Studies towards the Synthesis of Steroids with unusual Ring Systems: Synthesis of Calvatianone and Advanced Precursors of Spiroseoflosterol and Euphorstranol B

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Abstract

Calvatianone, a novel steroid displaying a unique ergosterol-related 6/5/6/5-fused ring skeleton with a contracted tetrahydrofuran B-ring, was isolated in 2014 from the mushroom *Calvatia nipponica*, a rare puffball mushroom collected in South Korea. *Calvatia* species are widespread used as food and in traditional medicine, and calvatianone has been found to suppress cell viability in MCF-7 breast cancer cells as a possible ERα antagonist. This work describes the first chemical synthesis of calvatianone. Starting with ergosterol as a readily available starting material, the challenging B ring formation was achieved by oxa-Michael addition after oxidative functionalization and cleavage of ring B. Along the optimized (scalable) synthetic sequence, the target natural product was obtained in 4 steps in 8% overall yield.

The second part of this thesis describes studies towards the synthesis of spiroseoflosterol and spiroconyone A, two rearranged steroids only differing in their side chain and displaying an unusual spiro[4,5]-decan ring skeleton. Spiroseoflosterol was isolated from the fruiting bodies of *Butyriboletus roseoflavus* in China and proved to be against the hepatoma cell lines HepG2 and Huh7/S (sorafenib-resistant Huh7) with IC_{50} values of 9.1 and 6.2 μM, respectively. Starting from cholesteryl acetate, the devised key intermediate (keto aldehyde) was successfully synthesized in seven steps (model series with a simplified side chain). However, the planned key intramolecular aldol reaction to set-up the spirocyclic ring substructure could not be achieved.

The third part of this thesis concerns the synthesis of the natural product euphorstranol B, a rare 9,11-seco tetracyclic triterpenoid featuring a cyclic enol-hemiacetal moiety. It was isolated in 2021 from *Euphorbia stracheyi* and found to exhibit significant cytotoxicity against the breast cancer cell line MDA-MB-468. After protecting the side chain double bond of lanosterol by epoxidation/reduction, acetylation of the C(3)-OH group and double allylic oxidation, the selective removal of the C(7) oxo group was achieved by Wolff-Kishner reduction. However, the final conversion of prepared advanced intermediate into the target structure (e.g. by Baeyer-Villiger reaction and subsequent lactone reduction) could not be achieved.

Kurzzusammenfassung

Calvatianon, ein neuartiges Steroid mit einem einzigartigen, dem Ergosterol verwandten 6/5/6/5-kondensierten Ringskelett und einem kontrahierten Tetrahydrofuran-B-Ring, wurde 2014 aus dem Pilz *Calvatia nipponica* isoliert. Calvatia-Arten sind als Lebensmittel und in der traditionellen Medizin weit verbreitet, und es wurde festgestellt, dass Calvatianon als möglicher ERα-Antagonist die Vitalität von MCF-7-Brustkrebszellen inhibiert. Die vorliegende Arbeit beschreibt die erste chemische Synthese von Calvatianon. Ausgehend von Ergosterol als leicht verfügbarem Ausgangsmaterial wurde die anspruchsvolle B-Ringbildung, nach oxidativer Funktionalisierung und Spaltung von Ring B, durch eine Oxa-Michael- Addition erreicht. Entlang der optimierten (skalierbaren) Synthesesequenz wurde das Zielmolekül in 4 Schritten in 8 % Gesamtausbeute erhalten.

Der zweite Teil dieser Arbeit beschreibt Untersuchungen zur Synthese von Spiroseoflosterol und Spiroconyone A, zwei umgelagerten Steroiden, die ein ungewöhnliches Spiro[4,5]decan-Ringgerüst aufweisen. Spiroseoflosterol wurde aus den Fruchtkörpern des Pilzes *Butyriboletus roseoflavus* isoliert und erwies sich als cytotoxisch gegen die Hepatomzelllinien HepG2 und Huh7/S (IC_{50} = 9.1 bzw. 6.2 µM). Ausgehend von Cholesterylacetat gelang die Synthese einer fortgeschrittenen Zwischenstufe über sieben Stufen. Die angestrebte intramolekulare Aldol-Reaktion zum Aufbau des spirocyclischen Ringsystems konnte jedoch nicht realisiert werden.

Der dritte Teil dieser Arbeit befasst sich mit der Synthese von Euphorstranol B, einem tetracyclischen 9,11-Seco-Triterpenoid, das eine cyclische Enol-Hemiacetal-Einheit aufweist. Es wurde 2021 aus *Euphorbia stracheyi* isoliert und zeigt eine Cytotoxizität gegen die Brustkrebszelllinie MDA-MB-468. Nach Schutz der Seitenkettendoppelbindung von Lanosterol durch Epoxidierung/Reduktion, Acetylierung der C(3)-OH-Gruppe und doppelter allylischer Oxidation wurde die selektive Entfernung der C(7)-Oxogruppe durch eine Wolff-Kishner-Reduktion erreicht. Die finale Umwandlung des hergestellten fortgeschrittenen Zwischenprodukts in die Zielstruktur (z. B. durch Baeyer-Villiger-Oxidation und anschließende Lactonreduktion) konnte jedoch nicht erreicht werden.

ACKNOWLEDGEMENT

I am delighted to have finally reached the stage of acknowledging my doctoral dissertation. Looking back a few years ago, when I set out from Beijing to Cologne with dreams and passion, I was both nervous and excited. Everything was unknown to me, but I was ready to embrace it all. Time has flown by, and today I have the incredible opportunity to celebrate the end of my doctoral studies. Looking back on all these years of studying, I have faced difficulties, challenges, failures, but also gained so much in the form of new friendships, care and encouragement.

I'm so grateful to my professor Schmalz, who made this journey so memorable. I'll always remember the first time I met him, even though it was snowing outside, his warm smile made me feel at ease right away. I'm thrilled to have started my doctoral career here at the University of Cologne with such an inspiring professor. Every journey has its challenges, but they're all part of the adventure. The differences between China and Germany are fascinating too – the systems, the labs, the experiments. I had a hard time adapting at first, but I'm so grateful to have a group of good colleagues who helped me learn the rules little by little and who welcomed me into their big family.

I could go on and on, but when I write down my feelings, I feel so much. I don't know how to express all my feelings in detail, but I can use one sentence to replace thousands of words. Thank you very much, AK Schmalz, I love you!

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I love you Köln, I love you Germany!

Table of Content

1 Theoretical Section

1.1 Introduction

Steroids are complex tetracyclic organic molecules that serve many roles and functions in multicellular organisms. They are structural components of cell membranes, exemplified by the important dietary steroid cholesterol, and have many functional regulatory roles as endogenous endocrine hormones. In all organisms, hormones in *vivo* play key regulatory roles in mediating communication and regulation of important functions and processes within and between cells, and across tissues, to connect all organs of the body.[1] Endocrine hormones circulate in the bloodstream and allow communication between cells and organs separated by relatively large distances. Hydrophilic or water-soluble hormones act primarily at the cell surface by binding to protein receptors embedded in the plasma membrane. In contrast, hydrophobic hormones circulate primarily bound to carrier plasma proteins and are able to freely defuse across cell membranes to activate specific intracellular hormone receptors. And in this context, steroid hormones, which are all biosynthetically derived from cholesterol, play a key role.[2]

Over the last century, the chemical synthesis of natural products has become one of the most important disciplines in science. The multi-stage synthesis of complex organic molecules, starting from simple, commercially available starting materials, is often referred to as “total synthesis”. This method is often used when the target compound is only available in small quantities from natural sources, when the structure has not yet been secured or when a large amount of the compound is required. The process of total synthesis can be quite demanding and requires careful planning and execution of several synthesis steps. Often, protecting groups must be used to prevent unwanted reactions and to ensure that the right functional groups are introduced at the right time and in the right place.[3]

Semi-synthesis, or partial synthesis, is a type of chemical synthesis that involves the modification of existing compounds, which are often natural products. This process takes a naturally occurring precursor and modifies it chemically to create the desired compound. This method is particularly useful when the structure of the target molecule is similar to that of an available natural product. It can be a more efficient way to produce complex molecules

compared to total synthesis, as fewer synthetic steps may be needed. One of the key advantages of semi-synthesis is that it can sometimes be used to make changes to a natural product that would be difficult to achieve with total synthesis. It is also often used in the production of pharmaceutical drugs, where a natural product is modified to improve its potency, selectivity, or pharmacokinetic properties. In both cases, thousands of natural products and their derivatives have been synthesized, either by total synthesis or semi-synthesis, and many of the most prominent organic chemists have been involved in this endeavor during the past decades.

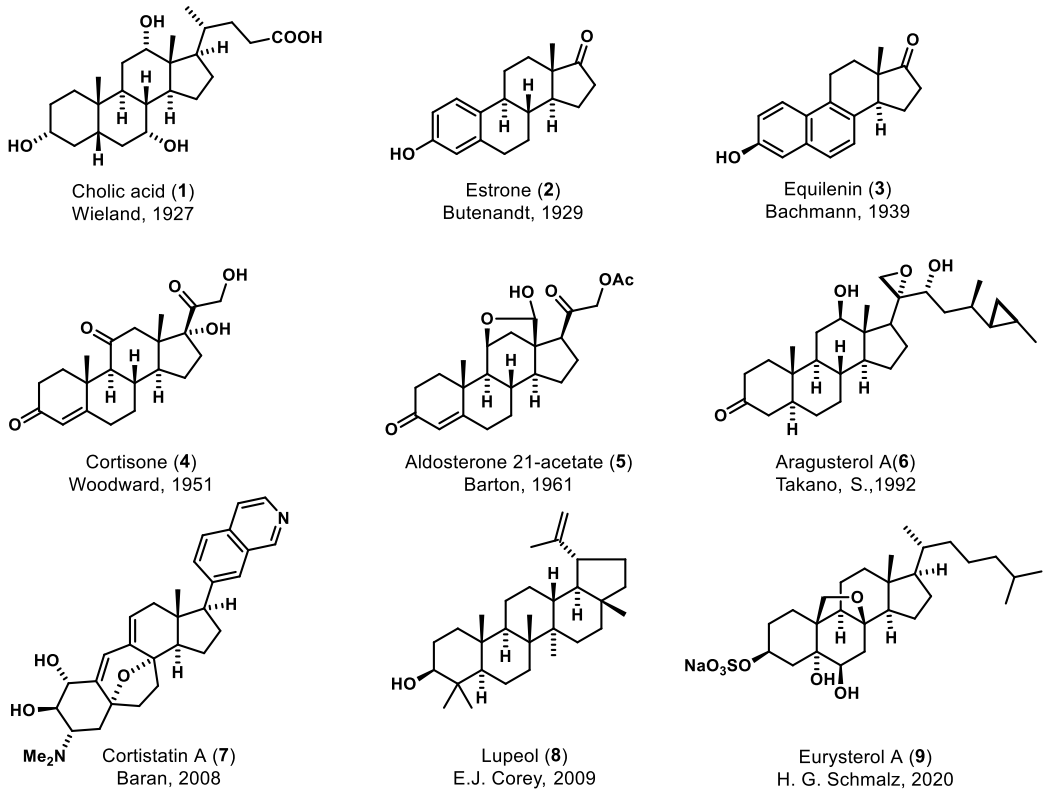
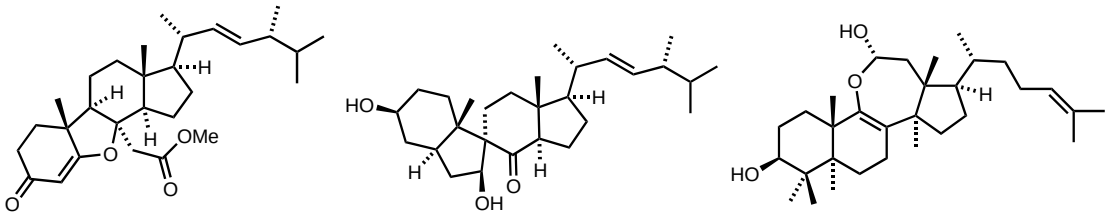


Figure 1. Structures of selected important steroids.

Due to their unique structural features and exciting biological properties, steroids are a particularly relevant class of substances that has attracted great interest among chemists. Some selected examples are shown in Figure 1. In 1927, the German chemist Heinrich Wieland (1877-1957) won the Nobel Prize in Chemistry for his achievements in the study of the steroidal compound cholic acid (**1**) extracted from animal liver and the structure of related compounds.[4] In the 1930s, the Chinese chemist Wang You was engaged in the synthesis of bile acids and

sterols under the guidance of Wieland. He found an improved method of introducing conjugated double bonds into the steroid ring system and succeeded to synthesize cholestadienone and cholestadienol. Estrone (**2**) was the first steroid hormone isolated independently by Doisy and Butenandt from the urine of pregnant women in 1929.[5] Equilenin (**3**) is a naturally occurring steroidal estrogen extracted from the urine of pregnant mares and used as a n conjugated estrogens. A milestone in the history of steroids was the total synthesis of equilenin by Bachmann[6] in 1939, the first steroid to be produced in the laboratory. Ten years later, cortisone (**4**), a metabolite of the anti-inflammatory and antistress hormone cortisol, was synthesized by Nobel laureate R. B. Woodward as another landmark of total synthesis.[7] Another key player in the field of steroid chemistry was Derek Barton, who developed conformational analysis as a tool to understand the three-dimensional structure of steroids.[8] In 1961, Barton also pioneered the use of radical reaction in organic chemistry and discovered several reactions, such as the Barton deoxygenation protocol for the reductive removal of unwanted hydroxyl groups. Barton employed his nitrite ester method for the synthesis of aldosterone-21-acetate (**5**) from corticosterone acetate.[9] Steroid resarch has witnessed and contributed to the development of synthetic methodologies, and chemists have learned to synthesize steroids with increasing structural complexicity and novel pharmacological properties. For example, aragusterol A (**6**) was synthesised by S. Takano in 1992 and showed potent in viva antitumor activity.[10] In more recent years, several research groups have focused on the synthesis of steroids with unusual oxidation and ring patterns by finding ways to manipulate the classical aliphatic carbon backbone. For instance, cortistatin A (**7**) was synthesized by P. Baran in 2008,[11] lupeol (**8**) was total synthesized by E. J. Corey in 2009,[12] and a synthesis of eurysterol A (**9**) was reported in 2020 by H.- G. Schmalz and Ö. Taspinar.[13] While the classic steroids with a condensed 6/6/6/5-ring skeleton have long been used as lead structures in drug research, it remains interesting to see whether the rearranged derivatives will also find pharmaceutical application in the future. Steroids continue to capture the attention of the (bio)synthetic community like no other class of natural products, and further exciting developments can be expected in the future. Another example of a steroid with a unique structure and interesting biological properties is calvatianone (**10**), which is shown in Figure 2. It is a novel sterol isolated from the fruiting bodies of *C. nipponica* (South Korea) in 2020, which is used in traditional medicine and is

potentially effective against breast cancer cell lines. The molecule shows an unusual 6/5/6/5-fused ring system with a tetrahydrofuran as B-ring.[14]



calvatianone (**10**) spiroseoflosterol (**11**) euphorstranol B (**12**)

Figure 2. Structures of calvatianone and spiroseoflosterol and euphorstranol B.

In this thesis, the first synthesis of calvatianone (**10**) will be described which exploits an intramolecular oxa-Michael addition to set up the tetrahydrofuran ring. A second project of the thesis concerns studies towards the synthesis of spiroseoflosterol (**11**), a 7(8→9)-abeo-ergostane steroid with a unique spiro[4.5]decan-6-one C/B-ring system, which was isolated from the fruiting bodies of *Butyriboletus roseoflavus* in China 2020.[15] A third project concerns investigations aiming at the synthesis of euphorstranol B (**12**), a 9,11-seco euphane or lanostane triterpenoid featuring a cyclic enol-hemiacetal functionality, which was isolated in 2019 in China from the pant *Euphorbia stracheyi*.[16]

1.2 Background

1.2.1 The Chemistry of Steroids: Origins, Development and Importance

As already mentioned above, steroids form a particular important class of natural products. As a special structural feature, most steroids display the typical cyclopentano-perhydro-phenanthrene carbon skeleton, which is also known as the steroid nucleus. Natural steroids exhibit diverse, complex structural patterns and a broad spectrum of biological activities. Research into steroids, from extraction, isolation, structure elucidation, synthesis and pharmaceutical application, has become a very active area of drug development in the 20th century. Steroids are among the most thoroughly researched drugs and have made a special contribution to human health as pharmaceuticals. In addition, steroid chemistry has played a

central role in the history of the development of organic chemistry, starting with the development of synthetic methods, stereochemistry and the mechanistic understanding of reactions and their stereochemical progression.[17]

And the chemistry of steroids has a long history (Figure 3). As early as 1769, De La Salle discovered a lipoid substance in bile, and in 1815 the chemist Chevreul named this lipoid substance cholesterol. In 1775, the British physician Withering found that dried digitalis leaves had a very good effect on rheumatism and swelling, and later he found that they had a cardiotonic effect on the weakened heart, and the main components of digitalis leaves include digoxin and digitoxin as well as other cardiac glycoside steroid components. The main constituents of digitalis leaves include digoxin and digitoxin, which are cardiac glycoside steroids. The German pharmacist Schmiedeberg isolated a pure digitoxin product in 1875, which was later used clinically by the French pharmacist Nativelle.[18] In 1927, the German chemist Heinrich O. Wieland was awarded the Nobel Prize in Chemistry for his work on bile acids and sterols.[4] The following year, Adolf O. R. Windhaus was also awarded the Nobel Prize in Chemistry in recognition of his work on the structure of sterols, which lasted over a quarter of a century.[19] He was called the "father of steroid chemistry". Despite these honors, the recognized centerpiece of their research, the structure of cholesterol, was misattributed. It was not until 1932 that the English scientist John D. Bernal used the new and groundbreaking technique of X-ray crystallography to decipher the structure of ergosterol.[20] And after this, the structures of other steroids, such as digoxin (**13**) and cholesterol (**14**) could be correctly assigned as well (Figure 4). In the decades that followed, steroids became an obsessive object of study for scientists who recognized the central role of these molecules in controlling human biology, particularly in relation to male and female reproductive mechanisms.[21]

1775 — The English physician **Withering** discovered that dried digitalis leaf was very effective against rheumatic swelling, and was later found to be cardiotonic for a weakened heart.

1815 — In 1769, **De La Salle** discovered a lipid-like substance in bile, and in 1815, the chemist Chevreul named this lipid-like substance cholesterol.

1875 — The German pharmacist **Schmiedeberg i**solated a pure form of digitalis glycoside, which was later used clinically by the French pharmacist Nativelle.

1927 — **Heinrich O. Wieland** wins the Nobel Prize in Chemistry for his work on sterols and bile acids.

1928 — **Adolf O. R.Windhaus** wins the Nobed Prize in Chemistry for his work on the constitution of the sterols, however, the structure for cholesterol is incorrect.

1929 — **Androsterone, Equilenin, Equilin, Progesterone, Estradiol and Testosterone** were isolated in the 1930s, however, the structure remained unknown.

1932 — **John D.Bernal** publishes x-ray structure of ergosterol which is only slightly mistaken in its positioning of the pendant hydroxyl group.

1930s — **Edward A. Doisy and Aldof F. Butenandt** independently reported the first isolation of the steroid hormone,

1939 — **Werner E. Bachmann** completes the total synthesis of equilenin, the first steroid to be made in the laboratory.

1950 — **Edward C. Kendall, Tadeus Reichstein, and Phillp S. Hench** win the Nobel Prize in Physiology or Medicine for " their discoverys relating to the hormones of the adrenal cortex, their structure and biological function."

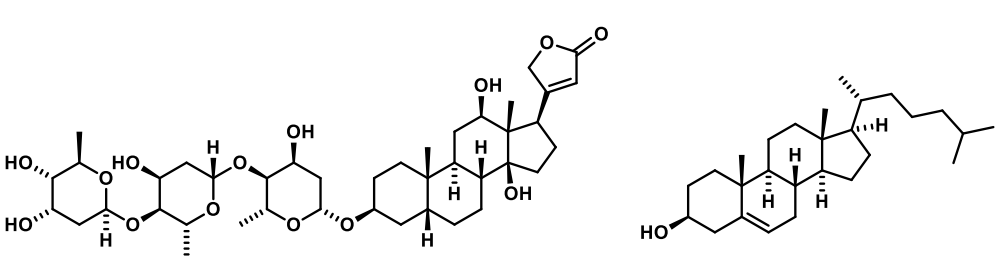
1951 — **Robert B. Woodward** complete the synthesis of cortisone and chelosterol.

1953 — **Sir Robert Robinson and Sir John W. Cornforth** complete the synthesis of *epi*androsterone.

1971 — **William S. Johnson** completes the biomimetic synthesis of progesterone.

1977 — K. **Peter C. Vollhardt** completes the cobalt catalyzed total synthesis of estrone.

Figure 3. Landmarks of the history of steroid chemistry.



Digoxin (**13**) Cholesterol (**14**)

Figure 4. Structures of digoxin and cholesterol.

In 1950, the British chemist Sir Derek H. R. Barton published one of the most influential papers in organic chemistry entitled “The Conformation of the Steroid Nucleus”.[8] In this seminal work, he described his theory of conformational analysis, and every organic chemist who followed him has applied these principles in one way or another in their work. Barton's work was inspired by the fundamental and methodological work of Odd Hassel from Oslo, with whom he later shared the Nobel Price, on the three-dimensional shapes (chair and boat) of cyclohexane rings.[22] The rigid conformation of the sterol skeleton is shown below (Figure 5) in a perspective drawing of hydrocortisone (**15**).

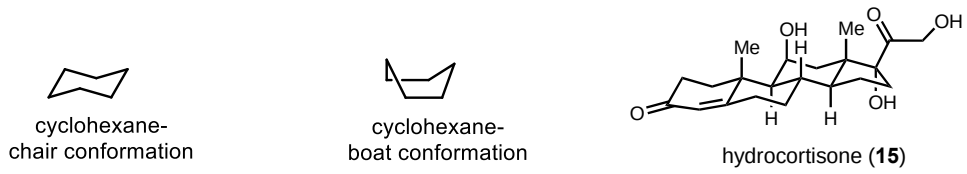


Figure 5. 3D-Structures of cyclohexane (chair and boat conformation) and of hydrocortisone.

The following year (1952), Robert B. Woodward completed his seminal synthesis of cortisone and cholesterol after carrying out a relentless assault on the limits of what was possible in synthetic organic chemistry, tackling molecules whose complexity was then considered unattainable in the laboratory.[23] The ideas and concepts he pursued bear the stamp of artistry and sheer genius. These qualities were recognized by Nobel Prize in Chemistry in 1965 “for his outstanding achievements in the art of organic synthesis”.[24] In 1971, W. S. Johnson synthesized progesterone (**16**) employing a stunning biomimetic cascade polyene cyclization in which three new carbon-carbon bonds and three rings are formed stereoselectively in a single step, an event that has remained as a milestone in the history of total synthesis. [25] This result was followed by K. P. C. Vollhardt's brilliant synthesis of estrone (**2**) in 1977, using a cobalt-catalyzed key reaction.[26]

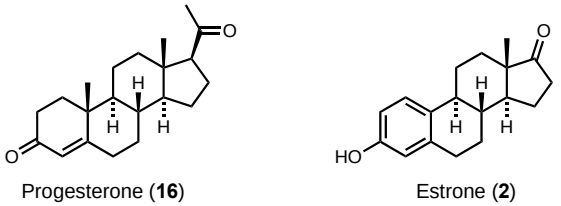


Figure 6. Structures of Progesterone and Estrone.

Another highly distinguished chemist who substantially contributed to steroid chemistry is E. J. Corey from Harvard University. In recognition of his contributions to the design of multistep synthesis (retrosynthesis) and the development of synthetic methods he was awarded the Nobel Prize in Chemistry in 1990.[27]

To summarize this section: During the historical development of steroid chemistry, many significant discoveries and advances have been made over the last hundred years. These include:

- Structure elucidation of important bioactive molecules;
- Development of new synthetic methods and strategies;
- Understanding steroid hormone receptors and the role of steroids in various physiological processes and diseases;
- Medical use of steroids as hormones, and as drugs against inflammatory, cardiovascular and autoimmune diseases, osteoporosis, immune and neurological disorders, and even cancer.

In any case, steroids have a profound impact on human society. Besides the pharmaceutical applications, they are play a role in functional foods, dietary supplements and agriculture, e.g. to lower cholesterol levels through plant sterol-enriched foods and to improve plant and animal health.

1.2.2 Steroid Classes and Biosynthesis

As mentioned before, steroids are characterized by a basic carbon skeleton (nucleus) consisting of three six-membered rings (A, B, C) and one five-membered ring (D). The remarkably stable and rigid tetracyclic core structure is composed of 17 carbon atoms which are numbered as shown in Figure 7.[28]

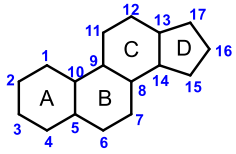


Figure 7. The core of all classical steroids with IUPAC-approved atom numbering and ring lettering

In most steroids, the decalin system formed by rings **A** and **B** has a *trans*-junction (5α-gonane, **17**) albeit rings A and B may also be *cis*-fused (5β-gonane, **18**) (Figure 8). α and β are stereo descriptors originally devised for steroid nomenclature. The substituents "below" the plane of the steroids are described as α and those "above" the plane as β substituents. The hydrogen atoms at the bridge-head centers must be clearly indicated to avoid confusion. Due to the chair-like conformation of six-membered rings in steroids, substituents can also be classified as being axial or equatorial with respect to the ring plane.[28]

Figure 8. 5*a*-gonane (**17**) and 5*b*-gonane (**18**).

Steroids are further divided into subclasses (Figure 9) differing in their constitution and the number of carbon atoms. These are: estranes C_{18} (**19**), androstanes C_{19} (**20**), pregnanes C_{21} (**21**), cholanes C_{24} (**22**), cholestanes C_{27} (**23**), ergostanes C_{28} (**24**) and stigmastanes C_{29} (**25**).

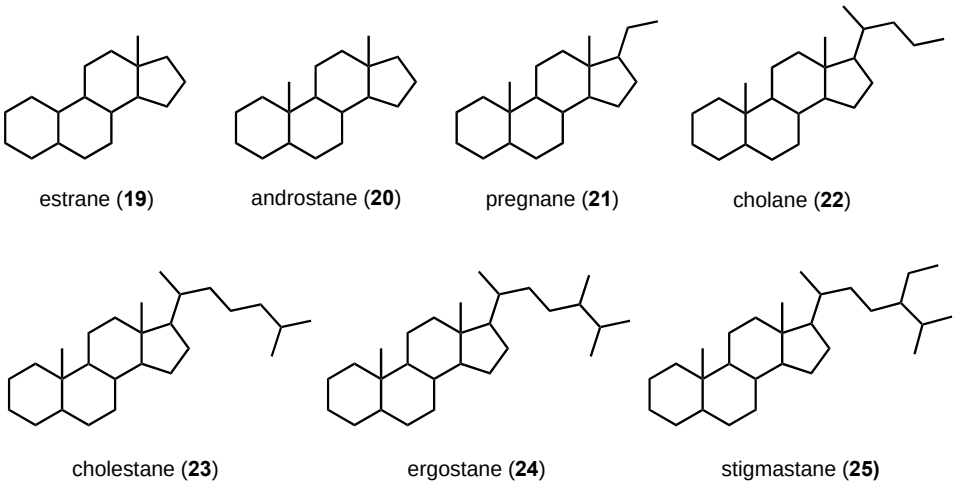
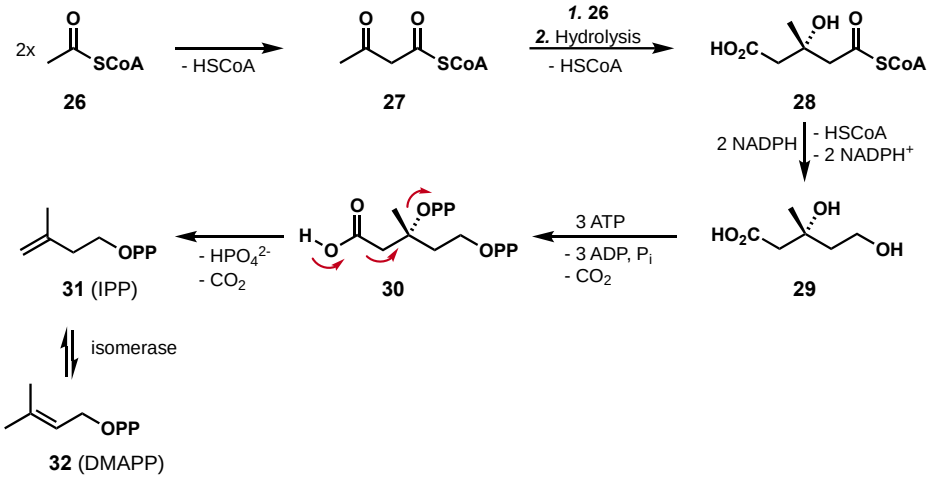


Figure 9. Skeletal structure of important steroid subclasses**.**

The differences between steroids result not only from the variation of the carbon skeleton, but also from the type and stereochemical arrangement of functional groups. This results in a great diversity with regard to the structure and biological activity of steroids. In addition to the

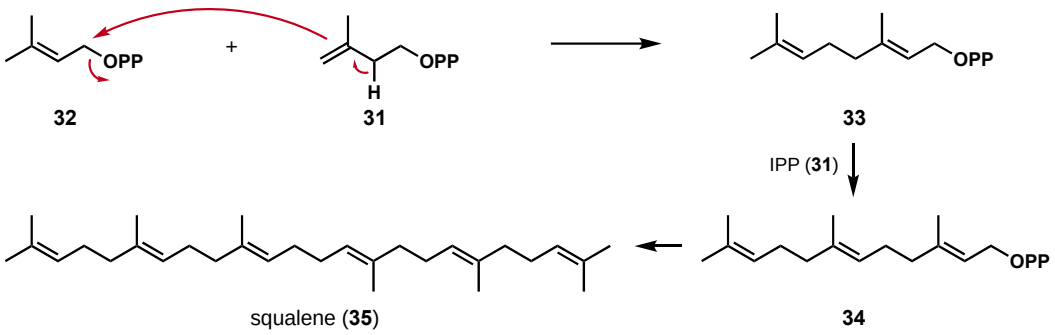
naturally occurring compounds, an enormous number of derivatives and analogs have been synthesized which, as hormones, drugs or signaling molecules, have a wide variety of effects in the human body or other organisms. In addition, steroids and steroid-containing phospholipids are components of cell membranes.

The biosynthesis of steroids can be divided into three reaction sequences (Schemes 1 to 3), all of which proceed via enzymatic reactions.[29] In the so-called mevalonate pathway (Scheme 1), two molecules of acetyl coenzyme A (AcSCoA) (**26**) first condense in a Claisen condensation to form acetoacetyl-SCoA (**27**), which reacts with a third acetyl-SCoA unit to hydroxymethylglutaryl-SCoA (**28**). An enzymatic reduction then yields mevalonate (**29**), from which, after phosphorylation and decarboxylation, isopentyl pyrophosphate (IPP, **31**) is formed, which is in enzymatic equilibrium with its isomer dimethylallyl pyrophosphate (DMAPP, **32**).



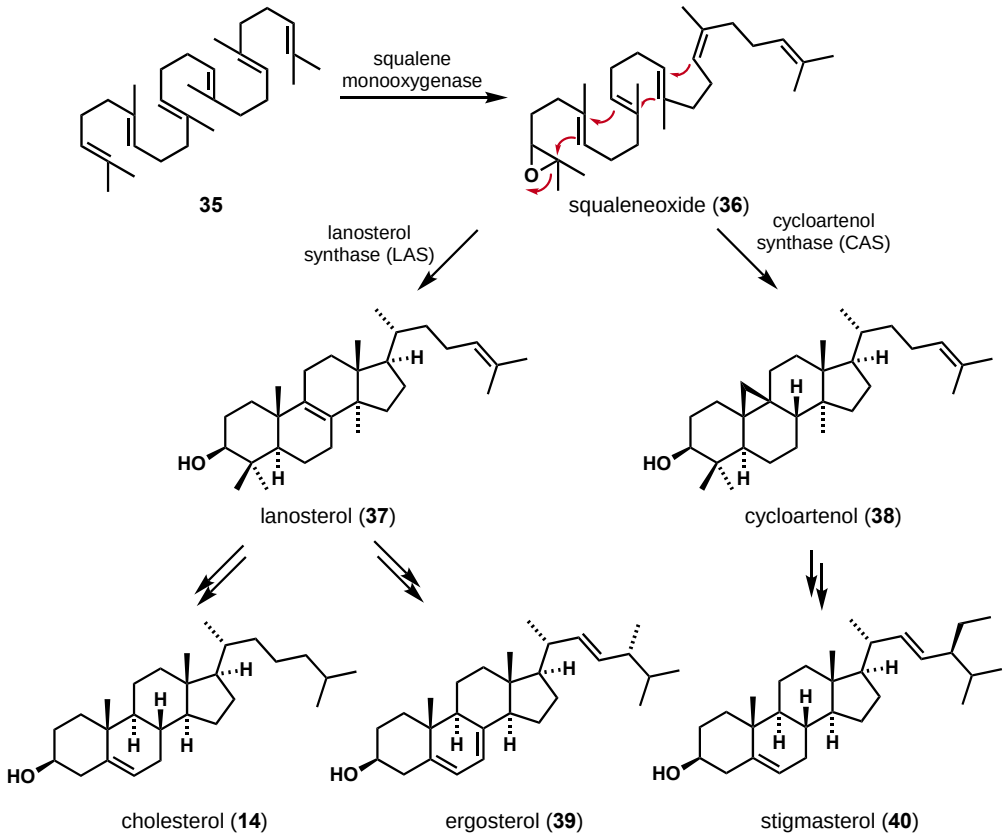
Scheme 1. Mevalonate pathway.

These two C_5 building blocks can now react with each other by IPP (**31**) attacking the allylic position of DMAPP (**32**) to form geranyl pyrophosphate (GPP, **33**), which further condenses with another DMAPP (**32**) to form farnesyl pyrophosphate (FPP, **34**). The final step of this biosynthetic (enzymatic) sequence is the reductive tail-to-tail condensation of two molecules of **34** to form squalene (**35**), a central C_{30}-isoprenoid synthesized from six C_5 units.



Scheme 2. Biosynthesis of squalene from the C_5 building blocks **31** and **32**.

The last sequence begins with an epoxidation of squalene (**35**) to the "activated" (S)-2,3-epoxy-squalene (squalene oxide, **36**), which then is cyclized to either lanosterol (**37**) or cycloartenol (**38**) (Scheme 3). In animals and fungi steroid biosynthesis then further proceeds via lanosterol (**37**), while cycloartenol (**38**) serves as an intermediate in plants. These central metabolites are further converted in multiple steps to the main steroids cholesterol (**14**), ergosterol (**39**) or stigmasterol (**40**).



Scheme 3. Steroid biosynthesis via squalene oxide cyclization to lanosterol (**37**) or cycloartenol (**38**).

1.2.3 Unusual Steroids

While "classical" steroids have attracted the attention of science and industry for many decades, several "unusal" steroids have only gained interest in more recent years. Most of these modified or rearranged steroids are formed by cleavage, expansion, or contraction of the tetracyclic carbon backbone. Notably, the modified geometries and altered functionalities are also associated with new promising bioactivities. The following IUPAC nomenclature rules are used to specify such compounds:[28] When a bond in the classical tetracyclic scaffold is broken, the compound is termed a *seco*-steroid. For instance, when the bond between C5 and C6 is cleaved, the compound should be named a 5,6-*seco*-steroid. The term *abeo* is used when one or more bond migrations have taken place. The extension or expansion of a ring or a substituent by insertion of a C_1-unit is indicated by the prefix *homo* while the prefix *nor* is used to indicate elimination or contraction of a ring or substituent by a C_1-unit. Additional rings formed within the nuclear skeleton are indicated by the prefix *cyclo*, and the 3,5-cyclosteroids in particular are often referred to as *i*-steroids (*iso*). A selection of such "unusal" steroids are shown in Figure 10.

