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Chemo-Enzymatic Total Synthesis of Sorbicillinoid Natural Products

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• **A. Sib**, T. A. M. Gulder, Chemo-enzymatic Total Synthesis of Oxosorbicillinol, Sorrentanone, Rezishanones B and C, Sorbicatechol A, Bisvertinolone, and (+)-Epoxysorbicillinol, *Angew. Chem. Int. Ed.* **2018**, *57*, 14650 – 14653, DOI: 10.1002/anie.201802176.

To my family, who taught me to:

„ ALWAYS LOOK ON THE BRIGHT SIDE OF LIFE“

-ERIC IDLE-

Contents

Abstract

The sorbicillinoids are a polyketide family with over 50 members, which possess highly complex structures and a diverse range of potent bioactivities. Because of the high density of functional groups and specific stereoinformation, the chemical enantioselective synthesis of these natural products is hardly possible. Investigations on the biosynthesis of sorbicillinoids revealed a highly efficient pathway of nature to the targeted molecules. By oxidative dearomatization of the polyketide precursor sorbicillin with the enzyme SorbC, a reactive intermediate in sorbicillinoid biosynthesis, named sorbicillinol, is produced. SorbC is a FAD-dependent oxidoreductase and acts by hydroxylating the sorbicillin ring structure at the C4-position, thus leading to dearomatization. With this knowledge we decided to focus on the chemo-enzymatic total synthesis of these fascinating scaffolds in this thesis. By using recombinant monooxygenase SorbC and chemically accessible sorbicillin-type synthetic precursors, enantioselective, one-pot synthetic routes to a broad range of sorbicillinoids were developed.

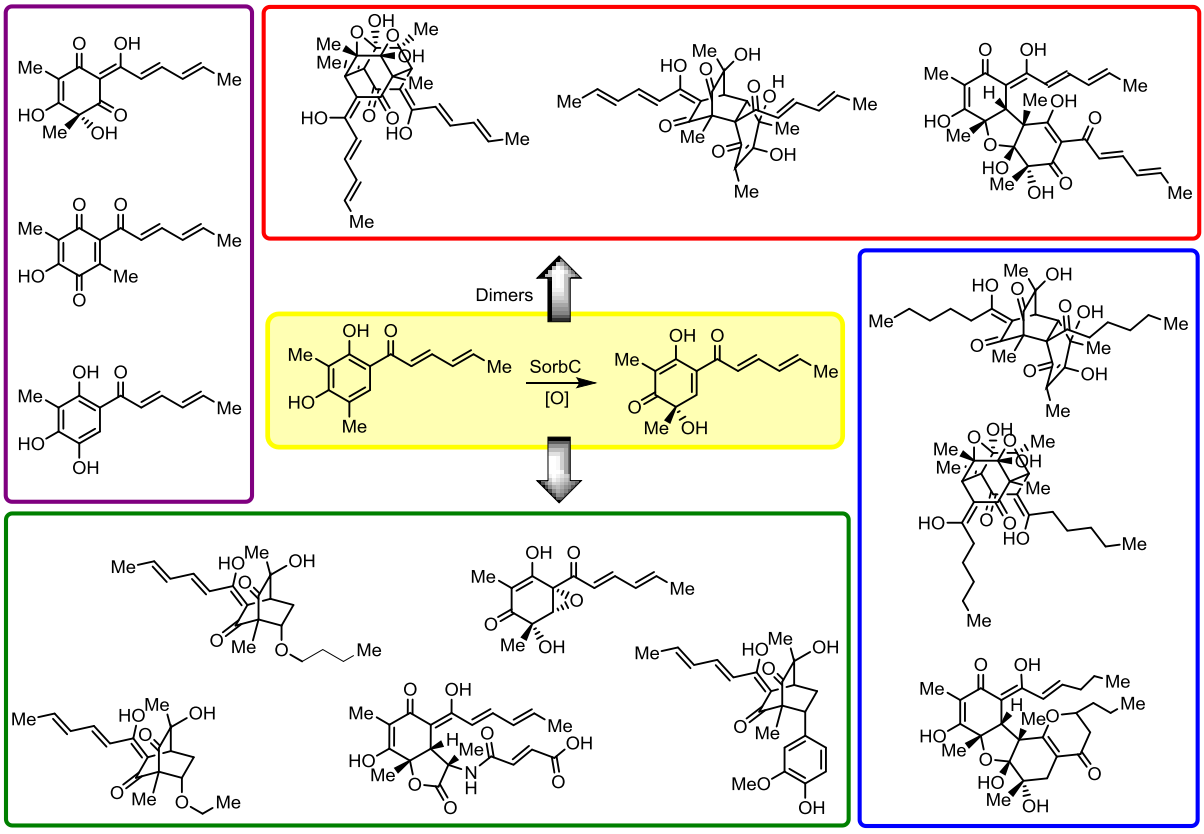
Focusing first on dimeric compounds, a co-solvent dependent enzymatic transformation did either lead to a [4+2] cycloaddition of two sorbicillinol moieties to bicycle[2.2.2]octandione frameworks such as bisorbicillinol by the use of acetone as co solvent. Alternatively, by using a more polar co-solvent like DMF, the kinetically slower Michael-addition with subsequent ketalization gave the cage like structure of trichodimerol. In addition to sorbicillinol dimers, we were also able to produce heterodimers. Adding sorbicillin and pyridine to semi-purified sorbicillinol lead to the first total synthesis of sorbiquinol, a Diels-Alder product formed by reaction of the cyclic core of sorbicillinol with the side chain of sorbicillin. Through the addition of oxosorbicillinol and pyridine to semi-purified sorbicillinol in DMF the [6.5.6]tricyclic core of the last dimer group, the bisvertinols, was made accessible.

The next aim was to examine the substrate specificity of monooxygenase SorbC by the chemical synthesis of different side chain and core analogs. Using the saturated hexyl side chain the corresponding bisorbicillinol and trichodimerol derivatives were formed. A 2-hexenyl side chain analog formed through oxidative dearomatization by SorbC a bisvertinol core with an attached dihydro-4-pyranone, the latter being formed by an additional Michael addition of an oxygen function onto the α,β- unsaturated side chain. Derivatisation of the core system was mainly made at the C4- and C5-postion, aiming to change the substrate right at the site of the enzymatic oxidation. We found that demethylsorbicillinol was converted to an aromatic triol, 2,5-dimethylsorbicillin lead to the formation of the monomeric sorbicillinoid natural product sorrentanone, and 5-hydroxysorbicillinol formed oxosorbicillinol by transformation with SorbC. Although not very promiscuous, SorbC is thus able to transform a variety of different precursors.

Having synthesized a broad range of monomeric and dimeric sorbicillinoids, we set out for the synthesis of functionalized sorbicillinoids. By inverse electron demand Diels Alder cycloadditions the natural products rezishanone B and C as well as anti-viral sorbicatechol A got accessible. Further we were able to establish the first enantioselective total synthesis of (+)-epoxysorbicillinol by a Weitz-Schaeffer epoxidation of sorbicillinol.

As a start for biological activity screening, we decided to derivatize the catechol portion of sorbicatechol A. We selected this molecule because of the facile access to derivatization by Diels-Alder cycloaddition with different dienophiles and the already reported good anti-viral activity of sorbicatechol A. The catechol moiety was exchanged by substituted aromatic systems, alkanes or even heterocycles. Following this concept we synthesized 14 derivatives and tested them for their anti-viral activity against HIV and additionally against influenza. We were able to detect one promising derivative active against HIV with an IC_{50} value of 32.2 ± 2.52 µM.

Taken together, we introduced the enantioselective total syntheses of 10 sorbicillinoid natural products, which were either the first or superior to all established synthesis and produced 17 selected sorbicillinoid derivatives. We therefore significantly contributed to research on sorbicillinoid natural products by simplifying and providing synthetic routes to monomeric, dimeric and functionalized sorbicillinoids as well as by providing material for further screening on biological activity or derivatization of compounds of this highly intriguing class of fungal natural products.



Zusammenfassung

Die Sorbicillinoide sind eine Polyketidfamilie mit über 50 Mitgliedern, die hoch komplexe Strukturen und eine vielseitige Bandbreite höchst potenter Bioaktivitäten besitzen. Aufgrund der hohen Dichte an funktionellen Gruppen und spezifischer Stereoinformation ist die chemische enantioselektive Synthese dieser Naturstoffe nur schwer möglich. Die Untersuchung der Biosynthese der Sorbicillinoide deckte den effizienten Weg der Natur zur Synthese der Zielmoleküle auf. Durch oxidative Dearomatisierung des Polyketidvorläufers Sorbicillin mit dem Enzym SorbC wird das reaktive Intermediat Sorbicillinol hergestellt. SorbC ist eine FAD-abhängige Oxidoreduktase und führt durch Hydroxylierung der Ringstruktur von Sorbicillin an der C4-Position zur Dearomatiserung des Moleküls. Auf Basis dieses Wissens entschieden wir uns, den Fokus dieser Arbeit auf die chemo-enzymatische Totalsynthese dieser faszinierenden Molekülgerüste zu legen. Mithilfe der rekombinanten Monooxygenase SorbC und chemisch-synthetisch zugänglichen sorbicillinartigen Vorläufermolekülen, konnte eine enantioselektive, Ein-Topf Syntheseroute zu einem breiten Spektrum an Sorbicillinoiden etabliert werden.

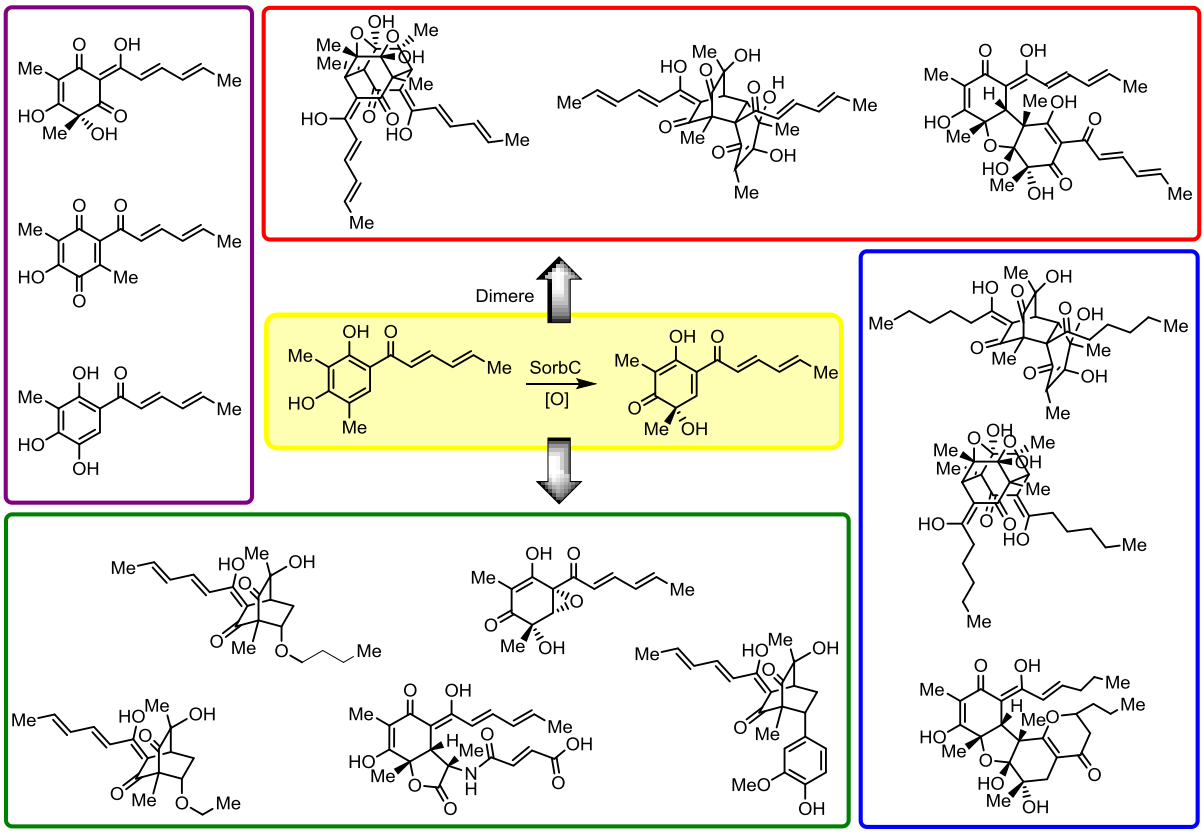
Der Fokus wurde zuerst auf die dimeren Substanzen gelegt. Hierbei führte die Cosolvent-abhängige enzymatische Umwandlung bei Nutzung von Aceton als Cosolvenz zu einer [4+2] Cycloaddition von zwei Sorbicillinoleinheiten zu einem Bicyclo[2.2.2]octandiongerüst wie in Bisorbicillinol. Alternativ konnte bei Verwendung eines polareren Lösungsmittels, wie DMF, die kinetisch langsamere Michael-Addition mit folgender Ketalisierung bevorzugt werden, wobei das käfigartige Trichodimerolgerüst entstand. Neben den Sorbicillinoldimeren, konnten wir zudem Heterodimere herstellen. Indem wir Sorbicillin und Pyridin zu teilgereinigtem Sorbicillinol gaben, war es uns möglich, die erste Totalsynthese von Sorbiquinol, einem Diels-Alder Produkt aus dem Ringsystem von Sorbicillinol mit der Seitenkette von Sorbicillin, zu etablieren. Durch die Zugabe von Oxosorbicillinol und Pyridin zu teilgereingtem Sorbicillinol in DMF konnte zudem der [6.5.6]trizyklische Kern der letzten Dimergruppe, der Bisvertinole, zugänglich gemacht werden.

Das nächste Ziel war die Untersuchung der Substratspezifitiät der Monooxygenase SorbC durch die chemische Synthese verschiedener Seitenketten- und Ringderivate. Durch die Verwendung einer gesättigten Hexylseitenkette an Sorbicillin wurden Bisorbicillinol und Trichodimerolderivate gebildet. Das 2-Hexenylseitenkettenderivat von Sorbicillin bildete bei der oxidativen Dearomatisierung durch SorbC ein Bisvertinolgerüst mit einem erweiterten Dihydro-4-pyranon durch eine zusätzliche Michael Addition einer Sauerstofffunktion an die α,β- ungesättigte Seitenkette. Die Derivatisierung des Ringsystems wurde hauptsächlich an der C4- und C5 Position vorgenommen, um das Substrat genau an der Position der enzymatischen Oxidation zu variieren. Wir fanden heraus, dass

Demethylsorbicillinol zu einem aromatischen Triol umgewandelt wurde, 2,5-Dimethylsorbicillin zur Bildung des monomeren sorbicillinoiden Naturstoff Sorrentanone führte und 5-Hydroxysorbicillin durch Einfluss von SorbC zu Oxosorbicillinol reagierte. Obwohl SorbC keine große Substratpromiskuität zeigte, konnten also eine Reihe verschiedener Vorgänger erfolgreich umgesetzt werden. Nachdem wir so eine Vielzahl an monomeren und dimeren Sorbicillinoiden hergestellt hatten, nahmen wir die Synthese von funktionalisierten Sorbicillinoiden in Angriff. Durch inverse Diels Alder Cycloaddition konnten die Naturstoffe Rezishanon B und C, sowie das anti-viral wirkende Sorbicatechol A zugänglich gemacht werden. Des Weiteren war es uns möglich, die erste enantioselective Totalsynthese von (+)-Epoxysorbicillinol durch eine Weitz-Schaeffer Epoxidierung von Sorbicillinol zu etablieren.

Als Anfang für ein Screening der biologischen Aktivität entschlossen wir uns, die Catecholeinheit von Sorbicatechol A zu derivatisieren. Wir entschieden uns für diese Molekül, aufgrund der guten Zugänglichkeit der Derivate durch Diels-Alder Cycloaddition mit verschiedenen Dienophilen und der bereits publizierten guten antiviralen Aktivität. Die Catecholeinheit wurde mit substituierten aromatischen Systemen, Alkanen oder sogar Heterocyclen ausgetauscht. Diesem Plan folgend, synthetisierten wir 14 Sorbicatechol A Derivate und testeten sie gegen HIV und gegen Influenza A. Es gelang uns eine vielversprechende Substanz mit Aktivität gegen HIV mit einem IC_{50} Wert von 32.2 ± 2.52 µM zu entwickeln.

In dieser Arbeit stellen wir die enantioselektive Totalsynthese von 10 sorbicillinoiden Naturstoffen vor, wobei die Syntheserouten entweder die ersten sind, oder deutliche Verbesserung im Gegensatz zu bekannten Routen zeigen, sowie die Herstellung von 17 sorbicillinoiden Naturstoffderivaten. Wir haben einen signifikanten Beitrag zur Forschung an den sorbicillinoiden Naturstoffen geleistet, indem wir neue Synthesemöglichkeiten von zuvor synthetisch unzugänglichen monomeren, dimeren und funktionalisierten Sorbicillinoiden bereitgestellt haben, sowie bereits etablierte Routen vereinfachten. So ist es nun möglich, Material für weitere Untersuchungen auf biologische Aktivität oder Derivatisierung von Substanzen dieser höchst beeindruckenden Klasse fungaler Naturstoffe zu Verfügung zu stellen.


Dimere
SorbC
[O]

1. Introduction

1.1 Pharmacological relevance of natural products

To ensure selection advantages and survival, living organisms such as plants, fungi and bacteria produce secondary metabolites. These chemical compounds often show impressive pharmacological activity and - in contract to primary metabolites - it is believed that secondary metabolites are not essential for organism growth but instead provide specific ecological advantages. Secondary metabolites can fulfill a range of roles within the producing organisms. For example, they may act as chemical signaling molecules for communication between different organisms, they can inhibit growth of competitors, or work as repellents or attractants.[1] The majority of these compounds, which are often referred to as natural products, also have a range of bioactivities against diverse human pathogenic organisms. The extensive spectrum of bioactivities is possible due to the broad structural variety and complexity. Over 60% of the drugs that have been available on the market or were in clinical trials between 1981-2002 were natural products or their derivatives.[2]

In the evolutionary process, these biomolecules developed a huge structural diversity and complexity, which is extremely challenging for synthetic chemists to replicate. The high degree of functionalization and the defined complex stereo-structures often pose the greatest difficulties to be achieved with conventional chemical total syntheses. Therefore, chemo-enzymatic reactions, that is the transformation of a synthetic starting material by use of enzymes, gain increasing importance in the synthesis of complex natural products, because they often overcome the challenges of total syntheses. Biocatalysts have many advantages, for example, most enzymatic reactions are highly stereospecific and proceed under mild conditions. In addition, protective group chemistry can be reduced to a minimum. This results in increased overall efficiency by potentially increasing the total yield of the synthetic route by reducing the number of reaction steps. It is often even possible to combine multi-step sequences to a one-pot-reaction, saving solvents and chemicals, thus leading to an environmental friendly alternative and responsible treatment of nature and its resources, which is in general referred to as “green chemistry”.[3]

An interesting example is the oxidative dearomatization of phenols.[4] Many potent natural products contain substituted cyclohexene or cyclohexadiene units, biosynthetically originiating from oxidative dearomatization of the earlier phenyl ring by oxygenases.[4] Dearomatizations in general are reactions where the aromatic system is repealed by overcoming the resonance energy of the system

thereby activating the remaining double bonds for further functionalization. In the following sections, different ways of dearomatization are introduced.

1.2 Synthetic approaches for dearomatization

Aromatic molecules are a large group of organic compounds that constitute perfect starting materials for more complex and synthetically versatile intermediates. This is often required in natural product synthesis or medicinal chemistry, where a high degree of functionalization and specific stereoselectivity is absolutely necessary.[5] In addition to benzene, also heteroaromatic compounds such as thiophene, pyrrole and furan can undergo dearomatizations. In comparison, the resonance stability of these heteroarenes is much lower, making benzene the supreme target in monocyclic dearomatization.[6]

The broad spectrum of simple, cheap and readily available arenes and the high value of the complex products resulting from dearomatization arose the interest of chemists all over the world. Many different strategies have been explored, varying from reductive or oxidative dearomatization to nucleophilic or radical addition mechanisms, as well as transition-metal-mediated or -catalyzed dearomatizations, arenophile-mediated reactions or dearomative cycloadditions.[7] Each of these research fields has brought great advances in developing new methodologies in chemical synthesis, especially natural product total synthesis. An impressive example for a dearomative reduction is the synthesis of the antiviral drug oseltamivir phosphate (**1**, Tamiflu™, Scheme 1a) by Hoffmann-La Roche.[8] Under exposure of hydrogen and a ruthenium on alumina catalyst the aromatic starting material is completely saturated by *cis*-selective hydrogenation in a yield of 82%. The subsequent enzymatic desymmetrization introduces the correct stereoinformation, thus leading to the desired anti-viral oseltamivir phosphate (**1**) in a few additional steps with an overall yield of ~30%.

A nucleophilic route is used in the total synthesis of the shellfish toxin isodomic acid B (**2**), where a lithiated nucleophile attacks a benzene derivative resulting in dearomatization (Scheme 1b).[9] In contrast, the addition of carbon-centered radicals to arenes can build up aryl cyclitols (e.g., **3**) by radical dearomatization with subsequent oxidation (Scheme 1c).[10] Cyclitols such as **3** are carbasugars that have many pharmacological effects, for example D-pinitol is efficient in lowering blood glucose level and acts as an antioxidant agent.[11]

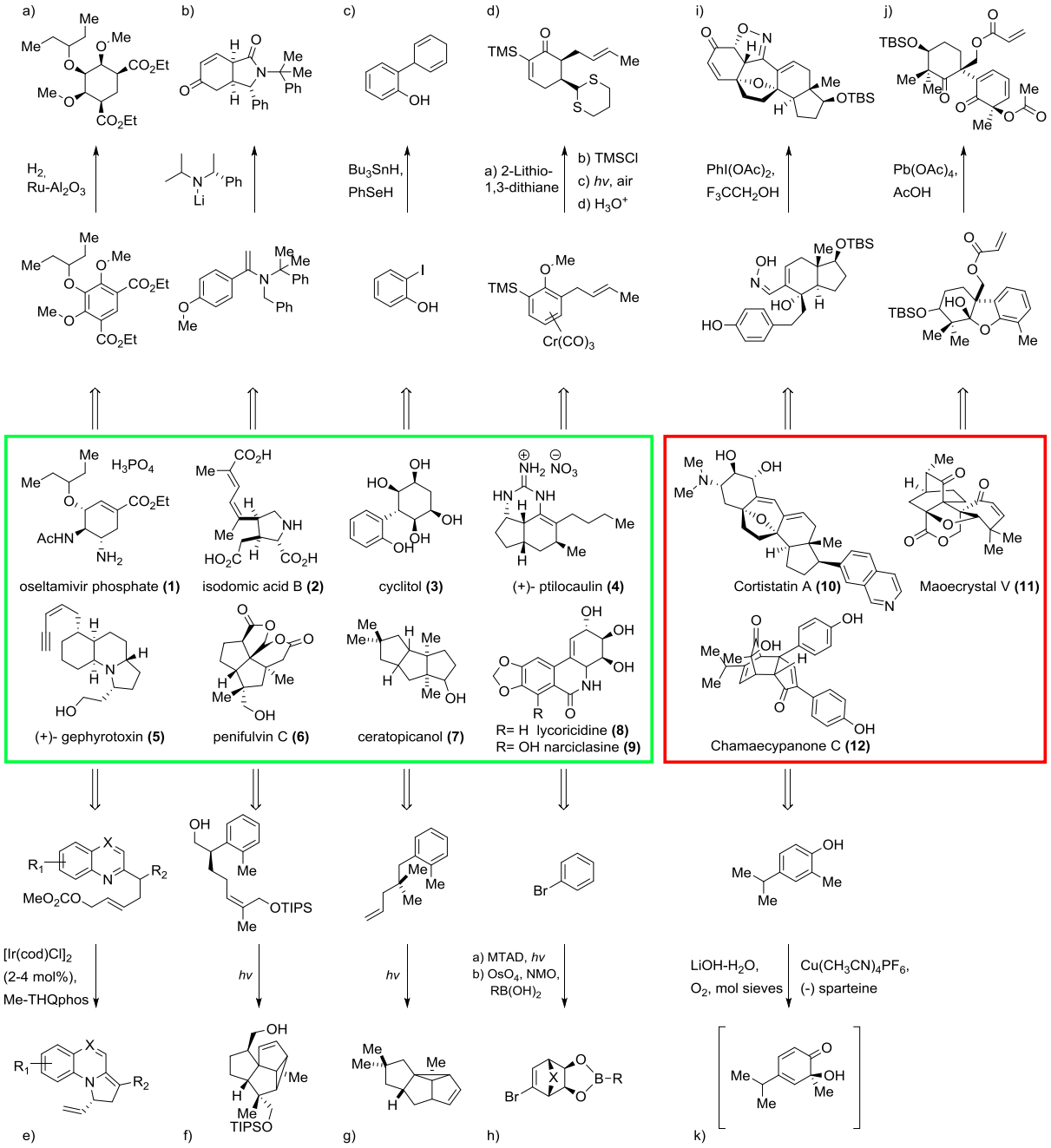
A more recent approach is the use of transition-metal-mediated dearomatization. By η^2-complexation with a metal the arene gets polarized, leading to a negative charge at the opposite position of the coordinating metal binding site. Consequently, an electrophilic attack is facilitated,

leaving a cationic intermediate, which in the following reacts with a nucleophile, thus regenerating the neutral complex. After decomplexation from the metal, the 1,2- or 1,4 substituted dearomatized products are accessed with defined stereo- and regioselectivity.[12] A nice example for this dearomatization route is the total synthesis of antimicrobial and cytotoxic (+)-ptilocaulin (**4**) where a prochiral chromium-arene complex is nucleophilically attacked by a lithiated dithiane with subsequent neutralization of the complex by acidic hydrolysis of the methoxy group, giving the corresponding enone in 45% yield and >99:1 *er* (Scheme 1d).[12]

The transition-metal catalyzed dearomatization, also known as catalytic asymmetric dearomatization (CADA), works by insertion of a coordinated ligand into the aromatic system. By Iridium CADA of an electron deficient arene, the facile synthesis of the key intermediate for the synthesis of (+)-gephyrotoxin (**5**), a frog toxin that works as a muscarinic antagonist, was accessible in quantitative yield and 99% *ee* (Scheme 1e).[13]

A popular method is dearomative cycloaddtion, where the structural complexity is highly increased by formation of bridged or fused bicyclic systems. Photochemical activation is often used for either [2+2], [2+3] or [2+4] cycloadditions, leading to *ortho*-, *meta*- or *para*-substituted bicycles, which are widespread natural product core structures. The key steps in the total syntheses of the insecticidal penifulvin C (**6**)[14] (Scheme 1f) or the triquinane sesquiterpene ceratopicanol (**7**)[15] (Scheme 1g) are both performed by a alkene-arene *meta* cycloaddition.

A relatively new field is the arenophile-mediated dearomatization. Arenophiles are visible-light photoactivable 2π compounds. Because of their smaller HOMO-LUMO gap when compared to arenes, photoexcitation with visible light is possible in these compounds, while the arene stays in ground state during the reaction.[16] By *para*-cycloaddition an isolation of two π-bonds in the aromatic ring is achieved, making further olefin-type reactions possible, such as *syn*-dihydroxylation.[7] The total syntheses of the alkaloids lycoricidine (**8**) and narciclasine (**9**) by Sarlah et al.[17] are impressive examples for this new methodology (Scheme 1h).



Scheme 1. Selection of natural products accessible by dearomatization. Green box: overview of different strategies: a) reductive dearomatization to oseltamivir phosphate (**1**), b) nucleophilic dearomatization to isodomic acid B (**2**), c) radical dearomatization to cyclitol **3**, d) transition metal mediated to (+)- ptilocaulin (**4**), e) transition metal catalyzed to (+)-gephyrotoxin (**5**), f) and g) photochemical cycloaddition to penifulvin C (**6**) and ceratopicanol (**7**) and h) arenophil mediated dearomatization to lycoricidine (**8**) and narciclasine (**9**). The red box shows oxidative dearomatizations with different reagents: i) hypervalent iodine for synthesis of cortistatin A (**10**), j) $Pb(OAc)_4$-oxidation to maoecrystal V (**11**) and k) copper II sparteine mediated reaction to chamaecypanone (**12**).

In Nature, dearomatization is commonly achieved by oxidative reactions. Chemists have attempted to emulate this approach by using different reagents such as I^{III}, I^{V}, Pb^{IV} or Cu^{I} complexes, to afford *ortho*-quinol products from phenols with formation of new *C*-*C*, *C*-*N*, *C*-halogen and *C*-*O* bonds.[4] Many outstanding total syntheses of natural products were completed thanks to the advances in this field. In the synthesis of the antiangiogenic steroid cortistatin A (**10**), for example, dearomatization is achieved by oxidation with bis(acetoxy)iodobenzene (PIDA), a I^{III} reagent, in 80% yield.[18] Using lead tetraacetate helped to build up the core structure of maoecrystal V (**11**),[19] and the synthesis of the microtubilin inhibitor (+)-chamaecypanone C (**12**) would not have been achieved without the oxidative dearomatization step with copper II sparteine.[20] However, there is still a lot of potential to increase applicability of this research field, as most reactions either have to be run with stoichiometric amounts of reagents and/or are not regio- or stereoselective.[4] Another problem is associated with the purification of the products, as these are often unstable due to potential dimerization, rearomatization or rearrangements. In case of natural product synthesis also another huge problem comes to mind, as most of these compounds are highly functionalized, leading to side and cross reactions with the metal reagents, making it difficult to produce pure compounds in acceptable yield. These limitations show the urgent need for new methods of enantioselective catalytic oxidative dearomatization.

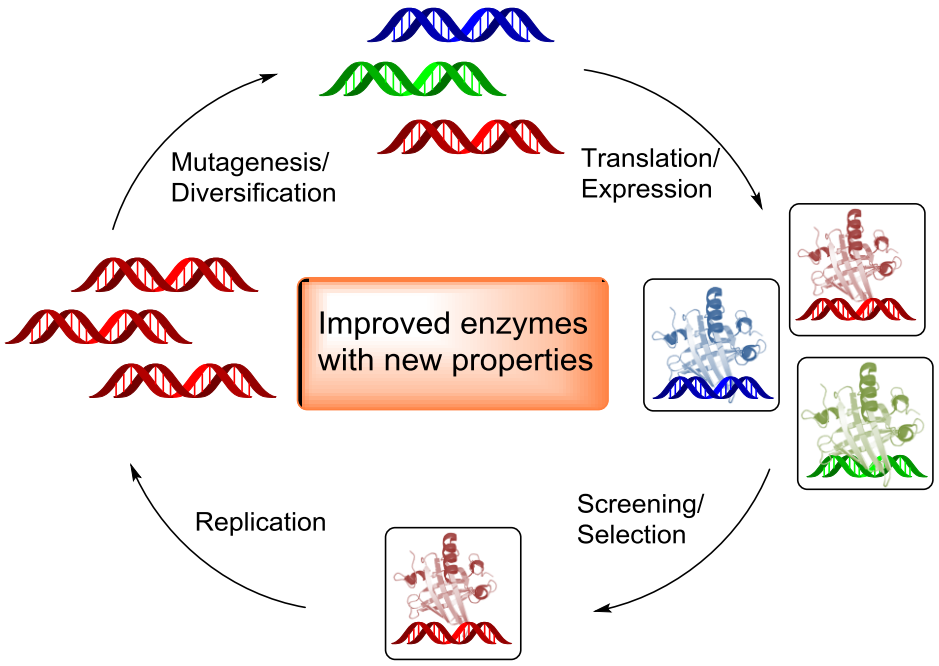
1.3 Biological approach for dearomatization reactions

Enzymes provide a new resource for dearomative transformations of small molecules and with the help of synthetic biology, a field that explores naturally occurring mechanisms and uses them to create new artificial systems that aim to simplify or enable synthesis of needed compounds, the reaction output can be competitively increased. An important example is the directed evolution of artificial enzymes, a research topic made famous by Frances H. Arnold, who won the Nobel Prize of Chemistry 2018 because of her great advances in this field.[21]

Directed evolution is based on the idea that advantageous mutations that are identified in screening libraries are used to create a new desired enzymatic function.[22] This approach can also have additional advantages, including increasing enzyme stability under harsh conditions, enabling activity in non-natural enzymatic environments, such as organic solvents, improving activity on non-native substrates, enhancing thermostability and changing enantioselectivity.[23][24] Remarkably, unnatural reactions can also be catalyzed by metalloproteins that are improved by directed evolution.

The process of directed evolution can be summarized in four steps: 1) Identification of a suitable starting protein (best with already low levels of the desired property), 2) diversification by

mutagenesis of its DNA sequence, 3) functional screening to identify improved variants. Subsequently steps 2 and 3 are repeated until the desired property is achieved and can be applied in step 4 (Scheme 2).[25]



Scheme 2. Directed evolution workflow. First the DNA sequence of the chosen enzyme is mutated, then activity assays or functional screening for the fitness differences are performed and subsequently the improved enzyme is selected and the steps are repeated until sufficient activity is achieved.[22]

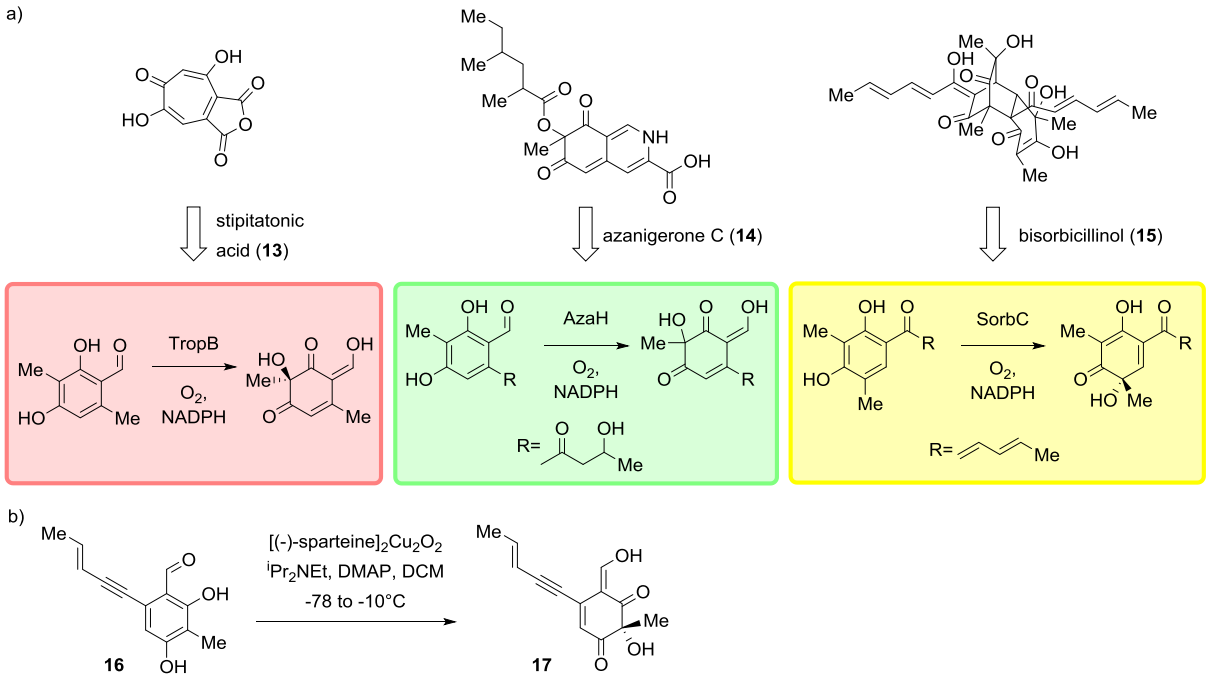
In the last decades, synthetic chemists have been inspired by nature, developing beautiful strategies to mimic natural reactions. Now the time seems to change and protein engineers are inspired by the creativity of these ideas and also the limitations of synthetic chemistry to generate enzymes that will improve or replace synthetic pathways.[23][24] The Nobel Prize winner even goes that far to predict that in the not-so-distant future DNA-programmable microorganism will be producing many of our chemicals.[23] And when looking at the advances that were made over the last few decades in molecular biology, such as the ability to write, cut and paste DNA and to have DNA read and translated into proteins in recombinant organisms, significant progress can be expected in this scientific field.

1.3.1 Enzymatic dearomatizations

In Nature dearomatization is mostly accomplished through the action of FAD-dependent monooxygenase enzymes. One attractive biocatalyst is TropB from the stipitatic acid biosynthetic pathway.[26] It was first discoverded by Cox et al.[26] in a *Penicillium stipitatum* strain and examined by

targeted gene knockout. They found that three genes are required to form the seven-membered ring of the tropolone core of, for example, stipitatonic acid (**13**). The gene *tropA* encodes a non-reducing polyketide synthase TropA that builds up 3-methylorcinaldehyde. The second gene, *tropB*, encodes an FAD-dependent monooxygenase TropB that catalyzes the oxidative dearomatization of 3-methylorcinaldehyde by hydroxylation at the C3 position (Scheme 3, red box). This reaction is performed regio- and stereoselectively to give the 3*R*-configured product. The last gene *tropC* encodes a non-heme Fe(II)-dependent dioxygenase TropC that is responsible for the oxidative ring expansion to the tropolone 7-ring core.[26]

Another interesting FAD-dependent monooxygenase is AzaH, found in *Aspergillus niger* ATCC 1015 by Tang et al.[27] It is part of a gene cluster responsible for the biosynthesis of azanigerones, for example azanigerone C (**14**). AzaH is homologous to the monooxygenase TropB with 43% identity and 61% similarity. It was thus suggested that they perform a similar reaction. To examine its role in the biosynthesis of azanigerones, the monooxygenase was expressed and purified from *E. coli* BL21(DE3). It was then incubated with 2,4-dihydroxy-6-(4-hydroxy-2-oxopentyl)-3-methylbenzaldehyde and the oxidative dearomatization to the hydroxylated product (Scheme 3, green box) was detected by HPLC analysis. By enzymatic transformation of at least 2 to a maximum of 6 further Aza-enzymes, the biosynthesis of all azanigerones can be completed.



Scheme 3. Enzymatic oxidative dearomatization of phenols. A) TropB (red box) and AzaH (green box) stereo- and regioselectively oxidize at C3 position, leading to the natural products stiptatonic acid (**13**) and azanigerone C (**14**). In contrast, SorbC (yellow box) catalyzes the stereoselective oxidation of C5, thus producing the sorbicillinoid dimer bisorbicillinol (**15**). b) Chemical copper-oxo-sparteine mediated oxidation of **16** to *o*-quinol **17**.

The oxidative dearomatizations by TropB and AzaH are strikingly similar to the chemical synthesis of azaphilones by Porco et al., using a copper-oxo-sparteine oxidant to form *ortho*-quinols from resorcinol precursors (Scheme 3b).[28] However, the synthetic reaction conditions are harsher, requiring cold temperatures (-70 to -10°C), hazardous chemicals and a 30 h reaction time. Using FAD-dependent monooxygenases here instead would bring huge advantages for the production of key synthetic intermediates for further synthetic routes to, for example, the azaphilones.

Another promising enzyme for oxidative dearomatization is the FAD-dependent monooxygenase SorbC, found in a *Penicillium chrysogenum* strain E01-10/3, which plays a major role in biosynthesis of the sorbicillinoid natural product family, e.g., the dimeric compound bisorbicillinol (**15**).[29]

1.4 The sorbicillinoid natural product class

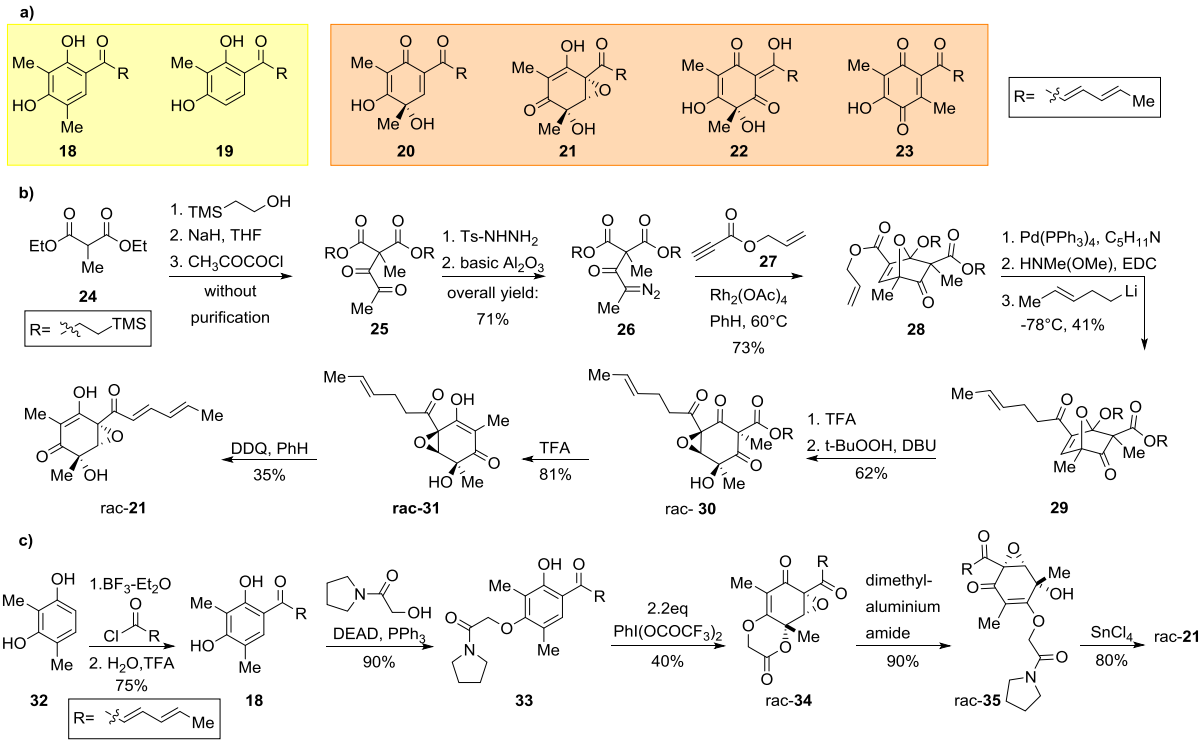
Sorbicillinoids are highly functionalized secondary metabolites as well as beautifully complex structures that belong to the class of hexaketide metabolites, originating from various marine and terrestrial fungi.[30] They were first described by Cram and Tishler in 1948, who reported the first isolation and characterization of sorbicillin (**18**) from the fungus *Penicillium notatum.*[31] After Dreiding et al.[32] also found sorbicillinoid natural products in the fungus *Verticillium intertextum* in 1981 the number of identified sorbicillinoids increased steadily and now the family consists of more than 50 compounds, all of which have the characteristic sorbyl or dihydrosorbyl side chain, high oxygen content and many are built up of polycyclic and bridged complex scaffolds. For characterization they can be divided into several subgroups, namely monomers, dimers, trimers and functionalized derivatives with non-sorbicillinoid attachments.

1.4.1 Monomeric sorbicillinoids

Members of the monomeric sorbicillinoids are the core molecules sorbicillin (**18**), demethylsorbicillin (**19**)[33] and sorbicillinol (**20**), but also further functionalized compounds such as epoxysorbicillinol (**21**), oxosorbicillinol (**22**)[33] and sorrentanone (**23**).[34] These natural products can be found in *Trichoderma sp*. and *Penicillium chrysogenum* strains and despite their small size many possess interesting pharmacological activity. Oxosorbicillinol (**22**), for example, shows DPPH radical scavenging potential with an ED_{50}-value of 87.7 µM[33] and sorrentanone (**23**) acts as an antimicrobial with moderate activity against gram-positive and gram-negative bacteria, with best result against *Streptococcus pyogenes* A9604 with an MIC of 16 µg/mL.[34]

While these compounds are the smallest of the sorbicillinoids, total syntheses of some of these molecules are extremely challenging for synthetic chemists. Currently, there are no stereoselective total syntheses of any of these introduced chiral monomeric sorbicillinoids reported. Two syntheses of (+/-)-epoxysorbicillinol (**21**) nicely show how difficult it is to introduce stereoselectivity even into these small molecules. In the first synthesis of Wood et al.[35] diethylmethylmalonate **24** was transesterified with TMS-ethanol, then deprotonated with NaH and under addition of pyruvoyl chloride diketone **25** was produced. By diazotization α-diazo ketone **26** was obtained, which then underwent a cycloaddition with allyl propiolate **27** to oxabicycle **28**. The deprotection of the allyl ester **28**, followed by coupling to Weinreb´s amine and subsequent addition of 3-pentenyllithium, gave ketone **29**. The cyclic acetal was opened by adding TFA and epoxidation was achieved with *t*-BuOOH and DBU to give a single diastereomer of epoxy alcohol *rac*-**30**. After decarboxylation with TFA to epoxide *rac*-**31**, the sidechain was dehydrogenated with DDQ to receive (+/-) epoxysorbicillinol (**21**) in about 3.7% yield after a 12 steps synthesis (Scheme **4b**).

The second totals synthesis to (+/-)-epoxysorbicillinol (**21**) was invented by Pettus and his group.[36] In this case, the employed starting material was sorbicillin (**18**), produced following a protocol of Nicolaou and his group[37] from resorcinol **32**, which was coupled with an α-hydroxy amide under Mitsunobu conditions to amide **33**. After exposition of **33** to 2.2 eq $PhI(OCOCF_3)_2$, the epoxide *rac*-**34** was produced as a single diastereomer and the lactone was opened employing a Weinreb procedure, resulting in amide *rac*-**35**. By dealkylation of the vinylogous ester with $SnCl_4$ and cleavage of the tin enolate with hydrochloric acid, (+/-)-epoxysorbicillinol (**21**) was obtained in approx. 19% yield after 6 steps. In comparison to the synthesis of Wood et al., Pettus made a large improvement, although the aim to develop a stereoselective route with minimal step count and better yield was not yet achieved.



Scheme 4. a) Variety of monomeric sorbicillinoid natural products. Unoxidized compounds sorbicillin (**18**) and demethylsorbicillin (**19**) (yellow box), intermediate sorbicillinol (**20**) and the oxidized monomers epoxysorbicillinol (**21**), oxosorbicillinol (**22**) and sorrentanone (**23**) (orange box). **b) Total synthesis of (+/-) epoxysorbicillinol (21) by Wood et al**.[35] **c) Total synthesis of (+/-) epoxysorbicillinol (21) by Pettus et al**.[36]

1.4.2 Dimeric sorbicillinoids

The dimers are the largest group of sorbicillinoids and it is presumed that they originate from different reactions of the highly reactive intermediate sorbicillinol (**20**). They can be divided into 3 main groups, the bisorbicillinols, the trichodimerols and the bisvertinols. Within these groups, the different congeners are mostly distinguished by a reduced versus intact double bond at the C2´-C3´position of the sorbyl side chain.

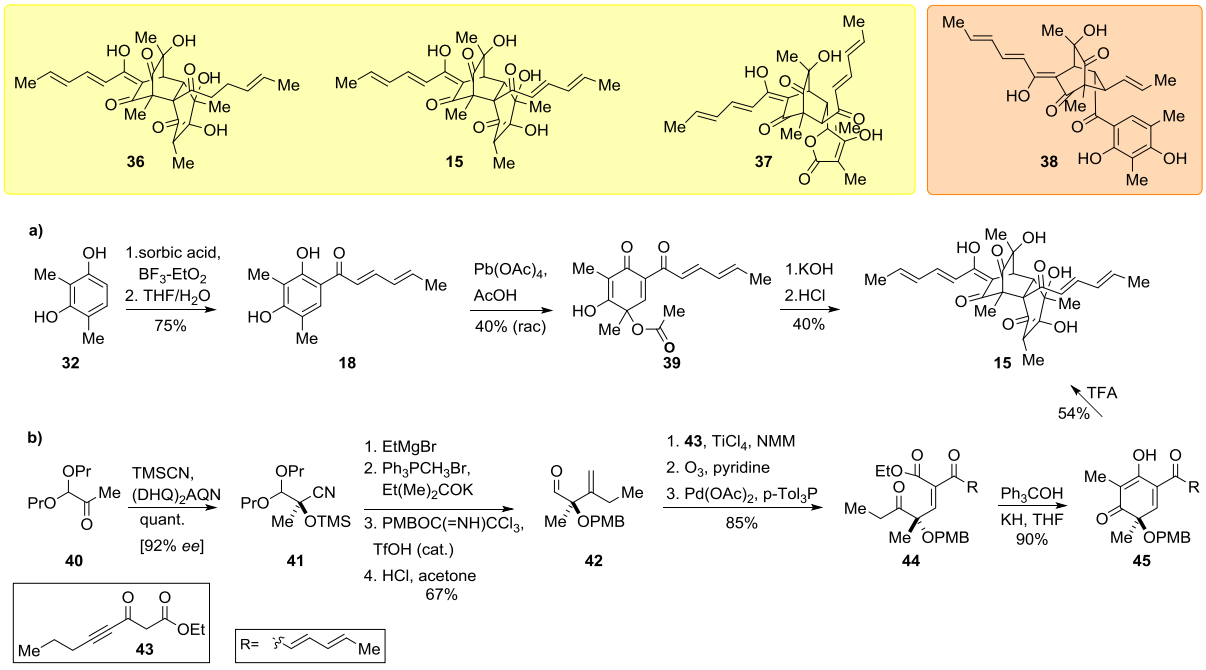
The first group are the bicyclo[2.2.2]octandiones that are formed by a [4+2]- cycloaddition from two units of sorbicillinol (**20**). Bisvertinoquinol (**36**) was the first isolated member of this subgroup, and was identified from the fungus *Verticillium intertextum* in 1981.[32] Bisorbicillinol (**15**) was identified in 1998, featuring an intact sorbyl side chain. It became the primary model for the group of bicylco[2.2.2]octandiones.[38] Other examples include bisorbibutenolid (**37**) and sorbiquinol (**38**), which differ in the attached scaffold. The structures of many bisorbicillinoids act as efficient radical scavengers for DPPH radicals. The most active bisorbicillinoid identified to date is bisorbicillinol (**15**) with an ED_{50}-value of 31.4 µM. This is comparable to established radical scavengers such as

butylhydroxytoluol (BHT), which has an ED_{50}-value of 27.0 µM. Free radicals can cause various diseases such as atherosclerosis, arthritis or cancer, making research for alternative treatment options by new antioxidants necessary and important.[30] Recent studies revealed that bisorbibutenolide (**37**) has notably good anti-cancer properties.[39] In contrast to many chemotherapeutics (around 80% of the chemotherapeutics on the market) that lead to the death of cancer cells by inducing apoptosis, bicyclo[2.2.2]octandione possesses cytostatic activity by suppressing proliferation and eventually causing cell death.[39] Following this mechanism, no resistance against pro-apoptosis drugs will be developed, thus giving an interesting alternative to conventional mechanisms of action. Bisorbibutenolide (**37**) has thus great potential as a lead structure against dangerous types of cancer, such as NSCL carcinoma, glioma, melanoma, pancreatic cancer, and metastases forming carcinoma (Table 1).[39]

Table 1. Results of the treatment of different cancer cell lines with bisorbibutenolide (37). [39]

Cancer cell line	IC_{50}-value [µM]	
U373 (glioblastoma)	4 (+/- 1)	shows apoptosis-resistance
A549 (NSCL-carcinoma)	11 (+/- 2)	shows apoptosis-resistance
SKMEL-28 (melanoma)	8 (+/- 1)	shows apoptosis-resistance
OE21 (esophageal cancer)	9 (+/- 1)	shows apoptosis-resistance
Hs683 (human oligodendroglioma)	22 (+/- 3)	apoptosis- sensitive
B16F10 (murine melanoma)	3 (+/- 1)	apoptosis- sensitive

Owing to these interesting biomedical properties, chemists likewise attempted to synthesize these complex and bioactive molecules. Especially bisorbicillinol (**15**) got into the focus and several attempts for its total synthesis have been made. Most remarkable are the total syntheses of Nicolaou et al.[37] and Deng et al.[40] (scheme 5). Nicolaou elaborated a very short biomimetic route with only four steps. 2,4-Dimethylresorcinol (**30**) is acylated via Friedel-Crafts acylation to sorbicillin (**18**), which is in the next step oxidatively dearomatized with $Pb(OAc)_4$ to acetylsorbicillinol (**39**), which via deprotection and dimerization directly delivers (+/-)-bisorbicillinol (**15**). While this route is very short and beautifully shows the potential of biomimetic syntheses, huge disadvantages once again are the formation of the racemic mixture of bisorbicillinols, low yields and the generation of an undesired regioisomer in the oxidation step that is tedious to be separated from the desired product.

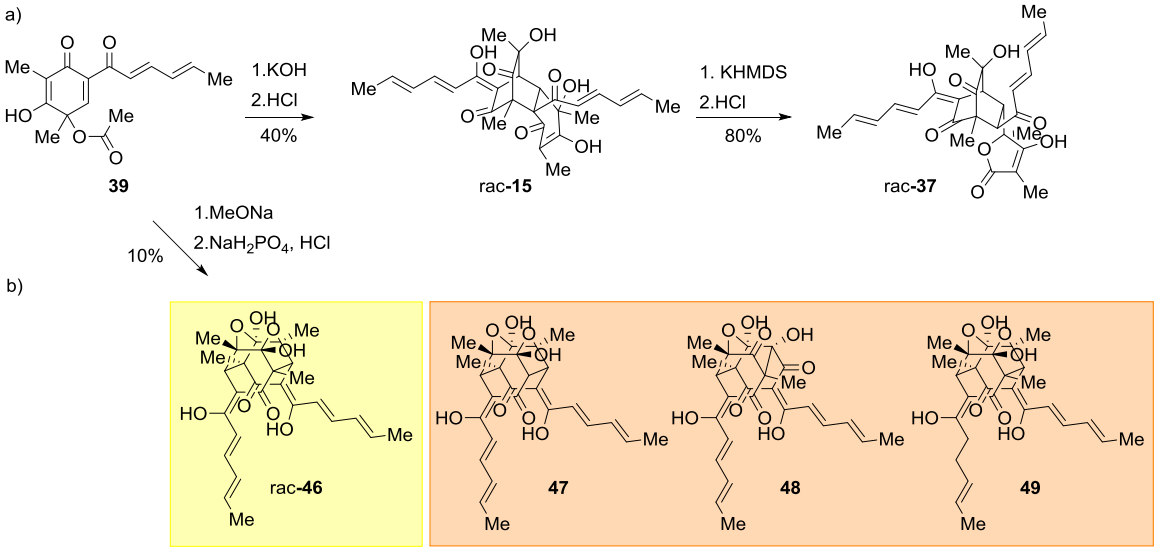


Scheme 5. Dimeric group of bicyclo[2.2.2]octandiones: bisvertinoquinol (**36**), bisorbicillinol (**15**) and bisorbibutenolid (**37**) (yellow box) as dimers of sorbicillinol (**20**) and sorbiquinol (**38**) (orange box) as dimer of sorbicillin (**18**) and sorbicilinol (**20**). a) **Total synthesis to (+/-) bisorbicillinol (15) by Nicolaou et al**.[37] b) **Stereoselective total synthesis of 15 by Deng et al**.[40]

In comparison, Deng's route[40] is significantly longer with a total of 11 steps and consequently significanlty more time and resource consuming. However, this approach constitutes the first stereoselective total synthesis of bisorbicillinol (**15**) and is also higher yielding with 27.5% overall yield. The reaction sequence starts with an enantioselective cyanosilylation of acetal ketone **40** to (*R*)-silylcyanohydrinether **41** and, via subsequent synthetic standard reactions such as Grignard, Wittig olefination, PMB-protection and deacetalisation, aldehyde **42** is produced. A Knoevenagel condensation of **42** with β-ketoester **43** with subsequent ozonolysis and isomerization gave tri-ketone **44**. In the penultimate step a Claisen-Vorländer cyclisation was conducted, which elegantly gave sorbicillinol derivative **45**. After PMB deprotection sorbicillinol (**20**) was dimerized to enantiomerically pure (+)-bisorbicillinol (**15**).

After the synthesis of bisorbicillinol (**15**) was established, Nicolaou and his group developed an anionic cascade rearrangement to transform **15** into the isomer bisorbibutenolide (**37**) (Scheme 6)[37] in only one additional step. Further, Corey *et al.* showed that another subgroup of dimers is also accessible. By dimerization of tautomeric forms of sorbicillinol (**20**), the cage like structures of the trichodimerols can be formed.[41] This group of natural products was first described by Ayer *et al*. in 1992, when he isolated trichodimerol (**46**) from the fungus *Trichoderma longbrachiatum*.[42] The first derivatives demethyltrichodimerol (**47**) and bisorbibetanone (**48**) of this impressive molecule were

found by Abe et al. at the end of the nineties in another *Trichoderma* strain.[38] With dihydrotrichodimerol (**49**) also a side chain derivative was found in 2005 from two different groups in different fungi.[43]



Scheme 6. a) Synthesis of bisorbibutenolid (**37**) out of bisorbicillinol (**15**) via anionic cascade reaction. **b) Trichodimerols.** Dimerization of sorbicillinol (**20**) to cage like structure of trichodimerol (**46**) (yellow box). Further trichodimerolic natural products found in fungi are demethyltrichodimerol (**47**), bisorbibetanone (**48**) and dihydrotrichodimerol (**49**) (orange box).

Trichodimerols have widespread pharmacological activities. An example is provided by trichodimerol (**46**) that, in addition to its good effects against P388 leucemia cells with an IC_{50} value of 0.33 µM,[30] also has further potential application areas, as it is an effective inhibitor of tumor necrosis factor α, a cytokine that plays a key role in immune answer and inflammation. With IC_{50} values of 0.2 µM against TNF-α production in macrophages and 0.6 µM in blood monocytes[44] it is highly active and might be used as lead structure for drugs against diseases like depression, Alzheimer's disease, arthritis or cancer. Dihydrotrichodimerol (**49**) was found to be an effective and selective agonist of peroxisome proliferators-activated receptor γ (PPAR-γ) with an ED_{50} value of 80 ng/mL. By activation of PPAR-γ the glucose metabolism as well as the insulin sensitivity is improved and by binding to the receptor further anti-inflammatory effects are achieved.[45] Compounds with these properties may be important against diseases like diabetes, inflammation and some types of cancer.[46]

The last subgroup of the bisorbicillinoids are the bisvertinols, with a characteristic [6.5.6]tricyclic core structure (Scheme 7). The first compounds of this group were isolated and described by Dreiding *et al*. in 1986, when he first characterized, i. a., bisvertinol (**50**) and bisvertinolone (**51**), found in the fungus *Verticillium intertextum*.[47] It took 6 more years until it was first possible to confirm the dimeric structure with the help of NOE experiments.[42] In 2007, isobisvertinol (**52**) was first isolated by Koyama et al. from *Aspergillus sp*. FK1-1746.[48] This compound is noteworthy because it is the

only known sorbicillinoid with an oxidized carbon atom in the *R*-configuration, thus implying that the fungal species are able to produce both enantiomers of sorbicillinol (**20**).[30]

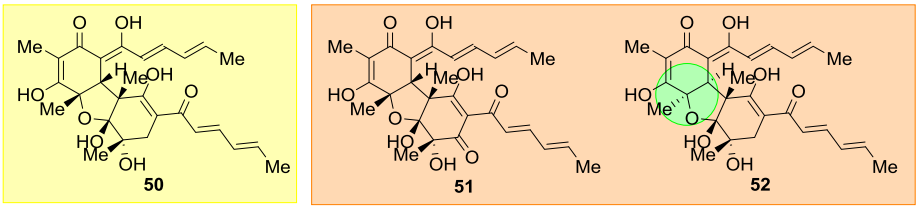
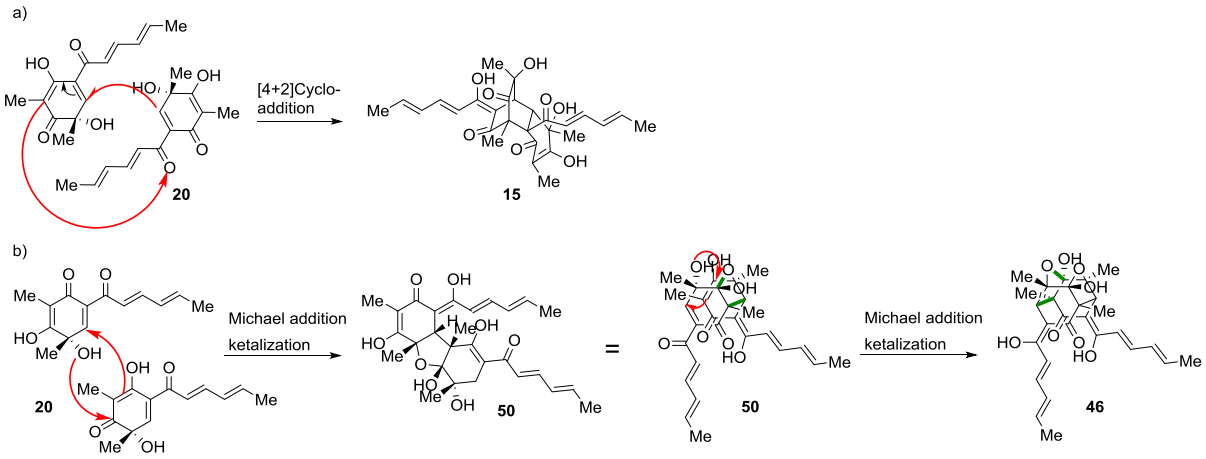


Figure 1. Third subgroup of bisorbicillinoids, the [6.5.6]tricyclic core structured bisvertinols: bisvertinol (**50**), bisvertinolone (**51**) and isobisvertinol (**52**), which has an inverted stereocenter in comparison to other sorbicillinoids (green circle).

Like the other bisorbicillinoids, bisvertinols have a range of interesting biological activities. Kontani and his group first described bisvertinolone (**51**) in 1994 with inhibitory activity against β-1,6-glucan biosynthesis.[49] It affects morphological changes in growing hyphae of *Phytophthora capsici*, which is a phytopathogenic microorganism. Antibiotic activity against the human pathogen *Staphylococcus aureus* 6538P was also observed with an MIC of 30 µg/mL. Because of increasing antibiotic resistance, the identification of new anti-microbial compounds continues to gain significance. Bisvertinolone (**51**) is a valuable compound in the search of antibiotics against multiple drug resistant pathogenic bacteria.[50] Another interesting compound of this group is isobisvertinol (**52**). Koyama published in 2007 that the synthesis of cholesteryl ester CE (IC_{50}= 2.5µM) and triacylglycerol TG (IC_{50}= 4.0 µM) in murine macrophages is inhibited by **52**.[48] By inhibiting the production of these molecules, lipid droplet[51] accumulation is prevented, as cholesteryl ester and triacylglycerol are stored in these organelles. Diseases such as atherosclerosis are the result of lipid droplets being deposited throughout the body, for example in veins, thus giving isobisvertinol (**52**) a pharmacological importance in this pathology. Despite their interesting bioactivity, currently no total synthesis of any bisvertinol has been reported, again showing the difficulties in producing these highly complex molecules via traditional organic chemistry.

The putative formation of the different bisorbicillinoid structures in nature is presented in Scheme 8. Bicyclo[2.2.2]octandiones such as bisorbicillinol (**15**) are formed via a Diels Alder cycloaddition. Their formation is possible because of the high inherent reactivity of sorbicillinol (**20**), which exhibits reactivity as both diene and dienophile (Scheme 8a). The [6.5.6]tricyclic core of bisvertinol (**50**) is accessible by a Michael addition, where one hexadiene double bond acts as a Michael donor and attacks the Michael acceptor system in the second sorbicillinol (**20**) unit (Scheme 8b). Subsequent ketalization by the attack of the hydroxy group to the ketone function leads to the formation of **50**. By repeating the exact steps on the other side of the molecule, the cage like structure of

trichodimerol (**46**) is formed. The ability of nature to build three blatantly different and highly complex structures from a single, relatively simple starting material is tremendously impressive.



Scheme 7. Dimerization mechanisms of bisorbicillinoids. a) [4+2] cycloaddition of two units sorbicillinol (**20**) bicyclo[2.2.2]octandione bisorbicillinol (**15**). b) Michael addition – ketalization sequence of two sorbicillinols (**20**) to bisvertinol (**50**), and another intramolecular Michael addition and ketalization from **50** to the cage like structure of trichodimerol (**46**).

1.4.3 Trimeric sorbicillinoids

When the bisorbicillinoids react with additional sorbicillinoid scaffolds, higher structures such as trimers are produced. Trisorbicillinol A (**53**) was first isolated in 2007 from the deep sea fungus *Phialocephala sp*. FL30r.[52] Derivatives of this molecule, known as Trisorbicillinol B (**54**) and Trisorbicillinol C (**55**), were found only a few years later in 2010 by Gu and his group in the same strain.[53] As they are relatively new, there is still a lot of research potential for these rather uninvestigated complex molecules.

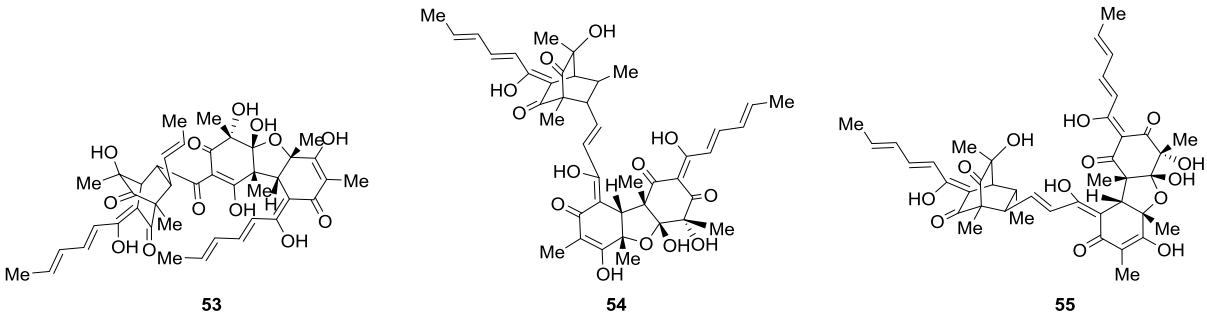


Figure 2. Structure of sorbicillinoid trimers: Trisorbicillinol A-C (**53-55**).

1.4.4 Further functionalized sorbicillinoids

The last group of this natural product family are the functionalized sorbicillinoids. They contain the sorbicillinol scaffold with an additional non-sorbicillinoid moiety, which leads to formation of a broad diversity of different molecular structures and bioactivities (Figure 3).

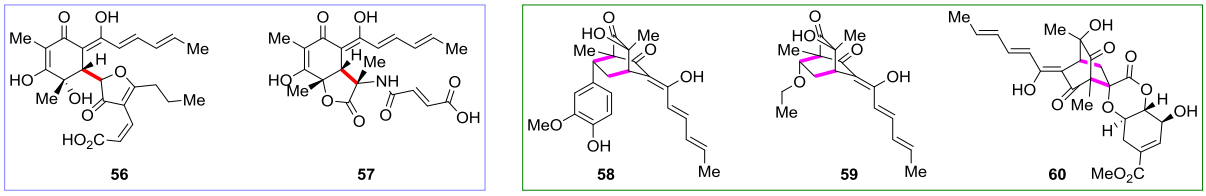


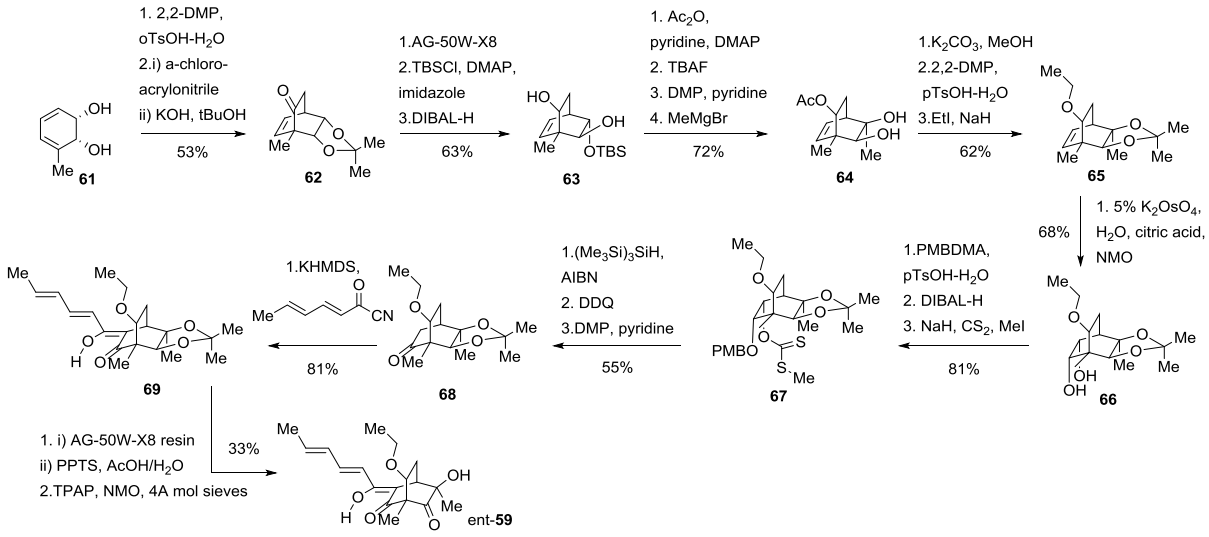
Figure 3. Structural variety of functionalized sorbicillinoids: sorbifuranone A (**56**) and sorbicillactone A (**57**) formed by Michael Addition (blue box, **red bond**) and sorbcatechol A (**58**), rezishanone C (**59**) and spirosorbicillinol A (**60**) formed via Diels-Alder [4+2] cycloaddition (green box, pink bonds).

In comparison to the dimeric compounds, the functionalized class was discovered only recently but continues to rapidly expand. Most common are functionalized sorbicillinoids produced via Michael addition or Diels Alder cycloaddition. Examples for Michael-addition-derived sorbicillinoids are sorbifuranone A (**56**) and sorbicillactone A (**57**). The latter was discovered in 2005 in *Penicillium chrysogenum* and is the first reported nitrogen containing sorbicillinoid.[54] **56** was first characterized in 2010 by Bringmann et al. who isolated sorbifuranone A (**56**) and its derivatives B and C from the same fungus *P. chrysogenum*.[55]

The other majority of functionalized sorbicillinoids are Diels-Alder-derived products of sorbicillinol (**20**) with an external dienophile. These can differ from simple styrene derivatives as in sorbicatechol A (**58**), to small alkylvinyl ethers such as ethylvinyl ether in rezishanone C (**59**), up to the addition of complex natural product portions such as scytolide in spirosorbicillinol A (**60**). Sorbicatechol A (**58**) was first found in 2014 in *P. chrysogenum* PJX- 17 strain by Li *et al.* who elucidated the structure and identified activity against the influenza H1N1 virus. Thus, **58** is the first sorbicillinoid found to have anti-viral activity with an IC_{50} value of 85 µM.[56] Rezishanone C is known under several names as it was isolated and characterized by different groups around the same time. It was first reported by Laatsch and his group in 2005 who found the compound in the fungus *Penicillium notatum.*[57a] In the same year Bringmann et al., who named the substance sorbivinetone,[54] and Lee et al.[43a] reported on the rezishanones. Until last year it was not clear if the published sorbivineton and rezishanone C have the same structure. Clarification that the two compounds were identical was finally given after Yan and his group[57b] succeeded in synthetically producing the enantionmeric **59** (Scheme 11). However, it is not completely clear if these compounds are true natural products or if they derive from side-reactions during the isolation procedure, for example from contamination of ethyl acetate with ethyl vinyl ether, which would then undergo a [4+2]-cycloaddition with sorbicillinol (**20**). The

complex structure of spirosorbicillinol A (**60**) was first described by Hirota and coworkers in 2009[58] who isolated **60** from a *Trichoderma st*. USF-4860 strain from soil. The scaffold of this spiro centered molecule indicates that it originates from a Diels Alder cycloaddition of sorbicillinol (**20**) with another natural product named scytolide.

As none of these sorbicillinoids are yet available by synthesis using classical organic chemistry, it has remained extremely challenging to obtain the quantities needed for bioactivity assays, thus inhibiting the possible application of these molecules as future pharmaceuticals. However, there have been some interesting and creative synthetic attempts, such as the total synthesis of *ent*-**59** by Yan and his group.[57b]

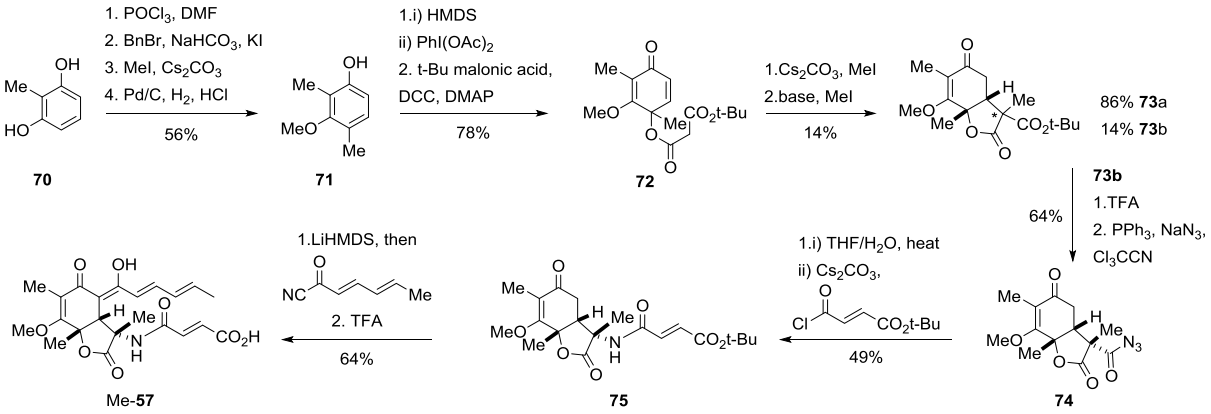


Scheme 8. Total synthesis of *ent*- 59 by Yan and his group.

The synthesis starts with converting commercially available, enzymatically derived *cis*-1,2-dihydrocatechol **61** into bicyclo[2.2.2]octenone **62** by oxidation with DMP and acetal formation of the hydroxyl functions by a Diels Alder reaction with α-chloroacrylonitrile. After cleavage of the acetonide moiety with acidified AG-50W-X8 resin, TBS-protection and reduction of the carbonyl function, diol **63** was obtained as an epimeric mixture. Separation was achieved by chromatography and the synthesis went on with acetylation of the hydroxyl groups, removal of the TBS moiety, oxidation of the deprotected hydroxyl group with DMP and subsequent Grignard reaction with MeMgBr to afford methylated diol **64**. After saponification of the ester and acetonide protection of the vicinal hydroxy groups the compound was alkylated with ethyl iodide and sodium hydride to gain ethyl ether **65**. In the next step, the double bond was oxidized with K_2OsO_4 to afford diol **66**, which was then converted into an epimerically pure acetal by treatment with *para*-methoxybenzaldehyde dimethylacetal and *para*-toluenesulfonic acid. After cleavage with DIBAL-H the compound was

converted into xanthate **67** via Barton-McCombie dioxygenation reaction. Reduction of **67** with TTMSS and AIBN with subsequent PMB deprotection and oxidation using DMP gave ketone **68**. *C*-Acylation with (3E,5E)-2-oxo-3,5 heptadienenitrile under basic conditions lead to alcohol **69** which was then hydrolyzed with acidified AG-50W-X8 resin and PPTS and oxidized with Ley-Griffith reagent to give the enantiomer of the natural rezishanone C (*ent*-**59**). The total synthesis contains 22 steps and an overall yield of around 1%, leaving much space for improvement.

Another very interesting approach to obtain a functionalized sorbicillinoid is the total synthesis of methylated sorbicillactone A (Me-**57**) by Harned and his group.[59] Taking 2-methylresorcinol (**70**) as starting material, the synthesis begins with the formylation, benzylation and methylation to protect the hydroxyl groups with subsequent removal of the benzyl group to afford alcohol **71**. In the next step an oxidative dearomatization took place utilizing hypervalent iodine chemistry and esterification of the emerging alcohol with *tert*-butyl malonic acid to ester **72**. Cyclization was achieved by adding Cs_2CO_3, followed by methylation with methyl iodine. Unfortunately, the desired diastereomer turned out to have the wrong configuration and can thus only be seen as a side product. The desired lactone **73b** was only obtained in 14% yield, thus tremendously reducing the efficiency of this synthesis. In the next step, the malonate of **73b** was deprotected and converted into an acyl azide **74** with sodium azide, followed by a Curtius rearrangement in THF/H_2O to produce an amine that could then be coupled with a fumarate to amide **75**. *C*-Acylation of **75** and subsequent deprotection of the carboxylic acid with TFA led to methylated sorbicillactone A (Me-**57**) in a 13 step synthesis and an overall yield of around 1%. All attempts to cleave off the remaining methyl group failed, thus unfortunately leaving the total synthesis of sorbicillacton A (**57**) incomplete.



Scheme 9. Total synthesis of methylated sorbicillactone A (**57**) by Harned *et al*.

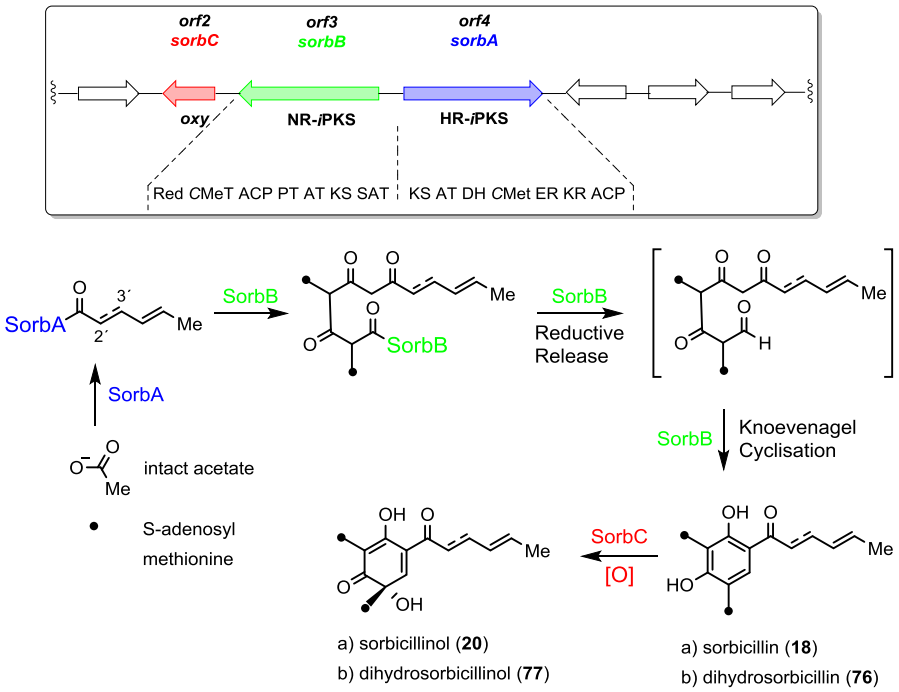
The synthesis of **57** is important and desirable for multiple reasons. First, **57** is of course a very interesting structure with many functional groups in close proximity and thus an interesting synthetic challenge. Second, sorbicillactone A (**57**) has several interesting bioactivities: it acts as a cell growth inhibitor on murine leukemic lymphoblasts (L5178y) with an IC_{50} value of 2.2 µg/mL. Furthermore, it

protects human T cells from HIV-1 (concentration range 0.3- 3.0 µg/mL) by inhibiting the expression of viral proteins. Last but not least, it was also found that by pre-incubation of neurons in sorbicillactone A (**57**) at 10 µg/mL, the effects of serotonin and L-glutamic acid on their intracellular calcium concentration was blocked, thus suggesting that **57** could serve as a potential lead structure in neuroprotective drug research.[30]

1.5 Biosynthesis of sorbicillinoids

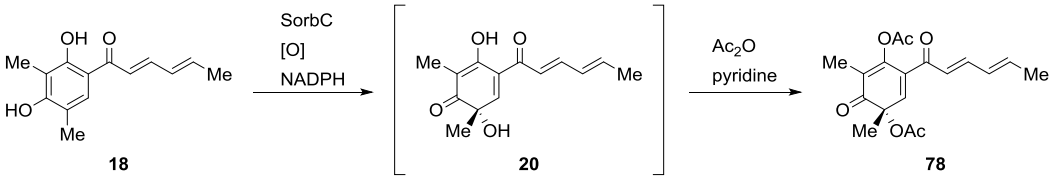
As this special natural product family fascinates chemists as well as biologists it is highly interesting to elucidate the biosynthesis of these diverse, complex and mostly bioactive compounds. Cox and his group revealed how sorbicillinol (**20**) is biosynthesized in nature,[29] which is the key intermediate in sorbicillinoid natural product formation. They took a closer look on the sorbicillinoid producing fungus *Penicillium chrysogenum* E01-10/3, and as the structure of sorbicillinoids strongly suggests a polyketidic origin, they screened gDNA by PCR using the reported KHKS2/KHKS3c primers that amplify polyketide genes.[60] Using this approach, three fragments of iterative PKS-genes were amplified and sequenced. In the next step, a clone fosmid library consisting of 4800 clones from *P. chrysogenum* E01-10/3 gDNA was built and screened by PCR with primers targeting these PKS genes. The largest of the thus identified fosmid clones was subjected to random shotgun sequencing and revealed to have a length of 48.8 kb and seven putative open reading frames. These encoded a transcriptional regulator, an FAD-dependent monooxygenase, a non-reducing iterative polyketide synthase (NR-iPKS), a highly-reducing iPKS (HR-iPKS), a transcription factor, an ABC transporter, and a second FAD-dependent oxidoreductase.

Domain analyses of the HR-iPKS showed the presence of a ketosynthase (KS), acyl transferase (AT), dehydratase (DH), *C*-methyl transferase (CMeT), ketoacylreductase (KR), enoylreductase (ER) and acyl carrier protein (ACP) domains, indicating that it might belong to the biosynthesis of the side chain of sorbicillin (**18**). The respective gene was consequently named SorbA. The NR-iPKS, named SorbB, contained a starter-unit acyl transferase (SAT), KS, AT, product template (PT), ACP, CMeT and a reductive release (Red) domain, which are required for the biosynthesis of the sorbicillin core structure (Scheme 13).



Scheme 10. Biosynthesis of sorbicillinol (20).[29]

Having identified the genes putatively encoding the core structure of sorbicllin (**18**), the group next focused on elucidating the proposed oxidative dearomatization step to **20**. A candidate gene *sorbC* was identified that encoded an FAD-dependent monooxygenase. The corresponding enzyme might well be involved in the oxidative dearomatization of (**18**), as similar enzymes were previously shown to be involved in this process in the biosynthesis of **13** and **14** (see Chapter 1.3.1 above). The gene *sorbC* was cloned into a suitable expression vector and over-expressed as soluble His-tagged fusion protein SorbC in *Escherichia coli*. Isolation and purification of SorbC and subsequent incubation with sorbicillin (**18**) or dihydrosorbicillin (**76**) showed conversion to an unstable intermediate, sorbicillinol (**20**) or dihydrosorbicillinol (**77**), which could then be trapped by bisacetylation with acetic anhydride in pyridine. After purification bisacetylsorbicillinol (**78**) was identified.[29]



Scheme 11. Enzymatic transformation of sorbicillin (**18**) with FAD-dependent monooxygenase SorbC to sorbicillinol (**20**). Stabilization of **20** by derivatization to bisacetylsorbicillinol (**78**) with acetic anhydride and pyridine.

The elucidation of the enzymatic dearomatization step in sorbicillinoid biosynthesis by the Cox group, was the precondition to study the respective enzymes. The main goal of this work thus was to utilize SorbC as a biocatalytic tool for the total synthesis of sorbicillinoid natural products.

2. Aims of this thesis

The emerging knowledge on the biosynthesis of the sorbicillinoids has created new opportunities towards the total synthesis of this class of natural products. In this thesis, we aim to develop short, biomimetic and stereoselective methods to produce these highly functionalized and complex molecules. By utilizing a chemo-enzymatic route, the first aim is to synthesize the precursor sorbicillin (**18**) and establish the reaction conditions under which the purified enzyme SorbC will efficiently transform it by oxidative dearomatization to the reactive sorbicillinol (**20**). Success of this method leads to new questions such as: Is the transformation truely stereoselective and what is the *ee*-value? How can we guide the reaction of unstable sorbicillinol (**20**) toward a desired product, e.g. by dimerization or further functionalization? And of vital importance, how can we purify our products? How can biochemical and physical data, such as substrate tolerance, enantioselectivity or enzyme kinetics of the monooxygenase SorbC, help us to exploit the full potential of this protein? Lastly, what can we find out about the structure-activity relationship of the desired small-molecule products? Answering these questions will lead us to a better understanding of this natural product family and enable us to build up chemo-enzymatic total syntheses to all sorbicillinoids structures. In summary, this leads to the following main research goals:

- Chemo-enzymatic and chemical synthesis of acetylsorbicillinol (**39**), for examination of the stereoselectivity of SorbC by determination of the *ee*-value by HPLC.
- Development and optimization of reaction conditions to bisorbicillinoid natural products. The aim is to find specific reaction routes to the three different backbones of dimeric sorbicillinoids: 1) bicyclo[2.2.2]octandiones, 2) cage-like trichodimerols, 3) [6.5.6] tricyclic cores of bisvertinols.
- Determination of substrate specificity of SorbC and design of derivatives for establishing a library of natural and unnatural potentially bioactive sorbicillinoids.
- Chemo-enzymatic total synthesis of functionalized sorbicillinoids, e. g. sorbicatechol A (**58**), epoxysorbicillinol (**21**), sorbifuranon A (**56**) and rezishanones.
- Derivatisation of anti-viral sorbicatechol A (**58**) and screening for anti-HIV and anti-H1N1 activity.
- Upscaling of enzymatic the reaction to enable the synthesis of more complex sorbicillinoids, e. g. sorbicillacton A (**57**), by opening the possibility to further alter the compound synthetically after enzymatic transformation.

3. Result and discussion

3.1 Stereoselective Total Synthesis of Bisorbicillinoid Natural Products by Enzymatic Oxidative Dearomatization/Dimerization

Based on: **A. Sib**, T. A. M. Gulder, Stereoselective Total Synthesis of Bisorbicillinoid Natural Products by Enzymatic Oxidative Dearomatization/Dimerization, *Angew. Chem. Int. Ed.* **2017**, *56*, 12888 – 12891; *Angew. Chem.* **2017**, *129*, 13068 – 13071.

Sorbicillinoids are natural products of significant structural and biomedical interest. Their biological activities range from anti-infective to radical-scavenging and cytotoxic properties. However, classical chemical total synthesis of members of the sorbicillinoid family is difficult to achieve because of their remarkable structural complexity. To avoid long and inefficient synthetic routes towards this natural product family, we established a chemo-enzymatic total synthesis of three bisorbicillinoids and three derivatives. The key step of our approach is the enzymatic oxidative dearomatization of the central precursor sorbicillin (**18**) to the highly reactive sorbicillinol (**20**), the key biosynthetic intermediate of all sorbicillinoids. This biocatalytic transformation proceeds with perfect regio- and stereocontrol (*ee*-value of ca. 99.5) and thus proves to be superior over all purely synthetic counterparts developed so far, particularly the oxidative dearomatisation with lead(IV)acetate.

Starting with sorbicillinol (**20**), a multitude of bisorbicillinoids is available by fast and controlled dimerization reactions. An interesting example is the assembly of bisorbicillinol (**15**) by *in-situ* dimerization by a Diels-Alder cycloaddition. When sorbicillinol (**20**) dimerizes via Michael-addition with subsequent ketalization, the cage like structure of trichodimerol (**46**) is formed. We found that by changing the co-solvent in the enzymatic transformation step, we could trigger either the formation of the bicyclo[2.2.2]octanedinoe **15** by use of acetone, or the synthesis of trichodimerol (**46**) by use of DMF. Further, when adding pyridine to the extracted DMF phase, the coupling product of sorbicillin (**18**) with sorbicillinol (**20**) sorbiquinol (**38**) was accessible for the first time.

Substrate specificity of SorbC is vital for its use in downstream chemo-enzymatic synthesis reactions. In this work, we synthesized sorbicillin derivatives with different side chains attached to the resorcinol core and tested their transformation with SorbC. Impressively, the saturated hexyl side chain and hexenyl side chain showed successful conversion. In the case of hexylsorbicillin, the expected dimeric derivatives hexylbisorbicillinol (**79**) and hexyltrichodimerol (**80**) were obtained by applying of the established reaction conditions.

Chemoenzymatic Synthesis

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Stereoselective Total Synthesis of Bisorbicillinoid Natural Products by Enzymatic Oxidative Dearomatization/Dimerization

*Anna Sib and Tobias A. M. Gulder**

Abstract: *Natural products are a virtually inexhaustible source of small molecules with spectacular molecular architectures and biomedical potential. Their structural complexity generates formidable challenges to total synthesis but often also precludes time- and resource-efficient, stereoselective synthetic access. Biosynthetically, nature frequently uses dimerization and oligomerization reactions to produce highly challenging frameworks from simple starting materials. Impressive examples are the bisorbicillinoids, a family of fungal natural products thought to originate from the polyketide precursor sorbicillin. Utilizing the recombinant oxidoreductase SorbC from the sorbicillin biosynthetic gene cluster, a robust, fully stereoselective synthesis of bisorbicillinoid natural products and unnatural side-chain analogues was developed.*

Ever since the discovery of penicillin, specialized metabolites from fungi have fascinated natural product chemists, medicinal chemists, and biochemists alike. The sorbicillinoids are a particularly intriguing class of fungal compounds.[1] This family comprises a large number of dimeric natural products with beautiful, stereochemically complex three-dimensional architectures and promising biological properties. Examples include the efficient radical scavenger bisorbicillinol (**1**),[2] the cage-like trichodimerol (**2**),[3] which inhibits production of TNF-α by targeting prostaglandin H synthase-2,[4] and the β-1,6-glucan biosynthesis inhibitor[5] bisvertinolone (**3**)[6] (Figure 1). Trimeric compounds such as trisorbicillinone A (**4**),[7] as well as divergently functionalized sorbicillinol derivatives such as the antileukemic sorbicillactone A (**5**),[8] further extend the structural variability of this intriguing class of natural products.

