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Bhiomicyn A: A new antibiotic

Bachelor's Thesis

Directed by Dr. Neumaier

Chemistry Bahelor's

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Abstract

The research in new medication is both in the past and in the present an important research camp around the world due the huge number of new bacterial, and the bacterial which develops defences against the current medication. The aim of this project is to be able to synthesize the bhimamycin A, a natural antibiotic which can be isolated from the *Streptomyces spin* a laboratory, using the Ethyl-L-lactate as a reagent that is commercially available.

The synthesis is composed by 3 parts, the first one, produced by Dr. Neumaier, is the synthesis of the 2-acetyl-8-methoxynaphthale-1,4-dione, which is a Michael acceptor, from O-methoxybenzoic acid. The second part is the synthesis of (*R*) -2- (benzyloxy) propanal from ethyl-L-lactate using oxidation and reduction reactions. Finally, the last part begins by adding the aldehyde to the Michael acceptor using the Corey-Seebach reaction or the Stetter reaction to make the 1,4- addition possible. Once this addition is made, a catalysis is necessary for the ciclation of the product, and a Lewis acid to deprotect the alcohol.

To be able to do this work, most of the reaction has been done under inert atmosphere, purified with flash chromatogaphy and characteritzed with NMR, HPLC-MS and TLC-MS.

1 Objective

The aim of this project is to synthesize bhimamcyn A an antibiotic, which is present in some natural plants. There are different kinds of bhimamcyn, all of them have a quinone structure in common. For the moment only the bhimamcyn B has successfully been synthesized according to the literature.[1]

2 Introduction

The Eberhard Karls University in Tübingen in the South of Germany is one of the most important universities in the country, both in terms of studies and research. In the past ages many Nobel prize winners went out from here.

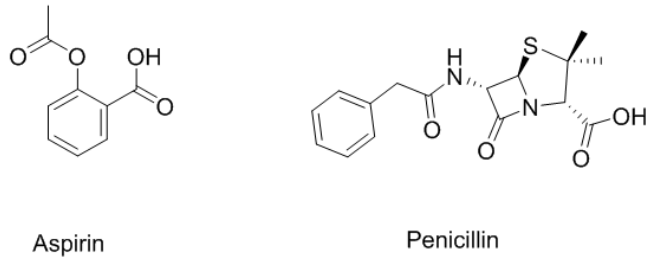
The research group of Professor Ziegle is specialized in carbohydrate chemistry, and how to synthesize saccharides, which are used for biochemical studies. They develop new synthetic strategies for complex saccharides including new protecting groups. They are also interested in enzyme reactions, combinatorial synthesis and heterocyclic chemistry.

In this project we will focus on natural medicaments, which have been present in the history of the humanity since approximately 3000 B.C., when the opium juice had been discovered as a powerful painkiller. Since then, it has been possible to find a large amount of natural products used in the ancient world which still have a big influence in modern life.[2]

These medicaments can be extracted from plants, fungi, marine environment, marine algae, marine sponges and marine sources.

A classic example of a semi-synthetic medicament is the aspirin (acetylsalicyclic acid) which is extracted from the *Salix alba*, a tree. The acetylsalicyclic acid from a natural product is an anti-inflammatory agent discovered in 1763 by Edward Stone, but it also has secondary effects to the stomach. Was in 1986 when Félix Hoffmann, a Bayer pharmaceutical discovered a shyntethical variant of the acid, which don’t have those secondary effects.[3]

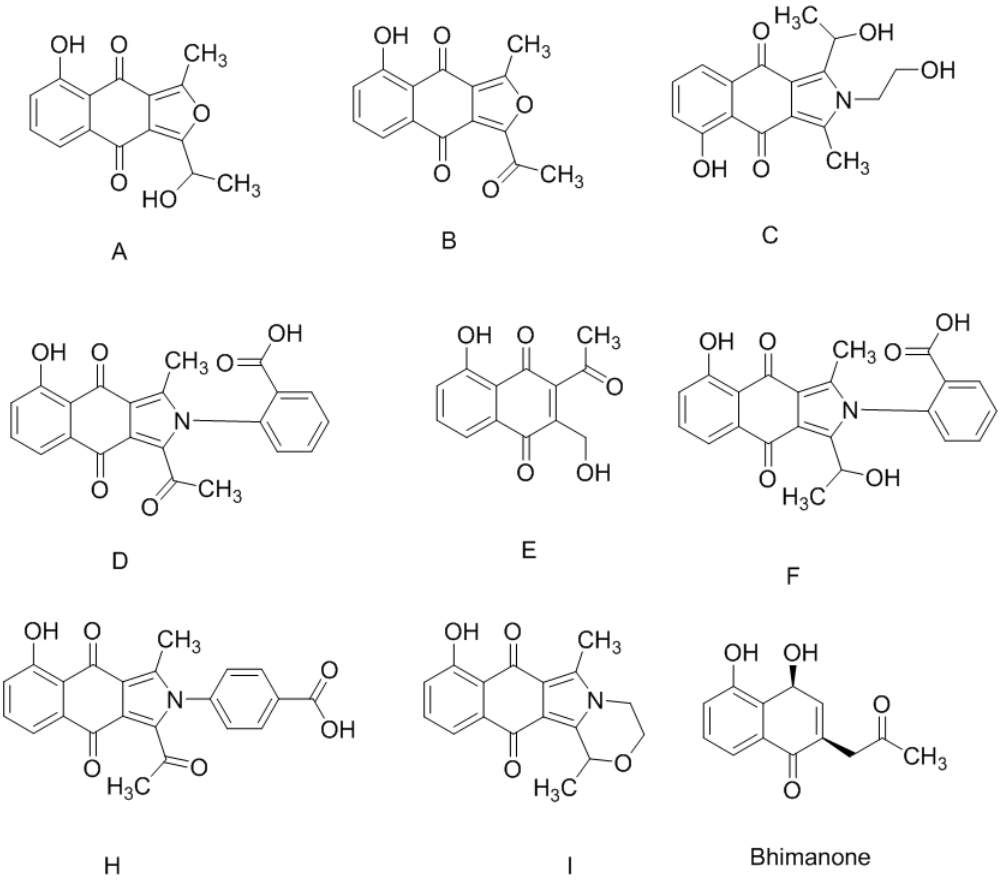
Another famous medicament is the Penicillin, extracted from the fungi *Penicilliumnotatum*. This antibiotic discovered in 1923 by Fleming was a big revolution in the world of medications, after this discovering the world started the research for new antibioticsand bioactive natural products, which can be isolated from microorganisms.[4]



Picture 1: Molecular formula of Aspirin and penicillin

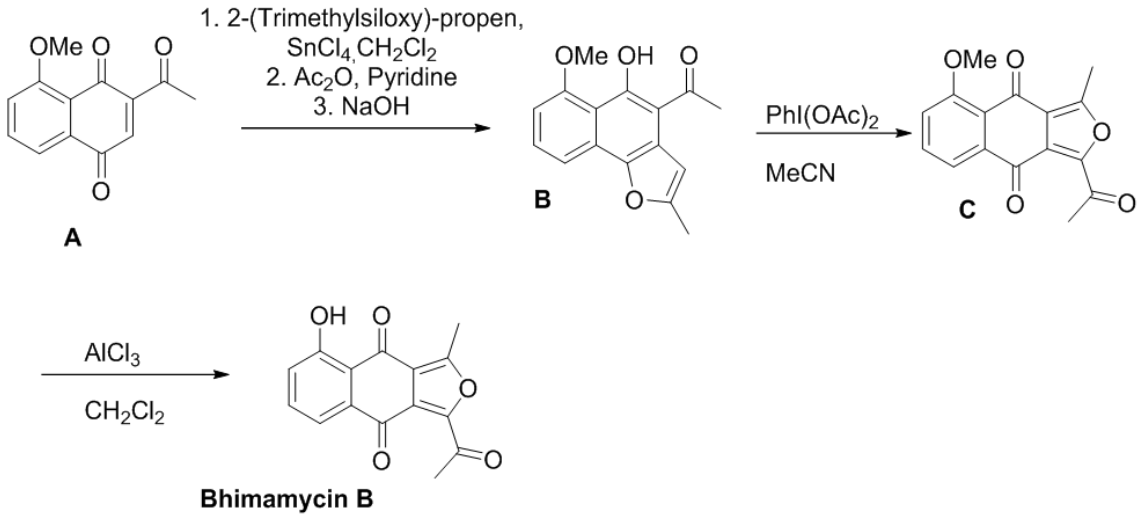
The target of this project will be the bhimamycin A antibiotic, which was isolated from *Streptomyces sp.* (an actinobacteri present in the grown and in the spores) and shows antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Streptomyces viridochromogenes*.

Nowadays there are nine different bhimamycins which had been isolated from the *Streptomyces sp*. Even if they have a different actibacterial activity, all of them have a quinone structure in common:[5,6]



Picture 2: The nine kinds of bhimamycin

For the moment, only the bhimamycin B has been possible to synthesize in the literature:[1]



Picture 3: Main reactions for the shynthesis of bhimamycin B

The synthesis started with the intermediary key **A**. The first step is the Lewis acid promoted reaction of **A** with the 2-(trimethylsiloxy)propene, the treatment with Ac_2O/pyridine and saponification with a solution of NaOH to afford **B.**After that, it had reacted with $PhI(OAc)_2$ in acetonitrile to give **C**, which will react with $AlCl_3$ in DCM to eliminate the benzyl group and afford bhimamycin B.

Despite that synthesis, the bhiamamcyn is not the unique quinone antibiotic. Nowadays, the quinone antibiotic is divided in several groups according to their presence in a huge amount of biological activity. The electrons transport throw the biological membrane is easily produced by redox reactions giving one or two electrons which have different mechanisms depending on the finale objective of the molecule, these ones will determinate in which group will be the molecule. There is a large number of functions for this compounds, such as antibacterial, anticancer agent or anti-hormone [7]

3 Main reactions

The main idea is to synthetize the bhimamcyn A from the ethyl-L-lactate, which is a natural product present in different kinds of food. In this section it will be explained the main reactions that are needed for the synthesis.

3.1 Stetter and Corey-Seebach reaction

The oxygen is the most electronegative atom in a carbonyl group, it means that it will be the nucleophyle of the reaction, but it makes very difficult to do a 1,2-, 1,4- and 1,6- heteroatoms substitutions.

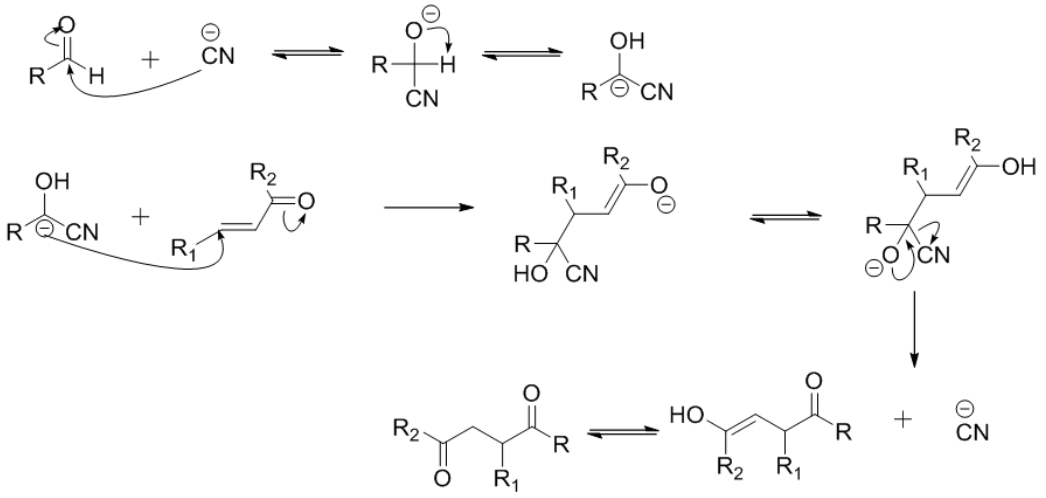
Seebach discovered the umpolung, in 1969, in order to resolve the previous situation.

Theumpolung was descrived for the first time as the substitute of the carbonyl group for a thioacetal and then, with a base, the deprotonation of the hydrogen next to the thioacetal will make the nucleophile carbonyl.[8]

3.1.1 Stetter reaction[9]

The Stetter Reaction was discovered by Hermann Stetter in January of 1976 ,and he published it in the AngewandteChemie.

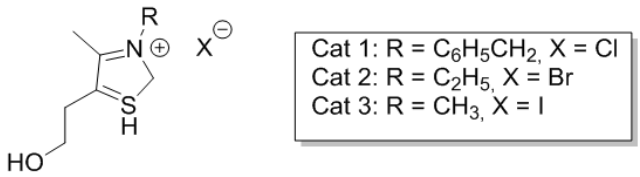
The addition of an aldehyde to an α,β-unsaturated system had been always a difficult reaction, not a very favourable one. For this reason, Stetter had been researching for a more favorable synthesis route to add aldehydes, esters, ketones and nitriles to a α,β-unsaturated system using the influence of cyanide ions or thiazolium salt to create an umpolung and be able to do the addition.



Picture 4: Reaction mechanism of stetter reaction

In the case of using cyanide, it will create cyanhydrin-anion, which will act as a nucleophile an attack the α,β-unsaturated system. After some equilibrion steps the aldehyde will getadded, and cyanide will leave the molecule.

In the case of the thiazolium salt, there are different options where the counter-ion and the residueat the nitrogen will change:

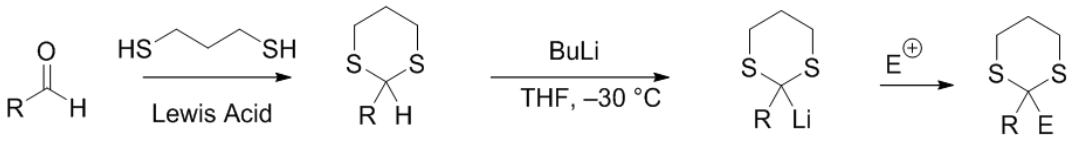


Picture 5: Different Stetter catalyst

In the orginal article of the reaction, the stetter reaction showed good yields when cyanide or thiazolium salt were used as a catalyst.

3.1.2 Corey-Seebach reaction[10]

Corey and Seebach discovered the reaction in 1997 and they published it in the Synthesis.
The Corey-Seebach reaction is used with the same aim than the Stetter reaction explained in the previus section.
In this case, the umpolung is made using 1,3-dithiane which is a protection group for aldehydes. Thioacetals are very good protective groups which need extrem conditions to be removed. This also means that we can do a huge range of reactions without exposing them to losetheir protection.
This group also need the deprotonation of the proton next to it to make it a nucleophile, which can be added to an α,β-unsaturated system:



Picture 6: Corey-Seebach reaction

3.2 Dess-Martin Periodinane[10]

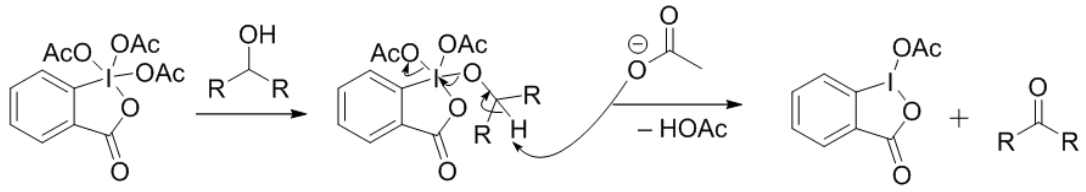
The Dess-Martin Periodinane (DMP) was discovered from Daniel Benjamin Dess and James Cullen Martin in 1983.

