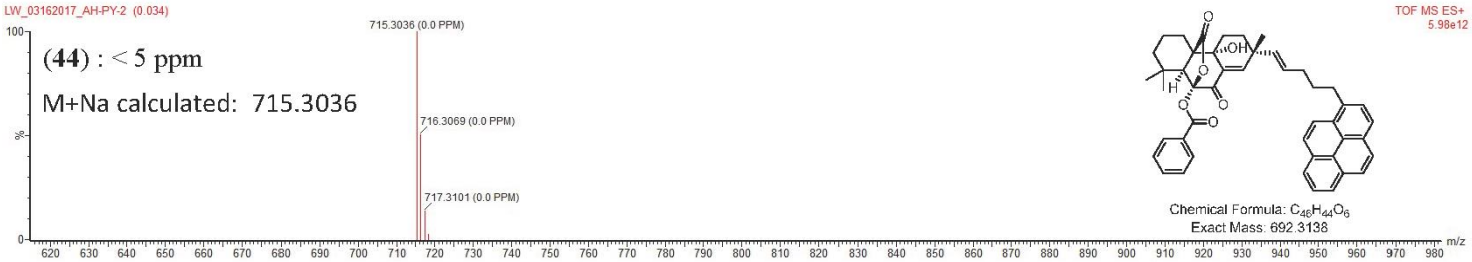

144.5755
133.8139
130.1575
128.9690
128.6814
112.9909
77.3255
77.0000
76.6905
40.6321
32.6029
29.5898
26.6223
24.3937
22.3860
17.7363
42
f1 (ppm)


0
RS_SP2_12062016_1 (0.034)
MSMS: 0.00000000
TOF MS ES+
7.35e12
(42) : < 5 ppm
M+Na calculated: 473.1940
473.1940 (0.0 PPM)
474.1974 (0.0 PPM)
475.2002 (0.0 PPM)
Chemical Formula: C27H30O6
Exact Mass: 450.2042
RS_SP2_12062016_1 26 (0.461)
TOF MS ES+
5.87e5
M+Na found: 473.1920
473.1920
474.1963
m/z

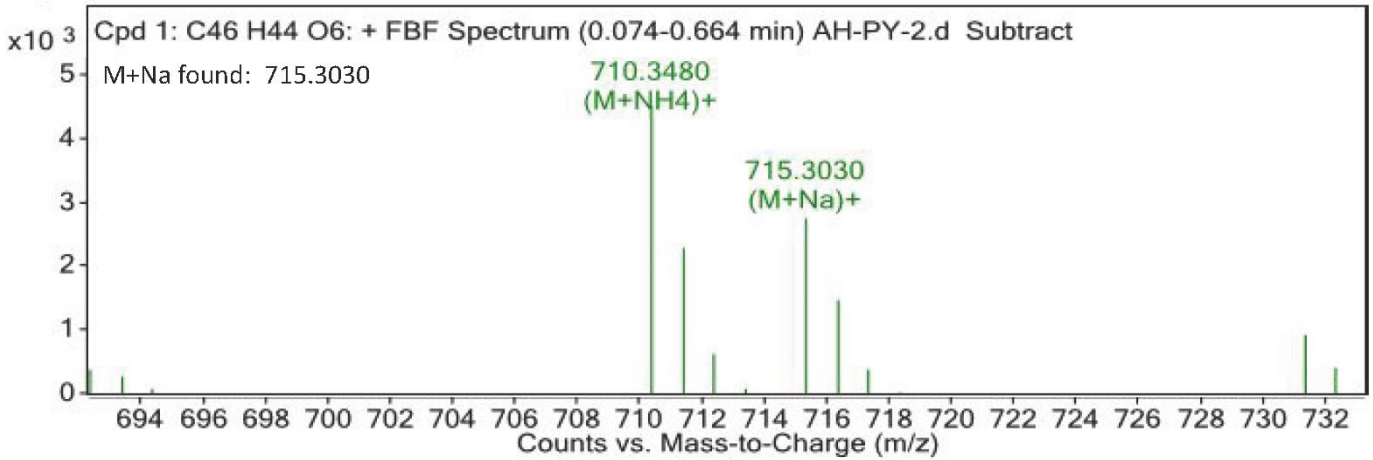

43
f1 (ppm)
7.2570
7.2485
6.6009
6.2098
6.2076
6.2012
6.1990
5.8669
5.8403
5.8232
5.7966
5.1114
5.0897
5.0675
5.0632
2.9944
2.4238
2.2832
2.2587
2.2497
1.3403
1.1741
1.1278
0.93
1.00
1.10
1.22
2.40
1.13
3.50
31.94


0
LW_03162017_RS-SP-22 (0.034)
100
%
(43) : < 5 ppm
M+Na calculated: 477.1889
477.1889 (0.0 PPM)
478.1923 (0.0 PPM)
479.1950 (0.0 PPM)
0
475
476
477
478
479
480
481
482
m/z
MSMS: 0.00000000
TOF MS ES+
7.41e12
OH
H
Chemical Formula: C26H30O7
Exact Mass: 454.1992
LW_03162017_RS-SP-22 15 (0.273)
TOF MS ES+
1.45e6
100
%
M+Na found: 477.1902
477.1902
477.3063
478.1939
0
475
476
477
478
479
480
481
482
m/z


8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
8.65
0.86
0.96
1.82
1.09
2.00
2.00
45.09
8.2602
8.1689
8.1657
8.1384
8.1287
8.1167
8.1110
8.1076
8.0450
8.0399
8.0016
7.8831
7.5188
6.6339
5.5984
5.5826
5.5671
5.5591
5.5432
5.5279
5.4991
5.4597
3.3772
3.3584
3.3389
1.2808
1.1959
1.1159
44
H
O
O
O
O
O
OH


LW_03162017_AH-PY-2 (0.034)
100
%
0
715.3036 (0.0 PPM)
(44) : < 5 ppm
M+Na calculated: 715.3036
716.3069 (0.0 PPM)
717.3101 (0.0 PPM)
620 630 640 650 660 670 680 690 700 710 720 730 740 750 760 770 780 790 800 810 820 830 840 850 860 870 880 890 900 910 920 930 940 950 960 970 980
m/z
TOF MS ES+
5.98e12
OH
H
Chemical Formula: C46H44O6
Exact Mass: 692.3138

MS Spectrum


x10 3
5
4
3
2
1
0
Cpd 1: C46 H44 O6: + FBF Spectrum (0.074-0.664 min) AH-PY-2.d Subtract
M+Na found: 715.3030
710.3480
(M+NH4)+
715.3030
(M+Na)+
694 696 698 700 702 704 706 708 710 712 714 716 718 720 722 724 726 728 730 732
Counts vs. Mass-to-Charge (m/z)

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