The bisorbicillinoids are all thought to biosynthetically originate from the dimerization of just one building block, sorbicillin (**6**). It has long been under debate whether **6** rather constitutes a stable biosynthetic intermediate on the way to bisorbicillinoids, or a metabolic end point derived from the reactive intermediate sorbicillinol (**7**).[8a,9] Recent work by the Cox group firmly established the order of biosynthetic events.[10] They conclusively showed that sorbicillin (**6**) is the direct product of a polyketide synthase (PKS). Compound **6** is

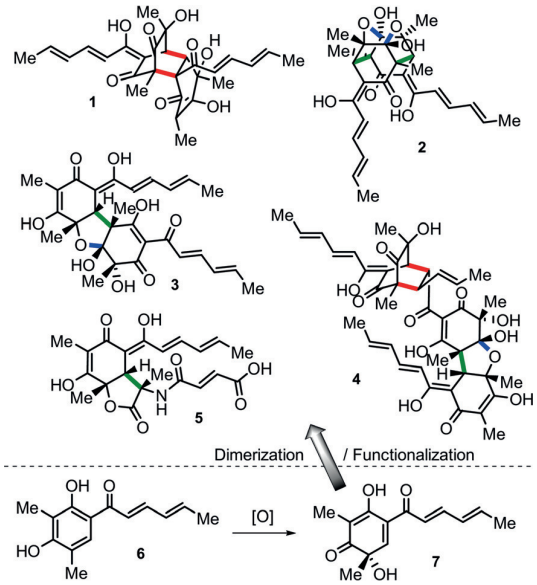

Figure 1. Structures of selected sorbicillinoid natural products **1**–**5** putatively formed after oxidation of **6** to the reactive sorbicillinol (**7**) with subsequent structural diversification by Diels–Alder cycloaddition (red bonds), Michael addition (green bonds), and ketalization (blue bonds) reactions.

the substrate of the enzyme SorbC, which catalyzes oxidative dearomatization to **7** as the final enzyme-derived product of this pathway. Sorbicillinol (**7**) might then serve as a common reactive precursor to all bisorbicillinoids through subsequent dimerization events by Michael addition, ketalization, and cycloaddition chemistry (Figure 1).

Owing to their structural complexity and biomedical potential, the bisorbicillinoids have attracted broad interest from the synthetic community, particularly bisorbicillinol (**1**) and trichodimerol (**2**; Scheme 1). The first independent total syntheses of **1** by Corey[11] and Nicolaou[12] et al. both relied on a biomimetic strategy, with oxidative dearomatization of **6** using $Pb(OAc)_4$ as the key step to give acetate *rac*-**8**. Resolution of this material by HPLC on a chiral stationary phase and subsequent O-deacetylation under suitable conditions yielded **1** or **2**. This resulted in impressively short routes to these highly complex compounds. However, the lack of good regiocontrol and any stereocontrol in the oxidative dearomatization reaction led to low overall yields. The first progress towards a stereoselective synthesis of **1** was reported by Pettus et al.[13] Using a chiral tether in **9** during oxidative dearomatization triggered by hypervalent iodine chemistry, sorbicillinol synthon **10** was obtained as a mixture of

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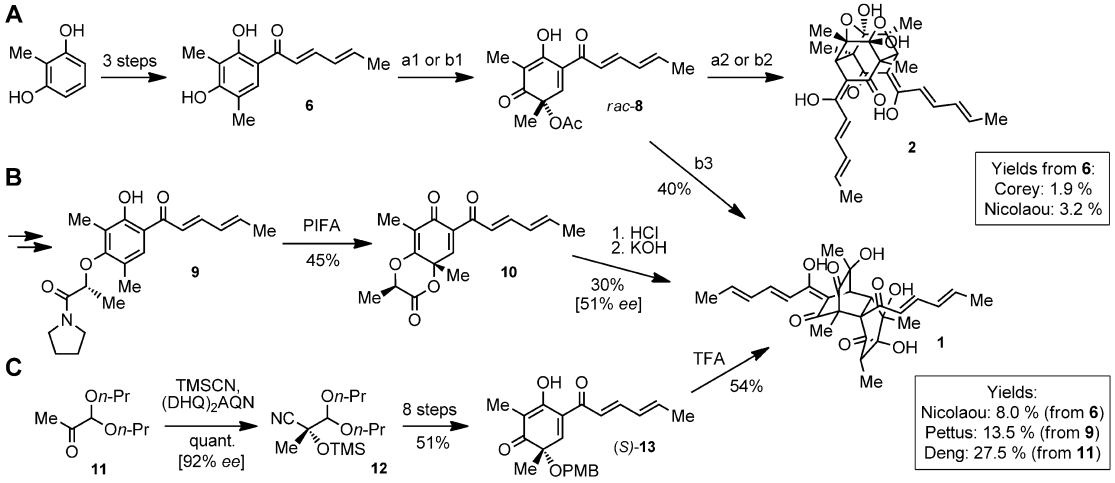


Scheme 1. Total synthetic routes to **1** and **2**. A) Corey et al (a1: $Pb(OAc)_4$, $AcOH/CH_2Cl_2$ (5:1), 23 °C, 30 min, 38% *rac*-**8**→max. 19% (*S*)-**8** isolated by HPLC on a chiral phase; a2: NaOMe, MeOH, then $NaH_2PO_4{\cdot}H_2O$, HCl/MeOH, 10%) and Nicolaou et al (b1: $Pb(OAc)_4$, $AcOH/CH_2Cl_2$ (1:1), 0 °C, 30 min, 40% *rac*-**8**→max. 20% (*S*)-**8** isolated by HPLC on a chiral phase, b2: $CsOH{\cdot}H_2O$, MeOH, then $NaH_2PO_4{\cdot}H_2O$, 16%; b3: KOH, THF/H_2O (9:1), then 1 N HCl, 40%). B) Pettus et al. C) Deng et al.

diastereomers that was further converted without separation into **1** with 51% *ee*. The only truly enantioselective synthesis of **1**, as published by Deng et al.,[14] introduces the stereochemical information into ketone **11** in the first step of the synthesis through enantioselective cyanosilylation to quantitatively deliver **12** in good 92% *ee*. However, this strategy required a subsequent eight-step sequence to deliver the PMB-protected sorbicillinol **13**, which, in analogy to above syntheses, was dimerized to **1** upon PMB removal. In summary, the impressively short biomimetic approaches by Corey, Nicolaou, and Pettus suffer from a lack of stereocontrol, while the enantioselective Deng strategy results in a comparably long synthetic route.

To combine high stereoselectivity with a most streamlined access, we set out to develop a biocatalytic approach using the cognate enzyme SorbC in the synthetic key step. The gene *sorbC*, as identified in the sorbicillactone A producing strain *Penicillium chryosgenum* E01-10/3 by Cox et al.,[10] was used in a codon-optimized version for expression in *E. coli*. The gene was cloned into a pET-28a(+) vector to facilitate heterologous production of SorbC equipped with an N-terminal hexahistidine tag, thereby enabling batch purification using Ni-NTA affinity chromatography (see Supporting Information). An initial screening of suitable conditions for the biocatalytic transformation revealed that 50 mM phosphate buffer at pH 8.0 supported high enzymatic activity of SorbC. Substrate **6** for the envisioned enzymatic oxidation was available in a three-step synthetic sequence[12] from 2-methyl resorcinol (see the Supporting Information). Due to the low solubility of **6** in the aqueous buffer system, acetone (up to 20% of the final volume) was used to dissolve the substrate prior to its addition to the reaction mixture. In addition, NADPH was shown to be replaceable by the cheaper NADH in vitro. To our delight, SorbC efficiently and selectively formed a single product with a significantly altered UV spectrum when compared to that of **6**, as expected for an oxidative dearomatization (Figure 2A). LC–MS analysis revealed a molecular mass consistent with **7**, which is in line with the functional data reported by Cox et al.[10] To firmly establish product identity, this reactive intermediate was also intercepted by O-acylation with acetyl chloride to give the corresponding stable acetate **8**. Having enzymatically produced **8** in hands facilitated to probe the enantioselectivity of the enzymatic transformation. Racemic **8** was prepared by oxidative dearomatization of sorbicillin (**6**) utilizing hypervalent iodine chemistry[15] (see the Supporting Information). The material was used to establish an analytical separation method by HPLC on CHIRALCEL OD-RH material, leading to the expected 1:1 ratio of the two chromatographically resolved enantiomers (Figure 2B). Analysis of **8** derived from the enzymatic oxidative dearomatization revealed virtually perfect stereocontrol by the enzyme with an *ee* > 99.5%.

We next turned our attention to the synthesis of the bisorbicillinoid target structures **1** and **2**. During the initial test assays with SorbC, compound **7** seemed to be rather stable in the aqueous buffer system. This is in sharp contrast to the observed high reactivity of **7** under the conditions reported in the total syntheses of **1** and **2** published so far (e.g., its dimerization to **1** in THF/H_2O = 9:1, see Scheme 1). It was thus expected that the reactivity of **7** towards dimerization will be dramatically increased by changing the solvent polarity. Indeed, when the enzymatic reactions were quenched with a large excess of dichloromethane (50:1), the selective formation of a single new product peak at the cost of **7** was observable by HPLC analysis. Sorbicillinol (**7**) was fully consumed within 40 min (Figure 2A). Isolation of the product by HPLC gave bisorbicillinol (**1**) in 27% yield (Scheme 2) based on consumed starting material.[16] Dimerization by Diels–Alder cycloaddition between two units of **7** thus proved to be very fast and did not permit the formation of any other dimeric analogues. We thus suspected that by choosing

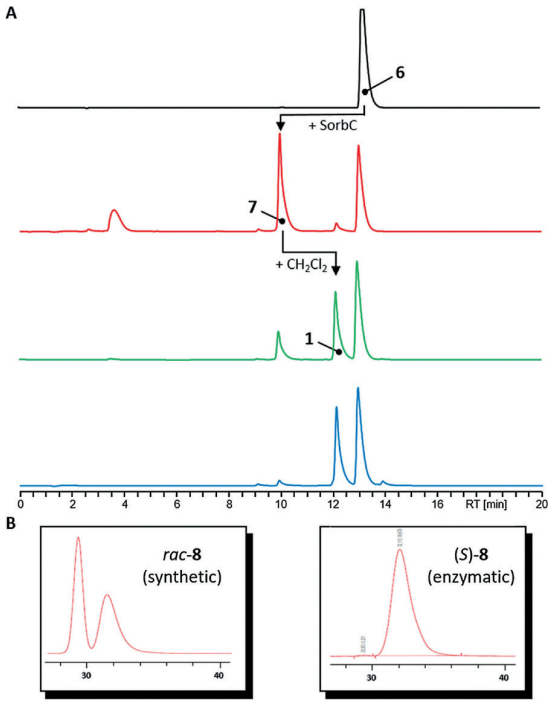
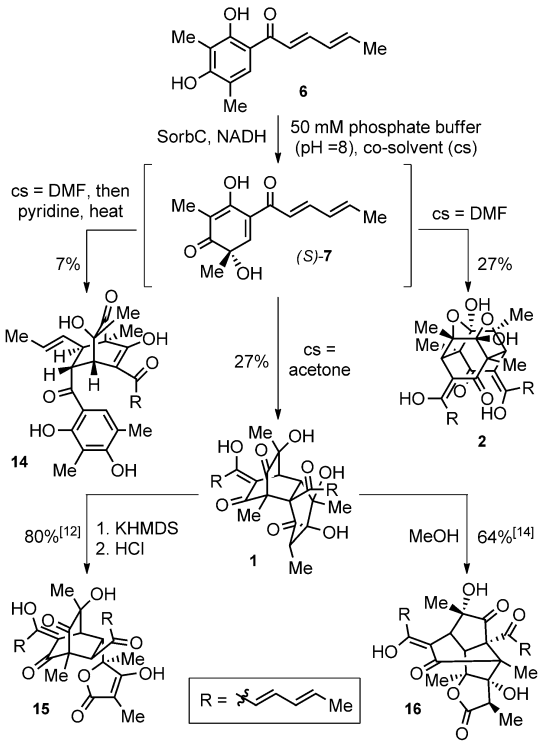


Figure 2. HPLC–UV traces of the Sorb C catalyzed synthesis of **1** by oxidative dearomatization of sorbicillin (**6**, black) to sorbicillinol (**7**, red), with subsequent quenching of the reaction using CH_2Cl_2 to give **1** (green and blue, 20 and 40 min after quenching, respectively). B) Stereoselectivity of the oxidative dearomatization reaction monitored by HPLC–UV on a chiral phase (OD-RH): separation of synthetic *rac*-**8** and analysis of enzymatically derived (*S*)-**8**.

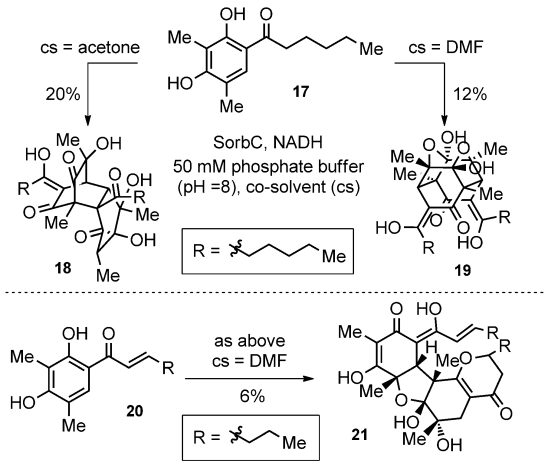


Scheme 2. Developed method for the stereoselective enzymatic total synthesis of the complex bisorbicillinoids bisorbicillinol (**1**), trichodimerol (**2**), and sorbiquinol (**14**), with formal routes also established to bisorbibutenolide (**15**) and bisorbicillinolide (**16**).

a suitable polar organic co-solvent for the enzymatic oxidation, the slower formation of alternative dimerization products might become possible. In fact, using DMF as organic co-solvent in the biocatalytic process with subsequent extraction with dichloromethane indeed allowed the production of trichodimerol (**2**) in 27% isolated yield as the main product, with an additional 20% of **1**. Intriguingly, the product spectrum was further extendable through extraction of the reaction set-ups containing DMF after 4 hours using dichloromethane, fast evaporation of the latter, and addition of pyridine to the remaining DMF followed by heating. This not only resulted in the formation of **1** (16%) and **2** (13%) but also gave sorbiquinol (**14**)[17] in 7% yield, which arises from a Diels–Alder reaction of **7** with the side chain of sorbicillin (**6**). To the best of our knowledge, this constitutes the first total synthesis of **14**. Through simple variation of the work-up of the enzymatic oxidation of **6**, three structurally diverse bisorbicillinoids formed by Michael addition/ketalization (**2**) as well as core-to-side-chain (**14**) or core-to-core (**1**) Diels–Alder cycloaddition are readily accessible in one-pot reactions from **6** (Scheme 2). Compound **1** can furthermore be rearranged to bisorbibutenolide (**15**) upon treatment with KHMDS followed by HCl,[12] or to bisorbicillinolide (**16**) through exposure to MeOH,[14] thus additionally concluding formal enantioselective total syntheses of these natural products.

To test the versatility of this biocatalytic approach, the preparation of unnatural analogues of **1** and **2** was attempted. Following the identical synthetic procedure as for the synthesis of **6** but using hexanoic instead of sorbic acid in the Friedel–Crafts acylation reaction (see the Supporting Information), the fully saturated sorbicillin side-chain analogue **17** was prepared. To our delight, SorbC accepted **17** as a substrate, permitting the synthesis of the respective side-chain-saturated bisorbicillinol derivative **18** in 20% yield of isolated product when using acetone as the organic co-solvent (Scheme 3). In analogy to the conversion with the natural substrate **6**, the respective trichodimerol derivative **19** was accessible in 12% yield when utilizing DMF as a co-solvent during oxidative dearomatization. Interestingly, when applying the (*E*)-2-hexenoic acid-derived sorbicillin derivative **20** as a substrate, the formation of the expected side-chain analogues of **1** and **2** was never observed, although substrate turnover to the oxidatively dearomatized reactive intermediate was clearly visible. The product formed rather corresponded to a novel structural framework containing the bisvertinol core (see Figure 1) formed by an initial Michael addition/ketalization sequence with an additional Michael addition of an oxygen function onto the α,β-unsaturated side chain to deliver the cyclic ether portion in **21** as a single diastereomer in 6% yield. While not yet reported, similar cyclic ethers might also be formed in the natural producers.

In conclusion, we developed a stereoselective, one-pot biocatalytic total synthesis of complex bisorbicillinoids using



Scheme 3. Chemoenzymatic synthesis of **18**, **19** and **21**, unnatural derivatives of **1** and **2** that are accessible by using the sorbicillin side-chain analogues **17** and **20** as enzymatic substrates.

an enzymatic oxidative dearomatization/dimerization cascade. This facilitates the direct preparation of sorbiquinol (**14**), trichodimerol (**2**), and bisorbicillinol (**1**), with additional formal total syntheses of bisorbibutenolide (**15**) and bisorbicillinolide (**16**) by single rearrangement reactions from **1**. The approach combines the strengths of the landmark biomimetic syntheses of Corey[11] and Nicolaou,[12] with the high stereoselectivity provided by the biocatalyst SorbC. It is therefore the most efficient synthetic access towards bisorbicillinoid natural products yet developed. The biocatalytic transformations were generally conducted at a 20–80 mg substrate scale, delivering up to 10 mg of the target molecules in high purity. The method is thus suitable for the fast preparation of the target compounds and unnatural analogues thereof in mg quantities sufficient to permit future in-depth structure–activity relationship studies. The uncatalyzed, spontaneous formation of bisorbicillinoids in vitro strongly suggests that these compounds are also assembled by non-enzymatic reactions in the natural producers. Since the product spectrum of bisorbicillinoids reported from different fungi varies, this raises questions on how nature potentially directs the selective formation of specific dimers. Investigations into the full synthetic potential and substrate promiscuity of SorbC and into the elucidation of factors that may govern product selectivity in nature are currently in progress in our laboratory.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: biocatalysis · enzyme catalysis · natural products · sorbicillinoids · total synthesis

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3.2. Chemo-enzymatic Total Synthesis of Oxosorbicillinol, Sorrentanone, Rezishanones B and C, Sorbicatechol A, Bisvertinolone, and (+)-Epoxysorbicillinol

Based on: **A. Sib**, T. A. M. Gulder, Chemo-enzymatic Total Synthesis of Oxosorbicillinol, Sorrentanone, Rezishanones B and C, Sorbicatechol A, Bisvertinolone, and (+)-Epoxysorbicillinol, *Angew. Chem. Int. Ed.* **2018**, *57*, 14650 – 14653.

In addition to the bisorbicillinoids, the two other main groups of this natural product class are the monomeric and further functionalized sorbicillinoids. This work shows the total synthesis of seven natural products and four unnatural analoges, containing the synthesis of different starting materials, the enzymatic oxidative dearomatisation with SorbC and subsequent functionalization.

For monomeric sorbicillinoid synthesis we examined the substrate specificity of SorbC altering the ring system of sorbicillin (**18**). By deleting the C4-methyl group from the sorbicillin scaffold, we observed oxidation of the C4 position by attachment of a hydroxylgroup with subsequent re-aromatisation. When shifting the methyl group from C4 position to C5, the natural product sorrentanone (**23**) was accessible by oxidative dearomatisation. We then set out to synthesize the phloroglucinol precursor hydroxysorbicillinol for SorbC oxidation, which was then transformed to the natural product oxosorbicillinol (**22**) in 21% yield.

Next we went on to produce functionalized compounds of this natural product class. We targeted the total synthesis of rezishanones B and C (**59**) as well as the antiviral sorbicatechol A (**58**) which all derive from an inverse electron demand Diels Alder cycloaddition (iedDA) with the sorbicillinol diene and an electron-rich dienophile. In the case of the rezishanones, simple vinyl ethers can be used, while sorbicatechol A (**58**) was accessible by iedDA with a 4-ethenyl-2-methoxyphenol portion. The reactions can either run with acetone as cosolvent with direct addition of diene to the extracted enzymatic reaction, or with semi purified sorbicillinol (**20**) in DMF. Further, we used two commercially available substituted styrenes and another vinyl ether to confirm the general applicability of this cycloaddition.

Regarding the total synthesis attempts to epoxysorbicillinol (**21**) from other groups[35/36] we next challenged ourselves with the task of further functionalization of sorbicillinol (**20**) via organic reactions after enzymatic transformation. Taking semi-purified sorbicillinol (**20**) in DMF, we achieved stereoselective epoxidation via precomplexing of potassium *tert*-butylperoxid to the C4-hydroxyl group of **20** to (+)- epoxysorbicillinol (**21**) following the Weitz-schaeffer protocol. Again, the high applicability and efficiency of SorbC catalysis for total synthesis of sorbicillinoids can be proved.

Total Synthesis

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Chemo-enzymatic Total Synthesis of Oxosorbicillinol, Sorrentanone, Rezishanones B and C, Sorbicatechol A, Bisvertinolone, and (+)-Epoxysorbicillinol

*Anna Sib and Tobias A. M. Gulder**

Dedicated to Professor Wolfgang A. Herrmann on the occasion of his 70th birthday

Abstract: *The sorbicillinoids are a large family of fungal natural products, many of which possess highly challenging molecular architectures. Depending on their individual structures they exhibit strong biological activities ranging from radical scavenging and anti-infective properties to cytotoxicity. Despite the resulting strong biomedical potential of these natural products and the interest of synthetic chemists owing to their fascinating structures, many sorbicillinoids are currently not synthetically accessible, thus hampering in-depth biological characterization and structural diversification. By using recombinant oxidoreductase SorbC and readily accessible sorbicillin-type synthetic precursors, we have developed enantioselective, one-pot chemo-enzymatic routes to a broad range of sorbicillinoids, thereby establishing total syntheses of oxosorbicillinol, sorrentanone, rezishanones B and C, sorbicatechol A, bisvertinolone, and (+)-epoxysorbicillinol.*

The sorbicillinoid family of fungal natural products stands out due to its exceptional diversity of molecular structures and selective biological activities, although all members are derived from the simple polyketide sorbicillin (**1**).[1] Aside from structural analogues of **1**, which are characterized, for example, by slightly different substitution patterns at the aromatic ring system or varying saturation of the sorbyl side chain, the sorbicillinoids biosynthetically originate from the oxidative dearomatization of **1** to sorbicillinol (**2**) by the monooxygenase SorbC.[2] This transformation is achieved with perfect regio- and stereocontrol.[3] Sorbicillinol (**2**) contains a highly reactive *cis* diene that is concurrently part of an activated Michael acceptor system. Much of the structural diversity of the sorbicillinoids results from dimerization of **2** guided by its inherent reactivity. This leads, inter alia, to the formation of the radical scavenger bisorbicillinol (**3**)[4] by Diels–Alder cycloaddition and the inhibitor of the prostaglandin-H-synthase-2 trichodimerol (**4**)[5] by a Michael addition/ketalization sequence (Figure 1). In addition, nature

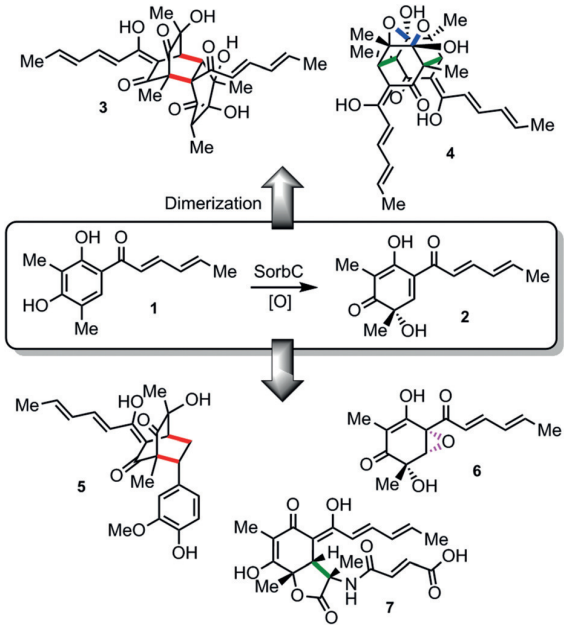

Figure 1. Structural diversity of sorbicillinoid natural products derived from the oxidative dearomatization of **1** to **2** with subsequent dimerization to give bisorbicillinoids, e.g., **3** and **4**, or further functionalization reactions, e.g., leading to **5**–**7**. Bond formation is achieved by Diels–Alder (red bonds), Michael addition (green bonds), ketalization (blue bonds), and oxidation (pink bonds) reactions.

is capable of taming the high reactivity of **2** sufficiently to also allow alternative functionalization pathways to occur. For example, a Diels–Alder reaction with a vinylphenyl dienophile leads to sorbicatechol A (**5**),[6] while epoxidation of **2** leads to (+)-epoxysorbicillinol (**6**),[7] and a Michael addition with an alanine-derived C-nucleophile paves the way for the formation of sorbicillactone A (**7**).[8] The catalytic activity of a single enzyme, SorbC, thus gives rise to the complete metabolic diversity of the sorbicillinoid family of natural products. This enzyme therefore bears exceptional potential as a biocatalytic tool for the total synthesis of these structurally challenging natural products starting from a single, readily available synthetic precursor molecule, sorbicillin (**1**). This is particularly rewarding due to the strong and selective biological activities that can be found in the sorbicillinoid natural product family. For example, **5** exhibits promising antiviral properties against the influenza A

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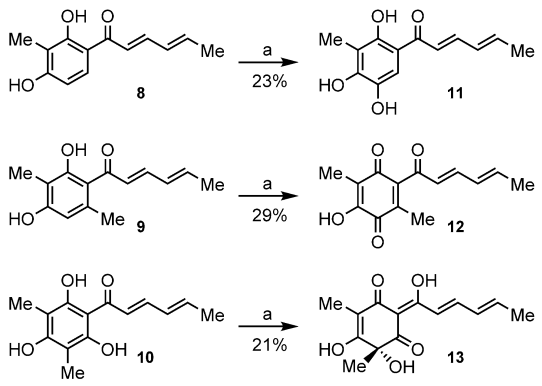
virus (H1N1), with IC_{50} values of 85 μM, thus in the range of the commercial antiviral agent ribavirin which is used as a control with an IC_{50} of 84 μM.[6] Sorbicillactone A (**7**) not only possesses strong anti-HIV activity by inhibiting viral protein expression and protecting human T lymphocytes against the cytopathic effect of HIV-1, but also has selective activity against L5178y murine leukemic lymphoblasts at an IC_{50} of 2.2 $\mu g\,mL^{-1}$.[8] Developing fast, unified synthetic approaches targeting this promising natural product family is thus of importance to facilitate further investigations into their biomedical value.

We have recently shown that SorbC can indeed be utilized for the fast and stereoselective total synthesis of bisorbicillinoids resulting from dimerization of **2**, for example, leading to **3** and **4**, whereas the Diels–Alder reaction of unreacted **1** with **2** facilitates the first total synthesis of sorbiquinol.[3] The enzyme also accepted structural analogues of **1** as substrates, allowing for the synthesis of unnatural bisorbicillinoid derivatives.[3] The catalytic activity of SorbC towards a larger set of substrates was recently evaluated by Narayan and co-workers, also including the monooxygenases TropB from stipitatonic acid biosynthesis[9] and AzaH from azaphilone biosynthesis[10] offering different site- and stereoselectivity. This work nicely showcases the broad synthetic potential of these biocatalysts for the oxidative dearomatization of α-acylated or -formylated phenols.[11]

Following our interest in utilizing SorbC for the total synthesis of functionalized sorbicillinoid natural products that do not derive solely from the reaction of **2** with **1** or **2**, we tested the catalytic activity of SorbC towards a set of further monomeric sorbicillin derivatives **8**–**10** which have different substitution patterns at the aromatic ring system. These substrates were prepared by $AlCl_3$-promoted Friedel–Crafts acylation of the corresponding phenols with sorbic acid chloride (see the Supporting Information). Interestingly, the natural product demethylsorbicillin (**8**)[12] was selectively oxidized to triol **11** in 23% yield,[13] while compound **9**, in which one methyl group is moved from C-5 to C-6 relative to sorbicillin (**1**), is oxidized to the corresponding *para*-quinone **12** in 29% yield (Scheme 1), a compound know as the natural product sorrentanone from *Penicillium chrysogenum*.[14] When the hexasubstituted phloroglucinol **10** was used as a substrate, conversion to the natural product oxosorbicillinol (**13**)[12] was achieved in 21% yield (*e.r.* = 95:5, see the Supporting Information). In contrast to the highly reactive sorbicillinol (**2**), all oxidation products **11**–**13** from these transformations are stable compounds. Exemplarily for the production of **12** we furthermore investigated in situ NADH co-factor regeneration using glucose dehydrogenase Gdh.[15] This facilitated the reduction of the amount of NADH from approximately 1.2 equivalents to 0.2 equivalents without affecting product yields (see the Supporting Information).

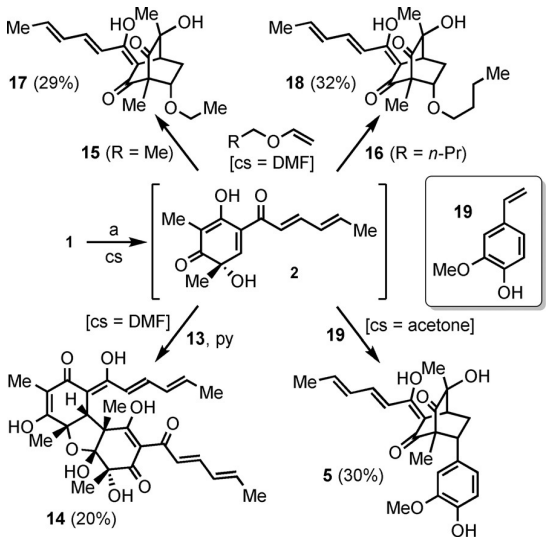
The oxidative dearomatization of **10** by SorbC not only concluded the first synthesis of the natural product **13**, but also promised direct access to the structurally challenging bisvertinolone (**14**), as this natural product likely results from a biosynthetic Michael addition/ketalization sequence of **2** with **13**.[16] As our previous work only permitted the fusion of sorbicillinol (**2**) with itself or sorbicillin (**1**),[3] a method for the



Scheme 1. Oxidation of sorbicillin analogues **8**–**10** to give triol **11** and the natural products sorrentanone (**12**) and oxosorbicillinol (**13**), respectively. a) Phosphate buffer (50 mM, pH 8.0), SorbC, NADH, room temperature; acetone was used as co-solvent (cs) for solubilization of substrates **8**–**10**.

extraction of **2** from the enzymatic transformation followed by a controlled reaction setup for further functionalization was required to pave the way for the chemo-enzymatic synthesis of further diversified sorbicillinoids such as **14**. Given the increased stability of **2** in polar solvents, such as H_2O and DMF, the use of significant amounts of DMF as a co-solvent in the aqueous enzymatic transformations followed by its extraction from the aqueous phase with CH_2Cl_2 and the fast removal of only the CH_2Cl_2 under reduced pressure was thought to give access to semi-purified **2** in DMF solution for further functionalization chemistry. While the formation of the dimeric bisorbicillinol (**3**) and other minor products resulting from the inherently high reactivity of **2** towards dimerization cannot be prevented entirely during this workup procedure, this strategy indeed enables the controlled reaction of **2** with alternative building blocks. This facilitated the first total synthesis of bisvertinolone (**14**) in 20% yield by treatment of the DMF solution of **2** with oxosorbicillinol (**13**) and pyridine, promoting a highly selective Michael addition/ketalization sequence (Scheme 2).

The extension of this methodology to the synthesis of various other sorbicillinoids, derived by the Diels–Alder reaction of **2** with different dienophiles, was subsequently evaluated. The two reported ether derivatives sorbivineton[8a] and rezishanone C served as interesting target compounds.[17] However, as recently shown by synthetic work by Yan et al.,[18] the structures of these compounds are indeed identical and correspond to **17**. This product was obtainable within our work by addition of ethyl vinyl ether (**15**) to **2** in DMF solution to give **17** in 29% yield (Scheme 2). While the above-mentioned synthesis of the unnatural *ent*-**17** by Yan et al. required >20 individual synthetic steps,[18] our biocatalytic approach delivered **17** in a single step from sorbicillin (**1**), with the latter being accessible in a simple additional three-step synthetic sequence.[3] Rezishanone B (**18**)[17b] was likewise accessible in 32% yield following this approach by using *n*-butyl vinyl ether (**16**) as the dienophile. As an alternative to the use of DMF and its extraction with CH_2Cl_2, the application of acetone as the co-solvent during the

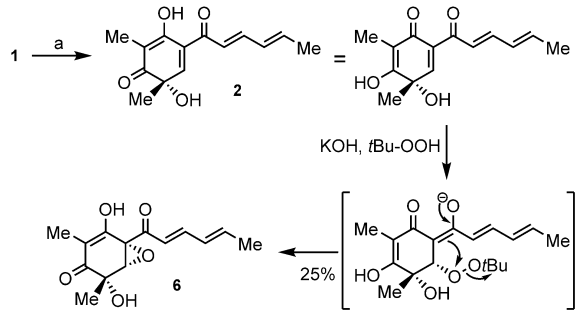


Scheme 2. Chemo-enzymatic total syntheses of bisvertinolone (**14**), rezishanone C (**17**), rezishanone B (**18**), and sorbicatechol A (**5**). a) cf. Scheme 1.

enzymatic synthesis of **2** with subsequent CH_2Cl_2 extraction followed by the direct addition of the desired dienophile to the combined organic phases and slow evaporation of the solvent was also feasible for nonvolatile enes. Using 4-ethenyl-2-methoxyphenol (**19**) as the reaction partner for **2** thereby facilitated the first total synthesis of the antiviral sorbicatechol A (**5**)[6] in 30% yield. Interestingly, while the NMR data of synthetic **5** perfectly matched the data reported for isolated **5**, optical rotation values were in disagreement, including opposite signs. This discrepancy was resolved by comparison of the CD spectra, revealing a perfect match of synthetic with isolated **5** (see the Supporting Information). As the optical rotations of unnatural analogues of **5** with variations in the aromatic portion (see below) were in a similar range, it is likely that an incorrect value was reported for **5** in the original isolation paper.

To further demonstrate the applicability of this chemo-enzymatic approach, we set out to prepare a set of unnatural sorbicillinoids derived from the Diels–Alder cycloaddition of **2** with vinyl ethers or styrenes. Use of phenyl vinyl ether, *tert*-butyl vinyl ether, 1-methyl-2-vinylbenzene, and 4-vinylaniline readily gave the expected addition products **20**–**23** in 15–30% yield (see the Supporting Information for details). These results emphasize that our chemo-enzymatic approach is suitable for the fast generation of mg quantities of sorbicillinoid Diels–Alder analogues sufficient for structure–activity relationship studies.

Encouraged by these results, we were interested in determining whether it is possible to functionalize **2** in stabilizing, polar solution using non-sorbicillinoid, external nucleophiles. An interesting target structure to this end was (+)-epoxysorbicillinol (**6**),[7] which is derived from regio- and stereoselective epoxidation of one of the double bonds in the cyclohexene unit of **2**. Despite considerable interest in the synthesis of **6**, the only two currently existing total synthetic routes by Pettus et al.[19] and Wood et al.[20] lead to a mixture of the enantiomers (+/−)-epoxysorbicillinol (**6**). We envisioned that selective functionalization of the desired double bond in **2** should be possible by conjugate addition due to its double activation (Scheme 3). Introduction of the required



Scheme 3. Chemo-enzymatic, stereoselective total synthesis of (+)-epoxysorbicillinol (**6**). a) cf. Scheme 1; cs = DMF.

epoxy function should thus be possible by conducting a Weitz–Scheffer epoxidation using KOH and *t*-butylperoxide. The tertiary alcohol function in **2** could thereby direct the potassium *t*-butylperoxide to preferentially attack from the same side by precomplexation. This would allow chirality transfer from the enzymatically installed stereogenic center to the epoxide unit. As a consequence, this should preferentially lead to the desired configuration at the newly formed stereogenic centers. When a DMF solution containing **2** was cooled to 0 °C and treated with KOH and *t*-butylperoxide, the exclusive formation of (+)-epoxysorbicillinol (**6**) in 25% yield as a single stereoisomer was indeed achieved.

Taken together, we have developed a chemo-enzymatic strategy for the stereoselective total synthesis of diverse sorbicillinoids and unnatural analogues that involves the partial purification of the highly reactive **2** in DMF solution to enable subsequent cycloaddition and conjugate addition chemistry. Through the use of different dienophiles, this paved the way for the first total syntheses of bisvertinolone (**14**), rezishanones C (**17**) and B (**18**), as well as sorbicatechol A (**5**). Utilizing a Weitz–Scheffer epoxidation furthermore gave the first stereoselective access to (+)-epoxysorbicillinol (**6**). In combination with our previous establishment of oxidative dearomatization/dimerization sequences towards bisorbicillinoids,[3] this chemo-enzymatic concept provides highly streamlined access to most sorbicillinoids. The application of this approach to the synthesis of focused compound libraries for the sorbicillinoid congeners with the most promising biological activities will now facilitate the evaluation of the biomedical potential of this natural product family. This work is currently in progress in our laboratory.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: biocatalysis · enzyme catalysis · natural products · sorbicillinoids · total synthesis

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3.3. Unpublished results

3.3.1 Chemo-Enzymatic Total Synthesis of Sorbicatechol Structural Analogs and Evaluation of Their Anti-Viral Potential

Based on: **A. Sib**, A. Herrmann, L. Oubraham, A. Pichlmair, R. Brack-Werner, T. A. M. Gulder, Chemo-Enzymatic Total Synthesis of Sorbicatechol Structural Analogs and Evaluation of Their Anti-Viral Potential, submitted.

The fungal natural product family of sorbicillinoids can be characterized as polyketides with intriguingly different molecular structures, often containing high oxygen content and highly diverse bioactivities. An interesting member of this outstanding family is sorbicatechol A (**55**), an anti-viral sorbicillinoid with activity against influenza virus A (H1N1) and with a complex architecture consisting of a 2-methoxyphenol moiety attached to the bicyclo[2.2.2]octandione core that is already known from the dimeric sorbicillinoid bisorbicillinol.

To enable structure-activity determination, derivatization of the catechol portion of sorbicatechol A (**58**) was performed. We developed a chemo-enzymatic synthesis with the recombinant oxidoreductase SorbC, sorbicillin (**18**), and a variety of commercially available styrenes and vinyl compounds, to a broad range of sorbicatechol-A derivatives. In total, 14 molecules were produced by Diels-Alder cycloaddition of sorbicillinol (**20**) with different dienophils, exchanging the catechol moiety of **58**. The attached scaffolds vary from substituted aromatic systems, made of the corresponding styrenes, to vinyl ethers with either alkyl or phenyl moieties. We also succeeded in attaching heterocycles, such as pyridine, pyrazine or thiazole to sorbicillinol (**20**), as well as a benzylic ester and an ethylketone.

Having produced a range of different sorbicillinoid structures, we set out to explore the pharmacological potential of this compound class. Sorbicatechol A (**58**) and the derivatives were tested for their anti-viral activity against HIV and Influenza A virus. The tests showed, that aromatic scaffolds attached to the sorbicillinoid core lead to anti-viral activity. The most active compound, a 2-methylphenyl side chain derivative, exhibited anti-HIV activity with an IC_{50} value of 32.2 ± 2.52 µM. These results indicate that the aromatic system is essential for anti-viral activity and show that even within a small library of sorbicatechol derivatives potent compounds can be found, thus proofing once again the great properties of this fascinating natural product class.

Chemo-Enzymatic Total Synthesis of Sorbicatechol Structural Analogs and Evaluation of Their Anti-Viral Potential

Anna Sib,[a] Alexander Herrmann,[c] Lila Oubraham,[d] Andreas Pichlmair,[d] Ruth Brack-Werner,[c] and Tobias A. M. Gulder*[a,b]

Abstract: The sorbicillinoids are fungal polyketides that are characterized by highly complex and diverse molecular structures with considerable stereochemical intricacy combined with a high degree of oxygenation. Many sorbicillinoids possess promising biological activities. An interesting member of this natural product family is sorbicatechol A, reported to have anti-viral activity, particularly against influenza A virus (H1N1). Utilizing a straightforward, one-pot chemo-enzymatic approach with the oxidoreductase SorbC that was recently developed in our group, we set out to structurally diversify the characteristic bicyclo[2.2.2]octane core of sorbicatechol by variation of its natural 2-methoxyphenol substituent. This facilitated the preparation of a focused library of structural analogs bearing substituted aromatic systems, alkanes, heterocycles and ethers. The fast access to this structural diversity provided an opportunity to explore the anti-viral potential of the sorbicatechol family.

Sorbicillinoids are a large polyketide natural product family consisting of more than 50 members.[1] They can be isolated from a diverse set of marine and terrestrial fungi and can be categorized into four different groups according to their molecular structures: the monomeric, dimeric, trimeric and the further functionalized sorbicillinoids. Biosynthetically, all sorbicillinoids derive from stereo- and regioselective oxidative dearomatization of sorbicillin (**1**) to the highly reactive sorbicillinol (**2**) by the oxidoreductase SorbC (cf. Figure 2).[2] The dimeric/trimeric sorbicillinoids result from subsequent dimerization/trimerization of **2** with **1** or **2** by Michael addition or Diels-Alder cycloaddition due to the inherent respective reactivity of **2**, leading to beautiful molecular architectures such as bisorbicillinol (**3**, resulting from a dimerization by Diels-Alder reaction)[3] or trichodimerol (**4**, double Michael addition/ketalization)[4] (Figure 1). Further functionalized sorbicillinoids are likewise formed by these transformations, instead of a simple dimerization, however, involving non-sorbicillinoid nucleophiles or dienophiles. Structurally and biomedically interesting examples of this class of sorbicillinoids include sorbicillactone A (**5**),[5] spirosorbicillinol A (**6**)[6] and sorbicatechol A (**7**).[7]

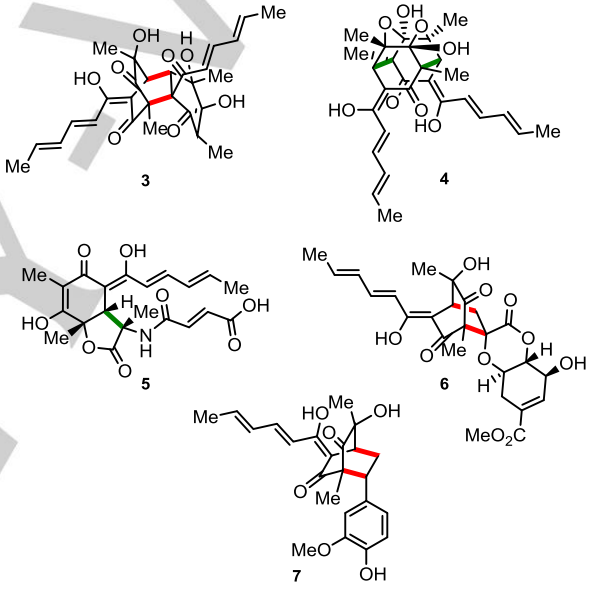

Figure 1. Examples of dimeric sorbicillinoid natural products bisorbicillinol (**3**) and trichodimerol (**4**) and of further functionalized congeners formed by Micheal addition (green bonds), e.g., sorbicillactone A (**5**), or Diels-Alder cycloaddition chemistry (red bonds), e.g., spirosorbicillinol A (**6**) and sorbicatechol A (**7**).

The reported biological activities of the functionalized sorbicillinoids are as diverse as their structures. Sorbicillactone A (**4**) is active against murine leukemic lymphoblast cell line L5178y with an IC_{50} of 2.2 mg/mL and protects human T cells from HIV-1 in a concentration range of 0.3 and 3.0 mg/mL.[5] Spirosorbicillinol A (**6**) shows DPPH-radical scavenging activity,[6] a property also typical for dimeric sorbicillinoids such as **3**.[3] While currently far less studied when compared to the above-mentioned compounds, sorbicatechol A (**7a**) was reported to exhibit promising anti-viral activity against H1N1.[7] With an IC_{50} value of 85 µM, **7** shows identical potential as the antiviral drug ribavirin (IC_{50} of 84 µM).

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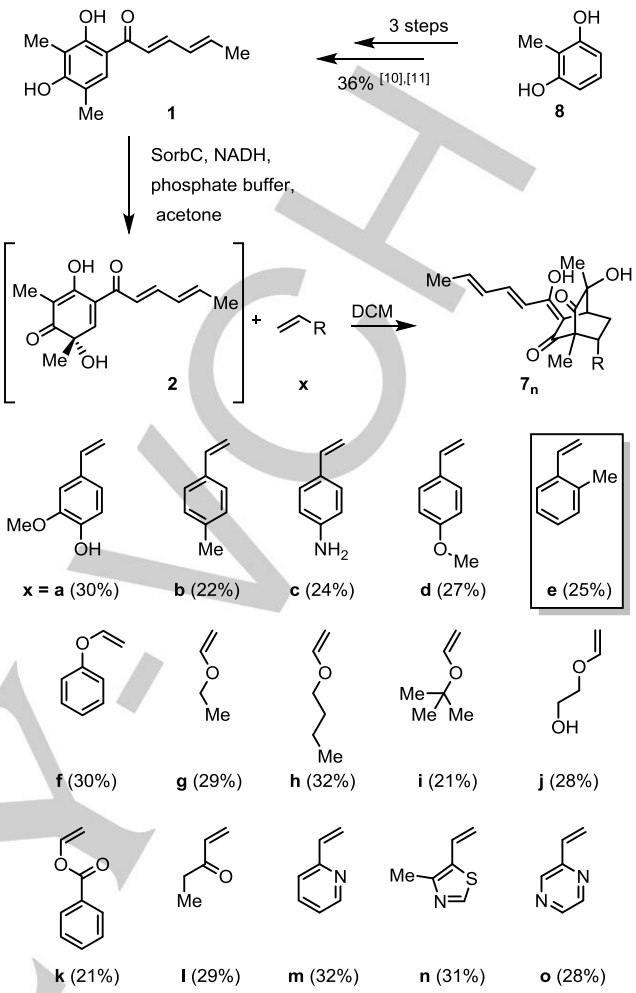
As the structural variability of any given type of natural sorbicillinoid core is rather limited and as the isolation of these compounds from the complex metabolic matrix of the fungal producer strains is tedious, synthetic strategies are required to facilitate biomedical studies on these fascinating molecules. While a number of elegant total synthetic routes towards dimeric sorbicillinoids such as **3** and **4** do exist, most of these are not stereoselective and/or rather lengthy.[8] In case of the sorbicatechol core structure, only a single total synthetic approach exists. This led to the synthesis of *ent*-rezishanone C bearing an ethoxy- instead of the 2-methoxyphenol substituent when compared to **7a**. Overall, the synthetic route contains >20 individual steps with a combined yield of less than 3%,[9] clearly evidencing the need for improved synthetic strategies towards the sorbicillinoids. To this end, we have recently developed chemo-enzymatic approaches for the efficient one-pot synthesis of dimeric soribcillinoids such as **3** and **4**, utilizing synthetically readily available sorbicillin (**1**) that gets oxidatively dearomatized employing SorbC to give sorbicillinol (**2**), which in term can be dimerized in a controlled manner depending on the organic cosolvent employed during the biocatalytic reaction.[10] This methodology was further extended to enable functionalization of **2** with external nucleophiles, e.g., leading to the first stereoselective total synthesis of epoxysorbicillinol, and dienophiles, yielding to the first synthetic access to rezishanones and sorbicatechol A (**7a**).[11] We herein present the application of this chemo-enzymatic toolkit for the straightforward preparation of a focused library of sorbicatechol-type structural analogs to enable the evaluation of their anti-viral potential.

The required synthetic starting material **1** for the biocatalytic reaction was prepared in three-steps starting from 2-methylresorcinol following our published procedure.[11] In a regio- and stereoselective oxidative dearomatization reaction using SorbC, **1** was transformed into **2**, the reactive precursor of all sorbicatechol derivatives. Quenching of the reaction solution containing **2** with a diverse set of dienophiles readily delivered the target compounds in yields ranging from 21% to 32% (Scheme 1), irrespective of the substitution of the ene-function. This is in the range of the typical product yields for all sorbicillinoids following this chemo-enzymatic strategy and can be explained by unreacted starting material **1** as well as by the formation of dimeric side-products, particularly **3**, that cannot be fully suppressed due to the intrinsic high reactivity of **2**. Besides the synthesis of sorbicatechol A (**7a**), sets of styrenes substituted at the aromatic portion (**b**-**e**), of vinyl ethers (**f**-**k**), ethyl vinyl ketone (**l**) and heteroaromatic building blocks (**m**-**o**) were employed to give the respective sorbicatechol analogs **7a**-**o**.

Because of the reported anti-viral activity of **7a** against H1N1, a set of the prepared sorbicatechol analogs **7a**-**o** was evaluated for its anti-viral activity against influenza A virus (IAV). Initial tests were performed using the gaussia luciferase reporter virus.[12] Abundance of gaussia luciferase activity that accumulates in the supernatant of cells infected with this virus can be used as a proxy for virus replication. A resazurine conversion assay was also performed in order to assess the cytotoxic effect of the compounds on the cells.



Scheme 1. Synthesis of sorbicatechol A (**7a**) and 14 structural analogs **7b**-**o**. Product yields for each analog are given in brackets.

Unfortunately, the tested compounds exhibited cytotoxicity to the viral host cells at concentrations below the observation of anti-viral effects. This effect thus not only made it impossible to assess any anti-viral effects against IAV, but also raises the question if the initially reported anti-viral activity of **7a**[7] is a true anti-viral effect or rather the result of the cytotoxicity of the compound to the host cells. To further broaden our anti-viral screening we thus decided to expand from only testing *Orthomyxoviridae* (IAV) to *Retroviridae* by including tests against HIV-1. This system was also chosen due to the significant need for finding compounds active against HIV. Despite the fact that current anti-HIV drugs can minimize virus replication and thereby prevent the outbreak of AIDS, a series of severe problems remain, including the rapid emergence of resistant viruses, high virus variability, high costs and adverse side-effects.[13] As test system for anti-HIV activity, the EASY-HIT assay was performed, which is based on the reporter cell line LC5-RIC that contains a stably integrated fluorescent reporter gene. Upon HIV infection these cells express the fluorescent marker dsRed which can be directly connected to the anti-HIV activity of a compound when reduced during treatment.[14] We

used the resazurine conversion assay, which is based on enzymatic reduction of resazurin,[15] to check for any signs of negative effects on the vitality and metabolism of the LC5-RIC cells that could appear during the course of the EASY-HIT assay. Out of the 15 sorbicatechol analogs evaluated for anti-HIV properties, six compounds showed activities beyond their respective cytotoxicity values as determined by the viability assay.[15] Interestingly, all active compounds were either derived of a styrene (**7a**-**e**) or equipped with an aromatic portion in close proximity to the ene functionality in the substrate, as in ethyl phenyl ether **f**. All other compounds did not exhibit a measureable activity, including all additional ethers and all heteroaromatic systems as well as the benzoylated derivate **7b** with only one additional keto function in between the alkene and the aromatic moiety when compared to **7f**. These results strongly indicate that an aromatic portion in close proximity to the bicyclo[2.2.2]octane core structure of the sorbicatechols is required for activity. The selectivity indices (equaling the CC_{50}/IC_{50}) for the active compounds are mostly in the range of 1.37 to 1.72 (Table 1). In this range, cytotoxic effects on the host cells might indeed influence the anti-viral activity values. For the *ortho*-methyl substituted analog **7e**, however, a significant increase in activity can be observed, along with a slight decrease of cytotoxicity. Overall, this leads to a selectivity index of 3.49 for **7e**. Interestingly, a simple repositioning of the methyl substituent from the *para* (as in **7b**) to the *ortho* position led to a 2 fold increase in activity.

Table 1. Anti-HIV activity given as IC_{50} values and cytotoxicity determined as CC_{50} in the viability assay.[15] The selectivity index is calculated as CC_{50}/IC_{50}. Emtricitabine (FTC) was used as HIV inhibition control.

7x	1st step Easy Hit IC_{50} [µM]	Viability assay CC_{50} [µM]	Selectivity index (CC_{50}/IC_{50})
a	65.9 ± 4.68	*102**	1.55
b	68.8 ± 6.97	*105.2**	1.53
c	75.9 ± 7.37	*125.2**	1.65
d	62.7 ± 10.08	*108.4**	1.72
e	32.2 ± 2.52	*112.3**	3.49
f	76.6 ± 4.28	*105.2**	1.37
FTC	0.7 ± 0.22	*>100.0*	>140

Taken together, we have synthesized 15 sorbicatechol derivatives **7a**-**o** by application of a chemo-enzymatic synthesis of sorbicillinol (**2**) followed by quenching of **2** with a diverse set of dienophiles. Evaluation of their activity against IAV has revealed their cytotoxicity against the host cell system rather than a true anti-viral effect. Screening of the compound library against HIV-1 showed that aromatic substitution as the bicyclo[2.2.2]octane substructure is required to obtain anti-viral activity, although it remains to be weak. The strongest activity was obtained with **7e** with an IC_{50} value of approx. 32 µM and a selectivity index of 3.49. The activity is thus about 50-fold lower when compared to the commercial virostatic drug emtricitabine (FTC) with a >40-fold decreased selectivity index. In contrast to previous reports, our findings did thus not reveal antiviral activity of the sorbicatechols in a range promising for further antiviral lead optimization.

Experimental Section

Experimental Details.

The synthesis of sorbicatechol A (**7a**) and its derivatives follows a two-phase procedure, which is described here exemplary for the production of the most active derivative **7e**. First, sorbicillin (**1**) is dissolved in acetone and added to phosphate buffer (50 mM, pH = 8) with the enzyme SorbC. The reaction starts upon addition of NADH and is incubated for 12 h at room temperature. Second, the produced sorbicillinol (**2**) is extracted with organic solvent and *o*-methylstyrene (**e**) is added. After slow evaporation of the organic solvent under reduced pressure, to increase the concentration of the dienophile slowly over time, the crude product is purified by preperative HPLC.

Acknowledgements

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Keywords: biocatalysis • IAV • HIV • sorbicillinoids • anti-viral bioactivity

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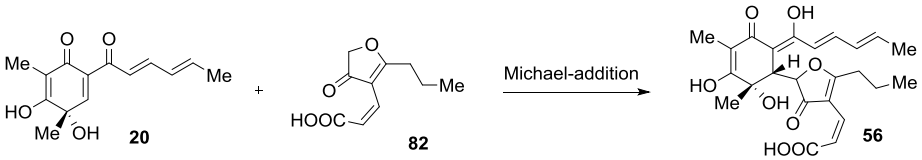
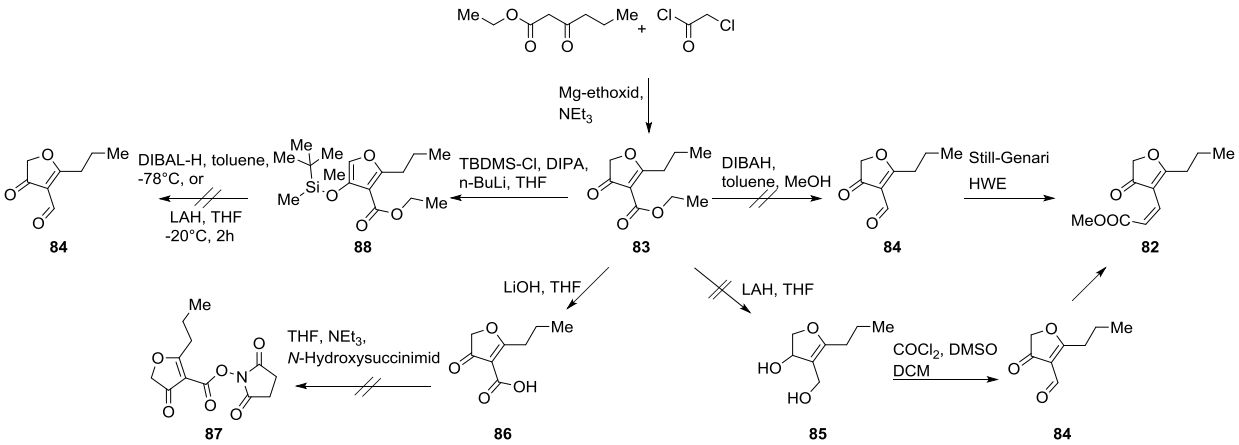
3.3.2 Synthesis of sorbifuranone A (56)

Sorbifuranone A (**56**) belongs to the functionalized sorbicillinoids, derived from a sorbicillinol (**20**) and a furanone moiety combined through a Michael addition.



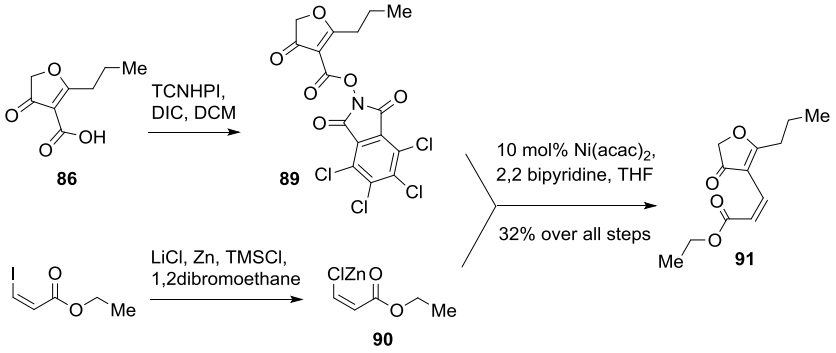
Scheme 12. Proposed synthesis of sorbifuranone A (**56**).

As sorbicillinol (**20**) is readily accessible using our optimized enzymatic dearomatization reaction with SorbC, only the synthesis of furanone **82** needed to be developed. We already knew from our synthesis of (+)-epoxysorbicillinol (**21**) that functionalization of enzymatically produced sorbicillinol (**20**) using external nucleophiles is possible. Thus, we assumed that coupling of **82** with sorbicillinol (**20**) can be performed analog to the addition of the peroxide in the synthesis of **21**. The first approach contained the synthesis of ester **83** by cyclisation of commercial available ethyl butyrylacetate and chloro acetylchloride with magnesiumethoxid and trietyhlamin. Having ester **83** in hand we planned to reduce the ester to an aldehyde function for further Still Genari HWE (Horner-Wadsworth Emmons) reaction to furanone **82**. Unfortunately, neither the reduction with DIBAL-H nor with LAH led to any product, but just to reisolation of starting material or degradation. We next thought of activating the ester by saponification of the ester bond with LiOH to acid **86** and subsequent synthesis of the *N*-hydroxysuccinimidester **87.** Unfortunately, it was not possible to purify and obtain the active ester **87**. The last attempt was to synthesize the silylenolether **88** to allow reduction of the ester to aldehyde **84** by using DIBAL-H or LAH. Again, only re-isolation of ester **83** was observed.



Scheme 13. Failed attempts to synthesize Michael-adduct 82.

We next turned our attention to the synthesis of furanone **82** by a protocol published by Baran and his group.[61] They developed a method to connect carbonic acid with iodo alkenes by cross coupling.



Scheme 14. Synthesis of furanone 91 via cross coupling.

The synthesis is divided into two steps: first the formation of TCNHPI-active ester **89** from acid **86** and second the production of zinc reagent **90** from commercially available *cis* ethyl-3-iodoacrylate. Both were used without further purification and we assumed a quantitative transformation. By combination of **89** and **90** and addition of 10% $Ni(acac)_2$ and 2,2- bipyridine in THF, furanone **91** can be obtained in 32% overall yield. After saponifaction of the ester of **91**, the synthesis of the desired furanone **82** would be completed.

As the starting material for the synthesis of **91** is expensive and yields were not yet optimized, we decided to first establish the Michael addition of a model furanone to sorbicillinol (**20**). As test substrates for establishing the required reaction conditions for the Michael addition we selected ester **83** (F) and silylether **88** (S) (Table 2). We decided to use three different strategies for Michael addition, first using organic bases for activation, second coordinative activation by Lewis acids, and third the use of a Brönstedt base. The first strategy was attempted using either pyridine, 2.5 lutidine, DBU, NMM, DIPA, NH_4OH or trimethylamine. As Lewis acid we decided on testing $FeCl_3$ and KOH as a Brönstedt base. The reaction temperatures were varied from room temperature to 100°C and reaction time differed between 8 and 16 hours (over night, oN).

Unfortunately, the desired adduct was not detectable with mass spectrosmetry or NMR analysis under none of the described reaction conditions. But we were able to detect the masses of furanone-sorbicillinol adducts with more than one furanone attached to sorbicillinol (**20**) by using the conditions labelled in red (Table 2), leading us to the conclusion that the problem was not insufficient reactivity during the Michael-addition, but rather too high reactivity, leading to multiple additions. We shortened reaction times and used stoichiometric amounts of furanone **83** and were able to ultimately detect the mass of the desired product by ESI- measurements (Figure 2). As the desired protected furanone **91** is already accessible and Michael addition conditions seem to work under the described conditions, the synthesis of sorbifuranone A (**56**) should have a foreseeable end.

Table 2. Reaction condition tested for Michael addition between sorbicillinol (20) and furanones 83 and 89.

Sorbicillinol (10mg/2mL DMF)	furanone (F)/ silylether (S)		Base / Lewis acid	solvent	temp.	time
2 mL	F,	5 eq	pyridine 0.5 mL	DMF	Rt	oN
2 mL	F,	5 eq	pyridin 1 mL	DMF	Rt	oN
2 mL	F,	5 eq	2,6- lutidin 0.5 mL	DMF	Rt	oN
2 mL	F,	5 eq	2,6 –lutidin 0.5 mL	DMF	100°C	8h
2 mL	F,	5 eq	pyridin 0.5 mL	DMF	100°C	8h
2 mL	F,	5 eq	DBU 0.5 mL	DMF	100°C	8h
2 mL	F,	5 eq	DBU 0.5 mL	DMF	Rt	oN
2 mL	F,	5 eq	NMM 0.5 mL	DMF	Rt	oN
2 mL	F,	5 eq	NMM 0.5 mL	DMF	100°C	8h
2 mL	F,	5 eq	DIPA 0.5 mL	DMF	Rt	oN
2 mL	F,	5 eq	DIPA 0.5 mL	DMF	100°C	8h
2 mL	F,	5 eq	NH4OH 0.5 mL	DMF	100°C	8h
2 mL	F,	5 eq	NH4OH 0.5 mL	DMF	Rt	oN
2 mL	F,	5 eq	triethylamine 0.5 mL	DMF	Rt	oN
2 mL	F,	5 eq	triethylamine 0.5 mL	DMF	100°C	8h
2 mL	F,	5 eq	FeCl3, cat.	DMF	100°C	8h
2 mL	F,	5 eq	FeCl3, cat.	DMF	Rt	oN
2 mL	S,	5 eq	FeCl3, cat.	DMF	100°C	8h
2 mL	S,	5 eq	FeCl3, cat.	DMF	Rt	oN
2 mL	F,	5 eq	KOH, 1M, 0.5 mL	DMF	100°C	8h
2 mL	F,	5 eq	KOH, 1M, 0.5 mL	DMF	Rt	oN
2 mL	S,	5 eq	KOH, 1M, 0.5 mL	DMF	100°C	8h
2 mL	S,	5 eq	KOH, 1M, 0.5 mL	DMF	Rt	oN
2 mL	S,	5 eq	pyridine, 0.5 mL	DMF	Rt	oN
2 mL	S,	5 eq	pyridine, 0.5 mL	DMF	100°C	8h
2 mL	**F,**	**1 eq**	**Pyridine, 0.5 mL**	**DMF**	**Rt**	**30 min**

Analytic data of the variety of Michael addition reactions were performed by ESI-mass spectroscopy. Using the green labelled reaction condition (Table 2) the mass of furanone (**83**) coupled to sorbicillinol (**20**) was detected (Figure 4).

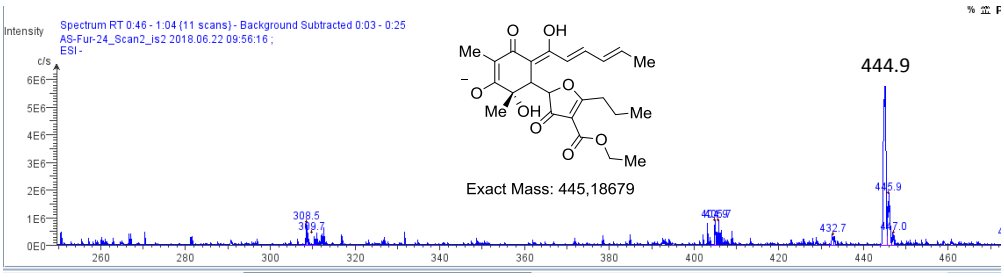
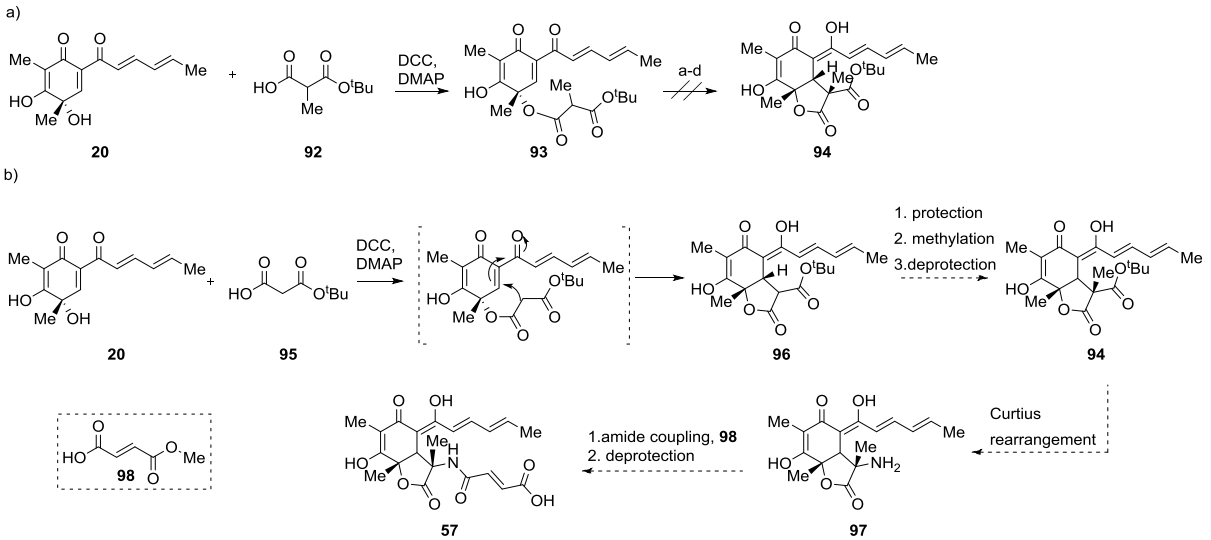


Figure 4. ESI-mass spectra of furanone 83 coupled to soribicillinol (20).

3.3.3 Synthesis of sorbicillactone A (57)

Sorbicillactone A (**57**) belongs to the class of functionalized sorbicillinoids and in addition to its special molecular structure possesses a range of intriguing bioactivities, as mentioned in chapter 1.4.4. We therefore developed a chemo-enzymatic, total synthetic strategy shown in Scheme 15 below. The proposed synthesis starts with esterification of mono-protected malonic acid or methyl malonic acid to sorbicillinol (**20**), followed by a Michael addition to form the five-membered ring of sorbicillactone A (**57**). After the core structure of **57** is completed, methylation followed by a Curtius rearrangement should be performed. In a last step, fumaric acid is attached by amide coupling, giving the desired product potentially with perfect stereoselectivity and within an efficient synthetic route.

In a first attempt, methyl malonic acid was mono-protected with *tert*-butanol to ester **92**, which was then coupled to sorbicillinol (**20**) using a standard esterification protocol to give ester **93**. Cyclisation by Michael addition of **93** to lactone **94** was attempted using different Lewis acids (Scheme 15). Unfortunately, none resulted in the desired outcome and we thus changed the starting point to malonic acid, because we envisioned that cyclization will be easier to achieve without the steric hinderence of the methyl group. Malonic acid was converted to ester **95** by the same procedure as described. After coupling of **95** to sorbicillinol (**20**), the Michael addition occured spontaneously giving lactone **96** in only one step. The next planed steps will be the introduction of the methyl group to lactone **94**, followed by a curtius rearrangement to amine **97** and subsequent amide bond formation with fumaric acid (**98**) to sorbicillactone A (**57**).



Scheme 15. Synthesis of sorbicillactone A (57). a) Esterification of sorbicillinol (**20**) with *tert*-butyl protected methyl-malonyl carboxylic acid (**92**) with subsequent attempts for cyclisation a-d. a) cat. $FeCl_3$-$6H_2O$, rt, 12h b) cat.$FeCl_3$ (dry), rt, 12h c) 1.0 eq. LiI, DME, 85°C, 12h and d) allox, 85°C, 2h.

b) Esterification of sorbicillinol (**20**) with *tert*-butyl protected malonyl carboxylic acid (**95**) and spontaneous cyclisation to lactone **96**. The next steps to sorbicillactone A (**57**) are protection, methylation and deprotection to **94**, which is then converted to amine **97** via curtius rearrangement. Finally monoprotected fumaric acid **98** is coupled to **97** and deprotectd to obtain **57**.

3.3.4 Upscaling of the SorbC enzymatic reaction

Enzymatic reactions can suffer from the huge disadvantage that the reaction scale is rather limited. When establishing a chemo-enzymatic synthesis containing the enzymatic step at an early stage of the synthetic plan, this can lead to difficulties regarding the amount of required material for further functionalization. It is thus important to provide an upscaling methodology for the efficient use of enzymes in total synthesis.

In our case, the further chemical functionalization of lactone **96** made it necessary to upscale the enzymatic reaction, as it was only possible to obtain mg quantities of semi-purified sorbicillinol via transformation in batch work. The idea was to immobilize SorbC by using the hexahistidine tag for attachment to a nickel column and to utilize this setup in a flow reactor. Using a test column with 1 mL volume, we examined if SorbC is still active when attached to solid phase. To our delight, the monooxygenase converted sorbicillin (**18**) completely to sorbicillinol (**20**) in the first run. Activity decreased after the second run, but by washing the column with phosphate buffer, the activity was restored (Figure 5). These preliminary results now set the stage for upscaling into larger columns until the need for gram quantities of semi-purified sorbicillinol (**20**) is satisfied.

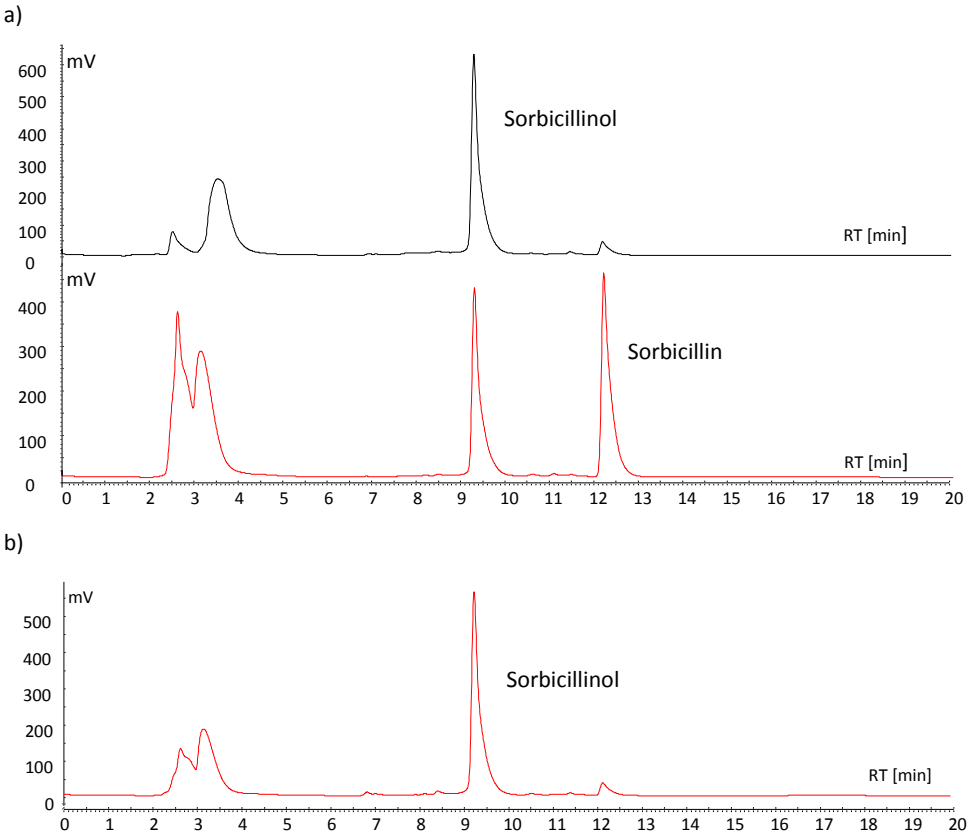


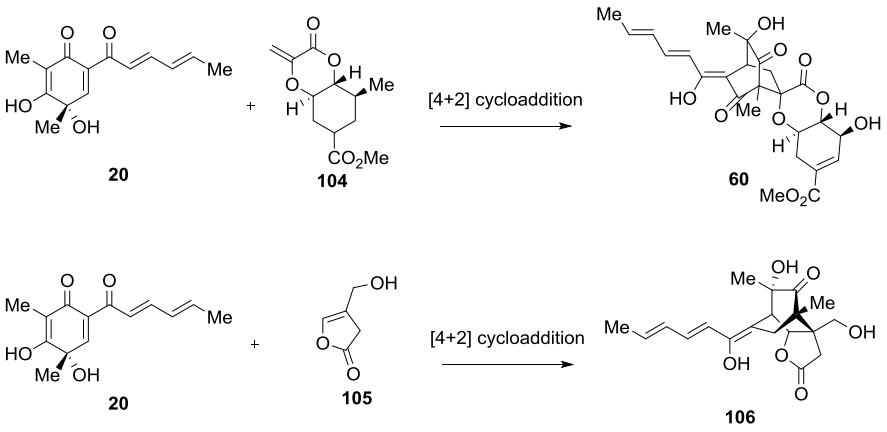
Figure 5. HPLC chromatogram of the sorbicillin (18) reaction solution oxidized by immobilized SorbC on nickel beads to sorbicillinol (**20**). a) first run, total conversion to **20**, second run, activity decreased to 50%, c) after a washing step with phosphate buffer, the activity of SorbC was regained.

4. Outlook

In this thesis we, established the first chemo-enzymatic total synthesis to a variety of sorbicillinoids. Following our procedure, we improved the synthetic access to enantiomerically pure bisorbicillinol (**15**) and trichodimerol (**46**) and developed the first total synthesis to bisvertinolone (**51**) and sorbiquinol (**38**). Further, we created derivatives of all dimeric core structures by variation of the attached side chain, thus proving the applicability of SorbC on different substrates. We then set out to the synthesis of functionalized sorbicillinoids and established an efficient chemo-enzymatic pathway to achieve the first enantioselective total synthesis of sorbicatechol A (**58**), rezishanone B and C (**59**). Further, tests on the substrate tolerance of SorbC by derivatization of the core structure of sorbicillin (**18**) lead to the first synthesis of the natural products sorrentanone (**23**) and oxosorbicillinol (**22**). Examining the possibility of further chemical functionalization of sorbicillinol (**20**), we developed the first enantioselective total synthesis of (+)-epoxysorbicillinol (**21**). As most sorbicillinoids exhibit highly fascinating biological activities we envisioned picking one of our functionalized compounds for further structural derivatization and screening for pharmacological use. We decided on sorbicactechol A (**58**), because of its anti-viral activity against Influenza A. Following our established route, we synthesized 14 different catechol-derivatives and tested them against HIV and H1N1, thus finding 5 active compounds with one superior structure containing a good anti-HIV activity with an IC_{50} value of 32.2 ± 2.52 µM.

The completion of the total synthesis of sorbifuranone A (**56**) (Scheme 12) and sorbicillactone A (**57**) (Scheme 15) are next steps for further work on the sorbicillinoids. While for **56** only two more steps are needed, the synthesis of **57** has just started. Having synthesized the core structure of lactone **96**, six further organic reaction steps need to be established.

Other interesting sorbicillinoids to be targeted for chemo-enzymatic synthesis are, for example, spirosorbicillinol A (**60**), which contains a highly interesting spiro scaffold, or rezishanone A (**106**) (Figure 6). Synthetic routes to lactone **104** and **105** are currently developed in our group.



Scheme 16. Synthesis of spirosorbicillinol A (60) and rezishanone A (106) by [4+2]-cycloaddition of enzymatically provided sorbicillinol (**20**) and a synthetically produced dienophile **104** or **105**.

Other very interesting structures for total synthesis are trisorbicillinone A (**107**) or trichodermanone A (**108**). Up to date, there have been no successful syntheses of these highly interesting compounds.

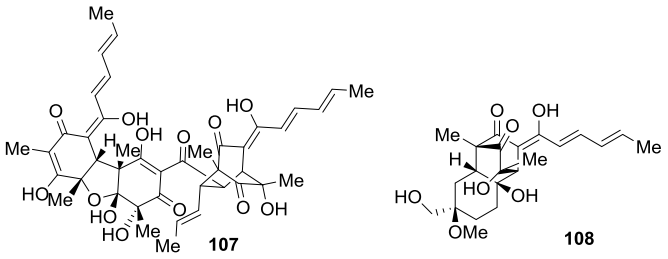


Figure 6. Structures of trisorbicillinone A (107) and trichodermanone A (108).

Further investigation on the biosynthesis of this natural product class still has high potential for new discoveries in sorbicillinoid research. Regarding the fact that commonly only one to a few sorbicillinoids are found in a single producer, the question arises how nature controls biosynthesis of specific sorbicillinoids. In this thesis we proofed that the sorbicillinoids are accessible from sorbicillinol (**20**) without the need of additional enzymes, but only by variation of the surrounding conditions. To find out how the different pathways to the variety of sorbicillinols are triggered, or if nature has additional tricks available for our use will be a crucial question for following research on this natural product class. Answering this question could help to improve the yields of the enzymatic transformations, as the formation of side products, especially of dimeric sorbicillinoids, could possibly be reduced, leading to an even higher efficacy of natural product synthesis.

Another highly important task will be the expansion of bioactivity tests. Before our work it was not possible to produce high amounts of pure compounds for pharmacological screening. Instead, most of the times only one test system was used and a lot of potential was left out. Now, as stereoselective production and chemical functionalization of sorbicillinoids is available, a new possibility for derivatization and drug design is provided. As shown in our total synthesis of (+)-

epoxysorbicillinol (**21**), sorbicillinol (**20**) can be chemically altered by external nucleophiles, thus opening the field for producing compound libraries for further testing. Despite the large number of already reported biological activities of sorbicillinoids, we are confident that a global screening of the now accessible compounds will bring an even larger pool of interesting pharmacological activities and a potentially applicable medicinal benefit.

Chemo-enzymatic as well as natural product total synthesis are strong and important research fields in organic synthesis. The work on sorbicillinoids proofs once again the advantages of combining these fields and learning from nature. For the future we can be sure that this intriguing class of sorbicillinoid natural products will continue to provide interesting and astonishing results, thus evidencing that there is still a huge potential to reveal and a lot to expect.

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List of Abbreviations

AcP	acyl carrier protein
AT	acyltransferase domain
AIBN	azobisisobutyronitrile
BHT	butylhydroxytoluol
CADA	catalytic asymmetric dearomatization
CE	cholesteryl ester
CMet	*C*-methyl transferase
DH	dehydratase
DNA	deoxyribonucleic acid
DMP	Dess-Martin periodinan
DBU	1,8-diazabicyclo[5.4.0]undec-7-en
DCM	dichloromethane
DCC	dicyclohexylcarbodiimid
DIBAL-H	diisobutylaluminium hydride
DMAP	4-dimethylaminopyridine
DMF	dimethylformamid
DPPH	2,2-diphenyl-1-picrylhydrazyl
ER	enoylreductase
FAD	flavin adenine dinucleotide
gDNA	genomic deoxyribonucleic acid
HOMO	highest occupied molecular orbital
HPLC	high-performance liquid chromatography
IPTG	isopropyl β-d-1-thiogalactopyranoside
KR	ketoreductase
KS	ketosynthase
LUMO	lowest unoccupied molecular orbital
MIC	minimal inhibitory concentration
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate

NSCL	non-small cell lung
NMR	nuclear magnetic resonance spectroscopy
NOE	nuclear Overhauser effect
PPAR-γ	peroxisomal proliferator-activated receptor γ
PIDA	Phenyliodine(III) diacetate / bis(acetoxy)iodobenzene
PP	phosphate buffer
PMB	*p*-methoxybenzyl
PKS	polyketide synthase
PCR	polymerase chain reaction
PT	product template
PPTS	pyridinium *p*-toluenesulfonate
Rac	racemic
Red	reductase
SAT	starter-unit acyl transferase
TBS	*tert*-butyl silyl
THF	tetrahydrofuran
TE	thioesterase domain
TG	triacylglycerol
TFA	trifluoric acid
TMS	trimethylsilyl
TTMSS	tris(trimethylsilyl)silane
TNF	tumor necrosis factor

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Appendix

A.1. Supplemental materials of publications

A.1.1. Stereoselective Total Synthesis of Bisorbicillinoid Natural Products by Enzymatic Oxidative Dearomatization/Dimerization – Sib *et al*. 2017

A.1.2. Chemo-enzymatic Total Synthesis of Oxosorbicillinol, Sorrentanone, Rezishanones B and C, Sorbicatechol A, Bisvertinolone, and (+)-Epoxysorbicillinol, Sib *et al.* 2018

A.1.3. Chemo-Enzymatic Total Synthesis of Sorbicatechol Structural Analogs and Evaluation of Their Anti-Viral Potential

A.2. Experimental part of sorbifuranone A synthesis

A.3. Experimental part of sorbicillactone A synthesis

A.4. NMR-data

Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Stereoselective Total Synthesis of Bisorbicillinoid Natural Products by Enzymatic Oxidative Dearomatization/Dimerization

*Anna Sib and Tobias A. M. Gulder**

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1. General Information

Chemistry: All solvents used in the reactions were p.A. grade. Solvents for chromatography were technical grade and distilled prior to use. Anhydrous dichloromethane and THF were obtained from an MBraun MB-SPS 800 solvent purification system. Commercial materials were purchased at the highest commercial quality from the providers abcr, Acros, Organics, Alfa Aesar, Carbolution, Carl Roth, Merck, Sigma Aldrich, VWR, Jena Biosciences and Thermo Fisher Scientific. These chemicals were used without further purification. Silica gel Geduran® Si 60 (particle size 0.40 – 0.60 mm) purchased from Merck, was used for flash column chromatography. Solvent mixtures are understood as volume/volume. For TLC analysis, TLC-silica gel 60 F254 plates were purchased from Merck. Applied substances were observed using a UV lamp at 254 nm. For UV-inactive substances, dyeing reagents, such as 0.36% ninhydrin solution in ethanol were used. NMR spectra were recorded on Bruker AVHD300, Bruker AVHD400, Bruker AVHD500 (only ^{1}H NMR spectra), or Bruker AV500-cryo spectrometers. The chemical shifts δ are listed as parts per million [ppm] and refer to δ(TMS) = 0. The spectra were calibrated using residual undeuterated solvent as an internal reference (δ($CDCl_3$) = 7.26 ppm, δ(C_6D_6) = 7.16 ppm, δ(methanol-d_4) = 3.31 ppm for ^{1}H NMR; δ($CDCl_3$) = 77.0 ppm, δ(C_6D_6) = 128.1 ppm, δ(methanol-d_4) = 49.0 ppm for ^{13}C NMR). The following abbreviations (or combinations thereof) are used to explain the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets, q = quartet, m = multiplet, br = broad. In addition, the following abbreviations for chemicals are used: EtOAc = ethyl acetate, Et_2O = diethylether, ACN = acetonitrile, TLC = thin layer chromatography, rt = room temperature.
For High Performance Liquid Chromatography (HPLC) analyses, a computer controlled Jasco system was used (UV-1575 Intelligent UV/VIS Detector, DG-2080-53 3-Line Degaser, two PU-1580 Intelligent HPLC Pumps, AS-1550 Intelligent Sampler, HG-1580-32 Dynamic Mixer). The analyses of the recorded chromatograms were performed using Galaxie-Chromatography-Software provided by Jasco. A Eurosphere II 100-3 C18 A (150 x 4.6 mm) column with integrated precolumn manufactured by Knauer was used for analytical separations with the following composition of the eluent: A = H_2O + 0.05% TFA and B = ACN + 0.05% TFA. The analytical method consisted of the following gradient: 0-1 min 5% B, 1-15 min 95% B, 15-18 min 95% B, 18-18.5 min 5% B, 18.5-20 min 5% B with a flowrate of 1 mL/min. This method was used for all analyses. The chiral resolution was carried out on CHIRALCEL OD-RH column material (150 x 4.6 mm) using isocratic conditions (33 % B) at a flow rate of 1 mL/min at 15°C. Isolation of the products was carried out by semi-preparative HPLC controlled by a Jasco HPLC system consisting of an UV-1575 Intelligent UV/VIS Detector, two PU-2068 Intelligent prep. Pumps, a MIKA 1000 Dynamic Mixing Chamber (1000 µL Portmann Instruments AG Biel-Benken), a LC-NetII/ ADC, and a Rheodyne injection valve. The system was controlled by the Galaxie-Software and the eluent system consisted of: A = H_2O + 0.05% TFA and B = ACN + 0.05% TFA. A Eurosphere II 100-5 C18 A (250 x 16 mm) column with precolumn (30 x 16 mm) provided by Knauer was used as the stationary phase. The individual gradient compositions are given below. After preparative separation of the product, the collected fractions containing the desired product were combined and the ACN was removed under reduced pressure. The remaining aqueous phases were freeze-dried in liquid nitrogen and the water removed by lyophilization (Alpha 2-4 *Christ* with Chemistry-Hybrid-Pump-RC6 pump). For medium pressure liquid chromatography (MPLC) the Reveleris® X2 MPLC system (Grace) was used together with Reverleris® Reverse Phase (RP) C18 columns (Grace) using UV-detection at 220 nm, 254 nm, and 280 nm. The eluent system was composed as follows: A= H_2O + 0.05% TFA and B= ACN + 0.05% TFA.

Biochemistry/Molecular Biology: Kits for plasmid isolation (peqGOLD Plasmid Miniprep Kit I C-Line, Peqlab), PD-10 columns, and Vivaspin 2 Hydrosart membrane columns (30,000 MWCO) were bought from VWR. The bacterial strain *Escherichia coli* SoluBL21 was acquired from Genlantis and used as a host for the production of the enzyme SorbC. *E.coli* were grown in 2xYT-Media. SorbC was produced as hexa-histidyl-tagged fusion protein. The vector pET28a (+), containing sorbC, KanR and a hexa-histidine tag were purchased from Genscript. The bacterial cells were transformed with the expression construct by electroporation. 100 ng of the plasmid DNA was suspended in 50 µL electrocompetent *E.coli* SoluBL 21 cell suspension and poured into a sterile electroporation cuvette. Electroporation was carried out using the program Ec2 bacteria (Biorad Micropulser). After the electroporation the cells were suspended in 1 mL SOC-Media and shaken for 60 min at 37°C. 100 µL of the bacterial suspension were streaked out onto a LB-Agar plate containing kanamycin sulfate and incubated over night at 37°C. Single clones were used to inoculate starting cultures for protein production. The transformants were grown at 25 °C in 2xYT medium containing 50 µg/ml kanamycin sulfate overnight as a preculture. These starter cultures were used to inoculate 2xYT (1:100) supplemented with antibiotic. When an OD_{600} = 0.6 was reached, protein production was induced by adding 0.5 mM isopropyl-β-D-thiogalactopyranosid (IPTG), followed by an overnight incubation at 25 °C with shaking (200 rpm). The cells were harvested by centrifugation and re-suspended in buffer A. After sonication on ice and subsequent centrifugation (10,000 rpm, 4 °C, 30 min), the supernatant was incubated with High Density Nickel Agarose (Jena Bioscience) with moderate shaking for one hour on ice. This suspension was applied onto a column, which was washed with buffer A. Finally, the protein was eluted with buffer B. A PD-10 column (GE Healthcare) was used to exchange the eluate's buffer to buffer C. The protein solution was then concentrated using a Vivaspin 2 Hydrosart membrane column (Sartorius). Final protein concentrations were determined photometrically using the Nanophotometer 330 (Implen) at 280 nm using the extinction coefficient of SorbC ε(280 nm) = 50920 M^{-1} cm^{-1}. Protein production, enrichment and purification were monitored by SDS-PAGE analysis (BioRad Mini Protean® Tetra System) using Unstained Protein MW Marker (Thermo Scientific). All buffers consisted of 50 mM Tris/Cl at pH 7.5, 150 mM NaCl, and 5% glycerol) with changing concentrations of imidazole (buffer A: 20 mM; buffer B: 250 mM; buffer C: no imidazole). The enzymatic oxidative dearomatization reactions were performed in phosphate buffer (50 mM, pH 8.0).

2. Expression construct and gene/protein sequences

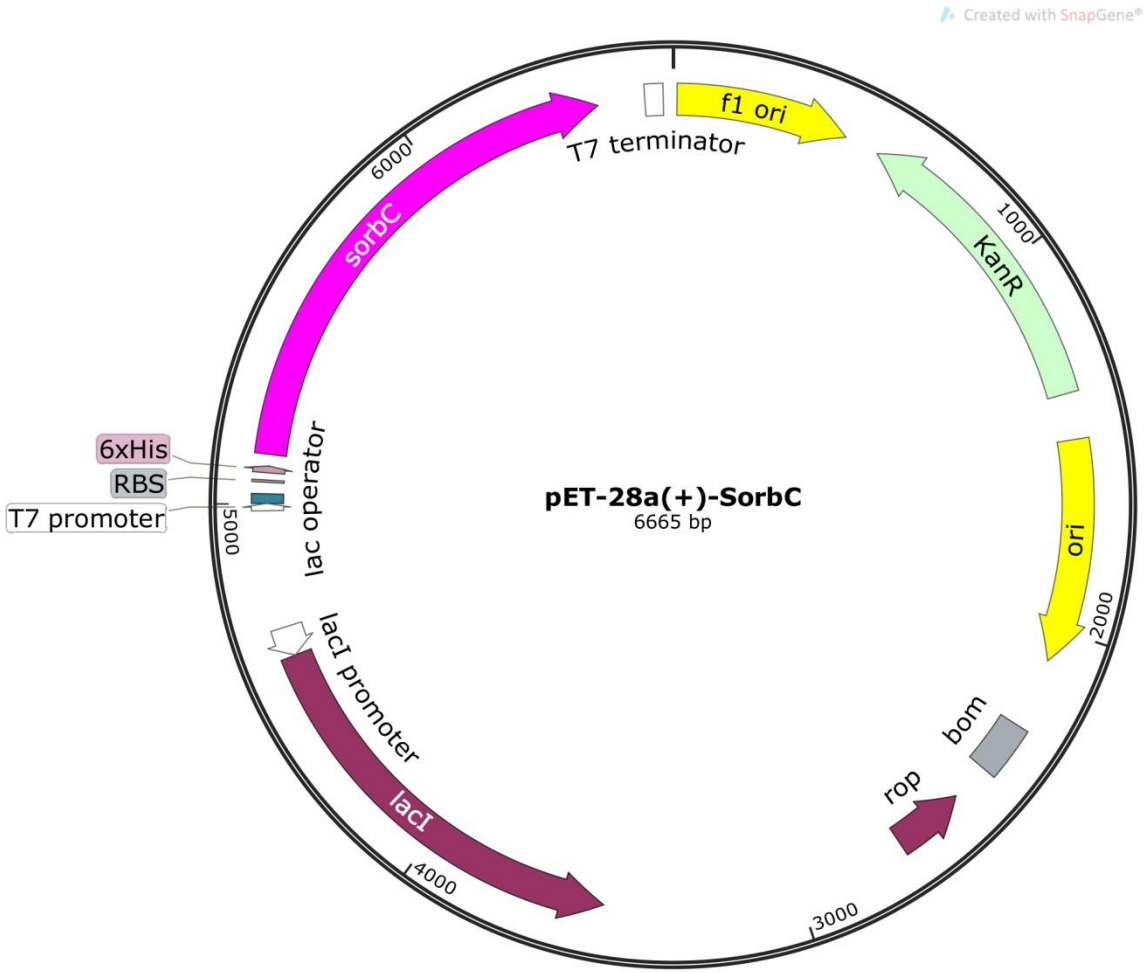


Figure S1. Vector map of the expression construct of s*orbC*.

