Tampere University of Applied Sciences

Oxidative C-C Cleavage of Quinic Acid Derivatives

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ABSTRACT

Tampereen ammattikorkeakoulu
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KOIVUPORRAS, ALISA:
Oxidative C-C Cleavage of Quinic Acid Derivatives

Bachelor's thesis 58 pages, appendices 19 pages
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This thesis was done in the Synthetic Chemistry team, which is part of the Tampere University research group Chemistry and Advanced Materials. The Synthetic Chemistry team was led by Nuno Rafael Candeias. This thesis was done as a part of doctoral student Suvi Holmstedt's study about the preparation quinic acid derivatives in spring 2020.

The objective of this thesis was to develop a method or methods for the oxidative cleavage of C-C bond for derivatives of quinic acid. The purpose of this thesis was to search information about the oxidative C-C bond cleavage and to synthesize the quinic acid derivatives by applying the methods of previous scientific publications. The objective was also to purify and characterize the synthesized products by column chromatography and Nuclear magnetic resonance spectroscopy (NMR).

In this thesis, three different quinic acid derivatives were studied and modified to a state in which oxidative cleavage would be possible. The syntheses were tested with various methods and the methods were modified by testing different conditions, for example, changing reaction temperature and reagents. The monitoring and the verification of pure product was studied with the abovementioned methods.

In this thesis, the oxidative C-C cleavage was successfully performed for all wanted quinic acid derivatives, and it was possible to create a practical method. In future, the methods (created in this study) should be optimized, and the oxidative cleavage method should be tested on other quinic acid derivatives or other cyclic compounds with cis vicinal diols. Chiral molecules can be used as building blocks because of their chirality and oxidative cleavage produces new building blocks with aldehyde/alcohol "handles". These building blocks could possibly be used in future in the pharmaceutical industry.

Key words: quinic acid, oxidative cleavage

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Energia- ja ympäristötekniikka
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Opinnäytetyö tehtiin Tampereen yliopiston Kemia- ja uudet materiaalit -tutkimusryhmässä synteettisen kemian tiimissä keväällä 2020. Tohtoriopiskelija Suvi Holmstedt on tutkinut kiinihapon johdannaisia vuodesta 2017 ja opinnäytetyön aihe liittyy vahvasti tähän tutkimukseen. Opinnäytetyön tavoitteena oli kehittää metodi/metodeja, jonka avulla hapettava hiili-hiilisidoksen katkaisu olisi mahdollista kolmessa eri kiinihapon johdannaisessa. Tarkoituksena oli aiempien tutkimuksien avulla soveltaa metodeja kiinihapon johdannaisille sopiviksi ja syntetisoida uusia molekyylejä. Tarkoituksena oli myös puhdistaa ja karakterisoida saadut tuotteet pylväskromatografiaa ja ydinmagneettista resonanssispektroskopiaa apuna käyttäen.

Tutkituille kiinihapon johdannaisille piti tehdä muokkauksia, jotta hapettava katkaisu voitiin tehdä, ja että tuotteen karakterisointi olisi luotettavasti mahdollista. Menetelmiä testattiin erilaisilla reagensseilla sekä parametreillä kuten lämpötilan muutoksilla. Reaktioiden onnistumista ja lopputuotteiden puhtautta tutkittiin aiemmin mainituilla menetelmillä. Työssä kaikille halutuille kiinihapon johdannaisille pystyttiin suorittaa hiili-hiilisidoksen katkaisu.

Toimiva menetelmä hapettavalle hiili-hiilisidoksen katkaisulle pystyttiin luomaan aiempien tutkimusten avulla. Luotuja metodeja pitäisi optimoida nostamalla synteesien saantoja, ja hapettavalle katkaisulle luotua metodia pitäisi testata useammille kiinihapon johdannaisille sekä muille biomolekyyleille. Hapettava hiili-hiilisidoksen katkaisu tuottaa uudenlaisia johdannaisia aldehydi/ketoni ”kahvoilla”, joita voidaan käyttää kiraalisina rakennuspalikoina osana suurempaa kokonaisuutta. Rakennuspalikoita voidaan käyttää esimerkiksi lääketeollisuudessa uusien lääkeaineiden syntetisoimisessa.

Avainsanat: kiinihappo, oksidatiivinen katkaisu

CONTENTS

ABBREVIATIONS AND TERMS

ACN	acetonitrile
Crude	Product without further purification
DAHP	3-desoxyheptulosonic acid
DCM	Dichloromethane
Deprotect	to remove protection group
DHQ	3-dehydroquinic acid
DMSO	Dimethyl sulfoxide
EtOAc	Ethyl Acetate
HCl	Hydrochloric acid
LTA	Lead tetra-acetate
MeOH	Methanol
NMR	Nuclear magnetic resonance spectroscopy
PCC	Pyridium chlorochromate
PDC	Pyridium dichromate
TBAF	Tetrabutylammonium fluoride
TBDMS/TBS	*tert*-Butyldimethylsilane
TBDPS	*tert*-Butyldiphenylsilane
TES	Triethylsilane
THF	Tetrahydrofuran
THP	Tetrahydropyran
TIPS	Triisopropylsilane
TLC	Thin layer chromatography
TMS	Trimethylsilane
TPAP	Tetraproylammonium perruthenate

1 INTRODUCTION

This thesis was done in the Tampere University's synthetic chemistry research group which is led by Nuno Rafael Candeias. The research behind this thesis about quinic acid derivatives was started in April 2017 by doctoral student Suvi Holmstedt. Quinic acid is a naturally occurring product which has been widely studied because it is cheap, natural and has chiral centres. Crude oil is one of the biggest sources of chemicals but is not a renewable natural resource. Because quinic acid is a natural product, it fulfils the idea of "green-chemistry" and can be an alternative option for crude oil.

Quinic acid can be modified into different derivatives and new small chiral molecules are created. These small chiral molecules can be used as building blocks in other syntheses targeting, for example, natural products or pharmaceutical products. In this thesis, the usability of deoxygenated quinic acid is demonstrated by oxidative cleavage of vicinal diols. The oxidative cleavage produces building blocks with "handles", which are either aldehydes/ketones, which allows the building blocks to be used as a part of larger entity.

The objective of this thesis is to develop a method or methods for the oxidative cleavage of C-C bond for derivatives of quinic acid. The purpose of this thesis was to search information about the oxidative C-C bond cleavage and to synthesize the quinic acid derivatives by applying the methods of previous scientific publications. Another purpose is to purify and characterize the synthesized products by column chromatography, thin layer chromatography (TLC) and Nuclear Magnetic resonance spectroscopy (NMR).

In this thesis, three different quinic acid derivatives, which are triols, are studied and the bond between two carbon atoms are broken to yield aldehydes. This thesis describes what kind of methods can be used for the oxidative C-C cleavage for three quinic acid derivatives.

2 THEORETICAL BACKGROUND

2.1 Quinic acid

Quinic acid is a natural product which was isolated by Vauquelin for the first time in the year 1806. After that, quinic acid has been widely studied and in the year 1932 Fischer and Dangschat found the structure and stereochemistry of it. It is a naturally occurring product and is found widely from plants such as coffee beans, cinchona bark and *urtica dioica.* Quinic acid is found as a free molecule or as esters, which are called chlorogenic acids. (Barco et al. 1997, Mulzer, Drecher & Enev 2008.)

Fischer and Dangschat studied the relationship between the biogenetic origin of shikimic acid, gallic acid and quinic acid (figure 1) because all the mentioned molecules are natural products. They found out that the acids have a common biogenetic origin and that quinic acids presence can be related to shikimate pathway, which is an important multi-step process of chemical reactions within a cell. Shikimate pathway is used in biosynthesis by bacteria, plants, archaea, and others. (Barco et al. 1997.)

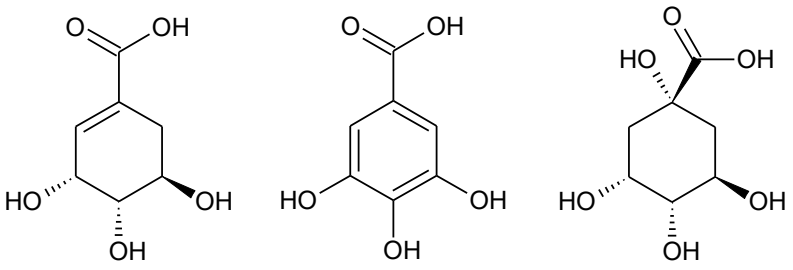


FIGURE 1. Natural products with common biogenetic origin.

The quinic acid is biosynthesized from D-glucose, which undergoes four steps with different enzymes (figure 2). The pathway starts from by converting D-glucose to D-erythrose-4-phosphate by transketolase. Phosphoenol pyruvate is added to the compound and they react and form 3-desoxyheptulosonic acid (DAHP). With DHQ synthase, the DAHP is converted to 3-dehydroquinic acid

(DHQ) which is then converted to quinic acid with dehydrogenase enzyme. (Barco et al. 1997; Mulzer et al. 2008; Clifford, Jaganath, Ludwig & Crozier 2017.)

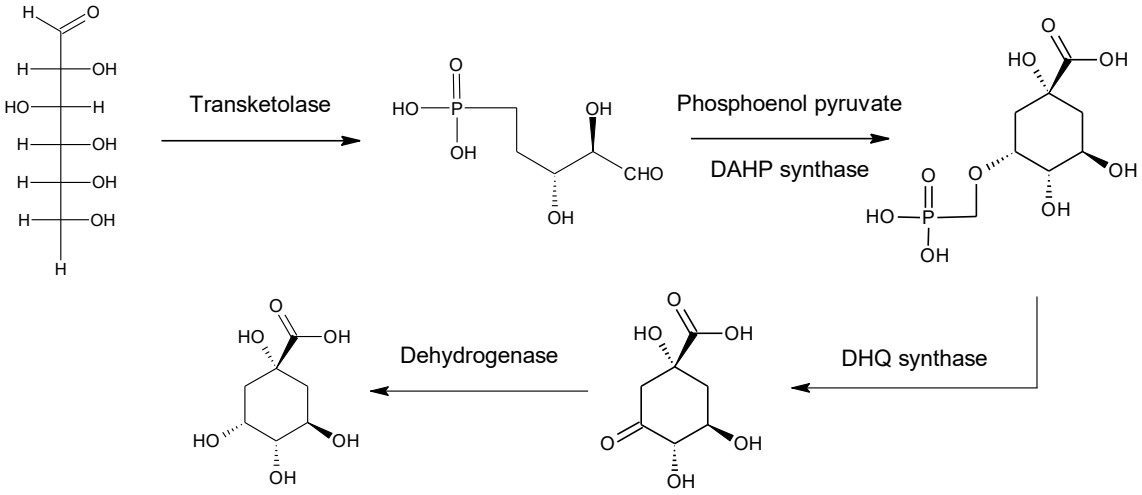


FIGURE 2. The biosynthesis of quinic acid from D-glucose.

The chlorogenic acids are a big group of natural compounds which are synthesized in plants. The chlorogenic acids are synthesized of hydroxycinnamate acids and D-(-)-Quinic acid. The most common hydroxycinnamates are coumaric acid, caffeic acid, sinapic acid and ferulic acid. (Mulzer et al. 2008; Clifford et al. 2017.)

In the year 1932 Fisher and Dangchat proposed that chlorogenic acid, ester, formed from quinic acid and caffeic acid would be called 5-*O*-caffeoylquinic acid. Later in the year 1950 Barnes reported that it is present in large quantities in coffee beans. Even though quinic acid is naturally available, the industrial preparation of quinic acid is necessary. Quinic acid can be produced by alkaline hydrolysis of the 5-*O*-caffeoylquinic acid or by fermentation of D-glucose. (Frost, Draths,& Ward 1998; Mulzer et al. 2008; Clifford et al. 2017.) The alkaline hydrolysis is presented in figure 3.

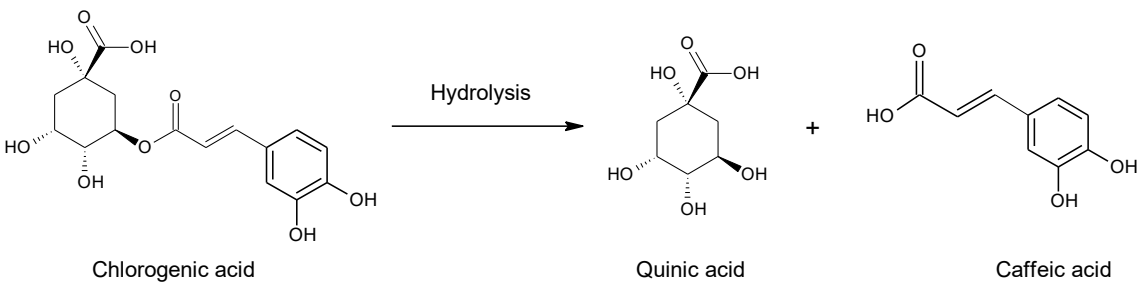


FIGURE 3. Hydrolysis of Chlorogenic acid

Quinic acid is one of the most widely studied natural products. Quinic acid is easily derivatized because it has good conformational properties and stereochemical variety with the defined functional groups. It is a diverse starting material for synthesizing chiral compounds because it has natural chirality. Quinic acid can be further functionalized by creating new bonds in the cyclohexane carbon skeleton. The advantage of the functional groups of quinic acid is that they influence the introduction of new functionalities, often making the process chemo-, regio-, and stereoselective. (Barco et al. 1997; Mulzer et al. 2008; Clifford et al. 2017.)

2.2 Oxidation and reduction agents

Most organic molecules contain multiple functional groups. In the molecule these groups can be the same or vary. The selectivity in these multifunctional organic molecules is different and it is important to predict which (chemoselectivity) functional group, where (regioselectivity) and how (stereoselectivity) it reacts (Clayden, Greeves & Warren 2012, 528). Earlier when talking about quinic acid, the good selectivity in all three selectivity types was mentioned.

Oxidations and reductions of organic compounds are often studied in regard to their selectivity. The oxidation-reduction reaction (redox reactions) are reactions in which transferring of electrons is involved. These reactions are used to synthesize many organic molecules. Redox reactions are producing energy and they are biologically important. General oxidation and reduction for different functional groups is presented in figure 4. (Bruice 2001, 789; Clayden et al. 2012, 528–529)

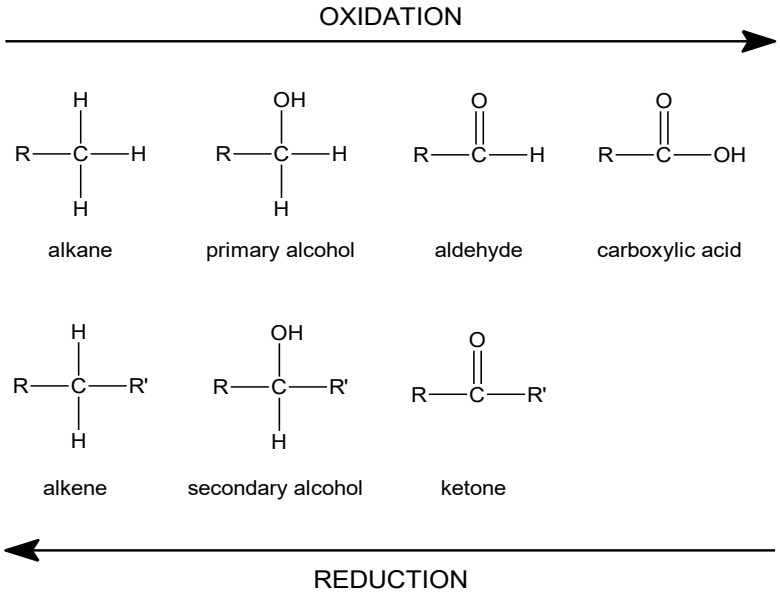


FIGURE 4. General oxidation and reduction of different functional groups. (Clayden et al. 2012, 544, modified)

Reduction happens when hydrogen is added to the molecule. This happens in a few ways which are described in this chapter. First type of reduction can happen by adding two hydrogen atoms to a molecule. These kinds of reductions are usually done with catalytic amount of metal, for example, reduction of alkenes and alkynes. Second type of reduction, called dissolving metal reductions, can happen by addition of a proton or an electron. In these reactions, an electron and a proton are donated from two different reagents. This is mostly used in the reduction of aromatics and alkynes and is usually done with liquid ammonia and sodium or lithium. (Bruice 2001, 791–795.)

Third type of reduction can happen when first, a hydride ion (H^-) is added, which forms an alkoxide ion, and second, a proton (H^+) is added to reduce the desired molecule completely. This reduction type is one of the most used in the manipulation of functional groups containing a carbonyl, because of its practical simplicity, good chemoselectivity and high yields. Two most generally used reducing agents for this reduction are borohydrides (e.g sodium borohydride, lithium borohydride) and lithium aluminum hydride. Borohydrides are milder reducing agents and they mostly reduce aldehydes to primary alcohols and ketones to secondary alcohols. Lithium aluminum hydride is a stronger reducing agent than borohydride and reduces carboxylic acids, esters and amides, which borohydride reduces really slowly or not at all. The milder conditions for the reaction are always better

because unwanted side reactions and decomposition of the molecule can be avoided. (Bruice 2001, 791-795; Clayden et al. 2012, 530-531.)

Out of borohydrides, sodium borohydride ($NaBH_4$) is mostly used because it is easy to handle (no unwanted side reactions), it can be used chemoselectively, and it tolerates water. Borohydrides are popular because the ketones and aldehydes are reduced into alcohols with a good yield. $NaBH_4$ can reduce only when protic solvents like ethanol or water are used. On the other hand, lithium borohydride ($LiBH_4$) can reduce when an aprotic solvent is used. The selection of the solvent or the used borohydride is important because the solvent or the electrophilic metal cation can provide the proton (H^+) needed for alkoxide, which eventually forms the alcohol. (Bruice 2001, 794–796; Clayden et al. 2012, 530–531.)

Lithium aluminum hydride ($LiAlH_4$) being a stronger hydride donor reacts exothermically with water which limits the usage of solvents to dry and aprotic. Usually $LiAlH_4$ is used in reductions which $NaBH_4$ cannot perform and it is a popular reagent just like $NaBH_4$. When reducing carboxylic acids, esters and amines with $LiAlH_4$, it first creates an aldehyde, which reacts further to primary or secondary alcohol, depending on the starting material. $LiAlH_4$ does not have the chemoselectivity that $NaBH_4$ has because the reduction happens much faster, which is why the choice between these two reductants needs to be considered. (Bruice 2001; Clayden et al. 2012, 530–531.)

Oxidation is the reverse reaction of reduction; it happens when hydrogen is removed from the molecule together with the bond electrons. Oxidation can happen with various methods and have chemoselective features, just like reduction. (Bruice 2001, 795–796; Clayden et al. 2012, 544.)

The most used methods for oxidizing alcohols and carbonyl compounds are oxidizing with metal-based reagents with high oxidation states, like chromium (VI) or manganese (VII). When oxidizing alcohols, the oxidation yields different products depending on the starting material. Primary alcohols oxidize to aldehydes and then further to carboxylic acids, but secondary alcohols oxidize to ketones. Chromium removes the hydrogen, for example, from the alcohol and is reduced from Cr(VI) to Cr(IV) in the process, which is why it is widely used with different

reagents. Cr(VI) can remove electrons from primary alcohols by creating a cyclic intermediate, and this is what happens, for example, with oxidizing agents pyridinium dichromate (PDC) and pyridinium chlorochromate (PCC). (Bruice 2001, 797–800; Clayden et al. 2012, 544–545.)

Jones reagent, which is formed from chromium trioxide and sulfuric acid, is used for oxidizing secondary alcohols to ketones. Its aqueous conditions lead to overoxidation of primary alcohols because aldehyde forms a hydrate that readily oxidizes to carboxylic acid. Oxidizing with PDC and PCC is suitable for primary alcohols because PDC and PCC are soluble to polar solvents, which can be used non-aqueously. One of the mildest conditions to oxidize alcohols to aldehydes, is by using catalytic amount of tetrapropylammonium perruthenate (TPAP). With TPAP using toxic chromium oxidations and heavy-metal waste are avoided. (Bruice 2001, 797–800, 804; Clayden et al. 2012, 544–545.)

Chromium oxidizing agents are not the strongest oxidizing agents, which is why over-oxidation can be avoided while using those oxidizing reagents. Sometimes it is preferable that the alcohol is over-oxidized straight into carboxylic acid. For this usage one of the strongest oxidizing agents, potassium permanganate, can be used. Potassium permanganate is used as acidic or basic aqueous solution, and the reaction follows the same pattern as with the chromium oxidizing agents. If the strong agent is not suitable for oxidizing aldehyde to carboxylic acid, milder reagents like silver oxide under basic conditions, or oxidation with sodium chlorite ($NaClO_2$) under mild acidic conditions can be used. (Bruice 2001, 804; Clayden, et al. 2012, 546.)

When performing an oxidation or reduction it is important to consider what kind of conditions the studied molecule endures. Some of the oxidizing and reducing agents requires strong acidic or basic conditions. The stereochemistry and the structure of the studied molecule can affect the oxidation or reduction because of the hindered positions. (Clayden et al. 2012.)

2.3 Protection groups and deprotection of alcohols

Sometimes when a molecule is in a reaction, it reacts in an unwanted way. When a selective reaction to one functional group is preferred in a compound, which contains other functional groups, the other groups needs to be temporarily blocked. To prevent the unwanted reactions in the other functional groups, it is important to consider what kind of protection can be provided to the functional groups. The protection group need to fulfil requirements about the selectivity, yield, stableness, easy deprotection and purification. (Wuts 2014, 1–4.)

The molecule needs to be stable for the desired conditions in the reaction performed, and the removal (deprotection) of the protection needs to be easy without decomposing the molecule. For example, the earlier mentioned reducing agent, lithium aluminum hydride (being highly reactive) can reduce esters but it reacts also with ketones and aldehydes, which might be undesirable. Then, the wanted functional group is protected with protection group that does not react with hydrides, and is removed after the reaction with the hydride is done. Mainly functional groups like hydroxyl groups, carboxylic acids, amines, phosphates and ketones usually need protection. Different kind of protection groups are used for all the functional groups. The protection of functional groups has an important role in multistep organic synthesis, because it provides chemoselectivity. (Wuts 2014, 1-4, 549–553). Earlier mentioned functions of quinic acid are mainly caused by hydroxy groups, why the focus in this chapter is on protecting and deprotecting of the alcohols.