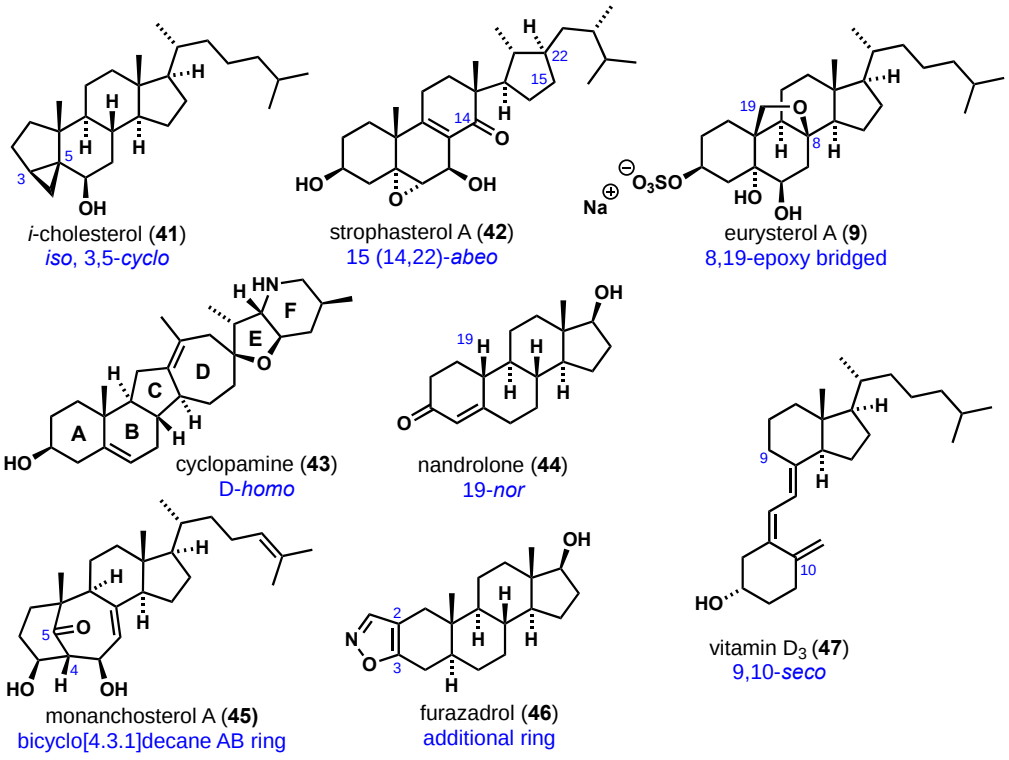


Figure 10. Selected examples for unusual steroids.

1.2.4 Fungi as a Source of Steroids and Related Triterpenoids

Fungi are a large group of eukaryotic organisms that include microorganisms such as yeasts, molds and the well-known mushrooms. Similar to all other eukaryotes, fungi cells have a nucleus, several membrane-bound organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus, etc., and their membranes contain steroids (mainly ergosterol). The ribosomes of fungi are the same size as those of other organisms and produce many sugars, including sugar alcohols (e.g. mannitol), disaccharides (e.g. alginate) and polysaccharides (e.g. glycogen, which is also found in animals). As eukaryotes, Fungi can also synthesize terpenoids from mevalonate.[30] Since the first isolation of ergosterol from ergot fungi by Tanret in 1889, a large number of triterpenoids and steroidal compounds have been identified from fungi.[31] However, these compounds are derived from only a few different triterpene skeletons, which is far less than the more than 100 triterpene skeletons found in plants. The most abundant triterpenoids from fungi are lanostanes (C_{30}) and ergostanes (C_{28}), cholestanes (C_{27}), pregnanesters (C_{21}), and androstanoids (C_{19-20}), which will be discussed in the following.

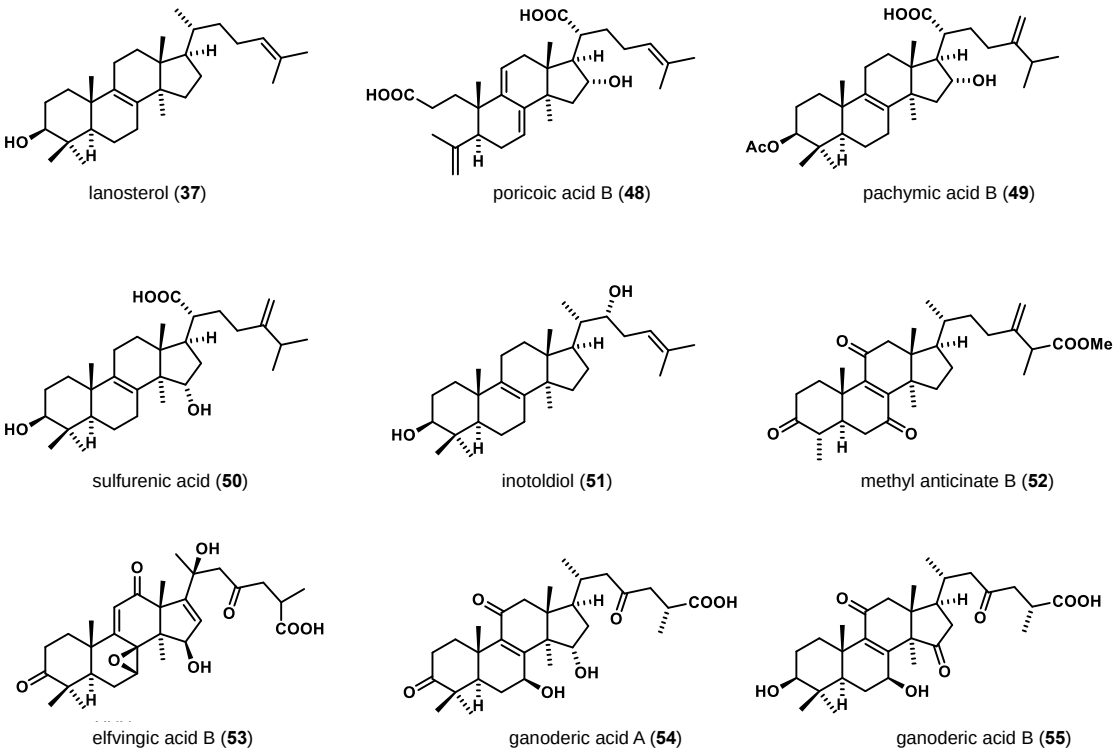


Figure 11. Selected examples for lanostane triterpenoids from fungi.

Selected representative lanostane triterpenoids are shown in Figure 11. Compounds **48** to **53** are produced by different macrofungi from lanosterol (**37**) through oxidation, acetylation, methylation, dehydration, ring opening or other reactions and show pronounced antitumor activities.[32] Other prominent compounds are ganoderic acid A (**54**) and ganoderic acid B (**55**) from *Ganoderma lucidum*, which exhibit hepatoprotective, and antitumoral activities.[33]

Ergostanes, derived from lanosterol by removal of three methyl groups at C(14) and C(4) and introduction of a methyl group at C(24), are most widely distributed in fungi, and are the main sterol components of higher ascomycetes and stramenopiles as well as of *Saccharomyces cerevisiae* (Figure 12).[34] Ergosterol (**39**) itself is a component of the cell membranes of most fungi and plays an important role in the maintenance of fungal life activities. Therefore, inhibition of ergosterol biosynthesis has become an important concept in the development of antifungal agents. In addition to ergosterol and its close relatives such as fecosterol (**56**) and episterol (**57**), a whole series of ergosterol metabolites, formed by oxidation, carbon-carbon cleavage, ring expansion or ring condensation, have been identified from fungi (Figure 12). Hirotani et al. isolated blazeispirol (**58**), the first ergosterol metabolite devoid of ring A, from the mycelium of *Agaricus blazei*.[35] Kikuchi et al. isolated eringiacetal A (**59**),[36] the C-ring cleavage product **60**,[37] and the B-ring rearranged compound pleurocin A (**61**)[38] from the ascospores of almond abalone mushroom (*Pleurotus eryngii*).[39] Hu et al. isolated the heavily rearranged steroids phomopsterones A (**62**) and B (**63**) from the fungus *Phomopsis sp*.[40] Han et al isolated, among other related compounds, gloeophyllins A (**64**) and I (**65**), which are both characterized by a cleavage of the bond connecting ring C and D, from a fungus of the genus Fusarium.[41] Recently, Luo et al. isolated the massively reconstructed steroid **66** from *Ganoderma theaecolum*, a fungus of the genus Ganoderma.[42] These examples demonstrate that ergosterol is not only a component of fungal cell membranes, but also an important precursor of various (bioactive) steroidal secondary metabolites from fungi.

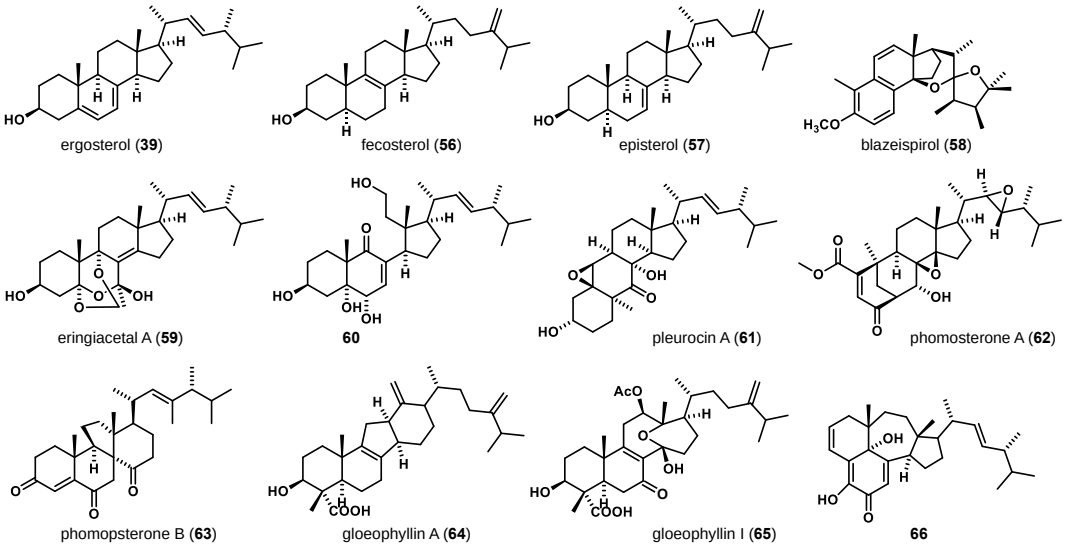


Figure 12. Selected examples for ergostane triterpenoids from fungi.

It has long been thought that ergosterol (**39**) is the only dominating steroid in all fungi, just as mammalian steroids are dominated by cholesterol (**14**). However, subsequent studies have shown that some lower fungi, such as the *phylum Potamococcus*, mainly use cholesterol (**14**) or 24-methylcholesterol (**67**) as the major sterols (Figure 13).[43] Some ascomycetes of the genera *Taphrina* and *Protomyces*, on the other hand, contain rapeseed sterol (brassicasterol, **68**) as the parent sterol component.[44] In addition, 24-ethylcholestanols have been detected in some higher ascomycetes, such as *Blumeria graminis f.*,[45] and stramenopiles. 24-ethyl-cholesta-7,24(28)-dienol (**69**) and 24-ethyl-cholesta-7-enol (**70**) were found to be the major sterols in fungi of the order *Sclerotinia*, but it is still unknown whether the C24 stereocenter in these sterols has the same configuration as in the phytosterols, or not.

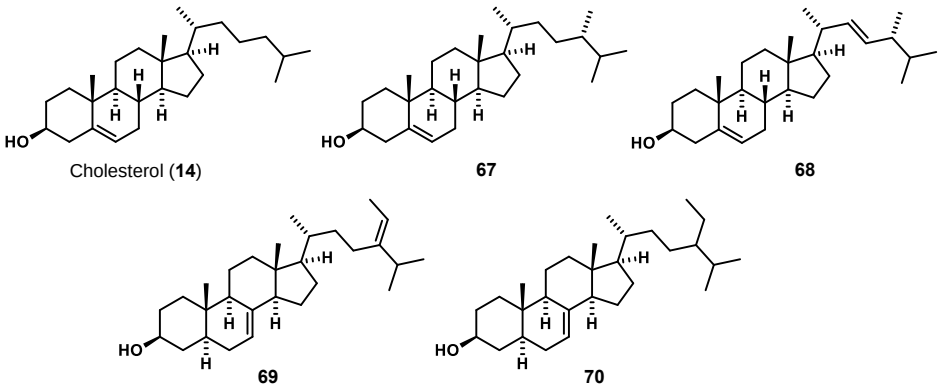


Figure 13. Selected examples of cholesterol-related steroids from fungi.

Thus, different fungi may use different types of parent steroids. However, it appears that during the evolution of fungi, there was a shift from cholesterol to ergosterol.[46]

Pregnenolones are a class of biologically important compounds that are formed from cholesterol (**14**) by C(20)-C(22) bond cleavage and usually consist of 21 carbon atoms. While pregnenolone (**71**) is an important precursor for the synthesis of progesterone, glucocorticoids, salinocorticoids and sex hormones in mammals, a number of pregnenolone derivatives such as **72** and **73** have also been identified in fungi (Figure 14).[47] Also derivatives with specialized structures have been found, such as 4α-carboxypregnenolone (**74**), isolated by Elsebai et al. from the symbiotic fungus *Phaeosphaeria spartinae* of a marine red alga.[48] 4α-Methylpregnenolone (**75**) and its ring A cleavage product **76** were isolated by Z. Qin et al. from the polyarthrospore fungus *Nodulisporium sp.*.[49] In 1986, the C(4)-C(6)-bridged pregnanolone virone (**77**) containing a furan ring was obtained from the green slime mold *G. virens*.[50] Later, the reduced analog 3-dihydrovirone (**78**) was isolated from *H. pseudoalbidus*.[51] Recently, Ding et al. identified two other furanopregnanolides, secovironolide (**79**) and epoxyvirone (**80**), from the endophytic fungus *T. wortmannii* LGT-4, of which compound **79** is the first furanopregnanolide with a contracted B-ring.[52]

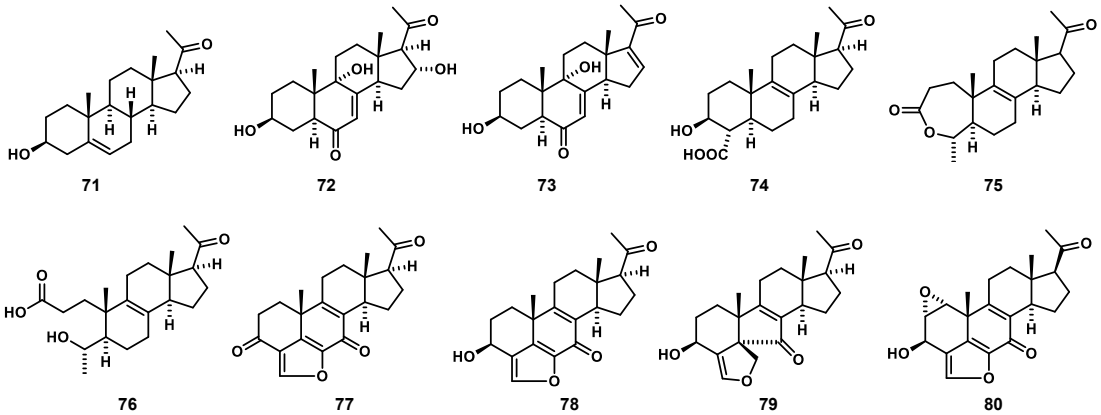


Figure 14. Selected examples of pregnenolone-related steroids from fungi.

Androstanes are another class of steroids formally derived from cholestanes or pregnenolones by oxidative removal of the side chain at C(17). At present, androstanoids found in fungi can be divided into two groups: furanosteroids with a furan ring bridging between C(4) and C(6), and androstanoids with a contracted B ring. Furanosteroids are biologically important fungal

secondary metabolites. Prominent representatives include viridin (**81**), viridiol (**82**), demethoxy-viridin (**83**), demethoxyviridiol (**84**) and wortmannin (**85**) (Figure 16).[53] These compounds display a variety of antibacterial, antifungal, and anti-inflammatory activities and are able to inhibit phosphatidylinositol-3-kinase (PI3K) at nmol concentrations.[54] Among them, the highly functionalized steroid wortmannin (**85**) has been widely used as a PI3K inhibitor in molecular biology. Recently, PX-886, a wortmannin derivative developed as an antitumor drug, has even entered phase II clinical trials.[55] The asterogynins **86** and **87** were discovered by Cao et al. from the vascular plant endophyte *Asterogyne martiana*. [56] These 18-nor-steroids are characterized by a 6/5/6/5 tetracyclic skeleton, which differs from that of the common androstane core by contraction of ring D.

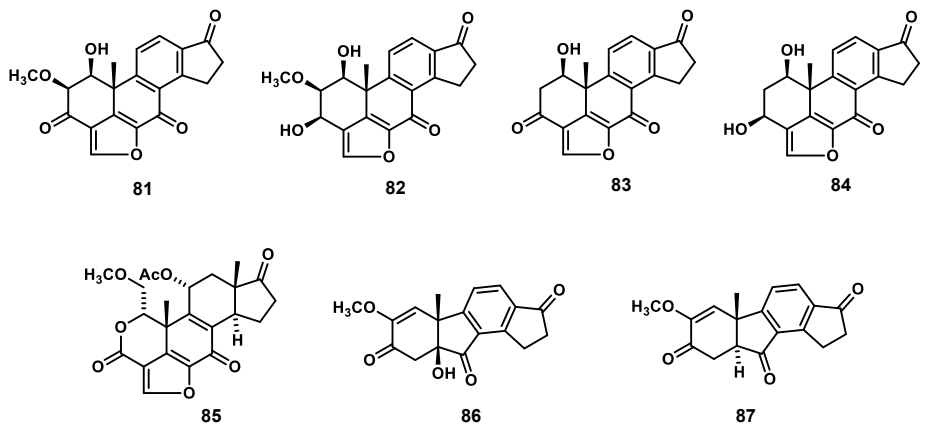


Figure 15. Selected examples of androstanoids from fungi.

Like lanosterol (**37**), protostadienol (**88**) (Figure 16) is formed by protonation-initiated cyclization of 2,3(S)-epoxysqualene in a chair-boat-chair configuration, but unlike in the biosynthesis of lanosterol, no Wagner-Meerwein rearrangement (after formation of a C(20) carbocation) takes place. Instead, proton elimination directly generates the C(17)=C(20) double bond of **88**. Three representative compounds are fusidic acid (**89**), helvolic acid (**90**) and cephalosporin P1 (**91**, also called acremonic acid) (Figure 16). These compounds form a class of triterpenoid antibiotics discovered from fungi, show pronounced anti-Gram-positive activity, and are widely used clinically for the treatment of drug-resistant *Staphylococcus aureus* infections.[57]

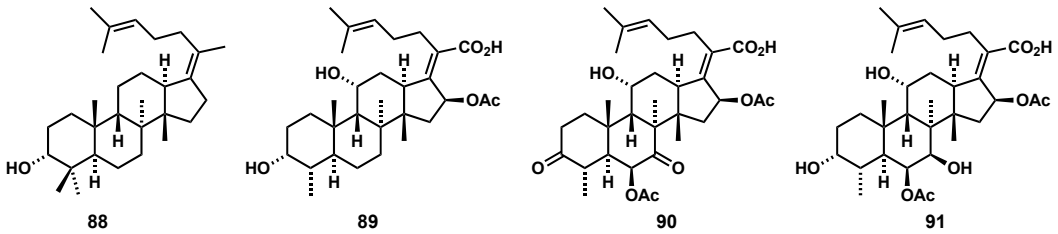


Figure 16. Selected examples of androstanoids from fungi.

Fernane (**92**) is the parent structure of a class of pentacyclic triterpenoid compounds (Figure 17) which are found only in very few fungi.[58] Shigematsu et al. isolated the triterpene glycoside WF11605 (**94**) from the fungal strain F11605.[59] Subsequently, enfumafungin (**95**)[60] and fuscoatroside (**96**)[61] were identified from the fungi *Hormonema sp.* and *Humicola fuscoatra* NRRL 22980, respectively. Deyrup et al. isolated kolokoside A (**97**) from the fungus *Xylaria sp.* of the genus Carbonobacteria. These compounds display prominent antifungal activity, but the mechanism of their biosynthesis is still unclear.[62] Recently, a series of pentacyclic triterpenoids such as **98** were isolated from the sponge-symbiotic fungi *Neosartorya fennelliae* KUFA 0811 and *Neosartorya tsunodae* KUFC 9213.[63]

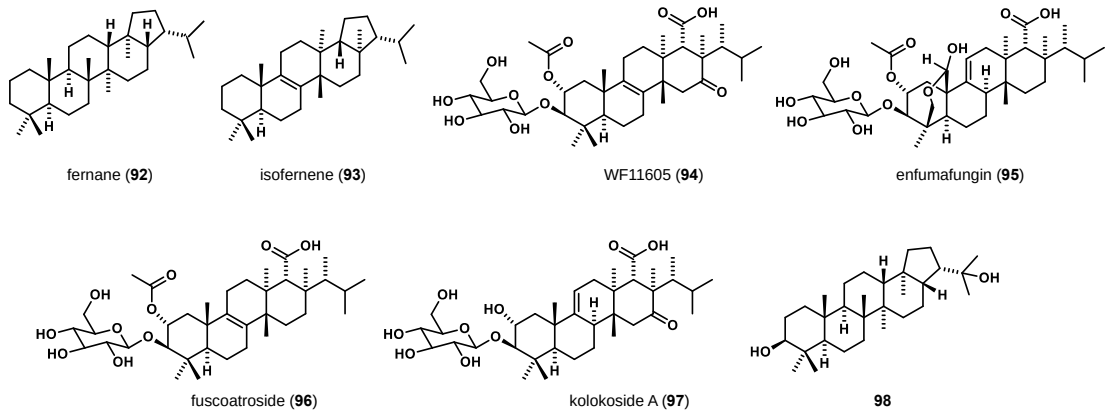


Figure 17. Selected examples of fernane-type triperpenoids from fungi.

The examples give in this section demonstrate that fungi produce a variety of interesting triterpenoids, and modern research tools such as genome mining will promote the discovery of additional fungal triterpenoids and steroids in the future and lead to an improved understanding of their biosynthesis.

1.2.5 Calvatianone - a novel steroid with an unusual skeleton

Calvatianone (**10**) was extracted and isolated as a colorless gum from the fruiting bodies of *Calvatia nipponica*, a rare Japanese giant puffball mushroom of the genus Pelargonium within the family Umbelliferae (Figure 18).[14] The surface of the object is white and soft, and the sphere consists of a skin (chitin) and a fleshy part. The crust consists of three layers, the outermost being a white film, the center a thick light yellow film, and the inner layer a tan colored ultra-thin film structure. The fleshy part of the mushroom (the basic body) is encased in this crust, which is white when young and is edible until the spores mature.[64]

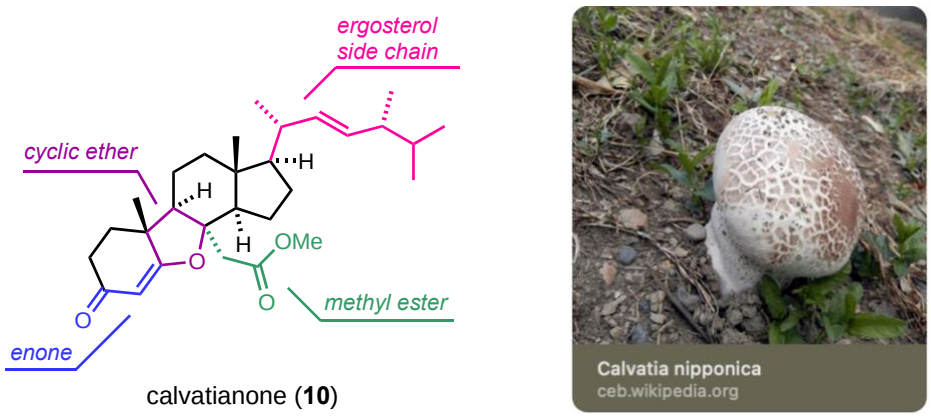


Figure 18. Structure of Calvatianone and a picture of the mushroom *Calvatia nipponica*.

Besides calvatianone, four other (known) steroids (**99**-**102**) were also isolated from the same extract (Figure 19).[14] These compounds also display the typical ergosterol side chain and reflect the biosynthesis of all compounds (including **10**) from ergosterol. The unique structure of **10** was elucidated by means of MS and NMR spectroscopic methods. It shows a replacement of the B ring of ergosterol by a tetrahydrofuran ring, a methyl acetate sidechain at C(9) and an enone functionality in ring A, which is actually part of a vinylogous ester.

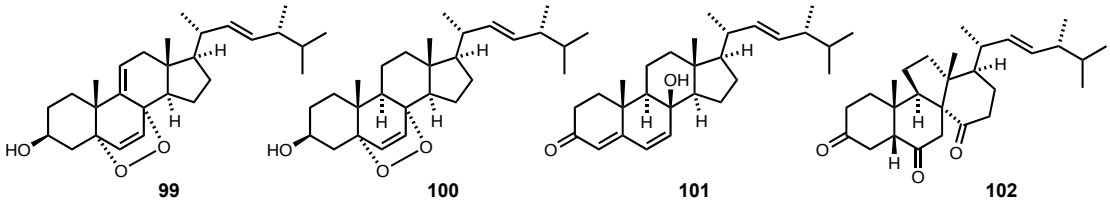


Figure 19. Structure of other ergosterol-derived metabolites isolated together with calvatianone (**10**).

Calvatianone (**10**) and its congeners **99**–**102** were evaluated for their cytotoxicity and antiestrogenic activity using the MCF-7 human breast cancer cell line. While compounds **100** and **101** exhibited significant antiestrogenic activity (suggesting that they may act as antagonists to the estrogen receptor ERα), the activity of calvatianone was only very weak (Figure 20).[14]

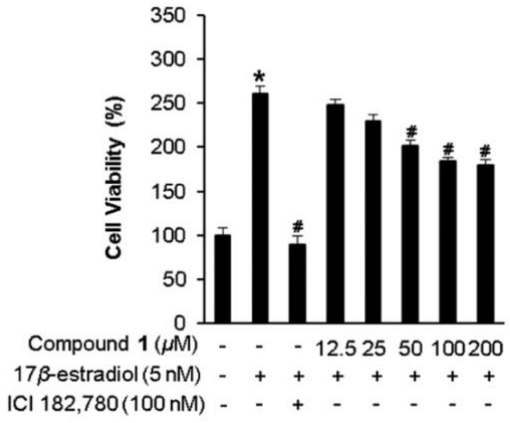


Figure 20. Antiestrogenic activity of calvatianone (**10**) in the absence or presence of 17β-estradiol in the MCF-7 human breast cancer cell line.[14]

Nevertheless, due to its unusual structure, calvatianone (**10**) represents an attractive target molecule for chemical synthesis, and it cannot be excluded that other interesting biological activities will be found for this or related structures in the future.

1.2.6 Spiroseoflosterol - a unique fungal spiro-steroid

The spirosteroids form a class of steroid compounds characterized by the presence of a spiro-cyclic unit, in which two rings share a common carbon atom. This substructure gives the compounds interesting chemical and biological properties. Spirosteroids (spirosterols) occur naturally in certain plants, fungi and marine organisms and play an important role in living organisms. These include functions such as maintaining cell membrane stability (especially in fungi) and participating in metabolic processes. In plants, they can act as resistance-inducing substances and help to ward off pathogens and damage resulting from environmental pollution. In recent years, spirosteroids have attracted attention due to their diverse biological activities, including their potential use as anti-tumoral, anti-inflammatory, antimicrobial and immuno- modulatory agents. These properties make them important research targets in the

fields of drug development, crop protection and functional foods. And in order to be able to further exploit the potential of these compounds in the future, advances in synthetic chemistry will be required.

A more recently discovered spirosteroid is spiroseoflosterol (**11**) a 7(8→9)-abeo-ergostane derivative displaying a unique 1-hydroxy-spiro[4.5]decan-6-on unit (instead of the usual B/C-ring system) and an ergosterol side chain. It was isolated from the fruiting bodies of *Butyriboletus roseoflavus* and its structure was carefully elucidated by J. J. Chen and coworkers using advanced spectroscopic methods and X-ray crystallography.[15]

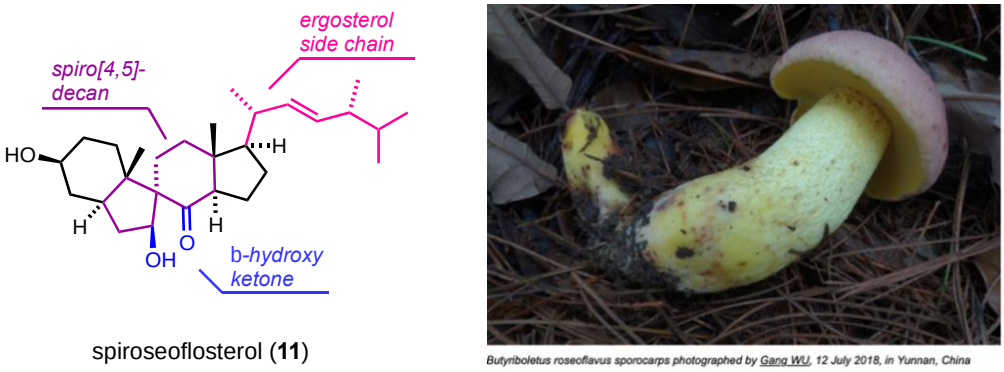


Figure 21. Structure of spiroseoflosterol and a picture of the mushroom *Butyriboletus roseoflavus*.

Interestingly, spiroconyone A (**103**), a natural product closely related to spiroseoflosterol (**11**), was independently isolated from the aerial parts of the plant *Conyza japonica* as the first rearranged phytosterol featuring an spiro[4,5]-decan ring system.[65] It shows a weak tyrosyl–DNA phosphodiesterase 1 (TDP1) inhibitory activity. The two compounds (**11** and **103**) differ only in the nature of the side chain as shown in Figure 22.

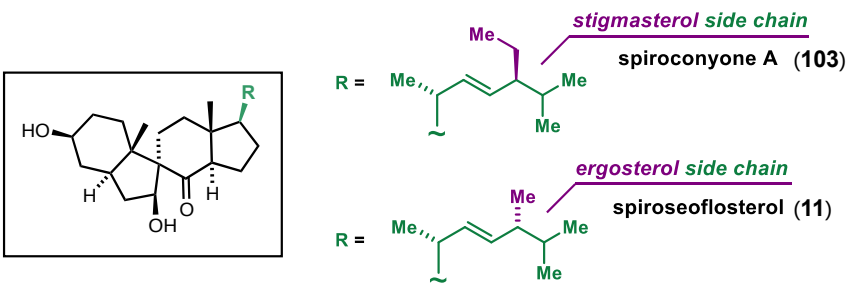


Figure 22. Structures of spiroconyone A and spiroseoflosterol in comparison.

The anti-hepatoma potency activity of spiroseoflosterol (**11**) was assessed using two hepatoma cell lnes, i.e. HepG2 and sorafenib-resistant Huh7 (Huh7/S), and L02 as a normal liver cell line. The result revealed that spiroseoflosterol (**11**) exhibited significant cytotoxicity against HepG2 (IC50 9.1 μM), comparable to sorafenib (IC50 5.5 μM) used as a positive control. More importantly, **11** showed cytotoxicity against Huh7/S with an IC_{50} value of 6.2 μM, indicating its potential for a treatment of sorafenib-resistant hepatoma. The lower cytotoxicity of **11** against L02 cells (IC_{50} = 22.8 μM), also in comparison to sorafenib (IC_{50} 9.1 μM), even suggests that spiroseoflosterol (**11**) might be considered as an anti-hepatoma drug candidate with improved selectivity.

It is worth mentioning the lengthy and tedious protocol for the isolation of spiro-seoflosterol (**11**) as described in reference [15]. A large amount (5.0 kg) of freshly collected fruiting bodies of *B. roseoflavus* was crushed and extracted three times for two days with 10 liters of 95% aqueous EtOH. The combined EtOH percolates were then concentrated in vacuo to obtain 155 g of crude residue. However, after silica gel column chromatography, additional fractionation on a Sephadex LH-20 gel column and final semi-preparative HPLC, only 3 mg of spiroseoflosterol (**11**) was obtained. It is obvious that such a complex and laborious extraction process to isolate only tiny amounts of the natural product does not provide a feasible basis for further research or even medical application of the compound. Thus, the development of a total- or semi-synthetic access to this interesting natural product would be a worthwhile endeavor.

1.2.7 Euphorol J, Euphorstranol A, and Euphorstranol B: Three novel 9,11-seco-triterpenoids featuring an enol-hemiacetal functionality

As another group of unsual triterpenoids, euphorol J (**104**), euphorstranol A (**105)**, and euphorstranol B (**12**) were recently isolated from the plant *Euphorbia stracheyi* by Yuan et al.[16] After careful structure elucidation bey means of a combination of spectroscopic, computational, chemical, and single-crystal X-ray diffraction methods, these compounds were identified as 9,11-seco-triterpenoids containing a rare cyclic enol-hemiacetal moiety (Figure 23). Interestingly, these compounds differ in the configuration of the stereocenters along ring D. While euphorol J (**104**) and euphorstranol A (**105)** display a configuration which suggest

them being biosynthetically derived from euphol (**106**), the isomeric compound euphorstranol B (**12**) reflects the configuration of lanosterol (**37**)

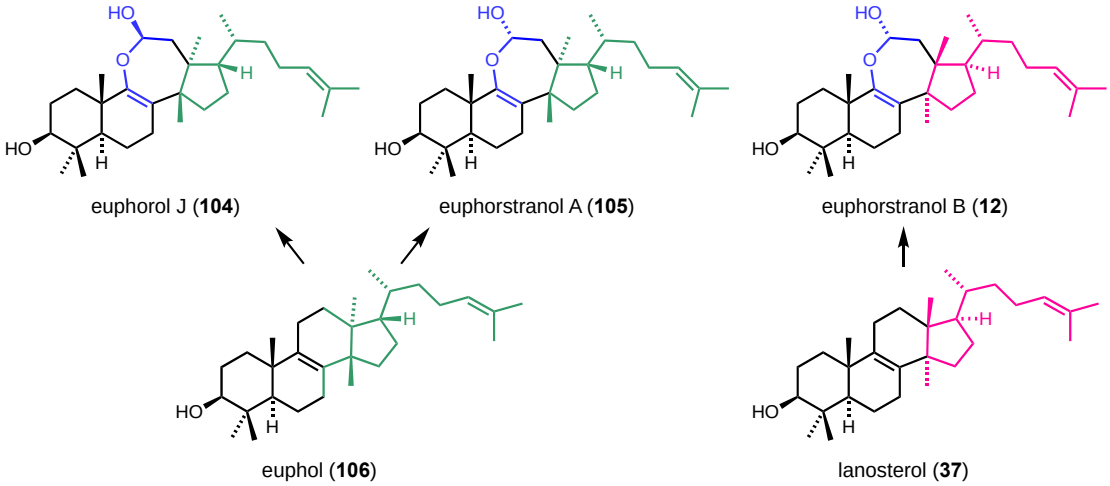


Figure 23. Structures of euphorol J and euphorstranols A and B and their proposed biosynthetic precursors euphol and lanosterol, respectively.

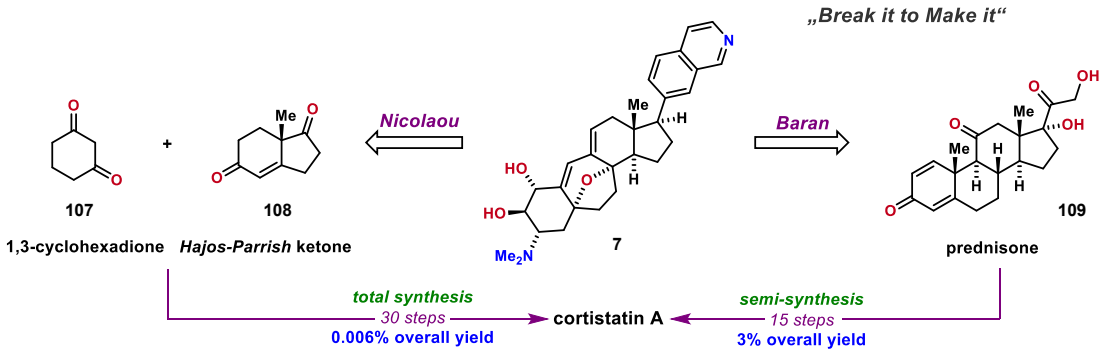
The antitumoral activity of compounds **104**, **105** and **12** was investigated using the MDA-MB-468 breast cancer cell line, and it was found that all three compounds showed significant cytotoxicity with IC_{50} values in the range of 2.9–3.9 μM. Together with their interesting structural properties, this makes these compounds attractive target molecules for chemical synthesis.

1.3 Motivation and Concept

Bioactive molecules are defined as small or large molecular compounds, including natural products, synthetic compounds, proteins and peptides, which have the capacity to influence physiological processes in living organisms. These molecules regulate vital life activities and signaling processes by interacting with specific biological targets, such as enzymes, receptors or nucleic acids. The use of bioactive molecules is wide-ranging and encompasses various applications, particularly in medicine (including disease diagnosis, treatment and prevention), crop protection, cosmetics, fragrances and the food industry.