DNA sequence of *sorbC* [1338 bp]:

ATGACGCGTTCGGCGAATAGCCCGTTTGAAGTCGCAATCGTGGGTGGTGGCATTACGGGTCTGGCACTGGCGGTCGGTCTGCT
GAAACGTAACGTTTCATTTACCATTTATGAACGCGCCGAAAATTTCGGTGAACTGGGTGTCGGCATCACCTTTACGCCGAACGCT
CAGCGTGCGATGGAAGCGCTGGACCCGTGCGTCCTGCAATCGTTTACCAACGTGGCAAGTGCTCCGTCCGGCGGTACGATTAA
TTTCGTCGATGGTGTGCGTGAACAGGGCTCAGAAGACCCGCGCACCTCGACCGCCGCACTGCTGTTTCAACTGCATGTGAAAG
GCGGTTATAAAGCATGCCGTCGCTGTGATTTCGTGGACCAGATTGTTCAACACATCCCGAAAGACTGTGTTCAGTACCGCAAATG
GCTGGATAGCATTGAAACCGACCATGAATCTGGTCGTGCGGTGCTGAAATTTCGCGATGGCGAAATCGCGCACGCCGATGTGGT
TATTGGTTGCGACGGCATCCGTAGCCAGGTTCGCGCTTCTATGTTCGGTACCGATGAACTGTGTCCGCGTGCGCAGTATAGTCA
TCAACTGGGTTACCGCGGCATGGTTCCGCTGGCACAAGCTACCGCCGTCCTGGGTCCGGAAAAAACCAGCTCTGCGGTGCTGC
ACACCGGTCCGGGTGCATTTGTGCTGACGATCCCGCTGGCAGAAGTTCATGCTATGCACATTGAAGCCTTCATCATGGATAAAG
AAGAATGGCCGGAAGTCCAGACCAGTTCCGACTCAAAACGTTACGTGCTGCCGGCTACCCGCAATGAAGCGACGAAAGCCTTTG
CAGAATTTGGCCCGACGGTCCGTTCAGCCGTGTCGATGTTCCCGGAAAAACTGGAAAAATGGGCAGTCTTTGATATGCTGGAAG
CCCCGGTGCCGACCTTCGCAAAAGGTCGCGTTTGCCTGGCTGGCGACGCAGCTCATGCCAGCACGCCGAACCAGGGCGGTGG
CGCGGGTTTTGGCATTGAAGATGCGCTGGTTCTGGCCGAAGTTCTGGCAGTCCTGGCTGAAGCGCCGAATGTTAGTGGTATCGT
CGCCTCCGAAGCCCTGGCAGTGTATAGTGAAGTTCGTTACGAACGCTCCCAGTGGCTGGTGCGTTCATCGCGTCGCACCGGTG
AACTGTGTACGTGGAAAGATCGTGATTGGGGCCTGGCCGCAGAAGAACTGAGCCGTGATATTATCAGCCGCTCTCATCAACTGT
GGGATCACGACACCGCGGGCATGGTCTCTGATGCACTGGCGATTCTGGGCGAACGTGTCCGCGGCGCTGATACGGCGTTCTGA

Protein sequence of *sorbC* [445 AA, 48.1 kDa]:

MTRSANSPFEVAIVGGGITGLALAVGLLKRNVSFTIYERAENFGELGVGITFTPNAQRAMEALDPCVLQSFTNVASAPSGGTINFVDGV
REQGSEDPRTSTAALLFQLHVKGGYKACRRCDFVDQIVQHIPKDCVQYRKWLDSIETDHESGRAVLKFRDGEIAHADVVIGCDGIRSQ
VRASMFGTDELCPRAQYSHQLGYRGMVPLAQATAVLGPEKTSSAVLHTGPGAFVLTIPLAEVHAMHIEAFIMDKEEWPEVQTSSDSKR
YVLPATRNEATKAFAEFGPTVRSAVSMFPEKLEKWAVFDMLEAPVPTFAKGRVCLAGDAAHASTPNQGGGAGFGIEDALVLAEVLAVL
AEAPNVSGIVASEALAVYSEVRYERSQWLVRSSRRTGELCTWKDRDWGLAAEELSRDIISRSHQLWDHDTAGMVSDALAILGERVRG
ADTAF

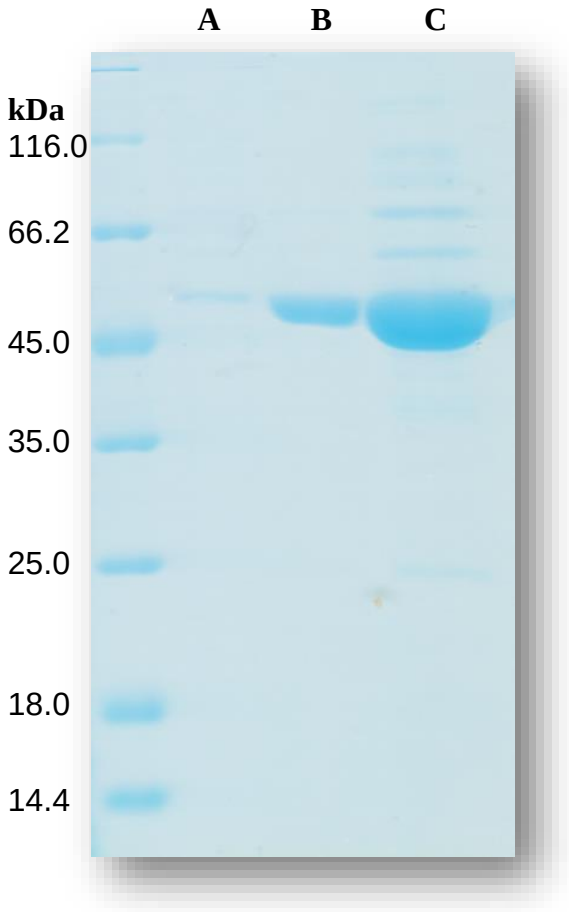


Figure S2. SDS-PAGE analysis of purified SorbC (50.3 kDa with tag). A: after elution from Ni NTA; B after PD-10 purification; C after final concentration.

3. Experimental Data

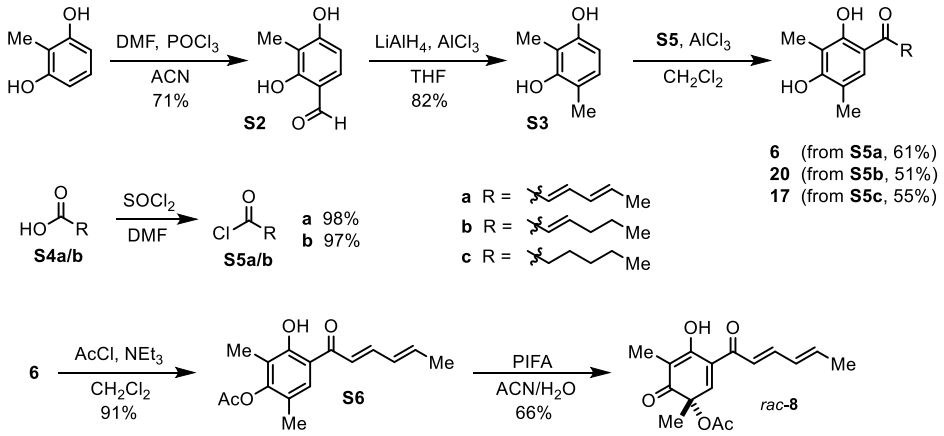


Figure S3. Synthesis of enzyme substrates **6**, **17**, and **20** as well as of *rac*-**8** for the development of a resolution method on a chiral HPLC phase.

3.1 Synthesis of 2,4-dihydroxy-3-methylbenzaldehyde (**S2**)

DMF (2.5 mL, 32.5 mmol, 1.3 eq.) was mixed with dry ACN (7.5 mL). A solution of phosphoryl chloride (2.5 mL, 26.7 mmol, 1.1 eq.) in ACN (2.5 mL) was added dropwise over 5 min. After stirring for 30 min at rt, the reaction mixture was cooled to 0 °C and 2-methylresorcinol (**S1**) (3.0 g, 24.2 mmol, 1.0 eq.) dissolved in dry ACN (7.5 mL) was added dropwise over 1 h. The reaction was finished after stirring at room temperature for 1 h. The product precipitated as white solid, was filtered off and recrystallized from H_2O. This furnished **S2** as white crystals (2.6 g, 17.1 mmol, 71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 11.67 (s, 1H), 9.68 (s, 1H), 7.28 (d, *J* = 8.5 Hz, 1H), 6.48 (d, *J* = 8.5 Hz, 1H), 5.75 (bs, 1H), 2.14 (s, 3H). ^{13}C NMR (300 MHz, $CDCl_3$) δ= 194.8, 162.4, 161.4, 133.1, 115.3, 111.3, 108.2, 7.2. MS (ESI-): m/z = 151.1 $[M-H]^-$. The physical and spectroscopic data were in agreement with that described in the literature.[1]

3.2 Synthesis of 2,4-dimethylresorcinol (**S3**)

S2 (1.0 g, 6.6 mmol, 1.0 eq.) was dissolved in abs. THF (60 mL) under argon atmosphere and cooled to 0°C. $AlCl_3$ (2.2 g, 16.5 mmol, 2.5 eq.) and $LiAlH_4$ (625.0 mg, 16.5 mmol, 2.5 eq.) were added slowly. The resulting slurry was refluxed for 3 h. After cooling of the reaction mixture to 0 °C, remaining reagents were quenched by careful addition of 1 N HCl and the acidified solution extracted with Et_2O (3 x 100 mL). The organic phases pooled, dried over $MgSO_4$, filtered and then solvent evaporated under reduced pressure. Column chromatography (silica gel, *n*-pentane/EtOAc (3:1), R_f = 0.62) gave the product as a white powder (0.74 g, 5.4 mmol, 82%). ^{1}H NMR (300 MHz, MeOD) δ= 6.69 (d, *J* = 8.1 Hz, 1H), 6.26 (d, *J* = 8.1 Hz), 2.11 (s, 3H), 2.08 (s, 3H). ^{13}C NMR (300 MHz, MeOD) δ= 155.0, 154.7, 128.2, 116.8, 112.6, 107.6, 16.2, 8.9. MS (ESI-): m/z = 137.1 $[M-H]^-$.The physical and spectroscopic data were in agreement with that described in the literature.[2]

3.3 Synthesis of sorbic acid chloride (**S5a**)

Under argon atmosphere sorbic acid (**S4a**) (5.0 g, 44.6 mmol, 1.0 eq.) was dissolved in $SOCl_2$ (6.5 mL, 89.6 mmol, 2.0 eq.) and DMF (50 µL) and then heated for 40 minutes at 80 °C. The reaction mixture was distilled under reduced pressure (40°C, 0.5 torr) to obtain the product as slightly yellow liquid (5.7 g, 43.8 mmol, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 7.43 (dd, *J* = 14.8, *J* = 10.7 Hz, 1H), 6.19-6.45 (m, 2H), 5.99 (d, *J* = 14.8 Hz, 1H), 1.93 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (300 MHz, MeOD) δ= 169.1, 146.5, 140.8, 130.8, 119.3, 18.7.The physical and spectroscopic data were in agreement with that described in the literature.[3]

3.4 Synthesis of (E)-2-hexenoic acid chloride (**S5b**)

Under argon atmosphere, (E)-2-hexenoic acid (**S4b**) (5.0 g, 43.0 mmol, 1.0 eq.) was dissolved in $SOCl_2$ (10.4 mL, 87.6 mmol, 2.0 eq.) and DMF (50 µL) and refluxed for 40 minutes. The reaction mixture was distilled under reduced pressure (40°C, 0.5 torr). A clear liquid was obtained (5.7 g, 42.3 mmol, 97%) and directly used in 3.16. ^{1}H NMR (400 MHz, MeOD) δ= 6.89 (dt, *J* = 15.7, 7.0 Hz, 1H), 5.78 (d, *J* = 15.7 Hz, 1 H), 2.15 (qd, *J* = 7.2, 1.6 Hz, 2 H) 1.44 (h, *J* = 7.4 Hz, 2H), 0.88 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (300 MHz, MeOD) δ= 168.2, 150.5, 121.9, 35.0, 22.2, 13.9.

3.5 Synthesis of sorbicillin (**6**)

S3 (700.0 mg, 5.1 mmol, 1.0 eq.) was dissolved in abs. CH_2Cl_2 under argon atmosphere and the solution cooled to 0°C. Freshly produced sorbic acid chloride (**S5**) (600.0 µL, 5.6 mmol, 1.1 eq.) and $AlCl_3$ (800.0 mg, 6.0 mmol, 1.2 eq.) were added. After 1 h of stirring at rt, the reaction mixture was acidified with 1 N HCl and extracted with CH_2Cl_2 (3 x 50 mL). The organic phases were pooled, dried over $MgSO_4$, filtered, and the solvent evaporated under reduced pressure. Purification of the raw material by MPLC (column: Reveleris C18- 40g, isocratic, H_2O+ 0.05% TFA / ACN+ 0.05% TFA = 50 / 50, flow rate: 30 mL/min, running time: 33.5 min, retention time: 22.0-27.5 min) gave bright yellow crystals of sorbicillin (**6**) (710 mg, 3.1 mmol, 61%). ^{1}H NMR (500 MHz, MeOD) δ= 7.56 (s, 1H), 7.41 (dd, *J* = 14.8, 11.0 Hz, 1H), 7.12 (d, *J* = 14.8 Hz, 1H), 6.50-6.26 (m, 2H), 2.19 (s, 3H), 2.08 (s, 3H), 1.91 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (125 MHz, MeOD) δ= 193.9, 163.6, 162.2, 145.3, 141.7, 132.0, 130.0, 123.4, 117.2, 113.9, 112.0, 18.9, 16.4, 8.0. MS (ESI+): m/z = 233.1 $[M+H]^+$.The physical and spectroscopic data were in agreement with that described in the literature.[4]

3.6 Synthesis of hexyl sorbicillin (**17**)

Under argon atmosphere, 2,4-dimethylresorcinol (**S3**) (100 mg, 724.3 µmol, 1.0 eq.) was dissolved in abs. CH_2Cl_2 (5 mL) and cooled to 0°C. Commercial hexyl acid chloride (109.0 µL, 796.0 µmol, 1.1 eq.) and $AlCl_3$ (106.1 mg, 796.0 µmol, 1.1 eq.) were added. After 1 h of stirring at rt the, reaction mixture was acidified with 1 N HCl and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. Purification via MPLC (column: Reveleris C18- 40g, isocratic elution with H_2O + 0.05% TFA / CAN + 0.05% TFA = 60:40, flow rate: 40 mL/min, running time: 29.0 min, retention time: 16.0-20.0 min, detection wavelength: UV1= 200 nm, UV2= 254 nm, UV3= 220 nm) gave bright yellow crystals of **17** (94.0 mg, 398.2 µmol, 55 %). ^{1}H NMR (300 MHz, MeOD) δ= 7.44 (s, 1H), 2.92-2.85 (m, 2H), 2.16 (s, 3H), 2.06 (s, 3H), 1.74-1.62 (m, 2H), 1.39-1.32 (m, 4H), 0.96-0.88 (m, 3H). ^{13}C NMR (125 MHz, MeOD) δ= 206.8, 162.5, 161.8, 130.4, 117.1, 113.3, 111.9, 38.6, 32.7, 26.0, 23.6, 16.4, 14.3, 8.0. MS (ESI-): m/z 235.4 $[M-H]^-$, MS (ESI+): m/z = 237.2 $[M+H]^+$. The physical and spectroscopic data were in agreement with that described in the literature.[6]

3.7 Synthesis of (E)-2-hexenyl sorbicillin (**20**)

Under argon atmosphere, 2,4-dimethylresorcinol (**S3**) (1.4 g, 10.1 mmol, 1.0 eq.) was dissolved in abs. CH_2Cl_2 (60 mL) and cooled to 0°C. Freshly produced (E)-2-hexenoyl chloride (1.2 mL, 11.1 mmol, 1.1 eq.) and $AlCl_3$ (1.5 g, 11.2 mmol, 1.1 eq.) were added. After 1 h of stirring at rt the reaction mixture was acidified with 1 N HCl and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. Column chromatographic purification (silica gel, *n*-pentane/EtOAc (3:1), R_f= 0,8) and purification via MPLC (column: Reveleris C18- 40g, isocratic, H_2O + 0.05% TFA/ACN + 0.05% TFA = 50/50, flow rate: 40 mL/min, running time: 25.5 min, retention time: 15.0 - 20.5 min, detection wavelengths: UV1= 200 nm, UV2= 254 nm, UV3= 220 nm) gave bright yellow crystals of **20** (1.2 g, 5.1 mmol, 51%). ^{1}H NMR (400 MHz, MeOD) δ= 7.52 (s, 1H), 7.14-6.99 (m, 2H), 2.36-2.26 (m, 2H), 2.17 (s, 3H), 2.07 (s, 3H), 1.56 (h, *J* = 7.4 Hz, 2H), 0.99 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, MeOD) δ= 193.9, 163.7, 162.2, 149.5, 130.3, 125.8, 117.2, 113.5, 112.1, 35.8, 22.7, 16.3, 14.1, 8.0. HRMS (ESI+): m/z = 235.1327 $[M+H]^+$, calc.: 235.1329.

3.8 Synthesis of *O*-acetyl sorbicillin **S6**

Sorbicillin (**6**) (70.0 mg, 302.0 µmol, 1.0 eq.) was dissolved in CH_2Cl_2 (3 mL) and the solution cooled to 0°C. Acetyl chloride (26.0 µL, 364.0 µmol, 1.2 eq.) and dry triethylamine (41.5 µL, 300.0 µmol, 1.0 eq.) were added to the reaction mixture. After stirring for 5 min at 0°C, the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel, 2-3% EtOAc in *n*-pentane, R_f = 0.5). This yielded **S6** as a yellow solid (75.0 mg, 274.0 µmol, 91%). ^{1}H NMR (300 MHz, MeOD) δ= 7.66 (s, 1H), 7.46 (dd, *J* = 14.8, 10.1 Hz, 1H), 7.11 (d, *J* = 14.8 Hz, 1H), 6.50 – 6.28 (m, 2H), 2.34 (s, 3H), 2.10 (s, 3H), 2.00 (s, 3H), 1.90 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (75 MHz, MeOD) δ= 195.1, 169.8, 162.3, 155.1, 147.0, 143.1, 131.9, 129.8, 123.0, 122.1, 120.5, 118.5, 20.3, 19.0, 16.0, 9.0. MS (ESI+): m/z = 275.02 $[M+H]^+$. The physical and spectroscopic data were in agreement with that described in the literature.[5]

3.9 Chemical synthesis of racemic *O*-acetyl sorbicillinol (**8**)

Sorbicillin ester **S6** (75.0 mg, 274.0 µmol, 1.0 eq.) was dissolved in ACN (6 mL) and H_2O (670 µL). At 0°C PIFA (142.5 mg, 331.0 µmol, 1.2 eq.) was added. The reaction mixture was stirred for 30 min at rt and extracted with EtOAc (23 mL). The organic phase was washed with H_2O and brine and dried over $MgSO_4$. After filtration, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography (silica gel, *n*-pentane/EtOAc (3:1), R_f = 0.13). Compound **8** (52.2 mg, 180.0 µmol, 66%) was obtained as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 11.84 (s, 1H), 7.44 (dd, *J* = 14.8, 10.6 Hz, 1H), 7.24 (s, 1H), 6.65 (d, *J* = 14.8 Hz, 1H), 6.43 – 6.18 (m, 2H), 2.13 (s, 3H), 1.91 (d, *J* = 6.4 Hz, 3H), 1.84 (s, 3H), 1.48 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ= 195.0, 193.4, 170.1, 162.6, 152.0, 148.4, 144.9, 130.2, 125.6, 120.4, 111.8, 78.3, 24.2, 20.6, 19.3, 7.3. MS (ESI+): m/z = 290.95 $[M+H]^+$. The physical and spectroscopic data were in agreement with that described in the literature.[5]

3.10 Enzymatic synthesis of *O*-acetyl sorbicillinol (**8**)

Sorbicillin (**6**) (20.0 mg, 86.0 µmol, 1.0 eq.) dissolved in acetone (4 mL) was added to phosphate buffer (20 mL, 50 mM, pH = 8) followed by addition of SorbC (0.5 mL, 13.1 mg/mL in Tris buffer). Die reaction was started by addition of NADH (100.0 mg, 150.5 µmol, 1.7 eq.) and incubated for 90 min at rt. To stop the enzymatic transformation ACN (40 mL) was added and the reaction mixture was centrifuged (10000 rpm) for 10 min. The supernatant was transferred to a new reaction vessel and the ACN was removed by bubbling argon through the solution. The reaction mixture was cooled to 0 °C and stirred for 30 min, pyridine (10 mL) was added, followed by addition of freshly distilled acetic anhydride (6 mL) over 10 min. The reaction mixture was stirred for 1 h at rt. The solvent was evaporated under reduced pressure and the crude product purified by preperative HPLC (Column: Eurospher II 100-5 C18 A (250 x 16 mm), gradient: 1-40 min 95% B, 40-43 min 95% B, 43-45 min 5% B, 45-50 5% B, flow rate: 15 mL/min, retention time of **8**: 22.0 min, detection wavelenght: 295 nm). Compound **8** was obtained as a yellow solid (2.0 mg, 6.9 µmol, 8%). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 11.89 (s, 1H), 7.45 (dd, *J* = 14.6, 11.0 Hz, 1H), 7.25 (s, 1H), 6.65 (d, *J* = 14.8 Hz, 1H), 6.42 – 6.24 (m, 2H), 2.14 (s, 3H), 1.92 (d, *J* = 6.5 Hz, 3H), 1.85 (s, 3H), 1.48 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 195.3, 193.3, 170.2, 162.9, 152.0, 148.5, 145.0, 130.2, 125.6, 120.3, 111.9, 78.3, 24.3, 20.7, 19.4, 7.3. MS (ESI+): m/z = 290.89 $[M+H]^+$. The physical and spectroscopic data were in agreement with that described in the literature.[6]

3.11 Enzymatic synthesis of bisorbicillinol (**1**)

Sorbicillin (**6**) (20.0 mg, 86.0 µmol, 1.0 eq.) dissolved in 4 mL acetone was added to phosphate buffer (20 mL, 50 mM, pH = 8) with the enzyme SorbC (0.5 mL, 12.4 mg/mL in Tris buffer). The reaction was started by addition of NADH (100.0 mg, 150.5 µmol, 1.7 eq.) and incubated for 90 min at rt. The reaction mixture was extracted with CH_2Cl_2 (3 x 150 mL) and the organic phase was dried under reduced pressure. The crude product was purified by preparative HPLC (for conditions, see 3.7, retention time of **1**: 25.0 min). The substrate sorbicillin (**6**) was re-isolated in 26% yield (5.2 mg, 22.4 µmol), based on which the desired product bisorbicillinol (**1**) was obtained in 27% isolated yield (4.3 mg, 8.7 µmol, 20% absolute yield). ^{1}H NMR (500 MHz, C_6D_6/MeOD=10:1) δ= 7.44 – 7.30 (m, 2H), 6.76 (d, *J* = 14.8 Hz, 1H), 6.43 (d, *J* = 15.0 Hz, 1H), 6.07-5.96 (m, 1H), 5.86 - 5.69 (m, 2H), 5.68-5.59 (m, 1H), 3.97 (s, 1H), 3.78 (s, 1H), 2.03 (s, 3H), 1.81 (s, 3H), 1.48 (s, 3H), 1.46 (d, *J* = 7.2 Hz, 3H), 1.38 (d, *J* = 6.2 Hz, 3H), 1.31 (s, 3H). ^{13}C NMR (125 MHz, C_6D_6/MeOD=10:1) δ= 208.6, 200.7, 196.4, 177.1, 169.7, 166.4, 147.4, 143.9, 142.9, 139.7, 131.5, 130.8, 124.7, 119.3, 111.1, 109.4, 75.5, 70.0, 68.7, 63.2, 48.3, 42.2, 33.5, 24.9, 18.9, 18.8, 10.9, 9.1. MS (ESI-): m/z = 495.2 $[M-H]^-$. The physical and spectroscopic data were in agreement with that described in the literature.[6]

3.12 Enzymatic synthesis of trichodimerol (**2**)

Sorbicillin (**6**) (20.0 mg, 86.0 µmol, 1.0 eq.) was dissolved in 4 mL DMF and added to phosphate buffer (20 mL, 50 mM, pH = 8) with the enzyme SorbC (0.5 mL, 13.0 mg/mL in Tris buffer). The reaction was started by addition of NADH (100.0 mg, 150.5 µmol, 1.7 eq.) and incubated for 6 h at rt. The reaction mixture was extracted with CH_2Cl_2 (3 x 50 mL) and the solvent was evaporated under reduced pressure. The crude product was purified by preperative HPLC (for conditions, see 3.7; retention time of **2**: 28.0 min). The substrate sorbicillin (**6**) was re-isolated in 25% yield (5.0 mg, 22.0 µmmol), based on which the desired product trichodimerol (**2**) was obtained in 27% isolated yield (4.3 mg, 8.7 µmol, 20% absolute yield), along with 20% bisorbicillinol (**1**) (3.1 mg, 6.3 µmol, 15% absolute yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 7.32 (dd, *J* = 14.8 Hz, 10.8 Hz, 2H), 6.33-6.25 (m, 2H), 6.23-6.16 (m, 2H), 6.14 (d, *J*= 14.8 Hz, 2H), 3.02 (bs, 2H), 2.98 (s, 2H), 1.89 (d, *J* = 6.7 Hz, 6H), 1.45 (s, 6H), 1.42 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 198.1, 176.0, 143.8, 140.6, 131.0, 118.6, 104.2, 102.9, 78.9, 58.9, 58.0, 21.4, 19.1, 18.8. MS (ESI-): m/z = 495.2 $[M-H]^-$. The physical and spectroscopic data were in agreement with that described in the literature.[6]

3.13 Enzymatic synthesis of sorbiquinol (**14**)

Sorbicillin (**6**) (80.0 mg, 344.7 µmol, 1.0 eq.) was dissolved in 16 mL DMF and added to phosphate buffer (80 mL, 50 mM, pH = 8) with the enzyme SorbC (2.5 mL, 13.0 mg/mL in Tris buffer). The reaction was started by addition of NADH (300.0 mg, 451.5 µmol, 1.3 eq.) and incubated for 12 h at rt. The reaction mixture was extracted with CH_2Cl_2 (3 x 100 mL) and the solvent was quickly evaporated under reduced pressure until only DMF was left. 8 mL pyridine were added and the reaction mixture was refluxed for 2 h. The solvent was evaporated under high vacuum. The crude product was purified by preperative HPLC (for conditions, see 3.7; retention time of **14**: 28.0 min). The substrate sorbicillin (**6**) was re-isolated in 30% yield (24.0 mg, 103.4 µmol), based on which the desired product sorbiquinol (**14**) was obtained in 7.2% isolated yield (4.2 mg, 8.7 µmol, 5% absolute yield), along with 13% bisorbicillinol (**1**) (8.0 mg,16.1 µmol, 9% absolute yield) and 16% trichodimerol (**2**) (9.6 mg, 19.4 µmol, 11% absolute yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 13.94 (s, OH), 12.60 (s, OH), 7.60 (s, 1H), 7.18 (dd, *J* = 14.8, 11.0 Hz, 1H), 6.09 (dd, *J* = 14.9, 7.0 Hz, 1H), 5.95 – 5.84 (m, 1H), 5.51 (d, *J* = 14.9 Hz, 1H), 5.44 (dd, *J* = 14.9, 6.5 Hz, 1H), 5.05-4.97 (m, 1H), 4.27 (dd, *J* = 6.6 Hz, *J* = 1.8 Hz, 1H), 3.30 (d, *J* = 1.8 Hz, 1H), 3.25 (dd, *J* = 9.9, 6.6 Hz, 1H), 2.28 (s, 3H), 2.11 (s, 3H), 1.82 (d, *J* = 6.8 Hz, 3H), 1.60 (d, *J* = 7.8 Hz, 3H), 1.20 (s, 3H), 1.16 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 211.6, 202.2, 198.1, 168.8, 162.0, 158.9, 142.5, 139.6, 130.8, 130.7, 129.1, 128.2, 117.3, 114.9, 112.2, 110.7, 106.8, 75.8, 63.2, 47.0, 46.7, 46.5, 24.5, 19.0, 18.0, 16.1, 10.1, 7.7. MS (ESI-): m/z 495.2 $[M-H]^-$. The physical and spectroscopic data were in agreement with that described in the literature.[7]

3.14 Enzymatic synthesis of hexyl bisorbicillinol (**18**)

Hexyl sorbicillin (**17**) (22.0 mg, 93.2 µmol, 1.0 eq.) was dissolved in 4 mL acetone and added to phosphate buffer (20 mL, 50 mM, pH = 8) with subsequent addition of he enzyme SorbC (2.0 mL, 9.5 mg/mL in Tris buffer). The reaction was started by addition of NADH (100.0 mg, 150.5 µmol, 1.7 eq.) and incubated for 12 h at rt. The reaction mixture was extracted with CH_2Cl_2 (5 x 50 mL) and the combined organic phases were dried under reduced pressure. The crude product was purified by preperative HPLC (for conditions, see 3.7; retention time: 27.0 min). The substrate sorbicillin (**6**) was re-isolated in 63% yield (14.0 mg, 59.0 µmol), based on which the desired product hexyl bisorbicillinol (**18**) was obtained in 20% isolated yield (1.8 mg, 3.6 µmol, 8% absolute yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 14.26 (s, 1H), 3.79 (s, 1H), 3.57 (s, 1H), 2.96-2.88 (m, 1H), 2.44-2.25 (m, 3H), 2.23-2.14 (m, 2H), 1.57 (s, 3H), 1.52 (s, 3H), 1.37-1.33 (m, 4H), 1.31 (s, 3H), 1.30-1.23 (m, 6H), 1.16 (s, 3H), 0.92 (t, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 207.0, 204.8, 203.8, 201.6, 193.5, 184.7, 108.1, 77.4, 75.4, 75.4, 71.8, 67.7, 63.3, 49.3, 42.9, 39.7, 32.6, 31.8, 31.0, 30.3, 25.1, 25.0, 23.4, 22.6, 22.5, 21.7, 14.0, 9.5. MS (ESI-): m/z = 503.5 $[M-H]^-$. The physical and spectroscopic data were in agreement with that described in the literature.[6]

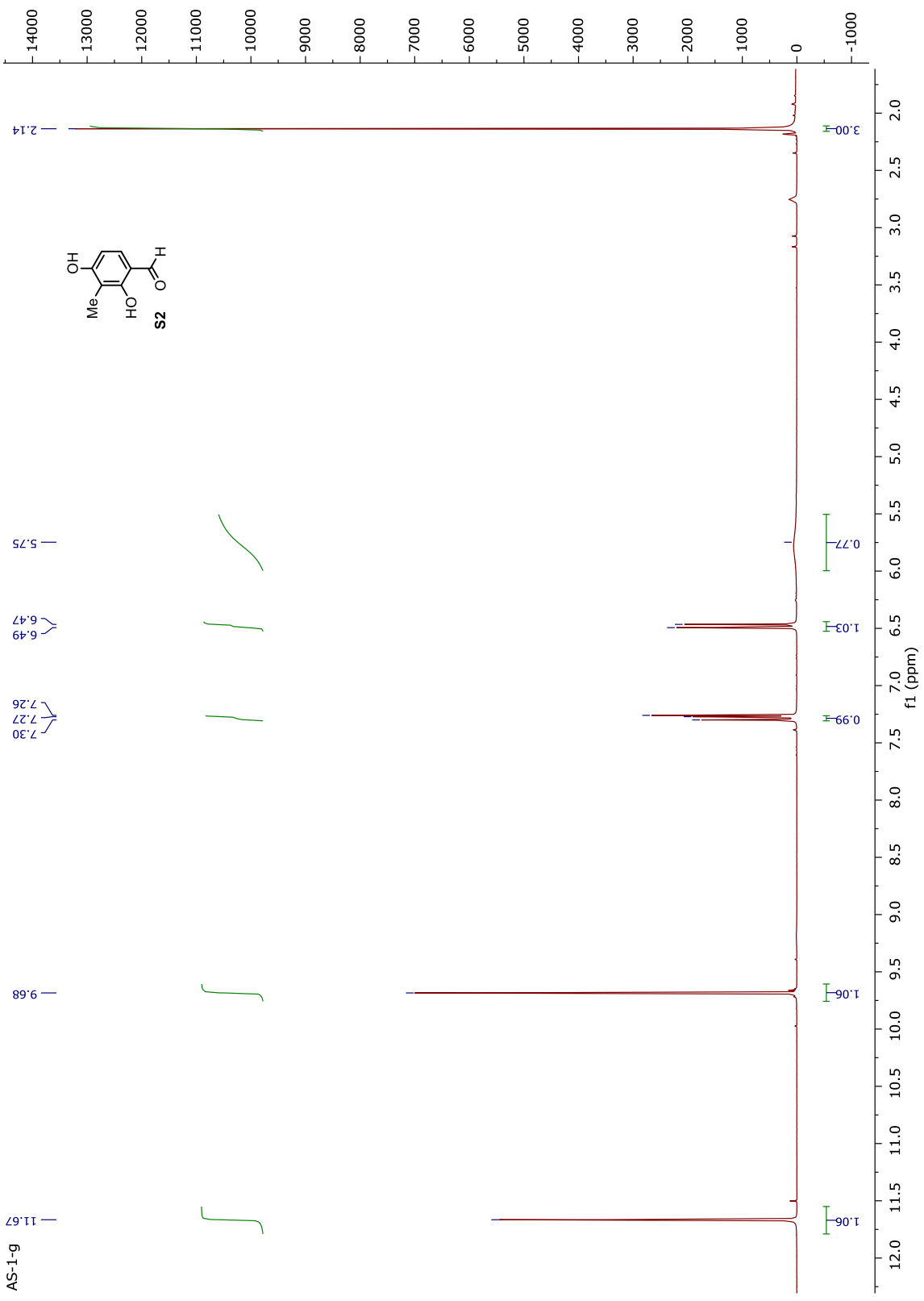
3.15 Enzymatic synthesis of hexyl trichodimerol (**19**)

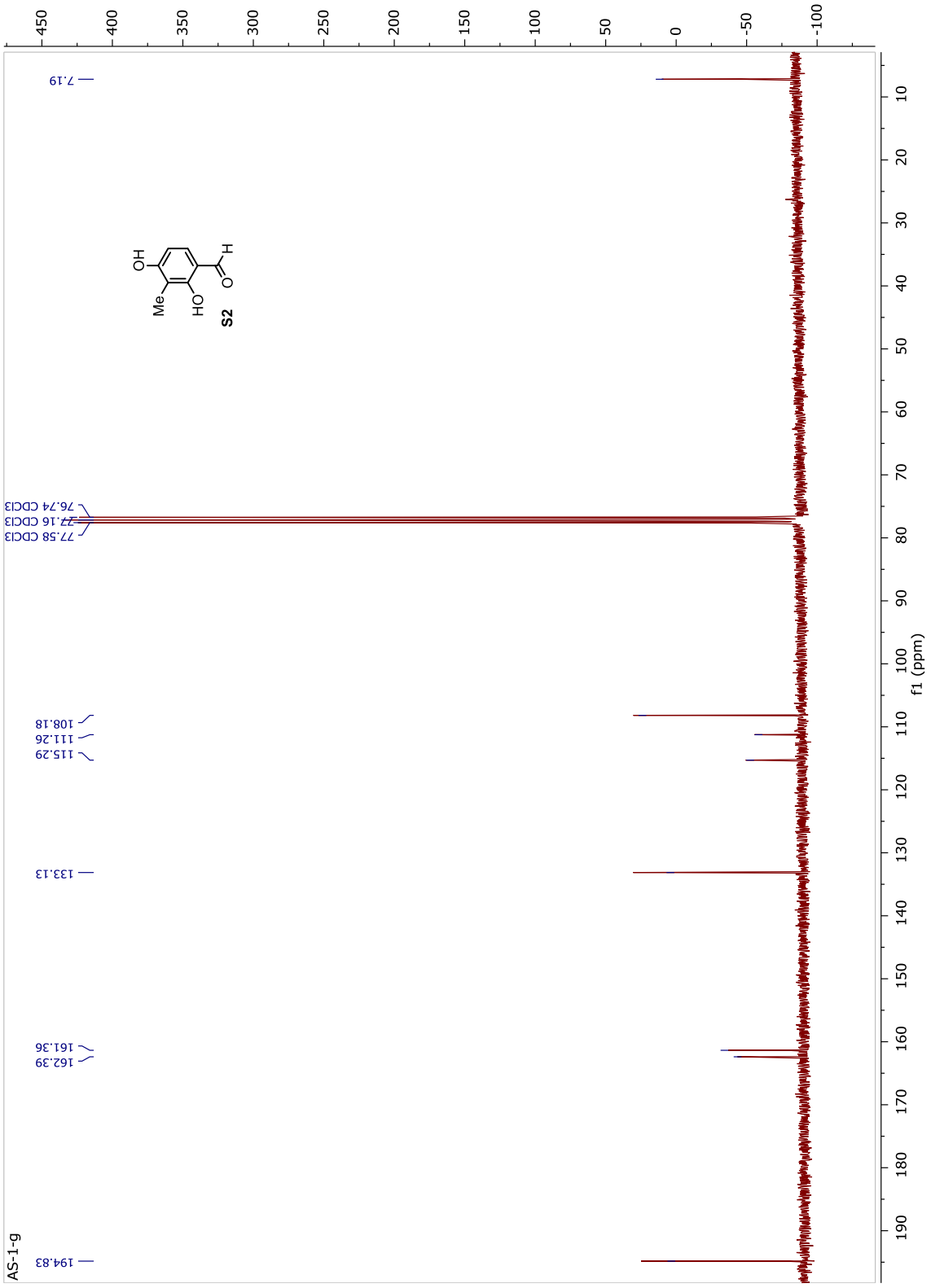
Hexyl sorbicillin (**17**) (20.0 mg, 84.7 µmol, 1.0 eq.) was dissolved in 4 mL DMF and added to phosphate buffer (20 mL, 50 mM, pH = 8) with the enzyme SorbC (1.5 mL, 13.0 mg/mL in Tris buffer). The reaction was started by addition of NADH (100.0 mg, 150.5 µmol, 1.7 eq.) and incubated for 12 h at rt. The reaction mixture was extracted with CH_2Cl_2 (3 x 50 mL) and the solvent was evaporated under reduced pressure. The crude product was purified by preperative HPLC (for conditions, see 3.7; retention time: 28.0 min). The substrate sorbicillin (**6**) was re-isolated in 57% yield (11.5 mg, 48.7 µmol), based on which the desired product hexyl trichodimerol (**19**) was obtained in 12% isolated yield (1.1 mg, 2.2 µmol, 5% absolute yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 3.06 (bs, 2H), 2.91 (s, 2H), 2.45 – 2.27 (m, 4H), 1.63 – 1.58 (m, 4H), 1.42 (s, 12H), 1.36 – 1.29 (m, 8H), 0.91 (t, *J* = 6.8 Hz, 6H). ^{13}C NMR (500 MHz, $CDCl_3$) δ= 193.6, 192.0, 104.0, 103.4, 78.8, 58.1, 57.4, 34.8, 31.7, 25.4, 22.6, 21.4, 18.9, 14.1. MS (ESI-): m/z = 495.2 $[M-H]^-$. The physical and spectroscopic data were in agreement with that described in the literature.[6]

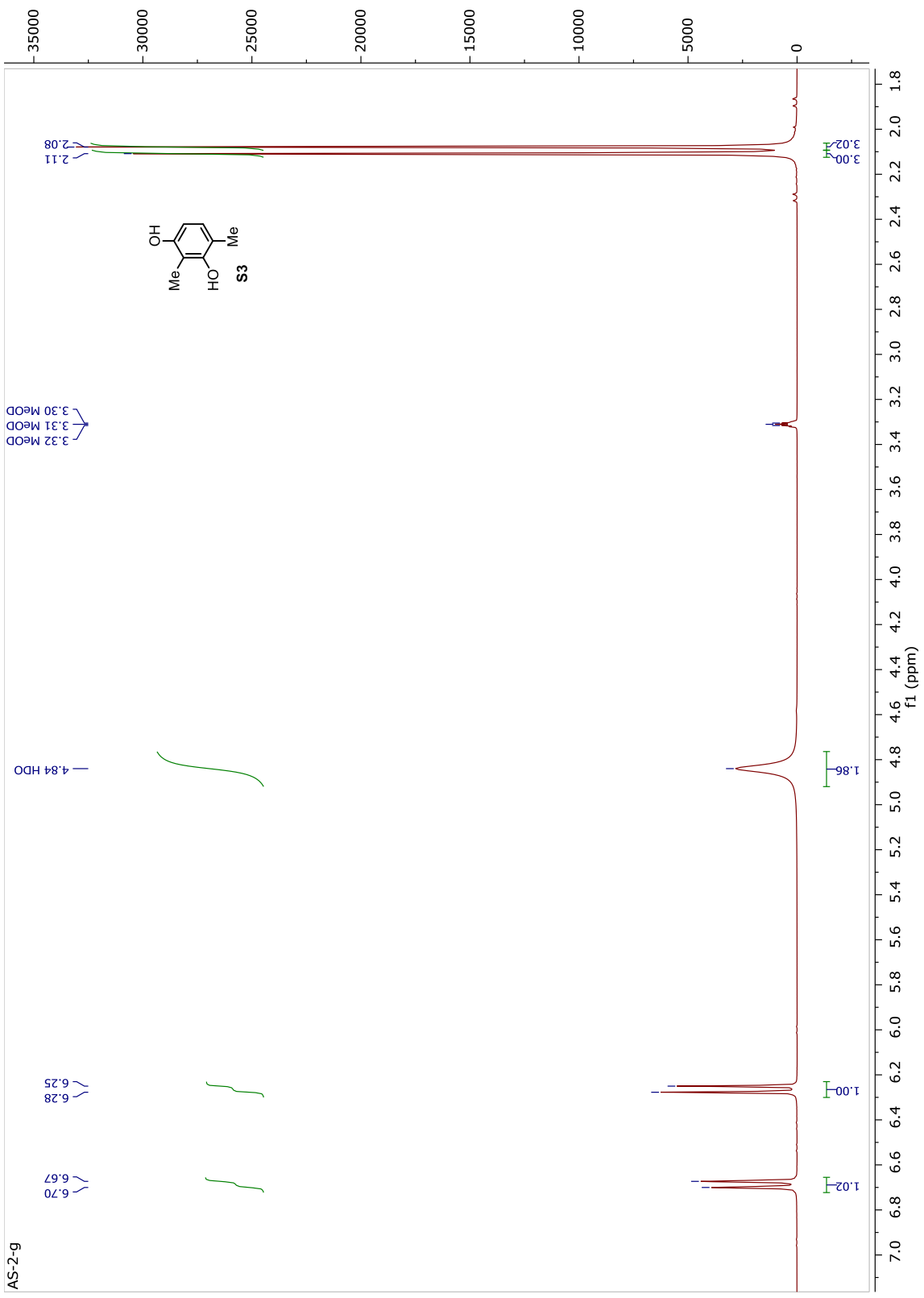
3.16 Enzymatic synthesis of dimer **21**

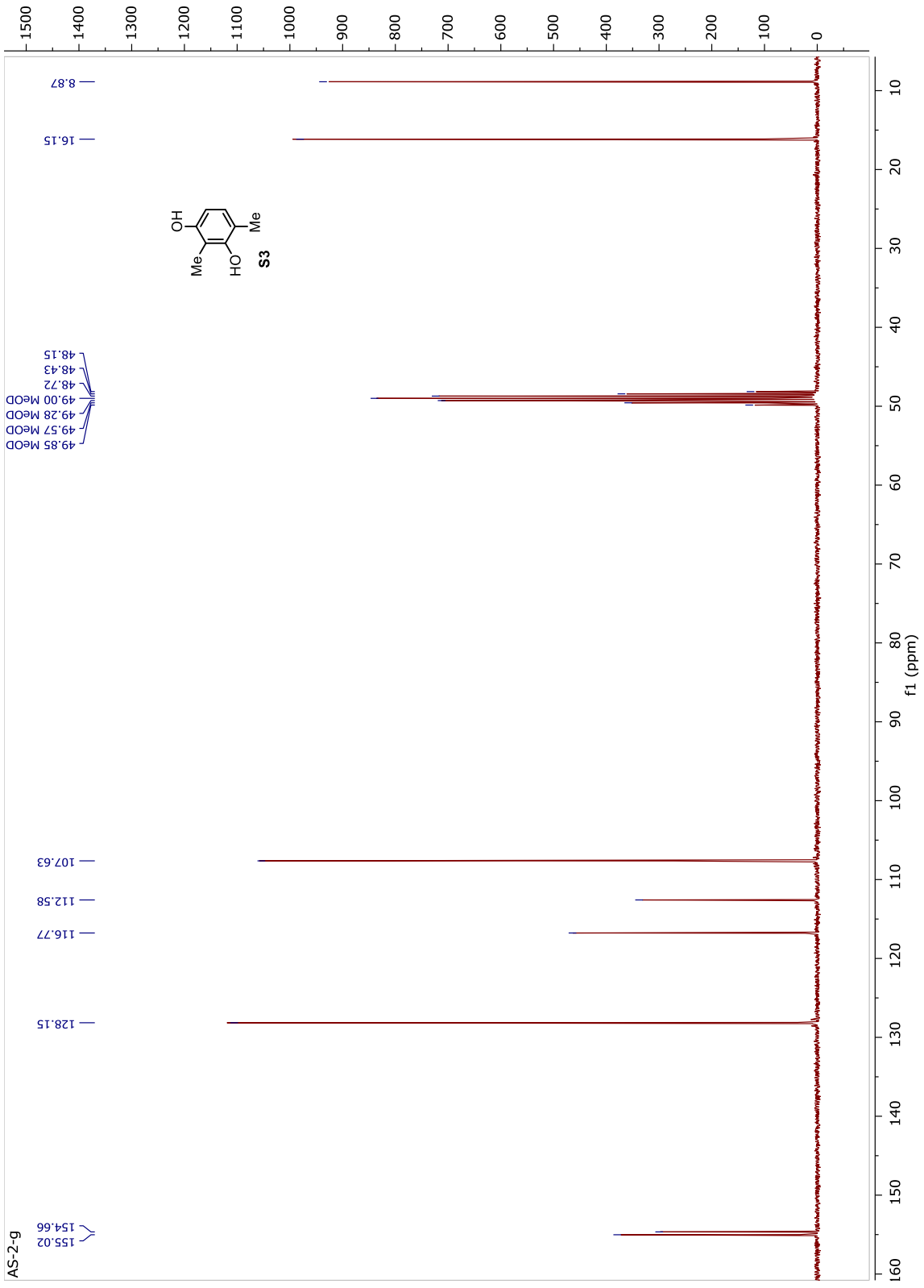
(E)-2-hexenyl sorbicillin (**20**) (20.0 mg, 85.4 µmol, 1.0 eq.) dissolved in 4 mL DMF were mixed with phosphate buffer (20 mL, 50 mM, pH = 6,7) and the enzyme SorbC (1.5 mL, 13.0 mg/mL in Tris buffer) was added. The reaction was started by addition of NADH (100 mg, 150.5 µmol, 1.7 eq.) and incubated for 48 h at rt. The reaction mixture was extracted with CH_2Cl_2 (3 x 50 mL) and the solvent was evaporated under reduced pressure. The crude product was purified by preparative HPLC (elution time: 28.0 min). The substrate sorbicillin (**20**) was re-isolated in 25% yield (5.1 mg, 21.8 µmol), based on which the new dimer **21** was obtained in 6% isolated yield (0.9 mg, 1.8 µmol, 4% absolute yield). For NMR data, see Table S1, page 46 below. HRMS (ESI-): m/z = 501.2494 $[M-H]^-$, calc.: 501.2494.

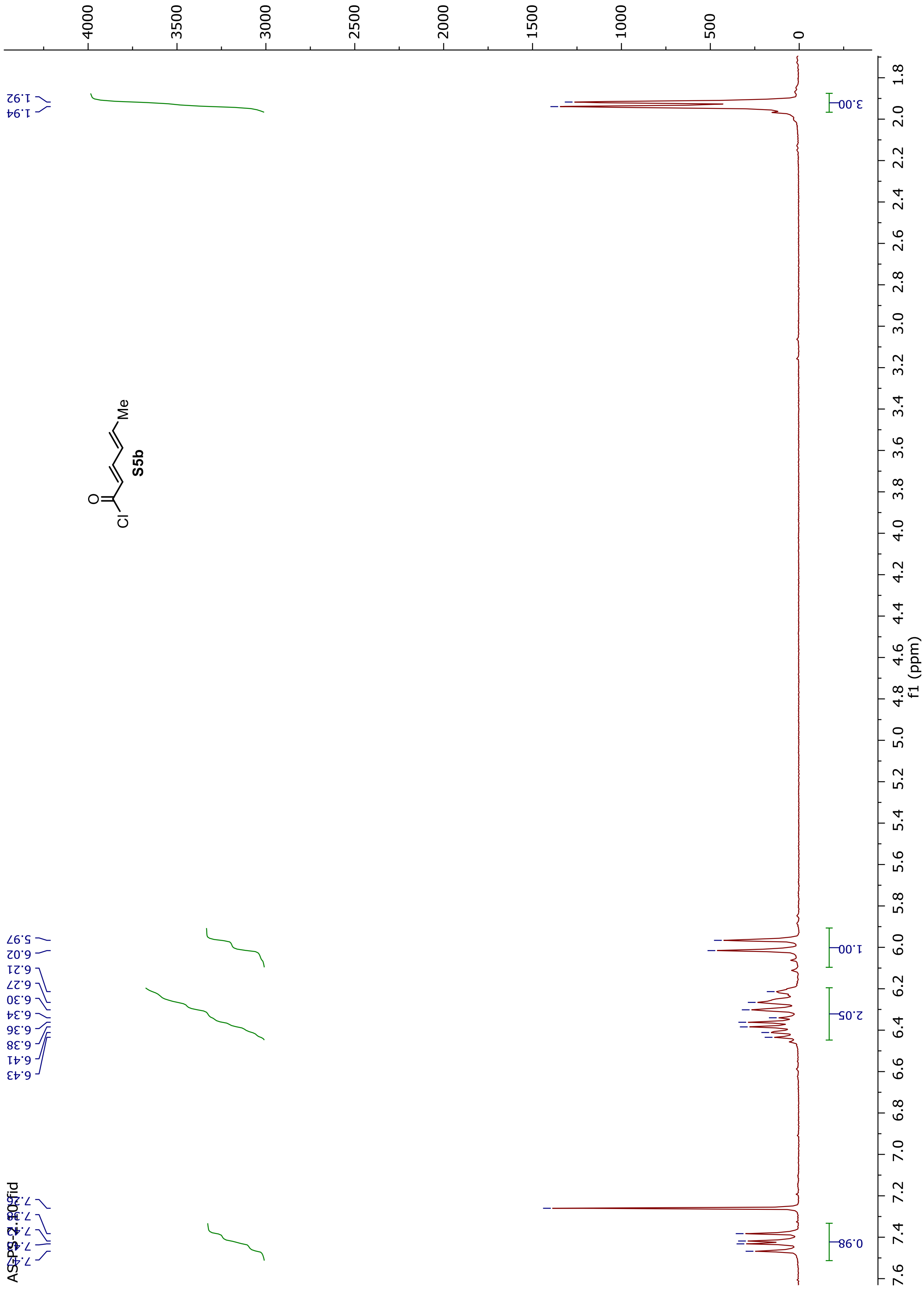
4. NMR Spectra

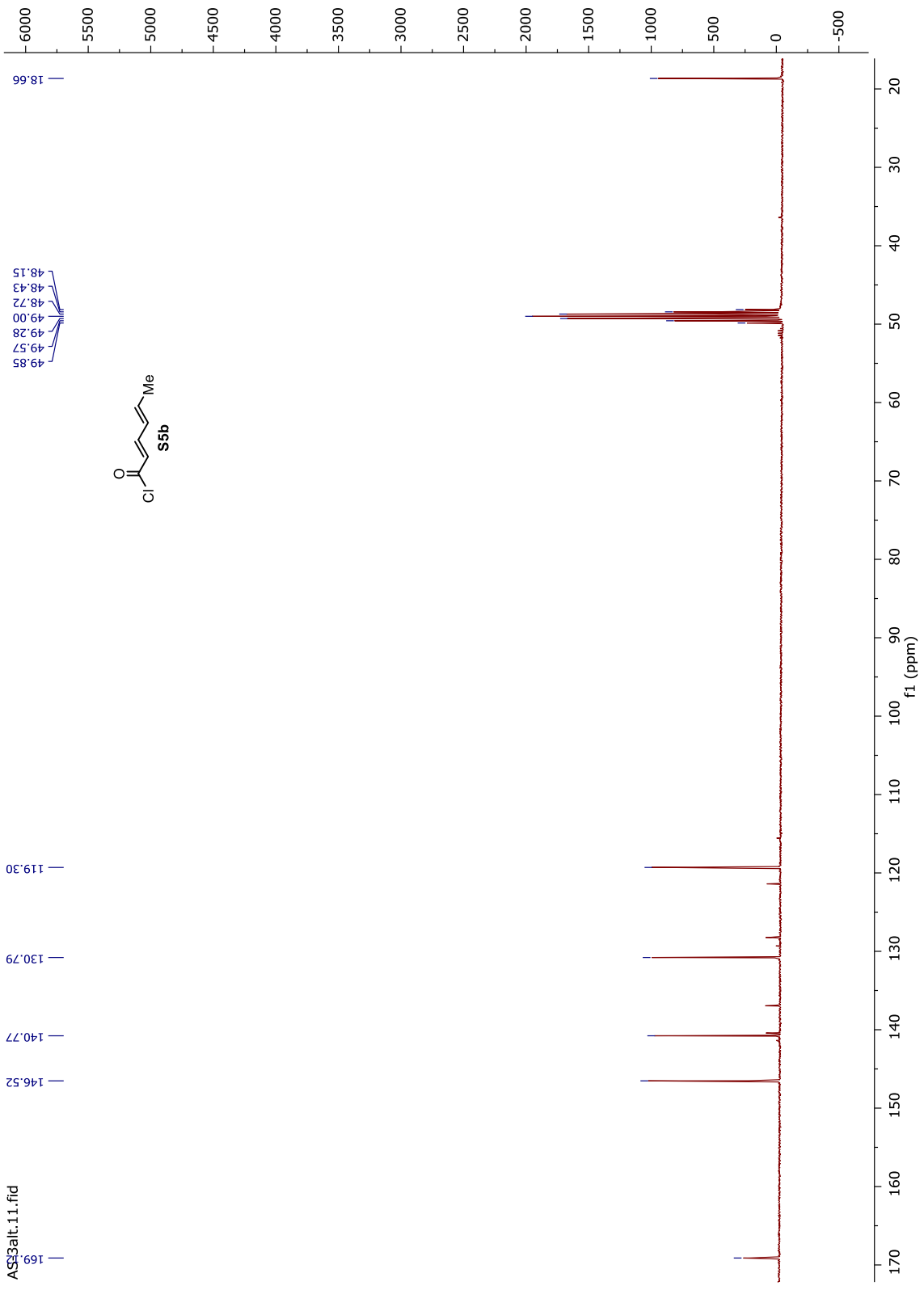


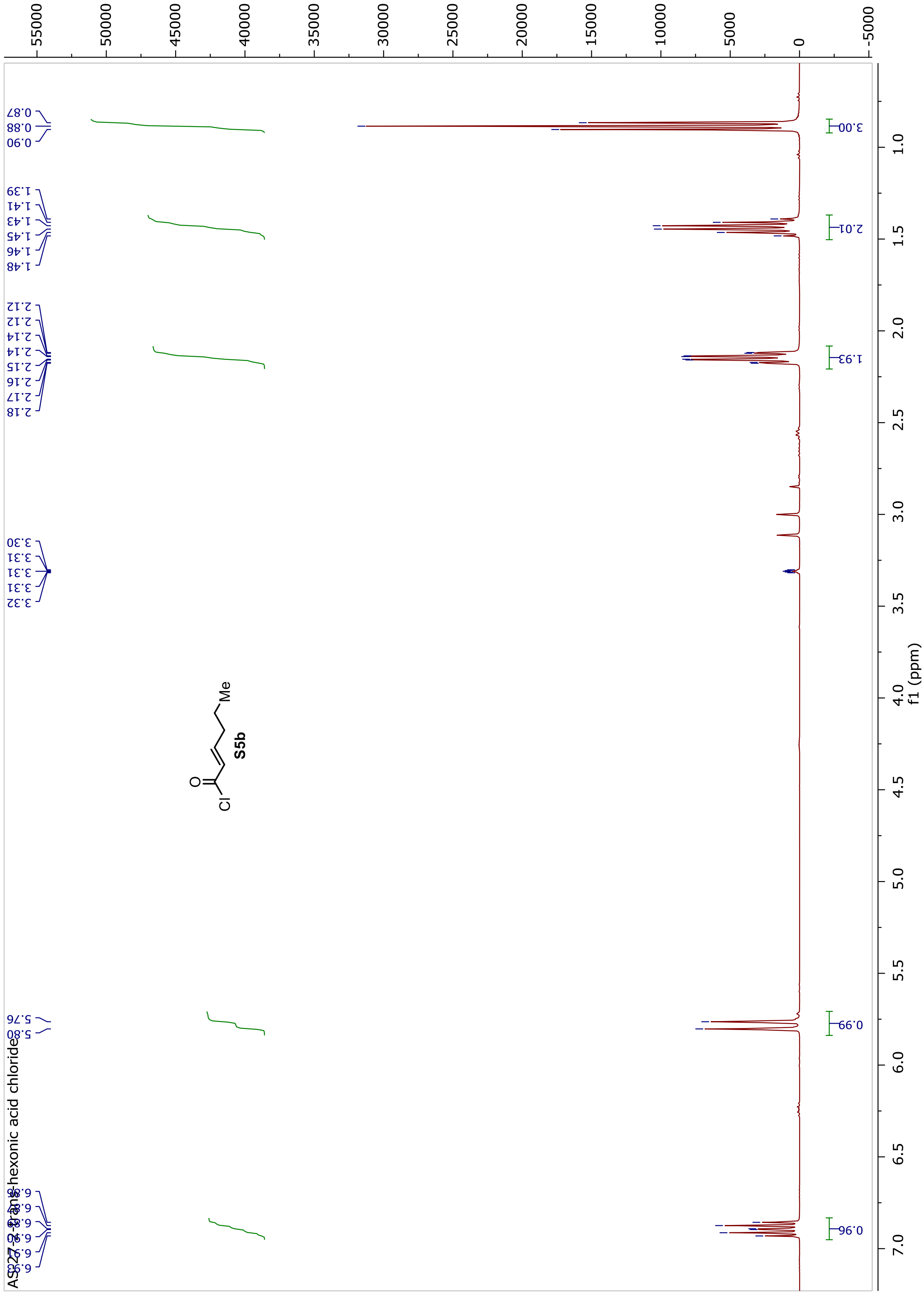

AS-1-g
194.83
162.39
161.36
133.13
115.29
111.26
108.18
77.58 CDCl3
77.16 CDCl3
76.74 CDCl3
7.19
OH
Me
HO
O
H
S2
f1 (ppm)


AS-2-g
6.70
6.67
6.28
6.25
4.84 HDO
3.32 MeOD
3.31 MeOD
3.30 MeOD
2.11
2.08
1.02
1.00
1.86
3.00
3.02
OH
Me
HO
Me
S3
f1 (ppm)


AS-2-g
155.02
154.66
128.15
116.77
112.58
107.63
49.85 MeOD
49.57 MeOD
49.28 MeOD
49.00 MeOD
48.72
48.43
48.15
16.15
8.87
OH
Me
Me
HO
S3
f1 (ppm)


S5b
Me
Cl
O
f1 (ppm)
ASPS-2.10.fid


S5b
Me
O
Cl
ASS3alt.11.fid
169.52
146.52
140.77
130.79
119.30
49.85
49.57
49.28
49.00
48.72
48.43
48.15
18.66
f1 (ppm)


AS27-2-trans-hexonic acid chloride
S5b
Cl
O
Me
f1 (ppm)
3.00
2.01
1.93
0.99
0.96
0.87
0.88
0.90
1.39
1.41
1.43
1.45
1.46
1.48
2.12
2.12
2.14
2.14
2.15
2.16
2.17
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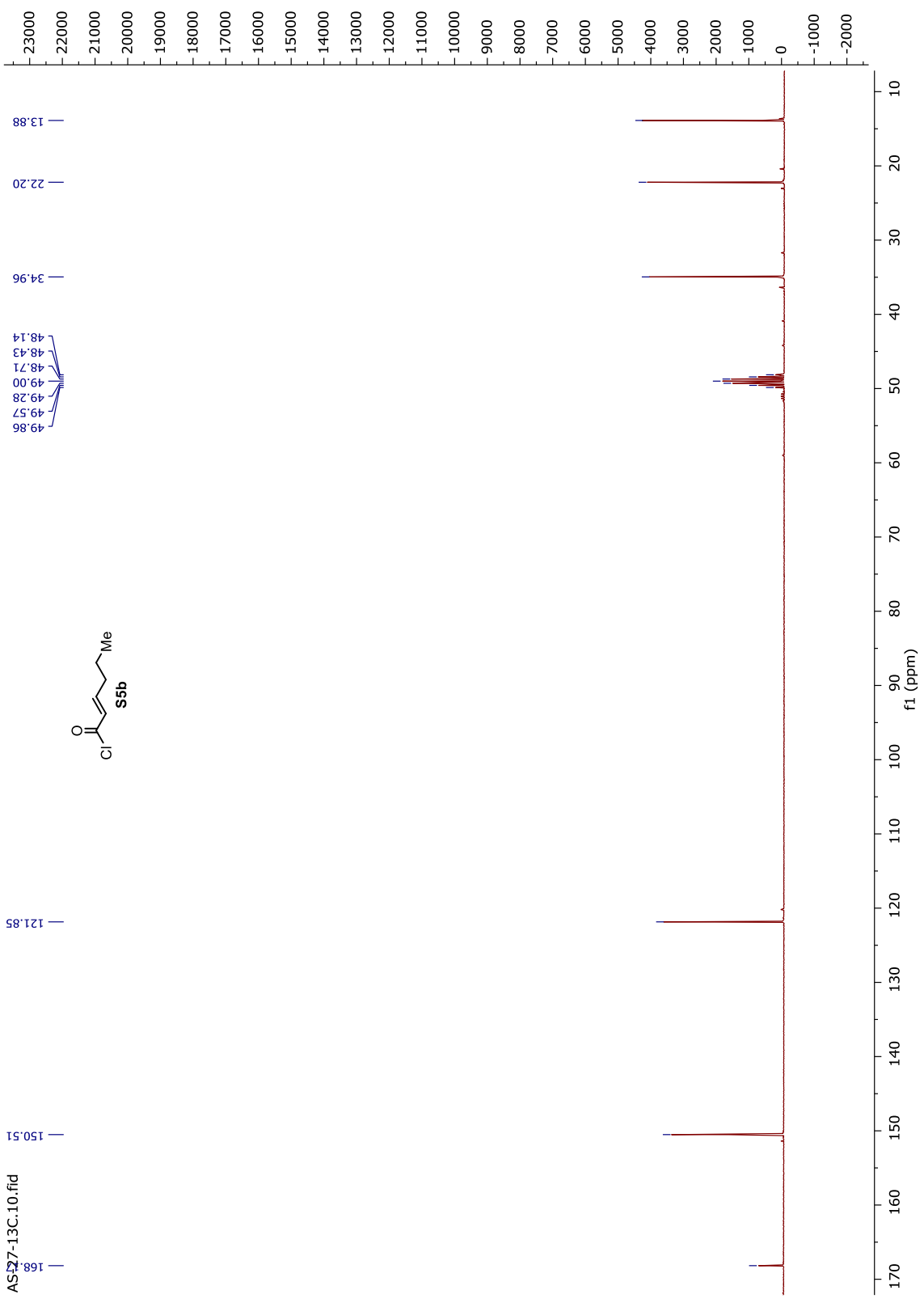
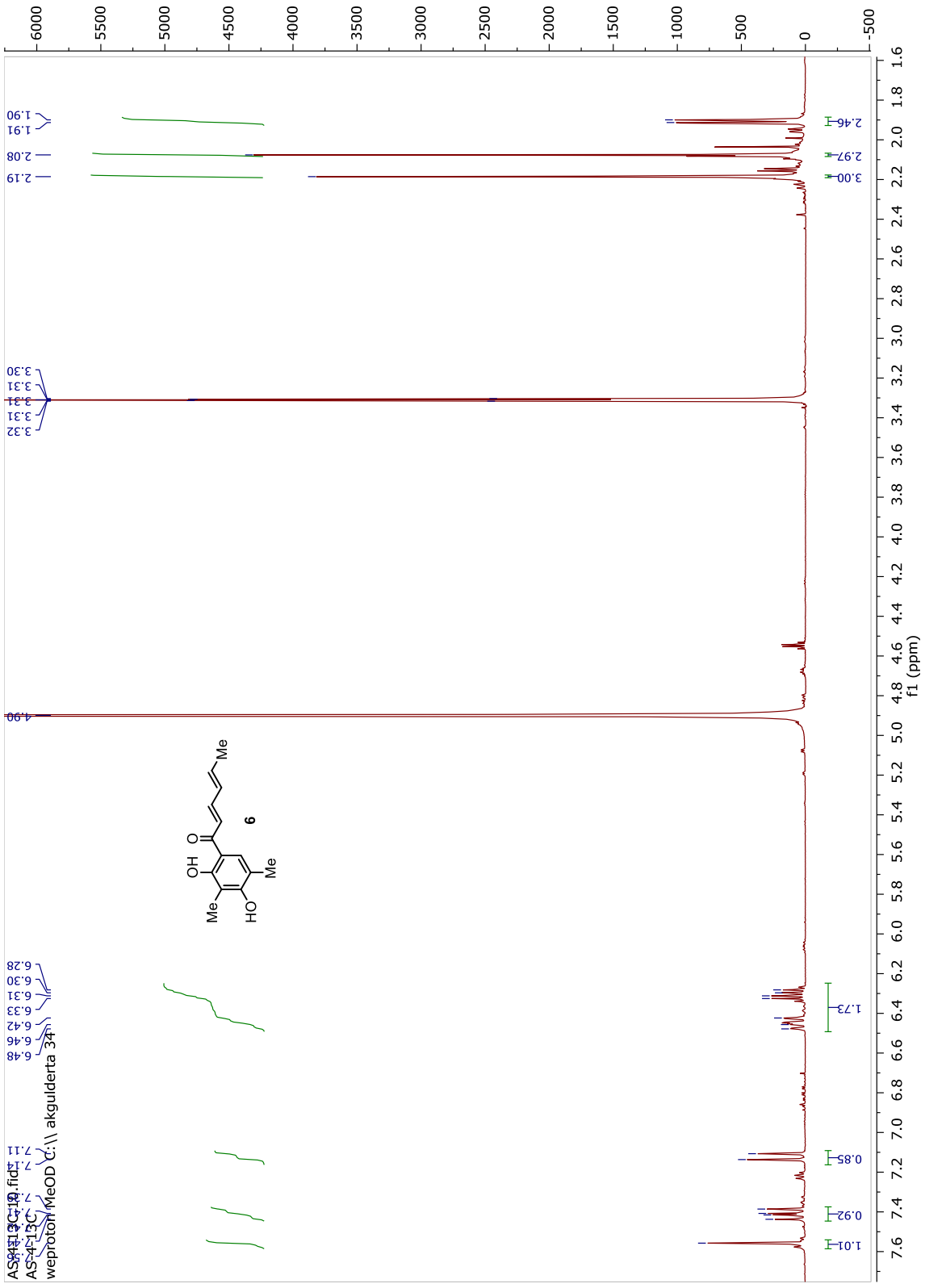
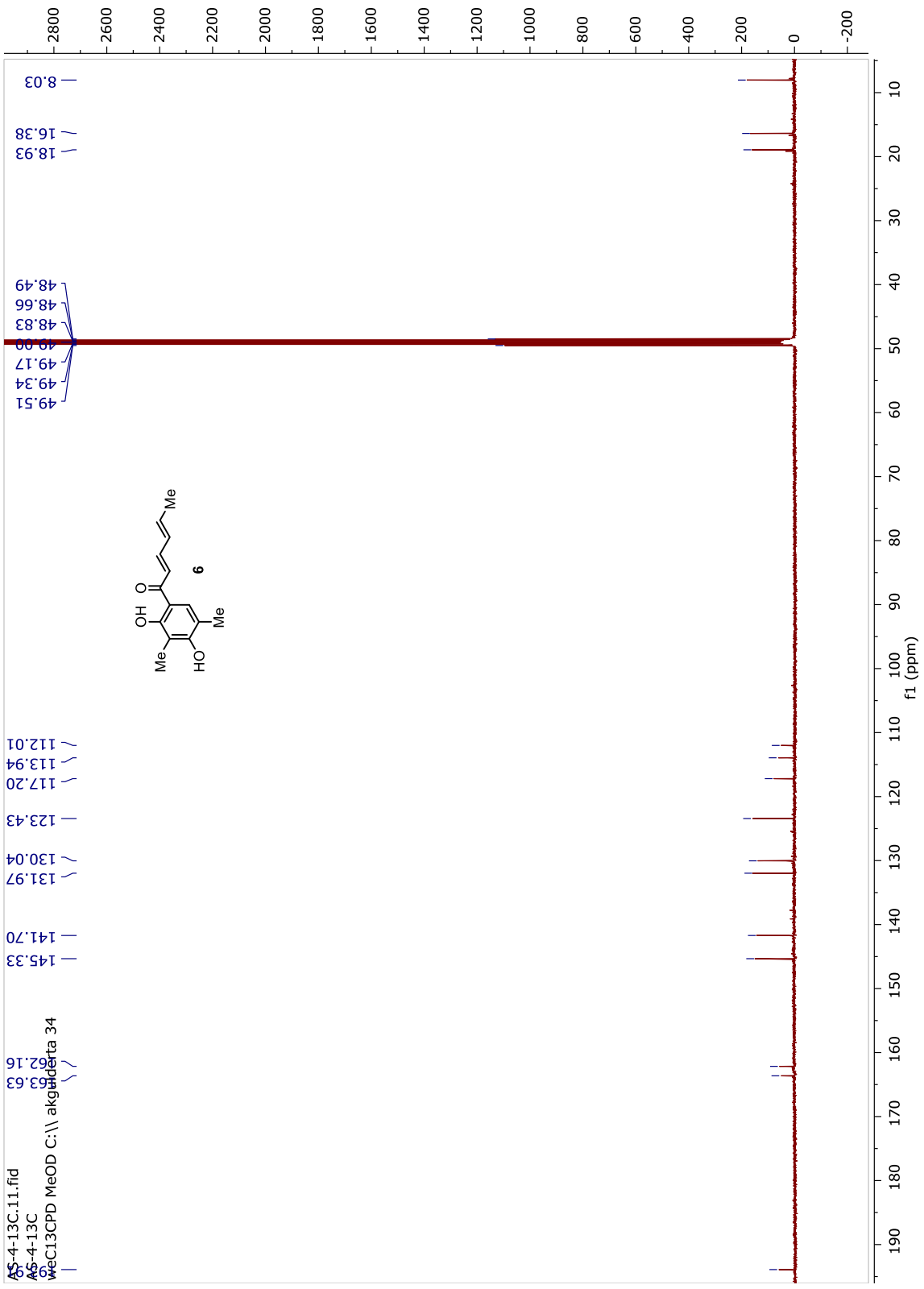
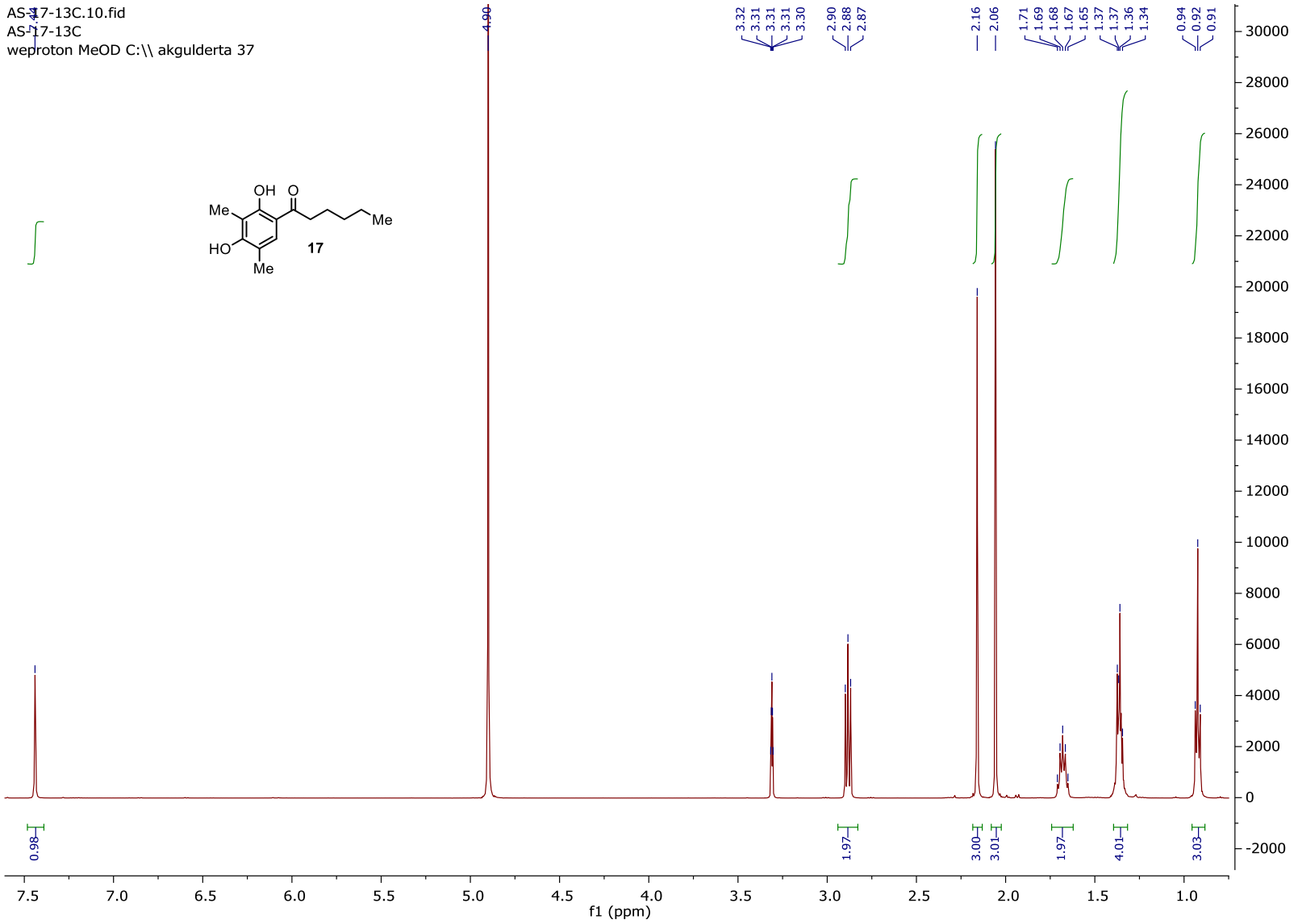
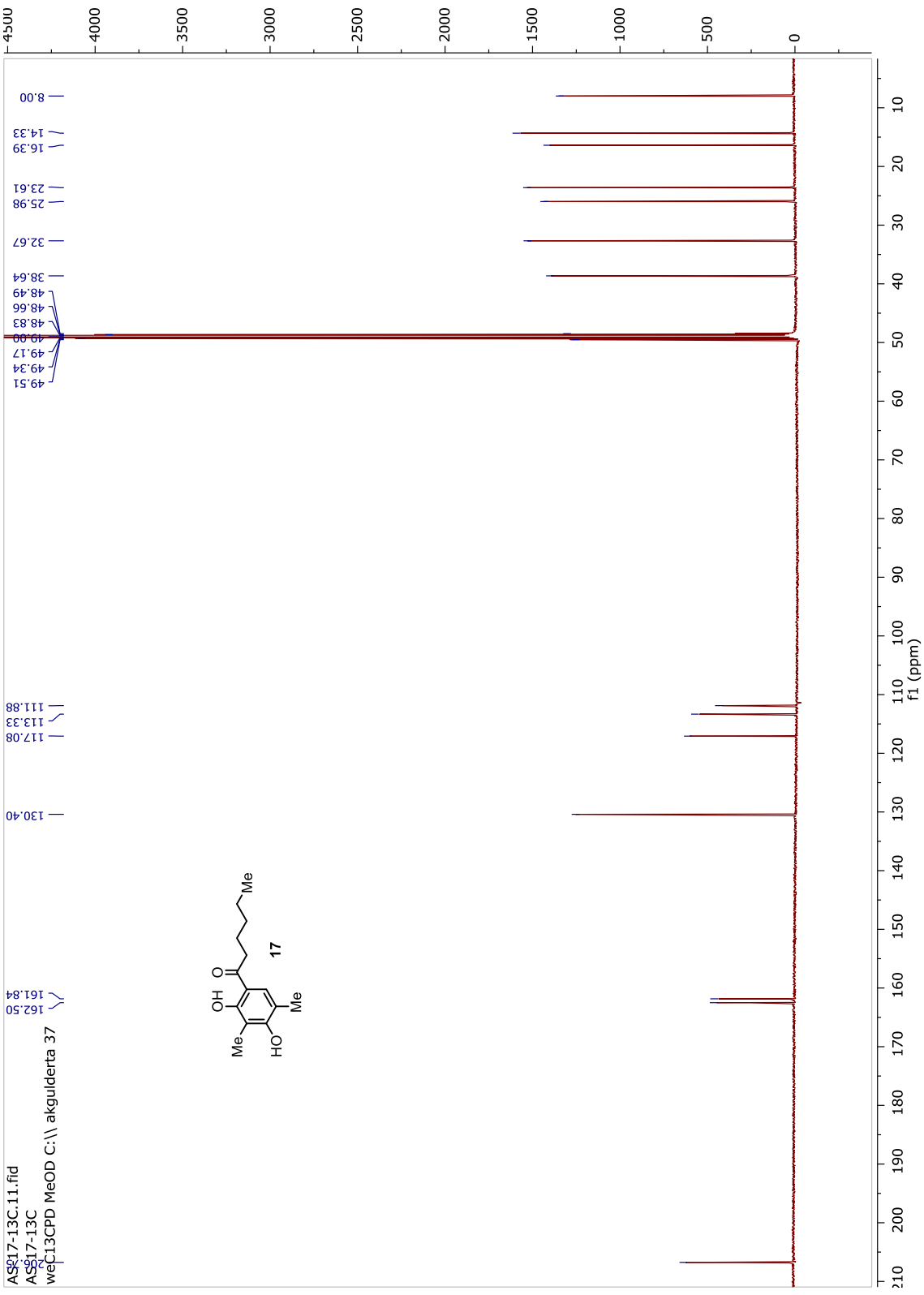
3.31
3.31
3.31
3.32
5.76
5.80

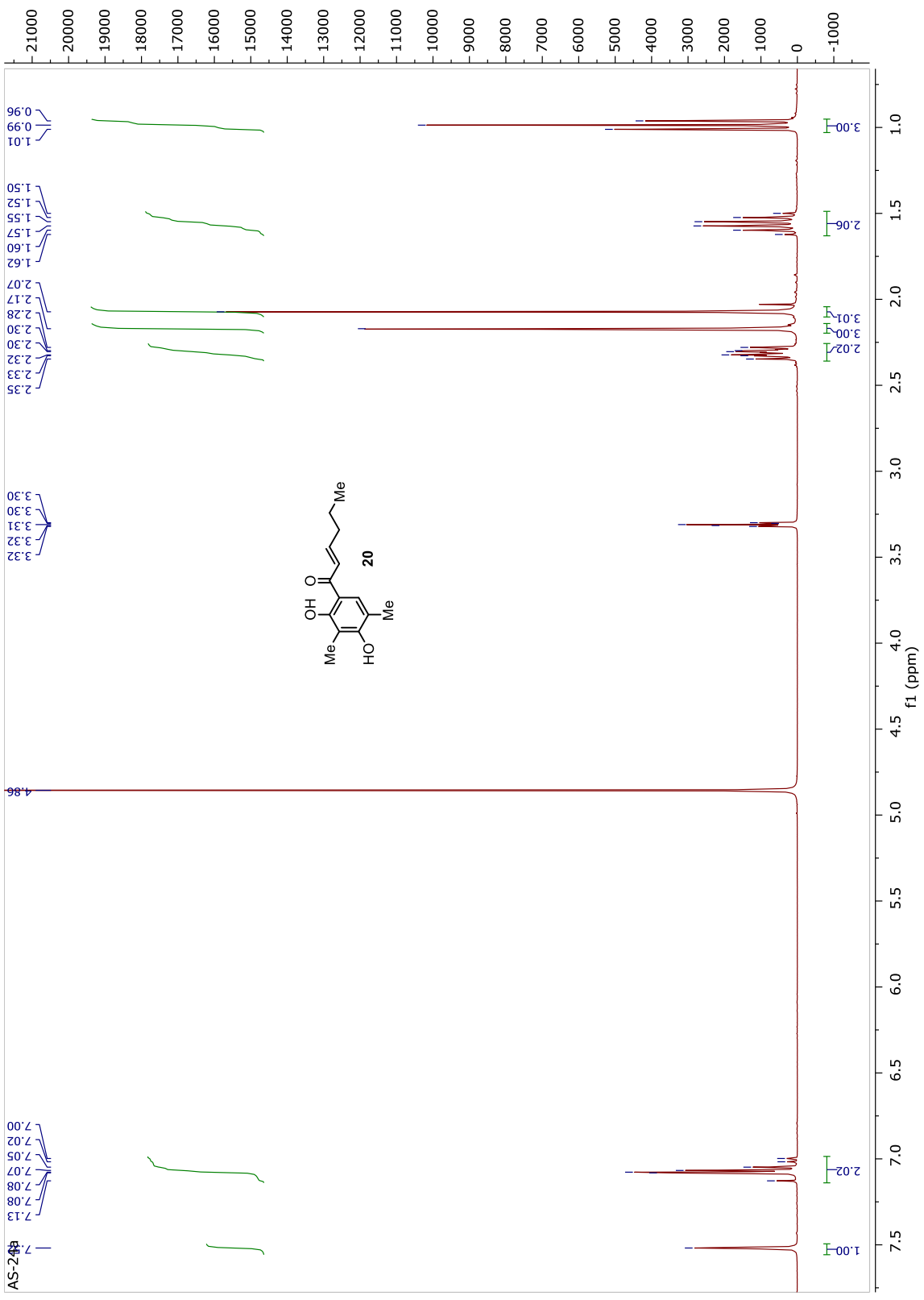

AS-27-13C.10.fid
168.27
150.51
121.85
49.86
49.57
49.28
49.00
48.71
48.43
48.14
34.96
22.20
13.88
Cl
O
Me
S5b
f1 (ppm)

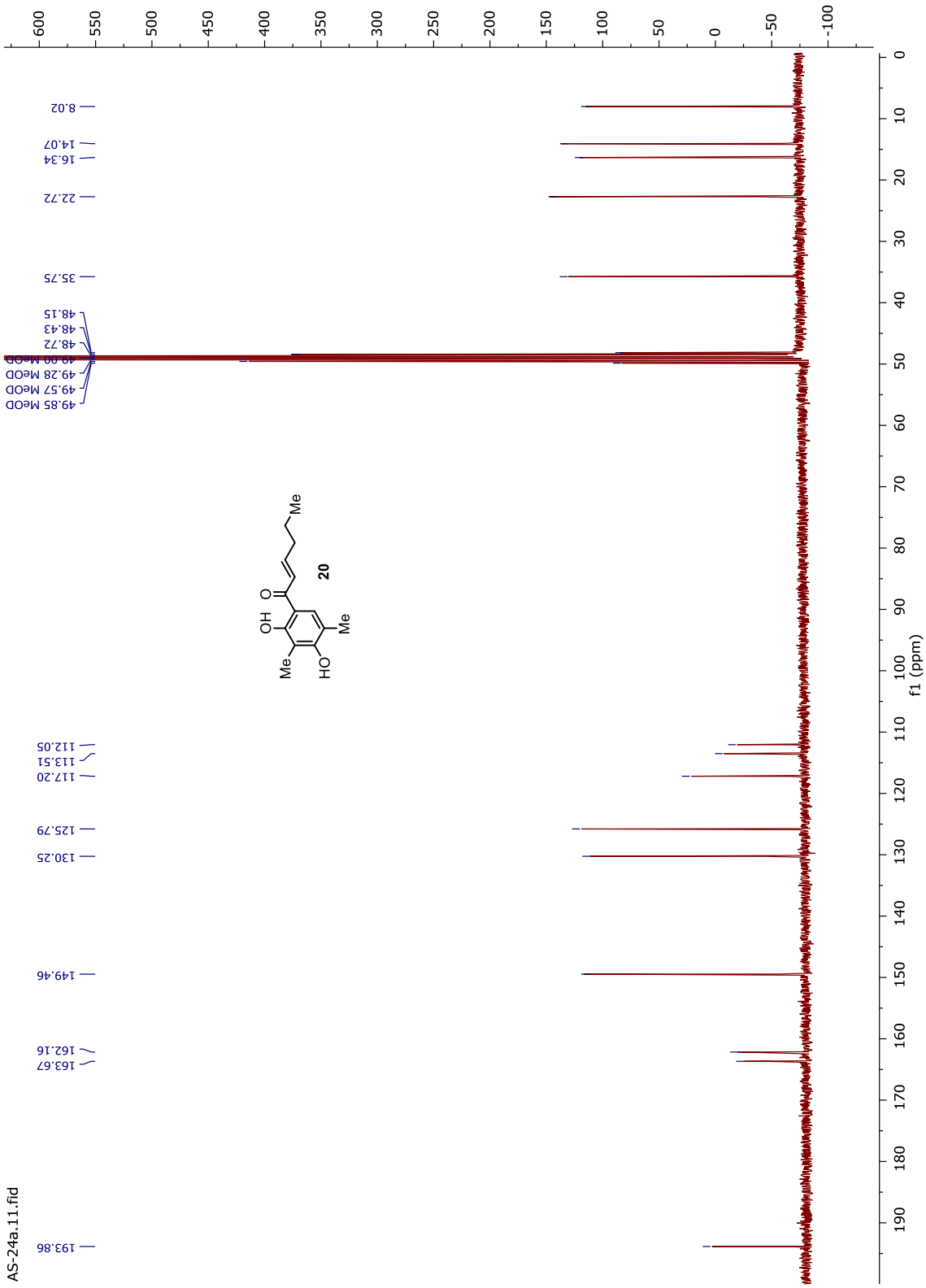

AS-4-13C-10.fid
AS-4-13C
weproton MeOD C:\\ akgulderta 34
6
OH
O
Me
Me
HO
Me
f1 (ppm)

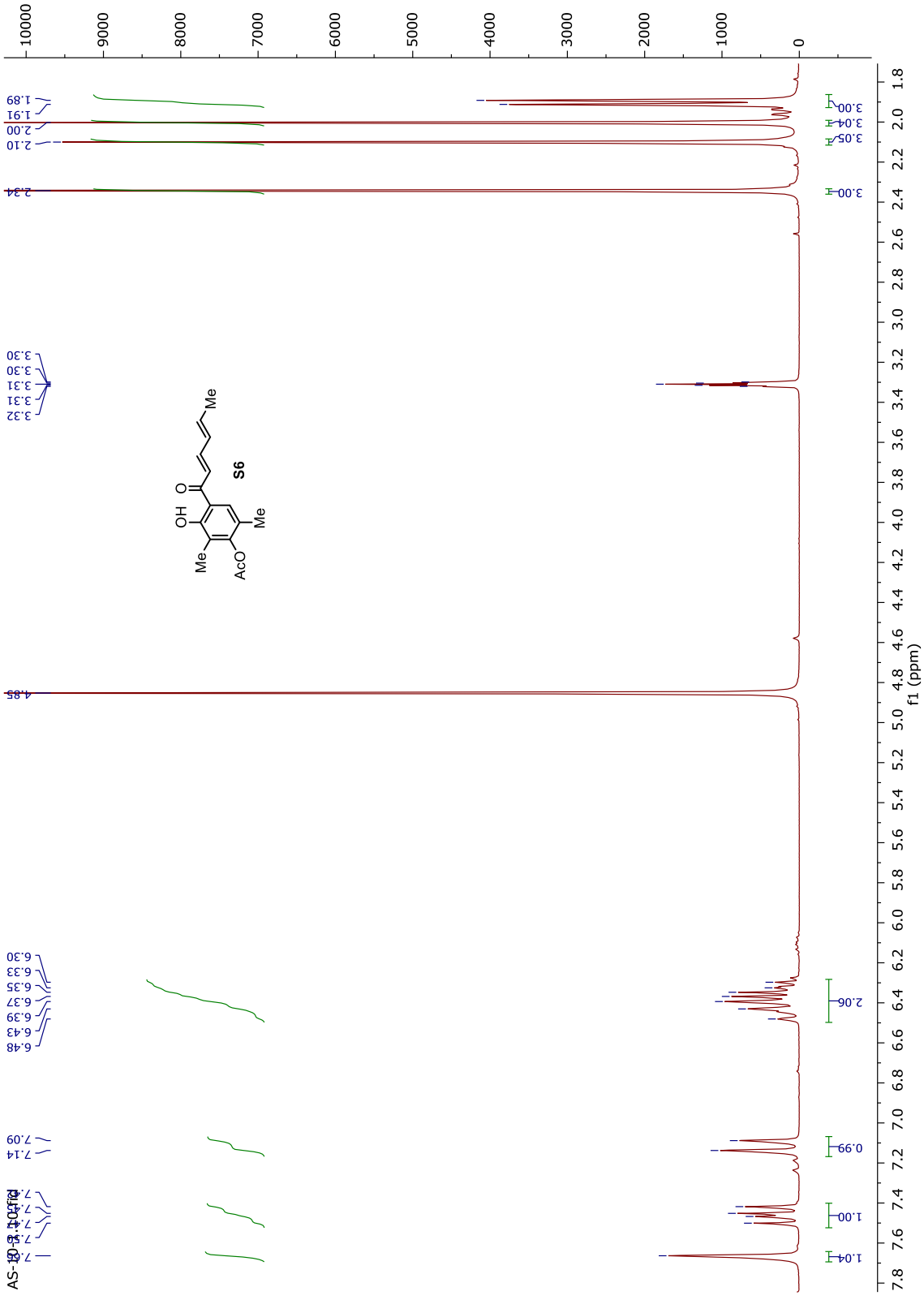

AS-4-13C.11.fid
AS-4-13C
weC13CPD MeOD C:\\ akguderta 34
8.03
16.38
18.93
48.49
48.66
48.83
49.00
49.17
49.34
49.51
112.01
113.94
117.20
123.43
130.04
131.97
141.70
145.33
162.16
163.63
OH
O
Me
Me
HO
Me
6
f1 (ppm)

