Triterpene Carboxylic Acids as Cortisol Lowering Agents and Synthesis of Hexadeuterated β-Ionone

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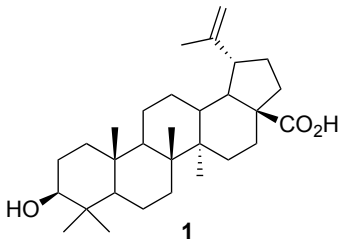
For Erin and James

Abstract

In part one, betulinic acid (**1**) was isolated from the American Sycamore (*Platanus occidentalis*) in 1.6% yield, while ursolic acid (**3**) was isolated from Fuji and McIntosh apple peels in 1.0% and 0.8% crude yields, respectively. Oleanolic (**4**) and dehydrocanophyllic (**6**) acids were previously available, along with several analogs. Additional analogs of **1**, **3** and **4** were prepared, including 9 new compounds, for a total of 51 compounds. Compounds were initially screened for cortisol lowering properties *in vitro* using a fish head kidney cell assay. Platanic acid (**43**) was selected for *in vivo* study in rats, along with **1** and a blend of *Platanus occidentalis* and *Souroubea sympetela*. No significant cortisol lowering was observed *in vivo*.



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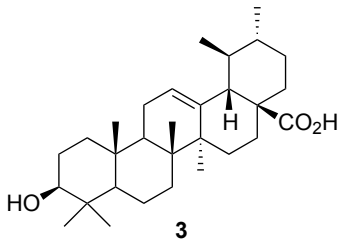

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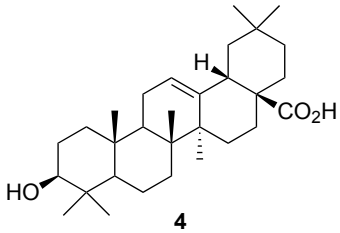

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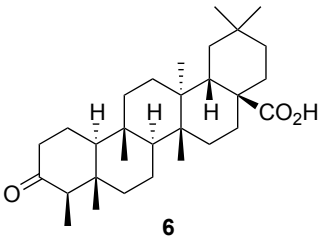

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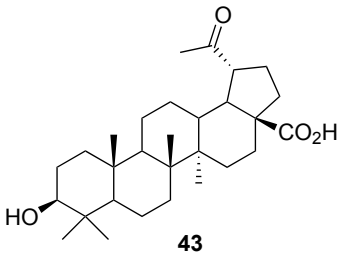

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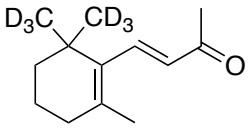
In part two, β-ionone-d_6 (**75**) was synthesized in 6.5% yield from ethyl 2-oxo-cyclohexane carboxylate (**77**). Total deuterium incorporation was 99.85%, with 0.03% d_0 analog. **75** was converted to retinoic acid-d_6 (**93**) in 2.2% yield.



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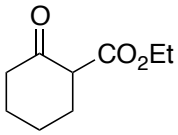

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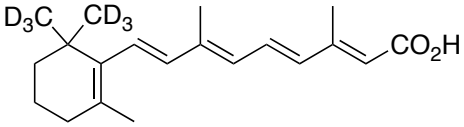

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List of Schemes

List of Abbreviations and Symbols

Å	angstrom
Ac	acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
ACTH	adrenocorticotropic hormone
aq	aqueous
Ar	argon
atm	atmosphere
Bn	benzyl
br	broad
BuLi	butyllithium
CAD	Canadian
CBT	cognitive behavioural therapy
CRH	corticotropin-releasing hormone
d	doublet
D	deuterium
δ	chemical shift
DCM	dichloromethane
DMAP	4-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	dimethyl formamide
EI	electron ionization
ESI	electrospray ionization
Et	ethyl
Et_2O	diethyl ether
EtBr	bromoethane
Et_3N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol

g	gram
GC	gas chromatography
HPA	hypothalamic-pituitary-adrenal
HPLC	high performance liquid chromatography
hr	hour
HRMS	high-resolution mass spectrometry
Hz	hertz
i-Pr_2NH	diisopropylamine
*i*PrOH	isopropanol
J	coupling constant
LAH	lithium aluminum hydride
LDA	lithium diisopropylamide
m	multiplet
mCPBA	*meta*-chloroperoxybenzoic acid
Me	methyl
MeCN	acetonitrile
MeOH	methanol
Me_2S	dimethyl sulfide
MHz	megahertz
min	minute
mmHg	millimeters of mercury
mmol	millimoles
MS	mass spectrometry
MW	molecular weight
m/z	mass to charge ratio
[M]+	molecular ion
NaOEt	sodium ethoxide
NMR	nuclear magnetic resonance
Ph	phenyl
$P(OEt)_3$	triethyl phosphite
PPTS	pyridinium *para*-toluenesulfonate

PTSA	*para*-toluenesulfonic acid
ppm	parts per million
q	quartet
rel. int.	relative intensity
rt	room temperature
Rf	retention factor
s	singlet
SAR	structure-activity relationship
sat'd	saturated
SE	standard error
t	triplet
THF	tetrahydrofuran
THP	tetrahydropyranyl
TLC	thin layer chromatography
TMS	tetramethylsilane **or** trimethylsilyl
TMSOTf	trimethylsilyl trifluoromethanesulfonate
UV	ultraviolet
UV-vis	ultraviolet-visible
vol	volume
wt	weight

Part I: Triterpene Carboxylic Acids as Cortisol Lowering Agents

-Chapter 1: Introduction-

1.1. "The Age of Anxiety"

The 20th century was referred to by the poet W.H. Auden as the "age of anxiety", and by the philosopher and author Albert Camus as "the century of fear". By the 1950's, issues such as the threat of war, the invention of the atomic bomb, and political and economic upheaval had the potential for creating a great deal of anxiety amongst humans.[1] A likely contributor to the general public's anxiety was the use of communications technology such as television. The images of war, riots, and natural disasters transmitted via this medium would have made these events seem much more plausible, and thus more threatening. Modern times are not much different. Humans today face many threats such as nuclear disaster, environmental issues, threats to privacy, drugs, violence and terrorism.[2] Technology has developed even further, bringing us instant communications tools such as the internet, social media, and personal mobile devices. A terrible incident such as an act of war or a nuclear disaster can be known worldwide within minutes, and some individuals may believe that something similar will happen to them, even if rational thought would suggest otherwise. In addition to these pressures, North Americans in particular live in a fast paced culture with busy schedules and demanding careers, which can contribute to a high level of stress and lead to anxiety.[3] Approximately 10% of Canadians[4] and 18% of American adults[5] have an anxiety disorder at any given time, making it the most common of all mental health problems.[4-6] Whereas the 20th century was called "the age of anxiety", anxiety is now being referred to as the disease of the 21st century.[7]

1.2. Anxiety Disorders

Anxiety is defined as, "an abnormal sense of fear, nervousness, and apprehension about something that might happen in the future".[8] While it is normal to feel anxious at

certain times, individuals with anxiety disorders have unrealistic, intense, prolonged feelings of fear and distress that interfere with their everyday lives.[9] Several factors have been identified as the cause of anxiety disorders, including biological, cognitive and environmental factors. Biological causes of anxiety include the influence of neurotransmitters and hormones, or possibly a genetic link, as children of adults with a disorder are at greater risk of developing one themselves. Cognitive factors include the belief of the individual that a certain situation will bring them harm or embarrassment, thus causing them distress. Environmental factors include any stressful experience that can cause a person to become fearful of that experience.[9]

Anxiety disorders can manifest as one of seven specific subtypes: Generalized Anxiety Disorder (excessive worrying for at least six months, with symptoms such as poor concentration and fatigue); Social Anxiety Disorder (fear or avoidance of social situations where the person may be observed, embarrassed or humiliated); Specific Phobia (a persistent fear of objects or situations such as heights, flying, or animals); Post-traumatic Stress Disorder (involving flashbacks, frightening thoughts or memories, and anger or irritation towards a past event where the individual was threatened or harmed); Obsessive-Compulsive Disorder (persistent, inappropriate thoughts or impulses that cause the individual distress, often accompanied with compulsive behaviour to counteract the stressful thoughts or impulses); Panic Disorder (recurring, unexpected panic attacks along with at least one month of worry about subsequent attacks, the consequences of the attack, or altered behaviour related to the attack), and; Agoraphobia (fear of being in a situation where it would be difficult or embarrassing to escape, or where help may not be available in the event of a panic attack).[9] Individuals who suffer from panic attacks can experience heart palpitations, sweating, trembling, shortness of breath, chest pain, feelings of choking, nausea, dizziness, chills or hot flashes, and a fear of losing control.[9]

People with anxiety experience a diminished quality of life, as they are likely to avoid situations that may precipitate their fears or symptoms. This can interfere with work, education, recreation and social life.[9] Individuals with one type of anxiety disorder are more likely to develop a second anxiety disorder, major depression, personality disorder, or substance abuse. Anxiety results in economic costs such as lost work productivity due to sick leave and increased disability claims, along with increased

pressure on the health care system.[9] One study estimated the total cost of anxiety disorders in the United States in 1990 to be $42.3 billion,[10] almost a third of the nation's total mental health bill of $148 billion.[5]

1.3. Treatments of Anxiety

Two of the most effective treatments for anxiety are cognitive behavioural therapy (CBT) and pharmacological treatment.[11,12] CBT is related to the idea that the mindset of the individual and how they react to situations is responsible for their anxiety. CBT aims to help the patient identify and challenge their way of thinking, in order to change their reaction towards perceived stressors. Pharmacological treatment with drugs such as the antidepressant and anti-anxiety classes may also be used.[9,11] Depending on the treatment regime determined by a healthcare professional, the above methods may be used on their own or in combination.[11,12] In the United States in 2010, 253.6 million prescriptions of antidepressants were dispensed, for a total spending of $11.6 billion. During the same time period, the anti-anxiety drug alprazolam of the benzodiazepine family accounted for 46.3 million prescriptions.[13] Given the impact of anxiety on our health and the number of drug prescriptions per year, any drug able to offer relief would seem to be quite valuable, and the creation of a new medicine in this area would be worthwhile.

1.4. A New Anxiolytic from Natural Sources

The search for a new medicine often begins with a naturally occurring compound, as many natural products have been used by the pharmaceutical industry as leads for drug development.[14-16] A study of the number of new approved drugs between 1983-1994 indicated that 78% of antibacterials were of natural origin, and 61% of anticancer drugs were either of natural origin or modeled on a natural product.[16] Medicinal plants in particular are a good source of natural products, and the knowledge of traditional medicinal uses of plants can be helpful in locating a plant with specific activity.[14,15] One such family of plants used for its anxiolytic properties is the Marcgraviaceae family of

tropical shrubs, found in South and Central America and the West Indies.[17] Several native groups of these lands have used a drink made from the leaves of Marcgraviaceae as a sleep aid or a tranquilizing agent. Others have used Marcgraviaceae to prepare a drink that relieves "susto", which is a fear or apprehension that usually results from the belief that hexing by an enemy has been successful.[17]

In an effort to discover a new medicine with anxiolytic properties, the 2004 Ph.D. thesis of E. Puniani (University of Ottawa) described the isolation of a natural compound from the leaves of two species of Marcgraviaceae: *Souroubea gilgi* and *Souroubea sympetala*.[18] In order to accomplish this, a series of bioassay-guided fractionations were performed, beginning with the separation of a *S. sympetala* leaf extract made from 95% ethanol. This initial extract was shown to reduce anxiety in rats using classical methods of assessment such as the Elevated Plus Maze and the Fear-Potentiated Startle assays.[18] Subsequent fractionation showed that the anxiolytic activity resided in the ethyl acetate soluble portion. This material was then fractionated by silica gel column chromatography. Individual fractions were fed to rats, and the rats' behaviour was assessed for anxiety and fear. The fraction that gave the least anxious rats was further purified, until eventually a single active compound was isolated. As a result of this work, the natural product betulinic acid was identified as the principal anxiolytic.

1.5. Betulinic Acid

Betulinic acid (**1; Fig. 1.1.**) is a pentacyclic triterpenoid[19] that can be found from many natural sources such as the bark of white birch (*Betula alba*)[20], the bark of the American sycamore (*Platanus occidentalis*)[21], and leaves of rosemary (*Rosmarinus officinalis* L.)[22]. It has been identified as having anti-diabetic[23], anti-cancer[24], and anti-HIV properties[25]. Many analogs of betulinic acid were subsequently prepared and patented;[26,27] however, no anxiolytic properties of betulinic acid were reported in the literature at the time. This discovery resulted in a patent, issued in 2009, for the use of Marcgraviaceae extracts as an anxiolytic, as well as the potential use of betulinic acid and many semisynthetic analogs for this purpose.[28]

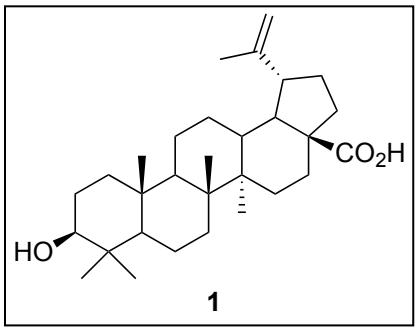

Fig. 1.1. Structure of betulinic acid (**1**).

In 2005, an experiment was conducted on the effects of feeding either betulinic acid or dried leaves of *Souroubea sympetala* to stressed, newly weaned juvenile pigs. It was observed that a diet containing either of these substances caused the piglets to have reduced levels of the stress hormone cortisol[29] in their blood plasma, compared to a control group[30]. Another experiment involved feeding restrained rats the methyl ester of betulinic acid. A significant decrease in the level of the major rodent stress hormone corticosterone[31,32] was observed.[18] The 2011 Ph.D. thesis of M. Mullally (University of Ottawa) also described the cortisol lowering effect of betulinic acid and *Souroubea sympetala* extracts on stressed rainbow trout.[33] All these experiments indicated that betulinic acid acted upon stressed animals by lowering the level of cortisol or corticosterone circulating in the blood.

1.6. Cortisol: The "Stress Hormone"

Cortisol is a naturally occurring hormone in humans with several important functions. It is involved in the regulation of glucose metabolism, maintenance of blood pressure, and suppression of the inflammatory response of the immune system.[1] In addition, cortisol has been called the "stress hormone", as it is important in helping the body respond to stress during the "fight or flight" response.[29,34,35] When the brain senses a threat, cortisol is released to help the body respond to it.[31,36,37] For example, an increase in cortisol will increase blood circulation and blood sugar levels to give extra energy for physical and mental activities, while lowering the body's sensitivity to pain.[29,37] When the stressful event is over, the body returns to a relaxed state, and circulating cortisol levels

return to normal. However, prolonged exposure to stressful situations can lead to a state of chronic stress and prolonged high levels of cortisol in the blood.[29] This can lead to problems such as hypertension, cardiovascular disease, blood sugar imbalance, suppressed immune and inflammatory response, impaired healing of wounds, suppressed thyroid function, decreased bone density and muscle tissue, deficits in cognitive function, and metabolic disorders.[29,31,36,38,39]

1.7. Cushing's Syndrome

One specific disease associated with excess cortisol is known as Cushing's Syndrome, which can develop in humans,[34] but is also known in dogs, cats and horses.[40] Humans with this disease can have symptoms such as upper body obesity, rounded face, easily bruised and poorly healing skin, weakened bones and muscles, fatigue, decreased sex drive, high blood pressure, high blood glucose, increased thirst and urination, anxiety, and depression.[34] Cushing's is a rare disease, usually affecting people between the ages of 20-50. It can occur in people taking steroids that are similar to cortisol, such as prednisone, for the treatment of asthma, rheumatoid arthritis, lupus, and other inflammatory diseases. Cushing's can also develop in individuals whose body produces too much cortisol.[34]

Cortisol release is controlled by the hypothalamic-pituitary-adrenal (HPA) system.[31] When the brain perceives a threat, it stimulates the release of corticotropin-releasing hormone (CRH) from the hypothalamus, which in turn stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary gland. ACTH then stimulates the release of cortisol from the adrenal glands.[31,34,41] Cortisol acts upon the hypothalamus by down-regulating the release of CRH, resulting in a negative feedback loop and controlled levels of cortisol.[31] When this regulatory system becomes damaged, excess cortisol can be released and an individual may develop Cushing's.[34]

Treatment of Cushing's in humans may involve several options, including surgery, radiation, chemotherapy, or the use of cortisol-inhibiting medication.[34] For dogs, medication is usually the treatment of choice, although surgery is also an option.[40]

1.8. Betulinic Acid as a Potential Anxiolytic and Cortisol Lowering Drug

To date, much research has been carried out by our research group on the use of whole plant materials from Marcgraviaceae shrubs such as *S.Sympetela* and *S.gilgi* for the reduction of anxiety. This has resulted in the preparation of a product that is close to being commercialized as an anxiolytic for calming companion animals that are prone to nervousness, such as dogs and horses. The anti-anxiety properties of betulinic acid were also investigated quite extensively. In addition, 11 analogs of betulinic acid were prepared, but with the exception of the methyl ester, none of these compounds were evaluated to any significant extent for their anti-anxiety properties.

There is also the potential to develop a cortisol-lowering treatment, using either raw plant material, an extract, or by the development of a single component drug based on betulinic acid as a drug lead. One area that has not been well studied thus far is the medicinal chemistry of betulinic acid with respect to its anxiolytic and cortisol lowering properties. It may be possible to get a more potent drug by preparing chemical analogs of betulinic acid. This is important from a commercial point of view, since less material would be required per dose of drug. Changes in the molecular structure of betulinic acid, along with bioactivity data, would give clues as to which parts of the molecule are most important for activity. Therefore, the goal of part one of this thesis was to prepare a library of betulinic acid analogues for biological screening to achieve the above objectives and provide one or more lead compounds for further drug development.

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-Chapter 2: Discussion and Results-

2.1. Triterpene Carboxylic Acids: SAR Study

As discussed in the previous chapter, betulinic acid (**1**) and its C-28 methyl ester (**2**) displayed cortisol lowering effects in both newly weaned piglets and restrained rats. It was therefore decided to investigate the effect of analogs of betulinic acid on the cortisol concentration as part of a classic Structure-Activity Relationship (SAR) study.

In addition, it was decided to prepare and evaluate analogs of the related triterpene carboxylic acids ursolic (**3**) and oleanolic (**4**). The investigation of ursolic acid analogs seemed especially relevant since ursolic acid and its 2α-hydroxy analog (**5**) were isolated from the leaves of Marcgraviaceae by E. Puniani.[1] Despite their presence in Marcgraviaceae, the possible anti-anxiety effects of ursolic and 2α-hydroxy ursolic acid have not been determined. In contrast, oleanolic acid gave no activity in an anti-anxiety assay.[2]

A fourth triterpene acid, dehydrocanophyllic acid (**6**), was available in our laboratory. Although this compound has a substantially different structure than the other three acids, it was considered worthwhile to investigate with several simple analogs as part of this project. The structures of the triterpene acids **1**, **3**, **4** and **6** are given in **Fig. 2.1.**

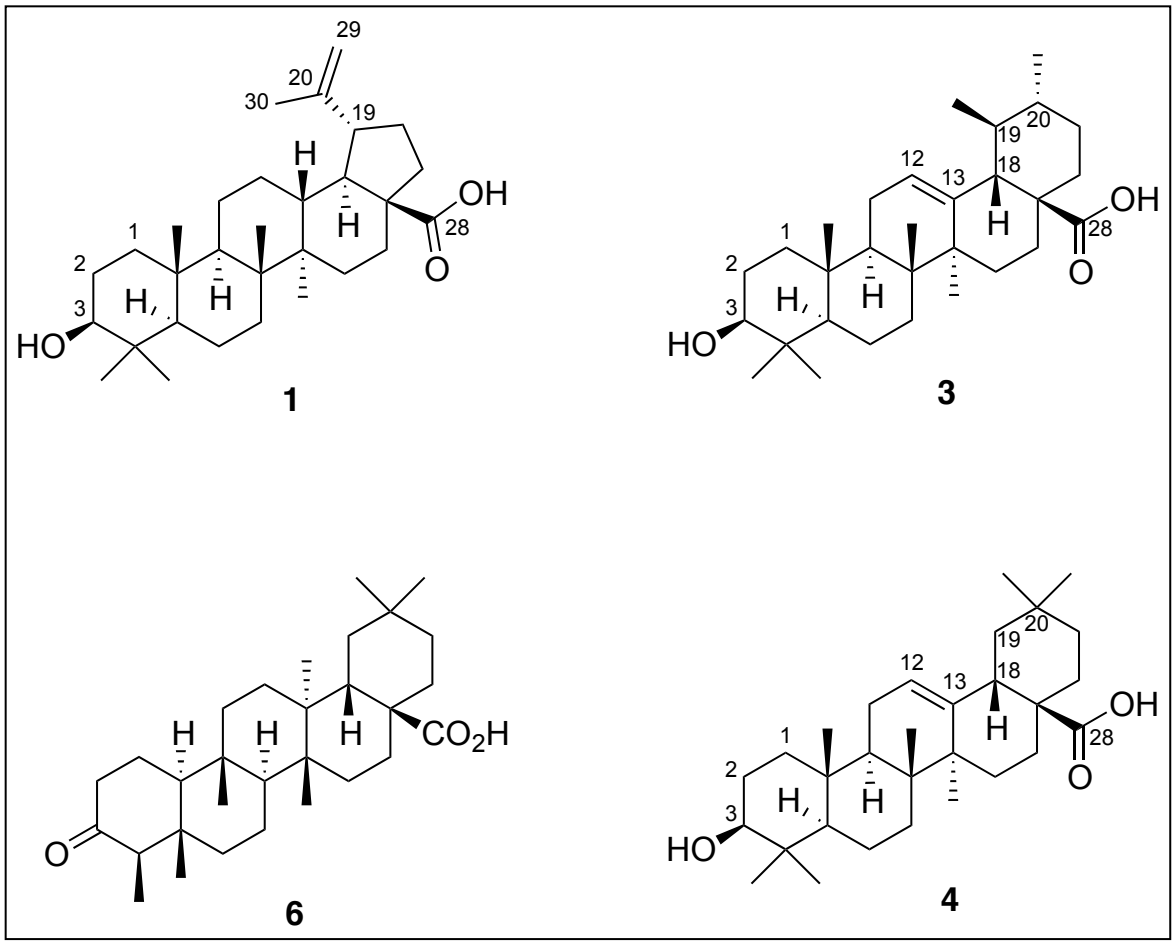


Fig. 2.1. Structures of triterpene carboxylic acids betulinic (**1**), ursolic (**3**), oleanolic (**4**) and dehydrocanophyllic (**6**) with selected carbons numbered.

2.2. Isolation of Triterpene Carboxylic Acids

For the purposes of the SAR study and the synthesis of triterpene acid analogs, gram quantities of starting materials were required. Betulinic acid (**1**) was quite expensive at the time of writing, costing $370 CAD for 0.5 grams of 90% pure material.[3] A less expensive alternative for obtaining betulinic acid was found by its extraction from natural sources. In particular, trees of the genus *Platanus* have been reported to have a betulinic acid content as high as 5.7% in the outer bark.[4-6] The bark of these trees is easily obtained, as it sheds readily and only needs to be collected.[6] One tree, 10 m in height, will provide several kilograms of bark per year.[6] For this work, bark was gathered from an American sycamore tree (*Platanus occidentalis*) at the Experimental Farm in Ottawa,

Ontario, Canada. Sporadic collection of the bark shed by this large, over one hundred year old tree provided over 35 kg of bark during the summer of 2011. American sycamore trees were also located in various cities across Southern Ontario, including Hamilton, London, Stratford, Sarnia, Leamington, Windsor and the surrounding area of Essex County.

The collected bark was ground into a powder, extracted by soaking overnight in EtOH, filtered, evaporated, and the crude extract purified by silica gel chromatography to give pure betulinic acid in gram quantities (1.6%). A more recent publication was later found describing an improved extraction that involved boiling the ground bark with water first and extracting with a solvent such as MeOH, EtOH or acetone.[4] The structure of betulinic acid was verified by comparison of its ^{1}H NMR spectrum with literature values.[1]

As with betulinic acid, gram quantities of ursolic acid (**3**) were required for the purposes of the SAR study. However, ursolic acid was also quite expensive, at a price of $273.50 CAD for 500 mg of 90% pure material at the time of writing.[3] Apple peels are a known source of ursolic acid, with a content of 0.71% in Fuji apple peels[7] and 0.15% in Red Delicious[8] apple peels. For this project, apple peels from Fuji and McIntosh apples were independently extracted by soaking overnight with EtOAc, filtering and evaporating the solvents to give a green extract. The Fuji extract was separated by silica gel chromatography to give crude, impure ursolic acid, with ^{1}H NMR confirming the structure.[9] A slightly different purification method was used on the McIntosh extract by stirring it vigorously in refluxing hexanes and filtering the crude material to remove non-polar impurities. The residue was dissolved in hot EtOH, stirred with charcoal, filtered and crystallized to give impure ursolic acid by ^{1}H NMR.[10] Repeated crystallizations from EtOH did not appear to improve the purity, even though a publication reported its purification by crystallization from EtOH.[11]

The remaining triterpene acids oleanolic (**5**) and dehydrocanophyllic (**6**) were already available in the lab in sufficient amounts, and there was no need to obtain any more from other sources.

2.3. Strategy for Preparation of Triterpene Acid Analogs

For the purposes of the SAR study, the strategy was to prepare a number of triterpene acid analogs using common, easily performed chemistry. This would allow a large number of analogs to be prepared relatively quickly, giving a broad range of analogs for biological testing. Also, any analog giving a "hit" would be easier and less expensive to produce as a drug compared to a more complex analog. It should be appreciated that the initial plan was to prepare several analogs and have them quickly screened for cortisol lowering ability using an *in vitro* fish head kidney cell bioassay, described in section 2.8. Feedback from the bioassays would then be used to guide the synthesis of new analogs. Unfortunately, the bioassays could not be carried out as easily as anticipated and thus to a considerable extent the analogs were prepared purely on speculation that they might be effective at lowering cortisol. As a result, the decision was made to prepare a representative number of analogs in each family; for example, a number of esters and amides of betulinic acid were prepared, along with a few analogs with modifications of the C20-C29 vinylidene group.

The solubility of the C-28 methyl ester of betulinic acid (**2**) made it much easier to work with than betulinic acid itself. Also, it was easier to administer in the bioassays and was shown to have the greatest potential of any of the analogs made by Puniani for lowering cortisol.[1] Thus, **2** was deemed to be the potential lead structure for further development and it was decided to include the C-28 methyl esters of all the structural families in this study (i.e. betulinic, oleanolic, ursolic, and canophyllic acids).

2.4. Betulinic Acid Analogs

The structure of betulinic acid (**1**) contains several sites for easy modifications, shown in **Fig. 2.2**. Esters and amides of the C-3 alcohol and C-28 carboxylic acid could readily be prepared. Elongation of the C-28 side chain could be achieved by alkylation of the corresponding alcohol, or by Wittig or Horner-Wadsworth Emmons reaction of the corresponding aldehyde. Introduction of substituents at C-2 could be achieved via enolate

chemistry on the corresponding C-3 ketone, and the C20-C29 vinylidene group could undergo various additions, or oxidation to the C-20 ketone.

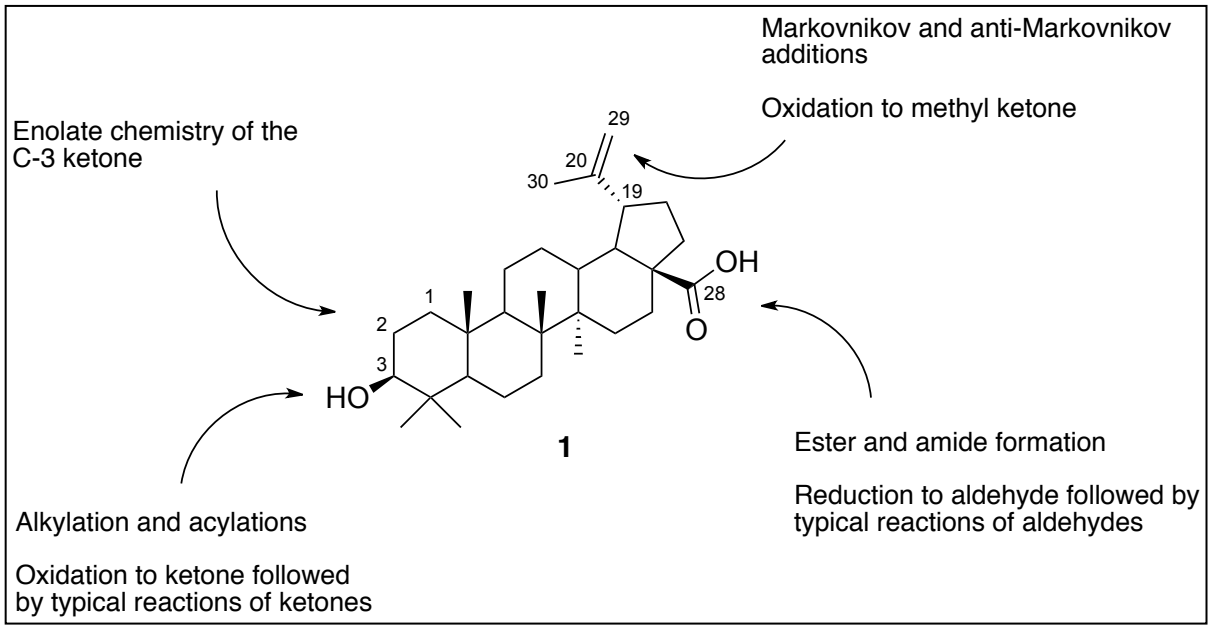


Fig. 2.2. Potential modifications of betulinic acid (**1**).

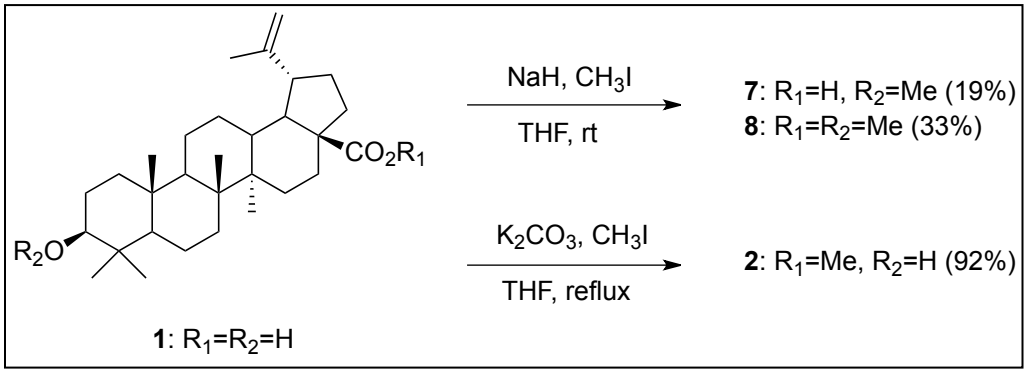
Most of the analogs prepared were purified by silica gel chromatography, unless otherwise specified. Any known compounds had their structures verified by comparison of their ^{1}H and/or ^{13}C NMR spectra to the literature, and references are given. New compounds had their structures verified by their NMR spectra (^{1}H and ^{13}C) and HRMS data.

2.4.1. Esters at C-3 and C-28

The first series of betulinic acid analogs were simple esters at the C-3 and C-28 positions. Initial attempts to prepare methyl betulinate (**2**) began by stirring **1** with NaH and CH_3I in THF at rt. The desired product was not observed, but two other products were isolated, identified as 3-methoxy betulinic acid (**7**) and 3-methoxy methyl betulinate (**8**) in 19% and 33% yield respectively (**Scheme 2.1**). The ^{1}H NMR of **7** gave a broad signal at 11.36 ppm (C-28 $CO_2\mathbf{H}$), with the 3-methoxy group appearing as a singlet at 3.35 ppm in ^{1}H and 88.7 ppm in the ^{13}C NMR spectrum. HRMS gave the mass as

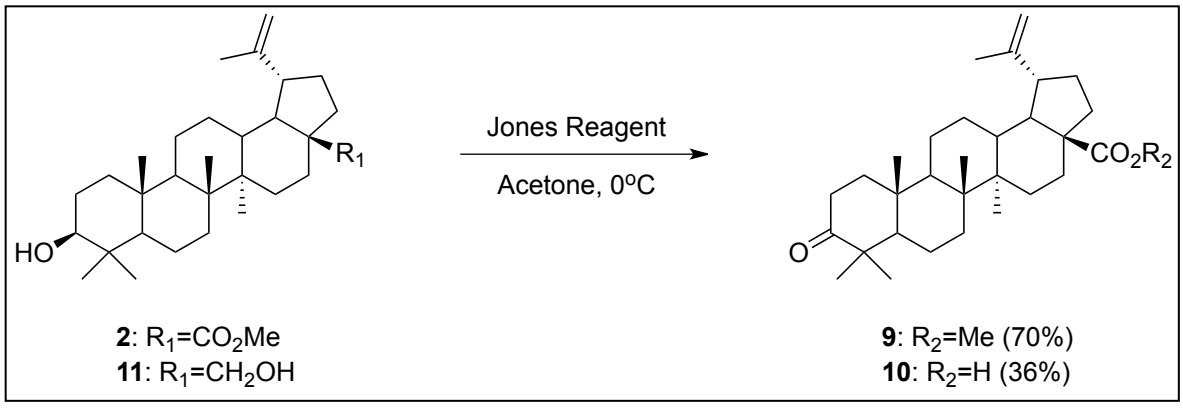
470.38018, compared to calculated 470.37600. In contrast, the ^{1}H NMR of **8** gave two singlets at 3.67 ppm (C-28 CO_2**Me)** and 3.35 ppm (C-3 O**Me**), with ^{13}C NMR giving signals at 51.3 ppm (C-28 CO_2**Me**) and 88.6 ppm (C-3 O**Me**). HRMS gave the mass as 484.39340, compared to calculated 484.39165. Compounds **7** and **8** have been reported[12-15], but the NMR data for these compounds has not been reported.

The preparation of methyl betulinate (**2)** was achieved in 92% isolated yield using the weaker base K_2CO_3 with CH_3I in refluxing THF (**Scheme 2.1**). NMR spectra (^{1}H and ^{13}C) confirmed the structure of the product.[1]



Scheme 2.1. Alkylation of betulinic acid (**1**) with CH_3I.

Oxidation of methyl betulinate (**2**) with the Jones reagent in acetone at 0 ^{o}C gave the C-3 ketone, methyl betulonate (**9**), in 70% isolated yield (**Scheme 2.2**). For comparison purposes, it was thought that the free acid of **9**, betulonic acid (**10)** should also be prepared. A large amount of the corresponding diol betulin (**11)** was available in the lab, which was oxidized under the above conditions to give betulonic acid **10** in 36% isolated yield (**Scheme 2.2.**). The structures of **9**[16,17], **10**[1] and **11**[1,18,19] were all confirmed by their ^{1}H and ^{13}C NMR spectra.



Scheme 2.2. Preparation of betulonic acid analogs **9** and **10** with the Jones Reagent.

Several other C-28 esters were available in our lab (**Fig. 2.3**). ^{1}H and ^{13}C NMR was used to confirm the structure and purity of the following C-28 esters: heptyl (**12**)[1], allyl (**13**)[1], benzyl (**14**)[1], and ethyl acetoxy (**15**)[1].

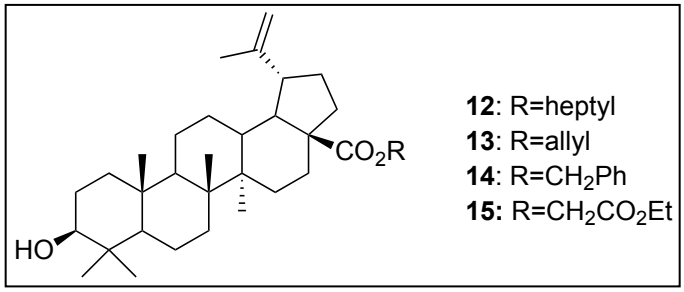
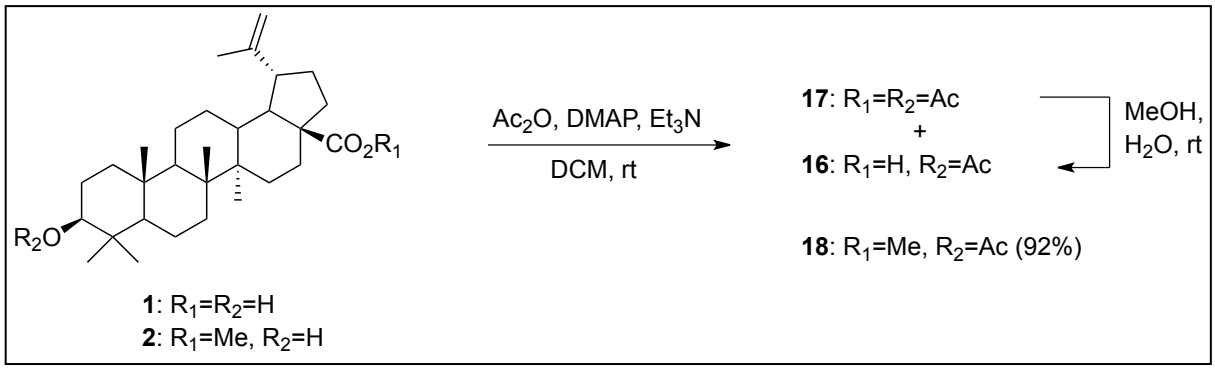


Fig. 2.3. Selected C-28 esters of betulinic acid.

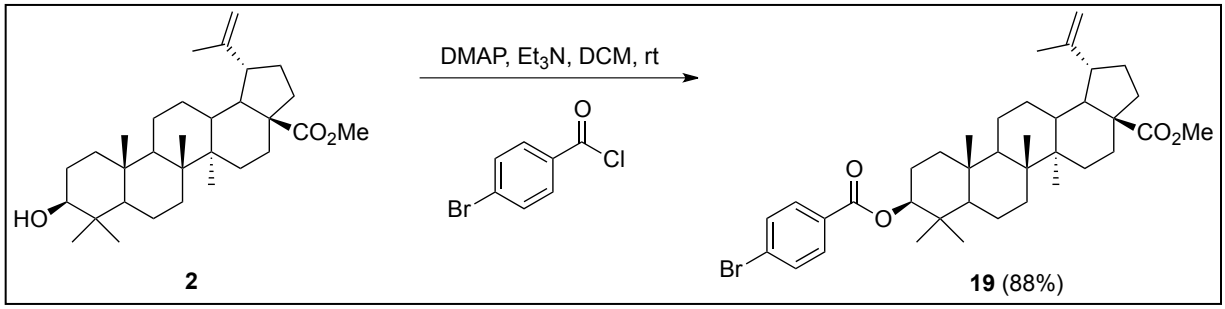
Esters at C-3 were also synthesized. Reaction of betulinic acid with Ac_2O, Et_3N and DMAP in DCM at rt gave 3-acetoxy betulinic acid (**16**) in 7% isolated yield as well as a 2:3 mixture of **16** and mixed anhydride **17** (19% and 29% respective yield by NMR integration; **Scheme 2.3**). This result was consistent with that reported by Pakrashi et al.[20] The mixed anhydride **17** was identified with ^{1}H NMR by the presence of an extra acetate singlet at 2.23 ppm integrating to 60%. Hydrolysis of the mixed anhydride was accomplished by stirring the mixture of **16** and **17** in $MeOH/H_2O$ at rt for several days, giving pure **16** in 79% yield (verified by its ^{1}H and ^{13}C NMR[1]).

A related analog, 3-acetoxy methyl betulinate (**18**), was prepared in 92% yield by stirring methyl betulinate (**2**) with Ac_2O, Et_3N and DMAP in DCM at rt (**Scheme 2.3**). It was also verified by its ^1H and ^{13}C NMR.[17,21]



Scheme 2.3. Acetylation of betulinic acid (**1**) and methyl betulinate (**2**).

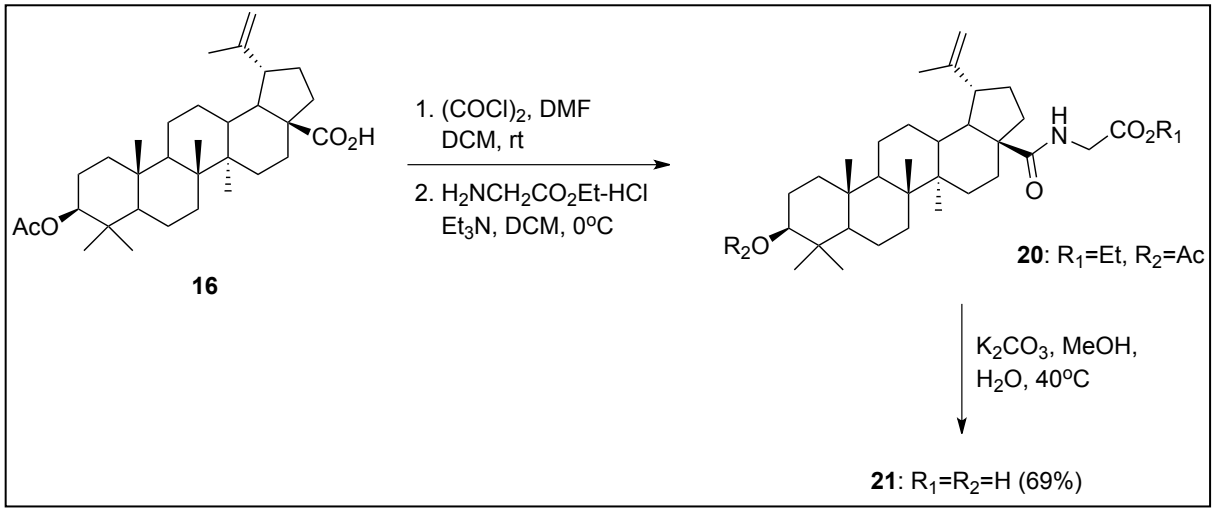
The novel compound 3-(4-bromo)-benzoyl methyl betulinate (**19)** was prepared in 88% yield by acylation of **2** with 4-bromobenzoyl chloride, Et_3N and DMAP in DCM at rt (**Scheme 2.4**). Its ^1H NMR gave multiplets at 7.91-7.87 ppm (2H) and 7.59-7.55 ppm (2H) for the phenyl ring. New ^{13}C signals were found at 165.6 ppm for the ester (Ph**C**O_2), and at 127.8, 129.9, 131.0 (doubled) and 131.6 ppm (doubled) for the phenyl ring. The HRMS was also consistent with the structure.



Scheme 2.4. Preparation of acylated analog **19**.

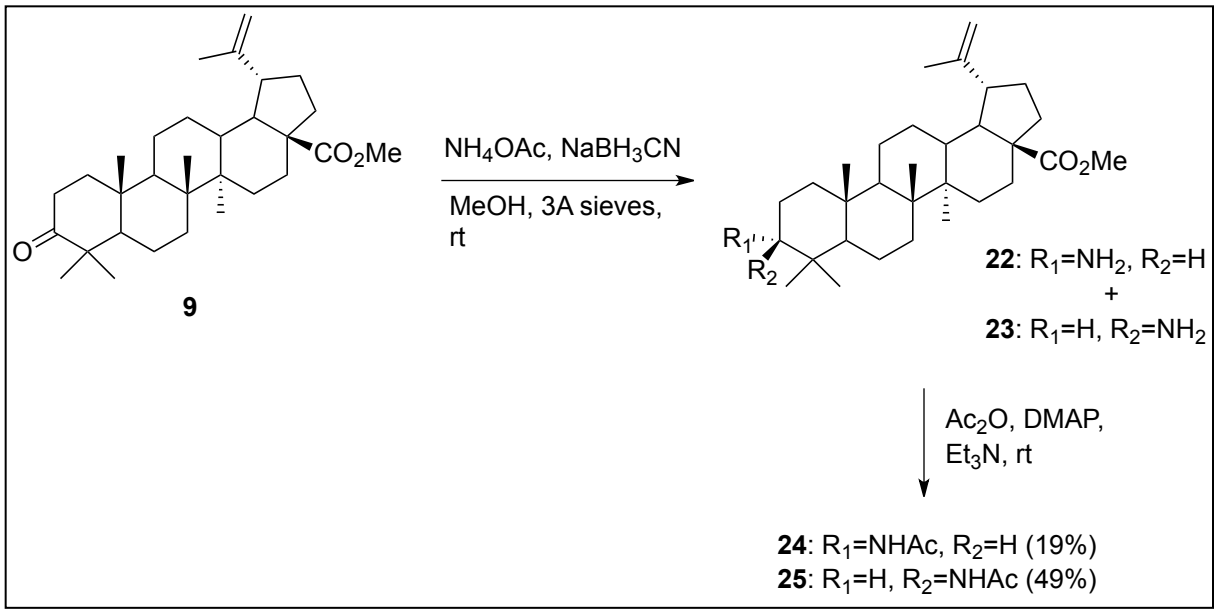
2.4.2. Amides at C-3 and C-28

Several amide analogs of betulinic acid were prepared, starting with a C-28 amide. To accomplish this, the acid chloride of **16** was first prepared by reacting it with oxalyl chloride and catalytic DMF in dry DCM at rt (**Scheme 2.5**).[1] Subsequent addition of the acid chloride to a solution of Et_3N and glycine ethyl ester hydrochloride in dry DCM at 0 ^oC gave the intermediate amide **20**. Amide **20** was then hydrolyzed by action of K_2CO_3, MeOH and H_2O at 40 ^oC to give glycine amide **21** in 69% yield from **16**, verified by its ^1H NMR.[22]



Scheme 2.5. Synthesis of the C-28 glycine amide **21**.

A series of C-3 amides were also prepared, generally accomplished by the reductive amination of methyl betulonate (**9**). The first reaction of **9** with NH_4OAc and $NaBH_3CN$ in MeOH with 3 Å molecular sieves at rt produced a mixture of intermediate amines **22** and **23** (**Scheme 2.6**). This mixture was acetylated with Ac_2O, Et_3N and DMAP at rt to give amides **24** (3-α) and **25** (3-β), isolated in 16% and 49% respective yields.



Scheme 2.6. Synthesis of C-3 amides **24** and **25**.

The ^{1}H NMR spectrum of **24** gave the amide proton (N-H) as a doublet at 5.71 ppm (J=9.8 Hz). The H-3 equatorial proton at 3.81 ppm had a 9.8 Hz coupling to the amide proton, and two 3.2 Hz couplings to the H-4 protons (equatorial-axial, equatorial-equatorial), confirming the 3α orientation of the amide. The acetate group gave a new singlet at 2.02 ppm (N-CO**CH$_3$**). ^{13}C NMR showed the disappearance of the C-3 ketone at 218.1 ppm with a new signal at 169.3 ppm (N-**C**OCH$_3$). The total number of carbon signals was 33, consistent with the structure. The HRMS was also consistent with the structure. Compound **24** was not previously reported in the literature. Conversely, compound **25** had been reported, but no NMR data was given.[23] Thus, the ^{1}H NMR of **25** showed the amide (N-H) proton at 5.27 ppm (J=10 Hz), and the H-3 axial proton at 3.64 ppm with a wide splitting pattern, partially buried under a singlet at 3.66 ppm (C-28 CO$_2$**Me**). The H-3 proton signal had a width of 26.6 Hz; subtracting the 10 Hz amide coupling left 16.6 Hz; subtracting one observed coupling of 4.4 Hz (H-3 axial to H-4 equatorial) left a 12.2 Hz coupling to H-4 axial, confirming the 3β orientation of the amide. A singlet at 1.98 ppm accounted for the acetate group (N-CO**CH$_3$**). ^{13}C NMR showed disappearance of the C-3 ketone at 218.1 ppm, with a new signal at 169.5 ppm (N-**C**OCH$_3$). The total number of 33 carbons was consistent with the structure, and the HRMS was also in agreement. It should be noted that the higher yield of 3β (**25)** was

likely caused by the greater ease of approach of the reducing hydride reagent from the bottom face, as the two axial methyl groups hindered the top face. The 3D representation of this is given in **Fig. 2.4**.

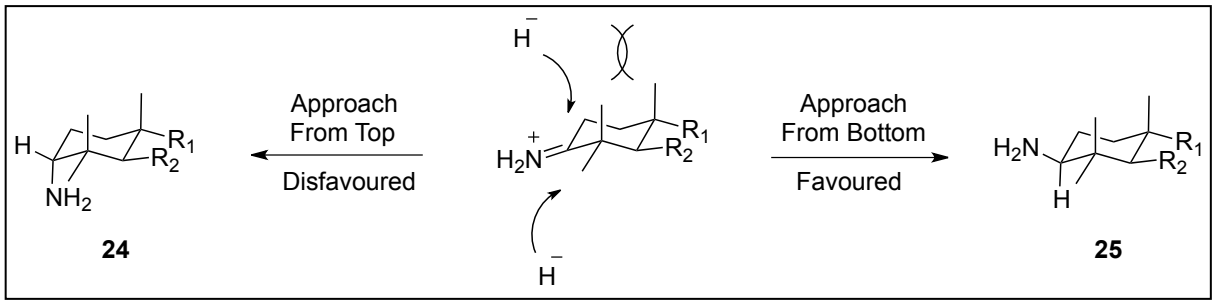
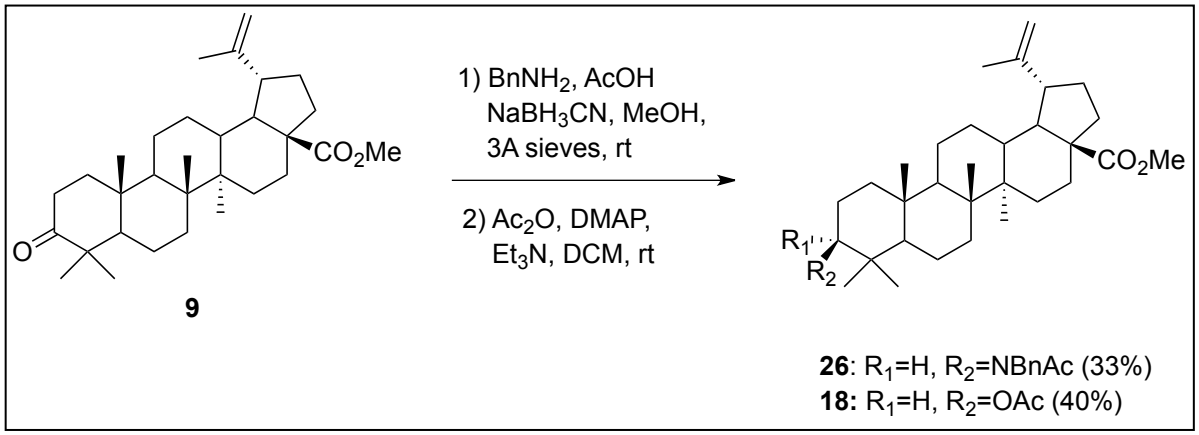


Fig. 2.4. Steric hindrance during reductive amination.

A more hindered amine was also prepared by reductive amination of methyl betulonate (**9)** with benzylamine using the same conditions as above. After acetylation, the novel amide **26** was isolated in 33% yield, along with 3-acetoxy methyl betulinate (**18**) in 40% yield (**Scheme 2.7**).



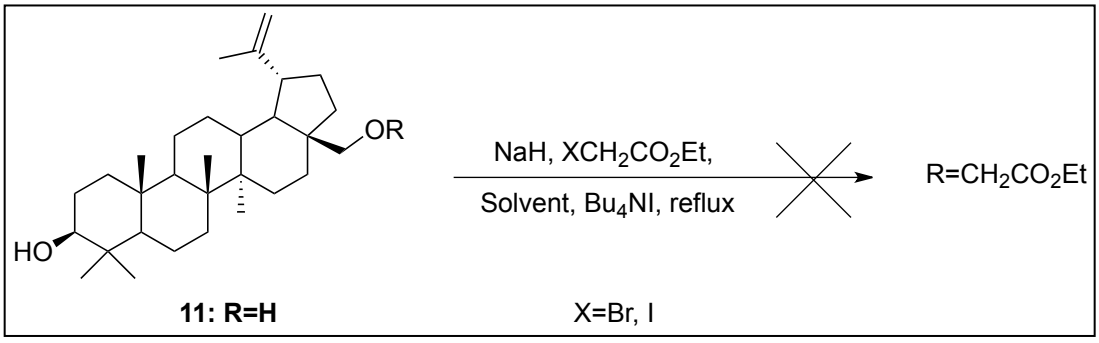
Scheme 2.7. Synthesis of C-3 benzylamide **26**.

The presence of **18** was attributed to unreacted ketone **9** being reduced to the alcohol and subsequent acetylation under the given reaction conditions. The low yield of amide was attributed to the steric hindrance of the condensation of benzylamine with the ketone in **9**. Analysis of the ^{1}H NMR spectrum of **26** indicated a complex mixure of two

rotamers in a ratio of 77:23. A multiplet at 7.38-7.13 ppm integrated cleanly for 5 phenyl hydrogens, The major rotamer gave a broad singlet at 4.59 ppm (N-**C**$\mathbf{H_2}$Ph) and a singlet at 1.97 ppm (N-CO**C**$\mathbf{H_3}$), while the minor rotamer gave doublets at 4.88 ppm (J=16.2 Hz) and 4.30 ppm (J=15.9 Hz) for N-**C**$\mathbf{H_2}$Ph, and a singlet at 2.27 ppm (N-CO**C**$\mathbf{H_3}$). The H-3 axial proton was observed in the major rotamer as doublet of doublets at 4.64 ppm with couplings of 12.5 and 3.1 Hz to H-4 protons, and in the minor rotamer as a doublet of doublets at 3.52 ppm with couplings of 12.6 and 3.5 Hz to H-4 protons. These observations confirmed the 3β orientation of the amide, and none of the 3α isomer was observed. HRMS of the product was consistent with the structure, and a ^{13}C NMR spectrum was not taken due to the anticipated complex spectrum of the two rotamers.

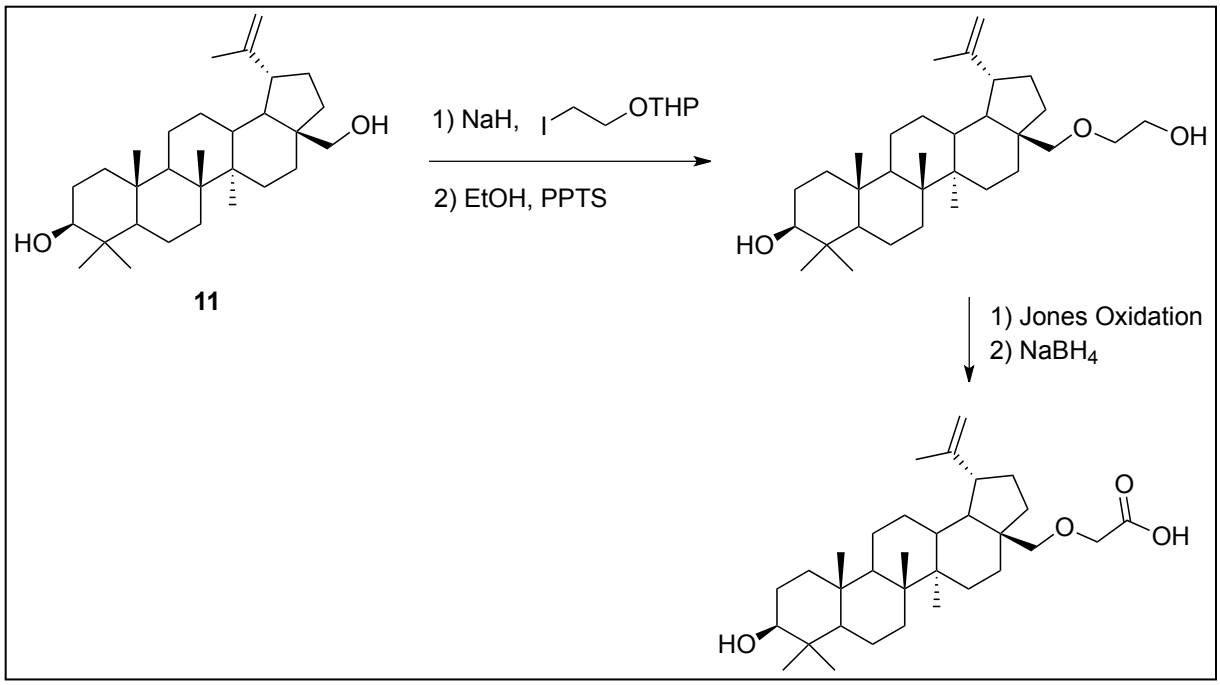
2.4.3. Extended C-28 Side Chains

The next series of analogs that were prepared had extended C-28 side chains. It was thought that alkylation of betulin (**11)** with ethyl bromoacetate could quickly provide such a compound. The two alcohols of **11** are primary (C-28) and secondary (C-3), so the alkylation should be favoured at C-28. However, multiple attempts at alkylation with ethyl bromoacetate and NaH failed to give the desired product, instead leading exclusively to acylation at C-28. This result was implied as hydrolysis of the product with NaOH in MeOH gave **11**. Solvents such as refluxing THF, 1,4-dioxane, and DMF were tried, as well as the phase transfer reagent Bu_4NI. The use of ethyl iodoacetate (without Bu_4NI) in THF led to a complex mixture of products (**Scheme 2.8**).



Scheme 2.8. Unsuccessful alkylation of betulin (**11**) with ethyl bromo/iodoacetate.

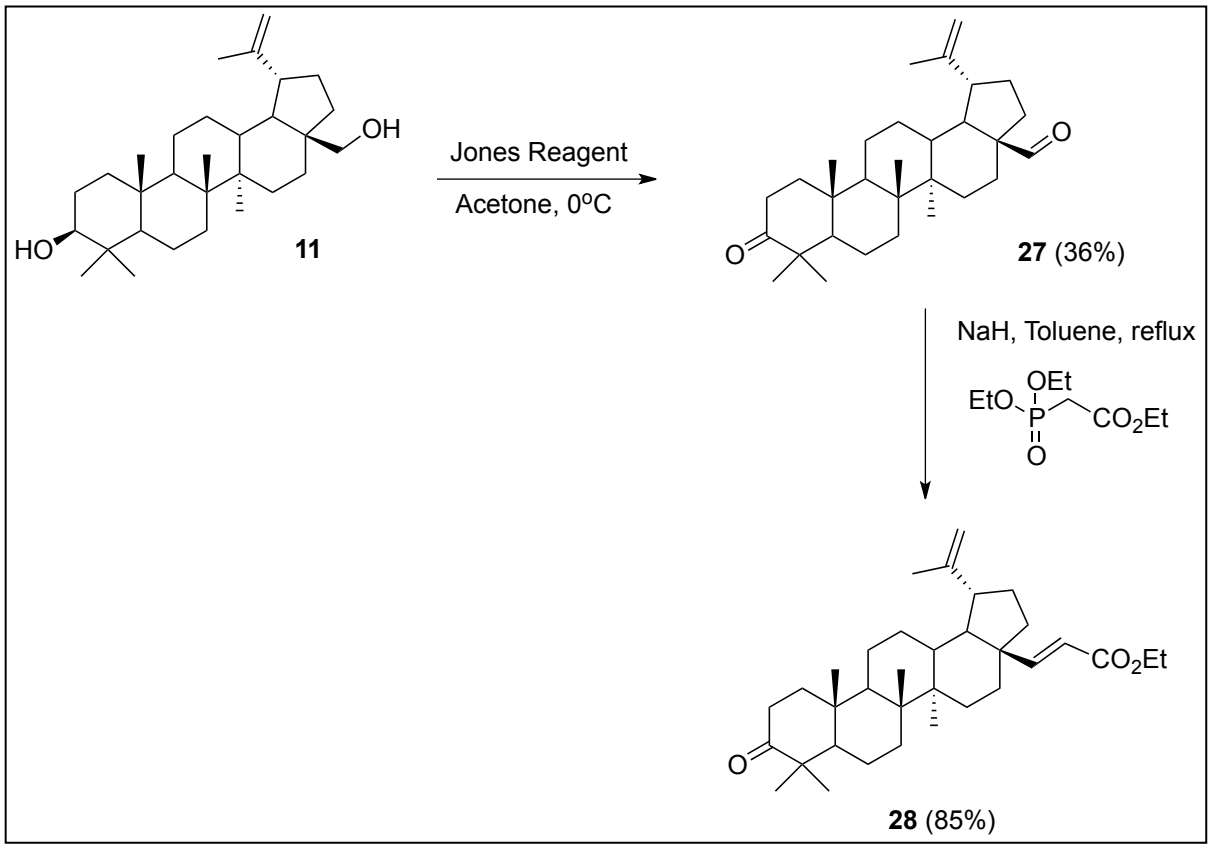
A publication by Mar and co-workers was later found, reporting the same result of preferred C-28 acylation over alkylation.[24] The authors note that this result was in direct contrast to a Russian patent, which described the successful alkylation of **11** with NaH and ethyl bromoacetate.[25,26] Mar and co-workers instead used the sequence shown in **Scheme 2.9**. This appeared somewhat long for our purposes, so a different approach was used.



Scheme 2.9. Alkylation of betulin (**11**) by Mar et al.[24]

It was thought that betulonic aldehyde (**27**) could preferentially react with a Wittig or Horner-Wadsworth-Emmons reagent to provide a C-28 extended product, given the greater reactivity of aldehydes over ketones. Thus, aldehyde **27** was prepared in 36% yield by oxidation of **11** with the Jones Reagent in acetone at 0 ^{o}C (**Scheme 2.10**). The low yield after chromatography was consistent with that reported by E. Puniani, and the structure was confirmed by ^{1}H and ^{13}C NMR.[1] Reaction of **27** with triethyl phosphonoacetate and NaH in dry toluene at reflux gave the novel ketoester **28** in 85% yield (**Scheme 2.10**), whose ^{1}H NMR showed the disappearance of the aldehyde proton at 9.66 ppm, along with the appearance of two olefinic protons at 7.25 and 5.89 ppm sharing a trans coupling constant of 16.2 Hz. The ethyl group appeared as a quartet at

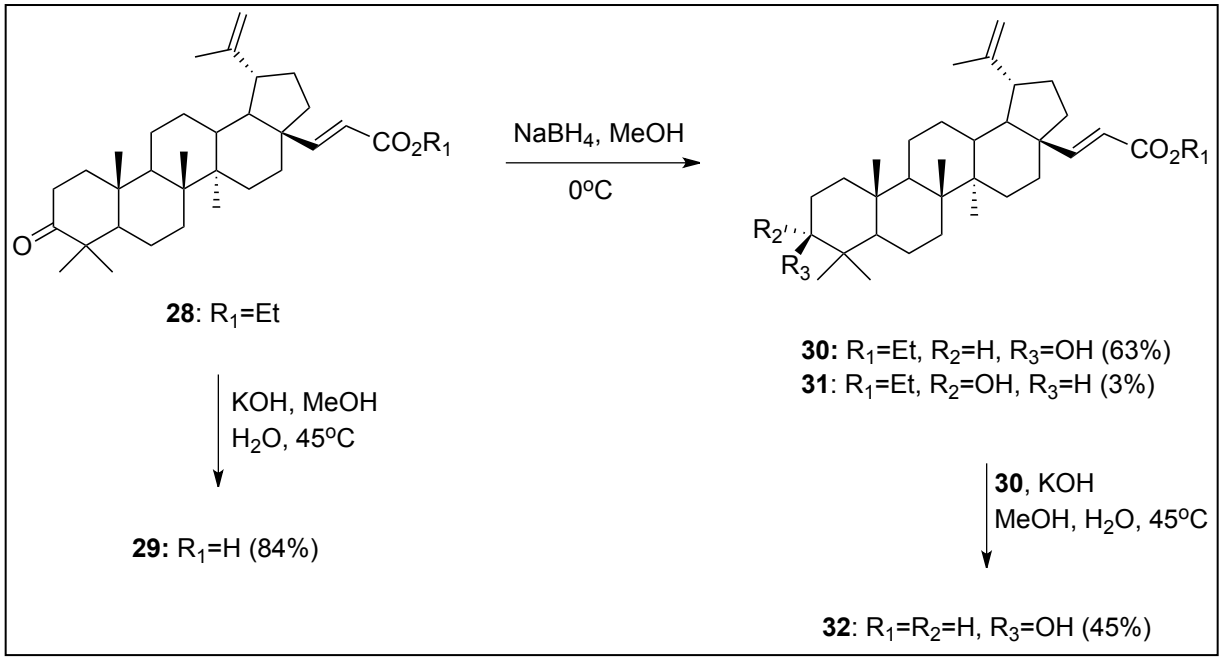
4.21 ppm (O**CH$_2$**CH$_3$, J=7.1 Hz) and a triplet at 1.21 ppm (OCH$_2$**CH$_3$**, J=7.2 Hz). ^{13}C analysis showed the C-3 ketone at 218.1 ppm, the ester at 167.1 ppm (**C**O$_2$Et), and two new olefinic carbons at 153.5 and 120.4 ppm. The total number of carbon signals in the ^{13}C NMR and the HRMS molecular ion peak were both consistent with the proposed structure.



Scheme 2.10. Preparation of C-28 extended side chain analog **28**.

Several other new compounds were prepared relatively quickly from ketoester **28**. Hydrolysis of the ester with KOH in MeOH/H$_2$O at 45 ^{o}C gave carboxylic acid **29** in 84% isolated yield (**Scheme 2.11**). The disappearance of the ethyl group was observed in the ^{1}H NMR, and the loss of two carbon signals from ^{13}C NMR (total of 32 carbons) was consistent with the structure. The carboxylate carbon also gave a shift from 167.1 ppm (**C**O$_2$Et) to 171.9 ppm (**C**O$_2$H), and the HRMS molecular ion was as expected.

Reduction of ketoester **28** with $NaBH_4$ in MeOH at 0 ^oC gave the novel alcohols 3β (**30**) and 3α (**31**), isolated in 63% and 3% yield, respectively (**Scheme 2.11**). The major 3β product was explained for the same reasons as above in the reductive amination, where the approach of the reducing hydride reagent was less hindered from the bottom face of the molecule. Analysis of the ^1H NMR spectrum of **30** showed the H-3 axial proton as a doublet of doublets at 3.18 ppm with coupling constants of 11.3 and 5.0 Hz to H-4 protons (axial-axial and axial-equatorial), thus confirming the 3β orientation of the alcohol. Its ^{13}C NMR showed the disappearance of the C-3 ketone peak at 218.1 ppm and the appearance of the C-3 alcohol peak at 79.0 ppm. In contrast, the ^1H NMR of **31** gave the H-3 equatorial proton as a triplet at 3.38 ppm with couplings of 2.4 Hz to H-4 protons (equatorial-equatorial, axial-equatorial), which was consistent with the 3α alcohol orientation. The disappearance of the C-3 ketone at 218.1 ppm and the appearance of the C-3 alcohol at 76.2 ppm in the ^{13}C NMR was in agreement with the expected structure change. Both new alcohols gave HRMS data that was consistent with their proposed structures.