The Dess-Martin periodinane is a selective oxidizing agent, which allows the reduction of primary alcohols to aldehydes and secondary alcohols to ketones.

This reaction consists in the addition of acetat groups to theiodine of the 2-iodoxybenzoic(IBX). This change provides the oxidaizing agent a better reactivity because the DMP is soluble in organic solvents while the IBX is not.

The DMP is also very used due the short time of reaction (0.5 – 2 h) and after the easily purification of the main product using a base to clean it up.

The mechanism of the oxidation starts with the substitution of one acetate group by the alcohol in the DMP. After this, the acetate group will take the proton, which will produce the expulsion of the ketone and another acetate group:

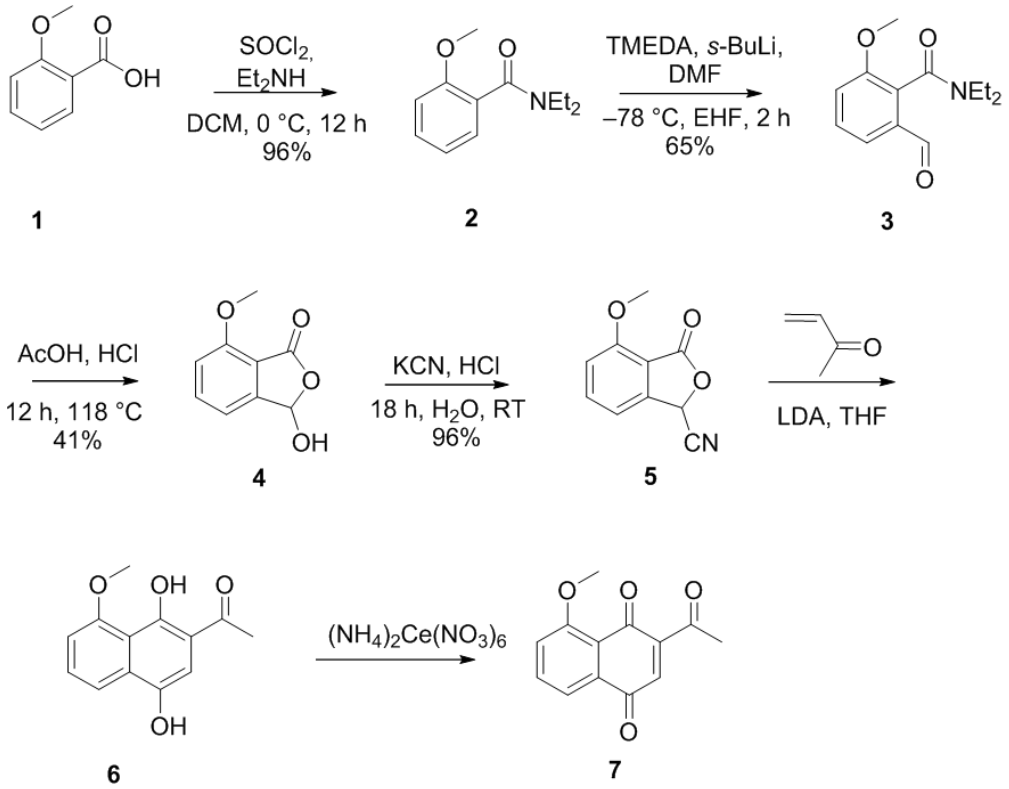


Picture 7: Reaction mechanism of dess-martin periodinane.

When Dess and Martin discrive the shyntesis of the DMP, the oxidation step to produce the IBX was made with $KBrO_3$ as anoxidazing agent which is a cancerigen agent and is difficult to manipulate. For this reasons, nowadays is substituted by the oxone, another oxidazing agent which doesn't present this kind of problems.[11]

3.1 Preparation of carbonyl 7

The Michael acceptor needed to do the Stetter and Corey-Seebach reaction had been synthesized before then for the Dr. Neumaier using the following synthetic route:

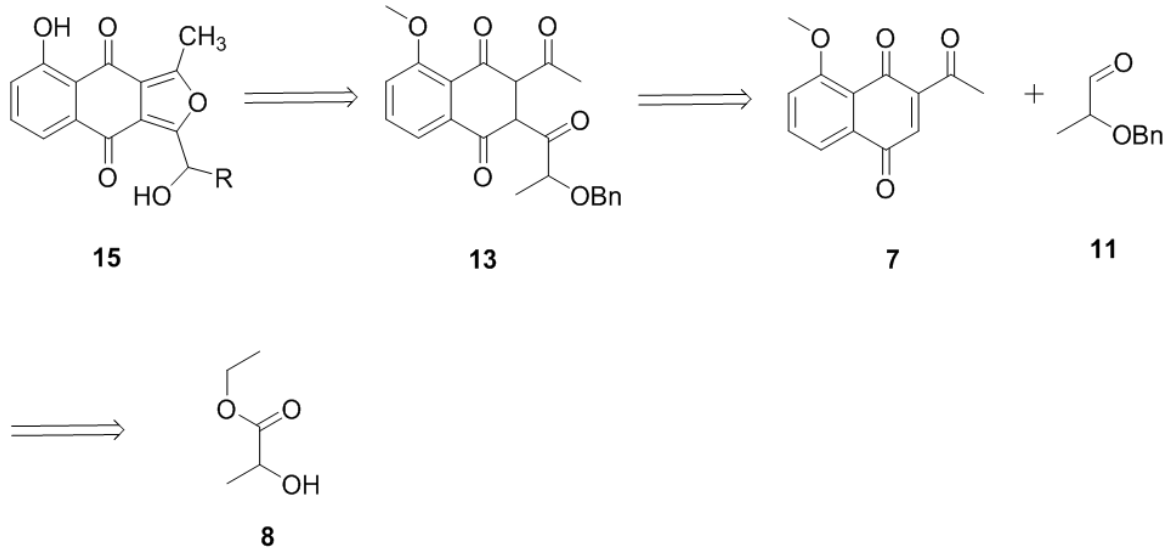


Picture 8: Synthesis route from 1 to 7

The synthesis started with the *O*-Anisic acid **1,** which is commercial aviable. The first step was the substution of the acid group for diethylamida group using $SOCl_2$ to make the intermedium and the second step from the intermedium to the final molecule was made with diethylamine as a reagent. The next step is an ortho-formylation of product **2** using tetramethylethylenediamine (TMEDA), *s*–Buli as the base and DMF as the solvent and reagent to obtain the product **3**. After using an acid catalysis with acetic acid in HCl is possible to close the ring creating the heterocycle **4**. The next reaction is the substitution of the alcohol for a cyanide, using potassium cyanide in HCl, a reaction type SN2 to produce **5,** which after that will do a Michael reaction with the but-3-en-2-one in LDA and THF to produce **6**. Finally, the oxidation of **6** with ceric ammonium nitrate (CAN) as an oxidazing reagent to synthesize **7.**

4 Results and discussions

4.1 Retrosynthesis

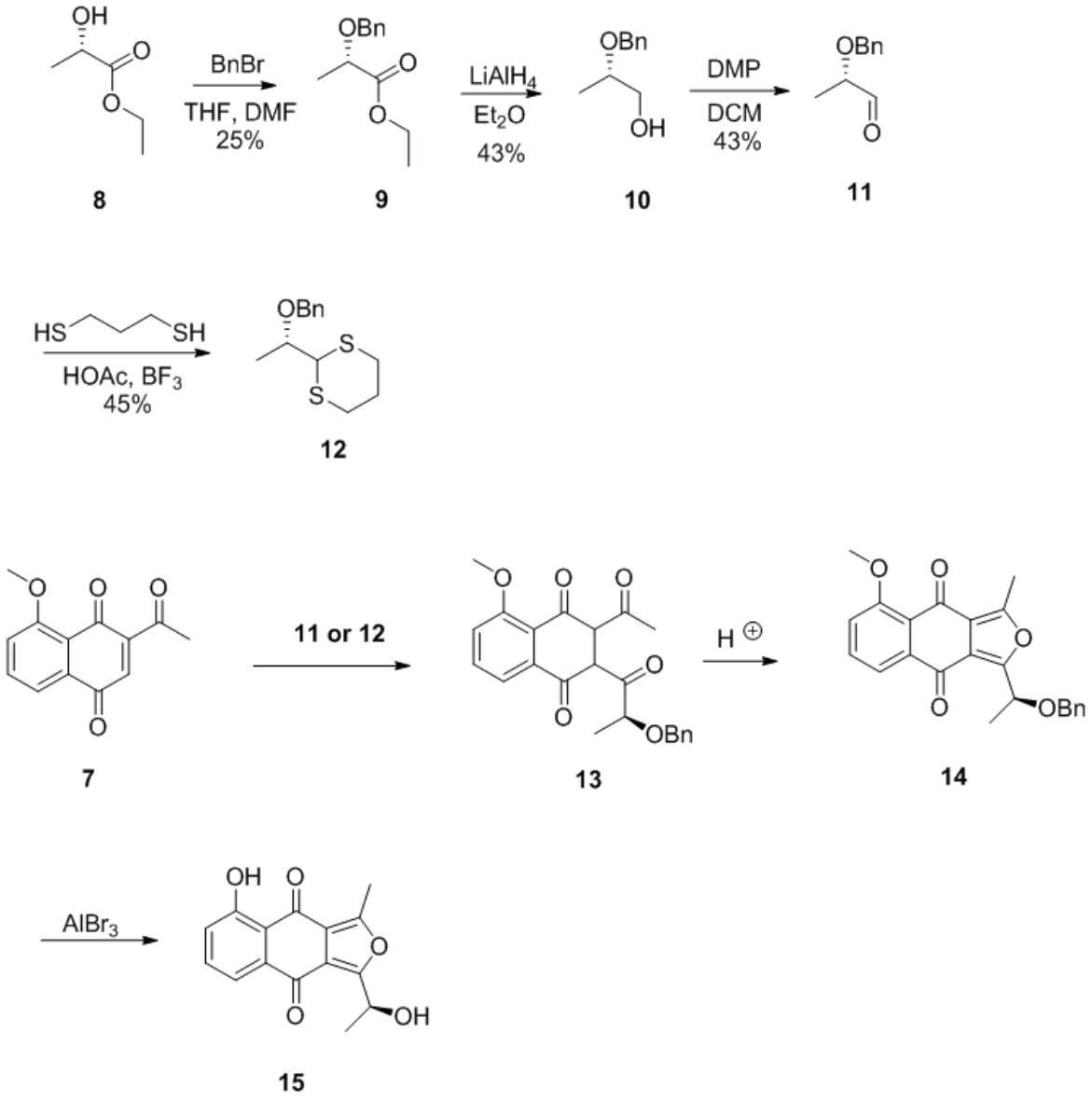


Picture 9: Retrosynthetic analysis

The previous picture shows the retrosynthetic analysis of the project, where **8** is commercial available, and is possible to obtain **11** (3 steps). After this it can react with **7**,which is already synthesized, to obtain **13** using the Stetter reaction (1 step) or Corey-Seebach reaction (3 steps) and finally, obtain the **15** (2 steps), which is the bhimamycin A.

4.2 Synthesis of the Bhimamycin A

The next synthesis rout is used to be able to synthesis the bhimamycin A:

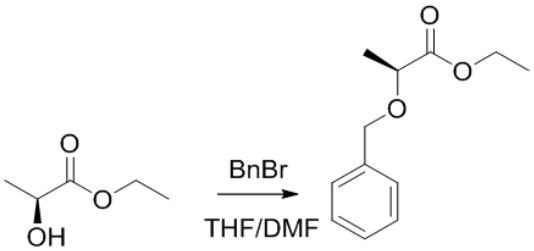


Picture 10: Synthetic plan.

This synthesis starts with the addition of a protecting group to **8,** which is commercial available. This addition is the benzylation of the alcohol using benzylbromide as a reagent to produce **9.** The next step is the reduction of the ester to an alcohol using litiumaluminiumhydrid ($LiAlH_4$) as a reductive agent with dry diethylether as a solvent to produce **10**. In the next step, the alcohol will be selectively oxidated to the aldehyde **11** with the Dess-Martin propinadine (DMP). It can be used directly to do the Stteter reaction an obtain **13** or can react with the 1,3-dithiolpropane in presence of boron trifluoride (BF_3) as the Lewis acid and acetic acid as the solvent to produce **12** which will react with **7** to produce **13**.

With an acid catalysis, it is possible to produce **14** from **13**. Finally, with a Lewis acid like aluminum bromide it could be possible to diprotect the alcohols and have the final molecule **15** (bhimamycin A).

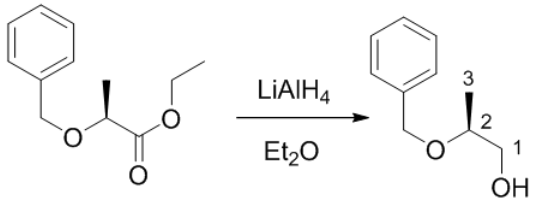
4.3 Display of compounds 8-13



8 **9**

This nucleophile addition of the benzylbromide is used to generate benzyl ethers. It starts with the deprotonation of the alcohol using sodium hydride as the base; this step produces hydrogen gas, so the base is added slowly to give time to the hydrogen to leave the reaction. After the oxygen will attack the $–CH_2$ of the benzyl bromide and the bromide, which is a very good leaving group, it will leave and make the salt with the sodium anion present in the reaction.

This reaction had been stirring at 0 °C during 5h and after that over night at RT. After the clean up, the TLC (30%EtOAc in PE) showed only the spot of **9** with aR_f of 0.50. The product was purificated using flash chromatography (5% EtOAc in PE), to obtain 4.59 g (25%) and a NMR which shows a high purity.



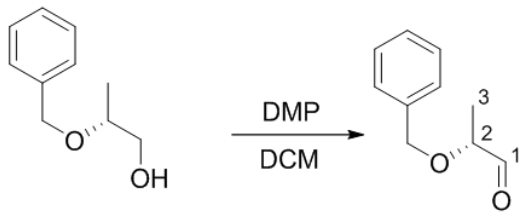
9 **10**

This reaction is the reduction of the ester to alcohol using the lithiumaluminiumhydrid, which is a powerful reducing agent, very used in the organic chemistry to reduce esters, carboxylic acids and amides.

The reaction was made under inert and dry atmosphere and using the dry diethyl ether as a solvent because the litiumaluminiumhydrid produces an explosive reaction with water.

The mixture of reagents was stirried during 1 h, and the solution was neutralized to eliminate the excess of $LiAlH_4$ (it was added carefully because it makes a violent reaction). After the cleaned up with ethyl acetate, the TLC (10% EtOAc in PE) showed only **10** with a R_f of 0.15, to obtain 0.35 g(43%).

The product was not purified with flash chromatography because the NMR shows a high purity and **10** could react with the silica. If it was done, the purity should be approximately the same, but the yield would decrease significantly.



10 **11**

This reaction is the oxidation of the alcohol to an aldehyde using the Dess-Martin periodinane and dichlormethane as the solvent. The Dess-Martin periodinane is thoroughly used in organic chemistry because is a selective oxidizing agent, which can oxidize selectively the alcohols to ketones or aldheydes.