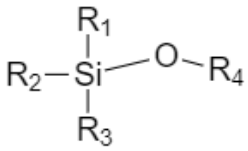
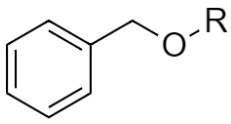
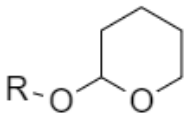
The hydroxy groups can be protected with various protection groups with different properties and a few examples are presented in table 1 (Clayden et al. 2012, 560). Silyl ethers are the most used protection groups against nucleophiles and bases. Silicon is one of the elements which has strong affinity for electronegative molecules. The silyl ethers are more stable against carbon and nitrogen bases and nucleophiles, but they are reacting with aqueous acid and fluoride salts. (Clayden et al. 2012, 550.)

Silyl ethers are a group of chemical compounds and they provide more than one selective compound for alcohol protection. Most seen silyl ethers are trimethylsilane (TMS), triethylsilane (TES), *tert*-butyldimethylsilane (TBDMS/TBS), triisopropylsilane (TIPS) and *tert*-butyldiphenylsilane (TBDPS) which are listed in size order. TBDPS being the bulkiest silyl ether group, is also the most stable and protects the alcohol better. The silyl ethers are added to a reaction as a silyl chloride. There are many methods for protecting alcohol with a silyl ether group, but the easiest and most common way is to add base and adjust the temperature from 0ºC to reflux, depending on hydroxy groups' position and the protection group. The deprotection of the silyl ether group can be done with aqueous acid (i.e. HCl) or fluoride salts (i.e. tetra-n-butylammonium fluoride (TBAF)). TMS group being the most labile can be removed simply by treating with methanol, but TBDPS group being the most stable requires acid or TBAF. (Clayden et al. 2012, 550; Wuts 2014, 26, 201, 549–553.)

When silyl ethers are not the best choice for being a protection group, for example, if the reaction is done in acidic conditions, benzyl ethers can be an optional choice. Benzyl ethers are great protection groups, because they are stable for most of the conditions that would normally yield alcohols, for example, bases, nucleophiles, reductants ($LiAlH_4$, $NaBH_4$) and oxidants (OsO_4, PDC/PCC). The usual method used for protecting alcohols with a benzyl group is by strong base and benzyl bromide. However, benzyl ether can do a C-O bond hydrogenolysis and can be removed gently with it. For example, hydrogenolysis with palladium catalyst cleaves the benzylic bonds. (Wuts 2014, 26–33, 146, 549–553.)

The third alternative option presented for protecting alcohol groups is tetrahydropyran (THP), which is also stable for most of the reagents, just like benzyl ether. THP is an acetal which is stable in basic conditions. THP is one of the earliest protection groups studied, and the protection of the hydroxyl group was first tried with an acid catalyzed reaction with dihydropyran. Unfortunately, this compound also has a weakness and it makes the THP vulnerable for hydrolysis in mildly acidic conditions, which is why this method is used for de-protection of the THP-group. (Clayden et al. 2012, 550; Wuts 2014, 2, 12, 549–553.)

TABLE 1. Common protection groups for alcohols (Clayden et al. 2012, 560, modified).

Protection group	Structure	Protects from
Silyl ethers (i.e. trimethylsilane)		Nucleophiles, C or N bases
Benzyl ether		Reductants, oxidants, nucleophiles, bases
Tetrahydropyranyl (THP)		Strong bases

Overall, the protection groups are very useful, but the usage of them extends the synthetic route of the molecule by two steps. They can be wasteful because the extra steps in the synthesis decrease the yield, because the yield is not usually 100 %.

2.4 Glycol cleavage

Glycol cleavage is an old oxidation method found by Léon Malaprade in 1928 and a few years later with further investigation by Rudolf Criegee in 1931. These oxidation reactions are valuable methods in synthetic chemistry, especially for oxygenated natural products. The Malaprade reaction is based on using periodic acid (HIO_4) and its salts to cleave C-C bond between atoms/carbons containing two hydroxyl groups in adjacent carbons. The cleavage of the C-C bond yields two aldehydes or/and ketones. The Criegee reaction is based on the same mechanism as the Malaprade reaction. The main difference is that lead tetra-acetate is used as the reagent to achieve the C-C bond cleavage. (Schmidt & Stark 2015.)

2.4.1 The Malaprade reaction

The first reported C-C cleavage oxidation in 1928 by periodic acid or its salts is named after its discoverer. The reagents of the oxidation are proved to be advantageous because of the good properties such as being stable, non-toxic and easy to handle (not hygroscopic etc.). The periodates are solids that can be used in all scales in reactions, usually the equivalent amount of the periodate is a bit excessive, for example, 1.2-1.5 equivalents. The periodates need water to perform the oxidation perfectly, which is why the generally used solvent mixtures are partly aqueous. This feature makes periodic acid and its salts comparable to other reagents. (Malaprade 1928; Pawar, Sankaranarayanan & Chattopadhyay 1995; Barthes & Grison 2012.)

The Malaprade reaction proceeds with a mechanism (type 1) that is presented in figure 5. Periodic acid reacts with the vicinal diol and creates a cyclic diester complex between the vicinal diol and periodic acid, assuming that the hydroxyl groups are placed close to each other in the molecule's conformation. This cyclic diester complex will collapse oxidatively to the two products. The ketone is formed when carbon with OH-group has two R-groups attached to it. The aldehyde is formed when an R-group and a hydrogen are attached to the carbon with the OH-group. (Schmidt & Stark 2015.)

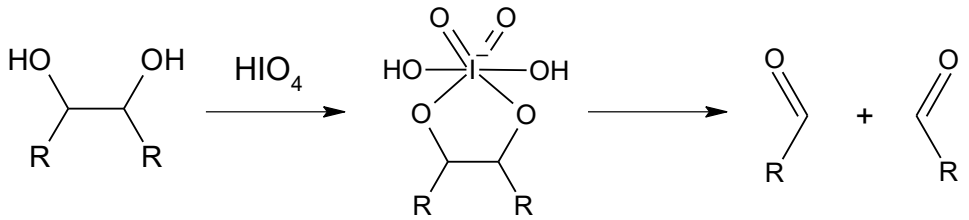


FIGURE 5. Type 1 mechanism of the Malaprade reaction (Schmidt & Stark 2015)

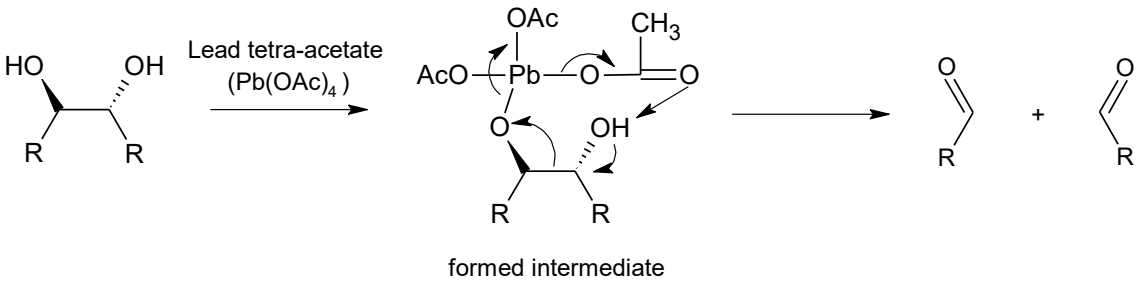
Periodate reactions are usually carried-out under pH neutral conditions which leads to high yield in products. If acidic functional groups are present in the reaction, the addition of a buffer should be considered to get neutral conditions. The previously mentioned mechanism type 1 is proved to be specific for 1,2-diols and high yielding but the mechanism has a few limiting features. The stereochemistry

of the molecules can affect the reaction rate, for example, periodate cannot operate fully with *trans*-diols because of the orientation of the reacting hydroxy group. (Schmidt & Stark 2015.)

2.4.2 The Criegee reaction

The Criegee reaction was published for the first time a few years after publishing the Malaprade reaction. Criegee oxidation has the same method as the Malaprade reaction making it a second option for cleaving the C-C bonds. In the Criegee reaction, the reagent used is not a periodate or its salt, but lead tetra-acetate (LTA). Lead tetra-acetate is a strong oxidant, but it is slightly milder than periodate, why the Criegee reaction might be a better option for reactions with sensitive molecules. Lead tetra-acetate has a wide range of applications in synthetic chemistry just like the periodate, but it is preferred when the reaction conditions need to be mild or non-aqueous. Periodates need an aqueous solvent mixture, which can limit the usage with non-water-soluble molecules. Lead tetra-acetate is not as selective as the periodate and when used as recommended stoichiometric amount the cleavage yields to aldehyde and/or ketones. (Reeves 1949; Schmidt & Stark 2015.)

The Criegee reaction proceeds more commonly with the mechanism (type 1) which is presented in figure 5, just like the Malaprade reaction. The lead tetra-acetate reacts with the vicinal diol, and a cyclic intermediate product is produced between them. The configuration of the diol affects the reaction mechanism of the lead tetra-acetate in some cases. Sometimes, if the conformation cannot operate with the mechanism type 1 it has an alternative mechanism (type 2) to undergo. This mechanism (type 2) is presented in figure 6. The mechanism type 2 is considerably slower than the mechanism type 1, because the cyclic intermediate product is strained. Mechanism type 2 undergoes usually for *trans*-diols. Same reaction limiting properties are affecting the Criegee reaction as the Malaprade reaction. (Schmidt & Stark 2015.)



formed intermediate

FIGURE 6. Type 2 mechanism of the Criegee reaction

2.5 Analytical methods

2.5.1 Nuclear magnetic resonance spectroscopy (NMR)

Nuclear magnetic resonance spectroscopy was developed by Felix Bloch and Edward Purcell in the end of 1940s (Bruice 2001). NMR is an analytical technique used for identifying molecule structures of organic compounds. NMR is a special technique and is different from other spectroscopic techniques used for identifying compounds. What separates NMR is that with it, besides identifying one specific atom's functionalization, NMR can also identify the functionalization of the neighbouring atoms. NMR is used in various applications and different fields of chemistry and physics in quality control and research. (Bruice 2001, 534–535; Keeler 2002.)

The principle behind NMR is in the nuclei of the molecule. All the molecules contain protons and neutrons and are electrically charged which leads to the fact that all nuclei have a spin state. The spin of the nuclei generates its own magnetic field which can be altered by an external magnetic field by placing the nuclei in there. (Keeler 2002.)

In a natural magnetic field, the spin state of an atom is randomly orientated. When identifying a compound with NMR spectroscopy, the spin states of the atoms align their magnetic moment in two states, depending on what spin state that nuclei is performing. When the spin of the atom is + ½, the spin state is orientated along the applied magnetic field and is called α spin state, the base energy level. In the

other hand when the spin is - ½, the spin state is aligned against the applied magnetic field and is called β spin state, the higher energy level. (Bruice 2001, 534–535.)

When the nuclei are oriented along or against the applied magnetic field, they create an energy transfer possibility between the different energy levels. When the nuclei shift from the base level to the higher level, it absorbs radiation on radiofrequency band and this phenomenon is called flipping the spin. When the spin is flipped from higher level to lower level the nuclei emits the energy. It is important to keep the magnetic field inside the spectrometer at constant, so that the short radiofrequency pulses excite all the nuclei at the same time. The spin states are flipping back and forth of the higher and lower energy states, and are creating signals whose frequency, depends on the energy difference between the α and β spin states. This kind of transfer produces signals and sine wave emission, which is measured and converted with Fourier transformation to intensity versus frequency information, to give an NMR spectrum. (Bruice 2001, 535–537; Keeler 2002.)

A spectrometer with a certain operating frequency can be tuned to different energies with various built in radiation sources, to measure different kind of nuclei, for example ^{1}H, ^{13}C and ^{15}N. The NMR spectrum obtained from the measurement gives peaks with the help of which, you can identify the measured compound. The way how the different peaks of the NMR spectrum are identified is the chemical shifts, which nuclei in a different chemical environment produce. The magnetic fields around the nuclei are affected by the different chemical environment. The chemical environment affects the spin flipping of the nuclei and the electromagnetic radiation measured. To identify these chemical shifts, a small amount of reference compound is added in the sample. The reference compound is usually TMS, which is usually added to the NMR solvent. Silicon is less electronegative than carbon. The positions of the peaks obtained from the sample are defined by comparing how far they are from the reference compound peak. Certain functionalities commonly appear in certain regions of the NMR spectrum, why the spectrum can be divided to regions by the functional groups (figure 7). (Braun, Kalinowski & Berger 1998; Bruice 2001, 535–537, 542; Keeler 2002.)

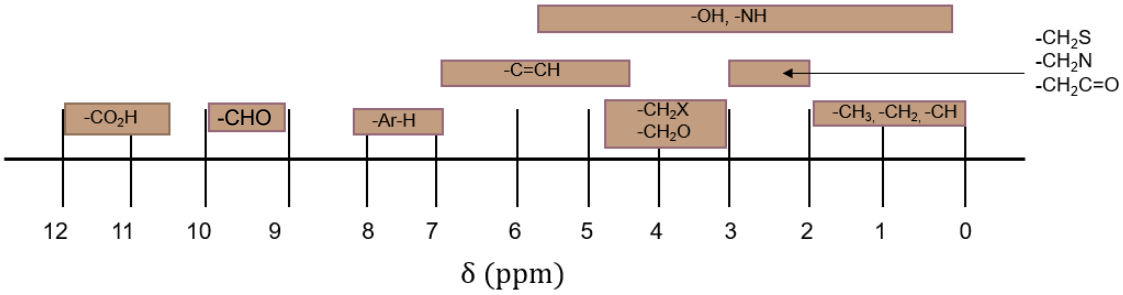


FIGURE 7. Chemical shifts of the functional groups in ^{1}H NMR (Bruice 2001, 543, modified)

With chemical shifts, the compound can be identified with its functionality. If all the nuclei were in the same environment, all the peaks in the spectrum would be at the same position. The thing that affects the nucleus in the same chemical environments is the shielding of the nuclei. The nuclei are surrounded by a cloud of electrons, and this cloud of electrons that circulates around the nuclei, affects the magnetic field in which the sample is placed. It causes a different effective magnetic field around the nuclei and modifies the environment where the nuclei are placed. Shielding separates the nucleus with the same functionality. When the nuclei are highly shielded, the energy difference between the spin states is bigger, and the nuclei need a higher frequency to flip the spin. When the nuclei are less shielded, the energy difference is lower, and the nuclei need a lower frequency of radiation to flip the spin. The electronegativity of the neighbouring atom effects how strong the shielding of the nuclei is. When nuclei are next to a highly electronegative atom, the nuclei are deshielded. (Bruice 2001, 537–541, 543; Keeler 2002.)

Example of ^{1}H spectrum of (1S,2R,4R)-cyclohexane-1,2,4-triol is presented in figure 8. Peaks around 1.0 to 2.0 ppm are peaks and in this case are coming from shielded protons, for example, $-CH_3$, $-CH_2$ and -CH groups. Peaks coming around 3.0 to 4.5 ppm come from less shielded protons which are next to highly electronegative atom, for example, next to -COH groups. The peak coming around 4.8 is the solvent residue peak of the NMR solvent, deuterium oxide (D_2O), the peak coming around 2.23 ppm is acetone (used as standard) and 0.00 ppm is trimethylsilane (TMS). (Bruice 2001, 542–547.)

The easiest way to predict if the molecule is the desired one is to calculate the number of protons that are next to highly electronegative atoms (deshielded) and protons that are not (Bruice 2001 549-551). In (1*S*, 2*R*, 4*R*)-4-(hydroxymethyl)cyclohexane-1,2-diol, seven protons are not next to electronegative atoms, but four protons are. The integration of the peaks is important because it gives a number value that represents the proton count (Bruice 2001, 549–551).

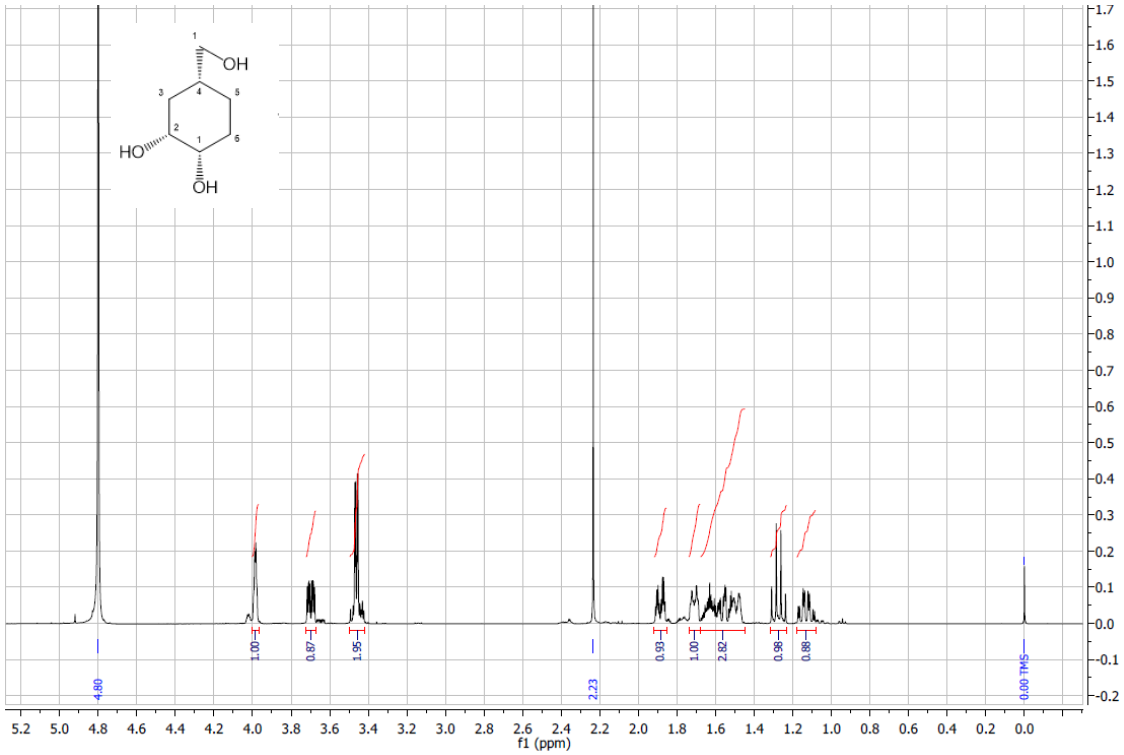


FIGURE 8. ^{1}H NMR spectrum from (1*S*, 2*R*, 4*R*)-4-(hydroxymethyl)cyclohexane-1,2-diol

When identifying more complex molecules, the peaks are harder to identify, which is why the shape of the peak is noted. Single peaks may have been split into clusters of peaks. One peak is a singlet, two peaks in the cluster means a duplet, three peaks in the cluster means a triplet and so on. In simple cases, the split of clusters is telling how many hydrogens are in the neighbouring atom. The amount of splits in a cluster is one more than the number of hydrogens attached to the neighbouring atom, for example, singlet does not have any hydrogen in the neighbouring atom, duplet has one hydrogen and triplet has two hydrogens. (Braun et al. 1998; Bruice 2001, 549–550)

The NMR spectrometer's most important compartments are sample holder, magnet, sweep generator, sweep coil, radiofrequency transmitter, receiver coil, amplifier, detector, and recorder (oscilloscope) (Braun et al. 1998). The instrumentation of the NMR spectrometer is seen from figure 9.

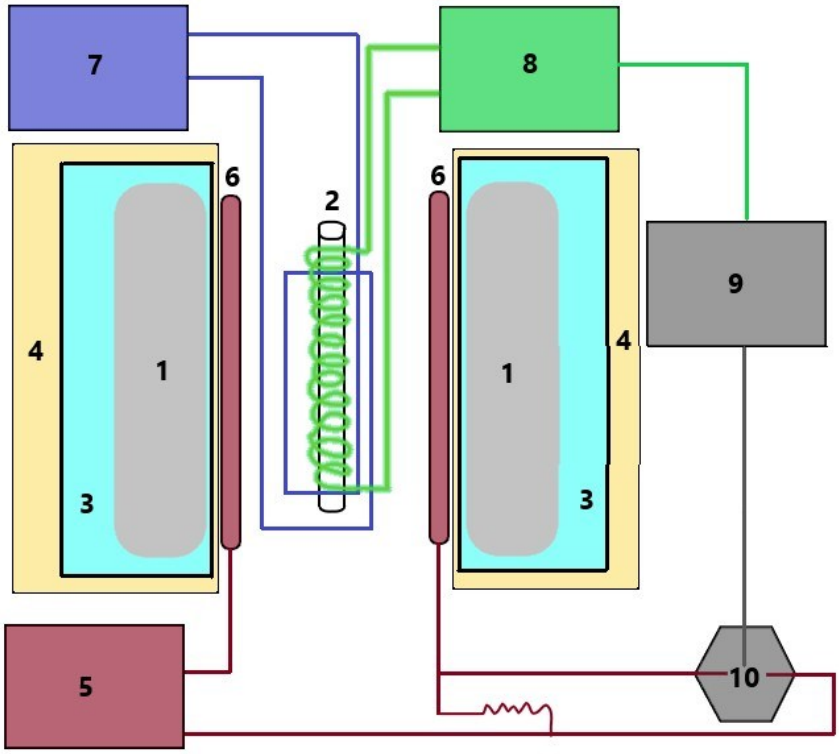


FIGURE 9. The instrumentation of the NMR spectrometer: 1. Magnets 2. Sample holder 3. Liquid helium 4. Liquid nitrogen 5. Sweep generator 6. Sweep coils 7. Radiofrequency transmitter 8. Amplifier 9. Detector 10. Recorder (oscilloscope)

The magnet (1 in figure 9) of the NMR spectrometer are the most important part because they create the applied magnetic field for the sample. The magnet can be one of the three types depending on what kind of frequency it is wanted to operate on. Conventional magnets can create 30-60 MHz frequency, permanent or electromagnets can create 60-100 MHz frequency and super conducting magnet can create even 1.1 GHz frequency. The most used magnet is the super conducting magnet. (Braun et al. 1998; Keeler 2002.)