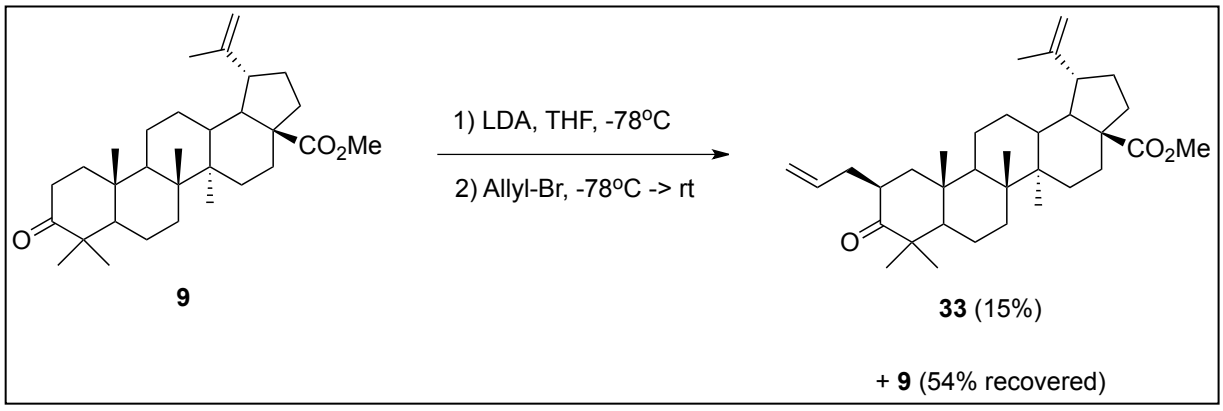


Scheme 2.11. Reduction and hydrolysis of C-28 extended side chain analogs.

One final compound in the series of C-28 extended carbon chain analogs was prepared, via hydrolysis of alcohol **30** with KOH in MeOH and H_2O at 45 ^oC. The novel carboxylic acid **32** was isolated in 45% yield (**Scheme 2.11**), which was somewhat low but was likely due to its observed low solubility during purification with silica gel chromatography. Disappearance of the ethyl group from ^1H NMR and two fewer carbons in ^{13}C NMR (total of 32) was consistent with the structure. An observed shift of the carboxylate carbon from 167.1 ppm (**C**O_2Et) to 171.6 ppm (**C**O_2H) was also noted.

2.4.4. Analogs With Substituents at C-2

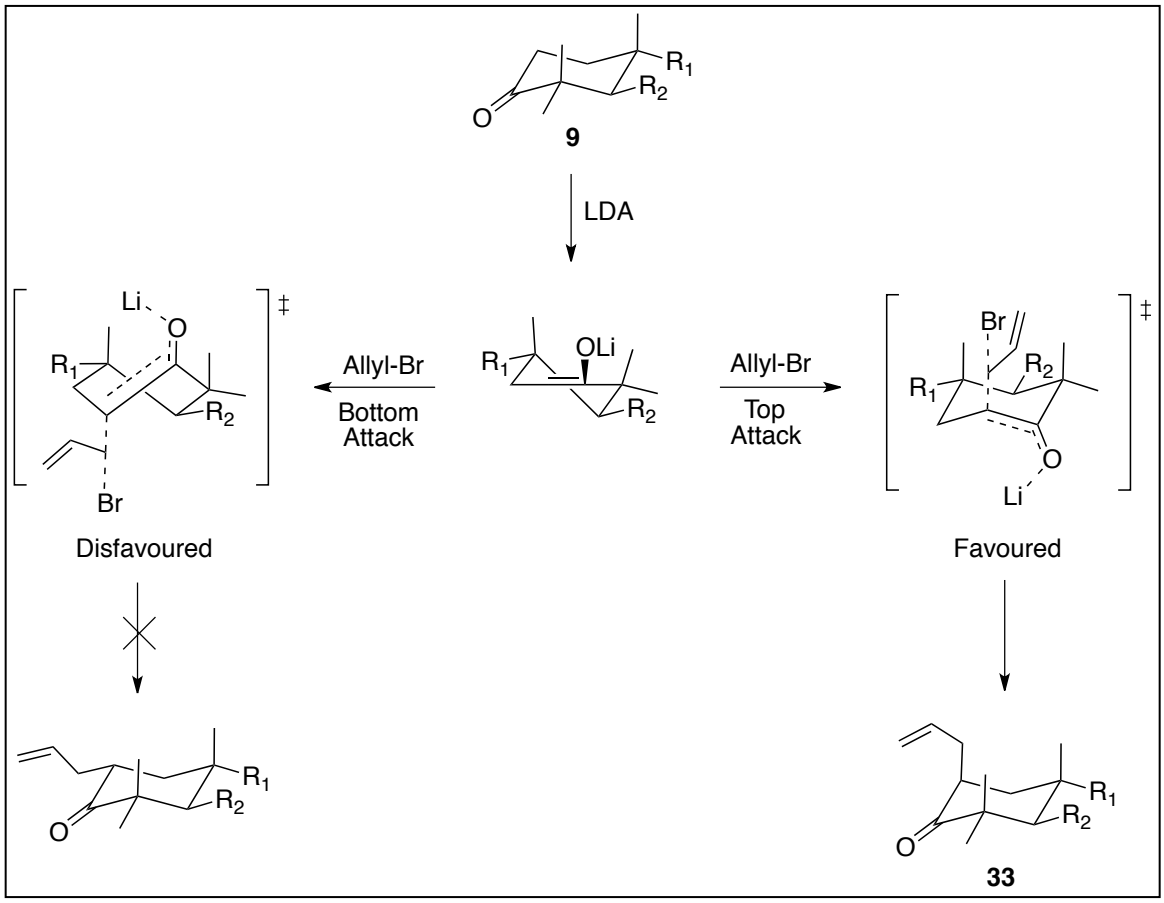
A series of betulinic acid analogs were prepared with C-2 substituents by using methyl betulonate (**9**) as starting material. The enolate of **9**, prepared from reaction with LDA in THF at -78 ^oC, was alkylated with allyl bromide at -78 ^oC to rt to give 2β-allyl methyl betulonate (**33)** in 15% yield with 54% recovered starting material (**Scheme 2.12**). At the time of preparation, no literature reports of **33** were found, but a subsequent 2011 publication reported its synthesis.[27] Comparison of the ^1H and ^{13}C NMR spectra of the product to the literature values gave a strong match, thereby confirming its structure.[27]



Scheme 2.12. Alkylation of methyl betulonate (**9**) with allyl bromide.

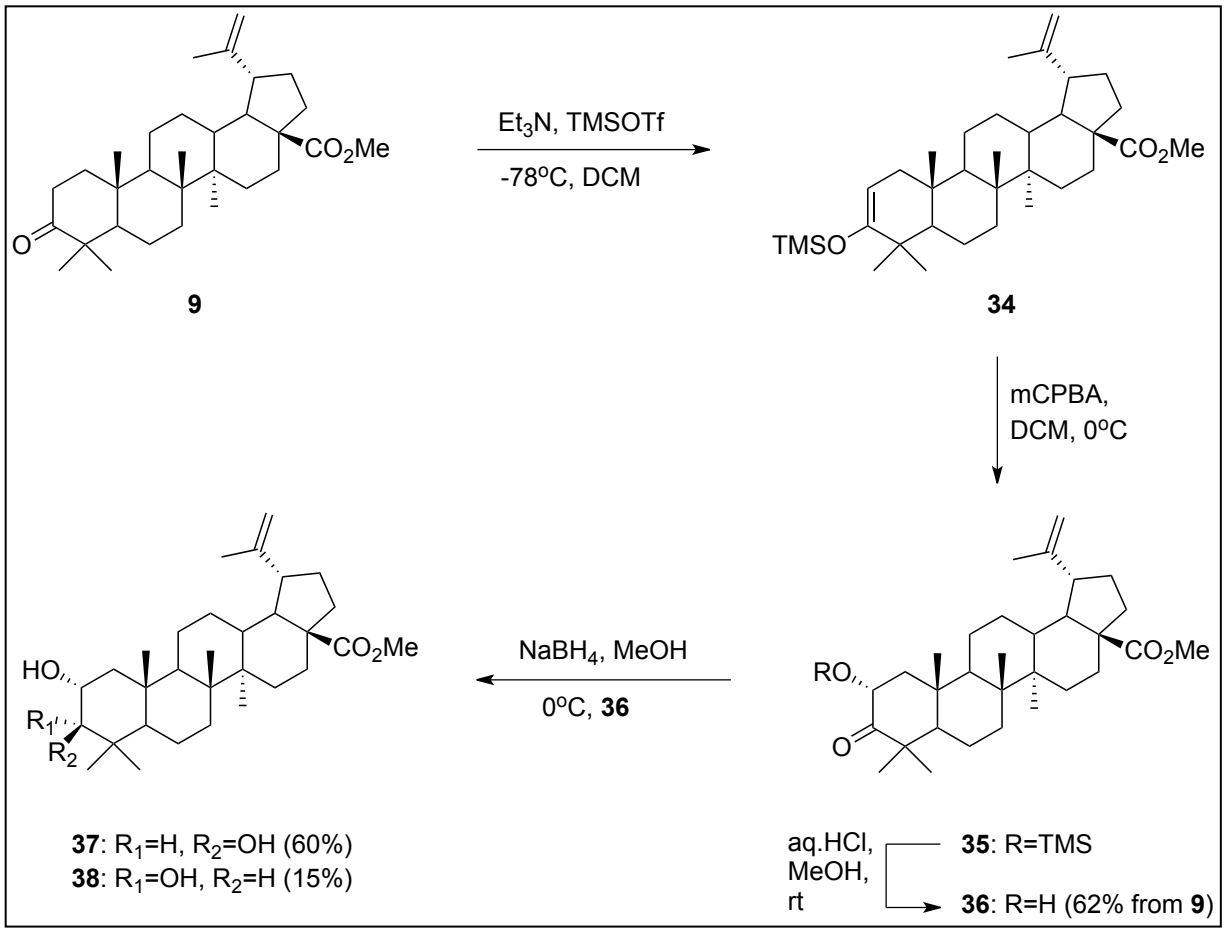
The exclusive formation of the 2β axial product can be explained by the transition states that would lead to the axial and equatorial products. Electrophilic attack of allyl bromide on the enolate from the bottom face would lead to a disfavoured twist boat

transition state of high energy. Alternatively, attack from the top face would lead to a low energy, chair-like transition state. A representation of this is given in **Scheme 2.13**.



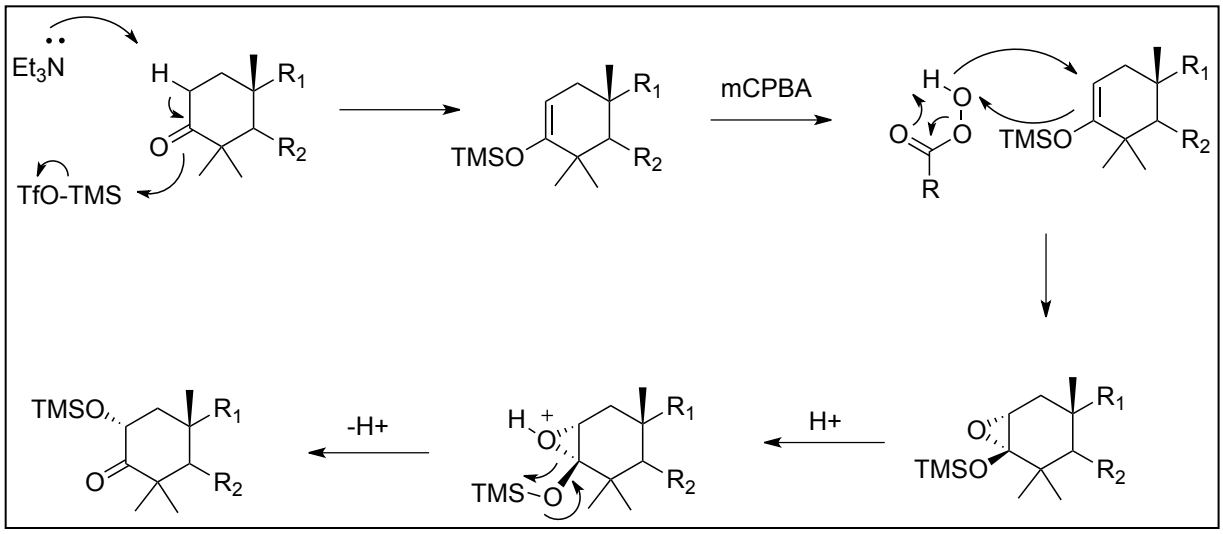
Scheme 2.13. Transition states for the alkylation of the enolate of **9**.

Analogs with 2α-hydroxy substituents were also prepared, following a procedure outlined by Deng and Snyder.[28] In this sequence, **9** was converted to intermediate silyl enol ether **34** with Et_3N and TMSOTf in dry DCM at -78 ^oC followed by oxidation with mCPBA in DCM at 0 ^oC to the intermediate silyl ether **35**. Hydrolysis with aqueous HCl in MeOH at rt provided the expected compound 2α-hydroxy methyl betulonate (**36**) in 62% yield from **9** (**Scheme 2.14**). The multiplicity of H-2 as a doublet of doublets at 4.52 ppm with couplings of 12.5 and 6.7 Hz to H-1 protons (axial-axial, axial-equatorial) confirmed the 2α orientation of the alcohol. The observed signal in the ^{13}C NMR of **36** at 69.3 ppm was assigned to the C-2 alcohol.



Scheme 2.14. Synthesis of 2α-hydroxy analogs of betulinic acid.

An explanation for the preferential 2α orientation of the alcohol was that approach of mCPBA occurred from the less hindered alpha side, due to steric hindrance on the beta side from the axially oriented methyl groups. A mechanism for this oxidation sequence is depicted in **Scheme 2.15.**

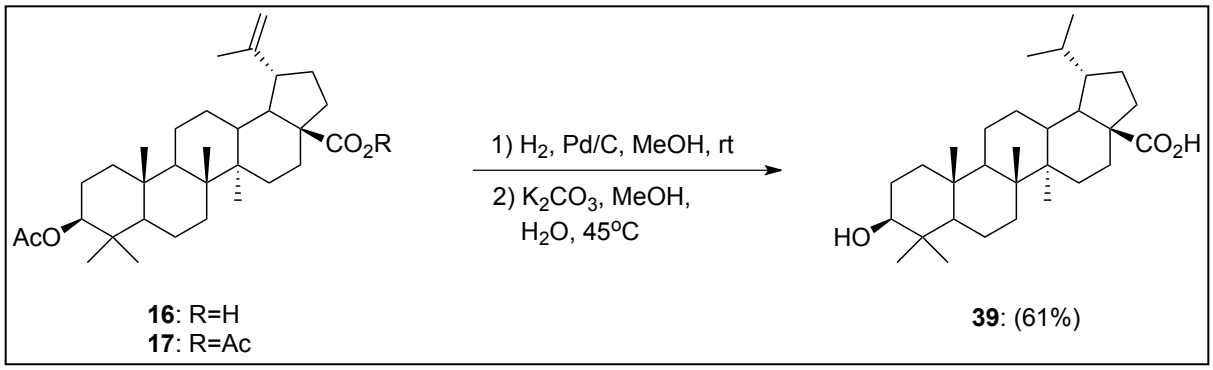


Scheme 2.15. Mechanism for the introduction of the 2α-silyloxy substituent.

The reduction of ketone **36** with $NaBH_4$ in MeOH at 0 ^oC gave a mixture of 3β (**37**) and 3α (**38**) isomers of 2α-hydroxy methyl betulinate in 60% and 15% respective yields (**Scheme 2.14**). The major 3β isomer was again explained by the greater ease of approach of the reducing hydride reagent from the less hindered alpha face of the molecule. Comparison of the ^1H NMR spectrum of **37**[29] and the ^1H and ^{13}C NMR spectra of **38**[30] to the literature confirmed their structures.

2.4.5. Modifications of the C20-C29 Vinylidene Group

Modifications of the C20-C29 vinylidene group provided several analogs of betulinic acid. Hydrogenation of a mixture of 3-acetoxy betulinic acid **16** and mixed anhydride **17** with H_2 and Pd/C in MeOH, followed by hydrolysis with K_2CO_3/H_2O/MeOH at 45 ^oC and crystallization from MeOH gave dihydrobetulinic acid (**39)** in 61% yield (**Scheme 2.16**). Both ^1H NMR and HRMS analysis were in agreement with the proposed product.[1]



Scheme 2.16. Synthesis of dihydrobetulinic acid (**39**).

Two closely related analogs, methyl dihydrobetulinate (**40**) and methyl 29-hydroxydihydrobetulinate (**41**), were also available in our lab (**Fig. 2.5**). They were both verified by ^{1}H NMR spectral analysis.[1]

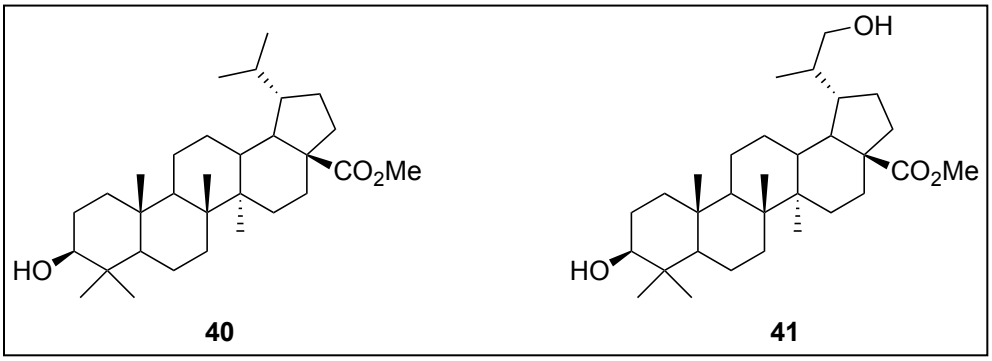
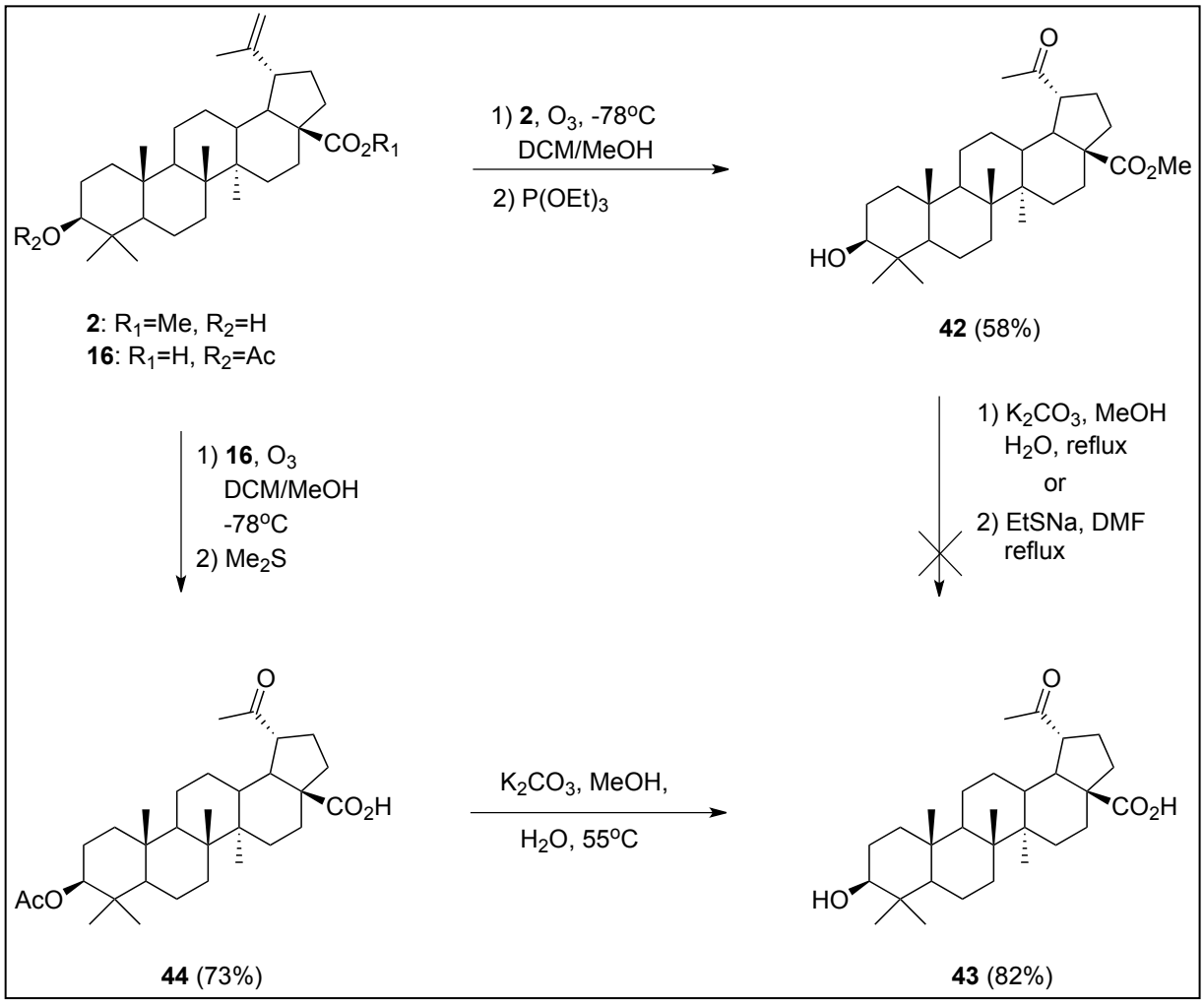


Fig. 2.5. Structures of methyl dihydrobetulinate **40** and methyl 29-hydroxydihydrobetulinate **41**.

It was thought that ozonolysis of the C20-C29 olefin could provide a set of readily prepared analogs. However, the low solubility of betulinic acid in many solvents, coupled with the low temperatures generally used in ozonolysis, would make it necessary to use a large amount of solvent. It was decided that methyl betulinate (**2**), with its greater solubility, could be ozonized to methyl platanate (**42**), followed by hydrolysis of the ester to provide platanic acid (**43**). Thus, ozonolysis of **2** in DCM/MeOH at -78 ^{o}C followed by workup with $P(OEt)_3$ gave methyl platanate (**42)** in 58% yield (**Scheme 2.17**), with ^{1}H and ^{13}C NMR values matching the literature.[1]

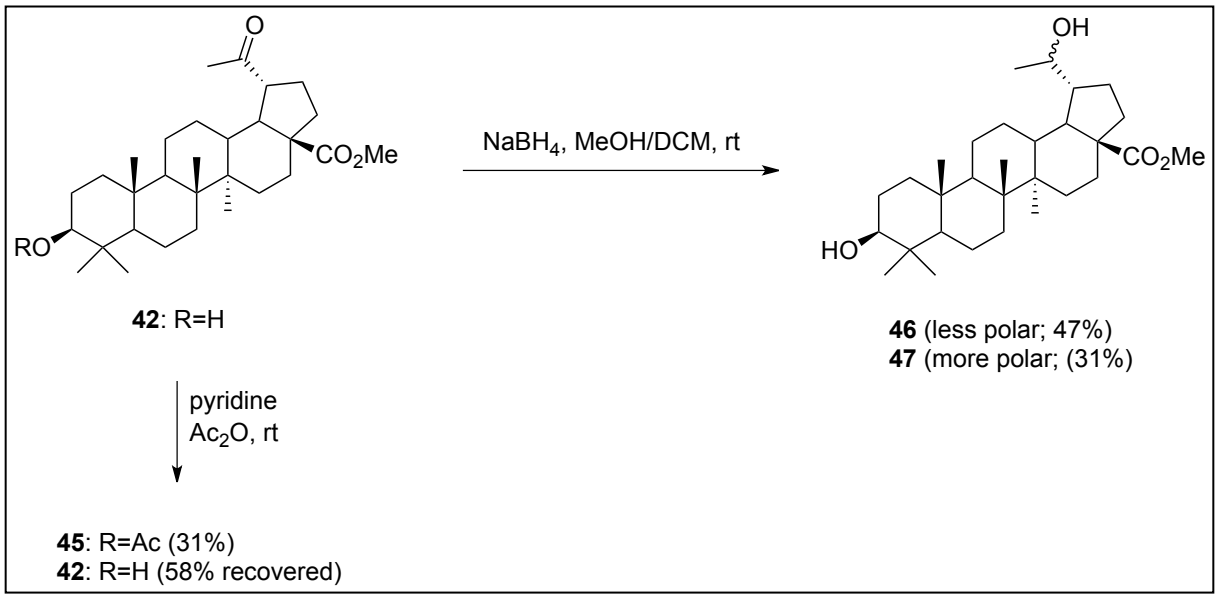
Subsequent hydrolysis of **42** to platanic acid (**43**) was first attempted using K_2CO_3/H_2O in MeOH, first at 40-50 ^oC for 16 hrs, then at reflux (75-80 ^oC) for 24 hrs, but no reaction was observed. It was thought that the steric hindrance of the C-28 ester (neopentyl) was responsible. Due to the potential for epimerization at C-19 via tautaumerization of the ketone, harsher conditions were not attempted. An alternative method to provide **43** was attempted by demethylation of the C-28 ester of **42** with EtSNa in DMF, but no reaction occurred at 100 ^oC after 2 hrs. Further heating to reflux for 2 hrs gave 4 spots on TLC. The major spot was starting material, with a tiny amount of what appeared to be **43** (by co-spotting with a reference sample). The remaining tiny spots were likely epimers of **42** and **43** due to their similar Rf values. This sequence of producing **43** was abandoned. Instead, it was decided to perform ozonolysis on 3-acetoxy betulinic acid (**16**) and hydrolyze the product obtained. This sequence would likely succeed, because the 3-acetoxy group had been hydrolyzed before in the preparation of dihydrobetulinic acid (**39)**, and **16** had good solubility. Thus, ozonolysis of **16** at -78 ^oC in DCM/MeOH, followed by workup with Me_2S gave the corresponding 3-acetoxy platanic acid (**44**) in 73% yield (**Scheme 2.17**). This product was successfully hydrolyzed with K_2CO_3/H_2O in MeOH at 55 ^oC to provide platanic acid **43** in 82% yield. Both compounds **43** and **44** were verified by comparison of their ^1H and ^{13}C NMR spectra to the literature.[1]



Scheme 2.17. Synthesis of platanic acid (**43**) and related esters.

Further modifications of methyl platanate **42** were carried out to provide several other analogs. Acetylation of **42** with Ac_2O in pyridine at rt gave 3-acetoxy methyl platanate (**45**) in 31% yield, with 58% recovered starting material (**Scheme 2.18**). The structure of **45** was confirmed by ^1H and ^{13}C NMR.[1] Reduction of **42** with $NaBH_4$ in MeOH/DCM at rt gave two isomers of the corresponding C-20 alcohol (**Scheme 2.18**). The less polar isomer (**46**) was isolated in 47% yield and found to be identical by ^1H and ^{13}C NMR to the isomer prepared by E. Puniani.[1] The more polar isomer (**47**) was isolated in 31% yield, prepared but not isolated or characterized by E. Puniani. Its ^1H NMR gave H-20 as a quartet of doublets at 4.03 ppm (J=6.3, 4.1 Hz), and the C-30 methyl group appeared as a doublet at 1.07 ppm (J=6.3 Hz, 3H). The ^{13}C NMR showed disappearance

of the C-20 ketone at 212.4 ppm and the newly formed C-20 alcohol was assigned at 69.7 ppm. HRMS analysis did not show an [M]+ peak, although an [M-18]+ peak was observed for the dehydration ion ([M-H_2O]+). The absolute stereochemistry of **46** and **47** was not determined, as it would only become important if either isomer emerged as a strong candidate after biological tests.

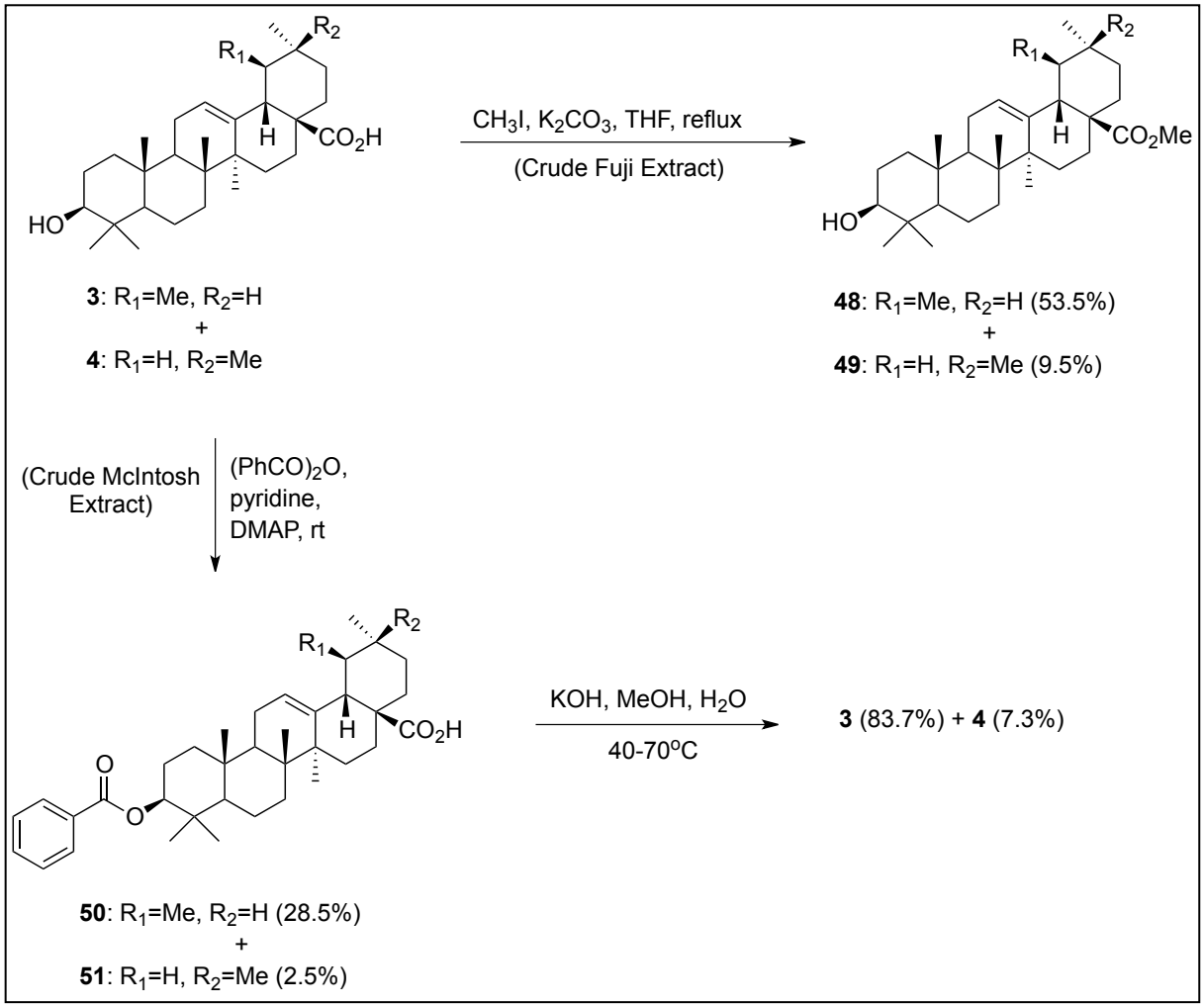


Scheme 2.18. Acetylation and reduction of methyl platanate (**42**).

2.5. Ursolic Acid Analogs

As discussed previously, crude ursolic acid (**3**) was obtained from the extracts of Fuji and McIntosh apple peels. The purity of these samples was inadequate for the bioassays, however; thus, it became important to find a method of obtaining pure material. The low solubility of **3** in many solvents made it difficult to handle, and it was thought that conversion of **3** to its methyl ester, methyl ursolate (**48**), would allow easier handling and purification. A simple hydrolysis of purified **48** could then provide pure **3**. Thus, the crude Fuji extract of **3** was converted to **48** with K_2CO_3 and CH_3I in refluxing THF (**Scheme 2.19**). A total yield of 63% was obtained, and the structure was verified by ^1H and ^{13}C NMR[1]; however, the product contained 15% of methyl oleanolate (**49),** implying that the crude Fuji extract had contained oleanolic acid (**4**). Distinguishing ^1H

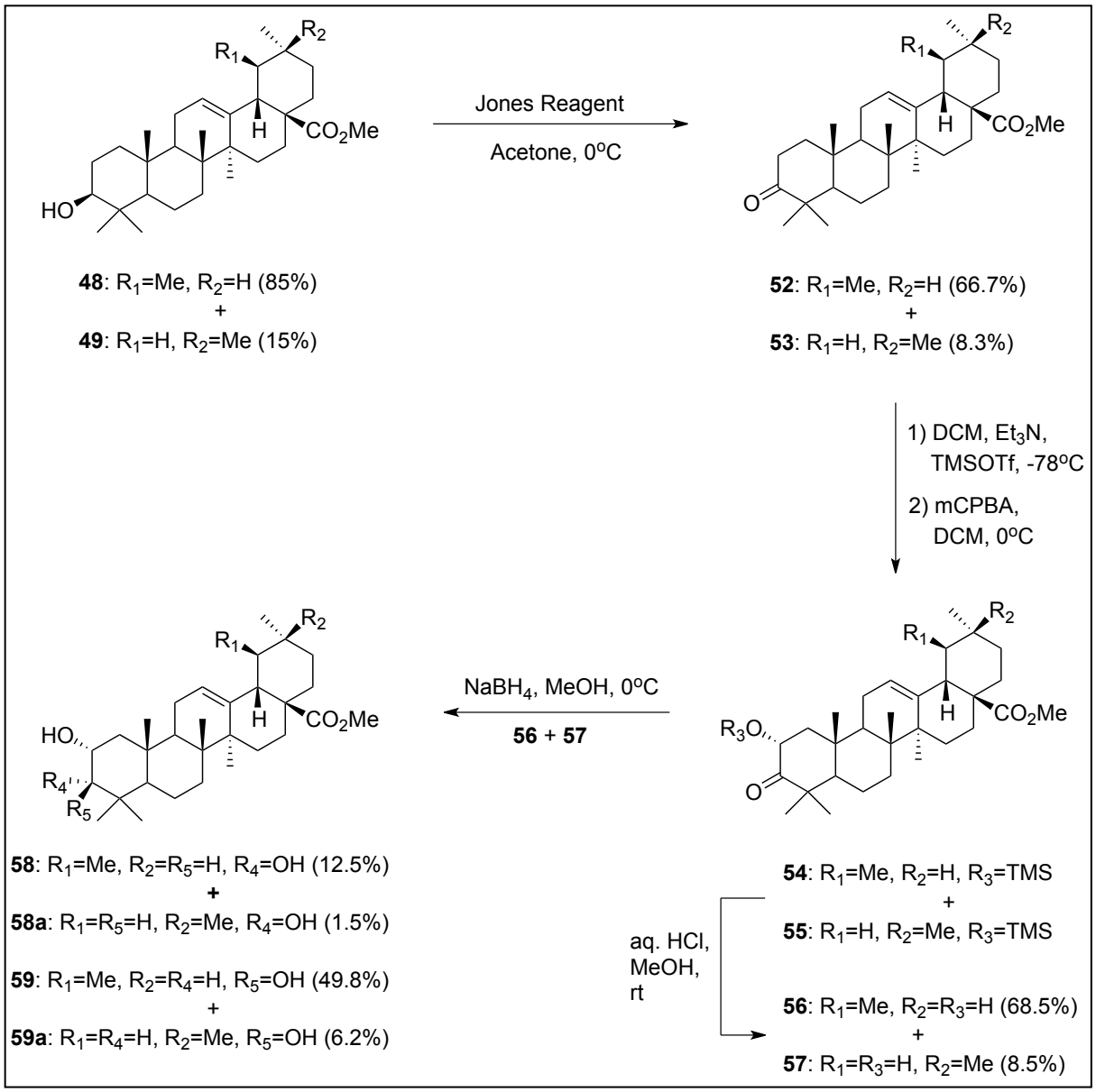
NMR signals between methyl oleanolate (**49**)[31] and ursolate (**48**)[1] included the methyl ester singlets at 3.62 ppm (**49**) and 3.60 ppm (**48**) and the H-12 olefinic proton triplets (J=3.5 Hz) at 5.28 ppm (**49**) and 5.24 ppm (**48**). Although methyl ursolate (**48**) was easier to handle, separating it from **49** was not easy. The structures of **48** and **49** are very similar, differing by the position of only one methyl group at C-19/C-20, so it would be expected that their separation be difficult. Silica gel chromatography of this mixture failed to give separation with various solvent systems (EtOAc/hexanes, acetone/hexanes, Et_2O/hexanes, DCM), and crystallization from several solvents (MeOH, EtOH, DCM/hexanes) did not improve the purity.



Scheme 2.19. Synthesis of methyl ursolate (**48**) and purification of ursolic acid (**3**).

As an alternative, it was thought that HPLC might give the desired purification. The equipment in our lab required a strongly absorbing functional group at 254 nm for HPLC, so the crude McIntosh extract of **3** was converted to 3-benzoyloxy ursolic acid (**50**) using benzoic anhydride, pyridine and DMAP at rt in 31% yield (**Scheme 2.19**). Removal of the side product benzoic acid was incomplete after chromatography, but it was fully removed by sublimation under vacuum at high temperature. ^{1}H NMR of **50** gave multiplets at 8.07-8.03 ppm, 7.57-7.53 ppm, and 7.46-7.42 ppm for 5 phenyl protons. ^{13}C NMR gave signals at 137.9, 132.7, 131.0, 129.5 (double intensity), 128.3 (double intensity), and 125.7 ppm to account for the eight olefinic/aromatic carbons in the structure. Signals at 183.7 and 166.3 ppm were found for the carboxylic acid (**C**O_2H) and ester (**C**O_2Ph) carbons, and all 37 carbons were accounted for in the spectra. The HRMS did not give an [M]+ peak, but a signal for [M-HCO_2H]+ was observed. It was found by ^{1}H NMR that the product had an oleanolic analog content of 8% (**51**), determined by integration of the H-12 olefin peaks at 5.30 ppm (**51**) and 5.25 ppm (**50**). This was deemed pure enough for the bioassays and thus HPLC was not required to remove the oleanolic analog. Hydrolysis of the mixture of **50** and **51** with KOH in MeOH/water at 40-70 ^{o}C followed by removal of benzoic acid via sublimation provided ursolic acid (**3**) in 91% total yield, containing 8% oleanolic acid (**4**). ^{1}H and ^{13}C NMR were used to verify the structure of **3**.[10]

After obtaining a sample of purified ursolic acid, it was decided to prepare several 2α-hydroxy analogs by following the sequence outlined previously in the synthesis of 2α-hydroxy methyl betulinate (**37**). Thus, methyl ursolate (**48**) (containing 15% of methyl oleanolate **49**) was oxidized with the Jones reagent at 0 ^{o}C in acetone to give the corresponding ketones of ursolate (**52**) and oleanolate (**53**) in 75% total yield (**Scheme 2.20**). The structure of **52** was verified by ^{1}H NMR,[32] and there was 11% oleanolate analogue (**53**) as determined by integration of its methyl ester singlet at 3.63 ppm and H-12 olefinic proton triplet at 5.30 ppm (J=3.6 Hz).



Scheme 2.20. Synthesis of 2α-hydroxy analogs of ursolic acid (**3**).

The ketone mixture was reacted with Et_3N and TMSOTf in dry DCM at -78 ^oC to give the corresponding silyl enol ethers, which were oxidized with mCPBA in DCM at 0 ^oC to give intermediate silyl ethers **54** and **55**. Hydrolysis of the silyl ethers with aqueous acid in MeOH at rt gave 77% total yield of 2α-hydroxy compounds **56** (ursolate) and **57** (oleanolate) as a mixture (**Scheme 2.20**). Compound **56** was found to be a new compound. The orientation of the 2α alcohol was determined by ^1H NMR, which gave H-2 axial as a doublet of doublets at 4.54 ppm with couplings of 12.6 and 6.6 Hz to H-1

protons (axial-axial, axial-equatorial). A new signal at 69.2 ppm was observed for the C-2 alcohol in the ^{13}C NMR, and the total of 31 carbon peaks was also consistent. The [M]+ peak from HRMS was also consistent with the proposed structure. It was determined that 11% of the product was oleanolate analog **57**, by integration of the ^{1}H NMR methyl ester singlet at 3.63 ppm and H-12 olefinic proton triplet at 5.29 ppm (J=3.5 Hz).

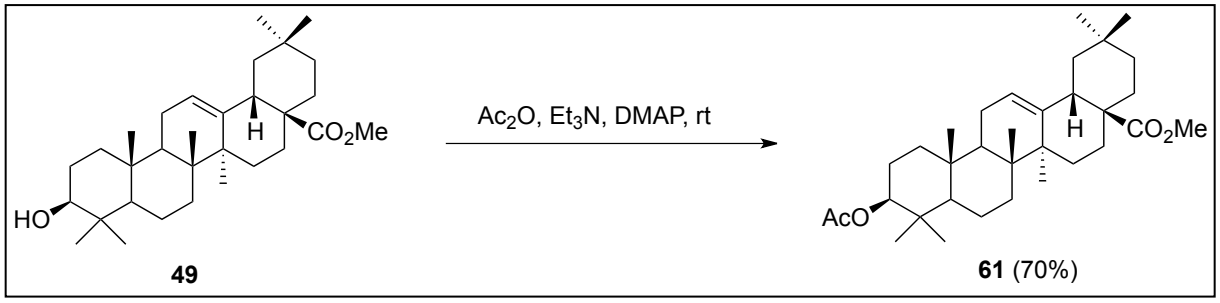
The mixture of ketones **56** and **57** were reduced with $NaBH_4$ in MeOH at 0 ^{o}C to give a mixture of 3α (**58)** and 3β (**59**) isomers of 2α-hydroxy methyl ursolate, in 14% and 56% respective total yields (**Scheme 2.20**). Compounds **58**[33] and **59**[1,33] were verified by analyzing their ^{1}H and ^{13}C NMR spectra. Both compounds contained 11% of their corresponding oleanolate analogs **58a** (3α) and **59a** (3β) as calculated by ^{1}H NMR by integration of the methyl ester singlets at 3.62 ppm and H-12 olefinic proton triplets at 5.28 ppm (J=3.5 Hz).

One final ursolic acid analog was obtained via acetylation of methyl ursolate **48** with Ac_2O, Et_3N and DMAP at rt (**Scheme 2.21**). The product 3-acetoxy methyl ursolate (**60**) was obtained in 63% total yield, containing 10% of 3-acetoxy methyl oleanolate (**61**) by integration of the methyl ester singlet at 3.62 ppm and H-12 olefinic proton triplet at 5.28 ppm (J=3.6 Hz). The ^{1}H and ^{13}C NMR spectra of **60** was used to verify its structure.[34,35]

Scheme 2.21. Acetylation of methyl ursolate (**48**).

2.6. Oleanolic Acid Analogs

A series of simple oleanolic acid analogs were also required for inclusion in the SAR study. As mentioned previously, oleanolic acid (**4**) was already available in the lab; methyl oleanolate (**49**) was also available, but it required purification by silica gel chromatography. Acetylation of **49** with Ac_2O, Et_3N, and DMAP at rt provided 3-acetoxy methyl oleanolate (**61**) in 70% yield (**Scheme 2.22**). The structures of **4**[36], **49**[31] and **61**[35] were all verified by their ^1H and/or ^{13}C NMR spectra.



49 **61** (70%)

Scheme 2.22. Acetylation of methyl oleanolate (**49**).

2.7. Dehydrocanophyllic Acid Analogs

As noted earlier, dehydrocanophyllic acid (**6**) was available in the lab for the SAR study. Several other analogs were also available, including the methyl ester of dehydrocanophyllic acid (**62**), canophyllol (**63**) and canophyllol acetate (**64**). The structures of **62-64** are given in **Fig. 2.6**.

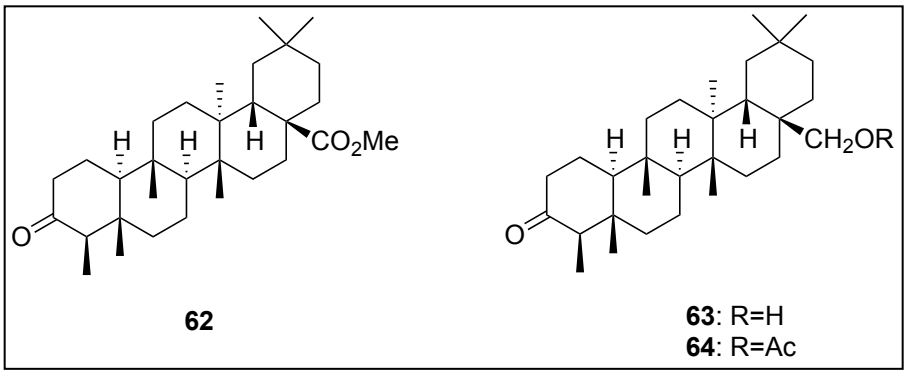


Fig.2.6. Structures of dehydrocanophyllic acid analogs **62**-**64**.

2.8. Screening for Cortisol Lowering Activity *in vitro*

Several of the above analogs were initially screened for their cortisol lowering ability using an *in vitro* cell tissue assay. This was to identify the most active compounds relatively quickly and inexpensively, as an *in vivo* assay of each would be costly and time consuming. After the most active compound was identified, an *in vivo* assay would confirm the results. The cell tissue assay was carried out using rainbow trout (*Oncorhynchus mykiss*), as described in the Ph.D. thesis of M. Mullally.[37] In rainbow trout, the interrenal tissue of the head kidney is responsible for the ACTH stimulated synthesis and release of cortisol (as opposed to the adrenal cortex in mammals). A preparation of rainbow trout head kidney cells was incubated with an appropriate amount of triterpene acid analog, and cortisol release was stimulated by addition of ACTH. The level of cortisol was measured using a standard radioimmunoassay and compared against a control sample. Cytotoxicity of the assay was also assessed using a lactate dehydrogenase assay.[38]

The writer would like to thank Dr. Martha Mullally for carrying out the fish cell assays. As noted earlier, due to unforeseen difficulties, only certain analogs were screened. However, all of the analogs are still available for future study. Results of the assay are given in **Fig.2.7**.

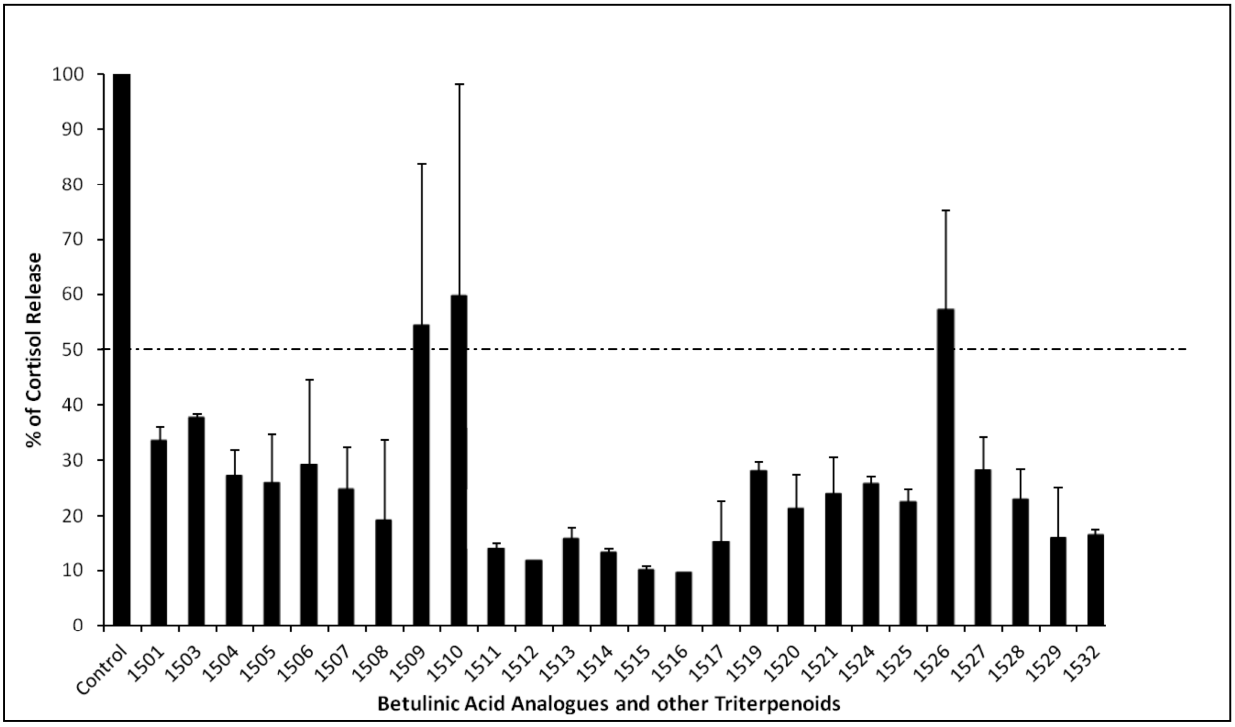


Fig. 2.7. Mean cortisol release, as compared to control, for head kidney cells incubated with triterpene acid analogs (60 µg/mL) following an ACTH challenge (1 U/mL). The dashed line represents a 50% reduction in cortisol response, as compared to the control. Compound **1516** (platanic acid) was identified as the most potent.

It should be noted that the numbers used in the above graph represent the numbering system used during this project. Corresponding numbers used in this thesis are available in Appendix A. However, for the purposes of discussion, several compounds are identified below.

The most effective cortisol-lowering analog was identified as platanic acid (**1516 / 43**). Other highly effective analogs were methyl 29-hydroxydihydrobetulinate (**1515 / 41**), ethyl acetoxy betulinate (**1512 / 15**) and methyl dihydrobetulinate (**1514 / 40**). The least effective compounds were allyl betulinate (**1510 / 13**), 3β-(N-benzyl)acetamido methyl betulinate (**1526 / 26**) and heptyl betulinate (**1509 / 12**). The only non-betulinic acid analog tested, oleanolic acid (**1532 / 4**), was found to have good activity as well. It should be noted that three of the top four analogs had modifications at the C20-C29 vinylidene group.

One note of caution concerning the above assay was that the principal compound betulinic acid was not included, so it was not possible to compare its relative activity to

the other analogs. This assay was carried out in July 2011, and a number of subsequent attempts to repeat it were unsuccessful. Thus, none of the initial results could be verified or repeated at lower concentration in order to obtain a greater differentiation of the activity of the various analogs. As mentioned earlier, this influenced potential progress towards identifying the most active analogs and there was no choice but to prepare a variety of analogs for eventual evaluation. Since platanic acid (**43**) was the most effective at lowering cortisol in the above assay, it was selected for further study *in vivo*.

2.9. Evaluation of Cortisol Lowering Activity *in vivo*

Live animal assays were conducted using only certain compounds due to the time and cost requirements. The animals used in these assays were male Sprague Dawley rats. Treatments included betulinic acid (2 mg/kg), platanic acid (2 mg/kg), a blend of dried raw Sycamore tree bark (*Platanus occidentalis*) and *Souroubea sympetela* leaves (55:45 respectively, 200 mg/kg), and a control vehicle (sweetened condensed milk). In addition to their regular diet, rats were fed the desired treatment, mixed with sweetened condensed milk, for three consecutive days prior to testing (2 days and 60 min). They were then subjected to a mild stressor by being placed inside a plastic cone restraint, and blood samples were collected at various time points thereafter. The blood samples were analyzed for levels of corticosterone with a standard radioimmunoassay. Results are depicted in **Fig 2.8**. The writer would like to thank Christian Cayer for conducting these assays.

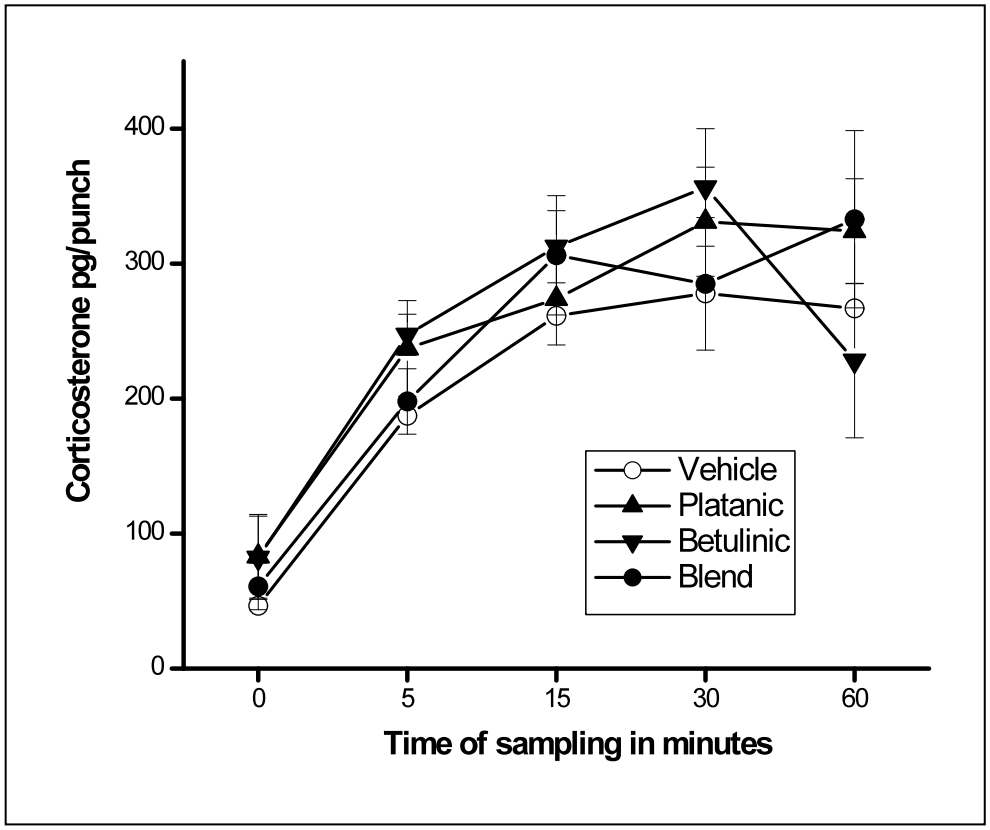


Fig. 2.8. Lineplot of corticosterone values (mean, SE) obtained from blood samples collected from all rats at baseline and 5, 10, 15, 30 and 60 minutes after acute mild restraint (*n* =31). The vehicle is compared with groups orally betulinic or platanic acid or a blend. A rise in corticosterone was observed due to the restraint. There were no significant differences between any of the groups at any time point.

There was a significant increase in corticosterone due to the restraint, but no significant differences in corticosterone levels was observed for any treatment compared to the control vehicle. Betulinic acid did show a trend to decline after 60 min, however. The critical issue may be the bioavailability of the treatment, as a previous study in the thesis of E. Puniani showed a significant decrease in corticosterone when methyl betulinate was used.[1] Methyl betulinate (**2**) is more bioavailable than betulinic acid (**1**), as it is more lipophilic and should be better able to cross cell membranes. Future *in vivo* assays should be carried out using methyl platanate (**42**) and methyl betulinate (**2**) to determine their relative cortisol lowering effects.

2.10. Conclusions and Future Directions

A variety of betulinic (**1**), ursolic (**3**), oleanolic (**4**) and dehydrocanophyllic (**6**) acid analogs were prepared for evaluation of their cortisol lowering ability. Not all of the analogs were tested *in vitro*, but several of them emerged as strong candidates. The most promising analog, platanic acid (**43**), was tested *in vivo* along with betulinic acid and a blend of Sycamore bark and *Souroubea Sympetela* leaves. No significant lowering of cortisol was observed, but this was likely due to the poor bioavailability of the acids.

There are several areas of this project that could be enhanced by future work. Modifications of the C20-C29 vinylidene group of betulinic acid provided the most potent cortisol lowering analogs *in vitro*, so the preparation of analogs with new modifications at this site may be worthwhile. For this project to proceed, the *in vitro* fish cell bioassay must be re-established as a working procedure, or an alternative bioassay must be developed. When a bioassay becomes established, any analogs not yet assessed *in vitro* should be, and the analogs should also be assessed at lower concentrations to better differentiate their cortisol lowering ability. This will provide guidance for the development of additional analogs. Once the most potent analogs are identified, *in vivo* studies should be carried out to confirm their cortisol lowering ability, and then toxicity and tolerance data should be collected.

The work described in part one of this thesis is currently under consideration for patent by Bioniche Lifesciences Inc. for a cortisol lowering drug. In addition, it may be worthwhile to evaluate the prepared analogs for their anxiolytic properties, since betulinic acid has been demonstrated to reduce anxiety in animals.[1] It is possible that an anxiolytic drug could emerge as a result of future studies.

2.11. References

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-Chapter 3: Experimental-

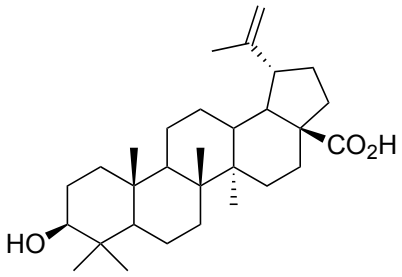
3.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 MHz or 400 MHz NMR Spectrometer, and were calibrated using the residual solvent peak unless otherwise indicated. NMR solvents were purchased from Sigma-Aldrich Inc. or Cambridge Isotope Laboratories Inc. HRMS spectra were recorded on a Kratos Concept-IIA mass spectrometer.

Reagents were obtained from Sigma-Aldrich and were used as is unless otherwise indicated. THF was distilled from sodium benzophenone ketyl prior to use. Dry DCM was obtained via distillation from CaH_2. Dry *i*-Pr_2NH was obtained by distillation from NaOH. Dry Et_3N was obtained from distillation from CaH_2. Molecular sieves were activated by heating to ~150 ^{o}C under vacuum for ~10 minutes. Dry toluene was obtained by drying overnight with activated 3 Å molecular sieves. Thin layer chromatography (TLC) was carried out using TLC Silica Gel 60 F_{254} sheets with aluminum backing from EMD Chemicals Inc., visualized by either UV irradiation or by heating after dipping in a solution of Hanessian's stain. Silica gel for chromatography was purchased from SiliCycle Inc, 40-63 μm.

3.2. Procedures and Spectral Data

Betulinic Acid (1)

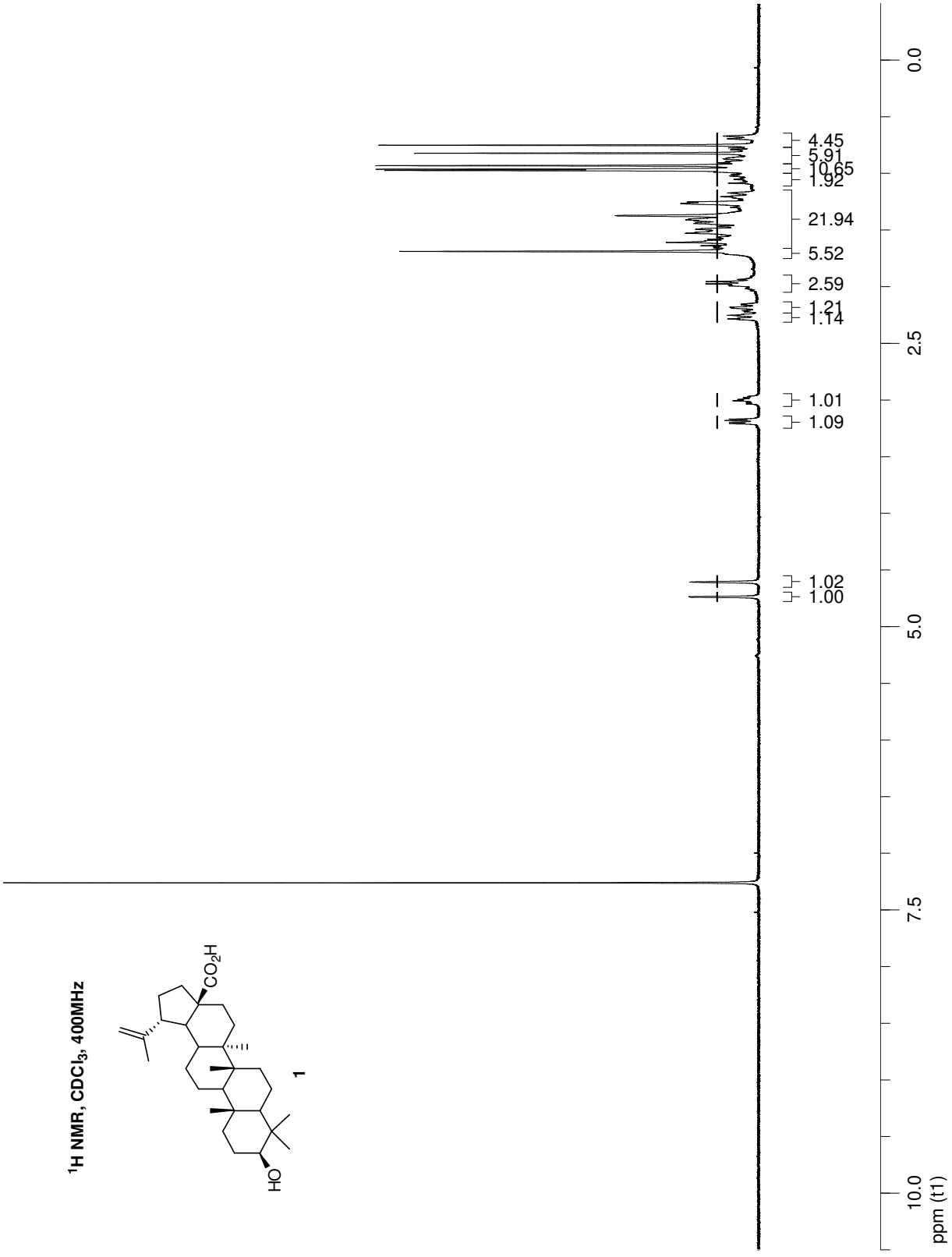


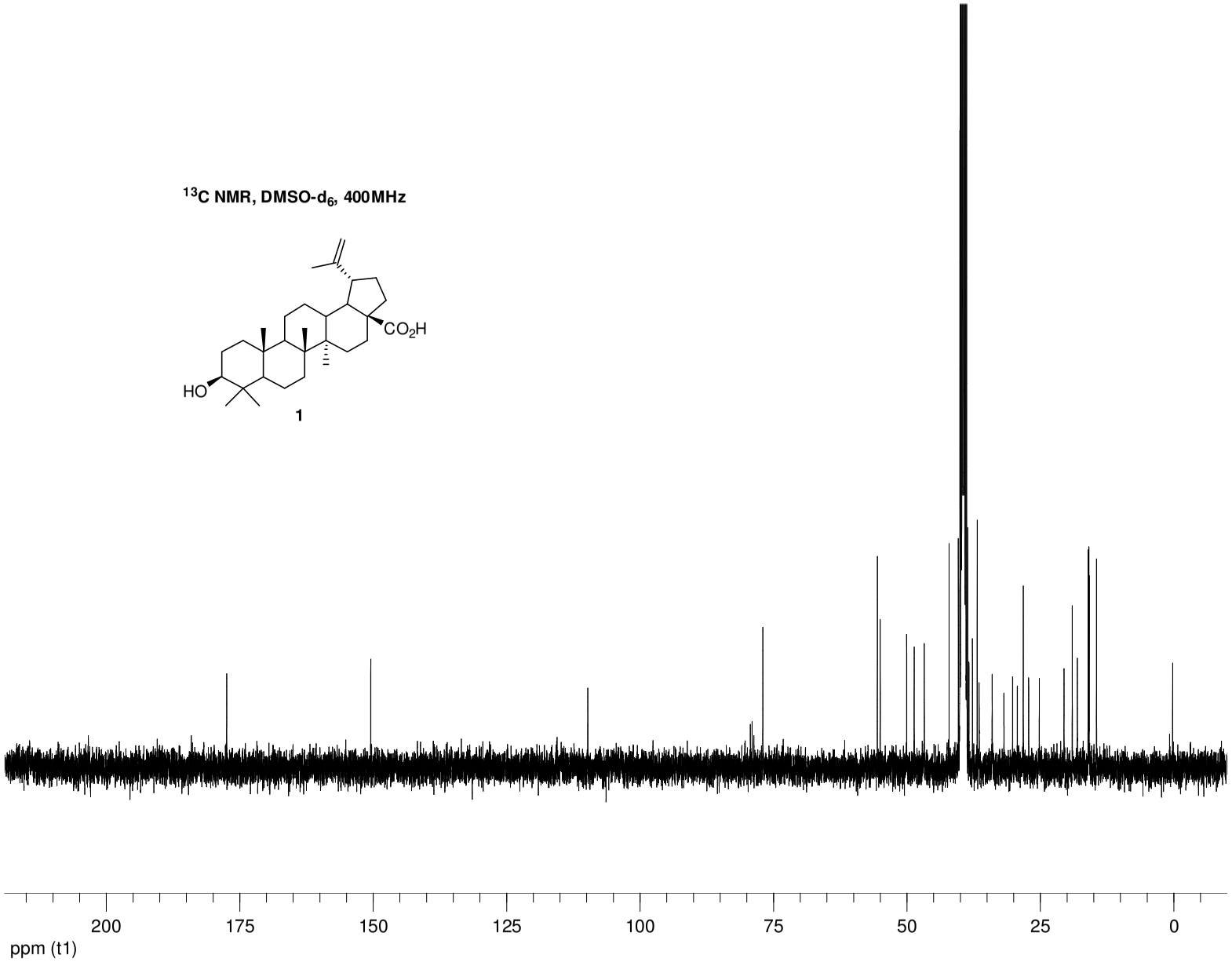
1

Ground bark from the American sycamore tree (9.11 g) was extracted by soaking overnight in EtOH (50 mL), filtering and evaporating solvent. This process was repeated once and the evaporated products were combined to give a yellow solid (410 mg). Purification of the residue by silica gel chromatography using a hexanes/EtOAc gradient gave pure **1** as a light yellow powder (148 mg, 1.6%). This process was repeated on a larger scale to provide gram quantities of **1** as required.

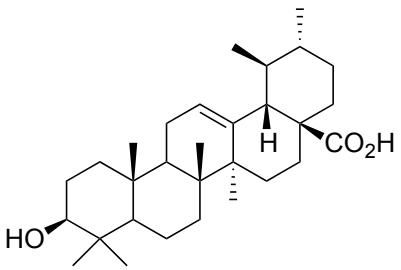
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.74 (s, 1H), 4.61 (s, 1H), 3.19 (dd, J=11.0, 5.0 Hz, 1H), 3.03-2.97 (m, 1H), 2.29-2.24 (m, 1H), 2.22-2.15 (m, 1H), 2.03-1.93 (m, 2H), 1.69 (s, 3H), 0.97 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H), 0.82 (s, 3H), 0.75 (s, 3H)

^{13}C NMR (DMSO-d_6, 400 MHz): δ (ppm) 177.4, 150.4, 109.8, 77.0, 55.5, 50.0, 48.6, 46.7, 42.1, 40.4, 38.6, 38.4, 37.7, 36.8, 36.5, 34.0, 31.8, 30.2, 29.3, 28.2, 27.2, 25.2, 20.6, 19.0, 18.1, 16.0, 15.9, 15.8, 14.5


^{1}H NMR, $CDCl_3$, 400MHz
CO_2H
HO
1
4.45
5.91
10.65
1.92
21.94
5.52
2.59
1.21
1.14
1.01
1.09
1.02
1.00
0.0
2.5
5.0
7.5
10.0
ppm (t1)


^{13}C NMR, DMSO-d$_6$, 400MHz
HO
CO$_2$H
1
200
175
150
125
100
75
50
25
0
ppm (t1)

Extraction of Ursolic Acid (3) from Apple Peels



3

Method A – Fuji Apples

Dried, ground Fuji apple peels (140.95 g) were soaked in EtOAc (400 mL) overnight and the liquid was filtered off. This process was repeated twice with the solid residue. The combined liquid extracts were evaporated to dryness, giving a green powder (3.88 g). The powder was separated by silica gel chromatography using a hexanes/EtOAc gradient to give crude, impure **3** as a light yellow powder (1.42 g; 1.0%). The crude material had the same Rf value as a reference sample on TLC (Rf=0.21, 3:1 hexanes:EtOAc).