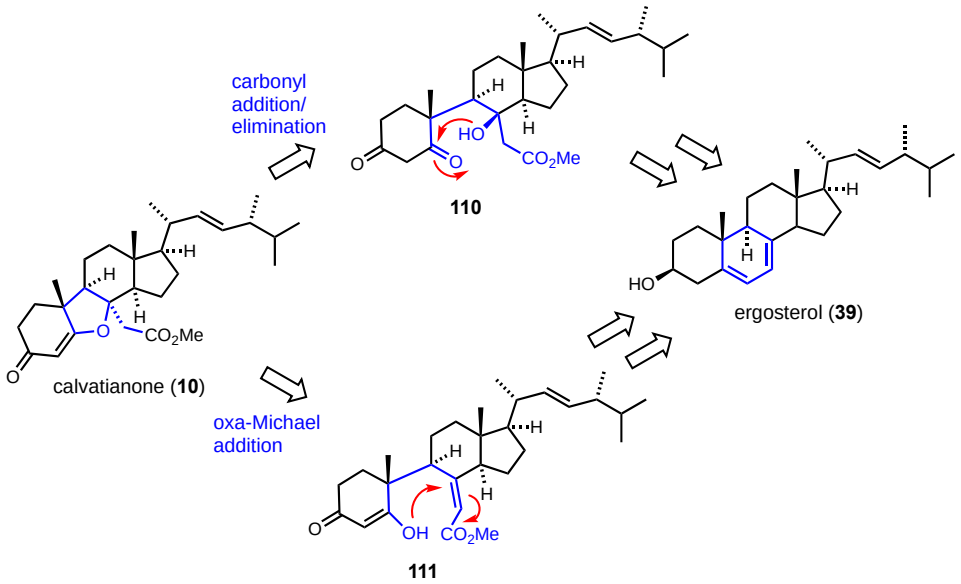
As described in the previous sections, steroids and related triterpenoids represent a particular relevant class of bioactive molecules with a wide range of different activities. Thus, the aim of this thesis was to contribute to the synthesis of some unusual steroids, which haven't been synthesized before.

In this context, a basic question was whether to strive for total synthetic or semi-synthetic approaches. Regarding the example of cortistatin A (**7**), the advance of semi-synthesis becomes obvious. Starting from the readily available building blocks **107** and **108**, the total synthesis of **7** by Nicolaou and coworkers required thirty steps and proceeded with an almost negligible overall yield (Scheme 4).[66] In contrast, Baran and coworkers followed a "*Break it to Make it*" strategy and succeeded to obtain the target molecule **7** by semi-synthesis in only fifteen steps in at least 3% yield.[11] Therefore, semi-synthetic strategies were also used in the course of this doctoral thesis.



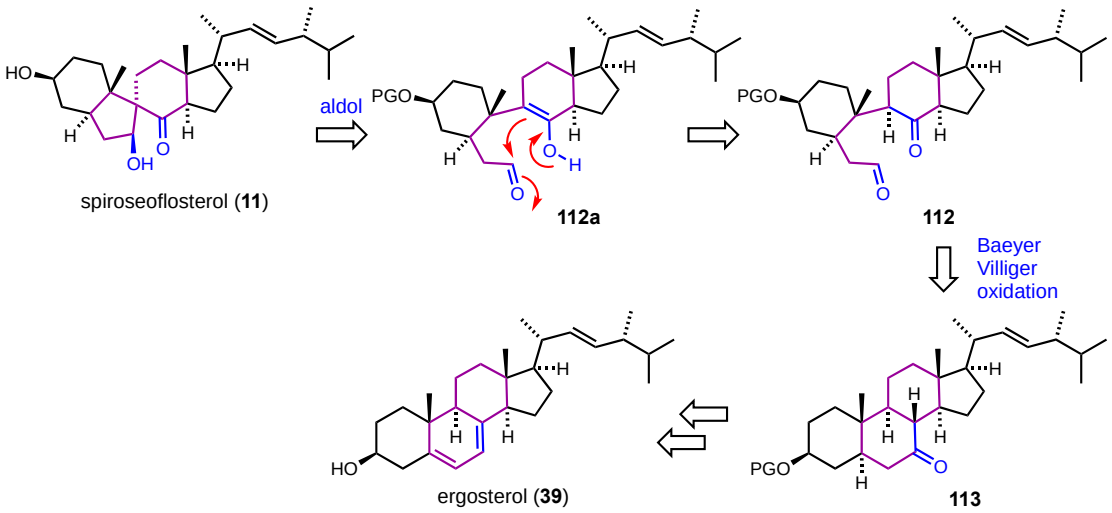
Scheme 4. Total synthesis versus semi-synthesis in the case of the usual steroid cortistatin A.

As a first target molecule, calvatianone (**10**) was selected (see section 1.2.5). According to the retrosynthetic analysis shown in Scheme 4, we envisioned that compound **110** could possibly be converted to **10** by vinylogous intramolecular esterification (via carbonyl addition and subsequent water elimination). Alternatively, the tetrahydrofuran ring could possibly be constructed by intramolecular oxa-Michael addition[67] from the unsaturated ester **111**. Both devised precursors should be accessible by semi-synthesis from readily available ergosterol (**39**) under oxidative opening of ring B.



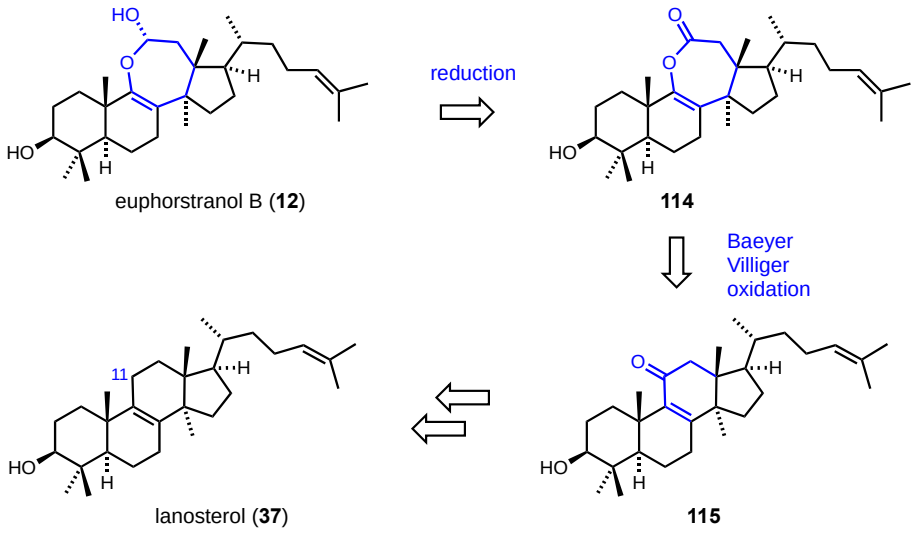
Scheme 5. Retrosynthetic analysis of calvatianone (**10**).

The second target molecule to be addressed in this thesis is spiroseoflosterol (**11**), which was introduced in section 1.2.6. In this case, it was envisioned that the spiro[4,5]decan substructure, which also represents a β-hydroxy-ketone functionality might eventually be obtained by intramolecular aldol addition from an enol (or enolate) **112a** derived from keto-aldehyde **112** (Scheme 6). This compound in turn might be prepared from the appropriately protected ketone **113** by Baeyer-Villiger Oxidation and subsequent lactone reduction/reoxidation. Again, a semi-synthetic strategy appeared most promising, as ketone **113** might be prepared from ergosterol (**39**) by selective functionalization of ring B after protection of the OH group at C(3) with a suitable protecting group (PG). To investigate the aldol addition as a most challenging key step of the planned synthesis, compounds with a saturated side-chain, readily derived from cholesterol (**14**), could be initially investigated as model compounds.



Scheme 6. Retrosynthetic analysis of spiroseoflosterol (**11**).

A third challenge to be investigated in the course of this thesis was the semi-synthesis of euphorstranol B (**12**) from lanosterol (**37**). In this case, the plan was to install the cyclic enol-hemiacetal moiety by reduction of the corresponding lactone (**114**), which in turn could possibly be prepared from enone **115** (Scheme 7). Another key challenge of the planned synthesis was the regioselective allylic oxidation of lanosterol (**37**) at position 11.

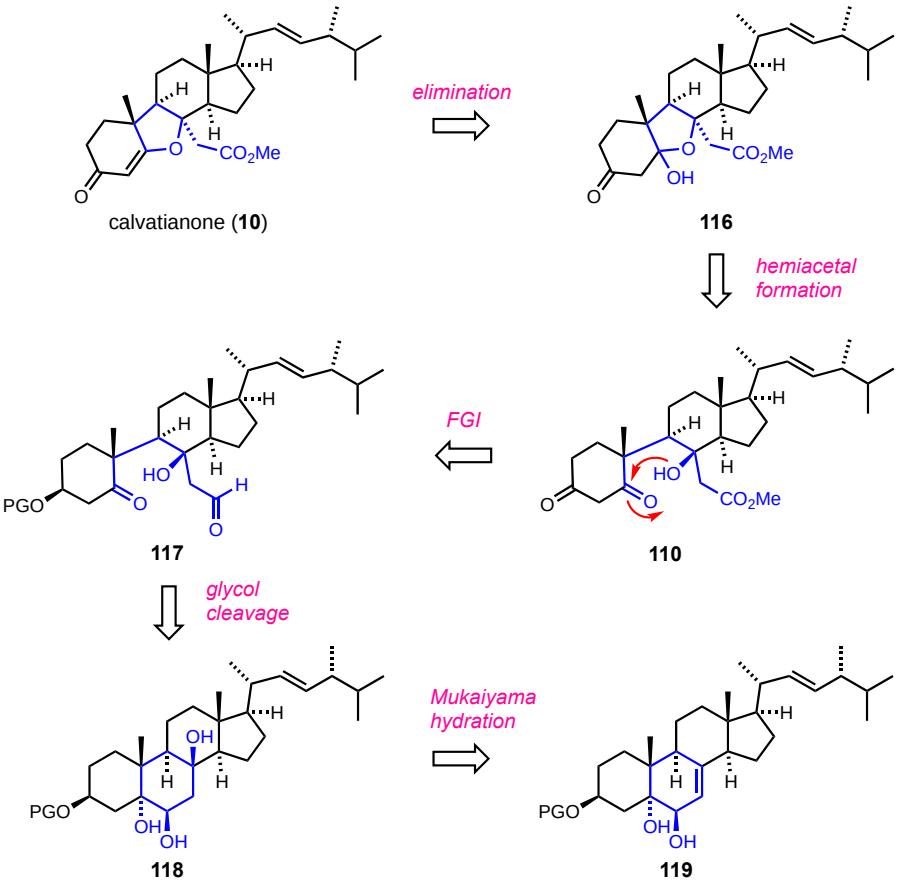


Scheme 7. Retrosynthetic analysis of euphorstranol B (**12**).

1.4 Results and Discussion

1.4.1 Synthesis of Calvatianone

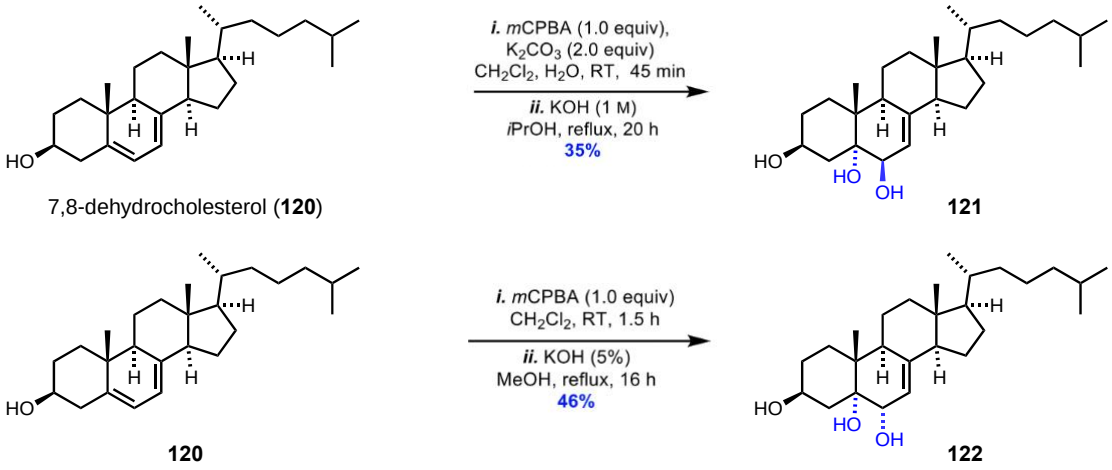
According to the concept outlined above (Scheme 5) a refined retrosynthetic analysis of calvatianone was developed (Scheme 8). The plan was to generate the target molecule by vinylogous esterification (via the hemiacetal intermediate **116**) from the precursor **110**, which should be prepared from a keto-aldehyde of type **117**. This intermediate could possibly be obtained by selective cleavage of the glycol moiety[68] in **118**, which in turn might be prepared by Mukaiyama hydration[69] of an ergosterol-derived diol of type **119**.



Scheme 8. First retrosynthetic analysis of calvatianone (Strategy A).

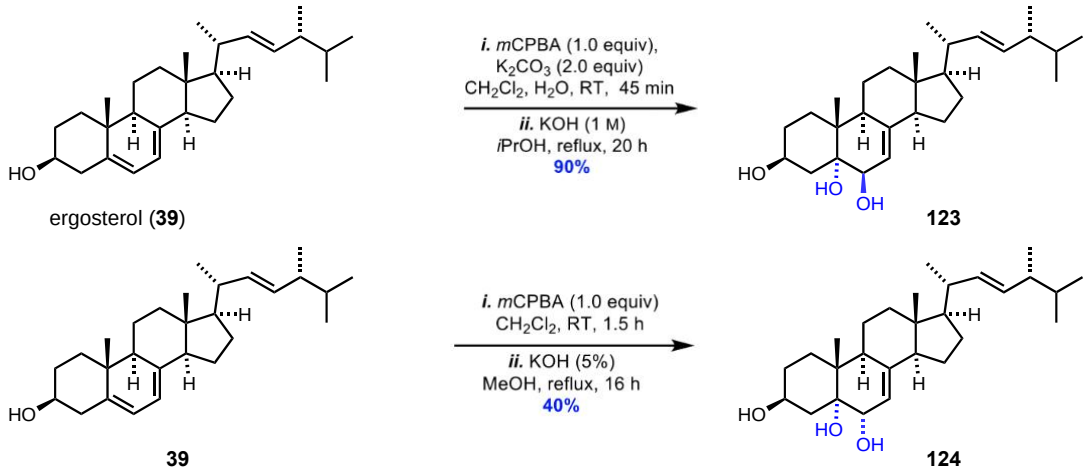
The experimental investigations were started by probing the regioselective dihydroxylation of ergosterol-related substrates. As a first test system, 7,8-dehydro-cholesterol (**120**) was employed in the reaction with *m*-CPBA and subsequent hydrolysis of the initially formed 5α-hydroxy-6-(3-chlorobenzoyl)oxy-substituted products. As shown in Scheme 9, either the *trans*-

or *cis*-selective dehydroxylated products **121** or **122**, respectively, were obtained as recently reported by Taspinar et al..[70]



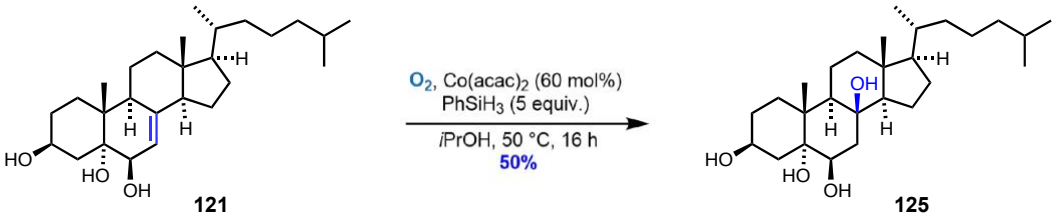
Scheme 9. Regio-and diastereoselective dihydroxylation of 7,8-dehydro-cholesterol.

Under the same conditions, ergosterol (**39**) afforded the dihydroxylation products **123** (Cerevisterol) and **124**, respectively (Scheme 10). Noteworthy is the selectivity of the reactions. In the presence of K_2CO_3 as a base, the initially formed 5,6-α-epoxide is directly opened in a S_N2-type fashion to afford the *trans*-product, epoxide opening occurs in the absence of a base in a S_N1 manner to give the cis product through attack of the benzoate from the less hindered face at the allylic cation. Remarkably, the (less electron-rich) side chain double bond of ergosterol (**39**) is not affected.



Scheme 10. Regio-and diastereoselective dihydroxylation of ergosterol.

While the planned Mukaiyama hydration could be successfully demonstrated with **121** as a model substrate to regio- and stereoselectively afford **125** (Scheme 11) initial attempts to employ the ergosterol-derived substrate **123** (with a second double bond in the side chain) failed.



Scheme 11. Synthesis of **125** by Mukaiyama hydration (model series).

In addition, in parallel investigations by Ö. Taspinar[70] it became evident, that the planned oxidative cleavage of a *trans*-5,6-diol unit in a cholestane or ergostane B ring (Figure 24) represents a rather difficult task.

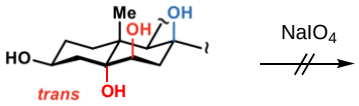
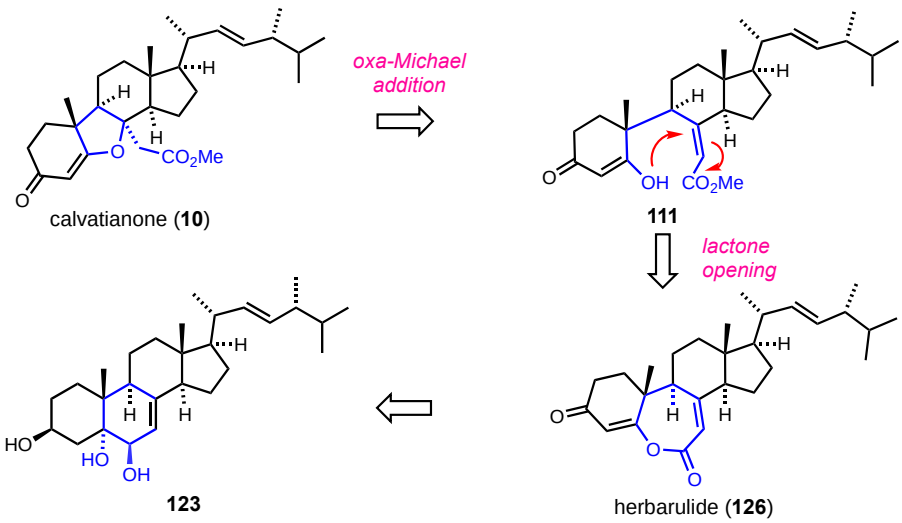


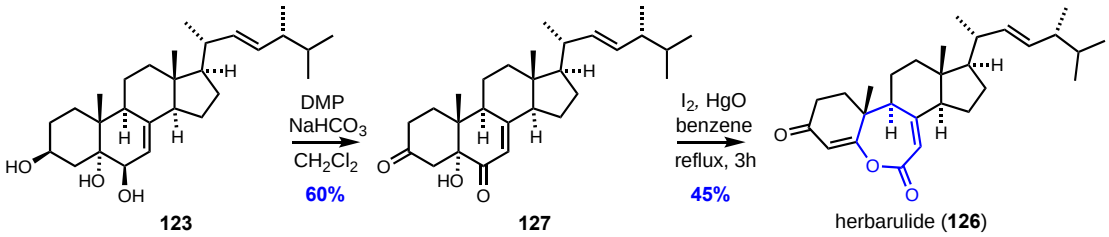
Figure 24.

At the same time, we became aware of a publication by P. Heretsch and coworkers,[71] who achieved the conversion of **123** to herbarulide (**126**). This prompted us to change the strategy and to consider an oxa-Michael addition for the construction of calvatianone (Scheme 12).



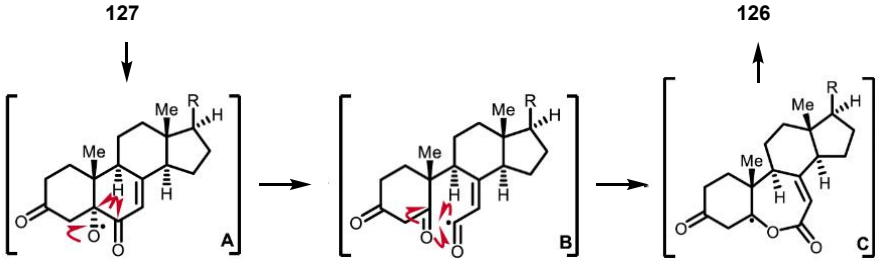
Scheme 12. Modified retrosynthetic analysis of calvatianone (Strategy B).

Following the protocol of Heretsch and coworkers, the synthesis of herbarulide (**126**) was achieved by oxidation of the triol **123** using the Dess-Martin periodinane (DMP)[72] and subsequent Baeyer-Villiger-type oxidation of the α-hydroxy-ketone unit of intermediate **127** (Scheme 13).



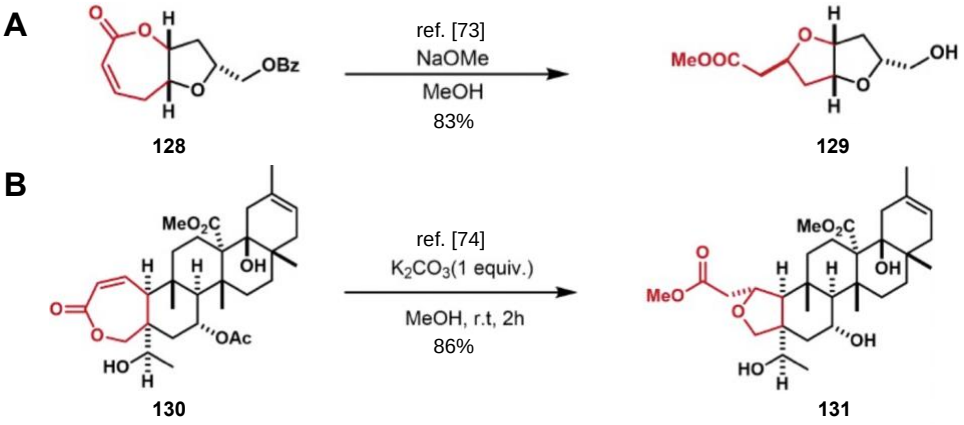
Scheme 13. Conversion of **123** to herbarulide (**126**) according to Heretsch.[71]

The latter transformation was performed using a combination of iodine and mercury oxide and assumed to proceed via a radical mechanism via intermediates A, B and C (Scheme 14), in analogy to the proposed biosynthesis of herbarulide.[71]



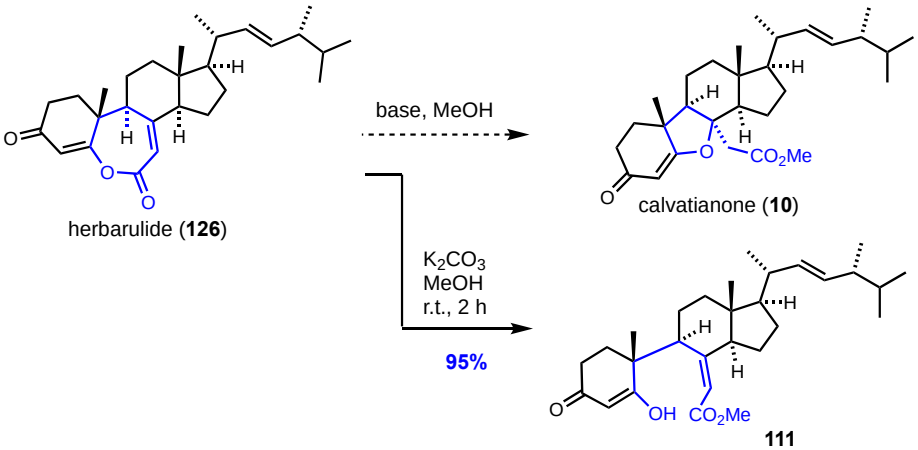
Scheme 14. Proposed radical mechanism of the formation of herbarulaide (**126**).

With herbarulide (**126**) in our hands, we next tackled its planned conversion to calvatianone (**10**). Literature reports even suggested that this task might be achieved in a single step, as related transformations, i.e. the conversion of α,β-unsaturated ε-lactones into tetrahydrofurans bearing a methyl acetate sidechain, had been achieved in high yield, for instance by L. E. Overman and coworkers[73] (Scheme 15A) or M. Herrera-Ruiz et al.[74] (Scheme 15B).



Scheme 15. Literature examples for the conversion of α,β-unsaturated ε-lactones.

Upon treatment of herbarulide (**126**) with K_2CO_3 in methanol for two hours, a product was formed in high yield, which, however, was not calvatianone (**10**) but the open-chain isomer **111** (Scheme 16). This behavior might be explained by considering that **126** actually represents a vinylogous anhydride, which readily undergoes methanolysis under the used conditions under formation of an α,β-unsaturated ester chain and a 1,3-diketone unit (ring A). The latter represents a vinylogous acid which preferentially exists in one of the two possible enolized tautomeric forms.



Scheme 16. Facile methanolysis of herbarulide (**126**) to yield **111**.

An X-ray crystal structure analysis of **111** (Figure 25) confirmed the expected) constitution and Z-configuration of the exocyclic double bond. Moreover, the structure of **132** in the crystalline state shows the A ring in a "turned around" conformation in which the oxygen atom at C(5) is

in a remote position with respect to the β-position of the enoate moiety to be attacked in the planned oxa-Michael addition (compare Scheme 12).

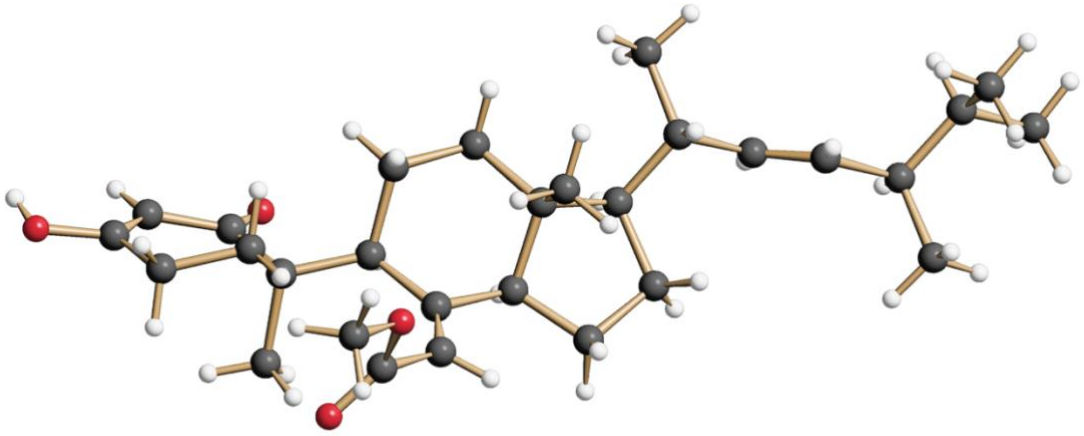
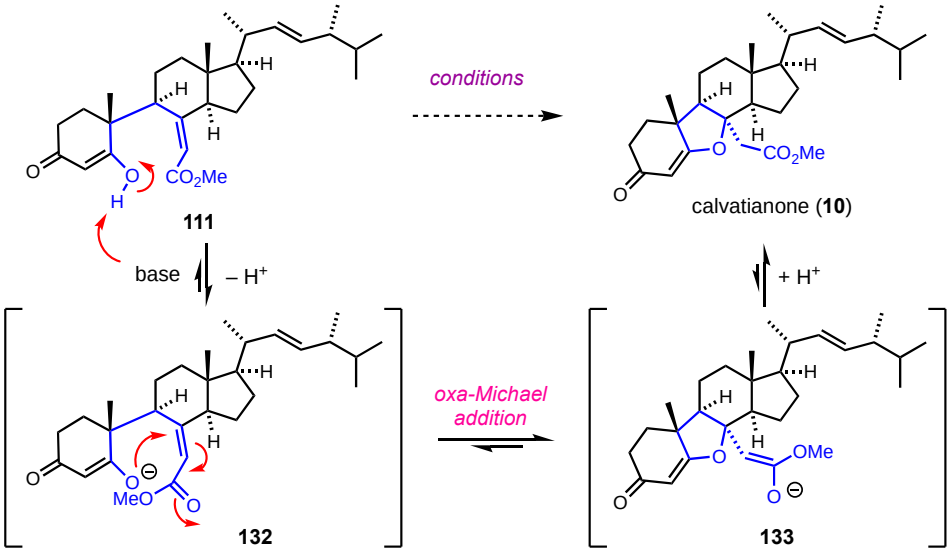


Figure 25. Structure of **111** in the crystalline state.

Nevertheless, the final key step of the planned synthesis of calvatianone (**10**) appeared mechanistically feasible (Scheme 17). Albeit the primarily formed anion **132** is strongly stabilized by resonance, it should be nucleophilic enough to undergo the intramolecular oxa-Michael addition step, which in principle is reversible. However, the resulting enolate **133** is comparably basic and thus expected to be rapidly protonated to give **10**.



Scheme 17. Mechanism of the planned conversion of **111** to calvatianone (**10**).

For this reason, various reaction conditions for the conversion of **111** to **10** were investigated. In a series of more than 100 experiments performed on a small scale and monitored by means of thin layer chromatography (TLC), various reaction parameters were screened. As bases, K_2CO_3, Et_3N, KO^{t}Bu, or DBU were tested, all of which in different solvents (MeOH, EtOAc, Et_2O, 1,4-dioxane, THF, DMF, DMSO, $CHCl_3$, toluene, or benzene). Reactions were run either at room temperature or at 50 – 100 °C for 12 hours. In addition, Takemoto's catalyst (**134**)[75] and the chiral squaramide catalyst **135**[76] were tested as hydrogen bonding organocatalysts. However, in all of these experiments, no conversion of **111** was observed.

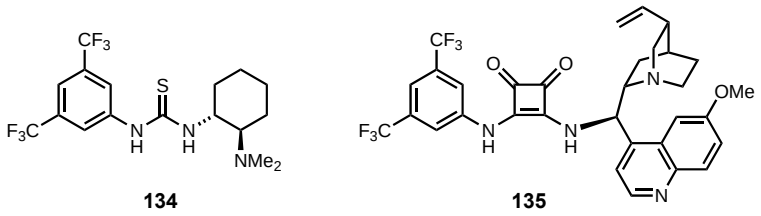
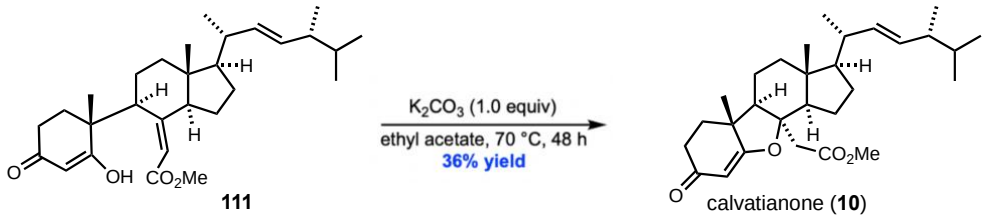


Figure 26. Hydrogen bonding catalysts tested for the conversion of **111** to **10**.

Much to our surprise, however, when an older sample of **111** was analyzed, i.e. a solution of **111** in ethyl acetate which had been stored over prolonged times (several weeks), we a new spot had appeared in the TLC. And indeed, this transformation could be reproduced by heating a solution of **111** in ethyl acetate in the presence of K_2CO_3 for 48 hours to give calvatianone (**10**) in at least 36% yield (Scheme 18).



Scheme 18. Successful conversion of **111** to calvatianone (**10**).

The identity of the synthetic sample of **10** with the natural product described by Lee et al.[14] was secured by comparison of the spectroscopic data. Figure 27 shows the ^{1}H NMR spectra of the synthetic and the natural samples, which clearly match in all details and additionally prove the high purity of the synthetic compound.

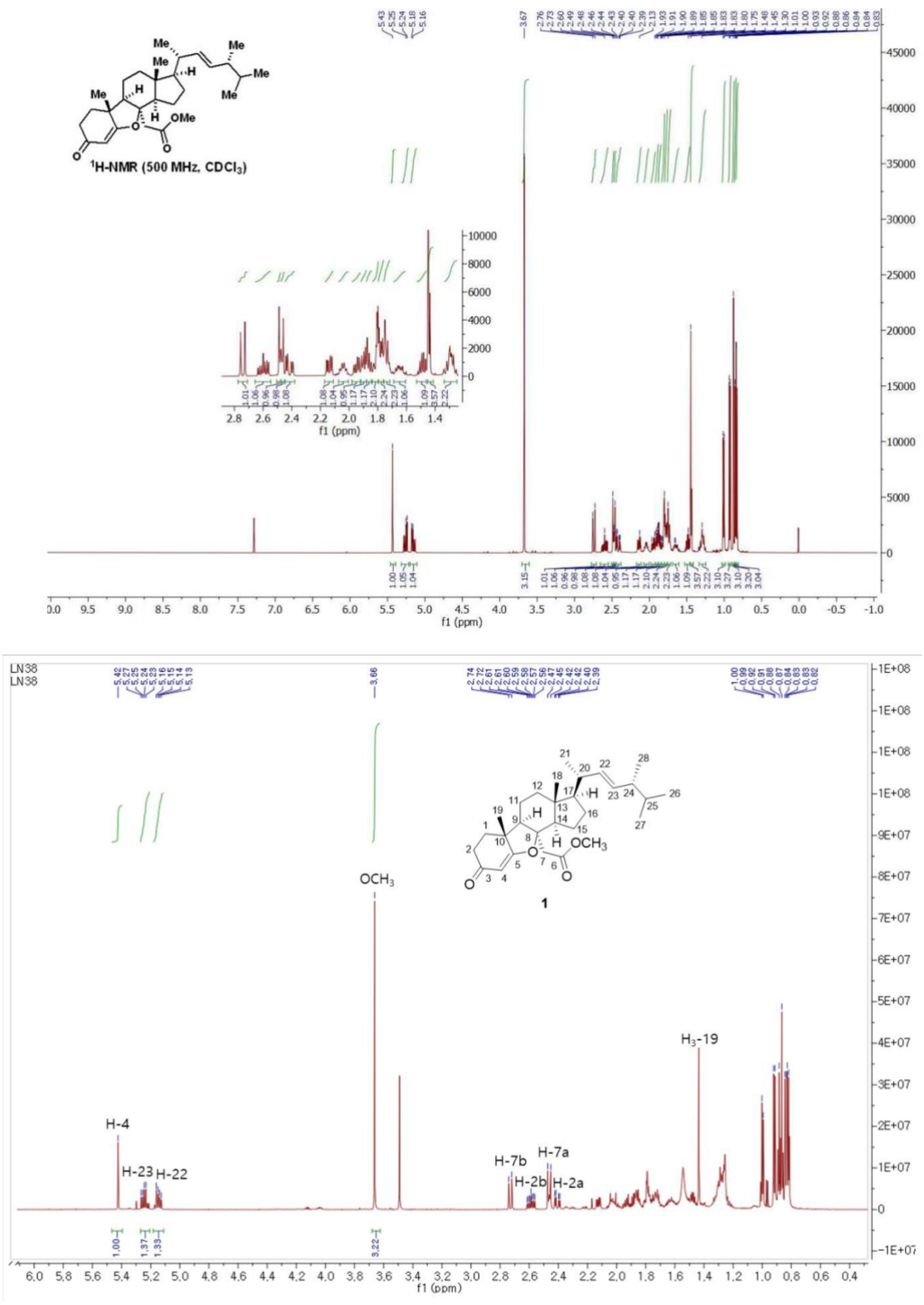
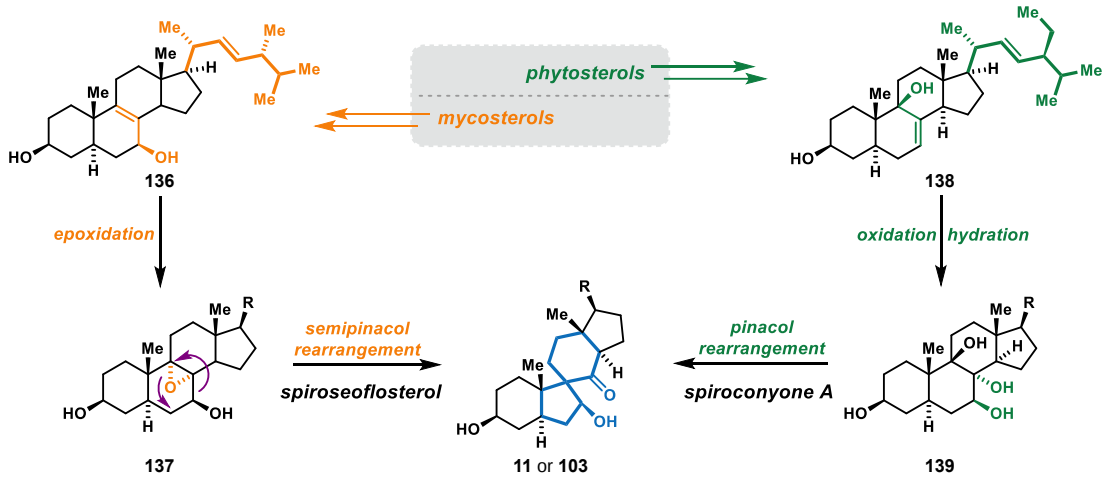


Figure 27. ^{1}H NMR spectrum of synthetic (top) and natural (bottom)[14] calvatianone.