The sample holder (2 in figure 9) is a plastic holder (1 in picture 1) in which a 5 mm glass tube (2 in picture 1) is slid in which is presented in picture 1. The sample is placed in the plastic holder (3 in picture 1) so that it is 20 cm deep. The plastic

holder with the glass tube is placed in a sample rack and the spectrometer will take the plastic holder with the sample tube inside the spectrometer. (Braun et al. 1998; Keeler 2002.)

PICTURE 1. The plastic sample holder in the measurement tube. 1. Plastic sample holder 2. Glass tube (sample) 3. Measurement tube

Liquid nitrogen (4 in figure 9) and liquid helium (3 in figure 9) are required for the instrumentation to reach extremely low temperature to keep the strong and constant magnetic field. The magnets are inside stainless steel or aluminum dewars, which contain the liquid helium. The liquid helium is contained by help of the liquid nitrogen. (Braun et al. 1998; Keeler 2002.)

Sweep generator (5 in figure 9) is conducting the sweep coils which are a set of Helmholtz coils. The sweep coils are altering the magnetic field which is created with the superconducting magnet. The sweep coils are located parallel to the magnet so that controlling the strength of the magnetic fields would be easy. (Braun et al. 1998; Keeler 2002.)

The radiofrequency transmitter (7 in figure 9) is a part of coil mounted perpendicularly to the path of magnetic field and the receiver coil. The transmitter is creating the short pulses of radio waves on radiofrequency which passes through the coils and towards the sample. The radiofrequency transmitter has usually more than one coil to create different intensities of radio waves. (Braun et al. 1998; Keeler 2002.)

The receiver coils are surrounding the sample and are connected to the amplifier (8 in figure 9). The receiver coils receive the electromagnetic radiation from the sample and the radiation goes to the amplifier which amplifies the electromagnetic radiation by 10^5 times. The signals received from the sample need to be amplified because they are weak. (Braun et al. 1998; Keeler 2002.)

The detector (9 in figure 9) detects the signal produced by the resonating nuclei by intensity versus time data. The detector moves this data for the recorder (10 in figure 9) (oscilloscope) which does the Fourier transformation of the data. The oscilloscope will produce the intensity versus frequency NMR spectrum. (Braun et al. 1998; Keeler 2002.)

3 METHODS

3.1 Applications of analytical methods

The reactions were monitored with thin-layer chromatography (TLC) with commercial silica gel plates with fluorescent indicator (Merck silica gel 60 F_{254}). The TLC plates were run with various mixtures of different eluents depending on the polarity of the reaction and the components. The TLC plates were visualized under ultra-violet light at 254 nm and by dyeing with staining reagent vanillin. The purification of the products was performed by flash column chromatography using silica gel 60 powder as the stationary phase.

The ^{1}H NMR spectra were recorded with 500 MHz JEOL Nuclear Magnetic Resonance ECZR series spectrometer (JNM-ECZ500R) which is presented in picture 2. The NMR samples were prepared by weighing 10-20 mg of the sample and dissolving to approximately 750 µl of deuterated solvent. Different deuterated solvents were used depending on the sample's polarity/solubility. The ^{1}H NMR spectrum of the samples are presented with chemical shifts of the sample in ppm referenced to the residual peaks of the used NMR solvent. In most cases, the used solvents were chloroform-d ($CDCl_3$) (residual peak at 7.26 ppm) and dimethyl sulfoxide (d6-DMSO) (residual peak at 2.50 ppm). When deuterium oxide (D_2O) (4.8 ppm) was used as the NMR solvent, a small amount of acetone was added and the ^{1}H NMR spectrum was referenced to the residual peak of acetone (2.22 ppm).

PICTURE 2. JEOL Nuclear Magnetic Resonance 500 MHz ECZR series spectrometer (JNM-ECZ500R)

3.2 Modification of the studied quinic acid derivatives

The three quinic acid derivatives, containing different degree of hydroxylation, were synthesized from commercially available quinic acid with multi-step synthesis process. These three quinic acid derivatives (**1**, **4**, and **7**) were prepared in house in practical training part of the studies before the thesis part begun and the synthesis routes for the preparing can be seen from appendix 1 figure 21.

The first quinic acid derivative that was studied in this thesis, (1*S*, 3*R*, 4*R*, 5*R*)-6-oxabicyclo[3.2.1]octane-1,3,4-triol (**1**), was further modified with two steps (figure 10). In the first step, the first derivative was synthesized to aldehyde (2*R*, 4*R*)-4-hydroxy-4-(2-oxoethyl)tetrahydrofuran-2-carbaldehyde (**2**) by performing the C-C cleavage, which was predicted to happen between carbons three and four with hydroxyl groups. In the second step, the aldehyde was synthesized to alcohol (3*S*, 5*R*)-3-(2-hydroxyethyl)-5-(hydroxymethyl)tetrahydrofuran-3-ol (**3**) by reduction of the free aldehyde groups.

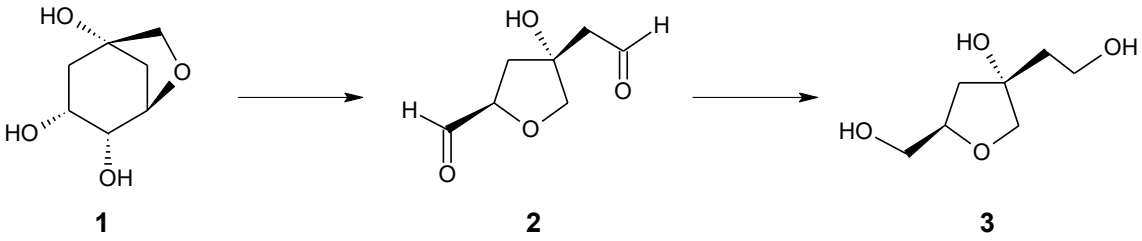


FIGURE 10. The synthetic route of modifying the first studied derivative (**1**)

The second quinic acid derivative that was studied in this thesis, (1*S*, 2*R*, 4*R*)-4-(hydroxymethyl)cyclohexane-1,2-diol (**4**), was also further modified with two steps (figure 11). In the first step, the second derivative was synthesized to alcohol (1*S*, 2*R*, 4*R*)-4-(((tert-butyldimethylsilyl)oxy)methyl)cyclohexane-1,2-diol (**5**) by protecting the primary alcohol with silyl ether. In the second step, the protected alcohol was synthesized to aldehyde (*R*)-3-(((tert-butyldimethylsilyl)oxy)methyl)hexanedial (**6**) by doing the C-C cleavage, which was also predicted to happen between carbons with hydroxyl groups.

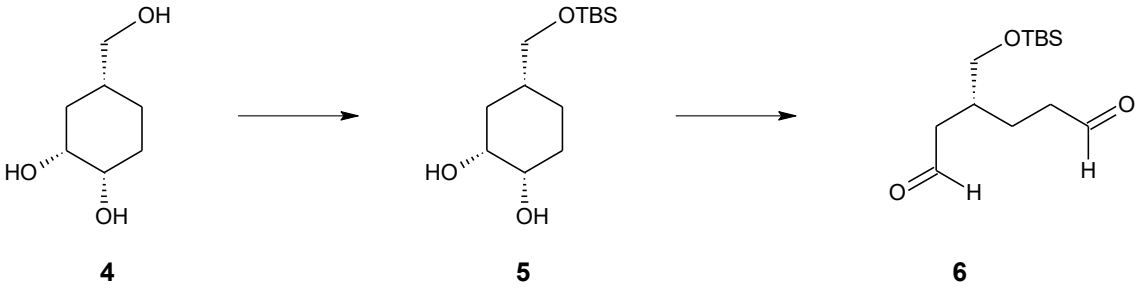


FIGURE 11. The synthetic route of modifying the second studied derivative (**4**)

The third quinic acid derivative (**7**) that was studied in this thesis was synthesized in four steps (figure 12). It is an intermediate for the synthesis of natural product homocitric acid. It was an exceptional molecule, because it could be modified into one previously studied molecule, on which the oxidative C-C cleavage had been successfully performed. This is why the C-C cleavage was not performed for the third derivative. Tavassoli, Duffy & Young (2005) reported that stereochemically different homocitrates and homocitrate lactones could be synthesized from shikimic acid. Shikimic acid and quinic acid having a common biogenetic origin and quinic acids presence being related to a shikimate pathway regulation gave a chance to modify the quinic acid derivative into a form where previously reported homocitrates were synthesized (Barco et al. 1997).

In the first step, the third derivative was synthesized to aldehyde (1*R*,3*R*,4*S*)-1,3,4-tris((tert-butyldiphenylsilyl)oxy)cyclohexane-1-carbaldehyde (**8**) by oxidizing the primary alcohol. In the second step, the aldehyde was synthesized to carboxylic acid (1*R*,3*R*,4*S*)-1,3,4-tris((tert-butyldiphenylsilyl)oxy)cyclohexane-1-carboxylic acid (**9**) by oxidation. In the third step, the carboxylic acid was synthesized into ester methyl (1*R*,3*R*,4*S*)-1,3,4-tris((tert-butyldiphenylsilyl)oxy)cyclohexane-1-carboxylate (**10**) by methylation. In the last and fourth step, the TBDPS protecting groups were removed to achieve ester with three hydroxyl groups methyl (1*R*,3*R*,4*S*)-1,3,4-trihydroxycyclohexane-1-carboxylate (**11**).

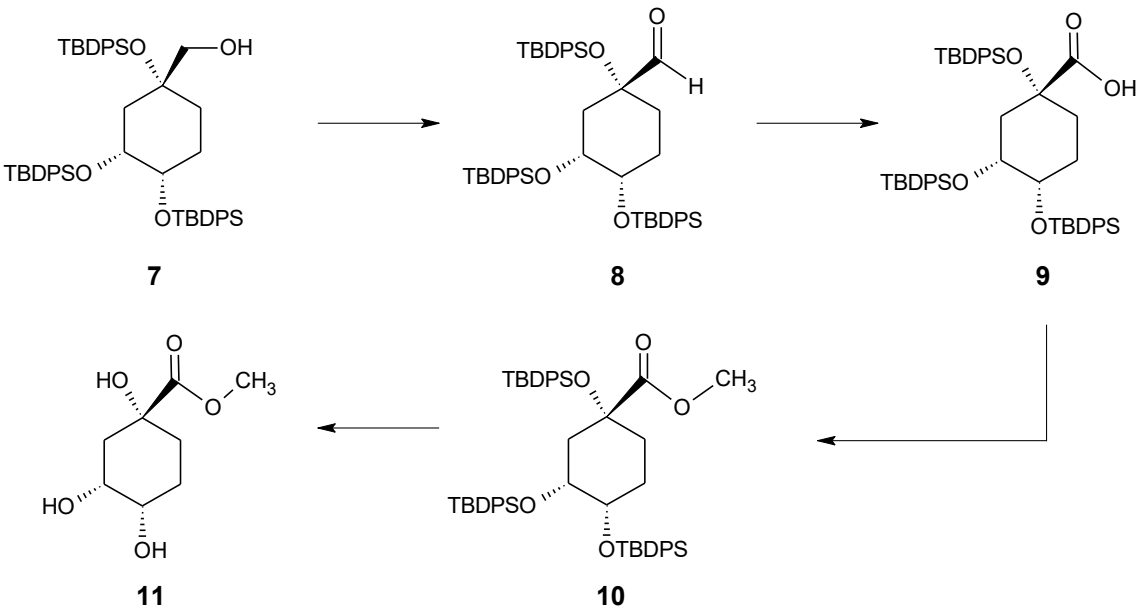
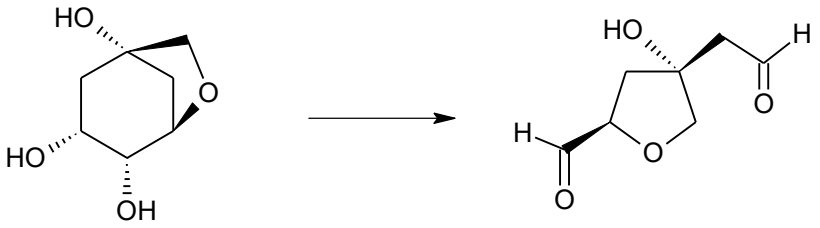


FIGURE 12. The synthetic route of modifying the third studied derivative (**11**)

3.2.1 Oxidative C-C cleavage of molecule 1

The first reaction of the quinic acid derivative that converts molecule **1** to molecule **2** is done by an oxidative C-C cleavage which is presented in figure 13. Two methods found in the literature were tested to find the right method. The experimental in the method is the same but the conditions vary. The two tested methods of the synthesis are described in this chapter.



1 **2**

FIGURE 13. The synthesis of molecule **2**

Method 1 – Test 1

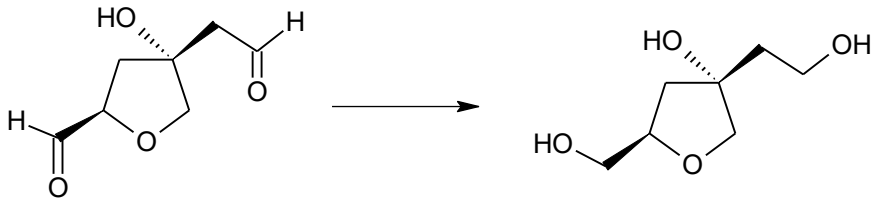
Starting material **1** (10 mg) was dissolved in acetonitrile(ACN)-water solution (3:2, 250 µl) at room temperature. The reaction mixture was cooled to 0°C and sodium periodate ($NaIO_4$) (16 mg, 0.07 mmol, 1.2 eq) was added. The mixture was left to stir at 0°C for 15 minutes before lifting to room temperature for 1 hour. The reaction mixture was filtrated through 5 cm silica batch with acetonitrile (20 ml). The residue was concentrated yielding a brownish solid. The obtained product was identified with NMR spectroscopy.

Method 2 – Test 1

Starting material **1** (10 mg) was dissolved in methanol(MeOH)-water solution (3:1, 200 µl) at room temperature. The reaction mixture was cooled to 0°C and $NaIO_4$ (15 mg, 0.07 mmol, 1.1 eq) was added and was left to stir at 0°C for 30 minutes. The reaction mixture was purified by dry-pack flash column chromatography using mixture of MeOH and ethyl acetate (EtOAc) (10:90) as eluent. The residue was concentrated yielding a brownish solid. The obtained product was identified with NMR spectroscopy.

3.2.2 Reduction of molecule 2

The second reaction of the first studied quinic acid derivative that converts molecule **2** to molecule **3** is done by reducing the aldehydes which is presented in figure 14. The detailed method of the synthesis is described in this chapter.



2 **3**

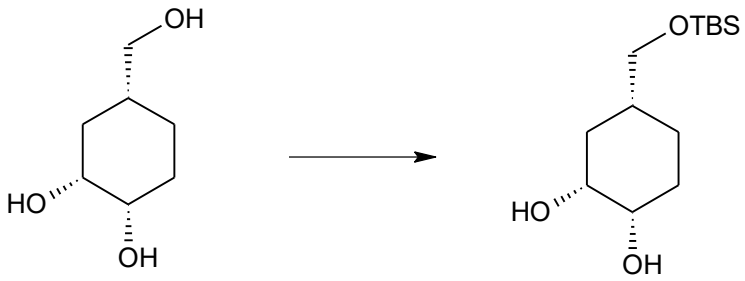
FIGURE 14. The synthesis of molecule **3**

Method 1 – Test 1

Starting material **2** (10 mg, 0.06 mmol) was dissolved in ethanol (0.3 ml) at room temperature. The reaction mixture was cooled to 0°C and $NaBH_4$ (70 mg, 0.2 mmol, 3 eq) was added portion-wise. It was left to stir at 0°C for 15 minutes before lifting the reaction mixture to room temperature for 30 min. The reaction was quenched with water (0.1 ml) and was left to stir at room temperature for 10 minutes. Reaction was diluted with EtOAc and the residue obtained was purified by dry-pack flash column chromatography using a mixture of EtOAc/MeOH (90:10) as eluent, yielding a white solid. The obtained product was identified with NMR spectroscopy.

3.2.3 Protection of the primary alcohol of molecule 4

The first reaction of the second quinic acid derivative that converts molecule **4** to molecule **5** was done by protecting the primary alcohol which is presented in figure 15. Silyl chlorides are widely used protection groups which led to the usage of TBS-Cl as a protection group (Clayden et al. 2012). Three different conditions were tested to get the desired product and the description of the methods are described in this chapter.



4 **5**

FIGURE 15. The synthesis of molecule **5**

Method 1 – Test 1

Starting material **4** (30 mg, 0.2 mmol) was dissolved in dry dichloromethane (DCM) (1 ml) at room temperature. The reaction mixture was cooled to 0°C followed by the addition of imidazole (25 mg, 0.4 mmol, 1.8 eq) and portion-wise addition of TBS-Cl (48 mg, 0.3 mmol, 1.55 eq). It was left to stir at 0°C for 15 minutes before lifting it to room temperature for 18 h. The reaction mixture was diluted with DCM and quenched with water (1 ml). The aqueous layer was extracted with DCM (3 x 10 ml) and the combined DCM extracts were dried over magnesium sulfate ($MgSO_4$) and filtered followed by the concentrating of the residue. The obtained residue was purified by flash column chromatography using a mixture of EtOAc/hexane (80:20) as eluent yielding a clear oil. The obtained product was identified with NMR spectroscopy.

Method 2 – Test 1

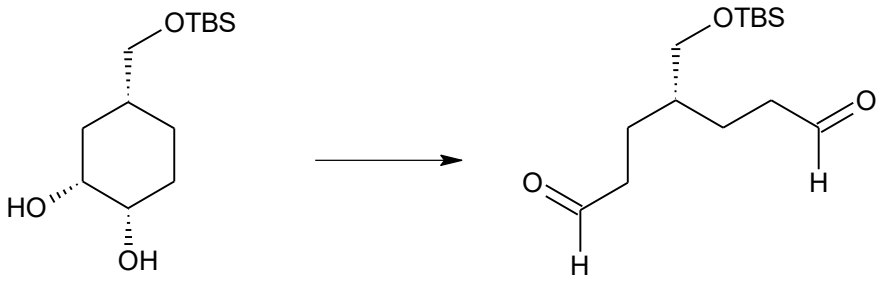
Starting material **4** (66 mg, 0.2 mmol) was dissolved in ACN (1.7 ml) at room temperature. The reaction mixture was cooled to 0°C followed by the addition of imidazole (52 mg, 0.8 mmol, 1.7 eq) and portion-wise addition of TBS-Cl (105 mg, 0.7 mmol, 1.55 eq). It was left to stir at 0°C for 15 minutes before lifting it to room temperature for 6 h. The reaction mixture did not proceed to the end and after 6 hours, starting material was still left. TBAF (47 mg, 1.8 mmol, 4 eq) was added to get the starting material back. The obtained product was purified by filtering through 3 cm silica batch. The obtained product was not identified.

Method 3 – Test 1

Starting material **4** (54 mg, 0.37 mmol) was dissolved in dry DCM (1.8 ml) at room temperature. The reaction mixture was cooled to 0°C followed by the addition of triethylamine (62 µl, 0.44 mmol, 2.4 eq) and 4-dimethylaminopyridine (3 mg, 10 mol%) and portion-wise addition of TBS-Cl (120 mg, 0.4 mmol, 2.2 eq). It was left to stir at 0°C for 5 minutes before lifting it to room temperature for 2.5 h. The reaction mixture was diluted with DCM and quenched with water (1 ml). The aqueous layer was extracted with DCM (3 x 10 ml) and the combined DCM extracts were dried over $MgSO_4$ and filtered followed by the concentrating of the residue. The obtained residue was purified by flash column chromatography using a mixture of EtOAc/MeOH (90:10) as eluent yielding a clear oil. The obtained product was identified with NMR spectroscopy.

3.2.4 Oxidative C-C cleavage of molecule 5

The second reaction of the second quinic acid derivative that converts molecule **5** to molecule **6** was done by doing the oxidative C-C cleavage which is presented in figure 16. The previously found method for the oxidative C-C cleavage for the first quinic acid was also used in this synthesis. The methods are described in this chapter.



5 **6**

FIGURE 16. The synthesis of molecule **6**

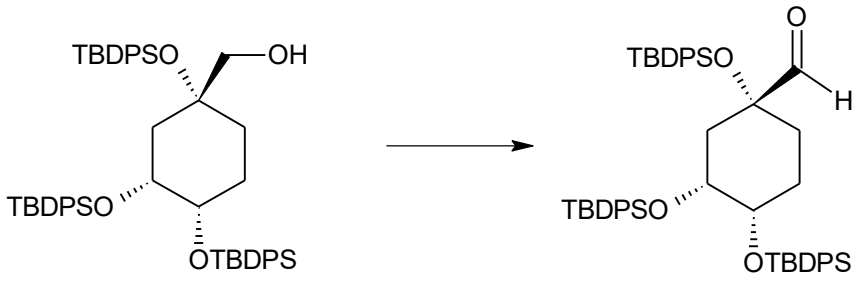
Method 1 – Test 1

Staring material **5** (20 mg, 0.08 mmol) was dissolved in ACN-water (ratio 3:2, 0.5 ml) solution at room temperature. The reaction mixture was cooled to 0°C and $NaIO_4$ (15 mg, 0.07 mmol, 1.2 eq) was added. The reaction mixture was left to

stir at room temperature for 1 hour and 15 minutes. The reaction was filtrated through 5 cm silica batch with ACN (20 ml). The residue was concentrated yielding a pinkish solid which was identified with NMR spectroscopy.

3.2.5 Oxidation of molecule 7

The first reaction of the third quinic acid derivative that converts molecule **7** to molecule **8** was done by oxidizing primary alcohol to aldehyde which is presented in figure 17. The oxidation of primary alcohol can occur by a variety of methods. The starting material did not react as desired and that is why a variety of methods and different conditions were tested. In total, seven different methods and conditions were tested. The methods and conditions are described in this chapter.



7 **8**

FIGURE 17. The synthesis of molecule **8**

Method 1 - Test 1

Starting material **7** (20 mg, 0.02 mmol) was dissolved in ACN-water solution (1:1, 114 µl) at room temperature followed by the addition of (diacetoxyiodo)benzene (BAIB) (16 mg, 0.05 mmol, 2.2 eq) and (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) (7 mg, 0.004 mmol, 0.2 eq). The reaction mixture was left to stir at room temperature for 2 hours. After 2 hours, the reaction mixture was concentrated and extracted with acid-base extraction with 1 M sodium hydroxide and 1 M hydrochloric acid (HCl) and DCM. The predicted product did not move to the organic layer and all the volatiles were removed from the aqueous layer. The white solid products obtained was identified by NMR spectroscopy.