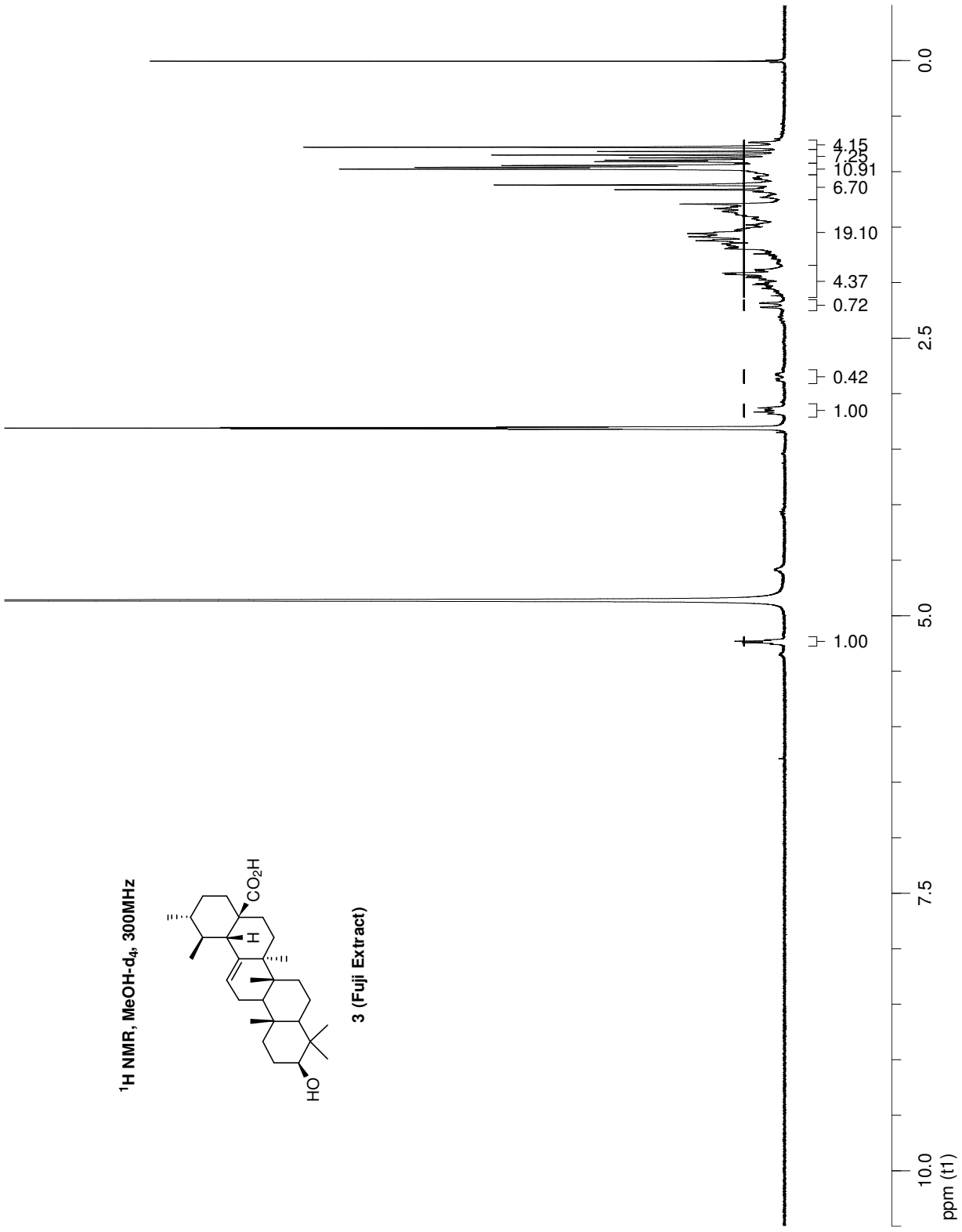
^{1}H NMR (MeOH-d_4, 300 MHz): δ (ppm) 5.26-5.22 (m, 1H), 3.18-3.12 (m, 1H), 2.20 (d, J=11.0 Hz, 1H), 1.12 (s, 3H), 0.97 (s, 3H), 0.96 (s, 3H), 0.95 (s, 3H), 0.78 (s, 3H).

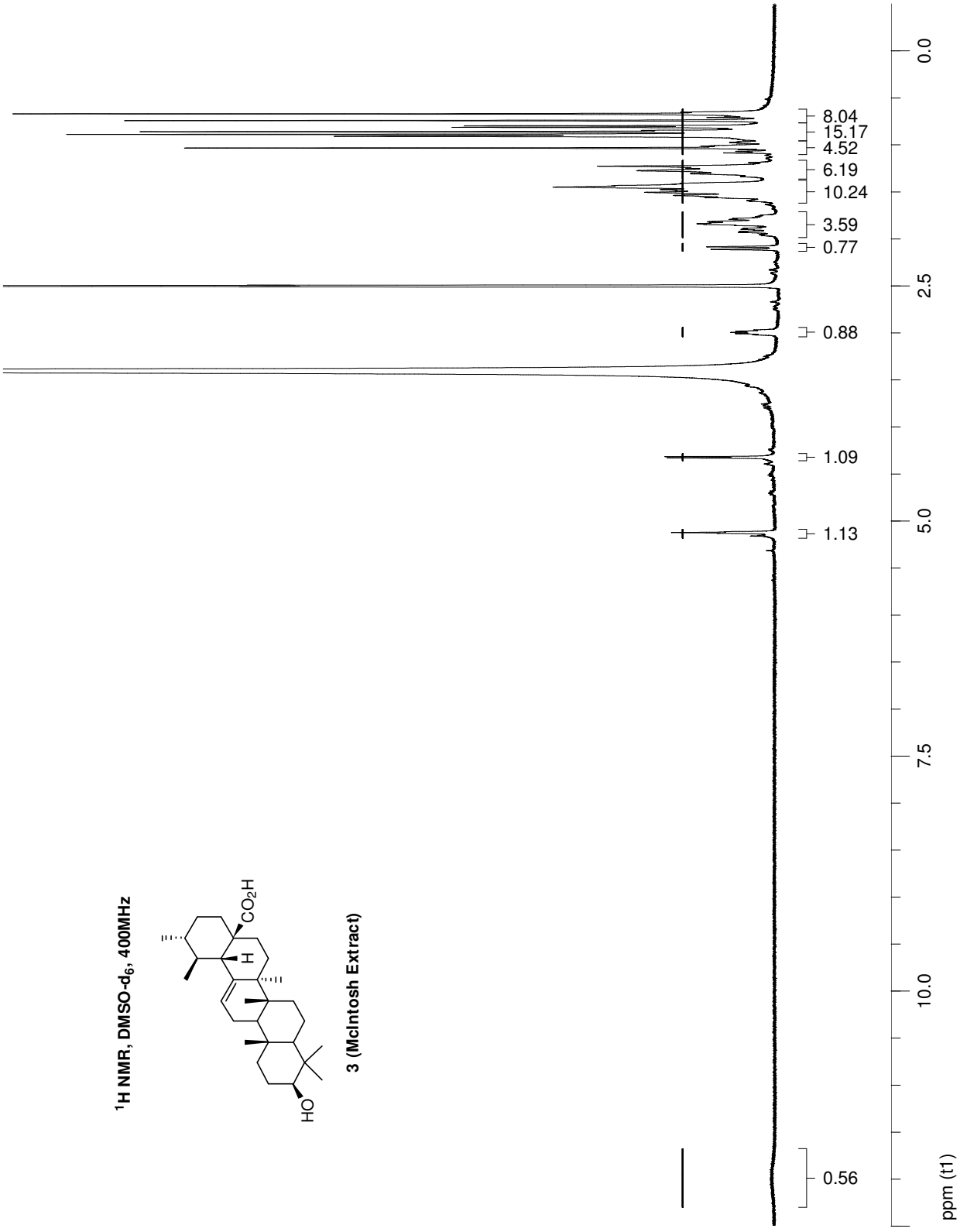
Method B – McIntosh Apples

Dried, ground McIntosh apple peels (147.75 g) were soaked in EtOAc (400 mL) overnight, and the liquid was filtered off. This process was repeated twice with the solid residue. The combined liquid extracts were evaporated to dryness, giving a green powder (4.11 g). The powder was covered with hexanes (80 mL) and stirred vigourously at reflux for 1 hr. The mixture was cooled to rt and filtered. The filtrate was evaporated to dryness, giving a green liquid containing only impurities by TLC. The solid residue was a greenish yellow solid (3.16 g) containing **3** with some polar impurities. The solid was dissolved in hot EtOH, charcoal was added and stirred, and the mixture was filtered hot. Crystallization of the solution upon cooling gave a white powder (597 mg). The mother liquor was re-crystallized to give more white powder (579 mg). The white powders were

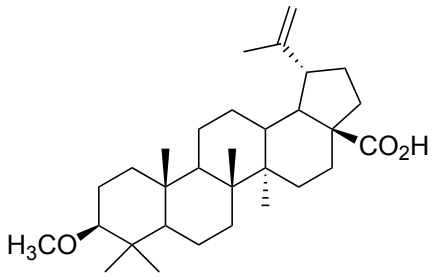
combined to give crude, impure **3 (**1.176 g; 0.8%), having the same Rf value as a reference sample on TLC (Rf=0.21, 3:1 hexanes:EtOAc).

^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 5.12 (t, J=3.5 Hz, 1H), 3.02-2.97 (m, 1H), 2.10 (d, J=11.3 Hz, 1H), 1.03 (s, 3H), 0.90 (d, J=8.1 Hz, 3H), 0.89 (s, 3H), 0.86 (s, 3H), 0.80 (d, J=6.4 Hz, 3H), 0.74 (s, 3H), 0.67 (s, 3H)


^{1}H NMR, MeOH-d$_4$, 300MHz
3 (Fuji Extract)
CO$_2$H
H
HO
4.15
7.25
10.91
6.70
19.10
4.37
0.72
0.42
1.00
1.00
0.0
2.5
5.0
7.5
10.0
ppm (t1)


^{1}H NMR, DMSO-d_6, 400MHz
3 (McIntosh Extract)
CO_2H
H
HO
8.04
15.17
4.52
6.19
10.24
3.59
0.77
0.88
1.09
1.13
0.56
0.0
2.5
5.0
7.5
10.0
ppm (t1)

3-Methoxy Betulinic Acid (7)



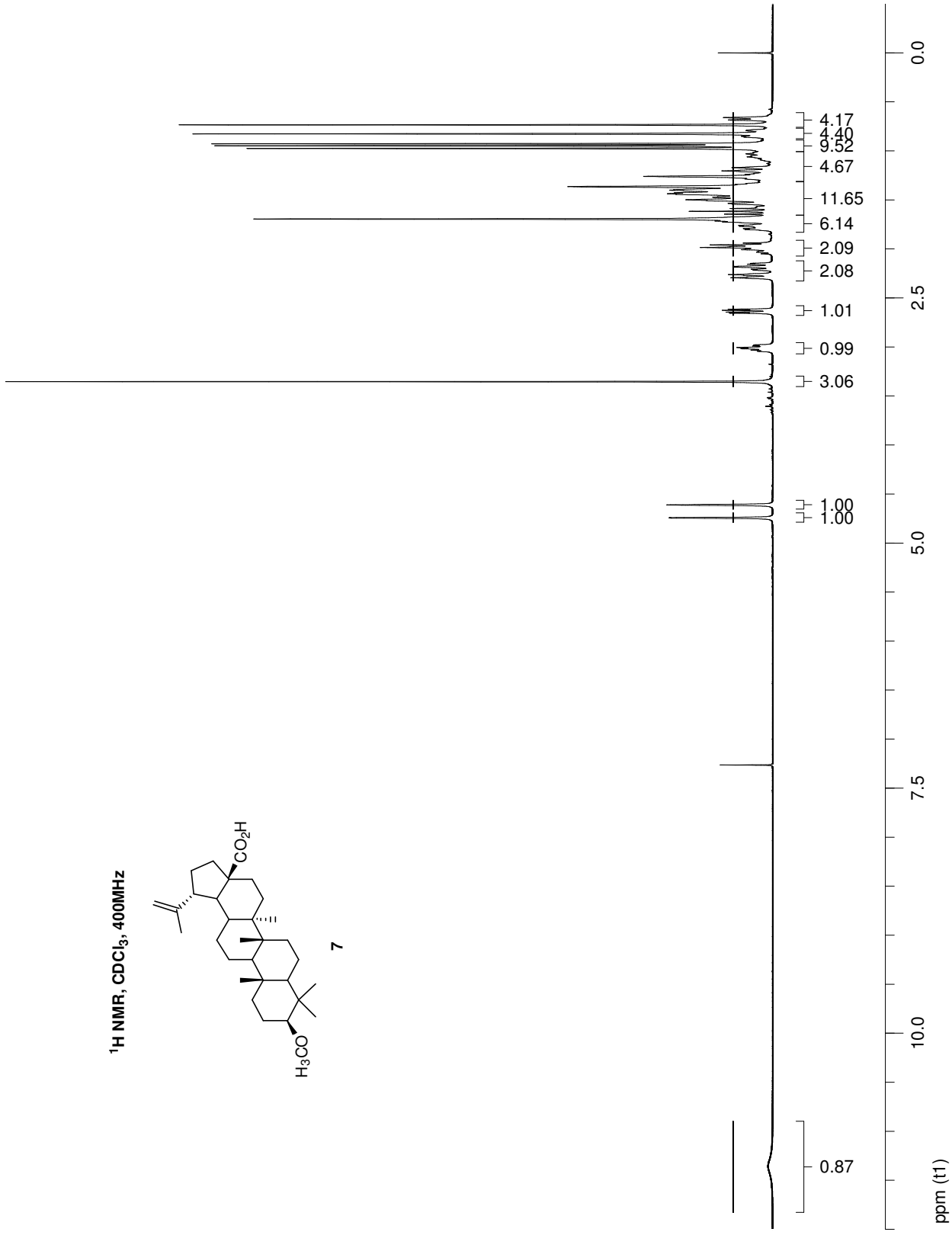
7

60% NaH in mineral oil (104 mg, 2.60 mmol) under N_2 was rinsed with dry THF (5 mL) and then suspended in dry THF (5 mL). A solution of **1** (200 mg, 0.44 mmol) in dry THF (9 mL) was added and stirred 30 min. CH_3I was added (0.33 mL, 5.30 mmol) and stirred overnight. Sat'd NH_4Cl solution (10 mL) and water (10 mL) was added and the solvent evaporated. The residue was extracted with EtOAc (3x15 mL), dried (Na_2SO_4), filtered and solvent evaporated to give a crude white solid (183 mg). Purification of the solid by silica gel chromatography using a hexanes/EtOAc gradient gave **7** as a white powder (39 mg, 19%).

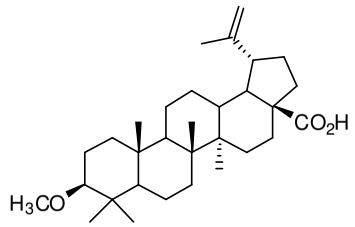
^{1}H NMR ($CDCl_3$, 400 MHz, centered on TMS): δ (ppm) 11.36 (br, 1H), 4.74 (d, J=1.4Hz, 1H), 4.61 (s, 1H), 3.35 (s, 3H), 3.04-2.98 (m, 1H), 2.64 (dd, J=11.7, 4.3 Hz, 1H), 2.30-2.25 (m, 1H), 2.22-2.15 (m, 1H), 2.05-1.94 (m, 2H), 1.69 (s, 3H), 0.97 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H), 0.83 (s, 3H), 0.73 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 182.5, 150.4, 109.7, 88.7, 57.5, 56.4, 55.9, 50.5, 49.3, 46.9, 42.4, 40.7, 38.8, 38.6, 38.4, 37.2, 37.0, 34.3, 32.2, 30.6, 29.7, 28.0, 25.5, 22.2, 20.8, 19.4, 18.1, 16.1, 16.0, 14.7

HRMS: Calculated for $C_{31}H_{50}O_3$, 470.37600; Found 470.38018

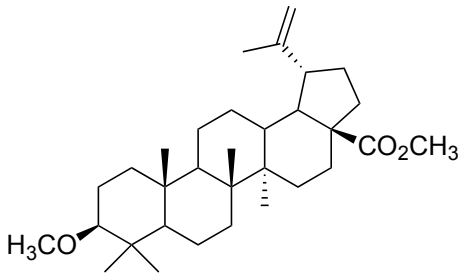

^{1}H NMR, $CDCl_3$, 400MHz
CO_2H
H_3CO
7
4.17
4.40
9.52
4.67
11.65
6.14
2.09
2.08
1.01
0.99
3.06
1.00
1.00
0.87
0.0
2.5
5.0
7.5
10.0
ppm (t1)

^{13}C NMR, $CDCl_3$, 400MHz



7

3-Methoxy Methyl Betulinate (8)



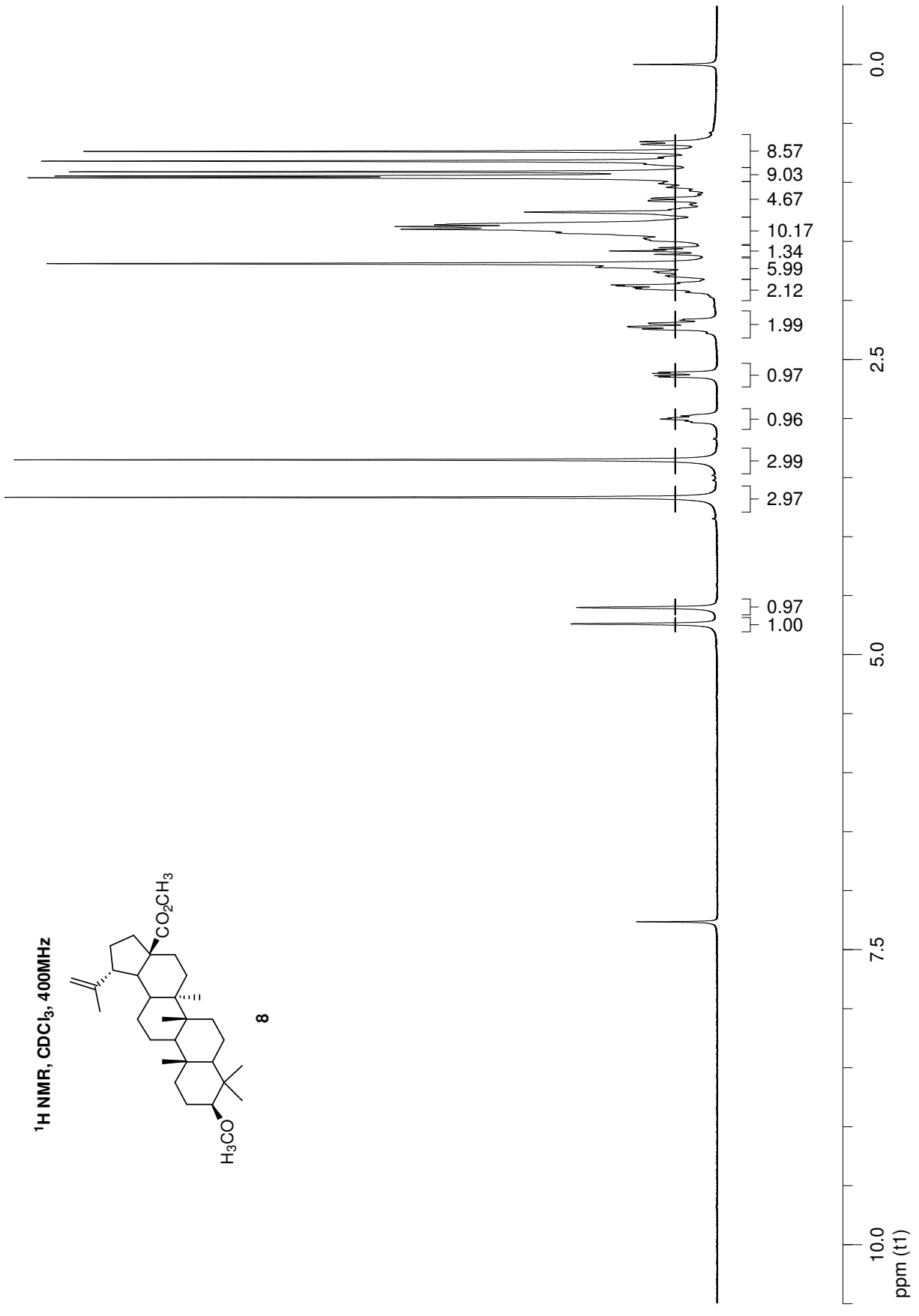
8

The procedure for **7** also gave 70 mg of **8** as a white powder after silica gel chromatography (33%).

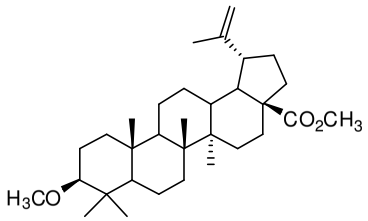
^{1}H NMR ($CDCl_3$, 400 MHz, centered on TMS): δ (ppm) 4.74 (s, 1H), 4.60 (s, 1H), 3.67 (s, 3H), 3.35 (s, 3H), 3.03-2.98 (m, 1H), 2.63 (dd, J=11.7, 4.1 Hz, 1H), 2.28-2.16 (m, 2H), 1.94-1.84 (m, 2H), 1.69 (s, 3H), 0.96 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H), 0.82 (s, 3H), 0.74 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 176.6, 150.6, 109.5, 88.6, 57.5, 56.6, 55.9, 51.3, 50.5, 49.5, 47.0, 42.4, 40.7, 38.8, 38.6, 38.3, 37.2, 37.0, 34.3, 32.2, 30.6, 29.7, 28.0, 25.5, 22.2, 20.9, 19.4, 18.2, 16.11, 16.07, 16.0, 14.7

HRMS: Calculated for $C_{32}H_{52}O_3$, 484.39165; Found 484.39340

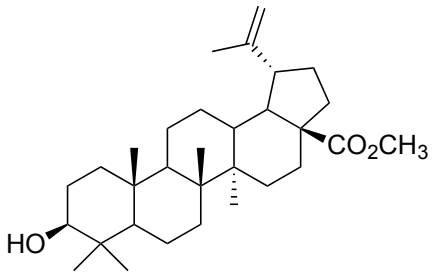

^{1}H NMR, $CDCl_3$, 400MHz
CO_2CH_3
H_3CO
8
8.57
9.03
4.67
10.17
1.34
5.99
2.12
1.99
0.97
0.96
2.99
2.97
0.97
1.00
0.0
2.5
5.0
7.5
10.0
ppm (t1)

^{13}C NMR, $CDCl_3$, 400MHz



8

Methyl Betulinate (2)

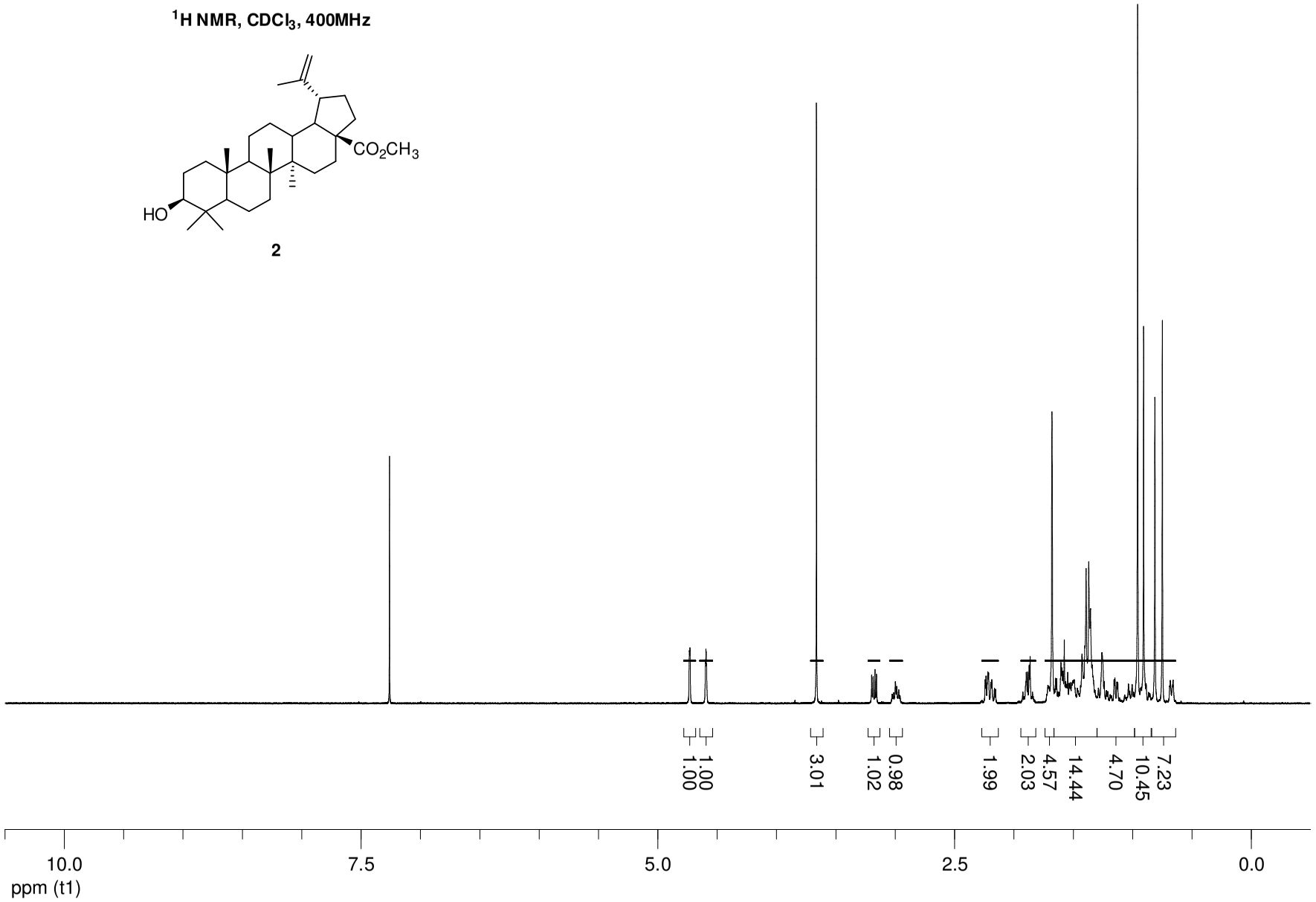


2

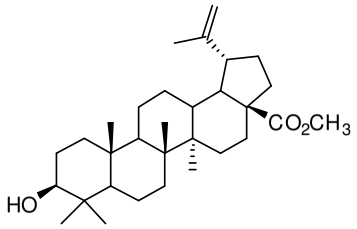
To a stirred solution of **1** (1.33 g, 2.92 mmol) in THF (67 mL) was added K_2CO_3 (4.03 g, 29.2 mmol) and CH_3I (0.91 mL, 14.6 mmol). The mixture was refluxed for 3 hrs then cooled to rt. Solvent was evaporated and water was added (80 mL). The mixture was extracted with EtOAc (3x60 mL), dried (Na_2SO_4), filtered and solvent evaporated to give **2** as a pale yellow powder (1.26 g, 92%) which did not require purification.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.73 (d, J=2.1 Hz, 1H), 4.59 (dd, J=2.2, 1.4 Hz, 1H), 3.66 (s, 3H), 3.18 (dd, J=11.2, 5.0 Hz, 1H), 2.99 (td, J=11.0, 4.5 Hz, 1H), 2.24-2.16 (m, 2H), 1.93-1.83 (m, 2H), 1.68 (s, 3H), 0.96 (s, 6H), 0.91 (s, 3H), 0.81 (s, 3H), 0.75 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 176.7, 150.6, 109.6, 79.0, 56.5, 55.3, 51.3, 50.5, 49.4, 47.0, 42.4, 40.6, 38.8, 38.7, 38.2, 37.2, 37.0, 34.3, 32.2, 30.6, 29.7, 28.0, 27.4, 25.5, 20.9, 19.4, 18.3, 16.1, 15.9, 15.3, 14.7

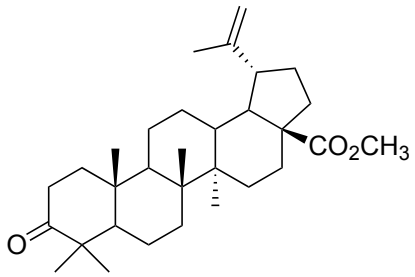

^{1}H NMR, $CDCl_3$, 400MHz
CO_2CH_3
HO
2
1.00
1.00
3.01
1.02
0.98
1.99
2.03
4.57
14.44
4.70
10.45
7.23
10.0
7.5
5.0
2.5
0.0
ppm (t1)

^{13}C NMR, $CDCl_3$, 400MHz



2

Methyl Betulonate (9)

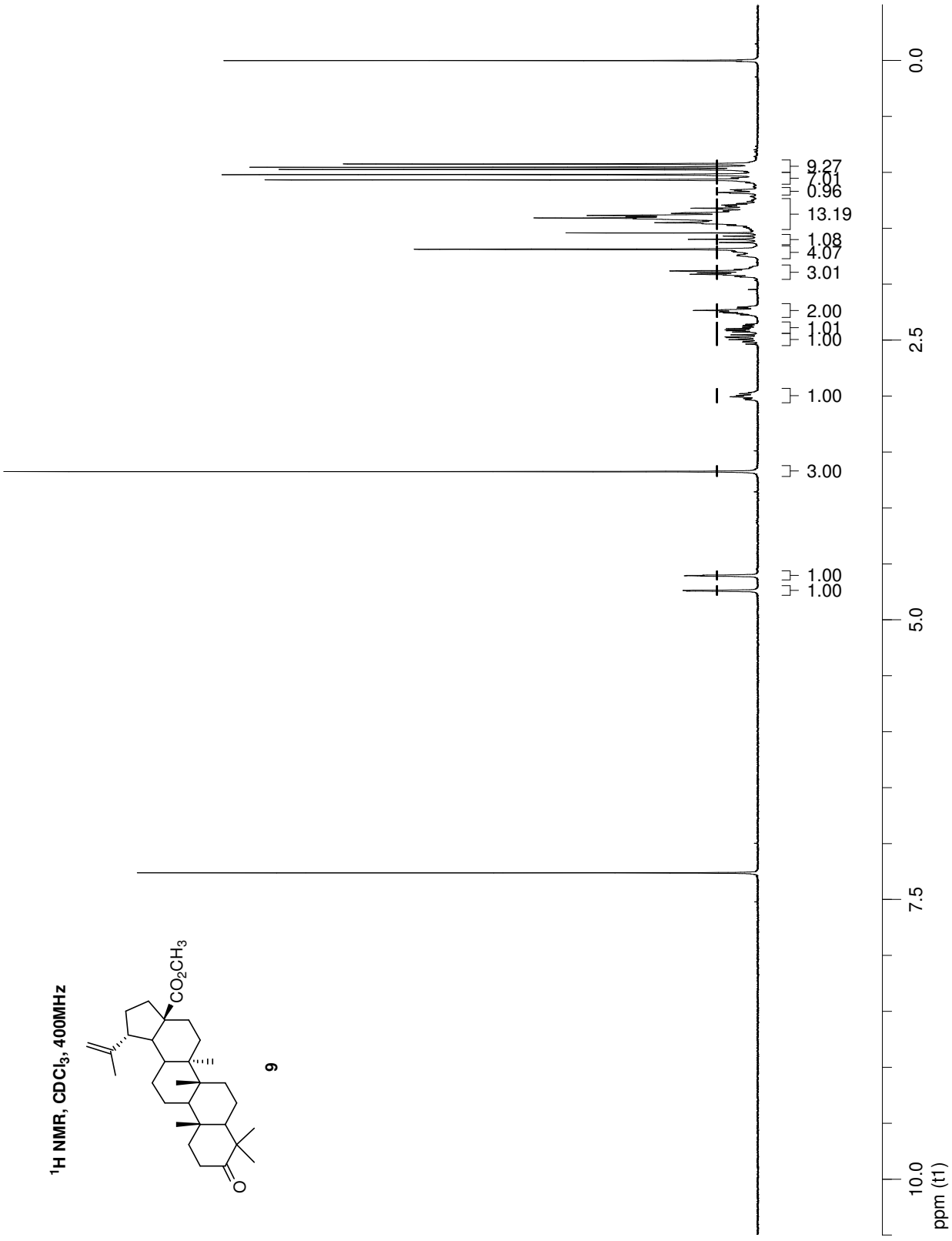


9

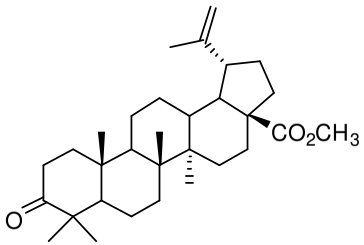
A solution of **2** (1.06 g, 2.25 mmol) in acetone (110 mL) was cooled to 0 ^{o}C. Fresh Jones reagent was prepared by dissolving CrO_3 (0.74 g) in H_2O (1.48 mL) and acidifying with H_2SO_4 (0.64 mL), and then added dropwise to the cooled acetone solution. After the orange colour persisted for 5 min, the reaction was quenched with MeOH (20 mL) and stirred 20 min. The solvent was evaporated and EtOAc added (150 mL). The mixture was washed with water (2x40 mL) and brine (40 mL), dried (Na_2SO_4), filtered and evaporated to give white foam (1.04 g). Purification of the foam by silica gel chromatography with a hexanes/EtOAc gradient gave **9** as a white powder (728 mg, 70%).

^{1}H NMR ($CDCl_3$, 400 MHz, centered on TMS): δ (ppm) 4.74 (d, J=1.9 Hz, 1H), 4.61 (dd, J=2.0, 1.4 Hz, 1H), 3.68 (s, 3H), 3.04-2.97 (m, 1H), 2.54-2.45 (m, 1H), 2.39 (ddd, J= 15.7, 7.6, 4.4 Hz, 1H), 2.28-2.20 (m, 2H), 1.69 (s, 3H), 1.60 (t, J=11.4 Hz, 1H), 1.07 (s, 3H), 1.02 (s, 3H), 0.97 (s, 3H), 0.95 (s, 3H), 0.92 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 218.1, 176.6, 150.5, 109.6, 56.5, 55.0, 51.3, 49.9, 49.4, 47.3, 46.9, 42.4, 40.6, 39.6, 38.3, 36.9, 34.1, 33.6, 32.1, 30.6, 29.6, 26.6, 25.5, 21.4, 21.0, 19.6, 19.4, 15.9, 15.7, 14.6

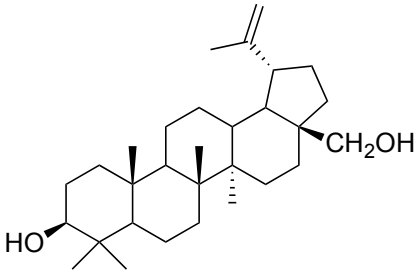

^{1}H NMR, $CDCl_3$, 400MHz
CO_2CH_3
O
9
9.27
7.01
0.96
13.19
1.08
4.07
3.01
2.00
1.01
1.00
1.00
3.00
1.00
1.00
0.0
2.5
5.0
7.5
10.0
ppm (t1)

^{13}C NMR, $CDCl_3$, 400MHz



9

Betulin (11)

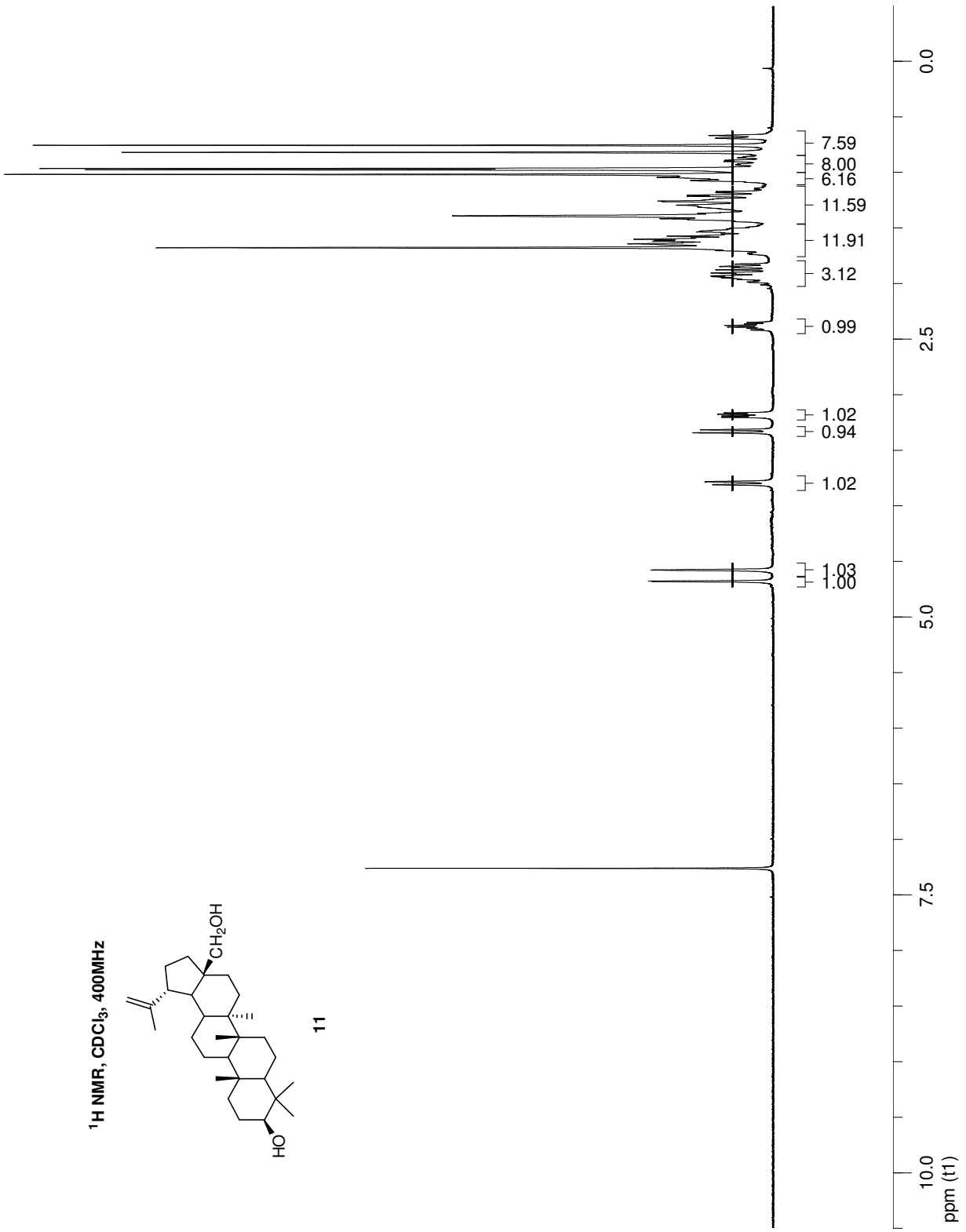


11

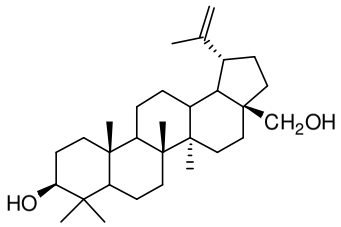
Betulin (**11**) was available in the lab, and its structure was verified by NMR data.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.68 (d, J=1.7 Hz, 1H), 4.58 (s, 1H), 3.80 (d, J=10.3 Hz, 1H), 3.33 (d, J=10.7 Hz, 1H), 3.18 (dd, J=11.1, 5.0 Hz, 1H), 2.38 (td, J=10.8, 5.9 Hz, 1H), 2.01-1.82 (m, 3H), 1.68 (s, 3H), 1.02 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.82 (s, 3H), 0.76 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 150.5, 109.7, 79.0, 60.5, 55.3, 50.4, 48.7, 47.8, 42.7, 40.9, 38.8, 38.7, 37.3, 37.1, 34.2, 33.9, 29.7, 29.1, 28.0, 27.4, 27.0, 25.2, 20.8, 19.1, 18.3, 16.1, 16.0, 15.3, 14.7

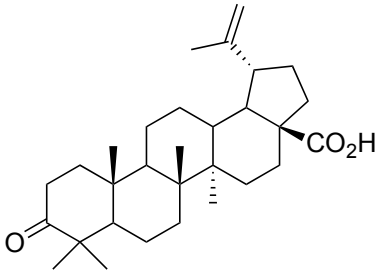

^{1}H NMR, $CDCl_3$, 400MHz
CH_2OH
HO
11
7.59
8.00
6.16
11.59
11.91
3.12
0.99
1.02
0.94
1.02
1.03
1.00
0.0
2.5
5.0
7.5
10.0
ppm (t1)

^{13}C NMR, $CDCl_3$, 400MHz



11

Betulonic Acid (10)

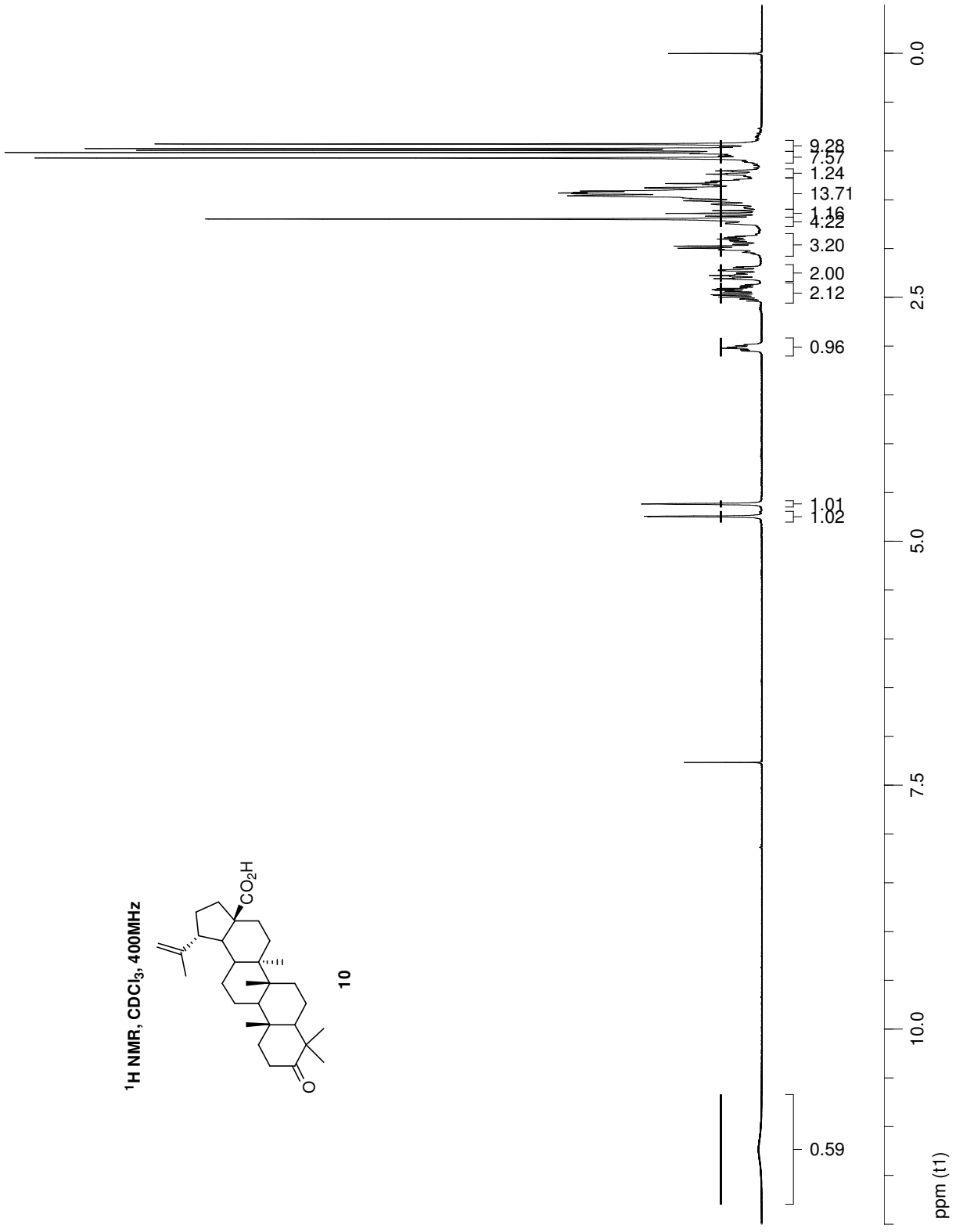


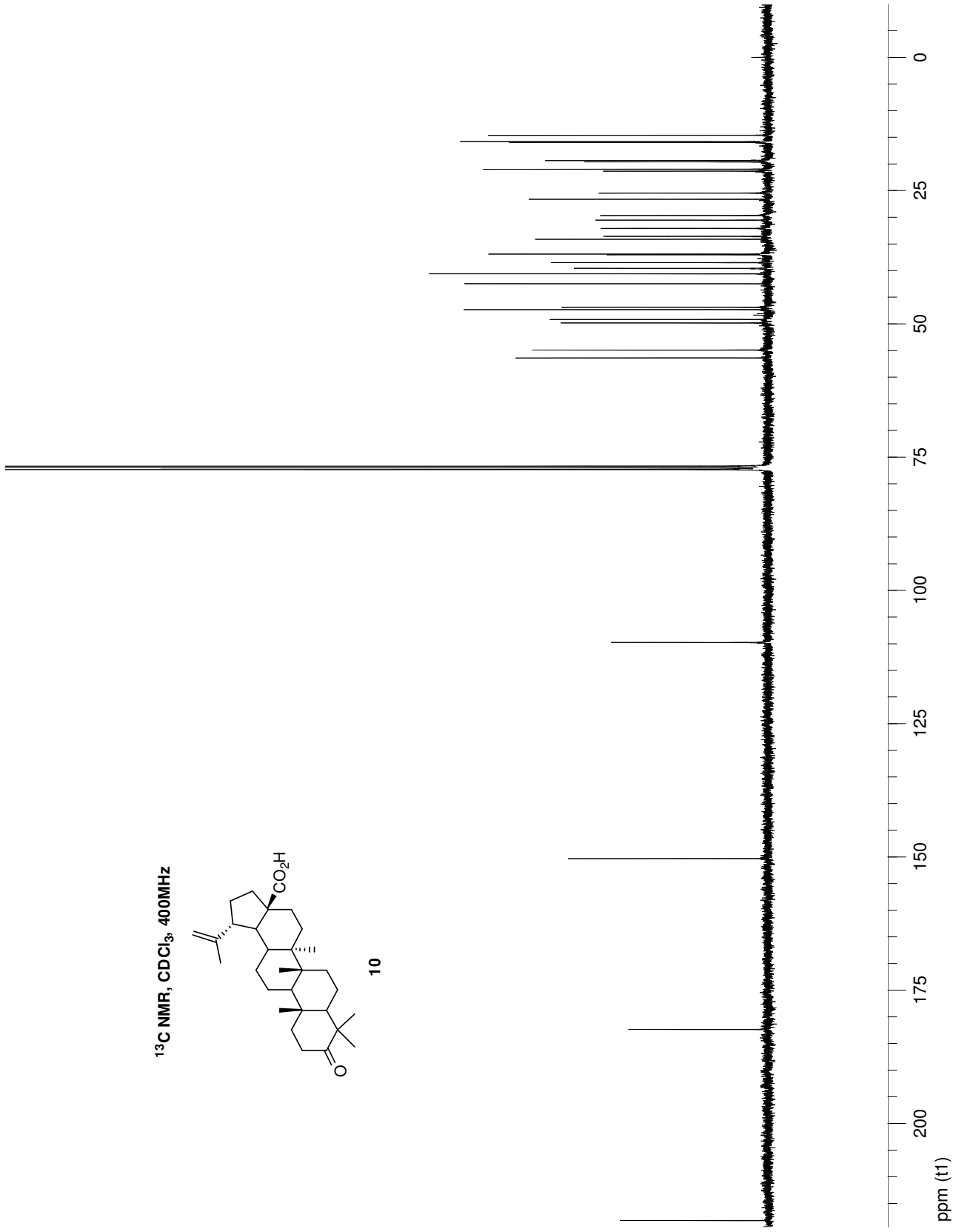
10

A solution of **11** (500 mg, 1.13 mmol) in acetone (45 mL) was cooled to 0 ^{o}C. Fresh Jones reagent was prepared by dissolving CrO_3 (0.56 g) in H_2O (1.10 mL) and acidifying with H_2SO_4 (0.48 mL), and then added dropwise to the cooled acetone solution. The reaction was monitored by TLC and quenched after 85 min with *i*PrOH (10 mL). After 20 min the solvent was evaporated and residue dissolved in EtOAc (80 mL). It was washed with H_2O (20 mL) and brine (20 mL), dried ($MgSO_4$), filtered and solvent evaporated to give pale yellow foam (441 mg). The foam was purified by silica gel chromatography using a hexanes/EtOAc gradient to give **10** as a white powder (187 mg, 36%).

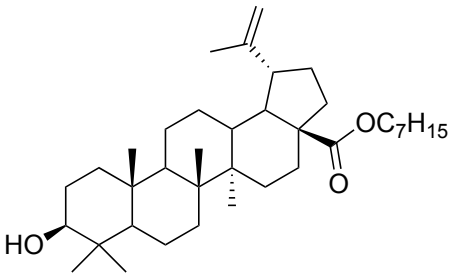
^{1}H NMR ($CDCl_3$, 400 MHz, calibrated with TMS): δ (ppm) 4.75 (d, J=1.7 Hz, 1H), 4.62 (br s, 1H), 3.05-2.98 (m, 1H), 2.54-2.45 (m, 1H), 2.40 (ddd, J=15.6, 7.6, 4.5 Hz, 1H), 2.32-2.27 (m, 1H), 2.26-2.19 (m, 1H), 2.06-1.96 (m, 2H), 1.93-1.87 (m, 1H), 1.70 (s, 3H), 1.07 (s, 3H), 1.02 (s, 3H), 0.99 (s, 3H), 0.98 (s, 3H), 0.93 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 218.2, 182.3, 150.3, 109.8, 56.4, 54.9, 49.8, 49.2, 47.3, 46.9, 42.5, 40.6, 39.6, 38.5, 37.0, 36.9, 34.1, 33.6, 32.1, 30.5, 29.7, 26.6, 25.5, 21.3, 21.0, 19.6, 19.3, 15.9, 15.8, 14.6


^{1}H NMR, $CDCl_3$, 400MHz
CO_2H
O
10
9.28
7.57
1.24
13.71
1.16
4.22
3.20
2.00
2.12
0.96
1.01
1.02
0.59
0.0
2.5
5.0
7.5
10.0
ppm (t1)


^{13}C NMR, $CDCl_3$, 400MHz
CO_2H
O
10
0
25
50
75
100
125
150
175
200
ppm (t1)

Heptyl Betulinate (12)

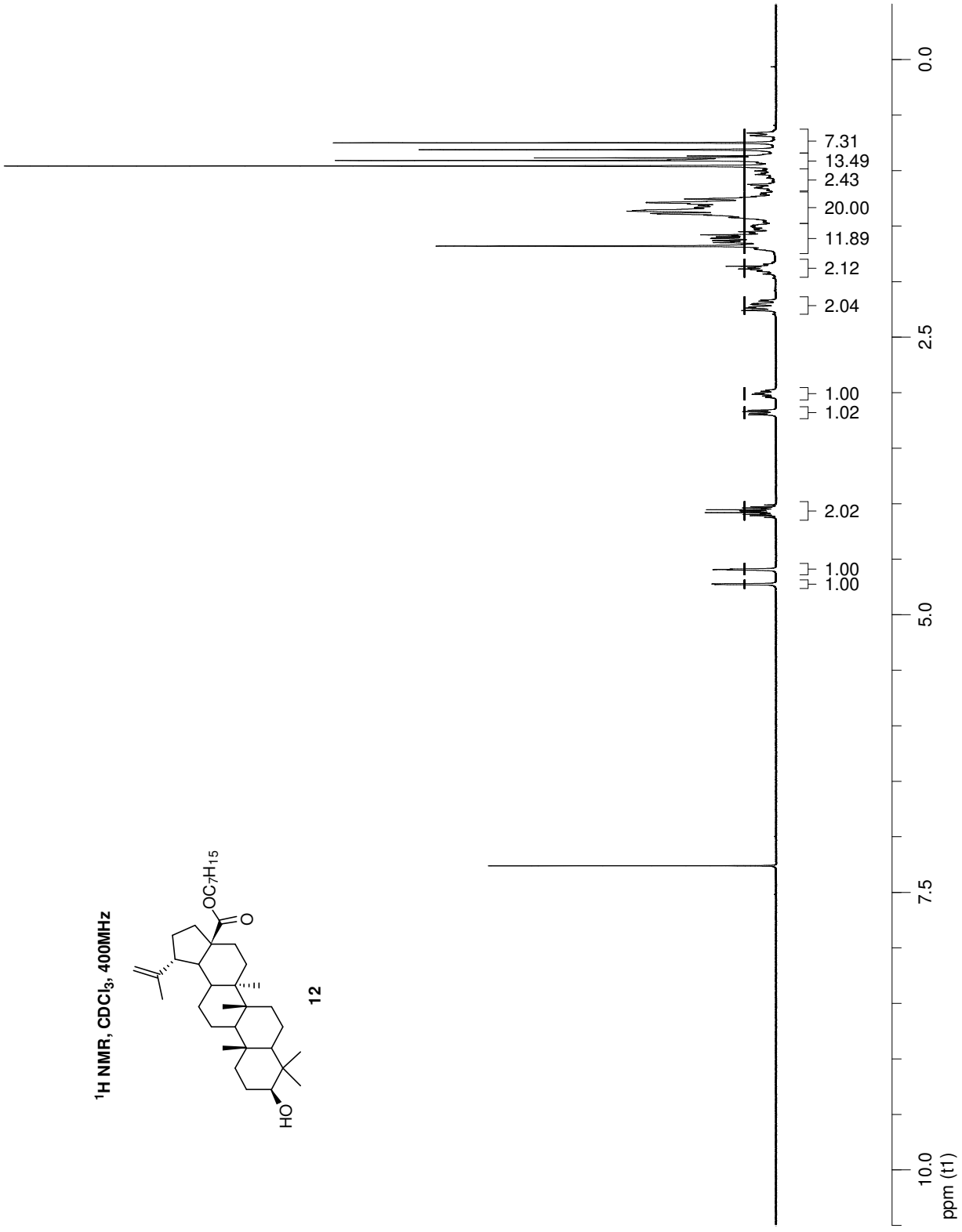


12

Heptyl betulinate (**12**) was available in the lab, and its structure was confirmed by NMR data.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.72 (d, J=2.2 Hz, 1H), 4.59 (dd, J=2.2, 1.3 Hz, 1H), 4.12-4.01 (m, 2H), 3.18 (dd, J=11.2, 5.0 Hz, 1H), 3.04-2.98 (m, 1H), 2.27-2.17 (m, 2H), 1.93-1.84 (m, 2H), 1.68 (s, 3H), 0.96 (s, 6H), 0.91 (s, 3H), 0.81 (s, 3H), 0.75 (s, 3H)

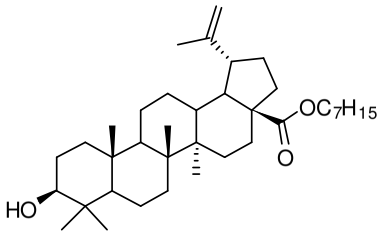
^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 176.3, 150.7, 109.5, 79.0, 64.0, 56.5, 55.3, 50.5, 49.4, 47.0, 42.4, 40.7, 38.8, 38.7, 38.3, 37.2, 37.1, 34.3, 32.2, 31.8, 30.6, 29.6, 28.9, 28.7, 28.0, 27.4, 26.1, 25.5, 22.6, 20.9, 19.4, 18.3, 16.1, 16.0, 15.3, 14.7, 14.0



^{1}H NMR, $CDCl_3$, 400MHz

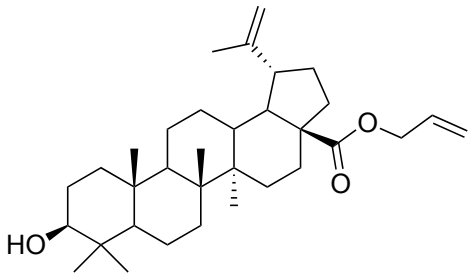
12

^{13}C NMR, $CDCl_3$, 400MHz



12

Allyl Betulinate (13)



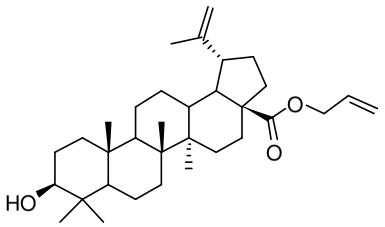
13

Allyl betulinate (**13**) was available in the lab, and its structure was confirmed by NMR data.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.97-5.87 (m, 1H), 5.33 (dd, J=17.2, 1.5 Hz, 1H), 5.23 (dd, J=10.4, 1.3 Hz, 1H), 4.73 (d, J=2.1 Hz, 1H), 4.63- 4.52 (m, 3H), 3.18 (dd, J=11.3, 5.0 Hz, 1H), 3.04-2.98 (m, 1H), 2.28-2.17 (m, 2H), 1.93-1.85 (m, 2H), 1.68 (s, 3H), 0.96 (s, 6H), 0.90 (s, 3H), 0.81 (s, 3H), 0.75 (s, 3H)

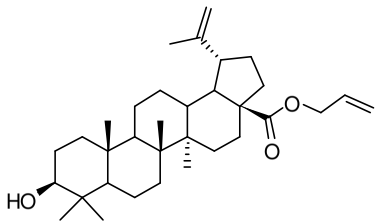
^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 175.7, 150.6, 132.5, 118.1, 109.6, 79.0, 64.6, 56.6, 55.3, 50.5, 49.4, 46.9, 42.4, 40.7, 38.8, 38.7, 38.2, 37.2, 37.0, 34.3, 32.1, 30.6, 29.6, 28.0, 27.4, 25.5, 20.9, 19.4, 18.3, 16.1, 15.9, 15.3, 14.7

^{1}H NMR, $CDCl_3$, 400MHz



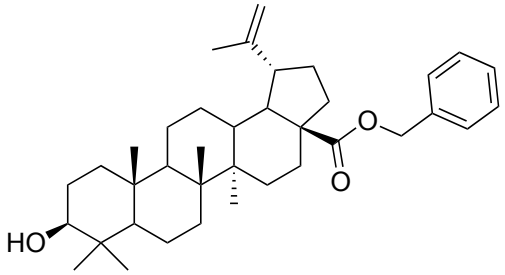
13

^{13}C NMR, $CDCl_3$, 400MHz



13

Benzyl Betulinate (14)

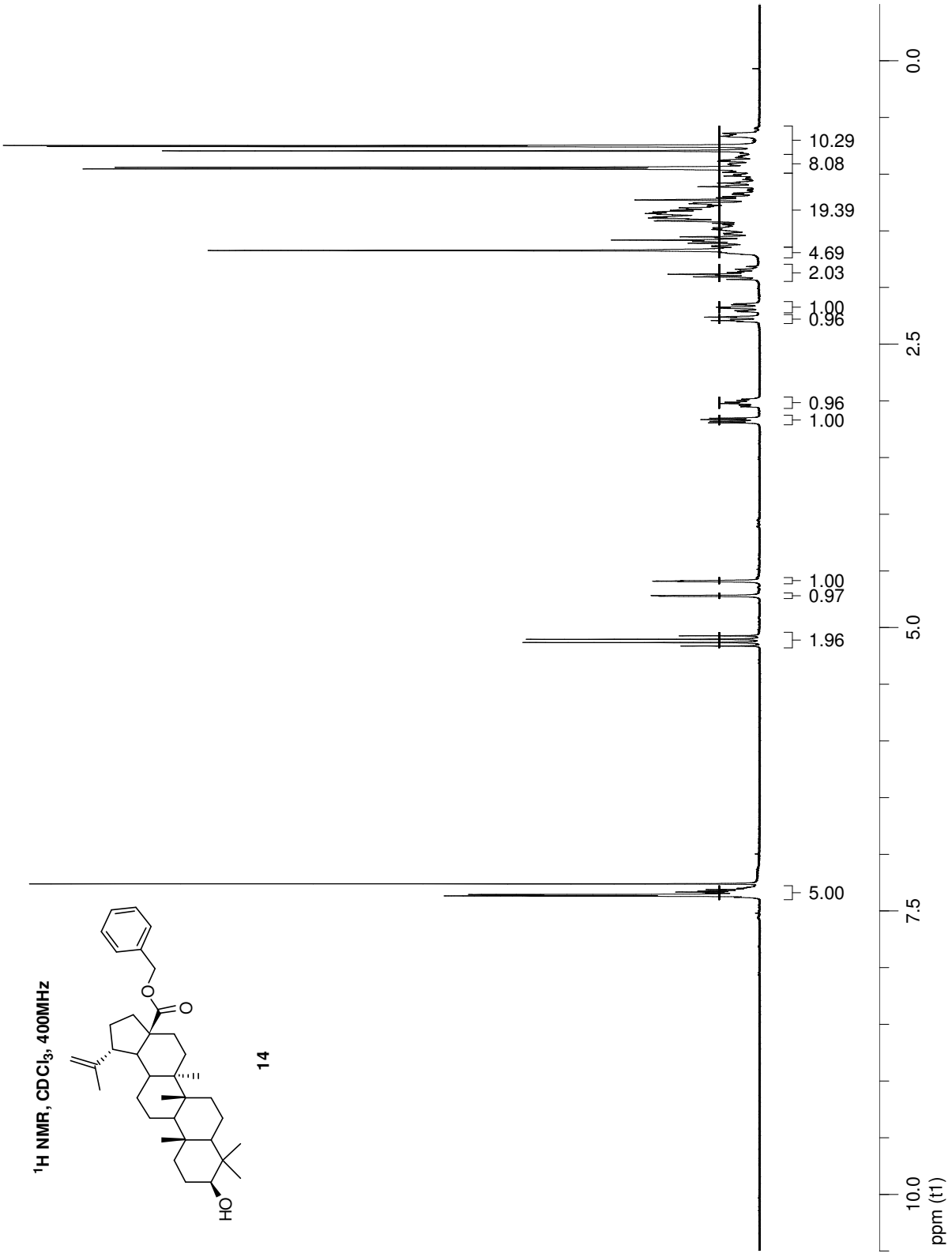


14

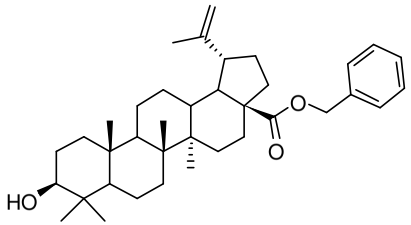
Benzyl betulinate (**14**) was available in the lab, and its structure was confirmed by NMR data.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.40-7.30 (m, 5H), 5.15 (d, J=12.3 Hz, 1H), 5.10 (d, J=12.3 Hz, 1H), 4.72 (d, J=2.1Hz, 1H), 4.59 (dd, J=2.2, 1.3 Hz, 1H), 3.17 (dd, J=11.3, 4.9 Hz, 1H), 3.05-2.98 (m, 1H), 2.30-2.25 (m, 1H), 2.21-2.14 (m, 1H), 1.93-1.82 (m, 2H), 1.67 (s, 3H), 0.95 (s, 3H), 0.94 (s, 3H), 0.79 (s, 3H), 0.76 (s, 3H), 0.75 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 175.8, 150.6, 136.5, 128.5, 128.2, 128.0, 109.6, 79.0, 65.7, 56.5, 55.3, 50.5, 49.4, 46.9, 42.4, 40.6, 38.8, 38.7, 38.2, 37.2, 36.9, 34.3, 32.1, 30.6, 29.6, 28.0, 27.4, 25.5, 20.9, 19.4, 18.3, 16.1, 15.8, 15.3, 14.7

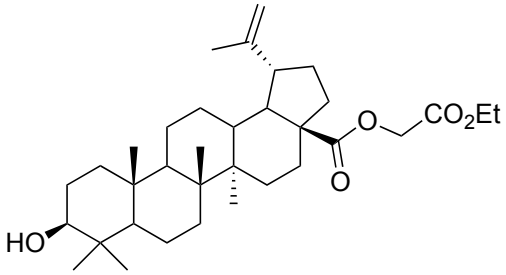

^{1}H NMR, $CDCl_3$, 400MHz
14
HO
O
O
10.29
8.08
19.39
4.69
2.03
1.00
0.96
0.96
1.00
1.00
0.97
1.96
5.00
0.0
2.5
5.0
7.5
10.0
ppm (t1)

^{13}C NMR, $CDCl_3$, 400MHz



14

Ethyl Acetoxy Betulinate (15)

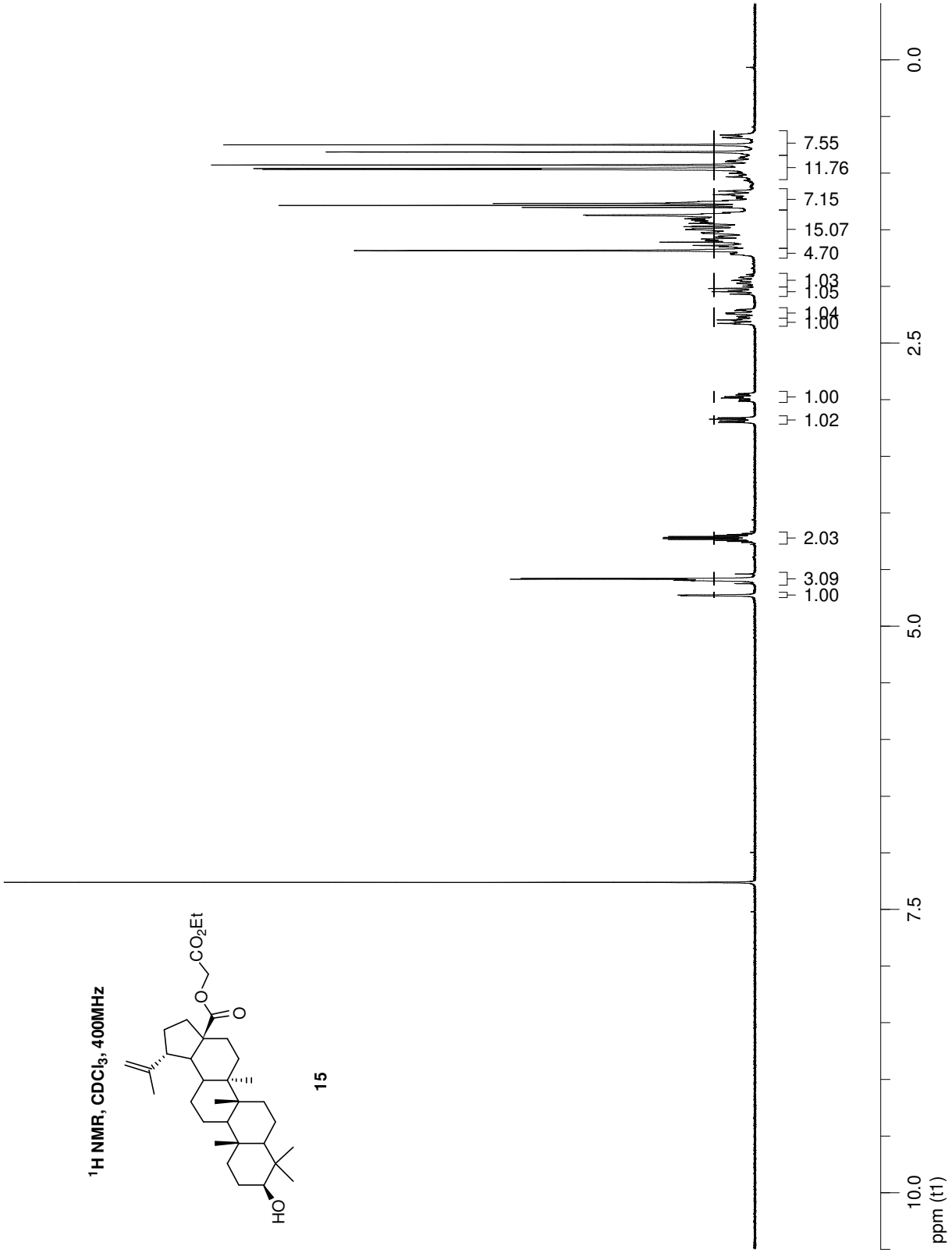


15

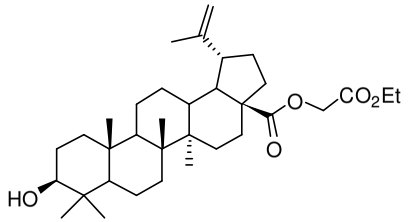
Ethyl acetoxy betulinate (**15**) was available in the lab, and its structure was confirmed by NMR data.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.73 (d, J=2.0 Hz, 1H), 4.59 (dd, J=2.2, 1.4 Hz, 1H), 4.60 (d, J=18.2 Hz, 1H), 4.57 (d, J=18.2 Hz, 1H), 4.26-4.18 (m, 2H), 3.18 (dd, J=11.2, 5.1 Hz, 1H), 3.01-2.95 (m, 1H), 2.33-2.29 (m, 1H), 2.27-2.20 (m, 1H), 2.07-2.02 (m, 1H), 2.00-1.90 (m, 1H), 1.68 (s, 3H), 0.97 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H), 0.81 (s, 3H), 0.75 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 175.4, 168.1, 150.5, 109.6, 79.0, 61.3, 60.3, 56.5, 55.3, 50.6, 49.4, 46.8, 42.4, 40.7, 38.8, 38.7, 38.1, 37.2, 36.9, 34.3, 31.9, 30.4, 29.5, 28.0, 27.4, 25.5, 20.9, 19.4, 18.3, 16.1, 15.9, 15.3, 14.7, 14.1

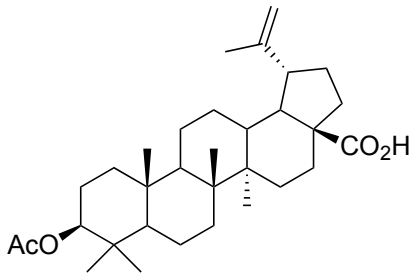

^{1}H NMR, $CDCl_3$, 400MHz
CO_2Et
O
HO
15
7.55
11.76
7.15
15.07
4.70
1.03
1.05
1.04
1.00
1.00
1.02
2.03
3.09
1.00
0.0
2.5
5.0
7.5
10.0
ppm (t1)

^{13}C NMR, $CDCl_3$, 400MHz



15

3-Acetoxy Betulinic Acid (16)



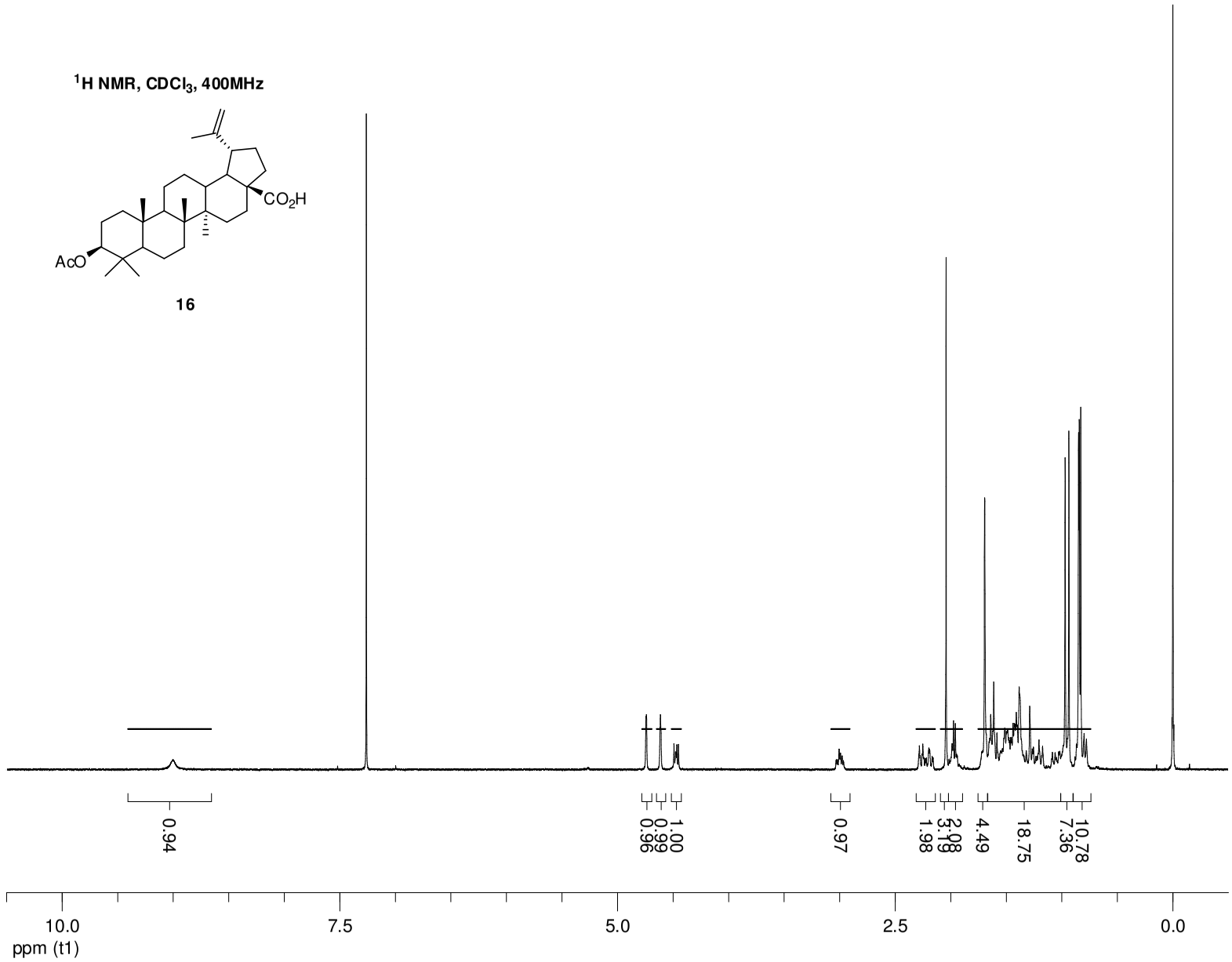
16

To a stirred mixture of **1** (1.10 g, 2.41 mmol) in DCM (80 mL) was added Et_3N (0.84 mL, 6.03 mmol), DMAP (10 mg) and Ac_2O (0.50 mL, 5.30 mmol). It was stirred over the weekend, then 0.5 N HCl was added (40 mL), stirred 30 min and the layers separated. The organic layer was washed with water (2x40 mL), dried (Na_2SO_4), filtered and solvent evaporated to give a light brown foam (0.98 g). Separation of the foam by silica gel chromatography using a hexanes/EtOAc gradient gave **16** as white powder (85 mg, 7%), and a 2:3 mixture of **16** and its C-28 mixed-anhydride **17** (0.60 g, 48%)

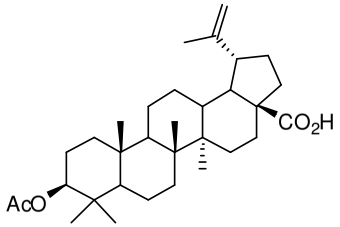
A 2:3 mixture of **16** and **17** (0.40 g, 0.76 mmol) was stirred in MeOH (30 mL) and H_2O (2 mL) for three days at rt. The solvent was evaporated and the residue purified by silica gel chromatography using a hexanes/EtOAc gradient to give pure **16** as a white powder (0.30 g, 79%).