To sum up, the projected semi-synthesis of calvatianone (**10**) has been successfully realized starting from ergosterol. The synthetic sequence requires six steps and proceeds in an overall yield of 8%.

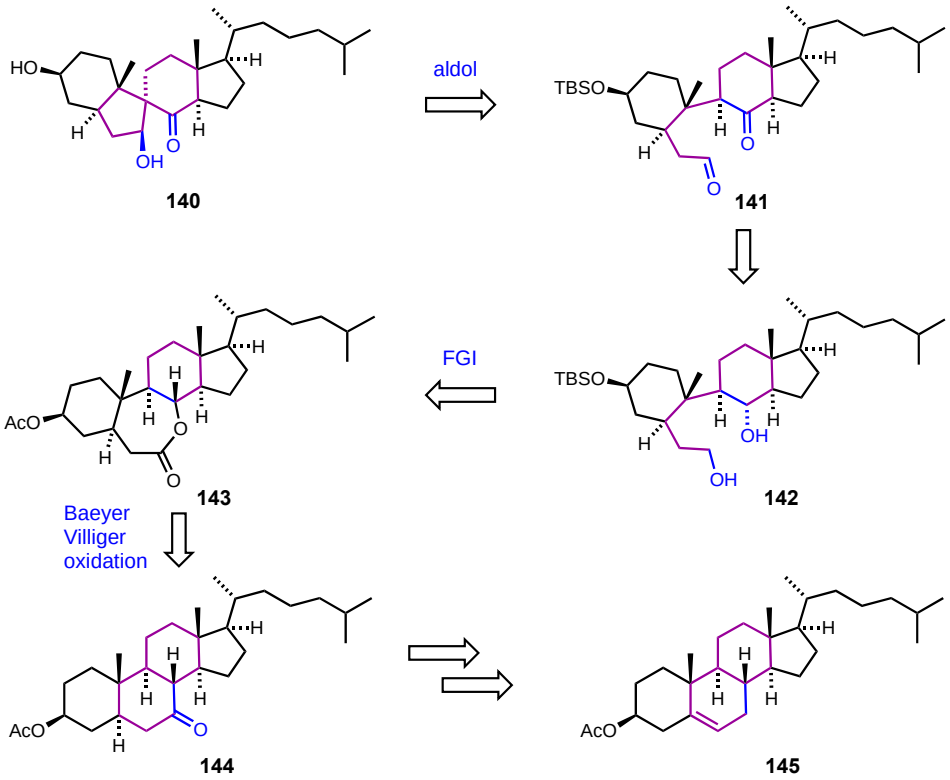
1.4.2 Studies towards the synthesis of Spiroseoflosterol

Having accomplished the synthesis of calvatianone (**10**), the second task of this thesis, i.e. the synthesis of spiroseoflosterol (**11**) and/or the related compound spiroconyone A (**103**) was tackled. Biosynthetically, these particular spirosteroids are supposedly derived by a pinacol or a semi-pinacol-type rearrangement from precursors of type **137** or **139**, respectively, which in turn are synthesized either from 7-hydroxy-lanosterol **136** (in fungi) or the related metabolite **138** (in plants) (Scheme 19).[77]



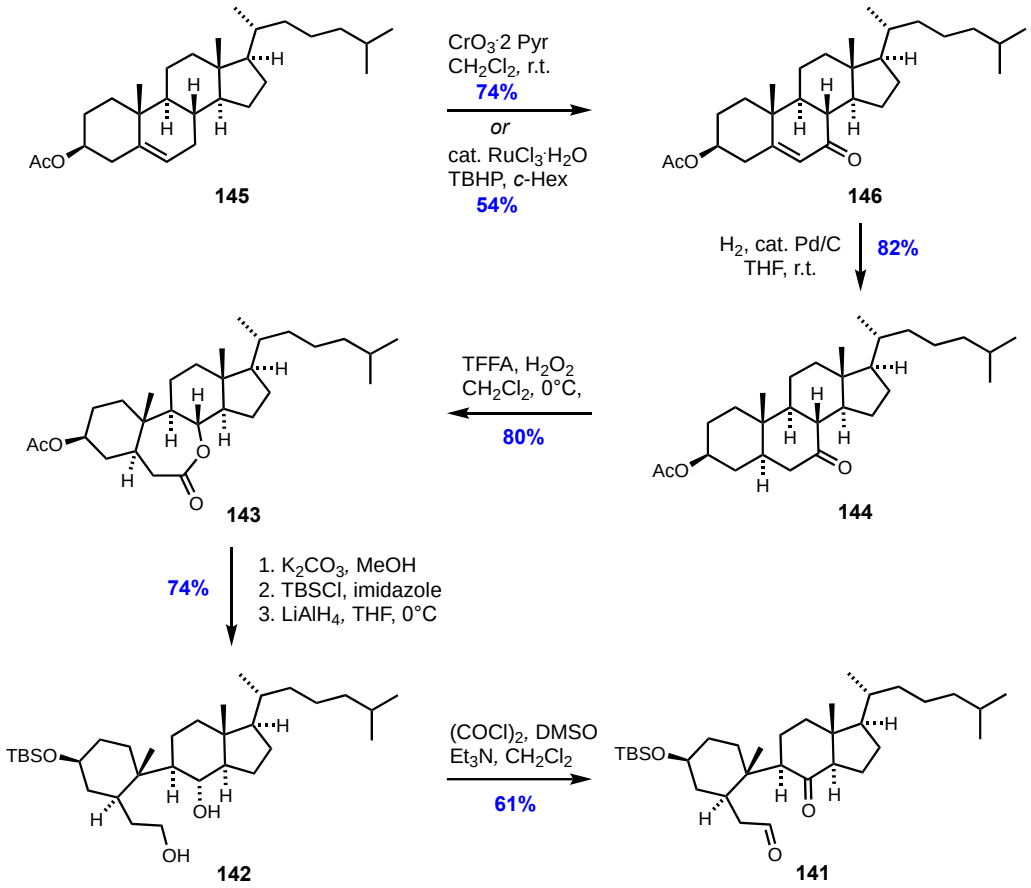
Scheme 19. Biosynthesis of spirosteroids such as spiroseoflosterol (**11**).

As already mentioned in section 1.3, it appeared feasible for the beginning to investigate the construction of the characteristic spirocyclic skeleton of spiroseoflosterol (**11**) using cholesterol-derived model compounds with a simplified (saturated) side-chain. According to the before-mentioned concept (compare Scheme 11), the idea was to generate the spiro[5.4]-decan substructure of **140** through an intramolecular aldol reaction (Scheme 20). The required keto-aldehyde **141** in turn would be obtained (via oxidation of diol **142)** from lactone **143**, which results from Baeyer-Villiger oxidation of ketone **144**. As a protecting group for the C(3) OH function, which is stable to both oxidation and reduction, a *tert*-butyldimethylsilyl (TBS) protecting group[78] was selected. Starting from cholesteryl acetate (**145**) the ketone **144** should be accessible via allylic oxidation and double bond reduction.



Scheme 20. Retrosynthetic analysis of the model spiro-steroid **140**.

Following this plan, cholesteryl acetate, which is commercially readily available, was subjected to an allylic oxidation using the chromium(VI)oxide-pyridine reagent in methylene chloride as a solvent[79] to give the enone **146** in 74% yield. As an environmentally more attractive protocol, the oxidation could also be performed with tert-butylhydroperoxide (TBHP) in the presence of catalytic amounts (0.7 mol%) of ruthenium chloride,[80] however, the yield was only 54% in this case. The subsequent catalytic hydrogenation of enone **146** to ketone **144** proceeded cleanly in THF as a solvent using 10 mol% of palladium on carbon as a catalyst. Reaction of ketone **144** with trifluoroperacetic acid (prepared in situ from the trifluoroacetic anhydride and H_2O_2) [81] afforded lactone **143** as the Baeyer-Villiger oxidation product with the expected regioselectivity. To avoid chemoselectivity problems in the upcoming oxidation step, the protecting group of the OH functionality at C(3) was changed at this point by methanolysis of the acetate function (K_2CO_3, MeOH) and reprotection of the liberated OH group as an TBS ether (TBSCl, imidazole). Subsequent lactone reduction using lithium aluminum hydride in THF afforded the diol **142**, which was finally oxidized under Swern conditions (oxalyl chloride, DMSO, Et_3N) to afford the target keto-aldehyde **141**.



Scheme 21. Synthesis of the keto-aldehyde **141** from cholesteryl acetate.

Notably, a by-product was obtained in one of the Swern oxidation experiments, which crystallized and was identified as the lactol **147** by X-ray crystallographic analysis (Figure 28).

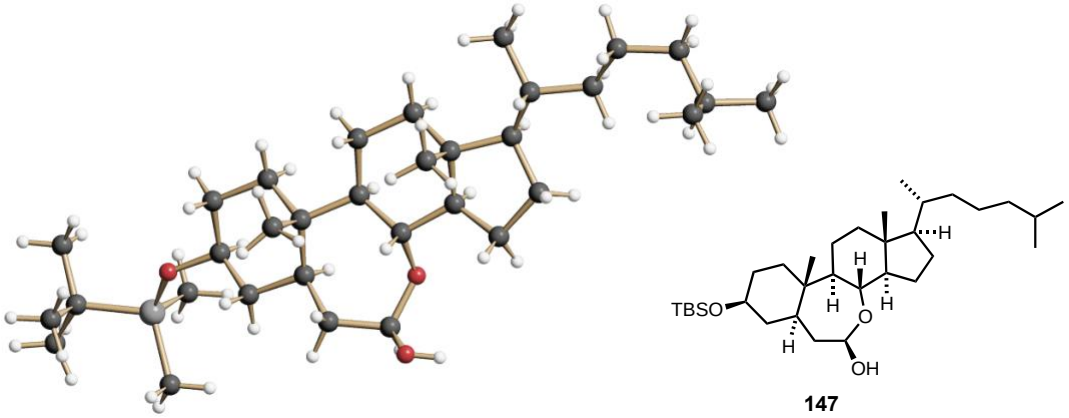
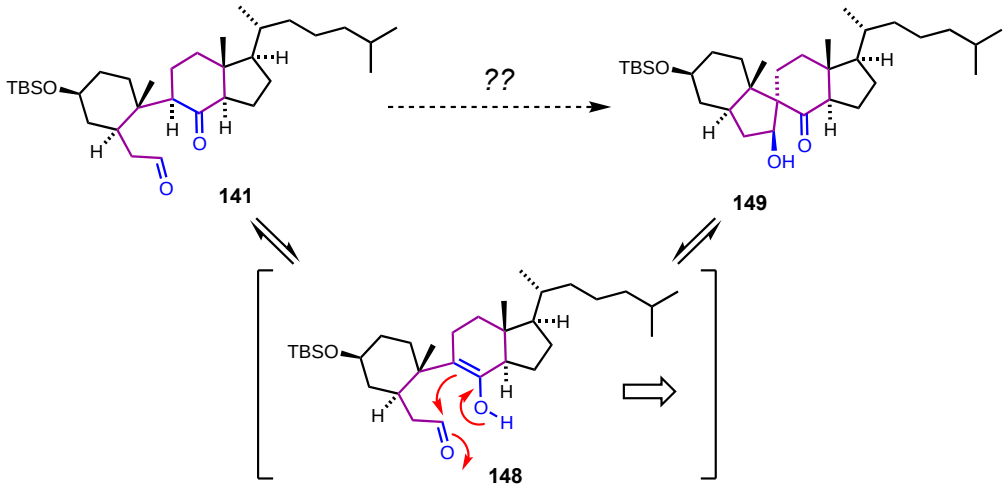


Figure 28. Structure of lactol **147** in the crystalline state.

Having successfully synthesized the keto-aldehyde **141**, several experiments were performed aiming at its conversion to the spiroseoflosterol-related spiro-steroid **149** (Scheme 22). The hope was, that under basic (equilibrium) conditions, the enol **148** would be generated, which could then react in an intramolecular aldol addition to give **149**.

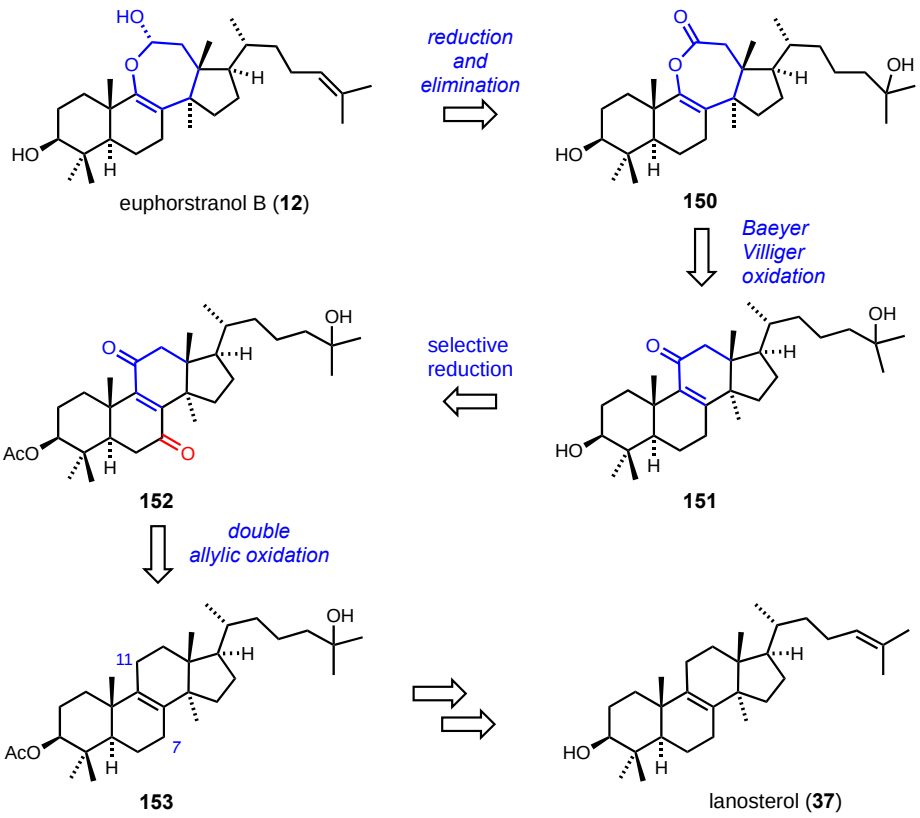


Scheme 22. Attempted spirocyclization of keto-aldehyde **141**.

However, under various conditions, such as K_2CO_3 in methanol (r.t. 16 h) or LiHMDS, THF (-78°C to r.t.), the desired transformation could not be realized. Of course, it cannot be excluded that the devised aldol cyclization to set-up the spirocyclic substructure of spiroseoflosterol (**11**) or spiroconyone A (**103**) can be achieved under special conditions. However, in the time frame of the present doctoral thesis, this task could not be achieved.

1.4.3 Studies towards the Synthesis of Euphorstranol B

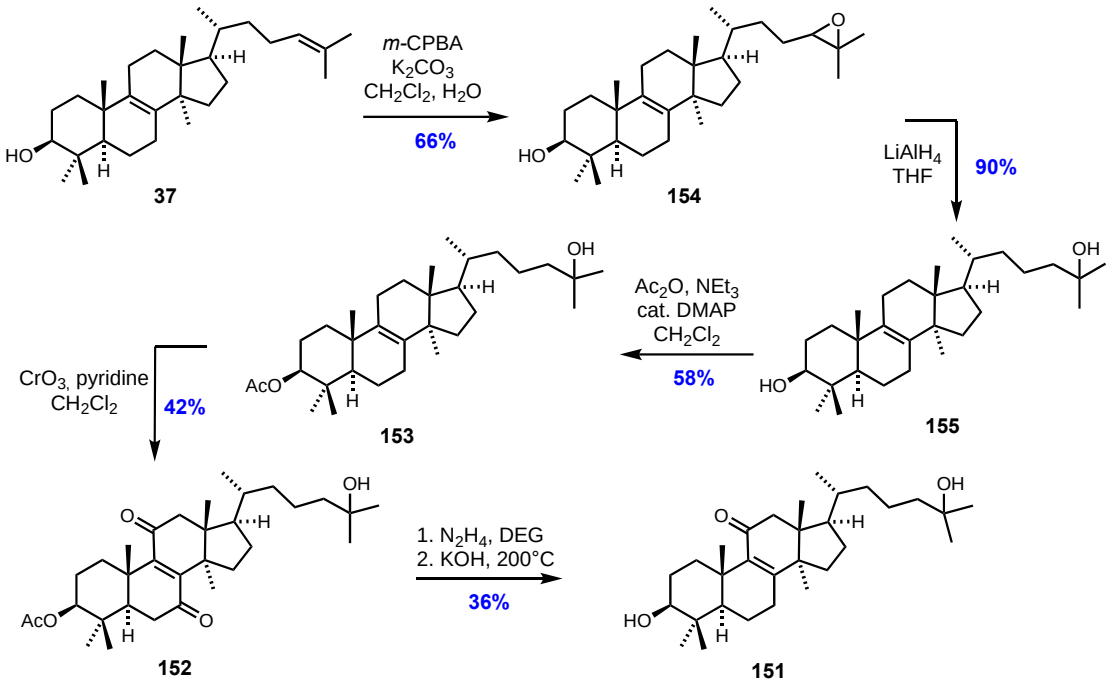
As already outlined in section 1.3, a third challenge was the question of whether the natural product euphorstranol B (**12**) could possibly be synthesized from lanosterol **37**. Key element of the strategy is the Baeyer-Villiger oxidation of an 11-oxo-lanosterol derivative (**151**) to an enol-lactone **150**. The introduction of the 11-oxo function could be achieved by double allylic oxidation of **153** to give **152**, followed by the selective removal of the more accessible 7-keto function in a Wolff-Kishner-type fashion. Notably, this strategy would resemble the proposed biosynthesis of euphorstranol B (**12**) from lanosterol (**37**).[16] However, to avoid problems (side reactions) associated with the presence of a double bond in the side chain, it seemed advisable to mask this double bond in the form of a tertiary alcohol at C(25)



Scheme 23. Refined retrosynthetic analysis for euphorstranol B.

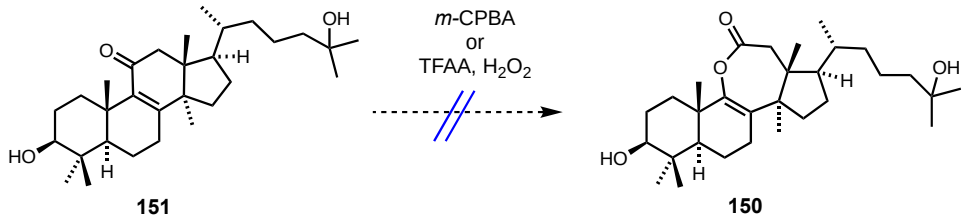
According to this plan, the investigation started with the epoxidation of lanosterol (**37**) with *m*-CPBA in the presence of K_2CO_3.[82] The resulting epoxide **154**, obtained as a mixture of diastereomers, was reduced with $LiAlH_4$ to give the tertiary alcohol **155** in high yield (Scheme 24). After protection of the C(3) hydroxy function by DMAP-assisted acetylation, the resulting

acetate **153** was reacted with chromium(VI) oxide in the presence of pyridine to give the 7,11-dioxo derivative **152**, Finally, reaction **152** with hydrazine (at the less hindered C(7) carbonyl group) and subsequent heating with KOH in diethylene glycol (DEG) afforded the Wolff-Kishner product **151** according to the spectroscopic data.



Scheme 24. Synthesis of **151** as an advanced intermediate for euphorstranol B.

However, several attempts to convert **151** by Baeyer-Villiger oxidation into the enol-lactone **150** under various common conditions (for instance using *m*-CPBA or trifluoroperacetic acid) did not lead to any success Scheme 25).



Scheme 25. Unsuccessful attempts to synthesize **150** by Baeyer-Villiger oxidation.

Only upon treatment of **151** with *m*-CPBA in the presence of trifluoromethylsulfonic acid, the formation of a new product (**156**) was observed (Scheme 26). However, as proven by X-ray crystal structure analysis (Figure **29**), this product did not display the desired lactone

substructure. Instead, a structural change in the sidechain had occurred, involving an oxidative 1,2-shift of the oxy-functionality from the C(25) to the C(24) position.

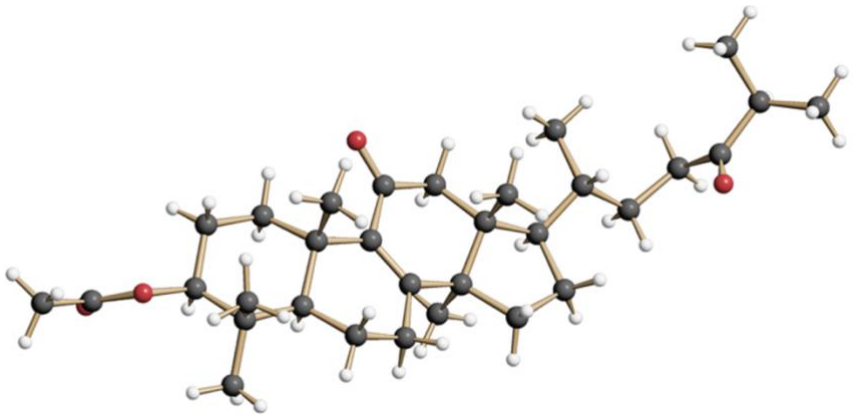
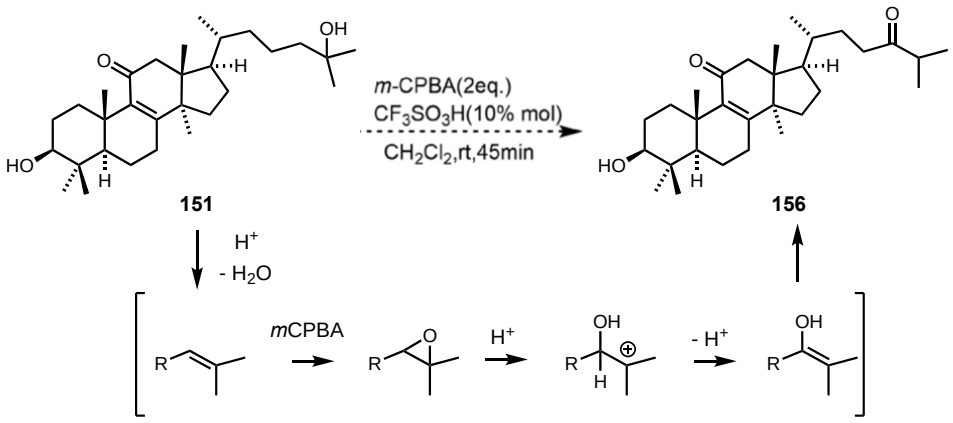


Figure 28. Structure of ketone **156** in the crystalline state.

The formation of the C(24) keto derivative **156** might explained by the mechanism shown in Scheme 26. It involves an acid-mediated E_1-type elimination to yield a double bond which is then epoxidized by the reagent (*m*-CPBA). The formed epoxide is finally converted to the isomeric ketone via formation of the more stable (tertiary) cation followed by proton elimination and tautomerization.



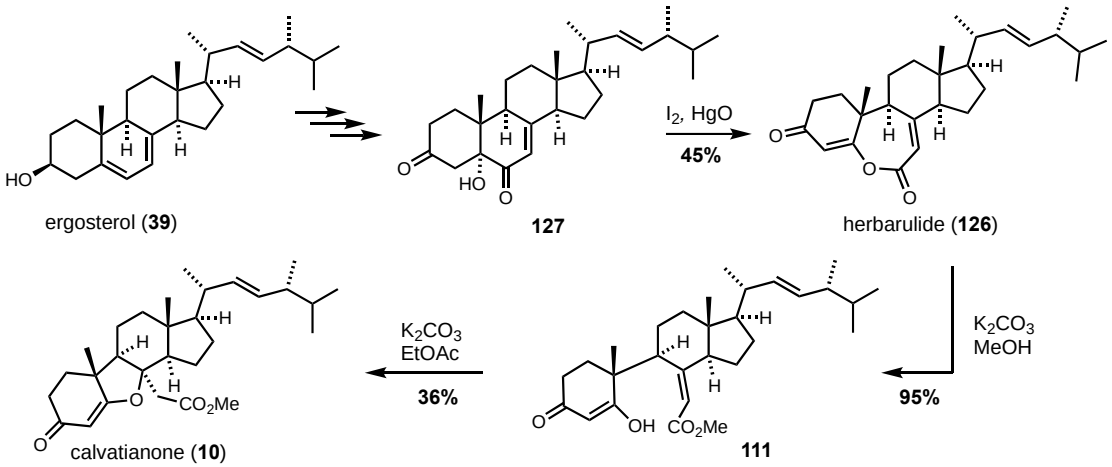
Scheme 26. Formation of ketone **156** as an undesired product.

Obviously, the devised strategy for the semi-synthesis of euphorstranol B (**12**) did not work out, and no further experiments toward this task were performed in the course of the present thesis.

1.5 Summary and Outlook

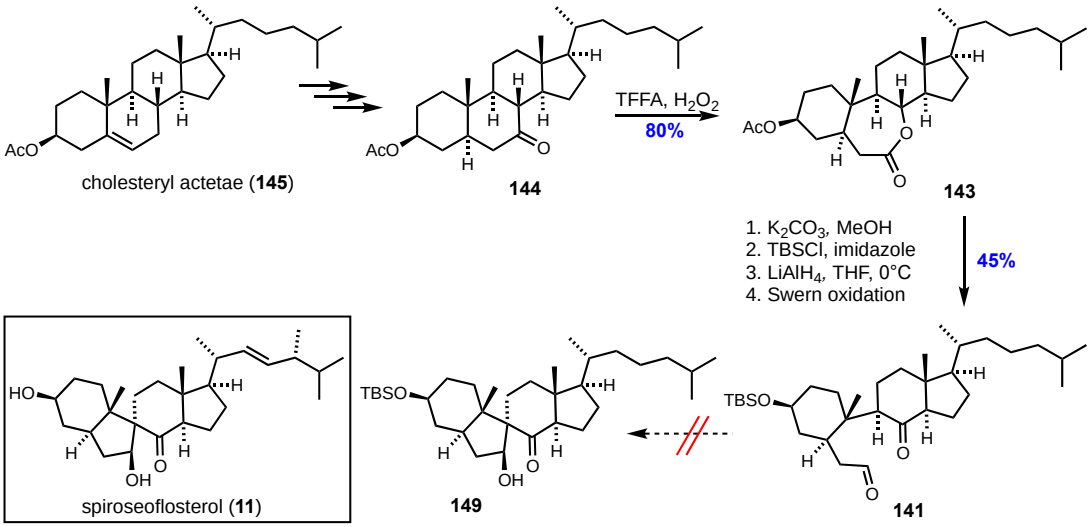
The general motivation for this thesis was the search for semi-synthetic approaches towards unusual steroids with rearranged or oxacyclic B or C rings, which are of potential pharmacological interest.

In a first part, a synthesis of the novel steroid calvatianone (**10**), which displays a unique contracted tetrahydrofuran B-ring, was successfully elaborated. Starting from ergosterol, herbarulide (**126**) was prepared in analogy to the route of Heretsch[71] via radical Bayer-Villiger-type oxidation of intermediate **127**. Methanolysis of the lactone moiety then afforded the enoate **111**, from which the target compound was obtained by oxa-Michael addition.



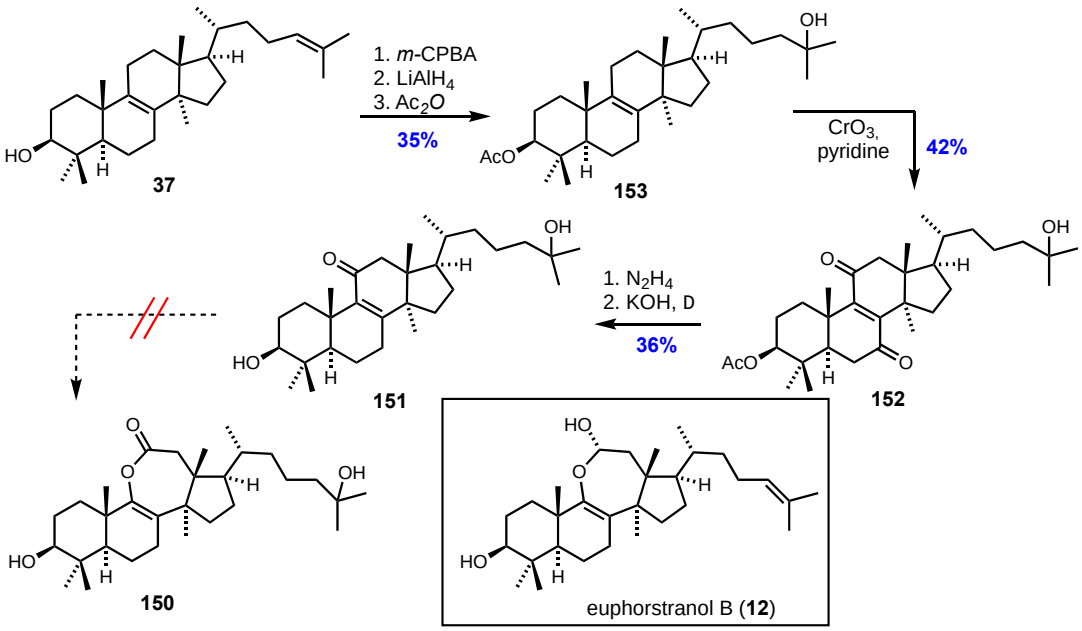
Scheme 27. Synthesis of calvatianone (**10**).

In a second part of this thesis, a possible approach towards the synthesis of spiro-seoflosterol (**11**), a rearranged steroid displaying a unique spiro[5.4]decan substructure, was investigated using model compounds with a saturated (cholesterol-derived) side chain (Scheme 28). In this context, cholesteryl acetate (**145**) was first converted into the 7-oxo-steroid (**144**), the B ring of which was cleaved by Baeyer-Villiger oxidation. The resulting lactone (**143**) was then transformed into the keto-aldehyde **141** as the projected pre-target molecule. However, all attempts to achieve the crucial final intramolecular aldol reaction (**141** to **149**) to set-up the spirocyclic ring system failed.



Scheme 28. Synthesis of ketoaldehyde **141** as a promising precursor of the spirocyclic steroid **149**.

The third part of this thesis concerns the semi-synthesis of the natural product euphorstranol B (**12**), a rare 9,11-seco tetracyclic triterpenoid featuring a cyclic enol-hemiacetal moiety. Starting from lanosterol (**37**), the side chain double bond was first masked by epoxidation/reduction, flowed by acetylation of the C(3)-OH group. After double allylic oxidation, the selective removal of the C(7) oxo group was achieved by Wolf-Kishner reduction to give the advanced intermediate **151**. However, the key Baeyer-Villiger reaction (conversion of **151** to **150**) could not be realized.



Scheme 29. Synthesis of enone **151** as a potential precursor of euphorstranol B (**12**)

2. Experimental Section

2.1 General Experimental Information

Nuclear magnetic resonance spectroscopy (NMR): ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ or CD_3OD at ambient temperature on a Bruker Avance II 500 (500 MHz), Bruker Avance III 500 (500 MHz) or Avance II+ 600 spectrometer (600 MHz). Chemical shifts for the ^{1}H and ^{13}C NMR spectra are given in parts per million (ppm) on δ scale from an internal standard of residual tetramethylsilane (TMS, 0 ppm). Signals are reported in relative to the residual signal of the nondeuterated solvent (^{1}H: 7.26 ppm and ^{13}C: 77.0 for $CDCl_3$; ^{1}H: 3.31 ppm and ^{13}C: 49.0 ppm for CD_3OD). Abbreviations of fine structure of ^{1}H NMR spectra used: s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, td = triplet of doublets, qd = quartet of doublets, t = triplet, dt = doublet of triplets, tt = triplet of triplets, m = multiplet, br = broad. Scalar coupling constants (*J*) are given in Hertz (Hz). All ^{13}C NMR spectra (except for eurysterol A) were recorded using APT sequence with complete proton decoupling. Abbreviations of fine structure of ^{13}C NMR spectra used: s for quaternary C, d for CH, t for CH_2 and q for CH_3. The non-trivial assignments were determined by 1H,1 H-COSY, ^{1}H,^{1}H-NOESY, ^{1}H,^{13}C-HSQCed/HMQC and ^{1}H, ^{13}C-HMBC spectra.

Fourier transform infrared spectroscopy (FT-IR): Infrared (IR) spectra were obtained in 4000 – 500 cm^{-1} range using a PerkinElmer Spectrum Two FT-IR spectrometer using ATR technique. Absorption bands are reported in cm^{-1}. Abbreviations of fine structure of spectra used: w = weak, m = middle, strong, s = strong, vs = very strong, bw = broad weak.

High resolution electrospray and electron impact ionization-mass spectrometry (HRMS):

High resolution mass spectra were performed on a Thermo Scientific LTQ Orbitrap XL spectrometer (ESI). The spray voltage (3.4 kV), capillary voltage (3.0 V), tube lens voltage (3.0 V) and capillary temperature of 275 °C were applied as ESI conditions. To generate a stable electrospray sheath gas and sweep gas were used (Nitrogen 5.0, Linde, ≥ 99.999%).

Specific optical rotation ($[\alpha]_{T\lambda}$): Optical rotation values were determined with an Anton Paar MCP 200 Polarimeter. The measurements were performered at ambient temperature in chloroform or methanol. The concentration c of the measured solution (g/100 mL) are preceded in squared brackets with optical rotation values. $[\alpha]_D$ represents the Fraunhofer d-lines at 589 nm.

Thin-layer chromatography (TLC): Reactions were monitored by means of TLC on silica gel 60 F254 (layer thickness 0.25 mm, Merck) and visualized with a solution of cerium ammonium molybdate (CAM) (2 g of molybdophosphoric acid, 1 g of cerium(IV) sulfate tetrahydrate in 10 mL of sulfuric acid and 90 mL of water) and subsequent heating.

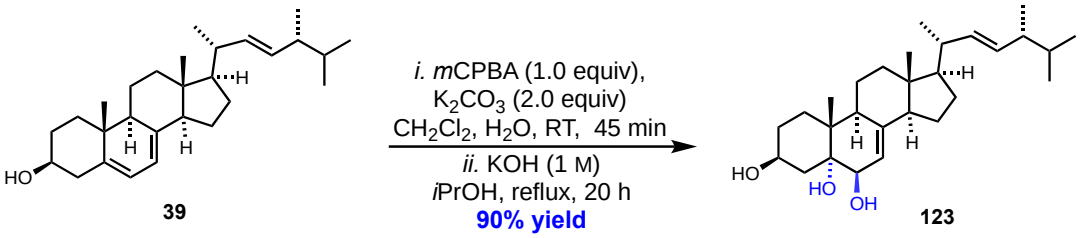
Column chromatography: Flash column chromatography was performed on silica gel 60 Å (230-400 mesh, Acros).

Melting-points: Melting points were determined with a Büchi Melting Point B-545 apparatus in open capillary tubes and are uncorrected.

Solvents and Reagents: All sensitive reactions were carried out under argon atmosphere in flame-dried glassware unless otherwise noted. Dichloromethane was distilled from calcium hydride under argon atmosphere. Diethyl ether, tetrahydrofuran and toluene were freshly distilled under argon atmosphere from sodium and benzophenone. All reagents were used as received from commercial sources without further purification unless otherwise noted. The solvents were distilled before use.

2.2 Experimental Procedures and Substance Data

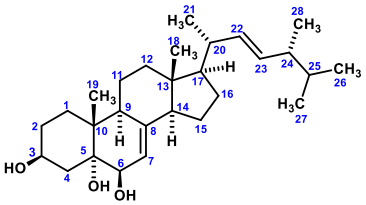
2.2.1 Synthesis of (22*E*)-Ergosta-7,22-diene-3*β*,5*α*,6*β*-triol (Cerevisterol) (123)



To a solution of ergosterol (**39**) (5.0 g, 12.6 mmol, 1.0 equiv) in CH_2Cl_2 (125 mL) was added a solution of K_2CO_3 (3.5 g, 25.1 mmol, 2.0 equiv) in 150 mL of H_2O. In a separate flask, 1.8 g (70%, 7.8 mmol, 1.0 equiv) of *m*-CPBA (70%) was suspended in CH_2Cl_2 (20 mL), and the suspension was then added dropwise to the vigorously stirred biphasic mixture containing **39** over a period of 5 min. After vigorously stirring the mixture for further 45 min at ambient temperature, the layers were separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with $NaHCO_3$ (sat. aq.) and brine, dried over $MgSO_4$, and the solvent was removed under reduced pressure to give a white solid, which was used in the next step without any purification.