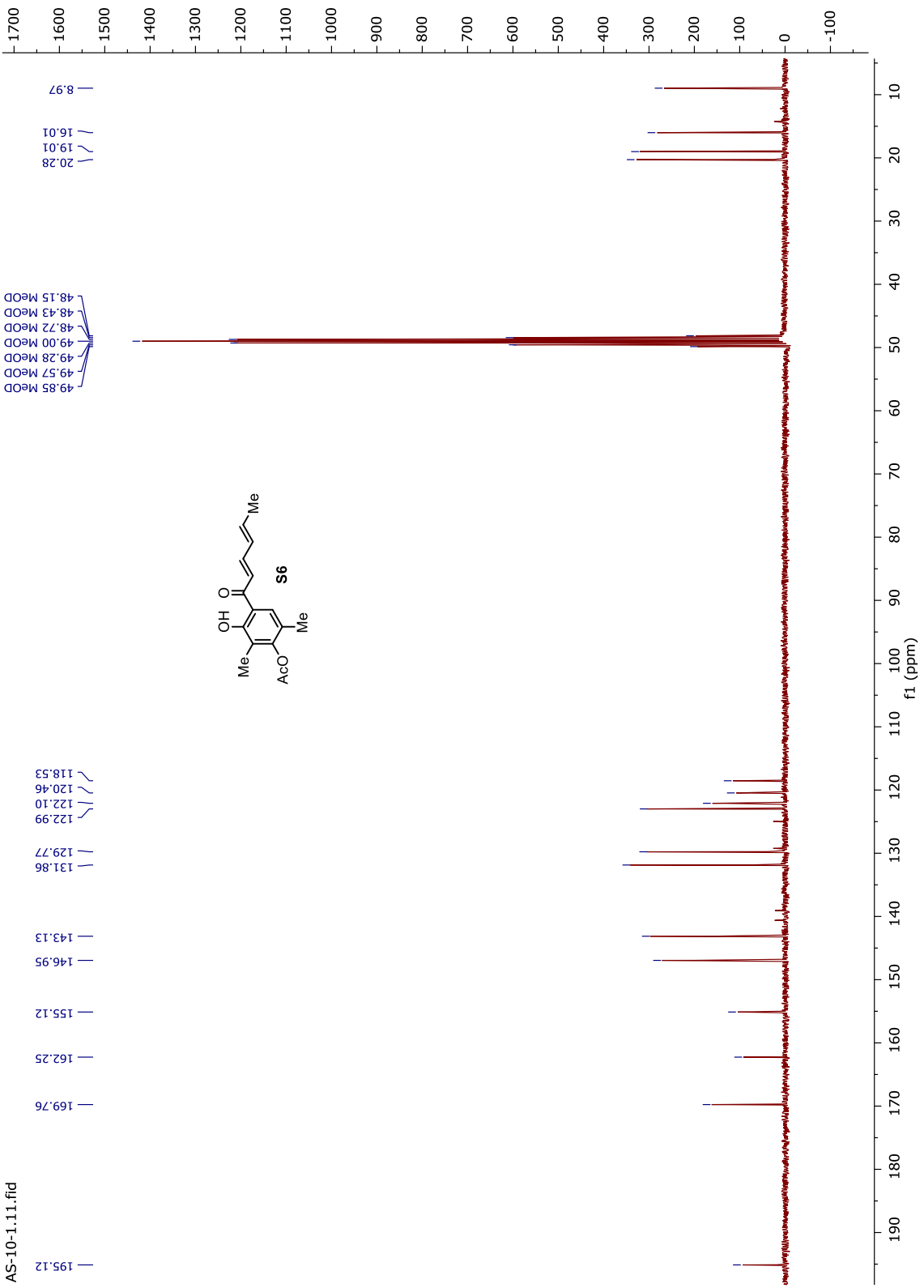

AS-17-13C.10.fid
AS-17-13C
weproton MeOD C:\\ akgulderta 37
OH
O
Me
Me
HO
Me
17
4.99
3.32
3.31
3.31
3.31
3.30
2.90
2.88
2.87
2.16
2.06
1.71
1.69
1.68
1.67
1.65
1.37
1.37
1.36
1.34
0.94
0.92
0.91
0.98
1.97
3.00
3.01
1.97
4.01
3.03
f1 (ppm)

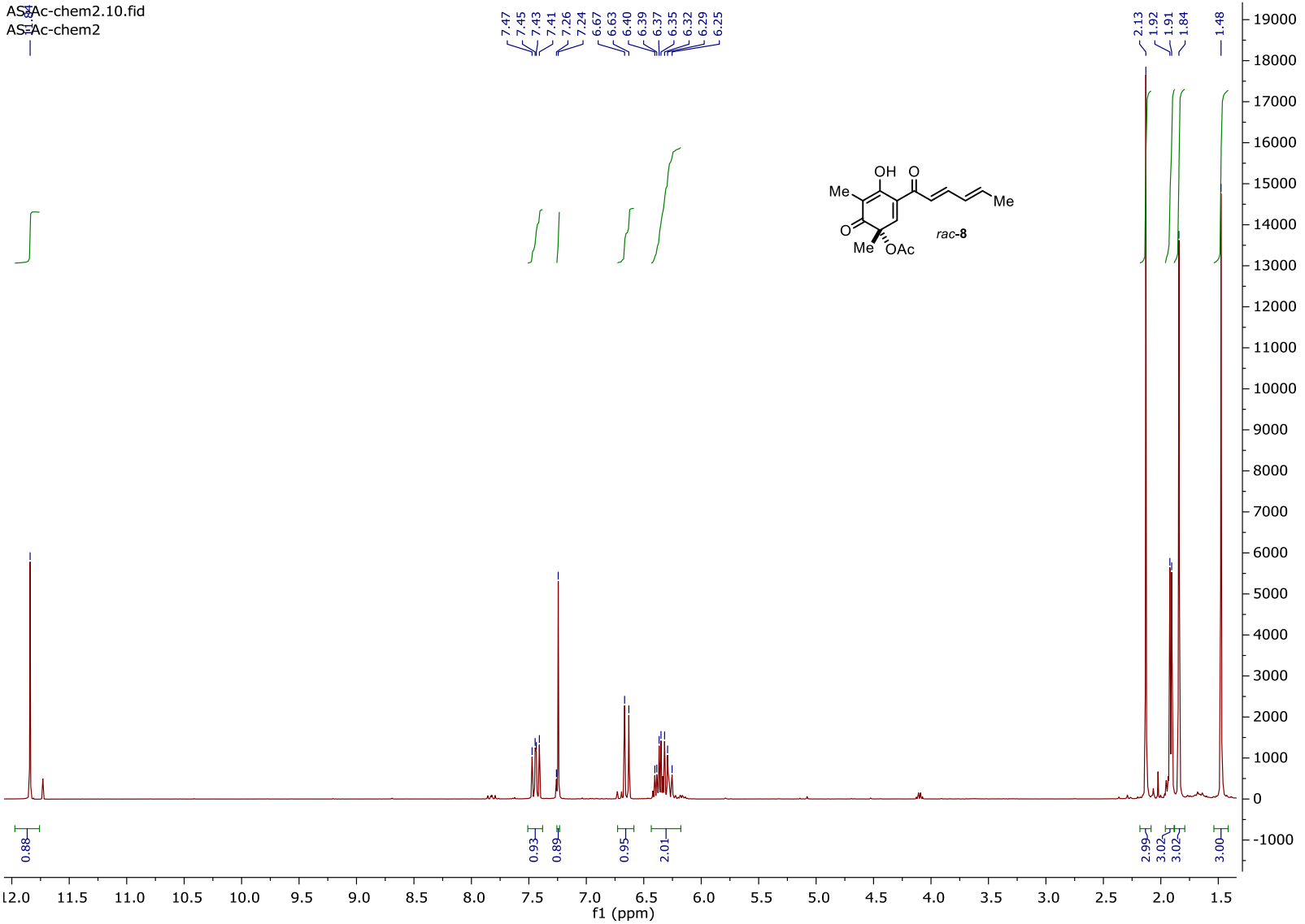

AS17-13C.11.fid
AS17-13C
weC13CPD MeOD C:\\ akgulderta 37
162.50
161.84
130.40
117.08
113.33
111.88
49.51
49.34
49.17
49.00
48.83
48.66
48.49
38.64
32.67
25.98
23.61
16.39
14.33
8.00
17
OH
O
Me
Me
HO
Me
f1 (ppm)

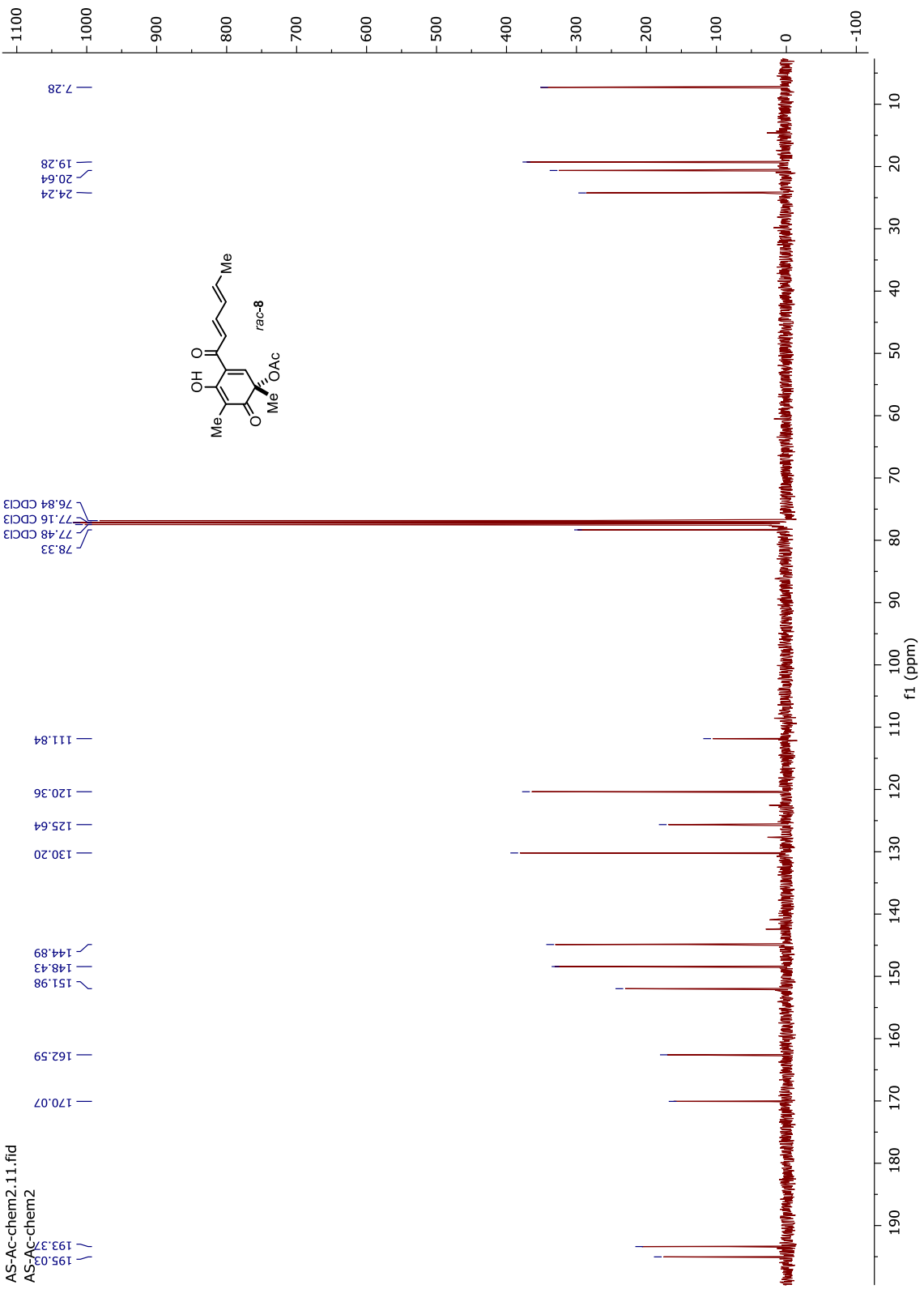

AS-24b
f1 (ppm)
20
OH
O
Me
Me
HO
Me

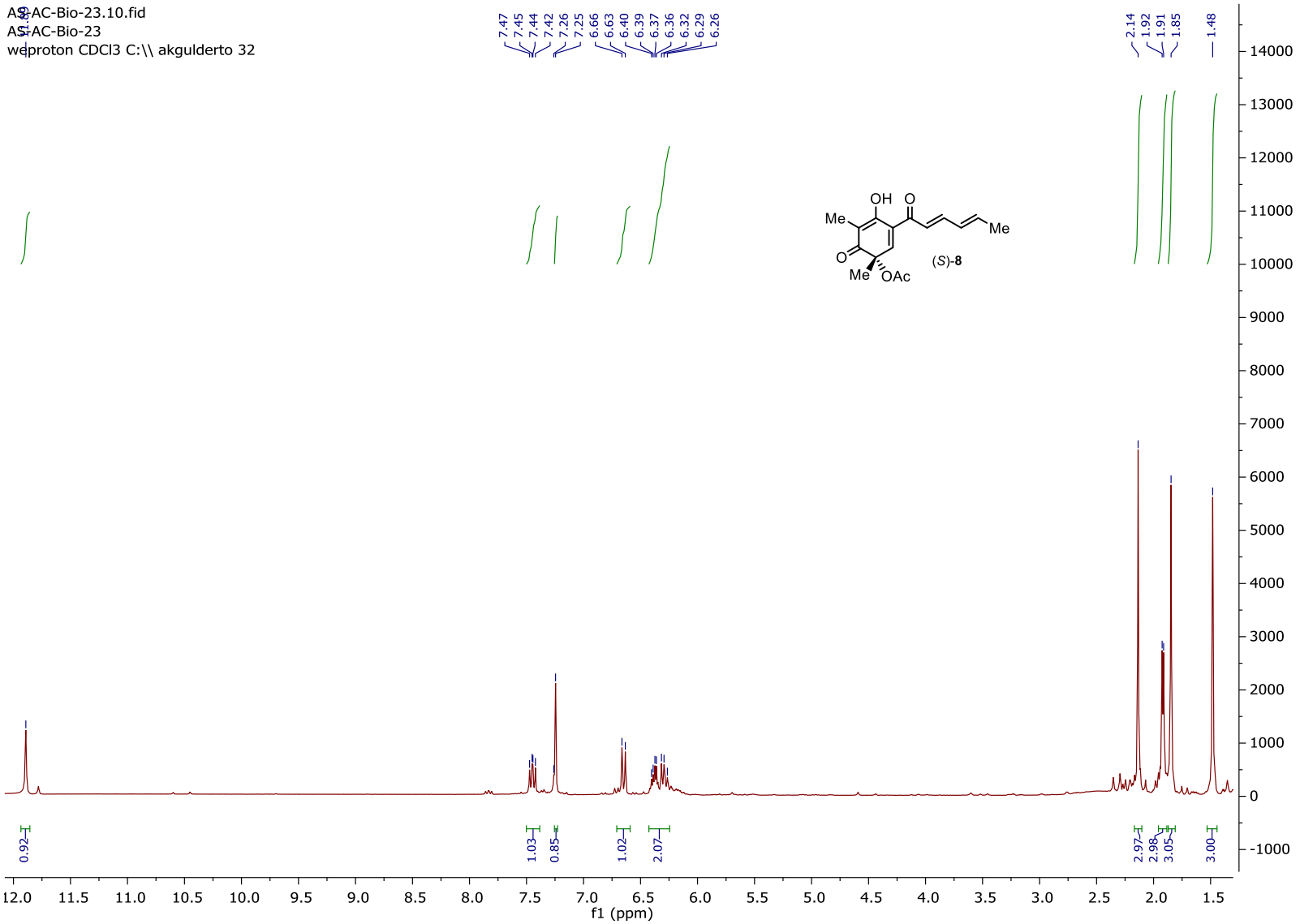

AS-24a.11.fid
193.86
162.16
163.67
149.46
130.25
125.79
117.20
113.51
112.05
49.85 MeOD
49.57 MeOD
49.28 MeOD
48.72
48.43
48.15
35.75
22.72
16.34
14.07
8.02
OH
O
Me
Me
HO
Me
20
f1 (ppm)

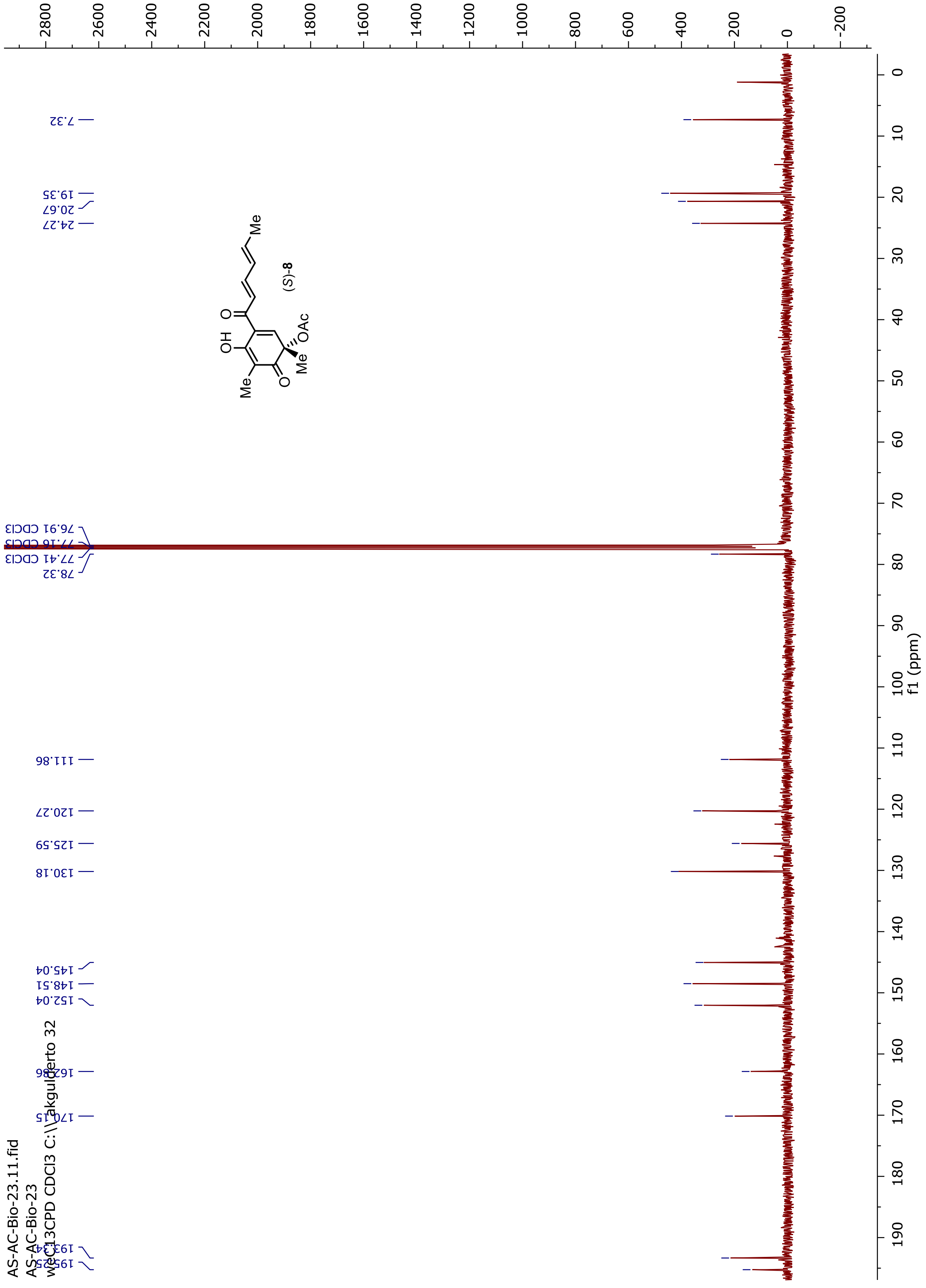

S6
OH
O
Me
Me
AcO
Me
f1 (ppm)


AS-10-1.11.fid
195.12
169.76
162.25
155.12
146.95
143.13
131.86
129.77
122.99
122.10
120.46
118.53
49.85 MeOD
49.57 MeOD
49.28 MeOD
49.00 MeOD
48.72 MeOD
48.43 MeOD
48.15 MeOD
20.28
19.01
16.01
8.97
S6
OH
O
Me
AcO
Me
Me
f1 (ppm)

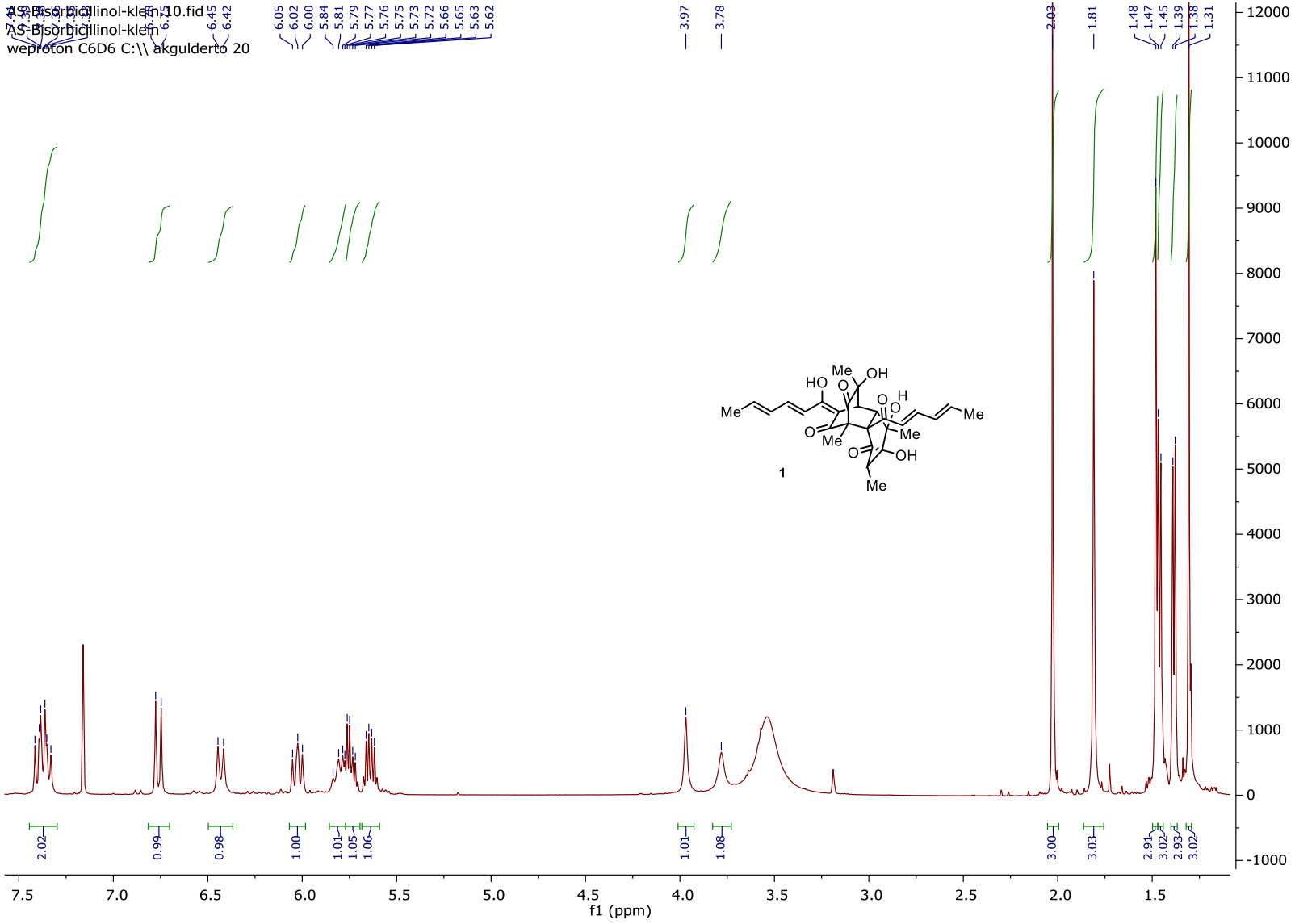

AS-Ac-chem2.10.fid
AS-Ac-chem2
7.47
7.45
7.43
7.41
7.26
7.24
6.67
6.63
6.40
6.39
6.37
6.35
6.32
6.29
6.25
2.13
1.92
1.91
1.84
1.48
OH
O
Me
Me
O
Me
OAc
rac-8
0.88
0.93
0.89
0.95
2.01
2.99
3.02
3.02
3.00
f1 (ppm)

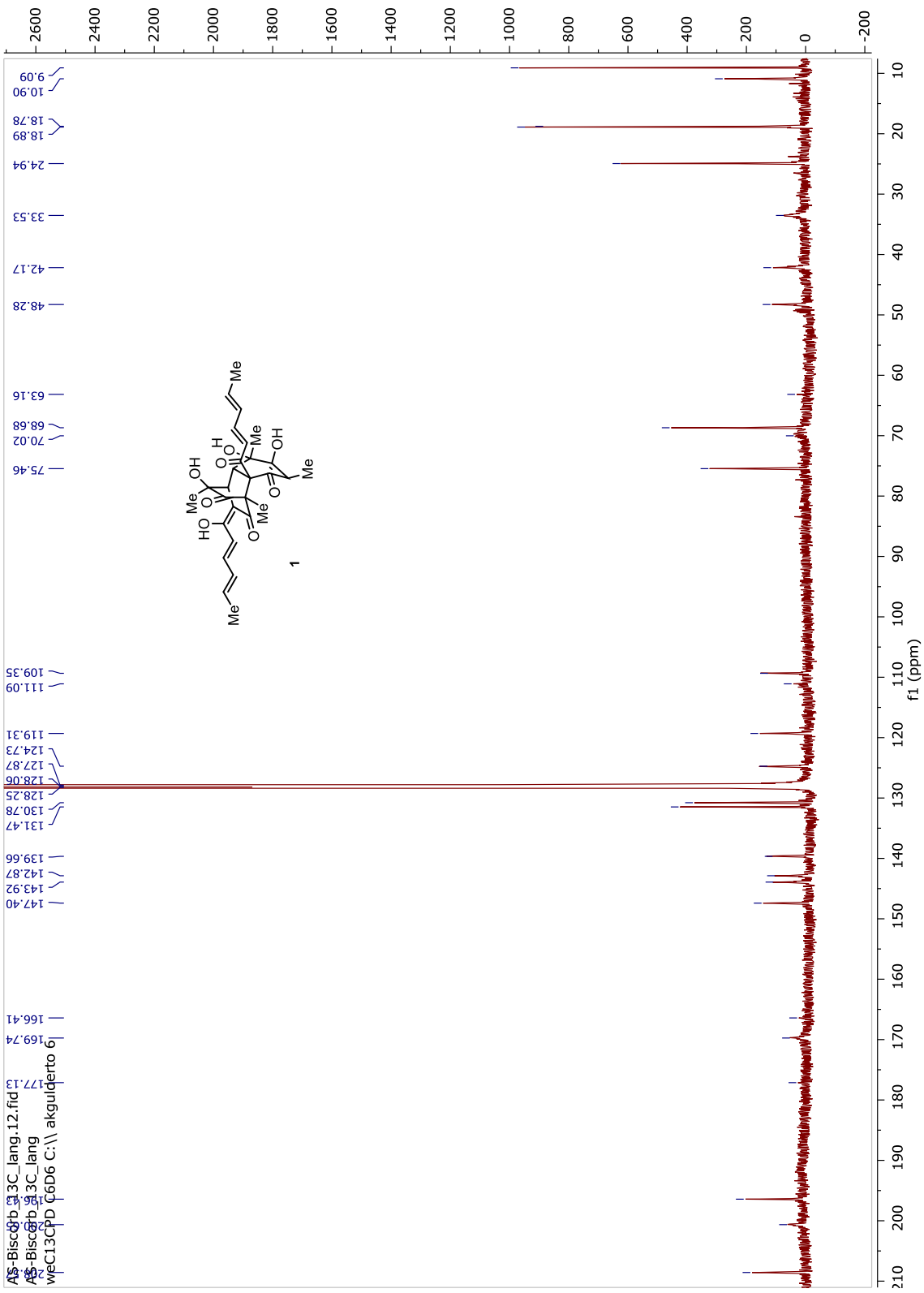

AS-Ac-chem2.11.fid
AS-Ac-chem2
195.03
193.37
170.07
162.59
151.98
148.43
144.89
130.20
125.64
120.36
111.84
78.33
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
24.24
20.64
19.28
7.28
rac-8
f1 (ppm)

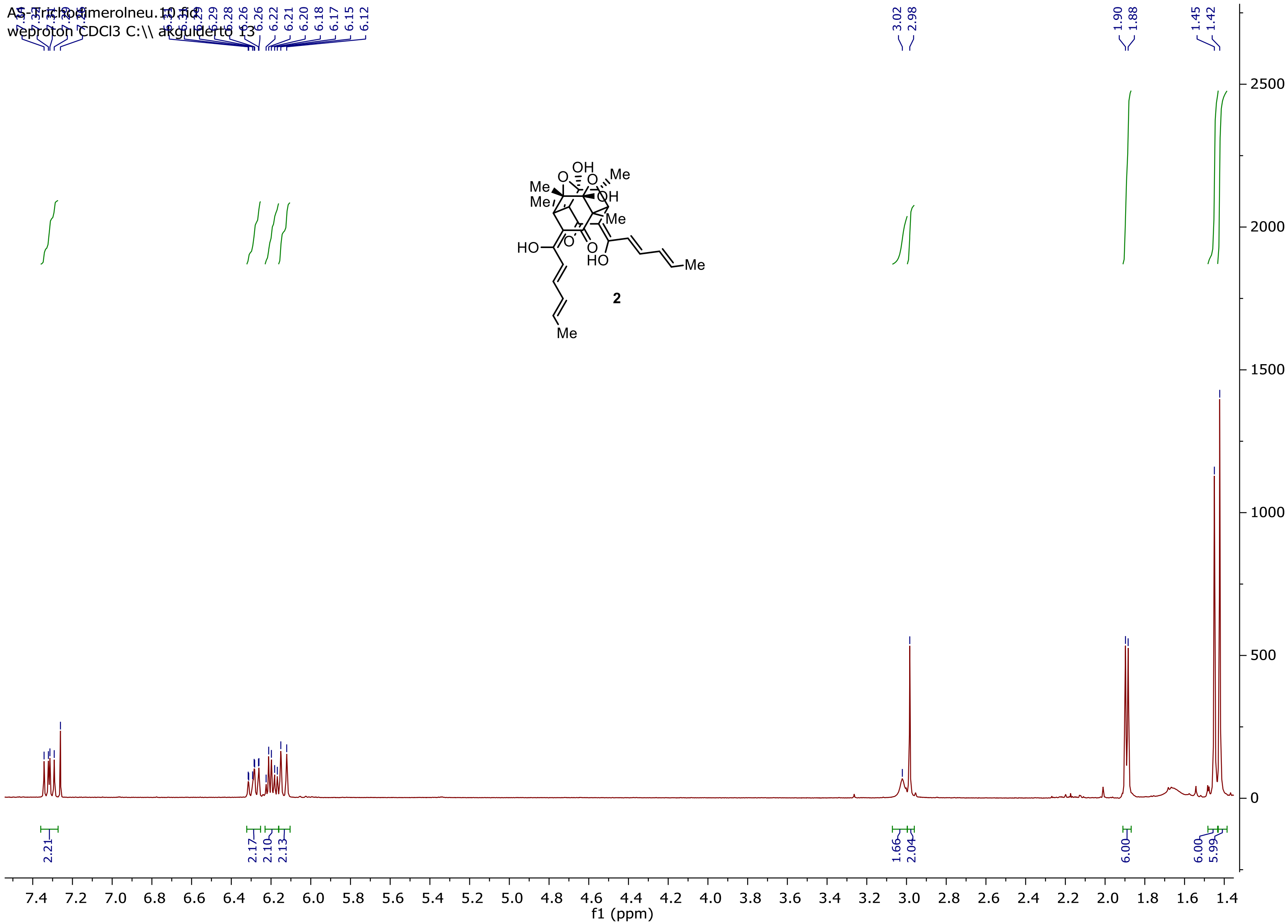

AS-AC-Bio-23.10.fid
AS-AC-Bio-23
wgproton CDCl3 C:\\ akgulderto 32
7.47
7.45
7.44
7.42
7.26
7.25
6.66
6.63
6.40
6.39
6.37
6.36
6.32
6.29
6.26
2.14
1.92
1.91
1.85
1.48
OH
O
Me
Me
O
Me
OAc
(S)-8
0.92
1.03
0.85
1.02
2.07
2.97
2.98
3.05
3.00
f1 (ppm)



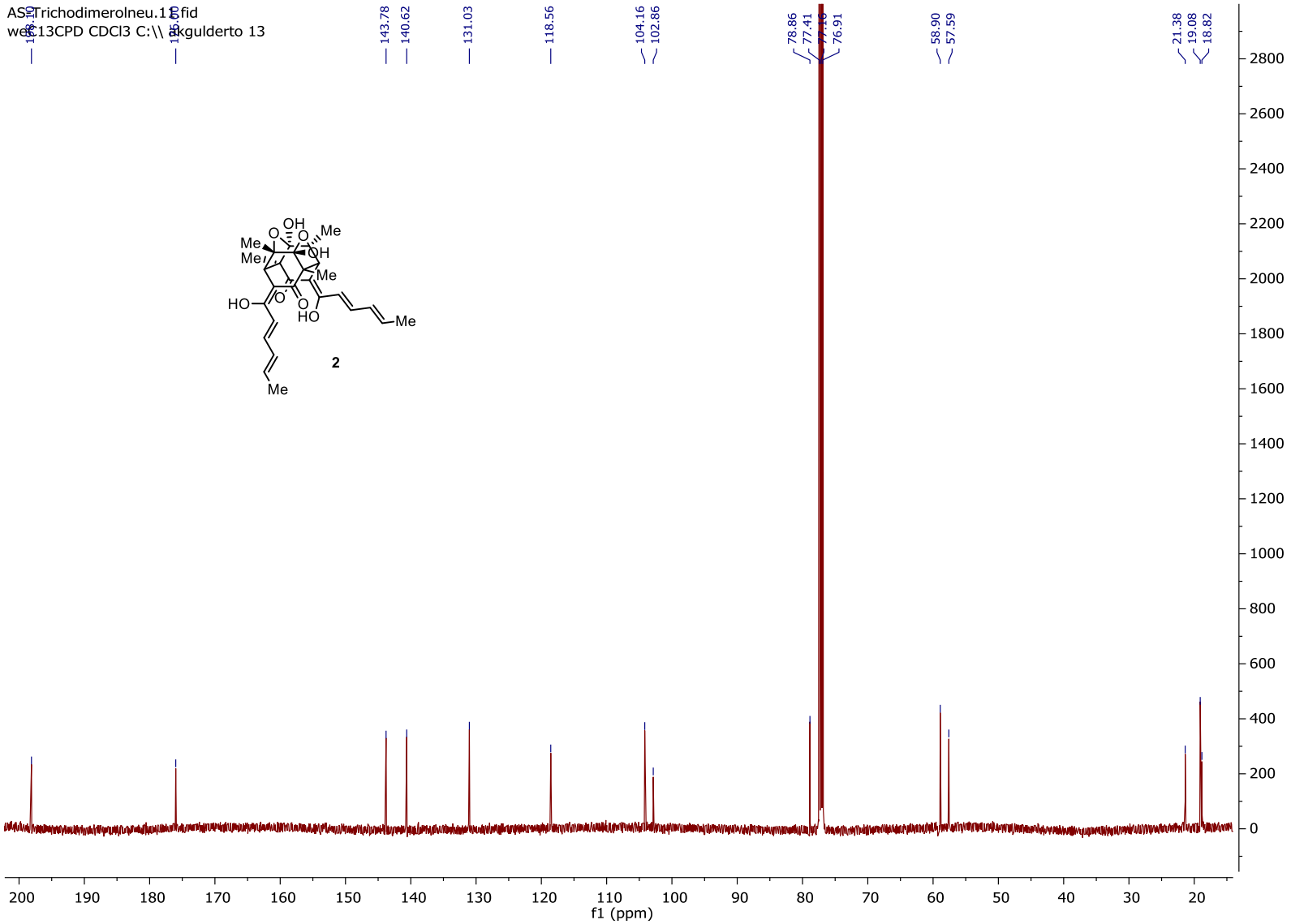
AS-AC-Bio-23.11.fid
AS-AC-Bio-23
CDCl3
7.32
19.35
20.67
24.27
76.91 CDCl3
77.16 CDCl3
77.41 CDCl3
78.32
111.86
120.27
125.59
130.18
145.04
148.51
152.04
170.15
(S)-8
f1 (ppm)

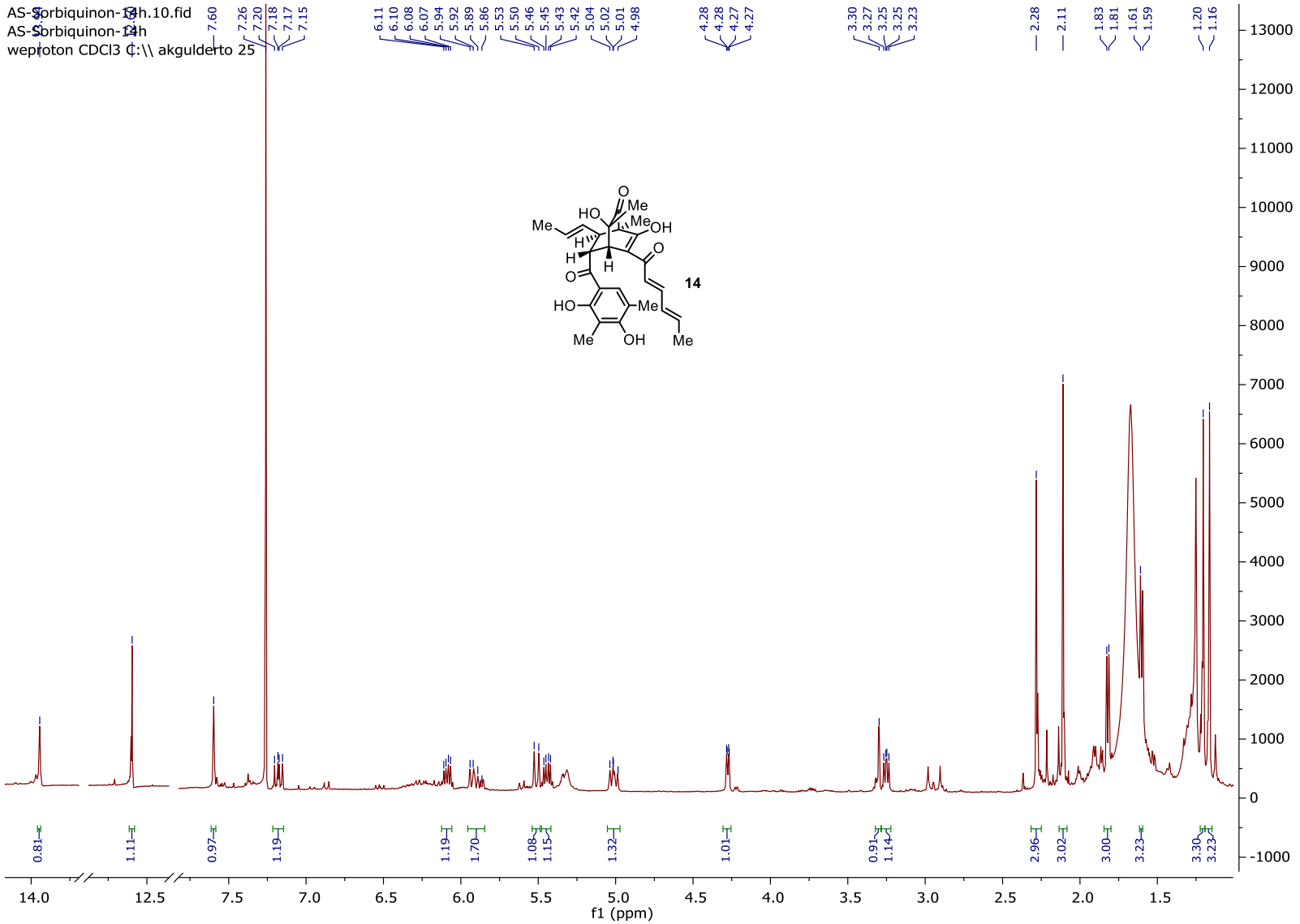

AS-Bisorbicillinol-klein-10.fid
AS-Bisorbicillinol-klein
weproton C6D6 C:\\ akgulderto 20
6.45
6.42
6.05
6.02
6.00
5.84
5.81
5.79
5.77
5.76
5.75
5.73
5.72
5.66
5.65
5.63
5.62
3.97
3.78
2.03
1.81
1.48
1.47
1.45
1.39
1.38
1.31
Me
OH
HO
O
O
H
Me
O
Me
Me
O
OH
Me
1
2.02
0.99
0.98
1.00
1.01
1.05
1.06
1.01
1.08
3.00
3.03
2.91
3.02
2.93
3.02
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)

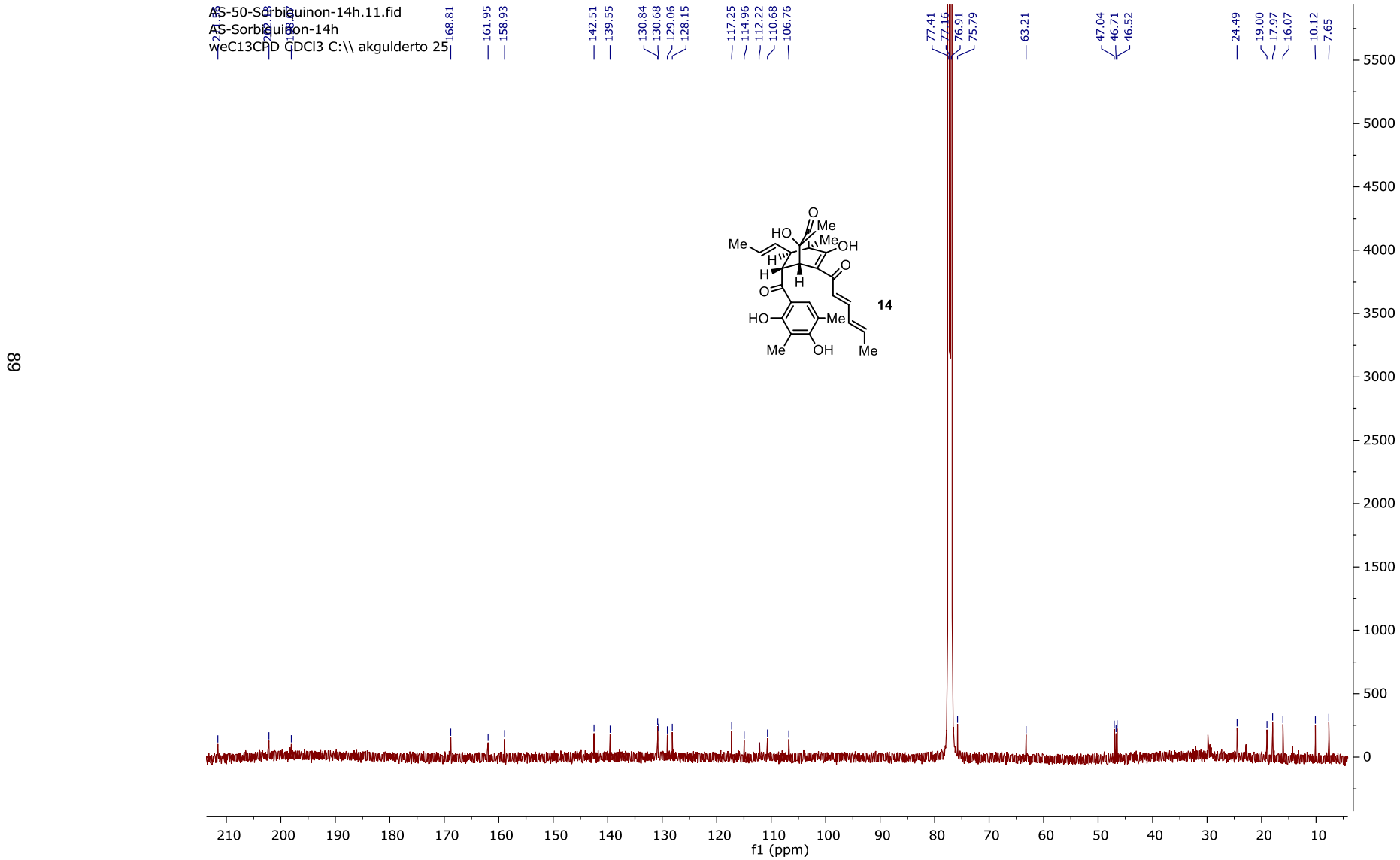

AS-Biscolb_13C_lang.12.fid
AS-Biscolb_13C_lang
weC13CPD C6D6 C:\\ akgulderto 6
9.09
10.90
18.78
18.89
24.94
33.53
42.17
48.28
63.16
68.68
70.02
75.46
109.35
111.09
119.31
124.73
127.87
128.06
128.25
130.78
131.47
139.66
142.87
143.92
147.40
166.41
169.74
177.13
f1 (ppm)
1



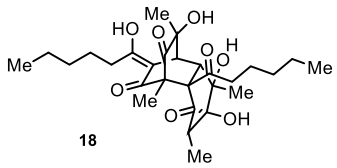
AS-Trichodimerolneu.10.fid
weproton CDCl3 C:\\ akgulderto 13
6.29
6.28
6.26
6.26
6.22
6.21
6.20
6.18
6.17
6.15
6.12
3.02
2.98
1.90
1.88
1.45
1.42
2
2.21
2.17
2.10
2.13
1.66
2.04
6.00
6.00
5.99
f1 (ppm)


AS-Trichodimerolneu.11.fid
wes C13CPD CDCl3 C:\\ akgulderto 13
188.11
165.65
143.78
140.62
131.03
118.56
104.16
102.86
78.86
77.41
77.16
76.91
58.90
57.59
21.38
19.08
18.82
OH
O
O
Me
Me
Me
OH
Me
HO
O
O
HO
Me
Me
2
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

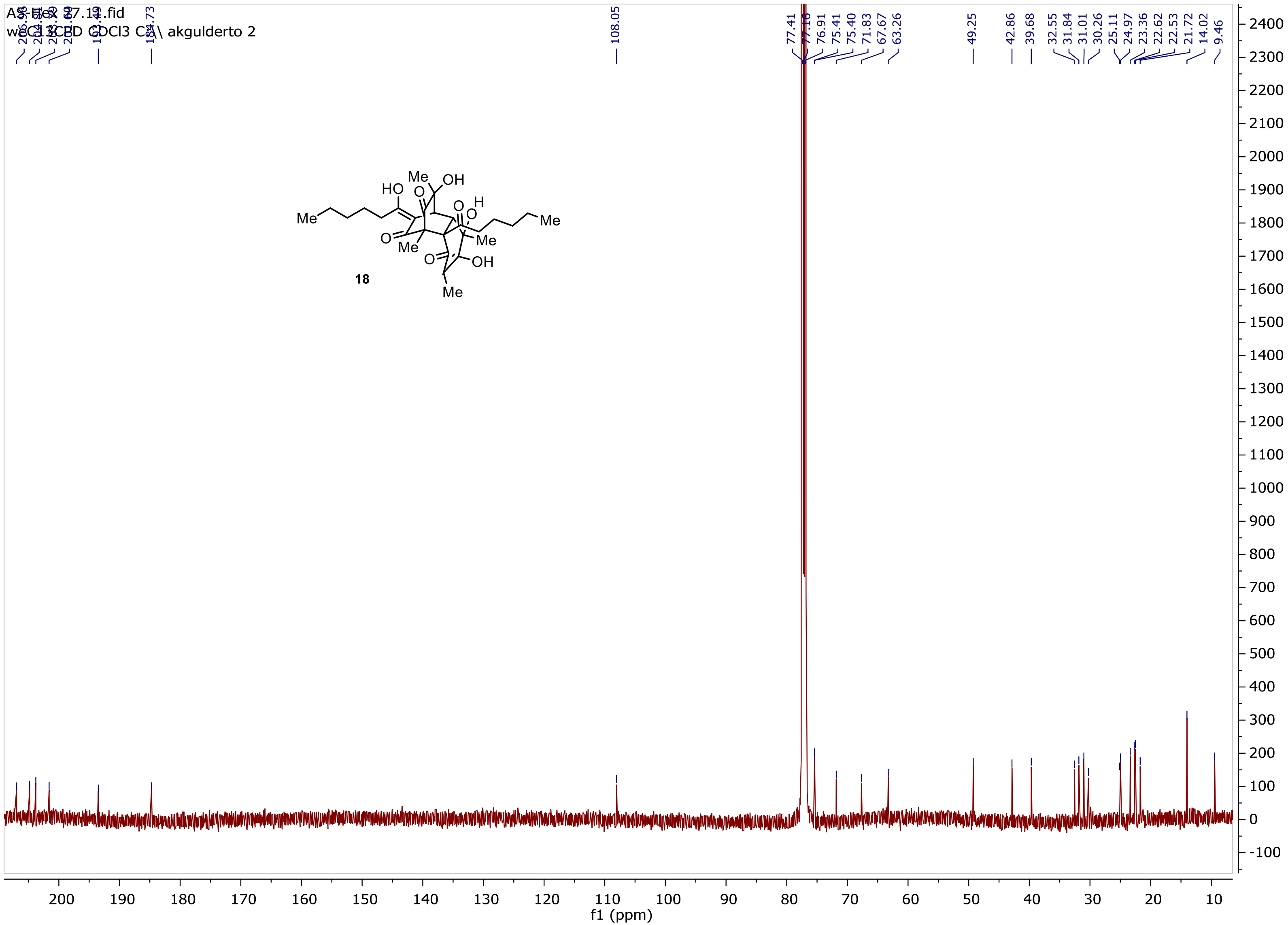

AS-Sorbiquinon-14h.10.fid
AS-Sorbiquinon-14h
weprloton CDCl3 C:\\ akgulderto 25
7.60
7.26
7.20
7.18
7.17
7.15
6.11
6.10
6.08
6.07
5.94
5.92
5.89
5.86
5.53
5.50
5.46
5.45
5.43
5.42
5.04
5.02
5.01
4.98
4.28
4.28
4.27
4.27
3.30
3.27
3.25
3.25
3.23
2.28
2.11
1.83
1.81
1.61
1.59
1.20
1.16
14
0.81
1.11
0.97
1.19
1.19
1.70
1.08
1.15
1.32
1.01
0.91
1.14
2.96
3.02
3.00
3.23
3.30
3.23
f1 (ppm)

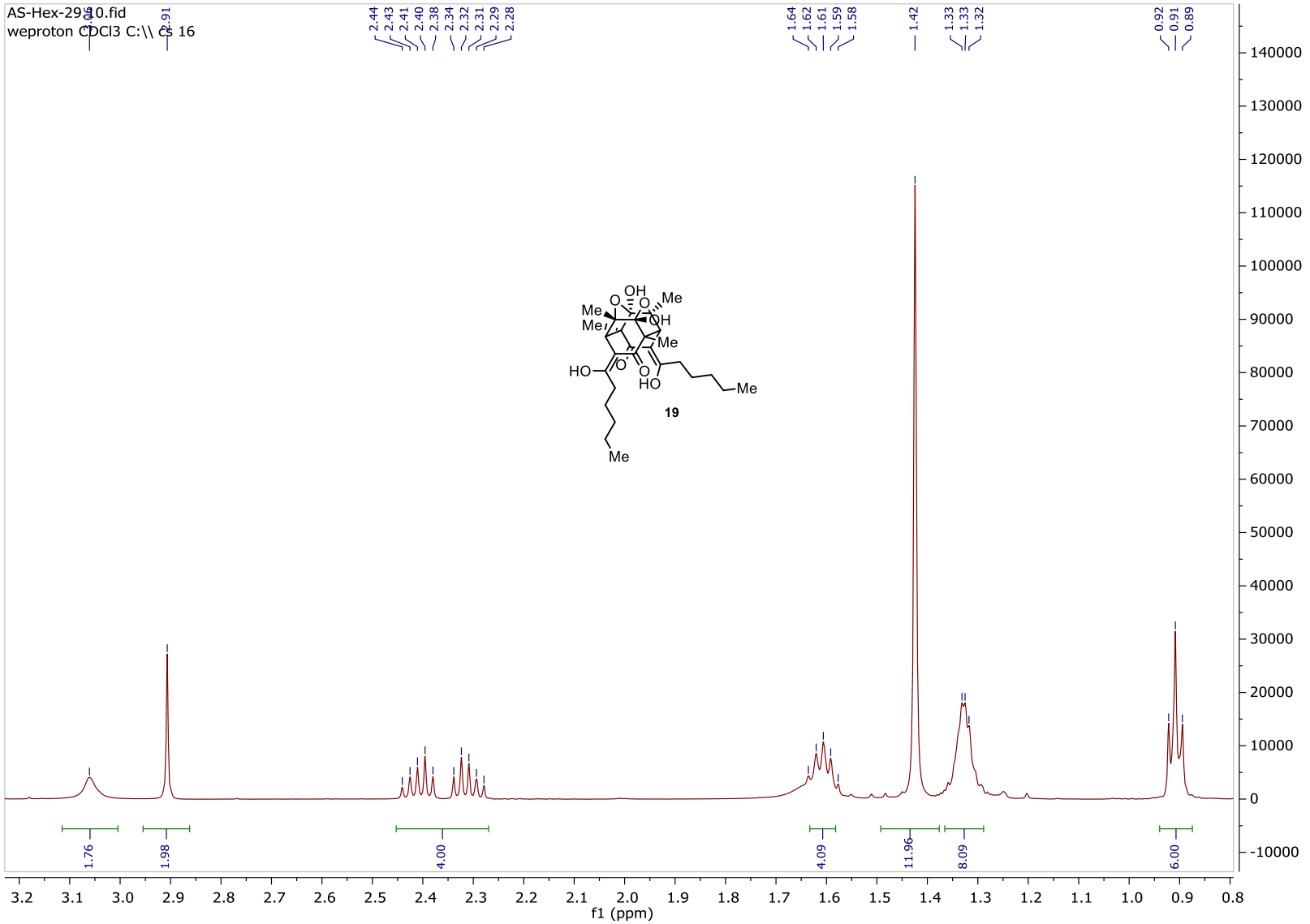

AS-50-Sorbiquinon-14h.11.fid
AS-Sorbiquinon-14h
weC13CPD CDCl3 C:\\ akgulderto 25
168.81
161.95
158.93
142.51
139.55
130.84
130.68
129.06
128.15
117.25
114.96
112.22
110.68
106.76
77.41
77.16
76.91
75.79
63.21
47.04
46.71
46.52
24.49
19.00
17.97
16.07
10.12
7.65
14
f1 (ppm)

AS-Hex 27.10.fid
weproton CDCl3 C:\\ akgulderto 2



18


AS-Hex 27.11.fid
wC13CPD CDCl3 C:\ akgulderto 2
108.05
77.41
77.16
76.91
75.41
75.40
71.83
67.67
63.26
49.25
42.86
39.68
32.55
31.84
31.01
30.26
25.11
24.97
23.36
22.62
22.53
21.72
14.02
9.46
184.73
f1 (ppm)
Me
HO
O
OH
H
Me
O
Me
OH
18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1.59
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1.33
1.32
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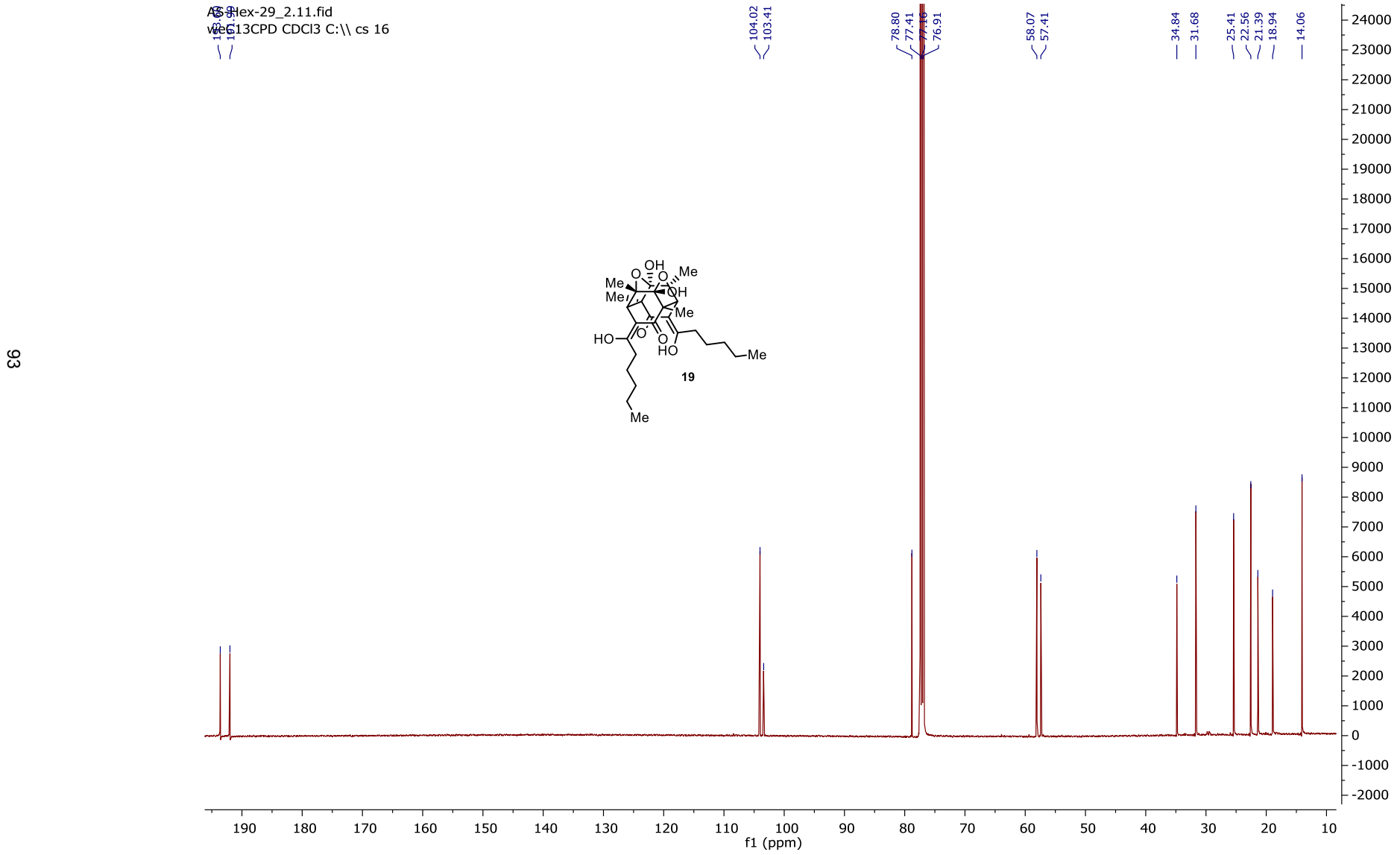
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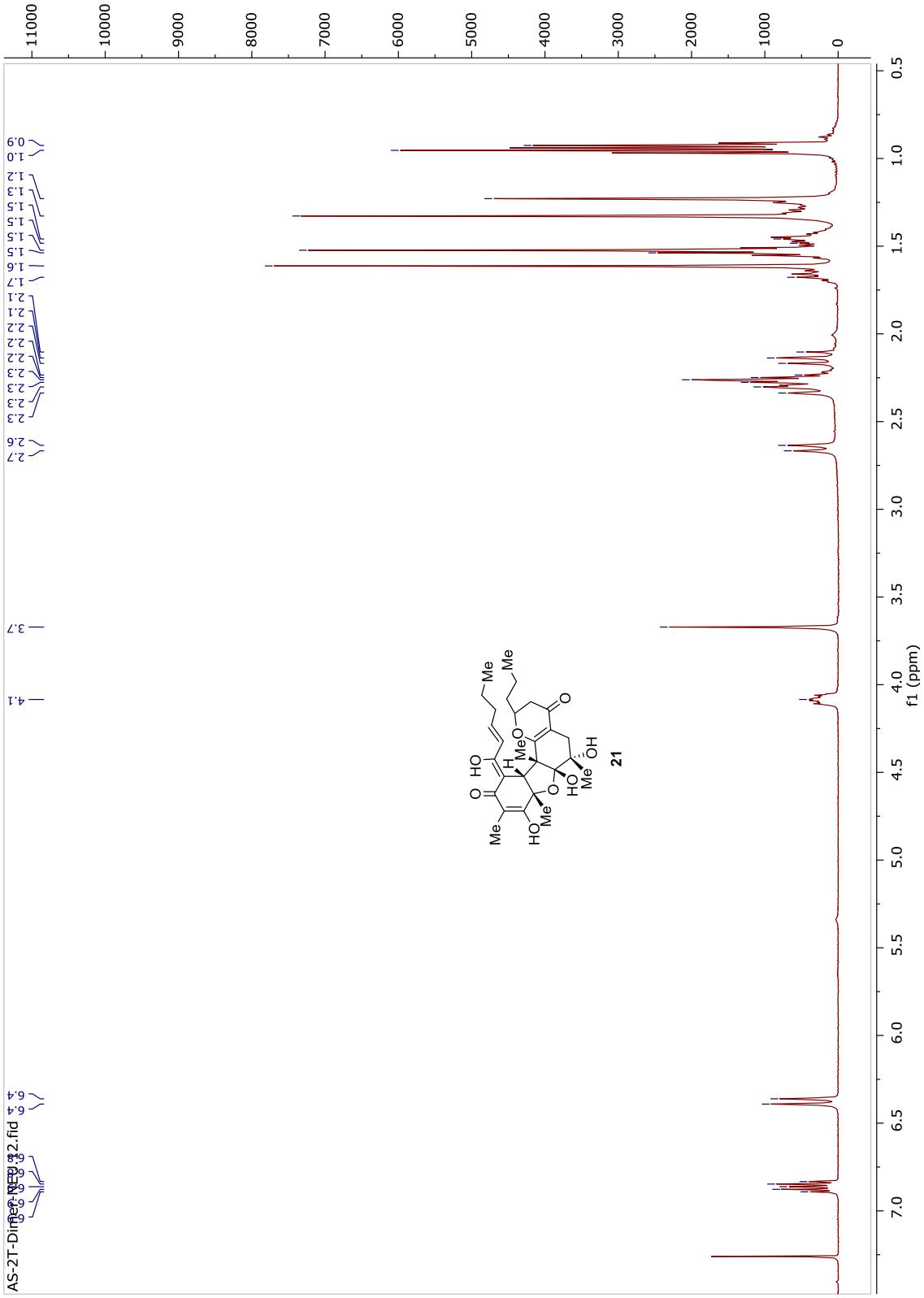
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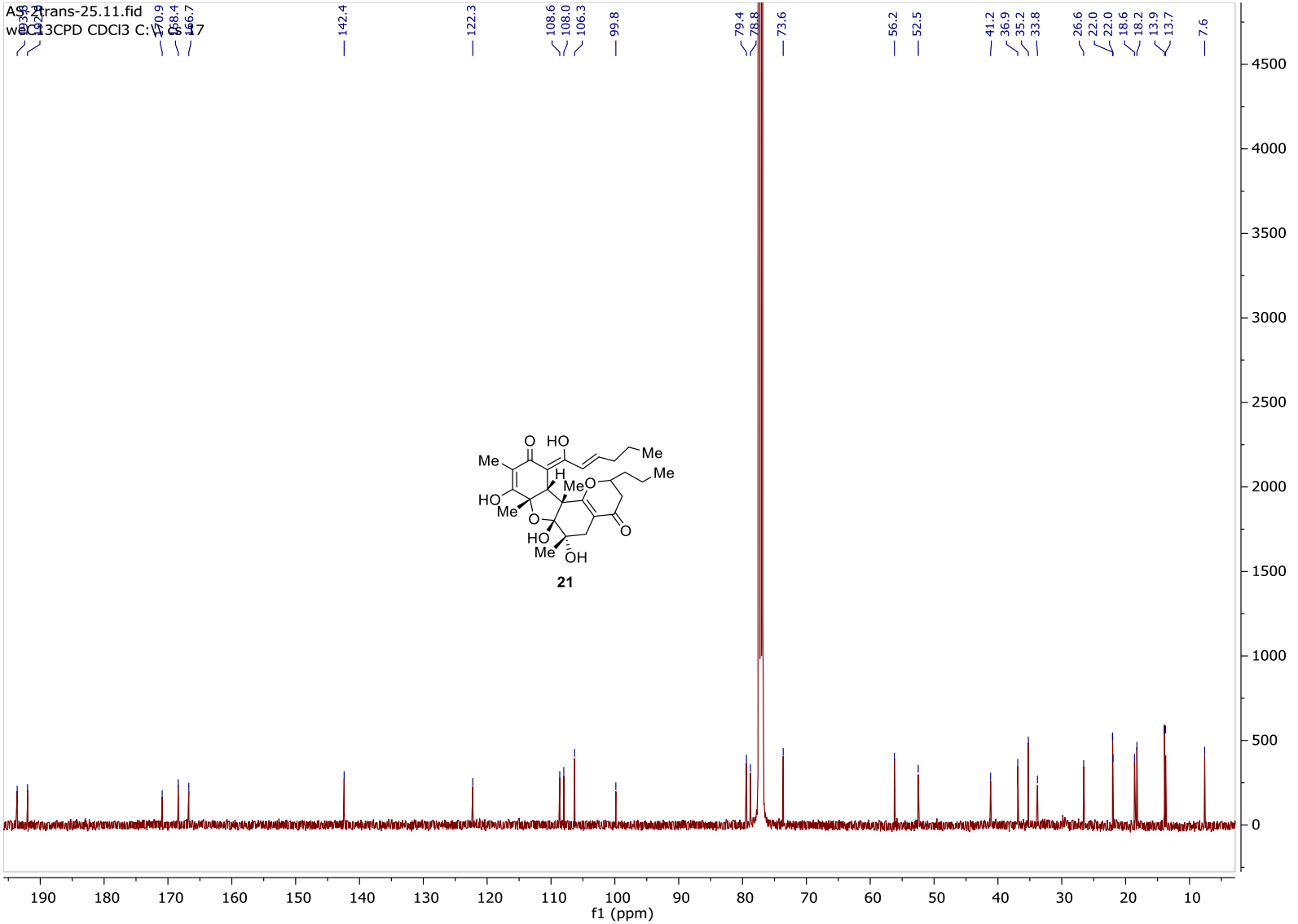
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13.7
7.6
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3500
3000
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2000
1500
1000
500
0
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f1 (ppm)

21

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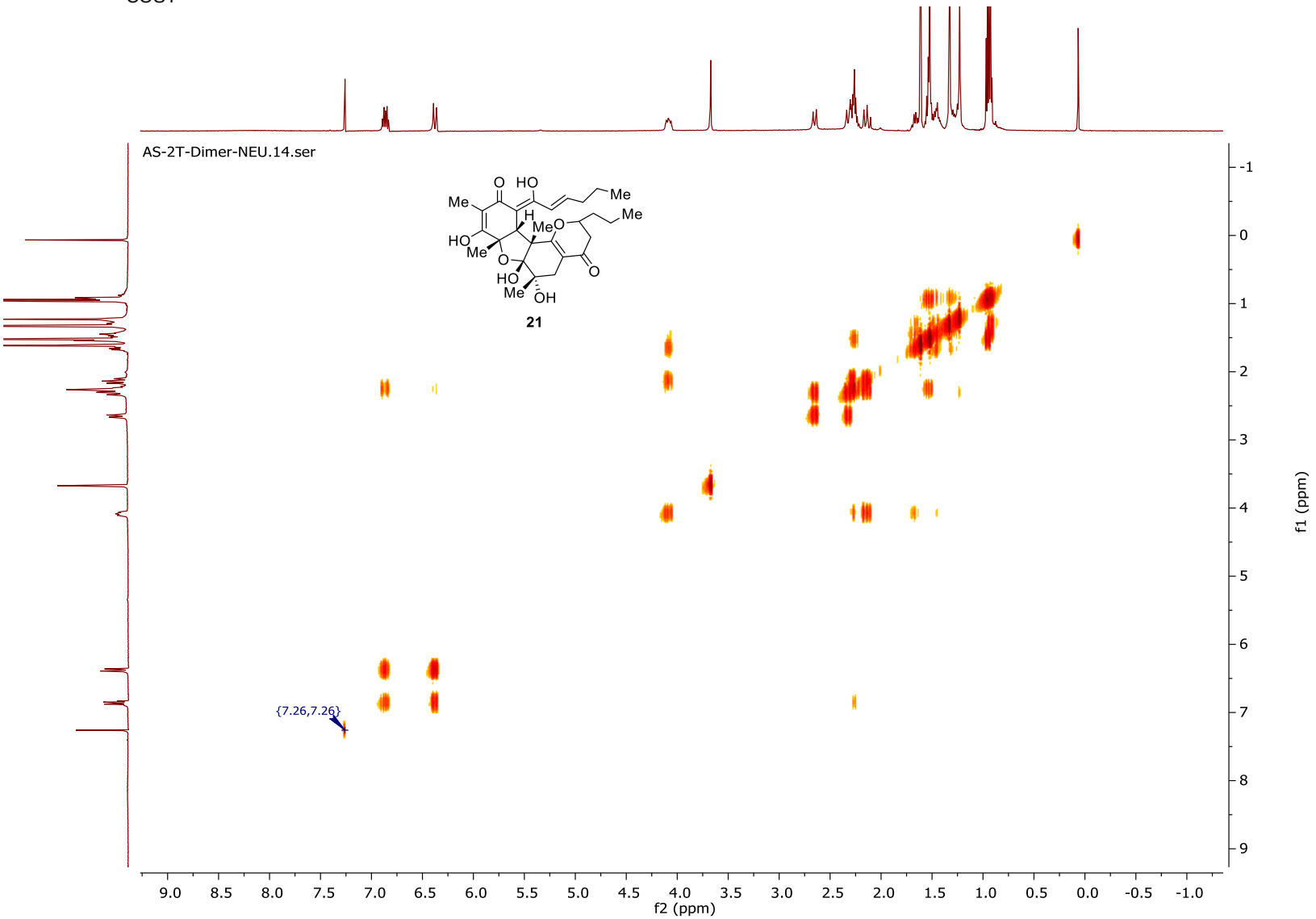
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HO
Me
Me
H
Me
Me
O
HO
Me
O
O
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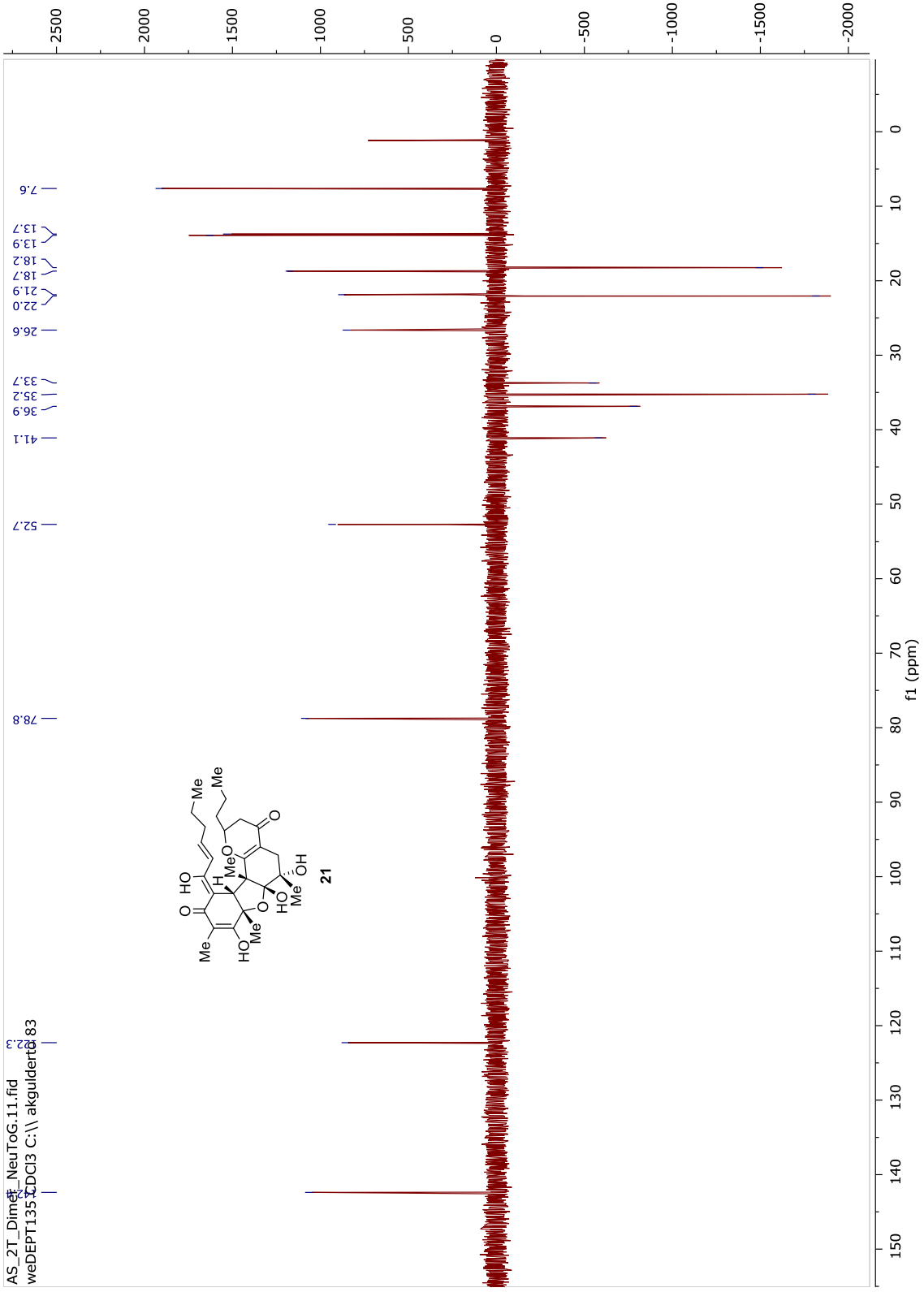
Me
OH

21

DEPT 135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f1 (ppm)
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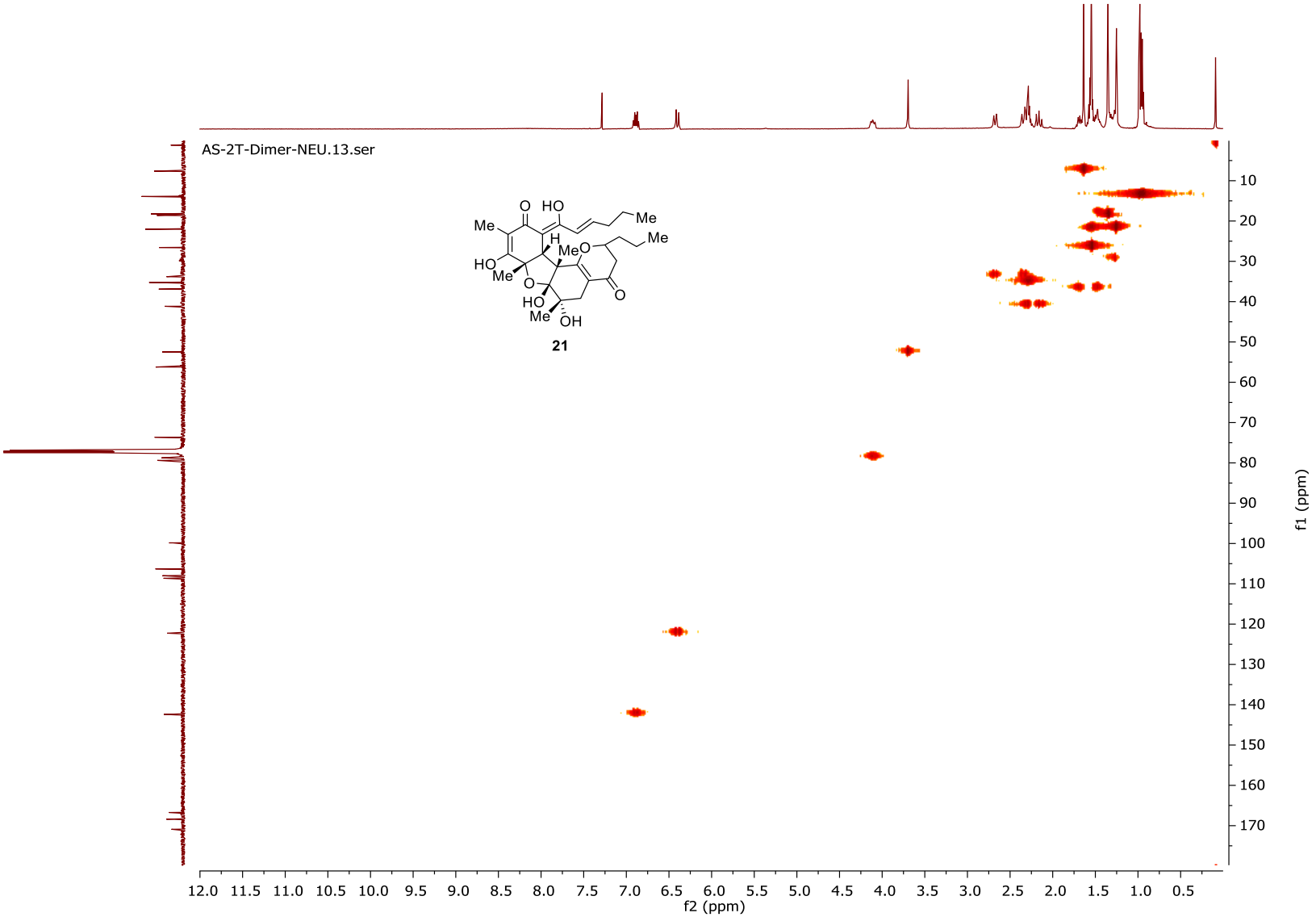

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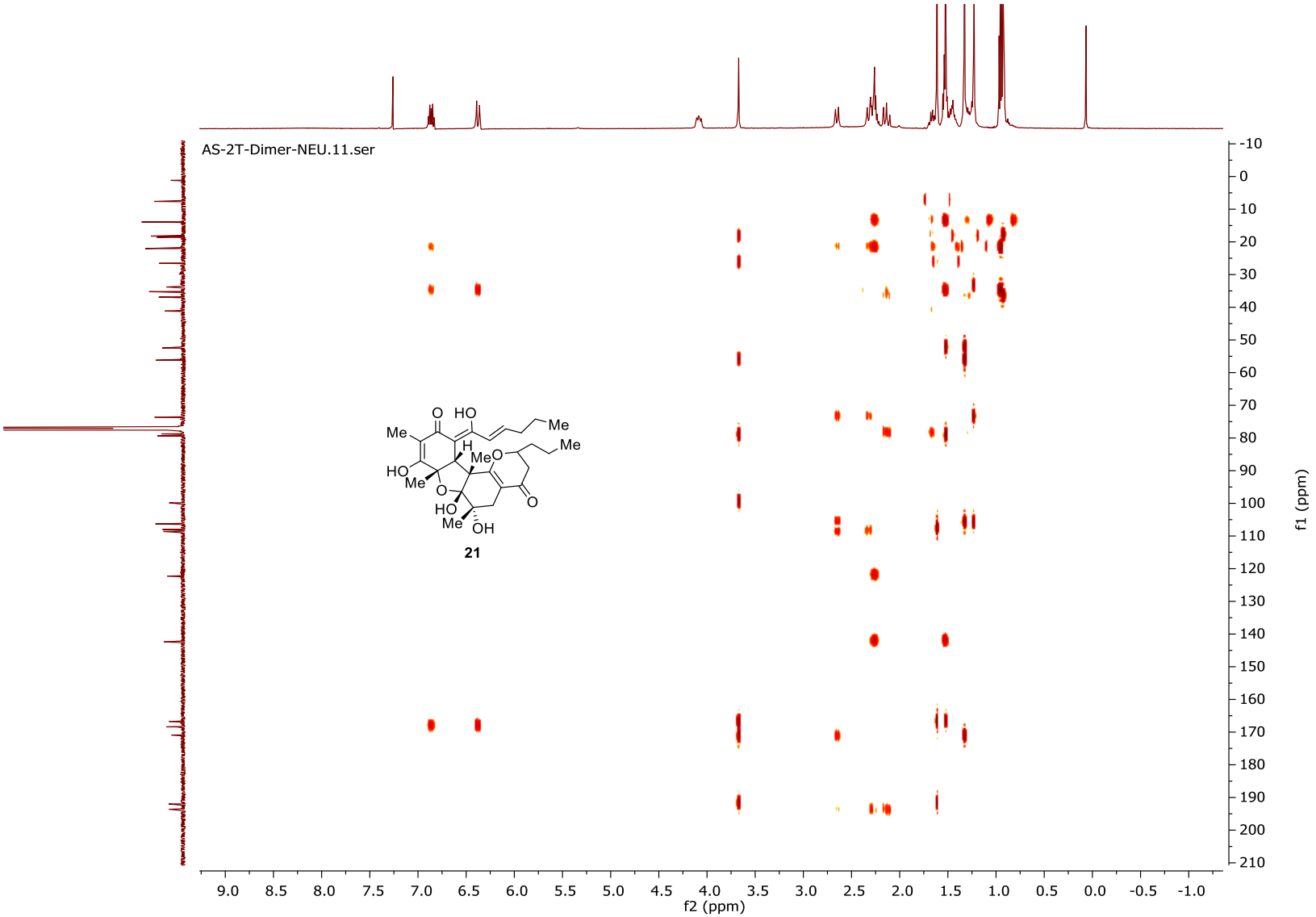
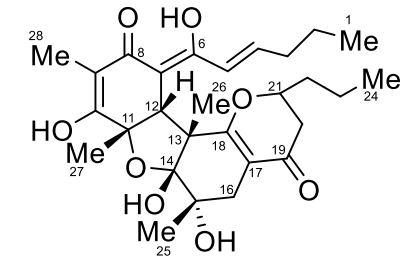
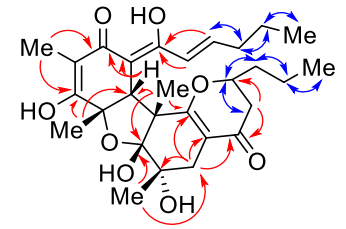


Table S1. NMR data of compound **21** (^{1}H at 500 MHz, ^{13}C at 125 MHz, $CDCl_3$).

	^{1}H	^{13}C	COSY	HMBC
1	0.95 (t, *J* = 7.3 Hz)	13.9	2	2,3
2	1.54 (m)	22.05	1, 3	1,3,4
3	2.27 (m)	35.2	2, 4	1,2,4,5
4	6.86 (dt, *J* = 15.0, 7.1 Hz)	142.4	3, 5	2,3,6
5	6.38(d, *J* = 15.0 Hz)	122.3	4	3,6
6		168.4		
7		99.8		
8		192.0		
9		108.0		
10		166.7		
11		79.4		
12	3.67 (s)	52.5		7,8,10,11,13,18,26,27
13		56.2		
14		106.3		
15		73.6		
16a	2.65 (d, *J* = 15.6 Hz)	33.8	16a <-> 16b	14,15,17,18,19,25
16b	2.33 (m)			
17		108.6		
18		170.9		
19		193.6		
20a	2.14 (pt, *J* = 15.4 Hz)	41.2	20a <-> 20b	19,21,22
20b	2.24 (m)		21	
21	4.08 (m)	78.8	20, 22	
22	1.68 (m) 1.46 (m)	36.9	21, 23	21,23,24
23	1.48 (m)	18.2	22, 24	22, 24
24	0.93 (t, *J* = 7.2 Hz)	13.7	23	22,23
25	1.23 (s)	21.98		14,15,16
26	1.33 (s)	18.6		12,13,14,18
27	1.52 (s)	26.6		10,11,12
28	1.61(s)	7.6		8,9,10

21





= selected HMBC interactions

= selected COSY interactions

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Angewandte Chemie
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Supporting Information

Chemo-enzymatic Total Synthesis of Oxosorbicillinol, Sorrentanone, Rezishanones B and C, Sorbicatechol A, Bisvertinolone, and (+)-Epoxysorbicillinol

*Anna Sib and Tobias A. M. Gulder**

anie_201802176_sm_miscellaneous_information.pdf

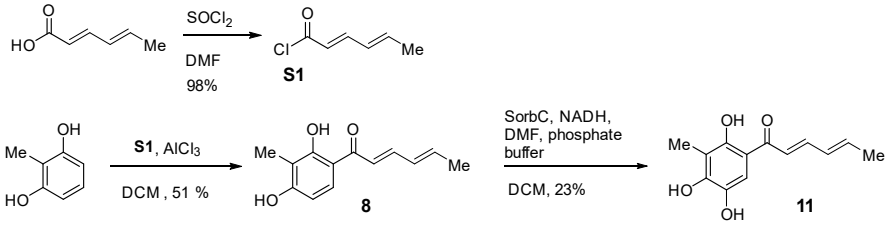
Table of Contents

1. General Information

Chemistry: All solvents used in the reactions were p.A. grade. Solvents for chromatography were technical grade and distilled prior to use. Anhydrous dichloromethane and THF were obtained from an MBraun MB-SPS 800 solvent purification system. Commercial materials were purchased at the highest commercial quality from the providers abcr, Acros, Organics, Alfa Aesar, Carbolution, Carl Roth, Merck, Sigma Aldrich, VWR, Jena Biosciences and Thermo Fisher Scientific. These chemicals were used without further purification. Silica gel Geduran® Si 60 (particle size 0.40 – 0.60 mm) purchased from Merck, was used for flash column chromatography. Solvent mixtures are understood as volume/volume. For TLC analysis, TLC-silica gel 60 F254 plates were purchased from Merck. Applied substances were observed using a UV lamp at 254 nm. For UV-inactive substances, dyeing reagents, such as 0.36% ninhydrin solution in ethanol were used. NMR spectra were recorded on Bruker AVHD300, Bruker AVHD400, Bruker AVHD500 (only ^{1}H NMR spectra), or Bruker AV500-cryo spectrometers. The chemical shifts δ are listed as parts per million [ppm] and refer to δ(TMS) = 0. The spectra were calibrated using residual undeuterated solvent as an internal reference (δ($CDCl_3$) = 7.26 ppm, δ(C_6D_6) = 7.16 ppm, δ(methanol-d_4) = 3.31 ppm for ^{1}H NMR; δ($CDCl_3$) = 77.0 ppm, δ(C_6D_6) = 128.1 ppm, δ(methanol-d_4) = 49.0 ppm for ^{13}C NMR). The following abbreviations (or combinations thereof) are used to explain the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets, q = quartet, m = multiplet, br = broad. In addition, the following abbreviations for chemicals are used: EtOAc = ethyl acetate, Et_2O = diethyl ether, ACN = acetonitrile, TLC = thin layer chromatography, rt = room temperature.
For High Performance Liquid Chromatography (HPLC) analyses, a computer controlled Jasco system was used (UV-1575 Intelligent UV/VIS Detector, DG-2080-53 3-Line Degaser, two PU-1580 Intelligent HPLC Pumps, AS-1550 Intelligent Sampler, HG-1580-32 Dynamic Mixer). The analyses of the recorded chromatograms were performed using Galaxie-Chromatography-Software provided by Jasco. A Eurosphere II 100-3 C18 A (150 x 4.6 mm) column with integrated precolumn manufactured by Knauer was used for analytical separations with the following composition of the eluent: A = H_2O + 0.05% TFA and B = ACN + 0.05% TFA. The analytical method consisted of the following gradient: 0-1 min 5% B, 1-15 min to 95% B, 15-18 min 95% B, 18-18.5 min to 5% B, 18.5-20 min 5% B with a flowrate of 1 mL/min. This method was used for all analyses. The chiral resolution was carried out on CHIRALCEL OD-RH column material (150 x 4.6 mm) using isocratic conditions (33 % B) at a flow rate of 0.7 mL/min at 15°C. Isolation of the products was carried out by semi-preparative HPLC controlled by a Jasco HPLC system consisting of an UV-1575 Intelligent UV/VIS Detector, two PU-2068 Intelligent prep. Pumps, a MIKA 1000 Dynamic Mixing Chamber (1000 µL Portmann Instruments AG Biel-Benken), a LC-NetII/ ADC, and a Rheodyne injection valve. The system was controlled by the Galaxie-Software and the eluent system consisted of: A = H_2O + 0.05% TFA and B = ACN + 0.05% TFA. A Eurosphere II 100-5 C18 A (250 x 16 mm) column with precolumn (30 x 16 mm) provided by Knauer was used as the stationary phase. General HPLC condition: gradient: 0-1 min 95% H2O + 0.05% TFA (A) / 5% acetonitrile + 0.05% TFA (B), 1-40 min 5% A / 95% B, 40-41 min 5% A / 95% B, 41-43 min 95% A / 5% B, 43-45 min 95% A / 5% B, flow rate: 12 mL/min, running time: 45 min The individual gradient compositions are given below. After preparative separation of the product, the collected fractions containing the desired product were combined and the ACN was removed under reduced pressure. The remaining aqueous phases were freeze-dried in liquid nitrogen and the water removed by lyophilization (Alpha 2-4 *Christ* with Chemistry-Hybrid-Pump-RC6 pump). For medium pressure liquid chromatography (MPLC) the Reveleris® X2 MPLC system (Grace) was used together with Reverleris® Reverse Phase (RP) C18 columns (Grace) using UV-detection at 220 nm, 254 nm, and 280 nm. General MPLC conditions: gradient: isocratic, H_2O+ 0.05% TFA /acetonitrile+ 0.05% TFA, proportion: 50:50, flow rate: 40 mL/min, running time: 20.0 min The eluent system was composed as follows: A= H_2O + 0.05% TFA and B= ACN + 0.05% TFA. For Electrospray ionization mass spectrometry (ESI-MS) a LCQ Fleet Ion Trap mass spectrometer attached to a UltiMate 3000 HPLC system (both *Thermo Scientific*) and controlled by Xcalibur software was used. The analyses of the recorded spectra were performed using Thermo Xcalibur Qual Browser 2.2 SP1.48 Software. For High resolution mass spectrometry (HRMS) a Thermo LTQ FT Ultra mass spectrometer was used and analyses of the recorded spectra were again performed using Thermo Xcalibur Qual Browser 2.2 SP1.48 Software. For CD measurements a Chirascan™ Circular Dichroism Spectrometer was used. The analyses of the recorded spectra were performed using Origin. The optical rotation value were measured by a Perkin-Elmer 241 MC polarimeter.

Biochemistry/Molecular Biology: PD-10 columns, and Vivaspin 2 Hydrosart membrane columns (30,000 MWCO) were purchased from VWR. Recombinant production and purification of SorbC was conducted as reported previously.[1] Final protein concentrations were determined photometrically using the Nanophotometer 330 (Implen) at 280 nm using the extinction coefficient of SorbC ε(280 nm) = 50920 M^{-1} cm^{-1}. Protein production, enrichment and purification were monitored by SDS-PAGE analysis (BioRad Mini Protean® Tetra System) using Unstained Protein MW Marker (Thermo Scientific). All buffers consisted of 50 mM Tris/Cl at pH 7.5, 150 mM NaCl, and 5% glycerol) with changing concentrations of imidazole (buffer A: 20 mM; buffer B: 250 mM; buffer C: no imidazole). The enzymatic oxidative dearomatization reactions were performed in phosphate buffer (50 mM, pH 8.0).