^{1}H NMR ($CDCl_3$, 400 MHz, calibrated with TMS): δ (ppm) 4.74 (d, J=1.7 Hz, 1H), 4.62 (s, 1H), 4.49-4.45 (m, 1H), 3.03-2.97 (m, 1H), 2.29-2.24 (m, 1H), 2.23-2.16 (m, 1H), 2.04 (s, 3H), 1.69 (s, 3H), 0.97 (s, 3H), 0.94 (s, 3H), 0.85 (s, 3H), 0.84 (s, 3H), 0.83 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 182.1, 171.1, 150.4, 109.7, 80.9, 56.4, 55.4, 50.4, 49.3, 46.9, 42.4, 40.7, 38.41, 38.37, 37.8, 37.1, 37.0, 34.2, 32.1, 30.6, 29.7, 27.9, 25.4, 23.7, 21.3, 20.8, 19.3, 18.1, 16.5, 16.2, 16.0, 14.6

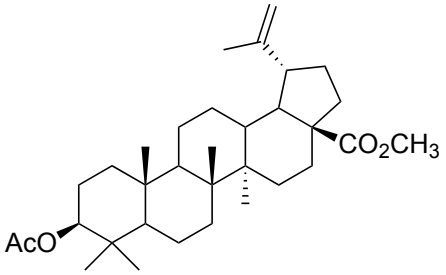

^{1}H NMR, $CDCl_3$, 400MHz
AcO
CO_2H
16
0.94
0.96
0.99
1.00
0.97
1.98
3.19
2.08
4.49
18.75
7.36
10.78
10.0
7.5
5.0
2.5
0.0
ppm (t1)

^{13}C NMR, $CDCl_3$, 400MHz



16

3-Acetoxy Methyl Betulinate (18)



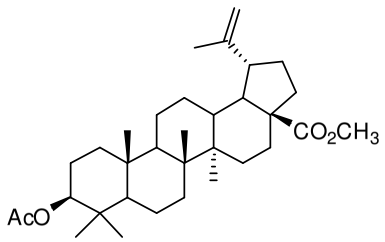
18

A solution of **2** (2.00 g, 4.25 mmol), DMAP (23 mg), Et_3N (2.07 mL, 14.9 mmol), and Ac_2O (1.20 mL, 12.8 mmol) in DCM (80 mL) was stirred overnight at rt. In the morning, 5% HCl was added (40 mL) and stirred 30 min. Layers were separated and aqueous phase extracted with DCM (80 mL). The combined DCM extracts were washed with H_2O (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and the solvent evaporated to give a yellow solid (2.17 g). The solid was purified by silica gel chromatography using a hexanes/EtOAc gradient to give **18** as a white powder (2.01 g, 92%).

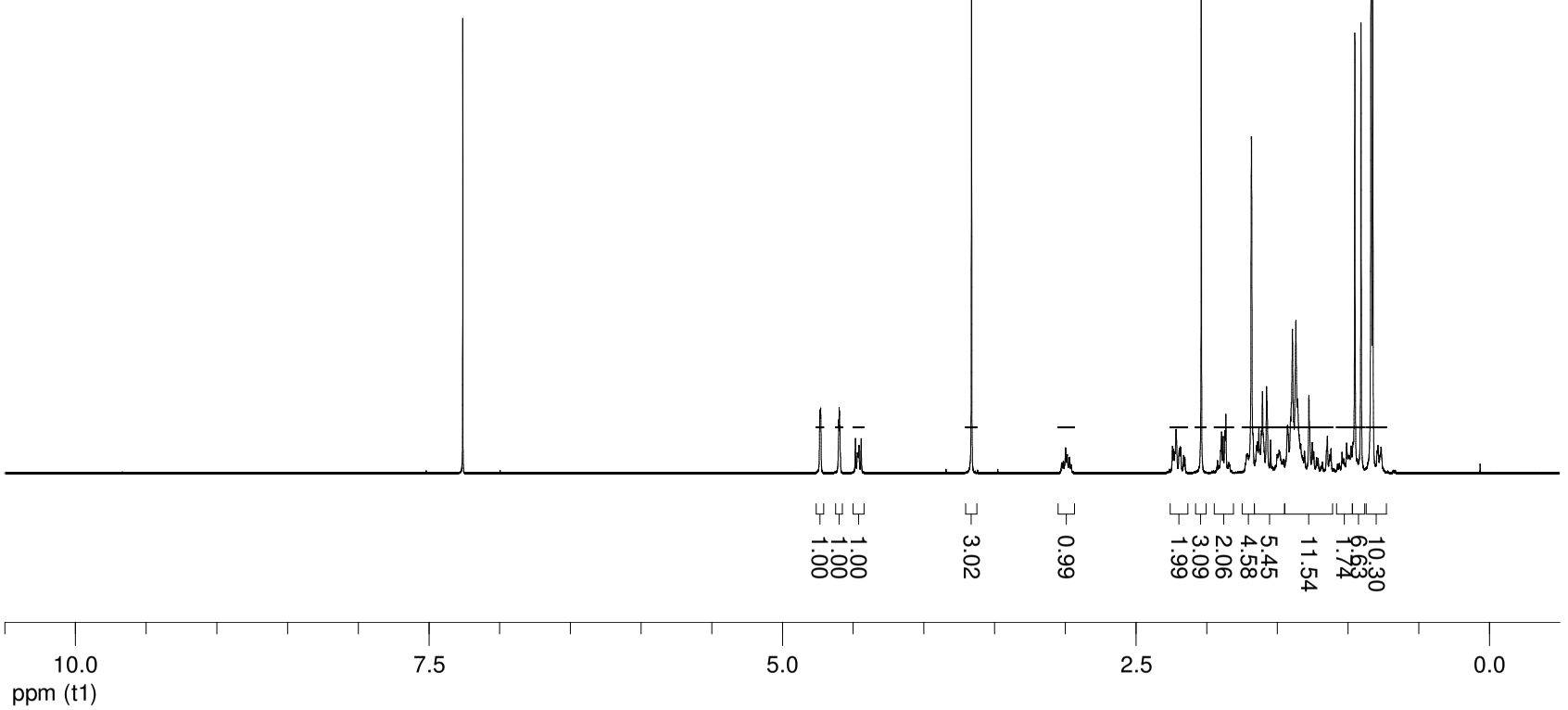
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.73 (d, J=2.2 Hz, 1H), 4.60 (dd, J=2.3, 1.4 Hz, 1H), 4.48-4.44 (m, 1H), 3.66 (s, 3H), 3.02-2.96 (m, 1H), 2.04 (s, 3H), 1.68 (d, J=0.5 Hz, 3H), 0.95 (s, 3H), 0.91 (s, 3H), 0.84 (s, 6H), 0.83 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 176.7, 171.0, 150.6, 109.6, 80.9, 56.5, 55.4, 51.2, 50.4, 49.4, 47.0, 42.4, 40.7, 38.4, 38.2, 37.8, 37.1, 36.9, 34.2, 32.1, 30.6, 29.6, 27.9, 25.4, 23.7, 21.3, 20.9, 19.3, 18.2, 16.5, 16.2, 15.9, 14.7

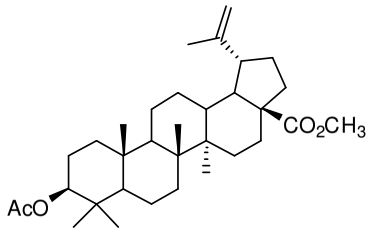
^{1}H NMR, $CDCl_3$, 400MHz



18

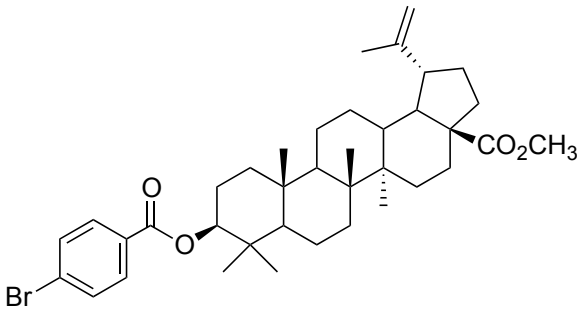


^{13}C NMR, $CDCl_3$, 400MHz



18

3-(4-Bromo)benzoyl Methyl Betulinate (19)



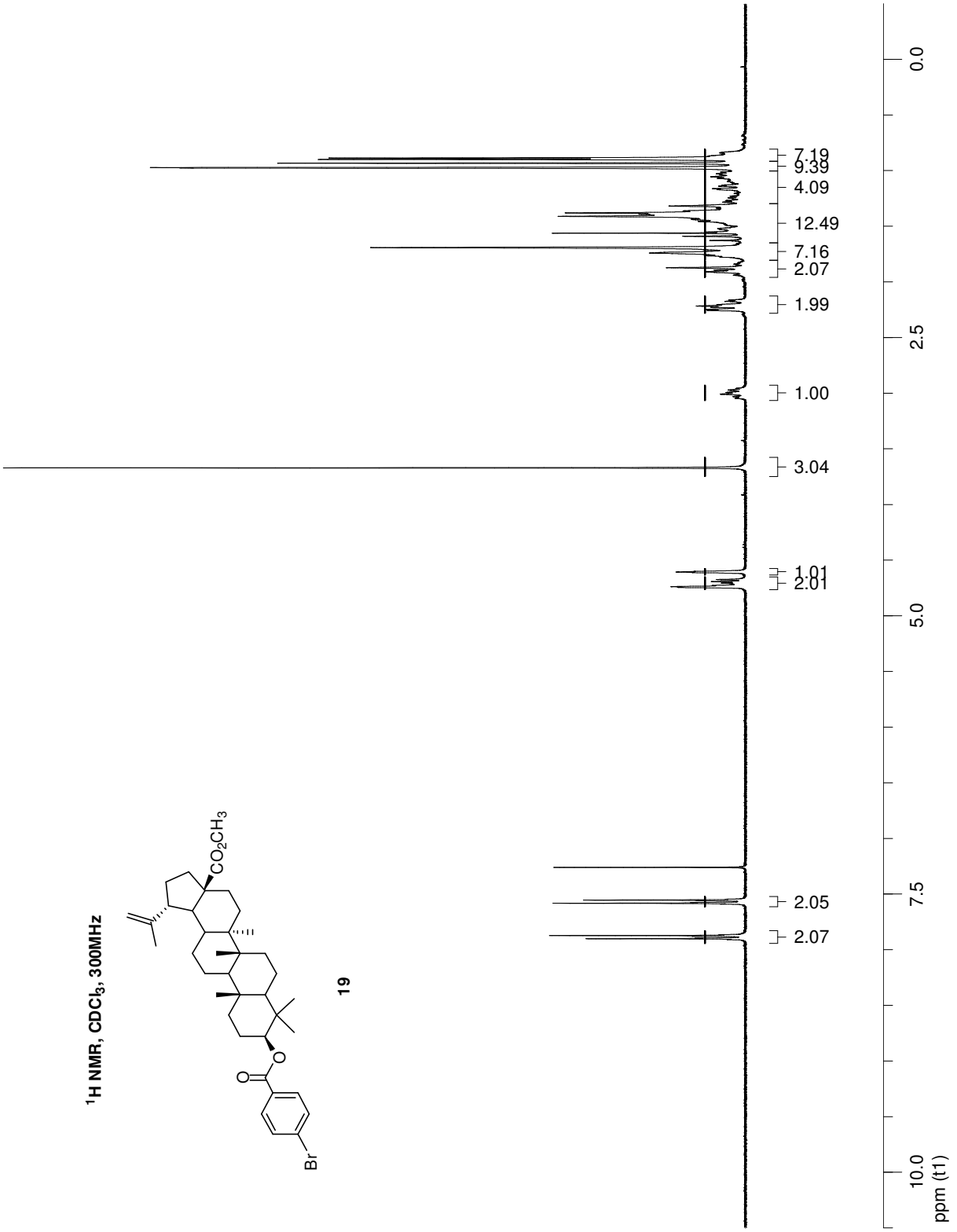
19

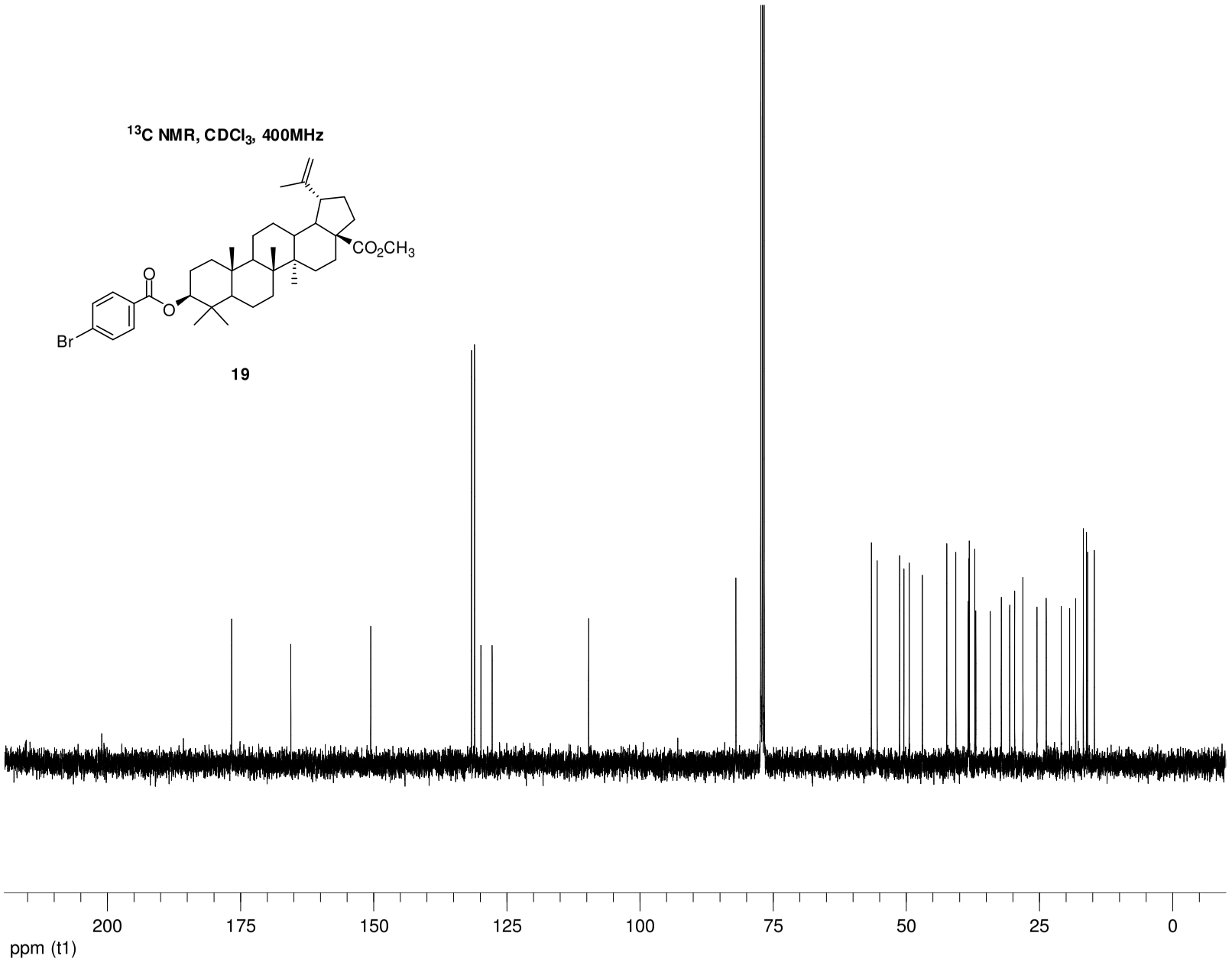
To a stirred solution of **2** (250 mg, 0.53 mmol) in DCM (5 mL) was added DMAP (10 mg), Et_3N (0.59 mL, 4.23 mmol) and 4-bromobenzoyl chloride (0.69 g, 3.14 mmol). The solution was stirred overnight, diluted with DCM (15 mL), washed with 5% HCl (10 mL) and H_2O (10 mL), dried ($MgSO_4$), filtered and the solvent evaporated. Purification of the crude material by silica gel chromatography using a hexanes/EtOAc gradient gave **19** as a white powder (304 mg, 88%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.91-7.87 (m, 2H), 7.59-7.55 (m, 2H), 4.74-4.67 (m, 2H), 4.61-4.60 (m, 1H), 3.67 (s, 3H), 3.05-2.96 (m, 1H), 2.26-2.16 (m, 2H), 1.69 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.93 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H)

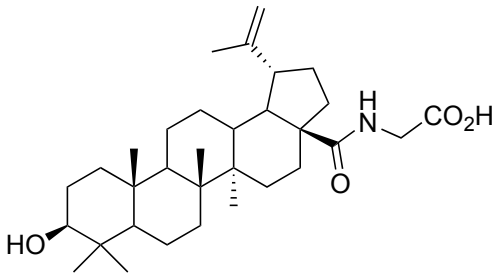
^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 176.7, 165.6, 150.5, 131.6, 131.0, 129.9, 127.8, 109.6, 82.0, 56.5, 55.5, 51.3, 50.5, 49.4, 47.0, 42.4, 40.7, 38.4, 38.24, 38.18, 37.1, 37.0, 34.2, 32.2, 30.6, 29.7, 28.1, 25.5, 23.7, 20.9, 19.3, 18.2, 16.8, 16.2, 16.0, 14.7

HRMS: Calculated for $C_{38}H_{53}BrO_4$, 652.31272; Found 652.31387


^{1}H NMR, $CDCl_3$, 300MHz
CO_2CH_3
O
Br
19
7.19
9.39
4.09
12.49
7.16
2.07
1.99
1.00
3.04
1.01
2.01
2.05
2.07
0.0
2.5
5.0
7.5
10.0
ppm (t1)


^{13}C NMR, $CDCl_3$, 400MHz
CO_2CH_3
O
O
Br
19
200
175
150
125
100
75
50
25
0
ppm (t1)

Betulinic Acid Glycine Amide (21)



21

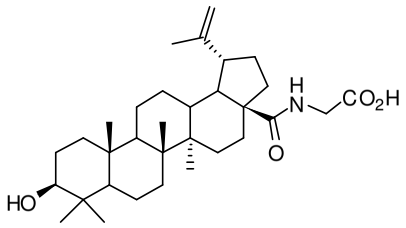
To a stirred solution of **16** (100 mg, 0.20 mmol) in dry DCM (10 mL) under N_2 was added DMF (10 μL) followed by dropwise addition of oxalyl chloride (34 μL, 0.40 mmol) at rt. After 6 hrs, the solvent was evaporated and the residue re-dissolved in dry DCM (2 mL). This solution was added dropwise to a stirred solution of glycine ethyl ester HCl (57 mg, 0.41 mmol), dry DCM (8 mL) and Et_3N (0.09 mL, 0.64 mmol) at 0 ^oC. It was warmed to rt overnight, then DCM (20 mL) and sat'd NH_4Cl (15 mL) was added. Layers were separated and the organic layer washed with H_2O (2x15 mL), dried (Na_2SO_4), filtered and solvent evaporated to give intermediate ester **20** as a crude white solid (108 mg).

Crude ester **20** was stirred overnight at 40 ^oC in MeOH (20 mL) with H_2O (1 mL) and K_2CO_3 (1.0 g). The solvent was evaporated and the residue acidified to pH~1 with 1 N HCl. H_2O was added (20 mL) and the mixture extracted with EtOAc (20 mL), washed with brine (15 mL), dried (Na_2SO_4), filtered and solvent evaporated. The residue was purified by silica gel chromatography using 9:1 $CHCl_3$:MeOH to give **21** as a white powder (71 mg, 69%).

^{1}H NMR ($CDCl_3$, 400 MHz, centered on TMS): δ (ppm) 6.11 (br s, 1H), 4.73 (s, 1H), 4.60 (s, 1H), 4.07 (dd, J=18.1, 5.2 Hz, 1H), 4.04 (dd, J=18.1, 5.2 Hz, 1H), 3.19 (dd, J=11.2, 5.0 Hz, 1H), 3.12-3.06 (m, 1H), 2.43-2.36 (m, 1H), 2.04-1.90 (m, 2H), 1.68 (s, 3H), 0.97 (s, 3H), 0.96 (s, 3H), 0.92 (s, 3H), 0.81 (s, 3H), 0.75 (s, 3H), 0.68 (br d, J=8.9 Hz, 1H)

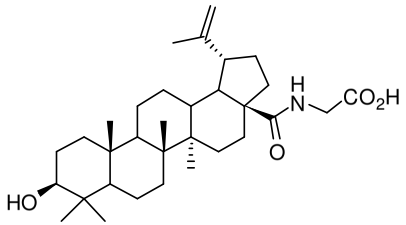
^{13}C NMR ($CDCl_3$, 400MHz): δ (ppm) 177.4, 173.5, 150.7, 109.5, 79.1, 55.8, 55.4, 50.6, 50.0, 46.7, 42.5, 41.4, 40.7, 38.8, 38.7, 38.3, 37.8, 37.2, 34.4, 33.7, 30.7, 29.4, 28.0, 27.4, 25.6, 20.9, 19.5, 18.3, 16.1, 16.0, 15.4, 14.6

^{1}H NMR, $CDCl_3$, 400MHz



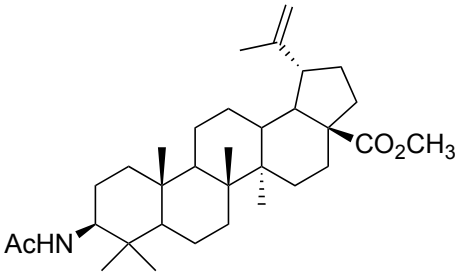
21

^{13}C NMR, $CDCl_3$, 400MHz



21

3β-Amido Methyl Betulinate (25)



25

A solution of **9** (100 mg, 0.214 mmol), NH_4OAc (165 mg, 2.14 mmol), $NaBH_3CN$ (9.4 mg, 0.15 mmol) and activated 3 Å molecular sieves in MeOH (10 mL) was stirred for two days. Additional $NaBH_3CN$ (10 mg, 0.16 mmol) was added and stirring continued for three more days. The solution was filtered and acidified to pH ~1 with 30% HCl. The solvent was evaporated and water (10 mL) and Et_2O (10 mL) were added. A white precipitate formed which was filtered off (49 mg). The layers were separated and aqueous layer extracted with EtOAc (2x10 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and solvent evaporated to give pale orange solid (75 mg).

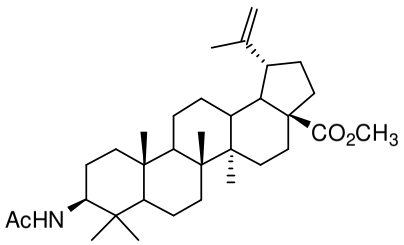
The orange solid and white precipitate were dissolved in DCM (6 mL) with Ac_2O (0.08 mL, 0.85 mmol), Et_3N (0.13 mL, 0.93 mmol) and DMAP (3 mg). After stirring overnight at rt, DCM (10 mL) and 5% HCl (10 mL) was added and stirred 1 hr. Layers were separated and the organic layer washed with water (10 mL) and brine (10 mL), dried ($MgSO_4$), filtered and solvents evaporated to give a white solid (142 mg). Separation of the solid by silica gel chromatography with a hexanes/EtOAc gradient gave **25** as a flaky white solid (54 mg, 49%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.27 (d, J=10.0 Hz, 1H), 4.73 (d, J=2.2Hz, 1H), 4.59 (dd, J=2.3, 1.4 Hz, 1H), 3.64 (ddd, J=12.2, 10.0, 4.4 Hz, 1H), 3.66 (s, 3H), 3.02-2.95 (m, 1H), 1.98 (s, 3H), 1.68 (s, 3H), 0.95 (s, 3H), 0.90 (s, 3H), 0.86 (s, 3H), 0.80 (s, 3H), 0.73 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 176.7, 169.5, 150.6, 109.6, 56.6, 56.5, 56.0, 51.2, 50.4, 49.4, 47.0, 42.4, 40.6, 39.1, 38.2, 37.7, 37.0, 36.9, 34.2, 32.1, 30.6, 29.6, 28.3, 25.6, 25.5, 23.7, 20.8, 19.3, 18.5, 16.4, 16.0, 15.9, 14.6

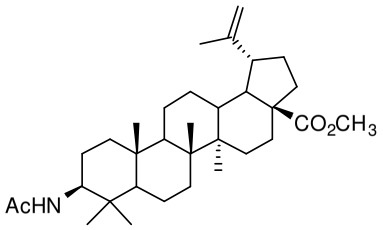
HRMS: Calculated for $C_{33}H_{53}NO_3$, 511.40254; Found 511.40075

^{1}H NMR, $CDCl_3$, 400MHz



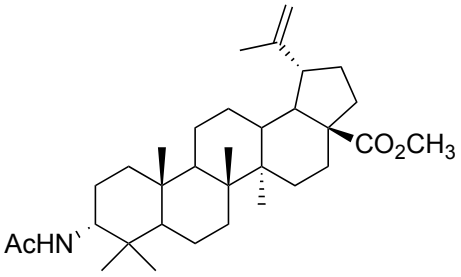
25

^{13}C NMR, $CDCl_3$, 400MHz



25

3α-Amido Methyl Betulinate (24)



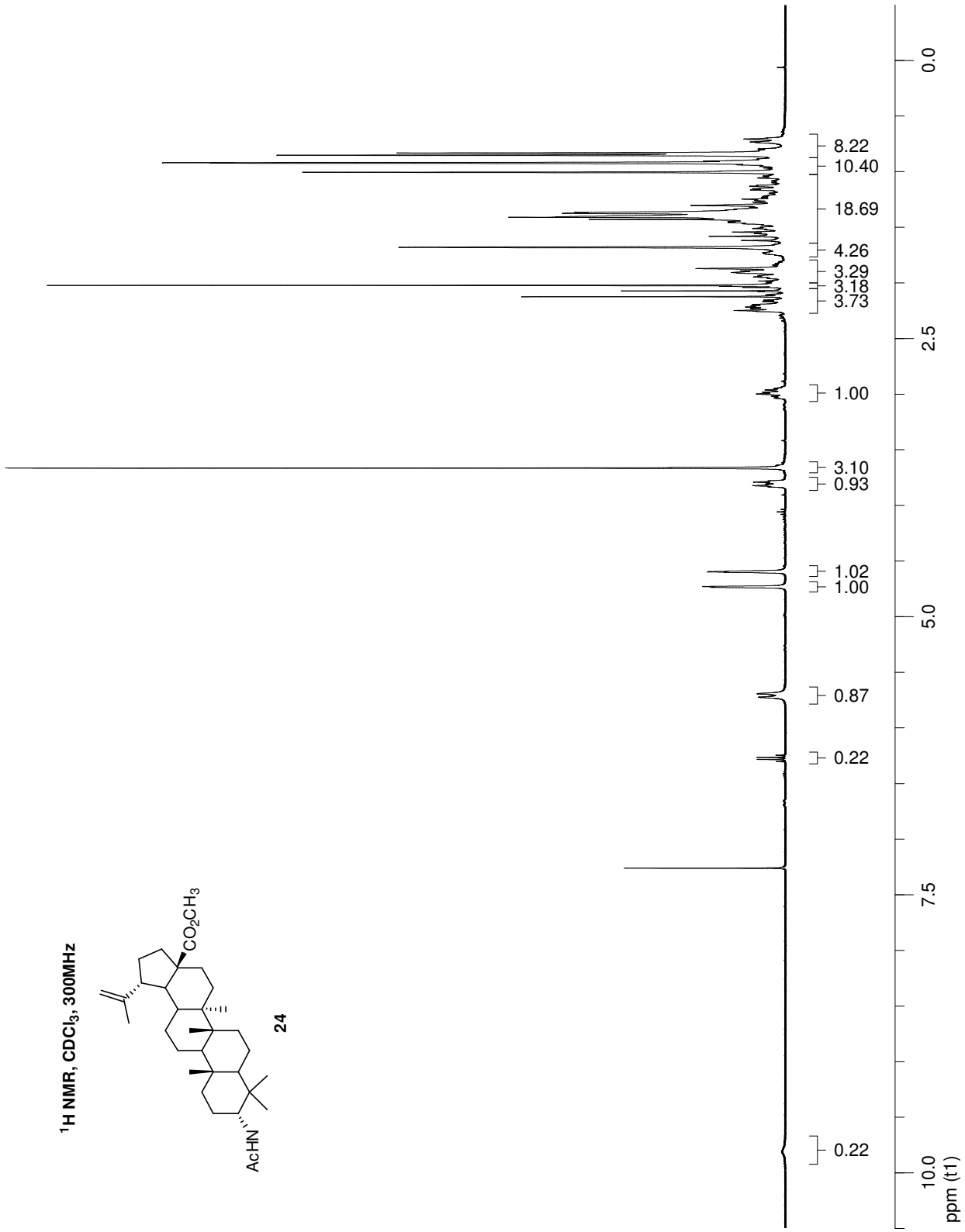
24

The procedure giving **25** also gave **24** as a white powder after silica gel chromatography (18 mg, 16%).

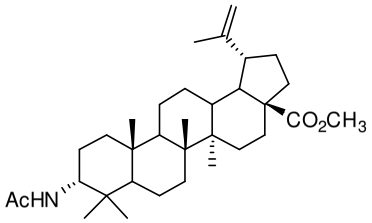
^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 5.71 (d, J=9.8 Hz, 1H), 4.73 (d, J=2.0 Hz, 1H), 4.60 (dd, J=2.1, 1.4 Hz), 3.81 (dt, J=9.8, 3.2 Hz, 1H), 3.66 (s, 3H), 3.04-2.95 (m, 1H), 2.02 (s, 3H), 1.68 (s, 3H), 1.01 (s, 3H), 0.923 (s, 3H), 0.919 (s, 3H), 0.85 (s, 3H), 0.83 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 176.6, 169.3, 150.5, 109.6, 56.5, 53.9, 52.0, 51.3, 50.8, 49.4, 46.9, 42.5, 40.8, 38.1, 37.4, 36.9, 36.4, 34.5, 34.1, 32.1, 30.6, 29.6, 28.3, 25.5, 23.8, 23.3, 22.9, 20.7, 19.4, 18.1, 16.02, 15.96, 14.9

HRMS: Calculated for $C_{33}H_{53}NO_3$, 511.40254; Found 511.40345

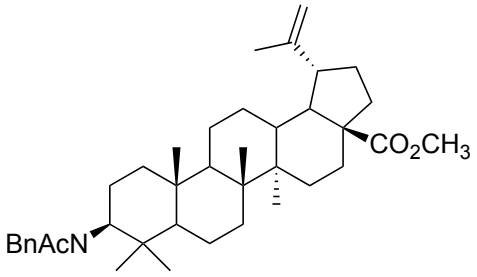

^{1}H NMR, $CDCl_3$, 300MHz
CO_2CH_3
AcHN
24
8.22
10.40
18.69
4.26
3.29
3.18
3.73
1.00
3.10
0.93
1.02
1.00
0.87
0.22
0.22
0.0
2.5
5.0
7.5
10.0
ppm (t1)

^{13}C NMR, $CDCl_3$, 400MHz



24

3β-(N-benzyl)acetamido Methyl Betulinate (26)



26

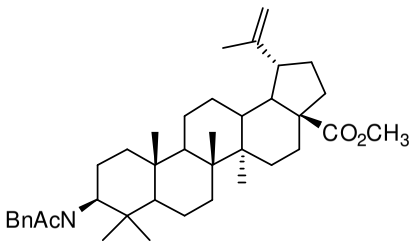
A solution of **9** (100 mg, 0.214 mmol), benzylamine (0.14 mL, 1.28 mmol), glacial acetic acid (0.04 mL, 0.75 mmol), $NaBH_3CN$ (9.4 mg, 0.15 mmol) and activated 3 Å molecular sieves in MeOH (10 mL) was stirred for two days. Additional $NaBH_3CN$ (10 mg, 0.16 mmol) was added and stirring continued for three days. The solution was filtered and acidified to pH ~1 with 30% HCl. The solvent was evaporated, water added (10 mL) and the mixture extracted with Et_2O (3x8 mL). The aqueous layer was made basic (pH 10) by adding K_2CO_3 and extracted with EtOAc (2x10 mL). All organic layers were combined, dried ($MgSO_4$), filtered and solvent evaporated. A white precipitate formed in the aqueous layer, which was filtered off and combined with the organic extracts to give a white solid (123 mg).

The white solid was dissolved in DCM (6 mL) with Et_3N (0.30 mL, 2.15 mmol), Ac_2O (0.16 mL, 1.70 mmol), and DMAP (3 mg). After stirring overnight at rt, DCM (10 mL) and 5% HCl (10 mL) was added and stirred 1 hr. Layers were separated and the organic layer was washed with H_2O (10 mL) and brine (10 mL), dried ($MgSO_4$), filtered and solvent evaporated to give a pale oil (142 mg). Separation of the oil by silica gel chromatography using a hexanes/EtOAc gradient gave **26** as a white powder (43 mg, 33%) and **18** as white powder (43 mg, 40%). ^1H NMR of **26** gave a 77:23 ratio of rotamers.

^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.36-7.13 (m, 5H), 4.88 (d, J=16.2 Hz, CH_2Ph minor rotamer, 1H), 4.72 (d, J=1.7 Hz, H-29, 1H), 4.64 (dd, J=12.5, 3.1 Hz, H-3 major rotamer, 1H), 4.59 (br s, H-29 [1H] and CH_2Ph major rotamer [2H]), 4.30 (d, J=15.9 Hz, CH_2Ph minor rotamer, 1H), 3.66 (s, CO_2CH_3 minor rotamer, 3H), 3.65 (s, CO_2CH_3 major rotamer, 3H), 3.52 (dd, J=12.6, 3.5 Hz, H-3 minor rotamer, 1H), 3.03-2.94 (m, 1H), 2.27 (s, $NCOCH_3$ minor rotamer, 3H), 1.97 (s, $NCOCH_3$ major rotamer, 3H), 1.68 (s, 3H), 0.96 (s, 3H), 0.89 (s, 6H), 0.86 (s, 3H), 0.79 (s, 3H)

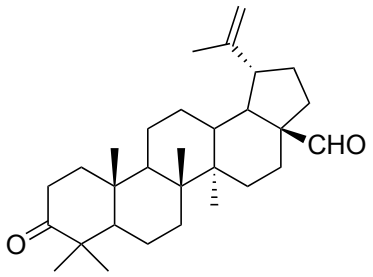
HRMS: Calculated for $C_{40}H_{59}NO_3$, 601.44949; Found 601.44734

^{1}H NMR, $CDCl_3$, 300MHz



26

Betulonic Aldehyde (27)

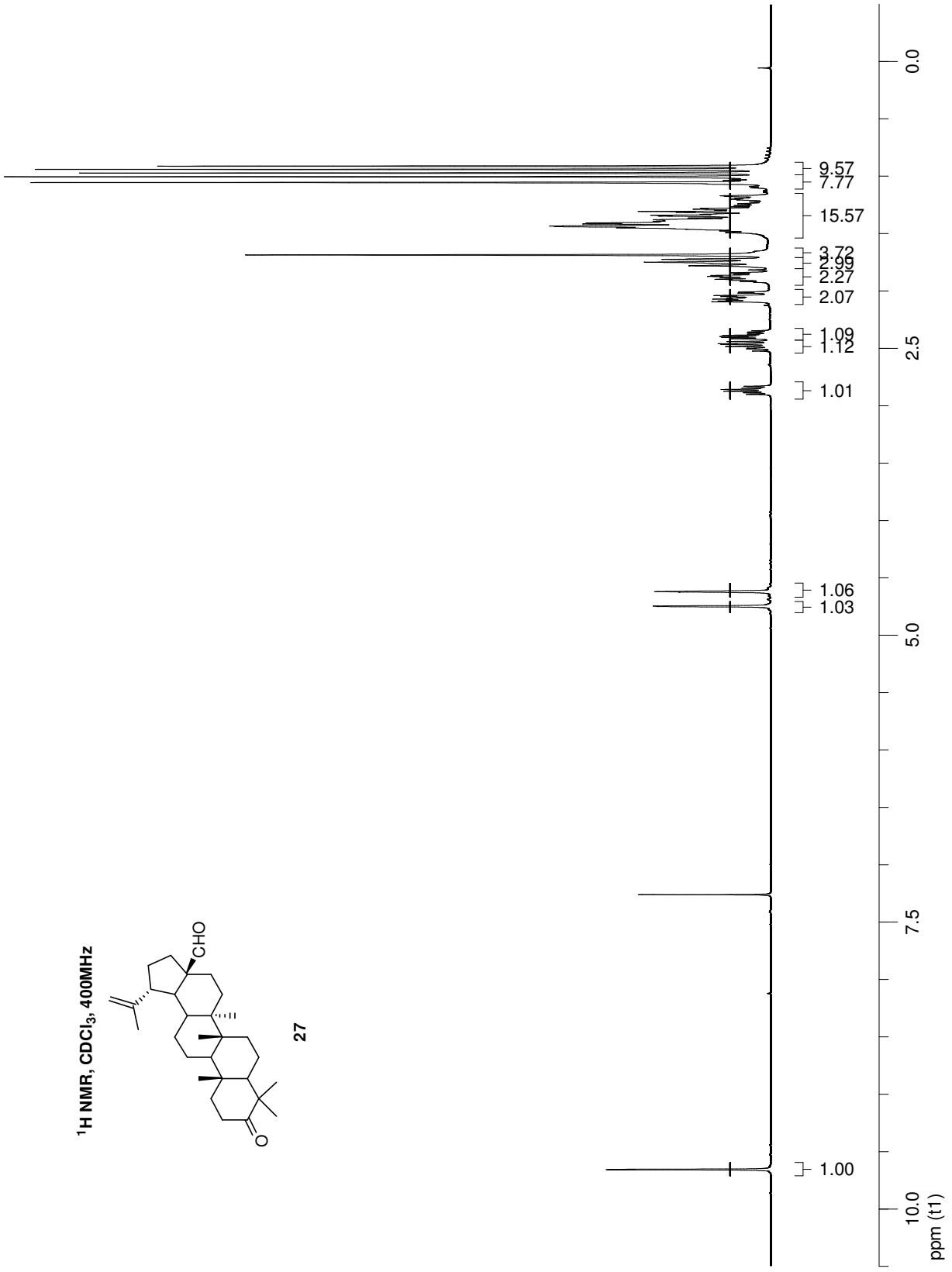


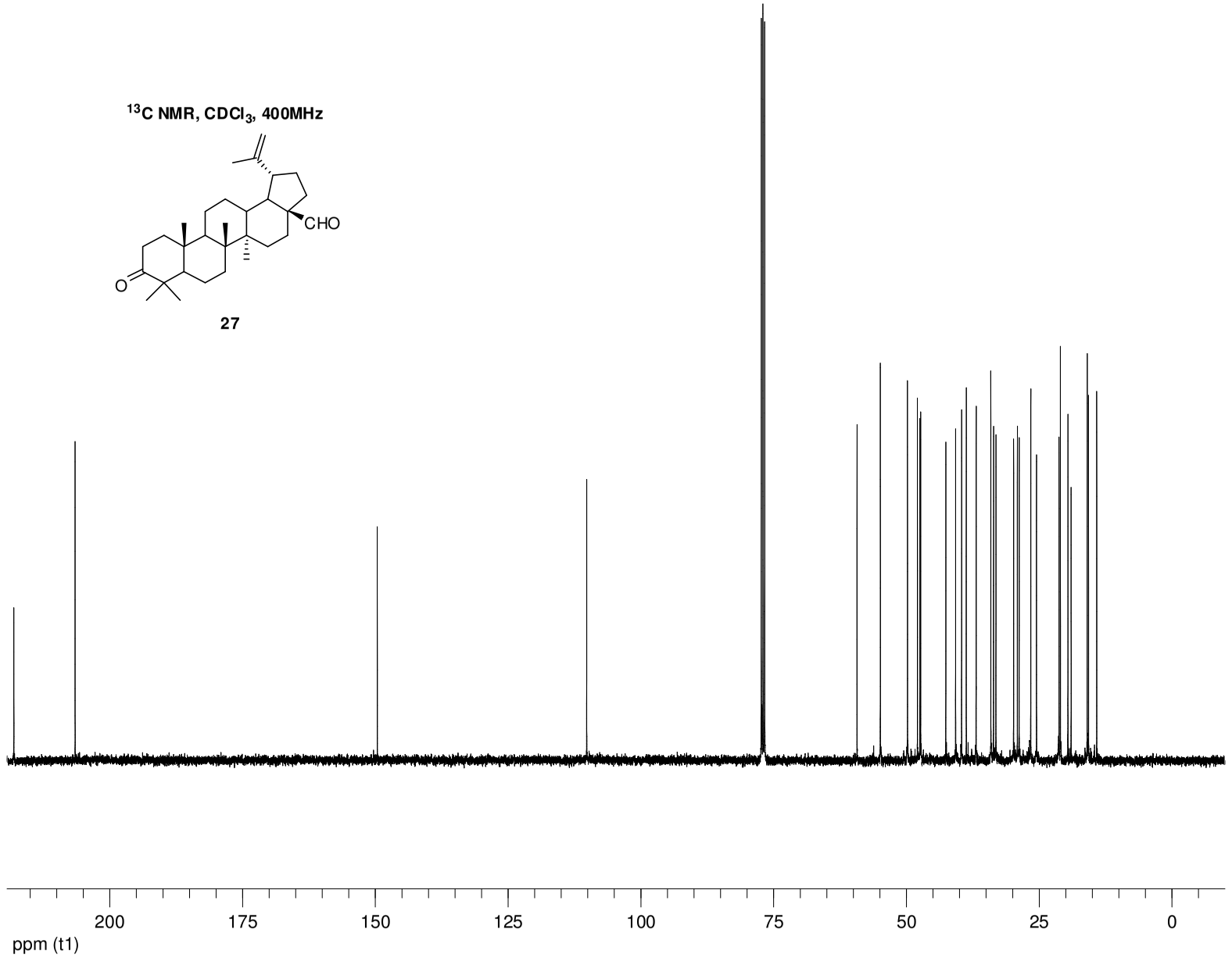
27

A solution of **11** (4.00 g, 9.04 mmol) in acetone (250 mL) was cooled to 0 ^{o}C. Fresh Jones reagent was prepared by dissolving CrO_3 (2.71 g, 27.1 mmol) in H_2O (5.3 mL) and acidifying with H_2SO_4 (2.32 mL), and then added dropwise to the cooled acetone solution. After an orange colour persisted for 5 minutes, the reaction was quenched with MeOH (15 mL) and stirred 20 min. The solvent was evaporated and the residue dissolved in EtOAc (200 mL). It was washed with H_2O (3x100 mL) and brine (50 mL), dried ($MgSO_4$), filtered and solvent evaporated to give an orange solid (4.00 g). The solid was purified by silica gel chromatography using 6.5% EtOAc in hexanes to give **27** as a white powder (1.43 g, 36%).

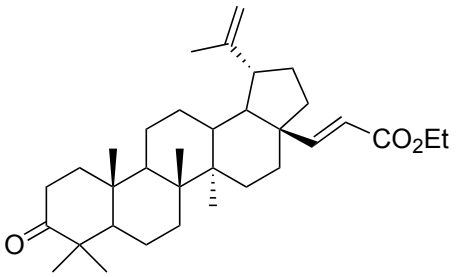
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.66 (d, J=1.5 Hz, 1H), 4.75 (d, J=1.7 Hz, 1H), 4.62 (br d, J=1.4 Hz, 1H), 2.87 (td, J=11.2, 5.8 Hz, 1H), 2.48 (ddd, J=15.7, 9.8, 7.5 Hz, 1H), 2.38 (ddd, J=15.7, 7.6, 4.4 Hz, 1H), 2.09-2.00 (m, 2H), 1.69 (s, 3H), 1.05 (s, 3H), 1.00 (s, 3H), 0.97 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 218.1, 206.5, 149.6, 110.2, 59.3, 54.9, 49.8, 47.9, 47.4, 47.3, 42.6, 40.7, 39.6, 38.7, 36.9, 34.1, 33.6, 33.1, 29.8, 29.1, 28.8, 26.6, 25.5, 21.2, 21.0, 19.6, 19.0, 15.9, 15.7, 14.2


^{1}H NMR, $CDCl_3$, 400MHz
CHO
O
27
9.57
7.77
15.57
3.72
2.99
2.27
2.07
1.09
1.12
1.01
1.06
1.03
1.00
0.0
2.5
5.0
7.5
10.0
ppm (t1)


^{13}C NMR, $CDCl_3$, 400MHz
CHO
O
27
200
175
150
125
100
75
50
25
0
ppm (t1)

C-28 Extended Ethyl Betulonate (28)



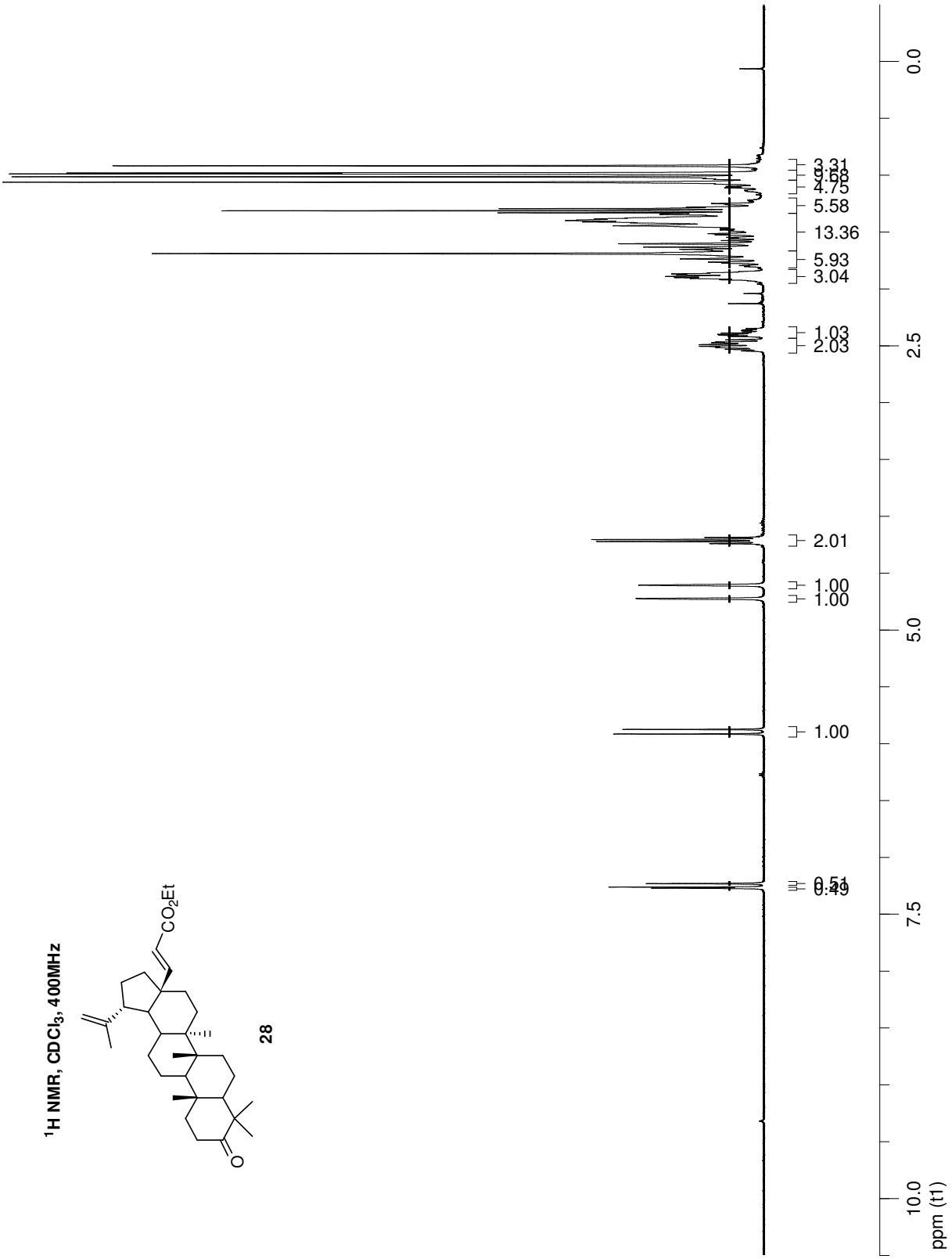
28

To a stirred suspension of 60% NaH in mineral oil (246 mg, 6.15 mmol) in dry toluene (8 mL) was slowly added triethyl phosphonoacetate (1.29 mL, 6.50 mmol). After 20 min, a solution of **27** (900 mg, 2.05 mmol) in dry toluene (7 mL) was added and heated to reflux for 4 hrs. The solution was cooled to 0 ^{o}C and quenched with saturated NH_4Cl solution (15 mL). It was diluted with hexanes (100 mL) and water (30 mL), shaken and layers separated. The organic extract was washed with water (50 mL) and brine (40 mL), dried ($MgSO_4$), filtered and solvent evaporated to give a colourless oil (1.29 g). Purification of the oil by silica gel chromatography using a hexanes/EtOAc gradient gave **28** as white foam (888 mg, 85%).