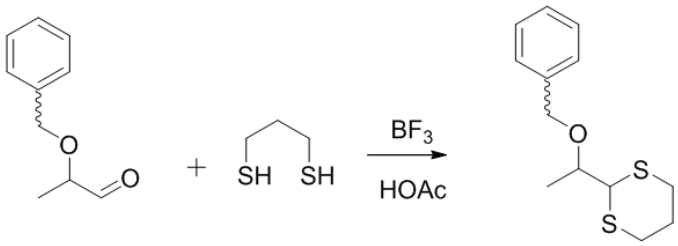
After the addition of the DMP to **10,** the mixture was stirring during 1 h or 24 h at RT, neutralized and the clean up was made to afford a TLC (10% EtOAc in PE) with only one spot with a Rf of 0.38. **11** was purified using flash chromatography and the NMR shows a high purity.

Table 1: Results of the reaction

Time (h)	Solvent	Yield (%)
1	DCM	43
24	DCM	26

If it stirred overnight, the IBX would appear as a precipitated, which is difficult to eliminate, even after filtrated, for this reason the yield decreases significantly.

After these results, it could be better to leave it stirring less time, even if it is still starting material, than leaving it stirring overnight.



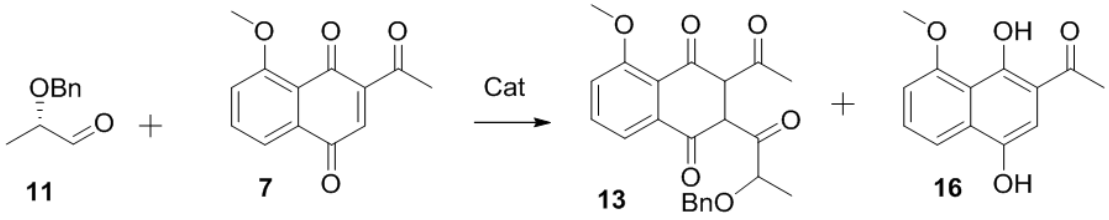
11 **12**

On one hand the addition of a dithiol to a carbonyl compound can be easily made and is useful as a protective group or to allow to create an umpolung (as in this case), but on the other hand it needs harder conditions for the deprotection of the aldehyde

To do this step it was necessary the presence of the dithiol (1,3propanedithiol), a Lewis acid catalyst (BF_3) and a carbonyl compound **11**, all of them stirried magnetically in acetic acid during 2.30 h. After the clean up was made to obtain a TLC (10%EtOAc in PE) with only one spot with a R_f of 0.38. **12** was purified using flash chromatography to afford 150 mg (45%).

The NMR shows 15% of impurities in the product,which was impossible to see in TLC, this is because **12** and **11** have approximately the same Rf. This fact makes it impossible to follow the reaction with the TLC.

The TLC after the column shows a double spotat the beginning, that maybe is the aldehyde and the product. Next time it would be better to try with more polar eluent as a 3% EtOAc in PE to try if it is possible to separate them completely.



11 **7** **13** **16**

To do this step, different condicions were tried:

Table 2: Results of the reaction

Number	Catalyst	Solvent	Base	Temperature (°C)	Color	Time(h)
1	NaCN	DMF	–	35	Brown/Black	1
2	Thiazolium	EtOH	Et_3N	70	Green/Black	24
3	Thiazolium	DMF	Et_3N	80	Red/Black	24
4	Thiazolium	DMF	Et_3N	80	Green/Black	24
5	–	DMF	$N(iPr)_2Et$	70	Brown/Black	24
6		DMF	–	70	Orange/Red	24

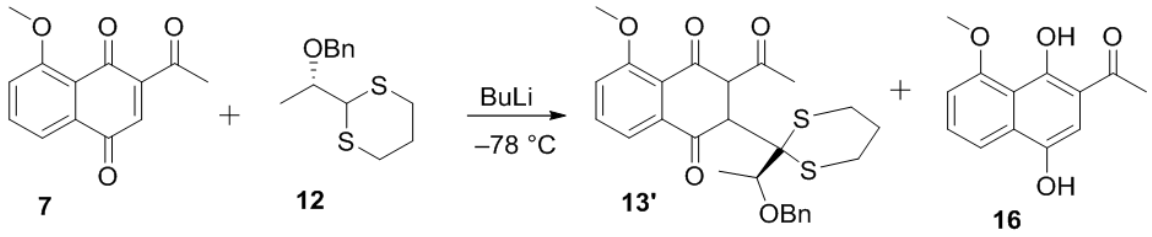
For the first option, all the reactive was added together using DMF as a solvent, NaCN as a catalyst without base and at 35 °C. The NMR showed an incorrect product, because there was no benzyl signal in it. The product that was characterized was the only one fluorescent in 365 nm as it had to be the product. For this reason, the next step is the synthesis of the Stetter catalyst which was synthesized according to the literature.[9]

The second and third option were to do the reaction using thiazolium salt as catalyst and trying with two different solvents, EtOH and DMF, but the compound **12** was not present in the HPLC-MS, only the reduced starting material **16.**

The forth option was to leave first the thiazolium salt stirred magnetically with thetriethyl amine to deprotonate it, and after thatthe addition of **7**, but this option also produces the reduced starting material **16.**

To see what was happening a test reaction was made, using only **7**, the thiazolium salt and the triethylamine. This test shows that when the base is added to the solution the Michael acceptor gets reduced and the solution gets darker, but if an oxidant reagent (CAN) is added, the solution becomes clearer. This means than in the moment of the addition of the base, for an unknown reason, **7** gets reduced to **16**.

The last option to do the Stetter reaction was to synthesize the 3-(benzyloxy)-2-hydroxybutanenitrile before the reaction. Then trying it with a soft base and without base, the fifth and sixth option respectively, but it did not work. In the solution with base **16** appears in the first second, and in the solution without base, there are no new spots until 24 h later when **16** appears.



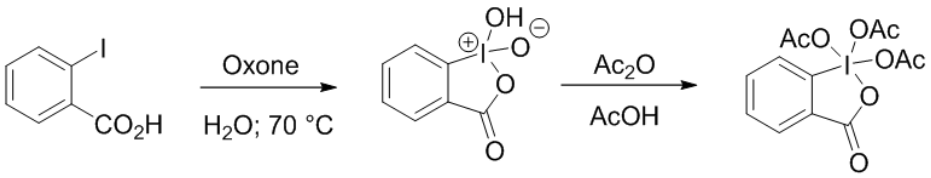
The Corey-Seebach reaction is another way to use the umpolung to do the addition to an α,β-unsaturated system, but using a thioacetal as it was explained in the section 3.1.2.

The reaction was made with base and without base to see if the base would affect **7** and if it would be possible to do it without base to avoid it. The characterization was done using TLC-MS.

The TLC shows different spots in the UV light of 365 nm:

In all the cases appears m/z = 255.1 which is the molecule weight of the reducted starting material **16** plus sodium as the anion. The others m/z are not possible to identify, but there is not m/z = 403.12 which belongs to **13'** plus sodium This means that the reaction was not done in the proposed direction.

4.3.1 Dess-Martin Periodinine(DMP)



Two steps were necessary for the synthesis of DMP from the 2-iodobenzoic. The first step was the synthesis of the IBX:

This reaction was done twice because the first time the ^{1}H NMR showed only a 62% of IBX, and 38% of 2-iodosobenzoic, the intermidiant of the reation. The becoming mixtured was oxidized again with 1.3 eq of oxone to afford a 96% of IBX and 2% of 2-iodosobenzoic.

The first result can be caused from the insufficient oxone in the reaction, it will be better to have 2 eq instead of 1,3 eq.

Table 3: Results of the reaction

Yield 1(%)	Yield 2(%)	Finally yield(%)
82	71	65

The yield is smaller because the reaction was done twice, the next time it will be better to do it from the first moment with two equivalents of oxone.

The second step of the reaction was the addition of the acetic group to the IBX to synthesize the DMP. This reaction was made with acetic anhydride as the reagent and glacial acetic acid as the solvent.

The reaction is a fast reaction (30 min) which have a yield of 79% and the NMR shows a huge purity.

5 Experimental part

5.1 General information

In this section it is possible to find the necessary equipment to do the purification and characterization of the products:

Thin layer chromatography (TLC)

To do the TLC, TLC POLYGRAM SIL G/UV_{254} was used, with a layer of 0.22 mm silica gel 60 with fluorescent indicator from the brand Macherey-Nagel.

Silica column

The silica column was manually prepared for all the purifications. There were different sizes of columns from 5 to 50 g. They were filled with silica 60 M with a diameter of 0.04-0.063 mm from the brand Macherey-Nagel.

The eluent was ethyl acetate and petrol ether 60/90, which were distillated before to be more pure, and the mixture was made in situ in the mixture pump.

Flash chromatography

The pumping system for the flash chromatography was composed of two pumps S1122 solvent delivery system from the brand Sykam

The product of the flash chromatography has been recollected automatically in vials using the LKB 2211 Super rac, an automatic collector from the brand LKB bromma.

The UV/ DAD detector is a S3210 UV/VIS detector from the brand Sykam, which uses the program ChromStar6.3 to process the datas.

Automatic spotting machine

To make some of the TLC after the flash chromatography, Auto-Spot Robot ASP 222 was used from the brand Intavisag.

Melting point

To determinate melting points, the Melting Point M560 from the brand Buchiwas used.

Mass spectrometry

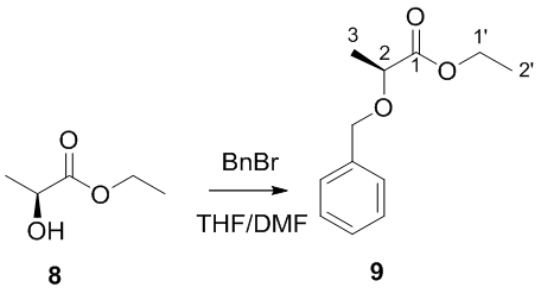
The mass spectrometry was done using the Esquire 3000 Plus from the brand Bruker Daltonics. This MS use the electron spray ionization (ESI)

Nuclearmagneticallyresonance (NMR)

The NMR was done with a Bruker Avance 400 equipped with a 5 mm ATMHead at 400.13 MHz (1H), 100.13 MHz (Fig. 13C). As the internal standard is tetramethylsilane. Respectively, the chemical shifts (δ) in ppm and the coupling constants (J) in Hz. The 13C spectra experimental are 1H-broad band decoupled. The respective signals were assigned by the recordings of 13C-DEPT, and correlation spectra such as H, H-COZY, HSQC and HMBC.

5.2 Experimental and analytical details

5.2.1 Ethyl (S)-2-(benzyloxy)propanoate)(9) [12]



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In a 250 mL round flask, ice-cooled bath and under inert atmosphere, sodium hydride (3.8 g, 94.4 mmol, 1.1eq) was slowly added to a solution of ethyl (*S*)-2-hydroxypropanoate (10 mL, 85.8 mmol, 1 eq), benzyl bromide (12.5 mL, 103 mmol, 1.2 eq) in DMF (50 mL) and THF(50 mL). The reaction was stirred magnetically at 0 °C during 5h and at room temperature overnight.

After 24h the TLC 30%EtOAc in PE as eluent, showed no starting material.

The reaction was quenched with water, extracted with ethyl acetate (3 × 25 mL), saturated sodium chloride solution (2 × 25 mL), dried with sodium sulfate,filtered and concentrated in vacuum.

The product was purified using a 20 g silica column with 5 % EtOAc in PE as eluent to obtain 4.59 g (25%) of a colourless oil.

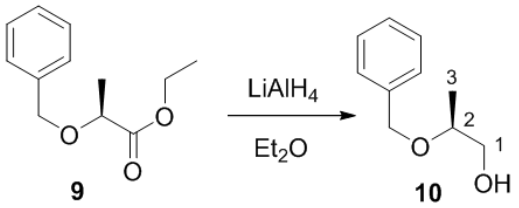
Yield = 25%

R_f= 0.50 (30 % EtOAc in PE)

^{1}H-NMR (400 MHz, $CDCl_3$): δ= 7.11–7.32 (m, 6H, arom.-H), 4.60 (d, *J* = 11.7 Hz, 1H, CH2-H), 4.36 (d, *J* = 11.9 Hz, 1H, CH2-H), 4.07–4.16 (m, 2H, 1'-H), 3.95 (q, *J* = 6.8 Hz, 1H, 2-H), 1.34 (d, *J* = 6.8 Hz, 3H, 3-H), 1.19 (t, *J* = 7.2 Hz, 3H, 2'-H) ppm.

^{13}C-NMR (101 MHz, $CDCl_3$): δ = 173.3 (C-1), 137.6 (C-arom.), 128.2 (C-arom.), 74.1 (C-2), 72.0 (C-CH2), 60.8 (C-1'), 18.7 (C-3), 14.3 (C-2') ppm.

5.2.2 (S)2-(benzyloxy)propanoat-1-ol(10)[12]



In a 100 ml round flask, under inert atmosphere,ethyl-2-(benzyloxy)propanoat (1.04 g, 5 mmol, 1 eq) was added to a suspension of Lithiumaluminiumhydride (0.49 g, 5 mmol, 1eq) in dry diethyleter (15 mL) using ice-cooled bath, and was stirred magnetically at 0 °C during 1 h. The reaction was monitoritzed with TLC (10% EtOAc in PE).

The solution was neutralized with a saturated ammonium chloride solution, and then filtered. The resulting solution was extracted with ethyl acetate (2 × 25 mL), dried with sodiumsulfat, filtered and concentrated in vacuum to obtain 0.35 g (43%) of a colourless oil.

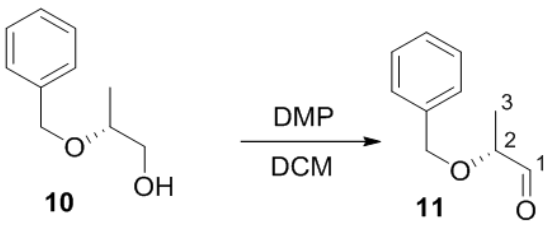
Yield = 43%

R_f= 0.15 (10 % EtOAc in PE)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.27–7.40 (m, 5H, arom.-H), 4.65 (d, *J* = 11.5 Hz, 1H, CH_2-benzyloxy), 4.49 (d, *J* = 11.6 Hz, 1H, CH_2-benzyloxy), 3.64–3.73 (m, 1H, 2-H), 3.61 (dd, *J* = 11.4 Hz, *J* = 3.4 Hz, 1H, 1-H), 3.50 (dd, *J* = 11.5 Hz, *J* = 7.1 Hz, 1H, 1-H), 2.20 (s, 1H, OH), 1.18 (d, *J* = 6.2 Hz, 3H, 3-H) ppm.

^{13}C-NMR (101 MHz, $CDCl_3$): δ = 138.5, 128.6, 127.8, 127.1 (C-arom.), 75.7 (C-2), 70.9 (C-7), 66.4 (C-1), 16.0 (C-3) ppm.

5.2.3 (*R*)-2-(benzyloxy)propanal (11)[13]



In a 25 mL round flask and inert atmosphere, Dess-Martin periodinane (201.2 mg, 0.4743 mmol, 1.4 eq) was added to a solution of 2-(benzyloxy)propanoat-1-ol (56.7 mg, 0.3410 mmol, 1 eq) in DCM (2 mL) and stirred magnetically during 1 h. The reaction was monitorized with a TLC (10% EtOAc in PE) until it was over.

The solution was quenched with sodium thiosulfate (0.33 g) and saturated sodium bicarbonate solution (1.4 mL). After 15 min the product was extracted with ethyl acetate (2 × 10 mL), saturate sodium chloride solution (10 mL), dried with sodium sulfate, filtered and concentrated in vacuum.