The white solid (epoxide) was dissolved in 50 mL of *i*PrOH and to this solution aqueous KOH (1 M, 50 mL) was added before the mixture was refluxed for 20 h. The reaction mixture was allowed to cool to ambient temperature and neutralized with HCl (1 M). The aqueous layer was extracted with CH_2Cl_2/*i*PrOH (4:1) and the combined organic layers were washed with brine, dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude material was finally purified by silica gel column chromatography (EtOAc/MeOH, 3:1) to give pure **123** (4.48 g, 10.41 mmol, 90%) as a white solid.

Formula:	$C_{28}H_{46}O_3$
Molecular Weight:	430.67 g/mol
m.p.:	246 °C – 248 °C.
TLC:	R_f = 0.15 (EtOAc), Cer reagent.



^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 5.20 (qd, *J* = 15.3, 8.6 Hz, 2H, H-22, H-23), 5.08 – 5.07 (m, 1H, H-7), 4.50 (d, *J* = 5.5 Hz, 1H, C6-OH), 4.23 (d, *J* = 5.6 Hz, 1H, C5-OH), 3.80 – 3.71 (m, 1H, H-3), 3.60 (s, 1H, C3-OH), 3.38 (d, *J* = 5.7 Hz, 1H, H-6), 2.03 – 1.78 (m, 6H, H-4, H-9, H-12, H-14, H-20, H-24), 1.69 – 1.58 (m, 2H, H-2, H-16), 1.51 – 1.38 (m, 6H, H-4, H-11, H-15, H-25), 1.35 – 1.20 (m, 6H, H-1, H-12, H-16, H-17), 0.99 (d, *J* = 6.6 Hz, 3H, H-27), 0.91 – 0.88 (m, 3H, H-21, H-26), 0.80 (t, *J* = 6.6 Hz, 3H, H-19, H-24), 0.54 (s, 3H, H-18).

^{13}C NMR (125 MHz, DMSO-d_6): δ [ppm] = 139.7 (C-8), 135.4 (C-22), 131.4 (C-23), 119.5 (C-7), 74.4 (C-5), 72.1 (C-6), 66.0 (C-3), 55.3 (C-17), 54.2 (C-14), 43.0 (C-13), 42.3 (C-9), 42.0 (C-24), 40.1 (C-4), 40.0 (C-20), 39.0 (C-12), 36.6 (C-10), 32.5 (C-25), 32.5 (C-1), 31.2 (C-2), 27.7 (C-16), 22.6 (C-15), 21.4 (C-11), 21.0 (C-27), 19.8 (C-26), 19.5 (C-21), 17.7 (C-28), 17.3 (C-19), 12.1 (C-18).

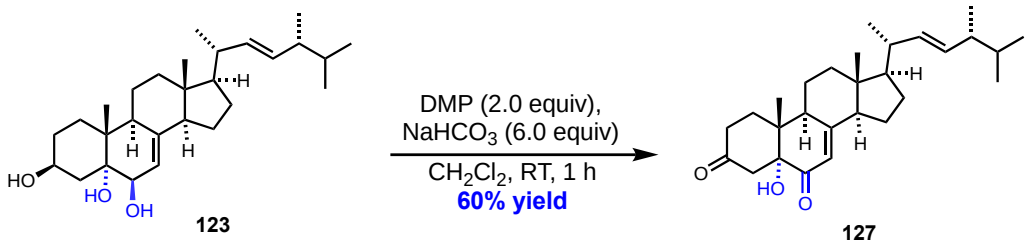
FT-IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3390 (br w), 2955 (m), 2870 (m), 1457 (m), 1371 (m), 1033 (m), 968 (m), 939 (w), 866 (w).

HR-MS: (EI, 70 eV) = *m/z* calcd. For: $C_{28}H_{44}O_2^+$ $[M\text{-}H_2O]^+$ 412.33358 u, found: 412.33333 u.

$[\alpha]_\lambda^T$: (c = 0.50 g/100 mL, $CHCl_3$) = $[\alpha]_{436}^{20}$: +270.5°, $[\alpha]_{546}^{20}$: +64.0°, $[\alpha]_{579}^{20}$: +48.2°, $[\alpha]_{D}^{20}$: +44.6°.

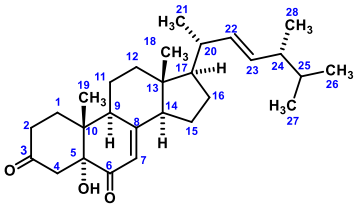
The NMR data are consistent with those reported in the literature.[71]

2.2.2 Synthesis of (22*E*)-5*α*-Hydroxy-ergosta-7,22-diene-3,6-dione (127)



To a solution of triol **123** (2.2 g, 5.1 mmol, 1.0 equiv) in CH_2Cl_2 (100 mL) were added 2.6 g (30.8 mmol, 6.0 equiv) of $NaHCO_3$ and 5 g (11.8 mmol, 2.0 equiv) of Dess-Martin periodinane

(DMP), and the mixture was stirred at ambient temperature for 1 h to give a yellow suspension. After diluting the reaction mixture with CH_2Cl_2 (150 mL), $Na_2S_2O_3$ (sat. aq., 150 mL) was added and stirring continued for 30 mins. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were washed with $NaHCO_3$ (sat. aq.), and brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (*c*-Hex/EtOAc, 3:1) to give **127** (1.3 g, 3.05 mmol, 60%) as a colorless solid.



Formula: $C_{28}H_{42}O_3$

Molecular Weight: 426.64 g/mol

m.p.: 250 °C – 252 °C (Lit.[71]: 230 – 232 °C).

TLC: R_f = 0.48 (*c*-Hex/EtOAc 1:1), Cer reagent.

^{1}H NMR (500 MHz, pyridine-d_5): δ [ppm] = 8.10 (s, 1H, H-7), 5.98 (s, 1H, C5-OH), 5.30 (dd, *J* = 15.3, 7.7 Hz, 1H, H-23), 5.21 (dd, *J* = 15.3, 8.5 Hz, 1H, H-22), 3.25 – 3.18 (m, 2H, H-4), 2.98 (ddd, *J* = 12.1, 7.1, 2.6 Hz, 1H, H-9), 2.61 – 2.43 (m, 3H, H-1, H-2), 2.08 – 2.03 (m, 3H, H-12, H-14, H-20), 1.93 – 1.87 (m, 2H, H-1, H-9), 1.76 – 1.63 (m, 3H, H-1, H-11, H-15), 1.54 – 1.47 (m, 3H, H-15, H-25), 1.42 – 1.29 (m, 3H, H-12, H-16, H-17), 1.25 (s, 3H, H-19), 1.08 (d, *J* = 6.6 Hz, 3H, H-21), 0.99 (d, *J* = 6.8 Hz, 3H, H-28), 0.90 (dd, *J* = 6.8, 4.3 Hz, 6H, H-26, H-27), 0.64 (s, 3H, H-18).

^{13}C NMR (125 MHz, pyridine-d_5): δ [ppm] = 210.8 (C-3), 199.0 (C-6), 165.4 (C-8), 136.3 (C-22), 133.0 (C-23), 120.8 (C-7), 81.1 (C-5), 56.6 (C-17), 56.4 (C-14), 45.8 (C-4), 45.2 (C-13), 44.7 (C-24), 43.6 (C-9), 42.1 (C-10), 41.1 (C-20), 39.5 (C-12), 38.4 (C-2), 33.9 (C-25), 33.0 (C-1), 28.8 (C-16), 23.3 (C-15), 22.8 (C-11), 21.9 (C-21), 20.7 (C-27), 20.4 (C-26), 18.4 (C-28), 16.1 (C-19), 13.3 (C-18).

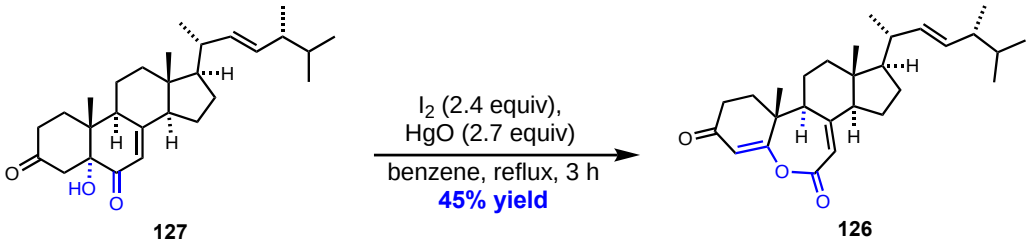
FT-IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3335 (br w), 2954 (s), 2870 (w), 1712 (s), 1671 (s), 1458 (w), 1369 (w), 1233 (m), 1160 (m), 968 (m), 872 (m), 522 (m).

HR-MS: (ESI, 70 eV) = *m/z* calcd. For: $C_{28}H_{42}O_3^+$ $[M+H]^+$ 426.31285 u, found: 426.31252 u.

$[\alpha]_\lambda^T$: (c = 0.50 g/100 mL, $CHCl_3$) = $[\alpha]_{436}^{20}$: +535.8°, $[\alpha]_{546}^{20}$: +260.5°, $[\alpha]_{579}^{20}$: +221.1°, $[\alpha]_{D}^{20}$: +210.4°.

The NMR data are consistent with those reported in the literature.[71]

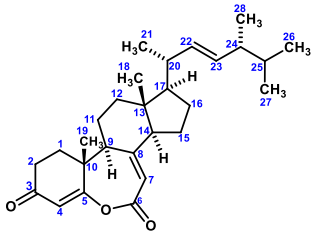
2.2.3 Synthesis of (22*E*)-5,6-Epoxy-5,9-seco-ergosta-4,7,22-triene-3,6-dione (herbarulide) (126)



To a solution of 1.2 g (2.8 mmol, 1.0 equiv) of **127** in benzene (300 mL) was bubbled argon for 10 mins, followed by the addition of I_2 (1.7 g, 6.7 mmol, 2.4 equiv) and 1.6 g (7.6 mmol, 2.7 equiv) of HgO. The reaction mixture was then heated to reflux for 3 h. After cooling the mixture to ambient temperature, the crude material was filtered through a short pad of silica and rinsed with EtOAc (100 mL). The organic layer was washed with $Na_2S_2O_3$ (sat. aq., 300 mL), brine (200 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue purified by silica gel chromatography (*c*-Hex/EtOAc, 5:1→3:1) to give herbarulide (**126**) (540 mg, 1.27 mmol, 45%) as a light-yellow solid.

Formula: $C_{28}H_{40}O_3$

Molecular Weight: 424.63 g/mol



m.p.: 158 °C – 160 °C.

TLC: R_f = 0.65 (EtOAc), Cerium reagent.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 5.74 (s, 1H, H-4), 5.72 (s, 1H, H-7), 5.24 (dd, *J* = 15.3, 7.8 Hz, 1H, H-23), 5.14 (dd, *J* = 15.3, 8.5 Hz, 1H, H-22), 2.55 – 2.40 (m, 3H, H-2, H-9), 2.21 – 1.94 (m, 5H, H-1, H-12, H-14, H-20), 1.88 – 1.58 (m, 5H, H-11, H-16, H-24), 1.51 – 1.33 (m,

5H, H-12, H-15, H-17, H-25), 1.23 (s, 3H, H-19), 1.02 (d, *J* = 6.6 Hz, 3H, H-21), 0.91 (d, *J* = 6.8 Hz, 3H, H-28), 0.82 (dd, *J* = 8.4, 6.8 Hz, 6H, H-26, H-27), 0.63 (s, 3H, H-18).

^{13}C NMR (125 MHz, $CDCl_3$): δ [ppm] = 199.1 (C-3), 174.0 (C-5), 162.6 (C-6), 159.6 (C-8), 134.8 (C-22), 133.0 (C-23), 114.8 (C-7), 113.4 (C-4), 58.2 (C-14), 56.5 (C-17), 47.3 (C-9), 47.1 (C-13), 43.0 (C-24), 40.5 (C-10), 40.4 (C-20), 39.3 (C-12), 34.1 (C-1), 33.3 (C-2), 33.2 (C-25), 27.8 (C-16), 25.5 (C-11), 22.7 (C-15), 21.2 (C-21), 20.1 (C-26), 20.1 (C-19), 19.8 (C-27), 17.7 (C-28), 12.6 (C-18).

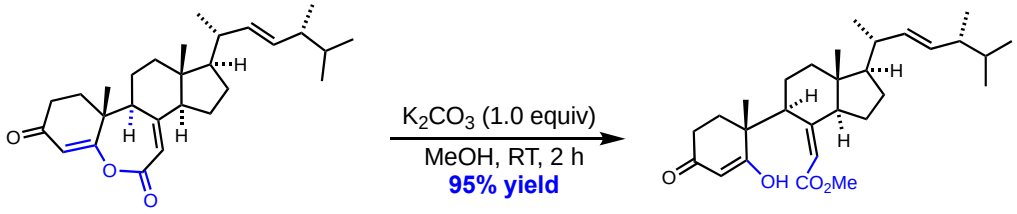
FT-IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2955 (s), 2870 (m), 1714 (s), 1672 (s), 1622 (m), 1458 (m), 1370 (m), 1234 (m), 1138 (m), 968 (m), 873 (m), 671 (w), 521 (w).

HR-MS: (ESI, 70 eV) = *m/z* calcd. For: $C_{28}H_{40}O_3^+$ $[M+H]^+$ 424.29720 u, found: 424.29691 u.

$[\alpha]_\lambda^T$: (c = 0.36 g/100 mL, $CHCl_3$) = $[\alpha]_{436}^{20}$: +471.6°, $[\alpha]_{546}^{20}$: +230.6°, $[\alpha]_{579}^{20}$: +196.4°, $[\alpha]_D^{20}$: +186.0°.

The NMR data are consistent with those reported in the literature.[71]

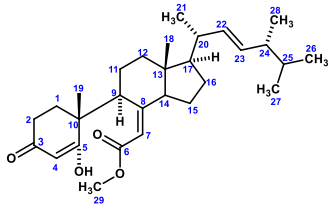
2.2.4 Synthesis of (7*Z*,22*E*)-3,5-Dioxo-5,6-seco-ergosta-7,22-diene-6-acid methyl ester (111)



126 111

To a solution of herbarulide **126** (500 mg, 1.18 mmol, 1.0 equiv) in MeOH (60 mL) was added 1.46 g (10.6 mmol, 9.0 equiv) of K_2CO_3 and the reaction mixture was stirred at ambient temperature. After 2 h, the mixture was quenched with H_2O and extracted three times with EtOAc. The combined organic layers were washed with $NaHCO_3$ (sat. aq.), brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by

silica gel chromatography (*c*-Hex/EtOAc, 5:1→2:1) to give **111** (370 mg, 0.80 mmol, 95%) as a colorless oil.



Formula: $C_{29}H_{44}O_4$

Molecular Weight: 456.67 g/mol

TLC: R_f = 0.21 (*c*-Hex/EtOAc 2:1), Cerium reagent.

^{1}H NMR (500 MHz, CDCl3): δ [ppm] =δ 5.51 (s, 1H,H-4), 5.20 (dd, *J* = 15.3, 7.8 Hz, 1H, H-23), 5.16 (dd, *J* = 15.3, 8.5 Hz, 1H, H-22), 3.54 (s, 3H, -OCH_3) , 3.44 (d, J = 10.0 Hz, 1H, H-2β), 3.23 (dd, *J* = 15.0, 5.0 Hz, 1H, H-2α), 2.71 – 2.62 (m, 1H, H-1β), 2.62 – 2.54 (td, *J* = 15.0, 5.0 Hz 1H, H-1α), 2.48-2.26 (m, 2H, H-11β, H-15β), 2.21 (dd, *J* = 10.0 , 5.0 Hz, 1H, H-9), 2.13 (d, J = 15.0 Hz, 1H, H-7), 2.08-2.00 (m, 2H, H-16β, H-20), 1.87-1.81 (m, 1H, H-24), 1.77-1.70(m, 1H, H-15α), 1.60-1.51 (m, 2H, H-12β, H-11α), 1.49-1.43 (m, 1H, H-25), 1.42 (t, *J* = 15.0, 10.0, 5.0Hz, 1H, H-14), 1.40 – 1.32 (m, 4H, H-17, H-19, H-12α, H-16α), 1.01 (d, J = 10.0 Hz, 3H, H-21), 0.91 (d, J = 10.0 Hz, 3H, H-28), 0.84-0.79 (m, 6H, H-26, H-27), 0.68 (s, 3H, H-18).

^{13}C NMR (125 MHz, CDCl3): δ [ppm] = 205.5 (C-3), 167.1 (C-6), 157.2 (C-5), 135.2 (C-22), 132.5 (C-23), 111.7 (C-4), 92.3 (C-8), 61.7 (C-9), 57.4 (C-7), 56.8 (C-19), 55.7 (C-2), 51.6 (C, -OCH3), 48.8 (C-10), 42.9 (C-24), 41.8 (C-1), 40.5 (C-20), 36.9 (C-1), 33.2 (C-25), 32.0 (C-11), 27.9 (C-16), 27.7 (C-15), 27.0 (C-13), 24.8 (C-17), 23.3 (C-12), 21.1(C-21), 20.7 (C-14), 20.0 (C-26), 19.8 (C-27) 17.8 (C-28), 12.6 (C-18).

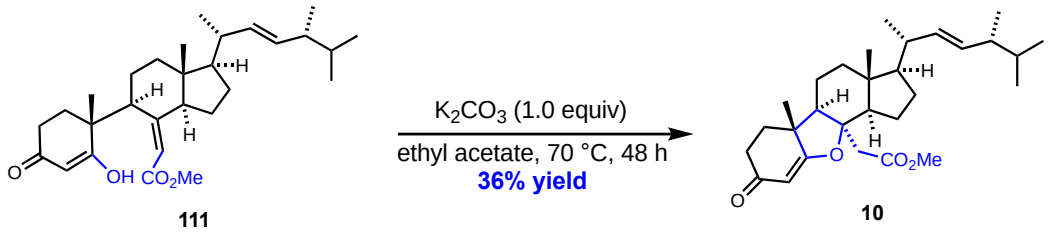
FT-IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2956 (s), 2872 (m), 1720 (s), 1627 (m), 1456 (w), 1381 (w), 1197 (s), 1084 (w), 1046 (m), 973 (w), 913 (w), 878 (w), 732 (m).

HR-MS: (ESI, 70 eV) = *m/z* calcd. For: $C_{29}H_{44}O_4Na$ $[M+Na]^+$ 479.31810 u, found: 479.31338 u.

$[\alpha]_\lambda^T$: (c = 0.50 g/100 mL, $CHCl_3$) = $[\alpha]_{436}^{20}$: +221.7°, $[\alpha]_{546}^{20}$: +110.7°, $[\alpha]_{579}^{20}$: +94.6°, $[\alpha]_{D}^{20}$: +89.2°.

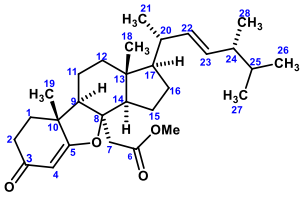
XRD: A single crystalline sample was obtained by recrystallization from $CHCl_3$/*n*-Hex (1:10).

2.2.5 Synthesis of Calvatianone (10)



111 **10**

To a stirred solution of 350 mg (0.76 mmol, 1.0 equiv) of **111** in EtOAc (50 mL) were added 525 mg (3.8 mmol, 5.0 equiv) of K_2CO_3 and the mixture was stirred at 70 °C for 48 h. The reaction mixture was quenched by addition of brine and extracted with EtOAc. The combined organic layers were washed with $NaHCO_3$ (sat. aq.), brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (Ag-doped; *c*-Hex/EtOAc, 15:1→2:1) to give calvatianone (**10**) (120 mg, 0.27 mmol, 36%) as a colorless oil.



Formula: $C_{29}H_{44}O_4$

Molecular Weight: 456.67 g/mol

TLC: R_f = 0.20 (*c*-Hex/EtOAc 2:1), Cerium reagent.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 5.41 (s, 1H, H-4), 5.24 (dd, *J* = 15.3, 7.8 Hz, 1H, H-23), 5.13 (dd, *J* = 15.6, 8.3 Hz, 1H, H-22), 3.66 (s, 1H, OCH_3), 2.72 (d, *J* = 14.9 Hz, 1H, H-7β), 2.58 (ddd, *J* = 18.6, 12.7, 6.0 Hz, 1H, H-2β), 2.45 (d, *J* = 14.7 Hz, 1H, H-7α), 2.41 (ddd, *J* = 18.7, 5.5, 1.0 Hz,1H, H-2α), 2.45 – 2.37 (m, 1H, H-9), 2.11 (ddd, *J* = 13.0, 6.0, 1.0 Hz, 1H, H-1α), 2.04 – 1.99 (m, 1H, H-20), 1.94 – 1.46 (m, 11H, H-1, H-11, H-12, H-14, H-16, H-17, H-24, H-25), 1.44 (s, 3H, H-19), 1.32 – 1.26 (m, 2H, H-15), 1.0 (d, *J* = 6.5 Hz, 3H, H-21), 0.92 (d, *J* = 6.5 Hz, 3H, H-28), 0.87 (s, 3H, H-18), 0.84 (d, *J* = 7.0 Hz, 3H, H-27), 0.82 (d, *J* = 7.0 Hz, 3H, H-26).

^{13}C NMR (125 MHz, $CDCl_3$): δ [ppm] = 199.1 (C-3), 186.3 (C-5), 170.7 (C-6), 135.0 (C-22), 132.6 (C-23), 104.1 (C-4), 92.3 (C-8), 57.4 (C-17), 49.7 (C-14), 45.5 (C-9), 45.7 (C-10), 45.1 (C-7), 42.9 (C-24), 40.2 (C-20), 37.5 (C-1), 35.3 (C-12), 34.1 (C-2), 33.2 (C-25), 27.7 (C-16), 27.0 (C-15), 21.6 (C-19), 20.8 (C-21), 20.7 (C-11), 20.1 (C-27), 19.8 (C-26), 17.7 (C-28), 16.4 (C-18).

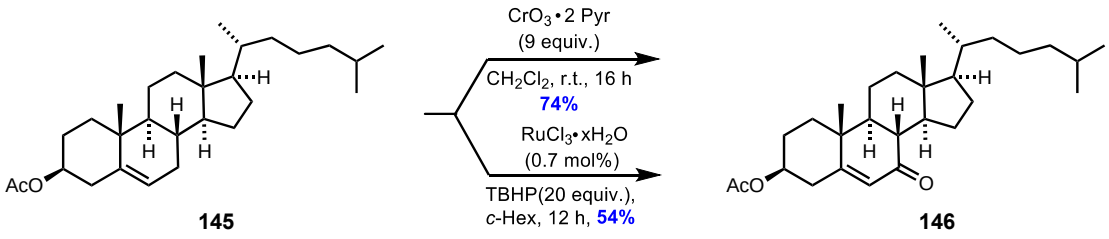
FT-IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3263 (br w), 2955 (m), 2871 (m), 1732 (m), 1626 (s), 1440 (m), 1342 (m), 1188 (s), 971 (m), 933 (w), 884 (m), 665 (w), 435 (w).

HR-MS: (ESI, 70 eV) = *m/z* calcd. For: $C_{29}H_{45}O_4$ $[M+H]^+$ 457.33123 u, found: 457.33154 u.

$[a]_\lambda^T$: (c = 0.47 g/100 mL, $CHCl_3$) = $[\alpha]_{436}^{20}$: +1103.3°, $[\alpha]_{546}^{20}$: +437.5°, $[\alpha]_{579}^{20}$: +360.0°, $[\alpha]_{D}^{20}$: +338.8°.

The analytical data are consistent with those reported for the natural product.[14]

2.2.6 Synthesis of 7-Oxo-cholesteryl acetate (146)



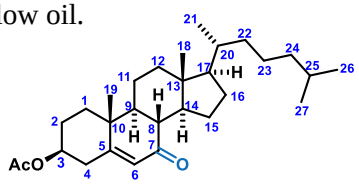
Procedure A:

To a rapidly stirred solution of pyridine (6.77 mL, 84 mmol, 18.0 equiv) in anhydrous CH_2Cl_2 (250 mL) was added under an atmosphere of dry argon 4.2 g (42 mmol, 9 equiv) of CrO_3. The red solution was stirred for 20 min at ambient temperature before a solution of 2.0 g (4.67 mmol, 1.0 equiv) of cholesteryl acetate (**145)** in CH_2Cl_2 (20 mL) was added in one portion. Stirring was continued at ambient temperature for 17 h. As TLC control indicated incomplete conversion after 18 h, an additional portion of CrO_3·2Pyr complex (prepared from 2.1 g (21 mmol, 4.5 equiv) of CrO_3 and 2.28 mL (28 mmol) of pyridine in 120 mL CH_2Cl_2) was added

to the reaction mixture and stirring was continued for further 7 h. The mixture was diluted with 500 mL of MTBE and washed with $NaHCO_3$ (sat. aq.), HCl (1 M, aq.), $NaHCO_3$ (sat. aq.) and brine. The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue (brown oil) was purified by silica gel column chromatography (c-Hex/EtOAc, 5:1) to yield enone **146** (1.553 g, 3.51 mmol, 71%) as a white solid.

Procedure B

To a solution of 2.0 g (4.67 mmol, 1.0 equiv) of **145** in *c*-Hex (200 mL) were added 6.78 mg (0.033 mmol, 0.7 mol%, 0.07 equiv) of $RuCl_3 \cdot xH_2O$ (suspension). Then TBHP (8.95 mL, 93.4 mmol, 20.0 equiv) was added dropwise and the resulting black clear solution was stirred at ambient temperature for 12 h. The reaction mixture was then quenched with Na_2SO_3 (sat. aq.). The aqueous layer was extracted with MTBE and the combined organic layers were washed with water, brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue (yellowish oil) was purified by silica gel column chromatography (c-Hex/EtOAc, 5:1) to give enone **146** (1.12 g, 2.533 mmol, 54%) as a light yellow oil.



Formula: $C_{29}H_{46}O_3$

Molecular Weight: 442.69 g/mol

TLC: R_f = 0.12 (*c*-Hex/EtOAc 2:1), Cer reagent.

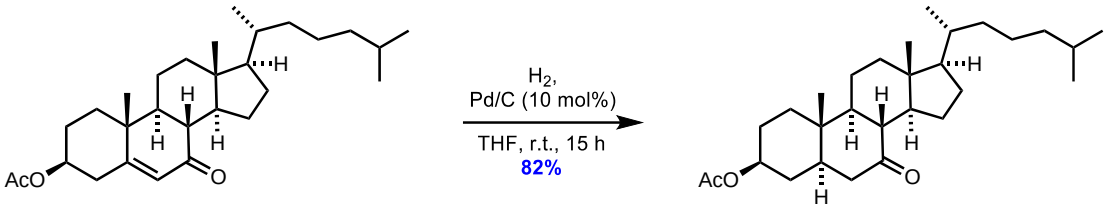
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] =δ 5.51 (s, 1H,H-4), 5.20 (dd, *J* = 15.3, 7.8 Hz, 1H, H-23), 5.16 (dd, *J* = 15.3, 8.5 Hz, 1H, H-22), 3.54 (s, 3H, $-OCH_3$) , 3.44 (d, J = 10.0 Hz, 1H, H-2β), 3.23 (dd, *J* = 15.0, 5.0 Hz, 1H, H-2α), 2.71 – 2.62 (m, 1H, H-1β), 2.62 – 2.54 (td, *J* = 15.0, 5.0 Hz 1H, H-1α), 2.48-2.26 (m, 2H, H-11β, H-15β), 2.21 (dd, *J* = 10.0 , 5.0 Hz, 1H, H-9), 2.13 (d, J = 15.0 Hz, 1H, H-7), 2.08-2.00 (m, 2H, H-16β, H-20), 1.87-1.81 (m, 1H, H-24), 1.77-1.70(m, 1H, H-15α), 1.60-1.51 (m, 2H, H-12β, H-11α), 1.49-1.43 (m, 1H, H-25), 1.42 (t, *J* = 15.0, 10.0, 5.0Hz, 1H, H-14), 1.40 – 1.32 (m, 4H, H-17, H-19, H-12α, H-16α), 1.01 (d, J = 10.0 Hz, 3H, H-21), 0.91 (d, J =

10.0 Hz, 3H, H-28), 0.84-0.79 (m, 6H, H-26, H-27), 0.68 (s, 3H, H-18).

^{13}C NMR (125 MHz, $CDCl_3$): δ [ppm] = 201.9 (C-7), 170.3 (Ac, -C=O), 163.8 (C-5), 126.7 (C-6), 72.2 (C-3), 54.8 (C-17), 49.9 (C-9), 49.8 (C-14), 45.4 (C-8), 43.1 (C-13), 39.5 (C-24), 38.7 (C-12), 38.3 (C-10), 37.7 (C-1), 36.2 (C-4), 36.0 (C-22), 35.7 (C-20), 28.5 (C-2), 28.0 (C-16), 27.3 (C-25), 26.3 (C-15), 23.8 (C-23), 22.8 (C-26), 22.5 (C-27), 21.2(C-11), 21.2(Ac, -CH_3), 18.9 (C-21), 17.2 (C-19) 12.0 (C-18).

HR-MS: (ESI, 70 eV) = *m/z* calcd. For: $C_{29}H_{47}O_3^+$ $[M+H]^+$ 443.35197 u, found: 443.35265 u, for: $C_{29}H_{46}O_3Na^+$ $[M+Na]^+$ 465.33391 u, found: 465.33400 u.

2.2.7 Synthesis of 7-oxo-cholestan-3*β*-yl acetate (144)



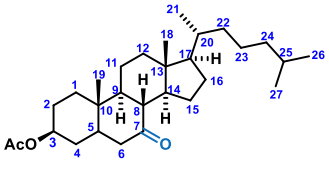
146 **144**

To a solution of 2.6 g of **146** (5.87 mmol, 1.0 equiv.) in 200 mL THF was added 125 mg of Pd/C (1.17 mmol, 0.2 equiv., ca. 20% w/w). After stirring for a short while, the flask was equipped with a hydrogen-filled balloon (1 atm) and purged with hydrogen. The mixture mixture was the stirred for 15 h at room temperature. After filtration, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (*c*-Hex/EtOAc, 5:1) to give compound **144** (2.13 g, 4.78 mmol, 82 %) as a white solid.

Formula: $C_{29}H_{48}O_3$

Molecular Weight: 444.70 g/mol

TLC: R_f = 0.13 (*c*-Hex/EtOAc 10:1), Cer reagent.

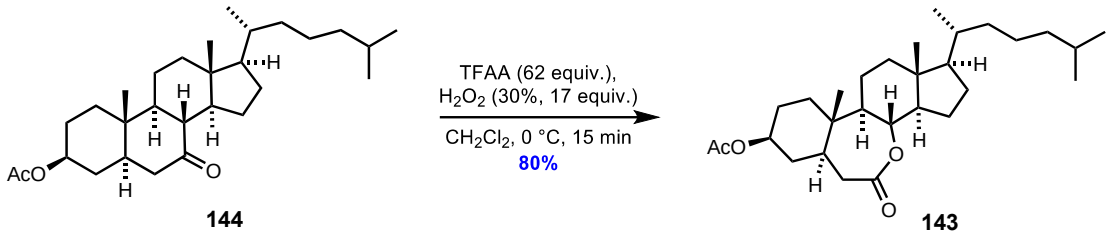


^{1}H NMR (500 MHz, CDCl3): δ [ppm] =δ 5.51 (s, 1H,H-4), 5.20 (dd, *J* = 15.3, 7.8 Hz, 1H, H-23), 5.16 (dd, *J* = 15.3, 8.5 Hz, 1H, H-22), 3.54 (s, 3H, -OCH_3) , 3.44 (d, J = 10.0 Hz, 1H, H-2β), 3.23 (dd, *J* = 15.0, 5.0 Hz, 1H, H-2α), 2.71 – 2.62 (m, 1H, H-1β), 2.62 – 2.54 (td, *J* = 15.0, 5.0 Hz 1H, H-1α), 2.48-2.26 (m, 2H, H-11β, H-15β), 2.21 (dd, *J* = 10.0 , 5.0 Hz, 1H, H-9), 2.13 (d, J = 15.0 Hz, 1H, H-7), 2.08-2.00 (m, 2H, H-16β, H-20), 1.87-1.81 (m, 1H, H-24), 1.77-1.70(m, 1H, H-15α), 1.60-1.51 (m, 2H, H-12β, H-11α), 1.49-1.43 (m, 1H, H-25), 1.42 (t, *J* = 15.0, 10.0, 5.0Hz, 1H, H-14), 1.40 – 1.32 (m, 4H, H-17, H-19, H-12α, H-16α), 1.01 (d, J = 10.0 Hz, 3H, H-21), 0.91 (d, J = 10.0 Hz, 3H, H-28), 0.84-0.79 (m, 6H, H-26, H-27), 0.68 (s, 3H, H-18).

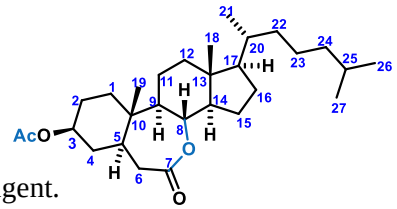
^{13}C NMR (125 MHz, $CDCl_3$): δ [ppm] = 175.0 (C-7), 170.4 (Ac,C=O), 79.8 (C-3), 72.2 (C-17), 56.1 (C-9), 54.3 (C-8), 52.2 (C-14), 43.1 (C-5), 40.9 (C-6), 39.4 (C-13), 38.4 (C-24), 38.3 (C-12), 37.6 (C-1), 36.8 (C-10), 36.0 (C-22), 35.9 (C-20), 35.5 (C-4), 28.0 (C-16), 27.9 (C-25), 26.8 (C-2), 25.1 (C-15), 23.9 (C-23), 22.8 (C-26), 22.8 (C-27), 22.5(Ac,-CH_3), 21.3 (C-11), 18.6 (C-21), 12.9 (C-18) 11.6 (C-19).

HR-MS: (ESI, 70 eV) = *m/z* calcd. for: $C_{29}H_{49}O_3^+$ $[M+H]^+$ 445.36762 u, found: 445.36718 u, for: $C_{29}H_{48}O_3Na^+$ $[M+Na]^+$ 467.34956 u, found: 467.34908 u.

2.2.8. Synthesis of 7-Oxo-7,8α-epoxy-7,8-seco-cholestan-3β-yl acetate (143)



To a stirred mixture of 30% aq. H_2O_2 (7.80 mL) and CH_2Cl_2 (30 mL) was slowly added TFAA (41.8 mL) at 0 °C. The mixture was stirred for 5 min at 0 °C before a solution of **144** (2.1 g, 4.49 mmol) in CH_2Cl_2 (30 mL) was slowly added. Stirring was continued for further 15 min and excess reagent was quenched by slow addition of aq. $NaHCO_3$ until neutral pH. The aqueous phase was extracted with CH_2Cl_2 (3×20 mL) and the organic phase was dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (*c*-Hex/EtOAc, 5:1) to give compound **143** (1.67 g, 3.62 mmol, 80 %) as a white solid.



Formula: $C_{29}H_{48}O_4$

Molecular Weight: 460.69 g/mol

TLC: R_f = 0.48 (*c*-Hex/EtOAc 10:1), Cer reagent.

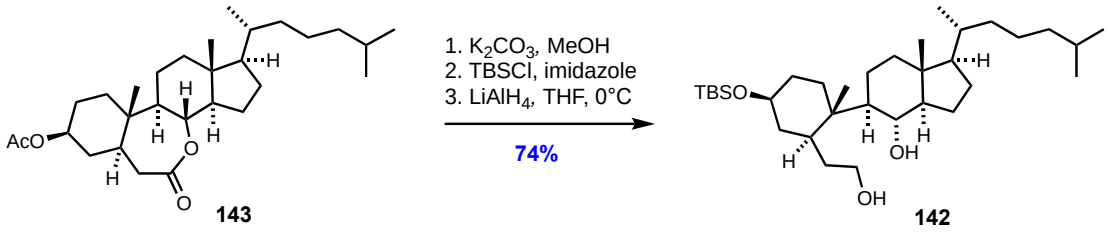
^{1}H NMR (500 MHz, CDCl3): δ [ppm] =δ 5.51 (s, 1H,H-4), 5.20 (dd, *J* = 15.3, 7.8 Hz, 1H, H-23), 5.16 (dd, *J* = 15.3, 8.5 Hz, 1H, H-22), 3.54 (s, 3H, -OCH_3) , 3.44 (d, J = 10.0 Hz, 1H, H-2β), 3.23 (dd, *J* = 15.0, 5.0 Hz, 1H, H-2α), 2.71 – 2.62 (m, 1H, H-1β), 2.62 – 2.54 (td, *J* = 15.0, 5.0 Hz 1H, H-1α), 2.48-2.26 (m, 2H, H-11β, H-15β), 2.21 (dd, *J* = 10.0 , 5.0 Hz, 1H, H-9), 2.13 (d, J = 15.0 Hz, 1H, H-7), 2.08-2.00 (m, 2H, H-16β, H-20), 1.87-1.81 (m, 1H, H-24), 1.77-1.70(m, 1H, H-15α), 1.60-1.51 (m, 2H, H-12β, H-11α), 1.49-1.43 (m, 1H, H-25), 1.42 (t, *J* = 15.0, 10.0, 5.0Hz, 1H, H-14), 1.40 – 1.32 (m, 4H, H-17, H-19, H-12α, H-16α), 1.01 (d, J = 10.0 Hz, 3H, H-21), 0.91 (d, J = 10.0 Hz, 3H, H-28), 0.84-0.79 (m, 6H, H-26, H-27)

, 0.68 (s, 3H, H-18).