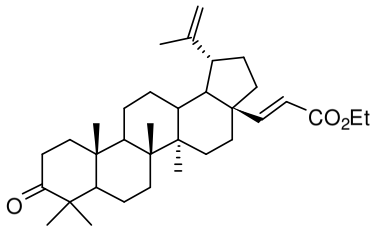
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.25 (d, J=16.2 Hz, 1H), 5.89 (d, J=16.2 Hz, 1H), 4.72 (d, J=1.2 Hz, 1H), 4.61 (s, 1H), 4.21 (q, J=7.1 Hz, 2H), 2.55-2.45 (m, 2H), 2.38 (ddd, J=15.6, 7.5, 4.4 Hz, 1H), 1.69 (s, 3H), 1.31 (t, J=7.2, 3H), 1.06 (s, 3H), 1.01 (s, 3H), 0.99 (s, 3H), 0.98 (s, 3H), 0.92 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 218.1, 167.1, 153.5, 149.7, 120.4, 110.1, 60.3, 54.9, 50.0, 49.7, 49.6, 47.6, 47.3, 42.9, 40.7, 39.6, 38.9, 38.7, 36.9, 34.1, 33.6, 33.3, 29.7, 27.7, 26.6, 25.2, 21.3, 21.0, 19.6, 19.3, 15.9, 15.8, 14.6, 14.3

HRMS: Calculated for $C_{34}H_{52}O_3$, 508.39165; Found 508.38972

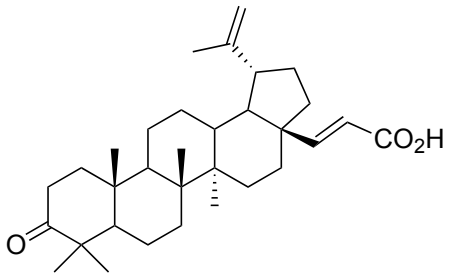

^{1}H NMR, $CDCl_3$, 400MHz
28
CO_2Et
O
3.31
9.68
4.75
5.58
13.36
5.93
3.04
1.03
2.03
2.01
1.00
1.00
1.00
0.51
0.49
0.0
2.5
5.0
7.5
10.0
ppm (t1)

^{13}C NMR, $CDCl_3$, 400MHz



28

C-28 Extended Betulonic Acid (29)



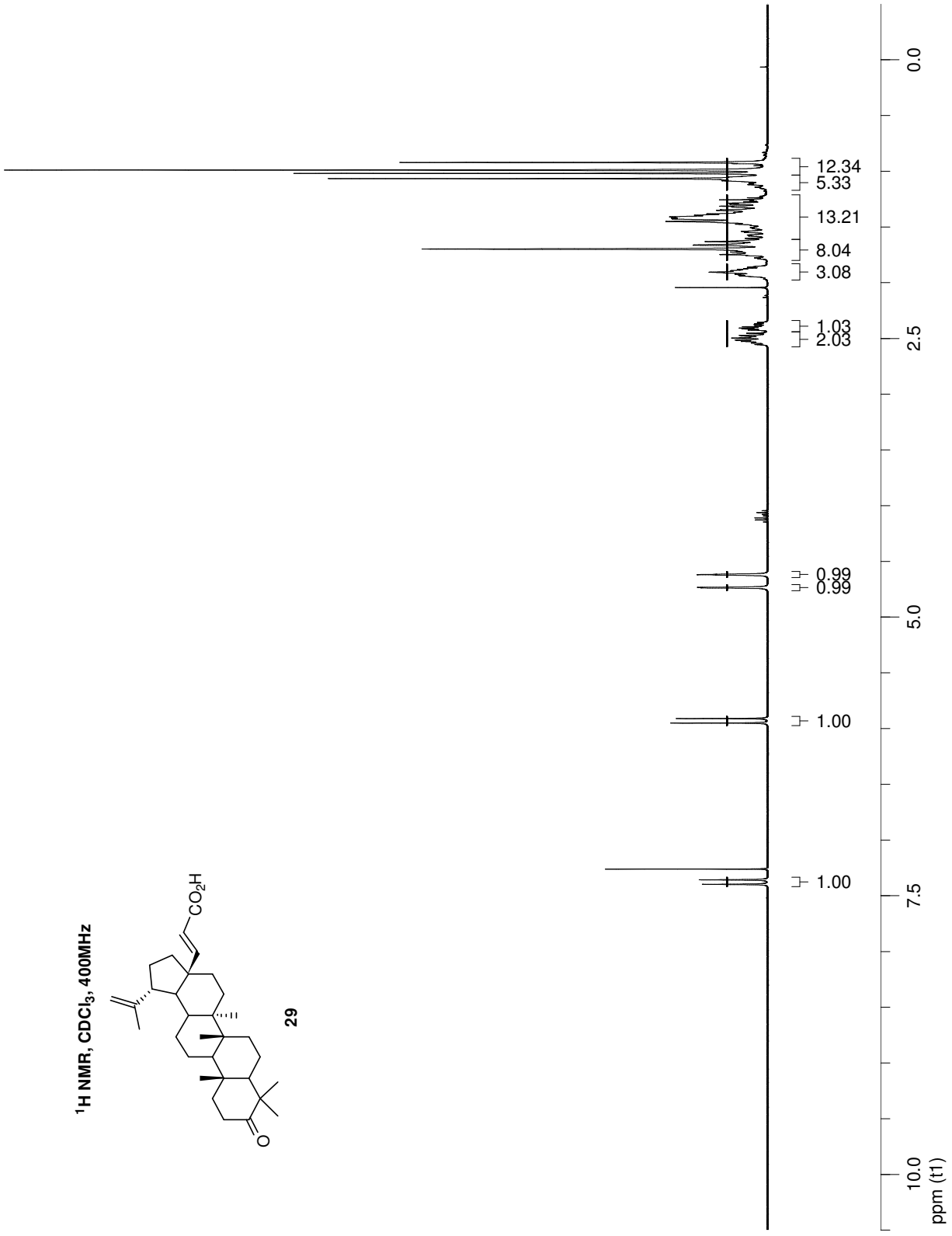
29

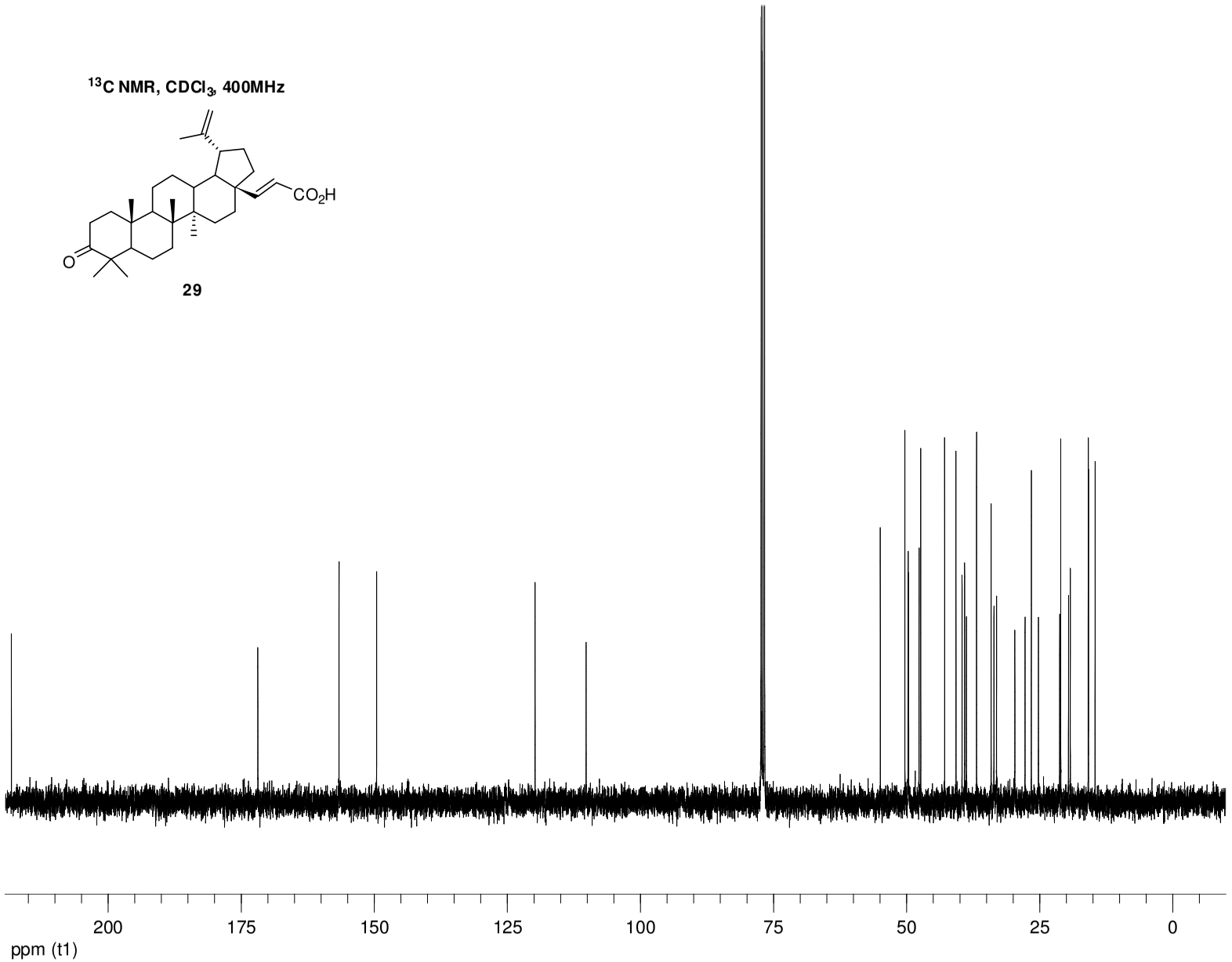
To a stirred solution of **28** (71 mg, 0.139 mmol) in MeOH (6 mL) was added water (0.2 mL) and KOH pellets (313 mg, 5.58 mmol). It was heated to 45 ^{o}C for 4 hrs until TLC showed complete disappearance of starting material (9:1 hexanes:EtOAc). The solution was cooled to rt, acidified with 30% HCl and solvent evaporated. It was diluted with water (5 mL) and extracted with EtOAc (3x15 mL). The organic extract was washed with brine (2x10 mL), dried ($MgSO_4$), filtered and solvent evaporated to give white foam (67 mg). Purification of the foam by silica gel chromatography using 4:1 hexanes:EtOAc gave **29** as white foam (56 mg, 84%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.38 (d, J=16.2 Hz, 1H), 5.93 (d, J=16.1Hz, 1H), 4.73 (d, J=1.7 Hz, 1H), 4.62 (d, J=1.4 Hz, 1H), 2.56-2.45 (m, 2H), 2.39 (ddd, J=15.6, 7.5, 4.3 Hz, 1H), 1.70 (s, 3H), 1.07 (s, 3H), 1.02 (s, 3H), 0.99 (s, 6H), 0.92 (s, 3H)

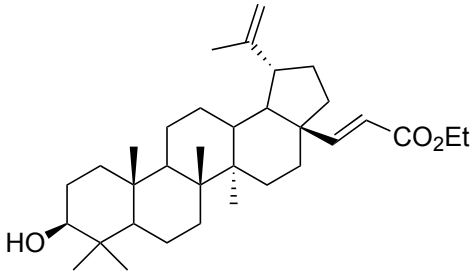
^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 218.2, 171.9, 156.6, 149.6, 119.8, 110.2, 54.9, 50.3, 49.7, 49.6, 47.7, 47.3, 42.9, 40.7, 39.6, 39.1, 38.8, 36.9, 34.1, 33.6, 33.1, 29.7, 27.8, 26.6, 25.2, 21.2, 21.0, 19.6, 19.3, 15.9, 15.8, 14.6

HRMS: Calculated for $C_{32}H_{48}O_3$, 480.36035; Found 480.35958


^{1}H NMR, $CDCl_3$, 400MHz
29
CO_2H
O
12.34
5.33
13.21
8.04
3.08
1.03
2.03
0.99
0.99
1.00
1.00
10.0
7.5
5.0
2.5
0.0
ppm (t1)


^{13}C NMR, $CDCl_3$, 400MHz
CO_2H
O
29
200
175
150
125
100
75
50
25
0
ppm (t1)

C-28 Extended Ethyl Betulinate (30)



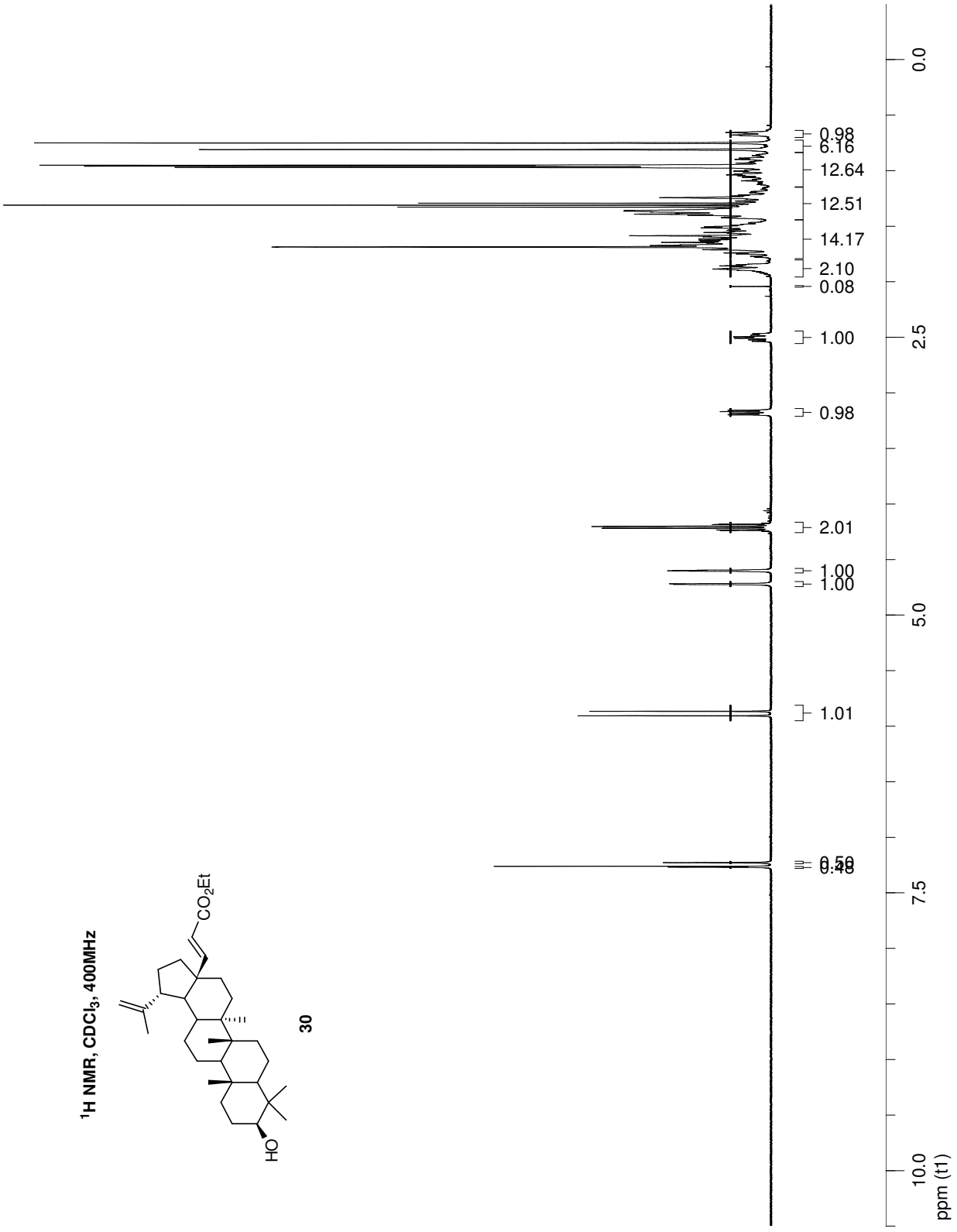
30

To a stirred solution of **28** (200 mg, 0.393 mmol) in MeOH (10 mL) at 0 ^{o}C was added $NaBH_4$ (15 mg). After 30 min, it was acidified with 10% HCl (10 mL), the solvent was evaporated and the residue extracted with EtOAc (2x20 mL). The extract was washed with water (15 mL) and brine (10 mL), dried ($MgSO_4$), filtered and solvent evaporated to give a white solid (185 mg). Purification of the solid by silica gel chromatography using a hexanes/EtOAc gradient gave **30** as a white powder (126 mg, 63%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.25 (d, J=16.2 Hz, 1H), 5.89 (d, J=16.2 Hz, 1H), 4.60 (dd, J=2.0, 1.6 Hz, 1H), 4.21 (qd, J=7.2, 1.1 Hz, 2H), 3.18 (dd, J=11.3, 5.0 Hz, 1H), 2.54-2.47 (m, 1H), 1.69 (d, J=0.5 Hz, 3H), 1.31 (t, J=7.1, 3H), 0.97 (s, 3H), 0.96 (s, 3H), 0.95 (s, 3H), 0.81 (s, 3H), 0.75 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 167.1, 153.6, 149.8, 120.3, 110.0, 79.0, 60.2, 55.3, 50.4, 50.0, 49.7, 47.7, 42.8, 40.8, 38.84, 38.75, 38.7, 37.1, 34.3, 33.4, 29.7, 28.0, 27.8, 27.4, 25.2, 20.7, 19.2, 18.3, 16.1, 16.0, 15.3, 14.7, 14.3

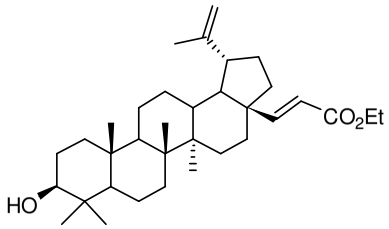
HRMS: Calculated for $C_{34}H_{54}O_3$, 510.40730; Found 510.40957



^{1}H NMR, $CDCl_3$, 400MHz

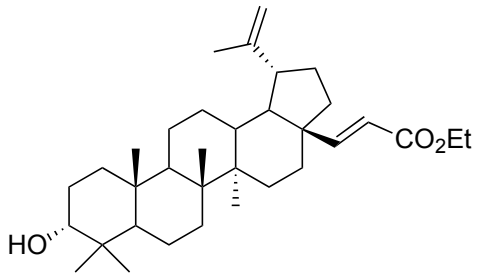
30

^{13}C NMR, $CDCl_3$, 400MHz



30

C-28 Extended Ethyl 3-Epi-Betulinate (31)



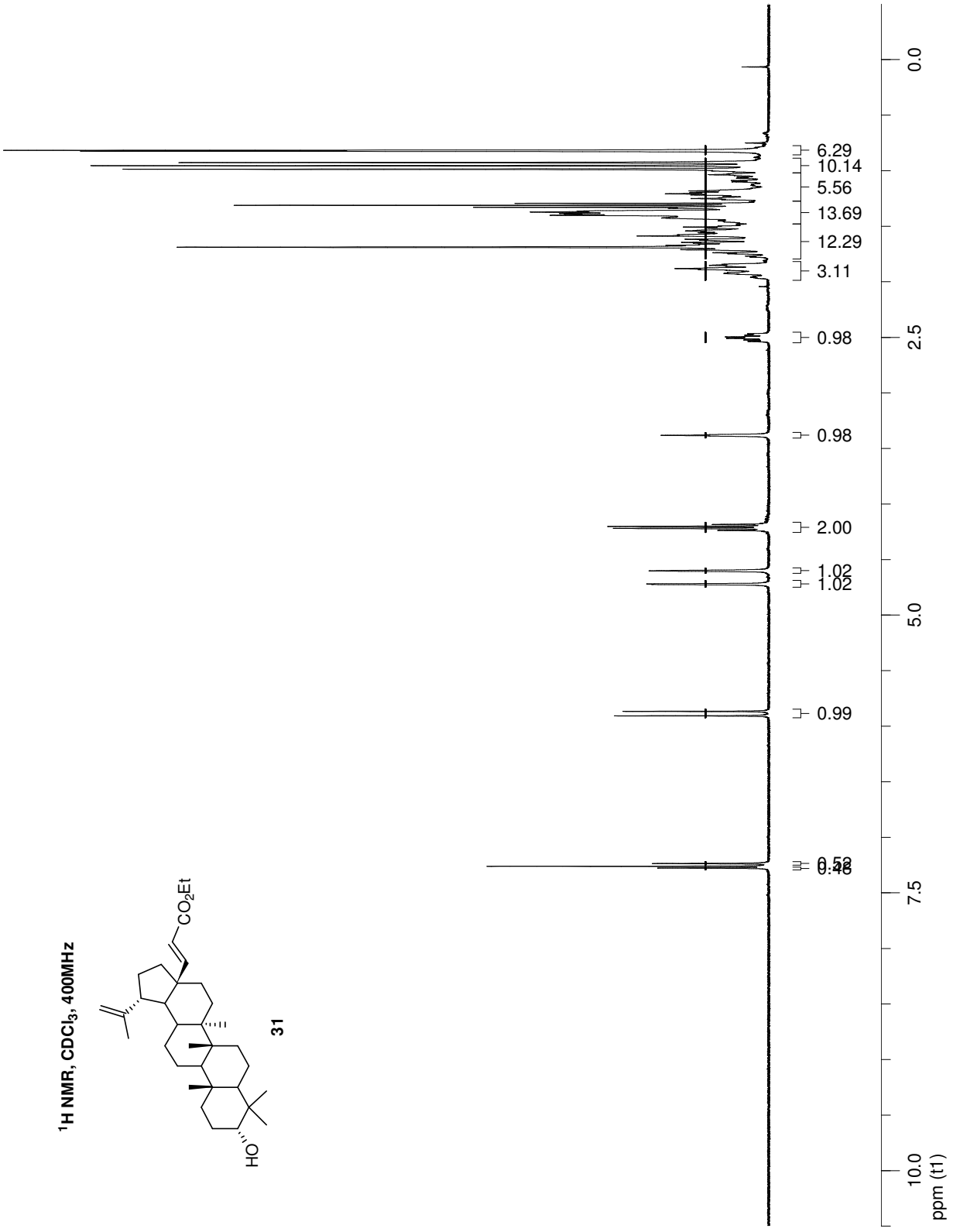
31

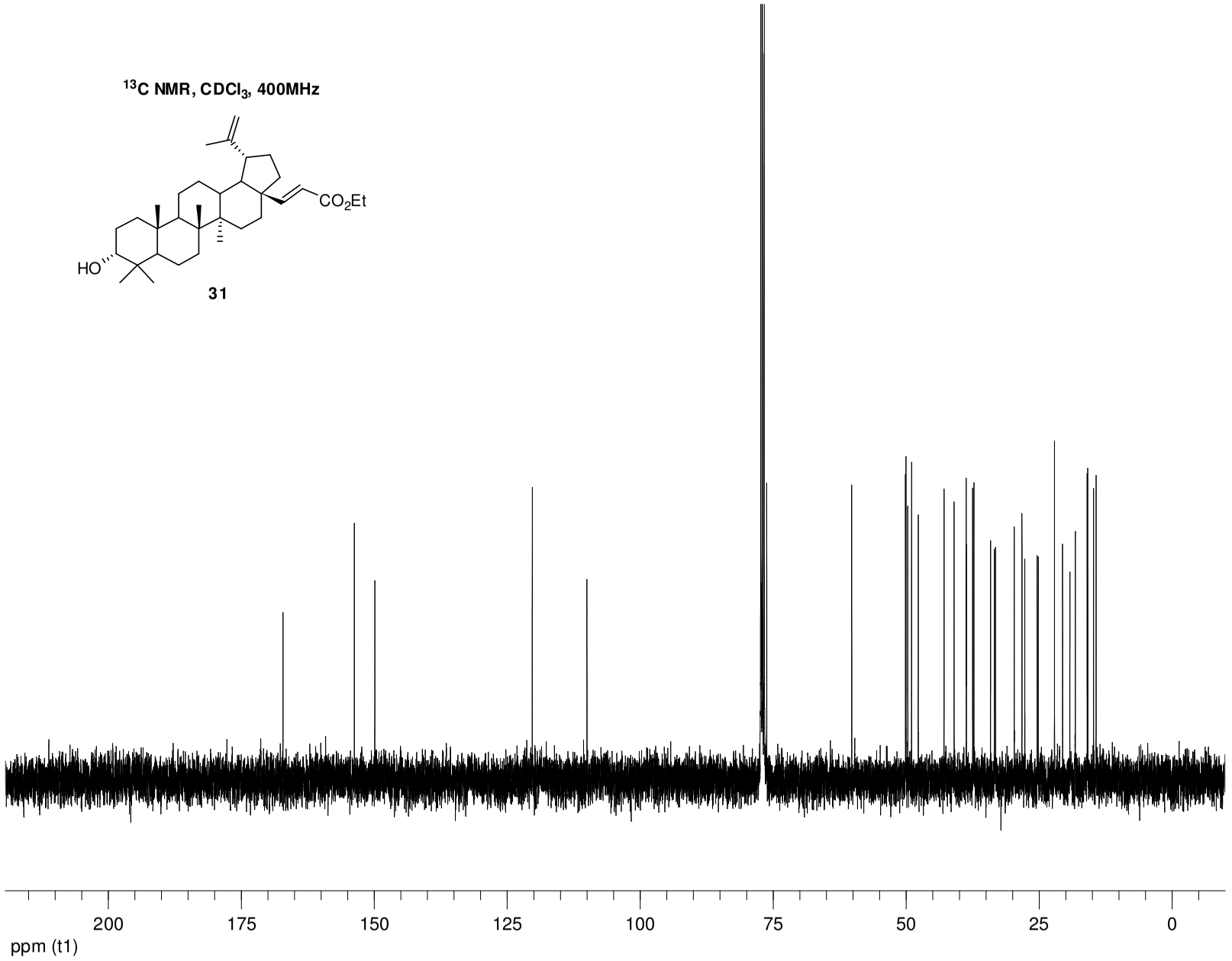
The procedure giving **30** also gave **31** as white foam after silica gel chromatography (7 mg, 3%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.26 (d, J=16.1 Hz, 1H), 5.89 (d, J=16.2 Hz, 1H), 4.72 (d, J=1.7 Hz, 1H), 4.60 (s, 1H), 4.21 (q, J=7.0 Hz, 2H), 3.38 (t, J=2.4 Hz, 1H), 2.54-2.47 (m, 1H), 1.68 (s, 3H), 1.31 (t, J=7.1, 3H), 0.99 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H), 0.82 (s, 3H), 0.82 (s, 3H)

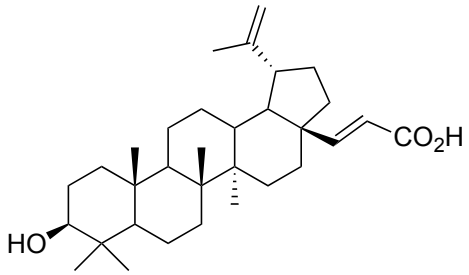
^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 167.1, 153.7, 149.9, 120.3, 110.0, 76.2, 60.2, 50.1, 50.0, 49.7, 49.0, 47.7, 42.9, 41.0, 38.71, 38.66, 37.5, 37.3, 34.1, 33.4, 33.2, 29.7, 28.2, 27.7, 25.4, 25.2, 22.1, 20.6, 19.2, 18.2, 16.0, 15.9, 14.8, 14.3

HRMS: Calculated for $C_{34}H_{54}O_3$, 510.40730; Found 510.40740


^{1}H NMR, $CDCl_3$, 400MHz
CO_2Et
HO
31
6.29
10.14
5.56
13.69
12.29
3.11
0.98
0.98
2.00
1.02
1.02
0.99
0.52
0.48
0.0
2.5
5.0
7.5
10.0
ppm (t1)


^{13}C NMR, $CDCl_3$, 400MHz
HO
CO_2Et
31
200
175
150
125
100
75
50
25
0
ppm (t1)

C-28 Extended Betulinic Acid (32)



32

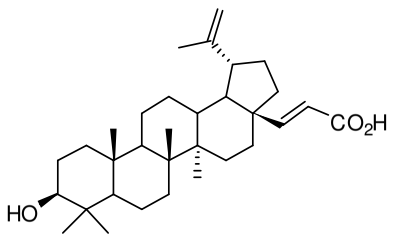
To a stirred solution of **30** (40 mg, 0.083 mmol) in MeOH (4 mL) was added water (0.1 mL) and KOH pellets (210 mg). It was heated to 45 ^{o}C for 5 hrs, cooled to rt, and solvent evaporated. The residue was acidified with 30% HCl, diluted with water (5 mL) and extracted with EtOAc (2x20 mL). The extract was washed with brine (10 mL), dried ($MgSO_4$), filtered and solvent evaporated. Purification of the residue by silica gel chromatography using a hexanes/EtOAc gradient gave **32** as a white powder (18 mg, 45%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.37 (d, J=16.2 Hz, 1H), 5.92 (d, J=16.1 Hz, 1H), 4.73 (d, J=1.7 Hz, 1H), 4.61 (dd, J=1.7, 1.4 Hz, 1H), 3.19 (dd, J=11.2, 5.0 Hz, 1H), 2.55-2.48 (m, 1H), 1.70 (s, 3H), 0.98 (s, 3H), 0.96 (s, 3H), 0.95 (s, 3H), 0.81 (s, 3H), 0.76 (s, 3H)

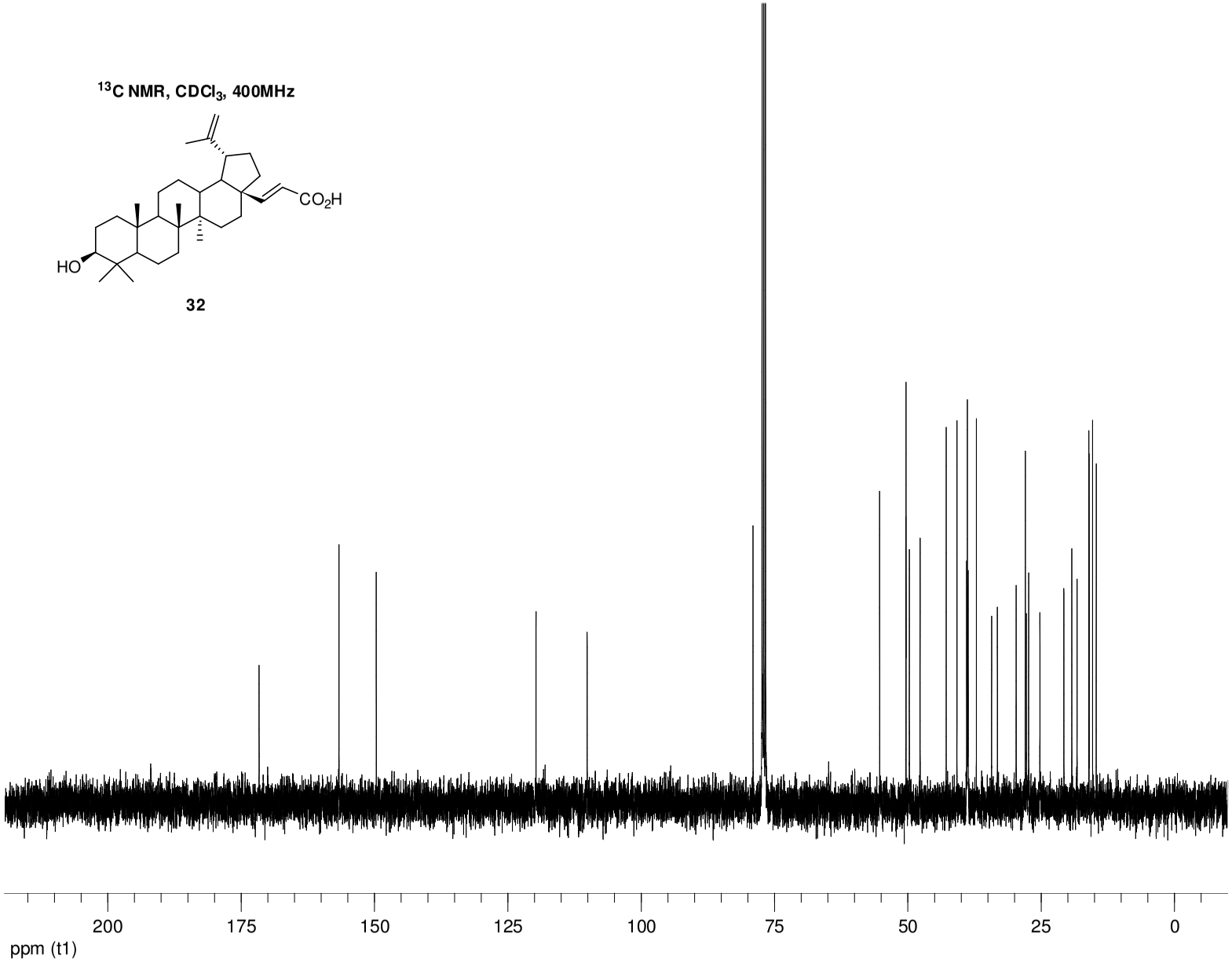
^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 171.6, 156.6, 149.7, 119.7, 110.1, 79.0, 55.3, 50.4, 50.3, 49.7, 47.7, 42.8, 40.8, 39.0, 38.84, 38.76, 38.68, 37.1, 34.3, 33.2, 29.7, 28.0, 27.8, 27.3, 25.2, 20.7, 19.3, 18.3, 16.1, 16.0, 15.4, 14.7

HRMS: Calculated for $C_{32}H_{50}O_3$, 482.37600; Found 482.37416

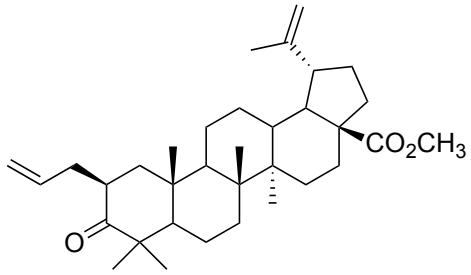
^{1}H NMR, $CDCl_3$, 400MHz



32


^{13}C NMR, $CDCl_3$, 400MHz
HO
CO_2H
32
200
175
150
125
100
75
50
25
0
ppm (t1)

2β-allyl Methyl Betulonate (33)

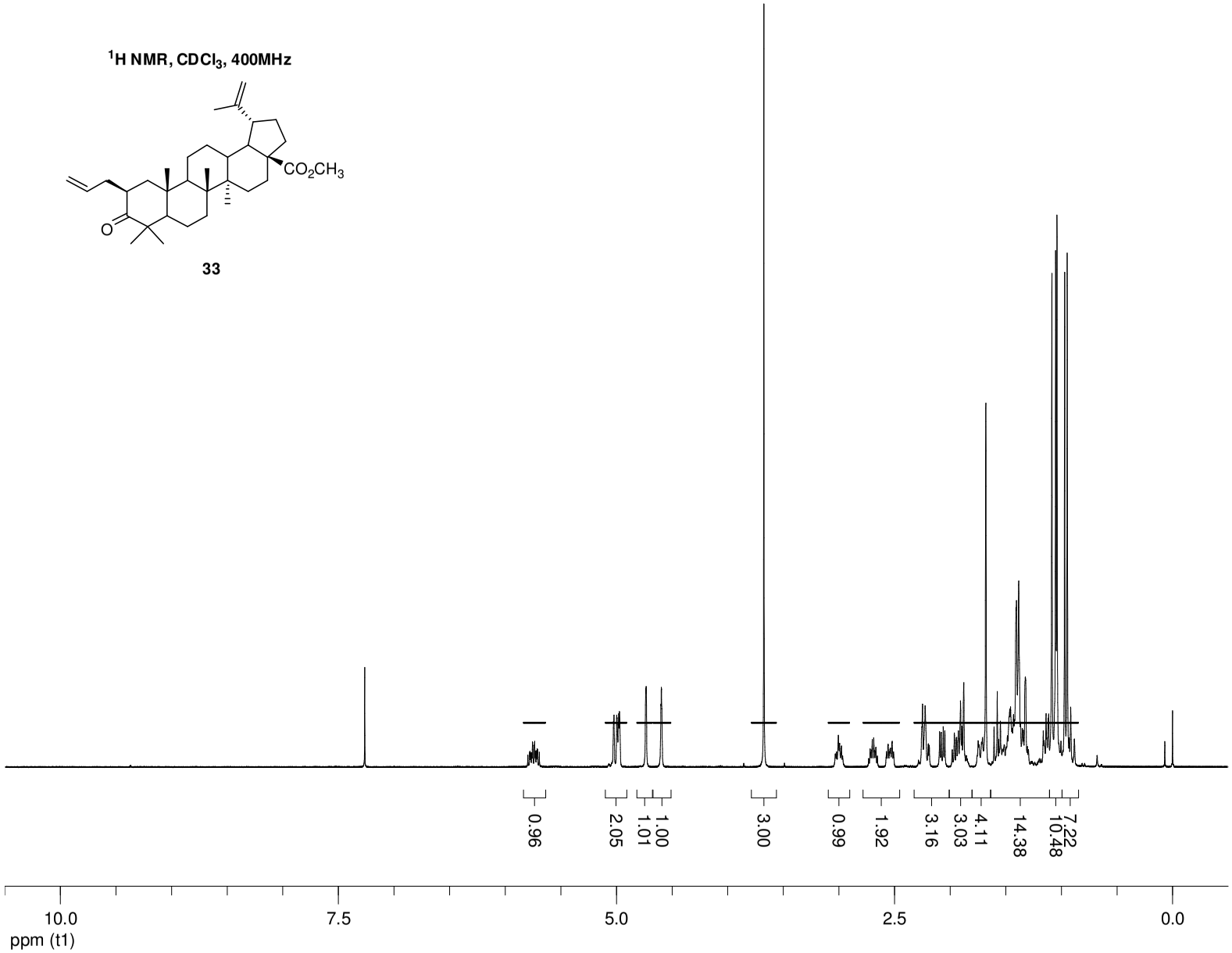


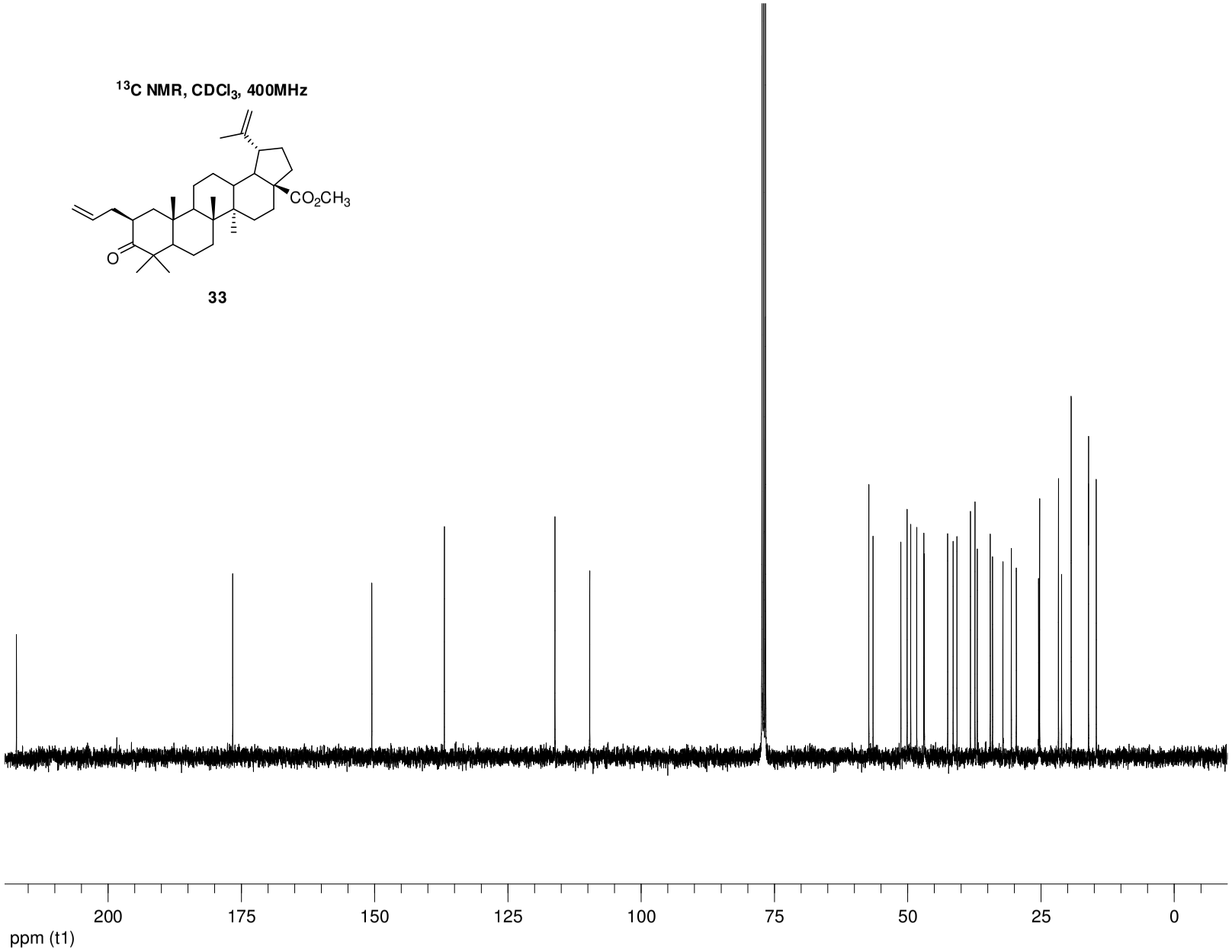
33

A solution of fresh LDA was prepared by dissolving dry *i*-Pr_2NH (25 μL, 0.18 mmol) in dry THF (4 mL) under N_2, cooling to -78 ^oC, adding 1.6 M n-BuLi in hexanes (109 μL, 0.17 mmol) and stirring for 15 min. To the LDA was added a solution of **9** (80 mg, 0.17 mmol) in dry THF (1 mL) under N_2. After 30 min, freshly distilled allyl bromide (16 μL, 0.19 mmol) was added and stirred 2 hrs before warming to 0 ^oC. After 2 hrs, the solution was warmed to rt overnight. It was then quenched with saturated NH_4Cl solution (10 mL) and solvent evaporated. The residue was dissolved in EtOAc (40 mL), washed with brine (2x10 mL), dried (Na_2SO_4), filtered and solvent evaporated to give a colourless oil (85 mg). Separation of the oil by silica gel chromatography using a hexanes/EtOAc gradient gave **33** as white foam (13 mg, 15%) along with recovered starting material **9** (43 mg, 54%)

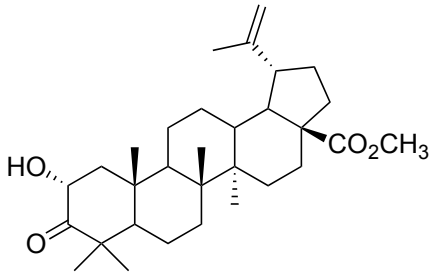
^{1}H NMR ($CDCl_3$, 400 MHz, centered on TMS): δ (ppm) 5.80-5.69 (m, 1H), 5.03-4.96 (m, 2H), 4.74 (d, J=2.0 Hz, 1H), 4.60 (dd, J=2.0, 1.4 Hz, 1H), 3.68 (s, 3H), 3.03-2.97 (m, 1H), 2.73-2.65 (m, 1H), 2.57-2.51 (m, 1H), 2.26-2.19 (m, 2H), 2.07 (dd, J=13.2, 5.7 Hz, 1H), 1.68 (s, 3H), 1.08 (s, 3H), 1.05 (s, 3H), 1.04 (s, 3H), 0.97 (s, 3H), 0.95 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 217.2, 176.6, 150.5, 136.9, 116.2, 109.7, 57.3, 56.5, 51.3, 50.1, 49.4, 48.3, 47.0, 46.9, 42.5, 41.5, 40.8, 38.2, 37.4, 37.0, 34.5, 34.1, 32.1, 30.6, 29.6, 25.4, 25.3, 21.7, 21.1, 19.3, 19.3, 16.10, 16.08, 14.6


^{1}H NMR, $CDCl_3$, 400MHz
CO_2CH_3
O
33
0.96
2.05
1.01
1.00
3.00
0.99
1.92
3.16
3.03
4.11
14.38
7.22
10.48
10.0
7.5
5.0
2.5
0.0
ppm (t1)


^{13}C NMR, $CDCl_3$, 400MHz
CO_2CH_3
O
33
200
175
150
125
100
75
50
25
0
ppm (t1)

2α-hydroxy Methyl Betulonate (36)



36

To a stirred solution of **9** (200 mg, 0.427 mmol) in dry DCM (8.7 mL) under N_2 at -78 ^oC was added dry Et_3N (0.60 mL, 4.27 mmol) followed by TMSOTf (0.39 mL, 2.14 mmol). After 1 hr the reaction was quenched with saturated $NaHCO_3$ solution (4 mL) and warmed to rt. Layers were separated and aqueous layer extracted with hexanes (3x5 mL). The organic extracts were combined, washed with brine (5 mL), dried ($MgSO_4$), filtered and evaporated to give crude silyl enol ether **34** (218 mg, 0.40 mmol; 95%) as a single spot on TLC (9:1 hexanes:EtOAc, Rf=0.79).

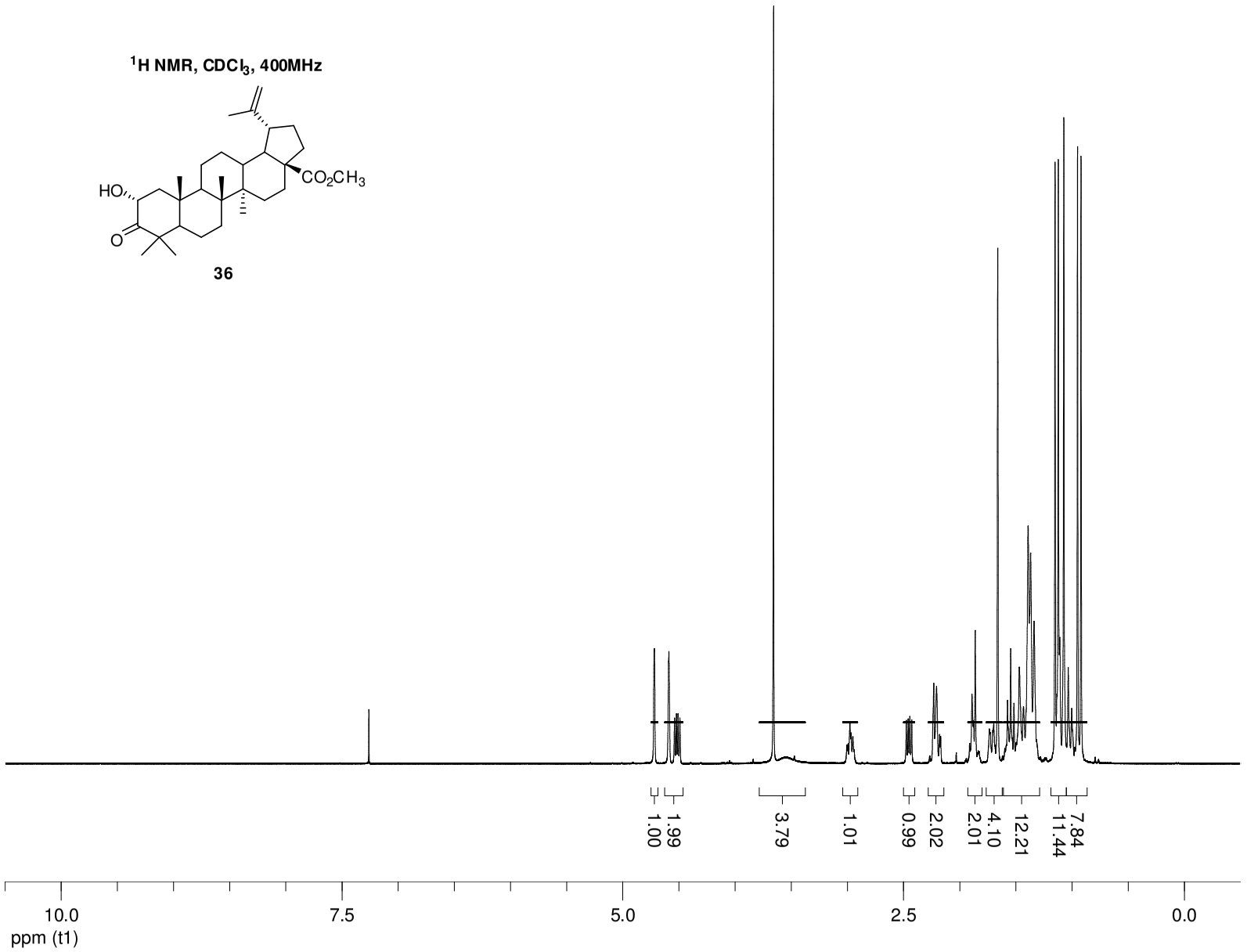
Crude **34** was dissolved in DCM (8 mL) and cooled to 0 ^oC. A solution of ~77% *m*CPBA (100 mg, 0.44 mmol) in DCM (10 mL) was cooled to 0 ^oC and added to **34**. After 1 hr 40 min TLC showed complete disappearance of starting material. The reaction was quenched with saturated $NaHCO_3$ (7 mL), stirred 30 min and layers separated. The organic layer was evaporated to give crude silyl ether **35**.

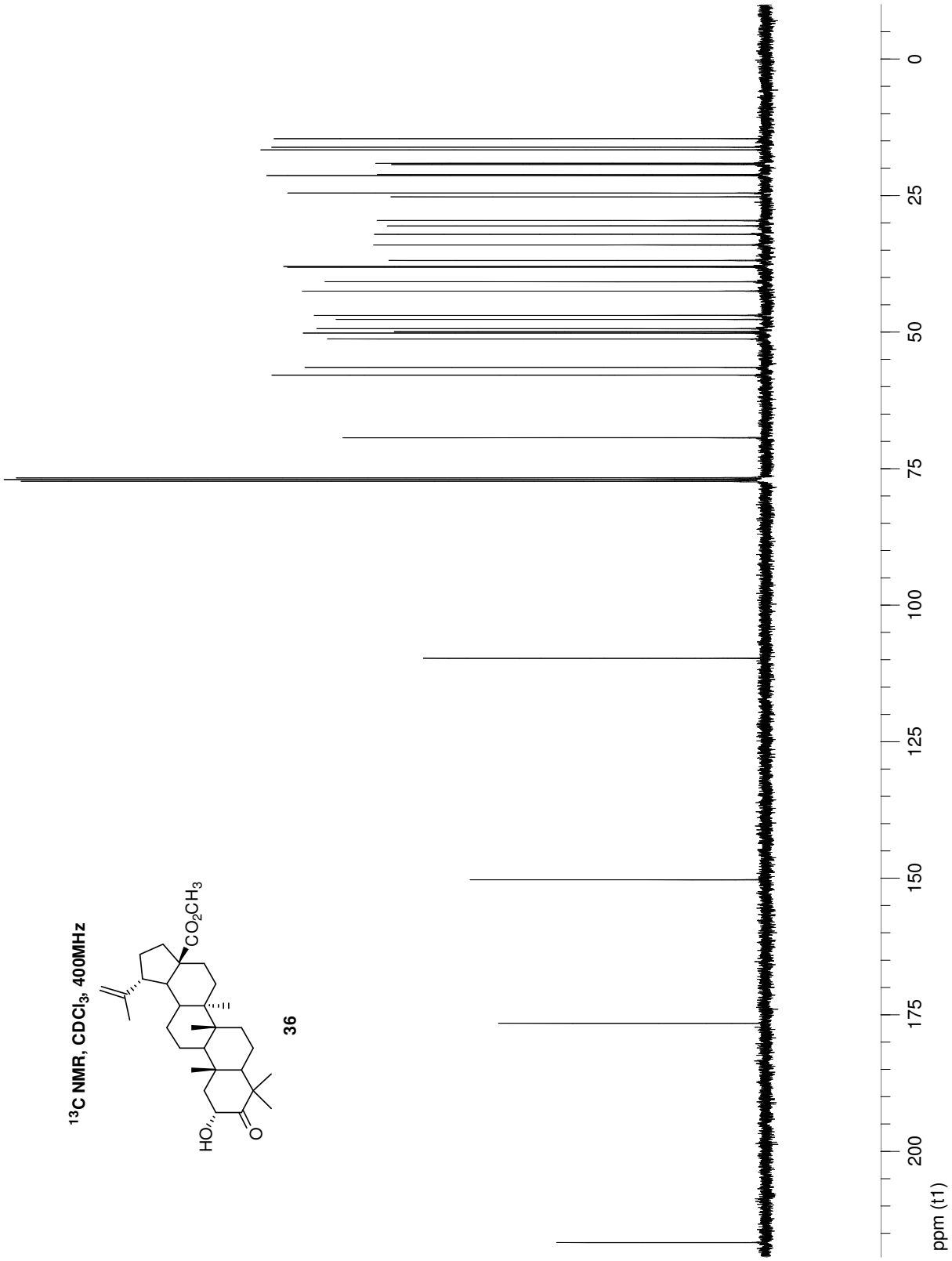
35 was dissolved in MeOH (10 mL) with 5% HCl (1 mL). After 30 min stirring, TLC showed hydrolysis was complete. Solvent was evaporated and the residue dissolved in EtOAc (20 mL). It was washed with water (8 mL) and brine (8 mL), dried ($MgSO_4$), filtered and solvent evaporated to give a colourless oil (203 mg). Separation of the oil by silica gel chromatography using 15% EtOAc in hexanes gave **36** as white foam (128 mg, 62%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.72 (br s, 1H), 4.59 (br s, 1H), 4.52 (dd, J=12.5, 6.7 Hz, 1H), 3.66 (s, 1H), 3.00-2.94 (m, 1H), 2.45 (dd, J=12.5, 6.7 Hz, 1H), 2.24-2.17 (m, 2H), 1.66 (s, 3H), 1.15 (s, 3H), 1.12 (s, 3H), 1.07 (s, 3H), 0.95 (s, 3H), 0.92 (s, 3H)

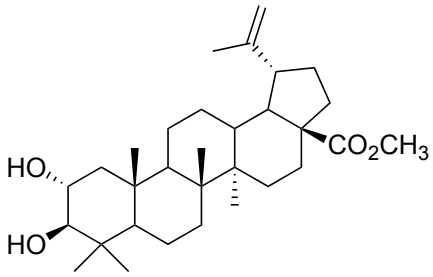
^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 216.7, 176.6, 150.3, 109.7, 69.3, 57.9, 56.4, 51.3, 50.2, 49.9, 49.4, 47.7, 46.9, 42.5, 40.8, 38.1, 38.0, 36.9, 34.0, 32.1, 30.5, 29.6, 25.2, 24.5, 21.3, 21.2, 19.3, 19.1, 16.6, 16.1, 14.6

HRMS: Calculated for $C_{31}H_{48}O_4$, 484.35526; Found 484.35454


^{1}H NMR, $CDCl_3$, 400MHz
HO
O
CO_2CH_3
36
1.00
1.99
3.79
1.01
0.99
2.02
2.01
4.10
12.21
11.44
7.84
10.0
7.5
5.0
2.5
0.0
ppm (t1)


^{13}C NMR, $CDCl_3$, 400MHz
CO_2CH_3
HO
O
36
0
25
50
75
100
125
150
175
200
ppm (t1)

2α-hydroxy Methyl Betulinate (37)



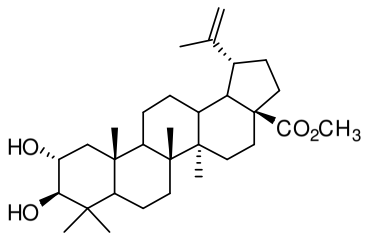
37

To a stirred solution of **36** (69 mg, 0.14 mmol) in MeOH (6 mL) at 0 ^{o}C was added $NaBH_4$ (22 mg, 0.58 mmol). After 30 min it was acidified with 10% HCl (5 mL) and the solvent evaporated. The residue was extracted with EtOAc (3x5 mL), washed with brine (6 mL), dried ($MgSO_4$), filtered and solvent evaporated to give a white powder (64 mg). Separation of the powder by silica gel chromatography using a hexanes/EtOAc gradient gave **37** as white foam (41 mg, 60%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.73 (s, 1H), 4.60 (s, 1H), 3.70-3.64 (m, 1H) 3.66 (s, 3H), 3.02-2.96 (m, 1H), 2.96 (d, J=9.5 Hz, 1H), 2.24-2.15 (m, 2H), 1.68 (s, 3H), 1.57 (t, J=11.3 Hz, 1H), 1.00 (s, 3H), 0.96 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.79 (s, 3H)

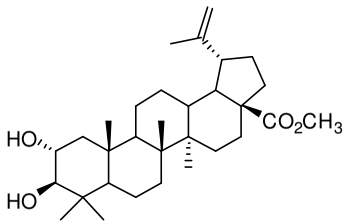
^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 176.6, 150.4, 109.6, 83.9, 69.2, 56.5, 55.5, 51.3, 50.5, 49.4, 47.0, 46.8, 42.4, 40.7, 39.2, 38.6, 38.2, 36.9, 34.2, 32.2, 30.6, 29.6, 28.5, 25.4, 21.0, 19.4, 18.3, 17.4, 16.5, 16.0, 14.7

^{1}H NMR, $CDCl_3$, 400MHz



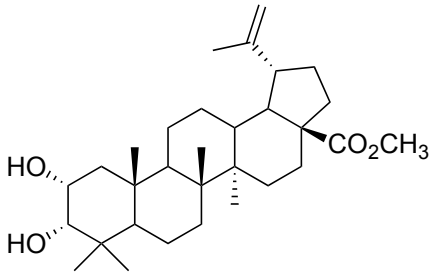
37

^{13}C NMR, $CDCl_3$, 400MHz



37

3-Epi-2α-hydroxy Methyl Betulinate (38)



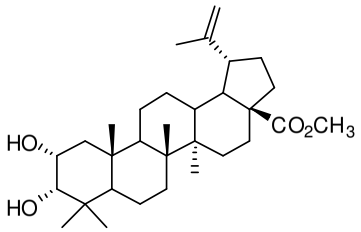
38

The procedure giving **37** also gave **38** as white foam after silica gel chromatography (10 mg, 15%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.73 (d, J=2.1Hz, 1H), 4.60 (dd, J=2.2, 1.3 Hz, 1H), 3.98 (br d, J=10.0 Hz, 1H), 3.41 (d, J=2.7 Hz, 1H), 3.02-2.96 (m, 1H), 2.24-2.15 (m, 2H), 1.68 (s, 3H), 1.57 (t, J=11.4 Hz, 1H), 0.99 (s, 3H), 0.97 (s, 3H), 0.90 (s, 3H), 0.86 (s, 3H), 0.83 (s, 3H)

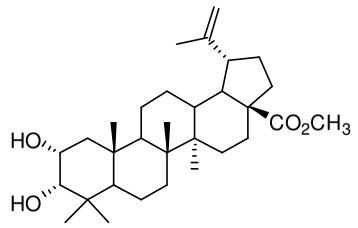
^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 176.7, 150.5, 109.6, 78.9, 66.7, 56.6, 51.2, 50.2, 49.4, 48.2, 47.0, 42.5, 42.2, 40.9, 38.6, 38.3, 38.2, 37.0, 34.1, 32.2, 30.6, 29.6, 28.5, 25.4, 21.6, 20.8, 19.3, 18.0, 17.1, 16.0, 14.8

^{1}H NMR, $CDCl_3$, 400MHz



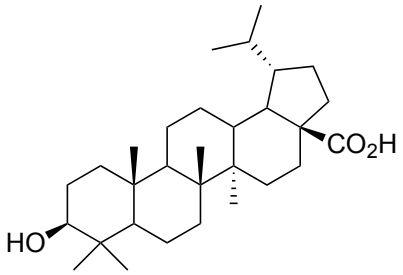
38

^{13}C NMR, $CDCl_3$, 400MHz



38

Dihydrobetulinic Acid (39)

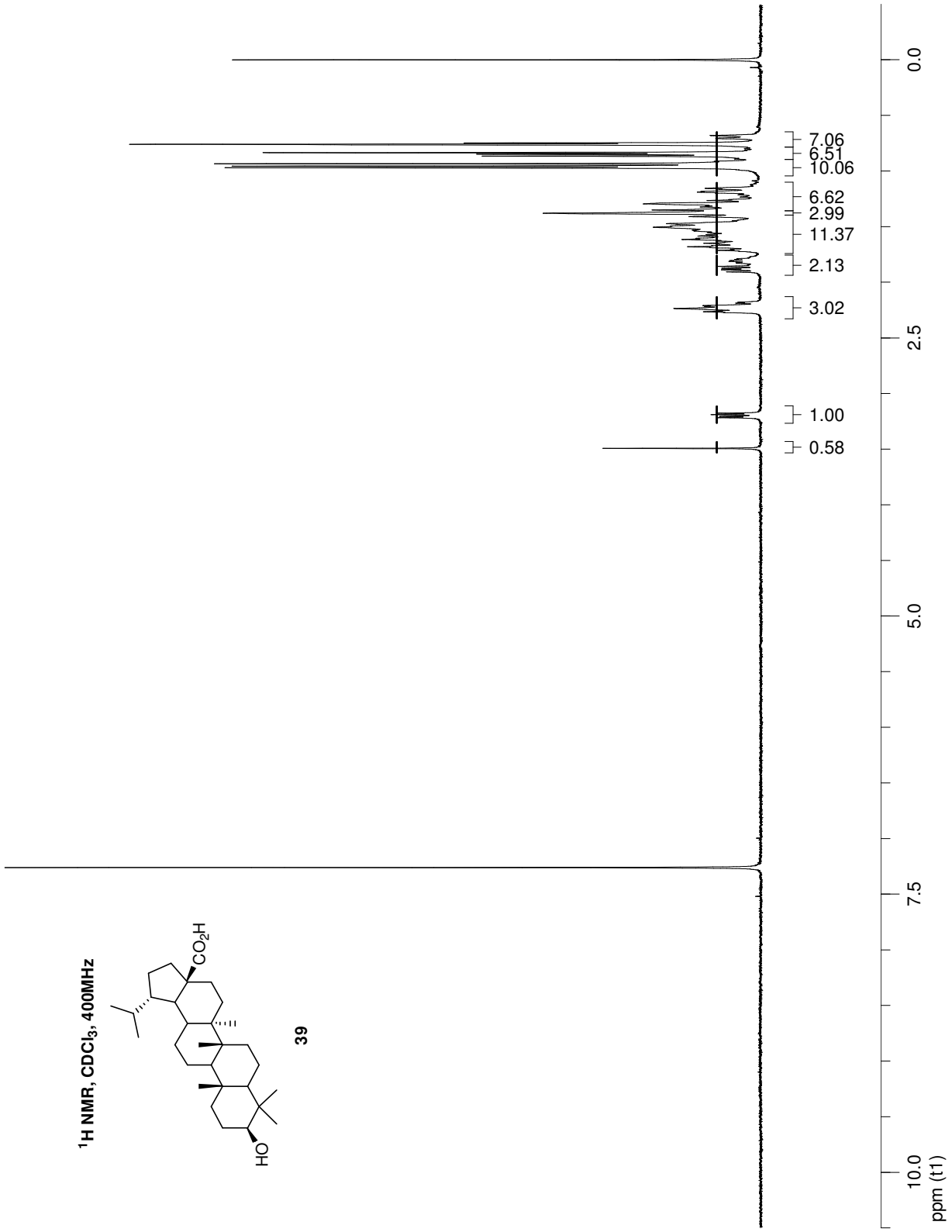


39

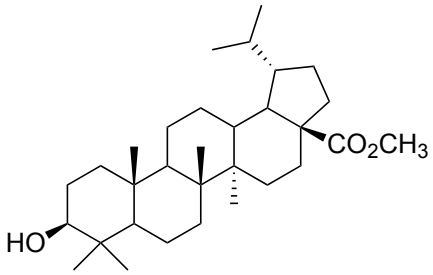
A 2:3 mixture of **16**:**17** (200 mg, 0.382 mmol) and 10% Pd/C (20 mg) in MeOH (30 mL) was degassed with H_2 and stirred under H_2 (1 atm) for 24 hrs. The mixture was filtered through celite and stirred at 40-45 ^oC with saturated K_2CO_3 solution (5 mL) for 18 hrs. The solvent was evaporated and the residue acidified with 1 N HCl to pH~1. It was extracted with EtOAc (4x25 mL), washed with brine (20 mL), dried (Na_2SO_4), filtered and solvent evaporated to give a white solid (154 mg). Recrystallization of the solid from hot MeOH gave **39** as white needles (107 mg, 61%).

^{1}H NMR ($CDCl_3$, 400 MHz, calibrated with TMS): δ (ppm) 3.20 (dd, J=11.2, 5.0 Hz, 1H), 2.27-2.18 (m, 3H), 1.91-1.86 (m, 1H), 1.83-1.79 (m, 1H), 0.97 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H), 0.86 (d, J=6.9 Hz, 3H), 0.84 (s, 3H), 0.76 (s, 3H)

HRMS: Calculated for $C_{30}H_{50}O_3$, 458.37600; Found 458.37801


^{1}H NMR, $CDCl_3$, 400MHz
CO_2H
HO
39
7.06
6.51
10.06
6.62
2.99
11.37
2.13
3.02
1.00
0.58
0.0
2.5
5.0
7.5
10.0
ppm (t1)

Methyl Dihydrobetulinate (40)

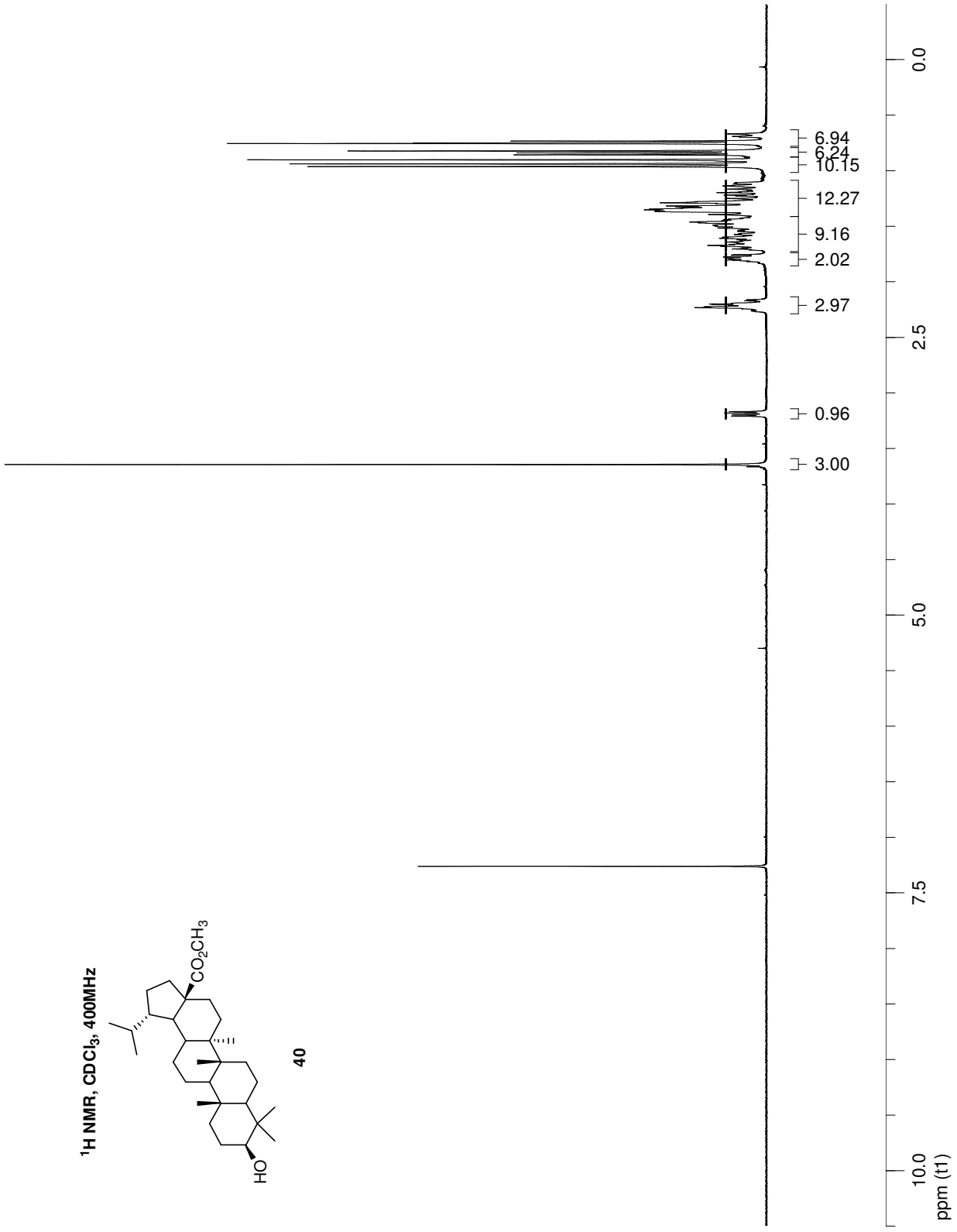


40

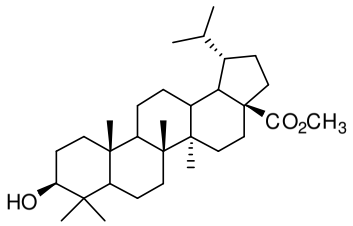
Methyl dihydrobetulinate (**40**) was available in the lab, and its structure was confirmed by NMR data.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 3.64 (s, 3H), 3.19 (dd, J=11.2, 5.0 Hz), 2.27-2.16 (m, 3H), 0.96 (s, 3H), 0.94 (s, 3H), 0.90 (s, 3H), 0.85 (d, J=6.9 Hz, 3H), 0.82 (s 3H), 0.76 (s, 3H), 0.74 (d, J=6.9 Hz, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 176.9, 79.0, 57.0, 55.3, 51.2, 50.3, 48.9, 44.2, 42.5, 40.7, 38.8, 38.7, 38.1, 37.3, 37.2, 34.4, 32.1, 29.73, 29.66, 28.0, 27.4, 26.9, 23.0, 22.8, 20.9, 18.3, 16.1, 16.0, 15.4, 14.7, 14.6

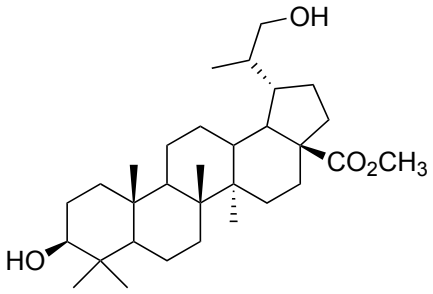

^{1}H NMR, $CDCl_3$, 400MHz
CO_2CH_3
HO
40
6.94
6.24
10.15
12.27
9.16
2.02
2.97
0.96
3.00
0.0
2.5
5.0
7.5
10.0
ppm (t1)

^{13}C NMR, $CDCl_3$, 400MHz



40

Methyl 29-Hydroxydihydrobetulinate (41)



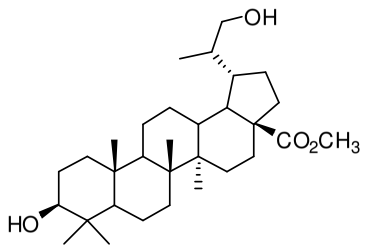
41

Methyl 29-hydroxydihydrobetulinate (**41**) was available in the lab, and its structure was verified by NMR data.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 3.78 (dd, J=10.4, 4.5 Hz, 1H), 3.64 (s, 3H), 3.41 (dd, J=10.3, 8.1 Hz, 1H), 3.19 (dd, J=11.3, 5.0 Hz, 1H), 0.96 (s, 3H), 0.96 (d, J=6.8 Hz, 3H), 0.94 (s, 3H), 0.90 (s, 3H), 0.82 (s, 3H), 0.75 (s, 3H)

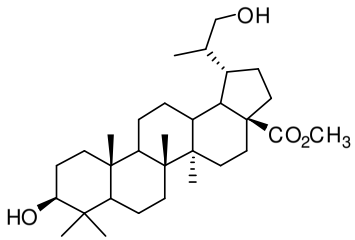
^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 176.6, 78.9, 64.3, 56.8, 55.3, 51.2, 50.2, 48.8, 43.2, 42.5, 40.6, 38.8, 38.7, 38.4, 38.2, 37.1, 37.0, 34.4, 32.0, 29.7, 28.0, 27.4, 27.2, 23.9, 20.9, 18.3, 18.1, 16.1, 15.9, 15.4, 14.6

^{1}H NMR, $CDCl_3$, 400MHz



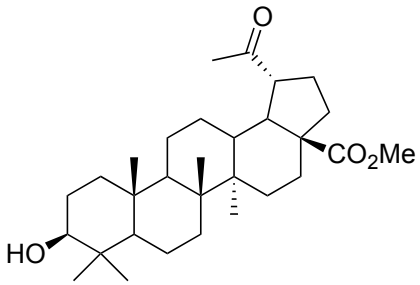
41

^{13}C NMR, $CDCl_3$, 400MHz



41

Methyl Platanate (42)



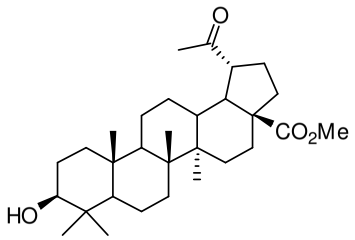
42

A solution of **2** (1.00 g, 2.13 mmol) in 9:1 DCM:MeOH (100 mL) was cooled to -78 ^{o}C and ozone was bubbled through until a faint blue colour was observed. The solution was purged with N_2 for 10 min and triethyl phosphite (1.80 mL, 10.5 mmol) was added. After 30 min, the solution was allowed to warm to rt overnight. The solvent was evaporated and the residue dissolved in EtOAc (100 mL), washed with water (40 mL) and brine (20 mL), dried ($MgSO_4$), filtered and solvent evaporated to give a white solid (1.35 g). Purification of the solid by silica gel chromatography using a hexanes/EtOAc gradient gave **42** as a white powder (589 mg, 58%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 3.67 (s, 3H), 3.28-3.22 (m, 1H), 3.18 (dd, J=11.2, 4.8 Hz, 1H), 2.27-2.22 (m, 1H), 2.17 (s, 3H), 0.98 (s, 3H), 0.95 (s, 3H), 0.88 (s, 3H), 0.81 (s, 3H), 0.75 (s, 3H)

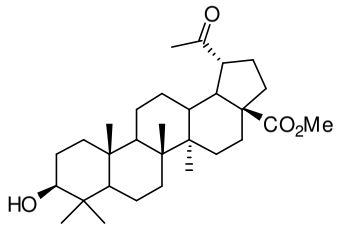
^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 212.4, 176.5, 78.9, 56.4, 55.3, 51.4, 51.2, 50.4, 49.4, 42.2, 40.5, 38.8, 38.6, 37.3, 37.2, 36.6, 34.2, 31.4, 30.1, 29.7, 28.3, 28.0, 27.3, 27.2, 20.9, 18.2, 16.1, 15.9, 15.3, 14.7

^{1}H NMR, $CDCl_3$, 400MHz



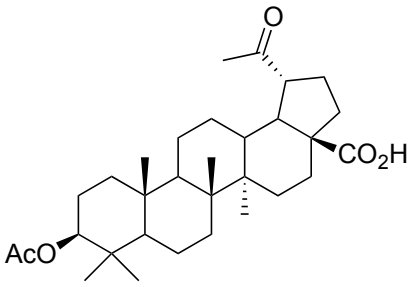
42

^{13}C NMR, $CDCl_3$, 400MHz



42

3-Acetoxy Platanic Acid (44)