The product was purified with a column of 10 g silica using 5 % EtOAc in PE as a eluent to obtain 21 mg (43%) of a colourless oil.

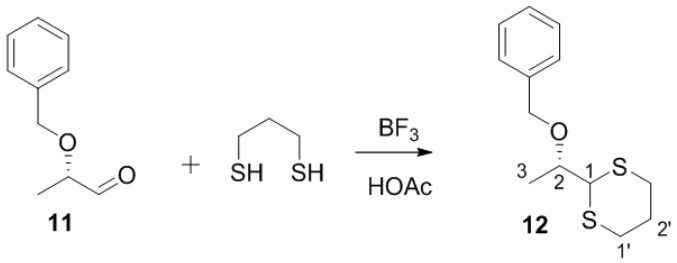
Yield = 43%

R_f= 0.38 (5 % EtOAc in PE)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.67 (d, *J* = 1.7 Hz, 1H, 1-H), 7.19–7.48 (m, 5H, arom.-H), 4.66 (d, *J* = 11.7 Hz, 1H, CH_2-benzyloxy), 4.62 (d, *J* = 11.7 Hz, 1H, CH_2-benzyloxy), 3.90 (qd, *J* = 6.9 Hz, *J* = 1.8 Hz, 1H, 2-H), 1.34 (d, *J* = 7.0 Hz, 3H, 3-H) ppm.

^{13}C-NMR (101 MHz, $CDCl_3$): δ = 203.5 (C-1), 137.5, 128.7, 128.2, 128.1 (C-arom.), 79.6 (C-2), 72.1 (CH_2-benzyloxy), 15.4 (C-3) ppm.

5.2.4 (*R*)2-(1-(benzyloxy)ethyl)-1,3-dithiane[14]



In a 25 ml round flask and inert atmosphere, 2-(benzyloxy)propanal (0.215 g, 1.31 mmol, 1eq), 1,3-propenedithiol (0.43 mL, 4.32 mmol, 3.3 eq) and BF_3 (0.5 mL) in HOAc (5mL) was added and stirred magnetically during 2.5 h at RT. The TLC 30 % EtOAc in PE showed no starting material.

The product has been solved in ethyl acetate (20 mL), clean up with $NaHCO_3$ (3 × 15 mL), saturated sodium chloride solution (2 × 15 mL), dried with sodium sulfate and concentred in vacuum.

The dried product was purified using the 20 g silica column with 30 % of EtOAc in PE as eluent to obtain 150 mg (45%) of a colourless oil.

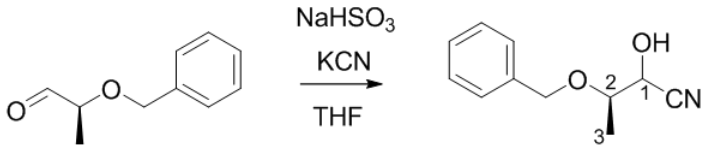
Yield= 45%

R_f= 0,38 (5 % PE: EtOAc)

^{1}H-NMR (400 MHz, $CDCl_3$): δ= 7.14–7.33 (m, 5H, arom.-H), 4.54 (s, 2H, CH2-H), 4.20 (d, *J* = 5.0 Hz, 1H, 1-H), 3.68 (qd, *J* = 6.4 Hz, *J* = 1.0 Hz, 1H, 2-H), 2.76–2.85 (m, 4H, 1'-H), 1.98–2.06 (m, 1H, 2'-H), 1.73–1.88 (m, 1H, 2'-H), 1.28 (d, *J* = 6.4 Hz, 3H, 3-H) ppm.

^{13}C-NMR (101 MHz, $CDCl_3$): δ = 138.2 (C-arom.), 128.2 (C-arom.), 71.4, 53.5, 30.5, 30.4, 26.3 17.8(C-3) ppm

5.2.5 3-(Benzyloxy)-2-hydroxybutanenitrile



In a 25 mL round flask, a saturated sodium sulfatesolution (0.55 mL), potassium cyanide (64.72 mg, 0.994 mmol, 1.2 eq) in THF (1 mL) was added to a 0 °C solution of 2-(benzyloxy)propanal (136 mg, 0.828 mmol, 1 eq) in THF (1 mL).

After 2 h, the TLC (30% EtOAc in PE) showed no starting material.

The product was extracted with ether (3 × 10 mL), saturated hydrochloric acid solution (2 × 10 mL), saturated sodium chloride solution (2 × 10 mL), dried with sodium sulfate, filtered and concentrated in vacuum to obtain 107 mg (68%) of a colourless oil.

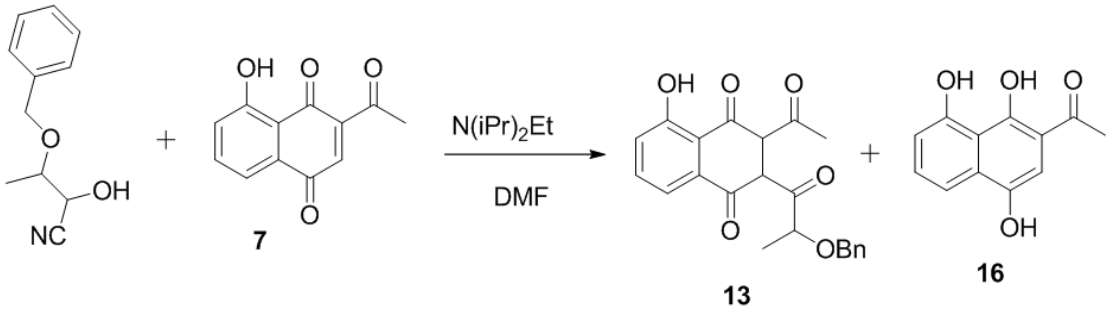
Yield = 68%

R_f= 0,18 (5 % EtOAc in PE)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.09–7.33 (m, 5H, arom.-H), 4.62 (q, *J* = 6.5 Hz, 1H, 2-H), 4.46 (dd, *J* = 34.6 Hz, *J* = 11.6 Hz, 1H, CH2-H), 3.63–3.75 (m, 1H, 1-H), 2.67 (s, 1H, OH-H), 1.23 (dd, *J* = 6.3 Hz, *J* = 2.6 Hz, 3H, 3-H) ppm.

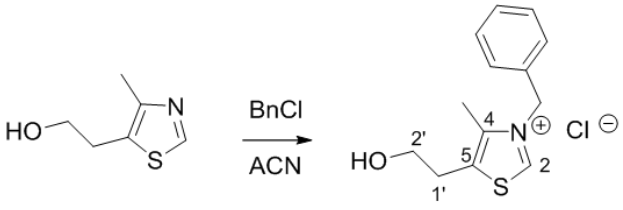
^{13}C-NMR (101 MHz, $CDCl_3$): δ = 136.9 (C-1), 128.1 (C-arom.), 74.8, 74.4, 71.6, 71.1, 65.0, 15.2 (C-4) ppm

5.2.6 3-Acetyl-2-(2-(benzyloxy)propanoyl)-5-methoxy-2,3-dihydronaphthalene-1,4-dione (13)



In 25 ml round flask and under inert atmosphere, $N(iPr)_2Et$ (0.123 mL, 0.479mmol, 3 eq) was added to a solution of 3-(benzyloxy)-2-hydroxybutanenitrile (30.51 mg, 0.160mmol, 1 eq) in DMF (1 mL). After 5 min the 2-acetyl-8-methoxynaphthalene-1,4-dione (38.45 mg, 0.1595 mmol, 1 eq) was added to the previous solution. The mixture was stirred magnetically at 70 °C during 24 h and monitoritzed with a TLC (50 % EtOAc in PE). No product was observed, only the reduced starting material **16**.

5.2.7 3-Benzyl-5-(2-hydroxyethyl)-4-methylthiazol-3-iumchloride[9]



In 25 ml round flask and under inert atmosphere, 2-(4-methylthiazol-5-yl)ethan-1-ol (2.5 mL, 20.95 mmol, 1 eq) and chloromethylbenzene (2.2 mL, 20.95 mmol, 1 eq) in acetonitrile (10 mL) was added. The solution was stirred magnetically during 48 h at 95 °C using a condenser. After it appeared a precipitated, which using a TLC (80 % EtOAc in PE) showed only the main product.

The product was filtrated and concentrated in high vacuum during 2 h to obtain 4.22g (75%) of a colourless solid.

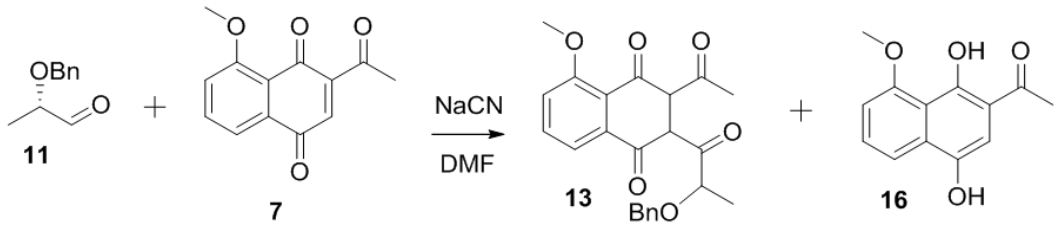
Yield = 75%

Melting point = 143 °C (Acetonitril)

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 7.28–7.55 (m, 5H, arom.-H), 5.83 (s, 2H, CH2-H), 5.36 (dd, *J* = 9.7 Hz, *J* = 4.5 Hz, 1H, OH-H), 3.63 (dd, *J* = 10.9 Hz, *J* = 5.5 Hz, 2H, 2'-H), 3.02 (t, *J* = 5.6 Hz, 2H, 1'-H), 2.35 (s, 3H, CH3-H) ppm.

^{13}C-NMR (101 MHz, DMSO-d_6): δ = 157.4 (C-1), 141.8, 136.4, 133.3, 128.9 (C-arom.), 59.8, 55.8, 29.7 (C-CH3), 11.7 ppm

5.2.8 3-Acetyl-2-(2-(benzyloxy)propanoyl)-5-methoxy-2,3-dihydronaphthalene-1,4-dione(13)[9]



In a 10 mL round flask and inert atmosphere, a solution of (*R*)-2-(benzyloxy)propanal (30.12 mg, 0.1827 mmol, 1 eq) in dry DMF (0.5 mL) was added to the solution of NaCN (3 mg, 0.0612 mmol, 0.34 eq) in DMF (0.5 mL) and stirred magnetically during 10 min at 35 °C.

After a solution of 2-acetyl-8-methoxynaphthalene-1,4-dione (32 mg, 0.1390 mmol, 0.75 eq) in DMF (0.5 mL) (orange solution) was added and stirred during 1h at 35°C (dark brown color).

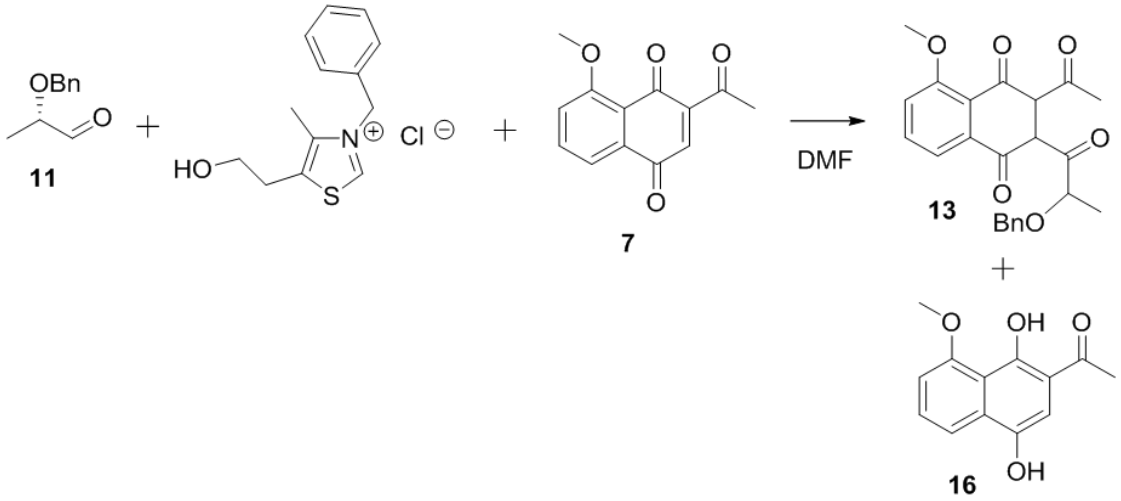
The reaction was monitorized with a TLC (50 % EtOAc in PE).

The reaction was quenched with water (2 mL) (brown chocolate color), was extracted with chloroform (3 x 10 mL) (red color), dried with sodium sulfate, filtered and concentrated in vacuum.

The product was purified using a 10 g silica column using 50 % EtOAc in PE as an eluent to obtain 40 mg of **16**

HPLC-MS (m/z): 255.1 (8.1min), 282.5 (8.6min), 297.2 (9.1 min) 328.5 (9.6min), 418.0 (11.2min),

5.2.9 3-Acetyl-2-(2-(benzyloxy)propanoyl)-5-methoxynaphthalene-1,4-dione(13)[9]

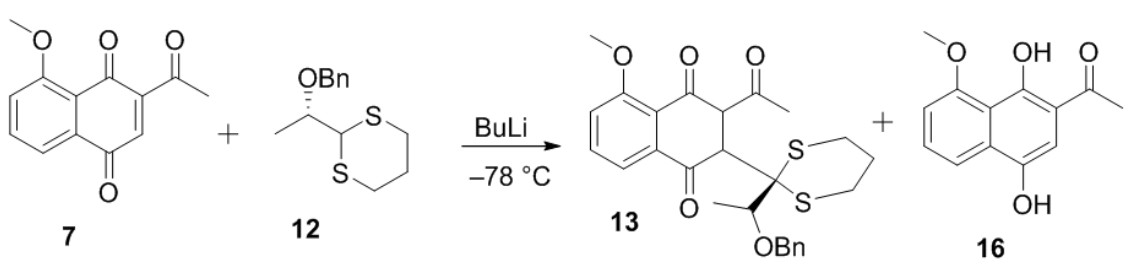


In a 25 mL round flask and under inert atmosphere, triethylamina (0.03 mL, 0.36 mmol, 2eq) in DMF (1 mL) was added to a solution of 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazol-3-ium chloride (4.93 mg, 0.0183 mmol, 0.1eq), (*R*)-2-(benzyloxy)propanal (30 mg, 0.183 mmol, 1 eq), 2-acetyl-8-methoxynaphthalene-1,4-dione (42 mg, 0.183 mmol, 1 eq) in ethanol (1 mL) and were stirred magnetically overnight at 70 °C.

The reaction was quenched with water (10 mL) and HCl solution (10 mL), was extracted with chloroform (3 × 10 ml) and sodium carbonate (2 × 10 ml), dried with sodium sulfate, filtered and concentrated in vacuum.

The product was purified using a 5 g silica column with 20 % of EtOAc in PE as eluent to obtain 3.56 mg of **16**.