Method 1 - Test 2

Starting material **7** (20 mg, 0.02 mmol) was dissolved in ACN-water solution (1:1, 114 µl) at room temperature followed by the addition of BAIB (16 mg, 0.05 mmol, 2.2 eq) and TEMPO (7 mg, 0.004 mmol, 0.2 eq). The reaction mixture was left to stir at room temperature for 2 hours. After 2 hours, the reaction mixture was concentrated and extracted with diethyl ether (3 x 10 ml). The predicted product did not move to the organic layer and all the volatiles were removed from the aqueous layer. The white solid product obtained was not identified.

Method 1 - Test 3

Starting material **7** (20 mg, 0.02 mmol) was dissolved in ACN-water solution (1:1, 114 µl) at room temperature followed by the addition of BAIB (16 mg, 0.05 mmol, 2.2 eq) and TEMPO (7 mg, 0.004 mmol, 0.2 eq). The reaction mixture was left to stir at room temperature for 2 hours. After 2 hours, the reaction mixture was quenched with saturated aqueous sodium carbonate followed by the addition of 4 M HCl to adjust the pH to acidic. The reaction mixture was extracted with DCM (3 x 10 ml). The combined DCM layers were dried over $MgSO_4$, filtered and concentrated. The white solid products obtained was identified by NMR spectroscopy.

Method 2 – Test 1

Starting material **7** (20 mg, 0.02 mmol) was dissolved in DCM-water solution (3:1, 1.1 ml) at room temperature followed by the addition of BAIB (18 mg, 0.06 mmol, 2.5 eq) and TEMPO (7 mg, 0.001 mmol, 0.3 eq). The reaction mixture was left to stir at room temperature for 18 hours. The reaction was not continued after 18 hours and no product was obtained.

Method 3 – Test 1

Starting material **7** (20 mg, 0.02 mmol) was dissolved in acetone (0.5 ml) at room temperature. The reaction mixture was cooled to -10ºC followed by the addition of Jones reagent (0.05 ml, 6 eq). The reaction mixture was left to rise to room temperature in 18 hours. The reaction was quenched with saturated aqueous sodium hydrogen sulfite (1 ml) followed by the extraction with diethyl ether (3 x 10 ml). The combined extraction layers were dried over $MgSO_4$, filtered and all

volatiles were evaporated. The white solid product obtained was identified by NMR spectroscopy.

Method 4 – Test 1

Starting material **7** (20 mg, 0.02 mmol) was dissolved in ACN-water solution (2:1, 228 µl) at room temperature. The reaction mixture was cooled to 0ºC followed by the addition of potassium permanganate (12 mg, 0.07 mmol, 3.5 eq) and sodium hydroxide (2 mg, 0.04 mmol, 2 eq). It was left to stir at 0ºC for 2 hours before diluting it with water. The reaction mixture was filtered with filter paper and the residues pH was adjusted with 1 M HCl to acidic. The extraction of the aqueous layer was done with EtOAc (3 x 10 ml) but the predicted product stayed in the water layer. All the volatiles were evaporated, and the obtained white solid product was not identified.

Method 5 – Test 1

Starting material **7** (20 mg, 0.02 mmol) was dissolved in acetone (0.5 ml) at room temperature. A solution of 50 % sulfuric acid (8 eq), water and potassium dichromate (13 mg, 0.04 mmol, 2 eq) was prepared at 0ºC before adding to a cooled mixture of starting material. The reaction mixture was left to stir at 0ºC for 10 min before lifting it to room temperature for 36 hours. The reaction mixture was quenched with water and diluted with DCM followed by the extraction of the aqueous layer with DCM (2 x 10 ml). The combined DCM layers were dried over $MgSO_4$, filtered and all volatiles were evaporated. The obtained solid product was not identified.

Method 6 - Test 1

Starting material **7** (20 mg, 0.02 mmol) was dissolved in dimethylformamide (175 µl) at room temperature followed by the addition of PDC (51 mg, 0.13 mmol, 6 eq). The reaction mixture was left to react at room temperature for 6 hours before lifting the temperature to 70°C and the reaction mixture was left to reflux overnight. After 18 hours the reaction was cooled to room temperature and was quenched with water (1 ml) and diluted with DCM. The reaction mixture was extracted with DCM (3 x 10 ml). The combined DCM layers were dried over $MgSO_4$ and filtered followed by the removing of all volatiles in vacuo. The residue was concentrated yielding a brown solid which was identified by NMR spectroscopy.

Method 6 - Test 2

Starting material **7** (20 mg, 0.02 mmol) was dissolved in dry dimethylformamide (0.2 ml) at room temperature followed by the addition of PDC (51 mg, 0.13 mmol, 6 eq). The temperature was raised to 35°C and the reaction mixture was left to stir overnight. After 18 hours, the reaction mixture was cooled to room temperature was quenched with water (1 ml) and diluted with DCM. The reaction mixture was extracted with DCM (3 x 10 ml) and the extracted DCM layers were washed with water (3 x 10 ml). The combined DCM layers were dried over $MgSO_4$ and filtered followed by the removing of all volatiles in vacuo. The residue was concentrated yielding a brown solid, which was identified by NMR spectroscopy.

Method 6 - Test 3

Starting material **7** (400 mg, 0.45 mmol) was dissolved in dry dimethylformamide (2.5 ml) at room temperature followed by the addition of PDC (1.03 g, 2.7 mmol, 6 eq). The temperature was raised to 70°C and it was left to reflux overnight. After 18 hours, the reaction mixture was cooled to room temperature and was quenched with water (30 ml) and diluted with diethyl ether (10 ml). The reaction mixture was extracted with diethyl ether (3 x 20 ml) and the combined diethyl ether layers were concentrated and washed with water (3 x 10 ml). The combined diethyl ether layers were dried over $MgSO_4$ and filtered followed by the removing of all volatiles in vacuo. The residue was concentrated yielding a white solid which was identified by NMR spectroscopy.

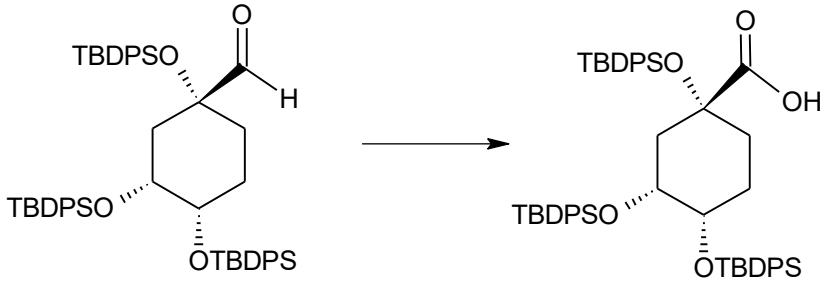
Method 7 – Test 1

Starting material **7** (20 mg, 0.02 mmol) was dissolved in DCM-water solution (1 ml) at room temperature followed by the addition of TEMPO (0.2 mg, 0.001 mmol, 0.05 eq), sodium hydrogen carbonate (4 mg, 0.05 mmol, 2.3 eq) and tetra-n-butylammonium bromide (0.6 mg, 0.002 mmol, 0.08 eq). The reaction mixture was cooled to 0ºC followed by the addition of aqueous solution of bleach (36 µl, 0.07 mmol, 3 eq) and sodium hydrogen carbonate (6 mg, 0.07 mmol, 2.9 eq). The reaction mixture was left to stir at 0ºC for 15 minutes before lifting it to room temperature for 4 hours. After 4 hours, the temperature was raised to 35ºC and the reaction mixture was left to stir at 35ºC for 14 hours. After 14 hours, it was quenched with 1 M HCl followed by the extraction with DCM (3 x 10 ml). The predicted product stays in aqueous layer and the layer was concentrated. The

obtained residue was purified by dry-pack flash column chromatography using a mixture of EtOAc/MeOH (80:20) as eluent yielding a clear oil. The obtained product was not identified.

3.2.6 Oxidation of the molecule 8

The second reaction of the third quinic acid derivative that converts molecule **8** to molecule **9** was done by oxidizing primary aldehyde to carboxylic acid by Pinnick oxidation which is presented in figure 18. The Pinnick oxidation occurs with certain reagents why variety of different conditions were tested. Three different methods and conditions were tested. The methods and conditions are described in this chapter.



8 **9**

FIGURE 18. The synthesis of molecule **9**

Method 1 - Test 1

Starting material **8** (50 mg, 0.06 mmol) was dissolved in solution of ACN-water (5:1, 420 µl) at room temperature. $NaClO_2$ (1 mg, 0.1 mmol, 2 eq) and sodium dihydrogen phosphate (34 mg, 0.2 mmol, 5 eq) were added at room temperature followed by the dropwise addition of 2-methyl-2-butene (60 µl, 0.6 mmol, 10 eq). The reaction mixture was left to stir at room temperature for 36 hours without proceeding. The reaction was not continued.

Method 1 - Test 2

Starting material **8** (70 g, 0.08 mmol) was dissolved in solution of ACN-water (5:1, 1 ml) at room temperature. The reaction mixture was cooled to 0ºC followed by

the addition of $NaClO_2$ (15 mg, 0.2 mmol, 2 eq) and sodium dihydrogen phosphate (50 mg, 0.4 mmol, 5 eq) and dropwise addition of 2-methyl-2-butene (80 µl, 0.8 mmol, 10 eq). The reaction mixture was left to stir at 0ºC for 30 minutes before lifting it to room temperature for 2 hours. After 2 hours, the reaction mixture was quenched with water (1 ml) followed by the extraction with EtOAc (3 x 10 ml). The combined EtOAc layers were dried over $MgSO_4$, filtered and concentrated. The obtained products were not identified.

Method 2 – Test 1

Starting material **8** (50 mg, 0.06 mmol) was dissolved in solution of acetone-water (5:1, 420 µl) at room temperature. $NaClO_2$ (20 mg, 0.22 mmol, 4 eq) and sodium dihydrogen phosphate (34 mg, 0.2 mmol, 5 eq) were added at room temperature followed by the dropwise addition of 2-methyl-2-butene (60 µl, 0.6 mmol, 10 eq). The reaction mixture was left to stir at room temperature for 36 hours. After 36 hours, it was quenched with saturated aqueous sodium hydrogen sulfite followed by the extraction with EtOAc (3 x 10 ml). The combined EtOAc layers were dried over $MgSO_4$, filtered and all volatiles were evaporated. The obtained residue was purified by dry-pack flash column chromatography using a mixture of EtOAc/MeOH (90:10) as eluent yielding a clear oil. The obtained product was identified by NMR spectroscopy.

Method 3 - Test 1

Starting material **8** (60 g, 0.06 mmol) was dissolved in solution of tert-butanol (t-BuOH) at room temperature. $NaClO_2$ (30 mg, 0.33 mmol, 5 eq) and sodium dihydrogen phosphate (55 mg, 0.05 mmol, 7 eq) were dissolved in 0.2 ml of water and added dropwise at 0ºC to the reaction mixture followed by the addition of 0.2 ml of 2-methyl-2-butene. The reaction mixture was left to stir at 0ºC for 3 hours before lifting it to room temperature for 18 hours. After 18 hours, it was quenched with a few drops of saturated aqueous ammonium chloride and diluted with EtOAc. The reaction mixture was extracted with EtOAc (3 x 10 ml) and the combined EtOAc layers were dried over $MgSO_4$ and filtered followed by the removing of all volatiles in vacuo. The obtained residue was purified by dry-pack flash column chromatography using a mixture of DCM/hexane (50:50) as eluent yielding a clear oil. The obtained product was not identified.

Method 3 - Test 2

Starting material **8** (160 mg, 0.2 mmol) was dissolved in mixture of t-BuOH and tetrahydrofuran (THF) (4:1, 2 ml) at room temperature. $NaClO_2$ (82 mg, 0.9 mmol, 5 eq) and sodium dihydrogen phosphate (153 mg, 1.3 mmol, 7 eq) were dissolved in 0.3 ml of water and added dropwise at 0ºC to the reaction mixture followed by the addition of 0.3 ml of 2-methyl-2-butene (15 eq). After 3 hours at 0ºC, the reaction mixture was quenched with a few drops of ammonium chloride and diluted with EtOAc. The reaction mixture was extracted with EtOAc (3 x 10 ml) and the combined EtOAc layers were dried over $MgSO_4$ and filtered followed by the removing of all volatiles in vacuo. The product was obtained without further purification as a yellowish solid foam. The obtained product was not identified.

3.2.7 Methylation of molecule 9

The third reaction of the third quinic acid derivative that converts molecule **9** to molecule **10** was done by methylating the carboxylic acid which is presented in figure 19. The methods and conditions are described in this chapter.

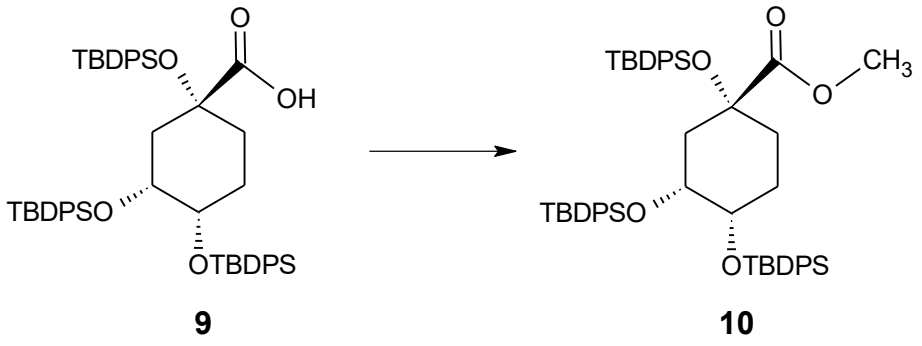


FIGURE 19. The synthesis of molecule **10**

Method 1 – Test 1

Starting material **9** (0.05 mmol) was dissolved in acetone (1 ml) at room temperature followed by the addition of potassium carbonate (30 mg, 0.2 mmol, 4 eq) and dimethyl sulfate (1 µl, 0.1 mmol, 2 eq) over 2 hours. After 18 hours at room temperature, the reaction was quenched with H_2O and diluted with EtOAc. The reaction mixture was extracted with EtOAc (3 x 10 ml) and the combined EtOAc layers were dried over $MgSO_4$ and filtered followed by removing of all volatiles in

vacuo. The obtained residue was purified by dry-pack flash column chromatography using a mixture of EtOAc/hexane (10:90) as eluent yielding a clear oil. The obtained product was identified by NMR spectroscopy.

Method 2 - Test 1

Starting material **9** (0.2 mmol) was dissolved in acetone (2 ml) at room temperature followed by the addition of potassium carbonate (114 mg, 0.8 mmol, 4 eq) and dimethyl sulfate (50 µl, 0.5 mmol, 2,5 eq). After 2 hours at room temperature, the reaction was quenched with water and diluted with EtOAc. The reaction mixture was extracted with EtOAc (3 x 10 ml) and the combined EtOAc layers were dried over $MgSO_4$ and filtered followed by removing of all volatiles in vacuo. The obtained residue was purified by dry-pack flash column chromatography using a mixture of EtOAc/hexane (5:95) as eluent yielding a clear oil. The obtained product was identified with NMR spectroscopy.

3.2.8 Deprotection of silyl groups of molecule 10

The fourth and last reaction of the third quinic acid derivative that converts molecule **10** to molecule **11** was done by deprotecting the silylated alcohols. The reaction scheme for this reaction is presented in figure 20. The deprotecting method was proved to work before in the practical training part, which is why at this synthesis, two methods were tested. The methods and conditions are described in this chapter.

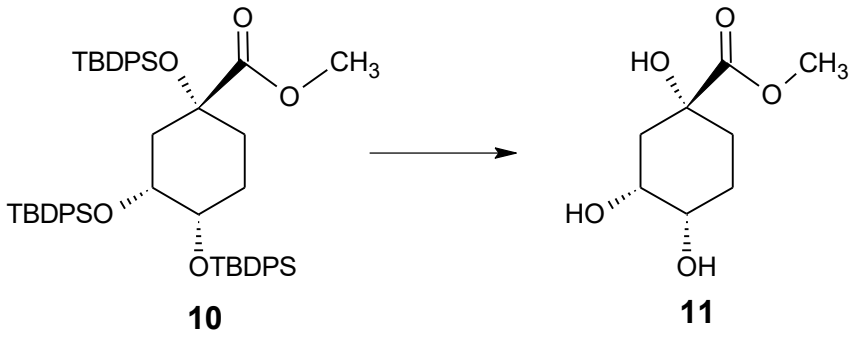


FIGURE 20. The synthesis of molecule **11**

Method 1 – Test 1

Starting material **10** (78 mg, 0.09 mmol) was dissolved in dry THF at room temperature followed by the addition of 230 mg of 4 Å molecular sieves. TBAF · H_2O (113 mg, 0.4 mmol, 5 eq) was added at room temperature followed by the rising of the temperature to 70ºC. After 2 hours of refluxing, the reaction was cooled to room temperature and was quenched with few drops of ammonium chloride followed by the removing of all volatiles in vacuo. The obtained residue was purified by dry-pack flash column chromatography using a mixture of EtOAc/MeOH (90:10) as eluent yielding a clear oil. The obtained product was identified by NMR spectroscopy.

Method 2 – Test 1

Starting material **10** (147 mg, 0.1 mmol) was dissolved in dry THF (1 ml) at room temperature followed by the addition of 230 mg of 4 Å molecular sieves. TBAF in 1.0 M solution of THF (116 µl, 0.4 mmol, 5 eq) was added at room temperature followed by the rising of the temperature to 40ºC for 2 hours and 70ºC for 2 hours. After 4 hours of refluxing, the reaction was cooled to room temperature and was quenched with few drops of ammonium chloride followed by the removing of all volatiles in vacuo. The obtained residue was purified by dry-pack flash column chromatography using a mixture of EtOAc/MeOH (90:10) as eluent yielding a clear oil. The obtained product was identified by NMR spectroscopy.

4 RESULTS AND CONCLUSIONS

4.1 Results for the oxidative cleavage of molecule 1

The oxidative cleavage of molecule **1** was tested three times. For the first quinic acid derivative (**1**) studied, the oxidative cleavage was possible to perform because of the structure of the molecule (**1**). The molecule (**1**), being a triol, has three hydroxyl groups.

Further investigation of the Malaprade reaction theory indicated that using a study of Pawar (1995) as a guideline for the first tests was a good opportunity. Barthes and Grison (2012) had reported oxidative cleavage with $NaIO_4$ but had used a different solvent.

The results of the syntheses with methods 1 and 2 are presented in table 2. Final product yield for purified compound is presented in milligrams and percent.

TABLE 2. The results of synthesis of molecule **1** with method 1 and 2.

Method	Test	Starting material (mg)	Product (mg)	Yield (%)
1	1	10	8	82
2	1	10	12	quant.

The TLC was checked under UV-light lamp and no UV-visible spots were detected. The TLC was dyed with vanillin to get the starting material sample and desired product sample to dye. In both methods, one major spot was formed, and the purified product was identified by ^1H NMR with JEOL 500 MHz spectrometer.

In appendix 2, spectra measured from the purified compound of method 1 test 1 product is presented. The peak at 0 ppm is a peak from an internal standard of TMS and peak at 4.8 ppm is peak from the solvent residue of water in D_2O, the NMR solvent used. One peak at 2.22 ppm is coming from acetone which was added for better calibration. The peaks around 9.5-10 ppm are coming from an aldehyde group and the three peaks around 3.5 ppm to 4.5 ppm are coming from

protons that are next to oxygen. The peaks around 2.0 ppm are coming from protons that are not next to oxygen.

The integrals of the spectra are showing bigger peaks and smaller peaks which indicates that the product from method 1 test 1 is a mixture of two products, despite that the product looked like one spot on TLC. Suspicions of causing the mixture was that the two products are hydrates caused by residue of the water which ends up to the product. In method 2, the purification method was changed.

In appendix 2, the spectrum measured from purified compound of method 2 test 1 product is presented. The peak at 0 ppm is a peak from the internal standard TMS and the peak at 2.50 ppm is a peak from the solvent residue of DMSO in d6-DMSO, the NMR solvent used. The spectrum from method 2 test 1 product is clearly a mixture of more than one compound because of the number of peaks. The integration of the peaks was too hard to get reliable information. The purification of the product was done by flash chromatography and the aldehyde products being not so stable, it was considered that maybe the aldehyde reacts in the silica and forms another product (Clayden et al. 2012).

4.2 Results for the reduction of molecule 2

The reduction of molecule **2** was tested once. To prove that the method worked and produced C-C cleavaged aldehyde, it was decided that the aldehyde which was present in two forms (**2**) would be reduced. The reaction for molecule **2** was done by mild reductant, sodium borohydride (Clayden et al. 2012).

Method 1 was tested once and the results for this synthesis are presented in table 3. The yield of the purified compound is presented in milligrams and percent.

TABLE 3. The results of synthesis of molecule **2** with method 1

Method	Test	Starting material (mg)	Product (mg)	Yield (%)
1	1	10	6	60

The TLC was dyed with vanillin to get the starting material sample and desired product sample to dye. In method 1 test 1, the TLC showed one major spot for the synthesis. The product was purified and identified by ^{1}H NMR with JEOL 500 MHz spectrometer.

In appendix 3, the spectrum measured from the purified compound of method 1 test 1 is presented. The peak at 0 ppm is a peak from TMS and peak at 4.8 ppm is peak from the solvent residue of water in D_2O, the NMR solvent used. One peak at 2.24 ppm is coming from acetone which was added for better calibration. The six peaks around 3.5 to 4.5 ppm come from protons that are next to oxygen. The two duplets around 3.7 ppm are coming from OH-groups. The peaks around 1.7 to 2.5 ppm are coming from protons that are not next to oxygen. The spectrum proved that two aldehyde groups had reduced to two hydroxyl groups and the desired product (**3**) was obtained.

4.3 Results from the protection of primary alcohol of molecule 4

The protection of the primary alcohol of molecule **4** was tested three times. The Malaprade reaction was first tested with unprotected diol but the characterization of the observed product was difficult due to acyclic structure and aldehyde functions' possibility to react with primary alcohol. It was decided that the primary alcohol would be protected with a silyl group.

The results of the methods are presented in table 4. The yields of the purified compounds are presented in milligrams and percent.