2. Experimental Data



Scheme S1. Synthesis of sorbicillin derivative **11**.

2.1 Synthesis of sorbic acid chloride (**S1**):

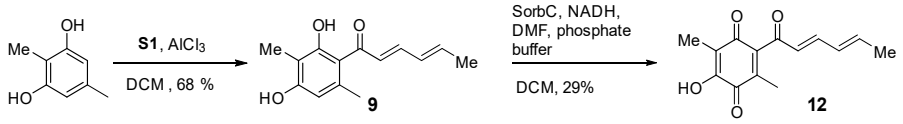
Sorbic acid (5.0 g, 44.6 mmol, 1.0 eq.) was dissolved in $SOCl_2$ (6.5 mL, 89.6 mmol, 2.0 eq.) and DMF (50 µL) under argon atmosphere and then heated for 40 minutes at 80 °C. The product was isolated by distillation under reduced pressure (40°C, 0.5 torr) to obtain the product **S1** as slightly yellow liquid (5.7 g, 43.8 mmol, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 7.43 (dd, *J* = 14.8, *J* = 10.7 Hz, 1H), 6.19-6.45 (m, 2H), 5.99 (d, *J* = 14.8 Hz, 1H), 1.93 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, MeOD) δ= 169.1, 146.5, 140.8, 130.8, 119.3, 18.7.The physical and spectroscopic data were in agreement with that described in the literature.[2]

2.2 Synthesis of demethylsorbicillin (**8**):

2-methylresorcinol (0.78 g, 6.3 mmol, 1.0 eq.) was dissolved in abs. dichloromethane (30 mL) under argon atmosphere and cooled to 0°C. Freshly produced sorbic acid chloride (**S1**) (0.8 mL, 6.9 mmol, 1.1 eq.) and aluminum chloride (0.9 g, 6.9 mmol, 1.1 eq.) were added. After 1 hour of stirring at room temperature, the reaction mixture was acidified with 1 N HCl and extracted with dichloromethane (3 x 50 mL). The combined organic phases were dried over magnesium sulfate and the solvent was evaporated under reduced pressure. Purification by MPLC (retention time: 11.0-14.0 min) gave bright yellow crystals (0.7 g, 3.2 mmol, 51%). ^{1}H NMR (500 MHz, MeOD) δ= 7.67 (d, *J* = 8.9 Hz, 1H), 7.41 (dd, *J* = 14.8, 10.9 Hz, 1H), 7.09 (d, *J* = 14.8 Hz, 1H), 6.45-6.25 (m, 3H), 2.04 (s, 3H), 1.89 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, MeOD) δ= 193.9, 165.3, 163.9, 145.4, 141.8, 131.9, 130.0, 123.3, 114.2, 112.4, 107.9, 18.9, 7.6. MS (ESI+): *m/z* = 219.07 $[M+H]^+$ The physical and spectroscopic data were in agreement with that described in the literature.[3]

2.3 Enzymatic Synthesis of (2*E*,4*E*)-1-(2,4,5-trihydroxy-3-methylphenyl)hexa-2,4-dien-1-one (**11**):

Demethylsorbicillin (**8**) (17.0 mg, 78.0 µmol, 1.0 eq.), dissolved in acetone (2 mL) was added to phosphate buffer (15 mL, 50 mM, pH = 8) with the enzyme SorbC (0,4 mL, 17.5 mg/mL in Tris-buffer). The reaction was started by addition of NADH (50.0 mg, 0.1 mmol, 1.3 eq.) and incubated for 120 min at room temperature. The reaction mixture was washed with dichloromethane (3 x 20 mL), then the aqueous phase was evaporated under reduced pressure. The crude product was dissolved in acetonitrile, filtered and purified by preperative HPLC (retention time: 21.0 min). The substrate demethylsorbicillin (**8**) was re-isolated in 28% yield (4.7 mg, 22.0 µmol), based on which the desired product triol **11** was obtained in 23% yield (3.0 mg, 13.0 µmol, 17% without re-isolation of **8**). ^{1}H NMR (500 MHz, MeOD) δ= 7.40 (dd, *J* = 14.8, 10.9 Hz, 1H), 7.13 (s, 1H), 6.98 (d, *J* = 14.8 Hz, 1H), 6.45 – 6.38 (m, 1H), 6.36 – 6.26 (m, 1H), 2.07 (s, 3H), 1.91 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 193.6, 159.8, 154.2, 145.2, 141.7, 138.5, 131.9, 123.4, 112.9, 112.4, 112.0, 18.9, 8.0. HRMS (ESI+): m/z = 235.0964 $[M+H]^+$, calc.: 235.0965.



Scheme S2. Synthesis of sorrentanone (**12**).

2.4 Synthesis of 2,5-dimethylsorbicillin (**9**):

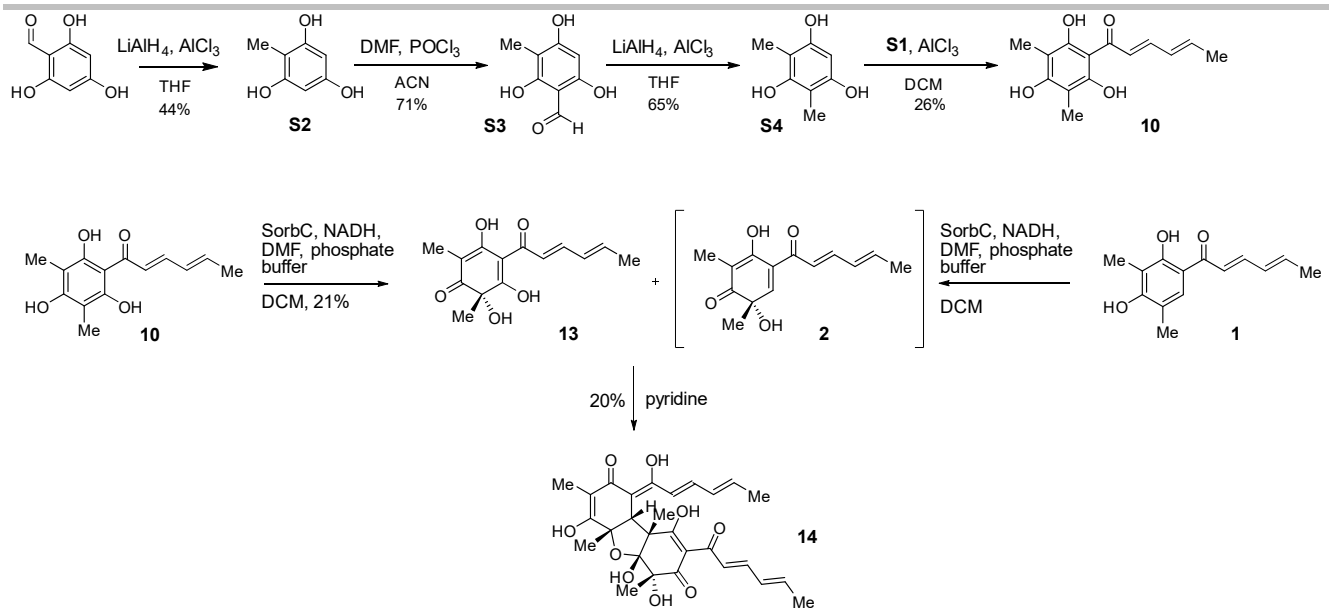
2,5-dimethylresorcinol (0.35 g, 2.5 mmol, 1.0 eq.) was dissolved in abs. dichloromethane (15 mL) under argon atmosphere and cooled to 0°C. Freshly produced sorbic acid chloride (**S1**) (0.3 mL, 2.7 mmol, 1.1 eq.) together with aluminum chloride (0.4 g, 2.7 mmol, 1.1 eq.) were added. After 1 hour of stirring at room temperature, the reaction mixture was acidified with 1 N HCl and extracted with dichloromethane (3 x 50 mL). The combined organic phases were dried over magnesium sulfate and the solvent was evaporated under reduced pressure. Purification by MPLC (retention time: 7.0-9.0 min) gave bright yellow crystals (0.4 g, 1.7 mmol, 68%). ^{1}H NMR (500 MHz, MeOD) δ= 7.20 (dd, *J* = 15.0, 10.4 Hz, 1H), 6.64 (d, *J* = 15.0 Hz, 1H), 6.40-6.25 (m, 2H), 6.24 (s, 1H), 2.35 (s, 3H), 2.00 (s, 3H), 1.88 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (125 MHz, MeOD) δ= 195.5, 163.0, 158.3, 143.6, 141.1, 138.0, 130.7, 128.6, 116.5, 110.6, 108.7, 23.9, 19.0, 7.7. HRMS (ESI+): *m/z* = 233.1172 $[M+H]^+$, calc.: 233.1172.

2.5 Enzymatic Synthesis of sorrentanone (**12**):

2,5 dimethylsorbicillin (**9**) (20.0 mg, 86.2 µmol, 1.0 eq.) dissolved in acetone (4 mL) was added to phosphate buffer (20 mL, 50 mM, pH = 8) with the enzyme SorbC (3.0 mL, 8.15 mg/mL in Tris-buffer). The reaction was started by addition of NADH (50.0 mg, 0.1 mmol, 1.3 eq.) and incubated for 120 min at room temperature. The reaction mixture was washed with dichloromethane (3 x 50 mL), then the aqueous and the organic phase were separately evaporated under reduced pressure. Purification by preperative HPLC (retention time: 19.0 min). The substrate 2,5-dimethylsorbicillin (**9**) was re-isolated in 34% yield (6.8 mg, 29.3 µmol), based on which the desired product **12** was obtained in 29% yield (4.1 mg, 16.6 µmol, 19% without re-isolation of **9**). ^{1}H NMR (500 MHz, MeOD) δ= 7.11 (dd, *J* = 15.7, 9.9 Hz, 1H), 6.37 – 6.34 (m, 2H), 6.19 (d, *J* = 15.7 Hz, 1H), 1.89 – 1.85 (m, 9H). ^{13}C NMR (75 MHz, MeOD) δ= 196.3, 188.0, 184.3, 155.1, 150.2, 144.4, 143.5, 138.6, 131.6, 129.3, 117.7, 19.1, 12.3, 7.7. HRMS (ESI+): m/z = 247.0964 $[M+H]^+$, calc.: 247.0965. The physical and spectroscopic data were in agreement with that described in the literature (Solvent not mentioned).[4]

2.5b Enzymatic Synthesis of sorrentanone (**12**) with cofactor recycling using Gdh:

2,5 dimethylsorbicillin (**9**) (20.0 mg, 86.2 µmol, 1.0 eq.), dissolved in acetone (4 mL) was added to phosphate buffer (20 mL, 50 mM, pH = 8) with the enzyme SorbC (3.0 mL, 7.6 mg/mL in Tris-buffer). Glucose (7.2 mg, 40.0 µmol) and the enzyme Gdh (0.261 mL, 3.0 mg/mL in Tris-buffer) were added and the reaction was started by addition of NADH (12.1 mg, 18.2 µmol, 0.2 eq.) and incubated for 120 min at room temperature. The reaction mixture was washed with dichloromethane (3 x 50 mL), then the aqueous and the organic phase were separately evaporated under reduced pressure. Purification by preperative HPLC (retention time: 19.0 min). The substrate 2,5-dimethylsorbicillin (**9**) was re-isolated in 35% yield (7.0 mg, 30.2 µmol), based on which the desired product **12** was obtained in 28% yield (3.8 mg, 15.5 µmol, 18% without re-isolation of **9**).



Scheme S3. Synthesis of hydroxysorbicillinol (**10**), oxosorbicillinol (**13**) and bisvertinolone (**14**).

2.6 Synthesis of 2-methylbenzene-1,3,5-triol (**S2**):

2,4,6-Trihydroxybenzaldehyde (2.8 g, 18.2 mmol, 1.0 eq.) was dissolved in abs. THF (170 mL) under argon atmosphere and cooled to 0°C. Aluminum chloride (6.1 g, 45.7 mmol, 2.5 eq.) together with lithiumaluminumhydride (1.7 g, 45.7 mmol, 2.5 eq) were added. After 3 hour of stirring at 78°C (reflux), the reaction mixture was cooled to 0°C, acidified with 1 N HCl and extracted with diethyl ether (3 x 200 mL). The combined organic phases were dried over magnesium sulfate and the solvent was evaporated under reduced pressure. Purification by column chromatography (EtOAc: pentane 1:1, R_f = 0.5) gave **S2** as a white powder (1.14 g, 8,0 mmol, 44%). ^{1}H NMR (300 MHz, MeOD) δ= 5.86 (s, 2H), 1.93 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ= 157.6, 156.6, 103.5, 95.4, 7.9. MS (ESI+): *m/z* = 140.93 $[M+H]^+$. The physical and spectroscopic data were in agreement with that described in the literature.[5]

2.7 Synthesis 2,4,6-trihydroxy-3-methylbenzaldehyd (**S3**):

DMF (0.9 mL, 11.6 mmol, 1.5 eq.) was mixed with abs. acetonitrile (2.7 mL) under argon atmosphere and a solution of phosphoroxychloride (0.9 mL, 9.9 mmol, 1.2 eq.) in acetonitrile (0.9 mL) was added. After 30 min of stirring at room temperature, the reaction mixture was cooled to 0°C and 2-methylbenzene-1,3,5-triol (**S2**) (1.1 g, 7.6 mmol, 1.0 eq.) in acetonitrile (4 mL) was added slowly. The reaction was completed after stirring for 1 hour at rt. The solid was filtered, recrystallized from water and **S3** was obtained as a white solid (0.9 g, 5.4 mmol, 71%). ^{1}H NMR (500 MHz, MeOD) δ= 10.00 (s, 1H), 5.87 (s, 1H), 1.90 (s, 3H). ^{13}C NMR (125 MHz, MeOD) δ= 192.6, 166.6, 163.7, 162.6, 106.2, 103.4, 94.4, 6.7. HRMS (ESI+): m/z = 169.0495 $[M+H]^+$, calc.: 169.0495.

2.8 Synthesis of 2,4-dimethylbenzene-1,3,5 triol (**S4**):

2,4,6-trihydroxy-3-methylbenzaldehyd (**S3**) (0.16 g, 1.0 mmol, 1.0 eq.) was dissolved in abs. THF (10 mL) under argon atmosphere and cooled to 0°C. Aluminum chloride (0.33 g, 2.5 mmol, 2.5 eq.) together with lithiumaluminumhydride (93.0 mg, 2.5 mmol, 2.5 eq) were added. After 3 hour of stirring at 78°C (reflux), the reaction mixture was cooled to 0°C, acidified with 1 N HCl and extracted with diethyl ether (3 x 50 mL). The combined organic phases were dried over magnesium sulfate and the solvent was evaporated under reduced pressure. Purification by column chromatography (EtOAc: pentane 1:1, R_f = 0.45) gave a white powder (0.1 g, 0.65 mmol, 65%). ^{1}H NMR (300 MHz, dmso-d_6) δ= 8.59 (bs, 2H), 7.73 (bs, 1H), 5.92 (s, 1H), 1.87 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 154.1, 153.2, 101.6, 94.5, 8.7. MS (ESI+): *m/z* = 154.96 $[M+H]^+$. The physical and spectroscopic data were in agreement with that described in the literature.[6]

2.9 Synthesis of 6-hydroxysorbicillin (**10**):

2,4-dimethylbenzene-1,3,5 triol (**S4**) (0.7 g, 1.9 mmol, 1.0 eq.) was dissolved in abs. dichloromethane (20 mL) under argon atmosphere and cooled to 0°C. Freshly produced sorbic acid chloride (**S1**) (0.23 mL, 2.1 mmol, 1.1 eq.) and aluminum chloride (0.3 g, 2.1 mmol, 1.1 eq.) were added. After 1 hour of stirring at room temperature, the reaction mixture was acidified with 1 N HCl and extracted with dichloromethane (3 x 100 mL). The combined organic phases were dried over magnesium sulfate and the solvent was evaporated under reduced pressure. Purification by MPLC (retention time: 7.0-9.0 min) gave orange crystals (125.0 mg, 0.5 mmol, 26%). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 7.41 (dd, *J* = 15.0, 10.0 Hz, 1H), 7.22 (d, *J* = 15.3 Hz, 1H), 6.35-6.19 (m, 2H), 2.08 (s, 6H), 1.89

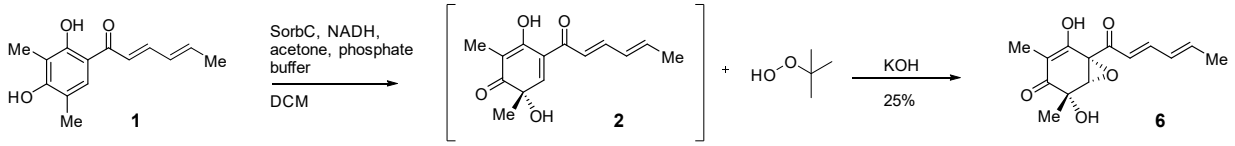
(d, *J* = 6.2 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 193.5, 158.6, 158.1, 144.3, 140.7, 131.0, 128.2, 105.6, 101.4, 19.0, 7.6. HRMS (ESI+): *m/z* = 249.1121 $[M+H]^+$, calc.: 249.1121.

2.10 Enzymatic Synthesis of oxosorbicillinol (**13**):

6-Hydroxysorbicillin (**10**) (20.0 mg, 80.3 µmol, 1.0 eq.), dissolved in acetone (3 mL) was added to phosphate buffer (20 mL, 50 mM, pH = 8) with the enzyme SorbC (2.0 mL, 8.15 mg/mL in Tris-buffer). The reaction was started by addition of NADH (50.0 mg, 0.1 mmol, 1.3 eq.) and incubated for 120 min at room temperature. The reaction mixture was extracted with dichloromethane (3 x 150 mL), the combined organic phases were dried over magnesium sulfate and the solvent was evaporated under reduced pressure. Purification by preperative HPLC (retention time: 22.0 min). The substrate 6-hydroxysorbicillin (**10**) was re-isolated in 23% yield (4.6 mg, 18.5 µmol), based on which the desired product oxosorbicillinol (**13**) was obtained in 21 % yield (3.4 mg, 13.0 µmol, 16% without re-isolation of **10**). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 7.55 (dd, *J* = 15.1, 10.7 Hz, 1H), 7.29 (d, *J* = 15.2 Hz, 1H), 6.48 – 6.22 (m, 2H), 1.91 (d, *J* = 6.5 Hz, 3H), 1.85 (s, 3H), 1.58 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 196.4, 192.4, 184.5, 167.6, 146.0, 142.1, 131.3, 122.5, 106.4, 104.6, 75.4, 30.7, 19.2, 7.3. HRMS (ESI+): m/z = 265.1069 $[M+H]^+$, calc.: 265.1070. $[\alpha]_D$= -28.0 (c = 0.3 in MeOH). The physical and spectroscopic data were in agreement with that described in the literature.[7]

2.11 Enzymatic synthesis of bisvertinolone (**14**):

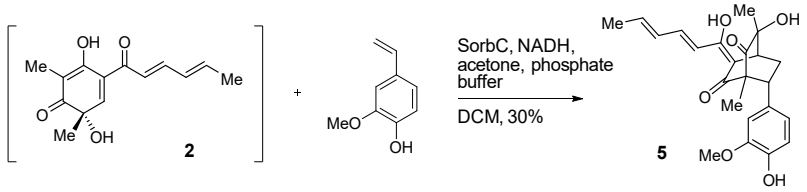
Sorbicillin (**1**) (20.0 mg, 86.2 µmol, 1.0 eq.), dissolved in DMF (4 mL) was added to phosphate buffer (40 mL, 50 mM, pH = 8) with the enzyme SorbC (3.0 mL, 10.0 mg/mL in Tris buffer). The reaction was started by addition of NADH (75.0 mg, 113.9 µmol, 1.3 eq.) and incubated for 12 h at rt. The reaction mixture was extracted with dichloromethane (3 x 150 mL) and the solvent was quickly evaporated under reduced pressure until only DMF was left. Oxosorbicillinol (**13**) (23.1 mg, 86.2 µmol, 1.0 eq.) and pyridine (4 mL, in excess) were added and the reaction mixture was stirred at rt over night. The solvent was evaporated under reduced pressure and the crude product was purified by preperative HPLC (retention time: 27.0 min). The substrate sorbicillin (**1**) was re-isolated in 20% yield (4.0 mg, 17.2 µmol), based on which the desired product bisvertinolone (**14**) was obtained in 20 % yield (7.0 mg, 13.7 µmol, 16% without re-isolation of **1**). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 7.58 (dd, *J* = 15.1, 10.0 Hz, 1H), 7.40 (d, *J* = 15.1 Hz, 1H), 7.33 (dd, *J* = 14.8, 11.0 Hz, 1H), 6.45 – 6.28 (m, 4H), 6.18 – 6.09 (m, 1H), 4.61 (bs, 1H), 4.19 (bs, 1H), 3.76 (s, 1H), 1.92 (d, *J* = 5.4 Hz, 3H), 1.89 (d, *J* = 6.6 Hz, 3H), 1.49 (s, 3H), 1.47 (s, 3H), 1.46 (s, 3H), 1.39 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 200.0, 196.7, 191.4, 185.8, 170.4, 163.7, 148.7, 144.5, 139.8, 137.8, 131.4, 131.1, 121.9, 120.1, 111.1, 107.3, 104.2, 100.0, 79.9, 79.3, 59.9, 54.5, 25.8, 23.1, 19.4, 19.0, 18.8, 7.1. HRMS (ESI+): m/z 513.2117 $[M+H]^+$, calc.: 513.2119. $[\alpha]_D$= -804.2 (c = 0.5 in MeOH). The physical and spectroscopic data were in agreement with that described in the literature.[8]



Scheme S4. Synthesis of epoxysorbicillinol (**6**).

2.12 Enzymatic synthesis of (+)-epoxysorbicillinol (**6**):

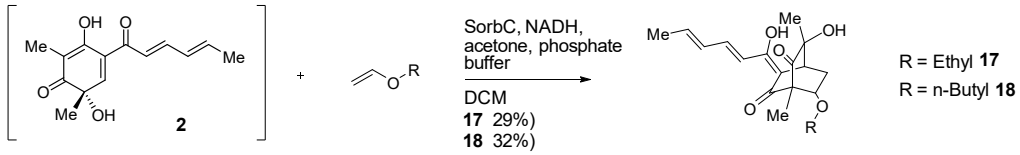
Sorbicillin (**1**) (80.0 mg, 344.8 µmol, 1.0 eq.) was dissolved in DMF (16 mL) and added to phosphate buffer (80 mL, 50 mM, pH = 8) with the enzyme SorbC (2.5 mL, 13.0 mg/mL in Tris buffer). The reaction was started by addition of NADH (300.0 mg, 451.5 µmol, 1.3 eq.) and incubated for 12 h at rt. The reaction mixture was extracted with dichloromethane (3 x 100 mL) and the solvent was quickly evaporated under reduced pressure until only DMF was left. 50 mg KOH were added and the reaction mixture was cooled to 0°C. *Tert*-butylhydroperoxide (5.5 M in nonan) (0.072 mL, 0.4 mmol, 1.1 eq.) was added and the reaction mixture was allowed to stir for 2h at 0°C. The reaction was quenched with 10 mL cold water and extracted with diethyl ether (3 x 100 mL). The organic phases were combined and the solvent was evaporated under reduced pressure. Purification by preperative HPLC (retention time: 16.0 min). The substrate sorbicillin (**1**) was re-isolated in 33% yield (26.4 mg, 113.8 µmol), based on which the desired product epoxysorbicillinol (**6**) was obtained in 25% yield (15.5 mg, 58.7 µmol, 17% without re-isolation of **1**). ^{1}H NMR (500 MHz, DMSO) δ= 7.16 (dd, *J* = 15.4, 10.3 Hz, 1H), 6.45 – 6.26 (m, 3H), 3.68 (s, 1H), 1.84 (d, *J* = 6.2 Hz, 3H), 1.56 (s, 3H), 1.43 (s, 3H). ^{13}C NMR (125 MHz, MeOD) δ= 194.5, 147.3, 144.4, 131.6, 124.5, 107.8, 70.4, 63.8, 62.8, 26.1, 19.0, 7.9. HRMS (ESI+): m/z 265.1070 $[M-H]^+$, calc.: 265.1070. $[\alpha]_D$= +29.7 (c = 0.3 in MeOH). The physical and spectroscopic data were in agreement with that described in the literature.[9]



Scheme S5. Synthesis of sorbicatechol A (**5**).

2.13 Enzymatic synthesis of sorbicatechol A (**5**):

Sorbicillin (**1**) (40.0 mg, 172.4 µmol, 1.0 eq.) was dissolved in acetone (8 mL) and added to phosphate buffer (40 mL, 50 mM, pH = 8) with the enzyme SorbC (1.75 mL, 13.0 mg/mL in Tris buffer). The reaction was started by addition of NADH (150.0 mg, 227.8 µmol, 1.3 eq.) and incubated for 12 h at rt. The reaction mixture was extracted with dichloromethane (3 x 100 mL), 4-ethenyl-2-methoxyphenol (72.3 mg, 482.0 µmol, 2.8 eq.) was added and the solvent was evaporated under reduced pressure, to increase the concentration of the dienophile slowly over time. Purification by preperative HPLC (retention time: 24.0 min). The substrate sorbicillin (**1**) was re-isolated in 43% yield (17.3 mg, 74.6 µmol), based on which the desired product sorbicatechol A (**5**) was obtained in 30 % yield (11.8 mg, 29.6 µmol, 17% without re-isolation of **1**). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 7.37 (dd, *J* = 15.0, 10.8 Hz, 1H), 6.78 (d, *J* = 8.1 Hz, 1H), 6.48 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.44 (d, *J* = 2.1 Hz, 1H), 6.37 – 6.17 (m, 3H), 3.78 (s, 3H), 3.29 (t, *J* = 2.8 Hz, 1H), 3.09 – 2.97 (m, 2H), 1.91 (d, *J* = 6.7 Hz, 3H), 1.86 -1.82 (m, 1H), 1.26 (s, 3H), 0.92 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 212.0, 198.0, 167.2, 146.6, 145.0, 142.6, 140.1, 133.3, 131.0, 121.6, 118.1, 114.4, 112.1, 110.4, 75.0, 65.2, 55.8, 47.8, 40.6, 31.5, 24.4, 19.1, 10.7. HRMS (ESI+): m/z 399.1802 $[M+H]^+$, calc.: 399.1802. $[\alpha]_D$= -62.0 (c = 1.2 in MeOH). The physical and spectroscopic data were in agreement with that described in the literature.[10]



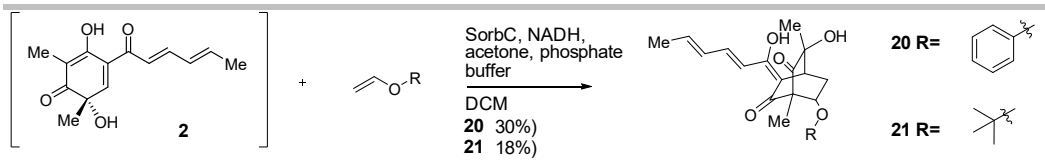
Scheme S6. Synthesis of rezishanone B (**18**) and C (**17**).

2.14 Enzymatic synthesis of rezishanone C (**17**):

Sorbicillin (**1**) (40.0 mg, 172.4 µmol, 1.0 eq.) was dissolved in DMF (8 mL) and added to phosphate buffer (40 mL, 50 mM, pH = 8) with the enzyme SorbC (1.75 mL, 13.0 mg/mL in Tris buffer). The reaction was started by addition of NADH (150.0 mg, 227.8 µmol, 1.3 eq.) and incubated for 12 h at rt. The reaction mixture was extracted with dichloromethane (3 x 100 mL) and the solvent was quickly evaporated under reduced pressure until only DMF was left. Ethyl vinyl ether (10 mL, 104.0 mmol, 603.2 eq. / in excess) was added and the reaction mixture was stirred at rt over night. The solvent was evaporated under reduced pressure. Purification by preperative HPLC (retention time: 24.5 min). The substrate sorbicillin (**1**) was re-isolated in 32% yield (13.0 mg, 56.0 µmol), based on which the desired product rezishanone C (**17**) was obtained in 29 % yield (10.8 mg, 33.8 µmol, 20% without re-isolation of **1**). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 7.29 (dd, *J* = 14.9, 10.8 Hz, 1H), 6.33 – 6.09 (m, 3H), 3.59 – 3.51 (m, 2H), 3.36 (dd, *J* = 9.6, 7.0 Hz, 1H), 3.15 (t, *J* = 3.0 Hz, 1H), 2.78 (ddd, *J* = 13.8, 8.4, 2.6 Hz, 1H), 1.89 (d, *J* = 6.8 Hz, 3H), 1.68 (dt, *J* = 13.8, 3.0 Hz, 1H), 1.31 (s, 3H), 1.20 (s, 3H), 1.12 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 211.1, 196.6, 166.5, 142.0, 139.4, 131.0, 118.1, 110.5, 79.3, 74.7, 67.1, 65.8, 39.9, 30.8, 24.5, 19.0, 15.2, 9.1. HRMS (ESI+): m/z 321.1695 $[M+H]^+$, calc.: 321.1696. $[\alpha]_D$= +285.7 (c = 0.7 in MeOH). The physical and spectroscopic data were in agreement with that described in the literature.[11]

2.15 Enzymatic synthesis of rezishanone B (**18**):

Sorbicillin (**1**) (40.0 mg, 172.4 µmol, 1.0 eq.) was dissolved in DMF (8 mL) and added to phosphate buffer (40 mL, 50 mM, pH = 8) with the enzyme SorbC (1.75 mL, 13.0 mg/mL in Tris buffer). The reaction was started by addition of NADH (150.0 mg, 227.8 µmol, 1.3 eq.) and incubated for 12 h at rt. The reaction mixture was extracted with dichloromethane (3 x 100 mL) and the solvent was evaporated under reduced pressure until only DMF was left. *N*-butyl vinyl ether (5 mL, 38.9 mmol, 226.4 eq. / in excess) was added and the reaction mixture was stirred at rt over night. The solvent was evaporated under reduced pressure. Purification by preperative HPLC (retention time: 28.0 min). The substrate sorbicillin (**1**) was re-isolated in 23% yield (9.0 mg, 38.8 µmol), based on which the desired product rezishanone B (**18**) was obtained in 32 % yield (15.0 mg, 43.1 µmol, 25% without re-isolation of **1**). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 7.29 (dd, *J* = 14.2, 10.0 Hz, 1H), 6.37 – 6.06 (m, 3H), 3.54 (dd, *J* = 8.3, 2.5 Hz, 1H), 3.48 (dt, *J* = 9.4, 6.4 Hz 1H), 3.32 – 3.23 (m, 1H), 3.15 (t, *J* = 3.0 Hz, 1H), 2.76 (ddd, *J* = 13.8, 8.3, 2.6 Hz, 1H), 1.89 (d, *J* = 6.7 Hz, 3H), 1.67 (dt, *J* = 13.7, 3.1 Hz, 1H), 1.51 – 1.41 (m, 2H), 1.32 (s, 3H), 1.35 – 1.25 (m, 2H), 1.20 (s, 3H), 0.86 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 211.1, 196.6, 166.4, 141.9, 139.3, 131.0, 118.2, 110.5, 79.6, 74.8, 70.1, 67.3, 39.9, 31.8, 30.6, 24.5, 19.4, 19.0, 14.0, 9.1. HRMS (ESI+): m/z 349.2008 $[M+H]^+$, calc.: 349.2010. $[\alpha]_D$= +253.4 (c = 0.7 in MeOH). The physical and spectroscopic data were in agreement with that described in the literature.[12]



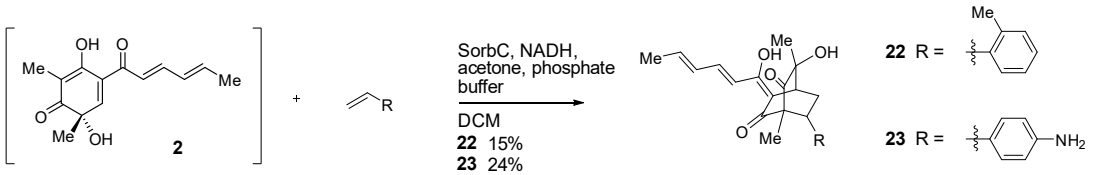
Scheme S7. Synthesis of rezishanone S1 (**20**) and rezishanone S2 (**21**).

2.16 Enzymatic synthesis of rezishanone S1 (**20**):

Sorbicillin (**1**) (40.0 mg, 172.4 µmol, 1.0 eq.) was dissolved in acetone (8 mL) and added to phosphate buffer (50 mL, 50 mM, pH = 8) with the enzyme SorbC (2.5 mL, 9.0 mg/mL in Tris buffer). The reaction was started by addition of NADH (150.0 mg, 227.8 µmol, 1.3 eq.) and incubated for 12 h at rt. The reaction mixture was extracted with dichloromethane (3 x 100 mL) and the solvent was evaporated under reduced pressure until only DMF was left. Phenyl vinyl ether (2.0 mL, 16.2 mmol, 94.4 eq. / in excess) was added and the reaction mixture was stirred at rt for 1 hour. The solvent was evaporated under reduced pressure. Purification by preperative HPLC (retention time: 27.0 min). The substrate sorbicillin (**1**) was re-isolated in 57% yield (23.0 mg, 99.1 µmol), based on which the desired product **20** was obtained in 30% yield (8.0 mg, 21.7 µmol, 13% without re-isolation of **1**). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 14.01 (s, 1H), 7.31 (dd, *J* = 14.9, 10.7 Hz, 1H), 7.26 – 7.22 (m, 2H), 6.95 (t, *J* = 7.4 Hz, 1H), 6.80 (d, *J* = 8.1 Hz, 2H), 6.29 – 6.14 (m, 3H), 4.42 (dd, *J* = 8.2, 2.2 Hz, 1H), 3.21 (t, *J* = 3.0 Hz, 1H), 3.01 (ddd, *J* = 14.2, 8.2, 2.5 Hz, 1H), 1.88 (d, *J* = 6.5 Hz, 3H), 1.79 (dt, *J* = 14.1, 2.9 Hz, 1H), 1.37 (s, 3H), 1.26 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 210.3, 195.9, 167.1, 157.0, 142.5, 139.8, 130.9, 129.7, 121.6, 117.9, 115.7, 110.2, 77.3, 74.7, 66.4, 39.9, 31.0, 24.5, 19.1, 9.2 . HRMS (ESI+): m/z 369.1695 $[M+H]^+$, calc.: 369.1697. $[\alpha]_D$= +232.6 (c = 0.8 in MeOH).

2.17 Enzymatic synthesis of rezishanone S2 (**21**):

Sorbicillin (**1**) (40.0 mg, 172.4 µmol, 1.0 eq.) was dissolved in acetone (8 mL) and added to phosphate buffer (50 mL, 50 mM, pH = 8) with the enzyme SorbC (2.5 mL, 9.0 mg/mL in Tris buffer). The reaction was started by addition of NADH (150.0 mg, 227.8 µmol, 1.3 eq.) and incubated for 12 h at rt. The reaction mixture was extracted with dichloromethane (3 x 100 mL) and the solvent was evaporated under reduced pressure until only DMF was left. *Tert*-butyl vinyl ether (2.0 mL, 15.2 mmol, 88.2 eq. / in excess) was added and the reaction mixture was stirred at rt for 1 hour. The solvent was evaporated under reduced pressure. Purification by preperative HPLC (retention time: 26.5 min). The substrate sorbicillin (**1**) was re-isolated in 35% yield (14.1 mg, 60.8 µmol), based on which the desired product **21** was obtained in 18% yield (7.0 mg, 20.1 µmol, 12% without re-isolation of **1**). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 14.00 (s, 1H), 7.31 (dd, *J* = 14.9, 10.7 Hz, 1H), 6.30 – 6.14 (m, 3H), 3.73 (dd, *J* = 8.5, 2.6 Hz, 1H), 3.11 (t, *J* = 3.0 Hz, 1H), 2.81 (ddd, *J* = 13.7, 8.5, 2.6 Hz, 1H), 1.89 (d, *J* = 6.8 Hz, 3H), 1.64 (dt, *J* = 13.7, 3.1 Hz, 1H), 1.24 (s, 3H), 1.19 (s, 3H), 1.11 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 211.8, 197.0, 166.2, 141.8, 139.2, 131.0, 118.2, 110.7, 74.7, 72.2, 66.9, 40.0, 34.8, 28.6, 28.5, 24.4, 19.0, 9.6. HRMS (ESI+): m/z 349.2011 $[M+H]^+$, calc.: 349.2010. $[\alpha]_D$= +145.3 (c = 0.7 in MeOH).



Scheme S8. Synthesis of derivatives **22** and **23**.

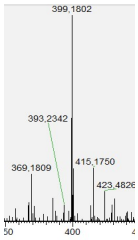
2.18 Enzymatic synthesis of derivative **22**:

Sorbicillin (**1**) (40.0 mg, 172.4 µmol, 1.0 eq.) was dissolved in acetone (8 mL) and added to phosphate buffer (50 mL, 50 mM, pH = 8) with the enzyme SorbC (3.0 mL, 8.2 mg/mL in Tris buffer). The reaction was started by addition of NADH (150.0 mg, 227.8 µmol, 1.3 eq.) and incubated for 12 h at rt. The reaction mixture was extracted with dichloromethane (3 x 100 mL) and the solvent was evaporated under reduced pressure until only DMF was left. 2-Methylstyrene (2.0 mL, 15.5 mmol, 89.7 eq. / in excess) was added and the reaction mixture was stirred at rt for 1 hour. The solvent was evaporated under reduced pressure. Purification by preperative HPLC (retention time: 27.0 min). The substrate sorbicillin (**1**) was re-isolated in 26% yield (10.2 mg, 44.0 µmol), based on which the desired product **22** was obtained in 15% yield (7.0 mg, 19.1 µmol, 11% without re-isolation of **1**). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 14.40 (s, 1H), 7.38 (dd, *J* = 15.0, 10.7 Hz, 1H), 7.15 – 7.08 (m, 3H), 6.86 (d, *J* = 7.4 Hz, 1H), 6.34 – 6.16 (m, 3H), 3.56 (dd, *J* = 10.7, 6.5 Hz, 1H), 3.29 (s, 3H), 3.01 (t, *J* = 10.6 Hz, 1H), 2.29 (s, 3H), 1.91 (d, *J* = 6.6 Hz, 3H), 1.77 (ddd, *J* = 13.5, 6.6, 2.5 Hz, 1H), 1.28 (s, 3H), 0.93 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 211.6, 198.0, 167.3, 142.6, 140.4, 139.9, 136.4, 131.0, 130.5, 127.1, 127.0, 127.0, 118.2, 112.2, 74.8, 65.1, 41.2, 40.5, 32.0, 24.5, 20.4, 19.1, 9.7. HRMS (ESI+): m/z 367.1902 $[M+H]^+$, calc.: 367.1904. $[\alpha]_D$= +86.8 (c = 0.7 in MeOH).

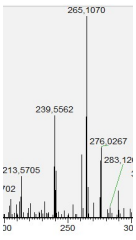
2.19 Enzymatic synthesis of derivative **23**:

Sorbicillin (**1**) (40.0 mg, 172.4 µmol, 1.0 eq.) was dissolved in acetone (8 mL) and added to phosphate buffer (50 mL, 50 mM, pH = 8) with the enzyme SorbC (3.0 mL, 8.2 mg/mL in Tris buffer). The reaction was started by addition of NADH (150.0 mg, 227.8 µmol, 1.3 eq.) and incubated for 12 h at rt. The reaction mixture was extracted with dichloromethane (3 x 100 mL) and the solvent was evaporated under reduced pressure until only DMF was left. 4-Aminostyrene (2.0 mL, 17.1 mmol, 99.2 eq. / in excess) was added and the reaction mixture was stirred at rt for 1 hour. The solvent was evaporated under reduced pressure. Purification by preperative HPLC (retention time: 17.0 min). The substrate sorbicillin (**1**) was re-isolated in 28% yield (11.0 mg, 47.4 µmol), based on which the desired product **23** was obtained in 24% yield (11.0 mg, 30.0 µmol, 17% without re-isolation of **1**). ^{1}H NMR (500 MHz, MeOD) δ= 7.39 (dd, *J* = 14.9, 11.0 Hz, 1H), 7.30 (d, *J* = 8.6 Hz, 2H), 7.17 (d, *J* = 8.6 Hz, 2H), 6.49 (d, *J* = 15.0 Hz, 1H), 6.43 (dd, *J* = 15.0, 11.0 Hz, 1H), 6.25 (dd, *J* = 14.8, 7.1 Hz, 1H), 3.36 – 3.33 (m, 1H), 3.29 (d, *J* = 6.2 Hz, 1H), 3.05 (ddd, *J* = 13.7, 10.6, 3.1 Hz, 1H),1.90 (d, *J* = 6.8 Hz ,3H), 1.82 (ddd, *J* = 13.5, 6.1, 2.7 Hz, 1H), 1.23 (s, 3H), 0.79 (s, 3H). ^{13}C NMR (125 MHz, MeOD) δ= 210.6, 199.1, 168.4, 161.7, 161.4, 144.7, 143.8, 140.6, 132.3, 131.3, 131.2, 124.2, 119.5, 113.6, 75.1, 65.8, 47.3, 42.2, 32.6, 23.9, 18.9, 11.3. HRMS (ESI+): m/z 368.1856 $[M+H]^+$, calc.: 368.1856. $[\alpha]_D$= -7.2 (c = 1.1 in MeOH).

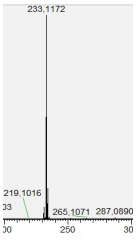
3. Screenshots of reported HR-MS analytical data



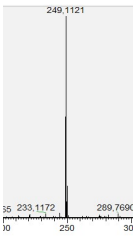
5



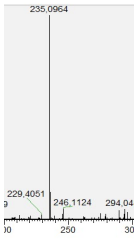
6



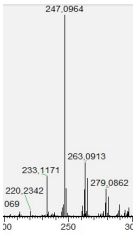
9



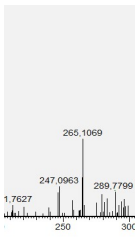
10



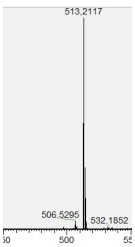
11



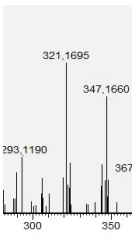
12



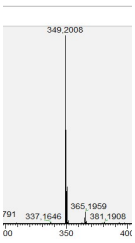
13



14

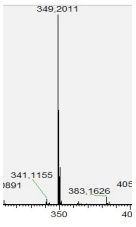


17

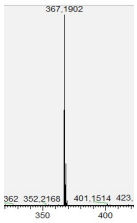


18

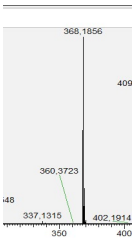
20



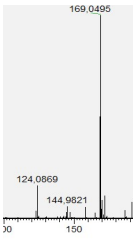
21



22



23



S3

4. Sorbicatechol A (5) – additional analytic data

4.1 CD- Spectra

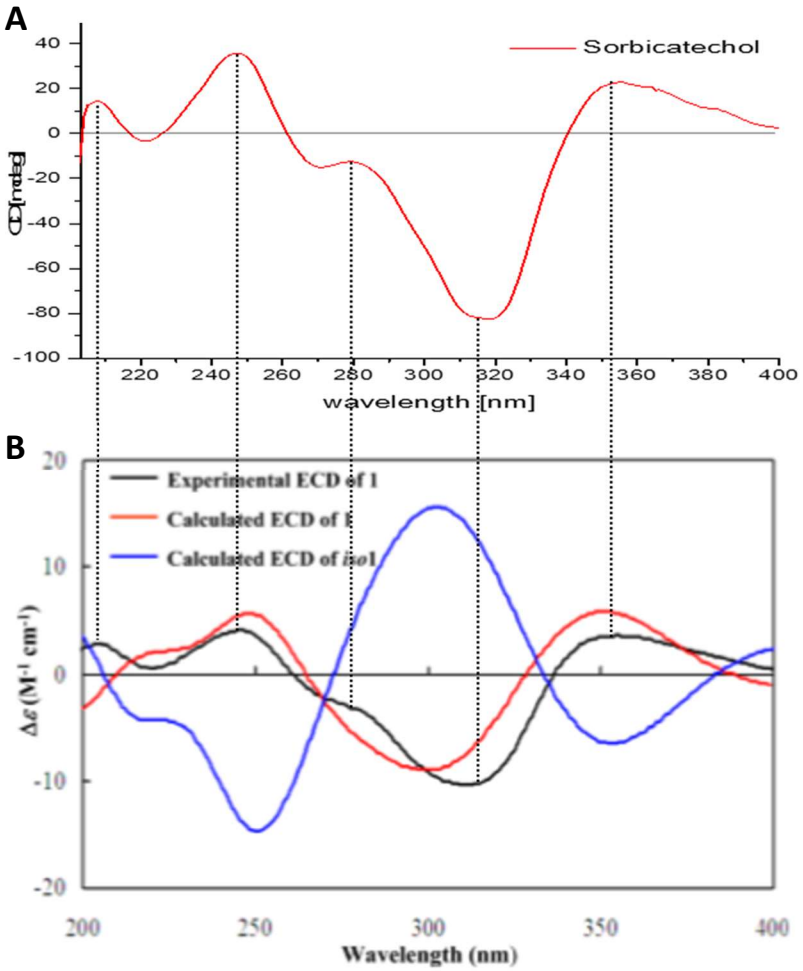


Figure S1. Comparison of ECD spectra (c = 0.88 mM in MeOH) of (**A**) synthetic sorbicatechol A (**5**) with (**B**) that provided in the literature[10] (black curve).

4.2 NMR- Comparison

Table S1. Comparison of the ^{13}C NMR data for sorbicatechol A (**5**) from the literature[10] to that of synthetic **5** prepared within this work. For better comparability of published and new NMR data, the $CDCl_3$ signal of the published NMR data was shifted from 7.0415 to 7.16 ppm (all NMR data in measured in $CDCl_3$ at 150 MHz and 125 MHz).

Signal	^{13}C, reference **5** [Lit]	^{13}C, synthetic **5**	Δ(ppm)
1	10.6	10.7	0.1
2	19.0	19.1	0.1
3	24.4	24.4	0
4	31.6	31.5	0.1
5	40.6	40.6	0
6	47.9	47.8	0.1
7	55.9	55.8	0.1
8	65.2	65.2	0
9	74.7	75.0	0.3
10	110.3	110.4	0.1
11	112.2	112.2	0
12	114.4	114.4	0
13	118.1	118.1	0
14	121.7	121.6	0.1
15	131.0	131.0	0
16	133.3	133.3	0
17	140.0	140.1	0.1
18	142.6	142.6	0
19	145.0	145.0	0
20	146.5	146.6	0.1
21	167.3	167.2	0.1
22	198.0	198.0	0
23	212.0	212.0	0

5. Determination of the stereoselectivity of the SorbC-catalyzed oxidation of 10 to 13

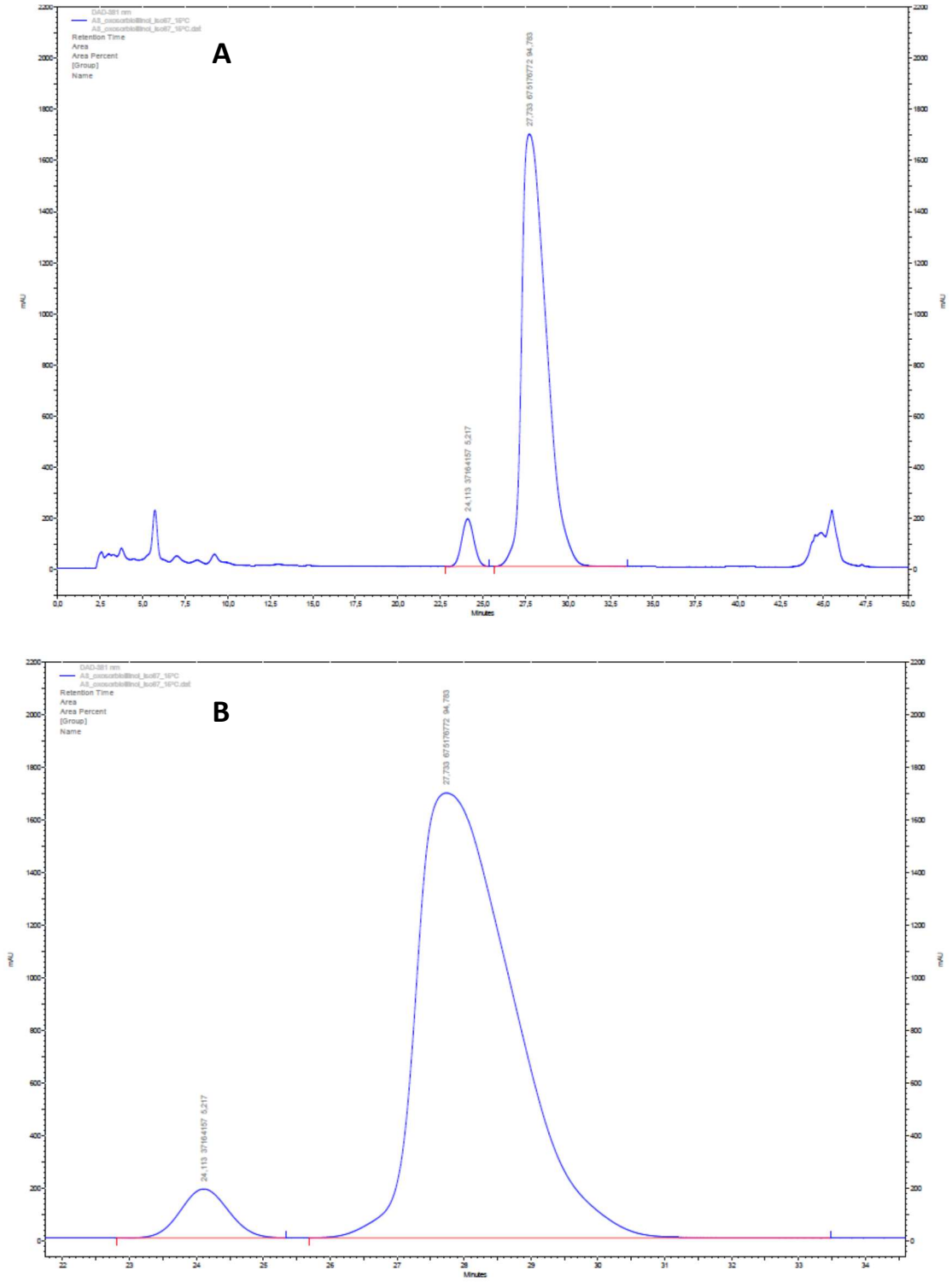
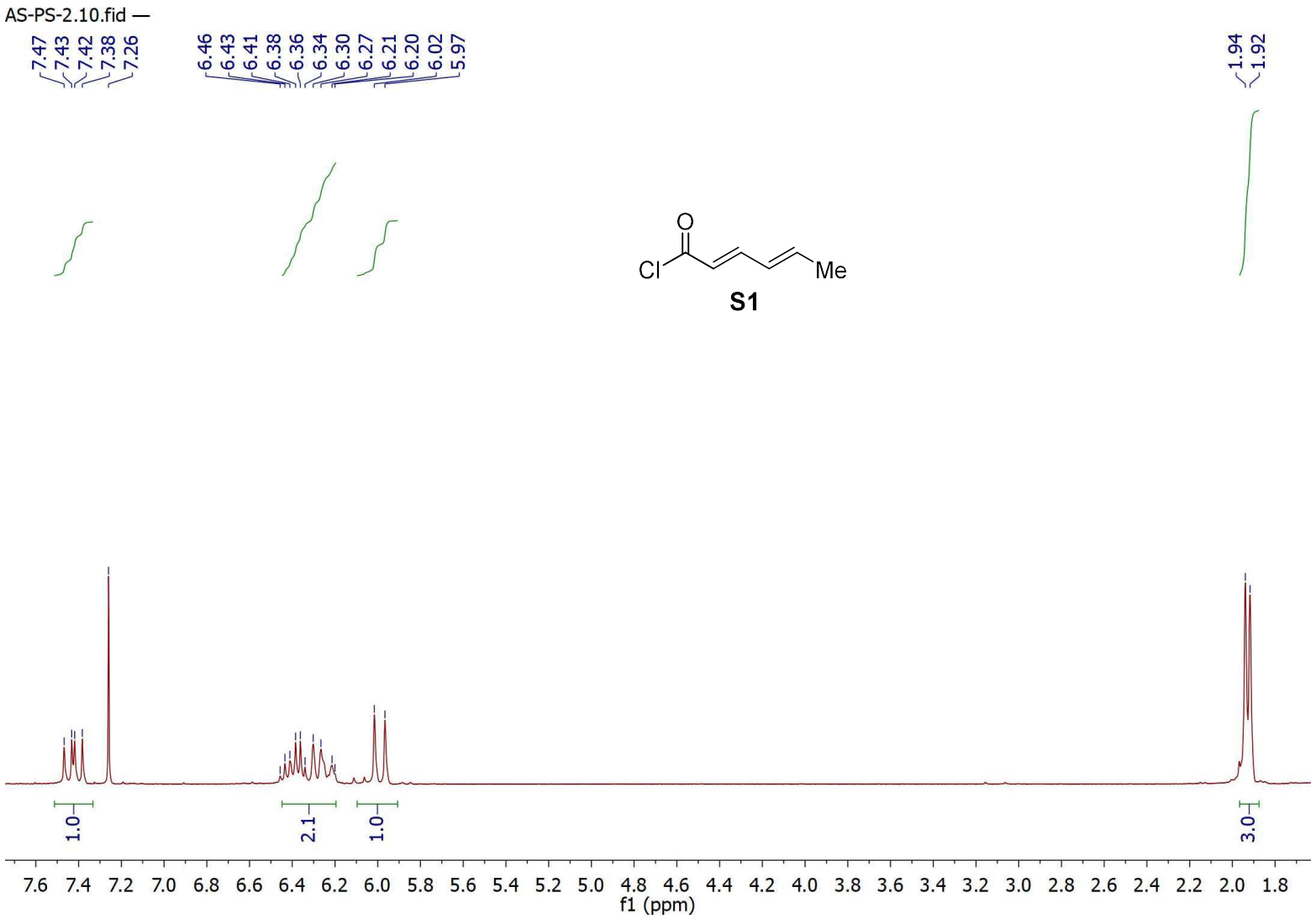
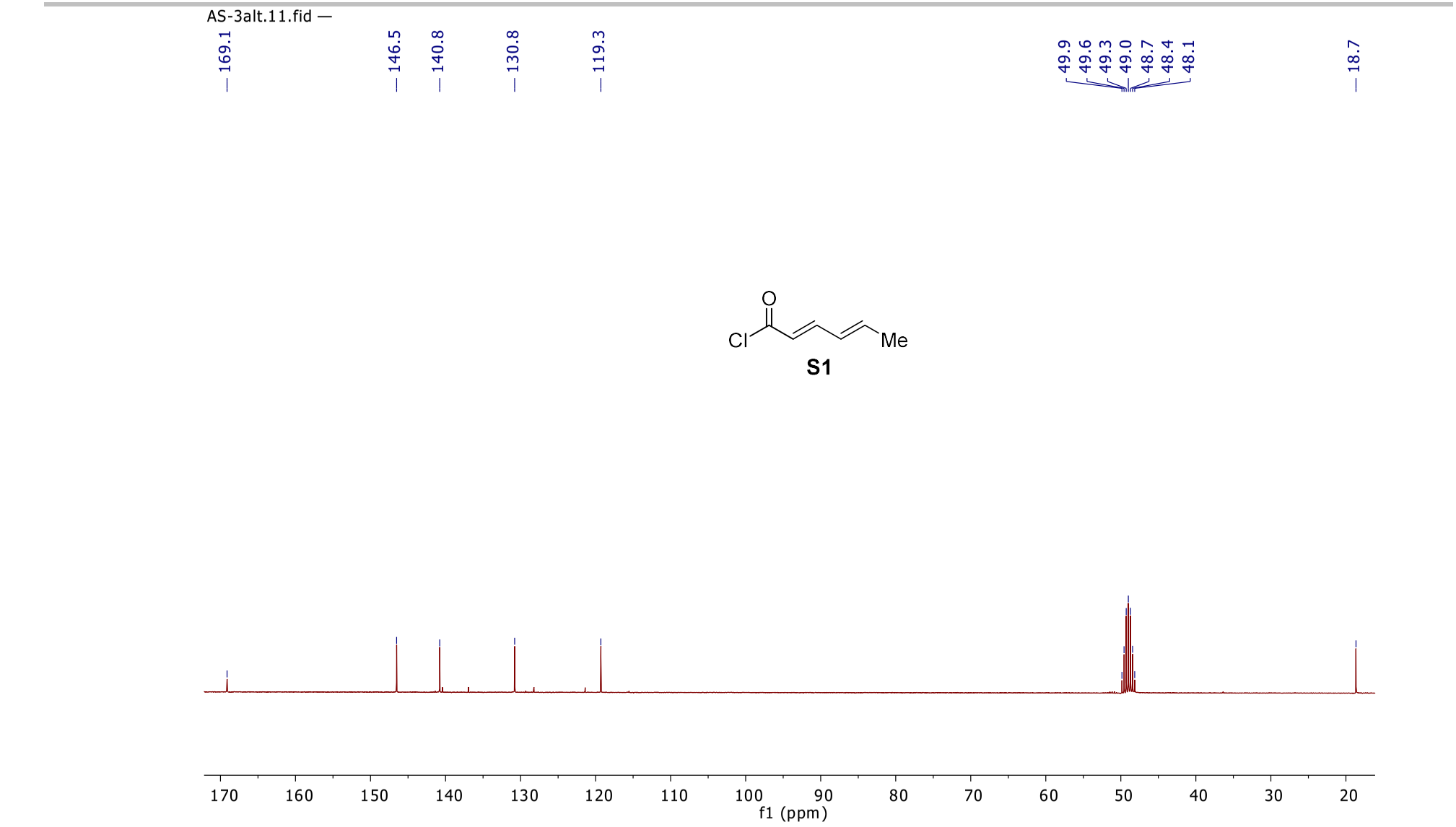


Figure S2. Determination of the enantiomeric ratio of oxosorbicillinol (**13**) obtained from SorbC-catalyzed oxidation of **10** by separation of the enantiomers by HPLC on a chiral phase. A: full chromatogram. B: zoom onto the signals corresponding to the separated enantiomers.

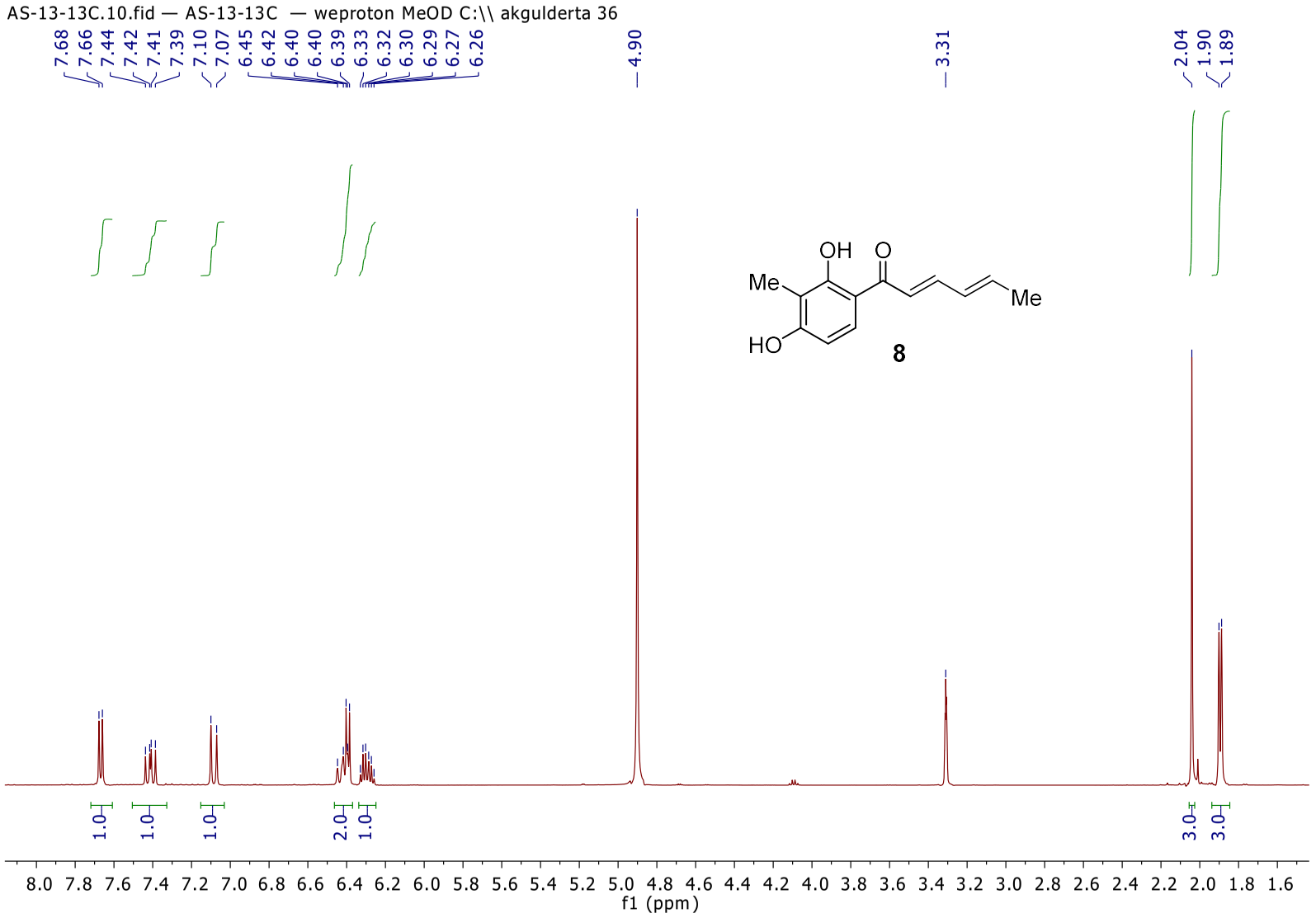
6 NMR Spectra

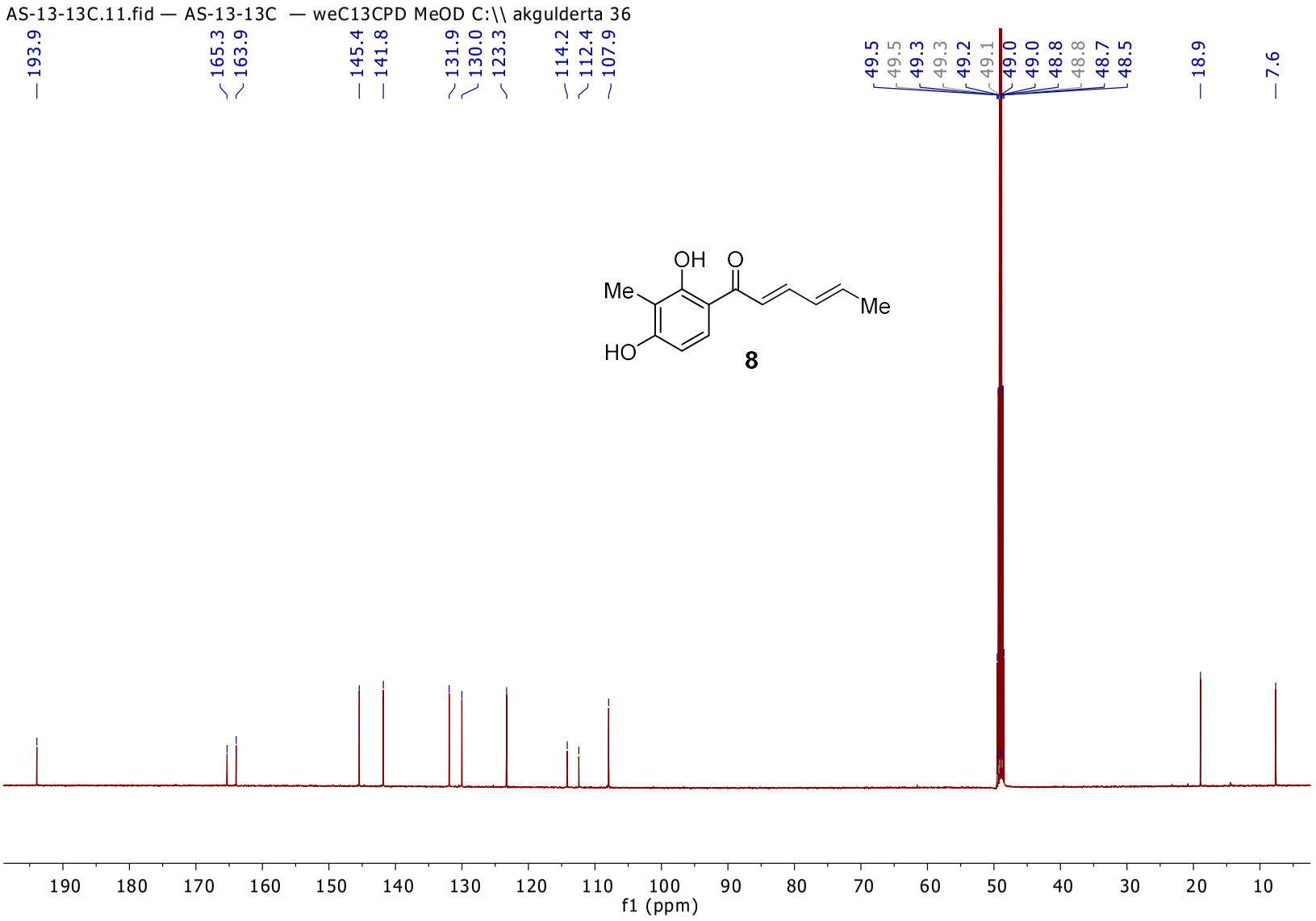
6.1 Sorbic acid chloride (**S1**)



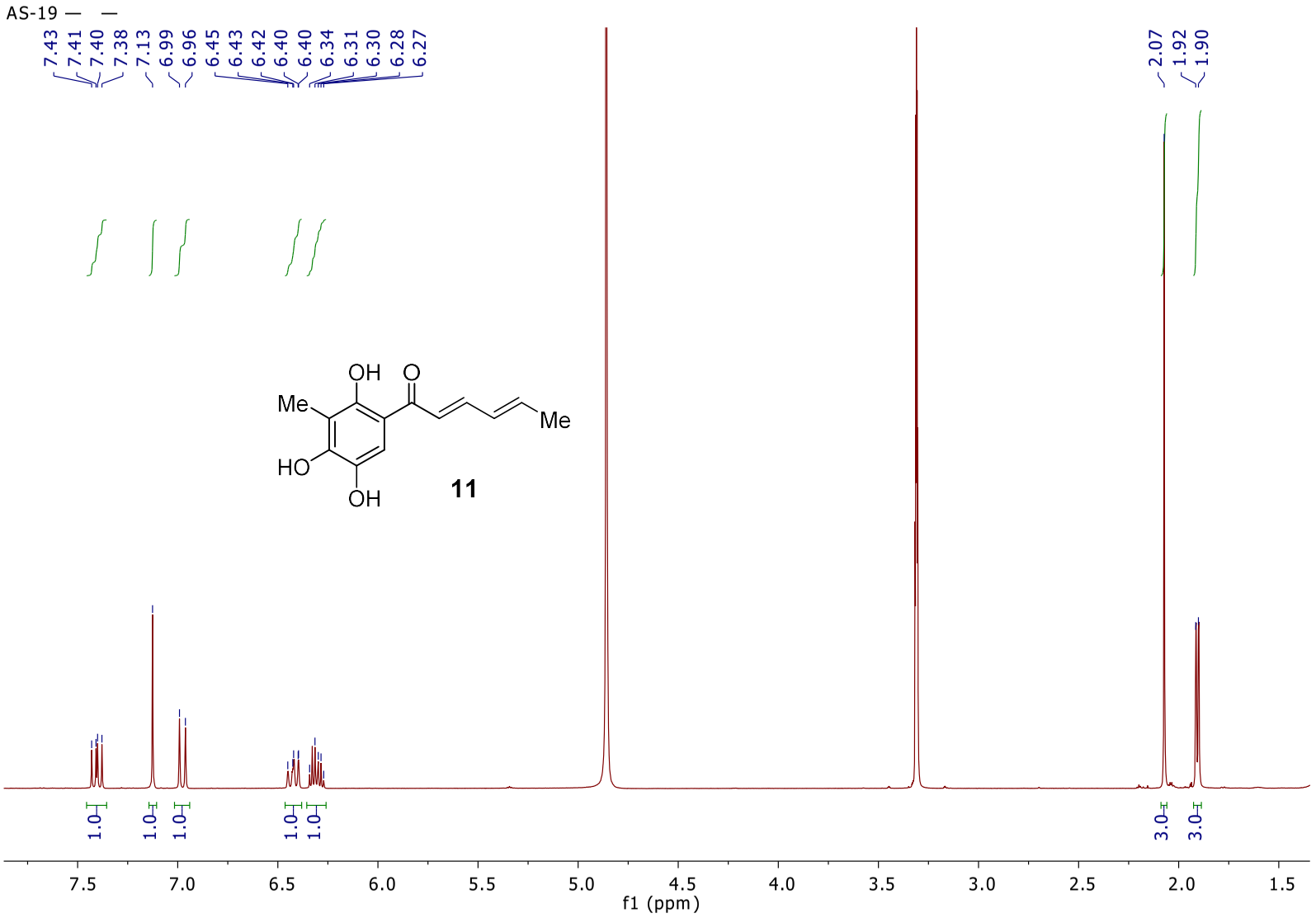

AS-3alt.11.fid
169.1
146.5
140.8
130.8
119.3
49.9
49.6
49.3
49.0
48.7
48.4
48.1
18.7
O
Cl
Me
S1
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

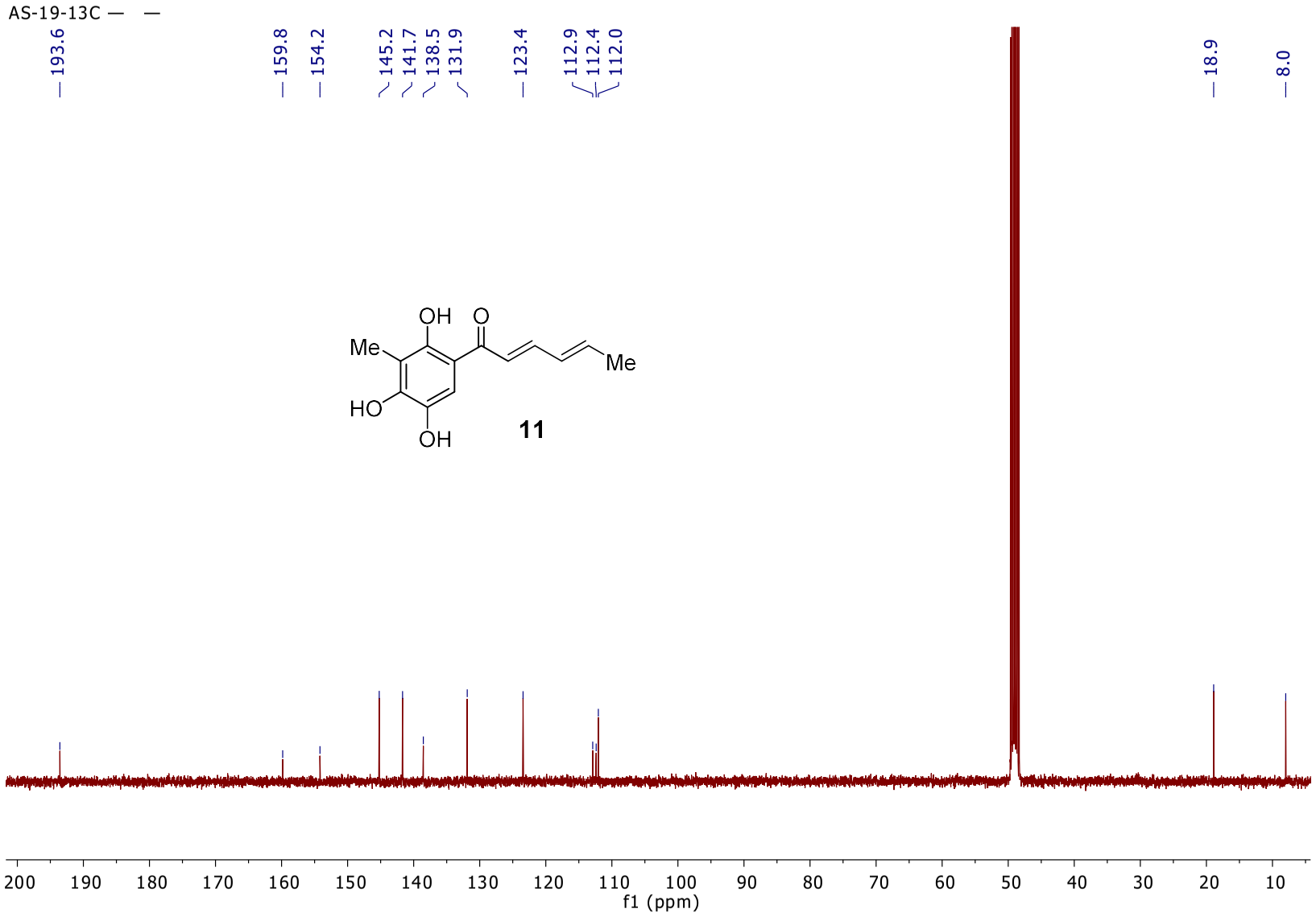
6.2 Demethylsorbicillin (**8**)



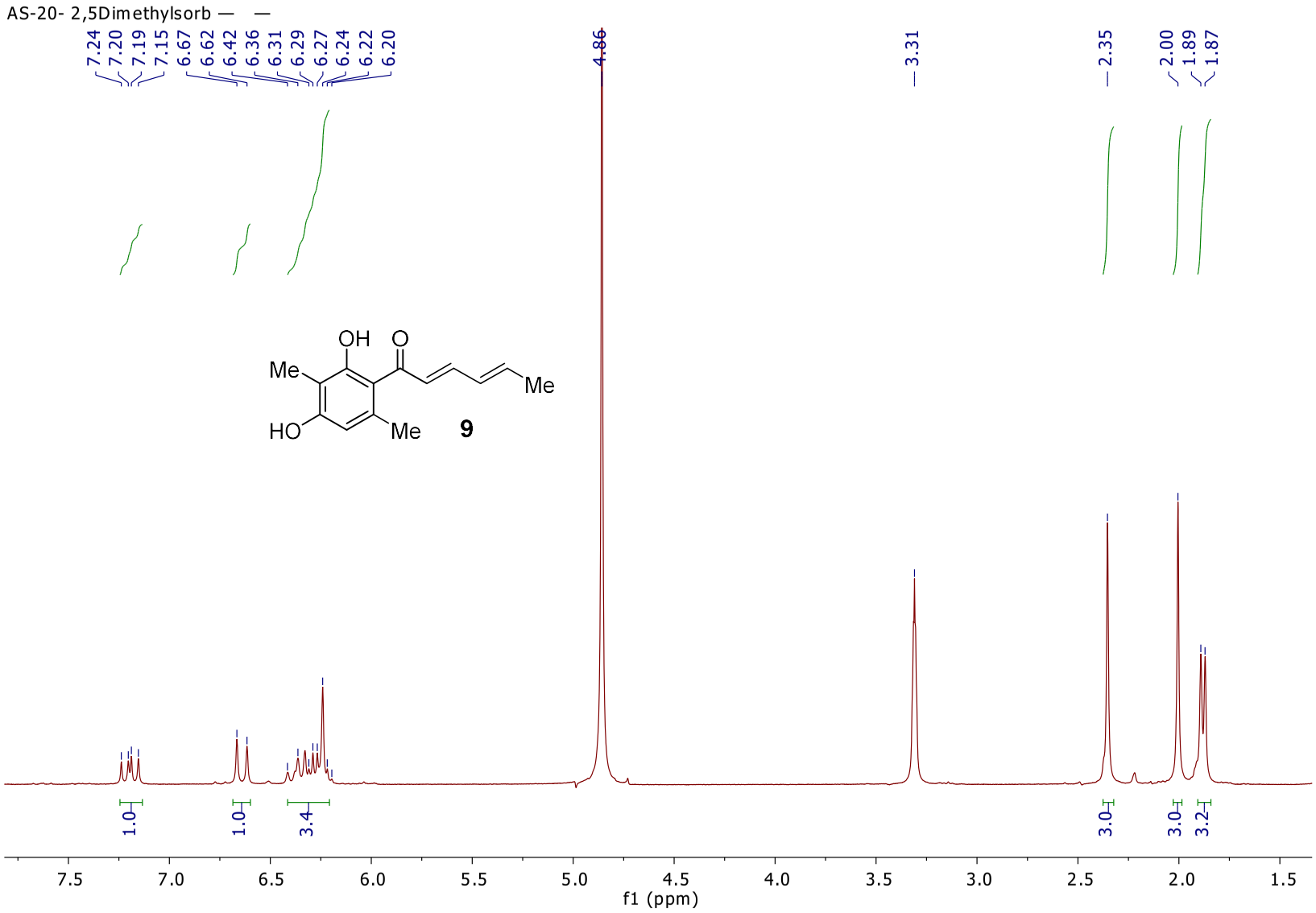

AS-13-13C.11.fid — AS-13-13C — weC13CPD MeOD C:\\ akgulderta 36
193.9
165.3
163.9
145.4
141.8
131.9
130.0
123.3
114.2
112.4
107.9
49.5
49.5
49.3
49.3
49.2
49.1
49.0
49.0
48.8
48.8
48.7
48.5
18.9
7.6
OH
O
Me
Me
HO
8
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

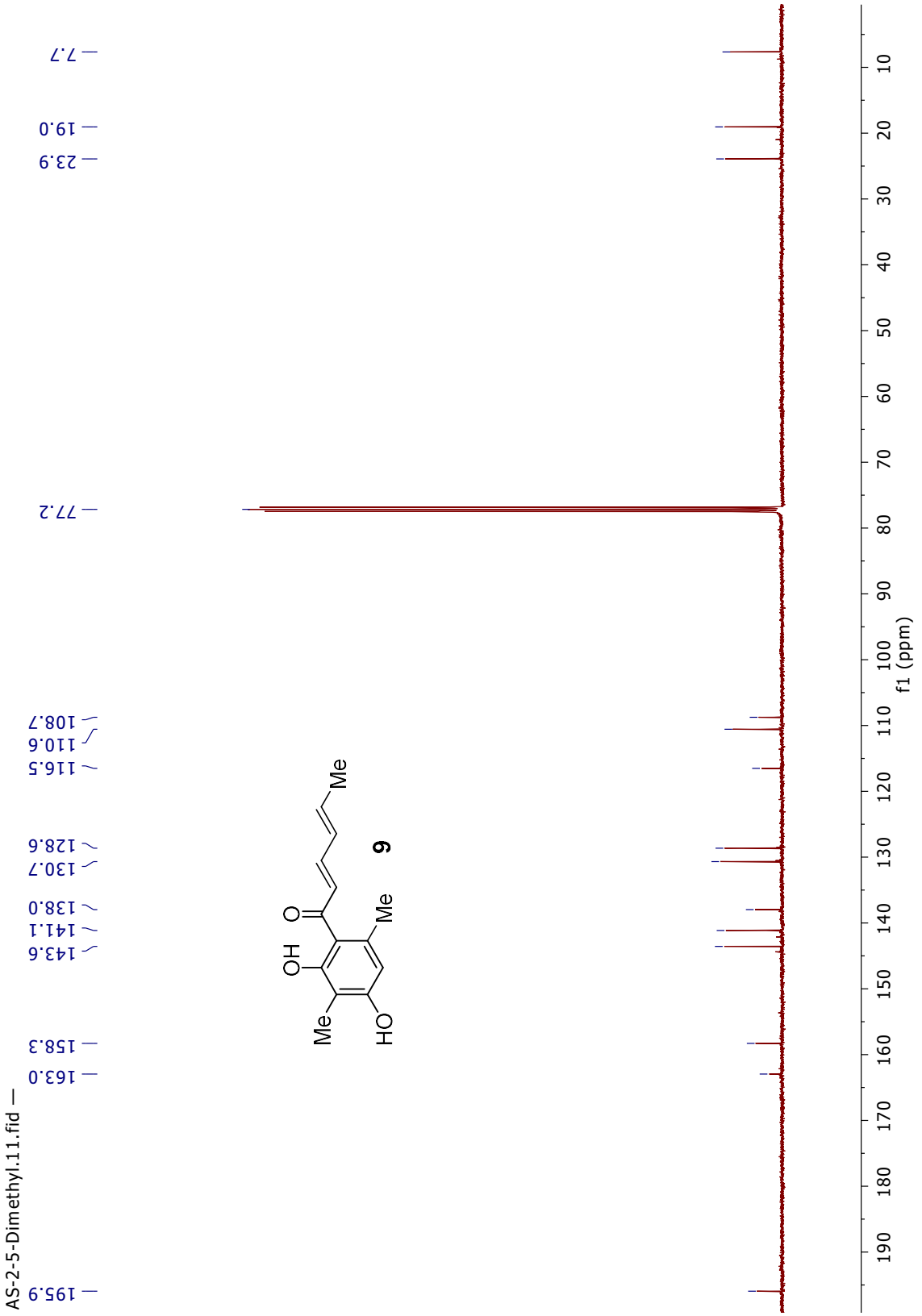
6.3 (2*E*,4*E*)-1-(2,4,5-Trihydroxy-3-methylphenyl)hexa-2,4-dien-1-one (**11**)



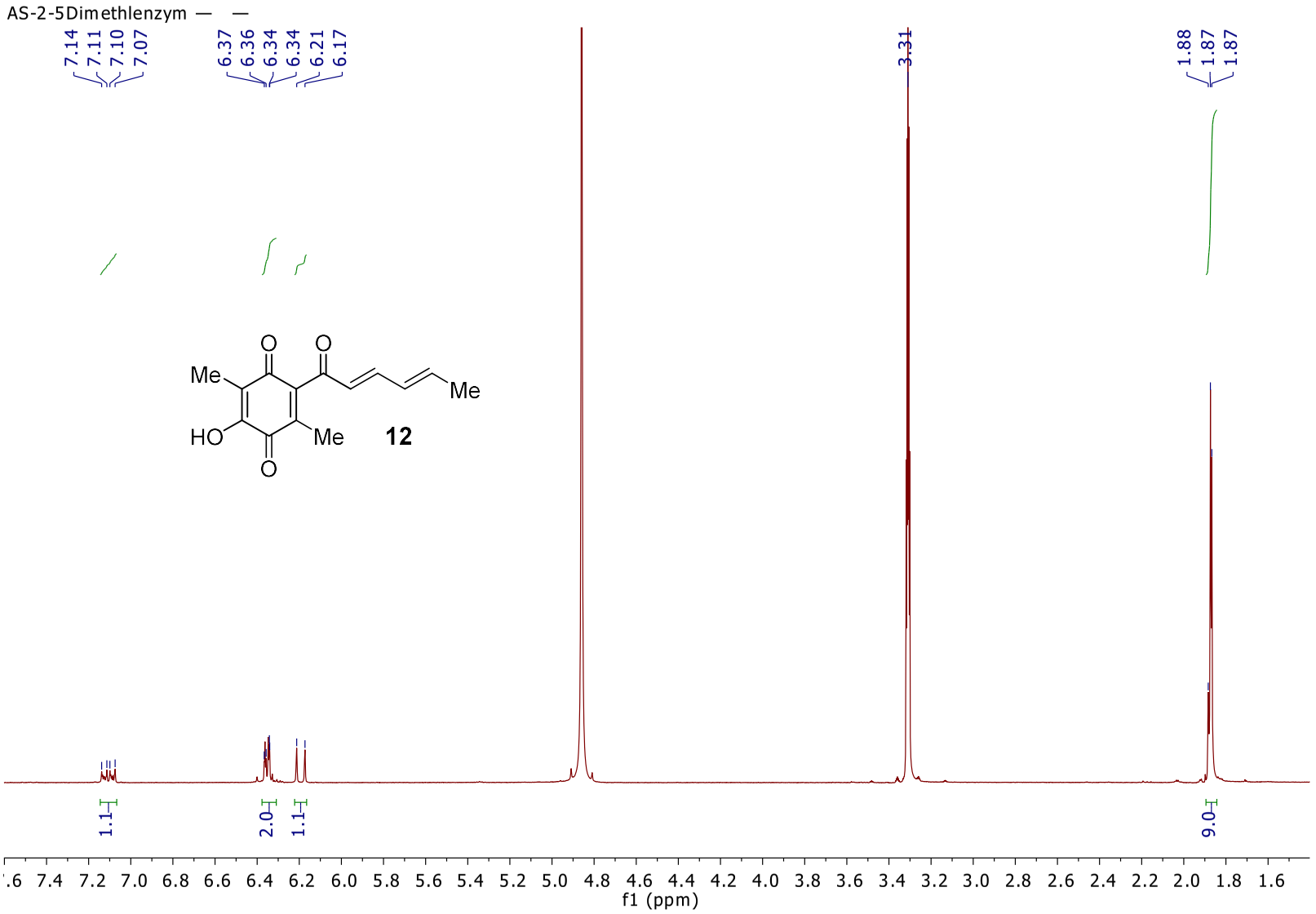

AS-19-13C
193.6
159.8
154.2
145.2
141.7
138.5
131.9
123.4
112.9
112.4
112.0
18.9
8.0
OH
O
Me
Me
HO
OH
11
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

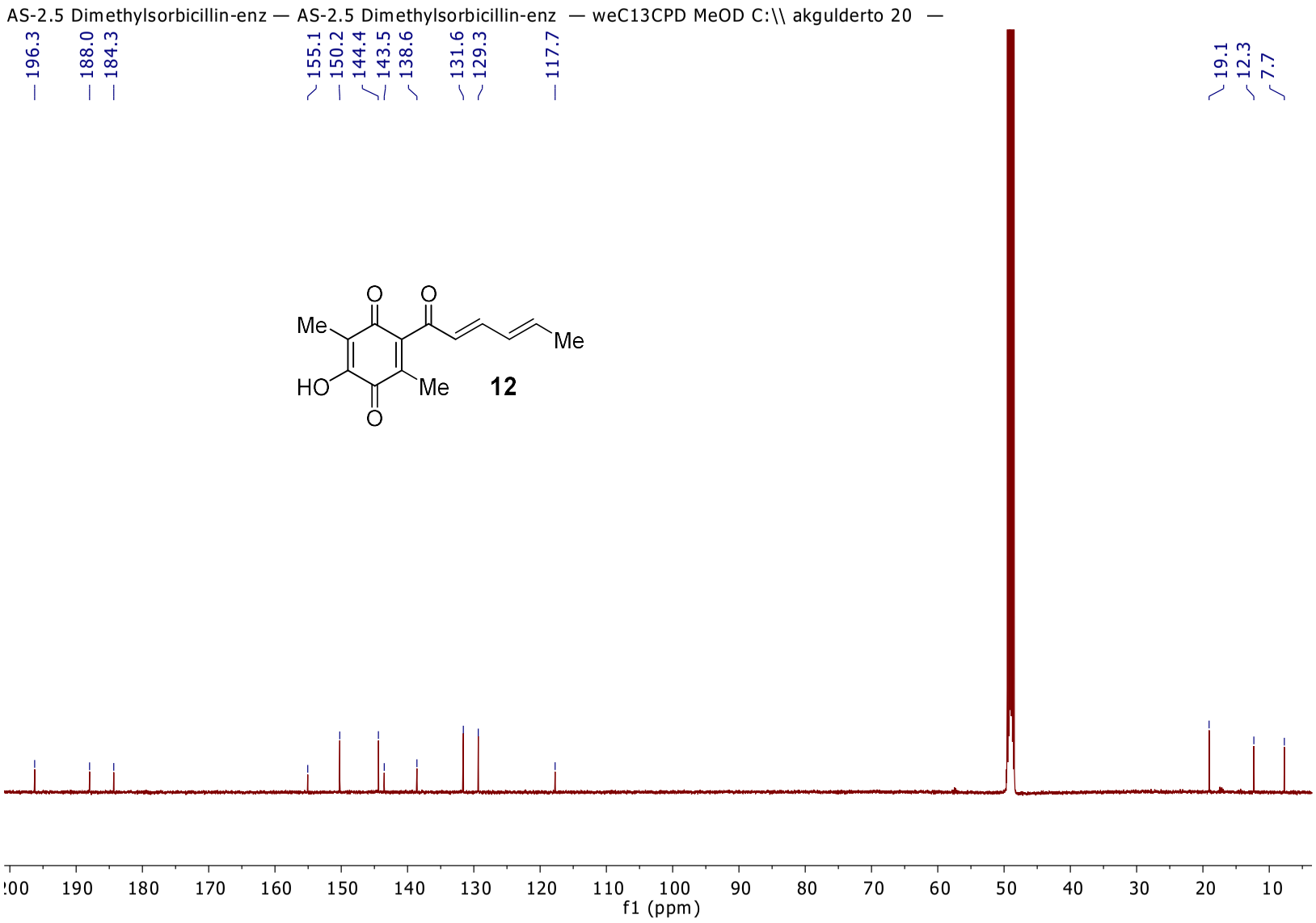
6.4 2,5-dimethylsorbicillin (**9**)