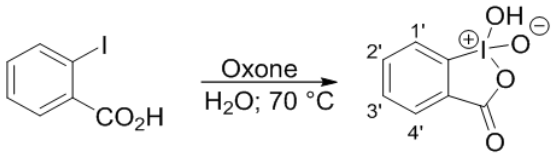
5.2.10 (*S*)-3-acetyl-2-(2-(1-(benzyloxy)ethyl)-1,3-dithian-2-yl)-5-methoxynaphthalene-1,4-dione(13)[15]



In a 10 mL round flask and under inert atmosphere, 2-acetyl-8-methoxynaphthalene-1,4-dione (13.3 mg, 0.0550 mmol, 1 eq), (*S*)-2-(1-(benzyloxy)ethyl)-1,3-dithiane (14 mg, 0.0550 mmol, 1 eq) and THF (1 mL) was added and stirred magnetically at – 80 °C during 10 min (yellow/browncolor). After that nBuLi (24 µL, 2.5 mol/L) was added carefully and stirred magnetically during 3.45 h (yellow/green color). The TLC 30% EtOAc in PE showed a big amound of starting material, so during 2 h it wasstirred magnetically at – 40 °C. After that another TLC showed the same result, therefore more nBuLi (24 µL, 2.5 mol/L) was added and it was stirred magnetically at room temperature during the weekend.
After the weekend, the solution had a black/brown colour and the TLC showed the same result.
The reaction was quenched using a saturated ammonium chloride solution (10 mL) and water (5 mL) (the solution became yellow). The water phase was extracted with diethyl ether (3 x 5 mL), dried with sodium sulfate, filtred and concentrated in vacuum.
The product was purified using a 10 g silica column with 30 % of EtOAc in PE as eluent to obtain 1.4 mg of **16**.

TLC-MS (m/z): 216.0, 372.3, 416.0, 472.5, 516.2, 560.2,604.2 648.2, 692.2 (2.8 min, Rf= 0.59), without base
TLC-MS (m/z):428.9 (2.4 min, Rf= 0.59) 282.9, 428.2 (3.3 min, Rf= 0.59) with base
TLC-MS (m/z):255.1, 298.4, 415.3, 454.2 (1.9 min, Rf= 0.49) with base.

5.2.11 Dess-Martin Periodinane (1 step, IBX)[11]



In a 2 L round flask, 2-iodobenzoic acid (50 mg, 0.2 mmol, 1eq) was added to a suspension of oxone (181 mg, 0.29 mmol, 1.3 eq) in deionized water (650 mL). It was magnetically stirring during 3 h at 73 °C after 1,5 h in an ice-cooled bath.
It was not possible to do a TLC because the product is not soluble in organic solvents.
The product was washed with water (6 x 100 mL), acetone (2 x 100 mL) and dried overnight in a crystallazing dish. The mixtured was again suspended in oxone (181 mg,

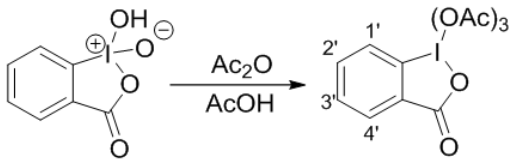
0.29 mmol, 1.3eq) in desionized water (650 mL) with the same conditions and procediment than before to obtain 36.76 g (65%) of a colourless solid.

The water residues were treated with Na_2SO_3 (70 g) and neutralized with sodium hydroxide (1 M).

Yield $_{\textbf{finally}}$ = 65%

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.15 (d, *J* = 7.8 Hz, 1H, 4'-H), 7.98–8.08 (m, 2H, 2',3'-H), 7.81–7.91 (m, 1H, 1'-H) ppm

5.2.12 Dess-Martin Periodinane (2n part).[16]



In a 1 L three-necked round flask and under inert atmosphere, IBX (36.76 g, 0.13 mol, 1 eq), acetic anhydride (125 mL) in glacial acetic acid (65 mL) was added and stirred magnetically during 30 min at 85 °C (internal temperature). After that it was stirred at room temperature for 24 h.

The precipitated was filtered to obtain 44.23 g (79%) of a coloureless solid.

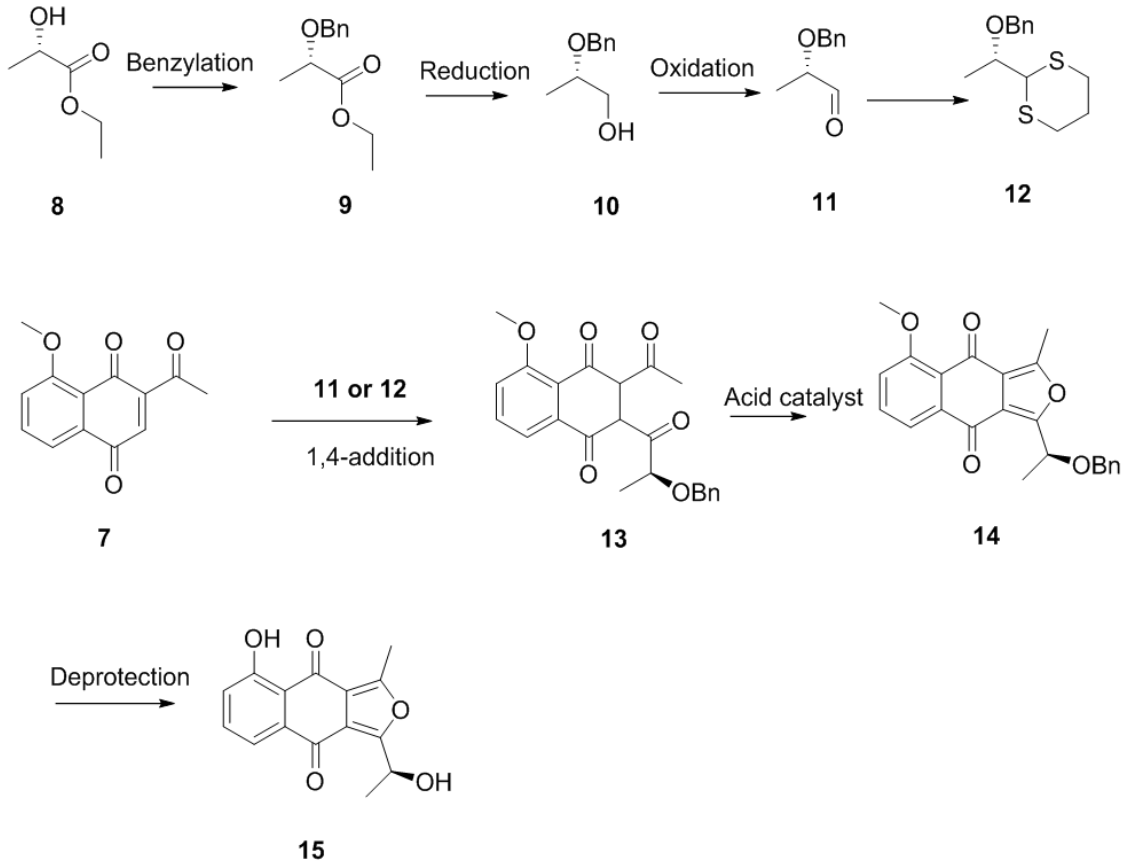
Yield = 79 %

^{1}H-NMR (400 MHz, $CDCl_3$): δ= 8.47 (dt, *J* = 7.7 Hz, *J* = 1.8 Hz, 2H, 2',3'-H), 8.25 (td, *J* = 7.8 Hz, *J* = 1.5 Hz, 1H, 4'-H), 8.08 (dt, *J* = 14.1 Hz, *J* = 7.5 Hz, 1H, 5'-H), 2.26 (s, 1H, -OAC), 2.17 (s, 5H, -OAc) ppm.

^{13}C-NMR (101 MHz, $CDCl_3$): δ = 176.1(C-OAc), 174.4(C-OAc), 166.5(C-OAc), 142.7 (C-arom.), 136.2 (C-arom.), 134.2 (C-arom.), 132.2 (C-arom.), 126.7 (C-arom.), 20.8(C-OAc) ppm.

6 Conclusions

The aim of this project is to be able to synthesize the bhimamycin A from the ethyl-L-lactate using a rout of 7 steps:



The first steps were the synthesis of the umpoluun to be able to do the 1,4-addition. This route did not have a high yield, but it had a high purifity.

The first route could be stopped in the aldehyde **11** to do the Stetter reaction. Until this moment, the first step had a worst yield with a 25% but the others were 43%. The overall yield of this rout was a 5%.

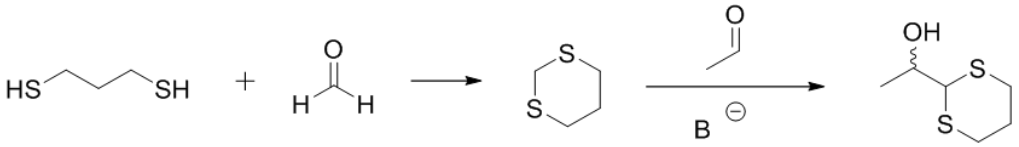
It was necessary to add 1,3-propanedithiol to do the Correy-Seebach reaction and it caused an additional step for the synthesis of **12** with a yield of 45%. This extra step made the yield decrease more, which meant a overall yield of 2%.

The next step should be the 1,4-addition, which had been tried using an umpolung in two different reactions: Stetter reaction and Corey-Seebach reaction. These reactions are very useful when an addition between two carbonyls wants to be made, but in this case, the problem was that in this addition it was necessary the presence of a base, which by an unknown reason, the Michael acceptor **7** got reduced. This is because **7** was also easily

reduced to **16** which contained a naphthalene, a more stable structure than the quinone. This is the main reason for the lack of success in this route, the impossibility of doing this addition.

In the future, it would be possible to try, for the last time, the Corey-Seebach reaction with a deprotected alcohol because maybe one of the problems was the benzoxy group can leave the molecule easily and the deprotonated alcohol is a bad leaving group. It had been tried to deptrotectit with palladium over carbon and with platinum, both under hydrogen atmosphere, but it did not react at all.

Another route to synthesize this molecule is:



This route is easier because there are only two steps, the first one is the addition of the 1,3-propendithiol to the formaldehyde, and the second one, it starts with the deprotonation of the dithiane, which after it will create an umpolung and will attack the acetaldehyde to produce the (*S*)-1-(1,3-dithian-2-yl)ethan-1-ol. This last molecule could do the Correy-Seebach reaction to produce **13**.

7 Literature

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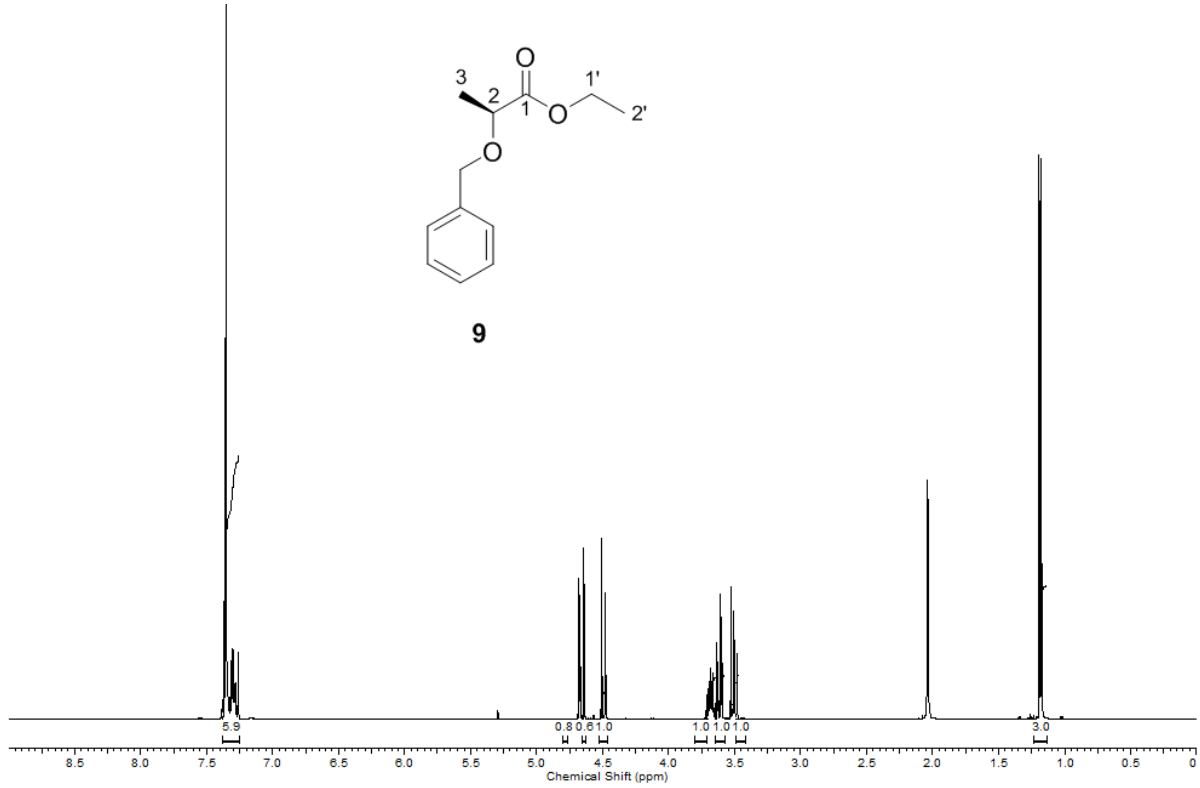
8 Annex

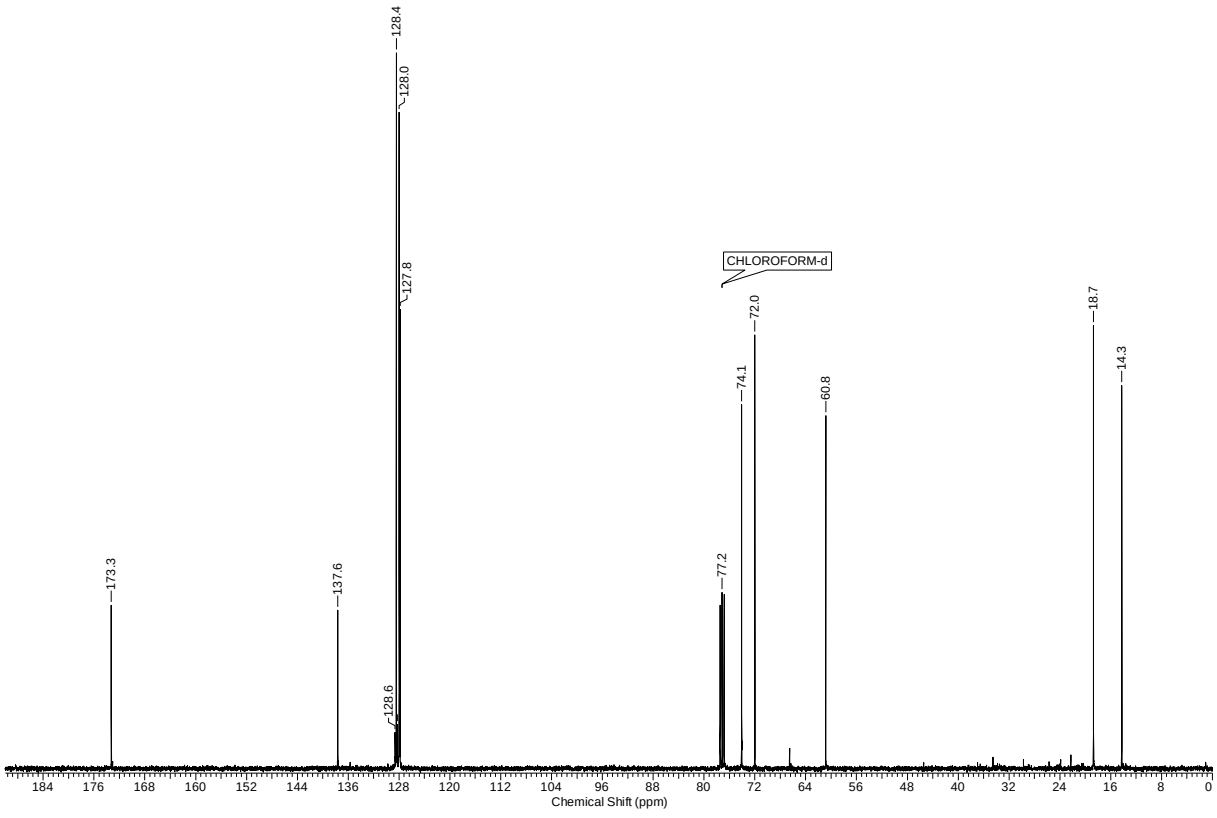
8.1 List of Abbreviations

n-BuLi	*n*-Butyllithium
d	Doublet
dd	Double doublet
DMF	Dimethylformamide
eq	Equivalents
EtOAc	Ethyl acetat
EtOH	Ethanol
g	Gram
h	Hour
HPLC-MS	High Performance Liquid Chromatography-Mass Spectrometry
J	Coupling constant
LDA	Lithium diisopropylamide
mL	Milliliter
mm	Millimeter
Mmol	Millimol
MW	Molecular weight
nm	Nanometres
NMR	Nuclear Magnetically Resonance
PE	Petrol ether
q	Quadruplet
R_f	Retention factor
RT	Room temperature
s	Singlet
t	Triplet
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TLC-MS	Thin layer chromatography- mass spectrometry
δ	Chemical shift