^{13}C NMR (125 MHz, $CDCl_3$): δ [ppm] = 211.6 (C-7), 170.5 (Ac,C=O), 72.8 (C-3), 70.2 (C-8), 55.0 (C-17), 50.0 (C-14), 48.9 (C-9), 46.5 (C-10), 45.9 (C-13), 42.5 (C-6), 39.5 (C-24), 38.7 (C-12), 36.1 (C-1), 36.0 (C-5), 35.8 (C-20), 35.7 (C-22), 33.8 (C-4), 28.4 (C-25), 28.0 (C-2), 27.1 (C-15), 25.0 (C-23), 23.8 (C-26), 22.8 (C-27), 22.6 (C-16), 21.8 (Ac, -CH_3), 21.4 (C-21), 18.8 (C-11), 12.1 (C-19) 11.7 (C-18).

HR-MS: (ESI, 70 eV) = *m/z* calcd. for: $C_{29}H_{49}O_4$ $[M+H]^+$ 461.3625 u, found: 461.3648 u, for: $C_{29}H_{48}O_4Na^+$ $[M+Na]^+$ 483.3444 u, found: 483.3418u.

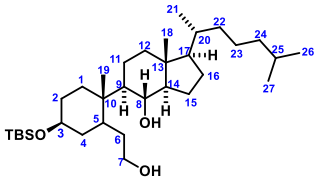
2.2.9. Synthesis of 3β-tert-Butyldimethylsiloxy-7,8-seco-cholestan-7,8α-diol (142)



To a stirred solution of K_2CO_3 (89.9 mg, 1.302 mmol, 2.0 equiv.) in methanol (10 mL) was added **143** (300 mg, 0.651 mmol) and the mixture stirred for 10 min. The mixture was then partitioned between EtOAc and $NaHCO_3$ (sat. aq.), and the organic layers were washed with water and brine. After drying the solution with $MgSO_4$ the solvent was removed under reduced pressure to give the deacetylated intermediate (269.4 mg, 98.5%) as a white solid.

To a stirred solution of the crude alcohol (269.4 mg, 0.65 mmol) in DMF (12 mL) were added 66.5 mg (0.97 mmol, 1.5 equiv.) of TBSCl followed by 294 mg (1.94 mmol, 3.0 equiv) of imidazole and stirring was continued at ambient temperature for 16 h. After addition of water the mixture was extracted with MTBE. The collected organic layers were washed with HCl (1 M, aq.), $NaHCO_3$ (sat. aq.), water and brine, dried over MgSO4 and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (c-Hex/EtOAc, 5:1) to give the TBS-protected lactone (270.2 mg, 0.51 mmol, 78%) as a white solid.

A solution of the TBS-protected lactone (12.2 mg, 0.049 mmol) in dry THF (2 mL) was added dropwise to a suspension of $LiAlH_4$ (6 mg, 0.16 mmol) in dry THF (1 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 1 h. The suspension was quenched by addition of water and a solution of sodium hydroxide (15%). The resulting mixture was filtered through a pad of Celite. The filter cake was washed with ethyl acetate, and the combined filtrates were concentrated in vacuo. The crude product was purified by thin layer chromatography on silica gel (hexane/ethyl acetate = 1/4) to afford **142** (9.7 mg, 0.038 mmol) as colorless oil.



Formula: $C_{33}H_{64}O_3Si$

Molecular Weight: 536.96 g/mol

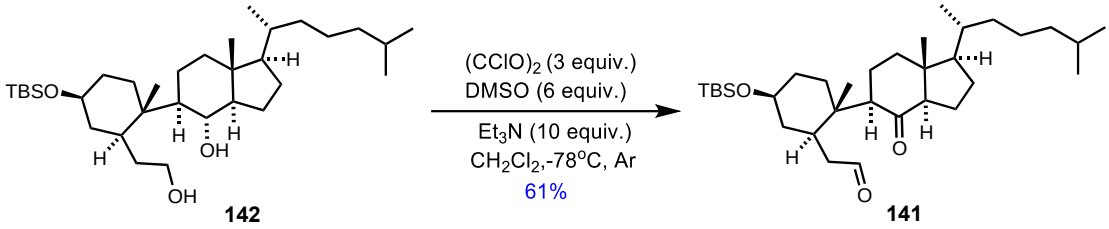
TLC: R_f = 0.87 (*c*-Hex/EtOAc 2:1), Cer reagent.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] =δ 5.51 (s, 1H,H-4), 5.20 (dd, *J* = 15.3, 7.8 Hz, 1H, H-23), 5.16 (dd, *J* = 15.3, 8.5 Hz, 1H, H-22), 3.54 (s, 3H, $-OCH_3$) , 3.44 (d, J = 10.0 Hz, 1H, H-2β), 3.23 (dd, *J* = 15.0, 5.0 Hz, 1H, H-2α), 2.71 – 2.62 (m, 1H, H-1β), 2.62 – 2.54 (td, *J* = 15.0, 5.0 Hz 1H, H-1α), 2.48-2.26 (m, 2H, H-11β, H-15β), 2.21 (dd, *J* = 10.0 , 5.0 Hz, 1H, H-9), 2.13 (d, J = 15.0 Hz, 1H, H-7), 2.08-2.00 (m, 2H, H-16β, H-20), 1.87-1.81 (m, 1H, H-24), 1.77-1.70(m, 1H, H-15α), 1.60-1.51 (m, 2H, H-12β, H-11α), 1.49-1.43 (m, 1H, H-25), 1.42 (t, *J* = 15.0, 10.0, 5.0Hz, 1H, H-14), 1.40 – 1.32 (m, 4H, H-17, H-19, H-12α, H-16α), 1.01 (d, J = 10.0 Hz, 3H, H-21), 0.91 (d, J = 10.0 Hz, 3H, H-28), 0.84-0.79 (m, 6H, H-26,27), 0.68 (s, 3H, H-18).

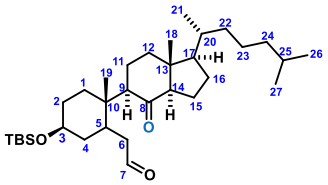
^{13}C NMR (125 MHz, $CDCl_3$): δ [ppm] = 72.5 (C-3), 71.6 (C-8), 61.3 (C-7), 58.5 (C-14), 56.7 (C-17), 52.0 (C-9), 44.0 (C-13), 39.6 (C-24), 39.5 (C-12), 37.0 (C-4), 36.3 (C-10), 36.2 (C-1), 36.0 (C-22), 35.5 (C-20), 32.0 (C-2), 31.6 (C-6), 31.1 (C-5), 28.3 (TBS,-C-), 28.0 (C-25), 26.0 (TBS, $-C(CH_3)_3$), 23.9 (C-15), 23.7 (C-23), 22.8 (C-26), 22.6 (C-27), 22.6 (C-16), 21.2 (C-21), 18.5 (C-11), 18.3 (C-19) 12.0 (C-18), -4.5 (TBS, $-Si-(CH_3)_2$).

HR-MS: (ESI, 70 eV) = *m/z* calcd. for: $C_{33}H_{65}O_3Si$ $[M+H]^+$ 537.4697 u, found: 537.4700 u, for: $C_{33}H_{64}O_3SiNa^+$ $[M+Na]^+$ 559.4516 u, found: 559.4516.

2.2.10 Synthesis of 7-Oxo-3*β*-tert-butyldimethylsiloxy-7,8-seco-cholestan-7-al (141)



A solution of oxalyl chloride (1.3 mL, 15.0 mmol) in dry CH_2Cl_2 (100 mL) was cooled to –78 ºC under an atmosphere of Ar. A solution of DMSO (1.75 mL, 24.8 mmol) in CH_2Cl_2 (10 mL) was added at a rate such that the reaction temperature remained below –65 ºC. After stirring for 5 min, a solution of **142** (3.23 g, 6.0 mmol) in CH_2Cl_2 (15 mL) was added slowly, and the resulting mixture was stirred for 15 min. Next, NEt_3 (8.4 mL, 60.7 mmol) was added slowly. After stirring the reaction for 10 additional min at –70 ºC, the cooling bath was removed and the reaction was allowed to warm for ca. 45 min. Upon reaching room temperature, water (100 mL) was added and stirring continued for 15 min. The reaction mixture was transferred to a separatory funnel and washed successively with 5% HCl (100 mL), saturated $NaHCO_3$ solution (100 mL), and brine (50 mL). The organic layer was dried ($MgSO_4$), the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (*c*-Hex/EtOAc, 3:1) to give compound **141** (1.95 g, 3.66 mmol, 61 %) as a white solid.



Formula: $C_{33}H_{60}O_3Si$

Molecular Weight: 532.43 g/mol

TLC: R_f = 0.5 (*c*-Hex/EtOAc 2:1), Cer reagent.

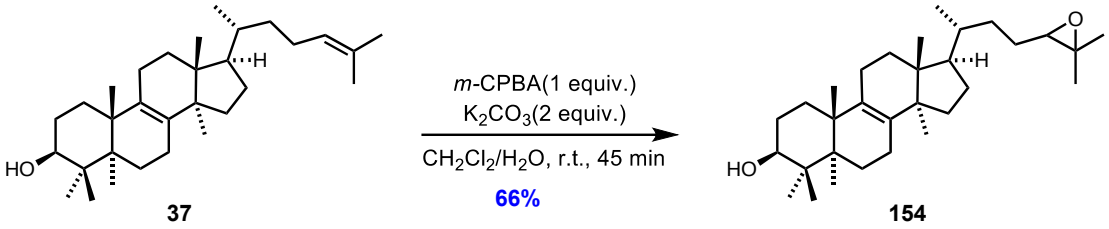
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] =δ 5.51 (s, 1H,H-4), 5.20 (dd, *J* = 15.3, 7.8 Hz, 1H, H-23), 5.16 (dd, *J* = 15.3, 8.5 Hz, 1H, H-22), 3.54 (s, 3H, -OCH_3) , 3.44 (d, J = 10.0 Hz, 1H, H-2β), 3.23 (dd, *J* = 15.0, 5.0 Hz, 1H, H-2α), 2.71 – 2.62 (m, 1H, H-1β), 2.62 – 2.54 (td, *J* = 15.0,

5.0 Hz 1H, H-1α), 2.48-2.26 (m, 2H, H-11β, H-15β), 2.21 (dd, *J* = 10.0 , 5.0 Hz, 1H, H-9), 2.13 (d, J = 15.0 Hz, 1H, H-7), 2.08-2.00 (m, 2H, H-16β, H-20), 1.87-1.81 (m, 1H, H-24), 1.77-1.70(m, 1H, H-15α), 1.60-1.51 (m, 2H, H-12β, H-11α), 1.49-1.43 (m, 1H, H-25), 1.42 (t, *J* = 15.0, 10.0, 5.0Hz, 1H, H-14), 1.40 – 1.32 (m, 4H, H-17, H-19, H-12α, H-16α), 1.01 (d, J = 10.0 Hz, 3H, H-21), 0.91 (d, J = 10.0 Hz, 3H, H-28), 0.84-0.79 (m, 6H, H-26, H-27), 0.68 (s, 3H, H-18).

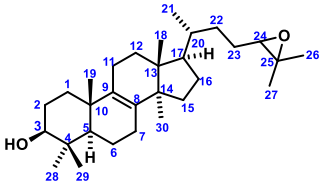
^{13}C NMR (125 MHz, $CDCl_3$): δ [ppm] = 210.6 (C-8), 202.3 (C-7), 70.9 (C-3), 63.4 (C-14), 56.8 (C-9), 56.1 (C-17), 50.6 (C-13), 44.3 (C-6), 39.4 (C-24), 39.4 (C-12), 37.6 (C-10), 36.4 (C-4), 35.9 (C-22), 35.5 (C-1), 34.6 (C-20), 31.2 (C-5), 28.6 (C-2), 28.0 (TBS,-C-), 27.9 (C-25), 25.9 (TBS, -C(CH_3)$_3$), 25.1 (C-16), 23.8 (C-23), 22.8 (C-15), 22.5 (C-26), 19.1 (C-27), 18.6 (C-11), 18.2 (C-21), 17.9 (C-19) 12.2 (C-18), -4.6 (TBS, -Si-(CH_3)$_2$).

HR-MS: (ESI, 70 eV) = *m/z* calcd. for: $C_{33}H_{61}O_3Si$ $[M+H]^+$ 533.4384 u, found: 537.4380 u, for: $C_{33}H_{60}O_3SiNa^+$ $[M+Na]^+$ 555.4203 u, found: 555.4204

2.2.11 Synthesis of 24,25-Epoxy-lanosterol (154)



4.00 g of lanosterol (**37**) (50% pure, 4.69 mmol, 1 eq.) was suspended in 60 mL of CH_2Cl_2. 1.33 g of K_2CO_3 (9.38 mmol, 2 eq.) was dissolved in 50 mL of water and the solution was added to the lanosterol suspension. 1.01 g of *m*-CPBA was dissolved in 20 mL of CH_2Cl_2 and added dropwise to the reaction mixture and stirring was continued for 45 minutes at room temperature. The phases were separated and the aqueous phase was extracted with dichloromethane three times. The combined organic phases were washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield 4.73 g (66% yield) of **154** as a pale-yellow crude product.



Formula:	$C_{30}H_{50}O_2$ white solid
Molecular Weight:	442.38 g/mol
m.p.:	126.8 - 128.8 °C.
TLC:	Rf = 0.47 (*c*-Hex/EtOAc 2:1), Cer reagent.
^{1}H NMR	(500 MHz, $CDCl_3$); δ [ppm] = 3.25 – 3.19 (m, 1H, H-3), 2.70 – 2.64 (m, 1H, H-24), 2.05 – 1.96 (m, 4H, H-7, H-11), 1.96 – 1.87 (m, 1H, H-16), 1.75 – 1.30 (m, 10H, H-1, H-2, H-6, H-12, H-15, H-16, H-17, H-20, H-22, H-23), 1.29 (d, J = 1.4 Hz, 3H, H-26 or 27), 1.25 (d, J = 0.8 Hz, 3H, H-26 or 27), 1.24 – 1.18 (m, 1H, H-1), 1.18 – 1.14 (m, 1H, H-15), 1.12 – 1.05 (m, 1H, H-22), 1.05 – 1.01 (m, 1H, H-5), 0.98 (s, 3H, H-28 or 29), 0.96 (s, 3H, H-19), 0.90 (d, J = 6.3 Hz, 3H, H-21), 0.86 (t, J = 1.2 Hz, 3H, H-30), 0.79 (s, 3H, H-28 or 29), 0.68 (d, J = 0.8 Hz, 3H, H-18)
^{13}C NMR	(125 MHz, CDCl3); δ [ppm] = 134.5 (C-9), 134.3 (C-8), 78.9 (C-3), 64.9 (C-24), 58.4 (C-25), 50.4 (C-5), 50.4 (C-17), 49.8 (C-14), 44.5 (C-13), 38.9 (C-4), 37.0 (C-10), 36.3 (C-20), 35.6 (C-1), 32.8 (C-22),

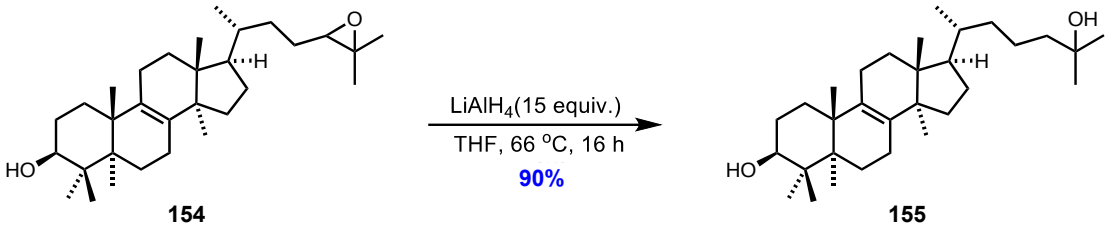
31.0 (C-12), 30.8 (C-15), 28.3 (C-16), 27.9 (C-2), 27.8 (C-7), 26.5 (C-23), 25.9 (C-30), 24.9 (C-26), 24.3 (C-27), 21.0 (C-28), 19.2 (C-29), 18.8 (C-11), 18.67(C-19), 18.3 (C-6), 15.8 (C-21), 15.4 (C-18).

FT-IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3499 (br), 2952 (s), 2930 (s), 2870 (s), 2835 (m), 1464 (m), 1455 (m), 1371 (s), 1321 (w),1287 (w), 1204 (w), 1189 (w), 1156 (w), 1134 (w), 1117 (w), 1099 (m), 1076 (m), 1029 (s), 1007 (m), 1000 (m), 932 (m), 685 (m).

HR-MS: (EI, 70 eV) = *m/z* calcd. for: $C_{30}H_{51}O_2^+$ $[M+H]^+$ 443.38005 u, found: 443.38891u .

$[\alpha]_\lambda^T$: (c = 0.48 g/100 mL, $CHCl_3$) = $[\alpha]_{436}^{20}$: +97.2°, $[\alpha]_{546}^{20}$: +57.3°, $[\alpha]_{579}^{20}$: +50.6°, $[\alpha]_{D}^{20}$: +48.7°.

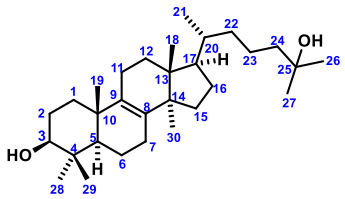
2.2.12 Synthesis of 24-H-25-Hydroxy-lanosterol (155)



154 **155**

Uner cooling in an ice/water bath, 683 mg of $LiAlH_4$ (18.0 mmol, 15 eq.) was suspended in 45 mL of THF before a solution of 532 mg of epoxide **154** (1.20 mmol, 1 eq.) in 30 mL of THF was added dropwise to the $LiAlH_4$ suspension at 0 °C. After the addition was completed the mixture was warmed to room temperature and then refluxed at 66 °C for 16 h. Excess reagent was quenched by dropwise addition of water under cooling at 0 °C, followed by dropwise addition of 10% NaOH solution. The mixture was diluted with ethyl acetate and the phases separated. The aqueous phase was extracted with ethyl acetate three times, the organic phases were combined and washed twice with saturated NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure to yield 481 mg (90%) of **155** as a white solid.

Formula: $C_{30}H_{52}O_2$ white solid



Molecular Weight: 444.40 g/mol

m.p.: 176.7 - 183.0 °C.

TLC: Rf = 0.65 (*c*-Hex/EtOAc 2:1) Cer reagent.

^{1}H NMR (500 MHz, $CDCl_3$); δ [ppm] = 3.25 – 3.19 (m, 1H, H-3), 2.70 – 2.64 (m, 1H, H-24), 2.05 – 1.96 (m, 4H, H7, H11), 1.96 – 1.87 (m, 1H, H-16), 1.75 – 1.30 (m, 10H, H-1, H-2, H-6, H-12, H-15, H-16, H-17, H-20, H-22, H-23), 1.29 (d, *J* = 1.4 Hz, 3H, H-26 or 27), 1.25 (d, *J* = 0.8 Hz, 3H, H-26 or 27), 1.24 – 1.18 (m, 1H, H-1), 1.18 – 1.14 (m, 1H, H-15), 1.12 – 1.05 (m, 1H, H-22), 1.05 – 1.01 (m, 1H, H-5), 0.98 (s, 3H, H-28 or 29), 0.96 (s, 3H, H-19), 0.90 (d, *J* = 6.3 Hz, 3H, H-21), 0.86 (t, *J* = 1.2 Hz, 3H, H-30), 0.79 (s, 3H, H-28 or 29), 0.68 (d, *J* = 0.8 Hz, 3H, H-18)

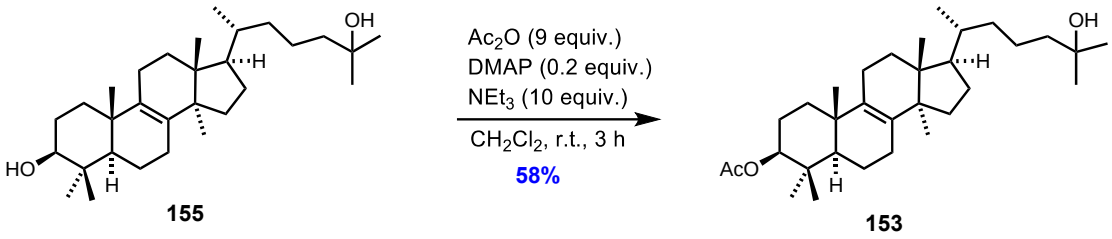
^{13}C NMR (125 MHz, CDCl3); δ [ppm] = 134.4 (C-9), 134.4 (C-8), 79.0 (C-3), 71.1 (C-25), 50.5 (C-17), 50.4 (C-5), 49.8 (14), 44.5 (C-13), 44.4 (C-24), 38.9 (C-4), 37.0 (C-10), 36.7 (C-22), 36.5 (C-20), 35.6 (C-1), 31.0 (C-16), 30.8 (C-26), 29.3 (C-27), 29.2 (C-12), 28.3 (C-16), 28.0 (C-2), 27.9 (C-7), 26.5 (C-30), 24.3 (C-28), 21.1 (C-29), 21.0 (C-11), 19.2 (C-23), 18.7 (C-19), 18.3 (C-6), 15.8 (C-21), 15.4 (C-18)

HR-MS: (EI, 70 eV) = *m/z* calcd. for: $C_{30}H_{53}O_2^+$ $[M+H]^+$ 445.396730 u, found: 445.40455 u .

$[\alpha]_\lambda^T$: (c = 0.50 g/100 mL, $CHCl_3$) = $[\alpha]_{436}^{20}$: +110.53°, $[\alpha]_{546}^{20}$: +66.24°, $[\alpha]_{579}^{20}$: +58.60°, $[\alpha]_{D}^{20}$: +55.93°.

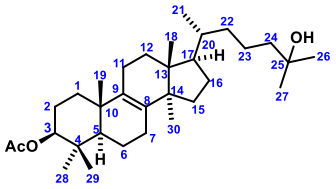
The NMR data are consistent with those reported in the literature83]

2.2.13 Synthesis of 24-H-25-hydroxy-lanosteryl acetate (155)



To a solution of 481 mg of crude **155** (1.08 mmol, 1 eq.) in 170 mL of CH_2Cl_2 were added 27 mg of DMAP (0.22 mmol, 0.2 eq.), 1.5 mL of triethylamine (108 mmol, 10 eq.) and lastly 0.41 mL of acetic anhydride (4.02 mmol, 4 eq.). The mixture was stirred at room temperature for 2 h, after which 0.5 mL of Ac_2O (5.3 mmol, 5 eq.) was added and stirring continued for 1 h. The reaction was quenched by adding methanol and then washed with water, saturated $NaHCO_3$ and NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography with a stepwise increase in solvent polarity (cHex : EtOAc = 10:1; 5:1; 3:1) affording 329 mg (58%) of **153** as a white solid.

Formula: $C_{32}H_{54}O_3$



Molecular Weight: 514.37 g/mol

m.p.: 166.8 - 168.0 °C

TLC: Rf = 0.40 (*c*-Hex/EtOAc 2:1), Cerium reagent.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 4.48 (dd, J = 11.8, 4.5 Hz, 1H, H-3), 2.03 (s, 3H, Ac-CH_3), 2.02 – 1.95 (m, 4H, C-7, H-11), 1.89 (dtd, J = 13.6, 9.4, 7.5 Hz, 1H, H-16), 1.75 – 1.25 (m, 16H, H-1, H-1, H-2, H-2, H-6, H-6, H-12, H-12, H-15, H-16, H-17, H-20, H-22, H-23, H-24, H-24), 1.19 (s, 7H, H-26, H-27, H-23), 1.16 (dt, J = 12.1, 2.8 Hz, 1H, H-15), 1.13 (dd, J = 12.6, 2.3 Hz, 1H, H-5), 1.03 – 0.95 (m, 4H, H-19, H-22), 0.88 (d, J = 6.5 Hz, 3H, H-21), 0.86 (s, 3H, H-28 or H-29), 0.86 (s, 3H, H-28 or H-29), 0.85 (s, 3H, H-30), 0.67 (s, 3H, H-18).

^{13}C NMR (125 MHz, $CDCl_3$): δ [ppm] = 171.0 (Ac-C=O), 134.5 (C-9), 134.3 (C-8), 80.9 (C-3), 71.1 (C-25), 50.5 (C-17), 50.5 (C-5), 49.8(C-14),

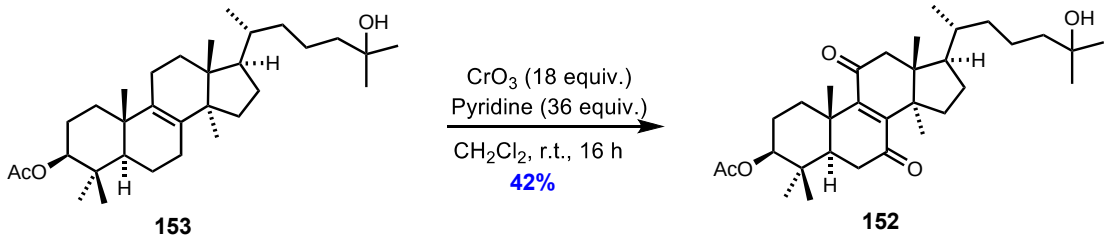
44.5 (C-13), 44.4 (C-24), 37.8 (C-4), 36.9 (C-10), 36.7 (C-22), 36.5 (C-20), 35.3 (C-1), 31.0 (C-15), 30.8 (C-26), 29.3 (C-27), 29.2 (C-12), 28.2 (C-16), 27.9 (C-7), 26.4 (C-30), 24.3 (C-2), 24.2 (C-28), 21.3 (C-29), 21.1 (C-11), 21.0 (C-23), 19.2 (Ac-CH_3), 18.7 (C-19), 18.1 (6), 16.5 (C-21), 15.8 (C-18)

FT-IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2955 (s), 2870 (m), 1714 (s), 1672 (s), 1622 (m), 1458 (m), 1370 (m), 1234 (m), 1138 (m), 968 (m), 873 (m), 671 (w), 521 (w).

HR-MS: (EI, 70 eV) = *m/z* calcd. for: $C_{32}H_{55}O_3^+$ $[M+H]^+$ 487.40730 u, found: 487.41512 u .

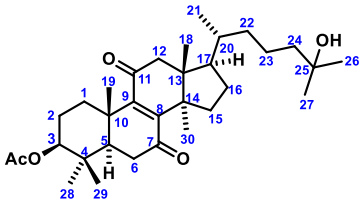
$[\alpha]_\lambda^T$: (c = 0.36 g/100 mL, $CHCl_3$) = $[\alpha]_{436}^{20}$: +108.9°, $[\alpha]_{546}^{20}$: +66.0°, $[\alpha]_{579}^{20}$: +58.1°, $[\alpha]_D^{20}$: +56.15°.

2.2.14 Synthesis of 24-*H*-25-Hydroxy-7,11-dioxo-lanosteryl acetate (152)



5.04 g of CrO_3 (50.4 mmol, 18 eq.) was added to an argon filled flask and suspended in 50 mL of CH_2Cl_2. 8.2 mL of pyridine (100.8 mmol, 36 eq.) was diluted with 5 mL of CH_2Cl_2 and added dropwise to the reaction flask while cooling to 0 °C. The contents were stirred for 10 minutes, followed by dropwise addition of 1.346 g of **153** (2.8 mmol, 1 eq.) in 10 mL of CH_2Cl_2. The reaction mixture was warmed and stirred at room temperature for 20 h. The reaction was worked up by adding saturated $NaHCO_3$ solution, separating the phases, extracting the aqueous phase with CH_2Cl_2 three times, combining the organic phases and washing them with saturated $NaHCO_3$ solution twice and saturated NaCl solution once, drying over $MgSO_4$ and removing the solvent under reduced pressure. The crude product was purified by column chromatography (cHex : EtOAc = 10:1; 5:1, 3:1) yielding 600 mg of **152** (42%) as a yellow solid.

Formula: $C_{32}H_{50}O_5$



Molecular Weight: 514.37 g/mol

m.p.: 195.4 - 202.6 °C (Lit.[8] 199 - 201 °C)

TLC: Rf = 0.63 (*c*-Hex/EtOAc 2:1) Cerium reagent.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 4.51 (dd, J = 11.7, 4.8 Hz, 1H, H-3), 2.88 (dt, J = 13.8, 3.7 Hz, 1H, H-1), 2.75 (dd, J = 15.9, 1.2 Hz, 1H, H-12), 2.63 – 2.58 (m, 1H, H-12), 2.52 – 2.41 (m, 2H, H-6, H-6), 2.12 (ddd, J = 12.3, 9.4, 2.2 Hz, 1H, H-15), 2.04 (s, 3H, H-Ac), 1.96 (dtd, J = 13.6, 9.5, 7.2 Hz, 1H, H-16), 1.81 – 1.59 (m, 5H, H-2, H-2, H-5, H-15, H-17), 1.49 – 1.32 (m, 6H, H-16, H-20, H-22, H-23, H-24, H-24), 1.31 (s, 3H, H-19), 1.28 – 1.22 (m, 1H, H-1), 1.20 (s, 7H, H-26, H-27, H-23), 1.16 (s, J = 1.0 Hz, 3H, H-30), 1.08 – 1.00 (m, 1H, H-22), 0.94 (s, 3H, H-28 or H-29), 0.90 – 0.88 (m, 6H H-21, H-28 or H-29), 0.78 (d, J = 1.0 Hz, 3H, H-18).

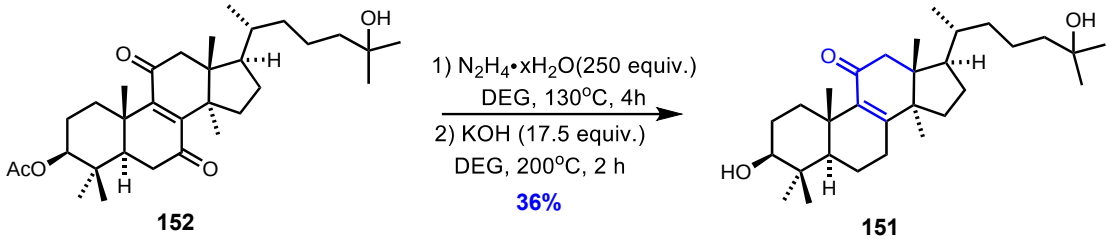
^{13}C NMR (125 MHz, $CDCl_3$): δ [ppm] = 202.4 (C-11), 201.9 (C-7), 170.8 (C=O, Ac), 151.7 (C-9), 150.6 (C-8), 79.3 (C-3), 71.0 (C-25), 51.6 (C-5), 50.2 (C-12), 49.1 (C-17), 49.0 (C-24), 47.5 (C-14), 44.3 (C-10), 39.6 (C-13), 37.7 (C-4), 36.5 (C-22), 36.3 (C-20), 36.2 (C-6), 33.8 (C-1), 32.2 (C-15), 29.4 (C-26 or 27), 29.2 (C-26 or 27), 27.8 (C-16), 27.4 (C-28), 25.9 (C-29), 23.9 (C-2), 21.2 (30), 21.0 (C-23), 18.6 (CH_3-Ac), 17.5 (C-19), 16.8 (C-21), 16.5 (C-18).

FT-IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3493 (br), 2973 (m), 2964 (m), 2930 (m), 2911 (m), 2868 (m), 1710 (s), 1683 (s), 1663 (s), 1379 (m), 1367 (m), 1331 (w), 1265 (s), 1239 (m), 1209 (m), 1182 (m), 1160 (m), 1118 (m), 1089 (w), 1036 (m), 1012 (m), 984 (m), 939 (m), 542 (m), 531 (m).

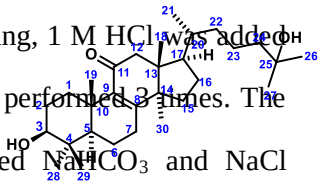
HR-MS: (ESI, 70 eV) = *m/z* calcd. for:(EI, 70 eV) = *m/z* calcd. for: $C_{32}H_{51}O_5^+$ $[M+H]^+$ 515.36583 u, found: 515.37465u .

$[\alpha]_{\lambda}^{T}$: (c = 0.36 g/100 mL, $CHCl_3$) = $[\alpha]_{436}^{20}$: +145.9°, $[\alpha]_{546}^{20}$: + 95.4°, $[\alpha]_{579}^{20}$: +88.3°, $\alpha]_{D}^{20}$: +85.0°.

2.2.15 Synthesis of 25-H-26-hydroxy-11-oxo-lanosterol (151)



100 mg of endione **152** (0.212 mmol, 1 eq.) was dissolved in 5 mL of diethylene glycol, to which 1.0 mL of hydrazine hydrate $NH_2NH_2 \cdot x\ H_2O$ (50-60% w/w) was added. The reaction was heated to 130 °C. After 1.5 h another 0.3 mL of $NH_2NH_2 \cdot xH_2O$ was added, 1 h after that an additional portion of 1.0 mL and finally after another 1 h 0.2 mL of $NH_2NH_2 \cdot xH_2O$ were added (in total 2.5 mL, 48.2 mmol, 227 eq.). Heating was stopped 20 minutes after the final $NH_2NH_2 \cdot XH_2O$ addition, the contents somewhat cooled and the excess hydrazine and water distilled under reduced pressure. 200 mg of KOH (3.56 mmol, 17 eq.) were added to the reaction mixture, followed by refluxing at 200 °C for 2 h. After cooling, 1 M HCl was added dropwise to neutralize the solution and extraction with ethyl acetate performed 3 times. The combined organic phases were washed sequentially with saturated $NaHCO_3$ and NaCl solutions, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (cHex : EtOAc = 10:1; 5:1; 2:1; 1.5:1; 1:1) affording 35 mg of **151** (76 mmol, 36%) as an white solid product.



Formula: $C_{30}H_{50}O_3$

Molecular Weight: 458.38 g/mol

m.p.: 173 - 174 °C

TLC: Rf = 0.45 (*c*-Hex/EtOAc 1:2), Cerium reagent.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 3.25 (dd, *J* = 11.3, 5.1 Hz, 1H, H-3), 3.03 (dt, *J* = 13.6, 3.6 Hz, 1H, H-1), 2.67 (dd, *J* = 16.4, 1.2 Hz, 1H, H-

12), 2.49 (d, *J* = 16.4 Hz, 1H, H-12), 2.37 (ddd, *J* = 20.2, 6.3, 1.3 Hz, 1H, H-7), 2.26 (ddd, *J* = 20.1, 11.1, 7.3 Hz, 1H, H-7), 1.98 (dtd, *J* = 14.1, 9.5, 7.3 Hz, 1H, H-15), 1.83 – 1.59 (m, 5H, H-2, H-2, H-6, H-16, H-17), 1.55 – 1.30 (m, 7H, H-6, H-15, H-16, H-20, H-22, H-23, H-24, H-24), 1.23 (s, 7H, H-23, H-26, H-27), 1.14 (s, 3H, H-19), 1.14 – 1.13 (m, 3H, H-30), 1.08 (dd, *J* = 13.0, 4.7 Hz, 1H, H-1), 1.04 (s, 3H, H-28 or 29), 0.93 (dd, *J* = 12.6, 1.7 Hz, 1H, H-5), 0.90 (d, *J* = 6.5 Hz, 3H, H-21), 0.84 (s, 3H, H-18), 0.84 (s, 3H, H-28 or 29)

^{13}C NMR (125 MHz, $CDCl_3$): δ [ppm] = 199.3 (C-11), 164.2 (C-8), 139.5 (C-9), 78.7 (C-3), 71.0 (C-25), 51.9 (C-5), 51.8 (C-12), 51.6 (C-14), 50.2 (C-17), 47.2 (C-13), 44.3 (C-24), 39.0 (C-10), 37.7 (C-4), 36.5 (C-22), 36.2 (C-20), 34.4 (C-1), 31.0 (C-15), 29.9 (C-26), 29.4 (C-27), 29.2 (C-7), 28.3 (C-16), 28.0 (C-2), 27.0 (C-28), 25.8 (C-29), 21.0 (C-23), 19.0 (C-30), 18.4 (C-19), 17.4 (C-21), 16.7 (C-6), 15.7 (C-18).

FT-IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3353 (br), 2961 (s), 2940 (s), 1654 (s), 1645 (s), 1587 (m), 1460 (m), 1419 (m), 1374 (s), 1338 (w), 1303 (m), 1283 (m), 1246 (m), 1217 (m), 1199 (m), 1185 (m), 1161 (m), 1149 (m), 1101 (m), 1031 (s), 1005 (m), 954 (m), 936 (m), 924 (m), 762 (m),), 661 (m), 626 (m), 600 (m), 539 (m).