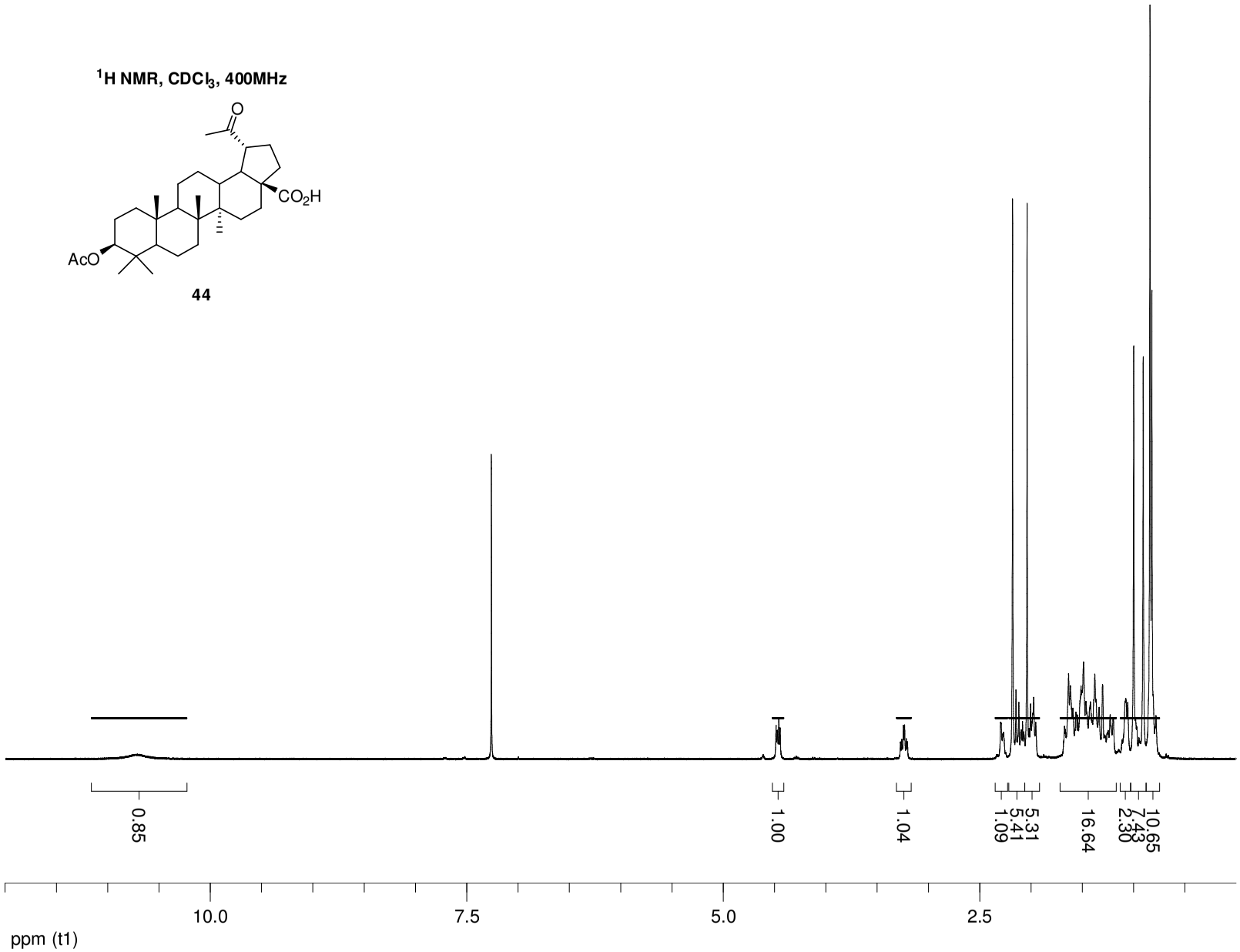


44

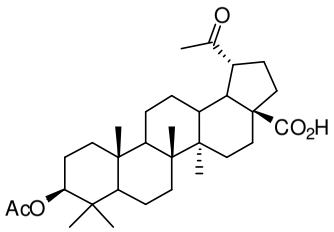
A solution of **16** (1.18 g, 2.37 mmol) in DCM (140 mL) and MeOH (10 mL) was cooled to -78 ^{o}C. Ozone was bubbled through until a faint blue colour was observed, and the solution was then purged by bubbling N_2 through for 20 min. Dimethyl sulfide (2.00 mL) was added, stirred 10 min and the solution was allowed to warm to rt. After 10 min the solvent was evaporated and the residue dissolved in EtOAc (200 mL). It was washed with water (2x40 mL) and brine (40 mL), dried ($MgSO_4$), filtered and solvent evaporated to give a white powder (1.17 g). Purification of the powder by silica gel chromatography using a hexanes/EtOAc gradient gave **44** as a white powder (863 mg, 73%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.47 (dd, J=10.8, 5.1 Hz, 1H), 3.27-3.21 (m, 1H), 2.18 (s, 3H), 2.04 (s, 3H), 1.00 (s, 3H), 0.91 (s, 3H), 0.84 (s, 6H), 0.82 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 212.1, 181.5, 171.0, 80.8, 56.2, 55.3, 51.2, 50.2, 49.2, 42.2, 40.6, 38.3, 37.8, 37.5, 37.1, 36.7, 34.1, 31.4, 30.1, 29.7, 28.2, 27.9, 27.1, 23.6, 21.3, 20.8, 18.1, 16.5, 16.1, 15.9, 14.7

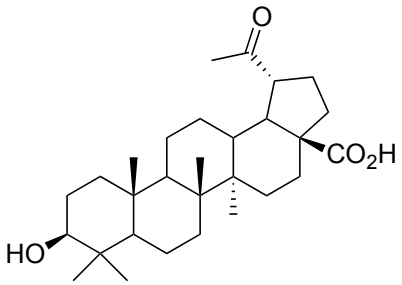

^{1}H NMR, $CDCl_3$, 400MHz
O
CO_2H
AcO
44
0.85
1.00
1.04
1.09
5.41
5.31
16.64
2.30
7.43
10.65
10.0
7.5
5.0
2.5
ppm (t1)

^{13}C NMR, $CDCl_3$, 400MHz



44

Platanic Acid (43)

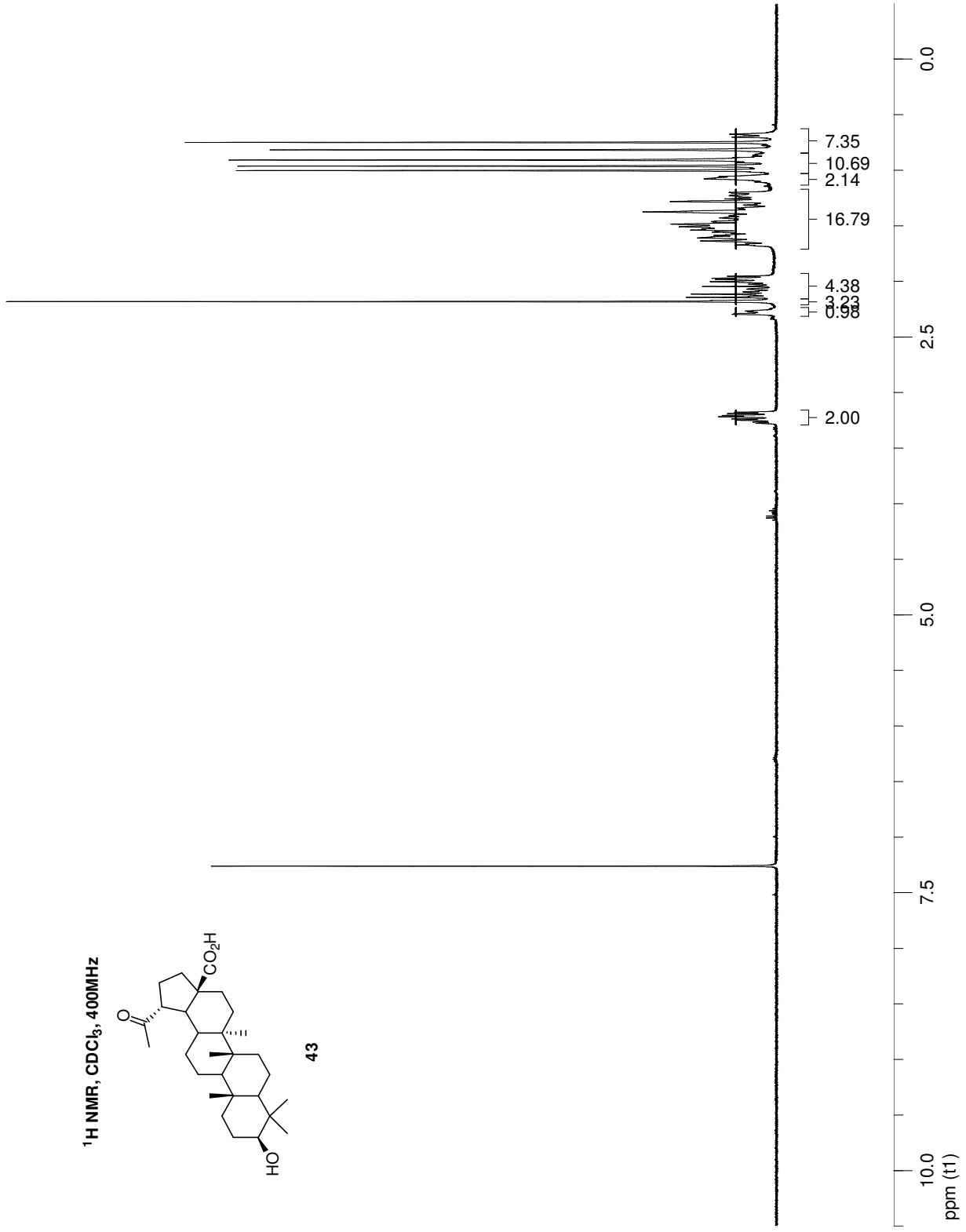


43

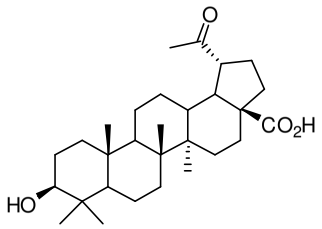
To a vigorously stirred solution of **44** (777 mg, 1.55 mmol) in MeOH (130 mL) was added K_2CO_3 (10.0 g) and H_2O (5.0 mL). The mixture was heated to 55 ^oC overnight, and in the morning the solvent was evaporated. The residue was dissolved in EtOAc (150 mL) and acidified with 10% HCl. Layers were separated and the organic extract was washed with brine (2x40 mL), dried ($MgSO_4$), filtered and solvent evaporated. The residue was purified by silica gel chromatography using a hexanes/EtOAc gradient to give **43** as a white powder (581 mg, 82%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 3.28-3.18 (m, 2H), 2.18 (s, 3H), 1.00 (s, 3H), 0.96 (s, 3H), 0.91 (s, 3H), 0.82 (s, 3H), 0.75 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 212.2, 181.2, 78.9, 56.2, 55.2, 51.1, 50.3, 49.2, 42.2, 40.5, 38.8, 38.6, 37.5, 37.2, 36.7, 34.2, 31.4, 30.1, 29.7, 28.2, 28.0, 27.3, 27.2, 20.8, 18.2, 16.1, 15.9, 15.3, 14.7

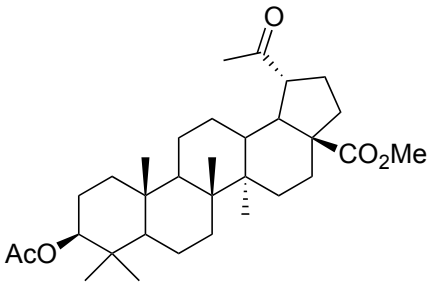

^{1}H NMR, $CDCl_3$, 400MHz
CO_2H
O
HO
43
7.35
10.69
2.14
16.79
4.38
3.23
0.98
2.00
0.0
2.5
5.0
7.5
10.0
ppm (t1)

^{13}C NMR, $CDCl_3$, 400MHz



43

3-acetoxy Methyl Platanate (45)

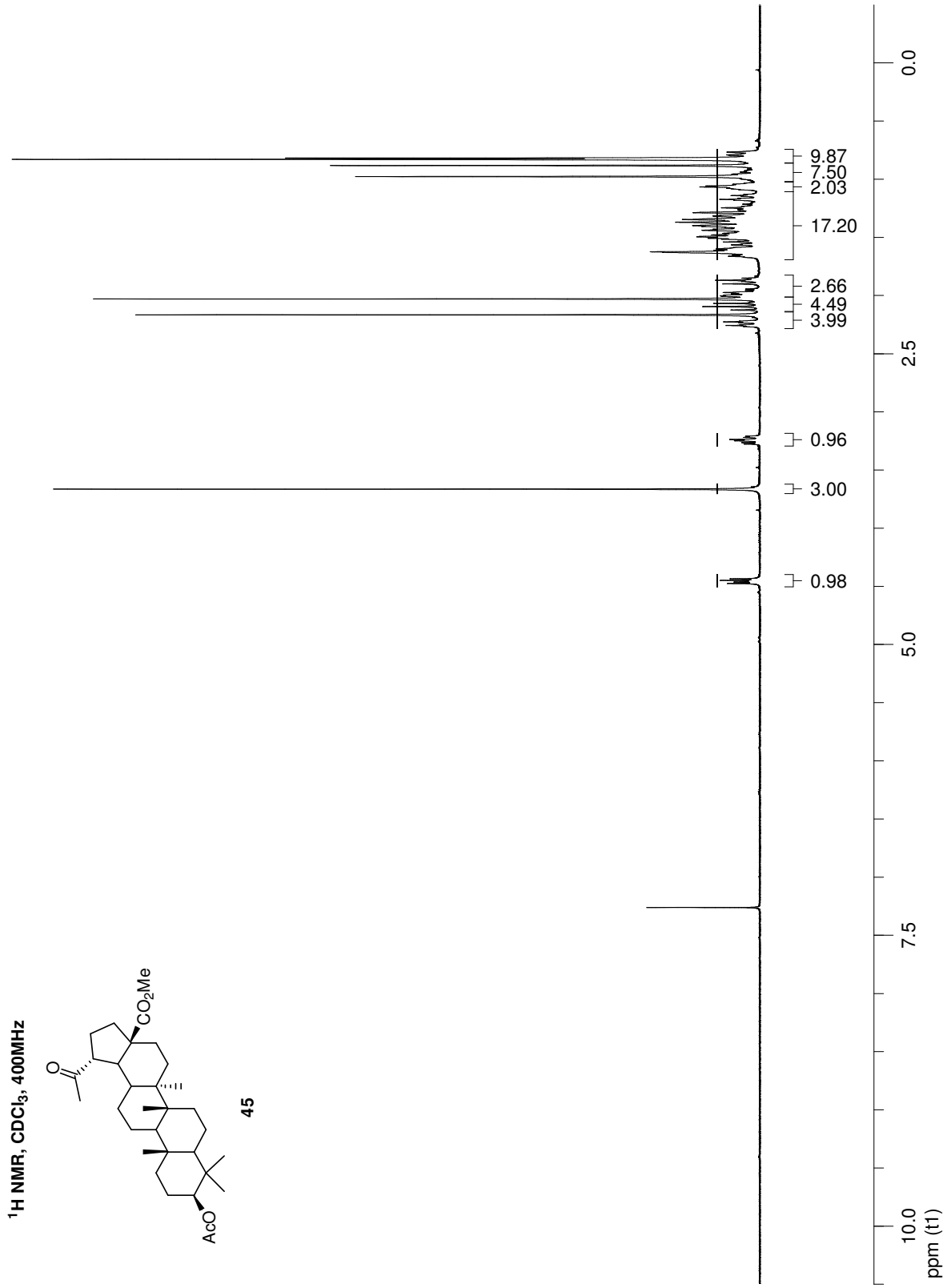


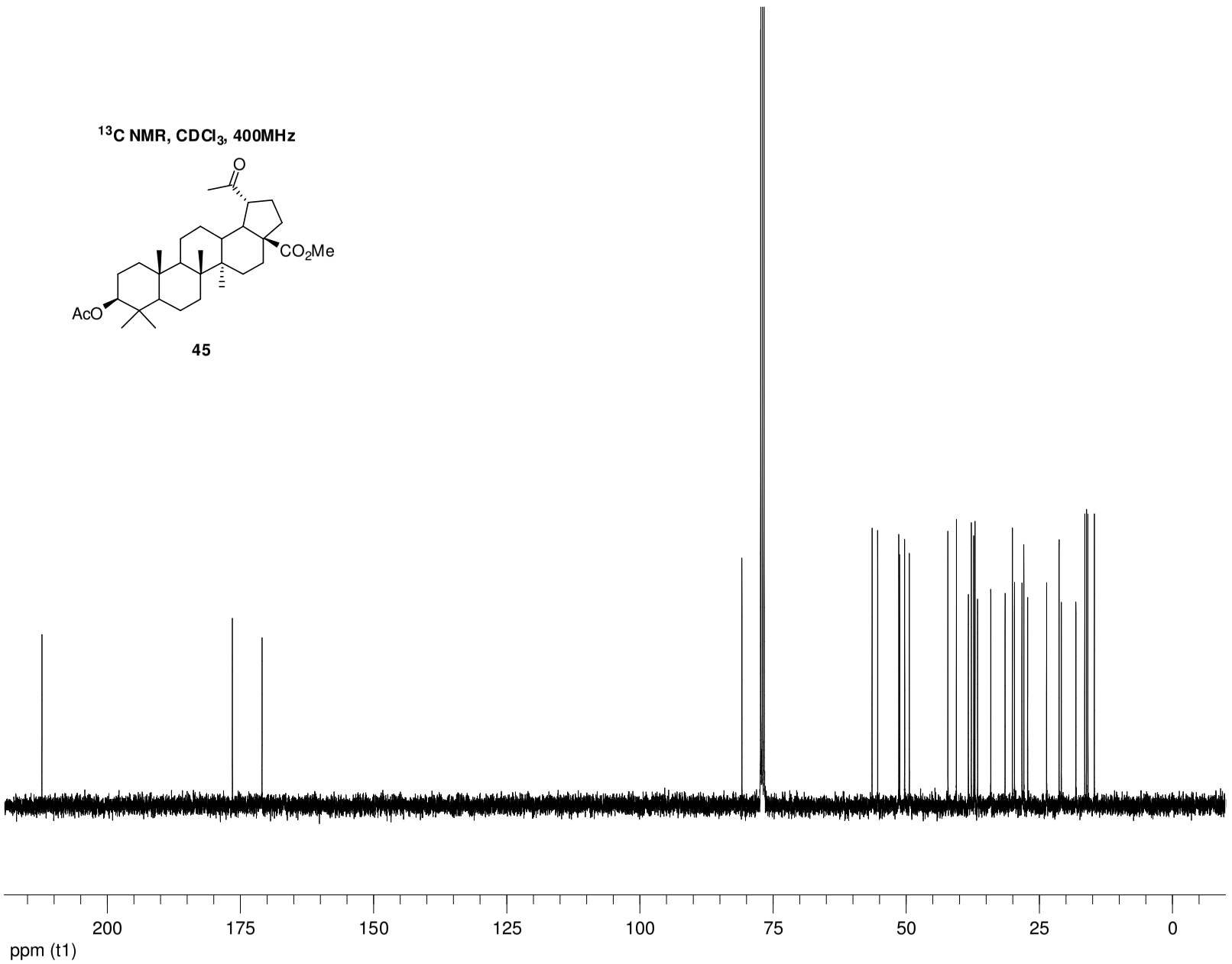
45

To a stirred solution of **42** (120 mg, 0.254 mmol) in pyridine (3 mL) was added Ac_2O (0.05 mL, 0.53 mmol). After stirring overnight at rt, 5% HCl (10mL) was added and stirred 30 min. The mixture was extracted with EtOAc (40 mL), washed with 5% HCl (2x15 mL) and brine (10 mL), dried ($MgSO_4$), filtered and solvent evaporated to give a white solid (139 mg). Purification of the solid by silica gel chromatography using a hexanes/EtOAc gradient gave **45** as a white powder (40 mg, 31%) along with recovered starting material **42** (70 mg, 58%).

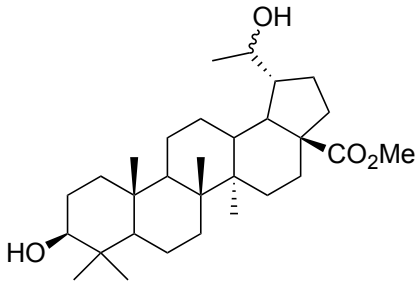
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.46 (dd, J=10.8, 5.2 Hz, 1H), 3.66 (s, 3H), 3.28-3.21 (m 1H), 2.27-2.22 (m, 1H), 2.17 (s, 3H), 2.03 (s, 3H), 0.98 (s, 3H), 0.88 (s, 3H), 0.83 (s, 6H), 0.82 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 212.3, 176.5, 170.9, 80.8, 56.4, 55.3, 51.4, 51.2, 50.3, 49.4, 42.2, 40.6, 38.3, 37.8, 37.3, 37.1, 36.6, 34.1, 31.4, 30.1, 29.7, 28.3, 27.9, 27.2, 23.6, 21.3, 20.9, 18.1, 16.5, 16.1, 15.9, 14.7


^{1}H NMR, $CDCl_3$, 400MHz
CO_2Me
O
AcO
45
9.87
7.50
2.03
17.20
2.66
4.49
3.99
0.96
3.00
0.98
0.0
2.5
5.0
7.5
10.0
ppm (t1)


^{13}C NMR, $CDCl_3$, 400MHz
O
CO_2Me
AcO
45
200
175
150
125
100
75
50
25
0
ppm (t1)

Methyl 3β,20-dihydroxy-lup-28-oate (Less Polar Isomer, 46)



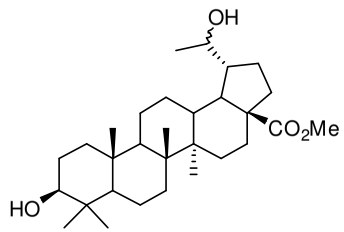
46

To a stirred solution of **42** (164 mg, 0.348 mmol) in MeOH (8.5 mL) and DCM (3.5 mL) was added $NaBH_4$ (197 mg, 5.21 mmol) at rt. After 2 hrs, it was acidified with 30% HCl, stirred 15 min and solvent evaporated. The residue was diluted with water (20 mL) and extracted with DCM (3x20 mL). The combined organic extracts were washed with brine (2x20 mL), dried ($MgSO_4$), filtered and the solvent evaporated to give a white foam (162 mg). Separation of the foam by silica gel chromatography using a hexanes/EtOAc gradient gave **46** as a white powder (77 mg, 47%; Rf=0.25 in 2:3 EtOAc:hexanes).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 3.89 (br q, J=6.3 Hz, 1H), 3.65 (s, 3H), 3.19 (dd, J=11.3, 5.0 Hz, 1H), 1.79 (dd, J=12.2, 7.2 Hz, 1H), 1.13 (d, J=6.4 Hz, 3H), 0.96 (s, 6H), 0.90 (s, 3H), 0.82 (s, 3H), 0.75 (s, 3H)

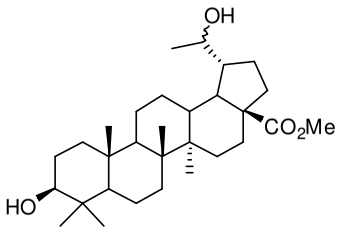
^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 176.9, 78.9, 68.9, 57.0, 55.3, 51.2, 50.2, 48.0, 45.6, 42.4, 40.6, 38.8, 38.7, 38.0, 37.1, 37.0, 34.3, 31.7, 29.7, 28.0, 27.4, 27.1, 23.3, 22.2, 20.9, 18.3, 16.1, 15.9, 15.4, 14.7

^{1}H NMR, $CDCl_3$, 400MHz



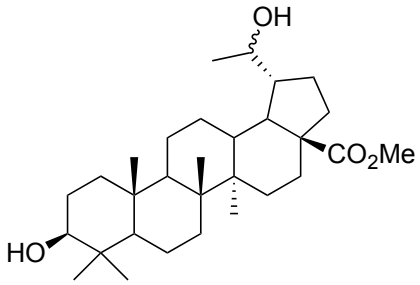
46 (less polar)

^{13}C NMR, $CDCl_3$, 400MHz



46 (less polar)

Methyl 3β,20-dihydroxy-lup-28-oate (More Polar Isomer, 47)



47

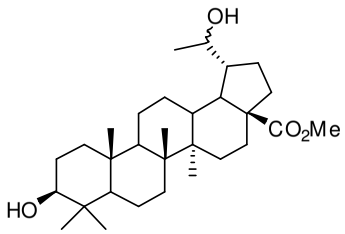
The procedure giving **46** also gave **47** as a white powder after silica gel chromatography (51 mg, 31%; Rf=0.14 in 2:3 EtOAc:hexanes).

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 4.03 (qd, J=6.3, 4.1 Hz, 1H), 3.65 (s, 3H), 3.19 (dd, J=11.3, 5.0 Hz, 1H), 2.55 (tt, J=10.6, 3.9 Hz, 1H), 2.26-2.17 (m, 2H), 1.85 (dd, J=12.4, 7.2 Hz, 1H), 1.07 (d, J=6.3 Hz, 3H), 0.96 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.82 (s, 3H), 0.75 (s, 3H)

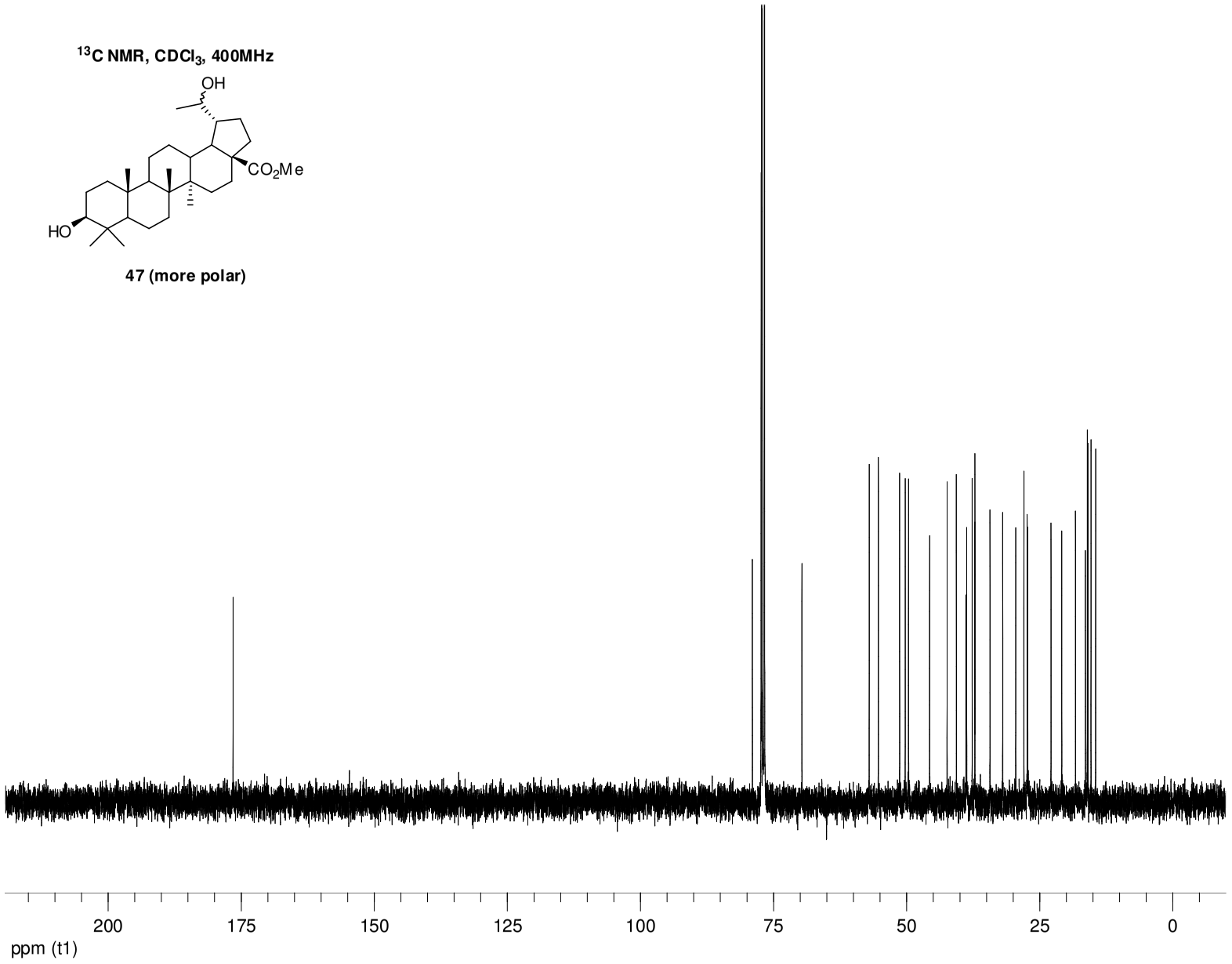
^{13}C NMR ($CDCl_3$, 400MHz): δ (ppm) 176.5, 79.0, 69.7, 57.0, 55.3, 51.3, 50.3, 49.6, 45.7, 42.4, 40.7, 38.9, 38.7, 37.7, 37.20, 37.15, 34.3, 31.9, 29.5, 28.0, 27.3, 27.2, 22.9, 20.9, 18.3, 16.4, 16.1, 15.9, 15.4, 14.5

HRMS: [M]+ not found; Calculated for $[M-H_2O]^+$, $C_{30}H_{48}O_3$, 456.3604;
Found $[M-H_2O]^+$ 456.3635

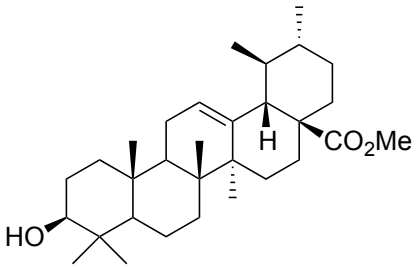
^{1}H NMR, $CDCl_3$, 400MHz



47 (more polar)


13C NMR, CDCl3, 400MHz
OH
CO2Me
HO
47 (more polar)
200
175
150
125
100
75
50
25
0
ppm (t1)

Methyl Ursolate (48)



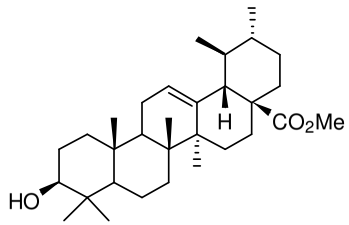
48

To a stirred solution of the crude Fuji apple extract of **3** (1.42 g, ~3.11 mmol) in THF (100 mL) was added K_2CO_3 (2.15 g, 15.5 mmol) and CH_3I (1.94 mL, 31.2 mmol). The mixture was stirred vigourously and heated to reflux for 3 hrs, then cooled to rt. The solvent was evaporated, the residue diluted with water (80 mL) and extracted with EtOAc (100 mL, then 2x40 mL). The combined extracts were dried ($MgSO_4$), filtered and solvents evaporated to give an orange foam (1.50 g). Purification of the foam by silica gel chromatography using a hexanes/EtOAc gradient gave **48** as a white powder (0.92 g, 63%) containing 15% methyl oleanolate (**49**).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.24 (t, J=3.5 Hz, 1H), 3.60 (s, 3H), 3.23-3.19 (m, 1H), 2.23 (d, J=11.5 Hz, 1H), 2.03-1.96 (m, 1H), 1.07 (s, 3H), 0.98 (s, 3H), 0.93 (d, J=6.1 Hz, 3H), 0.91 (s, 3H), 0.85 (d, J=6.5 Hz, 3H), 0.78 (s, 3H), 0.74 (s, 3H)

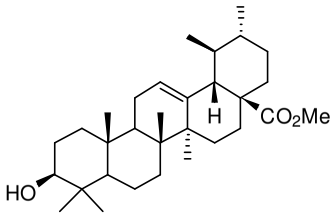
^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 178.1, 138.1, 125.5, 79.0, 55.2, 52.9, 51.4, 48.1, 47.5, 42.0, 39.5, 39.0, 38.9, 38.7, 38.6, 37.0, 36.6, 32.9, 30.6, 28.1, 28.0, 27.2, 24.2, 23.6, 23.3, 21.2, 18.3, 17.0, 16.9, 15.6, 15.4

^{1}H NMR, $CDCl_3$, 400MHz



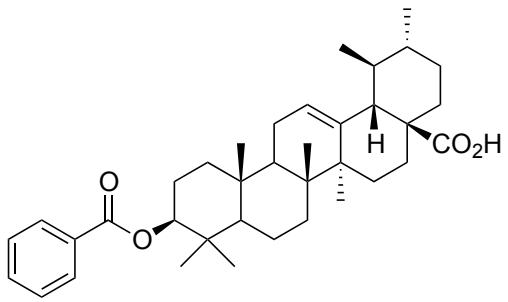
48

^{13}C NMR, $CDCl_3$, 400MHz



48

3-Benzoyloxy Ursolic Acid (50)



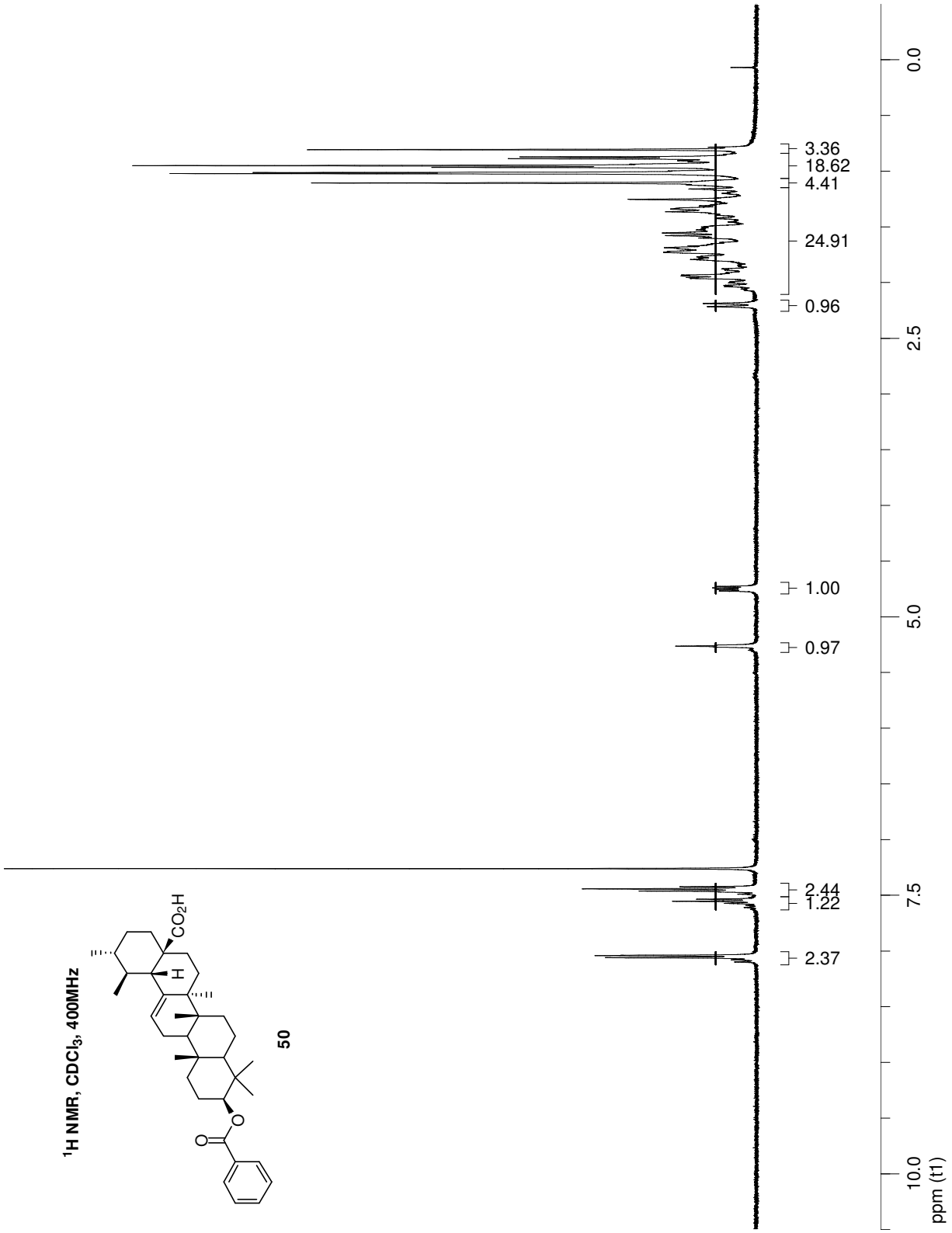
50

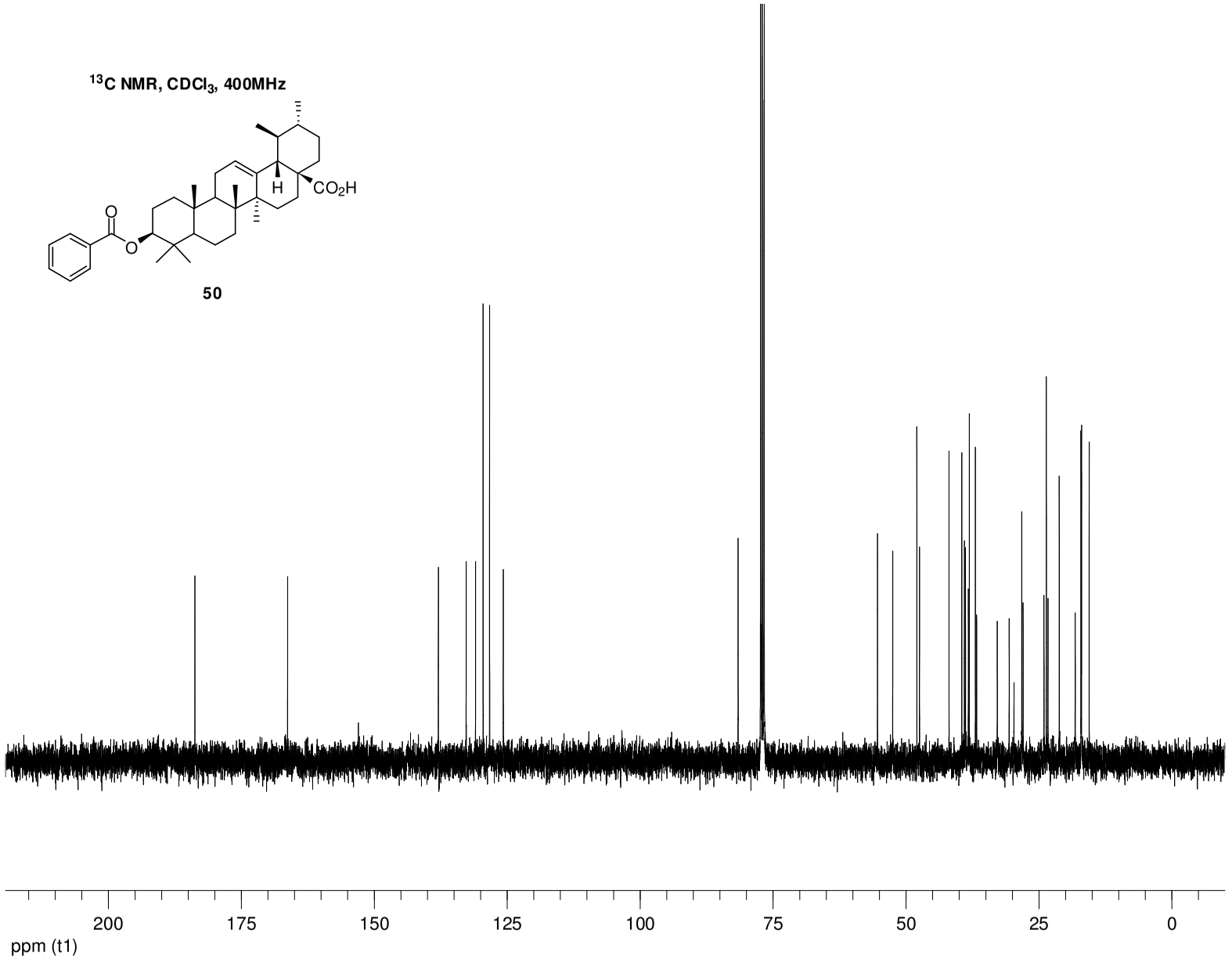
To a stirred solution of the crude McIntosh apple extract of **3** (561 mg, ~1.23 mmol) in pyridine (10 mL) was added DMAP (5 mg) and benzoic anhydride (1.39 g, 6.14 mmol). It was stirred for 3 days at rt, then acidified to pH ~4 with 10% HCl and stirred 30 min. The mixture was extracted with EtOAc (100 mL), washed with 5% HCl (40 mL), water (40 mL) and brine (40 mL), dried ($MgSO_4$), filtered and the solvent evaporated. The residue was purified using silica gel chromatography with a hexanes/EtOAc gradient to give impure **50** as a white solid containing benzoic acid. Heating the solid under vacuum removed benzoic acid by sublimation to give **50** as a white powder (216 mg, 31%) containing 8% 3-benzoyloxy oleanolic acid (**51**).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.07-8.03 (m, 2H), 7.57-7.53 (m, 1H), 7.46-7.42 (m, 2H), 5.25 (t, J=3.4 Hz, 1H), 4.75 (dd, J=10.9, 5.6 Hz, 1H), 2.19 (d, J=11.4 Hz, 1H), 1.10 (s, 3H), 1.024 (s, 3H), 1.018 (s, 3H), 0.96 (d, J=4.8 Hz, 3H), 0.95 (s, 3H), 0.88 (d, J=6.4 Hz, 3H), 0.80 (s, 3H)

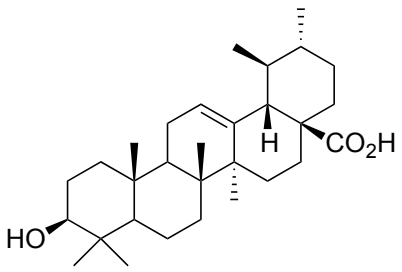
^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 183.7, 166.3, 137.9, 132.7, 131.0, 129.5, 128.3, 125.7, 81.6, 55.4, 52.5, 48.0, 47.5, 41.9, 39.5, 39.0, 38.8, 38.3, 38.1, 37.0, 36.7, 32.8, 30.6, 29.7, 28.2, 28.0, 24.0, 23.6, 23.3, 21.2, 18.2, 17.1, 17.02, 16.98, 15.5

HRMS: Found no [M]+; Calculated for [M-HCO_2H]+ as $C_{36}H_{50}O_2$, 514.3811;
Found [M-HCO_2H]+ as 514.3815


^{1}H NMR, $CDCl_3$, 400MHz
50
CO_2H
H
O
O
3.36
18.62
4.41
24.91
0.96
1.00
0.97
2.44
1.22
2.37
0.0
2.5
5.0
7.5
10.0
ppm (t1)


^{13}C NMR, $CDCl_3$, 400MHz
H
CO_2H
O
O
50
200
175
150
125
100
75
50
25
0
ppm (t1)

Ursolic Acid (3)

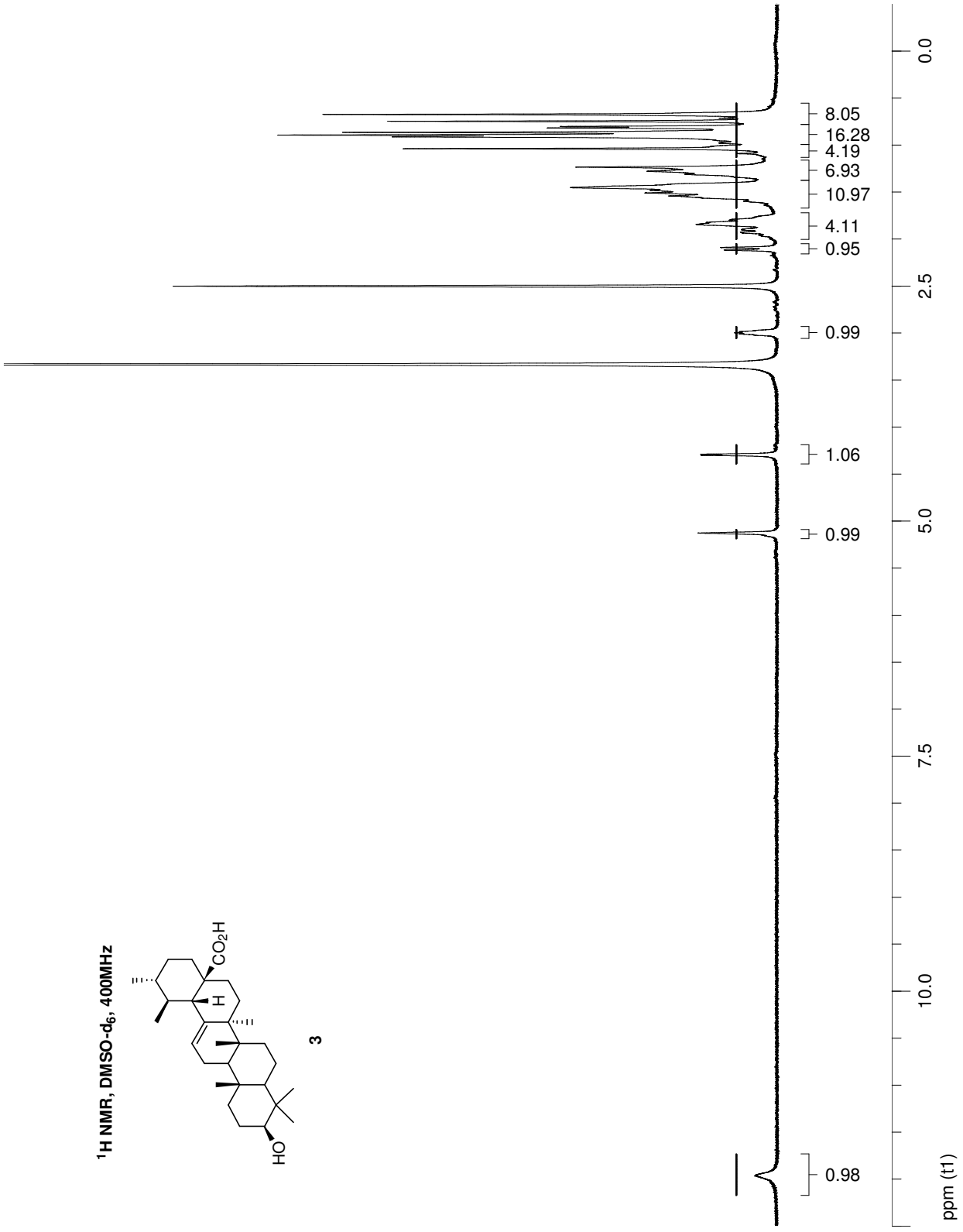


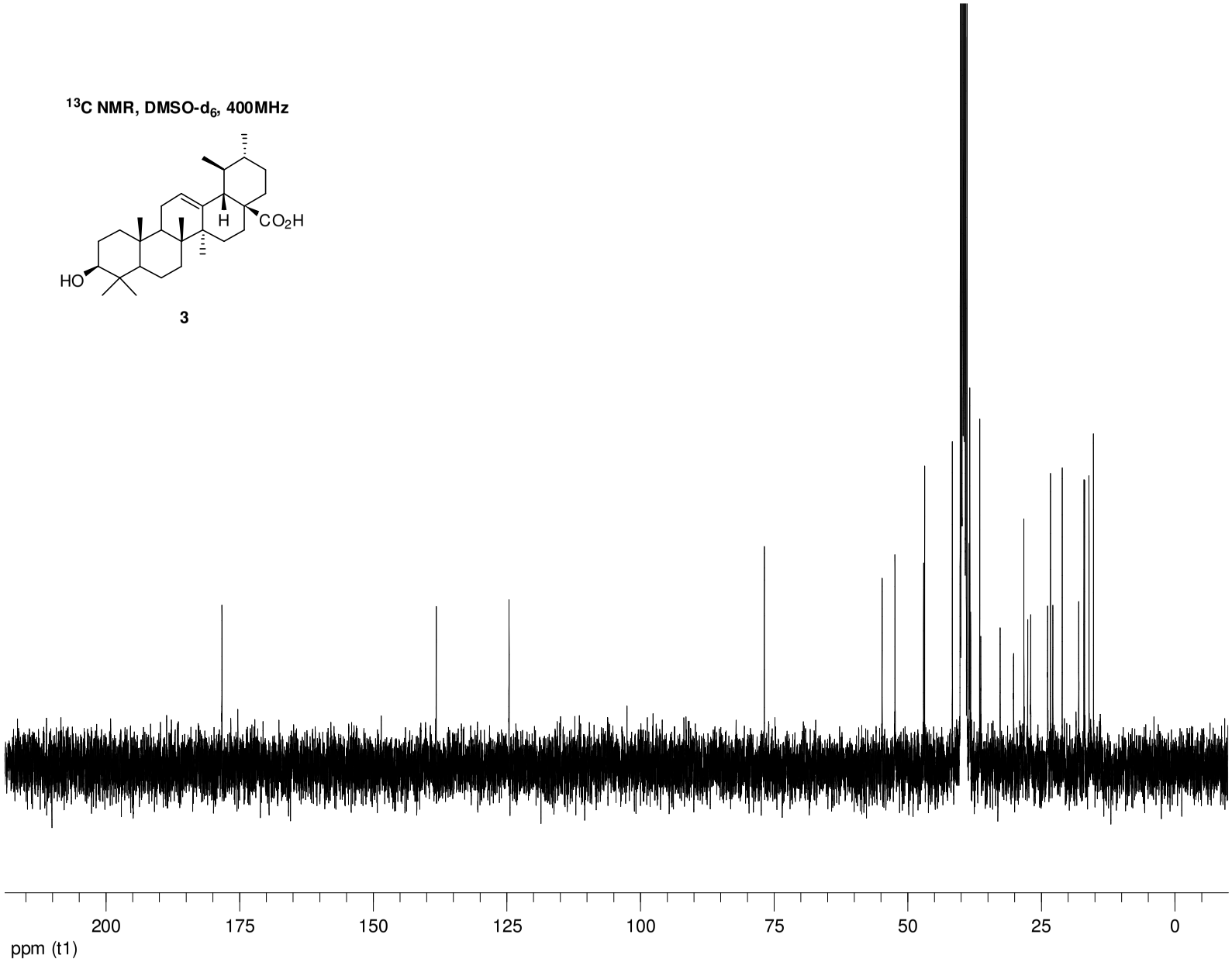
3

To a stirred solution of **50** (79 mg, 0.14 mmol) in MeOH (20 mL) and water (0.4 mL) was added KOH pellets (250 mg, 4.46 mmol). It was heated to 40 ^{o}C overnight, then to 70 ^{o}C for 2 hrs. The solvent was evaporated and the residue acidified with 30% HCl. It was diluted with water (15 mL) then extracted with EtOAc (2x35 mL). The combined extracts were washed with brine (2x25 mL), dried ($MgSO_4$), filtered and the solvent evaporated to give a white powder (78 mg). Heating the powder under vacuum removed benzoic acid by sublimation, leaving **3** as a white powder (58 mg, 91%) containing 8% oleanolic acid (**4**).

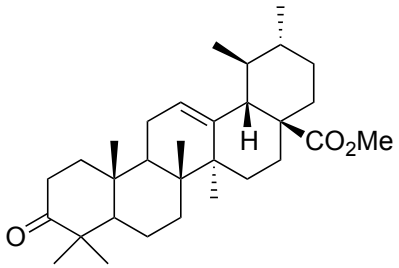
^{1}H NMR (DMSO-d$_6$, 400 MHz): δ (ppm) 5.13 (br s, 1H), 4.29 (d, J=4.8 Hz, 1H), 3.02-2.97 (m, 1H), 2.10 (d, J=11.2 Hz, 1H), 1.04 (s, 3H), 0.90 (d, J=7.4 Hz, 3H), 0.89 (s, 3H), 0.86 (s, 3H), 0.81 (d, J=6.2 Hz, 3H), 0.75 (s, 3H), 0.67 (s, 3H)

^{13}C NMR: (DMSO-d$_6$, 400 MHz): δ (ppm) 178.3, 138.2, 124.6, 76.8, 54.8, 52.4, 47.0, 46.8, 41.6, 38.5, 38.44, 38.39, 38.2, 36.5, 36.3, 32.7, 30.2, 28.3, 27.5, 27.0, 23.8, 23.3, 22.9, 21.1, 18.0, 17.0, 16.9, 16.1, 15.2


^{1}H NMR, DMSO-d_6, 400MHz
CO_2H
H
HO
3
8.05
16.28
4.19
6.93
10.97
4.11
0.95
0.99
1.06
0.99
0.98
0.0
2.5
5.0
7.5
10.0
ppm (t1)


^{13}C NMR, DMSO-d$_6$, 400MHz
HO
H
CO$_2$H
3
200
175
150
125
100
75
50
25
0
ppm (t1)

Methyl 3-Oxo-urs-12-en-28-oate (52)

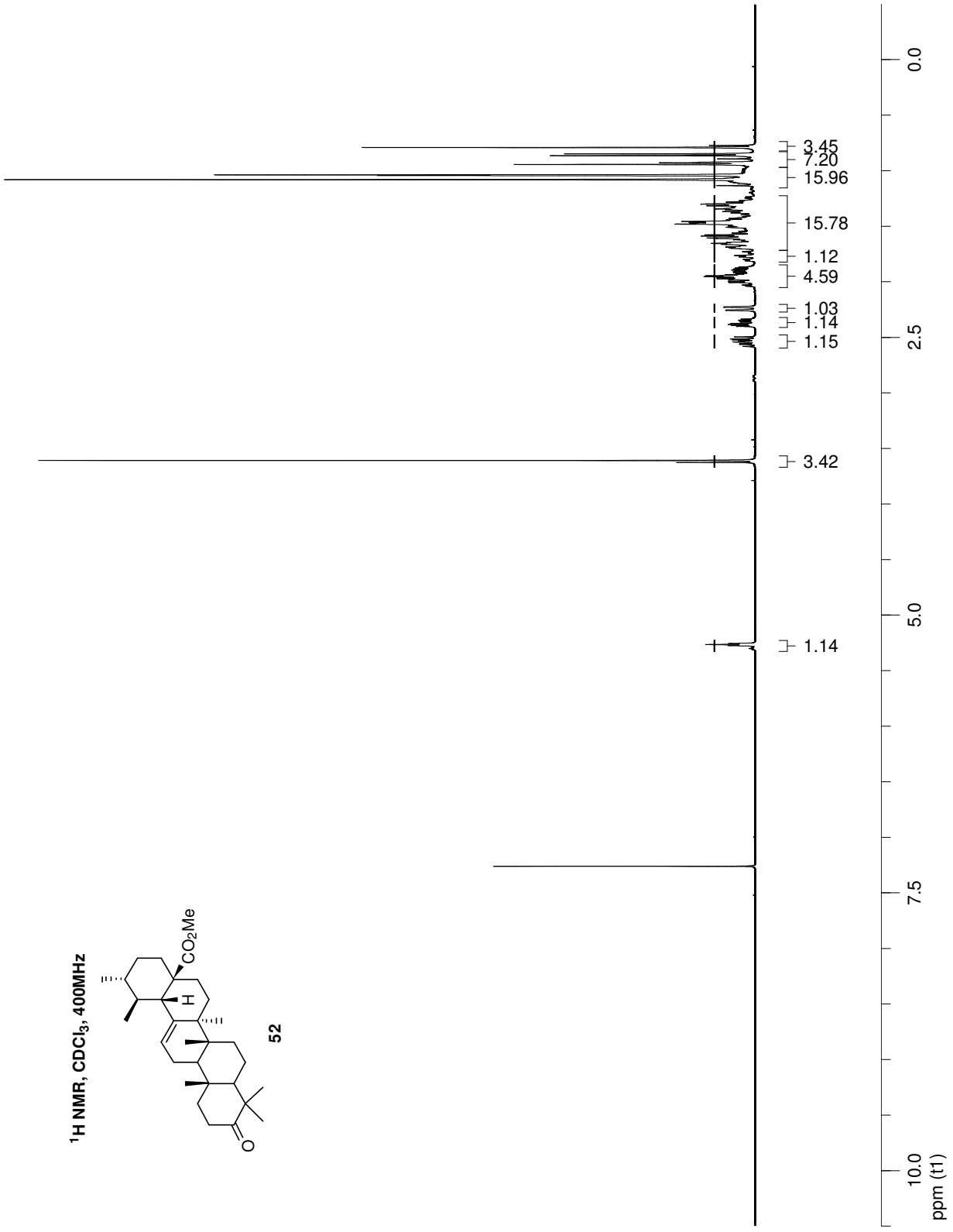


52

A stirred solution of **48** (753 mg, 1.60 mmol) in acetone (30 mL) was cooled to 0 ^{o}C. To this was added dropwise a solution of fresh Jones Reagent, prepared by dissolving CrO_3 (230 mg, 2.30 mmol) in water (0.50 mL) and acidifying with H_2SO_4 (0.20 mL). After an orange colour persisted for 5 min, the reaction was quenched with MeOH (10 mL), stirred for 20 min and the solvent evaporated. The residue was diluted with water (40 mL) and extracted with EtOAc (2x30 mL). The combined organic extracts were dried ($MgSO_4$), filtered and the solvent evaporated to give a white solid (660 mg). Purification of the solid by silica gel chromatography using a hexanes/EtOAc gradient gave **52** as a white powder (560 mg, 75%) containing 11% of oleanolate analog **53**.

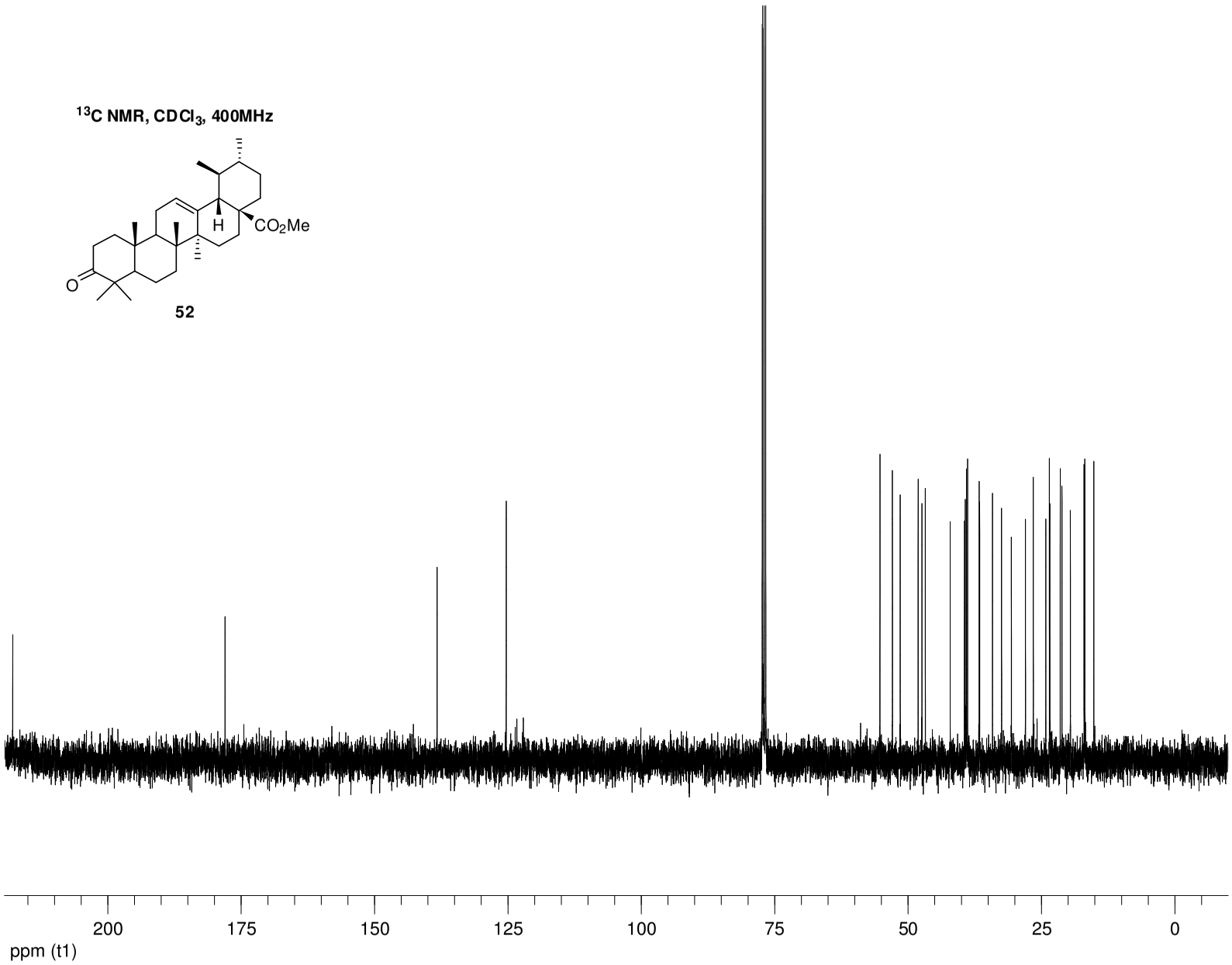
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.27 (t, J=3.6 Hz, 1H), 3.61 (s, 3H), 2.54 (ddd, J=15.9, 11.0, 7.3 Hz, 1H), 2.37 (ddd, J=15.9, 6.9, 3.7 Hz, 1H), 2.24 (dd, J=11.5, 0.9 Hz, 1H), 1.08 (s, 6H), 1.05 (s, 3H), 1.04 (s, 3H), 0.93 (d, J=6.2 Hz, 3H), 0.86 (d, J=6.5 Hz, 3H), 0.79 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 217.8, 178.0, 138.3, 125.3, 55.3, 52.9, 51.5, 48.1, 47.4, 46.8, 42.1, 39.4, 39.3, 39.0, 38.8, 36.7, 36.6, 34.2, 32.5, 30.6, 28.0, 26.5, 24.2, 23.5, 23.4, 21.5, 21.1, 19.6, 17.0, 16.9, 15.2

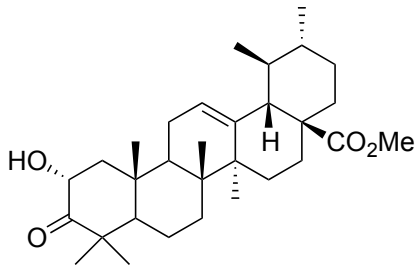


^{1}H NMR, $CDCl_3$, 400MHz

52


^{13}C NMR, $CDCl_3$, 400MHz
H
CO_2Me
O
52
200
175
150
125
100
75
50
25
0
ppm (t1)

Methyl 2α-Hydroxy-3-oxo-urs-12-en-28-oate (56)



56

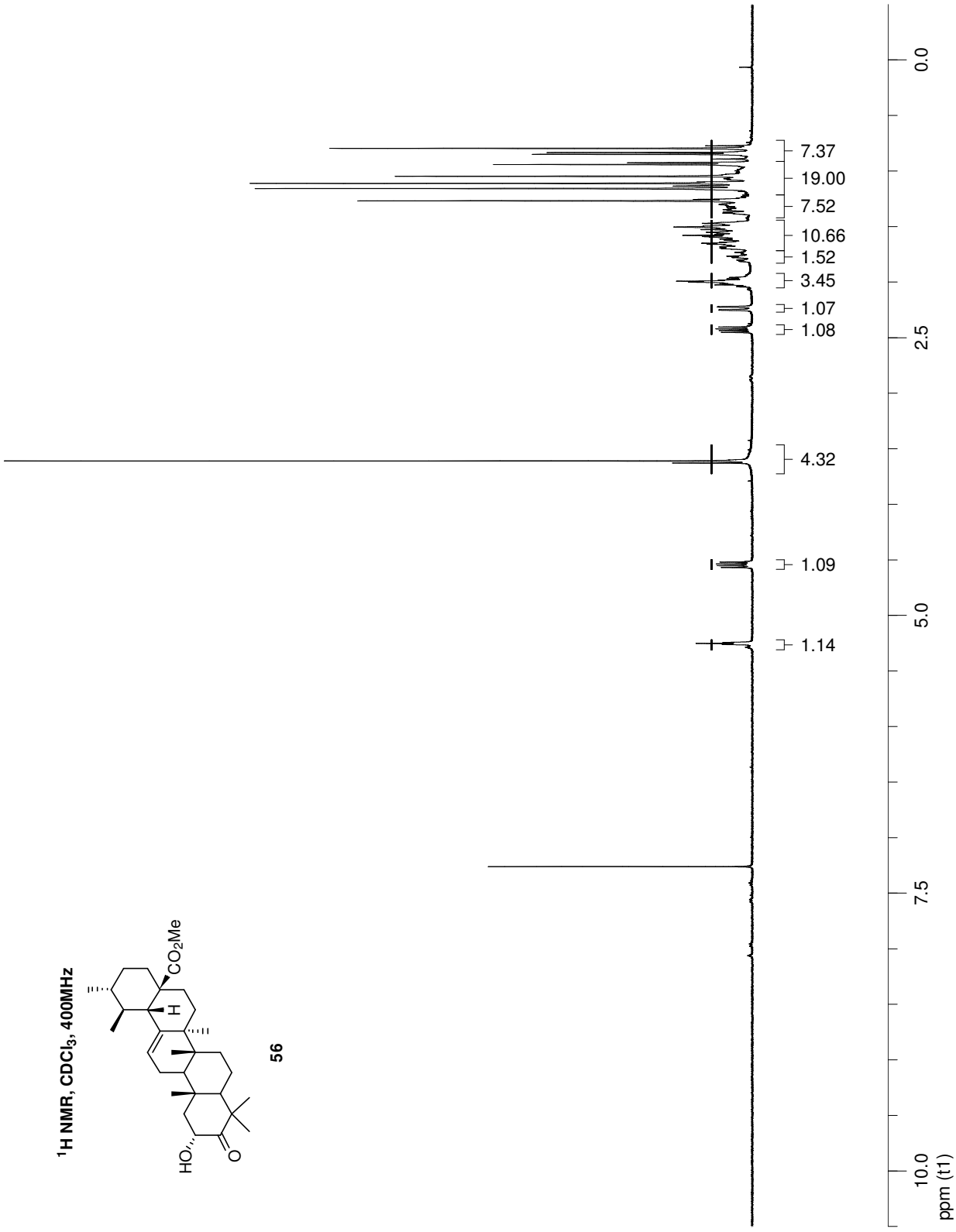
To a stirred solution of **52** (300 mg, 0.64 mmol) in dry DCM (13.1 mL) under N_2 at -78 ^oC was added dry Et_3N (0.89 mL, 6.4 mmol) followed by TMSOTf (0.58 mL, 3.2 mmol). After 1 hr, the reaction was quenched with saturated $NaHCO_3$ solution (6 mL) and warmed to rt. Layers were separated and the aqueous layer extracted with hexanes (3x5 mL). The combined organic extracts were washed with brine (8 mL), dried ($MgSO_4$), filtered and the solvent evaporated to give the crude silyl enol ethers as white foam (347 mg).