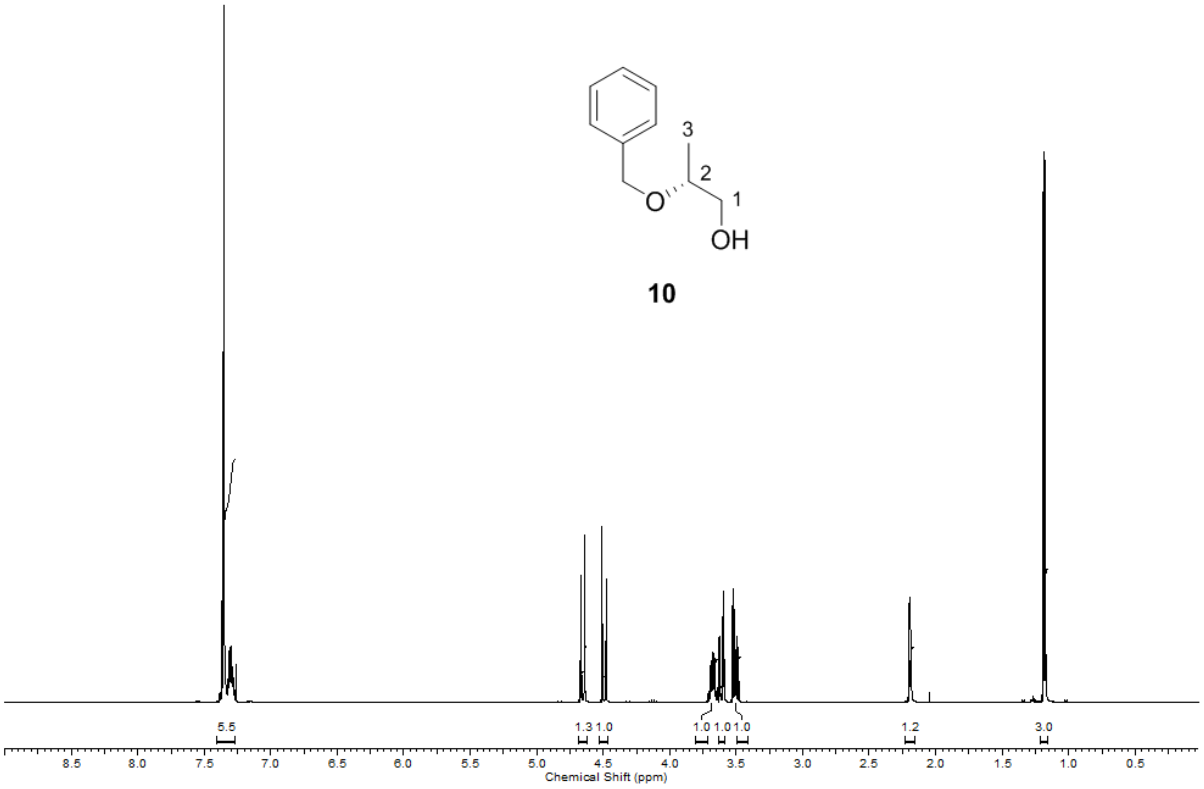
8.2 NMR-Spectrum

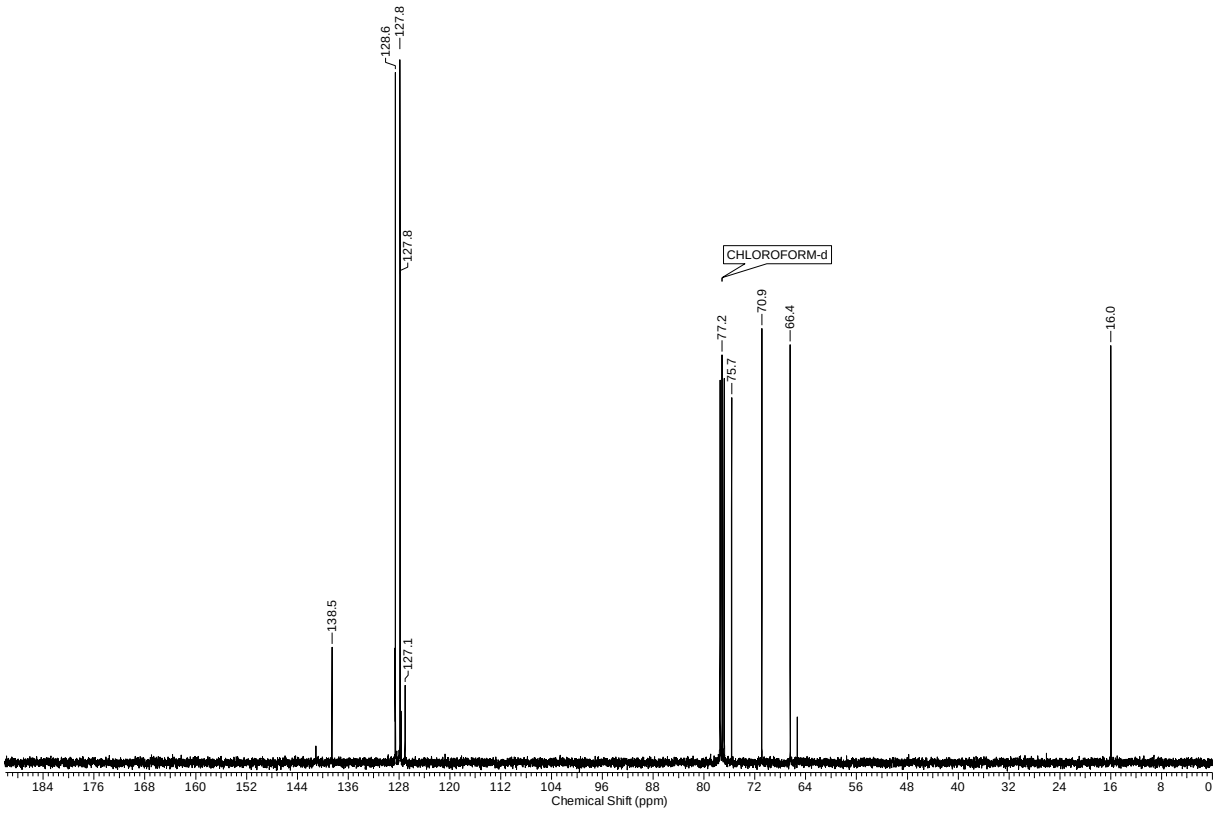
8.2.1 Ethyl (S)-2-(benzyloxy)propanoate)(9)



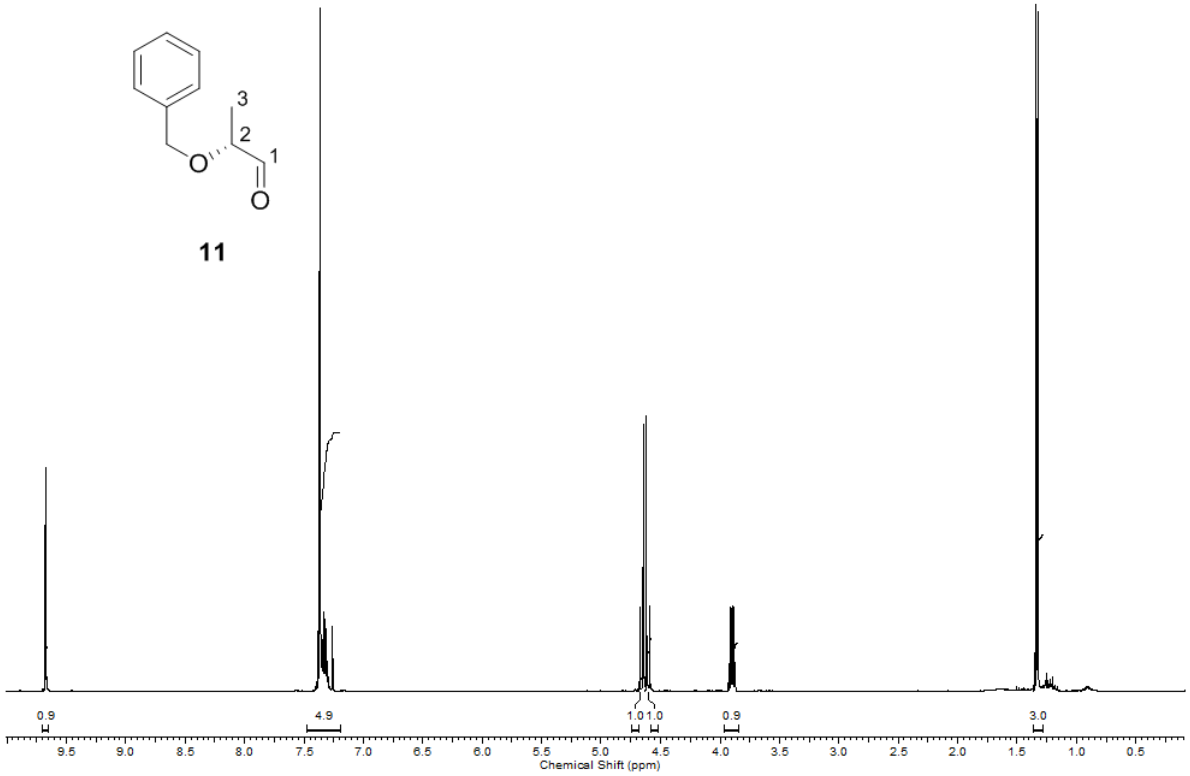


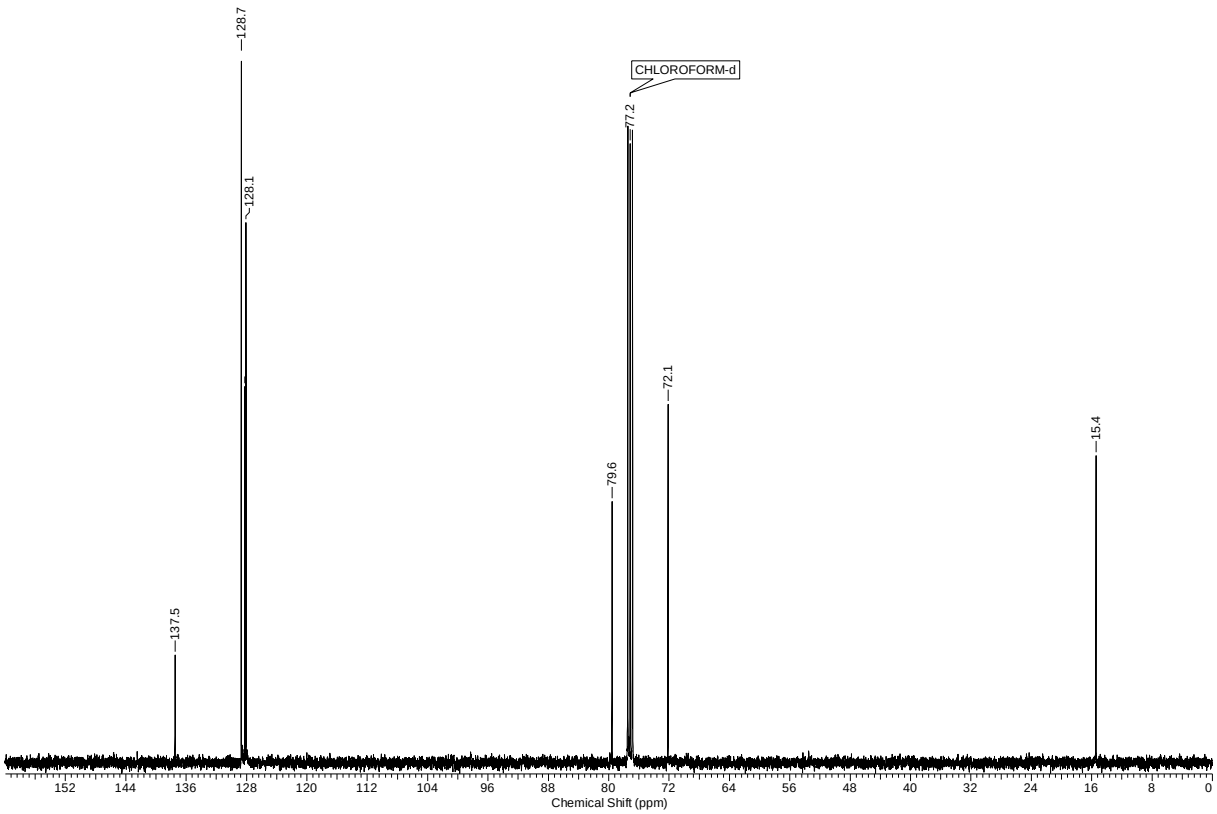
8.2.2 2-(Benzyloxy)propanoat-1-ol(10)