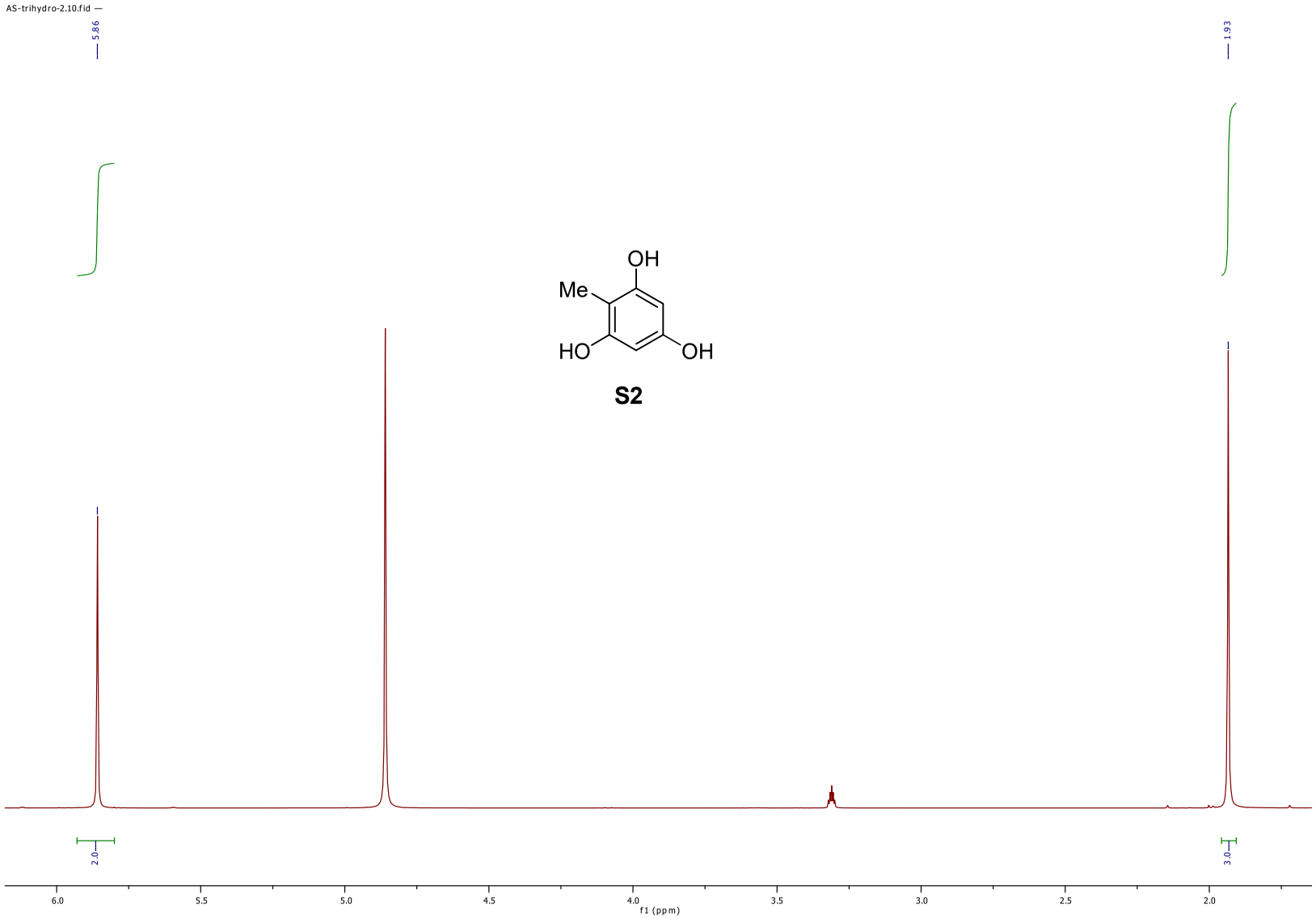

AS-2-5-Dimethyl.11.fid
195.9
163.0
158.3
143.6
141.1
138.0
130.7
128.6
116.5
110.6
108.7
77.2
23.9
19.0
7.7
OH
O
Me
Me
Me
HO
9
f1 (ppm)

6.5 Sorrentanone (**12**)

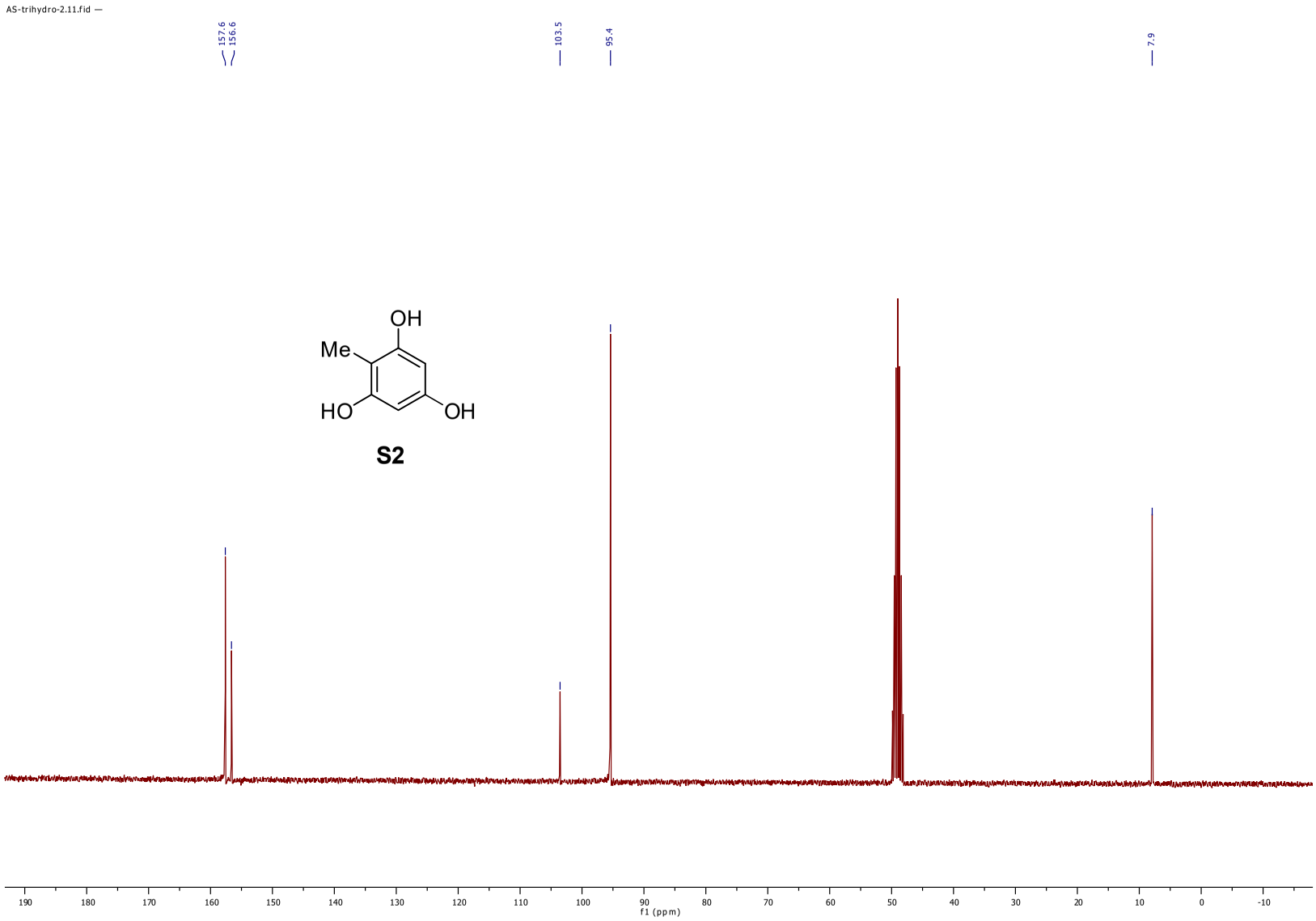



AS-2.5 Dimethylsorbicillin-enz — AS-2.5 Dimethylsorbicillin-enz — weC13CPD MeOD C:\\ akgulderto 20 —
196.3
188.0
184.3
155.1
150.2
144.4
143.5
138.6
131.6
129.3
117.7
19.1
12.3
7.7
Me
Me
HO
Me
O
O
O
12
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

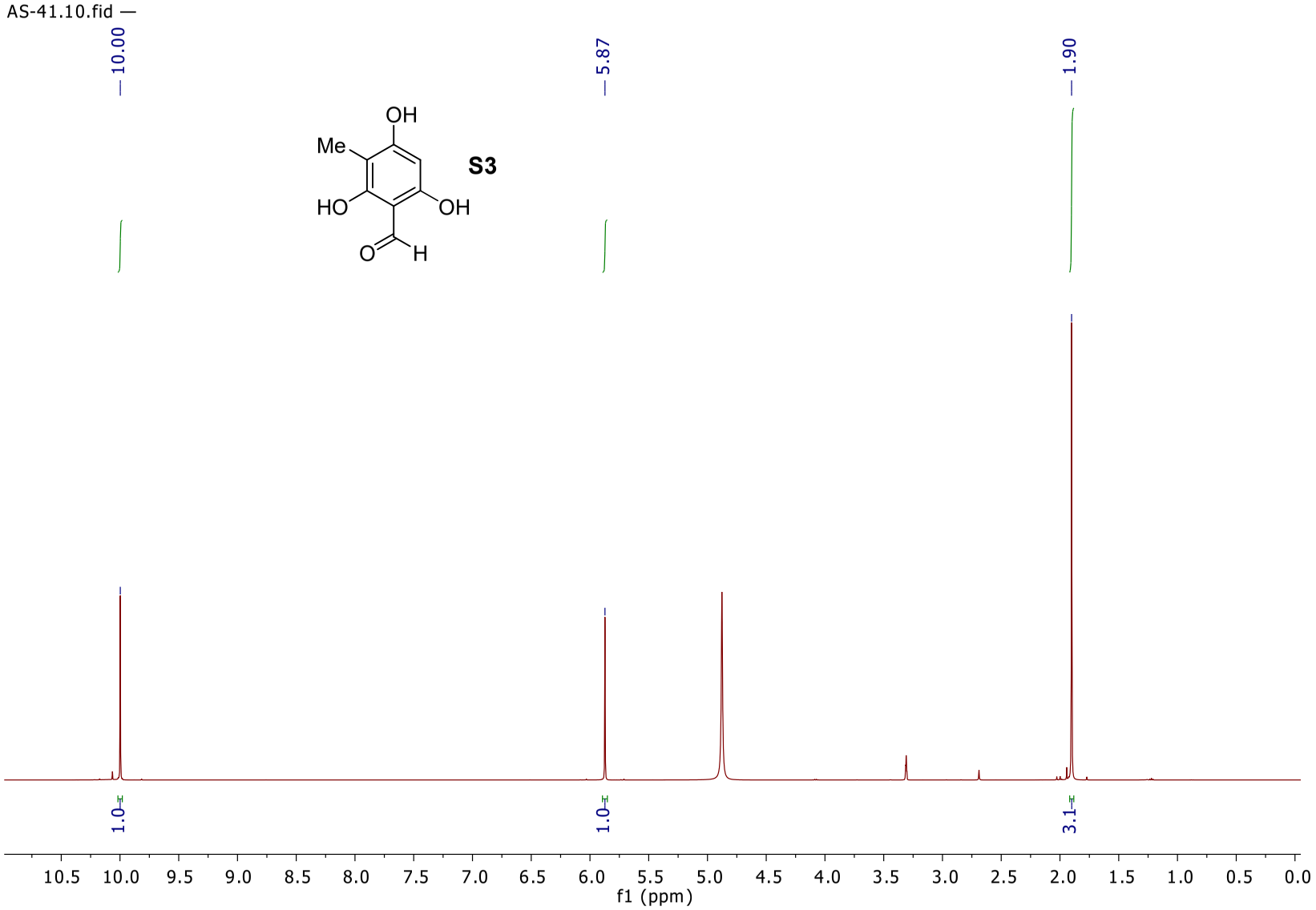
6.6 2-Methylbenzene-1,3,5-triol (**S2**)

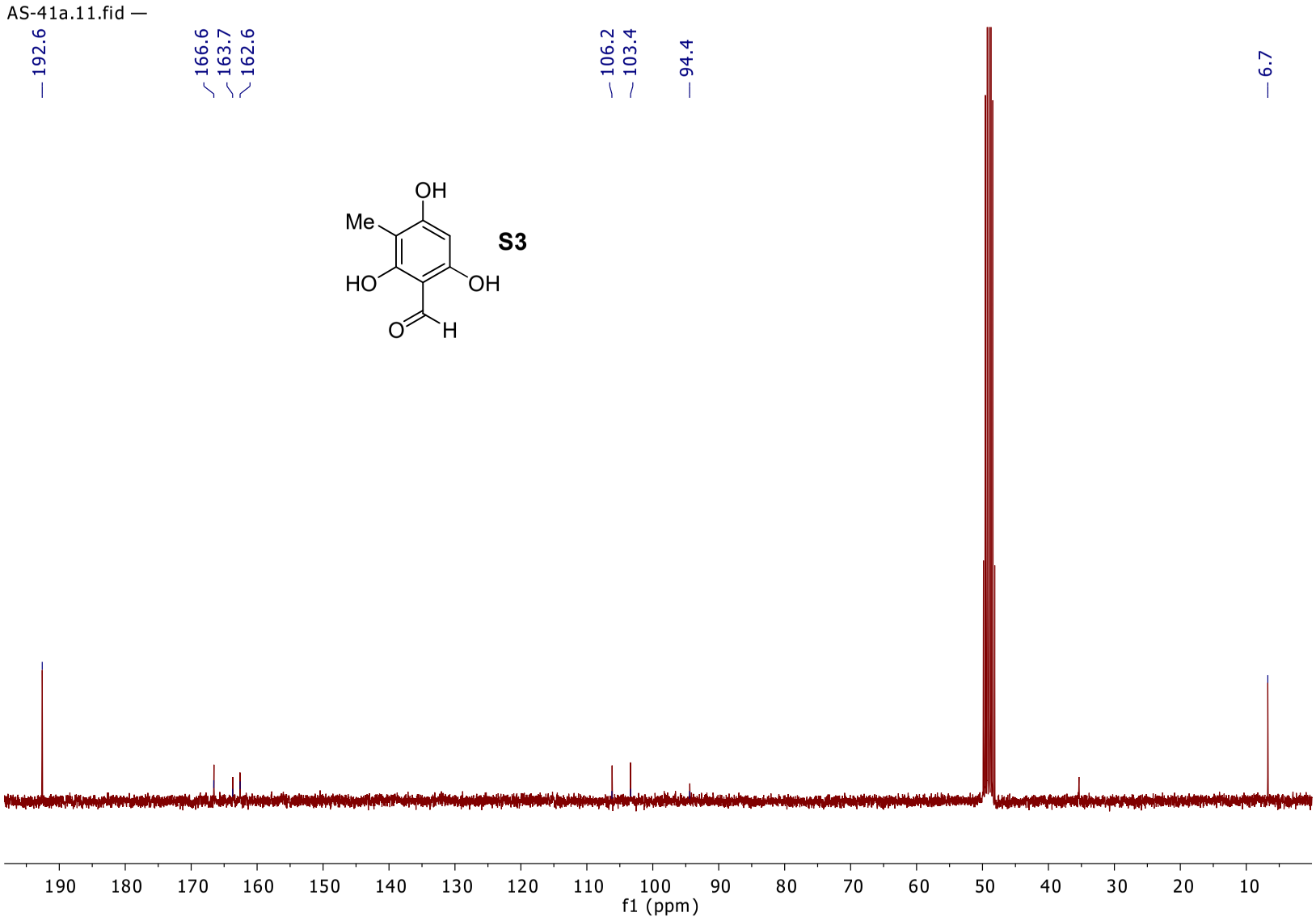


S2

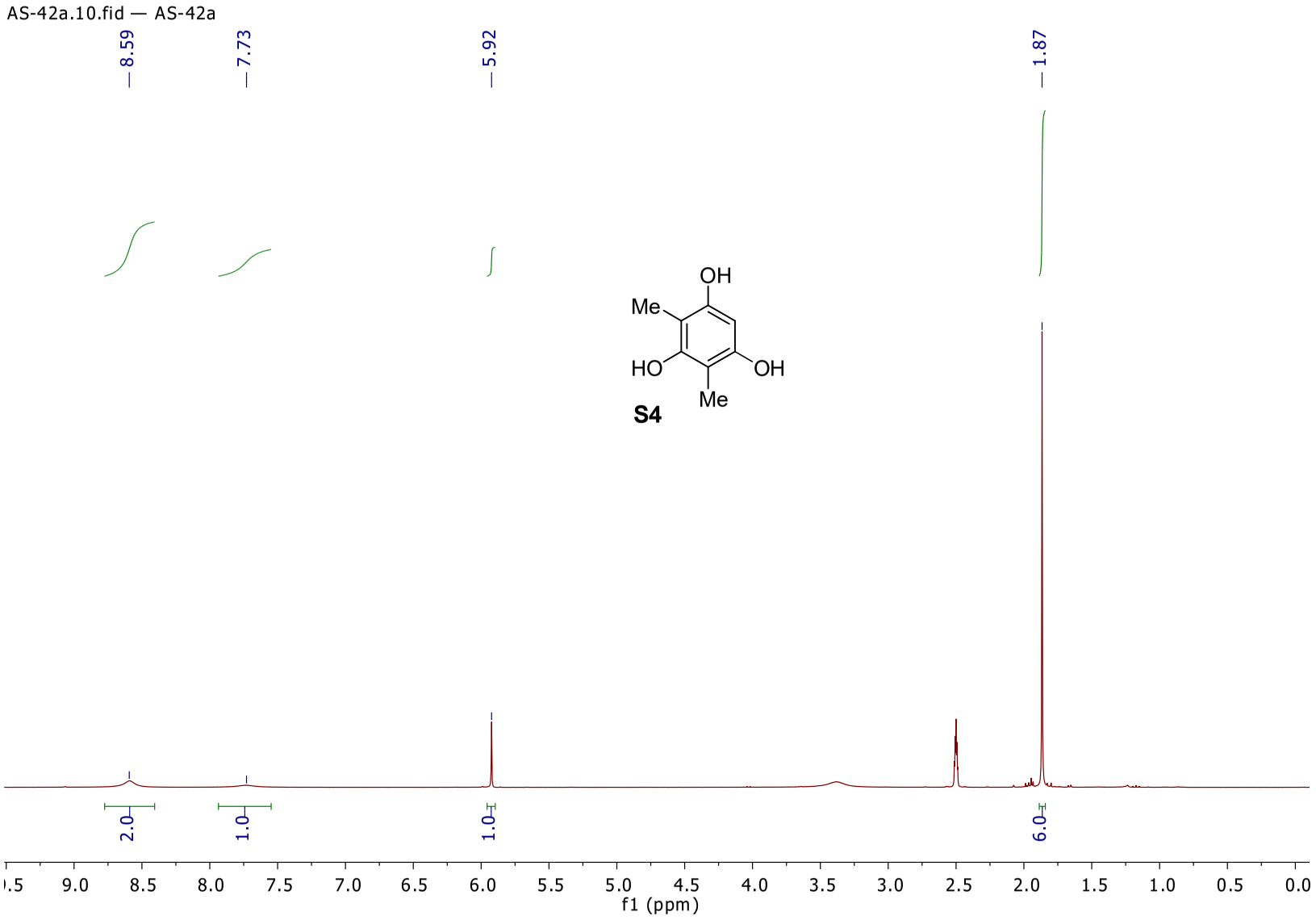

AS-trihydro-2.11.fid
157.6
156.6
103.5
95.4
7.9
OH
Me
HO
OH
S2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

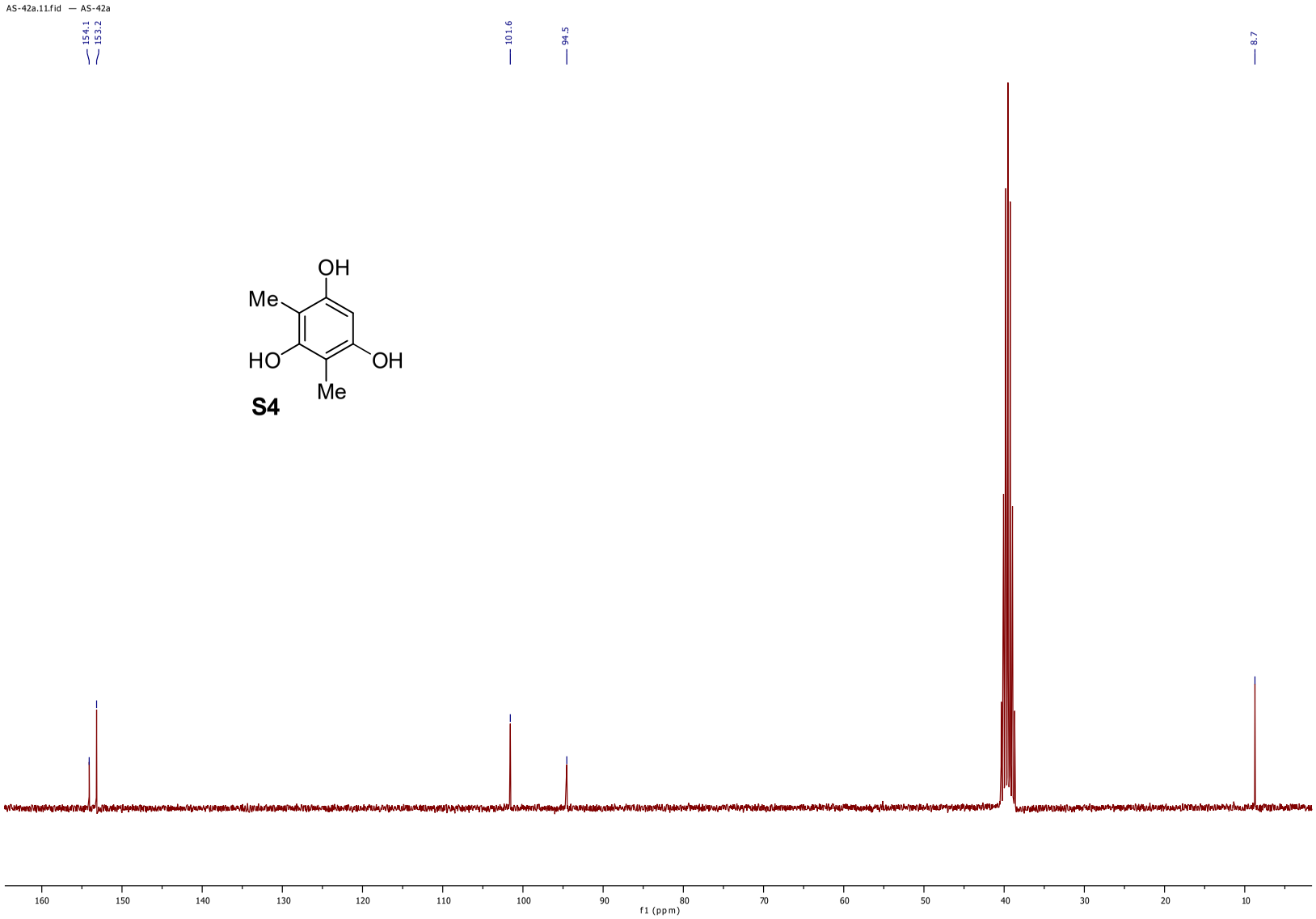
6.7 2,4,6-trihydroxy-3-methylbenzaldehyd (**S3**)



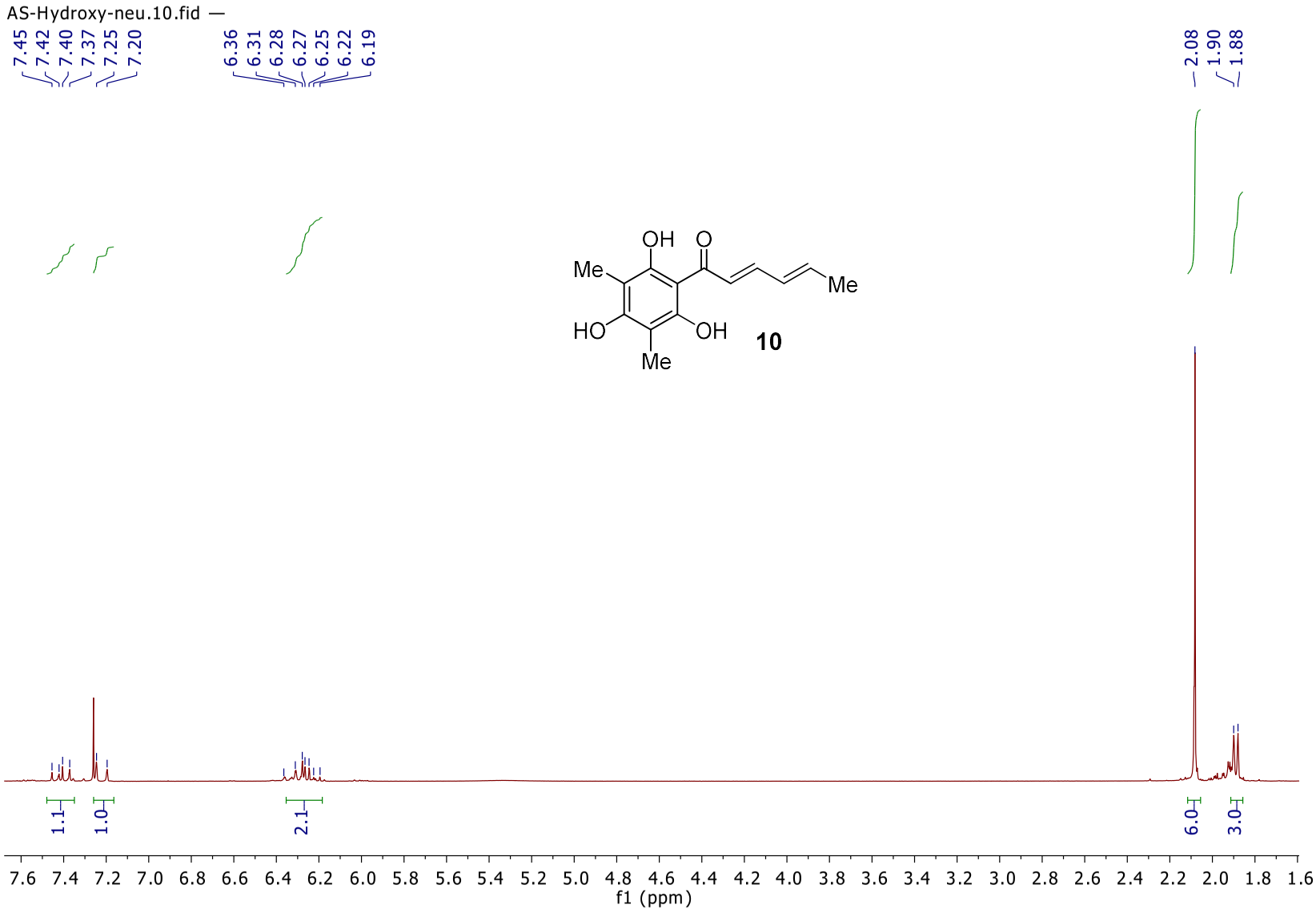

AS-41a.11.fid
192.6
166.6
163.7
162.6
106.2
103.4
94.4
6.7
OH
Me
HO
OH
O
H
S3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

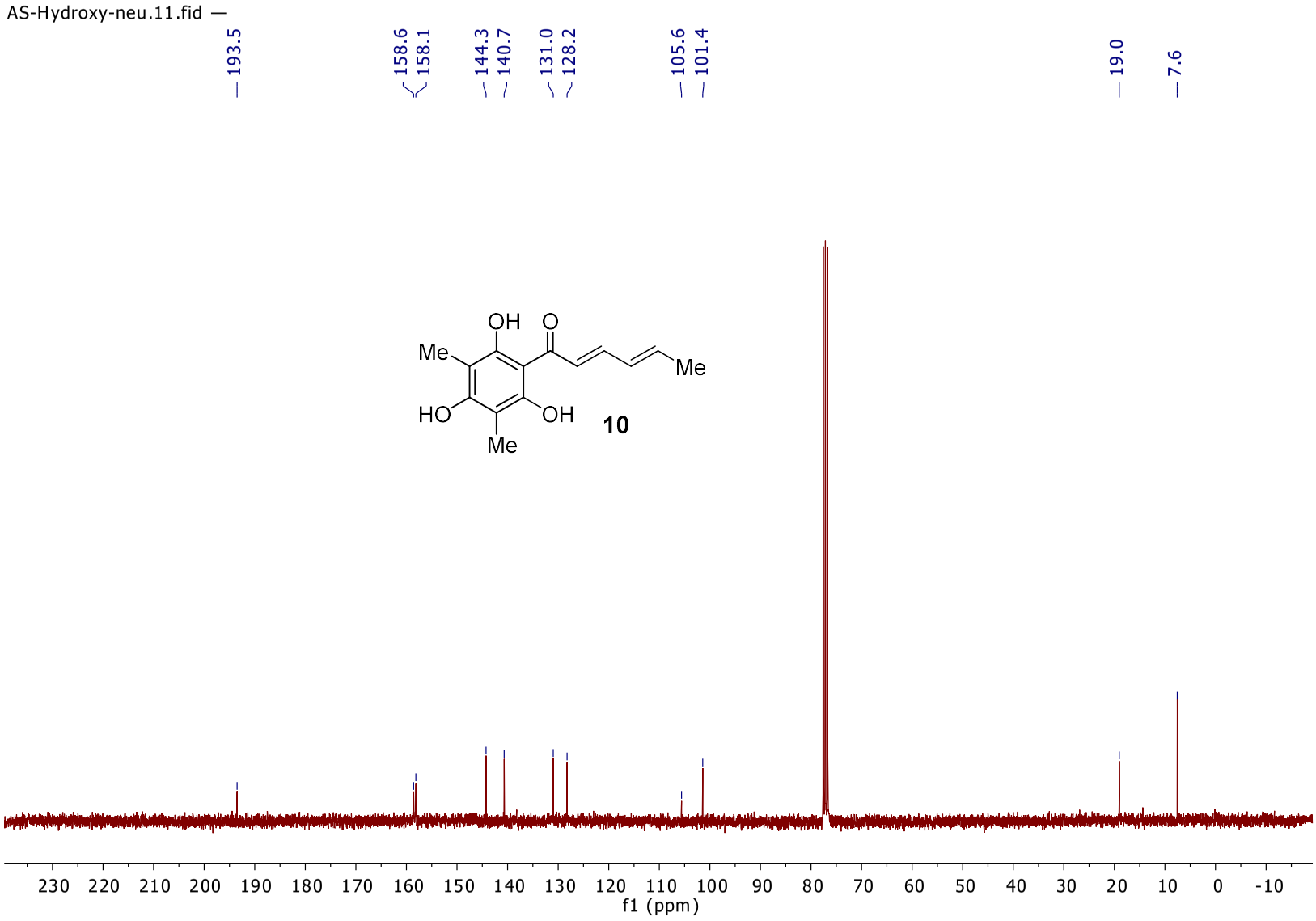
6.8 2,4-Dimethylbenzene-1,3,5 triol (**S4**)



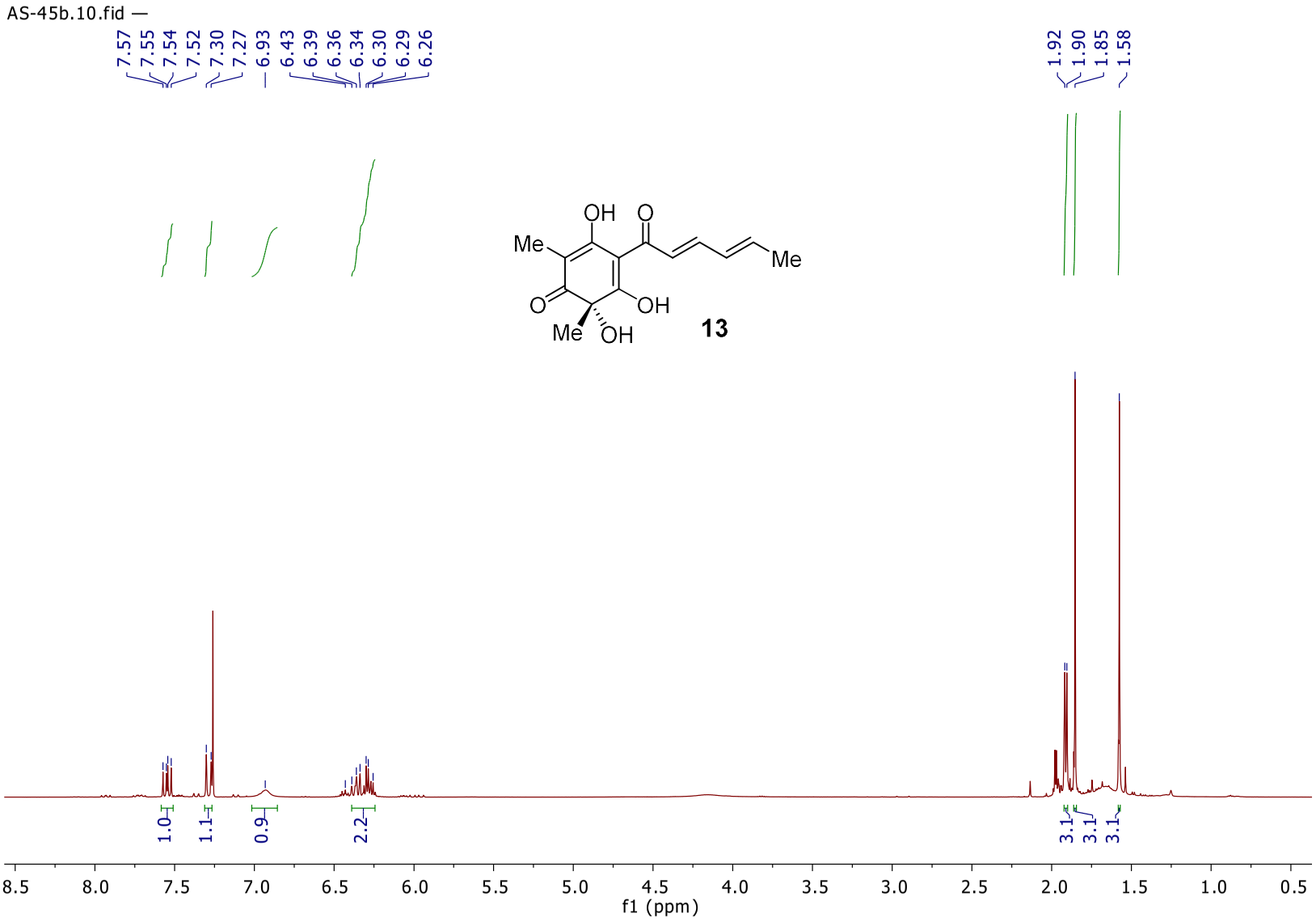

AS-42a.11.fid — AS-42a
154.1
153.2
101.6
94.5
8.7
OH
Me
HO
OH
Me
S4
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

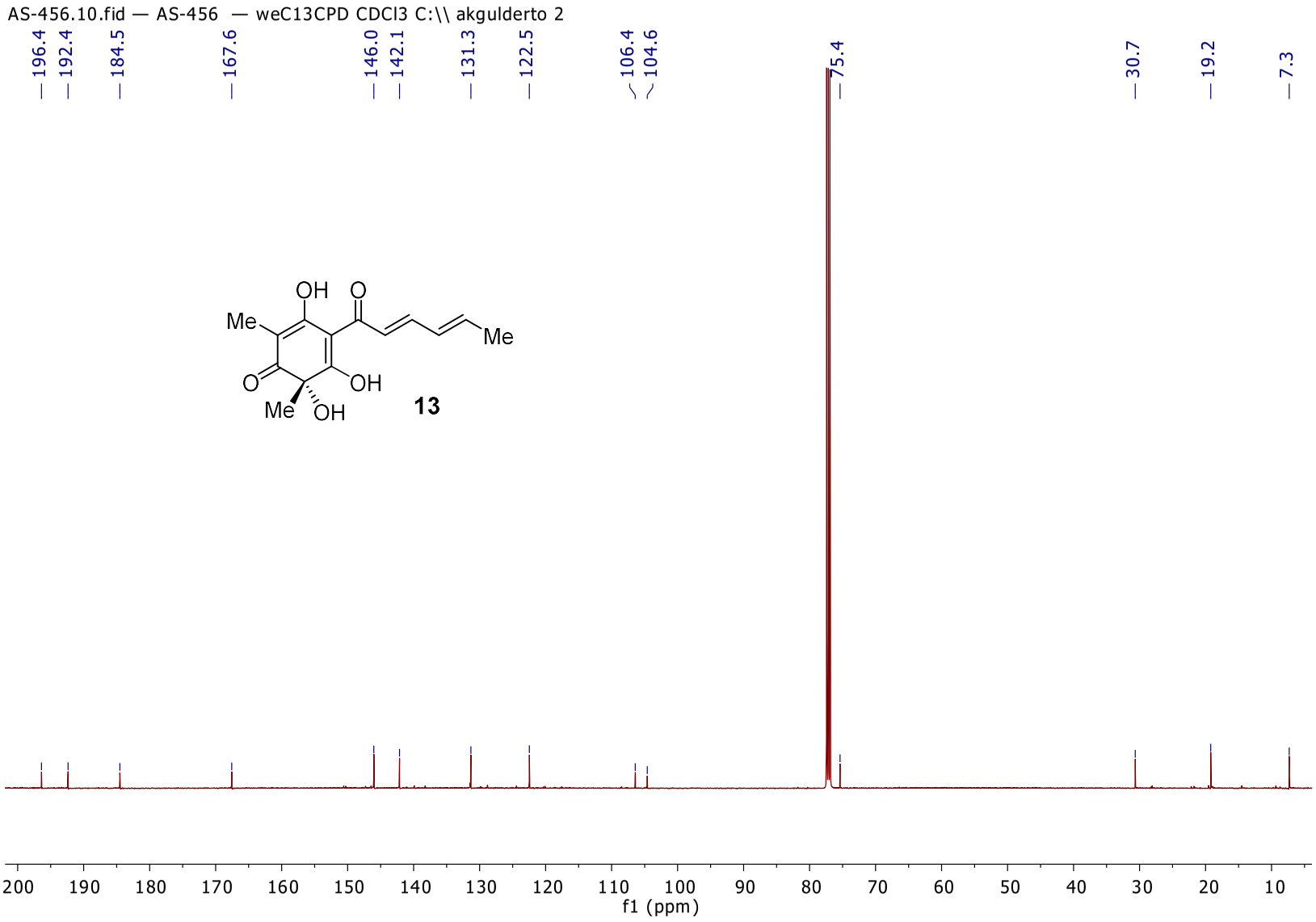
6.9 6-Hydroxysorbicillin (**10**)



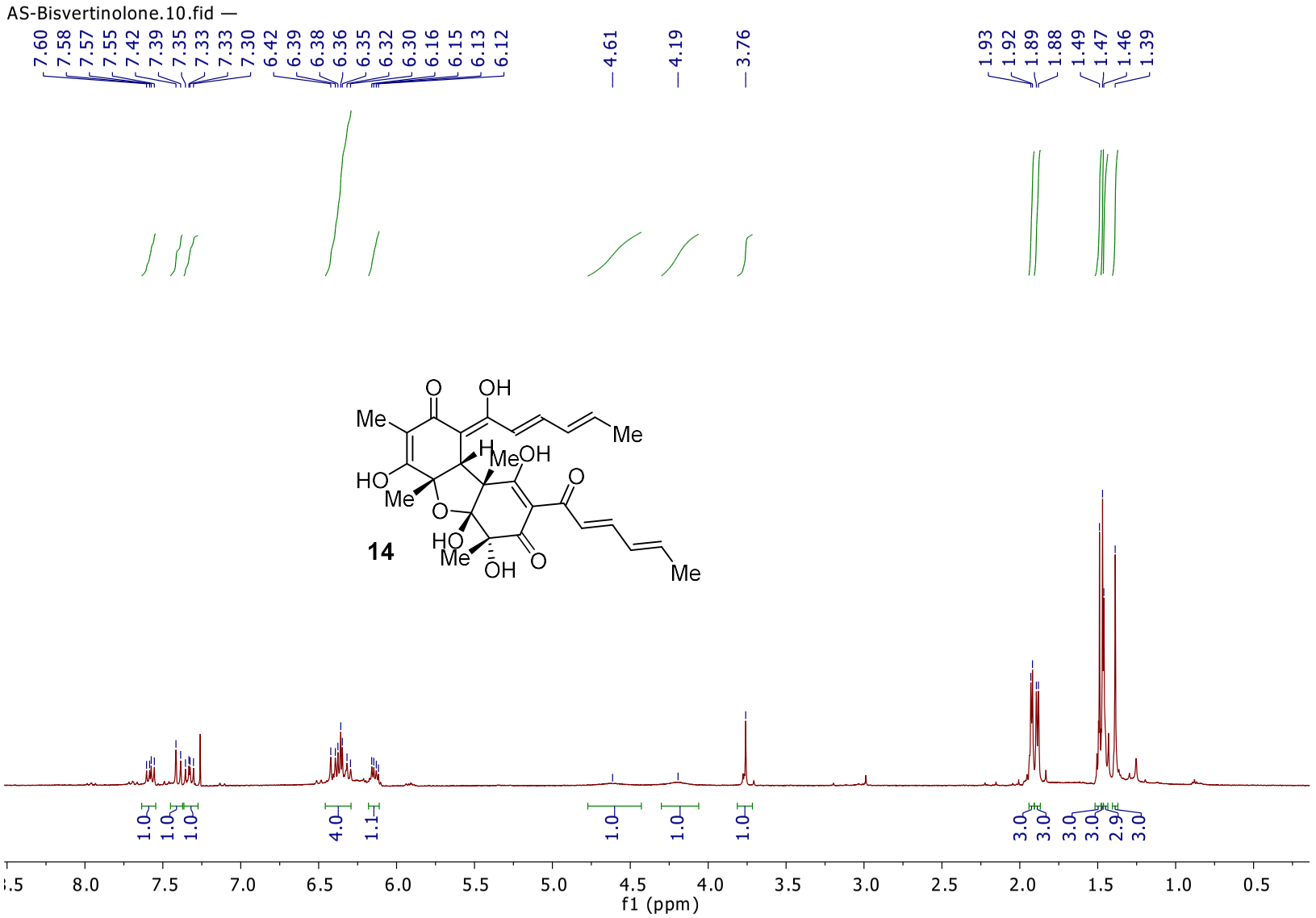

AS-Hydroxy-neu.11.fid
193.5
158.6
158.1
144.3
140.7
131.0
128.2
105.6
101.4
19.0
7.6
OH
O
Me
Me
HO
OH
Me
10
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

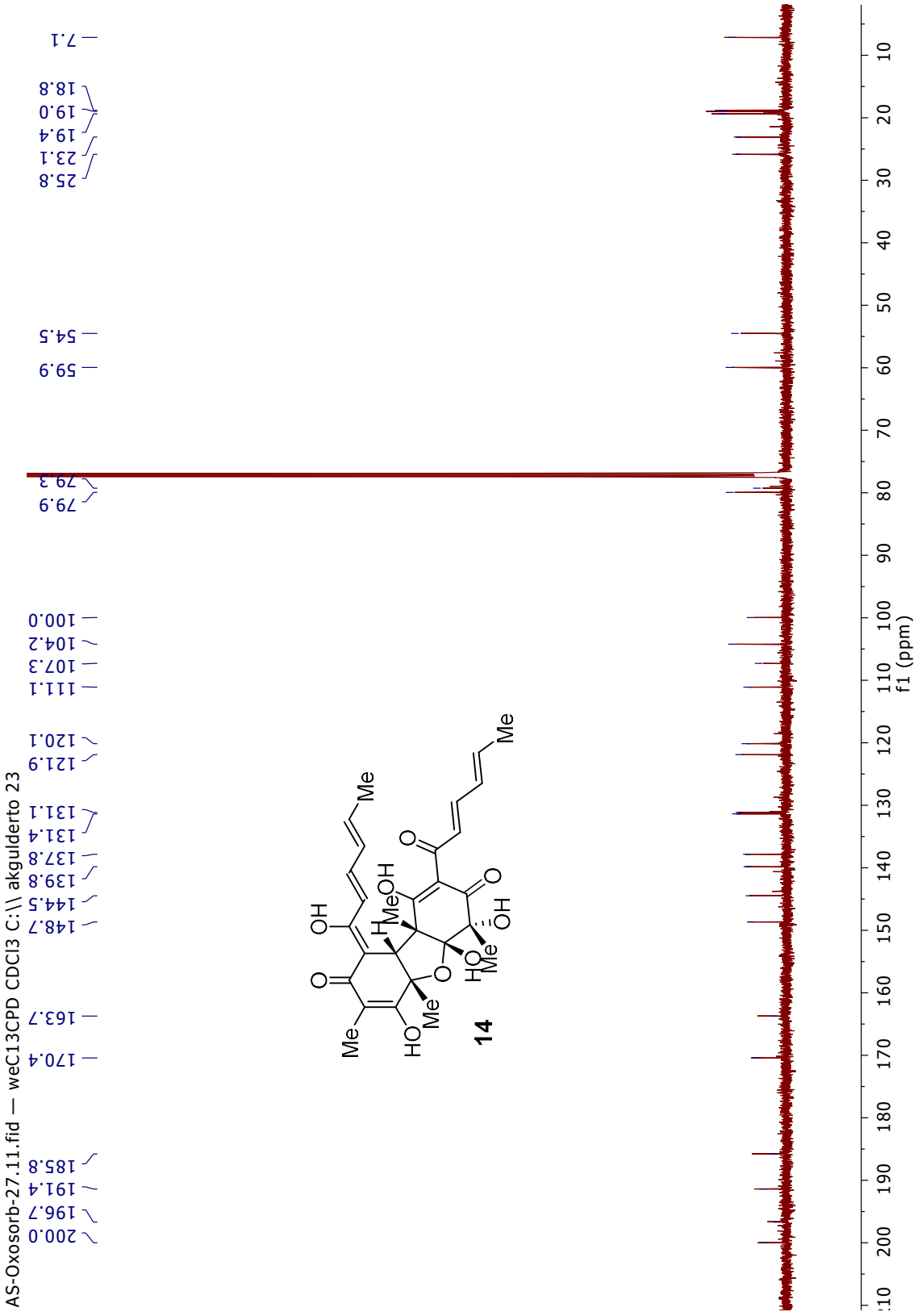
6.10 Oxosorbicillinol (**13**)



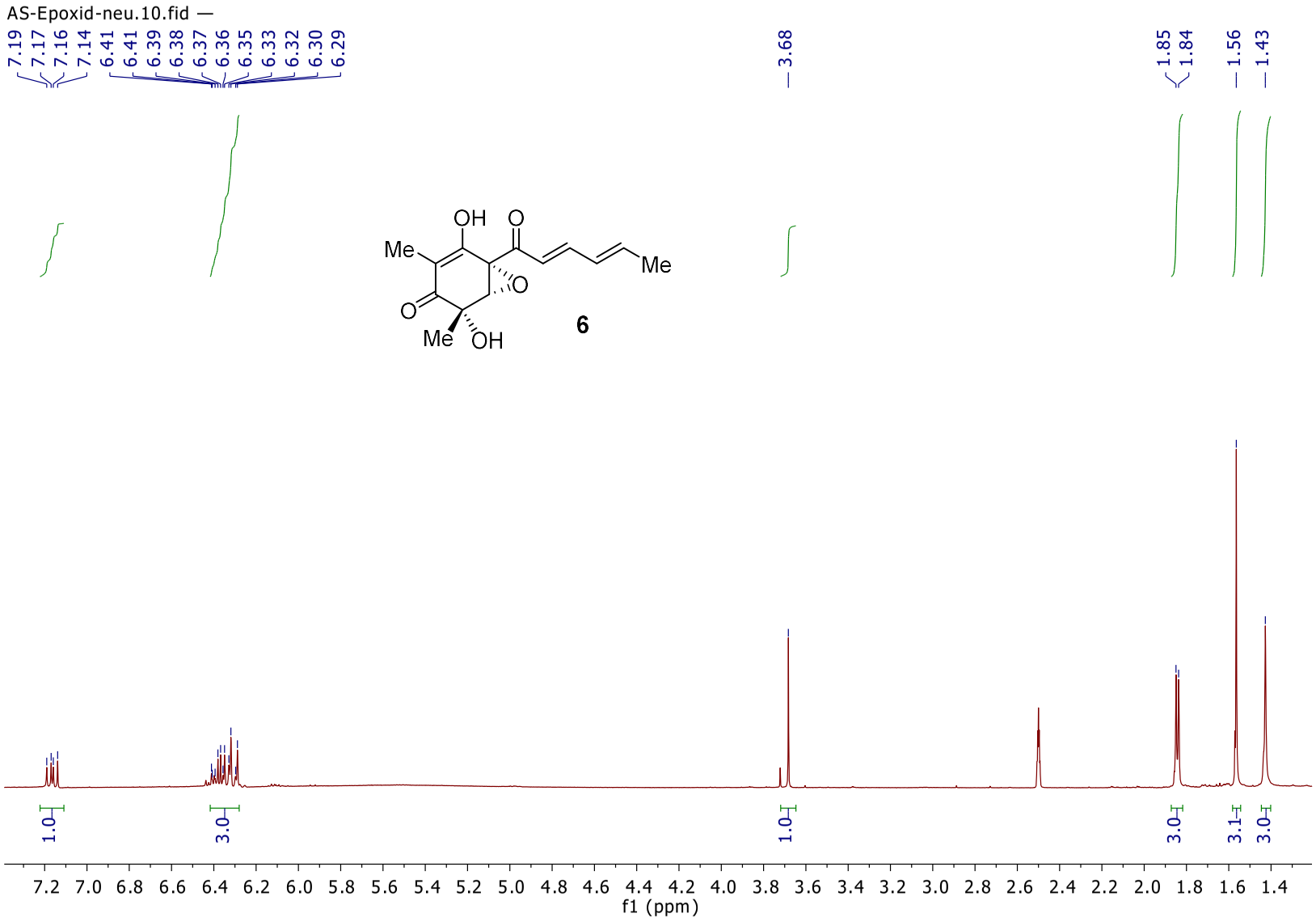

AS-456.10.fid — AS-456 — weC13CPD CDCl3 C:\\ akgulderto 2
196.4
192.4
184.5
167.6
146.0
142.1
131.3
122.5
106.4
104.6
75.4
30.7
19.2
7.3
OH
O
Me
Me
O
OH
Me
OH
13
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

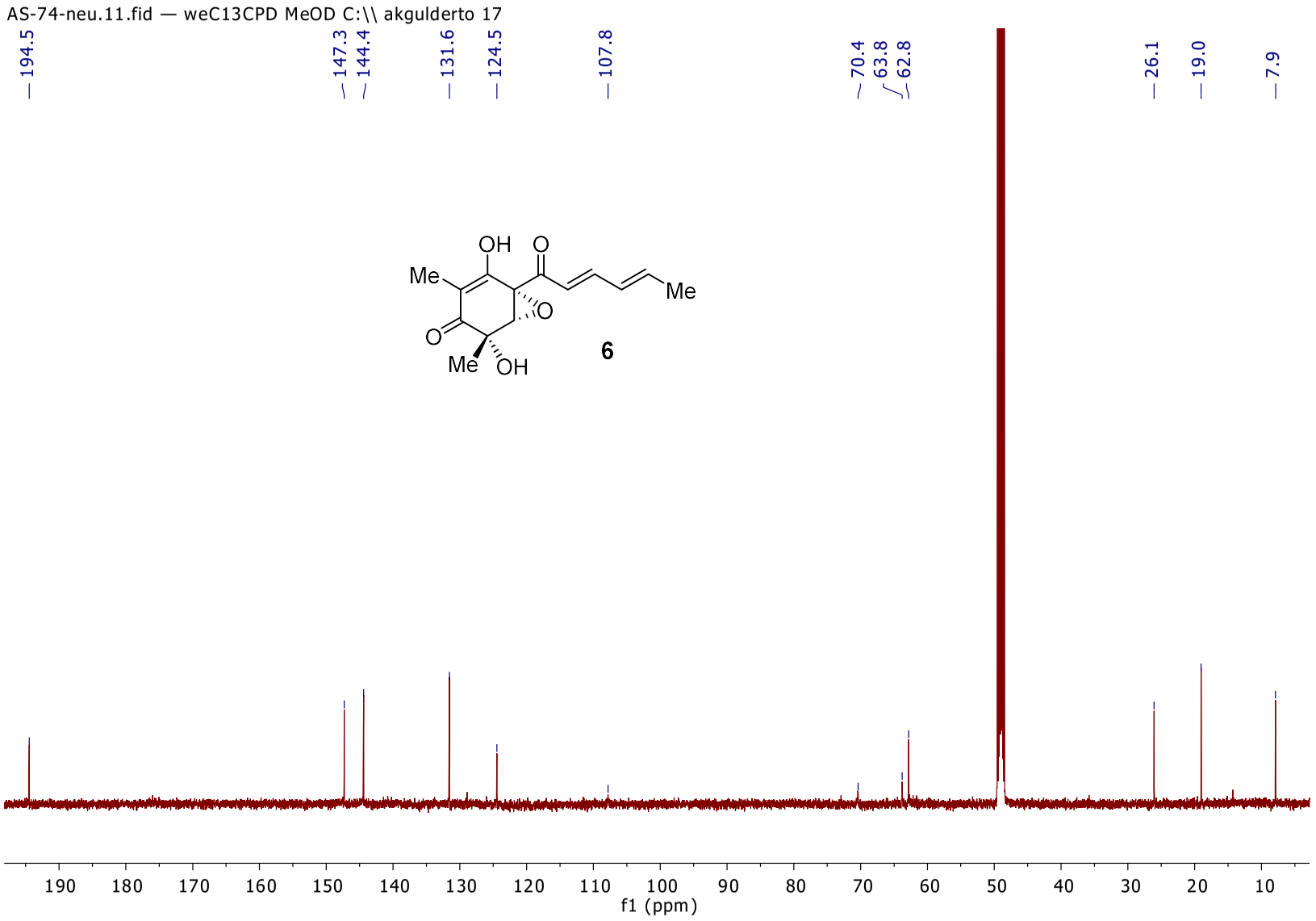
6.11 Bisvertinolone (**14**)



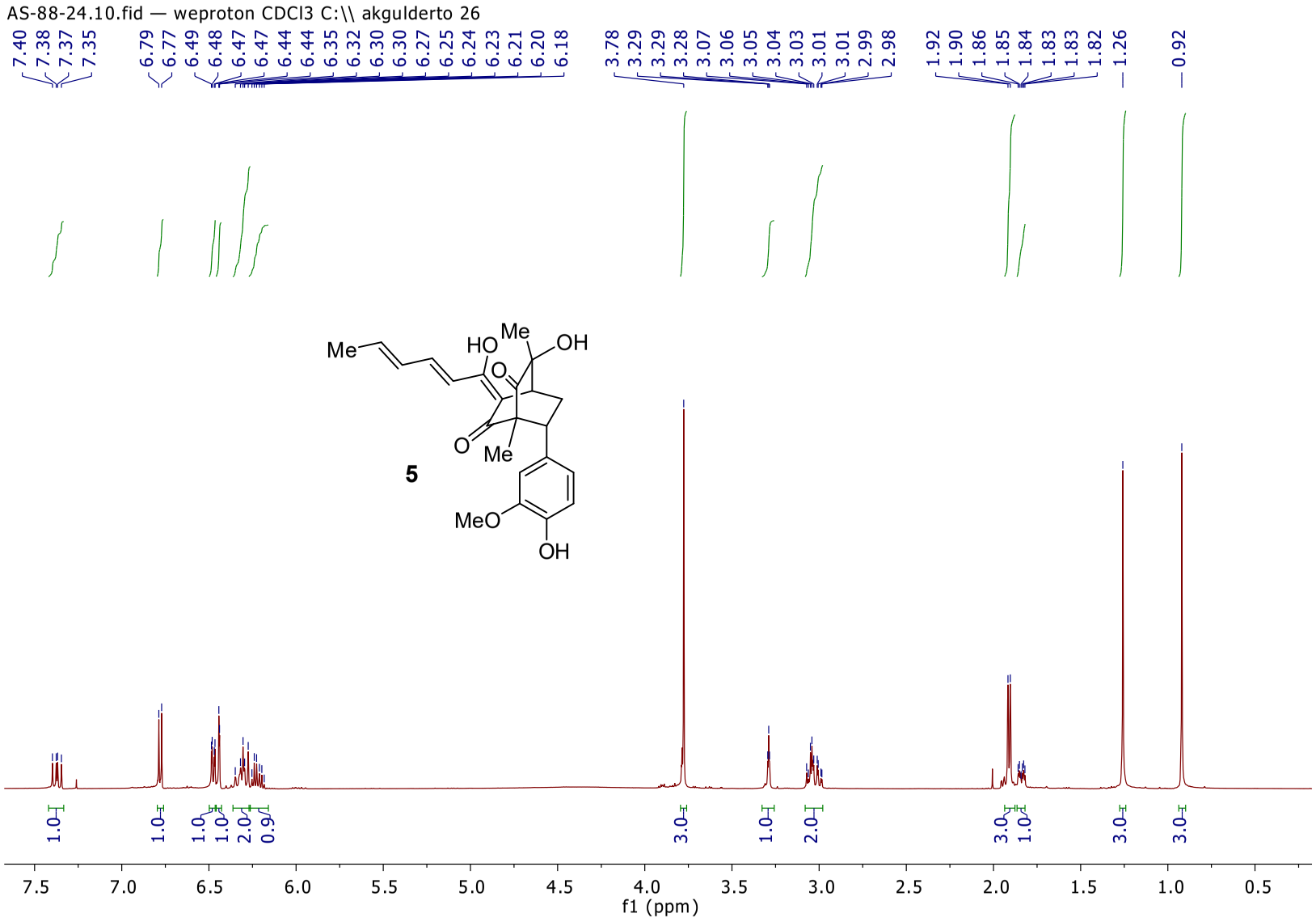

AS-Oxosorb-27.11.fid — weC13CPD CDCl3 C:\\ akgulderto 23
200.0
196.7
191.4
185.8
170.4
163.7
148.7
144.5
139.8
137.8
131.4
131.1
121.9
120.1
111.1
107.3
104.2
100.0
79.9
79.3
59.9
54.5
25.8
23.1
19.4
19.0
18.8
7.1
f1 (ppm)
14

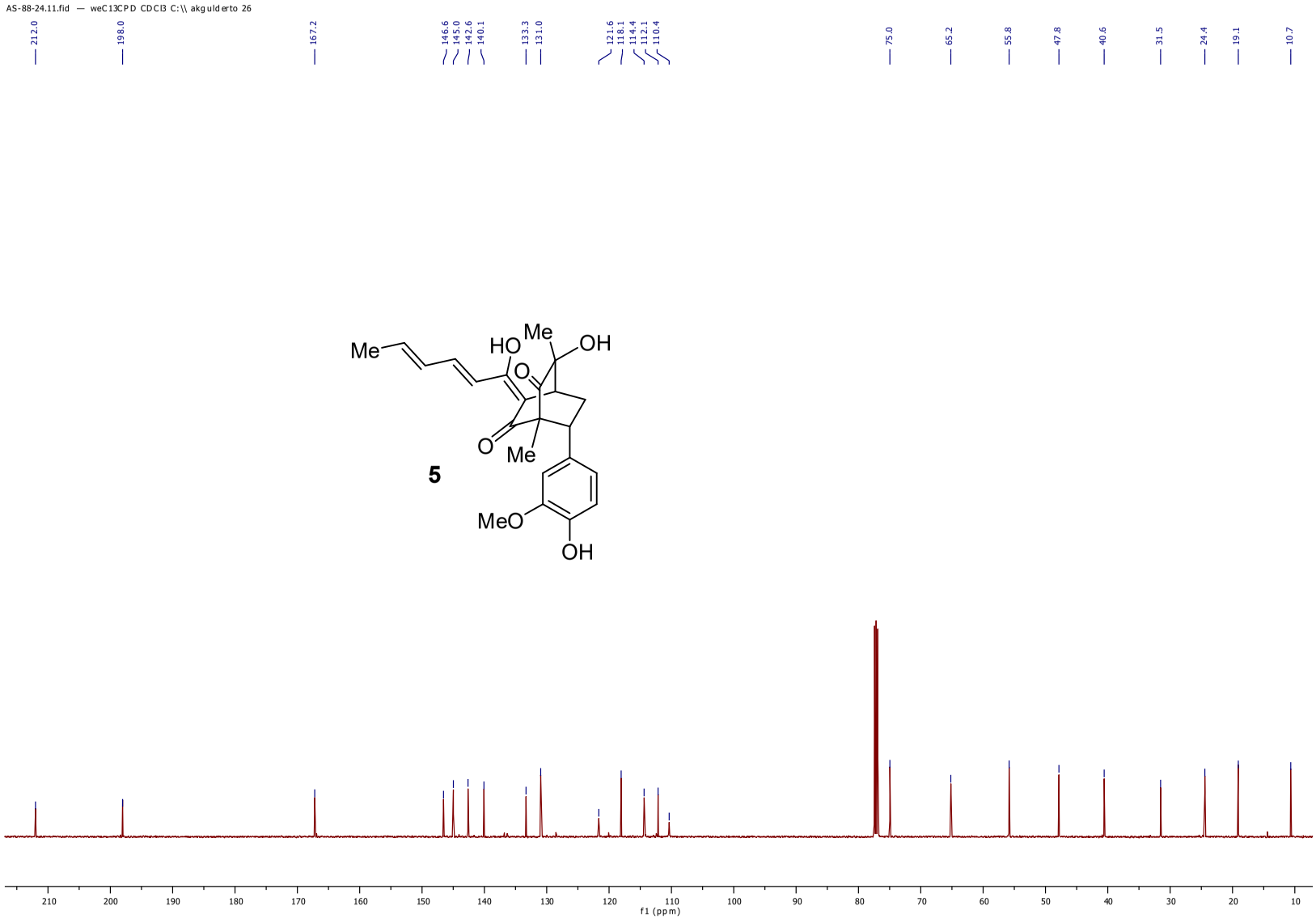
6.12 Epoxysorbicillinol (**6**)



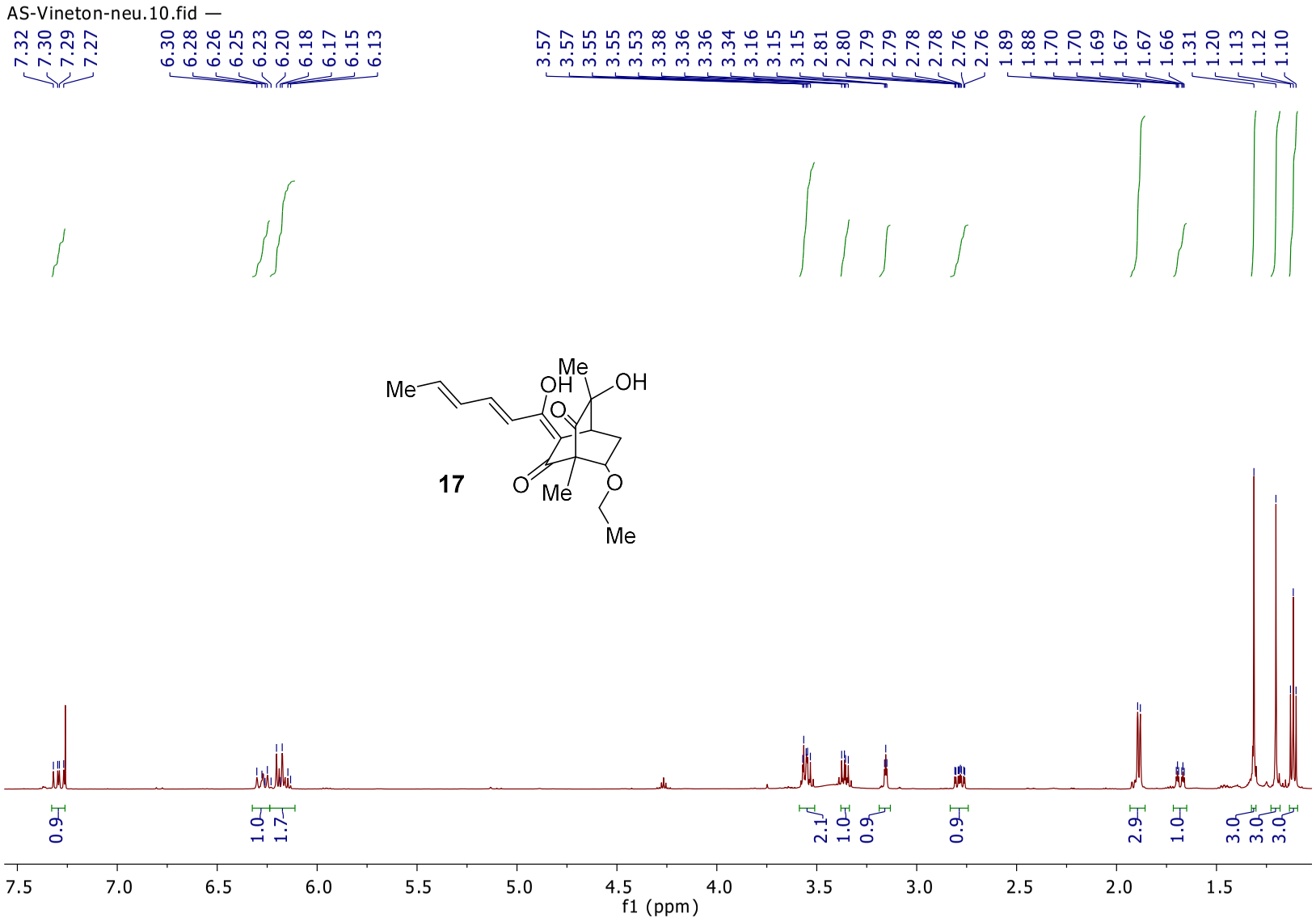

AS-74-neu.11.fid — weC13CPD MeOD C:\\ akgulderto 17
194.5
147.3
144.4
131.6
124.5
107.8
70.4
63.8
62.8
26.1
19.0
7.9
OH
O
Me
Me
O
O
Me
OH
6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

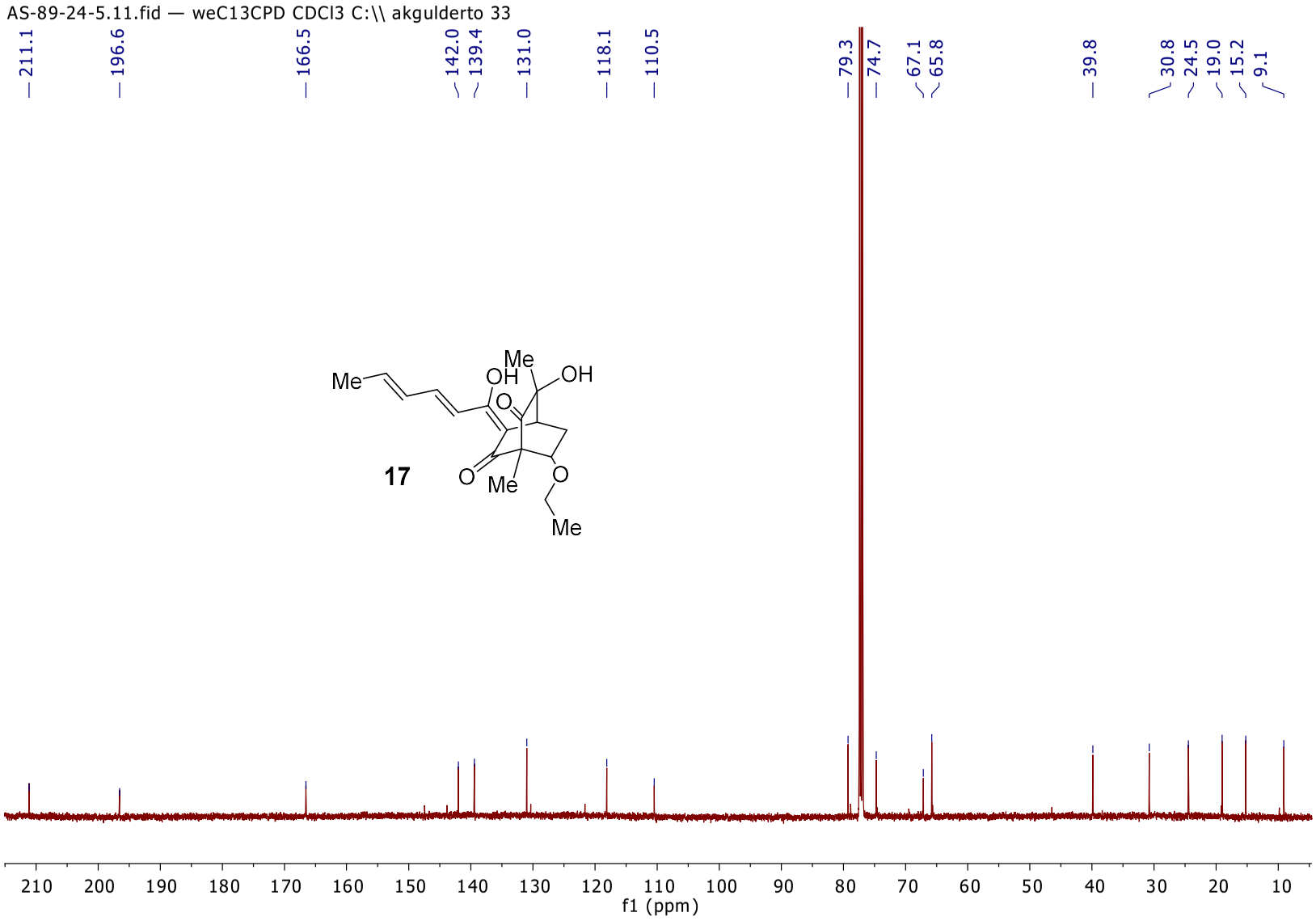
6.13 Sorbicatechol A (**5**)



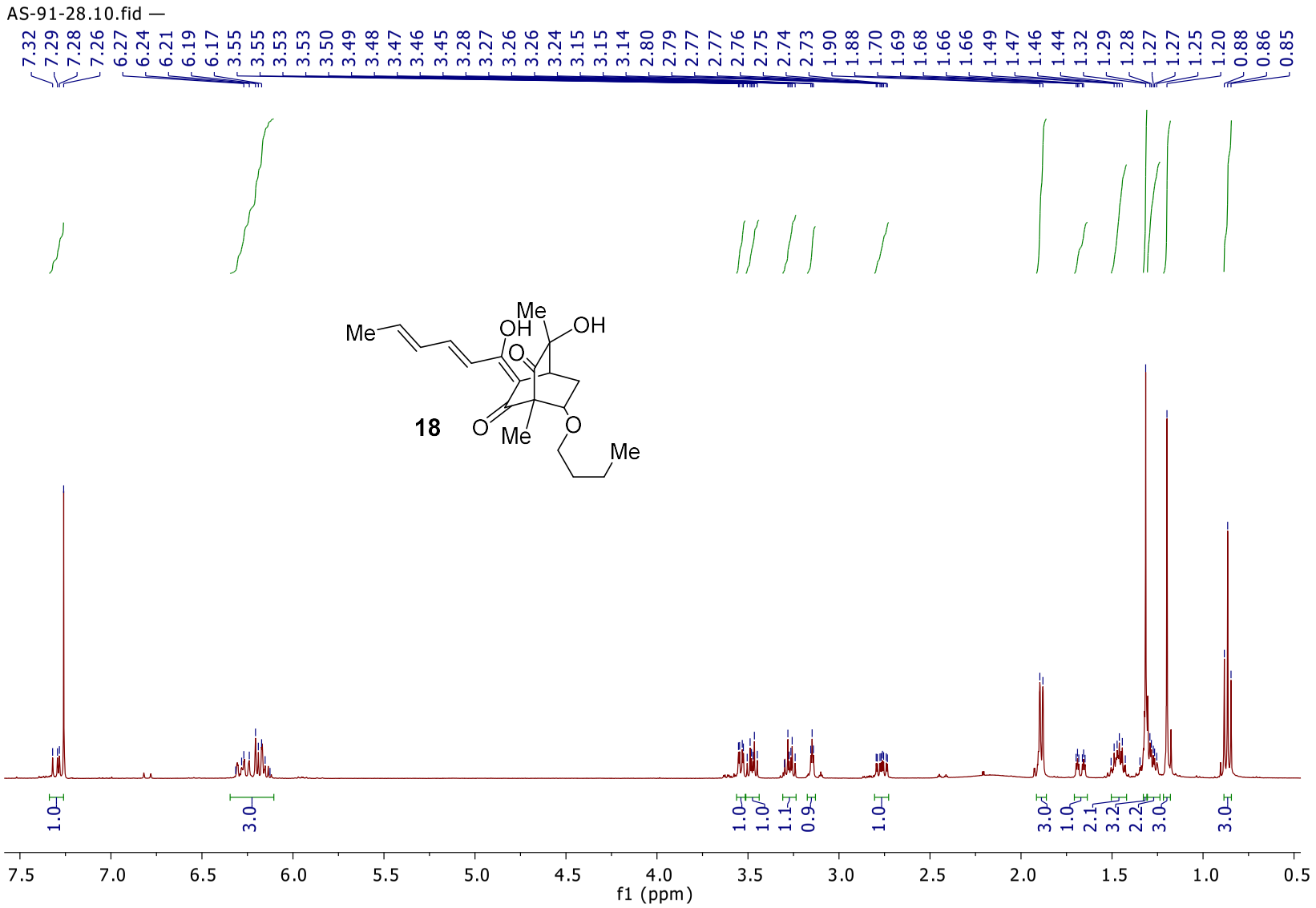

AS-88-24.11.fid — weC13CPD CDCl3 C:\\ akguld erto 26
212.0
198.0
167.2
146.6
145.0
142.6
140.1
133.3
131.0
121.6
118.1
114.4
112.1
110.4
75.0
65.2
55.8
47.8
40.6
31.5
24.4
19.1
10.7
Me
HO
Me
OH
O
O
Me
MeO
OH
5
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

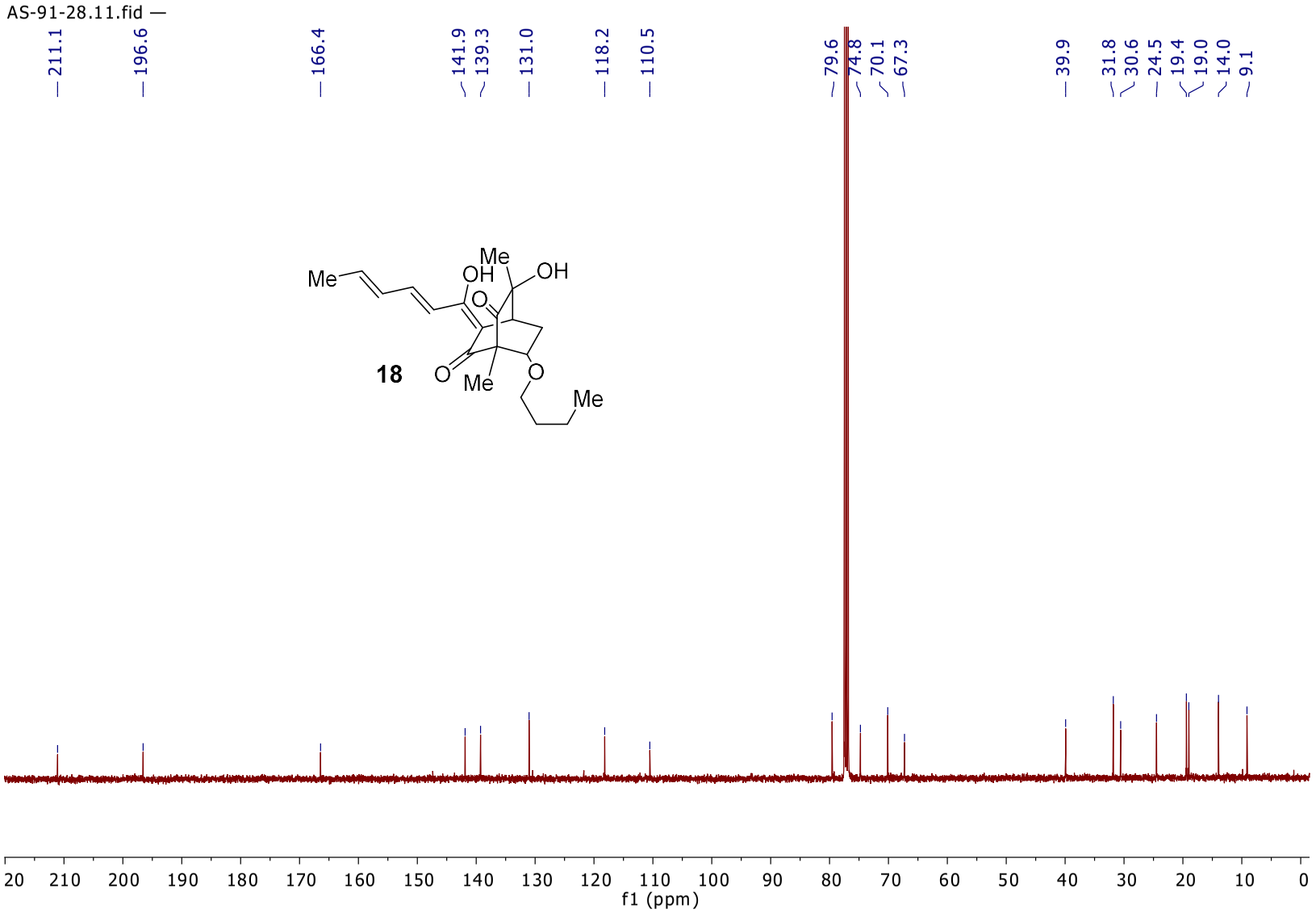
6.14 Rezishanone C (**17**)



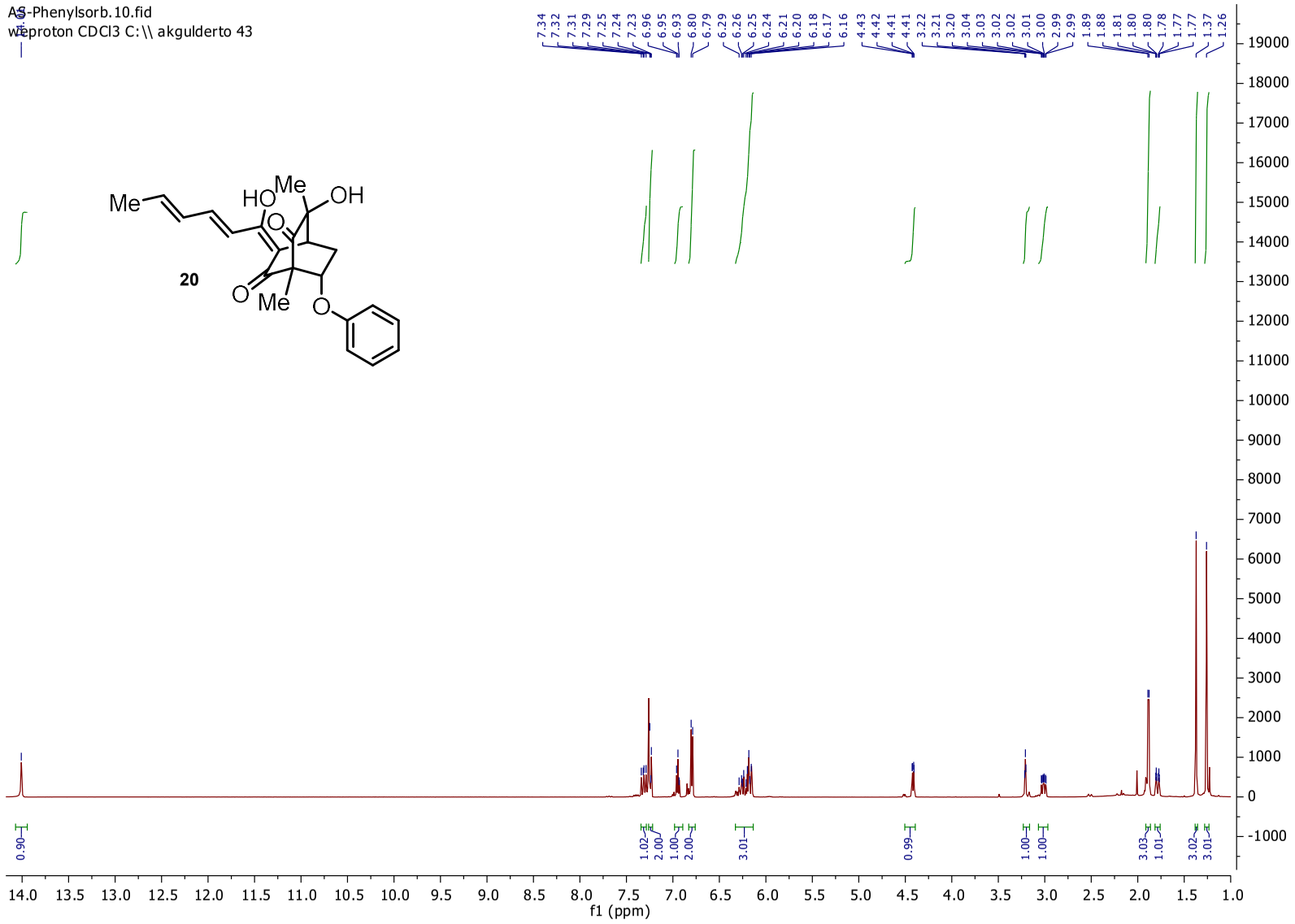

AS-89-24-5.11.fid — weC13CPD CDCl3 C:\\ akgulderto 33
211.1
196.6
166.5
142.0
139.4
131.0
118.1
110.5
79.3
74.7
67.1
65.8
39.8
30.8
24.5
19.0
15.2
9.1
Me
OH
OH
Me
O
O
Me
O
Me
17
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

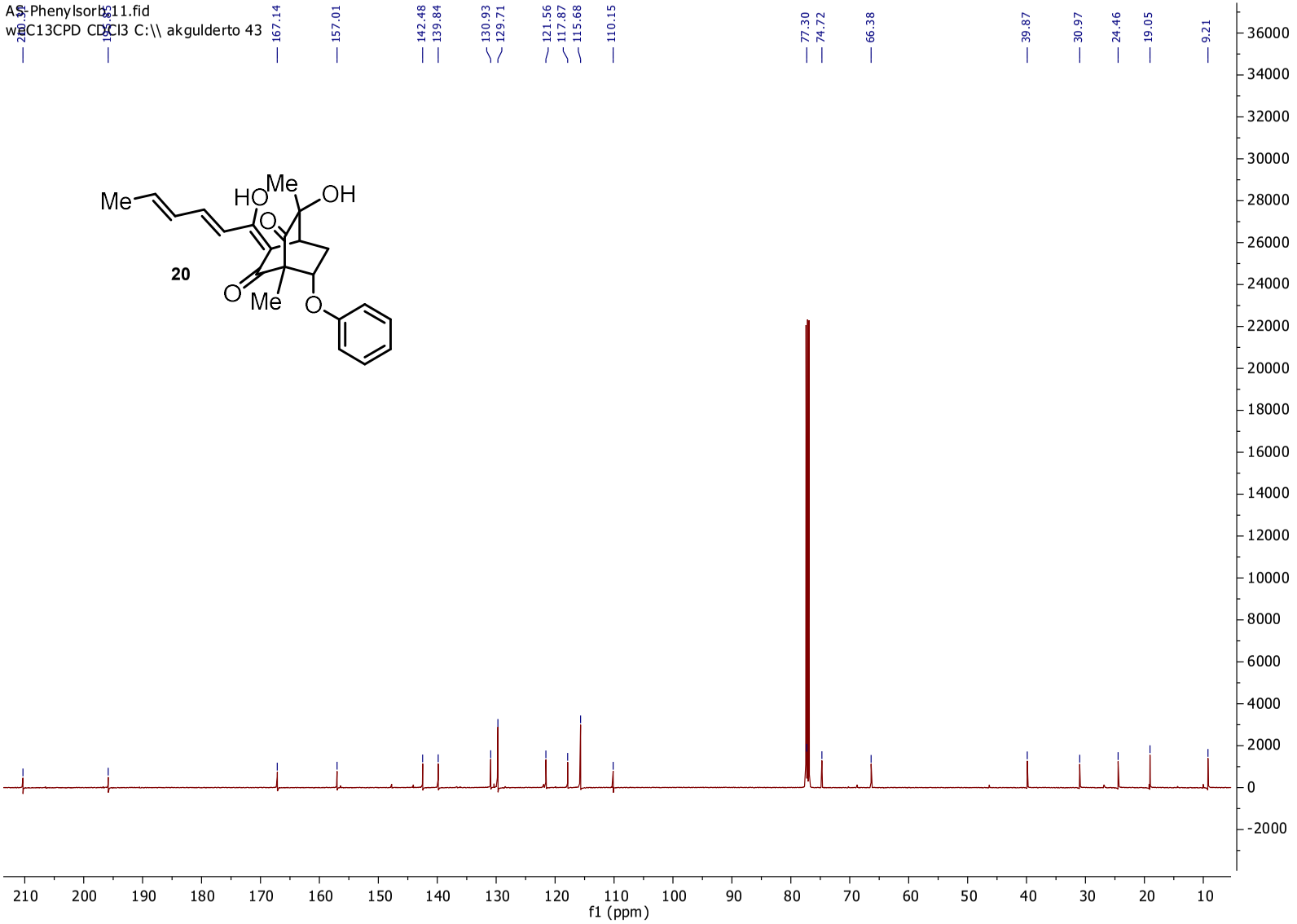
6.15 Rezishanone B (**18**)



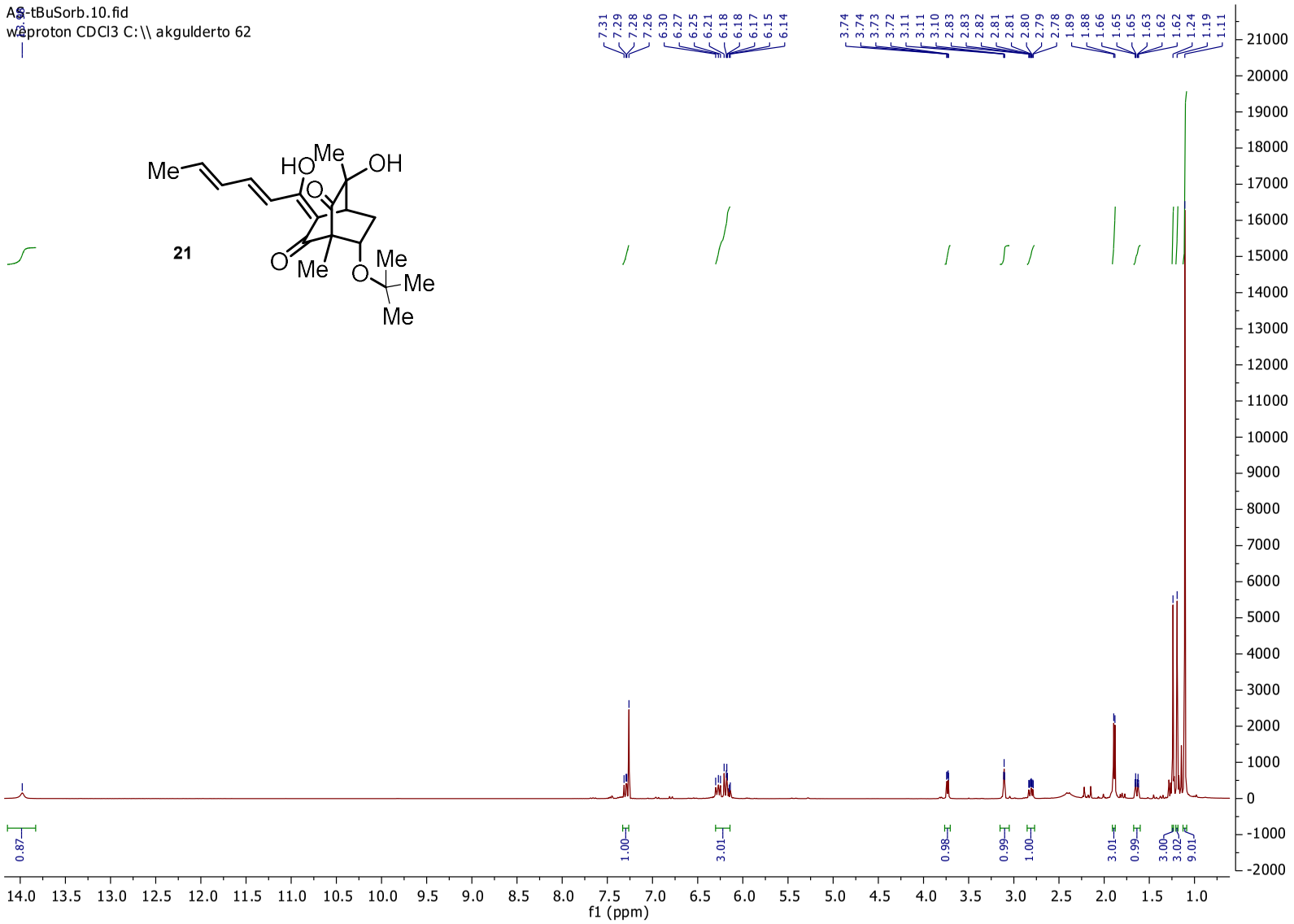

AS-91-28.11.fid
211.1
196.6
166.4
141.9
139.3
131.0
118.2
110.5
79.6
74.8
70.1
67.3
39.9
31.8
30.6
24.5
19.4
19.0
14.0
9.1
Me
OH
OH
Me
O
O
Me
O
Me
18
20 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

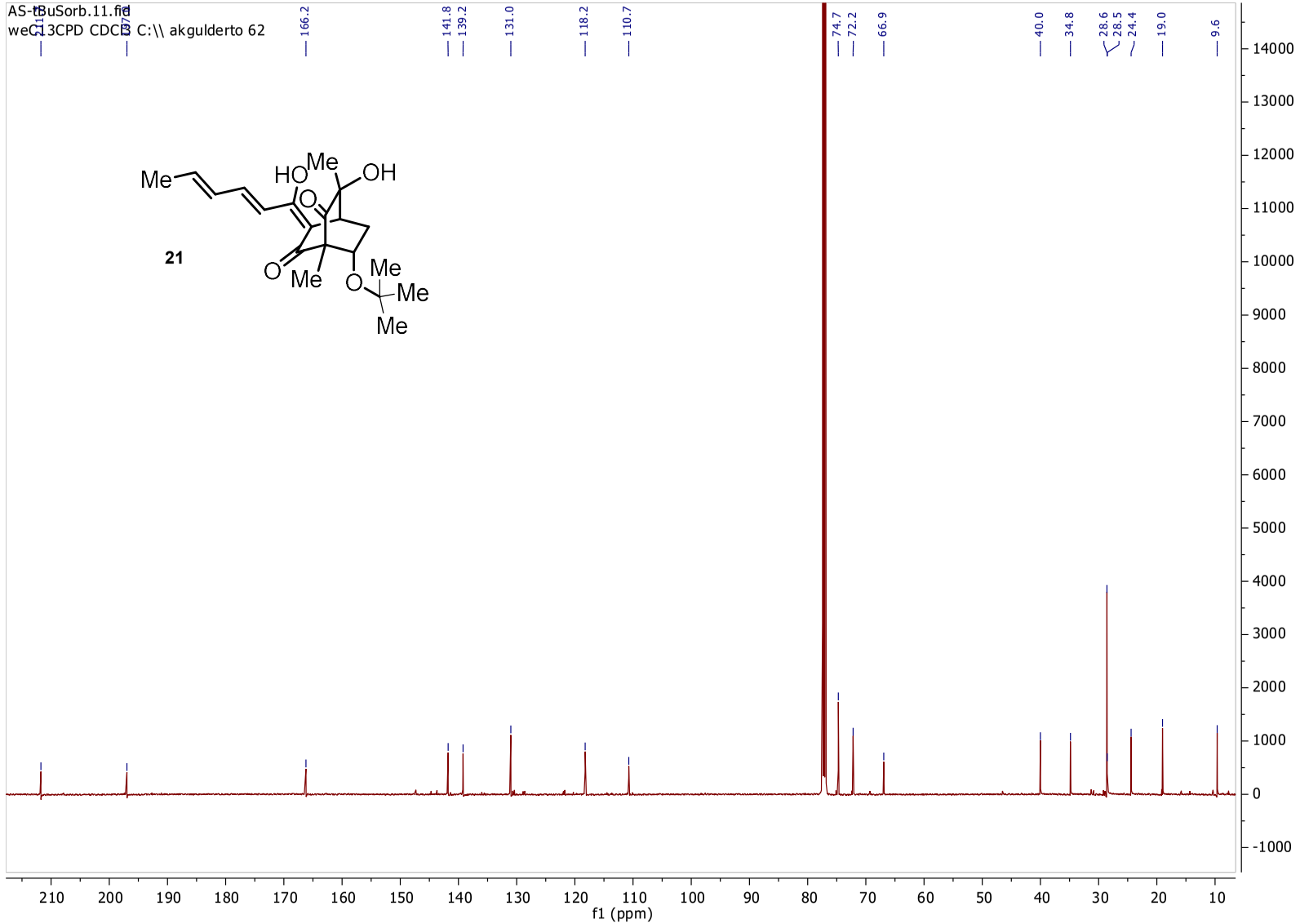
6.16 Rezishanone S1 (**20**)