HR-MS: (ESI, 70 eV) = *m/z* calcd. for:(EI, 70 eV) = *m/z* calcd. for: $C_{30}H_{51}O_3^+$ $[M+H]^+$ 458.36583 u, found: 458.37456u .

$[\alpha]_{\lambda}^{T}$: (c = 0.36 g/100 mL, $CHCl_3$) = $[\alpha]_{436}^{20}$: +179.8°, $[\alpha]_{546}^{20}$: +152.4°, $[\alpha]_{579}^{20}$: +137.0°, $[\alpha]_{D}^{20}$: +132.9°.

3 Appendix

3.1. List of Abbreviations

%	per cent
°C	Celcius degree
Å	Ångström
A549	adenocarcinomic human alveolar basal epithelial cells
DCM	Dichloromethane
DME	Dimethyl Ether
aq.	aqueous
brine	saturated NaCl solution
brsm	based on recovered starting material
C	concentration
CCDC	Cambridge Crystallographic Data Centre
c-Hex	cyclohexane
DEG	diethylene glycol
DMP	Dess Martin periodinane
d.r.	diastereomeric ratio
ent	enantiomer
equiv	equivalent
ESI	electrospray ionization
EtOAc	ethyl acetate
EWG	electron-withdrawing group
et al.	et alia (and others)
FT-IR	Fourier transform infrared spectroscopy
h	hour/s
HCT-116	human colon carcinoma cells
hv	light
hpi	hour/s post of the infection
DMSO	Dimethyl sulfoxide
IC_{50}	half maximal inhibitory concentration
IR	infrared spectroscopy
IUPAC	International Union of Pure and Applied Chemistry
MW	molecular weight
M	molar concentration
MIC	minimal inhibitory concentration

MHz	megahertz (10^6 Hz)
MOI	multiplicity of infection
m.p.	melting point
MTBE	methyl-tert-butyl ether
N	equivalent concentration
nm	nanometer (10^{-9} m)
NMR	nuclear magnetic resonance spectroscopy
PG	protecting group
ppm	parts per million
quant.	quantitative
R_f	retardation factor
RNA	ribonucleic acid
RT	room temperature
SAR	structure-activity relationship
sat.	Saturated
TBHP	Tert-butylhydroperoxide
TBS	tert.butyldimethylsilyl
TLC	thin-layer chromatography
THF	tetrahydrofuran
TRPV-1	transient receptor potential cation channel subfamily V-1
WT	wild-type
MeOH	methanol

3.2. ^{1}H and ^{13}C NMR spectra

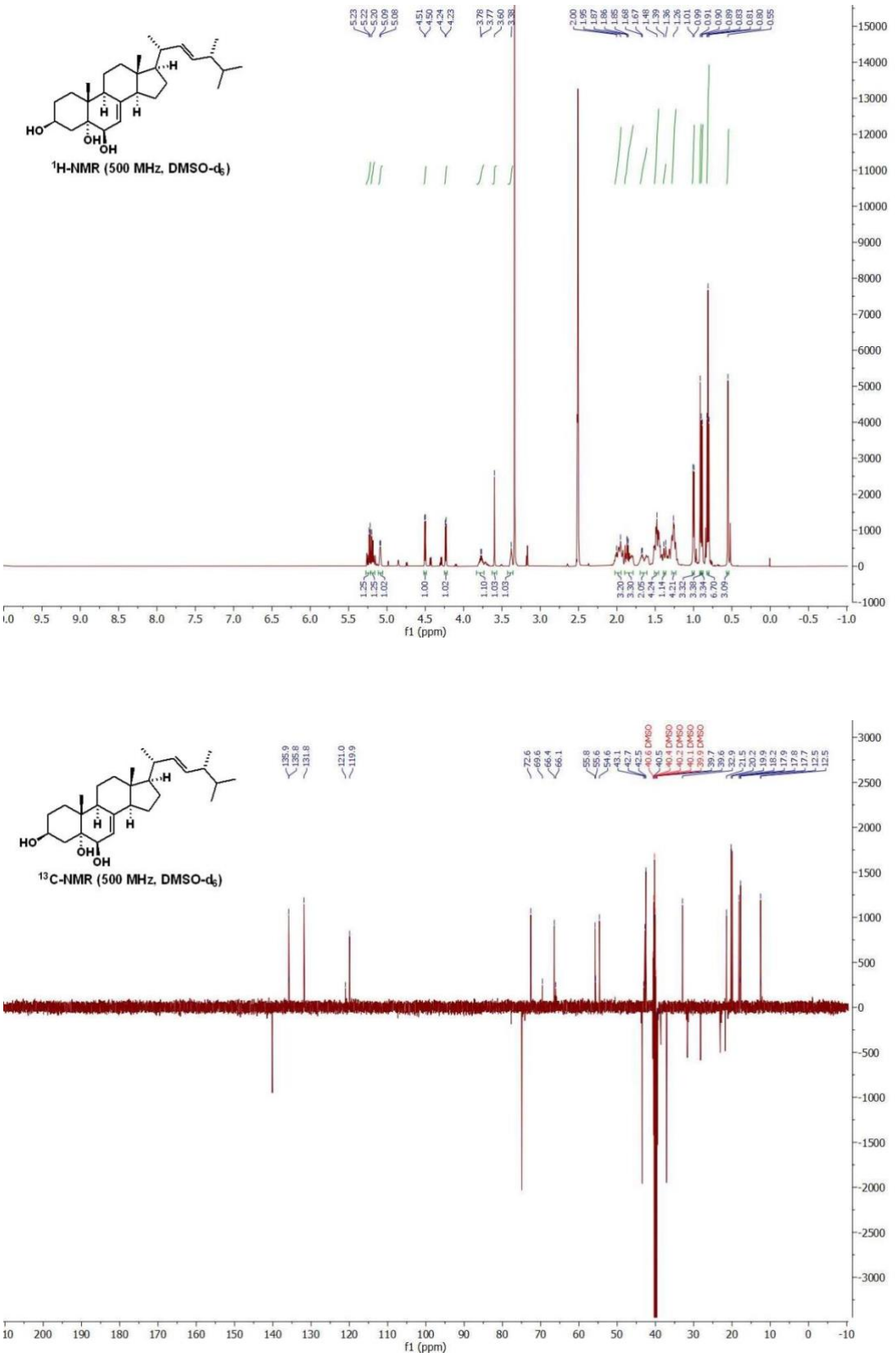


Figure 30: ^{1}HNMR and ^{13}C NMR of (22*E*)-ergosta-7,22-diene-3β,5α,6β-triol in DMSO-d6 (**123**)

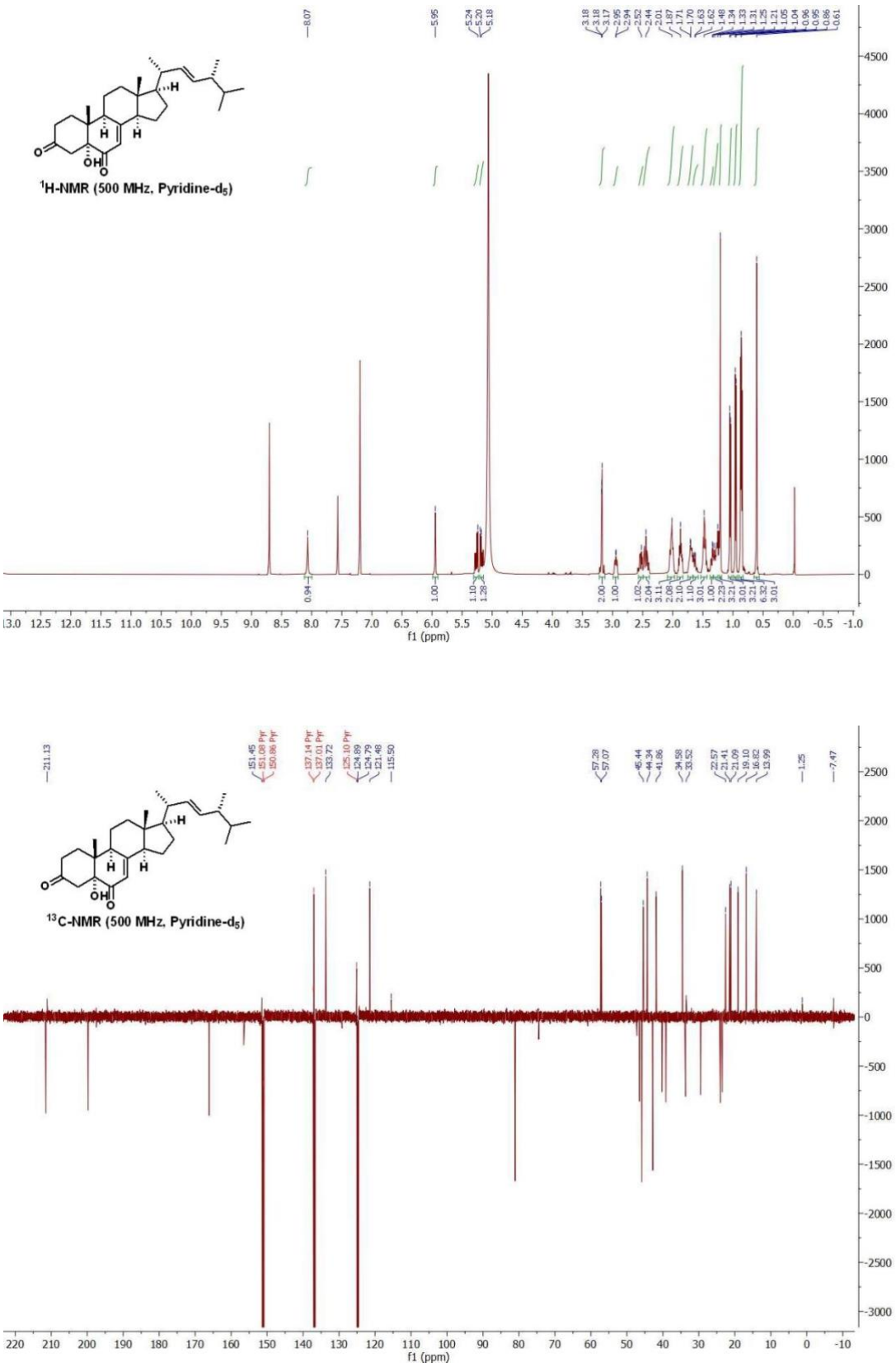


Figure 31: ^{1}HNMR and ^{13}C NMR of (22*E*)-5α-hydroxyergosta-7,22-diene-3,6-dione (**127**)

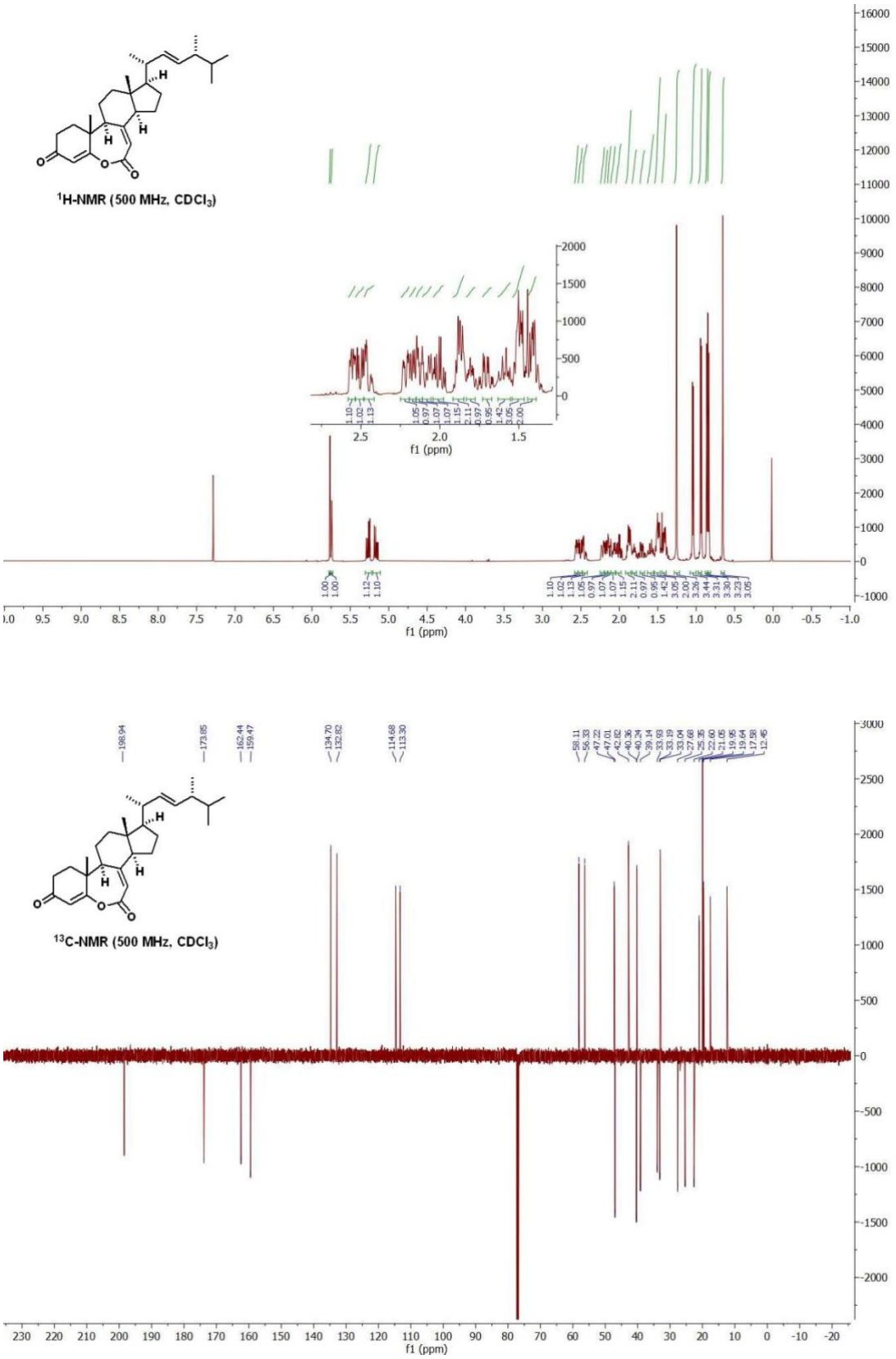


Figure 32: ^{1}HNMR and ^{13}C NMR of herbarulide (**126**) in $CDCl_3$.

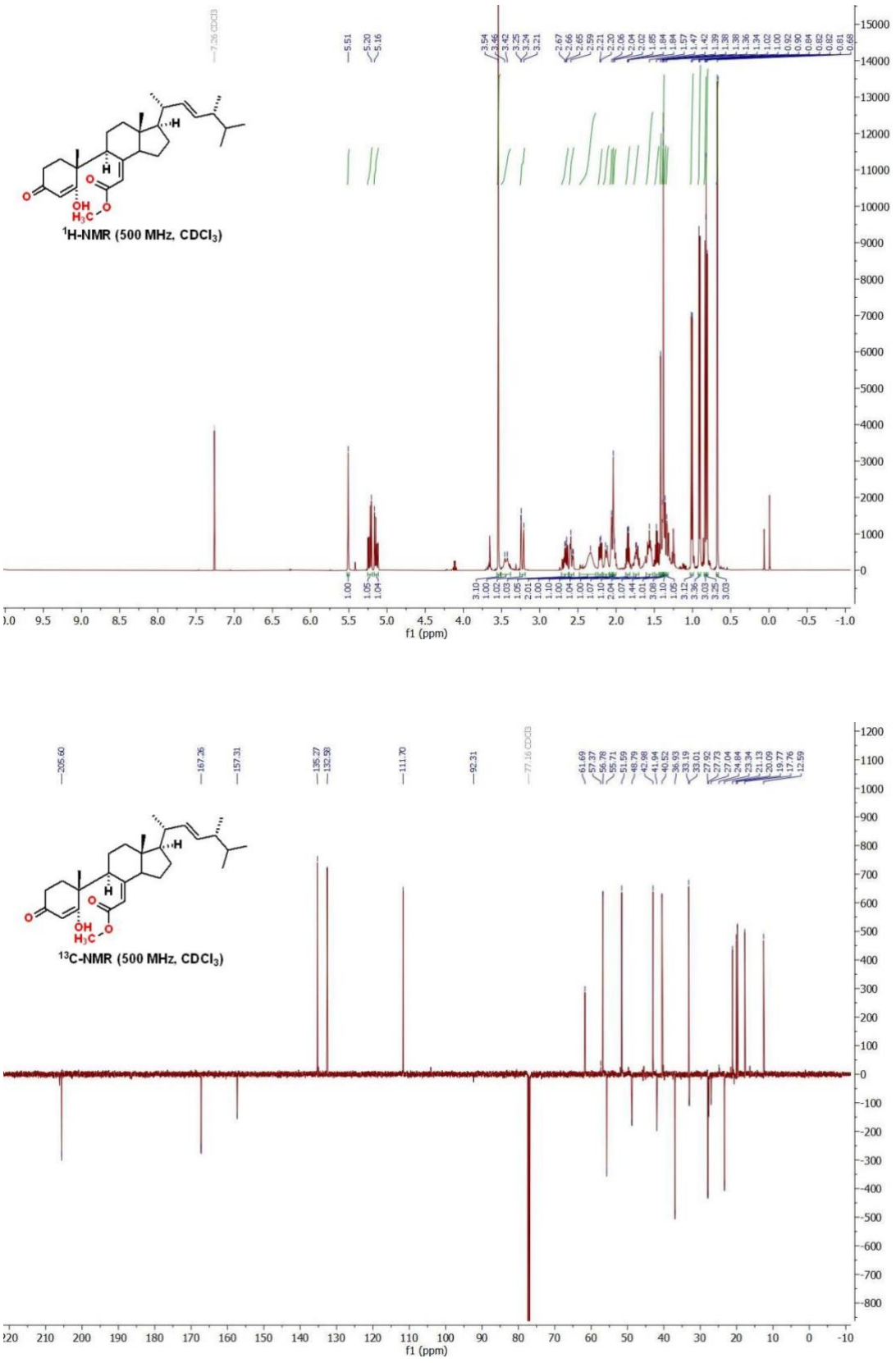


Figure 33: ^{1}H NMR and ^{13}C NMR of (22*E*)-5-hydroxy-3-oxo-ergosta-4,7,22-triene-6-methyl ester (**111**)

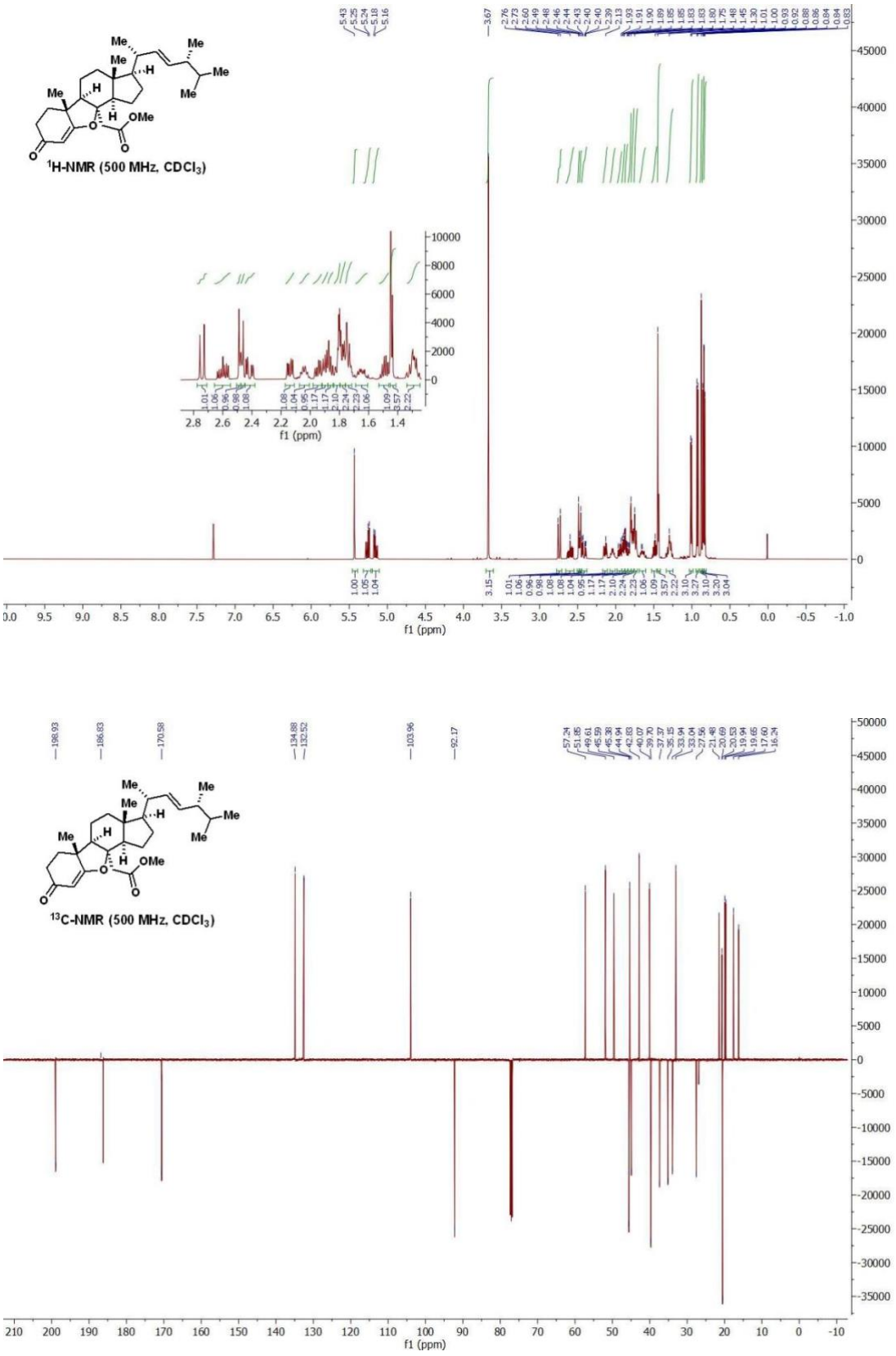


Figure 34: ^{1}HNMR and ^{13}C NMR of calvatianone (**10**) in $CDCl_3$.

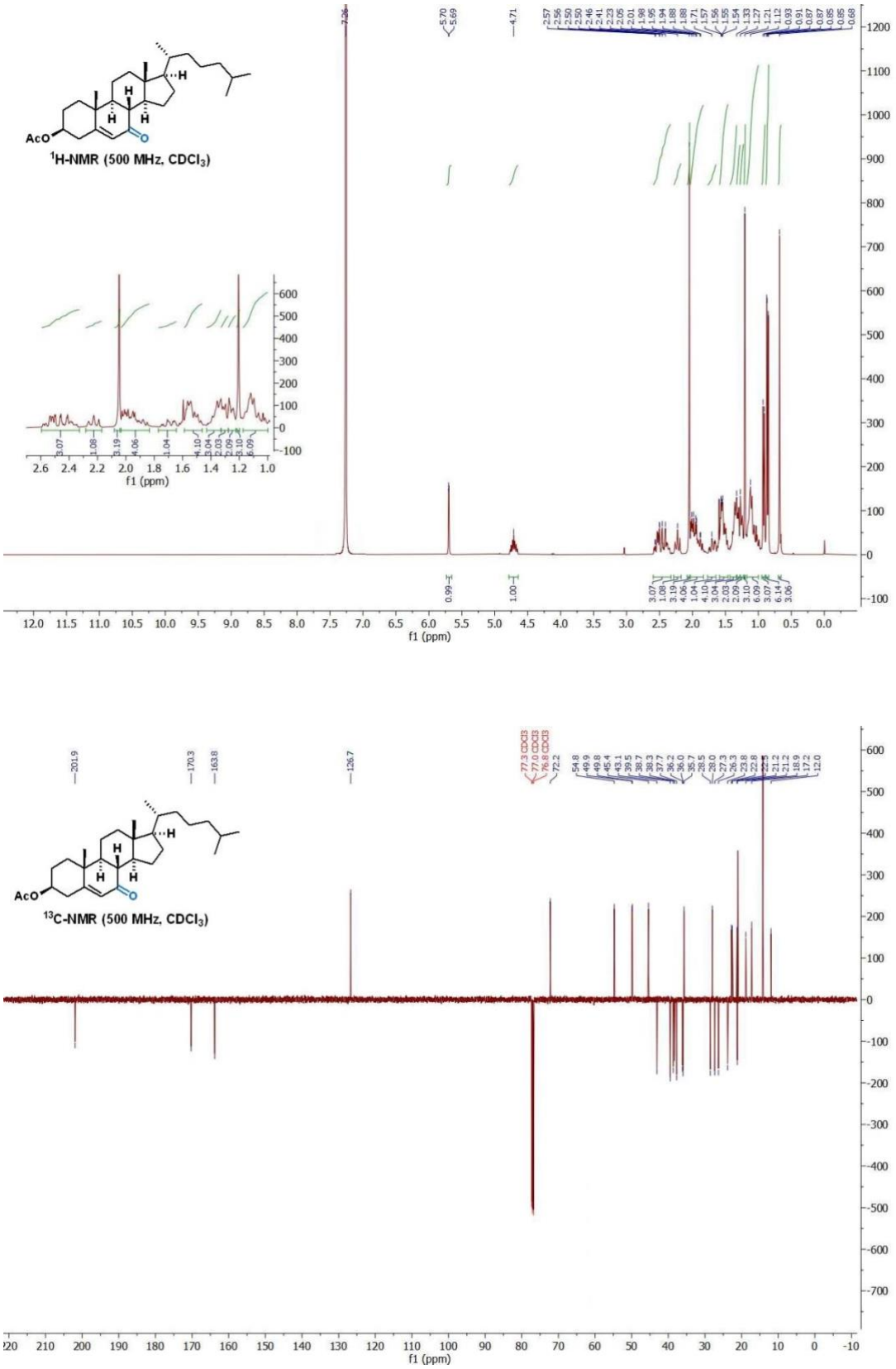


Figure 35: ^{1}HNMR and ^{13}C NMR of 7-oxo-cholest-5-en-3-yl acetate (**146**) in $CDCl_3$.

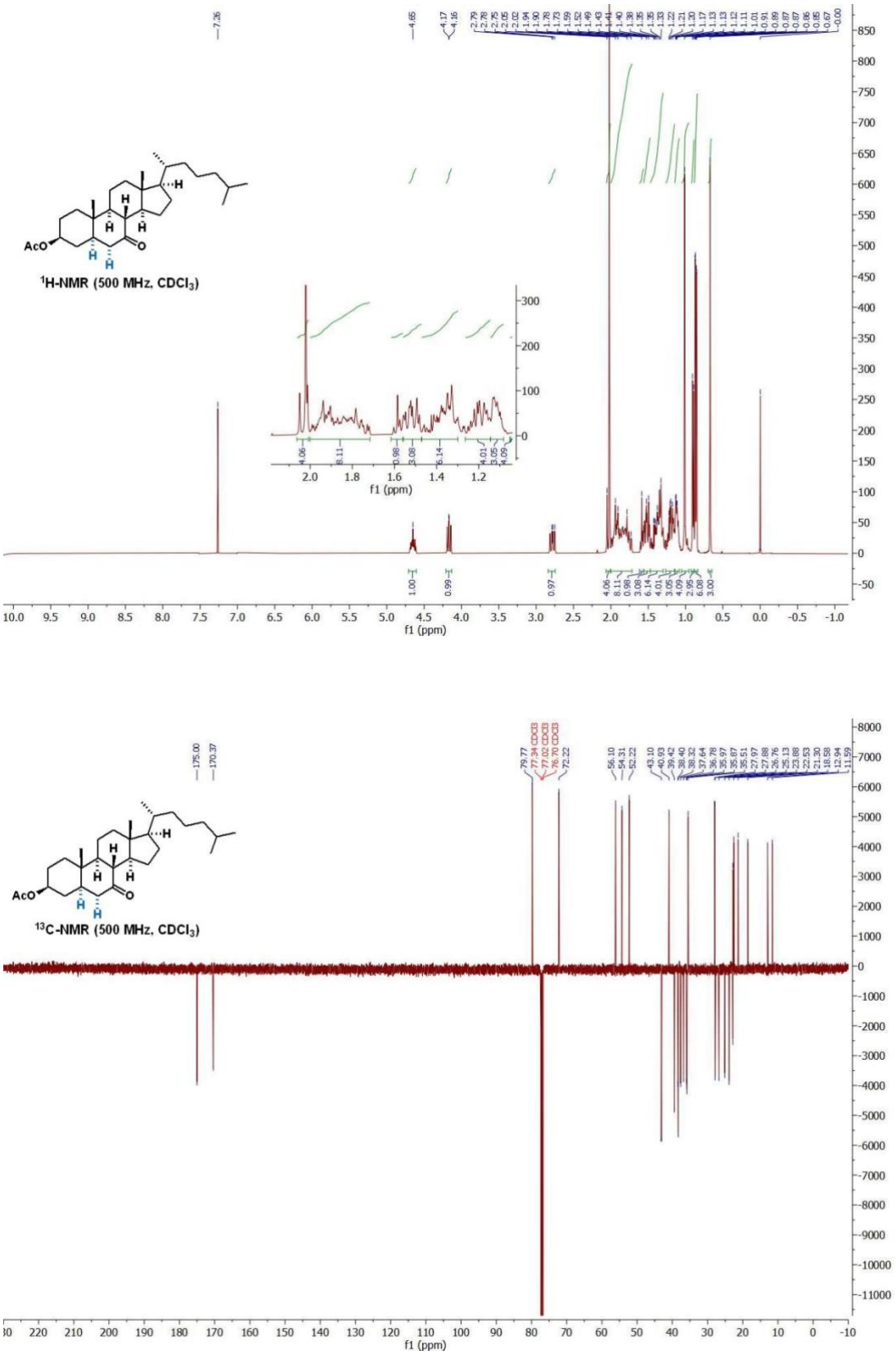


Figure 36. ^{1}H NMR and ^{13}C NMR **144** in $CDCl_3$.

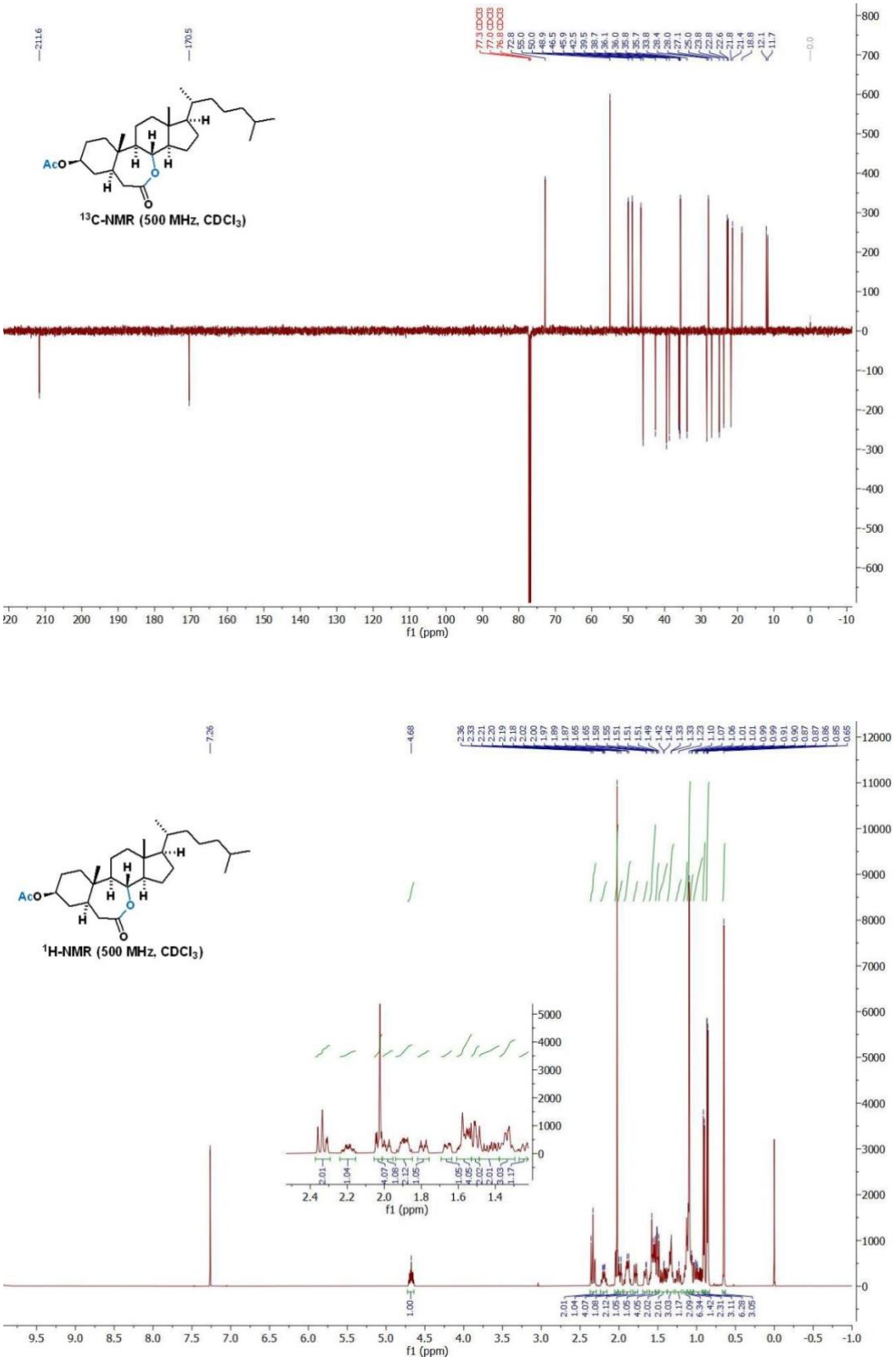


Figure 37. ^{1}HNMR and ^{13}C NMR of **143** in $CDCl_3$.