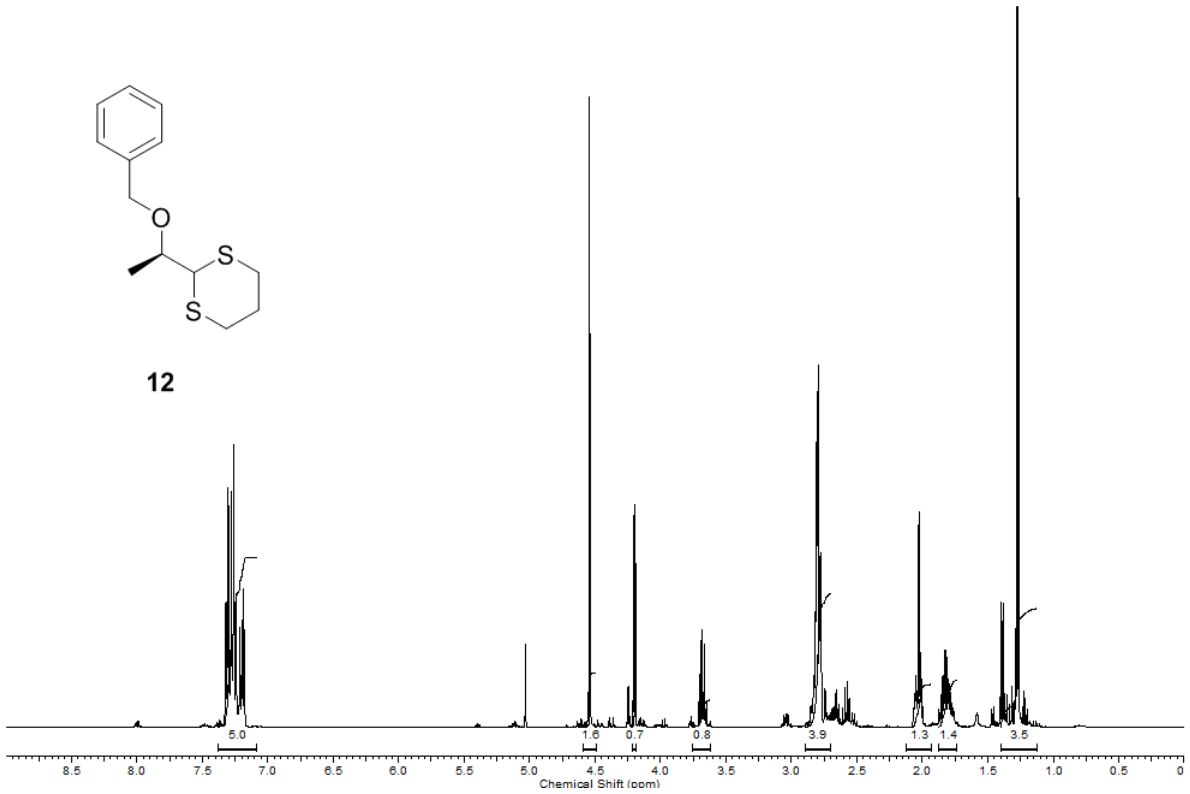


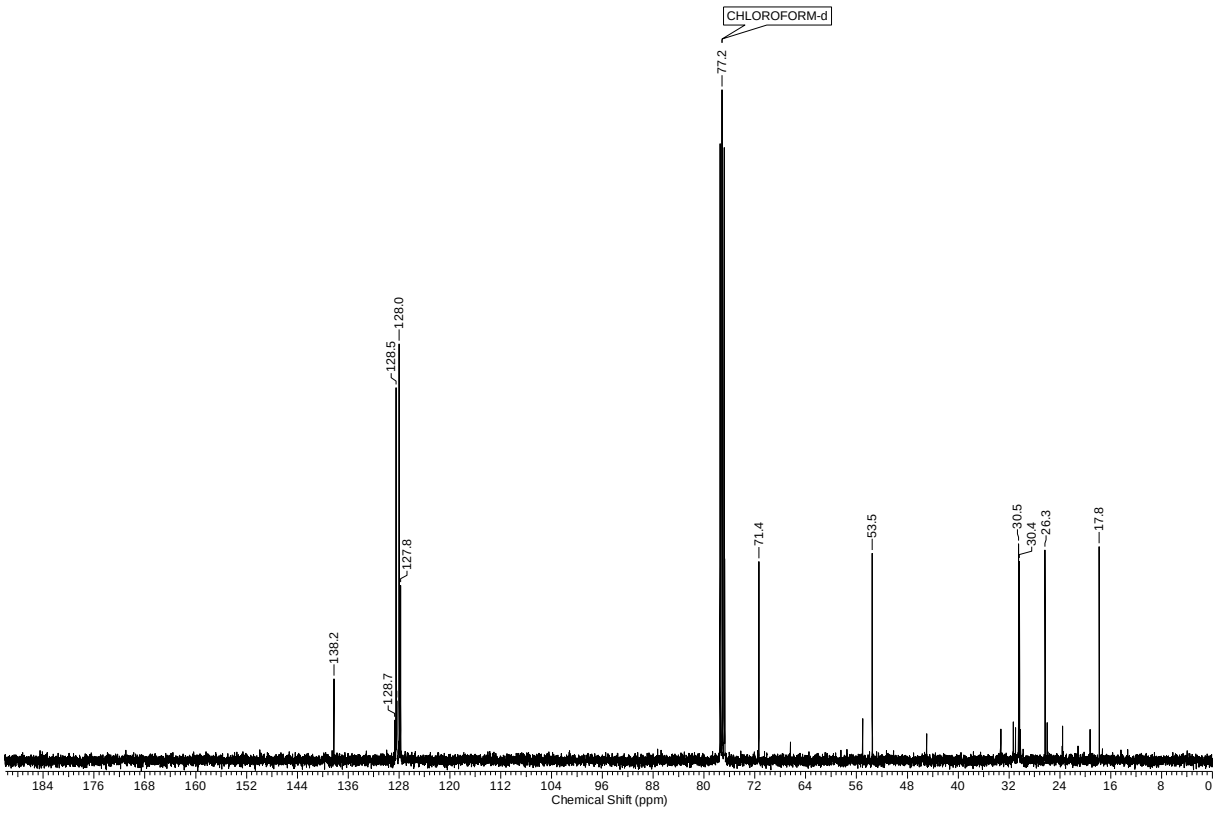
8.2.3 (*R*)-2-(Benzyloxy)propanal (11)



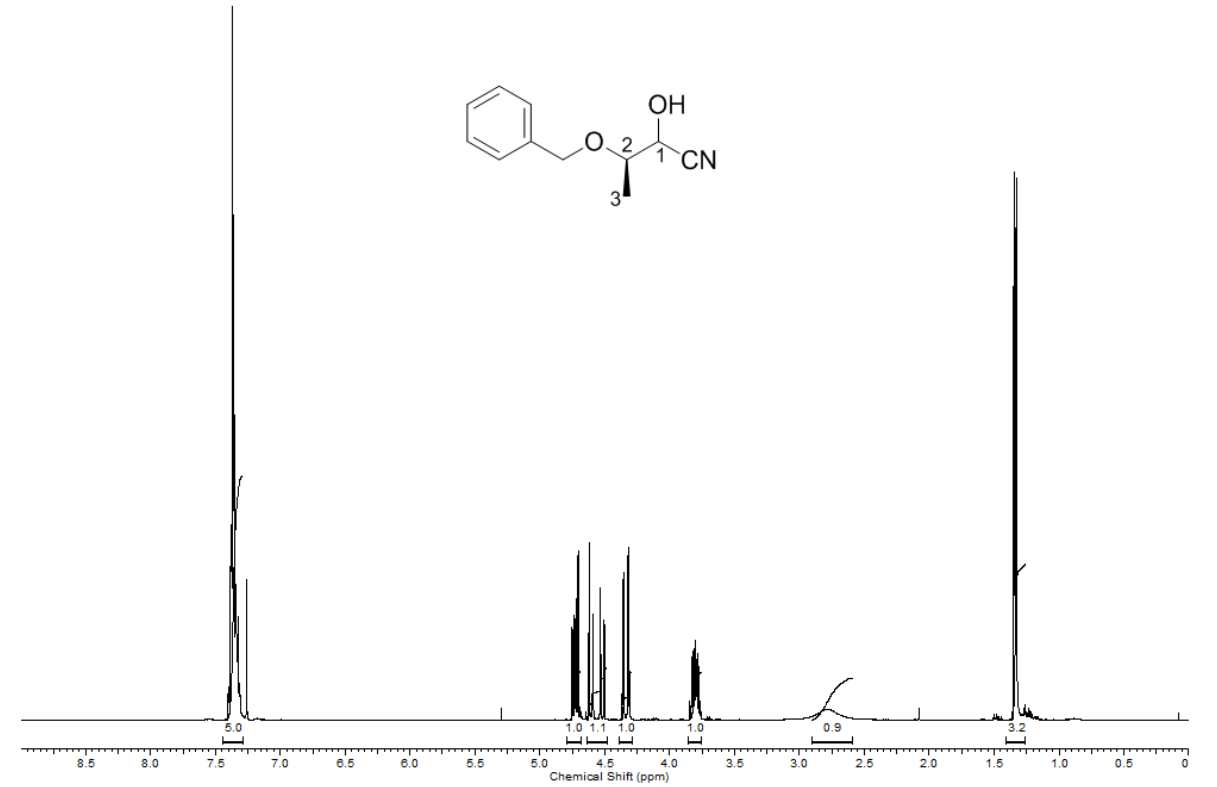


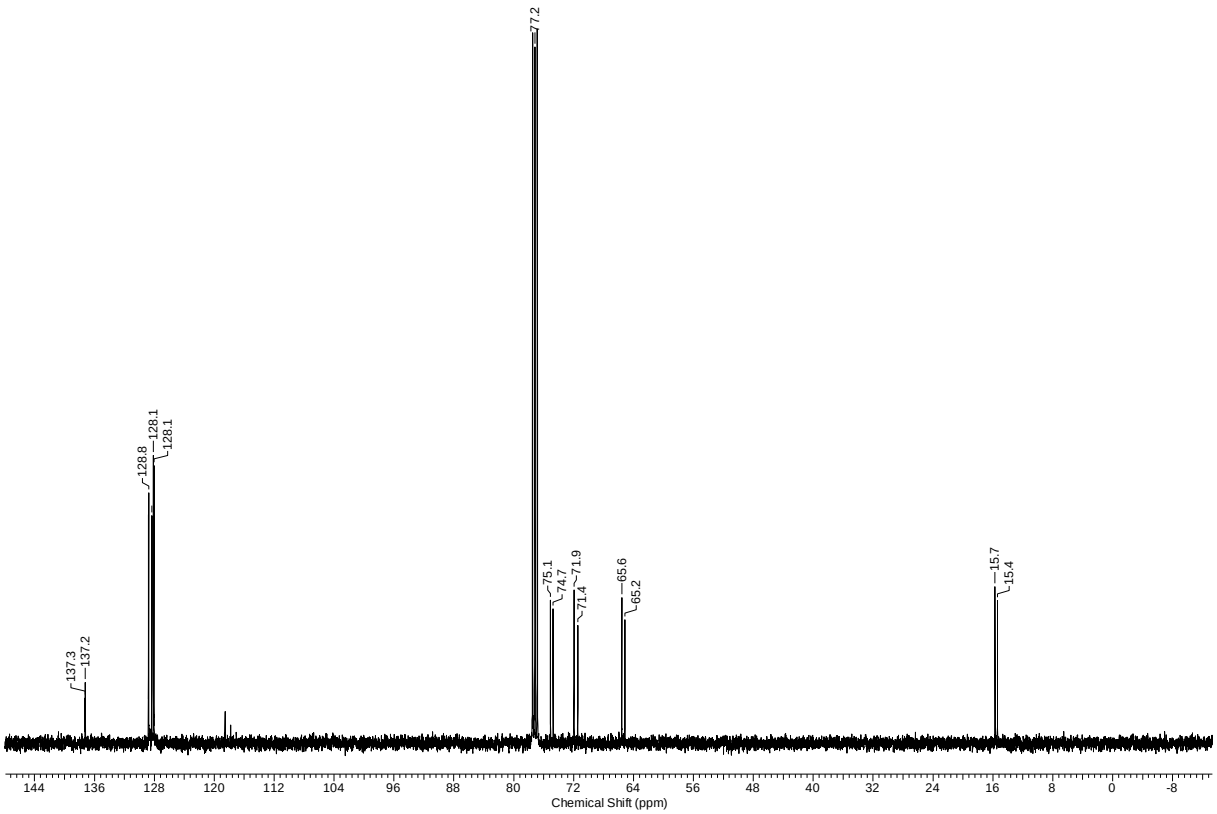
8.2.4 2-(1-(Benzyloxy)ethyl)-1,3-dithiane



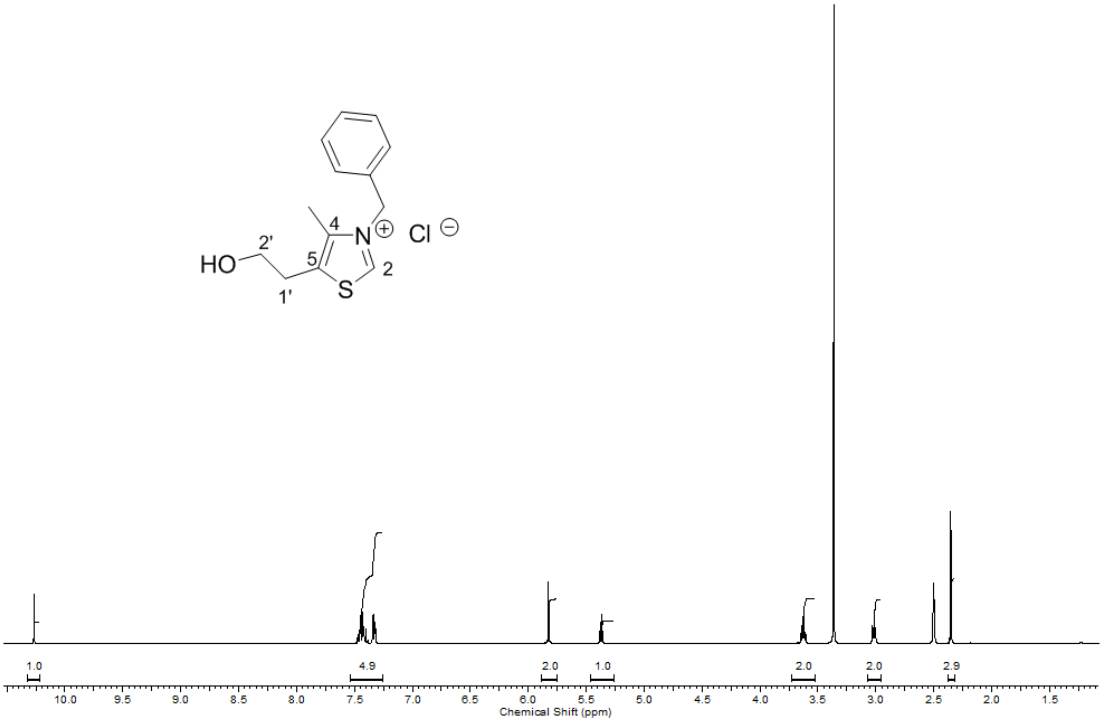


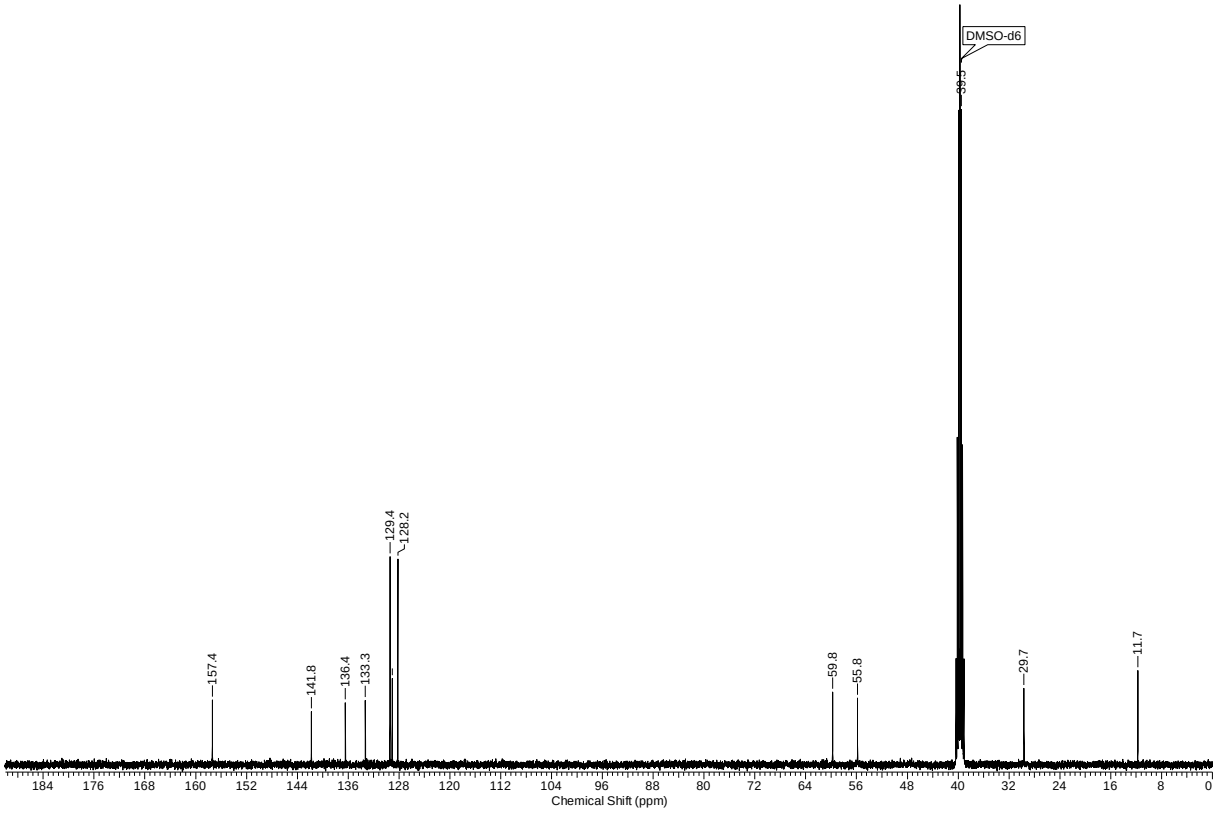
8.2.5 3-(Benzyloxy)-2-hydroxybutanenitrile