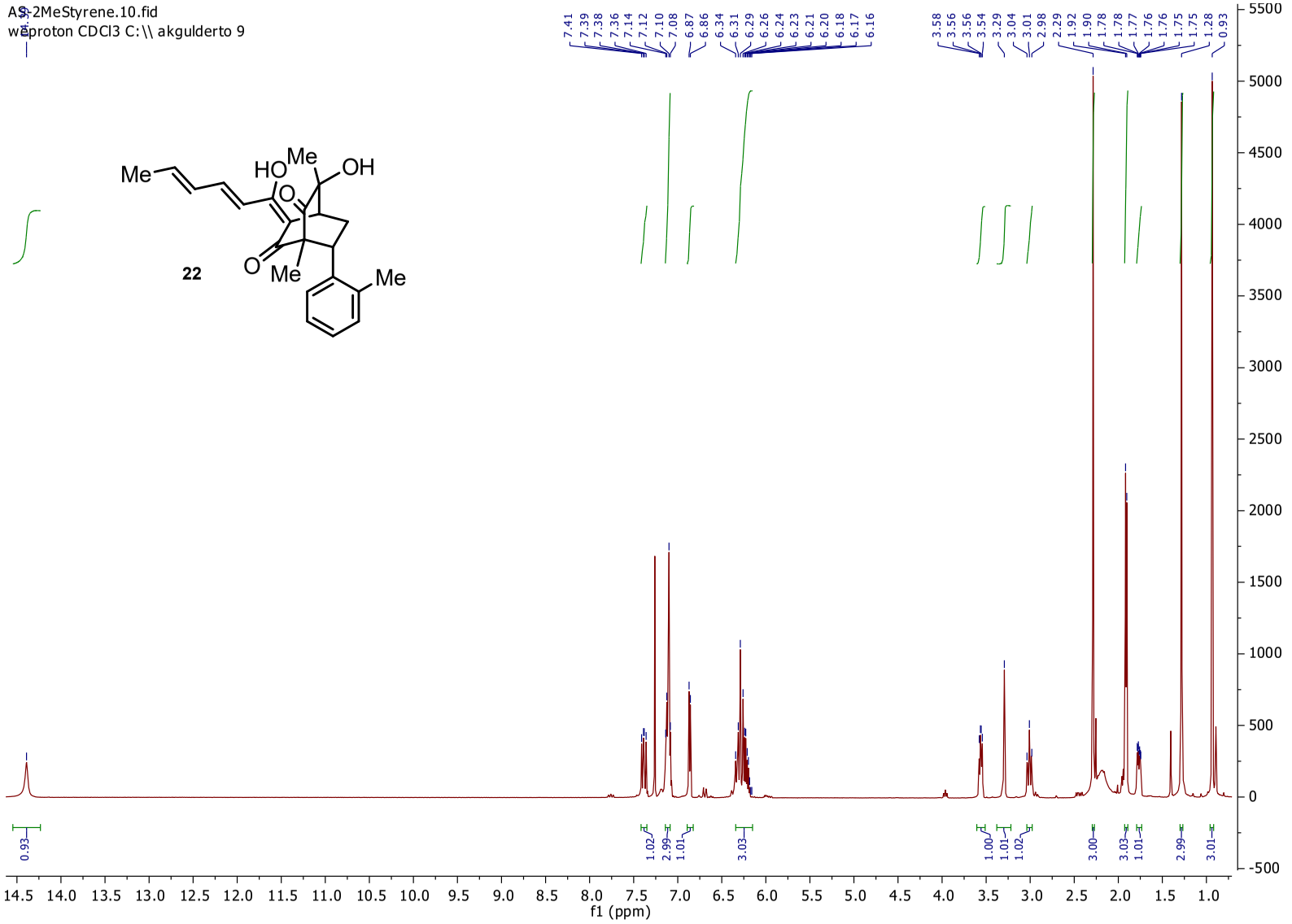

AS-Phenylsorb11.fid
wdC13CPD CDCl3 C:\\ akgulderto 43
167.14
157.01
142.48
139.84
130.93
129.71
121.56
117.87
115.68
110.15
77.30
74.72
66.38
39.87
30.97
24.46
19.05
9.21
Me
HO
Me
OH
O
O
Me
O
20
f1 (ppm)

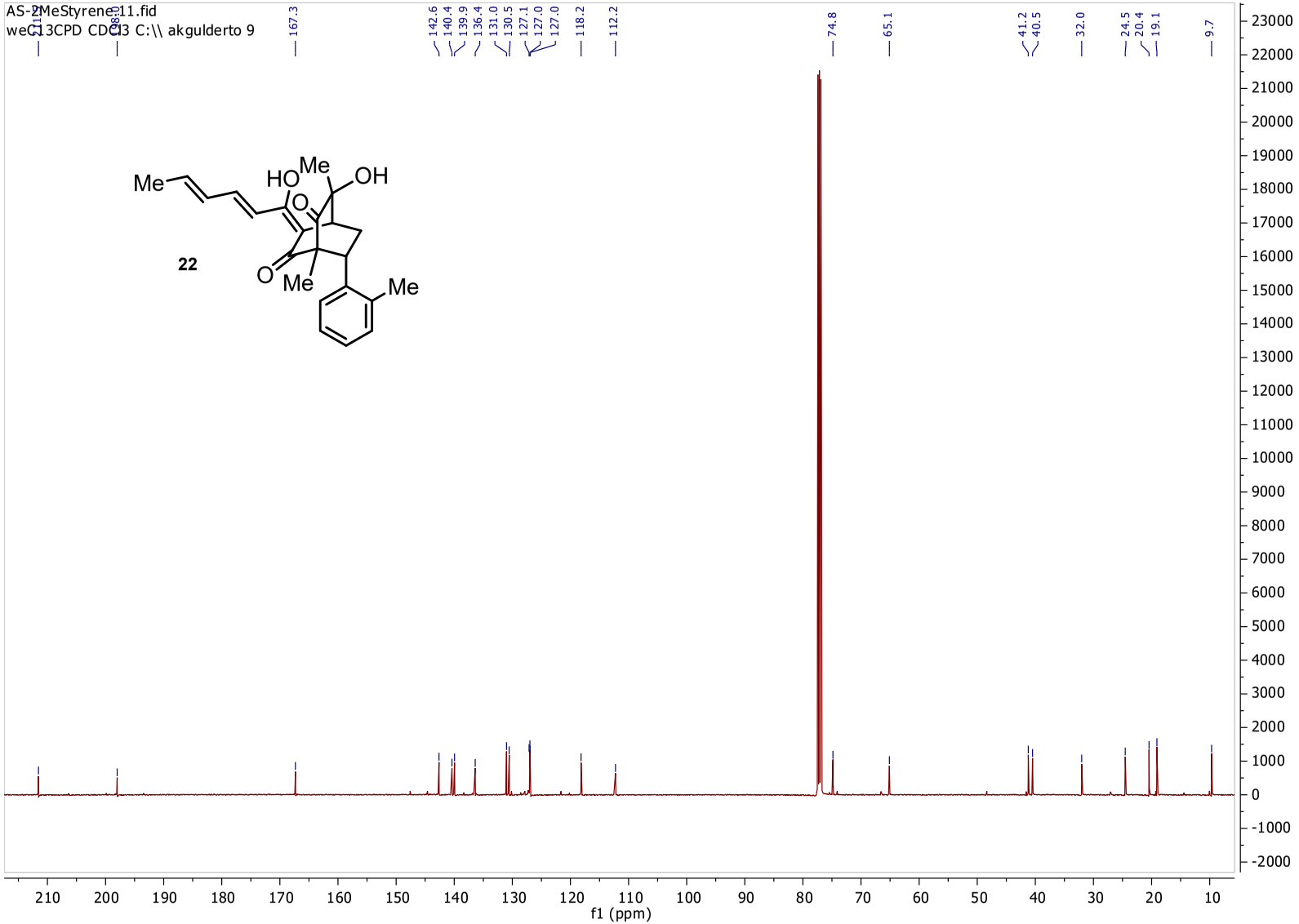
6.17 Rezishanone S2 (**21**)



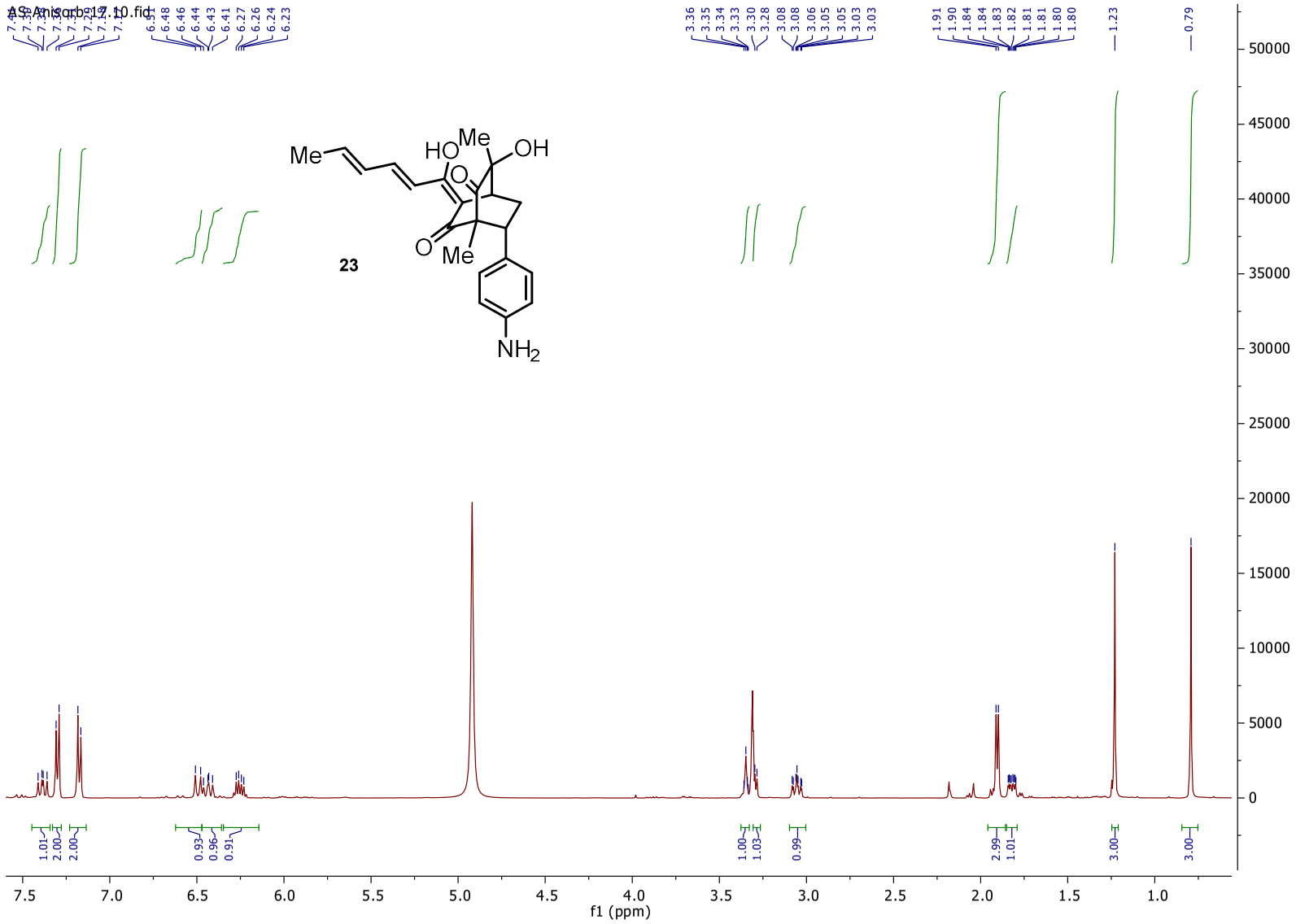

AS-tBuSorb.11.fid
weC13CPD CDCl3 C:\\ akgulderto 62
211.1
197.4
166.2
141.8
139.2
131.0
118.2
110.7
74.7
72.2
66.9
40.0
34.8
28.6
28.5
24.4
19.0
9.6
Me
HO
Me
OH
O
O
Me
O
Me
Me
Me
21
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

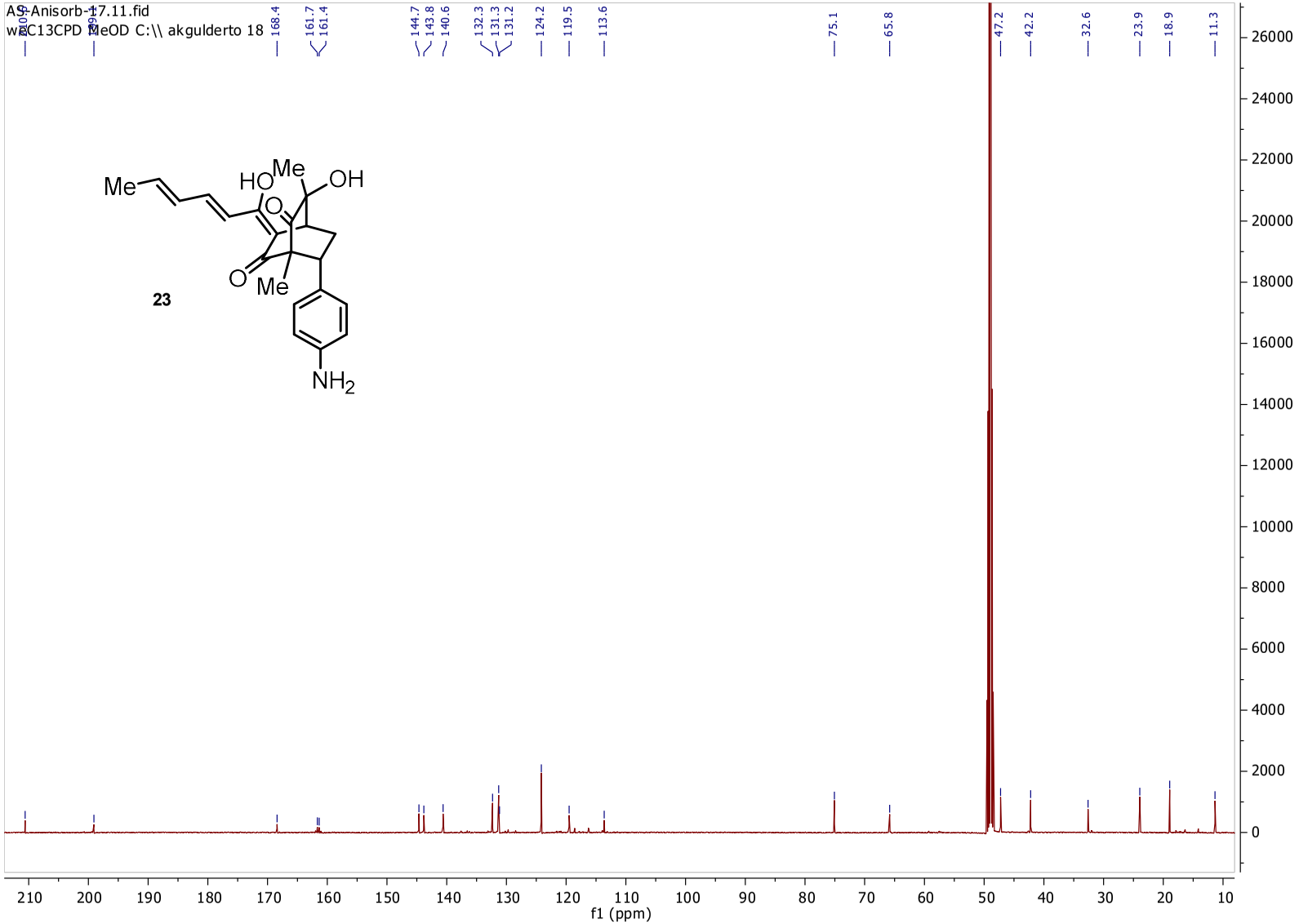
6.18 Compound **22**




AS-2MeStyrene-11.fid
weC13CPD CDCl3 C:\\ akgulderto 9
211.1
198.0
167.3
142.6
140.4
139.9
136.4
131.0
130.5
127.1
127.0
127.0
118.2
112.2
74.8
65.1
41.2
40.5
32.0
24.5
20.4
19.1
9.7
Me
HO
Me
OH
O
O
Me
Me
22
f1 (ppm)

6.19 Compound **23**




AS-Anisorb-17.11.fid
wa C13CPD MeOD C:\\ akgulderto 18
209.0
194.1
168.4
161.7
161.4
144.7
143.8
140.6
132.3
131.3
131.2
124.2
119.5
113.6
75.1
65.8
47.2
42.2
32.6
23.9
18.9
11.3
Me
HO
Me
OH
O
O
Me
NH2
23
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

7. References

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Chemo-Enzymatic Total Synthesis of Sorbicatechol Structural Analogs and Evaluation of Their Anti-Viral Potential

Anna Sib, Alexander Herrmann, Lila Oubraham, Andreas Pichlmair, Ruth Brack-Werner and Tobias A. M. Gulder *

Abstract: The sorbicillinoids are fungal polyketides that are characterized by highly complex and diverse molecular structures with considerable stereochemical intricacy combined with a high degree of oxygenation. Many sorbicillinoids possess promising biological activities. An interesting member of this natural product family is sorbicatechol A, reported to have anti-viral activity, particularly against influenza A virus (H1N1). Utilizing a straightforward, one-pot chemo-enzymatic approach with the oxidoreductase SorbC that was recently developed in our group, we set out to structurally diversify the characteristic bicyclo[2.2.2]octane core of sorbicatechol by variation of its natural 2-methoxyphenol substituent. This facilitated the preparation of a focused library of structural analogs bearing substituted aromatic systems, alkanes, heterocycles and ethers. The fast access to this structural diversity provided an opportunity to explore the anti-viral potential of the sorbicatechol family.

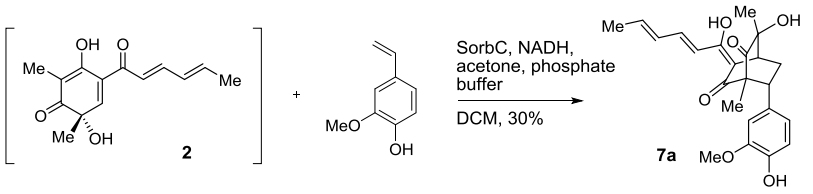
Table of Contents

1. General Information

Chemistry: All solvents used in the reactions were p.A. grade. Solvents for chromatography were technical grade and distilled prior to use. Anhydrous dichloromethane and THF were obtained from an MBraun MB-SPS 800 solvent purification system. Commercial materials were purchased at the highest commercial quality from the providers abcr, Acros, Organics, Alfa Aesar, Carbolution, Carl Roth, Merck, Sigma Aldrich, VWR, Jena Biosciences and Thermo Fisher Scientific. These chemicals were used without further purification. Silica gel Geduran® Si 60 (particle size 0.40 – 0.60 mm) purchased from Merck, was used for flash column chromatography. Solvent mixtures are understood as volume/volume. For TLC analysis, TLC-silica gel 60 F254 plates were purchased from Merck. Applied substances were observed using a UV lamp at 254 nm. For UV-inactive substances, dyeing reagents, such as 0.36% ninhydrin solution in ethanol were used. NMR spectra were recorded on Bruker AVHD300, Bruker AVHD400, Bruker AVHD500 (only ^{1}H NMR spectra), or Bruker AV500-cryo spectrometers. The chemical shifts δ are listed as parts per million [ppm] and refer to δ(TMS) = 0. The spectra were calibrated using residual undeuterated solvent as an internal reference (δ($CDCl_3$) = 7.26 ppm, δ(C_6D_6) = 7.16 ppm, δ(methanol-d_4) = 3.31 ppm for ^{1}H NMR; δ($CDCl_3$) = 77.0 ppm, δ(C_6D_6) = 128.1 ppm, δ(methanol-d_4) = 49.0 ppm for ^{13}C NMR). The following abbreviations (or combinations thereof) are used to explain the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets, q = quartet, m = multiplet, br = broad. In addition, the following abbreviations for chemicals are used: EtOAc = ethyl acetate, Et_2O = diethyl ether, ACN = acetonitrile, TLC = thin layer chromatography, rt = room temperature.
For High Performance Liquid Chromatography (HPLC) analyses, a computer controlled Jasco system was used (UV-1575 Intelligent UV/VIS Detector, DG-2080-53 3-Line Degaser, two PU-1580 Intelligent HPLC Pumps, AS-1550 Intelligent Sampler, HG-1580-32 Dynamic Mixer). The analyses of the recorded chromatograms were performed using Galaxie-Chromatography-Software provided by Jasco. A Eurosphere II 100-3 C18 A (150 x 4.6 mm) column with integrated precolumn manufactured by Knauer was used for analytical separations with the following composition of the eluent: A = H_2O + 0.05% TFA and B = ACN + 0.05% TFA. The analytical method consisted of the following gradient: 0-1 min 5% B, 1-15 min to 95% B, 15-18 min 95% B, 18-18.5 min to 5% B, 18.5-20 min 5% B with a flowrate of 1 mL/min. This method was used for all analyses. Isolation of the products was carried out by semi-preparative HPLC controlled by a Jasco HPLC system consisting of an UV-1575 Intelligent UV/VIS Detector, two PU-2068 Intelligent prep. Pumps, a MIKA 1000 Dynamic Mixing Chamber (1000 µL Portmann Instruments AG Biel-Benken), a LC-NetII/ ADC, and a Rheodyne injection valve. The system was controlled by the Galaxie-Software and the eluent system consisted of: A = H_2O + 0.05% TFA and B = ACN + 0.05% TFA. A Eurosphere II 100-5 C18 A (250 x 16 mm) column with precolumn (30 x 16 mm) provided by Knauer was used as the stationary phase. General HPLC condition: gradient: 0-1 min 95% H2O + 0.05% TFA (A) / 5% acetonitrile + 0.05% TFA (B), 1-40 min 5% A / 95% B, 40-41 min 5% A / 95% B, 41-43 min 95% A / 5% B, 43-45 min 95% A / 5% B, flow rate: 12 mL/min, running time: 45 min The individual gradient compositions are given below. After preparative separation of the product, the collected fractions containing the desired product were combined and the ACN was removed under reduced pressure. The remaining aqueous phases were freeze-dried in liquid nitrogen and the water removed by lyophilization (Alpha 2-4 *Christ* with Chemistry-Hybrid-Pump-RC6 pump). For medium pressure liquid chromatography (MPLC) the Reveleris® X2 MPLC system (Grace) was used together with Reverleris® Reverse Phase (RP) C18 columns (Grace) using UV-detection at 220 nm, 254 nm, and 280 nm. General MPLC conditions: gradient: isocratic, H_2O+ 0.05% TFA /acetonitrile+ 0.05% TFA, proportion: 50:50, flow rate: 40 mL/min, running time: 20.0 min The eluent system was composed as follows: A= H_2O + 0.05% TFA and B= ACN + 0.05% TFA.
For Electrospray ionization mass spectrometry (ESI-MS) a LCQ Fleet Ion Trap mass spectrometer attached to a UltiMate 3000 HPLC system (both *Thermo Scientific*) and controlled by Xcalibur software was used. The analyses of the recorded spectra were performed using Thermo Xcalibur Qual Browser 2.2 SP1.48 Software. For High resolution mass spectrometry (HRMS) a Thermo LTQ FT Ultra mass spectrometer was used and analyses of the recorded spectra were again performed using Thermo Xcalibur Qual Browser 2.2 SP1.48 Software.

Biochemistry/Molecular Biology: PD-10 columns, and Vivaspin 2 Hydrosart membrane columns (30,000 MWCO) were purchased from VWR. Recombinant production and purification of SorbC was conducted as reported previously.[1] Final protein concentrations were determined photometrically using the Nanophotometer 330 (Implen) at 280 nm using the extinction coefficient of SorbC ε(280 nm) = 50920 M^{-1} cm^{-1}. Protein production, enrichment and purification were monitored by SDS-PAGE analysis (BioRad Mini Protean® Tetra System) using Unstained Protein MW Marker (Thermo Scientific). All buffers consisted of 50 mM Tris/Cl at pH 7.5, 150 mM NaCl, and 5% glycerol) with changing concentrations of imidazole (buffer A: 20 mM; buffer B: 250 mM; buffer C: no imidazole). The enzymatic oxidative dearomatization reactions were performed in phosphate buffer (50 mM, pH 8.0).
Anti-viral assays were performed using plate reader (Infinite M200 Pro, Tecan), multispeed vortex (Kisker Biotech) and HeraSafe Laminar Flow Bank. Cell culture flask were provided by Sarstedt and CELLSTAR 96 well plates from Greiner Bio-one. For cell culture media, like antibiotic-antimycotic 100x, DMEM, fetal bovine serum, sodium pyruvate and trypsin-EDTA were obtained from Gibco.

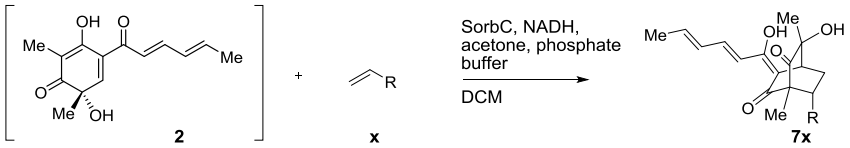
2. Chemo-enzymatic synthesis



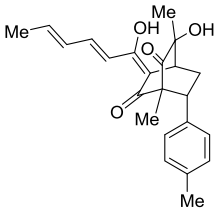
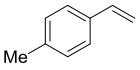
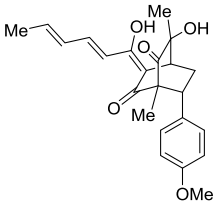
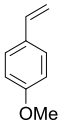
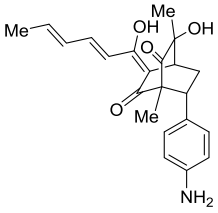
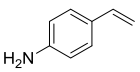
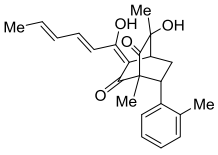
Scheme S1. Synthesis of sorbicatechol A (**7a**).

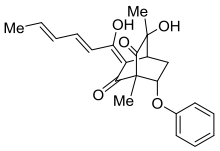
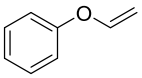
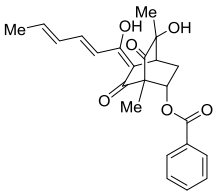
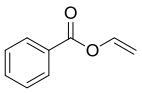
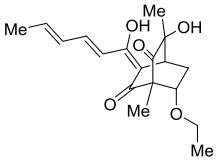
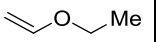
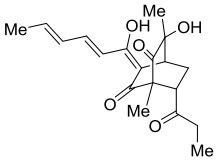
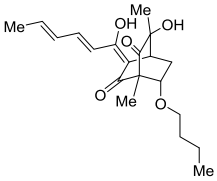
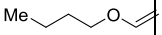
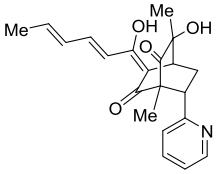
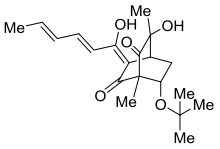
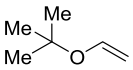
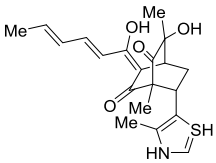
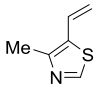
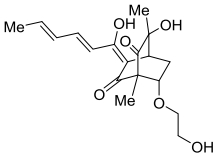
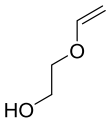
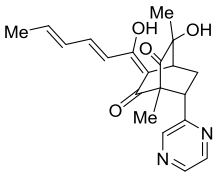
2.1 Enzymatic synthesis of sorbicatechol A (**7a**)[2]

Sorbicillin (**1**) (40.0 mg, 172.4 µmol, 1.0 eq.) was dissolved in acetone (8 mL) and added to phosphate buffer (40 mL, 50 mM, pH = 8) with the enzyme SorbC (1.75 mL, 13.0 mg/mL in Tris buffer). The reaction was started by addition of NADH (150.0 mg, 227.8 µmol, 1.3 eq.) and incubated for 12 h at rt. The reaction mixture was extracted with dichloromethane (3 x 100 mL), 2-Methoxy 4-vinylphenol (72.3 mg, 482.0 µmol, 2.8 eq.) was added and the solvent was evaporated under reduced pressure, to increase the concentration of the dienophile slowly over time. Purification by preperative HPLC (retention time: 24.0 min). The desired product sorbicatechol A (**7a**) was obtained in 30 % isolated yield (20.6 mg, 51.7 µmol, 30%). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 14.33 (s, 1H), 7.37 (dd, *J* = 15.0, 10.8 Hz, 1H), 6.78 (d, *J* = 8.1 Hz, 1H), 6.48 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.44 (d, *J* = 2.1 Hz, 1H), 6.37 – 6.17 (m, 3H), 3.78 (s, 3H), 3.29 (t, *J* = 2.8 Hz, 1H), 3.09 – 2.97 (m, 2H), 1.91 (d, *J* = 6.7 Hz, 3H), 1.84 (m, 1H), 1.26 (s, 3H), 0.92 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 212.0, 198.0, 167.2, 146.6, 145.0, 142.6, 140.1, 133.3, 131.0, 121.6, 118.1, 114.4, 112.2, 110.4, 75.0, 65.2, 55.8, 47.8, 40.6, 31.5, 24.4, 19.1, 10.7. HRMS (ESI+): m/z 399.1802 $[M+H]^+$, calc.: 399.1802. $[\alpha]_D$= -62.0 (c = 1.2 in MeOH). The physical and spectroscopic data were in agreement with that described in the literature.[3]



Scheme S2. Synthesis of Sorbicatechol derivertives.[2]

	Product		Educt n		Product		Educt n
7b		Yield: 22% R_t: 27 min		**7d**		Yield: 27% R_t: 26min	
7c		Yield: 24% R_t: 17min		**7e**		Yield: 22% R_t: 27min	

7f		Yield: 30% R_t: 27 min		**7k**		Yield: 21% R_t: 27min	
7g		Yield: 29% R_t: 23 min		**7l**		Yield: 29% R_t: 22min	
7h		Yield: 32% R_t: 27min		**7m**		Yield: 32% R_t: 18 min	
7i		Yield: 21% R_t: 26min		**7n**		Yield: 31% R_t: 22 min	
7j		Yield: 28% R_t: 18 min		**7o**		Yield: 28% R_t: 22 min	

2.2 Enzymatic synthesis of derivatives **7b-o**

Sorbicillin (**1**) (40.0 mg, 172.4 µmol, 1.0 eq.) was dissolved in acetone (8 mL), and added to phosphate buffer (50 mL, 50 mM, pH = 8) with the enzyme SorbC (3.0 mL, 8.2 mg/mL in Tris buffer). The reaction was started by addition of NADH (150.0 mg, 227.8 µmol, 1.3 eq.) and incubated for 12 h at rt. The reaction mixture was extracted with dichloromethane (3 x 100 mL), vinyl compound **n** (1.0 mL / in excess) was added and the solvent was evaporated under reduced pressure. Purification by preperative HPLC (retention time: R_t). The desired product **7n** was obtained in 21 -32% isolated yield.

7b: ^{1}H NMR (500 MHz, $CDCl_3$) δ= 14.31 (s, 1H), 7.38 (dd, *J* = 15.0, 10.9 Hz, 1H), 7.06 (d, *J* = 8.0 Hz, 2H), 6.84 (d, *J* = 8.1 Hz, 2H), 6.44- 6.16 (m, 3H), 3.29 (t, *J* = 2.9 Hz, 1H), 3.09 (dd, *J* = 10.7, 5.7 Hz, 1H), 3.00 (ddd, *J* = 13.6, 10.7, 3.0 Hz, 1H), 2.29 (s, 3H), 1.91 (d, *J* = 6.7 Hz, 3H), 1.85 (ddd, *J* = 13.4, 5.7, 2.8 Hz ,1H), 1.26 (s, 3H), 0.90 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 211.9, 197.9, 167.2, 142.5, 139.9, 138.4, 137.2, 131.0, 129.5, 129.5, 128.3, 128.3, 118.2, 112.2, 74.9, 64.9, 47.6, 40.6, 31.4, 24.5, 21.1, 19.1, 10.7. HRMS (ESI+): m/z 367.1905 $[M+H]^+$, calc.: 367.1904.

7c: ^{1}H NMR (500 MHz, MeOD) δ= 7.39 (dd, *J* = 14.9, 11.0 Hz, 1H), 7.30 (d, *J* = 8.6 Hz, 2H), 7.17 (d, *J* = 8.6 Hz, 2H), 6.49 (d, *J* = 15.0 Hz, 1H), 6.43 (dd, *J* = 15.0, 11.0 Hz, 1H), 6.25 (dd, *J* = 14.8, 7.1 Hz, 1H), 3.36 – 3.33 (m, 1H), 3.29 (d, *J* = 6.2 Hz, 1H), 3.05 (ddd, *J* = 13.7, 10.6, 3.1 Hz, 1H),1.90 (d, *J* = 6.8 Hz ,3H), 1.82 (ddd, *J* = 13.5, 6.1, 2.7 Hz, 1H), 1.23

(s, 3H), 0.79 (s, 3H). ^{13}C NMR (125 MHz, MeOD) δ= 210.6, 199.1, 168.4, 161.7, 161.4, 144.7, 143.8, 140.6, 132.3, 131.3, 131.2, 124.2, 119.5, 113.6, 75.1, 65.8, 47.3, 42.2, 32.6, 23.9, 18.9, 11.3. HRMS (ESI+): m/z 368.1856 $[M+H]^+$, calc.: 368.1856. $[\alpha]_D$= -7.2 (c = 1.1 in MeOH).

7d: ^{1}H NMR (500 MHz, $CDCl_3$) δ= 14.31 (s, 1H), 7.38 (dd, *J* = 15.0, 10.8 Hz, 1H), 6.88 (d, *J* = 8.7 Hz, 2H), 6.79 (d, *J* = 8.7 Hz, 2H), 6.39- 6.17 (m, 3H), 3.76 (s, 3H), 3.28 (t, *J* = 2.9 Hz, 1H), 3.09 (dd, *J* = 10.8, 5.5 Hz, 1H), 3.00 (ddd, *J* = 13.5, 10.7, 2.9 Hz, 1H), 1.91 (d, *J* = 6.7 Hz, 3H), 1.84 (ddd, *J* = 13.3, 5.6, 2.8 Hz ,1H), 1.26 (s, 3H), 0.90 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 211.9, 197.9, 167.2, 158.9, 142.6, 139.9, 133.4, 131.0, 129.5, 129.5, 118.2, 114.1, 114.1, 112.1, 74.9, 65.1, 55.4, 47.3, 40.6, 31.5, 24.5, 19.1, 10.7. HRMS (ESI+): m/z 383.1854 $[M+H]^+$, calc.: 383.1853.

7e: ^{1}H NMR (500 MHz, $CDCl_3$) δ= 14.40 (s, 1H), 7.38 (dd, *J* = 15.0, 10.7 Hz, 1H), 7.15 – 7.08 (m, 3H), 6.86 (d, *J* = 7.4 Hz, 1H), 6.34 – 6.16 (m, 3H), 3.56 (dd, *J* = 10.7, 6.5 Hz, 1H), 3.29 (s, 3H), 3.01 (t, *J* = 10.6 Hz, 1H), 2.29 (s, 3H), 1.91 (d, *J* = 6.6 Hz, 3H), 1.77 (ddd, *J* = 13.5, 6.6, 2.5 Hz, 1H), 1.28 (s, 3H), 0.93 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 211.6, 198.0, 167.3, 142.6, 140.4, 139.9, 136.4, 131.0, 130.5, 127.1, 127.0, 127.0, 118.2, 112.2, 74.8, 65.1, 41.2, 40.5, 32.0, 24.5, 20.4, 19.1, 9.7. HRMS (ESI+): m/z 367.1902 $[M+H]^+$, calc.: 367.1904. $[\alpha]_D$= +86.8 (c = 0.7 in MeOH).

7f: ^{1}H NMR (500 MHz, $CDCl_3$) δ= 14.01 (s, 1H), 7.31 (dd, *J* = 14.9, 10.7 Hz, 1H), 7.26 – 7.22 (m, 2H), 6.95 (t, *J* = 7.4 Hz, 1H), 6.80 (d, *J* = 8.1 Hz, 2H), 6.29 – 6.14 (m, 3H), 4.42 (dd, *J* = 8.2, 2.2 Hz, 1H), 3.21 (t, *J* = 3.0 Hz, 1H), 3.01 (ddd, *J* = 14.2, 8.2, 2.5 Hz, 1H), 1.88 (d, *J* = 6.5 Hz, 3H), 1.79 (dt, *J* = 14.1, 2.9 Hz, 1H), 1.37 (s, 3H), 1.26 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 210.3, 195.9, 167.1, 157.0, 142.5 139.8, 130.9, 129.7, 129.7, 121.6, 117.9, 115.7, 115.7, 110.2, 77.3, 74.7, 66.4, 39.9, 31.0, 24.5, 19.1, 9.2 . HRMS (ESI+): m/z 369.1695 $[M+H]^+$, calc.: 369.1697. $[\alpha]_D$= +232.6 (c = 0.8 in MeOH).

7g: ^{1}H NMR (500 MHz, $CDCl_3$) δ= 13.95 (s, 1H), 7.29 (dd, *J* = 14.9, 10.8 Hz, 1H), 6.33 – 6.09 (m, 3H), 3.59 – 3.51 (m, 2H), 3.36 (dd, *J* = 9.6, 7.0 Hz, 1H), 3.15 (t, *J* = 3.0 Hz, 1H), 2.78 (ddd, *J* = 13.8, 8.4, 2.6 Hz, 1H), 1.89 (d, *J* = 6.8 Hz, 3H), 1.68 (dt, *J* = 13.8, 3.0 Hz, 1H), 1.31 (s, 3H), 1.20 (s, 3H), 1.12 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 211.1, 196.6, 166.6, 142.0, 139.4, 131.0, 118.1, 110.5, 79.3, 74.7, 67.1, 65.8, 39.9, 30.8, 24.5, 19.0, 15.2, 9.1. HRMS (ESI+): m/z 321.1695 $[M+H]^+$, calc.: 321.1696. $[\alpha]_D$= +285.7 (c = 0.7 in MeOH). The physical and spectroscopic data were in agreement with that described in the literature.[4]

7h: ^{1}H NMR (500 MHz, $CDCl_3$) δ= 13.94 (s, 1H), 7.29 (dd, *J* = 14.2, 10.0 Hz, 1H), 6.37 – 6.06 (m, 3H), 3.54 (dd, *J* = 8.3, 2.5 Hz, 1H), 3.48 (dt, *J* = 9.4, 6.4 Hz 1H), 3.32 – 3.23 (m, 1H), 3.15 (t, *J* = 3.0 Hz, 1H), 2.76 (ddd, *J* = 13.8, 8.3, 2.6 Hz, 1H), 1.89 (d, *J* = 6.7 Hz, 3H), 1.67 (dt, *J* = 13.7, 3.1 Hz, 1H), 1.51 – 1.41 (m, 2H), 1.32 (s, 3H), 1.35 – 1.25 (m, 2H), 1.20 (s, 3H), 0.86 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 211.1, 196.6, 166.4, 141.9, 139.3, 131.0, 118.2, 110.5, 79.6, 74.8, 70.1, 67.3, 39.9, 31.8, 30.6, 24.5, 19.4, 19.0, 14.0, 9.1. HRMS (ESI+): m/z 349.2008 $[M+H]^+$, calc.: 349.2010. $[\alpha]_D$= +253.4 (c = 0.7 in MeOH). The physical and spectroscopic data were in agreement with that described in the literature.[5]

7i: ^{1}H NMR (500 MHz, $CDCl_3$) δ= 14.00 (s, 1H), 7.31 (dd, *J* = 14.9, 10.7 Hz, 1H), 6.30 – 6.14 (m, 3H), 3.73 (dd, *J* = 8.5, 2.6 Hz, 1H), 3.11 (t, *J* = 3.0 Hz, 1H), 2.81 (ddd, *J* = 13.7, 8.5, 2.6 Hz, 1H), 1.89 (d, *J* = 6.8 Hz, 3H), 1.64 (dt, *J* = 13.7, 3.1 Hz, 1H), 1.24 (s, 3H), 1.19 (s, 3H), 1.11 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 211.8, 197.0, 166.2, 141.8, 139.2, 131.0, 118.2, 110.7, 74.7, 72.2, 66.9, 40.0, 34.9, 28.6, 28.6, 28.6, 28.5, 24.4, 19.0, 9.6. HRMS (ESI+): m/z 349.2011 $[M+H]^+$, calc.: 349.2010. $[\alpha]_D$= +145.3 (c = 0.7 in MeOH).

7j: ^{1}H NMR (500 MHz, $CDCl_3$) δ= 13.93 (s, 1H), 7.31 (dd, *J* = 14.9, 10.8 Hz, 1H), 6.32- 6.16 (m, 3H), 3.69 – 3.59 (m, 4 H), 3.43 (ddd, *J* = 9.5, 5.7, 3.4 Hz, 1H), 3.17 (t, *J* = 3.2 Hz, 1H), 2.82 (ddd, *J* = 13.9, 8.3, 2.6 Hz, 1H), 1.89 (d, *J* = 6.5 Hz ,3H), 1.70 (dt, *J* = 13.8, 3.0 Hz, 1H), 1.34 (s, 3H), 1.21 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 210.5, 196.3, 166.9, 142.4, 139.8, 131.0, 117.9, 110.4, 80.0, 74.6, 71.4, 67.1, 61.9, 39.8, 30.5, 24.4, 19.1, 9.2. HRMS (ESI+): m/z 337.1646 $[M+H]^+$, calc.: 337.1646.

7k: ^{1}H NMR (500 MHz, $CDCl_3$) δ= 7.99 (dd, *J* = 8.3, 1.4 Hz, 2H), 7.59 (td, *J* = 7.3, 1.4 Hz, 1H), 7.45 (t, *J* = 7.8 Hz, 2H), 7.43 – 7.39 (m, 1H), 6.81 (d, *J* = 15.0 Hz, 1H), 6.39- 6.28 (m, 2H), 3.23 (ddd, *J* = 15.3, 9.4, 3.0 Hz, 1H), 3.15 (t, *J* = 2.9 Hz, 1H), 2.47 (dt, *J* = 15.3, 2.9 Hz, 1H), 1.92 (d, *J* = 5.8 Hz ,3H), 1.91 – 1.88 (m, 1H), 1.31 (s, 3H), 1.26 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 205.3, 197.0, 190.7, 165.5, 165.2, 148.0, 144.5, 130.3, 130.0, 130.0, 129.9, 129.2, 128.8, 128.8, 121.3, 74.5, 73.0, 67.6, 46.5, 27.2, 24.3, 19.3, 9.8. HRMS (ESI+): m/z 397.1646 $[M+H]^+$, calc.: 397.1646.

7l: ^{1}H NMR (500 MHz, $CDCl_3$) δ= 14.08 (s, 1H), 7.30 (dd, *J* = 14.9, 10.7 Hz, 1H), 6.29- 6.13 (m, 3H), 3.20 (t, *J* = 2.8 Hz, 1H), 3.04 (dd, *J* = 10.9, 5.8 Hz, 1H), 2.74 (ddd, *J* = 13.8, 11.0, 3.0 Hz, 1H), 2.42 (dd, *J* = 11.4, 7.2 Hz, 2H), 1.89 (d, *J* = 6.8 Hz ,3H), 1.58 (ddd, *J* = 12.8, 5.8, 2.8 Hz, 1H), 1.21 (s, 3H), 1.20 (s, 3H), 1.01 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 210.6, 210.0, 196.4, 167.0, 142.3, 139.6, 131.0, 118.0, 110.8, 74.9, 61.7, 50.7, 40.3, 37.6, 26.3, 24.3, 19.0, 10.4, 7.5. HRMS (ESI+): m/z 333.1698 $[M+H]^+$, calc.: 333.1697.

7m: ^{1}H NMR (500 MHz, $CDCl_3$) δ= 14.25 (s, 1H), 8.77 (d, *J* = 4.1 Hz ,1H), 8.14 (t, *J* = 7.5 Hz ,1H), 7.67 – 7.65 (m, 1H), 7.41 (dd, *J* = 14.8, 10.6 Hz, 1H), 7.35 (d, *J* = 7.9 Hz ,1H), 6.36- 6.23 (m, 3H), 4.00 (dd, *J* = 10.2, 5.3 Hz, 1H), 3.39 – 3.37 (m, 1H), 3.23 (t, *J* = 11.6 Hz, 1H),1.92 (d, *J* = 6.4 Hz ,3H), 1.87 – 1.83 (m, 1H), 1.31 (s, 3H), 0.97 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 208.0, 195.9, 168.8, 158.5, 143.9, 143.7, 143.6, 141.2, 131.0, 124.8, 124.7, 117.7, 111.8, 74.5, 63.2, 44.4, 40.6, 30.8, 24.2, 19.2, 10.4. HRMS (ESI+): m/z 354.1701 $[M+H]^+$, calc.: 354.1700.

7n: ^{1}H NMR (500 MHz, $CDCl_3$) δ= 14.32 (s, 1H), 8.97 (s, 1H), 7.42 (dd, *J* = 14.8, 10.1 Hz, 1H), 6.32- 6.22 (m, 3H), 3.60 (dd, *J* = 10.6, 4.8 Hz, 1H), 3.27 (t, *J* = 2.9 Hz, 1H), 3.16 (ddd, *J* = 13.4, 10.7, 2.5 Hz, 1H), 2,45 (s, 3H), 1.92 (d, *J* = 6.0 Hz ,3H), 1.71 (ddd, *J* = 13.8, 4.9, 3.1 Hz, 1H), 1.27 (s, 3H), 1.01 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 210.5, 196.0,

169.0, 152.8, 147.7, 143.9, 141.2, 135.6, 130.9, 117.6, 111.4, 74.8, 64.7, 40.3, 39.8, 33.4, 24.5, 19.2, 14.3, 10.1. HRMS (ESI+): m/z 374.1422 $[M+H]^+$, calc.: 374.1421.

7o: ^{1}H NMR (500 MHz, $CDCl_3$) δ= 14.10 (s, 1H), 8.57 (s, 1H), 8.43 (d, *J* = 2.5 Hz ,1H), 8.33 (d, *J* = 1.6 Hz ,1H), 7.36 (dd, *J* = 14.9, 10.8 Hz, 1H), 6.38- 6.16 (m, 3H), 3.37 (dd, *J* = 10.5, 6.0 Hz, 1H), 3.33 (t, *J* = 3.0 Hz, 1H), 2.94 (ddd, *J* = 13.4, 10.5, 3.2 Hz, 1H), 2.05 (ddd, *J* = 13.1, 6.1, 2.7 Hz, 1H), 1.91 (d, *J* = 6.7 Hz ,3H), 1.30 (s, 3H), 0.93 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 210.7, 196.4, 167.0, 157.1, 145.4, 143.7, 142.4, 142.3, 139.7, 131.0, 118.2, 111.9, 74.9, 63.5, 45.7, 40.5, 29.4, 24.4, 19.1, 10.7. HRMS (ESI+): m/z 355.1652 $[M+H]^+$, calc.: 355.1652.

3. Anti-viral Screening

3.1 HIV - Tests

I. HIV Full virus Screening (EASY-HIT). The EASY-HIT assay is based on HIV-1 susceptible reporter cells (LC5-RIC) that contain a stably integrated fluorescent reporter gene that is activated upon successful HIV-1 infection and expression of the early viral protein HIV-Rev and HIV-Tat. Briefly, LC5-RIC cells were seeded into black 96-well plates at a density of 10,000 cells per well 24 hours before infection and treatment of the cells. Compounds stocks dissolved at 100 mM in DMSO were screened at multiple concentrations from 3 to 100 µM at a final DMSO concentration of 0.1 % to establish IC_{50} curves. After compound addition, LC5-RIC cells were infected by adding HIV-1_{LAI} inoculum at an MOI of 0.5 to each well of the plate. Cells were incubated at 37 °C, 5 % CO_2 for 48 hours after infection and then measured for reporter expression. Reporter expression was determined by measuring the total fluorescent signal intensity of each well using a fluorescence microplate reader at an excitation filter wavelength of 552 nm and an emission filter wavelength of 596 nm.

II. Cell viability assays. Cell viability of LC5-RIC cultures exposed to HIV-1_{LAI} inoculum and test compounds was determined by performing a CellTiter-Blue® cell viability assay (Promega) and monitoring the ability of metabolically active cells to convert the redox dye resazurin into the fluorescent product resorufin. LC5-RIC cells were seeded into black 96-well plates at a density of 10,000 cells per well followed by overnight incubation at 37 °C, 5 % CO_2. Compounds stocks dissolved at 100 mM in DMSO were screened at multiple concentrations from 3 to 100 µM at a final DMSO concentration of 0.1 % followed by an additional 48 hours incubation. After the designated incubation time, CTB reagent (1:5 in cell culture medium) was added to each well. CTB containing plates were incubated for an additional hour after which fluorescence signal of resorufin was measured using a fluorescence microplate reader at an excitation filter wavelength of 550 nm and an emission filter wavelength of 600 nm.

3.2 Influenza – Tests

I. Influenza A inhibition and cell viability assay: A549 cells were seeded into 96-well-plates at a density of 20.000 cells per well 24 hours before infection and treatment on the cells. Compounds stocks (**5**, **5b**, **5m**, **5k**) dissolved at 500 mM in DMSO were screened at multiple concentrations from 5 mM serially diluted in DMEM to 0,25 µM (1:3). After compound addition, A549 cells were infected with Influenza A virus (strain SC35M) encoding Gaussia luciferase (PMID: 26068081) at an MOI of 0,01. Cells were incubated at 37°C, 5% CO_2 and gaussia luciferase accumulation was evaluated after 24, 48 and 72 hours. For testing cell viability, a reazurine reduction assay was performed at 48h post drug treatment (Citea PMID: 29255269).

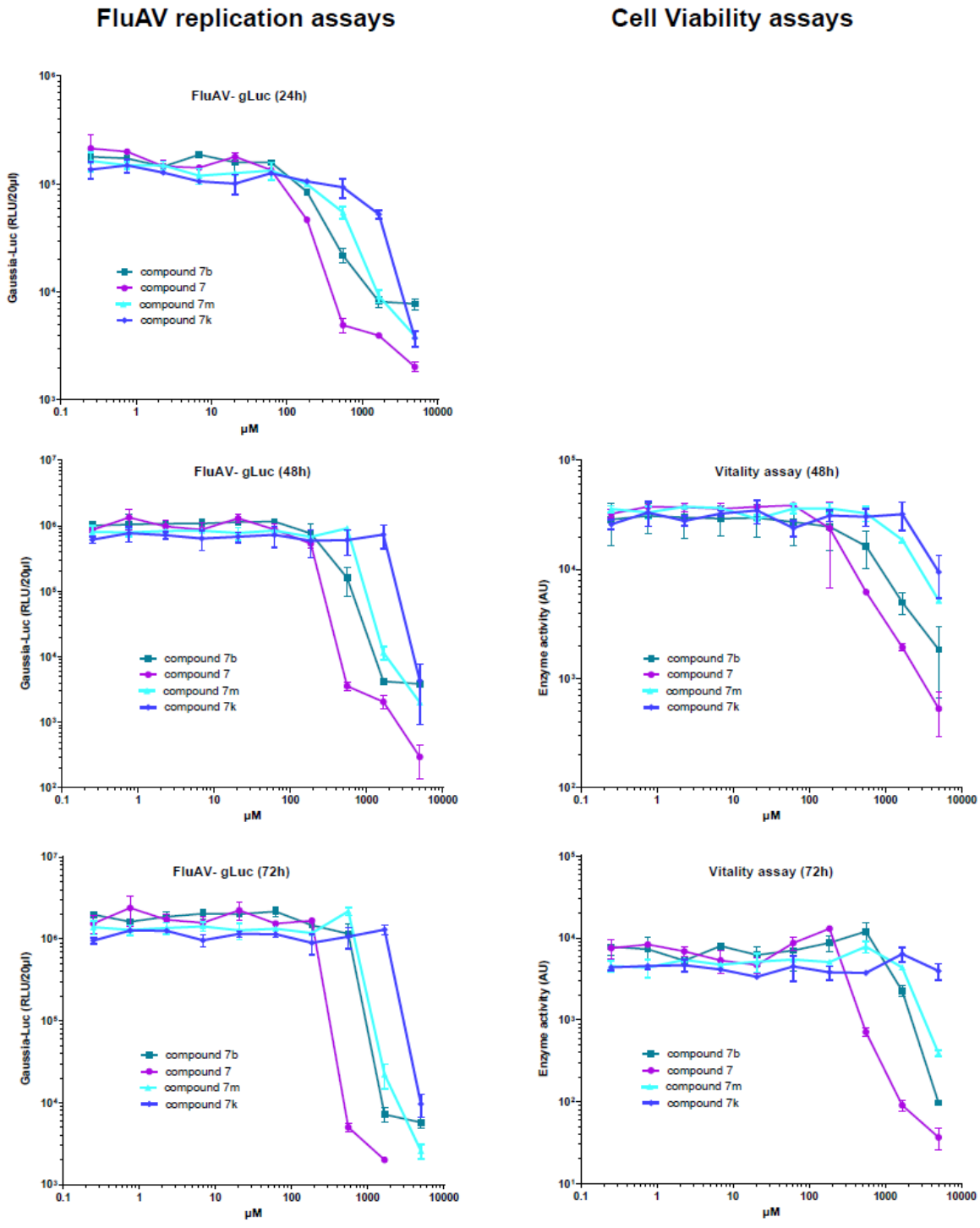
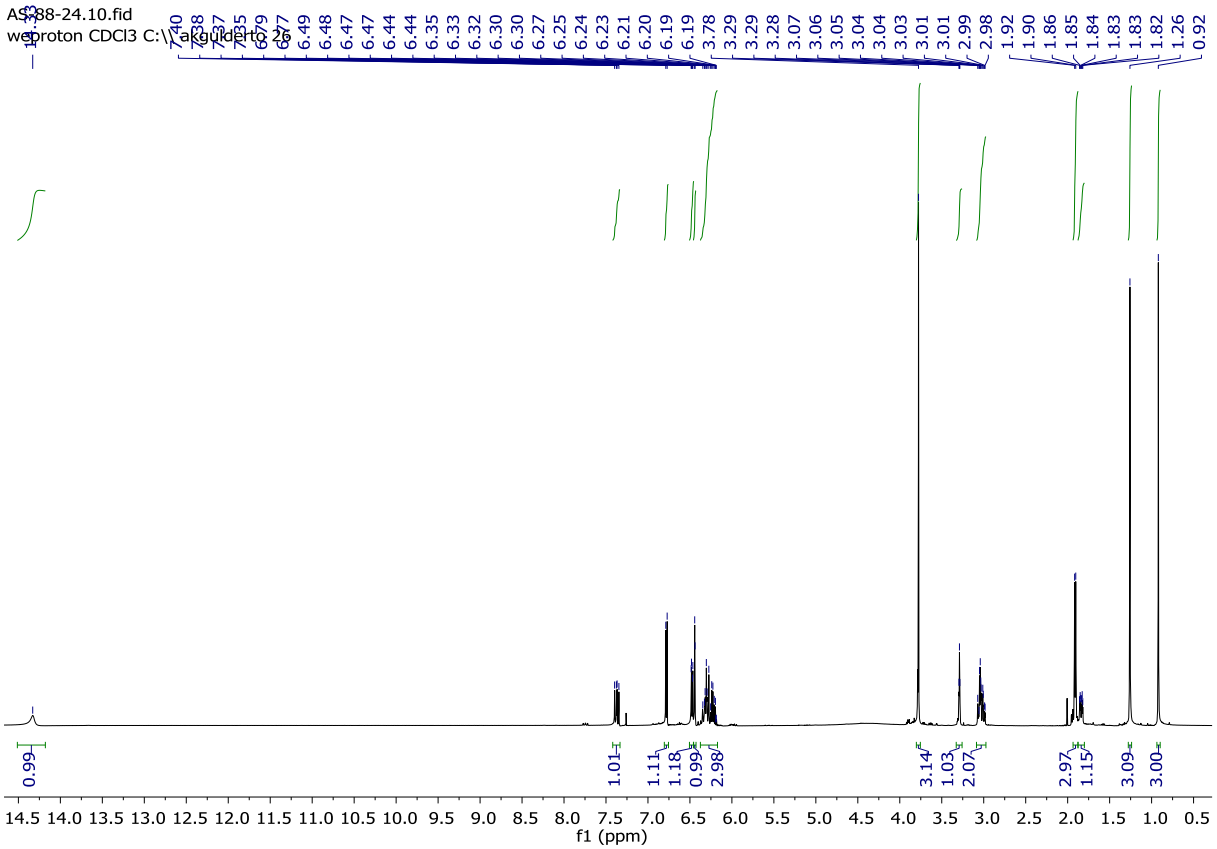
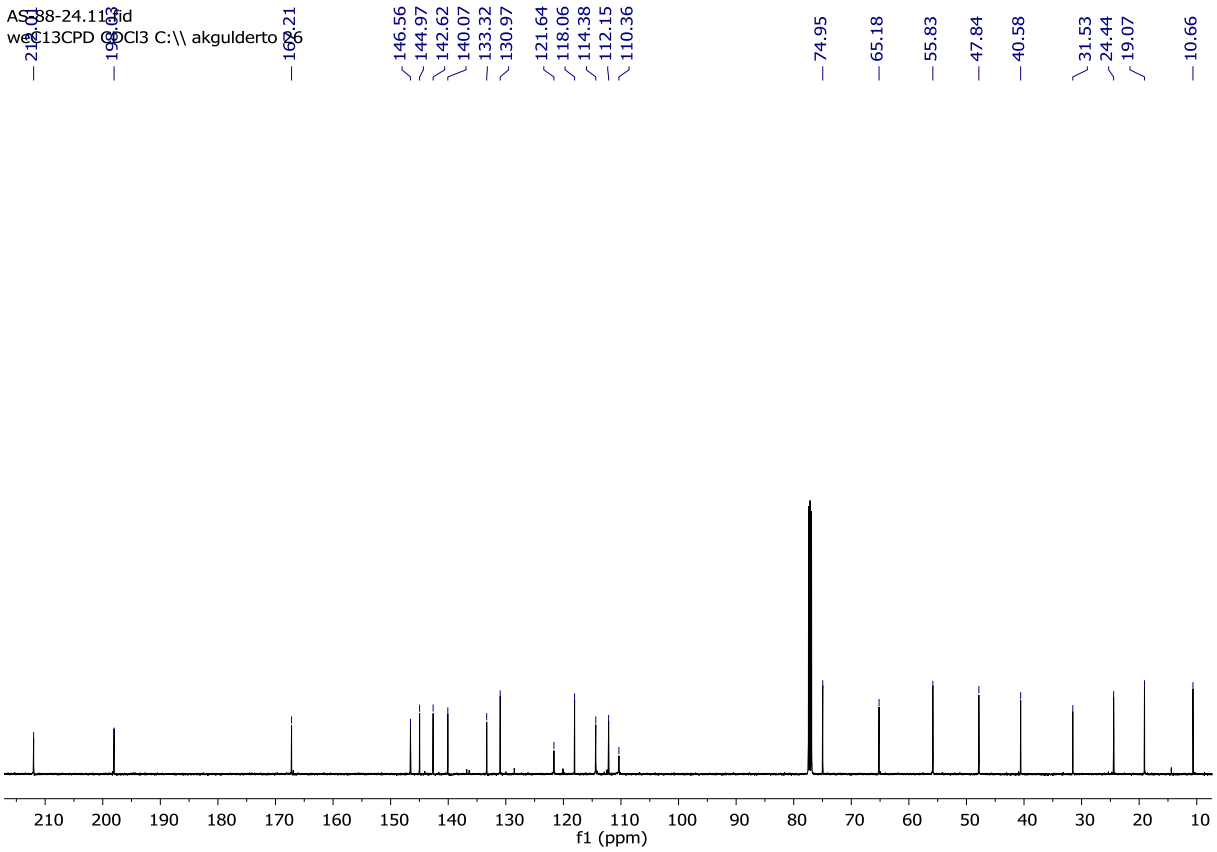


Figure S1. Activity of compounds on influenza A virus replication. Cells were treated with the indicated concentration of compound and infected with influenza A virus expressing gaussia luciferase. 24h, 48h and 72h later the supernatant was assayed for accumulation of gaussia luciferase (left). At 48h and 72h a resazurine conversion assay was used to test cell viability. Graph show representative experiments. Error bars: SD of duplicate measurements.

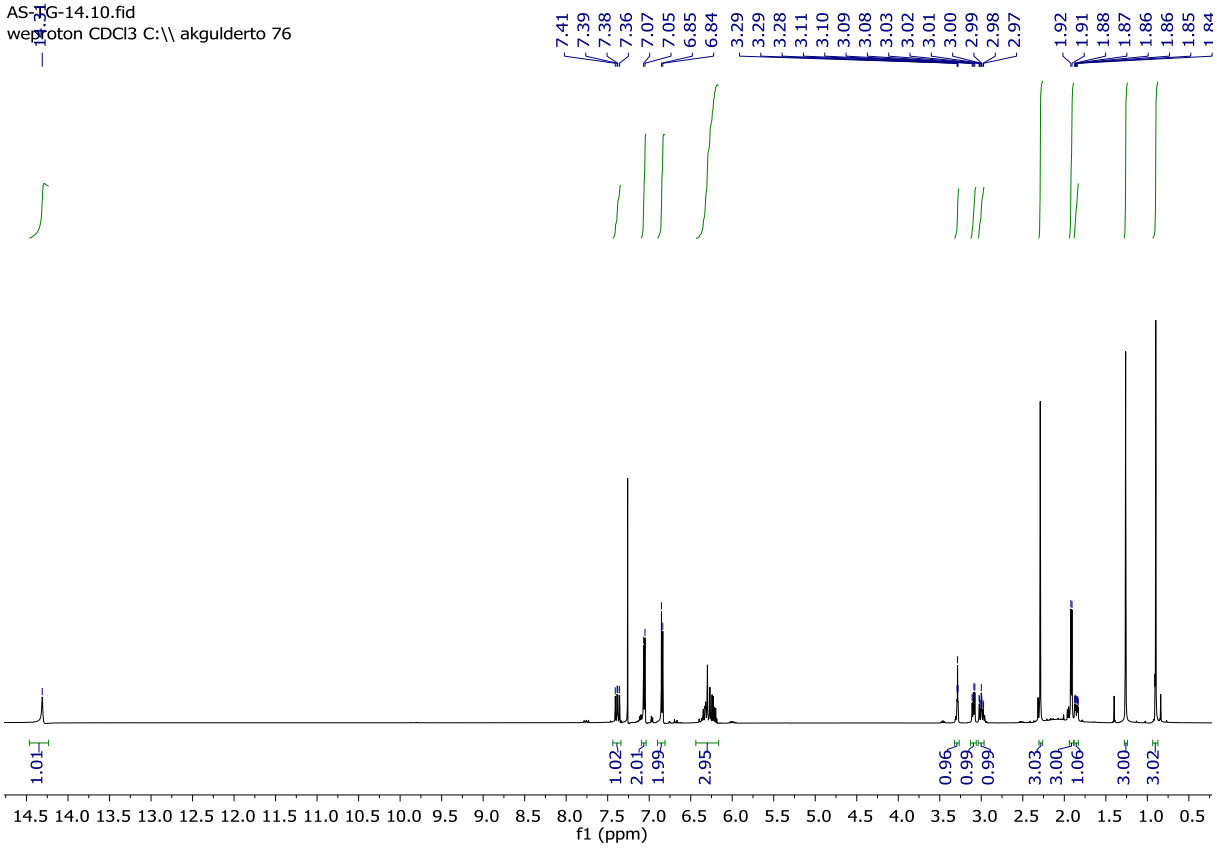
4. NMR-Spectra

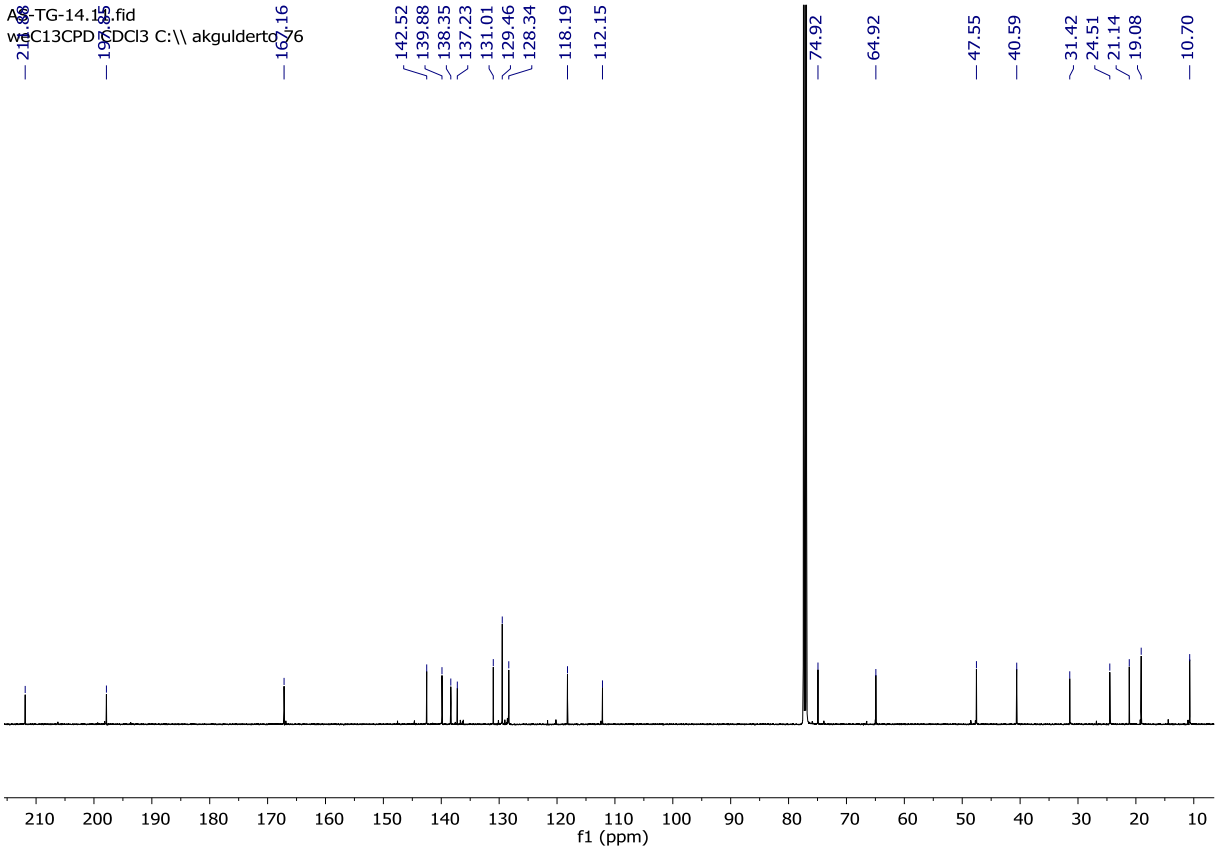
4.1 Sorbicatechol A (**7a**)