TABLE 4. The results of synthesis of molecule **4** with method 1 and 2.

Method	Test	Starting material (mg)	Product (mg)	Yield (%)
1	1	30	6	15
2	1	65	-	-
3	1	54	64	67

With the previous acquaintance with the protection of alcohols, it was decided that one of the silyl groups, TBS, would be used so that the protection reaction would be fast and easy.

The TLC was dyed with vanillin to get the starting material sample and desired product sample to dye. In method 1 test 1, the reaction was slow and even after overnight reacting there was starting material left.

In method 2 test 1, the solvent was changed to more polar because the very polar starting material had difficulties to dissolve in DCM. Still, the reaction was relatively the same as in method 1 test 1. Therefore, it was not continued and was restored back to the starting material.

In method 3 test 1, the same solvent was used as in method 1 test 1, but the used base was changed to stronger (triethylamine) and furthermore a bit of catalyst was added. Method 3 test 1 reacted to the end and formed one major spot. Products from methods 1 and 3 were identified by ^{1}H NMR with JEOL 500 MHz spectrometer.

In appendix 4, spectra from method 1 test 1 and method 3 test 1 purified compounds are presented. In method 1 test 1 spectrum, the peak at 0 ppm is a peak from TMS and peak at 7.26 ppm is peak from the solvent residue of $CHCl_3$ in $CDCl_3$, the NMR solvent used. The three peaks around 3.25 to 4.0 ppm come from protons that are next to oxygen. The peaks around 1.0 to 2.25 ppm are coming from protons that are not next to oxygen. The big singlet peak at 0.9 is coming from the TBS protection group, which indicated that the protection of the primary alcohol happened, but the reaction was still too slow and low yielding.

In method 3 test 1 spectrum, the TMS standard peak is coming also at 0.00 ppm but the NMR solvent used was d6-DMSO (residue of DMSO at 2.5 ppm). The four peaks around 3.25 to 4.5 ppm come from protons that are next to oxygen. One of the peaks is overlapping with the other peak which is coming from water at 3.33 ppm. The peaks around 1.0 to 1.75 ppm are coming from protons that are not next to oxygen. The big singlet peak at 0.8 is coming from the TBS protection group. The spectrum proved that the desired product (**5**) was obtained.

4.4 Results from oxidative cleavage of molecule 5

The oxidative cleavage of molecule **5** was tested once. The oxidative cleavage for the second derivative (**5**) could be performed with the previously created method. The results for the reaction are presented in table 5. The purified compound yield is presented in milligrams and percent.

TABLE 5. The results of synthesis of molecule **5** with method 1

Method	Test	Starting material (mg)	Product (mg)	Yield (%)
1	1	20	21	quant.

The TLC was dyed with vanillin to get the starting material sample and desired product sample to dye. In method 1 test 1, the starting material reacted to one major spot which was purified. Product was identified by ^{1}H NMR with JEOL 500 MHz spectrometer.

In appendix 5, spectrum measured from purified compound of method 1 test 1 is presented. The peak at 0 ppm is a peak from TMS and the peak at 7.26 ppm is a peak from the solvent residue of $CHCl_3$ in $CDCl_3$, the NMR solvent used. The two peaks around 3.25 to 3.75 ppm come from protons that are next to oxygen. The peaks around 1.5 to 2.5 ppm are coming from protons that are not next to oxygen. The peak at 1.54 is most likely coming from water. The big singlet peak at 0.85 is coming from the TBS protection group. The spectrum (appendix 5) proved that the desired product (**6**) was obtained.

4.5 Results for the oxidation of the molecule 7

The oxidation of the third quinic acid derivative (**7**) was tested eleven times in total with 7 different methods. For oxidizing alcohols, various methods were available which created opportunity to test different conditions.

The results for the methods are presented in table 6. The yields for the products apart from method 6 test 1, are presented without further purification. Method 6 test 1 product was purified with column chromatography.

TABLE 6. The results of synthesis of molecule **7** with seven methods.

Method	Test	Starting material (mg)	Product (mg)	Yield (%)
1	1	20	22	crude
1	2	20	3	15
1	3	20	24	crude
2	1	20	-	-
3	1	20	24	crude
4	1	20	-	-
5	1	20	-	-
6	1	20	22	quant.
6	2	20	3	15
6	3	400	322	82
7	1	20	-	-

The starting material of the synthesis was UV-visible, and the predicted product was also UV-visible. The TLC was dyed with vanillin to get the starting material sample, desired product sample and side products of the reaction to dye.

Method 1 was tested three times with different conditions and workups. When monitoring with TLC, it was thought that a very polar product was forming, and that is why different workups were tested. The reaction looked like it was giving a product but eventually was not reacting at all and the same thing happened with method 5. The oxidizing agent was not strong enough to oxidize the starting material.

In methods 2, 3, 4, 5 and 7, the reaction happened but the starting material did not react to the end, and when monitoring with TLC too many equal side products were formed. Any of the spots could not be purified and the methods were not continued.

The ^{1}H NMR were measured from the crudes of method 1 and 3 to see if the reactions had produced the desired product, but the crude spectra were too messy to identify any peaks reliably. The spectra are presented in appendix 6.

It was intended to oxidize the primary alcohol straight to carboxylic acid to lessen the steps, but the methods did not work. The third derivative was too hindered for the oxidation to happen. The third derivative had three big protection groups (TBDPS) in three oxygens. The surroundings of the primary alcohol might have been too hindered, which might be a result of the big protection groups. This might have prevented the reaction from taking place. It was necessary to change the plan and oxidize the alcohol to aldehyde first.

PDC is an oxidizing agent for primary alcohols, which is why the selective oxidation could happen (Clayden et al. 2012). In method 6, monitoring with TLC showed that only one spot was forming from the starting material, which was a good sign.

The workup of method 6 was difficult because the PDC was dissolving to the same organic solvents as the product, and the product was not fully pure of PDC after workup. A few different workups were tested, and the best work up was achieved by extracting with diethyl ether (test 3). The reagent, PDC, did not dissolve in diethyl ether, but unfortunately formed an emulsion which was hard to get out and why the yield decreased.

The spectrum measured in $CDCl_3$ from method 6 test 1 purified product gave a mixture of more than one product. The spectrum is presented in appendix 6. The spectrum indicated that the reactive aldehyde would decompose in the column. The spectrum from method 6 test 2 (appendix 6) has a peak in the area of aldehydes chemical shift at 8.7 ppm but the product is not pure enough and has residues of dimethylformamide (DMF) which can be seen from peaks at 8.01, 2.96, 2.88 ppm.

Method 6 test 3 product was obtained as quite pure without column purification. The spectrum (appendix 6) was measured in $CDCl_3$ (residue of $CHCl_3$ at 7.26 pm) and the peaks from 7.0 to 8.0 ppm are from aromatic groups. The single

peak at 8.7 ppm is coming from an aldehyde group. The two peaks around 3.25 to 4.0 ppm come from protons that are next to oxygen. The peak at 3.48 ppm comes from the residue of diethyl ether. The peaks around 1.25 to 2.5 ppm are coming from protons that are not next to oxygen. The spectrum (appendix 6) proved that the desired product (**8**) was obtained.

4.6 Results for oxidation of the molecule 8

The oxidation of the aldehyde (**8**) was tested five times in total with 3 different methods. After the aldehyde (**8**) was obtained, the next step was to oxidize it into carboxylic acid (**9**). The method tested was Pinnick oxidation because almost all available oxidation methods were already tested. Pinnick oxidation was tested with three methods.

The results for the methods are presented in table 7. The yields for the products are presented for purified compounds in milligrams and percent for method 2 and 3 test 1. The products which do not have yields presented have been used as a crude for next step and have not been weighed.

TABLE 7. The results of synthesis of molecule **8** with method 1, 2 and 3.

Method	Test	Starting material (mg)	Product (mg)	Yield (%)
1	1	50	-	-
1	2	73	-	-
2	1	50	24	48
3	1	58	4	< 5 %
3	2	160	-	crude

The starting material of the synthesis was UV-visible, and the predicted product was also UV-visible. The TLC was dyed with vanillin to get the starting material sample, desired product sample and side products of the reaction to dye.

Method 1 was tested two times with different conditions. Test 1 formed too many spots on TLC, and after 36 hours, none of the spots had not become major, which

is why the reaction was not continued anymore. In test 2, forming of too many spots was tried to prevent by adding the reagents at 0 ºC but the test ended up in the same situation as the first one.

In method 2 test 1, the solvent was changed and the oxidants ($NaClO_2$) amount was increased to 4 equivalents. The reaction proceeded first like in the first method, but one major spot was formed, and it was purified. The purified product was measured by ^1H NMR with JEOL 500 MHz spectrometer.

The spectrum measured from method 2 test 1 product is presented in appendix 7. The product was measured in d6-DMSO (residue of DMSO at 2.5 ppm) and the peaks around 7.0 ppm to 8.0 ppm are coming from aromatic groups. Two peaks around 3.75 ppm to 4.5 ppm are coming from protons that are next to oxygen. The peak around 3.36 is coming from residue of water and peaks at 2.9 and 2.7 are coming from impurity residues. The peaks around 1.25 to 2.0 ppm are coming from protons that are not next to oxygen. The spectrum showed hints about the desired product (**9**).

Method 2 test 1 was still modified because too many spots were forming on TLC. Method 3 was tested two times. In test 1, the solvent was changed to t-BuOH, the TLC looked the same as in the previous methods. The product was purified but the yield was too low to reliably identify it. In test 2, the used solvent was t-BuOH-THF and the solubility of starting material was better than in previous tests. The reagents were added as cooled solution and because the reactivity was lower the number of spots on TLC were decreased. Because the number of spots was decreased it was easier to continue to the methylation.

4.7 Results for methylation of the molecule 9

Methylation was tested two times with different methods. The results for the method are presented in table 8. The yields for the products are presented for purified compounds in milligrams and percent. The starting material was used as a crude from previous step, which is why it was not weighed. The presented yield is calculated by using the amount of previous step starting material.

TABLE 8. The results of synthesis of molecule **9** with method 1

Method	Test	Starting material (mg)	Product (mg)	Yield (%)
1	1	crude	3	< 5 %
2	1	crude	94	57

The starting material of the synthesis was UV-visible, and the predicted product was also UV-visible. The TLC was dyed with vanillin to get the starting material sample, desired product sample and side products of the reaction to dye.

In the method 1 test 1, the reaction was too slow because the reagents were added in two batches. First, only 2 equivalents of potassium carbonate and 1 equivalent of dimethyl sulfate were added, and when monitoring with TLC, nothing happened in 1 hour. Same amount of the reagents was added, and the reaction was left to stir overnight, but when the isolated spot was identified, peak from methyl was not seen.

In the method 2 test 1, the method was relatively the same but the total amount of reagents from test 1 was added in one batch. The reaction was faster, and the starting material reacted to the end. When monitoring with TLC, more than one spot was seen, but one spot was major, and it was purified.

The spectrum measured from method 1 test 1 cannot be integrated because the spectrum is too messy, and the concentration of the sample is too low. The spectrum can be seen from appendix 8.

The method 2 test 1 spectrum is presented in appendix 8. The spectrum was measured in $CDCl_3$ and the solvent residue of $CHCl_3$ is at 7.26 ppm. The two peaks around 3.25 ppm to 4.5 ppm are coming from protons that are next to oxygen. The peak around 1.55 is coming from residue of water. The peaks around 1.75 to 2.5 ppm are coming from protons that are not next to oxygen. The big peak around 2.75 ppm is coming from the methyl group. Three big peaks around 0.7 to 1.2 ppm are coming from the three TBDPS groups and the peaks from 1.2 to 1.4 ppm are coming from two hydrogens that are not next to oxygen

but are infused with the big peaks. The spectra (appendix 8) proved that the desired product (**10**) was obtained.

4.8 Results for deprotection of the silyl groups for molecule 10

Deprotection was tested with two different methods. After obtaining molecule **10,** there was only one step left before the previously reported (Tavassoli et al. 2005) molecule **11** was obtained. TBAF is a common reagent used for deprotecting silyl groups and it was used in both methods (Clayden et al. 2012). The results for the method are presented in table 9. The yields for the products are presented for purified compounds in milligrams and percent.

TABLE 9. The results of synthesis of molecule **10** with method 1 and 2

Method	Test	Starting material (mg)	Product (mg)	Yield (%)
1	1	87	12	67
2	1	147	21	68

The starting material of the synthesis was UV-visible, and the predicted product was not. The TLC was dyed with vanillin to get the starting material sample, desired product sample and side products of the reaction to dye. In both tests, one major UV-visible spot formed.

In the first method, solid TBAF · H_2O was used. The water in the reaction could react with the ester by hydrolysis when refluxing, why 4 Å molecular sieves were added. In the second method, dry TBAF in THF was used to see if it would work better and increase the yield. The reaction proceeded slower, but the UV-visible side product was not forming. The purified products were measured by ^{1}H NMR with JEOL 500 MHz spectrometer.

The method 1 test 1 was repeated two times and the spectra are presented in appendix 9. The peak at 0 ppm is a peak from TMS and peak at 7.26 ppm is peak from the solvent residue of $CHCl_3$ in $CDCl_3$, the NMR solvent used. The peaks around 3.5 to 4.5 ppm come from protons that are next to oxygen. The big peak

around 3.8 ppm is coming from methyl group. The four peaks around 1.5 to 2.5 ppm are coming from protons that are not next to oxygen.

The spectrum measured from method 2 test 1 is presented in appendix 9. Peaks of the spectrum are relatively same as in the method 1 test 1 spectra, but the purity of the sample varies. The measured spectra could be compared to the previously reported (Tavassoli et al. 2005) spectra. The spectra (appendix 9) proved that the desired product (**11**) was obtained.

5 DISCUSSION

The objective of this thesis was to develop a method or methods for the oxidative cleavage of C-C bond for derivatives of quinic acid. It was necessary to prove that the deoxygenated quinic acid could be used in further research where new chiral molecules would be created. The oxidative cleavage produced new molecules, building blocks with “handles”, in this case aldehydes. The objective was accomplished and a method for oxidative cleavage with sodium periodate was created.

Creating a method for the oxidative cleavage gave a chance to test different conditions and reagents which could be compared during the thesis work. Besides creating a method for oxidative cleavage of quinic acid derivatives, new methods for oxidizing, reducing, protecting and deprotecting were created, because the oxidative cleavage had limiting properties to succeed.

The execution of the thesis was accomplished as expected. The results are reliable because the obtained product was identified with NMR spectroscopy and the desired product was obtained. However, oxidative cleavage for the studied two first quinic acid derivatives has not been performed earlier and for deepening the reliability of the results, the method that worked the best should have been repeated more than once.

It was noticed that the reaction conditions and the used reagents have a great impact in the synthesis. One of the most adjusted conditions was temperature and it was noticed that in more than half of the reactions the reagents were needed to be added at 0 °C. Adding the reagents at 0 °C slows down the reaction and the formation of side products was decreased. Also, the inert conditions may have an impact in the formation of the side products, which is why the reactions not done in inert conditions should be done under inert conditions.

The results have a great impact for Tampere University and especially for Suvi Holmstedt’s dissertation research since by creating a new method, also new molecules were created. The obtained molecules and methods can be reported as

new results in part of the dissertation research. The usage of the deoxygenated quinic acid derivatives were proved and the created method could be used for other deoxygenated derivatives in future. The oxidative cleavage produced chiral molecules with aldehyde handles which could be used as chiral building blocks in a larger entity.

The created methods should be optimized by increasing the yield with finding the best amount of reagents and suitable temperature. The oxidative cleavage method should be tested on more quinic acid derivatives with vicinal diol properties to see if the method works for more than the three studied derivatives. The building block properties of the new produced molecules should be tested, so that the potential usage as part of pharmaceutical industry in the future could be proven.

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APPENDICES

Appendix 1. Preparing of the studied quinic acid derivatives

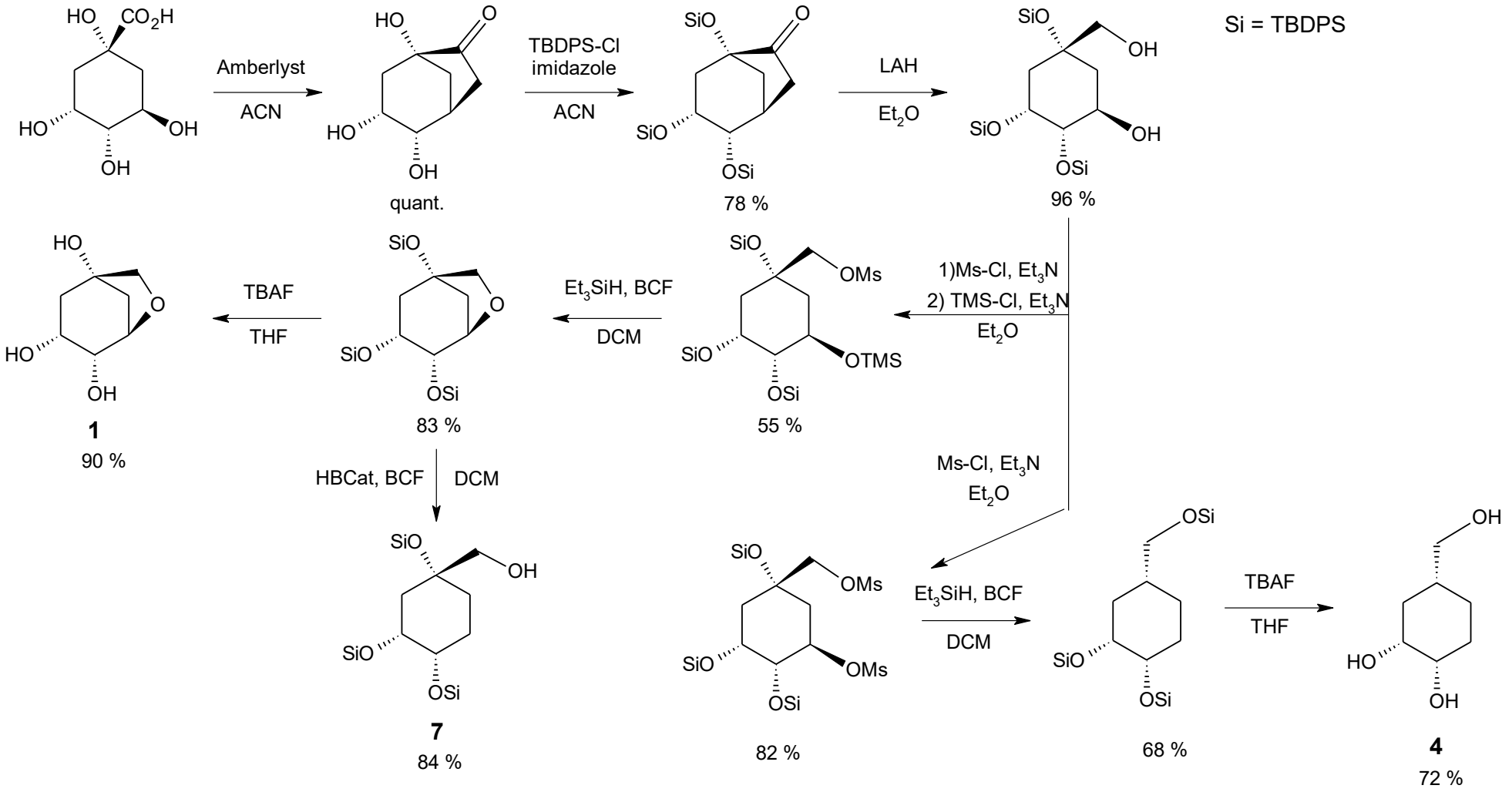
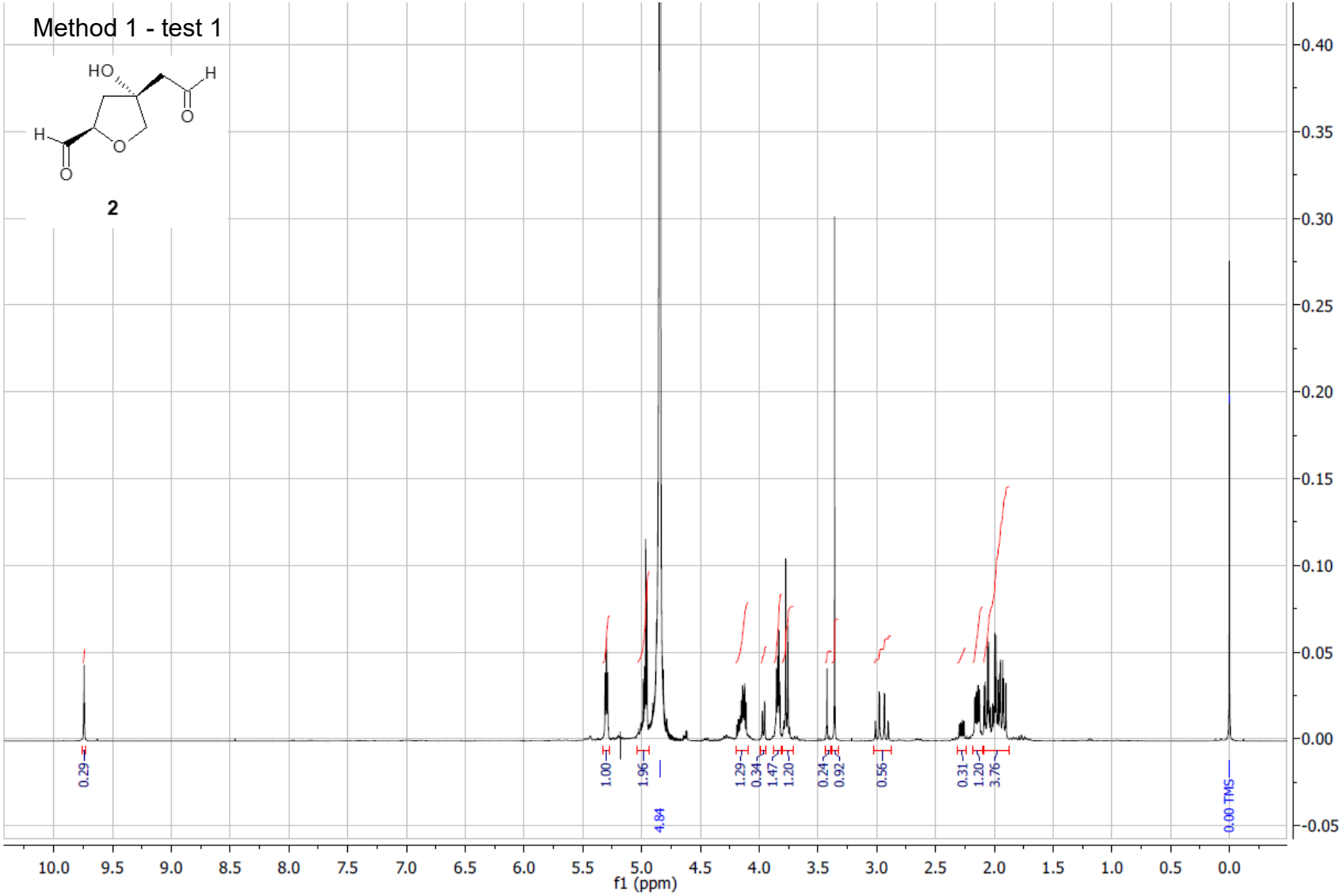