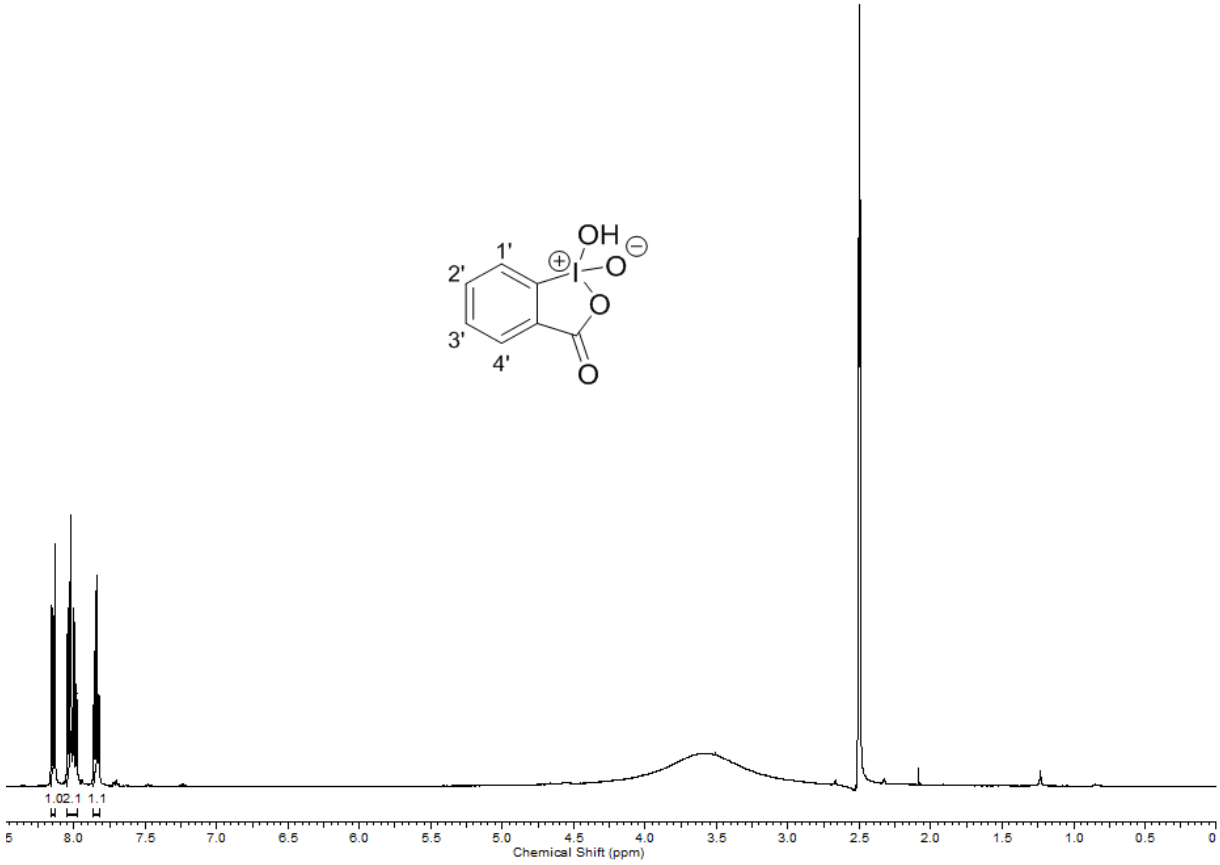


8.2.6 Benzyl-5-(2-hydroxyethyl)-4-methylthiazol-3-ium chloride [9]

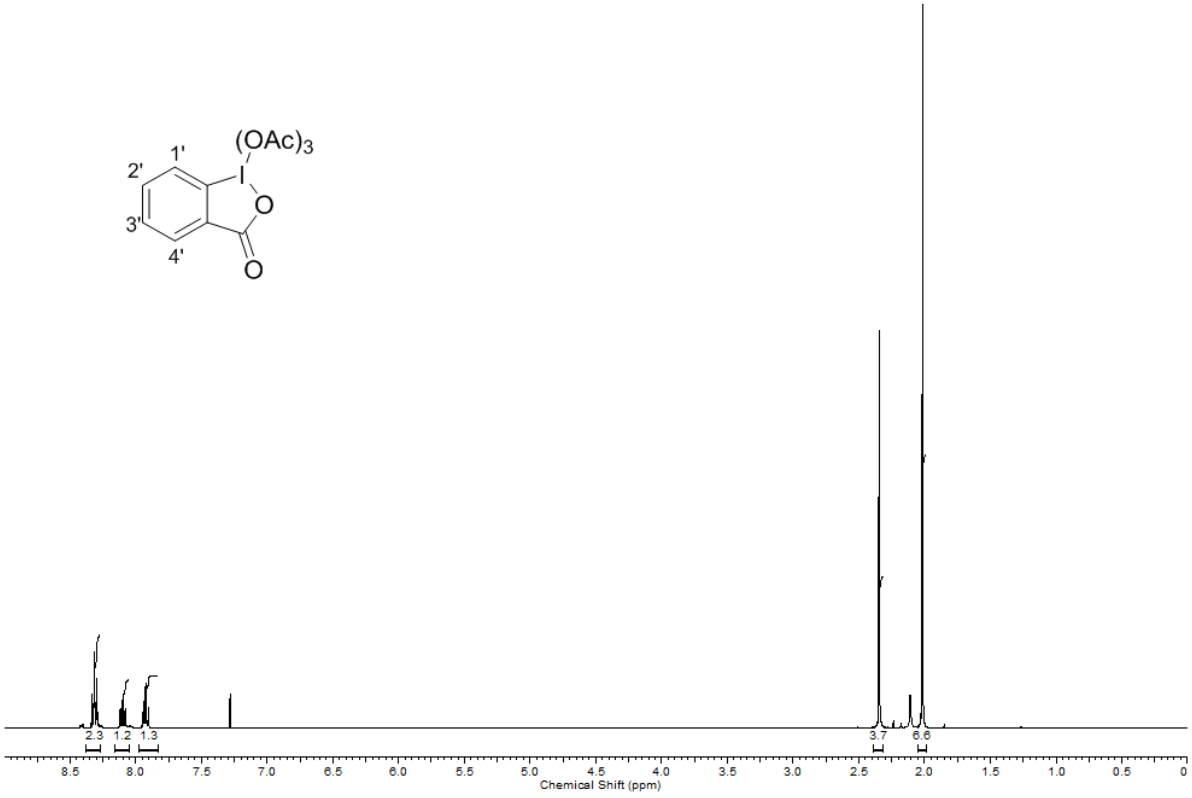


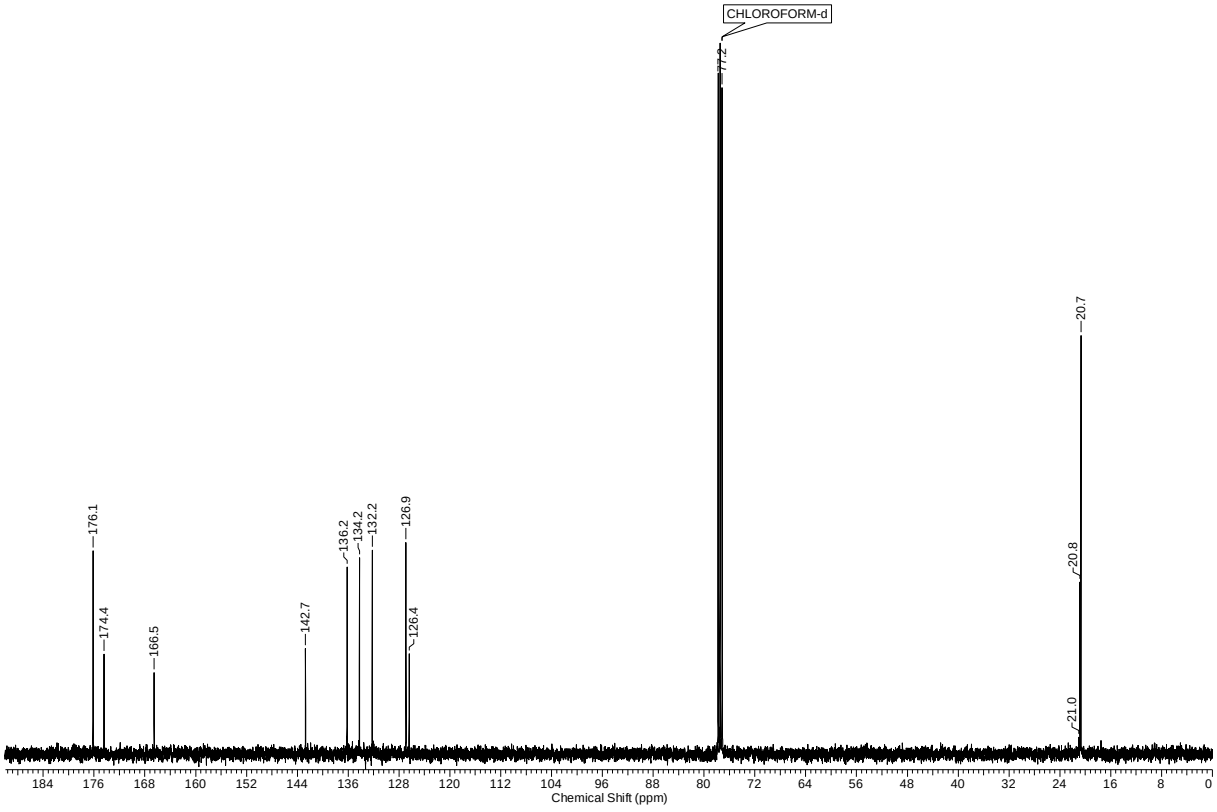


8.2.7 2-Iodoxybenzoic acid (IBX)



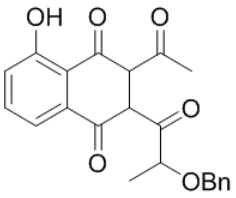
8.2.8 Dess-Martin Periodinine (DMP)





8.3 HPLC-MS

8.3.1 3-Acetyl-2-(2-(benzyloxy)propanoyl)-5-methoxy-2,3-dihydronaphthalene-1,4-dione (13)



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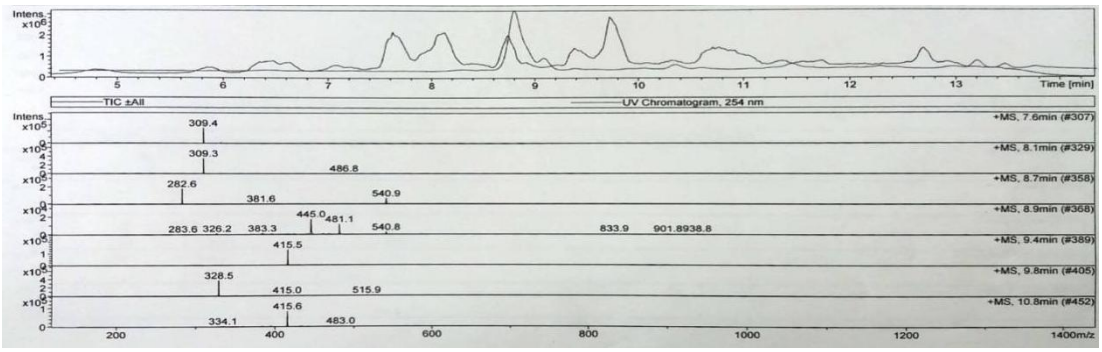
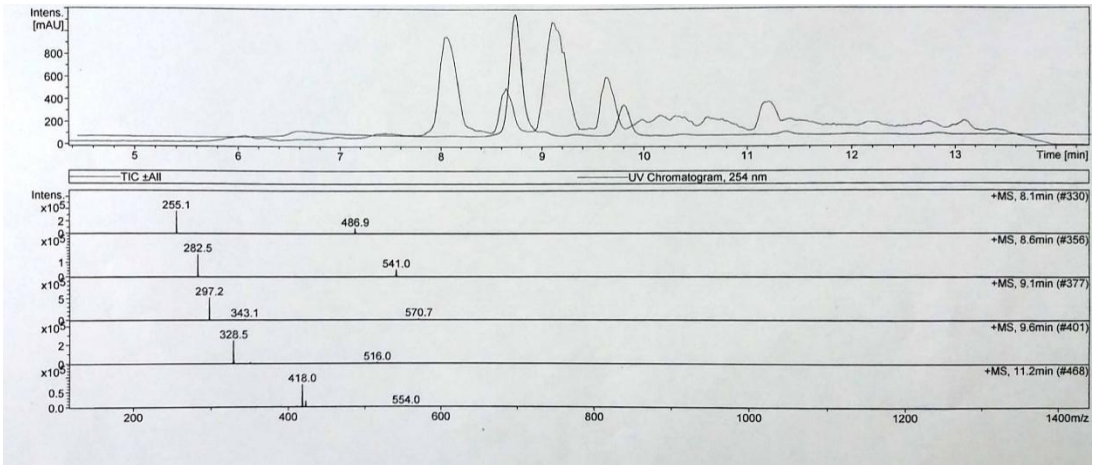
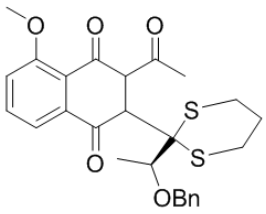




8.4 TLC-MS

8.4.1 (*S*)-3-acetyl-2-(2-(1-(benzyloxy)ethyl)-1,3-dithian-2-yl)-5-methoxynaphthalene-1,4-dione (13)



13'

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