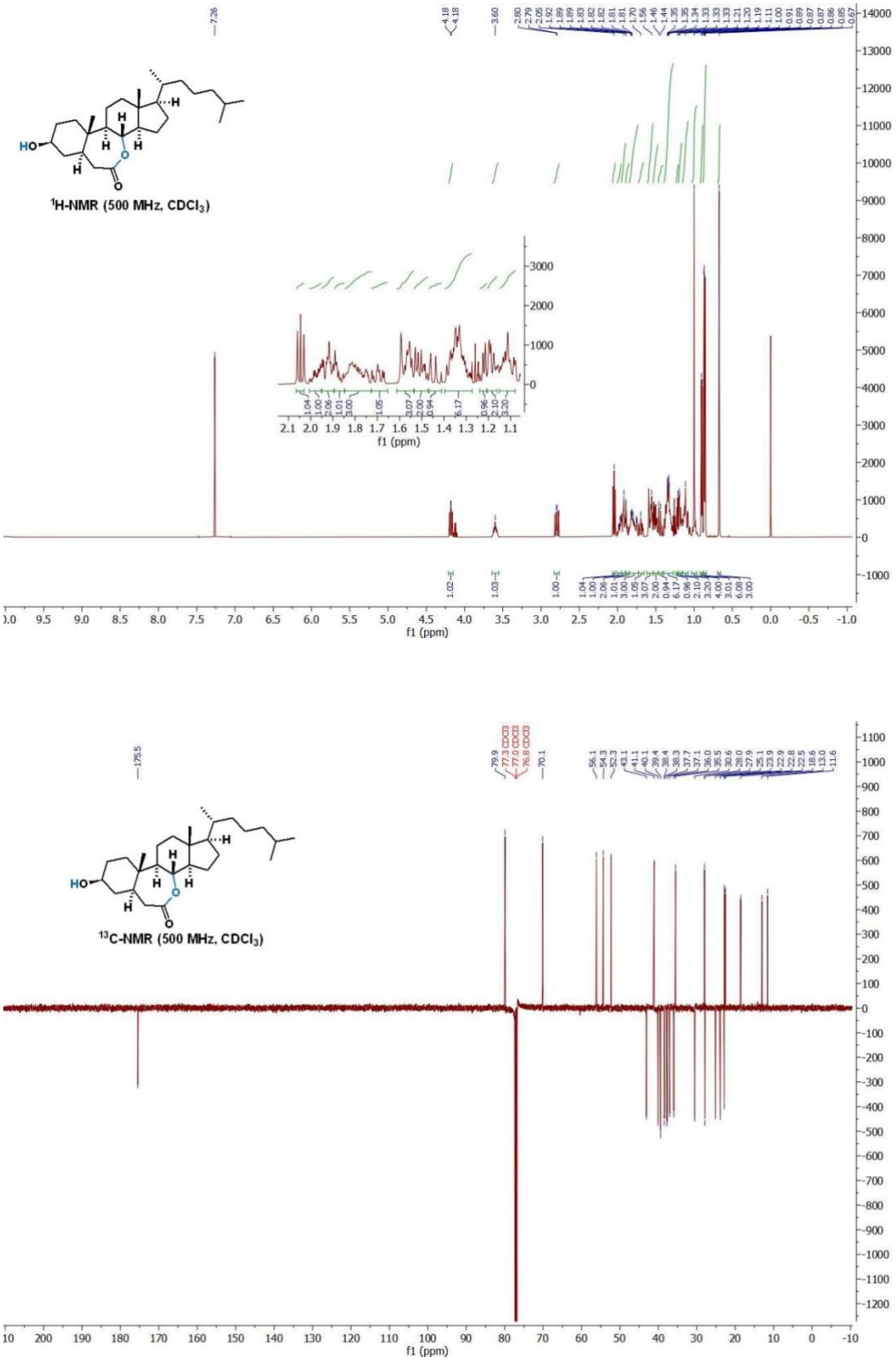
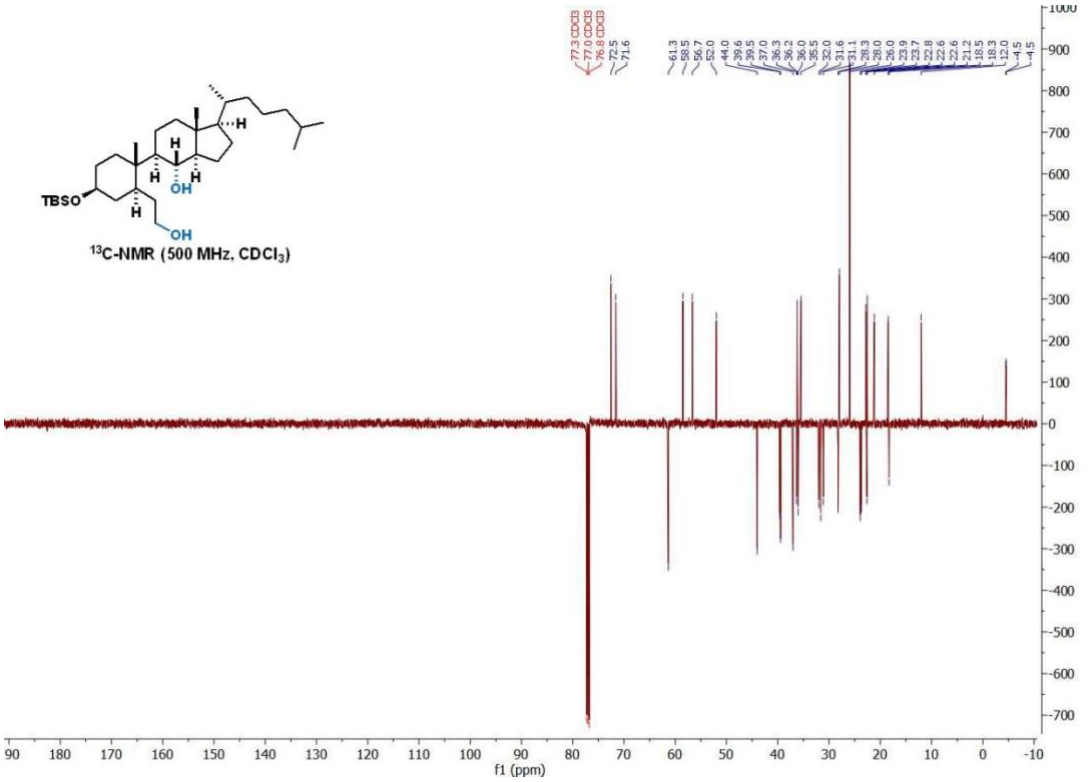


Figure 38. ^{1}H NMR and ^{13}C NMR des-acetyl-**143** in $CDCl_3$.



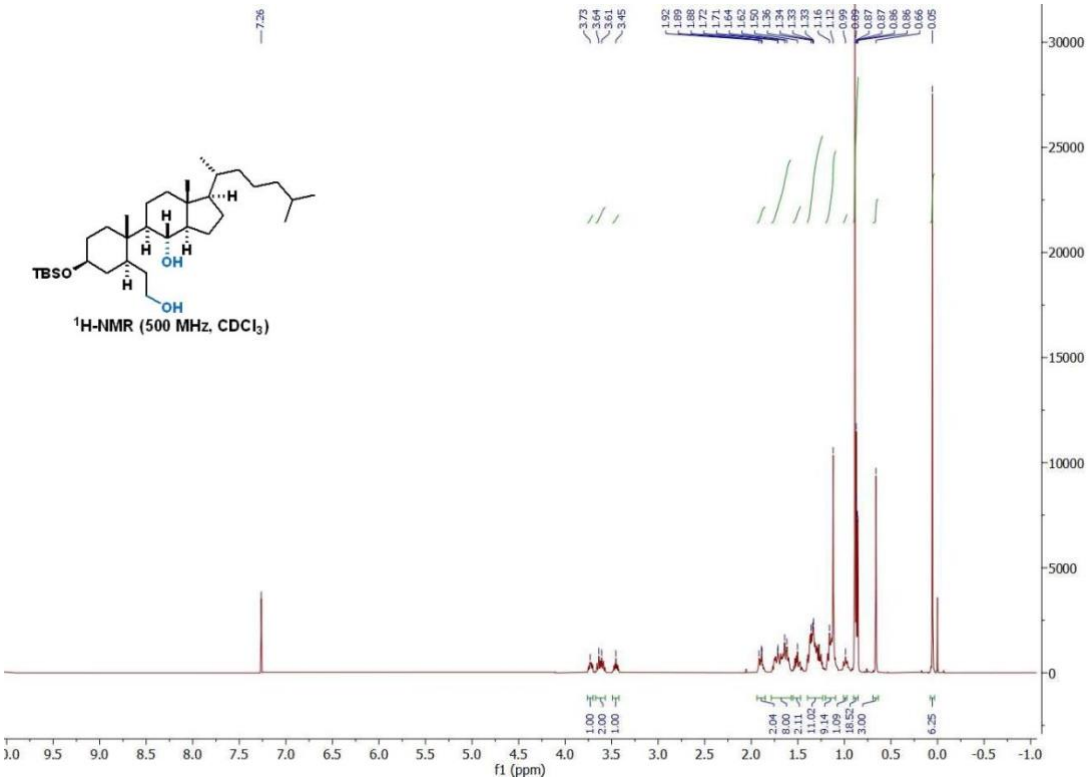


Figure **39**: ^{1}H NMR and ^{13}C NMR of **142** in $CDCl_3$.

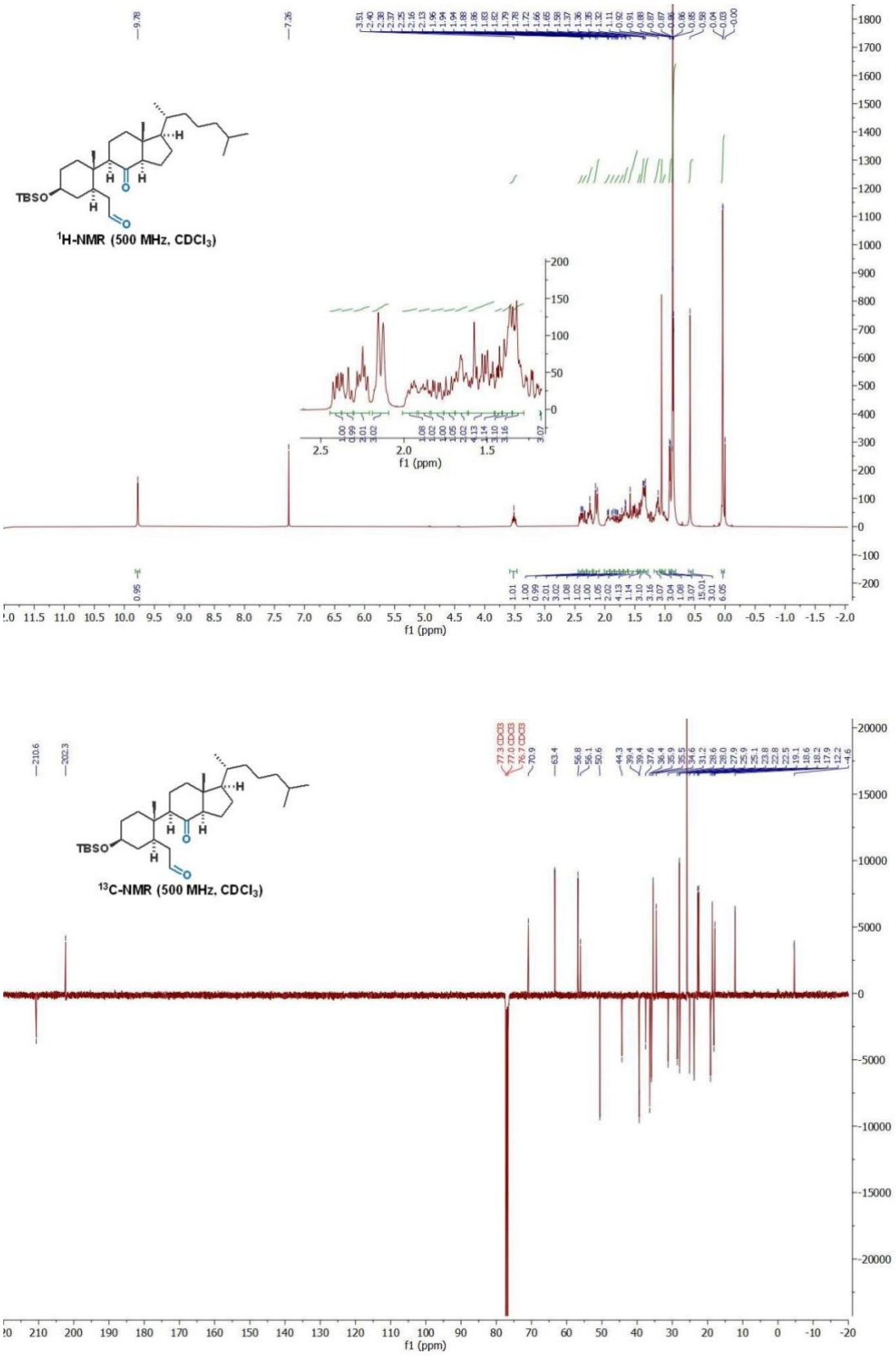


Figure 40. ^{1}H NMR and ^{13}C NMR of **141** in $CDCl_3$.

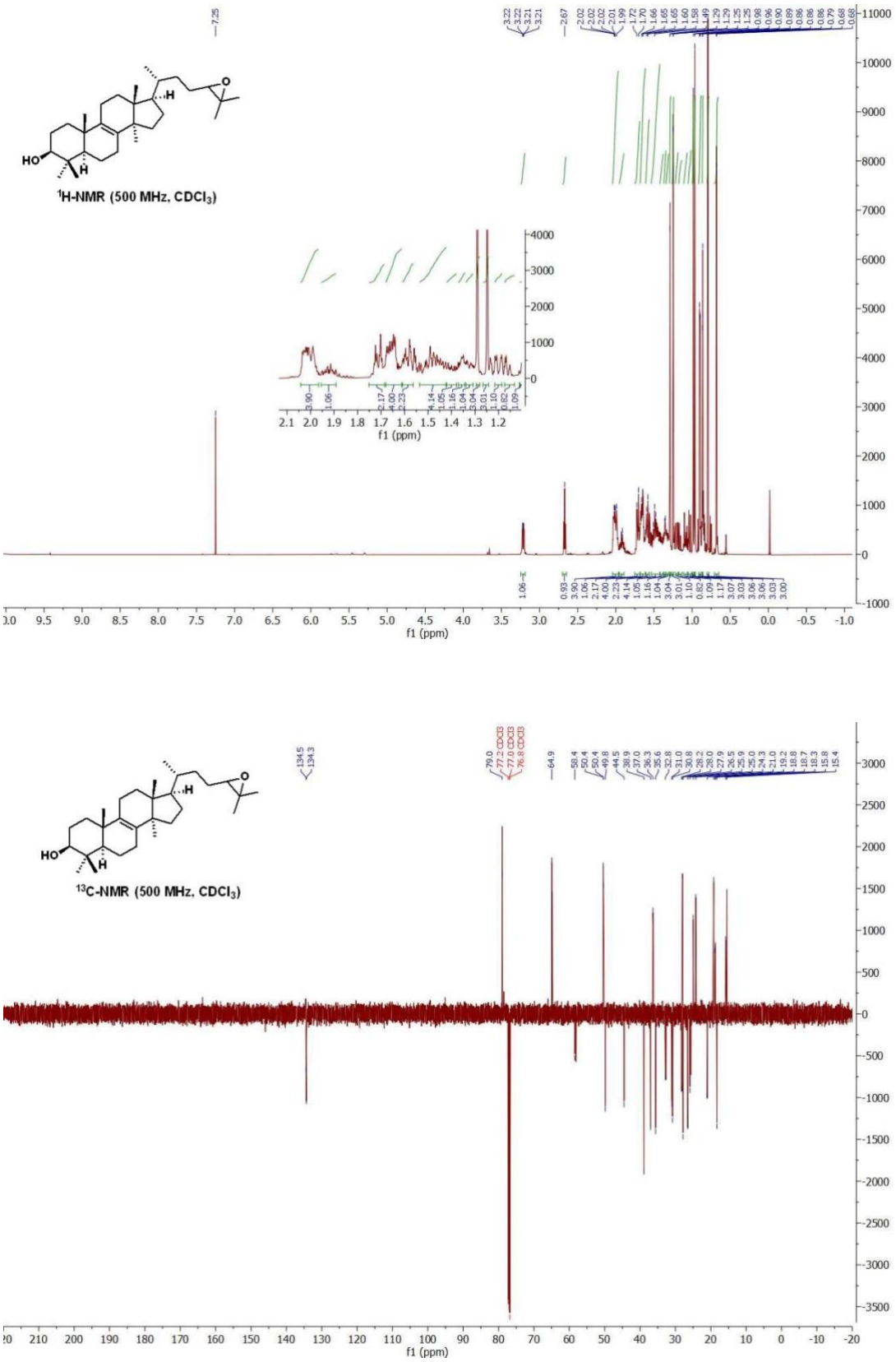


Figure 41. ^{1}HNMR and ^{13}C NMR of 25,26-epoxylanosterol (**154**) in $CDCl_3$.

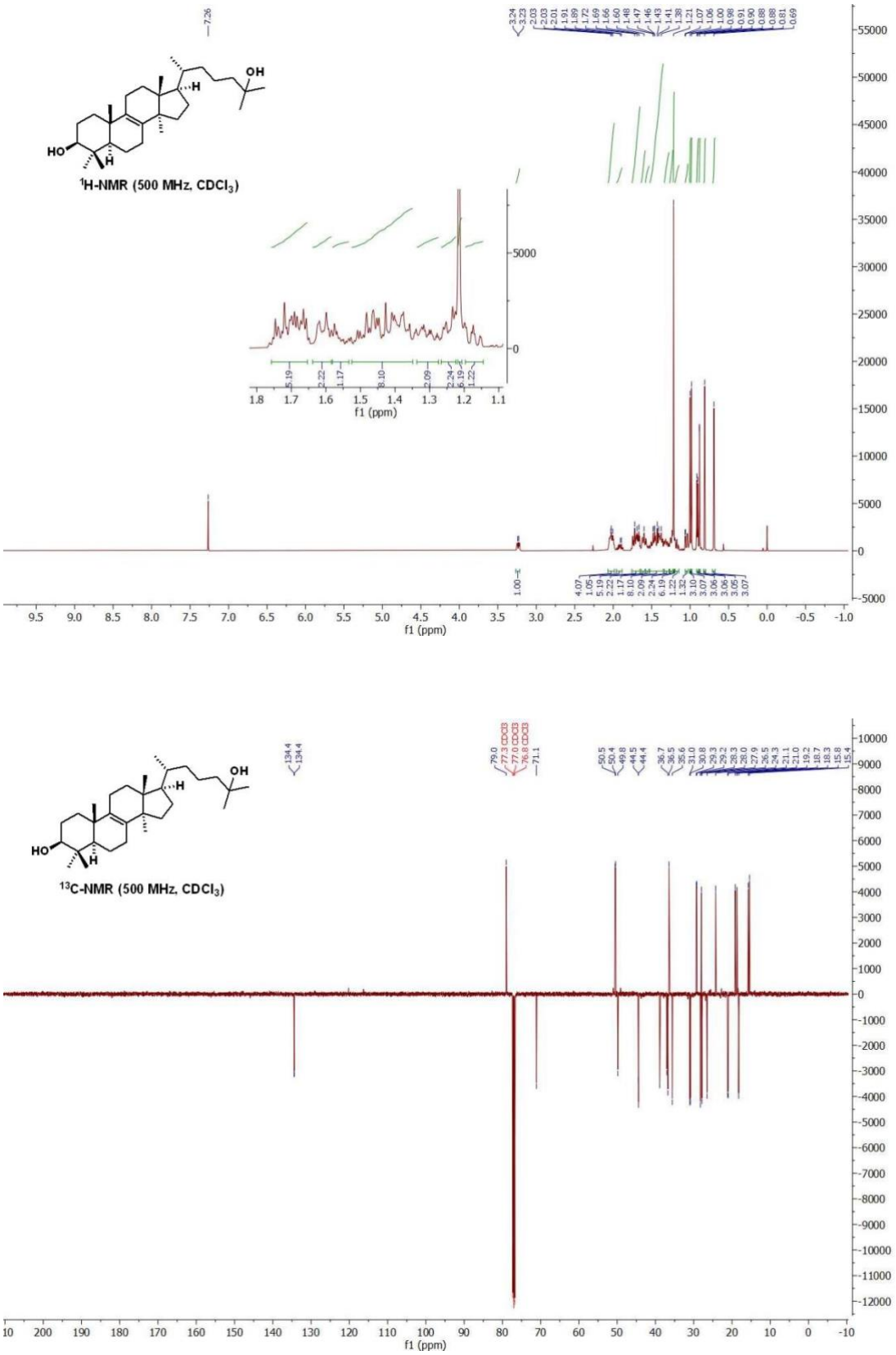


Figure 42. ^{1}H NMR and ^{13}C NMR of 25-H-26-hydroxy-lanosterol (**155**) in $CDCl_3$.

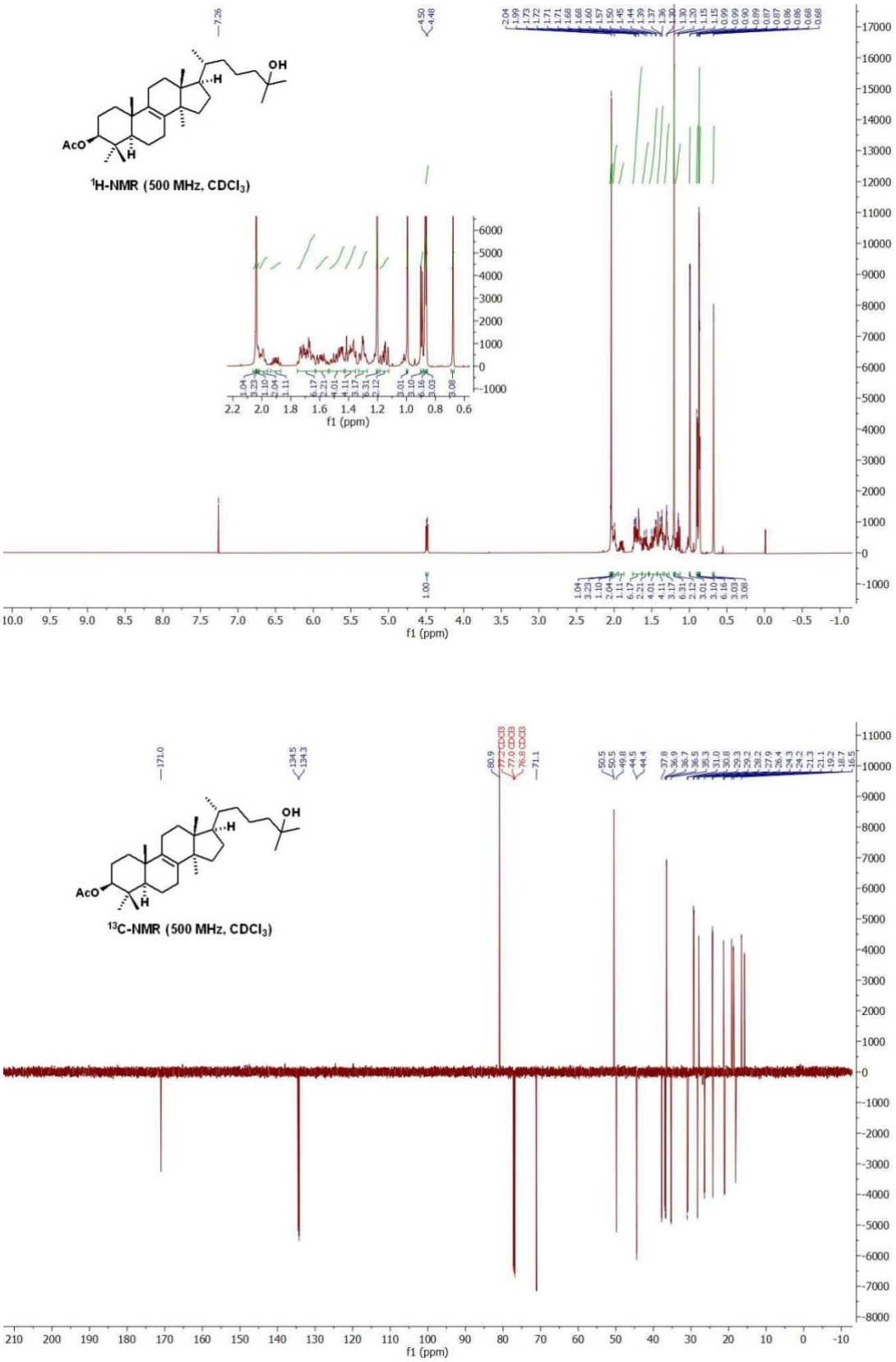


Figure 43. ^{1}H NMR and ^{13}C NMR of 25-H-26-hydroxylanosterol acetate (**153**) in $CDCl_3$.

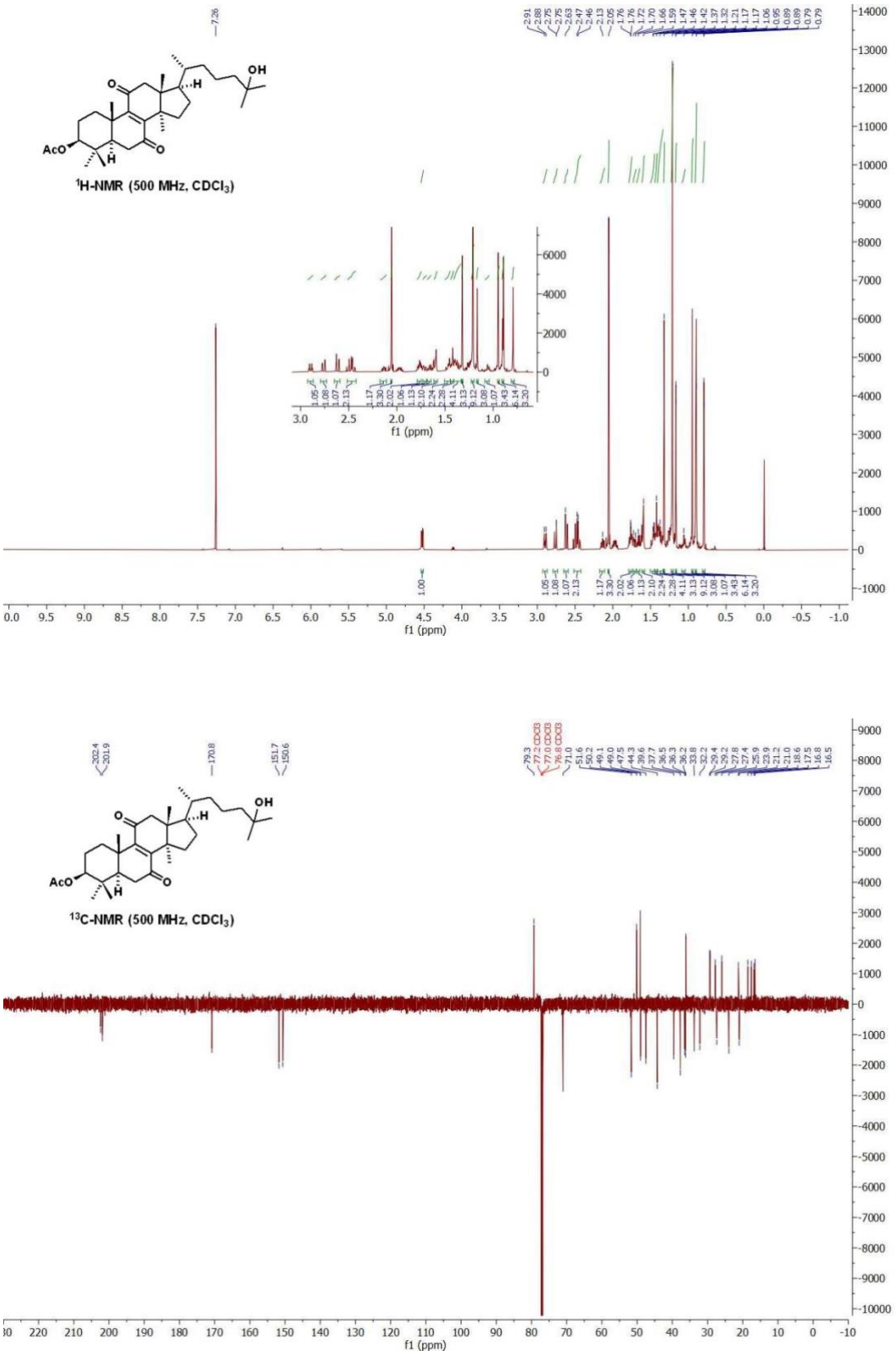


Figure 44. ^{1}H NMR and ^{13}C NMR of 25-H-26-hydroxy-7,11-dioxo-lanosterol acetate (**152**) in $CDCl_3$.

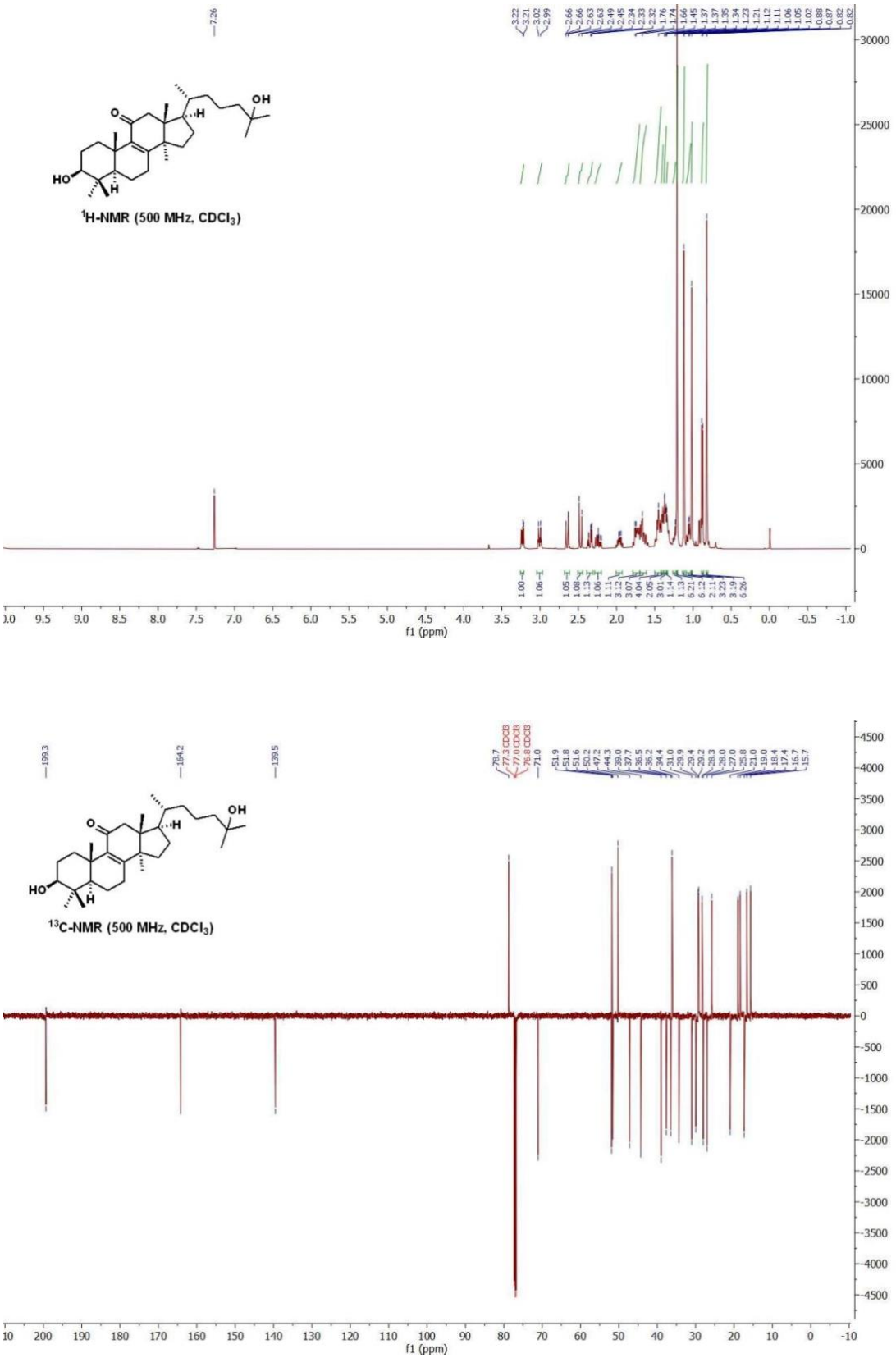
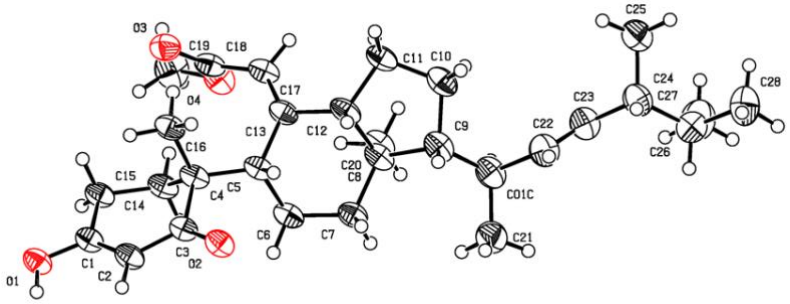


Figure 45. ^{1}H NMR and ^{13}C NMR of 25-H-26-hydroxy-11-oxo-lanosterol (**151**) in $CDCl_3$.

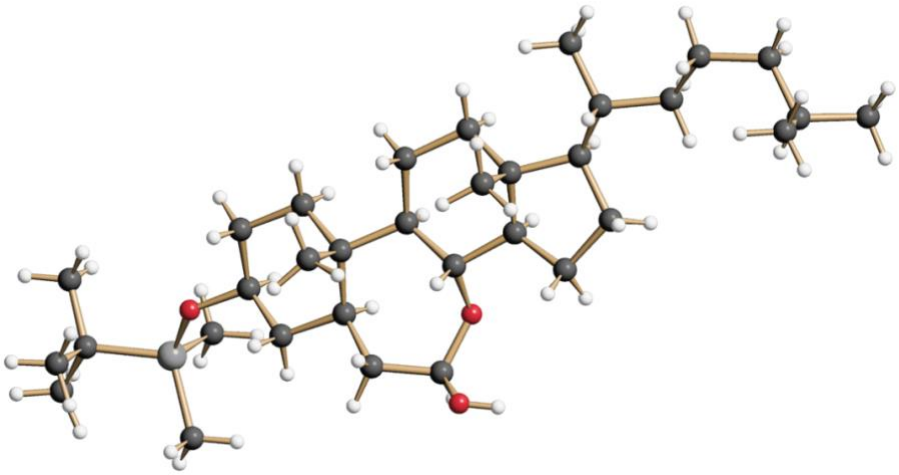
3.3 X-Ray Crystallographic Data

3.3.1 Crystal data and structure refinement for compound 111



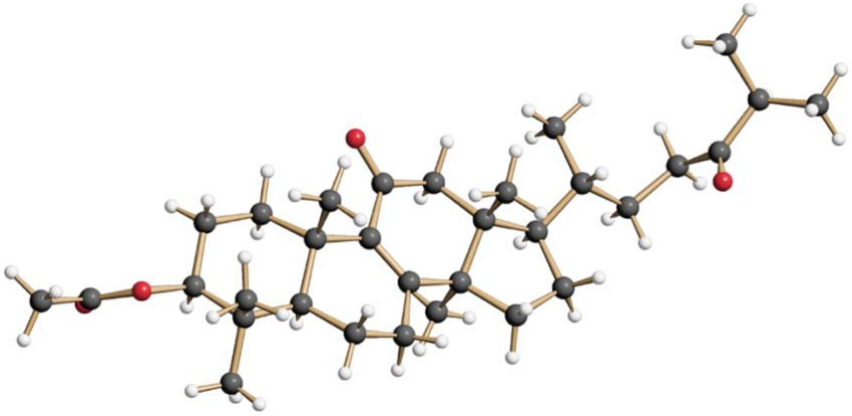
Empirical formula	$C_{29}H_{44}O_4$
Moiety formula	$C_{29}H_{44}O_4$
Formula weight	456.67 g/mol
Temperature	100(2) K
Wavelength	1.54178 Å
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	*a* = 6.7541(3) Å a= 90°.
	b = 22.7468(10) Å b= 90°.
	c = 39.272(2) Å g = 90°.
Volume	6033.6(5) $Å^3$
Z	4
Density (calculated)	1.102 Mg/m^3
Absorption coefficient	0.561 mm^{-1}
F(000)	2200
Crystal size	0.150 x 0.020 x 0.010 mm^3
Theta range for data collection	2.245 to 72.307°.
Index ranges	-8<=h<=7, -28<=k<=27, -48<=l<=48
Reflections collected	136358
Independent reflections	11935 [R(int) = 0.2135]
Completeness to theta = 67.679°	100.0 %
Absorption correction	Multiscan
Max. and min. transmission	0.7289 and 0.6144
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	11935 / 0 / 671
Goodness-of-fit on F^2	1.020
Final R indices [I>2sigma(I)]	R1 = 0.0660, wR2 = 0.1634
R indices (all data)	R1 = 0.0921, wR2 = 0.1860
Absolute structure parameter	-0.04(16)
Extinction coefficient	0.0051(4)
Largest diff. peak and hole	0.568 and -0.419 e.$Å^{-3}$

3.3.2 Crystal data and structure refinement for compound 147



Empirical formula	C33 H62 O3 Si [+ solvent]	
Moiety formula	C33 H62 O3 Si [+ solvent]	
Formula weight	534.91	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	C2	
Unit cell dimensions	a = 43.405(3) Å	a= 90°.
	b = 6.5064(6) Å	b= 98.213(3)°.
	c = 25.2087(17) Å	g = 90°.
Volume	7046.1(9) Å3	
Z	8	
Density (calculated)	1.008 Mg/m3	
Absorption coefficient	0.782 mm-1	
F(000)	2384	
Crystal size	0.300 x 0.070 x 0.050 mm3	
Theta range for data collection	2.057 to 72.430°.	
Index ranges	-53<=h<=53, -8<=k<=6, -31<=l<=31	
Reflections collected	115734	
Independent reflections	13512 [R(int) = 0.0433]	
Completeness to theta = 67.679°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7536 and 0.6747	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	13512 / 1 / 687	
Goodness-of-fit on F2	1.059	
Final R indices [I>2sigma(I)]	R1 = 0.0421, wR2 = 0.1238	
R indices (all data)	R1 = 0.0432, wR2 = 0.1258	
Absolute structure parameter	0.026(4)	
Extinction coefficient	n/a	
Largest diff. peak and hole	1.282 and -0.352 e.Å-3	

3.3.3. Crystal data and structure refinement for compound 156



Empirical formula	C32 H50 O4	
Moiety formula	C32 H50 O4	
Formula weight	498.72	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 7.22760(10) Å	a= 90°.
	b = 18.7106(4) Å	b= 108.8146(11)°.
	c = 11.2042(3) Å	g = 90°.
Volume	1434.21(5) $Å^3$	
Z	2	
Density (calculated)	1.155 Mg/m^3	
Absorption coefficient	0.575 mm^{-1}	
F(000)	548	
Crystal size	0.200 x 0.070 x 0.010 mm^3	
Theta range for data collection	4.168 to 71.997°.	
Index ranges	-7<=h<=8, -22<=k<=23, -13<=l<=13	
Reflections collected	29033	
Independent reflections	5520 [R(int) = 0.0482]	
Completeness to theta = 67.679°	98.5 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7536 and 0.6510	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5520 / 1 / 334	
Goodness-of-fit on F^2	1.026	
Final R indices [I>2sigma(I)]	R1 = 0.0332, wR2 = 0.0867	
R indices (all data)	R1 = 0.0341, wR2 = 0.0874	
Absolute structure parameter	0.13(7)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.232 and -0.162 e.$Å^{-3}$	

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