Figure 21. Synthetic routes for preparing of the studied quinic acid derivatives from quinic acid

Appendix 2. Spectra from the synthesis of molecule **2**



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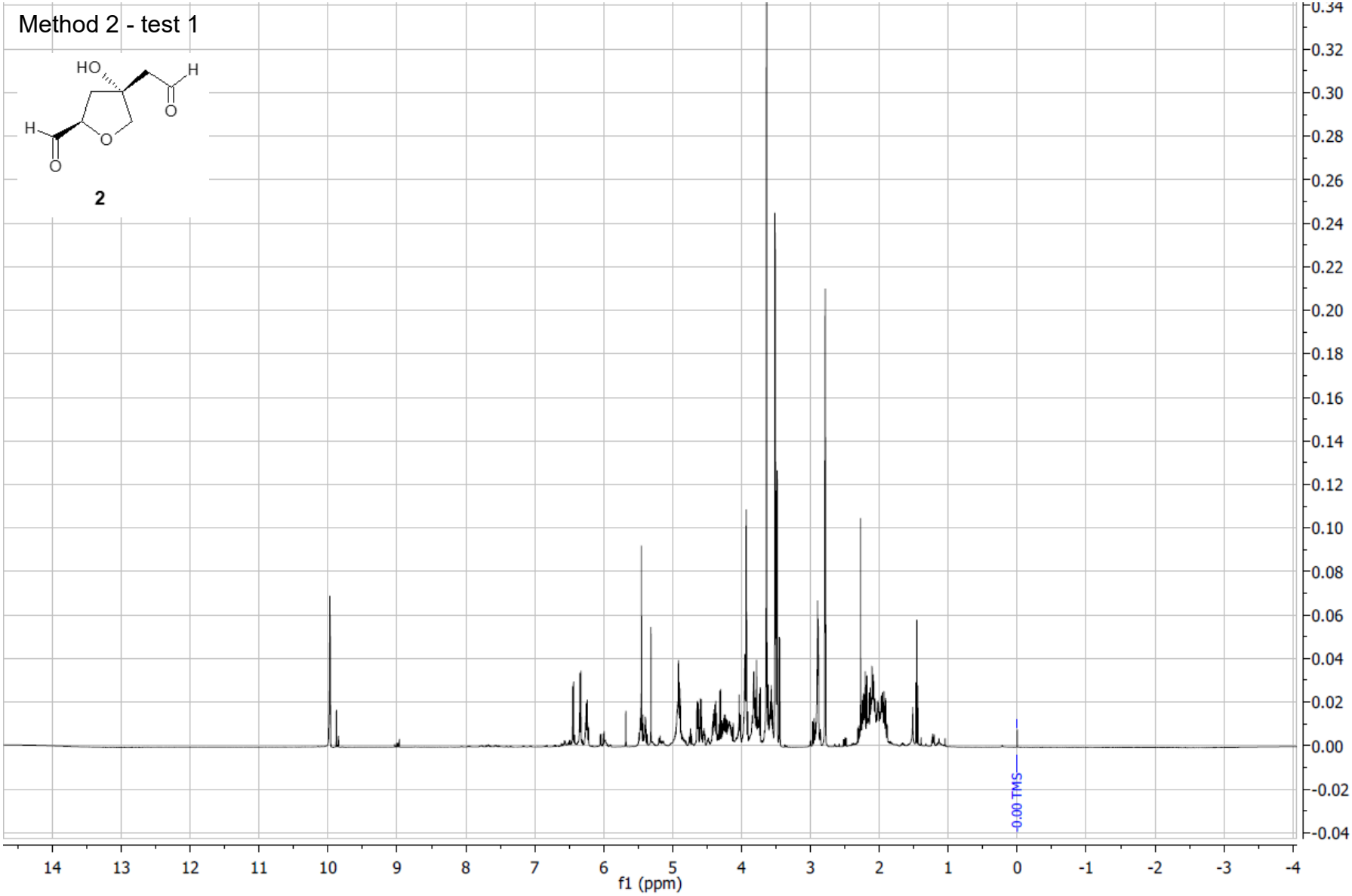
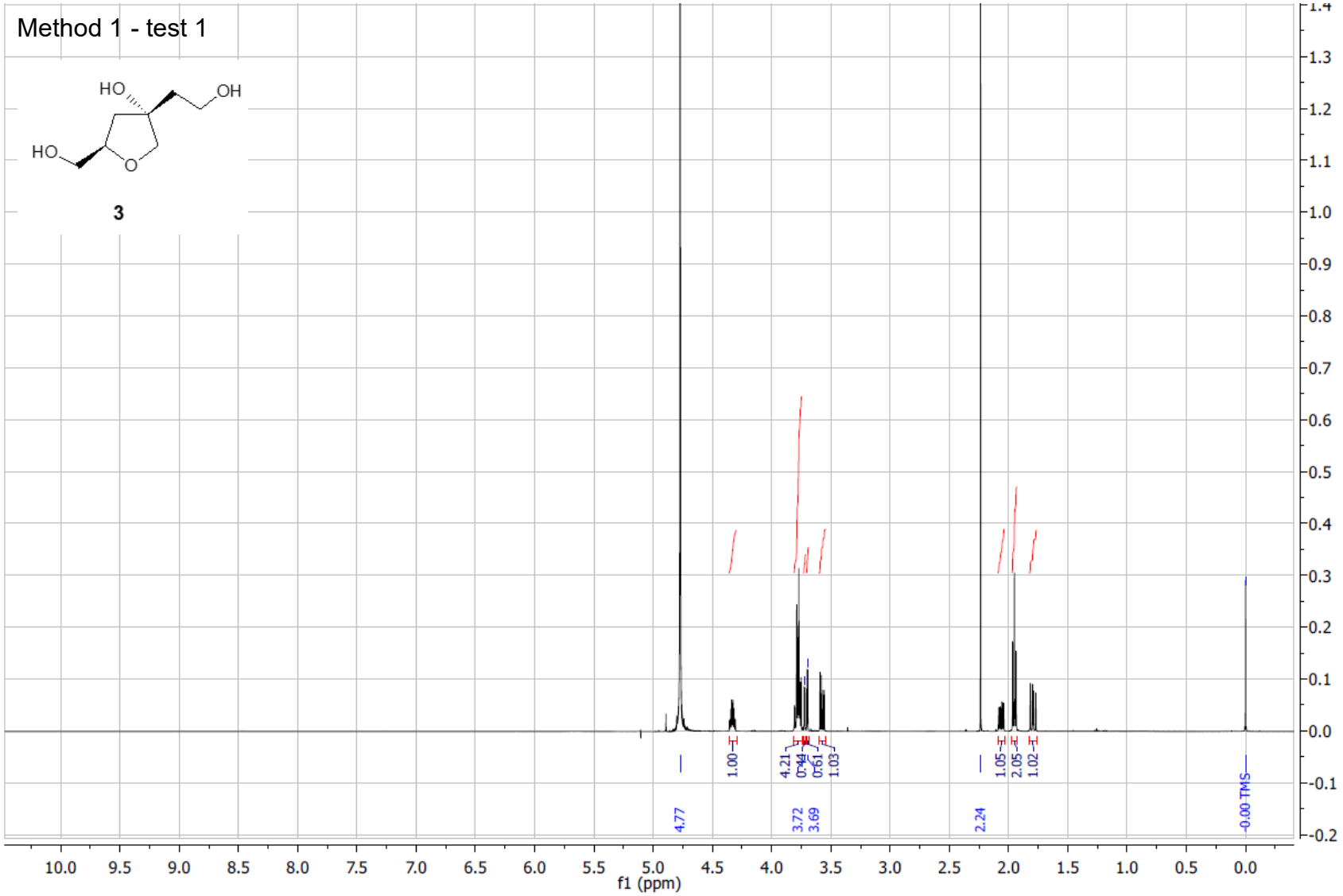
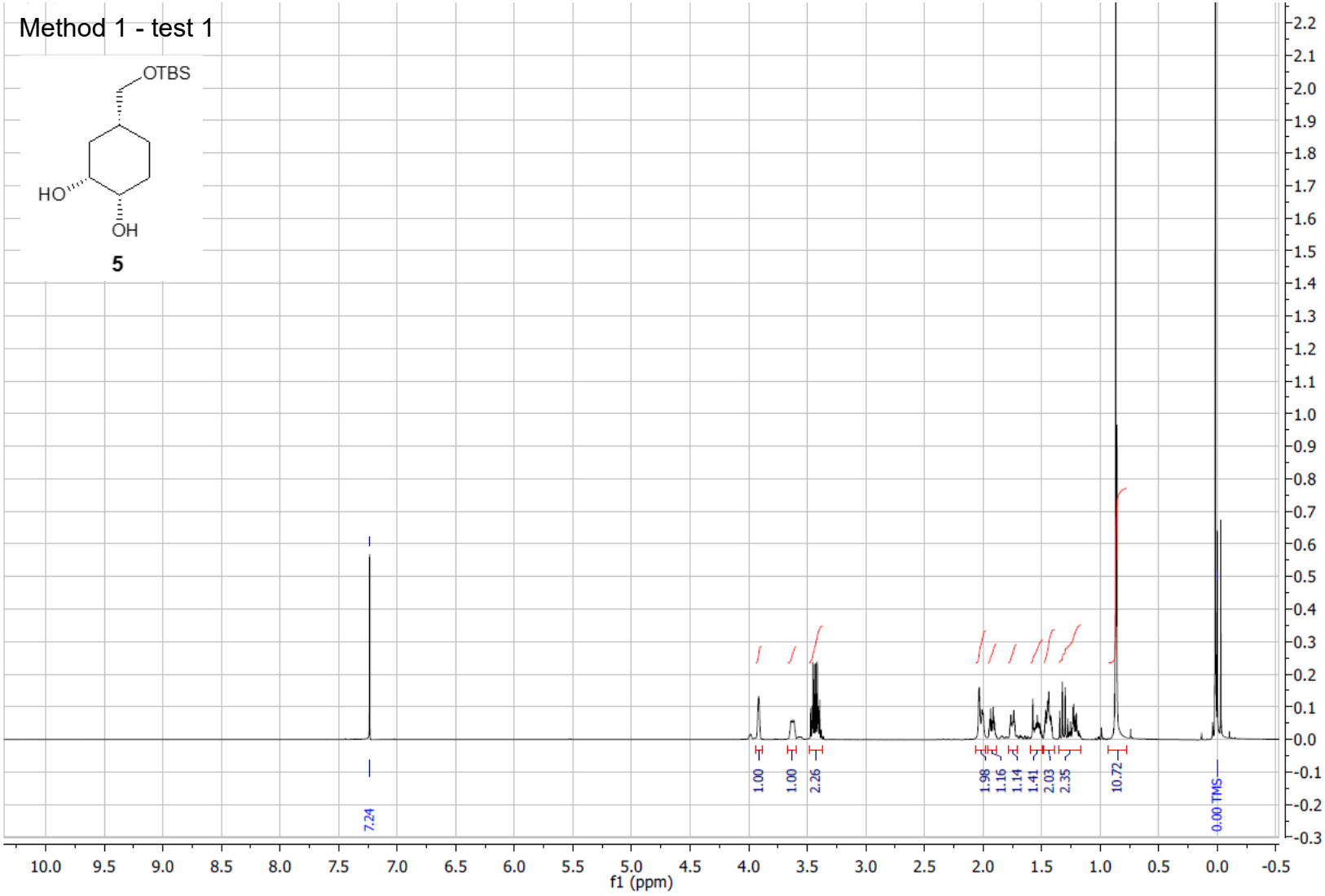

Method 2 - test 1
HO
H
O
H
O
O
2
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
0.00 TMS
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
f1 (ppm)

Appendix 3. Spectrum from the synthesis of molecule **3**



Appendix 4. Spectra from the synthesis of molecule **5**



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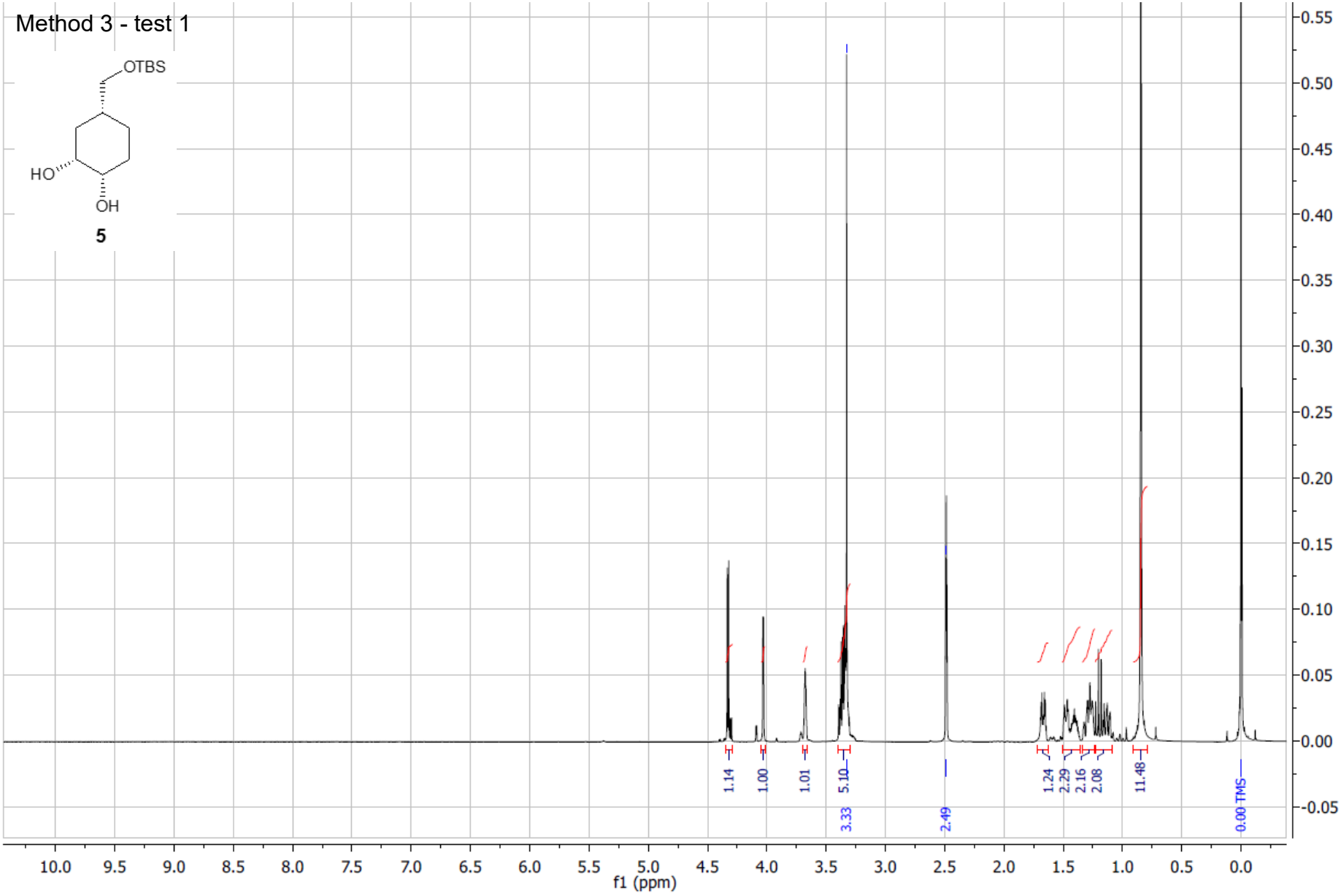
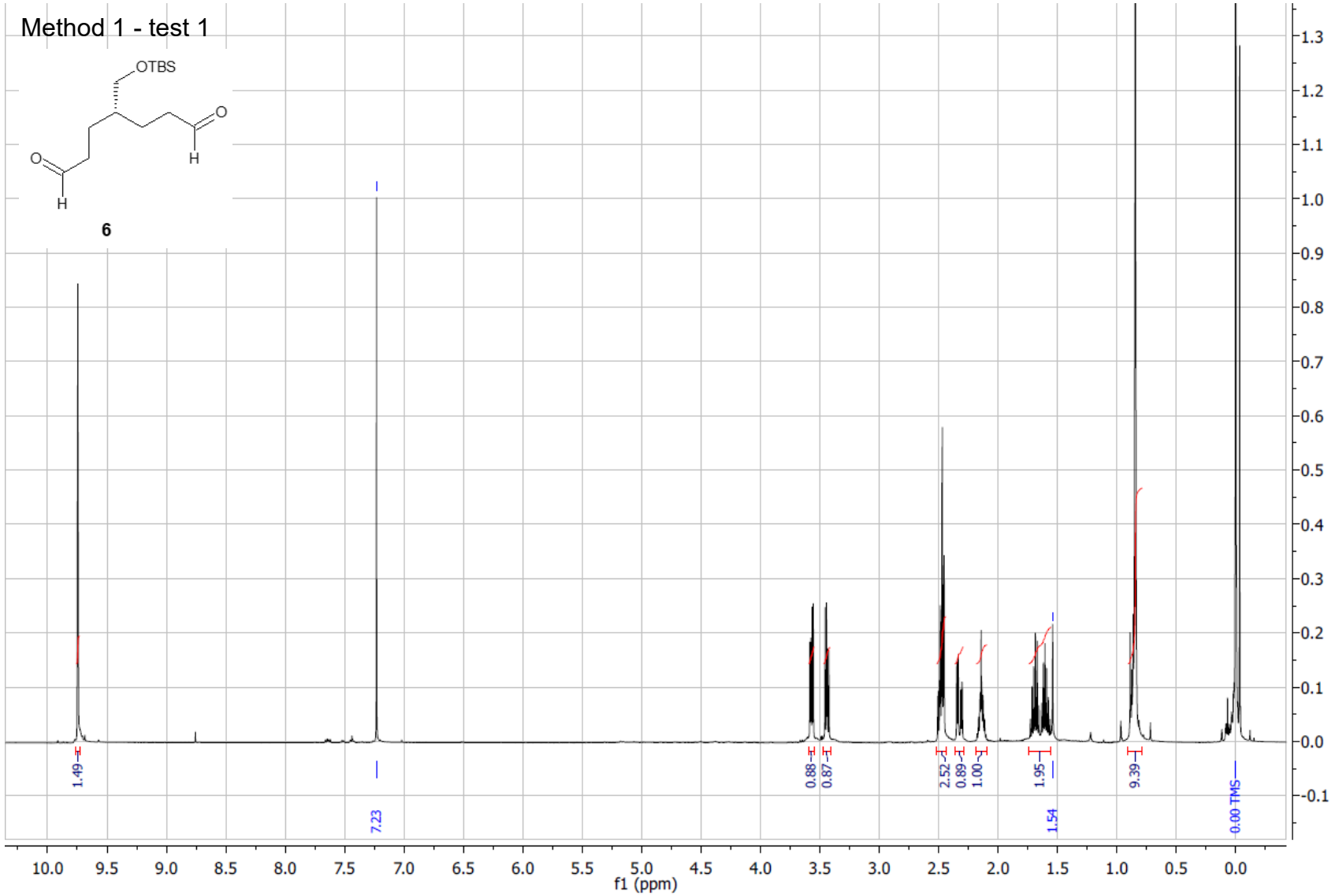
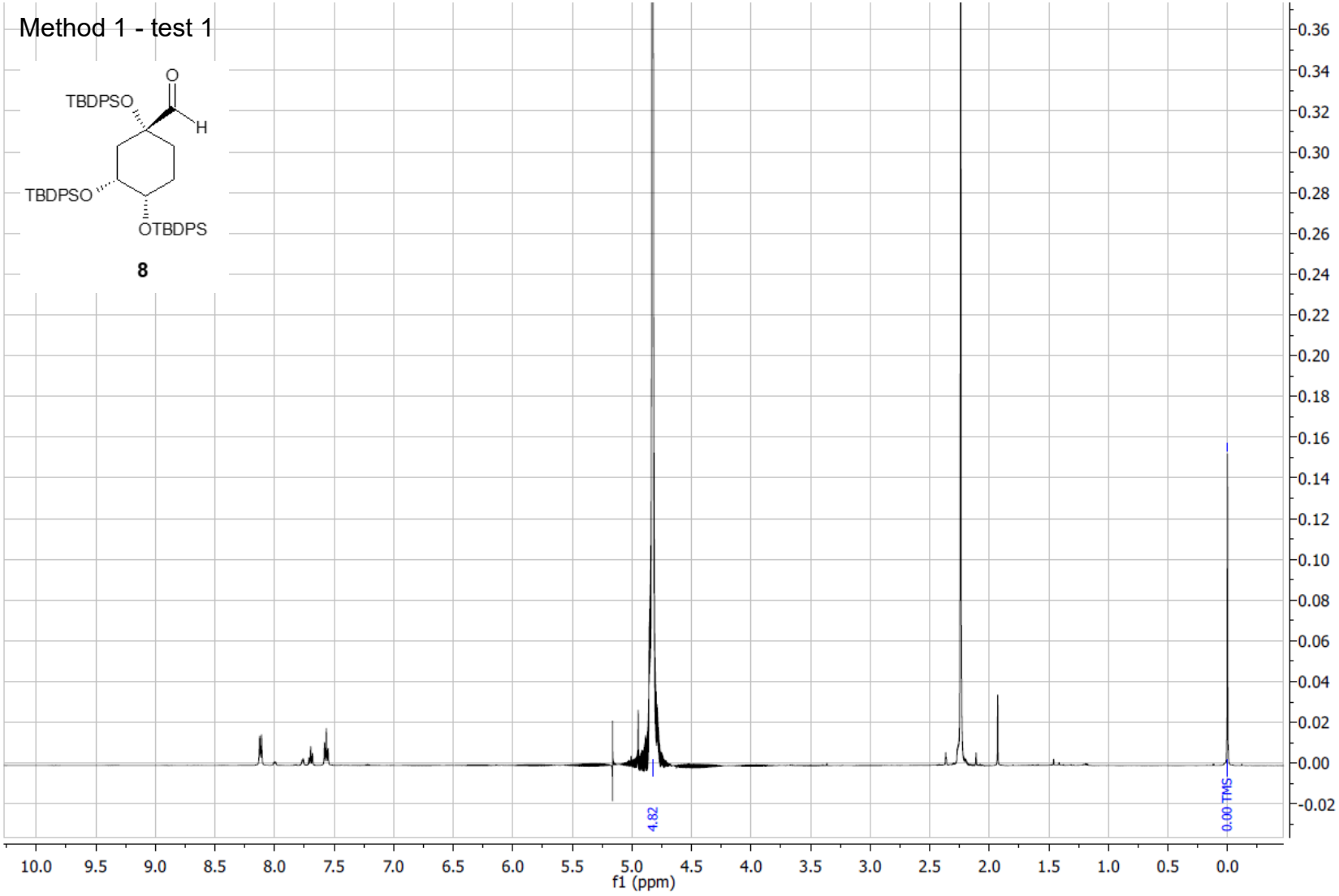