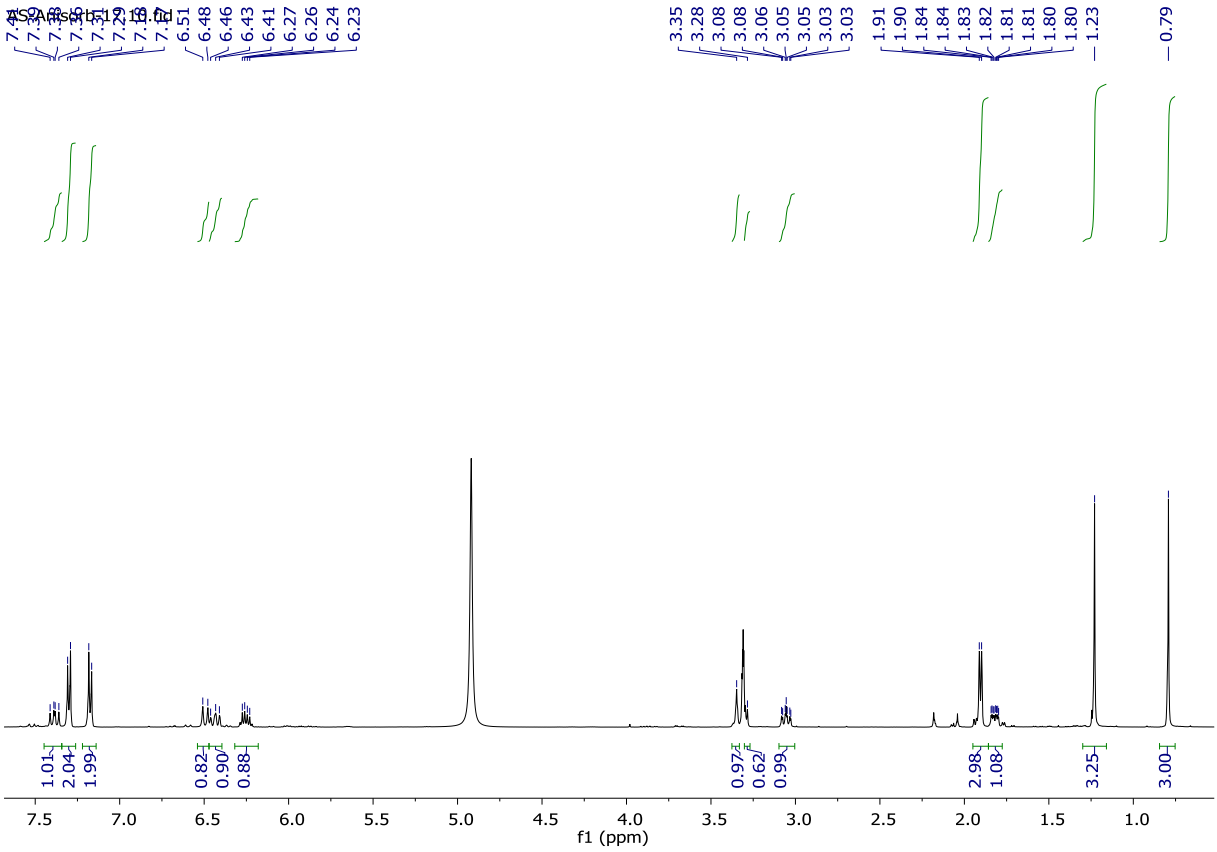


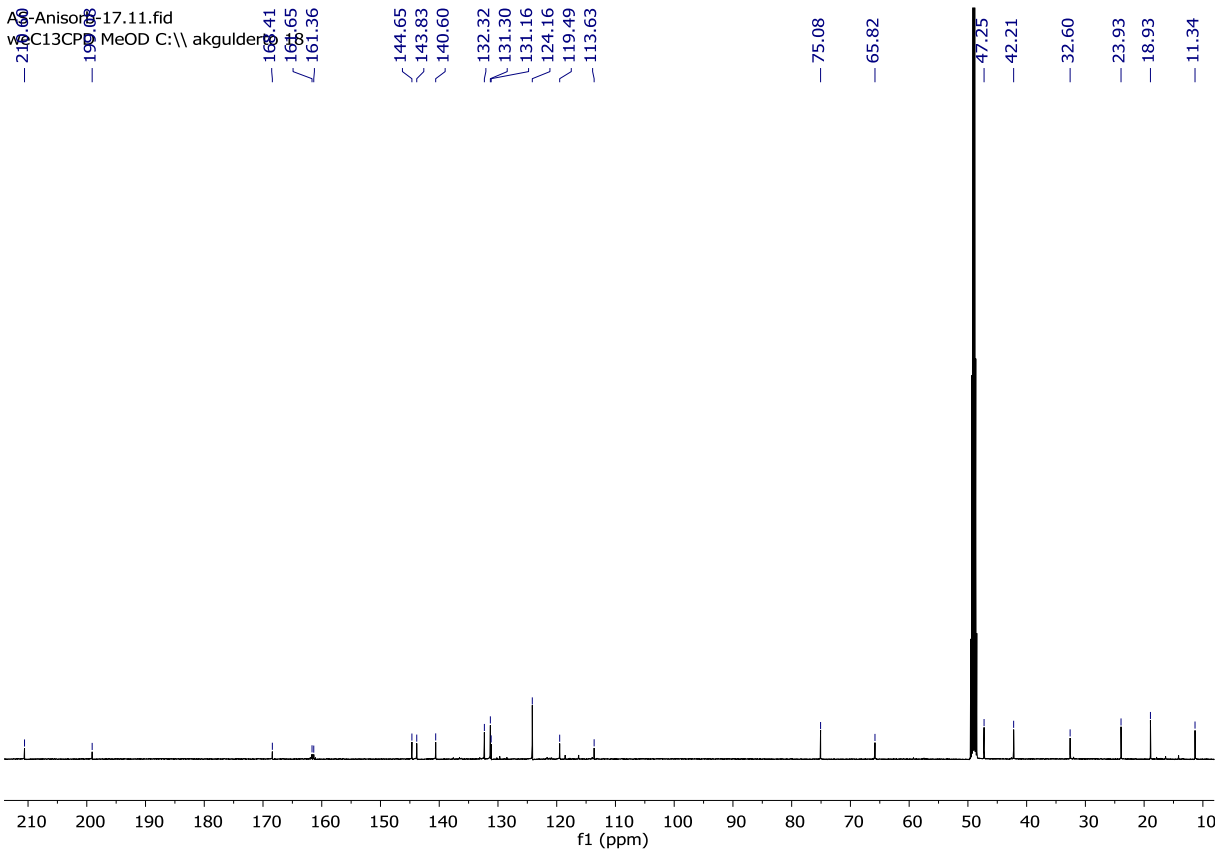
4.2 Compound **7b**



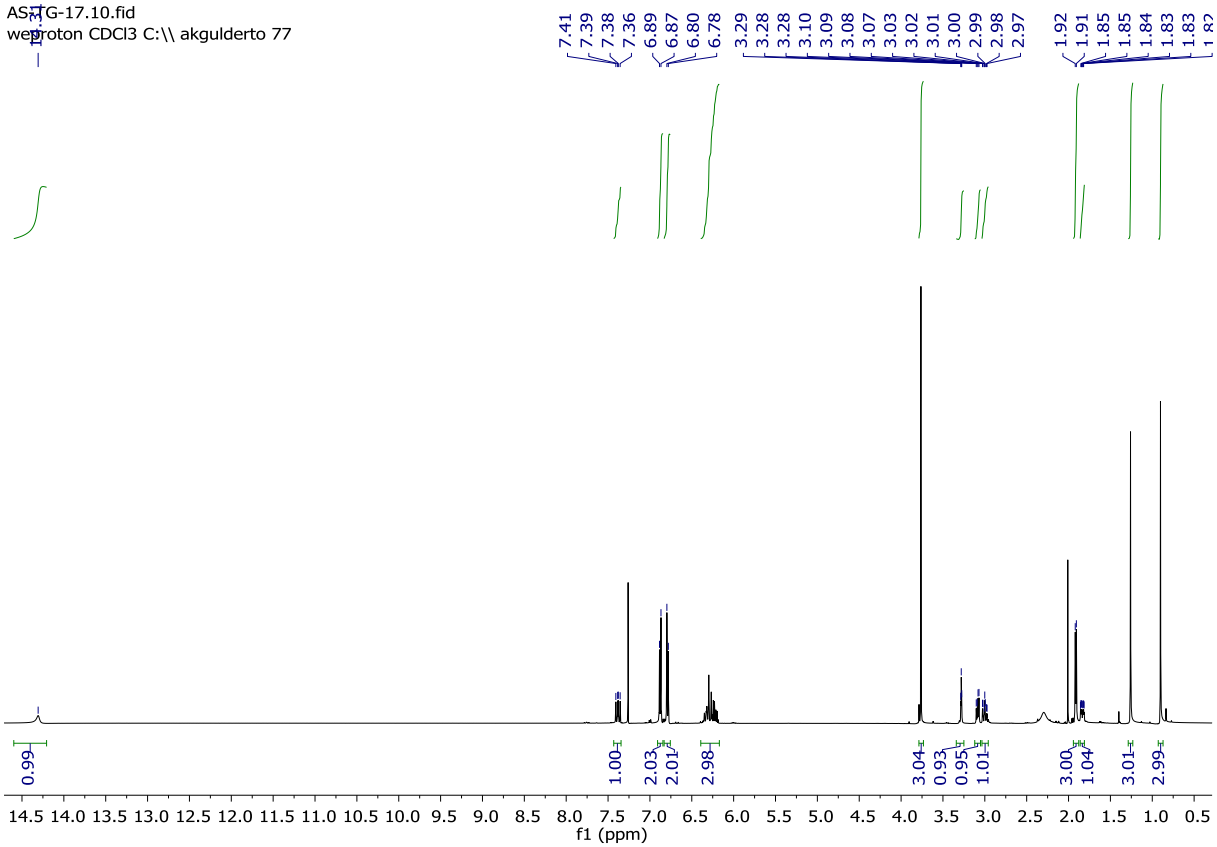


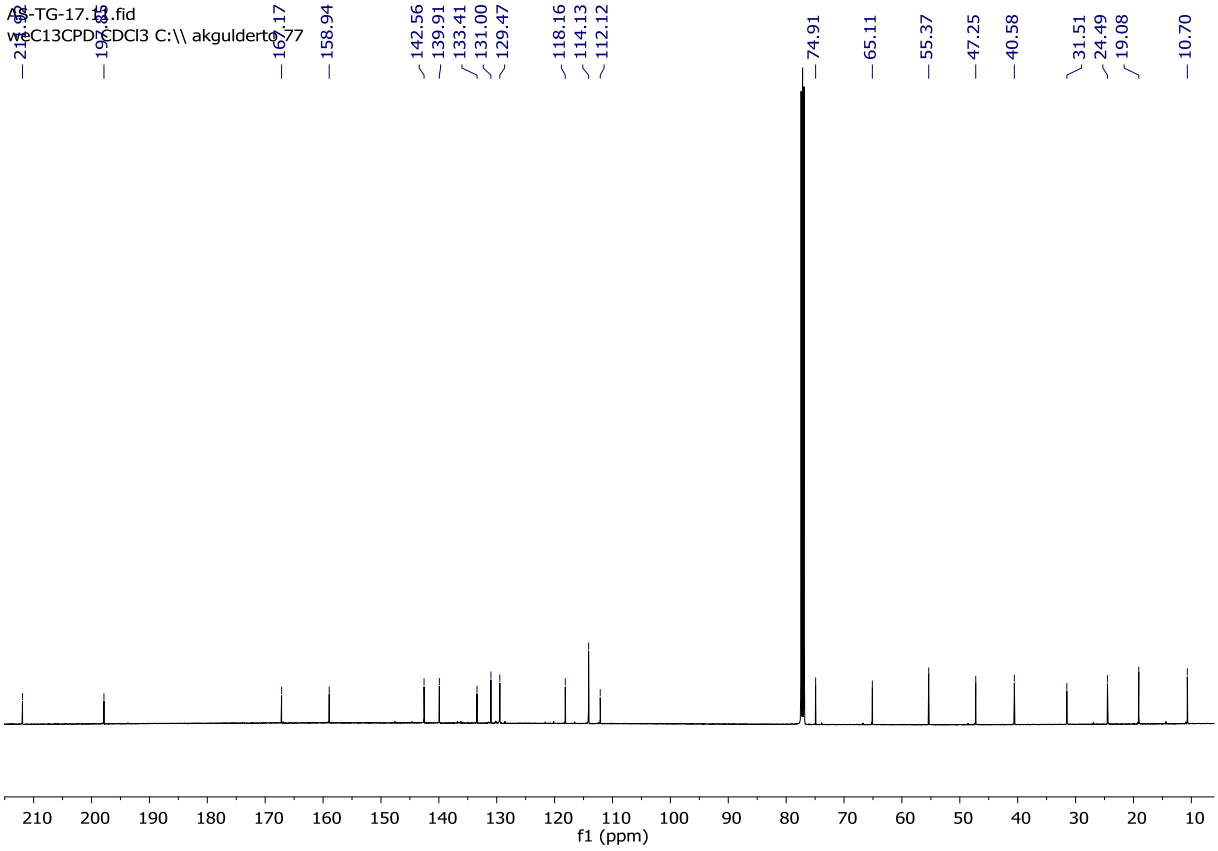
4.3 Compound **7c**



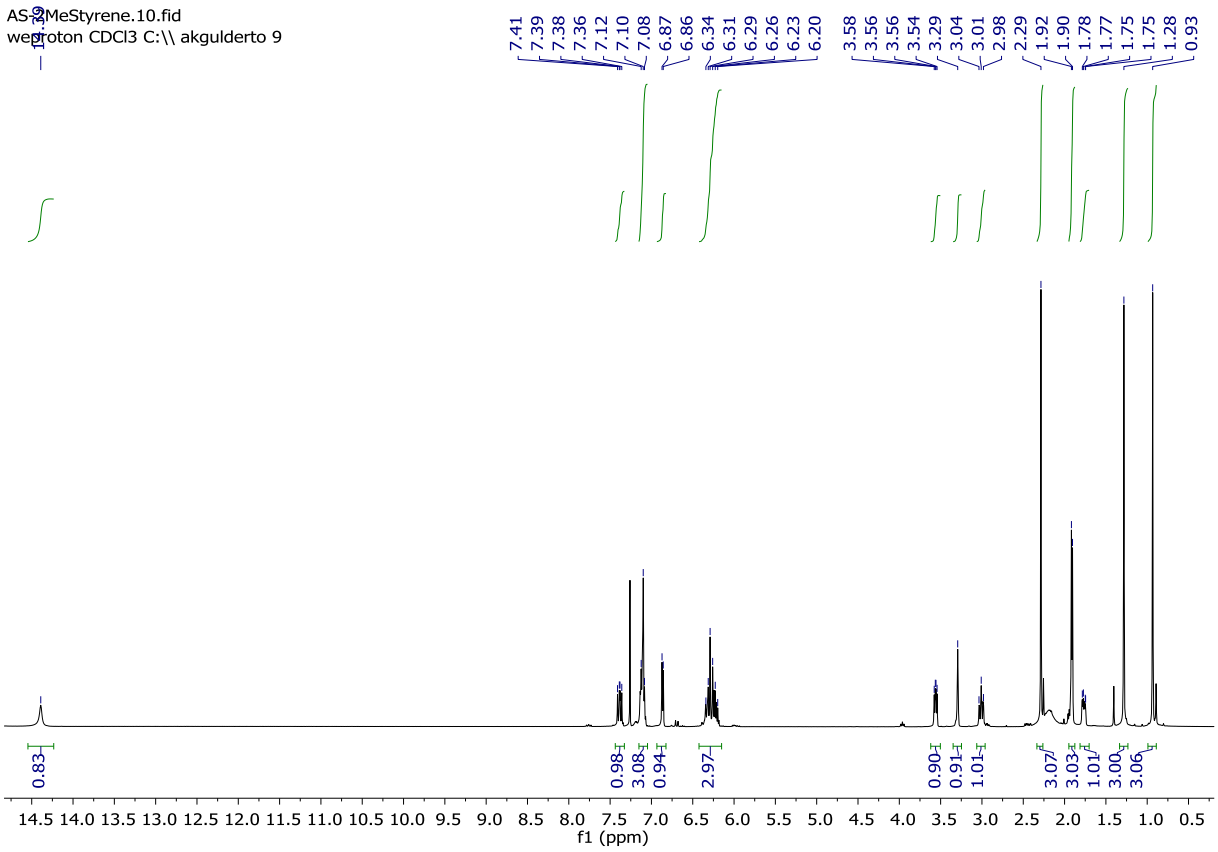


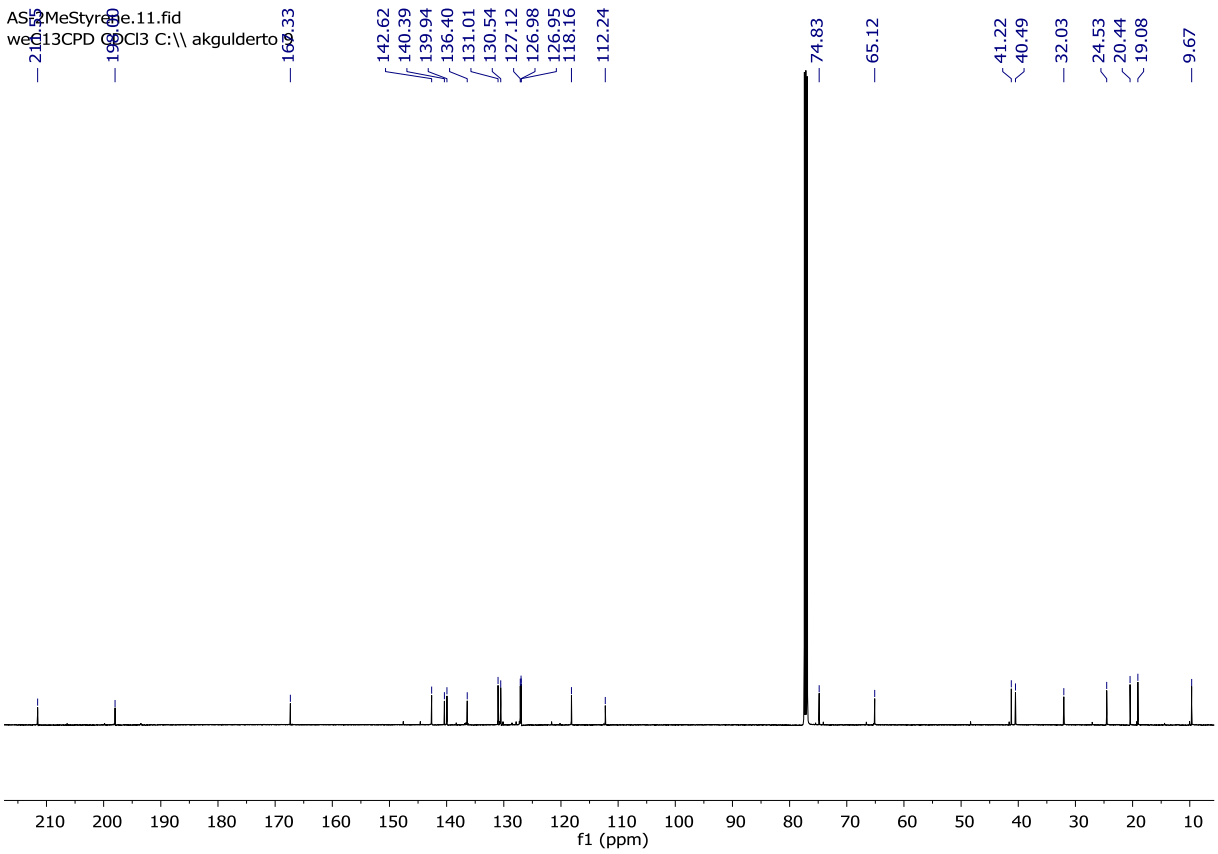
4.4 Compound **7d**



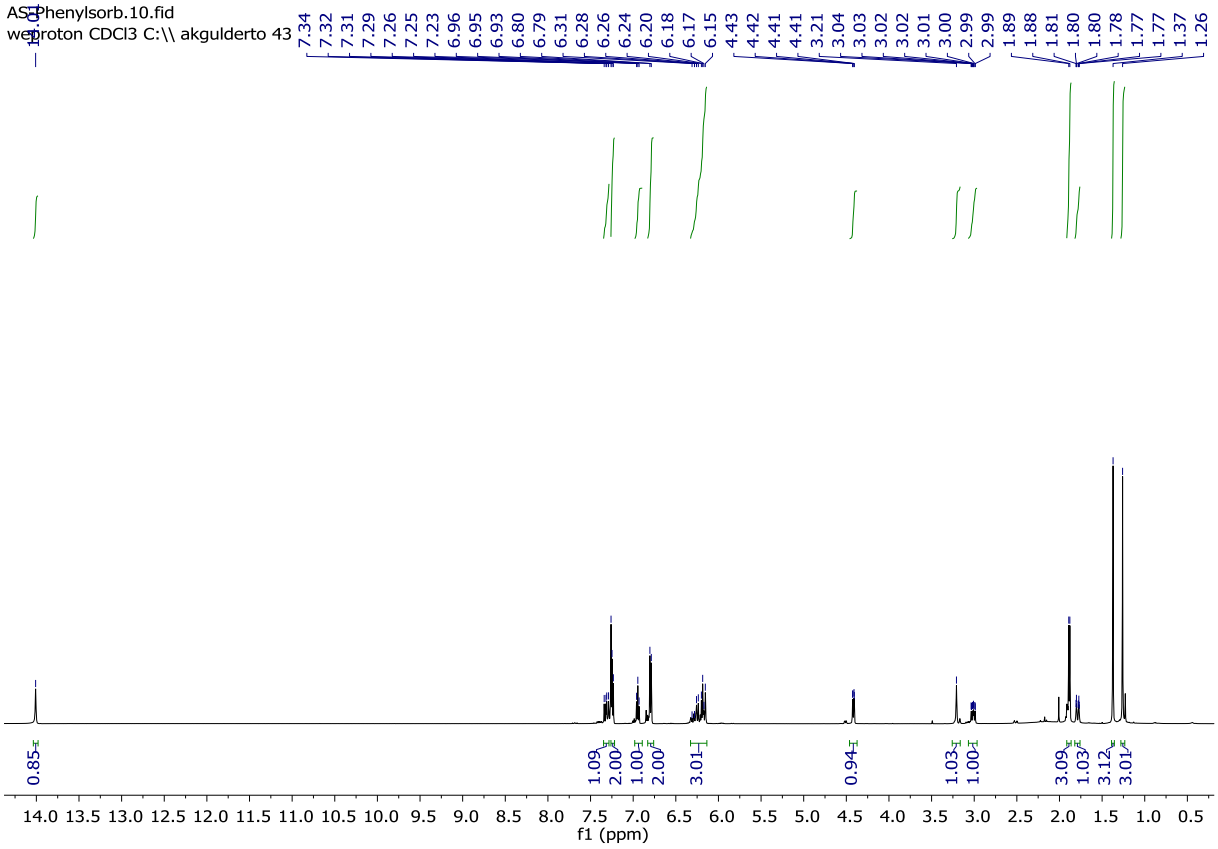


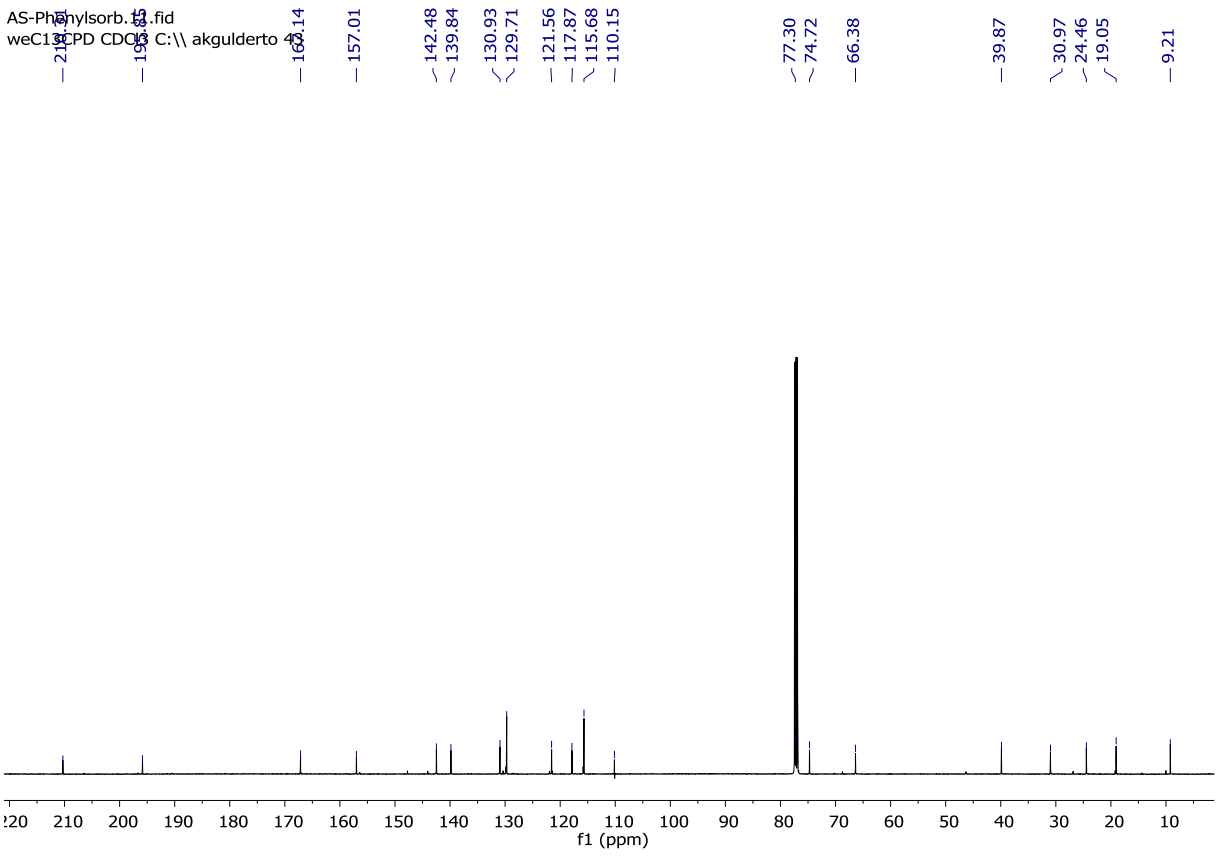
4.5 Compound **7e**



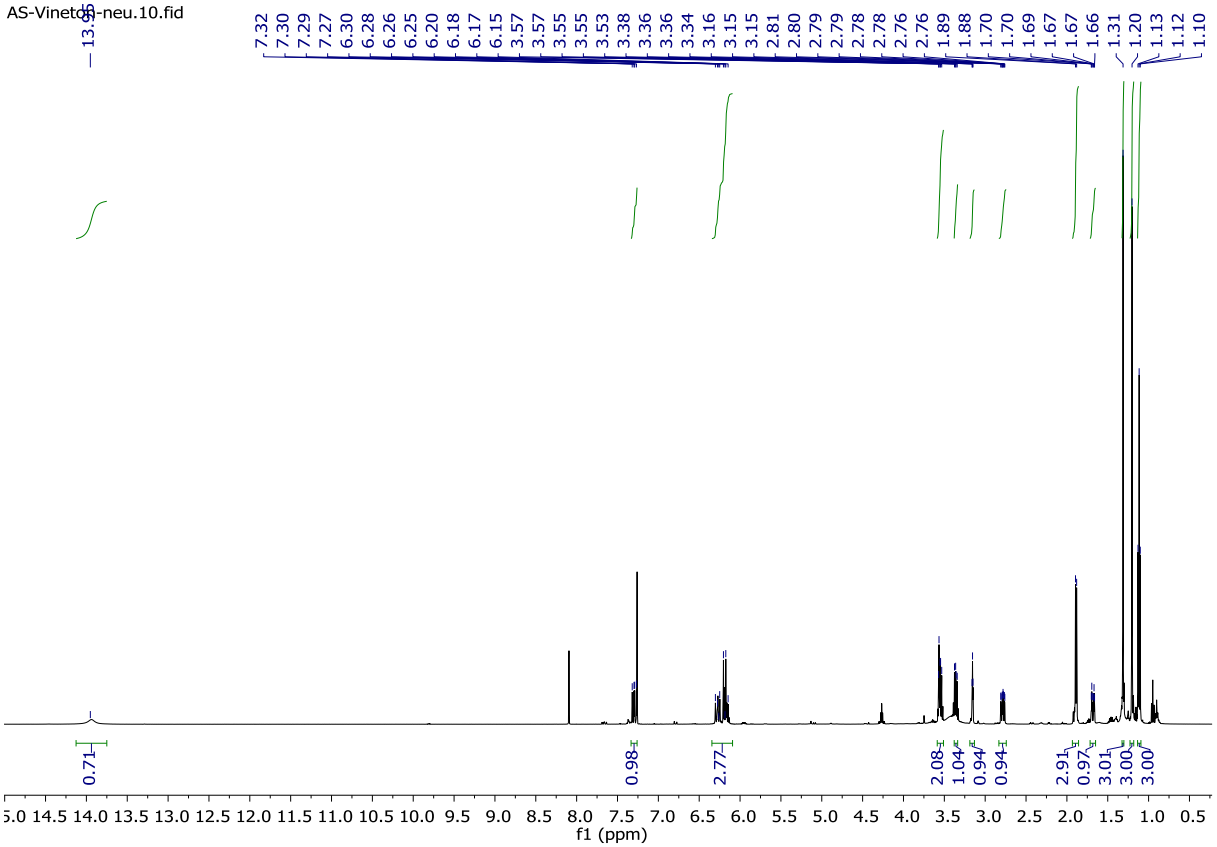


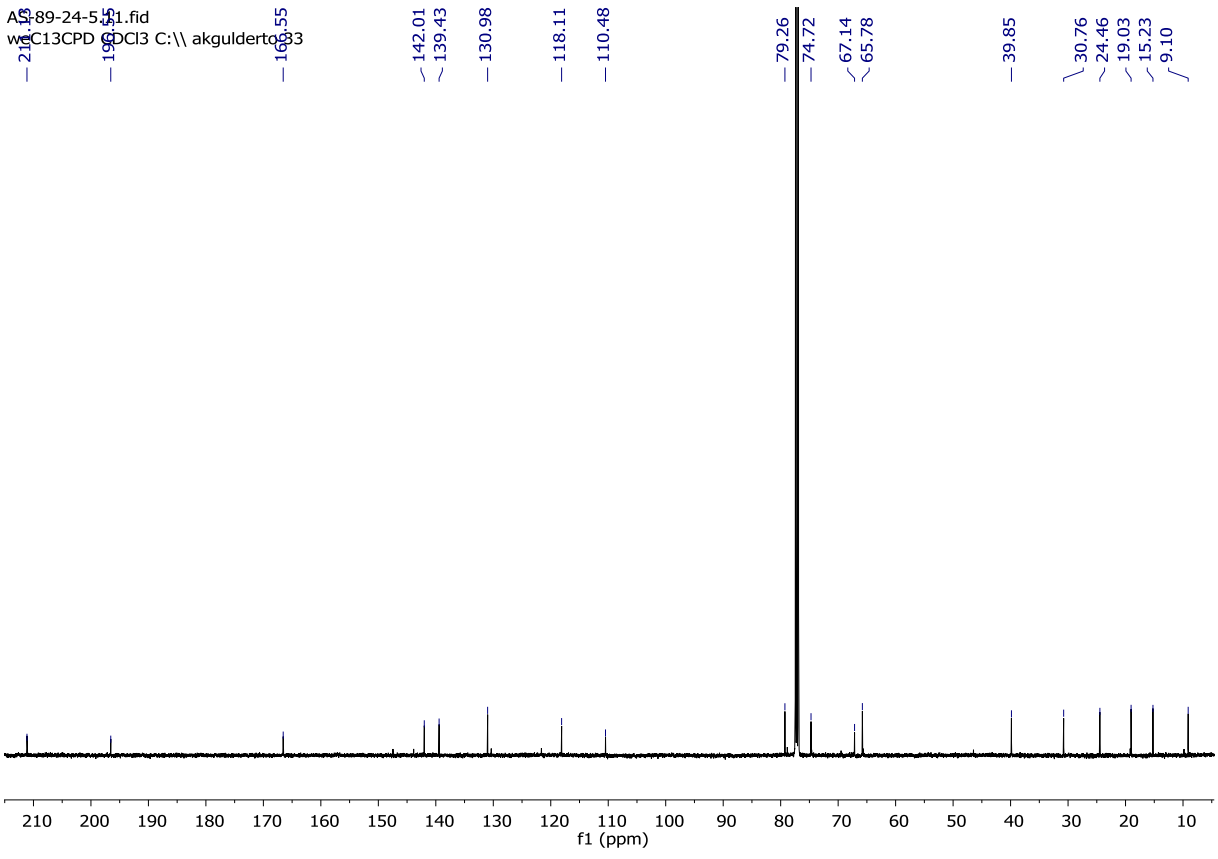
4.6 Compound **7f**



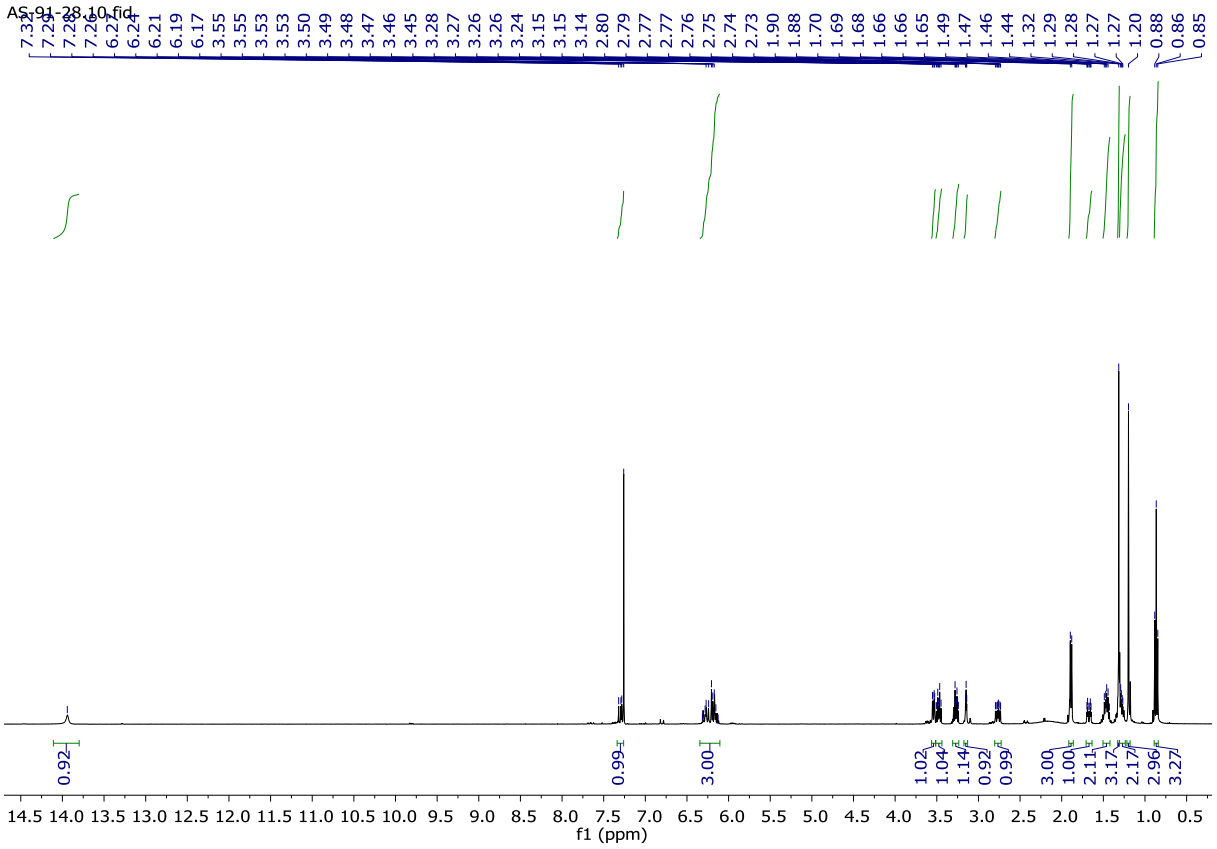


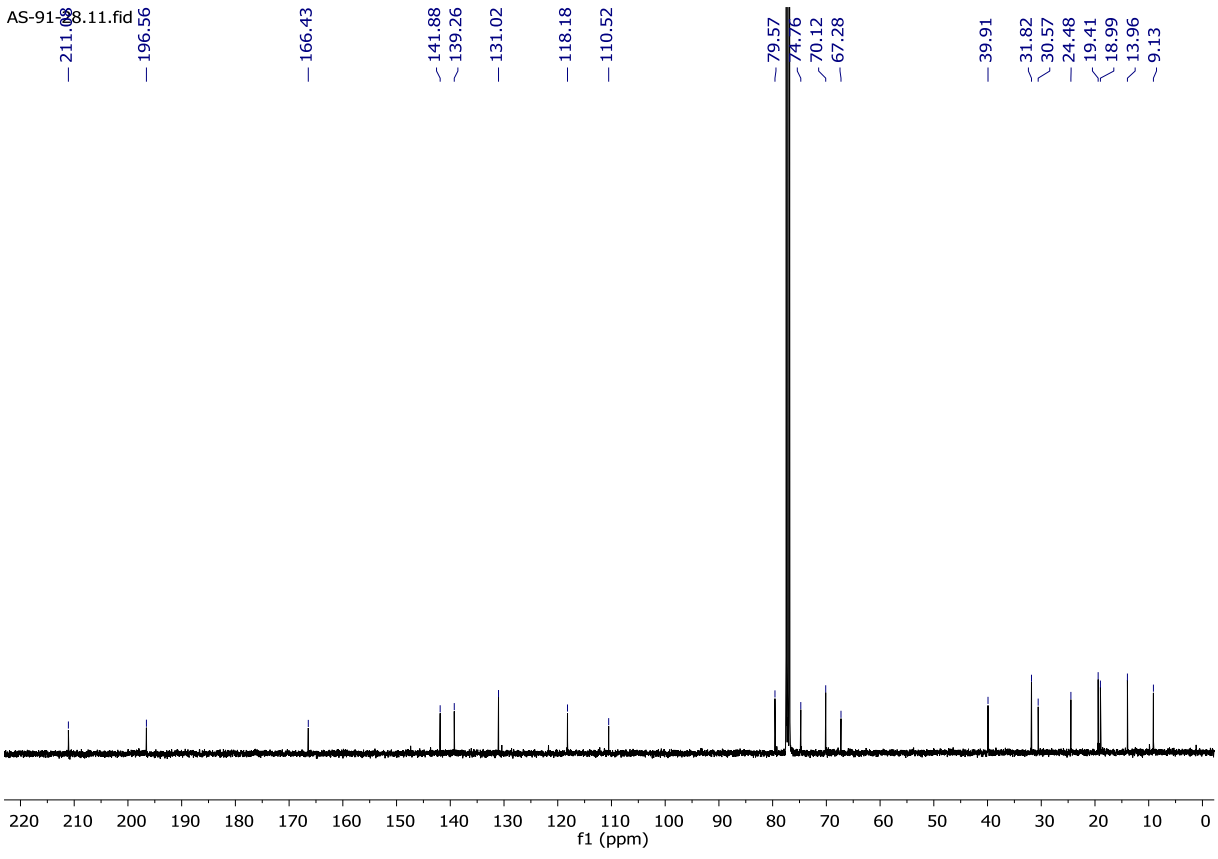
4.7 Compound **7g**



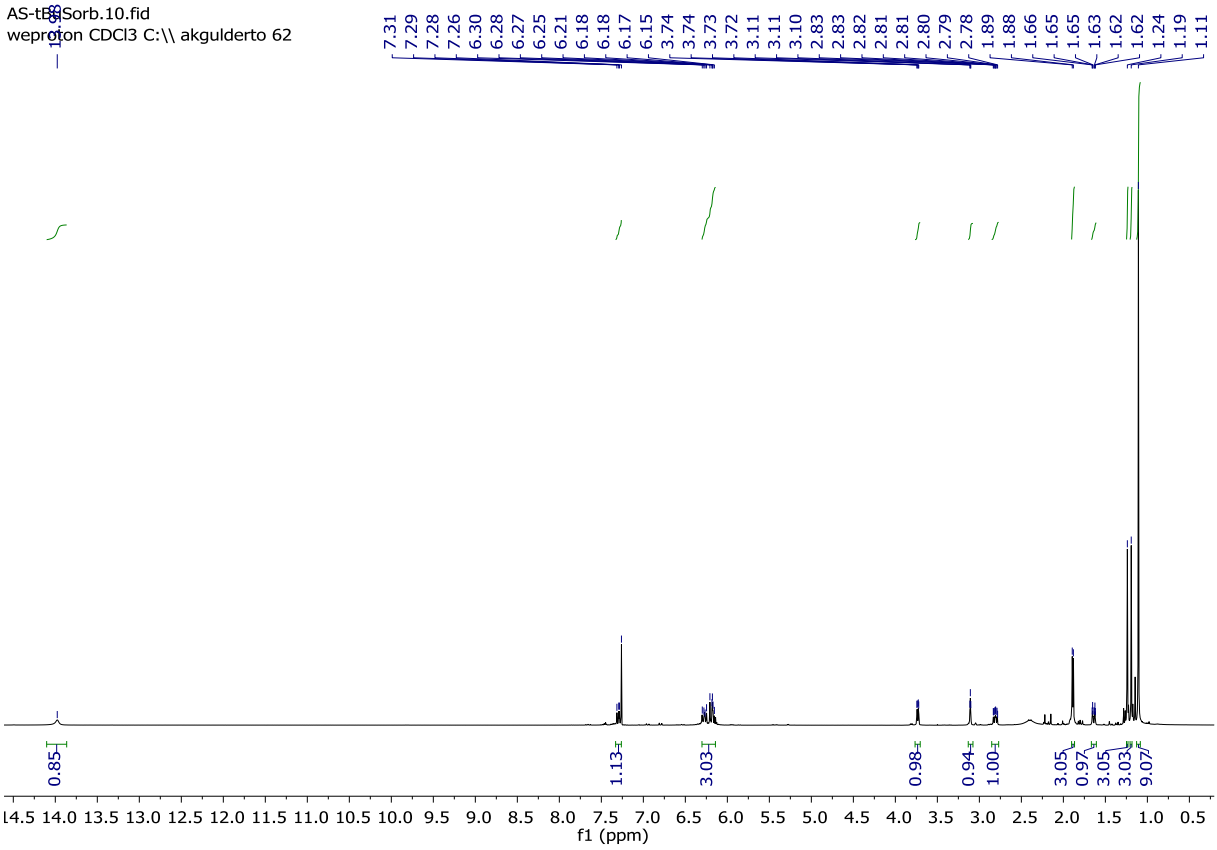


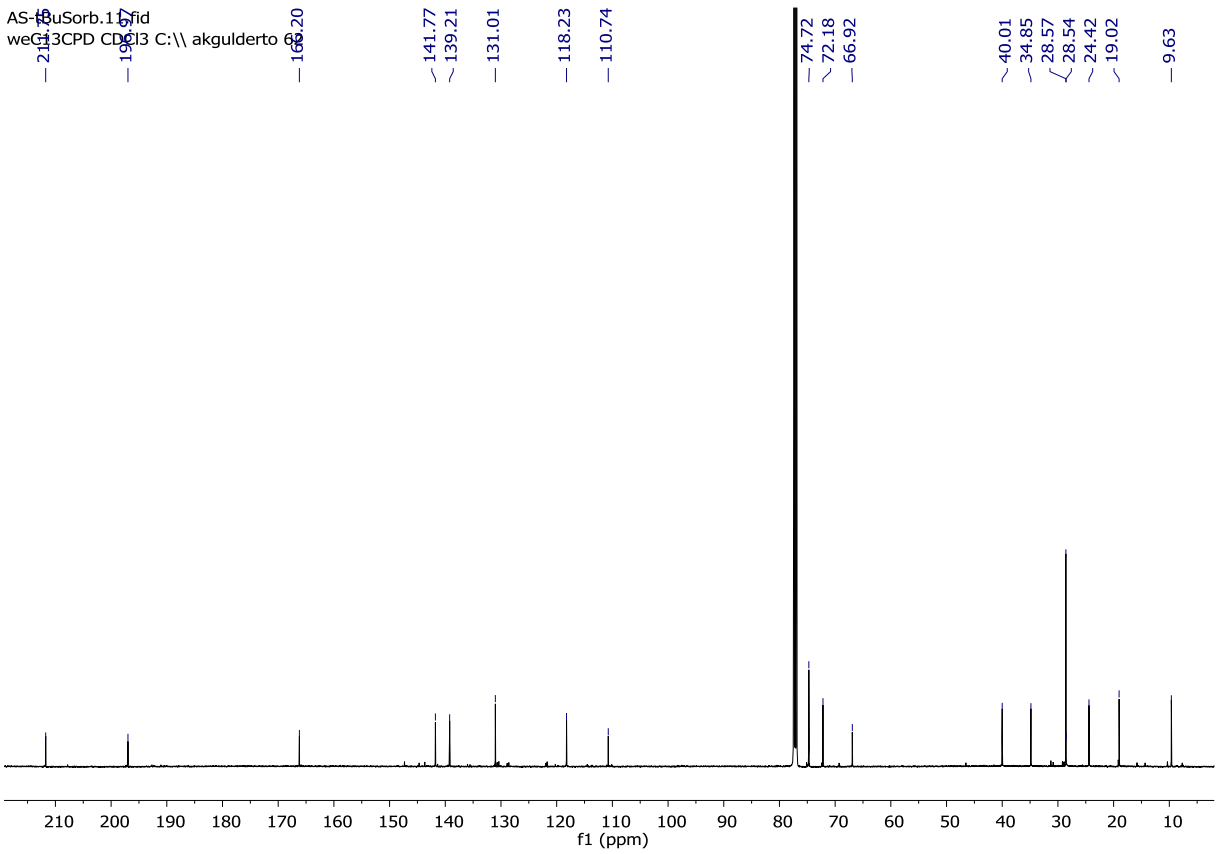
4.8 Compound **7h**



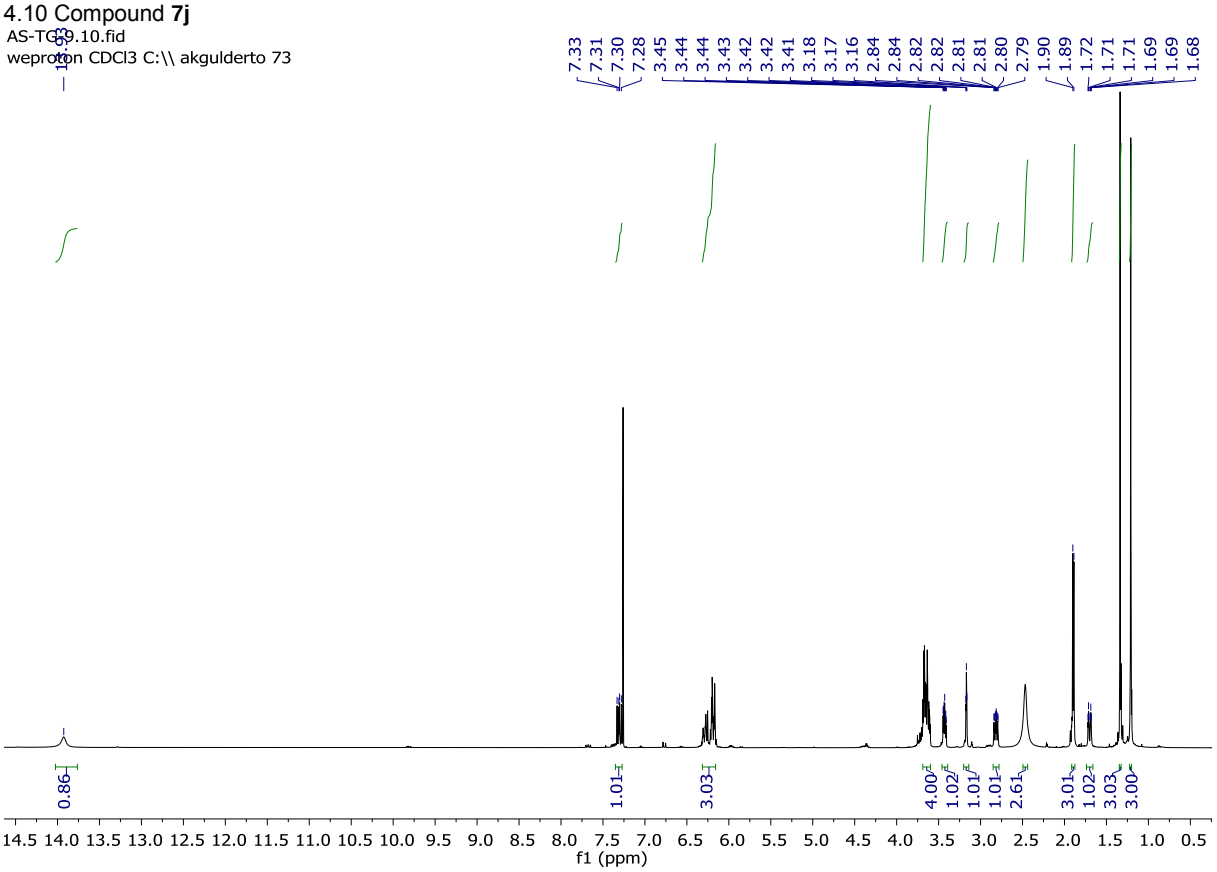


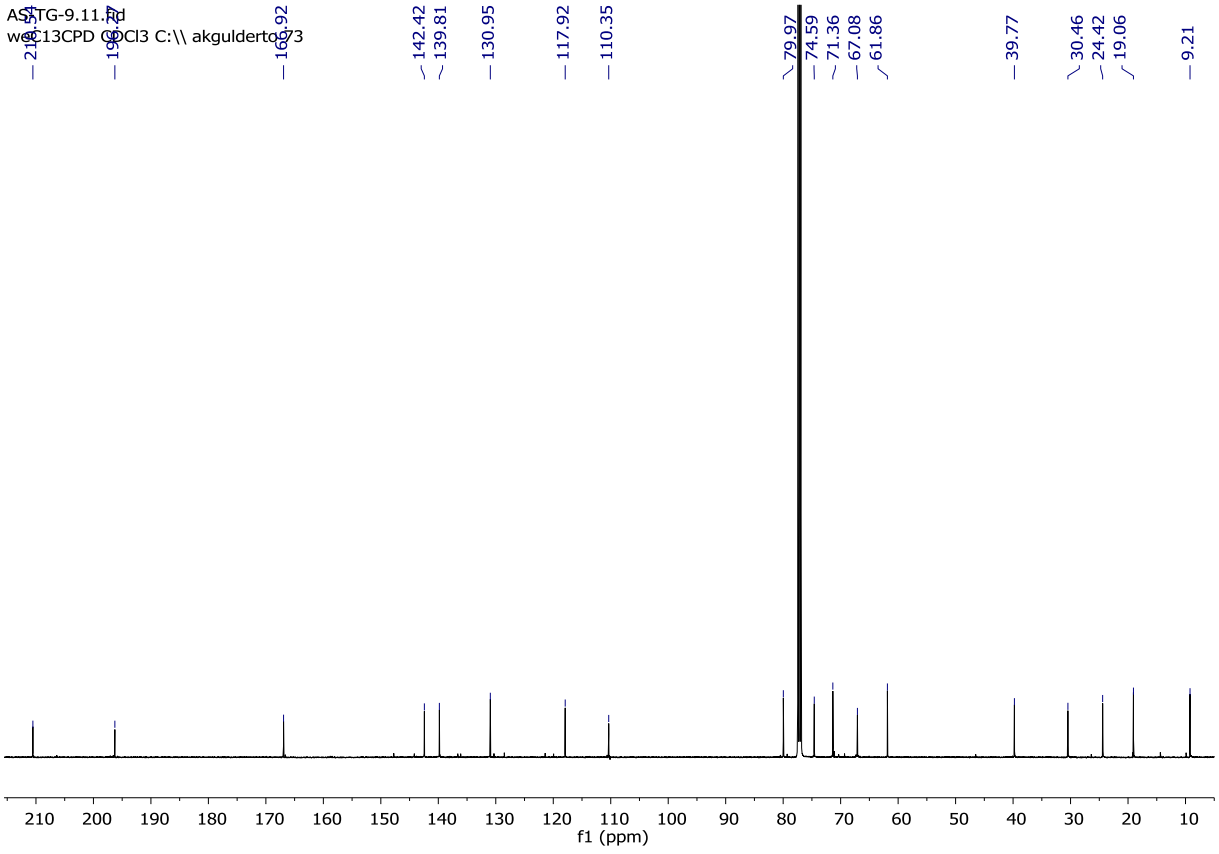
4.9 Compound **7i**



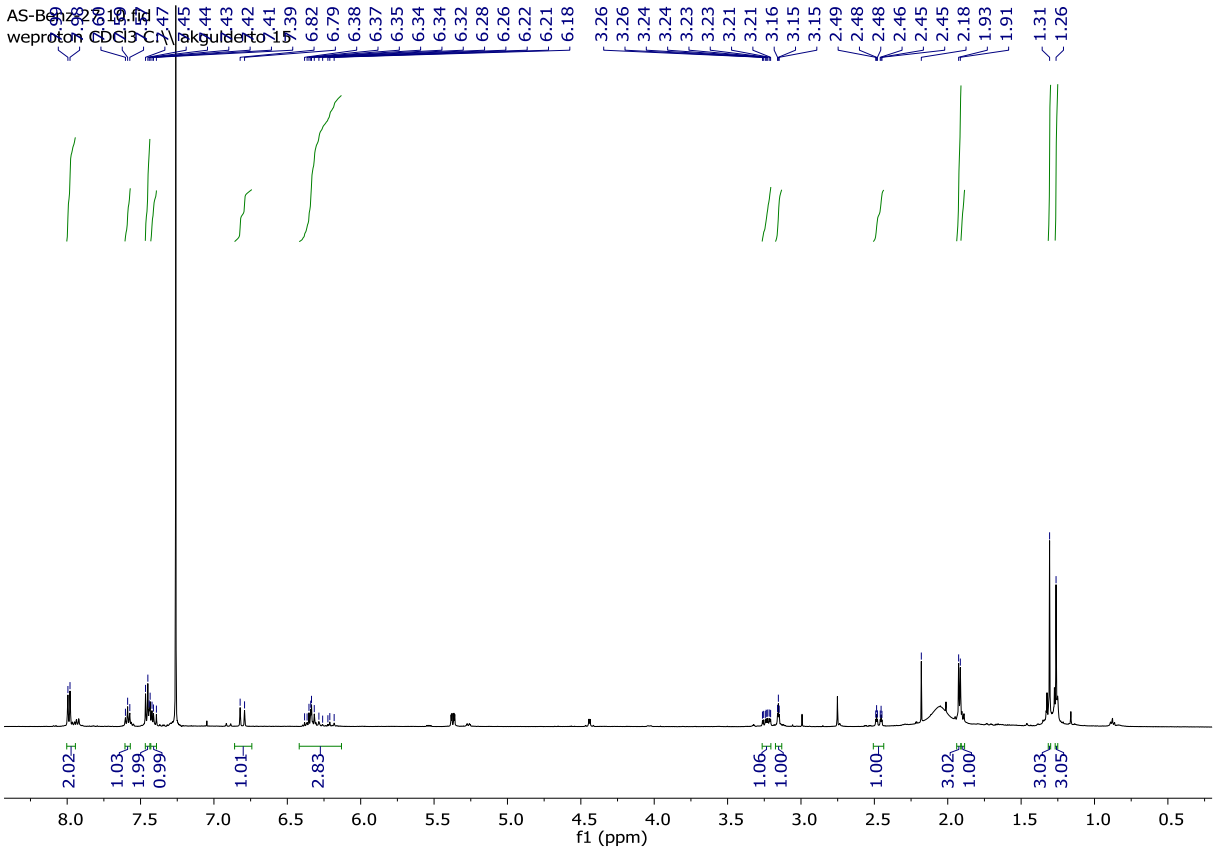


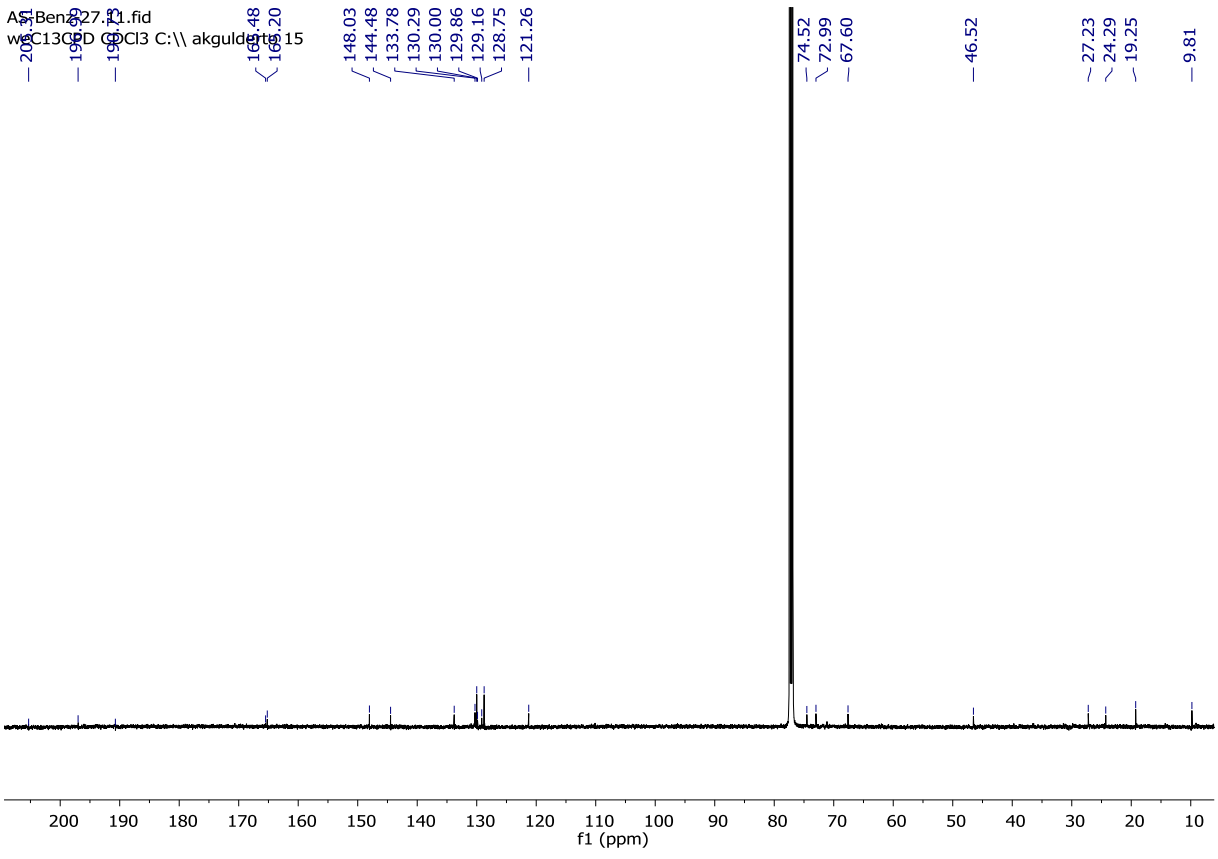
4.10 Compound **7j**



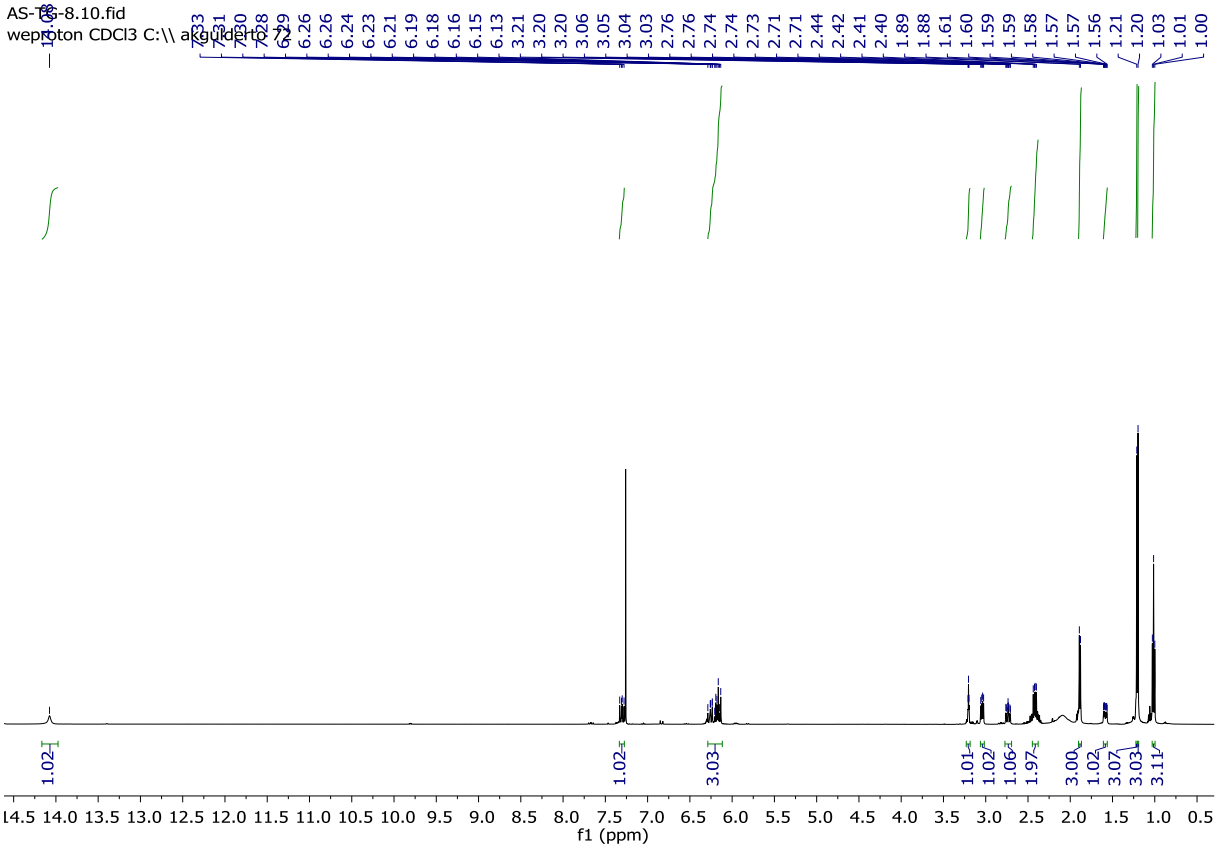


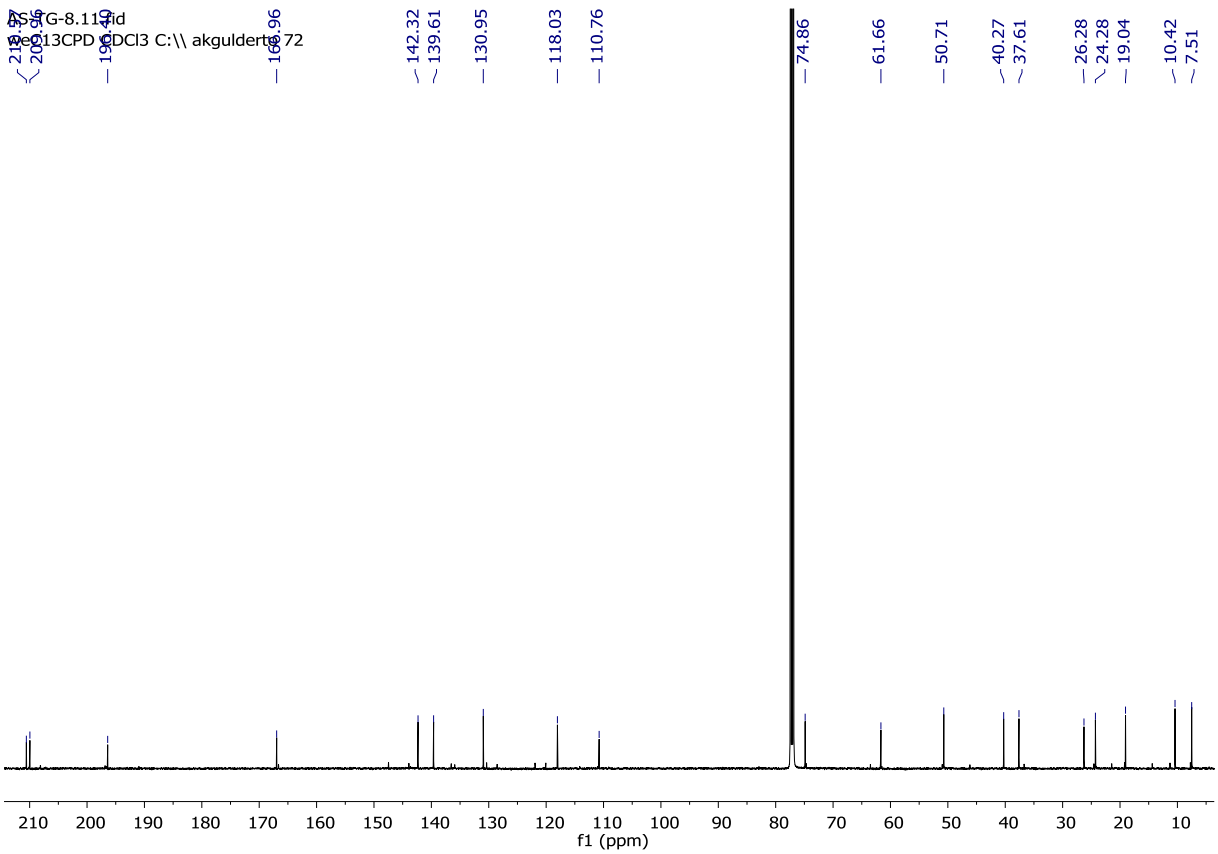
4.11 Compound **7k**



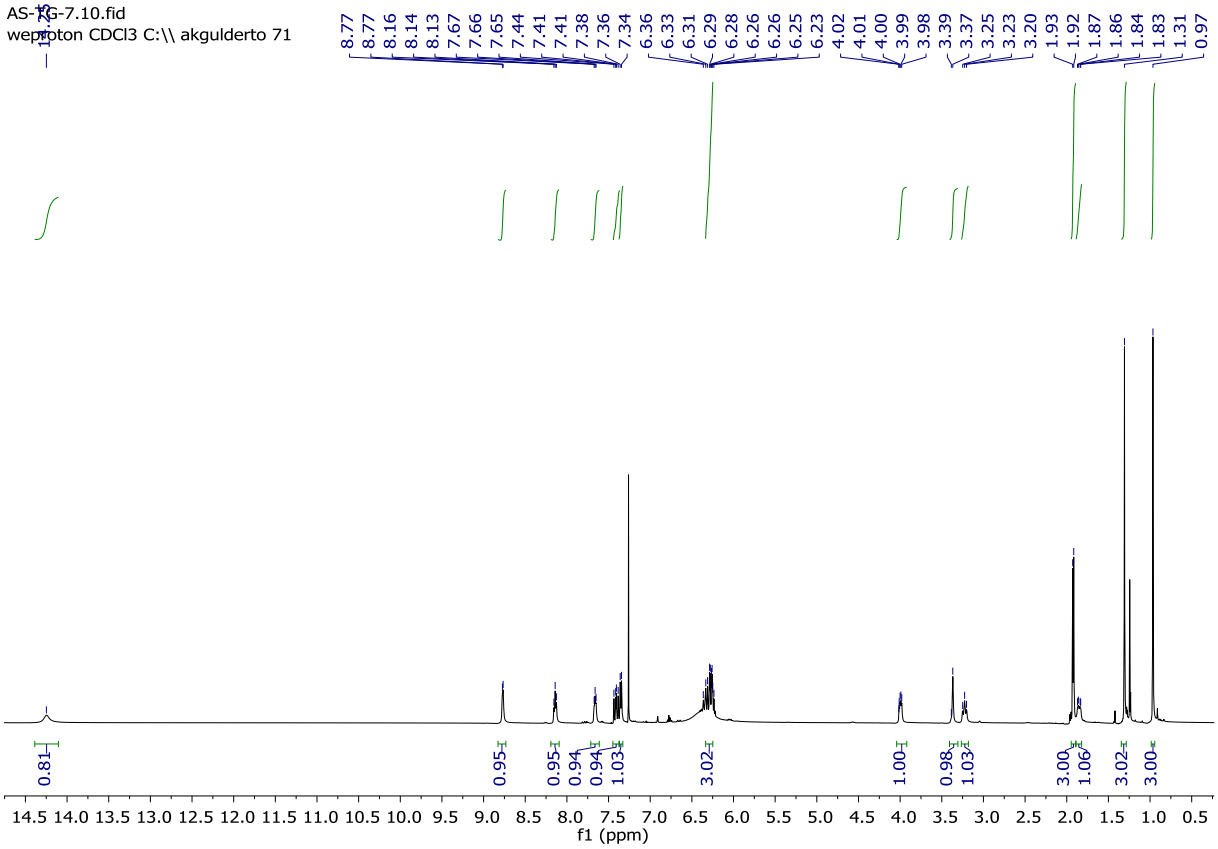


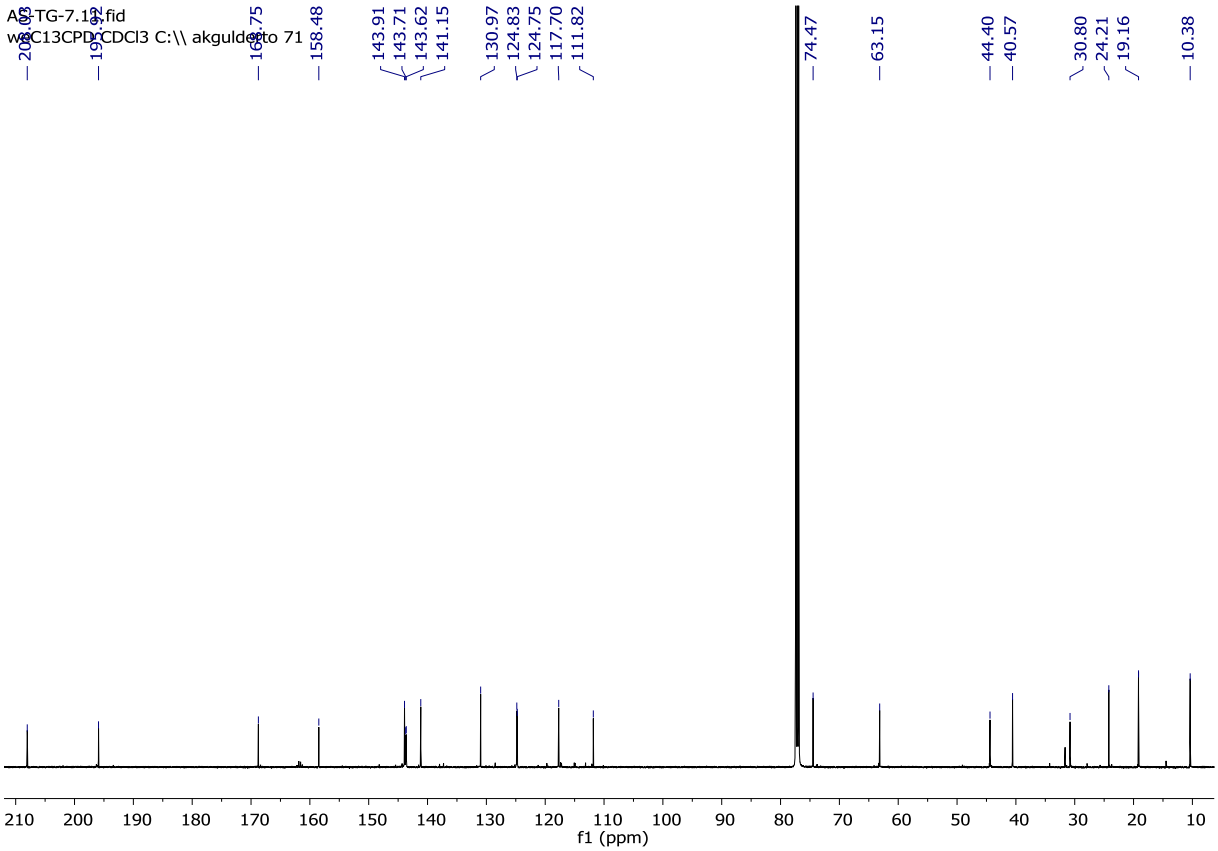
4.12 Compound **7l**



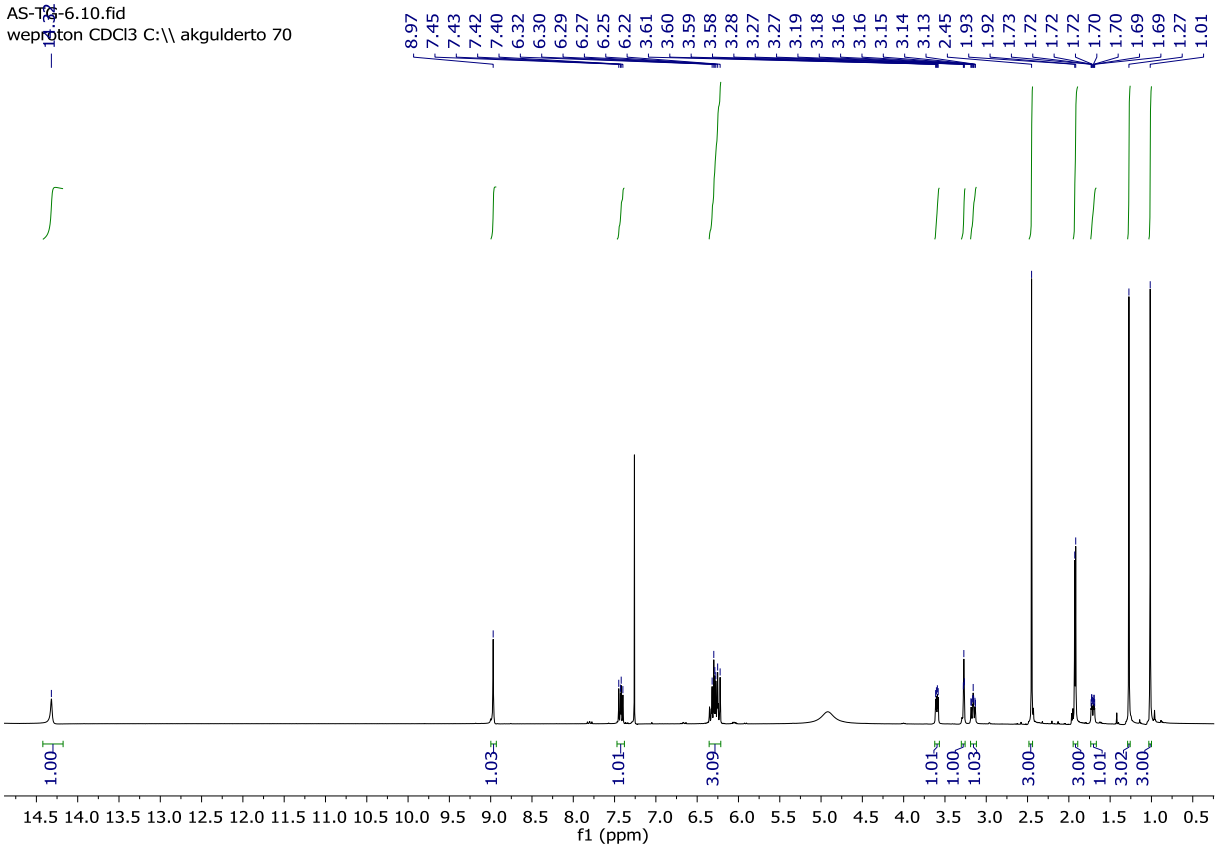


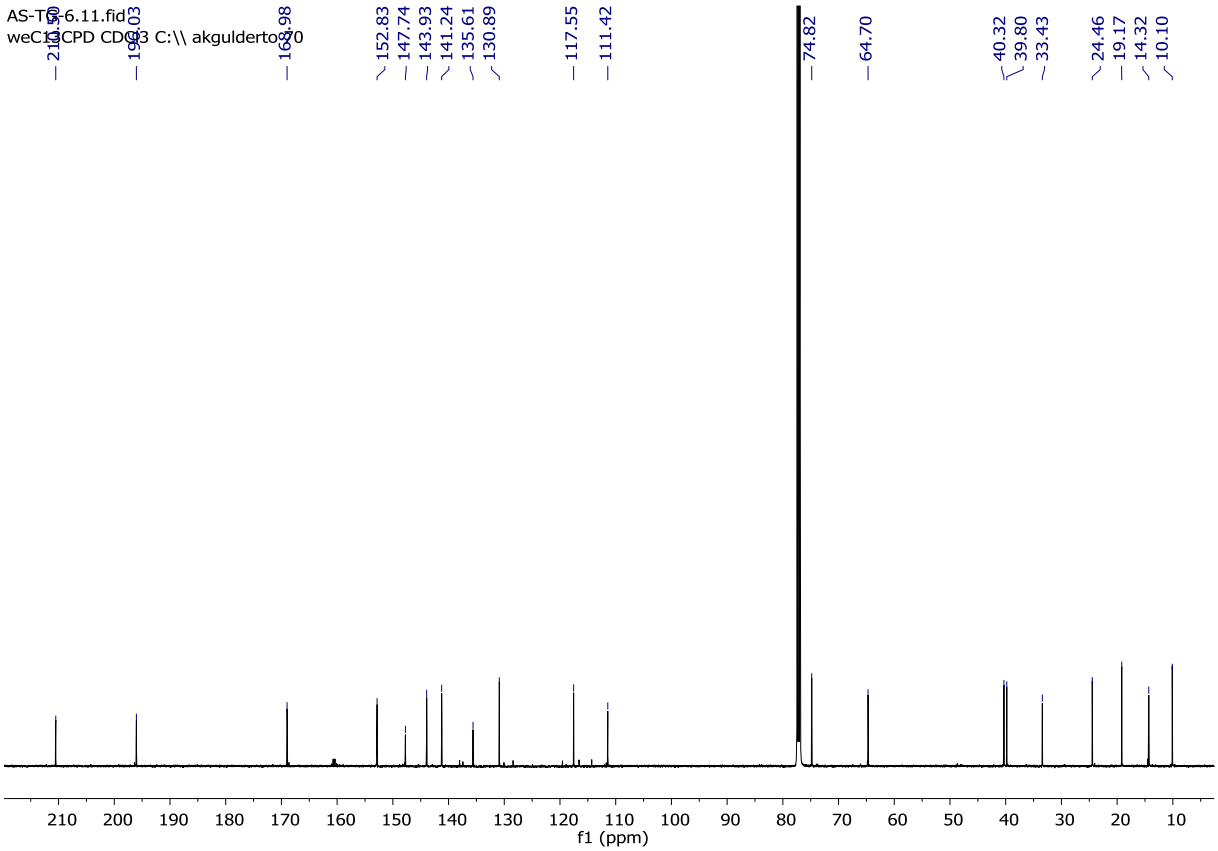
4.13 Compound **7m**



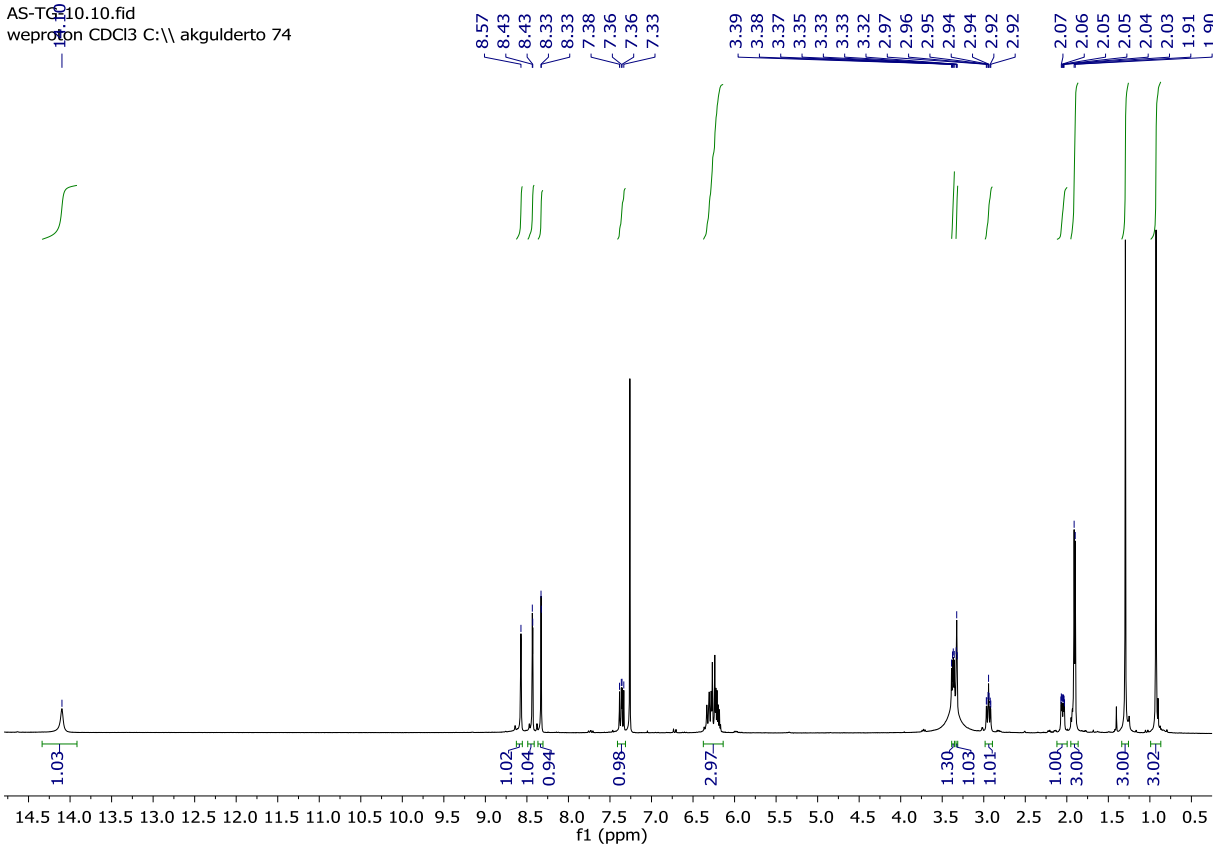


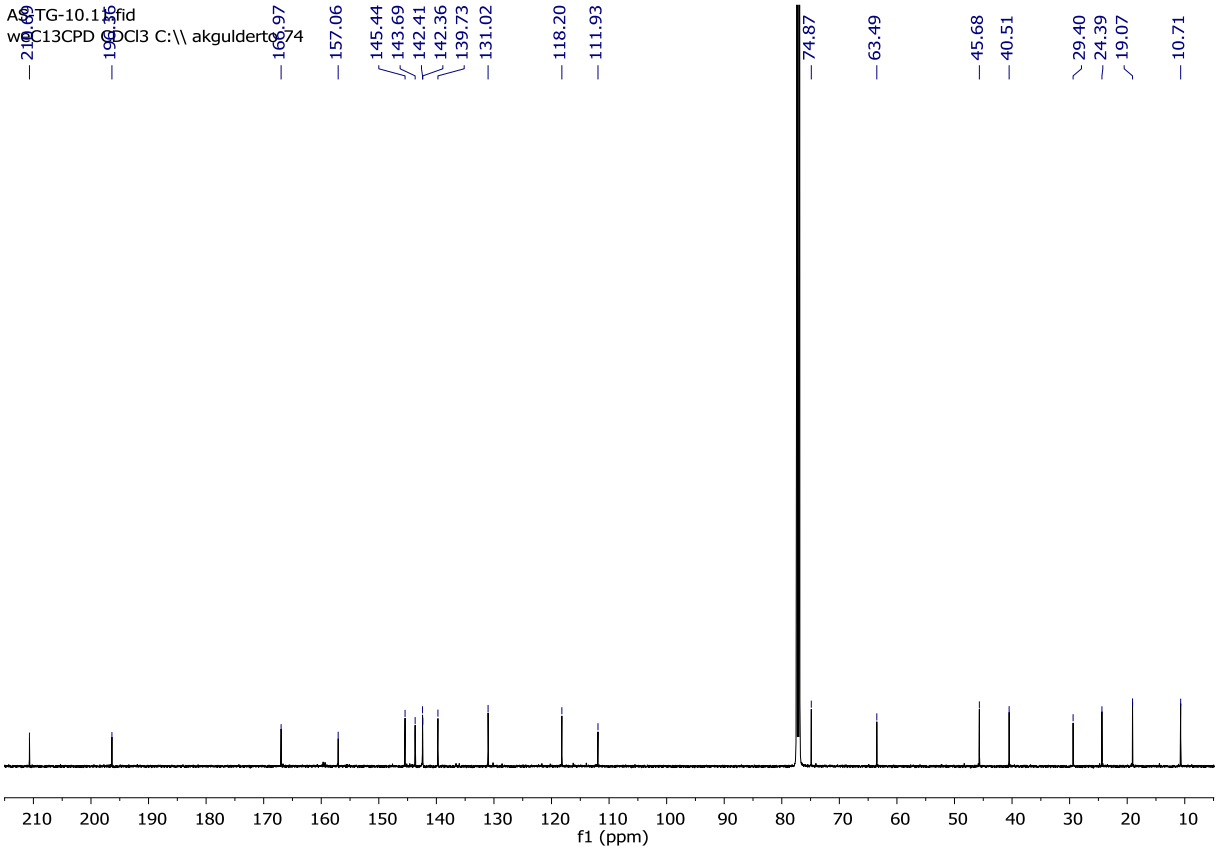
4.14 Compound **7n**





4.15 Compound **7o**





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[1] A. Sib, T.A.M. Gulder, *Angew. Chem. Int. Ed.* **2017**, *56*, 12888-12891.
[2] A. Sib, T.A.M. Gulder, *Angew. Chem. Int. Ed.* **2018**, *57*, 1-5.
[3] J. Peng, X. Zhang, L. Du, W. Wang, T. Zhu, Q. Gu, D. Li, *J. Nat. Prod*., **2014**, *77*, 424-428.
[4] D. Lee, J. H. Lee, X. F. Cai, J. C. Shin, K. Lee, Y.-S. Hong, J. J. Lee, *J. Antibiot.* **2005**, *58*, 615-620.
[5] R. P. Maskey, I. Gruen-Wollny and H. Laatsch, *J. Nat. Prod*., **2005**, *68*, 865-870.

A.2. Experimental part of sorbifuranon A (56) synthesis

This synthetic route towards **56** was so far only explorative and started only recently. Therefore full analytical data is not provided for every intermediate.

Synthesis of furanone **83**

Magnesium ethoxide (12.6 g, 110.0 mmol, 1.1 eq.) was dissolved in toluene (25.0 mL) and ethyl-3-oxohexanoate (16.0 mL, 15.8 g, 100.0 mmol, 1.0 eq.) was added. After stirring for 1 hour at room temperature acetonitrile (25.0 mL) was added and the reaction mixture was cooled to -10°C. Chloroacetylchloride (8.8 mL, 110.0 mmol, 1.1 eq.) was added dropwise and the solution was stirred at 0°C for 1 hour. The reaction mixture was poured into acidified water (100 mL H_2O with 6 mL conc. H_2SO_4) and extracted with diethylether (2x 100 mL). Organic phases were combined and dried over $MgSO_4$. Triethylamine (14 mL) was added to the reaction solution at 0°C and was then stirred at room temperature for 24 hours. The precipitated salt was filtered of and the solvent was evaporated. Purification was done by vacuum distillation with a heat gun. A yellow oil that crystallized after cooling was obtained in 90% yield (16.5 g, 90.0 mmol).

^{1}H NMR (500 MHz, $CDCl_3$) δ= 4.57 (s, 2H), 4.28 (q, *J* = 7.1 Hz, 2H), 2.95 (t, *J* = 7.4 Hz, 2H), 1.73 (h, *J* = 7.4 Hz, 2H), 1.32 (t, *J* = 7.1 Hz, 3H), 0.99 (t, *J* = 7.4 Hz, 3H).

The physical and spectroscopic data were in agreement with that described in the literature.[62]

Synthesis of carboxylic acid **86**

Furanone **83** (1.0 g, 5.0 mmol, 10.0 eq.) was dissolved in THF (5 mL) and LiOH (0.1 M, 150 mL) was added. It was stirred at room temperature over night or until complete consumption of **83** (determined by TLC). The solution was acidified to pH 3 with 1 M HCl and extracted with EtOAc. Organic phases were combined and the solvent was evaporated under reduced pressure. A yellow solid was obtained in quantitative yield (0.85 g, 5.0 mmol).

^{1}H NMR (500 MHz, $CDCl_3$) δ= 4.67 (s, 1H), 4.55 (s, 1H), 2.90 (t, *J* = 7.3 Hz, 2H), 1.75 (h, *J* = 7.4 Hz, 2H), 1.02 (t, *J* = 7.4 Hz, 3H). MS (ESI-): m/z = 169.01 $[M-H]^-$, calc. $[M-H]^-$: 169.05.

Synthesis of redox-active ester **89**

Under argon atmosphere carboxylic acid **86** (0.2 g, 1.2 mmol, 1.0 eq.), *N*-hydroxytetrachlorophthalimide (0.35 g, 1.2 mmol, 1.0 eq.) and DMAP (14.0 mg, 0.12 mmol, 0.1 eq.) were dissolved in DCM (10 mL) and stirred vigorously. DIC (0.16 g, 1.3 mmol, 1.1 eq.) was added dropwise and the mixture was allowed to stir until the acid was consumed (determined by TLC). Typical reaction times were between 8 to 12 hours. The mixture was filtered through a fritted funnel and rinsed with additional DCM. The solvent was removed under reduced pressure and the active ester was used further without purification.
The reaction can also be done with DCC instead of DIC.

Synthesis of zinc-reagent **90**

A flask was charged with LiCl (100.0 mg, 2.4 mmol, 2.0 eq.) and Zn dust (154.0 mg, 2.4 mmol, 2.0 eq.) and was carefully placed under vacuum and dried with a heat gun. After cooling the flask was filled with argon and THF (1.2 mL) was added. The mixture was stirred vigorously, then TMSCl (15 µL, 58.8 µmol, 0.05 eq.) was added and the reaction mixture was heated with a heat gun to the point of reflux. Upon cooling, the flask was placed in a 30°C water bath and cis-ethyl-3-iodoacrylate (265.0 mg, 1.2 mmol, 1.0 eq.) was added dropwise. The mixture was then stirred at ambient laboratory temperature for 1 hour resulting in a brown colored solution. By stopping stirring the remaining zinc settled to the bottom of the flask and the concentration can be determind by titration with iodine. It is further used without purification.
Note: If the color does not change within 5-10 min, it is likely that the zinc insertion reaction did not proceed. If this is the case GENTLY heat the reaction mixture with a heat gun.[61]

Synthesis of furanone **91**

Under argon atmosphere TCNHPI redox active ester (225.5 mg, 0.5 mmol, 1eq.) and $MgBr_2$ OEt_2 (258.0 mg, 1.0 mmol, 2.0 eq.) were added to a flask and cooled to 0°C. A solution of $Ni(acac)_2$ (13 mg, 0.05 mmol, 0.1 eq.) and 2.2 bipyridine (8.0 mg, 0.05 mmol, 0.1 eq.) in DMF (5.0 mL) was added and the mixture was stirred vigorously for 30 seconds. The mixture was removed from the ice bath and a solution of alkenylzinc reagent **90** (197.9 mg, 1.0 mmol, 2.0 eq.) in THF was added. The reaction was allowed to stir overnight at room temperature, then it was diluted with EtOAc, quenched with 1 M HCl and extracted first EtOAc and then DCM. After evaporation of the combined organic phases, it

was purified via column chromatography (3:1, pentane : EtOAc; R_f= 0.6). The desired product was obtained in 32% yield (35. 0 mg, 0.16 mmol).

^{1}H NMR (500 MHz, $CDCl_3$) δ= 6.24 (dt, *J* = 11.4, 7.4 Hz, 1H), 5.80 (dt, *J* = 11.4, 1.7 Hz, 1H), 4.15 (m, 4H), 2.95 (qd, *J* = 7.3, 1.7 Hz, 2H), 2.46 (t, *J* = 7.3 Hz, 2H), 1.27 (m, 6H).

A.3. Experimental part of sorbicillactone A (57) synthesis

This synthetic route towards **57** was so far only explorative and started only recently. Therefore full analytical data is not provided for every intermediate.

Synthesis of mono-*tert*-butyl malonic acid (**95**)

Malonic acid (1.0 g, 10.0 mmol, 1.0 eq.) and *tert*- butanol (1.9 mL, 20.0 mmol, 2.0 eq.) were dissolved in acetonitrile (50 mL), followed by the addition of DCC (2.3 g, 11.0 mmol, 1.1 eq.) in acetonitrile (10 mL). The mixture was stirred at room temperature for 30 min, filtered and the solvent was evaporated under reduced pressure. Purification was performed by column chromatography (3:2, pentane:EtOAc, R_f=0.5). The desired product was obtained as a colorless oil with a yield of 60% (0.96, 6.0 mmol).

^{1}H NMR (500 MHz, $CDCl_3$) δ= 3.35 (s, 2H), 1.49 (s, 9H).

The physical and spectroscopic data were in agreement with that described in the literature.[63]

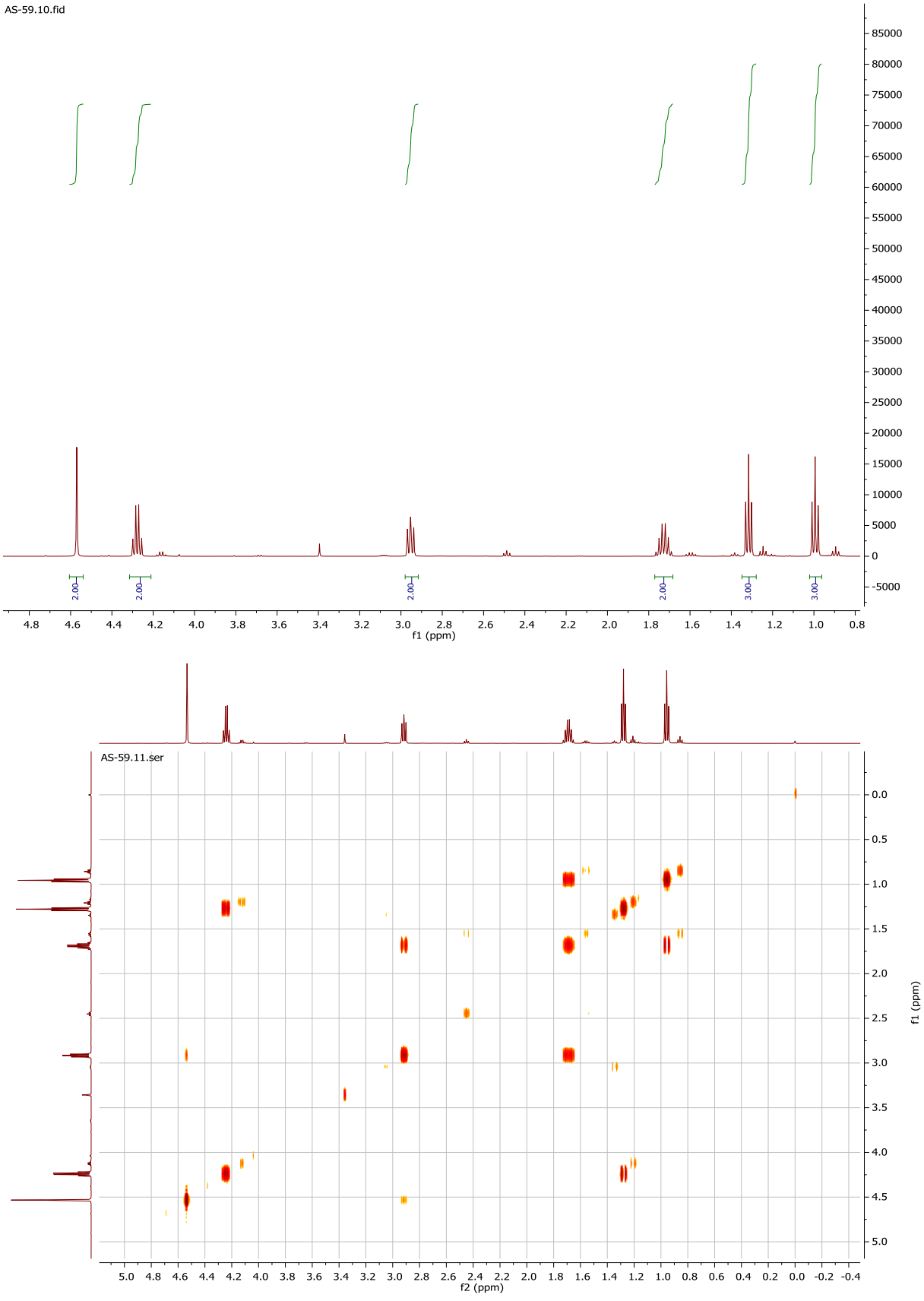
Synthesis of lactone **96**

A solution of semi-purified sorbicillinol (40.0 mg, 0.2 mmol, 1.0 eq.) in DMF (4 mL), DMAP (30.0 mg, 0.3 mmol, 1.5 eq.) and mono-*tert*-butyl malonic acid (30.0 mg, 0.2 mmol, 1.2 eq.) was cooled to 0°C and DCC (60.0 mg, 0.3 mmol, 1.5 eq.) was added. After stirring for 5 min the reaction mixture was warmed to room temperature and stirred for 12 hours. The precipitate was filtered of and the solvent was evaporated under reduced pressure. Purification was performed by preparative HPLC (retention time: 27 min, method see supporting info). The desired product was obtained as a yellow solid in 21% yield (16.4 mg, 0.04 mmol).

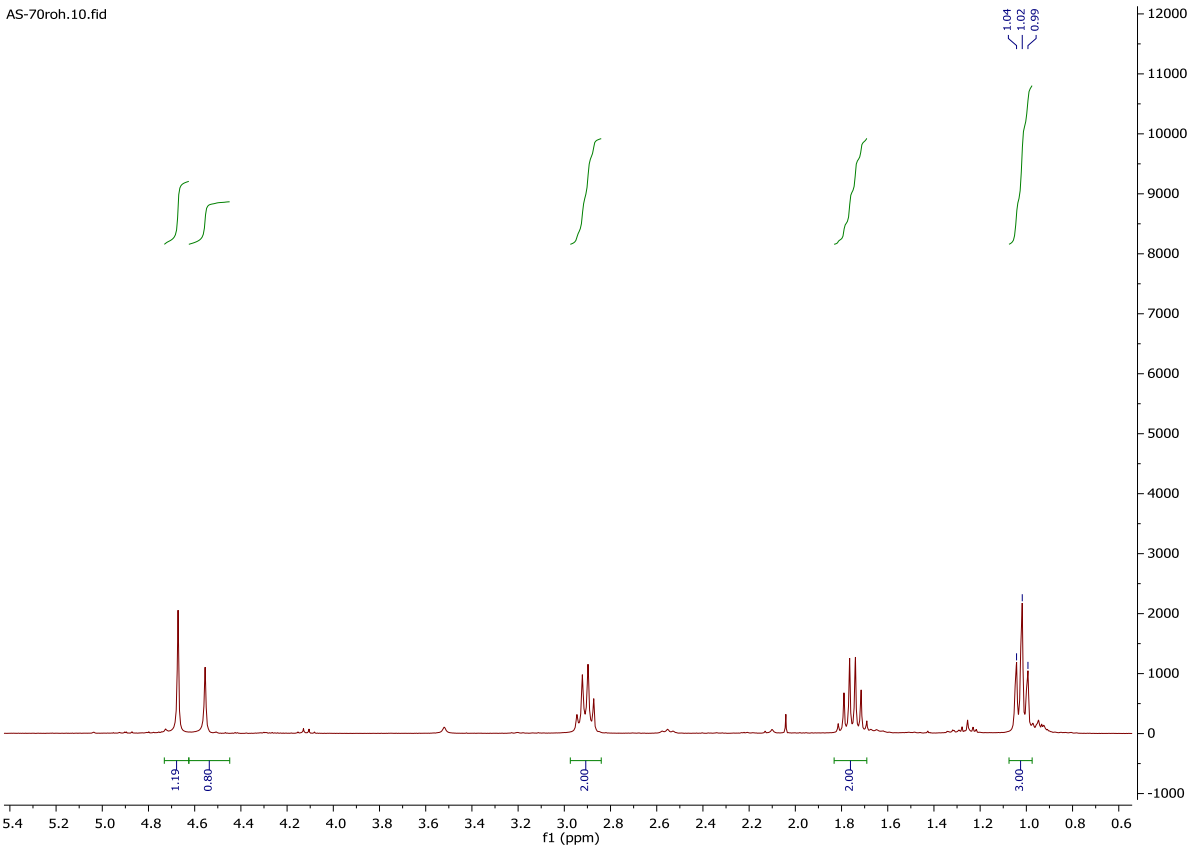
^{1}H NMR (500 MHz, $CDCl_3$) δ= 7.32-7.22 (m, 1H), 6.34-6.15 (m,3H), 3.94 (d, *J* = 11.6 Hz, 1H), 3.59 (d, *J* = 11.6 Hz, 1H), 1.89 (d, *J* = 6.2 Hz, 3H), 1.85 (s, 3H), 1.71 (s, 3H), 1.47 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 189.5, 172.1, 170.1, 166.5, 160.5, 141.5, 139.0, 131.0, 119.6, 111.8, 99.9, 84.1, 82.8, 55.3, 45.2, 28.0, 28.0, 28.0, 23.4, 18.9, 7.6. MS (ESI+): m/z = 391.06 $[M+H]^+$, calc. $[M+H]^+$: 391.17.

A.4. NMR-Data

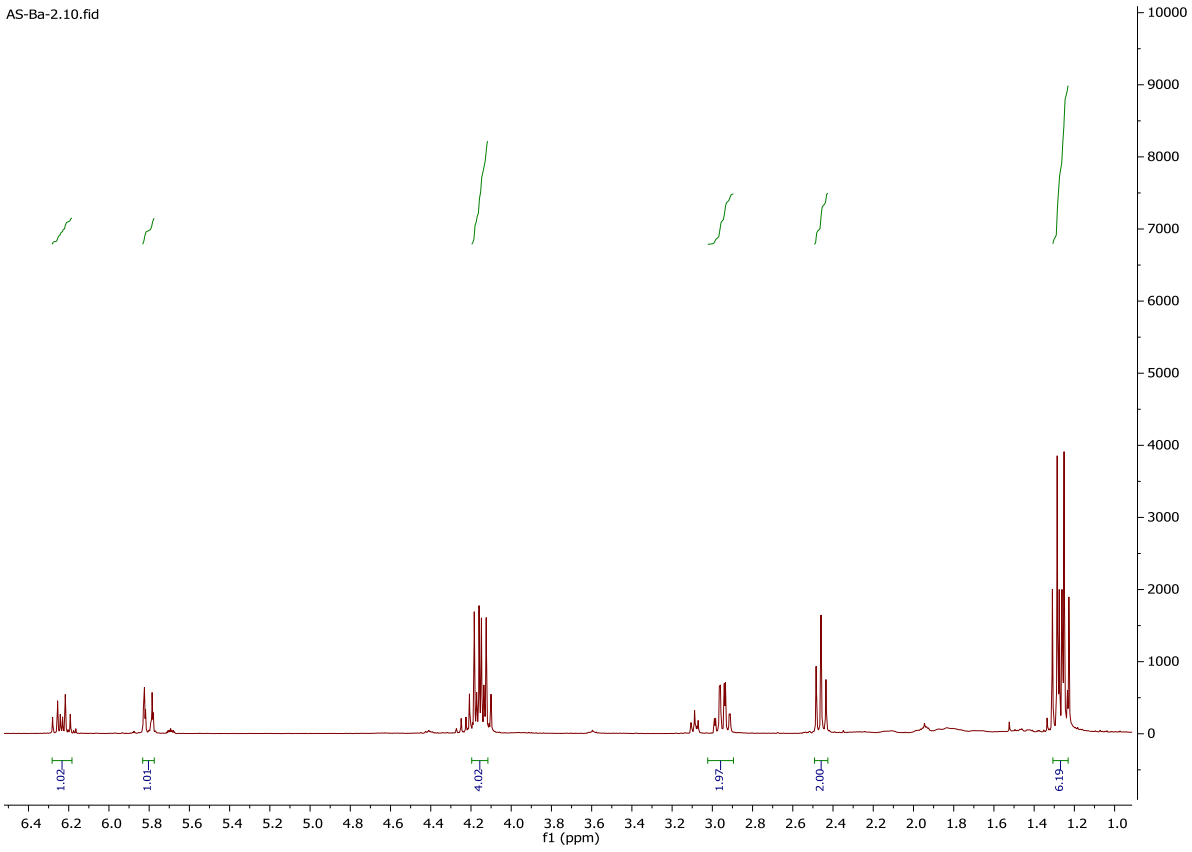
Furanone ester **83**



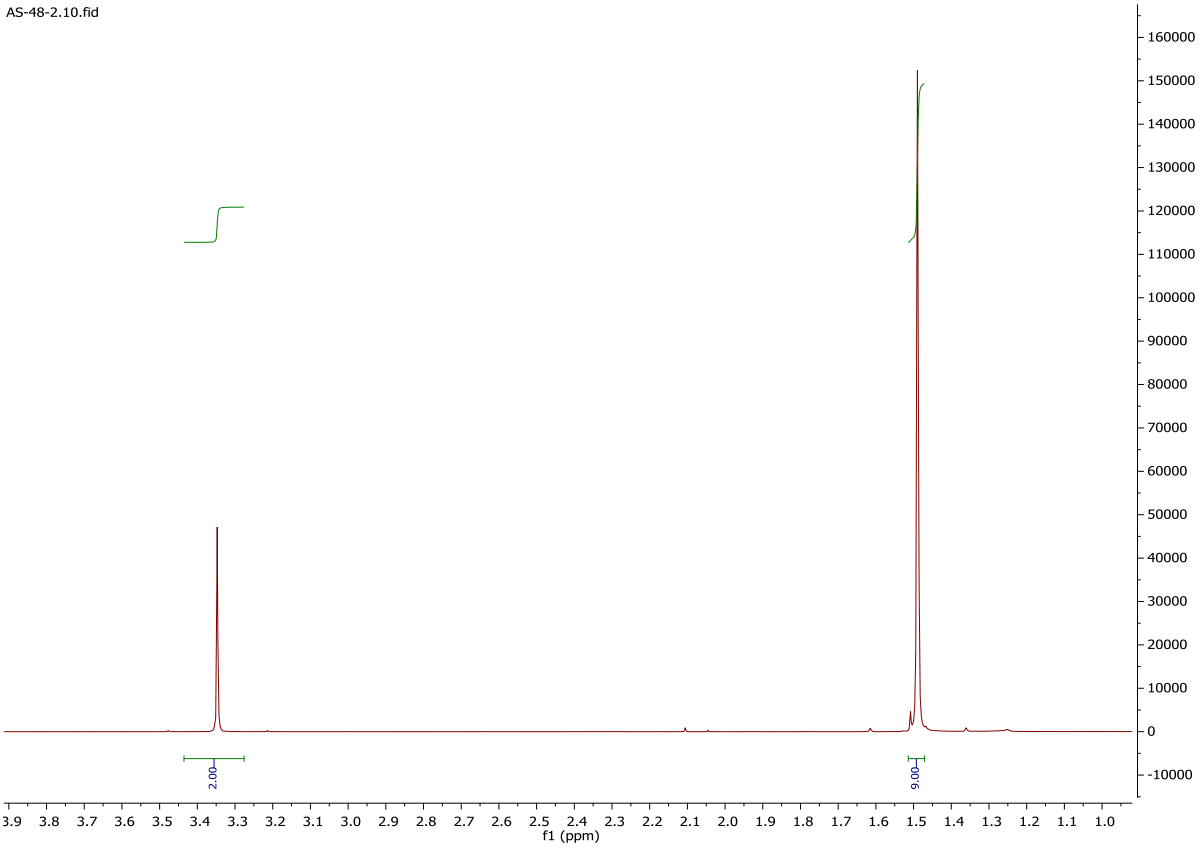
Furanone acid **86**



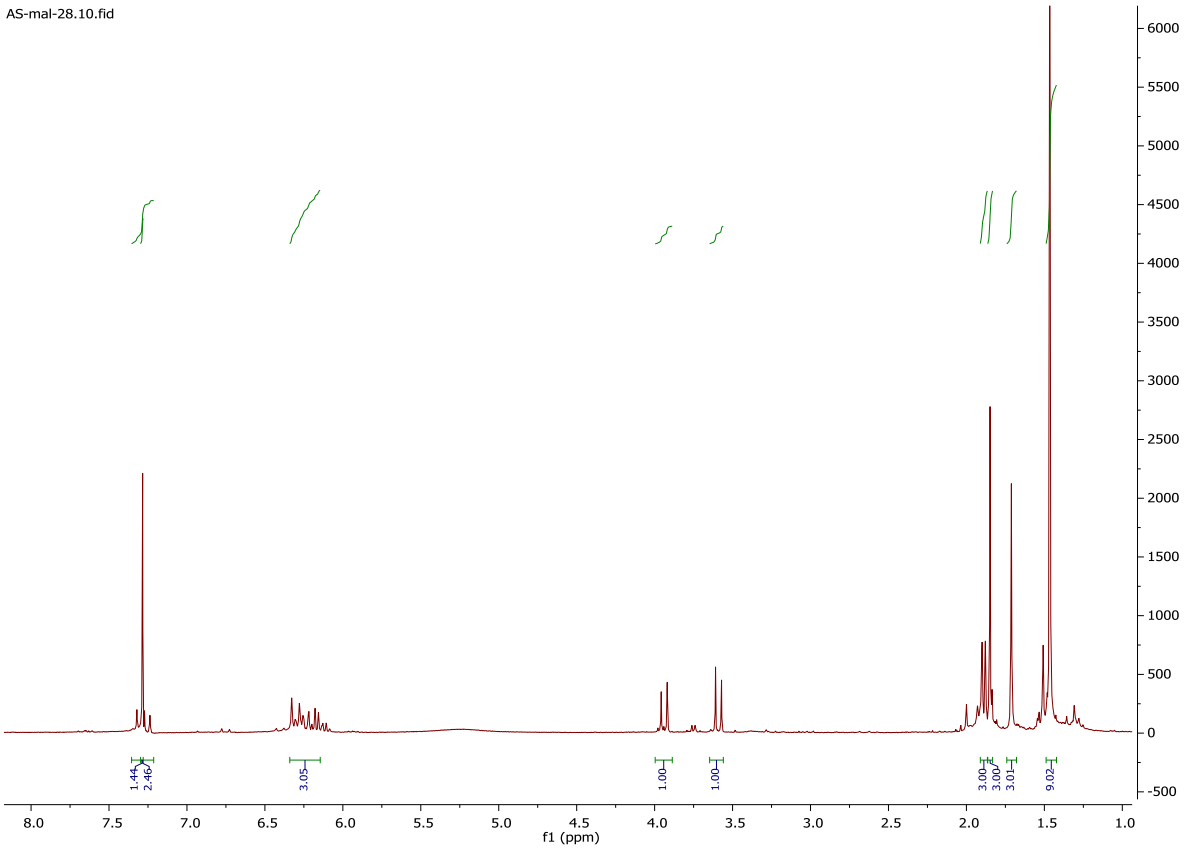
Ester **91**

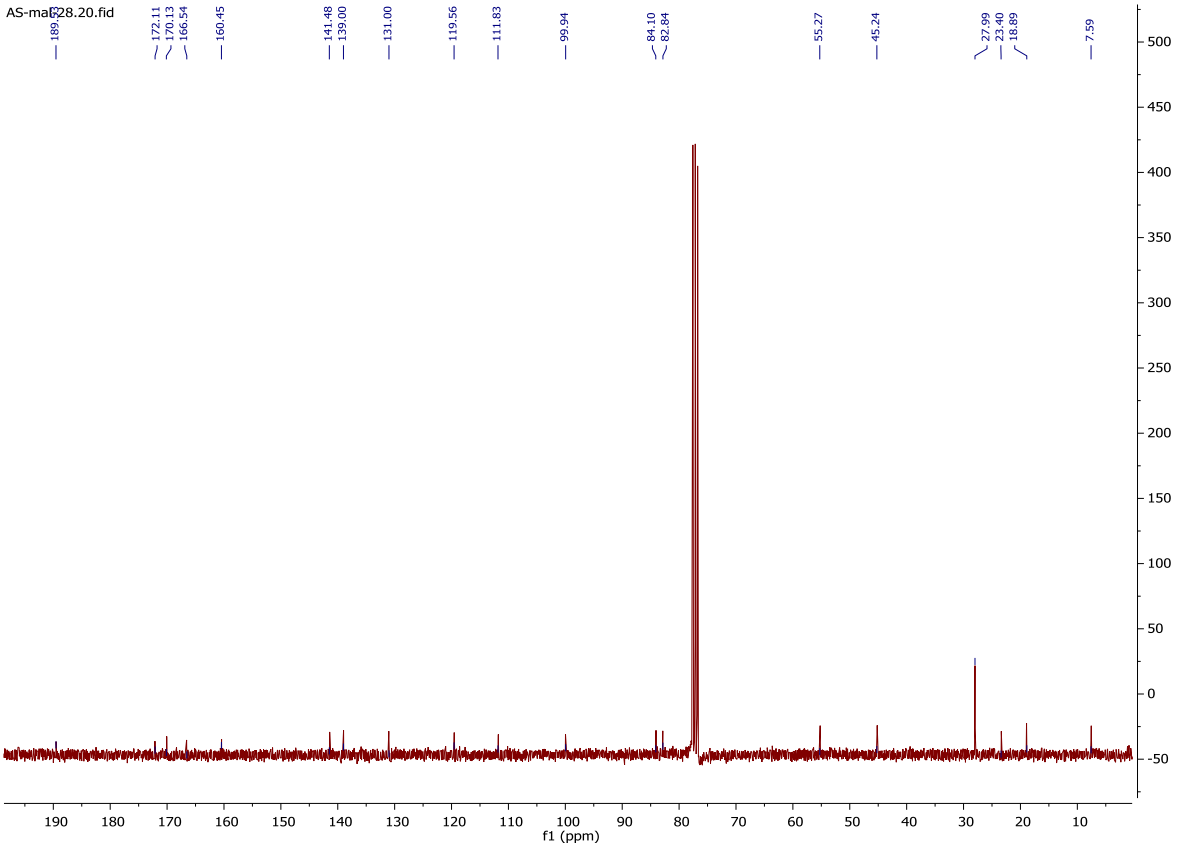


Mono-tert-butyl malonic acid **95**



Lactone **96**

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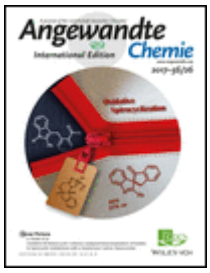

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