The crude silyl enol ethers were dissolved in DCM (12 mL) and cooled to 0 ^oC. To this was slowly added a solution of ~77% mCPBA (158 mg 0.70 mmol) in DCM (15 mL), pre-cooled to 0 ^oC. After 30 min, saturated $NaHCO_3$ solution (20 mL) was added and stirred 1 hr at rt. Layers were separated and the organic layer evaporated, giving silyl ethers **54** and **55**. The silyl ethers were dissolved in MeOH (20 mL) with 5% HCl (1 mL) and stirred 45 min at rt. The solvent was evaporated and the residue dissolved in EtOAc (50 mL). It was washed with water (15 mL) and brine (15 mL), dried ($MgSO_4$), filtered and the solvent evaporated to give a colourless oil (346 mg). The oil was separated by silica gel chromatography using a hexanes/EtOAc gradient to give **56** as a tacky white solid (239 mg, 77%) containing 11% oleanolate analog **57**.

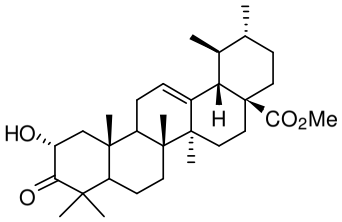
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.26-5.24 (m, 1H), 4.54 (dd, J=12.6, 6.6 Hz, 1H), 3.61 (s, 3H), 2.43 (dd, J=12.5, 6.6 Hz, 1H), 2.24 (d, J=11.5 Hz, 1H), 1.27 (s, 3H), 1.16 (s, 3H), 1.11 (s, 3H), 1.05 (s, 3H), 0.93 (d, J=6.3 Hz, 3H), 0.84 (d, J=6.5 Hz, 3H), 0.80 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 216.7, 178.0, 138.4, 125.0, 69.2, 57.7, 52.7, 51.5, 49.6, 48.0, 47.7, 47.2, 42.0, 39.6, 39.0, 38.8, 37.6, 36.6, 32.6, 30.6, 28.0, 24.7, 24.1, 23.6, 23.5, 21.6, 21.2, 19.1, 17.1, 17.0, 16.1

HRMS: Calculated for $C_{31}H_{48}O_4$, 484.35300; Found 484.35526

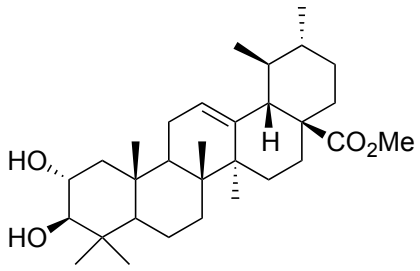

^{1}H NMR, $CDCl_3$, 400MHz
CO_2Me
H
HO
O
56
7.37
19.00
7.52
10.66
1.52
3.45
1.07
1.08
4.32
1.09
1.14
0.0
2.5
5.0
7.5
10.0
ppm (t1)

^{13}C NMR, $CDCl_3$, 400MHz



56

2α-Hydroxy Methyl Ursolate (59)



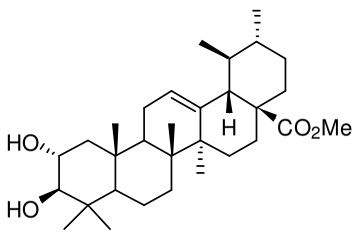
59

To a stirred solution of **56** (156 mg, 0.32 mmol) in MeOH (8 mL) at 0 ^{o}C was added $NaBH_4$ (25 mg, 0.66 mmol). After 1 hr, it was acidified with 30% HCl and the solvent was evaporated. The residue was diluted with water (10 mL) and extracted with EtOAc (3x8 mL). The combined extracts were washed with brine (8 mL), dried ($MgSO_4$), filtered and the solvent evaporated to give a white foam (149 mg). Separation of the foam by silica gel chromatography using a hexanes/EtOAc gradient gave **59** as white powder (87 mg, 56%) containing 11% of oleanolate analog **59a**.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.25 (t, J=3.5 Hz, 1H), 3.72-3.66 (m, 1H), 3.60 (s, 3H), 3.00 (d, J=9.5 Hz, 1H), 2.23 (d, J=11.3 Hz, 1H), 1.07 (s, 3H), 1.03 (s, 3H), 0.99 (s, 3H), 0.94 (d, J=5.8 Hz, 3H), 0.85 (d, J=6.4 Hz, 3H), 0.82 (s, 3H), 0.74 (s, 3H)

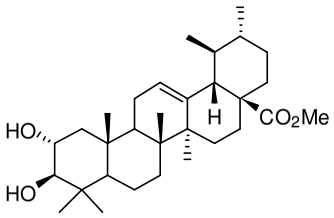
^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 178.0, 138.2, 125.3, 84.0, 69.0, 55.3, 52.8, 51.5, 48.0, 47.5, 46.6, 42.0, 39.5, 39.1, 39.0, 38.8, 38.2, 36.6, 32.8, 30.6, 28.6, 27.9, 24.2, 23.6, 23.3, 21.2, 18.3, 17.0, 16.9, 16.8, 16.7

^{1}H NMR, $CDCl_3$, 400MHz



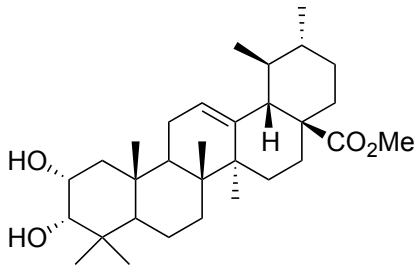
59

^{13}C NMR, $CDCl_3$, 400MHz



59

Methyl 2α,3α-dihydroxyurs-12-en-28-oate (58)

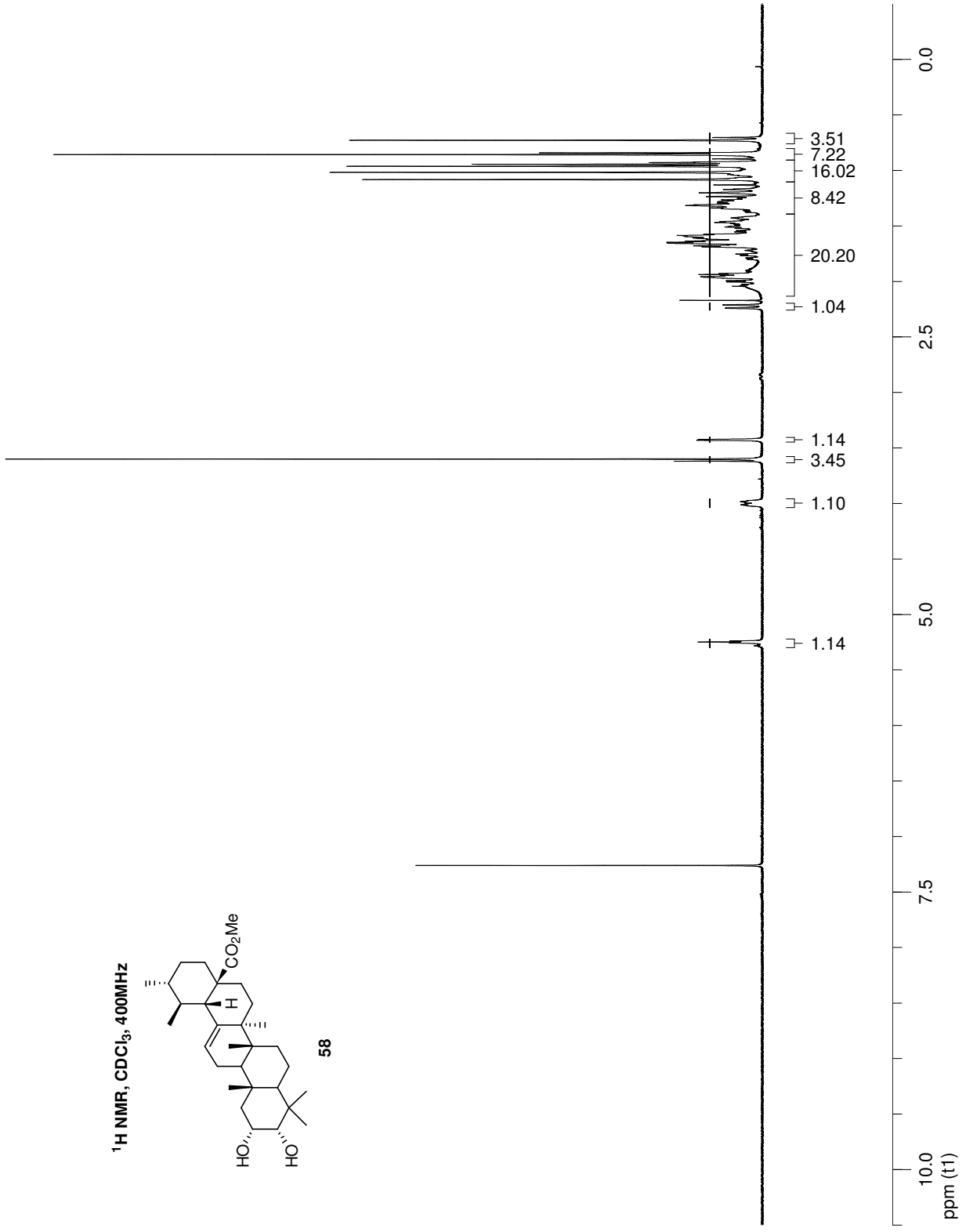


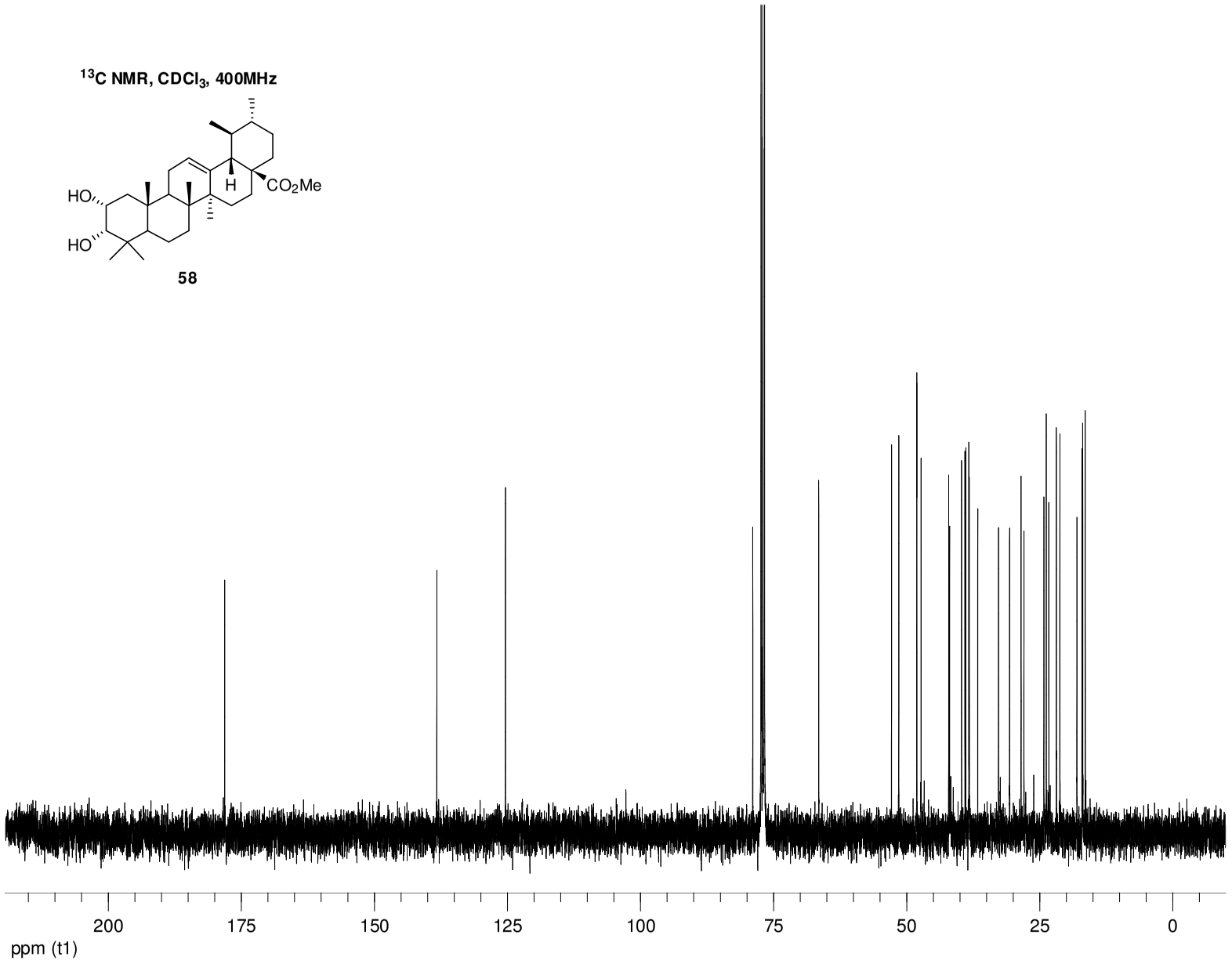
58

The procedure giving **59** also gave **58** after silica gel chromatography as a white powder (22 mg, 14%) containing 11% oleanolate analog **58a**.

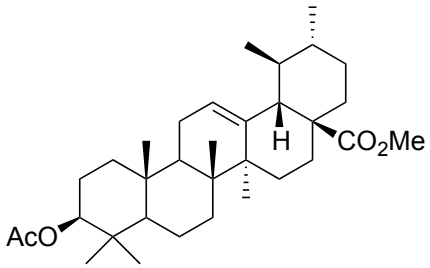
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.24 (t, J=3.5 Hz, 1H), 4.02-3.97 (m, 1H), 3.60 (s, 3H), 3.42 (d, J=2.5 Hz, 1H), 2.22 (d, J=11.2 Hz, 1H), 1.08 (s, 3H), 1.01 (s, 3H), 0.96 (s, 3H), 0.93 (d, J=6.0 Hz, 3H), 0.853 (s, 3H), 0.846 (d, J=6.2 Hz, 3H), 0.72 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 178.1, 138.3, 125.4, 78.9, 66.5, 52.8, 51.5, 48.09, 48.08, 47.3, 42.1, 41.9, 39.7, 39.0, 38.9, 38.3, 38.2, 36.6, 32.7, 30.7, 28.5, 28.0, 24.2, 23.8, 23.3, 21.9, 21.2, 18.0, 17.0, 16.9, 16.5


^{1}H NMR, $CDCl_3$, 400MHz
CO_2Me
H
HO
HO
58
3.51
7.22
16.02
8.42
20.20
1.04
1.14
3.45
1.10
1.14
0.0
2.5
5.0
7.5
10.0
ppm (t1)


^{13}C NMR, $CDCl_3$, 400MHz
HO
HO
H
CO_2Me
58
200
175
150
125
100
75
50
25
0
ppm (t1)

3-Acetoxy Methyl Ursolate (60)

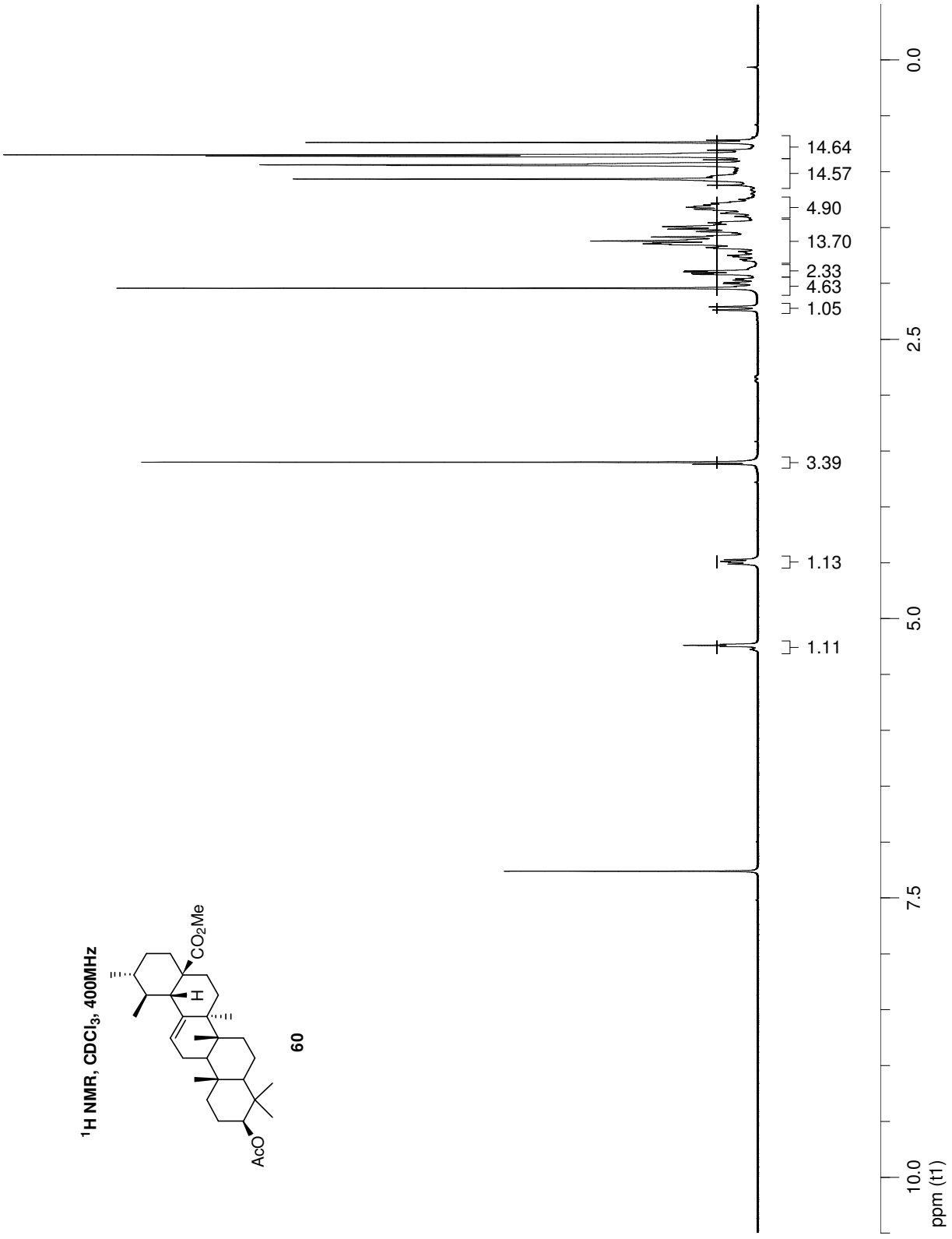


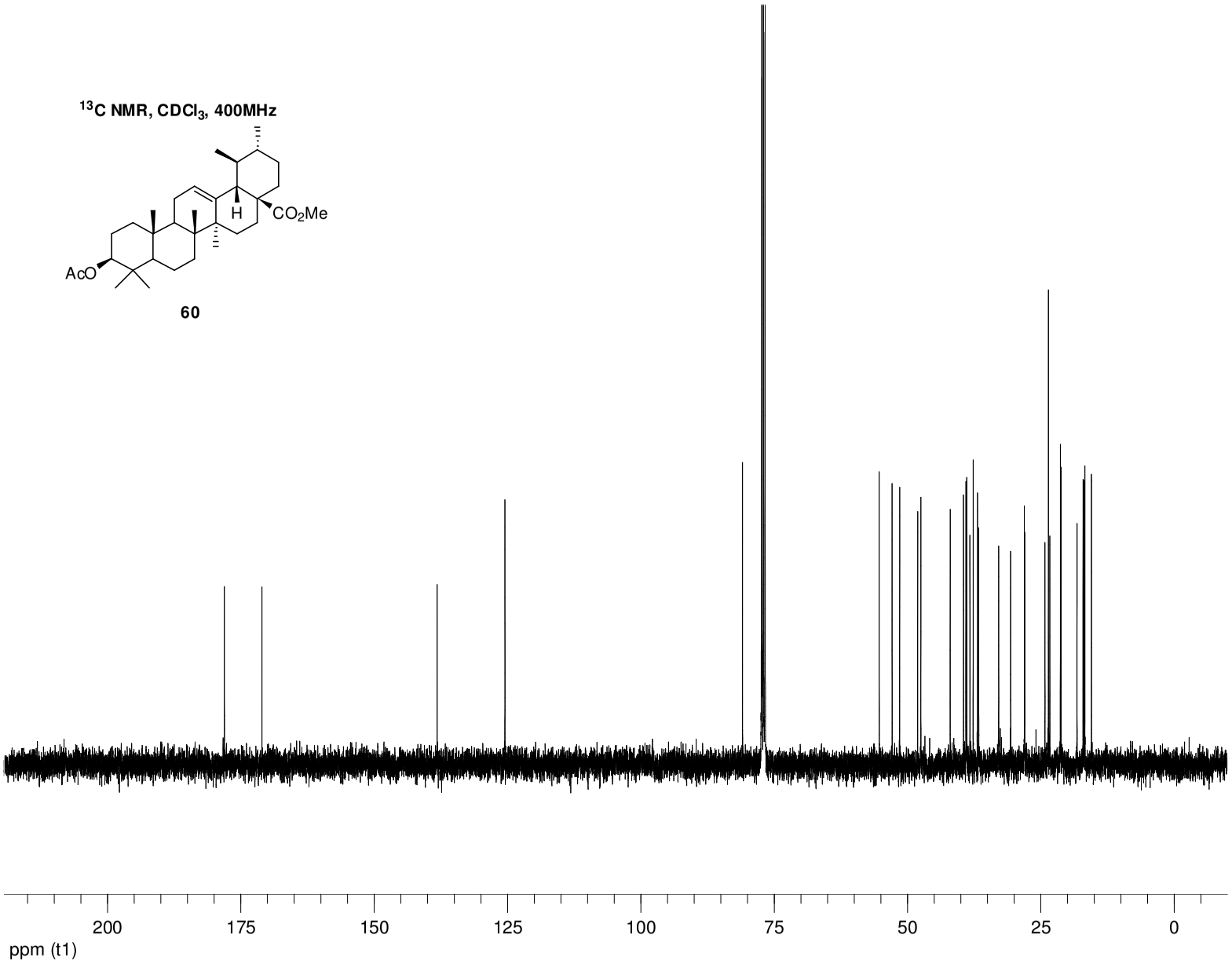
60

A solution of **48** (92 mg, 0.195 mmol), Et_3N (0.07 mL), DMAP (3 mg), and Ac_2O (0.04 mL) in DCM (5 mL) was stirred overnight at rt. In the morning, 5% HCl (10 mL) was added and stirred 30 min. The mixture was diluted with DCM (20 mL) and layers were separated. The organic extract was washed with brine (10 mL), dried ($MgSO_4$), filtered and the solvent evaporated to give a crude white solid (84 mg). Purification of the solid by silica gel chromatography using a hexanes/EtOAc gradient gave **60** as a white powder (63 mg, 63%) containing 10% of 3-acetoxy methyl oleanolate **61**.

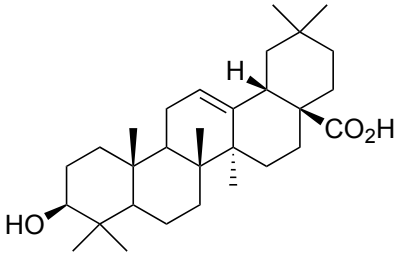
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.24 (t, J=3.5 Hz, 1H), 4.51-4.47 (m, 1H), 3.60 (s, 3H), 2.22 (d, J=11.3 Hz, 1H), 2.04 (s, 3H), 1.90 (dd, J=8.9, 3.6 Hz, 1H), 1.07 (s, 3H), 0.943 (d, J=5.4 Hz, 3H), 0.938 (s, 3H), 0.86 (s, 3H), 0.85 (d, J=4.5 Hz, 3H), 0.85 (s, 3H), 0.74 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 178.1, 171.0, 138.2, 125.4, 80.9, 55.3, 52.8, 51.4, 48.1, 47.5, 42.0, 39.5, 39.0, 38.9, 38.3, 37.7, 36.8, 36.6, 32.9, 30.6, 28.1, 28.0, 24.2, 23.5, 23.5, 23.3, 21.3, 21.2, 18.2, 17.1, 16.9, 16.7, 15.5


^{1}H NMR, $CDCl_3$, 400MHz
CO_2Me
H
AcO
60
14.64
14.57
4.90
13.70
2.33
4.63
1.05
3.39
1.13
1.11
0.0
2.5
5.0
7.5
10.0
ppm (t1)


^{13}C NMR, CDCl$_3$, 400MHz
H
CO$_2$Me
AcO
60
200
175
150
125
100
75
50
25
0
ppm (t1)

Oleanolic Acid (4)

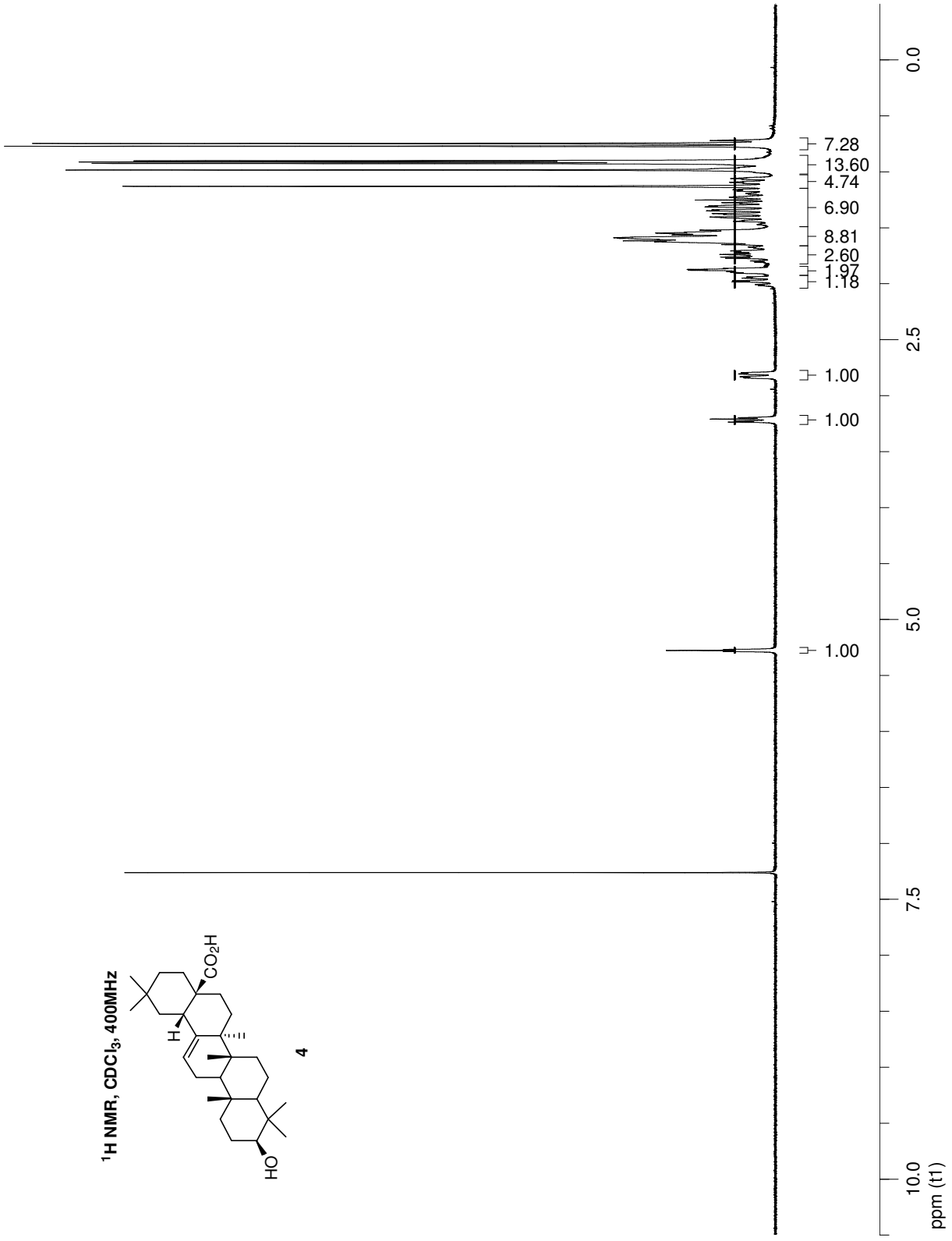


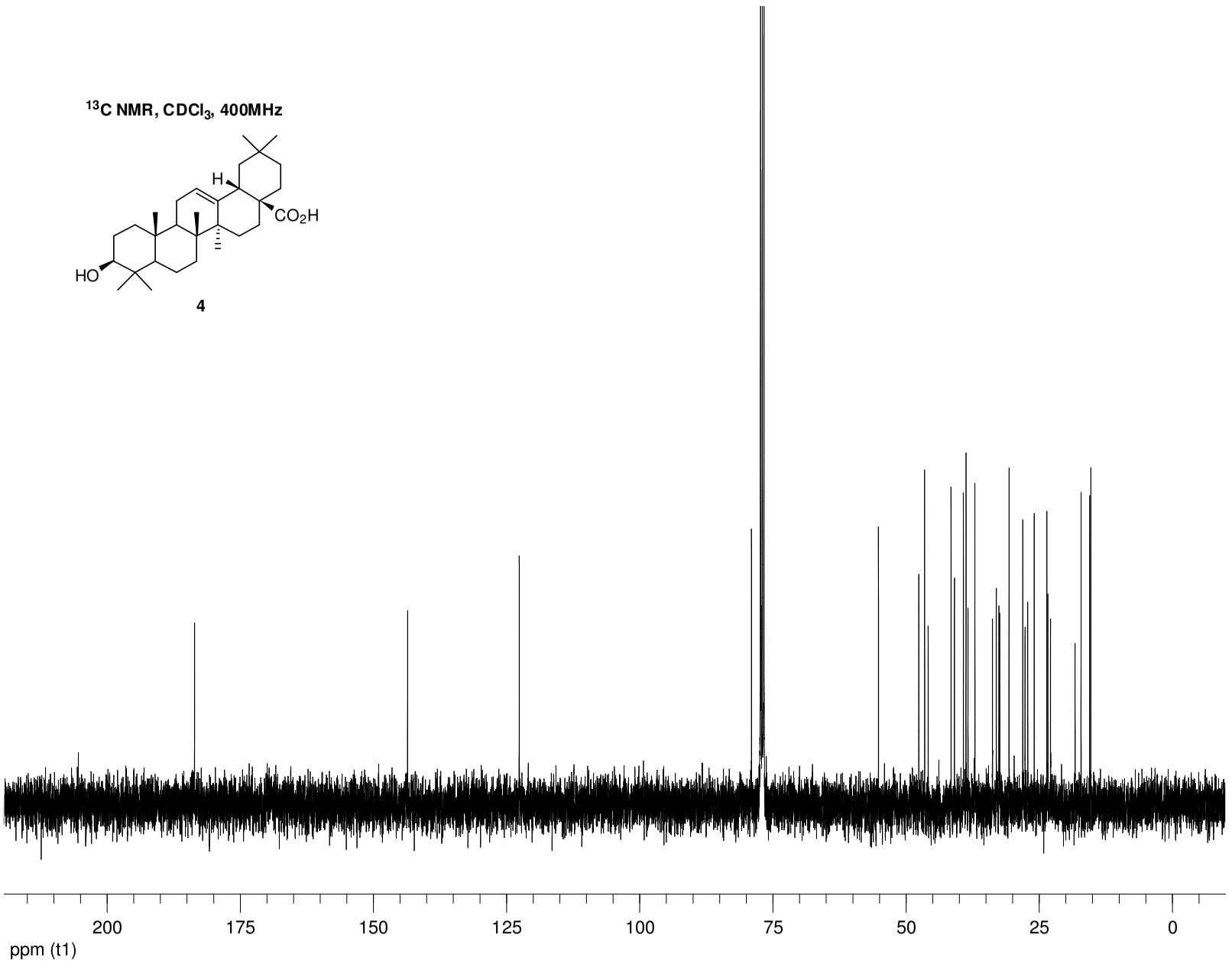
4

Oleanolic acid (**4**) was available in the lab, and its structure was confirmed by NMR data.

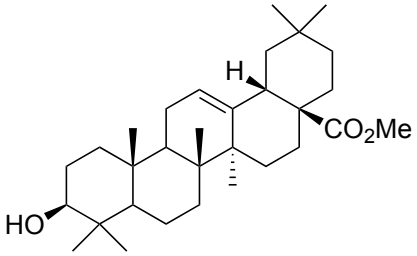
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.28 (t, J=3.6 Hz, 1H), 3.24-3.20 (m, 1H), 2.84-2.79 (m, 1H), 1.13 (s, 3H), 0.98 (s, 3H), 0.92 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.77 (s, 3H), 0.75 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 183.6, 143.6, 122.6, 79.0, 55.2, 47.6, 46.5, 45.8, 41.5, 40.9, 39.2, 38.7, 38.4, 37.1, 33.8, 33.1, 32.6, 32.4, 30.7, 28.1, 27.7, 27.2, 25.9, 23.6, 23.4, 22.9, 18.3, 17.1, 15.5, 15.3


^{1}H NMR, $CDCl_3$, 400MHz
CO_2H
H
HO
4
7.28
13.60
4.74
6.90
8.81
2.60
1.97
1.18
1.00
1.00
1.00
0.0
2.5
5.0
7.5
10.0
ppm (t1)


^{13}C NMR, $CDCl_3$, 400MHz
H
CO_2H
HO
4
200
175
150
125
100
75
50
25
0
ppm (t1)

Methyl Oleanolate (49)

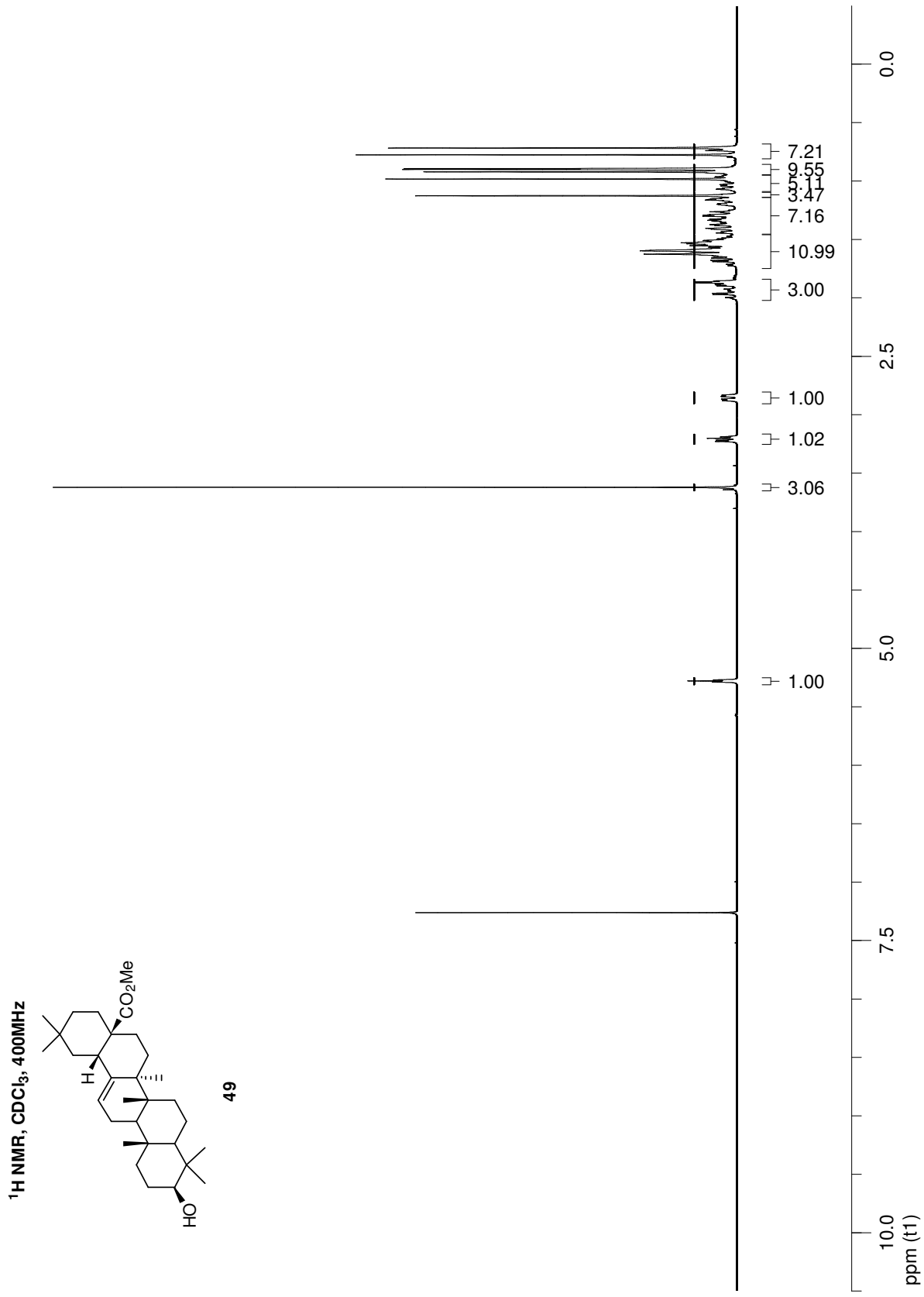


49

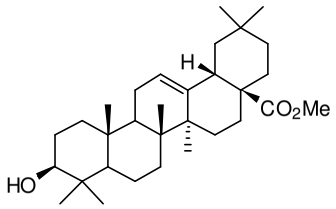
Methyl oleanolate (**49**) was available in the lab. It was purified by silica gel chromatography using a hexanes/EtOAc gradient.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.28 (t, J=3.5 Hz, 1H), 3.62 (s, 3H), 3.23-3.19 (m, 1H), 2.88-2.83 (m, 1H), 1.99-1.85 (m, 3H), 1.13 (s, 3H), 0.98 (s, 3H), 0.92 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.78 (s, 3H), 0.72 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 178.3, 143.8, 122.3, 79.0, 55.2, 51.5, 47.6, 46.7, 45.9, 41.6, 41.3, 39.2, 38.7, 38.4, 37.0, 33.8, 33.1, 32.6, 32.4, 30.7, 28.1, 27.7, 27.2, 25.9, 23.6, 23.4, 23.0, 18.3, 16.8, 15.6, 15.3

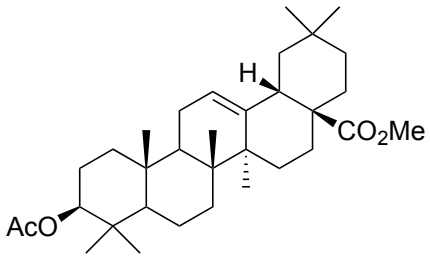

^{1}H NMR, $CDCl_3$, 400MHz
CO_2Me
H
HO
49
7.21
9.55
5.11
3.47
7.16
10.99
3.00
1.00
1.02
3.06
1.00
0.0
2.5
5.0
7.5
10.0
ppm (t1)

^{13}C NMR, $CDCl_3$, 400MHz



49

3-Acetoxy Methyl Oleanolate (61)

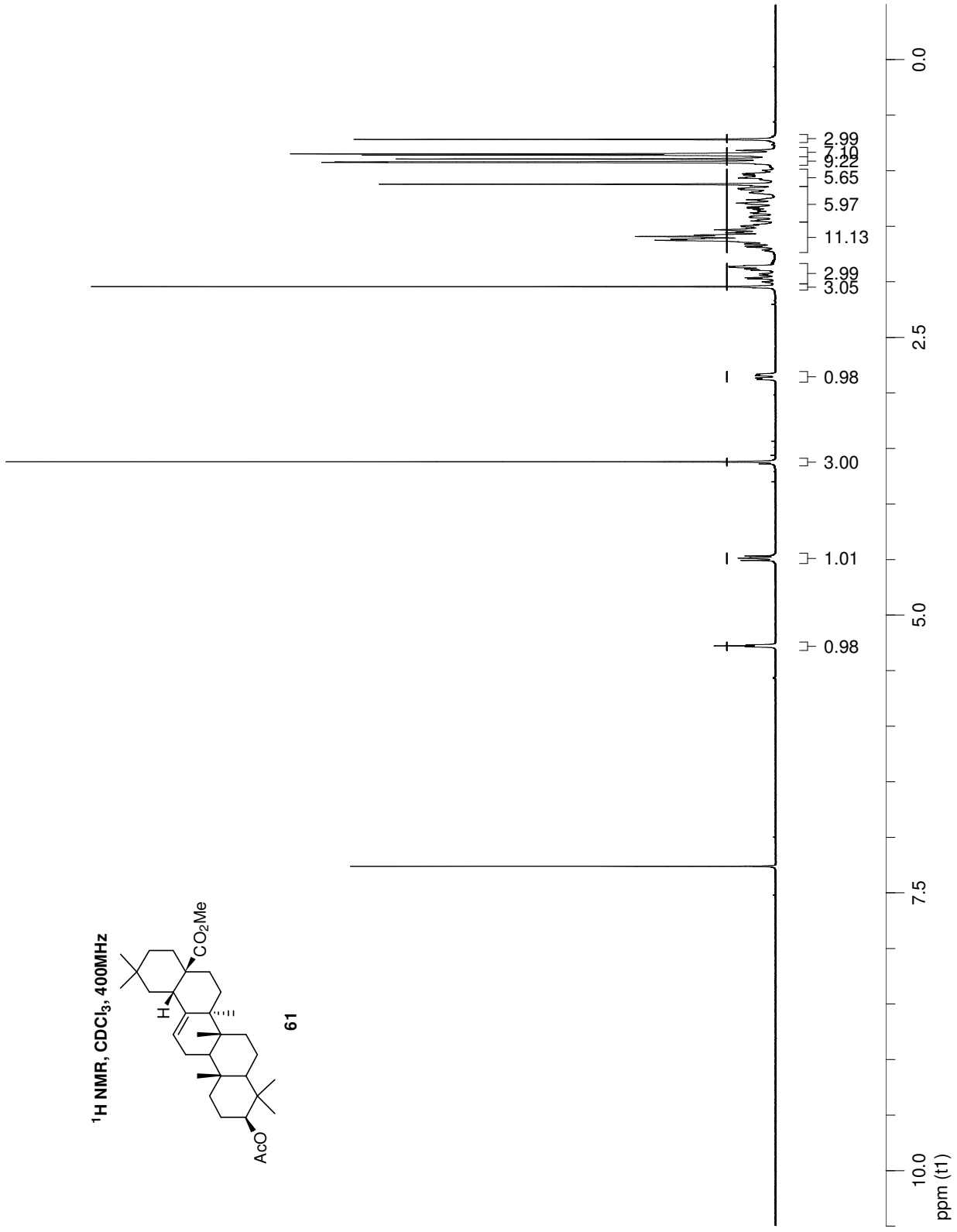


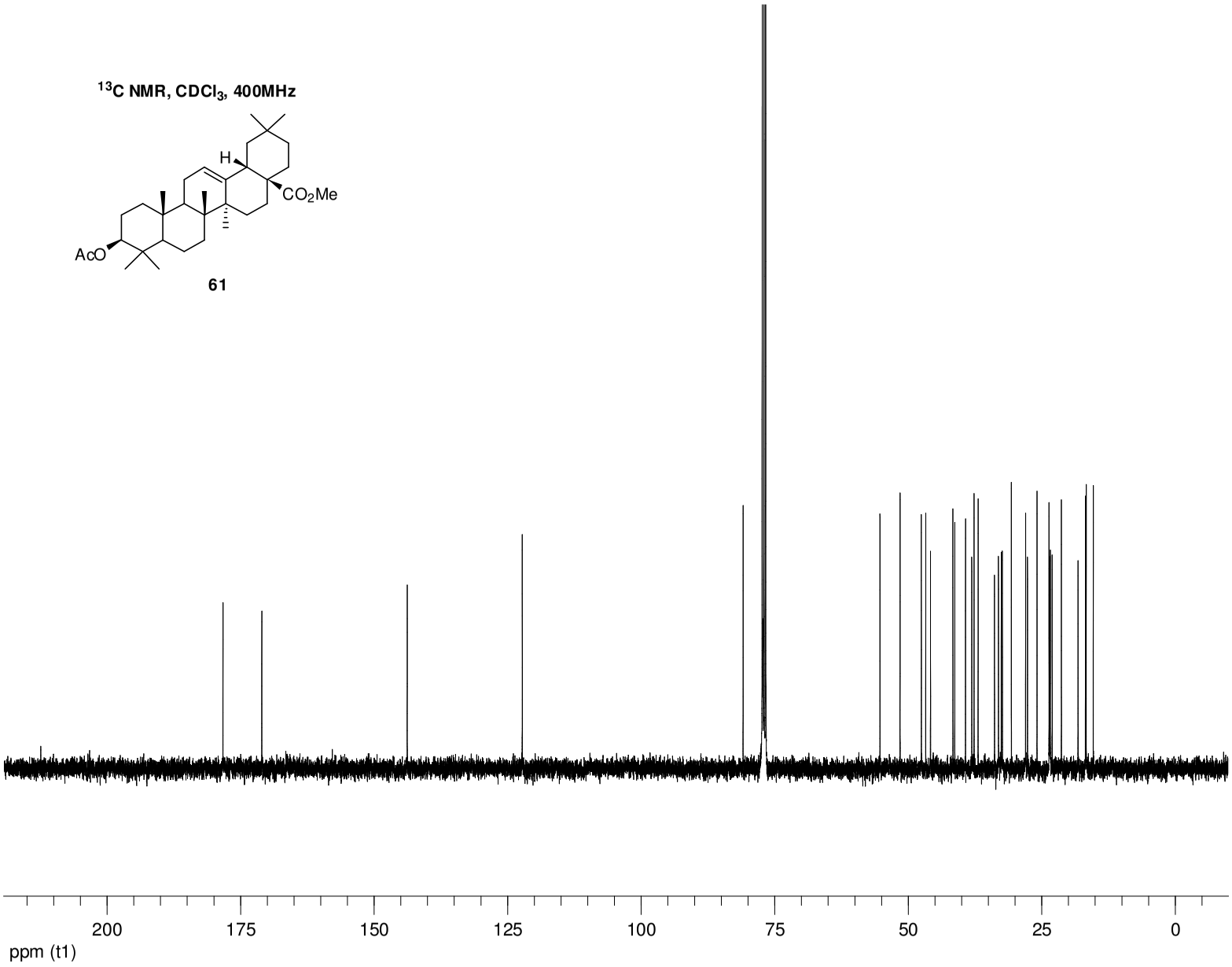
61

A solution of **49** (91 mg, 0.193 mmol), Et_3N (0.06 mL), Ac_2O (0.04 mL), and DMAP (3 mg) in DCM (4 mL) was stirred overnight at rt. In the morning, 5% HCl (10 mL) was added and stirred 30 min. The layers were separated and the aqueous layer extracted with DCM (2x15 mL). The organic extracts were combined, dried ($MgSO_4$), filtered and the solvent evaporated to give a crude white solid. Purification of the solid by silica gel chromatography using a hexanes/EtOAc gradient gave **61** as a white powder (69 mg, 70%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.28 (t, J=3.6 Hz, 1H), 4.51-4.47 (m, 1H), 3.62 (s, 3H), 2.85 (dd, J=13.9, 4.4 Hz, 1H), 2.04 (s, 3H), 2.00-1.93 (m, 1H), 1.90-1.86 (m, 2H), 1.12 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.86 (s, 3H), 0.85 (s, 3H) 0.72 (s, 3H)

^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 178.3, 171.0, 143.8, 122.3, 80.9, 55.3, 51.5, 47.5, 46.7, 45.8, 41.6, 41.3, 39.3, 38.1, 37.7, 36.9, 33.8, 33.1, 32.6, 32.4, 30.7, 28.0, 27.7, 25.9, 23.6, 23.5, 23.4, 23.0, 21.3, 18.2, 16.8, 16.7, 15.3


^{1}H NMR, $CDCl_3$, 400MHz
CO_2Me
H
AcO
61
2.99
7.10
9.22
5.65
5.97
11.13
2.99
3.05
0.98
3.00
1.01
0.98
10.0
7.5
5.0
2.5
0.0
ppm (t1)


^{13}C NMR, $CDCl_3$, 400MHz
H
CO_2Me
AcO
61
200
175
150
125
100
75
50
25
0
ppm (t1)

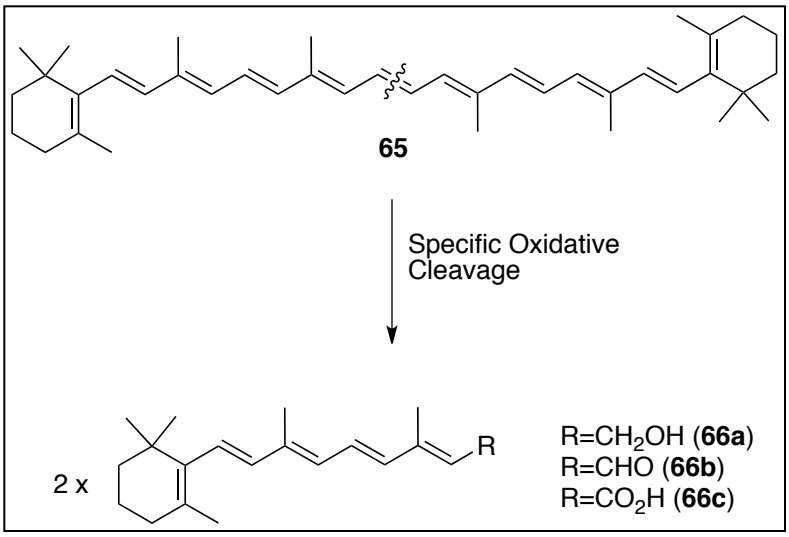
Part II: Synthesis of Hexadeuterated β-Ionone

-Chapter 4: Introduction-

4.1. Carotenoids

Carotenoids are a family of naturally occurring pigments that are widely dispersed in nature and are produced in a variety of plants, bacteria and fungi. They can be seen in the flowers, fruits and roots of many plants such as yellow daffodils, red tomatoes and orange carrots. In autumn, when the chlorophyll of green plant tissues becomes degraded, carotenoids can be seen in the brightly coloured foliage of autumn leaves.[1] Besides carotenoids' natural colouring ability, they possess several other important functions. They are highly effective at quenching the excited singlet state of oxygen to the lower energy triplet state, and may offer protection in living systems from singlet oxygen's damaging effects.[1-4] Carotenoids can quench excited state chlorophyll to help prevent damage during photosynthesis, but they can also transfer energy to chlorophyll for use in this process as well.[1,4,5] In mammals, carotenoids may offer some protection from lipid peroxidation.[2,6] Most carotenoids have a C_{40} backbone containing 3 to 15 conjugated double bonds,[1] and this extensive conjugation allows them to form highly resonance-stabilized carbon-centered radicals, which is the likely explanation for their antioxidant activity.[4,6]

One carotenoid of particular importance is β-carotene (**65**). In mammals, **65** is converted into compounds with Vitamin A activity such as retinol (**66a**), retinal (**66b**), and retinoic acid (**66c**), by specific oxidative cleavage of the central carbon-carbon double bond (**Scheme 4.1**).[1,2,4,7,8] Metabolites **66a-c** belong to a class of compounds known as retinoids, and they are particularly important for mammalian nutrition, vision, immune function and cell differentiation.[1,7,8] One aspect of carotenoids that has received less attention, but still appears to be important, is the mixture of products obtained from their complete and spontaneous oxidation with molecular oxygen.



Scheme 4.1. Specific oxidative cleavage of β-carotene (**65**) to retinol (**66a**), retinal (**66b**), or retinoic acid (**66c**).

4.2. Oxidized β-Carotene

Although the extensive conjugation in carotenoids allows them to form stable carbon-centred radicals, it also makes them susceptible to oxidative cleavage. β-carotene in particular has 11 conjugated double bonds, giving multiple sites for oxidation to occur and the potential for a large number of compounds to be formed. The propensity for carotenoids to oxidize, coupled with the presence of oxygen in the atmosphere, led researchers at Chemaphor Inc. to hypothesize that carotenoid oxidation products are responsible for non-vitamin A biological activity of carotenoids. It is noteworthy that fruits and vegetables contain a great variety of compounds derived from oxidized carotenoids, and that these compounds may be partially responsible for some of the beneficial effects of eating fruits and vegetables. In-depth studies on the products of complete, spontaneous oxidation of β-carotene with molecular oxygen were carried out, and the results were quite revealing. Many oxidation products were identified, classified as either low MW compounds (several are shown in **Fig. 4.1**) or high MW oxygen-rich oligomers of unknown structure.

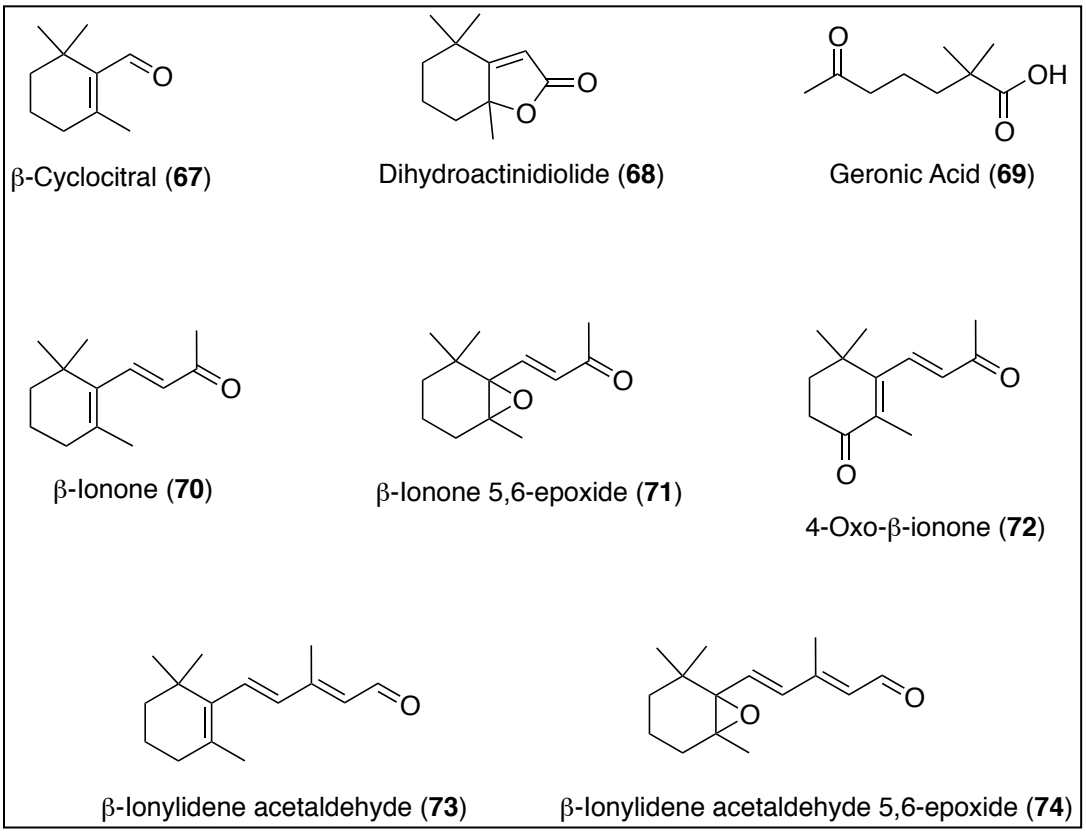


Fig. 4.1. Selected low MW products obtained from the spontaneous oxidation of β-carotene (**65**).

The whole product mixture of β-carotene oxidation was named "OxC-beta", and several biological applications were patented as a result of this research. First, it was shown that OxC-beta acted as both a cytostatic and anti-tumour agent.[9] OxC-beta was also found to enhance the immune system of animals by improving the early detection of pathogens and allowing the body to respond faster when challenged.[10] When pigs were fed a diet containing low amounts of OxC-beta (0.001% w/w), they grew approximately 8% faster and required approximately 8.5% less food to reach the same size as a control group.[11] Broiler chickens fed a diet containing 30 ppm OxC-beta were 4.3% heavier after 38 days when compared to a control group.[11] These results created an opportunity to develop a natural feed additive that could reduce infection and improve the growth of farmed livestock such as swine and poultry.

A major issue with farming livestock is the possibility of the animals becoming infected, which can be caused by the challenging conditions in which they are raised.[12]

The most expensive part of farming livestock is the cost of feed, estimated to account for 50-70% of the total cost.[11] Feed additives such as growth hormones and antibiotics have been used to improve animal growth and reduce the cost of feed. The growth hormone diethylstilbestrol was once commonly used, but it was banned in most countries after it was shown to be carcinogenic.[11] Antibiotics have now become commonly used by food producers,[11] with an estimated 70% of all antibiotics in the United States going towards livestock.[13] However, the overuse of antibiotics in this area permits the growth of antibiotic-resistant microorganisms, and the use of antibiotics in feeds has been banned in Europe for farming livestock since Jan. 1, 2006.[14] In the future, there may be a great demand for alternative feed additives that improve growth and reduce infection, and OxC-beta has the potential to meet this demand.

4.3. Unanswered Questions Concerning OxC-beta

Although much research has been done on OxC-beta, there are some areas that require further study. If it were to be used as a feed additive, it would be important to measure the content of retinoids **66a-c**. High levels of **66a-c** are toxic, and could cause problems if they entered the human food chain in high levels.[8] Preliminary studies have not detected these compounds, but in order to obtain government approval for sale, an accurate measurement must show **66a-c** to be within acceptable limits. At the same time, it would be useful to quantify the low MW compounds identified in **Fig. 4.1.** This would provide valuable information on the composition of OxC-beta.

Another area that would be worth studying further is the process of β-carotene oxidation. While general information such as reaction conditions, time and yield are available, little is known about the specific mechanisms of how the oxidation proceeds. One way to address these unanswered questions would be through the use of isotopically labelled compounds.

4.4. Isotopically Labelled Compounds

Compounds containing stable isotopes such as ^{13}C and D (deuterium) are quite useful in chemistry. For the quantification of analytes in a sample, they are an excellent choice as an internal standard. Internal standards are added prior to a sample's preparation and compensate for any losses of analyte during this process. Since a stable isotope of a compound would have nearly identical chemical and physical properties, the losses of internal standard and analyte would be very similar and the resulting measurement would be highly accurate.[15]

When quantifying analytes in a complex mixture or matrix, caution should be exercised if the measurements are recorded with UV or MS detectors, because the background may interfere and affect the accuracy.[15] To circumvent this problem, a combination of isotopic internal standards and highly selective MS/MS techniques has proven useful.[15,16] This combination allows the accurate quantification of analytes within a matrix with little or no sample preparation, and the amount of analyte is calculated using a ratio of analyte and isotope mass fragments given by the MS/MS detector.[15,16] Previous work on OxC-beta has demonstrated a matrix effect with MS and UV-Vis detectors. Thus, the combination of isotopically labelled internal standards with MS/MS detection would allow for the highly selective and accurate measurements of retinoids **66a-c** and the low MW compounds of OxC-beta.

Another use for isotopes is their ability to provide insight into reaction mechanisms. By inserting ^{13}C or D into a molecule, it is possible to elucidate a reaction mechanism by analyzing the reaction intermediates or products. In the case of OxC-beta, isotopically labelled β-carotene could be used to study the process of oxidation. Differences in NMR and mass spectra of the products could show how certain portions of β-carotene are transformed. A useful starting point to accomplish the above goals would be the preparation of a common isotopically labelled intermediate that could be converted into the desired compounds.

4.5. β-Ionone

A common synthetic intermediate for β-carotene, retinoids **66a-c**, and several of the low MW compounds of OxC-beta (**Fig. 4.1**) was identified as β-ionone (**70**) (**Fig. 4.2**). Through various chemical transformations such as oxidations and carbon chain extensions, it would be possible to convert **70** into the desired compounds. Thus, for part two of this thesis, a synthesis of isotopically labelled **70** was undertaken.

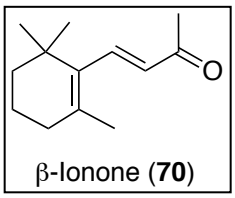


Fig. 4.2. β-Ionone (**70**), a common intermediate for the synthesis of low MW compounds of OxC-beta, retinoids **66a-c** and β-carotene (**65**).

4.6. References

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-Chapter 5: Discussion and Results-

5.1. Proposed Synthesis of Labeled β-Ionone

For the synthesis of isotopically labeled β-ionone, two choices of stable isotopes were identified as ^{13}C and D. It was thought that the use of D would be less expensive, so it was selected over ^{13}C. However, the site of incorporation and the synthetic sequence had to be carefully selected in order to avoid any exchange of deuterium with hydrogen, or "scrambling". For example, if deuterium was placed in an acidic position of the molecule, scrambling could occur and result in a poorer end product with a lower %D incorporation. The best way to avoid scrambling was to place the deuterium in a non-labile position. A literature search revealed several syntheses of β-ionone or similar compounds, so the strategy was to choose a synthetic intermediate that could be prepared with deuterium in a non-labile position and continue the synthesis towards labeled β-ionone. Based on this, it was proposed that β-ionone-d_6 (**75**) could be prepared from the common intermediate 2,2,6-trimethylcyclohexanone-d_6 (**76**), with deuterium incorporated at the geminal dimethyl group (**Fig. 5.1**).

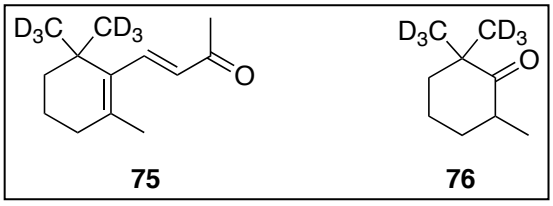
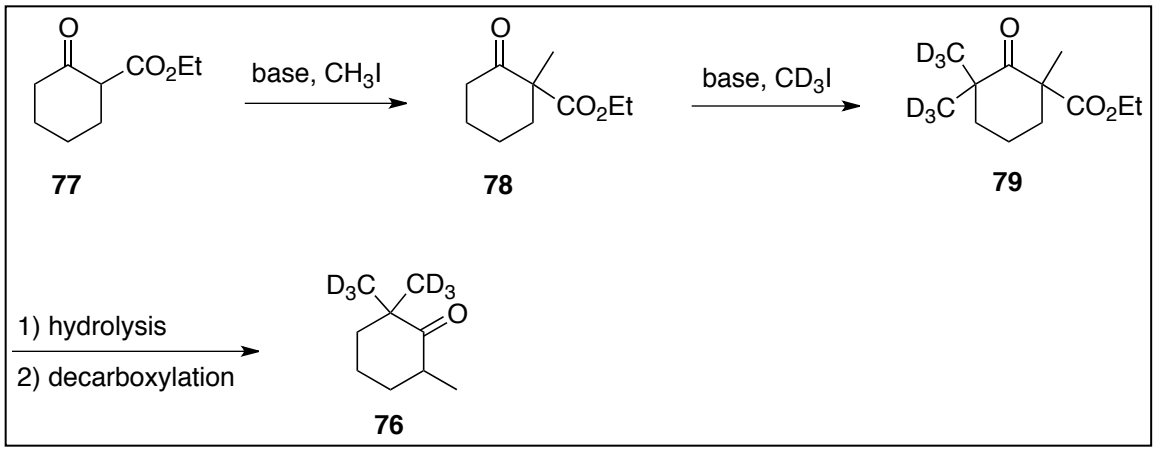


Fig. 5.1. Structures of β-ionone-d_6 (**75**) and 2,2,6-trimethylcyclohexanone-d_6 (**76**).

5.2. Synthesis of 2,2,6-Trimethylcyclohexanone-d_6

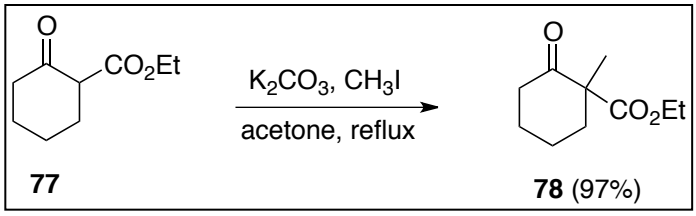
A general strategy for the synthesis of **76** was considered, and the following sequence was proposed (**Scheme 5.1**):



Scheme 5.1. General strategy for the synthesis of 2,2,6-trimethylcyclohexanone-d_6 (**76)**.

Commercially available ketoester **77** was selected as starting material because it was much less expensive than **78**, with a price of $100.50 CAD for 25 g vs. $315 CAD for 25 mg at the time of writing.[1] Also, **77** could be selectively converted to **78** by taking advantage of the difference in α-keto proton acidities. Alkylation of **78** at the remaining α-keto position with CD_3I would give deuterated ketoester **79**, and subsequent hydrolysis and decarboxylation would provide ketone **76**.

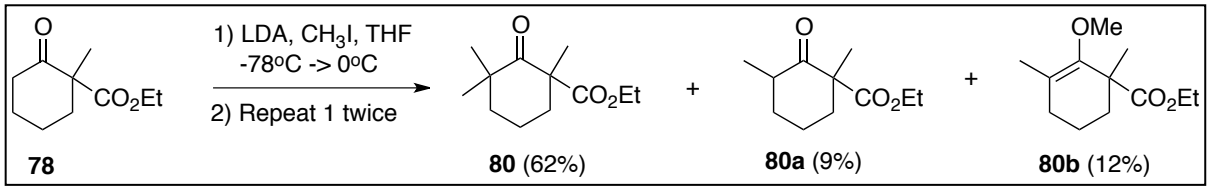
A literature procedure was found describing the reaction of **77** with CH_3I and NaOEt to give **78** in 60% yield after distillation.[2] A slightly modified procedure was attempted by dissolving Na in EtOH, adding **77** and CH_3I and heating to reflux. GC-MS analysis of this reaction showed a mixture of products, so this reaction product was discarded. It was thought that the use of the weaker base K_2CO_3 would give a selective, mild alkylation with few side products. Thus, reaction of **77** with K_2CO_3 and CH_3I in refluxing acetone gave complete, selective alkylation to provide **78** in 97% yield without purification (**Scheme 5.2**).



Scheme 5.2. Selective alkylation of ketoester **77**.

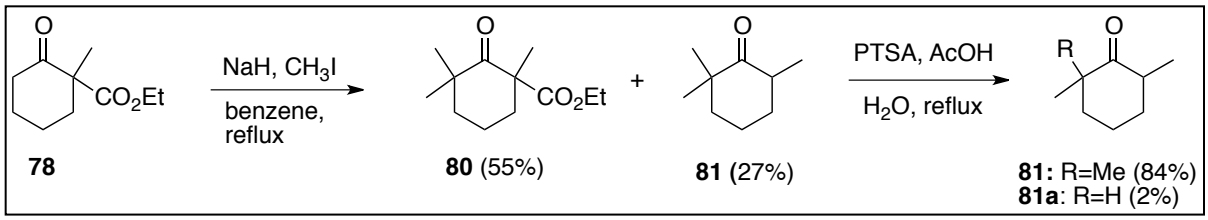
The mass spectrum of **78** gave the correct [M]+ peak of 184, and the ^{1}H NMR spectrum integrated for the correct total of 16 hydrogens with a methyl singlet at 1.29 ppm.