Method 3 - test 1
OTBS
HO
OH
5
1.14
1.00
1.01
5.10
3.33
2.49
1.24
2.29
2.16
2.08
11.48
0.00 TMS
f1 (ppm)

Appendix 5. Spectrum from the synthesis of molecule **6**



Appendix 6. Spectra from the synthesis of molecule **8**



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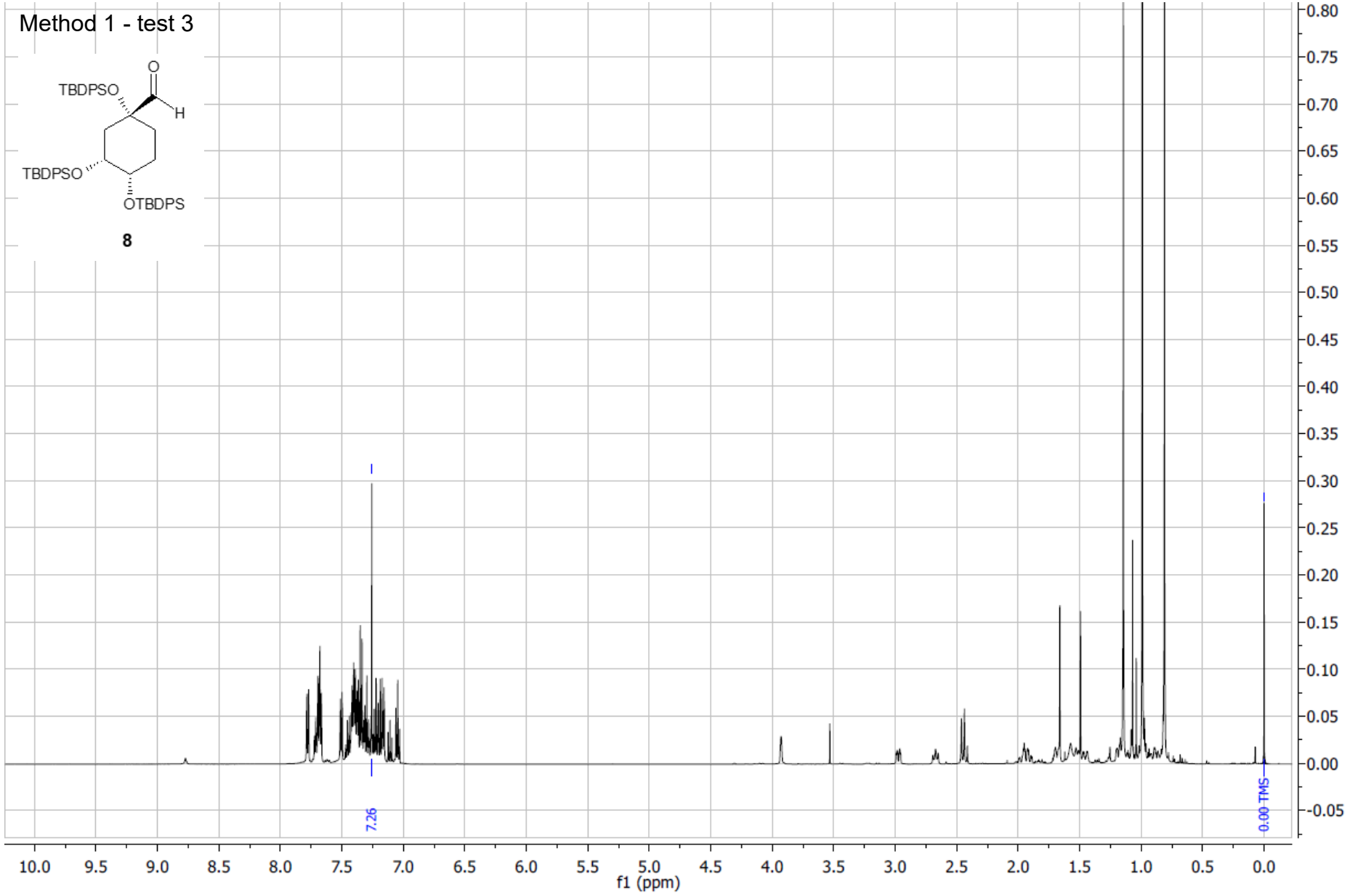
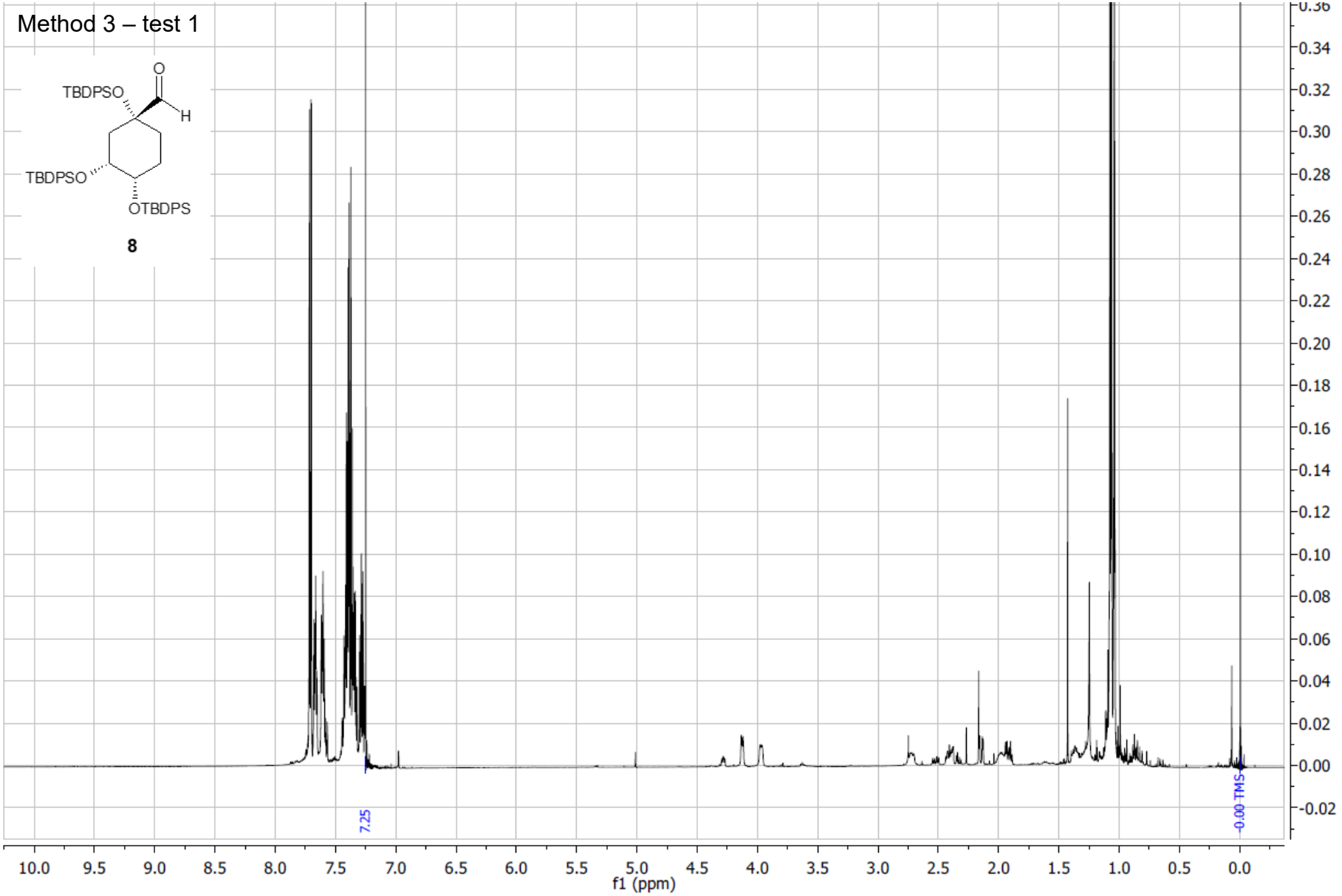
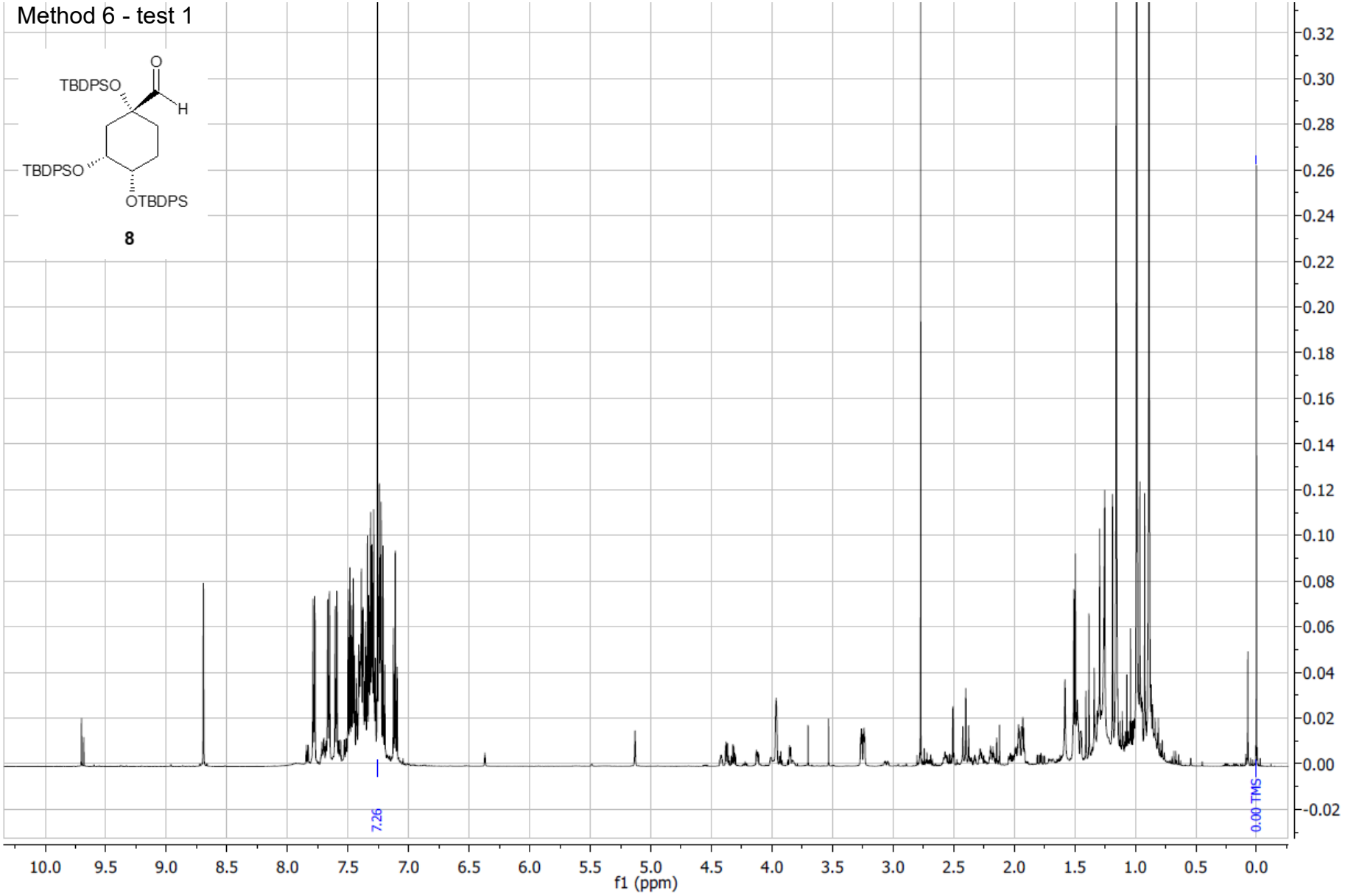
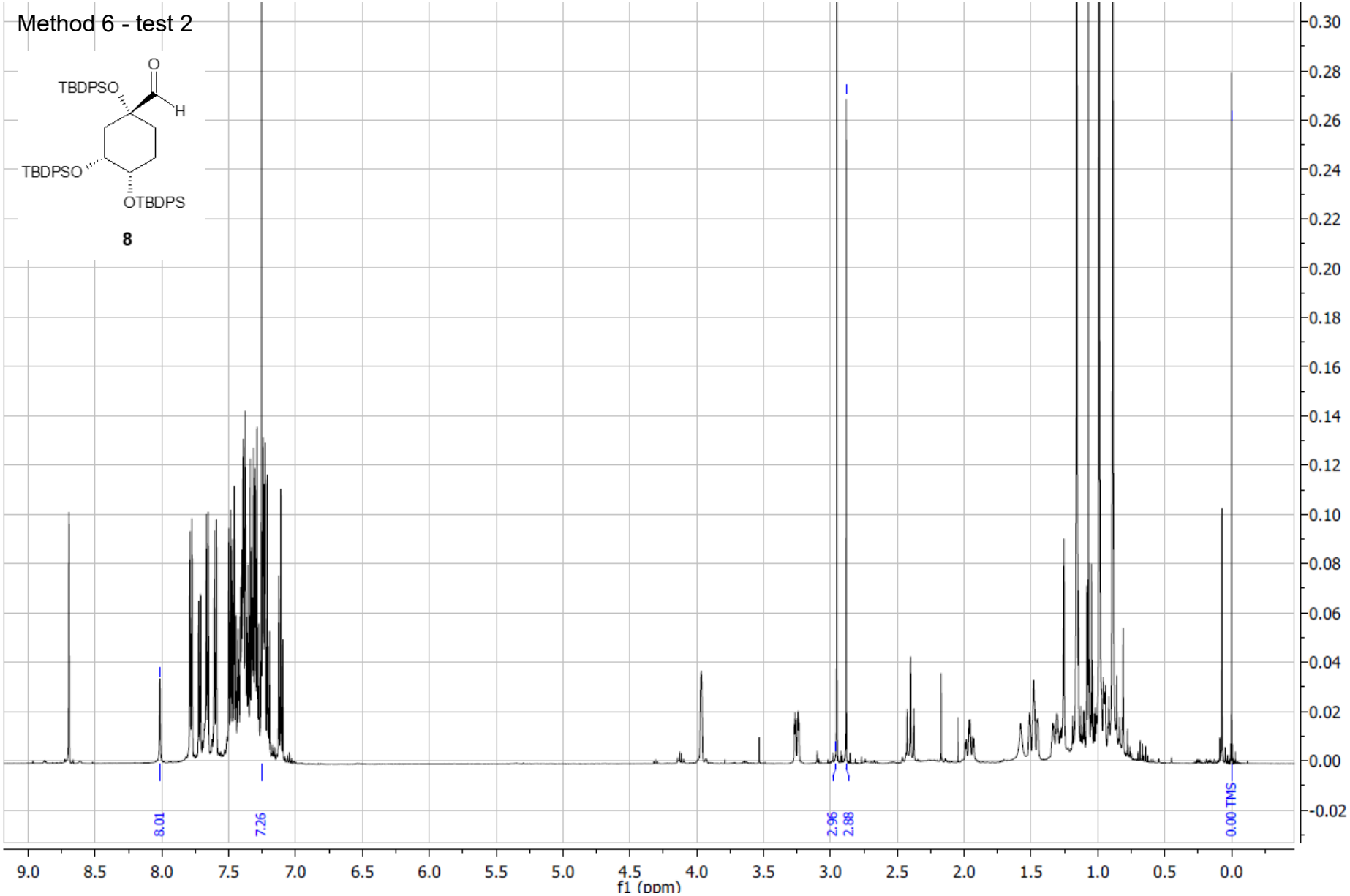

Method 1 - test 3
O
TBDPSO
H
TBDPSO
OTBDPS
8
7.26
0.00 TMS
f1 (ppm)

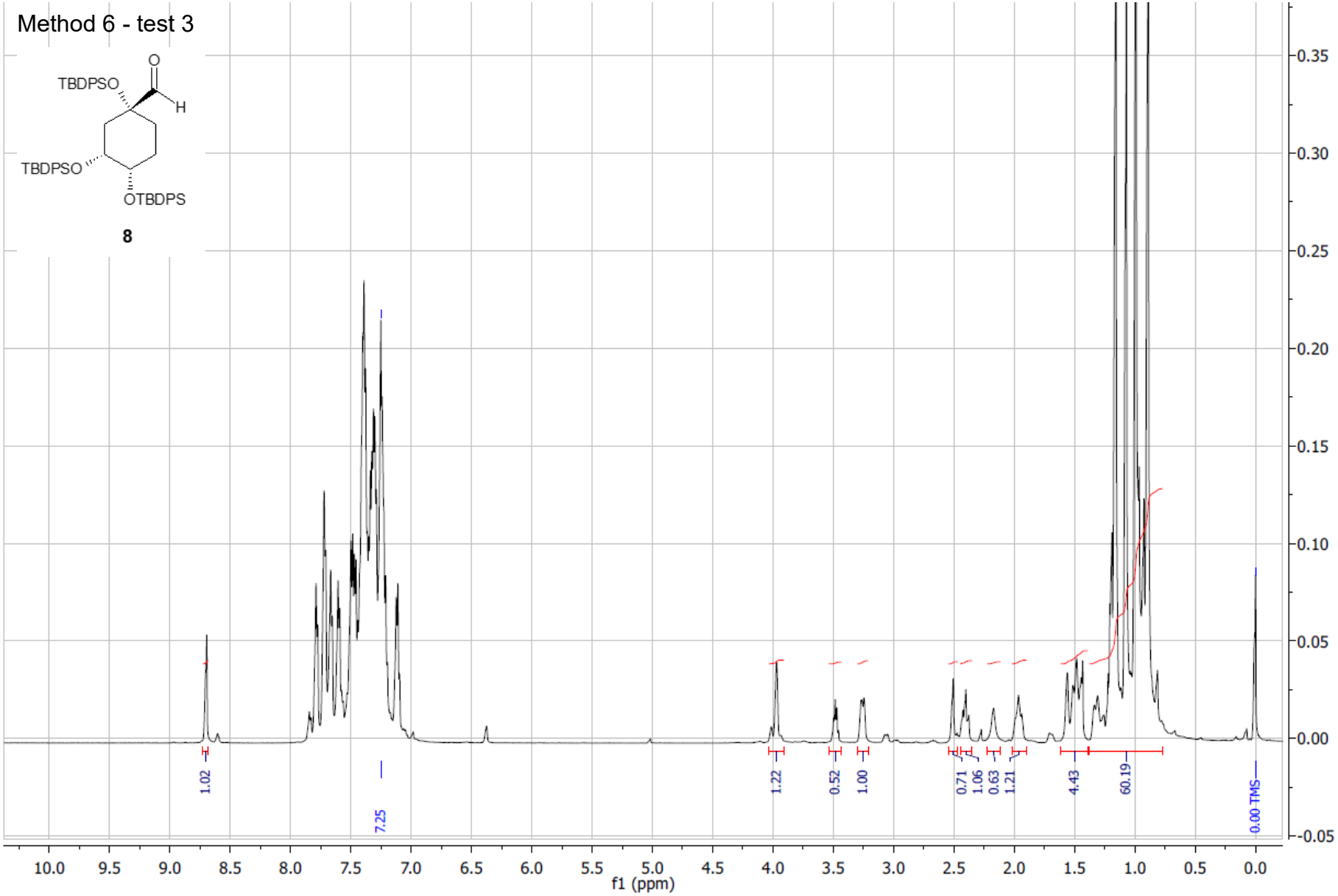
3 (6)



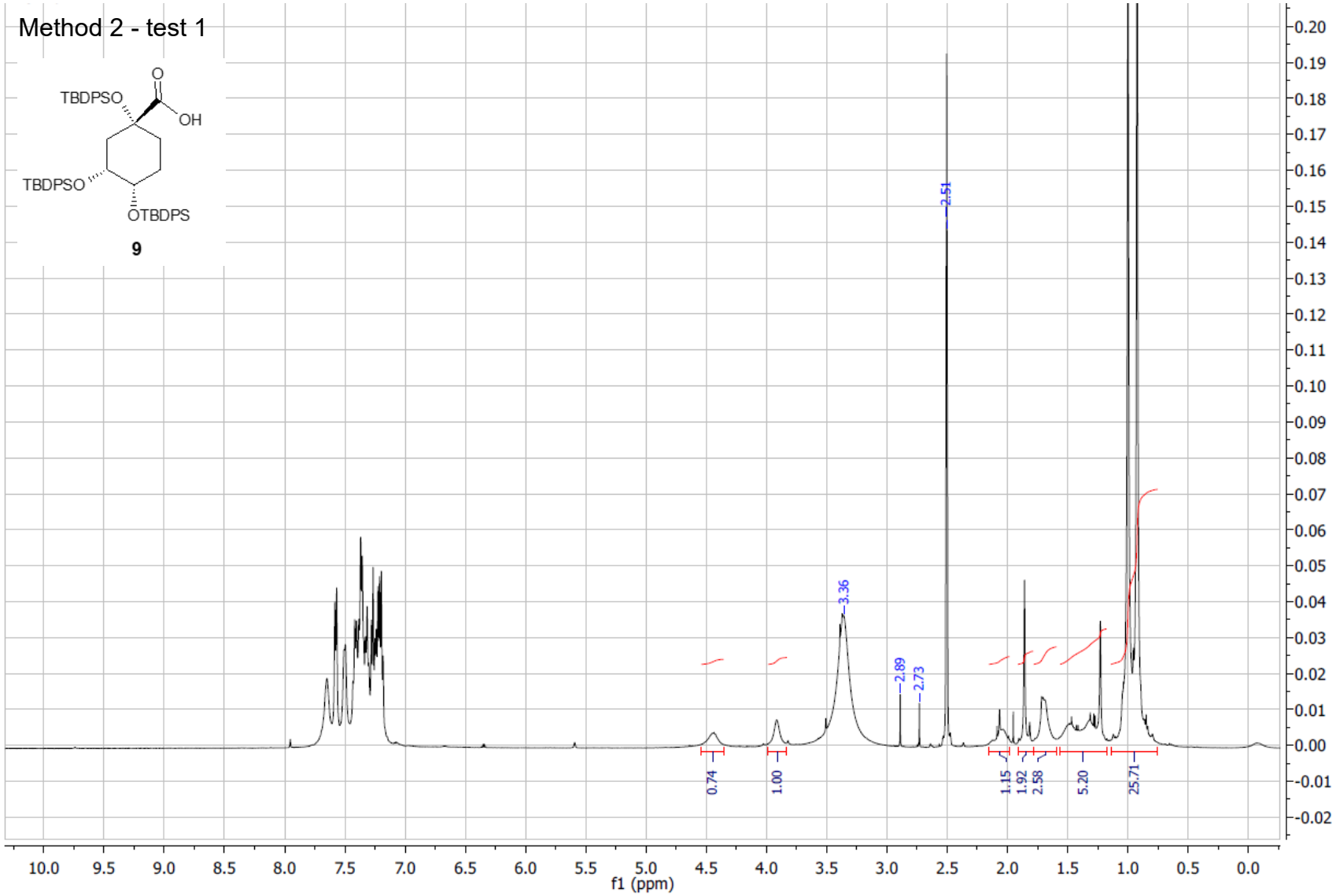

Method 6 - test 1
O
TBDPSO
H
TBDPSO
OTBDPS
8
7.26
0.00 TMS
f1 (ppm)

5 (6)

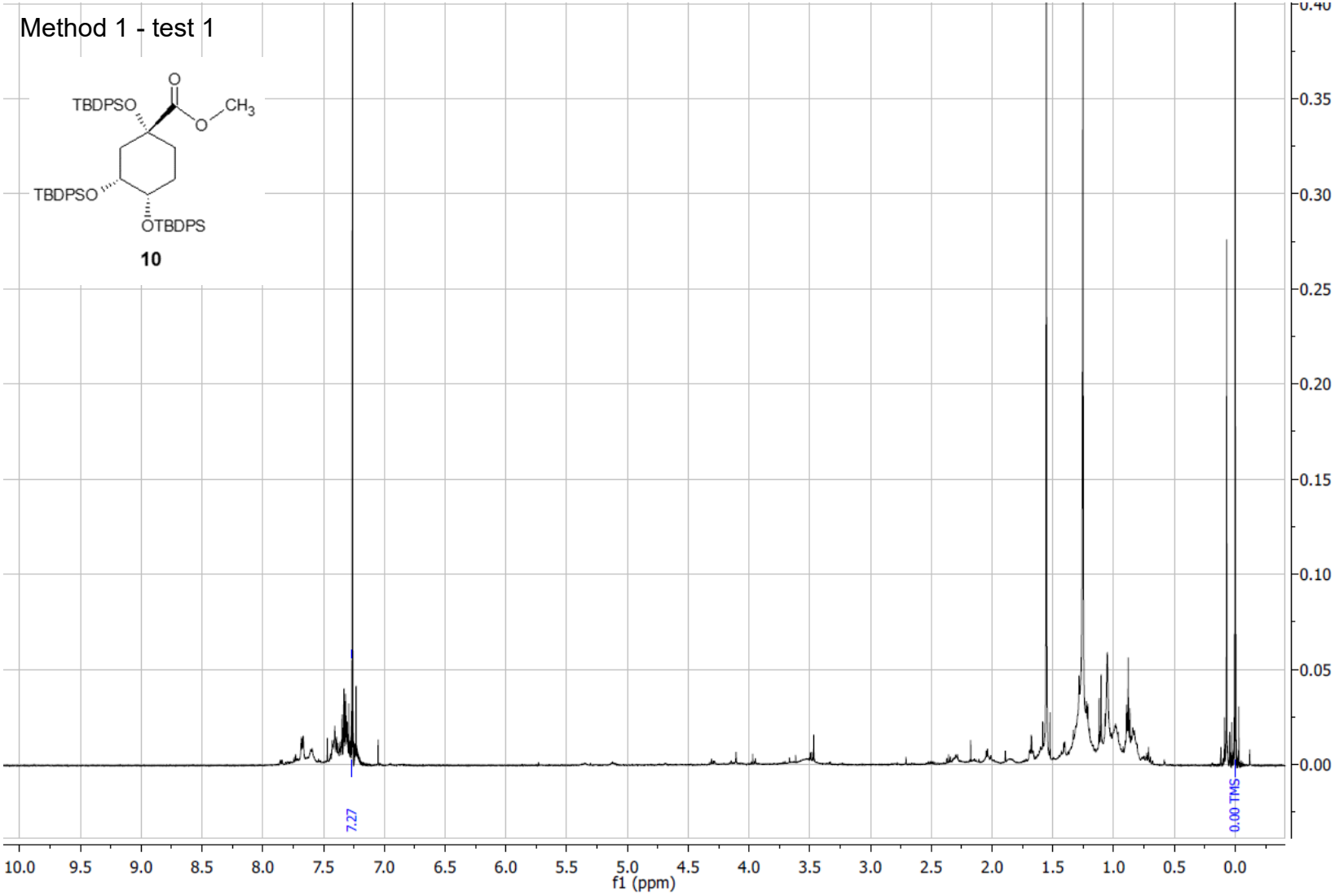

Method 6 - test 2
O
TBDPSO
H
TBDPSO
OTBDPS
8
8.01
7.26
2.96
2.88
0.00 TMS
f1 (ppm)


Method 6 - test 3
TBDPSO
O
H
TBDPSO
OTBDPS
8
1.02
7.25
1.22
0.52
1.00
0.71
1.06
0.63
1.21
4.43
60.19
0.00 TMS
f1 (ppm)

Appendix 7. Spectrum from the synthesis of molecule **9**



Appendix 8. Spectra from the synthesis of molecule **10**



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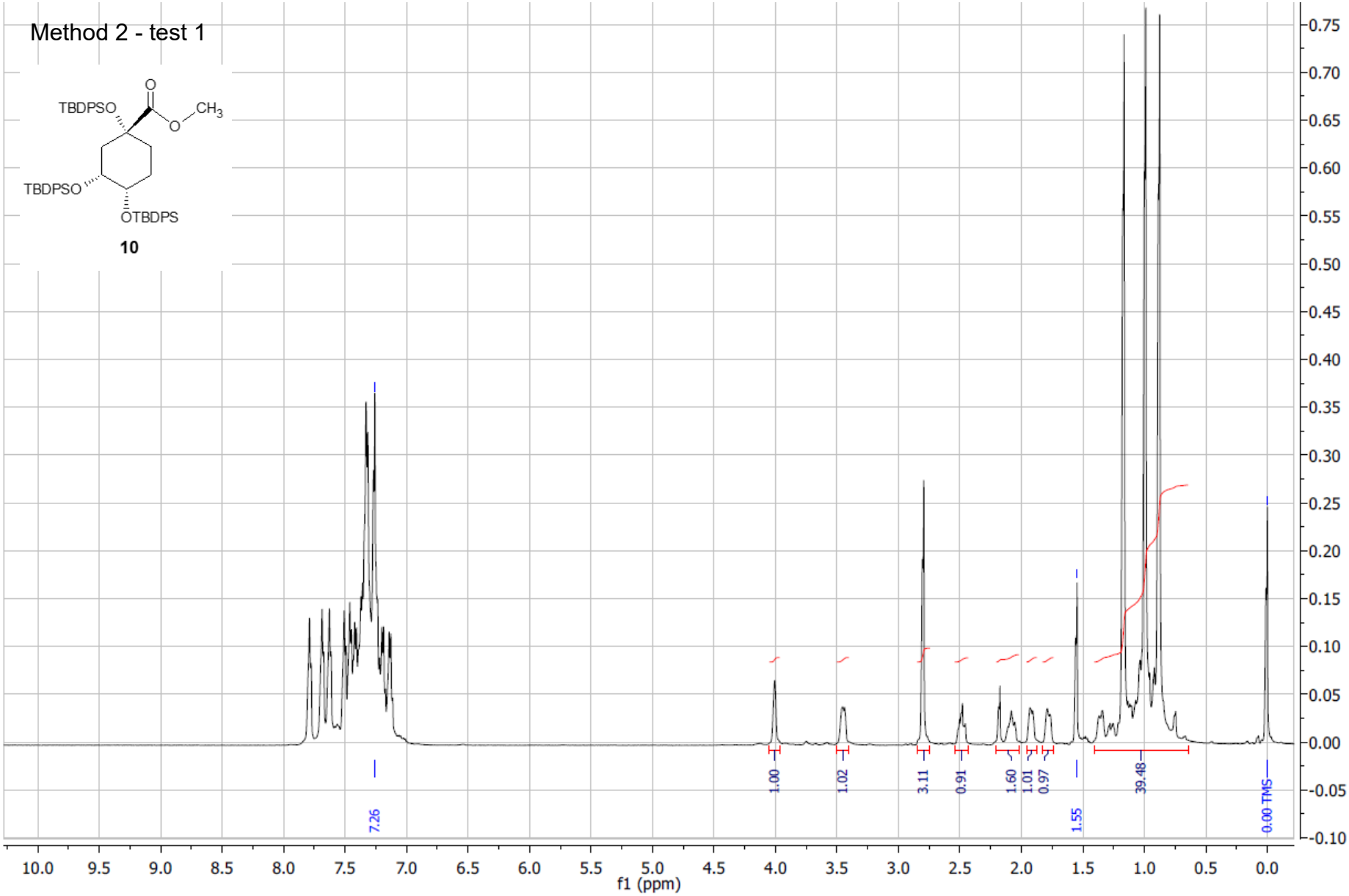
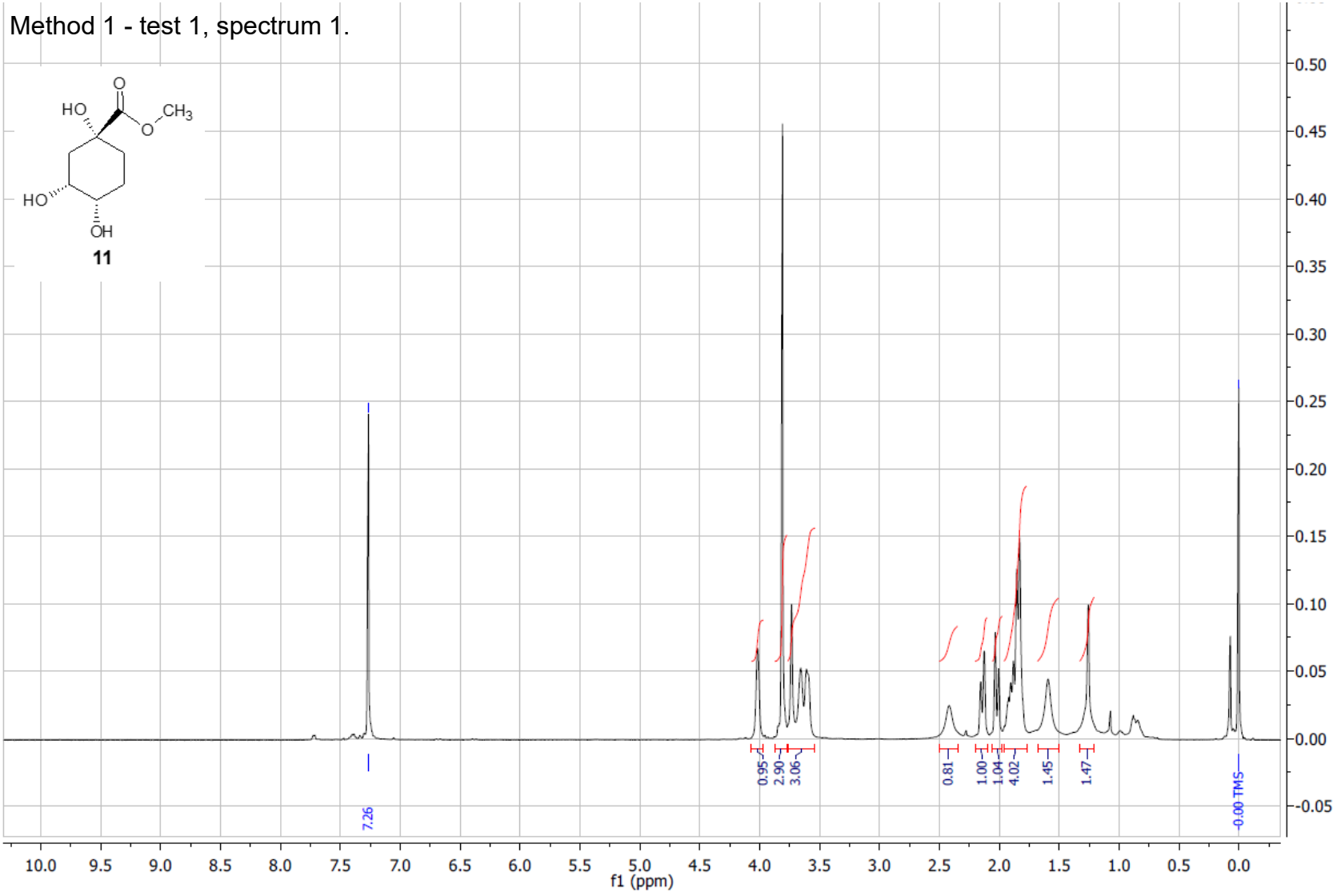
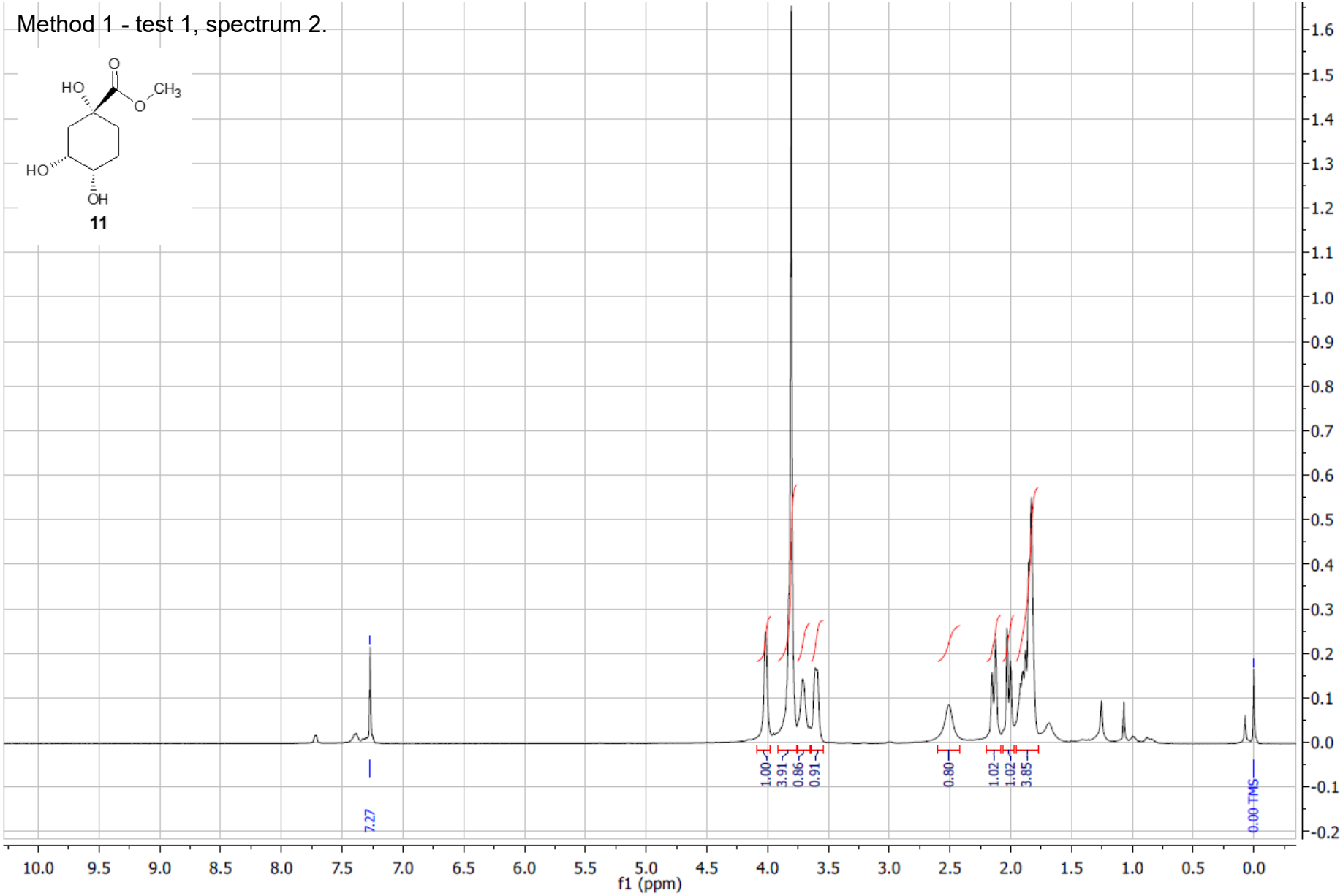

Method 2 - test 1
TBDPSO
O
O
CH3
TBDPSO
OTBDPS
10
7.26
1.00
1.02
3.11
0.91
1.60
1.01
0.97
1.55
39.48
0.00 TMS
f1 (ppm)

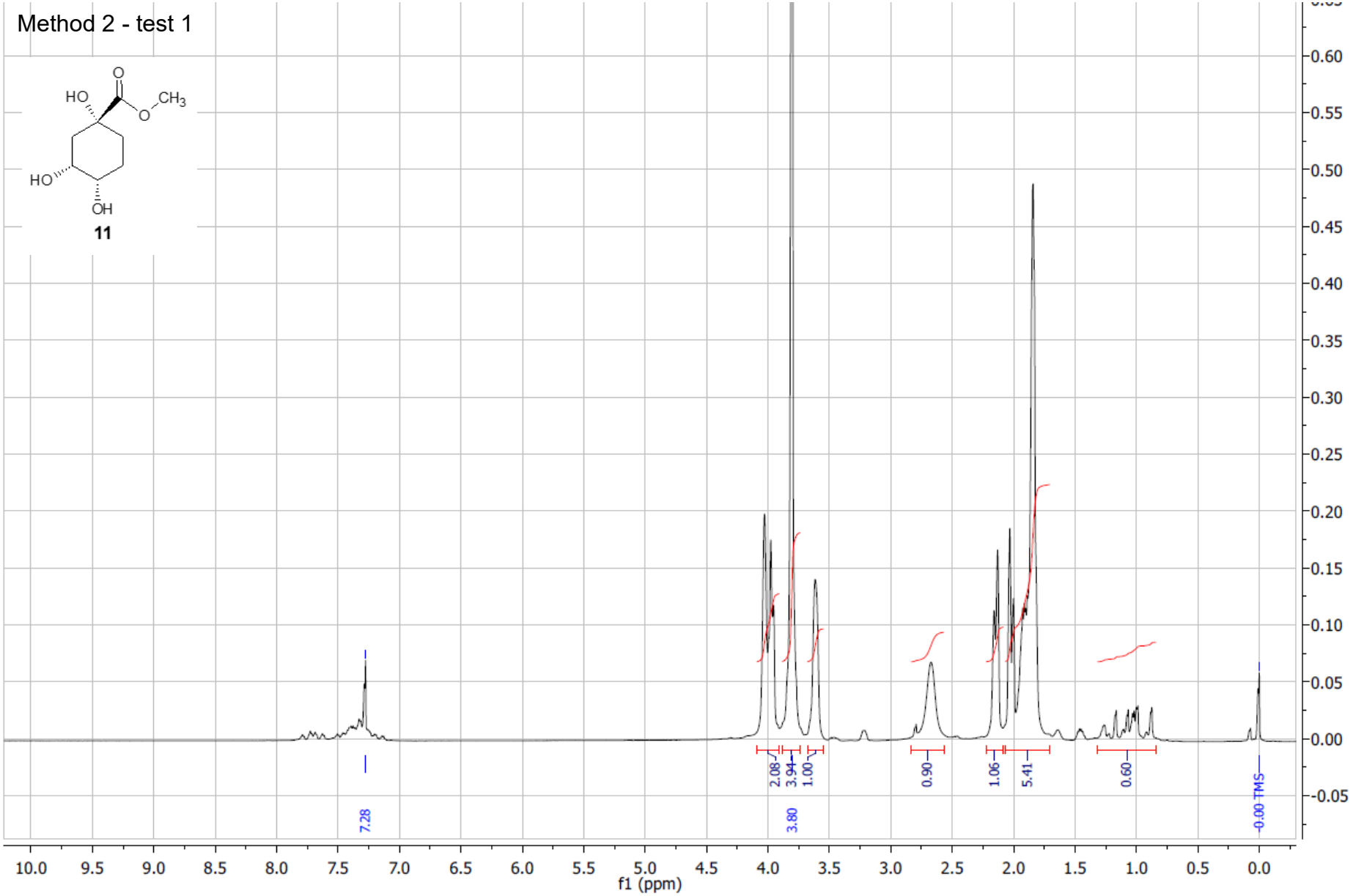
Appendix 9. Spectra from the synthesis of molecule **11**



continues

Method 1 - test 1, spectrum 2.




Method 2 - test 1
HO
O
CH3
O
HO
OH
11
7.28
2.08
3.94
1.00
3.80
0.90
1.06
5.41
0.60
0.00 TMS
f1 (ppm)