For the introduction of the geminal dimethyl group, some test reactions were carried out with CH_3I to determine the most effective conditions before using the more expensive CD_3I. The first reaction was a series of three alkylations with LDA and CH_3I in THF at -78 ^oC -> 0 ^oC. This method led to the desired product **80** (62%), but also gave the mono-methylated product **80a** (9%) and methyl enol ether **80b** (12%) by GC integration (**Scheme 5.3**).



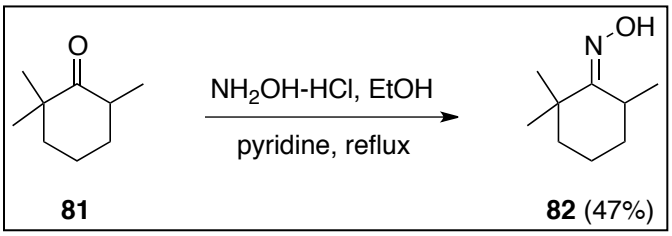
Scheme 5.3. Attempted alkylation of **78** with LDA/CH_3I.

The structures of these products were proposed based on their GC-MS [M]+ peaks of 198 (**80a**) and 212 (**80** and **80b**). Product **80b** was isolated after silica gel chromatography and its structure was determined by its ^{1}H NMR spectrum, which gave a distinct singlet at 3.60 ppm (O**C**$\mathbf{H_3}$). Since this method was time consuming and it gave a mixture of products, a better procedure was needed. A reference was found for the preparation of **80** that was both simple to perform and gave fewer side products.[3] Following this procedure, a mixture of **78,** NaH and CH_3I were refluxed in benzene to give a mixture of **80** (55%) and decarboxylation product **81** (27%) (**Scheme 5.4**). According to GC-MS, only traces of side products **80a** (0.9%) and **80b (**2.8%**)** were present. The decarboxylation product **81** was likely due to the use of old NaH that had partially hydrolyzed to NaOH. Under the reaction conditions, the ester could be hydrolyzed and the intermediate beta-keto acid would be readily decarboxylated.



Scheme 5.4. Preparation of 2,2,6-trimethylcyclohexanone **81**.

The mixture of **80** + **81** was decarboxylated following the procedure outlined by Stevens and Weinheimer[3] by refluxing with AcOH, PTSA and H_2O (**Scheme 5.4**). This gave **81** in 84% yield, along with 2% of mono-methylated ketone **81a** and traces of other impurities. The structures of products **81** + **81a** were determined by matching their mass spectra with the GC-MS spectral library. It was thought that removal of **81a** was important to avoid contamination of the final product β-ionone-d_6. Separation of **81** from **81a** would be difficult by silica gel chromatography due to their similar structures and polarity, but conversion of the oily mixture to a crystalline form could allow purification by crystallization. Thus, **81** + **81a** were converted to their corresponding oximes by reaction with NH_2OH-HCl and pyridine in 99% EtOH at reflux. The oximes were crystallized from hot hexanes to give pure oxime **82** in 47% yield (**Scheme 5.5**).

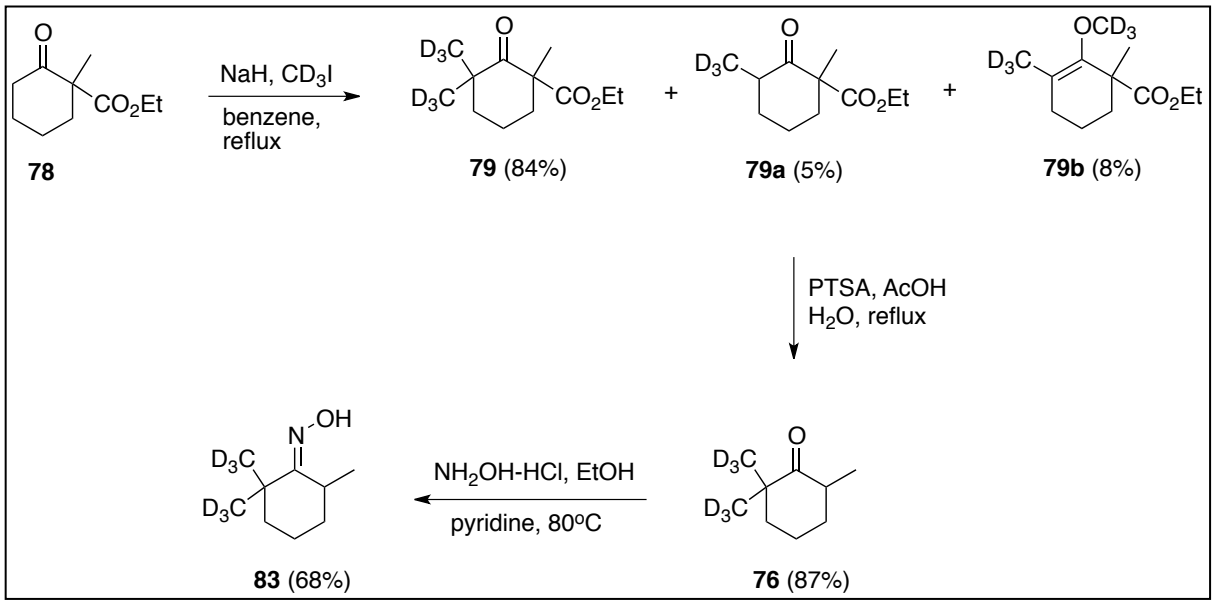


Scheme 5.5. Preparation of oxime **82**.

The GC-MS spectrum of **82** gave the expected [M]+ peak of 155, and the ^{1}H NMR spectrum integrated to 17H with three methyl groups as singlets at 1.16 and 1.19 ppm (6H) and a doublet at 1.20 ppm (J=7.2 Hz, 3H). The ^{13}C NMR spectrum was in agreement with one signal at 169.0 ppm and 9 carbons in total.

With a working procedure for the preparation of **81** and its purification as its oxime, the synthesis of a deuterated version was initiated. The alkylation of **78** with CD_3I

(99.9 atom %D) and NaH in refluxing benzene gave **79** in 84% yield after workup, although it was slightly less pure than before, as 5% of mono-methylated product **79a** and 8% of methyl enol ether **79b** was also obtained by GC integration (**Scheme 5.6**). It should be noted that the use of fresh NaH prevented the previously observed decarboxylation during this step.

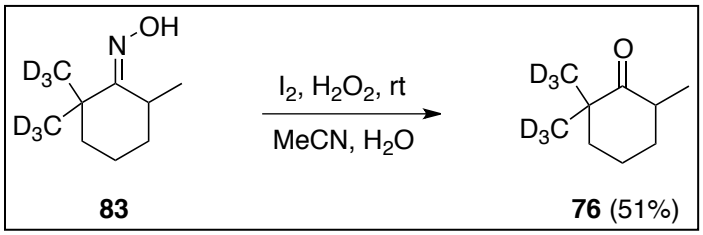


Scheme 5.6. Synthesis of deuterated ketone **76** and oxime **83**.

Decarboxylation of the product mixture with AcOH, PTSA and H_2O at reflux gave crude **76** as yellow oil (87%). This was converted to oxime **83** by stirring with NH_2OH-HCl, pyridine and 99% EtOH at 80 ^oC (**Scheme 5.6**). The crude white solid was crystallized four times from hot hexanes to give pure **83** as white crystals (68%). The GC-MS spectrum gave the correct [M]+ peak of 161, and comparison of the ^1H NMR spectrum to the non-deuterated oxime **82** showed complete disappearance of the methyl singlets at 1.16 and 1.19 ppm.

Removal of the oxime from **83** was first tested on a small scale to determine the optimum set of reaction conditions. Several methods were tested, including the use of MnO_2 in DCM at rt;[4] the use of $NaHSO_3$ in 1:1 EtOH:H_2O at 90 ^oC;[5] and the use of I_2 with 30% H_2O_2 in 9:1 MeCN:H_2O at rt.[6] These reactions were monitored by GC-MS and the latter set of conditions were found to be the cleanest, so they were selected. Thus,

conversion of **83** under the above conditions gave **76** after distillation at 74-75 ^{o}C / 35 mmHg (51% yield, 95% purity by GC integration) (**Scheme 5.7**).

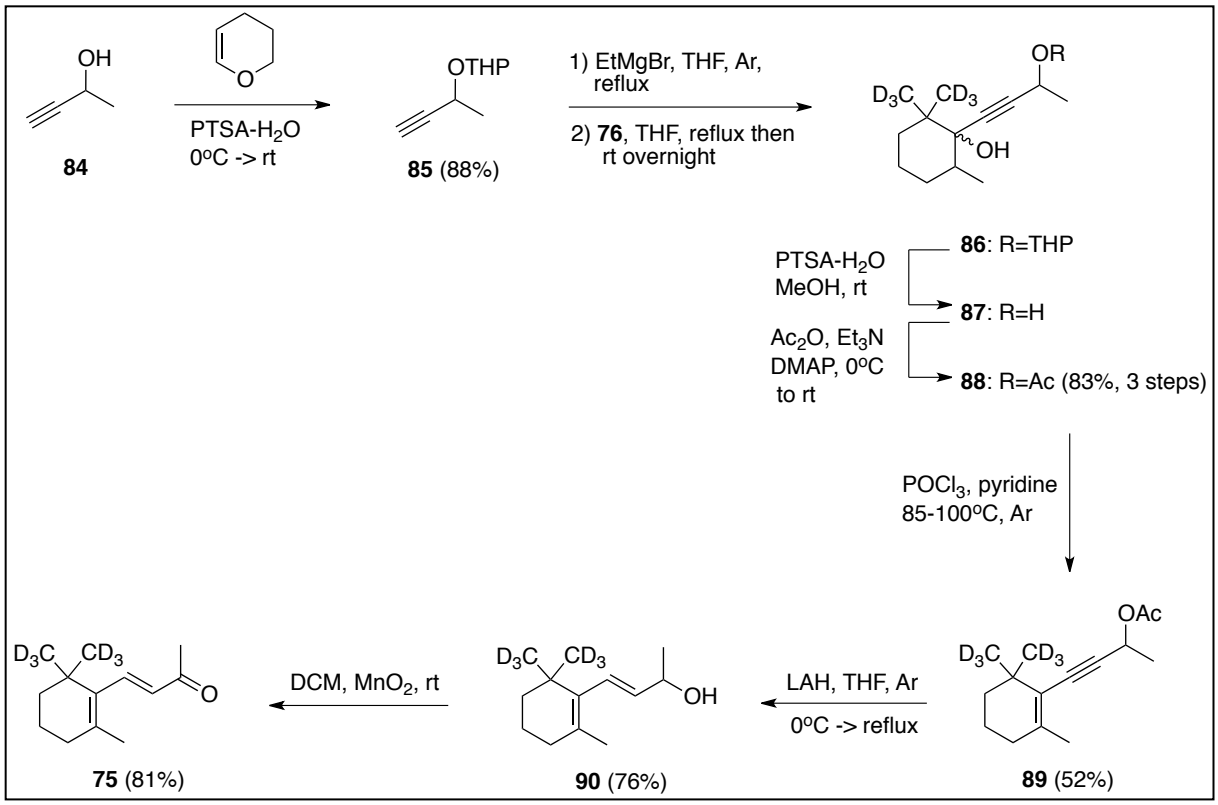


Scheme 5.7. Conversion of oxime **83** to 2,2,6-trimethylcyclohexanone-d_6 (**76**).

Analysis of the GC-MS spectum gave the correct [M]+ peak of 146, and the ^{1}H NMR spectrum integrated correctly for 10H with only one methyl group as a doublet at 0.99 ppm (J=6.4 Hz). The deuterium incorporation was calculated using GC-MS in selected ion monitoring mode, giving the total deuterium content as 99.85% with 0% d_0 ion ([M-6]+). With ketone **76** in hand, its conversion to β-ionone-d_6 could begin.

5.3. Synthesis of β-Ionone-d_6

As mentioned above, several literature procedures were available on the preparation of β-ionone analogs from compounds similar to **76**.[7-10] At this time, a procedure was also discovered describing the preparation of β-ionone-d_3 with one CD_3 at the geminal dimethyl position.[11] The following method was adapted from the literature[7-11] for the conversion of **76** to β-ionone-d_6 (**Scheme 5.8**):



Scheme 5.8. Conversion of ketone **76** into β-ionone-d_6 (**75**).

The sequence began by converting 3-butyn-2-ol **84** into its THP ether **85**. A general procedure by Robertson[12] was followed using PTSA-H_2O and 3,4-dihydro-2*H*-pyran from 0 ^oC to rt, giving 88% of a colourless oil after distillation (64 ^oC / 7 mmHg). The ^1H NMR spectrum showed two isomers due to the introduction of a second chiral center into the molecule. This was expected, and the ^1H NMR was consistent with that reported by Hickman and coworkers.[13]

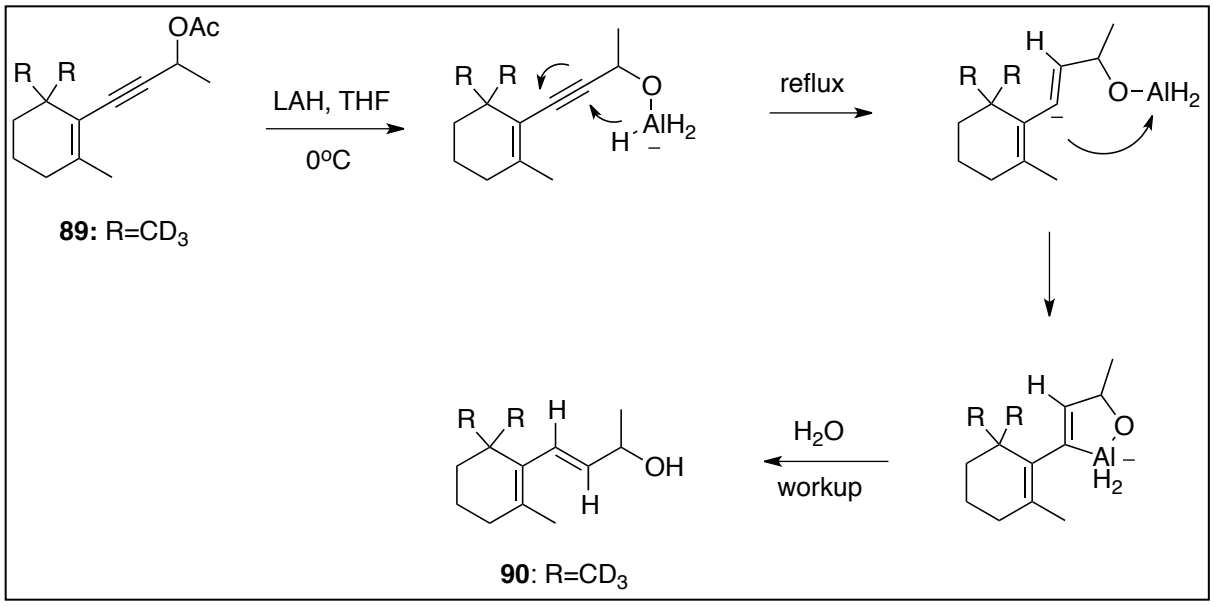
The Grignard reagent of **85**, prepared by reaction with EtMgBr, was added to a solution of ketone **76** to give THP ether **86** as a crude, dark reddish brown oil. It should be noted that the Grignard reagent of THP ether **85** was used instead of alcohol **84** due to its improved solubility and what appeared to be slightly better yields by comparison of the literature procedures.[7-12]

Removal of the THP ether of **86** was accomplished with PTSA-H_2O in MeOH at rt to give diol **87** as crude reddish brown oil. Acetylation of the crude oil using Ac_2O, Et_3N and DMAP from 0^oC to rt gave acetate **88** as a yellow oil after silica gel

chromatography (83% from **76**). The ^{1}H NMR was consistent with the non-deuterated analog of **88** reported by Hirai and coworkers, obtained as two diastereomers.[11]

The dehydration of acetate **88** with $POCl_3$ and pyridine at 85-100 ^{o}C was first tested on a small scale, and greatly different reaction times for the two isomers were observed by GC-MS. One isomer went to completion in hours, where the other took days. Modest yields were reported in various literature procedures (37-49%)[7-11], possibly due to one isomer not reacting in the short reaction times given (< 24 hrs). Thus, the large scale was run at 100 ^{o}C for 4 hours to give almost complete dehydration of one isomer, with the other relatively untouched. To prevent any decomposition of the product due to prolonged reaction times, this mixture was separated by silica gel chromatography to give **89** as a yellow oil (37%). The other isomer of **88** was re-subjected to the dehydration at 85-90 ^{o}C for almost 5 days before the reaction was complete, giving **89** as a yellow oil (15%). The total yield of 52% by this method was slightly higher than the literature procedures by 3-15%.[7-9,11] The ^{1}H NMR spectrum of **89** was consistent with its non-deuterated analog from the literature,[11] and the GC-MS spectrum gave a [M]+ peak of 240 in agreement with the expected value. Selected ion monitoring confirmed the deuterium incorporation as 99.83%, with 0.00% d_0.

Alkyne **89** was subjected to a reduction with LAH in THF from 0 ^{o}C to reflux to give the trans alkene **90** as a colourless oil after silica gel chromatography (76%). Its structure was confirmed by comparing the ^{1}H NMR spectrum against the literature; the trans coupling constant of 15.9 Hz for the olefinic protons confirmed the stereochemistry.[11] The mechanism of this selective reduction is given in **Scheme 5.9**.



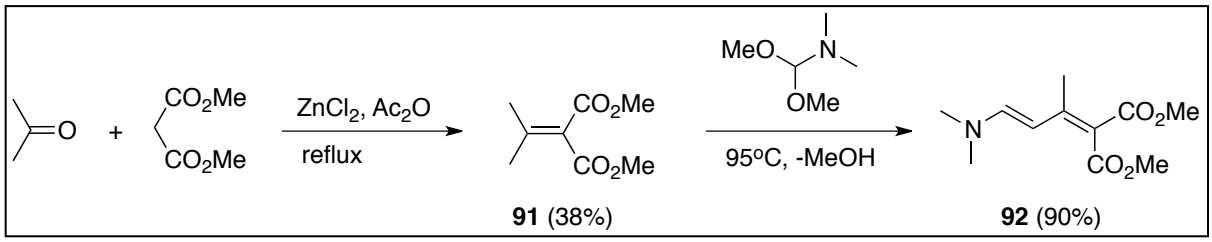
Scheme 5.9. Selective LAH reduction of alkyne **89** to trans alkene **90**.

The final step in this sequence was the oxidation of alcohol **90** with activated MnO_2 in DCM at rt. Purification of the product by silica gel chromatography gave the desired β-ionone-d_6 (**75**) as pale yellow oil in 81% yield. Its ^{1}H NMR spectrum was identical to a reference sample, except for the lack of the geminal dimethyl group at 1.07 ppm (s, 6H). Analysis of **75** for deuterium content was accomplished using electrospray ionization (ESI) as opposed to GC-MS, because only a weak [M]+ signal was observed in the latter. ESI gave 99.85% total deuterium incorporation and 0.03% d_0. The slight d_0 content may have been due to the background signal of the analysis. Regardless, β-ionone-d_6 was prepared with excellent deuterium incorporation, and some time was still available to try preparing other compounds from it.

5.4. Retinoic Acid-d_6

Amongst the many compounds that could be prepared from β-ionone-d_6, the most important were thought to be retinoids **66a-c**, since the quantification of **66a-c** in OxC-beta would be important for obtaining government approval for sale as a feed additive. An extensive literature search revealed a relatively recent literature procedure (2003) for a one-pot preparation of retinoic acid from β-ionone in 70% yield.[14,15] This was the

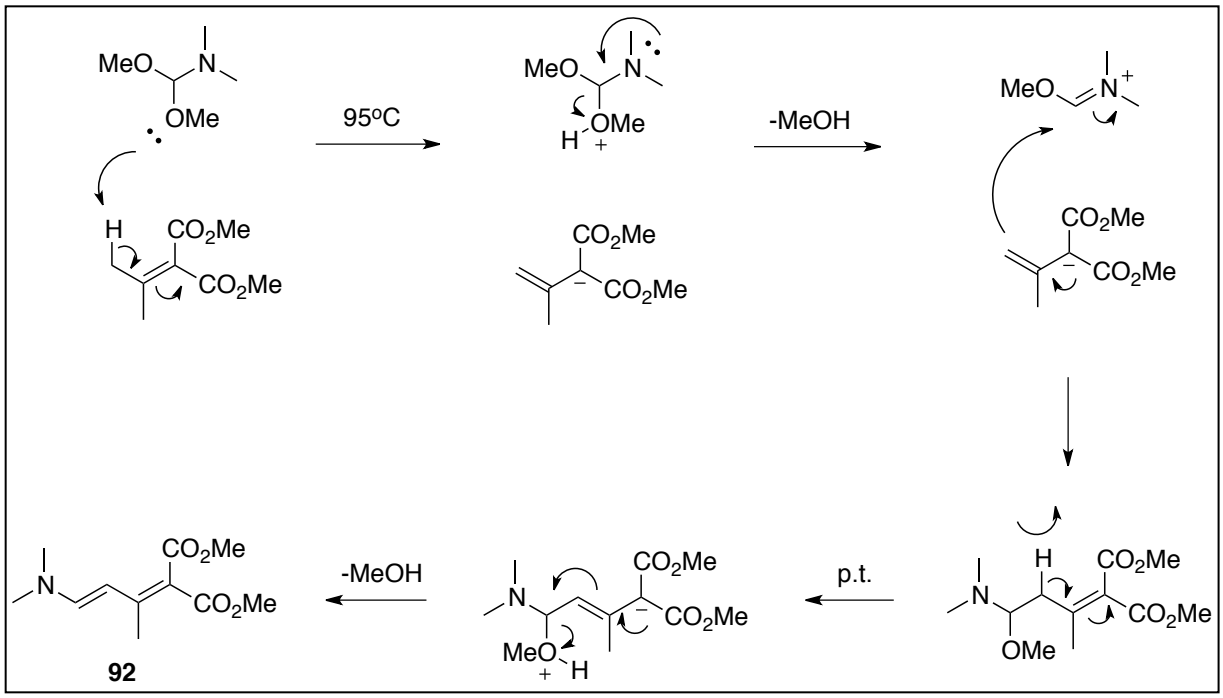
fastest way to obtain any of the retinoids **66a-c**, so it was decided to prepare retinoic acid-d_6 via this method. Before it could be attempted though, the one-pot reaction required the use of enamine **92**, which was prepared first via **Scheme 5.10**.



Scheme 5.10. Preparation of enamine **92** from acetone and dimethylmalonate.

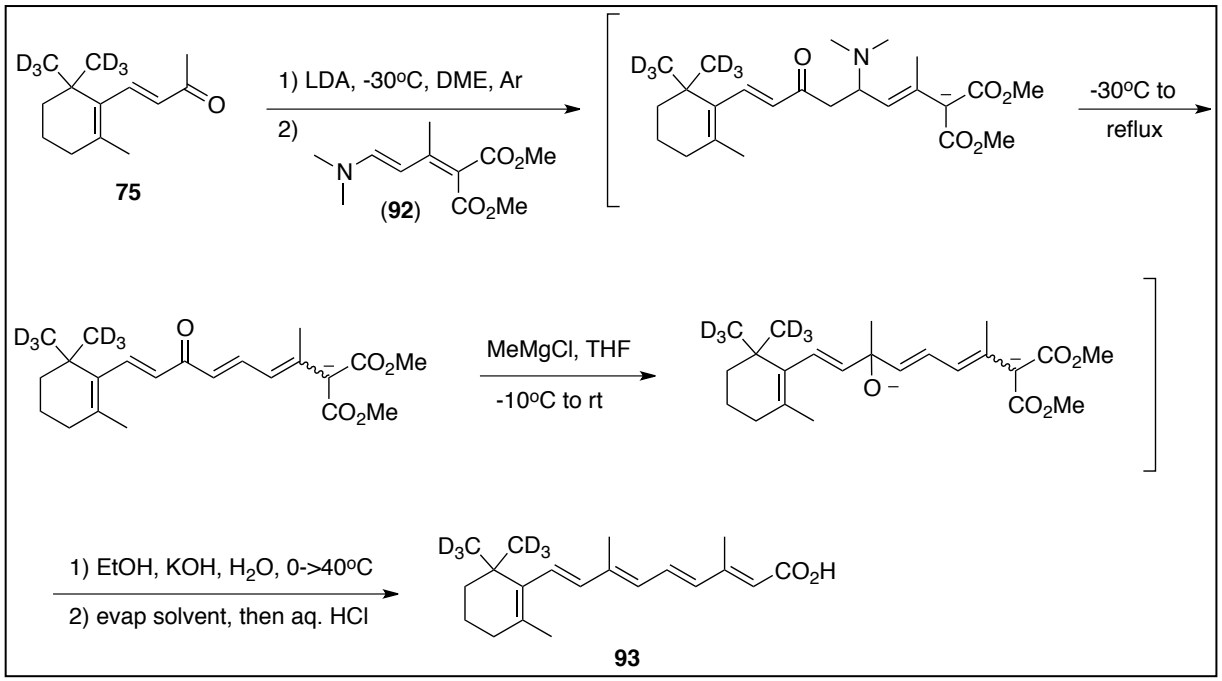
Following a slighty modified procedure by Eliel and coworkers,[16] the reaction of acetone, dimethylmalonate, Ac_2O, and anhydrous $ZnCl_2$ at reflux gave unsaturated malonate **91** as a colourless oil in 38% yield after distillation (88 ^oC / 1 atm). Both the GC-MS and ^1H NMR spectra were consistent with the product, with an [M]+ peak of 172 and two proton singlets at 2.07 and 3.77 ppm in a 1:1 ratio (6H each).

Enamine **92** was prepared by heating **91** with *N,N*-dimethylformamide dimethyl acetal at 95 ^oC, using a Dean-Stark apparatus to remove MeOH.[14] The product **92** was obtained as yellows crystals after crystallization from ether/pentane (90%), confirmed by matching its ^1H NMR spectrum with the literature.[14] The mechanism of this reaction is given in **Scheme 5.11**.



Scheme 5.11. Mechanism for formation of enamine **92**.

With enamine **92** in hand, the one-pot synthesis of retinoic acid-d_6 (**93**) was attempted. The general mechanism of the reaction is outlined in **Scheme 5.12**.



Scheme 5.12. One-pot synthesis of retinoic acid-d_6 (**93**) from β-ionone-d_6 (**75**).

A solution of **75** in DME was added to a solution of LDA in DME under argon at -30 ^{o}C to give the corresponding enolate. Addition of enamine **92** in DME gave attack by the enolate to form an intermediate amine with a stabilized negative charge due to conjugation with the diester. Heating the mixture to reflux forced the elimination of dimethylamine and created a newly conjugated double bond to the ketone. At this stage, addition of MeMgCl at -10 ^{o}C and warming to rt resulted in addition of the methyl group at the ketone. The anions were quenched by addition of EtOH at 0 ^{o}C and the diester hydrolyzed with KOH at 40 ^{o}C. Acidification of this mixture with HCl gave dehydration of the tertiary alcohol as well as spontaneous decarboxylation of the malonic acid group. Purification of the product by silica gel chromatography using 98:2 DCM:MeOH, followed by crystallization from MeCN and then MeOH gave pure **93** as yellow crystals. The ^{1}H NMR spectrum was an excellent match against a non-deuterated reference sample, and the deuterium incorporation was found to be excellent as evidenced by the lack of a methyl singlet at 1.03 ppm (geminal dimethyl group). The specific measurement of deuterium incorporation via GC-MS was not possible, as the product was not eluted from the column. When electrospray ionization was attempted, significant background was observed for both deuterated and non-deuterated retinoic acids under the conditions used, and the calculation of deuterium incorporation was unreliable. Although a firm number for deuterium incorporation cannot be given, no scrambling was observed by the ^{1}H NMR spectrum. In the future, it may be possible to modifying the operating conditions for electrospray ionization to remove the background, but no time was available to accomplish this.

It should be noted that the yield of this reaction was much lower than expected (2.2%). Unfortunately, there was partial oxidation of β-ionone-d_6 on storage, even though the storage flask was flushed with nitrogen. Extra care must be taken to completely purge the storage flask in the future, because the conjugated system of double bonds in β-ionone renders it susceptible to oxidation. Although the yield of the one-pot synthesis was low, enough retinoic acid-d_6 was made available to quantify its content in OxC-beta. Not enough time was available to perform this measurement, but the retinoic acid-d_6 is still available and more can be prepared via this method in the future if required.

5.5. Conclusions and Future Directions

The preparation of β-ionone-d_6 was successfully completed with an overall yield of 6.5% starting from ethyl 2-oxo-cyclohexane carboxylate (**77**). The final deuterium incorporation was 99.85% with 0.03% of the d_0 analog. β-Ionone-d_6 was also successfully converted to retinoic acid-d_6 by a one-pot procedure. Although no time was available for the quantification of its deuterium content or for the content of retinoic acid (**66c**) in OxC-beta, the deuterated standard is available for future analytical use.

Future directions for this project include the synthesis of the low MW compounds of OxC-beta in deuterated form, as well as the synthesis of deuterated retinol (**66a**) and retinal (**66b**). Once these compounds are prepared, they can be accurately quantified in OxC-beta by the use of MS/MS techniques.

Another project that would be highly worthwhile would be the conversion of β-ionone-d_6 to β-carotene-d_{12}, and literature is available on such a transformation.[17] This would be followed by its complete, spontaneous oxidation with molecular oxygen. Analysis of the products obtained from this oxidation could offer valuable insight into the specific mechanisms of β-carotene oxidation.

5.6. References

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-Chapter 6: Experimental-

6.1. General

^{1}H NMR spectra were recorded on a Bruker Avance 400 MHz NMR Spectrometer. NMR solvents were purchased from Sigma-Aldrich or Cambridge Isotope Laboratories Inc. NMR spectra were calibrated using TMS as an internal standard. TLC was carried out using TLC Silica Gel 60 F_{254} sheets with aluminum backing, from EMD Chemicals Inc, visualized by UV irradiation or by charring with phosphomolybdic acid solution (20% wt in EtOH), purchased from Sigma-Aldrich. Silica gel for chromatography was purchased from SiliCycle Inc., 230-400 mesh. Reagents were obtained from either Sigma-Aldrich or C/D/N Isotopes Inc. and were used as is. THF was distilled from sodium benzophenone ketyl prior to use. Dry acetone was distilled from calcium sulfate prior to use. Dry *i*-Pr_2NH was distilled from NaOH prior to use. Dry benzene was obtained by drying from activated 3 Å molecular sieves.

GC-MS data were recorded on an Agilent Technologies 5975B inert Series GC/MS System with Agilent Technologies 6890N Network GC System. The column used was an Agilent Technologies 19091S-102 HP-5ms Capillary GC Column, 25 m length, 0.2 mm I.D., 0.33 μm film. The NIST 05 Spectral Library was used for identification of certain compounds by comparison of their mass spectra. Selected ion monitoring mode for deuterium incorporation were carried out recording ions [M]+, [M-1]+, [M-2]+, [M-3]+, [M-4]+, [M-5]+, and [M-6]+. Two sets of operating conditions were used, described below as OC50x and OC100x.

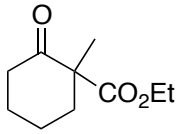
OC50x: start 50 ^oC for 1 min, +20 ^oC / min until 280 ^oC, hold 2.5 min; injector temp. 250 ^oC
OC100x: start 100 ^oC for 1 min, +20 ^oC / min until 280 ^oC, hold 5 min; injector temp. 250 ^oC

Electrospray mass spectrometry of β-ionone-d_6 (**75**) was performed on a ZQ 2000 (Waters-Micromass) mass spectrometer equipped with pneumatically-assisted

electrospray ionization source, operating in positive mode. The source temperature was set at 80 °C; an electrospray capillary was set at 3.5 kV with a cone voltage set at 10 V. Data were collected in single ion recording mode monitoring 7 ions: 193.4, 194.4, 195.4, 196.4, 197.4, 198.4, and 199.4 with dwell time 0.08 s. Alliance 2795 (Waters) liquid chromatograph was used as solvent pump. The solvent system was 1:1 acetonitrile:water with 0.1% formic acid with a flow rate of 200 mL per minute. Sample was introduced by loop injection.

6.2. Procedures and Spectral Data

Ethyl 1-methyl-2-oxocyclohexane carboxylate (**78**)



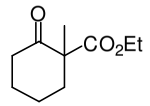
78

To a vigorously stirred solution of ethyl 2-oxo-cyclohexane carboxylate (20.00 g, 0.118 mol) in acetone (400 mL) was added K_2CO_3 (32.48 g, 0.235 mol) and CH_3I (9.16 mL, 0.147 mol). The mixture was heated to 57-58 ^oC, and then at 17 and 41 hours more K_2CO_3 (8.12 g) and CH_3I (2 mL) were added. After 63 hours, more K_2CO_3 (4.06 g) and CH_3I (1 mL) was added. After 70 hours, CH_3I (1 mL) was added and reflux continued over the weekend. The solvent was evaporated and the residue dissolved in Et_2O (400 mL). It was washed with water (2x200 mL) and brine (100 mL), dried (Na_2SO_4) filtered and the solvent evaporated. The residue was dissolved in DCM (20 mL), flushed through a small pad of silica gel and eluted with DCM (100 mL). Evaporation of the solvent gave **78** as pale yellow oil (20.93 g, 97%).

MS (EI) m/z (rel. int.): 184 $[M]^+$ (60), 156 (77), 141 (100), 139 (50), 113 (61), 111 (64), 110 (52), 83 (69), 82 (72), 55 (92)

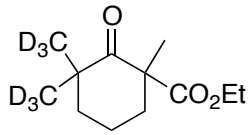
^{1}H NMR ($CDCl_3$): δ (ppm) 4.26-4.14 (m, 2H), 2.55-2.43 (m, 3H), 2.06-1.98 (m, 1H), 1.78-1.61, (m, 3H), 1.49-1.42 (m, 1H), 1.29 (s, 3H), 1.26 (t, J=7.1 Hz, 3H).

^{1}H NMR, $CDCl_3$, 400MHz



78

Ethyl 1,3,3-trimethyl-2-oxocyclohexane carboxylate-d_6 (79)

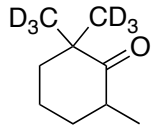


79

60% NaH in mineral oil (18.55 g 0.464 mol) was sealed under argon and washed with dry benzene (50 mL, then 2x30 mL). It was suspended in dry benzene (110 mL), cooled to 0 ^{o}C and **14** was added (25.23 g, 0.137 mol) over 10 min. It was stirred 10 min and then CD_3I added (17.07 mL, 0.274 mol; 99.9 atom %D). After 1 hour, the mixture was heated to 80 ^{o}C. After 2 hours, additional CD_3I was added (8.54 mL 0.137 mol; 99.9 atom %D) and heating continued overnight. The mixture was cooled to 0 ^{o}C and water (20 mL) was slowly added. The mixture was diluted with EtOAc (400 mL), washed with water (2x200 mL) and brine (100 mL), dried (Na_2SO_4), filtered and the solvent evaporated to give a yellow oil (29.37 g) as a mixture of **79** (84% yield), **79a** (5% yield) and **79b** (8% yield).

MS (EI) m/z (rel. int.): 218 $[M]^+$ (10), 190 (4), 173 (4), 145 (4), 117 (9), 116 (12), 115 (100), 88 (13), 87 (47), 76 (11), 69 (7), 55 (7)

1,1,3-Trimethylcyclohexan-2-one-d_6 (76)

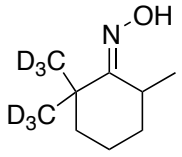


76

A mixture of crude **79** + **79a** + **79b** (29.37 g, 0.133 mol), glacial acetic acid (60 mL), H_2O (30 mL) and PTSA-H_2O (17.97 g, 0.0944 mol) was heated to reflux at 100 ^oC for 27 hours. A brownish grey solution formed, which was cooled to rt and diluted with H_2O (400 mL). It was extracted with pentane (200 mL, then 2x100 mL), and the combined pentane extracts were washed with 5% NaOH (150 mL), H_2O (100 mL), and brine (100 mL). The extract was dried (Na_2SO_4), filtered, and the solvent evaporated to give a crude yellow oil as **76** (18.85 g, 87% yield by GC integration).

MS (EI) m/z (rel. int.): 146 $[M]^+$ (31), 89 (10), 88 (100), 78 (6), 76 (9), 75 (26), 69 (5), 62 (23), 61 (9), 58 (12), 55 (6).

1,1,3-Trimethylcyclohexan-2-one-d_6 Oxime (83)

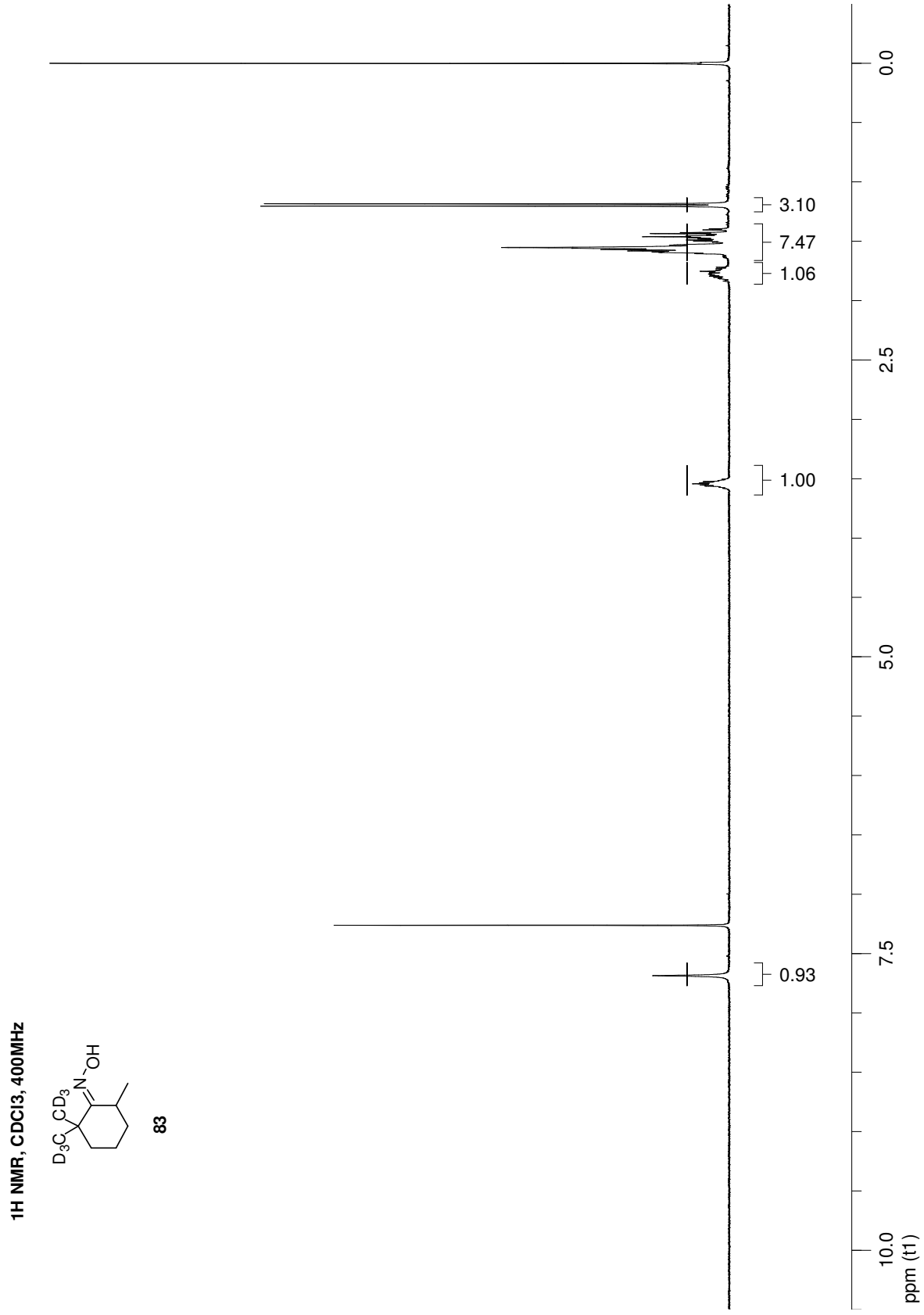


83

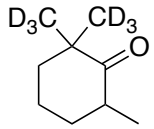
A stirred solution of crude ketone **76** (18.85 g, 0.115 mol), NH_2OH-HCl (19.00 g, 0.273 mol) and pyridine (19.43 mL, 0.240 mol) in 99% ethanol (190 mL) was heated to 80 ^oC for 1.5 hours. The mixture was cooled to rt, solvent was evaporated and the residue dissolved in DCM (300 mL). It is washed with 0.5 N HCl (200 mL), H_2O (150 mL) and brine (100 mL), dried (Na_2SO_4), filtered and the solvent evaporated to give a white solid (19.24 g). The solid was re-crystallized four times from hot hexanes (~50 mL per crystallization) to yield pure **83** as a fluffy white powder (12.57 g, 68%).

MS (EI) m/z (rel. int.): 161 $[M]^+$ (45), 146 (16), 144 (100), 129 (35), 128 (26), 106 (38), 89 (23), 86 (45), 76 (28), 73 (28), 58 (24), 55 (24)

^{1}H NMR ($CDCl_3$): δ (ppm) 7.69 (br, 1H), 3.59-3.50 (m, 1H), 1.84-1.71 (m, 1H), 1.65-1.51 (m, 3H), 1.51-1.39 (m, 2H), 1.19 (d, J=7.5 Hz, 3H).


1H NMR, CDCl3, 400MHz
D3C
CD3
N
OH
83
3.10
7.47
1.06
1.00
0.93
0.0
2.5
5.0
7.5
10.0
ppm (t1)

Removal of Oxime Functional Group of 83 to give Ketone 76



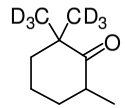
76

Oxime **83** (12.45 g, 77.3 mmol) was dissolved in 9:1 MeCN:H_2O (366 mL), followed by addition of I_2 (1.96 g, 7.73 mmol) and then 30% aqueous H_2O_2 (17.04 mL). After 5 hours, the reaction was quenched by addition of 10% sodium thiosulfate solution (60 mL). After stirring 10 min, the solvent was evaporated and the residue diluted with H_2O (100 mL). It was extracted with Et_2O (350 mL), washed with H_2O (100 mL) and brine (100 mL), dried (Na_2SO_4), filtered and the solvent evaporated. The residue was distilled under reduced pressure (35 mmHg / 75 ^oC) to collect **76** as a yellow oil (5.71 g, 51%; 95% purity by GC integration). Over time, the yellow colour faded and the product became colourless. The selected ion monitoring mode of GC-MS gave the overall deuterium incorporation as 99.85% with no d_0 ion observed.

MS (EI) m/z (rel. int.): see above preparation of **76**

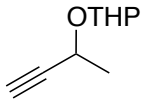
^{1}H NMR ($CDCl_3$): δ (ppm) 2.65 (septet, J=6.4 Hz, 1H), 2.09-2.01 (m, 1H), 1.88 (qt, J=13.4, 3.8 Hz, 1H), 1.79-1.73 (m, 1H), 1.68-1.61 (m, 1H), 1.54 (td, J=13.4, 4.2 Hz, 1H), 1.32 (qd, J=13.1, 3.9 Hz, 1H), 0.99 (d, J=6.4 Hz, 3H).

^{1}H NMR, $CDCl_3$, 400MHz



76

3-Butyn-2-ol THP ether (85)



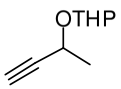
85

A stirred mixture of 3-butyn-2-ol **84** (11.76 mL 0.15 mol) and PTSA-H_2O (30 mg) was cooled to 0 ^oC and 3,4-dihydro-2-*H*-pyran (20.53 mL, 0.225 mol) was added over 25 min. After 30 min it was warmed to rt and stirred an additional 30 min. K_2CO_3 (2.00 g) was added and stirred overnight, and the pale yellow solution turned light orange. Filtration of the mixture, followed by evaporation of the solvent gave a yellow oil which was distilled under reduced pressure (7 mmHg / 64 ^oC) to give **85** as a colourless oil (20.38 g, 88%). Two isomers were visible in the ^1H NMR spectrum and GC chromatogram.

MS (EI) m/z (rel. int.): 154 $[M]^+$ (0.4), 153 (3), 125 (6), 111 (6), 101 (52), 93 (10), 85 (100), 67 (21), 57 (19), 56 (53), 55 (38), 53 (51)

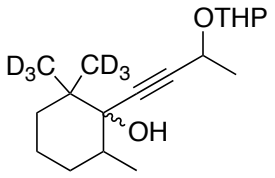
^1H NMR ($CDCl_3$): δ (ppm) 4.94 (dd, J= 4.3, 2.8 Hz, 1H, Isomer A), 4.78 (dd, J=3.4, 3.4 Hz, 1H, Isomer B), 4.55 (qd, J=6.7, 2.0 Hz, 1H, Isomer A), 4.47 (qd, J=6.6, 2.1 Hz, 1H, Isomer B), 4.02-3.96 (m, 1H, Isomer B), 3.85-3.80 (m, 1H, Isomer A), 3.57-3.50 (m, 1H each, Isomers A and B), 2.43 (d, J=2.2 Hz, 1H, Isomer B), 2.37 (d, J=2.0 Hz, 1H, Isomer A), 1.90-1.50 (m, 6H each, Isomers A and B), 1.48 (d, J=6.8 Hz, 3H, Isomer A), 1.45 (d, J=6.6 Hz, 3H, Isomer B)

^{1}H NMR, $CDCl_3$, 400MHz



85

4-(1'-Hydroxy-2,2,6-trimethylcyclohexyl)-3-butyn-2-ol-d_6 THP ether (86)

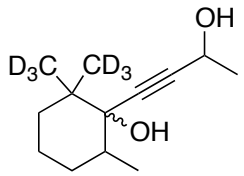


86

To a stirred solution of Mg turnings (2.04 g, 84.1 mmol) in dry THF (50 mL) under argon was slowly added EtBr (6.13 mL 82.1 mmol) at a rate that maintained gentle heating. The resulting grey solution was refluxed for 30 min and then cooled to rt. A solution of THP ether **85** (13.43 g, 87.2 mmol) in dry THF (25 mL) was slowly added at a rate that maintained gentle reflux. After the addition was complete, it was refluxed for 45 min and cooled to rt. A solution of ketone **76** (5.71 g, 39.1 mmol) in dry THF (25 mL) was slowly added to the Grignard reagent. It was refluxed for 1.5 hrs and left to stir overnight at rt (18 hrs). The solution was cooled to 0 ^{o}C, quenched with sat'd NH_4Cl solution (100 mL) and the solvent evaporated. The residue was extracted with Et_2O (300 mL), washed with sat'd NH_4Cl (100 mL) and brine (100 mL), dried (Na_2SO_4), filtered and the solvent evaporated to give a dark reddish brown oil (18.49 g). The crude product was used without purification in the next step.

MS (EI) m/z (rel. int.): 300 $[M]^+$ (3), 216 (24), 198 (87), 183 (34), 180 (52), 149 (31), 95 (27), 85 (100), 80 (56), 55 (34)

4-(1'-Hydroxy-2',2',6'-trimethylcyclohexyl)-3-butyn-2-ol-d_6 (87)

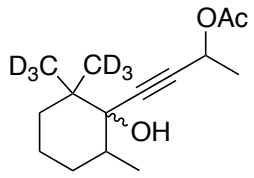


87

A solution of crude THP ether **86** (18.49 g) and PTSA-H_2O (0.30 g) in MeOH (300 mL) was stirred at rt for 16 hrs. It was quenched by addition of K_2CO_3 (3.0 g), stirred for 1 hr and the solvent was evaporated. The residue was dissolved in EtOAc (300 mL), washed with brine (2x100 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to leave **87** as a reddish-brown oil (8.45 g) that was used without purification in the next step.

MS (EI) m/z (rel. int.): 216 $[M]^+$ (19), 183 (54), 180 (100), 139 (37), 121 (79), 111 (55), 110 (37), 93 (88), 88 (87), 80 (51), 55 (37)

3-(1'-Hydroxy-2',2',6'-trimethylcyclohexyl)-1-methyl-2-propynyl acetate-d_6 (88)



88

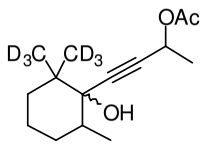
To a stirred mixture of crude **87** (8.45 g) and DMAP (48 mg) at 0 ^{o}C was added Et_3N (10.91 mL, 78.2 mmol) followed by Ac_2O (5.55 mL, 58.7 mmol). After 10 min it was warmed to rt and stirred for 1 hr, at which point TLC showed no more starting material (1:4 EtOAc:Hexanes). The solution was cooled to 0 ^{o}C and crushed ice (40 g) was added. After 30 min it was acidified to pH ~6 with 0.5 N HCl and extracted with EtOAc (3x90 mL). The combined extracts were washed with brine (2x80 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give a brown oil (9.93 g). Purification of the oil by silica gel chromatography (3:17 EtOAc:Hexanes) gave **88** as a yellow oil (8.32 g, 83% from **76**). A small sample of each of the two diastereomers was isolated by silica gel chromatography.

MS (EI) m/z (rel. int.): 258 $[M]^+$ (4), 216 (49), 198 (36), 183 (41), 180 (55), 149 (35), 121 (31), 80 (100), 79 (36)

^{1}H NMR ($CDCl_3$, Isomer A): δ (ppm) 5.48 (q, J=6.7 Hz, 1H), 2.07 (s, 3H), 1.90-1.80 (m, 1H), 1.84 (s, 1H), 1.60-1.39 (m, 4H), 1.50 (d, J=6.7 Hz, 3H), 1.33-1.22 (m, 2H), 1.00 (dd, J=6.5, 2.1 Hz, 3H).

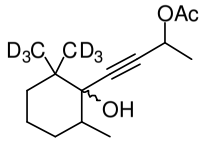
^{1}H NMR ($CDCl_3$, Isomer B): δ (ppm) 5.47 (qd, J=6.7, 1.1 Hz, 1H), 2.06 (s, 3H), 1.92-1.83 (m, 1H), 1.65 (s 1H), 1.62-1.39 (m, 4H), 1.48 (d, J=6.7 Hz, 3H), 1.33-1.16 (m, 2H), 1.03 (d, J=6.8 Hz, 3H).

^{1}H NMR, $CDCl_3$, 400MHz



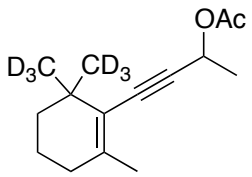
88 (Isomer A)

^{1}H NMR, $CDCl_3$, 400MHz



88 (Isomer B)

3-(2',2',6'-trimethylcyclohex-1'-enyl)-1-methyl-2-propynyl acetate-d_6 (89)



89

To a stirred solution of **88** (8.17 g 31.7 mmol) in pyridine (37.4 mL) under argon at -5 ^{o}C was added a solution of $POCl_3$ (10.16 mL, 0.108 mol) in pyridine (14.9 mL) over 30 min. The resulting mixture was heated to 100 ^{o}C, changing from yellow to dark brown over time. After 4 hours, it was cooled to rt and poured cautiously (exothermic rxn) onto a slurry of crushed ice (120 g) and cold water (150 mL). The mixture was extracted with Et_2O (4x100 mL) and washed with 0.5 N HCl (2x125 mL), H_2O (125 mL) and saturated $NaHCO_3$ (125 mL). The organic extract was dried (Na_2SO_4), filtered and concentrated *in vacuo* to give a light brown oil (6.65 g). Separation of the oil by silica gel chromatography using a hexanes/EtOAc gradient gave **89** as a yellow oil (3.33 g, 85% purity by GC integration; 37% yield) and recovered starting material **88** as a yellow oil (2.97 g).

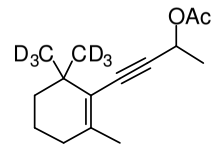
A solution of recovered **88** (2.60 g, 10.1 mmol) in pyridine (33.3 mL) was cooled to -5 ^{o}C under argon, followed by addition of $POCl_3$ (6.47 mL, 68.5 mmol) over 15 min. It was heated to 85-90 ^{o}C for 118 hrs, cooled to rt and poured cautiously over a slurry of crushed ice (65 g) and cold water (65 g). The black mixture was extracted with Et_2O (4x80 mL), washed with 0.5 N HCl (2x80 mL), H_2O (80 mL) and sat'd $NaHCO_3$ solution (80 mL). It was dried (Na_2SO_4), filtered and concentrated *in vacuo* to give a brown oil (1.83 g). Purification of the oil by silica gel chromatography (5% EtOAc in hexanes) gave **89** as a yellow oil (1.26 g, 91% pure by GC integration; 15% yield).

The selected ion monitoring mode of GC-MS gave the deuterium incorporation of **89** as 99.83% with 0.00% d_0 ion.

MS (EI) m/z (rel. int.): 240 $[M]^+$ (31), 198 (12), 197 (19), 183 (100), 180 (41), 162 (88), 118 (16), 117 (19)

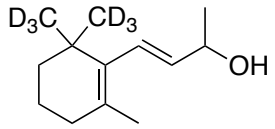
^{1}H NMR ($CDCl_3$): δ (ppm) 5.62 (q, J=6.6 Hz, 1H), 2.07 (s, 3H), 2.00 (br t, J=6.3 Hz, 2H), 1.85 (br s, 3H), 1.62-1.53 (m, 2H), 1.53 (d, J=6.6 Hz, 3H), 1.46-1.42 (m, 2H)

^{1}H NMR, $CDCl_3$, 400MHz



89

β-Ionol-d_6 (90)



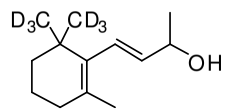
90

To a stirred suspension of LAH (2.54 g, 66.9 mmol) in dry THF (90 mL) under argon at 0 ^{o}C was added impure **89** (4.59 g, 16.6 mmol) in dry THF (20 mL) over 10 min. It was warmed to rt for 10 min, then heated to reflux for 3 hrs. The reaction was quenched at 0 ^{o}C by sequential addition of H_2O (2.54 mL), 15% wt/vol NaOH (2.54 mL), and H_2O (7.62 mL). After 30 min stirring, a white precipitate formed which was filtered and rinsed with EtOAc (3x10 mL). The filtrate was concentrated *in vacuo* to give a pale orange oil (3.83 g). Purification of the oil by silica gel chromatography (12% EtOAc in hexanes) gave **90** as a colourless oil (2.51 g, 76%).

MS (EI) m/z (rel. int.): 200 $[M]^+$ (10), 182 (75), 164 (83), 142 (35), 129 (35), 124 (100), 122 (48), 105 (44), 91 (35)

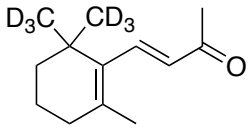
^{1}H NMR ($CDCl_3$): δ (ppm) 6.04 (d, J=15.9 Hz, 1H), 5.49 (dd, J=15.9, 6.7 Hz, 1H), 4.40-4.33 (m, 1H), 1.97 (br t, J=6.2 Hz, 2H), 1.67 (d, J=0.7 Hz, 3H), 1.63-1.56 (m, 2H), 1.49 (br, 1H), 1.45-1.42 (m, 2H), 1.32 (d, J=6.4 Hz, 3H)

^{1}H NMR, $CDCl_3$, 400MHz



90

β-Ionone-d_6 (75)



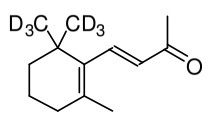
75

To a stirred solution of **90** (2.51 g, 12.6 mmol) in DCM (75 mL) was added activated MnO_2 (30 g). After 6 hrs, additional MnO_2 (30 g) was added, followed by another portion at 11 hrs (10 g). After stirring for 28 hrs, the mixture was filtered through celite and the filter cake rinsed with DCM (4x20 mL). The filtrate was concentrated *in vacuo* to give a pale yellow oil (2.31 g). Purification of the oil by silica gel chromatography (6% EtOAc in hexanes) gave **75** as a pale oil (2.02 g, 81%). The deuterium incorporation was determined by electrospray mass spectrometry to be 99.85%, with 0.03% d_0 ion.

MS (EI) m/z (rel. int.): 198 $[M]^+$ (3), 184 (12), 183 $[M\text{-}CH_3]^+$ (100), 180 (4), 136 (7), 135 (6), 122 (6), 121 (4), 110 (4), 93 (4)

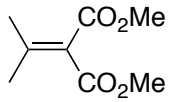
^{1}H NMR ($CDCl_3$): δ (ppm) 7.28 (d, J=16.4 Hz, 1H), 6.11 (d, J=16.5 Hz, 1H), 2.30 (s, 3H), 2.07 (br t, J=6.2 Hz, 2H), 1.77 (s, 3H), 1.65-1.59 (m, 2H), 1.50-1.46 (m, 2H)

^{1}H NMR, $CDCl_3$, 400MHz



75

Dimethyl Isopropylidenemalonate (91)



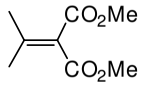
91

A stirred solution of dimethylmalonate (50.0 g, 0.379 mol), dry acetone (41.72 mL, 0.569 mol), Ac_2O (44.72 mL, 0.474 mol) and anhydrous $ZnCl_2$ (7.74 g, 56.9 mmol) was heated to reflux for 23 hrs. The resulting dark red solution was cooled to rt, diluted with benzene (250 mL) and stirred vigorously with H_2O (150 mL) for 30 min. The layers were separated and the organic layer was washed with H_2O (4x65 mL) and concentrated *in vacuo*. The residue was distilled under reduced pressure (4.5 mmHg / 88 ^oC) to give **91** as a colourless oil (27.01 g, 93% purity by GC integration; 38% yield).

MS (EI) m/z (rel. int.): 172 $[M]^+$ (2), 141 (72), 140 (100), 112 (54), 109 (52), 83 (12), 82 (82), 73 (18), 67 (43), 59 (18), 53 (11)

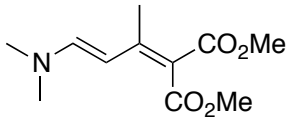
^{1}H NMR ($CDCl_3$): δ (ppm) 3.77 (s, 6H), 2.07 (s, 6H)

^{1}H NMR, $CDCl_3$, 400MHz



91

Dimethyl 2-[(2*E*)-3-(Dimethylamino)-1-methyl-2-propenylidene]malonate (92)



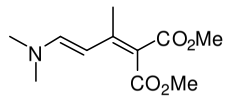
92

A stirred solution of impure **91** (9.92 g 53.4 mmol) and 94% *N,N*-dimethylformamide dimethyl acetal (9.09 mL 64.1 mmol) with attached reflux condenser and Dean-Stark apparatus was heated to 95 ^{o}C for 18 hrs, removing approximately 1 mL of MeOH. The Dean-Stark apparatus was removed and reflux continued for 2 hrs. The solution was cooled to rt and solvent evaporated to give a reddish yellow solid. Re-crystallization of the solid from ether/pentane gave **92** as dark yellow crystals (10.95 g, 90%).

MS (EI) m/z (rel. int.): 228 (11), 227 $[M]^+$ (90), 196 (99), 180 (20), 168 (24), 166 (22), 152 (27), 108 (43), 96 (100)

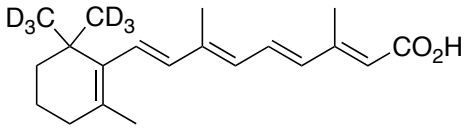
^{1}H NMR ($CDCl_3$): δ (ppm) 6.94 (d, J=13.3 Hz, 1H), 6.11 (d, J=13.4 Hz, 1H), 3.74 (s, 6H), 2.93 (s, 6H), 2.12 (s, 3H)

^{1}H NMR, $CDCl_3$, 400MHz



92

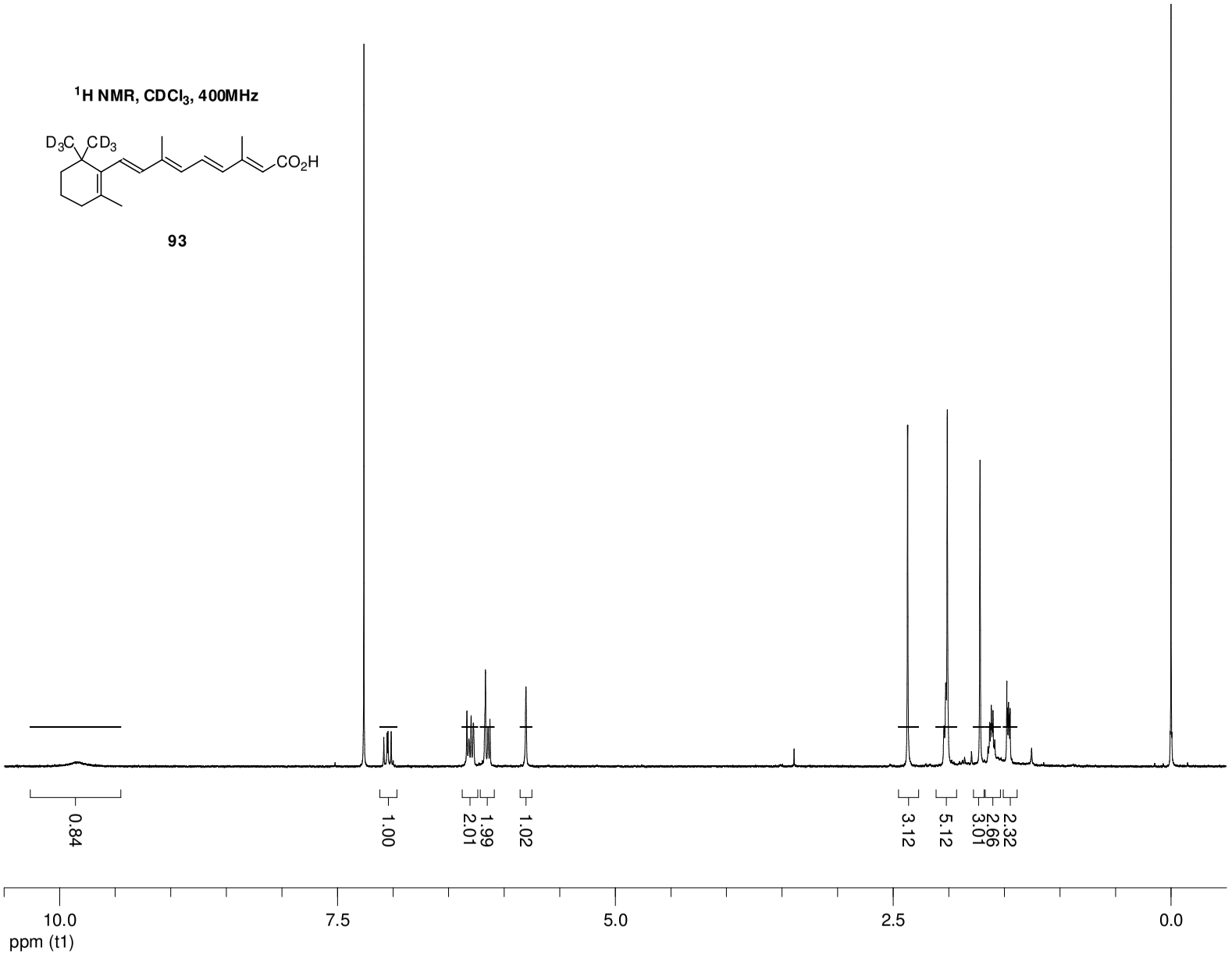
Retinoic Acid-d_6 (93)



93

A solution of fresh LDA was prepared at -25 °C by dissolving dry *i*-Pr_2NH (0.39 mL, 2.8 mmol) in dry DME (2.08 mL) under argon and adding 1.6 M n-BuLi in hexanes (1.68 mL, 2.69 mmol). The LDA was stirred 5 min and cooled to between -30 °C and -40 °C. To this was slowly added a solution of β-ionone-d_6 **75** (0.50 g, 2.53 mmol) in dry DME (2.08 mL). After 20 min, a solution of enamine **92** (0.62 g, 2.73 mmol) in dry DME (6.07 mL) was added. The cooling bath was removed and stirred 10 min before heating to reflux for 1 hr. The solution was cooled to between -10 °C and 0 °C and 3.0 M MeMgCl in THF (1.94 mL, 5.81 mmol) was slowly added. The mixture was warmed to rt for 30 min, then cooled again to 0 °C. EtOH (2.52 mL) was slowly added, followed by a solution of KOH (0.85 g, 15.2 mmol) in H_2O (7.6 mL). After 30 min at 0 °C, it was heated to 40 °C for 45 min. Solvents were then evaporated and the residue acidified with 1 N HCl and extracted with EtOAc (70 mL, then 2x40 mL). The combined extracts were washed with brine (2x30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give a dark red foam (764 mg). Separation of the foam by silica gel chromatography (98:2 DCM:MeOH) gave a reddish orange solid (318 mg) with the same Rf as a reference sample of retinoic acid (Rf=0.18, 98:2 DCM:MeOH). Re-crystallization of the solid from MeCN gave yellow crystals (50 mg), which were again re-crystallized from MeOH to give retinoic acid-d_6 (**93**) as yellow crystals (17 mg, 2.2%).

^{1}H NMR ($CDCl_3$): δ (ppm) 9.83 (br, 1H), 7.05 (dd, J=15.0, 11.5 Hz, 1H), 6.32 (d, J=15.1 Hz, 1H), 6.30 (d, J=15.1 Hz, 1H), 6.16 (d, J=9.3 Hz, 1H), 6.15 (d, J=16.1 Hz, 1H), 5.80 (s, 1H), 2.37 (d, J=0.7 Hz, 3H), 2.04-2.00 (m, 2H), 2.01 (s, 3H), 1.72 (s, 3H), 1.65-1.59 (m, 2H), 1.48-1.45 (m, 2H)


^{1}H NMR, CDCl$_3$, 400MHz
D$_3$C CD$_3$
CO$_2$H
93
0.84
1.00
2.01
1.99
1.02
3.12
5.12
3.01
2.66
2.32
10.0
7.5
5.0
2.5
0.0
ppm (t1)

Claims to Original Research

1) A number of analogs of betulinic, ursolic, and oleanolic acids were synthesized. New compounds include analogs **19**, **24**, **26**, **28**, **29**, **30**, **31**, **32**, and **36**.

2) Several triterpene acid analogs (**Fig. 2.7.**) were shown *in vitro* to possess cortisol lowering abilities.

3) A synthesis of β-ionone-d_6 (**75**) was carried out with an overall yield of 6.5% starting from ethyl 2-oxo-cyclohexane carboxylate (**77**). The final deuterium incorporation was 99.85% with 0.03% of the d_0 analog.

Appendix A: Table of Numbered Triterpene Acid Analogs

This table contains a list of the numbers used in the internal library of triterpene acid analogs. The corresponding numbers used in this thesis are given.

Library Number	**Thesis Number**
1500	1
1501	11
1502	27
1503	10
1504	2
1505	9
1506	16
1507	18
1508	19
1509	12
1510	13
1511	14
1512	15
1513	39
1514	40
1515	41
1516	43
1517	7
1518	8
1519	21
1520	33
1521	36
1522	37
1523	38
1524	24

1525	25
1526	26
1527	28
1528	29
1529	30
1530	31
1531	32
1532	4
1533	49
1534	3
1535	48
1536	52
1537	56
1538	58
1539	59
1540	42
1541	45
1543	6
1544	62
1545	63
1546	46
1547	47
1548	61
1549	60
1550	44